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Revision of the Rhopalomutillinae (Hymenoptera, Mutillidae): 1, generic review with descriptions of three new genera

Denis J. Brothers[1]

1 *School of Life Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville (Pietermaritzburg), South Africa*

Corresponding author: *Denis J. Brothers* (brothers@ukzn.ac.za)

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Abstract

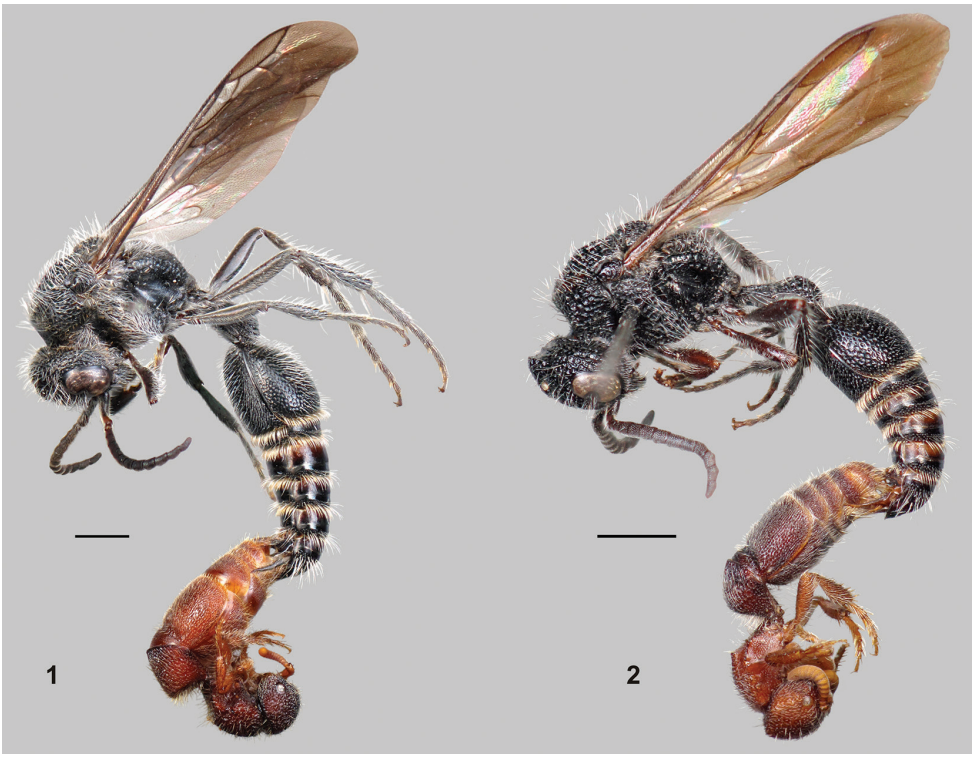
The generic classification of the species of Rhopalomutillinae is reviewed and shown to comprise four distinct genera, three of which are described as new. All genera are known from both sexes and can be distinguished primarily on the basis of differences in the male genitalia and the female mesosomal form. A key is provided to the genera (*Rimulotilla* Brothers, **gen. n.**; *Pherotilla* Brothers, **gen. n.**; *Rhopalomutilla* André, 1901; *Bischoffiella* Brothers & Nonveiller, **gen. n.**) and they are each characterised and discussed. The already-described species are placed in the appropriate genera and the new combinations indicated: *Ri. basalis* (Bischoff, 1920), **stat. n., comb. n.**; *Ri. conifera* (Bischoff, 1920), **comb. n.**; *Ri. tongaana* (Péringuey, 1909), **comb. n.**; *P. japhia* (Cameron, 1902), **comb. n.**; *P. mlanjeana* (Bischoff, 1920), **comb. n.**; *P. oceanica* (Mickel, 1935), **comb. n.**; *P. rufitincta* (Hammer, 1957), **stat. n.**, **comb. n.**; *P. striganovae* (Lelej, 2012), **comb. n.**; *B. cristata* (Bingham, 1912), **comb. n.** In addition, *Rh. javana* Pagden, 1949, **syn. n.**, is synonymized with *P. oceanica* (Mickel, 1935).

Keywords

Afrotropical, *Bischoffiella*, mutillid wasps, Oriental, *Pherotilla*, *Rhopalomutilla*, *Rimulotilla*

Introduction

The mutillid subfamily Rhopalomutillinae Schuster, 1949 is a relatively small taxon (about 40 species, including those as yet undescribed) distributed in the Afrotropical and Oriental Regions. Both sexes are very characteristic and unusual morphologically (see below). They exhibit true phoretic copulation, in which the male carries the female in flight while mating (Figs 1, 2) and visiting flowers, the only mutillids to do so apart from a few Myrmosinae (Brothers 1989); as a consequence females and males are collected together and most species are known from both sexes (Brothers in prep.), a highly unusual circumstance for the family. The females have almost never been collected in isolation, and it seems likely that they spend most of their adult life underground; this is supported by their relative scarcity in collections and their morphological similarity to those of species of the Australian genus *Ponerotilla* Brothers, 1994 which have been collected in the underground nests of ants (Brothers 1989, 1994). Although many mutillid species show size differences between the sexes or even within sexes depending on the size of the host, such differences are extreme in the Rhopalomutillinae (Brothers 1989); the maximum and minimum sizes (body lengths in mm) that I have recorded for specimens of *Rhopalomutilla anguliceps* (André, 1897) are 13.7 and 5.9 for males (n=210), and 6.4 and 3.8 for



Figures 1–2. Rhopalomutillinae, mating pairs, male above. **1** *Pherotilla rufitincta* (Hammer) **2** *Bischoffiella cristata* (Bingham). Scales = 1 mm.

females (n = 18) and I have seen no indication that large males preferentially mate with large females (or vice versa). No information exists about host relationships, but the variability and difference in size of both sexes and the probably subterranean existence of the females, has suggested that they may parasitize ants (Brothers 1989).

A species revision of the Rhopalomutillinae as a whole has long been in progress, initially with the collaboration of the late Guido Nonveiller (Zemun, Serbia). It has been delayed by many factors, not the least the initial paucity of specimens in collections, a circumstance which has more recently been remedied by extensive malaise-trap sampling programmes. However, inclusion of a realistic sampling of rhopalomutilline diversity in another project, an extensive morphological re-analysis of mutillid higher classification based on exemplars of both sexes for about 100 genera and subgenera in collaboration with Arkady Lelej (Vladivostok, Russia), has required the description of the new genera of Rhopalomutillinae at this time. The descriptions which follow include information derived from examination of many as-yet undescribed taxa and undescribed females of known taxa, which will formally be described in subsequent papers.

Materials and methods

Specimens of Rhopalomutillinae were kindly loaned by the curators of many institutions around the world (detailed acknowledgment of the relevant repositories will be done in subsequent papers) and have been examined using standard morphological methods. Photographs were taken with a Canon Powershot G10 digital camera adapted to a Wild M400 photomicroscope using a Clearshot 600 adapter kit (Alexis Scientific) and stacked using CombineZP software (Hadley 2010). Further image processing was done using CorelDRAW X4 and Corel PHOTO-PAINT X4. Drawings were made using a drawing-tube attachment on a Wild M7 stereomicroscope, inked on card or provided with texture using a black wax pencil on a textured board. Abbreviations are: F = flagellomere; S = sternum; T = tergum. In females the maxillo-labial complex is much reduced and the palps are normally concealed; examination of their structure usually necessitated relaxation of specimens and dissection, but the results were sometimes inconclusive because of their extremely small size, so that it is possible that palps may have been lost during dissection and even determination of segmentation was often uncertain.

Systematics

Rhopalomutillinae Schuster, 1949

Rhopalomutillinae Schuster, 1949: 121, 123, 125; Brothers 1975: 623, 1999: 244; Lelej and Nemkov 1997: 12.

Type genus. *Rhopalomutilla* André, 1901: 323, male & female.

Diagnosis. MALE. Fully winged; black (seldom with the mesosoma partly dark red), without bright pubescent patterns. *Head*: compound eye oval, inner margin deeply emarginate; antennal scrobe with dorsal carina and secretory tubercle (sometimes without both); postmandibular carina forming a simple blunt ridge; oral and mandibular fossae separated by anteriorly unfused depressed bridge; antennal scape without longitudinal carinae; mandible with oblique ventral lamellate expansion basally; maxillary and labial palps six- and four-segmented respectively. *Mesosoma*: pronotum with posterodorsal margin strongly concave; mesoscutum with notaulus present but anteriorly incomplete; parapsidal line/furrow evident but incomplete posteriorly (seldom complete); axilla simply rounded posteriorly; tegula entirely convex (sometimes weakly recurved posteriorly), scarcely elongate and reaching level of trans-scutal articulation; propodeal disc with three large fields delimited by strong longitudinal ridges, lateral margin carinate, disc and declivity abruptly differentiated (seldom distinct but merging); metasternal posterior median process shorter than coxal height, unidentate. *Wings*: fore wing with elongate broad pterostigma completely sclerotized and veins C and SC+R interrupted at its base, marginal cell with acute apex, third abscissa of vein RS without bulla, second submarginal cell broadly sessile anteriorly, crossvein *3r-m* without bulla; hind wing with crossvein *r-m* proximal and complete. *Legs*: each tarsomere 4 with a small oval median pulvillus posteroventrally; tarsal claw with basal lamella separated from acute apex by a deep cleft (lamella rarely highly reduced and apparently absent); fore tibial calcar with linear narrow blade with margin entire; hind coxa with small carinate tubercle dorsally; hind tibia without any apparent preapical secretory structure. *Metasoma*: first segment moderately petiolate, T1 gradually broadened posteriorly and about 0.5 × as wide and > 0.5 × as long as T2 (rarely slightly shorter than this), apically constricted; second metasomal segment without evident felt lines; T2–T6 and S2–S5 (sometimes S6 also) with apical fringes of moderate to strong semi-recumbent setae, but sparser and weaker on sterna and posteriad; T3 with large mediobasal stridulitrum; S7 short and concealed; hypopygium (S8) medially emarginate posteriorly (and usually with prominent process lateroventrally). *Genitalia*: paramere with inner basodorsal margin evenly curved, without parapenial lobe; volsellar digitus absent; penis valve with ventral tooth much reduced to weak rounded swelling distant from rounded apex; well developed eversible endophallus between penis valves.

FEMALE. Apterous; medium to dark brown without any bright patterns. *Head*: rounded, with oval to subcircular compound eye very small and flattened; antennal scrobe without any carina above; antennal tubercles with median transverse carina at base; postmandibular carina forming short blunt ridge; oral and mandibular fossae separated by anteriorly unfused depressed bridge; antenna clavate, short and stout, scape somewhat flattened and twisted with apex hooded over base of pedicel, pedicel and flagellomeres much wider than long; maxillary and labial palps each with two segments at most. *Mesosoma*: pronotum about as long as distance between pronotal and propodeal spiracle; metasternum with posterior median process longer than coxal height, acutely unidentate. *Legs*: short with laterally flattened tibiae and ventrally flattened or concave femora; without a pulvillus posteroventrally on any tarsomere; tarsal claws simple,

smoothly concave below; fore tibia with distinct obliquely oval to circular preapical pore on inner (anterior) surface (rarely without), calcar with linear narrow blade; fore basal tarsomere strongly curved, second to fourth tarsomeres short and broadly depressed; hind coxa with small carinate tubercle dorsally; hind tibia smooth and shining on inner (posterior) surface, without any apparent preapical secretory structure. *Metasoma*: no distinct posterior fringes on any segments; anterior and dorsal faces of T1 distinct but merging; T1 more or less parallel-sided posteriorly and almost half length of T2 or longer, almost as wide as T2 (sometimes much narrower); second segment without distinct felt lines; T3 without mediobasal stridulitrum; T6 with differentiated pygidial plate.

Comments. In the phylogenetic analyses of Brothers (1975, 1999) and also of Lelej and Nemkov (1997), the subfamily Rhopalomutillinae appears as originating fairly near the base of the mutillid tree, although its relationships with the "higher" subfamilies differ; at that time it had not been realised that several genera should be recognised, and it was effectively treated as monotypic. The current paper provides the basis for recognition of the four component genera and thus enables proper account to be taken of the diversity encountered in the group. A key to the genera is thus provided, for both sexes, and each genus is then characterised and discussed. It should be noted that where both sexes of a described species are indicated below as being known, both have often not yet been described; this will be done in subsequent papers.

Key to genera of Rhopalomutillinae

1 Male; macropterous 2
– Female; apterous **5**
2 Scutellum strongly protuberant, conical, with a short transverse carina apically; mandible apically bidentate; hypopygium (S8) weakly convex to flat, but with apparent posterior margin deeply incised medially to form a narrow notch; S6 (S7 concealed) with straight posterior margin..... ***Rimulotilla*** **Brothers, gen. n.**
– Scutellum pulvinate, not markedly protuberant, with simple apex; mandible apically tridentate; hypopygium with a prominent ventrally oriented process on each side, posterior margin shallowly excised to form a broad emargination; S6 (S7 concealed) with posterior margin deeply notched on each side, engaging processes of hypopygium..... **3**
3 Genital paramere simple, without a cluster of highly differentiated stout setae arising from beneath a dorsal lamelliform lobe, at most a few slender heavier setae present basodorsally (genal carina absent)... ***Pherotilla*** **Brothers, gen. n.**
– Genital paramere with an obvious cluster of highly differentiated very stout setae arising from beneath a dorsal lamelliform lobe and oriented posteromesally (genal carina present or absent) **4**
4 Penis valve with a strong dentate or lamellate projection on outer surface at about midlength ..... ***Bischoffiella*** **Brothers & Nonveiller, gen. n.**
– Penis valve with a smooth outer surface, evenly curved in dorsal view..... .....***Rhopalomutilla*** **André**

5 Mesosoma squat with dorsal face about as long as wide, dorsolateral margins posterior to propodeal spiracles often concave, disregarding posterolateral tooth, and usually diverging posteriorly (never converging) **6**

– Mesosoma elongate with dorsal face at least 1.3 × as long as wide, dorsolateral margins posterior to propodeal spiracles more or less straight, disregarding posterolateral tooth, and usually converging posteriorly (never diverging)... **7**

6 Mesosoma posteriorly with elevated broad sculptured median longitudinal ridge, strongly depressed posteriorly on either side of ridge, depressed areas smoothly merging with propodeal declivity; mesosoma narrower just posterior to propodeal spiracles than just anterior to spiracles .... ***Bischoffiella*** **Brothers & Nonveiller, gen. n.**

– Mesosoma more or less evenly convex dorsally, without any median elevated ridge or strong lateral depression posteriorly, propodeal declivity at a distinct angle to dorsal surface; mesosoma narrower just anterior to propodeal spiracles than just posterior to spiracles .....***Pherotilla*** **Brothers, gen. n.**

7 Posterodorsal margin of mesosoma with strong transverse scutellar scale; head slightly longer than wide, more or less parallel-sided; pygidium with apical margin deeply emarginate between a pair of strong apical spines..... ..... ***Rimulotilla*** **Brothers, gen. n.**

– Posterodorsal margin of mesosoma simple, without any distinct scutellar scale (rarely indicated as a slight acute tubercle); head more or less rounded, about as long as wide; pygidium with simple convex or straight apical margin..... .....***Rhopalomutilla*** **André**

Rimulotilla **Brothers, gen. n.**
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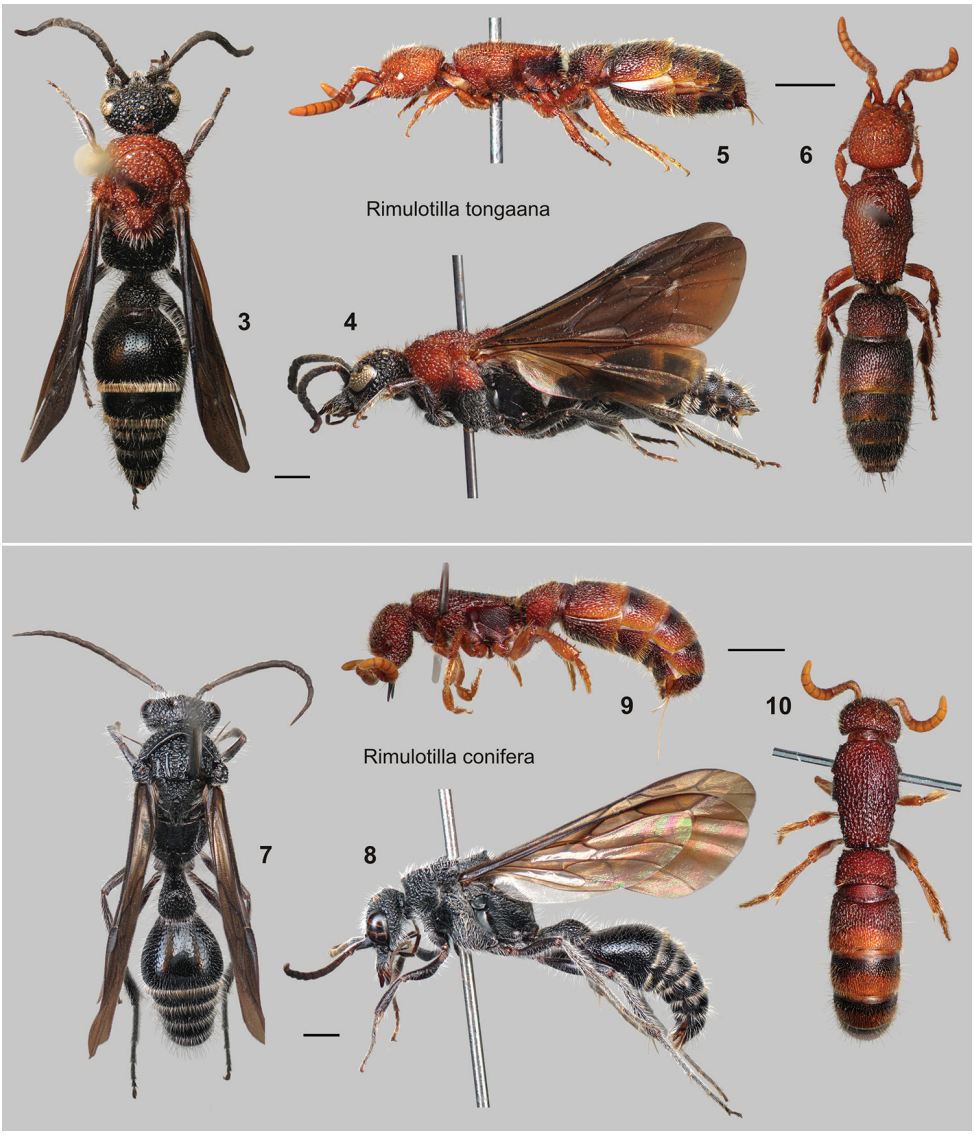
Figs 3–10, 31–32, 39–40

Type species. *Mutilla* (*Rhopalomutilla*) *tongaana* Péringuey, 1909: 386, male

Diagnosis. MALE. Head strongly transverse with vertex dorsally produced as an angle behind ocelli in anterior view; no genal carina; mandible bidentate; scutellum almost conical with strong transverse apical tubercle; S6 with posterior margin simple; S8 weakly and evenly convex, slightly elevated posteriorly with deep median notch in posterior margin; penis valves symmetrical, each almost triangular with many long setae along posterodorsal margin; paramere without any stronger setae. FEMALE. Head slightly longer than wide in anterodorsal view; mesosoma elongate with abrupt concave narrowing between metathoracic spiracle and propodeal spiracle, lateral margins of propodeum converging posteriad; disc of propodeum posteriorly with strong transverse median tubercle (scutellar scale) and small tooth at lateral angle; metasoma strongly elongate with T1 broad and long; pygidial plate broad and poorly defined, posterior margin deeply concave between strong apical teeth.



Figures 3–10. *Rimulotilla* gen. n. **3–6** *Ri. tongaana* (Péringuey) **3–4** male, dorsal & lateral views **5–6** female, lateral & dorsal views **7–10** *Ri. conifera* (Bischoff) **7–8** male, dorsal & lateral views **9–10** female, lateral & dorsal views. Scales = 1 mm.

Description. MALE. Body black (sometimes with mesosoma partly dark red), wings entirely infuscated (sometimes hyaline basally). *Head*: vertex more or less evenly rounded laterally but strongly elevated behind ocelli, posterodorsal margin of head in anterior view more or less straight on each side but forming a marked angle mesally; antennal scrobe above with diagonal transverse carina and secretory tuber-

cle; clypeus with sculptured median area, inner marginal teeth stronger than lateral teeth; genal carina absent (sometimes an irregular slight vertical ridge present), postgena slightly convex; postmandibular carina present, strong to weak; pleurostomal carina forming a ridge from posterior mandibular articulation to margin of oral fossa much anterior to half its length; mandible apically bidentate, subapical tooth smaller than apical tooth, ventral basal lamella scarcely developed. *Mesosoma*: mesoscutum with anteroadmedian lines weakly developed; notaulus incomplete anteriorly; parapsidal line incomplete posteriorly or complete; tegula convex, densely punctate; scutellum strongly convex, tapering posteriorly, with very strong elevated transverse tubercle; metanotal dorsellum with sides irregular but more or less straight, sculpture variable; propodeum with disc and declivity abruptly differentiated; lateral face of pronotum not strongly tapered, anteroventral margin carinate and continuous with anteroventral tooth; mesepisternum with transverse depression well developed and extending diagonally from scrobe towards ventral extremity of pronotum; mesosternum without any distinct projections. *Legs*: pubescence denser on tibiae and tarsi; claws deeply cleft apical to broad lamellate base; fore tibia without any apical secretory structure or with a vertically elongate preapical groove/pore on inner (anterior) surface; mid and hind tibiae with few inconspicuous preapical dorsal spines, and fewer very inconspicuous lateral spines (seldom absent). *Metasoma*: T2 widest posterior to midlength; T2 and S2 without any traces of lateral felt lines; pygidium (T7) with apical margin convex, edges slightly recurved (seldom not); S1 with a median tooth near base; S6 with posterior margin entire, with sparse apical fringe; hypopygium (S8) weakly convex, forming a slightly elevated lamelliform plate with deep median apical notch overlying true posterior margin, without any separate ventrally-projecting lateral lobes. *Genitalia*: basal ring very short; paramere almost straight with fairly broad apex, densely and finely setose on outer surface, without any differentiated strong setae; penis valves symmetrical, short, triangular, with a row of long fairly strong setae along posterodorsal margin.

FEMALE. *Head*: slightly elongate in anterodorsal view; sides behind eyes evenly and weakly convex, produced far beyond eye, well differentiated from posterodorsal margin of head; posterodorsal margin convex, without any distinct oblique depression at each side; clypeus with median lamellate tubercle dorsally, a median tubercle above obtusely triangular ventral concavity, a tooth at each side of concavity; gena broad, genal carina weak or absent; postmandibular carina weak and long or strong and short, separated from postgenal carina; pleurostomal carina forming a ridge from posterior mandibular articulation to margin of oral fossa; mandible more or less straight and evenly tapering distally, apically unidentate; maxillary palp unsegmented, cylindrical; labial palp two- or unsegmented, cylindrical; antennal scape with or without a weak blunt tubercle posterolaterally, flattened anteromesal surface not delimited by any carinae; pedicel without any distinct tuft of fine setae. *Mesosoma*: elongate, very distinctly longer than wide; anterodorsal margin distinct with short anterior face; humeral angle blunt; lateral margin fairly even and smooth, anteriorly

gently rounded and weakly convex, very abruptly and strongly converging and concave to base of propodeum then almost straight to posterolateral angle with short tooth; disc of propodeum posteriorly with small tubercles on each side of strong smooth tubercle (scutellar scale) slightly overhanging margin; posterior face of propodeum nearly vertical; lateral face of pronotum with anterior oblique carina absent or scarcely indicated, a straight carina running along anteroventral margin, ventral margin straight, anteroventral extremity blunt; mesepisternum weakly and evenly convex. *Legs*: fore leg with femur flattened below, tibia with prominent preapical oval to arcuate secretory pore on inner (anterior) surface, tibial calcar with blade finely pectinate on margin; mid and hind femora longitudinally concave below, each with a narrow elongate preapical lamella anteroventrally; mid and hind tibiae with preapical dorsal spines strong and fairly easy to distinguish, preapical lateral spines fairly weak and moderately difficult to distinguish. *Metasoma*: slender; T1 with anterior face meeting dorsal face at a rounded right angle, dorsal face long, broad and somewhat transverse, almost as wide as T2, sides weakly convex and weakly diverging from base; T2 < 0.75 × length T3–T6, with broad deep basal depression weakly convex posteriorly, sides beyond basal depression diverging then weakly convex and scarcely converging posteriorly, no trace of felt-line patch, posterior margin weakly concave to straight; T3 with posterior margin straight; T5 without any lateral tuft of long setae; pygidium (T6) with pygidial plate broad, with an irregular lateral bounding ridge basally, apical margin forming a semicircular concavity between two strong acute teeth; S1 with a short simple median carina anterior to a broad flat triangular area elevated anteriorly and becoming somewhat depressed posteriorly; S2–S4 with simple posterior margins; S5 with posterior margin lobed (sometimes tuberculate) on each side, with a posterolateral cluster of denser setae (sometimes without); S6 with apex acute, sides carinate, no flattened strong setae.

Species included. *Rimulotilla basalis* (Bischoff, 1920), male & female?, stat. n., comb. n.; *Ri. conifera* (Bischoff, 1920), male & female?, comb. n.; *Ri. tongaana* (Péringuey, 1909), male & female, comb. n.; two undescribed species, one male only, the other male & female.

Distribution. Central, eastern and southern Africa (Burundi, Democratic Republic of Congo, Kenya, Malawi, Mozambique, Namibia, South Africa, Tanzania, Zambia, Zimbabwe).

Etymology. From the Latin noun *rimula*, a small cleft (referring to the form of S8), combined with *-tilla*, a common suffix derived from the genus *Mutilla*; gender feminine.

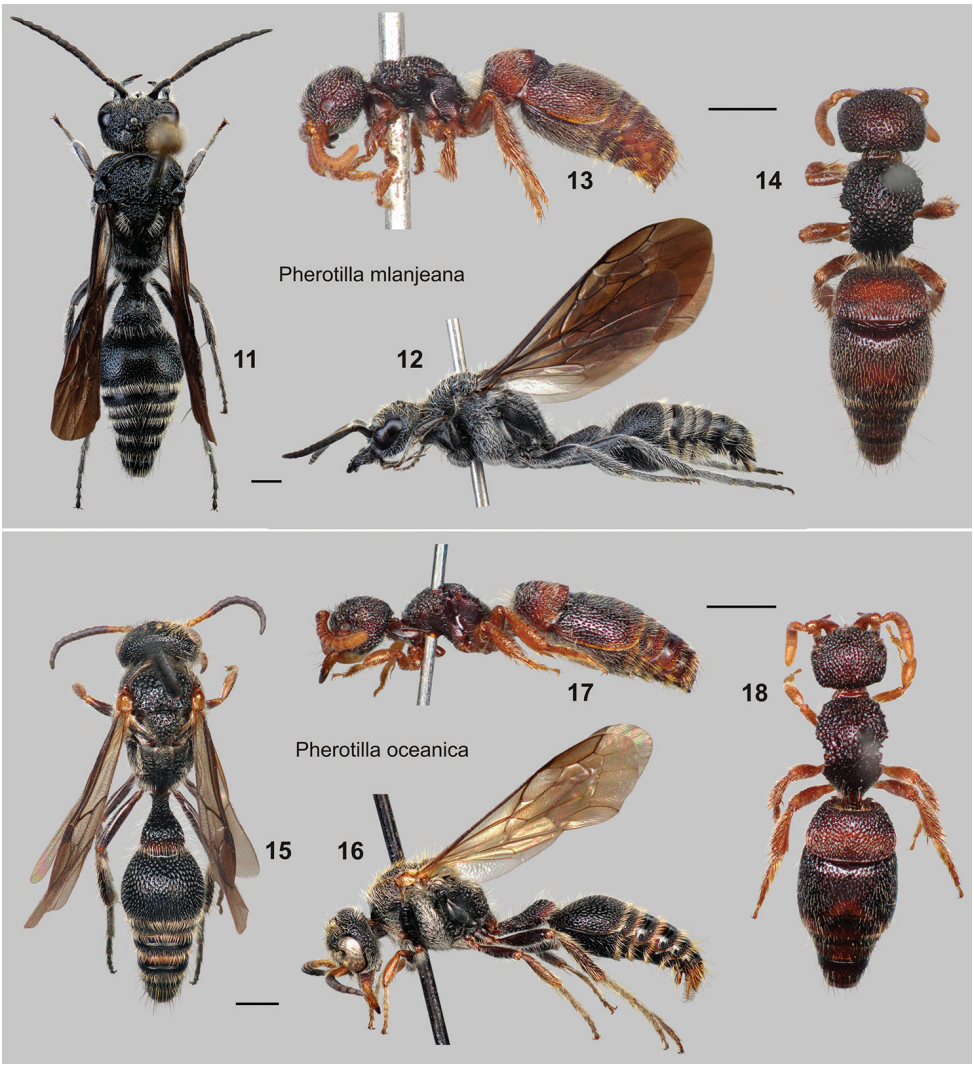
Comments. Phoretic copulation in this genus probably has a shorter duration than in the other genera of Rhopalomutillinae since there are few recorded copulating pairs in collections and the apical sterna of the males are less modified than in the other genera. The only species for which I have seen directly associated male and female specimens (and actually collected a mating pair myself) is *Ri. tongaana*, hence its designation as the type species. Other sex associations have mainly been based on collection of both sexes in malaise traps at similar times and places.

***Pherotilla* Brothers, gen. n.**
http://zoobank.org/B716E7F3-C2A0-4CF9-81A3-10F101AE05CD
Figs 1, 11–18, 33–34, 41–42

Type species. *Rhopalomutilla mlanjeana* Bischoff, 1920: 180, male & female (name determined under Article 61.4 of the Code (ICZN 1999))

Diagnosis. MALE. Head transverse with vertex evenly rounded in anterior view; no genal carina; mandible tridentate; scutellum pulvinate, evenly swollen; S6 with posterior margin deeply notched laterally; S8 strongly sculptured with prominent peg-like process laterally and posterior margin broadly and unevenly emarginate; penis valves asymmetrical with right valve larger than left (sometimes symmetrical), each elongate without any setae along posterodorsal margin, outer surface smoothly convex; paramere without cluster of very strong setae arising under a flange dorsobasally but often with a few slightly stronger long setae near base. FEMALE. Head rounded in anterodorsal view, about as wide as long; mesosoma squat, lateral margin with several teeth or tubercles, strongly convex anteriorly, with gradual concave narrowing between metathoracic spiracle and propodeal spiracle, lateral margins of propodeum diverging posteriorly; disc of propodeum posteriorly indistinct, without any median tubercle and with strong tooth at lateral angle; metasoma slightly elongate with T1 broad; pygidial plate oval, bounded by carina ventrolaterally, surface covered by dense recumbent setae, posterior margin convex between weak apical teeth.

Description. MALE. Body black (seldom with tegula and/or legs brown); wings hyaline basally (sometimes almost entirely infuscated). *Head*: transverse with vertex more or less evenly rounded without any elevation behind ocelli, posterodorsal margin of head in anterior view more or less evenly curved; antennal scrobe above with transverse carina separated from secretory tubercle; clypeus with ventral marginal teeth; genal carina completely absent, postgena slightly convex; postmandibular carina weak and short or moderate and long (rarely absent), pleurostomal carina absent (sometimes a ridge from posterior mandibular articulation to margin of proboscidal fossa slightly posterior to half its length); mandible tridentate with middle apical tooth smaller than other two, ventral basal lamella gradually narrowed apicad. *Mesosoma*: mesoscutum anteroadmedian lines separated by a longitudinal ridge; notaulus incomplete anteriorly; parapsidal line very short and incomplete posteriorly; tegula evenly convex or slightly recurved posteriorly; scutellum pulvinate, evenly swollen without any posterior tubercle; metanotal dorsellum variable in form and sculpture; propodeum with disc and declivity very weakly differentiated, evenly merging; lateral face of pronotum fairly strongly tapered, anteroventral margin a weak ridge continuous with undeveloped anteroventral tooth (sometimes tooth moderately developed); mesepisternum with transverse depression moderately developed to imperceptible; mesosternum with a short crenulate transverse carina on each side about halfway between anterior margin and mid coxa or without any distinct projections. *Legs*: pubescence denser on tibiae and tarsi; claws deeply cleft apical to broad lamellate base; fore tibia without any apical secretory structure (sometimes with a vertically elongate preapical groove/pore on inner



Figures 11–18. *Pherotilla* gen. n. **11–14** *P. mlanjeana* (Bischoff) **11–12** male, dorsal & lateral views **13–14** female, lateral & dorsal views **15–18** *P. oceanica* (Mickel) **15–16** male, dorsal & lateral views **17–18** female, lateral & dorsal views. Scales = 1 mm.

(anterior) surface); mid and hind tibiae with preapical dorsal and lateral spines few and difficult to distinguish. *Metasoma*: fairly slender; T2 widest beyond midlength; T2 and S2 without any traces or with dispersed traces of lateral felt lines; pygidium (T7) with apical margin convex, edges not recurved (sometimes slightly so, rarely flangelike); S1 without any tooth; S6 with posterior margin deeply notched on each side; hypopygium (S8) strongly sculptured with prominent peg-like process laterally and posterior margin broadly and unevenly emarginate. *Genitalia*: basal ring moderate; paramere curved with narrow apex, without any differentiated very strong setae except some-

times with a few long thicker setae along basodorsal margin; penis valves asymmetrical (sometimes symmetrical) with right valve slightly larger than left, without any setae.

FEMALE. *Head*: rounded in anterodorsal view; sides produced far behind eye, poorly to fairly well differentiated from posterodorsal margin of head; posterodorsal margin convex, without any distinct depression at each side; clypeus with a median tooth above small triangular area, a broad smooth ventral concavity, an acute lamellate tooth at each side of concavity; gena broad, without or with a weak posterior ridge; postmandibular carina irregular running from mandibular base to level posterior to posterior margin of oral fossa then obsolete; pleurostomal carina forming a ridge from posterior mandibular articulation to margin of oral fossa; mandible evenly curved, with inner margin expanded into an obtuse triangular lamella about one-third length from base, apically weakly bidentate; maxillary palp two- or unsegmented, weakly fusiform; labial palp two- or unsegmented, slightly broadened apically; antennal scape with a lamellate rounded tooth posterolaterally, flat anteromesal surface delimited dorsally by a weak carina basally or not at all; pedicel sometimes with ventral tuft of fine setae. *Mesosoma*: squat, no longer than wide or only slightly so; anterodorsal margin indistinct with fairly short anterior face at an obtuse angle to dorsal face and merging with it; humeral angle dentate or tuberculate; lateral margin uneven and tuberculate, anteriorly strongly convex, gradually converging to notch at base of propodeum then diverging to posterolateral angle with strong tooth; disc of propodeum posteriorly poorly discernible, fairly smooth and without any median tubercle; posterior face of propodeum strongly oblique; lateral face of pronotum flattened with no (or a weak) anterior oblique carina, a curved low ridge running along anteroventral margin, ventral margin fairly straight, anteroventral extremity obtuse; mesepisternum strongly convex, with or without a vertical ridge above or anterodorsal to mid coxa. *Legs*: fore leg with femur flattened below, tibia with preapical elongate to oval secretory pore on inner (anterior) surface, tibial calcar with blade smooth on margin; mid and hind femora flat to weakly longitudinally concave below, each with a basally broad preapical lamella anteroventrally; mid and hind tibiae with preapical dorsal spines very strong and easy to distinguish, preapical lateral spines strong and fairly easy to distinguish. *Metasoma*: fairly slender; T1 with anterior face meeting dorsal face at a rounded right angle, dorsal face long, very broad and transverse, almost as wide as T2, sides broadly convex anteriorly then convex and somewhat diverging posteriorly; T2 about as long as T3–T6, broad deep basal depression strongly convex posteriorly, sides beyond basal depression diverging then convex and converging, small indefinite felt-line patch anterolaterally (sometimes absent), posterior margin straight to weakly concave; T3 with posterior margin straight (sometimes very weakly concave medially); T5 without (seldom with) a tuft of fine setae at posterolateral angle; pygidium (T6) with pygidial plate oval, with a strong lateral bounding carina ventrally, sculpture concealed by dense setae, apical margin convex between two blunt teeth; S1 with a short median carina anterior to a broad flattened triangular area bounded by ridges and elevated anteriorly, narrow lateral marginal depression on each side posteriorly; S4 with posterolateral angle simple (seldom produced); S5 with posterolateral angle not (seldom scarcely) produced,

without (sometimes with) a tuft of setae posterolaterally; S6 entirely slightly convex, apex acute, sides weakly angled to carinate, no flattened strong setae.

Species included. *Pherotilla japhia* (Cameron, 1902), male, comb. n.; *P. mlanjeana* (Bischoff, 1920), male & female, comb. n.; *P. oceanica* (Mickel, 1935), male & female, comb. n.; *P. rufitincta* (Hammer, 1957), male & female, stat. n., comb. n.; *P. striganovae* (Lelej, 2012), male & female, comb. n.; five undescribed species, four male only, one male & female.

Distribution. Southern to eastern Africa (Kenya, Malawi, Mozambique, Namibia, Tanzania), southern to southeastern Asia (India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Sri Lanka, Thailand, Vietnam).

Etymology. From the Greek verb φέρω (*phero*), to carry (referring to their phoretic copulation), combined with *-tilla*, a common suffix derived from the genus *Mutilla*; gender feminine.

Comments. Both sexes have already been described for only one of the Afrotropical species (*P. mlanjeana*); although only a single female specimen is known to me, I have designated this as the type species since the male is more typical for the genus than the most commonly collected Afrotropical species (*P. rufitincta*, for which several specimens of both sexes are known). This is the only genus of Rhopalomutillinae which is found outside the Afrotropical Region, occurring also in the Oriental Region, for which there is no evident explanation since we know nothing about their ecology. Although two species have been described from far south-east Asia (*Rh. oceanica* Mickel, 1935 from Borneo and *Rh. javana* Pagden, 1949 from Java), these differ only in coloration and I have seen several specimens from neighbouring islands with intermediate colour patterns but essentially identical morphology (including the male genitalia), so have concluded that only one variably coloured species is involved, and *Rh. javana* **syn. n.** should be synonymized with *Rh. oceanica*; a detailed justification will be provided in a subsequent paper.

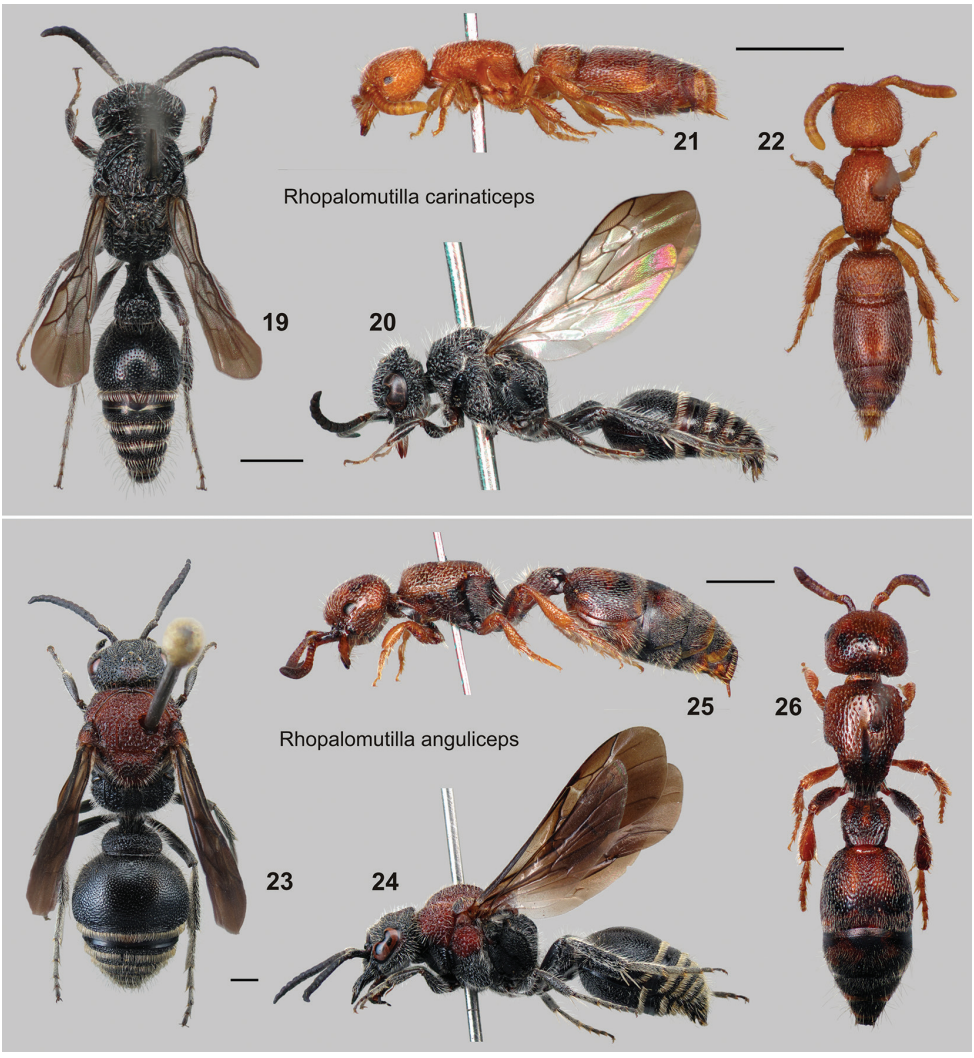
Rhopalomutilla André, 1901

Figs 19–26, 35–37, 43–45

Mutilla (*Rhopalomutilla*) André, 1901: 323.
Rhopalomutilla; André 1902: 23; Bischoff 1920: 176; Bradley and Bequaert 1928: 76; Lelej and Brothers 2008: 53.

Type species. *Mutilla* (*Rhopalomutilla*) *clavicornis* André, 1901: 323, male & female (monotypy).

Diagnosis. MALE. Head transverse with vertex straight, curved or angled in anterior view; genal carina present or absent; mandible tridentate; scutellum pulvinate, evenly swollen; S6 with posterior margin deeply notched laterally; S8 strongly sculptured with prominent peg-like process laterally and posterior margin broadly and unevenly emarginate; penis valves asymmetrical with right valve larger than left,



Figures 19–26. *Rhopalomutilla* André. **19–22** *Rh. carinaticeps* Bischoff **19–20** male, dorsal & lateral views **21–22** female, lateral & dorsal views **23–26** *Rh. anguliceps* André **23–24** male, dorsal & lateral views **25–26** female, lateral & dorsal views. Scales = 1 mm.

each elongate without any setae along posterodorsal margin, outer surface smoothly convex; paramere with cluster of very strong setae arising under a flange dorsobasally. FEMALE. Head rounded in anterodorsal view, about as wide as long; mesosoma elongate, lateral margin smooth, convex anteriorly, with concave narrowing between metathoracic spiracle and propodeal spiracle, lateral margins of propodeum parallel or converging posteriorly; disc of propodeum posteriorly without any median tubercle (rarely with very small acute median tooth) and with small tooth at lateral angle; metasoma slightly elongate with T1 broad or narrow; pygidial plate narrowly oval, bounded

by lateral carina along entire height (sometimes not reaching dorsal extremity), surface mostly smooth and shining (sometimes with a few longitudinal carinae and/or micropunctate and finely pubescent dorsally), posterior margin convex between weak apical teeth (sometimes without teeth).

Description. MALE. Body black (seldom with mesosoma partly dark red); wings basally hyaline and apically infuscated (sometimes entirely hyaline or entirely infuscated). *Head*: transverse with vertex flattened, rounded or medially produced behind ocelli, posterodorsal margin of head in anterior view straight, curved or angled; antennal scrobe above with transverse carina separated from conical secretory tubercle (seldom continuous with it, or carina or tubercle absent); clypeus with median tubercle (seldom without), 4 marginal teeth; genal carina strongly carinate, ridgelike or absent, postgena concave to convex; postmandibular carina varied from strong and extending from mandibular base to occipital foramen to weak and extending from mandibular base to a point slightly anterior to posterior extremity of oral fossa (carina rarely absent); pleurostomal carina forming a curved ridge from posterior mandibular articulation to margin of proboscidal fossa at about half its length (carina seldom barely distinguishable); mandible tridentate, middle apical tooth smaller than other two, ventral basal lamella well developed (sometimes weak, rarely absent), gradually or abruptly narrowed apicad. *Mesosoma*: with mesoscutum anteroadmedian lines distinct (seldom indistinct), forming two smooth longitudinal lines/depressions on either side of a slight ridge; notaulus deep and broad on posterior half or less, absent anteriorly; parapsidal line a short longitudinal scar distant from posterior border; tegula evenly convex (sometimes with posterior margin weakly recurved); scutellum pulvinate, evenly swollen without any posterior tubercle; metanotal dorsellum variable in form and sculpture; propodeum with disc and declivity abruptly differentiated; lateral face of pronotum tapered, anteroventral margin blunt (seldom ventrally carinate) and continuous with anteroventral tooth; mesepisternum with transverse depression weak (seldom well developed); mesosternum without any distinct projections (sometimes with a weak short transverse carina on each side about halfway between anterior margin and mid coxa). *Legs*: tarsal claws with basal lamella separated from acute apex by a deep cleft (sometimes lamella much reduced and apparently absent); fore tibia with an elongate or oval preapical groove/pore on inner (anterior) surface (seldom no discernible secretory structure); mid and hind tibiae with few inconspicuous preapical dorsal and lateral spines (sometimes absent on hind leg). *Metasoma*: fairly slender; T2 widest on posterior half (rarely at about midlength); T2 and S2 without any traces of lateral felt lines; pygidium (T7) with apical margin convex (seldom straight mesally), edges slightly recurved (rarely not recurved); S1 without any tooth; S6 with posterior margin deeply notched on each side; hypopygium (S8) strongly sculptured with prominent peg-like process laterally and posterior margin broadly and unevenly emarginate. *Genitalia*: basal ring moderate; paramere weakly curved to almost straight, with narrow apex, with cluster of differentiated very strong setae obliquely oriented and arising below flange on inner basodorsal margin; penis valves asymmetrical with right valve slightly larger than left, without any setae.

FEMALE. *Head*: rounded in anterodorsal view; sides produced far behind eye, poorly (seldom well) differentiated from posterodorsal margin of head; posterodorsal margin entirely moderately convex (seldom with a slight depression on each side); clypeus with strong median tooth dorsally, a tooth at each side of triangular ventral area; gena broad, no genal carina (seldom distinguishable but weak); postmandibular carina weak and irregular running from mandibular base more or less parallel to oral fossa to level slightly posterior to posterior margin of oral fossa; pleurostomal carina forming a weak fairly straight ridge from posterior mandibular articulation to margin of oral fossa at about half (seldom one-third) its length; mandible evenly curved, inner margin expanded into a weak obtuse long triangular lamella about one-third length from base, apically weakly bidentate; maxillary palp two- or unsegmented, apically narrowed; labial palp two- or unsegmented, curved and more or less cylindrical to clavate (rarely apparently absent); antennal scape with lamellate rounded tooth posterolaterally, anteromesal surface delimited dorsally by a weak carina basally (seldom a weak additional ventral carina or no carinae); pedicel without any distinct tuft of fine setae. *Mesosoma*: elongate; anterodorsal margin indistinct with fairly long anterior face; humeral angle bluntly rounded to weakly carinate; lateral margin fairly even and smooth to tuberculate, anteriorly weakly convex, then strongly converging and concave to base of propodeum, then almost straight and weakly converging to posterolateral angle with strong (seldom small) acute tooth; disc of propodeum posteriorly rounded, fairly smooth, without any median tubercle (rarely with very small acute median tooth); posterior face of propodeum moderately oblique; lateral face of pronotum flattened with no (or a weak) anterior oblique carina, anteroventral margin not carinate, ventral margin almost straight, anteroventral extremity obtuse to rectangular; mesepisternum strongly convex, with or without a vertical ridge above or anterodorsal to mid coxa. *Legs*: fore leg with femur flattened below, tibia with preapical oval to circular secretory pore (rarely absent) on inner (anterior) surface, calcar with blade smooth on margin; mid and hind femora longitudinally concave or flattened below, each with an elongate preapical lamella anteroventrally; mid and hind tibiae preapically with a few strong dorsal spines easy to distinguish, a few weak lateral spines fairly difficult to distinguish. *Metasoma*: fairly slender; T1 with anterior face meeting dorsal face at a rounded right to obtuse angle, dorsal face long and broad or fairly short and narrow but somewhat transverse, almost as wide as T2 or much narrower, sides weakly convex and slightly diverging from base or almost straight and slightly converging from base; T2 about as long as T3–T6, with broad deep basal depression weakly convex posteriorly, sides beyond basal depression diverging then weakly or strongly convex and scarcely converging posteriorly; a small indefinite felt-line patch anterolaterally (sometimes absent), posterior margin strongly concave to straight; T3 with posterior margin strongly concave to straight; T5 with a strong diagonal tuft of long fine setae (rarely without such setae) at posterolateral angle; pygidium (T6) with pygidial plate oval, with a strong lateral bounding carina (sometimes only ventrally), smooth (rarely with a few longitudinal ridges) and shining, but sometimes sparsely micropunctate and setose dorsolaterally or almost entirely, apical margin convex between two small teeth; S1 with a short median

carina (rarely scarcely developed) anterior to a flattened triangular area slightly (rarely not at all) elevated anteriorly; S4 with posterolateral angle produced, often with a tuft of setae; S5 with posterolateral angle produced, often with a small tuft of strong setae; S6 convex but often weakly depressed on each side, apex acute, sides carinate, rarely with a few long flattened setae posterolaterally.

Species included. *Rhopalomutilla anguliceps* (André, 1897), male & female; *Rh. carinaticeps* Bischoff, 1920, male & female; *Rh. clavicornis* (André, 1901), male & female; *Rh. punctinoda* (Cameron, 1910), male only; 18 undescribed species, 11 male & female, 7 male only.

Distribution. Sub-Saharan Africa (Angola, Botswana, Burkina Faso, Burundi, Cameroon, Chad, Côte d'Ivoire, Democratic Republic of Congo, Gabon, Guinea, Kenya, Malawi, Mozambique, Namibia, Nigeria, Senegal, South Africa, Tanzania, The Gambia, Zambia, Zimbabwe).

Etymology. Not stated by André (1901), but undoubtedly from the Greek noun ῥόπαλος (*rhopalos*), a club or cudgel (with reference to the clavate antenna of the female), combined with *Mutilla*; gender feminine.

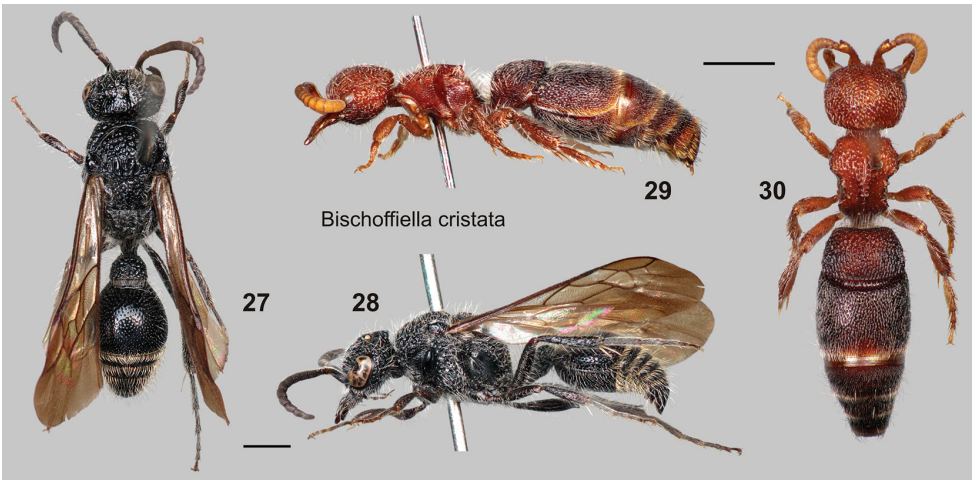
Comments. The only species for which both sexes have as yet been described is the type species (*Rh. clavicornis*). This is the largest genus in the subfamily, with the broadest African distribution, species being found in most of the sub-Saharan region except for the densely forested areas and the southernmost parts. It includes two groups based on females, with T1 either about as broad as T2 or much narrower, and also two groups based on males, with the penis valves either simple ventrally or with variably produced lobes; unfortunately, these groups have different members so that a simple subdivision of the genus is not feasible.

Bischoffiella Brothers & Nonveiller, gen. n.

http://zoobank.org/B42AEC7C-B2B2-4AA4-99C9-7174F20446EE
Figs 2, 27–30, 38, 46

Type species. *Mutilla cristata* Bingham, 1912: 536, male.

Diagnosis. MALE. Entirely black. Head transverse with vertex curved or medially protuberant in anterior view; genal carina present or absent; mandible tridentate; scutellum pulvinate, evenly swollen; S6 with posterior margin deeply notched laterally; S8 strongly sculptured with prominent rounded peg-like process laterally and posterior margin broadly and unevenly emarginate or weakly produced; penis valves symmetrical (rarely weakly asymmetrical with right valve scarcely larger than left), each elongate without any setae along posterodorsal margin, outer surface with dentate to lamellate protuberance; paramere with cluster of very strong setae arising under a flange dorsobasally. FEMALE. Head rounded in anterodorsal view, about as wide as long; mesosoma squat, lateral margin fairly smooth, strongly convex anteriorly, with strong concave narrowing between metathoracic spiracle and propodeal spiracle, lateral margins of propodeum weakly diverging posteriorly; disc of propodeum posteriorly



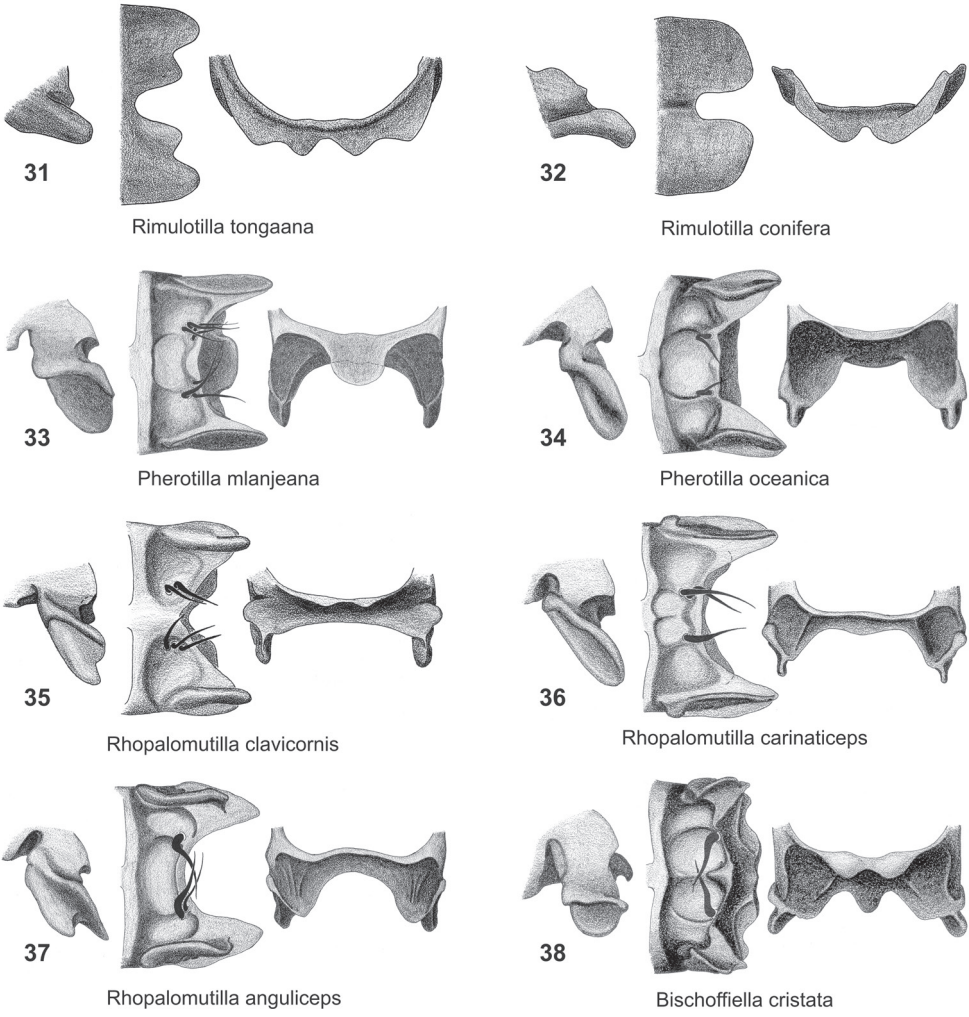
Figures 27–30. *Bischoffiella cristata* (Bingham). **27–28** male, dorsal & lateral views **29–30** female, lateral & dorsal views **29–32**. Scales = 1 mm.

indistinct, with a very strong median longitudinal ridge elevated and broadened posteriorly and ending abruptly in a vertical face (appearing as an enlarged but narrow scutellar scale) and with a very strong tooth at lateral angle; metasoma slightly elongate with T1 broad; pygidial plate oval, longitudinally sculptured, bounded by carina laterally, surface covered by dense semirecumbent setae, posterior margin convex between rounded extremities of lateral carinae.

Description. MALE. Body black; wings moderately infuscated but hyaline on about basal third. *Head*: vertex ending abruptly posteriorly, posterodorsal margin of head in anterior view evenly rounded or medially protuberant; antennal scrobe with irregular convex dorsal carina more or less continuous with weak lateral secretory tubercle; clypeus with median area flattened, sometimes with dorsal tubercle; genal carina absent or strong, postgena convex to concave; postmandibular carina evident only laterally; pleurostomal carina distinct; mandible apically tridentate, with middle apical tooth smaller than other two, ventral basal lamella poorly developed. *Mesosoma*: mesoscutum with anteroadmedian lines shallow, separated by a longitudinal ridge; notaulus deep and broad on posterior half, absent anteriorly; parapsidal line a broad short longitudinal scar distant from posterior border; tegula evenly convex, more or less evenly and finely punctate, punctures coarser anteriorly than posteriorly with a restricted smooth area anteromedially; scutellum with reticulate punctation finer than that on scutum; metanotal dorsellum rectangular to trapezoidal; propodeum with disc and declivity abruptly differentiated; lateral face of pronotum tapered, anteroventral margin blunt, continuous with anteroventral tooth (sometimes tooth absent); mesepisternum with transverse depression almost indistinguishable; mesosternum with a short crenulate transverse carina on each side about halfway between anterior margin and mid coxa. *Legs*: tarsal claws with basal lamella separated from acute apex by a deep

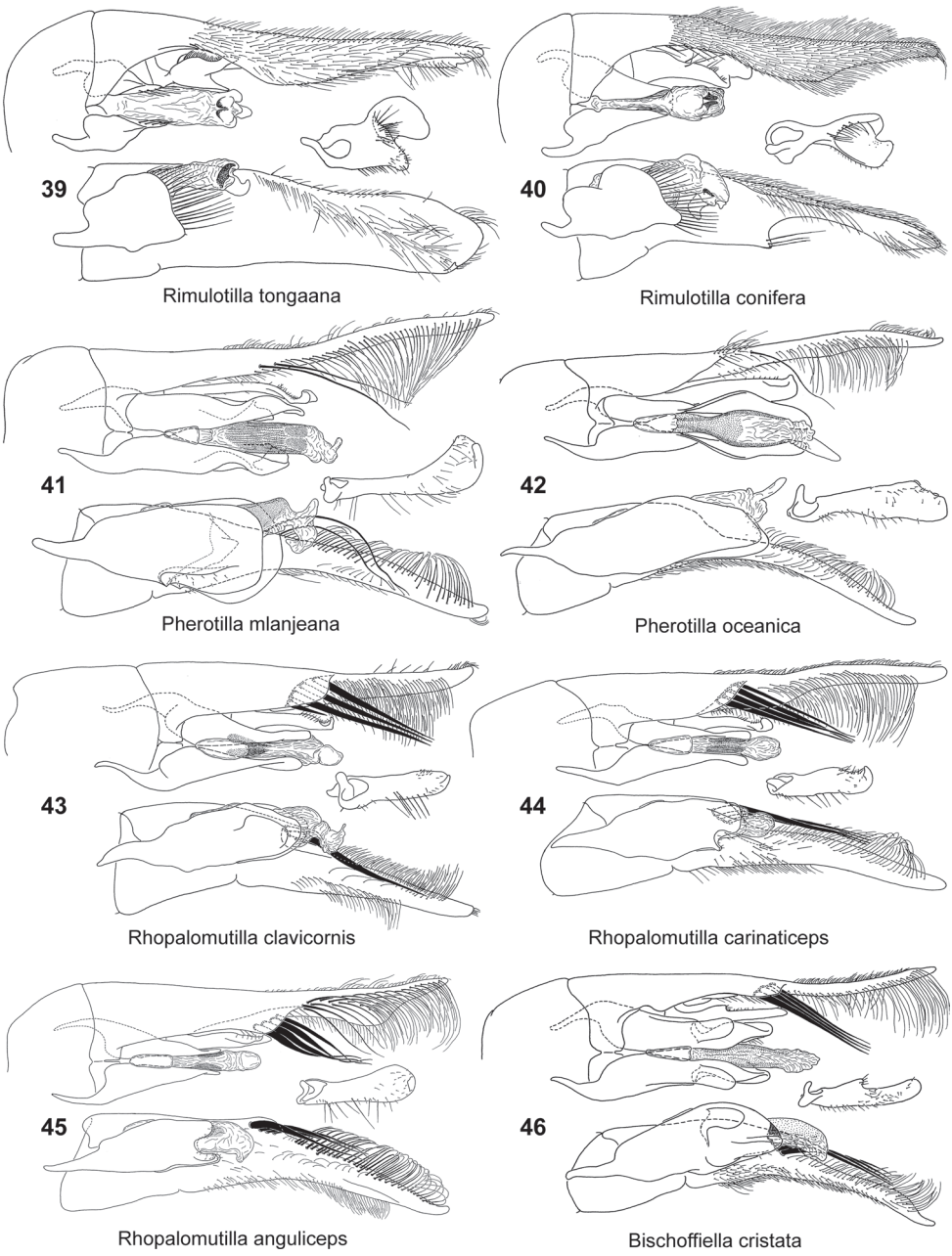
cleft; fore tibia with an obliquely oval preapical pore on inner (anterior) surface; mid and hind tibiae with preapical dorsolateral spines difficult to distinguish. *Metasoma*: fairly slender; T2 widest posterior to midpoint; T2 and S2 with inconspicuous dispersed traces of linear felt lines; pygidium (T7) with apical margin weakly convex, edges scarcely recurved (sometimes not recurved); S1 with a distinct paired longitudinal carina diverging posteriorly; S6 with posterior margin deeply notched on each side and posteriorly expanded medially; hypopygium (S8) strongly sculptured with prominent apically broadly rounded peg-like process laterally and posterior margin broadly and unevenly emarginate to medially produced. *Genitalia*: basal ring moderate; paramere curved with narrow apex, with cluster of differentiated very strong setae obliquely oriented and arising below flange on inner basodorsal margin; penis valves symmetrical (rarely weakly asymmetrical with right valve scarcely larger than left), without any setae, with rounded tooth or lamellate lobe on outer surface.

FEMALE. *Head*: rounded in anterodorsal view but vertex slightly longitudinally raised in the middle posteriorly, sides behind eyes weakly convex and somewhat converging posteriorly, produced more than twice length of eye, well differentiated from posterodorsal margin of head; posterodorsal margin convex medially; clypeus with very strong acute median tooth above small acute tooth flanked by a small acute tooth on each side at dorsomedial extremity of fairly strong dorsolateral bounding carina of smooth obtusely triangular depressed area, a strong acute lamellate tooth at ventrolateral extremity of carina; gena broad, without any genal carina; postmandibular carina moderate and fairly regular running from mandibular base to level somewhat posterior to posterior margin of oral fossa; pleurostomal carina forming a fairly distinct straight ridge from posterior mandibular articulation to margin of oral fossa at about one-third its length; mandible apically very weakly bidentate, inner margin expanded into a weak very obtuse long triangular lamella about one-third length from base; maxillary palpus two-segmented, basal segment elongate and weakly broadened apically, apical segment much narrower and cylindrical; labial palpus two-segmented, basal segment short and cylindrical; antennal scape with a lamellate narrowly rounded tooth posterolaterally, flattened anteromesal surface delimited dorsally by a weak carina over about basal third; pedicel without any ventral tuft of fine setae. *Mesosoma*: weakly elongate; anterodorsal margin extremely indistinct with long anterior face smoothly merging at a very obtuse angle with dorsal face; humeral angle rounded; lateral margin uneven but fairly smooth, anteriorly rounded, diverging from humeral angle to rounded protuberance just anterior to prothoracic spiracle then angled and convex, then converging and margin angled and strongly concave and converging to base of propodeum, lateral margin of propodeum concave and converging then diverging to posterolateral angle with a very strong acute flattened tooth; propodeal disc fairly long, with a very strong median longitudinal ridge elevated and broadened posteriorly and ending abruptly in a vertical face (appearing as an enlarged but narrow scutellar scale); posterior face of propodeum strongly oblique; lateral face of pronotum without any anterior oblique carina, anteroventral extremity narrowly rounded; mesepisternum strongly convex with a weak short vertical ridge immediately anterodorsal to mid coxa. *Legs*: fore leg



Figures 31–38. Rhopalomutillinae, hypopygium (S8): left = lateral view, anterior to left; centre = ventral view, anterior to left; right = posterior view. **31–32** *Rimulotilla* gen. n. **31** *Ri. tongaana* (Péringuey) **32** *Ri. conifera* (Bischoff) **33–34** *Pherotilla* gen. n. **33** *P. mlanjeana* (Bischoff) **34** *P. oceanica* (Mickel) **35–37** *Rhopalomutilla* André **35** *Rh. clavicornis* (André) **36** *Rh. carinaticeps* Bischoff **37** *Rh. anguliceps* André **38** *Bischoffiella cristata* (Bingham).

with femur flattened below, tibia with preapical oval secretory pore on inner (anterior) surface, calcar with blade smooth on margin; mid and hind femora distinctly longitudinally concave below, each with a very broad preapical lamella anteroventrally; mid and hind tibiae preapically with a few strong dorsal spines easy to distinguish, a few (several on mid tibia) lateral spines fairly easy to distinguish. *Metasoma*: fairly slender; T1 with anterior face weakly concave, meeting dorsal face at a fairly narrowly rounded



Figures 39–46. Rhopalomutillinae, male genitalia: upper = dorsal view; middle = left volsella, exterolateral view; bottom = sagittal view, penis valves and right paramere (right volsella not shown). **39–40** *Rimulotilla* gen. n. **39** *Ri. tongaana* (Péringuey) **40** *Ri. conifera* (Bischoff) **41–42** *Pherotilla* gen. n. **41** *P. mlanjeana* (Bischoff) **42** *P. oceanica* (Mickel) **43–45** *Rhopalomutilla* André **43** *Rh. clavicornis* (André) **44** *Rh. carinaticeps* Bischoff **45** *Rh. anguliceps* André **46** *Bischoffiella cristata* (Bingham).

angle, dorsal face very broad and transverse, more than half as long as wide, almost as wide and more than half as long as T2, sides convex and diverging posteriorly; T2 about as long as T3–T6, with broad deep basal depression strongly convex posteriorly, sides beyond basal depression somewhat diverging then converging, small indefinite felt-line patch anterolaterally; T3 with posterior margin very weakly concave medially; T5 without any tuft of setae at posterolateral angle; pygidium (T6) with pygidial plate broadly oval, with a strong lateral bounding carina along most of ventral height, sculpture somewhat concealed by dense semirecumbent setae, apical margin strongly convex between rounded extremities of lateral carinae; S1 with a short simple median elevation anterior to a broad flattened pentagonal area bounded by moderate irregular ridges and elevated anteriorly, narrow lateral marginal depression on each side over posterior half; S4 with a very weak tuft (sometimes absent) of fine setae at posterolateral angle which is very slightly produced; S5 with posterolateral angle produced and with a tuft of bent setae laterally; S6 slightly convex but with a longitudinal median ridge posteriorly, apex acute, sides carinate, no flattened strong setae.

Species included. *Bischoffiella cristata* (Bingham, 1912), male & female, comb. n.; two undescribed species, both male & female.

Distribution. Eastern and southern Africa (Angola, Botswana, Kenya, Malawi, Mozambique, Namibia, South Africa, Tanzania, Zambia, Zimbabwe).

Etymology. Named, at the suggestion of the late Guido Nonveiller, in recognition of the fundamental contributions of Hans Bischoff (Berlin) to the study of African Mutillidae, and validating this name which was found attached to a female specimen (misidentified as *Rh. clavicornis* by Bischoff) in the Royal Museum for Central Africa, Tervuren, Belgium by J. Chester Bradley who had recognized that it represented a different genus (see Bradley and Bequaert 1923: 216–7).

Comments. The type species is the only one yet described, from the male only; both sexes are known for all three included species, however.

Acknowledgments

The many curators and colleagues who made specimens available for this protracted study are thanked for their assistance and patience; the persons and institutions involved will be fully acknowledged in the papers dealing with the species. Information shared and generous collaboration provided by the late Guido Nonveiller (Zemun, Serbia) are gratefully acknowledged. Funding was provided by the University of KwaZulu-Natal Research Office.

References

André E (1897) Description de trois nouvelles espèces de Mutilles de l'Afrique orientale appartenant au Musee royal de Belgique. Bulletin de la Société Zoologique de France 22: 17–22.

André E (1901–1902) Matériaux pour servir à la connaissance des Mutillides d'Afrique. Zeitschrift für Systematische Hymenopterologie und Dipterologie (1901) 1: 279–288, 305–352; (1902) 2: 17–48.

André E (1902 ["1903"]) Fam. Mutillidae. Genera Insectorum. Wytsman, P, Bruxelles, Fasc. 11, 1–77, pl. 1–3.

Bradley JC, Bequert J (1923) Studies in African Mutillidae. Revue Zoologique Africaine 11: 211–258.

Bradley JC, Bequert J (1928) A synopsis of the Mutillidae of the Belgian Congo. Bulletin of the American Museum of Natural History 58(2): 63–122.

Bingham CT (1912 ["1911"]) South African aculeate Hymenoptera in the Oxford Museum. Transactions of the Entomological Society of London 1911: 528–550.

Bischoff H (1920–1921) Monographie der Mutilliden Afrikas. Archiv für Naturgeschichte (1920) 86A(1–3): 1–480; (1921) 86A(4–5): 481–830.

Brothers DJ (1975) Phylogeny and classification of the aculeate Hymenoptera, with special reference to Mutillidae. University of Kansas Science Bulletin 50: 483–648.

Brothers DJ (1989) Alternative life-history styles of mutillid wasps (Insecta, Hymenoptera). In: Bruton MN (Ed.) Alternative Life-History Styles of Animals. Perspectives in Vertebrate Science 6: 279–291. doi: 10.1007/978-94-009-2605-9_14

Brothers DJ (1994) A new genus and four new species of Mutillidae associated with *Brachyponera lutea* Mayr (Formicidae) in Western Australia (Hymenoptera). Journal of the Australian Entomological Society 33: 143–152. doi: 10.1111/j.1440-6055.1994.tb00941.x

Brothers DJ (1999) Phylogeny and evolution of wasps, ants and bees (Hymenoptera, Chrysidoidea, Vespoidea and Apoidea). Zoologica Scripta 28: 233–249. doi: 10.1046/j.1463-6409.1999.00003.x

Cameron P (1902) Descriptions of new genera and species of Hymenoptera collected by Major C. S. Nurse at Deesa, Simla and Ferozepore. Part I. Journal of the Bombay Natural History Society 14(2): 267–293, 1 pl.

Cameron P (1910) 8. Hymenoptera. 7. Fossores. Wissenschaftliche Ergebnisse der Schwedischen Zoologischen Expedition nach dem Kilimandjaro, dem Meru und den Umgebenden Massaisteppen Deutsch-Ostafrikas 1905–1906 unter Leitung von Prof. Dr. Yngve Sjöstedt 2(8): 197–294.

Hadley A (2010) CombineZP software. http://www.hadleyweb.pwp.blueyonder.co.uk

Hammer K (1957) Beiträge zur Kenntnis der Insektenfauna Ostafrikas, insbesondere des Matengo-Hochlandes. X. Hymenoptera: Mutillidae. Annalen des Naturhistorischen Museums in Wien 61: 232–237.

International Commission on Zoological Nomenclature (ICZN) (1999) International Code of Zoological Nomenclature. Fourth Edition. ITZN, London, i–xxx, 306 pp.

Lelej AS (2012) A new species of *Rhopalomutilla* André, 1901 (Hymenoptera: Mutillidae) from South Vietnam. Russian Entomological Journal 21: 143–145.

Lelej AS, Brothers DJ (2008) The genus-group names of Mutillidae (Hymenoptera) and their type species, with a new genus, new name, new synonymies, new combinations and lectotypifications. Zootaxa 1889: 1–79.

Lelej AS, Nemkov PG (1997) Phylogeny, evolution and classification of Mutillidae (Hymenoptera). Far Eastern Entomologist 46: 1–24.

Mickel CE (1935) The mutillid wasps of the islands of the Pacific Ocean (Hymenoptera: Mutillidae). Transactions of the Entomological Society of London 83: 177–312. doi: 10.1111/j.1365-2311.1935.tb01207.x

Pagden HT (1949) Description and records of Austro-Malaysian Methocidae and Mutillidae (Hymenoptera). Transactions of the Royal Entomological Society of London 100: 191–231. doi: 10.1111/j.1365-2311.1949.tb01431.x

Péringuey L (1909) New or little known South African Mutillidae (Hymenoptera). Annals of the South African Museum 5: 385–409.

Schuster RM (1949) Contributions toward a monograph of the Mutillidae of the Neotropical Region, III. A key to the subfamilies represented and descriptions of several new genera (Hymenoptera). Entomologica Americana (NS) 29: 59–140.

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RESEARCH ARTICLE

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Multimodal defensive strategies in larvae of two *Hemichroa* sawfly species

Jean-Luc Boevé[1]

1 *O.D. Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences, Rue Vautier 29, 1000 Bruxelles, Belgium*

Corresponding author: *Jean-Luc Boevé* (jean-luc.boeve@naturalsciences.be)

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Abstract

The two European sawfly species in the genus *Hemichroa* are a contrast in behaviour and appearance, since *H. crocea* is gregarious and brightly coloured, whereas *H. australis* is solitary and cryptic. Here, their defensive strategies are compared by integrating further components. In both species, ventral glands are minute, and no distinctive volatiles were detected by chemical analysis; hence, these exocrine glands are probably irrelevant in defence. Ethanol extracts of body parts were feeding deterrent to ant workers of *Myrmica rubra*, especially the integument of *H. australis* which was more deterrent than that of *H. crocea*. Single, living larvae of *H. crocea* were also attacked more frequently by ants. In contrast, single larvae of *H. crocea* are reluctantly taken by the bird *Parus major* that readily feeds on *H. australis*. The larvae of both species jerk their abdomen to physically defend themselves and/or to increase their (visual) warning signal (*H. crocea*). The larvae of *H. crocea* can scratch the host plant leaf with the tip of their abdomen to produce a sound assumed to convey information in intraspecific communication. However, this behaviour was also elicited from *H. australis*, when disturbed, which suggests that it may have another function. The defensive strategy is multimodal in both species. The principal differences are the reliance on gregariousness in *H. crocea*, as opposed to the use of integumental chemicals in *H. australis*.

Keywords

Hymenoptera, Tenthredinidae, defence strategy, ants, behaviour, sounds, chemical ecology

Introduction

The sawfly genus *Hemichroa* (Hymenoptera, Tenthredinidae, Nematinae) constitutes a small group of 13 recognized species (Taeger et al. 2010). The larval stage is described only for the species *H. australis* (Serville, 1823), *H. crocea* (Geoffroy, 1785), and *H. militaris* (Cresson, 1880) (Lorenz and Kraus 1957, Smith 1975). The geographic distribution is Palaearctic for *H. australis*, Palaearctic, Nearctic and Oriental for *H. crocea*, and Nearctic for *H. militaris* (Taeger et al. 2010). Only the two former species occur in Europe, where they are quite common. Both species feed mainly on *Alnus* (Betulaceae) (Taeger et al. 1998). Other host-plant genera of *Hemichroa* are *Betula* (Betulaceae), *Carpinus*, *Corylus* (Corylaceae), *Amelanchier*, *Crataegus*, and *Prunus* (Rosaceae) (Smith 1975). The phylogenetic position of *Hemichroa* is closest to *Platycampus* (Nyman et al. 2006) that feeds on *Alnus* and has extremely cryptic larvae (Boevé and Angeli 2010). The species *H. australis* is cryptic and solitary, whereas *H. crocea* is brightly coloured, gregarious (Lorenz and Kraus 1957, Boevé and Pasteels 1985; Fig. 1) and sometimes a serious pest (Escherich 1940–1942, Kriegl 1964).

Nematinae larvae are characterized by the presence of ventro-abdominal exocrine glands which are turned inside out to emit volatiles used in defence (Boevé and Pasteels 1985). The glands vary in size across species, but they are clearly reduced in *Hemichroa* (with a glandular surface of 0.03 mm^2; see Boevé and Pasteels 1985), and their chemical composition remains unknown. A unique facet of *H. crocea* larvae is their ability to scratch the leaf's surface with protuberances on their caudal segment, producing a stridulatory sound (Hopping 1937). These sounds are thought to maintain cohesion of the larval group, and to direct individuals to profitable, fresh leaves (Hograefe 1984). Similar communication by vibrational signals via a substrate is known for other sawflies such as the pergid *Perga affinis* (Carne 1962, Fletcher 2008).

It is likely that the defensive strategy of *Hemichroa* larvae is multimodal, combining behavioural, visual, chemical, and acoustic traits. This paper examines two aspects of their defence by using a comparative approach. The principal purpose was to determine whether or not *H. australis* and *H. crocea* – which display opposite appearance and gregariousness – also differ in other (behavioural and chemical) traits, and in the consequent effectiveness of their defensive strategies. Another aspect was to test whether and how acoustic cues are involved in defence.

Methods

Larvae of *Hemichroa* were collected in Belgium and identified following Lorenz and Kraus (1957). Voucher specimens are kept in the Royal Belgian Institute of Natural Sciences. Throughout the text, the sawfly collection reference number is given between square brackets.

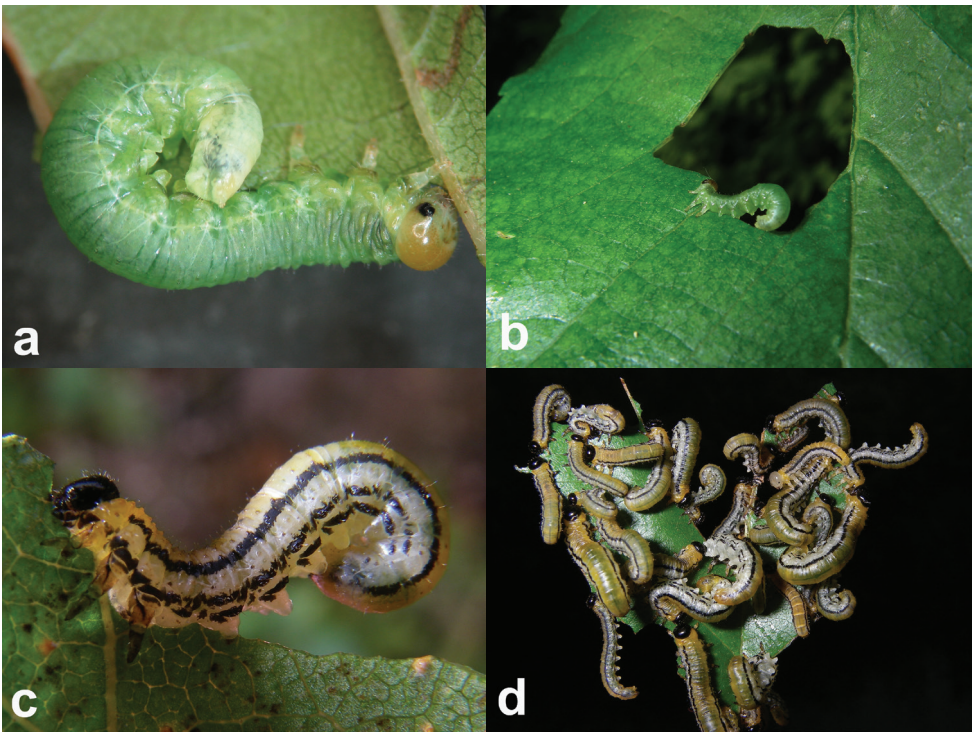

Figure 1. Pictures of larvae of the two studied *Hemichroa* species. **a, b** *H. australis*, solitary (body length ca. 18 mm) **c, d** *H. crocea*, gregarious (body length ca. 20 mm). Field host-plant [sawfly collection reference number]: **a** *Alnus glutinosa* [P2553] **b** *A. glutinosa* [P3999] **c** *Betula verrucosa* [P3225] **d** *A. glutinosa* [P3230]

Field observations were performed and documented with Pentax Optio W10 and Nikon Coolpix P300 cameras. An audio file was obtained in indoor conditions with a Zoom H4n digital recorder, its microphones being placed a few cm from a leaf harbouring a group of *H. crocea* larvae.

Ventral glands were dissected from larvae preserved in 70 % ethanol, then mounted between glass slides and plates. Glands were also dissected from larvae stored at -30 °C and thawed, to be analysed via solid sample injections by gas chromatography-ion-trap mass spectrometric detection (GC-ITD) as described in Boevé et al. (1992).

Hemolymph was collected with glass capillaries from live larvae. Afterwards, the larvae were frozen and the thawed specimens dissected to isolate integument and internal organs (mainly the digestive tract). The three samples from a batch of larvae were extracted in ethanol, then filtered, dried, and dissolved in sugar water. The laboratory, dual-choice bioassay consisted of comparing the number of ant workers of *Myrmica rubra* feeding on sugar water *versus* sugar water plus extract. Another bioassay consisted of placing a single live larva in the presence of 20 ants; the number of ants attacking the larva was counted, and the behavioural interactions were noted. All experimental procedures are detailed in Boevé (2010).

Results

Behaviour

The larvae of *H. crocea* and *H. australis* settle on the edge of a leaf, firmly gripping with their thoracic legs. Younger larvae make a hole in the leaf, thus feeding on the inner leaf edge, whereas older larvae feed on the outer edge, which is especially the case for *H. crocea* (Hopping 1937).

If disturbed, the larva places its body on the leaf side opposite to the source of disturbance, especially so in *H. australis*. Larvae can also perform defensive movements with the abdomen. These movements are either hearable scratch sequences (see Introduction; Suppl. material 1), or single quite violent jerks. Both abdominal movements were observed in *H. crocea* and *H. australis*. Larvae performed jerks when disturbed by approaching and attacking ant workers, or when I approached them, or when I directed my finger towards them. Since *H. crocea* is gregarious, jerking by one individual could be imitated by others, leading to 'waves' of jerks within a group (Suppl. material 2). This was elicited by an external disturbance as much as by an internal one in that the larvae were disturbing each other. When many larvae settled on one leaf and consumed a major part of it, scratching behaviour was virtually impossible because there was almost no leaf surface available. A few times in the field, my approach provoked scratching in a larva of *H. australis* (i.e. different larvae, locations, and dates; Suppl. material 3) and, if the environment was calm, I could hear it from nearly a meter away. After a while, however, the larva stopped scratching, probably do to habituation or fatigue.

Morphology and chemistry of ventral glands

The minute and flattened ventral glands of *H. crocea* and *H. australis* are associated with only one pair of retractor muscles. The secretory layer is composed of only about 25 glandular cells on each side of the pouch.

Only small amounts of chemical compounds were detected by analysing a whole ventral gland. These were alkanes with an odd number of carbon atoms from 23 to 27 in *H. crocea*, and 21 to 27 in *H. australis*.

Defensive efficiency

All extracts at a starting-test concentration of 8 mg DW extract / ml sugar water significantly deterred ants (Tab. 1). Internal organs proved to be the most deterrent body parts in both *Hemichroa* species. At this concentration, the total number of feeding ants was not significantly different between the two species, neither by comparing the hemolymph extract with the control solution ($P = 0.742$, Fisher exact probability test, two-tailed), nor by similarly considering the internal organs ($P = 0.617$). However, it

Table 1. Feeding deterrence rates of extracts of *Hemichroa* larvae against *M. rubra* ants.

Species	Extract	8.0 mg DW/ml	2.6 mg DW/ml	0.8 mg DW/ml
H. australis	Hemolymph	61** (93)	7 (198)	8 (141)
	Integument	65** (126)	24* (124)	6 (128)
	Internal organs	87** (169)	39** (154)	9 (200)
H. crocea	Hemolymph	57** (139)	36** (115)	3 (218)
	Integument	31* (283)	-2 (182)	–
	Internal organs	91** (129)	45** (131)	6 (175)

The deterrence rate is the percentage of (C-T)/(C+T), where C and T are the total numbers of ants feeding on the control and test solution, in a 12-replicated test. Values for C+T are given between parentheses. The starting extract solution was tested also in two logarithmic dilutions. For each test, the paired number of ants was compared with the Wilcoxon signed-rank test, two-tailed: (*) $P < 0.05$, (**) $P < 0.01$. (–) Not tested.

was significantly different for the integument ($P < 0.001$) with *H. australis* being more deterrent than *H. crocea*. Testing dilutions of the starting concentration confirmed these results for the integument and internal organs, but indicated that the hemolymph may be more deterrent in *H. crocea* (Tab. 1).

A single living larva of *H. australis* was significantly less likely to be attacked (by a mean ± SD of 3.5 ± 2.6 ants) than one of *H. crocea* (6.5 ± 1.8) ($P < 0.05$, Mann-Whitney test, two-tailed). Both sawfly species made violent body movements while being attacked. Conversely, *H. australis* provoked clearer signs of distress in the ants than *H. crocea*.

Discussion

Anti-predator defensive mechanisms often act in concert, but are dynamically modulated so as to produce specific responses to threats that vary in type, time, and intensity (Rowe and Harpin 2013). This is well illustrated in the two studied *Hemichroa* species in which visual, chemical, behavioural, and possibly acoustic components were revealed.

Ventral glands are greatly reduced compared to other Nematinae species. The detected alkanes, from heneicosane to heptacosane, were not unique to *Hemichroa* or the Nematinae, but correspond to those hydrocarbons generally occurring on the cuticular surface of insects (Blomquist and Bagnères 2010). As far as known, they are devoid of any particular interspecific repellent effect. Thus, the chemical defence in both species does not rely on a volatile glandular secretion, contrasting the situation in other Nematinae species (e.g. Boevé et al. 1992). However, another type of chemical defence exists because the extracts of all body parts proved to be deterrent to ants, notably the integument of *H. australis* (Tab. 1). This result was in accordance with the greater defensive efficiency of individual larvae of the latter species when confronted with 20 ants. Moreover, the mechanical resistance of the integument of *H. australis* is twice as

high as that of *H. crocea* (U. Schaffner and Boevé, unpublished results). The physical barrier is of particular importance in defending against invertebrate predators such as ants. But, the bird *Parus major* readily feeds on single larvae of *H. australis*, while only reluctantly accepting those of *H. crocea* (see Boevé and Pasteels 1985). Thus, a single *H. crocea* is better defended against birds than a single *H. australis*, the reverse being true against ants. In natural conditions, gregariousness of *H. crocea* probably enhances its defence against birds and may compensate for the relatively low defence efficiency of each individual against ants.

Both sawfly species exhibit similar abdominal movements. A larva can switch between jerking and scratching within a short period. The jerks are a common defensive behaviour among Nematinae larvae, and they can knock down a foraging ant or parasitoid, but are inefficient against birds as a physical defence (Boevé and Pasteels 1985). However, the intensity of the warning signal is increased by the gregarious behaviour of *H. crocea* larvae. The scratching behaviour is unusual among insects. In *Hemichroa*, there is good evidence that it plays a role in defence such as an acoustic and/or vibrational warning signal (Suppl. materials 1 and 3) that may function against birds as well as predatory invertebrates. It is reported here in *H. australis* for the first time. This solitary species obviously does not use scratching to communicate with conspecifics. It seems that this behaviour is used less frequently than jerking, and only when first encountering an antagonist, which may explain why Hograefe (1984) considered it as non-existent in this species. Interestingly, the larva of *H. militaris* does not possess caudal protuberances (Smith 1975), which raises the question whether it performs scratching or not. Furthermore, Dyar (1895: p. 305) says of the gregarious larvae of *Nematus ventralis* Say, 1824 (Nematinae): "The larvae scratch the leaf with their anal prongs and make a rasping sound". In *H. crocea*, the use of scratching in intraspecific communication is plausible, although counterarguments to the conclusions of Hograefe (1984) would be that undamaged foliage not only supposes leaves with higher nutritional quality, but also a larger leaf surface on which the behaviour can be executed, independently of its function. Moreover, it remains unclear why the larvae on the heavily eaten leaf perform frequent scratch sequences at the beginning of the experiment; the experiment itself possibly disturbed the larvae that may have responded by a 'defensive' behaviour.

Conclusion

Scratching is known to be a way of inter-individual communication in *H. crocea*. However, it is concluded here that the behaviour may be part of the defensive strategy in this gregarious species as well as in the solitary *H. australis*. There are gradual, behavioural responses to increasing levels of disturbance, with hiding (behind the leaf) followed by scratching, and finally jerking. The defensive arsenal is multimodal, involving behavioural traits as well as visual (gregariousness; brightly coloured *versus* cryptic integument), chemical (water-soluble chemical compounds), and acoustic (sounds by

scratching) traits. The divergence between the two defensive strategies is gregariousness in *H. crocea* and integumental chemicals in *H. australis*. The identity of these chemicals remains unknown. They may be plant-derived since the digestive tract (as main part of the internal organs) was overall the most active extract tested. The comparison of the defensive strategies between the two *Hemichroa* species reveals, 1) obvious contrasts in larval appearance and gregariousness, 2) points of similarity in jerking, scratching and in the absence of functional ventral glands, and 3) different defensive efficiencies against ants and birds, with single larvae of *H. crocea* being better defended against birds, whereas *H. australis* against ants.

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References

Blomquist GJ, Bagnères A-G (2010) Insect Hydrocarbons. Biology, Biochemistry, and Chemical Ecology. Cambridge University Press, 492 pp. doi: 10.1017/CBO9780511711909

Boevé J-L (2010) Bioassays to elucidate chemical defence strategies in sawfly larvae. In: Zhang W, Liu H (Eds) Behavioral and Chemical Ecology. Nova Science Publishers, NY, 135–153.

Boevé J-L, Angeli S (2010) Ecophysiology of dorsal versus ventral cuticle in flattened sawfly larvae. Naturwissenschaften 97: 595–599. doi: 10.1007/s00114-010-0668-9

Boevé J-L, Dettner K, Francke W, Meyer H, Pasteels JM (1992) The secretion of the ventral glands in *Nematus* sawfly larvae. Biochemical Systematics and Ecology 20: 107–111. doi: 10.1016/0305-1978(92)90097-W

Boevé J-L, Pasteels JM (1985) Modes of defense in nematine sawfly larvae. Efficieny against ants and birds. Journal of Chemical Ecology 11: 1019–1036. doi: 10.1007/BF01020672

Carne PB (1962) The characteristics and behaviour of the saw-fly *Perga affinis affinis* (Hymenoptera). Australian Journal of Zoology 10: 1–34. doi: 10.1071/ZO9620001

Dyar HG (1895) On the larvae of some nematoid and other saw-flies from the northern Atlantic States. Transactions of the American Entomological Society 22: 301–312.

Escherich K (1940–1942) Hymenoptera (Hautflügler) und Diptera (Zweiflügler). Paul Parey (Berlin) 5: 1–746.

Fletcher LE (2008) Cooperative signaling as a potential mechanism for cohesion in a gregarious sawfly larva, *Perga affinis*. Behavioral Ecology and Sociobiology 62: 1127–1138. doi: 10.1007/s00265-007-0541-9

Hograefe T (1984) Substrat-Stridulation bei koloniebildenden Blattwespenlarven von *Hemichroa crocea* (Geoff.) (Hymenoptera: Tenthredinidae). Zoologischer Anzeiger 213: 234–241.

Hopping GR (1937) Sawfly biologies No. 2, *Hemichroa crocea* Geoffroy. Canadian Entomologist 69: 243–249. doi: 10.4039/Ent69243-11

Kriegl M (1964) Zur Biologie und Parasitierung der Blattwespe *Hemichroa crocea* (Geoffr.) (Hymenopt., Nematinae), eines Schädlings der Grünerle in den Alpen. Anzeiger für Schädlingskunde 37: 153–156. doi: 10.1007/BF01813049

Lorenz H, Kraus M (1957) Die Larvalsystematik der Blattwespen (Tenthredinoidea und Megalodontoidea). Abhandlungen zur Larvalsystematik der Insekten (Berlin) 1: 1–339.

Nyman T, Farrell BD, Zinovjev AG, Vikberg V (2006) Larval habits, host-plant associations, and speciation in nematine sawflies (Hymenoptera: Tenthredinidae). Evolution 60: 1622–1637. doi: 10.1111/j.0014-3820.2006.tb00507.x

Rowe C, Halpin C (2013) Why are warning displays multimodal? Behavioral Ecology and Sociobiology 67: 1425–1439. doi: 10.1007/s00265-013-1515-8

Smith DR (1975) The sawfly genus *Hemichroa* Stephens: A review of species (Hymenoptera: Tenthredinidae). Entomologica Scandinavica 6: 297–302. doi: 10.1163/187631275X00145

Taeger A, Altenhofer E, Blank SM, Jansen E, Kraus M, Pschorn-Walcher H, Ritzau C (1998) Kommentare zur Biologie, Verbreitung und Gefährdung der Pflanzenwespen Deutschlands (Hymenoptera, Symphyta). In: Taeger A, Blank SM (Eds) Pflanzenwespen Deutschlands (Hymenoptera, Symphyta). Kommentierte Bestandsaufnahme. Goecke and Evers, Keltern, 49–135.

Taeger A, Blank SM, Liston AD (2010) World Catalog of Symphyta (Hymenoptera). Zootaxa 2580: 1–1064.

Supplementary material 1

Audio of scratch sequences performed by a group of larvae of *H. crocea*

Author: Jean-Luc Boevé

Data type: audio

Explanation note: Audio (mp2; duration 3min25sec) of scratch sequences performed by a group of larvae of *H. crocea*. Larvae were collected in the field (Ave-et-Auffe, 08.X.2013) [P3799], but sounds recorded in indoor conditions (10.X.2013, between 9 and 10 PM). For clarity, the original audio was 20 dB amplified, followed by a 24dB background noise reduction. Note that the third scratch sequence is quite loud. Following Hograefe (1984), a scratch sequence comprises 3–5 behavioural units lasting 224±6 milliseconds each.

Supplementary material 2

Group of larvae of *H. crocea* performing rather synchronized jerks with their abdomen

Author: Jean-Luc Boevé

Data type: video

Explanation note: Video (m4v; duration 19sec) in field conditions of a group of larvae of *H. crocea* performing rather synchronized jerks with their abdomen. The larvae are those shown in Fig. 1d.

Supplementary material 3

Larva of of *H. australis* performing the leaf scratching behaviour

Author: Jean-Luc Boevé

Data type: video

Explanation note: Video (m4v; duration 40sec) in field conditions of a larva of *H. australis* performing the leaf scratching behaviour. Larva is the one shown in Fig. 1b.

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A rare ant on Samoa: first record of the cryptic subfamily Proceratiinae (Hymenoptera, Formicidae) and description of a new *Proceratium* Roger species

Cong Liu[1], Georg Fischer[1], Evan P. Economo[1]

1 *Okinawa Institute of Science and Technology Graduate University, Okinawa, 904-0495, Japan*

Corresponding author: *Cong Liu* (cong.liu@oist.jp)

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Abstract

In this study we present a taxonomic update for the Oceanian *Proceratium*. A recent ant biodiversity survey in Samoa collected an unknown *Proceratium* species, which we describe here as *Proceratium silisili* **sp. n.** This new species also presents the first record of this genus, as well as the whole subfamily Proceratiinae, in Samoa. *Proceratium silisili* is clearly distinguishable from the other Oceanian *Proceratium* species based on the differences in petiole node shape, number of mandible teeth, shape of the abdominal segment IV, as well as the surface sculpture on the head. A detailed description of *P. silisili,* high-quality specimen images, as well as an identification key to Oceanian species and a diagnostic discussion are provided.

Keywords

Oceania, *Proceratium*, Samoa, taxonomy, *Proceratium silisili*

Introduction

Proceratium is the type genus of the Proceratiinae subfamily which also includes the genera *Discothyrea* Roger, *Probolomyrmex* Mayr, as well as the extinct *Bradoponera* Mayr. Within the subfamily, *Proceratium* represents the genus with the highest species diversity of currently 82 extant species (*Discothyrea*: 34, *Probolomyrmex*: 26 valid species; Bolton 2015). All three genera are more or less globally distributed, although comparatively patchily. *Discothyrea* and *Probolomyrmex*, however, both seem to be absent from temperate climates and most of the Nearctic and Palaearctic regions. Many species live hypogaeically, nest in soil, leaf litter, rotten wood, under deep-set stones (Brown 1958a, Baroni Urbani and de Andrade 2003, Fisher 2005), but also in tree branches (Brown 1974). Due to cryptic lifestyles and local rarity uncertainties about their biologies and biogeographic distributions are high (Baroni Urbani and deAndrade 2003, Hita Garcia et al. 2014). Specialised predatory behaviour has been documented in both *Discothyrea* and *Proceratium*. Workers were carrying and feeding on arthropod (probably spider) eggs and storing them in their nests (Brown 1958a, 1958b, 1974, 1980, Dejean and Dejean 1998, Dejean et al. 1999, Fisher 2005b, Katayama 2013).

According to Hita Garcia et al. (2014) the taxonomy of the genus is in a moderately good condition, with a relatively recent global revision by Baroni Urbani and de Andrade (2003) providing a valuable basis for smaller taxonomic updates and regional revisions (Bharti and Wachkoo 2014, Fisher 2005b, Hita Garcia et al. 2014, Hita Garcia et al. 2015, Xu 2006). For Japan Onoyama and Yoshimura (2002) provided a taxonomic revision for the genus, raising the number of *Proceratium* species from three to four. With increasing use of subterranean sampling techniques, more new taxa can be expected in the future. For example, due to recent intensive collection efforts on Madagascar and neighboring islands (Fisher 2005a), 11 undescribed species have been recorded for the Malagasy region (Hita Garcia and Fisher, unpublished – see www.antweb.org) – which is a large number for this relatively small genus.

In the Oceanian region, nine species are presently known, eight of them recognized by Baroni Urbani and de Andrade (2003): *Proceratium austronesicum* De Andrade, 2003, *P. ivimka* De Andrade, 2003 and *P. snellingi* Baroni Urbani & de Andrade, 2003 in Papua New Guinea, *P. caledonicum* De Andrade, 2003 and *P. politum* De Andrade, 2003 in New Caledonia, *P. oceanicum* De Andrade, 2003 and *P. relictum* Mann, 1921 in Fiji, and *P. papuanum* Emery, 1897 in Malaysia, Indonesia, Papua New Guinea, Philippines, and the Solomon Islands. Earlier this year, Hita Garcia et al. (2015) described *P. vinaka* as a third Fijian *Proceratium* species (=*P.* sp FJ01 in Sarnat and Economo (2012)).

In the present publication, *Proceratium silisili* sp. n., the tenth Oceanian species, is described. It was collected on Samoa and represents the first record for both, the genus *Proceratium* and the subfamily Proceratiinae on these islands (Wetterer and Vargo 2003). Morphologically, it is very distinct from the other species found across the region and shows a unique combination of characters that distinguishes it from all other Oceanian *Proceratium* species. Thus, it seems likely that *P. silisili* sp. n. is not a

member of the *silaceum* clade as defined by Baroni Urbani and de Andrade (2003). This clade currently includes all other Oceanian species (Hita Garcia et al. 2015) and 20 species with very different geographic distributions. Judging by the presence of mostly ancestral character states and an absence of real synapomorphies in the majority of species belonging to this clade (Baroni Urbani and de Andrade (2003) – see strict consensus tree, fig. 29), it is possible that the *silaceum* clade is not a phylogenetic unit and instead comprises several unrelated clades. The outer morphology of *P. silisili* sp. n. more closely resembles that of several Neotropical *Proceratium* species in the *micrommatum* clade – in particular with mandibles often containing less than six teeth (e.g. *P. micrommatum* & *P. mexicanum* with 4 teeth), petiole node stoutly nodiform, not squamiform as in *silaceum* clade, ventral petiole process small triangular, and abdominal segment IV strongly recurved. However, since the different clades were defined on the basis of presence-absence analyses of 62 morphological characters, it seems prudent to exercise caution in equating them with true phylogenetic relationships. A phylogenetic analysis including molecular data would be the next logical step in untangling the taxonomy of this phylogenetically basal ant genus.

Abbreviations of depositories

The collection abbreviation follows Evenhuis (2015). The holotype of the new species will be deposited at the following institution:

OSAKA Osaka Museum of Natural History (OMNH), Osaka, Japan

Material and methods

The holotype of the new species was collected during an inventory of the ant fauna of Samoa in 2015 by C. Liu and E.M. Sarnat. Morphological observations and measurements were performed with a Leica M165 C stereomicroscope equipped with an orthogonal pair of micrometres at a magnification of 100×. Measurements were recorded in millimetres to three decimal places and rounded to two decimal places for presentation. The measurements and indices used in this study follow Hita Garcia et al. (2014, 2015) who introduced a few new measurements and indices to *Proceratium* taxonomy:

EL Eye length: maximum length of eye measured in oblique lateral view.

HL Head length: maximum measurable distance from the mid-point of the anterior clypeal margin to the mid-point of the posterior margin of head, measured in full-face view. Impressions on anterior clypeal margin and posterior head margin reduce head length

HLM Head length with mandibles: maximum head length in full-face view including closed mandible

HW Head length: Maximum head width directly behind the eyes, measured in full-face view

HFeL Hind femur length: maximum length of hind femur measured along its external face

HTiL Hind tibia length: maximum length of hind tibia measured along its external face

HBaL Hind basitarsus length: maximum length of hind basitarsus measured along its external face

LT3 Abdominal tergum III length: maximum length of abdominal tergum III (= length of segment III) in lateral view

LS4 Abdominal sternum IV length: maximum length of abdominal sternum IV following Ward (1988)

LT4 Abdominal tergum IV length: maximum length of abdominal tergum IV following Ward (1988)

PeL Petiolar length: maximum length of the petiole in dorsal view including its anterior prolongation

PeW Petiolar width: maximum width of petiole in dorsal view

SL Scape length: maximum length of scape shaft excluding basal condyle

TL Total body length: combined length of HLM + WL + PeL + LT3 + LT4

WL Weber's length: diagonal length of mesosoma in lateral view from the anterior-most point of pronotal slope (excluding neck) to posterovental margin of propodeal lamella or lobe

CI Cephalic index: HW / HL × 100

OI Ocular index: EL / HW × 100

SI Scape index: SL / HL × 100

DPeI Dorsal petiole index: PeW / PeL × 100

ASI Abdominal segment index: LT4 / LT3 × 100

IGR Gastral reflexion index: LS4 / LT4

Results

Identification key to workers of Oceanic islands *Proceratium* (adapted from Baroni Urbani and de Andrade (2003), not including Papua New Guinea)

1 Dorsal face of petiole node sub-rectangular, almost as wide as long (DPeI 128) (Fig. 1A); mandibles with 4 distinct teeth; body covered with short, dense, decumbent pilosity, longer erect or suberect hairs completely absent; abdominal segment IV strongly recurved (IGR 0.25) (Fig. 1B) (Samoa) ..... ***P. silisili***

– Dorsal face of petiole node narrow and transversally compressed, distinctly wider than long (DPeI>150) (Fig. 1C); mandibles with 6 or more teeth or denticles; longer erect or suberect hairs present in addition to short decumbent pilosity; abdominal segment IV distinctly less recurved (IGR 0.46–0.54) (Fig. 1D)..... **2**

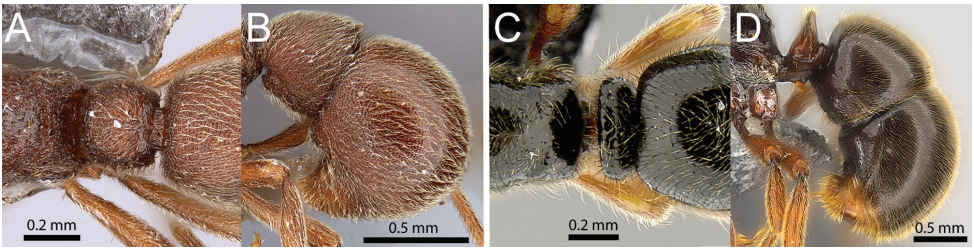

Figure 1. *Proceratium silisili* sp. n. (CASENT0741888). **A** Petiole in dorsal view **B** Gaster in profile. *Proceratium oceanicum* (CASENT0171053) **C** Petiole in dorsal view. *Proceratium relictum* (CASENT0194740) **D** Petiole in profile.

2 Subpetiolar process reduced and rounded without any distinct projections (Fig. 2A) (Fiji) ..... ***P. vinaka***
– Subpetiolar process not reduced and rounded, either with spiniform or subtriangular / subrectangular lamellate projections (Fig. 2B, C, D) **3**
3 Petiole shape in profile squamiform, dorsally distinctly narrower than at the base and anterior face oblique (Fig. 2B, C) **4**
– Petiole shape in profile flattened subrectangular, at apex not distinctly narrower than at the base and anterior face vertical or subvertical (Fig. 2D)..... **5**
4 Subpetiolar process spiniform (Fiji) (Fig. 2B) ..... ***P. oceanicum***
– Subpetiolar process lamellate, subtriangular (Fiji) (Fig. 2C)..... ***P. relictum***

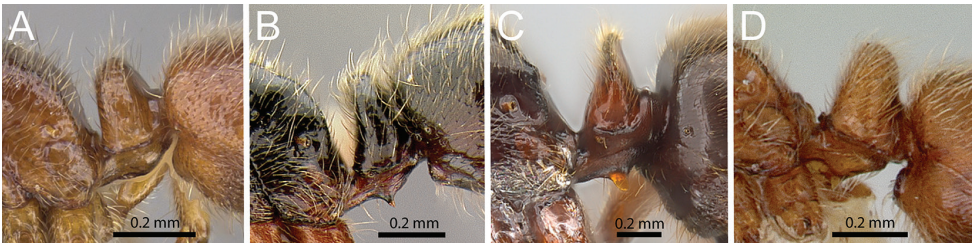

Figure 2. Petiole and subpetiolar process in profile view. **A** *Proceratium vinaka* (CASENT0187587) **B** *Proceratium oceanicum* (CASENT0171053) **C** *Proceratium relictum* (CASENT0194740) **D** *Proceratium caledonicum* (CASENT0172099).

5 Frontal lobes very small, each lobes' surface area covering less than clypeal area in between frontal lobes (New Caledonia) (Fig. 3A) ..... ***P. caledonicum***
– Frontal lobes larger than clypeal area between frontal lobes (Fig. 3B, C)..... **6**
6 Larger species (HL 0.69–0.74, WL 0.91–1.00); body smooth and shiny, head minutely punctate (New Caledonia) (Figs 3B, 4A) ..... ***P. politum***
– Distinctly smaller species (HL 0.55–0.60, WL 0.66–0.70); head and body weakly punctate (Fig. 3C, 4B) (Indonesia, Malaysia, Papua New Guinea, Philippines, Solomon Islands)..... ***P. papuanum***

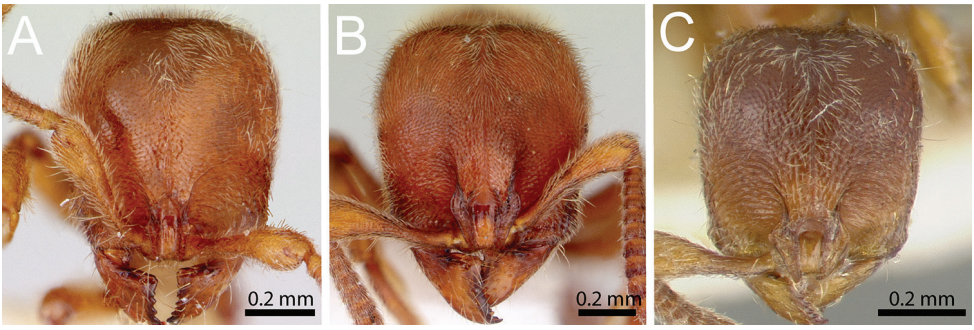

Figure 3. Head in full-face view. **A** *Proceratium caledonicum* (CASENT0172099) **B** *Proceratium politum* (CASENT0172113) **C** *Proceratium papuanum* (CASTYPE06965).

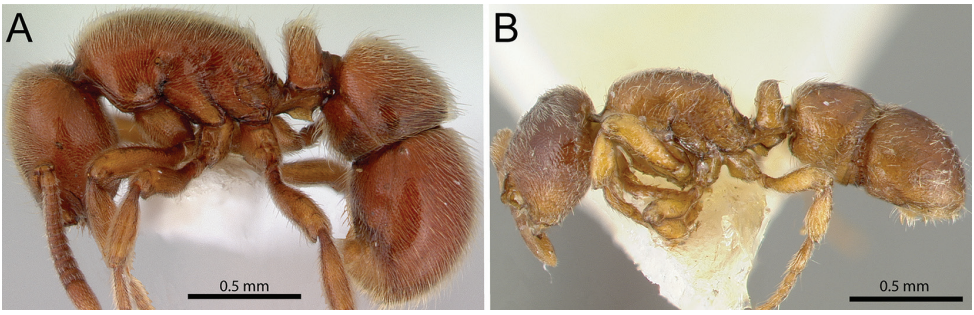

Figure 4. Body in profile view. **A** *Proceratium politum* (CASENT0172113) **B** *Proceratium papuanum* (CASTYPE06965).

***Proceratium silisili* Liu, Fischer & Economo, sp. n.**
http://zoobank.org/9AB0E143-16D2-4006-AEC1-F356097C9F03
Fig. 5

Type material. Holotype. Pinned worker, Samoa, Savai, 5.4km SSW A'opo vil, Mt. Silisili, 13°38'10"S, 172°30'23"W, 1200m, montane rainforest, leaf litter, 22.iv.2015 (*E. Sarnat & C. Liu*) (**OSAKA**: CASENT0741888).

Diagnosis. *Proceratium silisili* differs from the other Oceanian members of *Proceratium* by the following combination of characters: mandible with 4 distinct teeth; petiole node in dorsal face-view subrectangular, almost as long as wide (DPeI 128); abdominal segment IV in profile view strongly recurved (IGR 0.25), highly rounded and almost spherical in its appearance; whole body very densely punctate, except for small smooth and shiny spot posterior of frontal lobes; pilosity dense, uniformly short and decumbent, long and erect or suberect hairs completely absent. Using the above character combination, *P. silisili* can also be distinguished easily from its geographically closest congeners in Fiji. *Proceratium oceanicum*, *P. relictum* and *P. vinaka* all have elongate-triangular mandibles with relatively long masticatory margins and more than six teeth

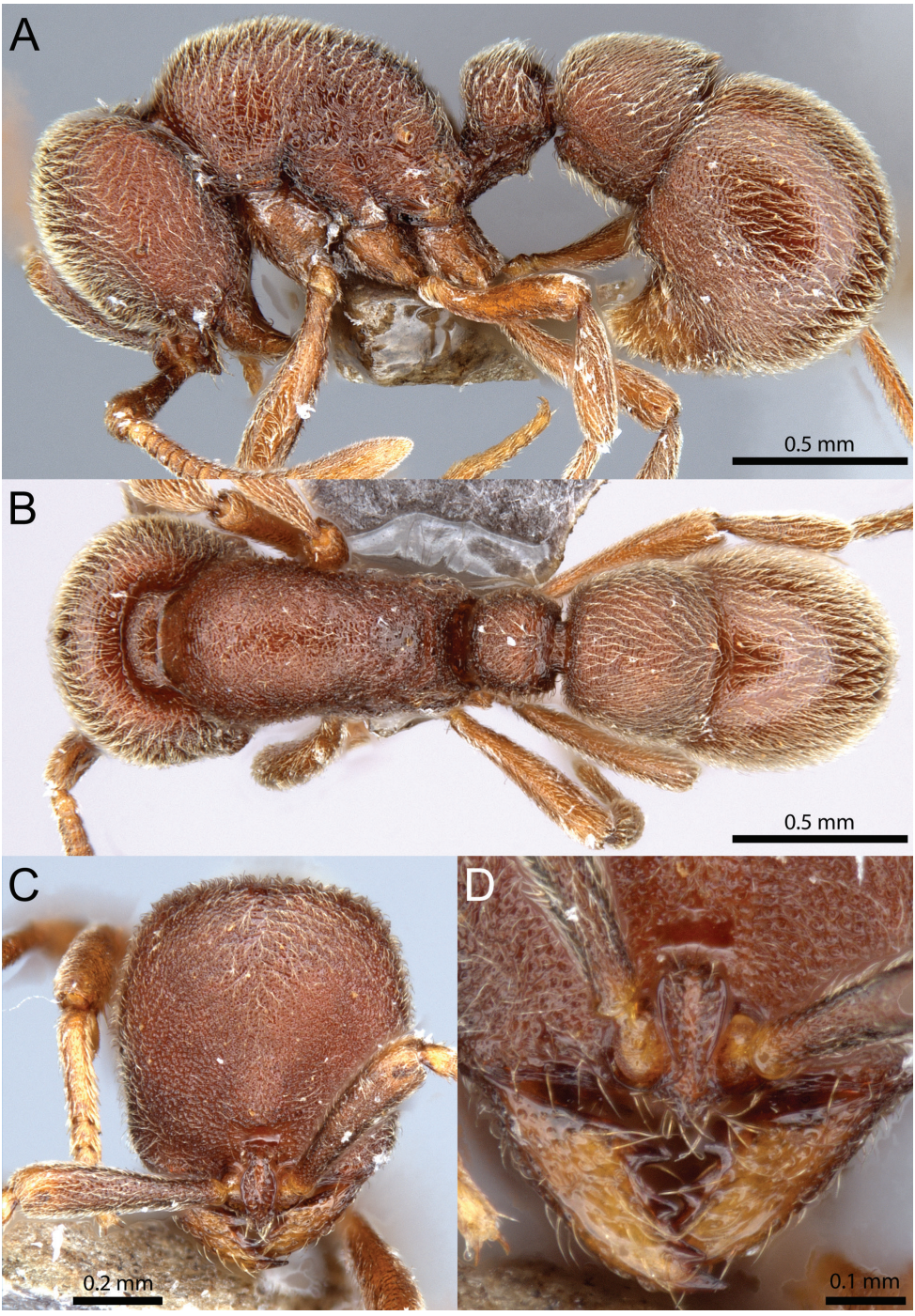

Figure 5. *Proceratium silisili* sp. n. (CASENT0741888). **A** Body in profile **B** Body in dorsal view **C** Head in full-face view **D** Mandible in frontal view.

or denticles, petiole in profile either squamiform or narrow, transversally compressed subrectangular, abdominal segment IV not strongly recurved (IGR > 0.45), and long standing hairs present.

Worker measurements (N=1). TL 3.36; EL 0.04; SL 0.56; HL 0.83; HLM 0.99; HW 0.75; WL 0.97; HFeL 0.65; HTiL 0.52; HBaL 0.27; PeL 0.24; PeW 0.31; DPeL 128; LT3 0.4; LS4 0.2; LT4 0.8; OI 6; CI 90; SI 74; IGR 0.25; ASI 204.

Worker description. In full-face view, head subrectangular, longer than wide (CI 90), sides and posterior head margin convex. Mandibles with four distinct, well developed teeth, curved triangular with short masticatory margin. Clypeus strongly reduced, anteromedially with a small, triangular projection, anterolaterally reduced to extremely narrow with a thin wall in front of antennal sockets. Frontal carinae absent or vestigial, frontal lobes narrow, not covering the antennal sockets, posteriorly strongly convergent, ending just after posterior limit of antennal sockets. Eyes very small (OI 6), consisting of single ommatidium.

Mesosoma in profile convex, almost as long as maximum head length including mandibles. Lower mesopleuron with well impressed sutures, propodeum without posterior teeth, propodeal lobes small, reduced and blunt, posterior declivity relatively steep, in posterolateral and posterodorsal view separated from lateral propodeum by a distinct margin, propodeal spiracle circular and facing posterior end of mesosoma, situated slightly above mid height. Front and hind tibia with pectinate spur present, both without calcar of strigil, mesotibial spur absent, pretarsal claws simple, arolia absent. Petiole node in profile about as high as long, anterior face almost vertical, the dorsum almost flat, anteriorly and posteriorly weakly rounded, in dorsal view subrectangular with convex sides and slightly wider than long (DPeL 128), ventral process a small, blunt tooth.

Abdominal segment III in dorsal view anteriorly wider than petiole, posteriorly diverging, in profile abdominal sternite III anterolaterally with small, angulate anterior projection on either side of shallow median depression. Constriction between abdominal segments III and IV distinctly impressed. Abdominal segment IV strongly recurved (IGR 0.25), highly rounded and almost spherical in its appearance, abdominal tergum IV about twice as long as abdominal tergum III (ASI 204). Remaining abdominal segments reduced and comparatively inconspicuous, curved forwards.

Whole body in profile and in dorsal view covered with uniform dense layer of short, decumbent hairs, longer erect hairs completely absent.

Sculpture on mandibles irregularly punctate, on remainder of body very densely punctate, except for small smooth and shiny spot posterior of frontal lobes. Punctation also less strongly developed on abdominal segment IV, tergum IV appearing more shiny.

Body color dark red, legs and flagella of lighter, reddish brown coloration.

Distribution and ecology. At present, the new species is only known from Savai island in Samoa, and is likely endemic to Samoa. The type locality is a montane rainforest on Mt. Silisili, situated at an elevation of 1200m. Only one single worker of the new species was collected through leaf litter extraction. The genus *Proceratium* has not been previously reported from Samoa according to the GABI database (Guénard et

al. in review). There is no additional information about its ecology due to the limited available material.

Taxonomic notes. The identification of *P. silisili* within the Oceanian region can be easily performed with the character combination given in the diagnosis. The new species is morphologically distinct from all the other members in the Oceanian region. It is thus possible that the Samoa species has a different origin than the other species in the region and that it is a descendent of a New World ancestor from the *micrommatum* clade. Several of the observed morphological characters are in support of this hypothesis: the mandibles have four teeth only, clypeus medially narrow with triangular projection, and mesotibiae without pectinate spur present. Also the subrectangular shape of the petiole and the absence of a lamellate ventral process, as well as the strongly recurved and almost spherical shape of the abdominal segment IV point in the same direction, although a triangular to strongly reduced ventral process can also be observed in the *Proceratium* species present on Fiji. A more definitive placement of the new species within the genus phylogeny, however, has to be postponed until more conclusive (e.g genetic) data can be analysed.

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References

Baroni Urbani C, de Andrade ML (2003) The ant genus *Proceratium* in the extant and fossil record (Hymenoptera: Formicidae). Museo Regionale di Scienze Naturali – Monografie 36: 1–480.

Bharti H, Wachkoo A (2014) New synonymy of *Proceratium williamsi* Tiwari (Hymenoptera, Formicidae). ZooKeys 388: 69–72. doi: 10.3897/zookeys.388.6972

Bolton B (2015) An online catalog of the ants of the world. Available from http://antcat.org [accessed 1 August 2015]

Brown WL (1958a) Predation of arthropod eggs by the ant genera *Proceratium* and *Discothyrea*. Psyche 64: 115. doi: 10.1155/1957/45849

Brown WL (1958b) Contributions toward a reclassification of the Formicidae. II. Tribe Ectatommini (Hymenoptera). Bulletin of the Museum of Comparative Zoology 118: 173–362.

Brown WL (1974) A remarkable new island isolate in the genus *Proceratium* (Hymenoptera: Formicidae). Psyche 81: 70–83. doi: 10.1155/1974/90949

Brown WL (1980) A remarkable new species of *Proceratium*, with dietary and other notes on the genus (Hymenoptera: Formicidae). Psyche 86: 337–346. doi: 10.1155/1979/78461

Dejean A, Dejean A (1998) How a ponerine ant acquired the most evolved mode of colony foundation. Insectes Sociaux 45: 343–346.

Dejean A, Grimal A, Malherbe MC, Suzzoni JP (1999) From specialization in spider egg predation to an original nesting mode in a "primitive" ant: a new kind of lestobiosis. Naturwissenschaften 86(3): 133–137. doi: 10.1007/s001140050585

Evenhuis NL (2015) The insect and spider collections of the world website. Available from http://hbs.bishopmuseum.org/codens [accessed 1 August 2015]

Fisher BL (2005a) A model for a global Inventory of ants: A case study in Madagascar. In: Jablonski NG (Ed.) Biodiversity: A Symposium Held on the Occasion of the 150th Anniversary of the California Academy of Sciences June 17–18, 2003. Proceedings of the California Academy of Sciences, ser. 4 56(Suppl. 1): 78–89.

Fisher BL (2005b) A new species of *Discothyrea* Roger from Mauritius and a new species of *Proceratium* Roger from Madagascar (Hymenoptera: Formicidae). Proceedings of the California Academy of Sciences 56: 657–667.

Guénard B, Weiser M, Gomez K, Narula N, Economo EP (in review) Revealing the distribution of ants: the Global Ant Biodiversity Informatics database.

Hita Garcia F, Hawkes PG, Alpert GD (2014) Taxonomy of the ant genus *Proceratium* Roger (Hymenoptera, Formicidae) in the Afrotropical region with a revision of the *P. arnoldi* clade and description of four new species. ZooKeys 447: 47–86. doi: 10.3897/zookeys.447.7766

Hita Garcia F, Sarnat EM, Economo EP (2015) Revision of the ant genus *Proceratium* Roger (Hymenoptera, Proceratiinae) in Fiji. ZooKeys 475: 97–112. doi: 10.3897/zookeys.475.8761

Katayama M (2013) Predatory behaviours of *Discothyrea kamiteta* (Proceratiinae) on spider eggs. Asian Myrmecology 5: 121–124.

Onoyama K, Yoshimura M (2002) The ants of the genus *Proceratium* (Hymenoptera: Formicidae) in Japan. Entomological Science 5: 29–50.

Sarnat EM, Economo EP (2012) The ants of Fiji. University of California Publications in Entomology 132: 1–398.

Ward PS (1988) Mesic elements in the Western Nearctic ant fauna: taxonomic and biological notes on *Amblyopone*, *Proceratium*, and *Smithistruma* (Hymenoptera: Formicidae). Journal of the Kansas Entomological Society 61: 102–124.

Wetterer JK, Vargo DL (2003) Ants (Hymenoptera: Formicidae) of Samoa. Pac Sci 57: 409–419. doi: 10.1353/psc.2003.0036

Xu ZH (2006) Three new species of the ant genera *Amblyopone* Erichson, 1842 and *Proceratium* Roger, 1863 (Hymenoptera: Formicidae) from Yunnan, China. Myrmecologische Nachrichten 8: 151–155.

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RESEARCH ARTICLE

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Colony distribution and prey diversity of *Cerceris fumipennis* (Hymenoptera, Crabronidae) in British Columbia

Troy Kimoto[1], Josie Roberts[2], Richard L. Westcott[3], Eduard Jendek[4], Matthias Buck[5], David Holden[1], Philip D. Careless[6]

1 *Canadian Food Inspection Agency, 4321 Still Creek Drive, Burnaby, British Columbia, Canada, V5C 6S7* **2** *Canadian Food Inspection Agency, 506 West Burnside Road, Victoria, British Columbia, Canada, V8Z 1M5* **3** *Oregon Department of Agriculture, 635 Capitol NE, Salem, Oregon, United States of America, 97301-2532* **4** *Canadian Food Inspection Agency, 960 Carling Avenue, Ottawa, Ontario, Canada, K1A 0Y9* **5** *Royal Alberta Museum, 12845-102nd Avenue, Edmonton, Alberta, Canada, T5N 0M6* **6** *Toronto, Ontario, Canada, M4R 1H9*

Corresponding author: *Troy Kimoto* (troy.kimoto@inspection.gc.ca)

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Abstract

Cerceris fumipennis Say, 1837 (Hymenoptera: Crabronidae) is a wasp that provisions its subterranean nests with jewel beetles (Coleoptera: Buprestidae). At 3 newly discovered colonies in British Columbia (BC), *C. fumipennis* prey were collected by excavating the subterranean nests, using sweep nets to capture paralyzed prey in the grasp of a female returning to her nest, or collecting prey discarded at the nest entrance. In total, 9 species were collected: *Acmaeodera idahoensis* Barr, *Agrilus crataegi* Frost, *Agrilus granulatus populi* Fisher, *Anthaxia* (*Haplanthaxia*) *caseyi caseyi* Obenberger, *Chrysobothris laricis* Van Dyke, *Chrysobothris leechi* Barr, *Phaenops drummondi* (Kirby), *Phaenops gentilis* (LeConte) and *Phaenops intrusa* (Horn). *Anthaxia caseyi caseyi* was the smallest beetle (4.2 mm) while *C. leechi* was the largest (12.0 mm). The average size of all buprestid prey taken by females from all 3 colonies was 8.8 mm. These represent the first prey records for *C. fumipennis* in BC and with the exception of *P. drummondi* are new prey records for this wasp. A single *Harpalus affinis* (Schrank) (Coleoptera: Carabidae) was discovered within a brood cell containing *Acmaeodera* spp. elytra, but it is unclear if this beetle was placed in the cell by a female wasp.

Keywords
Cerceris fumipennis, Hymenoptera, Crabronidae, biosurveillance, pest detection

Introduction

Cerceris fumipennis Say, 1837 (Hymenoptera: Crabronidae) is a ground-nesting wasp in which females provision their nests with paralysed buprestid beetles in jelly bean shaped subterranean cells (Scullen 1965; Hook and Evans 1991; Marshall et al. 2005). In Canada, colonies have been discovered in western Québec and throughout southern Ontario (Buck 2004; Marshall et al. 2005; Careless et al. 2009; Careless 2010). In 2012 and 2013, a total of 1 male and 4 females were captured by sweep net at a colony in Merritt, British Columbia (BC) (Kimoto and Buck 2015). This represents the first time *C. fumipennis* has been recorded from BC since 1935 (2 females collected by R.H. Beamer on 3 August 1935; University of Kansas Natural History Museum, Lawrence, Kansas, US) and also represents the first recorded colony.

Female *Cerceris fumipennis* are adept at capturing a wide variety of buprestid beetles, and have been the source for various new provincial, state and national records (Marshall et al. 2005). Therefore, it has been used in eastern North America by the Canadian Food Inspection Agency (CFIA) and other departments as a biosurveillance tool to detect the non-indigenous emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) (Marshall et al. 2006; Careless et al. 2009; Nalepa et al. 2012; Careless et al. 2014). In the eastern US, citizen-scientist programs have been established whereby colonies are "adopted" and monitored by the public. In 2012, an adopted *C. fumipennis* colony was responsible for the first state record of emerald ash borer in Connecticut (Rutledge et al. 2013). It is the intention of the senior author to establish citizen-scientist programs in BC to detect non-indigenous buprestid beetles, but additional colonies need to be discovered before this can occur.

In 2013 and 2014, additional sites from the Greater Vancouver Region, the Fraser Canyon, and the Okanagan Valley were examined for the presence of *Cerceris fumipennis* colonies. This paper outlines the variety of sites examined, the location of two new colonies and first prey records for *C. fumipennis* in BC.

Methods and materials

Searching for *Cerceris* colonies

In 2013, 13 sites in Ashcroft, Spence's Bridge, Cache Creek, Skihist, Boston Bar, Vernon, and West Kelowna were examined. In 2014, 85 sites in Kamloops, West Kelowna, Coldstream, Vernon, Osoyoos, Oliver, Okanagan Falls, Penticton, Summerland, Chilliwack, Merritt, Logan Lake, Lytton, Hope, Coquihalla Highway, Kane Valley Road, Cloverdale, Lillooet, Duffy Lake Road, Richmond, Ashcroft, Cache Creek, Clinton, Hat Creek, and Pavilion were examined.

Google Maps (2014) was used to find baseball fields and other areas with bare patches of soil exposed to full sun. The ground at each site was examined for the presence of circular holes and tumuli. Where possible, the central location of the Merritt colony was used to reinforce the search image of nest entrance shape and size. When entrance holes approximately 5–7 mm in diameter were discovered, clear plastic cups were placed over these holes and returning wasps were captured in sweep nets. Specimens were placed in glass vials with 75% ethanol and submitted to Matthias Buck for identification and deposited at the Royal Alberta Museum in Edmonton.

Collecting *Cerceris fumipennis* prey at nest entrances

Beetles were collected at *Cerceris fumipennis* colonies during 4 days in July and 2 days in August 2014. Clear plastic cups were placed over entrance holes and a sweep net was used to collect female wasps returning with prey. The nest entrances and completely enclosed tumuli were also examined for dropped prey.

Cerceris fumipennis nest excavation

On 25 July 2014, a single nest at the north end of St. Georges Road, Lytton was excavated. Using a trowel, soil around the nest entrance was removed and placed onto a cleared area. The soil was carefully broken into smaller pieces. Upon discovering a cell containing buprestid beetles or wasp cocoons, a tape measure was used to determine the depth below the surface. The contents of each cell was described and recorded. On 7 August 2014, a single nest that was at least 2 feet away from any other visible *Cerceris fumipennis* nest entrances was excavated at the Central Park colony in Merritt. Methodology followed that used in Lytton. On August 11, an additional 4 nests were excavated in Merritt, but the depth at which buprestid specimens were discovered was not recorded.

All beetles collected at the nest entrances and during excavation were placed in 100% USP/FCC propylene glycol and sent to the CFIA entomology laboratory in Victoria where they were identified and their length measured, to the nearest one tenth mm, using a Leica microscope (DFC495) along with Leica Applications Suite 4.1. These specimens were pinned, labelled and then sent to Eduard Jendek and Richard Westcott for confirmation of species determination.

Soil analysis

One soil sample immediately adjacent to (Lillooet) or within (Merritt, Lytton) a known *Cerceris fumipennis* nest was collected from each of the 3 colonies. Approximately 50 g of air-dried soil was submitted to the British Columbia Ministry of Environment to assess soil texture (sand, silt and clay composition) and organic content (2 mm sieve pass, loss on ignition).

Results

New colonies

Thirteen sites were examined in 2013 and 85 sites were examined from 30 June to 29 July 2014 (Table 1). Thirty percent of all sites were baseball fields (red shale), 22% were dirt roads (compact soil), 8% were baseball fields (compact soil) and 6% were parking lots (compact soil). On 11 July 2014, one female *Cerceris fumipennis* was captured in a sweep net in a dirt parking lot at the north end of St. Georges Road in Lytton. This colony occurs a few hundred metres north of the Stein Valley Nlakapamux School in compact sand adjacent to a baseball field (Fig. 1). A few ant nests but no *C. fumipennis* nests were observed in this baseball diamond. Two females were also captured on 17 July 2014 in a sweep net at a dirt parking lot, comprised of compact sand, adjacent to P'egp'ig'lha Community Centre in Lillooet (Fig. 2). Table 2 and Figure 3 provide additional information and show the relative location of each colony. As per the previous description of *C. fumipennis* specimens from BC (Kimoto and Buck 2015), the average fore wing length of the Lytton and Lillooet specimens is approximately 10.5 mm which

Table 1. List of sites examined for *Cerceris* colonies in BC, excluding Central Park, Merritt.

Year	Site Type	Substrate	Number of sites examined	*C. fumipennis* colonies
2013	Dirt roads, parking lots	Gravel	1	0
	Baseball fields	Red shale	4	0
	Baseball fields	Compact sand	2	0
	Baseball fields	Loose sand	1	0
	Running ovals	Gravel	2	0
	School yard	Gravel	2	0
	Field	Compact sand	1	0
2014	Dirt roads, parking lots	Gravel	5	0
	Dirt road	Compact sand	22	0
	Parking lot	Compact sand	6	2
	Baseball fields	Red shale	25	0
	Baseball fields	Compact sand	6	0
	Baseball fields	Loose sand	3	0
	Running ovals	Gravel	3	0
	Running ovals	Compact sand	1	0
	Equestrian centre	Loose sand or gravel	3	0
	Airstrip	Gravel or compact sand	2	0
	Campground	Gravel	4	0
	Picnic site	Gravel	1	0
	Beach (lake)	Loose sand	1	0
	Cemetery	Gravel, compact sand	1	0
	Natural area (desert)	Covered in vegetation	1	0
	Misc. open area	Loose sand	1	0

Figure 1. *Cerceris fumipennis* colony north of the Stein Valley Nlakapamux School, Lytton, BC.

Table 2. Site description of the 3 *Cerceris fumipennis* colonies in BC.

Site name	City	Date	Site type	Geographic coordinates (DD)	Elevation (m)
Central Park	Merritt	3 August 2012	Dirt path	50.11875N, -120.78348W	599
Stein Valley Nlakapamux School	Lytton	11 July 2014	Parking lot	50.27161N, -121.60358W	220
P'egp'ig'lha Community Centre	Lillooet	17 July 2014	Parking lot	50.67669N, -121.94610W	1136

Figure 2. *Cerceris fumipennis* colony adjacent to the P'egp'ig'lha Community Centre, Lillooet, BC.

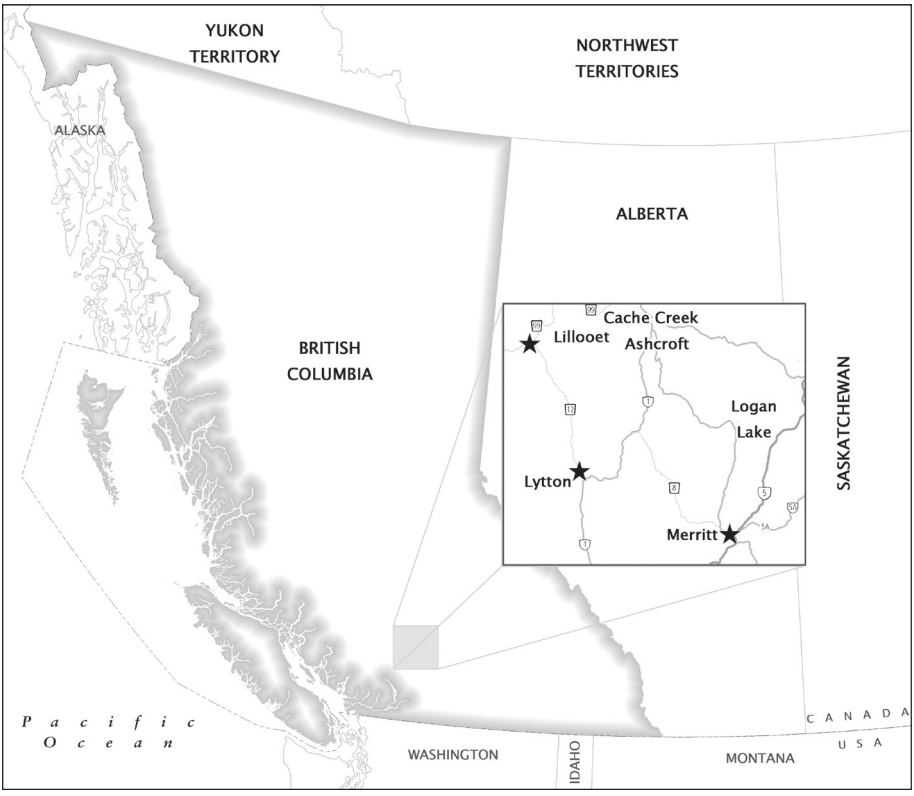

Figure 3. Map showing the location of the 3 *Cerceris fumipennis* colonies.

is within the lower range of eastern specimens. Lytton and Lillooet occur within the Ponderosa Pine biogeoclimatic zone which is characterized by very warm, dry summers and cool winters with light snow cover (Anonymous 2007). Large diameter (+45 cm dbh) ponderosa pine, *Pinus ponderosa* Douglas ex. Lawson and C. Lawson (Pinaceae), is the most common tree at both sites, with declining and dead pine observed near the Lytton colony. Some hardwood trees were observed within a few hundred metres of the Lytton colony, while both sites also contained woody shrubs. The Lytton colony occurs at the junction of the northern portion of the Cascade Mountains and the eastern edge of the Coast Mountains. The Lillooet colony occurs within the eastern limits of the Coast Mountains while the Merritt colony lies within the Thompson Plateau (Holland 1976). Including the Merritt colony, a total of 99 sites was examined but only 3 *C. fumipennis* colonies have been discovered in BC resulting in a 3% success rate.

The following wasps were also collected in sweep nets at the 3 *Cerceris fumipennis* colonies. Crabronidae: *Bembix americana* Fabricius (Lytton), *Cerceris nigrescens* Smith (Merritt), *Ectemnius dilectus* (Cresson) (Merritt), *Philanthus multimaculatus* Cameron (Lytton), *Tachytes sayi* Banks (Lytton), and *Zanysson texanus* (Cresson) (Lillooet). Chrysididae: *Parnopes edwardsii* (Cresson) (Lillooet). Halictidae: *Agapostemon* sp. (Merritt). Megachilidae: *Coelioxys* sp. (Lytton). Sphecidae: *Ammophila azteca* Cameron (Lytton). Vespidae: *Polistes dominula* (Christ) (Merritt, Lytton). However, *Cerceris californica* Cresson, another wasp known to prey upon buprestids (Scullen 1965) and previously recorded from British Columbia, was neither observed nor collected at any of the examined sites.

Prey collected at nest entrances

The majority of jewel beetles were collected at the Merritt colony and included *Chrysobothris leechi* Barr (Fig. 4), *C. laricis* Van Dyke, *Agrilus crataegi* Frost, *Phaenops drummondi* (Kirby) and *P. intrusa* (Horn). *Anthaxia caseyi caseyi* Obenberger was collected at the Lillooet colony. This specimen was discovered lying within a collapsed nest entrance covered with soil. Two other collapsed nest entrances were also covered by soil, but buprestid beetles were not observed.

Nest excavation

A 30.5 × 30.5 × 20 cm hole was dug at the Lytton site. Between ground level and 10 cm below grade, fly pupae and other insect larvae were collected within 4 cells. Based on the presence of wasp cocoons and/or buprestids, *Cerceris fumipennis* cells were discovered from a depth of 10 to 20 cm. In total, 17 *C. fumipennis* cells were discovered, with most occurring 14 to 18 cm below the surface. Eleven cells contained a single *C. fumipennis* cocoon, but only fragments remained in the other cells. All cells with the exception of one located at a depth of 15.25 cm only contained buprestid body parts; primarily

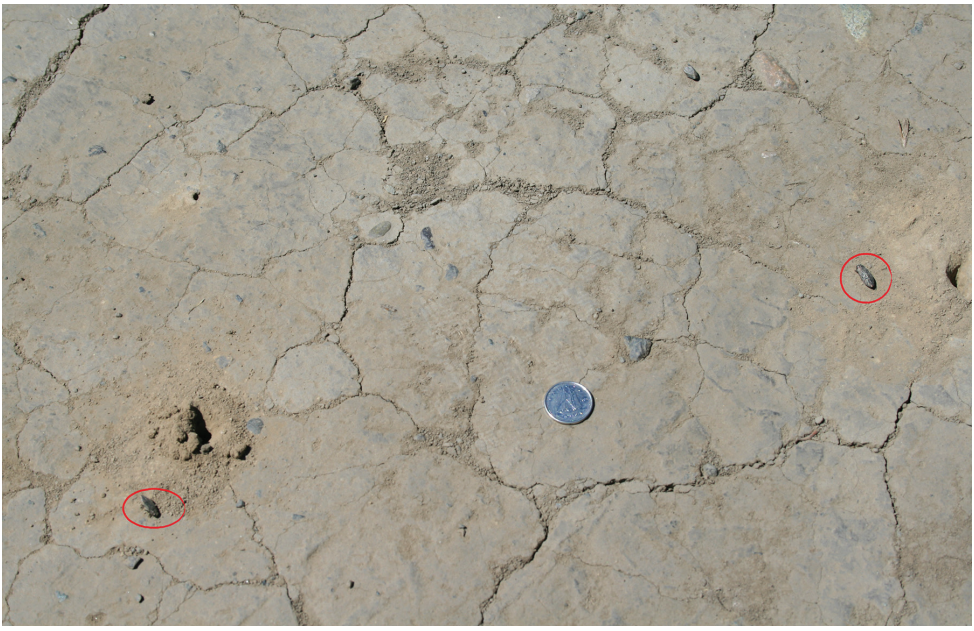

Figure 4. Two discarded *Chrysobothris leechi* at 2 different nest entrances, 11 July 2014, Merritt, BC.

Acmaeodera spp. and *Phaenops* spp. elytra were uncovered. The jelly bean-shaped cell at 15.25 cm contained 4 buprestids wrapped together of which only 3 could be identified as *Phaenops gentilis* (LeConte). *Acmaeodera idahoensis* Barr and *P. drummondi* were also collected during excavation of this nest. One *Harpalus affinis* (Schrank) (Coleoptera: Carabidae) was discovered within another cell at 15.25 cm that also contained *Acmaeodera* spp. elytra. A similar sized hole was dug on 7 August 2015 at the Central Park colony. The first *C. fumipennis* cell was uncovered at a depth of 9.5 cm and the last cells were 15.3 cm below grade. Half the cells were located between 12.7 and 15.3 cm beneath the surface. In total, 23 *C. fumipennis* cells were uncovered of which 13 had intact cocoons (2) or cocoon fragments (11). Only the cells at 12.7 and 14 cm below grade contained intact buprestids, *Anthaxia caseyi caseyi*. Otherwise all other cells contained elytra or other buprestid body parts primarily belonging to *Agrilus* and *Anthaxia* spp. During nest excavation on 11 August 2014, intact specimens of *Agrilus granulatus populi* Fisher, *A. caseyi caseyi*, *Chrysobothris leechi* and *P. drummondi* were collected.

In total 9 buprestid species were collected at the Merritt, Lillooet and Lytton colonies (Table 3). The smallest beetle was *Anthaxia caseyi caseyi* (4.2 mm) while the largest was *Chrysobothris leechi* (12.0 mm). The average size of all buprestid prey from all 3 colonies is 8.8 mm. All beetles have been archived in the Pacific Forestry Centre Arthropod Collection (PFCA, Victoria, BC) which is part of Natural Resources Canada-Canadian Forest Service.

Silt comprised 66.6% of the soil from the Merritt colony, while sand comprised 59.6 and 80.9% of the soil from the Lillooet and Lytton colonies, respectively. Clay

Table 3. Average length of *C. fumipennis* prey from largest to smallest. Measurements were not recorded for *A. idahoensis* and *P. gentilis* as their heads were missing. *New prey record for *C. fumipennis*.

Species	Average length (mm)
*Chrysobothris leechi**	10.5 (n=10)
*Chrysobothris laricis**	9.8 (n=1)
*Agrilus granulatus populi**	8.7 (n=4)
Phaenops drummondi	8.2 (n=5)
*Phaenops intrusa**	7.5 (n=1)
*Agrilus crataegi**	6.7 (n=1)
*Anthaxia caseyi caseyi**	4.7 (n=3)
*Acmaeodera idahoensis**	N/A (1 specimen)
*Phaenops gentilis**	N/A (3 specimens)

Table 4. Soil characteristics of the Lillooet, Lytton and Merritt colonies.

Site	Soil Texture			2 mm Sieve Pass %	Loss on Ignition %
	% Sand	% Silt	% Clay		
Merritt	11.6	66.6	21.8	100	2.2
Lillooet	59.6	34.1	6.3	99.3	2.0
Lytton	80.9	15.3	3.8	99.0	2.0

comprised 21.8% of the soil from Merritt, but only 3.8 and 6.3% of the soil from Lytton and Lillooet, respectively. All of the soil components from Merritt were less than 2 mm in any dimension, while 99 and 99.3% of soil constituents from Lytton and Lillooet passed through the 2 mm sieve. The organic content of all 3 soils is similar with 2.0 – 2.2 % loss on ignition (Table 4).

Discussion

Lytton and Lillooet represent 2 newly discovered *Cerceris fumipennis* colonies in BC. Eleven other species of wasps, including *C. nigrescens*, were collected at the Merritt, Lillooet and Lytton colonies; yet *C. californica* was not among them. A tremendous amount of time and resources were used to examine multiple sites from 2012 to 2014, yet only 3 *C. fumipennis* colonies have been discovered, resulting in a 3% success rate. Compared to a 22% success rate in finding *C. fumipennis* colonies in Connecticut, North Carolina and Maine (Nalepa et al. 2012), it appears that colonies are less common in BC. The northernmost colony in Ontario is in Parry Sound District (MacDougall Public School, 45.39861°N) (P.D. Careless, personal observation) which is substantially further south than all 3 BC colonies. Lillooet (50.67669°N) represents the most northern North American colony to date. The annual number of degree days above 5 °C in Lillooet is 2387.8 compared to 1702.4 in Beatrice, ON, which is the weather station closest to Parry Sound (Anonymous 2015). As colonies can occur at

Figure 5. Red shale typically found in baseball fields in southwestern BC. Note 2 ant nests.

sites in Ontario that are cooler than Lillooet, there may be additional colonies further north in BC.

Unlike *Cerceris californica* in Washington state (Looney et al. 2014) or *C. fumipennis* in eastern North America (Nalepa et al. 2012; Careless et al. 2014), none of the BC colonies were discovered in baseball fields. Most baseball fields in southwestern BC are comprised of red shale containing many rock fragments which is likely a poor nesting substrate for *C. fumipennis* (Fig. 5). All 3 colonies occur within compact sand parking lots (Lytton and Lillooet) or compact silt pathways (Merritt) with full sun exposure throughout the day (Figs 1, 2). The colonies occur in the 2 biogeoclimatic zones, Bunchgrass and Ponderosa Pine, with the warmest and driest summers in BC. The Bunchgrass zone is generally characterized by widely spaced *Pseudoroegneria spicata* (Pursh) Á. Löve (bluebunch wheatgrass) and *Artemisia tridentata* Nuttall (big sagebrush) although ponderosa pine and Douglas-fir, *Pseudotsuga menziesii* (Mirbel) Franco (Pinaceae), also occur in this zone. The Ponderosa Pine zone consists of very open, park-like stands of ponderosa pine with an understory of bluebunch wheatgrass (Anonymous 2007). Both zones have a sparse distribution of trees, allowing many areas to receive full sun throughout many summer days. The nesting areas in Merritt and Lytton both occur on a slight incline that assists in drainage. There is very little similarity in soils between the 3 sites. Merritt is predominantly silt (66.6%) and has

the largest component of clay at 21.8%. Lytton is primarily sand (80.9%), and Lillooet is 59.6% sand and 34.1% silt. Further analysis is required to determine if there are specific constituents required for nesting or if there are other factors that play a more significant influence in nest site selection. Three additional sites in Merritt with suspect *Cerceris* entrance holes were excavated, but *Cerceris* nests were not present and the substrate consisted of substantially more and larger pebbles.

Recently, archived *Cerceris fumipennis* specimens have been discovered in the Wallis-Roughley Museum (University of Manitoba) and the Strickland Museum (University of Alberta). These specimens were collected in Spruce Woods Provincial Park, MB and Writing-on-Stone Provincial Park, AB and resemble the eastern race of *C. fumipennis* in size and colouration. Both sites are further south than the Lillooet colony. Perhaps *Cerceris fumipennis* is more cold tolerant than *C. californica*, thus explaining its distribution within many of Canada's provinces whereas the latter occurs in western North America where winters are relatively short and mild.

A total of 9 buprestid species were collected at the 3 *Cerceris fumipennis* colonies. *Chrysobothris leechi* was the most common intact beetle collected, followed by *Phaenops drummondi*, *Agrilus granulatus populi*, *Anthaxia caseyi caseyi*, and *P. gentilis*. Single specimens of *A. crataegi*, *P. intrusa*, *C. laricis* and *Acmaeodera idahoensis* were also found at these *C. fumipennis* colonies. During nest excavation, buprestid-filled *C. fumipennis* cells occurred between 10 and 20 cm below the surface which is similar to nests in eastern North America (Careless et al. 2009). Since wasp larvae feed on paralyzed prey, body parts were collected more often than intact beetles during nest excavation thereby making species-level identification difficult if not impossible. Many *Chrysobothris*, *Anthaxia* and *Acmaeodera* elytra were found in the cells which could alter the actual ratio of species preyed upon by *C. fumipennis*. Population size, predatory avoidance behaviour, size of the beetle, and other factors will affect whether or not a beetle is suitable prey for *C. fumipennis*.

The size of *Cerceris fumipennis* prey ranged from 4.2 mm (*Anthaxia caseyi caseyi*) to 12.0 mm (*Chrysobothris leechi*). *Chrysobothris leechi* was not only the most common intact beetle collected, but on average it was the largest species at 10.5 mm. In comparison, *Phaenops intrusa* comprised over 70% of the prey taken by *Cerceris californica* Cresson in southcentral Washington (Looney et al. 2014). In New York State, *C. fumipennis* captured prey ranging in size from 4.1 to 18.9 mm (Hellman and Fierke 2014); the latter is 57% larger than the *C. leechi* found at the Merritt colony. *Buprestis aurulenta* (Linnaeus) is a relatively common and large buprestid (12–20 mm) occurring in southern BC that breeds within Douglas-fir and ponderosa pine (Furniss and Carolin 1977), yet it was not collected at any of the *C. fumipennis* colonies. Although only a handful of *C. fumipennis* specimens have been collected in BC, females appear to be within the lower size range (wing length 9.5–10.5 mm; n = 6) of their eastern counterparts (wing length 9.5–13.5 mm; n = 75) (Kimoto and Buck 2015). There is a positive linear relationship between the size of *Cerceris arenaria* L. and *C. halone* Banks with the size of the prey weevils collected (Byers 1978; Polidori et al. 2005). The smaller size *C. fumipennis* from BC has likely contributed to the smaller prey items captured by provisioning females.

Table 5. Distribution and host records for *C. fumipennis* prey. Unless otherwise noted the information is based on Nelson et al. (2008). Some host names have been changed according to The Plant List (www.theplantlist.org/, accessed 3 March 2015).

Species	Distribution	Larval hosts
Acmaeodera idahoensis	BC, WA, OR, CA, ID, NV, MT, WY, UT	*Celtis occidentalis* *Cercocarpus ledifolius* *Crataegus douglasii* *Quercus garryana* *Adults occur on a variety of flowers, notably in the family Asteraceae.
Agrilus crataegi	transcontinental	*Amelanchier alnifolia* *Crataegus douglasii* (Westcott 2005)
Agrilus granulatus populi	NV (Solomon 1995), AB, BC, WA, OR, CA, ID, MT	*Populus trichocarpa* *P. nigra*
Anthaxia (*Haplanthaxia*) *caseyi caseyi*	BC, WA, OR, CA, ID, MT, NV, AZ, UT	*Pinus coulteri* *P. ponderosa* *P. sabiniana*
Chrysobothris laricis	NWT, BC, AB, WA, OR, ID, MT, WY, UT, CO, NM, AZ	No larval host recorded; however, adults found on a variety of trees in the family Pinaceae.
Chrysobothris leechi	BC, AB, WA, OR, CA, ID, NV, MT	*Pinus aristata* *P. ponderosa*
Phaenops drummondi	transcontinental	A wide variety of trees in the family Pinaceae (MacRae and Westcott 2012).
Phaenops gentilis	BC, Rocky Mountain and Pacific States, NE, SD	*Pinus* spp.
Phaenops intrusa	BC, WA, OR, CA, ID, NV, MT, CO, AZ, NE, SD	*Larix occidentalis* *Pinus attenuata* *P. flexilis* *P. lambertiana* *P. ponderosa*

All of these beetles represent the first prey records for *Cerceris fumipennis* in BC. *Phaenops drummondi* is a known prey of eastern *C. fumipennis* (Paiero et al. 2012); however, all the other species and subspecies (i.e. *Agrilus granulatus populi*) are new prey records for this wasp. *Agrilus granulatus populi* and *P. intrusa* are also prey of *C. californica* in Washington state (Looney et al. 2014). Based on the list of prey species it is almost certain that female *C. fumipennis* forage on shrubs, conifers, deciduous trees and possibly flowers in BC (Table 5).

One ground beetle, *Harpalus affinis* was collected in a cell at 15.23 cm below grade along with the elytra of *Acmaeodera* spp. As other carabids were not discovered anywhere else in the nest it is uncertain if *Cerceris fumipennis* intentionally captured and provisioned the cell with this beetle. In 2009 and 2010, female *C. fumipennis* in Connecticut, Maine and New York captured 3 chrysomelids, *Neochlamisus bebbianae* (Brown), *Bassareus mammifer* (Newman), *Leptinotarsa decemlineata* (Say); 1 scarab, *Popillia japonica* Newman; and 2 cerambycids, *Saperda discoidea* F., *Oberea schaumii*

LeConte (Rutledge et al. 2011). *Neochlamisus bebbianae*, *B. mammifer* and *P. japonica* are shiny and similar in appearance to many jewel beetles, while *S. discoidea* and *O. schaumii* occur in tree canopies where *C. fumipennis* will forage. Therefore, collection of these non-prey items is understandable. *Harpalus affinis* is similar in length to some buprestids, however it looks different than most jewel beetles. Although adult *H. affinis* can fly, this specimen may have wandered into the nest entrance searching for prey or shelter. Until female wasps are intercepted carrying *H. affinis* back to their nests, this species can not be considered a prey item of *C. fumipennis*.

Conclusions

Despite a significant amount of time spent searching many sites, only 3 *Cerceris fumipennis* colonies have so far been discovered in BC; they seem to be less common than colonies in eastern North America. A total of 9 buprestid species are recorded here as prey items of BC *C. fumipennis* and with the exception of *P. drummondi*, are all new prey records for this wasp. The prey ranged in size from 4.2 to 12.0 mm and seem to be smaller than prey collected by eastern wasps. *Cerceris fumipennis* in BC appear to be smaller than specimens occurring east of the Rocky Mountains which may contribute to the difference in size of prey collected.

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References

Anonymous (2007) Biogeoclimatic Ecosystem Classification Program. British Columbia Ministry of Forests, Lands and Natural Resource Operations. http://www.for.gov.bc.ca/hre/becweb/resources/classificationreports/subzones/index.html [accessed 11 May 2015]

Anonymous (2015) Canadian Climate Normals 1981–2010 Station Data. Environment Canada. http://climate.weather.gc.ca/climate_normals/index_e.html [accessed 22 May 2015]

Buck M (2004) An annotated checklist of the spheciform wasps of Ontario (Hymenoptera: Ampulicidae, Sphecidae and Crabronidae). Journal of the Entomological Society of Ontario 134 [2003]: 19–84.

Byers GW (1978) Nests, prey, behavior and development of *Cerceris halone* (Hymenoptera: Sphecidae). Journal of the Kansas Entomological Society 51: 818–831.

Careless PD (2010) Working with *Cerceris fumipennis* [online]. http://www.cerceris.info [accessed 2 October 2014]

Careless PD, Marshall SA, Gill B, Appleton E, Favrin R, Kimoto T (2009) *Cerceris fumipennis* – A Biosurveillance Tool for Emerald Ash Borer. Canadian Food Inspection Agency, Ottawa, 16 pp.

Careless P, Marshall SA, Gill BD (2014) The use of *Cerceris fumipennis* (Hymenoptera: Crabronidae) for surveying and monitoring emerald ash borer (Coleoptera: Buprestidae) infestations in eastern North America. The Canadian Entomologist 146: 90–105. doi: 10.4039/tce.2013.53

Furniss RI, Carolin VM (1977) Western Forest Insects. U.S. Department of Agriculture, Forest Service. Miscellaneous publication No. 1339, 654 pp.

Hellman WE, Fierke MK (2014) Evaluating buprestid preference and sampling efficiency of the digger wasp, *Cerceris fumipennis*, using morphometric predictors. Journal of Insect Science 14(4): 1–18. doi: 10.1673/031.014.04

Holland SS (1976) Landforms of British Columbia. A Physiographic Outline. Bulletin 48. The Government of the Province of British Columbia, 138 pp. [accessed 12 May 2015]

Hook AW, Evans HE (1991) Prey and parasites of *Cerceris fumipennis (*Hymenoptera: Sphecidae) from central Texas, with description of the larva of *Dasymutilla scaevola* (Hymenoptera: Mutillidae). Journal of the Kansas Entomological Society 64: 257–264.

Kimoto T, Buck M (2015) Rediscovery of *Cerceris fumipennis* (Hymenoptera: Crabronidae) in British Columbia, with notes on geographic variation and nesting habits. The Canadian Entomologist 147: 419–424. doi: 10.4039/tce.2014.66

Looney C, Hellman WE, Westcott RL (2014) Sampling Buprestidae (Coleoptera) in Washington state with *Cerceris californica* Cresson (Hymenoptera, Crabronidae). Journal of Hymenoptera Research 39: 83–97. doi: 10.3897/jhr.39.8026

MacRae TC, Westcott RL (2012) Nomenclatural history of *Melanophila drummondi* ab. *nicolayi* Obenberger, 1944 (Coleoptera: Buprestidae), a change of authorship and synonymy under *Phaenops drummondi* (Kirby 1837), and a new distribution record and summary of larval hosts for the species. The Pan-Pacific Entomologist 88(1): 87–91. doi: 10.3956/2012-27.1

Marshall SA, Paiero SM, Buck M (2005) Buprestid sampling at nests of *Cerceris fumipennis* (Hymenoptera: Crabronidae) in southern Ontario: the first Canadian records of three buprestids (Coleoptera: Buprestidae). The Canadian Entomologist 137: 416–419. doi: 10.4039/n05-016

Marshall SA, Paiero SM, Buck M, Gill BD (2006) Using *Cerceris fumipennis* wasps to monitor the spread of emerald ash borer. In: Mastro V, Reardon R, Parra G (Eds) Emerald Ash Borer Research and Technology Meeting, Pittsburgh, September 26–27, 2005. U.S. Department of Agriculture, Forest Health Technology Enterprise Team.

Nalepa CA, Teerling C, Rutledge CE, Swink W, Arellano C (2012) Ball diamonds as habitat for nests of *Cerceris fumipennis* (Hymenoptera: Crabronidae): Comparisons among three states. Journal of the Kansas Entomological Society 85(3): 219–225. doi: 10.2317/JKES120418.1

Nelson GH, Walters Jr. GC, Haines RD, Bellamy CL (2008) A catalog and bibliography of the Buprestoidea of America north of Mexico. Coleopterists Society Special Publication 4: 1–274.

Paiero SM, Jackson MD, Jewiss-Gaines A, Kimoto T, Gill BD, Marshall SA (2012) Field Guide to the Jewel Beetles (Coleoptera: Buprestidae) of Northeastern North America. Canadian Food Inspection Agency, 411 pp.

Polidori C, Boesi R, Isola F, Andrietti F (2005) Provisioning patterns and choice of prey in the digger wasp *Cerceris arenaria* (Hymenoptera: Crabronidae): the role of prey size. European Journal of Entomology 102: 801–804. doi: 10.14411/eje.2005.111

Rutledge CE, Hellman W, Teerling C (2011) Two novel prey families for the buprestid-hunting wasp *Cerceris fumipennis* Say (Hymenoptera: Crabronidae). The Coleopterists Bulletin 65(2): 194–196. doi: 10.1649/072.065.0223

Rutledge CE, Fierke MK, Careless PD, Worthley T (2013) First detection of *Agrilus planipennis* in Connecticut made by monitoring *Cerceris fumipennis* (Crabronidae) colonies. Journal of Hymenoptera Research 32: 75–81. doi: 10.3897/jhr.32.4865

Scullen HA (1965) Review of the genus *Cerceris* in America north of Mexico (Hymenoptera: Sphecidae). Proceedings of the United States National Museum 116: 333–548. doi: 10.5479/si.00963801.116-3506.333

Solomon JD (1995) Guide to insect borers of North American broadleaf trees and shrubs. Agriculture Handbook 706. U.S. Department of Agriculture, Forest Service, Washington, DC, 735 pp.

Westcott RL (2005) A new species of *Chrysobothris* Eschscholtz from Oregon and Washington, with notes on other Buprestidae (Coleoptera) occurring in the United States and Canada. Zootaxa 1044: 1–15.

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RESEARCH ARTICLE

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New species of *Plesiocoelus* van Achterberg and *Mesocoelus* Schulz (Hymenoptera, Braconidae) from Brazil

Marco Aurélio Bortoni[1], Angélica Maria Penteado-Dias[1]

1 *Universidade Federal de São Carlos, Programa de Pós-Graduação em Ecologia e Recursos Naturais, CP 676, CEP 13 565-905, São Carlos, SP, Brazil*

Corresponding author: *Marco Aurélio Bortoni* (marcoabortoni@yahoo.com.br)

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Abstract

Three new species from Brazil, *Plesiocoelus anomalus* **sp. n.**, *P. areolatus* **sp. n.** and *Mesocoelus lobatus* **sp. n.**, are described and illustrated, and new records for *Plesiocoelus bassiformis* van Achterberg are also included. A key to the species of both genera is provided.

Keywords

Biodiversity, Ichneumonoidea, Neotropical Fauna, Taxonomy

Introduction

Plesiocoelus bassiformis van Achterberg, 1990 is the type species and the only described species of the genus. It is characterized by the apically reduced fore wing venation and the merging of discal and first submarginal cells. It has been recorded from Colombia, Ecuador and Honduras (Yu et al. 2012). There are no biological data for the genus.

The genus *Mesocoelus* Schulz, 1918 is recorded from Cuba and Saint Vincent (Yu et al. 2012) and the two known species so far are parasitoids of *Acrocercops* sp. and *Chilocampylla psidiella* Busck (Lepidoptera, Gracillariidae) (van Achterberg 1990). It has reduced apical wing venation, as in *Plesiocoelus*, but differs by the absence of veins 1m-cu and 2RS in the fore wing (Sharkey 2006).

Those morphological similarities led van Achterberg (1990) to revalidate the status of the subtribe Mesocoelina Viereck, also including the genus *Aneurobracon* Brues, 1930. Despite these similarities the subtribe is not consistent phylogenetically. The clade *Aneurobracon* + *Mesocoelus* was not recovered in some analyses in Sharkey et al (2006), but the morphological similarities led the authors to propose them as sister groups (Shakey et al. 2006, 2009). The genus *Plesiocoelus* was recovered in some analyses as closely related to some clades of *Bassus* s.l. (Sharkey et al. 2006). The generic concept of *Bassus* s.l. has gone through changes and split into other genera; now *Plesiocoelus* is thought to be the sister group of *Zacremnops* Sharkey & Wharton and *Therophilus* Wesmael (Sharkey et al. 2009)

In this paper, we describe two new species of *Plesiocoelus* and a new species of *Mesocoelus* and we include new distribution records for *P. bassiformis*. A key to the species of both genera is provided.

Methods

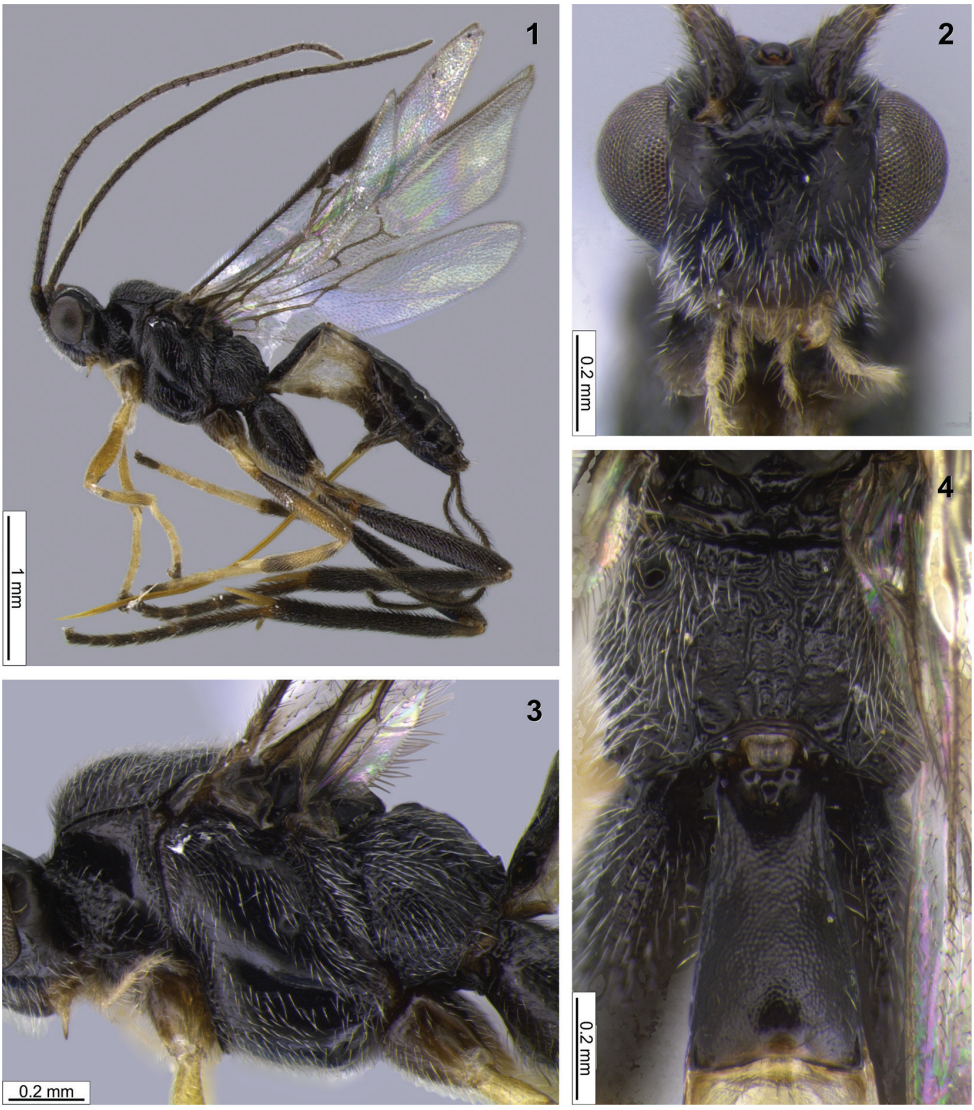
The examined specimens are deposited in the DCBU Collection (Departamento de Ecologia e Biologia Evolutiva da Universidade Federal de São Carlos, São Carlos, SP, Brazil); all were collected in Brazil in the States of Amazonas, Minas Gerais and São Paulo with Malaise traps. The genera were identified using the key by Sharkey (2006) and the species were compared with the description and illustrations in van Achterberg (1990). The morphological terminology follows Wharton (1997), except for "precoxal sulculs" which replaces "sternaulus". The colour photographs were taken and edited with a Leica® M205C with LAS image software.

Results

Plesiocoelus bassiformis van Achterberg, 1990

Figs 1–4

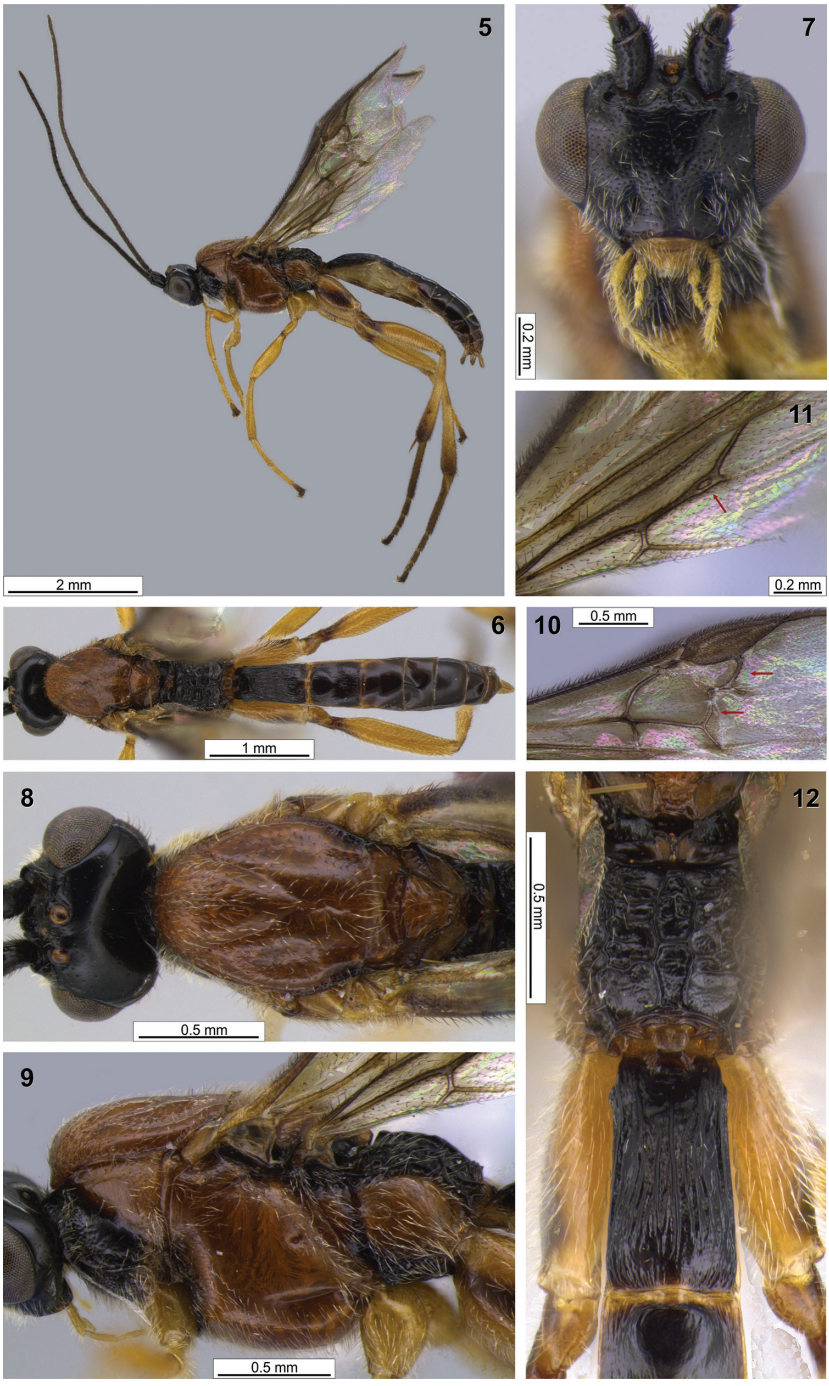
New record. *Plesiocoelus bassiformis* is recorded for the first time from Brazil. One female (DCBU 51443) "Bom Repouso, MG, Brasil, Serra dos Garcias, Armadilha Malaise, S22°29'25.6", W46°11'25.8", 04.V.2010, I.F. Melo col." One female (DCBU 51444) "Ribeirão Grande, SP, Brasil, Pq. Estadual de Intervales, Armadilha Malaise, S24°16'28.8", W48°25'20.6", 22.III.2010, N.W. Perioto e eq. cols."



Figures 1–4. *Plesiocoelus bassiformis* van Achterberg, 1990; **1** Habitus, lateral view **2** Head, frontal view **3** Mesosoma, lateral view **4** Propodeum and T1, dorsal view.

***Plesiocoelus anomalus* sp. n.**
http://zoobank.org/ACECDA73-0CDA-486B-BD0D-0691905BA02D
Figs 5–12

Material examined. Holotype – male. (DCBU 51445) "São Luiz do Paraitinga, SP, Brasil, Pq. Estadual da Serra do Mar, Núcleo Santa Virgínia, 22.XI.2010, Armadilha Malaise, S23°19'27.1", W45°5'38.4", N.W. Periotto e eq. col."



Figures 5–12. *Plesiocoelus anomalus* sp. n.; **5** Habitus, lateral view **6** Body, dorsal view **7** Head, frontal view **8** Head, mesonotum and scutellum, dorsal view **9** Mesosoma, lateral view **10** Fore wing, arrows indicating vein 2RS and 1m-cu present **11** Vein 1M of hind wing, arrow indicating widened part **12** Propodeum and T1, dorsal view.

Description of holotype. Body length: 4.0 mm. Fore wing length: 3.9 mm.

Head. Antenna with 33 segments, whitish setose, length of third segment equal to fourth; length of third, fourth and penultimate segments 3.6, 3.6 and 1.25 times their width, respectively. Maxillary palp with 5 segments and 0.6 times height of head. Length of eye in dorsal view 2.2 times temple. OOL: diameter of ocellus: POL = 15:10:15. Head completely smooth with long whitish setae on lateral parts of face, length of malar space 1.5 times basal width of mandible.

Mesosoma. Length of mesosoma 1.8 times its height. Propleuron sparsely punctate. Pronotum smooth but anteriorly punctate. Mesopleuron smooth, with precoxal sulcus faintly impressed and smooth. Mesonotum smooth, with notauli weakly impressed, crenulate anteriorly and smooth posteriorly. Scutellum smooth. Propodeum areolate.

Fore wing. Mostly infumate and hyaline near apex, length of pterostigma: R1 = 30:40, 1-CU1:2-CU1 = 3:20.

Hind wing. Mostly infumate and hyaline near apex, vein CUb present and tubular, vein 1M widened and with small cell (Fig. 11).

Legs. All legs smooth, length of femur, tibia and basitarsus of hind leg 5.0, 10.7, 8 times their width, respectively. Apex of hind tibia with 15 pegs. Length of hind spur 0.25 times hind basitarsus.

Metasoma. T1 striate, T2 with weakly granulate sculpture, remaining tergites smooth. T1 2.1 times longer than its apical width.

Colour. Head black, except ocelli dark yellow; clypeus, mandible, maxillary and labial palpi and glossa yellow. Mesosoma brown, but propleuron, basal half of pronotum, metanotum, propodeum and ventral margin of metapleuron black. Legs yellow except for brownish to black fore and mid telotarsi; lateral area of hind coxa, hind trochanter, hind trochantellus, base of femur, apex of tibia, hind basitarsus and hind tarsus brownish. Metasoma brown to black, but ventrally yellowish.

Diagnosis. This species differs from all other species of *Plesiocoelus* by the mostly brown mesosoma, completely yellowish legs and striate T1.

Etymology. This species is named after the unique shape of vein 1M of the hind wing (Fig. 11).

Biology. Unknown.

Distribution. Only known from the type locality in Brazil.

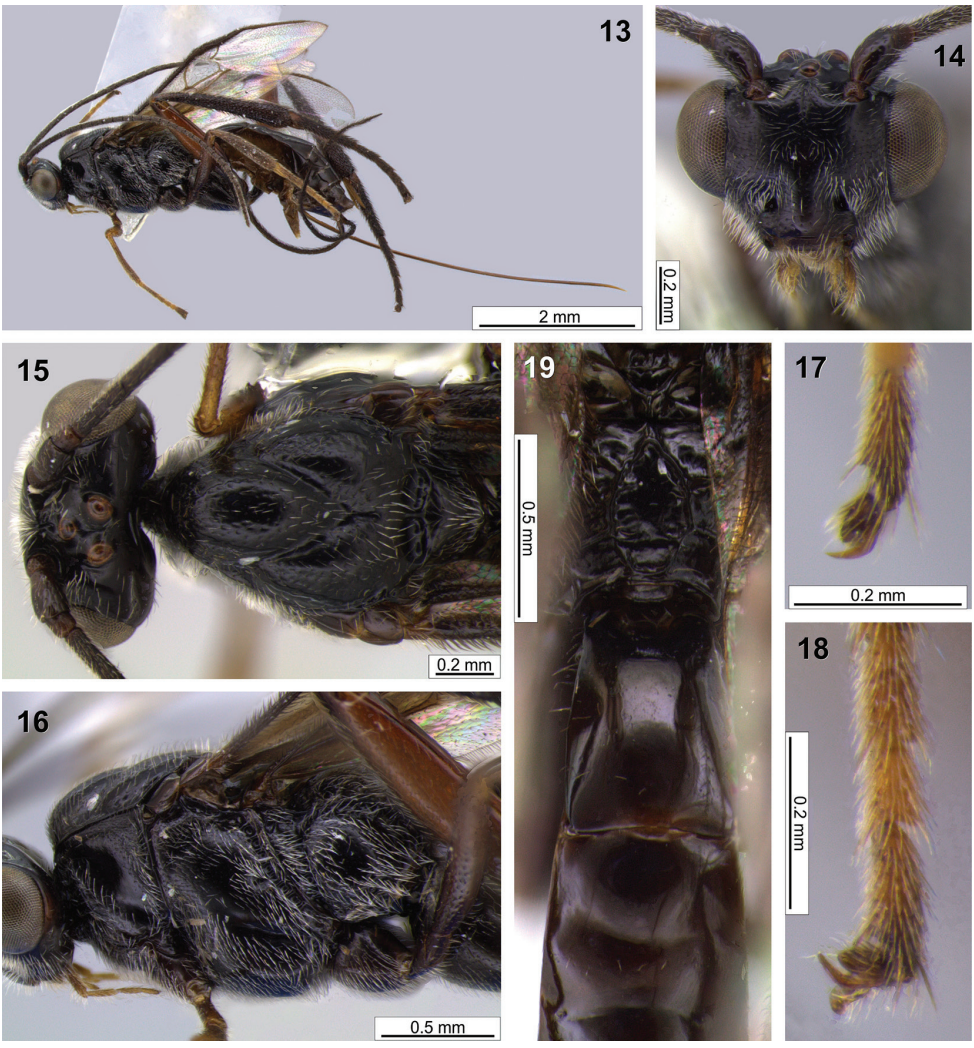
***Plesiocoelus areolatus* sp. n.**

http://zoobank.org/8D4AA3C4-02F6-4937-9864-86DCBD02940C

Figs 13–19

Material examined. Holotype – female. (DCBU 51038) "Manaus, AM, Brasil, Reserva Km 41, trilha, 24-25-XI-2004, S03°05'07", W60°02'4.92", Armadilha Malaise suspensa, R.B.Q. Silva col."

Description of holotype. Body length: 4.5 mm. Fore wing length: 3.2 mm



Figures 13–19. *Plesiocoelus areolatus* sp. n.; **13** Habitus, lateral view **14** Head, frontal view **15** Head, mesonotum and scutellum, dorsal view; **16,** Mesosoma, lateral view **17** Fore tarsus **18** Hind tarsus **19** Propodeum and T1, dorsal view.

Head. Antenna with 34 segments, whitish setose, length of third segment equal to fourth, length of third, fourth and penultimate segments 3.0, 3.0 and 1.5 times their width, respectively. Maxillary palp with 5 segments and its length 0.5 times height of head. Length of eye in dorsal view 2.1 times temple. OOL: diameter of ocellus: POL = 25:12.5:15. Head completely smooth, with long whitish setae on lateral parts of face, length of malar space 1.5 times basal width of mandible.

Mesosoma. Whitish setose, length of mesosoma 2.0 times its height. Propleuron sparsely punctate. Pronotum smooth, but apical and dorsal sides finely punctate. Mesopleuron smooth, with precoxal sulcus smooth. Mesonotum smooth, with notauli

smooth, crenulate anteriorly with apical third joining. Scutellum smooth. Propodeum smooth with large propodeal areola medially.

Fore wing. Infuscate, hyaline apically, length of pterostigma: R1 = 40:70, 1-CU1:2-CU1 = 2:13.

Hind wing. Hyaline, vein CUb present as a very short stub. Vein 1M normal.

Legs. All legs smooth, length of femur, tibia and basitarsus of hind leg 5.0, 10.0, 6.0 times their width, respectively. Length of hind spur 0.5 times length of hind basitarsus.

Metasoma. All terga completely smooth, T1 1.2 times as long as its apical width. Length of ovipositor about 1.4 times fore wing.

Colour. Black, except pedicellus and base of scape dark brown; mandibles, maxillary and labial palps yellowish; fore and mid legs dark brown, hind leg dark brown with femur orange brownish and tibial spurs yellowish. Tegula brownish. Metasoma ventrally yellowish, ovipositor sheath dark brown.

Diagnosis. This species is closely related to *Plesiocoelus bassiformis* but it differs by the anteriorly crenulate notauli, the completely smooth precoxal sulcus, the larger propodeal areola (covering most of the length of the propodeum) and the longer ovipositor sheath (about 1.4 times fore wing).

Etymology. This species is named after the large propodeal areola.

Biology. Unknown.

Distribution. Only known from the type locality in Brazil.

***Mesocoelus lobatus* sp. n.**

http://zoobank.org/25795B99-0CE1-425F-9235-F78E86441F9C

Figs 20–26

Material examined. Holotype – female. (DCBU 51446) "Palestina, SP, Brasil, Faz. Boa Vista, S20°17'18", W49°30'01", 18.VIII.2008, Armadilha Malaise, Noll, F. e eq. col."

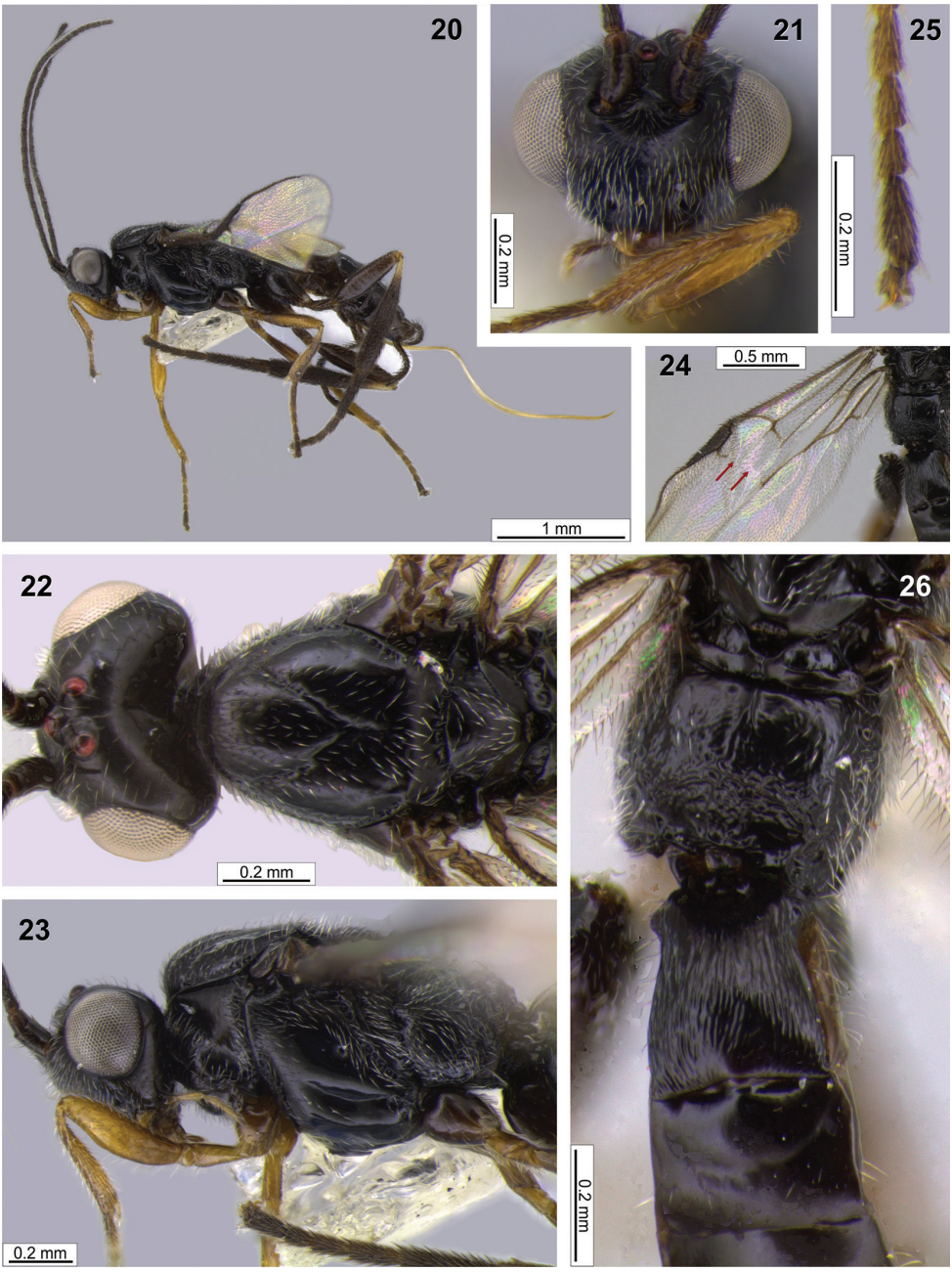
Description of holotype. Body length: 2.8 mm. Fore wing length: 2.7 mm.

Head. Antenna with 25 segments, whitish setose, length of third segment equal to fourth, length of third, fourth and penultimate segments 2.0, 2.0 and 1.2 times their width, respectively. Maxillary palp with 5 segments and its length 0.7 times height of head. Length of eye in dorsal view 2.2 times temple. OOL: diameter of ocellus: POL = 25:10:20. Head completely smooth with long whitish setae on lateral parts of face; length of malar space 1.5 times basal width of mandible.

Mesosoma. Length of mesosoma 2.0 times its height. Propleuron sparsely punctate. Pronotum smooth. Mesopleuron smooth, with precoxal sulcus smooth. Mesonotum smooth, with notauli crenulate anteriorly and smooth posteriorly, joining at apical third. Scutellum smooth. Propodeum rugose medially, apically faintly rugulose and with median longitudinal carina.

Fore wing. Hyaline, length of pterostigma: R1 = 40:70, 1-CU1:2-CU1 = 2:13.

Hind wing. Hyaline.



Figures 20–26. *Mesocoelus lobatus* sp. n.; **20** Habitus, lateral view **21** Head, frontal view **22** Head, mesonotum and scutellum, dorsal view **23** Mesosoma, lateral view **24** Wings, arrows indicating veins 2RS and 1m-cu absent **25** Hind tarsus **26** Propodeum and T1, dorsal view.

Legs. All legs smooth, length of femur, tibia and basitarsus of hind leg 5.0, 10.0, 6.0 times their width, respectively. Length of hind spur 0.25 times hind basitarsus. Tarsal claws with a basal lobe.

Metasoma. T1 striate, remainder of metasoma smooth. T1 length 1.1 times its apical width. Length of ovipositor sheath about 2.0 times hind tibia.

Colour. Black, except antenna dark brown; mandible, maxillary and labial palpi yellowish. Fore and mid legs yellowish with coxae and telotarsi brown. Hind leg dark brown. Metasoma ventrally yellowish, ovipositor sheath dark brown.

Diagnosis. This species differs from the two previously described species by the presence of notauli on the mesoscutum and the mid and hind tarsal claws with basal lobes.

Etymology. This species is named after the presence of tarsal lobes

Biology. Unknown.

Distribution. Only known from the type locality in Brazil.

Key to species of *Mesocoelus* and *Plesiocoelus*

(modified from Sharkey 2006 and van Achterberg 1990).

1 Discal and first submarginal cells of fore wing combined, closed by veins 1m-cu and 2RS (Fig. 10)..... **2**

– Discal and first submarginal cells of fore wing open, veins 1m-cu and 2RS absent (Fig. 24)..... **4**

2 Vein 1M of hind wing widened and with a small cell (Fig. 11). Mesosoma mostly brown (Figs 6, 8, 9). Hind femur yellow. T1 striate (Fig. 12)..... ..... ***P. anomalus* sp. n.**

– Vein 1M of hind wing normal. Mesosoma black (Figs. 3, 15, 16). Hind femur black or brownish. T1 smooth or punctate (Figs 4, 19)..... **3**

3 Propodeum with a large areola medially (Fig. 19). Ovipositor sheath about 1.4 times as long as fore wing. Notauli crenulate anteriorly. Precoxal sulcus smooth posteriorly..... ***P. areolatus* sp. n.**

– Propodeum mostly rugose medially and without distinct areola (Fig. 4). Ovipositor sheath about 0.8 times as long as fore wing. Notauli completely smooth. Precoxal sulcus crenulate posteriorly..... ***P. bassiformis* van Achterberg**

4 Mesonotum with notauli present (crenulate anteriorly and smooth posteriorly; Fig. 22). Mid and hind tarsal claws with basal lobes (Fig. 25)..... ..... ***M. lobatus* sp. n.**

– Mesonotum without notauli. Mid and hind tarsal claws simple, without basal lobes **5**

5 Mesoscutum with round depression medio-posteriorly. Hind basitarsus about 5 times as long as wide. Epistomal suture absent medially. Hind leg largely yellowish. Ovipositor sheath as long as hind tibia. Area between antennal sockets evenly convex ..... ***M. laeviceps*** **(Ashmead)**

– Mesoscutum without round depression medio-posteriorly. Hind basitarsus about 9 times its width. Epistomal suture shallowly impressed medially. Hind leg largely dark brown or blackish. Ovipositor sheath about 0.8 times as long as hind tibia. Area between antennal sockets with a pair of weak crests .... ***M. acrocercopsis*** **Muesebeck**

Acknowledgments

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References

Sharkey MJ (2006) Two new genera of Agathidinae (Hymenoptera: Braconidae) with a key to the genera of the new world. Zootaxa 1185: 37–51.

Sharkey MJ, Laurenne NM, Sharanowski B, Quicke DLJ, Murray D (2006) Revision of the Agathidinae (Hymenoptera: Braconidae) with comparisons of static and dynamic alignments. Cladistics 22: 546–567. doi: 10.1111/j.1096-0031.2006.00121.x

Sharkey MJ, Yu DS, van Noort S, Seltman K, Penev L (2009) Revision of the Oriental Genera of Agathidinae (Hymenoptera, Braconidae) with emphasis on Thailand including interactive keys in three different formats. Zookeys 21: 19–54. doi: 10.3897/zookeys.21.271

Sharkey MJ, Wharton RA (1997) Morphology and terminology. In: Wharton RA, Marsh PM, Sharkey MJ (Eds) Manual of the New World genera of the family Braconidae (Hymenoptera). International Society of Hymenopterists (Special Publication) 1: 21–40.

van Achterberg C (1990) Revision of the subtribe Mesocoelina Viereck (Hymenoptera: Braconidae). Zoologische Mededelingen Leiden 64(4): 31–57.

Yu DSC, van Achterberg C, Horstmann K (2012) World Ichneumonoidea 2011. Taxonomy, biology, morphology and distribution. CD/DVD. Vancouver.

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Protection against herbivory in the mutualism between *Pseudomyrmex dendroicus* (Formicidae) and *Triplaris americana* (Polygonaceae)

Adriana Sanchez[1], Edwin Bellota[2]

1 *Programa de Biología, Universidad del Rosario, Carrera 24 No. 63C-69, Bogotá, Colombia* **2** *Department of Entomology, Texas A&M University, College Station, Texas, U.S.A.*

Corresponding author: *Adriana Sanchez* (adriana.sanchez@urosario.edu.co)

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Abstract

Herbivory significantly impacts the growth and reproduction of plants. Many plants have developed ways to defend against herbivores and one common strategy is to associate with ants. In many ant-plant interactions, ants are known to protect their host. However, in the Neotropical ant-plant genus *Triplaris,* the benefits provided by associated ants have never been tested. Many *Pseudomyrmex* spp. ants are obligate inhabitants of *Triplaris* spp. trees. In this study, *Triplaris americana* was studied in association with *Pseudomyrmex dendroicus*, an ant highly specific to its host (it has not been collected from any other species of *Triplaris*). Ant exclusion experiments were carried out to assess the protective effect of ants. In addition, ant behavior was monitored in control plants to study the mechanisms by which ants might confer protection against herbivory. Ant removal led to a more than 15-fold increase in herbivory. *Pseudomyrmex dendroicus* are active at all times of day and night and aggressively and efficiently remove insect herbivores from their host.

Keywords

Ant-plant interaction, defense, herbivory, mutualism, myrmecophyte, Peru

Introduction

Herbivory can have significant negative effects on plant fitness. Both the incidence and the impact of damage can vary with leaf ontogeny and the specific tissue being attacked, and herbivory can result in decreased survivorship and reproductive output (Marquis 1984, Spotswood et al. 2002). Since herbivory confers often high costs to plant fitness, and its extent is largely dependent on the plant's defense investment (Coley and Kursar 1996, Strauss and Agrawal 1999, Strauss et al. 2002), plants have evolved several ways to protect themselves or to compensate for the effects of attack. The protective strategies plants use include structural defenses, toxins, digestibility-reducing compounds, and mutualistic relationships with natural enemies of herbivores, such as ants (Gong and Zhang 2014).

Myrmecophytes (i.e., plants sheltering the colonies of a limited number of 'plant-ant' species in hollow structures called domatia and sometimes also providing them with food in the form of extrafloral nectar and food bodies) are pervasive and very diverse in the Tropics (Chomicki and Renner 2015). This is a mutualistic association as, in return for being housed and sometimes fed, plant-ants protect their host myrmecophyte from encroaching vegetation, herbivores and pathogens, and/or provide them with nutrients (i.e., myrmecotrophy) (Rico-Gray and Oliveira 2007, Mayer et al. 2014).

Several studies have shown that herbivory increases when ants are excluded from their host (e.g., Janzen 1967, 1972, Agrawal 1998, Michelangeli 2003). However, the degree of protection may vary with the identity of the ant partner (Bruna et al. 2004, Djiéto-Lordon et al. 2004, Frederickson 2005, Dejean et al. 2006), and in some cases the ants provided no protection against herbivory (Fowler 1993, Frederickson and Gordon 2007).

Myrmecophytic-plant genera that have received much attention in the literature include *Acacia, Cecropia, Macaranga, Duroia,* and *Cordia*. However, several other genera remain to be studied. One such case is the myrmecophyte *Triplaris.* Some aspects of the ecological interaction between *Triplaris* and its associate ants have been addressed, but they have been limited to understanding the pruning behavior of *Pseudomyrmex* (Davidson et al. 1988, Larrea-Alcazar and Simonetti 2007), the taxonomy of the *Azteca* that inhabit *Triplaris melaenodendron* (Bertol.) Standl. & Steyerm. (Longino 1996), the foraging behavior of some of the ants associated with *Triplaris surinamensis* (= *T. weigeltiana* (Rchb.) Kuntze; Brandbyge 1986) in Brazil (Oliveira et al. 1987), and host discrimination following chemical cues (Weir et al. 2012).

Questions regarding the effectiveness with which ants associated with *Triplaris* protect the plant against herbivores remain unexplored. The association between *T. americana* L. (Polygonaceae) and *P. dendroicus* Forel (Formicidae) is particular in that it displays high levels of specificity. After examining more than 200 collection records of *Triplaris* and its associated ants (A. Sanchez unpublished data), it was clear that *P. dendroicus* only colonizes individuals of *T. americana*, even when other species of *Triplaris* occur in sympatry. Therefore, in this study we expected a dramatic reduction in herbivory afforded by *Pseudomyrmex* ants compared to an ant-exclusion experiment and high and effective levels of protection.

Methods

Study site

This study was carried out at Los Amigos Biological Station (12°34'9"S, 70°6'0.40"W; ca 250 m) in the department of Madre de Dios in southeastern Peru. Los Amigos conservation area is a private conservation concession established in 2000 by the Peruvian government in conjunction with the Amazon Conservation Association (ACA) and the Asociación para la Conservación de la Cuenca Amazónica (ACCA). The station comprises more than 145.000 ha of lowland Amazonian forest between 250-320 m in altitude, at the confluence of the Madre de Dios and Los Amigos rivers, and protects pristine ecosystems including wetlands, seasonally inundated and terra firme forests, and palm swamps. The climate is characterized by a single dry and wet season each year. The area receives most of its estimated 2000 mm of annual rainfall during the wet season, which typically lasts from November to May (Pitman et al. 1999). During the dry season, from May to November, temperatures are usually at their lowest point, and can be as low as 10 °C. This study was conducted during 42 days of the dry season, in August-September 2008. During our study, the precipitation was sporadic, with only a few days with rainfall amounts greater than 10 mm. The minimum temperature recorded for that period was 13.3 °C, the maximum 35.3 °C, and the average temperature was 23.4 °C (http://atrium.andesamazon.org/).

Study system

The species chosen for this study were *Triplaris americana* and *Pseudomyrmex dendroicus*. *Triplaris americana* is the most common and widespread species in the genus. It is found from Panama to Bolivia and Brazil, usually in lowlands and disturbed areas close to water (Brandbyge 1986). The stems of *T. americana* are hollow (Schremmer 1984, A. Sanchez pers. obs.) and harbor an entire colony of ants. Although these plants produce no food bodies or extrafloral nectaries, rewards to the ants are provided by a third symbiont – scale insects (Coccoidea, Hemiptera) in the form of honeydew (Schremmer 1984, Davidson and McKey 1993, Ward 1999). It has also been suggested that another symbiont – fungi is involved and may also provide food (Schremmer 1984, Defossez et al. 2009, Blatrix et al. 2013). *Pseudomyrmex* (Formicidae, Pseudomyrmecinae) is a genus that comprises ca. 200 species and is distributed in the New World (Ward and Downie 2005). The ants are characterized by large conspicuous eyes, a well-developed post-petiole and a well-developed sting (Ward 1990) with potent venoms (Pan and Hinks 2000, Touchard et al. 2014). Several different clades within *Pseudomyrmex* are associated with distantly related myrmecophytes such as *Acacia*, *Cordia*, *Tachigali*, and *Triplaris* (Ward 1999). Among other characteristics, *Pseudomyrmex dendroicus* is recognized by the coloration of its workers, with a light brown body that contrasts with a dark brown head (Ward 1999). *Pseudomyrmex dendroicus* is an obligate symbiont of the

myrmecophyte *Triplaris* and nests exclusively on *T. americana*. However, *T. americana* can be found in association with other species of *Pseudomyrmex* and with ants of other genera such as *Crematogaster* and *Azteca* (Longino 1996, Ward 1999; A. Sanchez pers. obs.). A single *T. americana* plant nearly always hosts only one ant colony (A. Sanchez pers. obs.).

Ant removal and its effect on herbivory

Prior to conducting the ant-exclusion experiments we explored two methods of exclusion, in order to determine which was the most effective. Following previous ant-exclusion experiments (e.g., Stanton and Palmer 2011), we applied a sticky resin in two separate branches per individual (Tangle-trap, Tanglefoot Company, Grand Rapids, MI). We applied the resin directly on the branch and over duct tape. However, the resin was not effective because of the formation of ant bridges (ants connected to each other in order to get over the barrier), and additionally bees ate the resin. Therefore, two to three days after the application of the resin, ants were already moving freely across the barrier and patrolling the leaves from which we intended to exclude them. We then used 0.5% Permethrin (Vidagro, Lima) to kill the entire colony. The use of insecticides has proven to be effective in previous ant-exclusion experiments (Stanton and Palmer 2011, Frederickson et al. 2012). The Permethrin was carefully injected through the prostoma of each internode avoiding contact with other parts of the plants. The prostoma are small unlignified zones between 6 to 10 mm long by which queens gain entry to make nesting sites (Schremmer 1984, Sanchez in review). Prostoma are present on twigs, branches, and the main stem (except at the base of the stem; A. Sanchez pers. obs.). Since adult plants of *Triplaris* can grow more than 15 m high, we chose saplings of less than 2 m tall for this study to ensure appropriate monitoring of herbivory and ant behavior. A total of 22 plants of similar sizes were used, with 11 replicates for each treatment (control and ant-exclusion). The Permethrin had no apparent effects on the plants' viability and growth during the study. The insecticide was used at low concentration (0.5%), and was only applied to the interior of the stem through the prostoma to minimize effects on the plants and on the potential herbivores.

To quantify the effects of ants on herbivory, two fully expanded leaves per sapling were monitored, always choosing the third leaf from the apical meristem from two adjacent branches. Prior to ant exclusion, photographs of every leaf were taken using a digital camera. A transparent sheet subdivided in grids of 1 square cm, each with 25 equidistant points within, was placed on top of the leaf, and photographs were always taken from the same distance. Percentage of herbivory was calculated by counting all the points that fell on areas where there was herbivory and divided by the total number of points that covered the leaf area. Photographs of each leaf were taken every two weeks for a total of six weeks.

We conducted a non-parametric Mann-Whitney *U* test for two independent samples to compare percentage of herbivory between control and experimental plants, us-

ing SigmaPlot version 12.5 (Systat Software Inc, San Jose, CA). We compared average herbivory after two, four and six weeks. The two leaves per sapling were used to calculate an average percentage of herbivory for each individual. A non-parametric ANOVA for repeated measures (Friedman ANOVA) was also conducted, to test if there was an increase of herbivory through time in the control and the ant-excluded plants.

Observations on ant behavior

Prior to taking the photographs, we conducted observations on the ants' behavior, recording their patrolling activities and their interactions with potential herbivores and with other ants that occasionally visit *Triplaris*. Plants were monitored for approximately 5 to 10 min every visit. Since ants seem to have patrolling activities that span the 24-hour cycle, observations were also recorded for some plants before dusk (between 1600 h and 1700 h) and at night (between 2100 h and 2200 h).

Results

Ant exclusion

Removal of ant colonies resulted in an increase in the percentage of herbivory. In each time interval (after 2, 4, and 6 weeks) there was a significant increase in herbivory compared to the control ($U = 14$, $P < 0.05$; $U = 21$; $P < 0.05$; $U = 14.5$, $P < 0.01$ respectively; Fig. 1). There was also a higher percentage of herbivory in the ant-excluded plants through time (ANOVA $\chi^2_{N=11,\ df=2} = 15.8$, $P < 0.001$; Fig. 1), but not in the control plants (ANOVA $\chi^2_{N=11,\ df=2} = 4$, $P > 0.05$).

Seven out of eleven control plants had zero percent herbivory during the six weeks of the experiment. The four other control plants suffered some herbivory by weeks 4 and 6 (~ 1%). In contrast, of the ant-excluded plants, six plants had more than 3% herbivory, having as high as 16% leaf damage (outlier not shown; Fig. 1). By the end of the experiment, the average percent of leaf damage was 3.2 in ant-excluded plants and 0.19 in controls. Plants with ants excluded had, on average, more than 15 times more herbivory than plants with resident ants. It was clear that the effect of herbivory was more pronounced as time progressed, especially in the experimental group (Fig. 1).

Ant behavior

Pseudomyrmex dendroicus actively patrolled their hosts, at all times of day and night, even when the temperature was as low as 13 °C. Whenever the plant was disturbed, they efficiently recruited other workers, and were very aggressive against any intruder. During their patrolling activities they removed any debris found on top of the leaves.

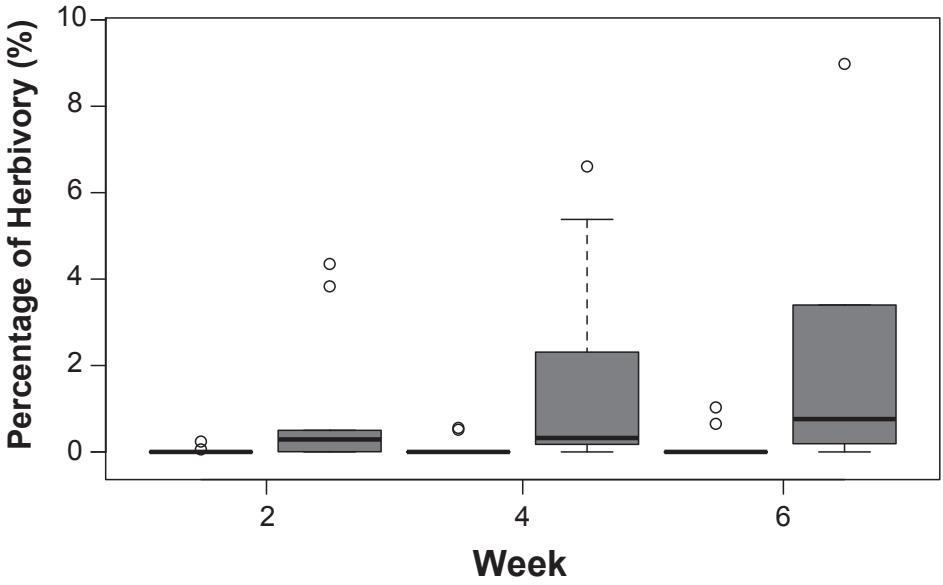

Figure 1. Percentage of herbivory with time for the control and ant-excluded plants (grey). Removing the ant colonies resulted in a significant increase in the percentage of herbivory after 2, 4, and 6 weeks ($U = 14$, $P < 0.05$; $U = 21$; $P < 0.05$; $U = 14.5$, $P < 0.01$ respectively). There was also a significant increase in herbivory through time in the ant-excluded plants (ANOVA $\chi^2_{N=11,\ df=2} = 15.8$, $P < 0.001$), but not in the control (ANOVA $\chi^2_{N=11,\ df=2} = 4$, $P > 0.05$).

Figure 2. Interaction between ant workers of *Pseudomyrmex dendroicus* and caterpillars visiting *Triplaris americana*.

They repeated this cleaning process constantly, on all the leaves of their host. In all saplings studied, the leaves had no signs of mosses, fungi or lichens growing them, and no sign of accumulated debris.

From our observations, the most common herbivores were grasshoppers (unidentified; Orthoptera) and caterpillars of the lepidopteran genera *Lophocampa* (Arctiidae, subfamily Arctiinae) and *Hylesia* (Saturniidae). When an ant encountered a caterpillar, a worker approached and detected it with its antennae, and then recruited more workers (Fig. 2). Typically more than 10 workers recruited around the intruder in less than five minutes. Several workers harassed the herbivore by stinging or biting, until it dropped off the plant. The caterpillars usually hung by a silk thread and attempted to move back onto the plant. However, individuals of *Pseudomyrmex* continued to chase them until they dropped again. This cycle was repeated several times. Other herbivores found in *Triplaris* included some Coleoptera. Most of the visitors frequented the plants at night or dawn. In all instances, *Pseudomyrmex* attacked the herbivores aggressively by biting and stinging.

Discussion

Herbivory

This is the first study to report that *Pseudomyrmex* provides benefits to *Triplaris* by reducing herbivory. Our results indicated a significantly higher percentage of herbivory on the plants where ants were excluded (more than 15 times more; Fig. 1), suggesting that the ants play an important protective role against herbivores. This has also been demonstrated in numerous other myrmecophytes, proving that there is a benefit from the effectiveness of the ant defense against herbivores (e.g., Chamberlain and Holland 2009, Rosumek et al. 2009, Trager et al. 2010). Frequent observations of the experimental plants revealed that herbivory tends to occur in a few concentrated events. As discussed by Michelangeli (2003), plants usually remain unharmed for a few weeks or days, but once they are discovered by herbivores, the damage occurs in a short time, often only a few hours.

According to the optimal defense theory (McKey 1974, 1979, Sagers and Coley 1995), defense should be concentrated on young shoots and leaves, since they constitute the most valuable and vulnerable parts of the plant (McKey 1974, Coley and Kursar 1996). In many plant species the nitrogen content in young leaves is very high, due to active growth, making them highly appealing to herbivores (Coley 1982, Kursar and Coley 1991). Vulnerability in young leaves is usually a result of being less tough, fibrous, and the lack of a protective cuticle (Coley and Kursar 1996). Since most of the herbivory damage a tropical plant will suffer throughout its lifetime is accumulated in juvenile leaves (Coley and Kursar 1996), having protection on these vulnerable areas constitute a great advantage. As expected, in several myrmecophytes, patrolling ants concentrate on these younger parts (Janzen 1972, McKey 1984, Fiala et al. 1994, Heil et al. 2001), though they also provide protection for mature leaves. In the association between *T. americana* and *P. dendroicus*, the ants actively guard young leaves and stipules, as well as mature leaves (A. Sanchez pers. obs.).

It has also been suggested that the protective role of ants extends to protecting the host against pathogenic fungi (Heil et al. 2001). Although we did not quantify fungal colonization directly, *Pseudomyrmex dendroicus* constantly removes debris, which could result in protection against fungi and other pathogens (García-Guzmán et al. 2001).

Ants constitute a rapid and direct line of defense, which can mobilize where they are required (McKey 1984, Fiala and Maschwitz 1990, Gaume et al. 1997, Agrawal 1998, Itioka et al. 2000, Michelangeli 2003). *Pseudomyrmex* behaves aggressively against other insects (Janzen 1967, Fonseca 1994, This study), viciously attacking any herbivore on the host, until it leaves the tree. This ant genus is also characterized by possessing potent venoms (Pan and Hink 2000, Touchard et al. 2014), which may allow them to effectively attack herbivores. We observed that *P. dendroicus* workers patrol their host at all times of day and night, even when temperatures are low. Workers do not forage outside the plant and do not eat the insect herbivores they attack (Fonseca 1994; A. Sanchez and E. Bellota pers. obs.). Although in some cases *P. triplarinus* Weddell and *P. dendroicus* take termite bait and/or tuna into the colony (Oliveira et al. 1987; E. Bellota pers. obs.), based on our observations, other larger insects such as caterpillars or Orthoptera do not seem to be preyed upon (as seen in other ant-plant *Pseudomyrmex* by Dejean et al. 2014).

The effects of herbivory may also extend beyond growth, ultimately affecting reproductive success and fitness of the host. Decreased energy spent on reparative growth could translate into increased energy allocation towards reproduction (Maron and Crone 2006). In addition, by protecting young individuals, survivorship would increase enhancing a plant's chances of living into adulthood, even if the production of domatia is costly (Brouat and McKey 2000, 2001). In *T. americana*, queens colonize plants as little as 30 cm tall and the first brood of workers was seen in seedlings ranging between 40 to 50 cm (Schremmer 1984, Sanchez in review). Therefore ants could play a fundamental role in the establishment and success of *Triplaris*.

Conclusion

Ant-exclusion experiments revealed that the myrmecophyte *Triplaris americana* is significantly affected by herbivory in the absence of its symbiotic associate *Pseudomyrmex dendroicus*. Ants actively patrol their host at all times of day and night, and rapidly recruit when an herbivore is encountered. Even though caulinary domatia are costly to produce (Brouat and McKey 2000, 2001), *T. americana* hosts queens of *P. dendroicus* as young seedlings. The specificity of *P. dendroicus* gives a clear difference in the potential survival of these plants. Therefore, following the development of seedlings through time and measuring the impact of herbivory before an ant colony establishes, would be fundamental to understanding how significant ant protection is during plant establishment. Short-term experiments give an idea of herbivore damage, but long-term experiments have revealed how vital ants can be for the plant's survival and the importance of ants on plant vitality, growth, and reproductive success (Heil et al. 2001). In addition,

since our experiment was conducted during the dry season, experiments comparing herbivory in the wet versus dry seasons would be beneficial, since higher herbivory rates can occur during the rainy season (Coley 1982).

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References

Agrawal AA (1998) Leaf damage and associated cues induced aggressive ant recruitment in a neotropical ant-plant. Ecology 79: 2100–2112. doi: 10.1890/0012-9658(1998)079[2100:LDAACI]2.0.CO;2

Blatrix R, Debaud S, Salas-Lopez A, Born C, Benoit L, McKey DB, Attéké C, Djiéto-Lordon C (2013) Repeated Evolution of Fungal Cultivar Specificity in Independently Evolved Ant-Plant-Fungus Symbioses. PLoS ONE 8: e68101. doi: 10.1371/journal.pone.0068101

Brandbyge J (1986) A revision of the genus *Triplaris* (Polygonaceae). Nordic Journal of Botany 6: 545–570. doi: 10.1111/j.1756-1051.1986.tb00454.x

Brouat C, McKey D (2000) Origin of caulinary ant domatia and timing of their onset in plant ontogeny: evolution of a key trait in horizontally transmitted ant-plant symbioses. Biological Journal of the Linnean Society 71: 801–819. doi: 10.1006/bijl.2000.0477

Brouat C, McKey D (2001) Leaf-stem allometry, hollow stems, and the evolution of caulinary domatia in myrmecophytes. New Phytologist 151: 391–406. doi: 10.1046/j.0028-646x.2001.00197.x

Bruna EM, Lapola DM, Vasconcelos HL (2004) Interspecific variation in the defensive responses of obligate plant-ants: experimental tests and consequences for herbivory. Oecologia 138: 558–565. doi: 10.1007/s00442-003-1455-5

Chamberlain SA, Holland JN (2009) Quantitative synthesis of context dependency in ant-plant protection mutualisms. Ecology 90: 2384–2392. doi: 10.1890/08-1490.1

Chomicki G, Renner SS (2015) Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. New Phytologist 207: 411–424. doi: 10.1111/nph.13271

Coley PD (1982) Rates of herbivory on different tropical trees. In: Leigh EG, Rand AS, Windsor DM (Eds) The ecology of a tropical forest: seasonal rhythms and long-term changes. Smithsonian Press, 123–132.

Coley PD, Kursar TA (1996) Anti-herbivore defenses of young tropical leaves: physiological constraints and ecological tradeoffs. In: Mulkey SS, Chazdon R, Smith AP (Eds) Tropical forest plant ecophysiology. Chapman and Hall, New York, 305–336. doi: 10.1007/978-1-4613-1163-8_11

Davidson DW, McKey D (1993) The evolutionary ecology of symbiotic ant-plant relationships. Journal of Hymenoptera Research 2: 13–83.

Davidson DW, Longino JT, Snelling RR (1988) Pruning of host plant neighbors by ants: an experimental approach. Ecology 69: 801–808. doi: 10.2307/1941029

Defossez E, Selosse MA, Dubois MP, Mondolot L, Faccio A, Djieto-Lordon C, McKey D, Blatrix R (2009) Ant-plants and fungi: a new threeway symbiosis. New Phytologist 182: 942–949. doi: 10.1111/j.1469-8137.2009.02793.x

Dejean A, Delabie JHC, Cerdan P, Gibernau M, Corbara B (2006) Are myrmecophytes always better protected against herbivores than other plants? Biological Journal of the Linnean Society 89: 91–98. doi: 10.1111/j.1095-8312.2006.00660.x

Dejean A, Labrière N, Touchard A, Petitclerc F, Roux O (2014) Nesting habits shape feeding preferences and predatory behavior in an ant genus. Naturwissenschaften 4: 323–330. doi: 10.1007/s00114-014-1159-1

Djiéto-Lordon C, Dejean A, Gibernau M, Hossaert-McKey M, McKey D (2004) Symbiotic mutualism with a community of opportunistic ants: protection, competition, and ant occupancy of the myrmecophyte *Barteria nigritana* (Passifloraceae). Acta Oecologica 26: 109–116. doi: 10.1016/j.actao.2004.03.007

Fiala B, Maschwitz U (1990) Studies on the South East Asian ant-plant association *Crematogaster borneensis*/*Macaranga*: Adaptations of the ant partner. Insectes Sociaux 37: 212–231. doi: 10.1007/BF02224049

Fiala B, Grunsky H, Maschwitz U, Linsenmair KE (1994) Diversity of ant-plant interactions: Protective efficacy in *Macaranga* species with different degrees of ant association. Oecologia 97: 186–192. doi: 10.1007/BF00323148

Fonseca CR (1994) Herbivory and the long-lived leaves of an Amazonian ant-tree. Journal of Ecology 82: 833–842. doi: 10.2307/2261447

Fowler HG (1993) Herbivory and assemblage structure of myrmecophytous understory plants and their associated ants in the central Amazon. Insectes Sociaux 40: 137–145. doi: 10.1007/BF01240702

Frederickson ME (2005) Ant species confer different partner benefits on two neotropical myrmecophytes. Oecologia 143:387–395. doi: 10.1007/s00442-004-1817-7

Frederickson ME, Gordon DM (2007) The devil to pay: a cost of mutualism with *Myrmelachista schumanni* ants in 'devil's gardens' is increased herbivory on *Duroia hirsuta* trees. Proceedings of the Royal Society B 274: 1117–1123. doi: 10.1098/rspb.2006.0415

Frederickson ME, Ravenscraft A, Miller GA, Arcila Hernández LM, Booth G, Pierce NE (2012) The direct and ecological costs of an ant-plant symbiosis. The American Naturalist 179: 768–778. doi: 10.1086/665654

García-Guzmán G, Dirzo R (2001) Patterns of leaf-pathogen infection in the understory of a Mexican rain forest: incidence, spatiotemporal variation, and mechanisms of infection. American Journal of Botany 88: 634–64. doi: 10.2307/2657063

Gaume L, McKey D, Anstett MC (1997) Benefits conferred by "timid" ants: active anti-herbivore protection of the rainforest tree *Leonardoxa africana* by the minute ant *Petalomyrmex phylax*. Oecologia 112: 209–216. doi: 10.1007/s004420050302

Gong B, Zhang G (2014) Interactions between plants and herbivores: A review of plant defense. Acta Ecologica Sinica 34: 325–336. doi: 10.1016/j.chnaes.2013.07.010

Heil M, Fiala B, Maschwitz U, Linsenmair KE (2001) On benefits of indirect defence: short- and long-term studies of antiherbivore protection via mutualistic ants. Oecologia 126: 395–403. doi: 10.1007/s004420000532

Itioka T, Nomura M, Inui Y, Itino T, Inoue T (2000) Difference in intensity of ant defense among three species of *Macaranga* myrmecophytes in a Southeast Asian dipterocarp forest. Biotropica 32: 318–326. doi: 10.1111/j.1744-7429.2000.tb00475.x

Janzen DH (1967) Interaction of the bull's horn acacia (*Acacia cornigera* L.) with an ant inhabitant (*Pseudomyrmex ferruginea* F. Smith) in eastern Mexico. University of Kansas Science Bulletin 47: 315–558.

Janzen DH (1972) Protection of *Barteria* (Passifloraceae) by *Pachysima* ants (Pseudomyrmecinae) in a Nigerian rain forest. Ecology 53: 885–892. doi: 10.2307/1934304

Kursar TA, Coley PD (1991) Nitrogen content and expansion rate of young leaves of rainforest species: implications for herbivory. Biotropica 23: 141–150. doi: 10.2307/2388299

Larrea-Alcázar DM, Simonetti JA (2007) Why are there few seedlings beneath the myrmecophyte *Triplaris americana*? Acta Oecologica 32: 112–118. doi: 10.1111/j.1744-7429.2011.00786.x

Longino JT (1996) Taxonomic characterization of some live-stem inhabiting *Azteca* (Hymenoptera: Formicidae) in Costa Rica, with special reference to the ants of *Cordia* (Boraginaceae) and *Triplaris* (Polygonaceae). Journal of Hymenoptera Research 5: 131–156.

Maron JL, Crone E (2006) Herbivory: effects on plant abundance, distribution and population growth. Proceedings of the Royal Society B 273: 2575–2584. doi: 10.1098/rspb.2006.3587

Marquis RJ (1984) Leaf herbivores decrease fitness of a tropical plant. Science 226: 537–539. doi: 10.1126/science.226.4674.537

Mayer VE, Frederickson ME, McKey D, Blatrix R (2014) Current issues in the evolutionary ecology of ant–plant symbioses. New Phytologist 202: 749–764. doi: 10.1111/nph.12690

McKey D (1974) Adaptive patterns in alkaloid physiology. American Naturalist, 305–320. doi: 10.1086/282909

McKey D (1979) The distribution of secondary compounds within plants. In: Rosenthal GA, Janzen DH (Eds) Herbivores: their interactions with secondary plant metabolites. Academic press, New York, 55–133.

McKey D (1984) Interactions of the ant-plant *Leonardoxa africana* (Caesalpiniaceae) with its obligate inhabitants in a rainforest in Cameroon. Biotropica 16: 81–99. doi: 10.2307/2387840

Michelangeli FA (2003) Ant protection against herbivory in three species of *Tococa* (Melastomataceae) occupying different environments. Biotropica 35: 181–188. doi: 10.1111/j.1744-7429.2003.tb00277.x

Oliveira PS, Oliveira Filho AT, Cintra R (1987) Ant foraging on ant-inhabited *Triplaris* (Polygonaceae) in western Brazil: a field experiment using live termite baits. Journal of Tropical Ecology 3: 193–200. doi: 10.1017/S0266467400002066

Pan J, Hink WF (2000) Isolation and characterization of myrmexins, six isoforms of venom proteins with anti-inflammatory activity from the tropical ant, *Pseudomyrmex triplarinus*. Toxicon 38: 1403–1413. doi: 10.1016/S0041-0101(99)00233-0

Pitman NCA, Terborgh J, Silman MR, Nuñez P (1999) Tree species distribution in an upper Amazonian forest. Ecology 80: 2651–2661. doi: 10.1890/0012-9658(1999)080[2651:TSDIAU]2.0.CO;2

Rico-Gray V, Oliveira PS (2007) The ecology and evolution of ant-plant interactions. University of Chicago Press, Chicago, 346 pp. doi: 10.7208/chicago/9780226713540.001.0001

Rosumek FB, Silveira FA, Neves FDS, Barbosa NPDU, Diniz L, Oki Y, Pezzini F, Fernandes GW, Cornelissen T (2009) Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. Oecologia 160: 537–549. doi: 10.1007/s00442-009-1309-x

Sagers CL, Coley PD (1995) Benefits and costs of plant defense in a Neotropical shrub. Ecology 76: 1835–1843. doi: 10.2307/1940715

Sanchez A (in review) Establishing an ant-plant mutualism: Foundress queen mortality and acquiring the third partner. Insectes Sociaux.

Schremmer F (1984) Untersuchungen und Beobachtungen zur Ökoethologie der Pflanzenameise *Pseudomyrmex triplarinus*, welche die Ameisenbäume der Gattung *Triplaris* bewohnt. Zoologische Jahrbücher. Abteilung fur Systematik, Ökologie und Geographie der Tiere 111: 385–410.

Spotswood E, Bradley KL, Knops JMH (2002) Effects of herbivory on the reproductive effort of 4 prairie perennials. BMC Ecology 2: 2. doi: 10.1186/1472-6785-2-2

Staton ML, Palmer TM (2011) The high cost of mutualism: effects of four species of East African ant symbionts on their myrmecophyte host tree. Ecology 92: 1073–1082. doi: 10.1890/10-1239.1

Strauss SY, Agrawal AA (1999) The ecology and evolution of plant tolerance to herbivory. Trends in Ecology and Evolution 14: 179–185. doi: 10.1016/S0169-5347(98)01576-6

Strauss SY, Rudger JA, Laua JA, Irwina RE (2002) Direct and ecological costs of resistance to herbivory. Trends in Ecology and Evolution 17: 278–285. doi: 10.1016/S0169-5347(02)02483-7

Touchard A, Labrière N, Roux O, Petitclerc F, Orivel J, Escoubas P, Koh JMS, Nicholson GM, Dejean A (2014) Venom toxicity and composition in three *Pseudomyrmex* ant species having different nesting modes. Toxicon 88: 67–76. doi: 10.1016/j.toxicon.2014.05.022.

Trager MD, Bhotika S, Hostetler JA, Andrade GV, Rodriguez-Cabal MA, McKeon CS, Osenberg CW, Bolker BM (2010) Benefits for plants in ant-plant protective mutualisms: a meta-analysis. PLoS ONE 5: e14308. doi: 10.1371/journal.pone.0014308

Ule E (1906) Ameisenpflanzen. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 37: 335–352.

Ward PS (1990) The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): generic revision and relationship to other formicids. Systematic Entomology 15: 449–489. doi: 10.1111/j.1365-3113.1990.tb00077.x

Ward PS (1999) Systematics, biogeography and host plant associations of the *Pseudomyrmex viduus* group (Hymenoptera: Formicidae), *Triplaris*- and *Tachigali*-inhabiting ants. Zoological Journal of the Linnean Society 126: 451–540. doi: 10.1111/j.1096-3642.1999.tb00157.x

Ward PS, Downie DA (2005) The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): phylogeny and evolution of big-eyed arboreal ants. Systematic Entomology 30: 310–335. doi: 10.1111/j.1365-3113.2004.00281.x

Weir TL, Newbold S, Vivanco JM, van Haren M, Fritchman C, Dossey AT, Bartram S, Boland W, Cosio EG, Kofer W (2012) Plant-inhabiting ant utilizes chemical cues for host discrimination. Biotropica 44: 246–253. doi: 10.1111/j.1744-7429.2011.00786.x

Wheeler WM (1942) Studies of Neotropical ant-plants and their ants. Bulletin of the Museum of Comparative Zoology at Harvard College 90: 41–65.

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RESEARCH ARTICLE

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A new species of *Encarsia* (Hymenoptera, Aphelinidae) developing on ficus whitefly *Singhiella simplex* (Hemiptera, Aleyrodidae) in China and Taiwan

Chiun Cheng Ko[1], Yuan Tung Shih[1], Stefan Schmidt[2], Andrew Polaszek[3]

1 *Department of Entomology, National Taiwan University, Taipei 106, Taiwan* **2** *SNSB-Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 Munich, Germany* **3** *Department of Life Sciences, the Natural History Museum, London SW7 5BD, UK*

Corresponding author: *Stefan Schmidt* (hymenoptera@zsm.mwn.de)

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Abstract

Encarsia singhiellae Polaszek & Shih, **sp. n.**, is described and illustrated. It is known so far from Taiwanand China. All specimens were reared from the ficus, or fig, whitefly *Singhiella simplex* (Singh), an Asian species recently attaining pest status in California, Colombia, and Florida.

Keywords

Parasitoid, invasive species, potential biocontrol agent

Introduction

The ficus or fig whitefly *Singhiella simplex* was described from India (Singh 1931), and appears to have a natural distribution across South and Southeast Asia that includes China, India, Myanmar and Taiwan (EPPO 2014, Natural History Museum, London, unpublished data). It was first recorded as an invasive species established in Florida (2007) and California (2012), has since become widespread in Central and South America and the Caribbean, and was recorded from Cyprus in 2014. It appears to be restricted to developing on several *Ficus* species, and is commonly recorded from

F. benjamina L. and *F. microcarpa* L.f. Some *Ficus* species appear to be unsuitable for its development (EPPO 2014).

There are three published records of parasitoids of this species, all apparently from Florida. Hodges (2007), recording *S. simplex* for the first time from Florida, cited *Encarsia tricolor* Förster, 1878 a species that occurs nowhere in the New World, nor anywhere within the natural Old World distribution of *S. simplex*. We therefore consider this parasitoid record to be a misidentification. *Encarsia protransvena* Viggiani, 1985, and *Amitus bennetti* Viggiani & Evans, 1992 were recorded from *S. simplex* in Florida (Avery et al. 2011) and, although no identification authority was cited, these seem to have been identified correctly. Given the origin of *S. simplex* in the Old World tropics, it seems likely that a native parasitoid, screened for a reasonable degree of host-specificity, might be a good candidate for classical biological control of *S. simplex* in the New World. Such a possible candidate is described below. Terminology follows Huang and Polaszek (1998).

Abbreviations

FAFU Fujian Agriculture and Forestry University, Fuzhou, CHINA.
NHM Natural History Museum, London, U.K.
NTU National Taiwan University, Taipei, TAIWAN.

Material and methods

A single series of reared specimens collected in September 2010 by the second author (YTS) was studied in detail for taxonomically useful morphological characters by the second and fourth (AP) authors. A single specimen reared one month later from the same host from mainland China proved to be morphologically identical. DNA was successfully sequenced from four individuals from the original sample, using the protocol described in detail by Polaszek et al. (2013). This "non-destructive" extraction method has proven extremely effective for the smallest parasitoids. Sequence data for the ribosomal 28S-D2 region were aligned using MUSCLE (Edgar 2004) and analysed using RAxML (Stamatakis 2014) by the third author.

Results

***Encarsia singhiellae* Shih & Polaszek, sp. n.**
http://zoobank.org/3E0E06D7-3280-44E4-B2C3-56BED3E54000
Figs 1–15

Description of female. Colour: Head yellow, antenna yellow, slightly darker towards apex. Mesosoma yellow except following light brown: pronotum, posterior margin of

mesoscutum, anterior margin of scutellum, axillae and sides of propodeum. Metasoma yellow except T5 dark brown in strong contrast. Base of T1, and T4, infuscate centrally. Fore wing slightly infuscate below marginal vein. Legs yellow.

Morphology: Mandibles each with three small teeth. Stemmaticum with five robust setae and reticulate surface sculpture. Antennal formula 1,1,4,2. F1, F2, F3 approximately equal in length, with any of the three antennomeres the longest in different specimens. Pedicel with two robust setae dorsally. F4 0.9 times F1 (0.85 in holotype); F5 0.7–0.9× F1 (0.73 in HT); F6 0.8–1.0times F1 (0.9 in HT); funicle length 2.5times clava length (2.3 in HT). F1–F6 with the following numbers of multiporous plate sensilla: F1:0; F2:2; F3:2; F4:3; F5:3 F6:3. Mid lobe of mesoscutum with 4 or 5 pairs of setae, 1 lateral pair and 3–4 centrally (one central seta unpaired in holotype); side lobes with three setae. Scutellar sensilla closely placed, separated by less than the maximum width of one sensillum. Distance between posterior pair of scutellar setae 2 times distance between posterior pair (2.1 times in HT). Fore wing 2.8 times maximum width of disc (2.84 in HT). Marginal fringe 0.26 times maximum width of disc (0.25 in HT). Submarginal vein with 3 setae; marginal vein anteriorly with 7–9 setae (8+9 in HT). Basal cell with 9–15 setae (11+12 in HT). Tarsal formula 5-5-5. Mid tibial spur 0.56 times corresponding basitarsus. Mid tibia with a prominent spine-like seta apically. Metasomal tergites with the following numbers of setae: T1: 0, T2: 2, T3: 2, T4: 2, T5: 4, T6: 4, T7: 4. Ovipositor 1.2 times mid tibia; 2nd valvifers 3.8 times third valvulae (3.7 in HT).

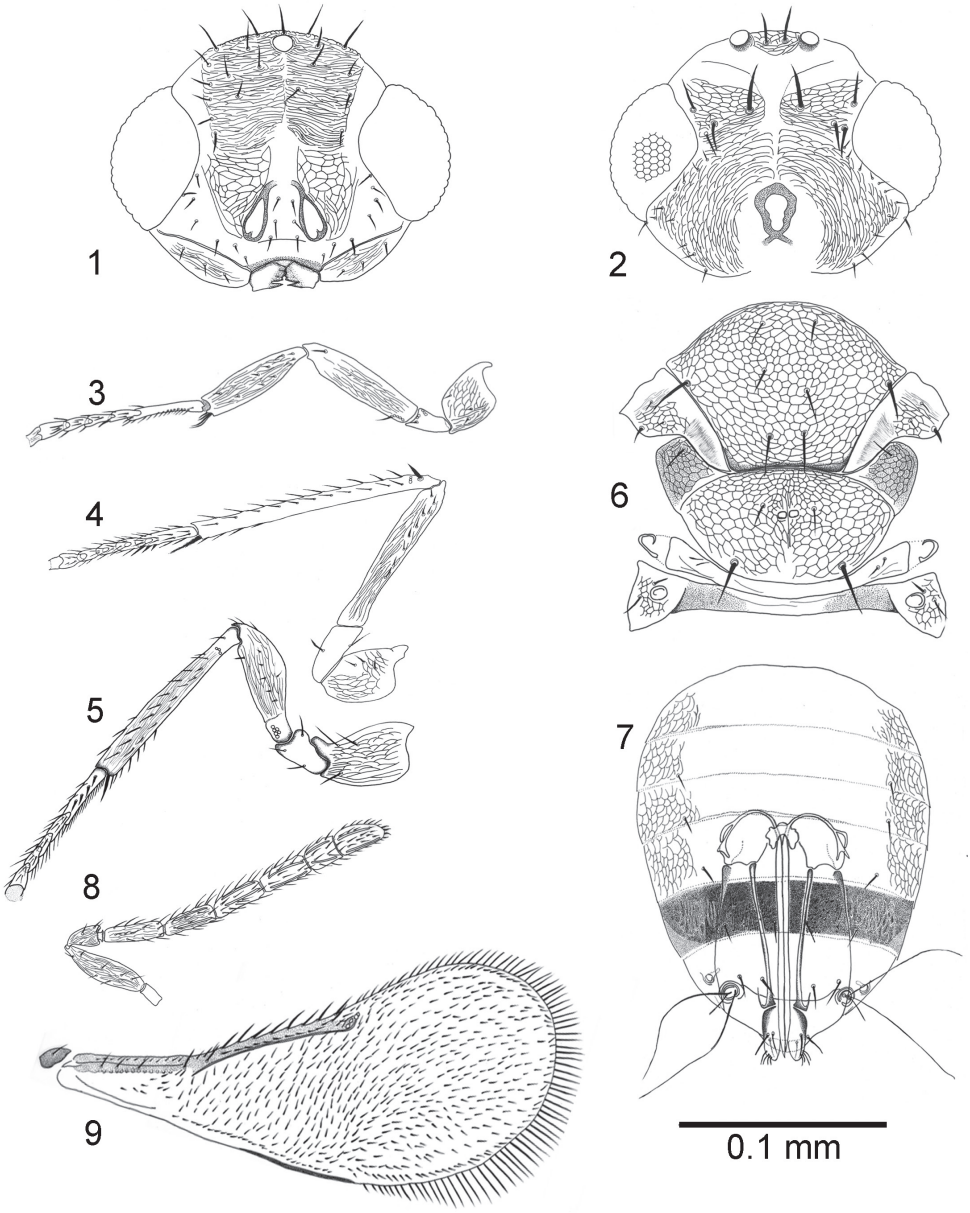
Male. Unknown.

Material examined. Holotype female (NHM) on slide, labelled "TAIWAN: Taoyuan, Kuanyin (25.034°N, 121.113°E), 07 July 2011, ex *Singhiella simplex* on *Ficus microcarpa* Y.T. Shih col. Holotype Encarsia singhiellae Shih & Polaszek"; paratypes: 9 females, same data as holotype (NHM, NTU). **CHINA:** Fujian, Xiamen (24.481°N, 118.089°E), 6.x.2010 ex *Singhiella simplex* on *Ficus microcarpa*, J Huang, A Polaszek, Z-H Wang col. (1 female, FAFU).

Species group placement. The close proximity of the scutellar sensilla, coupled with three setae on the submarginal vein might suggest placement of the new species in the *E. strenua* group, but the shape of the stigma vein indicates that this placement would be incorrect. *E. strenua* group species have a distinct constriction between the marginal and stigmal veins. DNA analysis of the 28S D2 region places *E. singhiellae* sp. n. far away from the monophyletic *E. strenua* group, in an assemblage that includes *E. tricolor* Foerster, *E. tachii* (Polaszek & Hayat), and *E. mineoi* Viggiani (S. Schmidt, unpublished data). *Encarsia singhiellae* is therefore currently unplaced with respect to any known species group of *Encarsia*. The sequence has been deposited in GenBank under accession number KT279403.

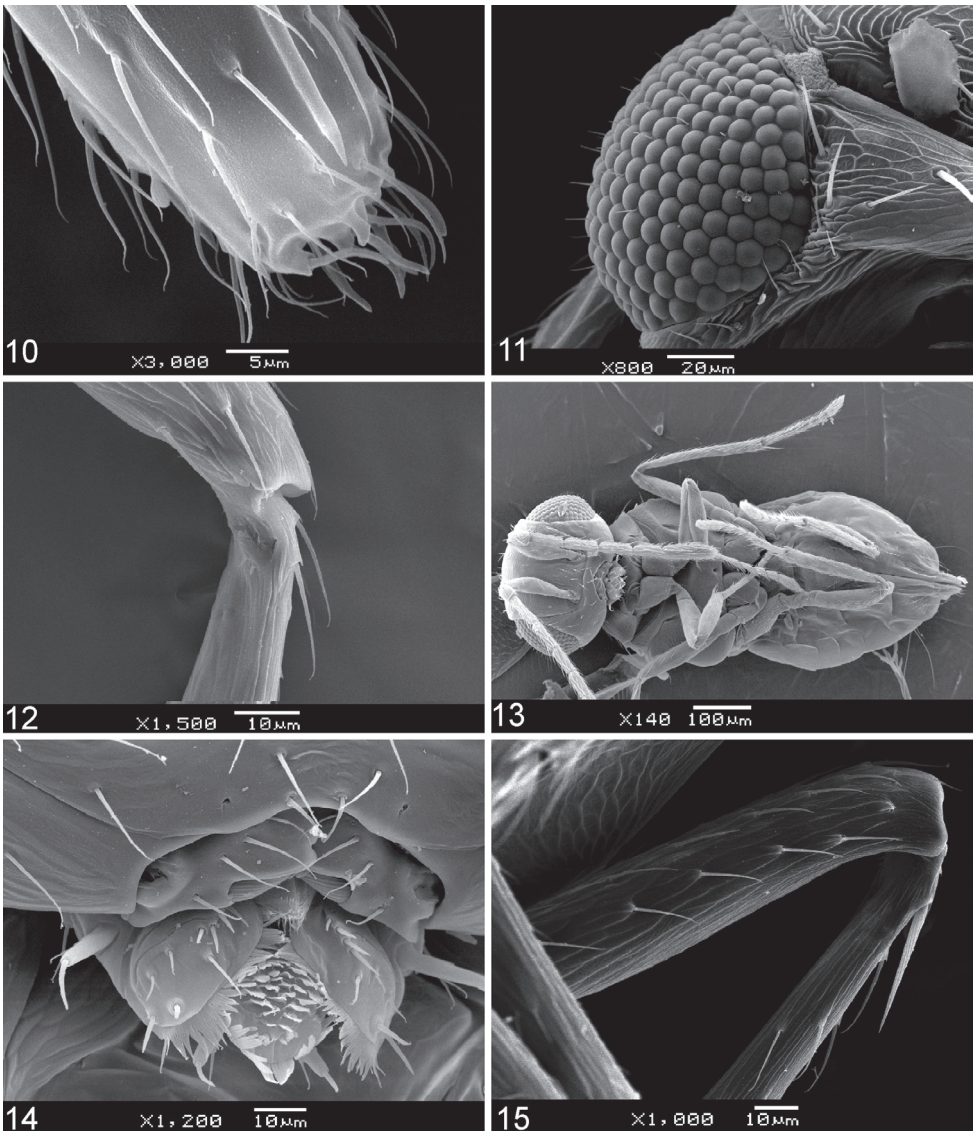
In the key to Chinese *Encarsia* species (Huang and Polaszek 1998), *E. singhiellae* sp. n. keys to *E. noyesana* Huang & Polaszek, 1998. It can be easily distinguished from that species by the three setae on the submarginal vein (two in *E. noyesana*); 2-segmented clava (3-segmented in *E. noyesana*); and the distinct colour pattern of the metasoma.

Host. *Singhiella simplex* (Singh) (Hemiptera: Aleyrodidae).



Figures 1–9. *E. singhiellae* sp. n.: **1** Head, frontal view **2** Head, back view **3** Fore leg **4** Mid leg **5** Hind leg **6** Dorsal mesosoma **7** Dorsal metasoma **8** Antenna **9** Fore wing.

Remarks. *Encarsia singhiellae* sp. n. is not closely related to any known *Encarsia* species, either in the Oriental Region or elsewhere. It has several unusual characters as follows: antenna with two robust setae on the pedicel, and F1 having distinct sculpture; anterior apex of mid tibia with one distinct long spine-like seta. The following



Figures 10–15. *E. singhiellae* sp. n.: **10** Apex of antenna **11** Detail of compound eye **12** Articulation of tibia and femur, fore leg **13** Ventral habitus **14** Mouth **15** Articulation of tibia and femur, mid leg.

character states place the new species in the genus *Encarsia*: fore and hind tarsi five-segmented, eight antennomeres (excluding radicle), scutellum with two pairs of setae, marginal vein longer than submarginal vein, stigmal vein very short and postmarginal vein absent.

It is the first recorded parasitoid of *Singhiella simplex* in Asia, and appears to show a high degree of host specificity, as there are no host records from other whitefly species. The species is currently only known from the type locality.

Acknowledgements

This study was funded by the Taiwan Ministry of Science and Technology (Project No. 102-2628-B-002-019-MY3 to CCK and YTS). Field work in China by AP was facilitated by a grant from the Chinese Academy of Sciences to Prof Huang Jian, Fujian Agricultural and Forestry University. We are grateful to the following for useful comments on earlier versions of the MS: John Heraty, John Huber, John Noyes, JimWoolley.

References

Avery PB, Mannion CM, Powell CA, McKenzie CL, Osborne LS (2011) Natural enemies managing the invasion of the Fig Whitefly, *Singhiella simplex* (Hemiptera: Aleyrodidae), infesting a *Ficus benjamina* Hedge. Florida Entomologist 94(3): 696–698. doi: 10.1653/024.094.0338

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32(5): 1792–1797. doi: 10.1093/nar/gkh340

EPPO (European and Mediterranean Plant Protection Organisation) (2014) *Singhiella simplex* (Hemiptera: Aleyrodidae) Ficus whitefly. http://www.eppo.int/QUARANTINE/Alert_List/insects/singhiella_simplex.htm [accessed March 2015]

Hodges G (2007) The Fig Whitefly Singhiella simplex (Singh) (Hemiptera: Aleyrodidae): A New Exotic Whitefly Found on Ficus Species in South Florida. Pest Alert DPI-FDACS. http://www.freshfromflorida.com/pi/enpp/ento/Singhiella%20simplex.html [accessed March 2015]

Huang J, Polaszek A (1998) A revision of the Chinese species of *Encarsia* Foerster (Hymenoptera: Aphelinidae): parasitoids of whiteflies, scale insects and aphids (Homoptera: Aleyrodidae, Diaspididae, Aphidoidea). Journal of Natural History 32: 1825–1966. doi: 10.1080/00222939800770911

Polaszek A, Ayshford T, Yahya BE, Fusu L (2013) *Wallaceaphytis*: an unusual new genus of parasitoid wasp (Hymenoptera: Aphelinidae) from Borneo. Journal of Natural History, special issue celebrating the Wallace100 centenary in 2013: 17–29.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. doi: 10.1093/bioinformatics/btu033

Singh K (1931) A contribution towards our knowledge of the Aleyrodidae (Whiteflies) of India. Memoirs of the Department of Agriculture in India 12: 1–98.

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RESEARCH ARTICLE

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Doryctobracon areolatus (Hymenoptera, Braconidae) a parasitoid of early developmental stages of *Anastrepha obliqua* (Diptera, Tephritidae)

Félix D. Murillo[1], Héctor Cabrera-Mireles[1], Juan F. Barrera[1], Pablo Liedo[1], Pablo Montoya[2]

1 *El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km 2.5, Tapachula, 30700 Chiapas, Mexico*
2 *Programa Moscafrut SENASICA-SAGARPA. Camino a los Cacaotales S/N, Metapa de Domínguez, CP 30860, Chiapas, México*

Corresponding author: *Félix D. Murillo* (fmurillo@ecosur.edu.mx)

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Abstract

Natural parasitism of *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae) on various development stages of *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) attacking *Spondias mombin* L. fruits was studied under field conditions. We collected 120 fruits of *S. mombin* from which we got 495 *A. obliqua* larvae of different instars. A total of 88% of these larvae were parasitized. Within the parasitized cohort, the first-instar of *D. areolatus* was frequently detected in all 3 larval stages (L1 = 94.3%, L2 = 98.1%, and L3 = 100%), and the rest (i.e., L1 = 5.7%, L2 = 1.8%) corresponded to the presence of eggs. In fruits with controlled infestation and cage-induced parasitism under field conditions, *D. areolatus* oviposited in mature eggs and recently hatched larvae of *A. obliqua* with comparable frequencies. Seven preimaginal stages of *D. areolatus* were observed during their development, which was completed in 27 days. It is concluded that *D. areolatus* has the capacity to oviposit in embryo eggs and neonate larvae of *A. obliqua* and that its first-instar larvae (with three distinct sizes) are capable of synchronizing their development with the development of the host larvae. This finding represents the first report of a native parasitoid attacking eggs or neonate larvae of a tephritid in the Neotropics. The implications of this finding are discussed within the context of the competitive interactions of this species with other parasitoid species under sympatric conditions, as well as the relevance for developing laboratory rearing methods for biological control purposes.

Keywords
Egg parasitoid, laboratory breeding, interspecific competition, morphology, fruit flies, biological control

Introduction

The native parasitoid guild that attacks fruit flies of the genus *Anastrepha* Schiner in the Neotropics is mainly composed of a group of solitary koinobiont endoparasitoids (primarily Braconidae and Figitidae) that oviposit in the host larvae and emerge from the pupae. The genus *Doryctobracon* constitutes 27% of the parasitoid species and shares a closely related evolutionary history with *Anastrepha* (López et al. 1999, Ovruski et al. 2000).

Another smaller group of *Anastrepha* parasitoids are pupal idiobionts that attack their hosts when they are in the soil and is represented by five species within the genera *Coptera* and *Trichopria* (Diapriidae) and three polyphagous species, *Pachycrepoideus vindemiae* (Rondani), *Spalangia cameroni* Perkins, and *S. endius* Walker (Pteromalidae) (Ovruski et al. 2000). The parasitoid guild of *Anastrepha* spp. in the Neotropics represents an important source of species with potential to be used in biological control programs against native tephritids (Aluja et al. 2003). However, native *Anastrepha* parasitoids attacking eggs or early larval developmental stages have not been reported. The only report of a parasitoid attacking *Anastrepha* eggs in the Americas correspond to Wharton et al. (1981), who found a small level of parasitism of the introduced *Fopius arisanus* (Sonan), apparently on *A. striata* (Schiner) eggs.

According to Aluja et al. (2009) species of the genera *Doryctobracon*, such as *D. crawfordi* (Viereck) and *D. areolatus* (Szépligeti), exhibit great potential as biological control agents and should be examined from the point of view of mass rearing projects. *D. areolatus* has been reported as a solitary koinobiont endoparasitoid that attacks third instar larvae of *Anastrepha* spp., both in native and exotic commercial fruits, with a wide distribution from Florida to Argentina (Hernández-Ortiz et al. 1994, López et al. 1999, Ovruski et al. 2000, Sivinski, et al. 2000, Aluja et al. 2003, 2009). This species frequently shows field dominance among concurrent parasitoid species attacking *Anastrepha* spp. (López et al. 1999, Sivinski et al. 1997, 2000; Aluja et al. 2003, Ovruski et al. 2004). The presence of diapause (Aluja et al. 1998, Ovruski et al. 2004) and an extrinsic capacity to find patches with low host density (Sivinski et al. 1998) allows an ample distribution in regions with low plant diversity (Eitam et al. 2004).

In the central region of Veracruz, Mexico, *D. areolatus* is the most abundant parasitoid species attacking *Anastrepha obliqua* (Macquart) in *Spondias* spp. (Cabrera et al. 2006), where it has frequently been observed at the beginning of the fruiting season. This suggests that this species could parasitize earlier developmental stages in relation to sympatric parasitoid species. Normally, parasitoids attacking eggs and first instar larvae of their hosts become more competitive than those attacking later stages (Wang and Messing 2002, Wang et al. 2003, Wang et al. 2008, Argov et al. 2011), showing

important potential to be used as biocontrol agents. Therefore, our aims in this study were: 1) to determine if *D. areolatus* parasitize immature stages of *A. obliqua* and 2) to characterize its developmental stages during each phase of host development.

Materials and methods

Study area

The study was conducted in the coastal region of central Veracruz, which is characterized by high densities of *A. obliqua* hosts, such as mango (*Mangifera indica* L.), native *Spondias* species and guavas (*Psidium guajava* L.). Fruit samples were collected from trees located in backyard orchards and marginal zones, which provide resources for the presence of flies and parasitoids all year round. This zone is located between 19°00' and 18°55' North latitudes and 96°10' and 96°13' West longitudes, with a mean altitude of 18.5 m.a.s.l. The climate is semi-humid, with a mean annual rainfall of 1,358 mm and a very marked rainy season from June to September. The highest mean monthly temperature (29.1 °C) occurs in the month of June and the lowest mean monthly temperature (21.4 °C) occurs in the month of January (SMN 2010).

Determination of the natural parasitism of *A. obliqua* larvae by *D. areolatus* under field conditions

From September to October 2013, hog plum (*Spondias mombin* L.) fruits were collected from four sites, three in the locality of "El Copital" and one in "El Mangal", municipality of Medellín de Bravo, from four to five trees per site. Fruits were collected directly from the trees (36 fruits, 30%) and from the ground surrounding the trees (84 fruits, 70%). Each sample consisted of 10 fruits per site. Samples were in three sampling dates separated by seven days to cover the fruiting season of *Spondias* spp. A total of 120 fruits were dissected.

Anastrepha obliqua larvae were extracted from each of the fruits the same day they were collected. Larval instars were categorized based on the width of the cephalic capsule and the body length (mean ± SE) (Carroll and Wharton 1989). Larvae were dissected immediately after collection, and the frequencies of the immature stages of *D. areolatus*, or any other parasitoid species, were recorded following descriptions by Aluja et al. (2013) and Murillo et al. unpublished data.

Photographs were captured with a Motic Plus 2.0® camera connected to a Carl Zeiss Smz -168® stereomicroscope. The *D. areolatus* immatures inside the *A. obliqua* larvae were measured using Motic Imagen Plus 2.0 ® software. The percentage of parasitized larvae was calculated, and frequencies of immature *D. areolatus* stages per larval instar of *A. obliqua* were determined.

Induction of *D. areolatus* parasitism on *A. obliqua* eggs and recently hatched larvae

Wild *A. obliqua* flies were collected as larvae from infested *S. mombin* fruits in the field. Upon completion of their development, the larvae were placed in containers with sterile sand for pupation. They were maintained under these conditions until adult emergence. Adults were maintained with water and food (sugar plus hydrolyzed yeast in a 3:1 ratio) until they were sexually mature.

Hog plum (*S. mombin*) fruits were previously protected from natural infestation by bagging clusters of young fruits using 30 × 20 cm brown paper bags. A total of 30 bags (≈10 fruits/bag) were used to protect ≈300 fruits. The fruits were subsequently collected, taking their maturity into account to allow for experimental infestation.

Infestation on the previously protected fruits was induced by exposing the fruits to *A. obliqua* gravid females (8–10 days old) in Plexiglass cages (20×20×20 cm) placed on a table in the field. Two clusters with five to eight fruits were placed in each cage together with 10 female flies and remained in the field at a mean temperature of 28.2 °C (range: 23.2–36.1) and a mean RH of 81.6% (range: 55.1–95.3). Flies were maintained for six hours in each cage and dead flies were replaced.

Anastrepha obliqua eggs were exposed to the parasitoid in the same type of cages 24, 48, and 72 hours after fly oviposition in the fruit (≈egg age), in order to cover the different egg stages before larval hatching. Twenty 7-day-old *D. areolatus* females were placed in each cage for three hours, time enough to locate and oviposit in the exposed eggs. Immediately after exposure, 35 *A. obliqua* eggs and 15 newly hatched larvae were extracted from the fruits. The eggs were characterized as either yolk-egg or embryo-egg (after Chapman 2013, pp: 358–407). All of the *A. obliqua* individuals in the egg and larval stages were dissected to characterize and record the immature stages of *D. areolatus*.

Characterization of *D. areolatus* development and morphological changes

Development of *D. areolatus* eggs and larvae was individually photographed and measured. To follow the development of *D. areolatus* in *A. obliqua* pupae, mature *A. obliqua* larvae were obtained from presumably infested fruit collected in the field. These larvae were placed in 100-ml plastic containers with sterile sand as a substrate to facilitate pupation. Three cohorts of 50 *A. obliqua* pupae were examined, and 3 to 5 pupae per day were dissected from 0 to 12 days of growth. *D. areolatus* individuals and their developmental stages were recorded for each *A. obliqua* pupa.

The frequencies of the different immature developmental stages and the characteristics of *D. areolatus* were calculated for each immature stage of *A. obliqua*. All of the observations of the organisms were conducted using the above-mentioned microscope.

Data analysis

Chi-square test was used to compare the number of *D. areolatus* individuals observed at each developmental stage with the expected number, using SPSS Statistic 17.0. (SPSS Inc., 2008). Measurements of the cephalic capsules of *A. obliqua* larvae are given as the mean ± (SE). The proportions of the immature stages of *D. areolatus* for each *A. obliqua* egg and larval stages are presented as observed numbers, and *D. areolatus* development is presented as numbers of individuals and percentages.

Results

Natural parasitism in the field

From the 120 fruits that were sampled, 495 *A. obliqua* larvae were extracted; 85, 115 and 295 of these were L_1, L_2 and L_3 larvae, respectively, and 69 (82%), 104 (90%) and 264 (89%) of these larval stages were parasitized, respectively (mean parasitism = 88 ± 5.2%).

D. areolatus was the dominant parasitoid species (93.1%), and only *Utetes anastrephae* (Viereck) (5.4%) was found as the second most dominant parasitoid (Figure 1a). The remaining 1.5% (third-instar larvae) was parasitized by both species and no apparent advantage was observed for either species, except for the occasional larger size of *D. areolatus* larvae (Figure 1b). *D. areolatus* and *U. anastrephae* larvae found together were first-instar larvae, which were easily distinguishable from each other, primarily because of the larger sizes of the cephalic capsule and the jaws of *U. anastrephae* (see Fig. 1).

The mean ± (SE) of the widths of the cephalic capsules and the body lengths, respectively, of *A. obliqua* larval instars were 0.09 ± 0.001 mm and 0.90 ± 0.05 mm for the L_1, 0.37 ± 0.03 mm and 4.67 ± 0.3 mm for the $L_{2,}$ and 0.63 ± 0.004 mm and 9.16 ± 0.3 mm for the L_3.

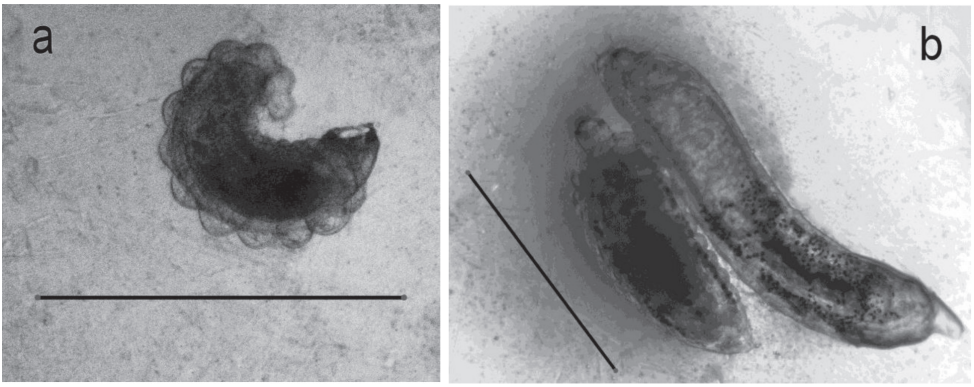

Figure 1. Parasitoids found in naturally parasitized *A. obliqua* larvae. **a** First instar larva of *U. anastrephae* found in a third instar larva of *A. obliqua* and **b** Larvae of *U. anastrephae* and *D. areolatus* found together in a third instar larva of *A. obliqua*. Scale bars = 1 mm.

Table 1. Numbers of observed individual stages of development of *D. areolatus* recorded in the different larval stages of *A. obliqua* extracted from field-collected hog plums (*S. mombin*).

D. areolatus	*A. obliqua*		
	L1	**L2**	**L3**
Egg	3	2	0
L_1 Early	66	5	0
L_1 Intermediate	0	97	2
L_1 Late	0	0	262

L1 Early = First instar larva small (≈ 0.8 mm long); L1 Intermediate = First instar larva medium (≈ 1.4 mm long); L1 Late = First instar larva large (≈ 1.7 mm long).

The numbers of the developmental stages of *D. areolatus* recorded in the various stages of naturally parasitized *A. obliqua* are given in Table 1. Embryo-eggs and a high frequency of early first-instar larvae were detected in *A. obliqua* first-instar larvae. In second-instar larvae, the presence of *D. areolatus* eggs was minimal, with a higher frequency of intermediate first-instar larvae. In the third-instar larvae, nearly all of the *D. areolatus* were late first-instar larvae. The relationship between the immature stages of *D. areolatus* and the larval states of *A. obliqua* was significant (χ^2_4= 800.9, $P < 0.0001$).

Presence of *D. areolatus* eggs in recently hatched *A. obliqua* larvae

In the controlled infestation experiment, *A. obliqua* yolk-eggs were not parasitized, which indicates that *D. areolatus* did not parasitize eggs without a formed embryo (Table 2). However, parasitism was detected in *A. obliqua* embryo-eggs. A recently laid *D. areolatus* egg (with yolk in its interior) on an *A. obliqua* embryo can be observed in Figure 2 and is folded in the embryo's interior, given that both structures are of a similar length. Seven recently hatched *A. obliqua* larvae that were parasitized by *D. areolatus* eggs were dissected; five were still in the yolk stage and two in the embryo stage (Figure 3a).

Table 2. Numbers of *D. areolatus* developmental stages found in *A. obliqua* eggs and first instar larvae when *S. mombin* fruits were infested in a controlled manner.

D. areolatus	*A. obliqua*			
	Eggs		**First instar larvae**	
	Yolk	**Embryo**	**Newly emerged**	**Mature**
Egg (yolk)	0	3	5	0
Egg (embryo)	0	0	2	2
L_1 Early	0	0	0	13

L1 Early = First instar larva small (≈ 0.8 mm long).

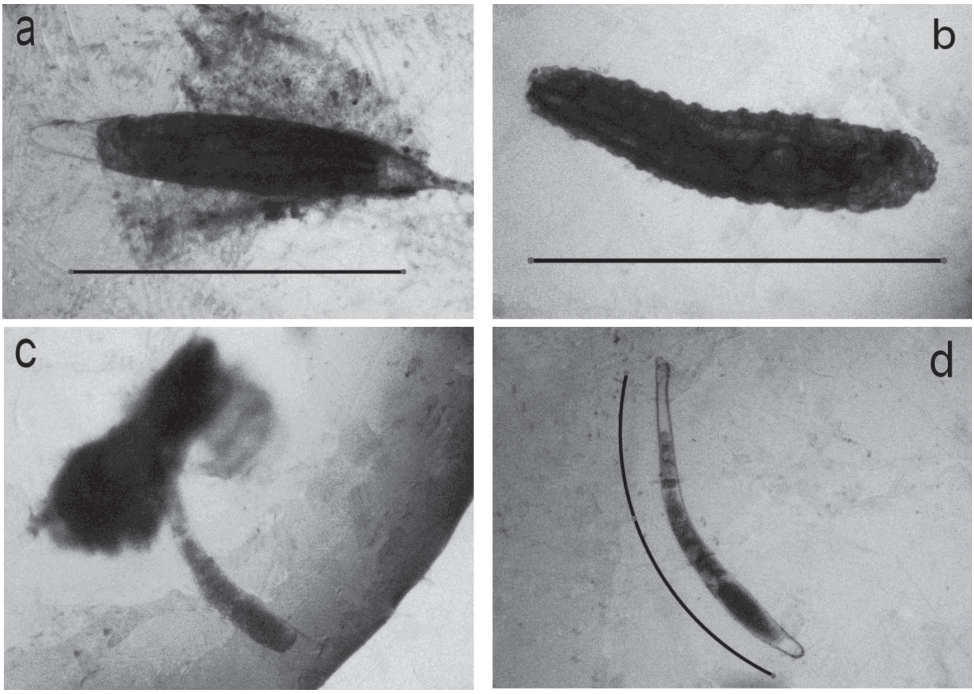

Figure 2. *D. areolatus* parasitizing an *A. obliqua* embryo. **a** *A. obliqua* egg embryo **b** *A. obliqua* embryonic egg removed **c** *D. areolatus* egg extracted from *A. obliqua* embryo, and **d** *D. areolatus* egg. Scale bars = 1 mm.

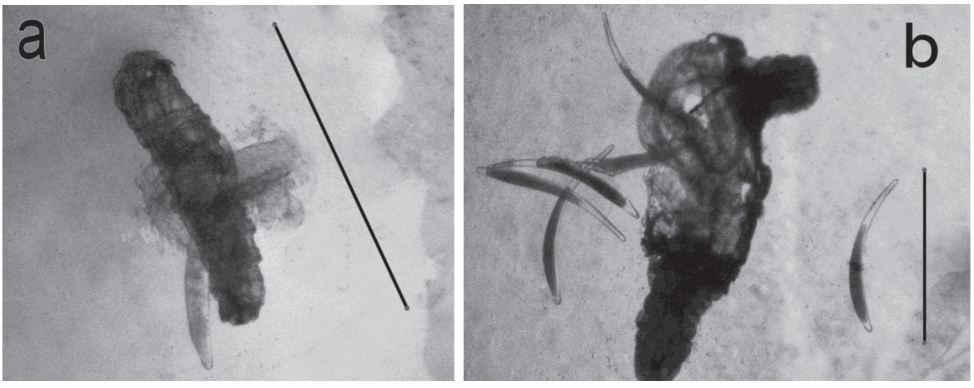

Figure 3. Parasitized *A. obliqua* newly hatched larvae **a** with a *D. areolatus* egg inside and **b** with six eggs of *D. areolatus* inside. Scale bars = 1 mm.

Superparasitism by *D. areolatus* was recorded in two out of seven recently hatched *A. obliqua* larvae, and six parasitoid eggs were recorded from one larva (Figure 3b).

Fifteen mature first-instar *A. obliqua* larvae were dissected. Of these, 13 were parasitized with early first-instar larvae and 2 with embryo-eggs of *D. areolatus*. Yolk-eggs of *D. areolatus* were not found (Table 2). The relationship between the immature stages of *D. areolatus* and the immature stages of *A. obliqua* was significant ($\chi^2_4 = 22.4$, $P < 0.0001$).

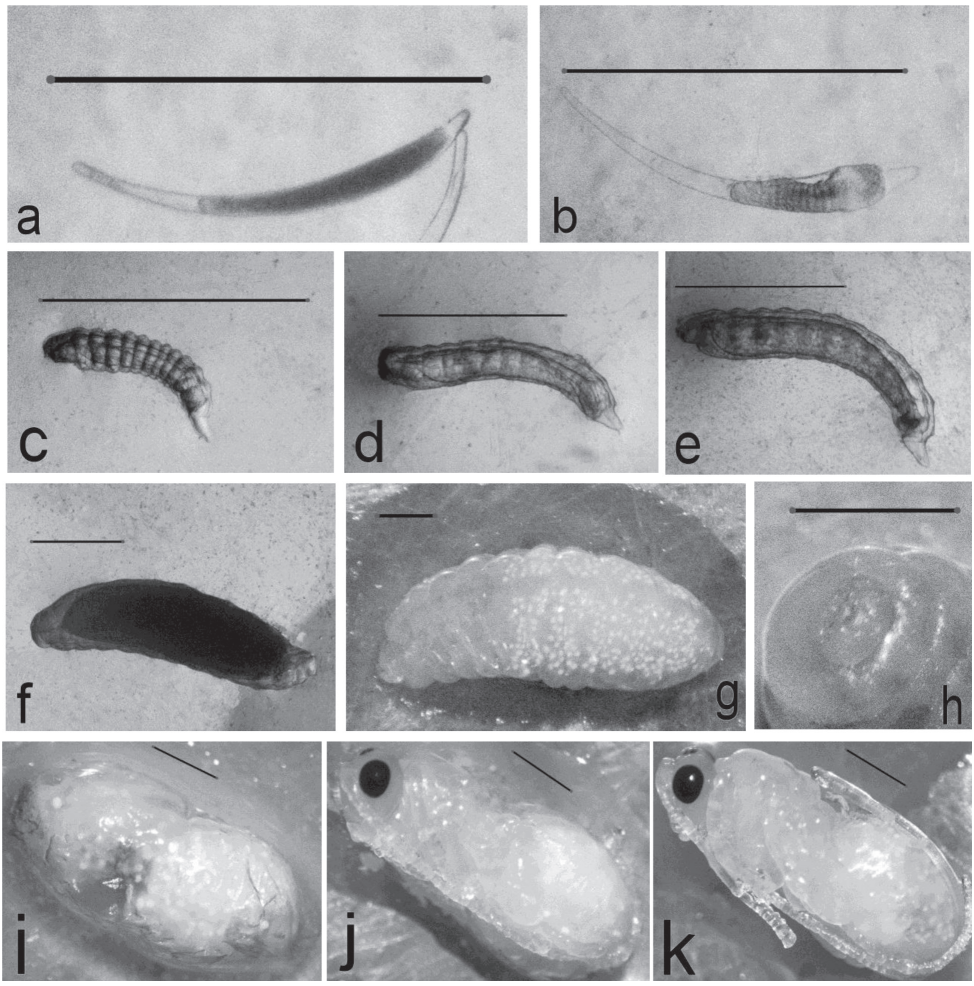

Figure 4. Development of immature stages of *D. areolatus.* **a** egg yolk **b** egg embryo **c** early first instar larva **d** intermediate first instar larva **e** late first instar larva **f** second instar larva **g** third instar larva **h** jaw of third instar larva **i** prepupa **j** male pupa, and **k** female pupa with her ovipositor. Scale bars = 1 mm.

Characterization of *D. areolatus* development

The *D. areolatus* egg measures ≈ 1 mm long and has an elongated shape and a whitish color with a dark yolk. After 24 hours, the embryo is formed with a claviform appearance and measures ≈ 0.5 mm in length (Table 3 and Figure 4).

The embryo of *D. areolatus* becomes an early first-instar larva within 24 to 36 hours of oviposition and measures ≈ 0.8 mm in length. After three to four days, an early first-instar larva grows into an intermediate first-instar larva, with a length of ≈ 1.4 mm. After another three to four days, the larva grows into a late first-instar larva measuring ≈ 1.7 mm in length. This larva almost immediately changes to a second-instar once the host pupa has formed, increasing in size and changing its shape (Table 3 and Figure 4).

Table 3. Numbers (and percentages) of individuals of each developmental stage of *D. areolatus* during the various developmental stages of *A. obliqua* in *S. mombin*.

D. areolatus	*A. obliqua*																		n (state Biological)
	Egg		Larva			Pupa													
	Yolk	Embryo	L_1	L_2	L_3	0	1	2	3	4	5	6	7	8	9	10	11	12	13	
Egg	0 (0)	3 (60)	2 (40)																	5
Embryo			5 (100)																	5
L_1 Early			66 (93)	5 (7)																71
L_1 Intermediate				97 (98)	2 (2)															99
L_1 Late					262 (84)	5 (16)														267
L_2						1 (5)	10 (45)	6 (27)	5 (23)											22
L_3										8 (57)	6 (43)									14
Prepupa												10 (48)	8 (38)	3 (14)						21
Pupa														17 (47)	8 (22)	7 (19)	4 (11)			36
Pharate adult																		5 (23)	17 (77)	22
Total 562																				

L_1 Early = First instar larva small (≈ 0.8 mm long), L_1 Intermediate = = First instar larva medium (≈ 1.4 mm long) and L_1 Late= First instar larva large (≈ 1.7 mm long).

In recently formed *A. obliqua* pupae, a higher frequency of late first-instar larvae of *D. areolatus* in the process of transformation to the second instar stage were observed. In 1-day old pupae, the *D. areolatus* larva had changed completely to the second instar stage, measuring ≈ 3.5 mm long, losing the cephalic capsule and occupying more than a third of the host pupa. In 3-to-4-day old pupae, the larva develops into the third instar stage, measuring ≈ 6.0 mm long, changing body shape, and occupying all of the host pupa. In 6 day-old *A. obliqua* pupae, *D. areolatus* pre-pupae that exhibit eye development have formed. In 8-day old host pupae, the parasitoid pupae are already observed with well-defined structures and genitalia. In 12-day old and older *A. obliqua* pupae, the parasitoids are found as their complete adult structure (Table 3 and Figure 4).

Discussion

The presence of eggs and larvae of *D. areolatus* in the interior of eggs and recently hatched *A. obliqua* larvae represent a novel finding within the native parasitoid guild that attack fruit flies in the Neotropics, because there have been no previous reports of any native parasitoid covering this ecological niche (López et al. 1999, Ovruski et al. 2000).

Among the particular observations regarding this finding under forced conditions, it was notable that *D. areolatus* oviposit inside embryo-eggs of *A. obliqua*, depositing a flexible egg that can fold inside the interior of the host embryo, and that the first-instar larvae present a prolonged development with three distinct sizes that synchronize with the development of the host larva and pupa. The low number of embryo-eggs found with egg parasitoids could be explained by the short developmental time of eggs (less than 24 hours).

In Mexico, *D. areolatus* has been reported to be closely associated with *A. obliqua* in fruit hosts of the genus *Spondias* (López et al. 1999, Ovruski et al. 2000, Sivinski et al. 2000), which most likely is favored by the presence of semiochemicals that could allow it to attack early stages of *A. obliqua* (eggs and recently hatched larvae). This scenario seems similar to that of *F. arisanus*, which detects marking pheromones and kairomones that emanate from the eggs of its host or from the interaction of the fruit and the host egg (Rousse et al. 2005, 2007, Pérez et al. 2013).

The finding of the parasitism of *D. areolatus* on eggs and recently hatched larvae of *A. obliqua* sheds light on two relevant aspects of its role as a natural enemy and biological control agent of fruit flies: 1) its competition and coexistence with other opiine parasitoids, highlighting the exotic species *Diachasmimorpha longicaudata* (Ashmead) and the native species *U. anastrephae* (García-Medel et al. 2007, Sivinski et al. 1998, Paranhos et al. 2013, Aluja et al. 2013), and 2) the promising development of its mass rearing under laboratory conditions (Eitam et al. 2003, Aluja et al. 2009).

It has been argued that *D. areolatus* is an inferior competitor compared to *D. longicaudata* (Sivinski et al. 1998, Eitam et al. 2004) and to *U. anastrephae* (Aluja et al. 2013). It has also been suggested that *D. longicaudata*, given its larger ovipositor,

could cause a local extinction by displacing *D. areolatus* when deprived of free space left by its competitors, as it has been suggested to explain the reduction of the dispersion range in Florida, USA, in the presence of *D. longicaudata* (Sivinski et al. 1998). For *U. anastrephae*, it has been suggested that the historic sympatry of *D. areolatus* and *U. anastrephae* depends on the ability of *D. areolatus* to avoid competition with the intrinsically superior competitor by exploiting hosts in larger fruits that are out of the reach of the smaller ovipositor of *U. anastrephae* (Sivinski et al. 1997, Aluja et al. 2013). However, our new findings suggest that both hypotheses can be reformulated in relation to the biology and oviposition behavior of *D. areolatus*.

An early action of *D. areolatus* against immature *A. obliqua* could represent an ecological advantage that prevents its displacement or local extinction by other competitors such as *U. anastrephae* and *D. longicaudata*, since these latter species invariably will attack mature larval stages that could already be parasitized by *D. areolatus*. According to Wang et al. (2003, 2008), in *F. arisanus* this earlier attack increases the probability to suppress the invasive larva through starvation or suffocation mechanisms. Field observations seem to support this assertion. Even though *D. longicaudata* has become established in numerous sites in Mexico where it has been released, its presence in *Spondias* species is inferior to that of *D. areolatus* (López et al.1999, Sivinski et al. 2000, Montoya et al. unpublished data). Our data showed that this species also competes successfully against *U. anastrephae* by parasitizing eggs and recently hatched larvae, which enables *D. areolatus* to become the dominant parasitoid species in these hosts.

Laboratory studies have reported that *D. areolatus* is an inferior competitor relative to *D. longicaudata* and *U. anastrephae* (Paranhos et al. 2013, Aluja et al. 2013) because larvae of *D. longicaudata* and *U. anastrephae* kill larvae of *D. areolatus* during competition through sequential exposures. However, these studies were conducted using mature host larvae (3rd instar), which, according to our results, presents a disadvantage to *D. areolatus*.

One difficulty in rearing *D. areolatus* has apparently been the oviposition stimuli in oviposition units (artificial devices with third-instar host larvae mixed with food) (Eitam et al. 2003), which has not been a problem in the case of *D. longicaudata* because this species detects its host by larval vibrations when feeding or moving (Lawrence 1981). However, in light of our new findings, new perspectives on laboratory rearing of this species are realized. The use of late-stage eggs or neonate larvae, similar to *Fopius arisanus* rearing (Harris et al. 1991, Zenil et al. 2004, Rousse et al. 2005, Montoya et al. 2009), should be tested.

Unsuccessful attempts have been made to rear *D. areolatus* using fruits with third instar host larvae (Eitam et al. 2003). This could be because *D. areolatus* females need different stimuli, such as chemical signals emitted by host (eggs or young larvae) interacting with fruit volatiles, as has been demonstrated for *F. arisanus* (Rousse et al. 2007, Pérez et al. 2013).

The preimaginal development of *D. areolatus* in *A. obliqua* required approximately 27 days, and 7 preimaginal stages were classified: egg, three larval stages, prepupa, pupa, and pharate adult. The morphological observations of the preimaginal stages of

D. areolatus are in agreement with what has been reported for *F. arisanus* and *D. longicaudata* (Rousse et al. 2005, Carabajal-Paladino et al. 2010). However, the development of *D. areolatus* is more akin to *F. arisanus* because both exhibit a facultative synchronization between their larval development and that of its host, which is reflected in the long period of time for the first-instar larva, which finally changes when its host reaches the prepupal stage (Rousse et al. 2005). This type of development allows the second or third instars of *F. arisanus* (and probably *D. areolatus*) to occupy most of the available space inside the pupa, which facilitate the elimination of competing larvae (Wang et al. 2003, 2008).

Our study shows that *D. areolatus* can parasitize *A. obliqua* eggs and recently hatched larvae, giving an advantage over other parasitoids that attack the later-stage larvae. This finding represents a novel report regarding the oviposition behavior of this species, suggesting that it may occupy an ecological niche that was previously thought empty in the Americas. These findings also open new perspectives for the biological control of fruit flies. If mass rearing methods are developed, this will allow release of the most dominant fruit fly parasitoid species in the Neotropics.

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References

Aluja M, López M, Sivinski J (1998) Ecological evidence for diapause in four native and one exotic species of larval-pupal fruit fly (Diptera: Tephritidae) parasitoids in tropical environments. Annals of the Entomological Society of America 91: 821–833. doi: 10.1093/aesa/91.6.821

Aluja M, Rull J, Sivinski J, Norrbom AL, Wharton RA, Macías-Ordoñez R, Díaz-Fleischer F, López M (2003) Fruit flies of the genus *Anastrepha* (Diptera: Tephritidae) and associated native parasitoids (Hymenoptera) in the tropical rainforest biosphere reserve of Montes Azules, Chiapas, Mexico. Environmental Entomology 32: 1377–1385. doi: 10.1603/0046-225X-32.6.1377

Aluja M, Sivinski J, Ovruski S, Guillén L, López M, Cancino J, Torres-Anaya A, Gallegos-Chan G, Ruiz L (2009) Colonization and domestication of seven species of native New World hymenopterous larval-prepupal and pupal fruit fly (Diptera: Tephritidae) parasitoids. Biocontrol Science and Technology 19: 49–79. doi: 10.1080/09583150802377373

Aluja M, Ovruski SM, Sivinski J, Córdova-García G, Schliserman P, Núñez-Campero SR, Ordano M (2013) Inter-specific competition and competition-free space in the tephritid parasitoids *Utetes anastrephae* and *Doryctobracon areolatus* (Hymenoptera: Braconidae: Opiinae). Ecological Entomology 38: 485–496. doi: 10.1111/een.12039

Argov Y, Blanchet A, Gazit Y (2011) Biological control of the Mediterranean fruit fly in Israel: Biological parameters of imported parasitoid wasps. Biological Control 59: 209–214. doi: 10.1016/j.biocontrol.2011.07.009

Cabrera MH, Murillo CFD, Ortega ZDA (2006) Dinámica del parasitismo larva-pupa en *A. obliqua* en frutales hospederos en la región central costera de Veracruz. [Dynamics of the larva-pupa parasitism in *A. obliqua* in fruit hosts in the coastal region of central Veracruz] Proceedings of the XXIX National Congress of Biological Control. Manzanillo, Colima, 171–175.

Carabajal-Paladino LZ, Papeschi AG, Cladera JL (2010) Immature stages of development in the parasitoid wasp, *Diachasmimorpha longicaudata*. Journal of Insect Science 10: 56. doi: 10.1673/031.010.5601

Carroll L, Wharton RA (1989) Morphology of the Immature Stages of *Anastrepha ludens* (Diptera: Tephritidae). Annals of the Entomological Society of America 82(2): 201–214. doi: 10.1093/aesa/82.2.201

Chapman RF (2013) In: Simpson SJ, Douglas AE (Eds) The Insects: Structure and Function. (5th edition), Cambridge University Press, 358–407.

Eitam A, Holler T, Sivinski J, Aluja M (2003) 'Use of host fruit chemical cues for laboratory rearing of *Doryctobracon areolatus* (Hymenoptera: Braconidae), a parasitoid of *Anastrepha* spp. (Diptera: Tephritidae)'. Florida Entomologist 86: 211–216. doi: 10.1653/0015-4040(2003)086[0211:UOHFCC]2.0.CO;2

Eitam A, Sivinski J, Holler T, Aluja M (2004) Biogeography of braconid parasitoids of the Caribbean fruit fly (Diptera: Tephritidae) in Florida. Annals of the Entomological Society of America 97: 928–939. doi: 10.1603/0013-8746(2004)097[0928:BOBPOT]2.0.CO;2

García-Medel D, Sivinski J, Díaz-Fleischer F, Ramírez-Romero R, Aluja M (2007) Foraging behavior by six fruit fly parasitoids (Hymenoptera: Braconidae) released as single- or multiple-species cohorts in field cages: Influence of fruit location and host density. Biological Control 43: 12–22. doi: 10.1016/j.biocontrol.2007.06.008

Harris EJ, Okamoto RY (1991) A method for rearing *Biosteres arisanus* (Hymenoptera: Braconidae) in the laboratory. Journal of Economic Entomology 84: 417–422. doi: 10.1093/jee/84.2.417

Hernández-Ortiz V, Pérez-Alonso R, Wharton R (1994) Native parasitoids associated with the genus *Anastrepha* (Diptera: Tephritidae) in Los Tuxtlas, Veracruz, Mexico. Entomophaga 39: 171–178. doi: 10.1007/BF02372355

Lawrence PO (1981) Host vibration- a cue to host location by the parasite, *Biosteres longicaudatus*. Oecologia 48: 249–251. doi: 10.1007/BF00347971

López M, Aluja M, Sivinski J (1999) Hymenopterous larval-pupal and pupal parasitoids of *Anastrepha* flies (Diptera: Tephritidae) in Mexico. Biological Control 15: 119–129. doi: 10.1006/bcon.1999.0711

Montoya P, Suarez A, López F, Cancino J (2009) *Fopius arisanus* oviposition in four *Anastrepha* fruit fly species of economic importance in Mexico. BioControl 54: 437–444. doi: 10.1007/s10526-008-9193-6

Ovruski S, Aluja M, Sivinski J, Wharton R (2000) Hymenopteran parasitoids on fruit-infesting Tephritidae (Diptera) in Latin America and the southern United States: diversity, distribution, taxonomic status and their use in fruit fly biological control. Integrated Pest Management Reviews 5: 81–107. doi: 10.1023/A:1009652431251

Ovruski S, Schliserman P, Aluja M (2004) Indigenous parasitoids (Hymenoptera) attacking *Anastrepha fraterculus* and *Ceratitis capitata* (Diptera: Tephritidae) in native and exotic host plants in northwestern Argentina. Biological Control 29: 43–57. doi: 10.1016/S1049-9644(03)00127-0

Paranhos BJ, Sivinski J, Stuhl C, Holler T, Aluja M (2013) Intrinsic competition and competitor-free-space influence the coexistence of parasitoids (Hymenoptera: Braconidae: Opiinae) of neotropical Tephritidae (Diptera). Environmental Entomology 42: 717–723. doi: 10.1603/EN12282

Pérez J, Rojas JC, Montoya P, Liedo P, Castillo A (2013) *Anastrepha* egg deposition induces volatiles in fruits that attract the parasitoid *Fopius arisanus*. Bulletin of Entomological Research 103: 318–325. doi: 10.1017/S0007485312000739

Rousse P, Harris EJ, Quilici S (2005) *Fopius arisanus*, an egg–pupal parasitoid of Tephritidae. Overview. Biocontrol News and Information 26(2): 59–69.

Rousse P, Chiroleu F, Veslot J, Quilici S (2007) The host and microhabitat olfactory location by *Fopius arisanus* suggests a broad potential host range. Physiological Entomology 32: 313–321. doi: 10.1111/j.1365-3032.2007.00575.x

Sivinski J, Aluja M, López M (1997) Spatial and temporal distributions of parasitoids of the Mexican *Anastrepha* species (Diptera: Tephritidae) within the canopies of fruit trees. Annals of the Entomological Society of America 90: 604–618. doi: 10.1093/aesa/90.5.604

Sivinski J, Aluja M, Holler T, Eitam A (1998) 'Phenological comparison of two braconid parasitoids of the Caribbean fruit fly (Diptera: Tephritidae)'. Environmental Entomology 27: 360–365. doi: 10.1093/ee/27.2.360

Sivinski JM, Piñero J, Aluja M (2000) The distributions of parasitoids (Hymenoptera) of *Anastrepha* fruit flies (Diptera: Tephritidae) along an altitudinal gradient in Veracruz, Mexico. Biological Control 18: 258–269. doi: 10.1006/bcon.2000.0836

SMN (2010) National Meteorological Service (Servicio Meteorológico Nacional). Normal values (1971-2000) for Medellín and Bravo, Veracruz (Valores normales de 1971-2000 para Medellín de Bravo, Veracruz). available: http://smn.cna.gob.mx/ [accessed 22 October 2013]

SPSS Inc. (2008) SPSS Statistics for Windows, Version 17.0. SPSS Inc., Chicago.

Wang XG, Messing RH (2002) Newly imported larval parasitoids pose minor competitive risk to the extant egg–larval parasitoid of fruit flies in Hawaii. Bulletin of Entomological Research 92: 423–429. doi: 10.1079/BER2002181

Wang XG, Messing RH, Bautista RC (2003) Competitive superiority of early acting species: a case study of opiine fruit fly parasitoids. Biocontrol Science and Technology 16: 391–402. doi: 10.1080/0958315031000104514

Wang XG, Bokonon-Ganta AH, Messing RH (2008) Intrinsic inter-specific competition in a guild of tephritid fruit fly parasitoids: Effect of co-evolutionary history on competitive superiority. Biological Control 44: 312–320. doi: 10.1016/j.biocontrol.2007.10.012

Wharton RA, Gilstrap FE, Rhode RH, Fischel-M M, Hart WG (1981) Hymenopterous egg-pupal and larval-pupal parasitoids of *Ceratitis capitata* and *Anastrepha* spp. (Dip.: Tephritidae) in Costa Rica. Entomophaga 26: 285–290. doi: 10.1007/BF02371878

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RESEARCH ARTICLE

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A new species of subgenus *Neodoryctes* Szépligeti (Hymenoptera, Braconidae) from China, with a key to Oriental and Palaearctic species

Tao Li[1], Ying Zhang[1], Cornelis van Achterberg[2,3]

1 *General Station of Forest Pest Management, State Forestry Administration, Shenyang 110034, P. R. China* **2** *Department of Life Sciences, Northwest University, 229 North Taibai Road, Xi'an, Shaanxi 710069, P. R. China* **3** *Department of Terrestrial Zoology, Naturalis Biodiversity Center, Postbus 9517, 2300 RA Leiden, The Netherlands*

Corresponding author: *Tao Li* (litao200105@163.com)

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Abstract

A new species of subgenus *Neodoryctes* Szépligeti (Hymenoptera: Braconidae), *Doryctes* (*Neodoryctes*) *henanensis* Li & van Achterberg, **sp. n.**, is described and illustrated. This species is associated with *Pterolophia* sp. (Coleoptera: Cerambycidae), a twig-boring pest of *Broussonetia papyrifera* (L.) L'Hert. et Vent. (Moraceae), in Henan (Central China). A key to the Palaearctic species of the subgenus *Neodoryctes* is provided.

Keywords

Doryctinae, Doryctini, *Doryctes*, *Neodoryctes*, new species, parasitoid

Introduction

The subgenus *Neodoryctes* Szépligeti, 1914 of the genus *Doryctes* Haliday, 1836 (Hymenoptera: Braconidae: Doryctinae: Doryctini) includes eight described species, of which six species are known from the Afrotropical Region (Yu et al. 2012). The other two species are known from China (Taiwan) and Japan (Belokobylskij 1996, Belokobylskij

and Maetô 2008). The subgenus *Neodoryctes* can be separated from the nominate subgenus by the relative length of vein 1-M of the hind wing (1.3–1.8 times as long as vein M+CU in *Neodoryctes;* 0.7–1.0, rarely up to 1.1, times in *Doryctes*). In addition, the hind tibia has long erect setae and tergites II+III have sublateral longitudinal grooves. The biology of the species of this subgenus is unknown but members of the subgenus *Doryctes* Haliday are ectoparasitoids of mainly Cerambycidae and Buprestidae (Yu et al. 2012). The subgeneric status of *Neodoryctes* is doubtful according to the study of Zaldívar-Riverón et al. (2008); *Doryctes* and *Neodoryctes* appeared distantly related according to the molecular analysis thus supporting a generic status for *Neodoryctes*.

Here we describe the third non-Afrotropical species of *Neodoryctes*, *D.* (*Neodoryctes*) *henanensis* Li & van Achterberg, sp. n., from Henan (Central China).

Materials and methods

Twigs of heavily infested *Broussonetia papyrifera* (L.) L'Hert. et Vent. trees were collected, brought to the laboratory and maintained in a large nylon cage at room temperature. Distilled water was sprayed over the trunks and twigs twice a week in order to prevent desiccation and the emerged insects were collected daily. After the emergence of hosts and parasitoids was complete, all remaining twigs were dissected to record their condition (i.e. status of hosts, and parasitism). The associated host was identified by Dr. Guang-Lin Xie (Yangtze University, Jingzhou, Hubei, China).

For the morphological terminology used in this paper see van Achterberg (1993) and Harris (1979). The descriptions, measurements and figures were made with a Leica M205A microscope. Focused photographs were combined using Leica DFC550 with Leica Application Suite (Version 4.5.0).

The type specimens and hosts are deposited in the Insect Museum, General Station of Forest Pest Management (GSFPM), State Forestry Administration, Shenyang, China. One paratype is deposited at the Naturalis Biodiversity Center (RMNH), Leiden, The Netherlands.

Key to Palaearctic and Oriental species of the subgenus *Neodoryctes* Szépligeti

1 Mesoscutum mainly striate or rugose (Fig. 5) and dorsal face of propodeum reticulate-rugose (Fig. 7); dorsal tooth on hind coxa absent (Fig. 8); vein m-cu of hind wing straight posteriorly (Fig. 12); second metasomal suture hardly impressed (Figs 1, 13) 2

– Mesoscutum and dorsal face of propodeum smooth or granulate; small dorsal tooth on hind coxa present; posteriorly vein m-cu of hind wing curved distally; second metasomal suture distinctly impressed **3**

2 Apical half of vein M+CU1 of fore wing strongly curved (Fig. 12); vein 1-M of hind wing 1.5–1.6 × as long as vein M+CU (Fig. 12); vein 3-SR of fore

wing about 1.6 × as long as vein m-cu; medial part of tergite II (between sublateral grooves) distinctly transverse (about twice wider than long medially); ovipositor sheath about as long as fore wing; propodeum weakly areolate .... ***D. henanensis* Li & van Achterberg, sp. n.**

– Apical half of vein M+CU1 of fore wing slightly curved; vein 1-M of hind wing about 1.3 × as long as vein M+CU; vein 3-SR of fore wing about 1.2 × as long as vein m-cu; medial part of tergite II about 1.2 × wider than long medially; ovipositor sheath 0.60–0.75 × as long as fore wing; propodeum distinctly areolate ..... ***D. slavianka* Belokobylskij, 1996**

3 Mesoscutum and dorsal face of propodeum smooth; setae on dorsal surface of hind tibia long and erect; hind coxa with distinct submedian dorsal tooth; vein SR of hind wing largely absent; apical width of tergite I 1.2–1.4 × its basal width..... ***D. denticoxa* Belokobylskij, 1996**

– Mesoscutum and dorsal face of propodeum granulate; setae on dorsal surface of hind tibia short and semi-erect; hind coxa with minute and hardly protruding dorsal tooth; basal half of vein SR of hind wing present; apical width of tergite I 1.5–1.8 × its basal width ..... ***D. makiharai* Belokobylskij & Maetô, 2008**

Description

***Doryctes* (*Neodoryctes*) *henanensis* Li & van Achterberg, sp. n.**
http://zoobank.org/726282A2-3F85-42B8-A264-2E130E8C8EBB
Figs 1–13

Type material. Holotype, ♀ (GSFPM), China: Henan, Xinxiang, 3.vi.2013, Mao-Ling Sheng. Paratypes (RMNH, GSFPM): 1 ♀ + 1 ♂, same data as holotype.

Diagnosis. Frons weakly concave behind antennal sockets, with 3–4 furrows. POL 1.2 × Od, 0.5 × OOL. Width of face 0.8 × height of eye, 1.2 × height of face and clypeus combined. Apical half of vein M+CU1 of fore wing strongly curved, vein 3-SR of fore wing about 1.6 × as long as vein m-cu and vein 1-M of hind wing 1.5–1.6 × as long as vein M+CU. Tergite II with oblique lateral depressions, medial part of tergite II (between sublateral grooves) distinctly transverse (about twice wider than long medially). Ovipositor sheath 1.3 × as long as metasoma and as long as fore wing.

Holotype. Female, length of body 5.8 mm, and of fore wing 4.8 mm (Fig. 1).

Head (Figs 2–3). Head width 1.3 × its median length in dorsal view, 1.1 × width of mesoscutum. Temple behind eye (dorsal view) roundly narrowed. Frons (Fig. 3) weakly concave behind antennal sockets, with 3–4 furrows. Stemmaticum (ocellar triangle) situated before middle of head. Ocelli medium-sized, and lateral areas weakly concave. POL 1.2 × Od, 0.5 × OOL. Eyes glabrous, slightly concave near antennal sockets, 1.2 × higher than wide. Diameter of antennal sockets 0.8 × distance between antennal sockets, 2.3 × distance between socket and eye. Face (Fig. 2) uniformly convex, with fine rugae and long setae, medially glabrous under sockets, rugae weaker

Figure 1. *Neodoryctes henanensis* Li & van Achterberg, sp. n., female, holotype. Habitus, lateral aspect.

laterally; width of face 0.8 × height of eye, 1.2 × height of face and clypeus combined. Length of malar space 0.4 × height of eye and 1.1 × basal width of mandible. Malar suture absent. Clypeus suture complete. Hypoclypeal depression round, equal to distance from depression to eye and 0.4 × as wide as face. Occipital carina complete dorsally, ventrally remaining separated from hypostomal carina. Length of maxillary palp 1.3 × height of head. Antenna (Figs 1, 4) broken (holotype), remaining antennal articles 34, setiform and slender. Scape 1.6 × longer than its maximum width (Fig. 4). Third article 5.5 × longer than its apical width (4.8 × in paratype) and 1.2 × as long as fourth article (as in paratype). Paratype with 39 antennal articles and antenna 1.3 × longer than body. Penultimate antennal article of paratype 3.1 × longer than its maximum width and about as long as apical segment.

Mesosoma (Figs 5–6). Mesosoma 2.4 × longer than high. Pronotum convex dorsally and distinctly concave medially, with irregular rugae. Mesoscutum largely covered with curved rugae (Fig. 5), interspaces smooth and strongly shiny and middle lobe of mesoscutum distinctly convex with fine median furrow, its posterior third glabrous. Notauli complete, distinctly crenulate anteriorly but posterior half shallowly crenulate and in a medio-posterior rugose area. Scutellar sulcus wide, deep, with a coarse median carina, almost smooth, 0.4 × as long as scutellum. Scutellum slightly convex and smooth, but rugulose posteriorly, its lateral carinae almost complete. Metanotum dorso-medially with three distinct carinae. Subalar depression shallow and wide, with short striae. Mesopleuron (Fig. 6) with rugose area anteriorly and with smooth inferior areas. Precoxal sulcus distinctly impressed, smooth and straight. Propodeum convex and reticulate-rugose, vaguely areolate and costulae indistinctly developed (Fig. 7); its median carina about 0.5 × as long as propodeum.

Figures 2–13. *Neodoryctes henanensis* Li & van Achterberg, sp. n., female, holotype, but 6 and 11 of female paratype. **2** Head, front aspect **3** Head, dorsal aspect **4** Basal anternal segments **5** Mesosoma, dorsal aspect **6** Mesosoma, lateral aspect **7** Propodeum, dorsal aspect **8** Hind coxa **9** Hind femur **10** Hind tibia **11** Hind tarsus **12** Wings **13** Metasoma, dorsal aspect.

Wings (Fig. 12). Fore wing: length about 4.0 × as long as its maximum width. Pterostigma 4.3 × as long as its maximum width. Vein M+CU1 strongly curved; vein r-m present, weakly oblique; vein 2-M present; vein r arising from near middle of pterostigma; 2-SR 1.4 × as long as r, 0.7 × as long as 3-SR, 0.3 × as long as SR1, 1.5 × as long as r-m; 1-SR+M curved; 1-CU1 0.2 × as long as 2-CU1; 3-CU1 0.5 × as long as m-cu. Hind wing: vein 1-M 1.6 × as long as M+CU; vein m-cu straight posteriorly; vein SR present basally.

Legs (Figs 6, 8–11). Fore tibia with 7-8 strong spines arranged in almost single straight row. Hind coxa (Fig. 8) 1.6 × as long as wide, rugose dorsally and without tooth; hind femur 2.6 × as long as wide and with long erect setae (Fig. 9); hind tibia with medium-sized erect setae (Fig. 10); hind tarsus (Figs 10–11) almost as long as to hind tibia; basitarsus (Fig. 11) 0.9 × as long as second-fifth segments combined; second segment of hind tarsus 0.4 × as long as basitarsus, 1.5 × as long as telotarsus (without arolium).

Metasoma (Fig. 13). Length 1.1 × as long as head and mesosoma combined. Tergite I 1.1 × longer than wide apically, with large dorsope, its surface with distinct, uniform and complete striae, and with minute transverse sculpture between striae; mediposteriorly weakly convex and smooth; dorsal carinae conspicuous and half as long as tergite I; apical width 1.6 × its basal width. Tergite II with oblique lateral depressions, medial part of tergite II (between sublateral grooves) distinctly transverse (about twice wider than long medially), tergites II+III 0.9 × as long as its basal width, 1.8 times as long as tergite IV in lateral view; its surface with distinct, uniform and complete striae in basal 0.7, smooth in apical 0.3; second suture hardly impressed, striate and curved. Basal half of tergites IV and V with fine sculpture, and apically (as remaining tergites) smooth. Ovipositor sheath 1.3 × as long as metasoma and as long as fore wing.

Colour. Black. Head brown but stemmaticum and apical half of mandible blackish brown, median part of face and scape yellowish brown with some reddish, apical half of antenna rather dark brown; pronotum, mesoscutum, mesopleuron (apically dark brown) and mesosternum reddish brown; tegula, fore leg (but femur and tibia blackish brown), mid leg (but coxa, femur and tibia blackish brown), hind trochanters and base of tibia, yellowish brown; pterostigma (except for yellowish brown base and apex) and veins blackish brown; sublateral striae of tergite II dark reddish brown.

Male. Length of body 4.2 mm, and of fore wing 2.9 mm. Antennal articles 29, antenna 1.1 × longer than body. Length of mesosoma 2.8 × longer than high. Length of tergite I 1.5 × as long as its apical width and apical width 1.7 × its basal width. Length of tergites II+III 1.8 × its basal width, 2.2 × as long as length of tergite IV. Head (but stemmaticum and apical half of mandible blackish brown; basal half of antenna yellow, apical half brown), fore leg, mid leg (but telotarsus and claws blackish brown), hind leg (but coxa and most of femur yellowish brown with some reddish; telotarsus and claws blackish brown), pterostigma and veins yellowish brown; pronotum and mesoscutum yellowish brown with some reddish; propodeum and metasoma reddish brown and partly blackish.

Variation. Female paratype has length of body 4.6 mm, and of fore wing 3.7 mm. Head (but stemmaticum and apical half of mandible blackish brown; apical half of an-

tenna brown), legs except claws blackish brown, tegulae, yellowish brown; pterostigma (but basally and apically yellowish brown) and veins brown; pronotum and mesoscutum yellowish brown with some reddish; scutellum, propodeum, hind coxa and metasoma (but tergites II+III yellowish brown with some reddish), dark reddish brown.

Biology. Presumably larval parasitoid of *Pterolophia* sp. (Coleoptera: Cerambycidae) boring in *Broussonetia papyrifera* (L.) L'Hert. et Vent. (Moraceae).

Distribution. Palaearctic China (Henan).

Remarks. The new species belongs to the subgenus *Neodoryctes* Szépligeti because of the short vein 1-M of the hind wing. It can be separated from other Palaearctic and North Oriental species of this subgenus by having vein 3-SR of fore wing about 1.6 × as long as vein m-cu, apical half of vein M+CU1 of fore wing strongly curved and vein 1-M of hind wing 1.5–1.6 × as long as vein M+CU (vein 3-SR of fore wing about 1.0–1.5 × as long as vein m-cu, apical half of vein M+CU1 of fore wing slightly curved and vein 1-M of hind wing about 1.3–1.4 × as long as vein M+CU in the remaining species). It shares with *D. slavianka* Belokobylskij, 1996, from Korea and Far East Russia, the lack of a dorsal tooth on the hind coxa and the striate or rugose mesoscutum; however, it differs from the latter species by having the medial part of tergite II (between the sublateral grooves) distinctly transverse (about twice wider than long medially; about 1.2 × in *D. slavianka*), the ovipositor sheath about as long as fore wing (0.60–0.75 ×), the propodeum weakly areolate (distinctly areolate) and vein M+CU1 of fore wing distinctly curved (nearly straight).

Etymology. The specific name is derived from the locality of the holotype.

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References

Bekokobylskij SA (1996) Nine new species of Braconidae (Hymenoptera) from the Russian Far East. Journal of Natural History 30: 1661–1681. doi: 10.1080/00222939600770981

Belokobylskij SA (1996) A contribution to the knowledge of the Doryctinae of Taiwan (Hymenoptera: Braconidae). Zoosystematica Rossica 5(1): 153–191.

Belokobylskij SA, Maetô K (2008) Doryctinae (Hymenoptera: Braconidae) of Ogasawara Islands (Japan). Annales Zoologici Warsaw 58(1): 125–166. doi: 10.3161/067.058.0107

Haliday AH (1836) Essay on parasitic Hymenoptera. Entomological Magazine 4(1): 38–59.

Harris RA (1979) A glossary of surface sculpturing. Occasional Papers in Entomology of the California Department of Food and Agriculture 28: 1–31.

Szépligeti G (1914) Afrikanische Braconiden des Königl. Zoologischen Museums in Berlin. Mitteilungen aus dem Zoologischen Museum in Berlin 7: 153–230.

van Achterberg C (1993) Illustrated key to the subfamilies of the Braconidae (Hymenoptera: Ichneumonoidea). Zoologische Verhhandeligen Leiden 283: 1–189.

Yu DS, van Achterberg C, Horstmann K (2012) Taxapad 2012–World Ichneumonoidae 2011. Taxonomy, Biology, Morphology and Distribution. On USB flash drive. Ottawa, Ontario, Canada. http://www.taxapad.com

Zaldívar-Riverón A, Belokobylskij SA, León-Regagnon V, Briceño-G R, Quicke DLJ (2008) Molecular phylogeny and historical biogeography of the cosmopolitan parasitic wasp subfamily Doryctinae (Hymenoptera: Braconidae). Invertebrate Systematics 22(3): 345–363. doi: 10.1071/IS07028

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The hosts of *Ophion luteus* (Linnaeus) (Hymenoptera, Ichneumonidae, Ophioninae) in Europe

Gavin R. Broad[1], Heinz Schnee[2], Mark R. Shaw[3]

1 *Department of Life Sciences, the Natural History Museum, Cromwell Road, London SW7 5BD, UK* **2** *Birkenweg 18, 04416 Markkleeberg, Germany* **3** *National Museums of Scotland, Chambers Street, Edinburgh, EH1 1JF, UK*

Corresponding author: *Gavin R. Broad* (g.broad@nhm.ac.uk)

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Abstract

A widespread European nocturnal ichneumonid, *Ophion luteus*, is shown to be a parasitoid of at least two species of noctuid moth, *Agrotis exclamationis* and *A. segetum*, probably most frequently a parasitoid of the former. The taxonomy, nomenclature and diagnostic features of this species are discussed. Possible explanations for a spring-flying generation, usually referred to as the '*distans*' morph, are discussed.

Keywords

Host-parasitoid, rearing, Lepidoptera, Noctuidae, Noctuinae

Introduction

In Europe, *Ophion luteus* (Linnaeus) is a widely distributed, often common, nocturnal ichneumonid, and a conspicuous visitor to light traps in August and September. But despite its near ubiquity in Britain (and probably much of north-west Europe) (Brock 1982; Natural History Museum and National Museums of Scotland collections), its biology has remained almost entirely unknown. Establishing the hosts of *O. luteus* is of interest because it is such a widespread (Fig. 1) and often common species, yet is one of very few ophionines that had not been reared in Britain. Population modelling work

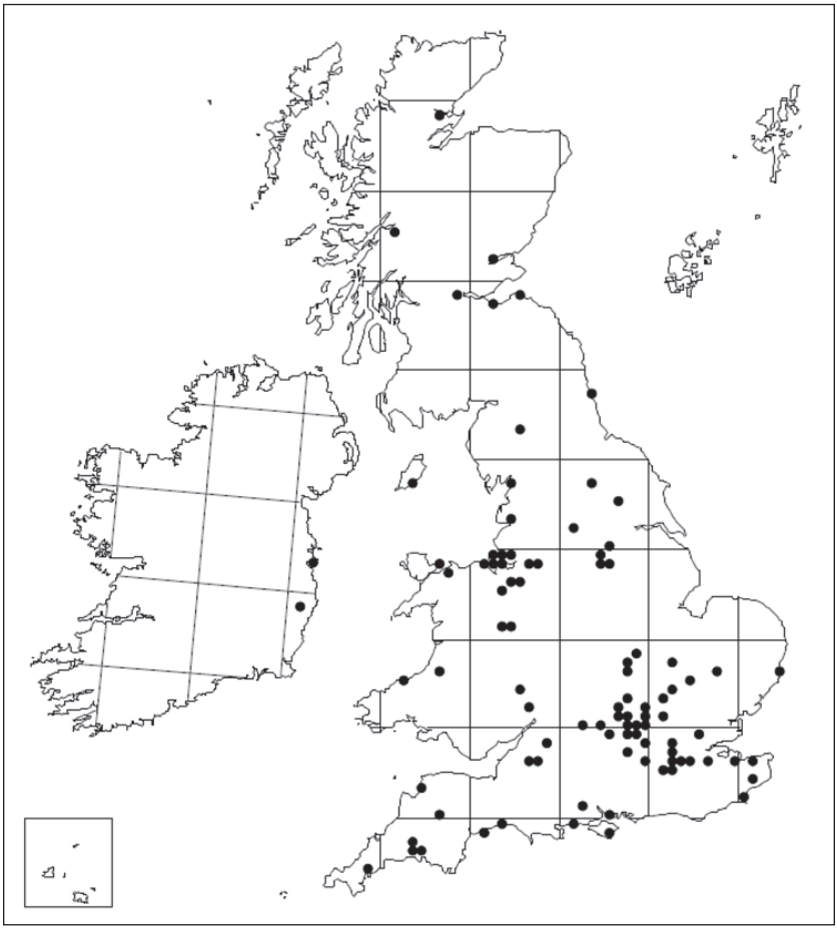

Figure 1. Preliminary distribution map of *Ophion luteus* in Britain and Ireland (Northern Isles displaced southwards), based on 475 occurrence records held in the database of the Nocturnal Ichneumonoidea Recording Scheme; note that many specimens in BMNH and NMS have not yet been databased and this map is presented only to give an impression of the range of the species, bearing in mind bias in coverage and omissions. Map plotted using DMAP, developed by Alan Morton (www.dmap.org).

in Finland has appeared to implicate parasitism by *O. luteus* as the cause of population cycles of boreal *Xestia* spp. (Noctuidae: Noctuinae) (Várkonyi et al. 2002), although *O. luteus* has never been reared from *Xestia*, neither in the wild nor by experimental exposure.

As Brock (1982) had suggested that the usual host of *O. luteus* is a "noctuid larva of such frequent occurrence that it is seldom reared by lepidopterists", GRB and MRS surmised that *Agrotis exclamationis* (Linnaeus) (Noctuidae: Noctuinae), the Heart & Dart, must be a good candidate for the usual host of *O. luteus*, given its wide distribution and suitable phenology. *Agrotis exclamationis* flies mainly from May to July, with well-grown larvae occurring in late summer to early autumn before overwintering

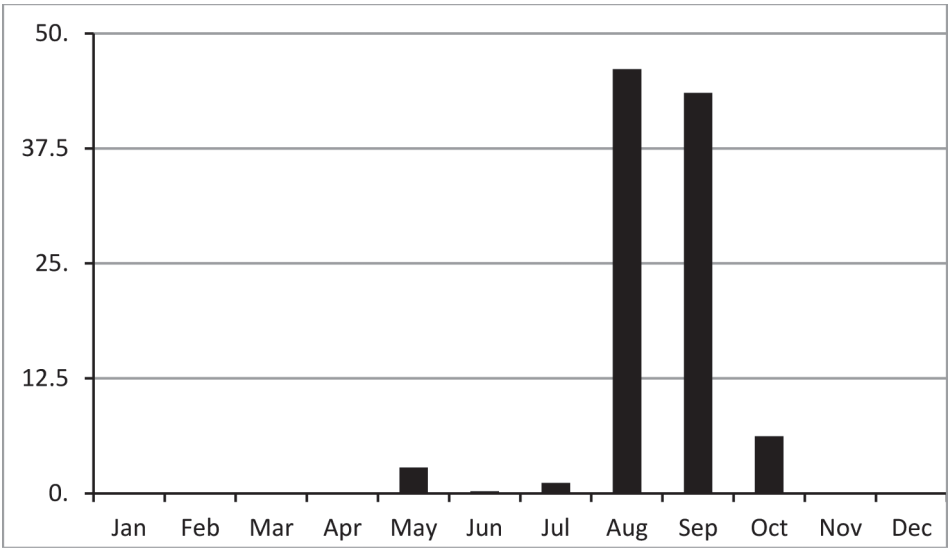

Figure 2. Phenology of adult *Ophion luteus* in Britain (percentages per month of 887 specimens from the Nocturnal Ichneumonoidea Recording Scheme database). Note the tiny May emergence.

fully fed to pupate the following spring. Adult *O. luteus* specimens have been collected in Britain mainly (90% of specimens seen) in August and September (Fig. 2) which would mean they are adult at a time coincident with suitable *A. exclamationis* larval hosts. Ophioninae almost always oviposit into well-grown Lepidoptera larvae (e.g. Price 1975; Vickery 1929; MRS pers. obs., see below) and many of the British species attack Noctuidae (Brock 1982 and collections of NMS); but larvae of common noctuids feeding partly subterraneously, such as *A. exclamationis*, are not often reared by lepidopterists. An opportunity to rear *O. luteus* (including experimental exposure to the parasitoid) arose when GRB collected several final instar larvae of *A. exclamationis* at night on 10.x.2012 in his garden, in Aldbury, Hertfordshire, England (51°47'59"N, 0°35'59"W), where *O. luteus* is frequent at the light trap. While these caterpillars were being reared, MRS and HS coincidentally reported further rearing records for *O. luteus*. The rearings by HS took place as a result of large populations in 1976 and some subsequent years of *Agrotis segetum* (Denis & Schiffermüller) and, to a lesser extent, *A. exclamationis* in Saxony and other regions of Central Germany (specimens retained from Dübener Heide and Eisleben), where the larvae (particularly of *A. segetum*) caused significant damage to crops.

Materials and methods

Specimens are deposited in the Natural History Museum, London (BMNH), National Museums of Scotland (NMS) and the Heinz Schnee collection (HSC). Rearing by

Table 1. Rates of parasitism of two *Agrotis* spp. by *Ophion luteus* in Central Germany.

Agrotis exclamationis			*Agrotis segetum*		
Year	**No. larvae**	**Parasitism (%)**	**Year**	**No. larvae**	**Parasitism (%)**
1976/7	138	0.7	1976/7	585	0.5
1977/8	100	11	1979/80	115	0.9
1981/2	48	2.1	1981/2	75	1.3
1983/4	64	7.6	1983/4	200	0.5
1984/5	44	6.8	1984/5	69	4.3
1985/6	52	7.7	1986/7	148	0.7
1990/1	58	1.7	1988/9	388	0.3
			1991/2	90	1.1

GRB of *A. exclamationis* larvae collected in the wild in their final instar was carried out in a shed at ambient temperatures throughout the winter, with livestock brought indoors, to a shaded environment, in the summer. Two wild-collected adult female *Ophion luteus* were fed on diluted honey and six final instar *A. exclamationis* larvae were exposed to them overnight, around 10.x.2012. *Ophion luteus* adults were introduced to large tubes containing single *A. exclamationis* larvae in the evening and observed from about 20:00-22:00. The two *O. luteus* were then separately left overnight with multiple *A. exclamationis*. In Germany, larvae of *A. exclamationis* and *A. segetum* were collected in October and November over several years (see Table 1), mainly from potato and sugar beet fields but also from corn and carrot. Larvae were reared individually in Petri dishes under long daylight conditions (18 hours light / 6 hours darkness) until emergence of adult moths, parasitoid larvae or death of the larvae. Parasitoid cocoons were over-wintered outdoors. Photographs of specimens were taken with a Canon EOS 450D digital camera attached to a Leica MZ12 stereomicroscope and partially focused images were combined using Helicon Focus v. 4.80 software. A photograph of the early cocoon was taken with a Samsung Galaxy SII mobile phone.

Results and discussion

The *Agrotis exclamationis* larvae collected in Aldbury in October 2012 were exposed to *O. luteus* females; during the period of observation, from about 20:00-22:00. *Ophion luteus* females paid no attention to the *Agrotis* larvae, even when walking over them. In light of the subsequent parasitoid emergence, they could already have been parasitized, or oviposition may have subsequently occurred in the dark, when they were not under observation. It proved difficult to overwinter the *A. exclamationis* larvae, which bury themselves in soil but are periodically restless. Most larvae succumbed to fungi but one survived the winter. On 19.iv.2013, it was found that a large parasitoid larva had emerged from this, probably prepupal, caterpillar and had begun to spin a cocoon below the host remains. Cocoon formation took about seven days, with the cocoon

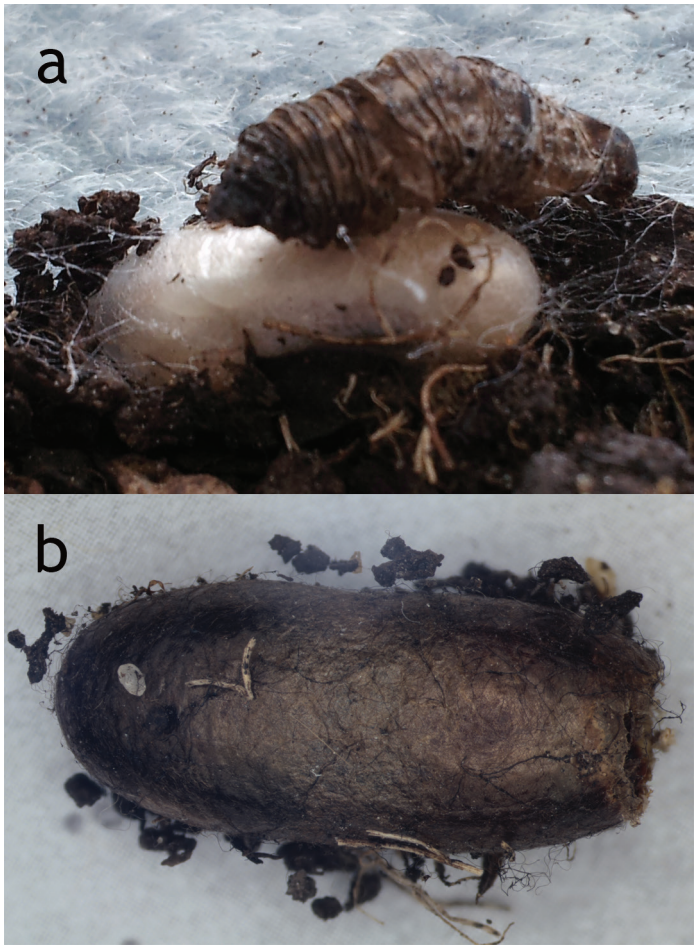

Figure 3. Cocoon of *Ophion luteus* ex *Agrotis exclamationis* (Aldbury, Herts.); **a** early stage in cocoon formation, 20.iv.2013 **b** same cocoon after emergence of adult *O. luteus*.

initially composed of loose, white silken threads (Fig. 3a) but gradually darkening and taking on the characteristic appearance of an *Ophion* cocoon, dark brown, slightly paler centrally (Fig. 3b). An adult female *O. luteus* emerged in the night of 2-3.ix.2013 (specimen deposited in BMNH). At around this time, *O. luteus* were regularly caught in GRB's light trap.

At about the same time as this specimen was pupating, MRS identified a female *O. luteus* reared from a fully grown *Agrotis* ?*vestigialis* (Hufnagel) larva, or prepupa, collected from under moss at Braunton Burrows, North Devon (SS4437) on 8.v.2007 (coll. B.P. Henwood) (specimen in NMS). The exact dates of cocoon formation and emergence of the adult parasitoid are not recorded, but were later in 2007.

HS also reported his previously unpublished rearing records from 1976–1992, when he reared 43 *O. luteus* from both *Agrotis exclamationis* and *A. segetum* field-col-

lected larvae in Germany (Table 1) (12 reared *O. luteus* specimens deposited in HSC). Unlike GRB's rearing, the *O. luteus* larvae in HS's rearings all emerged from their host larvae in the year of collection, although it should be noted that these emergences took place under unnatural light conditions, as detailed in Materials and Methods. HS also found some *Ophion* cocoons in the soil in November; however, emergence of these *O. luteus* adults was almost invariably between July and early September in the following calendar year, except for two that emerged in May. The phenology of both hosts and *O. luteus* may differ in Germany, at least in particularly warm years (such as 1977) but in Britain, *A. exclamationis* usually overwinters as a prepupa in the soil, pupating the following spring.

Host records for *O. luteus* in the literature cover a very wide range of Lepidoptera across ten families, including many noctuids, and even a tenthredinid sawfly (Yu et al. 2012). Most can be dismissed out of hand, especially as in the past almost any large yellowish ichneumonid was liable to be referred to *O. luteus*. However, Meyer's (1927) and Győrfi's (1943, 1944) references to, respectively, *Agrotis segetum* and *A. vestigialis* as hosts are, in retrospect, entirely plausible.

Almost all *O. luteus* records from GRB's Nocturnal Ichneumonoidea Recording Scheme are from August and September (and into early October) (Fig. 2), which is when large larvae of *A. exclamationis* and some other *Agrotis* species are available. However, as discussed by Brock (1982), there is a distinct, though rarely collected, cohort of apparent *O. luteus* on the wing in May. These specimens are typically smaller than normal *O. luteus*, with fewer antennal flagellomeres and more buccate heads (there is a distinct ocellar-ocular space and the temples are long in dorsal view). These have informally been referred to as the '*distans*' morph. The taxonomy of this morph is rather complex; the lectotype of *Ophion distans* Thomson, 1888 is very similar to that of *Ophion luteus* (Linnaeus, 1758) (Brock 1982) so, although this spring generation is informally called the '*distans*' morph, if there were found to be two species (spring and autumn-flying) then the '*distans*' morph would actually take the name *Ophion luteus*, with the autumn generation taking the next available name, *Ophion slaviceki* Kriechbaumer, 1892 (Brock 1982). However, Schwarzfeld et al. (submitted) have found that all British specimens of *O. luteus* sequenced for Cytochrome Oxidase I, including two '*distans*' specimens collected in May, cluster together as apparently one monophyletic species.

Brock (1982) reports that *O. luteus* of the '*distans*' form have been reared from *Ochropleura praecox* (Linnaeus) (Noctuidae: Noctuinae) on sand dunes, based on specimens in Claude Morley's collection, but the location of these specimens is now unknown. Four specimens in BMNH, reared by Claude Morley but with no host names given, seem to be '*distans*' although three of these have atypical mandibles, having the acute internal angle between the teeth that is a feature of most *Ophion* species other than *O. luteus*. The lectotype of *Ophion luteus*, and other '*distans*' specimens, have mandibles typical of the common and widespread form of *O. luteus*.

Agrotis exclamationis can have a small second brood (as occurred in 2013 in southern England), and it is plausible that the early-flying '*distans*' form results from late summer or autumn-pupating host individuals, with the parasitoid's early cocoon for-

mation triggered by hormonal changes in the host. These could give rise to adult *O. luteus* emerging in the late spring. However's HS's autumn-cocooning *O. luteus* are of the usual, autumn-flying morphology, including the two specimens that emerged in May (possibly as a result of unnatural rearing conditions). There are also, perhaps more pertinently, closely related dune-inhabiting noctuid species with this different phenology. Both factors mean that alternative hosts may be available in some areas for an early summer brood of *O. luteus*.

There are no reliable rearing records of *O. luteus* from any *Xestia* species. *Xestia* spp. and *O. luteus* have non-synchronous life cycles in Britain, with fully-grown *Xestia* larvae unavailable at the time for oviposition by *O. luteus*. The available evidence is that *O. luteus* is a parasitoid of *Agrotis* larvae, contrary to Várkonyi et al. (2002). Várkonyi et al.'s (2002) population modelling of boreal *Xestia* species implicitly assumes that *O. luteus* is a parasitoid attacking early instar host larvae, switching between two cohorts of *Xestia* spp. (which have a two year life history at high latitudes), because *Xestia* are not fully grown in Finland when *O. luteus* is on the wing. Várkonyi et al. (2002) also report *O. luteus* rearings from *Euxoa* species, which over-winter as small larvae, and use this as evidence for *O. luteus* ovipositing in small larvae of *Xestia* spp. This is contrary to our findings, as *O. luteus* adults are only on the wing when their *Agrotis* hosts are well-grown, and oviposition in young larvae would be unique within the genus. MRS has experience of rearing at least ten British *Ophion* species, always from Lepidoptera larvae collected as late (probably final) instars and never from larvae collected young, which he collects as often as possible for the sake of rearing Microgastrinae and Rogadinae (Braconidae) that kill hosts young. Furthermore, common *Xestia* species are easily collected as larvae in Britain, and have repeatedly been reared in large enough numbers for it to be implausible for regular parasitism by *O. luteus* to have been overlooked in Britain. There is a possibility that the Scandinavian and British specimens identified as *O. luteus* represent separate species (G. Várkonyi pers. comm.) although there has been no published evidence to support this. Three specimens of *Ophion* reared from *Euxoa* in Scandinavia have been identified as *O. luteus* by J.P. Brock (Brock, pers. comm.), which again raises the question of whether *O. luteus* as currently defined actually conceals more than one species, assuming that the host identification as *Euxoa* is correct (these specimens were not available to us). Despite these outstanding questions, we can now say that the common European species that we call *Ophion luteus* is most likely a frequent parasitoid of the noctuid *Agrotis exclamationis* and congeners and the hosts of any cryptic species within this complex should be found within noctuid larvae that are almost certainly late instars at the time the *Ophion* species is/are on the wing.

Identification of *Ophion luteus*

As with many species of *Ophion*, *O. luteus* is predominantly testaceous/pale orange and many *Ophion* in collections (and online images) are misidentified as *O. luteus*, which has generally been used as a catch-all name. However, Brock (1982) clarified the

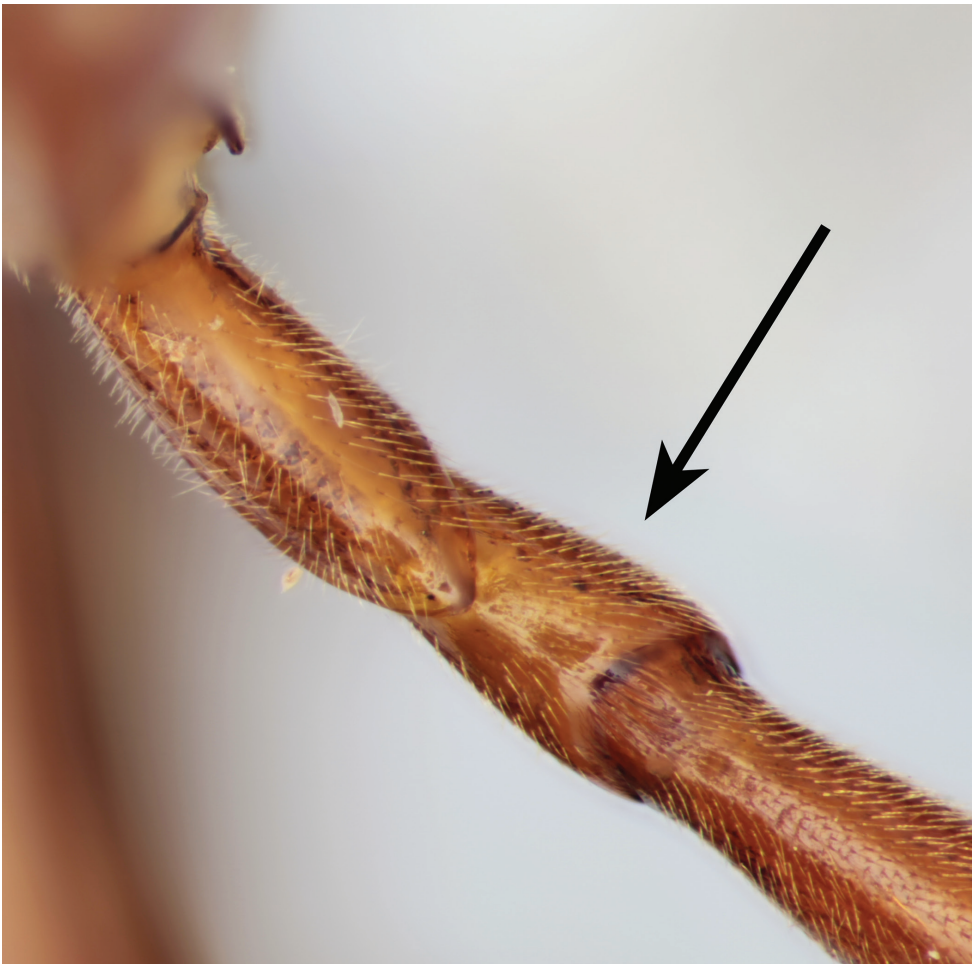

Figure 4. Hind leg of *O. luteus* with arrow pointing to trochantellus (leg parts as follows from top left to bottom right: coxa, trochanter, trochantellus, femur).

identification of *O. luteus* in his comprehensive key to the British *Ophion* species. This key can be difficult to use (because they are on the whole difficult species to identify), but *O. luteus* is usually relatively straightforward to identify. With the exception of very aberrant specimens, such as those reared by Morley (see above), *O. luteus* (including all specimens of the "ordinary" form and most of the "*distans*" form) can be identified by the combination of the long hind trochantellus (Fig. 4) and the usually characteristic mandibles (Fig. 5), which have simply tapered internal edges to the mandibular teeth, lacking internal angles at the base of the teeth (compare with the mandible of *Ophion crassicornis* Brock in Fig. 6), and often show considerable wear. Very occasionally, *Ophion crassicornis* may have very worn mandibles too, when the internal angles of the teeth are then not visible. Additional useful recognition features for *O. luteus* are the strongly sinuous fore wing vein *Rs* and the very short ramellus on fore wing vein

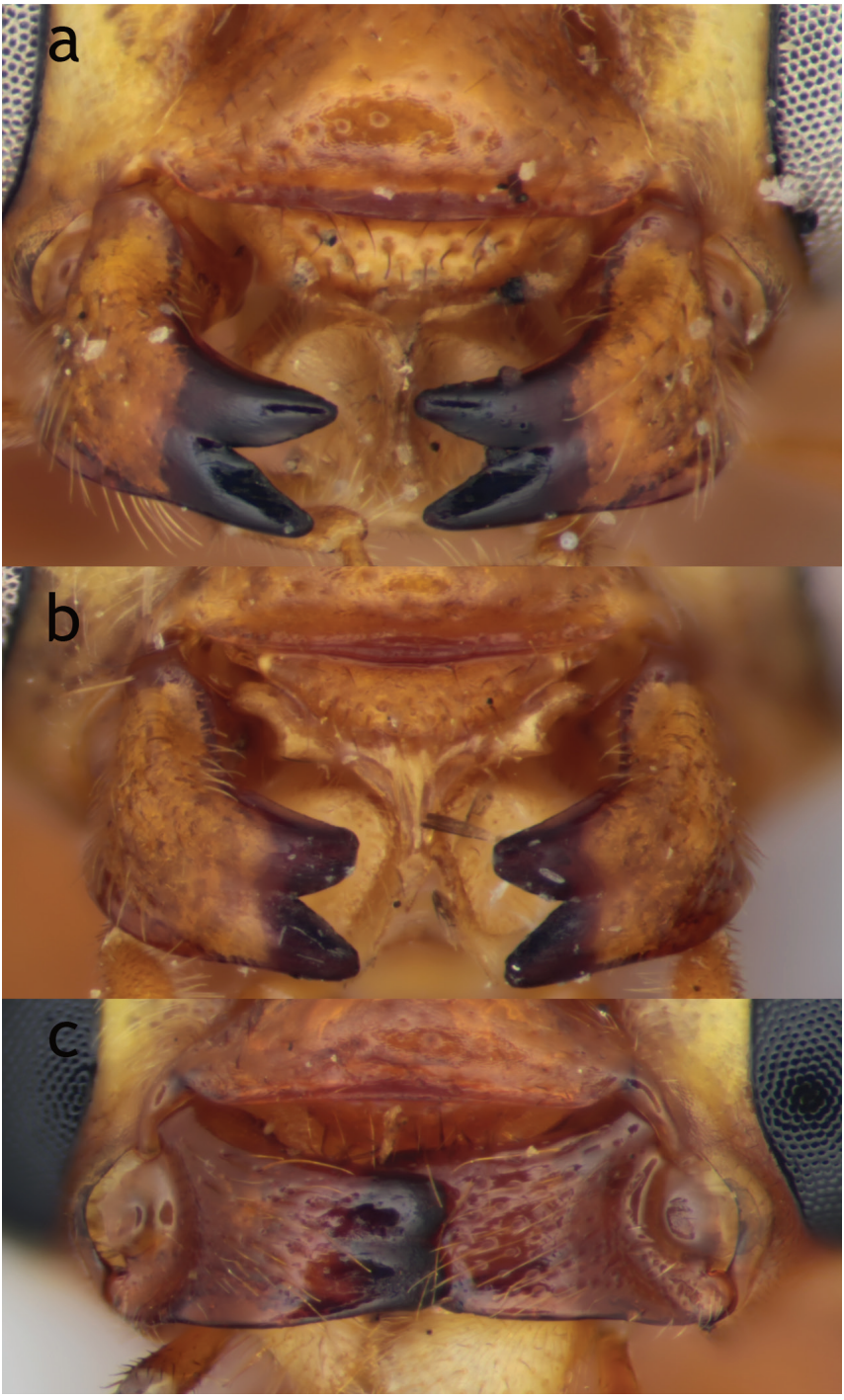

Figure 5. Mandibles of female *O. luteus*, showing differing degrees of abrasion; **a** Bath University, 14.x.2005 (coll. D. Watts) **b** Silwood Park, Berks., 16.viii.2000 (coll. G.R. Broad) **c** Aldbury, Herts., 13.ix.2013 (coll. G.R. Broad); all specimens in BMNH.

Figure 6. Mandible of *Ophion crassicornis* (Westcott, Bucks., 21.v.2009, coll. D. Wilton, BMNH).

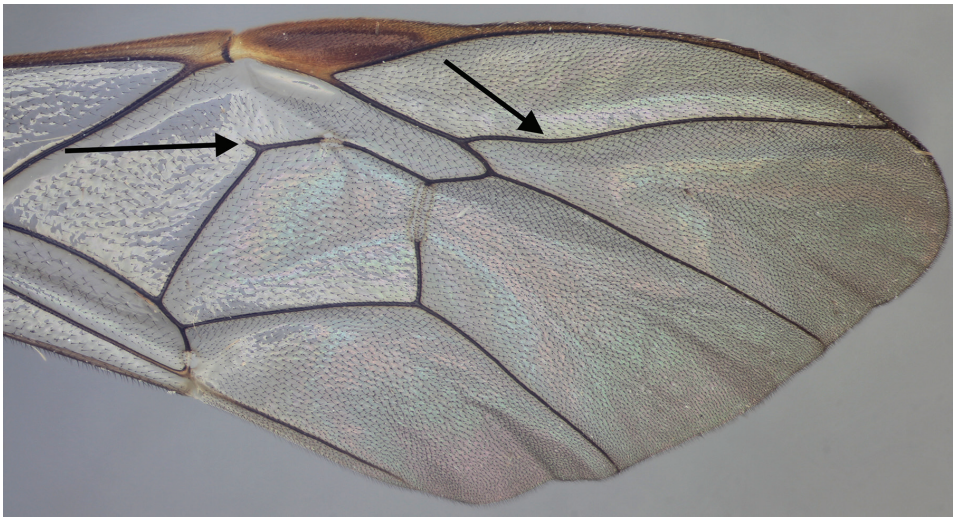

Figure 7. Fore wing of *Ophion luteus* (Aldbury, Herts., 29.ix.2012, coll. G.R. Broad) with arrows pointing to the ramellus (left) and vein *Rs* (right).

1*m-cu* (Fig. 7), together with the sparsely punctate mesopleuron (densely punctate in *O. crassicornis*) and very weak median longitudinal carinae on the propodeum (strong centrally in *O. crassicornis*). The mandibular teeth of *O. luteus* are frequently granulose in sculpture and the teeth may be worn down; the range of mandibular abrasion is shown in Fig. 5. Presumably the mandibles are abraded when the *Ophion* adults emerge from the soil, as this is seen in males as well as females and the mandibles of our reared specimens are unabraded and shiny. Some females have particularly strongly abraded mandibles (Fig. 5c), which still requires explanation – perhaps the soil that the adults need to tunnel through from their subterranean cocoons can become very strongly compacted and hard following a dry summer period, but males in general do not exhibit such extreme wear. *Agrotis* larvae are found above the soil surface at night, when they are presumably attacked by *O. luteus*, and *O. luteus* does not exhibit any modifications typically seen in ichneumonoids parasitising soil-inhabiting Lepidoptera, such as robust legs and short antenna.

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Mike Fitton provided access to the lectotype of *Ophion luteus* in the collections of the Linnean Society. B. P. Henwood kindly donated his reared *O. luteus* to MRS for identification and deposition in NMS. We are grateful for the constructive criticism offered by the small army of reviewers.

References

Brock JP (1982) A systematic study of the genus *Ophion* in Britain (Hymenoptera, Ichneumonidae). Tijdschrift voor Entomologie 125: 57–97.

Győrfi J (1943) Beiträge zur Kenntnis der Wirte von Schlupfwespen. Zeitschrift für Angewandte Entomologie 30: 79–103. doi: 10.1111/j.1439-0418.1944.tb00588.x

Győrfi J (1944) Beiträge zur Kenntnis der Ichneumoniden Ungarns. III. Fragmenta Faunistica Hungarica 7: 103–107.

Meyer NF (1927) [Parasites (Ichneumonidae and Braconidae) bred in Russia from injurious insects during 1881–1926.] Izvestiya Otdela Prikladnoi Entomologii 3: 75–91. [In Russian]

Price PW (1975) Reproductive strategies of parasitoids. In: Price PW (Ed.) Evolutionary strategies of parasitic insects and mites. Springer, New York and London, 87–111. doi: 10.1007/978-1-4615-8732-3_5

Várkonyi G, Hanski I, Rost M, Itämies J (2002) Host-parasitoid dynamics in periodic boreal moths. Oikos 98: 421–430. doi: 10.1034/j.1600-0706.2002.980306.x

Vickery RA (1929) Studies on the fall armyworm in the Gulf Coast district of Texas. United States Department of Agriculture. Technical Bulletin No.138: 63 pp.

Yu DS, Achterberg C van, Horstmann K (2012) Taxapad 2012, Ichneumonoidea 2011. Database on flash-drive. www.taxapad.com, Ottawa, Ontario, Canada.

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Geographic distribution of *Leptogenys elongata* (Buckley) and *Leptogenys manni* Wheeler (Hymenoptera, Formicidae, Ponerinae)

James K. Wetterer[1]

1 *Wilkes Honors College, Florida Atlantic University, 5353 Parkside Drive, Jupiter, Florida 33458 USA*

Corresponding author: *James K. Wetterer* (wetterer@fau.edu)

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Abstract

Leptogenys elongata and *Leptogenys manni* (Hymenoptera, Formicidae, Ponerinae), the only described *Leptogenys* known from the continental US, were recently included on a list of exotic ants established in North America. To evaluate this possibility, I compiled and mapped published and unpublished specimen records of *L. elongata* and *L. manni*. *Leptogenys elongata* records have an essentially continuous distribution through central and eastern Texas (65 counties), southern Oklahoma (one county), and western Louisiana (eight parishes), south through much of Mexico (nine states), plus one questionable record from Colorado and one almost certainly erroneous record from the District of Columbia. *Leptogenys manni* records are known from much of Florida (22 counties), plus one isolated record from Georgia that needs corroboration. I found no credible evidence that *L. elongata* or *L. manni* have established populations anywhere beyond their native ranges.

Keywords

Drimys, Encyrtidae, Eulophidae, *Gaiadendron*, Neotropical, *Phyllocnistis*, pupal morphology, serpentine leaf miner, taxonomy, *Tropaeolum*

Introduction

More than forty ant species have well-established populations in multiple areas of both the Old World and the New World, spread through human commerce (Wetterer 2015). In earlier papers, I have reviewed the worldwide spread of many of these cosmopolitan ant species, including some that have become major global pests, incurring great ecological and economic damage, e.g., *Anoplolepis gracilipes* (Smith), *Linepithema humile* (Mayr), *Pheidole megacephala* (Fabricius), *Solenopsis geminata* (Fabricius), *Solenopsis invicta* Buren, and *Wasmannia auropunctata* (Roger) (Wetterer 2005, 2011, 2012, 2013a, b, Wetterer et al. 2009).

Recently, Wittenborn and Jeschke (2011) investigated characteristics of exotic ant species by comparing species that they classified as native to North America with those they classified as exotic. Two species that Wittenborn and Jeschke (2011; supplementary material) considered exotic in North America were *Leptogenys elongata* (Buckley) and *Leptogenys manni* Wheeler. Of the 307 described *Leptogenys* species (Bolton 2015), *L. elongata* and *L. manni* are the only ones known from the continental US. Both *L. elongata* and *L. manni* appear to be specialist predators of terrestrial isopods (Wheeler 1904, Trager and Johnson 1988). Here, I examine the geographic ranges of *L. elongata* and *L. manni* and evaluate evidence concerning whether these species have any exotic populations in North America or elsewhere.

When a cosmopolitan ant species occurs in both the Old World and the New World, it is almost always clear that one of these ranges is entirely exotic. Within a species' native hemisphere, however, it can be difficult to determine what geographic area constitutes the native range and what area, if any, constitutes the exotic range. When evaluating the native and exotic ranges of a species, researchers consider a spectrum of distributional, historical, evolutionary, ecological, and genetic information (see Wetterer 2008). For example, evidence considered indicative of a species' native range includes 1) older records largely confined to a single continuous region, 2) occurrence in inland native communities, and 3) proximity to the ranges of closely related species. In contrast, evidence indicative of a species' exotic range includes 1) sudden appearance and spread of the species through an area discontinuous with other known populations, 2) occurrence exclusively in coastal and highly disturbed environments, and 3) geographic isolation from closely related species.

Taxonomy

Buckley (1866) described *Ponera elongata* (= *L. elongata*) from near Austin, Texas. Wheeler (1902) designated *Ponera texana* Buckley (described from Archer County, Texas) and *Lobopelta septentrionalis* Mayr (described from "Districte Columbia") as junior synonyms of *L. elongata*. Lattke (2011) designated *Leptogenys mexicana* Mayr (described from Mexico) as a junior synonym of *L. elongata*.

Wheeler (1923) described *Leptogenys elongata manni* (= *Leptogenys manni*) from Florida. Trager and Johnson (1988) raised *L. manni* to full species status.

Lattke (2011) placed *Leptogenys elongata* and *Leptogenys manni* in the *elongata* species group, along with nine other species (including seven new species), all known from Central America, and two with ranges extending into Colombia: *Leptogenys bifida* Lattke (known from Honduras), *Leptogenys chamela* Lattke (known from Jalisco, Mexico), *Leptogenys foraminosa* Lattke (known from Costa Rica, Panama, and Colombia), *Leptogenys honduriana* Mann (known from Honduras), *Leptogenys oaxaca* Lattke (known from Oaxaca, Mexico), *Leptogenys peninsularis* Mann (known from Baja California, Mexico), *Leptogenys sianka* Lattke (known from Veracruz, Chiapas, and Quintana Roo, Mexico), *Leptogenys sonora* Lattke (known from Sonora, Mexico), and *Leptogenys volcanica* Lattke (known from Costa Rica, Panama, and Colombia).

Materials and methods

Using published and unpublished records, I documented the known ranges of *Leptogenys elongata* and *Leptogenys manni.* I obtained unpublished site records from museum specimens in the collections of the Archbold Biological Station (ABS), the Museum of Comparative Zoology (MCZ), and the Smithsonian Institution (SI). In addition, I used on-line databases with collection information on specimens by Antweb (www.antweb.org). I obtained geo-coordinates for collection sites from published references, specimen labels, maps, or geography web sites (e.g., earth.google.com and www.tageo.com).

If a site record listed a geographic region rather than a "point locale," and I had no other record for this region, I used the coordinates of the largest town within the region or, in the case of small islands and natural areas, the center of the region. In the only exception, I mapped Deyrup et al.'s (1989) record of *L. manni* from Monroe County, Florida to a site in northeastern part of the mainland part of the county rather than the largest town (Key West) because Trager and Johnson (1988) and others wrote that *L. manni* is not known from the Florida Keys.

I was unable to map individually many records of *L. elongata* from caves because the site locations are kept secret to avoid vandalism. For example, Reddell and Cokendolpher (2001) and Cokendolpher et al. (2009) listed records of *L. elongata* from seven caves in Bexar County, Texas, including two caves on Camp Bullis Military Training Reservation; I included all these records as just a single site record, mapped near the center of Camp Bullis.

Results

In total, I mapped 139 site records of *Leptogenys elongata* (including 106 from Texas) and 27 site records of *Leptogenys manni* (all but one from Florida) (Fig. 1; Table 1).

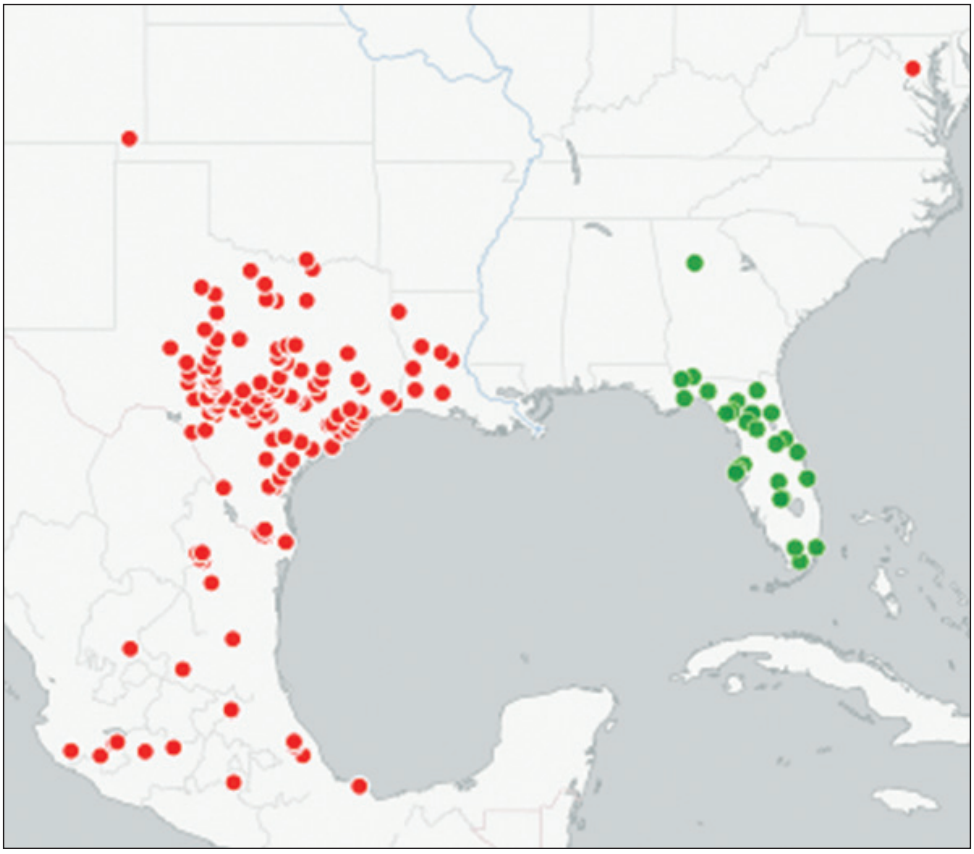

Figure 1. Site records of *Leptogenys elongata* (red) and *Leptogenys manni* (green).

Sites records of *L. elongata* in Texas came from 65 counties: Archer, Bandera, Bastrop, Bell, Bexar, Blanco, Brazoria, Brazos, Brown, Burleson, Burnet, Calhoun, Cameron, Comal, Concho, Coryell, Dallas, DeWitt, Edwards, Fayette, Fort Bend, Galveston, Gillespie, Grayson, Guadalupe, Harris, Hays, Hidalgo, Houston, Irion, Jack, Jefferson, Jones, Karnes, Kendall, Kerr, Kimble, Kinney, Kleberg, Liberty, Live Oak, Matagorda, McLennan, Menard, Milam, Nueces, Palo Pinto, Parker, Real, Refugio, Robertson, Runnels, San Jacinto, San Patricio, Schleicher, Stonewall, Sutton, Taylor, Travis, Uvalde, Victoria, Washington, Webb, Wharton, and Williamson (Buckley 1866, O'Keefe et al. 2000, Reddell and Cokendolpher 2001, Calixto Sanchez 2008, Cokendolpher et al. 2009, Lattke 2011, antweb). The northernmost Texas records came from Archer County and Grayson County (both ~33.6°N; Buckley 1866, O'Keefe et al. 2000). The westernmost Texas record came from Irion County (~101.2°W; Cokendolpher and Francke 1990).

The eight site records of *L. elongata* in Louisiana came from eight different parishes: Acadia, Avoyelles, Beauregard, Caddo, Calcasieu, Natchitoches, Rapides, and

Table 1. Earliest known records for *Leptogenys elongata* and *Leptogenys manni* in the US and Mexican states. * = probably erroneous. Site information given for unpublished records.

Leptogenys elongata	**Earliest record**
Texas	≤1866 (Buckley 1866)
Mexico	≤1870 (Mayr 1870 as *Lobopelta mexicana*)
Washington DC*	≤1886 (Mayr 1886 as *Lobopelta septentrionalis*)
Colorado*	≤1894 (Emery 1894 as *Leptogenys septentrionalis*)
Louisiana	1908 (collector unknown, Smithsonian Institution): Marksville
Veracruz	1941 (Lattke 2011)
Michoacán	1952 (Lattke 2011)
Tamaulipas	1964 (Lattke 2011)
Hidalgo	1965 (Lattke 2011)
Jalisco	1966 (Lattke 2011)
Nuevo León	1972 (R.E. Gregg, antweb): Horsetail Falls
San Luis Potosí	≤2005 (Vásquez-Bolaños 2005)
Morelos	≤2007 (Quiroz-Robledo and Valenzuela-González 2007 as *L. mexicana*)
Zacatecas	≤2007 (Navarrete-Heredia et al. 2007)
Oklahoma	2014 (K. Roeder, J. Trager, pers. comm.): Kingston
Leptogenys manni	**Earliest record**
Florida	≤1908 (Wheeler 1908 as *Leptogenys elongata*)
Georgia	≤1947 (Smith 1947 as *Leptogenys elongata*)

Vernon (Dash 2004, Lattke 2011, antweb). The northernmost Louisiana record came from Shreveport in Caddo Parish (32.5°N; Lattke 2011). The easternmost Louisiana record came from Marksville in Avoyelles Parish (92.1°W; Lattke 2011).

James Trager (pers. comm.) provided one unpublished record of *L. elongata* from Marshall County in southernmost Oklahoma (33.9°N; Table 1).

The 22 site records of *L. elongata* in Mexico came from nine states (Table 1). The two southernmost Mexico record came from Los Tuxtlas, Veracruz (18.6°N; Quiroz Robledo and Valenzuela González 2003 as *L. mexicana*) and Ticuman, Morelos (18.7°N; Quiroz Robledo and Valenzuela González 2007 as *L. mexicana*). The westernmost Mexico record came from Atenquiqui in Jalisco state (103.5°W; Lattke 2011).

Two of the new species in the *elongata* group that Lattke (2011) described have distributions in Mexico that overlap with that of *L. elongata* (*L. sianka* in Veracruz and *L. chamela* in Jalisco). Lattke (2011) re-identified specimens reported as *L. mexicana* from Quintana Roo on the Yucatan Peninsula (Dejean et al. 1995; Dejean and Olmsted 1997) as *L. sianka*. Lattke (2011) confirmed species identification for 12 of the 22 Mexican *L. elongata* site records. It is possible that some Mexican specimens not examined by Lattke (2011) that are currently considered *L. elongata* are actually a different species in the *elongata* group.

The 26 site records of *L. manni* in Florida came from 22 counties: Alachua, Baker, Brevard, Columbia, Dixie, Gadsden, Gilchrist, Highlands, Indian River, Leon, Levy, Marion, Miami-Dade, Monroe, Orange, Pasco, Pinellas, Polk, Putnam, Taylor,

Volusia, and Wakulla (Deyrup et al. 1989, Lattke 2011, antweb, ABS collection). The northernmost Florida record came from Tall Timbers Research Station in Leon County (30.7°N; ABS). There is a single, isolated record of *L. manni* from Georgia (33.8°N; see below).

Problematic records

There are three problematic reports of *L. elongata* (from Colorado, the District of Columbia, and Maryland). Creighton (1950) wrote: "Records for *elongata* have been reported from Colorado, the District of Columbia and Maryland. It is unlikely, but not impossible, that *elongata* occurs in southeastern Colorado. But records from the District of Columbia and Maryland seem plainly impossible."

Wheeler (1908) reported *L. elongata* from Texas, Florida, Colorado, and Maryland, though Wheeler (1908) cited Pergande and Mayr for the Maryland record and therefore this must refer to Mayr's (1886) record of Pergande's specimens reportedly from the District of Columbia. Wheeler (1923) wrote that *L. elongata* "occurs in the Gulf States from Texas to Florida and north to Colorado and the District of Columbia." Records of *L. elongata* from Florida were actually *L. manni* (see above), and it seems likely that published records of *L. elongata* from Colorado and the District of Columbia did not come from outdoor populations in these areas. Both southernmost Colorado (37.0°N) and the District of Columbia (38.9°N), have considerably higher latitudes (and much cooler climate) than the next highest latitude records for *L. elongata* (33.9°N, see above).

The single record of *L. elongata* from Colorado was based on specimens that Ezra T. Cresson collected (Emery 1894 as *L. septentrionalis*). Cresson made substantial collections of Hymenoptera in the Western US for the Academy of Natural Sciences of Philadelphia. Cresson collected in Colorado, but he also worked extensively in Texas (see Cresson 1872). Even though the validity of this record is questionable, I mapped it to Campo in southeastern Colorado.

The single record of *L. elongata* from the District of Columbia was based on specimens that Theodore Pergande collected (Mayr 1886 as *L. septentrionalis*). Pergande worked for the Federal Bureau of Entomology in Washington DC. All other ant specimen records I have found for Pergande from Washington DC were from inside houses and greenhouses (e.g., *Monomorium pharaonis*, *Paratrechina longicornis*, and *Tapinoma melanocephalum* in the Smithsonian collection). It is possible that the specimens Mayr (1886) reported came from a greenhouse. It seems more likely, however, that the specimens were mislabeled or their provenance was misunderstood. Pergande (1893, 1894, 1896) collected ants extensively in Mexico and Texas, which are more likely sources of his specimens. Lattke (2011) similarly concluded that this record was "an obvious error as the District of Columbia lies far beyond the range of *L. elongata*."

Smith (1947) wrote that he saw specimens of *elongata* labeled "Stone Mt., Decatur, Georgia." These specimens are at the Smithsonian and Lattke (2011) identified them

as *L. manni*. Lattke (2011) wrote: "The specimens labeled as from Georgia constitute a single series and lack a date, though the state of the label suggests the early 1900's. *L. manni* is now known to range into northern Florida, but over 300 kilometers separate Decatur from the nearest *L. manni* collection sites in Florida. The Georgia locality is a possibility but needs corroboration."

Discussion

Trager and Johnson (1988) wrote that *Leptogenys elongata* occurs in "western Louisiana, Texas, and northeastern Mexico." However Lattke's (2011) designation of *Leptogenys mexicana* as a junior synonym of *L. elongata* extends the reported range of this species south into central Mexico (Fig 1). Despite the paucity of records, *L. elongata* appears to show an uninterrupted range across much of Texas (65 counties) into southernmost Oklahoma (one county), east to central Louisiana (eight parishes), and south through much of Mexico (nine states) (Fig. 1). This continuous range gives no indication that *L. elongata* is exotic to any part of this region. The one questionable record from Colorado could be an isolated northern extension of this native range, but seems more likely to be an error, and the one record of *L. elongata* from the District of Columbia is almost certainly an error. The relatively recent first records of *L. elongata* from some states in Mexico (Table 1) are likely to be due to the scarcity of specimens and the difficulty of distinguishing *L. elongata* from the 16 other *Leptogenys* species known from Mexico (Antweb 2015), including five other members of the *elongata* species group (*L. chamela*, *L. oaxaca*, *L. peninsularis*, *L. sianka*, and *L. sonora*), rather than recent expansion into parts of Mexico.

Trager and Johnson (1988) listed *Leptogenys manni* only from Florida and wrote that *L. manni* "must be added to the growing list of Florida's endemic ants." *Leptogenys manni* records are now known from much of Florida (22 counties), plus one isolated record from a natural area in central Georgia that could be based on a labeling error and needs confirmation.

Leptogenys elongata and *L. manni* are large, distinctive ants that have been rarely collected, perhaps due to largely subterranean habits. In fact, Reddell and Cokendolpher (2001) and Cokendolpher et al. (2009) found *L. elongata* relatively often when surveying caves in Texas. Trager and Johnson (1988) speculated: "in south Florida, *L. manni* may be entirely subterranean." Virtually all records of *L. elongata* and *L. manni* come from relatively undisturbed natural areas, a character not normally indicative of an exotic species. In fact, I found no credible evidence that *L. elongata* or *L. manni* have established exotic populations beyond their native ranges. I conclude that Wittenborn and Jeschke (2011) were wrong in classifying *L. elongata* and *L. manni* as exotic to North America.

In documenting the ranges of other species on Wittenborn and Jeschke's (2011) list of North American exotics, I have found that numerous other species also appear to be misclassified. For example, Wittenborn and Jeschke (2011) categorized *Gnamptogenys*

hartmani (Wheeler), *Labidus coecus* (Latreille), and *Pachycondyla harpax* (Fabricius) as exotics in North America, but all three species have apparently continuous ranges from South America, through Central America, and into Mexico and the southern US and give no indications of being exotic in any part of their ranges (Wetterer 2014, in press a, Wetterer and Snelling 2015). Similarly, *Cephalotes varians* (Smith), *Tapinoma litorale* Wheeler, *Temnothorax allardycei* (Mann), and *Trachymyrmex jamaicensis* (André), also on the list of exotics, appear to be native throughout their seemingly continuous ranges in the West Indies and southern Florida (Wetterer in press b, in prep.). It is unfortunate that native North American ant species wound up on Wittenborn and Jeschke's (2011) list of exotics. Exotic species are an important ecological problem around the world. In order to protect native species and minimize the negative impacts of exotic species, it is essential to distinguish which species are native and which are exotic.

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References

Bolton B (2015) An online catalog of the ants of the world. http://www.antcat.org/catalog/430052 [accessed 4 September 2015]

Buckley SB (1866) Descriptions of new species of North American Formicidae. Proceedings of the Entomological Society of Philadelphia 6: 152–172.

Calixto Sanchez AA (2008) Implications of relative ant abundance and diversity for the management of *Solenopsis invicta* Buren with broadcast baits. PhD Thesis. Texas A and M University, College Station.

Cokendolpher JC, Francke OF (1990) The ants (Hymenoptera, Formicidae) of western Texas. Part II. Subfamilies Ecitoninae, Ponerinae, Pseudomyrmecinae, Dolichoderinae, and Formicinae. Special Publications, The Museum, Texas Tech University 30: 1–76.

Cokendolpher JC, Reddell JR, Taylor SJ, Krejca JK, Suarez AV, Pekins CE (2009) Further ants (Hymenoptera: Formicidae) from caves of Texas. Texas Memorial Museum, Speleological Monographs 5: 151–168.

Creighton WS (1950) The ants of North America. Bulletin of the Museum of Comparative Zoology 104: 1–585.

Cresson ET (1872) Hymenoptera Texana. Transactions of the American Entomological Society 4: 153–292. doi: 10.2307/25076272

Dash ST (2004) Species diversity and biogeography of ants (Hymenoptera: Formicidae) in Louisiana, with notes on their ecology. M.S. thesis, Louisiana State Univ., 290 pp.

Dejean A, Olmsted I (1997) Ecological studies on *Aechmea bracteata* (Swartz) (Bromeliaceae). Journal of Natural History 31: 1313–1334. doi: 10.1080/00222939700770741

Dejean A, Olmsted I, Snelling RR (1995) Tree-epiphyte-ant relationships in the low inundated forest of Sian Ka'an Biosphere Reserve, Quintana Roo, Mexico. Biotropica 27: 57–70. doi: 10.2307/2388903

Deyrup M, Johnson C, Wheeler GC, Wheeler J (1989) A preliminary list of the ants of Florida. Florida Entomologist 72: 91–101. doi: 10.2307/3494972

Emery C (1894) Studi sulle formiche della fauna Neotropica. Bollettino della Societa Entomologica Italiana 26: 137–241.

Lattke JE (2011) Revision of the New World species of the genus *Leptogenys* Roger (Insecta: Hymenoptera: Formicidae: Ponerinae). Arthropod Systematics and Phylogeny 69(3): 127–264.

Mayr G (1870) Neue Formiciden. Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien 20: 939–996.

Mayr G (1886) Die Formiciden der Vereinigten Staaten von Nordamerika. Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien 36: 419–464.

Navarrete-Heredia JL, Quiroz-Rocha GA, Vásquez-Bolaños M, Cortés-Aguilar J, Gómez-Flores V, López-Contreras E, Labrador-Chávez G (2007) Coleópteros y hormigas (Coleoptera e Hymenoptera: Formicidae) de algunas localidades del sur de Zacatecas. Entomología Mexicana 6(2): 1317–1320.

O'Keefe ST, Cook JL, Dudek T, Wunneburger DF, Guzman MD, Coulson RN, Vinson SB (2000) The distribution of Texas ants. Southwestern Entomologist 22 (Supplement): 1–93.

Pergande T (1893) On a collection of Formicidae from Lower California and Sonora, Mexico. Proceedings of the California Academy of Sciences (2) 4: 26–36.

Pergande T (1894) Formicidae of Lower California, Mexico. Proceedings of the California Academy of Sciences (2) 4: 161–165.

Pergande T (1896) Mexican Formicidae. Proceedings of the California Academy of Sciences (2) 5: 858–896.

Quiroz Robledo L, Valenzuela González J (2003) Las hormigas ponerinas y cerapaquinas (Formicidae: Ponerinae y Cerapachyinae) de las Estación de Biología Tropical "Los Tuxtlas", Veracruz, México. Folia Entomológica Mexicana 42: 381–395.

Quiroz Robledo L, Valenzuela González J (2007) Distribution of poneromorph ants (Hymenoptera: Formicidae) in the Mexican state of Morelos. Florida Entomologist 90: 609–615. doi: 10.1653/0015-4040(2007)90[609:DOPAHF]2.0.CO;2

Reddell JR, Cokendolpher JC (2001) Ants (Hymenoptera: Formicidae) from the caves of Belize, Mexico, and California and Texas (U.S.A.). Texas Memorial Museum, Speleological Monographs 5: 129–154.

Smith MR (1947) A generic and subgeneric synopsis of the United States ants, based on the workers. American Midland Naturalist 37: 521–647. doi: 10.2307/2421469

Trager JC, Johnson C (1988) The ant genus *Leptogenys* (Hymenoptera: Formicidae, Ponerinae) in the United States. In: Trager JC (Ed.) Advances in myrmecology. E. J. Brill, Leiden, 29–34.

Vásquez-Bolaños M (2005) Hymenoptera: Formicidae. In: Navarrete-Heredia JL, Guerrero-Vázquez S (Eds) Colecciones Zoológicas del Centro de Estudios en Zoología. Universidad de Guadalajara, Guadalajara, 23–35

Wetterer JK (2005) Worldwide distribution and potential spread of the long-legged ant, *Anoplolepis gracilipes*. Sociobiology 45: 77–97.

Wetterer JK (2008) Worldwide spread of the longhorn crazy ant, *Paratrechina longicornis* (Hymenoptera: Formicidae). Myrmecological News 11: 137–149.

Wetterer JK (2011) Worldwide spread of the tropical fire ant, *Solenopsis geminata* (Hymenoptera: Formicidae). Myrmecological News 14: 21–35.

Wetterer JK (2012) Worldwide spread of the African big-headed ant, *Pheidole megacephala* (Hymenoptera: Formicidae). Myrmecological News 17: 51–62.

Wetterer JK (2013a) Exotic spread of *Solenopsis invicta* (Hymenoptera: Formicidae) beyond North America. Sociobiology 60: 53–63. doi: 10.13102/sociobiology.v60i1.50-55

Wetterer JK (2013b) Worldwide spread of the little fire ant, *Wasmannia auropunctata* (Hymenoptera: Formicidae). Terrestrial Arthropod Reviews 6: 173–184. doi: 10.1163/18749836-06001068

Wetterer JK (2014) Geographic distribution of *Gnamptogenys hartmani* (Hymenoptera: Formicidae), an "agro-predator" that attacks fungus-growing ants. Terrestrial Arthropod Reviews 7: 147–157. doi: 10.1163/18749836-07021078

Wetterer JK (2015) Geographic origin and spread of cosmopolitan ants (Hymenoptera: Formicidae). Halteres 6: 66–78.

Wetterer JK (in press a) Geographic distribution of *Pachycondyla harpax* (Hymenoptera, Formicidae). Sociobiology.

Wetterer JK (in press b) Geographic distribution of *Trachymyrmex jamaicensis* (Hymenoptera, Formicidae). Florida Entomologist.

Wetterer JK, Snelling GC (2015) Geographic distribution of *Labidus coecus* (Hymenoptera, Formicidae), a subterranean army ant. Journal of Hymenoptera Research 44: 31–38. doi: 10.3897/JHR.44.4672

Wetterer JK, Wild AL, Suarez AV, Roura-Pascual N, Espadaler X (2009) Worldwide spread of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). Myrmecological News 12: 187–194.

Wheeler WM (1902) A consideration of S. B. Buckley's "North American Formicidae." Transactions of the Texas Academy of Sciences 4: 17–31.

Wheeler WM (1904) A crustacean-eating ant (*Leptogenys elongata* Buckley). Biological Bulletin 6: 251–259. doi: 10.2307/1535818

Wheeler WM (1908) The ants of Texas, New Mexico and Arizona. (Part I.). Bulletin of the American Museum of Natural History 24: 399–485.

Wheeler WM (1923) The occurrence of winged females in the ant genus *Leptogenys* Roger, with descriptions of new species. American Museum Novitates 90: 1–16.

Wittenborn D, Jeschke JM (2011) Characteristics of exotic ants in North America. NeoBiota 10: 47–64 (in supplementary material). doi: 10.3897/neobiota.10.1047

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Redescription of *Conostigmus albovarius* Dodd, 1915 (Hymenoptera, Megaspilidae), a metallic ceraphronoid, with the first description of males

Carolyn Trietsch[1], Andrew R. Deans[1], Istvàn Mikó[1]

1 *Frost Entomological Museum, University Park, PA 16802, United States of America*

Corresponding author: *Carolyn Trietsch* (cut162@psu.edu)

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Abstract

Conostigmus albovarius Dodd, 1915 (Hymenoptera: Megaspilidae) is a species previously known by a single female holotype. Here, we provide a redescription of this peculiar ceraphronoid based on several female specimens and describe the male of the species for the first time. Intraspecifically-variable morphological traits such as female antenna color pattern are documented and discussed. A phenotype bank of morphological characters is provided for use in future megaspilid taxonomic treatments. We also provide phenotypic data in a semantic form to allow for ease of data integration and accessibility, making taxonomic data more accessible to future systematic efforts.

Keywords

Ceraphronoidea, morphology, systematics, taxonomy

Introduction

Conostigmus Dahlbom, 1858 (Hymenoptera: Ceraphronoidea; Megaspilidae) is a relatively small genus of parasitoid wasps that has been largely neglected by modern taxonomic efforts, though they are commonly collected and are worldwide in distribution (Johnson and Musetti 2004). Relatively little is known about the biology of *Conostigmus*

(Graham 1984, Bijoy et al. 2014). However, the superfamily Ceraphronoidea is known to contain both endoparasitoids and ectoparasitoids, with examples of both known to occur even within the same genus (Mikó et al. 2013; Broad and Livermore 2014).

Ceraphronoids have been reared from hosts spanning a large variety of orders, including Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Mecoptera, Neuroptera, Thysanoptera, and Trichoptera (Schaffner 1959; Dessart 1967; Graham 1984; Dessart 1992; Goulet and Huber 1993; Mikó and Deans 2009). Information about the specific hosts of *Conostigmus* is limited: Kamal (1926) reports two species, *Conostigmus triangularis* Thomson, 1858 and *C. timberlakei* Kamal, 1926, as reared from Syrphidae (Diptera) puparia. Vidal (2003) reports that *Conostigmus rufescens* Kieffer, 1907 is known to parasitize the eggs and larvae of the brassica pod midge, *Dasineura brassicae* Winnertz, 1853 (Diptera: Cecidomyiidae), which is a pest of rapeseed (*Brassica napus* L. 1753). While species such as *C. rufescens* could be economically important, research efforts are hampered because so little work has been done on the systematics of Ceraphronoidea (Vidal 2003; Mikó et al. 2013).

Alan P. Dodd described the species *Lygocerus albovarius* from a single female specimen he captured in Australia (Dodd 1915). Dodd later expressed doubts on being able to distinguish between females of *Conostigmus* and *Lygocerus* after discovering the male of another Australian species he had described from a female specimen, *Conostigmus unilineatus* Dodd 1915. He advised that all of the Australian species he had described from female specimens, including *L. albovarius*, were more likely to be *Conostigmus* (Dodd 1916). Paul Dessart initially transferred *Lygocerus albovarius* from *Lygocerus* to *Dendrocerus* (Dessart, 1972), but later transferred the species to *Conostigmus* following the suggestion of Dodd (Dessart 1995; Dessart 1997).

Materials and methods

Air-dried point-mounted specimens were obtained from the Canadian National Collection of Insects (CNC). To prepare specimens for dissection, air-dried point-mounted specimens were removed from their points, cleared with BioQuip Specimen Clearing Fluid #6373, and subsequently kept in glycerol for dissection and storage. Dissections were performed with #2 insect pins and #5 forceps.

Air-dried point-mounted specimens and glycerol-stored specimens were examined using an Olympus SZX16 stereomicroscope with an Olympus SDF PL APO 1X PF objective (115×) and an Olympus SDF PL APO 2X PFC objective (230× magnification). Blue-Tack (Bostik, Inc., Wauwatosa, Wisconsin, USA) was used to stabilize specimens while making observations and images. Measurements were taken using a KR 851 stage micrometer (1mm in 100 divisions) attached to the same microscope. Bright field images were taken with an Olympus DP71 digital camera attached to an Olympus ZX41 compound microscope. Images were subsequently aligned and stacked using Zerene Stacker Version 1.04 Build T201404082055.

For the male and female species descriptions, 87 morphological characters were scored based on observations of air-dried point mounted specimens and glycerol stored specimens. Following the Phenotypic Quality Ontology (PATO; available at http://obofoundry.org/), the preferred label "count" was used instead of its synonyms, including "presence". It logically follows that the character "presence" should not include "absent" as a state because the correct use of this syntax would be "presence/absence: absent", which would over-complicate descriptions. Specimen data, OTU concepts, natural language phenotypes and images of specimens were compiled in the MX database (http://mx.speciesfile.org). This software was used to render the Diagnosis, Description, and Material Examined sections. Definitions and descriptions of the morphological terms and structures were mapped to classes in the following phenotype-focused ontologies: Hymenoptera Anatomy Ontology (HAO), Phenotypic Quality Ontology (PATO), Biospatial Ontology (BSPO), OBO Relation Ontology (RO), Ontology for Biomedical Investigations (OBI), and Information Artifact Ontology (IAO), all available at http://www.ontobee.org/ (Xiang et al. 2011).

Semantic statements were generated in Protégé Version 5.0.0 (Build beta-17) to build an ontology where phenotypes are represented semantically. Standardizing taxonomic data through ontology-based semantic representation could benefit future systematic work by allowing taxonomic data sets from different sources to be easily integrated, expediting computerized searches across these data sets (Deans et al. 2012; Balhoff et al. 2013; Mikó et al. 2014). Statements were written in OWL Manchester syntax, modeled after examples set by Balhoff et al. (2013), Mikó et al. (2014), and Mikó et al. (2015). A list of the semantic statements generated is presented in Supplementary file 1: Appendix 1. The data matrix file, the NEXUS file corresponding to the data matrix and the semantic annotations file are available at http://dx.doi.org/10.6084/m9.figshare.1539620, http://dx.doi.org/10.6084/m9.figshare.1544551, and http://dx.doi.org/10.6084/m9.figshare.1539621.

Taxonomy

***Conostigmus albovarius* (Dodd, 1915)**
Figs 1–6

Lygocerus albovarius: Dodd, A. P. 1915: 453 (original description)
Conostigmus albovarius: Dodd, A. P. 1916: 18
Dendrocerus albovarius: Dessart, P. 1972: 291
Conostigmus albovarius: Dessart, P. 1995: 320
Conostigmus albovarius: Dessart, P. 1997: 7, 133

Diagnosis. *Conostigmus albovarius* differs from all other Megaspilidae by the presence of metallic coloration and foveolate sculpturing.

Redescription. Color and sculpture. Color hue pattern male: cranium, mesosoma, F1-9, pedicel, distal region of hind femur, abdomen brown; scape, forelegs and midlegs, tibia of hind leg yellow; hind coxa and petiole neck white. Color hue pattern female: cranium except supraclypeal depression and mesosoma except posteroventral region metallic brown/purple; F9, distal and proximal region of scape, supraclypeal depression, abdomen, dorsal proximal regions of femur and tibia brown; F1-F5, pedicel, scape except distal and proximal regions, posteroventral region of mesosoma, petiole neck white; F6-F8 variable white or brown. Foveolate sculpture on body count: present on mesosoma and frons; present on frons. Occipital carina sculpture: crenulate.

Head morphometrics. Cephalic size (csb): Mean: 300–450 μm. Head height (lateral view) vs. eye height (anterior view): HH:EHf = 1.0–2.0. Head height vs. head length: HH:HL = 1.0–1.5. Head width vs. interorbital space: HW:IOS = 2.0–2.5. Head width vs. head height: HW:HH = 1.0–1.5. Male ocular ocellar line vs. lateral ocellar line: OOL:LOL = 1.0–2.0. Male ocular ocellar line vs. posterior ocellar line: OOL:POL = 0.5–2.0. Female ocular ocellar line vs. lateral ocellar line: OOL 1.5–2.5 × as long as LOL.

Head and antenna. Median flange of occipital carina count: absent. Submedial flange of occipital carina count: absent. Dorsal margin of occipital carina vs dorsal margin of lateral ocellus in lateral view: occipital carina is ventral to lateral ocellus in lateral view. Preoccipital lunula count: present. Preoccipital carina count: present . Preoccipital furrow count: present. Preoccipital furrow anterior end: Preoccipital furrow ends inside ocellar triangle. Postocellar carina count: absent. Transversely reticulate region on frons count: present. Transversely reticulate region on frons extent: restricted to lateral branches of supraclypeal depression. Rugose region on frons count: absent. White, thick setae on frons count: present. Ventromedian setiferous patch and ventrolateral setiferous patch count: absent. Antennal scrobe count: absent. Facial pit count: no external corresponding structure present. Supraclypeal depression count: present. Supraclypeal depression structure: absent medially, represented by two grooves laterally of facial pit. Intertorular area count: present. Intertorular carina count: present. Median region of intertorular area shape: flat. Transverse frontal carina count: absent. Ventral margin of antennal rim vs dorsal margin of clypeus: not adjacent. Torulo-clypeal carina count: present. Subtorular carina count: absent. Mandibular tooth count: 2. Female first flagellomere length vs pedicel: F1 as long as pedicel (1.0–1.1). Female ninth flagellomere length: F9 less than F7+F8; F9 = F7+F8. Male first flagellomere length vs male second flagellomere length: 1.0–1.1. Length of setae on male flagellomere vs. male flagellomere width: setae shorter than width of flagellomeres; setae as long as width of flagellomeres. Sensillar patch of the male flagellomere pattern: F5–F9.

Mesosoma and metasoma. Ventrolateral invagination of the pronotum count: present. Anterior mesoscutal width vs. posterior mesoscutal width: AscW/PscW = 0.8–0.9. Mesoscutal length vs anterior mesoscutal width: MscL/AscW = 1.0–2.0. Weber length: WL = 400–550 μm. Notaulus posterior end location: adjacent to transscutal articulation. Median mesoscutal sulcus posterior end: adjacent to transscutal articulation. Scutoscutellar sulcus vs transscutal articulation: adjacent. Axillular carina count:

absent. Speculum ventral limit: not extending ventrally of pleural pit line. Epicnemial carina count: complete. Epicnemium posterior margin shape: anterior discrimenal pit absent; epicnemial carina curved. Sternaulus count: present. Sternaulus length: short, not reaching 1/2 of mesopleuron length at level of sternaulus. Mesometapleural sulcus count: present. Metapleural carina count: present. Transverse line of the metanotum-propodeum vs. antecostal sulcus of the first abdominal tergum: adjacent sublaterally. Lateral propodeal carina count: present. Lateral propodeal carina shape: straight (left and right lateral propodeal carinae compose a carina that is not broken medially). Anteromedian projection of the metanoto-propodeo-metapecto-mesopectal complex count: absent. Posterior margin of nucha in dorsal view shape: concave. Transverse carina on petiole shape: concave.

Abdomen and male genitalia. S1 length vs. shortest width: S1 wider than long. Distal margin of male abdominal sternum 9 shape: straight. Proximolateral corner of abdominal sternum 9 shape: blunt. Cupula length vs. gonostyle-volsella complex length: cupula less than 1/2 the length of gonostyle-volsella complex in lateral view. Proximodorsal notch of cupula count: present. Proximodorsal notch of cupula shape: arched. Distodorsal margin of cupula shape: straight. Proximodorsal notch of cupula width vs length: wider than long. Proximolateral projection of the cupula shape: blunt. Distoventral submedian corner of the cupula count: absent. Dorsomedian conjunctiva of the gonostyle-volsella complex count: present. Dorsomedian conjunctiva of the gonostyle-volsella complex length relative to length of gonostyle-volsella complex: dorsomedian conjunctiva extending 2/3 of length of gonostyle-volsella complex in dorsal view. Distal end of dorsomedian conjunctiva of the gonostyle-volsella complex shape: acute. Parossiculus count (parossiculus and gonostipes fusion): present (not fused with the gonostipes). Apical parossiculal seta number: one. Distal projection of the parossiculus count: absent. Distal projection of the penisvalva count: absent. Dorsal apodeme of penisvalva count: absent. Harpe length: harpe shorter than gonostipes in lateral view. Distodorsal setae of sensillar ring of harpe length vs. harpe width in lateral view: setae as long or shorter than harpe width. Distodorsal setae of sensillar ring of harpe orientation: medially. Sensillar ring area of harpe orientation: medially. Lateral setae of harpe count: present. Lateral setae of harpe orientation: oriented distally.

Material examined. Specimens (3 males, 7 females): AUSTRALIA: 3 females. PSUC_FEM 35246, 45237, 83872. AUSTRALIA: Queensland: 3 males, 4 females. PSUC_FEM 36035, 45221, 45227, 45257, 84276, 91442, 98392. A full list of the locality data for each specimen is provided in Table 1.

Specimens will be deposited at the Canadian National Collection of Insects (CNC), Ottawa, ON, Canada and at the Frost Entomological Museum (FEM), University Park, PA, USA.

Comments. *Conostigmus albovarius* stands out from other species of *Conostigmus* due to its unique, white color pattern, for which the species was named (*albus* as in "white" and *varius* as in "variegated") (Dessart 1997). Dessart (1997) was intrigued by the stark white color present on the back of the mesosoma and on portions of the legs and antennae. He attributed the unique color of the antenna and legs to a lack of pig-

Figure 1. Bright field image of *Conostigmus albovarius* (Dodd, 1915) female. **A** Head and antennae, lateral view showing antennae and scape coloration (see http://dx.doi.org/10.6084/m9.figshare.1539641) **B** Habitus lateral view showing a variation in antennae coloration (see http://dx.doi.org/10.6084/m9.figshare.1539639).

ment in these areas, but voiced concerns whether the color of the mesosoma could be an artifact due to damage of the sole holotype specimen (Dessart 1997). With the discovery of ten new specimens, it is now clear that this coloring of the mesosoma is not an artifact, and that it is a phenotype shared by both females and males of the species (Figs 1, 2, 3).

Another phenotype that both males and females share is the presence of foveolate sculpturing, a feature which has not been described before in *Conostigmus*. Most mem-

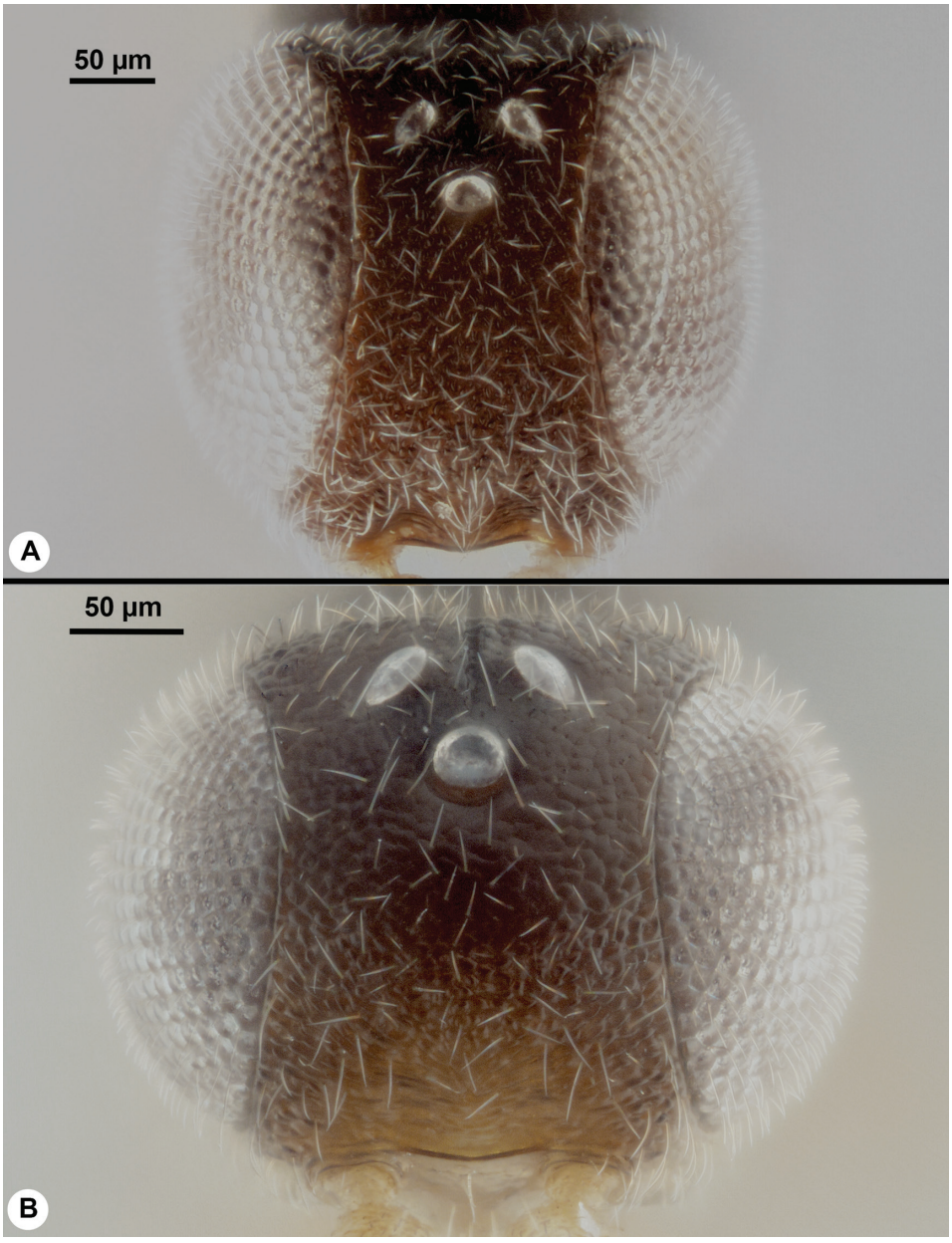

Figure 2. Bright field images of *Conostigmus albovarius* (Dodd, 1915). **A** Female head and frons, anterior view (see http://dx.doi.org/10.6084/m9.figshare.1539640) **B** Male head and frons, anterior view (see http://dx.doi.org/10.6084/m9.figshare.1539642).

bers of *Conostigmus* exhibit reticulate sculpturing (Yoder et al. 2010), which refers to the polygonal microreticulation of the cuticular surface that is most likely based on the pattern of epithelial cells (Krell 1994).

Figure 3. Bright field images of *Conostigmus albovarius* (Dodd, 1915) male habitus, lateral view (see http://dx.doi.org/10.6084/m9.figshare.1539644).

Table 1. A list of the locality data for the specimens examined.

Materials examined	
Specimen ID	**Locality data**
PSUC_FEM 98392	Australia: QLD: Wooroonooran National Park 17°34'09"S; 145°46'35"E: 375m, 25.ix.2004 s.s. L. Masner, rainforest, Q-28
PSUC_FEM 45257	Australia: QLD: Wooroonooran National Park 17°34'09"S; 145°46'35"E: 375m, 25.ix.2004 s.s. L. Masner, rainforest, Q-28
PSUC_FEM 91442	Australia: QLD: Wooroonooran National Park 17°34'09"S; 145°46'35"E: 375m, 25.ix.2004 s.s. L. Masner, rainforest, Q-28
PSUC_FEM 45237	Australia: QLD: Tully River Falls Road Misty Mountains Trail 12.IX.2004, s.s. Q-11 L. Masner, rainforest
PSUC_FEM 36035	Australia: QLD: Wooroonooran National Park: 17°34'06"S; 145°42'21"E: 500m, 9-14.ix.2004 YPT L. Masner, rainforest, Q-7a
PSUC_FEM 35246	Australia: QLD: Tully River Falls Road Misty Mountains Trail 12.IX.2004, s.s. Q-11 L. Masner, rainforest
PSUC_FEM 83872	Australia: QLD: Tully River Falls Road Misty Mountains Trail 12.IX.2004, s.s. Q-11 L. Masner, rainforest
PSUC_FEM 45221	Australia: QLD: Wooroonooran National Park: 17°34'06"S; 145°42'21"E: 500m, 9-14.ix.2004 YPT L. Masner, rainforest, Q-7a
PSUC_FEM 84276	Australia: QLD: Wooroonooran National Park: 17°34'06"S; 145°42'21"E: 500m, 9-14.ix.2004 YPT L. Masner, rainforest, Q-7a
PSUC_FEM 45227	Australia: QLD: Ella Bay Nat. Park 17°28'14"S, 146°03'48"E 21–23.IX.2004 YPT L. Masner, rainforest, Q-23

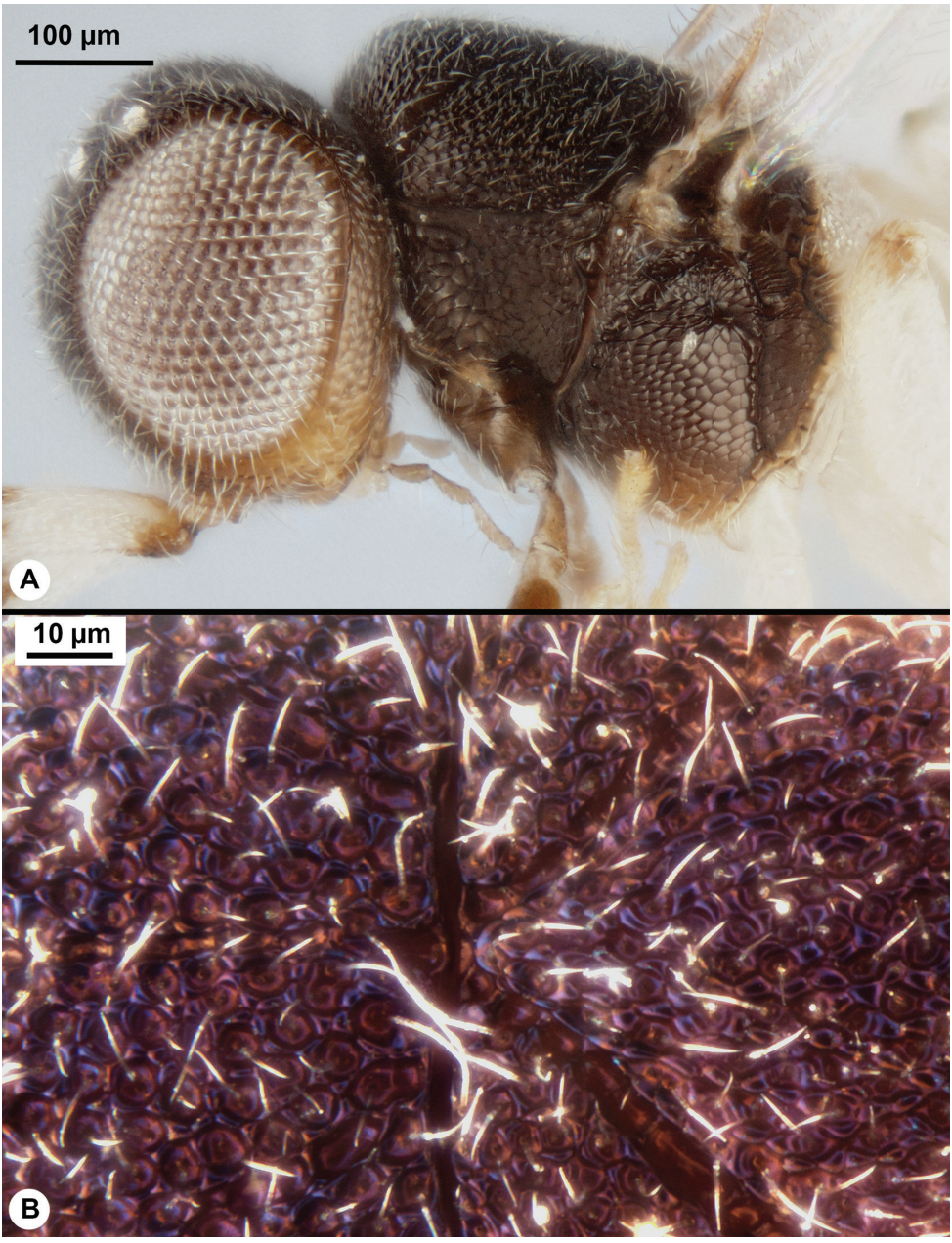

Figure 4. Bright field images of *Conostigmus albovarius* (Dodd, 1915) female. **A** Head and mesosoma, lateral view (see http://dx.doi.org/10.6084/m9.figshare.1539643) **B** Posterior mesoscutum and anterior mesoscutellum showing areolate sculpture and metallic coloration (see http://dx.doi.org/10.6084/m9.figshare.1539645).

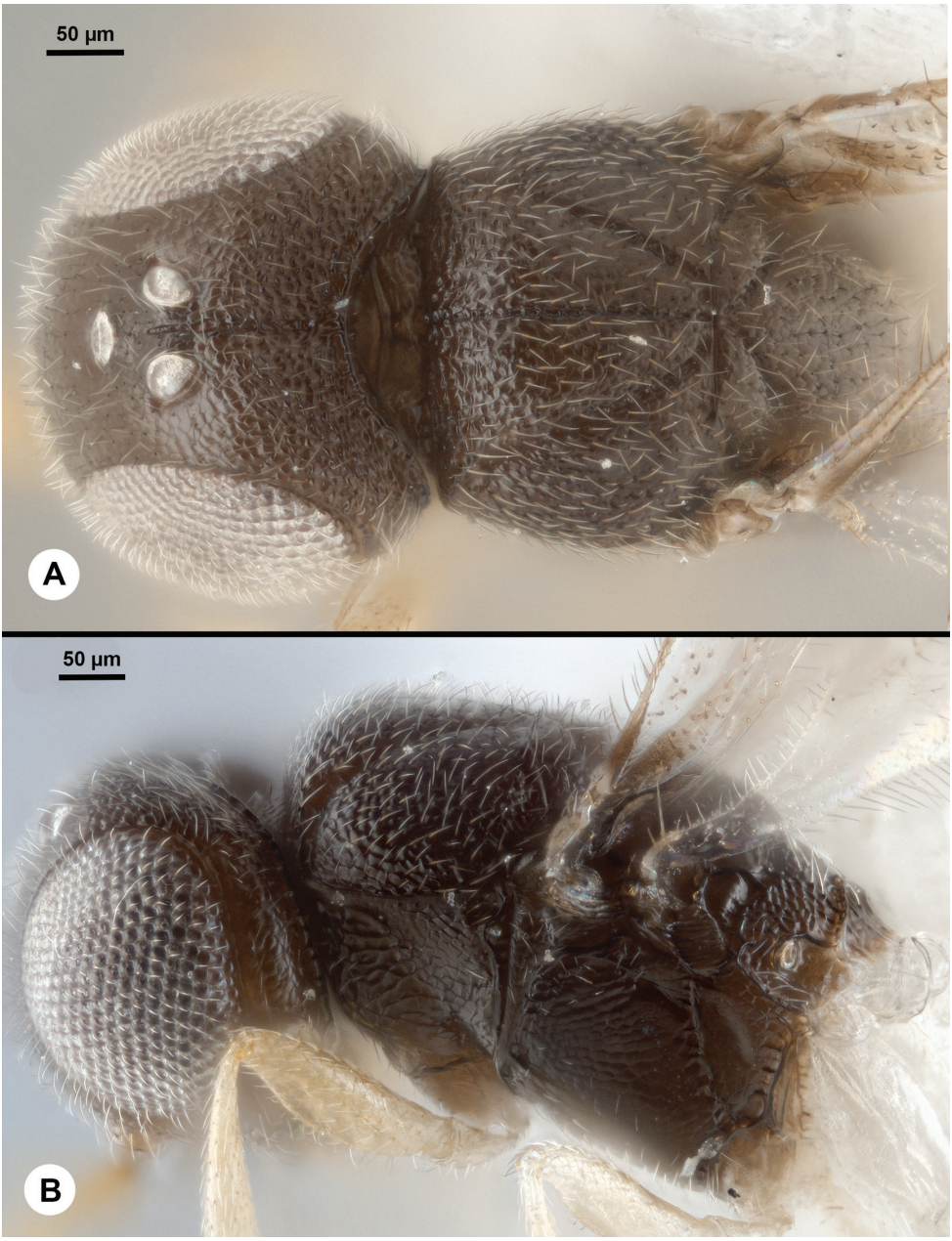

Figure 5. Bright field images of *Conostigmus albovarius* (Dodd, 1915) male. **A** Head and mesosoma, dorsal view (see http://dx.doi.org/10.6084/m9.figshare.1539647) **B** Head and mesosoma, lateral view (see http://dx.doi.org/10.6084/m9.figshare.1539646).

Figure 6. Bright field images of *Conostigmus albovarius* (Dodd, 1915) male genitalia. **A** Dorsal view (see http://dx.doi.org/10.6084/m9.figshare.1539648) **B** Ventral view (see http://dx.doi.org/10.6084/m9.figshare.1539649).

C. albovarius, in contrast, exhibits foveolate sculpturing. Our definition of foveolate sculpturing is based on Harris (1979), where the cuticle is divided into irregular pits with raised edges and a single seta is present at the center of each pit. In females, foveolate sculpturing is present on the head and mesosoma, while in males the foveolate sculpturing is only present on the frons. In females, the areas with foveolate sculpturing are also present with a metallic coloration ranging from a bronze sheen to a deep iridescence (Fig. 4). This metallic coloration is absent from males.

In comparing the antennae of different female specimens, it was observed that there was variation in the coloration of the apical flagellomeres (Fig. 1). Whereas F9 is always melanized and F1 through F4 always have transparent cuticle (it appears white because of the soft tissue, e.g. fat bodies and muscles, underneath), F5 through F8 vary

in whether melanization is present or not. When melanization is present, it is always present in the apical flagellomeres after the melanized flagellomere, such that if F5 is melanized, then F6–9 will also be melanized. It is unclear whether this intraspecific phenotypic variation in color is influenced by genetic or environmental factors, such as temperature (Quicke 1997). Females from different sampling events in different areas sometimes shared the same pattern of melanization on the antennae, though females collected from the same sampling event sometimes had different patterns.

It is known that the antennae play important roles in the courtship of parasitic wasps in general (Ayasse et al. 2001; Romani 2008). In *Cotesia rubecula* (Hymenoptera: Braconidae), females use their antennae to signal their receptivity to the males (Field and Keller 1993). It is possible that the melanization seen in female *C. albovarius* antennae could be used for visual signaling to males during courtship.

Author contributions

Conceived the project: IM. Character concept generation, semantic statement generation, specimen visualization and creation of plates: CT, IM. Specimen measurements: CT. Wrote the manuscript: CT. Commented on the final stage of the manuscript: ARD, IM.

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References

Ayasse M, Paxton RJ, Tengö J (2001) Mating behavior and chemical communication in the order Hymenoptera. Annual Review of Entomology 46(1): 31–78. doi: 10.1146/annurev.ento.46.1.31

Balhoff JP, Mikó I, Yoder MJ, Mullins PL, Deans AR (2013) A semantic model for species description, applied to the ensign wasps (Hymenoptera: Evaniidae) of New Caledonia. Systematic Biology 62(5): 639–659. doi: 10.1093/sysbio/syt028

Bijoy MC, Rajmohana K, Kumar R (2014) First report of the genus *Conostigmus* Dahlbom (Hymenoptera: Ceraphronoidea: Megaspilidae) from India with description of a new species. Biodiversity Data Journal 2: e991. doi: 10.3897/BDJ.2.e991

Broad GR, Livermore L (2014) Checklist of British and Irish Hymenoptera-Ceraphronoidea. Biodiversity Data Journal 2: e1167. doi: 10.3897/BDJ.2.e1167

Deans AR, Yoder MJ, Balhoff JP (2012) Time to change how we describe biodiversity. Trends in Ecology and Evolution 27: 78–84. doi: 10.1016/j.tree.2011.11.007

Dessart P (1967) Description de *Dendrocerus* (*Macrostigma*) *noumeae* sp. nov. de Nouvelle Calédonie [Ceraphronoidea Megaspilidae]. Entomophaga 12(4): 343–349. doi: 10.1007/BF02376920

Dessart P (1972) Revision des especes europeenes du genre *Dendrocerus* Ratzeburg, 1852 (Hymenoptera Ceraphronoidea). Memoires de la Société Royale Belge d'Entomologie 32: 310.

Dessart P (1992) Revision d'*Aphanogmus fulmeki* Szelényi, 1940 (Hymenoptera, Ceraphronoidea, Ceraphronidae) avec remarques biologiques. Bulletin de l'Institute Royal des Sciences Naturelles de Belgique 62: 83–91.

Dessart P (1995) Quelques considerations morphologiques pour l'etude des Hymenopteres Ceraphronoidea. Bulletin et Annales de la Societe Royale Belge d'Entomologie 131: 319–326.

Dessart P (1997) Les Megaspilinae ni européens, ni américains 1. Le genre *Conostigmus* Dahlbom, 1858 (Hym. Ceraphronoidea Megaspilidae). Mémoires de la Société Royale Belge d'Entomologie 37: 3–144.

Dodd AP (1915) Australian Hymenoptera Proctotrypoidea. No. 3. Transactions and Proceedings of the Royal Society of South Australia 39: 384–454.

Dodd AP (1916) Australian Hymenoptera Proctotrypoidea. No. 4. Transactions and Proceedings of the Royal Society of South Australia 40: 9–32.

Field SA, Keller MA (1993) Courtship and intersexual signaling in the parasitic wasp *Cotesia rubecula* (Hymenoptera: Braconidae). Journal of Insect Behavior 6(6): 737–750. doi: 10.1007/BF01201673

Goulet H, Huber JT (1993) Hymenoptera of the world: an identification guide to families. Research Branch, Agriculture Canada.

Graham MDV (1984) A new species of *Conostigmus* (Insecta: Hymenoptera, Ceraphronoidea) from Madeira. Bocagiana 77: 1–5. http://publications.cm-funchal.pt/handle/100/1487

Harris RA (1979) A glossary of surface sculpturing. Occasional papers in entomology/Laboratory Services, Division of Plant Industry, California Department of Food and Agriculture 28: 1–31. http://agris.fao.org/agris-search/search.do?recordID=US201300583954

Johnson NF, Musetti L (2004) Catalog of systematic literature of the superfamily Ceraphronoidea (Hymenoptera). Contributions of the American Entomological Institute 33(2): 59–83.

Kamal M (1926) Four new species of parasites from aphidophagous Syrphidae (Hymenoptera). The Canadian Entomologist 58 (11): 283–286. doi: 10.4039/Ent58283-11

Krell FT (1994) Phylogenetic and taxonomic considerations on the variability of cuticular surface micromorphology within one species, *Aphodius* (*Nialus*) *varians* Duftschmid (Insecta:

Coleóptera: Scarabaeidae: Aphodiinae). Revue suisse de Zoologie 101(1): 265–287. doi: 10.5962/bhl.part.79907

Mikó I, Deans AR (2009) *Masner*, a new genus of Ceraphronidae (Hymenoptera, Ceraphronoidea) described. Advances in the Systematics of Hymenoptera.: Festschrift in honour of Lubomír Masner. ZooKeys 20: 127–153. doi: 10.3897/zookeys.20.119

Mikó I, Masner L, Johannes E, Yoder MJ, Deans AR (2013) Male terminalia of Ceraphronoidea: morphological diversity in an otherwise monotonous taxon. Insect Systematics & Evolution 44(3–4): 261–347. doi: 10.1163/1876312X-04402002

Mikó I, Copeland RS, Balhoff JP, Yoder MJ, Deans AR (2014) Folding Wings like a Cockroach: A review of transverse wing folding ensign wasps (Hymenoptera: Evaniidae: *Afrevania* and *Trissevania*). PLoS ONE 9(5): e94056. doi: 10.1371/journal.pone.0094056

Mikó I, Yoder MJ, Balhoff JP, Deans AR (2015) Generating semantic phenotypes. Figshare, 9 pp. doi: 10.6084/m9.figshare.1314904

Quicke DL (1997) Parasitic wasps. Chapman & Hall Ltd, 470 pp.

Romani R, Rosi MC, Isidoro N, Bin F (2008) The role of the antennae during courtship behaviour in the parasitic wasp *Trichopria drosophilae*. Journal of Experimental Biology 211(15): 2486–2491. doi: 10.1242/jeb.022400

Schaffner JV (1959) Microlepidoptera and their parasites reared from field collections in the Northeastern United States. US Government Printing Office. doi: 10.5962/bhl.title.7899

Vidal S (2003) Identification of Hymenopterous parasitoids associated with oilseed rape pests. Biocontrol of Oilseed Rape Pests, 161–180. doi: 10.1002/9780470750988.ch11

Xiang Z, Mungall C, Ruttenberg A, He Y (2011) Ontobee: A Linked Data Server and Browser for Ontology Terms. Proceedings of the 2nd International Conference on Biomedical Ontologies (ICBO), July 28–30, 2011, Buffalo, NY, 279–281. http://ceur-ws.org/Vol-833/paper48.pdf

Yoder MJ, Miko I, Seltmann KC, Bertone MA, Deans AR (2010) A gross anatomy ontology for Hymenoptera. PLoS ONE 5(12): e15991. doi: 10.1371/journal.pone.0015991

Supplementary material 1

Semantic representation of phenotypic character states

Authors: Carolyn Trietsch, Andrew R. Deans, Istvàn Mikó

Data type: Table

Explanation note: A complete list of the phenotypic character states used and their corresponding semantic representations.

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Developing a paired-target apparatus for quantitative testing of nest defense behavior by vespine wasps in response to con- or heterospecific nest defense pheromones

Sean McCann[1], Onour Moeri[1], Sebastian Ibarra Jimenez[1], Catherine Scott[1], Gerhard Gries[1]

1 *Simon Fraser University, Department of Biological Sciences, 8888 University Dr., Burnaby, BC, Canada V5A 1S6*

Corresponding author: *Sean McCann* (smmccann@sfu.ca)

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Abstract

Social wasps commonly exhibit impressive, pheromone-mediated nest defenses with stinging attacks on potential vertebrate nest predators. Studying this type of nest defense and comparing results across studies is challenging because there is no standardized method for quantifying defense intensities. For that reason, we developed a simple, paired-target apparatus coupled with easy and inexpensive data recording and analysis technologies. Each target is formed by two conjoined black plastic weigh boats that generate distinct percussive sounds when struck by attacking wasps. A battery-powered microphone inside each target converts the sounds into electrical signals that are transferred to a digital audio recorder. These audio files are then split into left- and right-channel files, saved as 16-bit WAV files, and the strikes to each target are counted using the open-source software SoundRuler. Using this apparatus, we show that workers of *Vespula pensylvanica*, *V. alascensis*, and *V. germanica* strike targets that are treated with conspecific venom sac extract more frequently than paired control targets. We also show that workers of *V. alascensis*, *V. pensylvanica* and *V. germanica* strike targets that are treated with heterospecific extracts more frequently than paired control targets, indicating that the wasps recognize nest alarm pheromones from congeners. These data provide evidence for conserved nest defense pheromones among some *Vespula* wasps and proof of concept that our technology is capable of quantifying the intensity of pheromone-mediated nest defense behavior in *Vespula* and other large and formidable social wasps.

Keywords
Vespula, yellowjackets, alarm pheromone, defense

Introduction

Nest defense is an integral life history trait of social insects. Many social wasps and bees are capable of coordinated, massed stinging attacks against potential vertebrate nest predators. During nest defense, large numbers of workers are mobilized and engage in stinging, biting or venom spraying to dissuade potential nest predators (Smith et al. 2001). These defense tactics are trademarks of many vespine wasps and have earned them a fearsome reputation.

Nest defense is often coordinated by alarm and marker pheromones released by worker wasps. The alarm pheromone recruits nest mates out of the nest, and the marker pheromone that is deposited on potential predators directs the attacking workers toward them (Verheggen et al. 2010). In social insects with autotomizing stings, the (barbed) stinger and its associated venom sac becomes severed from the stinging insect and then remain attached to the host. If the venom sac contains marker pheromone, the stung predator is effectively marked for attack (Hermann 1971, Overal et al. 1981, Mulfinger et al. 1992). In other social species, such as hornets (*Vespa*) and some yellowjackets (*Vespula*), alarm and marker pheromones are present in the venom sac, and may be deposited on a predator's skin during stinging or venom-spraying (Veith et al. 1984, Landolt et al. 1995).

Pheromone-mediated nest defense seems to be widespread in vespines (Bruschini et al. 2010). Several vespines reportedly orient toward nest defense pheromones extracted from the venom sac of conspecific workers, and attack targets treated with such extract at a greater rate than similar untreated targets (Landolt et al. 1995). With southern yellowjackets, *V. squamosa* (Drury), and eastern yellowjackets, *V. maculifrons* (Buysson, 1905), sharing at least one alarm pheromone component [3-methyl butylacetamide (Heath and Landolt 1988, Landolt et al. 1995)], it is conceivable that *V. squamosa* and *V. maculifrons* recognize and respond to each other's alarm pheromones. The ability to recognize heterospecific nest defense pheromones may be adaptive in that worker wasps may recognize the approach of a wasp nest predator marked with heterospecific alarm pheromone and then quickly stage a defense even before the nest has come under attack.

Studying nest defense of vespines is destined to reveal complex and intricate communication systems, but these studies are challenging in that nest defense is difficult to quantify. Moreover, results of previous studies are difficult to compare because they were obtained using rather different recording technologies and experimental designs. Our objectives were (1) to design an apparatus for quantifying nest defense behavior, and (2) to test experimentally whether *Vespula* workers respond to nest defense pheromones from both con- and heterospecifics.

Materials and methods

Experimental insects

We worked with western yellowjackets, *Vespula pensylvanica* (de Saussure), common yellowjackets, *V. alascensis* Packard, and German yellowjackets, *V. germanica* (Fabricius) which are the most prevalent of the ground nesting vespines in suburban British Columbia (BC). All three species nest either underground or in cavities (Boieeie 1983). Workers hunt small prey and scavenge for carrion to feed larval brood in their nests. The habit of scavenging makes workers very evident in late summer, when they enter yards and dwellings in search of food.

The experimental nests we studied in behavioral tests were located near Vancouver in the municipalities of Langley, Burnaby, and Richmond. We sourced workers for pheromone extraction at separate nests in these municipalities.

Extraction of venom sacs

We captured worker wasps emerging from their nests by placing a 4-litre glass jar with a steel-mesh cone over the nest entrance. We immediately killed and froze captured wasps by crumbling powdered dry ice into the capture jar, emptied the frozen wasps into polyethylene bags in an icebox, and transported them back to the laboratory for dissection. We excised their venom sacs, placed them in acetonitrile, macerated the tissue with a clean metal rod, and filtered the extract through glass wool to remove tissue fragments. We kept extracts frozen at a concentration of one venom sac per 10 µl, and transported extracts in an ice chest to the field for testing.

Design of the paired-target apparatus

The design of our paired-target apparatus and its recording technology was inspired by Visscher and Vetter (1995) who engineered a device for counting strikes against a target using microphones as transducers. Unlike Visscher and Vetter (1995), we did not use an electronic counter but instead recorded audio files and analyzed them later with automated counting software. We designed our apparatus so that it (*i*) required only inexpensive parts for assembly, (*ii*) recorded two channels, and (*iii*) allowed data analyses with open-source software. The paired design was inspired by Visscher and Vetter (1995), who built a paired apparatus, but never used it, and suggested it for alarm pheromone studies.

The paired-target, tripod-mounted apparatus consists of a crossbar supporting two targets separated by 1 m (Fig. 1a). The crossbar bearing the microphones is mounted to the quick-release plate of the tripod which is placed near the entrance of a *Vespula* nest

Figure 1. Graphical illustration (**a**) and photograph (**b**) of the paired-target apparatus and the recording technology deployed to bioassay pheromone-mediated nest defense in *Vespula* wasps. In b, the apparatus is placed near the entrance of a subterranean vespine nest.

(Fig. 1b), with the two targets equidistant to the nest entrance. Each of the two targets is formed by two black plastic weigh boats (13.97 cm^2; Big Science Inc., Huntersville, NC, USA) that are conjoined with adhesive tape to act as a percussive medium, generating distinct sounds when struck by attacking wasps. Each target houses a Sony ECM-T6 tie-clip microphone (Sony Corporation, NY, NY, USA) powered by lithium watch cells (Fig. 1). Each of the two microphones is connected to a stereo digital audio recorder (Edirol R-09 HR, Roland Canada Ltd., Richmond, BC, Canada), with the microphone leads secured by tape along the crossbar, and fed into a two-mono-to-one stereo adapter plug. A stereo extension cable with a 3.5-mm jack extends the stereo signal cable to a convenient and safe distance from the nest for monitoring the recording event. Higher-quality shielded cables may be necessary depending on the length of the cable run and the ambient RF environment. For recording the signal from the microphones, we used an Edirol R-09 HC (Roland Corporation, Richmond BC) stereo field recorder. Other recorders may be used, but it is crucial that the two channels not be mixed in the final stereo signal.

General experimental design for testing nest defense

We studied nest defense behaviour with nests of *Vespula pensylvanica* in August 2010, and with nests of *V. alascensis* and *V. germanica* in August and September of 2011 and 2012. Invariably, we wore bee suits and veils, and retreated after disturbing a nest. We placed the paired-target apparatus 1 m from a nest entrance and recorded the wasps' responses for 1 min. We then disturbed the nest by tapping the nest entrance three times with a stick and recorded for 9 min. We repeated the test with new plastic targets, alternating the left-right position of treatment and control targets (see Supplemental Video 1).

Specific experiments

Exp. 1: Effect of target color on wasp responses

To assess the suitability of our paired-target apparatus and its recording technology for quantifying nest defense responses by vespines, we repeated the experiment by Visscher and Vetter (1995), using target color as the test variable, and a paired design. We worked with nests of *Vespula pensylvanica* following the general bioassay design, alternating the position of a white target and a black target on the paired-target apparatus.

Expts. 2–10: Effect of nest defense pheromones on wasp responses

We ran nest defense pheromone experiments the same way as the color experiment, except that (*i*) both targets of the apparatus were black, and (*ii*) and one target was treated with venom sac extract, hereafter "VSE" (at 5 venom sac equivalents), the other

with an equivalent amount of acetonitrile (50 µl) . Rather than randomly assigning the treatment and control stimulus to the left or right target in each test, we alternated their position between replicates, thus avoiding the possibility of a side bias.

In experiments 2, 5, 8 and 9, we tested the response of nest mates to VSE of conspecific workers (Exp. 2: *Vespula pensylvanica*; Exp. 5: *V. alascensis*; Expts. 8, 9: *V. germanica*) (Table 1), predicting that the target treated with VSE would receive a greater number of strikes by wasps than the solvent control target. In experiments 3, 4, 6, 7 and 10, we tested the response of nest mates to VSE of heterospecific workers. Specifically, we tested (*i*) responses of *V. pensylvanica* nests to VSE of *V. alascensis* workers (Exp. 3) and to VSE of *V. germanica* workers (Exp. 4), (*ii*) responses of *V. alascensis nests* to VSE of *V. pensylvanica* workers (Exp. 6) and to VSE of *V. germanica* workers (Exp. 7), and (*iii*) responses of *V. germanica* nests to VSE of *V. pensylvanica* workers.

Audio file processing and counting

For each replicate, we used Audacity (Audacity Team) to split the audio file into a left and right channel, and saved them as mono 16-bit WAV files under appropriate filenames. We then opened each file in SoundRuler (Gridi-Papp 2007), a free and open-source audio analysis software. Opening only the first 4 min of each recording, we analyzed the strikes against the target that show as sharp pulses and that correspond to percussive sounds as wasps struck the target (Fig. 2). We counted percussions using the "call recognition" ability and "auto" function of SoundRuler, and saving results as CSV text files. Filtering sounds above 900 Hz with the software's bandpass filter, we improved the signal-to-noise ratio of percussive strikes. We set strike recognition parameters as follows: amplitude peak: 1.1 ± 0.7 Pa; duration: 50 ± 0.4 ms; and interval: 500 ± 0.4 ms (see Appendix). Our settings file is available for download as Supplemental File 1 (see Data Resources). For all experiments except 9 and 10, we set the Soundruler to amplify the source file by 300%. For data analyses of experiments 9 and 10 which we ran in a noisy suburban construction setting, we set SoundRuler to amplify the source file by only 100%, thus avoiding automated counts of extraneous noise. As each file in any experimental series was assigned a unique name, it could be analyzed in one session of SoundRuler, parsing detailed results later using spreadsheet software.

Statistical analyses

Because our protocol produced paired data, we compared proportions of strikes on treatment and control targets in each replicate. In initial tests, we found a high variation in the total number of strikes on treatment and control targets between replicates, but the proportions of strikes on treatment targets was almost invariably higher, so a treatment effect became evident as a higher proportion of strikes on the treatment target than on the control target. We used a Wilcoxon Signed Rank test to determine whether the proportion of strikes on the treatment target differed from 0.5, and we

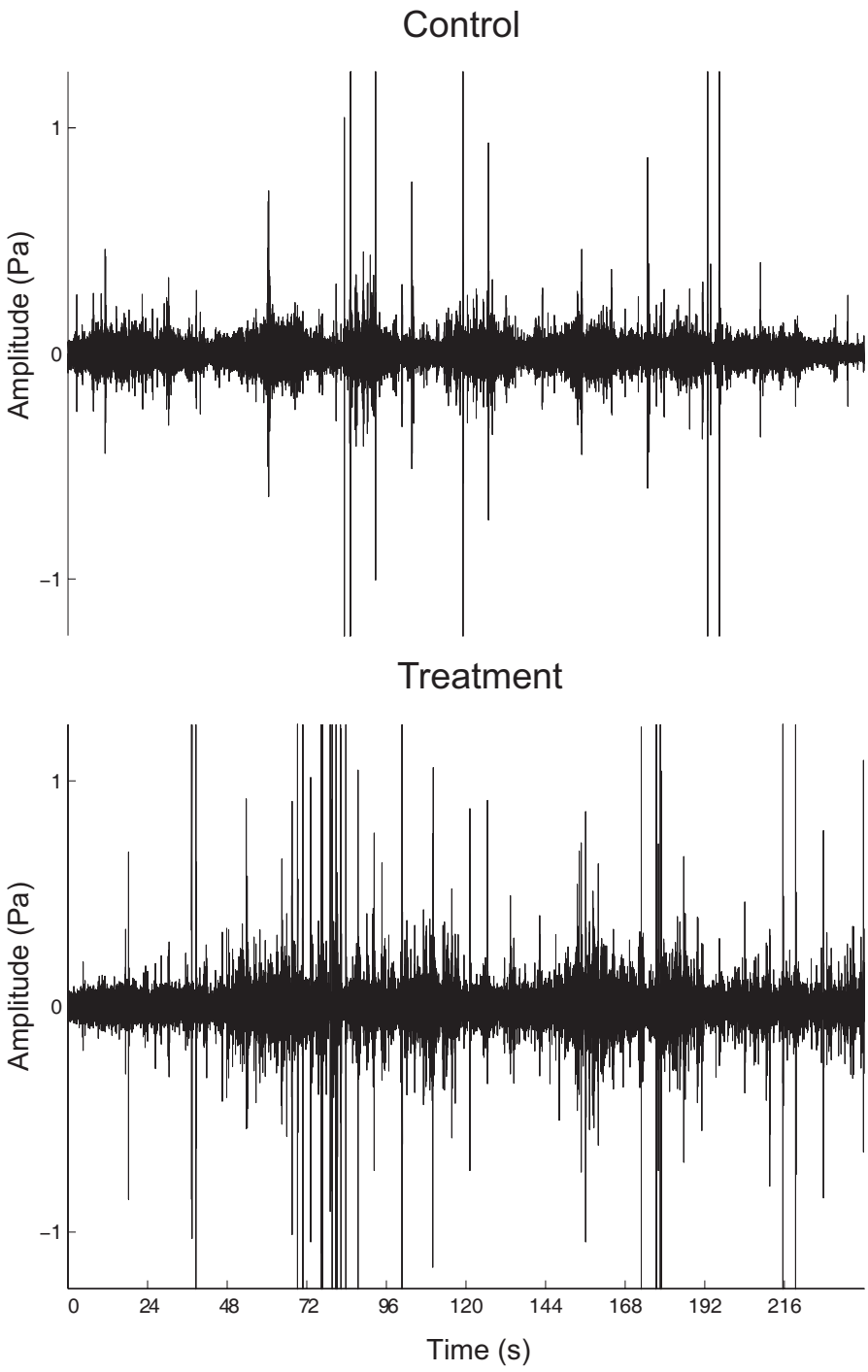

Figure 2. Representative example of paired oscillograms (obtained during replicate 6 of Exp. 2), depicting strikes caused by wasps hitting the control and treatment target of the bioassay apparatus (Fig. 1). Venom gland extract of worker wasps and acetonitrile at equivalent amount as a solvent control were applied to the treatment and control target, respectively (see methods for details).

also report the results of parametric paired T-tests for the same data. The more conservative Wilcoxon test has lower power because it uses ranks and discards ties, however, in all but one experiment the results agreed with those of the parametric tests.

Data resources

The audio data underpinning the analyses reported in this paper, as well as supplemental videos, figures and a SoundRuler settings file, are deposited at http://figshare.com at http://dx.doi.org/10.6084/m9.figshare.1581525

Results

Exp. 1: Effect of target color on wasp responses

Worker wasps of *Vespula pensylvanica* nests disproportionately struck black targets more often than white targets (Fig. 3, Table 1). The mean (± SE) number of strikes on black and white targets was 16.2 (± 12.75) and 0.1 (± 0.31), respectively, revealing a significant effect of target color on the wasps' responses.

Exps. 2–10: Effect of nest defense pheromones on wasp responses

Worker wasps of *Vespula pensylvanica*, *V. alascensis* and *V. germanica* nests struck targets treated with VSE of conspecific workers at a greater rate than control targets (Fig. 3, Table 1; Exp. 2: $P>0.05$; Exps. 5, 8, 9: $P<0.05$), indicating recognition of nest defense pheromone on treatment targets. We attribute the lack of statistical significance in experiment 2 to a lower than usual number of replicates (9 instead of 10 or 12; Table 1).

There was also recognition of nest defense pheromones from heterospecifics, as evident by nest mates striking targets treated with VSE of heterospecific workers at a greater rate than control targets. We demonstrated this phenomenon for (*i*) workers of *Vespula pensylvanica* nests responding to VSE of *V. alascensis* or *V. germanica* (Fig. 3, Table 1; Expts. 3, 4), (*ii*) workers of *V. alascensis* nests responding VSE of *V. pensylvanica* or *V. germanica* (Fig. 3, Table 1; Expts. 6, 7), and (*iii*) workers of *V. germanica* nests responding to VSE of *V. pensylvanica* workers (Fig. 3, Table 1; Expt. 10).

Discussion

Our experimental data coupled with personal observations in field experiments indicate that the paired-target apparatus meets all the criteria to effectively quantify nest defense behavior by vespine wasps in response to nest defense pheromones.

Table 1. The effect of color of two paired targets (Fig. 1), and of venom sac extract (VSE) or acetonitrile (CH_3CN) solvent applied to targets, on the number of strikes (mean ± SE) by *Vespula* (*V*) congeners in nest defense experiments (see text for details).

			Color stimulus		Olfactory stimulus					P < 0.05	
Exp.	**Date**	**n**	**S1**	**S2**	**S1**	**S2**	**Species tested**	**Strikes on S1**	**Strikes on S2**	**t-test**	**WSR[3]**
1	21Aug10	10	Black	White	None	None	*V. pensylvanica*	16.2 ± 12.75	0.1 ± 0.31	Y	Y
2	22Aug10	9	Black	Black	VSE[1] of *V. pensylvanica*	CH_3CN[2]	*V. pensylvanica*	70.7 ± 33.9	51.3 ± 46.4	N	N
3	7Sep11	10	Black	Black	VSE of *V. alascensis*	CH_3CN	*V. pensylvanica*	12.0 ± 11.1	3.5 ± 5.5	Y	Y
4	13Sep11	12	Black	Black	VSE of *V. germanica*	CH_3CN	*V. pensylvanica*	28.8 ± 20.5	10.2 ± 10.6	Y	Y
5	20Sep11	12	Black	Black	*V*SE of *V. alascensis*	CH_3CN	*V. alascensis*	122.1 ± 133.3	43.3 ± 120.8	Y	Y
6	12Sep11	10	Black	Black	VSE of *V. pensylvanica*	CH_3CN	*V. alascensis*	143.6 ± 152.6	18.0 ± 18.1	Y	Y
7	12Sep11	10	Black	Black	VSE of *V. germanica*	CH_3CN	*V. alascensis*	46.1 ± 48.2	6.6 ± 9.9	Y	Y
8	12Sep11	12	Black	Black	VSE of *V. germanica*	CH_3CN	*V. germanica*	24.8 ± 26.1	8.0 ± 11.2	Y	N
9	13Sep12	10	Black	Black	VSE of *V. germanica*	CH_3CN	*V. germanica*	6.1 ± 3.2	1.4 ± 1.1	Y	Y
10	9Sep11	12	Black	Black	VSE of *V. pensylvanica*	CH_3CN	*V. germanica*	31.2 ± 23.0	13.8 ± 9.5	Y	Y

[1] Venom sac extract (VSE) of 5 worker wasps in 50 µl of acetonitrile (CH_3CN)

[2] 50 µl of acetonitrile (CH_3CN)

[3] Wilcoxon Signed Rank test

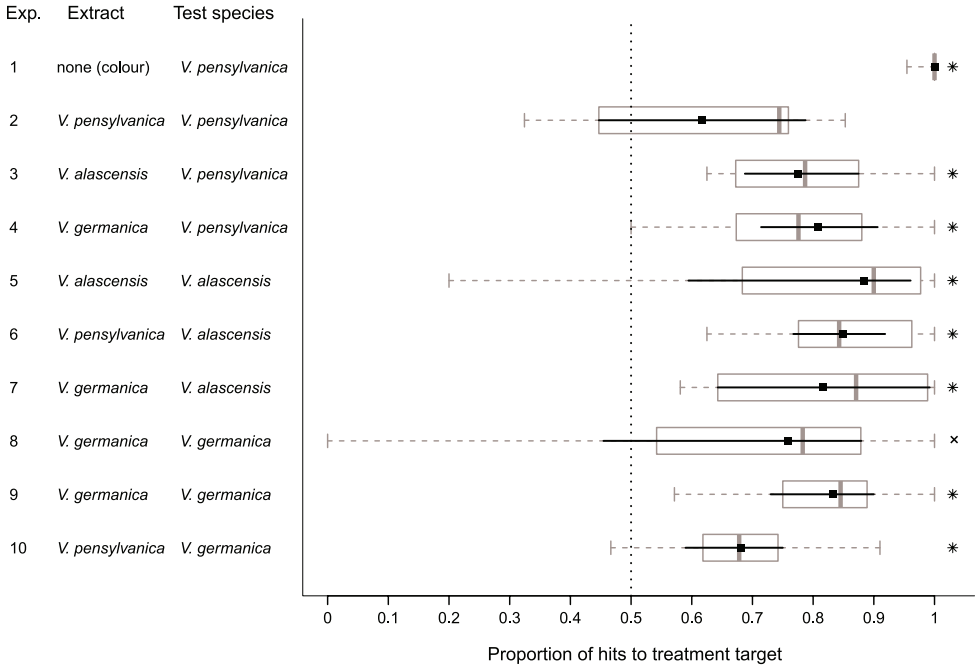

Figure 3. Proportion of strikes by various *Vespula* wasps on white or black targets in color discrimination experiment 1, and on pheromone-treated or control targets in experiments 2-10. In all experiments except 1, the control target was treated with acetonitrile. Gray boxplots show the medians (vertical lines), interquartile ranges (boxes), and ranges (whiskers). Pseudomedians and 95% confidence intervals are shown in black. Asterisks indicate pseudomedians and means that are significantly different from 0.5 using Wilcoxon Signed Rank tests and t-tests, respectively. The X in experiment 8 indicates that only the mean number of strikes on the treated target in was significantly different from 0.5.

The apparatus is assembled from inexpensive parts, its light weight facilitates transport to and from test sites, and the tripod-mount with height adjustment of the paired targets allows easy placement in uneven terrain. The conjoined plastic weigh boats serving as paired targets have surprisingly good resonant properties, thus facilitating recordings of the percussive sounds when they are struck by attacking wasps, with each strike becoming a quantifiable data point. The weigh boat targets are easily treated with test stimuli and can be readily replaced between replicates, thus avoiding the need to repeatedly clean the apparatus in a series of trials. The microphone and the digital audio recorder were sufficiently sensitive to record the wasps' strikes on targets, and "band-pass filtering" further improved the signal-to-noise ratio of these strikes. As a result, the number of strikes could be accurately counted by a software program (Sound Ruler), provided that the strike recognition parameters (amplitude, duration and inter-strike intervals) were finely tuned. Because the microphones also picked up sounds from a nearby construction site, it is advisable though to seek nests in quiet settings for data recording.

Automated counting of strikes has the advantage of expedient data processing, which is helpful when quantitative data are needed to decide on the composition of

test stimuli in follow-up experiments. The ability to run multiple sets of experiments in rapid succession is particularly critical in nest-defense pheromone research, where often many experiments are required to unravel the composition of complex pheromone blends (Veith et al. 1984, Dani et al. 2000, Wager and Breed 2000, Fortunato et al. 2004, Ibarra et al., unpubl.), and where research progress is tied each year to the few weeks during which vespine nests are in defense mode.

Exposing nests to paired rather than single targets provided the option to compare and analyze proportions, instead of absolute numbers, of strikes on treatment and control targets. This option proved valuable because a nest's propensity to defend in response to a test stimulus varied between days or replicates, a fact that renders the absolute number of strikes as an assessment criterion for the potency of a test stimulus more difficult to interpret.

Our data support evidence for the presence of nest defense pheromones in *Vespula pensylvanica*, *V. alascensis* and *V. germanica* (Fig. 3, Table 1, Exp. 2, 5, 8, 9). Alarm pheromone activity has previously only been noted for one of these species, *Vespula germanica* (Maschwitz 1964). Much greater rates of attack by *V. pensylvanica* workers on black targets than on white targets (Fig. 3, Table 1, Exp. 1) support similar results from a previous study (Visscher and Vetter 1995), and suggest that vespines in nest-defense mode respond to visual cues associated with potential nest predators.

Intriguingly, our data also provide evidence that vespines respond not only to their own nest defense pheromones but also to those of heterospecifics. Workers of *Vespula alascensis*, *V. germanica* and *V. pensylvanica* all struck targets treated with VSE of heterospecifics more frequently than paired control targets (Fig. 4, Table 1), indicating that they recognize nest defense pheromones from congeners. The underlying mechanisms are likely one or more pheromone components that are shared between congeners. *N*-3-methylbutylacetamide, for example, is an alarm pheromone component of both southern yellowjackets, *V. squamosa* (Drury), and eastern yellowjackets, *V. maculifrons* (Buysson). There are also common acetamides in VSEs of *V. alascencis*, *V. germanica* and *V. pensylvanica* (McCann et al., unpubl. data), one or more of which may have a nest defense pheromone function and may have contributed to the emerging evidence for conserved alarm pheromone chemistry in *Vespula* (Fig. 3, Table 1).

Each species will likely respond most vigorously to its own nest defense pheromone, because the alarm message is released by nest mates when they sense an immediate threat to the nest and when concerted defense by nest-mates is needed to protect the nest's offspring. Nonetheless, the recognition of nest defense pheromones from *Vespula* congeners seems advantageous because congeners sometimes nest in close proximity. If a nest were to be attacked by a vertebrate predator, and marked with nest defense pheromone while being stung by defending nest mates, then this "marked" predator could be sensed from a distance by worker wasps of a congener nest allowing nest mates to stage a defense well before the predator has even reached the nest and initiated an attack.

In conclusion, we have described a paired-target apparatus that facilitates the quantification of pheromone-mediated nest defense behavior by vespine wasps, and provide evidence that some *Vespula* species respond to nest defense pheromones of both con- and heterospecifics. This work provides the means and incentive to study this phenomenon more closely and to chemically identify the defense pheromones of *Vespula* species.

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References

Boieeie WO (1983) Control of yellowjackets (Hymenoptera: Vespidae) in British Columbia. MPM Thesis. Simon Fraser University, Burnaby, British Columbia.

Bruschini C, Cervo R, Turillazzi S (2010) Pheromones in social wasps. In: Vitamins and Hormones. Elsevier Inc., 447–492. doi: 10.1016/S0083-6729(10)83019-5

Dani FR, Jeanne RL, Clarke SR, Jones GR, Morgan ED, Francke W, Turillazzi S (2000) Chemical characterization of the alarm pheromone in the venom of *Polybia occidentalis* and of volatiles from the venom of *P. sericea*. Physiological Entomology 25: 363–369. doi: 10.1111/j.1365-3032.2000.00205.x

Fortunato A, Dani FR, Sledge MF, Fondelli L, Turillazzi S (2004) Alarm communication in *Ropalidia* social wasps. Insectes Sociaux 51: 299–305. doi: 10.1007/s00040-004-0744-2

Gridi-Papp M (2007) SoundRuler: Acoustic Analysis for Research and Teaching. http://soundruler.sourceforge.net

Heath RR, Landolt PJ (1988) The isolation, identification and synthesis of the alarm pheromone of *Vespula squamosa* (Drury) (Hymenoptera: Vespidae) and associated behavior. Experientia 44: 82–83. doi: 10.1007/bf01960257

Hermann HH (1971) Sting autotomy, a defensive mechanism in certain social Hymenoptera. Insectes Sociaux 18: 111–120. doi: 10.1007/BF02223116

Landolt PJ, Heath RR, Reed HC, Manning K (1995) Pheromonal mediation of alarm in the eastern yellowjacket (Hymenoptera: Vespidae). The Florida Entomologist 78: 101–108. doi: 10.2307/3495673

Maschwitz UW (1964) Alarm substances and alarm behaviour in social Hymenoptera. Nature 204: 324–327. doi: 10.1038/204324a0

Mulfinger L, Yunginger J, Styer W, Guralnick M, Lintner T (1992) Sting morphology and frequency of sting autotomy among medically important vespids (Hymenoptera: Vespidae) and the honey bee (Hymenoptera: Apidae). Journal of Medical Entomology 29: 325–328. doi: 10.1093/jmedent/29.2.325

Overal W, Simoes D, Gobbi N (1981) Colony defense and sting autotomy in *Polybia rejecta* (F)(Hymenoptera: Vespidae). Revista Brasiliera de Entomologia 25: 41–47.

Smith AR, Donnell SO, Jeanne RL (2001) Correlated evolution of colony defence and social structure: A comparative analysis in eusocial wasps (Hymenoptera: Vespidae). Evolutionary Ecology Research 3: 331–344.

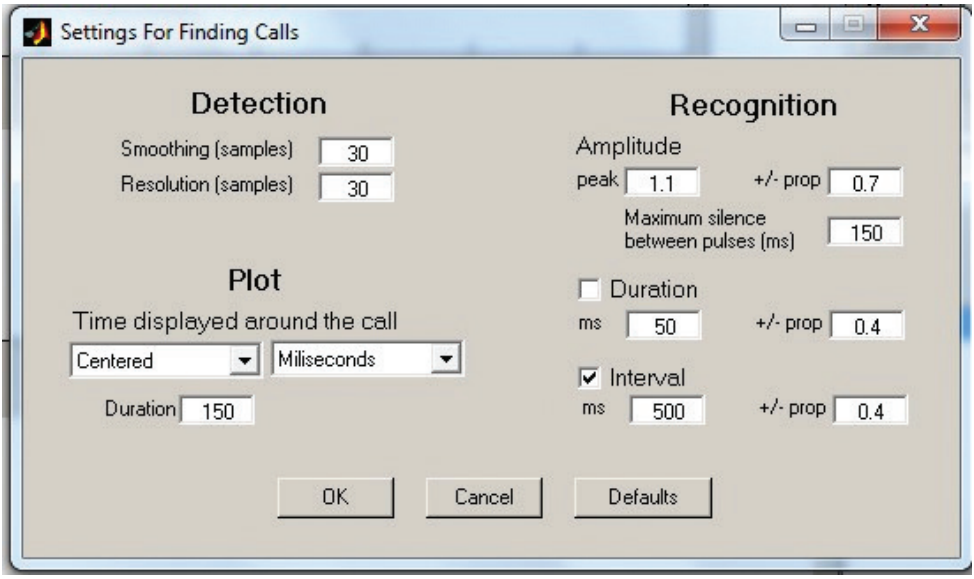
Veith HJ, Koeniger N, Maschwitz U (1984) 2-Methyl-3-butene-2-ol, a major component of the alarm pheromone of the hornet *Vespa crabro*. Naturwissenschaften, 328–329. doi: 10.1007/bf00396622

Verheggen FJ, Haubruge E, Mescher MC (2010) Alarm pheromones-chemical signaling in response to danger. Vitamins and hormones 83: 215–39. doi: 10.1016/S0083-6729(10)83009-2

Visscher PK, Vetter RS (1995) Smoke and target color effects on defensive behavior in yellowjacket wasps and bumble bees (Hymenoptera: Vespidae, Apidae) with a description of an electronic attack monitor. Journal of Economic Entomology 88: 579–583. doi: 10.1093/jee/88.3.579

Wager B, Breed M (2000) Does honey bee sting alarm pheromone give orientation information to defensive bees? Annals of the Entomological Society of America 93: 1329–1332. doi: 10.1603/0013-8746(2000)093[1329:DHBSAP]2.0.CO;2

Apppendix



Call recognition parameters used in SoundRuler software for detecting and counting wasp strikes against plastic targets. In addition to these settings, we also set a bandpass filter of 900 Hz.

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SHORT COMMUNICATION

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Paracyphononyx scapulatus (Hymenoptera, Pompilidae), a koinobiont ectoparasitoid of *Trochosa* sp. (Araneae, Lycosidae)

Hebert da Silva Souza[1], Yuri Fanchini Messas[1], Fabiana Masago[2], Eduardo Fernando dos Santos[3], João Vasconcellos-Neto[1]

1 *Universidade Estadual de Campinas, Instituto de Biologia, Departamento de Biologia Animal, Rua Monteiro Lobato, 255, Campinas, São Paulo, Brazil* **2** *Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências, Departamento de Farmacologia, Distrito de Rubião Júnior, s/n, Botucatu, São Paulo, Brazil* **3** *Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências, Letras e Ciências Exatas, Departamento de Biologia Animal, Rua Cristóvão Colombo, 2265, São José do Rio Preto, São Paulo, Brazi*

Corresponding author: *Hebert da Silva Souza* (hssouza.bio@gmail.com)

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Abstract

The genus *Paracyphononyx* Gribodo, 1884 (Pompilidae) contains species that act as koinobiont parasitoids of cursorial spiders. Here, we record a new parasitism interaction involving the pompilid wasp *Paracyphononyx scapulatus* (Bréthes) and the hunter spider *Trochosa* sp. (Lycosidae), and we describe how the wasp develops on the spider. This study contributes new information about the interaction between koinobiont ectoparasitoid wasps and spiders, which probably arose independently in different groups of wasps.

Keywords

Atlantic forest, Lycosidae, Hunter spider, Larval development, Parasitoid wasp

Introduction

The Pompilidae family contains about 5000 known wasp species worldwide. In the Neotropical region (Central and South America), around 1000 species have been described, belonging to 60 genera and five subfamilies (Pitts et al. 2006). In North America, 282 species have been documented in detail, belonging to 40 genera (Goulet and Huber 1993). In South America, this family has been studied in Brazil, Argentina, Uruguay, Paraguay, and Chile (Banks 1946, 1947, Bradley 1944) and, recent studies are being conducted in Colombia (Fernández 1995, 2000). Fernández (2000) reported 50 genera belonging to four subfamilies in South America; however, most of these genera require detailed taxonomic revision.

All species of Pompilidae use spiders as a food source for larval development, leading to their common name of "Spider Wasps" (Brothers and Finnamore 1993, Brothers 1995). Many species of this wasp family are idiobiont ectoparasitoids of cursorial spiders, building their nests in pre-existing holes in the soil or burrows dug by the wasps (Martins 1991). However, some koinobiont ectoparasitoids, belonging to the genus *Paracyphononyx* Gribodo, 1884 (Pompilidae), allow the host to resume activities after paralyzing it and laying a single egg on the spider's abdomen (Grout and Brothers 1982, Conley 1985, El-Hennawy 1996).

Paracyphononyx has been recorded in the tropics and warmer temperate regions throughout the world (Evans 1966), with nine species recorded in Brazil (Fernández 2000). Only three species of *Paracyphononyx* are known to use spiders as hosts, notably those belonging to the Lycosidae. These interactions were recorded in the Afrotropical region for *P. africanus* (Radoskowski, 1881), *P. funereus* (Lepeletier, 1845) in the Nearctic, and *P. ruficrus* (Klug, 1834) in the Palearctic.

The Lycosidae is distributed worldwide, containing small species (4–10 mm body length) that roam freely among stones or low vegetation, and larger species (10–20 mm body length) that typically dig burrows (Foelix 2011). Besides the records of Lycosidae used by Pompilidae as hosts, recent studies have presented data of a lycosid parasitized by an acrocerid fly (koinobiont endoparasitoid; Toft et al. 2012) and egg sacs parasitized by wasps of the families Ichneumonidae and Platygastridae (Cobb and Cobb 2004, Bowden and Buddle 2012). The lycosid genus *Trochosa* comprises small to medium sized spiders (5.8–13.0 mm body length) which are often found at the edge of woods and in woodland habitats (Dreyer and Brady 2008). They usually are nocturnal in the adult stage (Workman 1978).

Here, we record the occurrence of a new parasitic interaction involving *Paracyphononyx scapulatus* (Bréthes) and the hunter spider *Trochosa* sp. (Lycosidae), with a description of wasp development.

Methods

At 11:30 on July 3, 2014, we found a lycosid spider (*Trochosa* sp.) with a *Paracyphononyx scapulatus* egg on its abdomen (Fig. 1A) in a fragment of Atlantic Forest in the

municipally of Iracema do Oeste, Paraná, Brazil (24°29'11"S, 53°21'14"W). We collected the spider alive, and placed it in a plastic container (12 × 12 × 10 cm). We fed the spider with *Musca domestica* Linnaeus, 1758 (Muscidae) and kept the individual at natural conditions of temperature (21 °C) and photoperiod (13 h of light and 11 h of darkness). We then observed the behavior of the spider as the parasitoid developed from a larva to an adult wasp. In parallel, we photographed the spider daily, to obtain a time-series of the development of the parasitoid. We recorded the period of each developmental stage, cocoon construction, and adult wasp emergence. We measured the length and width of the larva throughout its development by analyzing the photographs using the software ImageJ (National Institute of Health).

Results and discussion

The *Paracyphononyx scapulatus* egg (1.4 mm length and 0.8 mm width) was deposited on the anterior-dorsal region of the abdomen of a juvenile *Trochosa* sp. (abdomen: 9 mm length and 6 mm width; cephalotorax: 7 mm length and 5.8 mm width; Fig. 1A), and hatched after seven days. The host remained active (resting during the day and walking at night), while the attached larva gradually fed on the spider's hemolymph in the abdomen. This result indicates that *P. scapulatus* is a koinobiont parasitoid, like other species of *Paracyphononyx* for which the biology is known (Grout and Brothers 1982, Conley 1985, El-Hennawy 1996). Conley (1985) documented *P. funereus* (Lepeletier 1845) as a koinobiont parasitoid of the spider *Geolycosa rafaelana* (Chamberlin, 1928), causing high mortality to adult females during the winter. Grout and Brothers (1982) observed *P. africanus* paralyzing its lycosine host temporarily, depositing a single egg transversely on the concave anterodorsal surface of the spider abdomen, and then abandoning the host, which subsequently recovered normal activity. El-Hennawy (1996) recorded the wasp *P. ruficrus* manipulating the behavior of a lycosid spider, inducing the host to construct a silken cocoon around itself.

The larval development of *Paracyphononyx scapulatus* includes five instars, evidenced by marked increase in size of the larva between each stage (Fig. 1B–F), doubling its length between the fourth and fifth instars (Fig. 2). The fifth instar larva killed the spider in the morning, 20 days after the egg hatched. The spider body was then completely consumed over the next two days. On the second day, the larva spun in different locations on the ground of the plastic container looking for suitable substrate to construct a cocoon (Fig. 1G). The larva located a suitable site on the afternoon of the second day (16:00), and required about 16 hours to construct the cocoon (cocoon: 16 mm length and 6 mm width). We observed the presence of meconium after six days in the portion of the cocoon that was fixed onto the substrate (Fig. 1H, arrow). An adult female wasp (15 mm length) emerged 32 days after cocoon construction, cutting its apical region (Fig. 1I–J). Fifty-four days was required for the development period from the egg hatching to adult emergence. This period is higher than that observed by El-Hennawy (1996) for the congeneric wasp *P. ruficrus*, which had a 30-day total development period.

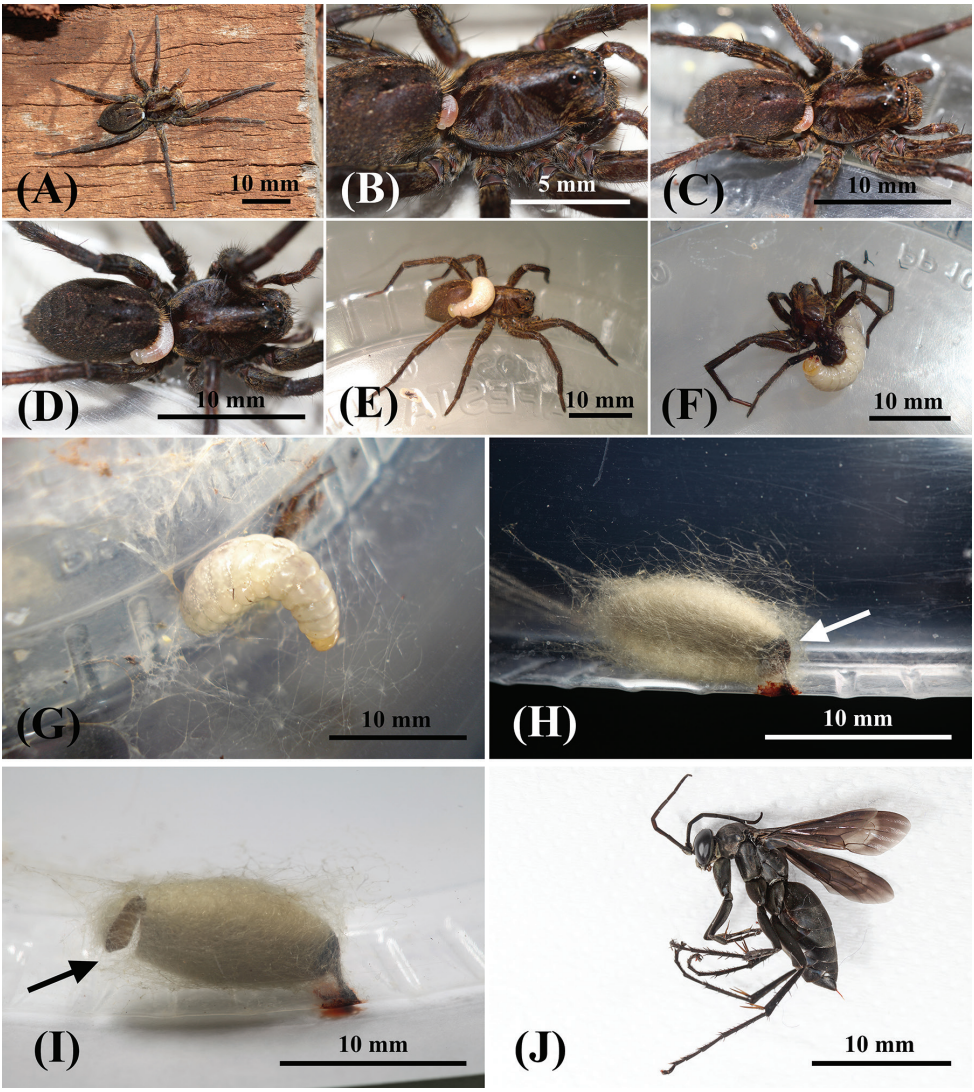

Figure 1. Juvenile *Trochosa* sp. spider parasitized by the wasp *Paracyphononyx scapulatus* and containing on its abdomen: **A** the egg of the wasp; the **B** first **C** second **D** third, and **E** fourth instar larva of the wasp **F** the fifth instar larva of the wasp starting the consume the spider abdomen **G** the fifth instar larva constructing the cocoon **H** the cocoon containing the meconium (arrow) **I** the cocoon after adult wasp emergence (arrow indicates the location where the wasp emerged) **J** the adult female wasp. Photography: Hebert da Silva Souza (**A–D**), Eduardo Messas Junior (**E–G**), and Yuri Fanchini Messas (**H–J**).

To date, all observations involving *Paracyphononyx* as the koinobiont ectoparasitoid (Grout and Brothers 1982, Conley 1985, El-Hennawy 1996), including the present study, have shown that this wasp genus only uses spiders belonging to the Lycosidae as hosts. This observation indicates the existence of host-parasitoid specificity.

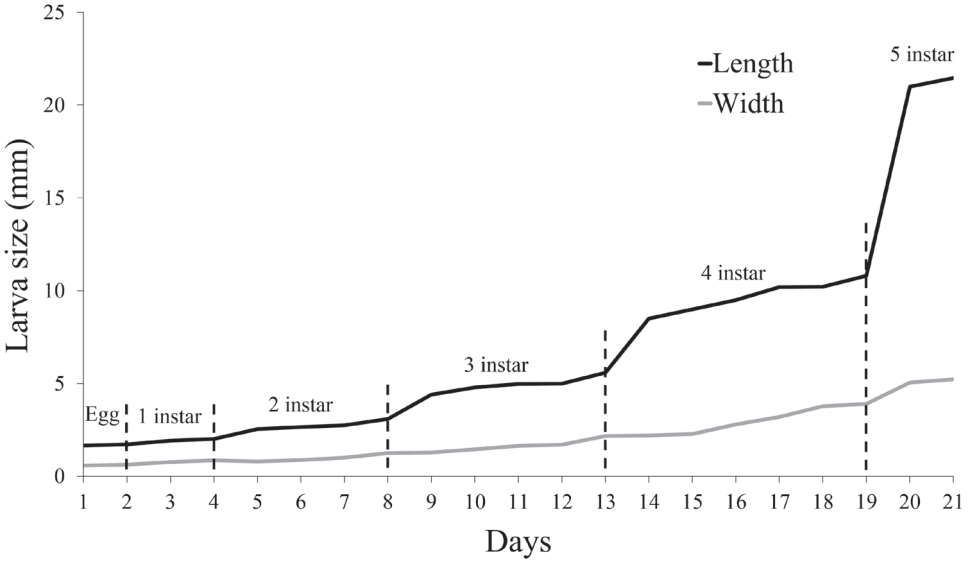

Figure 2. Size of *Paracyphononyx scapulatus* larva on the abdomen of the *Trochosa* sp. (length and width) during their development.

Koinobiont ectoparasitoids of the genus group *Polysphincta* (Hymenoptera: Ichneumonidae, Pimplinae) exhibit high parasitoid-host specificity, using only one species or groups of spider species that share common characteristics as hosts (Matsumoto and Konishi 2007, Sobczak et al. 2009, Gonzaga and Sobczak 2011, Takasuka and Matsumoto 2011, Eberhard 2013, Sobczak et al. 2014). Furthermore, parasitic wasps tend to select intermediate-sized hosts that have sufficient biomass for larval development, but do not represent a threat to the wasp during the attack (Gonzaga and Sobczak 2011). Few records exist of parasitism by *Paracyphononyx*; consequently, knowledge about the ecology and natural history of this genus remain limited. Therefore, we encourage future studies to elucidate the type of host selection used by this group of wasps.

Paracyphononyx scapulatus attacked the *Trochosa* sp. during winter, which is also when Conley (1985) recorded 50–60% mortality of adult *Geolycosa rafaelana* females caused by *P. funereus*. The same author observed that the parasitism rates were not strongly related to reduced-density populations of *G. rafaelana*, indicating that *P. funereus* selectively forages in areas with higher spider density (Conley 1985). Thus, there may be a phenological adjustment between *P. scapulatus* and *Trochosa* sp., the period of wasp attacks coincides with the period when the number of juvenile spiders is at its peak, which provide sufficient biomass for adequate larval development.

We did not observe any behavioral modification by the larva on the parasitized *Trochosa* sp. After killing the spider, the *Paracyphononyx scapulatus* larva started cocoon construction much faster than that observed for *P. ruficrus* by El-Hennawy (1996). However, in the previous study the *Lycosa* host spun a silken chamber, clos-

ing itself and the *P. ruficrus* wasp larva inside. Then, the wasp larva continued to feed on the spider inside the silken shelter, killing the host and building its cocoon. Similar behavior was observed by Day (1981) for *Pompilus cinereus* (Fabricius). Most known behavioral manipulation interactions induced by parasitoid wasps target web building spiders, with behavior modification tending to include the construction of modified webs which may exhibit a tridimensional tangle structure, absence of sticky spirals, central hub and stabilimentum, reduced number of radial lines and refuges that protect the cocoon and increase parasitoid survival (Eberhard 2013, Korenko et al. 2015). Data about the behavioral manipulation of pompilid hosts remains limited. In this study, having the spider caged in a featureless container did not allow us to test if the parasite tried to manipulate the spider behavior (e.g. induce it to hide itself and consequently hide the wasp cocoon). Thus, new records and more conclusive studies are necessary to understand the behavioral modification induced by Pompilidae species.

Many Pompilidae species hunt wandering spiders on the ground; consequently, immobilized spiders are exposed to predators, such as ants. Thus, these wasp species may dig burrows or use pre-existing holes to hide the paralyzed spider with its egg. In comparison, the type of koinobiont parasitism adopted by *Paracyphononyx scapulatus* (i.e., allowing the spider to continue normal activity), may be an alternative strategy to prevent the exposure hosts to predation. According to Korenko et al. (2011), most interactions between koinobiont parasitic wasps and spiders are host-specific, with the life histories of both wasp and spider being tightly coupled. Therefore, we would expect *P. scapulatus* to kill the host during the day at the end of the larval development under natural conditions, which is when the spider is typically resting in a refuge.

The genus-group *Polysphincta* is well-known to contain exclusively koinobiont ectoparasitoids of spiders (Gauld and Dubois 2006). Yet, knowledge about the host-parasite interactions of Pompilidae family have been summarized in a few studies that provide limited and brief information. Further investigations detailing these interactions may provide a promising path towards understanding the evolution of koinobiont ectoparasitism, which probably arose independently in different groups of wasps.

Aknowledgements

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References

Banks N (1946) Studies of South American Psammocharidae Part I. Bulletin of the Museum of Comparative Zoology 96: 311–525.

Banks N (1947) Studies of South American Psammocharidae Part II. Bulletin of the Museum of Comparative Zoology 99: 371–486.

Bowden JJ, Buddle CM (2012) Egg sac parasitism of Arctic wolf spiders (Araneae: Lycosidae) from northwestern North America. Journal of Arachnology 40: 348–350. doi: 10.1636/P11-50.1

Bradley JC (1944) A preliminary revision of the Pompilinae (Exclusive of the tribe Pompilini) of the America (Hymenoptera: Pompilidae). Transactions of the American Entomological Society 70: 23–157.

Brothers DJ (1995) The vespoid families (except vespids and ants). In: Hanson PE, Gauld ID (Eds) The Hymenoptera of Costa Rica. Oxford University Press, Oxford, 504–560.

Brothers DJ, Finnamore AT (1993) Superfamily Vespoidea. In: Goulet H, Huber JT (Eds) Hymenoptera of the World: an identification guide to families. Research Branch Agriculture Canada Publication (Otawa): 161–27.

Cobb LM, Cobb VA (2004) Occurrence of parasitoid wasps, *Baeus* sp. and *Gelis* sp., in the egg sacs of the wolf spiders *Pardosa moesta* and *Pardosa sternalis* (Araneae, Lycosidae) in southeastern Idaho. The Canadian Field-Naturalist 118: 122–123.

Conley RC (1985) Predation versus resource limitation in survival of adult burrowing wolf spiders (Araneae: Lycosidae). Oecologia 67: 71–75. doi: 10.1007/BF00378453

Day MC (1981) A revision of *Pompilus* Fabricius (Hymenoptera: Pompilidae), with further nomenclatural and biological considerations. Bulletin of the British Museum (Natural History), Entomology 42: 1–42.

Dreyer MD, Brady AR (2008) *Trochosa sepulchralis*, a senior synonym of *Trochosa acompa,* and the restoration of *Trochosa abdita* (Araneae, Lycosidae). Journal of Arachnology 36: 65–75. doi: 10.1636/H07-19.1

Eberhard WG (2013) The polysphinctine wasps *Acrotaphus tibialis*, *Eruga* ca. *gutfreund*, and *Hymenoepimecis tedfordi* (Hymenoptera, Ichneumonidae, Pimplinae) induce their host spiders to build modified webs. Annals of the Entomological Society of America 106: 652–660. doi: 10.1603/AN12147

El-Hennawy HK (1996) A spider (*Lycosa*? sp.) (Araneida: Lycosidae) providing a shelter for its predator *Paracyphononyx ruficrus* (Klug, 1834) (Hymenoptera: Pompilidae). Revue Suisse de Zoologie 1: 185–188.

Evans HE (1966) A revision of the Mexican and Central American spider wasps of the subfamily Pompilinae (Hymenoptera: Pompilidae). Memoirs of the American Entomological Society 20: 1–442.

Fernández F (1995) La biodiversidad de los Hymenoptera en Colombia. In: Rangel JO (Ed.) La Diversidad Biológica en Colombia. Universidad Nacional de Colombia, Bogotá, 373–442.

Fernández F (2000) Avispas Cazadoras de Arañas (Hymenoptera: Pompilidae) de la Región Neotropical. Biota Colombiana 1: 3–24.

Foelix RF (2011) Biology of Spiders. Oxford University Press, Oxford, 419 pp.

Gauld ID, Dubois J (2006) Phylogeny of the *Polysphincta* group of genera (Hymenoptera: Ichneumonidae; Pimplinae): a taxonomic revision of spider ectoparasitoids. Systematic Entomology 31: 529–564. doi: 10.1111/j.1365-3113.2006.00334.x

Gonzaga MO, Sobczak JF (2011) Behavioral manipulation of the orb-weaver spider *Argiope argentata* (Araneae, Araneidae) by *Acrotaphus chedelae* (Hymenoptera, Ichneumonidae). Entomological Science 14: 220–223. doi: 10.1111/j.1479-8298.2010.00436.x

Goulet H, Huber JT (1993) Hymenoptera of the World: an identification guide to families. Research Branch Agriculture Canada Publication, Ottawa, 668 pp.

Grout TG, Brothers DJ (1982) Behaviour of a parasitic pompilid wasp (Hymenoptera). Journal of the Entomological Society of South Africa 45: 217–220.

Korenko S, Michalková V, Zwakhals K, Pekár S (2011) Host Specificity and Temporal and Seasonal Shifts in Host Preference of a Web-Spider Parasitoid *Zatypota percontatoria*. Journal of Insect Science 11: 1–12. doi: 10.1673/031.011.10101

Korenko S, Satrapová J, Zwakhals K (2015) Manipulation of araneid spider web architecture by the polysphinctine parasitoid *Zatypota picticollis* (Hymenoptera: Ichneumonidae: Pimplinae). Entomological Science 18: 383–388. doi: 10.1111/ens.12132

Martins RP (1991) Biologia e comportamento de comunidades de vespas escavadoras (Hymenoptera: Aculeata). PhD thesis. Universidade Estadual de Campinas, São Paulo.

Matsumoto R, Konishi K (2007) Life histories of two ichneumonid parasitoids of *Cyclosa octotuberculata* (Araneae): *Reclinervellus tuberculatus* (Uchida) and its new sympatric congener (Hymenoptera: Ichneumonidae: Pimplinae). Entomological Science 10: 267–278. doi: 10.1111/j.1479-8298.2007.00223.x

Pitts JP, Wasbauer MS, Dohlen CD (2006) Preliminary morphological analysis of relationships between the spider wasp subfamilies (Hymenoptera: Pompilidae): revisiting an old problem. Zoologica Scripta 35: 63–84. doi: 10.1111/j.1463-6409.2005.00217.x

Sobczak JF, Loffredo APS, Penteado-Dias AM, Gonzaga MO (2009) Two new species of *Hymenoepimecis* (Hymenoptera: Ichneumonidae: Pimplinae) with notes on their spider hosts and behaviour manipulation. Journal of Natural History 43: 2691–2699. doi: 10.1080/00222930903244010

Sobczak JF, Moura-Sobczak JCMS, Messas YF, Souza HS, Vasconcellos-Neto J (2014) A new record of a host-parasitoid interaction: *Hymenoepimecis veranii* Lofredo & Penteado-Dias, 2009 (Hymenoptera: Ichneumonidae) parasitizing *Araneus orgaos* Levi, 1991 (Araneae: Araneidae). Journal of Insect Behavior 27: 753–758. doi: 10.1007/s10905-014-9467-z

Takasuka K, Matsumoto R (2011) Lying on the dorsum: unique host-attacking behaviour of *Zatypota albicoxa* (Hymenoptera, Ichneumonidae). Journal of Ethology 29: 203–207. doi: 10.1007/s10164-010-0263-8

Toft S, Nielsen BO, Funch P (2012) Parasitoid suppression and life-history modifications in a wolf spider following infection by larvae of an acrocerid fly. Journal of Arachnology 40: 13–17. doi: 10.1636/P11-28.1

Workman C (1978) Life cycle and population dynamics of *Trochosa terricola* Thorell (Araneae: Lycosidae) in a Norfolk grass heath. Ecological Entomology 3: 329–340. doi: 10.1111/j.1365-2311.1978.tb00934.x

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SHORT COMMUNICATION

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Sphecid wasp larvae feeding on large-sized cockroaches in a dead wood cavity

Masato Ito[1], Takumi Oshio[1], Hironobu Handa[2], Kyohei Watanabe[3]

1 *Laboratory of Insect Biodiversity and Ecosystem Science, Kobe University, Rokkodaicho 1–1, Nada, Kobe, Hyogo 657–8501, Japan* **2** *Saitama Museum of Natural History, Nagatoro 1417–1, Chichibu, Saitama 369–1305, Japan* **3** *Kanagawa Prefectural Museum of Natural History, Iryuda 499, Odawara, Kanagawa 250–0031, Japan*

Corresponding author: *Masato Ito* (fixsenia@hotmail.co.jp)

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Abstract

We investigated the nest site and prey items of *Isodontia auripygata* (Hymenoptera: Sphecidae) in a subtropical forest on Iriomote Island, ca. 200 km east of Taiwan. *I. auripygata* used the cavity of a dead branch as their nest site, and the nest was composed of two cells that were divided by wood offcuts. Inside the branch, we found large-sized adult cockroaches, *Rhabdoblatta formosana* (Blattodea: Epilampridae), *R. formosana* adults were fed to an *I. auripygata* larva in each cell. To our knowledge, this is the third record of species of the genus *Isodontia* that prey on cockroaches.

Keywords

Sphecidae, Iriomote Island, *Isodontia auripygata*, cockroach prey, *Rhabdoblatta formosana*

Introduction

Sphecid wasps (Sphecidae s. str. Latreille, 1802) are a diverse and cosmopolitan group, consisting of 19 genera and 736 species (Pulawski 2015). Each wasp species hunts a limited range of arthropod groups, e.g., Hymenoptera, Lepidoptera, Coleoptera, Orthoptera, or Araneae (Bohart and Menke 1976), and uses particular substrates as its nest site, e.g., plant cavities or ground nests (Bohart and Menke 1976).

Isodontia Patton, 1880 is a medium-sized genus of the family Sphecidae (Hymenoptera), which contains 61 described species that are distributed worldwide (Pulawski 2015). Of these, 23 species are recorded from the Oriental region, 14 from the Neotropical region, seven from the Nearctic region, six from the Palearctic region, five from the Ethiopia region, four from the Oceania region, and three from the Australia region (Pulawski 2015).

In the genus *Isodontia*, prey items for only 13 species (21%) have been recorded; 11 of these species (85%) use orthopterans (Pulawski 2015). Only two species, *I. diodon* (Kohl, 1890) and *I. formosicola* (Stand, 1913), have been reported to use cockroaches (Blattodea) as food for their larvae (Barthélémy 2010; Iwata 1939).

Five species of *Isodontia, I. auripygata* (Strand, 1913), *I. boninensis* (Tsuneki, 1973), *I. harmandi* (Perez, 1905), *I. maidli* (Yasumatsu, 1938), and *I. nigella* (Smith, 1856), have been recorded from Japan. Among these, the nesting biology (cavity of dead plants) and prey items (Orthoptera) are known for only *I. harmandi*, *I. maidli*, and *I. nigella* (Murota, 1999). Nest sites and prey items of the other two species have not previously been reported. One of them, *I. auripygata* was originally described from Taiwan (Strand 1913). In Japan, this species was first collected from Iriomote Island, ca. 200 km east of Taiwan, in 1978 (Nagase 2005). However, only four individuals have been collected since the first record (Takahashi 2005).

In late May 2014, we observed at least 10 individuals of *Isodontia auripygata* visiting flowers of *Psychotria rubra* (Lour.) Poir. (Rubiaceae) in the subtropical forest of broad-leaved evergreen trees on Iriomote Is. (24.340137°N, 123.913752°E). They frequently visited flowers between 12:00 and 15:00. In the forest, one individual was observed to go out of a small hole (diameter: about 10.0 mm) on a dead branch of a live tree (height: about 5 m), suggesting that a nest of *I. auripygata* was formed in the branch. Therefore, we examined the inside of the branch to find the nest and prey.

Materials and methods

We used a knife to dissect the branch from which a wasp went out. The structure of the nest and the prey items were examined. The insects collected from the nest were preserved in petri dishes (diameter: 85 mm, height: 20 mm) and observed under laboratory conditions.

To clarify whether the wasp larvae found in the nest were of *Isodontia auripygata*, we compared DNA sequences of the mitochondrial COI gene between the larva and

an *I. auripygata* adult. DNA was extracted from the larva found in the nest, and an adult collected from *Psychotria rubra* flowers. The detailed method of DNA extraction is described in Ito et al. (2014), who used the primer set (LCO1490, HCO2198) designed by Folmer et al. (1994). All the DNA sequences obtained are deposited in the DDBJ/EMBL GenBank database.

Result

We found that the nest was composed of two cells in the branch. The cells were delimited by wood fragment partitions (each cell length: about 50 mm, tunnel diameter: about 1.5 mm (Fig. 1b, c)). We found anesthetized adults of the cockroach *Rhabdoblatta formosana* (Shiraki, 1906) (Epilampridae) in these cells: one female and one male cockroach were found in the upper cell (cell A), while two female and two male cockroaches were found in the lower cell (cell B; Fig. 1b, c). We found one wasp larva on the base of the fore coxa of a cockroach in each petri dish four days after the collection (May 16 2014).

The wasp larva collected from cell B consumed three cockroaches. The COI sequence of the wasp larva was identical to the sequence of *I. auripygata* adult (570 bp, 100% sequence homology).

The wasp larva collected from cell A consumed two cockroaches. This larva spun a cocoon 10 days after the collection. This individual pupated on 17 April 2015, and emerged on 6 May 2015, suggesting one generation per year in this species. (One assumes this individual was an Isodontia auripygata adult – perhaps a male given the lesser number of prey item.) (Since the nest was provisioned in May 2014 but the mature larva did not pupate until April 2015, this suggests extended larval diapause and fewer than one generation per year (although the extended diapause could be an artefact)).

Discussion

Wasps of the genus *Isodontia* are known to use cavities of dead plants as nest sites (e.g. Iwata 1939; Barthélémy 2010; Murota 1999). Similarly, in the present study, *I. auripygata* nests were found in the cavities of dead tree branch. The cavity shape was similar to that of nests of the carpenter bee *Xylocopa appendiculata circumvolans* (Smith, 1873) (Sugiura 1995). Because *X. appendiculata circumvolans* does not occur on Iriomote Is., it is likely that *I. auripygata* may have used an empty nest of *X. albinotum* Matsumura, 1926, a similar carpenter bee which does occur there. (one might indicate that both belong to the same subgenus, *Alloxylocopa*).

Iwata (1939) reported that *Isodontia formosicola* used adults of an unidentified cockroach species (body length, ca. 35 mm) as food for its larvae. Barthélémy (2010) reported that *I. diodon* used adults and nymphs of the small-sized cockroach genera *Balta* and *Blattella* (5–15 mm) as food. In this study, we found *I. auripygata* used

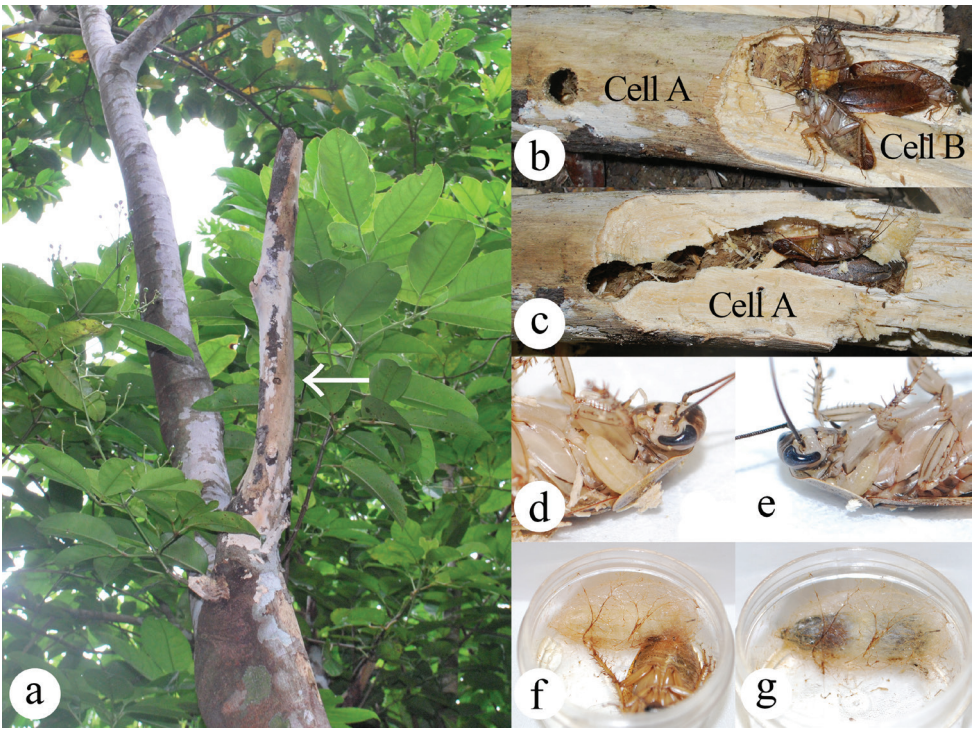

Figure 1. A dead branch of a live tree (**a**), inside of cell B (**b**), inside of cell A (**c**), wasp larva of cell B (**d**), wasp larva of cell A (**e**), prepupa of wasp in cell A (**f**), and cocoon of wasp in cell A (**g**).

adults of the large-sized cockroach *R. formosana* (23–33 mm; Asahina 1991). Female body length differed among *I. formosicola* (17–26 mm; Hensen, 1991), *I. diodon* (14–19 mm; Hensen 1991), and *I. auripygata* (30 mm; in this study). The widely differing body sizes of *Isodontia* wasps is a likely basis for the differences in prey (cockroach) size.

Nymphs of *Rhabdoblatta formosana* were not found among the prey items of *I. auripygata* detected by us. The habitats of *R. formosana* nymphs and adults are known to differ; nymphs are frequently being found under stones along forest streams, while adults are found on ferns in forests (Bell et al. 2007). While our sample size is small (1 incomplete nest), *Isodontia auripygata* adults are thus far known to prey only on *R. formosana* adults, but not nymphs.

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References

Barthélémy C (2010) Nesting Biology of *Isodontia diodon* (Kohl, 1890) (Hymenoptera: Sphecidae), a predator of cockroaches, in Hong Kong. Journal of Hymenoptera Research 19: 201–216.

Asahina S (1991) Blattaria of Japan. Nakayama shoten, 253 pp.

Bell WJ, Roth LM, Nalepa CA (2007) Cockroaches Ecology, Behavior, and Natural History. The John Hopkins University Press, 230 pp.

Bohart RM, Menke AS (1976) Sphecid wasps of the world: a generic revision. University of California Press, 695 pp.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–297.

Hensen RV (1991) Review of Malesian Sphecina (Hymenoptera, Sphecidae, Sphecinae). Tijdschrift Voor Entomologie 134: 9–30.

Iwata K (1939) Habits of some solitary wasps in formosa (IV). The journal of the Natural History Society of Taiwan 29: 161–178.

Murota T (1999) On the nesting behavior of *Isodontia maidli* Yasumatsu (Hymenoptera) and the Fukui prefectural distribution. Entomological Journal of Fukui 24: 17–24.

Nagase H (2005) New record of Sphecid wasp from Japan. Tsunekibachi 4: 26.

Pulawski WJ (2015) Catalog of Sphecidae sensu lato: http://www.calacademy.org/scientists/projects/catalog-of-sphecidae [updated 18 Mar. 2015]

Sugiura N (1995) Burrow construction by the Japanese carpenter bee, *Xylocopa appendiculata circumvolans* Smith, for Overwintering (Hymenoptera: Anthophoridae). Journal of the Kansas Entomological Society 68: 116–119.

Strand E (1913) H. Sauter's Formosa-Ausbeute. Crabronidae und Scoliidae. I. (Die Gattungen *Sphex*, *Sceliphron* und *Ammophila*, nebst Literaturverzeichnis als Nachtrag zu Dalla Torres Katalog.). Archiv für Naturgeschichte 79A (3): 76–87.

Takahashi H (2005) Records of Isodontia auripygata (Hymenoptera, Sphecidae) in Japan and Taiwan. Tsunekibachi 5: 17–18.