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Revision of Canadian Eurytomidae (Hymenoptera, Chalcidoidea) associated with galls induced by cynipid wasps of the genus *Diplolepis* Geoffroy (Hymenoptera, Cynipidae) and description of a new species

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Abstract

Eurytomids are small parasitic wasps associated with many communities of phytophagous insects. In most cases, the accurate identification of eurytomids is impeded by inadequate species descriptions that do not include figures of diagnostic features, and keys that are difficult to use. Here, diagnostic features and redescriptions are provided for both sexes of the eurytomids associated with galls induced by cynipid wasps of the genus *Diplolepis* Geoffroy found on shrub roses across Canada. Consequently, six species of *Eurytoma* Illiger, along with *Tenuipetiolus ruber* Bugbee, are dealt with. One new species, *Eurytoma shorthousei* Zhang & Gates, **sp. n.**, is described. Two species are synonymized, *E. hebes* Bugbee, 1973 and *E. spina* Bugbee, 1951 under *E. longavena* Bugbee, 1951, **syn. n.** Several new host and distribution records are reported. A dichotomous key is provided for both sexes of all seven species using photographs and scanning electron microscopy images.

Keywords

Eurytomidae, *Diplolepis*, *Eurytoma*, *Tenuipetiolus*, Canada

Introduction

The interaction between insect herbivores and their associated natural enemies is one of the key factors in understanding the origin and evolution of multi-trophic systems. One particularly species-rich, ecologically-closed model system for studies of host-parasitoid relationships is the community of gall wasps (Hymenoptera: Cynipidae) and their associated inquilines (e.g. *Periclistus* spp., Cynipidae: Diastrophini) and parasitoids (primarily Hymenoptera: Ichneumonoidea, Chalcidoidea) on oaks and roses (Csóka et al. 2005, Nicholls et al. 2010, Ronquist et al. 2015). Inquilines have lost their ability to induce galls, but still retain the ability to modify gall tissue (Brooks and Shorthouse 1997, Ronquist et al. 2015, Pujade-Villar et al. 2016). While entirely phytophagous, inquilines usually result in the death of inducers either through adult oviposition, or through competition for the same resources in the gall (Shorthouse 1973, 2010, Pujade-Villar et al. 2016). Parasitoids associated with cynipid galls feed either internally or externally as the host continues to develop (koinobionts), or when development of the host has been arrested by stinging prior to oviposition as for idiobionts (Csóka et al. 2005). Approximately 200 species of hymenopteran parasitoids are known from cynipid galls in Europe and North America, most of which are gall-specific (Csóka et al. 2005). For instance, parasitoids associated with galls induced by *Diplolepis* Geoffroy, in particular the European species *D. rosae* L. (Stille 1984, Lázsló and Tóthmérész 2006, 2011, Lotfalizadeh et al. 2007c), the North American species *D. polita* (Ashmead), *D. nodulosa* (Beuttenmüller) (Brooks and Shorthouse 1997, Shorthouse 1973, 2010), and *Diplolepis fructuum* (Rübsaamen) in Iran (Lotfalizadeh et al. 2012) have been well studied. However, the taxonomy and ecology of many of these inquilines and parasitoids associated with *Diplolepis* are poorly known due to their small size and morphological conservatism (Shorthouse 2010).

Members of the family Eurytomidae (Hymenoptera: Chalcidoidea) are one of the most common parasitoids associated with cynipid galls on roses in Canada, often comprising up to 40% of the component community (Shorthouse 2010). Bugbee (1951a, b) reported 12 species of *Eurytoma* Illiger and one species of *Tenuipetiolus* Bugbee known from galls of *Diplolepis*, and suggested that most species are monophagous. Little is known about the host specificity of eurytomids in galls of the 14 species of *Diplolepis* found in Canada, but all species of *Diplolepis* are host to at least one species of *Eurytoma* (Shorthouse 2010, Zhang et al. 2014).

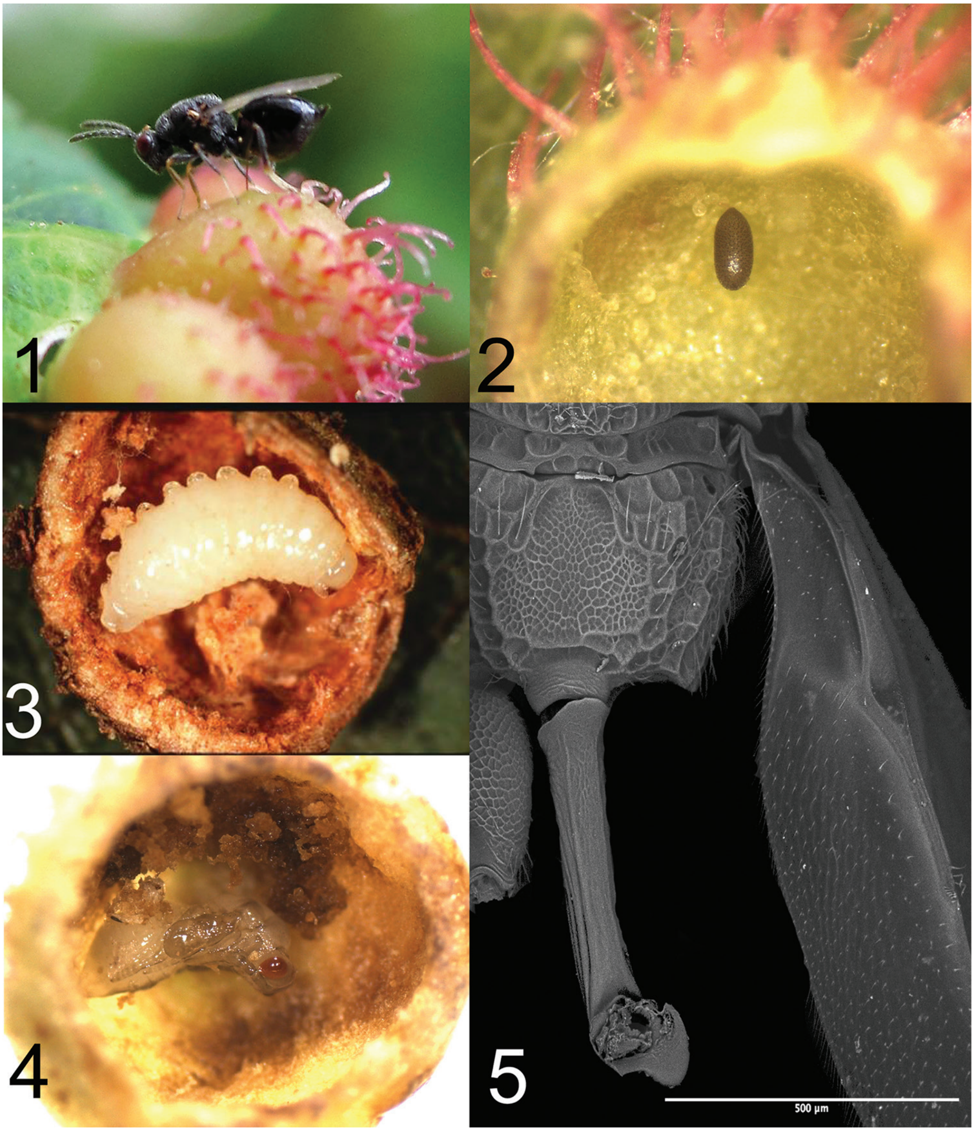
Recent phylogenetic analyses redefined Eurytomidae as a monophyletic group (Lotfalizadeh et al. 2007b, Heraty et al. 2013). A gradual and mosaic evolution with large levels of homoplasy was observed within Eurytominae based on the study by Lotfalizadeh et al. (2007b). The genus *Tenuipetiolus* Bugbee was grouped with *Prodecatoma* Ashmead based on the following derived states: 1) Adscrobal area with a dorsal depression or areola; 2) Epicnemium with a large and circular median areola dorsally; 3) Precoxal carinae close to anterior margin of metapleuron; 4) Submedian carinae close to each other. However, no formal synonymization has been proposed and all four species of *Tenuipetiolus* are found restricted within Nearctic, associated with galls of cynipids and cecidomyiids (Bugbee 1951a).

All species with a carinate gena and showing no other outstanding characters were redefined as *Eurytoma sensu stricto* (*s.s.*) with the following derived states: 1) Postgenal lamina present and raised ventrally over the surface of the postgena; 2) Postgena with a ventral depression between the posterior margin of the gena and the hypostomal fossa, with the depression delimited dorsally by a ridge or a step; 3) Gena with posterior margin slightly angulate above oral fossa (Lotfalizadeh et al. 2007b). *Eurytoma s.s.* is divided into 11 species groups including 700 nominal species worldwide, with ~100 Nearctic species north of Mexico (Lotfalizadeh et al. 2007b). *Eurytoma* associated with cynipid gall inducers have been placed under the *rosae* group, characterized by the presence of a precoxal tooth formed by the adscrobal carina (Lotfalizadeh et al. 2007b, see arrow in Fig. 11). Members of the *rosae* group often include cryptic species, which were morphologically indistinct or similar but with genetic and biological differences (Claridge and Askew 1960, Ács et al. 2002, Lotfalizadeh et al. 2007a, Gómez et al. 2011). The most recent published key to Nearctic *Tenuipetiolus* (Bugbee 1951a) and *Eurytoma* (Bugbee 1951b, 1967) is difficult to use due to the overlapping character states and the lack of illustrations. Additionally, current identification keys are limited to females, and thus the males are unidentifiable to species level.

Biology of eurytomids associated with galls induced by Diplolepis

Most eurytomids associated with galls induced by *Diplolepis* are univoltine. They feed throughout the summer on larvae of the gall inducer, inquilines, or on other parasitoids, overwinter as larvae within gall chambers, pupate in the spring and turn into adults (Shorthouse 1973). The emergence period of adults is synchronized with that of their host, which occurs soon after the immature galls appear such that the ovipositing females (Fig. 1) can reach the chambers of developing galls in this narrow window of opportunity (Shorthouse 2010). Eurytomid eggs are brown to black, and with an elongated egg body, and a curved peduncle which may be used to attach the egg to the inside surface of the developing gall chambers or the body of its host (Fig. 2) (Vårdal et al. 2016).

Eurytomid larvae in gall chambers with immature *Diplolepis* feed as koinobionts, keeping the inducer alive until the larva is fully grown and then consume it. This is necessary since the eurytomid larva grows to the same size as its single host. Eurytomids commonly feed on gall tissues along the inside surface of galls after the inducer is consumed (Fig. 3), and as a result, maturing galls become lined with frass (Fig. 4), a characteristic sign of this parasitoid (Shorthouse 1973). Eurytomid larvae having emerged from eggs deposited in galls with immature *Periclistus* larvae feed as predators consuming many inquilines before the inquiline form chambers (Shorthouse 1973, Brooks and Shorthouse 1997). Eurytomids in *Periclistus*-modified galls then chew into the *Periclistus* chambers to consume larvae.



Figures 1–5. *Eurtyoma longavena* **1** Female ovipositing into immature gall **2** Egg deposited on the inside surface of gall chamber **3** Mature larva inside a gall showing frass as a result of feeding on gall tissue **4** Pupa overwintering inside the gall before exiting the following year. *Tenuipetiolus ruber* **5** Propodeum + petiole. Photos 1–4 credit Brandy L. Fenwick.

Fully grown eurytomid larvae can be distinguished from larvae of other parasitoids in *Diplolepis* galls by their cylindrical body shape and the presence of dorsal protuberances (Shorthouse 1973). While the larvae of Ormyridae also have protuberances, they can be readily separated by their single toothed mandible that is not externally visible, as opposed to the bidentate, partially visible external mandibles of eurytomids (Fig. 3)

(Gómez et al. 2011). In late summer, eurytomids overwinter within the gall chamber at the pupal stage (Fig. 4). The coloration of the pupa darkens with the approach of adult emergence, the imago chewing through the gall wall to exit and repeat the life cycle.

The objective of this study is to describe both male and female eurytomids associated with rose galls in Canada, as well as updating morphological characters, hosts, and distributional records following the molecular study using *COI* (Zhang et al. 2014). Additionally, a dichotomous key is provided for both sexes of all seven species.

Materials and methods

The eurytomids used for this study were from the collection of J. D. Shorthouse (JDS), previously deposited at Laurentian University in Sudbury, Ontario. Upon JDS's retirement, pin-mounted specimens were deposited in the Canadian National Collections of Insects, Arachnids, and Nematodes (CNCI) in Ottawa. Specimens stored in alcohol and emerged from thousands of galls that were collected across Canada over the past 45 years, are deposited at Edinburgh University in Scotland in the laboratory of Graham Stone. In most cases, the eurytomids, along with all other gall inhabitants, were obtained by storing mature galls in glass jars in the laboratory and aspirating adults daily when they appeared. In other cases, the larvae of gall inhabitants were removed from mature galls either in autumn or spring and placed in pin-mounted gelatin capsules. Adults obtained in this manner could be associated with the larval stage and were cleaner than those that had chewed their way out of galls. Most specimens from these collections for this study were chemically dried using hexamethyldisilazane (HMDS), following the protocol of Heraty and Hawks (1998), before they were point- or card- mounted. A full list of specimens examined is listed under each redescription. Additional specimens were also borrowed from the following institutions:

CNCI	Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada (John T. Huber, curator);
DEBU	University of Guelph Insect Collection, Guelph, Ontario, Canada (Stephen A. Marshall, curator);
USNM	National Museum of Natural History, Washington D.C., USA (Michael W. Gates, curator).

The specimens were photographed using a Canon 7D Mark II with either a Canon MP-E 65mm F/2.8 Macro Photo Lens, or with a Mitutoyo M Plan Apo 10x objective mounted onto the Canon EF Telephoto 70–200 mm zoom lens, and the Canon MT-24EX Macro Twin Lite Flash with custom made diffusers to minimize hot spots. Scanning electron microscopy (SEM) micrographs were taken using methods described by Gates and Pérez-Lachaud (2012). Composite descriptions were based on a combination of all examined specimens with DNA vouchers. Terminologies used for surface sculptures follow Harris (1979), while the morphology follows Gibson (1997), Lotfalizadeh et al. (2007b), and Gates and Pérez-Lachaud (2012). The following acronyms are used: **LOL** (Lateral ocellar line), **OOL** (Ocello-ocular line), **POL** (Posterior ocellar line), **F**$_{\mathbf{n}}$ (Funicular segment #), **Gt**$_{\mathbf{n}}$ (Gastral tergite #). Measurements were taken using an ocular micrometer, and size range given is based on the largest and smallest specimens in the material examined. Image processing and plate assembly was completed with Photoshop CC and Illustrator CC. Distribution maps were produced using Simplemappr (Shorthouse 2017, www.Simplemappr.net).

Taxonomy

Key to species of Eurytomidae associated with galls induced by *Diplolepis* in Canada

1 Female. Gt$_1$ and Gt$_2$ fused dorsally. Petiole longer than metacoxa (Fig. 5). Male. F$_3$–F$_5$ with one row of setae . Petiole about 2× as long as metacoxa .... ***Tenuipetiolus ruber***

– Female. Gt$_1$ and Gt$_2$ not fused dorsally. Petiole subequal in length to metacoxa (Fig. 11). Male. F$_3$–F$_5$ with 2–3 rows of setae (Figs 29, 30). Petiole less than 2× as long as metacoxa........ ***Eurytoma* Illiger 2**

2 Tegula, scape and pedicel yellow (Figs 12, 13), propodeal spiracle with raised rim anteriorly (Fig. 19) .... ***E. shorthousei* sp. n.**

– Tegula black to brown, scape and pedicel black with yellow restricted to basal region (Fig. 9), propodeal spiracle not without raised rim anteriorly........ **3**

3 Pro- and mesocoxa yellow to brown, never entirely black (Fig. 7) ..... ***E. iniquus***

– Pro- and mesocoxa entirely black (Fig. 8)........ **4**

4 Female. Gaster dorsal outline S-curve shaped in lateral view, longer than head plus mesosoma, ovipositor sheath upturned (Fig. 27). Male. Funicular segments longer than wide, with 2 rows of longitudinal sensilla and adpressed setae (Fig. 29) .... ***E. discordans***

– Female. Gaster not S-curve shaped (Fig. 28), shorter or equal to head plus mesosoma, ovipositor sheath not upturned. Male. Funicular segments as long as wide, with 1 row of longitudinal sensilla and erect setae (Fig. 30) **5**

5 Scape entirely black (Fig. 8) .... ***E. longavena***

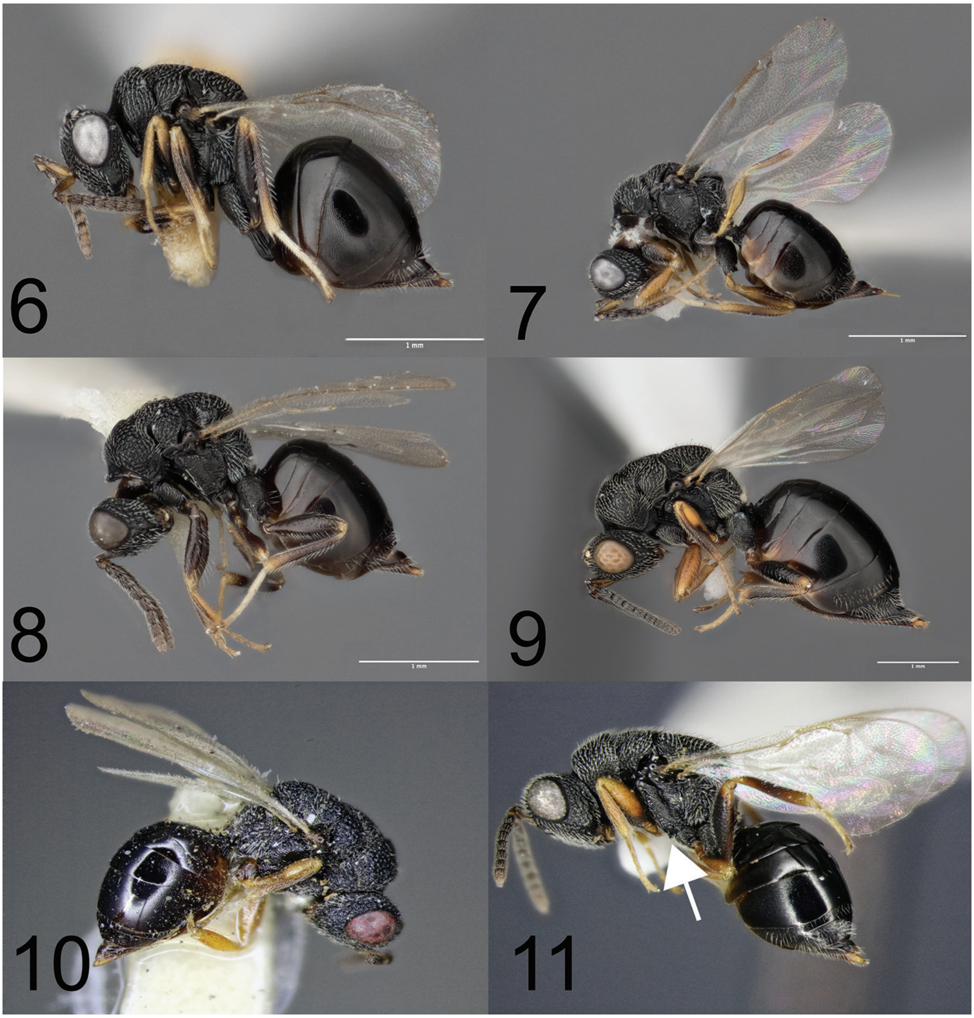
– Scape black with yellow at extreme base or along entire anterior surface **6**

6 Female metasoma small, oval shaped (Fig. 10) .... ***E. imminuta***

– Female metasoma large, elongate (Fig. 11)........ ***E. spongiosa***

Tenuipetiolus ruber Bugbee, 1951
Figs 5, 6, 31

Tenuipetiolus rubra Bugbee, 1951a: 39–42. Holotype female (USNM). Type data: USA, Indiana [Bloomington]; reared from galls induced by *Diplolepis rosae* (L.) on species of *Rosa*, March 28, 1939.



Figures 6–11. *Tenuipetiolus ruber* **6** Female habitus. *Eurytoma iniquus* **7** Female habitus. *E. longavena* **8** Female habitus. *E. discordans* **9** Female habitus. *E. imminuta* **10** Female habitus (length 2.0 mm). *E. spongiosa* **11** Female habitus (length 3.2 mm), arrow pointing to precoxal tooth formed by the adscrobal carina.

Females. Body length 2.0–3.6 mm. Color: Black except yellow to brown on basal half of scape, pedicel, and funicular segments, apices of all femora, pro-, meso- and metatibia, tip of ovipositor sheath, tegula, wing venation; all tarsomeres 1–4 white (Fig. 6).

Head. Head 1.2× as broad as high, umbilicate punctured with small tentorial pits. Genal carina present. Malar space 0.8× eye height, clypeus emarginate and supraclypeal area superficially rugose. Toruli positioned dorsad lower ocular line. Antenna with funicular segments subequal in length; pedicel chalice-shaped; funiculars fusiform with one row longitudinal sensilla and two whorls of setae; F_1 slightly narrowed basally. Ratio LOL:OOL:POL as 1:1:2.5. Head posteriorly without postgenal lamina or postgenal depression. Postgena sparsely setose.

Mesosoma. About 1.2× as long as broad; notauli incomplete, shallow. Epicnemium imbricate, flattened. Mesepisternum anterior to femoral depression umbilicate; mesepimeron reticulate ventrally, striolate or smooth dorsally, with longitudinal rugae originating from the posterior margin. Propodeum concave, superficially punctate, bordered mediolaterally by numerous carinae forming irregular setose cells, median furrow not delimited (Fig. 5). Procoxa imbricate, lacking setation proximally. Metacoxa asetose anteriorly and one row of setae on the posterior apical margin. Forewing hyaline, marginal vein distinctly longer than postmarginal vein.

Metasoma. Gaster 1.5× as long as mesosoma in lateral view; smooth, anterior edge of gastral tergites microreticulate. Petiole 2× length of metacoxa (Fig. 5). Gaster laterally compressed, triangular shaped and strongly convex in lateral view, ovipositor parallel to horizontal axis. Gt_1 and Gt_2 fused dorsally, Gt_{1-4} glabrate, Gt_{5-8} and apex of ovipositor sheaths setose.

Male. Body length: 1.4–2.5 mm. Color as described for female. Sculpture as described for female. Antennae with funicular segments pedunculate, F_2–F_5 each with 2 rows of erect setae and 1 row of longitudinal sensilla; scape without ventral plaque. Petiole in lateral view cylindrical, in dorsal view about 3× as long as greatest width, 2× as long as metacoxa; strigose laterally.

Remarks. This species is likely a generalist that is not restricted only to *Diplolepis* galls. Rearing records reported in the original description include cynipids of the genus *Diastrophus*.

Biology. Reared from field populations of galls induced by *Diplolepis bassetti* on *Rosa woodsii* Lindl.; *D. polita*, *D. nodulosa*, and *D. triforma* on *Rosa acicularis* Lindl.

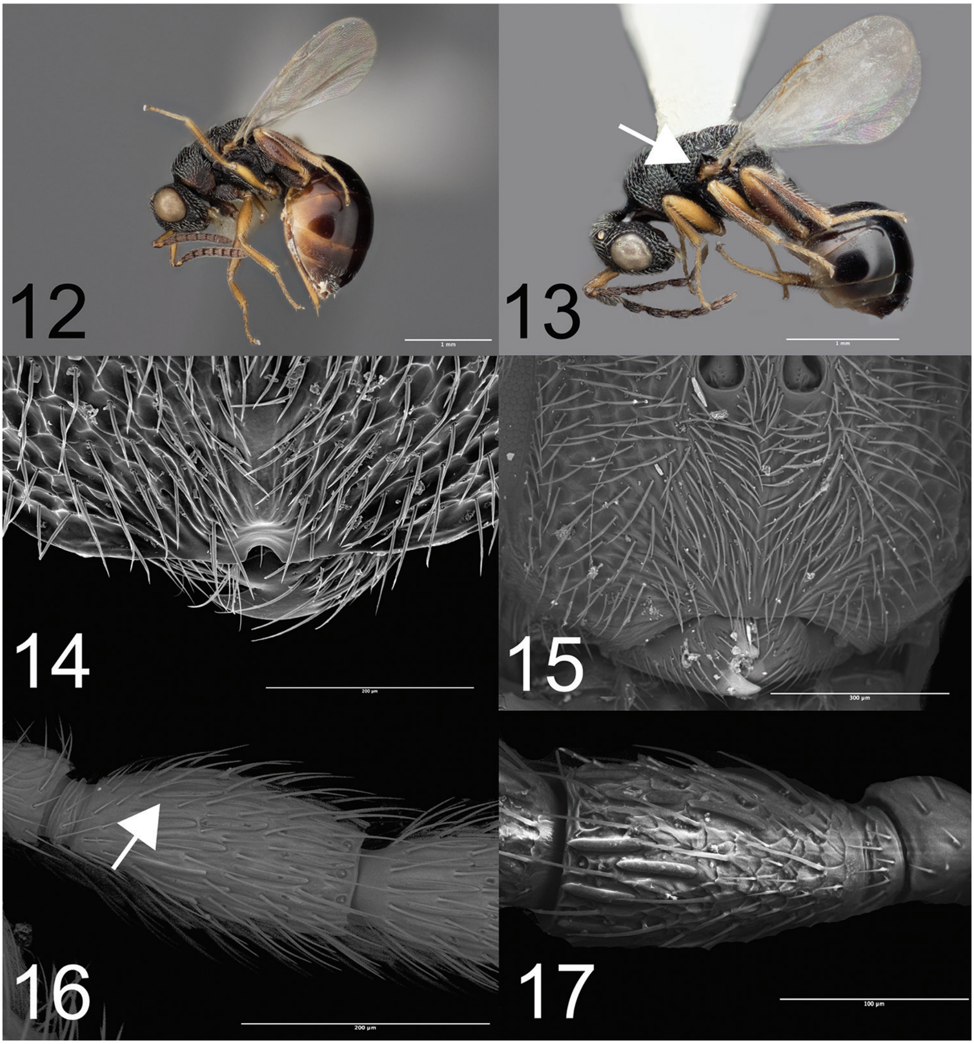
Material examined (27 females, 12 males). CANADA: British Columbia: Osoyoos, 14.V.2003, J.D. & M.R. Shorthouse, ex *Diplolepis bassetti* on *Rosa woodsii* (3F, 3M, CNCI). **Ontario**: Chelmsford, 5.V.1994, S.E. Brooks, ex *Periclistus pirata*-modified galls of *Diplolepis nodulosa* (3F, 1M, CNCI); Cochrane, 24.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex *Diplolepis triforma* on *Rosa acicularis* (9F, 2M, CNCI); Manitoulin Island, 2.V.2009, J.D. Shorthouse & J.D. Renelli, ex *Diplolepis triforma* on *Rosa acicularis* (5F, 1M, CNCI). **Québec:** La Sarre, 13.VII.2010, B.L. Smallwood & Y.M. Zhang, ex *Diplolepis polita* on *Rosa acicularis* (7F, 5M, CNCI).

Distribution. Disjunctive populations have been found in Western Canada in British Columbia and Eastern Canada in Ontario and Québec (Fig. 31).

Eurytoma shorthousei Zhang & Gates, sp. n.

http://zoobank.org/1EAC204E-372C-41ED-8C38-F653CA15A392
Figs 12, 13, 15, 17, 19, 21, 32

Etymology. This species is named for Joseph D. Shorthouse, honoring his contribution to the understanding of *Diplolepis* galls and their associated inhabitants, as well as the collector of the type specimens.



Figures 12–17. *Eurytoma shorthousei* sp. n. **12** Female habitus **13** Male habitus, arrow pointing to tegula **14** Clypeus **17** Female funicular segment 1. *E. obtusilobae* **15** Clypeus **16** Female funicular segment 1, arrow pointing to multiporous plate sensilla (MPS).

Diagnosis. This species differs from other eurytomids studied in the yellow or brown scape and tegula, with supraclypeal area strigose (Fig. 15). Propodeal spiracle with raised rim anteriorly (Fig. 19).

Holotype female. Body length 3.2 mm. Color: Black except brown funicular segments, apices of procoxa and metafemur, metatibia excluding apex, and yellow scape, pedicel, pro- and mesotibia, mesofemur, apices of metatibia and metafemur, all tarsomeres 1–4, tip of ovipositor sheath, tegula, wing venation (Fig. 12).

Head. 1.3× as broad as high, 2.5× as broad as long in dorsal view, umbilicate punctured. Malar space 0.5× eye height, malar carina present, raised in ventral half, becoming impressed line in dorsal half (Fig. 24). Gena entirely umbilicate punctured, minutely strigose posterad malar carina in ventral half, umbilicate posterad dorsal half (Fig. 24). Genal carina raised, forming blunt angle above oral fossa. Clypeus receding to median emargination and lower face strigose (Fig. 14), median longitudinal glabrous supraclypeal area (Fig. 14). Toruli positioned about ~1.5 torular diameters above lower ocular line. Lateral margin of antennal scrobes carinate, forming a raised lobe just above toruli. Intertorular projection approximately quadrate, dorsally truncate, with 2 rows of setae. LOL:OOL:POL is 1:1.4:2.3. Antennal segment ratios as: 55:15:3:25:20:18:15:15:35; pedicel chalice-shaped; funicular segments fusiform, subequal, with 2 rows of longitudinal sensilla and 2–3 rows adpressed setae; F1 lacking longitudinal sensilla in the basal third (Fig. 17).

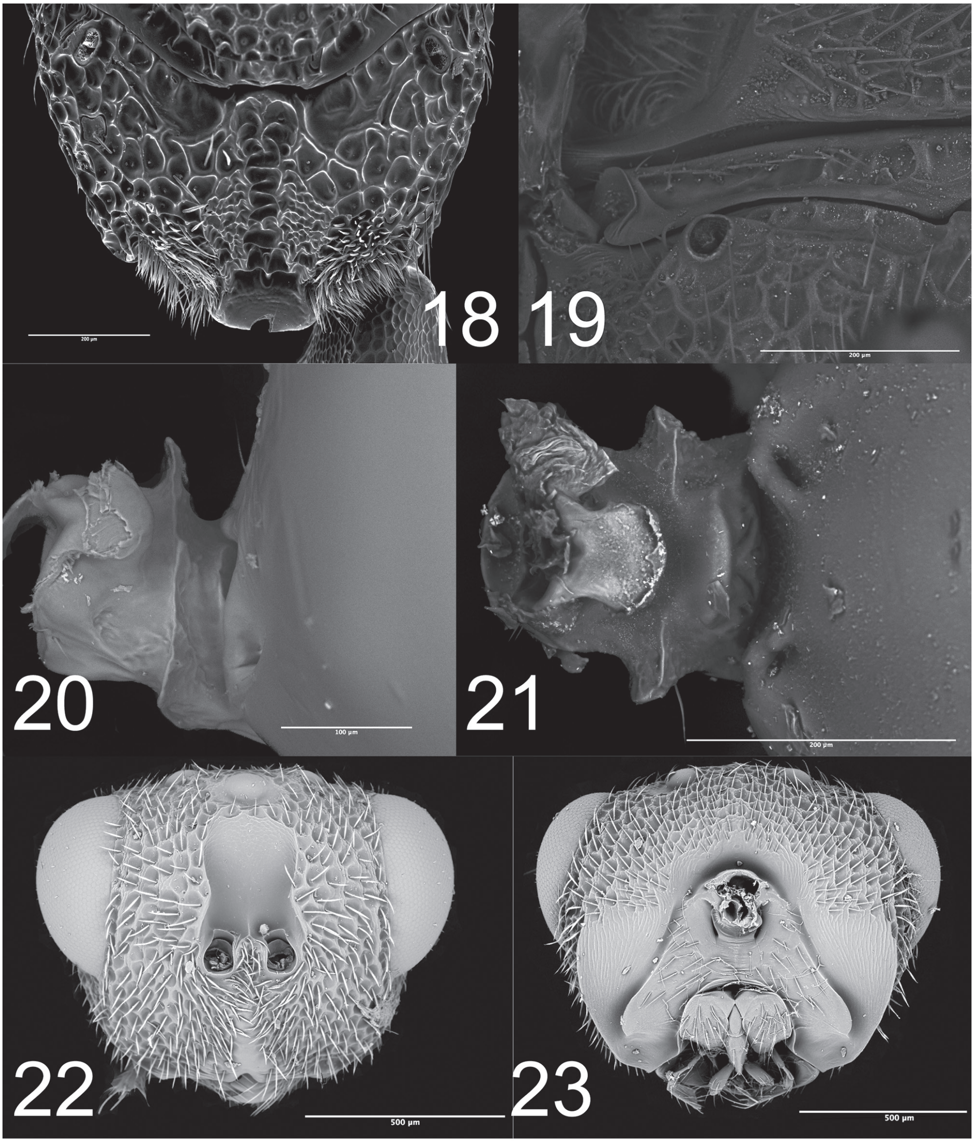
Mesosoma. About 1.2× as long as broad; notauli impressed, shallow. Epicnemium imbricate, flattened. Mesepisternum anterior to femoral depression, umbilicate; mesepimeron reticulate ventrally, striolate or smooth dorsally, with longitudinal rugae originating from the posterior margin. Precoxal tooth formed by raised adscrobal carina present in lateral view. Procoxa imbricate, lacking setation proximally, with oblique groove and S-like basal ridge. Metacoxa sparsely setose anteriorly and one row of setae on the posterior apical margin. Mesocoxal lamella absent. Lateral panels of propodeum and callus with umbilicate punctures distinctly delimited from median area by carinae forming irregular asetose cells, median furrow delimited, forming 1–2 rows of irregular foveae. Propodeal spiracle with raised rim anteriorly (Fig. 19). Forewing hyaline, setation dark, marginal vein as long as postmarginal vein, basal cell bounded by distinct basal and cubital setal lines, with sparse row setae parallel to submarginal vein; costal cell with single row of setae dorsally in apical half, speculum present and approximates width parastigma.

Metasoma. Gaster 1.5× as long as mesosoma in lateral view. Length 81 (valvulae excluded), height 55, relative lengths of Gt_{1-4} measured along dorsomedial line: 8:9:20:25; syntergum 7. Smooth, anterior edge of gastral tergites microreticulate. Petiole with three separate protuberances, one dorsomedial and two anterolateral (Fig. 21). Gaster laterally compressed, oval shaped and convex in lateral view, ovipositor parallel to horizontal axis. Gt_{1-4} glabrate, Gt_{5-8} and apex of ovipositor sheaths setose. Posterior margin of Gt_4 convex ventrally, straight dorsally. Posterior margin of Gt_5 weakly emarginate.

Male. Body length 3.0 mm. Color: Black, yellow and brown areas as described for female. Sculpture as described for female. Antennal segment ratios as: 52:14:3:36:27:31:27:27:42; funicular segments longer than wide, pedunculate, F_2–F_5 each with 3 irregular rows of appressed setae and two irregular rows of longitudinal sensilla; scape with ventral plaque in apical half (Fig. 13). Gaster 0.9× as long as mesosoma in lateral view. Length 40, height 30, relative lengths of Gt_{1-4} measured along dorsomedial line: 7:10:34:10; syntergum 1. Petiole 2.0× as long as broad, rugulose dorsally, mostly glabrous laterally and ventrally.

Variation. Body length ranges from 2.5–3.2 mm in females, 1.7–3.0 mm for males. Occasionally, brownish area on anterior pronotum extends laterally onto collar.

Remarks. This species was originally mistakenly identified as *Eurytoma obtusilobae*, described by Ashmead in 1885 based on four specimens, "bred from an unidentified



Figures 18–23. *Eurytoma obtusilobae* **18** Propodeum **20** Petiole, lateral view. *E. shorthousei* sp. n. **19** Propodeal spiracle **21** Petiole, dorsal view. *E. discordans*: **22** Head, anterior view **23** Head, posterior view.

cynips gall on *Quercus obtusiloba* [now *stellata*; post oak]" from Jacksonville, Florida. The only specimen remaining from this series is a female designated and labeled as a lectotype by Bugbee (1967). Bugbee (1951b) had previously redescribed "*obtusilobae*" based on the types and included 5 females and 10 males collected by J. C. Bridwell in Vienna, Virginia 1941 (ex *Rhodites radicum* on *Rosa palustris*), but the latter belong to *E. shorthousei*. Only 4 pointed females and males could be located, though there is a gelatin capsule with 30 individuals from the same collecting event were not examined.

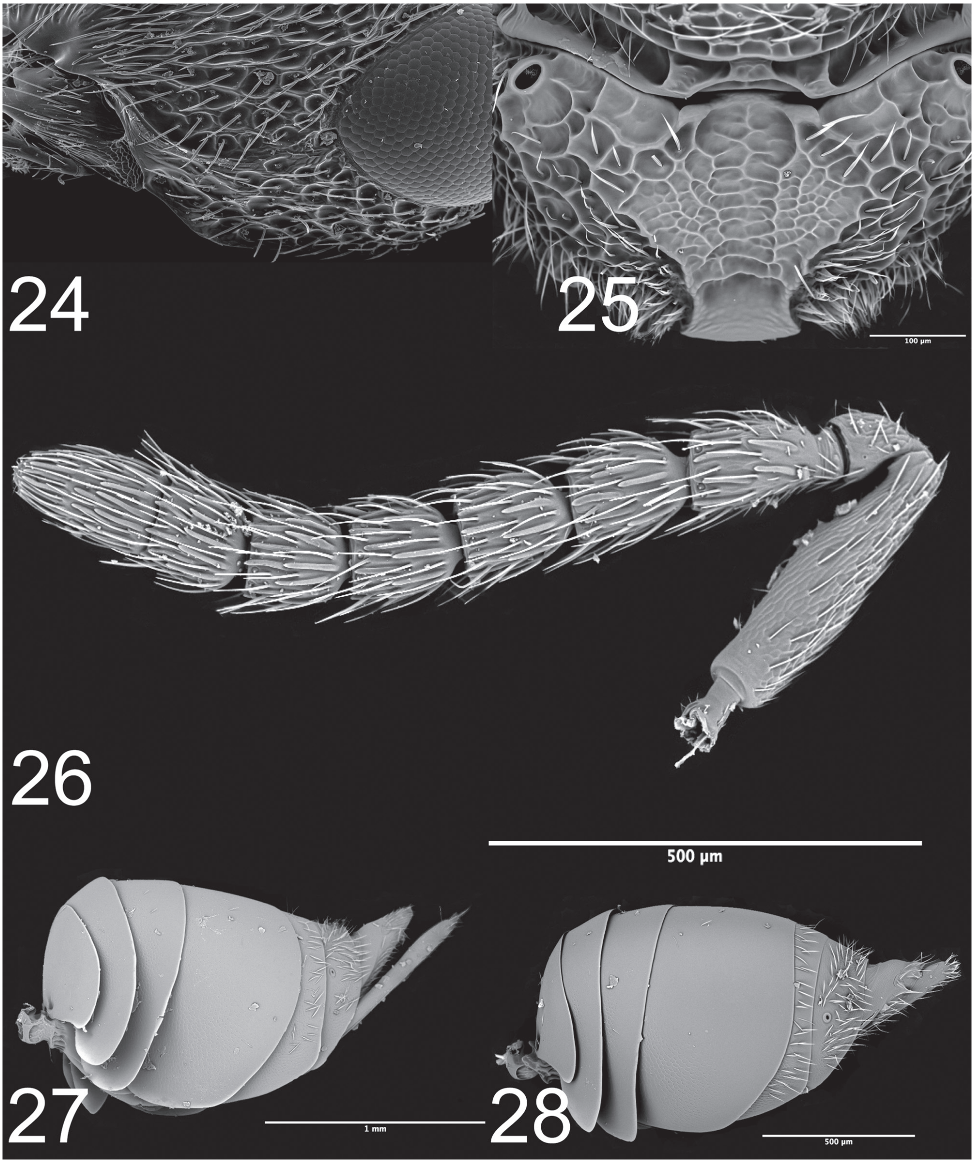


Figure 24–28. *Eurytoma shorthousei* **24** Face in lateral view. *E. longavena* **25** Propodeum **28** Female metasoma, lateral view. *E. discordans* **26** Female antenna **27** Female metasoma.

Despite the fact that the lectotype from Florida was reared from cynips on post oak, Bugbee (1967: 460) refers to *E. obtusilobae* as being restricted to *Diplolepis* galls on rose. He speculated that Ashmead erred either in gall determination or incorrectly associated *E. obtusilobae* with oak. We suspect that *E. shorthousei* was incorrectly identified as conspecific with the *E. obtusilobae* type series, given the affinities of the latter (petiole, F_1 MPS, propodeal spiracular rim, etc.) with oak associated *Eurytoma*, namely *E. sphaera*

Bugbee. Further, it appears that *E. obtusilobae* falls within the range of variation of *E. sphaera*, a species associated with post oak throughout the eastern United States. Bugbee (1951b) incorrectly referred to a holotype and neotypes of both sexes (of *E. obtusilobae*) deposited in the USNM and the Bugbee Collection (now at USNM).

The lectotype of *E. obtusilobae* is not conspecific with *E. shorthousei* as noted below although the two species do resemble each other in general habitus. They were confused due because sharing the supraclypeal striae and similar sculpture and coloration. They may be separated as *E. shorthousei* has medially notched clypeal margin (Fig. 14) versus un-notched (Fig. 15); MPS lacking in basal half F1 (Fig. 17) versus present (Fig. 16); propodeal spiracle with raised rim anteriorly (Fig. 19) versus not raised (Fig. 18); petiole anteriorly with three separate protuberances, one dorsomedial and two anterolateral (Fig. 21) versus anterodorsally produced as sharp lamina (Fig. 20). The three separate petiolar protuberances are commonly encountered in various forms across *Eurytoma* (and Eurytomidae) so are seen in all *Eurytoma* treated herein. The petiolar production as in the *E. obtusilobae* lectotype is common in Eurytomidae and is germane given it is seen in other species attacking oak-associated cynipids (e.g. *Quercus californica*, *Q. querciglobuli*, and *Q. studiosa*). However, much additional work across all *Eurytoma* associated with cynipids on oaks must be done before morphological trends are solidified.

Found at only one site in this study, *E. shorthousei*'s distribution is wide in North America given the specimens reported by Bugbee (1951b) as *E. obtusilobae*: VA, King George; MA, Gloucester; OR, La Grange; UT, Price; and CAN: Manitoba. The series Bugbee reported from New York and Minnesota could not be located. The series from Glencoe, Illinois is not *E. shorthousei*. The overall dearth of rearing records corresponds to the difficulty in locating their host galls induced by *D. radicum*, which are at ground level and often covered with soil and detritus (Shorthouse 2010). *Eurytoma shorthousei* resembles *E. discordans* in gaster shape, but can be distinguished from the latter by coloration patterns and shape and size of female gaster.

Biology. Reared from field populations of galls induced by *Diplolepis radicum* on *Rosa carolina*, *R. palustris,* and *R. woodsii*.

Material examined. Holotype. Female, **CANADA: British Columbia:** Central Okanagan; Kelowna 2 km S.E. of Kelowna airport 49.952N – 119.381W; 344m; J.D. Shorthouse & R.G. Lalonde; 14/10/1999. ex *Diplolepis radicum* on *Rosa woodsii* (USNM). Paratypes. 6F, 3M; 4M, 3F, same label data as holotype (all USNM); 2F, **CANADA: British Columbia:** Sandilands, 15–V–1967, J.C. Melvin, host gall on rose (CNCI).

Distribution. Collected in British Columbia and Manitoba (Fig. 32).

Eurytoma iniquus Bugbee

Figs 7, 33

Eurytoma iniquus Bugbee, 1951b: 253–254. Holotype female (USNM). Type data: USA, Colorado [Manitou]; associated with galls induced by *Diplolepis neglectus* (Gillette) on species of *Rosa*, April 24, 1920.

Diagnosis. This species is similar to *E. discordans*, it can be distinguished by the yellow infuscation on the inner side of the pro- and mesocoxae (Fig. 7), whereas all other species have entirely black coxae.

Females. Body length 2.2–3.0 mm. Color: Brown to black except for the following yellow to brown: inner faces of procoxa, pro- and mesofemur and tibia, apices of hindleg, protibia laterally, tip of ovipositor sheaths, all tarsomeres 1–4, wing veins (Fig. 7).

Head. 1.3× as broad as high, umbilicate punctured with small tentorial pits. Genal carina present; malar space 0.8× eye height; clypeus truncate and supraclypeal area smooth (Fig. 22). Toruli positioned about half way above lower ocular line. Intertorular space acute dorsally, with 2 rows setae. Ratio of LOL:OOL:POL is 1:1:2. Funiculars subequal in size; pedicel chalice-shaped; funicular segments fusiform; F1 slightly narrowed basally, funiculars with 2 rows of longitudinal sensilla and 3 whorls of setae; clava 2-segmented.

Mesosoma. Largely umbilicate, 1.4× as long as broad; notauli complete, shallow. Epicnemium imbricate, flattened. Mesepisternum anterior to femoral depression umbilicate; mesepimeron reticulate ventrally, striolate or smooth dorsally, with longitudinal rugae originating from the posterior margin. Precoxal tooth formed by raised adscrobal carina present in lateral view. Lateral panels of propodeum and callus umbilicately punctate, distinctly delimited from median area by carinae forming irregular setose cells, median furrow delimited, forming 2 rows of irregular foveae (Fig. 25). Procoxa imbricate, lacking setation proximally. Mesocoxal lamella absent. Metacoxa sparsely setose anteriorly and one row of setae on the posterior apical margin. Forewing hyaline, marginal vein subequal to postmarginal vein in length. Basal cell with one row of setae.

Metasoma. Gaster 1.8× as long as mesosoma in lateral view; smooth, anterior edge of gastral tergites microreticulate (Fig. 7). Petiole 0.5× as long as broad in dorsal view, with projecting lateral teeth as well as mediodorsal prong. Gaster laterally compressed, oval shaped and convex in lateral view, ovipositor slightly upturned dorsad the horizontal axis. $Gt_{1–4}$ glabrate, $Gt_{5–8}$ and apex of ovipositor sheaths setose. Gt_4 strongly emarginate on posterior margin dorsally.

Male. Body length: 1.7–2.8 mm. Color: Black, yellow areas as described for female. Sculpture as described for female. Antennae with funicular segments pedunculate, F_2–F_5 each with 2 rows of erect setae and 1 row of longitudinal sensilla (Fig. 30); scape with ventral plate in apical half. Gastral petiole in lateral view cylindrical, in dorsal view length about 1.8× as long as greatest width, 0.6× times length of metacoxa; evenly reticulate dorsally and ventrally, obliterated laterally.

Remarks. This species is likely a predator of inquiline *Periclistus* rather than the gall inducer, as they are reared from hosts that have a high rate of inquilism (Zhang et al. 2014). *Eurytoma iniquus* is particularly abundant in *Periclistus*-modified galls induced by *D. nodulosa*, which are morphologically distinct from unmodified galls (Shorthouse 2010). As the inducer larvae are killed during oviposition by the inquiline, the only inhabitants that are abundant within these galls are inquiline larvae. *Eurytoma iniquus* are morphologically distinct from the lectotype of *Eurytoma nigri-*

coxa Provancher, which has yellow coxae. The type of *E. nigricoxa* has the front and middle coxae orange-yellow similar to the remainder of the legs. The hindleg has the coxa darkish brown or dark orange-brown, somewhat lighter than the black meso- and metasoma, but much darker than the rest of the hindleg, which is similar in color to the fore- and midlegs (Gibson, pers. comm.). The CNCI has a single specimen with a Bugbee determination label as *E. nigricoxa* from Aylmer, Quebec that is reared from *Periclistus*-modified gall, but it has all the coxae black (Gibson, pers. comm.). *Eurytoma nigricoxa* is the only Nearctic species recorded in association with *Periclistus*, however this is likely an error as the lectotype lacks any biological information. Specimens from CNCI identified as *E. nigricoxa* are actually *E. iniquus* (Bugbee 1967). Closely resembles *E. longavena*, but can be distinguished from the latter by the yellow infuscation on of the pro- and mesocoxae.

Biology. Reared from galls induced by *Diplolepis bicolor* on *Rosa blanda*; *D. nodulosa* on *R. virginiana*; *D. polita, D. rosaefolii* on *R. acicularis*; and *D. variabilis* on *R. woodsii*.

Material examined (33 females, 20 males). CANADA: Alberta: Peace River, 16.VIII.1970, J.D. & M.R. Shorthouse, ex *Diplolepis polita* fall/spring emergence (4F, 2M, CNCI); Waterton Lakes National Park, 11.V.2011, J.D. & M.R. Shorthouse, ex *Diplolepis bicolor* on *Rosa blanda* (2F, CNCI). **British Columbia:** Kelowna, 20.V.2008, R.G. Lalonde, ex *Diplolepis variabilis* on *Rosa woodsii* (1M, CNCI). **Ontario:** Chelmsford, 5.V.1994, S.E. Brooks, ex *Periclistus pirata*-modified galls of *Diplolepis nodulosa* (6F, CNCI); Manitoulin Island, 29.IV.2011, J.D. Shorthouse, B.L. Smallwood & Y.M. Zhang, ex *Diplolepis nodulosa* modified by *Periclistus* sp. (4F, 2M, CNCI); Red Lake, 18.V.2002, J.D. Shorthouse & S.T. Offman, ex *Diplolepis rosaefolii* on *Rosa acicularis* (2M, CNCI); Thunder Bay, 15.V.2002, J.D. Shorthouse & S.T. Offman, ex *Diplolepis bicolor* on *Rosa blanda* (14F, 10M, CNCI). **Prince Edward Island:** Eldon, J.D. & M.R. Shorthouse, 23.VIII.1992, ex *Periclistus pirata*-modified galls of *Diplolepis nodulosa* on *Rosa virginiana* (3F, 3M, CNCI).

Distribution. British Columbia, Alberta, Ontario, Québec, and Prince Edward Island (Fig. 33).

Eurytoma longavena Bugbee

Figs 8, 25, 28, 30, 34

Eurytoma longavena Bugbee, 1951b: 249–250. Holotype female (USNM). Type data: CANADA, British Columbia [Terrance]; associated with galls induced by *Diplolepis bicolor* (Ashmead) on species of *Rosa*, 1927.

Eurytoma hebes Bugbee, 1973: 13–14. Holotype female (USNM). Type data: CANADA, Alberta [Peace River]; associated with galls induced by *Diplolepis polita* (Ashmead) on species of *Rosa*, August 16, 1970. **Syn. n.**

Eurytoma spina Bugbee, 1951b: 250–251. Holotype female (USNM). Type data: USA, Oregon [La Grande]; bred from *Diplolepis tuberculatrix versicolor* on *Rosa* species, April 12, 1920. **Syn. n.**

Diagnosis. This species differs from other eurytomids in the wholly brown to black scape, legs (except apices of femora and tibiae) (Fig. 8). Additionally, the females have large Gt_4 that covers most of Gt_5.

Females. Body length 3.0–3.7 mm. Color: Black, except for the following yellow to brown: apices of all legs, protibia laterally, tip of ovipositor sheaths, tarsomeres, wing venation (Fig. 8).

Head. 1.25× as broad as high, umbilicate punctured with small tentorial pits. Genal carina present; malar space 0.8× eye height; clypeus weakly emarginate and supraclypeal area smooth (Fig. 22). Ratio of LOL:OOL:POL is 1:1.6:2.5. Head posteriorly with postgenal lamina and postgenal grooves ridged, delimited ventrally by postgenal depression. Postgena sparsely setose. Toruli dorsad, positioned about dorsad to lower ocular line. Funicular segments subequal in size; pedicel chalice-shaped; funicular segments fusiform; F_1 slightly narrowed basally, funicular segments with 2 rows of longitudinal sensilla and 3 whorls of setae.

Mesosoma. Largely umbilicate, 1.2× as long as broad; notauli complete, shallow. Epicnemium imbricate, flattened. Mesepisternum anterior to femoral depression umbilicate; mesepimeron mesepimeron reticulate ventrally, striolate or smooth dorsally, with longitudinal rugae originating from the posterior margin. Precoxal tooth formed by raised adscrobal carina present in lateral view. Lateral panels of propodeum and callus umbilicately punctate, distinctly delimited from median area by carinae forming irregular setose cells, median furrow delimited, forming 2 rows of irregular foveae (Fig. 25). Procoxa imbricate, lacking setation proximally. Mesocoxal lamella absent. Metacoxa sparsely setose anteriorly and one row of setae on the posterior apical margin. Forewing hyaline, marginal vein subequal to postmarginal vein in length. Basal cell with one row of setae.

Metasoma. Gaster 1.3× as long as mesosoma in lateral view; smooth, anterior edge of gastral tergites microreticulate (Fig. 28). Petiole 0.6× as long as broad in dorsal view, with projecting lateral teeth as well as mediodorsal prong. Gaster laterally compressed, oval shaped and convex in lateral view, ovipositor parallel to horizontal axis. $Gt_{1–4}$ glabrate, $Gt_{5–8}$ and apex of ovipositor sheaths setose. Gt_5 emarginate to expose Gt_6 spiracle. Gt_4 weakly emarginate in dorsal view.

Male. Body length: 1.7–2.2 mm. Color: Black, yellow areas as described for female. Sculpture as described for female. Antennae with funicular segments pedunculate, F_2–F_5 each with 2 rows of erect setae and 1 row of longitudinal sensilla (Fig. 30); scape with ventral plate in apical half. Gastral petiole in lateral view cylindrical, in dorsal view length about 1.5× as long as greatest width, 0.6× times length of metacoxa; evenly reticulate dorsally and ventrally, obliterated laterally.

Remarks. This widespread species is found from galls of 7 native species of *Diplolepis* that induce galls on leaves. Additionally, it is collected from stem galls of *D. fusiformans*, a species that is closely related to *D. rosaefolii*, which induces galls on leaves (Shorthouse 2010, Plantard et al. 1998). Two generations of *E. longavena* have been recorded as "fall emergents" exit spring-induced galls (e.g. *D. polita*) as early as mid-summer to early fall, while "spring emergents" overwinter and exit from galls the following year (Shorthouse 1973, 2010). It is likely that fall emergents attack late-summer induced galls

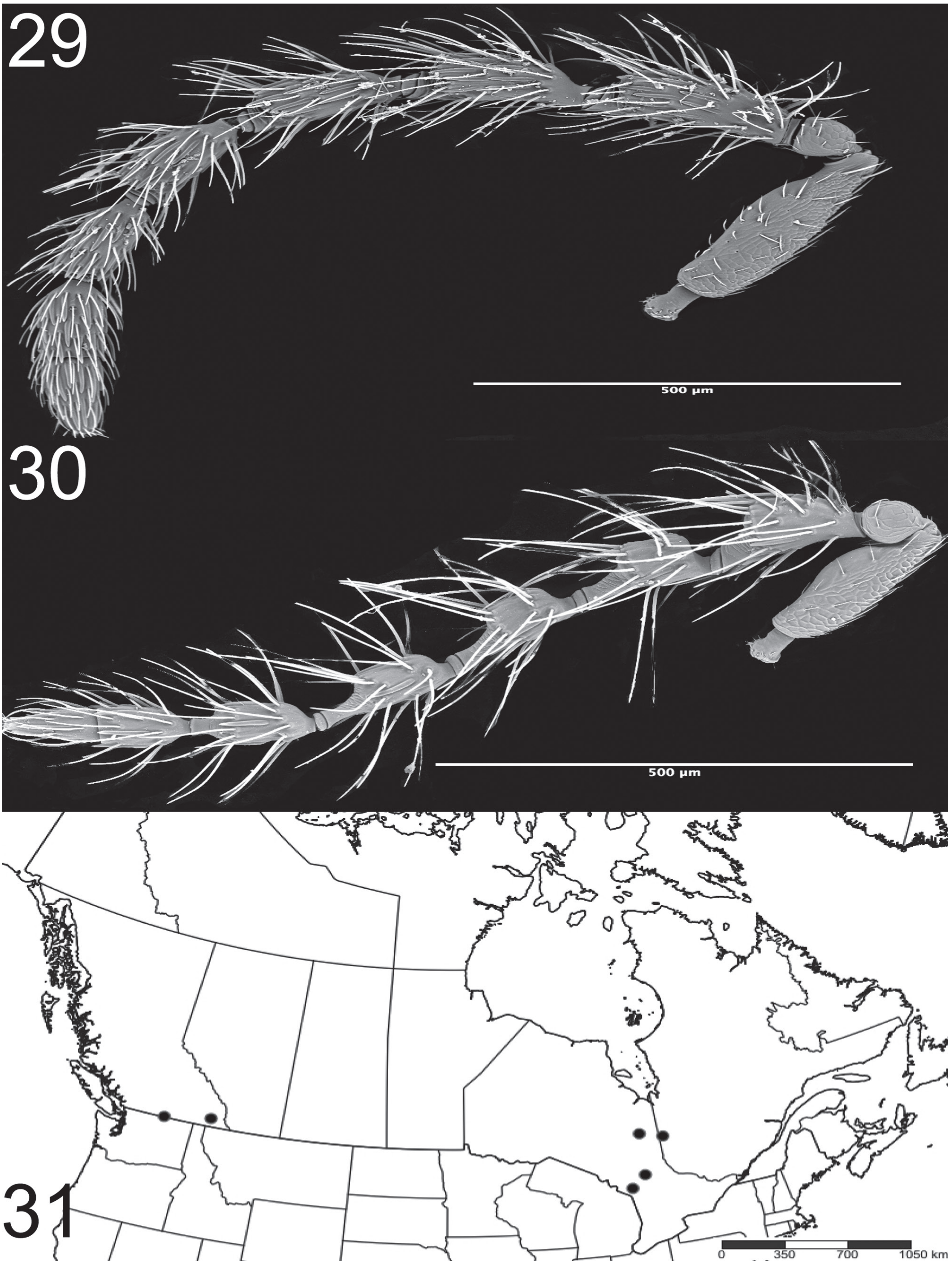


Figure 29–31. *Eurytoma discordans* **29** Male antenna. *E. longavena* **30** Male antenna **31** Known localities of *Tenuipetiolus ruber* in Canada.

(e.g. *D. nebulosa*), or late appearing galls of *D. polita* (Shorthouse 1973); however, it is unknown what factors determine this bivoltinism. *E. hebes* shares all the distinguishing characters of *E. longavena* but are smaller and brown, thus they are synonymized under the latter. This species closely resembles *E. iniquus*, but can be distinguished by the wholly black pro- and mesocoxae.

Biology. Reared from galls induced by *Diplolepis bassetti*, *D. bicolor*, *D. fusiformans* on *R. blanda*; *D. gracilis* on *R. woodsii*; *D. nebulosa* on *R. blanda*; *D. polita*, *D. rosaefolii* on *R. acicularis* and *R. woodsii*; and *D. variabilis* on *Rosa* sp.

Material examined (52 females, 22 males). CANADA: Alberta: Coaldale, 24.X.2002, J.D. Shorthouse, ex *Diplolepis nebulosa* on *Rosa woodsii* (2F, 1M, CNCI); Peace River, 16.VIII.1970, J.D. & M.R. Shorthouse, ex *Diplolepis polita* fall/spring emergence (19F, 3M, CNCI). **British Columbia:** Kelowna, 19.X.1999, R.G. Lalonde, ex *Diplolepis rosaefolii* on *Rosa woodsii* (7F, 3M, CNCI). **Ontario:** Cochrane, 24.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex *Diplolepis rosaefolii* on *Rosa acicularis* (8F, 7M, CNCI); Chelmsford, 1.X.1995, J.D.Shorthouse (2F, CNC); Manitoulin Island, 4.IX. 2010, J.D. & M.R. Shorthouse, ex *D. nebulosa* on *Rosa blanda* (2F, 4M, CNCI); Moose Factory Island, J.D. Shorthouse & M.G. St. John, ex *Diplolepis polita* on *Rosa acicularis* (4F, CNCI); Renfrew, 15.IV.2000, J.D. Shorthouse, ex *Diplolepis fusiformans* on *Rosa blanda* (2M, CNCI). **Québec:** La Sarre, B.L. Smallwood & Y.M. Zhang, 13.VII.2010, ex *D. polita* on *R. acicularis* (5F, 1M, CNCI). **Saskatchewan:** Douglas Provincial Park, J.D. & M.R. Shorthouse, 26.IX.1999, ex *D. gracilis* on *Rosa woodsii* (2F, 1M, CNCI); Pike Lake Provincial Park, J.D. & M.R. Shorthouse, 29.IX.1999, ex *D. gracilis* on *Rosa woodsii* (1F, CNCI).

Distribution. Widespread, from British Columbia, Alberta, Saskatchewan, Ontario, and Québec (Fig. 34).

Eurytoma discordans Bugbee

Figs 9, 22, 23, 26, 27, 29, 35

Eurytoma discordans Bugbee, 1951b: 220–223. Holotype female (USNM). Type data: USA, Indiana [Howe]; associated with galls induced by *Diplolepis globuloides* (Beutenmuller) = (*Diplolepis variabilis* (Bassett)) on species of *Rosa*, Dec 20, 1930.

Eurytoma acuta Bugbee, 1951b: 223–234. Holotype female (USNM). Type data: USA, Utah [Price]; associated with galls induced by *Diplolepis tuberculatrix xerophila* (Cockerell) on *Rosa*, April 20, 1920. Zhang et al. 2014 (synonymy under *Eurytoma discordans*).

Eurytoma calcarea Bugbee, 1951b: 240–249. Holotype female (USNM). Type data: USA, Utah [Wellsville]; associated with galls induced by *Diplolepis variabilis* (Bassett), September 6, 1927. Zhang et al. 2014 (synonymy under *Eurytoma discordans*).

Diagnosis. Females are distinguished from other species by the S-curved metasoma that is larger than head plus mesosoma (Fig. 27) and the sharply upturned ovipositor dorsad the horizontal axis of the metasoma. Males have elongated funicular segments and yellow fore- and midlegs similar to *E. obtusilobae*, however, it differs in the black pedicle and tegulae.

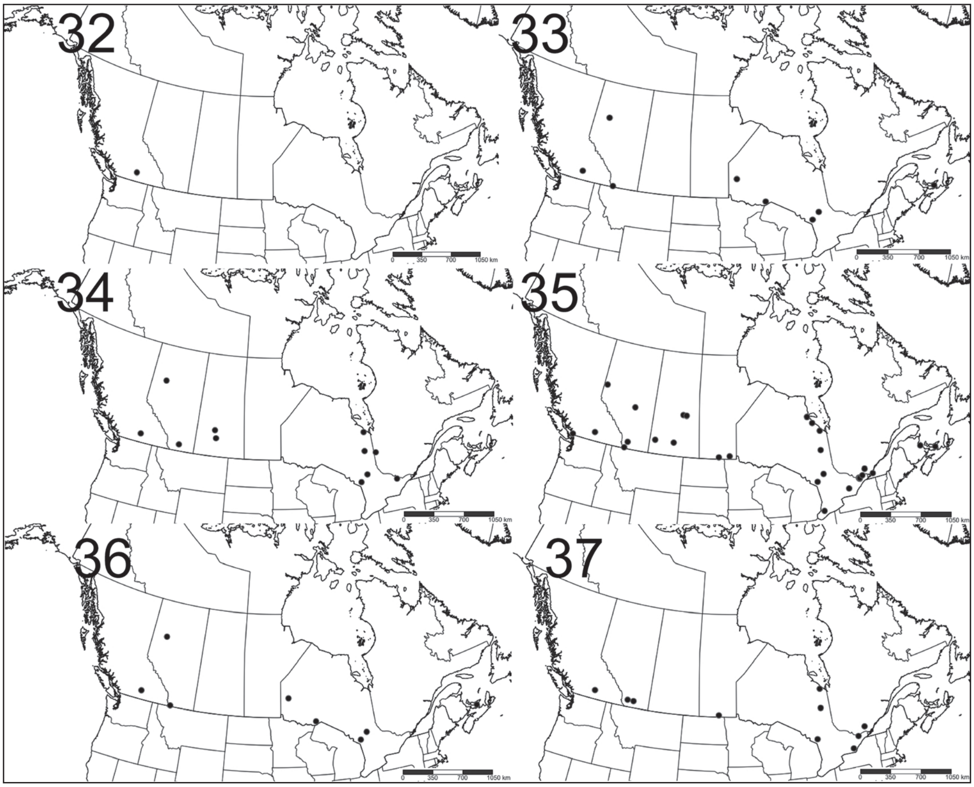


Figure 32–37. 32 Known localities of *Eurytoma shorthousei* in Canada **33** Known localities of *E. iniquus* in Canada **34** Known localities of *E. longavena* in Canada **35** Known localities of *E. discordans* in Canada. **36.** Known localities of *E. imminuta* in Canada **37** Known localities of *E. spongiosa* in Canada.

Females. Body length 2.1–5.0 mm. Color: Black except for the following yellow – basal half of scape, pro- and mesofemur, basal pro- and mesotibia, apex of metatibia, tip of ovipositor sheaths, tarsomeres 1–4, wing veins (Fig. 9).

Head. 1.2× as broad as high, umbilicate punctured (Fig. 22). Genal carina present, evenly sculptured; malar space 0.7× eye height, clypeus strongly emarginate and supraclypeal area smooth (Fig. 22). Toruli positioned slightly above lower ocular line. Intertorular space acute dorsally, with 2 rows setae. Ratio of LOL:OOL:POL is 1:1.3:2. Head posteriorly with postgenal lamina and postgenal grooves ridged, delimited ventrally by postgenal depression. Postgena evenly setose (Fig. 23). Funicular segments subequal in size, longer than wide; pedicel chalice-shaped; funicular segments fusiform; F_1 slightly narrowed basally, funicular segments with 2 rows of longitudinal sensilla and 3 whorls of setae (Fig. 26).

Mesosoma. Largely umbilicate, 1.5× as long as broad; notauli complete, shallow. Epicnemium imbricate, flattened, with superficial submedial, shallow depressions to receive procoxa. Mesepisternum anterior to femoral depression umbilicate; mesepimeron reticulate ventrally, striolate or smooth dorsally, with longitudinal rugae originating from the posterior margin. Precoxal tooth formed by raised adscrobal carina present in lateral view. Lateral panel of propodeum and callus with umbilic punctures, distinctly delimited from median area by carinae forming irregular setose cells, median furrow delimited, forming 2 rows of irregular foveae (Fig. 25). Procoxa imbricate, lacking setation proximally. Mesocoxal lamella absent. Metacoxa densely setose along anterior margins, glabrate with one row of setae along posterior distal margin. Forewing hyaline, marginal vein and postmarginal vein subequal in length. Basal cell evenly setose.

Metasoma. Gaster 1.8× as long as mesosoma in lateral view; smooth, anterior edge of gastral tergites microreticulate (Fig. 27). Petiole 0.7× as long as broad in dorsal view, with projecting lateral teeth as well as mediodorsal prong. Gaster laterally compressed, S-curve shaped and not convex in lateral view, ovipositor upturned dorsad horizontal axis. Gt_{1-3} glabrate, Gt_4 with 1–4 setae lateromedially, Gt_{5-8} and apex of ovipositor sheath densely setose. Gt_4 strongly emarginate on posterior margin dorsally.

Male. Body length: 1.7–3.1 mm. Color: Black, yellow areas as described for female. Sculpture as described for female (Fig. 29). Antennae with funicular segments longer than wide, pedunculate, F_2–F_5 each with 2 or more rows of apressed setae and 2 rows of longitudinal sensillae (Fig. 29); scape with ventral plaque in apical half. Gastral petiole in lateral view cylindrical, in dorsal view length about 1.5× as long as greatest width, 0.7× times length of metacoxa; evenly reticulate dorsally and ventrally, obliterated laterally.

Remarks. This is a widespread and morphologically variable species. Bugbee (1951b) originally divided this species into 3 based on subtle morphological differences and distribution: the Eastern populations as *E. discordans* and western populations as *E. acuta* (5 subspecies), and those smaller in size as *E. calcarea* (6 subspecies). Upon examining additional materials it was noted that the degree of infuscation on legs and scape are variable within this species and thus cannot be used as reliable distinguishing characters. The molecular evidence presented in Zhang et al. (2014) also support the monophyly of this group, albeit with the highest intra-specific divergence compared to other eurytomids. Considering there are no distinct geographical or host differences that support three distinct species, *E. acuta* and *E. calcarea* along with their associated subspecies were synonymized under *E. discordans* pending further molecular or ecological studies. It is also likely that *Eurytoma incerta* Fullaway is the senior synonym of *E. discordans* given the similar description by Bugbee (1951b). Closely resembles *E. obtusilobae*, but can be distinguished from the latter by the coloration of the tegulae, scape, and shape of the female metasoma.

Biology. Reared from field populations of galls induced by *D. bicolor* on *R. blanda*; *D. nodulosa* on *R. woodsii*; *D. spinosa* on *R. blanda* and *Rosa rugosa* Thunb.; *D. radicum* on *R. acicularis*; *D. tumida* on *R. woodsii*; *D. variabilis* on *R. woodsii*. Also reared from galls of *Diastrophus nebulosus* (Osten Sacken) on *Rubus* spp..

Material examined (197 females, 120 males). CANADA: Alberta: Beaverlodge, 1933 (1F, CNC); Head-Smashed-In Buffalo Jump, 10.V.2011, J.D. & M.R. Shorthouse, ex *Diplolepis tumida* on *Rosa woodsii* (3F, 4M, CNCI); Edmonton, 20.IV.1942, R.W.Salt (1F, CNC); Edmonton, 6.VI.1946, R.M.Mason, ex rose gall (5F, CNC); Waterton Lakes National Park, 9.V.2007, J.D. & M.R. Shorthouse, ex *Diplolepis bicolor*/*Diplolepis nodulosa* modified by *Periclistus* sp. on *Rosa woodsii* (24F, 20M, CNCI). **British**

Columbia: Kelowna airport, 20.V.2008, R.G. Lalonde, ex *Diplolepis variabilis* on *Rosa woodsii* (2F, 1M, CNCI); Summerland, 2.V.1959, R.E. Leech, ex *Rosa* (3F, CNC); Surrey, 9.IV.1954. K. Yamanaka, ex *Rubus* (4F, 2M, CNC). **Manitoba:** Morden, 1.IX.1986, J.D. Shorthouse, ex *Rosa woodsii* (7F, 3M, CNCI); Sandlands F.R., em 1.VI.1944, F.I.Survey, stem gall on raspberry (2F, CNC). **New Brunswick:** Kouchibouguac National Park, 8.VIII.1977, S.J. Miller (3F, 9M, CNC). **Ontario:** Attawapiskat, 18.V.2005, M.J.T. Bodnar, ex *Diplolepis spinosa* on *Rosa blanda* (22F, 20M, CNCI); Bell's Corners, 13.V.1940. O. Peck, Host *Diastrophus nebulosus* (1F, CNC); Chelmsford, 5.V.1994, S.E. Brooks, ex *Periclistus pirata*-modified galls of *Diplolepis nodulosa* (4F, 2M, CNCI); Cochrane, 24.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex *Diplolepis spinosa* on *Rosa blanda* (5F, 5M, CNCI); Fort Albany, 28.V.2005, M.J.T. Bodnar, ex *Diplolepis radicum* on *Rosa acicularis* (4F, 2M, CNCI); Jockvale, 8–27.V.1955, O. Peck, *Rosa blanda*/*rugosa* (42F, 19M, CNC); Manitoulin Island, 29.IV.2011, J.D. Shorthouse, B.L. Smallwood & Y. M. Zhang, ex *Diplolepis nodulosa* modified by *Periclistus* sp. (2F, 1M, CNCI); Marmora, 20.VI.1945. G.R.Hammond. Cynipid gall on wild rose (4F, CNC); Merivale, 17.VI.1954. O. Peck, Host gall *Periclistus pirata* ex *Rosa blanda* (3F, 1M, CNC); Moose Factory Island, 23.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex *Diplolepis spinosa* on *Rosa blanda* (5F, 5M, CNCI); One Sided Lake, em XII.1960. S.M. Clark (2F, CNC); Ottawa, 17–25.V.1955, O. Peck, ex *Rosa rugosa* (19F, 4M, CNC); Thamesville, 4.VII.1962. S.M.Clark, ex gall of wild rose (3F, CNC); Rockcliffe, 27.V.1959. S.M.Clark. gall on *Rosa* sp. (3M, CNC). **Prince Edward Island:** Eldon, J.D. & M.R. Shorthouse, 23.VIII.1992, ex *Periclistus pirata*-modified galls of *Diplolepis nodulosa* on *Rosa virginiana* (4F, 3M, CNCI). **Québec:** Aylmer, VIII.1939. E.G. Lester, Host *Periclistus sylvestris* (2F, 5M, CNC); Chrysostome, 23.VI.1986, ex Cynipidae on *Rosa* sp. (1F, CNC); Lac Mercier, 7.VIII.1937. G.S. Walley. (1F, CNC); Montréal, O. Peck, 22.V–11.VI.1962, ex *D. radicum* (6F, 2M, CNC). **Saskatchewan:** Caron, em 20–23.II.1951, F.I.Survey, rose gall (1F, 3M, CNC); Great Sand Hills, 22.IX.1999, J.D. & M.R. Shorthouse, ex *Diplolepis radicum* on *Rosa woodsii* (6F, 6M, CNCI); Snowden, 18.VII.1944, O. Peck (1F, CNC); White Fox, 17.VI.1944, O. Peck (3F, CNC).

Distribution. Widespread, from British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Québec, New Brunswick, and Prince Edward Island (Fig. 35).

Eurytoma imminuta Bugbee

Figs 10, 36

Eurytoma imminuta Bugbee, 1951b: 259–260. Holotype female (USNM). Type data: USA, Nevada [Pyramid Lake]; associated with galls induced by *Diplolepis variabilis* (Bassett) on *Rosa puberulenta*, May 20, 1929.

Diagnosis. *Eurytoma imminuta* can be distinguished from most *Eurytoma* species by black tegula and scape. *E. imminuta* differs from *E. spongiosa* by their small, oval metasoma that is not laterally compressed (Fig. 10).

Females. Body length 1.9–4.0 mm. Color: Black except for the following yellow – basal half of scape, apical fifth of pro- and mesofemur, basal pro- and mesotibia, apex of hindlegs, tip of ovipositor sheaths, tarsomeres 1–4, wing veination (Fig. 10).

Head. 1.2× as broad as high, umbilicate punctured with small tentorial pits. Genal carina present; malar space 0.8× eye height, supraclypeal area smooth (Fig. 22). Toruli positioned slightly above lower ocular line. Intertorular space obtuse dorsally, with 2 rows setae. Ratio of LOL:OOL:POL is 1:1.2:2.4. Head posteriorly with postgenal lamina and postgenal grooves ridged, delimited ventrally by postgenal depression. Postgena sparsely setose. Funicular segments subequal in size, fusiform; pedicel chalice-shaped; F_1 slightly narrowed basally, funicular segments with 2 rows of longitudinal sensilla and 3 whorls of setae; clava 2-segmented.

Mesosoma. Largely umbilicate, 1.5× as long as broad; notauli complete, shallow. Epicnemium imbricate, flattened. Mesepisternum anterior to femoral depression umbilicate; mesepimeron mesepimeron reticulate ventrally, striolate or smooth dorsally, with longitudinal rugae originating from the posterior margin. Precoxal tooth formed by raised adscrobal carina present in lateral view. Lateral panel of propodeum and callus with umbilicate punctures, distinctly delimited from median area by carinae forming irregular setose cells, median furrow delimited, forming 2 rows of irregular foveae (Fig. 25). Procoxa imbricate, lacking setation proximally. Mesocoxal lamella absent. Metacoxa densely setose along anterior margins, glabrate with one row of setae along posterior distal margin. Forewing hyaline, marginal vein and postmarginal vein subequal in length.

Metasoma. Gaster 1.2× as long as mesosoma in lateral view; smooth, anterior edge of gastral tergites microreticulate (Fig. 10). Petiole 0.5× as long as broad in dorsal view, with projecting lateral teeth as well as mediodorsal prong. Gaster not laterally compressed, oval shaped and convex in lateral view, ovipositor parallel to horizontal axis. $Gt_{1–3}$ glabrate, Gt_4 with 1–4 setae lateromedially, $Gt_{5–7}$ and apex of ovipositor sheath densely setose. Gt_4 strongly emarginate on posterior margin dorsally.

Male. Body length: 1.7–2.0 mm. Color: Black, yellow areas as described for female. Sculpture as described for female. Antennae with funicular segments pedunculate, F_2–F_5 each with 2 rows of erect setae and 1 row of longitudinal sensilla (Fig. 30); scape with ventral plate in apical half. Gastral petiole in lateral view cylindrical, in dorsal view length about 1.5× as long as greatest width, 0.5× times length of metacoxa; irregularly reticulate dorsally and ventrally, obliterated laterally.

Remarks. *E. imminuta* in the USNM collection can be separated from *E. spongiosa* by the metasomal character noted. It is best to keep them as separate species based on the examination of hundreds of *E. spongiosa* in the collection in which there are smaller specimens resembles the *E. imminuta* in metasomal shape. The profemur leg coloration character is inconsistent within *E. spongiosa*.

Biology. Reared from galls induced by *Diplolepis ignota* on *R. woodsii*, *D. nebulosa*, *D. polita*, *D. spinosa* on *R. blanda*, *R. rugosa*; *D. rosae* on *R. canina*, *D. triforma* on *R canina*; *D. tumida* on *R. woodsii*, and *D. variabilis*.

Material examined (101 females, 50 males): CANADA: Alberta: Coaldale, 12.V.2007, J.D. & M.R. Shorthouse, ex *Diplolepis ignota* on *Rosa woodsii* (2F, CNCI);

Head-Smashed-In Buffalo Jump, 10.V.2011, J.D. & M.R. Shorthouse, ex *Diplolepis tumida* on *Rosa woodsii* (3F, 2M, CNCI). **British Columbia:** Kelowna, 20.V.2008, R.G. Lalonde, ex *Diplolepis variabilis* on *Rosa woodsii* (3F, 1M, CNCI). **Ontario:** Cochrane, 24.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex *Diplolepis spinosa* on *Rosa blanda*, (5F, CNCI); Kanata, 19.IV.2006, J.D. & M.R. Shorthouse, ex *Diplolepis spinosa* on *Rosa rugosa* (2F, 6M, CNCI); Manitoulin Island, 2.V.2009, J.D. Shorthouse & J.D. Renelli, ex *Diplolepis triforma* on *Rosa canina* (7F, 3M, CNCI); Manitoulin Island, 3.V.2009, J.D. Shorthouse & J.D. Renelli, ex *Diplolepis rosae* on *Rosa canina* (5F, 1M, CNCI); Manitoulin Island, 6.V.2010, J.D. Shorthouse & Y.M. Zhang, ex *Diplolepis triforma* on *Rosa canina* (9F, 5M, CNCI); Moose Factory Island, 23.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex *Diplolepis spinosa* on *Rosa blanda*, (5F, 5M, CNCI); Picton, 28.IV.2003, J.D. Shorthouse, ex *Diplolepis mayri/D. rosae* on *Rosa canina* (12F, 7M, CNCI). **Manitoba:** Mordon, 1.IX.1986, J.D. Shorthouse, ex *Diplolepis spinosa* on *Rosa woodsii* (31F, 18M, CNCI). **Québec:** Mt. Tremblant, 23.XII.2004, M.J.T. Bodnar, ex *Diplolepis spinosa* on *Rosa rugosa* (17F, 2M, CNCI).

Distribution. Widespread, from British Columbia, Alberta, Manitoba, Ontario, and Québec (Fig. 36).

Eurytoma spongiosa Bugbee

Figs 11, 37

Eurytoma spongiosa Bugbee, 1951b: 254–258. Holotype female (USNM). Type data: USA, Indiana [Bloomington]; associated with galls induced by *Diplolepis rosae* (L.) on species of *Rosa*, April 14, 1933.

Diagnosis. Similar to *E. imminuta*, but metasoma larger and more elongated (Fig. 11).

Females. Body length 1.0–4.0 mm. Color: Black except for the following yellow - basal half of scape, posterior half profemur, mesofemur, basal pro- and mesotibia, apex of hindlegs, tip of ovipositor sheaths, tarsomeres 1–4, wing veination (Fig. 11).

Head. 1.3× as broad as high, with umbilicate punctures with small tentorial pits. Genal carina present; malar space 0.8× eye height; supraclypeal area smooth (Fig. 22). Toruli positioned slightly above lower ocular line. Intertorular space obtuse dorsally, with 2 rows setae. Funicular segments subequal in size, fusiform; pedicel chalice-shaped; F_1 slightly narrowed basally, funicular segments with 2 rows of longitudinal sensillae and 3 whorls of setae; clava 2-segmented. Ratio of LOL:OOL:POL is 1:1.2:2.5. Head posteriorly with postgenal lamina and postgenal grooves ridged, delimited ventrally by postgenal depression. Postgena sparsely setose.

Mesosoma. Largely umbilicate, 1.5× as long as broad; notauli complete, shallow. Epicnemium imbricate, flattened. Mesepisternum anterior to femoral depression umbilicate; mesepimeron reticulate ventrally, striolate or smooth dorsally, with longitudinal rugae originating from the posterior margin. Precoxal tooth formed by raised adscrobal carina present in lateral view. Lateral panels of propodeum and callus with umbilicate punctures, distinctly delimited from median area by carinae forming irregular setose cells, median furrow delimited, forming 2 rows of irregular foveae (Fig. 25). Procoxa imbricate, lacking setation proximally. Mesocoxal lamella absent. Metacoxa densely setose along anterior margins, glabrate with one row of setae along posterior distal margin. Forewing hyaline, marginal vein and postmarginal vein subequal in length.

Metasoma. Gaster 1.2× as long as mesosoma in lateral view; smooth, anterior edge of gastral tergites microreticulate (Fig. 11). Petiole 0.5× as long as broad in dorsal view, with projecting lateral teeth as well as mediodorsal prong. Gaster not laterally compressed, oval shaped and convex in lateral view, ovipositor parallel to horizontal axis. Gt_{1-3} glabrate, Gt_4 with 1–4 setae lateromedially, Gt_{5-8} and apex of ovipositor sheath densely setose. Gt_4 strongly emarginate on posterior margin dorsally.

Male. Body length: 1.7–2.2 mm. Color: Black, yellow areas as described for female. Sculpture as described for female. Antennae with funicular segments pedunculate, F_2–F_5 each with 2 rows of erect setae and 1 row of longitudinal sensillae (Fig. 30); scape with ventral plaque in apical half. Gastral petiole in lateral view cylindrical, in dorsal view length about 1.5× as long as greatest width, 0.6× times length of metacoxa; irregularly reticulate dorsally and ventrally, obliterated laterally.

Remarks. Few consistent morphological differences were found between *E. imminuta* and *E. spongiosa* (identified in Zhang et al. 2014 as *E. spongiosa* 1, and *E. spongiosa* 2, respectively) despite deep divergence in *COI* sequences and differences in host records (Zhang et al. 2014). Considering there are examples of other members of the *Eurytoma rosae* species group having been identified as genetically distinct but morphologically indistinguishable (Ács et al. 2002, Gómez et al. 2011), the two species are therefore considered as distinct. Variation in size and degrees of infuscation on legs. Bugbee (1951b: 259) indicated that *E. flavicruensa* may represent "the extreme western equivalent of *E. spongiosa*." Only the holotype is intact and all of the paratypes are lacking metasoma, much of their legs, and antennae. He indicates the pro- and mesocoxae are yellow, but they are actually blackish on their lateral surfaces. Given the paucity of material of *E. flavicruensa*, evaluation of its species status must await the collection of additional topotypical material.

Biology. Reared from galls induced by *D. fusiformans* on *R. blanda*; *Diplolepis ignota* on *R. arkansana* Porter; *D. nebulosa* on *R. blanda*; *D. polita* on *R. acicularis*; *D. triforma* on *R. acicularis* and *R. canina*; and *D. variabilis*.

Material examined (28 females, 25 males). CANADA: Alberta: Coaldale, 12.V.2007, J.D. & M.R. Shorthouse, ex *Diplolepis ignota* on *Rosa arkansana* (4F, 2M, CNCI). **Ontario:** Cochrane, 24.IV.2010, J.D. Shorthouse & Y.M. Zhang, ex *Diplolepis triforma* on *Rosa acicularis*, (11F, 7M, CNCI); Manitoulin Island, 2.V.2009, J.D. Shorthouse & J.D. Renelli, ex *Diplolepis triforma* on *Rosa acicularis*, (1F, 3M, CNCI); Manitoulin Island, 6.V.2010, J.D. Shorthouse & Y.M. Zhang, ex *Diplolepis triforma* on *Rosa canina*, (1F, 5M, CNCI); Manitoulin Island, 4.IX.2010, J.D. & M.R. Shorthouse, ex *Diplolepis nebulosa* on *Rosa blanda*, (1F, CNCI); Moose Factory Island, 13.VIII.1998, J.D. Shorthouse & M.G. St. John, ex *Diplolepis polita* on *Rosa acicularis* (1M, CNCI);

Renfrew, 15.IV.2000, J.D. Shorthouse, ex *Diplolepis fusiformans* on *Rosa blanda* (1M, CNCI). **Québec:** La Sarre, 13.VII.2010, B.L. Smallwood & Y.M. Zhang, ex *Diplolepis polita* on *Rosa acicularis* (5F, 1M, CNCI). **Saskatchewan:** Maple Creek, 10.V.2003, J.D. & M.R. Shorthouse, ex *Diplolepis ignota* on *Rosa arkansana* (5F, 5M, CNCI).

Distribution. From Alberta, Saskatchewan, Ontario, and Québec (Fig. 37).

Discussion

Taxonomic recognition of chalcid wasps of the family Eurytomidae is notoriously difficult, as is the case with members within the genus *Eurytoma* associated with galls of *Diplolepis* (Shorthouse 2010). All species associated with galls of *Diplolepis* are conservative in regards to adult morphology. The distinguishing features presented by Bugbee (1951a, 1951b, 1967, 1973) are unfortunately often ambiguous due to overlapping measurements and intermediate character states. While the results of the current study indicate that morphological characters on the posterior head capsule, male antennae, and petiole are particularly useful in species delimitation, they are often obscured on intact specimens and cannot be seen clearly unless dissections are performed.

The overall morphological similarities between the Canadian species of *Eurytoma* with other members of the *rosae* species group found in Europe suggest that these species shared a common evolutionary line. As the *rosae* group is most diverse in Europe (Lotfalizadeh et al. 2007b, Delvare pers. comm.), it seems likely that the six *Eurytoma* species found in Canada represent an extension of this species group, although the low number of Nearctic *rosae* group species could simply be the result of insufficient taxonomic study.

Although eurytomids are known from a wide variety of hosts (Lotfalizadeh et al. 2007b), those examined as part of this project were only found associated with galls induced by *Diplolepis* on native wild rose species and the introduced Japanese rose *Rosa rugosa* and the European rose *R. canina*. It is difficult to see overall patterns of host specificity by eurytomids associated with rose galls, as recorded in this paper; however, now that the species are more clearly delineated, a more detailed examination of the specimens collected from across Canada can be undertaken. A good start would be the wet collections of JDS now stored at Edinburgh University. There are thousands of eurytomids in this collection all associated with host galls, the host wild rose, and collection localities.

Even without this more extensive analysis, some trends are already apparent. Firstly, the abundance of eurytomids in the galls of all species of *Diplolepis* from across Canada indicate that eurytomids are so closely associated with galls of *Diplolepis* that some species, or certain populations of these species, are now restricted to rose galls. Eurytomids attack both galls initiated in the spring (*D. polita* and *D. spinosa*) and those initiated later in the season (*D. nebulosa* and *D. ignota*) indicating that the emergence periods of eurytomids are lengthy enabling them to track different periods of gall initiation. Some species such as *T. ruber* and *E. discordans* attack both leaf and stem galls.

Eurytoma iniquus and *E. discordans* attack both galls inhabited only by an inducer where they feed as koinobionts, along with inquiline-modified galls of the same *Diplolepis* where they feed as predators on immature *Periclistus* and then chew into several *Periclistus*-induced chambers to consume larvae (Shorthouse 1973). Some species such as *T. ruber*, *E. imminuta* and *E. spongiosa* feed on inhabitants of the *D. rosae* gall which is a species introduced from Europe (Shorthouse 2001). All three species of *Diplolepis* introduced from Europe are naturalized in Canada, along with their European host roses (Shorthouse 2001) and are inhabited by eurytomids. It is not known if these eurytomids came from Europe with galled host plants or if the endemic eurytomids have moved onto the European galls once they became established in Canada. We suspect *E. imminuta* and *E. discordans* have followed *D. spinosa* and *D. triforma* onto introduced *R. rugosa* which are grown in urban gardens where they are just as heavily attacked by eurytomids as are galls growing on wild roses in their natural habitat.

Three of the most widely distributed galls in Canada are those induced by *D. polita*, *D. spinosa* and *D. triforma* and all are heavily attacked by eurytomids. These observations suggest that eurytomids are highly plastic in their choice of hosts, ability to locate roses and their galls in all parts of the range of each, feed as predators or koinobionts on all species of gall inhabitants, tolerate cold and dry conditions of northern Canada, and in the case of *E. longavena*, have two generations per year when populations of the same gall appear both in the spring and mid-summer (Shorthouse 1973). Obtaining large numbers of eurytomids from rose galls over long distances is easy for once the mature galls are collected in the spring, gall inhabitants are emerged in the laboratory. Collecting galls induced by one species of *Diplolepis* in one habitat and emerging the adults, accurately establishes the species of inhabitants at that locality.

While the distribution of eurytomids in this study only includes localities within Canada, it is likely representative of the Nearctic fauna even though most diversity of wild roses occurs within Canada (Shorthouse 2010). As part of the contribution of this study, we have reported an expansion of known localities for seven species of eurytomids. While the full ranges of these eurytomids are still to be determined, we suspect their distribution mirrors that of their hosts. The exceptions are *E. obtusilobae* and *T. ruber* which apparently are only found in disjunctive populations in Canada, although past literature suggests it is widespread within USA (Bugbee 1951a, 1951b). As a result of our study, new provincial records were made from British Columbia to Prince Edward Island since the last revision (Bugbee 1951a, 1951b). Bugbee (1951b, 1967 and 1973) used distributions as an important criterion for delimiting species of *Eurytoma*. The range of expansions in our study joins the previously disjunctive populations and along with molecular data (Zhang et al. 2014) supports the synonymization of these species. In addition to the galls of *Diplolepis*, *E. discordans* and *T. ruber* have also been collected from galls of *Diastrophus* spp. on raspberry. Given the polyphagous nature of many of these eurytomid species, the full host range is likely much wider than currently known. Thus, using host records alone to distinguish morphologically similar species is error-prone and a source of confusion when identifying eurytomids (Lotfalizadeh et al. 2007b).

The systematic placement of the Eurytomidae within the superfamily Chalcidoidea has been controversial in past studies (Lotfalizadeh et al. 2007b, Gates 2008, Munro et al. 2011, Heraty et al. 2013). The species treated by Bugbee (e.g. 1951a, 1951b, 1967) undoubtedly includes many synonymous species, and a revision of all Nearctic eurytomids is needed. Details of the evolutionary relationships of *Diplolepis* with their host roses and the relationships between *Eurytoma* and other gall inhabitants using modern molecular techniques, remain to be undertaken. With further insight into the taxonomy of eurytomids associated with cynipid rose galls, a new and exciting approach has been provided for future phylogenetic studies of the whole superfamily Chalcidoidea.

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RESEARCH ARTICLE

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New species and designation of primary types in Neotropical carpenter bees of the genus *Xylocopa* Latreille (Hymenoptera, Apidae)

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Abstract

We describe and figure two distinctive new species of Neotropical carpenter bees of the genus *Xylocopa*, *X.* (*Schonnherria*) *barbarae* **sp. n.** from Bolivia, and *X.* (*Schonnherria*) *bigibbosa* **sp. n.** from Peru. To stabilize the names, we designate a neotype for *X.* (*Schonnherria*) *dimidiata* Latreille and lectotypes for the following species: *X.* (*Neoxylocopa*) *andica* Enderlein, *X.* (*Neoxylocopa*) *columbiensis* Pérez, *X.* (*Neoxylocopa*) *mendozana* Enderlein, and *X.* (*Neoxylocopa*) *rufidorsum* Enderlein. For each species, we provide comparative diagnosis, taxonomic comments, and summarize its distribution.

Keywords

Anthophila, Bolivia, Peru, taxonomy

Introduction

Carpenter bees of the genus *Xylocopa* Latreille (Apidae: Xylocopini) are a cosmopolitan, commonly collected group of promising pollinators. The group contains more than 470 species in 31 subgenera worldwide (Michener 2007), with some species effectively used as pollinators of diverse crops, including passion fruit, squash, tomato, Brazilian nut tree, and eggplant (e.g., Keasar 2010, Giannini et al. 2015). As for many other bees, the species-level systematics of *Xylocopa* remains troubling, with many species known either only from the type specimen, from small number of specimens, or from a single sex. Identification keys to species are also lacking for many subgenera or regions, and basic biological aspects, such as nesting behavior, are known from common species only (Gonzalez et al. 2013, Lucia et al. 2014, 2015). Such obstacles not only hinders the true diversity of these bees, but also interferes with current conservation and sustainable use efforts on these pollinators.

The purpose of this paper is to address the nomenclature of some species of the subgenera *Xylocopa* (*Neoxylocopa*) Michener and *X.* (*Schonnherria*) Lepeletier de Saint Fargeau, the two most species-rich groups in the Western Hemisphere. We describe two new species of *X.* (*Schonnherria*) and designate primary types for five species in both subgenera to stabilize their names. We also provide comments on the taxonomy and distribution of each species.

Material and methods

Morphological terminology generally follows that of Hurd and Moure (1963) and Michener (2007). Species descriptions emphasize shapes and dimensions of structural features that appeared to be reliable in species recognition. Measurements were taken with an ocular micrometer attached to an Olympus SZ-60 stereomicroscope. We measured total body length from the head to the apex of metasoma in lateral view; forewing length from the apex of the costal sclerite to the wing apex; mesosoma width as the maximum distance between the outer borders of tegulae; and metasoma width as the maximum width of the second tergum. We used the following abbreviations: T, S, and OD for metasomal tergum and sternum, and maximum diameter of the median ocellus, respectively. Supraorbital line refers to the upper ocular tangent and we used it to indicate the position of the lateral ocelli in frontal view. We prepared photomicrographs using a Canon 7D digital camera attached to Infinity K-2 long-distance microscope lens, and assembled them with the Combine ZM software package. We reproduced information on label and locality data as they appear on each label, with a slash (/) separating portions appearing on different labels attached to the same specimen. Annotations to clarify information as it appears on labels are indicated in square brackets.

We used the following institutional acronyms for repositories holding material: **NHML**, Natural History Museum, London, England (David Notton); **MNHN**, Museo National D'Histoire Naturelle, Paris, France (Agniėle Touret-Alby); **MIZ**, Museum i Instytut Zoologii Polskiej Akademii Nauk, Warsaw, Poland (Tomasz Hufleit); **MLP**, Museo de La Plata, La Plata, Argentina (Analia Lanteri, Alberto Abrahamovich); **SEMC**, Snow Entomological Collection, Division of Entomology, University of Kansas Natural History Museum, Lawrence, Kansas, USA (Michael S. Engel, Zach Falin, Jennifer Thomas); and **USNM**, National Museum of Natural History, Washington, DC, USA (Sean Brady, Brian Harris).

Systematics

Genus *Xylocopa* Latreille, 1802
Subgenus *Neoxylocopa* Michener, 1954

Xylocopa (*Neoxylocopa*) *andica* Enderlein, 1913
Fig. 1

Xylocopa (*Neoxylocopa*) *andica* Enderlein, 1913: 160 (Lectotype: USNM; ♀, Peru, Chanchamayo) (new lectotype designation).

Diagnosis. This species can be distinguished by the combination of the following features: large body size (body length ~23 mm); pubescence black; integument dark brown to black throughout, basal three terga with weak, dark brown bands; wings dark brown with green highlights apically, violet basally; labrum basally with three longitudinally elongated tubercles; gena and supraocular areas sparsely punctate; mesoscutellum with subhorizontal dorsal surface smooth and shiny, declivitous posterior surface weakly imbricate, dull, not separated by a border or carina; T2 and T3 sparsely punctate on discs, punctures separated by at least 2–3 times a puncture width; apical terga with denser punctures laterally; discs of T2 and T3 medially with very short, black, simple setae, each seta barely exiting puncture, increasing in length laterally and on apical terga.

Comments. The female lectotype of *Xylocopa andica* is morphologically similar to the female of *X. nigrocincta* Smith, a species that occurs in Brazil, Argentina, and Paraguay. It differs from *X. nigrocincta* in the coloration of the terga and wings. In *X. nigrocincta*, all terga are basally reddish brown, except T6, and the wings are dark brown with strong violet highlights. In *X. andica*, the basal three terga have weak, dark brown bands, and the wings are dark brown with green highlights apically, violet basally. Because integumental coloration of the metasomal terga and wings are variable in other species of *Xylocopa*, it is possible that both species are conspecifics. For example, specimens of *X. frontalis* (Olivier) from Central America and northern South America have terga uniformly black and wings with strong green highlights. In contrast, specimens from southern South America (Brazil, Argentina, and Paraguay) have light reddish brown bands on the basal terga and wings with strong violet highlights (Hurd 1978: 80–81). Furthermore, we examined two female specimens from the same locality and collector in Argentina (Virginia, nr. La Ramada, Dpto. Burruyacu, III-27-1967 / Cucurbita maxima / USNM) in which the coloration of the wings is similar to that of *X. andica* in one of them, and to *X. nigrocincta* in the other.

Another species that might be conspecific with *X. nigrocincta* is *X. suspecta* Moure and Camargo, a species recorded from Argentina, Bolivia, Brazil, and Paraguay (Moure 2007). Male and female specimens from Bolivia (El Beni) standing in SEMC under *X. suspecta* are undistinguishable from *X. nigrocincta*, including in the male genitalia. Doubtless, future studies are necessary to assess the species limits of these species.

Figure 1. Dorsal habitus of the female lectotype of *Xylocopa* (*Neoxylocopa*) *andica* Enderlein.

Xylocopa andica was described from five specimens (two females and three males) and no holotype was designated. According to T. Hufleit (MIZ), these syntypes were borrowed by the late J.S. Moure in 1959 and were never returned. These specimens could not be located at the Universidade Federal do Paraná, Curitiba, Brazil (G. Melo, pers. comm.). However, we found a female specimen of this type series in the general bee collection of the USNM, which we here designate as the lectotype to stabilize the name. The label data for this specimen are as follows: Peru Chanchamayo [Wilhelm] Hoffmanns [green label] / Co = Typus [yellow label] / Xylocopa andica Enderl. ♀ Type Dr. Enderlein det.1913 [handwritten] / Lectotype, *Xylocopa andica* Enderlein des. M. Lucia & V.H. Gonzalez. This specimen is currently in USNM.

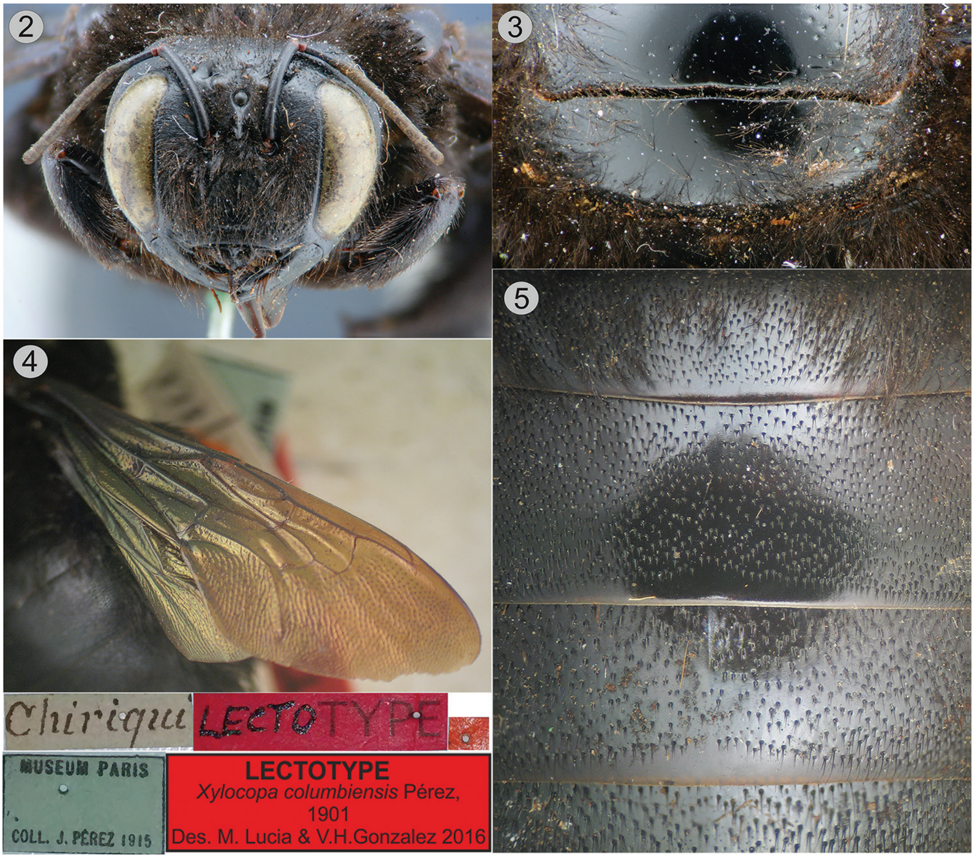
Distribution. Peru (Chanchamayo).

***Xylocopa* (*Neoxylocopa*) *columbiensis* Pérez, 1901**

Figs 2–5

Xylocopa (*Neoxylocopa*) *columbiensis* Pérez, 1901: 94 (Lectotype: MNHN; ♀, Panama, Chiriquí) (new lectotype designation).

Diagnosis. This species can be recognized by the combination of the following features: large body size (body length ~25 mm); pubescence black; integument dark brown to black throughout, with coxae and femora, apical margins of sterna, and T2–T4 brown; tegula black; wings dark brown with coppery highlights (Fig. 4); labrum basally with three longitudinally elongated tubercles; gena and supraocular areas sparsely punctate, punctures separated by ~2–3 times a puncture width; mesoscutellum (Fig. 3) weakly angled in profile; T1 and T2 densely punctate on discs, punctures small, separated by



Figures 2–5. Female lectotype of *Xylocopa* (*Neoxylocopa*) *columbiensis* Pérez. **2** Facial view **3** detail of mesoscutellum **4** Right fore and hind wings **5** detail of T1–T3.

~1–2 times a puncture width, punctures denser laterally; T3 sparsely punctate on disc, punctures small, separated by 2–3 times a puncture width (Fig. 5); T4–T6 with large punctures on discs, punctures separated by a puncture width; T1 with short, plumose and simple setae intermixed; discs of T2 and T3 with long (0.15–0.50 × OD), simple setae; T4–T6 with long (0.8–4.0 × OD), simple setae.

Comments. *Xylocopa columbiensis* superficially resembles *X. andica*, *X. nigrocincta*, and *X. suspecta*. It can be separated from those species by the coloration and punctation of terga, as well as the coloration of terga and wings, as indicated in the diagnosis.

Xylocopa columbiensis was described from six specimens (three females and three males) and no holotype was designated. We examined these specimens, which are in the MNHN, and chose one of the females as the lectotype to stabilize the name. The label data for this specimen are as follows: Chiriqui [white label, handwritten] / Museum Paris, Coll. J. Pérez / Lectotype [red label] / Lectotype *Xylocopa columbiensis* Peréz des. M. Lucia & V.H. Gonzalez.

Distribution. Panama (Chiriquí), Peru (Moure 2007).

***Xylocopa* (*Neoxylocopa*) *mendozana* Enderlein, 1913**
Fig. 6

Xylocopa (*Neoxylocopa*) *mendozana* Enderlein, 1913: 160 (Lectotype: USNM; ♀, Argentina, Mendoza) (new lectotype designation).

Diagnosis. This species can be recognized by the combination of the following features: large body size (body length ~27 mm); pubescence black; integument dark brown to black throughout, with legs and apical margins of terga light brown; tegula black; wings dark brown with green highlights; labrum basally with three longitudinally elongated tubercles; gena and supraocular areas densely punctate, punctures separated by ~1–1.5 times a puncture width; mesoscutellum with basal, smooth and shiny surface gently continuing onto distal, punctate surface; T2 densely punctate on disc, punctures small, separated by a puncture width, punctures denser laterally; T3 with sparse punctures on disc, punctures small, separated by 1–2 times a puncture width; T4–T6 with large punctures on discs, punctures separated by a puncture width; T1 with very short, plumose and simple setae intermixed; discs of T2 and basally on T3 with very short (0.2–0.4 × OD) simple setae, increasing in length laterally and on apical terga; T3 apically and T4–T6 with long (1–4 × OD), simple setae. The male can be easily recognized by the distinctive punctation and pubescence of T2 and T3, which are finely and densely punctate, with very short setae, each seta barely exiting the puncture. Thus, these terga appear largely bare at low magnifications when compared with remaining terga, which are densely covered by very long setae.

Comments. Specimens of *X. mendozana* were erroneously identified as *X. ordinaria* Smith by Hurd (1978: 67), a species that does not occur in Argentina (Lucia et. al 2014). *Xylocopa mendozana* is most similar to *X. atamisquensis* Lucia & Abrahamovich from Argentina but it can be easily separated by the large body size (25–29 mm), wings with strong green highlights, and T2 and T3 with short, simple setae contrasting with the long setae on the remaining terga. *Xylocopa atamisquensis* is smaller (21–23 mm), have wings with strong violet-green highlights, and T2–T6 with setae uniformly long. The male of *X. mendozana* can be distinguished from the male of other species occurring in Argentina by the features listed in the diagnosis.

Xylocopa mendozana was described from eight specimens (four females, three males and one gynandromorph) and no holotype was designated. In 1959, these syntypes were loaned to the late J.S. Moure and have never returned to the MIZ (T. Hufleit, pers. comm.). We were able to locate two females, two males, and the gynandromorph specimen in the general bee collection of the USNM. The remaining two females and one male could not be located at the Universidade Federal do Paraná, Curitiba, Brazil (G. Melo, pers. comm.). To stabilize the name, we chose one of the females as the lectotype. The label data for this specimen are as follows: Argentinien, Mendoza, Jensen-Haarup S. 1.1.07 [green label] / Type [red label] / Xylocopa mendozana Enderl. ♀ Type Dr. Enderlein det.1913 [handwritten] / Mus. Zool. Polonicum. Warszowa. 12/45/ Lectotype Xylocopa mendozana Enderlein P. D.Hurd '60 / Lectotype *Xylocopa*

Figure 6. Dorsal habitus of the female lectotype of *Xylocopa* (*Neoxylocopa*) *mendozana* Enderlein.

mendozana Enderlein des. M. Lucia & V.H. Gonzalez. This specimen as well as the others are currently in USNM.

Distribution. Argentina: Catamarca, Córdoba, La Pampa, La Rioja, Mendoza, Rio Negro, Salta, San Juan, San Luis, Santiago del Estero, and Santa Fe (Lucia et al. 2014).

Xylocopa (*Neoxylocopa*) *rufidorsum* Enderlein, 1913

Fig. 7

Xylocopa (*Neoxylocopa*) *rufidorsum* Enderlein, 1913: 160 (Lectotype: USNM; ♀, Peru, Chanchamayo) (new lectotype designation).

Diagnosis. This species can be recognized by the combination of the following features: medium body size (body length, ~21 mm); pubescence black, except dorsum of mesosoma densely covered with ferruginous setae; integument dark brown to black throughout, except tegula reddish brown; wings dark brown with strong violet highlights; labrum basally with three longitudinally enlongated tubercles; gena and supraocular areas sparsely punctate; mesoscutellum with subhorizontal dorsal surface smooth and shiny, declivitous posterior surface weakly imbricate, dull, not separated by a border or carina; T2 densely punctate on disc, punctures separated by 1–1.5 times a puncture width, punctures denser laterally; T3 sparsely punctate on disc, punctures separated by 2–3 times or more a puncture width; T1 with plumose and very short, simple setae intermixed; discs of T2 and T3 with very short (0.2–0.7 × OD), simple setae, increasing in length laterally and on apical terga; T4 and T5 with long (1.0–1.5 × OD), simple setae.

Comments. As indicated by Moure (1949), *X. rufidorsum* belongs to the group of species that includes *X. similis* Smith, *X. tacanensis* Moure, *X. eximia* Perez, and *X. aurulenta*

Figure 7. Dorsal habitus of the female lectotype of *Xylocopa* (*Neoxylocopa*) *rufidorsum* Enderlein.

(Fabricius), which share the ferruginous pubescence on the dorsum of mesosoma. *Xylocopa similis* can be easily recognized by the presence of ferruginous setae on T1 (T1 with black setae in *X. rufidorsum*); *X. aurulenta* by the wings light brown with coppery highlights (dark brown with strong violet highlights in *X. rufidorsum*); *X. eximia* by the presence of long (≥1.5 × OD), simple setae on discs of T2 and T3 (discs of T2 and T3 with short [0.2–0.7 × OD], simple setae in *X. rufidorsum*); and *X. tacanensis* by the gena and T3 more densely punctate, punctures separated by 1–1.5 a puncture width (at least 2 or 3 times a puncture width in *X. rufidorsum*).

Xylocopa rufidorsum was described from three females and no holotype was designated. As in the case of *X. andica* and *X. mendozana*, these syntypes were also loaned to the late J.S. Moure and have never returned to the MIZ (T. Hufleit, pers. comm.). We only found one of these females in the general bee collection of the USNM; the remaining two females could not be located at the Universidade Federal do Paraná, Curitiba, Brazil (G. Melo, pers. comm.). To stabilize the name, we chose the female we found as the lectotype. The label data for this specimen are as follows: Peru, Chanchamayo [Wilhelm] Hoffmanns [green label] / Co=Typus [yellow label] / Xylocopa rufidorsum Enderl. ♀ Type Dr. Enderlein det.1913 [handwritten] / Mus. Zool. Polonicum. Warszowa. 12/45 / Lectotype, *Xylocopa rufidorsum* Enderlein des. M. Lucia & V.H. Gonzalez. This specimen is currently deposited in USNM.

Distribution. Bolivia (Cochabamba) and Peru (Chanchamayo) (Moure 2007).

Subgenus *Schonnherria* Lepeletier de Saint Fargeau, 1841

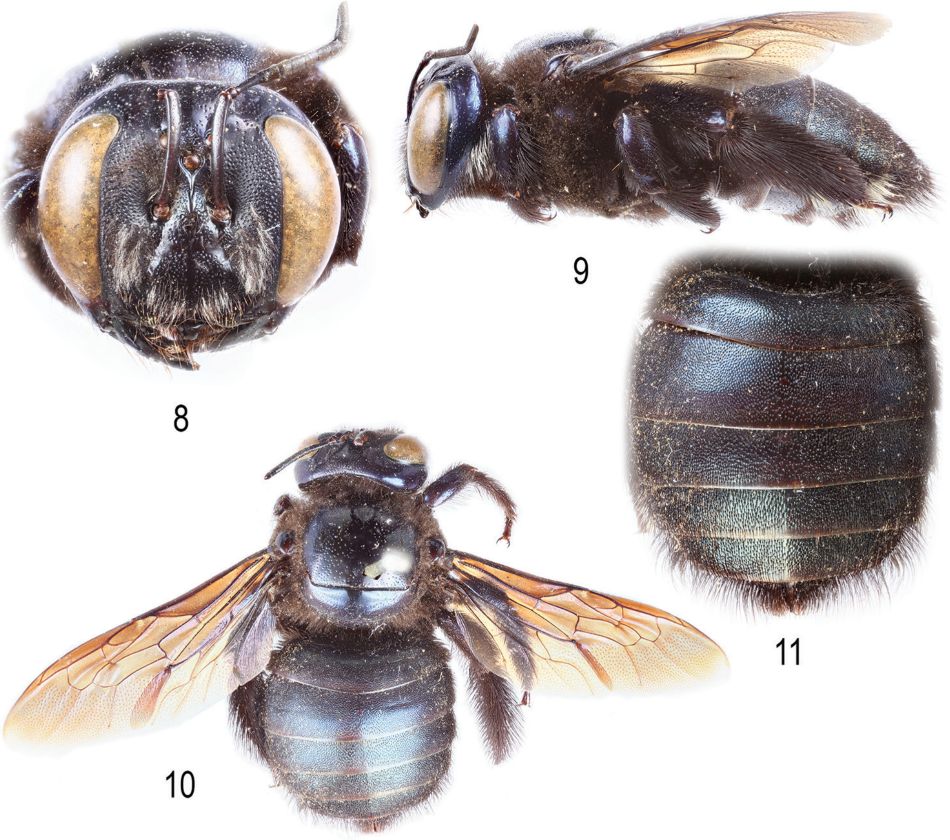
Xylocopa (*Schonnherria*) *barbarae* sp. n.

http://zoobank.org/2B534E11-D150-48DE-A7FE-82A4961710C4
Figs 8–11

Diagnosis. This species, known only from the female sex, can be easily distinguished from other species of *Schonnherria* by the combination of the following features: medium body size (body length, 19–22 mm); integument dark brown to black with blue highlights throughout and green metallic highlights on discs of terga; wings dark brown with strong violet highlights throughout; labrum basally with a distinct, large, single capitate tubercle; sides of T2–T4 and sides of S2–S4 with white setae; and discs of T2–T4 with dark brown to black setae, 0.5–1.0 × OD.

Xylocopa barbarae is most similar to *X. dimidiata* Latreille (see below), sharing a similar body size, metallic highlights on all tagmata, wings dark brown with violet highlights, and a labrum basally with a distinct, large, single capitate tubercle. However, *X. barbarae* can be easily distinguished by the longer and denser pubescence of terga (T2–T4 with setae 0.5–1.0 × OD in *X. barbarae* vs. setae very short, barely exiting puncture in *X. dimidiata*), presence of white setae on sides of T2–T4 (absent in *X. dimidiata*), and wings with violet highlight throughout (with green highlights apically in *X. dimidiata*).

Description. ♀, Body length 22.0 mm; head width 6.9 mm; mesosoma width 8.3 mm; metasoma width 9.9 mm; forewing length 17.0 mm. *Coloration*. Integument dark brown to black throughout, with weak blue metallic highlights on gena, vertex, mesoscutum except on disc, mesoscutellum, tegula, outer surfaces of tibiae, and T1 and T2; sterna with strong blue metallic highlights; T2 preapically and discs of remaining terga with weak green metallic highlights. Wings dark brown with strong violet highlights, pterostigma and veins dark brown to black. *Pubescence.* Dark brown to black, except face, gena, sides of T2–T4, and sides of S2–S4 with white setae. Discs of



Figures 8–11. Female holotype of *Xylocopa* (*Schonnherria*) *barbarae* sp. n. **8** Facial view **9** Lateral habitus **10** Dorsal habitus **11** Detail of metasomal terga.

terga with decumbent to semierect simple setae, shortest on T1 (0.2–0.5 × OD), progressively increasing in density and length towards apical terga, longest on T6 (5.0–6.0 × OD), sides of terga with semierect, mostly branched setae, denser and longer than discs. *Sculpturing*. Weakly imbricate to smooth and shiny. Vertex and upper gena with scattered punctures, largely impunctate. Mesoscutum with scattered, coarse punctures on anterior margin and lateral sides, impunctate otherwise; mesoscutellum largely impunctate, with large, scattered punctures on posterior half. Terga with circular to ovoid setiferous punctures, about as large and coarse as those on mesoscutellum, submedially with punctures separated at most by 1–2 times a puncture width, punctures denser and coarser laterally and on apical terga; S1 with small, ovoid, dense punctures, remaining sterna with elongate punctures on discs, denser and coarser preapically. *Structure*. Head 1.3 times broader than long; compound eyes with inner margins parallel or nearly so; inferior interocular distance 1.2 times superior interocular distance; lateral ocelli just below supraorbital line; ocellocular distance 2.5 times OD; ocellocipital distance 4.8

times OD; clypeotorular distance 1.3 times longitudinal diameter of antennal torulus; clypeus 2.3 times longer than broad; labrum basally with a distinct, large, single capitate tubercle, about as large as OD. Mesoscutum gently convex in profile; mesoscutellum convex, without carina or border between dorsal and posterior surfaces; metanotum and propodeum vertical.

Holotype. Bolivia: ♀, Chapare [Department of Cochabamba], 14.xi.1945 [14 November 1945], R. Zischka (MLP).

Paratypes. (*n* = 5) Three paratypes with same data as the holotype but collected on 11 and 12 November, 1945 (MLP); 1♀, Chapare (Yungas), I-49 [January 1949] (MLP); 1♀, Dpto. La Paz, Alto Marani, N. of Rurrenbaque, 10 Nov. 1956 (L. Peña) / SEMC1232254 (SEMC).

Etymology. The specific epithet is a matronym honoring Mrs. Bárbara Defea, loving and supporting wife of the senior author.

Distribution. *Xylocopa barbarae* is known from the departments of Cochabamba and La Paz in Bolivia.

***Xylocopa* (*Schonnherria*) *bigibbosa* sp. n.**

http://zoobank.org/F6275DE2-C404-4FB2-9C94-22FAFADD21B6
Figs 12–16

Diagnosis. The female of this species superficially resembles that of *X. splendidula* Lepeletier de Saint Fargeau and other small species of *Schonnherria*, such as *X. lucida* Smith and *X. muscaria* (Fabricius). However, *X. bigibbosa* can be easily separated from those species and from any other species of the subgenus by the mesoscutum anteriorly with two distinct round tubercles (Fig. 15).

Description. ♀, Body length 16.7 mm; head width 5.6 mm; mesosoma width 6.8 mm; metasoma width 7.5 mm; forewing length 14.3 mm. *Coloration*. Integument dark brown to black throughout, with strong blue metallic highlights on gena, vertex, mesoscutum lateral and anterior to tubercles, and sterna; outer surfaces of tibiae with weak blue metallic highlights; terga with weak greenish metallic highlights. Wing membranes dark brown with strong violet highlights, pterostigma and veins dark brown to black. *Pubescence*. Dark brown to black, except face, gena, and sides of T2–T5 with white setae. Discs of terga with decumbent to semierect simple setae, shortest on T1 (< 0.5 × OD), progressively increasing in density and length towards apical terga, longest on T6 (~5.0 × OD), sides of terga with semierect, mostly branched setae, denser and longer than discs. *Sculpturing*. Weakly imbricate to smooth and shiny. Vertex and upper gena with scattered punctures, largely impunctate. Mesoscutum with scattered, coarse punctures on anterior margin and lateral to tubercles, impunctate otherwise; mesoscutellum largely impunctate, with large, scattered punctures on posterior half. Terga with circular to ovoid setiferous punctures, as large and coarse as those on mesoscutellum, mostly separated by 1–2 times a puncture width, denser laterally and on apical terga; sterna with elongate punctures on discs, denser and coarser preapically.

Figures 12–16. Female holotype of *Xylocopa* (*Schonnherria*) *bigibbosa* sp. n. **12** Facial view **13** Lateral habitus **14** Dorsal habitus **15** Detail of mesoscutum in profile with arrow pointing to tubercles **16** Detail of T1–T3.

Structure. Head 1.2 times broader than long; compound eyes with inner margins parallel or nearly so; inferior interocular distance 1.1 times superior interocular distance; lateral ocelli just below supraorbital line; interocellar distance 1.5 times OD, 0.8 times ocellocular distance; ocellocipital distance 3.7 times OD; clypeotorular distance about as long as longitudinal diameter of antennal torulus; clypeus about twice longer than broad; labrum basally with a distinct, large, single capitate tubercle, about as large as OD. Mesoscutum anteriorly with two round tubercles; mesoscutellum gently convex; metanotum and propodeum vertical or nearly so.

Holotype. Peru: ♀, Yurac, 67 mi. E. of Tingo Maria., 350 m, XII-11-54 [November 11, 1954] / E.I. Schlinger & E.S. Ross collectors / SEMC1232253 (SEMC).

Etymology. The specific epithet refers to the two distinctive humps on the mesoscutum of this species. The novelty of this species was recognized by P.D. Hurd and J.S. Moure who intended to use this name. We retained this designation.

Distribution. This species is known only from the type locality in eastern Peru.

Xylocopa dimidiata Latreille, 1809

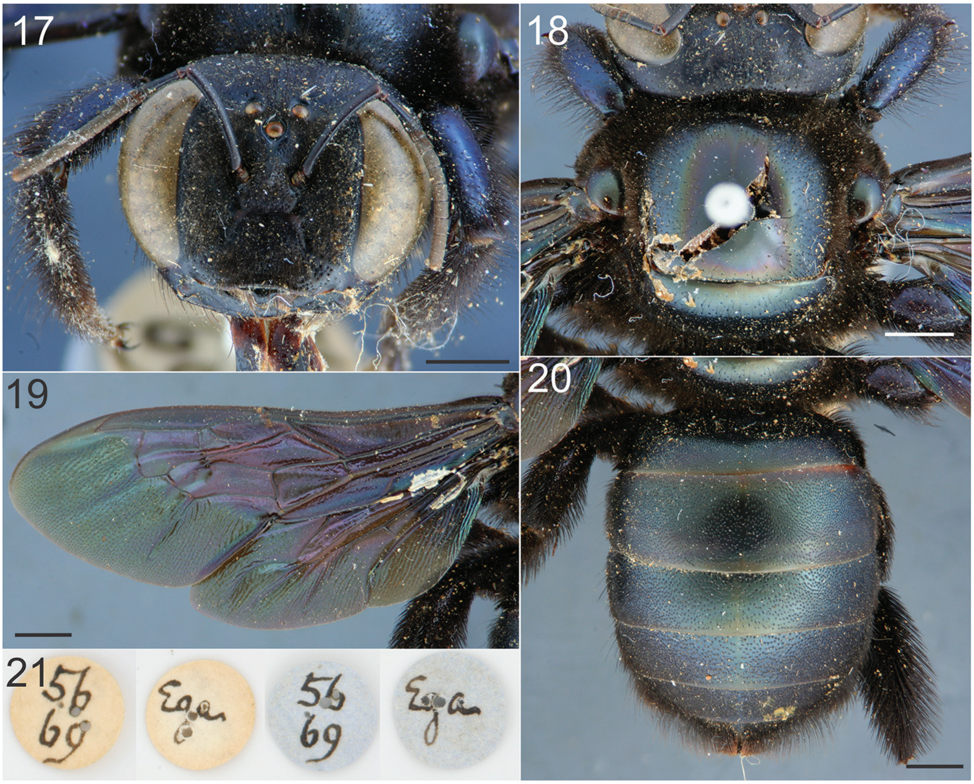
Figs 17–21

Xylocopa dimidiata Latreille, 1809: 95, pl. 38. (Neotype: BMNH; ♀, Brazil, Ega [Tefé]) (new neotype designation).

Xylocopa batesi Cockerell, 1907: 228, new name for "*Xylocopa dimidiata* Smith", *nomen invalidum* (not *X. dimidiata* Latreille, 1809 and *X. dimidiata* Lepeletier de Saint-Fargeau, 1841 [= *X. ocularis* Pérez, 1901]).

Diagnosis. This species can be recognized by the combination of the following features: medium body size (body length 23 mm); integument black, with distinct metallic green highlights on metasoma and mesosoma, particularly on mesoscutellum, and head with blue metallic highlights (Figs. 17, 18, 20); wings dark brown with violet highlights except apex green (Fig. 19); pubescence black; labrum basally with a distinct, large, single capitate tubercle, about as large as OD; T1 with plumose and simple setae; discs of T2 and T3 with very short, simple setae, each seta barely exiting puncture (Fig. 20); and T4–T6 with long setae, 1–2 times longer than those on T2 and T3.

Comments. The identity of *Xylocopa dimidiata* proposed by Latreille (1809) has been a mystery and its current concept encompasses more than one species (Fig. 22). Latreille proposed *Xylocopa dimidiata* from a single female collected somewhere along Alexander von Humboldt and Aimé Bonpland's voyage and whose whereabouts are unknown (Hurd 1978, Moure 2007). More than 60 years after Latreille's description, Smith (1874) identified and briefly described a female specimen from Ega [Tefé], Brazil, as what he interpreted to represent *X. dimidiata* Latreille. Subsequent authors followed Smith's concept of *X. dimidiata* and even incorrectly attributed the name to him, despite Smith making a clear reference to Latreille when using the name (e.g., Schrottky 1902). In fact, because Cockerell (1907) thought that Smith had proposed *X. dimidiata* in 1874, which then would result in homonym with Latreille's *X. dimidiata* as well as with one established by Lepeletier (1841), he proposed to replace Smith's name with *X. batesi* Cockerell (Hurd 1959). However, because Smith (1874) did not establish a name, Cockerell's replacement name for an unavailable name is therefore invalid under the ICZN (*nomen invalidum*). For the same reasons, the synonym of *X. batesi* under *X. dimidiata* Latreille listed by Hurd and Moure (1963), Hurd (1978), and Moure (2007) are unavailable.



Figures 17–21. Neotype of *X. dimidiata* Latreille. **17** Facial view **18** Dorsal view of head and mesosoma **19** Left fore and hind wings **20** Detail of metasomal terga **21** Associated labels (NHML).

The specimen identified as *X. dimidiata* by Smith is in the NHML (Figs 23–26), along with the specimen from the same locality herein designated as the neotype of that species. The second specimen is in better condition than that identified by Smith and, judging by the handwriting of the labels, it appears to be from the same collection event. Given the confusing identity of *X. dimidiata*, the designation of a neotype is necessary to stabilize the name. For practical reasons, we chose as the neotype a specimen that matches Smith's concept of *X. dimidiata* Latreille. Although this proposed taxonomic action resolves the identity issue of *X. dimidiata*, there is no reason to assume that Smith's interpretation of Latreille's *X. dimidiata* is correct. First, Smith's specimen is from the Amazon Basin, in a locality well outside of the route taken by Humboldt and Bonpland. These explorers traveled through large areas of Venezuela and the Magdalena River Valley in Colombia and through the Andes southward to Lima, Peru, and Guayaquil, Ecuador, before traveling to Mexico (e.g., Sandwith 1925, 1926, Papavero 1971). Thus, Latreille's specimen could have been collected from any of these areas bordering the Amazon Basin, unless it came from San Carlos de Río Negro in Venezuela's Amazonas state. Second, specimens standing in collections under *X. dimidiata* actually belong to multiple species, distinct from Smith's material. This is the case for the Bolivian specimens described

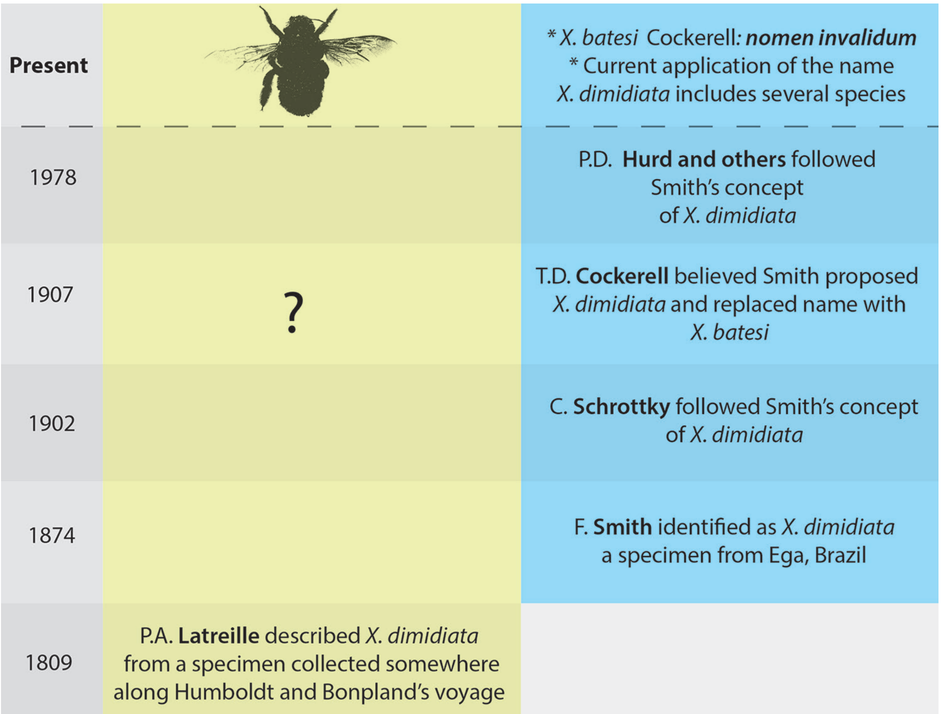
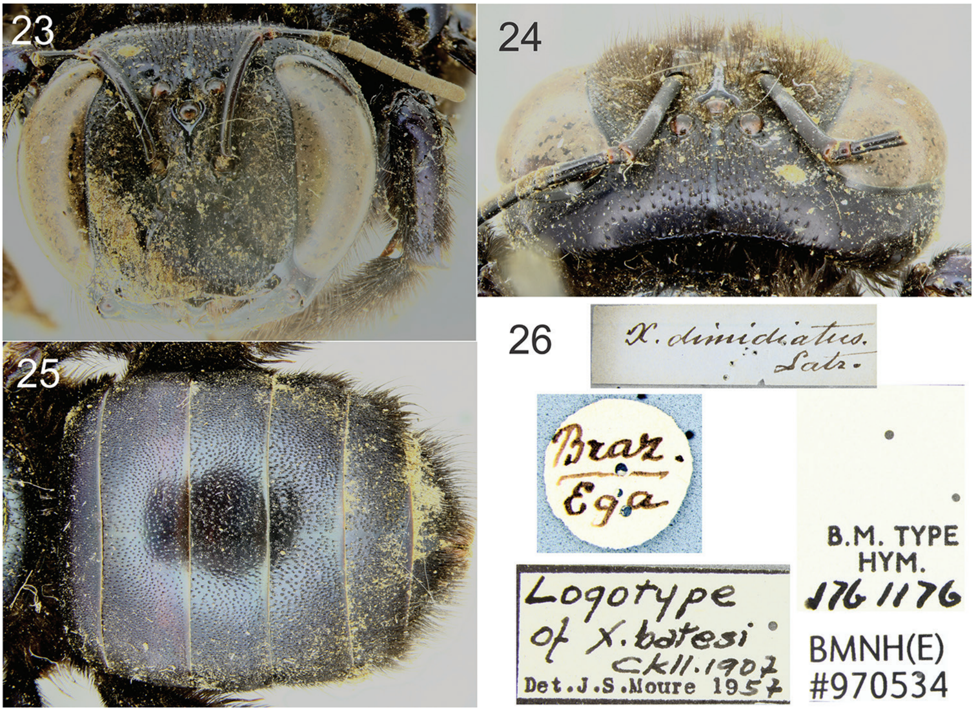


Figure 22. Schematic representation of the historical changes in the species concept and nomenclature surrounding *X. dimidiata* Latreille (see text for details). The yellow and blue columns each represent a species concept.

herein as new (*X. barbarae*), which superficially fall within the historical concept of Smith's *X. dimidiata*, but differing in the longer and denser pubescence of terga, presence of white setae on sides of T2–T4, and wings with violet highlights throughout. Thus, Smith's concept of Latreille's *X. dimidiata* is questionable, and the current application of this name includes several species.

Given the problems outlined above, we initially thought that it would be best to regard *X. dimidiata* Latreille as a *nomen dubium*, meaning that future workers would have the task to fix this name to one of those biological species overlapping Humboldt and Bonpland's route. *Xylocopa* (*Schonnherria*) is the second most species-rich group of *Xylocopa* in the Western Hemisphere, containing about 30 species ranging from southern United States to southern Argentina (Michener 2007). Because taxonomic issues like these are best resolved in a context of a revision, a study of such a magnitude might take several years to complete, if done at all. Thus, despite all problems surrounding the identity of *X. dimidiata*, we chose to designate a neotype for this species following Smith's interpretation. This might not be Latreille's concept of *X. dimidiata* but we believe this taxonomic action now would be beneficial to the advancement of the taxonomy of the group. The label data for the specimen here designated as the neotype are as follows: 5669 / Ega / 5669 / Ega / Neotype, *Xylocopa dimidiata* Latreille des. M. Lucia V.H. Gonzalez ♀.



Figures 23–26. Specimen of the NHML identified as *X. dimidiata* Latreille by F. Smith. **23** Facial view **24** Dorsal view of head **25** Detail of terga **26** Associated locality and determination labels (Photographs by David Notton, NHML).

Acknowledgments

We are indebted to each of the curators listed by institution in the Material and Methods for the loan of the specimens. We are thankful to Amy Comfort for considerable assistant and support throughout the work and Michael S. Engel for insightful suggestions and comments on the manuscript, particularly on addressing the identity of *X. dimidiata*. Claus Rasmussen and Rodrigo Gonçalves for comments and suggestions that improved this manuscript. This work was supported by the National Science Foundation's REU program (DBI 1560389).

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Cotesia icipe sp. n., a new Microgastrinae wasp (Hymenoptera, Braconidae) of importance in the biological control of Lepidopteran pests in Africa

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Abstract

A new species of Microgastrinae, *Cotesia icipe* Fernández-Triana & Fiaboe, **sp. n.**, is described from eastern Africa. It was reared in Kenya as a solitary parasitoid from two major amaranth pests, *Spodoptera littoralis* (Boisduval, 1833) and *S. exigua* (Hübner, 1808); study of specimens in collections also revealed its presence in four other countries in the Afrotropical region (Madagascar, Saudi Arabia, South Africa, and Yemen). Morphological, molecular and biological characters are used to describe the new species and to distinguish it from all 12 previously described species of Afrotropical *Cotesia*. *Cotesia icipe* shows potential in the biological control of key Lepidopteran pests in small scale farming conditions in Africa.

Keywords

Microgastrinae, *Cotesia*, *Spodoptera*, biological control, Africa

Introduction

The subfamily Microgastrinae (Hymenoptera, Braconidae) is the single most important group of parasitoid wasps attacking caterpillars, with many species used or being considered as biocontrol agents against Lepidopteran pests in agriculture and forestry

(Whitfield 1997). It comprises more than 2,700 described species (Yu et al. 2016) with many thousands of additional, undescribed ones (Rodriguez et al. 2013). The genus *Cotesia* is currently the second largest, with almost 300 described species worldwide (Yu et al. 2016) but many hundreds awaiting description.

There are 12 species of *Cotesia* recorded from the entire Afrotropics (Rousse and Gupta 2013, Yu et al. 2016, Kaiser et al. 2017). We do not include in that total *Cotesia plutellae* (Kurdjumov, 1912), which was recently removed from synonymy with *Cotesia vestalis* (Haliday, 1834) and considered as a valid species by Rousse and Gupta (2013). Rousse and Gupta based their decision on a paper from Rincon et al. (2006) that found that "*Cotesia plutellae*" seemed to comprise two partially incompatible population aggregates. While we agree that specimens previously identified as "*Cotesia plutellae*" might comprise a complex of morphologically cryptic species –as is the case with many other species of Microgastrinae (e.g., Fernández-Triana 2010, Fernández-Triana et al. 2014, Kaiser et al. 2017), without further study, especially of the type specimens involved, there is no real basis for raising that name from synonymy. It is clear from both papers (Rincon et al. 2006, Rousse and Gupta 2013) that those authors never studied the pertinent material, and at present there is no sound evidence for a conclusion as to which type specimens (if any) are associated with their segregates. Therefore, we consider here the name *Cotesia plutellae* (Kurdjumov, 1912) to be just a synonym of *Cotesia vestalis* (Haliday, 1834).

This paper describes a new species of *Cotesia* from Kenya (also present in other Afrotropical countries), which has been found to be important in the biological control of two major amaranth pests, *Spodoptera littoralis* and *S. exigua* (Lepidoptera, Noctuidae). The parasitoid was found during various field studies carried out by the International Centre of Insect Physiology and Ecology (*icipe*), in the scope of developing an IPM programme against amaranth pests in East Africa. In Kenya, specimens of the new species were collected from different locations where the project activities are carried out and there is a potentially much wider distribution. The new species is compared to the other 12 species of *Cotesia* previously recorded from the Afrotropical region, and diagnostic characters to recognize it are provided.

Methods

Specimens of the new species were collected from different locations in Central Kenya: Yatta, (01.23044°S; 37.45789°E) and Mwea (0.6309°S; 37.35117°E); Kitengela (1.6°S; 36.85°E) and Thika (1.00269°S; 37.07858°E). The material has been deposited in the International Centre of Insect Physiology and Ecology, Nairobi, Kenya (*icipe*), the Canadian National Collection of Insects, Ottawa (CNC) and the National Museums of Kenya (NMK).

Morphological terms and measurements of structures follow those used by Mason (1981), Huber and Sharkey (1993), Whitfield (1997), Karlsson and Ronquist (2012), and Fernández-Triana et al. (2014). The abbreviations T1, T2, and T3 refer to metasomal mediotergites 1, 2, 3; and F1-F16 refer to antennal flagellomeres 1 to 16. The description of the new species contains ratios commonly used in taxonomic studies of Microgastrinae (e.g., ovipositor sheaths length/metatibia length); we also provide the raw measurements of morphological structures (in mm) as they allow for additional ratios to be explored in the future, if needed.

Seven specimens (including the holotype and paratypes, with voucher codes CNC507547–CNC507553) were sampled for DNA barcodes (the 5' region of the cytochrome c oxidase I (CO1) gene, Hebert et al. 2003). DNA extracts were obtained from single legs using a glass fibre protocol (Ivanova et al. 2006). Total genomic DNA was re-suspended in 30 µl of dH2O, a 658-bp region near the 5' terminus of the CO1 gene was amplified using standard primers (LepF1–LepR1) following established protocols (http://v4.boldsystems.org/index.php), and a composite sequence was generated for all successful amplifications. All information for the sequences associated with each individual specimen can be retrieved from the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007).

Photos were taken with a Keyence VHX-1000 Digital Microscope, using a lens with a range of 10–130 ×. Multiple images were taken of a structure through the focal plane and then combined to produce a single in-focus image using the software associated with the Keyence System. Plates were prepared using Microsoft PowerPoint 2010.

A map with the distribution of the species was generated using SimpleMappr (Shorthouse 2010).

Original descriptions and/or authenticated specimens of the 12 *Cotesia* species previously recorded for the Afrotropical region were checked. In the Detailed diagnosis section below, the new species is compared individually with all other species previously described from the Afrotropics, and diagnostic characters to separate it from all of them are presented. Morphological comparisons are based mostly on female specimens, as male Microgastrinae often are difficult to identify (e.g., Whitfield 1997, Fernández-Triana et al. 2014).

A neighbor-joining tree (K2P) of all specimens in the Barcoding of Life Data System (BOLD) currently associated with the new species (BIN BOLD:ABZ7318) was generated using the capabilities available in BOLD (for an explanation of the BIN concept see Ratnasingham and Hebert 2007). A second neighbor-joining tree (K2P) was generated for all Afrotropical species of *Cotesia* with available sequences over 500 base pairs in BOLD (the data was accessed on September 2017).

Results

Cotesia icipe **Fernández-Triana & Fiaboe, sp. n.**

http://zoobank.org/E697611F-08A4-4CA7-B300-E35B50675BD9

Holotype. Female, Kenya, NMK. Holotype locality: Yatta. Holotype labels: KENYA, Yatta, 1°13'49.59"S 37°27'28.41"E, 1184m, coll. ICIPE. Voucher code: CNC507547.

Paratypes. 3 females (CNC507548, CNC507549, CNC507553), 3 males (CNC50750, CNC50751, CNC50752); same locality of holotype. Paratypes deposited in the CNC, ICIPE and NMK.

Other specimens considered as this species. There are 10 DNA barcodes in BOLD from Madagascar, Saudi Arabia, South Africa and Yemen (Fig. 4) whose sequences match those of the Yemen holotype and paratypes and thus we consider them all as conspecific. However we only include in the type series those specimens from Kenya that we were able to examine.

Diagnosis. Metasoma with T3 dark brown to black centrally, yellow laterally; hind legs mostly yellow, except for metacoxa (mostly black, with small yellow spot on apical 0.1), brown spots on apical 0.1 of metafemur (dorsally), apical 0.1 of metatibia dark brown, and metatarsus entirely dark brown; tegula and humeral complex yellow; fore wing with most veins brown. Besides coloration, *C. icipe* has scuto-scutellar sulcus with eight carinae; T1 almost parallel-sided, very slightly widening towards posterior margin; T2 relatively small, quadrate and not covering the entire surface of the tergum, and T3 1.3x as long as T2 length. The above combination of characters is sufficient to separate the species from all other described species of Afrotropical *Cotesia*, but see the Detailed diagnosis section below for one-to-one comparisons of *C. icipe* versus every other species. Beyond morphological characters, from a molecular perspective there is also sufficient information to recognize the new species. Eight out of the 12 previously described Afrotropical species of *Cotesia* have DNA barcodes available in BOLD (Fig. 5), and they are all clearly different from *C. icipe*.

Description. Female (Fig. 1). Metasoma brown dorsally, except for T3 (which is centrally brown, laterally yellow); most of laterotergites and sternites yellow, hypopygium brown; F2 length 1.78–2.00 × F14 length; metafemur length 3.60–3.80 × metafemur width; metatibia inner spur length 1.17 × metatibia outer spur length; metatibia inner spur length 0.52 × first segment of metatarsus length; T1 entirely sculptured with coarse punctures and a polished knob centrally on posterior margin; T1 very slightly widening towards posterior margin (width at posterior margin 1.1–1.2 × its width at anterior margin); T1 length centrally 1.5–1.7 × its width at posterior margin; T2 entirely sculptured, with coarse punctures along all margins and longitudinal striation centrally; T2 more or less rectangular, width at posterior margin 2.0 × its length centrally; T3 smooth, with rows of setae that are more dense on posterior half of tergite; T3 length centrally 1.3 × T2 length centrally; ovipositor sheaths length 0.16–0.19 × metatibia length. ***Body measurements*** (all in mm). Body length: 2.20–2.50; fore wing length: 2.20–2.50; ovipositor sheaths: 0.12–0.15; metafemur length 0.65; metafemur width: 0.17–0.18; metatibia length: 0.76–0.80; metatibia inner spur: 0.21; metatibia outer spur: 0.18; first segment of metatarsus: 0.40. T1 length centrally: 0.35–0.37; T1 width at anterior margin: 0.19; T1 maximum width: 0.25; T1 width at posterior margin: 0.22–0.24; T2 width at posterior margin: 0.32; T2 length centrally: 0.16; T3 length centrally: 0.21; length of F1: 0.17–0.19; length of F2: 0.16–0.18; length of F3: 0.16–0.17; length of F14: 0.09; length of F 15: 0.08; length of F16: 0.10.

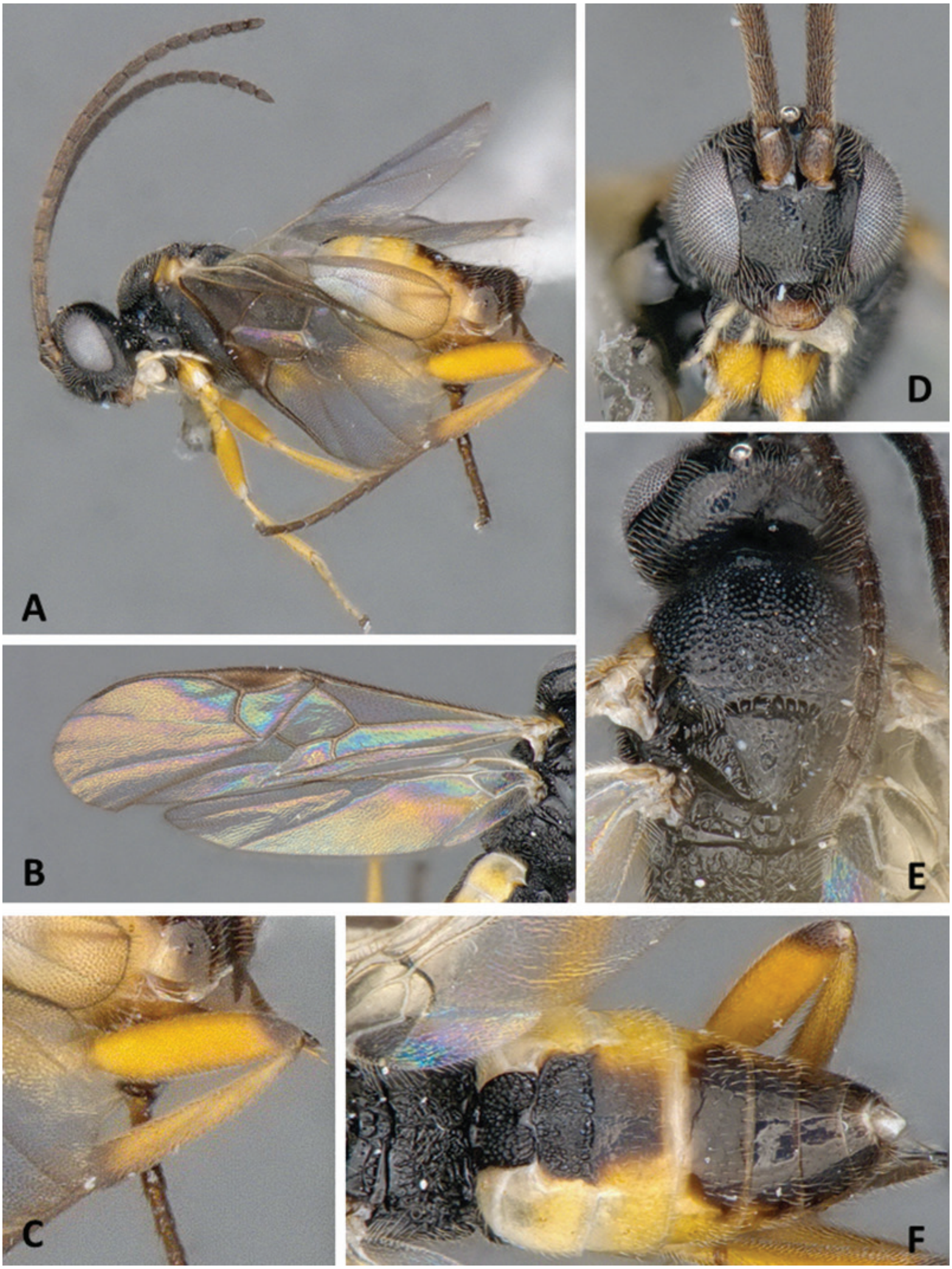

Figure 1. *Cotesia icipe*, female holotype. **A** Habitus lateral **B** Wings **C** Hind leg, hypopygium and ovipositor **D** Head frontal **E** Head and mesosoma dorsal **F** Propodeum and metasoma dorsal.

Male (Fig. 2). As female but with darker metasoma dorsally, sometimes with T3 entirely dark brown to black.

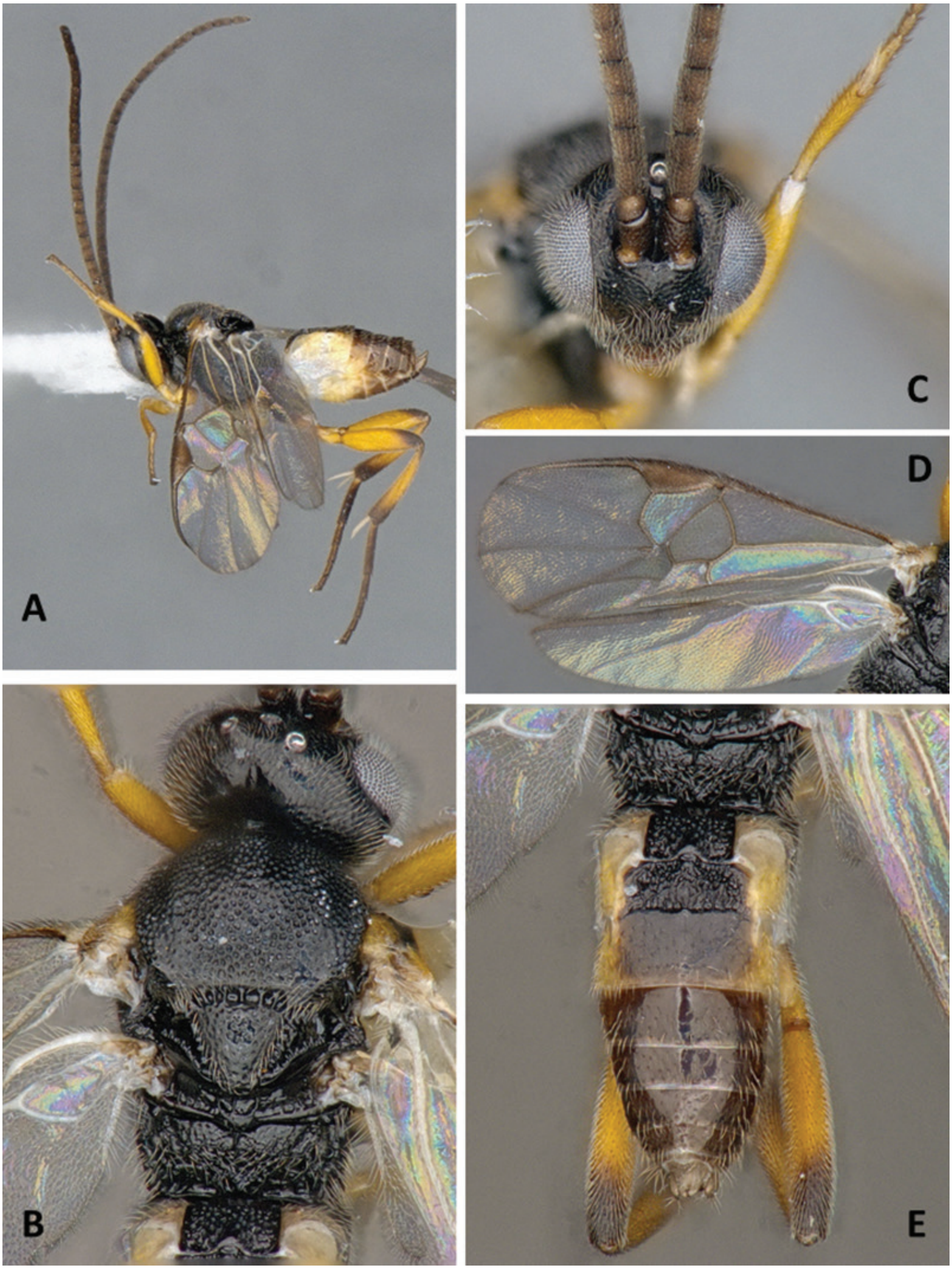

Figure 2. *Cotesia icipe*, male paratype. **A** Habitus lateral **B** Head and mesosoma dorsal **C** Head frontal **D** Wings **E** Propodeum and metasoma dorsal.

Biology. Based on 4,000+ parasitism cases observed under laboratory condition at *icipe* in Kenya, *C. icipe* is a solitary larval endoparasitoid of *Spodoptera littoralis* (Boisduval, 1833) and *Spodoptera exigua* (Hübner, 1808) (Lepidoptera, Noctuidae). It was

Figure 3. Known distribution of *Cotesia icipe* in the Afrotropical region. Blue dots show distribution based on specimens of the type series (Kenya), red dots show distribution based on specimens with DNA barcodes matching those of the Kenya series.

successfully reported to attack those two noctuid species tested on amaranth. However, it failed to parasitize three Crambidae species tested on the same host plant: *Spoladea recurvalis* (Fabricius, 1775), *Udea ferrugalis* (Hübner, 1796) and *Herpetogramma bipunctalis* (Fabricius, 1794). Further studies of host range are warranted to explore the full potential of the new species in the biological control of key pests in small scale farming conditions in Africa. The female prefers ovipositing on second instar host larvae. The development time from egg to adult is two weeks and ovipositing females fed with honey can live more than two weeks at 25°C (our unpublished data).

Distribution. Afrotropical: Kenya, Madagascar, Saudi Arabia, South Africa, Yemen (Fig. 3).

DNA barcodes. Currently (as of September 2017) there are in BOLD 19 available sequences from five Afrotropical countries: Kenya (9 sequences), Madagascar (1), Saudi Arabia (3), South Africa (1) and Yemen (5). Although we could not examine the specimens from Saudi Arabia or South Africa, their sequences are identical to the rest, and thus we consider them all to represent the species *Cotesia icipe*. They belong to BIN BOLD:ABZ7318; however, that BIN is likely to contain more than one species, as the sequences currently assigned to it are grouped in different clusters (Fig. 4). The Afrotropical sequences are in a single cluster, separated by more than 1% different

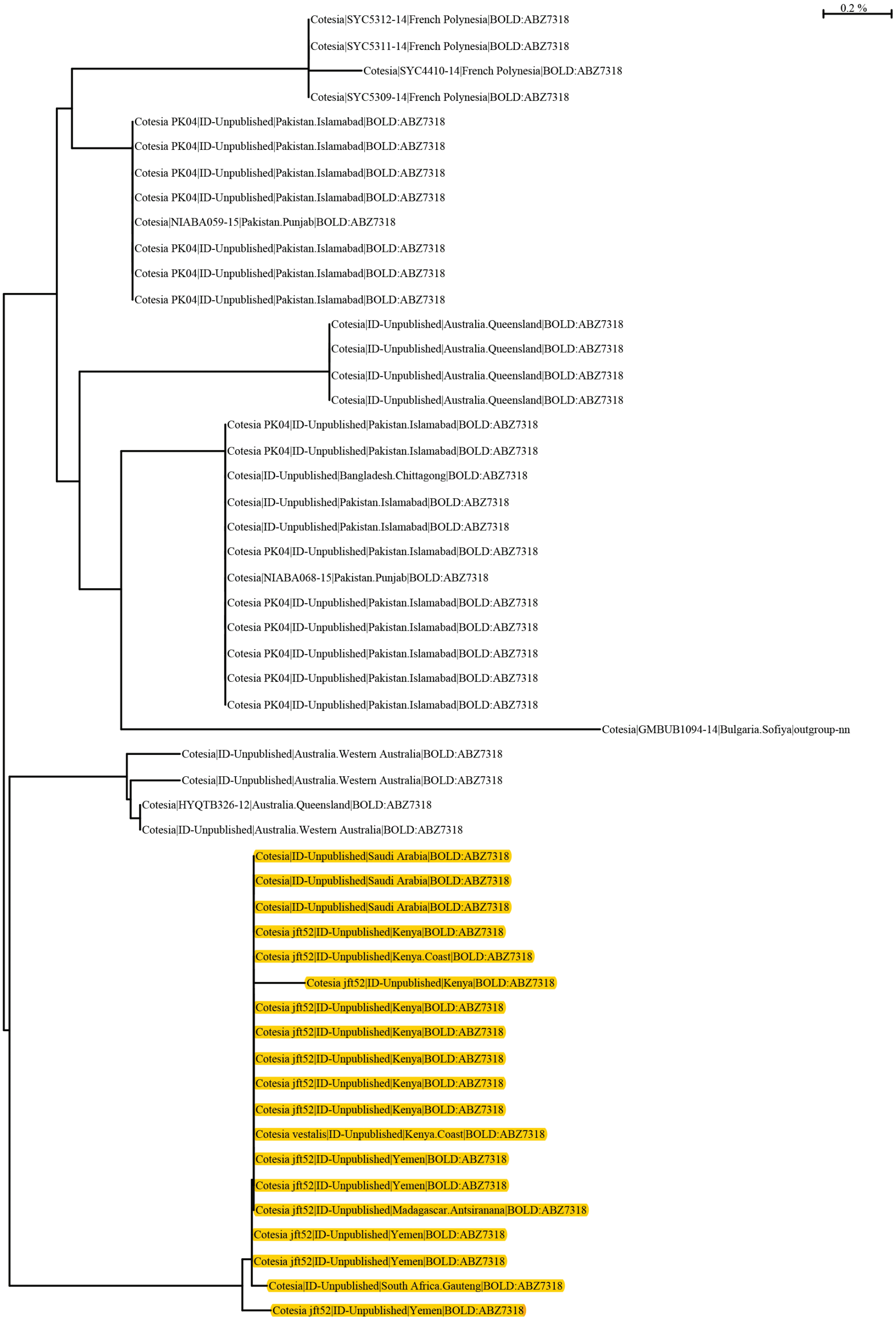

Figure 4. Neighbor-joining tree (K2P) of all specimens in the Barcoding of Life Data System (BOLD) currently associated with BIN BOLD:ABZ7318 (for explanation on the BIN concept see Methods of this paper). The cluster highlighted in Yellow in the tree shows the specimens considered in the present paper as *Cotesia icipe*.

Figure 5. Neighbor-joining tree (K2P) of Afrotropical species of *Cotesia* in the Barcoding of Life Data System (BOLD) with over 500 base pairs; data accessed on September 2017. Specimens named as "*Cotesia* jft52" in BOLD actually belong to the species *Cotesia icipe* (cluster highlighted in Yellow), and specimens named as "*Cotesia* jft68" in BOLD actually belong to the species *Cotesia chrysippi* (Viereck, 1911).

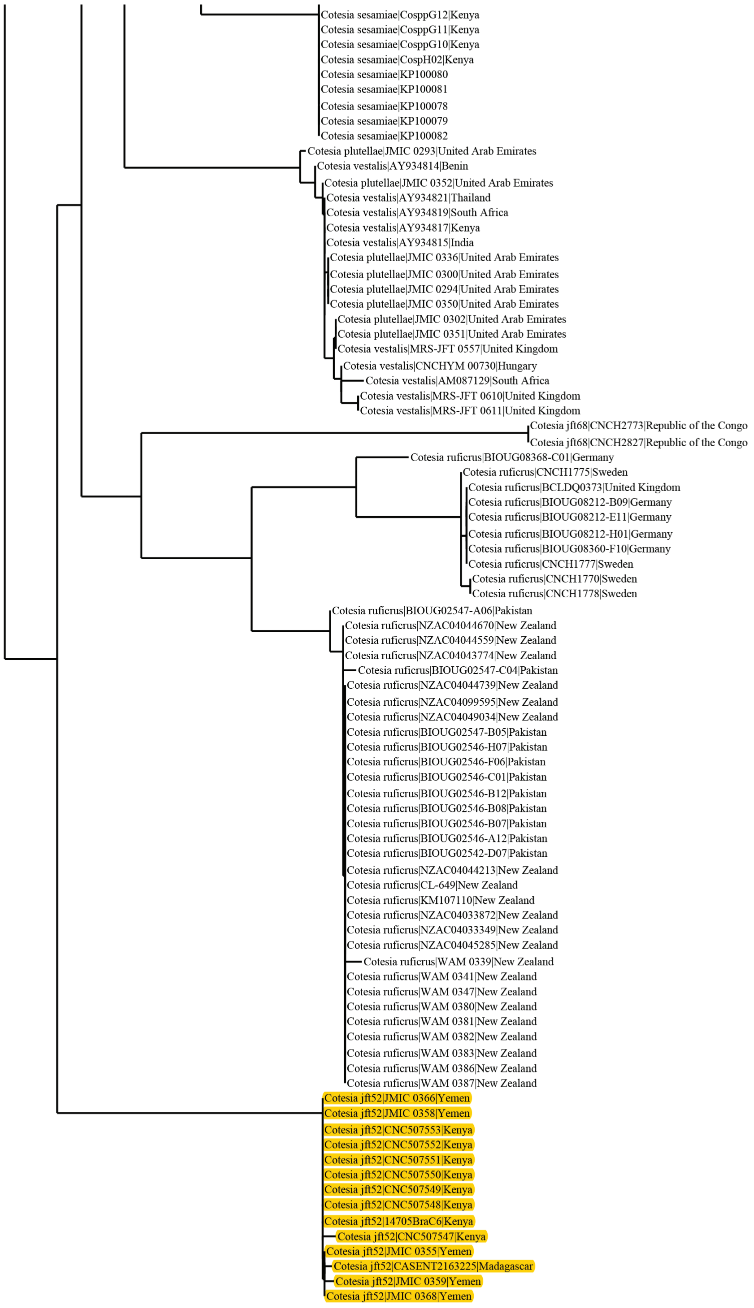

Figure 5. Continued. Neighbor-joining tree (K2P) of Afrotropical species of *Cotesia* in the Barcoding of Life Data System (BOLD) with over 500 base pairs; data accessed on September 2017. Specimens named as "*Cotesia* jft52" in BOLD actually belong to the species *Cotesia icipe* (cluster highlighted in Yellow), and specimens named as "*Cotesia* jft68" in BOLD actually belong to the species *Cotesia chrysippi* (Viereck, 1911).

base pairs from the other sequences in that BIN (from Australia, French Polynesia and Pakistan, all in separate clusters). Solving the species limits for this BIN is beyond the scope of this paper, but for the time being we consider that only the Afrotropical sequences represent *Cotesia icipe*.

Etymology. We dedicate this species to the "International Centre of Insect Physiology and Ecology (*icipe*)" for its long-term promotion of Integrated Pest Management and for building the capacity of thousands of African young scientists over the years in insect sciences.

Detailed diagnosis. Additionally, and to facilitate future work on the group, we detail below how each of the other 12 species of *Cotesia* previously described from the Afrotropics individually differ from *C. icipe*.

Cotesia bignellii (Marshall, 1885) has metasoma (entirely) and hind legs (mostly) dark brown, very different from the body color of *C. icipe* as detailed above. *C. bignellii* also parasitizes a different family of Lepidoptera hosts (Nymphalidae) and it is mostly distributed in Europe (Yu et al. 2016).

Cotesia chrysippi (Viereck, 1911) parasitizes a different family of Lepidoptera hosts (Nymphalidae) (Yu et al. 2016) and it has a very different color, with a reddish-brown metasoma dorsally, dark brown tegula and humeral complex and fore wing with most veins white.

Cotesia decaryi (Granger, 1949) is only known from Madagascar and probably represents a local endemic from that country. It parasitizes a different family of Lepidoptera hosts (Lasiocampidae) (Yu et al. 2016). It has metacoxa mostly smooth (rugose-punctate in *C. icipe*), T1 clearly widens towards posterior margin, T2 covers most of the tergum dorsally, and T3 is about the same length than T2 (T3 1.3 × as long as T2 length in *C. icipe*).

Cotesia flagellator (Wilkinson, 1930) is only known from Uganda (Yu et al. 2016); it has scuto-scutellar sulcus with 4–5 (very rarely 6) major carinae (*C. icipe* has 8–9, rarely 7 major carinae); metacoxa mostly smooth, with only indefinite coarse punctures anterodorsally (metacoxa mostly with shallow punctures in *C. icipe*); T2 2.2 × as long as wide at posterior margin (2.0 × in *C. icipe*); T3 1.5 × as long as T2 length (1.3 × in *C. icipe*).

Three related species, ***Cotesia flavipes*** Cameron 1891**,** ***Cotesia sesamiae*** (Cameron, 1906) and ***Cotesia typhae*** Fernández-Triana, 2017, have all been recently revised in Kaiser et al. (2017), including extensive illustrations. They are all part of the *flavipes* complex and can be distinguished by relatively short antennae (much shorter than body length, usually not surpassing the length of head and mesosoma; whereas the antenna of *C. icipe* is comparatively much longer, about as long as body), body relatively depressed, and metasoma extensively to slightly pale in coloration (yellow, orange or light brown) but clearly much lighter in color than *C. icipe*. Beyond the substantial morphological differences these three species parasitize different host species within the families Crambidae and Noctuidae (Yu et al. 2016, Kaiser et al. 2017).

Cotesia pistrinariae (Wilkinson, 1929) has a strongly narrowed T1 centrally, which is unique among all known species of *Cotesia*; extensive illustrations of *pistrinariae* can be found in Gupta et al. (2016). It also parasitizes a different family of Lepidoptera hosts (Pieridae) (Yu et al. 2016).

Cotesia vestalis (Haliday, 1834) has T1 clearly widening towards posterior margin and T2 covering most of the tergum dorsally, both tergites are also much more coarsely sculptured than in *C. icipe*; the coxae (especially pro- and mesocoxae) are also darker colored as compared to *icipe*. There are many host records attributed to this species (Yu et al. 2016), some of them dubious; they include mostly Erebidae, Nymphalidae and Plutellidae, with a couple of Noctuidae species different from those parasitized by *C. icipe.*

Cotesia vanessae (Reinhard, 1880) is mostly a Palearctic species, recently found to have spread into the Nearctic (Hervet et al. 2014), and only marginally distributed in Africa (Yu et al. 2016). It has tegula and humeral complex dark brown to black, metasoma entirely black dorsally, and legs mostly dark brown. Numerous host records, some of them certainly inaccurate, are attributed to this species (Yu et al. 2016).

Of all described Afrotropical species ***Cotesia ruficrus*** (Haliday, 1834) looks most similar to *C. icipe* from a morphological perspective; however, it has T1 clearly wider at posterior margin as compared to anterior margin (almost the same width in *C. icipe*); T2 covers most of the tergum surface; and vein R1 in fore wing is comparatively much shorter, about the same length of pterostigma, and with the distance between end of vein R1 and the end of vein 3RSb being more than 0.4 × the length of vein R1 (in *C. icipe* the vein R1 in fore wing is comparatively much longer, clearly longer than pterostigma length, and with the distance between end of vein R1 and the end of vein 3RSb being less than 0.2 × the length of vein R1); also, the overall coloration is lighter and more reddish than in *C. icipe*. Furthermore, from a biological point of view, *C. ruficrus* is a gregarious parasitoid while *C. icipe* is a solitary parasitoid. Numerous host records, some of them certainly inaccurate, are attributed to *C. ruficrus* (Yu et al. 2016).

Cotesia rugosa (Szépligeti, 1914) presents the most difficult case to assess, as it is only known from the male holotype, collected in 1912 at an altitude of 2,000 m, on the western side and near of Mount Kenya (Szépligeti 1914, Papp 2008). *C. icipe* has been collected not too far from that locality, at close to 1,200 m. However, and according to the original description, *C. rugosa* has different colored legs, including many red areas (legs mostly yellow in *C. icipe*, with a few areas brown); tegula reddish-brown (yellow in *C. icipe*); T2 as long as T3 (0.7–0.8 × in *C. icipe*); T1 is described as 'almost transversal' (Szépligeti 1914: 184) which, within the context of the Microgastrinae species described in that paper, seems to indicate that T1 is strongly widened towards posterior margin, i.e., T1 width at posterior margin is about as long as T1 length medially (whereas T1 is 1.5 × as long as wide at posterior margin in *C. icipe*).

Discussion

The finding of the new species *C. icipe* is of particular interest because it successfully attacks Noctuids of the genus *Spodoptera* (Agbodzavu et al. in press), which includes notorious pest species. The tropical and subtropical genus *Spodoptera* includes several economically important species in Africa (Brown and Dewhurst 1975). The most destructive ones reported in the Afrotropical regions include *S. littoralis*, *S. exigua*, *S. exempta* (Brown and Dewhurst 1975) and *S. frugiperda* (Goergen et al. 2016). The first two have been confirmed as suitable hosts for *C. icipe*, which therefore represents a new component in the environmental friendly management of both pests in small scale farming systems (Agbodzavu et al. in press). *Spodoptera littoralis* and *S. exigua* are herbivores with over 90 known host plants including cotton, tobacco, soybean, cabbage, amaranth, coffee, potato and wheat, among others (Abdullah et al. 2000, Agbodzavu et al. in press, Azidah and Sofian-Azirun 2006, Clarke-Harris et al. 2004, Saeed et al. 2017, Santiago-Alvarez and Ortiz-Garcia 1992). Young larvae of both pests feed on terminal leaf clusters, seedlings, and stems of host crops, leading to skeletonized plants (Bohmfalk et al. 1999, East et al. 1989, McDougall et al. 2013). Further studies are also warranted to assess the performance of *C. icipe* on *S. exempta* and *S. frugiperda*. The African armyworm *S. exempta* is a serious pest of maize and rice, the most important staple crops across Africa (Grzywacz et al. 2008, Okello-Ekochu and Wilkins 1996, Sithole 1989) while the fall armyworm *S. frugiperda* is a newly reported invasive pest on the continent, causing currently the highest damage on maize, rice, sorghum and millet (Cock et al. 2017, Goergen et al. 2016). *Cotesia icipe* represents therefore a potential candidate for natural control of pests of these staple crops.

Acknowledgements

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Supplementary material 1

Tree for Barcode Index Number – BOLD:ABZ7318

Authors: Komi K.M. Fiaboe, Jose Fernández-Triana, Faith W. Nyamu, Komi M. Agbodzavu

Data type: molecular data

Link: https://doi.org/10.3897/jhr.61.21015.suppl1

Supplementary material 2

Tree Result – Search: Sample IDs; Seq Length(500 bp); Include public records (170 records returned) (170 records selected)

Authors: Komi K.M. Fiaboe, Jose Fernández-Triana, Faith W. Nyamu, Komi M. Agbodzavu

Data type: moleculara data

Link: https://doi.org/10.3897/jhr.61.21015.suppl2

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RESEARCH ARTICLE

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Additions to the xiphydriid woodwasp (Hymenoptera, Xiphydriidae) fauna of New Caledonia

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Abstract

Calexiphyda marystellae Villemant & Smith, **sp. n.**, is described from New Caledonia. This is the sixth species of Xiphydriidae known from New Caledonia. A key to the six species is given, the female of *C. crocea* Smith is described, and a new locality for this species and for *C. caledonia* Smith is given.

Keywords

New Caledonia, high-altitude forest, *Calexiphyda*, Xiphydriidae

Introduction

Jennings et al. (2007) were the first to record Xiphydriidae from New Caledonia, a single male described as *Rhysacephala novacaledonica* Jennings and Austin. Smith (2008) treated five species from New Caledonia, *Lissoxiphyda tripotini* Smith and four species in the new genus *Calexiphyda*. Two of the *Calexiphyda* species were based on females and two on males. Because so many xiphydriids are based on single specimens and because of the extreme sexual dimorphism, it is difficult to associate sexes. Smith (2008) cited a number of unassociated males which did not fit the described species and need to await confirmed association of sexes through collections of long series or by rearing.

Three additional specimens were recently collected in New Caledonia by CV. One is an undescribed species, one is the female of a previously described species, and one was previously described but from another locality. Here, we add these discoveries to the xiphydriid fauna and give an updated key to species on the island. There are now six species known from New Caledonia, five based on females and one based on a male. New Caledonia now has the largest xiphydriid fauna of any of the Pacific islands. Three are known from New Zealand (Ward and Goulet 2011), one from Lord Howe Island (Jennings and Austin 2009), and one from Fiji (Smith 2008). The only other species of Symphyta known in New Caledonia is the introduced *Phylacteophaga frottatti* Riek (Pergidae), a leafminer of *Eucalyptus* spp. (Jennings et al. 2013).

Where known, larvae of xiphydriids are wood borers in weakened or dying limbs of woody plants. Nothing is known of the biology of the New Caledonian species (Smith 2008). Two New Zealand xiphydriids, *Moaxiphia decepta* (F. Smith) and *M. duniana* (Gourlay), are recorded from *Coprosma robusta* Raoul (Rubiaceae) and *Nothofagus* spp. (Nothofagaceae), respectively (Ward and Goulet 2011).

Materials and methods

Specimens were collected by CV in the course of the "*Our Planet Reviewed*" project (http://laplaneterevisitee.org/en) during the "New Caledonia 2016-2017" expedition. All are deposited in the Muséum National d'Histoire Naturelle, Paris, France.

Insect collection was performed in November 2016 in the southeastern part of the island, where the mountains exposed to the Pacific Ocean have been little explored, hence the name of "Côte Oubliée" given to the coastal massif. Three stations, two in the mountains (Comboui and Kwakwé) and one in the Ouinné Valley, were inventoried using Malaise traps placed for ten days in five plots of every station. In addition, height groups of three yellow pan traps were set up in the Comboui station in or close to the Malaise trap sites. This station was located 12 km from the coast, near Mount Bwa Bwi (1,214 m) above a forest valley whose streams flow into the Néma River. Traps were located along a transect set up from a dense scrub habitat on the Bwa Bwi slope to a tropical high-altitude forest on the crest and a dense humid forest of low and medium altitude on igneous substrate in the forest bottom (Muzinger and Bruy, pers. com.).

The three xiphydriids collected during this mission were caught in yellow pan traps located on the crest in the dense and humid high-altitude forest. This forest, showing a typical facies with lichens, bryophytes and Hymenophyllaceae, was rather low, with a canopy about 12 m high and some emerging trees up to 15 m (Muzinger and Bruy, pers. com.).

Images were acquired through an EntoVision micro-imaging system. This system included a Leica M16 with a JVC KY-75U 3-CCD digital video camera or a GT-Vision

Lw11057C-SCI digital camera attached that fed image data to a notebook or desktop computer. The program Cartograph 6.6.0 was then used to merge an image series into a single in-focus image.

Morphological terminology follows Huber and Sharkey (1993).

Results

Key to the Xiphydriidae of New Caledonia

1 Female ... **2**

– Male ... **6**

2 Hind wing with cells Rs and M absent; hind tarsal claw simple; mesopleuron shiny; mesonotum rugose, without carinae (antennomeres 1 and 2 orange; legs orange with apical tarsomeres black) (see Smith 2008, figs 27–30) ... ***Lissoxiphyda tripotini*** **Smith**

– Hind wing with cells Rs and M present; hind tarsal claw with inner tooth; mesopleuron usually rugose (except *C. crocea*); mesonotum usually with longitudinal or transverse carinae (except *C. crocea*) (*Calexiphyda*) ... **3**

3 Black with mid and hind tibiae, mid and hind tarsi, and sheath orange (Fig. 13); anteromedian part of mesonotum and inner half of mesonotal lateral lobes with distinct longitudinal carinae (Fig. 15) ... ***C. caledonia*** **Smith**

– Some yellow markings on head (Figs 4, 10) and sometimes thorax; legs various, all light orange, black with tibiae and tarsi light orange or almost entirely black; sheath black (Figs 1, 7); anteromedian part of mesonotum and mesonotal lateral lobes rugose, with faint longitudinal or transverse carinae or without sculpture (Figs 3, 9) ... **4**

4 Head with two longitudinal stripes on vertex, inner orbits, spots between antennae, and genae yellow; thorax with posterior margin of pronotum yellow; legs black with inner surfaces of tibiae reddish brown; anteromedian part of mesonotum with transverse carinae (see Smith 2008, figs 34–38) ... ***C. blanki*** **Smith**

– Head mostly black with yellow spots on lower inner orbits and between antennae (Figs 4, 10); thorax black (Figs 3, 9); legs yellow orange or black with tibiae and tarsi yellow orange (Figs 1, 7); anteromedian part of mesonotum with punctures, longitudinal carinae, or shiny (Fig. 9) ... **5**

5 Anteromedian part of mesonotum shiny with very fine punctures and microsculpture (Fig. 9); legs with coxae and trochanters white, rest of legs orange (Fig. 7); white spot at base of sheath; antennomeres 1 and 2 orange (Figs 10, 12); antennae more than 2× head width, with antennomeres 2× or more longer than broad (Fig. 12); head strongly narrowed behind eyes (Fig. 11) ... ***C. crocea*** **Smith**

– Anteromedian part of mesonotum with faint, irregular, longitudinal carinae on posterior half (Fig. 3); legs black with tibiae and tarsi yellow orange

(Figs 1, 2); antenna and sheath black; antenna about 1.7× head width, antennomeres less than 2× longer than broad (Fig. 6); head broad behind eyes (Fig. 5) .... ***C. marystellae*** **sp. n.**

6 Mostly orange with black mesonotal lateral lobes; head and body shiny (see Smith 2008, figs 39–41) ....***C. crocea*** **Smith**

– Mostly black; mesonotum with longitudinal carinae, frons and mesopleuron rugose (see Jennings et al. 2007, figs 2–9) .... ***C. novacaledonica*** **Jennings & Austin**

Calexiphyda marystellae Villemant & Smith, sp. n.

http://zoobank.org/5E4830D4-B307-47E6-B4E4-09A590E7AD05

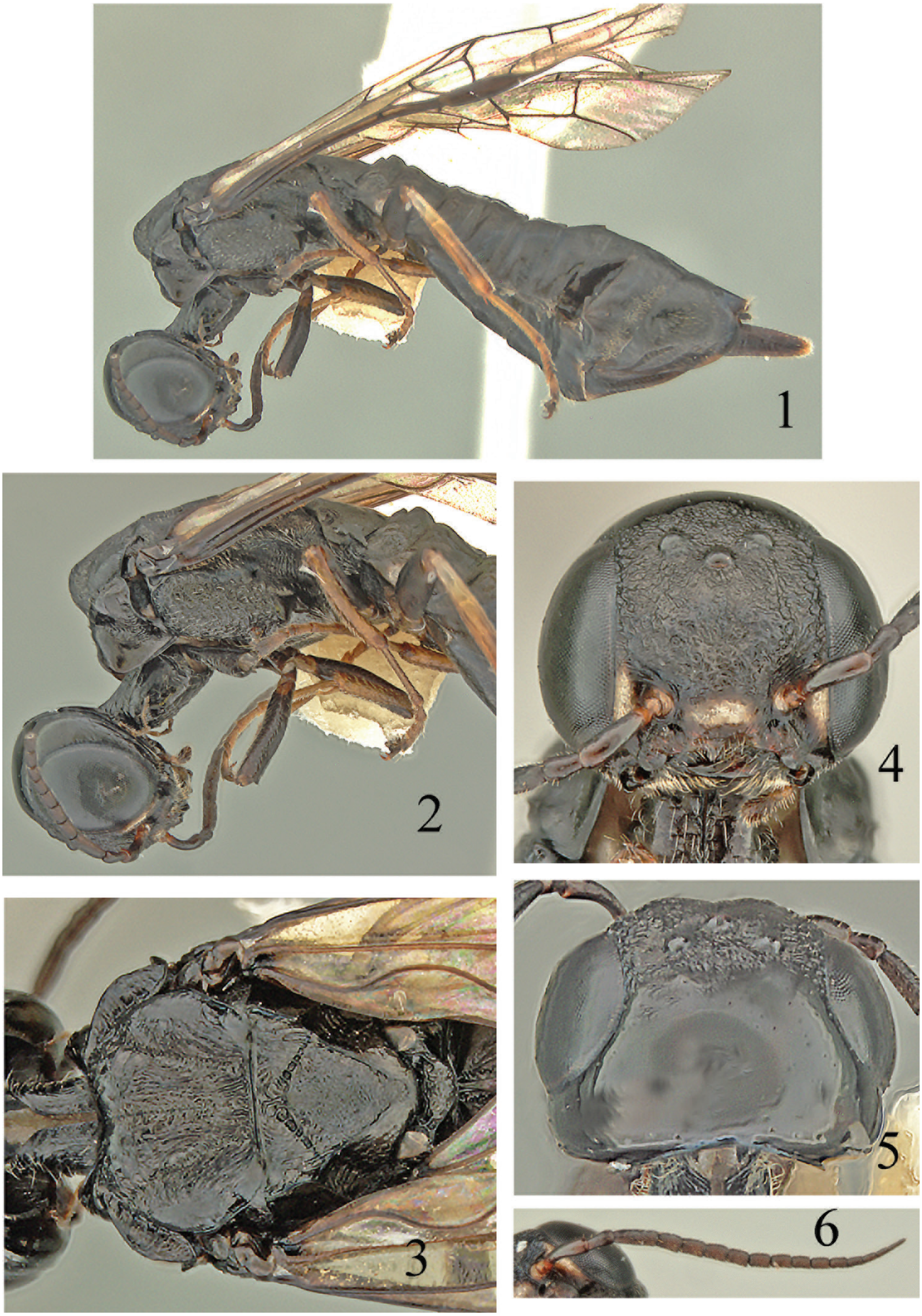
Figs 1–6

Description. Female: Length, 7.0 mm. Black; head with yellow spots between antenna and eye and between antennae (Fig. 4). Thorax black. Legs black with tibiae and tarsi pale yellow. Abdomen black with lateral white spots on first tergite (Figs 1, 2). Wings hyaline, veins and stigma black. *Head*: Antenna (Fig. 6) with 17 antennomeres, 1.7× head width; lengths of scape, pedicel, and first flagellomere as 1.0:0.5:0.8; flagellomeres less than 2× longer than broad. Frons, interocellar area, and area just above ocelli rugose; vertex and gena shiny, almost impunctate; few punctures on lower gena and few indistinct carinae at center of gena. Lower interocular distance subequal to eye height, in front view, eyes very slightly diverging below (Fig. 4). In dorsal view, head broad behind eyes, distance behind eyes less than 2× eye length (Fig. 5). Labial palpus with 4 palpomeres, maxillary palpus with 7 palpomeres. *Thorax*: Propleuron with fine longitudinal carinae dorsally, shiny laterally. Pronotum shiny with central area of fine carinae. Mesonotum (Fig. 3) with shallow notauli, with closely set pits; anteromedian part of mesonotum with fine longitudinal carina on posterior half, more finely rugose centrally and anteriorly; lateral lobes with fine longitudinal carinae on inner halves, shiny on lateral halves; axillae with longitudinal carinae; mesoscutellum finely rugose. Mesepisternum rugose, with narrow stripe along anterior margin shiny, impunctate; mesepimeron with cross carinae; metapleuron rugose (Fig. 2). Hind tarsal claw with small inner tooth near base, fore and mid tarsal claws with long inner tooth, more than half length of outer tooth, near center. Hind basitarsus subequal in length to remaining tarsomeres combined. *Abdomen*: Shiny with very fine microsculpture; tergum 1 duller, with denser microsculpture than rest of abdomen. Tergum 1 subequal in length to tergum 2. Sheath to basal plates as 1.0:1.8.

Male: Unknown.

Holotype. Female, "Nouvelle Calédonie, Province Sud, Thio, Comboui, 21.77742S, 166.29495E, 1037 m, NC-COM-YPT3 12-18.XI.2016, yellow pan trap, C. Villemant rec."

Etymology. This species is described in honor of Mary-Stella Guelemé, a New Caledonian participant to the "*Our Planet Reviewed*" expedition who contributed with



Figures 1–6. *Calexiphyda marystellae*. **1** Lateral **2** Head and thorax, lateral **3** Thorax, dorsal **4** Head front **5** Head dorsal **6** Antenna.

another manager of the Comboui camp to maintain morale of the team when bad weather conditions retained it longer than expected on the Bwa Bwi slope.

Remarks. The black head and body with only three white spots on the front of the face and one white spot laterally on tergite 1, light orange tibiae and tarsi, and faint, irregular, longitudinal sculpture of the mesonotum (Fig. 3) will distinguish this species.

Calexiphyda crocea Smith

Figs 7–12

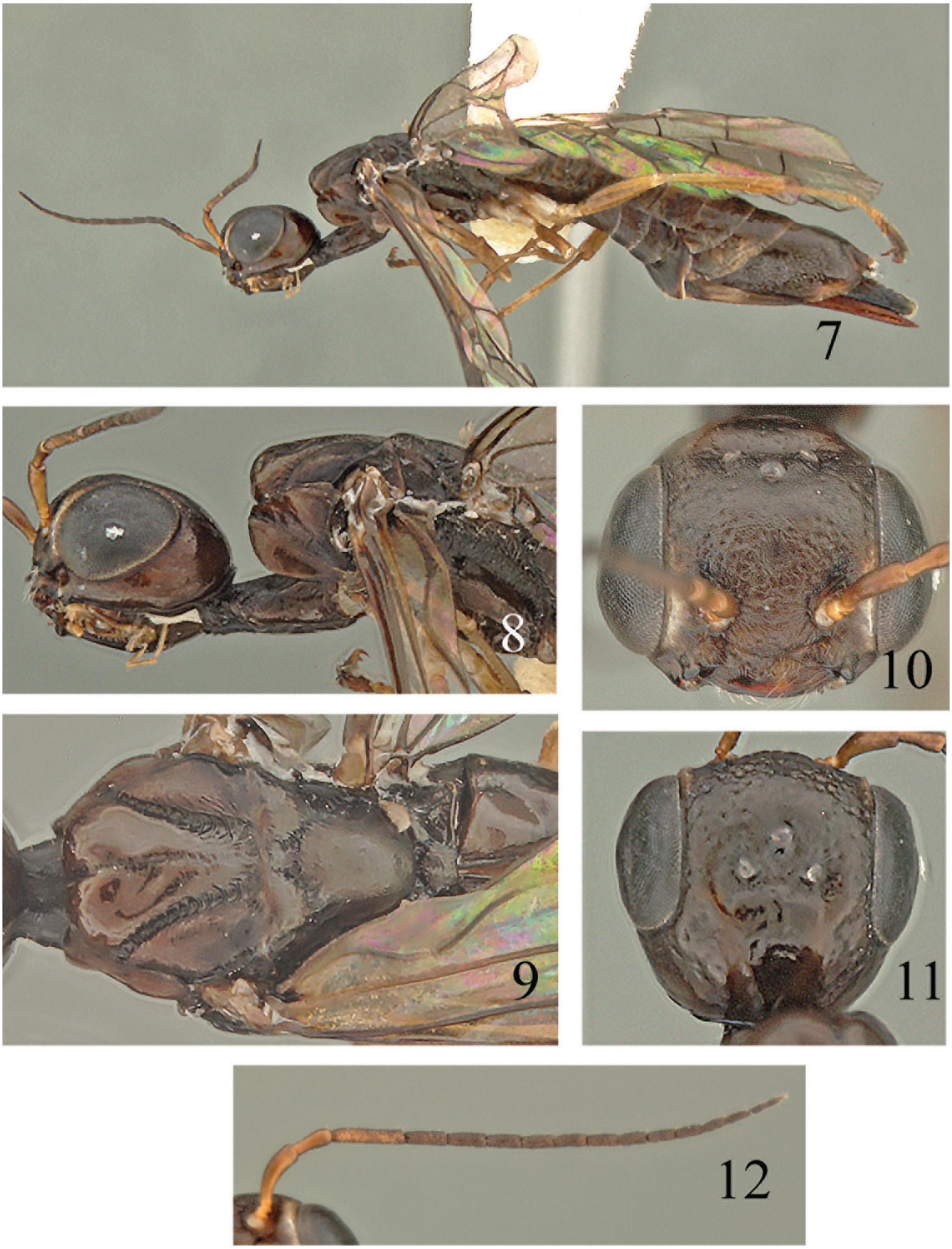
Calexiphyda crocea Smith 2008: 35.

Description. Female. Length, 7.0 mm. Head black with lower inner orbits narrowly white and two pale orange stripes on vertex (Figs 8, 10, 11); antenna black with antennomeres 1–3 orange (Figs 8, 10, 12). Legs with coxae and trochanters white, rest of legs orange (Fig. 7). Abdomen black with white spot at base of tergum 1, indistinct white spots laterally on tergites 8 and 9, and apical white spot on 8th tergite. Wings uniformly hyaline, veins and stigma black. *Head*: Antenna (Fig. 12) 2.1× head width, with 17 antennomeres; lengths of scape, pedicel, and first flagellomere as 1.0: 0.8:1.2; pedicel more than 2× longer than broad, flagellomeres 2 to apex 2× or more longer than broad. Frons finely rugose to just above ocelli; vertex and gena shiny. Lower interocular distance 1.1× eye height; in front view, eyes slightly diverging below (Fig. 10). Head, in dorsal view, long and strongly narrowed behind eyes (Fig. 11); length behind eyes about half eye length. Genal carina distinct to top of eyes (Fig. 8). Labial palpus with 4 palpomeres, maxillary palpus with 7 palpomeres. *Thorax*: Propleuron shiny, finely rugose anteriorly and dorsally. Pronotum shiny, with central, diagonal groove, finely rugose in groove; posterior lateral corners rounded, without carinae. Anteromedian part of mesonotum shiny, finely rugose at extreme posterior portion; lateral lobes with fine longitudinal carinae on inner third, shiny on outer portions; axillae finely rugose with faint longitudinal carinae; mesoscutellum shiny, finely rugose; metascutellum rugose; notauli deep, broad, with distinct deep pits (Fig. 9). Mesepisternum smooth and shiny, mesepimeron with cross carinae; metapleuron shiny with fine microsculpture. Hind tarsal claw with small inner tooth near base, fore and mid tarsal claws with long inner tooth, more than half length of outer tooth, near center. *Abdomen*: Shiny. Tergum 1 1.3× length of tergum 2. Length of sheath to basal plates as 1.0:1.8.

Male: Length 6.5 mm. Color and structure similar to female (see Smith 2008).

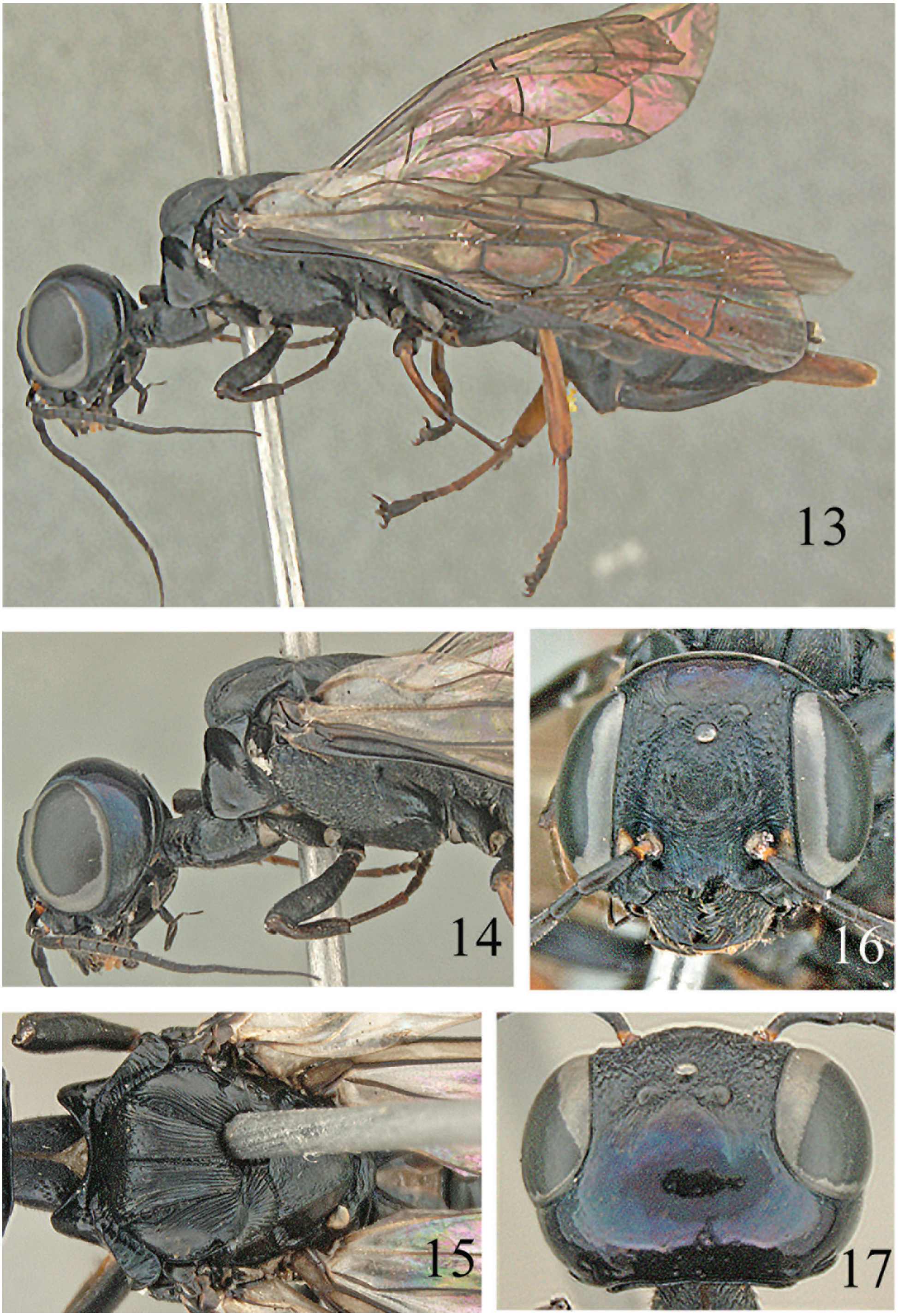
Specimen examined. Female, "Nouvelle Calédonie, Province Sud, Thio, Comboui, 1037 m (21.77742S, 166.29495E) NC-COM-YPT-3, 12-18.XI.2016, yellow pan trap, C. Villemant rec."

Remarks. Although descriptions of unassociated males are discouraged, Smith (2008) described *C. crocea* because of its unusual shiny appearance and stated that association with the female should not be difficult. The characteristics of this female are so similar to the male described as *C. crocea* that it must be the female of the species.



Figures 7–12. *Calexiphyda crocea*, female. **7** Lateral **8** Head and thorax, lateral **9** Thorax dorsal **10** Head front **11** Head dorsal **12** Antenna.

The shiny appearance, long antennae, rounded pronotum, deep, broad notauli with deep punctures, and the long first tergite, 1.3× length of the second tergite, are characters common to both sexes.



Figures 13–17. *Calexiphyda caledonia*. **13** Lateral **14** Head and thorax lateral **15** Thorax dorsal **16** Head front **17** Head dorsal.

Calexiphyda caledonia Smith

Figs 13–17

Calexiphyda caledonia Smith 2008: 33.

Diagnosis. Female: Length, 21 mm. Black with mid and hind tibiae and tarsi and sheath orange, white spots laterally on first tergite (Fig. 13). Frons finely punctate to rugose; short, indistinct longitudinal carinae between antennae (Fig. 16, 17). Anteromedian part of mesonotum, inner half of mesonotal lateral lobes, and axillae with longitudinal or diagonal carinae, those on anteromedian part of mesonotum nearly entire length (Fig. 15).

Male: Unknown.

Specimen examined. Female, "Nouvelle Calédonie, Province Sud, Thio, Comboui, 1040 m (21.77805S, 166.29472E), NC-COM-YPT8 17-19.XI.2016, yellow pan trap, C. Villemant rec."

Remarks. This species is recognized by its black color with the contrastingly orange tibiae, tarsi, and sheath (Fig. 13), and the distinct longitudinal carinae on the mesonotum (Fig. 15). This specimen represents a new record for the species. It was previously recorded from Rivière Bleue Province Park and Mt. Khogis, in Province Sud, 17 km NNE Nouméa (Smith 2008).

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RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH
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A new species of *Hexacladia* Ashmead (Hymenoptera, Encyrtidae) and new record of *Hexacladia smithii* Ashmead as parasitoids of *Dichelops furcatus* (Fabricius) (Hemiptera, Pentatomidae) in Argentina

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Abstract

Pentatomid adults of the species *Dichelops furcatus* (F.), collected on stubble of soybean, *Glycine max* (Linnaeus) Merril, in Santa Fe province of Argentina, were found parasitized by two encyrtid wasp species (Hymenoptera: Encyrtidae). One of the encyrtids is described as *Hexacladia dichelopsis* Torréns & Fidalgo, **sp. n.,** from both sexes, and the other species *H. smithii* Ashmead, is recorded for the first time from *D. furcatus* in Argentina. Both species are gregarious endoparasitoids which carry out the whole development (larval and pupal) in their living hosts; they emerge as imagoes, by cutting their way out through the dorsal wall of the abdomen. Including the newly described *H. dichelopsis*, seven species of the genus are recorded from South America, and an identification key to separate them is presented.

Keywords

Hexacladia, new species, parasitoids, *Dichelops furcatus*

Introduction

Members of the genus *Hexacladia* Ashmead are gregarious parasitoids of the late nymphs or adult stages of Pentatomidae, Coreidae, Pyrrhocoridae and Scutelleridae (Hemiptera) (Burks 1972, Noyes 2010), emerging from the living host. *Hexacladia* is unique amongst Encyrtidae in having a relatively slender, distinct petiole; the very high placement of the antennae, a dome-like scutellum, characteristic infuscation and setation of the fore wing and branched antenna of the males makes the genus very easy to recognize (Noyes 2010).

Six species of *Hexacladia* have been recorded from South America: *H. blanchardi* De Santis (Argentina and Brazil), *H. linci* Rasplus (Peru), *H. smithii* Ashmead (Argentina, Brazil and Venezuela), *H. townsendi* (Crawford) (Peru, Ecuador and Venezuela), *H. supina* Noyes (Ecuador), *H. impiros* Noyes (Ecuador and Peru) (De Santis 1963, 1979, 1980, De Santis and Fidalgo 1994, Rasplus et al. 1990, Noyes 2010, 2017). *H. smithii* is the species with many hosts mentioned in the literature, and also the most widely distributed being found in Argentina, Brazil, Costa Rica, México, Nicaragua, Puerto Rico, Trinidad and Tobago, USA and Venezuela (Gordh 1979, Noyes 2010, 2017).

Dichelops furcatus (F.) known as "chinche furcada" or "chinche de los cuernos" is considered a corn pest because it injects toxins into the stem during feeding. These toxins damage the vigor of the seedlings producing a detention of its growth, malformations in some cases and even death by intense attacks in the most susceptible stages (Canale and Ferreira 2013). *D. furcatus* has been recorded as the primary host of two encyrtids, *Ooencyrtus submetallicus* (Noyes 2010), and *Hexacladia smithii* in Southern Brazil (Panizzi and Da Silva 2010).

Herein we describe *Hexacladia dichelopsis* sp. n. and provide a key to separate the seven species of *Hexacladia* now known from South America. We also present a new record of *H. smithii* as parasitoid of *D. furcatus* in Argentina.

Methods

Both species of *Hexacladia* emerged from diapausing adults of *D. furcatus* on soybean stubble. The material was collected in the Experimental field J. Villarino of the Faculty of Agronomic Sciences in Zavalla, province of Santa Fe (Argentina). The collection date was August 2015, March and May 2016. In total, 33 females and 2 males of *H. dichelopsis* sp. n. and 4 females and 4 males of *H. smithii* were collected. The material was preserved in alcohol and then mounted; some of them were slide-mounted in Canada balsam.

For morphological terms and species identification we followed Burks (1972), Noyes (2010) and Cuezzo and Fidalgo (1997). The abbreviations used are: Fn = flagellar segment n; LOL = shortest distance between posterior ocellus and anterior ocellus in frontal view, OOL = shortest distance between posterior ocellus and eye margin in dorsal view and POL = shortest distance between posterior ocelli in dorsal view.

Specimens are deposited in the Instituto Fundación Miguel Lillo, Tucumán, Argentina (IFML); Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires, Argentina (MACN); and the Entomology collection of CRILAR, Anillaco, La Rioja (CRILAR-En).

Results

Key to the South American species of *Hexacladia* Ashmead (modified from Burks 1972)

1 Male; funicular segments of antenna with branches (Fig. 9)........................**2**
– Female; funicular segments of antenna without branches (Fig. 10) **5**
2 Fore wings comparatively short, 1.3× as long as mesosoma (Figs 1, 2) **3**
– Fore wings normally developed, nearly 2.5× as long as mesosoma (Figs 11, 12)..**4**
3 Fore wings with marginal fringe uneven, largely or completely absent.......... ....***linci* Rasplus**
– Fore wings with marginal fringe present and complete except perhaps for a short strip immediately distad of apex of venation or towards anal angle (Fig. 7)..***dichelopsis* sp. n.**
4 Branch of F6 extremely short, 0.1× length of segment itself. Female unknown ....***blanchardi* De Santis**
– Branch of F6 at least as long as segment itself ....***smithii* Ashmead**
5 Fore wings comparatively short, 1.3× as long as mesosoma **6**
– Fore wings normally developed, nearly 2.5× as long as mesosoma...............**7**
6 Fore wings with marginal fringe uneven, largely or completely absent.......... ....***linci* Rasplus**
– Fore wings with marginal fringe present and complete except perhaps for a short strip immediately distad of apex of venation or towards anal angle (Fig. 8)..***dichelopsis* sp. n.**
7 Fore wings with marginal fringe uneven, largely or completely absent.........**8**
– Fore wings with marginal fringe present and complete except perhaps for a short strip immediately distad of apex of venation or towards anal angle.....**9**
8 Costal cell of fore wing dorsally naked, or virtually so; with at most only one seta ventrally. Male unknown.. ***supina* Noyes**
– Costal cell of fore wing with up to 9 setae dorsally and 7 ventrally............... ....***smithii* Ashmead**
9 Each lateral projection of hypopygium broadened, flattened and rounded apically, paddle-like. Male unknown.....................................***impiros* Noyes**
– Hypopygium apically with a pair of long, curved lateral processes either side of a deep, median invagination. Male unknown........ ***townsendi* (Crawford)**

Hexacladia dichelopsis Torréns & Fidalgo, sp. n.

http://zoobank.org/83FD4672-A94D-437C-8F6E-68F4673E84E6

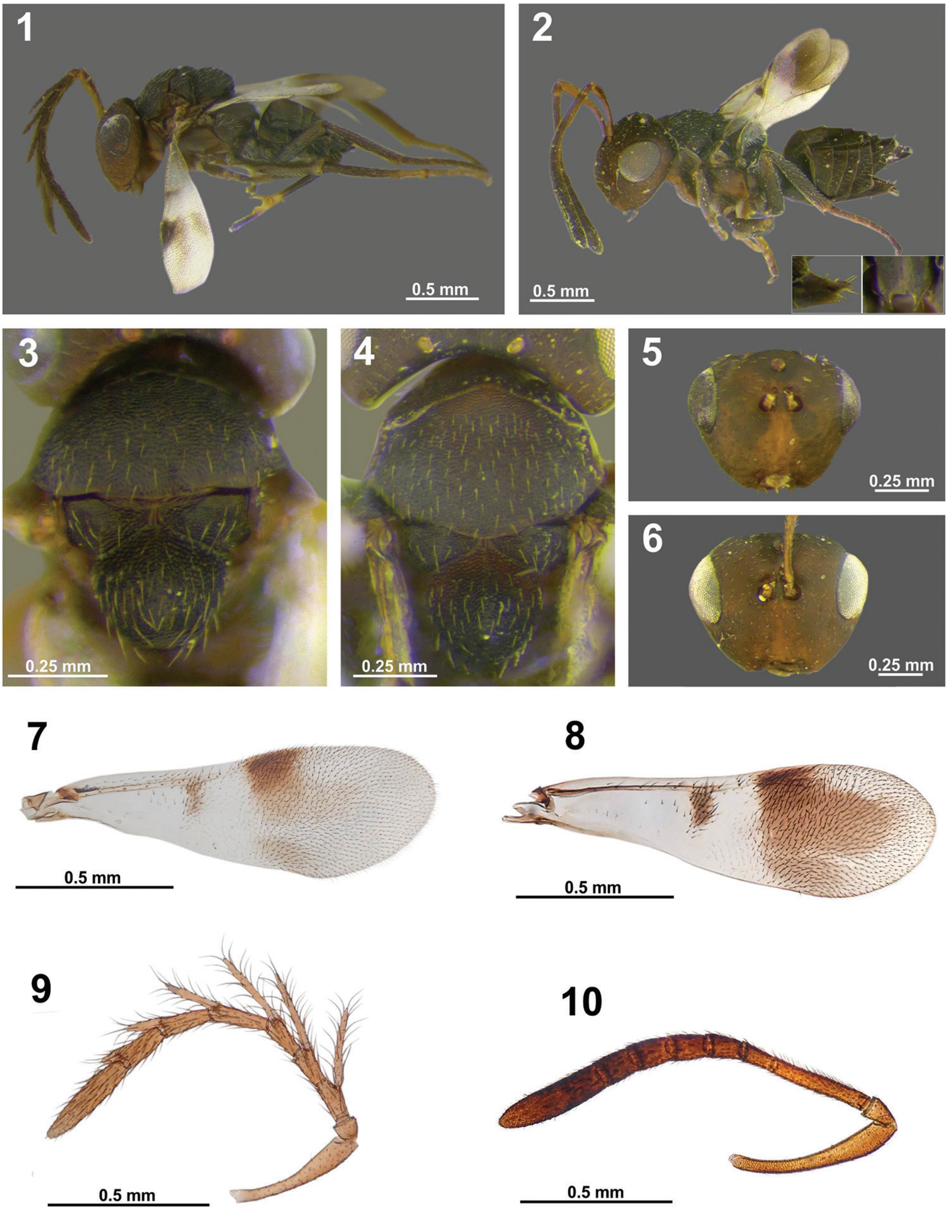
Figs 1–10

Diagnosis. Distinguished from other species by the following combination of characters: general body color dark brown to black (Figs 1, 2); small wings, fore wing 1.3× as long as length of mesosoma (Figs 1, 2); female with costal cell with four or five setae, distal third of the upper margin without marginal setae, dark areas of the wing disc well evident; male with costal cell with abundant setae (25 to 27), basal third with eight to nine setae, distal third of the upper margin with marginal setae and dark areas of the wing disc poorly defined; male with F7 2.0× as long as F6, branch of F6 0.5× length of F6.

Description. Female (holotype): length, 2.3mm. General body color dark brown to black dorsally, being paler toward head, scape and pedicel, axilla, metanotum, apex of tibiae and tarsi, propodeum and base of first tergite of gaster; entire body lighter ventrally, femora and metasoma slightly darker than the rest; setae on dorsum of mesosoma translucent (Figs 2, 6). Wings largely hyaline, but fore wing with two brown areas, the first and smaller behind base of parastigma, with about 40 strong dark brown setae, the distal area is most extensive, begins behind marginal vein and occupies most of wing apex, alar disc between the two brown areas with light setae (Fig 8).

Head 1.3× as broad as high. POL 2.1× LOL; POL 1.3× OOL. Head with fine, raised, imbricate-reticulate sculpture, well-marked and moderately deep on frons and vertex, and slightly marked and irregular on face and gena; anterior ocellus connected to toruli by a shallow groove; interantennal area narrow and smooth; frontovertex and face with inconspicuous, decumbent setae, each slightly shorter than diameter of anterior ocellus (Fig. 6). Eyes separated by 1.7× their height. Malar space 0.9× height of eye. Antenna 9-segmented, flagellar segments cylindrical. Flagellum 1.6× as long as head height; length of F1 0.6× length of scape, and 2.8× as long as broad; clava 3.4× as long as broad. Antenna (Fig. 10) apically with slightly curved setae in all segments, more dense on funicle; funicle with linear sensilla only on F3–F6 (Fig. 10).

Mesosoma with fine, raised, polygonally-reticulate to imbricate-reticulate sculpture on mesoscutum (Figs 2, 4). Pronotum with small and decumbent setae, head overlapping the pronotum. Mesoscutum 1.7× as broad as long, with small and decumbent setae (Fig. 4). Scutellum 1.1× as long as maximum width; axilla and scutellum with coarse reticulate sculpture slightly deeper than that on mesoscutum, and with semi-erect and longer setae than the mesoscutum; scutellum humped, strongly and fairly evenly curved in profile (Fig. 4); propodeum with a group of about 11–14 setae outside spiracle. Fore wing 3.1× as long as broad, costal cell with seven or eight setae dorsally, linea calva broad and open posteriorly, basal cell with five to eight setae, submarginal vein with 11 bristle-like setae, postmarginal vein slightly shorter than stigmal vein, marginal fringe present, stigmal vein without uncus and with three or four placoid sensilla; hind wing 3.7× as long as broad.



Figures 1–10. *Hexacladia dichelopsis* sp. n.: **1** habitus (male) **2** habitus (female) (magnified apex of hypopygium, lateral and ventral view) **3** mesosoma (male, dorsal) **4** mesosoma (female, dorsal) **5** head (male, frontal) **6** head (female, frontal) **7** fore wing (male, lateral) **8** fore wing (female, lateral) **9** antenna (male, lateral) **10** antenna (female, lateral).

Metasoma 1.5× as long as broad. Hypopygium very nearly reaching apex of metasoma; apically with a pair of short lateral processes either side of a wide, shallow, median invagination; with about 6 hypopygial hairs (Fig. 2). Ovipositor not observed.

Variation. The length of the female varies between 2.2–2.6 mm. The coloration in some specimens are darker than holotype, otherwise very little variation in material examined.

Male. Similar to female except for following: The length of the male varies 1.7–1.9 mm. General body color and head darker than female (Figs 1, 3, 5), fore wing with areas paler, with costal cell with abundant setae (25 to 27), basal third with eight to nine setae, stigmal vein with four or five placoid sensilla (Fig. 7) ; antenna with funicle segments longer than broad and with a elongate dorsal branch (Fig. 9); F1 a little longer than F2 and F3; F4 longer than F5 and F6; F7 is the longest flagellomere; branch of F1 2.0× as long as F1 and as long as F1 and F2 together; branch of F6 0.5× length of F6. Length:width of the antennal segments in the following relationship: scape (48:7), pedicel (9:8), F1 and branch of F1 (14:5 and 29:3), F2 and branch of F2 (13:5 and 31: 2,5), F3 and branch of F3 (13:5 and 30:2), F4 and branch of F4 (19:5 and 20:2), F5 and branch of F5 (16:5 and 14:2), F6 and branch of F6 (16:6 and 9:2), F7 (35:7).

Material examined. Holotype ♀: ARGENTINA, Santa Fé, Zavalla, Campo experimental Villarino, Fac. Cs. Agrarias, 2.v.2016., E. Punschke, ex. diapausing adult of *Dichelops furcatus* on soybean stubble (MACN). Paratypes (11 ♀♀ and 23 ♂♂): same locality as holotype, 2.viii.2015 (MACN, 11 ♀ and 2 ♂), 2.v.2016 (IFML, 11♀♀, IFML; CRILAR-En, 10 ♀♀).

Hexacladia smithii **Ashmead, 1891**

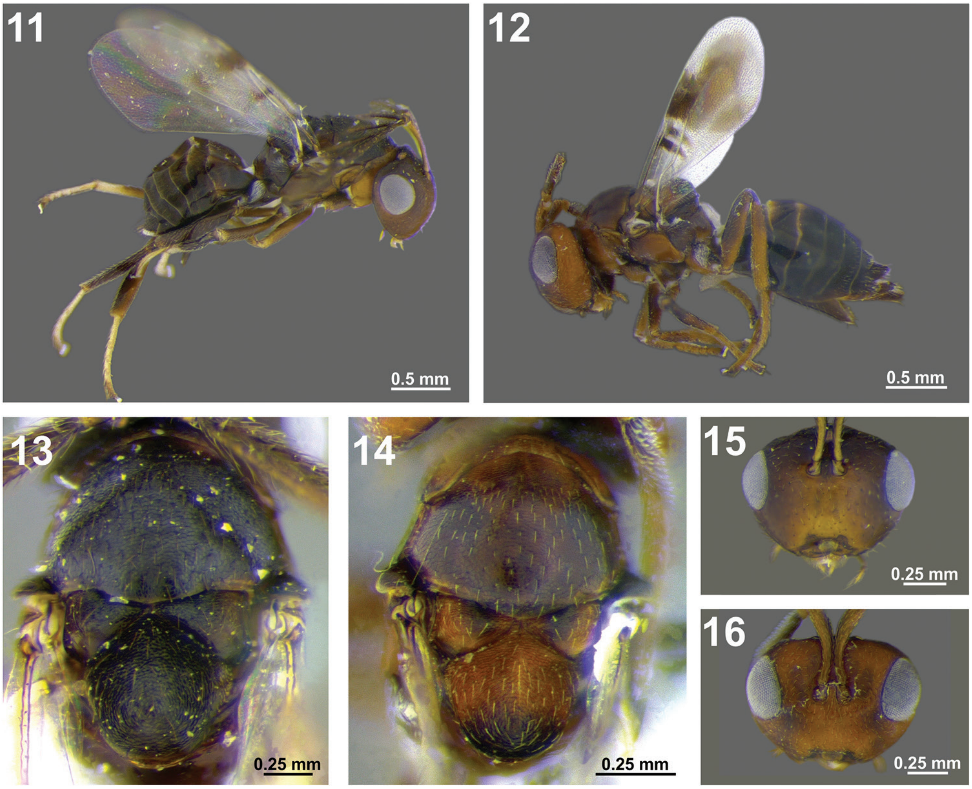
Figs 11–16

Hexacladia smithii Ashmead, 1891:457, lectotype designated by Burks (1972).

Hexacladia mexicana Girault, 1917, lectotype designated by Cuezzo and Fidalgo (1997). Synonymized under *smithii* by Cuezzo and Fidalgo (1997).

Diagnosis. Distinguished from other species by the following combination of characters: female with general body color and head mostly dark, reddish brown (Figs 12, 14, 16); presence of subapically curved and slightly flattened setae dorsally on F1; fore wing fully developed, reaching apex of gaster, with marginal fringe absent, dark areas of the wing disc well evident; costal cell dorsally with fewer than 10 setae and ventrally with at least 2 or 3; hypopygium apically with a pair of short lateral processes either side of wide, shallow, median invagination. Male similar to female but body color mostly dark brown (Figs 11, 13, 15); antenna with all funicle segments branched, branch of F6 at least as long as segment itself; fore wing with marginal fringe present, although occasionally slightly reduced.

Material examined. ARGENTINA, Santa Fé, Zavalla, Campo experimental Villarino, Fac. Cs. Agrarias, 2.viii.2015, E. Punschke, ex. diapausing adult of *Dichelops furcatus* on soybean stubble (CRILAR-En, 1♀ and 1♂; MACN, 1♀ and 1♂; IFML, 2♀♀ and 2♂♂). ARGENTINA, Tucumán, S. M. de Tucumán, 3.v.1993, P. Fidalgo (IFML, 1♀).



Figures 11–16. *Hexacladia smithii*: **11** habitus (male) **12** habitus (female) **13** mesosoma (male, dorsal) **14** mesosoma (female, dorsal) **15** head (male, frontal) **16** head (female, frontal).

Discusion

Hexacladia dichelopsis sp. n. differs from *H. linci* (morphologically the closest species) in: general body color dark brown to black, including head; funicle including clava and scape dark brown (versus general body color brown, pleura yellowish to brown, yellowish face and funicle, the first two flagellomeres and scape dorsally darker, apex of clava black); antenna of the female with F1 as long as following three segments, with slightly curved setae (versus F1 almost as long as following four segments, with straight setae), antenna of the male with branch of F6 0.5× length of F6 (branch of F6 as long as F6 or longer than the flagellomere); fore wing with continuous marginal fringe (versus absent or discontinuous marginal fringe), basal third of wings with setae present (versus basal third of wings without setae). Following the description of Rasplus et al. (1990) the shape of distal margin of hypopygium is similar in both species.What about shape of distal margin of hypopygium.

Two species were previously recorded from Argentina, *Hexacladia smithii* and *H. blanchardi*; *H. dichelopsis* differ from *H. smithii* mainly by its coloration, length and setation of fore wing. While the male differ from that of *H. blanchardi* in: antenna of male with branch of F1 two times longer than the F1 and branch of F6 0.5× length of the F6 (versus branch of F1 11 to 12 times longer than F1 and branch of F6 rudimentary); F7 two times longer than F6 (versus F7 1.2× longer than F6); fore wings 1.1–1.2 mm, reaching half of metasoma (versus 1.9 mm, reaching to the apex of metasoma); basal third of wings with eight to nine setae (versus basal third of wings densely setose).

Concerning the parasitoidism data, it was not possible to separate the parasitoidism of both species; there were few occasions when the host were found so the rate of parasitoidism is only an estimation and correspond to both species combined: five to eight adults per diapausing *Dichelops furcatus* emerged and reached up to 10% during 2015 and up to 7.5% in 2016. The development from collection of parasitized host to emergency of adult parasitoids took approximately 30 days.

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SHORT COMMUNICATION

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Online image databases as multi-purpose resources: discovery of a new host ant of *Rickia wasmannii* Cavara (Ascomycota, Laboulbeniales) by screening AntWeb.org

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Abstract

Public awareness has been raised on the importance of natural history and academic collections for science and society in a time when reduced financial support and staff cuts are prevalent. In the field of biology, new species and new interspecies associations are constantly discovered by making use of museum collections, digitalised materials or citizen science programs. In our study, the *Myrmica* Latreille, 1804 image collection of AntWeb.org was screened for fungal ectoparasites. A total of 397 imaged specimens from 133 species were visually investigated. A single specimen of *M. hellenica* Finzi, 1926, collected in Greece by U. Sahlberg, showed a conspicuous fungal infection. The parasite was identified using microscopic methods as *Rickia wasmannii* Cavara, an ectoparasitic fungal species specialised to *Myrmica* ants. This finding represents a new country record and a new *Myrmica* species for the host spectrum of *R. wasmannii*. According to our results, online entomological databases can be screened relatively easily for ectoparasitic fungal infections from new hosts and new regions. However, depending on quality of the insect voucher photos, additional investigation of the material could be needed to confirm the identity of the parasite.

Keywords

Biological collections, database, *Myrmica hellenica*, distribution, Greece

Introduction

Natural history collections have served as the fundament of taxonomic and biogeographical research over centuries. These biological collections could be important to reveal new geographical distributions of several species (Ponder et al. 2001, Suarez and Tsutsui 2004, Solow and Roberts 2006, Pyke and Ehrlich 2010). Examining biological samples in these collections may also reveal previously unrecorded associations between different species (Frey et al. 1992), e.g. in the case of parasites that remain observable and identifiable after the host is conserved and deposited (Haelewaters et al. 2015a). Among the Fungi, this approach in recording parasites' distributions and host associations have been applied in the case of plant-infecting fungi (e.g. Denchev and Denchev 2016) and insect ectoparasites. The latter category includes works describing new species (e.g. Thaxter 1924, Haelewaters and Rossi 2015, 2017, Santamaria et al. 2016, Wang et al. 2016) or presenting new distributional and host association records (e.g. Weir and Hammond 1997, Báthori et al. 2014, 2015, Haelewaters et al. 2014, 2015a, 2017). Records of parasitic fungi have also originated from photo-sharing websites such as Flickr and iNaturalist (e.g. fungi parasitizing *Harmonia axyridis* see Haelewaters et al. 2017) and the image collections of further citizen science projects may similarly be utilized (e.g. Lost Ladybug Project in the USA, the UK Ladybird Survey and iSpot in South Africa, see also Roy et al. 2016). Regarding the family of ants (Formicidae), our group has recently found that the thorough screening of museum specimens can lead to new distributional and host-association records of the otherwise rarely observed ectoparasites in the order Laboulbeniales, providing the first Central European records of the species *Laboulbenia camponoti* Batra (Báthori et al. 2014) and that of *Rickia lenoirii* Santam. (Báthori et al. 2015).

Natural history collections experienced a decline in funding in the last decade with growing concern over the vast potential of biological information waiting to be uncovered from these resources (Pyke and Ehrlich 2010). On the other hand, digitization of conserved material and the possibility to remotely access an ever-growing number of high resolution photographs from collections around the globe has opened up previously unimaginable possibilities (e.g. Beaman and Cellinese 2012) in recent years. Although most researchers are in agreement that new species, taxa and their distributions should not be described merely on digital photos under most circumstances (Amorim et al. 2016, Krell 2016), digital collections can serve as a powerful resource leading to their discoveries as they can point to the material for later direct examination scattered over collections, or direct to the sampling localities.

In the present study, we extend our previous work by screening preserved ant specimens for fungal ectoparasites utilizing the AntWeb website, currently the world's largest online database of images, specimen records, and natural history information on ants (AntWeb 2017). Our aim was to test whether the relatively easily recognizable fungus *Rickia wasmannii* Cavara, an ectoparasite of *Myrmica* Latreille, 1804 ants, can be observed on AntWeb's collection of digitized material of this species-rich and widespread ant genus, thereby providing new distributional and host records for the fungus.

Material and methods

To survey *R. wasmannii* infections, all the specimens in the genus *Myrmica* (workers, males, and queens) digitized in the online database of AntWeb.org were examined. Based on their current statistics (ver. 6.58), 594,399 specimen records and 199,352 total specimen images can be found in this online database from all over the world. In the case of the genus *Myrmica*, 263 species are present in the database, but only 133 species have imaged specimens. In total 397 specimens of these *Myrmica* spp. (44 queens, 30 males, 323 workers) are represented in the database with 1823 photographs (1409 if images of specimen labels are excluded), originating from Europe, Africa, Asia and North America (see Supplementary file 1). Eleven specimens were not identified to species level and one specimen (FOCOL0709) was misplaced.

We examined all the available images of the genus *via* opening each in a web browser. Identifying imaged specimens as being infected was based on the comparison with specimens in our collection of dried infected *Myrmica* ants. The thalli of *R. wasmannii* are relatively easy to identify on the basis of their morphology (De Kesel et al. 2016). Their appearance is conspicuously whitish, hyaline in the case of dried host material, therefore one can quickly differentiate it from hairs. The microscopic examination of slide mounted material and the comparison with the detailed morphological description and illustrations by De Kesel et al. (2016) and Báthori et al. (2017) is sufficient for species identification of the fungal parasite.

As described previously (e.g. Haelewaters et al. 2015a), the strong attachment of Laboulbeniales thalli to the host (Tragust et al. 2016) may last for decades even in the case of dried host specimens. To test whether the mode of host collection and initial conservation affects the appearance of the fungal infection, we killed infected hosts previously collected in Hungary (Pfliegler et al. 2016) by freezing or with ethanol or chloroform then mounted them on pins. No substantial loss of thalli was observed during the procedure and the appearance and density of thalli was unchanged after months of subsequent storage. The fungal infection remained easily observable on photographs taken of these dried specimens (Fig. 1). Thus, we concluded that the image collection of AntWeb can reliably be used to screen for *R. wasmannii* infections, including older specimens.

From the infected host material identified during the survey, a single thallus (Fig. 2) was removed with an entomological pin dipped into gylcerol. The thallus was slide-mounted into PVA medium and deposited at Conservatoire and Jardin botaniques de Genève (CJBG, deposition number: G00562301). Photographs of the mounted thallus were made using an Olympus BD40 microscope equipped with an Olympus 100× Phase Contrast lens and an Olympus DP-70 digital microscope camera, with the DP Controller (Olympus) software. Subsequently, image background was removed in Photoshop CS6 (Adobe). Morphological species identification was conducted using available descriptions (De Kesel et al. 2016) and comparison with the slide collection of the Dept. of Evolutionary Zoology and Human Biology, University of Debrecen.

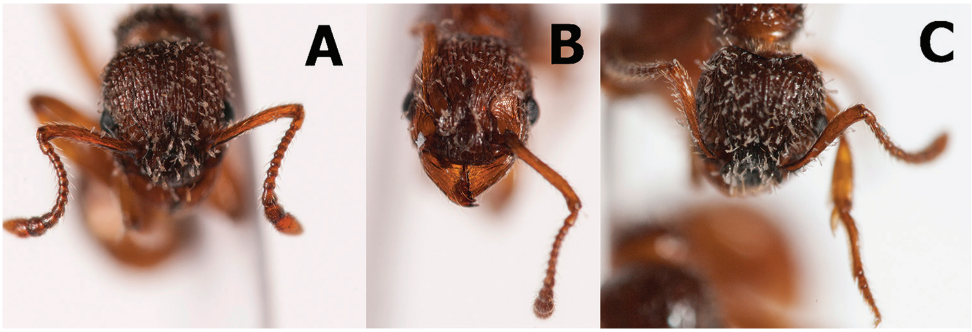

Figure 1. Appearance of the fungal infection on mounted specimens: *Rickia*-infected *Myrmica scabrinodis* hosts killed by freezing (**A**) or with ethanol (**B**) or chloroform (**C**), photographed one month after mounting and dry storage.

Figure 2. Photograph of a slide-mounted *Rickia wasmannii* thallus (deposition number: G00562301) from *Myrmica hellenica* host, recorded on the AntWeb (specimen: CASENT0907653).

Results and discussion

Most of the 397 examined *Myrmica* spp. specimens were represented by multiple photographs showing the ants from different angles in the AntWeb database. Due to the presence of important taxonomic characters, the head was imaged for all specimens. This is advantageous property of the database for our work, as the number of thalli is usually the highest on the head (Haelewaters et al. 2015b, De Kesel et al. 2016). This, together with the image quality and resolution in the database enabled us to identify any infected specimen.

We have identified a conspicuously infected *M. hellenica* Finzi, 1926 worker (Photo by Zach Lieberman, https://www.antweb.org/bigPicture.do?name=casent0907653&shot=h&number=1; Image: AntWeb 2002–2017. Licensing: Creative Commons Attribution License; downloaded on 09.05.2017) (Fig. 3) among the 397 available *Myrmica* specimens (0.25% of the investigated samples). The other 396 *Myrmica* specimens (99.75%) did not exhibit signs of *R. wasmannii* infection. Due to the occasional presence of dust particles (e.g. on photograph of specimen CASENT0900350) and the fact that host ants are occasionally parasitized by a very low number of thalli (Markó et al. 2016), which may be obscured in photographs, our method of screening the AntWeb database does not enable us to completely rule out infection among these specimens.

The single unequivocally infected specimen identified by us was collected in Greece, thus it represents a new country record and simultaneously a new host species for the fungus. The collection data of the specimen is as follows: Greece: Patras (approx. 38°14'47"N, 21°44'4"E), collected by U. Sahlberg. The fungal thalli covered the ant's head and body in high density. No other specimens with the same collection data were available in AntWeb. Further specimens of the host, *M. hellenica*, collected in Northern Greece and Iran were present in the database, but these were not photographed or did not show infection, respectively.

With our new record, the number of countries this fungus is recorded in is now increased from 17 to 18: Spain, France, Great Britain, Belgium, Netherland, Luxembourg, Germany, Switzerland, Austria, Italy, Czech Republic, Slovakia, Poland, Hungary, Romania, Slovenia, Bulgaria (Santamaria 2001, Espadaler and Santamaria 2012, Csata et al. 2013, De Kesel et al. 2016) and Greece (this paper). The known host species of the fungus is raised from nine: *M. gallienii* Bondroit, 1920; *M. rubra* (Linnaeus, 1758); *M. ruginodis* Nylander, 1846; *M. sabuleti* Meinert, 1861; *M. scabrinodis* Nylander, 1846; *M. slovaca* Sadil, 1952; *M. specioides* Bondroit, 1918; *M. spinosior* Santschi, 1931; and *M. vandeli* Bondroit, 1920 (De Kesel et al. 2016, Haelewaters et al. 2015a, 2015c) to ten by *M. hellenica* (this paper).

Our observations have proven that online image databases can be exploited to record parasitic infections, extend the distribution and host spectrum of parasitic species, thereby supplementing the direct examination of specimens in non-digitized collections. Making more high-quality micrographs and SEM images (see: Tragust et al. 2016) available for the public would facilitate similar efforts. Furthermore, raising

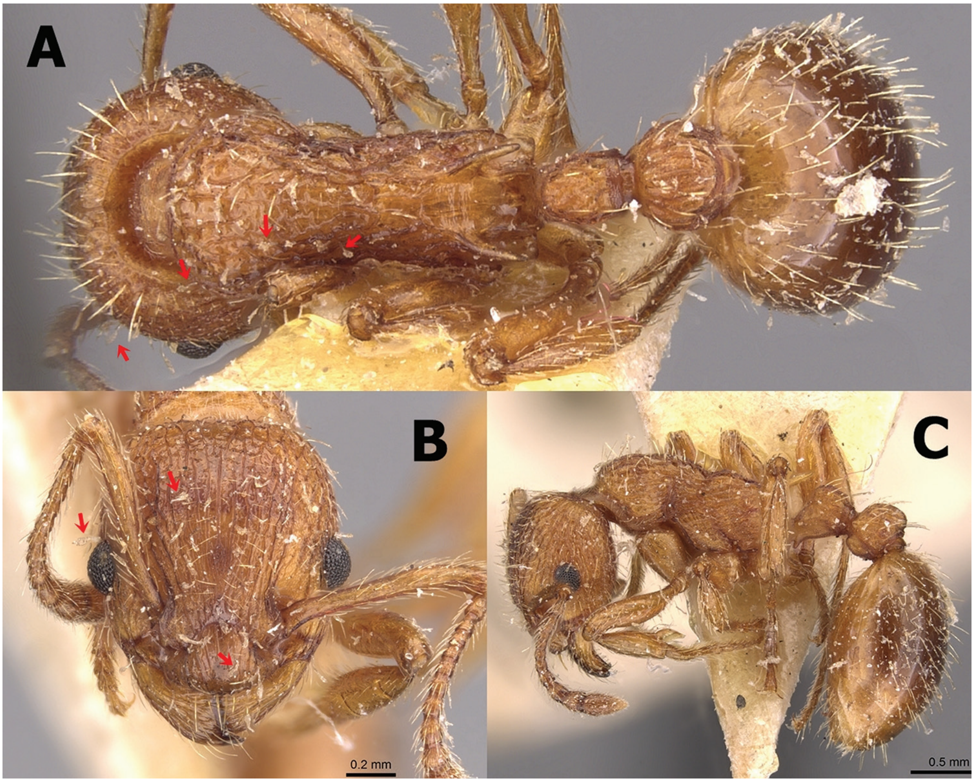

Figure 3. Dorsal (**A**), head (**B**) and profile (**C**) view of the *Rickia wasmannii* infected *Myrmica hellenica* worker, recorded on AntWeb (specimen: CASENT0907653), arrows indicate some clearly identifiable *R. wasmannii* thalli.

the number of photographs available for individual specimens would be beneficial to conduct fungal infection screenings (see: Fig. 3C where the infection is not clearly visible). Depending on the quality of the host photos, additional investigation of the material might be still needed to assess the identity of the fungal parasite due to their small size.

Conclusion

Our results highlight the possibility of using digitized collections to uncover host-parasite associations. The study of insect-Laboulbeniales associations have recently also benefited from the use of digital photo- and biological observation sharing websites (Flickr and iNaturalist; Haelewaters et al. 2016), further emphasising the new possibilities and non-trivial uses of resources in the digital era.

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Supplementary material 1

Online image databases as multi-purpose resources: *Rickia wasmannii* Cavara (Ascomycota, Laboulbeniales) on a new host ant from a new country by screening AntWeb.org

Authors: Ferenc Báthori, Walter P. Pfliegler, Carl-Ulrich Zimmerman, András Tartally

Data type: occurence

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Link: https://doi.org/10.3897/jhr.61.20255.suppl1