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Phylogeny of the subfamilies of Ichneumonidae (Hymenoptera)

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Abstract

A combined morphological and molecular phylogenetic analysis was performed to evaluate the subfamily relationships of the parasitoid wasp family Ichneumonidae (Hymenoptera). Data were obtained by coding 135 morphological and 6 biological characters for 131 exemplar species of ichneumonids and 3 species of Braconidae (the latter as outgroups). The species of ichneumonids represent all of the 42 currently recognized subfamilies. In addition, molecular sequence data (cytochrome oxidase I "DNA barcoding" region, the D2 region of 28S rDNA and part of the F2 copy of elongation factor 1-alpha) were obtained from specimens of the same species that were coded for morphology (1309 base pairs total). The data were analyzed using parsimony and Bayesian analyses. The parsimony analysis using all data recovered previously recognized informal subfamily groupings (Pimpliformes, Ophioniformes, Ichneumoniformes), although the relationships of these three groups to each other differed from previous studies and some of the subfamily relationships within these groupings had not previously been suggested. Specifically, Ophioniformes was the sister group to (Ichneumoniformes + Pimplformes), and Labeninae was placed near Ichneumoniformes, not as sister group to all Ichneumonidae except Xoridinae. The parsimony analysis using only morphological characters was poorly resolved and did not recover any of the three informal subfamily groupings and very few of the relationships were similar to the total-evidence parsimony analysis. The molecular-only parsimony analysis and both Bayesian analyses (total-evidence and molecular-only) recovered Pimpliformes, a restricted Ichneumoniformes grouping and many of the subfamily groupings recovered in

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the total-evidence parsimony analysis. A comparison and discussion of the results obtained by each phylogenetic method and different data sets is provided. It is concluded that the molecular characters produced results that were relatively consistent with traditional, non-phylogenetic concepts of relationships between the ichneumonid subfamilies, whereas the morphological characters did not (at least not by themselves). The inclusion of both molecular and morphological characters using parsimony produced a topology that was the closest to the traditional subfamily relationships. The method of analysis did not greatly affect the overall topology for the molecular-only analyses, but there were differences between Bayesian and parsimony results for the total-evidence analyses (especially near the root of the tree). The Bayesian results did not seem to be altered very much by the inclusion of morphological characters, unlike in the parsimony analysis. In summary, the following groups were supported in multiple analyses regardless of the characters used or method of tree-building: Pimpliformes, higher Ophioniformes, higher Pimpliformes, (Claseinae + Pedunculinae), (Banchinae + Stilbopinae), Campopleginae, Cremastinae, Diplazontinae, Ichneumoninae (including *Alomya*), Labeninae, Ophioninae, Poemeniinae, Rhyssinae, and Tersilochinae sensu stricto. Conversely, Ctenopelmatinae and Tryphoninae were never recovered without inclusion of other taxa. Based on the hypothesis of relationships obtained by the total-evidence parsimony analysis, the following formal taxonomic changes are proposed: Alomyinae Förster (= *Alomya* Panzer and *Megalomya* Uchida) is once again synonymized with Ichneumoninae and is now considered a tribe (Alomyini **rev. stat.**); and *Notostilbops* Townes is transferred from Stilbopinae to Banchinae, tribe Atrophini.

Keywords
Ichneumonidae, phylogeny, parsimony, Bayesian, classification, taxonomy

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Introduction

The catalogue of Yu et al. (2016) listed 25,285 described species of ichneumonids in 1601 genera. In terms of the subfamily classification within Ichneumonidae, there is general consensus for the taxonomic limits of most subfamilies; however, some authors disagree on a small minority (see Table 1). Townes (1969) established the modern subfamily classification recognizing 25 subfamilies. Morphological studies by several authors between 1969 and 2002 increased this number gradually to 37 (Wahl 1990, 1993; Gauld 1991; Porter 1998; Gauld et al. 2002a). The latter study recognized 37 subfamilies including the Pedunculinae, but not the Claseinae (both proposed by Porter 1998). Thus the total number recognized by morphology-based studies alone is 38 (see column 1 of Table 1).

From 1998–2009, several studies used single gene molecular evidence (the D2–D3 region of 28S ribosomal DNA) to examine ichneumonid subfamily relationships, either combined with morphological characters (Quicke et al. 2005; Quicke et al. 2009) or using molecular characters alone (Belshaw et al. 1998; Laurenne et al. 2006). It was not until Quicke et al. (2005) that formal changes to the subfamily classification were proposed based on studies using molecular data. This study proposed two additional subfamilies: Nesomesochorinae and Nonninae for three genera previously included in Campopleginae. Further, Laurenne et al. (2006) proposed the resurrection of Alomyinae (included in Ichneumoninae in previous classifications) (Wahl and Mason 1995). Subsequently, Quicke et al. (2009) evaluated sequences of 28S D2–D3 ribosomal DNA from 1001 species of Ichneumonidae and proposed the resurrection of one subfamily and the synonymy of three others (see column 2 of Table 1) (for a total of 39 subfamilies). The state of knowledge in ichneumonid systematics was summarized by Quicke (2015) and Broad et al. (2018).

More recently, Broad (2016) reversed one of the synonymies proposed by Quicke et al. (2009) by once again recognizing Neorhacodinae. Santos (2017), using both morphology and multi-gene sequence data, studied the relationships within Cryptinae

Table 1. Comparison of extant subfamilies of Ichneumonidae recognized by recent studies. Column 1: subfamilies recognized by morphological studies alone (up to 2002). Column 2: most recent analysis of all subfamily relationships using both morphological and molecular data. Column 3: subfamilies recognized following current study. *Brachyscleromatinae is now known as Sisyrostolinae (Bennett et al. 2013). Neorhacodinae was resurrected by Broad (2016) (considered part of Tersilochinae by Quicke et al. 2009). Ateleutinae and Phygadeuontinae were raised to subfamily status from within Cryptinae by Santos (2017).

Gauld et al. (2002a) + Porter (1998)	Quicke et al. (2009)	Current study (after formal changes)
Acaenitinae	Acaenitinae	Acaenitinae
Adelognathinae	Adelognathinae	Adelognathinae
Agriotypinae	Agriotypinae	Agriotypinae
	Alomyinae (previously part of Ichneumoninae)	
Anomaloninae	Anomaloninae	Anomaloninae
		Ateleutinae (previously part of Cryptinae)
Banchinae	Banchinae	Banchinae (including *Notostilbops*)
Brachycyrtinae	Brachycyrtinae	Brachycyrtinae
	Brachyscleromatinae* (previously part of Phrudinae)	
Campopleginae	Campopleginae	Campopleginae
Claseinae	Claseinae	Claseinae
Collyriinae	Collyriinae	Collyriinae
Cremastinae	Cremastinae	Cremastinae
Cryptinae	Cryptinae	Cryptinae
Ctenopelmatinae	Ctenopelmatinae	Ctenopelmatinae
Cylloceriinae	Cylloceriinae	Cylloceriinae
Diacritinae	Diacritinae	Diacritinae
Diplazontinae	Diplazontinae	Diplazontinae
Eucerotinae	Eucerotinae	Eucerotinae
Hybrizontinae (previously Paxylommatinae)	Hybrizontinae (previously Paxylommatinae)	Hybrizontinae
Ichneumoninae	Ichneumoninae	Ichneumoninae (including Alomyini)
Labeninae	Labeninae	Labeninae
Lycorininae	Lycorininae	Lycorininae
Mesochorinae	Mesochorinae	Mesochorinae
Metopiinae	Metopiinae	Metopiinae
Microleptinae	Microleptinae	Microleptinae
Neorhacodinae		Neorhacodinae
	Nesomesochorinae (including Nonninae of Quicke et al. (2005)	Nesomesochorinae
Ophioninae	Ophioninae	Ophioninae
Orthocentrinae	Orthocentrinae	Orthocentrinae
Orthopelmatinae	Orthopelmatinae	Orthopelmatinae
Oxytorinae	Oxytorinae	Oxytorinae
Pedunculinae	Pedunculinae	Pedunculinae
Phrudinae		
		Phygadeuontinae (previously part of Cryptinae)
Pimplinae	Pimplinae	Pimplinae
Poemeniinae	Poemeniinae	Poemeniinae
Rhyssinae	Rhyssinae	Rhyssinae
		Sisyrostolinae*
Stilbopinae	Stilbopinae	Stilbopinae (excluding *Notostilbops*)
Tatogastrinae	Tatogastrinae	Tatogastrinae
Tersilochinae	Tersilochinae (including Neorhacodinae + part of Phrudinae)	Tersilochinae
Tryphoninae	Tryphoninae	Tryphoninae
Xoridinae	Xoridinae	Xoridinae

and raised Phygadeuontinae (from the tribe Phygadeuontini) and Ateleutinae (from the tribe Cryptini, subtribe Ateleutina). In summary, 42 ichneumonid subfamilies were recognized prior to this study. Since Santos (2017), two other studies have been published which discuss ichneumonid subfamily relationships but neither of these proposed changes to the subfamily classification. Broad et al. (2018) provided an updated handbook to the ichneumonids of Britain and Ireland including discussion of all subfamilies, and Klopfstein et al. (2019) used transcriptomes and target DNA enrichment to examine the relationships of Pimplinae and related subfamilies.

In terms of the relationships of the subfamilies, prior to about 1990, relationships could only be inferred by the their relative arrangement in Henry Townes's Genera of Ichneumonidae (Townes 1969, 1970a, 1970b, 1971), a classification that was largely based on the supporting evidence (especially larval) in the character outline of Townes (1969) (pp. 29–35). As Townes (1969) wrote (p. 29): "Examination of the larval characters gave final proof of the basic faults in the old system and helped in the formulation of a new one". Since 1990, phylogenetic hypotheses have been proposed for several groupings of subfamilies within Ichneumonidae. The informal name Pimpliformes was proposed for five subfamilies putatively related to Pimplinae (Wahl, 1990), and this group was further divided into eight subfamilies by Gauld (1991) through establishment of Diacritinae, Poemeniinae and Rhyssinae. Further, Wahl (1991) proposed the name Ophioniformes for eight subfamilies and Wahl (1993a) hypothesized that the two large subfamilies Cryptinae and Ichneumoninae were related and proposed the informal name Ichneumoniformes to comprise these two subfamilies along with Brachycyrtinae. The studies by Quicke and colleagues have for the most part, upheld these three groupings. Their studies also suggested placement for other subfamilies, for example, Quicke et al. (2009) placed an additional 8 subfamilies within the Ophioniformes (for a total of 16), 3 additional subfamilies in Ichneumoniformes (for a total of 5), and 1 additional subfamily (Collyriinae) in Pimpliformes (for a total of 9). Still, the study of Quicke et al. (2009) had two subfamilies with uncertain affinity (Eucerotinae and Microleptinae). Their study included the most comprehensive taxon sampling of any phylogenetic study of Ichneumonidae, including species from all subfamilies, but the analysis included only one gene and morphology was coded mostly at the subfamily and tribal levels (88 terminal taxa). More recent studies (Santos 2017 which sampled heavily within Ichneumoniformes and Klopfstein et al. 2019 that focused on Pimpliformes) included much more molecular data, but with a more limited taxonomic scope. The overall purpose of this study is to examine the relationships of the subfamilies of Ichneumonidae based on phylogenetic analyses using both morphological and molecular sequence data. The study is of interest because it includes the largest morphological data set for Ichneumonidae that has been coded for individual exemplar species. Novel morphological characters are introduced, especially for larvae, and the nuclear gene elongation factor 1-alpha is sequenced across the entire family for the first time. The results will be compared to previous hypotheses of relationships (e.g., Wahl and Gauld 1998, Quicke et al. 2009, Santos 2017, Klopfstein et al. 2019) and will provide another hypothesis of relationships against which future studies can be compared.

For the purposes of this study, the following definitions for the subfamily groupings are used:

1) Ophioniformes includes the following 18 subfamilies: Anomaloninae, Banchinae, Campopleginae, Cremastinae, Ctenopelmatinae, Hybrizontinae, Lycorininae, Mesochorinae, Metopiinae, Oxytorinae, Neorhacodinae, Nesomesochorinae, Ophioninae, Sisyrostolinae, Stilbopinae, Tatogastrinae, Tersilochinae and Tryphoninae. This concept is the same as that of Quicke et al. (2009)–the 16 subfamilies listed under Ophioniformes in their table 4 plus Neorhacodinae resurrected by Broad (2016) and Tatogastrinae which was presumably accidentally omitted from their table. Note that this group includes the eight subfamilies included by Wahl (1991) when the name was proposed. Gauld et al. (1997) included nine subfamilies: the eight of Wahl (1991) and Tersilochinae. Gauld et al. (1997) also coined the name Tryphoniformes to comprise Adelognathinae, Eucerotinae, Tryphoninae and Townesioninae (the latter now synonymized with Banchinae). Adelognathinae and Eucerotinae are now believed more closely related to Ichneumoniformes (Quicke et al. 2009, Santos 2017) and Tryphoninae is thought to belong to Ophioniformes (Quicke et al. 2009), as sister group to all other subfamilies, therefore we do not use the term Tryphoniformes. Within Ophioniformes is a group of closely related subfamilies that Quicke et al. (2009) called the "higher Ophioniformes" which we define as Anomaloninae, Campopleginae, Cremastinae, Nesomesochorinae and Ophioninae.
2) Pimpliformes includes the following nine subfamilies: Acaenitinae, Cylloceriinae, Diacritinae, Diplazontinae, Orthocentrinae, Pimplinae, Poemeniinae, Rhyssinae and in addition, Collyriinae. This definition corresponds to the concept of Quicke et al. (2009), which was the same as Wahl and Gauld (1998), except for the inclusion of Collyriinae in Quicke et al. (2009). The higher Pimpliformes is comprised of Pimplinae, Poemeniinae and Rhyssinae (Quicke et al. 2009). Note that the term "higher Pimpliformes" was used previously by Gauld (1991) for a group comprised of Diplazontinae, Orthocentrinae and Microleptinae, but this is not the definition used in the current study. Gauld (1991) believed that Rhyssinae was sister subfamily to all others in Pimpliformes, followed by a polytomy of Pimplinae, Poemeniinae and Acaenitinae leading to the koinobiont, endoparasitoid fly parasitoids (Cylloceriinae and his "higher Pimpliformes"). The sister group relationship of Rhyssinae to the rest of Pimpliformes was not upheld by the studies of Wahl and Gauld (1998) and Quicke et al. (2009), but the grouping of Rhyssinae, Poemeniinae and Pimplinae was strongly supported in both studies.
3) Ichneumoniformes is considered in two senses: a) in the strict sense (Ichneumoniformes sensu stricto) as originally proposed by Wahl (1993a) and including only Brachycyrtinae, Cryptinae (including Ateleutinae and Phygadeuontinae) and Ichneumoninae (including Alomyinae), and b) in the broad sense (Ichneumoniformes sensu lato) comprised of Ichneumoniformes s.s. plus seven related subfamilies which will be further described and discussed in the Results and discussion section.

In addition, Quicke et al. (2009) defined the following higher groups:

4) Xoridiformes comprised of Xoridinae
5) Labeniformes comprised of Labeninae
6) Orthopelmatiformes comprised of Orthopelmatinae
7) Brachycyrtiformes comprised of Brachycyrtinae, Claseinae and Pedunculinae.

See the Results section on Support/ relationships of subfamilies (below) for discussion on the support/ placement of these groups in the current study.

Methods

Outgroups

There is very strong evidence that Braconidae is the sister group of Ichneumonidae based on morphology (Rasnitsyn 1988; Sharkey and Wahl 1992) as well as combined morphological and molecular evidence (Heraty et al. 2011; Sharkey et al. 2012; Peters et al. 2017; Branstetter et al. 2017). The three exemplar species (from three subfamilies) chosen for this study were species used in the studies of Heraty et al. (2011) and Sharkey et al. (2012) which was done to aid comparison between these studies and to ensure inclusion of a range of life strategies in our outgroups: *Doryctes erythromelas* (Brullé) (Doryctinae) (idiobiont ectoparasitoid); *Aleiodes terminalis* Cresson (Rogadinae) (koinobiont endoparasitoid) and *Rhysipolis* sp. (Rhysipolinae) (koinobiont ectoparasitoid).

Ingroup

Ingroup taxa were chosen in order to provide complete coverage of all currently recognized subfamilies based on the most recent study of ichneumonid relationships (Quicke et al. 2009) as well as to try to include the widest range of morphological variation among the species in each subfamily. In addition, some equivocally placed taxa were deliberately included (see Results and Discussion). Apart from taxonomic coverage and choosing species to maximize morphological divergence within subfamilies, the following additional criteria were used for choosing ingroup exemplar taxa (in order of importance): a) availability of fresh specimens from which all three DNA regions could be obtained; b) knowledge of the larva; c) knowledge of biological (life-history) traits; d) knowledge of the egg. All taxa used in this study are listed in the morphological character matrix (Table 2) as well as the list of taxa examined (Appendix 2).

Voucher specimens and larval slides are deposited at the Australian National Insect Collection, Canberra, Australia (ANIC) (J. Rodriguez), the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Canada (CNC) (A. Bennett), the

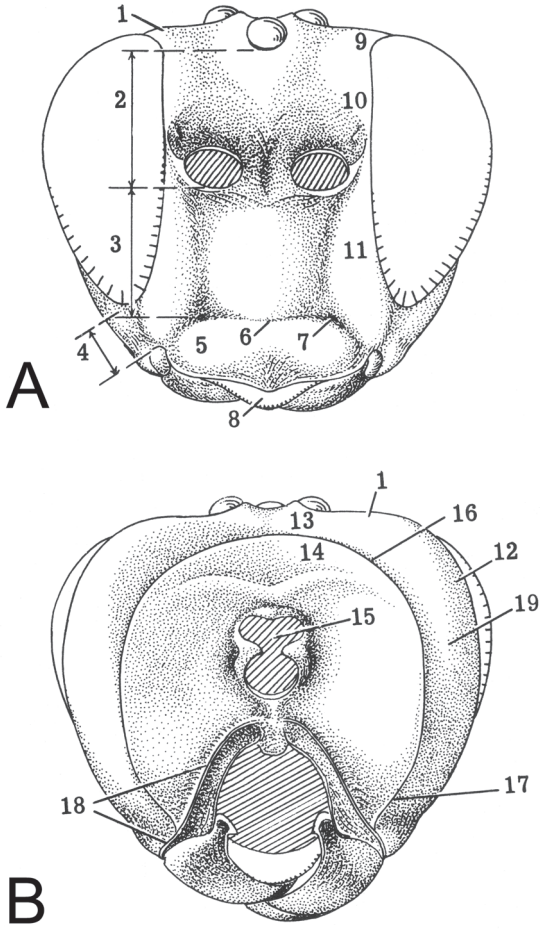
Entomology collection, Department of Biology, Utah State University, Logan, Utah, USA (EMUS) (D. Wahl), the Smithsonian Institute, Washington, DC (NMNH) (R. Kula) and the University of Kentucky, Lexington, Kentucky, USA (UKY) (E. Chapman).

Morphological character coding

We used a species exemplar approach in this study, rather than coding morphological characters at the tribal or subfamily level as has been done in other studies on family and subfamily relationships within Hymenoptera (Brothers and Carpenter 1993 for Aculeata; Quicke and van Achterberg 1990 for Braconidae; Quicke et al. 2009 for Ichneumonidae). Whereas the species exemplar approach is very time-consuming for large datasets, it is preferable (if possible) in order to make the character coding as objective and reproducible as possible. It also permits testing of the monophyly of the subfamilies and tribes (evidence of monophyly is provided when all constituent taxa cluster together). For a comparison of the exemplar and generic abstraction approaches, see Gauld et al. (2002b), which included both types of coding for Pimplinae. In all cases, coding of adult morphology was done with reference to the actual specimen from which DNA was obtained, as well as other authoritatively identified, conspecific specimens used to ensure that the DNA voucher was representative of the species. Larval characters were coded directly from conspecific larval slides of our exemplar species (when known), or if the larva of our exemplar species was not known, coding was done with reference to slides and or literature figures of species within the same genus. In cases in which there was no larval knowledge of any species within the genus, the larval characters were coded as missing. The same strategy was used for the egg and biological characters.

Morphological terms, measurements and photography

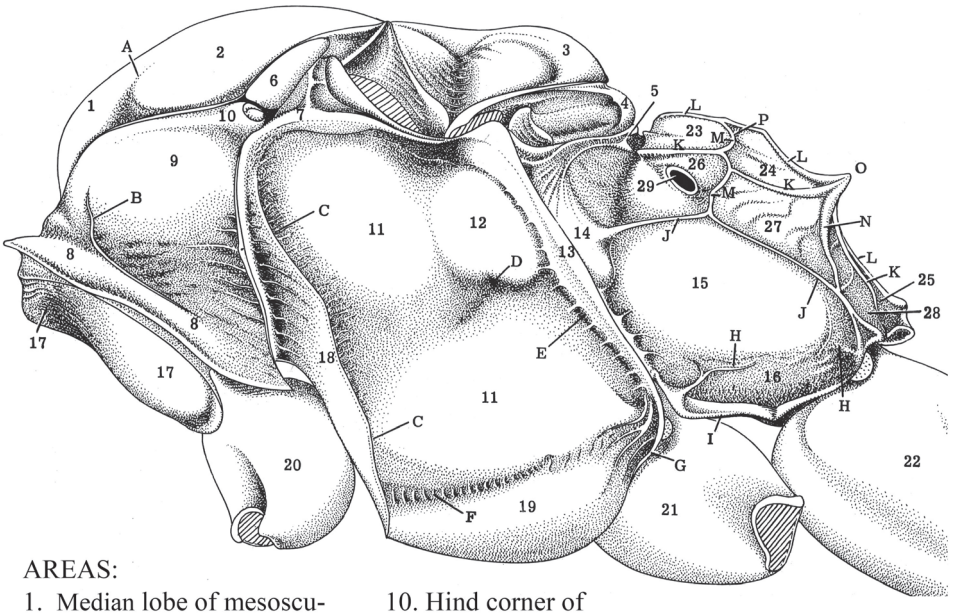
All terms of ichneumonid morphology follow Townes (1969) with the following modifications: hypostomal carina (Fig. 1, structure #18) for 'oral carina', supra-antennal area (Fig. 1, structure #2) for 'frons', supraclypeal area (Fig. 1, structure #3) for 'face', gena (Fig. 1, structure #19) for 'temple', occiput (Fig. 1, structure #14) for 'postocciput', malar space (Fig. 1, structure #4) for 'cheek', epicnemial carina (Fig. 2C) for 'prepectal carina', laterotergites (Fig. 79) for 'epipleura', gonoforceps (Fig. 92) for 'claspers', and hypopygium (Fig. 86) for 'subgenital plate'. The term 'mesosoma' is used for the body region that includes the thorax and first abdominal segment (the propodeum). The term 'metasoma' is used for the apparent abdomen, with MS1, MS2, etc. referring to metasomal segments 1, 2, etc., T1, T2, etc. referring to the corresponding tergites; L1 and L2, etc. referring to the laterotergites and S1, S2, etc. referring to the sternites. The term T2+ refers to tergite 2 and all tergites posterior to T2. Terms of relative position of the body follow Goulet and Huber (1993). Wing venation terms follow the Comstock-Needham system as updated by Ross (1936) and incorporate



1, 13. Vertex
2. Supra-antennal area
3. Supraclypeal area
4. Malar space (= cheek)
5. Clypeus
6. Epistomal suture
7. Anterior tentorial pit (= clypeal fovea)
8. Labrum
9, 10, 11. Paraocular area
12. Genal orbit
14. Occiput
15. Foramen magnum
16, 17. Occipital carina (17 = genal carina)
18. Hypostomal carina (= oral carina)
19. Gena

Figure 1. Head of an ichneumonid. **A** anterior view **B** posterior view (modified from Townes 1969).

the recommendations of Goulet and Huber (1993) except for naming of the vein that forms the distal edge of fore wing cell *1+2Rs* (the 'areolet' of Townes 1969). This vein is of uncertain origin and is here referred to as 'vein *3rs-m*' (Fig. 4A) in conformity with Wahl and Gauld (1998) (= *3r-m* vein of Sharkey and Wahl 1992). The following terms for specialized structures are defined: epomia (Fig. 2b): a raised ridge (carina) on the pronotum (Figs 30–31); glymma (Fig. 5, structure #11): the lateral depression



AREAS:

1. Median lobe of mesoscutum
2. Lateral lobe of mesoscutum
1, 2. Mesoscutum
3. Scutellum
4. Metanotum (= postscutellum)
5. Hind margin of metanotum
6. Tegula
7. Subalar ridge (= subtegular ridge)
8. Collar
8, 9, 10. Pronotum
10. Hind corner of pronotum
11, 12, 18. Mesopleuron [mesepisternum]
12. Hypoepimeron (= speculum)
13. Mesepimeron
14. Upper division of metapleuron
15. Lower division of metapleuron
16. Juxtacoxal area
17. Propleuron
18. Epicnecium (= prepectus)
19. Mesothoracic venter
20. Fore coxa
21. Middle coxa
22. Hind coxa
23-28. Propodeum
23. First lateral area
24. Second lateral area
25. Third lateral area
26. First pleural area
27. Second pleural area
28. Third pleural area
29. Propodeal spiracle

CARINAE AND GROOVES:

A. Notaulus
B. Epomia
C. Epicnemial carina (= prepectal carina)
D. Scrobe (= mesopleural fovea)
E. Mesopleural groove
F. Sternaulus
G. Posterior transverse carina of mesothoracic venter
H. Juxtacoxal carina
I. Submetapleural carina
J. Pleural carina
K. Lateral longitudinal carina of propodeum
L. Median longitudinal carina of propodeum
M. Anterior transverse carina of propodeum (= basal transverse carina)
N. Posterior transverse carina of propodeum (= apical transverse carina)
O. Propodeal apophysis or crest
P. Costula [part of anterior transverse carina]

Figure 2. Mesosoma of an ichneumonid, lateral view (modified from Townes 1969).

sub-basally on T1; notaulus (Fig. 3A): a longitudinal groove sublaterally on the mesoscutum; sternaulus (Fig. 2F): a longitudinal groove subventrally on the mesopleuron (not to be confused with the mesopleural groove which is more dorsal (see Fig. 30).

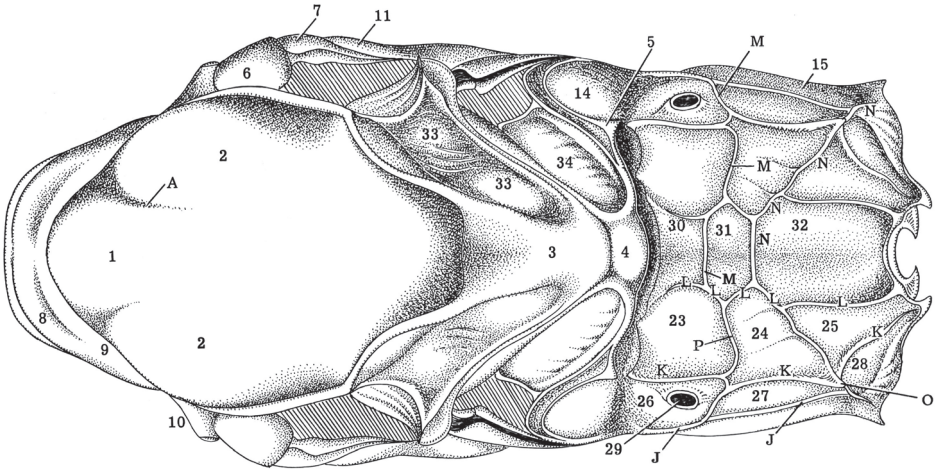
The carinae on the propodeum (see Figs 3, 4) can present difficulties for scoring of their presence and absence, depending on interpretation of their homology. For example, if only one transverse carina is present near the longitudinal middle of the propodeum, there is ambiguity with respect to whether this should be considered the anterior or posterior transverse carina. To help make scoring of the propodeal carinae more objective, the following conventions were used:

1) In cases in which there is ambiguity between the anterior and posterior transverse carinae, the posterior transverse carina was given precedence.
2) In cases in which there is ambiguity between the medial and lateral longitudinal carinae, the medial longitudinal carinae were given precedence.
3) In cases in which longitudinal and transverse carinae run obliquely together across the propodeum, the transverse carina was given precedence over the longitudinal carinae.
4) In cases in which an abscissa of a carina is incomplete (e.g., the costula of many specimens of Campopleginae), the abscissa was considered present if it extended half the way across the area in which it is located or greater, or if this could not be determined (e.g., no other carinae are present on the propodeum), one sixth of the length (for longitudinal carinae) or width (for tranverse carinae) of the propodeum.

Digital photos at the CNC were taken using a Leica MZ16 stereomicroscope with motorized focus drive attached to a Leica DFC420 digital camera. Photos were combined and edited using Leica Application Suites Montage Multifocus software V3.8, Auto-Montage Pro 5.01 and Adobe Photoshop CS4. Photos taken at EMUS were taken with an EntoVision micro-imaging system. This system consists of a Leica M16 zoom lens attached to a JVC KY-75U 3-CCD digital video camera that feeds image data to a desktop computer. The program Archimed 5.3.1 was used to merge an image series (representing typically 15–30 focal planes) into a single in-focus image. Lighting was provided by an EntoVision dome light.

Molecular protocols

Most sequences in this study were obtained by sequencing specimens at the Canadian National Collection of Insects, Arachnids and Nematodes (CNCI), except as noted in Appendix 2 (11 sequences downloaded from Genbank: the nine outgroup sequences and 28s DS for *Poecilocryptus nigromaculatus* Cameron and *Hellwigia obscura* Gravenhorst). GenBank accession numbers are listed in Appendix 2. All sequences, including those downloaded from GenBank, were compared to published sequences of putatively related taxa to verify sequence veracity using the nucleotide BLAST tool (Altschul et al. 1990) through GenBank (Benson et al. 2017).



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6. Tegula
7. Subalar ridge (= subtegular ridge)
8. Collar
8, 9, 10. Pronotum
10. Hind corner of pronotum
11. Mesopleuron [mesepisternum]
14. Upper division of metapleuron
15. Lower division of metapleuron
23-32. Propodeum
23. First lateral area
24. Second lateral area
25. Third lateral area
26. First pleural area
27. Second pleural area
28. Third pleural area
29. Propodeal spiracle
30. Basal area
31. Areola
32. Petiolar area
33. Axillary trough of mesonotum
34. Axillary trough of metanotum

CARINAE AND GROOVES:

A. Notaulus
B. Epomia
C. Epicnemial carina (= prepectal carina)
D. Scrobe (= mesopleural fovea)
E. Mesopleural groove
F. Sternaulus
G. Posterior transverse carina of mesothoracic venter
H. Juxtacoxal carina
I. Submetapleural carina
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P. Costula [part of anterior transverse carina]

Figure 3. Mesosoma of an ichneumonid, dorsal view (modified from Townes 1969).

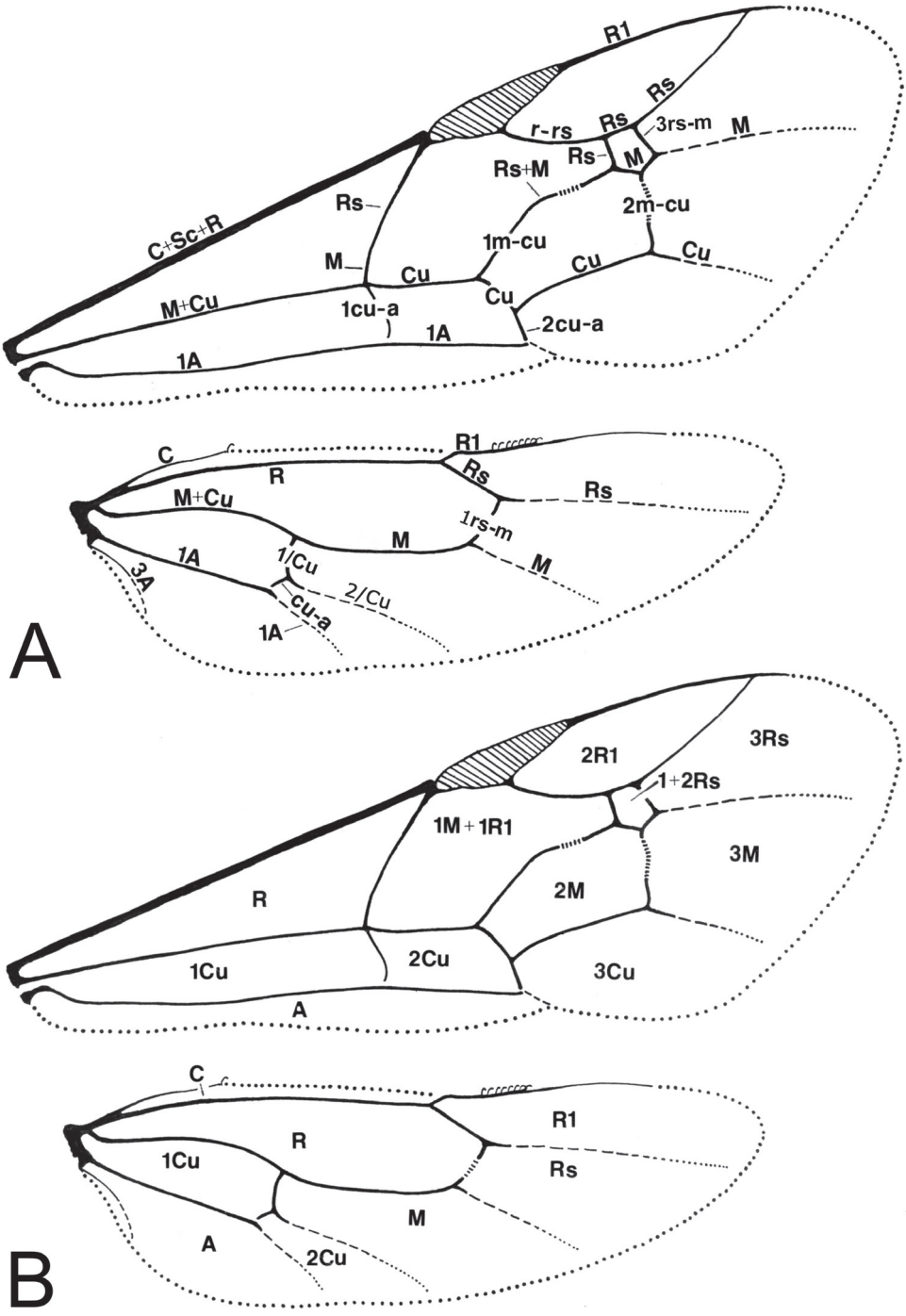
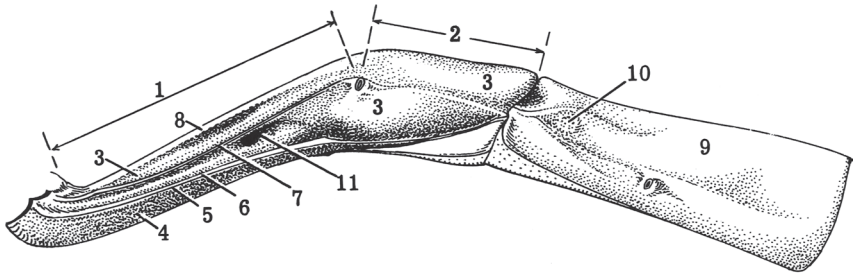


Figure 4. Fore and hind wings of an ichneumonid (Ross system). **A** wing veins **B** wing cells (modified from Townes 1969).



1. Petiole
2. Postpetiole
3. Tergite 1
4. Sternite 1
5. Tergosternal suture
6. Ventrolateral carina
7. Dorsolateral carina
8. Median dorsal carina
9. Tergite 2
10. Thyridium
11. Glymma

Figure 5. First and second metasomal tergites of an ichneumonid, lateral view (modified from Townes 1969).

Molecular extracts were obtained using standard protocols outlined in DNeasy Blood and Tissue extraction kit instructions (Qiagen, Gaithersburg, MD, USA). A single mid leg was macerated except for small specimens in which a hind and mid leg were used. PCRs were carried out using 50 µl reactions containing 4 µl DNA extract, 1 µl of each primer, 5 µl PCR buffer, 1 µl DNTPs, 1 µl $MgCl_2$, 0.25 µl Taq and 36.75 µl RNase-free water. Primers were as follows: Cytochrome oxidase I (COI) (F) (LCO1490): GGTCAACAAATCATAA AGATATTGG; (R) (HCO2198): TAAACTTCAGGGTGACCAAAAAATCA (Folmer et al. 1994); 28S (F) (3665): AGAGAGAGT TCAAGAGTACGTG; (R) (4068): TTGGTCCGTGTT TCAAGACGGG (Belshaw et al. 1998); F2 copy of elongation factor 1-alpha (EF1a) (F): AGATGGGYAAR GGTTCCTTCAA; (R): AACATGTTGTCDCCGTGCCATCC (Belshaw and Quicke 1997). Verification that only the F2 copy was present was done by comparing our sequences to published F1 and F2 sequences from Klopfstein and Ronquist (2013). PCR protocols followed the studies listed above for each molecule. The PCR products were purified using the QIAquick PCR Purification Kit Protocol (Qiagen). Problems with PCR amplification of EF1a resulting from multiple gene copies were resolved through gel-cutting. All centrifugation steps were performed for 60 s and samples were left to incubate for two minutes in Buffer PE (to reduce salt concentration), and five minutes in Buffer EB. Sequencing reactions were performed in a total reaction volume of 10 µl, with 1 µl ddH2O, 3µl of sequencing buffer, 1 µl of primer with concentrations ranging from 51.8 to 69.9 nmol, 1 µl of BigDye Terminator (PE Applied Biosystems, Foster City, CA, USA), and 4 µl of purified PCR product. Sequencing was performed in both directions at the Agriculture & Agri-Food Canada, Eastern Cereal and Oilseed Research Centre Core Sequencing Facility (Ottawa, ON, Canada) on an Applied Biosystems Incorporated PRISM 3100-Avant Genetic Analyzer.

Sequence alignment

Preliminary alignment of coding genes (COI and EF1-a) was done using ClustalW (Thompson et al. 1994) followed by alignment with reference to amino acid coding using MacClade 4.0 (Maddison and Maddison 2001) and Mesquite 2.75 (Maddison and Maddison 2011). For COI, third codon positions were excluded from analysis because of saturation. Alignment of 28S used the secondary structure model for Ichneumonoidea (Gillespie et al. 2005). The following invariant or unalignable regions were excluded from analyses: NHR(1), REC(1'), RAA(2), RAA(3), NHR(2), REC(2'), RAA(7), REC(3'), RAA(8), REC(4), REC(5), REC(6), RAA(10), REC(5'), RAA(15). The number of aligned bases were as follows: COI: 441; 28s D2: 448; EF1a: 417.

Aligned sequences are available in Suppl. materials 1–4 and by request from the corresponding author.

Phylogenetic analyses

Parsimony analysis was performed using TNT v. 1.1 (Goloboff et al. 2003). All characters were treated as unordered and equally weighted in order to avoid placing subjective, pre-conceived notions on the direction and relative importance of particular transitions. The optimal score was found 20 times using the default settings of "xmult" plus 10 cycles of tree-drifting. Optimal trees found were then imported into Winclada 1.00.08 (Nixon 2002) for tree-viewing and were then exported to NONA 2.0 (Goloboff 1999) for additional branch-swapping using the command "max" to find the complete set of equally parsimonious cladograms for the starting cladograms. NONA did find additional trees and this step is highly recommended when doing parsimony searches with TNT. Strict consensus cladograms were produced using WinClada. The taxonomy depicted in all cladograms (Figs 117–124) represents classification prior to this study. Taxa for which formal nomenclatural changes are made are indicated with an asterisk (see text of respective taxa for details). Cladograms showing optimization of individual characters (Figs 122–124) were produced using MacClade 4.03 (Maddison and Maddison 2001) and Mesquite 2.75 (Maddison and Maddison 2011). Nodal support on parsimony cladograms is shown by optimization of morphological characters using ACCTRAN which favours reversals over parallelisms as well as by decay index values (Bremer 1994) calculated using TNT (shown as a boxed number under each node or taxon) (see Fig. 117). Parsimony analyses were run for the total-evidence data set (Fig. 117), morphological characters only (Fig. 118) and sequence data only (Fig. 119).

Bayesian analyses were conducted using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Model testing was done in JModelTest 0.1.1 (Posada 2008) with the best-fitting models for each partition as follows: COI (TIM+I+G); 28S (GTR+F); EF1a (K80+I+T), morphology (discrete model with gamma). Bayesian analysis was run with all parameters unlinked across partitions. In addition for 28S, secondary structure stems versus loops were analyzed using separate models. Eight independent runs

of 4 chains each were run for 10 million generations, with trees sampled every 1000 generations. Chains were run until the average standard deviation of split frequencies went below 0.01. For each analysis, the trees in a burn-in period were excluded, and the remaining post-burn-in trees were used to construct a maximum clade credibility tree. Nodal support is indicated using Bayesian Posterior Probabilities (BPP) shown to the right of each node. Bayesian analyses were run for the total-evidence data set (Fig. 120) and sequence data only (Fig. 121). Trees from Bayesian analyses were made using FigTree 1.4.3 (Rambaut 2016).

Results and discussion

Morphological characters (see Table 2 for matrix)

In total, 141 morphological and biological characters were included in the analyses: 104 adult, 28 larval, 3 egg and 5 biological.

1. Clypeus: 0) comprised of one part (Fig. 1); 1) differentiated into basal and apical parts (Fig. 8).
2. Clypeus and supraclypeal area: 0) separated by groove or indentation (Fig. 1); 1) without any impression separating them – more or less flat or uniformly convex (Fig. 9).
3. Clypeal margin in anterior view: 0) simple, truncate to slightly convex or slightly concave (Fig. 12); 1) medially with apical denticle or denticles (not bilobed) (Fig. 10); 2) apically bilobed (strongly concave medially) (median denticle may be present or absent) (Fig. 11).
4. Clypeal margin vestiture: 0) with small scattered setae or lacking setae; 1) with regularly spaced strong setae (Fig. 12).
5. Mandibles: 0) bidentate, gradually to strongly tapering (Fig. 1); 1) unidentate, chisel shaped (Fig. 13); 2) unidentate, wide apically and twisted (Fig. 14); 3) reduced to flap (Fig. 15); 4) tridentate (dorsal tooth divided in two) (Fig. 16).
6. Subocular sulcus: 0) absent to indistinct (Fig. 12); 1) present (Fig. 17).
7. Apical flagellomere of female: 0) simple; 1) with projections arising at outgrowth from surface (see Wahl and Gauld 1998: Fig. 2); 2) flattened apically and without setae (Fig. 18)
8. Antennal color of female: 0) more or less unicolorous; 1) with distinct light coloured median band.
9. Flagellum of male: 0) central flagellomeres lacking tyloids; 1) central flagellomeres with elliptical (Fig. 19) or raised, longitudinal ridge-like tyloids on ventral surface (Fig. 20).
10. Inner margin of eye: 0) not or only weakly emarginate (Figs 10, 12); 1) strongly emarginate near antennae.
11. Lateral ocellus: 0) small, separated from eye by 0.5× its diameter or greater; 1) enlarged, touching, or almost touching eye.

12. Gena: 0) simple; 1) denticulate (Fig. 21).
13. Occiput with medial notch near foramen magnum: 0) absent; 1) present (see Wahl and Gauld 1998: Figs 8–9).
14. Occipital carina: (0) joining hypostomal carina at a distance from mandible that is less than basal width of mandible (Fig. 1); 1) joins hypostomal carina at a distance from mandible greater than basal width of mandible; 2) runs directly to mandibular base.
15. Foramen magnum: 0) simple (Fig. 1); 1) laterally expanded (see Wahl and Gauld 1998: Fig. 11).
16. Maxillary palpus: 0) 5-segmented; 1) 4-segmented; 2) 3-segmented.
17. Maxillary palpus: 0) normal; 1) elongate, reaching to or beyond middle coxa.
18. Labial palpus: 0) 4-segmented; 1) 3-segmented.
19. Propleuron: 0) without lateroventral posteriorly-projecting lobe (Fig. 2); 1) with lateroventral posteriorly-projecting lobe (Fig. 22).
20. Epomia: 0) present as ridge ventro-anteriorly that crosses furrow dorsally (Fig. 2); 1) reduced to short central ridge across furrow (Fig. 23); 2) absent.
21. Mesoscutum: 0) smooth (Fig. 2); 1) with transverse rugae (Fig. 24).
22. Notaulus: 0) shallow or vestigial; 1) deeply impressed anteriorly (Fig. 2A); 2) absent.
23. Notaulus: 0) extending to 0.5× length of mesoscutum or less; 1) extending posteriorly past centre of mesoscutum but not joining other notaulus; 2) extending posteriorly past centre and joining either other notaulus or rugose medial area (Fig. 25); 3) absent.
24. Notaular crest or carina anterolateral to notaulus: 0) absent; 1) present (arrow in Fig. 26).
25. Epicnemial carina: 0) not curving anteriorly, vertical to around middle of pronotum; 1) curving to anterior of mesopleuron, around middle of pronotum (arrow in Fig. 27); 2) extending all the way to subtegular ridge (Fig. 2C); 3) present ventrally only (not extending dorsally to ventral edge of pronotum); 4) absent.
26. Sternaulus length: 0) present, less than 0.7× length of mesopleuron; 1) present, greater than or equal to 0.7× length of mesopleuron (Figs 2F, 28); 2) entirely absent.
27. Sternaulus curvature: 0) short, not reaching posterior end of mesopleuron (posterior curvature not scoreable); 1) posterior end ending dorsal to posterolateral corner of mesopleuron (Fig. 2F); 2) posterior end ending anterior to posterolateral corner of mesopleuron (arrow in Fig. 28).
28. Foveate groove of mesopleuron: 0) absent; 1) present (arrow in Fig. 29).
29. Mesopleural groove: 0) absent to incomplete; 1) complete to posterior margin of mesopleuron ("mg" in Fig. 30).
30. Posterior transverse carina of mesothoracic venter: 0) absent; 1) interrupted near anterior of middle coxa (gap indicated by arrow in Fig. 31); 2) complete (arrow in Fig. 32).
31. Metapostnotum: 0) posterolateral triangles present (Figs 3, 33); 1) posterolateral triangles absent.

32. Propodeum base: 0) without median tubercle; 1) with small, median tubercle (arrow in Fig. 34).
33. Propodeal spiracles: 0) separated from pleural carina by about minimum diameter of spiracle or more (Fig. 2); 1) separated from pleural carina by less than minimum diameter.
34. Propodeal spiracles: 0) round to sub-circular (less than 1.5× as long as high) (Figs 34, 35); 1) ovoid to elongate (1.5× as high as long or more) (Figs 2, 36).
35. Lateral profile of propodeum: 0) angulate with separate dorsal and posterior faces (Fig. 2); 1) rounded to flattened, without separate dorsal and posterior faces (Fig. 35).
36. Anterior transverse carina of propodeum: 0) complete (medially and sublaterally) (Fig. 3); 1) medial abscissa present, sublateral abscissae (= costulae) absent (Fig. 36); 2) median abscissa absent, sublateral abscissae present (Fig. 37); 3) completely absent (Fig. 40). Note: condition of the lateral abscissa was not coded because the presence of the spiracle makes scoring the condition in this region problematical.
37. Anterior transverse carina of propodeum: 0) medially angled (Fig. 3); 1) forming more or less smooth arc (Fig. 38). Note: if carina absent or incomplete so that angulation/curvature could not be scored, character is coded as "?"
38. Posterior transverse carina of propodeum: 0) complete (medially and sublaterally) (Figs 3, 36–38, 40); 1) medial abscissa present, sublateral abscissae absent; 2) median abscissa absent, sublateral abscissae present (areolar area confluent with petiolar area) (Fig. 39); 3) completely absent/ indistinguishable.
39. Posterior transverse carina of propodeum: 0) as strongly developed as other carinae; 1) conspicuously stronger than other carinae (Fig. 33). Note, if carina absent, coded as "?"
40. Posterior transverse carina: 0) angled (Fig. 3); 1) more or less continuous arc (Fig. 40). Note: if carina absent or angulation uncertain, coded as "?"
41. Anterior abscissa of medial longitudinal carina: 0) present, not fused (Fig. 3); 1) present, fused for entire length (arrow in Fig. 41); 2) absent (Fig. 40).
42. Median abscissa of medial longitudinal carina: 0) present, not fused (Fig. 3); 1) present, fused for entire length; 2) absent (Fig. 40).
43. Posterior abscissa of medial longitudinal carina: 0) present, not fused (Fig. 3); 1) present, fused for entire length; 2) absent (Fig. 40).
44. Anterior abscissa of lateral longitudinal carina: 0) present (Fig. 3); 1) absent (Fig. 38).
45. Median abscissa of lateral longitudinal carina: 0) present (Fig. 3); 1) absent (Fig. 40).
46. Posterior abscissa of lateral longitudinal carina: 0) present (Fig. 3); 1) absent (Fig. 37).
47. Propodeal surface reticulation: 0) smooth, without reticulation (Fig. 3); 1) mostly covered by reticulation that obscures carinae (Fig. 42).

48. Submetapleural carina: 0) complete, anterior section unmodified to slightly broadened (arrow in Fig. 43); 1) complete, anterior section abruptly broadened into a lobe (arrow in Fig. 44); 2) anteriorly present, not lobe-like (arrow in Fig. 45), posteriorly absent; 3) completely absent.
49. Height of ventral edge of metasomal foramen ("mf" in Figs 46, 47): 0) ventral in relation to dorsal edge of hind coxal foramen ("cf" in Fig. 46): 1) dorsal in relation to dorsal edge of hind coxal foramen ("cf" in Fig. 47).
50. Metacoxal cavity posteromedially: 0) separated from metasomal insertion by sclerotized bridge (Fig. 46); 1) confluent with metasomal insertion (arrow in Fig. 48 points to unsclerotized region joining metasomal and coxal foramina).
51. Fore wing vein *2m-cu*: 0) present (Fig. 4); 1) absent (Fig. 49).
52. Fore wing vein *2m-cu* posteriorly: 0) vertical (joining vein *Cu* at right angle) to slightly reclivous (Fig. 4); 1) inclivous (joining *C*u at acute angle) (Fig. 50).
53. Fore wing vein *2m-cu*: 0) with two discrete bullae separated by small length of tubular vein (arrows in Fig. 50); 1) with single bulla (Figs 52–54).
54. Fore wing cell *1+2Rs* (areolet): 0) obliquely rhombic to subtriangular (Fig. 51); 1) rhombic to subrhombic (Fig. 52); 2) with vein *Rs* absent so only cross vein is distad *2m-cu* (Fig. 53); 3) with *3rs-m* absent so only cross vein is basad *2m-cu* (Fig. 50); 4) obliterated (vein *Rs* touching vein *M* so that veins *Rs* and *3rs-m* cannot be scored (Fig. 55); 5) symmetrically pentagonal (Fig. 4A) (vein *3rs-m* may be present or absent, but if absent, pentagonal nature of cell is evident) (e.g., Fig. 50); 6) large, roughly square, distal edge as high as proximal edge (Fig. 56); 7) irregularly pentagonal (Fig. 57).
55. Fore wing cell *1+2Rs* (areolet) veins: 0) smilar thickness to other fore wing veins (Fig. 4); 1) abscissae of vein Rs and vein M that comprise areolet thickened relative to other fore wing veins (Fig. 58).
56. Fore wing cell *1+2Rs* (areolet): 0) sessile anteriorly (Figs 4A, 56–57); 1) petiolate anteriorly (Figs 51–52).
57. Antero-medial fore wing flexion line basally: 0) splitting anterior to *M* and anterior fold running anterior to *M* (forming bulla indicated by arrow) (Fig. 49); 1) anterior fold running posterior to *M* (creating bulla(e) in vein *2m-cu*) (Figs 50–57). Note: In ichneumonids (state 1), the flexion line never creates a bulla in the basal cross-vein of the areolet (whether open, closed or as in Ophioninae); whereas in braconids (state 0), this bulla is generally present (unlabelled arrow in Fig. 49).
58. Fore wing cell *2R1* (radial cell) posterior angle: 0) greater than 100 degrees (Fig. 4); 1) 90 degrees or less (Fig. 58).
59. Fore wing cell *1M+1R1*: 0) uniformly hirsute; 1) with small to large glabrous area that may have free sclerites (Fig. 59).
60. Fore wing vein *Rs+M* (ramellus): 0) complete (extending across all of cell 1M + 1R1) (Fig. 49); 1) incomplete (present only as short vein or stub) (Fig. 53); 2) completely absent (Fig. 4).

61. Fore wing vein *1cu-a*: 0) opposite, slightly proximal or slightly distad vein *M* (Fig. 4); 1) distad vein *M* by 0.5× length of *1cu-a* or greater (Fig. 58); 2) proximal to vein *M* by 0.3 length of *1cu-a* or greater (Fig. 60).
62. Hind wing vein *1rs-m*: 0) basal to separation of veins *R1* and *Rs* (Fig. 49); 1) opposite or apical to separation of veins *R1* and *Rs* (Fig. 4).
63. Hind wing vein *2/Cu*: 0) equidistant between *A* and *M*, closer to *A* or slightly closer to *M* (Fig. 4); 1) much closer to *M* than *A* (Fig. 61) or appearing to originate from *M*; 2) absent (Fig. 49).
64. Hind wing vein *M+Cu*: 0) complete (Fig. 62); 1) basal 0.6 spectral (Fig. 58).
65. Hind wing vein *M+Cu*: 0) straight to weakly arched (Fig. 61); 1) strongly arched (Fig. 62).
66. Hind wing basal hamuli: 0) distant from wing base on membrane or spectral vein; 1) close to wing base on spur of tubular vein; 2) absent.
67. Hind wing distal hamuli: 0) 1–3; 1) 4 or greater.
68. Apex of fore tibia: 0) without tooth; 1) with distinct tooth on dorsal margin (Fig. 63).
69. Fore tibia dorsal surface: 0) covered with uniform thickness of setae; 1) with uniform setae and sparse, much stouter spines (Fig. 64).
70. Middle tibial spurs: 0) two; 1) one.
71. Apex of middle and hind tibiae: 0) with common area of insertion for spurs and basitarsus; 1) with sclerotized bridge separating insertion areas (Fig. 65).
72. Female hind coxa: 0) simple, without furrow; 1) with inner surface near base with vertical basal furrow (Fig. 66).
73. Hind tibia: 0) covered with uniform thickness of setae; 1) with uniform setae and sparse, much stouter spines (Fig. 67).
74. Hind tibia: 0) with posterior face simple (not smooth and shining) and with moderately sparse fringe of setae (Fig. 68); 1) with posterior face smooth and shining and thick fringe of setae (Fig. 69).
75. Hind tibial spurs: 0) present; 1) absent.
76. T1 petiolar cross-section (measured at petiolar midpoint): 0) not petiolate or if petiolate not wider than high (height/ width = 1–1.2×); 1) wider than high (height/ width = 0.6–0.7×).
77. T1 spiracle location: 0) at or anterior to 0.6× length of segment; 1) posterior to 0.6× length of segment.
78. T1 glymma: 0) present and shallow (Fig. 70); 1) relatively deep, almost meeting at midline (Fig. 71); 2) absent.
79. First metasomal segment shape: 0) non-petiolate: more or less evenly broadened from near base to posterior end or, if more or less parallel-sided throughout, then clearly depressed and less than 2.0× as long as posteriorly wide (Fig. 72); 1) petiolate: anteriorly more or less parallel-sided and broadened posterior to spiracles or, if parallel-sided throughout, then more or less cylindrical and greater than 2.0× as posteriorly wide. (Fig. 73).
80. S1 length: 0) 0.6× length of T1 or less; 1) longer than 0.6× length of T1.

81. S1 fusion apically: 0) not fused to T1 (Fig. 74); 1) fused, but suture visible (Fig. 75); 2) fused, suture not visible.
82. Thyridium shape: 0) present, ovoid (Fig. 76); 1) present, linear ("th" in Fig. 77); 2) absent.
83. Thyridium location: 0) less than one length or diameter distant from anterior edge of T1 (Fig. 77); 1) greater than or equal to one length, width or diameter distant from anterior edge of T1 (Fig. 76).
84. Gastrocoelus: 0) absent; 1) present ("gs" in Fig. 77).
85. Pseudothyridium of T2: 0) present ("ps" in Fig. 78); 1) absent.
86. T2 sculpture: 0) smooth to granulate; 1) with clearly defined longitudinal rugulae.
87. T2 and T3 fusion: 0) separate, with flexion line allowing movement (Fig. 2); 1) fused, without a flexion line.
88. MS2 laterotergites: 0) creased and curved mesad under metasoma ("L2" in Fig. 79); 1) not separated by a crease (Fig. 80).
89. MS3 laterotergite: 0) separated by crease, often partially turned under ("L3" in Fig. 79, arrow points to crease); 1) not separated by crease (Fig. 80).
90. MS4 laterotergite: 0) separated by crease (at least basally), often turned under; 1) not separated by crease (Fig. 80).
91. T2–T4 sculpture: 0) each tergite uniformly sculptured; 1) posterior 0.2 of each tergite sculptured differently than anterior 0.8 (arrows in Fig. 81).
92. Apical segment of female metasoma: 0) short; 1) elongate with horn or boss ("h" in Fig. 82); 2) elongate without horn or boss (arrow in Fig. 83).
93. Posterior sternites of female: 0) without ovipositor guides; 1) with tuberculate ovipositor guides (arrows in Fig. 84).
94. Female hypopygium in lateral profile: 0) inconspicuous and uniformly sclerotized; 1) moderate length, regularly triangular in profile, uniformly sclerotized (Fig. 85); 2) moderate length, regularly triangular in profile, medially membranous (Fig. 86, arrow points to medial, membranous region); 3) extending far beyond apex of posterior tergites, strongly triangular in profile, uniformly sclerotized (Fig. 87).
95. Hind margin of female hypopygium: 0) simple; 1) with median apical notch (arrow in Fig. 88).
96. Ovipositor length: 0) longer than apical height of metasoma but shorter than metasoma; 1) shorter than or equal to apical height of metasoma; 2) longer than length of metasoma.
97. Ovipositor ventral valve: 0) with teeth apically (Fig. 89); 1) without teeth apically.
98. Ovipositor ventral valve: 0) not enclosing dorsal valve; 1) enclosing dorsal valve.
99. Ovipositor dorsal valve, apically: 0) simply tapered (Fig. 2); 1) with dorsal, subapical notch (arrow in Fig. 90); 2) slender and needle-like (Fig. 85); 3) simple with nodus (Fig. 91, arrow points to nodus); 4) weakly sclerotized.
100. T8 or T8+9 of male: 0) medially longitudinally divided (Fig. 92, arrow points to line of longitudinal division); 1) not medially, longitudinally divided.

101. Hypopygium of male: 0) short and apically truncate; 1) elongate and scoop-like (Fig. 93).
102. Gonoforceps: 0) simple ("gon" in Fig. 92; 1) apically long and rod-like (Fig. 94).
103. Apical 0.5 of aedeagus: 0) subcylindrical and slightly to strongly clubbed (Fig. 95); 1) strongly flattened (Fig. 96).
104. Ovaries: 0) bohrertypus of Pampel (1913) (i.e., relatively low number of ovarioles and short lateral oviducts); 1) Ophion-typus of Pampel (large number of ovarioles and lateral oviducts 1–2 times as long as ovaries) (see Wahl 1991: Fig. 2).
105. Larval epistomal suture: 0) unsclerotized (Fig. 97); 1) partially sclerotized, medially incomplete (Fig. 6r); 2) completely sclerotized forming epistomal band (Figs 99, 101).
106. Larval hypostoma and pleurostoma: 0) not laterally expanded (Fig. 6a, b); 1) laterally expanded (Fig. 99).
107. Larval pleurostoma location: 0) inferior mandibular process dorsad or opposite to dorsal margin of labial sclerite (Fig. 6a, b); 1) shifted ventrally inferior to mandibular process opposite labial palpus (Fig. 100).
108. Larval labral sclerite: 0) present (Figs 6f, 7f); 1) absent (Fig. 97).
109. Larval mandible shape: 0) triangular, auxiliary tooth present or absent (Figs 6m, 7m); 1) cone shaped and with small apical tooth (Fig. 111, mandible in upper left); 2) bidentate, teeth subequal (Fig. 101); 3) reduced so that only apex of blade is present (Fig. 100); 4) absent.
110. Larval mandible with accessory teeth: 0) absent (Fig. 97); 1) present (Fig. 6m).
111. Larval mandible sclerotization: 0) uniformly sclerotized (Fig. 6m); 1) blade weakly sclerotized (base only or all except apex) ("m" in Fig. 100).
112. Larval mandibular blade denticles: 0) present on entire dorsal and ventral margins (Fig. 102); 1) present only on entire dorsal margin (Fig. 98); 2) absent on both dorsal and ventral margins (Fig. 97); 3) restricted to apex of dorsal and ventral margins; 4) mandible completely absent (Fig. 106).
113. Larval mandible, spines at base of blades: 0) juncture of base and blade without long, horizontal spines (Figs 97, 98); 1) juncture of base and blade with long (> 2× blade length) horizontal spines (Fig. 6m).
114. Larval posterior struts of inferior mandibular processes: 0) short (as long as dorsal struts) and not connected by band (Fig. 6k); 1) long (> 2× length of anterior struts) and connected by band (the latter indicated by an arrow in Fig. 103).
115. Larval hypostoma length: 0) long (>2 × as long as hypostomal spur) (Fig. 6b relative to 6c); 1) reduced (1–1.5 × as long as hypostomal spur); 2) absent or present only as a rudimentary stub (Fig. 103).
116. Larval hypostoma, lateral end: 0) simple/ undivided (Fig. 6b) divided as two bands, or with one upcurved extension; 1) divided into two bands, ventral band long, robust, downcurved (arrow in Fig. 104).
117. Larval hypostomal spur: 0) normal, about 2× as long as basal width or longer (Fig. 6c); 1) reduced, about as long as basal width (Fig. 97); 2) absent or only a rudimentary stub (Figs 99, 100).

118. Larval hypostomal spur meeting stipital sclerite: 0) near middle to lateral end of stipital sclerite (Fig. 6c, 6d); 1) near median end of stipital sclerite (Fig. 102); 2) fused and forming hypostomal-stipital plate (Fig. 105); 3) hypostomal-stipital plate reduced to narrow strip (Fig. 106); 4) fused in L-shaped structure (Fig. 107); 5) absent (Fig. 112).
119. Larval stipital sclerite orientation: 0) oriented so that lateral end at about right angle to labial sclerite (Figs 97, 98); 1) dorso-ventrally angled, lateral end near or on hypostoma (Fig. 99); 2) absent.
120. Larval stipital sclerite lateral end: 0) unmodified (Fig. 6d); 1) with plate-like expansion (Fig. 108).
121. Larval cardo: 0) unsclerotized (Fig. 6j); 1) present as lightly sclerotized oval (Fig. 104).
122. Larval maxillary apex: 0) unsclerotized (Fig. 6p); 1) sclerotized.
123. Larval maxillary and labial palpi sensilla: 0) maxillary bearing one to two, labial bearing one to three (Fig. 6i, 6h); 1) each bearing three (Fig. 103); 2) each bearing four to five (Fig. 109).
124. Larval labial sclerite shape: 0) quadrate (Fig. 6e); 1) circular (Fig. 104) to elongate-ovoid (Fig. 97); 2) absent (Fig. 109).
125. Larval labial sclerite dimensions: 0) about as long as wide, length of ventral portion/ total length = 0.2–0.3 (Fig. 98); 1) 1.4–1.7× as long as wide, length of ventral portion/ total length = 0.4–0.7× (Fig. 97).
126. Larval labial sclerite ventral margin: 0) relatively unmodified (may be lobes, minor scalloping, etc.) (Fig. 6e); 1) produced as spine (Fig. 100).
127. Larval prelabial sclerite: 0) absent (Fig. 6); 1) present as transverse band (arrow in Fig. 110); 2) present as triangular to Y-shaped structure ("ps" in Fig. 97).
128. Larval prelabium, number of sensilla: 0) 6 (Fig. 108); 1) 8 or more (Fig. 104).
129. Larval sclerotized plate ventral to labial sclerite: 0) absent (Fig. 6); 1) present ("sp" in Fig. 98).
130. Larval clypeolabral plate location: 0) absent (Fig. 6); 1) present, not contacting pleurostoma ("cl" in Fig. 100); 2) touching pleurostoma (Fig. 109).
131. Larval antenna: 0) present and with central papillus present (Figs 6n, 102); 1) present and lacking central papillus (Figs 104, 108); 2) absent.
132. Larval spiracles: 0) present, closing apparatus separated from atrium by section of trachea (Fig. 103b); 1) present, closing apparatus adjacent to atrium (Fig. 109); 2) present, closing apparatus absent; 3) completely absent.
133. Larval salivary orifice: 0) transverse or ovoid (Figs 105, 107); 1) u-shaped (Figs 6g, 97).
134. Egg: 0) without stalk; 1) with chorionic stalk (Fig. 113); 2) egg with a wide, pedunculate, ventral protrusion and an apical, sinuous stalk (Fig. 114); 3) stalk formed from hardened secretion from female (Figs 115, 116).
135. Egg stalk anchor: 0) anchor absent or entire stalk absent; 1) with tryphonine-like anchor (Fig. 113); 2) with eucerotine disk-like anchor (Fig. 116).
136. Exit of egg from body: 0) egg travels down lumen of ovipositor; 1) egg exits from hole ventral to ovipositor, stalk goes down ovipositor; 2) entire egg exits from hole ventral to ovipositor.

137. Biological mode (timing of larval maturation): 0) idiobiont; 1) koinobiont.
138. Biological mode (location of development): 0) ectoparasitoid; 1) endoparasitoid; 2) inside hind gut with final ectoparasitoid phase AND pupate inside host cocoon.
139. Host/ source of larval nutrition: 0) phytophagous (at least facultatively after consuming insect host); 1) Hymenoptera; 2) Lepidoptera; 3) Coleoptera; 4) Diptera; 5) Araeneae; 6) Trichoptera; 7) Neuroptera; 8) egg predators.
140. Oviposition location: 0) through lignified plant tissue; 1) through non-lignified plant or gall tissue; 2) in leaf rolls, cases and other plant tissues held by silk; 3) in silken bags or sacs; 4) into or onto exposed larval/ pupal hosts; 5) into ground nests; 6) into trichopteran tubes; 7) into exposed eggs; 8) on to leaf; 9) into host in host.
141. Oviposition/emergence stages: 0) eggs (predators); 1) larval-larval (not last instar); 2) pupa-pupa; 3) egg-larval; 4) immature spider - adult spider; 5) larval-larval (last instar); 6) larval-pupal; 7) egg-pupal; 8) leaf-pupa.

Phylogenetics

Parsimony analysis

Total-evidence

The total-evidence parsimony analysis found 1728 equally parsimonious trees of length 9917 (C.I. = 0.15, R.I. = 0.44). In the strict consensus tree (Fig. 117), 14 nodes collapsed. Table 3 (column "Pars. (all)") provides a summary of the taxa that were recovered as unequivocally monophyletic. In summary, of the 29 groupings included in Table 3, 15 were monophyletic and a further 8 groupings would be monophyletic with inclusion or exclusion of one or more problematical/ equivocally classified taxa as described in footnotes. *Aplomerus* (Xoridinae) was sister group to all other Ichneumonidae, (*Odontocolon* + *Xorides*) was sister group to all except for *Aplomerus*, and Orthopelmatinae (*Orthopelma mediator* Thunberg) was sister group to all taxa except the three Xoridinae. The remainder of the subfamilies were arranged in three groupings. The sister group to the other two was the Ophioniformes of Quicke et al. (2009) comprised of 17 subfamilies. The second group was equivalent to the Pimpliformes of Quicke et al. (2009). The third group was the Ichneumoniformes s.l., which was comprised of Ichneumoniformes s.s. of Wahl (1993a) i.e., Brachycyrtinae + Cryptinae (including Ateleutinae and Phygadeuontinae) + Ichneumoninae (including *Alomya*), as well as Adelognathinae, Agriotypinae, Claseinae, Eucerotinae, Labeninae, Microleptinae and Pedunculinae. Placement and relationships of subfamilies within these three groupings will be discussed in the section Support/ relationships of taxa (below). The average consistency indices for the different character types were as follows: adult (0.24), larval (0.41), egg (0.80), biological (0.22) and molecular (0.04).

Table 2. Data matrix for morphological character states of Ichneumonidae and outgroup taxa. Key to the letters in matrix below: a=0/1; b=0/2; c=0/3; d=1/2; e=1/3; f=1/4; g=1/5; h=2/3; k=2/4; m=2/6; n=3/4; p=3/7; q=5/6; r=6/7; s=0/1/3; t=1/5/6; u=2/6/7; v=5/6/7; w=2/5/6/7; x=3/5/6/7.

Taxon	1									2										3										4										5										6										7									
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
Doryctes erythromelas	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	1	2	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	3	?	3	?	?	1	0	0	0	0	0	1	0	0	1	1	?	?	6	0	0	0	0	0	0	1	0	2	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0
Rhysipolis sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	1	2	0	0	0	0	0	0	0	0	1	0	1	0	1	3	?	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	?	?	6	0	0	0	0	0	0	1	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0
Aleiodes terminalis	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	2	0	0	2	0	1	0	0	0	0	0	1	0	1	0	1	3	?	3	?	?	1	1	1	1	1	1	1	1	0	0	1	1	?	?	6	0	0	0	0	0	0	1	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0
Spilopteron occiputale	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	2	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	h	0	?	1	0	0	2	b	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	2	0	0
Coleocentrus rufus	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	2	0	3	0	0	0	0	0	0	0	0	1	1	0	3	?	1	0	1	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	2	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Adelognathus sp.	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	3	?	3	?	?	2	2	2	1	1	0	0	0	0	0	0	0	1	3	0	?	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	2	0
Agriotypus armatus	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	1	0	1	2	0	0	0	0	0	1	1	0	0	0	0	1	1	3	?	3	?	?	0	0	0	0	0	0	0	0	0	0	0	1	3	0	?	1	1	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	2	1
Anomalon picticorne	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	2	0	1	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	2	2	1	1	1	1	0	0	0	0	0	1	2	0	?	1	0	0	2	0	1	2	0	1	1	1	0	0	1	0	0	0	0	0	0	1	2	1
Therion texanum	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	1	3	?	3	?	?	2	2	2	1	1	1	1	1	0	0	0	0	1	3	0	?	1	0	0	2	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	1	2	1
Lissonota scutellaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	3	0	0	2	0	0	0	0	1	1	0	1	0	0	3	?	0	0	1	2	2	2	1	1	0	0	1	0	0	0	0	1	0	0	1	1	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Exetastes bioculatus	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	2	3	0	0	0	0	0	0	0	1	1	0	?	0	0	3	?	0	0	1	2	2	2	1	1	0	0	1	0	0	0	0	1	0	0	1	1	0	0	2	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0
Apophua simplicipes	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	3	0	0	2	0	0	0	0	1	0	0	0	0	0	2	?	0	0	1	0	0	2	0	0	1	0	1	0	0	0	0	3	0	?	1	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Sphelodon phoxopteridis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	2	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	3	0	?	1	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Brachycyrtus wardae	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0	0	2	0	?	1	0	0	0	0	0	0	0	0	0	0	0	3	0	?	1	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2	1
Bathyplectes infernalis	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	2	3	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	0	?	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	0	2	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	1	2	0
Campoletis sonorensis	0	1	1	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	1	1	0	2	3	0	1	0	0	0	0	0	2	0	0	1	0	0	0	0	2	0	?	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0
Campoplex sp.	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	2	0	0	2	0	1	0	0	0	0	0	2	0	0	0	0	1	0	0	2	0	?	0	0	0	0	0	1	1	0	0	0	0	0	0	1	c	0	1	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	2	1
Casinaria grandis	0	1	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	2	1	0	?	1	1	1	0	3	?	?	0	0	2	1	1	1	1	0	0	0	0	0	1	0	0	1	1	0	0	2	0	1	0	0	0	2	1	0	0	0	0	0	0	0	0	1	2	1
Dusona egregia	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	2	3	0	1	0	0	0	0	2	0	0	0	1	1	0	0	2	0	?	0	2	2	1	1	1	0	0	0	0	0	0	1	0	0	1	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	2	1
Hyposoter sp.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	2	3	0	1	0	0	0	0	2	1	0	0	0	0	0	0	2	0	?	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	0	1	0	1	0	0	1	2	1	0	0	0	0	0	0	0	1	0	0
Olesicampe sp.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	2	3	0	1	0	0	0	0	2	0	0	0	0	0	0	0	2	0	?	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	0	2	0	1	0	0	1	2	1	0	0	0	0	0	0	0	1	0	0
Rhimphoctona macrocephala	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	1	2	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	1	1	0	0	2	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0	0
Clasis sp. nov.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	1	0	2	2	2	1	1	1	0	0	0	0	0	0	5	0	0	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	2	1
Collyria catoptron	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	2	0	d	2	0	0	0	0	1	0	0	1	1	3	?	3	?	?	0	0	0	0	0	0	0	2	0	0	0	0	0	3	0	?	1	0	0	2	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0
Eiphosoma pyralidis	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	2	0	1	0	0	1	0	1	0	0	0	0	1	2	1
Xiphosomella setoni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	1	0	0	0	0	2	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	2	0
Agonocryptus chichimecus	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	2	0	0	1	1	0	?	0	1	0	1	2	0	?	2	2	2	2	1	1	1	0	0	0	0	0	0	1	5	0	0	1	0	0	2	2	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	2	1
Ateleute sp. nov.	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	1	0	1	0	1	0	1	0	0	4	1	0	0	0	2	1	0	0	0	1	3	?	1	?	?	2	2	0	1	1	1	0	0	0	0	0	1	1	6	0	0	1	0	0	2	0	1	2	0	1	0	a	0	0	0	0	0	0	0	0	2	0
Baryceros texanus	0	0	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	1	1	0	0	1	1	0	0	3	?	?	0	2	2	1	1	1	0	0	0	0	0	0	1	5	0	0	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	2	1
Diapetimorpha brunnea	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	2	0	0	1	1	0	0	0	1	0	0	2	0	?	2	2	2	2	1	1	1	0	0	0	0	0	0	1	5	0	0	1	0	0	2	2	1	0	0	1	2	1	0	0	0	0	0	0	0	0	1	2	1
Echthrus reluctator	0	0	1	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	1	2	0	0	1	0	0	0	1	0	0	3	?	0	1	1	0	0	2	0	0	0	0	0	0	0	0	0	1	5	0	0	1	0	0	1	2	1	0	0	0	2	1	0	0	0	0	0	0	0	0	0	2	0
Lymeon orbus	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2	0	1	1	0	2	1	2	0	0	1	1	0	0	0	1	0	0	3	?	?	0	2	2	1	1	1	0	0	0	0	0	0	1	5	0	0	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	1	2	0

Taxon	1										2										3										4										5										6										7									
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
Polytribax contiguus	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	2	0	0	1	0	0	0	1	1	0	a	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	5	0	0	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	2	0
Rhytura pendens	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	1	2	0	0	1	0	0	0	0	0	0	0	h	?	b	1	0	0	0	0	0	0	0	0	0	0	0	1	5	0	0	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	2	0
Acrolyta sp.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	1	0	0	0	0	0	1	0	5	0	0	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	2	0
Endasys patulus	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	1	0	1	0	0	2	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	5	0	0	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	2	0
Mastrus sp.	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	2	0
Ctenopelma sanguineum	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	s	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	2	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0
Xenoschesis limata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	1	0	1	3	?	3	?	?	2	2	2	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	2	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	2	0
Euryproctus sentinis	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	1	0	1	3	?	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	3	0	?	1	0	0	2	0	1	0	0	1	0	1	1	0	0	0	0	1	0	0	1	0	2	0
Mesoleptidea decens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	1	0	1	3	?	3	?	?	0	0	0	1	1	0	0	0	0	0	0	0	0	3	0	?	1	0	0	2	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	2	0
Barytarbes honestus	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	2	0	0	0	0	0	0	1	0	1	3	?	3	?	?	2	2	2	1	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	2	1	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
Himerta luteofacia	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	3	?	3	?	?	2	b	d	1	1	1	0	0	0	0	0	0	1	3	0	?	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Lathrolestes asperatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	a	0	1	0	0	0	0	0	0	1	0	0	1	1	0	0	2	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0
Perilissus concolor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	b	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	2	0	1	1	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0
Rhorus bartelti	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	0	2	0	1	0	0	1	2	1	1	0	0	0	0	0	0	0	0	0	0	0
Sympherta fucata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	?	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	3	0	?	1	0	0	2	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	2	0
Seleucus cuneiformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	2	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	2	1
Onarion sp.	0	0	1	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	1	0	2	3	0	0	2	0	0	0	0	1	0	?	0	1	3	?	3	?	?	2	2	2	1	1	1	0	0	0	0	0	0	1	3	0	?	1	0	0	2	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
Westwoodia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	2	0	0	0	0	0	0	0	0	1	3	?	3	?	?	2	2	0	1	1	1	0	2	0	0	0	0	1	0	0	1	1	0	0	2	1	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
Ctenopelmatinae Genus NZ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0	1	1	0	0	0	0	0	0	0	1	0	1	3	?	0	0	0	2	2	0	1	1	1	0	0	0	0	0	0	1	3	0	?	1	1	0	2	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Cylloceria melancholica	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2	0	1	0	0	0	0	0	0	0	0	1	0	1	3	?	0	0	?	0	0	d	0	0	0	0	0	0	0	0	0	3	0	?	1	0	0	2	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0
Diacritus incompletus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	1	1	0	0	0	0	1	0	0	0	0	0	3	?	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	2	1
Diplazon laetatorius	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	3	?	2	0	0	0	0	0	0	0	0	0	0	1	0	0	1	3	0	?	1	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Woldstedtius flavolineatus	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	3	0	1	2	0	0	0	0	1	0	0	0	1	3	?	3	?	?	2	2	2	1	1	1	0	0	0	1	0	0	1	3	0	?	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Euceros sp. nov.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	1	1	0	0	2	0	0	0	0	1	0	0	1	0	3	?	0	1	0	0	0	2	1	1	0	0	0	0	0	0	1	3	0	?	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Hybrizon rileyi	0	0	0	0	3	0	0	0	0	0	0	0	0	?	0	2	0	1	0	1	0	2	3	0	3	2	0	0	0	0	1	0	0	1	1	3	?	3	?	?	2	2	1	1	1	0	0	3	1	0	1	?	?	3	0	?	1	0	0	2	1	1	2	1	?	2	1	0	0	0	0	0	0	0	0	2	1
Alomya debellator	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	3	?	0	0	1	2	2	2	a	a	0	0	2	0	1	0	0	0	5	0	0	1	0	0	2	0	1	0	0	0	2	1	0	1	0	0	0	0	0	0	0	2	1
Centeterus euryptychiae	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5	0	0	1	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	2	1
Phaeogenes hebrus	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	0	d	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	1	0	0	2	0	1	0	0	0	1	1	0	0	0	0	0	0	0	1	2	1
Stenodontus sp. nov.	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	d	0	0	0	0	1	0	1	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	1	0	0	2	0	1	0	0	1	2	1	0	0	0	0	0	0	0	1	2	1
Coelichneumon eximius	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	d	0	0	0	0	1	0	0	1	1	1	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	5	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	2	1
Protichneumon grandis	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	1	0	0	1	1	1	e	0	0	0	0	0	0	0	a	a	0	0	0	0	0	0	0	0	5	0	0	1	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	1	2	1
Barichneumon neosorex	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	2	1
Cratichneumon w-album	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	1	0	0	2	0	1	0	0	0	0	1	0	1	0	0	0	0	0	1	2	1
Orgichneumon calcatorius	0	1	0	0	0	0	0	1	1	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	1	2	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	b	0	0	0	0	0	0	0	0	5	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	2	1
Patrocloides montanus	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	2	1

Taxon	1										2										3										4										5										6										7									
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
Plagiotrypes concinnus	0	1	0	0	0	0	0	1	1	0	0	0	0	2	0	0	0	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	5	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2	1
Dilopharius otomitus	0	1	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	1	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2	1
Cyclolabus impressus	0	1	0	0	0	0	0	1	1	0	0	0	0	2	0	0	0	0	1	0	1	0	0	2	0	0	0	0	1	0	0	0	0	0	a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	2	1
Linycus exhortator	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	2	1
Grotea anguina	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	2	0	2	3	0	1	2	0	0	0	0	0	0	1	1	1	3	?	0	0	0	0	0	2	0	0	0	0	0	0	0	0	7	0	0	1	0	0	2	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	2	1
Labium sp.	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	1	b	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	7	0	0	1	0	0	2	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1	2	1
Apechoneura sp.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	3	0	0	2	0	0	0	1	0	0	0	0	1	1	3	?	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	2	0	1	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	2	0
Labena grallator	1	0	0	0	0	0	0	2	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1	2	0	0	1	0	1	0	0	0	1	1	0	0	a	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	7	0	1	1	0	0	2	0	1	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	2	0
Poecilocryptus nigromaculatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	1	0	0	0	0	1	0	1	0	0	1	0	0	0	2	0	?	0	0	0	1	0	0	0	0	0	0	0	1	1	7	0	0	1	0	0	2	0	1	2	0	1	1	1	0	0	0	0	0	0	0	0	0	2	1
Lycorina glaucomata	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	0	1	0	0	0	0	0	1	0	?	0	1	3	?	c	0	1	2	2	2	1	1	1	0	1	0	0	0	0	1	3	0	?	1	0	0	2	0	1	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Astiphromma sp. nov.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	h	?	0	0	0	0	0	0	0	0	0	a	0	0	0	1	0	0	1	1	0	1	1	0	0	2	1	1	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0
Chineater masneri	0	1	0	0	0	0	0	0	?	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	2	0	0	0	0	2	0	0	0	0	1	0	0	0	1	1	1	0	1	1	0	0	2	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0
Cidaphus paniscoides	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	2	2	1	0	0	1	0	1	1	1	0	0	0	0	0	0	0	1	1
Lepidura collaris	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	?	0	0	0	2	2	0	1	1	1	0	0	0	0	1	1	1	0	1	1	0	0	2	0	1	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	1
Mesochorus sp.	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	2	0	1	2	0	1	0	1	1	0	0	0	0	0	0	0	1	1
Exochus semirufus	0	1	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	3	0	?	1	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Metopius pollinctorius	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	3	0	d	1	1	0	0	1	1	0	1	1	1	3	?	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	7	0	0	1	0	0	2	0	1	1	0	0	2	1	1	0	1	0	0	0	0	0	0	0	0	0
Scolomus sp.	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	2	3	0	0	2	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	?	0	2	0	0	1	1	0	0	0	0	0	0	1	0	0	1	1	0	0	2	0	1	2	0	1	0	1	0	0	0	0	0	0	0	0	1	0
Seticornuta terminalis	0	1	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	2	0	2	3	0	2	2	0	0	0	1	1	0	0	1	0	3	?	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	2	1	1	0	0	1	2	1	0	0	0	0	0	0	0	0	0	0	0
Microleptes sp.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	d	0	0	0	0	1	0	0	0	0	0	3	?	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	3	0	?	1	0	0	2	0	1	0	0	1	0	1	1	1	0	0	0	0	0	1	0	0	0	2	0
Neorhacodes enslini	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2	0	2	3	0	0	0	0	0	0	0	1	0	0	0	0	3	?	0	0	0	0	0	2	0	0	0	0	0	0	0	0	?	?	4	?	?	?	0	0	2	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Chriodes sp.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	1	0	0	0	0	2	1	0	1	1	1	0	0	0	0	0	0	0	0	2	0	0	1	0	1	0	0	0	1	3	0	?	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	2	1
Nonnus sp.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	1	0	1	0	2	0	0	0	0	0	0	0	0	0	0	2	1	0	1	1	3	?	0	1	1	2	2	2	1	1	1	0	0	0	0	0	0	1	7	0	0	1	0	0	2	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	2	1
Enicospilus flavostigma	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	1	0	0	1	1	0	1	3	?	?	2	0	2	1	1	1	0	0	0	0	0	0	1	2	0	?	1	0	1	2	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1	2	1
Hellwigia obscura	1	0	0	0	0	0	0	0	?	1	0	0	0	1	0	1	0	0	1	2	0	2	3	0	0	0	0	0	0	2	1	0	?	1	1	3	?	3	?	?	2	2	2	1	1	1	0	0	0	0	0	0	1	2	0	?	1	0	0	2	1	1	0	0	1	1	0	1	0	0	0	1	0	0	0	1	2	1
Ophion sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	1	2	0	0	0	0	1	0	0	1	1	0	1	2	0	?	2	0	1	1	1	0	0	0	0	0	0	0	1	2	0	?	1	0	1	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	2	1
Skiapus sp.	0	0	0	0	2	1	0	0	0	1	0	0	0	1	0	0	0	0	1	2	0	2	3	0	1	2	0	0	0	2	1	0	0	1	1	0	1	0	0	1	2	2	2	1	1	0	0	0	0	0	0	1	1	2	0	?	1	0	0	2	1	1	1	0	0	1	1	0	1	0	0	0	1	0	0	0	1	2	1
Thyreodon sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	1	2	0	0	0	1	1	0	1	1	3	?	3	?	?	2	2	2	1	1	1	0	0	0	0	0	0	1	2	0	?	1	0	0	2	1	1	1	0	0	2	1	0	0	0	0	0	0	0	0	1	2	1
Megastylus sp. nov.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	1	1	3	?	0	0	0	2	2	2	1	1	0	0	0	0	0	0	1	0	3	1	?	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	2	0
Orthocentrus sp.	0	1	0	0	0	1	0	0	0	0	0	0	0	?	0	0	0	0	0	2	0	0	0	0	1	1	2	0	0	0	0	0	1	0	0	3	?	0	1	0	0	0	2	0	0	0	0	0	0	0	0	1	1	3	0	?	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Proclitus speciosus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0	3	?	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	n	0	?	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	2	0
Orthopelma mediator	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2	0	1	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	3	0	?	1	1	0	2	0	1	2	0	1	0	1	0	0	0	0	0	0	0	0	0	2	0
Oxytorus albopleuralis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	2	1
Pedunculus sp. nov.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	5	0	0	1	0	0	2	0	1	2	0	1	0	1	0	0	0	0	0	0	1	0	0	0	2	1
Perithous divinator	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	3	?	0	1	1	2	2	2	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	0	1	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0

Taxon	1										2										3										4										5										6										7									
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
Acrotaphus wiltii	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	2	0	1	1	0	0	2	0	0	0	0	1	0	?	0	1	3	?	3	?	?	2	2	2	1	1	0	0	3	0	0	0	1	0	3	0	?	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Clistopyga recurva	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	0	2	0	0	0	0	1	0	1	0	1	3	?	3	?	?	2	2	2	1	1	0	0	0	0	0	0	0	0	3	0	?	1	0	0	1	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
Dolichomitus irritator	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	1	0	2	0	0	0	0	0	0	1	1	1	3	?	3	?	?	b	b	2	1	1	0	0	2	0	0	0	0	0	0	1	1	0	0	2	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Zaglyptus pictilis	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	2	0	0	0	0	1	0	1	0	1	0	3	?	3	?	?	2	2	2	1	1	0	0	2	0	0	0	0	0	3	0	?	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Pimpla annulipes	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	1	0	1	3	?	3	?	?	2	2	2	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	2	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0
Theronia bicincta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	1	3	?	0	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	1	1	0	0	2	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0
Neoxorides caryae	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	2	0	1	2	0	4	2	0	0	0	0	1	0	1	0	1	3	?	0	0	?	2	2	2	1	1	0	0	0	0	0	0	0	0	3	0	?	1	0	0	1	0	1	1	0	0	1	1	0	1	0	0	0	0	0	0	2	0
Poemenia albipes	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	1	2	0	4	2	0	0	0	0	1	0	0	0	1	3	?	3	?	?	2	2	2	1	1	0	0	2	0	0	0	0	a	0	0	1	1	0	0	1	0	1	1	0	1	1	1	0	1	0	0	0	0	0	0	0	2	0
Megarhyssa greenei	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2	0	0	0	0	1	0	1	1	1	3	?	3	?	?	2	2	2	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	2	0
Rhyssa crevieri	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2	0	0	0	0	0	0	0	1	1	3	?	3	?	?	2	2	2	1	1	1	0	3	0	0	0	0	0	0	0	1	1	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	2	0
Rhyssella nitida	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2	0	0	0	0	0	0	0	1	1	3	?	3	?	?	2	2	2	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	2	0
Brachyscleroma sp.	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Erythrodolius calamitosus	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	2	0	1	3	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	?	1	0	0	2	0	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0
Notostilbops sp. nov.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Stilbops vetulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	2	1	1	0	0	1	0	1	0	0	0	0	0	0	0
Tatogaster nigra	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	?	?	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1	0	0	2	2	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	2	1
Allophrys sp.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	3	?	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	1	3	1	?	1	1	0	2	1	1	2	1	1	2	0	0	0	0	0	0	0	1	2	1
Peucobius fulvus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	3	0	?	1	0	0	2	a	1	0	0	1	0	1	1	0	0	0	0	0	0	1	0
Phrudus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	2	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	2	0	1	2	1	1	0	1	0	0	0	0	0	0	0	0	2	1
Stethantyx nearctica	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	0	0	0	0	2	2	0	1	0	0	0	0	1	0	0	3	?	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	1	3	0	?	1	0	0	2	1	1	2	1	1	0	a	0	0	0	0	0	0	0	1	0	1
Tersilochus sp.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	0	0	0	0	1	2	0	1	0	0	0	0	1	0	0	3	?	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	1	3	1	?	1	1	0	2	1	1	2	1	1	2	a	0	0	0	0	0	0	0	1	0	1
Eclytus sp.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	3	?	0	0	0	2	0	1	0	0	0	0	0	0	1	0	0	0	3	0	?	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Idiogramma longicauda	0	0	0	1	0	?	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	?	3	?	?	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	2	0	1	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0
Zagryphus nasutus	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	3	0	?	1	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0
Netelia sp.	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2	0	1	1	0	0	0	0	0	0	0	0	0	1	1	1	3	?	3	?	?	2	2	2	1	1	1	0	0	0	1	0	0	0	0	0	1	1	0	0	2	1	1	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	1	0
Phytodietus vulgaris	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	b	0	0	0	0	0	0	?	0	1	3	?	3	?	?	2	2	2	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0
Cteniscus sp.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	2	0	1	0	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	0
Cycasis rubiginosa	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	2	0	1	0	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0
Polyblastus sp.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	?	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	2	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Aplomerus sp.	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	2	0	2	2	0	0	0	0	0	0	1	1	?	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	3	0	?	1	0	0	2	2	1	0	0	0	1	1	0	1	0	0	0	0	0	0	2	0
Odontocolon albotibiale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	2	0	0	0	0	0	0	1	0	0	0	0	0	3	?	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	3	0	?	1	0	0	1	2	1	0	0	1	1	1	0	1	0	0	0	0	0	0	2	0
Xorides stigmapterus	1	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	2	0	1	2	0	2	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	3	0	?	1	0	0	1	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	2	0

Table 2. Continued. Data matrix for morphological character states of Ichneumonidae and outgroup taxa. Key to the letters in matrix below: a=0/1; b=0/2; c=0/3; d=1/2; e=1/3; f=1/4; g=1/5; h=2/3; k=2/4; m=2/6; n=3/4; p=3/7; q=5/6; r=6/7; s=0/1/3; t=1/5/6; u=2/6/7; v=5/6/7; w=2/5/6/7; x=3/5/6/7.

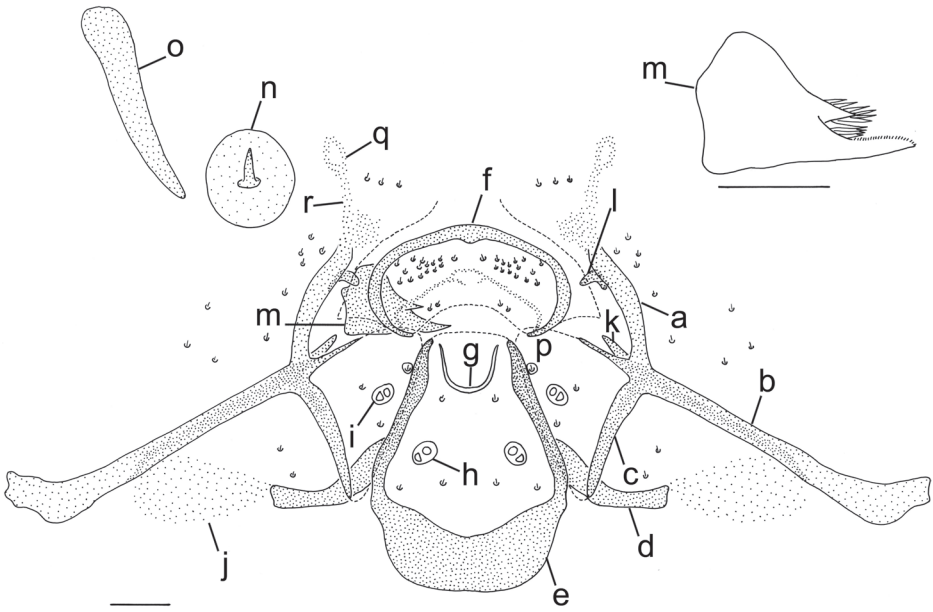
Taxon																					1										1										1										1										1	
	8										9										0										1										2										3										4	
	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1
Doryctes erythromelas	0	1	2	?	0	0	1	1	0	0	1	0	2	0	1	0	2	0	0	0	1	0	0	1	?	2	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1
Rhysipolis sp.	0	0	2	?	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	3	1	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	?	?	?	0	0	0	1	0	2	1	5
Aleiodes terminalis	0	0	2	?	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	1	?	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	?	0	0	0	0	1	1	2	4	5
Spilopteron occiputale	0	1	0	0	0	0	0	0	0	0	0	0	0	0	3	0	2	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	2	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	?	0	0	?	?	0	0	0	?	?	?	3	0	?
Coleocentrus rufus	0	0	0	0	0	0	0	0	0	0	0	0	2	0	3	0	2	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	1	0	0	0	0	1	0	0	0	?	0	0	1	1	0	0	0	?	?	?	3	0	?
Adelognathus sp.	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	?	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0	0	1	k	1
Agriotypus armatus	1	2	2	?	0	0	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	6	6	2
Anomalon picticorne	1	2	2	?	0	1	0	0	0	1	1	0	0	0	0	0	0	1	0	1	1	0	0	0	1	2	0	0	1	0	0	0	1	0	0	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	1	?	?	0	1	1	h	k	6
Therion texanum	1	2	2	?	0	1	0	0	0	1	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	2	1	0	1	0	0	0	2	0	0	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	1	2	0	0	1	1	2	4	6
Lissonota scutellaris	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	2	0	1	2	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	1	1	1	0	0	0	1	1	2	2	5
Exetastes bioculatus	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	1	1	0	1	0	0	0	0	1	2	0	0	1	0	0	0	2	0	1	0	0	0	0	0	0	0	1	1	1	0	0	1	1	0	0	1	1	1	0	0	0	1	1	2	k	5
Apophua simplicipes	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	1	1	0	0	0	1	1	2	2	5
Sphelodon phoxopteridis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	2	1	0	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	1	1	2	2	5
Brachycyrtus wardae	1	2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	?	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	?	0	1	?	?	?	?	0	7	?	w
Bathyplectes infernalis	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	?	0	0	0	1	0	0	0	2	0	0	0	0	1	0	0	1	0	0	0	1	0	0	2	0	0	0	1	1	1	0	0	0	1	1	3	4	q
Campoletis sonorensis	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	2	0	0	0	0	1	0	0	1	0	0	0	1	0	0	2	0	0	0	1	1	1	0	0	0	1	1	2	4	1
Campoplex sp.	1	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	2	0	0	0	0	1	0	0	1	0	0	0	1	0	0	2	0	0	0	1	1	1	0	0	0	1	1	2	2	g
Casinaria grandis	1	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	2	0	0	0	0	1	0	0	1	0	0	0	1	0	0	2	0	0	0	1	1	1	0	0	0	1	1	2	4	1
Dusona egregia	1	2	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	2	0	0	0	0	1	0	0	1	0	0	0	1	1	0	2	0	0	0	1	1	1	0	0	0	1	1	2	4	q
Hyposoter sp.	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	2	0	0	0	0	1	0	0	1	0	0	0	1	0	0	2	0	0	0	1	1	1	0	0	0	1	1	2	4	1
Olesicampe sp.	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	2	0	0	0	0	1	0	0	1	0	0	0	1	0	0	2	0	0	0	1	1	1	0	0	0	1	1	1	4	q
Rhimphoctona macrocephala	0	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	?	0	0	0	1	0	0	0	2	0	0	0	0	1	0	0	1	0	0	0	1	0	0	2	0	0	0	1	1	1	?	?	0	1	1	3	0	g
Clasis sp. nov.	1	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	3	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Collyria catoptron	0	0	2	?	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	4	0	0	0	?	2	5	2	0	?	0	?	?	?	0	0	?	0	0	2	1	?	0	0	0	1	1	1	1	3
Eiphosoma pyralidis	1	2	2	?	0	1	0	0	1	1	1	0	0	0	0	0	0	1	0	1	1	0	0	0	?	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	2	2	5
Xiphosomella setoni	0	1	0	1	0	1	1	0	0	1	1	0	0	0	0	0	0	1	0	1	?	0	0	0	?	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	1	0	0	2	0	0	0	1	1	1	?	?	?	?	?	2	2	?
Agonocryptus chichimecus	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	3	a	?

Taxon																					1										1										1										1										1	
	8										9										0										1										2										3										4	
	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1
Ateleute sp. nov.	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	2	2	?
Baryceros texanus	1	2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	2	4	?
Diapetimorpha brunnea	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	2	4	2
Echthrus reluctator	0	1	0	1	0	0	0	0	0	0	0	0	2	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	?	3	0	?
Lymeon orbus	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	?	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	?	?	?	?	?	d	?	?
Polytribax contiguus	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	?	0	2	?	m
Rhytura pendens	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Acrolyta sp.	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	?	0	0	0	0	0	0	0	0	?	0	0	?	0	0	0	0	0	0	?	0	?	0	0	0	0	0	0	0	1	?	?	0	0	0	1	4	2
Endasys patulus	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	4	2
Mastrus sp.	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	d	f	2
Ctenopelma sanguineum	0	0	2	?	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	h	p
Xenoschesis limata	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	3	t
Euryproctus sentinis	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	4	?
Mesoleptidea decens	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	?	1	1	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	?	?	?	1	1	1	4	?
Barytarbes honestus	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	1	4	?
Himerta luteofacia	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	?	1	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	4	?
Lathrolestes asperatus	0	0	2	?	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	?	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	d	1	q
Perilissus concolor	0	0	2	?	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	1	2	1	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	4	?
Rhorus bartelti	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	2	0	0	0	0	?	1	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	1	1	0	0	0	1	1	1	4	6
Sympherta fucata	1	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	2	0	0	0	0	1	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	4	?
Seleucus cuneiformis	0	1	2	?	0	1	0	0	1	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	2	?
Onarion sp.	0	0	2	?	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Westwoodia sp.	0	0	1	0	0	1	0	0	1	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	1	1	4	t
Ctenopelmatinae Genus NZ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	2	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0	?	?	1	2	?	u
Cylloceria melancholica	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2	1	0	0	1	0	0	0	?	0	0	0	0	0	0	0	2	0	0	0	0	0	2	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	?	?	?	?	1	4	?	?
Diacritus incompletus	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Diplazon laetatorius	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	1	1	0	0	1	1	3	0	1	2	0	0	2	0	2	?	2	0	0	0	0	0	0	1	0	0	0	1	2	0	0	0	0	0	1	1	4	4	7
Woldstedtius flavolineatus	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	1	?	0	0	1	1	0	0	1	2	0	0	2	0	2	?	2	0	0	0	1	0	0	1	0	0	0	1	2	?	?	?	?	?	?	?	4	4	?
Euceros sp. nov.	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	4	0	0	0	0	0	1	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	3	2	0	1	1	1	8	8

Taxon																					1										1										1										1										1	
	8										9										0										1										2										3										4	
	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1
Hybrizon rileyi	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	2	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	4	q
Alomya debellator	0	1	2	?	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	?	2	0	0	1	0	0	0	2	0	0	0	0	2	?	2	0	0	0	2	1	0	0	0	0	0	0	?	0	1	?	?	0	1	1	2	4	5
Centeterus euryptychiae	1	1	2	?	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	?	2	0	0	1	0	0	0	2	0	0	0	?	2	?	2	0	0	0	2	2	?	?	0	0	0	2	?	1	1	?	?	?	?	1	2	?	?
Phaeogenes hebrus	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0	0	0	0	2	0	0	1	0	0	0	2	0	0	0	?	2	?	2	0	0	0	2	2	?	?	0	0	0	2	1	1	1	0	0	0	?	1	2	k	u
Stenodontus sp. nov.	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	2	?	?
Coelichneumon eximius	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	3	0	1	0	0	0	2	1	0	1	0	0	0	2	0	0	0	?	2	?	2	0	0	0	2	2	?	?	0	1	0	1	1	1	1	0	0	0	?	1	2	4	u
Protichneumon grandis	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	1	0	1	0	0	0	2	0	0	0	?	2	?	2	0	0	0	2	2	?	?	0	1	0	1	1	1	1	0	0	0	?	1	2	4	u
Barichneumon neosorex	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	?	2	0	0	1	0	0	0	2	0	0	0	?	2	?	2	0	0	0	2	2	?	?	0	0	0	1	1	1	1	0	0	0	?	1	2	4	u
Cratichneumon w-album	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	1	0	0	0	2	0	0	1	0	0	0	2	0	0	0	?	2	?	2	0	0	0	2	2	?	?	0	0	0	1	?	1	1	0	0	0	0	1	2	4	2
Orgichneumon calcatorius	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	3	0	1	0	0	?	2	0	0	1	0	0	0	2	0	0	0	?	2	?	2	0	0	0	2	2	?	?	0	?	0	1	?	1	1	?	?	0	1	1	2	4	r
Patrocloides montanus	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	?	2	1	0	1	0	0	0	2	0	0	0	?	2	?	2	0	0	0	2	2	?	?	0	?	0	1	?	1	1	?	?	0	1	1	2	4	r
Plagiotrypes concinnus	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Dilopharius otomitus	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	3	?	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Cyclolabus impressus	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	?	1	0	0	1	0	0	0	2	0	0	0	?	2	?	2	0	0	0	2	2	?	?	0	0	0	1	?	1	1	0	0	0	1	1	?	4	6
Linycus exhortator	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	?	1	0	0	1	0	0	0	2	0	0	0	?	2	?	2	0	0	0	2	2	?	?	0	0	0	1	?	1	1	?	?	0	?	1	2	4	?
Grotea anguina	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	?	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0
Labium sp.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	?	0	0	0	0	0	0	0	2	0	0	0	0	0	4	0	0	0	1	2	0	0	0	0	0	0	0	1	0	0	?	?	?	?	0	1	5	?
Apechoneura sp.	0	2	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	1	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	0	?
Labena grallator	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	1	0	0	0	?	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	3	0	1
Poecilocryptus nigromaculatus	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	1	0	0	0	?	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	?	1	0	?	?	?	0	0	0	1	?
Lycorina glaucomata	0	0	2	?	0	1	0	0	0	0	0	0	0	0	2	0	2	0	0	3	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	1	1	0	1	2	2	4	6
Astiphromma sp. nov.	0	1	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	1	0	2	0	0	1	0	?	0	0	0	1	0	0	1	2	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	?	1	1	?	?	0	1	1	1	9	v
Chineater masneri	0	1	2	?	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	2	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Cidaphus paniscoides	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	2	0	0	1	0	?	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	?	?	0	1	1	1	9	v
Lepidura collaris	1	1	0	1	0	1	0	0	0	1	1	0	0	0	1	0	1	1	0	2	0	0	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Mesochorus sp.	0	1	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	2	0	0	1	0	1	0	0	0	1	0	0	1	2	0	0	2	0	2	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	9	5
Exochus semirufus	0	0	2	?	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	2	0	0	0	0	?	2	1	0	1	0	0	0	2	0	0	0	0	2	?	1	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	2	k	6
Metopius pollinctorius	0	0	2	?	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	2	1	0	0	0	1	2	1	0	1	0	0	0	2	0	0	0	0	2	?	1	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	2	4	6
Scolomus sp.	0	1	2	?	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0	2	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?

Taxon	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141
Seticornuta terminalis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	?	2	1	0	1	0	0	0	2	0	0	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	1	?	?	0	1	1	2	2	u
Microleptes sp.	1	1	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	1	0	0	1	0	0	0	?	0	0	0	1	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	?	1	?	?	?	?	1	4	?	?
Neorhacodes enslini	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	?	0	0	?	?	0	0	0	1	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	?	?	?	?	1	1	?	?
Chriodes sp.	1	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Nonnus sp.	1	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	2	1	0	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Enicospilus flavostigma	1	2	0	1	0	1	0	0	0	1	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	0	1	0	1	0	0	0	2	0	0	0	1	0	0	0	0	1	0	0	1	1	0	2	1	0	0	1	1	1	0	0	0	1	1	2	4	q
Hellwigia obscura	1	2	0	0	0	1	0	0	0	1	1	0	0	0	0	0	1	1	0	1	1	1	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	2	?	?
Ophion sp.	1	2	0	0	0	1	0	0	0	1	1	0	0	0	0	0	1	1	0	1	1	0	0	0	1	0	1	0	1	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	1	1	0	2	1	0	0	1	1	1	0	0	0	1	1	h	4	q
Skiapus sp.	1	2	2	?	0	1	0	0	0	0	1	0	0	0	1	0	1	1	0	0	1	0	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Thyreodon sp.	1	2	0	1	0	1	0	0	1	1	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	1	1	0	2	1	0	0	1	1	1	0	0	0	1	1	2	n	5
Megastylus sp. nov.	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0	1	?	0	0	0	1	4	?	?	4	?	0	1	0	0	3	2	?	0	0	0	0	0	0	0	0	0	0	1	1	0	?	?	0	1	1	4	f	x
Orthocentrus sp.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1	0	0	1	?	0	0	0	1	1	0	1	2	0	0	2	0	0	3	2	?	0	0	?	1	0	?	0	?	0	0	?	0	0	?	?	?	?	1	4	?	?
Proclitus speciosus	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	2	1	0	1	1	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	4	?	?
Orthopelma mediator	1	1	2	?	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	?	0	0	0	1	0	1	0	2	0	0	0	?	2	?	2	?	0	0	?	2	0	0	0	0	0	0	1	?	0	?	?	0	?	1	1	1	?
Oxytorus albopleuralis	1	1	0	0	0	1	0	0	1	1	1	0	0	0	0	0	1	1	0	1	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Pedunculus sp. nov.	1	2	0	0	0	1	0	1	0	1	1	0	0	0	0	0	1	1	0	3	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Perithous divinator	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2
Acrotaphus wiltii	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	?	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	?	1	0	5	4	4
Clistopyga recurva	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	?	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	?	0	0	8	3	0
Dolichomitus irritator	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	1	?	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1
Zaglyptus pictilis	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	0	0	0	1	?	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	0	0	0	8	3	0
Pimpla annulipes	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	2	0	0	1	0	0	0	2	0	0	2	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	1	2	4	2
Theronia bicincta	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	2	4	2
Neoxorides caryae	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	1	0	1	0	0	1	?	1	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	0	0	0	e	0	1
Poemenia albipes	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	1	0	0	1	1	0	0	0	0	0	0	0	2	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	?	?	0	0	0	1	a	1
Megarhyssa greenei	0	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	2	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1
Rhyssa crevieri	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1
Rhyssella nitida	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	2	0	0	0	0	0	0	1	?	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	?	0	0	0	0	0	0	1	0	1
Brachyscleroma sp.	0	1	2	?	0	1	0	0	0	1	1	0	0	0	0	0	2	1	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	3	2	?

Taxon																					1										1										1										1										1	
	8										9										0										1										2										3										4	
	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1
Erythrodolius calamitosus	0	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	2	0	0	3	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Notostilbops sp. nov.	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	2	1	0	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Stilbops vetulus	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	?	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	1	0	1	?	?	0	1	1	2	2	7
Tatogaster nigra	1	1	2	?	0	1	0	0	1	1	1	0	0	0	0	0	1	1	0	1	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Allophrys divaricata	1	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	3	1	?
Peucobius fulvus	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	2	1	0	0	?	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Phrudus sp.	1	1	2	?	0	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	?	?	?	0	?	1	0	0	2	0	?	0	0	0	0	0	0	?	0	0	1	0	0	0	0	0	?	?	?	1	?	?	?	?	?	3	?	?
Stethantyx nearctica	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0	2	1	0	1	0	0	0	0	?	0	0	0	1	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	1	1	?	?	0	1	1	3	1	6
Tersilochus sp.	0	1	0	0	0	1	0	0	1	1	1	0	0	0	0	0	2	1	0	1	0	0	0	0	?	0	0	0	1	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	1	h	1	6
Eclytus sp.	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	1	0	0	1	0	0	0	?	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	4	6
Idiogramma longicauda	0	0	0	0	0	1	0	0	1	1	1	0	0	0	1	0	2	0	0	3	0	0	0	0	?	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	1	1	1	1	1	0	1	1	6
Zagryphus nasutus	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	?	1	?	?	?	?	?
Netelia sp.	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	1	0	1	1	0	2	4	6
Phytodietus vulgaris	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	2	0	0	3	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	1	0	2	2	6
Ctenisucs sp.	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	?	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	0	1	4	6
Cycasis rubiginosa	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	1	1	0	0	1	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	1	?	?	1	4	?
Polyblastus sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	1	1	1	0	1	4	6
Aplomerus sp.	0	0	2	?	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Odontocolon albotibiale	1	1	2	?	0	0	0	0	0	0	0	0	2	0	0	0	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	3	0	1
Xorides stigmapterus	0	1	2	?	0	1	0	0	0	0	0	0	2	0	0	0	2	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	3	0	1



a. pleurostoma
b. hypostoma
c. hypostomal spur
d. stipital sclerite
e. labial sclerite
f. labral sclerite
g. salivary orifice
h. labial palpus
i. maxillary palpus
j. cardo
k. inferior mandibular processes
l. superior mandibular process
m. mandible
n. antenna (with central papillus present)
o. parietal band (= ocular line)
p. maxillary apex
q. anterior tentorial pit
r. epistoma

Figure 6. Cephalic sclerites of final larval instar, *Xorides* sp. Scale bars: 1mm.

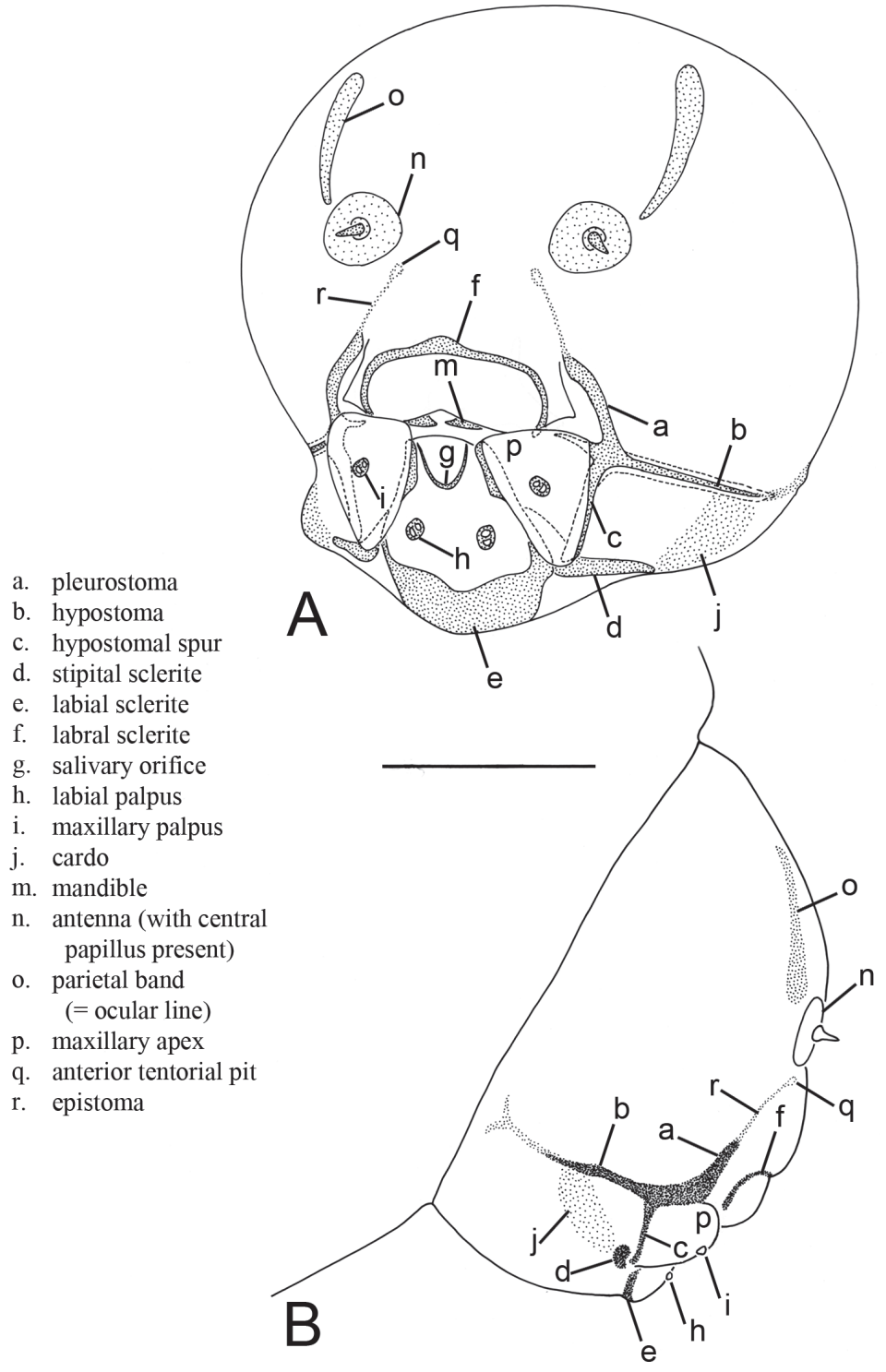
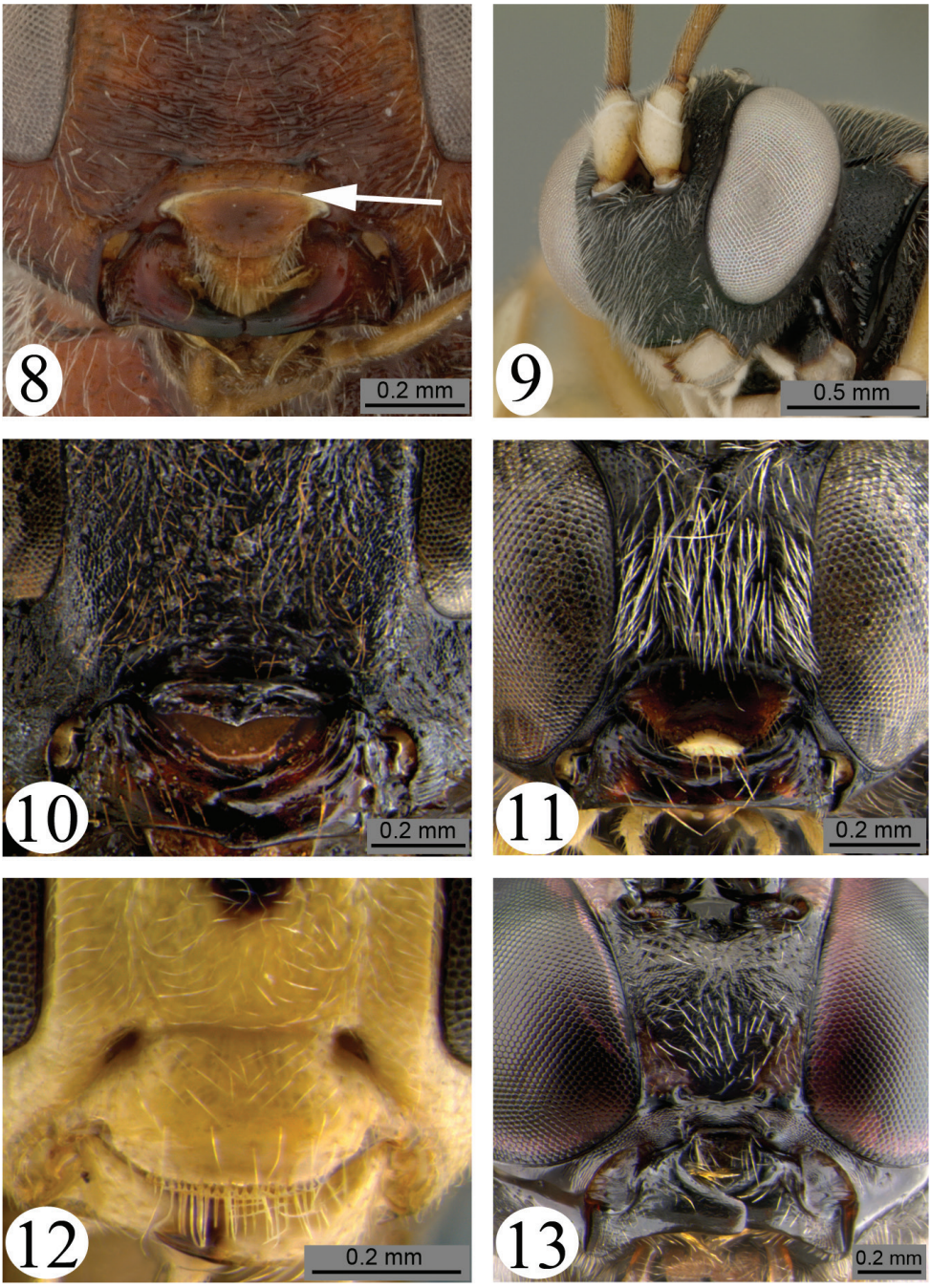
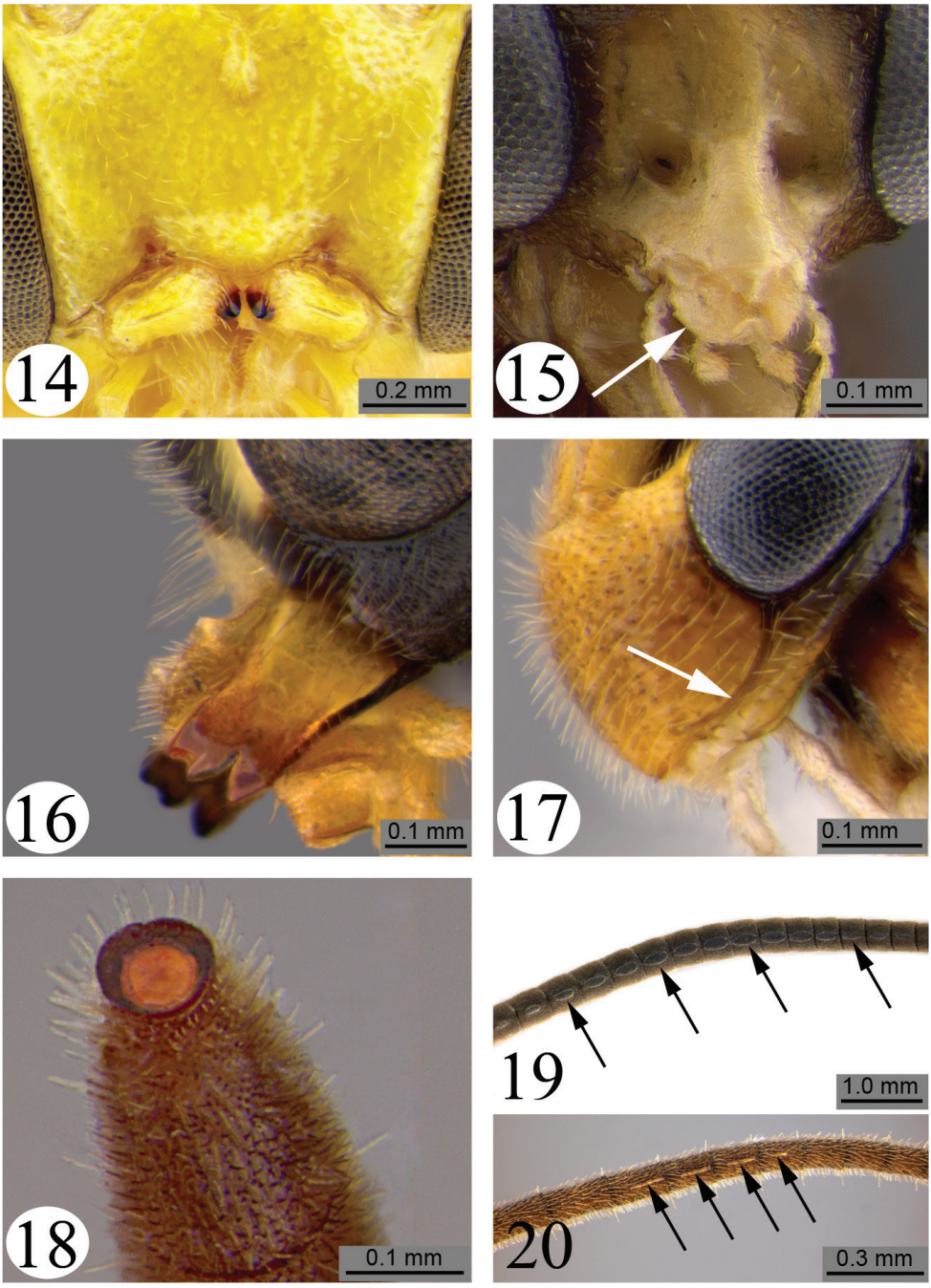


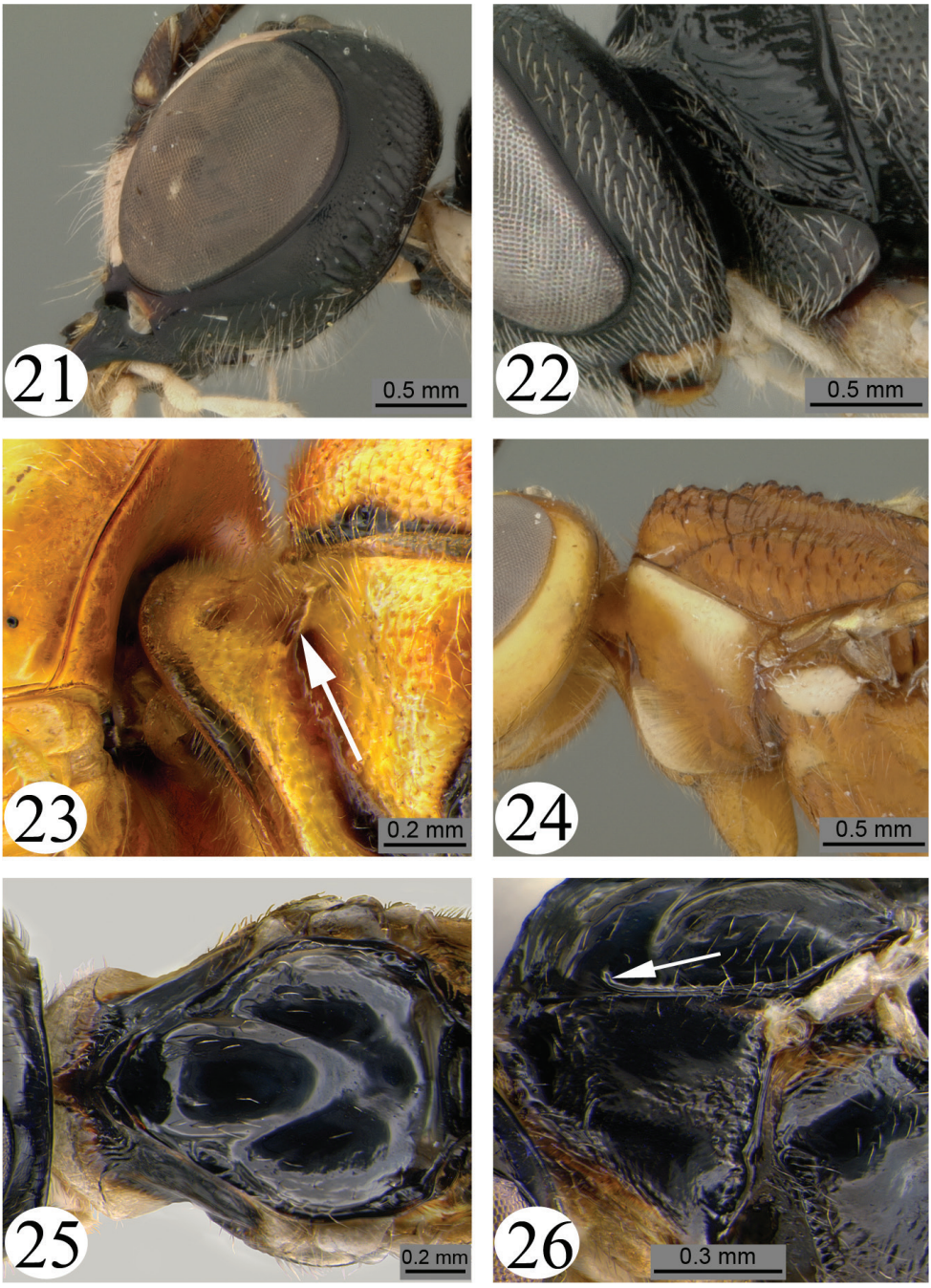
Figure 7. Final larval instar, anterior of whole larva, *Xorides* sp. **A** anterolateral view **B** lateral view.



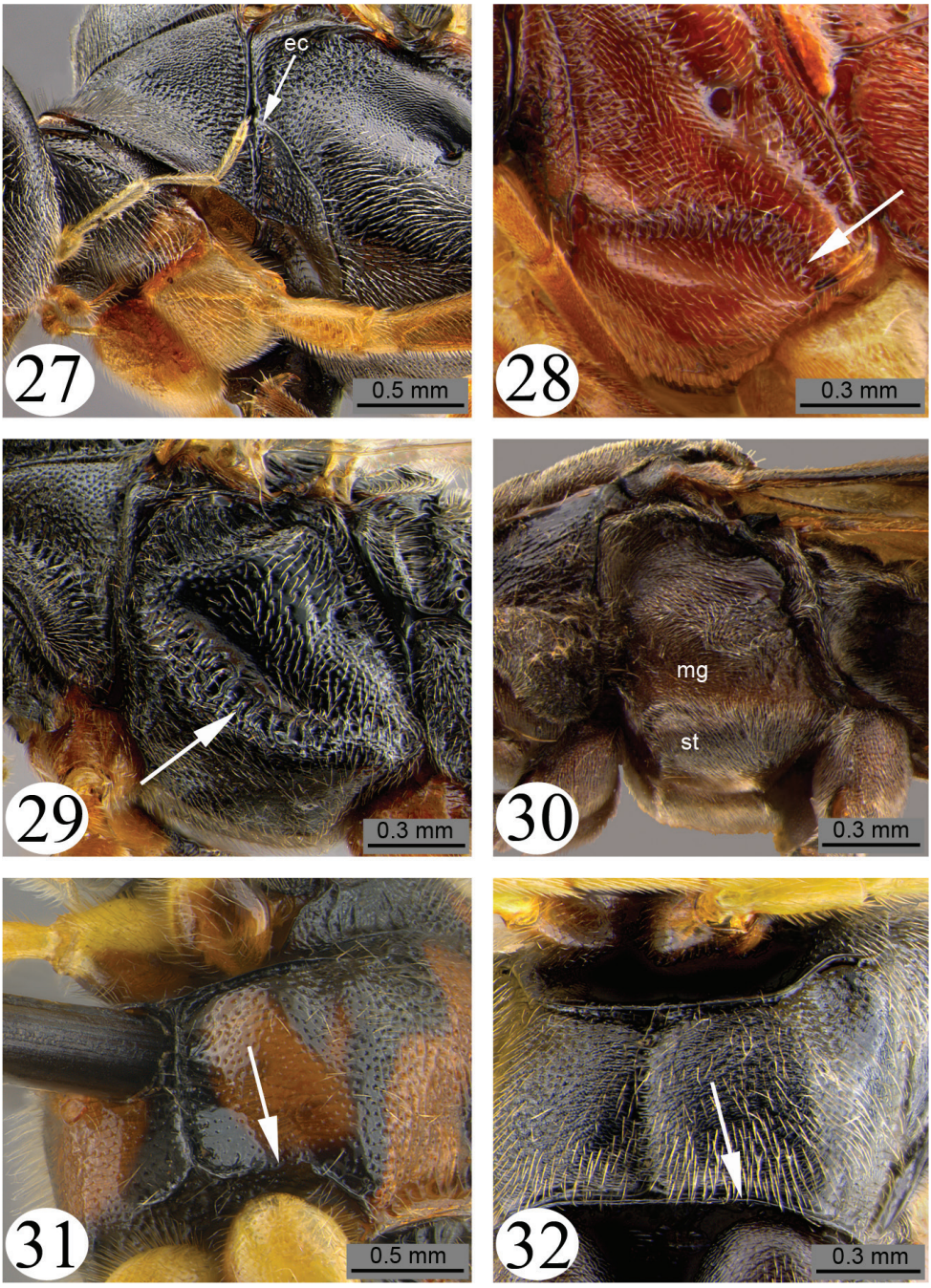
Figures 8–13. Head. **8** *Xorides stigmapterus*, anterior view. Arrow indicates division of clypeus into basal and apical parts **9** *Hyposoter* sp., anterolateral view **10–13** Anterior view **10** *Echthrus reluctator* **11** *Dolichomitus irritator* **12** *Hercus fontinalis* **13** *Neoxorides caryae*.



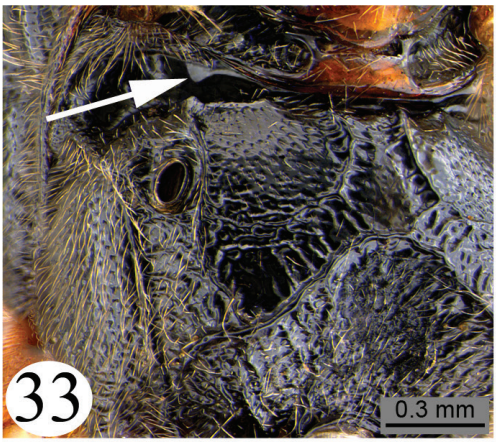
Figures 14–20. 14–15 Head, anterior view **14** *Skiapus* sp **15** *Hybrizon rileyi* Arrow indicates reduced mandible **16–17** Head, lateral view **16** *Diplazon laetatorius* **17** *Orthocentrus* sp. Arrow indicates subocular groove **18–20** Antennae **18** *Labena grallator*, apical flagellomere **19–20** Flagellum, lateral view, arrows indicate longitudinal tyloids **19** *Protichneumon grandis* **20** *Lymeon orbus* (Say).

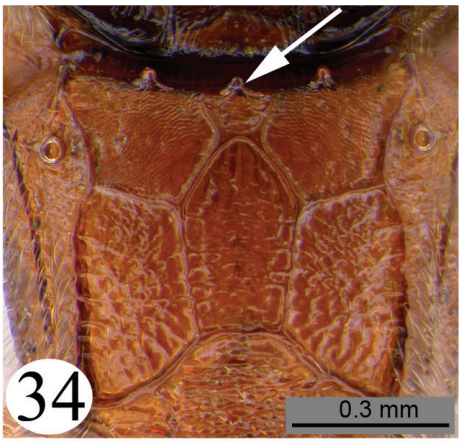


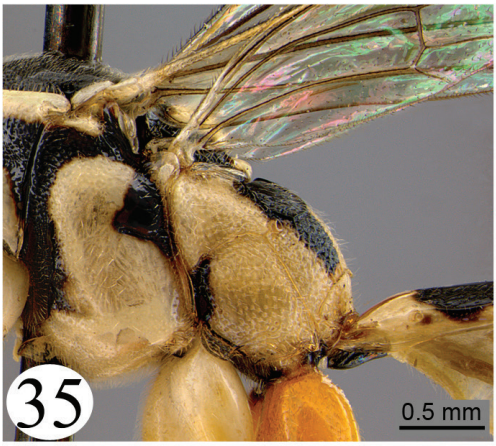
Figures 21–26. 21 *Podoschistus vittifrons* (Cresson), head, lateral view **22** *Venturia sokanakiakorum* (Viereck), head and mesosoma, lateral view, showing ventral lobe of propleuron **23** *Labena grallator*, pronotum, lateral view. Arrow indicates epomia **24** *Rhyssella perfulva* Porter, head and mesosoma, lateral view **25–26** *Diacritus incompletus* **25** Mesosoma, dorsal view **26** Mesosoma, lateral view. Arrow indicates notaular crest.

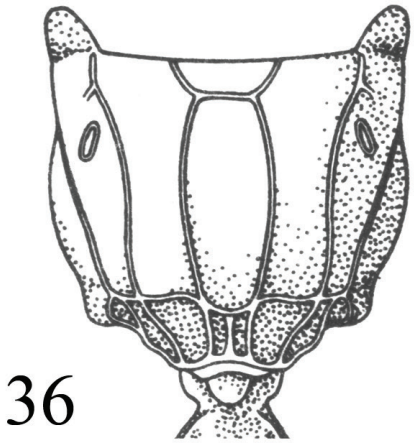


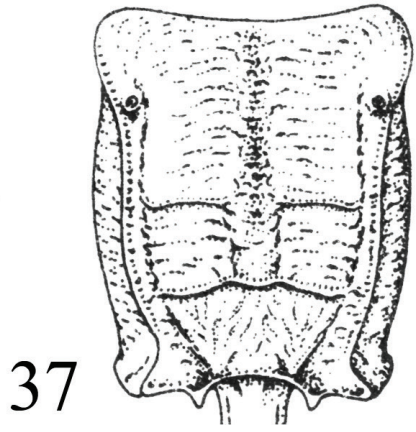
Figures 27–32. 27–30 Mesosoma, lateral view **27** *Rhimphoctona macrocephala. ec* = epicnemial carina **28** *Diapetimorpha brunnea* Townes. Arrow indicates sternaulus curving ventrally anterior to posterolateral corner **29** *Stethantyx nearctica.* Arrow indicates foveate groove **30** *Agriotypus armatus. mg* = mesopleural groove, *st* = sternaulus **31–32** Mesosternum, ventral view **31** *Therion longipes* (Provancher). Arrow indicates incomplete posterior transverse carina **32** *Dusona egregia.* Arrow indicates complete posterior transverse carina.

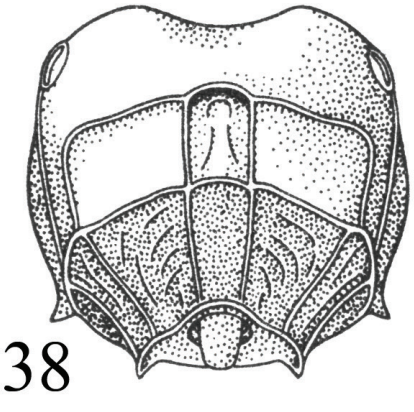




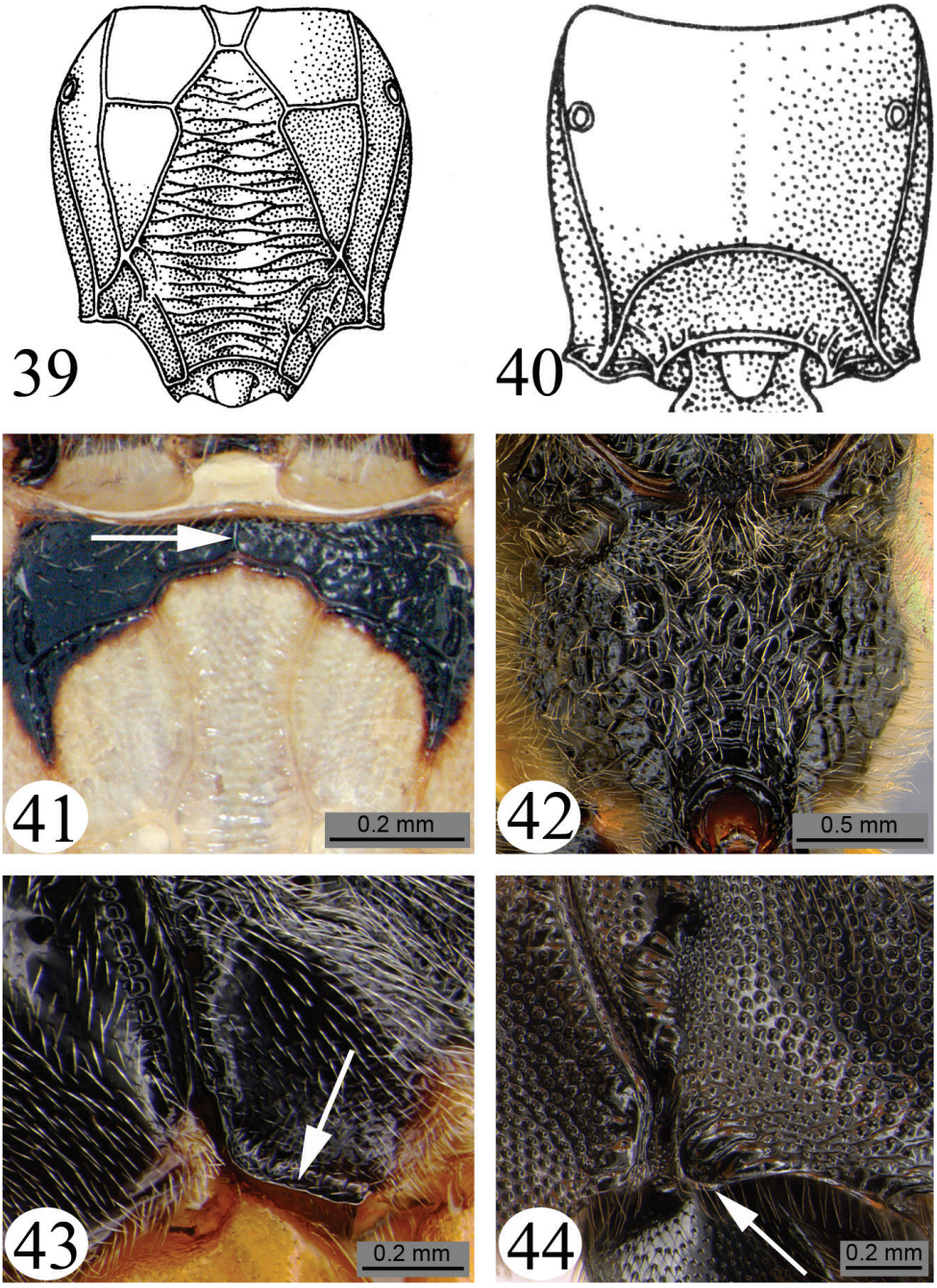




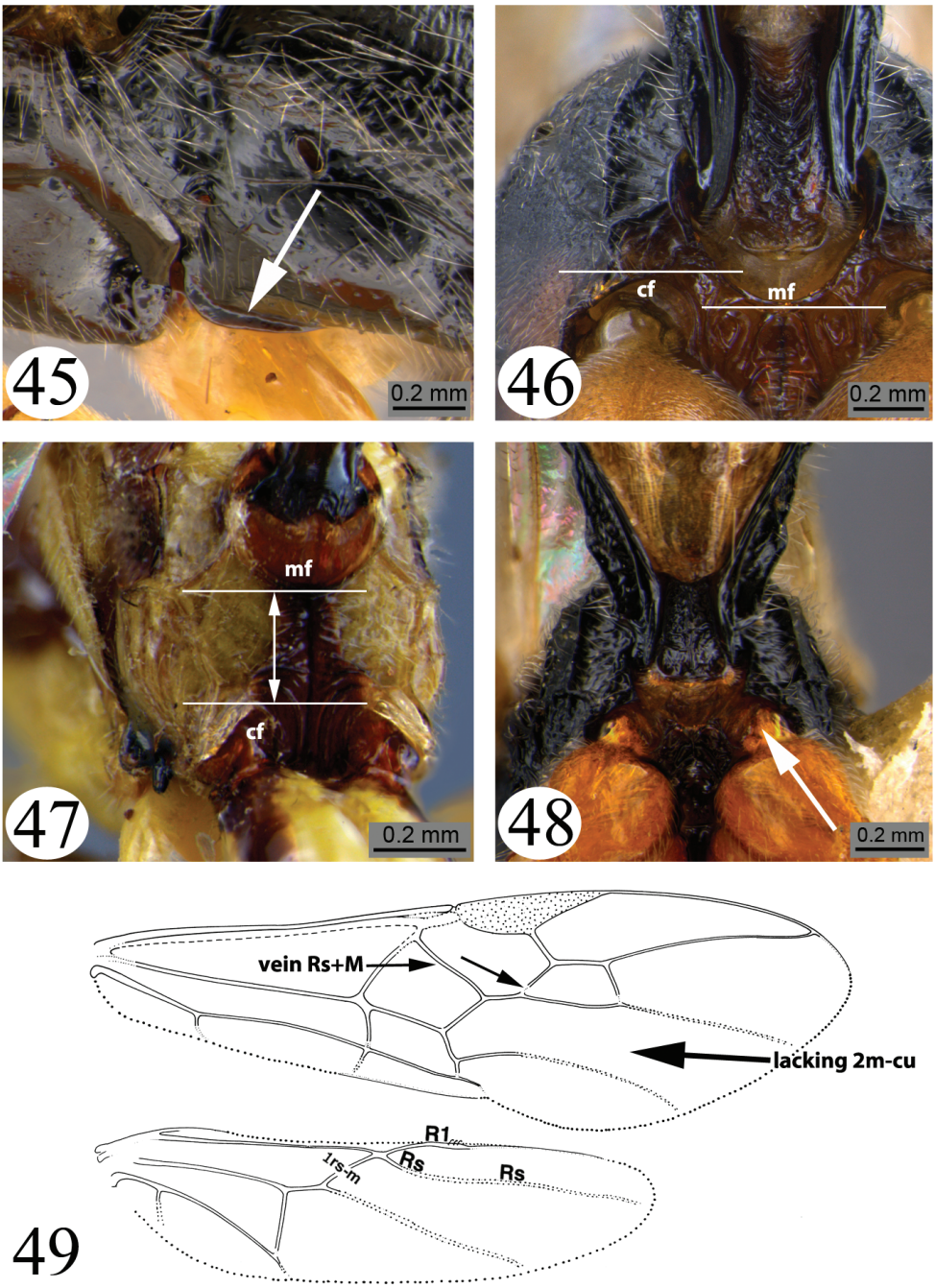




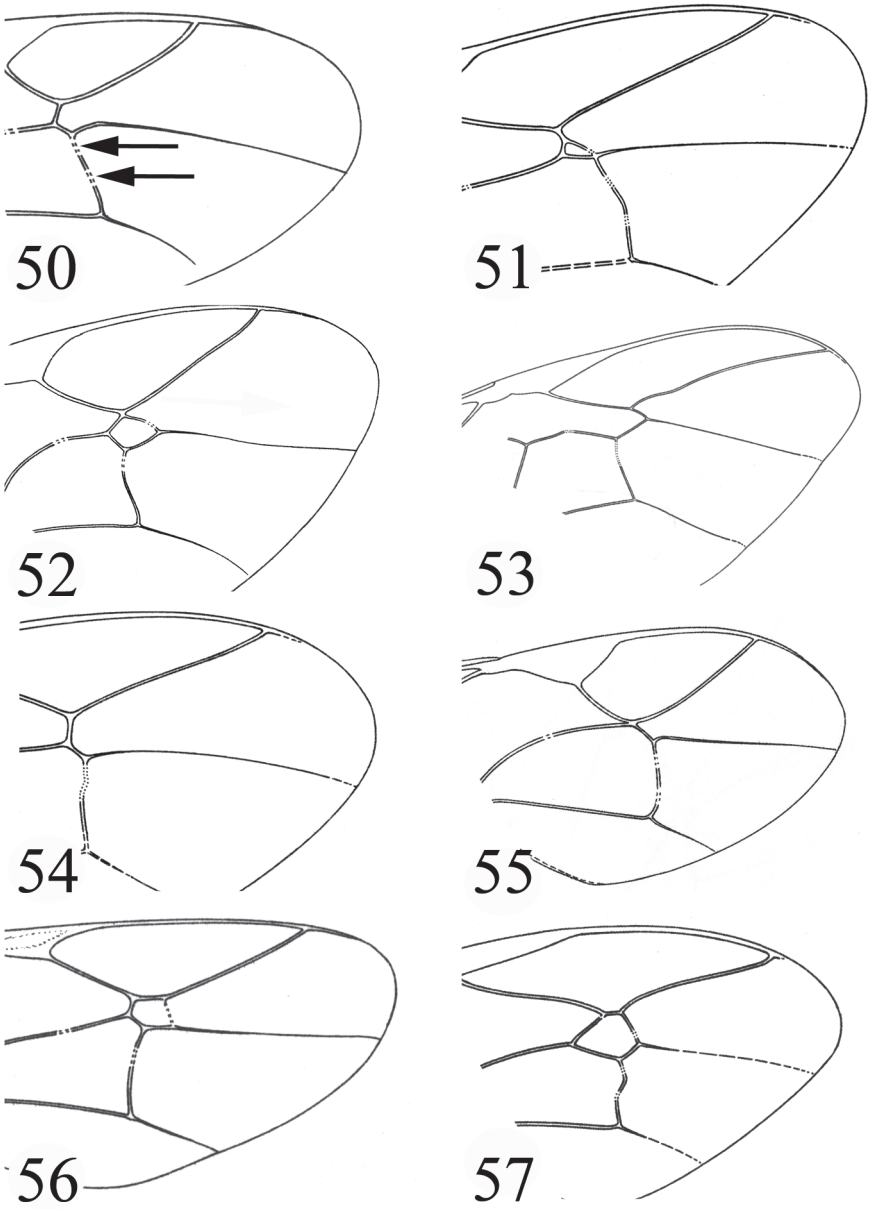
Figures 33–38. Propodeum. **33** *Polytribax contiguous* (Cresson), dorsolateral view. Arrow indicates posterolateral triangle of metanotum **34** *Centeterus euryptychiae*, dorsal view. Arrow indicates median tubercle at base of propodeum **35** *Sphelodon phoxopteridis*, lateral view **36–38** Dorsal view (re-drawn after Townes 1969, 1970a, 1971) **36** *Aplomerus tibialis* (Provancher) **37** *Ateleute tsiriria* (Seyrig) **38** *Ophion flavidus* Brullé.



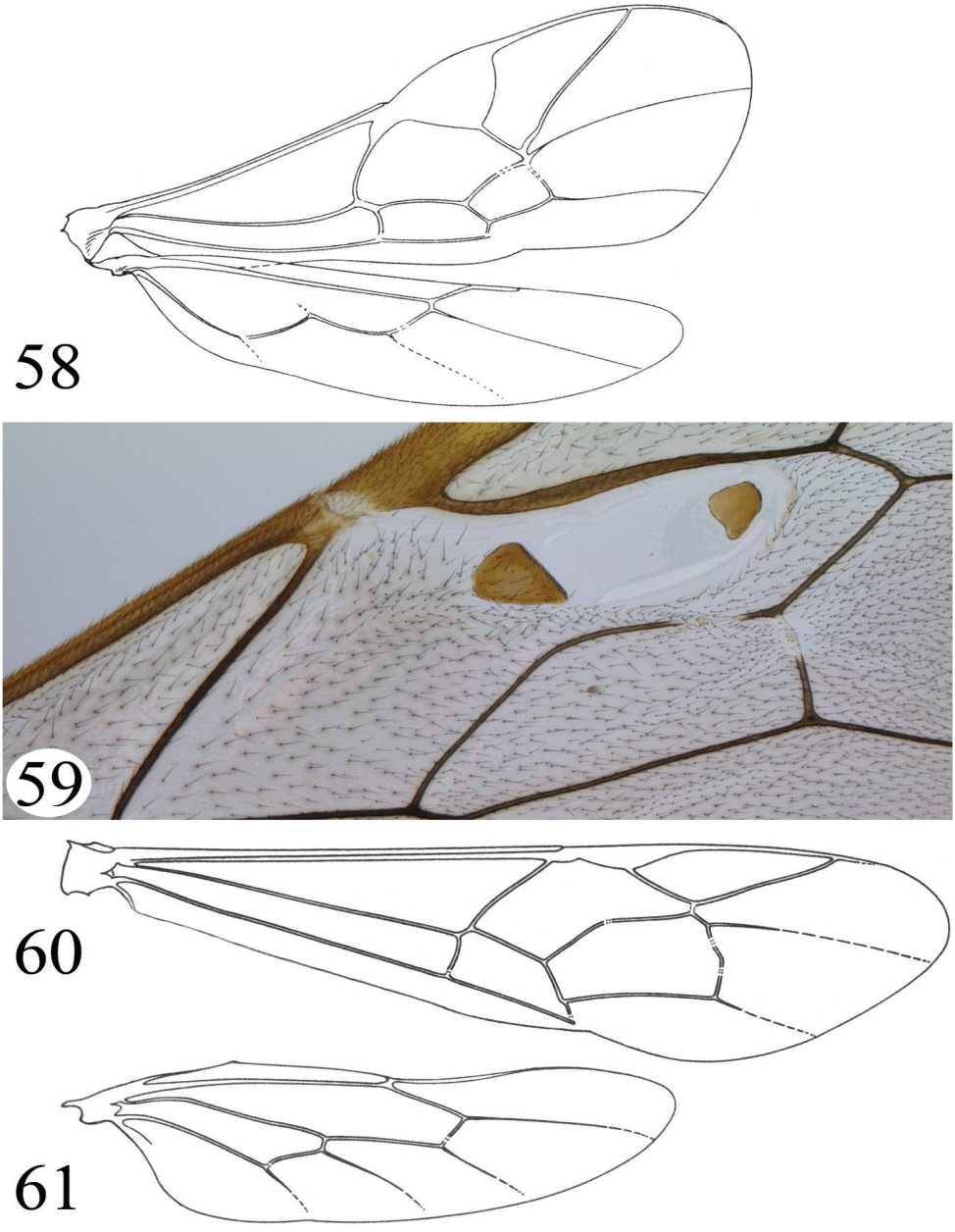
Figures 39–44. 39–42 Propodeum, dorsal view **39** *Pyracmon hyalinus* (re-drawn after Townes 1970b) **40** *Lissonota lineolaris* (Gmelin) (re-drawn after Townes 1970b) **41** *Brachycyrtus wardae.* Arrow indicates fused anterior abscissa of medial longitudinal carina **42** *Therion longipes* (Provancher) **43–44** Metapleuron, lateral view **43** *Pimpla aequalis* Provancher. Arrow indicates submetapleural carina **44** *Exetastes fornicator* (Fabricius). Arrow indicates anterior lobe of submetapleural carina.



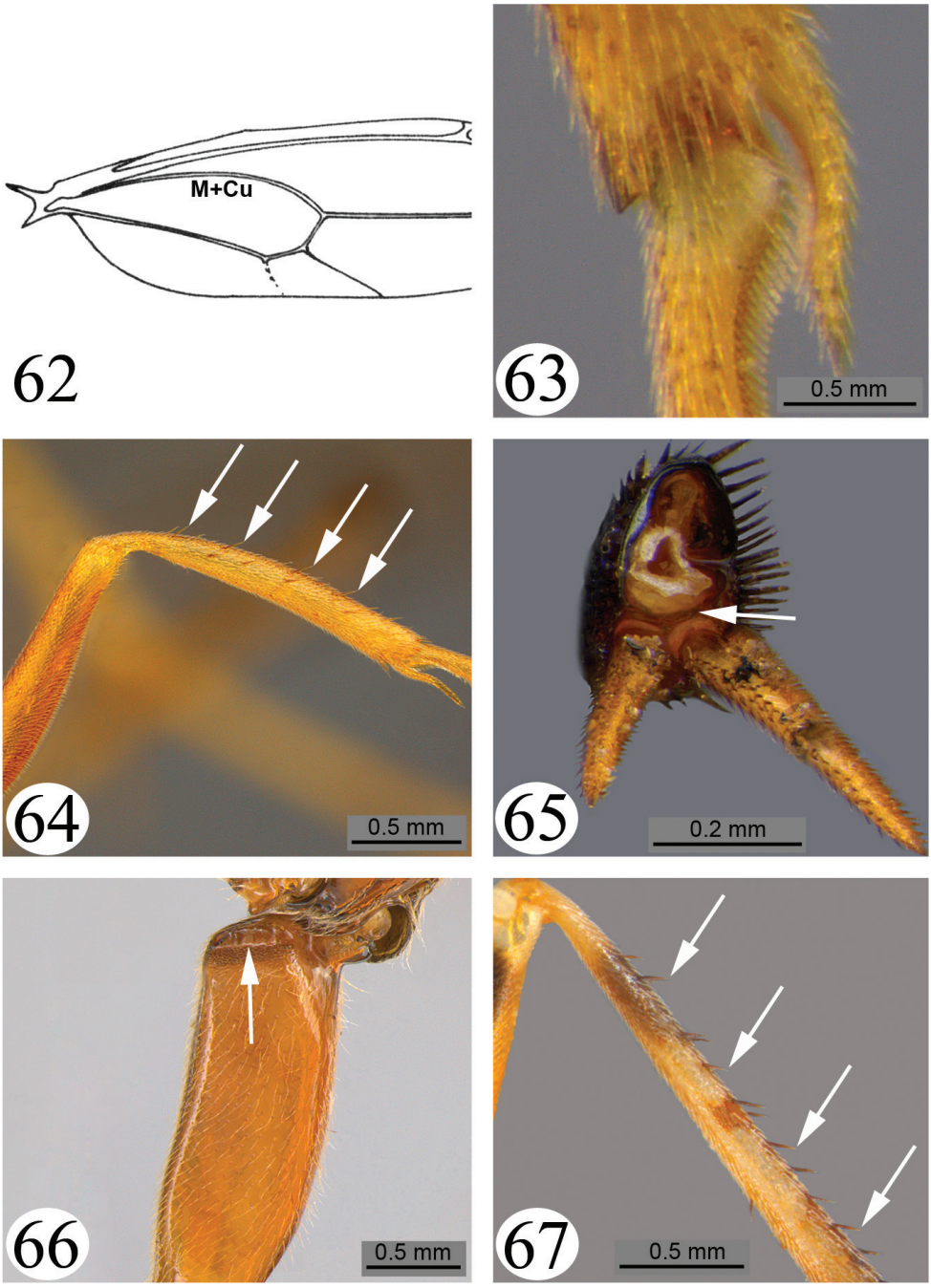
Figures 45–49. 45 *Dolichomitus irritator*, mesopleuron and metapleuron, lateral view. Arrow indicates submetapleural carina **46–48** Mesosoma and metasoma, ventroposterior view. Line above *cf* = dorsal edge of coxal foramen, line below *mf* = ventral edge of metasomal foramen **46** *Lissonota scutellaris* **47** *Apechoneura* sp. **48** *Polyblastus pedalis* (Cresson). Arrow in 48 indicates unsclerotized region joining metasomal and coxal foramina **49** Wings, *Helcon* sp. (Braconidae) (modified from Goulet and Huber 1993). Unlabeled arrow indicates antero-medial flexion line.



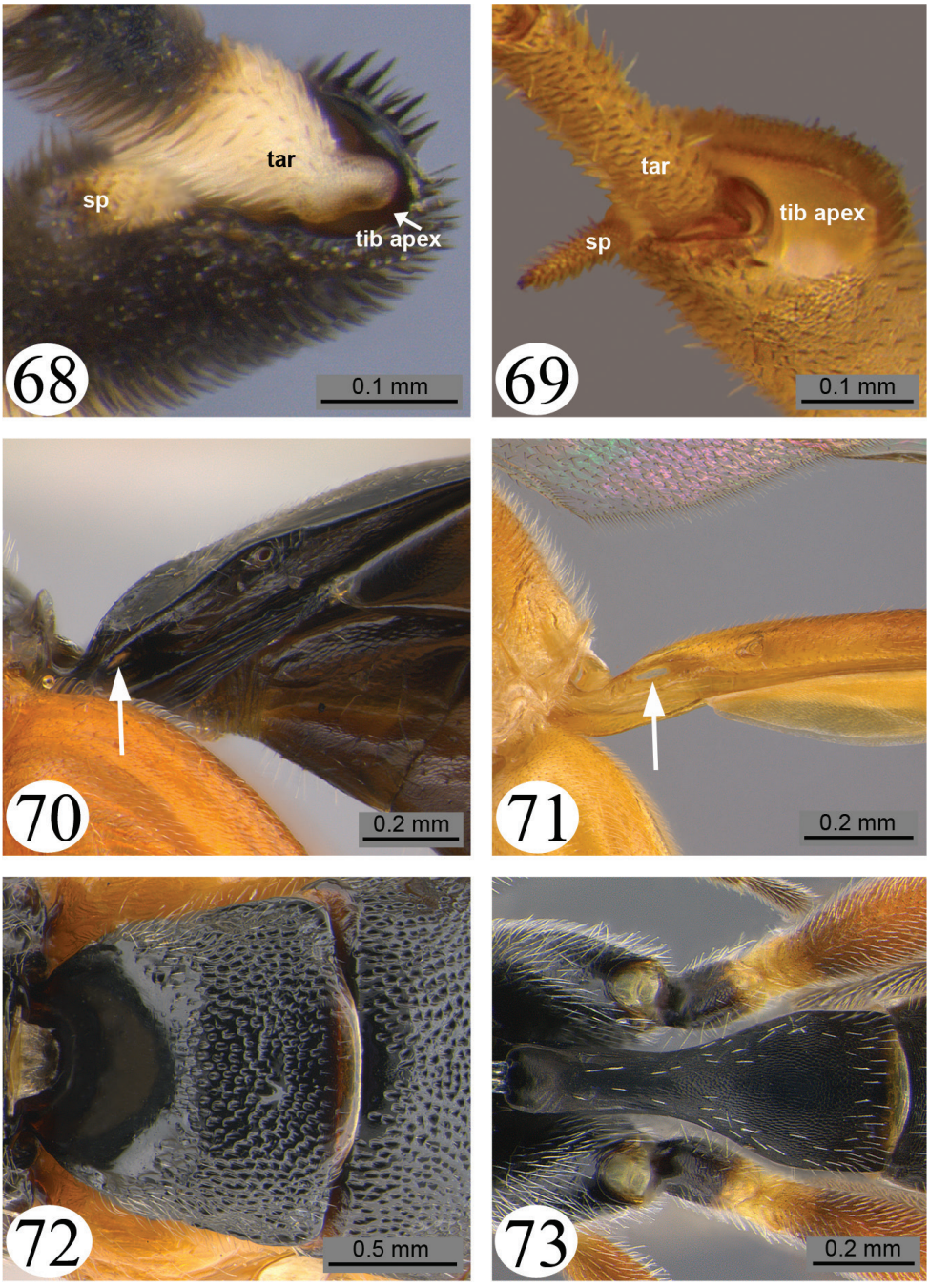
Figures 50–57. Fore wing (modified from Townes 1969, 1970a, 1970b, 1971) **50** *Mastrus acitulatus*. Arrows indicate bullae in vein *2m-cu* **51** *Phytodietus gelitorius* (Thunberg) **52** *Mesochorus* sp. **53** *Ophion flavidus* **54** *Glypta erratica* Cresson **55** *Proclitus* sp. **56** *Ateleute tsiririria* **57** *Labena grallator*.



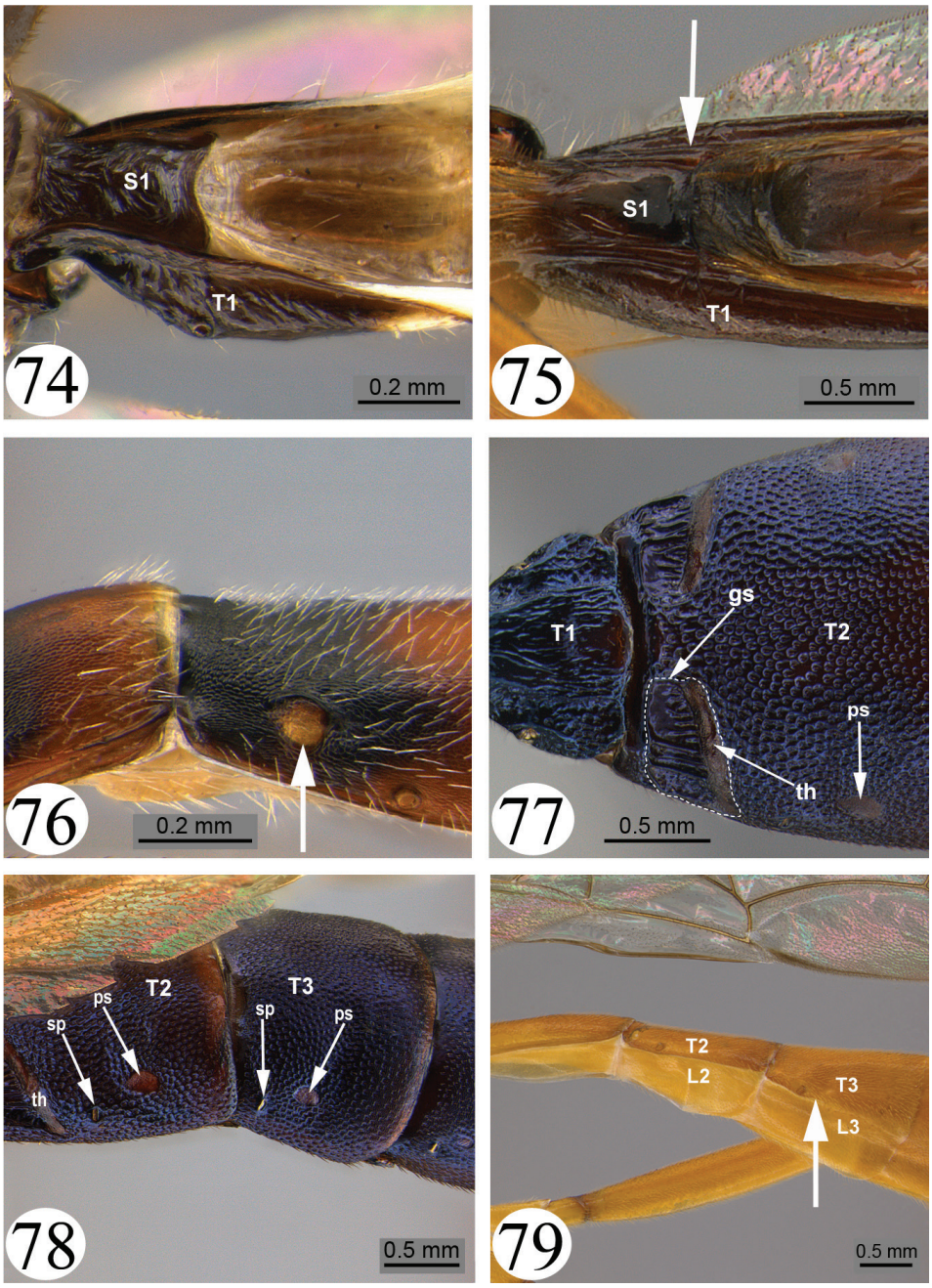
Figures 58–61. Wings. **58** *Allophrys oculata* (Ashmead), fore and hind wings **59** *Enicospilus purgatus* (Say), fore wing showing sclerites in cell *1M* + *1R1* **60** *Aplomerus tibialis*, fore wing **61** *Exetastes fornicator*, hind wing.



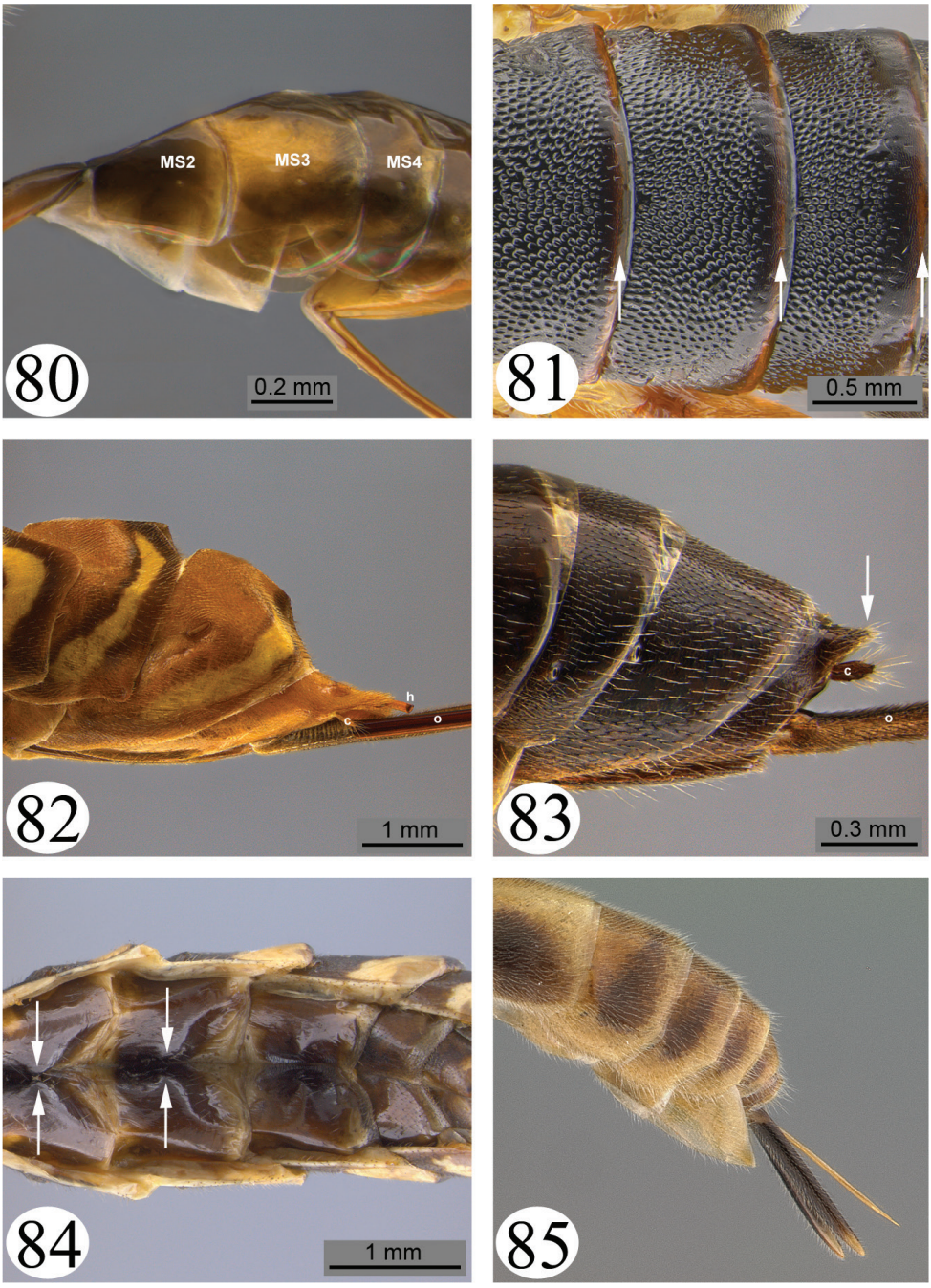
Figures 62–67. 62 *Ateleute tsiriria*, hind wing. **63–64** Fore tibia, lateral view **63** *Euryproctus sentinis* Davis (apex with distinct tooth) **64** *Phytodietus vulgaris*. Arrows indicate sparse, stout spines **65** *Eiphosoma pyralidis*, hind tibia, apical view. Arrow indicates sclerotized bridge between insertion points of spurs and basitarsus **66** *Labena grallator*, inner surface of hind coxa of female, lateral view. Arrow indicates furrow for bracing ovipositor during oviposition **67** *Phytodietus vulgaris*, lateral view. Arrows indicate sparse, stout spines.



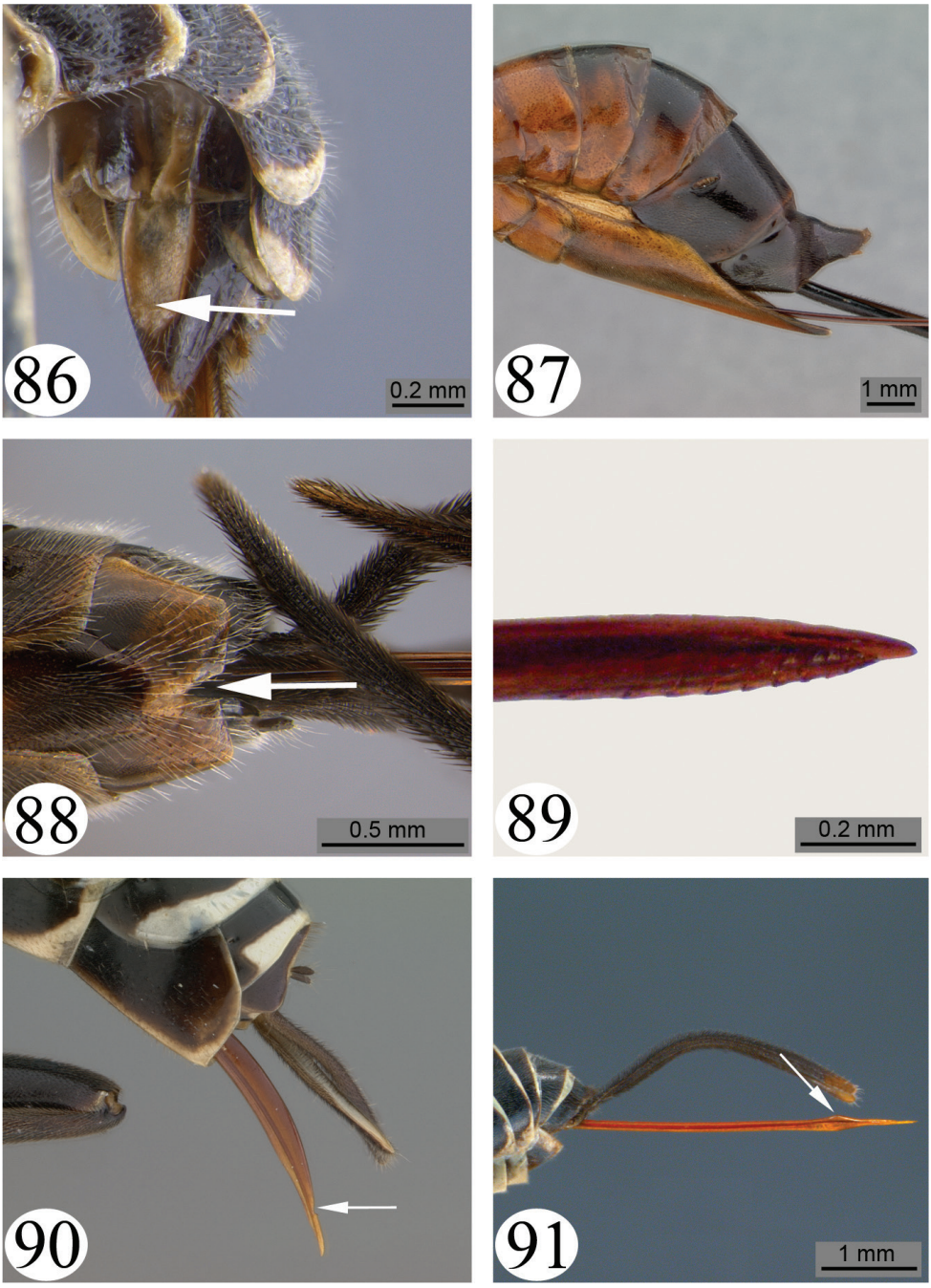
Figures 68–73. 68–69 Hind tibial apex, apical view. *sp* = spur, *tar* = tarsus, *tib apex* = tibial apex **68** *Polyblastus pedalis* (simple apex with sparse fringe of stout setae) **69** *Pedunculus* sp. (apex with apical face smooth and enlarged with thick fringe of fine setae) **70–71** Tergite 1 of metasoma, lateral view **70** *Phytodietus vulgaris* Arrow points to glymma **71** *Netelia sp.* Arrow points to deep glymma (sides of glymma separated medially by only a thin, translucent sclerite) **72–73** Tergite 1, dorsal view **72** *Pimpla aequalis* **73** *Campoletis sonorensis* (Cameron).



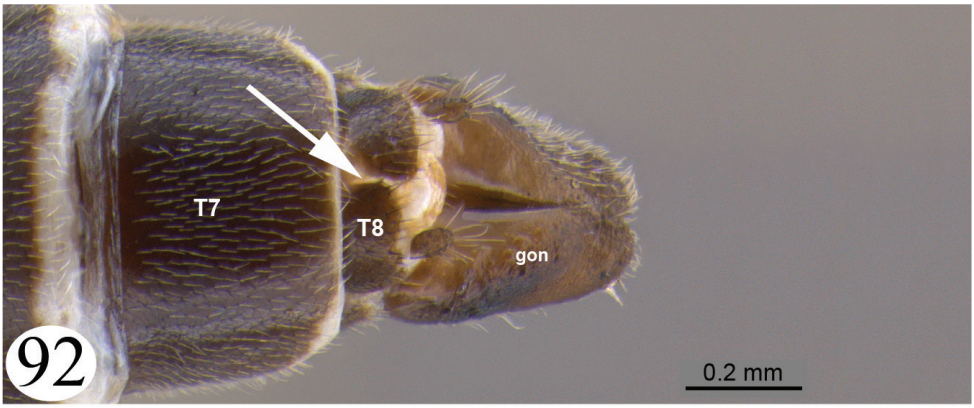
Figures 74–79. 74–75 Metasomal segment 1, ventral view. *S1* = sternite 1, *T1* = tergite 1 **74** *Rhyssa lineolata* (Kirby) **75** *Megarhyssa macrura* (Linnaeus). Arrow indicates S1 fused to T1 posteriorly **76–79** Anterior tergites of metasoma **76** Lateral view, *Olesicampe* sp. Arrow points to thyridium **77** Dorsal view, *Patrocloides montanus* (Cresson). *gs* = gastrocoelus (delineated by dotted line), *th* = thyridium (linear, posterior part of gastrocoelus), *ps* = pseudothyridium, *T2* = tergite 2 **78–79** Lateral view **78** *Patrocloides montanus. sp* = spiracle. *T3* = tergite 3 **79** *Netelia* sp. Arrow indicates crease separating tergite 3 and laterotergite 3. *L2* = laterotergite 2, *L3* = laterotergite 3.

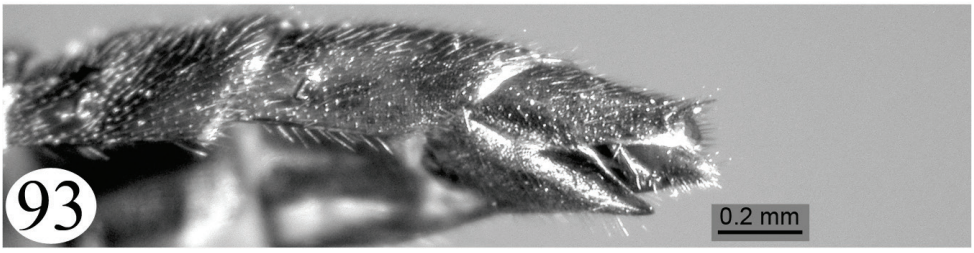


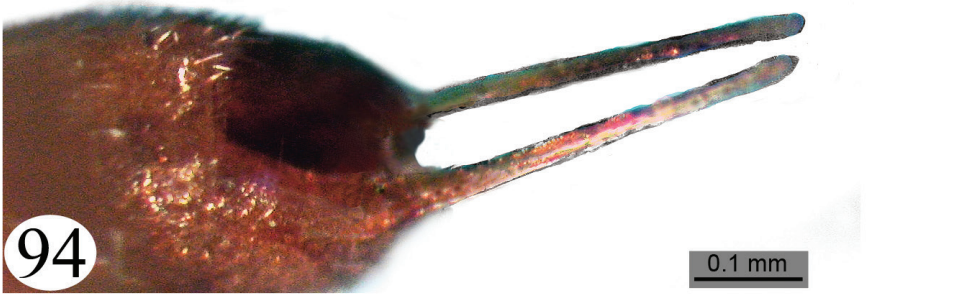
Figures 80–85. Metasoma. **80** Lateral view, *Allophrys divaricata* MS2, etc. = metasomal segment 2, etc. **81** Tergites 2 to 4, dorsal view, *Pimpla aequalis.* Arrows indicate different sculpture on posterior 0.2 of tergites **82–83** Posterior segments of female, lateral view **82** *Megarhyssa greenei*. *c* = cercus , *h* = horn, *o* = ovipositor **83** *Odontocolon albotibiale.* Arrow indicates lack of horn **84** Sternites of female, ventral view, *Rhyssa lineolata.* Arrows indicate tuberculate ovipositor guides **85** Posterior segments of female, lateral view, *Astiphromma splenium.*



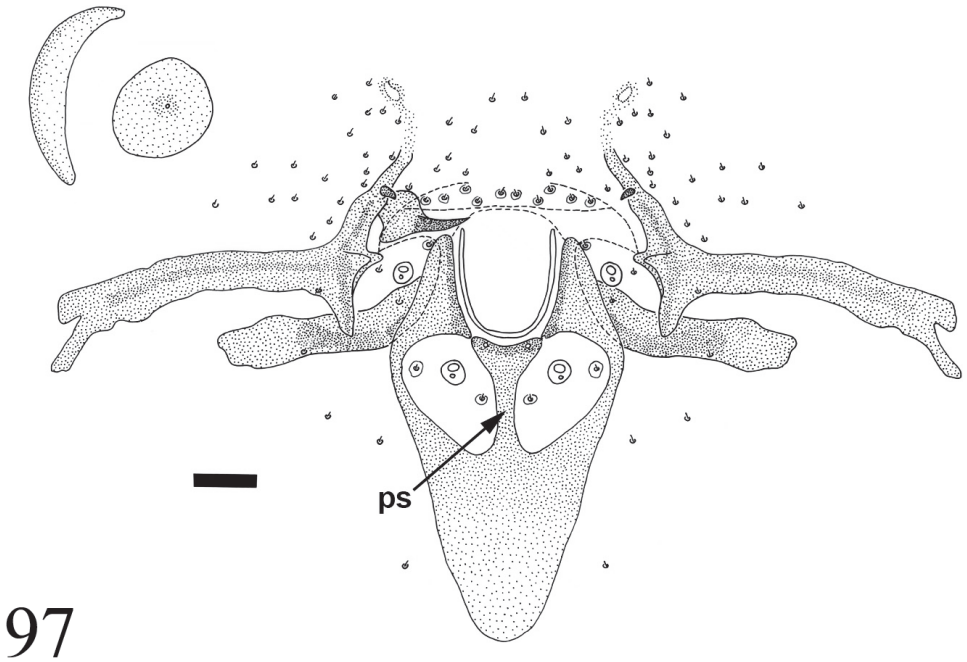
Figures 86–91. Posterior of female metasoma **86** Ventroposterior view, *Lycorina albomarginata* (Cresson). Arrow indicates medial, membranous area of hypopygium **87** Lateral view, *Coleocentrus occidentalis* Cresson **88** Ventral view, *Lissonota scutellaris*. Arrow indicates medial apical notch of hypopygium **89–91** Ovipositor, lateral view: **89** *Pimpla aquilonia* Cresson **90** *Exetastes* sp. nov. Arrow indicates dorsal, subapical notch **91** *Phytodietus burgessi* (Cresson). Arrow indicates dorsal, subapical nodus.







Figures 92–96. Posterior of male metasoma. **92** Dorsal view, *Phytodietus vulgaris.* Arrow showing longitudinal division of tergite 8. *T7* = tergite 7, *T8* = tergite 8, *gon* = gonoforceps **93** Ventrolateral view, *Pimpla* sp. **94** Ventrolateral view, *Mesochorus* sp. **95–96** Gonoforceps and aedeagus, lateral view **95** *Thyreodon* sp. **96** *Rhyssa crevieri.*



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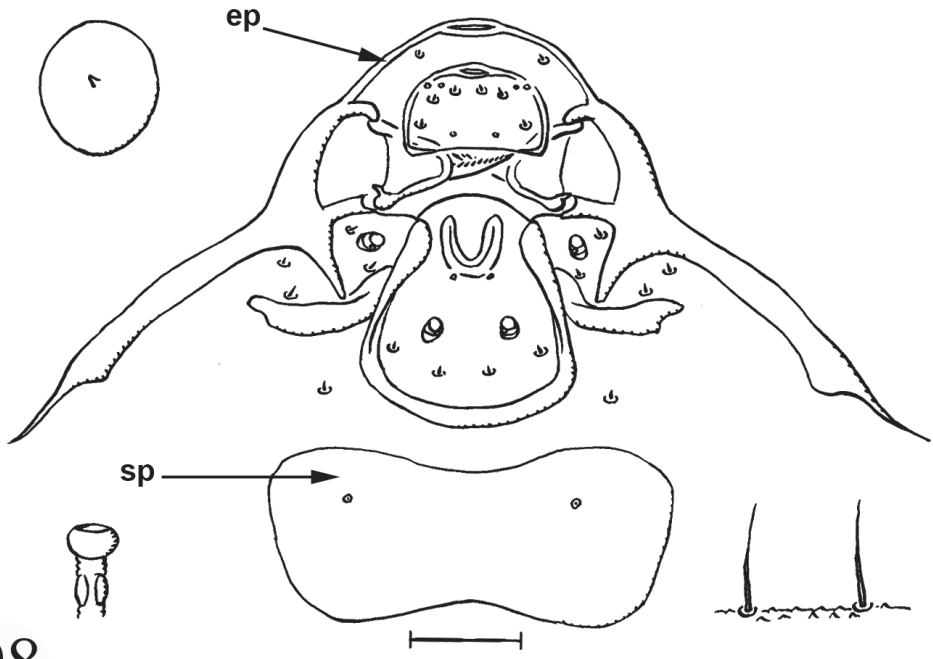
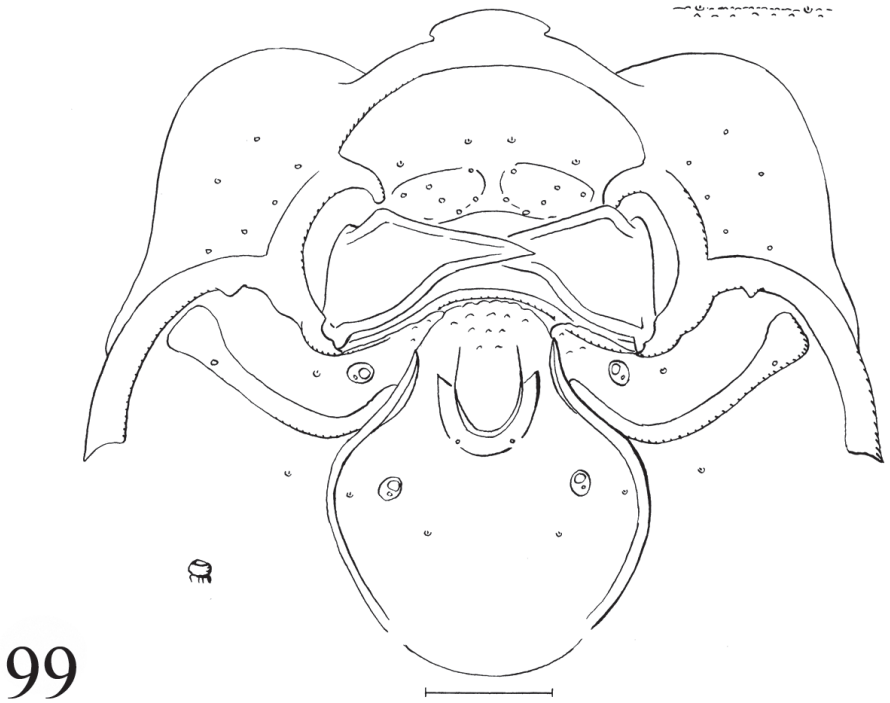


98

Figures 97–98. Cephalic sclerites of final larval instar **97** *Dusona* sp. (copied from Wahl 1990) *ps* = pre-labial sclerite **98** *Phytodietus polyzonias* Förster (copied from Short 1978) *ep* = epistomal band, *sp* = sclerotized plate ventral to labial sclerite.



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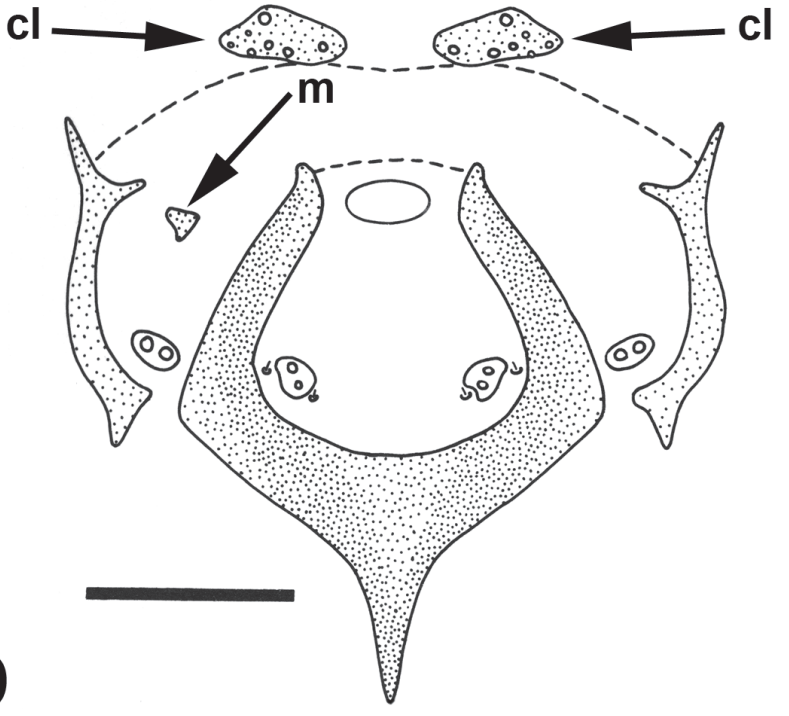
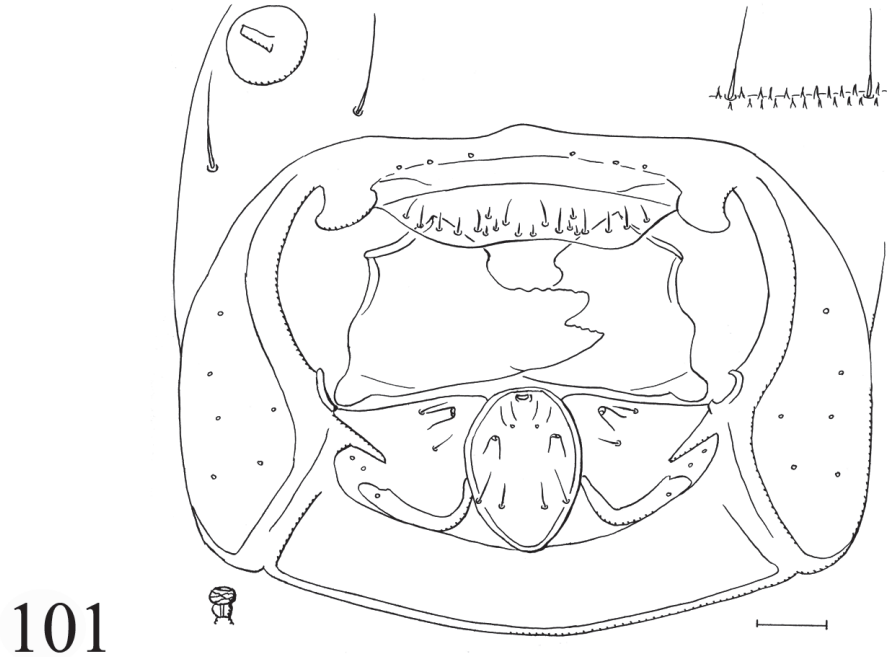
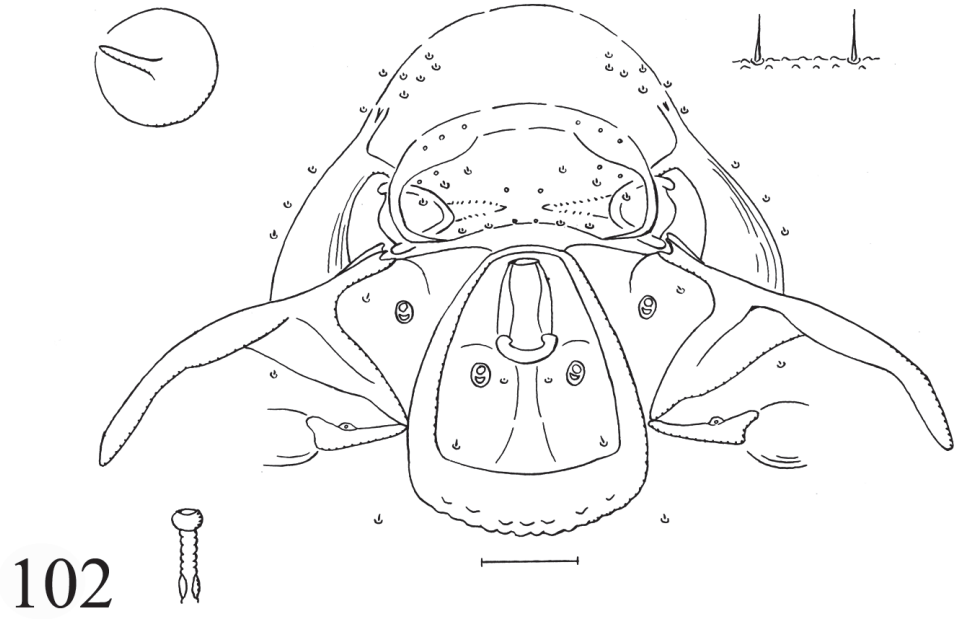


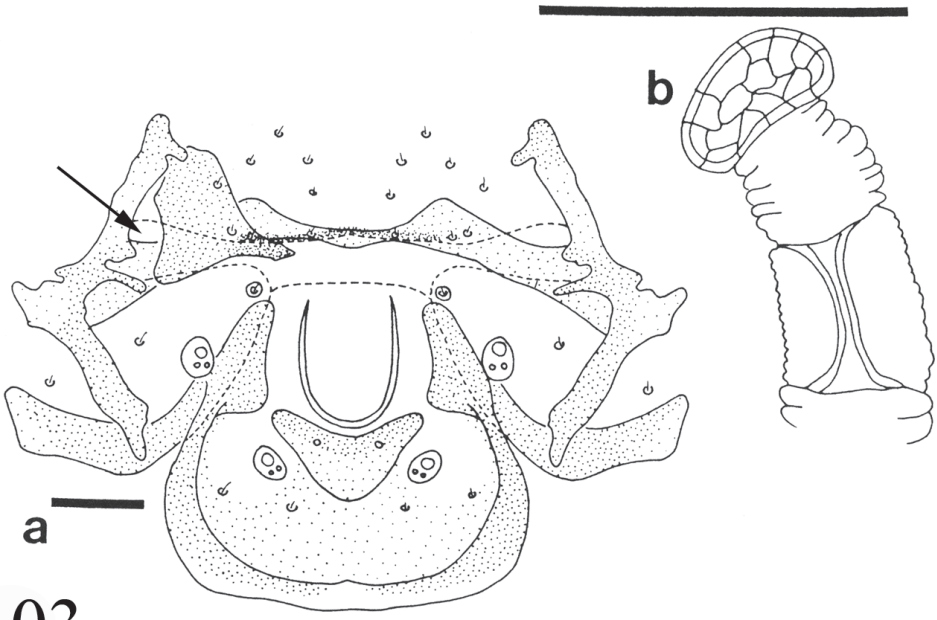
100

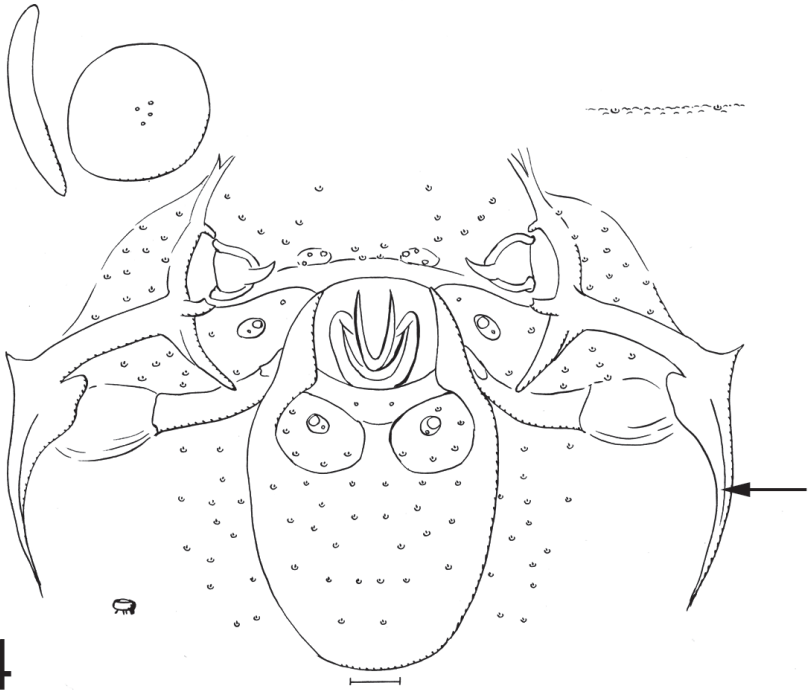
Figures 99–100. Cephalic sclerites of final larval instar **99** *Exochus albifrons* Cresson (copied from Short 1978) **100** *Diplazon laetatorius* (copied from Wahl 1990) *cl* = clypeolabral plates, m = mandible.



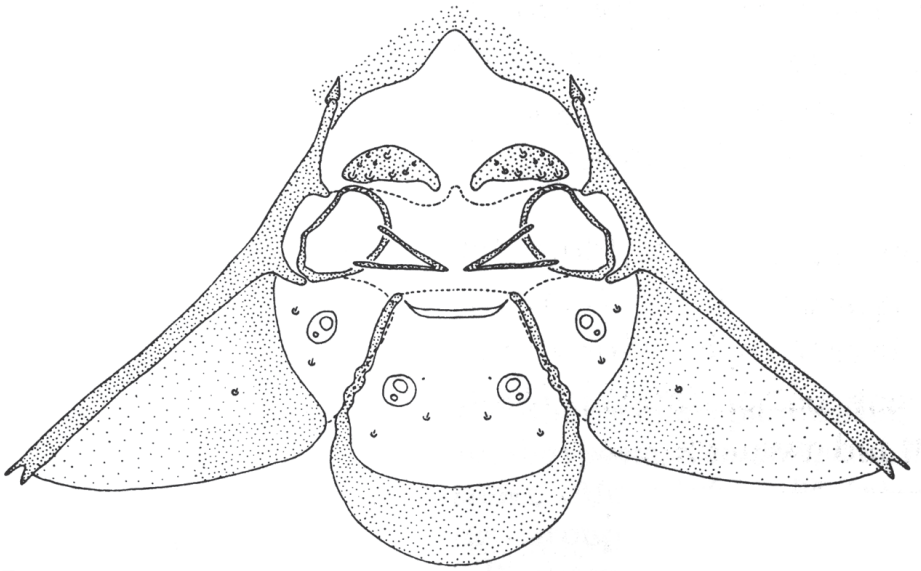


Figures 101–102. Cephalic sclerites of final larval instar **101** *Poecilocryptus nigromaculatus* (copied from Short 1978) **102** *Dolichomitus irritator* (copied from Short 1978).





Figures 103–104. Cephalic sclerites of final larval instar **103** *Lissonota occidentalis* (Cresson) (copied from Wahl 1988). Arrow points to lateral edge of lightly sclerotized band connecting posterior struts of inferior mandibular processes **104** *Enicospilus biharensis* Townes, Townes & Gupta (copied from Short 1978). Arrow points to ventral end of hypostoma.



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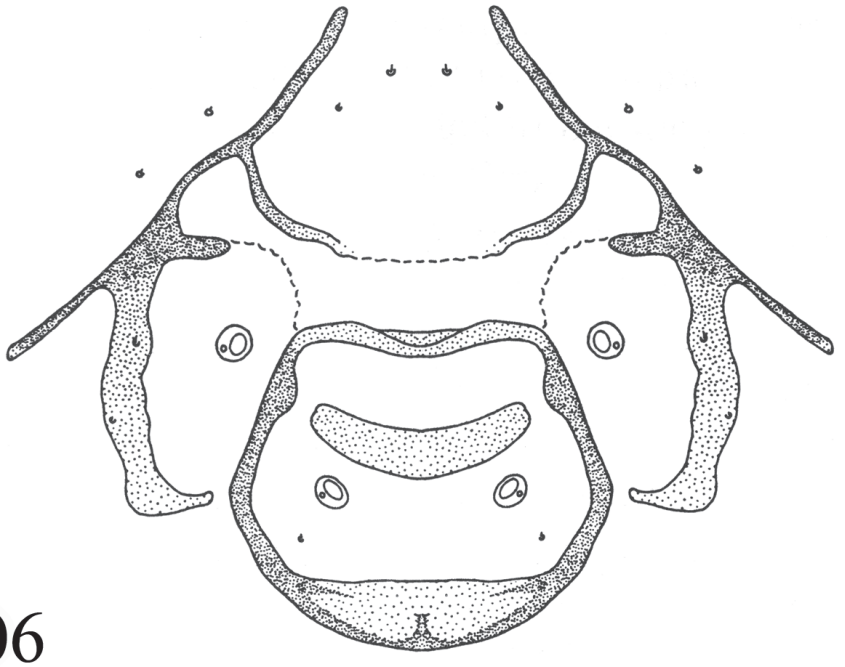

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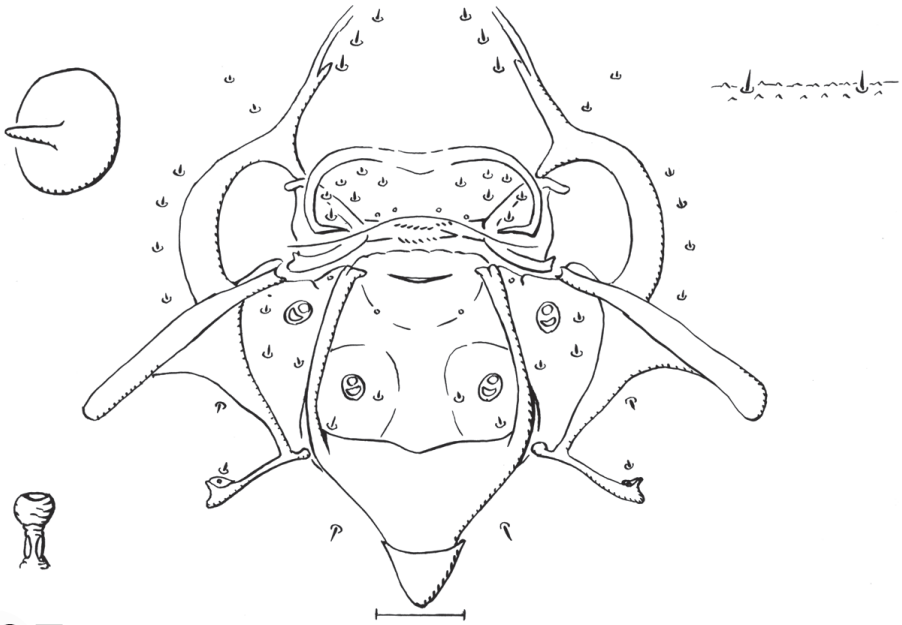
Figures 105–106. Cephalic sclerites of final larval instar **105** *Spilopteron* sp. (modified from Wahl 1990) **106** *Megastylus* sp. (modified from Wahl 1990).



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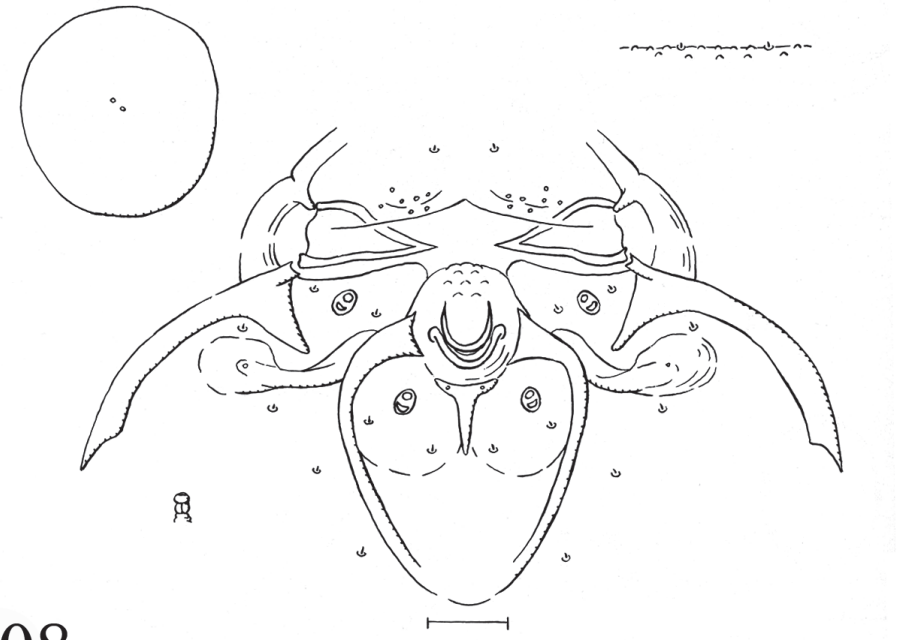

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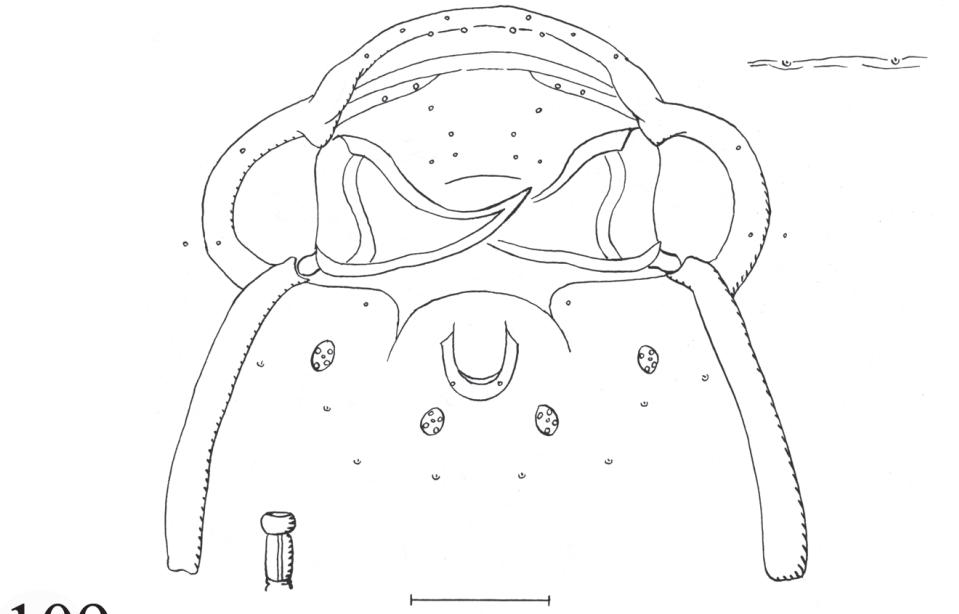
Figures 107–108. Cephalic sclerites of final larval instar **107** *Neoxorides borealis* (copied from Short 1978) **108** *Rhimphoctona aldrichi* (Davis) (copied from Short 1978).



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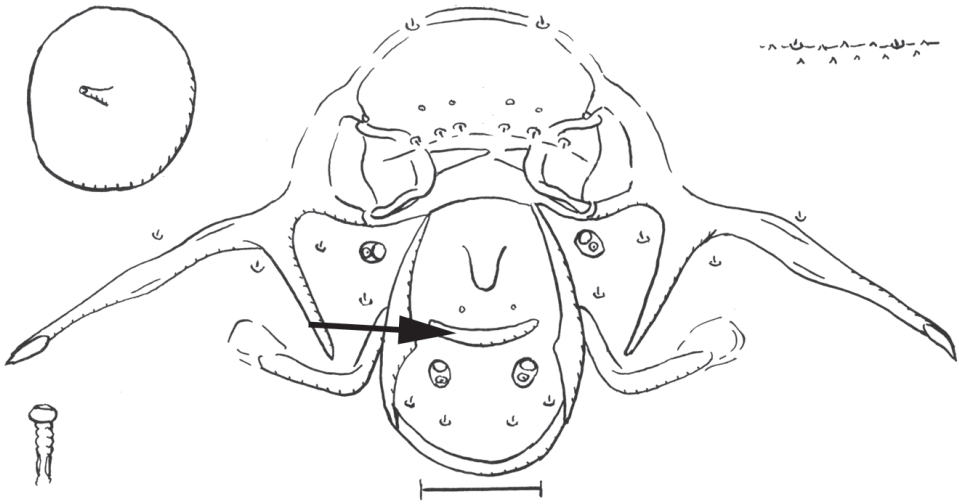
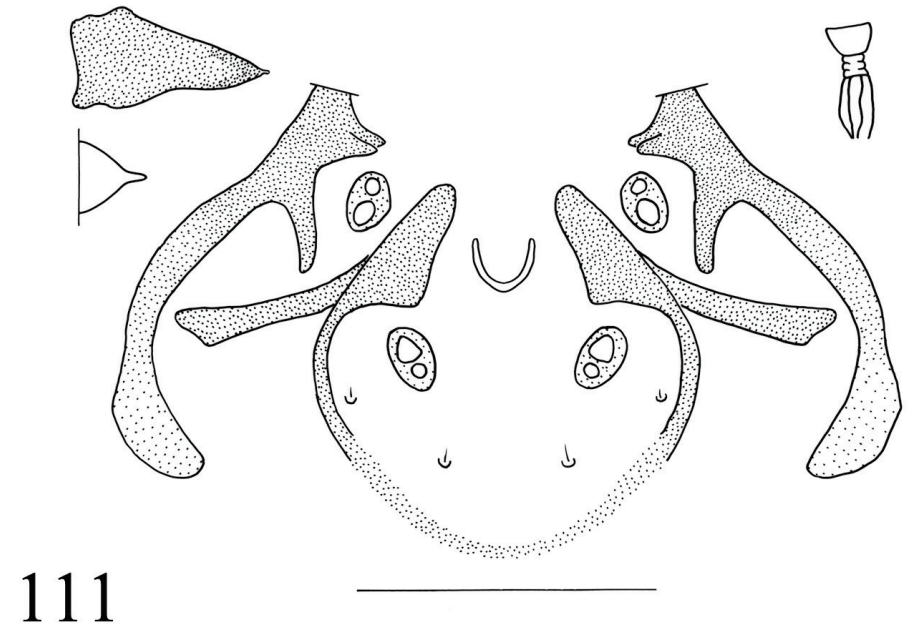
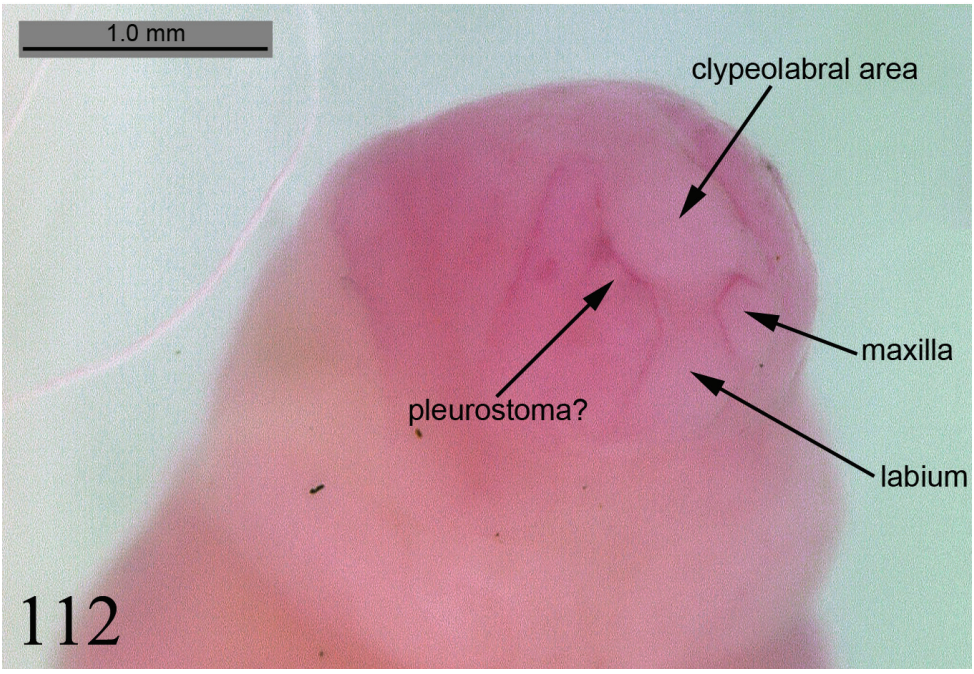


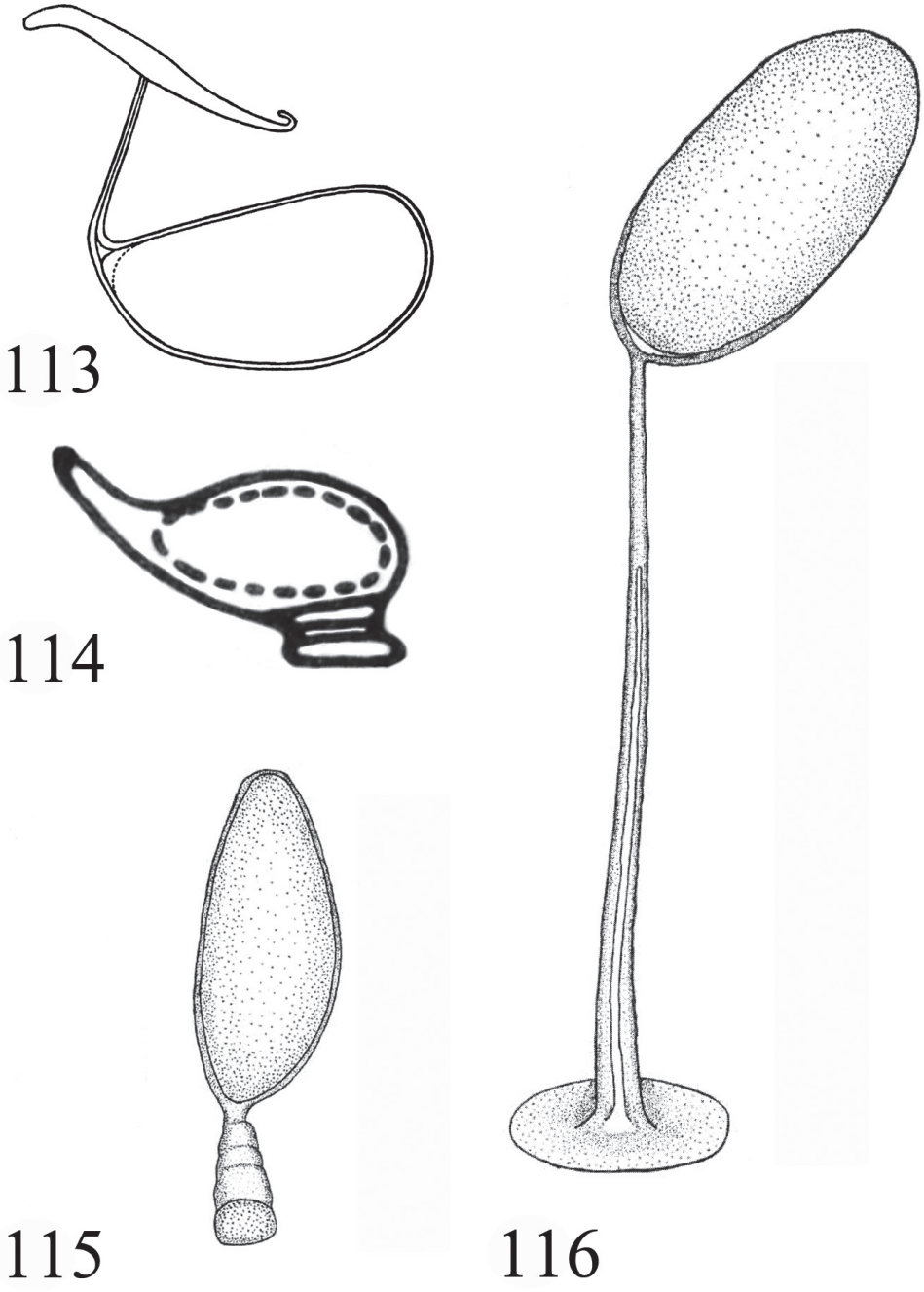
110

Figures 109–110. Cephalic sclerites of final larval instar **109** *Phaeogenes hebrus* (copied from Short 1978) **110** *Euceros serricornis* (Haliday) (copied from Short 1978). Arrow points to prelabial sclerite.





Figures 111–112. 111 Cephalic sclerites of final larval instar, *Phrudus* sp. Figures in upper left corner is whole mandible (upper) and close-up of apex of mandible (lower). Scale bar: 0.1 mm. Mandible and spiracle not same scale as main figure (spiracle is 27 μm in length) **112** Final larval instar, anterior of whole larva, *Collyria catoptron*, lateroventral view of head and thorax (stained with acid fuschin).



Figures 113–116. 113 Mature egg of *Ctenochira sanguinatoria* (Ratzeburg) (re-drawn after Kasparyan 1973) **114** Ovarian egg of *Therion* sp. egg (re-drawn after Iwata 1960) **115** Mature ovarian egg of *Euceros frigidus* Cresson (copied from Tripp 1961) **116** Egg of *Euceros frigidus* following oviposition (copied from Tripp 1961).

Morphological and biological characters only

The parsimony analysis using only the 141 morphological and biological characters produced 3872 equally parsimonious trees of 1527 steps (C.I. = 0.15, R.I. = 0.60). The strict consensus had 75 nodes collapsed (Fig. 118). Despite the lack of resolution in the "middle" of the tree, 14 of the 29 groupings considered in Table 3 were recovered as monophyletic in this analysis. This number did not include any of the three major subfamily groupings, which illustrates that our morphological data set was not good at resolving old divergences, but was relatively successful at supporting the monophyly of subfamilies. Agriotypinae (*Agriotypus armatus* Curtis) was always the sister group of all other Ichneumonidae. This analysis provided support for six subfamilies that were not monophyletic in the parsimony analysis with all characters (i.e., Acaenitinae, Banchinae, Mesochorinae, Metopiinae, Orthocentrinae and Xoridinae), but overall, it was much less well-resolved than the total-evidence parsimony analysis.

Molecular characters only

The parsimony analysis with molecular characters found 104 trees of 8126 steps (C.I. = 0.16, R.I. of 0.42). Ten nodes collapsed in the strict consensus cladogram (Fig. 119). Lycorininae (*Lycorina glaucomata* (Cushman)) was sister to all other Ichneumonidae, followed by *Neorhacodes enslini* (Ruschka) (Neorhacodinae), *Phytodietus vulgaris* Cresson and then *Netelia* sp. (both Tryphoninae). Nine of the 29 groupings in Table 3 were supported (including Pimpliformes) as well as 3 others that would be monophyletic if equivocally classified taxa were included or excluded (Banchinae, Metopiinae, and the *Phrudus* group of genera). Apart from the base of the tree, the overall topology more closely resembled the total-evidence parsimony analysis (Fig. 117), rather than the parsimony analysis with only morphological and biological characters (Fig. 118). This implies that the molecular data contributed more signal to the "middle" nodes of the tree than the morphological data.

Bayesian analysis

Total-evidence

The total-evidence Bayesian analysis majority credibility tree is shown in Figure 120. The topology at the base of the tree differed from the total-evidence parsimony analysis (Fig. 117). (Xoridinae was not sister to all other Ichneumonidae and Orthopelmatinae was not sister to all species except the xoridines). Instead, the Bayesian tree was similar to the parsimony analysis with only molecular characters (Fig. 119), in that its base had a grade of taxa: *Neorhacodes enslini* + (*Netelia* sp. + *Phytodietus vulgaris*) + (*Lycorina glaucomata* + *Idiogramma longicauda* (Cushman) + the remaining Tryphoninae). Apart from the base of the tree, there were some similarities between the parsimony and Bayesian total-evidence analyses. For the Bayesian analysis, 11 of the 29 groupings in Table 3 were unequivocally supported (BPP = 100) as well as 5 others (Banchinae,

Table 3. Comparison of the monophyly of selected taxa with different phylogenetic analyses and datasets. See footnotes and introduction for composition of selected taxa. "Yes" indicates that all taxa were supported in all equally parsimonious trees or had a support value of 100 in the Bayesian majority credibility tree. "No" indicates lack of monophyly in at least one most parsimonious tree or a support value of less than 100 in the Bayesian analysis. Pars. = parsimony; all = all characters; morph. = morphological and biological characters only; mol. = molecular characters only; Bayes = Bayesian analysis.

Taxon	Pars. (all)	Pars. (morph.)	Pars. (mol.)	Bayes (all)	Bayes (mol.)
Pimpliformes	Yes	No	Yes	Yes	Yes
Ichneumoniformes	No[1]	No	No	No	No
Ophioniformes	Yes	No	No	No	No
Acaenitinae	No	Yes	No	No	No
Anomaloninae	Yes	Yes	No	No	No
Banchinae	No[2]	Yes	No[2]	No[2]	No[2]
Campopleginae	Yes	No	Yes	Yes	Yes
Cremastinae	Yes	Yes	Yes	Yes	Yes
Cryptinae	Yes	No	No	Yes	Yes
Ctenopelmatinae	No[3]	No	No	No	No
Diplazontinae	Yes	Yes	Yes	Yes	Yes
Ichneumoninae	Yes	Yes	Yes[9]	Yes	Yes[9]
Labeninae	Yes	No	Yes	Yes	Yes
Mesochorinae	No[4]	Yes	No	No[4]	No[4]
Metopiinae	No[5]	Yes	No[5]	No[5]	No[5]
Nesomesochorinae	Yes	No	No	No	No
Ophioninae	Yes	Yes	No	Yes	No
Orthocentrinae	No	Yes	No	No	No
Phrudus group	No[6]	No	No[6]	No[6]	No[6]
Phygadeuontinae	Yes	Yes	No	No	No
Pimplinae	No	No	No	No	No
Poemeniinae	Yes	Yes	Yes	Yes	No
Rhyssinae	Yes	Yes	Yes	Yes	Yes
Sisyrostolinae	No	No	No	No	No
Stilbopinae	No	No	No	No	No
Tersilochinae s.l.	No[7]	No	No	No[7]	No
Tersilochinae s.s.	Yes	Yes	Yes	Yes	Yes
Tryphoninae	No[8]	No	No	No	No
Xoridinae	No	Yes	No	Yes	No

[1] Ichneumoniformes of Wahl (1993) ((Ichneumoninae including *Alomya*) + Cryptinae (including Phygadeuontinae) + Brachycyrtinae)) shared a common ancestor, but ancestor shared with *Clasis*, *Pedunculus*, Labeninae, *Agriotypus*, *Euceros*, *Adelognathus*, and *Microleptes* (this group called Ichneumoniformes s.l.).

[2] All Banchinae shared a common ancestor, but ancestor shared with *Notostilbops* (Stilbopinae).

[3] All Ctenopelmatinae shared a common ancestor, but ancestor shared with *Hybrizon*, *Lycorina*, *Oxytorus*, and *Tatogaster* as well as *Chineater* (Mesochorinae) and *Scolomus* (Metopiinae).

[4] All Mesochorinae shared a common ancestor except for *Chineater* (see [3], above).

[5] All Metopiinae shared a common ancestor except for *Scolomus* (see [3], above).

[6] *Phrudus* and *Peucobius* shared a common ancestor, but ancestor shared with *Erythrodolius* (Sisyrostolinae).

[7] All Tersilochinae and Sisyrostolinae shared a common ancestor (albeit with very low support).

[8] All Tryphoninae shared a common ancestor, but ancestor shared with *Neorhacodes* (Neorhacodinae).

[9] All Ichneumoninae shared a common ancestor, but *Alomya* was not sister group to this grouping.

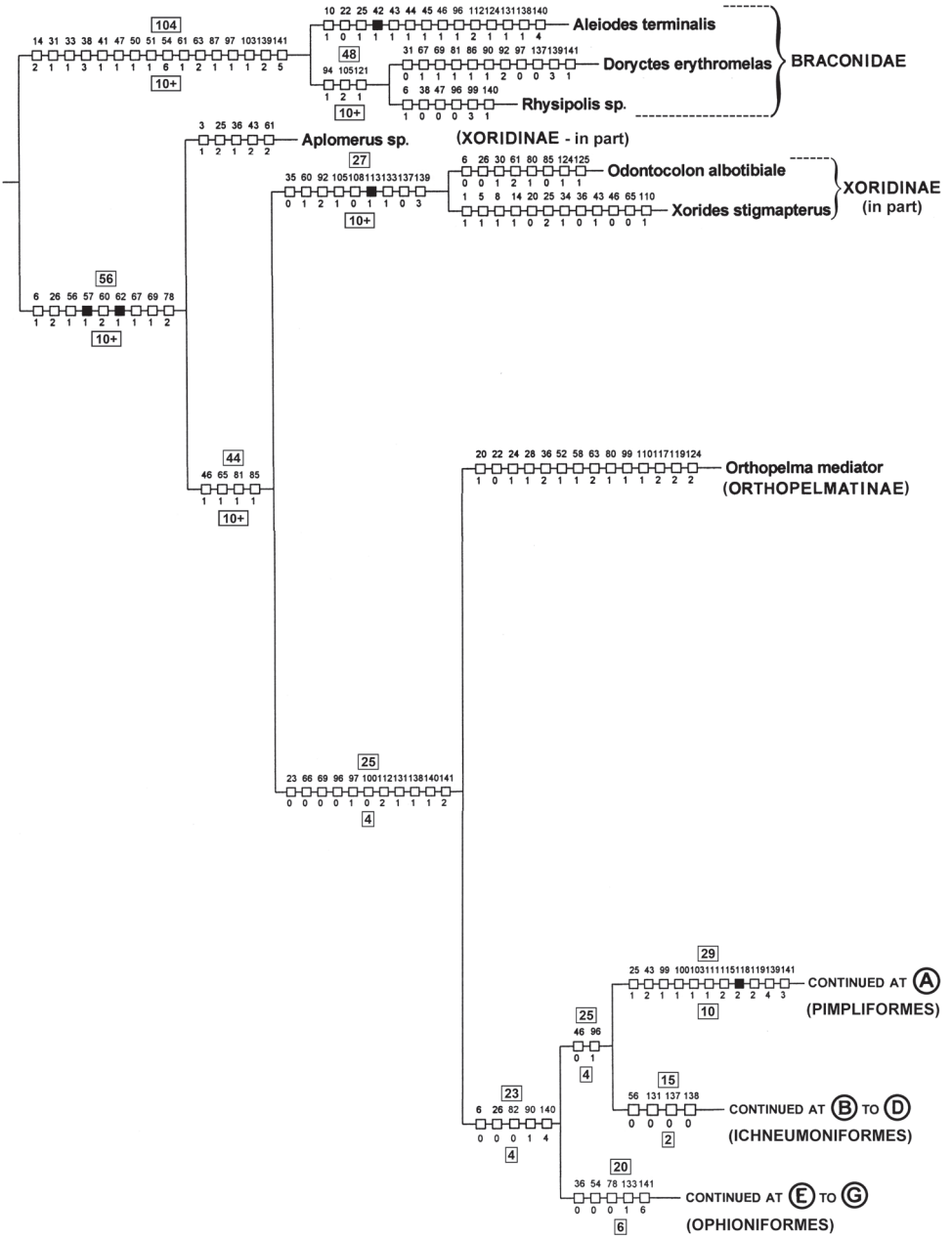


Figure 117. Part 1. Total-evidence parsimony analysis. Strict consensus cladogram (14 nodes collapse). Number of most parsimonious cladograms = 1728, length = 9917, CI = 0.15, RI = 0.44. Morphological characters optimized on tree using ACCTRAN. Open boxes are homoplasiously derived character states. Closed boxes are uniquely derived character states. Small numbers above boxes are character numbers. Small numbers below boxes are character states. Large numbers in squares above characters are total number of supporting characters (morphological and molecular). Large numbers in squares below characters are Bremer support values. Taxon names reflect classification prior to study (taxa with asterisks after name are formally re-classified in current study). "**A**" continued on part 2 of Fig. 117; "**B–D**" continued on part 3; "**E–G**" continued on part 4.

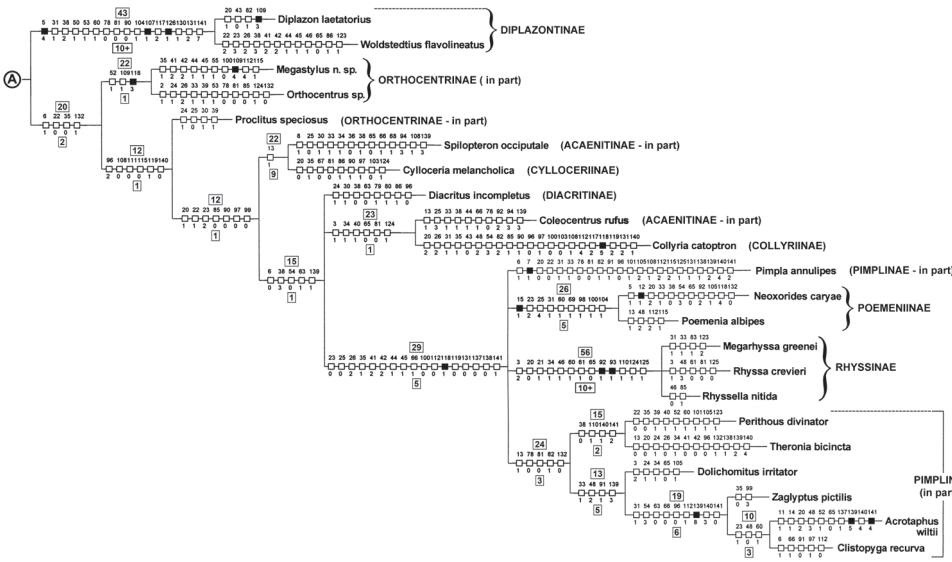


Figure 117. Part 2. Total-evidence parsimony analysis strict consensus cladogram continued from part 1 of Fig. 117. Pimpliformes group of subfamilies. See Fig. 117, part 1 figure heading for description of figure.

Mesochorinae, Metopiinae, the *Phrudus* group and Tersilochinae s.l.) that would be monophyletic with the inclusion or exclusion of equivocally classified taxa. Pimpliformes was unequivocally supported, but not Ichneumoniformes (either strictly or broadly defined) or Ophioniformes. Neither was there support for Pimpliformes + Ichneumoniformes s.l. This was because Labeninae was recovered as sister group to Pimpliformes + Ichneumoniformes s.l. The remainder of the subfamilies in Ichneumoniformes s.l., did group with Pimpliformes with a high support value (BPP = 98). In general, the basal nodes of the tree were weakly supported (BPP < 90), suggesting that more data are required for these regions of the tree.

Molecular characters only

Similar to the total-evidence Bayesian analysis and the parsimony analysis with only molecular characters, the base of the tree for the Bayesian analysis with only molecular characters (Fig. 121) consisted of a grade of Ophioniformes species (Neorhacodinae, Lycorininae and Tryphoninae). The analysis found unequivocal support for 8 of the 29 groupings in Table 3, including Pimpliformes. Banchinae, Mesochorinae, Metopiinae and the *Phrudus* group would also be supported with inclusion or exclusion of problematic taxa. Ichneumoniformes s.l. except for Labeninae was moderately well supported (BPP = 95) and was unequivocally the sister group of Pimpliformes. Labeninae was the sister of these two groupings with a much higher support value (BPP = 99) than the total-evidence Bayesian analysis (BPP = 66) (Fig. 120), suggesting that the morphological and molecular data were conflicting with respect to the placement of Labeninae.

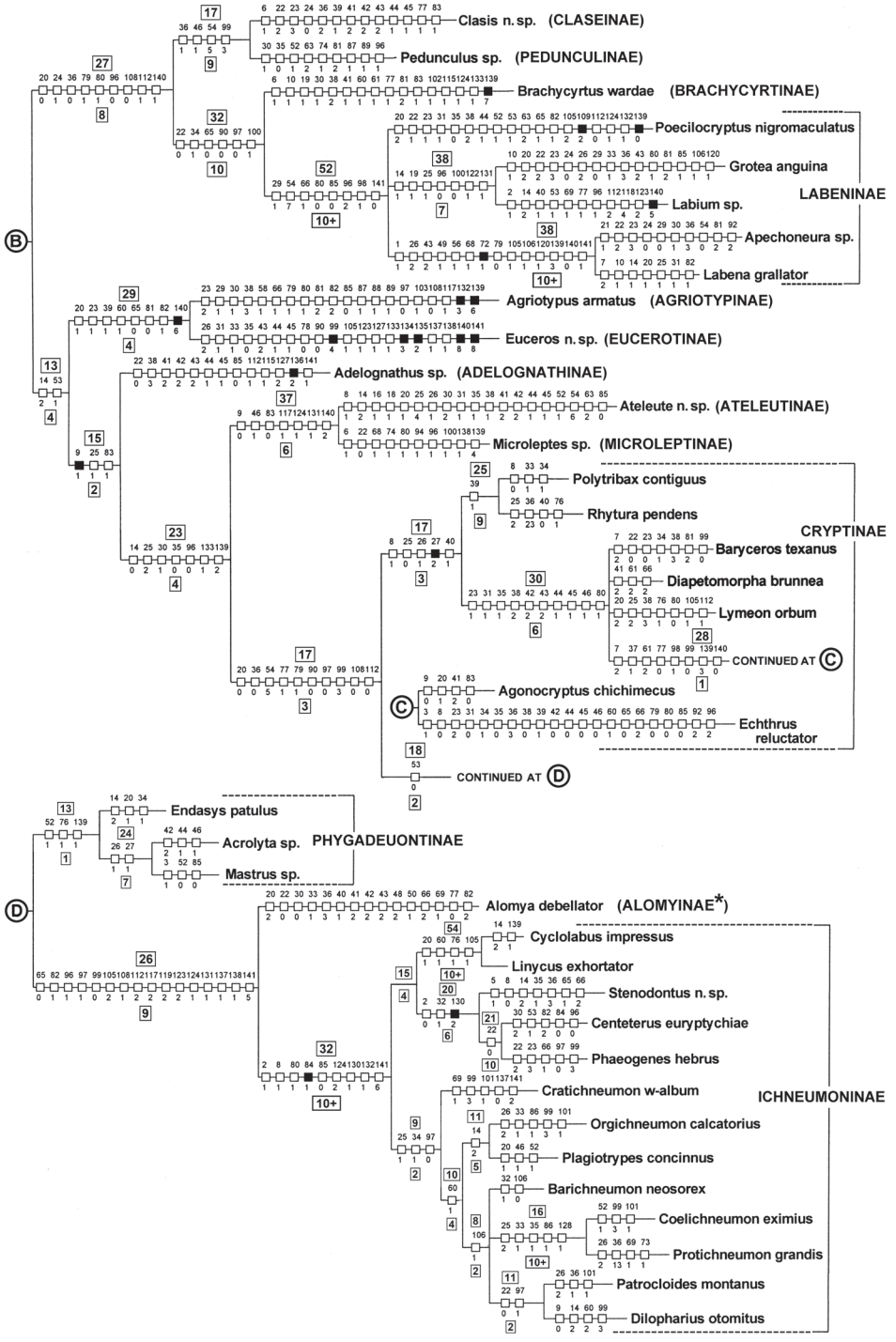


Figure 117. Part 3. Total-evidence parsimony analysis strict consensus cladogram continued from part 1 of Fig. 117. Ichneumoniformes s.l. group of subfamilies. See Fig. 117, part 1 figure heading for description of figure and Table 3 (footnote 1) for definition of Ichneumoniformes s.l.

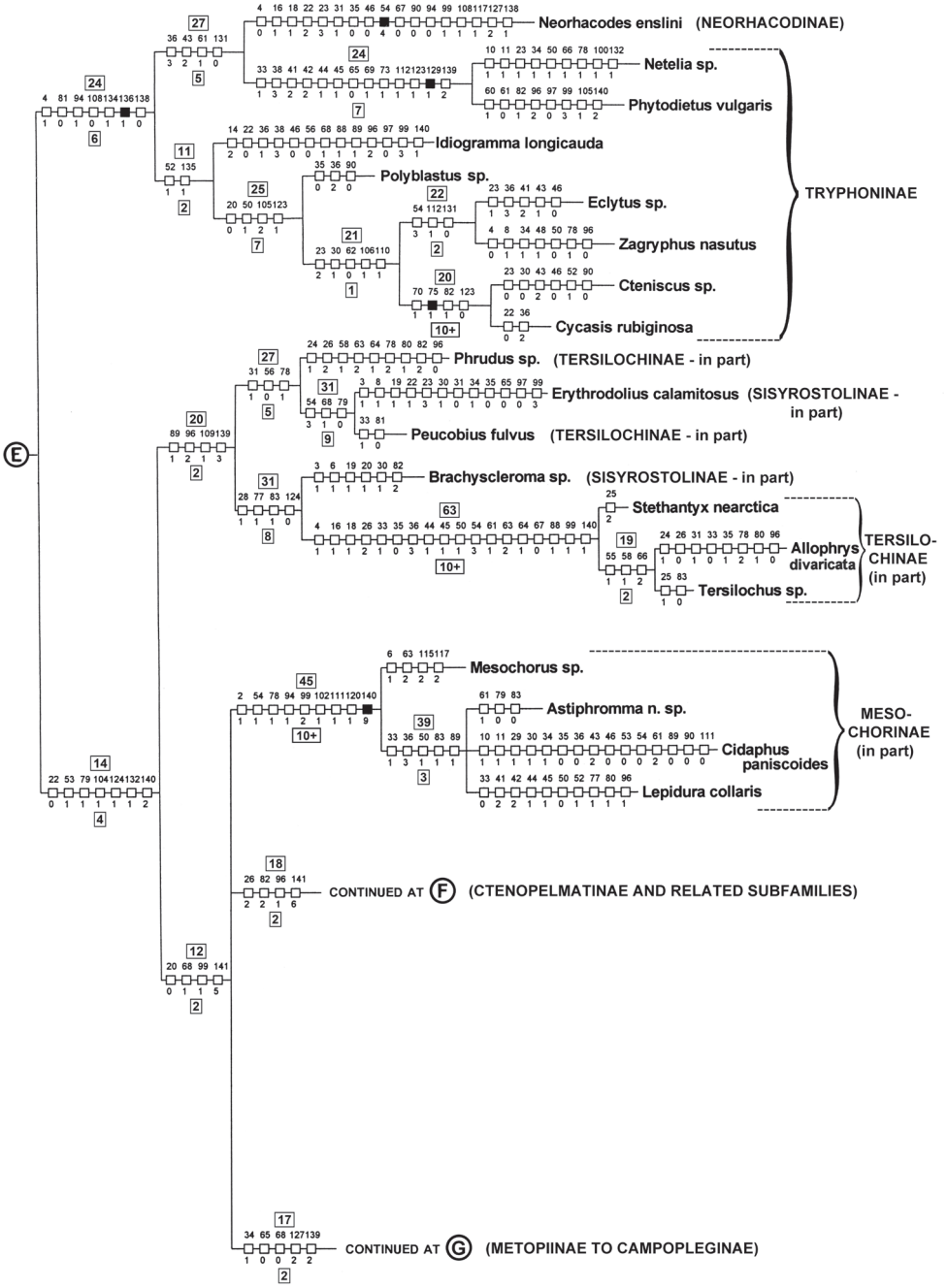


Figure 117. Part 4. Total-evidence parsimony analysis strict consensus cladogram continued from part 1 of Fig. 117. Ophioniformes group of subfamilies (base of clade). See Fig. 117, part 1 figure heading for description of figure. "F" continued on part 5 of Fig. 117; "G" continued on part 6 of Fig. 117.

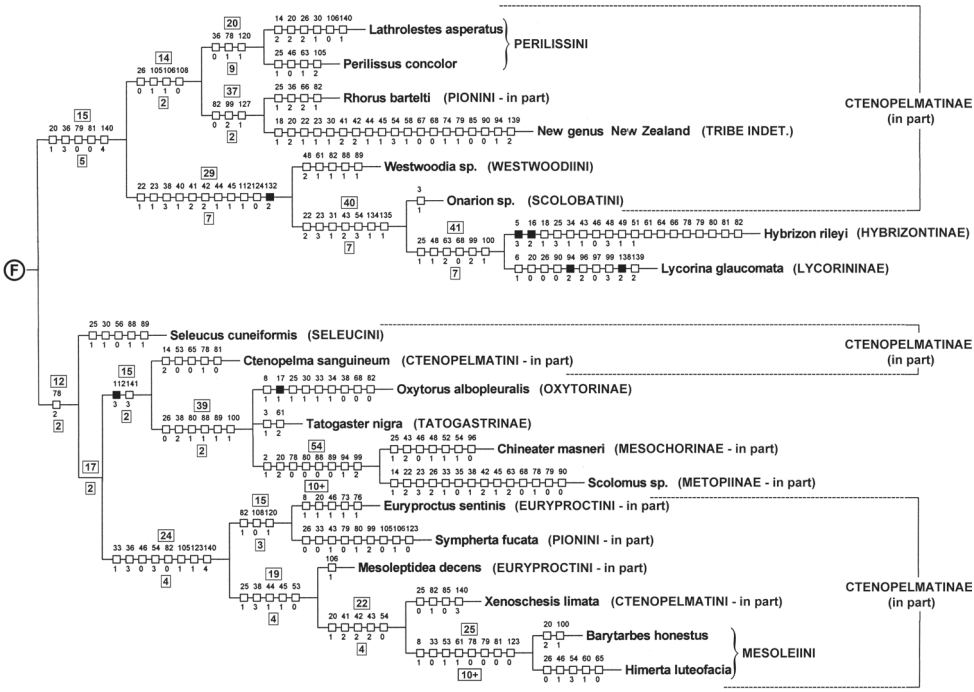


Figure 117. Part 5. Total-evidence parsimony analysis strict consensus cladogram continued from part 4 of Fig. 117. Ctenopelmatinae and related subfamilies. See Fig. 117, part 1 figure heading for description of figure.

Support/relationships of taxa

Ichneumonidae

It was not the purpose of this study to examine relationships outside of Ichneumonidae, therefore only minimal outgroup sampling was used; however, all analyses did recover a monophyletic Ichneumonidae (Figs 117–121). In the total-evidence parsimony analysis, Ichneumonidae was supported by a Bremer support of greater than 10 steps and 56 synapomorphies, 9 of which were morphological, including two that were uniquely derived: character 57, state 1: fore wing with antero-medial flexion line basally with the anterior fold running posterior to vein M (basal vein of areolet without a bulla); and character 62, state 1: hind wing vein *1r-m* opposite or apical to separation of veins *R1* and *Rs* (Fig. 4 compared to Fig. 49 for both of these characters).

Sister group to all other Ichneumonidae

The following taxa were sister group to all other Ichneumonidae depending on the analysis:

Aplomerus sp. (Xoridinae): total-evidence parsimony analysis (Fig. 117);
Agriotypus armatus (Agriotypinae): morphology-only parsimony analysis (Fig. 118);

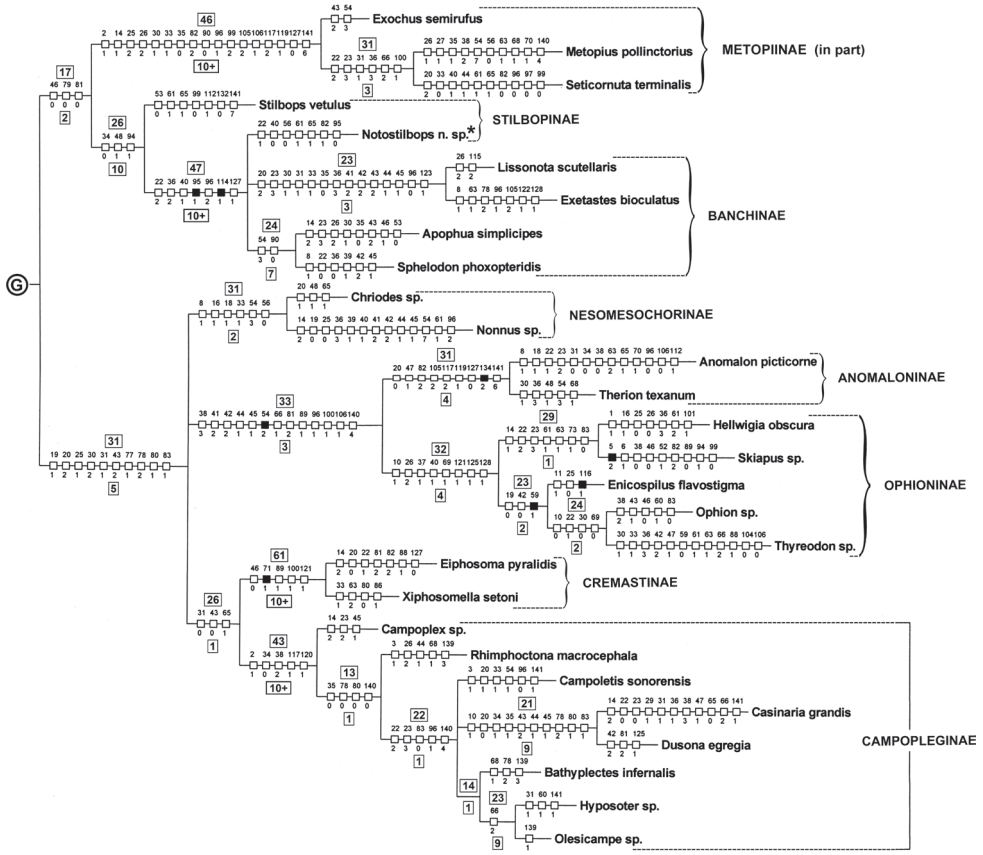


Figure 117. Part 6. Total-evidence parsimony analysis strict consensus cladogram continued from part 4 of Fig. 117. Metopiinae to Campopleginae. See Fig. 117, part 1 figure heading for description of figure.

Lycorina glaucomata (Lycorininae): molecular-only parsimony analysis (Fig. 119); *Neorhacodes enslini* (Neorhacodinae): Bayesian analyses (Figs 120–121).

Our total-evidence parsimony analysis found the three Xoridinae species at the base of Ichneumonidae; however, they were not monophyletic with *Aplomerus* Provancher sister group to all other ichneumonids and (*Odontocolon* Cushman + *Xorides* Latreille) sister to all the rest (Fig. 117). In contrast, Xoridinae was monophyletic in the morphology-only parsimony analysis (Fig. 118) and both Bayesian analyses (Figs 120–121), albeit not as sister to the rest of Ichneumonidae. The biology of Xoridinae (ectoparasitoids of wood-boring beetles) is consistent with the hypotheses of Handlirsch (1907) and Gauld (1988a) with respect to the evolution of parasitoidism beginning with idiobiont ectoparasitoids in concealed substrates.

In our morphological parsimony analysis, the clade comprising all ichneumonids except *Agriotypus armatus* was supported by 11 synapomorphies, of which one was uniquely derived: character 87(0) (T2 and T3 separate, with flexion line allowing move-

ment) (Fig. 78). The Bremer support value was only 1. In all of our other analyses, Agriotypinae was placed within Ichneumonidae, with the fusion of T2 and T3 evolving in parallel in Braconidae and Agriotypinae. Agriotypinae are idiobiont ectoparasitoids of pupal and pre-pupal Trichoptera in fast-running streams (Bennett 2001).

The evidence that Lycorininae is sister group to all other ichneumonids is based on 36 uniquely derived molecular substitutions that support the clade comprising all ichneumonids except *Lycorina glaucomata* in the molecular parsimony analysis (Fig. 119). Among our five analyses, the placement of Lycorininae was one of the least stable of any taxon. The total-evidence parsimony analysis placed *L. glaucomata* within the Ctenopelmatinae and relatives clade as sister to Hybrizontinae (see section on Hybrizontinae for details on support). In the two Bayesian analyses, it was placed within a grade near the bottom of the cladogram along with *Neorhacodes enslini* (Neorhacodinae) and various species of Tryphoninae (Figs 120–121).

The biology of *Lycorina* is not completely known, but species for which oviposition and development have been observed are koinobionts (Coronado-Rivera et al. 2004) that oviposit in the anus of Lepidoptera larvae and complete development feeding externally on the host and pupating inside the host cocoon (Shaw 2004) (our character 138, state 2).

Neorhacodinae was sister group to all other Ichneumonidae in both Bayesian analyses on the basis of posterior probabilities of 100 supporting the grouping comprised of all ichneumonids except *Neorhacodes enslini* (Figs 120–121). The parsimony analysis with only molecular characters placed *N. enslini* close to its placement in the Bayesian analyses, as the sister taxon to all Ichneumonidae except *Lycorina glaucomata* (Lycorininae) (part 1 of Fig. 119). Conversely, the total-evidence parsimony analysis placed *N. enslini* within Tryphoninae (as sister to Phytodietini) (part 4 of Fig. 117). The biology of Neorhacodinae is not completely known. *Neorhacodes* spp. have been reared from nests of aculeate Hymenoptera (Horstmann 1968; Danks 1971) and are probably endoparasitoids (Wahl 1993c), but it is not clear whether they are idiobionts or koinobionts.

In terms of previous studies, Quicke et al. (1999) using 28S D2–D3 sequence data analyzed with parsimony for a limited taxon sampling of Ichneumonoidea found that Xoridinae was sister group to all other Ichneumonidae. Similarly, the analyses of Klopfstein et al. (2019) using transcriptomes from 6 braconids and 19 ichneumonids found that *Xorides praecatorius* (Fabricius) was sister to all other ichneumonids and this same placement was also found in all of their anchored enrichment analyses of 84 taxa, including 10 non-ichneumonid ougroups. Quicke (2015) summarized his "best-guess" by placing both Xoridinae and Labeninae unresolved as sister taxa to all other Ichneumonidae, and this arrangement was also presented by Broad et al. (2018). Apart from Xoridinae and Labeninae, Agriotypinae (*Agriotypus* Curtis) has previously been accorded family group status within Ichneumonoidea (e.g., Ashmead 1900) which could be consistent with a sister group relationship with all other Ichneumonidae, although all recent studies have concluded that it belongs within Ichneumonidae and most likely as part of Ichneumoniformes sensu lato (Quicke et al. 2009; Santos 2017). Similarly, Hybrizontinae (*Hybrizon* Fallén and two fossil genera) has been given family group status within Ichneumonoidea (e.g., Marsh 1989), but more recent studies

Figure 118. Part 1. Parsimony analysis (morphological and biological characters only: Chs 1-141). Strict consensus cladogram (75 nodes collapse). Characters optimized on tree using ACCTRAN. Number of most parsimonious cladograms = 3872, length = 1527, CI = 0.15, RI = 0.60. See Figure 117 figure heading for description of character support. Taxon names reflect classification prior to study (taxa with asterisks after name are formally re-classified in current study). "**A**" continued on part 2 of Fig. 118.

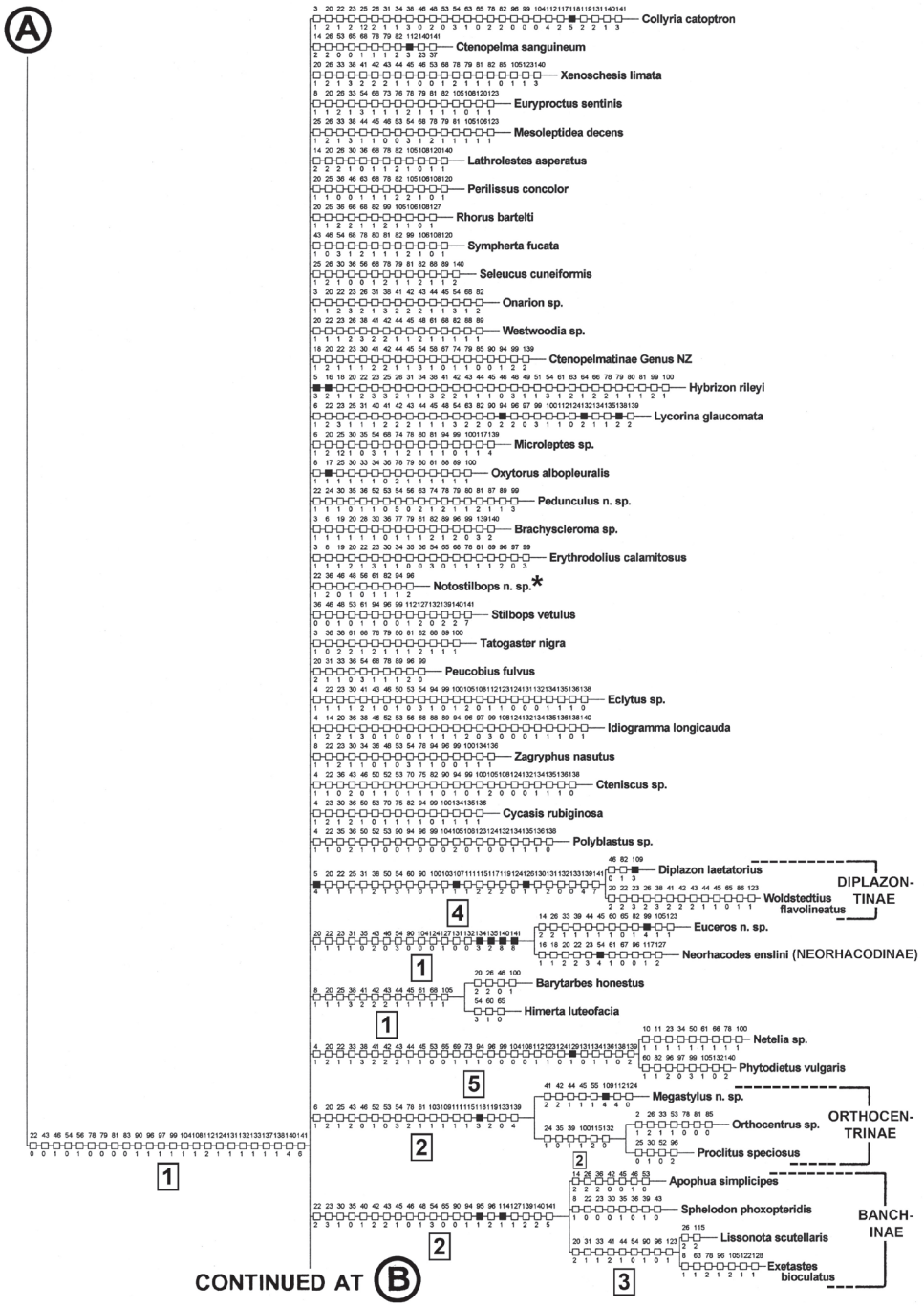


Figure 118. Part 2. Parsimony analysis (morphological and biological characters only) strict consensus cladogram continued from part 1 of Fig. 118. See Fig. 118, part 1 figure heading for description of figure. "**B**" continued on part 3 of Fig. 118.

Figure 118. Part 3. Parsimony analysis (morphological and biological characters only) strict consensus cladogram continued from part 2 of Fig. 118. See Fig. 118, part 1 figure heading for description of figure.

place it within Ichneumonidae and more specifically, within Ophioniformes (Quicke et al. 2009, Broad et al. 2018). There was no evidence in the current study that Hybrizontinae was sister group to the rest of Ichneumonidae. Neorhacodinae (currently comprised of *Neorhacodes* Hedicke, *Romaniella* Cushman and *Eremura* Kasparyan) has been classified as a subfamily in both Ichneumonidae (e.g. Townes 1969) and Braconidae (Fahringer 1936), but it has never been given family group status within Ichneumonoidea, nor has it ever been suggested that it is the sister group of all other Ichneumonidae. This is also the case for Lycorininae. Both of these taxa have most recently been placed within Ichneumonidae and, more specifically, within Ophioniformes (Quicke et al. 2009; Broad et al. 2018). In summary, the only previously hypothesized sister group to all other Ichneumonidae that was supported in the current study was Xoridinae, and the only analysis that supported this (total-evidence parsimony), did not recover the subfamily as monophyletic (Fig. 117). Future studies should include a greater number of outgroups from Braconidae and outside Ichneumonoidea, in order to examine this question.

Subfamily groupings

Ophioniformes

Ophioniformes (including Tryphoninae) was supported in the total-evidence parsimony analysis by 20 total characters, of which 5 were morphological: 36(0) anterior transverse carina of propodeum complete (Fig. 38); 54(0) areolet obliquely rhombic to subtriangular (Fig. 51); 78(0) glymma present and shallow (Fig. 5); 133(0) larval salivary orifice u-shaped (Fig. 7g); and 141(0) larval-pupal oviposition-emergence. The Bremer support value of this node was 6 steps (part 1 of Fig. 117). None of the other analyses supported Ophioniformes as monophyletic and in general, Ophioniformes species formed a grade leading to the Ichneumoniformes and Pimpliformes taxa (e.g., Bayesian total-evidence tree: part 1 of Fig. 120).

In terms of the arrangement within Ophioniformes in the total-evidence parsimony analysis, Tryphoninae (including *Neorhacodes enslini*) was sister to all other taxa (part 4 of Fig. 117), which was similar to the findings of Quicke et al. (2009), albeit the latter study had taxa of some other subfamilies clustering within Tryphoninae (i.e., Eucerotinae, Sisyrostolinae, Stilbopinae, but not Neorhacodinae). Our total-evidence parsimony analysis next found a grouping of Sisyrostolinae and Tersilochinae as sister to all remaining Ophioniformes, followed by a clade without resolution comprised of the following three groups: 1) Mesochorinae (except *Chineater masneri* Wahl) (part 4 of Fig. 117); 2) Ctenopelmatinae and related subfamilies (part 5 of Fig. 117); and 3) (Metopiinae + *Stilbops* + Banchinae including *Notostilbops*) + the higher Ophioniformes. Higher Ophioniformes was a clade of three groups with equivocal relationships among the equally parsimonious trees: Nesomesochorinae/ (Anomaloninae + Ophioninae)/ (Cremastinae + Campopleginae)) (part 6 of Fig. 117). In the total-evidence parsimony analysis, higher Ophioniformes was moderately well supported by 31 synapomorphies, of which 10

were morphological (none uniquely derived) and a Bremer support of 5. The group was also quite well-supported in the total-evidence Bayesian analysis (BPP = 98).

The topology within Ophioniformes in the current total-evidence parsimony analysis is similar to that of Quicke et al. (2009), with the following major exceptions: 1) Orthopelmatinae and Microleptinae never belonged to Ophioniformes in our study (equivocal placement in Quicke et al. 2009, but within Ophioniformes in both of their presented cladograms); 2) Tersilochinae was associated with Sisyrostolinae as opposed to related to Ctenopelmatinae and Mesochorinae in Quicke et al. (2009); 3) Mesochorinae and Metopiinae were not paraphyletic with respect to Ctenopelmatinae in our analysis; 4) Ctenopelmatinae could be monophyletic based on our results with the inclusion of four small subfamilies (Hybrizontinae, Lycorininae, Oxytorinae and Tatogastrinae) and inclusion of two enigmatic genera-*Chineater* Wahl (Mesochorinae) and *Scolomus* Townes & Townes (Metopiinae); 5) Hybrizontinae clustered with the Ctenopelmatinae exemplars in our study as opposed to related to *Skiapus* Morley (Ophioninae) and Anomaloninae in Quicke et al. (2009).

The anchored enrichment study by Klopfstein et al. (2019) included 12 exemplars of Ophioniformes with the following topology: Tryphoninae + (Banchinae + (Tersilochinae + (Mesochorinae + (Ctenopelmatinae + (Metopiinae + (Anomaloninae + (Cremastinae + (Ophioninae + Campopleginae))))))))). The main similarities between the current total-evidence parsimony analysis and the results of Quicke et al. (2009) and Klopstein et al. (2019) are: 1) Tryphoninae is sister to the rest of Ophioniformes; 2) Anomaloninae, Cremastinae, Ophioninae and Campopleginae cluster together as part of the higher Ophioniformes. The placement of the other subfamilies differed somewhat between these three studies and will be discussed in the relevant subfamily sections in *Support/ relationships of subfamilies* (below).

Pimpliformes

Pimpliformes was monophyletic in all analyses with the exception of parsimony using only morphological characters (Table 3). In the total-evidence parsimony analysis it was supported by 29 characters, of which 11 were morphological, including the uniquely derived character 118(2) (larval hypostomal spur fused to form a hypostomal-stipital plate) (part 1 of Fig. 117). The Bremer support value was 10 steps. Most previous analyses have also supported Pimpliformes using either morphological data (Wahl and Gauld 1998), sequence data (Belshaw et al. 1998; Klopfstein et al. 2019) or combined morphology and sequence data (Quicke et al. 2009).

In the current study, the topology within Pimpliformes was equivocal, depending on the method of analysis. The total-evidence parsimony and molecular parsimony analyses recovered Diplazontinae as sister to all other taxa, followed by the orthocentrine genera (not clustering together) and the rest of the taxa (part 4 of Fig. 117, part 2 of Fig. 119). In contrast, the Bayesian total-evidence analysis placed the taxa in two separate clades: 1) the "higher Pimpliformes" of Quicke et al. (2009) = Pimplinae, Poemeniinae and Rhyssinae; and 2) all other exemplars (part 2 of Fig. 120). Higher Pimpliformes was strongly supported in the parsimony total-evidence analysis with

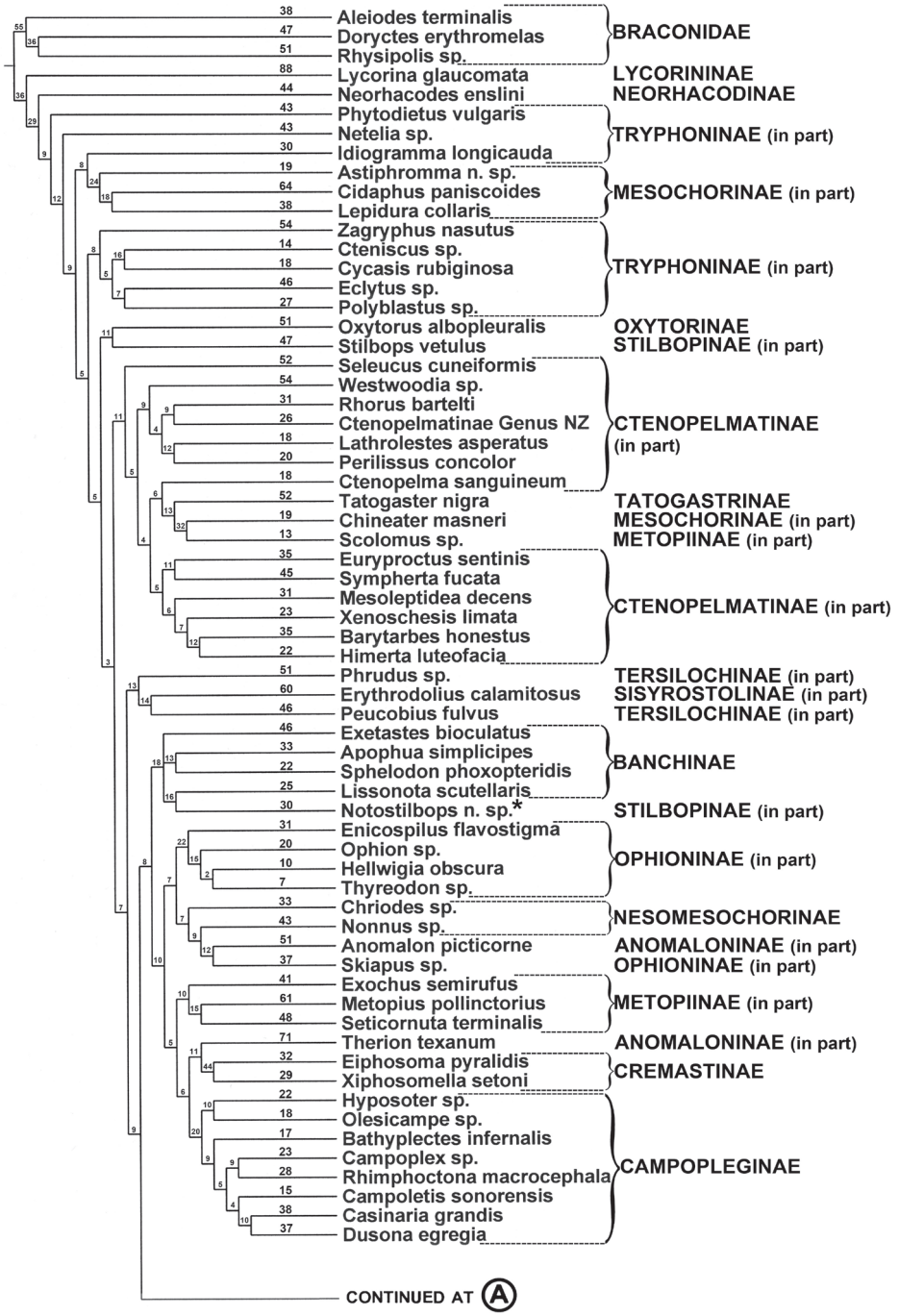


Figure 119. Part 1. Parsimony analysis (molecular characters only). Strict consensus cladogram (10 nodes collapse). Number of most parsimonious cladograms = 104, length = 8126, CI = 0.16, RI = 0.42. Numbers above branches are number of substitutions supporting each node or taxon. Taxon names reflect classification prior to study (taxa with asterisks after name are formally re-classified in current study). "**A**" continued on part 2 of Fig. 119.

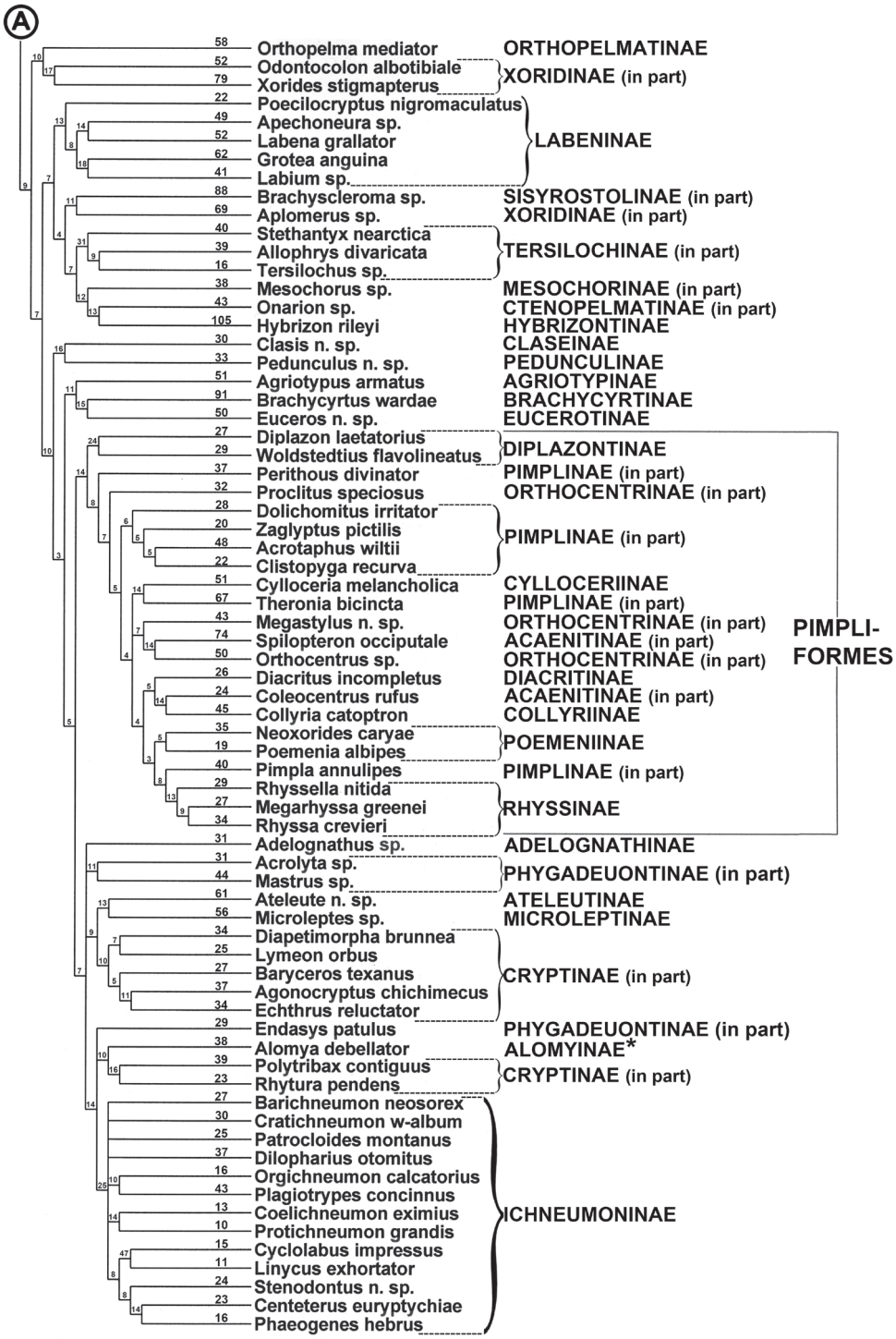


Figure 119. Part 2. Parsimony analysis (molecular characters only). Strict consensus cladogram continued from part 1 of Fig. 119. See Fig. 119, part 1 figure heading for description of figure.

Figure 120. Part 1. Total-evidence Bayesian analysis maximum clade credibility tree. Numbers to the right of nodes are posterior probabilities. "EN" = change from ectoparasitoid to endoparasitoid (character 138); "EC" = change from endoparasitoid to ectoparasitoid; "E2" = change from ectoparasitoid to development inside hind gut with final ectoparasitoid phase; "I" = change from koinobiont to idiobiont (character 137); "K" = change from idiobiont to koinobiont. Taxon names reflect classification prior to study (taxa with asterisks after name are formally re-classified in current study). "**B**" is continued on part 2 of Fig. 120.

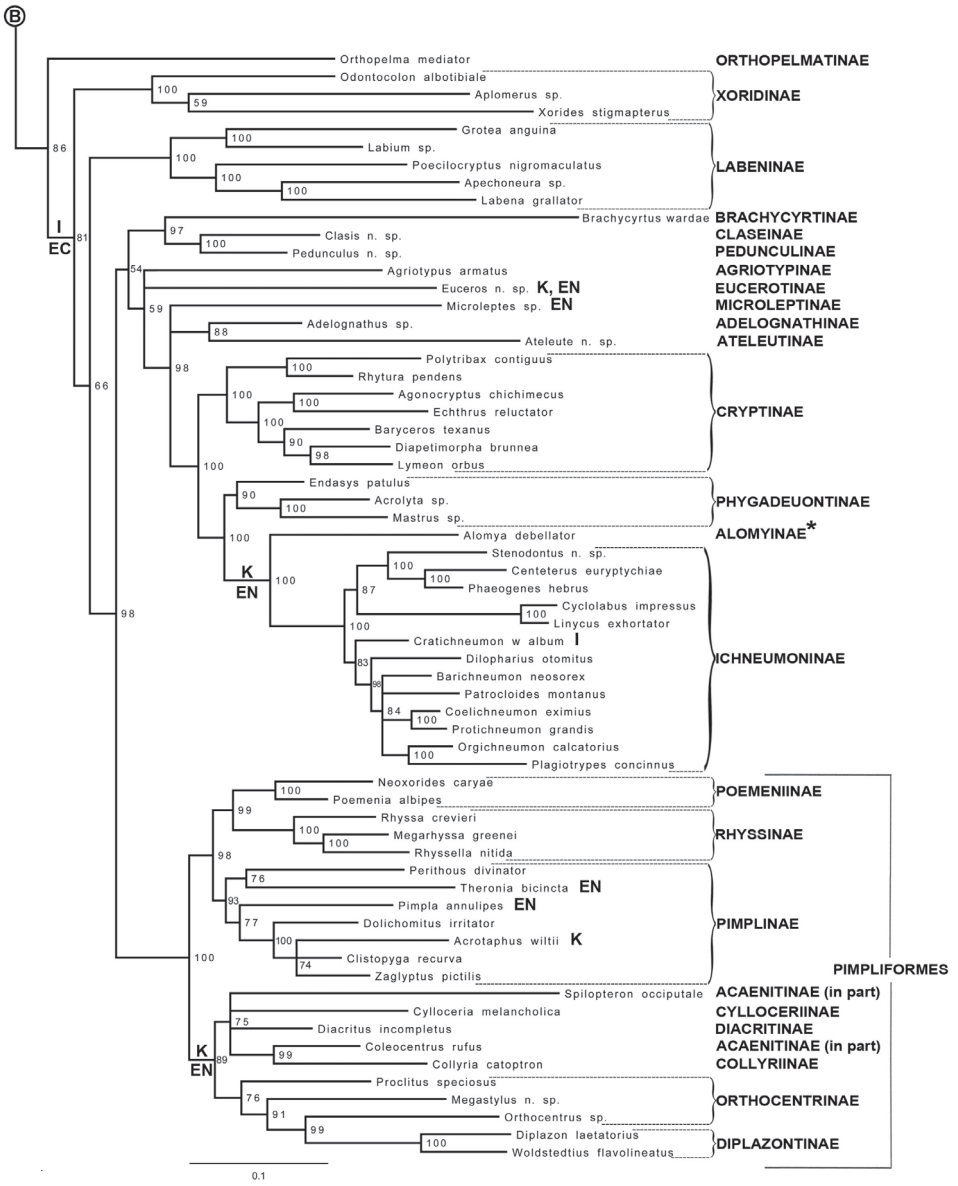


Figure 120. Part 2. Total-evidence Bayesian analysis maximum clade credibility tree continued from part 1 of Fig. 120. Numbers to the right of nodes are posterior probabilities. See Fig. 120, part 1 figure heading for description of figure.

29 synapomorphies, 17 of which were morphological and one of these was uniquely derived: character 118, state 1: larval hypostomal spur meeting stipital sclerite near median end of stipital sclerite (Fig. 102). The Bayesian analysis with only molecular characters was mostly unresolved for Pimpliformes (part 2 of Fig. 121).

In terms of previous hypotheses of internal Pimpliformes relationships, Wahl and Gauld (1998), using morphology-based parsimony recovered (Acaenitinae + (Diacritinae + (Cylloceriinae + (Diplazontinae + Orthocentrinae)))) + (Pimplinae + (Rhyssinae + Poemeniinae)). Quicke et al. (2009) had Diacritinae as sister group to all other taxa which were divided into two sister clades: 1) Acaenitinae + (Cylloceriinae + (Orthocentrinae + (Diplazontinae + Collyriinae))); 2) (Pimplinae + (Rhyssinae + Poemeniinae)). Finally, Klopfstein et al. (2019) had equivocal results depending on their analysis. Their transcriptome analysis recovered their diplazontine exemplar, *Syrphophilus tricinctorus* (Thunberg) as sister taxon to the other nine species of Pimpliformes. Some of the topologies found in their anchored enrichment analyses were similar to the transcriptome topology with Diplazontinae as sister to all other taxa, whereas others more resembled the current total-evidence Bayesian analysis and Wahl and Gauld (1998) with two main clades—the higher Pimpliformes and a clade including Acaenitinae and the fly-parasitizing subfamilies. Regardless of the analysis, Klopfstein et al. (2019) found very short branch lengths close to the base of Pimpliformes, which they hypothesized suggests a rapid radiation. The ambiguous nature of the current results and those of Klopfstein et al. (2019) indicate that resolving ancestral Pimpliformes relationships may be one of the most challenging aspects of future phylogenetic studies within Ichneumonidae.

Ichneumoniformes

Ichneumoniformes sensu stricto (of Wahl 1993a) was not recovered in any analyses (Table 3), although in the total-evidence parsimony analysis (part 3 of Fig. 117), these subfamilies (Brachcyrtinae, Ichneumoninae including Alomyinae, and Cryptinae including Ateleutinae and Phygadeuontinae) were recovered as part of a larger group that included seven other subfamilies (Adelognathinae, Agriotypinae, Claseinae, Eucerotinae, Labeninae, Microleptinae and Pedunculinae). This grouping is hereby called Ichneumoniformes sensu lato. None of the other analyses recovered either Ichneumoniformes s.s. or Ichneumoniformes s.l. In the total-evidence parsimony analysis, Ichneumoniformes s.l. was only supported by 15 synapomorphies, of which 4 were morphological: 56(0) areolet sessile anteriorly (Figs 4, 56); 131(0), larval antenna present and with central papillus (Fig. 7n); 137(0) idiobiont timing of maturation and 138(0) ectophagous location of development (part 1 of Fig. 117). The Bremer support value was only 2 and none of the four morphological characters had a consistency index above 0.16. With respect to specific topologies in our other analyses, in the molecular-only parsimony analysis (part 3 of Fig. 117) and the total-evidence Bayesian analysis (part 2 of Fig. 120), a subset of Ichneumoniformes s.l. was strongly supported that included the following: Microleptinae, Adelognathinae, Ateleutinae, Cryptinae, Phygadeuontinae, Alomyinae and Ichneumoninae) (BPP = 98 in the total-evidence Bayesian analysis). We have chosen our definition of Ichneumoniformes s.l. (which includes thirteen subfamilies) based on support for this grouping in the total-evidence parsimony analysis. Future studies may lead us to propose a more restricted definition of Ichneumoniformes s.l. (e.g., the subset listed above). More discussion on the rela-

tionships within Ichneumoniformes is made in the section Support/ relationships of subfamilies (below), especially with respect to Claseinae.

Apart from the study of Quicke et al. (2009), Ichneumoniformes has also been studied previously in a strictly molecular phylogenetic study of Laurenne et al. (2006) and more recently, by a combined morphological and seven gene study by Santos (2017). The latter study focused on the relationships of Cryptini, but included outgroup taxa from other tribes of Cryptinae as well as Pimpliformes, Ophioniformes, Xoridinae and all subfamilies in our Ichneumoniformes s.l except Alomyinae and Pedunculinae. The combined, maximum likelihood analysis of Santos (2017) found the following topology: Labeninae + (Claseinae + (Eucerotinae + Brachycyrtinae) + (Agriotypinae + ((Cryptini + Aptesini) + (Phygadeuontini including Ichneumoninae, *Hemigaster* Brullé, *Microleptes* Gravenhorst, *Adelognathus* Holmgren and *Ateleute* Förster))))). Accordingly, Santos (2017) raised Phygadeuontini and Ateleutina to subfamily status in order to maintain Ichneumoninae as a subfamily, and restricted Cryptinae to only two tribes. He moved *Hemigaster* to Phygadeuontinae, but did not sink *Microleptes* or *Adelognathus* within Phygadeuontinae. Furthermore, he stated that more study was required to determine the relationships of various phygadeuontine taxa relative to Ichneumoninae. Overall, the relationships within Ichneumoniformes s.l. in our study are quite similar to the results of Santos (2017), especially the finding that *Ateleute* does not cluster within Cryptini, as was classified prior to Santos (2017), and that Ichneumoninae were derived from within Cryptinae s.l., a notion first postulated by Gokhman (1988).

Relationship of Ophioniformes, Pimpliformes and Ichneumoniformes

The total-evidence parsimony analysis was the only one that recovered Pimpliformes, Ophioniformes and Ichneumoniformes s.l. as monophyletic (Table 3, Fig. 117) with Ophioniformes as the sister group to Ichneumoniformes s.l. + Pimpliformes. Both Bayesian analyses (Figs 120, 121) had a grade of Ophioniformes leading to a grouping as follows: Labeninae + (Ichneumoniformes s.l. (except Labeninae) + Pimpliformes). Therefore, apart from the lack of monophyly of Ophioniformes in the Bayesian analyses, the relative relationships of the three major subfamily groupings was similar in both our total-evidence analyses.

The study of Quicke et al. (2009) proposed the following topology: Xoridinae + (Labeninae + (Pimpliformes + ((Claseinae + (Pedunculinae + Brachycyrtinae) + (Ichneumoniformes + Ophioniformes))))). Therefore, Quicke et al. (2009) supported Pimpliformes as sister group to the other two groupings whereas Santos (2017) and Klopfstein et al. (2019) generally supported Ophioniformes as sister group to Pimpliformes + Ichneumoniformes albeit with a reduced number of outrgroups compared to the current study. The other main difference between the current study and Quicke et al. (2009) was the latter's placement of Labeninae as sister group to all ichneumonids except for Xoridinae. See section on Labeninae (below) for discussion of its equivocal placement in the current study.

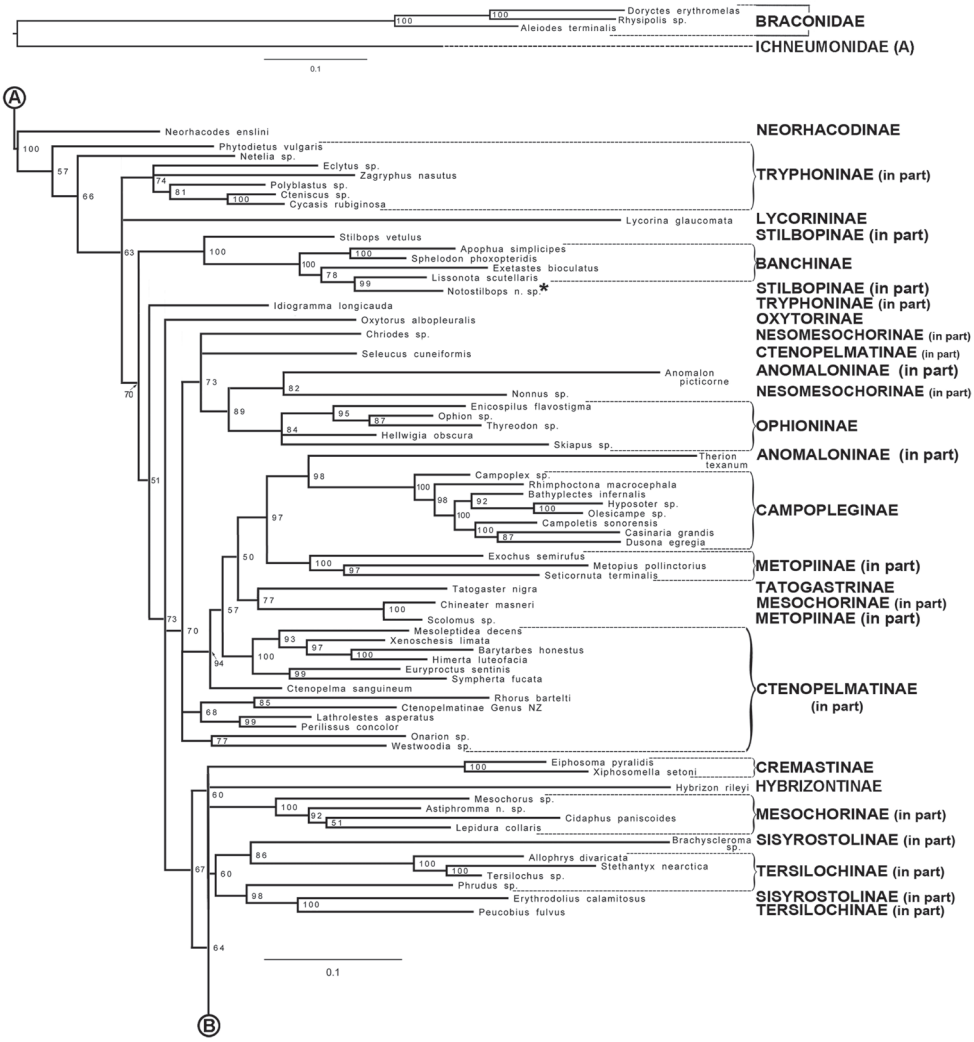


Figure 121. Part 1. Bayesian analysis (molecular characters only) maximum clade credibility tree. Numbers to the right of nodes are posterior probabilities. Taxon names reflect classification prior to study (taxa with asterisks after name are formally re-classified in current study). "**B**" is continued on part 2 of Fig. 121.

Support/ relationships of subfamilies

Acaenitinae

In the total-evidence analyses (parsimony and Bayesian), the two exemplars of Acaenitinae (*Spilopteron occiputale* (Cresson) and *Coleocentrus rufus* Provancher) did not cluster together. *Coleocentrus rufus* was sister to *Collyria catoptron* Wahl (Collyriinae), whereas *S. occiputale* had various placements within Pimpliformes, such as sister to *Cylloceria melancholica* (Gravenhorst) (Cylloceriinae) in the total-evidence parsimony analysis (part 2 of Fig. 117), with two of the orthocentrines in the molecular parsi-

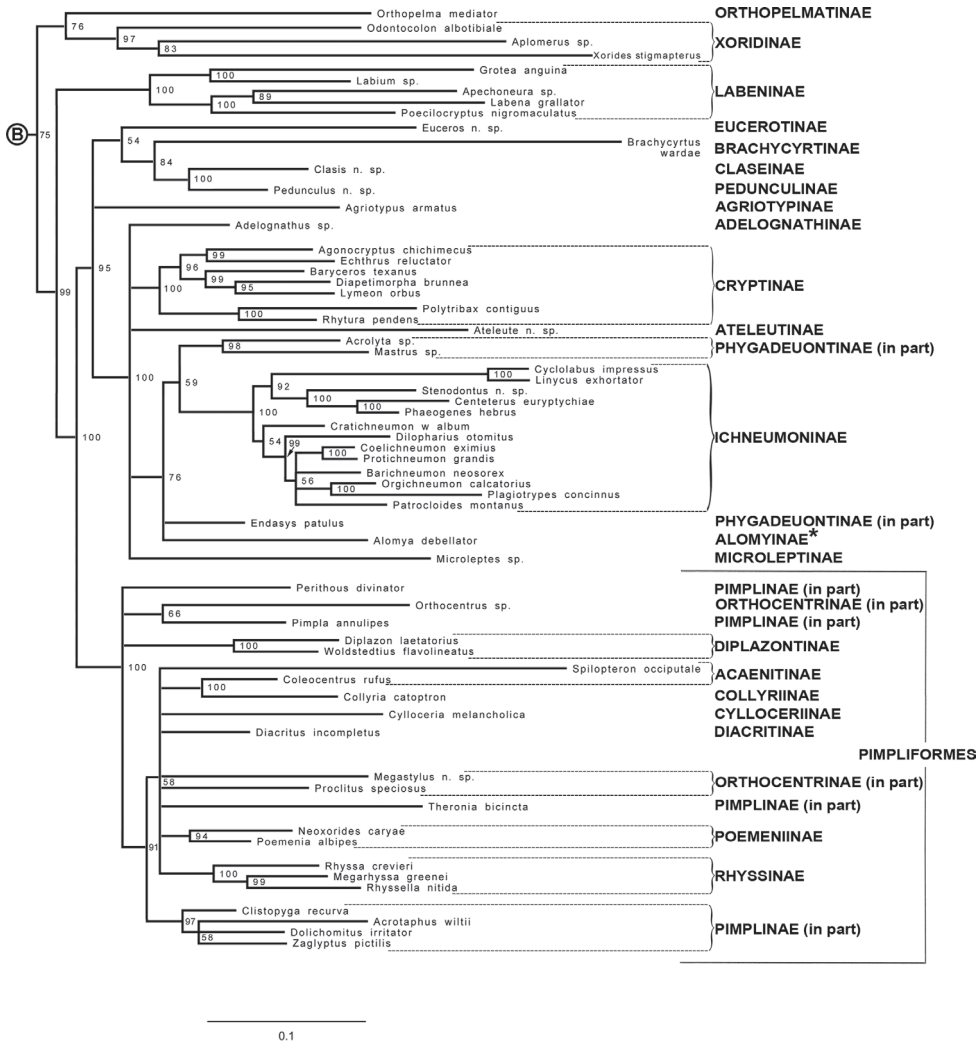


Figure 121. Part 2. Bayesian analysis (molecular characters only) maximum clade credibility tree continued from part 1 of Fig. 121. Numbers to the right of nodes are posterior probabilities. See Fig. 121, part 1 figure heading for description of figure.

mony analysis (part 2 of Fig. 119) and in a grouping with *C. melancholica*, *Diacritus incompletus* Momoi (Diacritinae) and the pair of *Coleocentrus rufus* and *Collyria catoptron* in the total-evidence Bayesian analysis (part 2 of Fig. 120). The only analysis in which the two Acaenitinae exemplars were sister taxa was in the morphological parsimony analysis (part 1 of Fig. 118). This grouping was supported by six morphological synapomorphies of which one was uniquely derived character 94(3) (female hypopygium extending far beyond apex of posterior tergites, strongly triangular in profile) (Fig. 87).

Wahl and Gauld (1998) stated that "the unusual and highly autapomorphic female hypopygium make the Acaenitinae one of the most distinctive of all ichneumonid subfamilies." Their analysis; however, did not test the monophyly of the subfamily as it was scored as a single line of code in their phylogenetic matrix. Quicke et al. (2009) analyzed the subfamilies as three groups: *Procinetus* Förster, *Coleocentrus* Gravenhorst and "other Acaenitinae". Their morphology-only analysis and total-evidence analysis recovered Acaenitinae as paraphyletic as follows: Diacritinae + (*Procinetus* + (((*Coleocentrus* + other Acaenitinae) + (Cylloceriinae + ((Diplazontinae + Collyriinae + *Hyperacmus* Holmgren) + Orthocentrinae))). They noted an additional morphological synapomorphy of the subfamily: three venom gland insertions (instead of one or two) (see fig. 9C, D in Quicke et al. 2009), although *Procinetus* was not coded for this character. Finally, Klopfstein et al. (2019) included five species of Acaenitinae in their anchored enrichment analysis and found strong support for monophyly of their four Acaenitini exemplars, but *Coleocentrus excitator* (Poda) clustered inconsistently with various groups within Pimpliformes, but almost never shared a unique, common ancestor with Acaenitini. So is Acaenitinae monophyletic?

The current analysis and Klopfstein et al. (2019) did not include *Procinetus*, therefore its placement within Acaenitinae cannot be commented on except subjectively to say that the female does have an elongate, triangular hypopygium, although as Townes 1971 observed, this structure has a deep, medial notch resembling the form in atrophine Banchinae. With respect to the other genera of Acaenitinae, the results of Quicke et al. (2009) generally supported their monophyly, whereas the current analysis and Klopfstein et al. (2019) generally do not. It must be stated that *Spilopteron* spp. are somewhat aberrant molecularly relative to other Acaenitinae (see Genbank sequences from Quicke et al. (2009); Ito et al. (2015)). For example, the D2 region of 28S of *S. occiputale* is only 74.5% similar to that of *C. rufus* (*C. rufus* is 96.6% similar to our *Perithous divinator* (Rossi) sequence and of comparable similarity to other species of Pimpliformes in our analysis). It is possible that use of a different Acaenitini exemplar instead of *S. occiputale* may have resulted in support of Acaenitinae in the current study, but the study of Klopfstein et al. (2019) included *Spilopteron occiputale* and it always clustered with the other three Acaenitini species, therefore monophyly of Acaenitinae appears to be more an issue of whether Coleocentrini and Acaenitini share a common ancestor, rather than monophyly of Acaenitini. More information on the hosts, biology and larvae of Acaenitinae may also help elucidate the monophyly of the family. Known hosts are stem or wood-boring beetles (e.g. Finlayson 1970; Shaw and Wahl 1989), but biology and larval morphology are unknown for most genera and substantiation of existing host records is required.

Adelognathinae

The total-evidence parsimony analysis found Adelognathinae to be the sister group to ((Microleptinae + Ateleutinae) + ((Aptesini + Cryptini including *Echthrus reluctator* (Linnaeus)) + (Phygadeuontinae + (*Alomya debellator* (Fabricius) + Ichneumoninae))) (part 3 of Fig. 117). This grouping was supported by 3 morphological synapomorphies

(15 total) including the uniquely derived character 9(1) (flagellum of male with elliptical or raised, longitudinal, ridge-like tyloids on ventral surface) (Fig. 19). Note that contrary to Townes (1969), some male *Adelognathus* species have tyloids, including our exemplar specimen. The total-evidence Bayesian analysis was also similar, although the relationships of *Adelognathus* sp., *Microleptes* sp. and *Ateleute* sp. nov. at the base of this clade were not unequivocally resolved (part 2 of Fig. 120). The morphology-only parsimony analysis provided no clear placement of *Adelognathus* sp. (it was unresolved near the base of the tree) (Fig. 118).

Townes (1969) implied that Adelognathinae was related to Pimplinae, Tryphoninae, Labeninae and Xoridinae. Presumably, this was because of the medial placement of the spiracle of T1 and the lack of a dorsal, subapical notch on the ovipositor. Both of these character states are plesiomorphic in Ichneumonidae, and therefore of no use in ascertaining phylogeny. Quicke et al. (2009) most usually found the following: Agriotypinae + (Adelognathinae + Cryptinae) and included these three subfamilies in their Ichneumoniformes grouping along with Alomyinae and Ichneumoninae. Most of our analyses placed Adelognathinae within Ichneumoniformes s.l., although its exact placement is equivocal at this point depending on the type of phylogenetic analysis and the characters used. Some of the analyses of Santos (2017) recovered *Adelognathus* among his Phygadeuontinae exemplars but none of our results supported this arrangement.

Agriotypinae

The total-evidence parsimony analysis found that *Agriotypus armatus* was sister species to *Euceros* sp. nov. (Eucerotinae) and these two species were sister group of the clade listed above under Adelognathinae , (i.e., Adelognathinae… to Ichneumoninae) (part 3 of Fig. 117). Agriotypinae + Eucerotinae was supported by 29 characters states, of which 8 were morphological, including one that was uniquely derived: oviposition into Trichoptera cases (character 140(6)) with a state change to oviposition on leaves for Eucerotinae (character 140(8)). The Bremer support value was 4 steps. The total-evidence Bayesian analysis was similar in that Agriotypinae and Eucerotinae were placed unresolved as sister group to a clade including Microleptinae, Adelognathinae, Cryptinae and Ichneumoninae, although this clade was very poorly supported (BPP = 59) (part 2 of Fig. 120). In stark contrast to both of the total-evidence analyses, the morphological parsimony analysis recovered Agriotypinae as the sister taxon to all other Ichneumonidae (Fig. 118).

The monotypic Agriotypinae is morphologically aberrant in that it has strongly sclerotized posterior metasomal tergites, which is an autapomorphy within Ichneumonoidea. They are also biologically unusual in that they are aquatic idiobiont ectoparasitoids of prepupae and pupae of Trichoptera in fast-running streams (Bennett 2001). Earlier classifications (e.g., Haliday 1838) placed *Agriotypus* Curtis within its own monotypic family. This family status was maintained by some relatively recent authors (e.g., Mason 1971; Chao 1992); however, Townes (1969) placed the genus within Ichneumonidae, and Sharkey and Wahl (1992) concurred, stating that *Agriotypus* shared the two autapomorphies of Ichneumonidae: the apical displacement of

vein Rs of the fore wing to form the characteristic ichneumonid areolet and the shortening or loss of vein Rs+M in the fore wing (our character 60, states 1 and 2) (Fig. 4). Wahl and Gauld (1998) recovered Agriotypinae in a clade as follows: Labeninae + (Agriotypinae + (Brachycyrtinae + (Cryptinae + Ichneumoninae))). Note that this study used a limited number of subfamilies as outgroups, their purpose being to establish character polarity within Pimpliformes. Bennett (2001) using a morphological cladistic analysis with limited outgroups found Agriotypinae to be sister group to Labeninae. Quicke et al. (2009) found various placements for Agriotypinae depending on the analysis, but concluded that it was likely part of Ichneumoniformes, or perhaps associated with Brachycyrtinae.

With respect to the putative relationship of Agriotypinae and Eucerotinae, of the eight morphological synapomorphies, five of them undergo transformations/ reversals in one taxon or the other. For example, the uniquely derived character 140(6) (oviposition into Trichoptera cases) which supports both taxa, changes to character 140(8) (oviposition on to leaves) in Eucerotinae. Given the extremely different morphology and biology of these two subfamilies, it appears likely that these two highly derived taxa have clustered together because of coincidental homoplasious traits and the lack of any phylogenetic signal linking them to another group. Having said that, both the parsimony and Bayesian total-evidence analyses placed Agriotypinae in relatively the same part of the tree (i.e. somewhere within Ichneumoniformes s.l.), which is similar to the conclusions of Quicke et al. (2009) and Santos (2017).

Alomyinae

In the total-evidence parsimony and Bayesian analyses (Figs 117, 120) and the morphological parsimony analysis (Fig. 118), *Alomya debellator* was sister taxon to Ichneumoninae. Both molecular-only analyses placed *A. debellator* close to Ichneumoninae (along with various cryptine exemplars), but not as its sister taxon (Figs 119, 121).

Since the beginning of the 20th century, *Alomya* has been recognized as closely associated with Ichneumoninae. It has been treated variously as a separate subfamily by Perkins (1959a, 1959b) and Constantineanu (1965), as an ichneumonine tribe consisting solely of *Alomya* (Schmiedeknecht 1902, Morley 1915, Kasparyan 1981), or as part of a tribe including *Phaeogenes* Wesmael and its relatives (Townes et al. 1961, Yu and Horstmann 1997). These decisions were based solely upon adult morphology in a non-phylogenetic framework. Hinz and Short (1983) reared and described the last instar larva commenting that "larval characters generally indicate an affinity with Ichneumoninae, particularly in the disc-shaped maxillary and labial palps each bearing five sensilla of about equal size" (our Character 123, state 2) (Figs 109, 125). They did; however, conclude that bearing in mind the unusual biology (mummification of the host), it should be placed in its own tribe (Alomyini) within Ichneumoninae. Wahl and Mason (1995), in communication with H.K. Townes, stated that *Alomya* was related to *Centeterus* Wesmael and *Colpognathus* Wesmael, genera placed within the ichneumonine tribe Phaeogenini which should therefore take the name Alomyini. More recently, Laurenne et al. (2006) performed a molecular parsimony analysis

including Cryptinae, Ichneumoninae, *Alomya* and a species of the putatively related *Pseudalomya* Telenga and found that *Alomya* was never recovered within Ichneumoninae, whereas *Pseudalomya* clustered with the Phaeogenini exemplars. They therefore formally re-established Alomyinae to encompass *Alomya* as well as the morphologically similar *Megalomya*. They did not include *Pseudalomya* within Alomyinae. The addition of adult morphological characters to these sequences by Quicke et al. (2009) found that *Alomya* was the sister group of Ichneumoninae (and therefore could be postulated to be included within Ichneumoninae. In the same study, *Pseudalomya* still clustered within Phaeogenini. What do our current results indicate about the placement and status of Alomyinae?

Our study, with three genes and a large morphological data set, generally places *Alomya* as the sister group of the Ichneumoninae (both total-evidence analyses and parsimony with only morphological characters). Based on these results, it would be possible to expand Ichneumoninae to encompass *Alomya* (as a tribe), although maintenance of Alomyinae is equally acceptable. We prefer to classify *Alomya* and the closely related genus *Megalomya* Uchida as a tribe within Ichneumoninae, and therefore formally synonymize Alomyinae with Ichneumoninae. Since both classifications are equally acceptable, there is no need for a discussion of similarities and differences between Ichneumoninae and *Alomya* as this would not justify the rank of Alomyini/ Alomyinae one way or the other. A detailed re-description of the larva of *Alomya* is presented in Appendix 1 (Fig. 125).

Anomaloninae

The two Anomaloninae exemplars (*Anomalon picticorne* (Viereck) and *Therion texanum* (Ashmead)) clustered together in the total-evidence and morphology-only parsimony analyses (Figs 117, 118). Thirty-one total characters supported Anomaloninae in the total-evidence parsimony analysis, including nine morphological, of which one was uniquely derived: character 134(2) (egg with a wide, pedunculate, ventral protrusion and an apical, sinuous stalk) (Fig. 114). The Bremer support was 4 steps. It should be noted that not all anomalonines have this egg structure and the egg of *A. picticorne* was scored as unknown. In terms of diagnostic characters for Anomaloninae, character 47(1) (propodeum with reticulate sculpture) (Fig. 42) was a synapomorphy for the subfamily, but this character state also evolved independently in *Thyreodon* sp. (Ophioninae) and *Casinaria grandis* Walley (Campopleginae), therefore it must be used in concert with other characters to distinguish Anomaloninae.

Anomaloninae was the sister group of Ophioninae in the total-evidence parsimony analysis, supported by 33 characters (13 morphological) including the uniquely derived character 54(2): cell *1 +2Rs* (areolet) of fore wing with vein *Rs* absent so that the only cross-vein is distad vein *2m-cu* (Fig. 53) (with a reversal in *Therion texanum*). The Bremer support was only 3. The Bayesian total-evidence analysis also recovered Anomaloninae, but only with a posterior probability of 84 (Fig. 120) and as sister group to *Nonnus* sp. (Nesomesochorinae) (posterior probability of 82). Anomaloninae was not supported in either of the molecular-only analyses (Figs 119, 121).

Previous morphological analyses (e.g., Gauld 1976) supported the monophyly of Anomaloninae. Quicke et al. (2009) coded morphology at the tribal level for Anomaloninae and found that Anomalonini was the sister group to Gravenhorstiiini in both their morphological and total-evidence parsimony analyses. Whereas it is possible that Anomalonini (i.e., *Anomalon picticorne*) and Gravenhorstiini (as represented by *Therion texanum*) are not sister taxa, the weight of evidence supports this relationship. Lack of congruence between our molecular and total-evidence analyses may be because of our choice of exemplars or genes which should be examined by addition of more Gravenhorstiini species and additional gene regions in future studies.

Ateleutinae

Ateleute sp. nov. was placed wihin Ichneumoniformes s.l. in all of our analyses, except parsimony with only morphological characters in which it was sister species to *Nonnus* sp. in a portion of the tree that lacked resolution (part 1 of Fig. 118). *Ateleute* was sister species to *Microleptes* sp. (Microleptinae) in the other two parsimony analyses (part 3 of Fig. 117, part 2 of Fig. 119), sister species to *Adelognathus* sp. (Adelognathinae) in the total-evidence Bayesian analysis (part 2 of Fig. 120) or in a clade that lacked internal resolution with *Adelognathus* sp., *Microleptes* sp., *Alomya debellator*, Cryptinae, Ichneumoninae and exemplars of Phygadeuontinae (Bayesian molecular-only analysis: part 2 of Fig. 121). In summary, in all analyses except for parsimony with only morphological characters, it grouped with exemplars of the six subfamilies listed above, but at no time did it group within Cryptinae or any other subfamily.

Previously, Townes et al. (1961) placed *Ateleute* within Phygadeuontini, but later moved it to Cryptini (Townes 1970a) within its own subtribe (Ateleutina). The molecular analysis of Laurenne et al. (2006) also found equivocal placement for *Ateleute*. Depending on alignment parameters, it was sometimes placed as sister to: ((most of Hemigastrini) + Cryptini), or in an unresolved clade of *Ateleute*/ Phygadeuontini/ (Hemigastrini + Cryptini), or even as sister to Ichneumoninae. The combined morphology and molecular analysis of Quicke et al. (2009) coded morphological characters for *Ateleute* separately from all other Cryptinae species and recovered *Ateleute* in a clade of five species that was sister to a clade including all Hemigastrini and Cryptini exemplars. In the latter analysis, *Ateleute* spp. clustered with exemplars of a second genus of Ateleutinae (*Tamaulipeca* Kasparyan) as well as *Handaoia* Seyrig and *Austriteles* Gauld (both Phygadeuontini). More recently, Santos (2017) recovered a clade of six exemplars of *Ateleute* and *Tamaulipeca* as sister group to Adelognathinae in his parsimony analysis and nested with some of his Phygadeuontini exemplars in his maximum likelihood analysis. On the basis of our parsimony and Bayesian analyses, we concur with the raising of Ateleutina to subfamily status by Santos (2017), but its exact placement within Ichneumoniformes s.l. is still equivocal.

Banchinae

The four exemplar species of Banchinae were closely related in all analyses. The two Glyptini species (*Apophua simplicipes* (Cresson) and *Sphelodon phoxopteridis* (Weed))

were always sister species and Atrophini (*Lissonota scutellaris* (Cresson)) was generally sister to Banchini (*Exetastes bioculatus* Cresson). In the total-evidence parsimony analysis, Glyptini, (Atrophini + Banchini) and *Notostilbops* sp. (Stilbopinae) were found in a clade that lacked internal resolution (part 6 of Fig. 117). This grouping was strongly supported: 47 total characters (7 morphological) of which 2 were uniquely derived-character 95(1) (hind margin of hypopygium with median apical notch) (Fig. 88); character 114(1) (larval posterior struts of inferior mandibular processes greater than two times length of anterior struts and connected by a band) (Fig. 103). The Bremer support was 10+ for this node. Moving *Notostilbops* sp. so that it was sister species to *Stilbops vetulus* resulted in a tree that was 13 steps longer (9930). Note that *Notostilbops* sp. was scored as 0 for character 95 (apical notch of hypopygium absent) and as unknown for character 114 (larva of *Notostilbops* Townes is not known). *Stilbops vetulus* (Gravenhorst) (Stilbopinae) was sister to the clade comprised of *Notostilbops*/ Atrophini + Banchini/ Glyptini. The total-evidence Bayesian analysis had *Notostilbops* sp. as the sister of the four banchine species (posterior probability of 100), but Banchinae was only supported with a posterior probability of 92 (part 1 of Fig. 120). Both analyses with only molecular characters found *Notostilbops* to be sister to *Lissonota*, clustering within Banchinae (Figs 119, 121). The morphological parsimony analysis was the only one that found unequivocal support for the monophyly of Banchinae (without *Notostilbops*) (Fig. 118). The strict consensus tree for this analysis had equivocal placement of *Notostilbops*, but in 98 % of the 3872 equally parsimonious trees, it was sister to *Stilbops vetulus* and in 88% of the trees, these two taxa formed a sister group to the eight Tryphoninae species (and no trees had either of the stilbopines sister group to Banchinae). To summarize our results, the parsimony analysis with only morphological characters supported Banchinae with no evidence that either stilbopine exemplar was related to Banchinae (Fig. 118); molecular evidence found that *Notostilbops* is a banchine (and *Stilbops* is sister to Banchinae) (Figs 119, 121) and this was the same result as the total-evidence analyses, except that the relationships in the *Notostilbops* + Banchinae clade was equivocally resolved (Figs 117, 120).

Wahl (1988) commented on the placement of *Notostilbops*. He stated that most *Notostilbops* females have a membranous region apically in the hypopygium where the notch is present in Banchinae, although some *N. fulvipes* (Townes) have a distinct notch. In terms of the ovipositor, all *Notostilbops* females have a distinct dorsal subapical notch which is present in Banchinae, but absent in the two other genera currently assigned to Stilbopinae: *Stilbops* and *Panteles* Förster. On the basis of these two apomorphic character states and the weight of evidence in our study, *Notostilbops* appears better placed in Banchinae (either within Atrophini or *incertae sedis*). We formally transfer *Notostibops* Townes to tribe Atrophini of Banchinae. Its tribal placement is based on the fact that it does not possess synapomorphies that would place it within Glyptini (e.g., T2–T4 of metasoma with chevron-shaped grooves) or Banchini (e.g., character 63, state 1: hind wing vein *2/Cu* much closer to vein M than A). The monophyly of Atrophini relative to Banchini and Glyptini has not been thoroughly tested with a morphological, cladistic analysis and this would be required to confirm monophyly of

Atrophini, and placement of *Notostilbops* within it. Wahl (1988) stated that knowledge of the larva of *Notostilbops* would help determine its placement (presence of the band connecting the larval mandibular processes would support its placement in Banchinae). Quicke et al. (2009) generally recovered Banchinae as monophyletic near the base of Ophioniformes, but not in a sister-group relationship with Stilbopinae (which clustered with Tryphoninae). They did not include *Notostilbops* in their analyses.

Brachycyrtinae

The placement of *Brachycyrtus wardae* Bennett varied somewhat between our analyses. Total-evidence parsimony placed it as the sister to Labeninae, supported by 32 characters (6 homoplasious morphological) with a Bremer support of 10 (part 3 of Fig. 117). This pairing was sister to (Claseinae + Pedunculinae) and together these taxa were sister to the rest of Ichneumoniformes s.l. The total-evidence Bayesian analysis recovered (Brachycyrtinae + (Claseinae + Pedunculinae)) with a posterior probability of 97 (part 2 of Fig.120). The exact relationships of this grouping to Agriotypinae, Eucerotinae and the rest of Ichneumoniformes s.l. is unclear because of very low posterior probabilities (54 to 59) for the nodes in this part of the tree. Brachycyrtinae was not related to Labeninae in the total-evidence Bayesian analysis, with Labeninae placed as sister group to a well-supported clade (posterior probability of 98) comprised of Pimpliformes + Ichneumoniformes s.l..

As noted in the Introduction, Wahl (1993a) placed Brachycyrtinae within his Ichneumoniformes s.s. as the sister group to (Cryptinae + Ichneumoninae). Our results do not support this precise relationship; however, our total-evidence parsimony analysis (part 3 of Fig. 117) suggests that Brachycyrtinae belongs within Ichneumoniformes s.l. Quicke et al. (2009) found that Claseinae and Pedunculinae usually clustered with Brachycyrtinae and sometimes also with Eucerotinae and Microleptinae. Their Brachycyrtiformes: (Claseinae + (Pedunculinae + Brachycyrtinae)), was not supported in our total-evidence parsimony analysis without Labeninae nested within as sister to Brachycyrtinae (Bremer support of 10 steps). The total-evidence Bayesian analysis did support it, but not unequivocally (BPP = 97). Since both our total-evidence parsimony and Bayesian analyses did not support the group unequivocally, we prefer not to recognize Brachycyrtiformes exactly as Quicke et al. (2009) proposed it until the relationship of Labeninae to these taxa can be more clearly defined. See the sections below for Claseinae, Labeninae and Pedunculinae for more discussion on the relationships of these taxa.

Campopleginae

Both total-evidence analyses strongly supported the monophyly of the eight exemplar species of Campopleginae. In the parsimony analysis, the subfamily was supported by 43 synapomorphies (5 morphological, none of which were uniquely derived) with a Bremer support value of 10+ (part 6 of Fig. 117). The Bayesian analysis also supported the subfamily with a posterior probability of 100 (part 1 of Fig. 120). Both molecular-only analyses supported the monophyly of Campopleginae (Figs 119, 121), whereas the morphological parsimony analysis found that *Casinaria grandis* and *Dusona egregia*

(Viereck) did not cluster with the other six species (Fig. 118). Cremastinae was the sister group to Campopleginae in both total-evidence analyses although the support in the parsimony analysis was relatively low (26 characters including 3 morphological of which none were uniquely derived and a Bremer support value of 1). Internally, both total-evidence analyses found the same topology, with *Campoplex* sp. sister to the other seven species and *Rhimphoctona macrocephala* (Provancher) sister to the rest.

Townes (1970b) divided Campopleginae into four tribes. Wahl (1991) commented on these tribes from a cladistic viewpoint and recommended suspension of Townes's tribal classification, instead recognizing five informal genus groups. He also hypothesized that Cremastinae was the sister group to Campopleginae. Miah and Bhuiya (2001) performed a morphological cladistic analysis finding that Townes's tribes Hellwigiini and Nonnini (= Nesomesochorini) did not belong in Campopleginae. Later, Quicke et al. (2005) based on morphology and molecular data transferred Hellwigiini (*Skiapus* and *Hellwigia* Gravenhorst) to Ophioninae and resurrected Nonninae for *Nonnus* Cresson and Nesomesochorinae for *Chriodes* Förster and *Klutiana* Betrem. Quicke et al. (2009) placed the latter two genera together within Nesomesochorinae. The latter study found that either Cremastinae or Nesomesochorinae was sister to Campopleginae, depending on how gaps were treated in the molecular data. In contrast, the anchored enrichment study of Klopfstein et al. (2019) recovered Cremastinae + (Ophioninae + Campoplegeinae).

The current results strongly support the monophyly of Campopleginae (i.e., the group comprised of the genera that were previously classified in Townes's Campoplegini and Porizontini). They also support the removal of Hellwigiini and Nesomesochorini from Campopleginae. In terms of the sister-group relationship, both total-evidence analyses support Cremastinae as sister to Campopleginae with Nesomesochorinae and (Ophioninae + Anomaloninae) also related, albeit more distantly. With respect to internal relationships, Townes's Campoplegini and Porizontini were not supported (*Campoplex* sp. was sister to all other genera, but the other Campoplegini exemplar, *Casinaria grandis*, clustered within the six Porizontini species. Likewise, there was no support for the genus groups of Wahl (1991). For example, the two exemplar species from Wahl's *Bathyplectes* genus group (*Bathyplectes infernalis* (Gravenhorst) and *Rhimphoctona macrocephala*) were not sister taxa. A more comprehensive analysis is required to define natural groups within the subfamily.

Claseinae

Claseinae (*Clasis* sp. nov.) was sister group to Pedunculinae (*Pedunculus* sp. nov.) in all analyses except the morphology-only parsimony analysis in which it was unresolved near the base of Ichneumonidae (part 1 of Fig. 118). In the total-evidence parsimony analysis, (Claseinae + Pedunculinae) was supported by 17 synapomorphies, 4 of which were morphological (none uniquely derived) and a Bremer support of 9 (part 3 of Fig. 117). The Bayesian total-evidence analysis supported this relationship with a posterior probability of 100 (part 2 of Fig. 120). See Brachycyrtinae (above) for more discussion of the relationships of these two subfamilies to other taxa.

Clasis Townes was originally placed within the cryptine tribe Phygadeuontini (Townes and Townes 1966) and then later, Townes (1969) moved it to Labeninae as a tribe (Clasini) along with the newly described monotypic *Ecphysis* Townes. Gauld (1983) moved Clasini back into Cryptinae as its own tribe. Porter (1998) raised the group to subfamily status. Quicke et al. (2009) found that Claseinae was the sister group of (Pedunculinae + Brachycyrtinae) in most of their analyses. Santos (2017) never recovered his three *Clasis* species within Phygadeuontini or Cryptinae but instead, they clustered together as sister to Agriotypinae, (Agriotypinae + Labeninae) or (Brachycyrtinae + Eucerotinae), depending on the method of analysis.

The fact that our total-evidence parsimony analysis supports the monophyly of Labeninae as it was defined by Townes (1969) (i.e., including *Clasis*, *Pedunculus* Townes and *Brachycyrtus* Kriechbaumer) is intriguing, considering how much work has been done studying and ultimately dividing this group. Certainly, the arrangement of Townes (1969) is not exactly the same as our parsimony results (e.g., Townes placed *Pedunculus*, *Brachycyrtus* and *Poecilocryptus* Cameron into a single tribe), but the fact that all eight of the species we chose from the Labeninae of Townes (1969) clustered together in our total-evidence parsimony analysis raises the question, should Labeninae be re-established to include Brachycyrtinae, Claseinae, Pedunculinae and Labeninae as it is currently defined? One of the arguments against this change is that our total-evidence Bayesian analysis recovered Labeninae (in the current sense) unrelated to (Brachycyrtinae + (Claseinae + Pedunculinae)) (as sister to Pimpliformes + Ichneumoniformes s.l. except for Labeninae). In addition, Townes (1969) did not provide any characters that define his concept of Labeninae. Since the relationships of Labeninae, Brachycyrtinae and (Claseinae + Pedunculinae) are equivocal among our five analyses, and the results of Santos (2017) were similarly equivocal, it seems prudent to maintain the subfamily status of all four of these subfamilies, rather than sink them back into Labeninae. Nevertheless, it is interesting that this study has recovered one of Townes's previously recognized groupings, similar to the study of Klopfstein et al. (2019) that moved *Pseudorhyssa* Merrill back into Pimplinae and resurrected tribe Theroniini.

Collyriinae

Both total-evidence analyses (Figs 117, 120) and both molecular-only analyses (Figs 119, 121) found that *Collyria catoptron* was sister species to *Coleocentrus rufus* (Acaenitinae) within Pimpliformes. *Coleocentrus rufus* was not however, closely related to the other acaenitine exemplar: *Spilopteron occiputale* (see Acaenitinae, above). The total-evidence parsimony analysis supported (*Collyria catoptron* + *Coleocentrus rufus*) based on 23 synapomorphies (6 morphological of which none were uniquely derived) with a Bremer support of only 1. The relative placement of this sister-group pairing within Pimpliformes is not clear because of major differences in the overall topology of the group depending on the phylogenetic method used (see Pimpliformes section, above). The placement of Collyriinae in the morphological parsimony analysis was also not clearly resolved (Fig. 118).

Historically, the taxonomic placement of *Collyria* has been contentious. Dalla Torre (1902) and Morley (1908) related it to *Acaenitus* Latreille and *Arotes* Gravenhorst (Acaenitinae), but the latter noted that previous British catalogues placed it within Ophioninae (i.e. Ophioniformes) related to *Pristomerus vulnerator* (Panzer) (Cremastinae). Cushman (1924) placed it in a new tribe, Collyriini within Pimplinae (i.e., Pimpliformes). Townes (1971) stated: "Neither the adult nor the larva, however, have any real resemblance to the acaenitines" and he placed *Collyria* in a separate subfamily, arranged close to *Orthopelma* Taschenberg (Orthopelmatinae) and Orthocentrinae (although this part of his Genera of Ichneumonidae seems to be where he placed taxa of uncertain affinity). Short (1978) simply stated it was "isolated on both larval and adult characters". Wahl and Gauld (1998) did not include it in their analysis of the Pimpliformes; however, Belshaw et al. (1998) and Quicke et al. (2009) recovered Collyiinae within Pimpliformes, the latter study placing it within the Diptera-parasitizing clade (Diplazontinae, Orthocentrinae and Cylloceriinae). Note that species of *Collyria* are egg-larval, koinobiont endoparasitoids of Cephidae (Hymenoptera) (Salt 1931; Wahl et al. 2007), whereas the few known records of Acaenitinae show that they are larval koinobiont endoparasitoids of Coleoptera (Shaw and Wahl 1989).

More recently, Kuslitzky and Kasparyan (2011) described a second genus of Collyriinae: *Aubertiella* Kuslitzky & Kasparyan, from the Middle East and Sheng et al. (2012) described a third genus (*Bicurta* Sheng, Broad & Sun) from southeastern China. The latter study also re-examined the phylogenetic analysis of Wahl and Gauld (1998) with the addition of *Collyria* and *Bicurta* and found that they clustered together within Pimpliformes, but in terms of their placement, relationships in their unweighted parsimony analysis were largely unresolved. Searching with implied weights (Goloboff 1993) with values ranging from k = 1 to k = 10 resulted in a single topology in which *Bicurta* + *Collyria* was the sister group to Rhyssinae. Sheng et al. (2012) did, however, note that Wahl and Gauld (1998) coded all Acaenitinae at the subfamily level in their matrix and suggested that re-coding all genera of Acaenitinae separately could reveal different patterns of relationships within Pimpliformes, especially relative to Collyriinae. They also noted some similar general characteristics between Collyriinae and some Acaenitinae and Poemeniinae namely, short antennae, median tubercle on the clypeus (our character 3, state 1), lack of transverse carinae of the propodeum (our characters 36 and 38, state 3) and hind wing vein *2/Cu* originating close to vein *M* (our character 63, state 1). Characters 3, 36 and 63 supported the grouping of *Coleocentrus* + *Collyria* in our total-evidence parsimony analysis (part 2 of Fig. 117). Most recently, the transcriptome analysis of Klopfstein et al. (2019) found a sister-group relationship of *Collyria trichophthalma* (Thomson) and *Coleocentrus excitator* (Poda), the only exemplars of these two subfamilies in this analysis. Their anchored enrichment analyses found the placement of *Collyria* to be unstable with some analyses placing it as sister to all other Pimpliformes and in others it was related to *Coleocentrus* or Acaenitini. In summary, the relationships of Collyriinae are still equivocal, but it may be related to Acaenitinae. Finally, it is noted that the description of the final larval instar cephalic

sclerites of *Collyria coxator* (Villers) by Short (1959) is inaccurate. We have examined the slide mount used by Short. The mandibles are completely absent, and there is no trace of the antenna. A whole larva of *Collyria catoptron* has been photographed (Fig. 112) and a revised description of the cephalic sclerites of the mature larva of *Collyria* is provided in Appendix 1.

Cremastinae

The two species of Cremastinae, *Eiphosoma pyralidis* Ashmead and *Xiphosomella setoni* Johnson, clustered together in all analyses with very strong support. The total-evidence parsimony analysis supported this grouping with 61 synapomorphies (5 morphological) and a Bremer support value of 10+ (part 6 of Fig. 117). Character 71, state 1 (apex of middle and hind tibiae with sclerotized bridge separating insertion areas) (Fig. 65) was uniquely derived. Cremastinae was sister group to Campopleginae in both total-evidence analyses (Figs 117, 120) and these two subfamilies grouped with Nesomesochorinae and (Anomaloninae + Ophioninae) in the higher Ophioniformes.

The monophyly of Cremastinae has generally gone unquestioned, supported largely by the unique synapomorphy (within Ichneumonidae) of the sclerotized bridge of the middle and hind tibiae separating the insertion points of the tarsus and tibial spurs (Gauld et al. 2000). Quicke et al. (2009) coded morphology separately for the *Belesica* group (*Belesica* Waterston and *Eurygenys* Townes) relative to "other Cremastinae". They stated that Cremastinae was monophyletic in all individually aligned analyses, but when they combined these data into a single data set (the elision strategy of Wheeler et al. 1995), *Eurygenys* sp. did not cluster with the other Cremastine, but was sister to a clade comprised of *Skiapus*, Hybrizontinae, Anomaloninae, Ophioninae, Cremastinae, Nesomesochorinae and Campopleginae. We were not able to include a representative of the *Belesica* group in our analysis, therefore the relationships of this group to other genera of Cremastinae remains ambiguous. See Campopleginae (above) for discussion of prior hypotheses of relationships of Cremastinae, Campopleginae and Nesomesochorinae.

Cryptinae sensu stricto and Cryptinae sensu lato

The seven species of Cryptinae sensu stricto, i.e., members of the tribes Cryptini and Aptesini (formerly Hemigastrini), grouped together in both of the total-evidence analyses and the Bayesian (molecular-only) analysis (Table 3). The total-evidence parsimony analysis had moderately low support for Cryptinae with 17 synapomorphies (5 morphological, including one uniquely derived: sternaulus posteriorly ending anterior to posterolateral corner of mesopleuron (character 27(2)) (Fig. 28). The Bremer support value was 3 (part 3 of Fig. 117). There was much stronger support for Cryptinae in the total-evidence Bayesian analysis (BPP = 100) (part 2 of Fig. 120).

In the analyses in which Cryptinae s.s. was monophyletic, it was always related to species in the subfamilies Ateleutinae, Microleptinae, Phygadeuontinae, Alomyinae, Ichneumoninae and Adelognathinae. The specific relationships of each of these subfamilies are discussed more fully in the section above on Ichneumoniformes, as well as in the respective subfamily sections.

The 11 species of Cryptinae sensu lato (species of Ateleutinae, Phygadeuontinae and Cryptinae s.s.) never shared a unique, common ancestor, regardless of the analysis. This was caused by: 1) placement of *Ateleute* sp. nov. (Ateleutinae) away from the other species; 2) paraphyly of the other 10 species of Cryptinae s.l. with respect to Alomyinae + Ichneumoninae. In summary, we found no evidence to support the monophyly of Cryptinae s.l. (= Gelinae) of Townes (1970a).

Regarding Phygadeuontinae, in the total-evidence parsimony analysis, the three species (*Endasys patulus* (Viereck) + (*Acrolyta* sp. + *Mastrus* sp.)) grouped together with weak support (13 synapomorphies and a Bremer support value of 1) (part 3 of Fig. 117). This group was also supported in the Bayesian total-evidence analysis, but not unequivocally (BPP = 90) (part 2 of Fig. 120). Both of these analyses placed Phygadeuontinae as the sister group to (Alomyinae + Ichneumoninae) (18 synapomorphies and a Bremer support value of 2 for the parsimony analysis and p.p of 100 for the Bayesian analysis). Therefore, in terms of the monophyly and placement of Phygadeuontinae (Phygadeuontini prior to Santos 2017), our analysis provided some support for its monophyly; however, we included only three species and the more rigorous study of Santos (2017) has shown that this group is most likely not monophyletic. Its raising to subfamily status by the latter study was necessitated by its paraphyly with Ichneumoninae, but it may be a "dumping ground" for all the "non-Aptesini" and "non-Cryptini" taxa that were previously placed within Cryptinae. See the section on Phygadeuontinae (below) for more discussion of this taxon.

With respect to the relationship between Cryptinae s.s. and Ichneumoninae, Townes (1969) stated that they were not related on the basis of differences in larval morphology. Gauld (1991) disagreed, placing the two families together in his "Phygadeuontoid" subfamilies. Wahl (1993a), in his discussion on the relationships of his Ichneumoniformes commented on the opinion of Townes (1969) stating: "The larvae are indeed very dissimilar but this is because, at the subfamilia level, Phygadeuontinae [= Cryptinae s.l.] larvae are plesiomorphic in almost every respect while larvae of Ichneumoninae are extremely specialized endoparasitoids. Larvae of Ichneumoninae can be easily derived from a Phygadeuontinae-like precursor." Nevertheless, Wahl (1993a) did not explicitly suggest paraphyly of Cryptinae and Ichneumoninae. The first author to suggest this was Gokhman (1988) who argued that the ichneumonine tribe Phaeogenini provided evidence for an "evolutionary pathway" between Ichneumoninae and Phygadeuontini.

In terms of more recent, sequence-based studies, some of the analyses of Laurenne et al. (2006) (with high gap costs) found Ichneumoninae nested within most of Phygadeuontini. The combined analysis of Quicke et al. (2009) had similar results, although their two presented cladograms propose Ichneumoninae as sister group to (Agriotypinae + (Adelognathinae + Cryptinae s.l.)).

Finally, in terms of support for the tribes of Cryptinae s.s., the total-evidence parsimony analysis had strong support for Cryptini (including *Echthrus reluctator*) with 30 synapomorphies and a Bremer support of 6, and Aptesini had 25 synapomorphies and a Bremer support of 9. The Bayesian total-evidence analysis also supported both tribes (BPP = 100 for each) (part 2 of Fig. 120).

Ctenopelmatinae

Ctenopelmatinae was never recovered as monophyletic (Table 3). The 14 species of Ctenopelmatinae did share a relatively recent common ancestor in the total-evidence parsimony analysis (part 5 of Fig. 117) along with the following small subfamilies and enigmatic genera nested in the same clade: *Hybrizon rileyi* (Ashmead) (Hybrizontinae); *Lycorina glaucomata* (Lycorininae); *Oxytorus albopleuralis* (Provancher) (Oxytorinae); *Tatogaster nigra* Townes (Tatogastrinae); *Chineater masneri* (Mesochorinae) and *Scolomus* sp. (Metopiinae). This grouping was relatively weakly supported by 18 synapomorphies (4 morphological of which none were uniquely derived) and a Bremer support of 2. In the strict consensus cladogram it was placed within Ophioniformes in a clade with equivocal relationships as follows: (Mesochorinae except *Chineater*) / (Ctenopelmatinae and related taxa) / ((Metopiinae + (*Stilbops* + Banchinae including *Notostilbops*) + higher Ophioniformes). Of interest, the clade was supported by only 12 synapomorphies including the presence of a tooth on the fore tibia (character 68(1)) (Fig. 63) with a reversal back to the lack of a tooth in Metopiinae to Campopleginae. In other words, the one morphological character that has been used to define Ctenopelmatinae in the past did not define Ctenopelmatinae (by itself) in our analysis. Sixty-seven percent of the most parsimonious trees supported a sister-group relationship of (Mesochorinae except *Chineater* + Ctenopelmatinae and related taxa). In comparison to the parsimony analysis, the total-evidence Bayesian analysis recovered a grouping that included all 14 Ctenopelmatinae species as well as *Hybrizon* and *Tatogaster* and all four species of Metopiinae, but this group did not include *Oxytorus* or *Lycorina* (part 1 of Fig. 120). The support for this clade, however, was very low (BPP = 52). None of the other analyses recovered Ctenopelmatinae as monophyletic, with or without Metopiinae, Mesochorinae or the exemplars of the small subfamilies.

In terms of support for the ctenopelmatine tribes, of the five for which multiple species were included, only two (Perilissini and Mesoleiini) had their species clustering together in the total-evidence parsimony analysis. The other three large tribes (Pionini, Euryproctini and Ctenopelmatini) were not recovered as monophyletic (part 5 of Fig. 117).

Previous studies have questioned the monophyly of Ctenopelmatinae. Gauld and Wahl (2006) stated that it is possible that Ctenopelmatinae may represent a basal grade within Ophioniformes. Our total-evidence parsimony analyses did not support this hypothesis, but the subfamily's paraphyly with respect to many other small subfamilies and the lack of resolution of most of the ctenopelmatine tribes agrees with the conclusions of Gauld et al. (1997) who stated that the classification of the group is the least satisfactory of any ichneumonid subfamily. Belshaw and Quicke (2002) using the 28S D2–D3 region found that their five ctenopelmatine exemplars were paraphyletic with respect to *Mesochorus* sp. (Mesochorinae) and *Colpotrochia cincta* (Scopoli) (Metopiinae). The much more extensive analysis of Quicke et al. (2009) found Tryphoninae as the sister group to a grade of Ctenopelmatinae leading to the rest of Ophioniformes. The position of the various Ctenopelmatinae groupings relative to themselves and the other Ophioniformes subfamilies varied greatly, depending on whether gaps were treated as informative or not. Based on the current and previous studies, is there a way forward to create a natural classification of the Ctenopelmatinae and related taxa?

Our total-evidence analyses indicate that the taxa currently comprising Ctenopelmatinae belong within Ophioniformes and yet not within the "higher Ophioniformes" (Anomaloninae, Campopleginae, Cremastinae, Nesomesochorinae, Ophioninae). Ctenopelmatinae may be related to Mesochorinae (part 4 of Fig. 117), (perhaps mesochorines could even be derived from within it) (part 1 of Fig. 120) and several small superfamilies may also need to be placed within it (e.g., Tatogastrinae, Oxytorinae) (part 5 of Fig. 117). It may be related to Metopiinae (part 1 of Fig. 120), or it may not (part 4 of Fig. 117). Beyond this, the subfamily is not defined by any morphological synapomorphy. Taxa in several other subfamilies have the fore tibial tooth including Mesochorinae (Gauld and Wahl 2006), Sisyrostolinae (Bennett et al. 2013) and some Tryphoninae (Bennett 2015), and the distinctiveness of the tooth varies within Ctenopelmatinae. The 28S D2–D3 gene by itself does not seem to provide clear resolution of this part of the ichneumonid phylogeny, as evidenced by the major differences in arrangement of ctenopelmatines based on differing gap treatments and costs in Quicke et al. (2009). Addition of COI barcoding region and EF1a in the current study may have helped us find molecular characters to support Ctenopelmatinae (including some of the small subfamilies), but the results of our total-evidence parsimony analysis may have also been affected by our relatively low number of exemplar ctenopelmatines. It is possible that adding more exemplar species may create instability in the topology as was seen in Quicke et al. (2009). In terms of monophyly of the tribes of Ctenopelmatinae, we think it likely that their monophyly in some of the analyses of Quicke et al. (2009) was an artefact of the coding of morphology at the tribal level for Ctenopelmatinae. The major differences in topology among our five analyses and previous studies corroborate Gauld et al. (1997) that internal relationships of Ctenopelmatinae and relationships of these taxa to other subfamilies are one of the least clearly resolved parts of the ichneumonid phylogeny.

Cylloceriinae

In the total-evidence parsimony analysis, Cylloceriinae (*Cylloceria melancholica*) was sister taxon to *Spilopteron occiputale* (Acaenitinae) in the middle of our Pimpliformes grouping (part 2 of Fig. 117). This relationship was supported by 22 synapomorphies, of which only one was morphological: character 13, state 1: occiput with medial notch present near foramen magnum, a trait evolved independently in *Poemenia albipes* (Cresson) (Poemeniinae), *Coleocentrus rufus* (Acaenitinae) and a clade containing most of Pimplinae. The Bremer support value was 9. The morphology-only parsimony analysis had *C. melancholica* as sister to *Diacritus incompletus* (Diacritinae) with these two species sister group to Acaenitinae (part 1 of Fig. 118). There were only three characters supporting *C. melancholica* + *D. incompletus* and the Bremer support value was only 1. Conversely, the molecular-only parsimony analysis supported a grouping of *C. melancholica* + *Theronia bicincta* (Cresson) (Pimplinae), based on 14 substitutions (part 2 of Fig. 119). In the total-evidence Bayesian analysis, *C. melancholica* was in a clade that lacked resolution with *S. occiputale*, *D. incompletus* and (*Coleocentrus rufus* + *Collyria catoptron*). The support for this grouping was low (BPP = 75). The Bayesian analysis with only molecular characters placed *C. melancholica* within Pimpliformes,

but the relationships at the base of this clade lacked resolution with low posterior probabilities (part 2 of Fig. 121).

Wahl and Gauld (1998) found the following relationship based on a morphological parsimony analysis: Acaenitinae + (Diacritinae + (Cylloceriinae + (Diplazontinae + Orthocentrinae). The grouping of Cylloceriinae + (Diplazontinae + Orthocentrinae) makes sense biologically because all species for which hosts are known are endophagous in Diptera (Wahl 1990). It was expected that our morphology-only parsimony analysis would find the same results as Wahl and Gauld (1998), but their study coded Acaenitinae and Diplazontinae at subfamily level and their character set was designed specifically to analyze Pimpliformes, not all of Ichneumonidae. It should be noted that when we analyzed only the morphological characters with a Bayesian approach, we found the following: Cylloceriinae + (a grade of Orthocentrinae + Diplazontinae), but with a posterior probability of only 69 (results not shown). More recently, most of the analyses of Klopfstein et al. (2019) found that Cylloceriinae formed a clade with Orthocentrinae and Diacritinae.

The combined analyses of Quicke et al. (2009) generally recovered Diacritinae + (the acaenitinae *Procinetus* + ((higher Pimpliformes) + (other Acaenitinae + (Cylloceriinae + (Orthocentrinae + (Diplazontinae + (Collyriinae + *Hyperacmus*))). As exemplars of Cylloceriinae, Quicke et al. (2009) included two species of *Cylloceria* and *Allomacrus arcticus* (Holmgren) and these species clustered together. They did not include any species of *Rossemia* Humala. Most of their analyses (including both cladograms they provide) recovered a sister-group relationship of *Hyperacmus* with Collyriinae, not Cylloceriinae. Only when high gap costs were applied (not shown) did *Hyperacmus* cluster with Cylloceriinae. Nevertheless, Quicke et al. (2009) formally transferred *Hyperacmus* to Cylloceriinae. One of their rationales for this move was based on the fact that the venom resevoir of *Cylloceria* and *Hyperacmus* possess the uniquely derived state of being comprised of two symmetric parts. In addition, Quicke et al. (2009) point out that the venation of the two genera is similar, males of both have concave tyloids on rather basal flagellomeres, and the propodeum is elongated with strong latero-median carinae (shared characters also summarized by Broad et al. 2018). Alternate placements for *Hyperacmus* include Orthocentrinae (Wahl and Gauld 1998) and related to *Microleptes* (Humala 2003). Unfortunately, we were not able to include sequence of *Hyperacmus* in the current study. In addition, the larva of *Hyperacmus* is not known and this knowledge would be instructive in confirming the placement of *Hyperacmus*. We were also not able to include specimens of *Rossemia* or *Allomacrus* Förster.

In our study, all the exemplars of Diacritinae, Acaenitinae, Collyriinae, Cylloceriinae, Diplazontinae and Orthocentrinae clustered together in only one analysis: the total-evidence Bayesian analysis (part 1 of Fig. 120) and only with weak support (BPP = 89). Neither of our total-evidence analyses recovered the relationship of Cylloceriinae with Orthocentrinae, Diplazontinae and Collyriinae, but this may have partly been because of the aberrant 28S sequence of *S. occiputale* which may have affected relationships in this region of the tree (see Acaenitinae, above). In summary, our analyses unequivocally confirm previous results that *Cylloceria* belongs to Pimpliformes (Wahl

and Gauld 1998; Quicke et al. 2009). Its precise placement within Pimpliformes is uncertain and depends on the characters used and the type of analysis, but it certainly appears more likely to be related to Acaenitinae, Collyriinae, Diacritinae, Diplazontinae and Orthocentrinae, as opposed to the higher Pimpliformes.

Diacritinae

The total-evidence parsimony analysis recovered our single exemplar of Diacritinae (*Diacritus incompletus*) in a clade within Pimpliformes with equivocal relationships as follows: *Diacritus*/ *Coleocentrus rufus* + *Collyria catoptron*/ all species of Pimplinae, Poemeniinae and Rhyssinae (part 2 of Fig. 117). This clade was supported by 15 synapomorphies, of which 5 were morphological, and a Bremer support value of only 1. See the section on Cylloceriinae (above) for more description of the placement of Diacritinae in our analyses.

Previous analyses have found Diacritinae as either sister to all Pimpliformes (Quicke et al. 2009), to all Pimpliformes except for Acaenitinae (Wahl and Gauld 1998), or related to Orthocentrinae and Cylloceriinae (Klopfstein et al. 2019). Our conclusions about the placement of Diacritinae are similar to that of Cylloceriinae: it most likely belongs to the grouping with Acaenitinae, Collyriinae, Cylloceriinae, Diplazontinae and Orthocentrinae, but the current study does not clarify its relationships within this group. Nothing is known of the biology or larva of any of the three included genera and this information could help to place Diacritinae more precisely.

Diplazontinae

The monophyly of Diplazontinae (*Diplazon laetatorius* (Fabricius) and *Woldstedtius flavolineatus* (Gravenhorst)) was supported in all of our analyses, regardless of the data used or the method of analysis (Table 3). In the total-evidence parsimony analysis (part 2 of Fig. 117), Diplazontinae was supported by 43 synapomorphies including 16 morphological of which 3 were uniquely derived: 5(4) mandibles tridentate (Fig. 16); 107(1) larval pleurostoma and mandible location shifted ventrally inferior to mandibular process opposite labial palpus (Fig. 100); and 126(1) larval labial sclerite with ventral margin produced as a spine (Fig. 100). The Bremer support value was more than 10 steps.

As described above in the section on Cylloceriinae, all previous studies have placed Diplazontinae within Pimpliformes, generally as sister group to Orthocentrinae (Gauld and Wahl 1998; Quicke et al. 2009). Our total-evidence parsimony analysis did not support this grouping as the arrangement of Pimpliformes was "upside-down" (with Diplazontinae as sister group to all other taxa) similar to the transcriptome and maximum likelihood anchored enrichment analyses of Klopfstein et al. (2019). The current Bayesian total-evidence analysis did recover the three species of Orthocentrinae in a clade with Diplazontinae, but Orthocentrinae formed a grade leading to Diplazontinae and support for the grouping was low (BPP = 76) (part 2 of Fig. 120). The generic relationships of Diplazontinae are relatively well-studied on the basis of a phylogenetic analysis using morphological characters and four genes (Klopfstein et al. 2011) as are the species concepts (e.g., Dasch 1964; Klopfstein 2014).

Eucerotinae

As described in the section above on Agriotypinae, in the total-evidence parsimony analysis part 3 of (Fig. 117), *Euceros* sp. nov. was sister taxon to *Agriotypus armatus*, these two taxa being sister group to the remainder of Ichneumoniformes sensu lato except for ((Claseinae + Pedunculinae) + (Brachycyrtinae + Labeninae)). This was similar to the molecular-only parsimony analysis except that *Euceros* sp. nov. was in a clade as follows: (*Agriotypus armatus* + (*Brachycyrtus wardae* + *Euceros* sp. nov.) (part 2 of Fig. 119). *Euceros* sp. nov. occupied a similar position in both Bayesian analyses: close to *A. armatus* and near the base of Ichneumoniformes s.l. except for Labeninae, Brachycyritinae and related subfamilies (part 2 of Fig. 120, part 2 of Fig. 121). The only different hypothesized relationship for *Euceros* sp. nov. was in our morphology-only parsimony analysis in which *Euceros* sp. nov. was sister to *Neorhacodes enslini* (Neorhacodinae) with 18 synapomorphies of which 4 of them were uniquely derived, although all of the uniquely derived character states described the egg and biology of *Euceros* sp. nov. with reversals/ transformations in *N. enslini* (part 2 of Fig. 118). Optimization of these characters using DELTRAN changed these four characters to be autapomorphies of *Euceros* sp. nov. There was no resolution in this part of the tree to be able to determine relationships of this pairing.

Euceros Gravenhorst is unique in Ichneumonidae in that species lay eggs on vegetation which hatch into planidial larvae (Tripp 1961). It has previously had an uncertain placement in Ichneumonidae. Viereck (1918) included *Euceros* within his Ctenopelminae (*Ctenopelma* Holmgren, etc.), but later (Viereck 1919) raised the taxon to subfamily status as "Eucerinae". Townes (1945) placed it as a tribe within Ctenopelmatinae, but later as a tribe in Tryphoninae (Townes et al. 1965, Townes 1969). Short (1959) placed *Euceros* as a tribe (Euceratini) in Ctenopelmatinae. Perkins (1959a) reverted the rank of *Euceros* to a subfamily ("Euceratinae") and Short (1978) concurred with this placement on the basis of larval characters. It is now recognized that the apparent similarities between *Euceros*, Tryphoninae and Ctenopelmatinae are based on symplesiomorphies such as the non-petiolate T1 (character 76, state 0) with spiracle placed anterior to middle (chareacter 77, state 0) (Fig. 72). Furthermore, it is not believed that the stalked egg of Tryphoninae (Fig. 113) is homologous to the stalked egg of Eucerotinae (Figs 115–116) (Gauld and Wahl (2002). The egg in Tryphoninae is an extension of the chorion (Kasparyan 1973); whereas, the stalk of *Euceros* is a secretion that hardens allowing the egg to be stuck to vegetation (Tripp 1961).

Previous phylogenetic studies have attempted to ascertain the relationships of Eucerotinae within Ichneumonidae. A morphological analysis by Gauld and Wahl (2002), using a limited number of outgroups, found that Eucerotinae was sister group to *Labium* (Labeninae) + *Brachycyrtus*. The combined morphological and molecular studies of Quicke et al. (2000a) (using 28S D2) and Quicke et al. (2009) (using 28S D2–D3) generally found that *Euceros* was sister to *Brachycyrtus* with this pairing often associated with Claseinae, Pedunculinae, and Microleptinae within Ichneumoniformes s.l. except Labeninae. Similar results were found by Santos (2017) with seven genes and morphology. Overall, our analyses generally concur with the findings of Quicke et al.

(2009) and Santos (2017) that *Euceros* is placed near the base of Ichneumoniformes s.l. There are no morphological characters that support this placement and the unique biology of *Euceros* also offers no clues as to whether it is correct.

Hybrizontinae

Hybrizon rileyi, the exemplar of Hybrizontinae in our study, was sister to *Lycorina glaucomata* (Lycorininae) in the total-evidence parsimony analysis and was nested within the Ctenopelmatinae and relatives clade (part 5 of Fig. 117). The pairing was supported by 41 synapomorphies, of which 6 were morphological with a Bremer support value of 7 steps. None of the morphological characters supporting this pair were uniquely derived or even had a consistency index above 0.2. The total-evidence Bayesian analysis was similar to the total-evidence parsimony analysis: *H. rileyi* was within the Ctenopelmatinae and relatives clade in a region with equivocal relationships: Perilissini/ (*Rhorus bartelti* Luhman + Ctenopelmatinae Genus NZ/ (*Westwoodia* sp. + (*Onarion* sp. + *H. rileyi*)) (part 1 of Fig. 120). The support for this clade was only 84 and *H. rileyi* had an extremely long branch length. The molecular-only parsimony analysis recovered *H. rileyi* in a clade as follows: (Labeninae + (*Brachyscleroma* sp. + *Aplomerus* sp.) + (Tersilochinae s.s. + (*Mesochorus* sp. + (*Onarion* sp. + *H. rileyi)*))) (part 2 of Fig. 119). Finally, the molecular-only Bayesian analysis placed *H. rileyi* in a similar position to the molecular-only parsimony analysis: not clustering with most members of Ctenopelmatinae, but rather unresolved with members of Tersilochinae, Mesochorinae, Sisyrostolinae and Cremastinae at the base of a clade containing Pimpliformes and members of Ichneumoniformes s.l. (part 1 of Fig. 121).

Similar to *Agriotypus*, *Hybrizon* Fallén and its relatives have had a varied placement over time: included in Braconidae (Achterberg 1976), Ichneumonidae (Gauld 1984), or as a family itself: Hybrizontidae or Paxylommatidae (Marsh 1971, 1989). The different family-level placements have been proposed because of wing venation, primarily because of the lack of fore wing vein *2m-cu* (character 51, state 1) (as in Fig. 49) in *Hybrizon*, a character that defines all of Braconidae except *Apozyx* Mason (Sharkey and Wahl 1992). Nevertheless, other ichneumonids lack *2m-cu* (e.g., *Neorhacodes* Hedicke) and the fossil hybrizontine *Tobiasites striatus* Kasparyan possesses this vein (Kasparyan 1988), indicating that presence of the vein may be the ground plan state for the group. Furthermore, Mason (1981) noted that *Hybrizon* does not have fusion of metasomal tergites 2 and 3, therefore excluding it from Braconidae. Currently, it is believed that *Hybrizon* and its relatives are correctly placed within Ichneumonidae as a subfamily (Sharkey and Wahl 1992) and our results support this: *H. rileyi* did not cluster within the outgroups, nor was it ever placed as sister to the rest of Ichneumonidae.

In terms of proposed placement within Ichneumonidae, Sharkey and Wahl (1992) suggested that on the basis of the lack of vein *2m-cu* and similar host biology (endoparasitoids of Aculeata), Hybrizontinae may be the sister of Neorhacodinae. *Hybrizon* spp. are known to parasitize ants (Donisthorpe 1913, Gómez Durán and Achterberg 2011), whereas *Neorhacodes* spp. parasitize stem-nesting Crabronidae (Horstmann 1968; Danks 1971). None of our results support this relationship. More recent studies

using sequence data and morphology (Quicke et al. 2009) often recovered a relationship of *Hybrizon* with *Lycorina* Holmgren (Lycorininae), but that study stated that the placement of *Hybrizon* was highly variable depending on the analysis (although generally related to Ophioniformes taxa). In the end, Quicke et al. (2009) suggested that *Hybrizon* may be a derived Anomaloninae. In summary, our combined analyses suggest Hybrizontinae may be related to Ctenopelmatinae, whereas our molecular-only analyses place it somewhere within Ophioniformes (but not the higher Ophioniformes). Certainly its biology does not lend support to the notion that it is a ctenopelmatine which are mostly parasitoids of sawflies, but neither does it suggest it is an anomalonine, which parasitize Lepidoptera (Gauld et al. 1997).

Ichneumoninae

The following discussion pertains to the 13 exemplar species of Ichneumoninae excluding Alomyini. See the section on Alomyinae (above) for the rationale for moving *Alomya* and *Megalomya* within Ichneumoninae (as Alomyini).

The 13 species of Ichneumoninae were monophyletic in all analyses (Table 3). The total-evidence parsimony analysis had 32 synapomorphies supporting Ichneumoninae, of which 9 were morphological, including the uniquely derived character state 84(1) gastrocoelus present (Fig. 77). The Bremer support value was greater than 10 steps. (Ichneumoninae + *Alomya debellator*) was the sister of Phygadeuontinae in both total-evidence analyses and these were sister group to Cryptinae s.s. As discussed in the discussion of Ichneumoniformes (above), various other taxa were associated with this grouping, including Adelognathinae, Agriotypinae, Brachycyrtinae, Claseinae, Eucerotinae, Microleptinae and Pedunculinae as well as Labeninae in a more distant relationship.

In terms of the tribes, all analyses recovered the two species of Platylabini together (*Cyclolabus impressus* (Provancher) + *Linycus exhortator* (Fabricius)) and the two species of Heresiarchini (*Coelichneumon eximius* (Stephens) + *Protichneumon grandis* (Brullé)) were also always monophyletic, although the latter pair was always nested within Ichneumonini (5 species), as was the single exemplar of Listrodromini (*Dilopharius otomitus* (Cresson)). All analyses except parsimony with only morphological characters recovered Phaeogenini as monophyletic (*Stenodontus* sp. nov. + (*Centeterus euryptychiae* (Ashmead) + *Phaeogenes hebrus* (Cresson))), and (Platylabini + Phaeogenini) was sister to all other species in the total-evidence and molecular-only parsimony analyses (Figs 117, 119).

In terms of tribal relationships, Quicke et al. (2009) summarized their findings by stating that they found Phaeogenini and Platylabini "in basal positions" within Ichneumoninae, although their consensus cladogram with gaps treated as informative had Platylabini in the middle of Ichneumoninae and their analysis with gaps treated as missing data had some members of Phaeogenini separated from the rest and nested within a clade comprised predominantly of Ichneumonini species. More recently, the maximum likelihood total-evidence analysis of Santos (2017) found that all five species of Phaeogenini clustered together as sister group to the other eight Ichneumoninae species, but this same analysis did not recover a monophyletic Platylabini. We accept the hypothesis

supported by the majority of our analyses, that Phaeogenini and Platylabini are sister groups and these two groups form a sister group to the rest of Ichneumoninae except for Alomyini. We do concede that our low taxon sampling may be presenting a simplified view that may not be supported by future studies with additional taxa.

With respect to monophyly and relationships of the other tribes of Ichneumoninae, none of the studies of Laurenne et al. (2006), Quicke et al. (2009), Santos (2017) or our analyses have found support for Heresiarchini relative to Ichneumonini. We did not include any of the morphologically distinct *Callajoppa* group (Sime and Wahl 2002), which would need to be done to study the relationships of Heresiarchini and Ichneumonini further. Sime and Wahl (2002), using morphology alone, recovered Heresiarchini as monophyletic, but only included one non-heresiarchine as an outgroup (*Ichneumon caliginosus* Cresson), therefore it could not draw conclusions regarding the monophyly of Heresiarchini relative to Ichneumonini as a whole. In summary, it appears as though more taxa, characters and knowledge of biology will need to be assessed to determine the internal structure of Ichneumoninae.

Labeninae

Labeninae was monophyletic in all analyses except parsimony using only morphology (Table 3). In the total-evidence parsimony analysis, Labeninae was strongly supported by 52 synapomorphies, of which 8 were morphological (none uniquely derived) and a Bremer support value of greater than 10 steps (part 3 of Fig. 117). Labeninae was sister to Brachycyrtinae (Bremer support of 10) and these two subfamilies were sister to (Claseinae + Pedunculinae) (Bremer support of 8). Together these four subfamilies were sister group to all other taxa within Ichneumoniformes s.l. In contrast, neither of the Bayesian analyses recovered a sister-group pairing of Brachycyrtinae and Labeninae but rather, Labeninae was sister group to a clade comprised of the remaining exemplars of Icheumoniformes s.l. + Pimpliformes (part 2 of Fig. 120, part 2 of Fig. 121). There was low support for this relationship in the total-evidence Bayesian tree (BPP = 66), but strong support in the molecular-only analysis (BPP = 99).

Some previous studies have suggested that Labeninae may be sister group to all ichneumonids except Xoridinae, for example, most analyses in Quicke et al. 2009, the unweighted parsimony analysis of Santos (2017) and two of five anchored enrichment analyses in Klopfstein et al. (2019). This placement was not supported by the current results. The latter study found a lack of stability in the placement of Labeninae, for example, their maximum likelihood analysis with amino acids placed Labeninae as sister to Ichneumoniformes s.l. + Pimpliformes, whereas the likelihood analysis analyzing all nucleotides placed Labeninae as sister to Pimpliformes. In terms of the relationship of Labeninae to Brachycyrtinae, Claseinae and Pedunculinae, Quicke et al. (2009) generally recovered the latter three subfamilies as monophyletic but as sister group to the rest of Ichneumoniformes, not Labeninae. This was similar to the maximum likelihood analysis of Santos (2017) who found Labeninae + (Claseinae + (Eucerotinae + Brachycyrtinae)) + (the rest of Ichneumoniformes). Note that both Santos (2017) and Klopfstein et al. (2019) only included one species of Labeninae in their analyses. In

summary, with respect to the placement of Labeninae, the current study either found that Labeninae is part of the sister group to Ichneumoniformes s.l. (with Brachcyrtinae, Claseinae and Pedunculinae) or as sister group by itself to a clade comprising Ichneumoniformes s.l. + Pimpliformes. Based on the equivocal placement of Labeninae in the current study, including apparent strong support as sister to Brachycyrtinae in the total-evidence parsimony analysis, we do not currently recognize the higher group Labeniformes of Quicke et al. (2009).

With respect to tribal relationships within Labeninae, our analysis only included exemplars of three tribes (no Xenothyrini exemplars). In the consensus tree of the total-evidence parsimony analysis, the tribes Labenini (*Labena grallator* (Say) + *Apechoneura* sp.), Orthognathelini (= Groteini) (*Grotea anguina* Cresson + *Labium* sp.) and Poecilocryptini (*Poecilocryptus nigromaculatus*) had equivocal relationships (part 3 of Fig. 117). The Bayesian analyses both supported the following topology: (Orthognathelini + (Poecilocryptini + Labenini)) which is ths same as the hypothesis of Gauld and Wahl (2000). This topology contradicts the relationships hypothesized by Wahl (1993a) and Quicke et al. (2009): Poecilocryptini + (Orthognathelini + Labenini).

Lycorininae

The placement of Lycorininae (*Lycorina glaucomata*) was one of the least stable of any taxon in our analyses. The total-evidence parsimony analysis placed *L. glaucomata* within the clade of Ctenopelmatinae and relatives as sister to Hybrizontinae (see section on Hybrizontinae for details on support). In the parsimony analysis with only molecular characters, *L. glaucomata* was sister to the rest of Ichneumonidae (part 1 of Fig. 119), and in the two Bayesian analyses it was placed within a grade along with *Neorhacodes enslini* (Neorhacodinae) and various exemplars of Tryphoninae (part 1 of Fig. 120, part 1 of Fig. 121).

Quicke et al. (2009) also found inconsistent placement of Lycorininae, including: 1) sister group to Hybrizontinae; 2) grouping with *Townesion* Kasparyan (Banchinae: Glyptini), and with these two taxa as sister group to the rest of Banchinae; and 3) grouping with Tersilochinae s.l. as sister group to Banchinae. They dismissed the association of Lycorininae with Hybrizontinae as "long-branch attraction", but did point to the presence of an aulaciform rod of the ovipositor (see Quicke et al. 1994) as evidence that Lycorininae belong to Ophioniformes.

What is known of the biology of *Lycorina* is that they are koinobionts (Coronado-Rivera et al. 2004) that oviposit in the anus of Lepidoptera larvae and complete development externally on the host and pupate inside the host cocoon (Shaw 2004) (our character 138(2)). The egg of *Lycorina* is stalked with an anchor, similar to most Tryphoninae, but Coronado-Rivera et al. (2004) note three differences between the eggs and method of oviposition. First, lycorinine eggs are much narrower than eggs of Tryphoninae (0.3 mm average width (for 10 species in 5 tribes) versus 0.1 mm for two species of *Lycorina*. Second, during oviposition, the stalk and/or anchor of almost all tryphonine eggs travel down the ovipositor while the body of the egg exits the female from the genital opening at the base of the ovipositor (character 136(1)). This process

was not witnessed in Lycorininae (Shaw 2004), and it is therefore likely that the entire egg travels down the ovipositor (character 136(0)). Third, the eggs of Tryphoninae have a more strongly sclerotized chorion than Lycorininae. Whereas our two Bayesian analyses placed *L. glaucomata* near Tryphoninae, Lycorininae was never nested within Tryphoninae, which agrees with the findings of Quicke et al. (2009). The current study and Quicke et al. (2009) therefore support the hypothesis that the stalked eggs of Lycorininae and Tryphoninae have evolved independently.

In summary, the majority of our analyses agreed with Quicke et al. (2009) that Lycorininae is related to Ophioniformes. Our total-evidence parsimony analysis suggested a relationship with Ctenopelmatinae, whereas Townes (1970b) and Quicke et al. (2009) support a relationship with Banchinae.

Mesochorinae

The five species of Mesochorinae only clustered together in the morphology-only parsimony analysis (part 3 of Fig. 118) based on 15 synapomorphies, of which one was uniquely derived: character 140(9): oviposition into host inside host (i.e., internal hyperparasitoid). In both Bayesian analyses and the total-evidence parsimony analysis, only four of the five mesochorine species clustered together, with *Chineater masneri* consistently paired with *Scolomus* sp. (Metopiinae) and associated with *Tatogaster nigra* (Tatogastrinae) and various ctenopelmatine species. *Chineater masneri* has not been included in a phylogenetic analysis since the morphological analysis of Wahl (1993b) when it was described, but the latter study included a generalized outgroup, thus forcing monophyly of Mesochorinae (including *C. masneri*). The current study suggests that it may be misplaced in Mesochorinae.

Chineater masneri does have a large, rhombic areolet (character 54(1)) (Fig. 52), which is characteristic of most mesochorines (although obliquely quadrangular in *Cidaphus paniscoides* (Ashmead)). A large, rhombic areolet is rare in Ctenopelmatinae (generally the areolet is obliquely quadrangular or open). The ovipositor of *C. masneri* is thin and needle-like (character 99(2)) (Fig. 85) as in all mesochorines, but it is relatively short for a mesochorine. Most ctenopelmatines have a dorsal, subapical notch on the ovipositor, but a thin, needle-like ovipositor is known in most Pionini (e.g., *Rhorus bartelti*) as well as some Perilissini and Ctenopelmatini, therefore this character does not help in the subfamily placement of *C. masneri*. As discussed under Ctenopelmatinae, the apical tooth of the fore tibia (character 68(1)) is not diagnostic of Ctenopelmatinae by itself and, in fact, this character state is present in all five of our mesochorine species, including *C. masneri*. What may help decide the placement of *C. masneri* would be knowledge of either: a) the male gonoforceps – if rod-like (character 102(1)), then this would support placement in Mesochorinae, or; b) oviposition location – if an internal hyperparasitoid (character 140(9)), then this would also support the hypothesis that *C. masneri* is correctly placed in Mesochorinae. Until one or both of these characters are known, we defer transfer of *Chineater* and maintain it within Mesochorinae.

With respect to the four other mesochorine species, the total-evidence parsimony analysis placed them in a clade with equivocal relationships within Ophioniformes as

follows: Mesochorinae except *Chineater*/ Ctenopelmatinae and relatives/ Metopiinae to Campopleginae (part 4 of Fig. 117). The clade "Mesochorinae except *Chineater*" was supported by 45 synapomorphies, of which 9 were morphological, including the uniquely derived character 140(9): oviposition into a host inside a host. The Bremer support was 10+ steps. Our Bayesian total-evidence analysis had a similar placement for the group (Mesochorinae except *Chineater*): related to a clade containing ((Ctenopelmatinae and relatives including Metopiinae) + the higher Ophioniformes) (part 1 of Fig. 120). In terms of relationships within Mesochorinae, in the total-evidence parsimony analysis, *Mesochorus* sp. was sister to the other three exemplars (*Astiphromma* sp. nov., *Cidaphus paniscoides* and *Lepidura collaris* Townes) that had equivocal relationships. This arrangement contradicts Wahl (1993b) who found that *Cidaphus* Förster was sister group to all other genera. The Bayesian total-evidence analysis favoured *C. paniscoides* as the sister to the other three species, but only with weak support (BPP = 69).

There have been some previous studies that suggested that Mesochorinae may render Ctenopelmatinae paraphyletic (Belshaw and Quicke 2002; Quicke et al. 2009), but this hypothesis was not supported in any of our five analyses. Neither was the hypothesis of Quicke et al. (2009) that *Tatogaster nigra* Townes (Tatogastrinae) belongs to Mesochorinae. Owing to the lack of resolution of natural groups within Ctenopelmatinae, future phylogenetic studies including Ctenopelmatinae, Mesochorinae, Metopiinae and Tatogastrinae are definitely warranted which will hopefully clarify the placement of Mesochorinae.

Metopiinae

Similar to Mesochorinae, all four species of Metopiinae only clustered together in the morphology-only parsimony analysis (part 3 of Fig. 118), supported by 20 synapomorphies (none uniquely derived) and a Bremer support of 2. In the other analyses, three of the four species (*Exochus semirufus* Cresson, *Metopius pollinctorius* (Say) and *Seticornuta terminalis* (Ashmead)) shared a common ancestor with strong support (Table 3), but *Scolomus* sp. was placed elsewhere within Ophioniformes. In the total-evidence parsimony analysis, the three Metopiinae species that clustered together were supported by 46 synapomorphies, including 17 morphological (none uniquely derived), and a Bremer support of greater than 10 steps (part 6 of Fig. 117). Metopiinae (except *Scolomus* sp.) was the sister group to (*Stilbops vetulus* + the unresolved clade (*Notostilbops* sp. nov./ *Lissonota scutellaris* + *Exetastes bioculatus*/ Glyptini). See sections on Banchinae and Stilbopinae for further description of these relationships. Together this grouping was the sister group to the higher Ophioniformes. In contrast, the total-evidence Bayesian analysis placed Metopiinae (except *Scolomus* sp.) in an unresolved clade as follows: Metopiinae (except *Scolomus*)/ *Ctenopelma sanguineum* (Provancher)/ (*Tatogaster nigra* + (*Chineater masneri* + *Scolomus* sp.)) and a cluster of six Ctenopelmatinae (part 1 of Fig. 120). This grouping was, however, very weakly supported (BPP = 71). Based on the support values of this part of the tree, the Bayesian analysis only tells us that Metopiinae belongs to Ophioniformes, but not within the higher Ophioniformes.

Gauld and Wahl (2006) suggested that Metopiinae may have arisen from within Ctenopelmatinae. Our total-evidence parsimony analysis refuted this hypothesis, but our Bayesian total-evidence analysis had some weak support for the relationship, albeit with a general lack of resolution in this part of the tree. In summary, the most well-resolved hypothesis of the placement of Metopiinae from the current study is that it is related to Stilbopinae and Banchinae, in contrast to the hypothesis of Gauld and Wahl (2006) and the results of Quicke et al. (2009). Biologically, Metopiinae, Stilbopinae and Banchinae share parasitism of Lepidoptera (Wahl 1993c) which is much less common in Ctenopelmatinae – a few Holarctic species of *Lathrolestes* Förster have been reared from Eriocraniidae (Heath 1961) and some species of *Megaceria* Szépligeti in Australia parasitize Geometridae (Morley 1913) and Notodontidae (Gauld 1984).

In terms of the placement of *Scolomus*, Gauld and Wahl (2006) synonymized the metopiine genus *Apolophus* Townes, 1971 with the ctenopelmatine genus *Scolomus* (tribe Pionini) with the name *Scolomus* Townes & Townes, 1950 having priority. Because the morphological evidence supporting placement in either subfamily was equivocal, they placed *Scolomus* within Metopiinae on the basis of the one host record for *Scolomus*: *S. borealis* (Townes) reared from *Schrekensteinia festaliella* (Hübner) (Lepidoptera: Schrekensteiniidae) (Broad and Shaw 2005). Quicke et al. (2009) stated that the placement of *Scolomus* was "associated with various Ctenopelmatinae" and in only a few analyses was it recovered as sister to Metopiinae. On the basis of all of our analyses except parsimony (morphology-only), the species of *Scolomus* that we included in our analysis does not seem to be well-placed with our other exemplars of Metopiinae. But where is it best placed?

The current total-evidence parsimony analysis placed *Scolomus* within the Ctenopelmatinae and relatives grouping (part 5 of Fig. 117) and not closely related to the other three Metopiinae species. The total-evidence Bayesian analysis was less clear as *Scolomus* was placed in a clade that included the three other metopiine species and seven ctenopelmatines (part 1 of Fig. 120), but with low support (BPP = 71). Whereas it is tempting to suggest that *Scolomus* should be moved to Ctenopelmatinae on the basis of the parsimony total-evidence results, the fact that the analyses are placing it next to several other enigmatic taxa makes this decision difficult, especially with respect to the decision of which ctenopelmatine tribe it should be placed. It is also possible that the synonymy of *Scolomus* and *Apolophus* by Gauld and Wahl (2006) was not correct. Our exemplar specimen would have been placed in *Apolophus* prior to 2006 on the basis of the lack of horns on the subtegular ridge. Prior to any decision on the subfamily placement of *Scolomus*, it would be prudent to undertake a revision of the genus (including description of new species that may be intermediate between *S. borealis* and *S. viridis* Townes & Townes) in order to confirm its monophyly. For now, we delay a decision on the placement of *Scolomus* until this work has been completed.

Unfortunately, we were not able to include any other of the problematic genera of Metopiinae in our analysis: *Bremiella* Dalla Torre, *Ischyrocnemis* Holmgren, and *Lapton* Nees; therefore we cannot comment on their relatedness to the four exemplar metopiines we analyzed. Quicke et al. (2009) found their placements unstable, but stated that

Ischyrocnemis was consistently recovered within Pimpliformes, *Bremiella* sometimes was a sister group to Metopiinae or associated with various Ctenopelmatinae and *Lapton* was sometimes recovered with a grouping including Ophioninae, Campopleginae, Anomaloninae and Mesochorinae.

Microleptinae

Microleptinae (*Microleptes* sp.) was sister to *Ateleute* sp. nov. (Ateleutinae) in the parsimony total-evidence analysis, supported by 37 synapomorphies (7 morphological, none of which were uniquely derived) and a Bremer support of 6. These two taxa were sister group of (Cryptinae + (Phygadeuontinae + (Alomyinae + Ichneumoninae))) (part 3 of Fig. 117). The Bayesian total-evidence analysis was similar, with *Microleptes* sp. in a well-supported clade (BPP = 98) that lacked internal resolution, but included (*Adelognathus* sp. + *Ateleute* sp.) and (Cryptinae + (Phygadeuontinae + (Alomyinae + Ichneumoninae))) (part 2 of Fig. 120).

Previous studies were equivocal in their placement of Microleptinae. Wahl (1986) described the larva stating that the reduced hypostomal spur, shape of the stipital sclerite and small size of the labial sclerite was similar to the larvae of Metopiinae and Anomaloninae (Ophioniformes), but stated that these similarities may be convergences based on similar larval behaviour (spinning flimsy cocoons inside the host pupa). The morphological character analysis in Quicke et al. (2000a) placed Microleptinae in a clade with Orthocentrinae and Diplazontinae (Pimpliformes) which is consistent with their biology as endoparasitoids of Diptera larva (*Microleptes* has been reared from Stratiomyidae: Wahl 1986). The combined morphology and 28S D2–D3 sequence analysis of Quicke et al. (2009) suggested that Microleptinae belonged to Ophioniformes as sister group to Orthopelmatinae, with these two taxa being the sister group to either Tersilochinae (gaps treated as missing) or (when gaps treated as informative), nested within Ctenopelmatinae in a clade as follows: Perilissini + (Oxytorinae + (Seleucini + (Microleptinae + Orthopelmatinae). Conversely, the molecular-only analysis of Quicke et al. (2009) placed Microleptinae as sister group to Eucerotinae near the base of the Ichneumoniformes (close to the placement of the current study). Santos (2017) also recovered *Microleptes* within Ichneumoniformes, its precise placement dependent on the method of analysis (parsimony versus maximum likelihood).

Our study provides support for the placement of Microleptinae near the base of Ichneumoniformes s.l. In terms of morphology, there are no compelling characters for this placement – the only uniquely derived character in this region of the tree is Character 9 (state 1): central flagellomeres of male with elliptical or longitudinal ridge-like tyloids which supports Adelognathinae + ((Microleptinae + Ateleutinae) + (Cryptinae + (Phygadeuontinae + (Alomyinae + Ichneumoninae)))). This character; however, has a reversal in Microleptinae + Ateleutinae (part 3 of Fig. 117). The support, therefore, comes mainly from the molecular characters. Both of our total-evidence analyses and both of our analyses with only molecular characters supported the monophyly of the seven subfamilies listed directly above (e.g., BPP of 100 in Bayesian analysis with only molecular characters) (part 2 of Fig. 121).

Neorhacodinae

Neorhacodinae (*Neorhacodes enslini*) was perhaps the most unstable taxon in our analyses. The total-evidence parsimony analysis placed *N. enslini* within Tryphoninae (as sister to Phytodietini) (part 4 of Fig. 117). The parsimony analysis with only molecular characters placed *N. enslini* as sister to all Ichneumonidae except *Lycorina glaucomata* (Lycorininae) (part 1 of Fig. 119) and the two Bayesian analyses were similar, except that *Neorhacodes enslini* was sister to all other Ichneumonidae (part 1 of Fig. 120, part 1 of Fig. 121).

Originally, Ruschka (1922) described *Rhacodes* Ruschka in its own subfamily in Braconidae. Hedicke (1922) noted that *Rhacodes* was preoccupied by the crustacean *Rhacodes* Koch and renamed the taxon *Neorhacodes* Hedicke, changing the subfamily name to Neorhacodinae. The reason why it was placed within Braconidae is the apparent lack of vein *2m-cu* of the fore wing. Roman (1923) using reflected light concluded that vein *2m-cu* was present and therefore moved *Neorhacodes* to Ichneumonidae, placing it within Pimplinae. Cushman (1940) described a second genus (*Romaniella* Cushman). Townes (1945) listed *Neorhacodes* under "genera of uncertain subfamily" before moving it to Banchinae as a tribe (Neorhacodini) (Townes and Townes 1951). Townes (1971) later raised Neorhacodini to subfamily status "placed between the Lycorininae and the Banchinae". More recently, Quicke et al. (2009) found that *Neorhacodes enslini* clustered with exemplars of Tersilochinae and the "*Phrudus* group" of Phrudinae and subsequently formally synonymized Neorhacodinae (and the *Phrudus* group) within Tersilochinae. Finally, Broad (2016) considered Neorhacodinae as a separate subfamily, rather than a synonym of Tersilochinae.

Given the equivocal placement of *Neorhacodes enslini* in the current study, we are not able to make any precise statements with respect to the relationships of Neorhacodinae. There are no compelling morphological or biological characters in our study that link Neorhacodinae to Tryphoninae. The egg of Neorhacodinae is not known, and determining whether it bears a stalk or not would help greatly in determining whether the sister-group relationship of Neorhacodinae and Phytodietini is artefactual or not. The current study coded the host of Neorhacodinae as Hymenoptera, which is similar to most Tryphoninae, although Phytodietini are parasitoids of Lepidoptera. Furthermore, one could argue that coding the hosts for *Neorhacodes* (aculeate Hymenoptera) (Horstmann 1968; Danks 1971) the same state as for most Tryphoninae (sawflies) (Kasparyan 1973; Bennett 2015) is not correct and they should be given different states for this character. Similarly, there was no evidence of a close relationship with *Neorhacodes* and Banchinae (as proposed by Townes and Townes 1951), Lycorininae (Townes 1971) or Tersilochinae (Quicke et al. 2009). The most precise statement of affinity that we can make for Neorhacodinae is that it appears to belong *incertae sedis* within Ophioniformes because *N. enslini* was most often associated with taxa belonging to Ophioniformes, and never clustered within Pimpliformes or Ichneumoniformes in our analyses.

Nesomesochorinae

Nesomesochorinae (*Nonnus* sp. and *Chriodes* sp.) was only recovered as monophyletic in the total-evidence parsimony analysis (part 6 of Fig. 117) (Table 3). It was sup-

ported by 31 synapomorphies (6 morphological) and a Bremer support of 2. Character 16 (reduction of maxillary palpomeres from 5 to 4) is a relatively rare synapomorphy in our data set (C.I. = 0.33) and therefore is a good character state supporting the monophyly of these two taxa. Having said this, the parsimony analysis with only morphological characters did not recover these two taxa together: *Chriodes* sp. was placed in the higher Ophioniformes (part 3 of Fig. 118), but the placement of *Nonnus* sp. was not clearly resolved (part 1 of Fig. 118). The other three analyses all had *Nonnus* sp. and *Chriodes* sp. clustering near each other along with the exemplars of Anomaloninae and Ophioninae, although *Nonnus* sp. and *Chriodes* sp. were never sister taxa to each other. In terms of their placement within Ichneumonidae, both total-evidence analyses placed *Nonnus* sp. and *Chriodes* sp. within the higher Ophioniformes (part 6 of Fig. 117; part 1 of Fig. 120) (posterior probability of 98 in the Bayesian tree). The relationships of Nesomesochorinae within the higher Ophioniformes were not precisely clear in the parsimony total-evidence analysis because this group was unresolved as follows: Nesomesochorinae/ Anomaloninae + Ophioninae/ Cremastinae + Campopleginae. Similarly, the Bayesian total-evidence analysis does not help resolve their placement, as they were placed as follows: (*Chriodes* sp. + ((*Nonnus* sp. + Anomaloninae) + Ophioninae)), but this clade only had a posterior probability of 61. All that is suggested by the Bayesian analysis is that *Chriodes* sp. and *Nonnus* sp. do not cluster within Campopleginae + Cremastinae because the sister-group relationship of these two subfamilies is very strongly supported (BPP = 99) (part 1 of Fig. 120).

Nesomesochorini was proposed by Ashmead (1905) to include *Nesomesochorus* Ashmead (= *Chriodes*). Nonnini was proposed by Townes et al. (1961), comprised of *Nonnus*. Townes (1970b) placed *Nonnus* and *Chriodes* together in Nonnini within Campopleginae. Miah and Bhuiya (2001) and later Quicke et al. (2005) showed that these two genera did not belong to Campopleginae. Quicke et al. (2009) established that they belonged together in one subfamily with the oldest family group name having priority (Nesomesochorinae). In terms of affinity, Quicke et al. (2009) found that Nesomesochorinae belonged to the higher Ophioniformes as either sister group of (Cremastinae + Campopleginae) or sister group of Campopleginae (depending on gap treatment). Our total-evidence analyses do not refute a sister-group relationship of Nesomesochorinae to (Cremastinae + Campopleginae), but the lack of resolution means that other relationships within the higher Ophioniformes are also possible (e.g., Anomaloninae + Ophioninae). Our results do not support a sister-group relationship of Nesomesochorinae + Campopleginae.

Ophioninae

Ophioninae (*Enicospilus flavostigma* Hooker, *Hellwigia obscura*, *Ophion* sp. *Skiapus* sp. and *Thyreodon* sp.) was supported in both total-evidence analyses and the parsimony analysis with only morphological characters (Table 3). The subfamily was supported in the total-evidence parsimony analysis by 32 characters (8 morphological) and a Bremer support of 4. Character 37 (state 1): anterior transverse carina of propodeum forming more or less smooth arc (Fig. 38) was a rare synapomorphy in our study (C.I. = 0.5). In addition, character 121 (1): larval cardo present as a slightly sclerotized oval

(Fig. 104) and character 128 (1): 8 or more sensilla on larval prelabium, both had C.I.s of 0.33; however these characters are not known for *Hellwigia obscura* or *Skiapus* sp. The parsimony analysis with only molecular characters supported Ophioninae except for *Skiapus* sp., which clustered with the nesomesochorines and *Anomalon picticorne*. (Anomaloninae) as the sister group to the other ophionines (part 1 of Fig. 119). The Bayesian analysis with only molecular characters had all five ophionines clustering together, but with a low posterior probability (BPP = 84) (part 1 of Fig. 121).

The monophyly of the majority of the genera of Ophioninae has long been established on the basis of the relatively rare (in Ichneumonidae) fore wing areolet lacking vein *Rs* so that the only cross vein is distad vein *2m-cu* (character 54, state 2) (Fig. 53), along with possession of pectinate claws and presence of hind wing vein *2/Cu* (Gauld 1988b). The inclusion of the genera *Hellwigia* and *Skiapus* within Ophioninae is more equivocal. Quicke et al. (2009) found that *Hellwigia* generally clustered with the other exemplars of Ophioninae, whereas the position of *Skiapus* was unstable – sometimes included within Ophioninae or recovered as the sister taxon to the rest of Ophioninae, or in a minority of analyses, clustering with *Anomalon* Panzer and Hybrizontinae. The majority of our analyses place *Skiapus* and *Hellwigia* within Ophioninae (e.g., in the total-evidence parsimony analysis, *Skiapus* sp. + *Hellwigia obscura* are sister group to the other three exemplars) (part 6 of Fig. 117). Knowledge of the larva of *Skiapus* and *Hellwigia* would provide additional evidence to support or refute this placement.

Orthocentrinae

Orthocentrinae (*Megastylus* sp. nov., *Orthocentrus* sp. and *Proclitus speciosus* Dasch) was only recovered as monophyletic in the parsimony analysis using only morphological characters (part 2 of Fig. 118) (Table 3). Orthocentrinae was supported by 18 morphological characters, of which one was uniquely derived: Character 188, state 3: larval hypostomal-stipital plate reduced to narrow strip (Fig. 106) (this character unknown for *P. speciosus*). See the section on Pimpliformes (above) for a description of the relationships of the orthocentrine species to the rest of Pimpliformes.

Previous definitions of Orthocentrinae have differed depending on whether authors considered *Orthocentrus* Gravenhorst and relatives (the *Orthocentrus* group of genera) to be related to *Helictes* Haliday and relatives (the *Helictes* group of genera). Townes (1971) placed the groups in two separate subfamilies. His Orthocentrinae was comprised of the *Orthocentrus* group and his Microleptinae included the *Helictes* group, although he stated that his Microleptinae was a "wastebasket" group and later studies removed several genera to other subfamilies (e.g., Gupta 1988; Wahl 1990). Wahl (1990), on the basis of larval synapomorphies, joined these two groups together in an expanded Orthocentrinae and stated that Orthocentrinae was closely related to Diplazontinae, both of which belonged to the informal grouping Pimpliformes. The morphological cladistic analysis of Pimpliformes by Wahl and Gauld (1998) supported the fact that the *Orthocentrus* and *Helictes* groups shared a common ancestor which was sister taxon to Diplazontinae. In addition, their analysis supported that Orthocentrinae and Diplazontinae belonged to the following grouping: Acaenitinae + (Diacritinae

+ (Cylloceriinae + (Orthocentrinae + Diplazontinae))). Quicke et al. (2009) also recovered Orthocentrinae as monophyletic (except they removed *Hyperacmus* to Cylloceriinae). In their study, Orthocentrinae had a placement within Pimpliformes as follows: (Acaenitinae except for *Procinetus* + (Cylloceriinae + (Orthocentrinae + (Diplazontinae + (Collyriinae + *Hyperacmus*)))). More recently, Klopfstein et al. (2017) found strong support for seven species of Orthocentrinae in their anchored enrichment analyses, although the two species of *Hemiphanes* Förster did not cluster with the other species and were subsequently formally transferred to Cryptinae. In the latter study, Orthocentrinae was most often sister group to Diacritinae and this pairing was sister group to Cylloceriinae.

Given the strong support of Orthocentrinae in other studies, it is likely that the lack of monophyly of Orthocentrinae is artefactual, perhaps because of low taxon sampling and, as discussed by Quicke et al. (2009), relatively high sequence divergence of the 28S D2–D3 region in Orthocentrinae. Addition of more taxa as well as knowledge of larvae including *Proclitus* spp. would help to determine whether Orthocentrinae is monophyletic as well as its relationships within Pimpliformes. In terms of internal relationships within Orthocentrinae, Wahl and Gauld (1998) found that the *Orthocentrus* group was nested within the *Helictes* group as follows: *Aperileptus* Förster + ((*Entypoma* Förster + *Orthocentrus* group) + (all other *Helictes* group genera)). None of our results supported a sister-group relationship of the *Orthocentrus* and *Helictes* groups (i.e., *Orthocentrus* sp. + (*Megastylus* sp. nov. + *Proclitus speciosus*). When two orthocentrine species clustered together, it was either *Orthocentrus* sp. + *Megastylus* sp. nov. (part 2 of Fig. 117) or *Orthocentrus* sp. + *Proclitus speciosus* (e.g., part 2 of Fig. 118).

Orthopelmatinae

The total-evidence parsimony analysis placed Orthopelmatinae as sister group to all other Ichneumonidae except the exemplars of Xoridinae (part 1 of Fig. 117). In the Bayesian total evidence analysis, Orthopelmatinae was sister group to Xoridinae + (Labeninae + (all exemplars of Ichneumoniformes and Pimpliformes)) (part 2 of Fig. 120).

Previous analyses have had trouble discerning the precise relationships of Orthopelmatinae. Barron (1977) suggested that it was the sister group to Cryptinae, and in turn these two taxa were sister group to Ichneumoninae. Gauld et al. (1997) placed Orthopelmatinae in his Labeniformes, which also included Xoridinae, Labeninae, Agriotypinae, Brachycyrtinae, Cryptinae and Ichneumoninae. Quicke et al. (2000a) using morphological and 28S D2 ribosomal DNA found Orthopelmatinae to be sister group of Ophioniformes, but with no morphological synapomorphies supporting this relationship, and they therefore proposed the informal higher group Orthopelmatiformes for the subfamily. The placement of Orthopelmatinae in Quicke et al. (2009) was unstable as the two combined cladograms placed it with Microleptinae and Oxytorinae either within Ctenopelmatinae (gaps treated as informative) or within Tersilochinae (gaps treated as missing). Quicke et al. (2009) also stated that the sister-group relationship of Orthopelmatinae and Ophioniformes was recovered "with many parameter combinations". Finally, their analysis with only morphological characters found the

following: (Oxytorinae + (Microleptinae + Orthopelmatinae) + (Neorhacodinae + Tersilochinae))) and this clade was sister group to the remainder of Ophioniformes. In summary, Quicke et al. (2009) suggested that Orthopelmatinae was either sister taxon to Ophioniformes or placed somewhat "basally" within Ophioniformes. Their summary table maintained Orthopelmatinae by itself within Orthopelmatiformes.

None of our analyses recovered a sister-group relationship of Orthopelmatinae and Ophioniformes, although it is noted that moving *O. mediator* as sister to Ophioniformes only lengthened the total-evidence parsimony tree by 9 steps (9926). The parsimony analysis with only morphological characters (part 3 of Fig. 118) found a relationship as follows: *Orthopelma mediator* + (*Phrudus* sp. + (*Allophrys divaricata* Horstmann + (*Stethantyx nearctica* Townes + *Tersilochus* sp.))). It was supported by 15 morphological characters of which 1 was relatively strong: character 28 (state 1): foveate groove of mesopleuron present (C.I. = 0.33) (Fig. 29). This character is one of the important diagnostic characters for Tersilochinae sensu stricto (Tersilochinae of Townes 1971); however it is also present in some of the *Phrudus* group (synonymized with Tersilochinae from Phrudinae by Quicke et al. 2009), as well as sporadically found in other taxa, including *Orthopelma mediator* (but not all species of *Orthopelma*) (Barron 1977). Biologically, Tersilochinae are koinobiont endoparasitoids most often associated with beetles (Khalaim and Broad 2013), but have also been reared from Xyelidae (Hymenoptera) (Khalaim and Blank 2011) and Eriocraniidae (Lepidoptera) (Jordan 1998). Considering the differences between the hosts of Tersilochinae and Orthopelmatinae (endoparasitoids of cynipid gall wasps) (Blair 1945), as well as the fact that the foveate groove is not present in all *Orthopelma* spp., it is likely that the relationship postulated between *O. mediator* and these exemplars of Tersilochinae is artefactual. Similarly, none of our results indicated a close relationship of Orthopelmatinae with Cryptinae or Ichneumoninae as postulated by Barron (1977). The placement of *O. mediator* as sister group to all ichneumonids except the exemplars of Xoridinae (part 1 of Fig. 117) or related to Ichneumoniformes s.l. + Pimpliformes (part 2 of Figs 120, 121) was not expected. In terms of whether to recognize the higher group Orthopelmatiformes, despite ambiguity in its precise relationships, both of our total-evidence analyses found Orthopelmatinae to be sister group to a large number of other subfamilies, therefore maintainence of the higher group Orthopelmatiformes seems valid, at least until such time that more corroborated evidence is found to place Orthopelmatinae more precisely.

Oxytorinae

Our analyses generally placed Oxytorinae (*Oxytorus albopleuralis*) with exemplars of Ophioniformes; however, its placement within this group changed depending on the analysis. In our total-evidence parsimony analysis, Oxytorinae clustered within a clade comprised of all 14 Ctenopelmatinae species as well as Hybrizontinae, Lycorininae, Tatogastrinae, *Chineater masneri* (Mesochorinae) and *Scolomus* sp. (Metopiinae) (part 5 of Fig. 117). See the discussion on Ctenopelmatinae (above) for supporting characters for this clade. In the total-evidence Bayesian analysis, Oxytorinae was placed unresolved in a large clade with exemplars of Ctenopelmatinae, Hybrizontinae, Meso-

chorinae, Metopiinae, Tatogastrinae and the higher Ophioniformes; however, the support for this group was very low (BPP = 53) (part 1 of Fig. 120).

Prior to Townes (1971), *Oxytorus* Förster was considered to be related to genera now classified within Ctenopelmatinae (e.g., within the Mesoleptini of Ashmead 1900). Townes (1971) placed it provisionally in his Microleptinae (questioning whether it belonged there), and Wahl (1990) proposed that it should be placed in its own subfamily because of the lack of characters linking it with other ichneumonid subfamilies. The larva and host are not known, but Wahl (1990) commented that H.K. Townes believed that its notched ovipositor suggested it is probably an endoparasitoid. Gauld et al. (1997) included Oxytorinae in their unplaced subfamilies. Molecular studies have consistently placed Oxytorinae within Ophioniformes. Belshaw and Quicke (2002) using the 28S D2–D3 region and a limited taxon sampling found *Oxytorus* sp. was sister to *Banchus volutatorius* (Linnaeus) (Banchinae) and related to exemplars of Tryphoninae, Lycorininae and Orthopelmatinae. Quicke et al. (2009) generally recovered Oxytorinae within Ophioniformes, related to Banchinae, Ctenopelmatinae, Metopiinae, Neorhacodinae, Stilbopinae, Tersilochinae and Tryphoninae. For example, their combined analysis with gaps treated as missing placed Oxytorinae as sister group to (Tersilochinae + (Microleptinae + Orthopelmatinae)) and more distantly related to Ctenopelmatinae, Tatogastrinae, Mesochorinae and Metopiinae). Our results agree with the placement of *Oxytorus* within Ophioniformes and likely most closely related to Ctenopelmatinae. A more precise placement of Oxytorinae can only be determined by a more comprehensive study of the relationships of Ctenopelmatinae and its relatives.

Pedunculinae

Pedunculinae (*Pedunculus* sp. nov.) was strongly supported as sister group to Claseinae (*Clasis* sp. nov.) in all analyses except the parsimony analysis with only morphological characters in which its relationships were unclear. In both total-evidence analyses, these two subfamilies were placed near the base of Ichneumoniformes s.l. See the sections on Brachycyrtinae, Claseinae and Labeninae (above) for discussion of support of this node and further relationships of Pedunculinae.

Townes (1969) described *Pedunculus*, placing it within Brachycyrtini (Labeninae). Wahl (1993a) raised Brachycyrtini (including *Pedunculus*) to subfamily status. Porter (1998) removed *Pedunculus* to its own subfamily and later, Gauld et al. (2000) included *Adelphion* Townes and *Monganella* Gauld in Pedunculinae. Pedunculinae appear to be supported by presence of a smooth posterior face of the hind tibia (Fig. 69), although this condition has evolved independently in other taxa including *Microleptes* (Microleptinae), some Phygadeuontinae (e.g., *Cisaris* Townes) and some Ctenopelmatinae. Quicke et al. (2009) found that Pedunculinae was sister group to Brachycyrtinae and these two subfamilies were sister group to Claseinae in most of their analyses. As was noted in the discussion of Claseinae, most of our analyses supported a relationship of (*Pedunculus* sp. nov. + *Clasis* sp. nov.) with *Brachycyrtus wardae*, and sometimes these three taxa grouped with Labeninae, but we maintain the subfamily status of all four because of differences in their relationships depending on the characters used and the method of analysis.

Phygadeuontinae

As discussed above in the section on Cryptinae, Phygadeuontinae (*Acrolyta* sp., *Endasys patulus* and *Mastrus* sp.) was supported in both total-evidence analyses (part 3 of Fig. 117, part 2 of Fig. 120), but only moderately so, and was not supported in any other analyses. When supported, it was the sister group of (Alomyinae + Ichneumoninae). The only morphological character supporting this grouping in the total-evidence parsimony analysis was character 53, state 0: fore wing vein *2m-cu* with two bullae (C.I. = 0.07) (Fig. 50) and the Bremer support was only 2 steps, but this sister-group relationship was unequivocally supported in the Bayesian total-evidence analysis (BPP = 100).

Our total-evidence analyses results, albeit with very limited sampling, concur with that of Santos (2017) that Phygadeuontinae (previously Phygadeuontini) is not a tribe within Cryptinae. We did not include specimens of *Hemigaster*, which Santos (2017) moved from Cryptinae (Hemigastrini) to Phygadeuontini, nor did we include specimens of *Helcostizus* Förster which Broad (2016) moved from Phygadeuontini to Cryptini. Whether Phygadeuontinae is monophyletic or needs to be further divided into smaller, natural groups will require future analyses with more taxa. Our analysis certainly concurs with Santos (2017) that Phygadeuontinae is closely related to Cryptinae, Ateleutinae and Ichneumoninae.

Pimplinae

The seven species of Pimplinae (*Perithous divinator*, *Acrotaphus wiltii* (Cresson), *Clistopyga recurva* (Say), *Dolichomitus irritator* (Fabricius), *Zaglyptus pictilis* Townes, *Pimpla annulipes* Brullé and *Theronia bicincta*) never clustered together with unequivocal support in any of our analyses (Table 3); although the total-evidence Bayesian analysis did have moderate support for the monophyly of the subfamily (BPP = 93) (part 2 of Fig. 120). In the total-evidence parsimony analysis, six of the seven species grouped together, the exception being *Pimpla annulipes* (Pimplini), which was part of an unresolved clade including Rhyssinae, Poemeniinae, and the six other species of Pimplinae (part 2 of Fig. 117). Examination of the 1728 equally parsimonious cladograms found that *P. annulipes* was either sister taxon to the rest of the higher Pimpliformes (33% of trees) or sister taxon to Rhyssinae (67% of trees). Moving *P. annulipes* so that it was sister species to the other six species of Pimplinae resulted in an increase in tree length of only three steps (9920). Both analyses using only molecular characters recovered a polyphyletic Pimplinae (part 2 of Figs 119, 121). Finally, the parsimony analysis with only morphological characters recovered the higher Pimpliformes, but the relationships among the Pimplinae species were equivocal (only *Pimpla* and *Theronia* were sister species in all trees) (part 1 of Fig. 118). Examination of the individual equally parsimonious trees found that Pimplinae was monophyletic in 87 % of the 3872 trees, as opposed to 13% that had *D. irritator* as sister taxon to Rhyssinae, the latter topology supported by characters associated with parasitism of wood-boring hosts, e.g., character 96(2): ovipositor longer than length of metasoma and character 140(1): oviposition through lignified tissue.

Historically, Pimplinae in the broad sense (i.e., including related taxa such as Poemeniinae and Rhyssinae) was one of the five traditional subfamilies of Ichneumonidae

(Holmgren 1857). The morphology-based classification of Townes (1969) used a tribal classification within Pimplinae (his "Ephialtinae") to attempt to define natural groups. Some of these tribes were later raised to subfamily status (e.g., Diacritinae, Poemeniinae, Rhyssinae) (Eggleton 1989, Gauld 1991). More recent morphological phylogenetic studies analyzed the relationships within Pimplinae (Wahl and Gauld 1998, Gauld et al. 2002b). Both of these studies provided strong support for the monophyly of Pimplinae. In contrast, recent molecular studies have found varied levels of support for Pimplinae. Belshaw et al. (1998) using a limited taxon sampling of Pimplinae never recovered the subfamily as monophyletic. The study of Quicke et al. (2009) does not explicitly comment on the monophyly of Pimplinae in their analyses with only molecular characters, but they do present data on the monophyly of Pimplini with differing gap extension and opening costs, and the majority of these molecular analyses did not recover Pimplini as monophyletic. Finally, the study of Klopfstein et al. (2019) had varied results. Their transcriptome analysis with five species of Pimplinae using amino acids recovered Pimplinae, but the similar analysis with nucleotides did not. In addition, none of their hybrid enrichment analyses recovered Pimplinae because *Xanthopimpla varimaculata* Cameron never clustered with all of the other 24 pimpline exemplars. Their three hybrid enrichment analyses with amino acids did recover the other 24 exemplars as monophyletic but the two analyses with nucleotides did not. Klopfstein et al. (2019) speculated that the placement of *Xanthopimpla* in their analyses may be because of a long branch of their exemplar species, or it may indicate an actual relationship requiring formal recognition of a new tribe or subfamily. In terms of formal changes in Pimplinae, Klopfstein et al. (2019) resurrected Theroniini, comprised of the *Theronia* group of genera, which did not generally cluster with Pimplini. In summary, most previous morphological analyses have supported Pimplinae, although the current analysis is equivocal. In contrast, most molecular analyses have not found strong support for Pimplinae, regardless of the genes used or the method of analysis.

With respect to the placement of Pimplinae in the current study, when there was some support for the family (in 87% of the trees in the morphology-only parsimony analysis and the Bayesian total-evidence analysis with BPP = 93), Pimplinae was sister to (Poemeniinae + Rhyssinae). This is the same as the relationship postulated by the morphological analysis of Wahl and Gauld (1998), the combined morphology and molecular analysis of Quicke et al. (2009) and all analyses using amino acids in Klopfstein et al. (2019).

Concerning tribal monophyly and their relationships, the following previous hypotheses have been postulated:

1) (Delomeristini (not including *Perithous* Holmgren) + (Ephialtini + (*Perithous* + Pimplini including the *Theronia* group)) (morphological parsimony analysis of Wahl and Gauld 1998);
2) (Pimplini + (Delomeristini including *Perithous*) + Ephialtini)) (morphological parsimony analysis of Gauld et al. 2002b);
3) (Delomeristini (including *Perithous*) + (Pimplini + Ephialtini)) (combined morphological and molecular parsimony analysis of Quicke et al. 2009);

4) (Delomeristini (including *Perithous* and *Pseudorhyssa*) + Theroniini) + (Pimplini + Ephialtini), with *Xanthopimpla* clustering outside Pimplinae) (anchored enrichment analysis using amino acids of Klopfstein et al. 2019).

The current study supported monophyly of Ephialtini in all studies except parsimony with only morphological data. For example, the Bayesian total-evidence analysis had the following topology: (*Perithous divinator* + *Theronia bicinta*) + (*Pimpla annulipes* + (*Dolichomitus irritans* + (*Acrotaphus wiltii*/ *Clistopyga recurva*/ *Zaglyptus pictilis*))). (part 2 of Fig. 120). The latter four species comprise Ephialtini and the support for this tribe was unequivocal (BPP = 100). The total-evidence parsimony analysis was similar but more resolved: (*D. irritator* + (*Z. pictilis* + (*A. wiltii* + *C. recurva*))) with 13 synapomorphies and a Bremer support of 5 steps (part 2 of Fig. 117). Relative to the four previous hypotheses of relationships listed above, the current Bayesian total evidence analysis supported the relationships of Klopfstein et al. (2019) in that *Theronia bicincta* (Theroniini) was sister to *Perithous divinator* (Delomeristini), not sister to *Pimpla annulipes* (Pimplini).

Poemeniinae

Poemeniinae (*Neoxorides caryae* (Harrington) and *Poemenia albipes*) was supported unequivocally in all analyses except the Bayesian analysis using only molecular characters (Table 3), and even the latter analysis still had moderate support with a posterior probability of 94 (part 2 of Fig. 121). The subfamily was supported by 26 synapomorphies (9 morphological) in the total-evidence parsimony analysis with a Bremer support of 5 steps (part 2 of Fig. 117). One of the morphological characters was uniquely derived: character 15(1): foramen magnum laterally expanded.

In terms of relationships, there was strong support for Poemeniinae being the sister group to Rhyssinae (e.g., Bayesian total-evidence, BPP = 99) (part 2 of Fig. 120). The parsimony total-evidence analysis did not contradict this grouping, although the strict consensus was unresolved in this part of the tree with the following clade with equivocal relationships: Poemeniinae/ *Pimpla annulipes*/ Rhyssinae/ other Pimplinae (part 2 of Fig. 117). Thirty-three percent of the shortest trees recovered Poemeniinae + Rhyssinae. Furthermore, most analyses placed Poemeniinae within the higher Pimpliformes (e.g., Bayesian total-evidence had a posterior probability of 98 for this grouping). See discussion of Pimpliformes (above) for support of this grouping in the parsimony total-evidence analysis.

Historically, *Poemenia* Holmgren was placed within the traditional "Pimplinae" of early authors, e.g., Holmgren (1860). Townes (1969) considered *Poemenia* and relatives as a tribe within Ephialtinae (= Pimplinae), and it was only with the study of Gauld (1991) based on the thesis of Eggleton (1989) that the group was raised to subfamily status. Wahl and Gauld (1998) confirmed the subfamily status within Pimpliformes with a phylogenetic analysis of morphological characters that found the following relationship: Pimplinae + (Rhyssinae + Poemeniinae). They also defined three tribes: Pseudorhyssini comprised of *Pseudorhyssa*, Rodrigamini comprised of *Rodrigama* Gauld and Poemeniini comprised of all other genera. The current study was only

able to include species of Poemeniini. Quicke et al. (2009) included exemplars of all three tribes. Their morphological analysis hypothesized that *Pseudorhyssa* belonged to Pimplinae, not Poemeniinae, a placement that was previously suggested (e.g., Townes 1969). In contrast, the combined morphology and sequence analyses of Quicke et al. (2009) had equivocal placement for *Pseudorhyssa*, including related to Pimplinae, Rhyssinae, or Poemeniinae, as sister to (Rodrigamini + Poemeniini)). They left *Pseudorhyssa* as unplaced in their summary table.

More recently, *Pseudorhyssa* clustered within Pimplinae in all of the full-data-set analyses of Klopfstein et al. (2019) which led them to formally move the genus back to Pimplinae. Since the current analysis did not include *Pseudorhyssa* or *Rodrigama*, we cannot comment on these taxa, but we can state that there was strong support for the monophyly of Poemeniini and for the relationship of Poemeniini (at least) to Rhyssinae.

Rhyssinae

Similar to Poemeniinae, Rhyssinae (*Megarhyssa greenei* Viereck, *Rhyssa crevieri* (Provancher) and *Rhyssella nitida* (Cresson) was well-supported – it was unequivocally monophyletic in all of our analyses (Table 3). In the total-evidence parsimony analysis, Rhyssinae was supported by 56 synapomorphies including 13 morphological, of which two were uniquely derived: character 92, state 1: apical segment of female metasoma elongate with a horn or boss (Fig. 82); and character 93 state 1: posterior sternites of female metasoma with tuberculate ovipositor guides (Fig. 84). The Bremer support was greater than 10 steps. Rhyssinae is likely the most well-supported subfamily within Ichneumonidae. All of our analyses placed Rhyssinae within the higher Pimpliformes (with Poemeniinae and Pimplinae) and when there was resolution within this group, the sister group for Rhyssinae was Poemeniinae (e.g., part 1 of Fig. 120).

As described above for Poemeniinae, *Rhyssa* Gravenhorst and its relatives were also historically placed in the traditional Pimplinae (Holmgren 1860) (= equals our higher Pimpliformes). Phylogenetic studies have confirmed this placement (Wahl and Gauld 1998; Quicke et al. 2009, Klopfstein et al. 2019, and most analyses in the current study). Based on the strength of evidence supporting the higher Pimpliformes, it could be argued that a reversion to a previous concept of Pimplinae is warranted (including poemeniines and rhyssines). Considering that the limits of Poemeniinae were just changed by the removal of *Pseudorhyssa* and the fact that we were not able to include *Rodrigama* in our analysis, we believe this move would be premature. Maintainence of these taxa in three subfamilies is more prudent until additional studies are able to confirm the current definitions and relationships of all groups within higher Pimpliformes.

Sisyrostolinae

Sisyrostolinae (*Brachyscleroma* sp. and *Erythrodolius calamitosus* Seyrig) was not recovered as monophyletic in any of our analyses (Table 3). Having said this, these two species often clustered in close proximity to each other along with exemplars of Tersilochinae. For example, in the total-evidence parsimony analysis, all five tersilochine species, together with the two sisyrostoline species formed a clade that was supported

by 20 synapomorphies (of which 4 were morphological), with a Bremer support of 2 (part 4 of Fig. 117). Two of these characters had a relatively high consistency index: character 109, state 1: larval mandible conical and with small, apical tooth (CI = 0.80) (Fig. 111), and character 139, state 3: Coleoptera host (CI = 0.27). Similarly, these seven species clustered together in the total-evidence Bayesian analysis, but with a low support value (BPP = 78) (part 1 of Fig. 120). The larvae of both *Brachyscleroma* Cushman and *Erythrodolius* Seyrig are unknown, as is the host of *Erythrodolius*.

The genera to which our two exemplars of Sisyrostolinae belong were, until recently, included in the subfamily Phrudinae (e.g., Townes 1971; Gauld et al. 1997). The relatedness of Tersilochinae and Phrudinae was implied by their placement next to each other in Townes (1971). The latter author did; however, express doubt regarding the monophyly of Phrudinae, and Gupta (1994) in his revision of *Brachyscleroma* stated "The Phrudinae contains a heterogenous assemblage of genera which are certainly not related." Conversely, Gauld et al. (1997) stated with respect to the small, temperate genera (i.e., the *Phrudus* group of genera) and the large, mostly Afrotropical *Erythrodolius* group of genera that they were "correctly associated" with each other. The latter study cited "the peculiarly narrow proboscidial fossa" as an autapomorphy of Phrudinae.

More recent phylogenetic studies investigated the monophyly of Phrudinae. Quicke et al. (2009) included 12 phrudine exemplars. In all their analyses, three *Erythrodolius* species clustered with their other two members of the *Erythrodolius* group (*Melanodolius* sp. and *Icariomimus* sp.) and the sister taxon of this group was *Brachyscleroma* sp. The relationship of this clade to the *Phrudus* group; however, was equivocal. In their combined analysis with gaps treated as missing, the *Phrudus* group was monophyletic and clustered with Tersilochinae, whereas (*Brachyscleroma* sp. + the *Erythrodolius* group) was placed separate to Tersilochinae (near Tryphoninae). In contrast, in their combined analysis with gaps treated as informative, exemplars of two genera of the *Phrudus* group (*Phrudus* Förster and *Astrenis* Förster) were sister group to *Brachyscleroma* sp. + the *Erythrodolius* group). Whereas these are only two of many analyses that Quicke et al. (2009) performed, it illustrates the fact that there may (or may not) be a relationship between the *Erythrodolius* group + *Brachyscleroma* and at least some of the *Phrudus* group. Despite this ambiguity, Quicke et al. (2009) formally divided the Phrudinae of Townes (1971), placing all of the *Phrudus* group within Tersilochinae and resurrecting Brachyscleromatinae Townes to accommodate *Brachyscleroma* and the *Erythrodolius* group as well as the Oriental and Eastern Palaearctic *Lygurus* Kasparyan. Since that time, Sheng and Sun (2011) described another genus in this group: *Laxiareola* Sheng & Sun from the Oriental region. Bennett et al. (2013) noted the priority of the name Sisyrostolinae for this higher group. Quicke et al. (2009) provided a diagnosis for Sisyrostolinae as follows: 1) sternites mostly sclerotized and laterotergites large; 2) scape cylindrical (rather long and narrow); 3) proboscidial fossa strongly narrowed; 4) ovipositor lacking notch; 5) hind wing vein *M + Cu* long relative to vein *1-M*.

Our analyses do not uphold the monophyly of Sisyrostolinae, nor its separate status from Tersilochinae (including the *Phrudus* group). It is likely that the *Erythrodolius* group is monophyletic (*Melanodolius* Saussure and *Icariomimus* Seyrig are very closely

related based on morphology, for example, the frons in all three genera bears a longitudinal ridge, and the latter may even be paraphyletic with respect to *Erythrodolius* (Bennett et al. 2013). Whether the *Erythrodolius* group is only distantly related to Tersilochinae or nested within Tersilochinae is unclear at present. The sister-group relationship of *Brachyscleroma* to the *Erythrodolius* group was not upheld by this study, and this needs to be re-examined. Similarly, the relationship of the *Phrudus* group to Sisyrostolinae needs to be studied (see Tersilochinae, below). This statement is based on the fact that all of our analyses and some of Quicke et al. (2009) did not recover a monophyletic *Phrudus* group, as well as the fact that some taxa putatively placed within the *Phrudus* group have never been assessed cladistically (e.g., *Notophrudus* Porter). Similarly, *Lygurus* and *Laxiareola* have not been sequenced or coded for morphology to examine where they fit. Finally, with respect to the five diagnostic characters that Quicke et al. (2009) used to define Sisyrostolinae, none of them are convincing autapomorphies of the group because all of them also occur in members of the *Phrudus* group, for example, most *Astrenis* spp. have much more sclerotized sternites than any member of Sisyrostolinae. It should be noted that despite careful examination of many specimens of the *Erythrodolius* group, the *Phrudus* group and the Tersilochinae sensu stricto, we were unable to code the proboscidial fossa character of Gauld et al. (1997). Because of the large amount of work still remaining to be done on this part of the tree, we refrain from making any formal changes at this time. We do, however, concur with Townes (1971) and Quicke et al. (2009) that Sisyrostolinae belongs to Ophioniformes, not Pimpliformes (= the traditional Pimplinae) as Seyrig (1932) proposed. Our study placed them within Ophioniformes, but not within the higher Ophioniformes which is in accordance with the findings of Quicke et al. (2009).

Stilbopinae

The two species of Stilbopinae (*Stibops vetulus* and *Notostilbops* sp. nov.) were never sister taxa in any of our analyses (Table 3). *Notostilbops* sp. nov. clustered with the four species of Banchinae in all analyses except the parsimony analysis with only morphological characters and consequently we have formally moved *Notostilbops* to Banchinae. See Banchinae section (above) for justification of this taxonomic change.

Tatogastrinae

Tatogastrinae (*Tatogastra nigra*) was one of the small subfamilies that clustered within the "Ctenopelmatinae and related subfamilies" clade in the total-evidence parsimony analysis (part 5 of Fig. 117). It was placed in a clade that lacked internal resolution as follows: Oxytorinae/ Tatogastrinae/ (*Chineater masneri* + *Scolomus* sp.) based on 39 synapomorphies (six of which were morphological) and a Bremer support of 2 steps. It occupied a similar position in the Bayesian total-evidence analysis (part 1 of Fig. 120), as sister species to (*Chineater masneri* + *Scolomus* sp.) in a very poorly supported group (posterior probability = 55). See the discussion on Ctenopelmatinae (above) for additional discussion of the relationships of *Tatogaster* to Ctenopelmatinae, Metopiinae and Hybrizontinae in the Bayesian total evidence analysis.

In terms of previous hypotheses concerning the relationships of Tatogastrinae, Wahl (1991) performed a morphological phylogenetic analysis and proposed that Tatogastrinae was the sister group of Ophioninae on the basis of four synapomorphies: 1) fore wing with spurious vein originating at distal end of vein *1A*; 2) glymma of T1 absent and T1 enveloping S1; 3) profile of propodeum not angulate; and 4) ovipositor short, about equal in length to the depth of the metasoma. The current analysis did not code the spurious vein. It is present in *Tatogaster* and in our five ophionine species including *Hellwigia obscura*, but apparently absent in *Hellwigia elegans* Gravenhorst. The vein also appears to be present in some taxa outside of Ophioninae, especially in larger-bodied species (e.g., *Metopius pollinctorius*, *Megarhyssa greenei*, *Netelia* sp.), and therefore it may be related to species size as much as phylogeny. The current analysis coded the fusion of T1 and S1 (character 81) for *Tatogaster* as state 1: fused, but suture visible, compared to Ophioninae as state 2: fused, suture not visible (i.e., character coded somewhat differently than in Wahl 1991). In terms of profile of the propodeum (character 35), the current analysis coded about two thirds of the exemplar taxa as state 1: rounded to flattened without separate dorsal and posterior faces and the consistency index of this character is very low (0.03) with multiple parallelisms and reversals which makes it difficult to determine its apomorphic state. Ovipositor length (character 96) has a similarly low consistency index (0.05). Moving *Tatogaster nigra* as sister group to Ophioninae increased the tree length to 9955 (+ 38 steps).

The morphological analysis of Quicke et al. (2009) and their combined morphology and 28S D2–D3 analysis found Tatogastrinae to be the sister group to Mesochorinae with both of these subfamilies related to various tribes of Ctenopelmatinae. In terms of morphology, the similarly large, rhombic areolet of Tatogastrinae and Mesochorinae was noted as a possible synapomorphy of these two taxa although several differences were also noted (presence of a dorsal notch in the ovipositor of Tatogastrinae, lack of a glymma, and lack of rod-like gonoforceps). Moving *T. nigra* as sister group to Mesochorinae (except for *Chineater masneri*) in our analysis increased the tree length to 9941 (+ 24 steps). In summary, the current analysis found no evidence supporting a sister-group relationship to Ophioninae or Mesochorinae (except the enigmatic genus *Chineater*), but it did suggest a relationship of Tatogastrinae with our exemplars of Ctenopelmatinae, albeit with weak support.

Tersilochinae

As discussed in Sisyrostolinae (above), the five species of Tersilochinae sensu lato (*Allophrys divaricata*, *Phrudus* sp., *Peucobius fulvus* Townes, *Stethantyx nearctica* and *Tersilochus* sp.) did not cluster together in any analyses, except in a grouping that also included the two species of Sisyrostolinae (in both total-evidence analyses) (Table 3). See the section on Sisyrostolinae for a discussion of the support for this grouping.

In contrast, the Tersilochinae sensu stricto, which is equivalent to the Tersilochinae of Townes (1971), is a well-supported group in all analyses (Table 3). For example, in the total-evidence parsimony analysis, this clade – *Stethantyx nearctica* + (*Allophrys divaricata* + *Tersilochus* sp.) was supported by 63 characters, 18 of which were mor-

phological, and a Bremer support of more than 10 steps. Despite the fact that none of the morphological synapomorphies were uniquely derived, several had a relatively high consistency index, for example, character 4, state 1: clypeal margin with uniform fringe of setae (C.I. = 0.25) (similar to Fig. 12); character 16, state 1: maxillary palpus four-segmented (C.I. = 0.33); and character 64, state 1: basal 0.6 of hind wing vein *M + Cu* spectral (C.I. = 0.33) (Fig. 58).

Our study generally supports a relatively close relationship of the *Phrudus* group and Tersilochinae sensu stricto; however, *Erythrodolius* and *Brachyscleroma* (Sisyrostolinae) clustered within this clade as follows: (*Phrudus* sp. + (*Erythrodolius calamitosus* + *Peucobius fulvus*)) + (*Brachyscleroma* sp. + Tersilochinae sensu stricto) (e.g., part 4 of Fig, 117, part 1 of Fig. 120). More work is needed to determine whether Tersilochinae sensu lato needs to be broadened to include some or all of the genera currently placed in Sisyrostolinae. Paramount in these studies is increased knowledge of the larva in this clade. The current study reports the first larval description of the *Phrudus* group of genera (Appendix 1) including a putative synapomorphy for the *Phrudus* group and Tersilochinae sensu stricto: character 109, state 1: larval mandible cone-shaped with small, apical tooth (C.I. = 0.8) (Fig. 111). Examination of the larva of other *Phrudus* group specimens and Sisyrostolinae would be very helpful to determine the relationships within this clade. Another character that requires additional examination in the *Phrudus* group, Tersilochinae sensu stricto and Sisytrostolinae are the modified sensory structures of the sub-basal flagellomeres reported by Vikberg and Koponen (2000), which were not coded in the current study. In our exemplar species, they are present in *Phrudus* sp., *Stethantyx nearctica* and *Tersilochus* sp. and possibly in *Allophrys divaricata* (hard to score because of setae and small size), but apparently absent from *Brachyscleroma* sp., *Erythrodolius calamitosus*, and *Peucobius fulvus*. Finally, our results agree with Broad (2016), that Neorhacodinae is not a synonym of Tersilochinae as Quicke et al. (2009) proposed, but should be treated as a separate subfamily (see Neorhacodinae, above).

Tryphoninae

Tryphoninae (*Eclytus* sp., *Idiogramma longicauda*, *Zagryphus nasutus* (Cresson), *Netelia* sp., *Phytodietus vulgaris*, *Cteniscus* sp., *Cycasis rubiginosa* (Gravenhorst) and *Polyblastus* sp.) was not supported in any of our analyses (Table 3); however, the total-evidence parsimony analysis did find that all eight tryphonine species shared a common ancestor, but also including *Neorhacodes enslini* (Neorhacodinae) (part 4 of Fig. 117). This grouping was supported by 24 synapomorphies, of which 7 were morphological, including one uniquely derived: character 136, state 1: egg exits body ventral to ovipositor and stalk travels down lumen of ovipositor. The Bremer support was 6 steps. Note that egg morphology and method of oviposition are unknown for *N. enslini* (and were therefore coded as "?"). The parsimony analysis using only morphology did not support any groupings within Tryphoninae, except for Phytodietini (*Phytodietus vulgaris* + *Netelia* sp.) (part 2 of Fig. 118). The other three analyses all placed the tryphonine exemplars near the base of the tree, often in a grade. For example, the total-evidence Bayesian analysis had *Neorhacodes enslini* as sister to all other Ichneumonidae, followed

by Phytodietini and then a clade of equivocal relationships comprised of *Lycorina glaucomata*/ *Idiogramma longicauda*/ (*Zagryphus nasutus* + (*Eclytus* sp. + (*Polyblastus* sp. + (*Cteniscus* sp. + *Cycasis rubiginosa*)))) (part 1 of Fig. 120).

Historically, Tryphoninae was one of the five major groups of Ichneumonidae (Holmgren 1857), but this included many taxa that were later removed to their own subfamilies (e.g., Ctenopelmatinae, Metopiinae, etc.) by Townes et al. (1961) and later authors. Townes et al. (1961) presumably based the definition of Tryphoninae sensu stricto on possession of stalked eggs, a trait recognized as an important indicator of phylogeny as early as Hartig (1837). Short (1978), studying the larva of *Euceros* spp. (Eucerotinae), confirmed the conclusions of Perkins (1959a) that Eucerotinae (which also has a stalked egg) does not belong to Tryphoninae. Later, Shaw (2004) and Coronado-Rivera et al. (2004) described the stalked egg of *Lycorina* spp. (Lycorininae), which raised the possibility that *Lycorina* should be moved to Tryphoninae. See the Lycorininae section (above) for the reasons against this move.

In terms of phylogenetic analyses, Belshaw et al. (1998) using the 28S D2 region found that *Netelia* sp. was paraphyletic with respect to the other five tryphonine exemplars. The later, more comprehensive, combined morphological and molecular analysis of Quicke et al. (2009) found that all of their 62 tryphonine species shared a common ancestor, but species of Sisyrostolinae, Stilbopinae, Eucerotinae and *Ischyrocnemis* (Metopiinae) were also nested in this clade. Bennett (2015) using only morphological characters and a limited number of outgroups, did recover Tryphoninae as monophyletic with three uniquely derived synapomorphies: the clypeal fringe of setae, the larval mandible lacking denticles on the dorsal surface of the blade and stalked eggs.

Based on the current study, Quicke et al. (2009) and Bennett (2015), there is some evidence for the monophyly of Tryphoninae; however, more knowledge is needed about taxa that may be related (or may belong) to Tryphoninae (e.g., Neorhacodinae, Lycorininae, Sisyrostolinae, Stilbopinae). Knowledge of whether the body of the egg travels down the lumen of the ovipositor in these taxa could alter their placement relative to Tryphoninae, as occurred previously for the genus *Acaenitellus* Morley (see Gupta 1988). In addition, an increase in our knowledge of Tryphoninae larval characters and hosts would also help clarify whether Tryphoninae is monophyletic (host order is only known for 29 of the 54 extant genera) (Bennett 2015). With respect to *Neorhacodes*, the known host of *N. enslini* (*Spilomena* spp.) (Hymenoptera: Crabronidae) was coded the same (character 139, state 1) as most tribes of Tryphoninae which parasitize sawflies. Perhaps coding this character differently for Aculeata as opposed to sawflies would be appropriate and would modify the placement of *Neorhacodes* relative to Tryphoninae.

With respect to the findings of Belshaw et al. (1998) that Phytodietini may be related to, but not included within Tryphoninae, the current study is equivocal. Both Bayesian analyses and the parsimony analysis with only morphological characters found that Phytodietini did not cluster with the other six exemplars of Tryphoninae, but the total-evidence parsimony analysis did. In order to accept the notion that Phytodietini do not belong to Tryphoninae, one has to postulate that evolution of the stalk

of the egg travelling down the lumen of the ovipositor during oviposition has evolved twice. A future study including the morphological characters of Bennett (2015) and sequence data obtained by next generation sequencing will address this issue.

Relationships within Tryphoninae based on the total-evidence parsimony analysis were as follows: (Neorhacodinae + Phytodietini) + (Idiogrammatini + (*Polyblastus* group of Tryphonini + (Eclytini + Oedemopsini) + *Exenterus* group of Tryphonini))) (part 4 of Fig. 117). Ignoring the placement of Neorhacodinae, the relationships differ from the morphological cladistic analysis of Bennett (2015) in two ways: 1) Phytodietini is the sister group to the rest of Tryphoninae (Idiogrammatini was the sister group in the latter study); 2) Tryphonini (including the *Exenterus* group) is not monophyletic (the *Exenterus* group of genera clustered within Tryphonini in the latter study). It is difficult to draw conclusions on relationships within Tryphoninae from the current analysis because of the low sample size (only 8 of 54 extant genera in five of seven tribes). Future studies will include a greater number of taxa including exemplars of Sphinctini and Ankylophonini.

Xoridinae

Xoridinae (*Aplomerus* sp., *Odontocolon albotibiale* (Bradley) and *Xorides stigmapterus* (Say)) was recovered as monophyletic in the parsimony analysis using only morphological characters, as well as both Bayesian analyses. In the morphological parsimony analysis (part 1 of Fig. 118), lack of resolution near the base of the tree precludes any statements about its placement within Ichneumonidae, except that it does not belong in the higher Ophioniformes. In both Bayesian analyses, Xoridinae was related to Ichneumoniformes and Pimpliformes, not Ophioniformes. The total-evidence Baysian analysis placed Xoridinae within the Ichneumoniformes/ Pimpliformes grouping as follows: Orthopelmatinae + (Xoridinae + (Labeninae + (remainder of Ichneumoniformes s.l. + Pimpliformes))) (part 2 of Fig. 120). The Bayesian analysis with only molecular characters had a similar topology as follows: (Orthopelmatinae + Xoridinae) + (Labeninae + (Ichneumoniformes s.l. except Labeninae + Pimpliformes)) (part 2 of Fig. 121). In contrast, the total-evidence parsimony analysis found that *Aplomerus* sp. was sister taxon to all other Ichneumonidae as follows: *Aplomerus* sp. + ((*Odontocolon albotibiale* + *Xorides stigmapterus*) + (*Orthopelma mediator* + all other Ichneumonidae))) (part 1 of Fig. 117).

With respect to the monophyly of Xoridinae, on the basis of morphology alone, Xoridinae is well-suppported, with 23 synapomorphies, including one uniquely derived: character 113, state 1: larval mandible with spines at base of blade (part 1 of Fig. 118). A previous morphological cladistic analysis supports this assertion (Gauld et al. 1997). Similarly, the combined morphological and molecular analysis of Quicke et al. (2009) recovered Xoridinae as monophyletic when treating gaps as missing or informative, although the latter analysis included specimens of *Xorides*, *Odontocolon* and *Ischnoceros* Gravenhorst, but not *Aplomerus*, which Gauld et al. (1997) found to be the sister group to the other three genera. Quicke et al. (2009) also coded morphology at the subfamily level for Xoridinae, and some of their analyses with only molecular data did not support the monophyly of the subfamily. In summary, based

on morphological evidence and the majority of our results, is it likely that Xoridinae is monophyletic. The lack of monophyly of Xoridinae in our total-evidence parsimony analysis was possibly because of how the parsimony algorithm dealt with the sequence data (as evidenced by the fact that Xoridinae was monophyletic in the Bayesian total-evidence analysis). Note that the larva of *Aplomerus* is unknown, and if it is confirmed that *Aplomerus* larvae also bear spines at the base of their mandible, this would help confirm the monophyly of the subfamily.

In terms of the placement of Xoridinae within Ichneumonidae, the total-evidence parsimony analysis provides some support that Xoridinae (or a subset of xoridine taxa) is the sister group of all other Ichneumonidae. Quicke et al. (1999) using only 28S DNA sequence data for 24 ichneumonoids and two aculeate outgroups found that *Xorides praecatorius* (Fabricius) was sister species to all other ichneumonids. Similarly, the transcriptome analysis of Klopfstein et al. (2019) with six braconid outgroups and their anchored enrichment analyses with six braconids and three non-ichneumonoids also had *X. praecatorius* as sister to all other ichneumonids. Therefore there is evidence that Xoridinae is sister-group to all other Ichneumonidae, but the results of our Bayesian analyses refute this, and therefore, future analyses involving only ichneumonid taxa should investigate the effects of different rootings on ingroup topology, rather than only root their trees with Xoridinae. In terms of the recognition of the higher group Xoridiformes, because both of our total-evidence analyes found that Xoridinae is the sister group to a large number of other subfamilies, this seems valid at this point.

Biological transitions

Inclusion of biological characters in our data matrix allows an examination of the evolution of these characters within Ichneumonidae, at least with respect to the exemplar taxa used in this analysis. In terms of the validity of this kind of analysis, we agree that biological characters can be complex and our unweighted, unordered analysis may not take into account differences in the likelihood of particular character state changes evolving relative to others. Despite this, we believe that there is value in this kind of analysis. An unweighted, unordered analysis is objective. It does not place pre-conceived notions on the direction of evolution, nor does it make subjective decisions on the relative importance of characters. Whether the current analyses of the evolution of biological characters in Ichneumonidae is realistic or over-simplistic is a question that will hopefully foster discussion on the evolution of these interesting traits, producing hypotheses that can be tested by future analyses.

Three biological characters have been optimized on to the total-evidence parsimony strict consensus cladogram as follows: character 137: timing of larval maturation (Fig. 122); character 138: location of larval maturation (Fig. 123); and character 139: host order/ source of larval nutrition (Fig. 124). In addition to optimization on these characters on the parsimony tree, a comparison is made of differences and similarities of the evolution of each character hypothesized by the total-evidence Bayesian analysis (Fig. 120).

Timing of larval maturation

In the total-evidence parsimony analysis, character 137, timing of larval maturation, had a length of 8 steps (Fig. 122). The following transitions occurred: koinobiosis to idiobiosis (five times): 1) *Doryctes erythromelas* (Braconidae); 2) at base of (*Odontocolon albotibiale* + *Xorides stigmapterus*); 3) at base of higher Pimpliformes; 4) at base of Ichneumoniformes s.l.; 5) *Cratichneumon w-album* (Cresson); idiobiosis to koinobiosis (three times): 1) *Acrotaphus wiltii*; 2) *Euceros* sp. nov.; 3) Ichneumoninae including *Alomya debellator*. Optimization of the character using ACCTRAN or DELTRAN did not change the number and placement of transitions.

The ancestral state for Ichneumonidae favours koinobiosis, although this requires some discussion. The state is unknown for *Aplomerus* sp. (Xoridinae) and is equivocal for the next node (the other two xoridines which are both idiobionts). There is strong morphological evidence that *Aplomerus* Provancher belongs to Xoridinae (Gauld et al. 1997) and therefore, it is likely to be an idiobiont. If this is true, then the ancestral state for Ichneumonidae would be idiobiosis for timing of larval maturation. Additional taxa, for example, a species of *Ischnoceros* Gravenhorst (Xoridinae) should be included to re-evaluate this question, and hopefully also additional knowledge of biology of our exemplar species.

In terms of the direction of evolution of this character in the parsimony analysis, it has transitions in both directions, slightly favoured in the direction of koinobiosis to idiobiosis (five times) compared to vice versa (three times). The transition from a supposedly more specialized koinobiont to a less specialized strategy (idiobiosis) was not hypothesized by Gauld (1988a). Considering the size and age of Ichneumonidae (at least 85 mya) (Kopylov 2012), it is, perhaps, not surprising that transitions appear to have arisen in both directions in this character over the course of evolution of the family. Estimates of the age at which parasitism first evolved within Hymenoptera, i.e., the age of the taxon *Vespina* (= Orussoidea + Apocrita) is at least 164 mya (Rasnitsyn and Zhang 2004; Ronquist et al. 2012). Based on these ages, there is no reason to assume that the koinobiont life history strategy had not evolved prior to the origin of Ichneumonoidea. In terms of a mechanism to explain transitions from koinobiosis to idiobiosis, this simply requires a change in the timing at which the larva commences feeding and/or a change in the host-searching behaviour of the female wasp. It has been argued that delay in the commencement of larval feeding should be advantageous to the parasitoid because it provides a larger host on which to feed and takes advantage of host behaviours such as finding a secure location for pupation away from predators and parasitoids (Gauld 1988a). It could be argued; however, that when there are high populations of parasitoids and predators that specialize on finding large, exposed, wandering larvae, reverting to an idiobiont strategy on younger larvae could be favourable in order to avoid this pressure. In addition, the evolution from late larval-pupal koinobionts to pupal-pupal idiobionts could easily evolve by the female wasp delaying and modifying its host-searching behaviour in order to search for pupae rather than late larvae/ pre-pupae. Whereas pupae are generally more concealed than larvae and therefore harder to find, they lack the ability to defend themselves physically and are generally

less setose/ spinose, so if they can be found, they may be easier to parasitize successfully. This type of transition appears to have evolved at least once in Ichneumonidae in our parsimony analysis in *Cratichneumon w-album* (green to red transition in Fig. 122).

Comparing the evolution of this character in the total-evidence Bayesian analysis, koinobiosis is favoured as plesiomorphic within Ichneumonidae (Fig. 120). The state of the sister taxon of all other Ichneumonidae (*Neorhacodes enslini*) is not known, but Phytodietini (sister taxon to all Ichneumonidae except *Neorhacodes*) are koinobionts, as are related taxa. The length of the character in the Bayesian analysis is 7 steps. Three transitions from koinobiosis to idiobiosis (indicated by the letter "I" in Fig. 120) occur as follows: 1) the outgroup *Doryctes erythromelas*; 2) *Cratichneumon w-album*; and 3) the ancestor of (Xoridinae + (Labeninae + (Ichneumoniformes s.l. + Pimpliformes))). Four changes occur from idiobiosis to koinobiosis: 1) *Euceros* sp.; 2) *Acrotaphus wiltii*; 3) the ancestor of Ichneumoninae (including *Alomya debellator*) and the ancestor of Acaenitinae, Orthocentrinae, Diplazontinae and related subfamilies (shown in Fig. 120 with a "K"). In summary, regardless of the method of phylogenetic analysis, transitions have occurred in both directions with respect to timing of larval maturation, and perhaps unexpectedly, koinobiosis appears more likely to be the plesiomorphic state within Ichneumonidae.

Location of larval maturation

Examination of the evolution of ectoparasitism versus endoparasitism (character 138) in the total-evidence parsimony analysis reveals that ectoparasitism is plesiomorphic in both Braconidae and Ichneumonidae (Fig. 123). As discussed for timing of larval maturation (above), our limited outgroups affected the plesiormorphic state for Braconidae (in this case with two ectoparasitoids versus one endoparasitoid) which in concert with the state for (*Odontocolon albotibiale* + *Xorides stigmapterus*) made the state at the base of Ichneumonidae unequivocally ectoparasitoidism. The length of the character is 12 steps. Unlike in character 137, the evolution of this character differed based on the type of character optimization used. Under ACCTRAN, transitions from ectoparasitism to endoparasitism occurred eight times: 1) *Aleiodes terminalis* (Braconidae); 2) ancestor of all Ichneumonidae except Xoridinae; 3) *Pimpla annulipes*; 4) *Theronia bicincta*; 5) *Euceros* sp. nov. 6) *Microleptes* sp.; 7) Ichneumoninae including *Alomya debellator*; 8) *Neorhacodes enslini*. Transitions from endoparasitism to ectoparasitism occurred three times: 1) ancestor of higher Pimpliformes; 2) ancestor of Ichneumoniformes s.l.; 3) ancestor of Tryphoninae (including *Neorhacodes enslini*). In addition, within Ophioniformes, Lycorininae (*Lycorina glaucomata*) had a transition from endoparasitism (state 1) to endoparasitism with a final ectoparasitoid phase followed by pupation within the host cocoon (Shaw 2004) (state 2, not shown in Fig. 123).

In contrast, under DELTRAN optimization (not shown), transitions from ectoparasitism to endoparasitism occurred ten times: 1) *Aleiodes terminalis* (Braconidae);

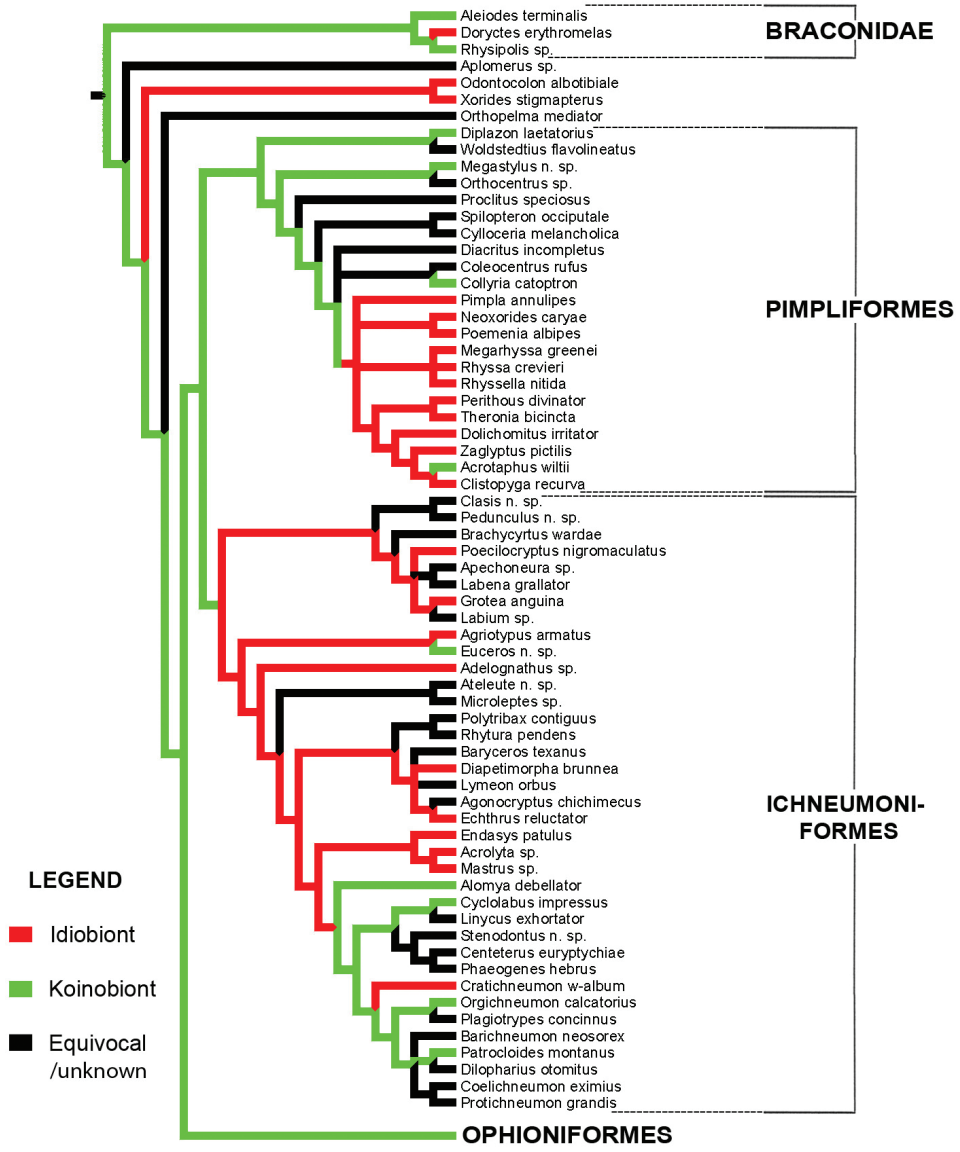


Figure 122. Optimization of character 137 (timing of larval maturation) on total-evidence parsimony analysis strict consensus cladogram using ACCTRAN. See Legend for description of colour coding for character states. Character length = 8 steps.

2) Orthopelmatinae; 3) ancestor of Pimpliformes; 4) *Pimpla annulipes*; 5) *Theronia bicincta*; 6) *Euceros* sp. nov. 7) *Microleptes* sp.; 8) Ichneumoninae including *Alomya debellator*; 9) *Neorhacodes enslini*; 10) ancestor of Ophioniformes. Only one transition from endoparasitism to ectoparasitism occurred in the ancestor of higher Pimpliformes. Lycorininae had the same transition to state 2 as for ACCTRAN.

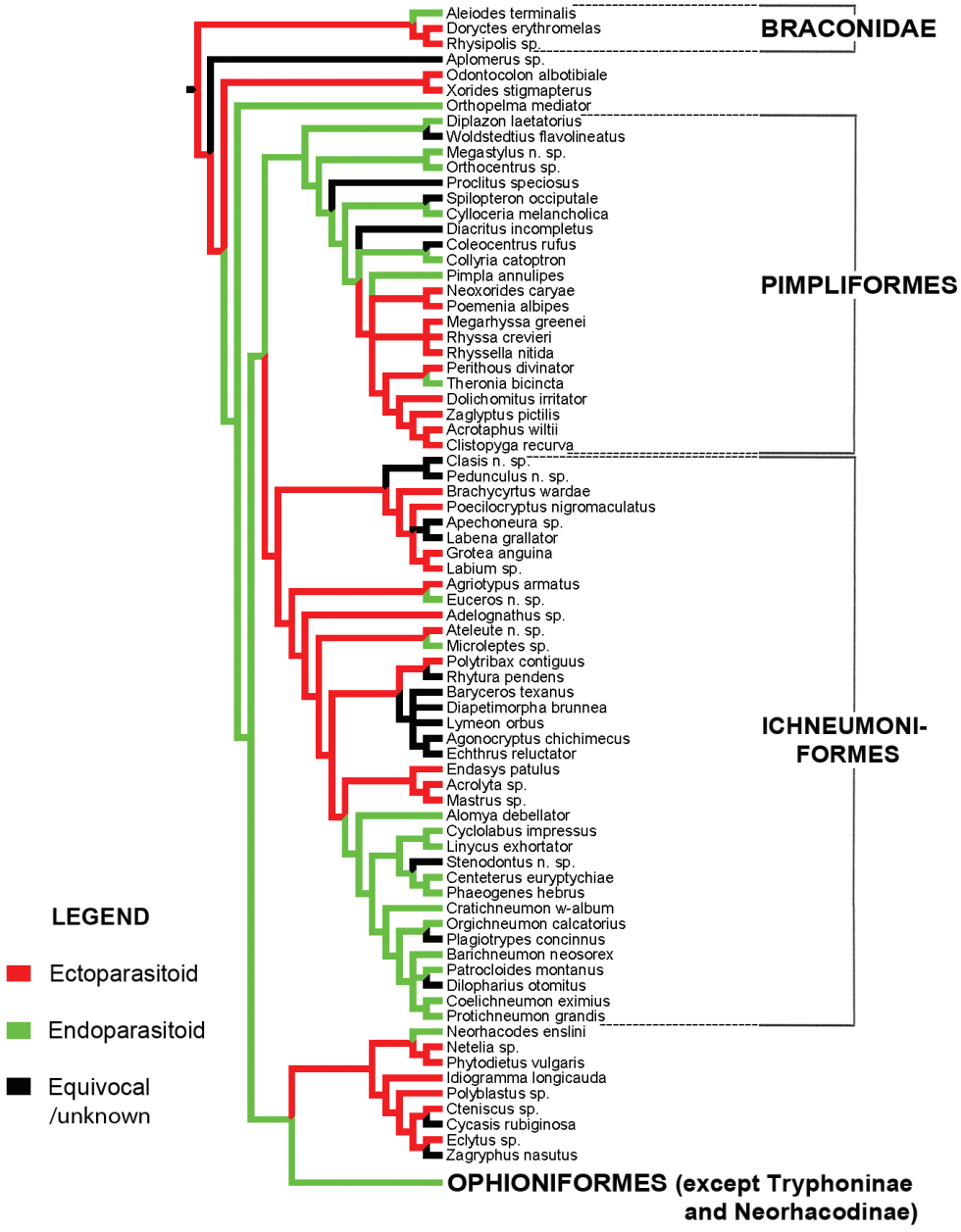


Figure 123. Optimization of character 138 (location of larval development) optimized on total-evidence parsimony analysis strict consensus cladogram using ACCTRAN. See Legend for description of colour coding for character states. Character length = 12 steps.

Gauld (1988a) provided several hypotheses regarding the evolution of endoparasitism, all of which postulated that it evolved from ectoparasitism. These hypotheses are mostly supported by the transitions observed in the total-evidence parsimony analysis.

Gauld (1988a) did not discuss the possibility that ectoparasitism could evolve via a reversal from an endoparasitoid state, although the results of Sharanowski et al. (2011) did question the hypothesis that ectoparasitism was plesiomorphic in Braconidae. Intuitively, the transition from endoparasitism to ectoparasitism appears more difficult to explain than a reversal from koinobiosis to idiobiosis. Evolution of endoparasitism can involve changes to the method of oviposition by the female wasp (Boring et al. 2009) and structure of the ovipositor (Belshaw et al. 2003; Quicke et al. 2000b), but also changes in the ovaries and egg morphology (Iwata 1960), venom properties (Moreau and Asgari 2015) and in some groups, co-evolution with polydnaviruses that alter host development via endocrinological changes (Tanaka and Vinson 1991; Pennacchio and Strand 2015), not to mention, major changes in larval wasp morphology (Short 1978; Wahl 1986, 1988, 1990). It is hard to imagine that any parasitoid lineage would be able to revert back to ectoparasitism following evolution of all the specialized attributes of an endoparasitoid lifestyle, although intuitively, some of the traits listed above may be just as easy or easier to lose, than to gain (e.g., association with polydnaviruses). Regardless, according to the parsimony total-evidence analysis, transitions from endoparasitism to ectoparasitism appears to have occurred at least once (DELTRAN optimization) or three times under ACCTRAN optimization. In comparison, the total-evidence Bayesian analysis contradicts the total-evidence parsimony analysis with respect to the topology of Pimpliformes. In the Bayesian analysis, the ectoparasitoid higher Pimpliformes is sister group to the remaining endoparasitoid subfamilies (Acaenitinae, Orthocentrinae, Diplazontinae, etc.), therefore there is no transition from endoparasitism to ectoparasitism in the Pimpliformes. That is not to say that the Bayesian analysis unequivocally supports the hypothesis that endoparasitism evolves from ectoparasitism and never the reverse. In Fig. 120, this character has 11 steps, of which there are nine transitions from ectoparasitism to endoparasitism (nodes or taxa indicated by "EN") and one transition from endoparasitism to ectoparasitism in the node that supports Xoridinae + (Labeninae + (all remaining Ichneumoniformes + Pimpliformes))) (indicated by "EC"). In addition, there is one change from ectoparasitism to the state in Lycorininae (indicated by "E2"). In summary, ectoparasitism is plesiomorphic within Ichneumonidae, regardless of the analysis. The transition from ectoparasitism to endoparasitism is far more common than the reverse, but there is at least one transition from endoparasitism to ectoparasitism hypothesized in both of our total-evidence analyses, despite the fact that the mechanism by which this transition could evolve is not easily explained intuitively.

Host/ source of larval nutrition

The order of host/ source of larval nutrition used by our exemplar taxa (character 139) is optimized on the total-evidence parsimony strict consensus tree in Fig. 124 using ACCTRAN. The length of the character is 28 steps and the ancestral state for Ichneumonidae is parasitism of Hymenoptera. Under DELTRAN optimization (not

shown), the ancestral state for Ichneumonidae is Lepidoptera. However, if the host of *Aplomerus* (Xoridinae) is coded as Coleoptera rather than unknown, based on its morphological similarity to known Xoridinae which are beetle parasitoids, the state at the base of Ichneumonidae changes to Coleoptera for the total-evidence parsimony analysis, regardless of the type of optimization. Finally, the Bayesian total-evidence analysis hypothesizes that Lepidoptera parasitism is plesiomorphic for Icheumonidae (Fig. 120, character optimization not shown). Even though the sister group of all other Ichneumonidae (*Neorhacodes enslini*: Neorhacodinae) is a parasitoid of aculeate wasps (Hymenoptera), the next two "basal" taxa in the Bayesian analysis parasitize Lepidoptera (Lycorininae and Phytodietini (Tryphoninae)) as do two of the three outgroups (*Aleiodes terminalis* and *Rhysipolis* sp.).

In terms of additional evidence supporting one of these three host orders as plesiomorphic for Ichneumonidae, all easily pre-date the origin of Ichneumonidae (at least 85 mya) (Kopylov 2012). Based on the fossil record, Coleoptera is the oldest of the three, first appearing in the Permian (290 mya) (Kukalová-Peck and Beutel 2012) and it was highly speciose and well-diversified by the upper Cretaceous (Smith and Marcot 2015). Hymenoptera is of similar age, with estimates that the order began to diversify 281 mya (Peters et al. 2017). In contrast, the oldest known Lepidoptera fossil is lower Jurassic (190 mya) (Whalley 1985). Therefore based on the fossil record, it has been assumed that Lepidoptera is the youngest of the insect orders and diversified with the radiation of angiosperms (Wahlberg et al. 2013), although the relatively recent origin and diversification has been questioned because of poor preservation of Lepidoptera relative to other orders (Sohn et al. 2015). Regardless, hosts of all three orders were present at the origin of Ichneumonidae, therefore any could have been the ancestral host order for Ichneumonidae.

A comparison of the relative frequencies of host use of the four major holometabolous orders in our parsimony analysis shows that Lepidoptera is the most prevalent host (for 35 % of our 131 exemplar ichneumonid species), compared to Hymenoptera (30 %), Coleoptera (12 %) and Diptera (5 %). These percentages are comparable to the known host use by ichneumonids at the subfamily level: Lepidoptera (18 subfamilies, 43 % of total); Hymenoptera (16 subfamilies, 38 %); Coleoptera (13 subfamilies, 31 %) and Diptera (8 subfamilies, 19%) (Wahl 1993c; Yu et al. 2016; Bennett, unpublished).

Examination of the different transitions that occur within this character in the parsimony total-evidence tree reveals that of the 28 state changes, there were 13 different types of transitions, (e.g., Lepidoptera to Coleoptera, Hymenoptera to Coleoptera, etc). The order that was most often plesiomorphic in the state changes was Hymenoptera with the following apomorphic states and number of changes: changes to Lepidoptera (8); Coleoptera (5); Diptera (1); Neuroptera (1); Trichoptera (1) and facultatively herbivorous (1). The next most common order that was plesiomorphic in these transitions was Lepidoptera, as follows: changes to Coleoptera (4); Hymenoptera (2) and Diptera (1). Thirdly, there were two transitions from Diptera: one to Coleoptera and one to Hymenoptera. Lastly, there was one transition from Coleoptera to egg predation and one change from egg predation to parasitization of spiders. Therefore,

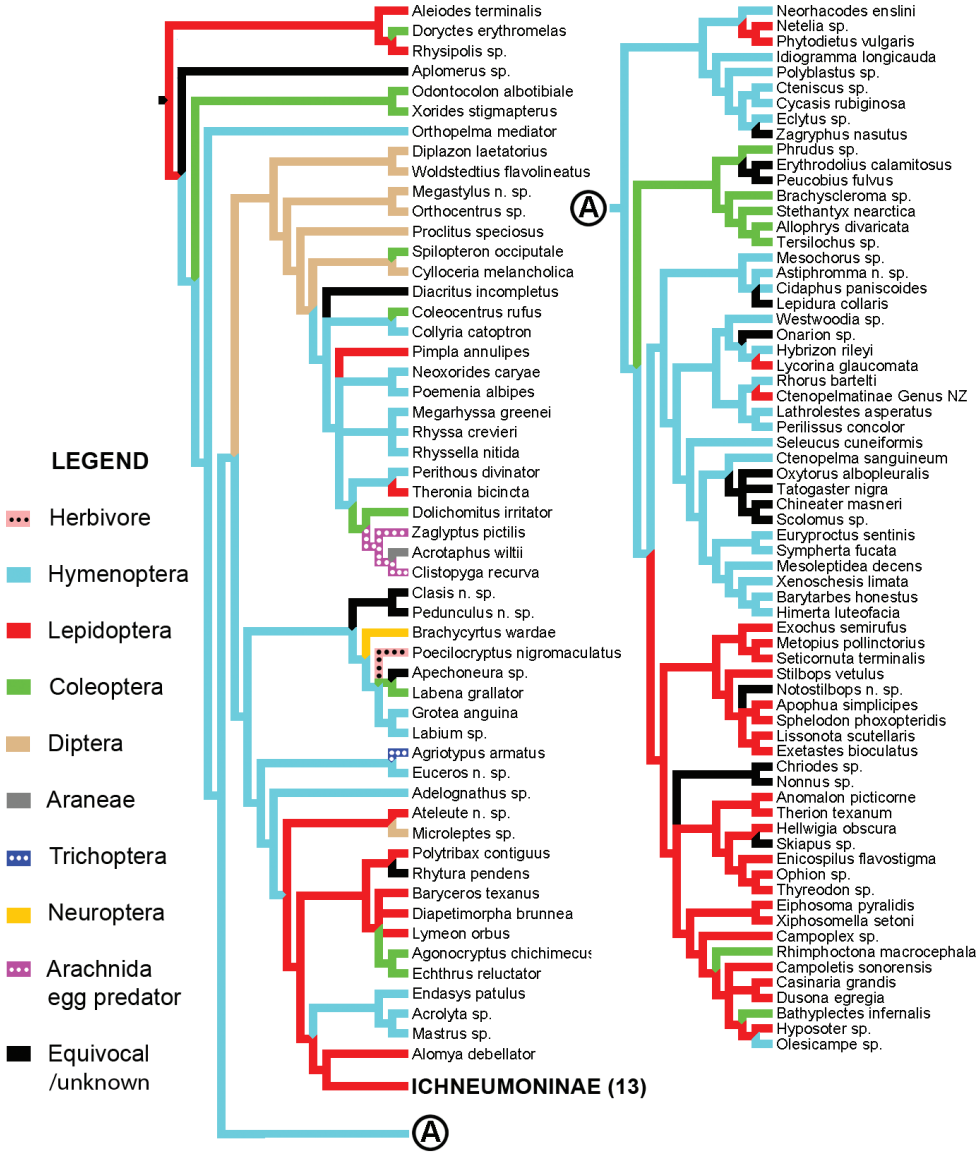


Figure 124. Optimization of character 139 (host) optimized on total-evidence parsimony analysis strict consensus cladogram using ACCTRAN. See Legend for description of colour coding for character states. Character length = 28 steps.

ichneumonids that parasitize Hymenoptera appear much more likely to switch to different host orders compared to, for example, an ichneumonid that parasitizes Lepidoptera, Diptera or Coleoptera. In fact, over half of all of the total host transitions (17 of 28 state changes) in Fig. 124 have Hymenoptera as the plesiomorphic state. This implies that when an ichneumonid group parasitizes Hymenoptera, it may somehow be better adapted to host-switch to another order; or stating this another way, there may be impediments to host switching when an ichneumonid evolves to parasitize, for

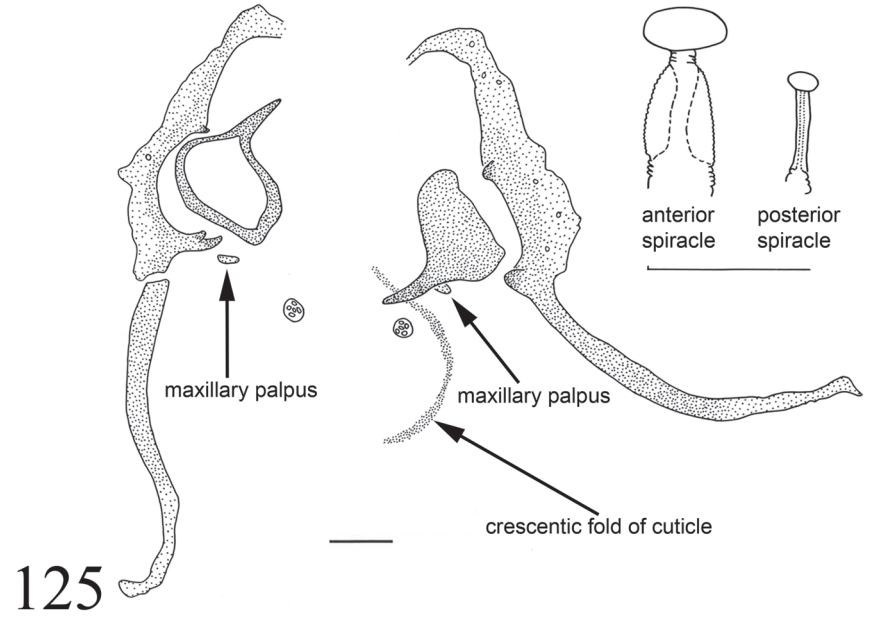


Figure 125. *Alomya semiflava* Stephens. Cephalic sclerites and spiracles of mature larva. Australian National Insect Collection. Scale bars: 0.1 mm. Structures are drawn as seen on slide (not duplicated bisymmetrically) because of distortion of the sclerites.

example, Diptera or Coleoptera. It is not clear what characters may be associated with the ability/ inability to switch hosts. Of course, this analysis is on a very broad scale and a more in-depth analysis including more species may reveal a different pattern.

Conclusions

Overall, the two total evidence analyses obtained the most resolution of relationships, followed by the molecular analyses and finally, the parsimony analysis with only morphological and biological characters. There was general congruence between the parsimony and Bayesian total-evidence analyses, except for at the base of Ichneumonidae (described below).

The relative support of different groupings within Ichneumonidae is shown in Table 3. Of the three major subfamily groupings, Pimpliformes was most well-supported (four of five analyses). A large portion of Ichneumoniformes s.l. (i.e., the group comprised of Cryptinae, Ichneumoninae, Phygadeuontinae, Ateleutinae, Adelognathinae and Microleptinae) was supported in both total evidence analyses, although the relationship to other subfamilies that have previously been placed in Ichneumoniformes (e.g., Agriotypinae) was equivocal, depending on the analysis. The Ophioniformes was only supported in the total-evidence parsimony analysis, whereas in the Bayesian total evidence analysis it formed a grade at the base of Ichneumonidae. With respect to internal arrangements within the three major groupings, the core of Ichneumoniformes

s.l. (listed above) was the most consistently recovered across analyses, whereas many of the relationships within Ophioniformes and especially, within Pimpliformes differed between the parsimony and Bayesian analyses. The ambiguity of relationships within Pimpliformes in the current study mirrored those of Klopfstein et al. (2019).

In both total evidence analyses, Pimpliformes was sister group to Ichneumoniformes s.l., which agrees with Santos (2017) and Klopfstein et al. (2019), but disagrees with Quicke et al. (2009) which supported Pimpliformes + (Ophioniformes + Ichneumoniformes s.l.). The sister group of all other Ichneumonidae was equivocal depending on the analysis. Xoridinae was only sister group in the total-evidence parsimony analysis and was not monophyletic in this analysis. Labeninae was related to / included in Ichneumoniformes s.l., not sister group to all other Ichneumonidae or sister to all ichneumonids except Xoridinae.

There were several other well-supported groupings of subfamilies including higher Pimpliformes, higher Ophioniformes, (Claseinae + Pedunculinae) and (*Stilbops* + Banchinae including *Notostilbops*). At the subfamily level, some subfamilies were well-supported across analyses: Banchinae (including *Notostilbops*), Campopleginae, Cremastinae, Diplazontinae, Ichneumoninae (including *Alomya*), Labeninae, Mesochorinae (excluding *Chineater*), Metopiinae (excluding *Scolomus*), Poemeniinae, Rhyssinae and Tersilochinae s.s. Moderate support (i.e., support in two to three analyses) was found for Anomaloninae, Cryptinae, Ophioninae, Phygadeuontinae and Xoridinae. Weak support (i.e., in only one analysis) was found for Acaenitinae, Nesomesochorinae and Orthocentrinae.

In contrast, the following subfamilies were never supported: Ctenopelmatinae, Pimplinae, Sisyrostolinae, Stilbopinae, Tersilochinae s.l. and Tryphoninae. Ctenopelmatinae was supported in the total-evidence parsimony analysis with the inclusion of Hybrizontinae, Lycorininae, Oxytorinae and Tatogastrinae. Tryphoninae was also supported in this analysis with the inclusion of Neorhacodinae. The most equivocally-placed subfamilies were Lycorininae, Neorhacodinae, Orthopelmatinae and Xoridinae.

Optimization of biological characters on the total evidence phylogenies hypothesized that for Ichneumonidae, ectoparasitism is plesiomorphic to endoparasitim. The ancestral state for timing of larval maturation is koinobiosis in both analyses; however, the lack of data for the sister taxon to all other ichneumonids in the parsimony analysis (*Aplomerus*) raises some doubt regarding this hypothesis. If *Aplomerus* is an idiobiont (as expected), then the parsimony analysis would support idiobiosis as plesiomorphic to koinobiosis. Finally, the ancestral host for Ichneumonidae is hypothesized to be Hymenoptera or Lepidoptera, although if the host of *Aplomerus* is determined to be Coleoptera (as expected), then Coleoptera would be the hypothesized ancestral host in one of the five analyses.

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Appendix 1

Taxonomic descriptions

Cephalic sclerites of mature larva of *Phrudus defectus* Stelfox.
Fig. 111

Cephalic sclerites mostly well-sclerotized. Epistomal suture (character 105): region distorted and not reconstructed in drawing, but possibly completely sclerotized and forming epistomal band (coded as "?"). Labral sclerite (character 108) & clypeolabral plates (character 130) unknown because labral region distorted (both scored as "?"). Stipital sclerite present, more or less horizontal and median end contacting labial sclerite (character 119. state 0) and without lateral plate-like extension (character 120, state 0). Pleurostoma only partially visible due to distortion; posterior struts of inferior mandibular processes not connected by band (character 114, state 0); inferior mandibular process dorsad to dorsal margin of labial sclerite (character 107, state 0); accessory pleurostomal area (character 106) not discernible (coded as "?"). Hypostoma well-sclerotized and long (character 115, state 0); lateral end simple, not divided or upcurved (character 116, state 0). Hypostomal spur present and long, about 2.0× as long as its basal width (character 117, state 0), meeting stipital sclerite near middle (character 118, state 0). Labial sclerite nearly circular (character 124, state 1), about as long as wide (character 125, state 0), not produced ventrally as a spine (character 126, state 0). Salivary orifice U-shaped (character 133, state 1). Prelabial sclerite absent (character 127, state 0). Sclerotized plate ventrad labial sclerite absent (character 129, state 0). Maxillary and labial palpi each bearing 2 sensilla (character 123, state 0). Mandible uniformly well-sclerotized (character 111, state 0), cone-shaped and apex with small, tooth-like projection (character 109, state 1) (Fig. 111, upper left); blade without denticles (character 112, state 2), without accessory teeth (character 110, state 0) and without basal spines (character 113, state 0). Antenna (character 131): unknown (coded as "?"). Spiracle with closing apparatus separated by section of trachea (character 132, state 0 - coded as "?" in matrix). Skin with numerous small triangular projections (10 μ long) and scattered elongate setae (32 μ long).

Material examined: *Phrudus defectus* Stelfox last larval instar exuvium slides: UNITED KINGDOM: Isle of Man, Laxey, Baldhoon Road, Crofton, SC4284; F.D. Bennett; from *Epuraea melanocephala* in sycamore flowers; exposed 1–2.vi.2008, adult emerged 4.v.2009 [DBW preparation 28.I.2012b] (EMUS); UNITED KINGDOM: Isle of Man, Laxey, Mooar Glen, SC43284; F.D. Bennett; from *Epuraea melanocephala* in sycamore flowers; collected 31.v.2008, adult emerged 11.v.2009 [DBW preparation 28.I.2012c] (EMUS).

Comments. Distortions of the two preparations (DBW preparations 28.I.2012b and 28.I.2012c) do not allow clear views of the dorsal portion of the cephalic capsule, and hence structures above the inferior mandibular processes are not shown. Figure 111 is a composite of the two preparations listed above. This is the first description of the larva of the *Phrudus* group of Tersilochinae which is of interest because prior to

Quicke et al. (2009), the *Phrudus* group was part of its own subfamily (Phrudinae). The shape of the mandible (apex with small, tooth-like projection) (character 109, state 1) is a synapomorphy for the *Tersilochus* and *Phrudus* groups of genera, thereby supporting the inclusion of these two groups in Tersilochinae.

Cephalic sclerites of mature larva of *Collyria* spp.
Fig. 112

Cephalic sclerites mostly weakly sclerotized or absent. Epistomal suture unsclerotized (character 105, state 0). Labral sclerite absent (character 108, state 1). Clypeolabral plates absent (character 130, state 0). Stipital sclerite absent (character 119. state 2) and without lateral plate-like extension (character 120, state 0). Pleurostoma difficult to discern, but apparently not laterally expanded (character 106, state 0) and posterior struts of inferior mandibular processes short and not obviously connected by band (character 114, state 0). Hypostoma absent (character 115, state 2). Hypostomal spur absent (character 117, state 2). Labial sclerite (character 124) difficult to discern ventrally, therefore shape scored as "?". Salivary orifice (character 133) not visible (scored as "?"). Prelabial sclerite absent (character 127, state 0). Sclerotized plate ventrad labial sclerite absent (character 129, state 0). Maxillary and labial palpi (character 123) not visible (scored as "?"). Mandible (scored for *C. coxator* from the description of Salt 1931): uniformly sclerotized (character 111, state 0) and triangular (character 109, state 0); blade without denticles (character 112, state 2), accessory teeth absent (character 110, state 0), basal spines absent (character 113, state 0). Mandible apparently absent in *C. catoptron* (Fig. 112). Antenna absent (character 131, state 2). Spiracle with closing apparatus separated by section of trachea (character 132, state 0). Skin with numerous small bubble-like projections and lacking setae.

Material examined: *Collyria coxator* (Villers) last larval instar exuvium slide mount: Locality unspecified. Label 1: Slide no. 284. Don G. Salt. Label 2: *Collyria calcitrator* (Grav.) JRTS 1955 (NMNH); *Collyria catoptron* Wahl slit and macerated last instar whole larva slide mount: CHINA, Gansu Province, Yuzhong County, ix.1998, ex. *Cephus fumipennis*, T. Shanower et al. [DBW preparation 3.I.2011] (EMUS).

Comments: The nature of the cephalic sclerites of the mature larva of *Collyria* is not straightforward. Salt (1931) gave a detailed description of the larval stages of *Collyria coxator* (Villers). The mature larval head was characterized as "not at all darkened or hardened, is without any noticeable facial rods, and appears to lack even mandibles." He went on to state: "Careful staining, however, shows that the mandibles and some of the usual facial rods are represented, but not well developed… The mandibular struts may be clearly distinguished, and there are vague sclerotic areas in the labral region, but all parts are so poorly represented that it is difficult to homologize them". Salt's drawing (his fig. 13b), shows only the pleurostomae and mandibles (the mandibles drawn with a finer line), all other cephalic sclerites being absent (the large square structure below the mandibles is the suspensorium of the hypopharynx, as pointed out by Short

(1959)). Short (1959) illustrated another specimen of *C. coxator* and gave a detailed description of it. Examination of the original slide (NMNH) reveals part of the drawing to be imaginary: only the pleurostomae and suspensorium of the hypopharynx are present, and the mandibles and antennae are absent. The mandibles in the drawing may have been added to conform to Salt's figure.

Between Salt's detailed study and Short's slide, *Collyria* appears to be quite unique amongst the Ichneumonidae for the drastic reduction of the cephalic sclerites. Reduction of the sclerites is associated with not spinning a cocoon (such as in the Anomaloninae, Ichneumoninae, Metopiinae, and Pimplini), but never to such an extent as in *Collyria*. Preserved larvae of a second species, *Collyria catoptron* Wahl, were available for comparison. A number of larvae were longitudinally slit and macerated in sodium hydroxide solution, with the resulting skins stained with acid fuschin and then slide mounted. Whole larvae were also stained with acid fuschin and then examined. No evidence of sclerotized structures could be found on the mounted skins. The stained whole specimens showed the general mouthpart regions as convexities and furrows but no sclerotized structures were present (Fig. 112).

In summary, *Collyria* lacks all cephalic sclerites except for the pleurostoma, part of the labium and maxilla and the mandibles (at least in *C. coxator*). It might be noted that Short depicted the spiracle's closing apparatus as extremely long and narrow. The spiracles in Short's slide and the new specimens of *catoptron* are not nearly as long or as narrow (his depiction of the closing apparatus being separated from the atrium is accurate). Short apparently did not use a camera lucida or ocular grid, and his drawings are often strikingly distorted.

Cephalic sclerites of mature larva of *Alomya semiflava* Stephens
Fig. 125

The following description is based on a re-examination of two larval slides prepared by J.R.T. Short (Hinz and Short 1983). Our figure of the last instar larva is depicted as it appears on the slide (i.e., not reconstructed as bilaterally symmetrical, as per usual practice) because the larva on the slide is distorted. In addition, the suspensorium of the hypopharynx is not shown, as its presence is universal within ichneumonids and its shape uniformative. See comments below for additional description of Short's slide preparation and how it was depicted in Hinz and Short (1983).

Cephalic sclerites with many prominent structures absent; remaining structures well-sclerotized. Epistomal suture completely sclerotized, uncertain if forming epistomal band because of distortion of larva, but coded as present (character 105, state 2). Labral sclerite absent (character 108, state 1); clypeolabral plates absent (character 130, state 0). Stipital sclerite absent (character 119, state 2). Pleurostoma well-sclerotized, not laterally expanded (character 106, state 0); posterior struts of inferior mandibular processes short and not connected by band (character 114, state 0). Hypostoma long and well-sclerotized (character 115, state 0), more or less straight, markedly angled ventrally towards cephalic

midline. Hypostomal spur absent (character 117, state 2). Labial sclerite absent (character 124), originally coded as present, (circular to ovoid: state 1), but re-assessed when manuscript in press" (see comments, below). Region near salivary orifice distorted, but presumably U-shaped (character 133, state 1). Prelabial sclerite absent (character 127, state 0). Plate ventrad labial sclerite absent (character 129, state 0). Maxillary and labial palpi each bearing 5 sensilla (character 123, state 2). Mandible large, uniformly well-sclerotized (character 111, state 0) and triangular (character 109, state 0); blade without denticles (character 112, state 2). Antenna (character 131) not visible (coded as "?"). Spiracle with closing apparatus adjacent to atrium (character 132, state 1, but coded as separated from atrium, state 0). Skin smooth with scattered short setae.

Material examined: 1 last larval instar slide mount, larva reared under laboratory conditions from mummified final larval instar of *Korscheltellus* (= *Hepialus*) *lupulinus* (Linnaeus, 1758), 1979, R. Hinz (ANIC). 1 penultimate stage larval slide mount. Same data as final instar (ANIC).

Comments: *Alomya semiflava*, like other species in its genus, attack species of Hepialidae (Lepidoptera) (Waterston 1926, Hinz and Short 1983). The wasp is a koinobiont endoparasitoid, with the unusual habit of pupating within the hardened remains of the host larva in contrast to most other ichneumonines which pupate within the host pupa (*Colpognathus* spp. are also known to pupate in the host larval remains – see Shaw and Bennett 2001). Short characterized both of his slide preparations as 'final larval instars', but one is clearly penultimate stage. Points of interest are as follows:

1) There is no trace of the antennal disc.
2) The region of the epistomal suture is distorted, and it is not possible to determine if an epistomal band is present (it could be there but thin and not well sclerotized). The band is present in the penultimate larva slide, but ichneumonid larvae often lose structures upon maturity (Wahl 1990).
3) Short reported cuticular folds bearing setae on the clypeolabrum but these could not be seen. He apparently thought these to be analogous to the narrow clypeolabral plates found in Phaeogenini.
4) The maxillary apices are distorted and could not be reconstructed as depicted by Short. Both maxillary palpi are rotated so that only lateral views were possible (Fig. 125) (and so the five sensilla in Short's figure were a reconstruction, albeit probably correct). Wrinkles in the cuticle led Short to depict the presence of stipital sclerites; they are not present.
5) The region of the salivary orifice is distorted and its shape cannot be determined (although it is presumably U-shaped). What Short depicts as the 'silk press' is the terminal end of the salivary duct.
6) Short shows the labial sclerite to be present, with the ventral section unsclerotized. The actual specimen has a crescentic structure on the right side in the vicinity of the labium (Fig. 125, crescentic fold of cuticle) but there is not a corresponding structure on the left side. Given the general distortion of the specimen, the crescentic structure is interpreted as an extended cuticular wrinkle.

7) The orientation of the epistoma+pleurostoma+hypostoma is difficult to determine, given the preparation's distortion. The left-side hypostoma has broken off from the pleurostoma. The slide of the penultimate instar has the orientation of Short's figure but the right side of the mature larval specimen belies that reconstruction. The actual arrangement is probably similar to that of *Thyrateles procax* (Cresson) or *Trogus pennator* (Fabricius) in Gillespie and Finlayson (1983).

In summary, the cephalic morphology of the final-instar larva of *A. semiflava* is that of a standard ichneumonine, lacking only clypeolabral plates.

Appendix 2

Taxa sequenced, countries of collection, specimen voucher numbers and Genbank accession numbers for molecular vouchers. All sequences were original to this study except as noted by superscripts indicating literature reference: [1]Heraty et al. (2011); [2]Quicke et al. (2006); [3]Quicke et al. (2009); [4]Belshaw and Quicke (2002); Taxonomy reflects nomenclature prior to current study.

Taxa	Country of collection	Voucher depository	Voucher number	Genbank accession numbers		
				COI	28s D2	EF1a
Braconidae						
Doryctes erythromelas (Brullé)	?	UKY	?	GQ374627[1]	GQ374709[1]	GQ410706[1]
Rhysipolis sp.	?	UKY	?	GQ374626[1]	GQ374708[1]	GQ410705[1]
Aleiodes terminalis Cresson	?	UKY	?	–	GQ374710[1]	GQ410707[1]
Aleiodes pictus (Herrich-Schäffer)	England	?	?	EF115464[2]	–	–
Ichneumonidae						
Acaenitinae						
Spilopteron occiputale (Cresson)	United States	CNC	CNC 422320	MK959483	MK851161	MK851398
Coleocentrus rufus Provancher	United States	CNC	CNC 422321	MK959401	MK851078	MK851315
Adelognathinae						
Adelognathus sp.	United States	CNC	CNC 422322	MK959374	MK851051	MK851288
Agriotypinae						
Agriotypus armatus Curtis	Czech Republic	CNC	CNC 422323	MK959376	MK851053	MK851290
Alomyinae						
Alomya debellator (Fabricius)	Switzerland	CNC	CNC 422374	MK959378	MK851055	MK851292
Anomaloninae						
Anomalonini						
Anomalon picticorne (Viereck)	United States	CNC	CNC 422324	MK959379	MK851056	MK851293
Gravenhorstiini						
Therion texanum (Ashmead)	United States	CNC	CNC 422325	MK959490	MK851168	MK851405
Ateleutinae						
Ateleute sp. nov.	United States	CNC	CNC 422344	MK959384	MK851061	MK851298
Banchinae						
Atrophini						
Lissonota scutellaris (Cresson)	United States	CNC	CNC 422326: (COI, 28S D2); CNC 422489: (EF1a)	MK959436	MK851113	MK851350

Taxa	Country of collection	Voucher depository	Voucher number	Genbank accession numbers		
				COI	28s D2	EF1a
Banchini						
Exetastes bioculatus Cresson	United States	CNC	CNC 422327	MK959424	MK851101	MK851338
Glyptini						
Apophua simplicipes (Cresson)	Canada	CNC	CNC 422328	MK959382	MK851059	MK851296
Sphelodon phoxopteridis (Weed)	United States	CNC	CNC 422329	MK959482	MK851160	MK851397
Brachycyrtinae						
Brachycyrtus wardae Bennett	Fiji	CNC	CNC 422490: (COI, 28S D2); CNC 422330: (EF1a)	MK959389	MK851066	MK851303
Campopleginae						
Bathyplectes infernalis (Gravenhorst)	United States	CNC	CNC 422331	MK959388	MK851065	MK851302
Campoletis sonorensis (Cameron)	United States	CNC	CNC 422332	MK959391	MK851068	MK851305
Campoplex sp.	United States	CNC	CNC 422333	MK959392	MK851069	MK851306
Casinaria grandis Walley	United States	CNC	CNC 422334	MK959393	MK851070	MK851307
Dusona egregia (Viereck)	United States	CNC	CNC 422335	MK959415	MK851092	MK851329
Hyposoter sp.	United States	CNC	CNC 422336	MK959429	MK851106	MK851343
Olesicampe sp.	United States	CNC	CNC 422491: (COI, 28S D2); CNC 422337: (EF1a)	MK959452	MK851129	MK851366
Rhimphoctona macrocephala (Provancher)	Canada	CNC	CNC 422338	MK959474	MK851151	MK851389
Claseinae						
Clasis sp. nov.	Chile	CNC	CNC 422339	MK959398	MK851075	MK851312
Collyriinae						
Collyria catoptron Wahl	China	CNC	CNC 422340	MK959402	MK851079	MK851316
Cremastinae						
Eiphosoma pyralidis Ashmead	United States	CNC	CNC 422341	MK959418	MK851095	MK851332
Xiphosomella setoni Johnson	United States	CNC	CNC 422342	MK959496	MK851174	MK851411
Cryptinae						
Aptesini						
Polytribax contiguus (Cresson)	Canada	CNC	CNC 422349	MK959471	MK851148	MK851386
Rhytura pendens Townes	United States	CNC	CNC 422350	MK959477	MK851155	MK851393
Cryptini						
Agonocryptus chichimecus (Cresson)	United States	CNC	CNC 422343	MK959375	MK851052	MK851289
Baryceros texanus (Ashmead)	United States	CNC	CNC 422345	MK959386	MK851063	MK851300
Diapetimorpha brunnea Townes	United States	CNC	CNC 422346	MK959411	MK851088	MK851325
Echthrus reluctator (Linnaeus)	Germany	CNC	CNC 422348	MK959416	MK851093	MK851330
Lymeon orbus (Say)	United States	CNC	CNC 422347	MK959438	MK851115	MK851352
Ctenopelmatinae						
Ctenopelmatini						
Ctenopelma sanguineum (Provancher)	United States	CNC	CNC 422354	MK959405	MK851082	MK851319
Xenoschesis limata (Cresson)	United States	CNC	CNC 422355	MK959495	MK851173	MK851410
Euryproctini						
Euryproctus sentinis Davis	United States	CNC	CNC 422356	MK959423	MK851100	MK851337
Mesoleptidea decens (Cresson)	United States	CNC	CNC 422357	MK959443	MK851120	MK851357
Mesoleiini						
Barytarbes honestus (Cresson)	United States	CNC	CNC 422358	MK959387	MK851064	MK851301
Himerta luteofacia Leblanc	United States	CNC	CNC 422359	MK959427	MK851104	MK851341
Perilissini						
Lathrolestes asperatus Barron	United States	CNC	CNC 422360	MK959433	MK851110	MK851347
Perilissus concolor (Cresson)	United States	CNC	CNC 422361	MK959461	MK851138	MK851375
Pionini						
Rhorus bartelti Luhman	Canada	CNC	CNC 422362	–	MK851152	MK851390
Sympherta fucata (Cresson)	United States	CNC	CNC 281120	MK959487	MK851165	MK851402

Taxa	Country of collection	Voucher depository	Voucher number	Genbank accession numbers		
				COI	28s D2	EF1a
Scolobatini						
Onarion sp.	Bolivia	CNC	CNC 422365	MK959453	MK851130	MK851367
Seleucini						
Seleucus cuneiformis Holmgren	Japan	CNC	CNC 422364	MK959479	MK851157	MK851395
Westwoodiini						
Westwoodia sp.	Australia	CNC	CNC 422366	MK959493	MK851171	MK851408
Tribe indet.						
Ctenopelmatinae Genus NZ	New Zealand	CNC	CNC 422367	MK959406	MK851083	MK851320
Cylloceriinae						
Cylloceria melancholica (Gravenhorst)	United States	CNC	CNC 422368	MK959409	MK851086	MK851323
Diacritinae						
Diacritus incompletus Momoi	Japan	CNC	CNC 422369	MK959410	MK851087	MK851324
Diplazontinae						
Diplazon laetatorius (Fabricius)	Canada	CNC	CNC 422370	MK959413	MK851090	MK851327
Woldstedtius flavolineatus (Gravenhorst)	United States	CNC	CNC 422371	MK959494	MK851172	MK851409
Eucerotinae						
Euceros sp. nov.	United States	CNC	CNC 422372	MK959422	MK851099	MK851336
Hybrizontinae						
Hybrizon rileyi (Ashmead)	United States	CNC	CNC 422373: (COI, 28S D2); CNC 422392: (EF1a)	MK959428	MK851105	MK851342
Ichneumoninae						
Heresiarchini						
Coelichneumon eximius (Stephens)	United States	CNC	CNC 422378	MK959400	MK851077	MK851314
Protichneumon grandis (Brullé)	United States	CNC	CNC 422379	MK959473	MK851150	MK851388
Ichneumonini						
Barichneumon neosorex Heinrich	United States	CNC	CNC 422380	MK959385	MK851062	MK851299
Cratichneumon w-album (Cresson)	United States	CNC	CNC 422381	MK959403	MK851080	MK851317
Orgichneumon calcatorius (Thunberg)	United States	CNC	CNC 422382	MK959455	MK851132	MK851369
Patrocloides montanus (Cresson)	United States	CNC	CNC 422383	MK959459	MK851136	MK851373
Joppocryptini						
Plagiotrypes concinnus (Say)	United States	CNC	CNC 422384	MK959468	MK851145	MK851382
Listrodromini						
Dilopharius otomitus (Cresson)	United States	CNC	CNC 422385	MK959412	MK851089	MK851326
Phaeogenini						
Centeterus euryptychiae (Ashmead)	Canada	CNC	CNC 422375	MK959394	MK851071	MK851308
Phaeogenes hebrus (Cresson)	United States	CNC	CNC 422376	MK959464	MK851141	MK851378
Stenodontus sp. nov.	United States	CNC	CNC 422377	MK959484	MK851162	MK851399
Platylabini						
Cyclolabus impressus (Provancher)	United States	CNC	CNC 422386	MK959408	MK851085	MK851322
Linycus exhortator (Fabricius)	United States	CNC	CNC 422387	MK959435	MK851112	MK851349
Labeninae						
Orthognathelini						
Grotea anguina Cresson	United States	CNC	CNC 422388	MK959426	MK851103	MK851340
Labium sp.	Australia	CNC	CNC 422389	MK959432	MK851109	MK851346
Labenini						
Apechoneura sp.	Bolivia	CNC	CNC 422390	MK959380	MK851057	MK851294
Labena grallator (Say)	United States	CNC	CNC 422391	MK959431	MK851108	MK851345
Poecilocryptini						
Poecilocryptus nigromaculatus Cameron	Australia	? (28s); CNC (EF1a)	? (28s); CNC 422392 (EF1a)	–	AJ302921[3]	MK851383

Taxa	Country of collection	Voucher depository	Voucher number	Genbank accession numbers		
				COI	28s D2	EF1a
Lycorininae						
Lycorina glaucomata (Cushman)	United States	CNC	CNC 422393	MK959437	MK851114	MK851351
Mesochorinae						
Astiphromma sp. nov.	United States	CNC	CNC 422394	MK959383	MK851060	MK851297
Chineater masneri Wahl	Chile	CNC	CNC 422395	MK959395	MK851072	MK851309
Cidaphus paniscoides (Ashmead)	United States	CNC	CNC 422396	MK959397	MK851074	MK851311
Lepidura collaris Townes	Chile	CNC	CNC 422397	MK959434	MK851111	MK851348
Mesochorus sp.	United States	CNC	CNC 422398	MK959442	MK851119	MK851356
Metopiinae						
Exochus semirufus Cresson	United States	CNC	CNC 422399	MK959425	MK851102	MK851339
Metopius pollinctorius (Say)	United States	CNC	CNC 422400	MK959444	MK851121	MK851358
Scolomus sp.	Chile	CNC	CNC 422401	MK959478	MK851156	MK851394
Seticornuta terminalis (Ashmead)	United States	CNC	CNC 422402	MK959480	MK851158	MK851396
Microleptinae						
Microleptes sp.	South Korea	CNC	CNC 422403	MK959445	MK851122	MK851359
Neorhacodinae						
Neorhacodes enslini (Ruschka)	Spain	CNC	CNC 422434	MK959446	MK851123	MK851360
Nesomesochorinae						
Chriodes sp.	Madagascar	CNC	CNC 422404	MK959396	MK851073	MK851310
Nonnus sp.	Argentina	CNC	CNC 422405	MK959449	MK851126	MK851363
Ophioninae						
Enicospilus flavostigma Hooker	United States	CNC	CNC 422406	MK959420	MK851097	MK851334
Hellwigia obscura Gravenhorst	France	?	?	–	AJ302858[4]	–
Ophion sp.	United States	CNC	CNC 422407	MK959454	MK851131	MK851368
Skiapus sp.	Mozambique	CNC	CNC 422408	MK959481	MK851159	–
Thyreodon sp.	Guyana	CNC	CNC 422409	MK959492	MK851170	MK851407
Orthocentrinae						
Megastylus sp. nov.	United States	CNC	CNC 422410	MK959441	MK851118	MK851355
Orthocentrus sp.	United States	CNC	CNC 422411	MK959456	MK851133	MK851370
Proclitus speciosus Dasch	United States	CNC	CNC 422412	MK959472	MK851149	MK851387
Orthopelmatinae						
Orthopelma mediator (Thunberg)	Sweden	CNC	CNC 422413	MK959457	MK851134	MK851371
Oxytorinae						
Oxytorus albopleuralis (Provancher)	United States	CNC	CNC 422414	MK959458	MK851135	MK851372
Pedunculinae						
Pedunculus sp. nov.	Chile	CNC	CNC 422415	MK959460	MK851137	MK851374
Phygadeuontinae						
Acrolyta sp.	United States	CNC	CNC 422351	MK959372	MK851049	MK851286
Endasys patulus (Viereck)	United States	CNC	CNC 422352	MK959419	MK851096	MK851333
Mastrus sp.	United States	CNC	CNC 422353	MK959439	MK851116	MK851353
Pimplinae						
Delomeristini						
Perithous divinator (Rossi)	Canada	CNC	CNC 422416	MK959462	MK851139	MK851376
Ephialtini						
Acrotaphus wiltii (Cresson)	United States	CNC	CNC 422417	MK959373	MK851050	MK851287
Clistopyga recurva (Say)	United States	CNC	CNC 422418	MK959399	MK851076	MK851313
Dolichomitus irritator (Fabricius)	United States	CNC	CNC 422419	MK959414	MK851091	MK851328
Zaglyptus pictilis Townes	United States	CNC	CNC 422420	MK959498	MK851176	MK851413
Pimplini						
Pimpla annulipes Brullé	Canada	CNC	CNC 422421	MK959467	MK851144	MK851381
Theronia bicincta (Cresson)	United States	CNC	CNC 422422	MK959491	MK851169	MK851406

Taxa	Country of collection	Voucher depository	Voucher number	Genbank accession numbers		
				COI	28s D2	EF1a
Poemeniinae						
Neoxorides caryae (Harrington)	United States	CNC	CNC 422423	MK959447	MK851124	MK851361
Poemenia albipes (Cresson)	United States	CNC	CNC 422424	MK959469	MK851146	MK851384
Rhyssinae						
Megarhyssa greenei Viereck	Canada	CNC	CNC 422425	MK959440	MK851117	MK851354
Rhyssa crevieri (Provancher)	Canada	CNC	CNC 422426	MK959475	MK851153	MK851391
Rhyssella nitida (Cresson)	Canada	CNC	CNC 422427	MK959476	MK851154	MK851392
Sisyrostolinae						
Brachyscleroma sp.	Kenya	CNC	CNC 422428	MK959390	MK851067	MK851304
Erythrodolius calamitosus Seyrig	Madagascar	CNC	CNC 422429	MK959421	MK851098	MK851335
Stilbopinae						
Notostilbops sp. nov.	Chile	CNC	CNC 422430	MK959450	MK851127	MK851364
Stilbops vetulus (Gravenhorst)	Hungary	CNC	CNC 422431	MK959486	MK851164	MK851401
Tatogastrinae						
Tatogaster nigra Townes	Chile	CNC	CNC 422432	MK959488	MK851166	MK851403
Tersilochinae						
Allophrys divaricata Horstmann	United States	CNC	CNC 422493: (COI, 28S D2); CNC 422433: (EF1a)	MK959377	MK851054	MK851291
Peucobius fulvus Townes	United States	CNC	CNC 422435	MK959463	MK851140	MK851377
Phrudus sp.	South Korea	CNC	CNC 422436	MK959465	MK851142	MK851379
Stethantyx nearctica Townes	United States	CNC	CNC 422437	MK959485	MK851163	MK851400
Tersilochus sp.	United States	CNC	CNC 422438: (COI, EF1a); CNC 422494: (28s D2)	MK959489	MK851167	MK851404
Tryphoninae						
Eclytini						
Eclytus sp.	United States	CNC	CNC 422495: (COI, 28S D2); CNC 422439: (EF1a)	MK959417	MK851094	MK851331
Idiogrammatini						
Idiogramma longicauda (Cushman)	United States	CNC	CNC 422440	MK959430	MK851107	MK851344
Oedemopsini						
Zagryphus nasutus (Cresson)	United States	CNC	CNC 422441: (COI, EF1a); CNC 422496: (28s D2)	MK959499	MK851177	MK851414
Phytodietini						
Netelia sp.	United States	CNC	CNC 422442	MK959448	MK851125	MK851362
Phytodietus vulgaris Cresson	United States	CNC	CNC 422443	MK959466	MK851143	MK851380
Tryphonini						
Cteniscus sp.	United States	CNC	CNC 422444	MK959404	MK851081	MK851318
Cycasis rubiginosa (Gravenhorst)	Switzerland	CNC	CNC 422445	MK959407	MK851084	MK851321
Polyblastus sp.	United States	CNC	CNC 422446	MK959470	MK851147	MK851385
Xordinae						
Aplomerus sp.	United States	UKY	CNC 681999 (COI, 28s D2), CNC	MK959381	MK851058	MK851295
		CNC	422447: (EF1a)			
Odontocolon albotibiale (Bradley)	United States	CNC	CNC 422497: (COI, 28s D2); CNC 422448: (EF1a)	MK959451	MK851128	MK851365
Xorides stigmapterus (Say)	Canada	CNC	CNC 422449	MK959497	MK851175	MK851412

Supplementary material 1

Ichneumonidae complete phylogenetic matrix, molecular characters coded as 0123
Authors: Andrew M.R. Bennett, Sophie Cardinal, Ian D. Gauld, David B. Wahl
Data type: phylogenetic data (nexus format)

Link: https://doi.org/10.3897/jhr.71.32375.suppl1

Supplementary material 2

Ichneumonidae complete phylogenetic matrix, molecular characters coded as 0123
Authors: Andrew M.R. Bennett, Sophie Cardinal, Ian D. Gauld, David B. Wahl
Data type: phylogenetic data (Hennig86 format)

Link: https://doi.org/10.3897/jhr.71.32375.suppl2

Supplementary material 3

Ichneumonidae phylogenetic matrix, molecular characters only, coded as ACGT
Authors: Andrew M.R. Bennett, Sophie Cardinal, Ian D. Gauld, David B. Wahl
Data type: phylogenetic data (nexus format)

Link: https://doi.org/10.3897/jhr.71.32375.suppl3

Supplementary material 4

Ichneumonidae phylogenetic matrix, molecular characters only, coded as ACGT
Authors: Andrew M.R. Bennett, Sophie Cardinal, Ian D. Gauld, David B. Wahl
Data type: phylogenetic data (Hennig86 format)

Link: https://doi.org/10.3897/jhr.71.32375.suppl4

Supplementary material 5

Total evidence parsimony trees
Authors: Andrew M.R. Bennett, Sophie Cardinal, Ian D. Gauld, David B. Wahl
Data type: Phylogenetic trees (Winclada format)

Link: https://doi.org/10.3897/jhr.71.32375.suppl5

Supplementary material 6

Morphology-only parsimony trees
Authors: Andrew M.R. Bennett, Sophie Cardinal, Ian D. Gauld, David B. Wahl
Data type: Phylogenetic trees (Winclada format)

Link: https://doi.org/10.3897/jhr.71.32375.suppl6

Supplementary material 7

Molecular-only parsimony trees

Authors: Andrew M.R. Bennett, Sophie Cardinal, Ian D. Gauld, David B. Wahl

Data type: Phylogenetic trees (Winclada format)

Link: https://doi.org/10.3897/jhr.71.32375.suppl7

Supplementary material 8

Bayesian total evidence trees

Authors: Andrew M.R. Bennett, Sophie Cardinal, Ian D. Gauld, David B. Wahl

Data type: Phylogenetic trees (Winclada format)

Link: https://doi.org/10.3897/jhr.71.32375.suppl8

Supplementary material 9

Bayesian molecular-only trees

Authors: Andrew M.R. Bennett, Sophie Cardinal, Ian D. Gauld, David B. Wahl

Data type: Phylogenetic trees (Winclada format)

Link: https://doi.org/10.3897/jhr.71.32375.suppl9

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SHORT COMMUNICATION

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Chromosomes of *Eupristina verticillata* Waterston, 1921 and an overview of known karyotypes of chalcid wasps of the family Agaonidae (Hymenoptera)

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Abstract

The karyotype of *Eupristina verticillata* Waterston, 1921 (Agaonidae) from Italy was studied for the first time using chromosome morphometrics. The present study showed that this species has n = 6 and 2n = 12, with five larger metacentrics and a smaller acrocentric chromosome in the haploid set. A brief overview of known karyotypes of chalcid wasps of the Agaonidae is given; certain features of karyotype evolution of this family are discussed.

Keywords

Chalcidoidea, Agaonidae, chromosomes, karyotypes

Introduction

Chalcidoidea are one of the most speciose, taxonomically complicated and economically important groups of parasitoid Hymenoptera (Godfray 1994, Quicke 1997). With its exceptionally high morphological and ecological diversity, this superfamily includes about 23 thousand known species (Huber 2017), but karyotypes of less than

200 members of this group are studied up to now (Gokhman 2013). For example, in the family Agaonidae, a highly specialized tropical/subtropical group of Chalcidoidea exclusively associated with fruits of the plant genus *Ficus*, chromosomes of only six species were previously studied (Gokhman et al. 2010, Liu et al. 2011, Chen et al. 2018). We have recently examined chromosomes of another member of this family, *Eupristina verticillata* Waterston, 1921 collected in Italy about 30 years after its introduction (Lo Verde et al. 1991, 2007). In addition, we briefly overview the current state of knowledge of the karyotypic diversity of Agaonidae.

Material and methods

Origin of the material studied

Green syconia of *Ficus microcarpa* Linnaeus, 1782 (Moraceae) containing immature stages of *E. verticillata* were collected by the senior author in July 2018 in the Villa Comunale (city park) of Napoli, Italy (40°49'58.4"N 14°14'3"E). Immediately after collection, the syconia were dissected in the lab, and wasp prepupae were extracted from the galls.

Preparation of chromosomes

Chromosomal preparations were obtained from cerebral ganglia of prepupae of *E. verticillata* generally following the protocol developed by Imai et al. (1988) with certain modifications. Ganglia were extracted from insects dissected in 0.5% hypotonic sodium citrate solution containing 0.005% colchicine. The extracted ganglia were then transferred to a fresh portion of the hypotonic solution and incubated for 30 min at room temperature. The material was transferred onto a pre-cleaned microscope slide using a Pasteur pipette and then gently flushed with Fixative I (glacial acetic acid: absolute ethanol: distilled water 3:3:4). The tissues were disrupted using dissecting needles in an additional drop of Fixative I. Another drop of Fixative II (glacial acetic acid: absolute ethanol 1:1) was applied to the center of the area, and the more aqueous phase was blotted off the edges of the slide. The slides were then dried for approximately half an hour and stored at room temperature. Chromosome preparations were stained with freshly prepared 3% Giemsa solution in 0.05M Sorensen's phosphate buffer (Na_2HPO_4 + KH_2PO_4, pH 6.8) for a few hours.

Image acquisition and analysis

Metaphase plates were analyzed under a Zeiss Axioskop 40 FL epifluorescence microscope (Carl Zeiss, Göttingen, Germany). Images of chromosomes were taken with Zeiss AxioCam MRc digital camera using Zeiss AxioVision software version

3.1. To prepare illustrations, the resulting images were arranged and enhanced with Adobe Photoshop 8.0. KaryoType version 2.0 software was also used for taking measurements from four diploid metaphase plates of *E. verticillata* having well-defined chromosome morphology. For each chromosome of the haploid set, we report both its relative length (100 × length of the chromosome divided by total length of the set) and centromeric index (100 × length of the shorter arm divided by total length of the chromosome). The chromosomes were classified into metacentrics (M), subtelocentrics (ST) and acrocentrics (A) following guidelines provided by Levan et al. (1964).

Results and discussion

The diploid karyotype of *E. verticillata* contains the largest pair of metacentric chromosomes, two pairs of shorter metacentrics, two other pairs of considerably smaller metacentric chromosomes, and the shortest pair of acrocentrics (Fig. 1; Table 1). On the other hand, its haploid karyotype contains six chromosomes which sizes and shapes are similar to those of the diploid chromosome set (not shown here); the chromosome number in this species is therefore n = 6 and 2n = 12.

The karyotype of *E. verticillata* is structurally similar to that of the only known member of the family Agaonidae with the same chromosome number (Table 2), *Blastophaga psenes* (Gokhman et al. 2010), although chromosome sets of these two species differ in some details. Specifically, diploid karyotypes of both *E. verticillata* and *B. psenes* contain five pairs of metacentrics, but the smallest pairs within their chromosome sets are acrocentric and subtelocentric respectively. In addition, the lengths of most metacentric chromosomes differ substantially in the former species compared to *B. psenes* (see Gokhman et al. 2010). Moreover, the smallest pair of chromosomes is much shorter than the preceding one in the latter species, in contrast to *E. verticillata*. These two chalcid wasps belong to different subfamilies of Agaonidae, Blastophaginae and Agaoninae respectively (Cruaud et al. 2010), and the similar karyotype structure of these species may be considered a plesiomorphic feature of the family in general (Gokhman et al. 2010). Furthermore, chromosome sets of all other studied members of Agaonidae, i.e. two species of the same genus *Eupristina* Saunders, 1882, *E. altissima* and *Eupristina* sp. with n = 5, as well as three members of the genus *Ceratosolen* Mayr, 1885 from the subfamily Kradibiinae, *C. solmsi*, *C. gravelyi*, and *C. emarginatus* with the same chromosome number (Table 2), contain only large metacentrics of similar size (Liu et al. 2011, Chen et al. 2018). Analogously to the situation observed in a few other chalcid families, e.g. Eulophidae, Torymidae and Ormyridae, results of the present study apparently confirm that the karyotypes with n = 5 resulted from the fusion between the smallest acrocentric/subtelocentric chromosome and a particular metacentric in an ancestral chromosome set with n = 6 (Gokhman 2013). If this is true, lower chromosome numbers of corresponding species could represent their putative synapomorphies within certain genera.

Figure 1. Diploid karyotype of *E. verticillata*. Scale bar: 10 μm.

Table 1. Relative lengths (RL) and centromeric indices (CI) of chromosomes of *E. verticillata* (mean ± SD).

Chr. no.	RL	CI
1	23.30 ± 1.35	42.38 ± 3.97
2	21.00 ± 0.77	44.53 ± 3.15
3	20.64 ± 0.76	43.11 ± 5.28
4	15.63 ± 0.31	46.72 ± 2.71
5	11.51 ± 0.78	46.21 ± 2.82
6	7.92 ± 0.46	0

Table 2. Characteristics of diploid karyotypes of studied species of Agaonidae.

Species	2n	Chromosome formula	Reference
Blastophaga psenes (Linnaeus, 1758)	12	10M + 2ST	Gokhman et al. 2010
Ceratosolen emarginatus Mayr, 1916	10	10M	Liu et al. 2011
C. gravelyi Grandi, 1916	10	10M	Liu et al. 2011
C. solmsi (Mayr, 1885)	10	10M	Liu et al. 2011
Eupristina altissima Balakrishnan & Abdurahiman, 1981	10	10M	Chen et al. 2018
E. verticillata Waterston, 1921	12	10M + 2A	Present study
Eupristina sp.	10	10M	Chen et al. 2018

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SHORT COMMUNICATION

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An unusual prey record for *Astata lugens* Taschenberg (Hymenoptera, Apoidea, Astatidae)

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Abstract

Astatid wasps are referred to in literature as specialized predators of hemipterans. We present an unusual prey record for the genus *Astata* in a Cerrado area (Savannah), at Chapada dos Veadeiros National Park, Goiás State, Brazil. We collected one specimen of *Astata lugens* Taschenberg carrying an immature cricket (Gryllidae) as prey.

Keywords

Apoid Wasps, Digger Wasp, Gryllidae, Neotropical Savannah, Orthoptera, Prey association

Introduction

Wasps of the family Astatidae are specialized predators of Hemiptera (Bohart and Menke 1976; Hanson and Menke 1995; O'Neill 2001; Amarante 2006). Females dig a nest in the soil, which ends in single or multiple cells. They hunt hemipterans that they completely paralyze, to feed their offspring (Evans 1957).

This family has 161 species described in four genera: *Astata* Latreille, 1796; *Diploplectron* Fox, 1893; *Dryudella* Spinola, 1843; and *Uniplectron* Parker, 1966 (Pulawski 2018). These four genera were traditionally placed in the subfamily Astatinae (Bohart and Menke 1976), however, Sann et al. (2018) elevated Astatinae to full family rank.

The only genus present in South America is *Astata*. This genus is distributed worldwide except absent in Australia. It is the most diverse genus in the family, with 82 described species (Pulawski 2018). Twenty-five species have been recorded from the Neotropical Region, ten species from South America, and at least six from Brazil (Parker 1968; Nascimento and Overall 1980; Amarante 2002, 2005).

The prey of this wasps are immature and adult hemipterans; of *Astata* mainly Pentatomidae (Evans 1957), but other Hemiptera families as well, e.g., Acanthososmatidae, Cydnidae, Lygaeidae, and Scutelleridae (Tsuneki 1947; Herting 1971; Krombein 1972; Bitsch et al. 2007; Gros 2008). There are no prey records for *Astata lugens*, and herein we present our observations on the prey of this species, which is highly unusual for the genus.

Material and methods

The observation was carried out on the border at Chapada dos Veadeiros National Park, São Jorge, Alto Paraíso de Goiás, Goiás State, Brazil (-14.160958° -47.791376°, 1,086 m a.s.l.) (Figure 1). The vegetation is composed mainly of tropical savannah (Cerrado). This area is considered a biodiversity hotspot (Cardoso da Silva and Bates 2002), with a high rate of endemism (Mendonça et al. 1998). According to Morrone (2014), this area is located in Cerrado Biogeographical Province, at Chacoan sub-region. Following the Köppen's (1936) classification, the predominant climate is tropical with dry winter (Aw) (Alvares et al. 2013). The annual average temperature is 25°C, and annual average precipitation is 1,600 mm. The rainy season runs from October to April, the dry season extends from May until September (Nimer 1989; Felfili et al. 2007).

Results

On April 07 2009, around sunset, on a trail in Cerrado *sensu stricto* formation, two of us (BMT and VC) observed a wasp carrying her prey. This individual was grasping the prey by the antennal base with her mandibles, walking forward, jumping, and flying short distances close to the soil level. The wasp and her prey were caught using a transparent plastic bag from a cigarette pack, the only thing that we had in our hands at that moment. We did not find the nest due to our anxiety to collect the prey. The voucher specimens of both specimens were pinned and deposited in the Hymenoptera Collection of the Museu da Biodiversidade at Universidade Federal da Grande Dourados (MuBio/UFGD), with number: Hym-00165-S.

The wasp was identified using the key of Parker (1968). The cricket specimen (Figures 2 A, B, and C) was determined as a juvenile gryllid with keys of Triplehorn and Johnson (2005). The identification was confirmed by Dr. Edison Zefa at Universidade Federal de Pelotas, Rio Grande do Sul State, Brazil.

The wasp agrees with Parker's (1962, 1968) diagnosis of *Astata lugens* Taschenberg, 1870 in the following: 1. second labial palpal segment asymmetrical (Figure 3A); 2.

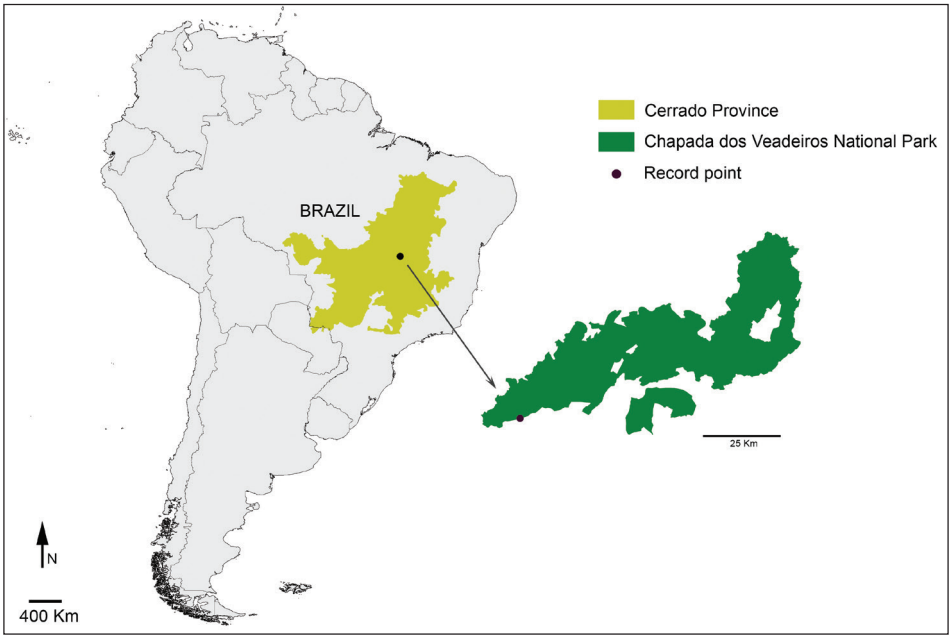

Figure 1. Map illustrating South America with Cerrado Province (Morrone 2014), and the observation point at Chapada dos Veadeiros National Park.

Figure 2. Habitus of juvenile Gryllidae, prey of *Astata lugens* Taschenberg, 1870; [BRASIL: Goiás, PN Chapada dos Veadeiros, -14.160958°, -47.791376°, 1.086 m, v.2009, Silvestre R. et al. col., MuBio-UF-GD Hym-00165-S] **A** head frontal, scale bar: 500 µm **B** dorsal, scale bar: 1 mm **C** lateral, scale bar: 1 mm.

Figure 3. *Astata lugens* Taschenberg, 1870 ♀ [BRASIL: Goiás, PN Chapada dos Veadeiros, -14.160958° -47.791376°, 1.086 m, v.2009, Silvestre R. et al. col., MuBio-UFGD Hym-00165-S] **A** labial palpus, scale bar: 200 µm **B** ocellar region, scale bar: 200 µm **C** propodeal enclosure, scale bar: 500 µm **D** pygidial plate, scale bar: 500 µm **E** thorax and abdomen in lateral view, scale bar: 1 mm.

interocellar area with about 50 punctures (Figure 3B); 3. propodeal enclosure irregularly reticulate-striate (Figure 3C); 4. pygidial plate bordered with conspicuous setae recurved posteriorly (Figure 3D); 5. body surface sparsely pitted (Figure 3E); 6. gaster

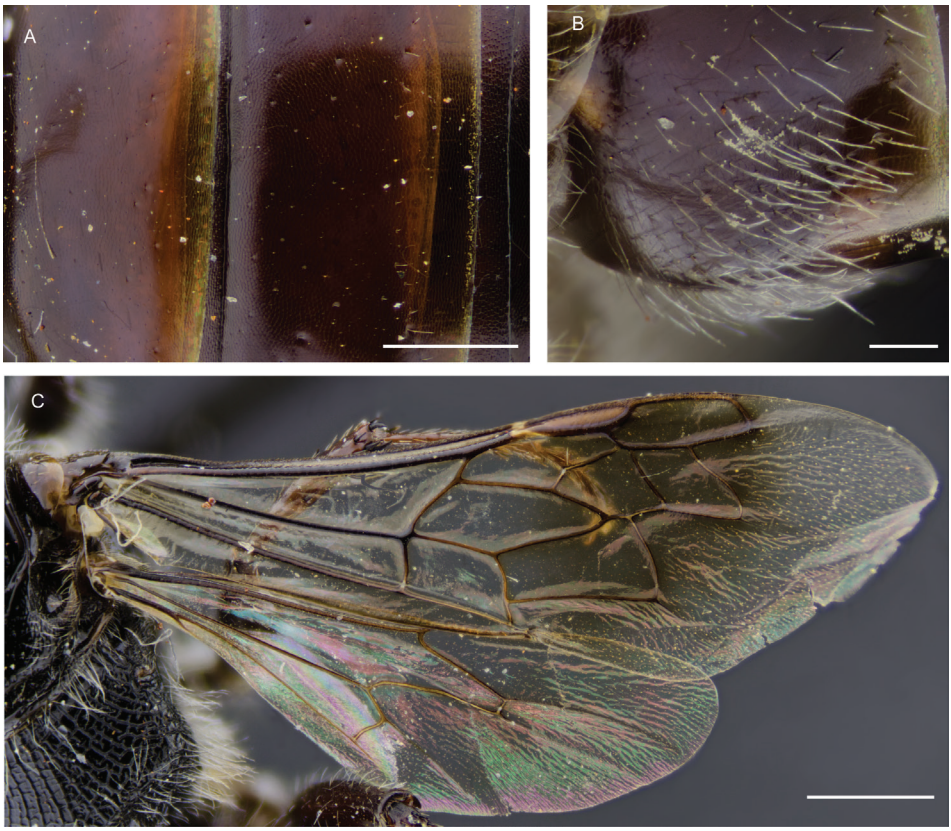

Figure 4. *Astata lugens* Taschenberg, 1870 ♀ [BRASIL: Goiás, PN Chapada dos Veadeiros, -14.160958° -47.791376°, 1.086 m, v.2009, Silvestre R. et al. col., MuBio-UFGD Hym-00165-S] **A** terga I and II, scale bar: 500 μm **B** sternum II, scale bar: 200 μm **C** wings, scale bar: 1 mm.

finely micropunctate (Figure 4A); 7. sternum II slightly humped (Figure 4B); 8. wings hyaline, forewing with a light brown band on marginal cell (Figure 4C); 9. prosternum, mesosternum, metasternum, coxae, sternum I and II densely covered with white long setae (Figure 3E). The only difference is that the gaster and legs are fuscous/brown, not completely black as described by Parker.

Discussion

The biological records for *Astata* species comes from North America, Central America and Eurasia. This prey record of a different order of insects is completely unusual for this genus; expose the lack of knowledge about the biology of Neotropical species.

This record may have been an occasional prey, or it is possible that in the Neotropical Region, this wasp genus exhibits different trophic interactions of species from Holarctic origin. To elucidate this hypothesis, objective studies must be carried out.

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RESEARCH ARTICLE

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A review and updated classification of pollen gathering behavior in bees (Hymenoptera, Apoidea)

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Abstract

Pollen is the primary protein and nutrient source for bees and they employ many different behaviors to gather it. Numerous terms have been coined to describe pollen gathering behaviors, creating confusion as many are not clearly-defined or overlap with existing terms. There is a need for a clear yet flexible classification that enables accurate, succinct descriptions of pollen gathering behaviors to enable meaningful discussion and comparison. Here, we classify the different pollen gathering behaviors into two main classes: active and incidental pollen collection. Active pollen collection is subdivided into six behaviors: scraping with the extremities, buzzing, rubbing with the body and/or scopae, rubbing with the face, tapping, and rasping. In addition to the active and incidental pollen gathering behaviors, many bees have an intermediate step in which they temporarily accumulate pollen on a discrete patch of specialized hairs. Each behavior is described and illustrated with video examples. Many of these behaviors can be further broken down based on the variations found in different bee species. Different species or individual bees mix and match these pollen collecting behaviors depending on their behavioral plasticity and host plant morphology. Taken together, the different behaviors are combined to create complex behavioral repertoires built on a foundation of simple and basic steps. This classification sets the groundwork for further research on various topics, including behavioral plasticity in different species, comparisons between generalists and specialists, and the relative effectiveness of different pollen gathering behaviors.

Keywords

Pollinators, pollen collection, foraging behavior, floral specialization, oligolecty, floral hosts, Anthophila

Introduction

Bees visit flowers primarily for nectar and pollen, which they use as provisions for their young and to meet their own energetic and nutritional requirements (Michener 2007; Cane et al. 2016). Pollen is the primary resource in the larval provisions of most species, and bees have evolved behaviors to efficiently collect large amounts of pollen (Thorp 2000). Unsurprisingly, not all bees gather pollen identically, and many different pollen gathering behaviors exist. For example, even on the same floral host, different bee species often use different pollen gathering behaviors (Thorp 1979). In addition, flexibility exists at the individual level, with individual bees using different pollen gathering behaviors depending on the floral host and pollen availability (Heinrich 1976b; Prosi et al. 2016; Russell et al. 2017). The types of behavior that bees use can influence their floral preferences and may impact their effectiveness as pollinators. Due to the importance of pollen gathering behavior and the growing interest in bee biology and pollination ecology, the number of research articles that describe or mention pollen gathering behavior has grown rapidly. As a result, it is necessary to produce a standardized terminology which both accurately describes the different behaviors and facilitates their effective communication.

Pollen-gathering behavior in bees has historically been divided into two main types: active and incidental (or passive) collection (Parker 1926; Doull 1970; Inouye et al. 1994; Westerkamp 1996; Thorp 2000). Active pollen collection refers to the purposeful uptake of pollen, generally through the use of the forelegs and mandibles, directly from anthers or other floral surfaces. Incidental (or passive) pollen collection refers to pollen that accumulates on bees as they forage for nectar – this pollen may either be packed into the pollen transporting structures or groomed and discarded (Doull 1970). However, such broad categories oversimplify the diversity of pollen gathering behaviors exhibited by bees and the line between active and incidental pollen collection is often unclear. Previous researchers have broken down active pollen gathering into numerous variants, but these attempts typically lack a broader framework and conflate, or overlap with, existing definitions. For example, a single species – *Osmia montana* Cresson (Megachilidae) – has had its pollen gathering behavior variously coined as "thumping," "tapping," "patting," and "drumming" (Rust 1974; Cripps and Rust 1989; Cane 2011; Cane 2017). Conversely, the same term can have multiple meanings, such as "scrabbling," which has been variously used to describe bees that scrape with the forelegs or run over a plane of flowers (Percival 1955; Thorp 2000). These numerous, overlapping, and poorly-defined terms illustrate the need for more precise and consistent terminology to describe pollen gathering behavior.

While pollen gathering behavior has previously been reviewed by others, these works tend to focus on such disparate fields as the phenological, chemical, and morphological adaptations of bees to flowers (Müller 1996b; Thorp 1979, 2000). In addition, the recent proliferation of high-speed, high-definition video technology enables the efficient study and communication of previously inaccessible or complex behaviors. Here, we build on the existing schema of active vs. incidental pollen gathering and propose a comprehensive terminology to categorize and describe the full range of pollen-gathering behaviors. Active pollen gathering is broken down into six types,

which can be further subdivided based on the nuances of particular bee groups and the morphology of floral hosts. Our updated classification systematizes pollen gathering behavior and facilitates the description and communication of behavioral observations. In addition, it provides a platform to better address questions on the breadth, limitations, evolution, and convergence of pollen gathering behaviors.

Methods

Pollen gathering videos were recorded by ZMP and MCO as well as gathered from other researchers or sources (e.g. youtube.com) and used with permission. To record behavior, we used a Sony A65 with a 90mm macro lens and a Pentax Optio WG-2. Scanning electron microscope images were taken with a Quanta FEG 650 Scanning Electron Microscope. Morphological terminology generally follows Michener (2007); as exceptions for accessibility, abdomen is used to refer to the metasoma and thorax is used to refer to the mesosoma. Classification generally follows Michener (2007) except eucerines follow Dorchin et al. (2018). Bees were identified using either collected specimens or still images from videos.

We broadly surveyed the literature for descriptions of pollen gathering behavior. We combined keyword searches in google scholar with manual searching of older literature from the literature collection of the Pollinating Insect Research Unit. Selecting appropriate terminology proved difficult because every pollen gathering behavior has been referred to by multiple different terms. In general, three main considerations were taken into account in selecting the most appropriate terminology for the different pollen gathering behaviors: priority (first known instance of use), usage (prevalence of a term in the scientific and popular literature), and accuracy (how well a term describes a given behavior). Exceptions were made for usage and accuracy, particularly when terms had conflicting or multiple meanings, or when a particular usage is widely accepted.

Results and discussion

Types of pollen gathering behavior

We define pollen gathering behaviors as the movements that bees use to acquire, actively or incidentally, pollen from anthers or other pollen presenting structures. Pollen gathering is related to, but separate from, pollen transport, which refers to the carrying of accumulated pollen back to the nest in specialized transport structures (Roberts and Vallespir 1978; Portman and Tepedino 2017). The different pollen gathering behaviors (listed below) may be used individually or in combination, concurrently or sequentially, creating complex repertoires from a set of simple, well-defined building blocks. Taken together, our definitions offer a useful guide to the full set of major behaviors used by bees when gathering pollen.

In foraging from flowers, bees have three possible primary purposes on each trip: to acquire nectar (or oil), pollen, or both. We term pollen gathering **active** when pol-

len is the primary objective or when nectar (or oil) and pollen are co-objectives. Active pollen gathering is broken down into six pollen gathering behaviors as described below. We term pollen gathering **incidental** when nectar is the primary objective and pollen is passively accumulated on the body. The bee then "decides," based on pollen and floral characteristics, and on need, to discard or keep the pollen. Bees may also incidentally collect pollen on non-target areas of the body while actively gathering pollen. Such pollen may or may not be gathered into the pollen transporting structures.

Active pollen gathering behaviors can be broken up into six types, listed loosely in order of prevalence:

- **Scraping with the extremities:** use of the legs or mouthparts to remove pollen directly from anthers, or, less commonly, to glean pollen from flowers.
- **Buzzing:** use of the flight muscles to vibrate a flower to assist with pollen release.
- **Rubbing with the body and/or scopa(e):** the gathering of pollen through direct and more or less continuous contact with the anthers by the main trunk of the body (thorax, abdomen) and/or the scopal hairs.
- **Tapping:** picking up pollen from anthers by a rapid up and down motion of the abdominal venter.
- **Rubbing with the face:** continuous and more or less direct contact with the anthers by the face.
- **Rasping:** rubbing of the thoracic dorsum against anthers, causing the anthers to vibrate and release the pollen.

Incidental pollen gathering (not broken down into types): the use of pollen that has accumulated on the body either through nectar gathering or on non-target areas as a result of a primary pollen gathering behavior. A major component of incidental behavior is the degree of movement by the bee, which influences the amount of pollen that adheres to the bee through contact.

Scraping with the extremities

Scraping with the extremities refers to the use of the legs and/or mouthparts (including biting with the mandibles) to gather pollen with a repetitive unidirectional motion (Figure 1; Suppl. material 1). The term "scraping" is generally accepted by most authors, though other terms, such as "brushing," "raking," "scrabbling," "stroking," and "wiping," have also been used (Casteel 1912; Jander 1976; Michener et al. 1978; Thorp 1979, 2000). Scraping can be performed with the forelegs, midlegs, hindlegs, mandibles, mouthparts, or combinations of these appendages, making it important to specify which are used and the manner of their use. Indeed, scraping with the extremities is something of a catchall category, and it could reasonably be divided into multiple distinct types. However, the diversity of behaviors, combined with the relative lack of detailed descriptions, made it infeasible to clearly and consistently split the category further.

Figure 1. *Andrena chlorogaster* Viereck (Andrenidae) biting with the mandibles and scraping with the forelegs **A** foreleg extended and **B** foreleg scraping towards the body. Note that the bee is also scraping the pollen from anthers by biting with the mandibles.

Scraping pollen directly from the anthers or other pollen-presenting structures with the basitarsal brushes of the forelegs is the most common pollen gathering method used by bees (Grinfel'd 1962; Michener et al. 1978; Westerkamp 1996; Michener 2007). The forelegs and mandibles are used together to scrape pollen, though the midlegs and/or hindlegs may also be used. The most typical form of the behavior is described in detail for *Lasioglossum zephyrum* (Smith) (Halictidae) (Batra 1966) and *L. lusorium* (Cockerell) (Bohart and Youssef 1976). The forelegs may also be used to glean pollen from other surfaces, such as flower petals, or even the scopae of other bees (termed "cleptolecty;" Thorp and Briggs 1980, Snelling and Stage 1995). One variation is seen in bees which "strike" poricidal anthers with the forelegs to loosen pollen. Striking has been observed in *Ptiloglossa* on *Solanum* (Linsley 1962), *Osmia ribifloris* Cockerell on blueberry (Torchio 1990), and *Apis mellifera* L. (Apidae) on cranberry ("drumming," Cane et al. 1993).

One term that refers to scraping with the forelegs – "scrabbling" – is particularly problematic because it has apparently adopted two different meanings. In the original, and most well-accepted usage, it refers to scraping with the forelegs and mandibles (Percival 1955; Harper 1957). However, scrabbling has also been defined as a more specific behavior whereby bees (and honey bees in particular) scrape with their forelegs and mandibles while *simultaneously* running across a plane of flowers (Doull 1970; Thorp 2000). Currently, scrabbling is almost exclusively used in the former sense, to refer only to scraping with the forelegs and mandibles (e.g. Cane and Dunne 2014; Muth et al. 2016; Russell et al. 2017). As a result of this confusion, the term scrabbling should either be avoided or explicitly paired with descriptions of the degree of movement.

The mouthparts are also frequently used to gather or loosen pollen. The mandibles are most often used to bite anthers to loosen the pollen in tandem with scraping with the forelegs (Sladen 1912; Thorp 1979), though some bees hang on by the mandibles while gathering with the forelegs (e.g. Batra 1966). Some bees, including *Apis*, *Colletes* (Colletidae) (Buchmann et al. 1977), *Bombus* (Apidae) (Todd 1882; Robertson 1890; Bowers 1975), and various halictines (Thorp and Estes 1975), use the mandibles in a

"milking" motion to gather pollen from poricidal flowers, such as *Cassia* (Fabaceae) and *Solanum* (Solanaceae) (however, the observations of *Bombus* from the late 1800's may be misinterpretations of buzzing behavior). This behavior is often combined with buzzing to release pollen (Thorp and Estes 1975). The mandibles can also be used to chew open anthers to render the pollen more accessible, a behavior observed in *Lasioglossum lustrans* (Cockerell) (Estes and Thorp 1975) and repeatedly in *Trigona* (Apidae) (Wille 1963; Inouye 1980; McDade and Kinsman 1980; Renner 1983, Telles et al. 2018).

The midlegs are generally used to assist the forelegs in scraping pollen (Westerkamp 1996). However, some megachilid bees have specialized hair brushes on the femur of the middle and/or hind legs that appear to be used to scrape pollen from Asteraceae flowers (Müller and Bansac 2004). Although the exact pollen gathering movements remain unclear, the mid or hind legs appear to bend at the femoral-tibial joint to scrape pollen from individual anthers, mirroring the movements used to groom pollen off the foreleg by the midleg (ZP, pers. obs).

Scraping movements are also made with the hind legs, particularly on keel-shaped flowers. In these cases, the hind legs appear to be used because the front and middle legs are occupied in obtaining purchase and spreading the wing petals to expose the anthers. Various *Andrena* (Andrenidae), *Anthophora* (Apidae), *Apis*, *Bombus*, and megachilids use these behaviors on keel-shaped flowers such as *Collinsia* (Plantaginaceae) and *Lupinus* (Fabaceae) (Rust and Clement 1977; Wainwright 1978). One particularly interesting example is *Samba turkana* Packer (Melittidae), which uses an enlarged and curved hind tibial spur to scrape pollen from anthers of *Crotalaria* (Fabaceae) (Packer and Martins 2015).

In bees that gather pollen primarily by rubbing with the body and/or scopae (discussed in a later section), the hind legs are often used in an accessory fashion to gather a clump of anthers and draw them to the body, as well as to help scrape pollen from the gathered anthers. This gathering and scraping behavior is seen in Megachilidae such as *Osmia cornuta* (Latreille) (Monzón et al. 2004) and *O. lignaria* Say (Rust and Clement 1977). It should be noted that scraping with the hind legs is distinct from rubbing with the scopae, which are often located on the hind legs. Scraping with the hind legs entails removal of pollen from anthers with a repeated, unidirectional movement where the pollen is then packed into another structure. In contrast, rubbing with hindleg scopae involves rubbing the scopal hairs back and forth on the anthers, and the continuous contact with the pollen works the pollen directly into the scopal hairs.

Bees that scrape with the extremities often possess morphological features that improve their efficiency. Many examples of hooked hairs on the forelegs and especially the foretarsi are associated with specialization on flowers with narrow corollas (Thorp 1979), although exceptions to the narrow-corolla association exist (Neff 2004). Examples have been reviewed by Thorp (1979, 2000); more recent examples include two species of European *Colletes* on hosts in Boraginaceae (Müller and Kuhlmann 2003), multiple species of North American *Colletes* on various hosts (Neff 2004), and multiple *Hoplitis* (Megachilidae) on various hosts (Sedivy et al. 2013).

Similar to the forelegs, the mouthparts (primarily the stipes, galea, and labial and maxillary palpi) can be modified, often with hooked hairs, to extract pollen from flowers

with narrow corollas. The presence and use of modified hairs on the mouthparts have been reviewed by Thorp (1979, 2000). More recent examples include various apid and halictid visitors to Pontederiaceae (Alves-dos-Santos 2003), *Ceblurgus longipalpis* Urban and Moure (Halictidae) on *Cordia* (Boraginaceae) (Milet-Pinheiro and Schlindwein 2010), multiple *Anthophora* on various hosts (Orr et al. 2018), *Haetosmia vechti* (Peters) (Megachilidae) on *Heliotropium* (Boraginaceae) (Gotlieb et al. 2014), and multiple Palaearctic *Hoplitis* on various hosts (Müller 2006; Sedivy et al. 2013). In addition to modified hairs, other bees have enlarged or elongated labial palpi, but it is not clear whether the function is to assist in the removal of pollen or the sucking of nectar. Examples include *Perdita heliotropii* Cockerell (Andrenidae), a specialist on *Heliotropium* (Timberlake 1958), and *Hesperapis* sp. (Stage manuscript name *"palpalis"*) (Melittidae), a specialist on Polemoniaceae (Stage 1966).

Buzzing

Buzzing, commonly referred to by the suboptimal terms "buzz pollination" and "floral sonication," is the use of the thoracic flight muscles to generate audible vibrations that aide in accessing and collecting pollen from a flower (Figure 2; Suppl. material 2; Buchmann 1983; Thorp 2000). Buzzing induces the release of pollen from the anthers, which the bee usually receives on the venter, where the adhesion of pollen is aided by the electrostatic attraction of the pollen to the bee's body and hairs (Buchmann and Hurley 1978; Corbet et al. 1982; Buchmann 1983; Vaknin et al. 2000). The legs are subsequently used to groom pollen from the body into the pollen transporting structures. A variant of buzzing, "buzz milking" (Cane and Buchmann 1989), is performed by *Protandrena mexicanorum* (Cockerell) (Andrenidae), which buzzes anthers of *Solanum* while gradually sliding up the upward-facing anthers before curling over the tips of the anthers to receive pollen. Another variant of buzzing, described as "head-banging," has been recorded for *Amegilla murrayensis* (Rayment) (Apidae), which repeatedly taps its head against anthers while buzzing rather than gripping with the mandibles (Switzer et al. 2016). Finally, buzzing can also be used in combination with rubbing with the face (Müller 1996a).

Here, we focus primarily on terminology because the extent and occurrence of buzzing behavior has been reviewed by others (see Buchmann 1983; Thorp 2000; Cardinal et al. 2018). Although it is clear what buzzing refers to, many different terms have been used to describe it (Table 1). Observations of buzzing have been made since at least 1902 (e.g. Lindman 1902; Schrottky 1908; Plath 1934; Rayment 1944; Meidell 1944; Osorno-Mesa 1947; Rick 1950; see Teppner 2018 for a discussion on the earliest mention), but it remained little-known and poorly-defined until Michener (1962) and Wille (1963) described the behavior in-depth and referred to it as "buzzing." Buchmann (1974, 1983) later codified the term "buzz pollination," though he and others have often used alternative terminologies, often within a single paper (Table 1). Starting in 1985, the term "floral sonication" has also been used to refer to this behavior (Cane 1985; Cane et al. 1985), though this term is a misnomer since me-

Figure 2. *Bombus impatiens* Cresson (Apidae) buzzing a *Solanum* (Solanaceae) flower. The pulsed vibrations expel the pollen from the anthers. Image adapted from Russell et al. (2016)

chanical vibrations, rather than sound waves, cause the pollen to be expelled. Due to their priority (Michener 1962; Wille 1963; Buchmann 1983) and widespread usage, we recommend using the term buzzing to refer broadly to the behavior, and variants of "buzz" (e.g. "buzzed," "buzzes") to refer to the specific act of buzzing flowers. Despite its wide usage, we do not recommend the term "buzz pollination" because it is also a misnomer as it does not necessarily effect pollination as the name implies. Others have proposed using the term "vibratile pollen harvesting" or "vibratory pollen collection" as these descriptive terms are more technically correct than "buzz pollination," but these terms have not been widely adopted (Neff and Simpson 1988; Teppner 2018).

Members of all bee families have been documented using buzzing behavior, though it is uncommon or rare in Andrenidae, Melittidae, and Megachilidae (Meidell 1944; Buchmann and Hurley 1978; Buchmann 1983; Houston and Thorp 1984; Cane et al. 1985; Neff and Simpson 1988; Thorp 2000). Interestingly, some of the most generalized bees, such as honey bees and *Trigona* spp., do not buzz flowers (Buchmann 1983; King and Buchmann 2003; Michener 2007). Although bees buzz a wide variety of plants (>72 families, reviewed in Buchmann 1983), this behavior was traditionally considered exclusive to plants with poricidal, or hidden, anthers (Michener 1962). However, there are many exceptions to this, and bees buzz a variety of flowers in the families Asteraceae, Cucurbitaceae, Fabaceae, Papaveraceae, Rosaceae, Plantaginaceae, and others (e.g. Suppl. material 2; Heinrich 1976a; Buchmann 1985; Bernhardt 1989; Russell et al. 2017).

Rubbing with the body and/or scopae

Rubbing with the body and/or scopae refers to the use of direct, more or less continuous contact between the anthers and the scopae and/or venter of the main body segments (thorax or abdomen). The defining character of rubbing with the body and/

Table 1. Terminology used to refer to buzzing in the literature. This list is not comprehensive; instead, it focuses on the first usage of terms and major works on buzzing.

Term used	Genus or Species	Floral Host	Citation
"einer gewaltsamen Vibration des Insektenkörpers und zugleich der ganzen Blüte" [a violent vibration of the insect body and at the same time the whole flower]	*Bombus* (Apidae)	*Senna* (Fabaceae)	Lindman 1902 (see Teppner 2018)
"die Bienen … versetzen die Blüte in starke Vibration, so dass der Pollen aus den Antheren herausgeschüttelt" [the bees… cause the flower to vibrate strongly, causing the pollen to be shaken out of the anthers]	*Augchlora* (Halictidae), *Oxaea* (Andrenidae), *Ptiloglossa* (Colletidae), *Xylocopa* (Apidae)	*Senna*, *Physalis* (Solanaceae), *Solanum* (Solanaceae)	Schrottky 1908 (see Teppner 2018)
"the worker seizes the anthers with her mandibles and first two pairs of legs, and shakes them, emitting an impatient buzz, as if angry because the stamens do not give up their pollen at once"	*Anthophora* (Apidae), *Bombus*	*Rosa* (Rosaceae), *Rubus* (Rosaceae)	Plath 1934
"vigorous whirrings" and "whirring method"	*Bombus*, *Megachile* (Megachilidae)	*Melampyrum* (Orobanchaceae)	Meidell 1944 (posthumously published notes)
"while…collecting pollen…she makes the continuous sound that has been compared with that of a honey-bee caught in a spider's web"	*Amegilla* (Apidae)	Not specified	Rayment 1944
"vibrar las anteras" ["vibrates the anthers"]	*Bombus*, *Xylocopa*	Not specified	Osorno-Mesa 1947
"vibrating the flowers with rapid leg movements accompanied by a high pitched hum"	Not specified	*Solanum* [as *Lycopersicon*] (Solanaceae)	Rick 1950
"buzz," "buzzing," "buzzing behavior," and "vibrate"	Various Andrenidae, Apidae, and Halictidae	*Cassia* (Fabaceae), *Solanum*	Michener 1962
"buzzing," "buzzing behavior," and "buzzing technique"	Various Apidae, Colletidae, and Halictidae	*Cassia*	Wille 1963
"vibrate the anthers"	Various Apidae, Andrenidae, and Colletidae	*Solanum*	Linsley and Cazier 1963
"vibratory pollen collection" and "wing vibration"	*Bombus*, various Halictidae	*Dodecatheon* (Primulaceae)	Macior 1964
"buzz," "buzzing," and "buzzing behavior"	*Agapostemon* (Halictidae)	*Chamaecrista* (Fabaceae), *Solanum*	Roberts 1969
"buzzed the anthers" and "vibrate the anthers"	*Caupolicana* (Colletidae), *Ptiloglossa*	*Datura* (Solanaceae), *Solanum*	Linsley and Cazier 1970
"vibrate their wings"	*Bombus terricola* Kirby	*Solanum*, *Spiraea* (Rosaceae)	Heinrich 1972
"buzz (vibratile) pollination," "buzzing," and "buzzing technique"	*Centris* (Apidae), *Melipona* (Apidae)	*Senna* [as *Cassia*]	Buchmann 1974
"vibratory behavior" and "vibrated anthers"	Various Apidae and Halictidae	*Chamaecrista* [as *Cassia*]	Thorp and Estes 1975
"vibration of the thorax"	*Anthophora*, *Augochlorella* (Halictidae), *Bombus*, *Psaenythia* (Andrenidae), *Xylocopa*	*Solanum*	Bowers 1975
"buzz," "buzzing," and "vibrated the anthers while emitting a buzzing sound"	*Bombus*	*Rosa*, *Solanum*, *Vaccinium* (Ericaceae)	Heinrich 1976b
"buzz," "buzz pollination," "buzzing," "buzzing behavior," and "vibratile pollination"	*Anthophora*, *Bombus*, *Colletes*, *Xylocopa*. Also, *Volucella* (Syrphidae)	*Solanum*	Buchmann et al. 1977
"buzz," "'buzz' pollination," "buzzing," "shivering," "vibratile pollination," and "vibrational pollination"	Not specified	*Solanum*	Buchmann and Hurley 1978
"wing vibration" and "wing vibration method"	*Bombus*	*Echeandia* (Asparagaceae)	Bernhardt and Montalvo 1979
"buzz pollination," "buzzing," "buzzing behavior," and "vibratory behavior,"	Not specified	Not specified	Thorp 1979
"buzzing" and "vibratile behavior"	Not specified	Not specified	Eickwort and Ginsberg 1980
"high frequency wing vibrations" and "vibrating"	*Bombus*	*Pedicularis* (Orobanchaceae)	Laverty 1980

Term used	Genus or Species	Floral Host	Citation
"buzz pollination," "buzzing," "floral vibration," "vibratile pollination," and "vibratile technique"	*Euglossa* (Apidae), *Paratetrapedia* (Apidae)	*Mouriri* (Melastomataceae)	Buchmann and Buchmann 1981
"buzz," "buzz pollination," "buzzing behavior," "buzzing, "vibratile pollen harvesting," "vibratile manipulation," "vibrational pollination," "vibrating," and "floral vibration"	Various Apidae, Andrenidae, Colletidae, Halictidae, Melittidae	Various plant families	Buchmann 1983
"thoracic vibration" and "vibrate"	*Lasioglossum* (Halictidae)	*Hibbertia* (Dilleniaceae)	Bernhardt et al. 1984
"buzzing technique"	Stenotritidae	Not specified	Houston and Thorp 1984
"buzz-pollination" and "floral sonication"	*Bombus*	"poricidally dehiscent, nectar-free flowers"	Cane 1985
"buzz," "buzz pollination," "buzz or vibratile pollen foraging" "buzzing," "buzzing behavior," "floral buzzing," and "floral sonication"	*Bombus*, *Melitta americana* (Smith) (Melittidae)	*Vaccinium*	Cane et al. 1985
"buzz," "buzz pollination," "buzzing," "buzzing behavior," "floral buzzing," "floral vibration," "vibratile methods," "vibratile pollen-collecting behavior," "vibratile pollen harvesting," "vibratile foraging behavior," "vibrating," "vibratory pollen-collecting behavior," "vibratory manner," and "vibratory pollen harvesting"	*Bombus*, *Eucera* [as *Xenoglossa*] (Apidae), *Megachile*, *Xylocopa*	Asteraceae, Cucurbitaceae, Papaveraceae, Rosaceae, Scrophulariaceae	Buchmann 1985
"buzz," "buzz pollination," "buzzing," and "vibratory pollen collection"	*Bombus*	*Actinidia* (Actidiniaceae), *Borago* (Boraginaceae), *Polygonatum* (Asparagaceae), *Symphtum* (Boraginaceae)	Corbet et al. 1988
"buzz," "buzzing," and "sonicate"	*Bombus*, *Habropoda* (Apidae)	*Vaccinium*	Cane and Payne 1988
"thoracic vibration," "vibratile behavior," and "vibratile pollen-harvesting"	*Megachile*	*Chamaecrista*	Neff and Simpson 1988
"buzz," "buzz-harvesting," "buzz-milking," "buzz pollination," "buzzing," and "sonicate"	*Protandrena mexicanorum* (Cockerell) (Andrenidae)	*Solanum*	Cane and Buchmann 1989
"buzz," "buzzing," "floral sonication," "sonicate," and "vibratile buzzes"	*Bombus*, *Ptiloglossa*	*Solanum*	Buchmann and Cane 1989
"buzzing," "buzz-collection"	Various	Various	Roubik 1989
"buzzing"	Not specified	Not specified	Westerkamp 1996
"floral sonication" and "sonicate"	Not specified	Not specified	Wcislo and Cane 1996
"buzz," "buzz pollination," "buzzing," "buzzing behavior," "sonication," "sonication behavior," "vibratile or buzz pollination," and "vibrating"	Various Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae, Melittidae, and Stenotritidae	Various floral hosts	Thorp 2000

or scopae is the curling of the abdomen, which generally moves in an up and down motion, or less often, a back-and-forth or telescoping motion (Figure 3; Suppl. material 3). This type of pollen gathering is here used in a broader sense than generally recognized by previous authors who largely restricted the definition to eucerine bees on sunflowers (e.g. Cane 2017). There are two variants of this behavior that depend on whether the scopae are located on the abdominal venter or the hind legs. Bees that transport pollen in abdominal scopa gather pollen directly into the scopal hairs, whereas bees that transport pollen with hind leg scopae must transfer pollen from the venter into the scopae. Despite this difference, the general pollen gathering movements are largely the same, and further, many bees straddle these categories because they have

Figure 3. Rubbing with the body and/or scopae. **A** *Andrena* sp. (Andrenidae) rubbing with the abdomen and scopae on *Camissonia* (Onagraceae) **B** *Ptilothrix bombiformis* (Cresson) (Apidae) rubbing with the abdomen and scopae on *Hibiscus* (Malvaceae) **C, D** *Melissodes* sp. (Apidae) rubbing with the abdomen on *Helianthus* (Asteraceae) **E, F** *Andrena helianthi* Robertson rubbing with the abdomen on *Helianthus* **G, H** *Macropis* sp. (Melittidae) rubbing with the venter of the thorax and abdomen on *Lysimachia* (Primulaceae).

diffuse scopa that cover both the ventral abdomen and hind legs (e.g. *Ptilothrix*). Rubbing behavior has been observed in a wide variety of species in every bee family (except Stenotritidae) on numerous floral hosts (Table 2) and is likely more common than the relatively few observations suggest.

Gathering pollen directly with the abdomen and/or scopae has been known since at least the late 1800's though it was not described in detail (Müller 1883; Robertson 1889, 1899; Stephen et al. 1969). Rubbing behavior has been referred to by a wide variety of inconsistent descriptions and terms and has often been referred to as, or lumped together with "tapping," which we consider a separate behavior. As a result, some examples which have been previously referred to as "tapping" fall under our definition of rubbing with the body and/or scopae (e.g. Cane 2017). In addition, observations of the same bee species rubbing on the same plant species often result in quite different descriptions: *Hoplitis anthocopoides* on *Echium vulgare* (Boraginaceae) has been reported as "she vibrates [the abdomen] back and forth rapidly against the anthers while her hind legs also move back and forth against the anthers and scopa" (Eickwort 1973) and alternatively described as "rapidly contracts and expands her abdomen, accordion fashion, over the anthers" (Strickler 1979). Similarly, *Dieunomia triangulifera*, while foraging on *Helianthus annuus* (Asteraceae) has been reported as "waggling the abdomen vigorously from side to side" (Cross and Bohart 1960), as well as "tapping the heads of the disc flowers with the ventral surface of the [abdomen]" (Minckley et al. 1994). We use the term "rubbing" to refer to these behaviors because the term is succinct, accurate, and paraphrases the earliest descriptions, which typically overlap with other more well-known behaviors (e.g. "scraping," "vibrating," and "waggling").

Most bees that gather pollen via rubbing take up the pollen directly with the abdominal scopa (e.g. Megachilidae) or by a combination of the hind leg scopae and abdomen (e.g. *Ptilothrix* and *Andrena* sp. – Figure 3A, B; Suppl. material 3). Rubbing is used by a wide variety of bees that perform additional variants at different speeds. For example, *Melissodes* spp. and *Andrena helianthi* both collect pollen from *Helianthus* by repeatedly rubbing the curled-over apex of their abdomen against the anthers, with the hind leg scopae periodically scraping the abdomen to transfer the gathered pollen (Figure 3C–D; Suppl. material 3). The basic movements of the legs and abdomen of the different species are more or less the same even though the speeds of the abdominal movements can be quite different. Further examples of the variation in rubbing behavior are seen in *Ptilothrix bombiformis* gathering pollen from *Hibiscus* as well as *Andrena* sp. gathering pollen from *Camissonia*, both of which curl the abdomen over a clump of anthers held by the legs (Figure 3A, B; Suppl. material 3). In addition, the venter of the thorax can be used for rubbing in tandem with the abdomen or scopae. Examples include bees in the genus *Macropis*, which use rubbing to accumulate pollen on corkscrew-shaped hairs on the venter of the thorax and abdomen (Cane et al. 1983; Vogel 1992; Schäffler and Dötterl 2011). Despite the variations in movement and speed, all of the aforementioned rubbing variants appear derived from the universal abdominal grooming and pollen-packing movements which involve tamping the legs against the sides and base of the abdomen (Michener et al. 1978), and any attempt to

Table 2. Rubbing with the body and/or scopae in the literature.

Genus or Species	Behavior Description	Floral Host	Citation
Family Andrenidae			
Protoxaea gloriosa (Fox)	Holding the anthers against the abdominal venter and hind legs and shaking them while rotating the body	*Kallstroemia* (Zygophyllaceae)	Cazier and Linsley 1974
Andrena erigeniae Robertson	"pollen was … rubbed from the anthers onto the bee's body and legs"	*Claytonia* (Montiaceae)	Davis and LaBerge 1975
Andrena sp.	Rubbing with abdominal and hind leg scopae	*Camissonia* (Onagraceae)	Figure 3A; Suppl. material 3
Family Apidae			
Eucera (*Tetralonia*) *fulvescens* (Giraud) [as *Tetralonia dufouri* [sic]]	"tummy-tapping"	Asteraceae	Westerkamp 1996
Eucerini spp.	"tummy-tapping"	Asteraceae	Simpson and Neff 1987
Melitoma spp.	"scraping [anthers] with the hind legs"	*Ipomoea* (Convolvulaceae)	Araujo et al. 2018
Ptilothrix bombiformis (Cresson)	"pollen was worked into the scopae"	*Hibiscus* (Malvaceae)	Rust 1980; Figure 3B; Suppl. material 3
Ptilothrix fructifera (Holmberg)	"brush the anthers [between the midlegs, hindlegs, and abdomen]"	*Opuntia* (Cactaceae)	Schlindwein and Wittmann 1997
Svastra obliqua (Say)	"tummy-tapping … with the distal portion of the abdominal venter"	Asteraceae	Simpson and Neff 1987
S. obliqua and *Melissodes agilis* Cresson	"rhythmically tapping … with the distal venter of their slightly decurved abdomen"	Asteraceae	Cane 2017
Family Colletidae			
Perditomorpha brunerii Ashmead	"pollen grains were also scraped directly from the anthers with the scopal setae on the abdominal sterna"	Malvaceae	Gaglianone 2000
Tetraglossula bigamica (Strand)	"rubbing the abdomen against the anthers"	*Ludwigia* (Onagraceae)	Gimenes 1997
Family Halictidae			
Dieunomia triangulifera (Vachal)	"waggling the abdomen vigorously from side to side"	*Helianthus* (Asteraceae)	Cross and Bohart 1960
D. triangulifera	"tapping the heads of the disc flowers with the ventral surface of the metasoma"	*Helianthus*	Minckley et al. 1994
Nomiinae spp.	"tummy-tapping"	Asteraceae	Simpson and Neff 1987
Systropha planidens Giraud	"they rapidly moved their abdomen up and down"	*Convolvulus*	Gonzalez et al. 2014; S. Burrows pers. comm.
Family Megachilidae			
Hoplitis anthocopoides (Schenck)	"she vibrates [the abdomen] back and forth rapidly against the anthers while her hind legs also move back and forth against the anthers and scopa"	*Echium* (Boraginaceae)	Eickwort 1973
H. anthocopoides, *H. producta* (Cresson), *Megachile relativa* Cresson, *Osmia caerulescens* (L.) [as *O. coerulescens* (L.)]	"rapidly contracts and expands her abdomen, accordion fashion, over the anthers"	*Echium*	Strickler 1979
O. lignaria Say, *Megachile* spp.	"filaments are held between the hind legs and … raked against the scopal hairs"	*Collinsia* (Plantaginaceae)	Rust and Clement 1977
Hoplitis simplex (Cresson)	"scrapes pollen directly from the anthers into her abdominal scopa using her hind legs while tapping the anthers with her abdomen"	*Nemophila* (Boraginaceae)	Neff 2009
Ochreriades fasciatus (Friese)	"repeatedly tap their metasomal scopa directly against the anthers"	*Ballota* (Lamiaceae)	Rozen et al. 2015; G. Pisanty pers. comm.
Family Melittidae			
Hesperapis regularis (Cresson)	"scraping [with the scopae]"	*Clarkia*	Burdick and Torchio 1959
H. regularis	"rapid lateral oscillations of the abdomen"	*Clarkia*	Stage 1966
Macropis nuda (Provancher)	"patting motions of the metasoma"	*Lysimachia* (Primulaceae)	Cane et al. 1983
M. fulvipes (Fabricius)	"females pressed the ventral side of the abdomen (by bending) against the anthers"	*Lysimachia*	Schäffler and Dötterl 2011; Schäffler pers. comm.

draw a clear dividing line between these variations will likely become increasingly futile as more observations are made.

Finally, various mentions in the literature suggest that rubbing behavior has been observed in additional species but precise enough descriptions for confirmation are lacking, and many of these could refer to tapping (see next section). This includes suggestions of rubbing by *Osmia lignaria*, *O. indeprensa* Sandhouse, and *O. kincaidii* Cockerell ("the anthers are drawn to the scopal hairs by the hind legs," Cripps and Rust 1989), *Megachile willughbiella* (Kirby) ("the scopa is brushed over the pollen presenting structures," Teppner 2005), as well as *O. bicornis* (L.) [as *O. rufa* (L.)] on *Ranunculus*, and *O. leaiana* (Kirby) and *O. caerulescens* on Asteraceae, which were observed "walking over the anthers so that the ventral scopa touched them while they probed the nectaries" (Raw 1974). In addition, various Megachilidae have been reported to gather pollen by "seesawing" the scopa directly against anthers: *Hoplitis robusta* (Nylander) on *Potentilla* (Rosaceae), *H. zandeni* (Teunissen and van Achterberg 1992), *Osmia* spp. on various hosts, *Pseudoanthidium eximium* (Giraud) on Asteraceae, and *Protosmia minutula* (Pérez) on Lamiaceae (Müller 1996a, 1996b, Müller and Mauss 2016). Lastly, various bees visiting *Clarkia* (Onagraceae) perform a "pollen dance" and "vibrate their bodies laterally" on the flowers and potentially rub their bodies and/or scopae to pick up pollen (MacSwain et al. 1973).

Tapping

Tapping refers to the act of picking up pollen through a rapid up and down motion of the abdominal venter directly against the anthers (Figure 4; Suppl. material 4). It is characterized by the horizontal orientation of the abdomen, the rapidity of movement, and the lack of continuous contact with the anthers (Cane 2017). The term "tapping" comes from what appears to be the original use by Pasteels and Pasteels (1974): "l'abdomen qui tapote de haut en bas sur étamines" ("the abdomen tapped up and down on the stamens"). In the same year, Rust (1974) used "thumping" to describe the behavior. However, Rust subsequently switched to the term "tapping" (Cripps and Rust 1989) to describe the behavior in the same species (Table 3).

Historically, "rubbing" and "tapping" with the abdomen have often been lumped together (e.g. Müller 1883; Simpson and Neff 1987; Westerkamp 1996; Thorp 2000). However, there are distinct differences between tapping and rubbing with the body and/or scopae: in tapping, the rate of movement is faster (Cane 2017), the abdomen is kept more rigid, and the orientation of the abdomen is parallel to the plane of anthers (compared to curled over in typical rubbing). Further, rubbing entails nearly continuous contact, while tapping is characterized by intermittent contact. In rubbing behavior especially, the legs are often used to grab a clump of anthers and rake them against the scopal hairs. Despite these differences, additional exploration of this behavior may reveal that rubbing and tapping fully intergrade, however, until that time, we follow Cane (2017) in splitting these behaviors. Currently, tapping has been observed solely in bees of the family Megachilidae (*Heriades*, *Lithurgus*, *Pseudoanthidium*, *Osmia*, *Megachile*, and *Trachusa*) on asteraceous hosts (Table 3).

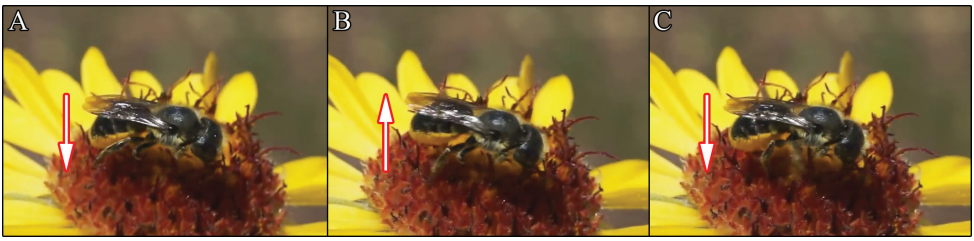

Figure 4. *Osmia* sp. tapping on Asteraceae. The directionality of the rapid movement of the abdomen is denoted by **A** down **B** up, and **C** down arrows.

Table 3. Tapping behavior in the literature. All bee species are in Megachilidae.

Genus or Species	Behavior Description	Floral Host (all Asteraceae)	Citation
Heriades spp.	"l'abdomen qui tapote de haut en bas sur les étamines" [the abdomen taps up and down on the stamens]	Asteraceae	Pasteels and Pasteels 1974
Osmia montana Cresson	"a rapid thumping movement of the abdomen"	*Helianthus*	Rust 1974
O. californica Cresson and *O. montana*	"very rapidly tapping the abdominal scopa against the composite disk anthers"	Asteraceae	Cripps and Rust 1989
O. californica	"tamp their abdomens"	*Balsamorhiza, Helianthella, Helianthus*	Williams 2003
Megachile melanopyga Costa and *M. octosignata* Nylander	"rapid up and down movements [of the abdomen]"	*Centaurea*	Müller and Bansac 2004
O. californica	"patting the flowers with their abdominal venters"	*Balsamorhiza*	Cane 2005
Heriades truncorum (L.)	"moving the abdomen rapidly up and down"	Asteraceae	Praz et al. 2008
O. californica and O. montana	"rapidly drumming or patting their abdomens up and down against the pollen-bearing floral styles"	*Balsamorhiza*	Cane 2011
O. californica, *O. coloradensis* Cresson, *O. montana*, *O. subaustralis* Cockerell, *Heriades cressoni* Michener (as *H. cressonii*)	"drumming"	Various Asteraceae	Cane 2017
Heriades crenulatus Nylander, *Lithurgus chrysurus* Fonscolombe, *Pseudoanthidium literatum* (Panzer), *Trachusa dumerlei* (Warncke)	"the rapid movement of their abdomen up and down"	*Centaurea*	Gonzalez et al. 2017; Gonzalez pers. comm.
Wainia eremoplana (Mavromoustakis)	"rapid up and down movements of the metasoma"	Asteraceae	Müller et al. 2018
Osmia sp.	Tapping with the abdominal scopa	Asteraceae	Figure 4; Suppl. material 4

Rubbing with the face

In this behavior, the face (anterior head) is used to gather pollen through direct, continuous rubbing contact with the anthers (Figure 5; Suppl. material 5). Rubbing with the face (reviewed in Müller 1996a; Thorp 2000) is found in numerous unrelated species in the families Apidae, Andrenidae, Halictidae, Megachilidae, and the wasp subfamily Masarinae (Vespidae). In most cases, the hairs on the clypeus and/or frons are thickened, hooked, or corkscrew-shaped, but they can also be unmodified (Müller 1996a; Prosi et al. 2016). Rubbing with the face is associated with the collection of pollen from nototribic flowers, which have the stamens and styles facing downwards from the dorsum of the corolla, thereby promoting contact with the dorsum of floral visitors (Müller

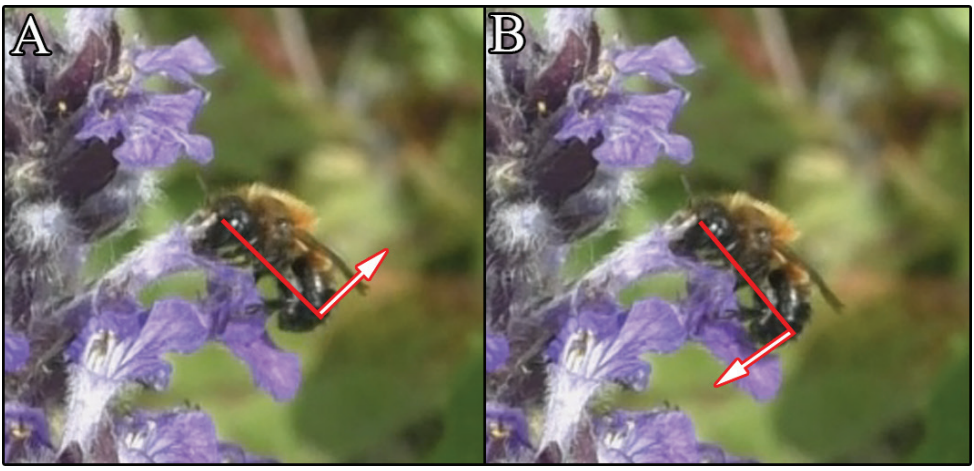

Figure 5. *Osmia pilicornis* Smith (Megachilidae) gathering pollen by rubbing with the face against the anthers of *Ajuga reptans* L. (Lamiaceae). The bee repeatedly jerks the entire body **A** up and **B** down. Image adapted from Prosi et al. (2016).

1996a; Thorp 2000). We follow the terminology of Müller (1996a), who referred to the behavior as "rubbing with the facial area" or "rubbing with the face."

Additional and recent examples of bees with modified facial pilosity that gather pollen from nototribic flowers include *Lasioglossum tropidonotum* McGinley (McGinley 1986); *Osmia brevis* Cresson, *O. cyaneonitens* Cockerell, and *O. ednae* Cockerell on *Penstemon penlandii* W.A. Weber (Plantaginaceae) (Tepedino et al. 1999); *Anthophora walteri* Gonzalez on *Salvia* (Lamiaceae) (Gonzalez et al. 2006); *O. calaminthae* Rightmyer, Deyrup, Ascher, and Griswold on *Calamintha* (Lamiaceae) (Rightmyer et al. 2011); *Megachile riyadhense* (Alqarni, Hannan, Gonzalez, and Engel) seen visiting *Blepharis* (Acanthaceae) (Alqarni et al. 2012); and *O. pilicornis* Smith on *Ajuga* and *Glechoma* (both Lamiaceae) (Prosi et al. 2016). Both *O. calaminthae* and *O. pilicornis* – two species for which detailed observations of pollen gathering are available – rapidly move their heads and bodies up and down against the anthers to remove pollen (Figure 5; Suppl. material 5; Rightmyer et al. 2011; Prosi et al. 2016). Interestingly, *O. pilicornis* also has hooked hairs on the mouthparts, which it uses to scrape pollen from flowers of *Pulmonaria* (Boraginaceae). In some cases, such as in *Rophites algirus* Pérez (Halictidae), rubbing with the face is combined with buzzing (Müller 1996a).

Rasping

Rasping is defined as rubbing anthers with the thoracic dorsum by moving the entire body in and out of the flower, causing vibrations which release pollen (Figure 6; Suppl. material 6). Rasping is a specialized behavior that has only been observed in bees foraging on *Penstemon* flowers that have partially concealed anthers with dentate tips (Table

Table 4. Rasping behavior in the literature.

Genus or species	Behavior Description	Floral Host	Citation
Pseudomasaris vespoides (Cresson) (Vespidae)	"[the anthers] rub against the thoracic surface"	*Penstemon* (Plantaginaceae)	Torchio 1974
Hymenoptera spp.	"the anthers have teeth that rasp against the back and wings of the pollinator"	*Penstemon*	Thomson et al. 2000
Osmia spp.	"the deliberate rubbing of their backs against the anthers"	*Penstemon*	Wilson et al. 2006
O. brevis Cresson	"rasping"	*Penstemon*	Cane 2014

4). Various Hymenoptera – including the pollen wasp *Pseudomasaris vespoides* (Cresson) (Vespidae), and mason bees *Osmia brevis*, *O. pentstemonis* Cockerell, and potentially other *Osmia* species – have been observed rubbing their thoracic dorsum against the dentate anthers to release the pollen (Torchio 1974; Wilson et al. 2006; Cane 2014). Rasping is associated with a roughened or punctate integument on the scutum of *P. vespoides*, which assists in vibrating the anthers (Torchio 1974; Cane 2014). Cane (2014) coined the term "rasping" due to the noise produced by the behavior. This noise was also observed by Torchio (1974), who reported the noise produced as a "clicking" sound. Interestingly, *O. brevis* also collects pollen from *Penstemon* by buzzing (Cane 2014) as well as by rubbing with the face (Tepedino et al. 1999). The different pollen gathering behaviors of *Osmia* on *Penstemon* may be related to the size of the *Penstemon* flowers, with rasping performed in larger flowers and other strategies used in smaller-flowered species (P. Wilson, pers. comm.). So far, rasping has only been observed on *Penstemon* flowers; it remains to be seen whether this pollen gathering is found in additional bee taxa or is performed on other nototribic flowers.

Incidental

When pollen accumulates on a bees body as a by-product of another behavior without any obvious deliberate pollen gathering movements related to that area, it is termed incidental (Suppl. material 7; Doull 1970; Buchmann and Shipman 1990; Thorp 2000). This pollen can either be discarded or packed into the pollen transporting structures (Hodges 1952; Doull 1970). The terms "incidental" and "passive" pollen collection have been used more or less interchangeably in the past (Thorp 2000); "incidental" has been more prevalent historically (e.g. Parker 1926; Doull 1970; Morse 1982) and "passive" has been used more often recently (e.g. Westerkamp 1996; Williams 2003). We prefer the term incidental because it is the earlier accepted term and "passive" implies that the pollen is picked up unintentionally, which is often not the case, since many bees engage in behaviors that appear to maximize the amount of pollen picked up incidentally.

Incidental pollen collection is always a secondary behavior that occurs when a bee performs a primary behavior such as nectar collecting, oil collecting, or another pollen collecting behavior. Incidental pollen gathering is further characterized by the accumu-

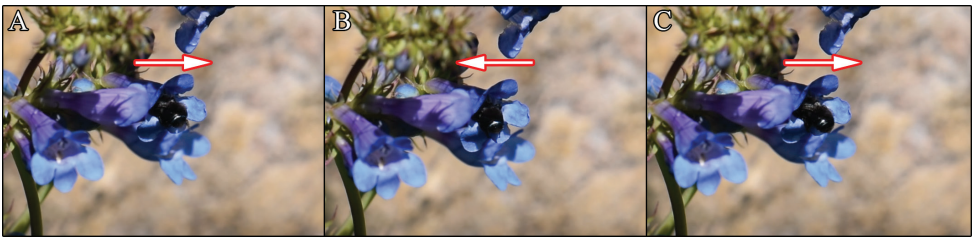

Figure 6. *Osmia* sp. (Megachilidae) performing rasping behavior on *Penstemon* (Plantaginaceae). The bee repeatedly jerks the whole body in and out to rasp the anthers, and then uses the midleg to scrape pollen from the dorsum of the thorax upon exiting the flower.

lation of pollen on generalized body hairs rather than specifically on a specialized brush or patch of hairs. This is particularly relevant when incidental buildup occurs during other primary behaviors such as rubbing or tapping, where pollen is incidentally accumulated on non-target areas. The amount of pollen actually picked up depends on the degree of contact with the anthers and the hairiness of the bee (Stavert et al. 2016). That the hairs specifically function to enhance pollen collection seems clear, since cleptoparasitic bees tend to undergo a reduction in overall hairiness along with the loss of their pollen-collecting behavior (Müller 1883; Robertson 1899; Stephen et al. 1969).

A major component of incidental pollen gathering is the degree of movement exhibited by the bee, since the act of moving over or through anthers invariably results in the incidental accumulation of pollen. The degree of movement represents a continuum, encompassing bees that move simply to reach the next anther or nectary and bees that seem to purposefully move as a means to rapidly accumulate additional pollen on their bodies. For example, on open flowers such as *Cornus* (Cornaceae), *Daucus* (Apiaceae), and *Spiraea* (Rosaceae), *Bombus* wade rapidly over flowers with their mid and hind legs and abdomen appressed in a way that maximizes the incidental collection of pollen, although the primary active mode of pollen gathering is still scraping with the forelegs (Heinrich 1976b; Morse 1982). Many different terms have been used to describe the movement of bees on flowers, e.g., "wallow," "run," "wade," "crawl," "sweep," "scramble," "walk," "scurry," "rotate," and "scrabble" (Malyshev 1936; Bohart and Nye 1960; Cross and Bohart 1960; Heinrich 1972; Houston 1975; Heinrich 1976b; Houston and Thorp 1984; Buchmann and Shipman 1990; Thorp 2000). We prefer the term "wade," since it is the earliest term to describe moving over or through a flower that also does not imply a specific speed (Bohart and Nye 1960). In addition, it accurately describes the behavior of how a bee moves both over and through a plane of floral structures.

Most bees appear to use incidental pollen collection to supplement their primary pollen gathering behaviors. For example, many groom off the incidentally accumulated pollen between flower visits and pack it into their pollen transport structures. However, some species appear to collect the majority of their pollen incidentally. For example, *Megachile fortis* Cresson gathers pollen by accumulating it on the body while foraging for nectar (Neff and Simpson 1990), while other species – including bees in the genera *Dasypoda* (Melittidae), *Panurgus* (Andrenidae), and *Protoxaea* (Andrenidae)

– "wallow" in anthers whilst nectaring to gather pollen, though these could potentially refer to rubbing with the abdomen and/or scopae (Malyshev 1936; Linsley and Cazier 1972). Incidental collection of pollen is often used by honey bees and bumble bees, which gather nectar without making any special effort to gather pollen, yet still pack accumulated pollen into the corbiculae (Suppl. material 7; Parker 1926; Hodges 1952; Heinrich 1976b; Morse 1982).

Temporary accumulation of pollen

In addition to the seven main pollen gathering behaviors, temporarily accumulating pollen on a specialized patch of hairs represents an important intermediate step in the pollen gathering behavior of many bee species (Suppl. material 8, Suppl. material 9). Traditionally, most bees are thought to gather pollen with the legs and immediately transfer it to the scopae, or, alternatively, take the pollen which has adhered to generalized body hairs and groom it into the scopae (Michener et al. 1978; Michener 2007). However, many bees perform a third method in which they temporarily accumulate large amounts of pollen onto a discrete patch of specialized hairs before transferring it to the scopae. These specialized hairs are simple (unbranched) rather than plumose and are often hooked or bent apically (Figure 7). The use of a temporary holding area does not appear to be limited to certain flowers, as bees with a temporary holding area collectively specialize on a wide array of floral families and flower types (Table 5). In addition, numerous species use a temporary holding area even though they gather pollen from open flowers such as *Sphaeralcea* (Malvaceae) or *Prosopis* (Fabaceae), meaning that a temporary holding area is not an adaptation to flowers that restrict pollinator movement and make it impossible to immediately transfer pollen to the scopa.

Temporarily accumulating pollen in a specialized hair patch is well-documented in Panurginae (Table 5) and appears to be used by a majority of species in the subfamily. Based on observations on species in the genera *Perdita* and *Macrotera* (Andrenidae), pollen is initially gathered by scraping with the forelegs and mandibles, which immediately load it into the discrete holding area on the venter of the thorax after each scrape. Once a sufficient quantity of pollen has accumulated in the holding area (which typically covers most of the thoracic venter), the forelegs transfer it to the scopae via the midlegs (e.g. Suppl. material 8; Eickwort 1977; Norden et al. 2003).

In addition to panurgines, other bee groups temporarily accumulate pollen but the behavior is not as well-documented. *Trigona* (Apidae) also load the specialized hair patch on the thoracic venter with the forelegs, but appear to transfer pollen to the corbiculae while hovering (Wille 1963; Michener et al. 1978; Renner 1983). Bees in the genus *Macropis* (Melittidae) also accumulate pollen on their venter, but use specialized corkscrew-shaped hairs that cover nearly their entire ventral body surface (Figure 3G–H; Suppl. material 9; Cane et al. 1983; Vogel 1992; Schäffler and Dötterl 2011). However, in this group, the pollen is loaded directly into the temporary holding area via rubbing with the thorax and abdomen. This behavior may have arisen in *Macropis*

Table 5. Panurgine (Andrenidae) bees documented to temporarily accumulating pollen.

Genus or species	Floral host	Citation
Anthemurgus passiflorae (Robertson)	*Passiflora* (Passifloraceae)	Neff and Rozen 1995; Neff 2003a
Calliopsis subalpina Cockerell	*Sphaeralcea* (Malvaceae)	Suppl. material 8
Macrotera mortuaria (Timberlake)	*Arctomecon* (Papaveraceae)	Suppl. material 8
M. opuntiae (Cockerell)	*Opuntia* (Cactaceae)	Bennett and Breed 1985
M. texana Cresson	*Opuntia*	Neff and Danforth 1991
Panurginus polytrichus Cockerell	Polylectic	Neff 2003b
Perdita floridensis Timberlake	*Ilex* (Aquifoliaceae)	Norden et al. 2003
P. gerhardi Viereck	*Monarda* (Lamiaceae)	Miliczky 1991
P. halictoides Smith	*Physalis* (Solanaceae)	Sullivan 1984
P. minima Cockrell	*Euphorbia* (Euphorbiaceae)	Suppl. material 8
P. multiflorae Parker	*Mentzelia* (Loasaceae)	Suppl. material 8
P. octomaculata (Say)	*Solidago* (Asteraceae)	Eickwort 1977
P. sphaeralceae Cockerell	*Sphaeralcea*	Suppl. material 8
P. spp.	*Prosopis* (Fabaceae)	Simpson et al. 1977
Rhophitulus anomalus (Moure and Lucas de Oliveira) [as *Cephalurgus anomalus*]	Malvaceae	Gaglianone 2000

because the legs are simultaneously occupied by dabbing up oil from the flowers (Vogel 1992). The presence of modified hair patches of hooked or corkscrew-shaped hairs on the venter of other bees whose pollen gathering behavior is unknown, e.g. *Monoeca* (Apidae) and various anthiidine bees (Müller 1996b; Michener 2007; Torretta and Roig-Alsina 2016), suggests that this method of temporarily accumulating pollen may occur in a wider variety of bee groups than is generally reported.

Finally, temporarily accumulating pollen in genal hair baskets has been observed in some bee groups, but it is not clear whether these baskets are loaded by directly scraping against pollen or are loaded with the forelegs. This is seen in *Perdita* subgenus *Heteroperdita*, specialists on *Tiquilia* (Boraginaceae) (Portman et al. 2016); *Hesperapis laticeps* Crawford, a specialist on *Mentzelia* (Suppl. material 9); and both species of *Xeralictus* (Halictidae) – *X. bicuspidariae* Snelling and Stage and *X. timberlakei* Cockerell – also specialists on *Mentzelia* (Snelling and Stage 1995).

Flexibility in pollen gathering behavior

The pollen gathering behaviors of bees are not rigid, stereotyped actions performed in isolation. Instead, bees combine different behaviors, mixing and matching them depending on their behavioral repertoires and the morphology of their host plants. The use of different behaviors on flowers with different morphologies has been well-demonstrated in *Apis* and *Bombus*, which will switch between collecting pollen either actively via scraping or incidentally depending on the floral host (Parker 1926; Heinrich 1976b). Another example is found in *Osmia pilicornis*, which uses the proboscis to gather pollen from *Pulmonaria* and rubs with the face on flowers of *Ajuga* and *Glechoma* (Prosi et al. 2016). Using different behaviors on different hosts is also found in specialist bees, such as the Asteraceae specialist *O. montana*, which taps for pollen on most asteraceous flowers, but scrapes with its legs on *Taraxacum* (Cane 2017). More impressively, *O. brevis*

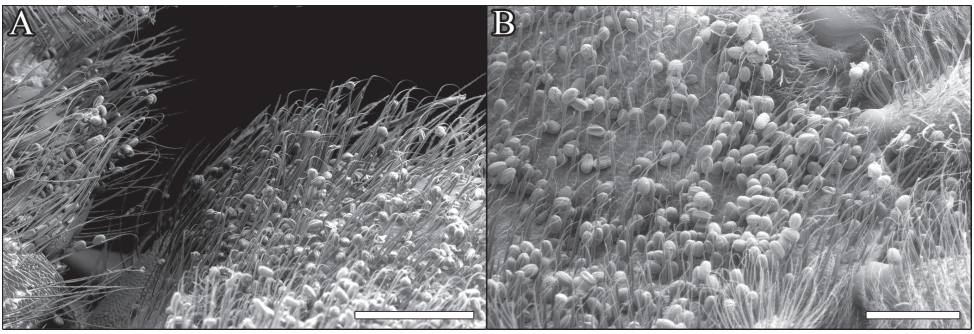

Figure 7. Specialized hairs on the venter of the thorax of **A** *Perdita turgiceps* Timberlake (Andrenidae) with specialized hairs on the fore-coxae (left) and ventral mesepisternum (right) and **B** *Protandrena maculata* Timberlake (Andrenidae) with specialized hairs on the ventral mesepisternum (left) and hind-coxae (far right). Specimens are positioned belly-up, with the head to the left. Scale bars: 200 micrometers.

uses three distinct behaviors when gathering pollen from *Penstemon* – rasping, buzzing, and rubbing with the face (Tepedino et al. 1999; Cane 2014). Another example is seen in *Tetraglossula bigamica* (Colletidae) a specialist on *Ludwigia* (Onagraceae); when anthers are closed, the bees open them with the mandibles and gather pollen by scraping with the fore- and mid-legs. However, when anthers are open, the bees gather pollen by rubbing the scopa directly against them (Gimenes 1997).

In addition to using different behaviors on the same or different hosts, many bees perform multiple pollen gathering behaviors simultaneously. For example, *Andrena helianthi* on *Helianthus* (Figure 3E–F; Suppl. material 3) simultaneously scrapes pollen with the forelegs and rubs the abdomen and scopa against the anthers, and *Osmia* sp. on Asteraceae (Figure 4; Suppl. material 4) taps with the abdomen while at the same time probing for nectar and incidentally picking up pollen on the face and body, which is subsequently groomed into the scopa during flight.

While some bees display flexibility in pollen gathering behavior, others have more limited behavioral suites. One might expect generalist bees to have a broader suite of pollen gathering behaviors than specialist bees. Social bees in particular are expected to be more versatile in their pollen gathering behavior compared to solitary bees because their colonies are active for longer periods and must therefore utilize a broad array of successively blooming plants (Heinrich 1976b). However, this hypothesis does not appear to be well-supported; instead of having a broad breadth of behaviors, many generalists and social bees have relatively limited behavioral repertoires and instead use variations on a small suite of basic behaviors to collect pollen from a variety of different host plants. For example, species in the highly generalist genus *Agapostemon* only use three behaviors: scraping with the extremities, buzzing, and incidental collection (Roberts 1969). Further, *Apis mellifera* and *Trigona* spp. – likely the most generalized bees – are even more restricted, since they do not buzz flowers (Wille 1963; Buchmann 1983). In contrast, the solitary specialist bee *O. brevis* has a much broader suite of behaviors, including scraping, buzzing, face rubbing, rasping, and incidental pollen collection (Tepedino et al. 1999; Cane 2014).

Ecological and evolutionary implications

The repertoire of pollen gathering behavior of bee species may influence their floral host choices. For example, some specialists have limited ability to collect pollen from alternative hosts even though their larvae can develop on alternative pollen sources (Cripps and Rust 1989; Williams 2003). This is particularly apparent in Megachilidae; for example, *Hoplitis anthocopoides* (which rubs directly with the scopa) only gathered pollen from *Echium* (Boraginaceae) and refused to gather pollen from either the related *Anchusa* (Boraginaceae) or alternative hosts in eight other plant families (Strickler 1979). In addition, the Asteraceae specialist *Heriades truncorum* (which taps with the scopa) refused to gather pollen from *Echium* (Praz et al. 2008). Similarly, Williams (2003) observed that the Asteraceae specialist *Osmia californica* (which taps with the scopa) refused to gather either *Phacelia* (Boraginaceae) or *Brassica* (Brassicaceae) pollen when it was the only host present, but, in an interesting twist, did gather some *Phacelia* pollen when its normal asteraceous host was also present. When *O. californica* gathered *Phacelia* pollen, it used the same tapping behavior it used on its normal Asteraceae host, but its attempts were clumsy and inefficient, particularly compared to the more generalized *O. lignaria* (Williams 2003). These examples support the hypothesis that the specialized behaviors of these bees (rubbing or tapping) mean that they are either incapable of gathering non-host pollen or can do so only inefficiently.

In contrast, some other specialist bees readily gather pollen from alternative hosts, particularly in times of pollen shortage (Linsley and MacSwain 1958). For example, an individual of *Diadasia australis* (Cresson) (Apidae), whose normal host is *Opuntia* (Cactaceae), was observed gathering pollen from *Phacelia* after the local cactus blooms were exhausted (Linsley and MacSwain 1958). Another *Diadasia* cactus-specialist, *D. rinconis* Cockerell, was experimentally induced to gather pollen from *Sphaeralcea*, but only after nesting was initiated by the temporary introduction of *Opuntia*, the preferred host (McIntosh 2001). Similarly, *Hesperapis pellucida* Cockerell (Melittidae) was observed gathering pollen from *Gilia capitata* Sims (Polemoniaceae) and other flowers when its normal host, *Eschscholzia californica* Cham. (Papaveraceae), was mowed (Stage 1966). When the normal host recovered and flowered a month later, the bees switched back to gathering pollen from it. *Andrena erythronii* Robertson gathers pollen from *Erythronium* (Liliaceaea) while it is in bloom, but readily switches to other sources once bloom has ended (Michener and Rettenmeyer 1956). Additional *Andrena* species, specialized on *Nemophila*, have also been documented switching to alternative pollen sources when host pollen was in short supply (Cruden 1972). Finally, Thorp (1969) reported multiple species of *Andrena* subgenus *Diandrena* gathering pollen from non-preferred hosts, particularly in times of pollen shortage. All of these bees that readily switch hosts apparently gather pollen primarily by scraping with the forelegs; this generalized pollen gathering behavior could allow them to more easily exploit non-preferred hosts.

Evidence suggests that there is a relationship between pollen gathering behavior and host plant preference. For example, rasping and rubbing with the face are associated with nototribic flowers (Müller 1996a; Table 4), and tapping is associated with

specialization on Asteraceae (Table 3). Similarly, in non-megachilid bees, rubbing with the body and/or scopae is repeatedly associated with floral hosts in the families Asteraceae, Malvaceae, and Onagraceae (Table 2). Plants in those families tend to have more adhesive pollen, either due to sticky viscin threads or spiny pollen with copious pollenkitt (Linsley et al. 1973; Williams 2003; Lunau et al. 2014; Portman and Tepedino 2017). The increased adhesiveness of these pollens may have facilitated the evolution of novel behaviors that take advantage of these pollen properties to efficiently uptake pollen. Particularly if bees have behavioral or morphological adaptations to certain floral or pollen morphologies, this could help explain broad-scale evolutionary patterns of floral host use in bees. This pattern has been repeatedly noted in bee species groups adapted to large or coarse pollen such as in the genera *Diadasia*, *Macrotera*, and *Eucera* (Michener 1944; Linsley and MacSwain 1958; Danforth 1996; Dorchin et al. 2018).

Although some behaviors are associated with particular host plants or floral types, the diversity of pollen gathering behaviors on a given host plant make clear that there is not necessarily a "right way" to gather pollen from a particular host plant. For example, on *Helianthus*, honey bees gather pollen incidentally while nectaring, generalist *Lasioglossum* gather pollen by scraping with the forelegs, *Osmia californica* gather pollen by tapping with the scopae, and *Melissodes* gather pollen by rubbing with the abdomen and scopae. These behavioral differences could potentially be explained by a number of reasons. For example, specialist behaviors such as tapping could be more efficient at particular hosts but less efficient at gathering from alternative hosts (Williams 2003). Conversely, more generalized behaviors could represent a compromise that allows for less efficient utilization of a wider variety of hosts. Lastly, different behaviors on the same host could represent different ecological niches, with some behaviors enabling bees to rapidly skim off accessible pollen while leaving the less accessible pollen untapped, whereas other behaviors could allow bees to meticulously gather the remaining pollen (Simpson et al. 1977).

Conclusion and future directions

Pollen gathering behavior in bees is a complex process that involves the mixing and matching of different behaviors depending on the behavioral repertoire of a given bee species and the floral morphology of host plants. Despite this complexity, pollen gathering can be broken down into two broad categories, "active" and "incidental," with active pollen gathering further divided into six subtypes. In addition, there is an intriguing intermediate step found in disparate groups that involves temporary accumulation of pollen on a discrete patch of specialized hairs. It is our hope that this updated classification of pollen gathering behavior will enable effective communication and comparison of future research, particularly given the rise of low cost, high definition video recording devices.

Despite the abundance of behavioral observations, the breadth and flexibility of pollen gathering behaviors remain poorly understood. Learning more about the behavior

of specific species can help shed light on the evolution of pollen gathering, particularly how behaviors such as rubbing with the abdomen and/or scopa(e), tapping, and rasping evolved and whether they are consistently associated with specific hosts or floral morphologies. Further, it is not clear why some specialist bees have broad behavioral flexibility, while others appear to have much more rigid repertoires. Similarly, it's not clear why some generalists have a smaller breadth of behaviors, particularly the apparent inability of *Apis* and *Trigona* to buzz flowers. Examining the tradeoffs between behavioral breadth and pollen gathering efficiency, as well as the genetic and physiological bases of these behavioral limitations, could shed light on these questions. Towards this end, a comprehensive dataset of the pollen-gathering behaviors of different bee species is needed.

One of the biggest unanswered questions is whether specialized behaviors are more effective at gathering pollen, either by increasing the efficiency of pollen uptake or by allowing bees to perform more than one behavior simultaneously, such as gathering pollen and nectar simultaneously (Strickler 1979). It seems probable that specialized behaviors (e.g. rubbing with the body/scopae or tapping) are more efficient than generalized behaviors (e.g. scraping with the forelegs) on the same host, but this largely remains to be experimentally tested. Conversely, more work is needed to understand whether pollen gathering behavior limits the floral hosts that bees can use.

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Supplementary material 1

Scraping with the extremities

Authors: Zachary M. Portman, Michael C. Orr, Terry Griswold

Data type: multimedia

Link: https://doi.org/10.3897/jhr.71.32671.suppl1

Supplementary material 2

Buzzing

Authors: Zachary M. Portman, Michael C. Orr, Terry Griswold

Data type: multimedia

Link: https://doi.org/10.3897/jhr.71.32671.suppl2

Supplementary material 3

Rubbing with the body and/or scopae

Authors: Zachary M. Portman, Michael C. Orr, Terry Griswold

Data type: multimedia

Link: https://doi.org/10.3897/jhr.71.32671.suppl3

Supplementary material 4

Tapping
Authors: Zachary M. Portman, Michael C. Orr, Terry Griswold
Data type: multimedia

Link: https://doi.org/10.3897/jhr.71.32671.suppl4

Supplementary material 5

Rubbing with the face
Authors: Zachary M. Portman, Michael C. Orr, Terry Griswold
Data type: multimedia

Link: https://doi.org/10.3897/jhr.71.32671.suppl5

Supplementary material 6

Rasping
Authors: Zachary M. Portman, Michael C. Orr, Terry Griswold
Data type: multimedia

Link: https://doi.org/10.3897/jhr.71.32671.suppl6

Supplementary material 7

Incidental pollen gathering
Authors: Zachary M. Portman, Michael C. Orr, Terry Griswold
Data type: multimedia

Link: https://doi.org/10.3897/jhr.71.32671.suppl7

Supplementary material 8

Temporary accumulation of pollen by panurgine bees
Authors: Zachary M. Portman, Michael C. Orr, Terry Griswold
Data type: multimedia

Link: https://doi.org/10.3897/jhr.71.32671.suppl8

Supplementary material 9

Temporary accumulation of pollen by melittid bees
Authors: Zachary M. Portman, Michael C. Orr, Terry Griswold
Data type: multimedia

Link: https://doi.org/10.3897/jhr.71.32671.suppl9

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RESEARCH ARTICLE

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Three new species of the genus *Zethus* Fabricius, 1804 (Hymenoptera, Vespidae, Eumeninae) from China, with an updated key to the Oriental species

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Abstract

Three new species, namely *Zethus striatus* **sp. nov.**, *Z. asperipunctatus* **sp. nov.** and *Z. nullimarginatus* **sp. nov.** from China are described and illustrated. *Z. tumidus* Nguyen & Carpenter, 2016 and *Z. angulatus* Nguyen & Carpenter, 2016 are newly recorded from China. An updated key to the Oriental species of the genus is provided.

Keywords

Zethus, Eumeninae, China, new species, new records

Introduction

Zethus Fabricius, 1804 is the most species-rich genus within the subfamily Eumeninae with 275 valid species, containing four subgenera: *Madecazethus* Giordani Soika, *Zethoides* Fox, *Zethus* Fabricius and *Zethusculus* Saussure, among

which *Madecazethus* is endemic to Madagascar with two species, both *Zethoides* and *Zethusculus* are restricted to the Neotropical and Nearctic regions, and the subgenus *Zethus* Fabricius is widely distributed worldwide excluding the Australian region (Bohart and Stange 1965; Giordani Soika 1979; Gusenleitner 2011; Pannure et al. 2016, 2018; Yeh and Lu 2017; Hermes and Lopes 2018; Tan et al. 2018). So far, there are 26 described species in the Oriental region. The first review on the Oriental *Zethus* concluding a key to six recognized species was given and then an updated key to eight species was provided (Giordani Soika 1941, 1958). In the following 60 years, eighteen more species were found and described, respectively (Giordani Soika 1995; Gusenleitner 1988, 2001, 2007, 2010; Gusenleitner and Gusenleitner 2013; Nguyen and Carpenter 2016, Nguyen and Xu 2017; Selis 2017, 2018; Yeh and Lu 2017; Tan et al. 2018). In our study of the genus *Zethus*, we systematically sorted out all of the references to the Oriental species. Based on the references and the specimens which were long-term collection throughout China by us and other Chinese museums, a key to the Oriental species are updated and three species are new to science. In addition, both *Z. tumidus* Nguyen & Carpenter and *Z. angulatus* Nguyen & Carpenter are recorded firstly from China.

Materials and methods

The pinned specimens examined were collected by using an insect net. Those specimens are deposited in Chongqing Normal University, Chongqing, China (CQNU), Central South University of Forestry and Technology, Changsha, China (CSUFT), Museum of Hebei University, Baoding, China (MHBU), Shanghai Entomological Museum C.A.S, Shanghai, China (SEM). Descriptions and measurements were made under a stereomicroscope (Olympus SZ61). All figures were taken with Keyence VHX-5000 digital microscope and Photoshop CS 6 was used to make the plates. Body length was measured from the anterior margin of the head to the posterior margin of metasomal tergum 2. For the density description of punctures, "sparse" means that distance is larger than punctures diameter and "dense" means less than the diameter. Terminology principally follows Bohart and Stange (1965) and Yamane (1990). The abbreviations used in this paper are shown as follows:

AE broadened apical expansion of metasomal sternum 1
BS slender basal stem of metasomal sternum 1
A1 for antennal segment 1,
A2 for antennal segment 2,
T1 for metasomal tergum 1,
T2 for metasomal tergum 2,
S1 for metasomal sternum 1,
S2 for metasomal sternum 2, and so on.

Taxonomy

Zethus Fabricius, 1804

Zethus Fabricius, 1804: xii, 282. **Type species**: "*Zethus coeruleo-pennis* Fab." [= *Vespa coeruleopennis* Fabricius, 1798], by subsequent designation of Latreille, 1810: 328, 438.

Zethus striatus Wang & Li, sp. nov.

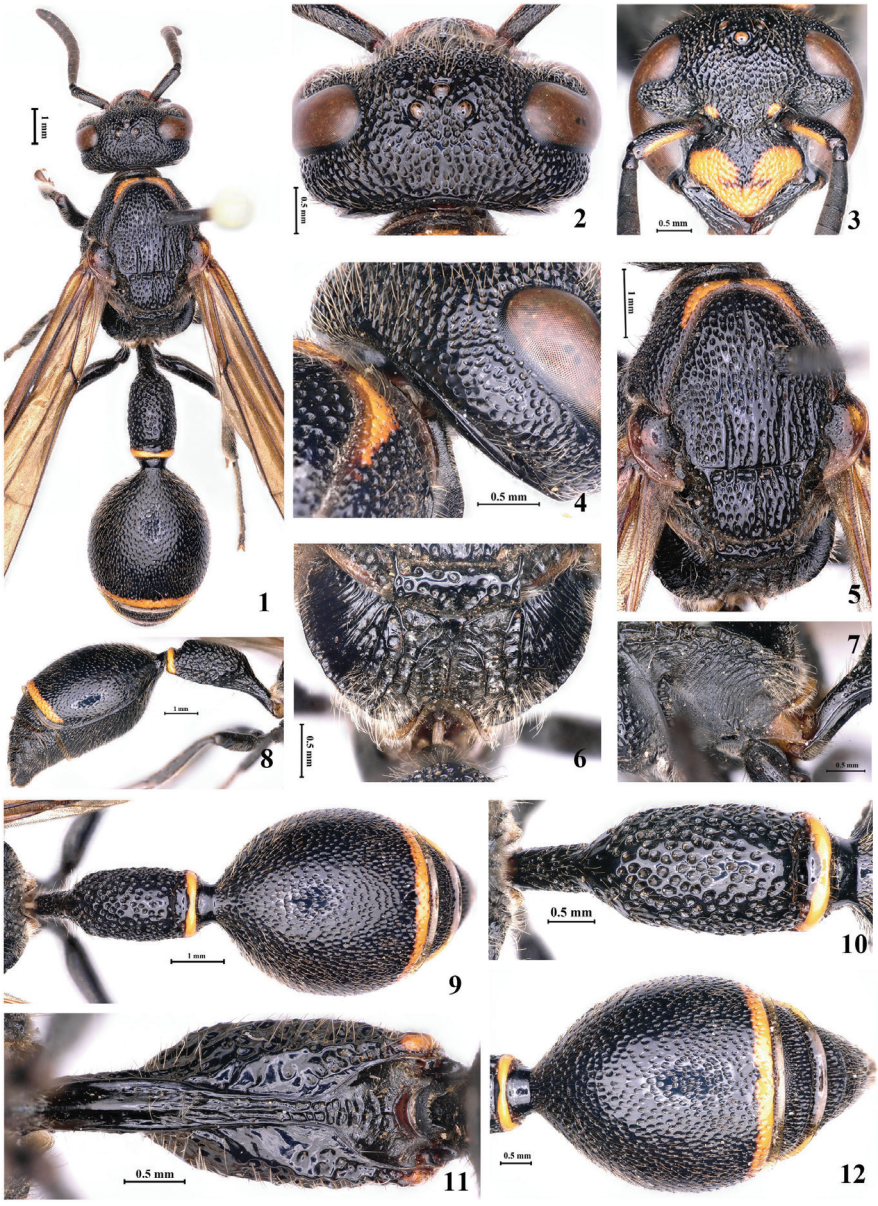
http://zoobank.org/AEB232FA-2A87-4419-8189-F50AFD58276E
Figs 1–12

Material examined. Holotype, ♀: CHINA, Guangxi, Baise City, Tianlin County, CengWangLaoShan National Nature Reserve, Dalongping, 24°29.361'N, 106°24.076'E, 24–25.VIII.2007, Xiang Li leg. (CQNU).

Description. Female: body length 15.3 mm (Fig. 1), fore wing length 13.7 mm. Black, the following parts yellow: clypeus except base and central middle, spot on dorso-inner margin of antennal socket, antennal scape ventrally, paired transverse middle spots on dorsal side of pronotum, apical bands on T1 and T2; tegula dark brown (Fig. 5).

Head. Head with short setae, their length slightly longer than posterior ocellar diameter; head about 2.1× wider than long in dorsal view (Fig. 2), wider than high in frontal view, about 1.2× as wide as high (Fig. 3); mandible with four blunt teeth and short setae; clypeus convex in lateral view and about 1.3× as wide as high in frontal view, with basal margin nearly straight, apical margin of clypeus frontally truncated and laterally cambered, clypeus punctate-reticulate with sparse setae (Fig. 3); frons with sparse setae and dense punctures; vertex and gena with dense punctures; gena in lateral view as wide as eye, without longitudinal carina; distance from posterior ocelli to apical margin of vertex 1.7× as long as distance from posterior ocelli to inner eye margin (Fig. 2); occipital carina developed laterally and weak dorsally (Fig. 4); antennal scape 3× as long as its maximum width, A3 1.4× as long as its maximum width, A4 as long as its maximum width, A5–11 wider than long, A12 bullet-shaped, as long as its basal width.

Mesosoma. Length of mesosoma about 1.3× as long as wide in dorsal view (Fig. 5); pronotal carina weak laterally and indistinct dorsally, pronotum with dense punctures and slightly reticulate dorsally; notaulix distinct on basal half of mesoscutum, mesoscutum with deep and dense punctures, area between punctures striate, in lateral view weakly convex; tegula smooth, with very sparse punctures, its posterior lobe triangular and well developed which exceeds parategula posteriorly; epicnemial carina distinct; mesopleura with deep and dense punctures, slightly sparse ventrally; scutellum flattened, with deep and dense punctures, area between punctures striate, longitudinal furrow on the middle of scutellum (Fig. 5); metanotum sloping down posteriorly, with deep punctures; metapleuron almost smooth; propodeum (Fig. 6) with strong



Figures 1–12. *Zethus striatus* sp. nov., holotype **1** habitus in dorsal view, ♀ **2** vertex, ♀ **3** head in frontal view, ♀ **4** vertex in dorsal-lateral view, ♀ **5** mesosoma, ♀ **6** propodeum in dorsal view, ♀ **7** propodeum in lateral view, ♀ **8** metasoma in lateral view, ♀ **9** metasoma in dorsal view, ♀ **10** T1, ♀ **11** S1, ♀ **12** T2, ♀.

striae and lateral carina, its dorsolateral surface with distinct and oblique striae and strong longitudinal carina along submedian carina, posterior surface of propodeum with transverse striae along median carina, submarginal carina produced into lamella above propodeal valvulae, its lateral surface with punctures along lateral carina and weak striae on its lower half (Fig. 7); orifice more or less angled dorsally (Fig. 6).

Metasoma. T1 about 2.5× as long as wide in dorsal view, gradually widening from one-third of the base, then nearly parallel-sided apically, with maximum width 3× its basal width, without medial carina, T1 with deep and dense macropunctures and sparse setae (Fig. 10), lateral carina of T1 present in ventral view (Fig. 11); BS of S1 with median longitudinal carina and narrow from its middle to apex, coarsely punctate on apical margin of BS and base half of AE (Fig. 11); T2 without petiole and with developed apical lamella, about 1.2× as long as wide in dorsal view, T2 with deep and dense punctures (Fig. 12); S2 swollen from the base to near midpoint, subsequent portion nearly straight in profile (Fig. 8), its apical margin with lamella; T3 with developed and raised apical lamella (Figs 8, 9, 12); subsequent terga and sterna with deep punctures, but slightly smaller than those on T2.

Male. Unknown.

Distribution. China: Guangxi.

Remarks. The species is similar to *Z. trimaculatus* Cameron, 1904 from Vietnam, Laos and India by the characters: body with dense punctures (Fig. 1), occipital carina developed laterally and weak dorsally (Fig. 4), pronotal carina weak laterally and indistinct dorsally (Fig. 5), S2 not tuberculate in profile (Fig. 8). It differs from *Z. trimaculatus* and all other members of the genus by the following character combination: apex of clypeus yellow, apical margin of clypeus frontally truncated (Fig. 3) and laterally cambered, BS of S1 with longitudinal carina (Fig. 11), propodeum with strong lateral carina and its dorsolateral and posterior surface with strong striae (Fig. 6).

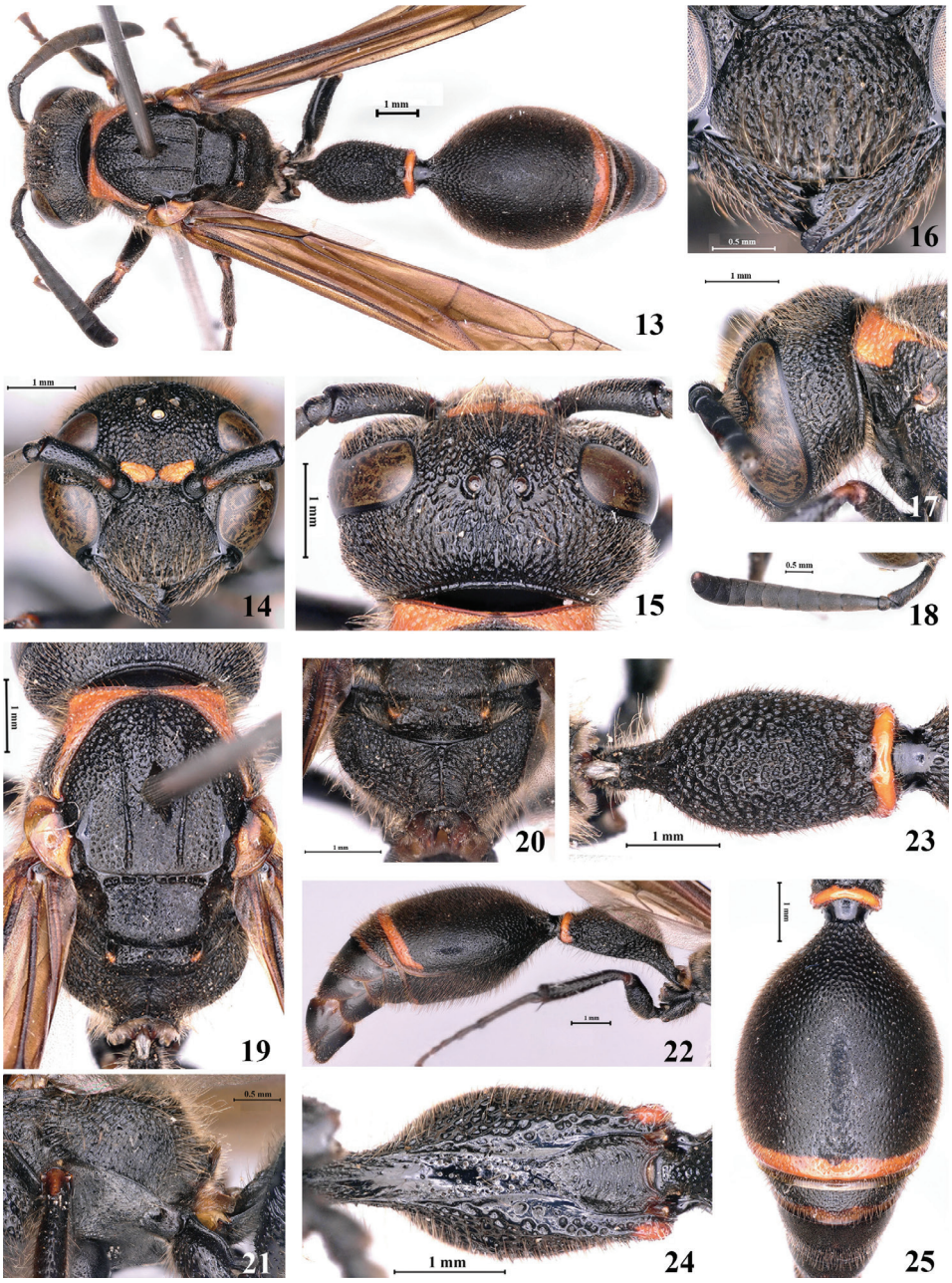
Etymology. The specific name *striatus* is derived from Latin word: *striatus*, referring to propodeum with strong striae in dorsal view.

***Zethus asperipunctatus* Wang & Li, sp. nov.**
http://zoobank.org/5CB359C4-C337-461C-8224-A42D274B3BD5
Figs 13–25

Material examined. Holotype, ♀: CHINA, Yunnan, Lijiang City, Yulong County, Hutiaoxia, 27°22.953'N, 99°53.157'E, 7.VI.2009, Meicai Wei leg. (CSUFT).

Description. Female: body length 16.2 mm (Fig. 13), fore wing length 13.7 mm. Black, the following parts ferruginous: marking on dorso-inner margin of antennal socket, antennal scape lower area ventrally, pronotum in dorsal view, tegula, dot on lateral of metanotum, apical bands on T1, T2, T3 and middle of S2 (Fig. 13).

Head. Head with long setae, their length distinctly longer than 2× posterior ocellar diameter; head about 1.3× as wide as high in frontal view (Fig. 14), about 1.7× wider than long in dorsal view (Fig. 15); mandible with four blunt teeth and dense setae, its outer surface with coarse punctures; clypeus convex in lateral view and about 1.3× as wide as high in frontal view, with basal margin almost straight, minutely bi-dentate apically, depressed space between teeth with a median carina, width of truncation 1/3× width of clypeus between inner eye margins, clypeus with dense and long setae and dense punctures (Fig. 16); frons with long setae and dense punctures; vertex and gena with dense punctures; gena slightly wider than eye in lateral view and without longi-



Figures 13–25. *Zethus asperipunctatus* sp. nov., holotype **13** habitus in dorsal view,♀ **14** head in frontal view, ♀ **15** vertex, ♀ **16** clypeus, ♀ **17** gena, ♀ **18** antenna, ♀ **19** mesosoma, ♀ **20** propodeum in dorsal view, ♀ **21** propodeum in lateral view, ♀ **22** metasoma in lateral view, ♀ **23** T1, ♀ **24** S1, ♀ **25** T2, ♀.

tudinal carina (Fig. 17); distance from posterior ocelli to apical margin of vertex 1.7× as long as distance from posterior ocelli to inner eye margin (Fig. 15); occipital carina complete (Fig. 15); antennal scape 2.7× as long as its apical width, A3 1.8× as long as

its maximum width, A4 slightly longer than its maximum width, A5–11 wider than long, A12 bullet-shaped, as long as its basal width (Fig. 18).

Mesosoma. Length of mesosoma about 1.3× as long as wide in dorsal view (Fig. 19); pronotal carina complete, pronotum with distinct humeral angle and reticulate punctures dorsally; notaulix complete, mesoscutum with dense punctures, gradually shallow from the anterior to posterior portion; tegula smooth, with dense and short setae on its anterior and posterior lobe; epicnemial carina distinct; mesopleura with deep and dense punctures; scutellum flattened, and with shallow punctures and middle longitudinal furrow almost invisible, distance between punctures about as wide as punctures diameter; punctures on metanotum like those on scutellum, but deeper; metapleuron almost smooth; propodeum dull, with lateral carina and without submedian carina, its dorsolateral surface with reticulate striae (Fig. 20), submarginal carina produced into lamella above propodeal valvulae, lateral surface of propodeum with sparse and fine punctures (Fig. 21); orifice angled dorsally (Fig. 20).

Metasoma. T1 about 2.2× as long as wide, with medial carina from basal margin to one-third of the tergum, gradually widening from one-fourth of the base, then narrowly toward apex, with maximum width about 3.3× its basal width, T1 with coarse and dense punctures (Fig. 23) and lateral carina of T1 present wholly (Fig. 24); BS of S1 without longitudinal carina and with sparse punctures, lateral portion of AE with weak striae and sporadic punctures (Fig. 24); T2 without distinct petiole, gradually swollen from the base to midpoint and nearly straight to apex in lateral view, with developed apical lamella, about 1.3× as long as wide in dorsal view, T2 with dense punctures, which gradually sparse from the base to apex, distance between punctures on the base distinctly less than its diameter and 1–2× larger than puncture diameter on apical margin (Fig. 25); S2 swollen from the base to near midpoint, subsequent portion nearly straight in profile (Fig. 22), punctures on the base of S2 large, punctures on subsequent portion like those on T2, but punctures on lateral portion dense, apical margin of S2 with lamella, T3 and S3 with dense and small punctures, T3 with thick apical lamella, and subsequent terga and sterna with small punctures, distance between punctures about as wide as its diameter.

Male. Unknown.

Distribution. China: Yunnan.

Remarks. This new species resembles *Z. nigerrimus* Gusenleitner, 2001 from China, Malaysia, Laos and Vietnam, with which it has the following common characters: clypeus minutely bi-dentate apically, depressed space between teeth with a median ridge (Fig. 16), T1 about 2.2× as long as wide (Fig. 23), T2 without distinct petiole (Fig. 25), S2 not tuberculate in profile (Fig. 22). It differs from *Z. nigerrimus* and all other members of the genus by the following character combination: body with ferruginous marking (Fig. 13), gena without carina (Fig. 17); BS of S1 with sparse punctures (Fig. 24), S2 blunt angulate in profile (Fig. 22), and propodeum and metasoma dull and with dense setae and punctures (Figs 13, 21, 22).

Etymology. The specific name *asperipunctatus* is derived from two Latin words: *asper* and *punctatus*, referring to metasoma dull and with dense punctures.

***Zethus nullimarginatus* Wang & Li, sp. nov.**
http://zoobank.org/AF713936-6F3B-48B5-9B43-1DA9BE727159
Figs 26–35

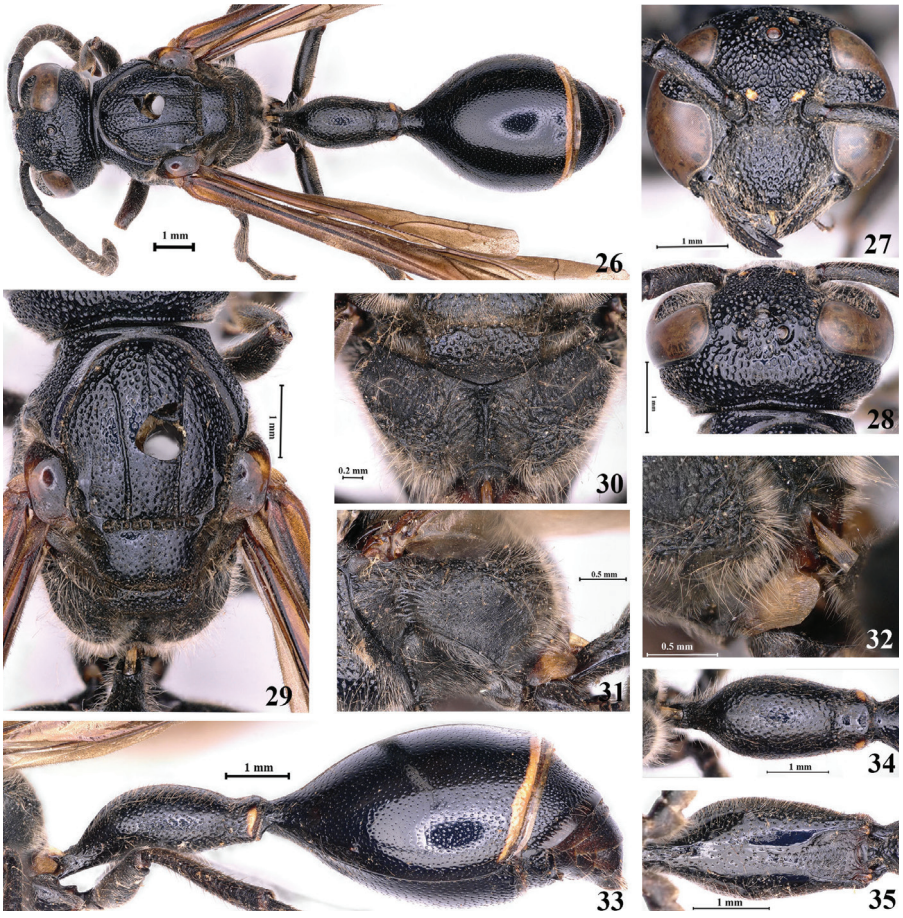
Material examined. Holotype, ♀: CHINA, Fujian, Longyan City, Changting County, Sidu Town, 25°39.126'N, 116°12.253'E, 1.V.1951, Gentao Jin & Yangming Lin leg. (SEM).

Description. Female: body length 15.2 mm (Fig. 26), fore wing length 13.5 mm. Almost black, the following parts yellow: spot on dorso-inner margin of antennal socket (Fig. 27), apical interrupted band on T1, apical complete band on T2; tegula dark brown (Fig. 29).

Head. Head wider than high, about 1.3× as wide as high in frontal view (Fig. 27), about 1.8× wider than long in dorsal view (Fig. 28); mandible with four teeth and dense setae, its outer surface with irregular punctures; clypeus convex in lateral view and about 1.6× as wide as high, with basal margin straight, apical margin near truncated, width of truncation 1/3× width of clypeus between inner eye margins, clypeus punctate-reticulate with dense long setae (Fig. 27); punctures on frons reticulate; vertex and gena with dense punctures; gena without longitudinal carina in lateral view; distance from posterior ocelli to apical margin of vertex 1.8× as long as distance from posterior ocelli to inner eye margin (Fig. 28); occipital carina complete (Fig. 28); antennal scape 2.8× as long as its maximum width, A3 1.7× as long as its maximum width, A4 slightly longer than its maximum width, A5–11 wider than long, A12 bullet-shaped, slightly longer than its basal width.

Mesosoma. Length of mesosoma about 1.4× as long as wide in dorsal view (Fig. 29); pronotal carina complete and developed, pronotum with coarse punctures dorsally, weak striae in lower lateral surface; notaulix complete, mesoscutum with dense punctures, gradually shallow from the anterior to posterior portion (Fig. 29); tegula smooth, with dense and short setae on its anterior and posterior lobe, its posterior lobe truncated (Fig. 29); mesopleura with dense punctures, slightly sparse ventrally; scutellum flattened, and with shallow punctures and weak middle longitudinal furrow; punctures on metanotum dense and coarse; metapleuron smooth; propodeum dull, with coarse surface in dorsal view and lateral carina very weak, without submedian carina (Fig. 30), its lateral surface with sporadic punctures and without striae (Fig. 31), submarginal lamella almost absent above propodeal valvulae (Fig. 32).

Metasoma. T1 about 2.5× as long as wide, with medial carina from basal margin to one-fourth of tergum, gradually widening from one-fifth of the base, then distinctly narrow toward apex, with maximum width 3× its basal width, T1 with punctures, distance between punctures about equal to the diameter (Fig. 34), lateral carina of T1 disappeared ventrally (Fig. 35); BS of S1 with sparse punctures, AE of S1 with weak and irregular striae and sporadic punctures (Fig. 35); T2 gradually swollen from the base to apical margin in lateral view, with developed apical lamella, about 1.2× as long as wide in dorsal view, T2 with sparse punctures, area between punctures with fine punctures, distance between punctures 1–2× larger than puncture diameter (Fig. 33); S2 gradually swollen from basal to apical margin in profile (Fig. 33); punctures on S2 slightly larger than those on T2, punctures on center area of S2 sparse, S2 with apical lamella; T3 with



Figures 26–35. *Zethus nullimarginatus* sp. nov., holotype **26** habitus in dorsal view, ♀ **27** head in frontal view, ♀ **28** vertex, ♀ **29** mesosoma, ♀ **30** propodeum in dorsal view, ♀ **31** propodeum in lateral view, ♀ **32** propodeum in dorsal-lateral view, ♀ **33** metasoma in lateral view, ♀ **34** T1, ♀ **35** S1, ♀.

dense punctures and thick apical lamella; apical margin of S3 with dense punctures like those on T3; subsequent terga and sterna with slightly sparse micropunctures.

Male. Unknown.

Distribution. China: Fujian.

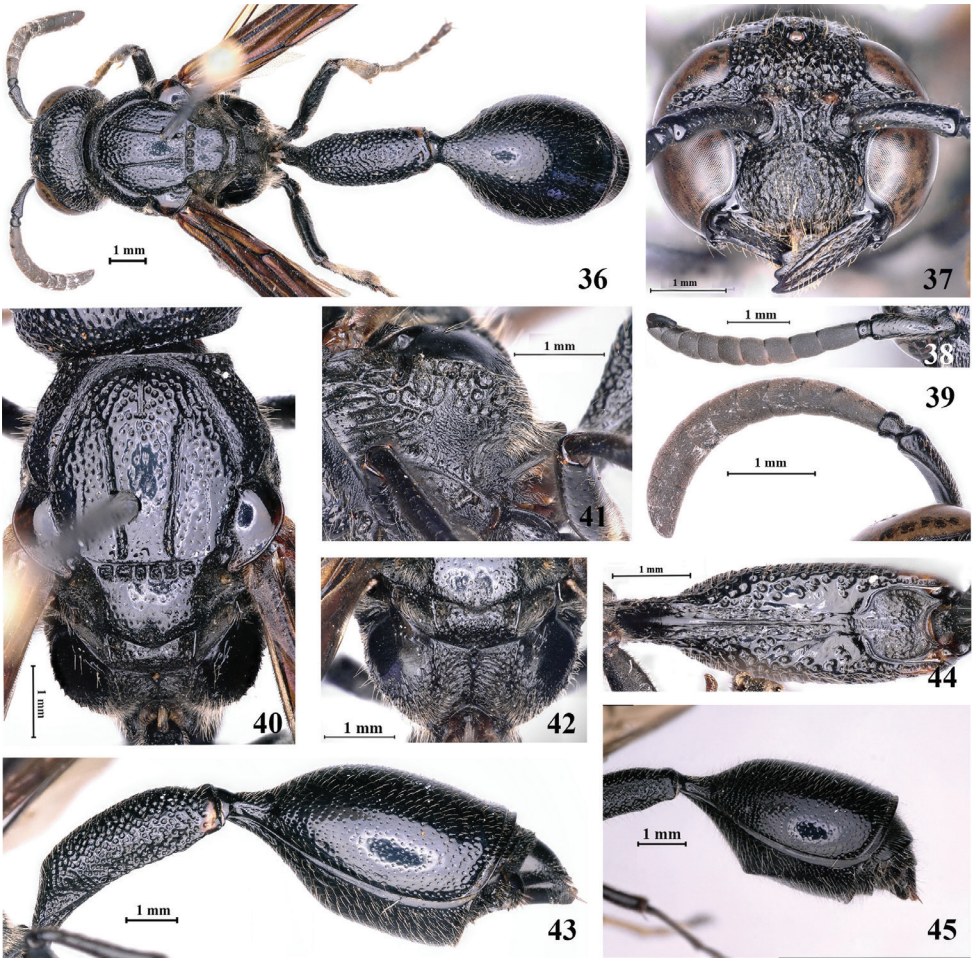
Remarks. The species resembles *Z. velamellatus* Tan, 2018 from Zhejiang, China by propodeum without submarginal lamella (Fig. 32), S2 gradually swollen from basal to apical margin in profile (Fig. 33). It differs from *Z. velamellatus* and all other members of the genus by the following character combination: apical margin of clypeus truncated (Fig. 27), the posterior lobe of tegula truncated (Fig. 29), propodeum without submarginal lamella (Fig. 32), T1 about 2.5× as long as wide, narrowly toward apex (Fig. 34), lateral carina of T1 disappeared ventrally (Fig. 35).

Etymology. The specific name *nullimarginatus* is derived from two Latin words: *null* and *marginatus*, referring to lateral carina of T1 disappeared ventrally.

Zethus angulatus **Nguyen & Carpenter, 2016, new record**
Figs 36–44

Zethus angulatus Nguyen & Carpenter, 2016: 27.

Material examined. 1♀: CHINA, Guangxi, Guiling City, Xingan County, Gaozhai Village, 25°50.901'N, 110°28.976'E, 19.VII.2015, Tingjing Li leg. (CQNU); 1♀: CHINA, Guangxi, Hechi City, Huanjiang County, Chuanshan Town, Linglang Village, 25°1.385'N, 108°5.761'E, 22.VII.2018, Qian Han et al. leg. (CQNU); 1♂: CHINA, Guangdong, Shaoguan City, Shixing County, CheBaLing National Nature Reserve, 24°43.503'N, 114°15.658'E, 22.VII.2018, Chengqiang Wang leg. (CQNU).



Figures 36–45. *Zethus angulatus* Nguyen & Carpenter, 2016 **36** habitus in lateral view, ♀ **37** head in frontal view, ♀ **38** antenna, ♂ **39** antenna, ♀ **40** mesosoma, ♀ **41** propodeum in lateral view, ♀ **42** propodeum in dorsal view, ♀ **43** metasoma in lateral view, ♀ **44** S1, ♀ **45**. *Zethus dolosus* Bingham, 1897, mesosomal segment 2 in lateral view, ♀.

Diagnosis. Female (Fig. 36): black, with the following parts yellow: spot on near dorso-inner margin of antennal socket and lateral carina of T1. Clypeus emarginated at basal and apical margin, clypeus punctate-reticulate and with long setae (Fig. 37); A12 bullet-shaped (Fig. 39); notaulix complete, punctures on mesoscutum gradually shallow from the anterior to posterior portion (Fig. 40); scutellum with punctures and middle longitudinal furrow; metapleuron with transverse striae; propodeum with lateral carina (Fig. 41) and transverse striae along median carina (Fig. 42), its lateral surface with large punctures in upper half (Fig. 41). T1 about 3× as long as wide in dorsal view, slightly narrow toward apical margin, T1 with coarse and deep punctures (Fig. 43); BS of S1 with longitudinal carina (Fig. 44), T2 with apical lamella, punctures on the near base of T2 denser and larger than other portion of tergum (Fig. 43); S2 with short apical lamella and obtuse angle in lateral view (Fig. 43); punctures on T3 and S3 dense and deep, T3 with thick apical lamella.

Male: structure as in female, but differing as follows: longitudinal pale yellow stripe on mandible, apical margin of clypeus deeply emarginated with the emargination forming a semicircle, A13 elongate, slightly curved (Fig. 38), propodeum with strong and oblique striae along median carina.

Distribution. China: Guangdong and Guangxi (**new record**); Vietnam.

Zethus tumidus Nguyen & Carpenter, 2016, new record

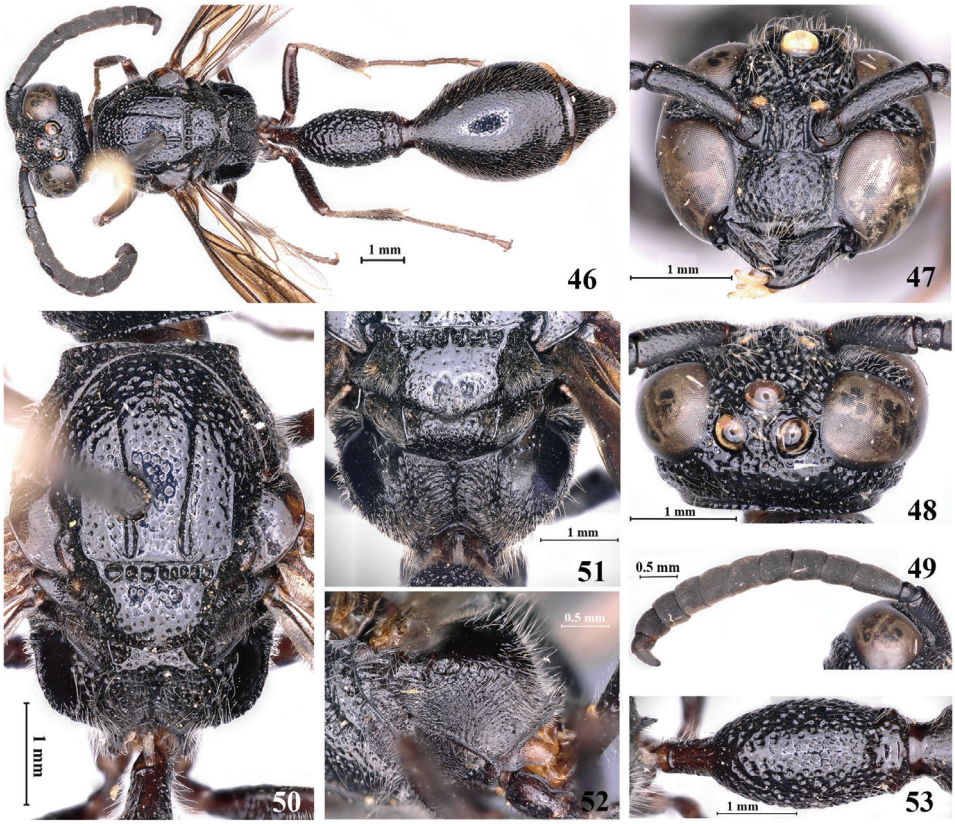
Figs 46–53

Zethus tumidus Nguyen & Carpenter, 2016: 29.

Material examined. 1♂: CHINA, Hainan, Changjiang County, Bawangling, 19°07.270'N, 109°05.009'E, 11–12.VII.2007, Yibin Ba & Juntong Lang leg. (MHBU).

Diagnosis. Male (Fig. 46): black, with yellow spot on dorso-inner margin of antennal socket and brown on S1 and the base of S2. Clypeus with reticulate punctures, apical margin distinctly emarginated (Fig. 47); ocelli large, its diameter as wide as antennal socket (Figs 47, 48); occipital carina complete (Fig. 48); A13 elongated (Fig. 49). Notaulix developed, mesoscutum with distinct punctures (Fig. 50); propodeum with lateral carina (Fig. 51), submarginal carina produced into developed lamella, its lateral surface without distinct punctures and striae and with deep and large punctures along lateral carina (Fig. 52). T1 2.4× as long as wide in dorsal view, maximum width 3× its basal width, then narrowly toward apical margin, with a medial carina which runs from basal margin to one-third of tergum, punctures on T1 large and dense (Fig. 53); BS of S1 with irregular and longitudinal striae; T2 with slightly dense punctures; T2 and S2 in lateral view gradually convex from basal to apical margin, with developed apical lamella; T3 and S3 with dense punctures; T3 with thick apical lamella.

Distribution. China: Hainan (**new record**); Vietnam.



Figures 46–53. *Zethus tumidus* Nguyen & Carpenter, 2016 **46** habitus in dorsal view, ♂ **47** head in frontal view **48** vertex **49** antenna **50** mesosoma **51** propodeum in dorsal view **52** propodeum in lateral view **53** T1.

Key to species of the genus *Zethus* from the Oriental region

1 T1 about 5× as long as wide in dorsal view ..... ***Z. fulgens*** **Gusenleitner, 2007**
– T1 at most 4.2× as long as wide in dorsal view (Figs 10, 23, 34, 43, 53) **2**
2 Head and mesosoma with dense and long golden orange setae **3**
– Head and mesosoma without dense golden orange setae **5**
3 Apical lamella of T3 indistinct; mesoscutum with large and dense punctures ..... ***Z. celebensis*** **Giordani Soika, 1958**
– Apical lamella of T3 distinct; mesoscutum with indistinct punctures **4**
4 S2 with developed lamella; in female, apical margin of S3 with a punctate median lobe; in male, antennae black, apical flagellomere rounded apically; apical margin of clypeus without teeth ..... ***Z. soikai*** **Selis, 2017**
– S2 without apical lamella; in female, apical margin of S3 without lobe; in male, antennae orange, apical flagellomere pointed apically; apical margin of clypeus with two triangular teeth ..... ***Z. luzonensis*** **Giordani Soika, 1941**

5 S2 not tuberculate in lateral view (Figs 8, 22, 33, 43).....**6**
– S2 tuberculate in lateral view (Fig. 45) **22**
6 Both T2 and T3 without lamellae .....
.....***Z. puehringeri*** **Gusenleitner & Gusenleitner, 2013**
– Both T2 and T3 with lamellae (Figs 12, 25, 33, 43, 45, 46).....**7**
7 Submarginal lamella of propodeum absent (Fig. 32) **8**
– Submarginal lamella of propodeum present (Figs 7, 21, 41, 52).....**9**
8 Apical margin of clypeus without depressed space and lateral tooth (Fig. 27); lateral carina of T1 disappeared in ventral view (Fig. 35)..... ***Z. nullimarginatus*** **sp. nov.**
– Apical margin of clypeus with depressed space between lateral tooth; lateral carina of T1 present in ventral view.....***Z. velamellatus*** **Tan, 2018**
9 Occipital carina developed laterally, weak dorsally (Fig. 4); pronotal carina almost indiscernible dorsally (Fig. 5) **10**
– Occipital carina and pronotal carina complete and visible (Figs 19, 29, 40, 50) ...**11**
10 Apical margin of clypeus emarginated; propodeum with weak lateral carina.....
.....***Z. trimaculatus*** **Cameron, 1904**
– Apical margin of clypeus frontally truncated and laterally cambered (Fig. 3); propodeum with strong lateral carina (Fig. 6).....***Z. striatus*** **sp. nov.**
11 Apical margin of clypeus almost truncated or rounded (Figs 16, 27).....**12**
– Apical margin of clypeus distinctly emarginated (Figs 47, 37).....**19**
12 T1 at least 3.5× as long as wide in dorsal view.....**13**
– T1 at most 2.6× as long as wide in dorsal view (Figs 23, 34, 53) **16**
13 Metapleuron with strong and short striae.....**14**
– Metapleuron without striae.....**15**
14 Scutellum with weak longitudinal furrow in middle .....
.....***Z. malayanus*** **Gusenleitner, 2010**
– Scutellum with deep longitudinal furrow on the apex behind .....
.....***Z. quadridentatus*** **Cameron, 1902**
15 Tegula and leg ferruginous; T1 narrowly toward apical margin .....
.....***Z. varipunctatus*** **Cameron, 1902**
– Tegula and leg black; T1 nearly parallel-sided apically.....
.....***Z. planiclypeus*** **Gusenleitner, 1988**
16 T2 without distinct petiole (Fig. 25) **17**
– T2 with petiole **18**
17 Mesosoma and metasoma almost black; gena with a longitudinal carina .....
.....***Z. nigerrimus*** **Gusenleitner, 2001**
– Mesosoma and metasoma with ferruginous marking (Fig. 13); gena without longitudinal carina (Fig. 17)..... ***Z. asperipunctatus*** **sp. nov.**
18 T1 gradually widening from one-sixth from the base, with medial carina from basal margin to near apical margin; submarginal carina of propodeum produced into pointed lamella.....***Z. nanlingensis*** **Nguyen & Xu, 2017**
– T1 gradually widening from one-fourth from the base, with medial carina from basal margin to one-fourth of tergum; submarginal carina of propodeum pro-

duced into round and short lamella..... ***Z. propodeus*** **Nguyen & Carpenter, 2016**

19 T1 about 3× as long as wide in dorsal view ..... ***Z. angulatus*** **Nguyen & Carpenter, 2016**

– T1 less than 2.5× as long as wide in dorsal view **20**

20 A3 2× as long as wide; ocellar diameter as wide as antennal socket (Fig. 47)..... ***Z. tumidus*** **Nguyen & Carpenter, 2016**

– A3 less than 2× its width; ocellar diameter narrower than antennal socket..... **21**

21 Gena without longitudinal carina; in male, mandible separated from middle tooth by a broad notch; clypeus black ..... ***Z. taiwanus*** **Yeh & Lu, 2017**

– Gena with a longitudinal carina; in male, mandible not separated by a broad notch; clypeus with yellow marking.... ***Z. tansoneus*** **Nguyen & Carpenter, 2016**

22 T1 swollen at the base, with lateral margins narrowly toward apex..... **23**

– T1, subcylindrical, nearly parallel-sided apically..... **25**

23 Propodeum without lateral carina ..... ***Z. bakeri*** **Giordani Soika, 1995**

– Propodeum with lateral carina **24**

24 A13 slightly curved; punctures on T2 large and slightly sparse; clypeus with yellow marking..... ***Z. mandibularis*** **Giordani Soika, 1995**

– A13 straight; punctures on T2 fine and dense; clypeus black..... ***Z. malabarica*** **Giordani Soika, 1995**

25 Apical lamella of the S2 not reaching the lateral margins of the same sternum .... ***Z. ceylonicus*** **de Saussure, 1867**

– Apical lamella of the S2 reaching the lateral margins of the same sternum..... **26**

26 Body glossy and almost without punctures.... ***Z. improcerus*** **Giordani Soika, 1995**

– Body with coarse and wide punctures **27**

27 Lateral surface of propodeum with oblique striae in the lower half..... ***Z. dolosus*** **Bingham, 1897**

– Lateral surface of propodeum smooth in lower half..... **28**

28 Metasomal segments 3–6 with long and dense setae..... ***Z. albopilosus*** **Giordani Soika, 1995**

– Metasomal segments 3–6 with short and sparse setae ..... ***Z. indicus*** **Giordani Soika, 1958**

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CATALOGUE

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Two new species of *Pirhosigma* Giordani Soika (Vespidae, Eumeninae), with an updated catalog for the genus

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Abstract

Two new species of eumenine wasps were described from Panama and Peru, namely *Pirhosigma abregoi* Garcete-Barrett & Hermes **sp. nov.** and *P. cambrai* Garcete-Barrett & Ferreira **sp. nov.** Lectotypes are designated for *Eumenes deformata barberoi* Bertoni and *Eumenes superficialis mondaiensis* Bertoni. *Pirhosigma mearimense putumayense* Giordani Soika **stat. nov.** is treated as a color variation of the typical *P. mearimense* (Zavattari). Additions to the key by Ferreira et al. (2017) are made and an updated catalog for species included in the genus is provided.

Keywords

New species, Panama, Peru, Potter wasps, Taxonomy

Introduction

Recently, the Eumeninae, the so-called potter wasps, have been receiving much attention in studies regarding the higher-level phylogenetic relationships of their main lineages, especially with the aid of novel molecular data (Hines et al. 2007; Pickett and Carpenter 2010; Hermes et al. 2014; Bank et al. 2017; Piekarski et al. 2018). The most recent results based on large-scale genomic datasets (Bank et al. 2017; Piekarski et al. 2018) have implied paraphyly of Eumeninae *sensu* Carpenter (1982), whose monophyly was corroborated by Pickett and Carpenter 2010 and Hermes et al. 2014.

Despite this recent phylogenetic progress, the Neotropical fauna of potter wasps remains little explored, a fact that is particularly noticeable by the poor representation of the group in most Latin American entomological collections. In fact, the group is not as abundant as the social vespids, and few experts are carrying out research within the group. Nevertheless, the number of described species has been increasing constantly for the Neotropics (e.g. Hermes 2012; Lopes and Noll 2014; Lopes and Hermes 2015; Cooper, 2016a, b; Lopes et al. 2017), indicating the need for more research to be carried out for the taxon.

Pirhosigma is a somewhat small genus of eumenine wasps, currently comprising nine described species. Of these, two were described within the last three years (Ferreira et al. 2015, Ferreira et al. 2017). Recently, Hermes et al. (2013) provided an insightful study on the nesting biology of two species of *Pirhosigma*, with special reference to the use of vegetable matter for potentially camouflaging the mud nests. Such biological strategies are scarcely known for the potter wasps as a whole, but recent available data are showing them to be very plastic regarding nesting strategies (e.g. Hermes et al. 2015, Auko et al. 2015). We hereby propose the description of two new species of *Pirhosigma*, along with an updated catalog for all species included in the genus.

Material and methods

Material from the following institutions was examined (the acronyms follow Evenhuis (2018) when available):

MIUP Museo de Invertebrados Graham B. Fairchild, Universidad de Panama, Panama (Dr. Roberto Cambra, Dr. Diomedes Quintero and Jean Carlos Ábrego);
MSNG Museo Civico di Storia Naturale "Giacomo Doria", Genova, Italy (Dr. Maria Tavano);
INPA Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (Dr. Márcio L. de Oliveira).

Additional acronyms mentioned in the catalog of species of *Pirhosigma* are:

AMNH American Museum of Natural History, New York, USA;
CMNH Carnegie Museum of Natural History, Pittsburgh, USA;

CUIC Cornell University, Ithaca, USA;
MHNG Muséum d´Histoire Naturelle, Geneva, Switzerland;
MNHNPY Museo Nacional de Historia Natural del Paraguay, San Lorenzo, Paraguay;
MSNVE Museo di Storia Naturale, Venice, Italy;
RMNH Naturalis Biodiversity Centre, Leiden, Netherlands.

Type specimens of species potentially closely related to the new species were examined from the cited institutions (details in the taxonomic catalog). All examined specimens were dry pinned preserved ones. Examination was undertaken with a Leica S8 APO stereomicroscope. Photographs of structures of interest were obtained with a Canon EOS Rebel T6 digital camera attached to the stereomicroscope. The same camera with a Canon Macro 100 mm lens and a Yongnuo 2x Extensor were used for photographing specimen habitus. All images were captured using Canon EOS Utility software and using a light dome modified from Kawada and Buffington (2016). The final stacking of multiple layers was carried out with the Helicon Focus software.

Body length is taken from the frons to the hind border of T2 and expressed as an approximate measurement, as body position can modify it more or less substantially. Wing length is taken from the humeral angle of the wing at the border of the tegula to the wing tip and expressed as an approximate measurement, as wing position can affect the total measurement.

Taxonomy

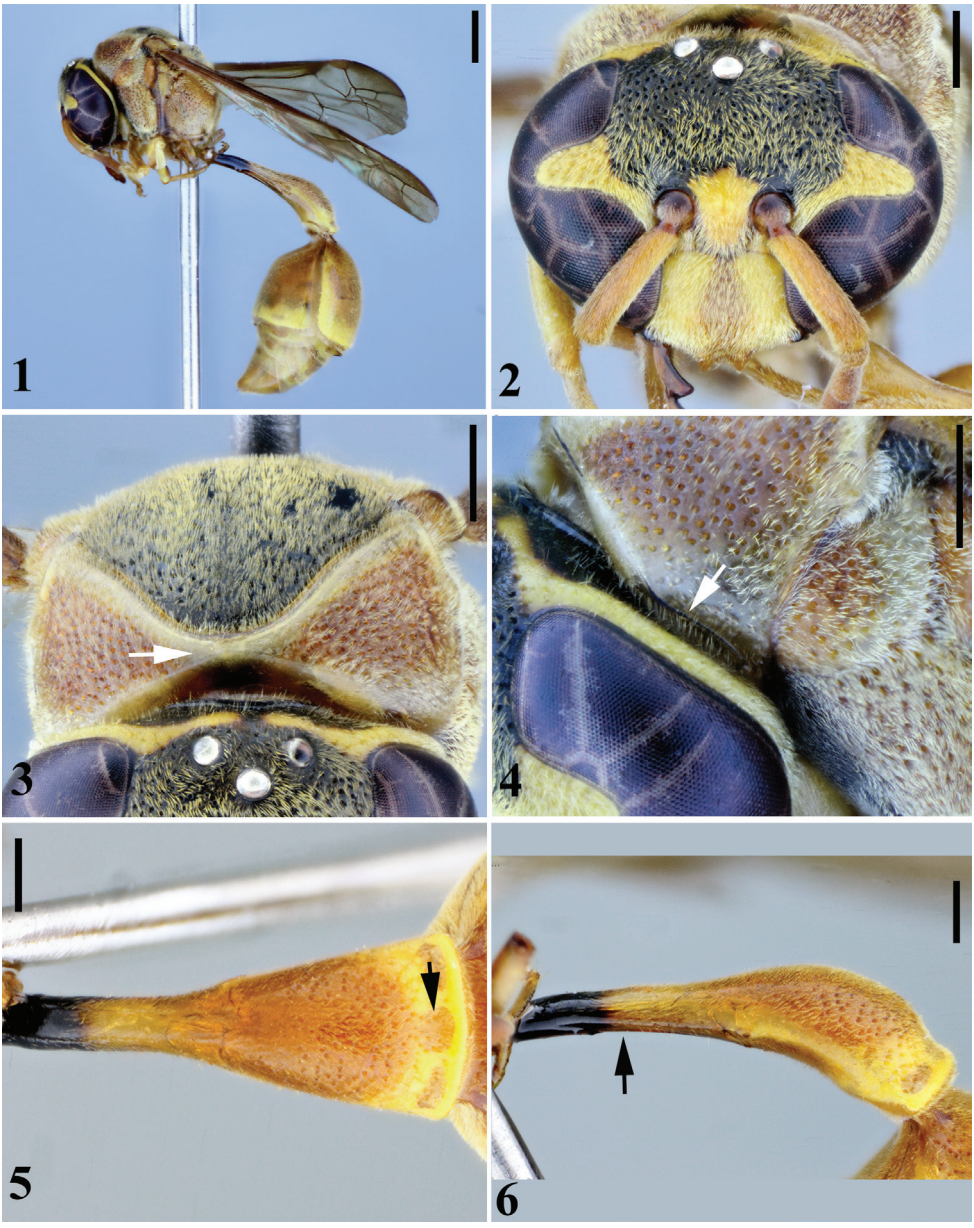
***Pirhosigma abregoi* Garcete-Barrett & Hermes, sp. nov.**
http://zoobank.org/A50BFD49-2125-49E6-88D2-873FDC30EEA9
Figs 1–7

Comments and diagnosis. This is the only species of *Pirhosigma* that does not present a preapical fossa in T1 (Fig. 5), which is present in all other species of this genus. However, this species presents all the other diagnostic features of *Pirhosigma*, such as the shape of T1, apically flask-shaped, with the apical lamella not preceded by a transverse swelling, and the basal portion with two laterally longitudinal carinae (Giordani Soika 1978; Carpenter and Vecht 1991) (Fig. 6). *Pirhosigma abregoi* differs from all other species of *Pirhosigma* by the following set of features: (i) absence of an evident preapical fossa on T1 (Fig. 5); (ii) pronotal carina in the shape of an inverted "V" in frontal view (Fig. 3), with a well-developed lateral lamella (Fig. 4); (iii) T2 oval, longer than wide, with evident, deep and spaced punctures (Fig. 7); (iv) lateral portion of the pronotum greatly shortened (Fig. 4); (v) short clypeus, wider than long (Fig. 2).

Description. *Holotype female*.

Measurements. Body length (from head to apex of T1): 5.5 mm; Forewing length (from mid tegula to apex): 6.07 mm.

Color. Body with predominantly brown-yellowish tegument. Yellow head, with a wide oval black mark on the frons, connected to a narrow black band extending to



Figures 1–6. *Pirhosigma abregoi* Garcete-Barrett & Hermes, holotype female **1** habitus **2** head, frontal view **3** pronotum, frontal view, arrow pointing to the pronotal carina in the shape of an inverted "V" **4** pronotum, lateral view, arrow pointing to the well-developed lateral lamella in the pronotal carina **5** T1, dorsal view, arrow pointing to the apical portion without a well-developed preapical fossa **6** T1, lateral view, arrow pointing to the well-developed longitudinal carina. Scale bars: 1 mm (**1**); 0.5 mm (**2–6**).

the occiput; brownish mark in the center of the clypeus. Mesosoma and metasoma with predominantly brown-yellowish tegument. Antennae with brownish scape and pedicel; progressively darker flagellum from the base to the apex. Mesoscutum totally

blackened. Scutellum with a central black-brown spot. Brownish propodeum. Black mark in the basal portion of T1. Yellow marks more prominent in the regions that follow: parategulae; apical margin of T1; lateral and apical margins of T2; apical margin of S1. Brown wings.

Structure. Labrum truncated. Clypeus broader than long, with short and emarginated apex; small and not carinate apical teeth present. Interantennal region without cariniform elevation. Pronotal carina well developed in all its extension, in the shape of an inverted "V" in frontal view, with a well-developed lateral lamella. Lateral surface of pronotum narrow, with the distance between pronotal fovea and the mesepisternum smaller than the size of the fovea itself; pronotal fovea slit-shaped. Pretegular carina absent. Parategulae triangular. Sulcus between the scutellum and metanotum obsolete. T1 elongated, with basal portion longer than the apical portion; two lateral longitudinal carinae present, not reaching half of the segment; preapical fossa absent. T2 oval, longer than wide, with lamella well developed. S2 without abrupt basal elevation.

Sculpture. Clypeus without evident punctation. Frons and vertex with deep, coarse and abundant punctures, with space between them smaller than the size of a puncture. Pronotum with granular punctation, with shallow, abundant and slightly thickened punctures, distance between them smaller than the size of a puncture. Mesepisternum with deep punctures, denser in its upper portion; shallow and slightly evident punctures in its lower portion. Mesoscutum, scutellum and propodeum with deep and coarse punctures. Apex of T1 with evident shallow punctation. T2 with well-marked deep punctation, distance between them smaller than the size of a puncture.

Pilosity. Golden pubescence covering the entire surface of the body. Bristles shorter, thick and abundant on clypeus, frons, vertex, and mesosoma. Elongated, delicate and thin bristles in the metasoma.

Male. Unknown.

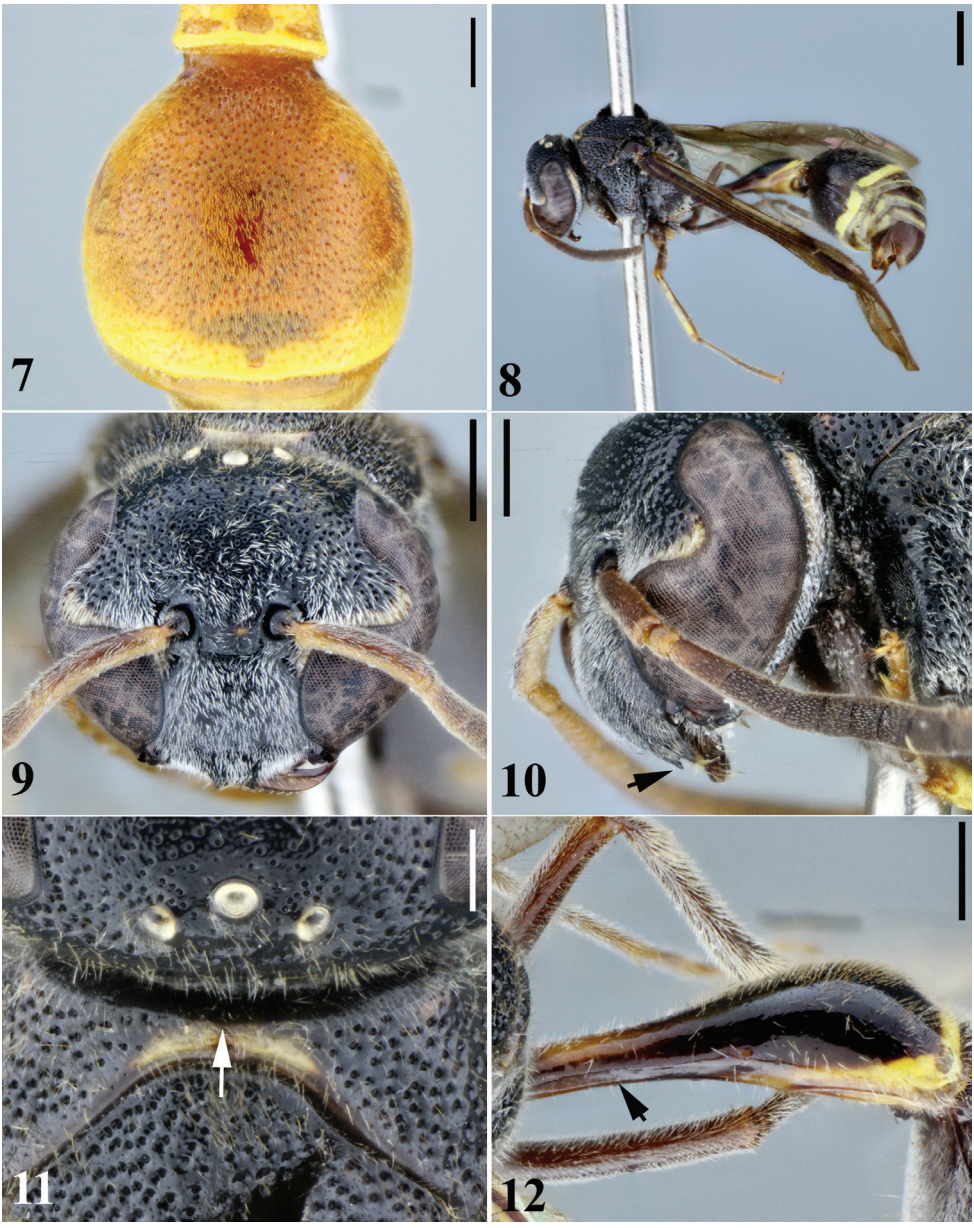
Type material. Holotype: PANAMA • 1 ♀; Peninsula Gigante, Barro Colorado Nature Monument; 30 Jul. 1990; A. Mena leg. (MIUP).

Type locality. Peninsula Gigante: Barro Colorado Nature Monument; Panama.

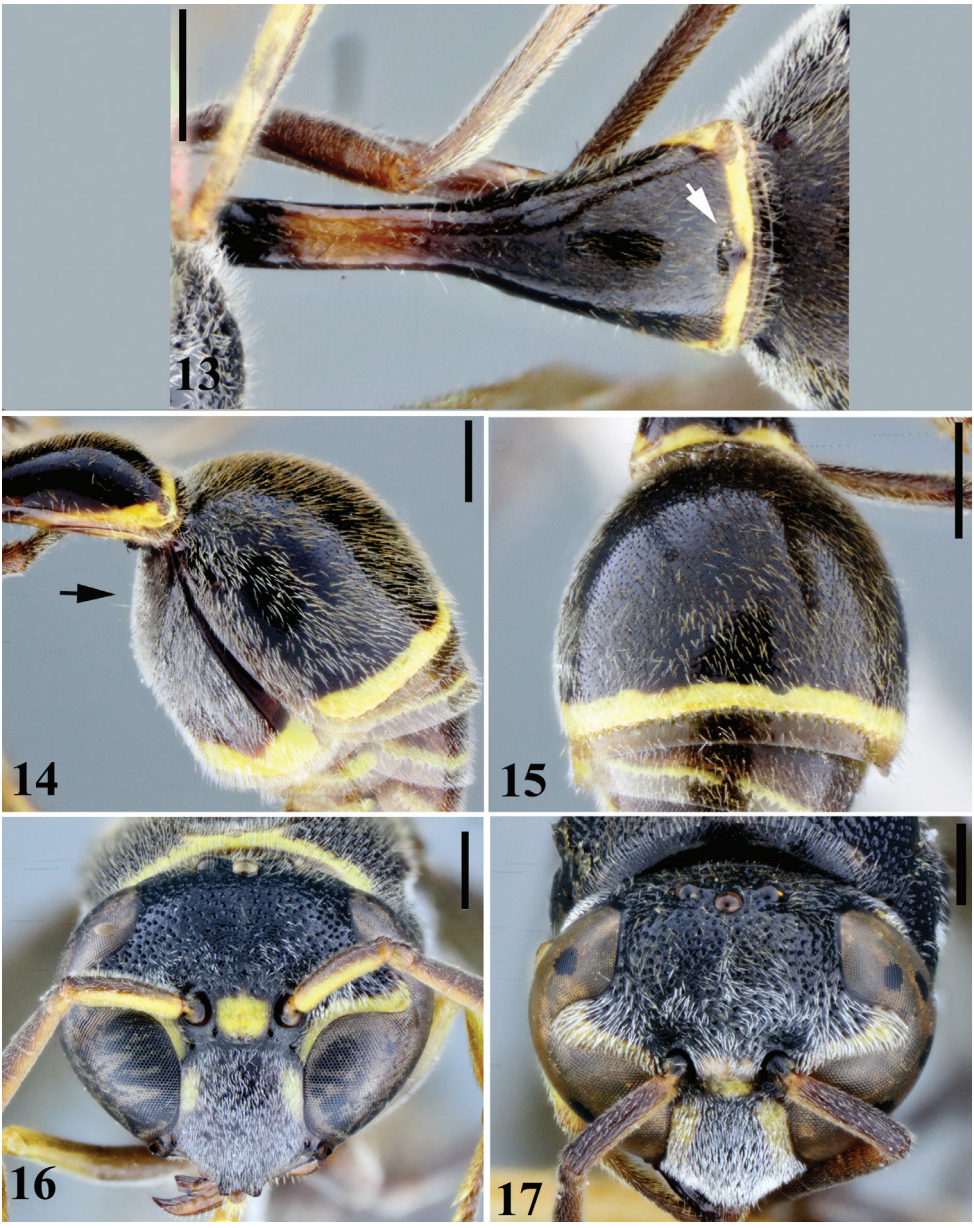
Etymology. This species is named after the Panamanian Biologist Jean Carlos Ábrego.

***Pirhosigma cambrai* Garcete-Barrett & Ferreira, sp. nov.**
http://zoobank.org/73F75F9A-9304-437D-A8F6-957E2A33BF33
Figs 8–15

Comments and diagnosis. *P. cambrai* is quite similar to *P. mearimense* (Zavattari) and *P. sulcata* Ferreira & Hermes, sharing with them the S2 without a basal slope followed by an elevation (Fig. 14); T1 distinctly filiform with basal region of greater length than the apical portion (Figs 12–13); T2 wider than long (Fig. 15); pronotal carina well developed dorsally (Fig. 11); and a black body color, with few yellow marks (Fig. 8). *Pirhosigma cambrai* is distinguished from *P. mearimense* and *P. sulcata*



Figures 7–12. *Pirhosigma abregoi* Garcete-Barrett & Hermes, holotype female **7** T2 with an evident punctuation, dorsal view. *Pirhosigma cambrai* Garcete-Barrett & Ferreira, holotype female **8** habitus **9** head, frontal view **10** head, lateral view, arrow pointing to the curved clypeus apex **11** pronotum, dorsal view, arrow pointing to the well-developed dorsally pronotal carina **12** T1, lateral view, arrow pointing to the well-developed longitudinal carina. Scale bars: 1 mm (**8**); 0.5 mm (**7, 9–12**).



Figures 13–17. Representatives of *Pirhosigma* species **13–15** *Pirhosigma cambrai* Garcete-Barrett & Ferreira, holotype female **13** T1, dorsal view, arrow pointing to the apex with a pre-apical fossa well-developed **14** T1 and S2, lateral view, arrow pointing to the S2 without a basal slope followed by an elevation **15** T2, dorsal view **16** *Pirhosigma mearimense* (Zavattari), female head, frontal view **17** *Pirhosigma sulcata* Ferreira & Hermes, holotype male head, frontal view. Scale bars: 0.5 mm (**13–17**).

Ferreira & Hermes by the presence of a short, wider than long clypeus (Fig. 9), curved backwards (Fig. 10).

Description. *Holotype female*.

Measurements. Body length (from head to apex of T1): 5.10 mm; Forewing length (from mid tegula to apex): 6.50 mm.

Color. Body with predominantly blackish tegument. Yellow marks as follows: stripes on inner margin of compound eyes; upper surface of the gena; narrow range in the antero-dorsal region of pronotum; narrow bands in the distal portions of the T1–T6 and S2–S6. S1 brownish. Antennae brownish. Yellow-brownish legs. Brownish wings.

Structure. Labrum rounded, narrow. Clypeus broader than long, apically curved backwards and with short, concave and emarginated apex; small and ecarinate apical teeth present, with small distance between each other. Interantennal region without cariniform elevation. Pronotal carina well developed dorsally, gently and roundly recurved in the humeral region. Lateral surface of pronotum narrow, with the distance between pronotal fovea and the mesepisternum smaller than the size of the fovea itself. Pretegular carina absent. Parategulae pointed. Sulcus between scutellum and metanotum obsolete. T1 distinctly filiform with basal region of greater length than the apical portion; two lateral longitudinal carina present; preapical fossa present. T2 wider than long, outlined as a half oval in dorsal view; well-developed apical lamella. S2 without basal slope followed by an elevation.

Sculpture. Clypeus without evident punctation. Frons and vertex with evident and abundant punctures, with distance between them approximately smaller than the size of a puncture; micro-punctation evident. Pronotum, upper portion of the mesepisternum, mesoscutum, scutelum, metanotum and propodeum with deep and abundant punctures, with distance between punctures smaller than the size of a puncture. Lower portion of the mesepisternum with shallow and sparse punctures, distance between them approximately greater than the size of a puncture. T1 unpunctate. T2 with micro-punctation evident.

Pilosity. Fine whitish pubescence covering the entire body. Whitish bristles covering the head, concentrated in the clypeus. Brownish, short and thin bristles on mesosoma. Brownish and long bristles on T1–T6 and S2–S6.

Male. Unknown.

Type material. Holotype: PERU • 1 ♀; Madre de Dios, Manu Reserve, Pakitza Station; 1–2 Mar. 1992; R. Cambra leg. (MIUP).

Type locality. Madre de Dios: Manu Reserve, Pakitza Station; Peru.

Etymology. This species is dedicated to the Panamanian entomologist Roberto Cambra.

Comments. The holotype of *P. cambrai* sp. nov. (female, MIUP) was compared with the holotypes of *P. mearimense* (Zavattari) (male, MSNG) and *P. sulcata* Ferreira & Hermes (male, INPA). Additional material of *P. mearimense*, two males and two females, were also analyzed (MSNVE). Unfortunately, the female of *P. sulcata* remains unknown, but by the uniformity of the clypeus between the sexes of *Pirhosigma*, we consider the comparison of this structure valid for the distinction between the species *P. sulcata*/*P. mearimense* from *P. cambrai*.

Additional examined material. *Pirhosigma mearimense* (Zavattari): Holotype: BRAZIL • 1 ♂; Miarim; Gribodo leg. (MSNG). SURINAME • 1 ♂; Republiek; 6 May. 1963; J. v. d. Vecht leg. (MSNVE). 1 ♀; Republiek; 24 Sep. 1963; D. C. Geyskes leg. (MSNVE) PERU • 1 ♂; El Campamiente, Colonia Perene; 21 Jun. 1920; Giordani Soika leg. (MSNVE). BOLIVIA • 1 ♀; Buenavista, Dep. Sta Cruz; alt. 450 m. (MSNVE). *Pirhosigma sulcata* Ferreira & Hermes: Holotype: BRAZIL • 1 ♂; Amazonas, KM31 AM-010, CEPLAC; 18 Jun. 1976; Joselita M. Santos leg. (INPA).

Updated key to the species of *Pirhosigma*, adapted from Ferreira et al. (2017)

The species *P. abregoi* is readily differentiated from all other species in the key of Ferreira et al. (2017), since it is the only species that does not present a preapical fossa in T1 – compare Fig. 5: *P. abregoi*, without a preapical fossa on T1; and Fig. 13: *P. cambrai*, with a preapical fossa on T1.

1’ Preapical fossa on T1 absent (Fig. 5) ..... ***P. abregoi* Garcete-Barrett & Hermes, sp. nov.**

– Preapical fossa on T1 present (Fig. 13) ....**(couplet 1 in Ferreira et al. 2017)**

The species *P. cambrai* runs to couplet 6 of Ferreira et al. (2017), which is modified as follows:

6 Pronotal carina well developed dorsally (Fig. 11); black, with a few yellow spots (Fig. 8) ..... ***6’***

– Pronotal carina not evident dorsally; yellowish with black marks and bands **8**

6’ Short clypeus, wider than long (Fig. 9) ..... ***P. cambrai* Garcete-Barrett & Ferreira, sp. nov.**

– Clypeus longer than wide or almost as long as wide (Figs 16, 17)..... **7**

7 Male with a well-marked groove between the metanotum and the scutellum [female unknown]..... ***P. sulcata* Ferreira & Hermes**

– Male without a well-marked groove between the metanotum and the scutellum ..... ***P. mearimense* (Zavattari)**

A Catalog of the genus *Pirhosigma* Giordani Soika (only taxonomic and nomenclatural procedures are indicated where they apply)

Pirhosigma Giordani Soika, 1978: 11, 229.

Type species: *Eumenes simulans* de Saussure, 1875, by original designation.

Tricomenes Giordani Soika, 1978: 10, 254.

Type species: *Eumenes pilosus* Fox, 1899, by original designation and monotypy.

Pirhosigma; Giordani Soika 1978: 10, 230–231. Carpenter and van der Vecht 1991: 230 (*Tricomenes* rejected, acting as first reviser).

Pirhosigma aenigmaticum Giordani Soika, 1978

Eumenes simulans; Zavattari 1906: 19; Zavattari 1912: 118. Misidentification.

Pirhosigma aenigmaticum Giordani Soika, 1978: 231, 250.

Type Data: Holotype female **RMNH**.

Type Locality: Valle Anchicaya, Cali, Colombia.

Pirhosigma aenigmaticum; Carpenter and van der Vecht 1991: 229 (probable synonym of *P. simulans* (de Saussure)). West-Eberhard et al. 1995: 574. Rodríguez--Palafox 1996: 480. Ferreira et al. 2017: 277.

Distribution: Mexico, Costa Rica, Panama, Colombia, Venezuela, Ecuador.

Pirhosigma deforme (Fox, 1899)

Eumenes deforma Fox, 1899: 453, 461.

Type Data: Lectotype female **CMNH**.

Type Locality: Corumbá, Mato Grosso do Sul, Brazil.

Eumenes deforma; Dalla Torre 1904: 22. Brèthes 1906: 335. Bertoni 1918: 206. Carpenter and van der Vecht 1991: 229 (designation of lectotype).

Eumenes deformata [!] *barberoi* Bertoni, 1926: 76.

Type Data: Lectotype female by present designation (**MNHNPY**).

Type Locality: Puerto Bertoni, Paraguay.

Eumenes deformata barberoi; Bertoni 1934: 112, 118.

Pirhosigma deforme; Giordani Soika 1978: 230, 231. Hermes and Köhler 2004: 74, 86. Somavilla et al. 2010: 260. Hermes et al. 2013: 434. Hermes et al. 2014: 456, 470, 471, 475. Ferreira et al. 2017: 276.

Distribution: Brazil, Paraguay.

Remarks. In the original description (Bertoni, 1926) of *Eumenes deformata barberoi*, the author did not mention how many specimens were part of the type series nor the locality where they were collected. Furthermore, Bertoni labelled seven specimens as *Eumenes deformata paranensis* (unpublished subspecific name), and the name *barberoi* was never attached to any specimen whatsoever. The lack of a locality, in this case, poses no issue, since all individuals bear a label with the locality "Puerto Bertoni", where the author lived for many years and collected many of his specimens. Also, the only subspecific name proposed by Bertoni under the specific name *deformata* is indeed *barberoi*, which leaves no doubt about the members of the type series. Finally, the description matches these specimens, and one well-preserved female was chosen as the lectotype and labelled accordingly; the remainder of the specimens (two males and four females) are to be treated as paralectotypes.

Here we have the opportunity to correct the date of Bertoni's paper "Hymenópteros nuevos o poco conocidos". Though considered as published in December of 1925, as suggested in the heading of its cover, issue 2(1) of the "Revista de la Sociedad Científica del Paraguay", was actually printed in 1926, as indicated in the foot of the very same cover. This paper contains the original descriptions of the species-level names *Zetamenes rufomaculata* ssp. *meridionalis* Bertoni, *Zetamenes filiformis* var. *costarricensis* Bertoni, *Discoelius strigosus* ssp. *costarricensis* Bertoni, *Pachymenes atra* var. *ornatissima*

Bertoni, *Eumenes deformata barberoi* Bertoni, *Amphimenes totonacus* var. *manateci* Bertoni, *Monobia paraguayensis* Bertoni, *Odynerus migonei* Bertoni and the generic name *Protozethus* Bertoni.

Pirhosigma limpidum Giordani Soika, 1978

Pirhosigma limpidum Giordani Soika, 1978: 230, 240.

Type Data: Holotype female **MSNVE**.

Type Locality: Espírito Santo, Brazil.

Pirhosigma limpidum; Hermes et al. 2013: 434. Hermes et al. 2014: 472. Ferreira et al. 2017: 272, 277.

Distribution: Brazil.

Pirhosigma mearimense (Zavattari, 1912)

Eumenes mearimensis Zavattari, 1912: 101.

Type Data: Holotype male **MSNG**.

Type Locality: Vitoria do Mearim, Maranhão, Brazil (Penati and Mariotti 2015).

Pirhosigma mearimense; Giordani Soika 1978: 231, 243. Ferreira et al. 2015: 118, 119. Ferreira et al. 2017: 277.

Pirhosigma mearimense mearimense; Santos et al. 2015: 41.

Pirhosigma mearimense putumayense Giordani Soika, 1978: 245. **New status**.

Type Data: Holotype female **CUIC**.

Type Locality: Putumayo, Peru.

Pirhosigma mearimense putumayense; Rasmussen & Asenjo, 2009: 42.

Distribution: Suriname, Brazil, Peru, Bolivia.

Remarks. It is widely acknowledged by the vespid experts that Antonio Giordani Soika was very fond of proposing subspecies based solely on coloration (see Carpenter (1987) for a good example). We hereby treat *P. mearimense putumayense* as a mere color variation of the typical form.

Pirhosigma pilosa (Fox, 1899)

Eumenes pilosa Fox, 1899: 454, 461.

Type Data: Lectotype female **CMNH**.

Type Locality: Chapada dos Guimarães, Mato Grosso, Brazil (15°26'S 55°45'W) (Papavero 1973, Carpenter and van der Vecht 1991).

Eumenes pilosa; Dalla Torre 1904: 24. Bertoni 1934: 112, 117.

Tricomenes pilosus; Giordani Soika 1978: 254 (inadvertent designation of lectotype).

Pirhosigma pilosa; Carpenter and van der Vecht 1991: 230. Ferreira et al. 2017: 277.

Distribution: Ecuador, Brazil.

Pirhosigma simulans (de Saussure, 1875)

Eumenes simulans de Saussure, 1875: 91.

Type Data: Lectotype female **MHNG**.

Type Locality: Orizaba, Mexico.

Zeteumenes simulans; Bertoni 1934: 110.

Pirhosigma simulans; Giordani Soika 1978: 231, 247. Rodríguez-Palafox 1996: 480. Ferreira et al. 2017: 277.

Distribution: Mexico.

Pirhosigma sulcata Ferreira & Hermes, 2015

Pirhosigma sulcata Ferreira et al. 2015: 118.

Type Data: Holotype male **INPA**.

Type Locality: Km 31 AM-010, Ceplac, Amazonas, Brazil.

Pirhosigma sulcata; Ferreira et al. 2017: 277.

Distribution: Brazil.

Pirhosigma superficiale (Fox, 1899)

Eumenes superficialis Fox, 1899: 441, 460.

Type Data: Lectotype female **CMNH**.

Type Locality: Chapada dos Guimarães, Mato Grosso, Brazil (15°26'S 55°45'W) (Papavero 1973, Carpenter and van der Vecht 1991).

Eumenes superficialis; Dalla Torre 1904: 25. Brèthes 1906: 335. Bertoni 1911: 106. Zavattari 1911: 49. Zavattari 1912: 99. Bertoni 1918: 206. Bertoni 1934: 113, 118. Carpenter and van der Vecht 1991: 225 (syn: *Pirhosigma superficiale impurum* Giordani Soika).

Eumenes superficialis mondaiensis Bertoni, 1934: 118.

Type Data: Lectotype female by present designation (**MNHNPY**).

Type Locality: Puerto Bertoni, Paraguay.

Pirhosigma superficiale; Giordani Soika 1978: 230, 236 (inadvertent designation of lectotype). Garcete-Barrett 1999: 8. Hermes and Köhler 2004: 74, 86. Somavilla et al. 2010: 260. Hermes et al. 2013: 433, 434. Hermes et al. 2014: 456, 472, 475. Ferreira et al. 2017: 270, 272, 276.

Pirhosigma superficiale impurum Giordani Soika 1978: 230, 239.

Type Data: Holotype female MCZ.

Type Locality: Oran, Abra Grande, Salta, Argentina (MCZ Type Database, http://140.247.96.247/mcz/Species_record.php?id=25550).

Pirhosigma superficiale impurum; Carpenter and van der Vecht 1991: 211, 225 (synonym of typical *P. superficiale*).

Distribution: Brazil, Paraguay, Argentina.

Remarks. Bertoni (1934) mentioned 20 specimens, both males and females, which he ramdomly chose to provide the description of *Eumenes superficialis mondaiensis*. Fifteen out of these twenty specimens were found at the MNHNPY to be part of the type series. Seven specimens are labelled as from Puerto Bertoni, two from Assuncion and five from Vista Alegre. These localities were all mentioned in the original description, except for the latter. Bertoni (1934) also mentioned having examined specimens from

"Amambái (Norte)" which undoubtedly correspond to Vista Alegre, which is in the upper part of the Aguaray Guazu river in the Amabay Department and, according to Brèthes (1924), on the approximate coordinates 23°40'S, 55°50'W (though Brèthes indicated 33 degrees for the coordinate south, which was no doubt just a *lapsus* ending in an inadvertent error of 10 degrees). One well preserved female from Puerto Bertoni was chosen as the lectotype and labelled accordingly; the remainder of the specimens (six males and eight females) are to be treated as paralectotypes.

Pirhosigma transfluvium Ferreira & Oliveira, 2017

Pirhosigma transfluvium Ferreira et al. 2017: 270, 277.

Type Data: Holotype male **AMNH**.

Type Locality: Beni: Rio Itenez, Bolivia.

Distribution: Bolivia.

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Alate gyne of the ant *Dolichoderus quadripunctatus* (L.) (Hymenoptera, Formicidae) follows foraging trail to aphids

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Abstract

The first observation of alate gyne of *Dolichoderus quadripunctatus* (L.) visiting aphids is described. A gyne walked along a foraging trail to the aphid *Panaphis juglandis* Goeze colony where it imbibed honeydew excreted on the leaf by the aphids, after which it returned to the trail. This recurred during two more days, always a single alate gyne at a time; hence the total number of gynes, one or more, remained open. The phenomenon, hitherto practically unknown in ants, is presented against the background of the biology of the species and discussed in the context of specific environmental circumstances and the colony dynamics.

Keywords

Ants, aphids, behavioural plasticity, colony dynamics, gyne foraging, life history, *Panaphis juglandis*

Introduction

In ants and other social insects, the basic division of tasks is such that workers take care of the colony tasks, whereas queens reproduce (Hölldobler and Wilson 1990). The variation around this basic theme is, however, large, depending on the species and specific circumstances. For example, workers of many species may produce male offspring (Bourke 1988). On the other hand queens may forage (see Discussion). Until now,

the published records on foraging gynes or queens have been on dealate individuals, in which plausible explanations are easily found, whereas observations on foraging alates seem to be lacking.

Here, we report our observations on this previously practically unknown behaviour, foraging of one or more alate ant gynes [though see Discussion on the microgynes of *Manica rubida* (Latr.)], and discuss it in a wider context of the biology and life history of the species in question, *Dolichoderus quadripunctatus* (L.).

The observations

Observations were made in the area with sparse single-family housing in Brwinów (52°08'N, 20°43'E) near Warsaw, Poland. The site of observation was in a garden growing, among others, a few common walnut trees (*Juglans regia* L.) next to a detached house with old wooden elements (elevation, pillars, balustrades) (Fig. 1). Nests of *D. quadripunctatus* were located in the walnut trees and in the wooden elements of the building and fences; the ants foraged mainly in the crowns of the trees. Observations were conducted irregularly in the years 2010–2019, with the most interesting finding recorded in July 2017.

At the beginning of July 2017, a colony of nymphs of the large walnut aphid *Panaphis juglandis* Goeze appeared on a leaf of a young walnut tree, on its upper surface, at a height of 1.1 m above the ground. On July 7, the aphids were discovered by *D. quadripunctatus* foragers from a nest in a wooden terrace pillar at a height of c. 4.5 m above the ground (Fig. 1). The ants formed a regular foraging trail from their nest to the aphids. The intricate trail measured at least 13 m (the ants came out of the crack of the pillar, but the location of the nest was unknown). The traffic intensity was low: one to two individuals per minute in each direction. During the day, depending on the weather, the aphids were accompanied by a few to nearly 30 workers. In the following days, two smaller *P. juglandis* colonies appeared on other leaves of the same tree. They were also visited by *D. quadripunctatus*, although with lower activity.

On July 16, an alate *D. quadripunctatus* gyne appeared at the aphids (Fig. 2). Like the workers, it licked the honeydew from the leaf surface in the immediate vicinity of the aphids. It did it less efficiently than the workers, with long breaks, thus spending much more time on the leaf than the workers. After about 15 minutes, the gyne left the leaf, following the foraging trail towards the nest. During occasional observations in the following days, the presence of a single alate gyne among foraging workers was recorded twice at the same aphid colony. Each time the gyne was seen at the aphids during sunny warm weather, when the foraging activity of the *D. quadripunctatus* workers was highest.

At the end of July, a ladybird (*Adalia bipunctata* L.) appeared at the aphid colony. Within three days, without being counteracted by the ants, it ate all the aphids. In the following days also the other two colonies of *P. juglandis* were consumed, which put an end to the observations. The lack of defense of the food resources by *D. quadripunctatus*

Figure 1. Site of observation; in the foreground, the young walnut with aphids, above (along the stairs), the black elder bush. The location of the *D. quadripunctatus* nest is indicated with an arrow. Photo taken on 16.07.2017 (Tadeusz Berliński).

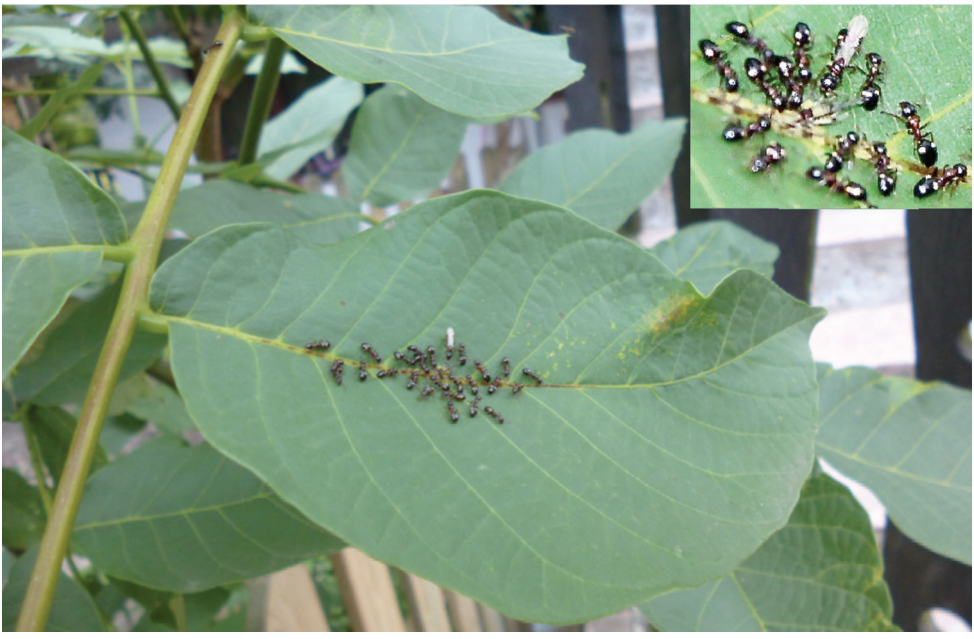

Figure 2. Aphids *Panaphis juglandis* visited by workers and alate gyne of *D. quadripunctatus*. Photo taken on 16.07.2017 (Tadeusz Berliński).

was also revealed by multiple observations of the syrphid fly which, not disturbed by ants, licked the honeydew in the immediate vicinity of them. Concurrently, elsewhere in the garden, *D. quadripunctatus* foragers used experimentally placed baits with diluted honey, but withdrew on the appearance of *Lasius niger* (L.) workers.

The foraging trail along which the alate gynes walked to the aphid colony on the young walnut, led, inter alia, through the trunk and twigs of a black elder (*Sambucus nigra* L.) shrub. The critical point of the route was the passage from the elder leaf to the handrail of the stairs (Fig. 1). When even a slight wind made the leaves touch the railing intermittently, the ants managed to overcome the gap.

Discussion

Foraging of inseminated dealate young gynes is not uncommon in ants. It is the norm in a lot of species which found new colonies semi-claustrally (e.g. Fridman and Avital 1983; Johnson 1996; Brown 1999; Brown and Bonhoeffer 2003; Peeters and Molet 2009), although the evolutionarily derived feature, claustral colony founding (where queens do not leave the nest), is the common mode in extant ants (Hölldobler and Wilson 1990). Species with semi-claustral colony founding with foraging dealate queens are known especially in the tropics and subtropics (e.g. Anderson et al. 2006, Brown 1999, Johnson 1996, 2002, Lenoir and Dejean 1994). In temperate regions, the dealate, mated fundatrices (founding queens) of *Manica rubida* and *Myrmica* species leave their nest to forage still after the first overwintering (Le Masne and Bonavita 1969; Elmes 1982; Lenoir et al. 2010).

In Dolichoderinae, foraging of queens is scarcely documented. It was only recently documented within the genus *Technomyrmex* Mayr which includes many tramp species; ergatoid (worker-like) queens of *T. vitiensis* Mann were foraging outside the nest with workers (Väänänen et al. 2018). In the light of this scarceness, the foraging of the alate *Dolichoderus quadripunctatus* gyne(s) is peculiar – overall, such foraging of alate ant gynes seems unusual (or at least unreported) in nature. The mating status and possible hibernation of the *D. quadripunctatus* gyne(s) observed by us remain unknown. A comparable case concerns the poorly known microgyne caste in *Manica rubida*, whose status also remains unrecognised. In the colony, the alates seem to fulfil the task of workers by foraging outside the nests (Lenoir et al. 2010).

In the following, we will discuss this new finding in *D. quadripunctatus* in the light of the biology and life history of the species. It is a Euro-West-Siberian xerothermophilic oligotope of warm bright forests, also found in old orchards, gardens and parks. It is a dendrobiontic species which forages almost exclusively on trees (mainly in canopies) and is well adapted to move around the leaves (Seifert 2008). The agility of the species to move in an unstable environment and ability to find alternative routes (including a few metres long nylon washing lines) was also evident in our long-standing observations.

The species avoids encounters with heterospecific ants (Torossian 1979; Czechowski et al. 2012; Seifert 2018), although a mere high number of workers may displace

other species to the periphery of its foraging area (Stukalyuk 2018). Our own observations on the non-aggressive behaviour of the *D. quadripunctatus* workers against the predatory ladybird beetle which consumed the aphids, and against the honeydew-consuming syrphid fly, may be interpreted such that the honeydew source was a relatively marginal one in the resource spectrum of this ant, which is basically a predatory species (Seifert 2018). Our *D. quadripunctatus* gyne(s) and workers were observed only to lick honeydew from the leaves, not to milk the aphids as it is the norm in true trophobiosis where the ants in return offer protection to the aphids (Hölldobler and Wilson 1990). Consumption of dropped honeydew without trophobiosis seems to be the usual way of aphid attendance for *D. quadripunctatus* (Seifert 2008) as for many other arthropods which feed on excrements of aphids on the leaves (Ulyshen 2011).

It is noteworthy that Stukalyuk (2018), while working on the supercolony of *D. quadripunctatus*, never observed alate gynes on the foraging trails, even though he saw them outside the nests (pers. comm. to WCz). This indicates that some specific circumstances may trigger the visits of *D. quadripunctatus* alate gynes to extra-nidal food sources. Here, we suggest two, not mutually excluding, explanations for this seemingly rare behaviour – remembering that seeming rarity is not synonymous with insignificance.

An evident first reason for the alate gyne(s) to leave the nest and follow worker trails to aphid colonies is an acute shortage of food in the nest. Indeed, in Poland the spring of 2017 was late and cool, and the summer generally rainy and unfavourable for insects, when alate gynes of *D. quadripunctatus* were observed at the aphids. The supposition that hunger drives alate gynes to forage is justified in light of the observation of Torossian (1968), who stated that the workers do not display special care for young gynes gathered together in queenless auxiliary nests from their emerging to mating time.

The impact of evident food shortage on the behaviour of ants, probably caused by an exceptionally dry summer, was reported by Vepsäläinen and Czechowski (2014). *Myrmica rugulosa* Nyl., a submissive species at the bottom of the ant competition hierarchy, temporarily took over groups of the aphid *Stomaphis quercus* (L.), which is obligately associated with the highest-level territorial ant species *Lasius fuliginosus* (Latr.). This unique intrusion lasted at least two weeks, during which *M. rugulosa* workers successfully brought honeydew milked from the *S. quercus* back to their nest(s). As in many other rare observations, one chooses the best explanation on indirect evidence. In this case, because the drought affected severely the herbs which harbour aphids normally used by *M. rugulosa*, the ants probably were forced to search for supplementary food. Here, the successful change of behaviour probably helped the colony to survive the drought period. As comes to the foraging of the alate gynes of *D. quadripunctatus*, the primary benefit was at the individual level.

To see how the foraging of alate gynes could contribute to the colony dynamics, an introduction to the social structure of a *D. quadripunctatus* colony is at place. Typically, it is monogynous polydomy with one main queenright nest and a few branch nests inhabited by workers with possible offspring. This structure is caused by the small size of individual nest spaces which most often can harbour maximally several tens of individuals; the entire colony usually consists of several hundred, rarely over 1000 adults

(Torossian 1960, 1967a; Seifert 2018). The scattered nest loci of the colony enable the species the following specific life-history features: (1) males arise only from eggs laid by workers; (2) worker oviposition is possible only away from the queen; (3) larvae can develop to gynes only without contact with the queen; (4) insemination of gynes frequently takes place inside or close to the nest; (5) to initiate a colony, the gyne needs to be adopted by queenless workers (Torossian 1967b, 1968, 1974; Seifert 2018).

Direct observations on how a gyne is able to find a queenless workforce and start a colony are lacking. It has been suggested that a group of workers may take the gyne to a queenless outpost nest (Torossian 1974). We suggest here an additional possibility, where gynes which visit aphid colonies might serendipitously take a trail back to a queenless nest and be accepted by its workers. The potential for success of such gyne behaviour could be highest in large supercolonies – like the one studied by Stukalyuk (2018) – where foraging trails of a high number of nests merge on a tree trunk, lead up to the crown, to spread there to a number of food sources (S. Stukalyuk, pers. comm. to WCz).

If our above suggestion has any value in the colony dynamics of *D. quadripunctatus*, the question remains, why alate gynes seem to be only very occasional visitors at aphid colonies. One evident reason is that the yearly number of gynes produced by a colony is low (Torossian 1974) and secondly, that the species is an arboreal ant which has usually most of its nests high up in the tree crowns, and which only exceptionally forages in the lower layers of vegetation (Seifert 2018). Simply, observations on what goes on in the main space of the colonies are needed in order to test our suggestion.

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