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Revision of *Dvivarnus* (Scelionidae, Teleasinae)

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Abstract

Two new species, *Dvivarnus elektrolythron* Talamas & Mikó, **sp. n.** and *D. mikuki* Talamas & Mikó, **sp. n.** are described. The genus is redescribed and a key is provided to separate *Dvivarnus* from other groups in Teleasinae with mesoscutellar spines.

Keywords

Teleasinae, *Dvivarnus*, *Trimorus*, *Gryonoides,* mesoscutellum

Introduction

The subfamily Teleasinae is well defined morphologically by wing venation (elongate marginal vein, short stigmal and postmarginal veins), the anterior pronotal process and, in most cases, a compact ocellar triangle. Generic classification within the subfamily is another matter and a thorough phylogenetic analysis is needed. The vast majority of species are found in *Trimorus* Förster, a genus whose limits are poorly defined with respect to many of the smaller genera. *Dvivarnus* Rajmohana & Veenakumari is a well defined teleasine genus that morphologically falls well outside of *Trimorus* and until now was monotypic.

We here expand knowledge about the species-level diversity in *Dvivarnus* with the addition of two new species. We also provide additional characters to those of Veenakumari et al (2011) for its diagnosis relative to two lineages in Teleasinae that also have mesoscutellar spines, *Gryonoides* Dodd and the *Trimorus carus* Nixon species group. The analysis of *Gryonoides* follows the examination of 12 species conducted as part of an active revision of this genus by the second author. Our treatment of the *Trimorus carus* species group is based on examination of the holotype of *T. carus* Nixon and two undescribed species from the Central African Republic that share the presence of a distally bifurcating metascutellar spine.

Materials and methods

The numbers prefixed with "USNMENT" or "OSUC " are unique identifiers for the individual specimens (note the blank space after some acronyms). Details on the data associated with these specimens may be accessed at the following link: purl.oclc.org/NET/hymenoptera/hol, and entering the identifier in the form. Persistent URIs for each taxonomic concept were minted by xBio:D in accordance with best practices recommended by Hagedorn et al (2013). Morphological terms were matched to concepts in the Hymenoptera Anatomy Ontology (Yoder et al 2010) using the text analyzer function. A table of morphological terms and URI links is provided in Suppl. material 1.

We represent natural language phenotypes in an Entity:Quality (EQ) format: Entity attribute: value. Semantic statements of natural language phenotypes (Suppl. material 2.) were composed in Protégé 5.0 (http://protege.stanford.edu/) using the OWL Manchester syntax (http://www.w3.org/TR/owl2-manchester-syntax/) following Balhoff et al (2013) and Mikó et al (2014). The full data set, represented in OWL (Web Ontology Language; http://www.w3.org/TR/owl2-overview/ last accessed February 4, 2014), was deposited as a Resource Description Framework (RDF)-XML file (http://www.w3.org/TR/REC-rdf-syntax/ in Figshare (https://dx.doi.org/10.6084/m9.figshare.2008203).

Taxonomic synopses and matrix-based descriptions were generated from the Hymenoptera Online Database (hol.osu.edu) and the online program vSysLab (vsyslab.osu.edu) (matrix title: Revision of *Dvivarnus*) in the format of character: state. Multiple states for a character are separated by a semicolon. Characters shared among the three species of *Dvivarnus* were exported as the generic description (OTU for generic characters: *Dvivarnus*), those that were not shared among all species were exported as species descriptions.

Photographs were captured with a Z16 Leica®™ lens with a JVC KY-F75U digital camera using Cartograph®™ software. Single montage images were produced from image stacks with the program CombineZP®™. In some cases, multiple montage images were stitched together in Photoshop®™ to produce larger images at high resolution and magnification. Full resolution images are archived at the image database at The Ohio State University (purl.oclc.org/NET/hymenoptera/specimage).

Scanning electron micrographs were produced with a Hitachi TM300 Tabletop Microscope. The specimen was disarticulated with a minuten probe and forceps and mounted to 12 mm slotted aluminum mounting stub (EMS Cat. #75220) using carbon adhesive tabs (EMS Cat. #77825-12) by means of a fine paint brush and sputter coated with approximately 70 nm of gold/palladium.

Character annotations

cly	clypeus (Figs 1–2)
ctk	central keel (Figs 15–16, 18)
epc	epomial carina (Fig. 25)
lpT3–T6	lateral patch on T3–T6 (Figs 21–24)
lpc	lateral propodeal carina (Figs 7, 9–10)
mc	mesopleural carina (Fig. 43)
mcsp	mesoscutellar spine (Figs 7–8)
mees	mesepimeral sulcus (Fig. 34)
mmsp	median mesoscutellar spine (Fig. 43)
ms	marginal setae (Fig. 9)
mns	metanotal trough (Figs 5–6)
msct	metascutellar spine (Figs 5–8)
nc	nuchal carina (Fig. 36)
nes	netrion sulcus (Fig. 4)
net	netrion (Figs 3–4, 34)
not	notaulus (Fig. 46)
plc	plica (Figs 6, 9–10)
ppp	posterior propodeal projection (Figs 5–6)
psu	posterior scutellar sulcus (Fig. 37)
pssu	prespecular sulcus (Fig. 34)
r	radicle (Figs 1–2, 17)
trt	torular triangle (Figs 15–16, 18)
vmc	ventral mesopleural carina (Fig. 43)

Specimens

This study is based on specimens from the following collections:

CNCI	Canadian National Collection of Insects, Ottawa, Canada
BPBM	Bernice P. Bishop Museum, Honolulu, HI, USA
ICIPE	International Centre of Insect Physiology and Ecology, Nairobi, Kenya
NMKE	National Museum of Kenya, Nairobi, Kenya

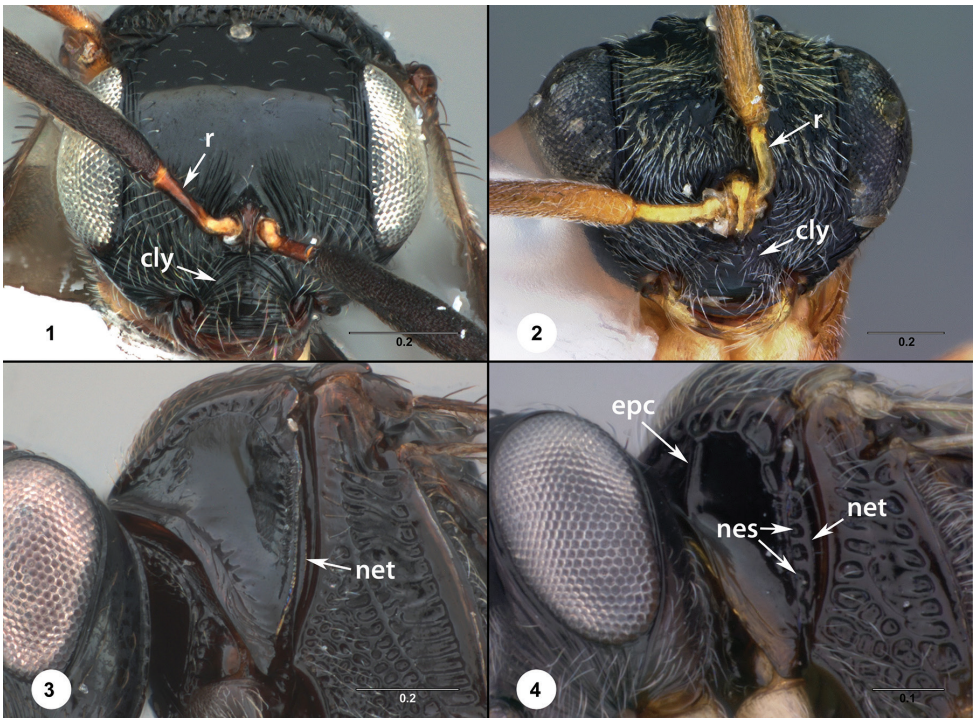

Figure 1–4. 1 *Trimorus* sp., female (OSUC 186090), head, anterior view **2** *Gryonoides pulchellus* Dodd, female (USNMENT00872146), head, anterior view **3** *Trimorus* sp., female (OSUC 192417), pronotum, anterolateral view **4** *Gryonoides glabriceps* Dodd, female (USNMENT00872142), pronotum, anterolateral view. Scale bars in millimeters.

OSUC C.A. Triplehorn Collection, The Ohio State University, Columbus, OH, USA
USNM Smithsonian National Museum of Natural History, Washington, DC, USA

Taxonomy

***Dvivarnus* Rajmohana & Veenakumari**
http://bioguid.osu.edu/xbiod_concepts/305672

Dvivarnus Rajmohana & Veenakumari, 2011: 40 (original description. Type: *Dvivarnus punctatus* Rajmohana & Veenakumari, by monotypy. Diagnosis, keyed).

Description. Number of basiconic sensilla on A7: 0. Number of basiconic sensilla on A8: 2. Color of radicle: yellow. Length of radicle: shorter than apical width of clypeus. Length of A3: as long as pedicel or longer. Number of basiconic sensilla on A12: 1. Number of mandibular teeth: 3. Mandibular teeth: ventral tooth the longest. Facial striae: present. Dorsal limit of facial striae: facial striae exceeding horizontal

plane at margin of anterior ocellus. Torular triangle: present. Height of torular triangle: less than height of clypeus. Central keel: present. Surface of dorsal frons in dorsal view: convex. Orbital carina: present. Genal patch: absent. Vertex patch: absent. Hyperoccipital carina: absent. Anterior margin of occipital carina dorsally: crenulate. Pronotal cervical sulcus: present. Sculpture of pronotal cervical sulcus: shallowly foveolate. Pronotal suprahumeral sulcus: present. Sculpture of pronotal suprahumeral sulcus: foveolate. Proximity of suprahumeral and pronotal cervical sulci: pronotal suprahumeral sulcus terminating before reaching pronotal cervical sulcus. Posterior pronotal sulcus: absent. Sculpture of propleural epicoxal sulcus: foveolate. Sculpture of posterior scutellar sulcus: foveolate. Sculpture of mesoscutal suprahumeral sulcus: foveolate. Length of mesoscutal suprahumeral sulcus: less than one half length the distance from the tegula to the anterior apex of mesoscutum. Sculpture of mesoscutal humeral sulcus: foveolate. Lateral scutoscutellar sulcus: reaching transaxillar carina. Transaxillar carina: present. Setae on lateral margin of mesoscutellum: present. Posterior scutellar sulcus: present. Acropleural sulcus: present. Length of acropleural sulcus: elongate. Subalar pit: present. Course of prespecular sulcus and mesepimeral sulcus: not continuous dorsally. Mesopleural pit: present. Sculpture of femoral depression: transversely rugose. Mesopleural carina: present. Proximity of ventral apex of mesopleural carina and ventral mesopleural carina: carinae adjacent. Sculpture of mesopleural epicoxal sulcus: foveolate. Sculpture of postacetabular sulcus: foveolate. Sculpture of mesopleuron below femoral depression: areolate rugose. Episternal foveae: indistinguishable from surface sculpture. Sculpture of mesepimeral sulcus: foveolate. Metascutellar spine: present. Shape of metascutellar spine in dorsal view: pointed. Length of metascutellar spine: longer than proximal striated region of metascutellum. Apical semitransparent lamella on metascutellar spine: absent. Sculpture of metascutellum: longitudinally striate throughout. Proximal striation of metascutellum: extending onto surface of metanotal spine.Setation of central propodeal area: present. Posterior propodeal projection: present. Metapleural sulcus: present. Setation of metapleuron: Area delimited posteriorly by paracoxal and vertical part of metapleural sulcus is covered with dense setae, remainder of metapleuron glabrous. Sculpture of paracoxal sulcus: foveolate. Dorsal margin of T1 in lateral view: concave. Length of pits on anterior T1: almost reaching posterior margin of tergite. Transverse line of pits on anterior T1: present. Transverse line of pits on anterior T2: present. Lateral patch on T2: present. Transverse line of pits on anterior T3: absent. Width of T3: as wide or slightly wider than mesoscutum. Sculpture of T3: punctate. Length of apical setae on T3: not longer than non-apical setae. Posterolateral patch on T3: present. Lateral patch on T4: present. Lateral patch on T6: present. Transverse line of pits on anterior S2: present. Felt field on S2: present. Transverse line of pits on anterior S3: absent.

Diagnosis. Per the characters presented by Veenakumari et al (2011), *Dvivarnus* can be differentiated from other teleasines by the combination of the dense punctation found throughout T3 and S3, the presence of paired mesoscutellar spines, the absence of lateral propodeal carina and the presence of an inverted U-shaped carina dorsally

surrounding the metasomal depression. Punctation on T3 can be found in some species of *Trimorus* (Fig. 20), but the punctation is surrounded by rugulae of varying intensity. In *Dvivarnus*, the punctation is uniform throughout most of the tergite and is not accompanied by additional sculptural elements. Specimen USNMENT01109195 (Fig. 20) also has spines derived from the metapleural carina, which are not present in *Dvivarnus*. Additional characters for the identification of *Dvivarnus* are presented in the key to teleasines with mesoscutellar spines.

Comments. The species of *Dvivarnus* are extremely similar in most pleural characters and differ primarily by features of the head, pronotum, and metasoma. Sexual dimorphism is exhibited mostly in the pattern of setation and striation of the frons. In males, the glabrous area above the interantennal process is less distinct and the density of setation throughout the frons varies greatly. The facial striae in males extend dorsally throughout the frons whereas in females the striation is absent from the center portion of the frons.

We examined two morphospecies of males that we were unable to unambiguously associate with the female of *D. elektrolythron*. One morphospecies (USNMENT01109164, Figs 46–50) shares with *D. elektrolythron* the pattern of striation on the lateral pronotum (Fig. 50) and the longitudinal furrow on the metanotal trough (Fig. 48). However, it has distinct notauli (Fig. 46) and *D. elektrolythron* has none, and the posterior margin of the mesoscutellum between the mesoscutellar spines is concave in USNMENT01109164, and medially pointed in *D. elektrolythron*. The other morphospecies (USNMENT01109212, Figs 40–45) has the opposite arrangement of characters: it shares with *D. elektrolythron* the absence of notauli and the presence of a pointed posterior margin of the mesoscutellum (Figs 40, 43) but it has a foveolate metanotal trough (Fig. 43) and the lateral pronotum is predominantly smooth (Fig. 45). In the absence of additional specimens that would allow us to thoroughly assess intraspecific variability in males, or molecular or biological data, we consider it best to document the morphology of these males and present them as undetermined at the species-level.

Key to teleasines with mesoscutellar spines

1 Radicle shorter than distal width of clypeus (Figs 15–19); lateral propodeal carina absent (Figs 36, 43, 48); metasomal depression surrounded dorsally by an inverted U-shaped carina (Figs 48, 43, 36); T3 and S3 densely punctate (Figs 21–23, 25, 29); metascutellar spine longitudinally striate throughout (Figs 5–6, 31, 36–37; T3 with lateral patch (Figs 21–23) ..... ***Dvivarnus***

– Radicle as long as distal width of clypeus (Figs 1–2); lateral propodeal carina present (Figs 9–10); metasomal depression not surrounded dorsally by continuous carina (Figs 9–10); T3 and S3 not densely punctate (Figs 12, 14, 24), or punctation with interstitial rugulae (Fig. 20); metascutellar spine smooth in distal half (Figs 7–8); T3 without lateral patch (Figs 12, 14, 24) **2**

2 Metasomal depression without marginal setae (Figs 8, 10); netrion sulcus distinct (Fig. 4); apex of metascutellar spine unbranched (Figs 8, 14) .... ***Gryonoides***

– Metasomal depression with marginal setae (Figs 7, 9); netrion sulcus not visible in lateral view (Fig. 3); metascutellar spine with bifurcate apex..... ..... ***Trimorus carus*** **species group**

Key to species *Dvivarnus* (females)

1 Frons with central keel separate from carinae of torular triangle (Fig. 15); mesoscutellum with posterior margin between mesoscutellar spines medially convex in dorsal view (Fig. 28, 31); pronotum with posterior portion transversely striate (Fig. 30); lateral patch on T4 present as a dense tuft of setae (Fig. 22); T5 with lateral patch present (Fig. 22) .... ***Dvivarnus elektrolythron*** **Talamas & Mikó, sp. n.**

– Frons with central keel extending to torular triangle (Figs 16–18); mesoscutellum with posterior margin concave between mesoscutellar spines in dorsal view (Figs 26, 33, 36); pronotum with posterior portion predominantly smooth (Figs 25, 32, 34–35); lateral patch on T4 broad (Figs 39, 21, 23) ; T5 without lateral patch (Figs 21, 23) **2**

2 Torular triangle setose (Figs 16–17); sulcus in metanotal trough foveolate (Fig. 5); lateral face of pronotum with epomial carina (Fig. 25) .... ***Dvivarnus agamades*** **(Kozlov & Lê)**

– Torular triangle without setation (Fig. 18); sulcus in metanotal trough present as an elongate furrow (Figs 6, 36–37); lateral face of pronotum without epomial carina (Fig. 34)..... ***Dvivarnus mikuki*** **Talamas & Mikó, sp. n.**

Dvivarnus agamades **(Kozlov & Lê)**
http://bioguid.osu.edu/xbiod_concepts/343746
Figures 5, 16–17, 23, 25–26

Gryonoides agamades Kozlov & Lê, 1986: 100 (original description); Lê, 2000: 218 (description, type information).

Dvivarnus punctatus Rajmohana & Veenakumari, 2011: 44 (original description); Talamas & Buffington, 2014: 104 (junior synonym of *Dvivarnus agamades* (Kozlov & Lê)).

Dvivarnus agamades (Kozlov & Lê): Talamas & Buffington, 2014: 104 (description, synonymy).

Description. Whorl of setae on flagellomeres in male: absent. Shape of A3–A11 in male: cylindrical.

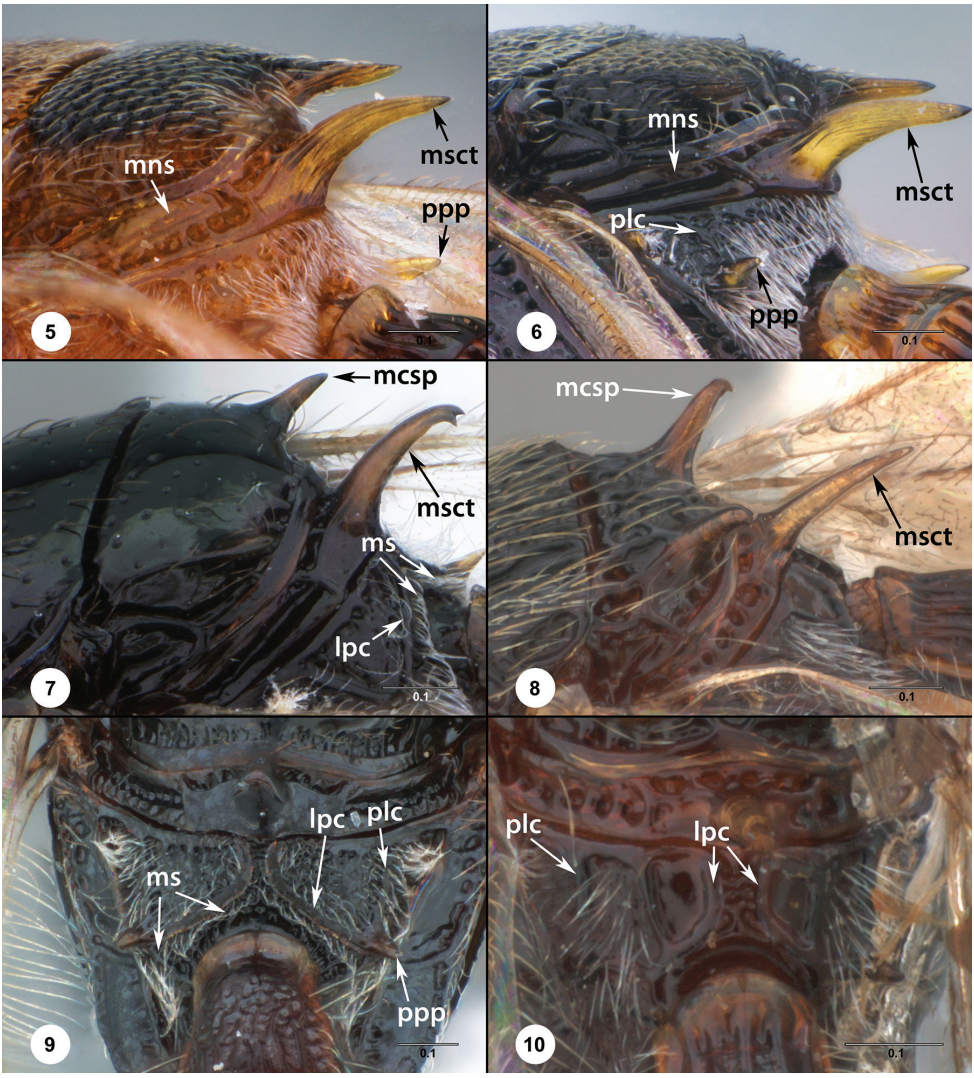

Figure 5–10. 5 *D. agamades*, female (USNMENT01109190), mesosoma, posterolateral view **6** *D. mikuki*, female (USNMENT01109213), mesosoma, posterolateral view **7** *Trimorus* sp., female (OSUC 186090), posterior mesosoma, dorsolateral view **8** *Gryonoides glabriceps* Dodd, female (USNMENT00872142), posterior mesosoma, dorsolateral view, anterolateral view **9** *Trimorus* sp., female (OSUC 1924417), mesosoma, posterior view **10** *Gryonoides glabriceps* Dodd, female (USNMENT00872142), mesosoma, posterior view. Scale bars in millimeters.

Color of antennae in female: A1–A2 orange, otherwise brown. Color of mesosoma: dorsal mesoscutellum black, otherwise orange. Color of head: black. Number of labial palpomeres: 1. Number of maxillary palpomeres: 4. Setation of torular triangle: present. Continuity of torular triangle and central keel: torular triangle closed dorsally, continuous complete central keel. Color of interantennal process: yellow. Setation of

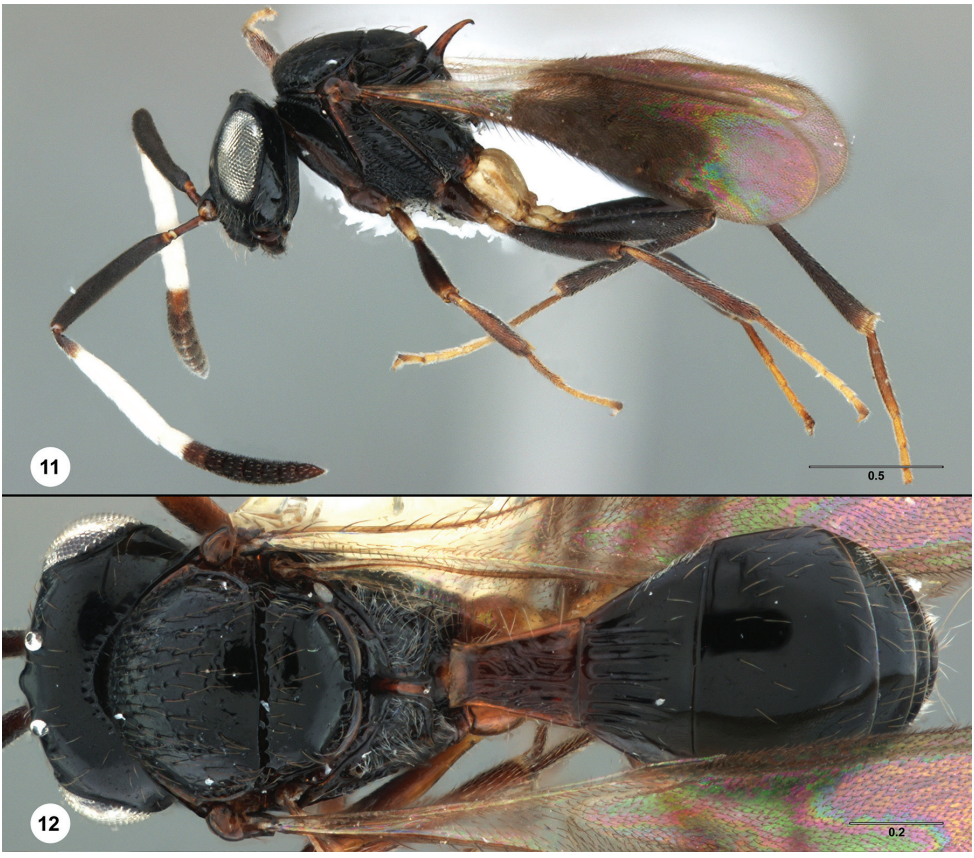

Figure 11–12. 11 *Trimorus* sp., female (OSUC 186090) head, mesosoma, metasoma, lateral view **12** *Trimorus* sp., male (OSUC 345677), head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.

frons: transverse strip directly above interantennal process glabrous, otherwise setose. Sculpture of dorsal frons: dorsoventrally strigose. Sculpture of vertex: rugulose; very finely punctate. Epomial carina: present. Netrion in lateral view: present only at ventral limit of pronotum. Sculpture of vertical face of pronotum: smooth. Ventral propleural area: smooth. Sculpture of propleural cervical sulcus: foveolate. Sculpture of mesoscutum: punctate to finely areolate, coarser in posterior half. Notaulus: weakly indicated posteriorly amid sculpture. Sculpture of scutoscutellar sulcus: smooth; smooth along posterior margin of mesoscutum, anterior margin of mesoscutellum with ridges laterally. Orientation of transaxillar carina: parallel to longitudinal axis of body. Sculpture of mesoscutellum: punctate to areolate; rugose. Density of setae on lateral margin of mesoscutellum: dense. Posterior margin of mesoscutellum: concave between mesoscutellar spines. Median mesoscutellar spine: present. Lateral extreme of posterior scutoscutellar sulcus: foveae extending to axillula. Sculpture of metanotal trough: foveolate. Sculpture of lateral propodeal area: irregularly rugose. Lateral propodeal carina: present. Plica: present. Forewing pattern in female: wing uniform in color. Forewing color

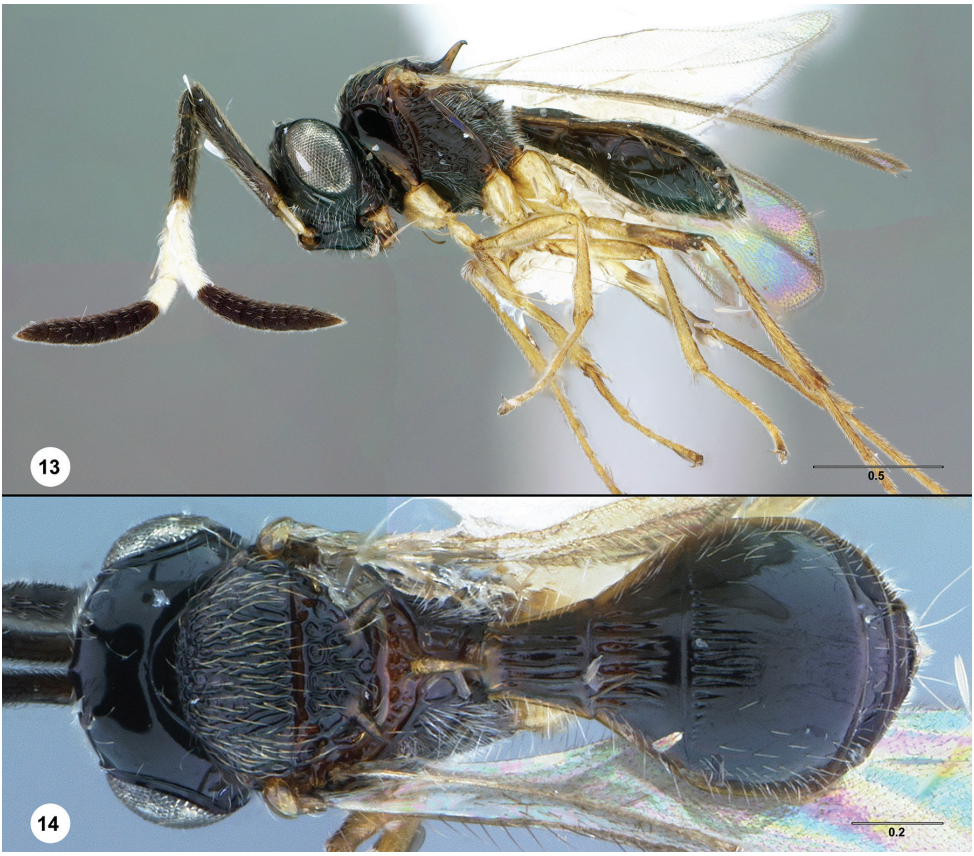

Figure 13–14. *Gryonoides glabriceps*, female (USNMENT00872142), **13** head, mesosoma, metasoma, lateral view **14** head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.

in male: uniform throughout. Sculpture of metapleural sulcus: smooth. Sculpture of dorsal metapleural area: smooth; transversely rugose. Sculpture of ventral metapleural area: transversely rugose. Number of setae on lateral T1: 2; 3; 4; many. Sculpture of T4: smooth. Lateral patch on T5: present.

Diagnosis. *Dvivarnus agamades* can be separated from *D. elektrolythron* and *D. mikuki* by the foveolate metanotal trough (Fig. 5). Females may be separated on the basis of the non-patterned forewing.

Link to distribution map. http://hol.osu.edu/map-large.html?id=343746

Material examined. *Paratype*: **VIETNAM**: Gia Lai Prov., K'Bang Dist., rice / sweet potato, Buôn Luói, 26.XI.1978, X. H. Lê (1 male, OSUC 184258 (ZIN)). *Other material*: (19 females, 8 males) **INDIA**: Delhi Union Terr., Indian Agricultural Research Institute (IARI) area, New Delhi, 26.X.1979, Boucek (1 male, USNMENT01109192 (CNCI)). Karnataka St., Bangalore, 21.VI-30.VI.1987, K. Ghorpade (1 female, USNMENT01109190 (CNCI)). Karnataka St., Indian Council of Agricultural Research (ICAR), Bangalore, XII-2003, Malaise trap, J. Poorani (1 male,

USNMENT01109189 (CNCI)). Karnataka St., grass / roadside, Malur, 28.IV.1988 (1 male, OSUC 230647 (OSUC)). Tamil Nadu St., Nilgiris Dist., Gudalur, 19.VI.1987 (1 male, OSUC 59262 (OSUC)). **LAOS**: Vientiane Prov., Gi Sion, Ban Na (Ban Tha Ngon Na), 7.II-21.II.1965 (1 female, USNMENT00877588 (BPBM)). **NEPAL**: Central Develop. Reg., nr. Birganj, MT #25, Lothar, 450ft, 29.VIII-5. IX.1967, Malaise trap (1 male, USNMENT01109175 (CNCI)). **TAIWAN**: Taiwan Prov., Nantou Co., Wushe, 1150m, 13.IV.1983, flight intercept trap, H. Townes (1 female, PSUC_000096246 (CNCI)). Taiwan Prov., Nantou Co., Wushe, 1150m, 22.V.1983, flight intercept trap, H. Townes (1 female, PSUC_000096141 (CNCI)). Taiwan Prov., Nantou Co., Wushe, 1150m, no date, Malaise trap, H. Townes & M. Townes (1 female, USNMENT01109188 (CNCI)). Taiwan Prov., Nantou Co., Wushe, 1150m, no date, H. Townes (4 females, USNMENT01109183, USNMENT01109184, USNMENT01109185, USNMENT01109187 (CNCI)). **THAILAND**: Chaiyaphum Prov., Taad Fah Waterfall, water supply station, T862, 245m, 15°56.468'N, 102°05.855'E, Tad Ton (Tat Tone) National Park, 8.IX-9.IX.2006, yellow pan trap, T. Jaruphan & O. Budsawong (1 female, OSUC 342789 (OSUC)). Chaiyaphum Prov., dry dipterocarp forest, T16, 250m, 15°59.037'N, 102°02.103'E, Tad Ton (Tat Tone) National Park, 28.VI.2006, Malaise trap, C. Nichumnan (1 female, OSUC 374197 (OSUC)). Kanchanaburi Prov., Khong Kraborg, # 4781, 210m, 14°29.972'N, 98°53.035'E, Khuean Srinagarindra National Park, no date, Malaise trap, Boonnam & Phumarin (1 male, USNMENT01109177 (CNCI)). Kanchanaburi Prov., Mae Kamint River, headquarters, # 3466, 14°38.123'N, 98°59.657'E, Khuean Srinagarindra National Park, no date, Malaise trap, Somboon & Daorueng (1 female, USNMENT01109173 (CNCI)). Kanchanaburi Prov., Mae Kamint River, tourist center, T4422, 210m, 14°38.312'N, 98°59.643'E, Khuean Srinagarindra National Park, no date, Malaise trap, Somboon & Daorueng (1 male, USNMENT01109169 (CNCI)). Khon Kaen Prov., Disturb (Moob Cave), T2, 296m, 16°44.837'N, 102°00.160'E, Phu Pha Man National Park, 13.VI-20.VI.2006, Malaise trap, R. Phatai (1 female, OSUC 374198 (OSUC)). Nakhon Si Thammarat Prov., TV aerial, T3108, 966m, 08°14.262'N, 99°48.289'E, Namtok Yong National Park, no date, Malaise trap, Yai & Amnad (1 female, USNMENT01109170 (CNCI)). Phetchabun Prov., Kaeng Krachan Nat. Park 12º32.141'N, 99º27.914'E T4540, no date, Malaise trap, Thongbai (1 female, USNMENT01109172 (CNCI)). Phetchabun Prov., helicopter landing ground, T266, 890m, 16°43.156'N, 101°35.118'E, Nam Nao National Park, 8.VII-9.VII.2006, pan trap, N. Hongyothi (1 female, OSUC 284994 (OSUC)). Phetchaburi Prov., Huai Palao Forest Unit 3, Pa La-U Waterfall, T4564, 12°32.149'N, 99°28.265'E, Kaeng Krachan National Park, no date, pan trap, Thongbai (1 female, USNMENT01109180 (CNCI)). Phetchaburi Prov., Huai Palao Forest Unit 3, Pa La-U Waterfall, T4566, 12°32.149'N, 99°28.265'E, Kaeng Krachan National Park, no date, Malaise trap, Thongbai (2 females, USNMENT01109174, USNMENT01109178 (CNCI)). Phetchaburi Prov., km33 / helipad, T4693, 735m, 12°50.177'N, 99°20.688'E, Kaeng Krachan National Park, no date, Malaise trap, Sirichai (1 male, USNMENT01109179 (CNCI)).

Dvivarnus elektrolythron **Talamas & Mikó, sp. n.**
http://zoobank.org/0CBCE486-CEBD-4924-9AF2-74EEA6A222B0
http://bioguid.osu.edu/xbiod_concepts/403212
Figures 15, 22, 27–31

Description. Color of antennae in female: brown throughout with dense white setae on A2–A4. Color of mesosoma: mesoscutellum and propodeum black, metascutellar spine brown, otherwise red. Color of head: except interantennal process, black. Setation of torular triangle: present. Continuity of torular triangle and central keel: torular triangle opened dorsally, not continuous reduced central keel. Color of interantennal process: yellowish brown. Setation of frons: transverse strip directly above interantennal process glabrous, otherwise setose. Sculpture of dorsal frons: strigose. Sculpture of vertex: strigose.

Epomial carina: indistinguishable from dorsoventral striation. Netrion in lateral view: extending dorsally to proximity of mesothoracic spiracle. Netrion sulcus: complete, extending dorsally to posterior margin of pronotum. Sculpture of vertical face of pronotum: dorsoventrally strigose anteriorly, longitudinal striate posteriorly. Sculpture of mesoscutum: finely punctate. Density of setation on medial mesoscutum: dense. Notaulus: absent. Sculpture of scutoscutellar sulcus: smooth. Orientation of transaxillar carina: projecting posterolaterally. Sculpture of mesoscutellum: finely areolate. Density of setae on lateral margin of mesoscutellum: sparse. Posterior margin of mesoscutellum: convex between mesoscutellar spines. Median mesoscutellar spine: present. Lateral extreme of posterior scutoscutellar sulcus: foveae terminating below mesoscutellar spine. Sculpture of metanotal trough: smooth with elongate furrow in ventral half. Lateral propodeal carina: absent. Forewing pattern in female: wing membrane and setae brown posterior to marginal vein and in distal third, separated by a band of hyaline membrane and white setae. Sculpture of metapleural sulcus: smooth. Sculpture of dorsal metapleural area: transversely rugose. Sculpture of ventral metapleural area: transversely rugose. Number of setae on lateral T1: 3. Sculpture of T4: punctate. Lateral patch on T5: present. Number of apical setae on T7: 2.

Diagnosis. *Dvivarnus elektrolythron may* be separated from females of *D. mikuki* and *D. agamades* by the incomplete central keel on the frons (Fig. 15), the form of the lateral patch on T4 (Figs 22, 29), and by the medially convex posterior margin of the mesoscutellum.

Etymology. The epithet for this species refers to the bright red color on the mesosoma of this species. It is derived from the words *elektron* which in Classical Greek means "amber" and, by extension in modern times, "electricity", and *lythron,* meaning "gore". The name is treated as a noun in apposition.

Link to distribution map. http://hol.osu.edu/map-large.html?id=403212

Material examined. Holotype, female: **IVORY COAST**: Savanes Rég., Korhogo Dept., Konborodougou, 18.III–21.III.1984, M. Matthews, USNMENT01109168 (deposited in CNCI).

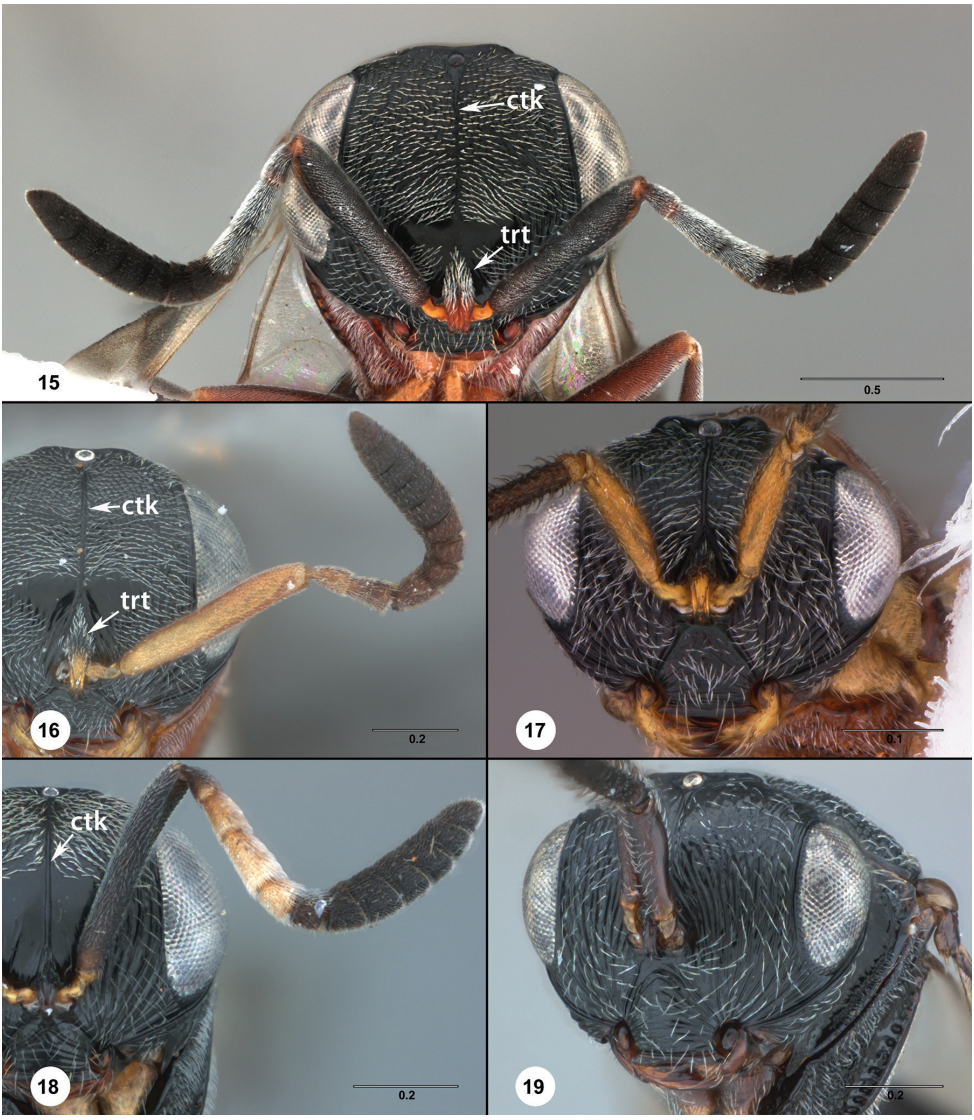

Figure 15–19. 15 *Dvivarnus elektrolythron*, female holotype (USNMENT01109168), head and antennae, anterior view **16** *D. agamades*, female (USNMENT01109190), head, anterior view **17** *D. agamades*, male (USNMENT01109177), head, anterior view **18** *D. mikuki*, female paratype (USNMENT01109214), head and antenna, anterior view **19** *D. mikuki*, male (USNMENT01109158), head, anterior view. Scale bars in millimeters.

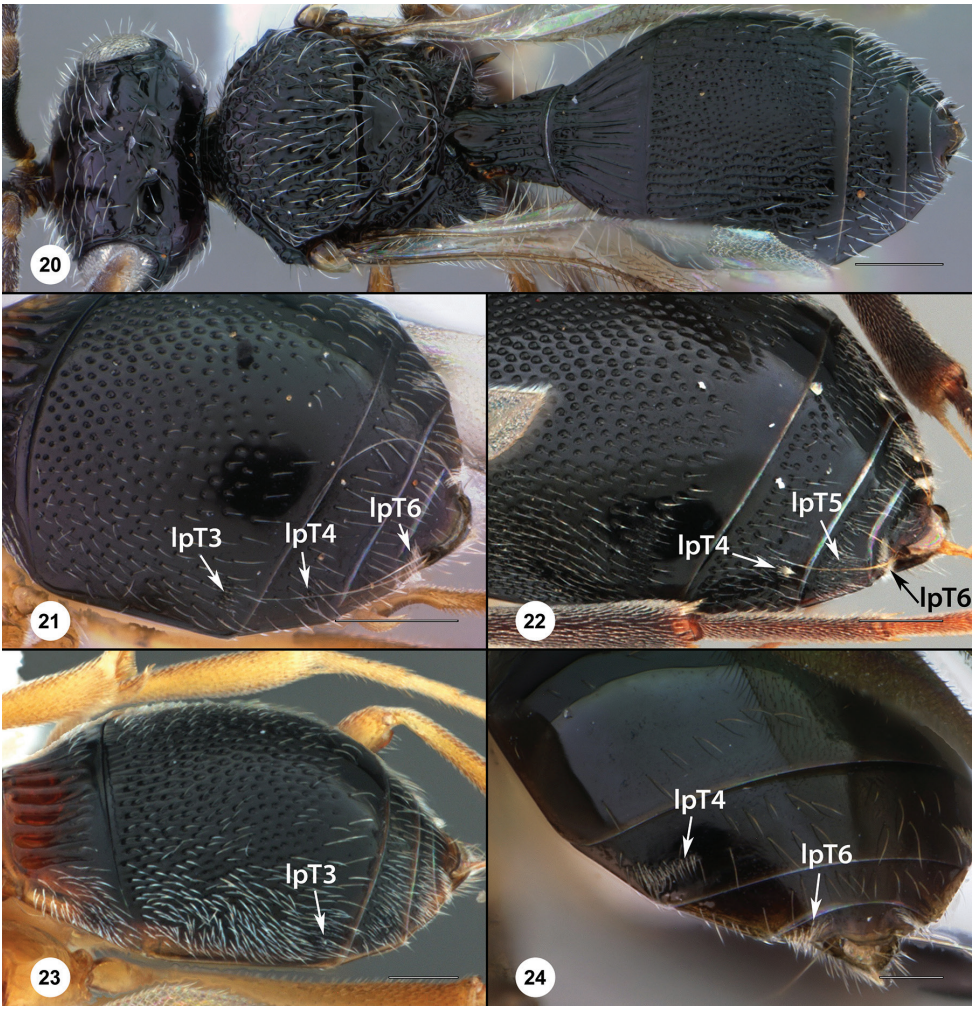

Figure 21–24. 20 *Trimorus* sp., female (USNMENT01109195), head, mesosoma, metasoma, dorsal view **21** *Dvivarnus mikuki*, female (USNMENT01109213), metasoma, dorsolateral view **22** *D. elektrolythron*, female (USNMENT01109168), metasoma, dorsolateral view **23** *D. agamades*, female (USNMENT01109174), metasoma, dorsolateral view **24** *Trimorus* sp., female (OSUC 186090), metasoma, posterodorsal view. Scale bars in millimeters.

Dvivarnus mikuki Talamas & Mikó, sp. n.

http://zoobank.org/1EC45732-63E1-40D5-94DE-453A560A9EEF
http://bioguid.osu.edu/xbiod_concepts/403211
Figures 6, 18–19, 21, 32–39

Description. Whorl of setae on flagellomeres in male: absent. Shape of A3–A11 in male: cylindrical. Color of antenna in male: brown. Color of antennae in female:

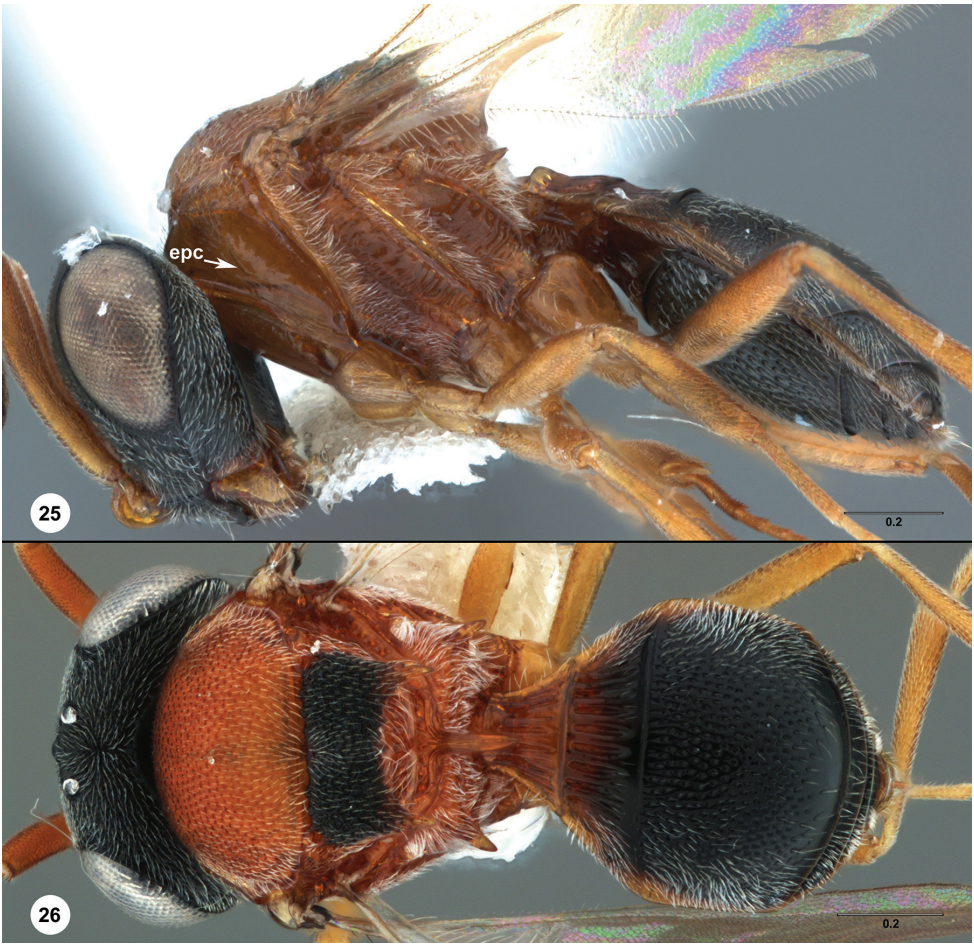

Figure 25–26. *Dvivarnus agamades.* **25** female (USNMENT01109183), head, mesosoma, metasoma, lateral view **26** female (USNMENT01109174), head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.

A1 brown, A2 brown to yellow, A3–A5 yellow with white setae, A6–A16 brown. Color of mesosoma: black, with yellow metascutellar spine. Color of head: black. Setation of torular triangle: absent. Continuity of torular triangle and central keel: torular triangle closed dorsally, continuous complete central keel. Setation of frons: area above interantennal process glabrous, otherwise setose. Sculpture of dorsal frons: dorsoventrally strigose. Sculpture of vertex: smooth; concentrically strigose. Epomial carina: absent. Netrion in lateral view: present only at ventral limit of pronotum. Ventral propleural area: smooth. Sculpture of propleural cervical sulcus: smooth. Sculpture of mesoscutum: punctate to finely areolate, coarser in posterior half. Density of setation on medial mesoscutum: dense. Notaulus: absent; weakly

Figure 27. *Dvivarnus elektrolythron*, female holotype (USNMENT0109168), head, mesosoma, metasoma, lateral view. Scale bar in millimeters.

indicated posteriorly amid sculpture. Sculpture of scutoscutellar sulcus: smooth. Orientation of transaxillar carina: parallel to longitudinal axis of body. Shape of axillula: bent ventrolaterally distally. Sculpture of mesoscutellum: punctate to areolate. Density of setae on lateral margin of mesoscutellum: sparse. Posterior margin of mesoscutellum: concave between mesoscutellar spines. Median mesoscutellar spine: absent. Lateral extreme of posterior scutoscutellar sulcus: foveae extending to axillula. Sculpture of metanotal trough: smooth with elongate furrow in ventral half. Lateral propodeal carina: absent. Plica: present. Forewing pattern in female: wing membrane and setae brown posterior to marginal vein and in distal third, separated by a band of hyaline membrane and white setae. Forewing color in male: uniform throughout. Sculpture of metapleural sulcus: transverse portion smooth, dorsoventral portions foveolate. Sculpture of dorsal metapleural area: smooth. Sculpture of ventral metapleural area: transversely rugose; smooth. Number of setae on lateral T1: 3; 4. Sculpture of T4: smooth. Lateral patch on T5: absent. Number of apical setae on T7: 4. Transverse line of pits on anterior S1: present.

Diagnosis. *Dvivarnus mikuki* can be separated from *D. agamades* and *D. elektrolythron* by the glabrous torular triangle and by the color of the mesosoma, which is entirely black except for the metascutellar spine. Additionally, *D. mikuki* can be separated from *D. agamades* by the absence of an epomial carina, the form of the metanotal trough, which is non-foveolate and is dorsoventrally divided by a transverse furrow,

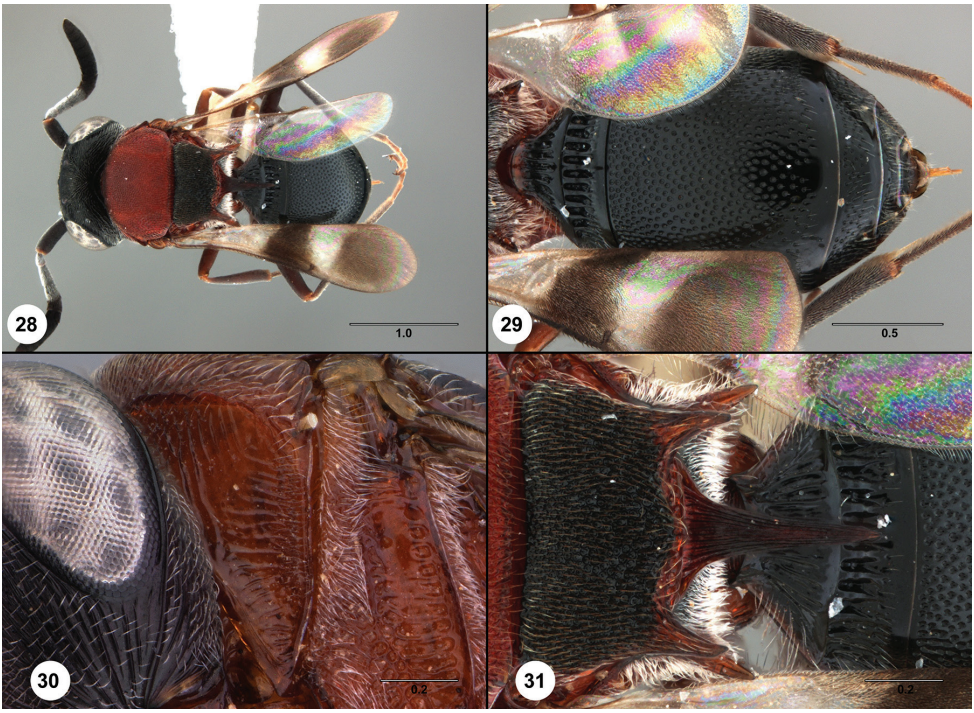

Figure 28–31. *Dvivarnus elektrolythron*, female holotype (USNMENT0109168) **28** head, mesosoma, metasoma, dorsal view **29** metasoma, dorsal view **30** pronotum, anterolateral view **31** posterior mesosoma, dorsal view. Scale bars in millimeters.

and by the banding pattern on the wings of females. From *D. elektrolythron* it can be separated by the broad lateral patch on T4 and the convex posterior margin of the mesoscutellum between the mesoscutellar spines.

Etymology. The word "mikuki" means "spears" in Swahili, the language of Kenya where the holotype specimen originates, and refers to the many spines found on the mesosoma. The name is treated as noun in apposition.

Link to distribution map. http://hol.osu.edu/map-large.html?id=403211

Material examined. Holotype, female: **KENYA**: Nairobi Co., International Centre of Insect Physiology and Ecology (ICIPE) campus, nr. stream / meadow / degraded shrub-grassland, 1600m, 01.22317°S 36.89653°E, Kasarani, 27.V-3.VI.2014, Malaise trap, R. Copeland, USNMENT01059120 (deposited in NMKE). *Paratypes*: (11 females, 8 males) **BENIN**: 25km N Cotonou, Abomey-Calavi, XII-1988, J. S. Noyes (1 male, USNMENT01109165 (CNCI)). **KENYA**: Kilifi Co., 3.30958° S 39.96538°E, Arabuko-Sokoke Forest 80 m, 22.VIII-5.IX.2014, Malaise trap, R. Copeland (1 male, USNMENT01109193 (USNM)). Kilifi Co., indigenous forest / secondary forest, 19m, 03.30946°S 40.01941°E, Gede Forest, 11.XII-25.XII.2011, Malaise trap, R. Copeland (2 females, USNMENT01059122-01059123 (USNM)).

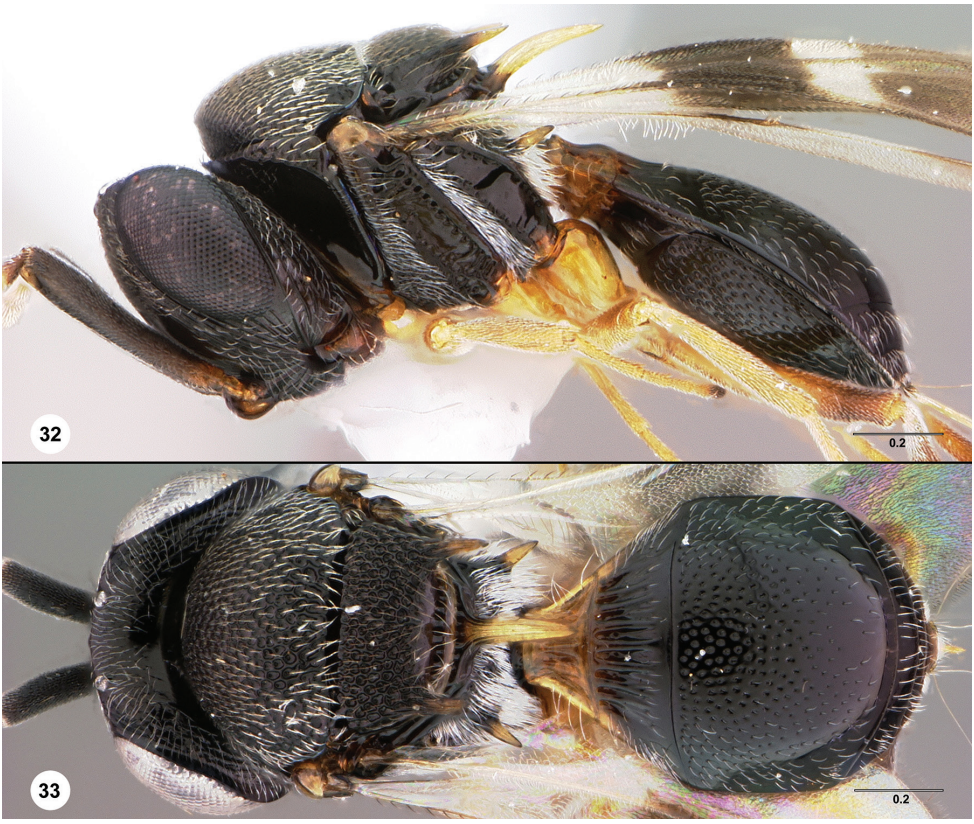

Figure 32–33. *Dvivarnus mikuki.* **32** female holotype (USNMENT01059120), head, mesosoma, metasoma, lateral view **33** female paratype (USNMENT01059121), head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.

Kwale Co., indigenous forest, 39.52462°E, Muhaka Forest, 41m 4.32664°S, 13.XII-27.XII.2014, Malaise trap, R. Copeland (1 female, USNMENT01109214 (NMKE)). Kwale Co., indigenous forest, Muhaka Forest, 41m, 4.32664°S, 39.52462°E, 27.XII-10.I.2015, Malaise trap, R. Copeland (1 female, USNMENT01109213 (NMKE)). Kwale Co., indigenous forest, 76m, 04.52814°S 39.24028°E, Marenje Forest, 11.VII-25.VII.2014, Malaise trap, R. Copeland (1 male, USNMENT01109194 (NMKE)). Kwale Co., indigenous forest, 76m, 04.52814°S 39.24028°E, Marenje Forest, 25.VII-8.VIII.2014, Malaise trap, R. Copeland (3 females, USNMENT01059121, 01059124, 01059135 (USNM)). **NIGERIA**: Oyo St., International Institute of Tropical Agriculture (IITA), Ibadan, X-1987, screen sweeping, J. S. Noyes (1 male, USNMENT01109162 (CNCI)). Oyo St., International Institute of Tropical Agriculture (IITA), Ibadan, no date, screen sweeping, J. S. Noyes (1 male, USNMENT01109163 (CNCI)). **ZIMBABWE**: Harare (Salisbury), no date, A. Watsham (1 female, 2 males,

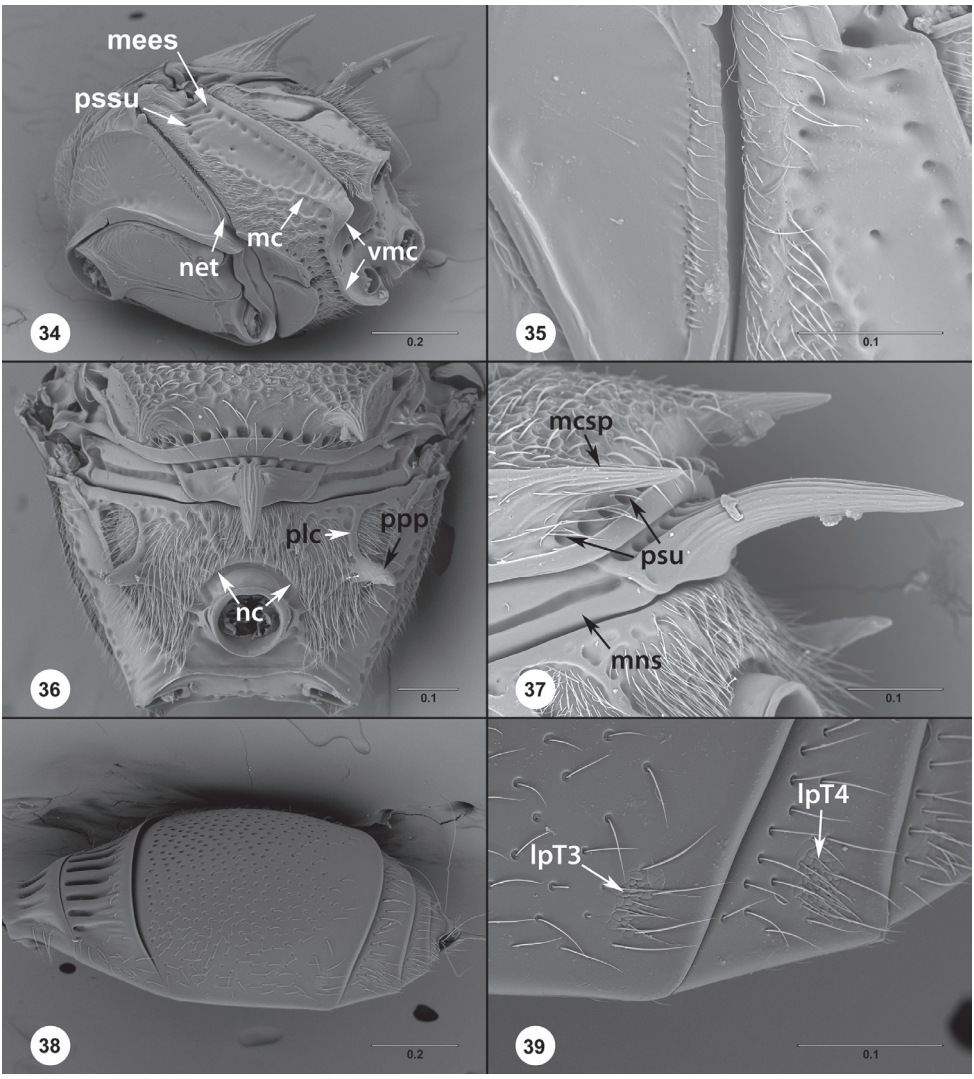

Figure 34–39. *Dvivarnus mikuki*, female paratype (USNMENT010591135) **34** mesosoma, anterolateral view **35** posterior pronotum, lateral view **36** mesosoma, posterior view **37** posterior mesosoma, posterolateral view **38** metasoma, dorsolateral view **39** T3–T5, dorsolateral view. Scale bars in millimeters.

USNMENT01109157, USNMENT01109158, USNMENT01109161 (CNCI)). Harare (Salisbury), no date, pan trap, A. Watsham (1 female, USNMENT01109159 (CNCI)). Harare (Salisbury), no date, yellow pan trap, A. Watsham (1 female, USNMENT01109156 (CNCI)). Harare (Salisbury), Chishawasha, no date, A. Watsham (1 female, USNMENT01109167 (CNCI)). Harare (Salisbury), Chishawasha, no date, pan trap, A. Watsham (1 male, USNMENT01109160 (CNCI)).

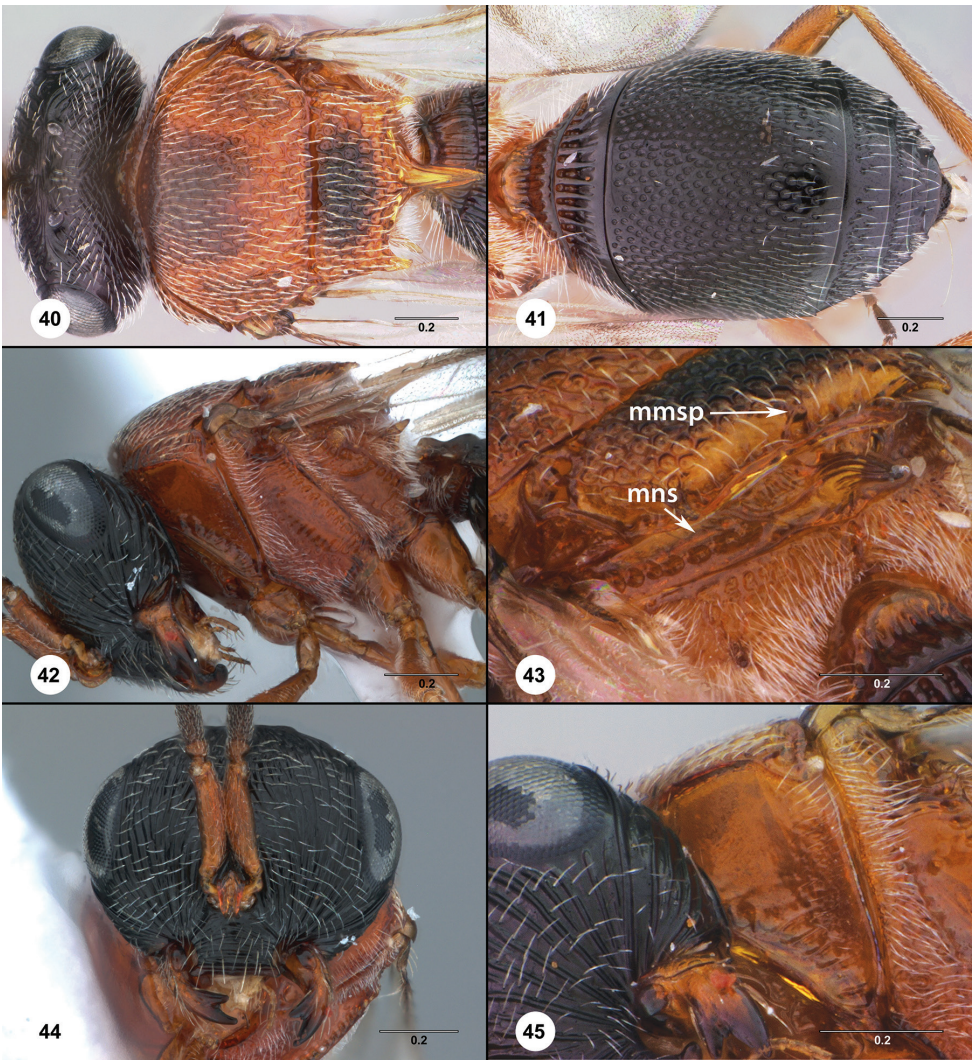

Figure 40–45. *Dvivarnus* sp., male (USNMENT01109212). **40** head and mesosoma, dorsal view **41** metasoma, dorsal view **42** head and mesosoma, lateral view **43** mesosoma, posterodorsal view **44** head, anterior view **45** pronotum, anterolateral view Scale bars in millimeters.

Dvivarnus sp., male

Figures 40–45

Dvivarnus sp., male

Figures 46–50

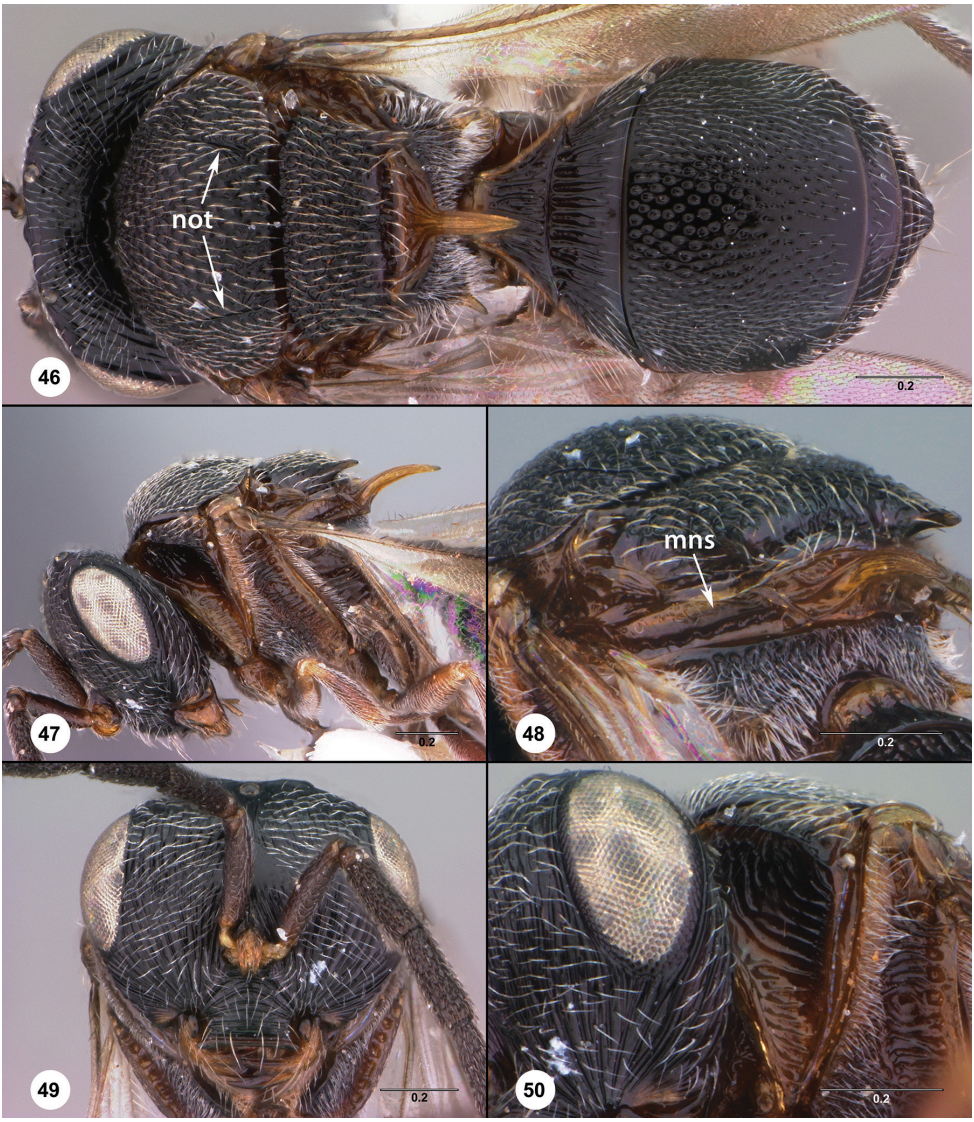

Figure 46–50. *Dvivarnus* sp., male (USNMENT01109164) **46** head, mesosoma metasoma, dorsal view **47** head and mesosoma, lateral view **48** mesosoma, posterolateral view **49** head, anterior view **50** pronotum, anterolateral view. Scale bars in millimeters.

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Supplementary material 1

URI table of HAO morphological terms

Authors: Elijah J. Talamas, István Mikó, Robert S. Copeland

Data type: Microsoft Excel Spreadsheet (.xls)

Explanation note: This table lists the morphological terms used in this publication and their associated concepts in the Hymenoptera Anatomy Ontology.

Supplementary material 2

Semantic representations of phenotypes in Manchester syntax format of *Dvivarnus*

Authors: Elijah J. Talamas, István Mikó, Robert S. Copeland

Data type: Microsoft Rich Text Format (.rtf)

Explanation note: Semantic representations of phenotypes in Manchester syntax format of the taxonomic treatment of World *Dvivarnus* (Hymenoptera: Platygastroidea: Teleasinae).

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RESEARCH ARTICLE

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Biogeography and demography of an Australian native bee *Ceratina australensis* (Hymenoptera, Apidae) since the last glacial maximum

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Abstract

The small carpenter bees, genus *Ceratina,* are highly diverse, globally distributed, and comprise the sole genus in the tribe Ceratinini. Despite the diversity of the subgenus *Neoceratina* in the Oriental and Indo-Malayan region, *Ceratina (Neoceratina) australensis* is the only ceratinine species in Australia. We examine the biogeography and demography of *C. australensis* using haplotype variation at 677 bp of the barcoding region of COI for specimens sampled from four populations within Australia, across Queensland, New South Wales, Victoria and South Australia. There is geographic population structure in haplotypes, suggesting an origin in the northeastern populations, spreading to southern Australia. Bayesian Skyline Plot analyses indicate that population size began to increase approximately 18,000 years ago, roughly corresponding to the end of the last glacial maximum. Population expansion then began to plateau approximately 6,000 years ago, which may correspond to a slowing or plateauing in global temperatures for the current interglacial period. The distribution of *C. australensis* covers a surprisingly wide range of habitats, ranging from wet subtropical forests though semi-arid scrub to southern temperate coastal dunes. The ability of small carpenter bees to occupy diverse habitats in ever changing climates makes them a key species for understanding native bee diversity and response to climate change.

Keywords

Climate change, dispersal, DNA barcoding, bayesian skyline plot, haplotype network, population structure

Introduction

Molecular studies have greatly increased our understanding of the antiquity of bees and their historical biogeography, especially with respect to centres of origin and subsequent dispersal routes (e.g. Fuller et al. 2005; Hines 2008; Chenoweth and Schwarz 2011; Rehan and Schwarz 2015). Other studies using museum collection data have implicated very recent climate change as a possible factor underlying changes in bee abundances (e.g. Cameron et al. 2011; Burkle et al. 2013), but there are surprisingly few studies that have attempted to infer changes in bee abundance beyond the last 200 years (but see Wilson et al. 2014). In the face of likely future climate change, it is important to understand how bees have responded to past climates so that we may better predict future trends.

Two recent studies (Groom et al. 2014a; López-Uribe et al. 2014) have used phylogeographic and coalescent Bayesian Skyline Plot analyses to examine changes in bee abundances for tropical halictine (Halictidae) and euglossine (Apidae) bees respectively. Both studies found a strong response to Pleistocene climates, suggesting that these two faunal groups have been impacted by glacial cycles despite their tropical distribution. Small carpenter bees, *Ceratina* (Apidae: Ceratinini), of the subgenus *Zadontomerus* in eastern North America also showed a rapid population expansion approximately 20kya, linked to post-glacial cycles (Shell and Rehan 2016). However, no studies have examined possible impacts of historical climates on bee species spanning temperate to xeric zones, beyond those using museum records.

The small carpenter bee genus *Ceratina* has a nearly global distribution, occurring on all continents except Antarctica (Rehan and Schwarz 2015). This includes recently colonized remote islands in the Southwestern Pacific and southern Indian Ocean, probably via accidental human agency (Rehan et al. 2010a; Groom et al. 2014b). *Ceratina* originated in Africa in the late Paleocene or early Eocene and showed rapid long distance dispersal events allowing it to eventually colonize the New World by the late Eocene (Rehan and Schwarz 2015). Interestingly, patterns of radiation in this tribe suggest that major long distance dispersal events have been rare and tended to occur more frequently in the early history of this tribe rather than later on, despite there being few geographical barriers to later dispersal events (Rehan and Schwarz 2015). Physical impediments to dispersal, such as water barriers, are actually believed to have decreased in the more recent history of this tribe (Rehan and Schwarz 2015).

Ceratina (*Neoceratina*) is the sister clade to all other *Ceratina* subgenera and originated from a dispersal from Africa to the Oriental region in the early Eocene, with some species extending into the Palearctic (Rehan and Schwarz 2015). Surprisingly, there is only a single ceratinine species in Australia, *Ceratina (Neoceratina) australensis* (Perkins, 1912). This species forms the sister lineage to all other *C.* (*Neoceratina*) species, and its stem age is dated to the middle Eocene. *Ceratina australensis* has become a model species for understanding simple forms of sociality where both solitary and social forms remain in sympatry (e.g. Rehan et al. 2010b, 2011, 2014). Solitary nests comprise about eighty-five percent of the population and are founded by females that

disperse from their natal nests (Rehan et al. 2014). Dispersal of females could facilitate gene flow between populations across Australia.

Michener (1962) recorded *Ceratina australensis* from subtropical and temperate regions of eastern Queensland and New South Wales but there have been no further studies of its distribution. Here we use haplotype variation at 677 bp of the mitochondrial 'barcoding' region of cytochrome *c* oxidase subunit 1 (CO1) from 102 specimens of *C. australensis*, obtained from southern Queensland, mid-New South Wales, northern Victoria and southern South Australia, to examine historical demography and geographical patterns in population genetic structure. Based on the dispersal of females from natal nests we predicted that there would be gene flow between populations, influencing the genetic structure and historical demography of the species.

Methods

Collecting localities for genetic samples

Specimens of *Ceratina australensis* were collected from four populations: (i) Mildura in northwestern Victoria; (ii) West Beach in metropolitan Adelaide, South Australia; (iii) the Cowra region in central New South Wales; and (iv) the Warwick region in southeastern Queensland (Figure 1). Our four study sites represent a substantial proportion of the geographic and climatic conditions for the species, covering warm temperate forest, semi-arid riverine woodland, mediterranean coastal dunes and central tablelands. GPS coordinates, collection dates and the number of barcoded specimens from each population are shown in Table 1. Nests, predominantly found in dead stems of plants from the genera *Cakile* (Brassicaceae), *Senecio* (Asteraceae), *Ferula* (Apiaceae) and the species *Verbena bonariensis* L. (Verbenaceae) were collected during early mornings or late evenings. This ensured that the bees would be present in the nest and not out foraging. Nests were collected, as this species is rarely observed on flowers (Michener 1962). Nests were stored on ice or at 4°C until processing. One randomly chosen adult from each nest was stored in 100% ethanol for DNA sequencing.

DNA extraction and sequencing techniques

One leg of each specimen was incubated overnight in arthropod lysis solution with proteinase K. Extractions proceeded using a glass fibre plate and a vacuum manifold to pull the eluates through the membrane, following the procedures detailed in Ivanova et al. (2006). The DNA extract was stored in 50µl TLE (10mM TRIS, 0.1mM EDTA pH8). Forward and reverse primers M070 (5'-TCC AAT GCA CTA ATC TGC CAT ATT A-3') and M080 (5'-TAC AGT TGG AAT AGA CGT TGA TAC-3') were used for PCR amplification of a 700 bp region of CO1. We used 27.5 µl reactions with 0.1 µl immolase as the active enzyme, 1 µl of each M070 and M080, 5 µl of MRT Buffer, 15.4

Table 1. Summary of samples collected from each population of *C. australensis*. Includes GPS coordinates, collection dates, total number of specimens sequenced and the number of unique haplotypes recovered.

Population	Latitude (S) / Longitude (E)	Collection Dates	Specimens Barcoded	Haplotypes
Cowra, New South Wales	33°52.78' / 148°45.73'	October 2015	11	8
Mildura, Victoria	34°09.25' / 142°09.58'	June 2013, October 2013, January 2014, April 2014	42	9
Warwick, Queensland	28°12.85' / 152°02.10'	January 2010	30	14
West Beach, South Australia	34°56.28' / 138°29.95'	June 2012, July 2014	19	1

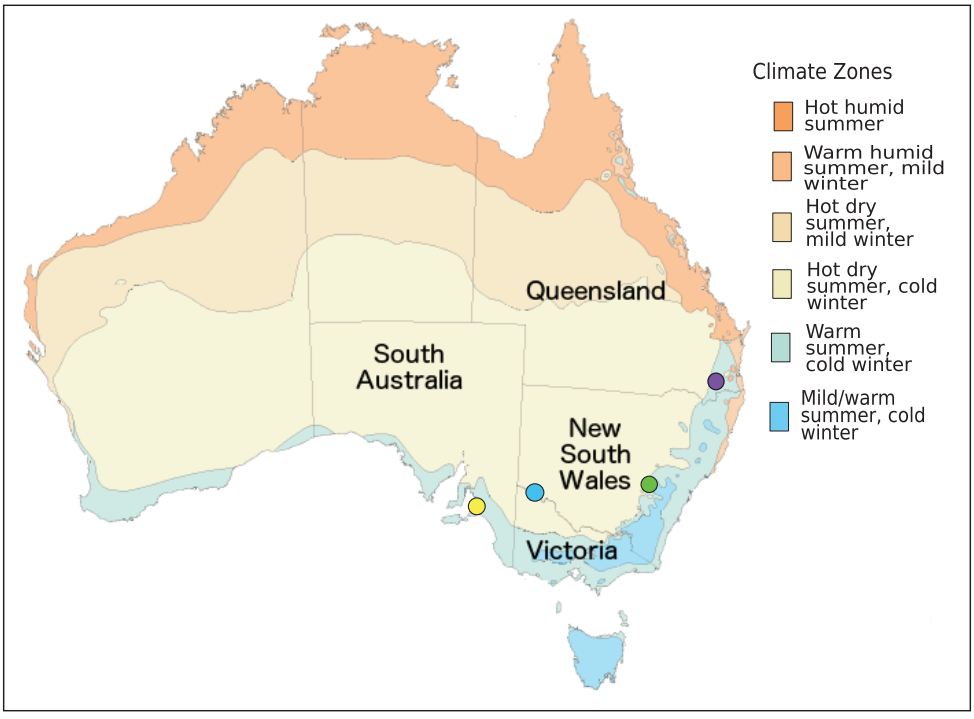

Figure 1. Map of Australia with overlaid temperature and humidity climate zones (Bureau of Meteorology 2012) showing sampling locations. New South Wales, green; Queensland purple; Victoria, blue; South Australia, yellow.

μl water and 5 μl DNA. The PCR cycle began with 10min of 94°C. The annealing stage had 5 cycles consisting of 60s at 94°C, 90s at 45°C and 90s at 72°C followed by 35 cycles of 60s at 94°C, 90s at 50°C and 60s at 72°C. Elongation was 10min at 72°C with a final 2min at 25°C. The PCR products were cleaned using a vacuum plate with 100 μl TLE, with the cleaned products stored in 30 μl TLE. Final forward and reverse sequencing of the cleaned PCR products was performed by the Australian Genome Research Facility.

Alignment and phylogenetic reconstruction

Sequences were imported into GENEIOUS v.6.1.6 (Kearse et al. 2012) for editing and alignment. Reverse and forward sequences were combined into a consensus sequence for each sample. As we were comparing individual base pair changes, no ambiguous or unknown base pairs (including at the end of sequences) could be left in the final alignment. We aimed to maximize both the sequence length and the number of samples. The sequence length was shortened, so that all samples had base pair data covering the same read length without any missing or ambiguous nucleotides, since missing data can lead to spurious results for coalescent analyses (Ho and Shapiro 2011). The final alignment consisted of 102 sequences of 677 bp in length, with 28 unique haplotypes. All edited sequences are submitted to GenBank (accession numbers KR824844-KR824934 and KU664337-KU664347).

An undescribed *Neoceratina* species from the Solomon Islands, *Ceratina (Neoceratina)* "Solomons sp." was included in the alignment as the outgroup to determine the root of the tree. This species has been shown to be phylogenetically distinct to *Ceratina australensis* (Rehan and Schwarz 2015). The sequences available for this species did not cover the full length of the 677 bp alignment, so the *C. australensis* sequences were shortened to 639 bp for this analysis. This sequence attenuation did not remove any of the unique haplotype information present within the *C. australensis* sequences. The phylogenetic tree was reconstructed in BEAST v.1.8.1 (Drummond and Rambaut 2007) with a Yule Process tree prior. The substitution model HKY+I+G model was identified as the most appropriate based on Akaike information criterion in JMODELTEST (Guindon and Gascuel 2003; Darriba et al. 2012). The analysis ran for 5 x 10^7 generations, logged every 1,000 trees, using a fixed mutation rate of 1.0, with all other parameters set to default. The log files were viewed in TRACER v.1.5 to confirm that the posterior had stabilized. A consensus tree was constructed with TREE ANNOTATER v.1.8.1 with a burn-in of 10,000 trees (i.e. 10 million iterations; Suppl. material 1).

The full alignment was then pruned to contain only unique haplotypes (28 sequences). The analysis was run following the conditions described above. TRACER again confirmed the posterior of the analysis had stabilised and a consensus tree with a burn-in of 10,000 trees was generated (Figure 2).

Haplotype networks

Analysis of Molecular Variance (AMOVA) was implemented in ARLEQUIN 3.11 (Excoffier et al. 2005) to compare genetic variation within and among Victoria, New South Wales, South Australia and Queensland populations. For these analyses we used all sequences and included all codon positions. All four populations were compared in the full model followed by pair-wise comparisons of each possible pairing in subsequent AMOVA analyses. The full alignment was imported into NETWORK (Fluxus

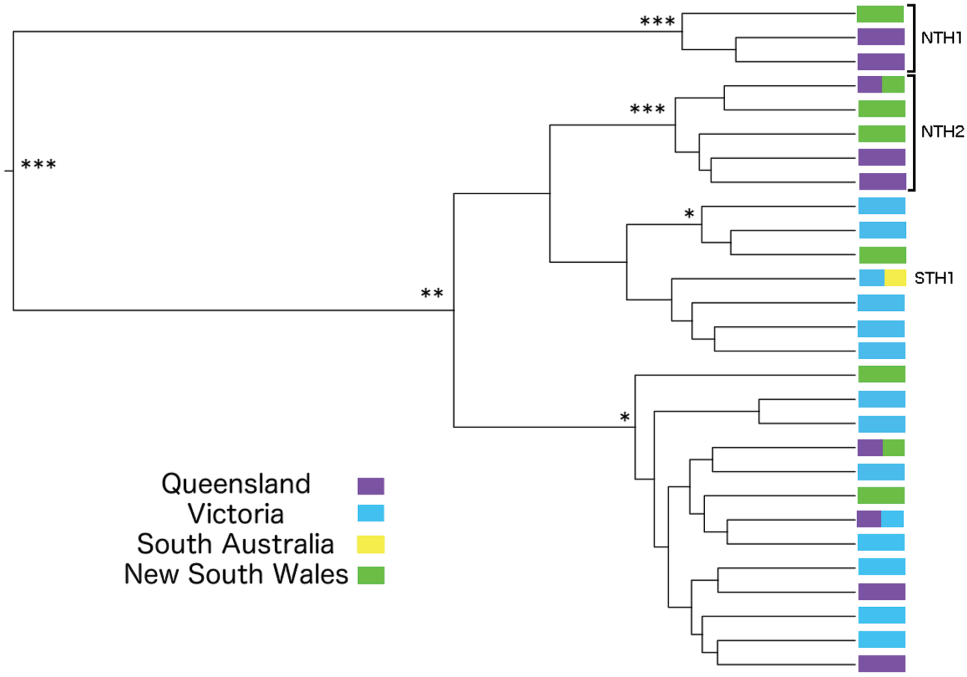

Figure 2. Maximum credibility tree of the *C. australensis* haplotypes from Bayesian BEAST analysis. Clades North 1 (NTH1), North 2 (NTH2) and South 1 (STH1) are indicated. Posterior probability values: *** = 1.0; ** ≥ 95; * ≥ 85.

Engineering 2016) and a haplotype network was constructed using a median-joining analysis with epsilon set as zero (Bandelt et al. 1999; Figure 3). HAPLOVIEWER confirmed the final network and was used to generate a publication quality figure (Salzburger et al. 2011).

Historical demography

We used Bayesian Skyline Plot (BSP) analyses implemented in BEAST and TRACER to explore historical changes in effective population size of *Ceratina australensis*. For these analyses we included all sequences available including duplicate haplotypes, as analyses of only unique haplotypes can give erroneous results (Grant 2015). BSP analyses assume that genetic markers evolve neutrally (Ho and Shapiro 2011). The very small number of amino acid changes in our alignment suggests that purifying selection may be operating on 1st and 2nd codon positions, so we restricted BSP analyses to 3rd codon positions only. In these analyses we used a GTR model for nucleotide substitutions, but did not include an invariant sites parameter (I) since 3rd codon positions should not be constrained by selection. Analyses were run for 100 million iterations, sampling every 10,000th iteration to reduce autocorrelation, and were repeated three

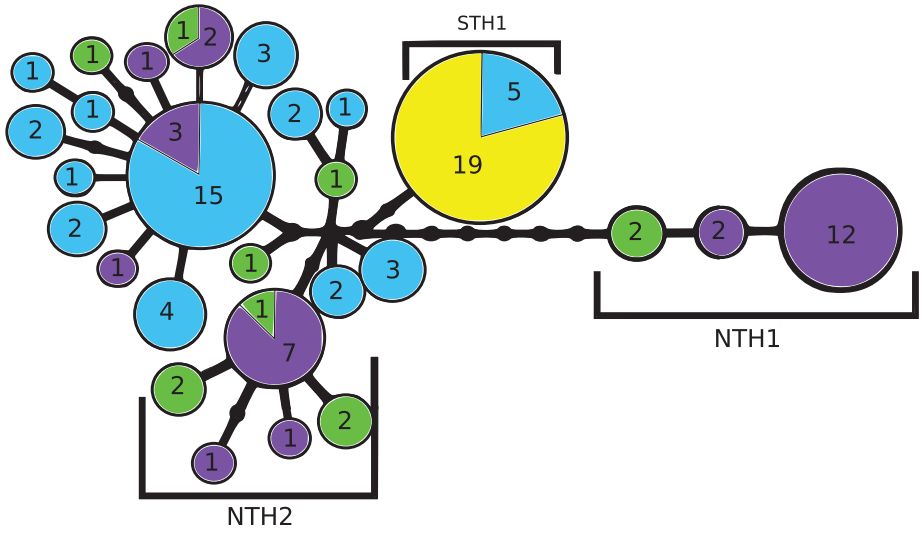

Figure 3. Haplotype network of *C. australensis* populations. Each circle represents a unique haplotype, with the numerals inside indicating the number of individuals sampled of that haplotype. Each step between haplotypes indicates one base pair substitution.

times to check for convergence. We implemented a strict molecular clock with rate of 1.0, which allows us to readily convert mutations per site per generation into chronological years.

We converted the Bayesian Skyline plot scale to chronological years through dividing it by mutation rate and the number of generations per year. We used the mitochondrial mutation rate observed in *Drosophila melanogaster* Meigen, viz. 6.2×10^{-8} mutations per site per generation as an estimate of the mutation rate for *Ceratina australensis* (Haag-Liautard et al. 2008). This method follows a previous study on demographic history in Fijian halictine bees in the genus *Lasioglossum* (Groom et al. 2014a) and North American *Ceratina* species (Shell and Rehan 2016). We note that the mitochondrial mutation rate for *Caenorhabditis elegans* (Maupas) is estimated as 9.7×10^{-8} mutations per site per generation, close to the rate for *D. melanogaster*, and that they have mitochondrial AT biases of 76% and 82% respectively. Previous studies have reported an AT bias of 74% for the same barcoding region as in our study (Groom et al. 2014a; Shell and Rehan 2016), and our *Ceratina* haplotypes had an AT bias of 78%. The number of generations was determined as two per year based on nest contents data from the Victorian and South Australian sites (Dew and Rehan, unpublished data), which also corresponds to detailed studies on the Queensland population (Rehan et al. 2010b, 2011, 2014).

In order to determine whether inferred changes in historical population size in the BSP analysis were significant we also carried out another coalescent analysis using the same parameter settings as our BSP analysis, but implementing a constant population size model. This was then compared to our BSP analysis using a Bayes Factor test.

Inferring ancestral distributions

Ancestral distributions were inferred using BEAST ancestral traits reconstruction. The full alignment of 102 sequences was used. Sample location for each sequence was coded as a discrete trait (either New South Wales, Queensland, South Australia or Victoria). The analysis ran for $2x10^8$ generations, logged every 1,000, with a Yule process tree prior. A HKY+I+G site model with a strict clock of rate 1.0 was employed. All parameters for phylogeny and ancestral trait reconstruction had reached stability, as viewed in TRACER with a burnin of $1x10^8$ generations. Using this burnin a consensus tree was constructed in TREE ANNOTATER.

Results

Haplotype lineages

In total 28 haplotypes of *Ceratina australensis* were found across the four field sites. The haplotype tree along with posterior probability values for node support from our BEAST analysis is given in Figure 2. This analysis indicates the presence of a clade comprising one New South Wales and two Queensland haplotypes, which is highly supported (PP = 1.0) as sister clade to the remaining haplotypes. Our haplotype network analysis (Figure 3) indicates that this clade, which we will refer to as NTH1 (due to their relative northern location), is separated from the common ancestor for the remaining haplotypes by seven nucleotide substitutions, none of which involve amino acid changes.

Both the haplotype tree in Figure 2 and the haplotype network in Figure 3 indicate geographical structuring of haplotypes. Firstly, the haplotypes in NTH1 were not recovered in any of the 99 specimens genotyped from the more southwestern sampling locations of Victoria and South Australia. Secondly, there was another clade comprising five haplotypes that were only recovered from the more northeastern localities of Queensland and New South Wales, and we refer to this clade as NTH2. Lastly, the haplotype, which we refer to as STH1 (due to its southern location), mostly comprised specimens from South Australia, but also some from Victoria, and it was not represented in any of the Queensland or New South Wales specimens. Two haplotypes are shared between Queensland and New South Wales, with one shared between Queensland and Victoria.

Population structure

Pair-wise comparisons among all individuals revealed significantly greater sequence divergence between populations than within populations for Victoria to New South Wales and Queensland, and for South Australia to all other populations (Table 2). Queensland

Table 2. *Ceratina australensis* regional population structure. Diagonal indicates average pairwise differences within populations, number in parentheses indicates total number of sequences for that region; above diagonal are average pairwise differences between populations; below diagonal are pairwise F_{ST} values. Significant values (p <0.05) indicated in bold.

Population structure	Queensland	New South Wales	Victoria	South Australia
Queensland	6.88736 (30)	6.5303 (0.49)	**6.93968 (<0.0001)**	**6.5 (<0.0001)**
New South Wales	0.0598 (0.19)	5.49091 (11)	**5.03247 (<0.0001)**	**5.09091 (<0.0001)**
Victoria	**0.35579 (<0.0001)**	**0.26487 (<0.0001)**	2.5331 (42)	**3.78571 (<0.0001)**
South Australia	**0.42823 (< 0.0001)**	**0.55586 (<0.0001)**	**0.58507 (<0.0001)**	0 (19)

Table 3. Tajima's D and Fu's F_S tests of neutrality within populations. Segregating sites (S), Tajima's D score and significance value (D p-value), and Fu's F_S value and significance values (F_S p-value) are presented. Values in bold are statistically significant (p <0.05).

Neutrality tests	Queensland	New South Wales	Victoria	South Australia
S	19	16	16	0
Tajima's D	1.36657	0.02304	-1.01646	0
D p-value	0.937	0.558	0.186	1.000
Fu's F_S	**-25.15767**	**-6.57395**	**-26.633**	$3.4x10^{38}$
F_S p-value	**0**	**0.001**	**0**	1

and New South Wales were not significantly different from one another. These results are mirrored by pairwise F_{ST} calculations (Table 2), suggesting that all populations except New South Wales and Queensland are genetically differentiated. There was only one fixed base pair difference identified between any of the populations. This was at base 486, which was a thymine in all Queensland and New South Wales samples but an adenine in all South Australia samples (Victorian samples varied at this base). Tajima's D value indicated neutral evolution between populations (Table 3).

Historical demography

Our Bayesian skyline plot (BSP) analysis is summarized in Figure 4 where it is temporally aligned with a graph summarizing two temperature proxies taken from Pahnke et al. (2003). In Figure 4 we have used two x-axis scales, one using mutations per site per generation and the other using years before present, based on a mutation rate of 6.2 x 10^{-8} mutations per site and two generations per year. Based on the current best estimate for mutation rate these plots suggest an increase in effective population size beginning approximately 20–18ka, with a peak rate of increase at about 15–8kya, and a plateauing after about 6kya. Our BSP plot shows a long period of stasis from approximately 64–20kya. This is an artifact of the analysis, where signals prior to the last major effective population size change are lost (Grant et al. 2012; Grant 2015). This period of stasis was trimmed in the final figure to show just the plot from 32kya.

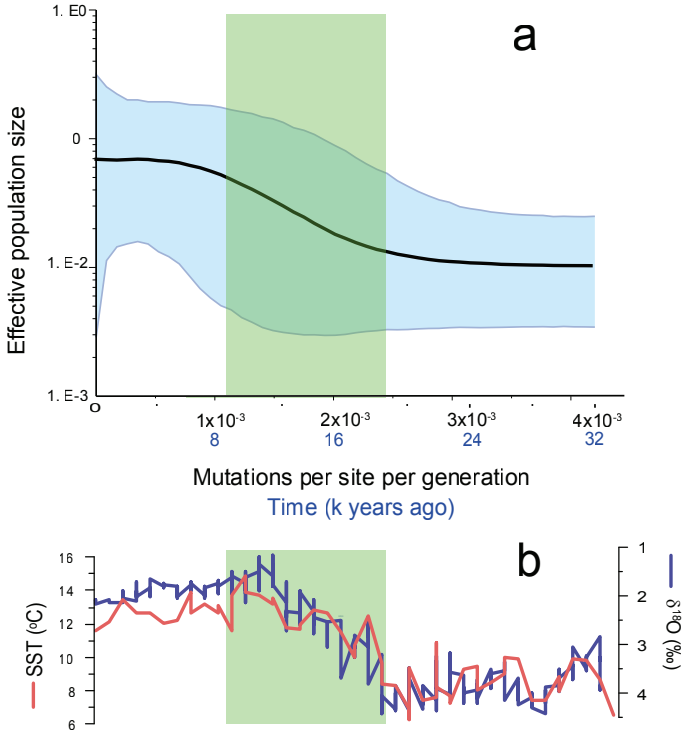

Figure 4. Bayesian skyline plot (**a**), and (**b**) graphs of two proxies for historical climate in the southern hemisphere (adapted from Pahnke et al. 2003). These proxies are δ18O ‰ and sea surface temperate (SST) based on Mg/Ca ratios. The boxes indicate the approximate period of elevated haplotype accumulation.

The 95% Highest Posterior Densities for the BSP plot in Figure 4 indicate a substantial level of uncertainty in how population size may have changed over time, although a general trend for logistic growth is clear. A Bayes Factor test comparing our BSP model with a constant population size model gave a Bayes factor of 6.164, indicating strong support for increasing population size over time.

Ancestral distribution

The reconstructed ancestral distribution of haplotypes is shown in Figure 5 with only the probability of the location reconstruction displayed for those branches with a probability below 0.99. The analysis supports a migration from further northeast moving southwest into South Australia. It suggests that there have been multiple dispersals between Victoria and Queensland but one strongly supported dispersal event between the New South Wales and Victorian populations. There is very low support for the ancestral distribution on all branches preceding the Queensland and New South Wales' clades, so we cannot infer directionality of dispersal between these clades and Victoria.

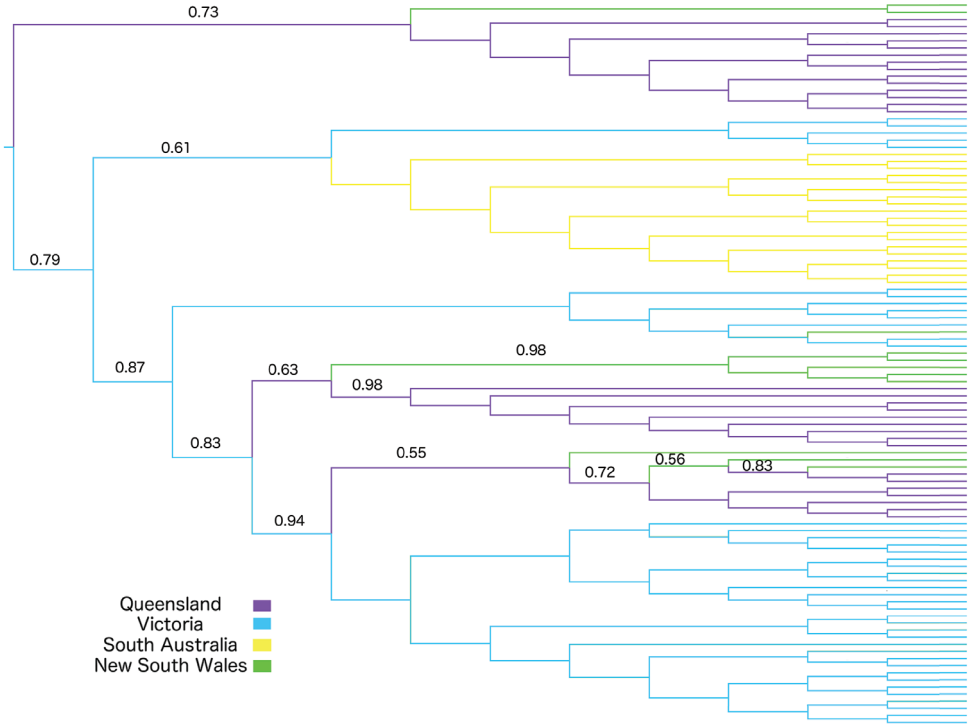

Figure 5. Cladogram showing inferred ancestral ranges of haplotypes from a BEAST traits analysis. Posterior probabilities of location reconstruction are shown only on those branches with support less than 0.99.

Discussion

Geographic structure

Our haplotype phylogeny, haplotype network and AMOVA analyses suggest geographic structure in haplotypes between the four sample sites. The NTH1 clade consisting of specimens from New South Wales and Queensland forms a sister clade to all other lineages (Figure 2), separated by a minimum of nine base pair mutations (Figure 3). Clade STH1 is restricted to the more southwestern populations of South Australia and Mildura. It is interesting that the South Australian population comprises only a single haplotype. It seems unlikely that a population bottleneck would remove all but one matriline in the population, however without further gene regions we cannot rule out this possibility. Another explanation is that the population has not been in place long enough for new haplotypes to arise and/or that there has been insufficient maternal gene flow from northern populations to promote haplotype diversity above that from a small founder population. The haplotype data are indicative of a southwestwards population expansion.

Interestingly, the second-most common haplotype in our sequences was found in both the Queensland and Victorian samples, and it has given rise to further haplotypes

in both regions and NSW. Our BSP phylogeny (Figure 4) suggests that these haplotypes arose recently, and this might indicate that vagility in *Ceratina australensis* has not been sufficient to completely erode geographical assortment of matrilines.

Historical demography

Our BEAST traits analysis also supports a more northeastern origin with a subsequent introduction into South Australia (Figure 5). The analysis is not able to discern between New South Wales, Queensland and Victoria as the likely origin of *Ceratina australensis* into Australia, but given that the subgenus *C.* (*Neoceratina*) is a primarily Oriental and Indo-Malayan clade (Rehan and Schwarz 2015), an origin in Queensland seems most likely. Pairwise comparisons indicate that the New South Wales and Queensland populations are not genetically distinct (Table 2). Interestingly the New South Wales population is about equidistant from both the Queensland and Victorian sites, however the Victorian population shows significant genetic distinction from New South Wales. The difference in gene flow between these populations may be due to fragmentation of suitable habitat for *C. australensis* in the semi-arid to arid zone separating the New South Wales and Victorian populations (Figure 1). The traits analysis indicates that multiple dispersals of matrilines between the Queensland-New South Wales populations and Victoria have occurred but the direction of movement between populations could not be resolved (Figure 5).

Regardless of when and where *Ceratina australensis* entered Australia, our BSP analyses provide strong support for an increase in effective population size beginning about 2.5×10^{-3} mutations/site ago then plateauing about 0.8×10^{-3} mutations/site ago. Assuming a mutation rate of 6.2×10^{-8} and two generations per year (Shell and Rehan 2016), these values correspond approximately to 20 kya and 6.5 kya respectively.

Our timescale indicates an increase in effective population size approximately 20 kya. This increase could be linked to reduced competition, expansion into a new niche or increased resource availability. To investigate these possibilities we need detailed historical reconstructions, which are not presently available for Australia. Climate reconstructions from 20 kya are available for the southern hemisphere (Pahnke et al. 2003). Climate change may act directly on species, or indirectly, for example by increasing flowering plants and therefore resource availability. In Figure 4 we have contrasted the BSP curve with two climate proxies (δ^{18} O isotopes and estimated sea surface temperatures, SST) for the southern hemisphere reported by Pahnke et al. (2003). These graphs suggest that the major period of increasing N_e for *Ceratina australensis* coincides with a major period of post-glacial warming, and that the more recent leveling off in N_e could correspond to a plateauing or slight decline in temperature since 6 kya.

Unfortunately, there are very few detailed studies of paleoclimates in Australia beyond a very small number of sites, limiting further analyses. In one of the most thorough studies, Ayliffe et al. (1998) reconstructed climate in the Naracoorte region, in the South East of Australia, over the past 500,000 years. This geographical location,

however, is well removed from our study sites. Reconstructions of Australia-wide paleoclimates are summarized by Byrne et al. (2008), and while this provides evidence for very broad changes in Australian climates, those studies do not permit reconstruction of paleoclimates in a way that permit refugial areas for *Ceratina australensis* during the last glacial maximum (LGM) to be identified with confidence. However, it seems likely that during the LGM, climatic regimes that now occur in southern Queensland would have had a more northerly distribution.

The inferred increase in N_e for *Ceratina australensis* coincides closely with the timing of dramatic increases in population size for three independent halictine bee clades in Vanuatu, Fiji and Samoa, each of which corresponded to interglacial warming (Groom et al. 2013, 2014a). It is difficult to imagine a factor other than global climate that would be able to influence isolated bee populations in a similar way across the southwestern Pacific (SWP) and Australia, especially when it is considered that *C. australensis* is in a different family to the SWP halictine bees and has very different nesting and floral-adaptation biologies to halictine bees (stem nesting versus ground nesting, and long-tongued versus short-tongued, respectively). On the other hand, we did not find evidence for a dramatic decline in N_e of *C. australensis* at the LGM, and this contrasts strongly with studies on SWP halictine bees (Groom et al. 2014a). It is possible that this contrast is due to *C. australensis* occurring on a continent, where it may have been able to persist in a wide range of refugial habitats, which would have not been as abundant for bee species restricted to small islands.

The expansion of population sizes in *Ceratina australensis* in the current interglacial is consistent with expectations for a Mediterranean or subtropical adapted species responding to warming climates in the southern hemisphere, where southern latitudes retreated from glacial conditions experienced at the LGM. This is also concordant with our historical biogeography analyses, which suggests a northeastern origin, followed by accumulation of haplotype diversity in the semi-arid population in northern Victoria and a recent dispersal to South Australia, indicated by the lack of haplotype diversity and BEAST ancestral traits reconstruction.

Conclusion

Our results suggest that *Ceratina australensis* has responded in major ways to climatic changes since the LGM, but there are two important questions that need resolution. Firstly, because bees are pollinators, historical changes in their diversity and abundance are likely to have impacted angiosperm reproduction in the past, and this may help understand current angiosperm communities. Secondly, if past climates have had large impacts on bee populations in the past, it is important to understand these so that we can anticipate the effects of future climate change. We can only interrogate museum records for impacts of climate change to very limited extents: for Australian insects this will be mostly limited to the last 200 years. In contrast, genetic methods can be used to examine changes well before the recent past and for species that were not covered by

early collectors. Our results suggest that genetic approaches to historical demographics of native bees may hold important insights for understanding how climate change has impacted pollinating biota and plant-pollinator relationships.

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Supplementary material 1

Cladogram including *Ceratina* (Neoceratina) Solomons sp. as the outgroup to root the tree

Authors: Rebecca M. Dew, Sandra M. Rehan, Michael P. Schwarz

Data type: EPS file

Explanation note: Cladogram including *Ceratina* (Neoceratina) Solomons sp. as the outgroup to root the tree. Posterior probability values: *** = 1.0; ** ≥ 95; * ≥ 85.

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SHORT COMMUNICATION

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Simultaneous detection of *Nosema* spp., *Ascosphaera apis* and *Paenibacillus larvae* in honey bee products

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Abstract

Honey bees are responsible for pollinating many native and cultivated plant species. These insects can be affected by many pathogens, including fungi and bacteria, both of which can form spores that are easily dispersed within the colony by means of the stored products, among other routes. The objective of this study was to develop a method to detect spores of the honey bee pathogens *Nosema apis*, *Nosema ceranae*, *Ascosphaera apis* and *Paenibacillus larvae* in samples of honey, bee pollen and royal jelly. The method was standardized for each product individually, and then analyzed by monoplex and multiplex PCR, which showed the same detection thresholds: 1.25 spores/mL of honey for *N. ceranae*; 7.5 spores/mL of honey for *A. apis*; and 0.4 spore/mL of honey for *P. larvae*, respectively. The standardized technique was effective and rapid for the detection of these pathogens in bee products and can be used for the establishment of official methods of sanitary control of bee products, considering the growing national and international trade of these products and the movement or migration of colonies between regions.

Keywords

Apis mellifera, Bee pathogens, Honey, Pollen, Royal Jelly, Detection of spores

Introduction

The honey bee, *Apis mellifera*, is essential to global agriculture by pollinating a wide range of food crops, besides supplying economically important products (Evans and Schwarz 2011). These insects can be affected by various pathogens (Bailey and Ball 1991), including the bacterium *Paenibacillus larvae* and the fungi *Ascosphaera apis*, *Nosema apis* and *Nosema ceranae*. These fungi stand out for their ease of transmission in the form of spores, which can remain viable in the environment for many years, transmitting diseases between colonies and among their individuals (Shimanuki and Knox 1994).

Within the colony, products like honey, pollen and royal jelly are susceptible to contamination by these pathogens, mainly through storage in honeycomb and trophallaxis (OIE 2014). In addition, some negligent hygienic practices by beekeepers, such as reuse of apiculture materials (Van der Zee 2010), exchange of combs containing the remains of diseased broods (OIE 2014), and reuse of contaminated wax (Malone and Gatehouse 1998; OIE 2014), among other practices (Higes et al. 2008; Aronstein and Murray 2010), are indicated as important routes for dissemination of these pathological agents (Hale and Menapace 1980; Higes et al. 2008; Giersch et al. 2009). For this reason, research has been carried out to develop methods to detect pathogens in bee products, mainly involving the use of polymerase chain reaction (PCR) (de Graaf et al. 2013).

Multiplex PCR is used if more than one pathogen is amplified simultaneously in a single reaction, resulting in considerable savings of both time and effort (Sigh et al. 2000), besides reducing costs of reagents and allowing fast analysis of a large number of samples. Thus, molecular biology techniques such as multiplex PCR are important tools for the detection of pathogens.

The aim of this study was to develop a method to detect spores of *N. apis*, *N. ceranae*, *A. apis* and *P. larvae* in honey, bee pollen and royal jelly samples.

Materials and methods

Solutions containing spores of *Nosema ceranae* and *Ascosphaera apis* were prepared from naturally contaminated bees according the protocols described by Teixeira and Message (2010) and Cantwell (1970). For the *Paenibacillus larvae* spore suspension, we were used a solution of ATCC 9545, supplied by the National Agriculture Laboratory - Ministry of Agriculture. The solutions were inoculated in different quantities of sterile bee products, previously submitted to cobalt irradiation, receiving an ion beam dose of approximately 960 Gy/10 cm: honey (5 mL, 10 mL and 20 mL), pollen (0.5 g, 1 g, 5 g and 10 g) and royal jelly (0.5 g, 1 g, 5 g and 10 g), which were submitted to different analytic processes (homogenization, filtration and centrifugation) to test the spore concentrations. The presence of pathogens was confirmed by PCR and sequencing before the tests.

For detection of the pathogens, the samples were weighed (10 g for pollen, 5 g for royal jelly and 20 mL for honey) in sterile tubes and diluted in 40 mL of sterile distilled water. The samples of pollen were diluted in 45 mL of sterile distilled water. The samples were vigorously homogenized and centrifuged at 12,500 *x* g for 40 min. The supernatant was discarded and the pellet was resuspended in 1 mL of sterile distilled water, with subsequent homogenization and centrifugation at 10,000 *x* g for 20 min. The supernatant was again discarded and the pellet was submitted to DNA extraction using the Qiagen DNeasy® Plant Mini Kit, following the manufacturer's instructions. In the case of bee pollen, after homogenization the samples were filtered with Whatman 1 filter paper using a vacuum pump and then the same procedure was followed as for the samples of honey and royal jelly.

Monoplex and multiplex PCR were carried out following Puker (2011) to confirm the detection of each pathogen in the products analyzed. These reactions were performed with a volume of 20 μL, composed of 10 μL of GoTaq® Green (Promega), 8 μL of DNAse free water, 1 μL of sample DNA and 1 μL of each primer (10 μM) (Piccini et al. (2002), Murray et al. (2005) and Martin-Hernandez et al. (2007) for *P. larvae*, *A. apis* and *N. apis*/*N. ceranae*, respectively). All analyses were carried out with positive and negative controls. Spores of *N. ceranae* removed from forage bees naturally infected with the microsporidian were used as positive control. The positive controls of *N. apis* was sent by the Bee Research Laboratory – USDA, considering the low prevalence of the pathogen in Brazil (0.39%) (Teixeira et al. 2013), causing it often not to be detected (Santos et al. 2014). As positive control of *P. larvae*, spores of ATCC 9545 supplied by Lanagro were used.

Decreasing spore concentrations were tested (2500, 250, 100, 50, 25 spores of *Nosema ceranae*; 3000, 300, 150, 75 spores of *Ascosphaera apis*; 1000, 100, 10, 8, 4, 1 spores of *Paenibacillus larvae*), until reaching the minimum detectable number, in order to identify the technique's sensitivity. All the analyses were performed at the Honey bee Health Laboratory of São Paulo State Agribusiness Technology Agency (LASA/APTA), located in Pindamonhangaba, São Paulo.

Results

Multiplex PCR allowed the simultaneous detection of *N. ceranae*, *A. apis* and *P. larvae* in different bee products, and the amplified product for each pathogen presented the expected fragment lengths (218 bp, 485 bp and 700 bp, respectively). Threshold values were the same for the monoplex and multiplex reactions (Figure 1). In samples of honey, we found 1.25 spores/mL of honey for *Nosema ceranae* (218 bp), 7.5 spores/mL for *Ascosphaera apis* (485 bp) and 0.4 spore/mL for *Paenibacillus larvae* (700 bp). For bee pollen we found 2.5 spores/g of pollen for *N. ceranae* (218 bp), 15 spores/g for *A. apis* (485 bp) and 0.8 spore/g for *P. larvae* (700 bp). For samples of royal jelly we found 5 spores/g of royal jelly for *N. ceranae* (218 bp), 30 spores/g for *A. apis* (485 bp) and 1.6 spores/g for *P. larvae* (700 bp).

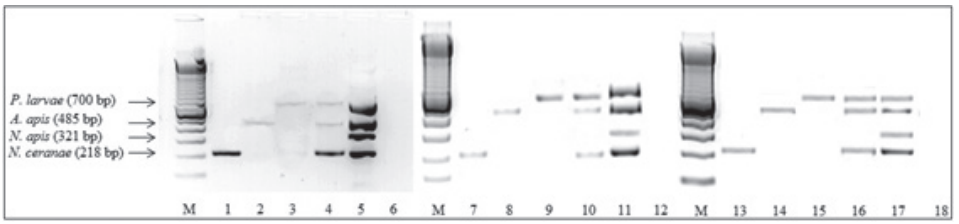

Figure 1. Agarose gel (2%) stained with SYBR® Safe of the monoplex and multiplex PCR products referring to the detection thresholds of the pathogens in honey bee products. M: 100 bp marker **1** 2.5 spores/g of pollen for *Nosema ceranae* **2** 15 spores/g of pollen for *Ascosphaera apis* **3** 0.8 spore/g of pollen for *Paenibacillus larvae* **4** multiplex PCR for *N. ceranae* (2.5 spores/g of pollen), *A. apis* (15 spores/g of pollen) and *P. larvae* (0.8 spore/g of pollen) **5** multiplex positive controls for *N. ceranae* (218 bp), *N. apis* (321 bp), *A. apis* (485 bp) and *P. larvae* (700 bp) **6** negative control. M: 100 bp marker **7** 1.25 spores/mL of honey for *N. ceranae* **8** 7.5 spores/mL of honey for *A. apis* **9** 0.4 spore/mL of honey for *P. larvae* **10** multiplex PCR for *N. ceranae* (1.25 spores/mL of honey), *A. apis* (7.5 spores/mL of honey) and *P. larvae* (0.4 spore/mL of honey) **11** multiplex positive controls for *N. ceranae* (218 bp), *N. apis* (321 bp), *A. apis* (485 bp) and *P. larvae* (700 bp) **12** negative control. M: 100 bp marker **13** 5 spores/g of royal jelly for *N. ceranae* **14** 30 spores/g of royal jelly for *A. apis* **15** 1.6 spores/g of royal jelly for *P. larvae* **16** multiplex PCR for *N. ceranae* (5 spores/g of royal jelly), *A. apis* (30 spores/g of royal jelly) and *P. larvae* (1.6 spores/g of royal jelly) **17** multiplex positive controls for *N. ceranae* (218 bp), *N. apis* (321 bp), *A. apis* (485 bp) and *P. larvae* (700 bp) **18** negative control.

Because *N. apis* occurs in very low prevalence (Teixeira et al., 2013), and is difficult to detect in naturally infected samples, spores of this species were not used in the standardization. We believe the detection threshold would be the same as that obtained for *N. ceranae* (Martín-Hernandez et al. 2007), where the amplification when compared to the positive control worked perfectly.

Discussion

Detection of *Nosema apis*, *N. ceranae*, *Ascosphaera apis* and *Paenibacillus larvae* in samples of bee products has been reported (Cox-Foster et al. 2007, Higes et al. 2008, Giersch et al. 2009), but few studies have reported the detection limits.

Puker (2011) centrifuged samples of honey at 10,000 *x* g for 40 min and reported detection limits of 10 and 100 spores/mL of honey for *Ascosphaera apis* in monoplex and multiplex reactions, respectively, 100 spores/mL of honey for *Nosema ceranae*, and 10 spores/mL of honey in monoplex and multiplex reactions for *P. larvae*. Our results showed lower limits of detection (1.25 spores / mL honey for *N. ceranae*, 7.5 spores / mL for *A. apis* mel and 0.4 spores / mL honey for *Paenibacillus larvae*, which can be attributed to the increase in the centrifugation speed and time.

Some studies have presented detection limits that corroborate this idea. Piccini et al. (2002) obtained a detection limit of 32 spores/mL of honey, analyzing 10 mL of honey and centrifuging the samples at 6,000 *x* g for 20 min. D'Alessandro et al. (2006)

also analyzed honey and found a detection limits of 20 spores/reaction, using 20 mL of honey and centrifuging the samples at 6,000 *x* g for 45 min. In these studies, the limits of detection decreased when the spin time was increased.

Although the technique presented here is similar to those employed by other authors, our tests showed that the samples centrifuged below 12,000 *x* g did not undergo sedimentation, and consequently a considerable number of spores were still present in the supernatant.

To assure that no spores remained in the supernatant, besides performing a centrifugation at 12,000 *x* g for 40 min, we also submitted the resuspended pellet obtained to new centrifuging at 10,000 *x* g for 20 min, for subsequent extraction of DNA. This factor is likely responsible for the technique's sensitivity.

For detection of the bacterium *Paenibacillus larvae*, various methods have been described, mainly using growth in culture medium and PCR (de Graaf et al. 2013; OIE 2014). The Manual of OIE (2014) describes detection of spores in suspension in samples of pollen and royal jelly, employing centrifuging at 6,000 *x* g during 30 min. This centrifuge force can be considered low compared to that used here, which may have left spores in the supernatant, thus hindering efficient detection of the pathogens contained in the samples.

According to the OIE (2014), for the analysis of *Paenibacillus larvae* in pollen, 1 g of the product in 10 mL of sterile distilled water or PBS should be filtered through Whatman 1 filter paper for subsequent microbiological analysis. We also suggest this filtration of pollen samples, because without this step the samples still contained a large quantity of material after centrifuging, which can have a negative effect on extraction of the genetic material and also on the PCR reactions.

Microbiological techniques have also been used to detect *Paenibacillus larvae*, but it is important to consider the time for analysis and efficacy in detecting this bacterium. According to the official technique specified in Brazil (Brasil 2003), it is necessary to wait five days for colony growth in PLA culture medium, plus over three days for confirmatory tests. In other suggested microbiological techniques, it is necessary to incubate the bacteria in culture medium for 6, 7 and 8 days (de Graaf et al. 2013; OIE 2014). The time frame thus varies from 5 to 11 days, placing a significant burden on beekeepers.

The protocol developed showed important results when used to analyze samples of bee products marketed in São Paulo state (in preparation), supporting the idea that PCR is a fast and reliable technique to diagnose pathogen infections (Martin-Hernandez et al. 2007; de Graaf et al. 2013).

The multiplex PCR method offers a significant cost-saving advantage, especially when large numbers of samples are analyzed (Sguazza et al. 2013). Furthermore, multiplex PCR is able to detect multiple target DNA sequences in a single reaction, with the simultaneous identification of two or more kinds of pathogens, simplifying the workflow and processing time, which are significantly reduced (Bilgic et al. 2013), among other advantages (Edwards and Gibbs, 1994).

The protocol presented here is useful to detect simultaneously *Nosema apis, Nosema ceranae, Ascosphaera apis* and *Paenibacillus larvae* in samples of honey, pollen and royal jelly and can support definition of official methods for surveillance actions.

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RESEARCH ARTICLE

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Influence of the reduction of urban lawn mowing on wild bee diversity (Hymenoptera, Apoidea)

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Abstract

To analyse the effects of reduced green space management in urban areas on the biodiversity of insects, we compared intensely mowed lawns (mowed 12 times per year) with meadows under reduced maintenance (mowed only twice per year) in the city of Tübingen (Baden-Württemberg, Germany). Over the entire field season, 177 wild bee individuals representing 43 species were caught using sweep nets. Areas with reduced maintenance showed significantly higher total species numbers and biodiversity indices. Our research supports the initiative "Bunte Wiese (Colourful Meadow) - Species Diversity in Public Greenspaces" of the University of Tübingen, which is campaigning for the enhancement of species diversity in public urban greenland areas by reorganising intensive mowing into a "twice a year" programme.

Keywords

Colourful meadow, conservation, grassland, meadow, mowing, urban ecology, urban green space, wild bees

Introduction

Urbanisation is one of the major environmentally relevant phenomena of our time. The expansion of urban areas is rapidly increasing. In western Germany, the areas settled by humans has increased by about 140% in the past fifty years (Kompakt 2011, Russel 2005). 13.6% of the area of Germany is covered by settlements and

infrastructure (Bundesamt 2016). Fragmentation and destruction of natural areas occurs as a result of these developments (BMU 2008). Even in the countryside, intense agricultural use leads to monocultures and the loss of biological diversity (Landschaften 2006). Green spaces usually have a wide range of flowering aspects and offer a large number of niches for animals (Briemle and Fink 1993). The loss of natural grassland endangers these functions (García 1992). The intensification of grasslands (fertilization, high mowing intensity, ploughing) has a negative influence on the diversity of flowering plants and the animals depending on these resources (Haufe et al. 2015, Schuch et al. 2012).

Urban areas can offer classic nature, providing a wide range of different small habitats with positive impacts on, for instance, flower-visiting insects (Matteson et al. 2013). This explains the current focus of conservationists on these easily introduced replacement biotopes (Bischoff 1996). According to Westrich (1989), half of the German wild bee species can be found in urban areas. For example, 258 bee species have been recorded in Stuttgart (Schwenninger 1999). Although urban natural spaces cannot provide the same functions, continuity, and habitat qualities as natural areas (Müller 2005), the protection of urban nature must form an important part of all biodiversity projects (Müller 2005).

We have investigated the influence of maintenance reduction on public grasslands in the city of Tübingen (Germany, Baden-Württemberg) on the occurrence of flower-visiting wild bees. We assume that the simple reduction of the lawn mowing frequency compared with an intense monthly mowing regime in cities can make an important contribution to the international efforts to reduce the loss of biodiversity (Ade et al. 2012, Hiller and Betz 2014, Kricke et al. 2014, Unterweger et al. 2012, Unterweger and Betz 2014).

If mowing events occur too frequently and at inappropriate times, they can cause significant harm on the biodiversity of both plants and insects (Landschaften 2006). In particular wild bees (Hymenoptera, Apoidea) are highly sensitive to mowing (Buri et al. 2014, Schweitzer et al. 2012). The fast and highly engineered mowing of a flowering meadow at a warm day can kill about 50% of an entire insect population (Hemmann et al. 1987, Oppermann and Claßen 1998) and, for example, kill up to 90,000 honeybees per hectare (Fluri et al. 2000). This is possible as highly engineered mowing entails huge machines, high mowing speeds mowing and a high ground coverage per hour.so the whole foraging ground of a bee population can be cut within a few hours. Mowing affects the life on a meadow by (1) the mowing process itself, (2) the preparation for loading (swathing) and (3) the loading process (Di Giulio et al. 2001). Moreover, (4) the change of the microclimate including humidity (Albrecht et al. 2010) after mowing also contributes to the loss of faunistic biomass on meadows.

Unimproved flower-rich grassland is one of the most important habitats for bumble bees. However, in western Europe, it has been largely lost to agriculture (Goulson et al. 2008). Its restoration can boost bumble bee populations (Carvell 2002, Carvell et al. 2007). In addition, the loss of food sources leads to a decline of wild bees as they

are no longer able to feed their offspring efficiently (Buri et al. 2014). Albrecht et al. (2010) have shown the positive effect on wild bees resulting from ecological improvements in meadows (transformation into two times/year mown hay meadows) that support species richness and ecological functions. Even in private gardens, more nature and less care provide more biodiversity by offering nesting sites for bees (Lindemann-Matthies and Marty 2013).

In 2010, both students and employees of the University of Tübingen founded a pressure group to support national and international aims to protect biodiversity (Unterweger et al. 2012). This group called "Initiative Bunte Wiese" ("The colourful meadow initiative") has been instrumental in persuading decision makers to improve the maintenance of inner urban green areas with respect to conservation issues. This improvement involves (1) the reduction of mowing events towards only twice a year, (2) the use of bar mowers and (3) the removal of the mown grass from the surface (Unterweger et al. 2013, Unterweger and Braun 2015). The results of this management reduction have been evaluated in several research projects. We have investigated grasshoppers (Hiller and Betz 2014), true bugs (Unterweger 2013), beetles (Ade et al. 2012) and butterflies (Kricke et al. 2014). All these investigations have revealed a significant positive impact of reduced grassland maintenance towards species diversity and the occurrence of rare or endangered species.

In the present work, we investigate the influence of a reduced mowing regime on the diversity of flower-visiting wild bees in urban green spaces. We hypothesize that (1) endangered species are strongly linked to areas with reduced maintenance and (2) the number of both species and individuals is significantly higher in these areas.

Methods

Presentation of sampling areas

The locations of the five sampling areas in the city of Tübingen (Baden-Württemberg, Germany) are shown in Figure 1. These sampling sites were carefully selected and represent typical urban green spaces. We think that effects caused by mowing and found on these areas are representative for other areas. Each sampling site was divided into two equal study sites (200–500m^2), i.e. one was treated as a lawn (mowed 12 times per year, a common maintenance practice performed by many public garden departments), whereas the other was mowed twice a year (first cut at the end of May, second cut at the end of September).

Climate

The climatic conditions in 2010 did not significantly differ from those of the past six years. The average annual temperature was about 8.7°C. In July, the average temperature

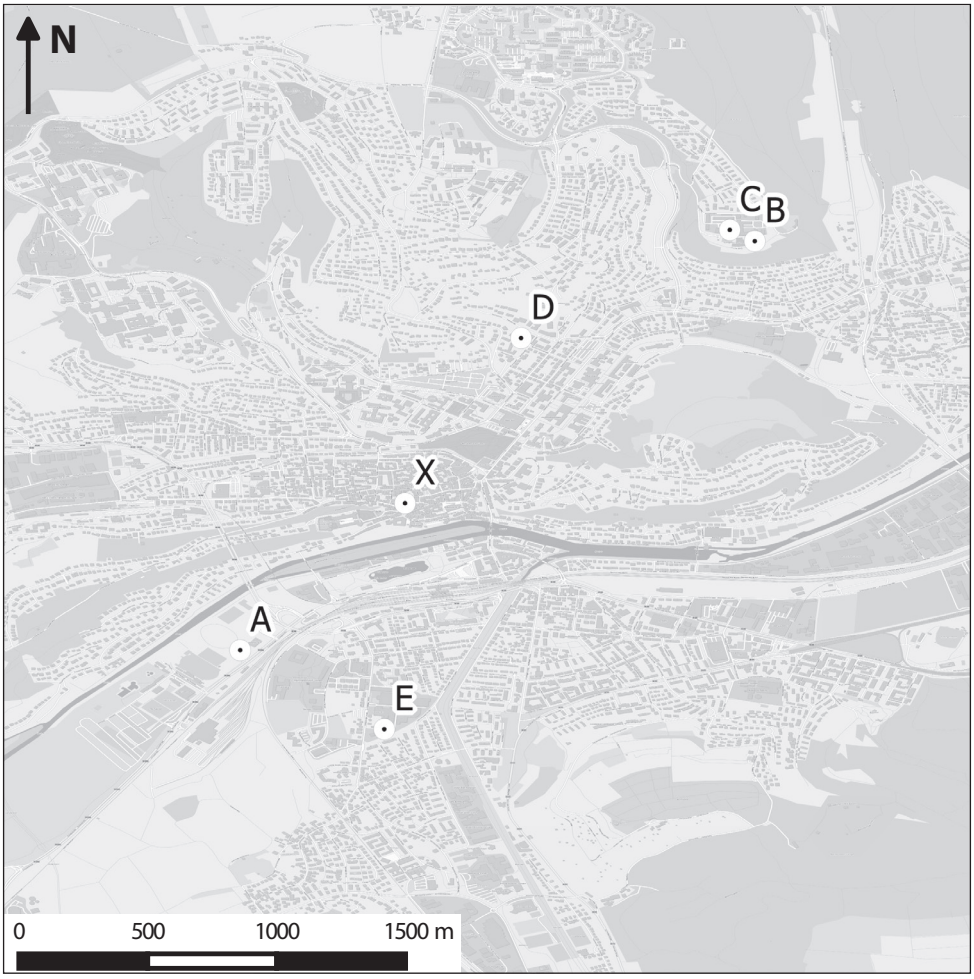

Figure 1. Map of the sampling sites in the urban area of Tübingen. A = Europastraße, B = Sand Süd, C = Sand Courtyard, D = university institute of political science, E = Julius-Wurster-Straße and X the city centre. Each area was divided into an intensely mowed lawn and an area with reduced maintenance (two cuts per year). Provided by OpenStreetMap contributors.

was 19.9°C. Rainfall was rare at the beginning of the year. However, this changed in May (99 mm m^{-2}). In June and July, the weather conditions were wetter than usual but returned to normal in September.

Sampling methods

We followed the sampling methods of Schwenninger (1992). Sweep nets were used to catch the individuals from the flowering plants and the whole area. Collecting time per study site amounted to one hour per date, and management type (honeybees were

not collected as the parent species of the domesticated honeybee is extinct in Europe (Amiet and Krebs 2012).) The sampling period was between April and September 2010 with collections performed on a monthly basis. The sampling dates corresponded to warm and sunny weather.

Preparation and determination

Bees that could not be determined to species in the field were killed with ethyl acetate and carefully prepared. Genitalia were prepared if necessary. Determinations were performed according to the five volumes of Amiet (1996) and the three volumes of Scheuchl and Schmid-Egger (1997) and the aid of the Stuttgart State Museum of Natural History. The systematic classification follows Michener (2007). For species names we followed Schwarz (1996), Gusenleitner and Schwarz (2002), Westrich et al. (2008, 2012), Westrich et al. (2000).

Statistical tests

To compare the two types of maintenance, we also used the Shannon Index and Evenness to evaluate the number of species and individuals (Mühlenberg 1993).

We performed Wilcoxon-tests to check the differences between the intensely mowed lawns and the areas with reduced maintenance. All statistical analyses were performed with SPSS (SPSS 22, IBM).

Results

Species data

Over the entire sampling time, on the five pairs of sampling sites, 177 wild bee individuals representing 43 species (Table 1) were found. The sampling sites (Fig.1) were equally distributed over the urban area of Tübingen. Threatened or declining species from both Germany (GER) and the state of Baden-Württemberg (BW) were only found on areas with reduced maintenance. In Table 1, they are shown with the IUCN (International Union for Conservation of Nature) and the BfN (German Federal Agency for Nature Conservation) standards. The number of individuals per species on the study sites varied from 1 to 21.

Plant data

The average number of dicotyledon species was to 13,6 species on the intensely mowed plots and to 15,8 species on the extensively mowed sites.

Table 1. List of bee species recorded from the five pairs of study plots. The red list categories correspond to the classifications of IUCN (3 = =vulnerable, V =near threatened). The numbers of captured individuals is also given (A = intense mowing, B = reduced management). The months of capture are marked in Roman numerals (I=January, II=February etc.). The dominance D_i and the sampling sites are presented in the last two columns. The sampling sites are named as in Figure 1. The mowing intensity is coded by numbers, i.e. 1 = intensely mowed lawn, 2 = reduced (twice a year) maintenance. Endangered species were only found on areas with reduced maintenance. The total number of caught species per month was IV = 15, V = 28, VI = 15, VII = 13, VIII = 20, IX = 11. The structure of dominance classifies species from 10–31.9% as dominant, 3.2–9.9% as subdominant, 1.0–3.1% as recendent and 0.32–0.99% as subrecendent (Igić 1999; Mühlenberg 1993).

Scientific name	Red List GER	Red List BW	No. of ind. A	No. of ind. B	month	Di	Sampling sites
Andrena cineraria (Linnaeus 1758)			3	4	IV,V	3.95	B1,B2,D1,D2,E1
Andrena gravida (Imhoff 1832)			5	1	IV,V	3.39	B1,E1,D1,D2
Andrena haemorrhoa (Fabricius 1781)			0	1	IV,V	0.56	E2
Andrena labiata (Fabricius 1781)			2	1	V	1.69	B1,C1,C2
Andrena minutula (Kirby 1802)			0	1	V,VI	0.56	B2
Andrena minutuloides (Perkins 1914)			0	5	VI,VII,VIII	2.82	A2,B2
Andrena nitida (Müller 1776)			2	0	IV,V	1.13	C1,D1
Andrena ovatula (Kirby 1802)			1	0	V	0.56	E1
Andrena strohmella (Stoeckhert 1928)			0	1	IV	0.56	D2
Andrena subopaca (Nylander 1848)			2	0	IV,V,VI	1.13	A1,B1
Andrena ventralis (Imhoff 1832)			2	0	IV	1.13	A1
Anthophora plumipes (Pallas 1772)			1	2	IV,V	1.69	A1,C2,D2
Bombus hortorum (Linnaeus 1761)			0	1	V-IX	0.56	D2
Bombus humilis (Illiger 1806)	vu, 3	vu, V	0	2	VI,VII,VIII	1.13	A2,B2
Bombus hypnorum (Linnaeus 1758)			0	3	V,VIII	1.13	E2,D2
Bombus lapidarius (Linnaeus 1758)			2	3	V-VIII	2.82	B2,C2,D1,D2
Bombus lucorum s.l. (Linnaeus 1761)			1	0	VIII	0.56	D1
Bombus pascuorum (Scopoli 1763)			6	15	V-IX	11.86	B2,A1,A2,C2,D1,D2
Bombus pratorum (Linnaeus 1761)			1	2	V	1.69	C1,C2,D2
Bombus sylvarum (Linnaeus 1761)	vu, V	vu, V	0	2	VIII,IX	1.13	B2,A2
Bombus terrestris s.l. (Linnaeus 1758)			0	5	V-VIII	2.82	B2,A2,C2
Chelostoma florisomne (Linnaeus 1758)			6	8	V,VI	7.91	B1,B2,C1,C2,D2

Scientific name	Red List GER	Red List BW	No. of ind. A	No. of ind. B	month	Di	Sampling sites
Colletes similis (Schenck 1853)		vu, V	0	1	V	0.56	B2
Eucera nigrescens (Pérez 1879)			0	2	V	1.13	E1
Halictus simplex (Blüthgen 1923)			0	2	V,VI,IX	1.13	B2,A2
Halictus tumulorum (Linnaeus 1758)			9	6	V-IX	5.65	B1,B2,D1,D2,E1,E2
Halticus scabiosae (Rossi 1790)		vu, V	0	3	V	0.56	B2
Heriades truncorum (Linnaeus 1758)			0	2	VI	1.13	D2
Hoplitis leucomelana (Kirby 1802)			0	2	VI	1.13	D2
Hylaeus communis (Nylander 1852)			0	3	VIII	1.69	B2,A2,D2
Hylaeus gredleri (Förster 1871)			0	1	VII-IX	1.13	A2
Hylaeus punctatus (Brullé 1832)			0	2	VIII	1.13	A2,B2
Lasioglossum calceatum (Scopoli 1763)			1	1	V,VIII,IX	1.13	C1,D2
Lasioglossum glabriusculum (Morawitz 1872)		vu, V	0	2	IV, VII-IX	1.13	A2
Lasioglossum laticeps (Schenck 1870)			2	3	IV,V,VII,VIII	2.82	B2,C1,C2
Lasioglossum leucozonium (Schrank 1781)			2	2	V,VIII	2.26	C1,D1,D2
Lasioglossum morio (Fabricius 1793)			4	5	IV,VII-IX	5.08	D1,D2
Lasioglossum pauxillum (Schenck 1853)			9	14	V-IX	7.91	B2,A2,E1,C1,C2,D2,E2
Lasioglossum villosulum (Kirby 1802)			1	0	VII-IX	1.69	B1,D1
Megachile circumcincta (Kirby 1802)	vu, V	vu, V	0	1	V,VI	0.56	B2
Melecta albifrons (Forster 1771)			1	1	IV,V	1.13	D1,D2
Osmia bicornis (Linnaeus 1758)			6	8	IV,V	9.60	B1,B2,C1,C2,D1,D2
Osmia cornuta (Latreille 1805)			0	1	IV	0.56	C2

Comparisons between intensely and extensively mowed study plots

The comparison of intensely mowed lawns with areas with reduced maintenance show significant differences, both at the species and at the individual level. Figure 2 shows that the number of species found on the intensely mowed lawns is significantly lower compared with the areas with reduced maintenance (RM) (paired t-test: $p < 0.05$, $n = 5$). The same trend (paired t-test: $p < 0.1$, $n = 5$) is visible with respect to the number of individuals (Fig. 3).

Diversity indices

The comparison of the Shannon Index revealed a significant difference between lawns and areas with reduced maintenance ($p < 0.05$). On all sampling sites, Evenness was between 0.81–0.95.

Discussion

Our results indicate that a reduction in mowing intensity has impacts on the wild bee fauna at various levels. Figure 2 shows that the reduced mowing regime results in a significant increase of wild bee species diversity. Buri et al. (2014), Goulson et al. (2008) and others have found that the abundance and richness of wild bee species significantly

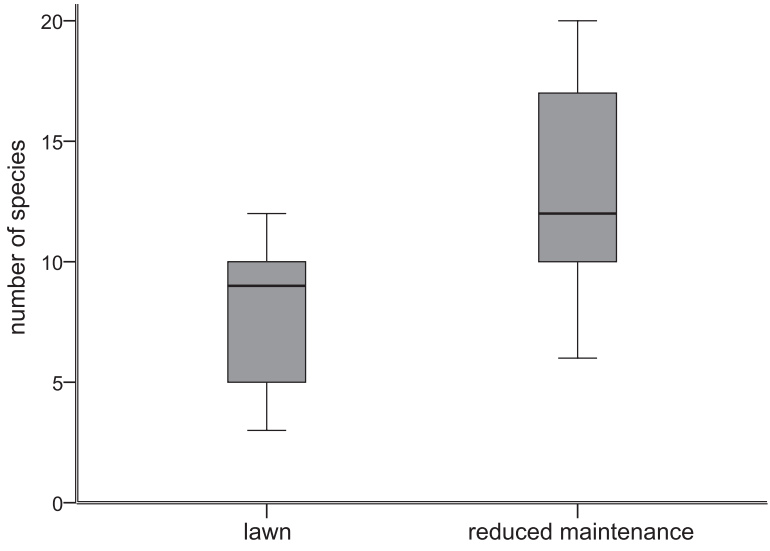

Figure 2. Boxplots showing a comparison of the number of species from intensely mowed lawns with that from areas of reduced maintenance . A Wilcoxon-test (N = 5) shows a significant difference between the numbers of species (p = 0.042).

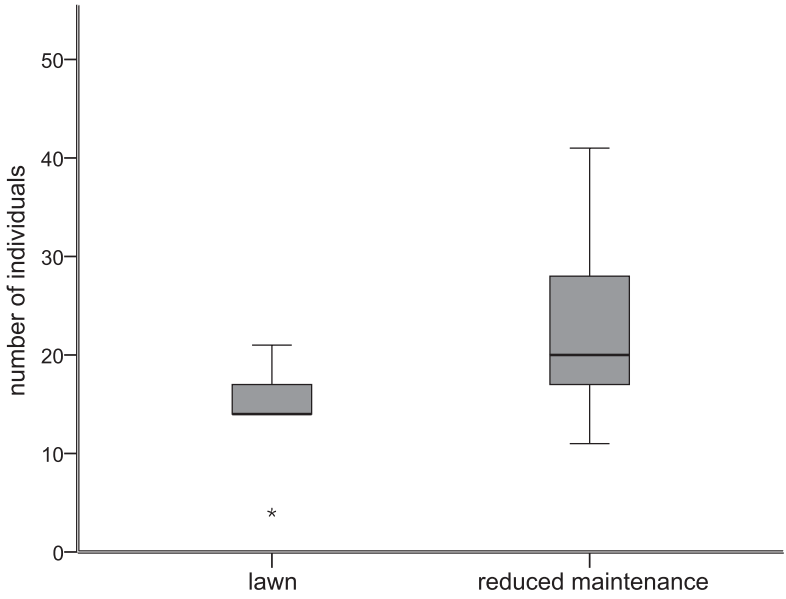

Figure 3. Boxplots showing a comparison of the number of individuals from intensely mowed lawns with that from areas with reduced maintenance. A paired t-test (N=5) shows a distinct trend toward significance (p = 0.08).

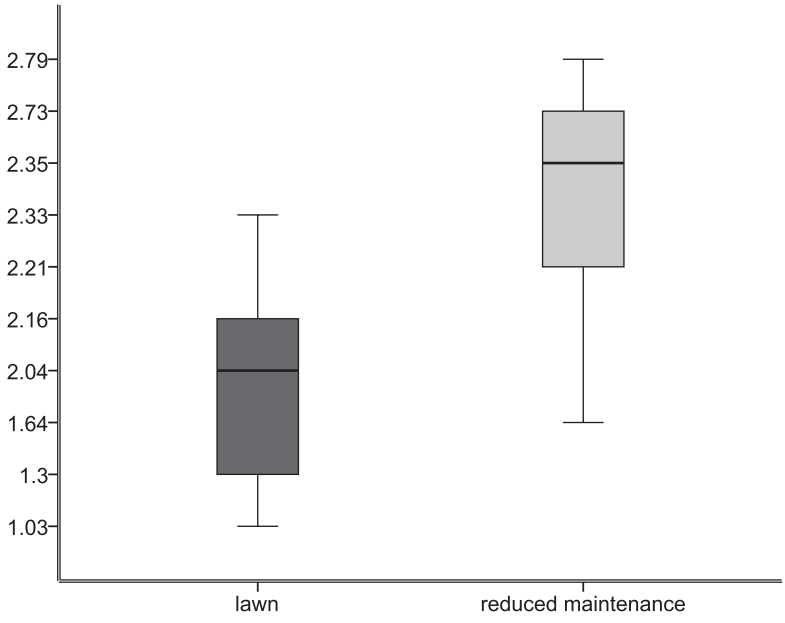

Figure 4. Boxplots showing the comparison of the Shannon Index of lawns and areas with reduced maintenance with paired t-tests shows a significant difference (p = 0.043). The Evenness values all lie between 0.81–0.95.

increases in meadows in which refuges were left uncut. The positive effects of undisturbed urban green spaces on wild bees has also been pointed out in Kutschbach-Brohl et al. (2010). This has also been confirmed for other insect orders such as beetles (Ade et al. 2012), grasshoppers (Hiller and Betz 2014), butterflies (Kricke et al. 2014) and true-bugs (Unterweger and Betz 2014). One reason for the observed differences between the intensely and extensively mowed sites can be seen in the direct effect that a single mowing event can have by reducing the number of individuals of wild bees by up to 50% (Hemmann et al. 1987, Oppermann and Claßen 1998).

Even the number of individuals of more common species can rapidly decline in areas of high mowing intensity. As seen in Table 1, single captures of species (i.e. species that could be detected only once during the investigation period) were made 12 times on areas with reduced maintenance and only four times on lawns over the whole sampling time. Six endangered species were found on the research areas and exclusively occurred on study plots with reduced maintenance. This shows the potential that a reduction of mowing intensity in public green areas can have for the conservation and maintenance of sub-populations of endangered wild bee species (Klaus 2013).

The number of sampled species had its maximum in May and August (IV = 15, V = 28, VI = 15, VII = 13, VIII = 20, IX = 11), a finding that indicates that mowing before the end of May has the greatest effect on urban wild bee populations. The importance of unmown summer meadows follows from the second peak in species richness in August. Wild bees often show a high preference for certain plant species and will benefit from the long-term reduction of maintenance (Weiner et al. 2011). It seems that cutting once per year would support wild bees the most. Nevertheless two mowing events per year has less impact than monthly mowing on the abundance and diversity of entomophilous flowering plants, which is important for nectar feeding insects.

Conclusion

Our results further support the conclusions drawn from studies of the initative “Bunte Wiese Tübingen” on other insect orders such as Orthoptera, Hemiptera, Coleoptera and Lepidoptera showing that a reduction of the maintenance of public urban green spaces supports the diversity of insects. Such actions are relatively easy to achieve in accord with local policy makers and form an effective way of meeting the demands of the global aspiration to stop the loss of biodiversity. The reduction of maintenance and the establishment of natural (infrequently, rather than intensely, mowed) green spaces and waysides can have a significant impact on mitigating the biodiversity crisis, even in our highly populated and highly degraded areas (low abundance and diversity numbers reported here indicate reduced mowing can lessen the impacts of urbanization but does not cure them) and, at the same time, increase the awareness of ecological problems occurring in urban human populations.

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RESEARCH ARTICLE

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First record of egg sac predation on a wall crab spider Selenopidae (Araneae) by the wasp *Camera lunavenatrix* sp. n. (Ichneumonidae, Cryptinae)

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Abstract

We report the first record of egg sac predation on the wall crab spider *Selenops cocheleti* by wasps of the genus *Camera* (Ichneumonidae: Cryptinae) with the description of a new species, as well as biological information on the wasp and the spider host. The rearing record and information presented herein are the first biological data for the genus.

Keywords

egg sac structure, egg predation, Hymenoptera, neotropical

Introduction

Interactions between adult spiders and predatory or parasitoid wasps are a well-known phenomenon. Wasps from the families Crabronidae, Sphecidae and Pompilidae paralyze adult or sub-adult spiders, which are taken as food for the larvae (Hanson and Gauld 2006). The transport of prey items to pre-made nests was hypothesized to be a pre-adaptation for the evolution of social behavior (Evans 1957, Evans and Shimizu 1996); as a consequence, such interactions have sparked much attention. Ichneumonid wasps from the *Polysphincta* group (Wahl and Gauld 1998) are koinobiont ectoparasitoids of various groups of spiders (Sobczak et al. 2012a, 2014, Gonzaga et al. 2015). Likewise, this has drawn much interest due to the phenomenon of host behavioral manipulation (Gonzaga and Sobczak 2011, Takasuka et al. 2015).

On the other hand, predators of spider eggs have received much less attention, particularly in regard to their host-seeking behavior and their influence on the fitness of spiders.

Some groups of wasps are egg parasitoids, including members of the families Encyrtidae, Eulophidae (Hieber 1984, Austin 1985, Schoeninger et al. 2015) and Platygastridae (Fitton et al. 1987, Godfray 1994, Stevens and Austin 2007, Bowden and Buddle 2012). In this case, each of these wasp larvae consumes only one egg of the spider host (LaSalle 1990). Other wasp lineages, including members of Eupelmidae, Eulophidae, Eurytomidae, Pteromalidae and Ichneumonidae (e.g. *Gelis festinans*), are known to act as predators in attacking and consuming successive eggs in the sacs (Fitton et al. 1987, Baarlen et al. 1994, 1996, Quicke 2015). In the temperate zone, several genera of Cryptinae (Ichneumonidae) are recorded preying on egg sacs of various groups of spiders, including *Agasthenes*, *Gelis*, *Hidryta*, *Idiolispa*, *Thaumatogelis* and *Trychosis* (Townes 1970, Schwarz and Shaw 1998, 1999, 2000). In the Neotropics, however, relatively few studies are aimed at spider egg predators (e.g. Sobczak et al. 2012b).

Spiders of the species *Selenops cocheleti* Simon 1880 (Araneae: Selenopidae) are sit-and-wait predators occurring on tree trunks, under bark or in crevices of rocks (Corronca 1998, Alayón-Garcia 2005, Valdez-Mondragón 2007). They are skilled hunters with nocturnal habits and fast movements to capture prey and escape from predators. When disturbed, individuals promptly hide in inaccessible places (Crews et al. 2008, Alayón-Garcia 2005), making observational studies rather difficult. As a result, knowledge of the ecological interactions involving these spiders is still sparse. In fact, there are no records of egg predation by wasps upon spiders of the family Selenopidae.

In this study we present information on the egg sac structure of *Selenops cocheleti*, as well as the first record of egg predation by wasps of the genus *Camera* (Ichneumonidae: Cryptinae), with the description of a new species. We also present biological information on the wasp and the spider host.

Methods

Study site

The *Serra do Japi* highlands area is a small mountainous region in southeastern Brazil, located in the west of the Atlantic Plateau in the state of *São Paulo*. This area has an altitudinal variation between 700m and 1300m above sea level. Average annual temperatures oscillate between 15.7°C and 19.2°C in the higher and lower parts of the highlands. A mesophyll seasonal forest covers most of the forest area of *Serra do Japi* (Morellato 1992). The material was collected in a farm near to the Base Ecológica at Serra do Japi (23°13'53.60"S, 46°52'47.01"W) in two sites denominated "Chácara do Lima" (23°13'53.60"S, 46°55'47.01"W) and "Monte Horebe" (23°14'01.17"S, 46°55'47.01"W).

Collection of *Selenops cocheleti* egg sacs

On September 28th and October 3rd of 2014, we searched at night (18:00 to 21:00) for egg sacs and for gravid adult females of *Selenops cocheleti* in trunks of *Plinia cauliflora* (Myrtaceae; commonly known as jaboticaba) and *Pinus elliottii* (Pinaceae) where these spiders are frequently seen.

The egg sacs and gravid adult females obtained in the field were photographed and taken to the laboratory where they were kept in plastic pots to inspect for potential larvae or pupae of parasitoids or predators. The attacked egg sacs were isolated and the emerging adult wasps collected. All egg sacs (attacked or not) were opened for structural analysis and description. Gravid adult females of *S. cocheleti* collected in the field were also taken and maintained under laboratory conditions. Then we collected the egg sacs laid by the females to estimate the number of eggs per sac and the development time of the early instars of this species of spider.

Taxonomic treatment

All methods and conventions, including morphological terminology and biometrics, follow Santos and Aguiar (2013), except that the cell 1+2Rs is called "areolet" for simplicity.

The studied specimens are deposited at DCBU (Departamento de Ecologia e Biologia Evolutiva da Universidade Federal de São Carlos, São Carlos, SP, Brazil) and MZUSP (Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo, Brazil). The numbers that follow the depository acronyms refer to the registration of each specimen in the institutions.

Results

Egg sac structure and number of eggs of *Selenops cocheleti*

Selenops cocheleti builds an egg sac consisting of three steps, with layers of web giving protection to the eggs: (1) The female at first produces a concave dish that is adhered to the bark of the tree where the eggs are laid; (2) later it builds a layer that will close the egg sac; and (3) when the egg sac is complete, a dense layer of silk threads is added. The egg sac can be found attached on the inner surface of loose bark on *Plinia* and *Pinus* tree trunks (Figure 1A). The two inner layers serve as a wrap for the eggs and the outermost layer is thicker and more resistant (Figure 1B). Adult females rest on the egg sac during the day, and leave their shelter at night to feed. The egg sac can contain up to 287 eggs (Figure 1C) when it is not attacked (average = 181.75; SD = 82.13; n = 4). The eggs hatched within 16 ± 2 days (n = 2) inside the egg sac (Figure 1D). During this period (approximately two weeks) adult females take care of the egg sac standing over it. About three days later, the spiderlings moulted for the first time (into their second instar) whilst still within the egg sac; they remain within the egg sac for on average 18 days, after which they perform a second moult and become more active. Two days after the second molt, spiderlings left the egg sac and scattered. Thus, development time from egg laying to the spiderlings leaving the egg sac was about 40 days. In the egg sacs (n=2) laid by females maintained in the laboratory and in two of the egg sacs collected in the field that were not attacked by *Camera lunavenatrix* sp. n. about 10% of the eggs remained without embryonic development, even after 18 days.

Egg predation

We collected five egg sacs in the field, of which three had *Camera lunavenatrix* sp. n. larva feeding on the eggs, in each case gregariously (Table 1). The wasps pupated inside

Table 1. Number of pupae of *Camera lunavenatrix* sp. n. (Ichneumonidae: Cryptinae) per egg sac of *S. cocheleti* recorded in the trees trunks of jaboticaba (*Plinia cauliflora*, Myrtaceae) and pine (*Pinus elliottii*, Pinaceae) during September (Sep.) and October (Oct.) of 2014 in the Ecological Station Serra do Japi, SP-Brazil.

	Month	Pupae	Males	Females	Survival rate of spiders
Egg sac 1	Sep.	3	1	-	0%
Egg sac 2	Sep.	4	2	2	0%
Egg sac 3	Sep.	0	0	0	88%
Egg sac 4	Oct.	2	0	2	0%
Egg sac 5	Oct.	0	0	0	91%
Total		**9**	**3**	**4**	

Figure 1. Egg sac of *Selenops cocheleti.* **A** In a tree trunk **B** Egg sac structure in layers **C** Eggs **D** Newly emerged spiderlings from the egg inside the egg sac (1st instar) and detail of one of the embryos **E** Second instar spiders **F** Spiderling (3rd instar) on tree trunk **G** Adult female on *Plinia cauliflora* (Myrtaceae) tree trunk in Serra do Japi, SP, Brazil.

the egg sac (Figure 2A). The adult wasps emerged 5–7 days after collection of the egg sacs. In one of the collected egg sacs, two wasp pupae did not emerge as adults.

After the emergence of the adult wasps, we dissected all attacked egg sacs and did not find spiderlings or exuviae inside, indicating that all of the eggs had been consumed. The wasp pupae were arranged next to each other in all the egg sacs attacked (Figure 2C). To leave the egg sac, the adult wasps cut a hole in the walls with their mandibles (Figure 2D).

Wasp behavior

Adult females of the wasp *Camera lunavenatrix* were observed in the field from19:50 PM to 20:25 PM inspecting cracks in the trunk as well as spaces under bark on *Plinia* trees trunks, possibly looking for egg sacs of *Selenops cocheleti*. On the trunks, the female wasps performed a "hammering" movement with the antennae on the trunk surface for about 15 minutes before flying to another trunk to continue searching.

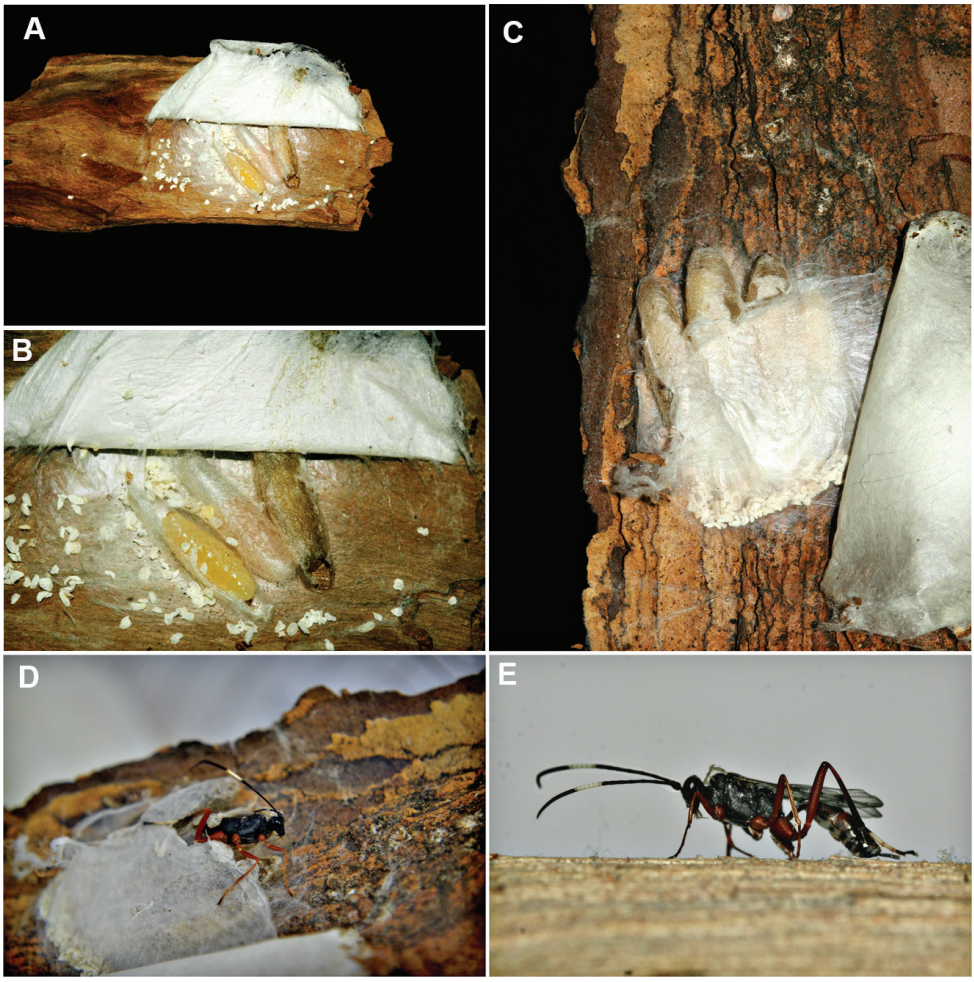


Figure 2. Pupae of *Camera lunavenatrix* sp. n. in egg sac of *Selenops cocheleti* (Selenopidae). **A, B** Egg sac collected under bark of jaboticaba (*Plinia cauliflora*, Myrtaceae) with wasp larvae developing; the white fragments correspond to consumed eggs **C** Egg sac collected under bark of pine (*Pinus elliottii*) **D, E** Adult female of *Camera lunavenatrix* emerging from the egg sac of *Selenops cocheleti*.

Taxonomy

Camera Townes 1962

Camera: Townes, 1962: 432. Type species: *Mesostenus euryapsis* (Cameron 1885), by original designation.

Diagnosis. Clypeus distinctly convex, subpyramidal in profile; malar space 0.80–1.00 as long as basal width of mandible. Mesoscutum only slightly convex; hind margin

of metanotum with distinct tooth-like projection. Propodeum short, about as long as maximum length of mesopleuron; anterior and posterior transverse carinae usually complete, posterior carina sometimes medially indistinct. Areolet moderately small, longer than wide, crossveins 2r-m and 3r-m subparallel or weakly convergent. First metasomal tergite with anterolateral tooth, its spiracle placed at posterior 0.4–0.3; ovipositor sheath 0.25–0.42 as long as hind tibia.

Comments. The new species *C. lunavenatrix* is the fifth species for the genus. There are three other Neotropical species, from Brazil, Mexico and Cuba, in addition to the type species, *Camera euryapsis*, which occurs from Texas to Mexico and also in Cuba. Species of *Camera* seem to be rarely collected by Malaise and yellow pan traps (e.g., none collected by the extensive sampling of Aguiar and Santos 2010), and examination of tens of thousands of specimens Neotropical Cryptini does not indicate a large number of undescribed species (unpublished data). *Camera* shares a number of features with Holarctic genera of Cryptini that attack spider eggs – such as *Trychosis*, *Idiolispa* and *Hidryta* – including a short ovipositor, relatively flat mesoscutum, strongly convex clypeus and a simple, unmodified tip of the apical flagellomere. The rearing record and information presented herein are the first biological data for the genus.

***Camera lunavenatrix* Santos & Onody, sp. n.**
http://zoobank.org/4394F1C7-E421-437D-8FF6-C75DE28527F5

Type material. Holotype ♀ (DCBU# 91323): BRAZIL, São Paulo, Jundiaí, Serra do Japi, 23°11'S, 46°52'W. Emerged on 24.x.2014 from egg sac of *Selenops cocheleti* (Araneae: Selenopidae) collected on 17.x.2014. G. Villanueva. Paratypes: 1 ♀ (MZUSP# 54697), same data as holotype; 2 ♀, 3 ♂ (DCBU# 91324-91326; MZUSP# 54698, 54699), same data as holotype, except egg sacs collected on 18.ix.2014 and emergences on 23–25.ix.2014.

Diagnosis. Head, mesosoma and metasoma almost entirely black except legs ferruginous; occipital carina incomplete ventrally; tergite 1 relatively short, almost as long as tergite 2; body covered with moderately dense white pilosity; ovipositor short, 0.25× as long as hind tibia; clypeus convex, in lateral view almost pyramidal; posterior transverse carina of propodeum medially arched forwards, raised laterally; crossveins 2r-m and 3r-m of areolet subparallel; vein 2m-cu almost straight, reclivous; forewing vein 1cu-a arising distinctly basad of 1M+Rs.

Female. Forewing 9.0 mm. *Head*. Densely pilose; mandible stout, apex 0.6× as wide as base; malar space 0.8× as long as basal width of mandible. Clypeus densely punctate, 2.0× as wide as high, in front view more or less trapezoidal, width at apex 1.4× of basal width, distinctly convex, apically abruptly truncate, in lateral view almost pyramidal; apical margin medially straight, laterally not projecting. Antenna with 33 flagellomeres; first flagellomere 6.5× as long as wide; apex of apical flagellomere uniformly tapered; area between antennae with small rounded tubercle. Supra-clypeal

Figures 3–4. *Camera lunavenatrix* sp. n. **3** Holotype habitus **4** Male paratype habitus. Scale bar: 0.5 mm.

area convex, distinctly rugose-punctate. Supra-antennal area distinctly rugose-punctate, medially with a distinct longitudinal depression. Vertex finely punctate. Occipital carina sharp, laterally sinuous, incomplete ventrally, not meeting hypostomal carina or base of mandible.

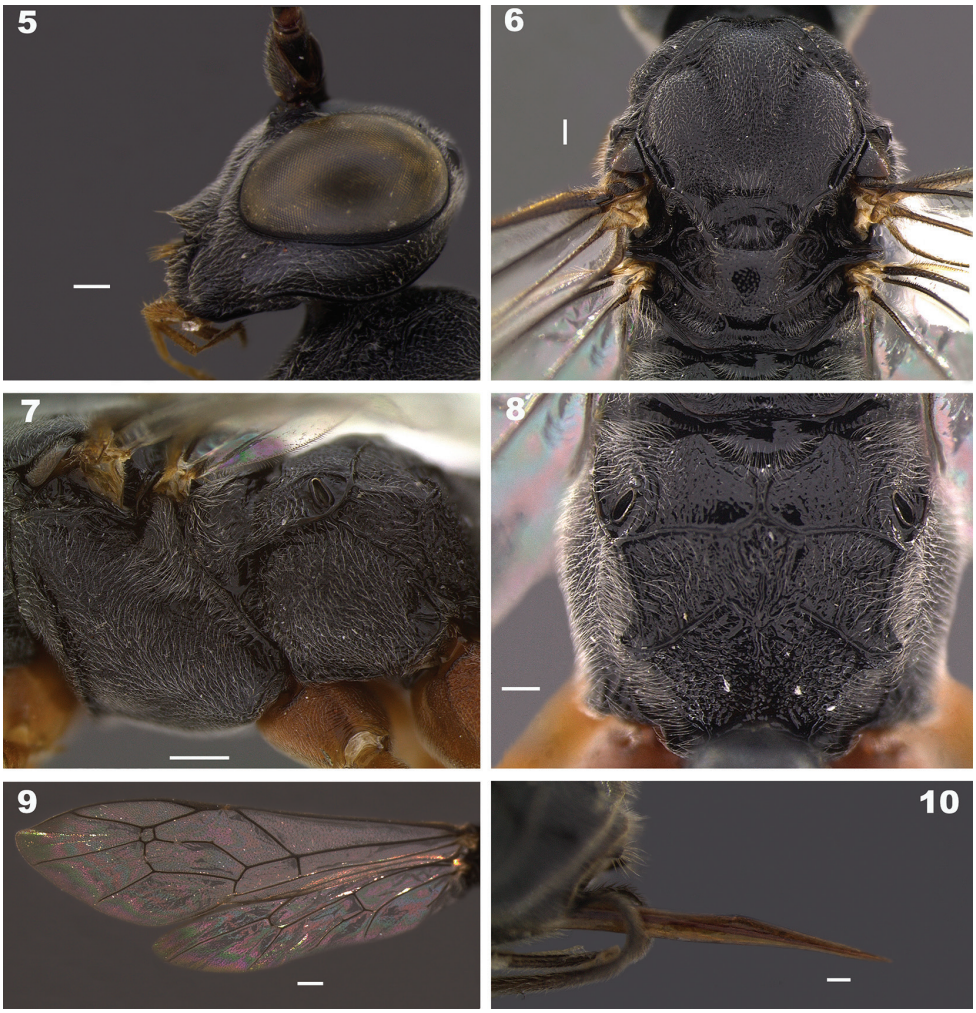
Thorax. Mostly mat, uniformly and densely pilose. Pronotum densely punctate, ventrally striate; epomia distinct, diverging from pronotal collar, dorsally straight, almost reaching dorsal margin of pronotum. Mesoscutum almost flattened, subcircular, 1.1× as long as wide, shiny, densely punctate; notaulus distinct over more than 0.5 of mesoscutum length, with transverse striae, strongly impressed anteriorly, weaker posteriorly; scutellum shiny, punctate; scuto-scutellar groove deep, anteriorly mostly smooth, posteriorly with longitudinal striae. Mesopleuron ranging from densely punctate to rugose-punctate; subalar ridge distinctly projecting, narrow; epicnemial carina reaching about 0.7 of distance to subalar ridge; sternaulus incomplete and weak, reaching 0.6 of distance to mid coxa. Transverse sulcus at base of propodeum deep, about 0.4× as long as anterior area of propodeum, medially smooth; metapleuron densely punctate, juxtacoxal carina absent. Fourth tarsomere not bilobed.

*Propodeum.*1.1× as long as wide; anterior margin medially concave, with distinct anterolateral projections; anterior area moderately punctate, shiny and smooth between punctures and slightly rugose on first lateral area, medially with distinct longitudinal carina; spiracle elliptic, spiracle 2.0× as wide as long; anterior transverse carina medially slightly arched forwards. Posterior area of propodeum rugulose; posterior transverse carina complete, medially distinctly arched forwards, raised laterally; median longitudinal carina of propodeum present between anterior and posterior transverse carinae.

Wings. Forewing vein 1-Rs+M and crossvein 1m-cu both almost straight; bulla of 1-Rs+M placed almost on its midlength; forewing crossvein 1cu-a arising distinctly basad of 1M+Rs; vein 2Cua 1.1× as long as crossvein 2cu-a; crossvein 2m-cu almost straight, reclivous, bulla placed on midlength; areolet of moderate size, 1.30 as long as pterostigma, pentagonal, 1.1x as long as wide; crossveins 2r-m and 3r-m subparallel, about same length; vein 3-M distinctly shorter than 2-M. Hind wing vein 1-M forming right angle with vein Cua; vein Cub weakly convex, forming right angle with vein Cua.

Metasoma. First tergite moderately short, almost as long as tergite 2, about 2.0× as long as maximum width; in cross section approximately depressed, mostly striate, sparsely pilose; apex 2.5× as wide as base; spiracle at posterior 0.4 of its length, distinctly prominent; ventrolateral and median dorsal carinae absent; dorsolateral carinae present, reaching 0.5 of tergite 1 length. Tergite 2 short, 1.1× as long as maximum width, in dorsal view trapezoidal, posterior width 1.7× anterior width, mostly strongly punctate, apically finely punctate; thyridium slightly longer than wide; tergites 3–8 finely pilose and punctate. Ovipositor very short, 0.25× as long as hind tibia, slender, straight, distinctly compressed, nodus slight but distinct; lower valve without conspicuous teeth.

Color. Black with ferruginous legs. Head: black; palpi ferruginous; antenna black, flagellomeres 6 apically and 7–13 entirely white. Mesosoma: black; legs mostly ferruginous; fore and mid legs basally slightly dark ferruginous, generally lighter towards



Figures 5–10. *Camera lunavenatrix* sp. n. (♀ holotype). **5** Head in lateral view **6** Mesoscutum in dorsal view **7** Mesopleuron and metapleuron in lateral view **8** Propodeum in dorsal view **9** Fore and hind wings **10** Ovipositor in lateral view. Scale bars: 0.25 mm (**5**); 0.2 mm (**6**); 0.5 mm (**7**); 0.25 mm (**8**); 0.5 mm (**9**); 0.1 mm (**10**).

apex except tarsomere 5 of foreleg and tarsomeres 3–5 of mid leg dark brown; hind leg with coxa, trochanters and femur dark ferruginous; hind tibia ferruginous on basal 0.65, apical 0.35 blackish; hind tarsomeres 1 basally, 3 apically and 4–5 dark brown to blackish, tarsomeres 1 apically and 2 entirely white. Metasoma: black, except very base of tergite 1 marked with ferruginous; ovipositor ferruginous and ovipositor sheath black; wings hyaline, apically infumate.

Male. Forewing 6.0–8.0 mm long. Generally similar to female except: vertex more sparsely punctate; first flagellomere shorter, about 3.5× as long as wide; tergite 1 apically

narrower, its maximum width about 1.3–1.5× minimum width; hind leg with tibia more extensively blackish and basal 0.25 of tarsomere 1 and tarsomere 5 entirely black.

Variation. Forewing 9–9.5 mm long. Two females have metasoma with tergites 2–8 having some dark ferrugineous areas.

Comments. *Camera lunavenatrix* sp. n. is similar in general morphology to the North American species *C. euryapsis* Cameron and *C. californica* Kasparyan and Ruíz-Cancino. The three species have the body approximately cylindrical, with the propodeum about as tall dorso-ventrally as the anterior part of the thorax, and a relatively short tergite 1. However, *C. lunavenatrix* can be readily distinguished from the other two species by the body almost entirely black (versus with extensive yellow marks); clypeus almost pyramidal in lateral view (versus only moderately convex, with rounded profile); posterior transverse carina of propodeum medially distinctly arched, bell-shaped (versus slightly and uniformly arched); and ovipositor quite short, only 0.25× as long as hind tibia (versus 0.42).

The Brazilian species *C. thoracica* Szépligeti is quite different from the other species of the genus in having the anterior part of the thorax rather stout, with the propodeum distinctly shorter in lateral view; furthermore, tergite1 is distinctly longer than tergite 2, very different from the short tergite1 of the other species. Additionally, *C. lunavenatrix* can be separated from *C. thoracica* by the black metasoma (versus bright orange); and forewing vein 1cu-a arising based to crossvein 1M+Rs (versus opposite).

Etymology. From the Latin *luna* ("moon") and *venatrix* ("huntress"). The name of the spider genus *Selenops* derives from *Selene*, the moon goddess, and the suffix *-ops*, Greek for "eye". Therefore the new species is deemed a "moon huntress" as it parasitizes egg sacs of *Selenops*.

Discussion

It is known that the egg sac works as a physical barrier isolating the eggs from the outside environment. In addition, maternal care provides additional protection by guarding of the eggs (Austin 1985). These protections do not provide absolute defense against predators such as *C. lunavenatrix* but can provide protection against a broad spectrum of opportunistic predators, e.g. ants and other spiders (Austin and Anderson 1978, Austin 1982, 1985).

The observation of adult females of *C. lunavenatrix* inspecting tree trunks between 19:50 PM and 20:25 PM seems to suggest some degree of nocturnal activity for the species. This is somewhat inconsistent with the general tendency of nocturnal ichneumonids to have pale body color and enlarged ocelli (Gauld 2006). It is unclear whether *C. lunavenatrix* may be also (or mostly) active during the day. Diurnal activity has been observed in other cryptine egg predators such as some European species of the genus *Trychosis, Hidryta* and *Gnypetomorpha* (M. Schwarz pers. comm.).

Females of *C. lunavenatrix* may search for egg sacs of *S. cocheleti* during the night because during this period the adult spider female leaves its shelter to feed, leaving the

egg sac vulnerable. On the other hand, while the (rather rapid) development of the wasp takes place, the female spider continues to guard the egg sac. This suggests that the spider may be unable to perceive any wasp activity or change inside the egg sac.

The larvae of *C. lunavenatrix* observed in this study consumed 100% of spider eggs. This is not unusual, as recorded for *Tromatobia* sp. (Ichneumonidae: Pimplinae) and *Aprostocetus* sp. (Ichneumonidae: Eulophidae) attacking *Araneus omnicolor* (Araneidae) (Sobczak et al. 2012b, 2015). However, other records show that egg consumption is not always complete; if the number of parasitoids in the egg sac is low (Cobb and Cobb 2004) some spiderlings may emerge (Fitton et al. 1988, Schwarz and Shaw 1999). Similar results were recorded for *Nephila edulis* (Nephilidae) when attacked by larvae of the moth *Anatrachyntis terminella* (Lepidoptera: Cosmopterigidae); in that case, even with low infestation (six larvae per egg sac), only up to 20% of the eggs hatched (Austin 1977).

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RESEARCH ARTICLE

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Illustrated notes on the biology of *Sphinctus serotinus* Gravenhorst (Hymenoptera, Tryphoninae, Sphinctini)

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Abstract

Field and experimental observations on the European ichneumonid *Sphinctus serotinus* as a koinobiont ectoparasitoid of the limacodid moth *Apoda limacodes* are recorded. The egg is anchored into the extremely thick cuticle of its host but not deeply enough so that it would survive host ecdysis. That may explain the late summer-early autumn flight time of the parasitoid, when practically all host larvae that remain are in their final instar, though avoiding competition from other parasitoids may also play a part. The tough cuticle of the host probably also underlies the lack of host feeding by the adult parasitoid. There is no ability to avoid superparasitism, though self-superparasitism is limited by post-oviposition flight to leave the host. The egg can be laid with its chorion still uncoloured when hosts are in plentiful supply, but such eggs become the usual pale brown colour within a short time. No dumping of eggs in the absence of hosts occurs. The egg normally doesn't hatch until after the host has prepared its cocoon, and the parasitoid larva, still anchored in the eggshell at first, feeds very slowly through the winter and early summer on the prepupal host. Development through the prepupal and pupal stages within the host cocoon similarly proceed slowly, with no evident diapause at any stage. The various stages of the life cycle are illustrated photographically.

Keywords

Life history, biology, egg, larva, pupa, superparasitism, *Apoda limacodes*, Netherlands, Veluwe

Introduction

Sphinctini is one of seven tribes recognised in the ichneumonid subfamily Tryphoninae (Bennett 2015) and contains 14 described species, distributed largely in the Palaearctic and Indo-oriental regions but also in the neotropics (Yu et al. 2012). According to Kasparyan (1992) all the temperate species known at that time fly in the late summer and early autumn. One species, *Sphinctus serotinus* Gravenhorst, occurs widely in Western Europe (Fauna Europaea 2015), and has long been recognised as a specialized parasitoid of the limacodid moth *Apoda limacodes* (Hufnagel). However, it is infrequently collected and it remains rather poorly known. Hinz (1976) reported in outline its oviposition behaviour and the unusual structure of the ovaries in the course of rearing a series in captivity on this host, in particular demonstrating that it is a koinobiont ectoparasitoid and securing its placement within Tryphoninae, but that paper was not illustrated and further aspects of the parasitoid's biology were not covered. The present account consists of both field observation in the Netherlands (JV) and experimentation (MRS), and gives some additional information and, particularly, some illustrative photographs.

Materials and methods

The source of wild *Apoda limacodes* and *Sphinctus serotinus* used to establish cultures for experiments in Edinburgh was the Veluwe (Gelderland) area in the Netherlands, where collections and field observations were made by JV. Host cocoons, including some resulting from larvae bearing eggs of the parasitoid, were sent to MRS in xi.2005, and the following year a culture of the host was set up in readiness for the emergence of the parasitoid adults in autumn. Once in Edinburgh, livestock was kept under semi-natural conditions in a detached, shaded and copiously ventilated rearing shed (cf. Shaw 1997), except for the brief periods of experimentation and photography undertaken indoors. At all times *A. limacodes* larvae were fed in closed plastic boxes (variously 18 x 12 x 6 and 14 x 8 x 6 cm) on *Quercus robur* leaves and, rather than using tissue paper liners, the boxes were frequently opened and wiped out to prevent excessive condensation of moisture instead. This protocol is vital because of the apodous nature of the larvae, and their reliance on secreted slime for locomotion.

When an adult female of *S. serotinus* emerged on 12.ix.2006 it was fed ad libitum on honey:water (1:3) and remained unmated. After six days it was offered cultured final instar larvae (the only instar by then available) of *A. limacodes* singly and under continuous observation at various times over the period until 26.ix.2006 (14 days after emergence), after which no further hosts were available. Initially the host larva, on the *Quercus* leaf on which it had been feeding or resting, and the parasitoid were confined under a transparent plastic container but, as the parasitoid's interest in the host increased, the cover was removed to allow unimpeded observation and photography. A further female of *S. serotinus* hatched on 27.ix.2006 from the 2005 field collections;

its general behaviour was similar, but no hosts were by then available for oviposition. Some observations and photographs involving a field-caught female in the Netherlands are included in the account below.

Parasitized hosts were allowed to continue feeding until cocoon formation, and (both from the wild-collected 2005 and experimental 2006 material) cocoons were opened at intervals during the ensuing winter and subsequent summer to follow the progress of the parasitoid within. As is well known (e. g. Porter 1997), *Apoda limacodes* passes the winter as a strongly cocooned prepupa and pupates in the spring.

One wild-collected host bearing an egg was fixed in 4% formaldehyde (ca 30 hr) and the relevant portion dehydrated with ethanol (50% to 96% in 5 stages) then degreased with 4 changes of xylol before being embedded and sectioned in paraffin wax. Sections (8 microns) were stained with Haematoxylin and Eosin, then slide-mounted in Malinol, in order to investigate details of the egg's attachment.

Photographic images were obtained digitally on a Konica Minolta Dynax 7D camera with Minolta 1-3 macro zoom lens and a ring flash, or on 35 mm Fuji colour transparency film using a standard SLR camera body and a Medical Nikkor 120 mm lens with automatic ring flash.

Results

Field observations

In the Veluwe, larvae of *A. limacodes* have, at least in recent years, occurred commonly on *Quercus robur* growing under a wide range of conditions, from quite dense woodland (where it is also common on *Fagus sylvatica*) to open heathland (Fig. 1), with 2004, 2005, 2008 and 2014 being years of particularly high abundance. Larvae, which exhibit some colour polymorphism not connected with parasitism (Figs 2, 3; final instar), were present in the field for a long period, from late May until early October, in variable stages of growth. By mid-September many *A. limacodes* larvae had become fully grown and spun their cocoons, and virtually all remaining were in their final instars. Attack from other parasitoids (in the area the campoplegine ichneumonid *Phobocampe alticollis* (Thomson), the microgastrine braconid *Dolichogenidea lacteicolor* (Viereck), and the rogadine braconid *Triraphis tricolor* (Wesmael)) was by then finished, so the *A. limacodes* larvae that remained were unparasitized. From late September onwards host larvae were increasingly found bearing eggs of *Sphinctus serotinus* (the first one seen on 25.ix.2005), but almost exclusively on relatively isolated *Q. robur* trees in open sunny heathland situations — in particular at Edese Heide (Fig. 1) where, as late September and the first half of October wore on, the majority of *A. limacodes* larvae were found to bear one or more eggs (Figs 2, 3) of this strictly solitary parasitoid. Good numbers of the host occured also in more shaded woodland in the Veluwe, on both *Q. robur* and *F. sylvatica*, but the parasitoid was essentially absent from this situation and none were found on hosts feeding on the latter tree (which was lacking in the open, xerothermic, areas favoured by the parasitoid).

Figure 1. Biotope at Edese Heide with *Quercus robur*.

Figures 2, 3. Final instar larva of *Apoda limacodes* with **2** one egg **3** four eggs of *Sphinctus serotinus*.

In the wild eggs of *S. serotinus* were seen on a large number of hosts, and appeared to be positioned more or less at random on the exposed surface of the host's body, including positions well down at the sides and near both anterior and posterior ends, though more central and dorsal positions were commonest. Many instances of two, three and even four eggs on a single host were seen (Fig. 3).

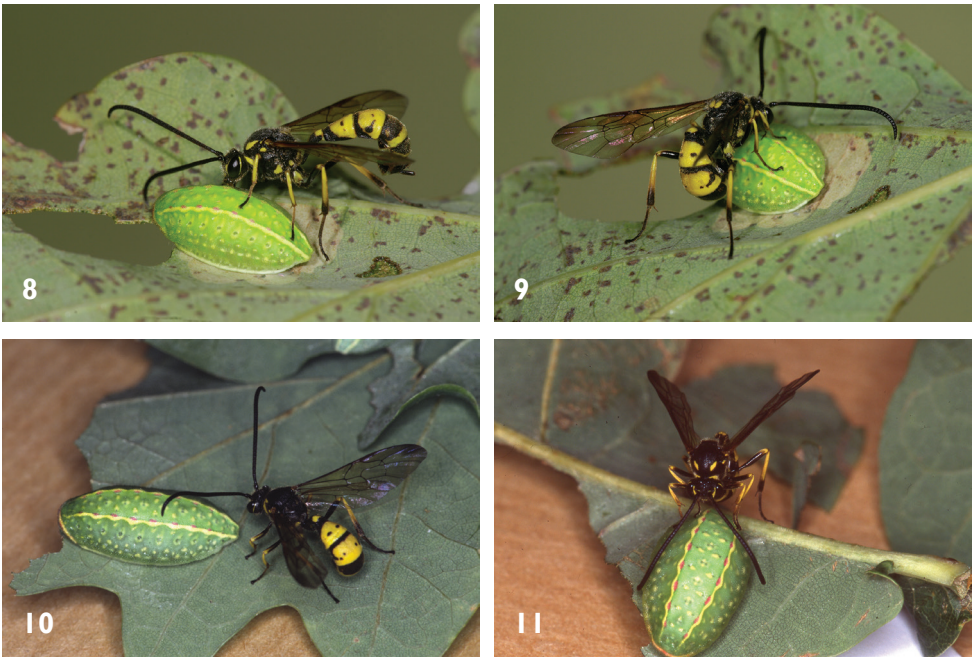
Behaviour and captive rearing

The adult female is about 14 mm long and adopts a fully-resting stance (i. e. when asleep; Figs 4, 5) that is rather unusual in Ichneumonidae, with wings spread away

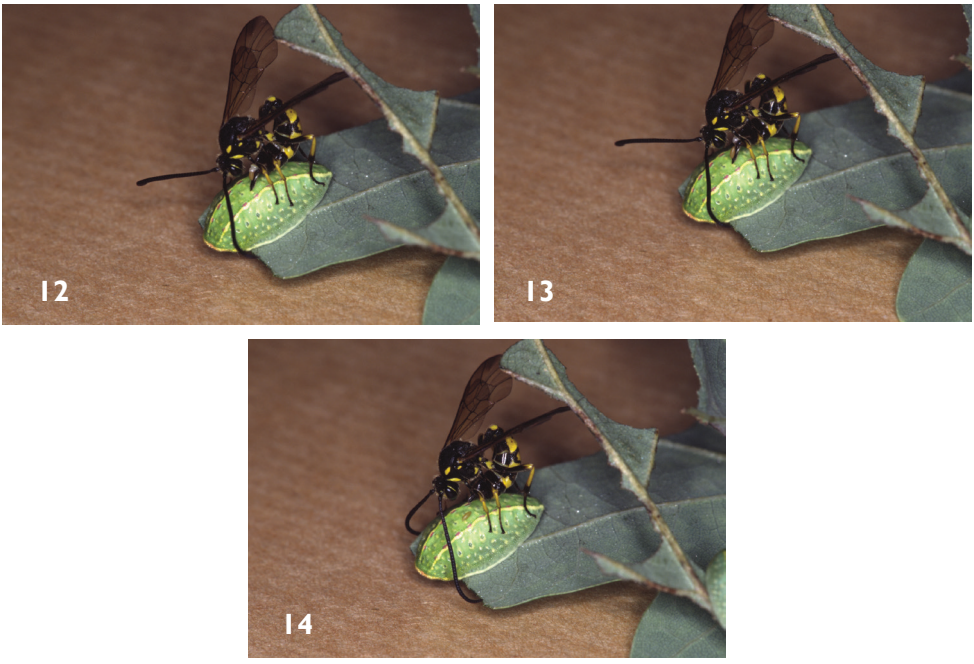
Figures 4–7. *Sphinctus serotinus*, **4, 5** asleep **6, 7** in motion.

from the metasoma, but which clearly accentuates its mimicry of vespid wasps (perhaps *Eumenes* in particular). This exposure of the metasoma is maintained while the parasitoid is actively searching on foot (Figs 6–8), though at such times the antennae are directed forwards and sideways and the wing tips are not flattened to the substrate. The experimental female fed avidly on dilute honey, but showed no tendency to host feed. Even when deprived of hosts, eggs were neither dumped nor carried externally at the apex of the ovipositor.

The female was first offered hosts six days after emergence, when she started to show weak interest in host traces (chewed leaf edges, and the slime trails by which the host moves). This interest became stronger on subsequent days. When a host was encountered (Figs 8, 9) it was often antennated with one antenna along each side of the host's long axis, which often (not always) caused the host to rock rhythmically from side to side (a behaviour that is provoked by various kinds of disturbance, including from another *A. limacodes* larva, and did not unduly delay the female parasitoid although a slight deterrent effect was evident). The position of the antennae suggested that the host was being measured: all hosts offered were final instar, but it was clear from all interactions and ovipositions that large (14 mm or more, female) hosts were much preferred to smaller ones (9–12 mm, male), though some ovipositions on the smaller size range did occur. Except that the antennae were curled downwards during actual oviposition and in the subsequent brief period of inspection (but usually not in contact with anything), they were not used for further investigation or manipula-



Figures 8–11. *Sphinctus serotinus*, approaching and accepting *Apoda limacodes* larva.

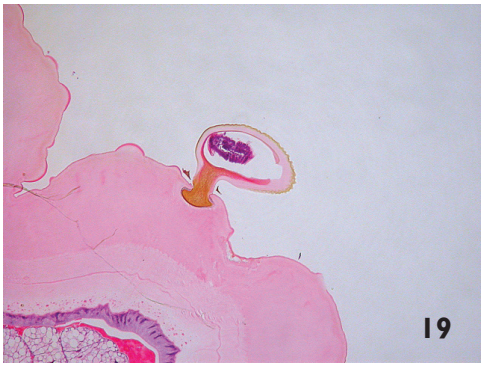


Figures 12–14. *Sphinctus serotinus*, oviposition sequence.

Figure 15. *Sphinctus serotinus*, priming for oviposition.

tion. Altogether 15 ovipositions were obtained, interspersed with rejections that were sometimes later reversed, on days 6 (2 ovipositions), 8 (1), 9 (1), 13 (7) and 14 (4) of this single female's adult life. When the female crawled, often rather slowly, onto the host (Fig. 10) she sometimes did so transversely and kept the hind legs on the substrate (Fig. 11), but usually she approached longitudinally and then used all six legs to perch upon it (Fig. 12). This difference accounts for the variable egg positions seen (respectively lateral and dorsal). The ovipositor was then brought forward between her legs to pierce the host integument, with the sheaths supporting the ovipositor. The main body of the egg issued from the genital opening caudal end first (Fig. 12) and slid down the outside of the ovipositor, with its anchor travelling down the shaft internally (Fig. 13) until oviposition was completed, leaving the egg on the surface of the host but its stalk implanted (Fig. 14). This process took about 10–15 (exceptionally 20) seconds, during which the host was usually completely quiescent, but appeared not to have suffered temporary paralysis. The female usually paused, apparently reviewing her achievement, for a few seconds after oviposition then, invariably, left purposefully — usually by flying away. A host already bearing an egg was accepted without any sign of hesitation or inhibition, so this prompt post-oviposition reflex departure seems to be the only way in which self-superparasitism is avoided. Normally the female fed avidly on honey:water between ovipositions, and there was sometimes a clear cocking and jerking motion at the apex of the metasoma suggestive of a new egg being moved down the oviduct to a position in the genital opening ready for oviposition as a host was approached (Fig. 15). The anchored egg is usually light brown at the time of oviposition

Figures 16–17. *Sphinctus serotinus*, laying an immature egg.



Figures 18–20. *Sphinctus serotinus*, egg on host **18** as laid **19, 20** sectioned.

and remains so until it hatches, but if more than two ovipositions occured in close succession the subsequent eggs were very pale yellowish green (Figs 16, 17), darkening to the usual light brown over a few hours. After the available hosts had run out the female was denied access to food, and died (undoubtedly of starvation) nine days later.

The matt, rather roughened egg of *S. serotinus* is 1.1 mm long and anchored into the host integument (Figs 18–20), as is typical for Tryphoninae (Bennett 2015). In this case the egg is fastened by a clear flange-like expansion at the distal end of its stalk. The anchor extends only into the already secreted cuticle which is abnormally thick in *Apoda*

Figure 21. *Sphinctus serotinus*, egg chorion splitting.

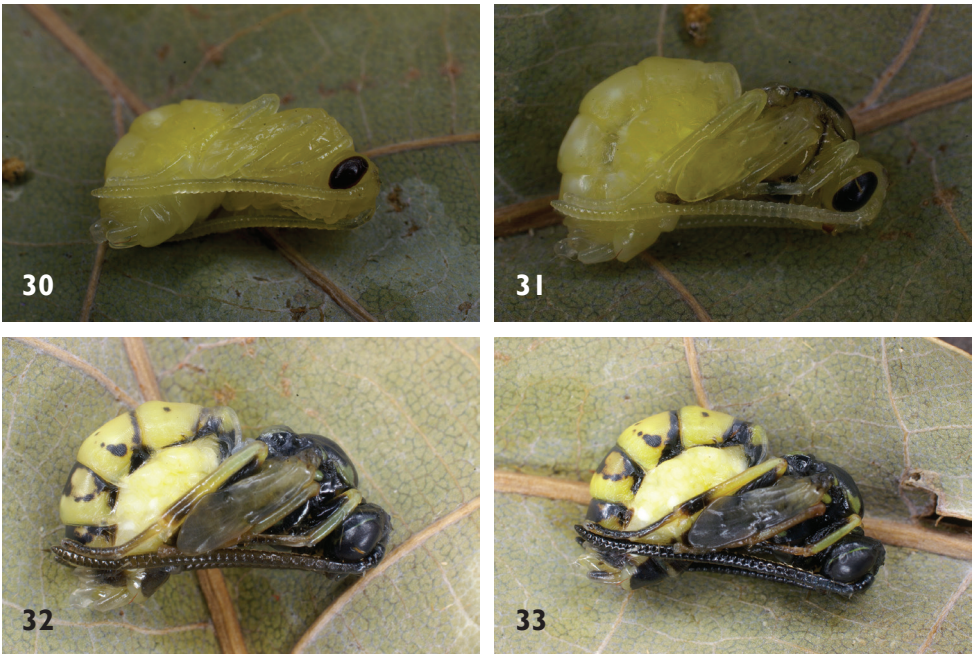
Figures 22–23. *Sphinctus serotinus*, first instar larva feeding.

(stained pink in Figs 19 and 20), and not through it into the deeper cell layers involved in secretion of new cuticle. The stalk of the egg appears to stain in a different way from the other collagens involved in the structure of the egg, but the stains used do not adequately characterise it (C. Gielis, pers. comm.). Normally the egg of *S. serotinus* does not hatch until the host has made its cocoon, but under artificially high humidity (closed plastic box) it may do so. The chorion splits (Fig. 21) at the anterior end (furthest from the anchor) but the setose first instar larva remains partly (caudally) in the eggshell as it starts to feed in the late autumn to early winter, as evidenced by a blackened feeding lesion (Figs 22, 23), with presumed urate patches already evident. The larva remains in this position, feeding slowly on the cocooned host prepupa, through the winter but by the second week of May a larva in a cocoon opened for observation had left the eggshell behind, grown considerably and moulted at least once, now moving freely across the body of the still living host prepupa and making new feeding lesions. The parasitoid larva was still setose and now very liberally dotted with presumed urate deposits. (In the milder early spring of 2007 the parasitoid larva in a cocoon opened on 19 March

Figures 24, 25. *Sphinctus serotinus*, intermediate instars **25** showing exuviae.

Figures 26–29. *Sphinctus serotinus*, prepupa **26, 27** in situ **27, 29** removed from host cocoon.

in the Netherlands had already reached this state (Fig. 24).) It is unclear at what point the supernumaries (in cases of superparasitism) are eliminated, or how, but presumably this cannot take place while the larvae are still confined by their eggshells. Growth continued slowly through the spring and early part of summer (in Edinburgh in 2006 the weather was particularly cold, with daytime temperatures in the rearing shed seldom exceeding 10°C during May). The larva had moulted at least twice, probably three or possibly even four times, leaving the rather robust cast skins split along a dorsal line (Fig. 25), before dying about half grown around 11.vi.2006 — probably largely a result of excessive disturbance, but no doubt exacerbated by cold weather.



Figures 30–33. *Sphinctus serotinus*, pupa **30** fresh **31–33** progressively developing adult colour.

A further host cocoon (ca 8.5 mm long) containing *S. serotinus* was opened on 25.vii.2006, and by this date the parasitoid had completed its feeding and was prepupal. (The Edinburgh weather had been much warmer during late June and July, with temperatures regularly in the low 20s°C.) The host cocoon now contained an area of coarse white silk, at its midlength, isolating the host remains between the host cocoon wall and a frail white diaphanous cocoon enclosing the *S. serotinus* prepupa (Figs 26–28). The isolating wall of silk was positioned against the venter of the parasitoid's cocoon, within which faeces were visible. The *Sphinctus* cocoon was easily removed (Fig. 28) and opened, but the prepupa (whose eye spots were clearly evident) could not be withdrawn without damage because it was anchored firmly, via its anus, to its faecal material by a tough viscous/elastic strand of brown material capable of supporting much more than its weight (Fig. 29). This individual was kept under observation and pupated on 1.viii.2006. Unlike the prepupa, the fresh pupa is easily removed from its cocoon (Fig. 30). One pupa was brought indoors on 25.viii.2006 after which it gradually developed colour (Figs 31–33), the mesoscutum being the first area to darken and the legs among the last.

On one host that had received an egg the parasitoid failed to develop beyond its first instar, and the host then progressed to the (pharate) adult stage, indicating that no venom component that interferes with the host's endocrine system had been injected to control host development.

Discussion

Despite its wide distribution (cf. Fauna Europaea 2015) *Sphinctus serotinus* is regarded as a rare insect in Europe, and indeed for several countries with historical records there are no recent reports: for example, the 19th century records for England given by Morley (1911) have not been repeated. However, despite its conspicuous colouring, it may be easily overlooked. The early autumnal flight time, appearance and behaviour of the adult would cause it to be easily passed over as a vespid, and perhaps given a wide berth even by entomologists at a time of year when stings from the numerous drowsy vespid workers are most likely to occur. Also, it seems not to be prominent in the field even where it is abundant: in the part of the Netherlands where it is so easy to find it in the egg stage, JV has seen only one adult during many hours of collecting its host, and indeed its eggs, during its flight time. Thus, like many parasitoids, its presence or absence may be much easier to investigate by finding its host than by depending on encountering adults, and even in Britain it may not be appropriate to regard it as extinct until a thorough search on that basis is made.

It was unexpected that the larval development was so gradual, and that there was no evident period of diapause, although the pupa was certainly rather slow to commence the process of adult cuticular development. Probably the timing of events was unduly affected by the cold Edinburgh climate (certainly a good deal colder than the Veluwe, or the areas of southern England where the host, and indeed *S. serotinus* in former times, have been recorded) but, significantly, there was no prolonged intact egg or pre-emergence adult stage, and the prepupal stage was reached only well into the summer.

The only contact assessment of the host that the female makes with her rather robust and scarcely flexing antennae appears to be in relation to host size, and the antennae seemed to be of low importance in tracking the host via leaf-surface traces and recognising it once found. However, any possible involvement of the antennae in courtship behaviour could not be investigated.

The egg evidently can be laid even when its chorion is sub-mature, and as many as seven were laid by the experimental female in one day. Although feeding on honey:water was quite ravenous on that day (between ovipositions) there was no tendency to host-feed — perhaps surprisingly in view of the host-feeding seen in other tryphonine parasitoids of Lepidoptera such as *Netelia*, in which the mandibles are used to make the necessary wound (Shaw 2001), until the very tough nature of the integument of *Apoda* larvae is considered. Host-feeding for *S. serotinus* may be simply too lengthy and risky a business to be viable. The anchored egg is embedded only in the host's outer cuticle, suggesting that only final instar hosts would be suitable for the parasitoid's development, as *S. serotinus* does not inject a development-changing venom and the egg would be easily sloughed off along with host cuticle at ecdysis. This is unlike the situation seen in the phytodietine tryphonine genus *Netelia* in which there is also no venom effect controlling host development (Shaw 2001). When a penultimate instar host is parasitized by *Netelia*, survival of host ecdysis by the egg tearing through the cuticle

being sloughed is readily achieved (Shaw 2001). The tough and thick cuticle of *Apoda limacodes* arguably limits the integumental tissues into which the egg can be anchored which may explain, at least as much as the lack of competition from other parasitoids, why *S. serotinus* has such a late flight time—in early autumn, when essentially all encountered host larvae will be in the final instar. This scenario also seems likely for other temperate *Sphinctus* species with similar limacodid hosts.

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RESEARCH ARTICLE

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Olfactory responses of *Theocolax elegans* (Hymenoptera, Pteromalidae) females to volatile signals derived from host habitats

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Abstract

The responses of female *Theocolax elegans* (Hymenoptera: Pteromalidae) to volatile signals derived from its host habitats were investigated in a static four-chamber olfactometer. Our results demonstrated that *T. elegans* females, irrespective of experience, were apparently attracted by the odors released from the faeces of *Sitophilus zeamais* larvae and adults, which has never been investigated in previous researches. Moreover, we compared the responses of female parasitoids to odors released from grains of rice damaged by *S. zeamais* larvae, *S. zeamais* males, *S. zeamais* females, and mechanically. Artificially damaged grains do not emit large amounts of the volatiles that attract experienced parasitoid females to grains damaged by *S. zeamais* larvae. Further experiments revealed that experienced *T. elegans* females were more strongly attracted to rice grains which had been infused with extract from the heads and thoraxes of weevil larvae than to rice grains that had been infused only with sodium phosphate. The behavior of *T. elegans* females to odors released from pheromone-releasing *S. zeamais* males on healthy grains and unmated *S. zeamais* females on healthy grains were observed. The results revealed that *S. zeamais* aggregation pheromones are not useful signals for *T. elegans* females, irrespective of experience. Based on these observations, *T. elegans* females used faeces to detect potential hosts. Our results revealed that head and thorax of *S. zeamais* larvae induces rice grains to release volatiles attractive to *T. elegans* females, particularly after experience.

Keywords

Chemical cues, Multitrophic interaction, Olfactory host finding, Parasitoids, *Sitophilus zeamais*, *Theocolax elegans*

Introduction

Many parasitoid insects are orientated to the chemical cues released by their target or its environment. The successive steps of the orientation process, or host-finding, have been described as host habitat location, host location, host recognition and host acceptance (Vinson 1998). The behaviours and the cues involved in these steps have been examined in numerous parasitoids (Godfray 1994; Quicke 1997). Parasitoids may use stimuli from different odor sources to locate phytophagous hosts. The host as well as its food plant can be sources of stimuli. Volatile compounds emitted by the host's food can elicit long-range attraction in parasitoids (Vinson 1985; Nordlund et al. 1988; Lewis et al. 1990) and it has been assumed that they play a crucial role in mediating host-habitat location (Vinson 1976). However, host location in parasitoids has been examined mostly in systems with hosts feeding on fruits, leaves, or stems of plants or on fungi (Dorn et al. 2002; Suverkropp et al. 2008). The attractiveness of plant seeds to parasitoids of seed feeders has rarely been studied so far (Steidle et al. 2005). The success of parasitic wasps in suppressing pest populations depends on their ability to locate hosts and, consequently, understanding the mechanisms governing host searching behaviour is critical to the successful implementation of biological control programs (Gardner et al. 2007; Germinara et al. 2009; Li et al. 1992).

The present paper is devoted to a tritrophic system consisting of the parasitoid *Theocolax elegans* (Westwood) (Pteromalidae), the maize weevil *Sitophilus zeamais* (Motschulsky) (Curculionidae), and grains of rice *Oryza sativa* L. (Poaceae). The beetle *S. zeamais* is one of the most destructive insect pests of stored cereals in tropical and sub-tropical regions (Ribeiro et al. 2014). *Sitophilus zeamais* is regarded as an internal feeder of grains. Adult females of *S. zeamais* cause damage by boring into the kernel and laying eggs (ovipositing). Then, larvae eat the inner parts of the kernel, resulting in a damaged kernel and reduced grain weight (Tang et al., 2008). Apart from weight losses, the damaged kernels have low nutritional value, low rates of germination, low commercial value, and increased susceptibility to fungal infestation (Nwosu et al. 2015; Guedes et al. 2006). *Sitophilus zeamais* causes extensive losses in quality and quantity of the grain in the field as well as in storage (Carvalho et al. 2014). *Sitophilus zeamais* utilizes male produced aggregation pheromones that attract both males and females. Release and perhaps production of the pheromones by *S. zeamais* males is closely tied to feeding or contact with food: males locate food, produce pheromones, attract females and other males, and mate; females oviposit at that site, where larvae ultimately develop (Walgenbach et al. 1983; Phillips and Throne 2010). *Theocolax elegans* is a solitary ectoparasitoid that parasitizes larvae and pupae of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), *Sitophilus* spp. (Coleoptera: Curculionidae), *Stegobium paniceum* (L.) (Coleoptera: Anobiidae), *Callosobruchus* spp. (Coleoptera: Bruchidae), and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) which develop inside cereal grains or legume seeds (Flinn et al. 1996). Its wide host range of grain-damaging beetles means that *T. elegans* has been shown to have wide potential as a biocontrol agent effective in controlling insect pests of stored products and with a positive impact

on the quality of stored cereal products. However, the sources of volatiles that attract the parasitoid to grains infested with the weevils were still unknown.

The present study investigates the sources of olfactory cues that *T. elegans* uses to locate infested rice grains. The potential sources investigated were the weevil larvae, their faeces, aggregation pheromone, and the grain material. We also studied the effect of experience with a host on the attractiveness of host-related stimuli to adult females of *T. elegans*.

Materials and methods

Insect cultures

All insect cultures were kept at 26±2 °C, 70±5% relative humidity (r.h.) and a photoperiod of L14: D10. To rear *T. elegans*, 50 newly emerged adult wasps were placed into Petri dishes (9 cm diameter, 1 cm high) with about 50g of rice grains infested by 3rd-4th instar larvae of *S. zeamais* and kept there until their death. After a developing time of 19–25 days, emerged parasitoids from the next generation were collected daily from each Petri dish. To rear *S. zeamais*, 30 adults were allowed to oviposit into 300ml of rice grains with about 14% moisture content in glass jars (8 cm diameter, 10 cm high). To obtain unmated males or females of *S. zeamais*, adults were separated by dimorphic rostral characteristics within 12 h of emergence (Halstead 1963).

Insects for bioassays

Parasitoids used in experiments were about 2 d old. To obtain experienced parasitoid females, recently emerged (< 24 hr old) wasps were placed in Petri dishes containing rice grains infested by weevil larvae and adults of *S. zeamais*. Females were allowed to mate and oviposit for 3 days. Subsequently they were removed and kept in Petri dishes with moistened filter paper until they were used in the experiments on the following day.

In accordance with Vet and Groenewold (1990), we define inexperienced parasitoids as insects which had no experience with the host beyond that which occurred during development within and eclosion from the host. To obtain naive parasitoids for bioassays, freshly emerged male and female parasitoids were collected from the infested grains within 1 hr of emergence and kept in Petri dishes on moistened filter paper in a climatic chamber without host odors under the same conditions as described above.

Static four-chamber olfactometer

The response of female parasitoids towards different odor samples was examined using a static four-chamber olfactometer as described by Ruther and Steidle (2000). The ol-

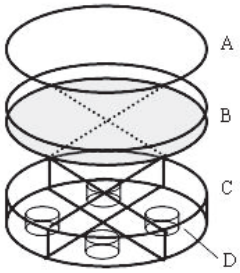

Figure 1. Static four-chamber-olfactometer used for all bioassays. For details see text.

factometer (Fig. 1) was made of acrylic glass and consisted of a cylinder (4 cm high, 19 cm diameter) divided by vertical plates into four chambers. On the top of the cylinder, a walking arena (1 cm high, 19 cm diameter) was placed consisting of plastic gauze (mesh 0.5 mm) with a rim of acrylic glass (0.9 cm high) and covered with a glass plate to prevent parasitoids from escaping. No airflow was generated. An odor sample was placed in a Petri dish (5.5 cm diameter) with brown filter paper (4 cm diameter) in one chamber or in two opposite chambers. Volatiles were allowed to diffuse through the gauze, resulting in an odor field in the walking arena above. The remaining chambers contained Petri dishes with brown filter paper only as controls.

General methods for bioassays

Evaluations were performed in a constant temperature and humidity room at 26±2 °C and 70±5% r.h., in darkness under red light to avoid distraction of parasitoids by light but to enable observations. Behavioural data were visually recorded using a stopwatch. To avoid biased results due to possible orientation preferences of the parasitoids, the position of the olfactometer was rotated clockwise by 90° after every insect. Contamination of the walking arena with sample odors or by possible pheromones of the parasitoids was avoided by cleaning the walking arenas and glass plates with ethanol and demineralized water before each insect. To avoid biased results due to possible human contamination of experimental material, disposable gloves were worn when carrying out the experiment. For all experiments, odor samples were renewed after five parasitoids each.

Fifty parasitoids were tested for each type of sample. Each individual parasitoid was used only once. At the start of each bioassay, the parasitoids were released individually in the center of the walking arena and their arrestment times in the four sectors above the arena were registered for 600 sec. The time the parasitoids spent walking in the areas directly above the Petri dishes with odor samples was compared to the areas with control Petri dishes and used to assess the arrestant effect of an odor sample. Parasitoids that walked for less than 50% of the total observation time were not included in the statistical analysis.

Responses of *T. elegans* females to *S. zeamais* faeces volatiles

Three different experiments were conducted using the static four-chamber olfactometer descried above. (1) 100 mg of faeces of *S. zeamais* larvae (LF) versus three empty Petri dishes (C); (2) 100 mg of faeces of adult *S. zeamais* males (MF) versus three empty Petri dishes (C); (3) 100 mg of faeces of adult *S. zeamais* females (FF) versus three empty Petri dishes (C).

Fifty parasitoids were tested for each experiment. Larval faeces from *S. zeamais* were obtained by sieving grain infested by 3rd-4th instar weevil larvae. Adult faeces from *S. zeamais* were obtained by sieving grain infested by unmated weevils.

Responses of *T. elegans* females to *S. zeamais* induced rice grains volatiles

We conducted a series of experiments to test the attraction of *T. elegans* females to herbivore-induced odors emitted from rice grains. (1) 50 grains infested by weevil larvae from which larvae, faeces, and egg plugs had been removed [infested grain only; (LIGO)] versus 50 healthy grains, which had been artificially damaged [artificially damaged grain; (AG)] and two empty petri dishes (C); (2) 50 grains infested by adult *S. zeamais* males from which weevils and faeces had been removed [infested grain only; (MIGO)] versus 50 healthy grains, which had been artificially damaged (AG) and two empty petri dishes (C); (3) 50 grains infested by unmated adult *S. zeamais* females from which weevils and faeces had been removed [infested grain only; (FIGO)] versus 50 healthy grains, which had been artificially damaged (AG) and two empty petri dishes (C).

Fifty parasitoids were tested for each experiment. The infested grain was obtained by dissecting grains infested by 3rd-4th instar weevil larvae, from which the larvae were removed, and removing faeces using a fine brush. Artificially damaged grains were cut with scissors, knives or needles in order to better mimic damage caused by the gnawing larvae or adults.

Responses of *T. elegans* females to extract from the heads and thoraxes of *S. zeamais* larvae and induced rice grains volatiles

Extract from the heads and thoraxes of *S. zeamais* larvae were prepared using the method described by Peiffer and Felton (2014) with slight modification: one hundred heads and thoraxes (3rd–4th instar weevil larvae) were ground with 5 ml of 0.05 M sodium phosphate (pH 8.0) (S) in order to maintain biological activity. The samples were centrifuged at 1000 r/min for 10 min. The resulting supernatant (E) was used for infusion (see below) within 12h.

A hole approximately 2 mm deep was drilled into the base of healthy rice grains with a 1 mm diameter drill. Some of the rice grains (EG) with dug holes were infused

with 2μl extract from the heads and thoraxes of *S. zeamais*, the remaining (SG) with dug holes were infused with 2μl of 0.05 M sodium phosphate (pH 8.0) alone. EG and SG were separately placed in petri dishes in humidifiers containing a saturated sodium chloride solution at 65%–70% r.h. for seven days before being used.

Two different experiments were conducted using the static four-chamber olfactometer descried above. (1) 1000μl extract from the heads and thoraxes of 3rd-4th instar weevil larvae (E) versus 1000μl sodium phosphate (S), and two empty petri dishes (C); (2) 50 EG that had been infused with 2μl extract from the heads and thoraxes of 3rd-4th instar weevil larvae (LEG) versus 50 SG, and two empty petri dishes (C). Fifty parasitoids were tested for each experiment.

Responses of *T. elegans* females to *S. zeamais* aggregation pheromone

Fifty parasitoids were tested for each experiment. One experiment was conducted using the static four-chamber olfactometer to test the attraction of *T. elegans* females to aggregation pheromone of *S. zeamais*. (1) 20 pheromone-releasing *S. zeamais* males on 100 healthy grains (GM) versus 20 unmated *S. zeamais* females on 100 healthy grains (GF) and two empty petri dishes (C).

Statistical analysis

The Friedman ANOVA was used to test for differences between the four areas. In case of significant differences the Wilcoxon-Wilcox-test for multiple comparisons was used to determine which sectors are different from each other.

Results

Responses of female parasitoids to faeces volatiles

Both naive and experienced parasitoid females spent significantly (Naive, LF, P=0.0009; Naive, MF, P=0.0015; Naive, FF, P=0.0006; Experienced, LF, P=0.0022; Experienced, MF, P=0.00016; Experienced, FF, P=0.0008) more time walking in the sector above the faeces than in the sectors with the control (Figs 2, 3). The results suggested that faeces of the host could be innately used as cues for habitat preference by *T. elegans*.

Responses of female parasitoids to *S. zeamais* induced rice grain volatiles

Both naive and experienced *T. elegans* females spent significantly more time in treatment odor fields compared to control odor fields in experiments involving either infested rice grains by *S. zeamais* from which weevil, faeces, and egg plugs had been

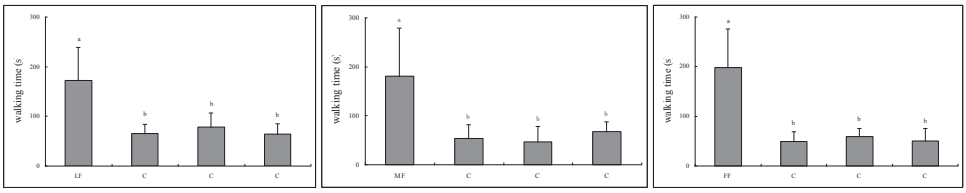

Figure 2. Mean walking time (± SD; n = 50) of naive females of *Theocolax elegans* in a four chamber olfactometer. LF: areas above Petri dishes with faeces of *S. zeamais* larvae, MF: areas above Petri dishes with faeces of *S. zeamais* males, FF: areas above Petri dishes with faeces of *S. zeamais* females, C: areas above control Petri dishes. Bars with different letters are significantly different at $P < 0.05$ (Friedman ANOVA followed by Wilcoxon-Wilcox-test for multiple comparisons).

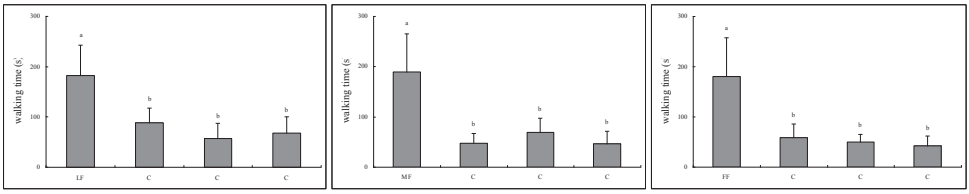

Figure 3. Mean walking time (± SD; n = 50) of experienced females of *Theocolax elegans* in a four chamber olfactometer. LF: areas above Petri dishes with faeces of *S. zeamais* larvae, MF: areas above Petri dishes with faeces of *S. zeamais* males, FF: areas above Petri dishes with faeces of *S. zeamais* females, C: areas above control Petri dishes. Bars with different letters are significantly different at $P < 0.05$ (Friedman ANOVA followed by Wilcoxon-Wilcox-test for multiple comparisons).

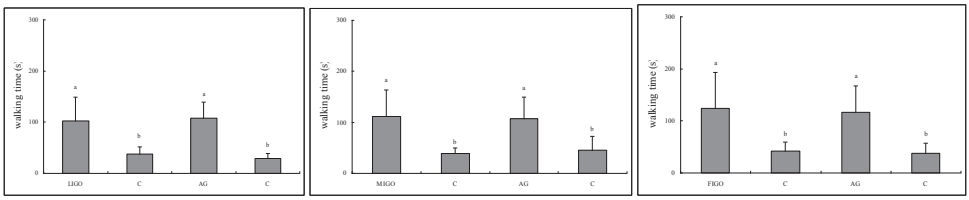

Figure 4. Mean walking time (± SD; n = 50) of naive females of *Theocolax elegans* in a four chamber olfactometer. LIGO: areas above Petri dishes with grains infested by weevil larvae from which larvae, faeces, and egg plugs had been removed, MIGO: areas above Petri dishes with grains infested by adult *S. zeamais* males from which weevils and faeces had been removed, FIGO: areas above Petri dishes with grains infested by unmated adult *S. zeamais* females from which weevils and faeces had been removed, AG: artificially damaged grains, C: areas above control Petri dishes. Bars with different letters are significantly different at $P < 0.05$ (Friedman ANOVA followed by Wilcoxon-Wilcox-test for multiple comparisons).

removed or artificially damaged rice grains (Figs 4, 5). The results suggested that *T. elegans* directs host location by using innate cues from host rice grains.

Naive *T. elegans* females showed no statistically significant difference (LIGO, P=0.2512; MIGO, P=0.1693; FIGO, P=0.2178) in choice between rice grains infested by *S. zeamais* and artificially damaged rice grains (Fig. 4). However, in contrast to inexperienced parasitoids, experienced *T. elegans* females were strongly (LIGO,

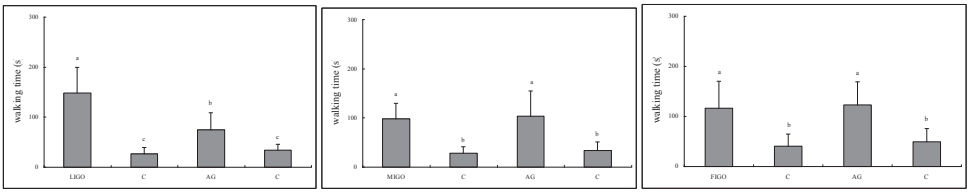

Figure 5. Mean walking time (± SD; n = 50) of experienced females of *Theocolax elegans* in a four chamber olfactometer. LIGO: areas above Petri dishes with grains infested by weevil larvae from which larvae, faeces, and egg plugs had been removed, MIGO: areas above Petri dishes with grains infested by adult *S. zeamais* males from which weevils and faeces had been removed, FIGO: areas above Petri dishes with grains infested by unmated adult *S. zeamais* females from which weevils and faeces had been removed, AG: artificially damaged grains, C: areas above control Petri dishes. Bars with different letters are significantly different at $P < 0.05$ (Friedman ANOVA followed by Wilcoxon-Wilcox-test for multiple comparisons).

P=0.0276) attracted to the rice grains infested by weevil larvae from which weevil, faeces, and egg plugs had been removed over the artificially damaged rice grains (Fig. 5). Experienced *T. elegans* females showed no statistically significant difference (MIGO, P=0.3729; FIGO, P=0.4745) in choice between rice grains infested by adult *S. zeamais* from which faeces had been removed and artificially damaged rice grains (Fig. 5). The results strongly suggested that experienced *T. elegans* females were attracted by the chemicals released from infested grains.

Responses of female parasitoids to extracts from heads and thoraxes of *S. zeamais* induced rice grain volatiles

Experienced *T. elegans* females showed no statistically significant difference (P=0.4659) in choice between areas containing extract from the heads and thoraxes of 3rd-4th instar weevil larvae (E) and sodium phosphate alone (S) (Fig. 6). This result confirmed that the extract from the heads and thoraxes of 3rd-4th instar weevil larvae was not directly responsible for attracting experienced *T. elegans* females.

Experienced *T. elegans* females were more strongly (LEG, P=0.0381) attracted to the rice grains which had been infused with 2μl extract from the heads and thoraxes of weevil larvae over the rice grains which had been infused only with 2μl sodium phosphate (Fig. 7). Based on these observations, it appeared that the extract from the heads and thoraxes of *S. zeamais* larvae induced the wounded rice grains to release volatile chemicals for attracting *T. elegans*.

Responses of female parasitoids to aggregation pheromone

Naive and experienced *T. elegans* females spent significantly more ($P < 0.05$) time in treatment odor fields compared to control fields of the olfactometer in all experiments (Figs 8, 9). Naive and experienced *T. elegans* females showed no statistically significant

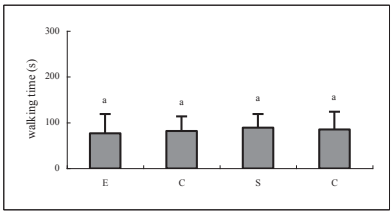

Figure 6. Mean walking time (± SD; n = 50) of experienced females of *Theocolax elegans* in a four chamber olfactometer. E: areas above Petri dishes with 1000μl extract from the heads and thoraxes of 3rd–4th instar weevil larvae, S: areas above Petri dishes with 1000μl of sodium phosphate alone, C: areas above control Petri dishes. Bars with different letters are significantly different at $P < 0.05$ (Friedman ANOVA followed by Wilcoxon-Wilcox-test for multiple comparisons).

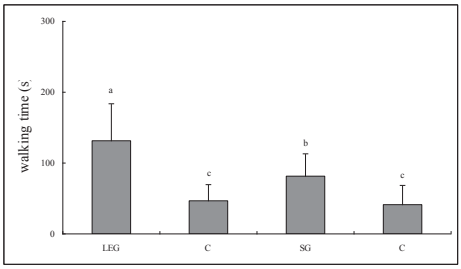

Figure 7. Mean walking time (± SD; n = 50) of experienced females of *Theocolax elegans* in a four chamber olfactometer. LEG: areas above Petri dishes with grains which had been infused 2μl extract from the heads and thoraxes of 3rd-4th instar weevil larvae, SG: areas above Petri dishes with grains which had been infused 2μl of sodium phosphate alone, C: areas above control Petri dishes. Bars with different letters are significantly different at $P < 0.05$ (Friedman ANOVA followed by Wilcoxon-Wilcox-test for multiple comparisons).

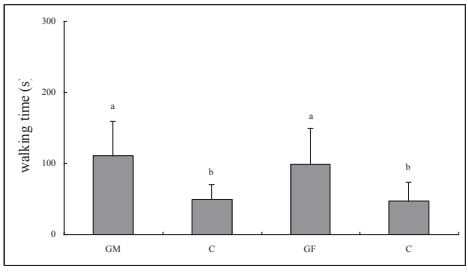

Figure 8. Mean walking time (± SD; n = 50) of naive females of *Theocolax elegans* in a four chamber olfactometer. GM: areas above Petri dishes with 20 pheromone-releasing *S. zeamais* males on 100 healthy grains, GF: areas above Petri dishes with 20 unmated *S. zeamais* females on 100 healthy grains, C: areas above control Petri dishes. Bars with different letters are significantly different at $P < 0.05$ (Friedman ANOVA followed by Wilcoxon-Wilcox-test for multiple comparisons).

difference (Naive, P=0.4879; Experienced, P=0.5127) in choice between 20 pheromone-releasing *S. zeamais* males on 100 healthy grains and 20 unmated *S. zeamais* females on 100 healthy grains (Figs 8, 9). The results suggested that *T. elegans* female response may not be mediated by aggregation pheromone.

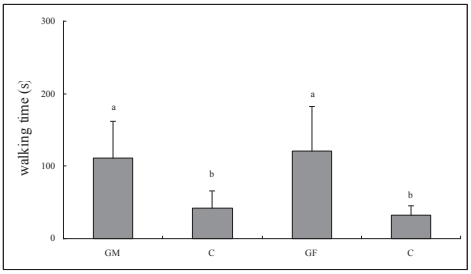

Figure 9. Mean walking time (± SD; n = 50) of experienced females of *Theocolax elegans* in a four chamber olfactometer. GM: areas above Petri dishes with 20 pheromone-releasing *S. zeamais* males on 100 healthy grains, GF: areas above Petri dishes with 20 unmated *S. zeamais* females on 100 healthy grains, C: areas above control Petri dishes. Bars with different letters are significantly different at $P < 0.05$ (Friedman ANOVA followed by Wilcoxon-Wilcox-test for multiple comparisons).

Discussion

The results show clearly that the naive and experienced *T. elegans* females can be attracted by faeces of host *S. zeamais*. Results similar to those for *S. zeamais* faeces are responsible for attracting female *Lariophagus distinguendus* (Tang et al. 2009). Faeces, as a reliable indicator of host presence, has been described as a widespread foraging cue for parasitoids (Hendry et al. 1973; Henson et al. 1977; Gross et al. 1975; Takabayashi and Takahashi 1989; Turlings et al. 1991; Agelopoulos et al. 1995; Alborn et al. 1995; Chiu-Alvarado et al. 2010). The present results suggest that faeces could be innately used as cues for habitat preference by *T. elegans* females. This aspect was neglected in earlier work on the innate use of kairomones for host-location in *T. elegans* (Germinara et al. 2009; 2016).

Parasitoids of phytophagous hosts can be attracted directly by infested host plants (Tumlinson et al. 1992; Godfray 1994; Kennedy 2003; Ode 2006; Hilker & Fatouros 2015). However, so far, little experimental evidence has been reported to support this, and the release of herbivore-induced synomones (HIS) has almost exclusively been demonstrated in somatic plant tissues. In 2005, Steidle *et al* concluded that grains have the ability to 'whisper for help' from the parasitoid *L. distinguendus*. Here, I provide the experimental data to demonstrate that experienced females of the parasitoid *T. elegans* are able to discriminate between artificially damaged grains and grains infested by *S. zeamais* from which weevil, faeces, and egg plugs had been removed. The attractiveness could be caused by emission of volatiles from the seeds due to phytochemical induction caused by the host. There is considerable evidence that the volatile "alarm signals" are induced by interactions of substances from the herbivore with the damaged plant tissue (Steowe et al. 1995). As demonstrated by Turlings et al. (1991) for the parasitoid *Cotesia marginiventris* (Cresson) and its host *Spodoptera exigua* (Hübner) and by Mattiacci et al. (2001) for *Cotesia glomerata*

(L.) and its host *Pieris brassicae* (L.), plant volatiles can be induced by the saliva of caterpillars. Experienced *L. distinguendus* females have been shown to be strongly attracted to grains to which had been applied protein substances from original regurgitants (Tang et al. 2013).

The experimental data provided here demonstrates that the behavior of experienced *T. elegans* females is not affected simply by extract from the heads and thoraxes of 3rd-4th instar weevil larvae or sodium phosphate. More interestingly, when the extracts from the heads and thoraxes of *S. zeamais* were applied to the artificially damaged grains, the *T. elegans* females can be strongly attracted, which apparently indicated that the specific defense chemical cues attracting *T. elegans* females were released as a result of the presence of *S. zeamais* extract. It appears that the extract from the heads and thoraxes of *S. zeamais* larvae induced the wounded grains to release volatile chemicals for attracting *T. elegans*. Thus it seems likely that specific parasitoid attracting volatiles are also induced by the saliva of the feeding weevil larvae.

It appears that many predatory and parasitoid arthropods are able to intercept the sex pheromone signals of their prey or hosts. For example, Bedard (as cited in Wood 1982) first reported the attraction of a parasitoid wasp, the pteromalid *Tomicobia tibialis* Ashmead, to volatiles produced by males of the bark beetle *Ips paraconfusus* (Le Conte) boring in ponderosa pine in 1965. Later, several hymenopterous parasitoids of the elm bark beetle, *Scolytus multistriatus* (Marsham), were found to be attracted to pheromones (Steowe et al. 1995). However, the *S. zeamais* aggregation pheromones are not useful signals for *T. elegans* females. Maybe it is because *T. elegans* is a broad spectrum ectoparasitoid that parasitizes larvae and pupae.

Under conditions whereby specific pest-derived chemical cues are used by natural enemies (Cox 2004), the strategy has been considered of applying semiochemicals during biological control to attract parasitoids or predators into a crop or to increase the amount of time they spend in a field. Therefore, increased understanding of the chemically mediated interactions between arthropod hunters and their victims will be very useful for the biological controls of pests on a crop. Our ultimate goal is to be able to develop an environmentally friendly method to control pest resurgence on a crop and reduce the currently heavy dependence on pesticides.

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Two newly recorded genera *Stenodyneriellus* and *Lissodynerus* with three new species from China (Hymenoptera, Vespidae, Eumeninae)

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Abstract

These two genera *Stenodyneriellus* Giordani Soika and *Lissodynerus* Giordani Soika are newly recorded from China. Three species, namely *Stenodyneriellus similiguttulatus* **sp. n.** from Yunnan, *S. maolanensis* **sp. n.** from Guizhou, and *S. depressus* **sp. n.** from Yunnan, are described and illustrated. *Stenodyneriellus guttulatus* (de Saussure, 1862) and *Lissodynerus septemfasciatus feanus* (Giordani Soika, 1941) are newly recorded and illustrated from China. A key to the Chinese species of *Stenodyneriellus* is provided.

Keywords

Hymenoptera, Eumeninae, *Stenodyneriellus*, *Lissodynerus*, new record, new species, China

Introduction

These two genera *Stenodyneriellus* and *Lissodynerus* were established by Giordani Soika in 1961 and 1994, respectively. Both of them are Oriental and Australian genera of potter wasps. Some species share the following characters: T2 with a lamella apically, shelf of propodeum developed dorsally, pronotal carina complete and rounded dor-

sally, T1 slightly narrower than T2. To date, *Stenodyneriellus* includes 59 species with five subspecies and *Lissodynerus* contains 17 species with eight subspecies worldwide (Giordani Soika 1961, 1993a, 1993b, 1994, 1995, 1996; Borsato 1994, 2003; Gusenleitner 2007, 2008, 2013; Girish Kumar and Carpenter 2015). Giordani Soika (1994) provided a good basis for further taxonomic study of *Stenodyneriellus* and *Lissodynerus*, and hereafter a few new species have been described by Borsato (2003), Gusenleitner (2007, 2008, 2013), and Girish Kumar and Carpenter (2015). Among the known species of these two genera, not one was reported from China. In our study of the eumenine wasps from China, four species of *Stenodyneriellus* and one species of *Lissodynerus* were collected. And as a result of careful comparisons with all known species, three new species of *Stenodyneriellus* are described and illustrated in detail, and *S. guttulatus* (de Saussure, 1862) and *L. septemfasciatus feanus* (Giordani Soika, 1941) are newly reported and illustrated from China. A key to the Chinese species of *Stenodyneriellus* is provided. The key was produced based on both the examination of specimens and the information extracted from the literature.

Materials and methods

The specimens examined are deposited in the Institute of Entomology and Molecular Biology, Chongqing Normal University, Chongqing, China (CQNU) and Department of Entomology, College of Plant Protection, Yunnan Agricultural University, Kunming (YNAU). Descriptions and measurements were made under a stereomicroscope (Nikon SMZ1500), and all figures were taken with a stereomicroscope (LEICA EZ4HD) attached to a computer using Leica Application Suite version 2.1.0 software. The ratios used throughout the descriptions were measured in the same magnifying multiple of stereomicroscope. All measurements were taken as the maximal length of body parts measured. Body length was measured from the anterior margin of the head to the posterior margin of metasomal tergum 2. For the density description of punctures, "sparsely" means that interspaces are larger than one puncture diameter, "moderately" means equal to the diameter, and "densely" means less than one diameter. Terminology principally follows Carpenter (1982) and Carpenter and Cumming (1985). The abbreviations used in the text are shown as follows:

A1 for antennal segment 1,
A2 for antennal segment 2,
POD for postocellar distance,
OOD for the minimum distance between the compound eye and posterior ocellus;
T1 for metasomal tergum 1,
T2 for metasomal tergum 2,
S1 for metasomal sternum 1,
S2 for metasomal sternum 2, and so on.

Taxonomy

Stenodyneriellus Giordani Soika, 1961

Stenodyneriellus Giordani Soika, 1961: 65, 71; Carpenter 1986: 85; van der Vecht and Carpenter 1990: 55; Giordani Soika 1994: 5, 48–49.

Type species. *Stenodyneriellus turneriellus* Giordani Soika, 1961, by original designation.

Diagnosis. Clypeus truncated or emarginated apically; length of thorax often slightly longer than its width, but in a few cases almost 2× its width; metanotum flat or only very weakly and regularly convex; dorsal surface of propodeum almost in the same horizontal plane as metanotum, sometimes more or less prolonged in a medial direction and joined together in the midline separating from the posterior surface of metanotum, shelf on propodeum absent, or well-developed and sometimes protruding upward so as to form two teeth behind metanotum; tegula generally wide and posterior lobe always small and short, but in some species tegula narrow and length nearly 2 × its width; parategula, legs and wings normal; generally, width of T1 approximately 2 × its length and slightly narrower than T2, but in some species T1 obviously narrower than T2, usually T2 without an apical lamella, but in some species with a translucent, thin blade-shaped lamella apically.

Distribution. Oriental and Australian regions.

Stenodyneriellus similiguttulatus sp. n.

http://zoobank.org/0EB8D6B0-ACBD-40BA-854E-3A148F22CBFA
Figs 1–9

Material examined. Holotype, ♀, China, Yunnan Province, Xishuangbanna State, Jinghong City, Manwai Village, 22°01'6.36"N, 100°50'30.65"E, 591-721m, 30.VII.2003, Qian Jiang, No. 1004061 (CQNU). Paratypes: 1♀1♂, same data as holotype, No. 1004062, 1004063 (YNAU).

Description. Female (Figs 1, 3–4, 6, 8): body length 8.0 mm, forewing length 7.0 mm. Black, with the following parts yellow: clypeus except medial goblet-shaped black spot (Fig. 3), mandible basally, scape ventrally, a large and wide band along inner eye orbit from basis of clypeus to upper frons occupying entire ocular sinus, interantennal spot, a spot on vertex, gena almost entirely, dorsal surface of pronotum anteriorly, a large dorsal spot and a small ventral spot on mesepisternum, tegula anteriorly and posteriorly, parategula, anterior half of scutellum, a large spot on dorsal surface of propodeum apically, a apical band on each of T1–T5 (Fig. 1) and S2, a round spot on the base of T2 laterally, irregular spots on lateral margin of S2 medially and apical margins of S3–S4 laterally (Fig. 8), elongated spots on front and mid femora inside, and all tibiae laterally; antenna except scape ventrally, legs except yellow parts, and tegula medially brown to dark brown. Wings lightly infuscate. Setae pale brown.

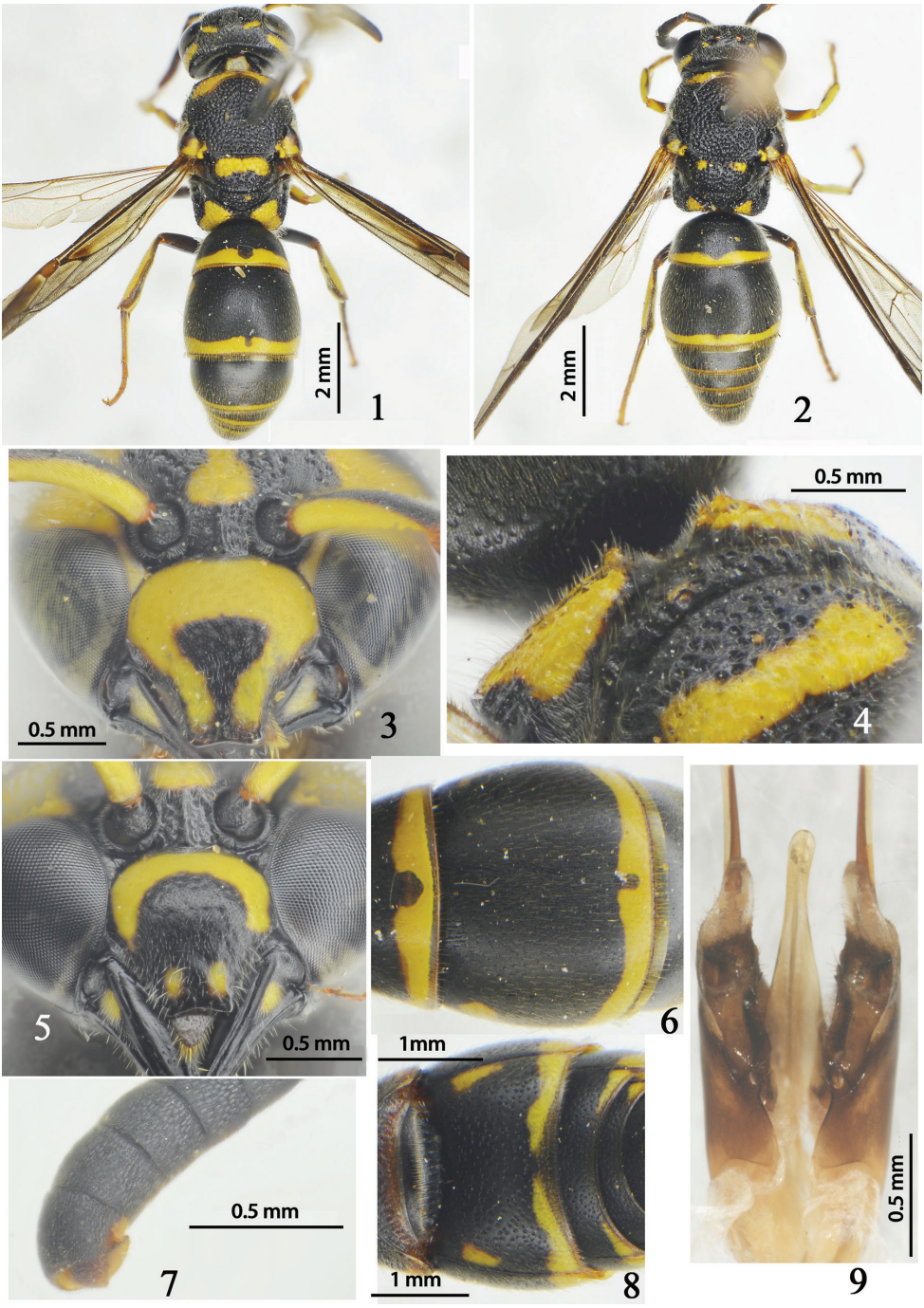
Head. Clypeus medially somewhat convex, with shallow, very thick and minute punctures, apex almost truncated and somewhat emarginated medially (Fig. 3), clypeal width 1.07 × its length, total width: apical width = 1.16: 0.4, apical width nearly equal to interantennal space; interantennal carina prominent; frons evenly convex and very coarsely punctate, interspaces between punctures with carinae and reticulate; vertex sparsely punctate, cephalic fovea obsolete; POD nearly as wide as OOD.

Mesosoma. Pronotum, mesoscutum, mesepisternum, mesoscutellum and metanotum very coarsely punctate and reticulate, these punctures distinctly deeper than those on frons, punctures on pronotum, mesoscutum and mesoscutellum somewhat sparser than those on mesepisternum and metanotum, and their interspaces with very small and shallow punctures. Pronotal carina complete, rounded dorsally and emarginated laterally; mesoscutum, mesepisternum, mesoscutellum normal; metanotum flat and only apically sloping; propodeum well-developed, dorsal surface almost in the same horizontal plane as metanotum, prolonged in a medial direction protruding upward so as to form two teeth behind metanotum (Fig. 4), well-separated from posterior surface, densely punctate, but obviously sparser and smoother than those on metanotum, interspaces between punctures polished; lateral surface of propodeum punctate and somewhat reticulate, punctures smaller and sparser than mesepisternum; posterior surface widely and deeply concave, smooth and with few punctures. Tegula wide, length slightly longer than its width, and posterior lobe small; parategula hooked and its apex just reaching the apex of tegula.

Metasoma. In dorsal view, T1 domed, its width 1.93 × length and 0.81 × width of T2, without a transverse carina anteriorly, with sparse punctures, punctures distinctly sparser and smaller than those on frons and mesosoma; T2 with a translucent, thin and blade-shaped lamella apically (Fig. 6), punctures on T2 more unobvious than those on T1; S2 widely depressed basally, and with moderate punctures (Fig. 8); visible part of T3–T6 and S3–S6 coriaceous and with minute punctures; the apical yellow bands on T1–T2 obviously wider than those on T3–T5 and with U-shaped gaps medially, the apical yellow band on S2 interrupted medially.

Male (Figs 2, 5, 7, 9). Body length 7.5 mm, forewing length 8.0 mm. Sculpture, punctuation, setae, and coloration as in female except as follows: yellow spots and bands on the body correspondingly smaller than those in female, clypeus largely black, a surrounding band basally and two spots on the apex laterally yellow (Fig. 5); T2 without a yellow spot laterally; clypeal width 0.97× its length, total width: apical width = 1.45: 0.6; A13 small, short, and backward only reaching the middle of A11 (Fig. 7); these two teeth on dorsal surface of propodeum sharper than those in female; width of T1 2.0 × its length and 0.86 × width of T2; genitalia as in Fig. 9, apical tip of penis valve somewhat oblong, volsella wide and blunt apically, and parallel spines elongate without setae; other characters same as those in female.

Remarks. This species resembles *S. guttulatus* (Saussure, 1862) by T2 with a translucent, thin, blade-shaped lamella apically (Fig. 6), and propodeum protruding upward so as to form two teeth behind metanotum dorsally (Fig. 4). It differs from *S. guttulatus*



Figures 1–9. *Stenodyneriellus similiguttulatus* sp. n. **1** habitus of holotype (dorsal view), ♀ **2** habitus of one paratype (dorsal view), ♂ **3** clypeus, ♀ **4** propodeum, ♀ **5** clypeus, ♂ **6** T2 (dorsal view), ♀ **7** apex of antenna, ♂ **8** S2-S4 (ventral view), ♀ **9** genitalia (front view), ♂.

and all other members of the genus by the following character combination: male A13 reaching the middle of A11 (Fig. 7); female scutum and T1 without yellow spots (Fig. 1); in genitalia, apical tip of penis valve somewhat oblong, and volsella wide and blunt apically (Fig. 9).

Distribution. China (Yunnan).

Etymology. The specific name *similiguttulatus* is named after the similar species *S. guttulatus* (Saussure, 1862), combined with the Latin word *similis* (= similar).

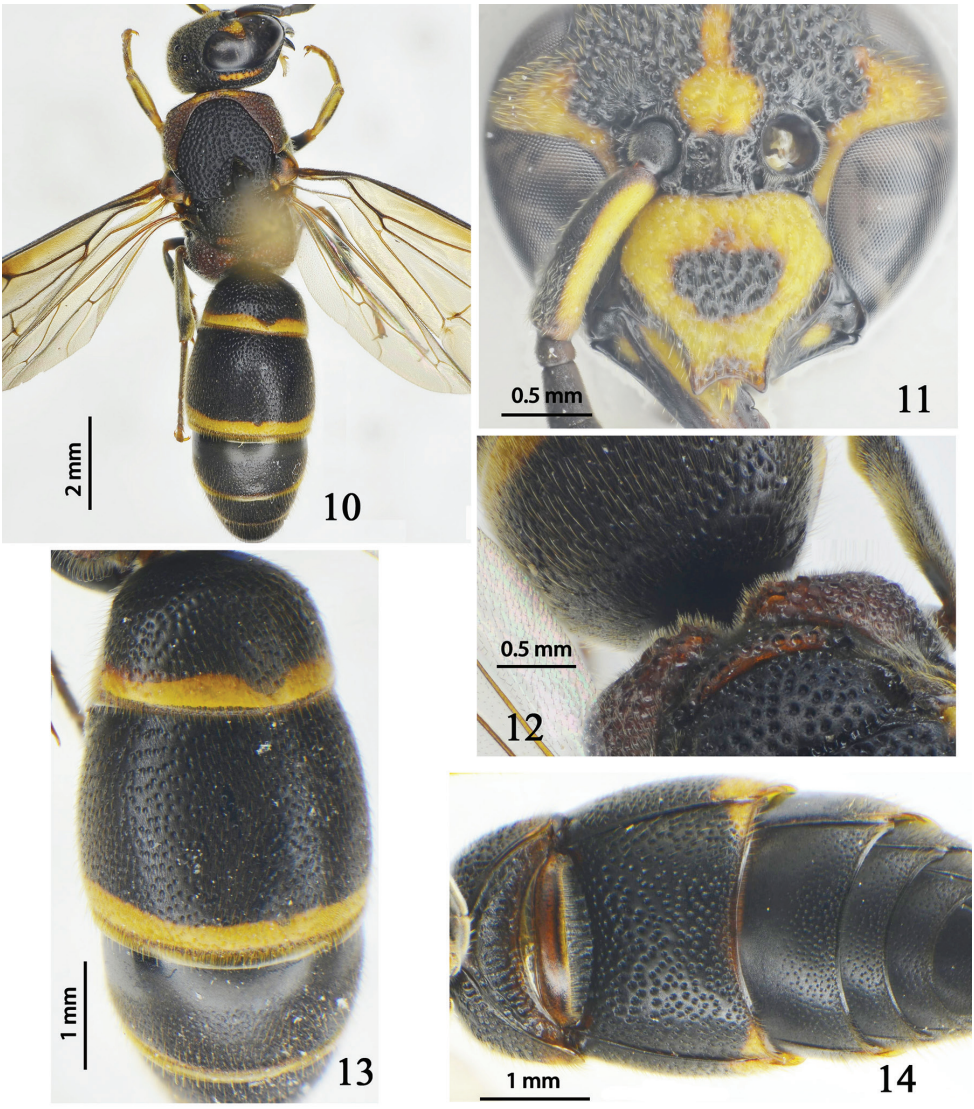
***Stenodyneriellus maolanensis* sp. n.**
http://zoobank.org/3A6E9C87-7746-4F8D-9359-052B88228FFA
Figs 10–14

Material examined. Holotype, ♀, China, Guizhou Province, Qiannan State, Libo County, Maolan National Nature Reserve, Dongdai Village, 25°14'54.88"N, 107°54'4.38"E, 782m, 21.VI.2015, Tingjing Li, No. 1004064 (CQNU).

Description. Female (Figs 10–14): body length 9.0 mm, forewing length 7.5 mm. Black; with the following parts dark ferruginous: pronotum except a thin yellow band medially (Fig. 10), tegula entirely, anterior half of metanotum, and entire dorsal surface of propodeum (Fig. 12); clypeus except medial black spot (Fig. 11), a small spot on mandible basally, scape ventrally, a large and wide band along inner eye orbit occupying entire ocular sinus, interantennal spot, a thin postocular band, a thin band on pronotum medially, parategula, fore tibia laterally, a apical band on each of T1–T3 (Fig. 13), and a lateral spot on apical margin of S2 yellow; fore femur apically, tibia mostly and tarsi dark brown. Wings lightly infuscate. Setae pale brown.

Head. Clypeus somewhat convex medially, coarsely punctate and somewhat reticulate medially, apex deeply emarginated medially (Fig. 11), clypeal width 1.28 × its length, total width: apical width = 1.19: 0.39, apical width approximately equal to interantennal space; interantennal carina prominent; frons evenly convex, very coarsely and densely punctate, and distinctly reticulate; punctures on vertex more sparsely than those on frons; cephalic fovea present; POD almost as wide as OOD.

Mesosoma. Pronotum, mesoscutum, mesepisternum, mesoscutellum, metanotum and propodeum very coarsely and deeply punctate, and reticulate, these punctures nearly as deep as and sparser than those on frons, punctures on mesepisternum and propodeum denser than those on pronotum, mesoscutum and mesoscutellum. Pronotal carina complete, somewhat rounded dorsally and emarginated laterally; mesoscutum, mesepisternum, mesoscutellum normal; metanotum with a short dorsal convex surface and sloping posteriorly. Propodeum well-developed; dorsal surface almost in the same horizontal plane as metanotum, prolonged in a medial direction protruding upward so as to form two teeth behind metanotum (Fig. 12), well-separated from posterior surface, coarser than lateral and posterior surfaces, and interspaces between punctures distinctly carinate; lateral surface somewhat reticulate; posterior surface widely and deeply concave, and punctate. Tegula wide, length slightly longer than its



Figures 10–14. *Stenodyneriellus maolanensis* sp. n. **10** habitus of holotype (dorsal view), ♀ **11** clypeus, ♀ **12** propodeum, ♀ **13** metasoma (dorsal view), ♀ **14** metasoma (ventral view), ♀.

width, and posterior lobe small; parategula hooked and its apex slightly exceeding the apex of tegula.

Metasoma. In dorsal view, T1 domed, width 1.81 × its length and 0.83 × width of T2, without a transverse carina anteriorly, distinctly punctate, interspace between punctures almost equal to one diameter, punctures distinctly smaller than those on frons and mesosoma; T2 with a translucent, thin, blade-shaped lamella apically (Fig. 13), punctures similar to those on T1; S2 somewhat depressed basally and distinctly punctate (Fig. 14), punctures distinctly larger and denser than those of T2, and gen-

erally smaller than those on head and mesosoma; visible parts of T3–T6 and S3–S6 coriaceous and with minute punctures; the apical yellow bands on T1–T2 distinctly wider than that on T3, and with U-shaped gaps medially (Fig. 13).

Male. Unknown.

Distribution. China (Guizhou).

Remarks. This species is easily distinguished from all other species of *Stenodyneriellus* by the following character combination: T2 with a translucent, thin, blade-shaped lamella apically (Fig. 13), T2 and S2 distinctly punctate (Fig. 13–14), propodeum protruding upward so as to form two teeth behind metanotum dorsally (Fig. 12), clypeus deeply emarginated apically (Fig. 11), and pronotum, metanotum and dorsal surface of propodeum dark ferruginous.

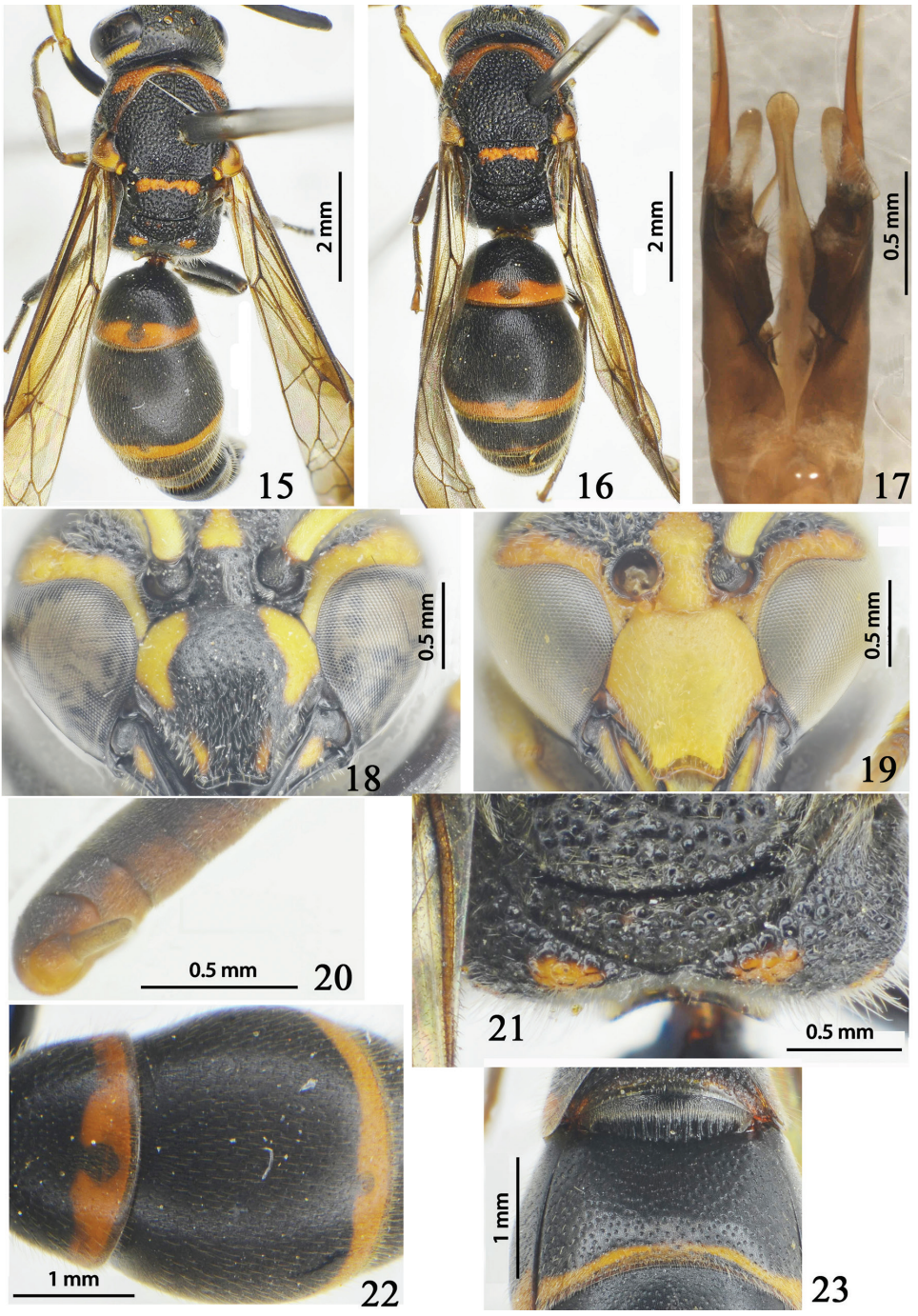
Etymology. It is named after the type locality of the species, Maolan National Nature Reserve in Guizhou of China.

***Stenodyneriellus depressus* sp. n.**
http://zoobank.org/19308E8E-9A2A-4032-9B94-F731447CDD0C
Figs 15–23

Material examined. Holotype, ♂, China, Yunnan Province, Baoshan City, Lujiang Town, Pumanshao, 25°01'32.16"N, 98°57'57.20"E, 773m, 17. VII. 2006, Rui Zhang, No. 1004065 (CQNU). Paratypes: 1♂; same data as holotype, No. 1004066 (YNAU); 1♀, China, Yunnan Province, Xishuangbanna State, Menghai County, Mangao Village, 22°01'10.11"N, 100°20'6.96"E, 716m, 26. VII. 2011, Xin Zhou, No. 1004067 (CQNU).

Description. Female (Fig 15, 18, 21–23): body length 8.5 mm, forewing length 7.0 mm. Black, with the following parts pale ferruginous (Fig. 15): dorsal surface of pronotum medially, a very small dorsal mesepisternal spot, a transverse band on the basis of scutellum, small spots on dorsal surface of propodeum medially and laterally, and a apical band on each of T1–T2 and S2; the following parts yellow: a wide arcuate band basal-laterally and a apical spot on clypeus laterally (Fig. 18), a spot on mandible basally, scape ventrally, a large and wide band along inner eye orbit from basis of clypeus to upper margin of ocular sinus (not occupying entire ocular sinus), interantennal spot, a long postocular band, tegula outside, parategula, fore and mid femora apically and tibiae laterally, and a spot on apex of mid coxa inside; antenna except scape ventrally and tegula except outside brown. Wings lightly infuscate. Setae pale brown.

Head. Clypeus medially convex, sparsely punctate and interspaces with shallow, very thick and minute punctures, apex moderately emarginated medially (Fig. 18), clypeal width approximately equal to its length, total width: apical width = 1.09: 0.4, apex wider than interantennal space; interantennal carina prominent; frons evenly convex and very coarsely punctate, interspaces between punctures with carinae and reticulate; vertex very sparsely punctate, cephalic fovea obsolete; POD nearly as wide as OOD.



Figures 15–23. *Stenodyneriellus depressus* sp. n. **15** habitus of one paratype (dorsal view), ♀ **16** habitus of holotype (dorsal view) (dorsal view), ♂ **17** genitalia (front view), ♂ **18** clypeus, ♀ **19** clypeus, ♂ **20** apex of antenna, ♂ **21** metanotum and propodeum, ♀ **22** T2 (dorsal view), ♀ **23** S2 (ventral view), ♀.

Mesosoma. Pronotum, mesoscutum, mesepisternum, mesoscutellum, metanotum, and dorsal and lateral surfaces of propodeum coarsely and deeply punctate and reticulate, these punctures distinctly deeper than those on frons, punctures on pronotum, mesoscutum, and mesoscutellum sparser than those on mesepisternum, metanotum and propodeum, and their interspaces with very small and shallow punctures. Pronotal carina complete, rounded dorsally and emarginated laterally; mesoscutum, mesepisternum, mesoscutellum normal; metanotum with a very short dorsal convex surface and sloping posteriorly (Fig. 21); dorsal surface of propodeum almost in the same horizontal plane as metanotum, without teeth behind metanotum, interspaces between punctures distinctly carinate (Fig. 21), and well-separated from posterior surface; posterior surface widely and deeply concave, very sparsely punctate and interspaces between punctures smooth. Tegula wide, length slightly longer than its width, and posterior lobe small; parategula hooked and its apex reaching the apex of tegula.

Metasoma. In dorsal view, T1 domed, width 1.47 × its length and 0.80 × width of T2, without a transverse carina anteriorly, sparsely punctate, interspaces between punctures larger than one diameter, punctures distinctly smaller than those on frons and mesosoma; tergum 2 without a lamella (Fig. 22) apically, punctures similar to those on T1; S2 distinctly depressed basally and moderately punctate (Fig. 23), punctures larger and denser than T2, and generally smaller than those on head and mesosoma; visible parts of T3–T6 and S3–S6 coriaceous and with minute punctures; the apical yellow bands on T1–T2 with gaps medially.

Male (Figs 16–17, 19–20). Body length 8.0 mm, forewing length 7.5 mm. Sculpture, punctuation, setae, and coloration as in female except as follows: clypeus entirely yellow (Fig. 19), interantennal spot orange–yellow, spots on mandible and interantennal space larger than those in female, propodeum without spots; clypeal width 0.93× its length, total width: apical width = 0.95: 0.41, apical emargination slightly deeper than that in female ; A13 backward reaching apical margin of A10 (Fig. 20); width of T1 1.60 × its length and 0.80 × width of T2; genitalia as in Fig. 17, apical tip of penis valve rounded, volsella wide and rounded apically, and parallel spines elongate without setae; other characters same as those in female.

Distribution. China (Yunnan).

Remarks. This species resembles *S. perpunctatus* Giordani Soika, 1994 from Malaysia by T2 without an apical lamella (Fig. 22), dorsal surface of propodeum not protruding upward so as to form two teeth behind metanotum, and propodeum with large dense punctures dorsally, interspaces between punctures carinate (Fig. 21). It differs from *S. perpunctatus* and all other members of the genus by the following character combination: clypeal apex moderately emarginated medially, apical width somewhat wider than interantennal space (Figs 18–19), S2 distinctly depressed basally (Fig. 23), and mesosoma and metasoma with pale ferruginous spots and bands (Fig. 15–16).

Etymology. The specific name is derived from two Latin words: *de-* and *pressus*, referring to S2 of the species being distinctly depressed basally.

***Stenodyneriellus guttulatus* (de Saussure, 1862)**
Figs 24–29

Odynerus guttulatus de Saussure, 1862: 200; van der Vecht 1937: 285.
Ancistrocerus megaspilus Cameron, 1907: 85; Giordani Soika 1994: 115.
Odynerus santabongensis Cameron, 1908: 562; Giordani Soika 1994: 115.
Odynerus megaspilus: Dover, 1931: 255.
Odynerus guttulatus var. *heterospilus* van der Vecht, 1937: 286.
Odynerus guttulatus var. *nigridorsus* van der Vecht, 1937: 287; Giordani Soika 1994 (syn. of *S. guttulatus* (de Saussure)).
Hylodynerus guttulatus: Gusenleitner, 1988: 180.
Stenodyneriellus guttulatus: Giordani Soika 1994: 57, 115; Gusenleitner, 2006: 689.

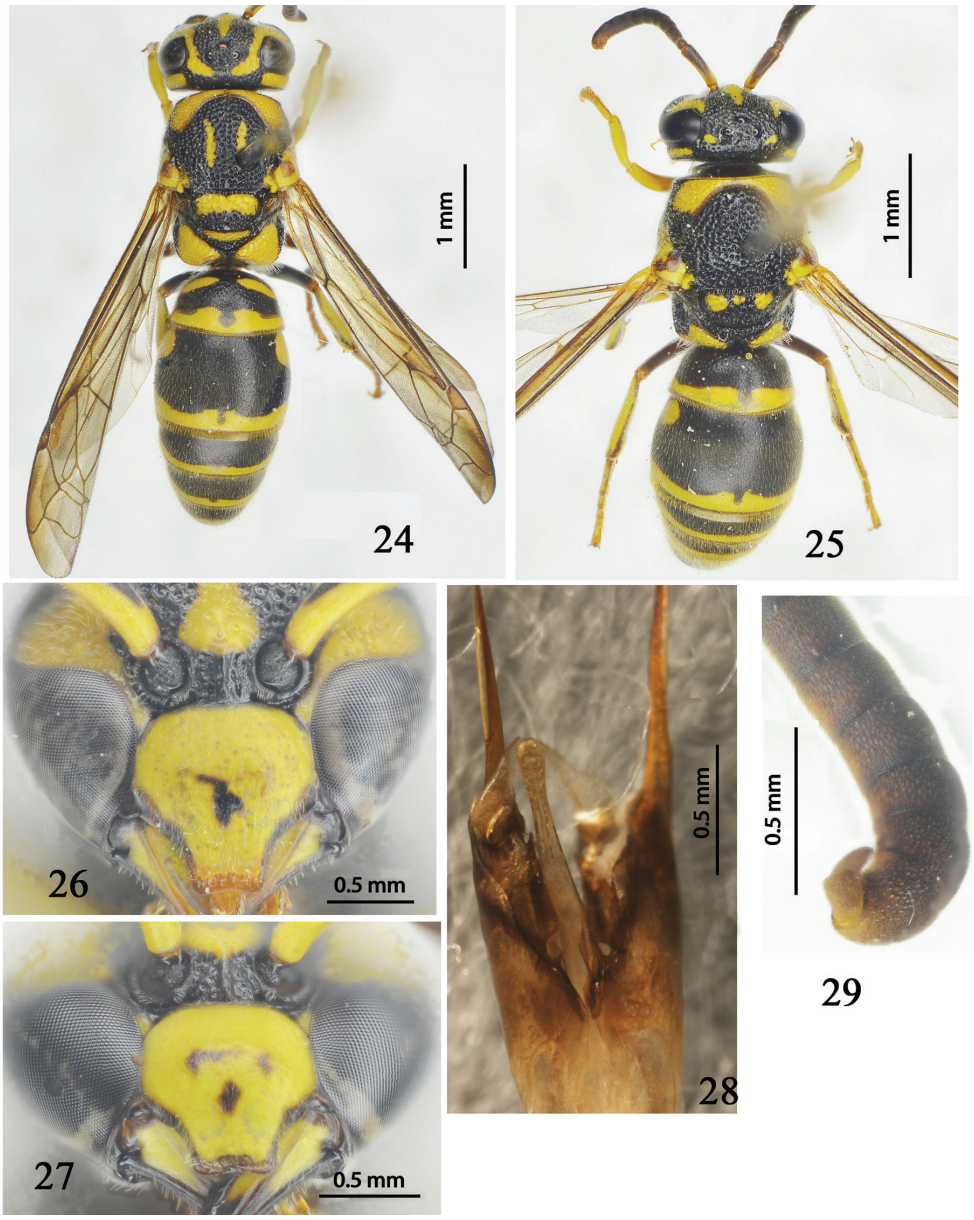
Material examined. 2♂♂1♀, China, Yunnan Province, Honghe State, Hekou County, southeast Nanxi Town, 21. VII. 2003, Qiang Li and Tingjing Li; 1♀, China, Yunnan Province, Honghe State, Hekou County, Nanxi Town, 18. VII. 2015, Lin Yang and Lingquan Zeng; 1♀, China, Yunnan Province, Honghe State, Hekou County, Nanxi Town, Duoyixia Village 19. VII. 2015, Chunfa Chen; 2♀♀, China, Yunnan Province, Honghe State, Hekou County, Former Fruit Factory, 12. VII. 2012, Jian Zhu.

Diagnosis. Clypeus truncated apically and almost entirely yellow (Figs 26–27); body with distinct yellow spots and bands (Figs 24–25), propodeum well-developed, prolonged in a medial direction protruding upward so as to form two teeth behind metanotum dorsally; T2 apically with a translucent, thin blade-shaped lamella; male A13 backward and reaching the base of A11 (Fig. 29); genitalia as in Fig. 28, apical tip of penis oblong, volsella narrow and acute apically, and parallel spines elongate without setae.

Distribution. China (new record: Yunnan); India; Thailand; Myanmar; Malaysia; Singapore; Indonesia.

Key to the Chinese species of *Stenodyneriellus*

1 T2 without an apical lamella (Fig. 22); dorsal surface of propodeum not protruding upward so as to form two teeth behind metanotum (Fig. 21) ..... ***S. depressus* sp. n.**
– T2 with a translucent, thin and blade-shaped lamella apically (Figs 6, 13); dorsal surface of propodeum protruding upward so as to form two teeth behind metanotum (Figs 4, 12) **2**
2 Clypeus deeply emarginated apically (Fig. 11); T2 and S2 densely and largely punctate (Figs 13–14); pronotum, metanotum and dorsal surface of propodeum dark ferruginous (Fig. 10) ..... ***S. maolanensis* sp. n.**
– Clypeus almost truncated or somewhat emarginated apically (Figs 3, 5, 26–27); T2 and S2 more sparsely and thinly punctate (Figs 6, 8); pronotum, metanotum and dorsal surface of propodeum yellow or black..... **3**



Figures 24–29. *Stenodyneriellus guttulatus* (de Saussure, 1862). **24** habitus (dorsal view), ♀ **25** habitus (dorsal view), ♂ **26** clypeus, ♀ **27** clypeus, ♂ **28** genitalia (front view), ♂ **29** apex of antenna.

3 Male A13 reaching the basis of A11 (Fig. 29); female scutum with two longitudinal yellow spots and T1 with two large, elongated and transverse yellow spots on anterior face (Fig. 24); in genitalia of male, volsella narrow and acute apically (Fig. 28) ..... ***S. guttulatus* (de Saussure, 1862)**

– Male A13 reaching the middle of A11 (Fig. 7); female scutum and T1 without yellow spots (Fig. 1); in genitalia of male, volsella wide and blunt apically (Fig. 9).....*S.* ***similiguttulatus*** **sp. n.**

Lissodynerus Giordani Soika, 1993

Lissodynerus Giordani Soika, 1993a: 135; Girish Kumar and Carpenter 2015: 7664–7667.
Lissodynerus Giordani Soika, 1973: 119, used as generic name for *Odynerus septemfasciatus* var. *feanus* Giordani Soika, 1941. Unavailable under Article 13.1.1 of the Code.
Trichodynerus Giordani Soika and Kojima, 1988: 178, used as a generic name in the combination *Trichodynerus agilis cursor* Giordani Soika and Kojima, 1988. Unavailable under Article 13.1.1 of the Code.

Type species. *Odynerus septemfasciatus* Smith, 1857, by original designation.

Diagnosis. Carina of pronotum well-developed, wide rounded, and regularly convex (Fig. 32) dorsally; shelf on the upper propodeum more or less developed, and sometimes confused with big and dense punctures on the interface between dorsal and posterior surfaces, dorsal and posterior surfaces clearly separated, posterior surface entirely concave (Fig. 33); T1 short, width approximately 2 × its length, with an vertical and slightly convex surface anteriorly, interface between anterior and dorsal surfaces with a transverse, smooth, and well-developed carina (Fig. 33); apex on each of T2-T3 or T2-T4 with a brown or black blade-shaped lamella (Fig. 35).

Distribution. Oriental and Australian Regions.

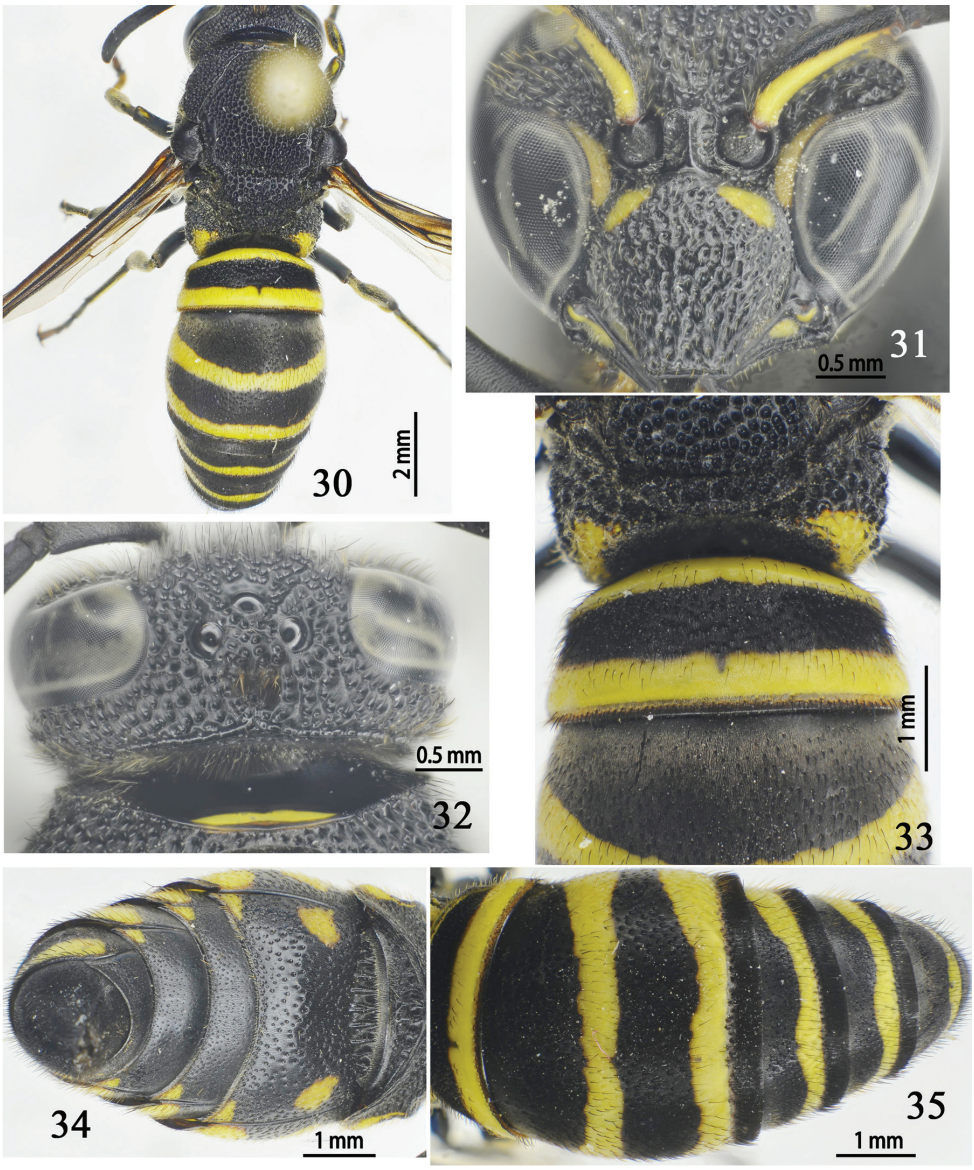
Lissodynerus septemfasciatus feanus (Giordani Soika, 1973), new record

Figs 30–35

Ancistrocerus septemfasciatus var. *feanus* Giordani Soika, 1941: 239.
Ancistrocerus septemfasciatus feanus: Giordani Soika 1973a: 29.
Lissodynerus septemfasciatus feanus: Giordani Soika 1973b: 119, 1994: 304, 315.

Material examined. 2♀♀, China, Guangxi Zhuang Autonomous Region, Laibin County, Dayao Mountain, 19. VII. 2015, Haixia Zhang and Yuting Hong.

Diagnosis. Female clypeus truncated apically (Fig. 31); vertex with big and moderately dense punctures; shelf of propodeum weak; apex on each of T2-T4 with a black blade-shaped lamella (Fig. 35); mesosoma almost entirely black and only with the following yellow parts: a short band on the anterior surface of pronotum medially (Fig. 32), a tiny spot on metanotum, rarely a spot on top of mesepisternum, and an apical band on dorsal surface of propodeum laterally (Fig. 33); metasoma largely with yellow



Figures 30–35. *Lissodynerus septemfasciatus feanus* (Giordani Soika, 1973). **30** habitus (dorsal view), ♀ **31** clypeus, ♀ **32** head and pronotum (dorsal view), ♀ **33** propodeum and T1 (dorsal view), ♀ **34** metasoma (ventral view), ♀ **35**. metasoma (dorsal view), ♀.

parts (Figs 30, 35): a wide band on the interface between anterior and dorsal surfaces of T1, a subapical wide band on each of T1–T5, a wide band in the middle of T2, a spot on the base of S2 laterally, and irregular spots on apical margins of S2–S4 laterally.

Distribution. China (new record: Guangxi); Myanmar; Vietnam; Malaysia.

Remarks. These female specimens of *Lissodynerus septemfasciatus feanus* from China are almost same as those from Myanmar, Vietnam and Malaysia (Giordani Soika 1994). Male specimens have not been found yet.

Discussion

It is well known that species of these two genera *Stenodyneriellus* and *Lissodynerus* occur in Australian or Oriental region. In China, southern and southwestern provinces typically belong to the Oriental region, including Guangdong, Guangxi, Hainan, Fujian, Guizhou, Sichuan, Chongqing and Yunnan. It proves that our results are reliable. We also believe that in future, more species or distributions of these two genera may be recorded from the provinces of China belonging to the Oriental region.

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RESEARCH ARTICLE

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Sawflies (Hymenoptera, Symphyta) newly recorded from Washington State

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Abstract

Examination of museum specimens, unpublished collection data, and field surveys conducted between 2010 and 2014 resulted in records for 22 species of sawflies new to Washington State, seven of which are likely to be pest problems in ornamental landscapes. These data highlight the continued range expansion of exotic species across North America. These new records also indicate that our collective knowledge of Pacific Northwest arthropod biodiversity and biogeography is underdeveloped, even for a relatively well known and species-poor group of insects. Notable gaps in the knowledge of Washington State's Symphyta remain for the Olympic Peninsula, the Cascade Mountain Range, and the arid interior of the state. Washington's shrub-steppe appears to be particularly poorly surveyed for sawflies.

Keywords

Exotic species, range expansion, state record, museum data

Introduction

Sawflies (Hymenoptera, Symphyta) comprise 14 families worldwide, with 12 of these and about 1,000 described species known from North America (Smith 1979a, Taeger et al. 2010). Eleven families and approximately 180 named species are currently recorded from Washington State (Smith 1979a, Gibson 1980, Goulet 1986, Smith 1989, Goulet 1996, Looney et al. 2012, Schiff et al. 2012). Most sawfly species are herbivores, including leaf- and stem-mining species, chewing defoliators, wood-borers, and leaf-tying defoliators. Species in the family Orussidae are external parasitoids of wood-boring insects (Powell and Turner 1975, Deyrup 1984), and adult *Tenthredo* (Tenthredinidae) are commonly observed feeding upon other arthropods (Pasteels and Gregoire 1984). Some sawflies are important forestry, horticultural and agricultural pests.

The 2009 discovery of the introduced alder-feeding sawfly *Monsoma pulveratum* (Retzius, 1783) in the Pacific Northwest (Looney et al. 2012) provided impetus to conduct a broad sawfly survey in Washington State from 2010 through 2012. Specimens collected during that survey revealed that the Pacific Northwest range of many sawfly species is incompletely delineated, and that relatively few contributions have been made towards understanding Pacific Northwest Symphyta during recent decades. Subsequent to the two year survey, we collected data from other researchers, museum specimens, and further serendipitous discoveries. Here we report 22 species not previously known from Washington or documented only in gray literature, expanding known ranges in some cases and filling gaps in others.

Methods

New sawfly records were compiled from many sources, including regional entomological collections and recent field surveys. More than 3,500 identified and unidentified sawflies in entomology collections at the Evergreen State College, the College of Idaho, Oregon State University, the University of Idaho, Washington State University, and Western Washington University were examined for species of interest.

Field surveys from 2010 through 2012 employed sticky traps and Malaise traps (Fig. 1), with subsequent targeted and opportunistic hand collections made through 2015. Double-sided yellow, green, or white sticky traps with hot-applied adhesive were placed in various woody host plants across Washington in 2010–2012. Host material surveyed included alder, poplar, hawthorn, mountain ash, cherry, pear, apple, elderberry, and various conifers, with approximately 105 sites surveyed in Washington. Nine Malaise traps were installed in Washington west of the Cascade mountain range. Three traps were installed near ports of entry, two along the Columbia River, two in mixed-use forest stands, and two in residential areas. One Malaise trap was installed in a prairie remnant surrounded by agricultural fields in eastern Washington. Traps were installed in April, 2012, and maintained through September 2012. Opportunistic

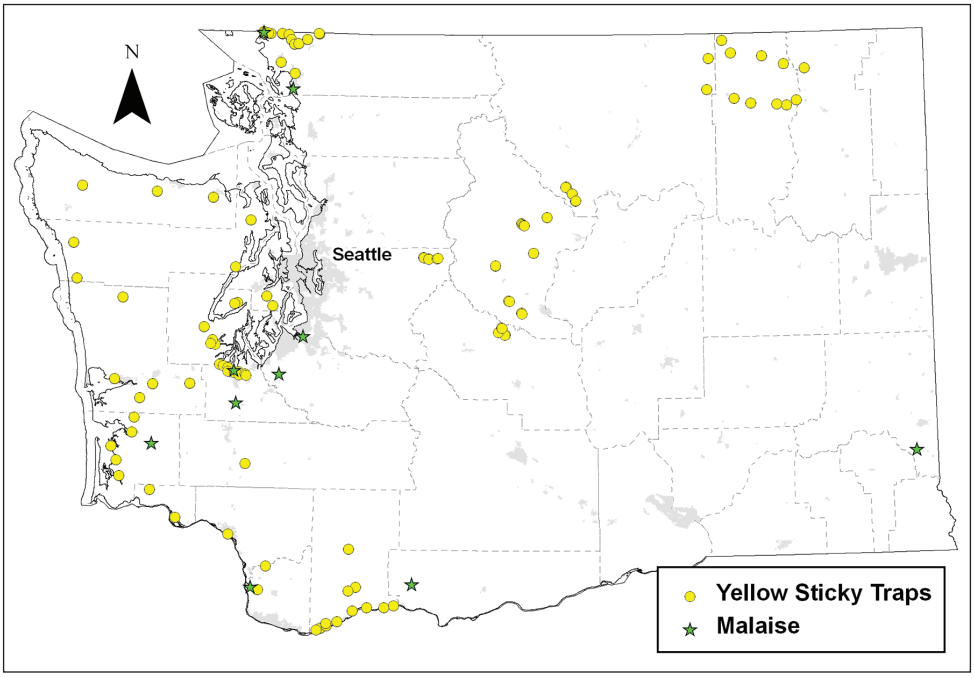

Figure 1. Locations of yellow sticky traps and Malaise traps deployed in Washington State in 2011.

hand collections of larvae and adults were made throughout 2010–2015 as part of this study. We also present novel data for leaf-mining sawflies in Washington collected during a survey conducted in 2006 (see also Digweed et al. 2009). Lastly, some of the species recorded here were first detected by citizens reporting new pest problems (e.g., *Neodiprion sertifer*).

Collection data were compiled for each species, and narratives were composed that briefly describe each species' natural history and other details. Species names follow Taeger et al. (2010). We have chosen to not alter two combinations in the Nematinae suggested by new work by Prous et al. (2014). Taxonomic changes for the North American fauna resulting from their research have not yet been made, and are best approached via a thorough examination of North American Nematinae rather than piecemeal in papers such as this. Probable combinations are noted in the narrative accompanying each species. There are numerous common names ascribed to many of the species reported on here, since so many of these species are pestiferous and conspicuous. We do not provide those names, but North American common names can be found at the websites for the Entomological Society of Canada (esc-sec.ca) and the Entomological Society of America (entsoc.org). Voucher specimens are deposited at the Northern Forestry Centre Arthropod Collection in Edmonton, Alberta (NFRC), the Washington State Department of Agriculture Collection (WSDAC), Western Washington University (WWUC), the Evergreen State College (TESC), and the National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM).

Results

Twenty-two species not previously documented in Washington State in peer-reviewed literature were detected in these field and museum surveys, primarily in western Washington (Table 1). One species was collected as by-catch in a survey for other pests, and seven species were first detected due to citizen complaints. Five species were first detected by examining unidentified material in museum collections, and the remainder were collected in sawfly surveys or general collecting. A map of collection localities for more than 1,200 sawfly specimens indicates that most collecting has been near developed towns and cities, or along major highways (Fig. 2). Remote and rugged areas are undersampled, and very few collections have been made in the arid interior shrub-steppe.

Xiphydriidae

Xiphydria prolongata (Geoffroy, 1785)

Xiphydria prolongata is a European species that is a wood-borer in small limbs of deciduous tree genera, including *Salix* L., *Quercus* L., and numerous Betulaceae (Smith 1983). Larvae generally bore in decaying wood, and are not pestiferous. This western European species was first documented in North America in Michigan and New Jersey in the early 1980s, bringing the known *Xiphydria* species in North America to nine (Smith 1983). Mudge et al. (2001) recorded the first west coast specimen from Multnomah County, Oregon, in 1989. The single specimen from Washington was found in a trap for Japanese beetle, near Boeing Field in King County (Fig. 3). The specimen is housed at WSDAC.

Diprionidae

Neodiprion sertifer (Geoffroy, 1785)

A member of Ross's (1955) *sertifer* species group, this species is the only *Neodiprion* native to Europe, where it is a forestry and nursery pest (Day and Leather 1997). Its primary hosts are *Pinus resinosa* Aiton and *P. sylvestris* L., although it will feed on most *Pinus* species and can be a significant source of damage in pine plantations during outbreak years (Alford 2012). Introduced to New Jersey, USA, in 1925 (Schaffner 1939), its more visible impacts have included feeding damage on Christmas trees, making them less valuable or unmarketable, and defoliation of older needles on ornamental and landscape plants (Wilson 1971). Economic damage in North America has been recorded on *P. strobus* L., *P. sylvestris*, and *P. mugo* Turra, well as several native North American *Pinus* spp. (Schaffner 1943, Craighead 1950, Benjamin et al. 1955, Griffiths 1959, Baker 1972). Since its introduction, the species has spread westward at least to North Dakota (Van Driesche et al. 2012) and Saskatchewan, Canada (Langor, unpublished data).

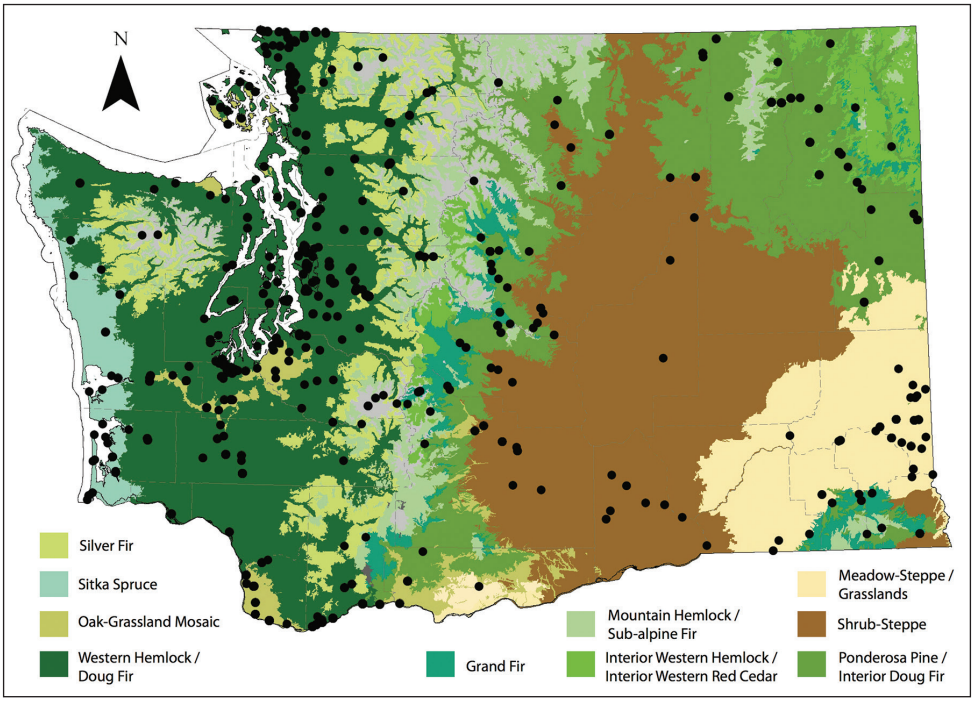

Figure 2. Map of specimen localities (black circles) for over 1,200 specimens collected in Washington State, including museum data and field data generated during this project. Vegetation zones are simplified from Washington's GAP Analysis project (Cassidy et al. 1997).

Neodiprion sertifer was detected in Washington State in 2008, when citizens in Bellingham, Washington, alerted pest control professionals to several defoliating outbreaks on ornamental pines. Larvae were observed again in 2012 on *P. sylvestris* and *P. mugo* in Bellingham, and adult males of *Neodiprion* sp. were collected in pheromone traps in the city that year. Although the latter were likely *N. sertifer*, male *Neodiprion* are not readily identifiable to species using external or genitalic morphology, or by mitochondrial DNA sequences (Linnen and Farrel 2012). Despite extensive surveys in 2010–2013, *N. sertifer* populations have to date only been found in Washington State within the Bellingham city limits (Fig. 3). The records from Washington State could represent a newer and separate introduction event in the Pacific Northwest, although transport on nursery stock seems to be the most likely introduction pathway. Specimens are housed at WSDAC.

***Diprion similis* (Hartig, 1836)**

Taeger et al. (2010) list 13 world species of *Diprion*. Most are Asian, but *D. similis* is one of two *Diprion* species native to Europe. Large populations of this solitary feeder

Table 1. Collection information for 22 sawfly species newly reported from Washington State.

County	Lat N	Long W	Date	Specimens	Collection Method	Collector
Xiphydria prolongata						
King	47.5374	122.3040	7 Aug 2012	1♀	Japanese beetle trap	D. Kitchen
Neodiprion sertifer						
Whatcom	48.7659	122.4518	30 May 2008, em. ~ 20 Sep 2008	6♀, 1♂	Rrd. ex *Pinus mugo*	L. Haines
Whatcom	48.7632	122.4505	May 2012	Mul. larvae	Obs. on *Pinus sylvestris*	C. Looney
Whatcom	48.7612	122.4482	May 2012, em. Aug 2012	2♂, 1♀	Rrd. ex *Pinus mugo*	C. Looney
Whatcom	48.7412	122.4745	25 Jul-4 Oct 2012	Multiple ♂	Wing trap w/ *Neodiprion* lure	C. Looney
Diprion similis						
Mason	47.1978	123.0995	26 Jul 2012, em. ~2 Aug 2012	3♂, 2♀	Rrd. ex *Pinus sylestris*	C. Looney
Thurston	47.0802	123.0203	4 Sep 2012	1 larva	Obs. on *Pinus contorta*	C. Looney C. Fate
Thurston	47.0799	123.0203	4 Sep 2012	3 larvae	Obs. on *Pinus monticola*	C. Looney C. Fate
Thurston	47.1056	123.0009	4 Sep 2012	2 larvae	Obs. on *Pinus monticola*	C. Looney C. Fate
Thurston	47.0902	123.0471	4 Sep 2012	1♂, 5 larvae	Wing trap w/ *D. pini* lure, Hand coll. on *Pinus monticola*	C. Looney C. Fate
Thurston	47.0540	122.9254	10 Sep 2012	1♀, 1 larva	Obs. on *Pinus monticola*	C. Looney A. Pelegrin
Whatcom	48.7412	122.4748	Aug 2012, em. 4 Jun 2013	1♂	Rrd. ex *Pinus monticola*	C. Looney M. Peterson
Gilpinia hercyniae						
Whatcom	48.7412	122.4745	31 Jul-6 Aug 2008; 9 Jul 2011, em Aug 2011; 8 Aug 2012	1♀; 1♀; 1♀	Hand coll.; Rrd. ex. *Picea abies*; White sticky trap, *Picea abies*	M. Peterson
Cladius grandis						
Thurston	47.0734	122.9767	9-16 May 1997	1♀	Malaise trap	J. Longino

County	Lat N	Long W	Date	Specimens	Collection Method	Collector
Cladius gregarious						
Okanogan	48.4204	119.7115	16 Sep 2010, em. spring 2011	1♀, 1♂	Rrd. ex *Populus tremuloides*	G. Kohler
Pristiphora geniculata						
King	47.7295	122.3045	25 Jul 2009	Mul. larvae	Obs. on *Sorbus aucuparia*	S. Collman
King	47.7295	122.3045	10 Aug 2010	Mul. adults	Obs. on *Sorbus aucuparia*	S. Collman
King	47.6808	122.1106	12 Jul 2011	Mul. larvae	Obs. on *Sorbus aucuparia*	A. Clarke
King	47.4857	121.7674	7 Jul 2011, em. 11-22 Aug 2011	15♀	Rrd. ex *Sorbus aucuparia*	K. Ripley
King	47.7724	122.3270	7 Aug 2012	Mul. larvae	Obs. on *Sorbus aucuparia*	S. Collman
King	47.4503	122.4908	6 June 2015	Mul. larvae	Obs. on *Sorbus aucuparia*	K. Ripley
King	47.4483	122.4836	6 June 2015	Mul. larvae	Obs. on *Crataegus douglasii*	K. Ripley
Snohomish	47.8626	121.8165	8 Aug 2009	Mul. larvae	Obs. on *Sorbus aucuparia*	S. Collman
Snohomish	47.9571	122.2318	1 Jul 2011	Mul. larvae	Obs. on *Sorbus aucuparia*	A. Jordan
Snohomish	47.8788	122.2240	9 Aug 2011	Mul. larvae	Obs. on *Sorbus aucuparia*	S. Collman
Snohomish	47.8788	122.2240	18 Jun 2012	Mul. larvae	Obs. on *Sorbus aucuparia*	S. Collman
Whatcom	48.7084	122.4433	11 Jul 2011	Mul. larvae	Obs. on *Sorbus aucuparia*	D. Wallesz
Whatcom	48.7427	122.4350	7 Jul 2013, em. 19-23 Aug 2013	7♀, 3♂	Rrd. ex *Crataegus douglasii*	T. Cahill
Pristiphora rufipes						
King	47.7295	122.3045	17 Sep 2010; 5 May 2012	Mul. larvae; Mul. larvae	Hand coll. on *Aquilegia* sp.	S. Collman
Snohomish	47.8656	121.9876	3 Sep 2013	Mul. larvae	Hand coll. on *Aquilegia* sp.	S. Collman
Snohomish	47.8788	122.2240	7 Sep 2013; 6 Nov 2014	Mul. larvae; Mul. larvae	Hand coll. on *Aquilegia* sp.	S. Collman
Thurston	47.0562	122.9250	24 Mar 2014	2♀, 6♂	Hand coll. on *Aquilegia* sp.	C. Looney
Craesus alniastri						
County	Lat N	Long W	Date	Specimens	Collection Method	Collector
Island	47.9590	122.3607	7-26 Jul 2011	1♀	Yellow sticky trap, *Alnus rubra*	K. Ripley
King	47.3809	122.2348	22 Sep 1976	1♀	Rrd. ex *Alnus rubra*	D. Rhoades
Kitsap	47.4400	122.9365	22 Jun-1 Jul 2011	1♀	Yellow sticky trap, *Alnus rubra*	K. Ripley

County	Lat N	Long W	Date	Specimens	Collection Method	Collector
Pierce	47.2500	122.3502	17 Jun-16 Aug 2010	1♀	Green sticky trap, *Alnus rubra*	D. Kitchen
Skagit	48.4204	122.4142	13 Sep 2010	1♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Skamania	45.5763	122.1917	20 May-23 Jun 2011	1♀	Yellow sticky trap, *Alnus rubra*	K. Sheehan
Skamania	45.6260	122.0241	15 Jun 2011	1♀	Yellow sticky trap, *Alnus rubra*	T. Murray
Skamania	45.5870	122.1595	2-23 Jun 2011	2♀	Yellow sticky trap, *Alnus rubra*	K. Sheehan
Skamania	45.7105	121.7801	30 Jun 2011	5♀	Yellow sticky trap, *Alnus rubra*	T. Murray
Skamania	45.5763	122.1917	30 Aug-6 Oct 2011	1♀	Yellow sticky trap, *Alnus rubra*	K. Sheehan
Snohomish	48.1987	122.1251	16 Aug-29 Aug 2011	2♀	Japanese beetle trap	R. Taylor
Whatcom	48.9970	122.2635	14-26 Jun 2012	2♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9940	122.6876	26 Jul-20 Aug 2012	1♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	49.0019	122.7547	26 Jul 2012	1♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	49.0013	122.7493	26 Jul 2012	1♀	Malaise trap	C. Looney
Whatcom	48.9988	122.2684	26 Jul-20 Aug 2012	4♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.7479	122.4343	1-21 Aug 2012	1♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9131	122.5741	8-20 Aug 2012	10♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9915	122.5294	8-20 Aug 2012	6♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9388	122.4443	8-20 Aug 2012	7♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9636	122.3675	8-20 Aug 2012	4♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9965	122.2632	8-20 Aug 2012	2♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9970	122.2635	8-20 Aug 2012	6♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9940	122.6878	20-30 Aug 2012	4♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9933	122.5874	20-30 Aug 2012	4♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9131	122.5741	20-30 Aug 2012	3♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9915	122.5294	20-30 Aug 2012	4♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9619	122.5091	20-30 Aug 2012	1♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9357	122.4817	20-30 Aug 2012	1♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9388	122.4443	20-29 Aug 2012	5♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9637	122.3675	20-30 Aug 2012	5♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9988	122.2684	20-30 Aug 2012	4♀	Yellow sticky trap, *Alnus rubra*	D. Maclean

County	Lat N	Long W	Date	Specimens	Collection Method	Collector
Nematus lipovskyi						
King	47.7295	122.3046	12 Jun 2008	Mul. larvae	Hand coll. on *Rhododendron mollis*	S. Collman
King	47.7632	122.3147	9 Jun 2008	Mul. larvae	Hand coll. on *Rhododendron occidentalis*	S. Collman
King	47.6367	122.2966	12 Jun 2011	Mul. larvae	Hand coll. on *Rhododendron occidentalis*	S. Collman
King	47.6569	122.2899	Jun 2013	Mul. larvae	Hand coll. on *Rhododendron* sp.	S. Collman
King	47.7632	122.3147	30 May 2014	Mul. larvae	Hand coll. on *Rhododendron occidentalis*	C. Looney A. Pelegrin
Thurston	47.0393	122.7985	26 Apr 1996	1♀	Hand coll.	B. Dightman
Thurston	47.0329	122.8992	28 May 2014	Mul. larvae	Obs. on *Rhododendron* sp.	C. Looney
Thurston	47.0384	122.8984	2 Jun 2014; 14 Apr 2015	Mul. larvae; 10♀	Hand coll. on *Rhododendron* sp.	C. Looney
Heterarthrus nemoratus						
Whatcom	48.8053	121.8936	22 Jul 1967	1♀	unknown	unknown
King	47.5085	122.3095	1 May 2007	1 sex unk.	Photograph, bugguide	C. Moorehead
Heterarthrus vagans						
Whatcom	48.9353	122.4817	2 Sep 2011	1 pupa	Hand coll. on *Alnus rubra*	W. Hellman
Whatcom	48.9939	122.6830	2 Sep 2011	1 pupa	Hand coll. on *Alnus rubra*	W. Hellman
Whatcom	48.9623	122.5085	2 Sep 2011	1 pupa	Hand coll. on *Alnus rubra*	W. Hellman
Whatcom	48.9636	122.3675	14-26 Jun 2012	3♂, 1♀	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9381	122.4428	26 Jun-12 Jul 2012	4♂	Yellow sticky trap, *Alnus rubra*	D. Maclean
Whatcom	48.9636	122.3675	8-20 Aug 2012	1♂	Yellow sticky trap, *Alnus rubra*	D. Maclean
Metallus lanceolatus						
Thurston	47.0734	122.9767	15-22 Aug 1997	1♀	Malaise trap	J. Longino
Thurston	47.0802	122.9749	Jun-Aug 2012	Mul. larvae	Mines in *Geum macrophyllum*	C. Looney
King	47.6538	122.1098	Jul 2014	Mul. larvae	Mines in *Geum macrophyllum*	C. Looney
King	47.5583	122.2503	22 Aug 2015	Mul. larvae	Mines in *Geum macrophyllum*	C. Looney
Fenusella nana						
Grays Harbor	47.0565	123.2739	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Grays Harbor	46.9826	123.6043	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor

County	Lat N	Long W	Date	Specimens	Collection Method	Collector
Grays Harbor	46.9755	123.8670	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
King	47.9820	122.1947	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
King	47.7449	122.3424	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
King	47.5067	122.2900	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
King	47.5285	121.8760	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
King	47.6579	122.1105	5 May 2013	2♀	Hand coll. on *Betula* sp.	C. Looney
King	47.4886	121.7946	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Pierce	47.2505	122.2896	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Skagit	48.4046	122.3315	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Skagit	48.4838	121.5991	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Skagit	48.5270	121.4420	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Snohomish	47.8562	121.6960	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Whatcom	48.9640	122.4625	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Whatcom	48.7949	122.4833	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Snohomish	48.2003	122.1266	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Snohomish	48.2462	121.6066	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Profenusa thomsoni						
King	47.5285	122.8760	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
King	47.4886	121.7946	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Skagit	48.4046	122.3315	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Skagit	48.5270	121.4420	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Snohomish	47.8562	121.6960	2006	Mul. larvae	Hand coll. on *Betula* sp.	D. Langor
Whatcom	48.8053	121.8936	15 Jul 1967	1♀	Unknown	Unknown
Profenusa inspirata						
Yakima	46.7157	120.8633	7 Jun 2015	Mul. larvae	Hand coll./obs. on *Quercus garryana*	C. Looney
Yakima	46.7441	120.7884	8 Jun 2015	Mul. larvae	Hand coll./obs. on *Quercus garryana*	C. Looney
Skamania	45.7182	121.4746	24 Sep 2015	Mul. larvae	Hand coll./obs. on *Quercus garryana*	C. Looney T. Murray
Lewis	46.6451	123.0198	2 Oct 2015	Mul. larvae	Hand coll./obs. on *Quercus garryana*	C. Looney M. Freeman

County	Lat N	Long W	Date	Specimens	Collection Method	Collector
Fenusa ulmi						
County	Lat N	Long W	Date	Specimens	Collection Method	Collector
King	47.6379	122.2961	20 Apr-9 May	Mul. ♀	Hand coll. near *Ulmus carpinifolia*	C. Scannell
Lewis	46.5523	122.8126	20 Jul-11 Aug 2011	3♀	Yellow sticky trap, *Alnus rubra*	D. Kitchen
San Juan	48.7017	122.9136	12 Jun 2015	Mul. larvae	Obs. on *Ulmus* sp.	C. Looney
Thurston	47.0415	122.8617	Jun 2012	Mul. larvae	Obs. on *Ulmus* sp.	C. Looney
Thurston	47.0379	122.8991	11 Jun 2014	Mul. larvae	Hand coll. on *Ulmus* sp.	E. Spurrier
Thurston	46.8716	122.9116	5-13 May 2010	1♀	Yellow sticky trap, *Alnus rubra*	E. LaGasa
Halidamia affinis						
Whatcom	48.9061	122.4991	6 Jun 1989	2♀	Hand coll.	E. LaGasa
Ferry	48.6091	118.138	6-27 Jun 2011	1♀	Yellow sticky trap, *Alnus rubra*	M. Johnson
San Juan	48.5514	123.0781	1 Apr 2010	1♀	Yellow sticky trap, *Alnus rubra*	T. Hanson
Clallam	48.0851	124.2636	29 Jun-19 Jul 2011	1♀	Yellow sticky trap, *Alnus rubra*	G. Kohler
Jefferson	47.9227	122.8156	18 May-7 Jun 2011	1 sex unk.	Yellow sticky trap, *Alnus rubra*	G. Kohler
King	47.4558	122.4529	5 May 2015	2 sex unk.	Hand coll	C. Looney
King	47.4473	122.4599	3-13 May 2010	1♀	Yellow sticky trap, Alnus rubra	K. Ripley
Kitsap	47.44	122.9365	26 May-4 Jun 2011	1 sex unk.	Yellow sticky trap, *Alnus rubra*	K. Ripley
Kitsap	47.4325	122.6126	26 May-4 Jun 2011	1 sex unk.	Yellow sticky trap, *Alnus rubra*	K. Ripley
King	47.3766	122.2418	12 Apr 2010	1♀	Sweep net	J. Cena
Pierce	47.2607	122.3513	2-16 May 2012; 16 May-7 Jun 2012	3 sex unk.; 1 sex unk.	Malaise trap	C. Looney
Thurston	47.0783	122.9732	18 May 2011	1♀	Sweep net	C. Looney
Thurston	47.0734	122.9767	9-16 May 1997	3 sex unk.	Malaise trap	J. Longino
Thurston	47.0231	122.9089	8 Jun-1 Aug 2011	1 sex unk.	Emerald Ash Borer trap	D. Kitchen
Thurston	47.0026	123.0002	24 May 2011	1 sex unk.	Yellow sticky trap, *Alnus rubra*	C. Looney
Grays Harbor	46.9738	123.2945	5-27 May 2010	2♀	Yellow sticky trap, *Alnus rubra*	G. Kohler
Thurston	46.8716	122.9116	10-27 May 2011; 2-14 Jun 2011	2 sex unk.; 3 sex unk.	Green sticky trap, *Alnus rubra*	E. LaGasa
Thurston	46.8207	123.1162	27 May-14 Jun 2010	8 sex unk.	Yellow sticky trap, *Alnus rubra*	K. Ripley
Pacific	46.5204	123.887	4-18 May 2011	2 sex unk.	Yellow sticky trap, *Alnus rubra*	D. Kitchen

County	Lat N	Long W	Date	Specimens	Collection Method	Collector
Lewis	46.4497	122.7989	18 May-1 Jun 2011	5 sex unk.	Yellow sticky trap, Alnus rubra	G. Kohler
Cowlitz	46.1103	122.8945	24 May-1 Aug 2011	1 sex unk.	Emerald Ash Borer trap	D. Kitchen
Clark	45.8623	122.7467	18 May-1 Jun 2011; 1-15 Jun 2011	8 sex unk.; 7 sex unk.	Yellow sticky trap, *Alnus rubra*	G. Kohler
Skamania	45.8473	121.4122	29 Jun 2012	1♀	Malaise trap	J. Markgraf
Skamania	45.8042	121.9348	19 May-23 Jun 2011	1 sex unk.	Yellow sticky trap, *Alnus rubra*	K. Sheehan
Clark	45.8004	122.6811	4 May-8 Jun 2011	1 sex unk.	Yellow sticky trap, *Alnus rubra*	K. Sheehan
Clark	45.7997	122.6818	31 Mar-4 May 2011; 4 May-8 Jun 2011	1 sex unk.; 6 sex unk.	Yellow sticky trap, *Alnus rubra*	K. Sheehan
Skamania	45.7106	121.6395	16 Jun 2011	1 sex unk.	Yellow sticky trap, *Alnus rubra*	T. Murray
Skamania	45.7105	121.7801	16-30 Jun 2011	1 sex unk.	Yellow sticky trap, *Alnus rubra*	T. Murray
Skamania	45.6257	122.0241	31 May 2011	3 sex unk.	Yellow sticky trap, *Alnus rubra*	T. Murray
Skamania	45.6142	122.115	19 May-23 Jun 2011	1 sex unk.	Yellow sticky trap, *Alnus rubra*	K. Sheehan
Clark	45.6053	122.5459	23 Mar 2010	1♀	Hand collected	A. Karankou
Skamania	45.587	122.1595	2-23 Jun 2011	2♀	Yellow sticky trap, *Alnus rubra*	K. Sheehan
Skamania	45.5763	122.1917	19 Apr-19 May 2011	2 sex unk.	Yellow sticky trap, *Alnus rubra*	K. Sheehan
Monophadnus pallescens						
Chelan	47.3120	120.2811	15 May 1999	1♀	Unknown	D. Knutson
Chelan	47.2802	120.1865	4 May 2003	1♀	Unknown	R. MacLean
Grays Harbor	46.9954	123.5951	28 Apr 2010	1♀	Hand coll.	C. Looney
King	47.3637	122.1202	20 Apr 1985	1♀	Unknown	P.E. Kalina
San Juan	48.4924	122.8944	10 May 1987	1♀	Unknown	D. Overdorff
Thurston	46.8716	122.9116	2-13 May 2012	2♀	Malaise trap	E. LaGasa
Thurston	47.0734	122.9767	18-25 Apr 1997; 25 Apr-2 May 1997; 2-9 May 1997; 9-16 May 1997	1♀; 1♀; 3♀; 1♀	Malaise trap	J. Longino
Whatcom	48.4534	122.2918	15 May 1986	1♀	Unknown	W. R. Buce
Whatcom	48.9465	122.4521	2 Jun 1967	2♀	Unknown	Unknown

County	Lat N	Long W	Date	Specimens	Collection Method	Collector
Whatcom	48.7502	122.4750	28 May 1975	1♀	Unknown	F. Robertson
Whatcom	48.9974	122.7278	18 Apr-11 May 2012	1♀	Yellow sticky trap, *Alnus rubra*	W. Hellman
Eupareophora parca						
King	47.6538	122.1098	5 May 2015	Mul. larvae	Hand coll. on *Fraxinus* sp.	C. Looney
Monostegia abdominalis						
King	47.5732	121.8856	26 Jun 2013	Mul. larvae	Obs. on *Lysimachia vulgaris*	K. Wal
King	47.6284	121.9334	Jun 2013	Mul. larvae	Obs. on *Lysimachia vulgaris*	K. Wal
King	47.5565	122.0735	11 Jun 2014, em. 13 Jul 2014	6♀	Reared ex. *Lysimachia vulgaris*	K. Wal
King	47.5701	122.0948	11 Jun 2014	Mul. larvae	Obs. on *Lysimachia vulgaris*	K. Wal
King	47.6532	122.1070	30 May 2014, em. 6 Jul 2014	3♀	Reared ex. *Lysimachia vulgaris*	C. Looney A. Pelegrin
Macrophya puntumalbum						
Whatcom	48.7596	122.4882	20 May 1977	1♀	Hand coll.	D. Manley

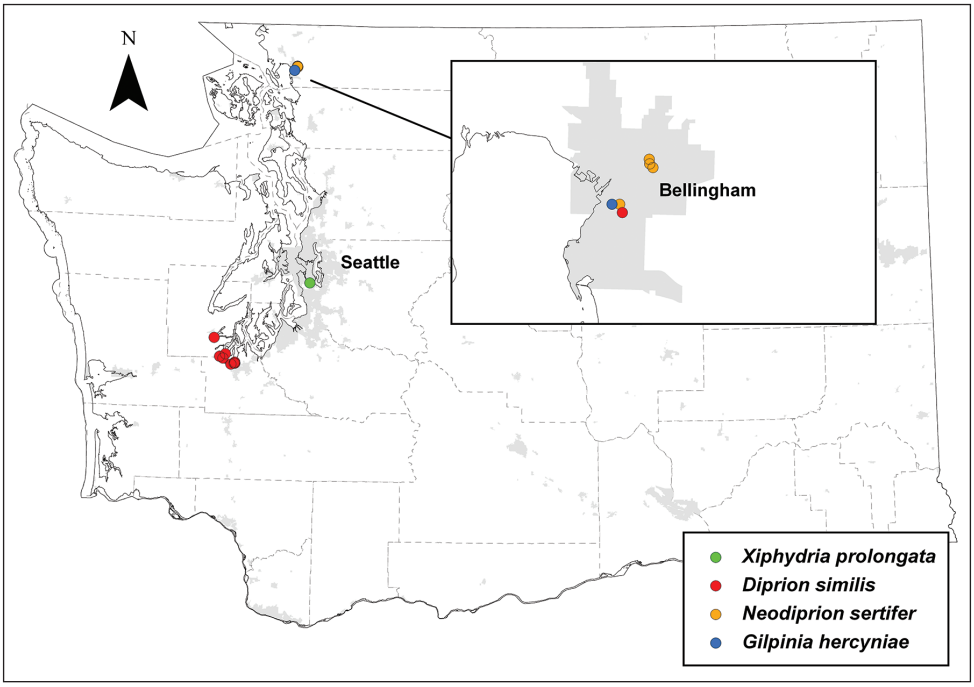

Figure 3. Localities of Xiphydriidae and Diprionidae newly detected in Washington State.

occasionally occur in production forestry, often in association with *D. pini* L., but it is typically not a serious pest (Taeger et al. 1998). The only *Diprion* recorded from North America (Taeger et al. 2010), *D. similis* was discovered in New Haven, Connecticut, in 1914, presumably introduced on imported European nursery stock or associated packing materials (Britton 1915). *Diprion similis* feeds upon multiple pine species, with marked oviposition preference for *P. strobus* observed in North America (Tsao and Hodson 1956). The species can potentially defoliate entire trees when populations are high; however, this appears to be rare in North America, perhaps due to control by weather events and introduced parasitoids (Wilson 1966, Van Driesche et al. 1996). It is known to occur from the northeastern states westward to the Great Lakes region, and south to North Carolina.

Specimens of *D. similis* were collected in 2012 when adult females emerged from *P. sylvestris* boughs collected in Shelton, Washington. Following this detection, yellow card traps were deployed in the south Puget Sound area and Whatcom County. Subsequent visual surveys for larvae were conducted in western Washington. The distinctive larvae are readily recognized, and were found at eight sites in three western Washington counties (Fig. 3). Larvae were most commonly found feeding on *P. monticola* Douglas ex D. Don, but also on *P. sylvestris* and *P. contorta* Douglas ex Loudon. A single male specimen was captured in a *Dipion pini* pheromone-baited trap, probably by chance

since the lure is not known to be attractive across species (O. Anderbrant, in litt.). Voucher specimens are deposited at WSDAC and WWUC.

Gilpinia hercyniae **(Hartig, 1837)**

Gilpinia comprises 37 described species native to Europe and Asia. *Gilpinia hercyniae* is a solitary spruce feeder, first detected in Ottawa, Ontario, Canada in 1922 and in New Hampshire in 1929 (Baker 1972). It quickly became a forest pest in the eastern United States and Canada (Balch 1939, Reeks and Barter 1951). Ambitious biological control programs during the 1930s imported and released several parasitoid species throughout the region. Concurrent with this, a nuclear polyhedrosis virus was inadvertently released which resulted in consistent region-wide control (Balch and Bird 1944). The species has not been an active management concern in North America for many decades (Nielon and Morris 1964, Kelleher and Hulme 1984). There are no published records of its occurrence west of Manitoba.

A specimen of *G. hercyniae* was collected in Bellingham in 2008 (although not identified until 2011) from *Picea abies* (L.) Karst. in a residential neighborhood. Wide-ranging visual and sticky-trap surveys in northwestern Washington failed to detect it beyond the original site, where more specimens were collected in 2011 and 2012 (Fig. 3). Specimens are housed at WWUC and WSDAC.

Tenthredinidae, Nematinae

Cladius grandis **(Serville, 1823)**

The earliest North American collections for this Palaearctic species are from Albany, New York, in 1887 (Smith 1974a). The species was presumably introduced separately to the west coast, with records from British Columbia in 1914 (Blackmore 1917). The specimens from British Columbia were first described as a new species, *Platycampus victoria* MacGillivray, 1920, reared from *Populus nigra* L. (MacGillivray 1920). In most previous literature, the species is known as *Trichiocampus viminalis* (Fallén, 1808). The most common host plants are *Populus* spp., although *Salix* (Benson 1958, Raizenne 1957) and *Alnus* P. Mill. (Smith 1974a) are also recorded. It has been reported as a minor pest of *Populus* L. in eastern North America and British Columbia (Béique 1961, Downes 1925). In Quebec, the species is bivoltine, with adults active in late May and early June, and again in late July through September (Béique 1961). There appears to be only one generation per year in British Columbia (Downes 1925). A single specimen from Washington was collected in a Malaise trap on the Evergreen State College campus in 1997 (Fig. 4), and detected while examining unidentified material in the college's natural history museum. The specimen is housed at WSDAC.

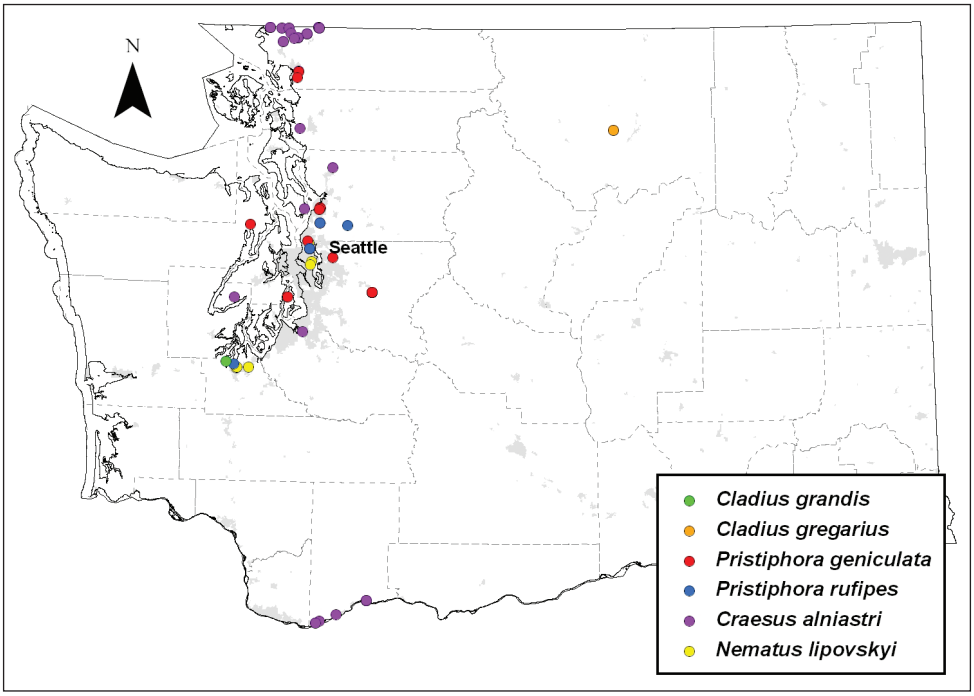

Fig 4. Localities of Nematinae (Tenthredinidae) newly detected in Washington State.

Cladius gregarius Dyar, 1895

This poplar-feeding species is native to North America, and is known from the northeastern United States and adjacent Canada, west to Michigan and Ontario (Smith 1974a, 1979a). Larvae feed on species of *Populus* in the spring, and adults can be found in late summer and fall. Two adult specimens were reared from larvae collected on *Populus tremuloides* Michx. in Okanogan County, Washington, 2010 (Figs 4–5). A visit to the same site in late summer 2013 failed to find more larvae, although similar feeding damage was seen on trees throughout the area. Specimens are housed at WSDAC.

Pristiphora geniculata (Hartig, 1840)

Pristiphora is a large, primarily Holarctic genus, although there are several described Neotropical and southern Asian species (Taeger et al. 2010). The genus is most diverse in Europe, with ca. 115 known species. Five European *Pristiphora* spp. introduced to North America are pestiferous, including *P. geniculata*. This species was first detected in the United States in Haines Falls, New York, and in Massachusetts in 1926, and now occurs throughout the northeastern states and provinces, and west to Minnesota and Ontario (Schaffner 1936, Smith 1979a). *Pristiphora geniculata*

Figure 5. *Cladius gregarius* larvae on poplar, Okanogan County WA.

feeds on *Sorbus aucuparia* L., *S. americana* Marshall, *S. decora* (Sarg.) C.K. Schneid., and the hybrid cultivar *Sorbaronia hybrida* (Moench) C.K. Schneid. (Forbes and Daviault 1964). Kunneman and Albers (1991) list *P. geniculata* as a pest of *Tilia* L., but this seems to be a misreporting of the "elm sawfly", *Cimbex americana* Leach, 1817 (Cimbicidae; Dirr 1983, Sinclair et al. 1987). Larvae are voracious feeders and can almost completely defoliate healthy trees; however, mortality of even repeatedly defoliated trees is infrequent (Forbes and Daviult 1964). Release and establishment of the ichneumonid *Olesicampe geniculatae* Quednau and Lim in the eastern states and provinces has resulted in diminished outbreaks of *P. geniculata* since the 1980s (Kelleher and Hulme 1984, Quednau 1990).

The species was first detected in Washington State in 2009, and is now common throughout the Puget Sound region (Fig. 4). Most specimens have been found via visual survey of defoliated trees, where larvae were readily apparent and subsequently reared. Several adults were reared from *Crataegus douglasii* Lindl. (a native hawthorn species) in 2011 and 2012, a new host record for the species (Fig. 6). The record in Washington probably represents a separate introduction event, either from its native range or a translocation from eastern North America. Specimens are housed at WSDAC.

Figure 6. *Pristiphora geniculata* larvae on *Crataegus douglasii*, Whatcom County, WA.

Pristiphora rufipes Serville, 1823

Pristiphora rufipes is native to central Europe, and spread to the United Kingdom in the mid-20th century (Benson 1947). It was first recorded in North America from Ottawa, Canada, in 1963 (MacNay 1964). The species is a significant pest of *Aquilegia* L. (Alford 2012). The species was previously known from the eastern states and provinces to the midwest. Most North American literature refers to the species by the synonym *Pristiphora aquilegiae* Snellen van Vollenhoven, 1866. It has been present in Washington since at least 1996, when Seattle-area gardeners began complaining of a new and voracious pest on columbine (Seattle Times 1996). Populations in Washington are currently known from Snohomish, King, and Thurston Counties (Fig. 4). The species has at least three generations per year in western Washington, with larvae present as late as November (Table 1). Specimens are housed at WSDAC.

Craesus alniastri (Scharfenberg, 1805)

Craesus alniastri is an alder-feeding nematine sawfly native to Europe, where it has been well-studied for its distinctive larval feeding aggregations (e.g., Boevé 1991). Buckle (1930) collected a single specimen of *C. alniastri* on parsnip flowers near Mt. Royal

(Montreal), Quebec, Canada, in 1926, and Kirby (1882) lists a single male specimen housed in the Natural History Museum, London, that was collected in Nova Scotia. Smith (1972) does not report any further specimens, and there appears to be no further literature discussing the species in North America. Prous et al. (2014) reassign species in this genus to *Nematus*.

Numerous specimens of *C. alniastri* were collected in western Washington while conducting surveys for alder-feeding sawflies throughout the Pacific Northwest in 2009–2011 (Fig. 4). Adult specimens were collected from May through August. Specimens were collected very near the British Columbia and Oregon borders, indicating that the species is likely widespread in the region. An additional female specimen collected near Seattle in 1976 is in the USNM; other voucher specimens are deposited at WSDAC.

Nematus lipovskyi **Smith, 1974**

This species was described from the eastern United States (Smith 1974b) and is known from Maine to Alabama, and west to Wisconsin. It is recently established in the Czech Republic (Macek and Šípek 2014). Larvae feed on deciduous azaleas in the *Pentanthera* subgenus and section of *Rhododendron* (e.g., *R. viscosum* (L.) Torr., *R. molle* (Blume) G. Don, *R. luteum* Sweet, and *R. calendulaceum* (Michx.) Torr.). Macek and Šípek (2014) also noted feeding damage on *R. obtusum* (Lindl.) Planch. inflorescences, but proposed that this was opportunistic feeding due to the proximity of more suitable host plants. This species is reassigned to *Euura* in Prous et al. (2014).

A 1996 specimen from Lacey, Washington, is the earliest record from the western USA. Larvae have been observed in several locations in King, Thurston, and Clark counties (Fig. 4). Adults were collected in Olympia, Washington, in April 2015, and are stored at WSDAC.

Tenthredinidae, Heterarthrinae

Heterarthrus nemoratus **(Fallén, 1808)**

Heterarthrus is a relatively small genus of leaf-mining sawflies native to Europe and Asia, which generally mine leaves of trees in Aceraceae, Betulaceae, and Salicaceae (Taeger et al. 2010). Two species have been introduced to North America, *H. nemoratus* and *H. vagans* (Fallén, 1808), which mine species of *Betula* L. and *Alnus*, respectively (Smith 1971, Taeger et al. 1998, Humble 2010). *Heterarthrus nemoratus* was first collected in North America in Pictou, Nova Scotia, in 1905 (Dowden 1941), and now has a transcontinental distribution in Canada (Digweed et al. 2009). Digweed et al. (2009) review the biology and provide detailed keys to mines, larvae, and adults of this and other birch-feeding Heterarthrinae in North America. The earliest known Wash-

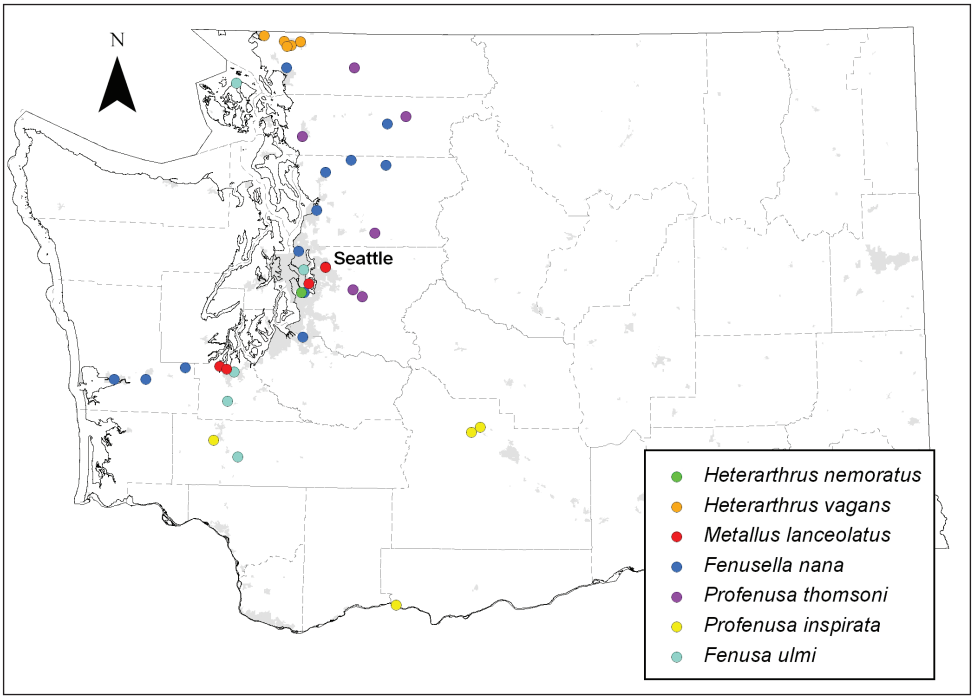

Figure 7. Localities of Heterarthrinae (Tenthredinidae) newly detected in Washington State.

ington specimen is a female collected in Whatcom County (the northern-most county in western Washington) in 1967, housed at WWUC. Specimens are also known from the Seattle area (Fig. 7), and the species is likely widespread in western Washington.

Heterarthrus vagans (Fallén, 1808)

Heterarthrus vagans was recently detected in North America, discovered in British Columbia in 2009 in numerous locations west of the Cascade Range and very close to the Washington State border (Humble 2010). Visual and sticky trap surveys in 2011 and 2012 detected the species at six sites in Whatcom County (Fig. 7). We have not yet found the species south of Whatcom County, but the widespread distribution of alder makes it likely that this species will continue to spread through Washington and into Oregon. This species' mines are identifiable by the round cocoon formed in the leaves of its host plants; other *Heterarthrus* spp. pupate outside of leaves (Humble 2010). Specimens are housed at WSDAC.

Metallus lanceolatus (Thomson, 1870)

Metallus lanceolatus (referenced as *M. gei* (Brishcke, 1883) in much of the European literature) is a leaf-miner of *Geum* L. The earliest North American specimens of this Palaearctic species were collected in British Columbia in 1933, and multiple specimens were collected

in the northeastern USA and Canada in the 1960s (Smith 1971). Smith (1971) described *M. bensoni* as a new species from the New York and British Columbia specimens; Koch (1989) subsequently recognized *M. bensoni* and *M. gei* as junior synonyms of the Palaearctic *M. lanceolatus*. *Metallus lanceolatus* forms coalescing blotch-mines in ornamental and wild *Geum*, and can be a pest of garden plants (Buhr 1941, Hoebeke and Wheeler 2005). It has apparently spread through southern Puget Sound (Fig. 7), where it was found attacking *Geum macrophyllum* Willd. in cultivated and wild conditions. Specimens have been hand-collected and captured in Malaise traps. The earliest mines in a large patch of *G. macrophyllum* near Olympia, Washington, were visible by early July in 2012 and 2013, and could be found on nearly every plant by mid-July of both years. Specimens are housed at WSDAC.

Fenusella nana (Klug, 1816)

This Palaearctic species is commonly recorded in the literature as *Messa nana* (see Taeger & Blank 1998 for nomenclatural discussion). *Fenusella nana* larvae form coalescing blotch mines in many birch species (e.g., Buhr 1941, Taeger et al. 1998, Digweed et al. 2009). The first North American records for this leafminer are from Maine in 1966 (Smith 1967). It has since spread across Canada and into Washington State (Digweed et al. 2009). Digweed et al. (2009) review its biology and provide detailed keys to mines, larvae, and adults of this and other birch-feeding Heterarthrinae in North America. This species has been collected as far southwest as Hoquiam, Grays Harbor County (Fig. 7). Voucher specimens are housed at the NRFC.

Profenusa thomsoni (Konow, 1886)

The first North American records for this birch leafminer are from Hamden, Connecticut in 1926. It was known from Maine, Ontario, Quebec, and Vermont by the 1960s (Smith 1971), and has since spread across Canada and into Alaska, the Yukon Territory, and Northwest Territories (Digweed and Langor 2004, Digweed et al. 2009, MacQuarrie et al. 2013). It is a significant pest in urban forests (Martin 1960, Drouin and Wong 1984, Snyder et al. 2007). Digweed et al. (2009) review its biology and provide detailed keys to mines, larvae, and adults of this and other birch-feeding Heterarthrinae in North America. The earliest Washington specimen was collected in 1967, in Whatcom County, and was discovered by examining specimens housed at WWUC. Numerous specimens have also been collected south to King County (Fig. 7) and are housed at NRFC.

Profenusa inspirata (MacGillivray, 1909)

Profenusa inspirata, a native North American species, is the only known sawfly leafminer of oaks in western North America. It creates blotch mines in the upper surface of oak leaves (Fig. 8), that can coalesce when multiple larvae are present in a leaf. The

Figure 8. *Profenusa inspirata* mines on *Quercus garryana*, Yakima County, Washington.

species was previously known from Nevada, California, and Oregon (Smith 1971). Multiple mines were observed in Garry oak (*Quercus garryana* Douglas ex Hook.) along the Tieton River, Yakima County, and in Skamania and Lewis counties in 2015 (Fig. 7). Larvae were identified using characters described in Smith (1971). Voucher specimens are at WSDAC.

Fenusa ulmi Sundevall, 1847

This Palaearctic elm leafminer was already well established in New York by 1898 (Felt 1898), and was certainly introduced with elms from Europe. It is known to attack several elm species, especially *Ulmus glabra* Huds. and its hybrids (Slingerland 1905, Liston 1993, Scannell 2000); Scannell (2000) added *U. pumilla* L. and *U. davidiana*

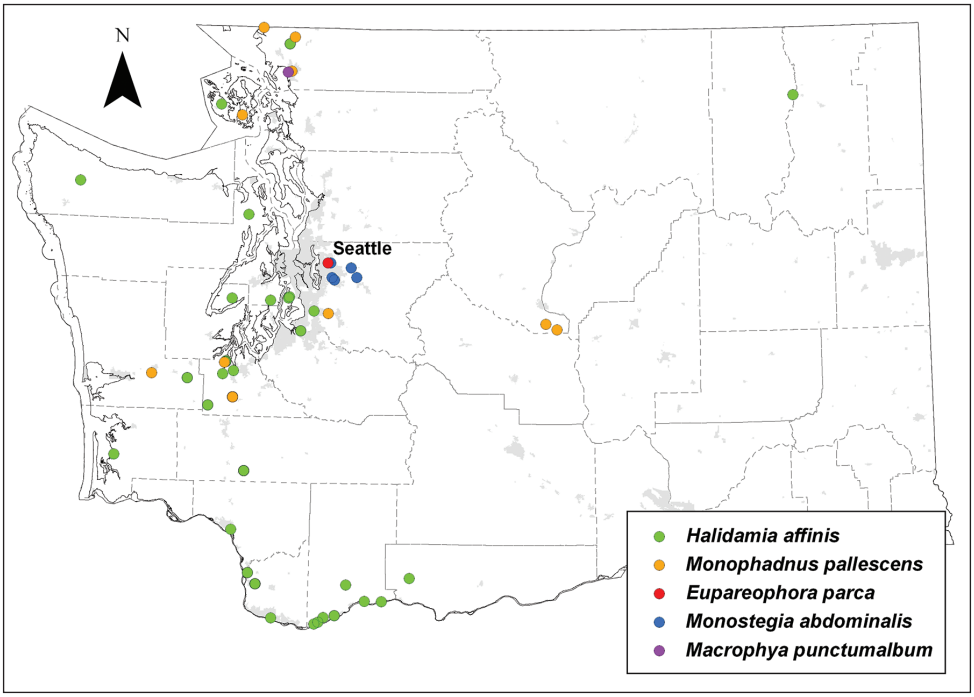

Figure 9. Localities of Blennocampinae, Allantinae, and Tenthredininae (Tenthredinidae) newly detected in Washington State.

Planch. to the known elm hosts. Early records of significant damage to *U. americana* L. seem to be unsubstantiated, with most authors reporting that larvae are unable to do more than initiate feeding (Slingerland 1905, Guries and Smalley 1994, Scannell 2000). Scannell (2000) provided detailed information on the life history and host preferences of this species in the Seattle area. The oldest known west coast specimens were collected in British Columbia in 1947, and are stored in the Canadian National Collection (H. Goulet, pers. comm.). There is anecdotal evidence that the species has been present in Washington since the early 1990s (Scannell 2000), and has since spread south through the Puget Sound area (Fig. 7). Washington State voucher specimens are at WSDAC.

Tenthredinidae, Blennocampinae

Halidamia affinis (Fallén, 1807)

This is an introduced European species that feeds on *Galium* L (Smith 1969, Taeger et al. 1998). The earliest North America records are 1931 from Cold Spring Harbor, New York, 1933 from Connecticut, and 1934 from New Jersey (Smith 1969). It has since spread to California and the Pacific Northwest. The species is common and was col-

lected as larvae from host plants and readily on yellow sticky traps and in Malaise traps in nearly every county surveyed (Fig. 9). Voucher specimens are at WSDAC.

Monophadnus pallescens (Gmelin, 1790)

A *Ranunculus*-feeding European introduction, this species has been present in the eastern United States and Canada since the late 1800s and in British Columbia since at least 1919 (Smith 1969). This species is widespread (Fig. 9), and was collected with sticky card traps, Malaise traps, and by hand. The earliest specimens discovered from Washington were collected in Whatcom County in the late 1960s, and were found in the material at WWUC. Numerous species of *Ranunculus* L. provide suitable hosts in western Washington, including the widespread invasive European weed *Ranunculus repens* L. Voucher specimens are housed at WWUC and WSDAC.

Eupareophora parca (Cresson, 1880)

The genus *Eupareophora* contains three species, with only *E. parca* native to North America. It is known from most of the northeastern and central states, and north and west to Alberta (Williams 2007). The species is not known from the Rocky Mountains, but has been collected from several northern California locations and Oregon (Smith 1969). The single historical specimen from Oregon was collected by Koebele, who collected in and around California in the 1880s. Most collection records for this western disjunct population are from California (Smith 1969), suggesting the species may have been native to eastern North America and spread west via human commerce. Williams (2007) similarly suggests that the species was not present in Alberta until the early 2000s. Several *Fraxinus* L. species are recorded hosts, as well as *Carya illinoinensis* (Wangenh.) K. Koch (Smith 1969) and *Chionanthus* L. (Dyar 1898), although Smith (1969) described the latter association as "dubious". Larvae are readily recognizable by the bristly appearance caused by numerous thick, bifurcate spines. Williams (2007) presented a very detailed account of larval and adult biology in Alberta. Heavy ash defoliation and shed skins of an unknown sawfly were noticed at a public park on the border of King and Snohomish counties in 2014, but no living animals were observed. A return visit in May 2015 found the easily identifiable larvae feeding on *Fraxinus latifolia* Benth. throughout the park (Fig. 9). Voucher specimens are at WSDAC.

Tenthredinidae, Allantinae

Monostegia abdominalis (Fabricius. 1798)

Monostegia abdominalis is a European sawfly pestiferous on *Glaux* L., *Lysimachia* L. and *Anagallis* L. (Price 1970, Taeger et al. 1998). It was first collected in North America

in Massachusetts in 1899, and described as *M. martini* MacGillivray, 1908 (Smith 1979b). In late summer of 2013, noxious weed management staff in King County noticed heavy defoliation of populations of *Lysimachia vulgaris* L., regulated as a noxious weed in Washington State, by unknown sawfly larvae. Larvae were subsequently collected and reared in the lab on a *Lysimachia* cultivar in 2014. Specimens are known from several *Lysimachia* infestations in western Washington (Fig. 9). Voucher specimens are at WSDAC.

Tenthredinidae, Tenthredininae

***Macrophya punctumalbum* (Linnaeus, 1767)**

This adventive European species was first recorded in North America from Toronto, Ontario, in 1932, from British Columbia in 1934 (Gibson 1980), and later from New York (Hoebeke and Johnson 1985). Larvae feed on *Ligustrum* L., *Syringa* L., and *Fraxinus* (Gibson 1980). A specimen collected in 1977 in Bellingham, Washington, and housed at WWUC is the only record from Washington State (Fig. 9).

Discussion

In addition to expanding regional knowledge of an ecologically interesting and economically important group of insects, these data highlight the continual intra-continental spread of introduced species. Eighteen of the 22 sawflies reported here represent range expansions for exotic sawflies introduced to North America long ago. Most of these species were first recorded from eastern states or provinces, likely introduced with nursery stock. Five species may have been first or simultaneously introduced on the west coast based on their historical detection data - *Cladius grandis*, *Heterarthrus vagans*, *Metallus lanceolatus*, *Monophadnus pallescens*, and *Macrophya punctumalbum*. It is unknown how most of the introduced species became established in the west. Certainly, commerce from eastern North America could explain the distribution of some species. For some pests of common cultivated plants, such as *Pristiphora rufipes* and *Neodiprion sertifer*, it seems probable that insects were transported inadvertently with nursery trade or by home gardeners, although natural dispersal by adults can not be discounted.

The movement of other species, such as *Halidamia affinis* and *Monostegia abdominalis*, is more mysterious – *Galium* is not commonly cultivated, and *Lysimachia vulgaris* is a noxious weed. *Halidamia affinis* has likely spread of its own accord, expanding through the immense range of its host plant, *Galium*. *Monostegia* may have moved with other cultivars of *Lysimachia* that are commercially sold, although one would expect that such voracious and notable sawfly larvae on garden plants would have been reported. The reports of previously more southern species, such as *Profenusa inspirata* and *Eupareophora parca* could indicate northward range expansion concurrent with in-

creasingly moderate winters. *Profenusa inspirata* in particular seems suggestive of such new expansion, since Garry oak conservation and ecology have long been studied in the Puget Sound region. However, it is certainly possible that *P. inspirata* has been present but undetected in Washington for decades.

The older specimens recorded here from collections made decades ago emphasize the value of institutional insect collections as repositories of valuable biodiversity information. It is noteworthy that for several of the species discussed herein, the first records for the state were found in the holdings of the insect collections at regional universities, providing evidence that many species had been transported west earlier than was previously known, or were perhaps derived from separate introduction events. Thus, despite the relatively small size of such collections compared to those at land grant universities, these collections fill a valuable role in documenting shifts in regional species composition. As a whole, the data from all museum specimens examined during this research also demonstrate gaps in our regional knowledge of sawflies. Unsurprisingly given remoteness and lack of access, the arid Columbia Basin and rugged mountain ranges in Washington are less frequently collected than other parts of the state (Fig. 2). Undoubtedly, other sawfly species occur in Washington that as yet are undetected and perhaps limited to these under-surveyed habitats. For example, the juniper-feeding genus *Susana* Rohwer and Middleton, 1932 occurs in states and provinces surrounding Washington but has not been recorded from the state.

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