Fossil Platygastroidea in the National Museum of Natural History, Smithsonian Institution

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Abstract

Platygastroid wasps preserved in Dominican amber and oil shale from the Kishenehn formation (Montana, USA) in the National Museum of Natural History are catalogued. Compression fossils in Kishenehn oil shale yield a specimen of Fidiobia, a specimen of Telenominae, and a specimen with a Scelio-type ovipositor system. Twenty-five described genera are documented from Dominican amber, all of which are known from the extant fauna: Allostemma Masner & Huggert, Anadophagus Ashmead, Calliscelio Ashmead, Calotelea Westwood, Dutia Nixon, Embidobia Ashmead, Embioctonus Masner, Fidiobia Ashmead, Gryon Haliday, Idris Förster, Inostemma Haliday, Leptacis Förster, Leptoteleia Kieffer, Macroteleia Kieffer, Odontacolus Kieffer, Opisthacantha Ashmead, Parabaeus Kieffer, Paridris Kieffer, Platygaster Latreille, Plaumannion Masner & Johnson, Probaryconus Kieffer, Psilanteris Kieffer, Spiniteleia Masner, Telenomus Haliday, and Triteleia Kieffer. Fourteen of these genera do not have previously published fossil records and are here documented for the first time. Plaumannion fistulosum Talamas, sp. n., and Paridris yumai Talamas, sp. n. are described as new species. A phylogenetic analysis of Paridris including P. yumai is presented. A male specimen belonging to an undescribed scelionine genus is documented and illustrated, but not described, as the best features for circumscribing this taxon are found in the female, and monographic work on this group is currently underway by other workers. Four specimens from Baltic amber, belonging to Leptacis, Platygaster, and Sembilancera Brues are presented for comparison to extant specimens and inclusions in Dominican amber.

Keywords

Platygastroidea, Platygastrinae, Scelioninae, Telenominae, Sceliotrachelinae, amber, Miocene, Eocene, taxonomy
Introduction

Our study of the amber collection of the National Museum of Natural History is preceded and enabled by the efforts of Alexandr Rasnitsyn, who identified hymenopteran inclusions to family in 1989. Here we continue this process and provide a finer taxonomic resolution for specimens of Platygastroidea. For most of the genera presented we did not describe the species as new for multiple reasons. First, examples exist of extant species that are found in amber (e.g. Palaeogryon musebecki Masner) and we choose not to operate under the assumption that the species covered here are new simply because they are fossil specimens, particularly because Dominican amber is relatively young. Second, quality morphology-based taxonomy requires examination of primary types and specimens from a broad geographical range to provide a context for interpreting morphology and intraspecific variation. Without synthetic work that provides a sound basis for accurate identification, the description of new species is of little use to taxonomy and can result in the proliferation of unstable species names, which are ultimately detrimental to understanding biodiversity and evolutionary history. Lastly, specimen location and orientation, whether within an amber matrix or a compression fossil, often prevent a complete examination. From our perspective, it makes little sense to describe a new species (or genus) based on specimens with limited assessable morphology without knowing from examination of other species that it can be reliably identified.

We think it is noteworthy that more than half of the fossil platygastroid genera were described by authors who have not published on the extant fauna of Platygastroidea (Meunier 1917, Starz 1938, Schlüter 1978, Carpenter 1992, Nel and Azar 2005, Nel and Prokop 2005, Poinar and Buckley 2012, Ortega-Blanco et al. 2014). Such disjunction does not preclude these works from being useful, but a lack of experience with morphological diversity and character systems within the group is at times evident. We are particularly critical of Ortega-Blanco et al. (2014) who erected 12 new genera, many on the basis of characters that commonly vary within extant genera (notaulus, number of clavomeres, presence of an occipital carina, metasoma length). Perhaps most disappointing in Ortega-Blanco et al. (2014) is the lack of discussion or examination of previously described fossil taxa. Such purely additive taxonomy, without revision of pre-existing concepts, rapidly leads to a foggy classification, increasing the burden on future taxonomists. Furthermore, if intermediate stages of evolution are to be identified that link the stem and crown groups, then the terminals must first be understood well enough to make sense of the fossils and produce meaningful hypothesis about the evolution of this group.

In recent decades very few fossil species have been described from extant genera, and with varying degrees of quality. Perrichot et al. (2014) described a new species of Macroteleia, diagnosed on the basis of Musebeck’s (1977) revision of this genus in the New World. Their treatment provides excellent photographs and a reasonable description, but does not mention the examination of any specimens of Macroteleia other than the fossil at hand, despite the availability of Musebeck’s material in
North American collections. We compared their images of *M. yaguarum* to the lectotype of *M. surfacei* (USNMENT00989887) and note that the diagnosis provided by Perrichot et al. (2014) lacks what we consider to be the most obvious characters to separate *M. yaguarum* and *M. surfacei*: the structure of the metascutellum and the sculpture of the posterior mesoscutum. The small amount of effort required to borrow relevant specimens would have greatly increased the diagnostic value of their work and, in our opinion, illustrates an unnecessary split between the taxonomy of the fossil and extant fauna. To facilitate future studies in *Macroteleia*, photographs of Muesebeck’s primary types deposited in the USNM, and representatives of all other species identified by him for which we have material at hand, are now publicly available in Specimage.osu.edu.

Buhl (2002) described three species of platygastids from Baltic amber with descriptions, diagnoses and illustrations that are inadequate for species-level identification for these speciose genera, and will eventually require redescriptions, reillustration, and rediagnosis. This kind of casual taxonomy, providing little more than a name that must be formally dealt with by future taxonomists, is proving to be a great hurdle in the advancement of biodiversity science.

The careful circumscription of fossils has taken on an even greater importance in systematics with recent advancements in phylogeny dating techniques (reviewed by Brady 2011). In some cases of higher-level phylogenetic reconstruction, a solid genus-level identification of a fossil group can have a profound impact on determining the date of particular nodes in the resulting chronogram; in fact, some fossil calibration points can affect rather distant nodes in the tree (Buffington et al. 2014). For the purpose of dating phylogenies, generic identification alone is a useful contribution, and in cases where morphology is included in phylogenetic reconstruction (Ronquist et al. 2012), the morphology of the specimens can be used for node dating without formal designation of a name. It should be pointed out that, again, for a fossil to be included within the dating analysis as a terminal, the taxon itself should be well circumscribed and based on specimens with sufficiently visible characters. An error at this stage, due to taxonomic error or misinterpretation of a character, could result in misleading conclusions.

Our goals here are to provide generic identifications to facilitate incorporation of these fossils in thorough species-level revisionary work, including photographs that will allow a first-pass assessment of the specimens. Our photographic efforts focused on specimens that were well preserved, easily photographed, and of morphological interest. We here include images for all genera that we identified, even in cases where the images leave much to be desired. Our philosophy is that an imperfect photograph is better than none at all. Taking the above statements into account regarding haphazard description of fossil species, we are confident in assigning names to two new species discovered through the course of this research.

*Plaumannion fistulosum* Talamas, sp. n. and *Paridris yumai* Talamas sp. n., are herein described. *Plaumannion* has only two previously described species (Masner et al. 2007), and the New World fauna of *Paridris* was recently revised by Talamas et al. (2012). High quality images for all species were provided by both Masner et al. (2007)
and Talamas et al. (2012), enabling us to determine that these species are both new to science and identifiable by the characters presented here.

Specimens that fit easily into extant genera are presented here simply as fossil records at the generic level. For other taxa, nuanced commentary is warranted and can be found after the heading for each taxon.

**Family-level classification**

Classification in Platygastroidea at the family-level has undergone changes in the past decade, enacted by workers who do not specialize on the systematics of this taxon and who provided no new analysis of relationships within it (Sharkey 2007, McKellar and Engel 2012). McKellar and Engel (2012), without explanation, treated Platygastridae as having a single subfamily. In the absence of analysis, formal or otherwise, we reject this change to the classification and here present fossil specimens organized by subfamily: Platygastrinae and Sceliotrachelinae in Platygastridae, and Scelioninae and Telenominae in Scelionidae.

**Materials and methods**

**Collection**

All specimens, excluding those in Baltic amber, are housed in the Department of Paleobiology, National Museum of Natural History, Smithsonian Institution. Inquiries regarding examination or loan of material should be directed to Dr. Conrad Labandeira (labandec@si.edu). The material in Baltic amber was sent to us for identification, and where the specimens will ultimately be deposited is presently unclear, but can be tracked via the specimen CUIDs.

**Informatics**

Collecting unit identifiers (CUIDs) were assigned only to the specimens that we photographed, which includes all specimens treated taxonomically (Paridris yumai sp. n. and Plaumannion fistulosum sp. n.). Suppl. material 1 provides the complete list of specimens examined, taxonomic determinations, USNM catalog numbers, and CUIDs, when applicable. The collection data for these specimens can be found in the Hymenoptera Online Database (http://purl.oclc.org/NET/hymenoptera/hol) by entering the specimen identifier (CUID) in the search form.

Occurrence records were exported from xBio:D in a Darwin Core Archive, with records to specimen images included, using Audubon Core vocabularies. These
records were subsequently loaded into the xBio:D IPT <http://xbiod.osu.edu/ipt/> as a new resource titled, “Fossil Platygastroidea of the USNM.” The xBio:D IPT uses the GBIF Integrated Publishing Toolkit software (Robertson et al. 2014) to disseminate biodiversity with the research community via Darwin Core Archives and is the preferred data harvesting method for many biodiversity aggregators, e.g., iDigBio, GBIF, etc.

Photography

Images were produced using a Microvision Instruments imaging system with Cartograph software, a Z16 Leica lens and a JVC KY-F75U digital camera. Single montage images were produced from image stacks with the program CombineZP. In some cases, multiple montaged images were stitched together in Photoshop to produce larger images at high resolution and magnification. Full resolution images, and additional photographs of the taxa treated here, are archived in Specimage, the image repository associated with the Hymenoptera Online Database (http://purl.oclc.org/NET/hymenoptera/specimage) and MorphBank (http://www.morphbank.net).

Images in Morphbank are organized according to the their level of identification. Links to collections of the full resolution images are provided following the header for each taxon and include many images not included in the figures. In some cases, the photographs in the figures are flipped horizontally to provide consistent views of the specimens, with the head to the left. The images in Morphbank and Specimage are presented in their original orientations.

The images in Specimage may be retrieved by searching the CUID or the taxon name, and are organized in the project “Fossils of Platygastroidea in the USNM”.

Morphological terms

Our terminology largely follows Mikó et al. 2007, with a notable exception. We found that striation radiating from the pleurostomal condyle anterior to the malar sulcus and striation posterior to the malar sulcus are independent characters. We here provide an alternative concept for facial striae: “the anatomical cluster anterior to the malar sulcus that is composed of carinae radiating from the pleurostomal condyle”. Similarly, malar striae are defined as “the anatomical cluster posterior to the malar sulcus that is composed of carinae radiating from the pleurostomal condyle”. For specimens in which striation exists but there is no malar sulcus, we apply the term “periepistomal striation”. These terms, and others used in the text, are matched to anatomical concepts in the Hymenoptera Anatomy Ontology (HAO) (Yoder et al. 2010). A full list of morphological terms, definitions, and URLs to additional information in the HAO is provided in Suppl. material 2.
Character annotations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>bs</td>
<td>basiconic sensillum (Figs 44–45)</td>
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<tr>
<td>eps</td>
<td>episternal foveae (Fig. 94)</td>
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<td>fs</td>
<td>facial striae (Fig. 95)</td>
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<td>mees</td>
<td>mesepimeral sulcus (Fig. 94)</td>
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<td>mp</td>
<td>mesopleural pit (Fig. 94)</td>
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<td>ms</td>
<td>malar striae (Figs 88, 90–91)</td>
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<td>pssu</td>
<td>prespecular sulcus (Fig. 94)</td>
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<tr>
<td>sc</td>
<td>submedian carina on T1 (Fig. 80)</td>
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<tr>
<td>shms</td>
<td>mesoscutal suprahumeral sulcus (Fig. 80)</td>
</tr>
<tr>
<td>sk</td>
<td>sublateral keel (Fig. 80)</td>
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<td>sv</td>
<td>submarginal vein (Fig. 12)</td>
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**Platygastrinae**

*Allostemma* Masner & Huggert
http://bioguid.osu.edu/xbiod_concepts/7818
Figures 1–4; Morphbank¹

**Comments.** Figures 1 and 3–4 illustrate specimens of *Allostemma* for which all of the characters were congruent with the diagnostic characters of Masner and Huggert (1989), with the exclusion of the tibial spurs, which we were unable to observe. The form of the extruded ovipositor system provides additional support for our generic determination (Fig. 2), given that this particular form of the ovipositor system is not known to us from other platygastrine genera. This specimen has a distinct break in the submarginal vein of the fore wing, directly proximal to the forked terminus— a character state that we have not yet observed in extant specimens of this genus.

*Inostemma* Haliday
http://bioguid.osu.edu/xbiod_concepts/7832
Figure 7; Morphbank²

**Comments.** Our determination of this specimen as *Inostemma* is based primarily on the 4-merous antennal clava and the submarginal vein of the fore wing terminating in a knob; the presence or absence of the felt field on metasomal S2 was not observable. The terminus of the submarginal vein is slightly bilobed in this specimen, a condition that we have not seen previously in *Inostemma*, but which we here attribute to intrageneric variability.
**Leptacis Förster**

http://bioguid.osu.edu/xbiod_concepts/7836

Figure 5–6; Morphbank⁴

**Comments.** *Leptacis* in Baltic amber (Fig. 6), as far as we can observe, exhibits characters used to identify this genus that appears entirely congruent with concepts of this genus based on extant specimens.

**Platygaster Latreille**

http://bioguid.osu.edu/xbiod_concepts/7843

Figures 8–13; Morphbank⁴

**Comments.** Hypotheses about the evolution of *Platygaster* are hampered by the nebulous limits of this genus and the huge number of species in the group that have yet to be divided into meaningful subgeneric groups. We found no characters among the species of *Platygaster* in Dominican amber that cannot be found in extant species. Examination of a specimen in Baltic amber (Figs. 12–13), which we here classify as *Platygaster*, exhibits two noteworthy characters: the submarginal vein in the fore wing is well defined and extends for about one quarter of the wing’s length (Fig. 12) and A8–A9 strongly project anteroventrally (Fig. 13), similar to A7–A8 in *Allotropa* (Fig. 14).
Figure 3–5. 3 *Allostemma*, female (USNMENT01059432), head, mesosoma, metasoma, dorsal view 4 *Allostemma*, female (USNMENT01059432), head, mesosoma, ventrolateral view 5 *Leptacis*, female (USNMENT00903167), head, mesosoma, metasoma, posterodorsal view.

Unplaced Platygastrinae
Morphbank

Sceliotrachelinae

*Fidiobia* Ashmead
http://bioguid.osu.edu/xbiod_concepts/7866
Figures 15–18; Morphbank

Comments. *Fidiobia* was the most abundant genus in the material we examined, undoubtedly the result of their association with the eggs of phytophagous beetles, Chrysomelidae and Curculionidae (Masner and Huggert 1989), many of which are associated with trees.
Figure 6–7. 6 Leptacis, female (USNMENT01109283b), head, mesosoma, metasoma, dorsolateral view
7 Inostemma, female (USNMENT00764965), head, mesosoma, metasoma, dorsolateral view. Scale bars in millimeters.

Parabaeus Kieffer
http://bioguid.osu.edu/xbiod_concepts/7872
Figure 19; Morphbank

Telenominae

Telenomus Haliday
http://bioguid.osu.edu/xbiod_concepts/605
Figures 20–24; Morphbank
**Telenominae**  
Figure 25; Morphbank^9

**Comments.** We interpret specimen USNMENT00877715 to be the oldest known telenomine based on T2 as the largest tergite, broad laterotergites loosely attached to the sternites, 11-merous antenna, and wing venation that is typical for Telenominae.

Brues (1940) described *Telenomus (Dissolcus) electrus* from Baltic amber with some characteristics that are not found in extant telenomines- 12-merous antennae with a 7-merous clava, neither of which are known to us from the extant fauna. These may represent plesiomorphic states for the subfamily or may indicate that *Telenomus electra* belongs to a scelionine genus with which these characters are consistent.
Scelioninae

*Aradophagus* Ashmead

http://bioguid.osu.edu/xbiod_concepts/451

Figure 27; Morphbank

**Comments.** The presence of *Aradophagus* in amber is not surprising, given their association with spiders (Vetter et al. 2012), which is the presumed reason for the abundance of *Idris* in amber.
Figure 12–14. 12 Platygaster, female (USNMENT01109284), head, mesosoma, metasoma, lateral view
13 Platygaster, female (USNMENT01109284), head and antennae, lateral view 14 Allotropa, female (OSUC 404951), head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.

Calliscelio Ashmead
http://bioguid.osu.edu/xbiod_concepts/461
Figures 31–32; Morphbank11

Calotelea Westwood
http://bioguid.osu.edu/xbiod_concepts/462
Figures 33–36; Morphbank12
Comments. *Calotelea* is abundant in Dominican amber, represented by perhaps half a dozen species in the USNM collection. Consequently, there are many beautifully preserved specimens that will be very useful for work at the species level when the Neotropical fauna of this genus is revised.

*Duta* Nixon

http://bioguid.osu.edu/xbiod_concepts/474

Figures 37–38; Morphbank

Comments. Figures 37–38 illustrate what we consider to be a typical form of *Duta*. The robust skaphion, absence of malar and facial striae, and simple metascutellum are all useful diagnostic characters. Elongation of A3–A5 is commonly found in this genus, and is expressed here in a moderate form.
Figure 18. *Fidiobia*, female (USNMENT00877716), head, mesosoma, metasoma, lateral view. Scale bars in millimeters.

Figure 19. *Parabaeus*, female (USNMENT01059078), head, mesosoma, metasoma, lateral view. Scale bars in millimeters.
Figure 20. *Telenomus*, female (USNMENT00989561), head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.

Figure 21–24. *Telenomus*, 21 female (USNMENT01059075), head, mesosoma, metasoma, lateral view 22 female (USNMENT00989564), head, mesosoma, metasoma, dorsolateral view 23 female (USNMENT00989562), head, mesosoma, metasoma, ventrolateral view 24 female (USNMENT01059092), head, mesosoma, metasoma, ventral view. Scale bars in millimeters.
Figure 25–26. 25 Telenominae, female (USNMENT008777715), head, mesosoma, metasoma, lateral view. 26 Scelioninae, female (USNMENT00979592), head, mesosoma, metasoma and ovipositor, dorsal view. Scale bars in millimeters.

**Embidobia Ashmead**

http://bioguid.osu.edu/xbiod_concepts/478
Figures 28, 39–42, 45, 47; Morphbank

**Comments.** The 3-merous clava of the specimen illustrated in Figures 39–42 initially led us to identify it as *Palaeogryon*. Figure 40 illustrates our first view of the specimen, including the wing venation, the stout bristles along the submarginal vein, and the 3-merous clava that are shared by *Embidobia* and *Palaeogryon*. Upon cutting the amber to fully observe the number of antennomeres it became clear that this species belongs to *Embidobia*, and we here reevaluate some of the characters used to diagnose these genera.
The clava in platygastroids has been defined in two ways, by width of the antennomere and by the presence of basiconic sensilla (Bin 1981). Masner (1976) used size to define clavomeres, and in most *Embidobia* A8 is distinctly larger than A7, resulting in its designation as a clavomere. The presence of basiconic sensilla provide an unambiguous means of designating clavomeres but can be challenging to observe in small specimens. Despite this, we prefer the latter definition, and clarify that all *Embidobia* that we have examined have a 3-merous clava based on the presence of basiconic sensilla. Clavomeres tend to be larger than non-clavomeres, and so size is sometimes a useful, but imperfect, proxy for the presence of sensilla when the sensillar structures are not within view.

Figure 48 illustrates the metascutellum of *Palaeogryon*, which is sufficiently developed posteriorly to be unreliable, in our opinion, for separating this genus from *Embidobia* (compare to Figures 40, 42). Masner (1976) also used the frontal depression (antennal scrobe) to separate *Embidobia* from *Palaeogryon*. We do not consider the dorsal limit of the antennal scrobe to be sufficiently defined to warrant its use in
separating these genera (see Figs 46–47). However, *Embidobia* and *Palaeogryon* remain easily identifiable by the number of antennomeres, 9 in *Palaeogryon* and 11 in *Embidobia*, and so reliance on these subtly differing characters is not necessary.

*Palaeogryon* is remarkably similar to *Sembilanocera* Brues (Baltic amber, Figures 49–50) with which it shares 9-merous antennae with a 3-merous clava, and a sharp angle in the submarginal vein at its intersection with the basal vein (or remnants of
the basal vein). The two may be separated by characters used in the generic description of *Palaeogryon* (Masner 1969): postmarginal vein much longer than stigmal vein; submarginal, marginal, and postmarginal veins with large bristles; frontal depression present; ocelli distant from inner orbits of eye. In *Sembilanocera*, the postmarginal and stigmal vein are approximately equal in length; the wing veins lack bristles; the frontal depression is absent, and the ocelli are close to the inner orbits.
Figure 35–36 *Calotelea* 35 female (USNMENT01059376), head, mesosoma, metasoma, lateral view 36 female (USNMENT01109099), head, mesosoma, metasoma and ovipositor, dorsal view. Scale bars in millimeters.
Figure 37–38. *Duta*, female (USNMENT01059071) 37 head, mesosoma, metasoma and ovipositor, dorsolateral view 38 head, mesosoma, metasoma and ovipositor, ventrolateral view. Scale bars in millimeters.

*Embioctonus* Masner
http://bioguid.osu.edu/xbiod_concepts/479
Figures 29, 43–44; Morphbank¹⁵

Comments. In addition to the characters presented by Masner (1980), *Embioctonus* may be separated from *Embidobia* by the incomplete or absent malar sulcus (Fig. 43).

*Gryon* Haliday
http://bioguid.osu.edu/xbiod_concepts/487
Figures 51–54; Morphbank¹⁶
Figure 39–40. *Embidoobia*, female (USNMENT01109100) 39 head, anterior view; mesosoma and metasoma, ventrolateral view 40 head, mesosoma, metasoma, posterolateral view. Scale bars in millimeters.
Figure 41–42. *Embidobia*, female (USNMENT01109100) 41 head, lateral view; mesosoma and metasoma, ventrolateral view 42 head, mesosoma, metasoma, anterodorsal view. Scale bars in millimeters.

**Idris Förster**
http://bioguid.osu.edu/xbiod_concepts/496
Figures 55–62; Morphbank.

**Comments.** Two generic concepts exist for *Idris*. One includes species in which females have T1 expanded dorsally into a horn and the other concept excludes these species, placing them in *Ceratobaeus* Ashmead. Masner and Denis (1996) treated *Ceratobaeus* as a jun-
Figure 43–48. 43 *Embioctonus*, female (USNMENT01059112), head, ventrolateral view 44 *Embioctonus*, female (USNMENT01059113), antennal clava, lateral view 45 *Embidobia*, female (USNMENT01059111), antennal clava, ventral view 46 *Palaeogryon muezebecki*, holotype female (USNMENT01059240), head and antennae, anterolateral view 47 *Embidobia urichi*, paralectotype female (USNMENT01109249), head and antennae, anterior view 48 *Palaeogryon*, female (OSUC 404926), head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.
ior synonym of *Idris* based on their analysis of Nearctic *Idris* in which they stated that the horn of T1 is variable within multiple species groups of *Idris*, and thus the horn does not correspond to a monophyletic group. Iqbal and Austin (2000) resurrected *Ceratobaeus* on more utilitarian grounds. They argued that *Ceratobaeus* provided a useful means of categorizing species for identification and that the possible polyphyly of *Idris* was justification for maintaining a polyphyletic *Ceratobaeus*. The molecular phylogeny of the tribe Baeini by Carey et al. (2007) retrieved both *Idris* and *Ceratobaeus* as polyphyletic, a state that could be resolved by treating *Ceratobaeus* and *Hickmanella* Austin as junior synonyms.

We here follow the classification of Masner and Denis (1996) and treat species with a horn on T1 as part of *Idris*. We are not making nomenclatural acts in either *Idris* or *Ceratobaeus* and without comprehensive study of them do not wish to do so, particularly because a phylogenetic study of generic relationships in Platygastroidea, conducted as part of the Platygastroidea Planetary Biodiversity Inventory (PBI) is currently in its final stages and will provide the most thorough analysis of the group to date.

**Leptoteleia Kieffer**
http://bioguid.osu.edu/xbiod_concepts/501
Figure 63; Morphbank

**Macroteleia Westwood**
http://bioguid.osu.edu/xbiod_concepts/504
Figure 64; Morphbank

**Odontacolus Kieffer**
http://bioguid.osu.edu/xbiod_concepts/525
Figure 30; Morphbank

**Opisthacantha Ashmead**
http://bioguid.osu.edu/xbiod_concepts/527
Figures 66–69; Morphbank

**Paridris yumai Talamas, sp. n.**
http://zoobank.org/0BB0E67F-8B31-4DFC-93E9-CCEED5CD3FFB
http://bioguid.osu.edu/xbiod_concepts/386112
Figures 70–73; Morphbank
Figure 49–50. *Sembilanocera*, female (USNMENT01109280) **49** habitus, dorsolateral view **50** habitus ventrolateral view. Scale bars in millimeters.

Transverse pronotal carina: present in posterior half of pronotum. Shape of pronotal shoulder in dorsal view: narrow and striplike. Form of pronotal suprhumeral sulcus: areolate.


Form of metascutellum in female: bispinose. Form of metascutellum in male: bispinose.


**Diagnosis.** *Paridris yumai* can be separated from most species of *Paridris* by the smooth clypeus that is narrower than the width across the toruli (Fig. 72, as in Fig. 77). In the vast majority of species the clypeus is distinctly wider than the distance across the toruli and ventrally serrate. This narrow form of the clypeus is shared in the New World by just one other species, *P. armata* Talamas (Fig. 77). *Paridris lemente* Talamas & Masner is similar in that the clypeus is not wider than the distance across the toruli, but the ventral margin is serrate (Fig. 76).
In *P. yumai* the metascutellum is posteriorly emarginate, forming two lateral spines, separating it from *P. lemete* in which the posterior margin of the metascutellum is straight, forming a transverse strip (Fig. 74). Females of these species may also be separated by the basiconic sensilla on A8: two are present in *P. lemete* and one in *P.*
Paridris yumai is known only from a male specimen, which can be separated from males of *P. yumai* by the coarsely foveate mesoscutellum (Fig. 75).

**Etymology.** This species is named for djembe teacher and herbalist, Yuma "Dr Yew" Bellomee, as an expression of appreciation. Yuma’s influence has increased the mental and physical health of the first author, in turn producing a positive effect on scientific productivity and general happiness.

**Phylogenetic analysis.** We coded the morphology of *P. yumai* in the matrix provided in Talamas et al. (2013) and reran the combined analysis of molecular and morphological
data using the same tree search parameters in TNT (Goloboff et al. 2008). Inclusion of *P. yumai* decreased the resolution between species within *Trichoteleia* Kieffer, and in *Paridris*, but not within the *nephta* and *pallipes* species groups (Fig. 78). The characters of *Paridris yumai* that we were unable to code due to artifacts associated with the amber matrix are likely culprits in adding ambiguity to the phylogenetic matrix, and thus the decreased resolution.

**Link to distribution map.** [http://hol.osu.edu/map-large.html?id=386112](http://hol.osu.edu/map-large.html?id=386112)

Figure 74–77. 74 Paridris lemete, paratype female (OSUC 334091), mesosoma and T1, dorsolateral view 75 Paridris armata, holotype male (OSUC 181352), head and mesosoma, dorsal view 76 Paridris lemete, holotype female (OSUC 334096), head, anterior view 77 Paridris armata, holotype male (OSUC 181352), head, anterior view. Scale bars in millimeters.

Plaumannion fistulosum Talamas, sp. n.
http://zoobank.org/C32D000A-111C-4E96-B0EC-4C1DBFA649AF
urn:lsid:biosci.ohio-state.edu:osuc_concepts:344197
Figures 79–82; Morphbank23

Description. Female body length: 1.40 mm (n=1). Sculpture of head: finely rugulose throughout. Occipital carina: complete. Cells along anterior margin of occipital carina: increasing in size ventrally.

Figure 78. Strict consensus of 1001 most parsimonious trees. Bootstrap values above 50 indicated on tree.
Figure 79. *Plaumnann fistulosum*, holotype female (USNMENT00903996), head, mesosoma, metasoma, ventral view. Scale bar in millimeters.


**Diagnosis.** *Plaumannion fistulosum* is closest to *P. fritzi* with which it shares a submedian carina on T1, coarse sculpture of the mesonotum and tergites, and a distinguishable mesoscutal suprahumeral sulcus. These two species can readily be separated by the sculpture of the head: finely rugulose in *P. fistulosum* and coarsely areolate in *P. fritzi*; and by the sculpture in the dorsal part of the lateral pronotum: finely striate in *P. fistulosum* and smooth in *P. fritzi*. *Plaumannion fistulosum* is best separated from *P. yepezi* by the coarse and deep sculpture of the mesoscutum and mesoscutellum, the distinct mesoscutal suprahumeral sulcus, and the presence of submedian carinae on T1. *Plaumannion fistulosum* has 6 visible sternites, whereas *P. yepezi* and *P. fritzi* each have 5. We here expand the generic concept of *Plaumannion* to include species with 6 sternites.

**Etymology.** The epithet fistulosum, meaning “full of holes” refers to the deeply areolate rugose sculpture found on this species.

**Link to distribution map.** [http://hol.osu.edu/map-large.html?id=344197]

**Material examined.** Holotype, female: DOMINICAN REPUBLIC: USNMENT00903996 (deposited in USNM).

**Comments.** *Plaumannion* is an exceptionally rare genus, known from only a handful of specimens. Its presence in amber suggests the possibility that this genus was more common in the past, and perhaps its biology, although unknown, predisposes it to preservation in tree resin.
Figure 80–82. *Plaumannion fistulosum*, holotype female (USNMENT00903996) 80 head, mesosoma, metasoma, dorsolateral view 81 head and mesosoma, ventrolateral view 82 head and mesosoma, lateral view. Scale bars in millimeters.

*Psilanteris* Kieffer
http://bioguid.osu.edu/xbiod_concepts/549
Figure 65; Morphbank²⁵

*Sembilanocera* Brues
http://bioguid.osu.edu/xbiod_concepts/560
Figures 49–50; Morphbank²⁶
Figure 83–84. *Probaryconus* 83 female (USNMENT01109098), head, mesosoma, metasoma, lateral view 84 male (USNMENT01059379), head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.

**Spiniteleia Masner**
http://bioguid.osu.edu/xbiod_concepts/563
Figures 87–92; Morphbank

**Comments.** We here expand the concept of *Spiniteleia* to include a species without a spine on the posterior of the mesoscutellum. Figures 87–89 illustrate a specimen that exhibits nearly all of the diagnostic characters for *Spiniteleia*: malar striae present; facial striae absent; skaphion absent; metascutellum present as a simple smooth strip; *Scelio*-type ovipositor. The dorsal head, mesosoma and anterior metasoma were sufficiently visible for us to observe the smooth and simple mesoscutellum, and the mesoscutum on which we saw no evidence of a skaphion. However, turbidity of the amber precluded us from capturing photographs of these characters. Before expanding the concept of *Spiniteleia*, we considered three similar genera: *Duta, Holoteleia* Kieffer, and *Masnerel-
la Özdikmen. *Masnerella* is similar in its lack of a skaphion (Fig. 93), but we excluded this genus because it has facial striae (Fig. 95), the mesepimeral and prespecular sulci on the mesopleuron are comprised of elongate cells (Fig. 94), and the episternal foveae extend dorsally to the mesopleural pit (Fig. 94). None of these are found in the amber specimen. We excluded it from *Duta* because this genus has a skaphion and lacks malar striae, and excluded *Holoteleia* because this genus lacks malar striae.

This species keys to couplet 36 in Masner’s (1980) key to Holarctic Scelioninae, where it agrees with all of the characters listed for *Spiniteleia*, excluding the spine on the posterior of the mesoscutellum. The mesoscutellar spine of *Spiniteleia* is presumably derived, and thus it is not unreasonable to discover a specimen with the characters of *Spiniteleia* that predates the emergence of the spine. Alternatively, the spine may simply be variable within the genus. Our placement of this species in *Spiniteleia* is sup-
ported by a study of external head morphology in Platygastroidea currently being conducted by the first author. Among the specimens and genera analyzed in this project, *Spiniteleia* alone has the combination of malar striae present, facial striae absent, and a *Scelio*-type ovipositor system. To accommodate the fossil species, either the concept of

Figure 87–91. *Spiniteleia* 87 female (USNMENT01059070), head, mesosoma, metasoma and ovipositor, ventral view 88 female (USNMENT01059070), head, anteroventral view 89 female (USNMENT01059070), head and mesosoma, lateral view 90 female (USNMENT00989622_1), head, anterior view 91 *Spiniteleia campbellii*, paratype female (USNMENT01029349), head, anterolateral view. Scale bars in millimeters.
Holoteleia would be expanded to include species with malar striae, or Spiniteleia would be expanded to include species without a mesoscutellar spine. We rejected expanding the concept of Holoteleia because the state of lacking both facial and malar striae is found in many genera in addition to those mentioned above (Calliscelio, Telenomus, Gryon, Trissolcus, Macroteleia, Palpoteleia Kieffer) and we prefer to lean in the direction of diagnosability when making taxonomic decisions.
Figure 96–97. *Triteleia*, female (USNMENT01059083) 96 head, mesosoma, metasoma, lateral view
97 head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.

*Triteleia* Kieffer
http://bioguid.osu.edu/xbiod_concepts/575
Figures 96–97; Morphbank

Unplaced Scelioninae
Figures 98–99; Morphbank

Ovipositor specimen
Figure 26; Morphbank

Specimen USNMENT00979592 (Fig. 26) is not identifiable to genus but it is a useful record of a *Scelio*-type ovipositor system, which we determined by the following char-
acteristics: The length that the ovipositor extends beyond the apex of the metasoma is as long as the metasoma itself, but the ovipositor itself is visible only in the distal half. Second, near the tip of the ovipositor is a dark area that corresponds to the typical location of sclerotized T7+8 when it is extruded with the ovipositor. Lastly, exsertion of the ovipositor at the time of death occurs commonly in extant scelionines with a Scelio-type ovipositor, but in scelionines with a Ceratobaeus-type ovipositor this is rare, and when it does occur it is not so far extruded.

Among described fossil species with a Scelio-type ovipositor, Chromoteleia theobaldi Maneval, described from Baltic amber, and Macroteleia renatae Szabó & Oehlke, 1986 are potentially the oldest. However, use of these determinations for dating a phylogenetic node may be problematic for multiple reasons. First, Baltic amber is typically dated at 40–60 million years of age, which is a very broad range, and the precise age is
debated (Buffington et al. 2014). The Kishenehn formation has a relatively narrow age range, with the largest window of error spanning from 38.6 to 48.4 mya, providing a significantly more accurate date (Constenius 1996, Greenwalt et al. in press). Second, we have reservations about accepting this determination of *Chromoteleia theobaldi* at face value. Neither the description nor the illustration of this species indicates the presence of a metascutellar plate, a defining character of this genus. The illustration also shows seven visible tergites whereas T7 is internal in extant *Chromoteleia*, unless extruded with the ovipositor system. Maneval’s (1938) illustration may document the preservation of a partially extruded T7 or may simply be incorrect. Alternatively *Chromoteleia* may have exhibited an alternative arrangement of tergites, or this species may belong to a different genus.

The phylogeny of Platygastroidea by Murphy et al. (2007) retrieved genera with a *Scelio*-type ovipositor (*Nyleta* Dodd, *Probaryconus*, *Neoscelio* Dodd) at the base of the main scelionid clade that contains *Idris*, known from Baltic amber, and Telenominae, now known from Kishenehn shale. *Archaeoteleia*, which also has a *Scelio*-type ovipositor system but was not included in the analysis of Murphy et al. (2007), retains many plesiomorphies that suggest its location in the phylogeny is even closer to the split between Platygastridae and Scelionidae. Specimen USNMENT00979592 can easily be distinguished from *Archaeoteleia* on the basis of wing venation and antennal characters (see Early et al. 2007). This record of a *Scelio*-type ovipositor at 45 mya is not particularly illuminating given the age of the group, but it demonstrates the kind of information that a specimen can provide without assigning a taxonomic name.

**Discussion**

With the advent of increasingly sophisticated methods of ancestral state reconstruction, phylogeography and divergence time estimation, the importance of accurate recognition and description of fossil insect specimens continues to increase. Hand in hand, inaccurate paleontological research can undermine these advances, as the methods rely solely on the accuracy of various aspects of calibration and morphological interpretation. This concern is amplified by the fact that fossil species are represented by a paucity of specimens, some of which can be exceedingly difficult to obtain for research purposes (and hence, the universe of characters for examination is very limited). We hope that future work with fossils of this group, as well as all insect paleontology, will be done in concert with research on the extant fauna. Parallel, or even worse, divergent, insect systematics programs lead to a waste of time and resources, neither of which are in surplus in the current research climate.

This publication also highlights the wealth of data potentially available in a research museum, data that is unlocked by the keen eye of trained researchers. In this case, the eyes were separated by a generation and over 25 years, yet without the combined efforts
of A. Rasnitsyn and the authors of this publication, all of the specimens surveyed here would remain in the dark, hopefully awaiting future discovery. This would be a loss for platygastroid systematics, as now we have data indicating that generic limits and ovipositor systems were well formed, in some cases, more than 40 million years ago, and these data may inform current research programs on platygastroids that are of considerable economic importance, e.g. *Paratelonomus* (Gardner et al. 2013) and *Trissolcus* (Talamas et al. 2015). As in all taxonomic research, fossils are a work in progress, and we hope future contributors to the field will find this work useful.

**Acknowledgements**

We extend our thanks to: Mark Florence, Jonathan Wingerath, Dale Greenwalt, Alan Rulis, Conrad Labandeira and Jorge Santiago-Blay (USNM) for making fossil specimens available; Norman Johnson (The Ohio State University) for taxonomic input and identification of *Sembilanocera*; Joe Cora (The Ohio State University) for continual database support and creating the IPT; Lubomír Masner (Canadian National Collection of Insects) for taxonomic input; Andrew Austin (University of Adelaide) for commentary on the systematics of *Idris* and *Ceratobaeus*; Elizabeth Alvarez, Pei Luo, Ashton Smith, Annika Salzberg, and Samantha Fitzsimmons-Schoenberger for photographic contributions; and Laetitia Plaisance (USNM) for translation. This work was made possible by funding from the Systematic Entomology Laboratory, USDA-ARS. The USDA does not endorse any commercial product mentioned in this research. USDA is an equal opportunity provider and employer.

**References**


Endnotes

1 http://www.morphbank.net/?id=853932
2 http://www.morphbank.net/?id=854209
3 http://www.morphbank.net/?id=854215
4 http://www.morphbank.net/?id=854258
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24 http://www.morphbank.net/?id=854275
25 http://www.morphbank.net/?id=854242
Supplementary material 1

URI table of HAO morphological terms
Authors: Elijah J. Talamas, Matthew L. Buffington
Data type: Microsoft Excel Spreadsheet (.xls)
Explanation note: This table lists the morphological terms used in this publication and their associated concepts in the Hymenoptera Anatomy Ontology.
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Supplementary material 2

Specimen determinations
Authors: Elijah J. Talamas, Matthew L. Buffington
Data type: Microsoft Excel Spreadsheet (.xls)
Explanation note: This table lists the determinations and specimen identifiers for fossil specimens of Platygastroidea housed in the National Museum of Natural History.
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Supplementary material 3

Phylogenetic matrix
Authors: Elijah J. Talamas, Matthew L. Buffington
Data type: TNT matrix (.tnt)
Explanation note: This matrix is that of the combined morphological and molecular data in Talamas et al. (2013) with the inclusion of Paridris yumai.
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Nest architecture and pollen hosts of the boreoalpine osmiine bee species Hoplitis (Alcidamea) tuberculata (Hymenoptera, Megachilidae)

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Abstract

Although Hoplitis tuberculata is a rather common bee species in the upper montane and subalpine zone of the Alps, its biology is only fragmentarily known. In the present publication, both nest architecture and pollen host spectrum are described. H. tuberculata nests in insect borings in dead wood, where one to several brood cells are built in a linear series. Examination of four nests obtained from trap nests revealed three peculiar characteristics of its nest architecture: i) the 0.3-0.5 cm thick partitions between the brood cells are three-layered consisting of two walls built from masticated leaves which enclose an interlayer that is densely packed with pebbles, earth crumbs and other small particles; ii) in the majority of the nests, a vestibule varying in length from 2.2-8.9 cm and loosely filled with small particles is present between the outermost cell partition and the nest plug; iii) the nest is sealed by a 1.2-1.9 cm long plug consisting of two walls of masticated leaves which enclose a space that is densely packed with small particles and divided up by one to three additional walls. The nest architecture of H. tuberculata is unique among Palaearctic osmiine bees; however, it corresponds to that of three North American species closely related to H. tuberculata. Microscopical analysis of female pollen loads and brood cell provisions revealed that H. tuberculata is polylectic with a strong preference for Fabaceae. Among the Fabaceae, Lotus and Hippocrepis were by far the most important pollen hosts. Non-Fabaceae taxa represented by substantial proportions in pollen loads or cell provisions were Helianthemum (Cistaceae), Vaccinium (Ericaceae) and Rubus (Rosaceae).
Keywords
Apiformes, *Hoplitis tuberculata* species group, *Monumetha*

Introduction

Osmiine bees are famous for their very diverse and often spectacular nest building behaviours as well as for their high proportion of species that exhibit narrow host plant specializations (Friese 1923, Malyshev 1937, Westrich 1989, O’Toole and Raw 1991, Müller et al. 1997, Cane et al. 2007, Sedivy et al. 2008, 2013a,b,c, Gotlieb et al. 2014, Haider et al. 2014, Müller 2015). While the biology of most Central European osmiine bee species is well known, gaps of knowledge exist for several species mainly occurring in the Alps.

*Hoplitis (Alcidamea) tuberculata* is a boreoalpine species, which has a disjunct distribution area encompassing the Alpine arc from France to Austria and some neighbouring mountains such as Jura and Schwarzwald on the one hand and the boreal zone from Scandinavia and northeastern Europe to easternmost Asia on the other hand (Tkalců 1977, Müller 2015). It belongs to a clade of six species, which were formerly treated as members of the subgenus *Monumetha* (Michener 2007, Ungricht et al. 2008), but recently merged as *Hoplitis tuberculata* species group into the large subgenus *Alcidamea* (Sedivy et al. 2013c, Müller 2015). While *H. tuberculata* is restricted to the Palaearctic region, all the other species occur in North America with some species reaching as far north as arctic Alaska (Michener 1947, 2007, Hurd and Michener 1955, Ascher and Pickering 2015), clearly suggesting a nearctic origin of the *Hoplitis tuberculata* species group (Sedivy et al. 2013c).

*Hoplitis tuberculata* is rather common in the upper montane and subalpine zone of the Alps, where it inhabits open forests, forest edges or windfalls between 900m a.s.l. and the timberline (Amiet et al. 2004). Its nesting biology is only fragmentarily known. While the species has repeatedly been observed to nest in insect burrows in dead wood (Giraud 1861, Frey-Gessner 1880, Friese 1923, Stoeckhert 1933, Grünwaldt 1939, Käpylä 1978, Westrich 1989, Amiet et al. 2004; Fig. 1), its nest architecture is unknown and available information on the material used to build cell partitions and nest plug is contradictory. According to Käpylä (1978), a combination of small stones, masticated leaves and pieces of rotten wood is used to seal the nest. In contrast, Westrich (1989) considered mud to be the exclusive nest building material, which - according to Amiet et al. (2004) - is sometimes combined with masticated leaves. Although current knowledge suggests that *H. tuberculata* is a pollen generalist collecting pollen from the flowers of at least seven plant families (Käpylä 1978, Westrich 1989), its host plant preferences in Central Europe have never been analyzed in detail.

Based on the investigation of four nests recently discovered in the Swiss Alps and the microscopical analysis of 87 pollen loads of females collected across the Alpine arc, the present publication aims to fill the knowledge gaps still existing on both the nesting biology and the flower preferences of *Hoplitis tuberculata*. 
Material and methods

In spring 2014 and 2015, a total of 20 trap nests were fixed at a height of 0.2-1.5 m to sun exposed dead wood in an open subalpine forest above Sedrun (Grisons, Switzerland) between 1650 m and 1800 m a.s.l., where *Hoplitis tuberculata* had been found to be common. Each trap nest consisted of a bundle of about 30 hollow bamboo sticks. The diameter of the burrows in the bamboo sticks varied between 3mm and 8mm. During the flight period of *H. tuberculata*, which lasted from the beginning of June to the end of July, the trap nests were checked twice for sealed nests or for nests still being provisioned. Sealed nests were opened in the laboratory by splitting them longitudinally with a knife, before nest architecture and nest building material were analyzed. To get additional information on the material used to construct nest plugs, trunks and stumps of dead trees in the vicinity of the trap nests were searched for sealed nests of *H. tuberculata*.

To uncover the pollen host preferences of *Hoplitis tuberculata*, the scopal pollen contents of 87 female specimens collected at 87 different localities in Switzerland (n=67), Austria (n=11), Liechtenstein (n=3), Germany (n=3) and Italy (n=3) from 1905 to 2015 were microscopically analyzed using the method outlined by Westrich and Schmidt (1986). Before removing pollen from the metasomal scopae, the degree to which they were filled was estimated. The amount of pollen in the scopae was assigned to five classes, ranging from 5/5 (full load) to 1/5 (filled to one-fifth). The pollen grains were stripped off the scopae with a fine needle and embedded in glycerol gelatin on a slide. When a pollen load was composed of different pollen types, their percentages were estimated by counting the grains along two transects chosen randomly across the cover slip at a magnification of 400x. Pollen types represented by less than 5% of the counted grains were excluded to prevent a potential bias caused by contamination. For pollen loads consisting of two or more different pollen types, the percentages of the number of pollen grains were corrected by their volume. After assigning different weights to scopae according to their degree of filling (full loads were weighted five times more strongly than scopae filled to only one-fifth), the estimated percentages were summed up over all pollen samples. The pollen grains were identified at a magnification of 400x with the aid of the literature cited in Westrich and Schmidt (1986), Beug (2004) and an extensive reference collection. In addition, the pollen provisions in six brood cells of two nests detected at the study site were analysed. To estimate the proportion of the different pollen types in the provisions, the amount of each pollen type was assigned to one of five quantity classes.

Results

Nest architecture

Four nests of *Hoplitis tuberculata* in three different trap nests were found. Five further nests were found in tree trunks and stumps in the vicinity of the trap nests (Figs 4, 5).
Figures 1–9. Nesting biology of *Hoplitis tuberculata*. 1 Female leaving her nest in a preexisting burrow in dead wood 2 Female transporting leaf pulp in her mandibles 3 Female collecting leaf pulp from the sepals of *Potentilla erecta* 4–5 Dead tree stumps with beetle burrows used as nesting sites (Sedrun, Grisons, Switzerland) 6–8 Sealed nests with outermost wall of nest plug with pebbles and earth crumbs embedded in the leaf pulp matrix 9 Sealed nest with outermost wall of nest plug consisting of leaf pulp only.
The maximal diameter of the nine burrows selected by the bees as nesting sites was 4 mm (n=1), 4.5 mm (n=6), 5 mm (n=1) and 6 mm (n=1).

All four nests built in the bamboo sticks of the trap nests had a similar structure and consisted of i) a basal wall that sealed the nest against the rear end, ii) a varying number of brood cells each delimited towards the nest entrance by a cell partition, iii) a (facultative) vestibule in front of the last cell and iv) a nest plug that closed the nest at the front end. The distance from the basal wall to the outermost wall of the nest plug was 6.5 cm, 7.6 cm, 8.3 cm and 10.9 cm.

The basal walls consisted of masticated leaves (“leaf pulp”) and had a width of about 1 mm. In two nests, a free space between pith and basal wall with a length of 0.4 cm and 3.5 cm, respectively, was present, which was loosely filled with small particles, such as pebbles, wood and leaf fragments, seeds or earth crumbs (Fig. 10). In the other two nests, the basal wall was constructed directly adjacent to the pith that filled the rear of the bamboo sticks (Fig. 14). Some of these particles, which were most probably transported into the nest by the female bees, partly adhered to the inside of the basal wall, suggesting that the females incorporated them into the leaf pulp matrix during the first steps of basal wall construction.

The four nests contained one (n=1), two (n=2) and four (n=1) linearly arranged brood cells, which had a length of 8.5-12.5 mm (Fig. 10, 14). In two nests, which were opened in late fall, the brood cells harboured postdefecating larvae spun in a semi-transparent, brownish-white cocoon, indicating that *Hoplitis tuberculata* overwinters as prepupa. The cell partitions had a width of 3-5 mm and invariably consisted of three layers (Fig. 11): two walls each of 0.5-1 mm width, which had been constructed from leaf pulp, enclosed an interspace of 2-4 mm length, which was densely packed with pebbles, earth crumbs, seeds, wood chips or fragments of leaves and needles. While the outer sides of both walls of the cell partitions were very carefully worked forming a plane surface, the inner sides were more irregular. Some of the particles of the inter-layer were partly incorporated into the leaf pulp matrix of the inside of the outer wall.

Three of the four nests contained a vestibule between the outermost cell partition and the nest plug measuring 2.2 cm, 8.1 cm and 8.9 cm in length (Fig. 10). The vestibule was loosely filled with a small amount of 20 to 30 particles, such as pebbles, earth crumbs, wood chips or fragments of leaves and needles (Fig. 13). In one nest, no vestibule was developed; instead, the outermost cell partition and the nest plug bordered directly at each other (Fig. 12, 14).

The nest plugs measured 1.2 cm, 1.3 cm, 1.4 cm and 1.9 cm in length. They consisted of one wall each at the rear and the front end, which enclosed a space that was divided up by one (n=1) or three (n=3) additional walls (Fig. 10, 12, 14). All interspaces between the walls were usually densely packed with small pebbles, earth crumbs, wood chips or fragments of leaves and needles. The walls of the nest plug had a width of 0.5-1 mm, were built from leaf pulp and partly contained foreign particles on their inside, which had been glued to the leaf pulp matrix during wall construction. In two nests, single pebbles and/or earth crumbs were embedded in the leaf pulp matrix of the outside of the front wall (Fig. 6, 7), whereas in the other two nests the front wall consisted
Figures 10–14. Nest architecture of *Hoplitis tuberculata*: 10 Opened nest in a hollow bamboo stick with – from left to right – i) short space filled with small particles followed by the basal wall, ii) two brood cells, which are delimited towards the nest entrance by a three-layered cell partition, iii) vestibule loosely filled with small particles, iv) nest plug consisting of one wall each at the rear and the front end enclosing a space that is filled with small particles and divided up by three additional walls (the low amount of particles in the space between the third and the fourth wall, the presence of only traces of the fourth and the outermost wall and the lack of particles between the two outermost walls is due to the loss of particles and walls during the splitting of the stick) 11 Three-layered cell partition composed of two walls enclosing an interlayer that is densely filled with particles 12 Three-layered cell partition being flush with the nest plug, which consists of several walls with densely packed small particles in between 13 Vestibule between outermost cell partition and innermost part of the nest plug loosely filled with small particles 14 Opened nest in a hollow bamboo stick with i) basal wall built directly adjacent to the pith in the rear of the stick, ii) four brood cells each delimited by a three-layered cell partition directly followed by iii) the nest plug.

exclusively of leaf pulp. This variability in the presence or absence of small particles incorporated into the front wall was also apparent in the five nests found in the vicinity of the trap nests: the front wall of two nests contained small particles on its outside (Fig. 8), whereas that of the other three nests was built of leaf pulp only (Fig. 9).

In summary, the females used two different materials for nest construction: i) leaf pulp to build all the walls within the nest (Fig. 2) and ii) small particles, which were amassed in interspaces between walls and occasionally also incorporated into the leaf pulp matrix of certain walls. The origin of the leaf pulp is not known in detail; one
female, however, was repeatedly observed to chew sepals of *Potentilla erecta* to collect leaf pulp (Fig. 3). The small particles were most probably all collected from the ground as judged by the observation that several females picked up small pebbles and earth crumbs from a small unpaved path.

**Pollen hosts**

The microscopical analysis of 87 female pollen loads revealed that *Hoplitis tuberculata* is polylectic harvesting pollen from the flowers of at least eight plant families (Tab. 1). However, pollen of Fabaceae strongly dominated constituting 84.5% of the total pollen grain volume, followed by pollen of Cistaceae represented by 8.9%. Pollen of all other plant families was recorded in small percentages only. The strong preference for Fabaceae pollen is also evident from the finding that all 87 pollen loads contained pollen of this plant family, 54 of which were pure Fabaceae pollen loads. Among the Fabaceae, *Lotus* was by far the most important pollen host (Tab. 1); its pollen represented 63.9% of the total pollen grain volume and was recorded in 84 out of 87 pollen loads, 35 of which were pure *Lotus* pollen loads. The second most important Fabaceae pollen host was *Hippocrepis*; its pollen represented 11.0% of the total pollen grain volume and was recorded in 31 out of 87 loads.

Pollen of *Lotus* was also the most important pollen type recorded in six brood cell provisions of two nests collected at the study site (Tab. 2). All cells contained large to very large amounts of *Lotus* pollen. The provisions of several cells, however, also contained considerable amounts of non-Fabaceae pollen, such as pollen of *Vaccinium* and *Rubus*.

**Discussion**

**Nest architecture**

As shown in the present study, *Hoplitis tuberculata* uses leaf pulp to construct the walls of both brood cells and nest plug. The erroneous assumption of mud as being the exclusive or predominant nest building material (Westrich 1989, Amiet et al. 2004) is probably based on the observation of females that collected earth crumbs or pebbles on the ground to amass them later in interspaces within their nests. In fact, among *Hoplitis* species of the large subgenus *Alcidamea* only two species have been recorded so far to use mud for nest construction, i.e. the stem-nesting Nearctic *H. grinnelli*, which constructs its cell partitions with clay while the nest plug consists of alternating layers of clay and pith (Davidson 1896, as *H. producta*), and the Palaearctic *H. fulva*, which seals its nest in preexisting cavities of loess scarps with a plug of mud whereas its brood cells are constructed from leaf pulp alone (Marikovskaya 1968).

Peculiar characteristics of the nest architecture of *Hoplitis tuberculata* include i) the three-layered cell partitions, ii) the presence of a vestibule loosely filled with small
Table 1. Pollen composition of female pollen loads of *Hoplitis tuberculata*. n=87 pollen loads from 87 different localities distributed across the Alps.

<table>
<thead>
<tr>
<th>Plant family</th>
<th>Plant genus/subfamily</th>
<th>% pollen grain volume</th>
<th>number (%) of loads with this pollen type</th>
<th>number (%) of pure loads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabaceae</td>
<td><em>Lotus</em></td>
<td>84.5</td>
<td>87 (100)</td>
<td>54 (62.1)</td>
</tr>
<tr>
<td>Fabaceae</td>
<td><em>Hippocrepis</em></td>
<td>63.9</td>
<td>84 (96.6)</td>
<td>35 (40.2)</td>
</tr>
<tr>
<td>Fabaceae</td>
<td><em>Onobrychis</em></td>
<td>11.0</td>
<td>31 (35.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fabaceae</td>
<td><em>Trifolium</em></td>
<td>2.9</td>
<td>3 (3.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fabaceae</td>
<td>unknown</td>
<td>5.2</td>
<td>7 (8.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cistaceae</td>
<td><em>Helianthemum</em></td>
<td>8.9</td>
<td>16 (18.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Boraginaceae</td>
<td><em>Echium</em></td>
<td>1.3</td>
<td>2 (2.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ericaceae</td>
<td><em>Vaccinium</em></td>
<td>0.9</td>
<td>2 (2.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Rosaceae</td>
<td><em>Potentilla</em></td>
<td>1.2</td>
<td>6 (6.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Rosaceae</td>
<td><em>Rubus</em></td>
<td>0.7</td>
<td>5 (5.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Rosaceae</td>
<td>unknown</td>
<td>0.5</td>
<td>1 (1.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ranunculaceae</td>
<td><em>Ranunculus</em></td>
<td>0.5</td>
<td>2 (2.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Cichorioideae</em></td>
<td>0.5</td>
<td>3 (3.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td></td>
<td>0.4</td>
<td>2 (2.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td><em>Lamioideae</em></td>
<td>0.4</td>
<td>1 (1.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td><em>Nepetoideae</em></td>
<td>0.05</td>
<td>1 (1.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>unknown</td>
<td></td>
<td>1.8</td>
<td>4 (4.6)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Table 2. Pollen composition of brood cells of *Hoplitis tuberculata*. n=6 brood cells from two nests collected near Sedrun (Grisons, Switzerland) on 18.7.2014. The amount of each pollen type was assigned to five quantity classes ranging from +=very small amount to ++++=very large amount.

| Nest 1 | Brood cell 1 |+++| ++| + | | Nest 2 | Brood cell 1 |+++| +++| ++| |
|--------|--------------|---|----|---| |--------|--------------|---|----|---| |
|        | Brood cell 2 |+++| +++|+++| |        | Brood cell 2 |+++| +++|+++| |
|        | Brood cell 3 |++| ++|+++|++| |        | Brood cell 3 |++| ++|+++|++|
|        | Brood cell 4 |++|+++|++| |        | Brood cell 4 |++|+++|++|

particles and iii) a nest plug that consists of densely packed layers of small particles sandwiched between at least three walls.

The construction of three-layered cell partitions composed of two walls with an interlayer of densely packed small particles in between seems to be unique among Palaearctic osmiine bees, which usually partition linearly arranged brood cells by single walls only (Müller 2015 and references therein). However, such three-layered cell partitions are known from several North American *Hoplitis* species, which are all members of the subgenus *Alcidamea* but differ in their preferred nesting sites. Species, which use preexisting cavities, such as *H. albifrons*, *H. fulgida* and *H. spoliata*, transport small par-
articles such as pebbles, wood chips, earth crumbs or fragments of conifer needles from outside into their nest to include them into the cell partitions (Fye 1965, Medler 1967, Clement and Rust 1976). Interestingly, these three species are closely related to *H. tuberculata* and also belong to the *Hoplitis tuberculata* species group (see Introduction). Among them, the construction of three-layered cell partitions seems to be the rule in *H. fulgida* and is most common in *H. albifrons*, while *H. spoliata* frequently omits the interlayer resulting in single leaf pulp walls between the brood cells (Fye 1965, Medler 1967, Clement and Rust 1976). In contrast, species, which excavate their own burrows in pithy stems, such as *H. hypocrita*, *H. pilosifrons* or *H. sambuci*, tightly pack particles of pith taken from the burrow walls in between the two leaf pulp layers (Michener 1955, Clement and Rust 1976). Although the latter three species are not members of the *Hoplitis tuberculata* species group, their shared habit of constructing three-layered cell partitions suggests a close relatedness to that group as shown by Sedivy et al. (2013).

The function of the three-layered cell partitions is counterintuitive at first sight as the thick nest plug built by *Hoplitis tuberculata* and its relatives (see below) is expected to already provide enough protection against the intrusion of nest predators. We hypothesize that these strong cell partitions might impede mobile larvae of predators, which already infested a brood cell before the nest was sealed, from invading adjacent cells. In fact, larvae of some *Trichodes* beetle species (Cleridae), which are antagonists of above-ground nesting megachilid bees, attack several brood cells in sequence by breaking through the cell walls (Carré 1980).

The presence of a vestibule filled with small particles seems to be another typical trait common to the members of the *Hoplitis tuberculata* species group except for *H. spoliata*, where the vestibule is empty (Fye 1965, Medler 1967, Clement and Rust 1976). As in *H. tuberculata*, vestibules may occasionally be absent in nests of *H. albifrons* and *H. fulgida* with the last provisioned cell being flush with the nest plug (Fye 1965, Clement and Rust 1976). In contrast to the three-layered cell partitions, which seem to be a unique character of only a few species of the subgenus *Alcidamea*, vestibules with amassed small particles are rather widespread among osmiine bees. They are known from some *Hoplitis* species of the subgenera *Anthocopa* and *Alcidamea* other than the *Hoplitis tuberculata* species group, from several *Osmia* species of the subgenera *Erythromia*, *Neosmia* and *Pyrosmia* as well as from *Wainia elizabethae* (Gess and Gess 1988, Müller 2015 and references therein).

The architecture of the nest plug varies both within and among the North American members of the *Hoplitis tuberculata* species group. The nest plug of *H. spoliata* usually consists of a single layer of leaf pulp, rarely of three or four layers with short empty spaces in between (Medler 1967). In contrast, the nest plug of *H. albifrons* and *H. fulgida* is usually three-layered with two walls enclosing an interspace tightly packed with small particles (Fye 1965, Clement and Rust 1976). In the latter species, one or two additional walls are occasionally present in the particle-filled space between the rear and the front wall of the nest plug, mirroring the situation found for *H. tuberculata* in the present study. As in *H. tuberculata*, small pebbles are often but not always cemented into the leaf pulp matrix of the front wall in *H. fulgida* and *H. spoliata* (Hicks 1926, Fye 1965, Medler 1967).
In summary, although the nest architecture of *Hoplitis tuberculata* is unique among Palaearctic osmiine bees, it corresponds to that of its closest North American relatives, indicating that nesting site, nest building material and nest structure are conserved traits within the *Hoplitis tuberculata* species group.

**Pollen hosts**

The present study shows that *Hoplitis tuberculata* is polylectic and collects pollen from the flowers of at least eight different plant families, among which Fabaceae clearly dominate. Fabaceae pollen was recorded in each pollen load and constituted almost 85% of the total pollen grain volume. In contrast, several brood cell provisions contained considerable amounts of non-Fabaceae pollen suggesting that pollen hosts other than Fabaceae may locally also play an important role for larval nourishment. Indeed, the significance of Fabaceae pollen as deduced from the analysis of pollen loads of females, which most probably all had been collected during flower visits, might have been overestimated. The probability that specimens of *H. tuberculata* are collected at flowers of *Lotus* or *Hippocrepis* rather than at flowers of e.g. *Vaccinium* or *Rubus* is likely higher because the conspicuously yellow Fabaceae flowers act as true magnet for each bee researcher due to the fact that they attract a multitude of different bee species. Considering this possible bias, the host plant spectrum of *H. tuberculata* recorded in the present study is similar to that found in Finland (Käpylä 1978). Here, pollen of seven different plant families represented by at least 3% per load was recorded in 16 pollen loads of females collected both at flowers and nesting sites. As in the present study, Fabaceae (*Caragana, Lathyrus, Lotus*), Ericaceae (*Vaccinium*), Rosaceae (*Geum, Potentilla, Rubus*), Ranunculaceae (*Anemone, Ranunculus*) and Asteraceae (*Leontodon*) were among the plant families exploited for pollen. In addition, flowers of Asparagaceae (*Convallaria, Polygonatum*) and Violaceae (*Viola*) also served as pollen hosts, the former being the most important pollen sources followed by flowers of the Fabaceae.

In summary, the pollen hosts of *Hoplitis tuberculata* known so far belong to ten different plant families, among which Fabaceae predominate but probably not to that large degree as might be expected from the analysis of the female pollen loads from the Alps alone.

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References


Emergence behaviour of adult *Trogus lapidator* (Fabricius) (Hymenoptera, Ichneumonidae, Ichneumoninae, Heresiarchini) from pupa of its host *Papilio machaon* L. (Lepidoptera, Papilionidae), with a comparative overview of emergence of Ichneumonidae from Lepidoptera pupae in Europe

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Abstract

Unusually for Ichneumonidae, *Trogus lapidator* emerges through a hole in the pupal wing case of its papilionid butterfly host that is made largely by a liquid secretion that softens and disintegrates the host tissue. The mandibles are deployed to help spread the secretion, but only towards the very end of the emergence process are they used (and then only in a minor way) to enlarge the hole. Links to video clips showing the emergence of *T. lapidator* are provided. Photographs illustrating the nature of emergence holes left in Lepidoptera pupae by a range of Ichneumonidae and some Chalcidoidea are presented and discussed, contrasting with the emergence hole left by *Trogus* and close allies.

Keywords

Pupal cuticle, eclosion, cuticular disintegration, staining, cap-cutting, mandibular structure
Introduction

One of the general functions of the mandibles of adult Hymenoptera is to aid emergence from the cocoon or other pupation site, although the wide range of variation of mandibular structure suggests that this is not always achieved in the same way, and indeed often indicates other functions for the mandibles too (undoubtedly including nest-building, prey manipulation, accessing hosts, and feeding; uses of the mandibles that are well-known in many relevant taxa). Many Ichneumonoidea pupate in well-defined cocoons, from which adults of various groups emerge by cutting a cap or strips with great precision, but others chew more or less irregular holes for the purpose. In contrast with Braconidae (the small subfamily Meteorideinae and a small and unusual subtribe, Aspidobraconina, of Braconinae aside), many Ichneumonidae emerge as adults from Lepidoptera pupae, whether as true pupal parasitoids (some Pimplinae, many Ichneumoninae, a few Cryptinae), or as larva-pupal parasitoids (Metopiinae, most Anomaloninae, many Ichneumoninae, occasional Campopleginae). In these cases it is usual for adult emergence to be either through a roughly shaped chewed hole, typically subapical, around which a scattering of bits of host cuticle can be found, or in many cases though the hole left by a more neatly cut and more or less detached apical cap (sometimes partly assisted by the host’s dorsal ecdysial sutures).

*Trogus lapidator* (Fabricius) is a widespread specialist koinobiont parasitoid of the Swallowtail Butterfly *Papilio machaon* Linnaeus (Papilionidae) in Europe, with rare confirmed records from the more restricted *Papilio alexanor* Esper (e.g. Sanetra 1998). The European nominal taxon *Trogus violaceus* (Mocsáry), which is confined to Corsica and Sardinia where it parasitizes both *P. machaon* and *P. hospiton* Guenée, is regarded as a synonym of *T. lapidator* by Wahl and Sime (2006). *Trogus* is a genus of about twelve species (Wahl and Sime 2006) of larva-pupal parasitoids of *Papilio* occurring in the Palearctic and New World, and all probably have similar biology, invariably emerging as adults through the wing case of the host pupa. Closely related taxa such as *Psilomastax pyramidalis* Tischbein, parasitizing *Apatura* spp. (Nymphalidae: Apaturinae) make similar emergence holes, presumably in the same way, though in the case of *Psilomastax* the emergence hole is substantially more ventral. According to Sime and Wahl (2002) in their cladistic analysis of the *Callajoppa* genus-group, emergence through the wing case (their character 58) is unique to the *Trogus*-subgroup, which contains several additional genera.

document the natural history of the French department of Var, the remarkable sequences showing the emergence of adult *Trogus lapidator* (https://www.youtube.com/watch?v=GrhKhsj00BE and https://www.youtube.com/watch?v=y_p8QcnHgjs) presented and discussed in this paper were brought to the attention of the first author. In order to set this in context, a brief illustrated overview of emergence from pupae of Lepidoptera by Ichneumonidae is given. When possible we have paid particular attention to those parasitizing butterflies which, by often being relatively exposed (and sometimes needing to persist for much of the year), may be expected to sometimes have relatively hard thick cuticle similar to that of *P. machaon*. Several groups of Chalcidoidea also emerge as adults from the pupae of Lepidoptera, and very brief mention is made of some of these.

**Materials and methods**

Observations on *Trogus lapidator*, which has up to three annual generations in the region, were made and filmed during spring and summer 2013 in a large garden (1.5 hectares) in southern France (Var: Callas), where natural populations of the swallowtail butterfly *Papilio machaon* and various of its parasitoids, including *T. lapidator*, occurred. In some cases ovipositions by captive *T. lapidator* into middle instar host larvae had been witnessed and, following their pupation a week later and after the unwitnessed emergence of an adult *T. lapidator* several weeks later from a different pupa, two hosts were watched carefully for signs of adult emergence. The emergence process took place in late morning over a period of about 45 minutes and both cases were filmed with a Canon XL2 with a 20× L IS zoom objectif XL 5.4-108mm with extender 72mm close-up lens 500D. The footage is registered on mini DV60 tapes. From the footage videos were made that can be seen at https://www.youtube.com/watch?v=GrhKhsj00BE and https://www.youtube.com/watch?v=y_p8QcnHgjs. Stills taken from these and another video resulting from a similar process were used for Figs 1–4 and 85.

The National Museums of Scotland (NMS) houses a large collection of western Palaeartic Ichneumonoidea, made special by the quantity of reared material it contains, particularly in respect of parasitoids of Lepidoptera. A survey of emergence patterns of adults from Lepidoptera pupae seen in the three main subfamilies of Ichneumonidae with this habit (Ichneumoninae, Anomaloninae and Metopiinae) as well as lesser groups (Pimplinae: Pimplini, and the few relevant taxa of Campopleginae and Cryptinae) was undertaken from this resource, with particular attention to emergence from butterfly hosts. We illustrate a particularly large number involving Ichneumoninae, as this subfamily has proved to be the most variable. The survey was briefly extended to Chalcididae and Pteromalidae. Digital photographs of host Lepidoptera pupal remains from which Ichneumoninae (or Chalcidoidea) had emerged were taken using a Nikon D80 with Medical Nikkor 120mm lens attachment.
Results

_Trogus lapidator_ and close relatives

From the two sequences of the emergence of _T. lapidator_ adults that were filmed (https://www.youtube.com/watch?v=GrhKhj00BE and https://www.youtube.com/watch?v=y_p8QcnHgis) it is clear that the emergence hole is made by a softening and dissociation of the host cuticle involving a wet process, presumably originating from a secretion (Figs 1–4). In the videos the mandibles can be seen partially protruding at various stages but, except possibly at the very end when their slight involvement in enlarging the hole is arguable, their role appears not to be to bite or chew, but rather to help spread fluid externally round the edge of the hole being created. The pupal remains of _P. machaon_ from which _T. lapidator_ has emerged, when they have dried, generally leave a clear sign of this process, both by the rim of the emergence hole having an almost molten appearance and by considerable staining (Figs 5–7). The pupal remains of _Apatura_ spp. (Nymphalidae) from which the related _Psilomastax pyramidalis_ have emerged typically show similar staining (Figs 8, 9).

Other groups of Ichneumonidae and Chalcidoidea that predominantly emerge from Lepidoptera pupae

Ichneumoninae

All Ichneumoninae are parasitoids of Lepidoptera, and virtually all emerge as adults from the host pupa (_Colpognathus_ is an exception: Shaw and Bennett 2001). Some taxa oviposit into the host in its larval stage, and are clear koinobionts, while others attack pupae or prepupae and are essentially idiobionts.

The most obvious group to survey for signs of similar modes of emergence is the tribe Heresiarchini, to which the _Trogus_-subgroup belongs. In all examined taxa (species of _Amblyjoppa, Callajoppa, Coelichneumon_ and _Syspasis_ of this tribe, other than the _Trogus_-subgroup, emergence has been by cutting a more or less neat cap from the anterior (capital) end of the host pupa (Figs 10–12), hereafter termed “type 1” emergence. There appears to be no sign of cuticular degradation or deformation, but slight staining is evident very narrowly around the extreme edge of the cut in the paler-coloured pupae (Fig. 10).

In the very large tribe Ichneumonini a surprising range of emergence patterns is seen. _Hoplismenus_, which are parasitoids of butterflies, appear always to display type 1 emergence, with sharp caps (Figs 13–16), and often (Figs 13–15) but not always (Fig. 16) there is clear staining at the margins of the cut. In certain other genera (e.g. _Virgichneumon_ (Figs 32, 33)) cap-cutting also seems to be the rule, although sampling within and between genera is not extensive. On the other hand, in _Ichneumon_ both type 1 cap-cutting (Figs 17–23) and subapical ventrally chewed holes (Figs 24–27), hereafter “type 2” emergence, are seen,
Emergence behaviour of adult Trogus lapidator (Fabricius)...

Figure 1–10. 1–4 Sequence in the emergence process of Trogus lapidator (Fabricius) from Papilio machaon Linnaeus (Papilionidae). 5–10 Host pupa following emergence of parasitoid. 5–7 Trogus lapidator from Papilio machaon 8, 9 Psilomastax pyramidalis Tischbein from Apatura iris (Linnaeus) (Nymphalidae) 10 Amblyjoppa proteus (Christ) from Deilephila elpenor (Linnaeus) (Sphingidae).
Figure 11–20. 11 Callajoppa cirrogaster (Schrank) from Mimas tiliae (Linnaeus) (Sphingidae) 12 Coelichneumon litoralis Horstmann from Conisania andalusica (Staudinger) (Noctuidae) 13, 14 Hoplistemens terrificus Wesmael from Nymphalis polychloros (Linnaeus) (Nymphalidae) 15, 16 Hoplistemens bispinatus (Thunberg) from Coenonympha pamphilus (Linnaeus) (Nymphalidae: Satyrinae) 17 Ichneumon cesator Müller from Aglais urticae (Linnaeus) (Nymphalidae) 18 Ichneumon stenocerus Thomson from Euphydryas aurinia (Rottemburg) (Nymphalidae) 19 Ichneumon quadrialbatus Gravenhorst from Colias croceus (Geoffroy) (Pieridae) 20 Ichneumon gracilicornis Gravenhorst from Boloria eunomia (Esper) (Nymphalidae)
Emergence behaviour of adult Trogus lapidator (Fabricius)... even within the species parasitizing butterflies. Usually, a given Ichneumon species is more or less consistent in habit (Figs 20–23; 24–26), though in some cases it seems clear that the origin of cap-cutting can be traced to eclosing through ventrally chewed slits, which may extend to detaching a cap (Figs 28, 29); i.e. there is a continuum between types 1 and 2. Both in Ichneumon (Figs 28, 29) and elsewhere in the tribe (Amblyteles armatorius Forster: Figs 30, 31) the two modes can sometimes be seen in the same species in different hosts, although there is insufficient evidence to decide whether this is genuinely host-related. Occasionally the process of detachment of an untidy (or small) cap may be aided by dorsal sutures in the host cuticle (Fig. 26), but this is by no means always the case and usually if a cap is detached it is chewed all round, even if untidily (Fig. 31). In the tribe as a whole cap-cutting ranges from very neat and complete (Figs 32–34) to haphazard (Fig. 31), and in many cases emergence through ventrally chewed holes does not lead to the detachment of a cap (e.g. Fig. 35). In uncommon situations, such as species parasitizing stem-inhabiting noctuids, the apical part of the pupa may be chewed away untidily (Figs 36, 37). Overall, the degree of staining seems rather inconsistent, though sometimes profound.

All members of the tribe Listrodromini parasitize lycaenid butterflies, and appear always to cut rather neat caps (type 1 emergence), seldom with much sign of staining (Figs 38–40).

Phaeogenini are all parasitoids of so-called microlepidoptera and, with the exception of the koinobiont Epitomus (Diller and Shaw 2014), they are essentially idiobionts. Colpognathus is exceptional in emerging from the mummified host larva (Shaw and Bennett 2001), but phaeogenines generally emerge from host pupae by chewing untidy subapical and more or less ventral holes. Any detachment of part of the pupa is incidental and usually ventro-apical, scarcely involving the dorsal part of the pupa, rather than fully apical (Figs 41–43), hereafter “type 3” emergence and regarded as relatively unspecialised, possibly reflecting the basal position of the tribe. However, in Heterischinus a facial cap is consistently and rather uniformly detached (Fig. 43). Staining is not usually evident.

In the koinobiont Platylabini, which all parasitize Geometridae or Drepanidae, rather variable cap-cutting (Figs 44–48) seems to predominate, though the origin as a ventral opening is sometimes clear when the cap has not been chewed all round and has failed to detach (Figs 49, 50), and sometimes the chewed hole is very untidy (Fig. 51). This is similar to the continuum between types 1 and 2 seen in Ichneumonini. A remarkable exception to the usual ventral position of emergence occurs in Cyclolabus axillatorius (Thunberg) (Figs 52, 53), in which all 3 examples seen have emerged through subdorsally chewed holes, with neither ventral damage to the host pupa nor detachment of a cap (other Cyclolabus species seen were unremarkable). Staining of the host pupal cuticle is generally absent.

Regarding the examined species in the small tribes, in Eurylabini an apical cap is sharply cut (Fig. 54), while in Goedartiini the adult emerges through a hole chewed in the apex of the pupa (Fig. 55). In Zimmerini the host pupal case of Cotiheresiarches dirus (Wesmael) is relatively frail and enclosed in a tough cocoon, and the problem of emerging from the latter causes the adult ichneumonid to leave the host pupa in a very damaged state, apparently a result of untidy and predominantly ventral tearing (Fig. 56).
Figure 21–30. 21–23 Ichneumon gracilicornis from 21 Melitaea athalia (Rottemburg) (Nymphalidae) 22 Maniola jurtina (Linnaeus) (Nymphalidae: Satyrinae) 23 Brenthis ino (Rottemburg) (Nymphalidae) 24–26 Ichneumon caloscelis Wesmael from 24 Hipparchia semele (Linnaeus) (Nymphalidae: Satyrinae) 25 Pyronia tithonus (Linnaeus) (Nymphalidae: Satyrinae) 26 Maniola jurtina (Linnaeus) (Nymphalidae: Satyrinae) 27 Ichneumon novemalbatus Kriechbaumer from Melanargia lachesis (Hübner) (Nymphalidae: Satyrinae) 28, 29 Ichneumon cf. exilicornis Wesmael from Agrodiaetus sp. (Lycaenidae) 30 Amblyteles armatorius (Forster) from ?Noctua sp. (Noctuidae)
Emergence behaviour of adult *Trogus lapidator* (Fabricius)...

**Figure 31–40.**
31 *Amblyteles armatorius* from *?Xestia* sp. (Noctuidae) 32 *Virgichneumon tergenus* (Gravenhorst) from *Satyrium w-album* (Knoch) (Lycaenidae) 33 *Virgichneumon albilineatus* (Gravenhorst) from *Spilosoma* sp. (Erebidae: Arctiinae) 34 *Thyrateles camelinus* (Wesmael) from *Vanessa cardui* (Linnaeus) (Nymphalidae) 35 *Diphyus palliatorius* (Gravenhorst) from *Diarsia* sp. (Noctuidae) 36, 37 *Chasmias paludator* (Desvignes) from *Nonagria typhae* (Thunberg) (Noctuidae) 38 *Anisobas seyrigi* Heinrich from *Glaucopsyche melanops* (Boisduval) (Lycaenidae) 39 *Neotypus intermedius* Mocsáry from *Lampides boeticus* (Linnaeus) (Lycaenidae) 40 *Listrodromus nycthemerus* (Gravenhorst) from *Celastrina argiolus* (Linnaeus) (Lycaenidae).
Figure 41–50. 41 Diadromus heteroneurus Holmgren from Ypsolopa vittella (Linnaeus) (Ypsolophidae) 42 Herpestomus brunnicornis (Gravenhorst) from Yponomeuta padella (Linnaeus) (Yponomeutidae) 43 Heterischmus truncator (Fabricius) from indet. Pterophoridae 44 Hypomecus quadriannulatus (Gravenhorst) from Cyclophora albipunctata (Hufnagel) (Geometridae) 45 Linycus exhortator (Fabricius) from Xanthorhoe fluctuata (Linnaeus) (Geometridae) 46 Platylabus dolerosus (Gravenhorst) from Chloroclysta sp. (Geometridae) 47 Platylabus vibratorius (Thunberg) from Eulithis testata (Linnaeus) (Geometridae) 48 Platylabus rufus Wesmael from Hydriomena furcata (Thunberg) (Geometridae) 49 Platylabus curtorius (Thunberg) from Phibalapteryx virgata (Hufnagel) (Geometridae) 50 Pristicerops infractorius (Linnaeus) from Cabera sp. (Geometridae).
Anomaloninae

In this koinobiont subfamily the hosts are always attacked as larvae but emergence is invariably from the host pupa.

All data presented here pertain to the tribe Gravenhorstiini, which is exclusively associated with Lepidoptera. Examined taxa of Aphanistes, Heteropelma, Therion and Trichomma make detached caps, though not very neatly (Fig. 57) from their relatively large hosts, mostly having fairly tough pupae. In Barylypa, Clypeocampulum and Erigorgus type 2 emergence seems the rule (Figs 58–60), though in the case of Clypeocampulum the hole is not always ventral even in the same species (Figs 59, 60). Within Agrypon and Habronyx (Camposcopus) emergence is in most cases type 2 (Fig. 61) but in the former an irregular cap sometimes develops, although probably more by force than by cutting (Figs 62, 63), and only in relatively frail short-lived pupae. In one species (Agrypon polyxenae Szépligeti: Figs 61, 64) the position of emergence through a chewed hole is very consistently dorsal in one host genus (Zerynthia (Fig. 64), identical in all 29 examples from these slender hosts) but variable in another (Archon, one ventral (Fig. 61) and one dorsal in the two available examples from this differently shaped host). In the subfamily as a whole, appreciable staining is only rarely seen (e.g. Fig. 59).

Metopiinae

Notwithstanding some uncertainly placed taxa of unknown biology, all metopiines are believed to be koinobiont larva-pupal parasitoids of Lepidoptera.

In the genera Chorinaeus, Hypsicera and Triclistus, which mostly parasitize “microlepidoptera” with relatively frail (most often cocooned) pupae, emergence is generally type 2, through a chewed ventral hole (Fig. 65, 66), sometimes leading to the detachment of a rough-edged cap (Fig. 67); in Scolomus and Synosis, however, type 1 behaviour seems to be more common or possibly the norm (Figs 68, 69). In Metopius a very neat detached cap (type 1) is seen in taxa of the subgenus Tylopius, parasitizing weakly concealed tough pupae (Fig. 71), but species in the subgenus Peltocharus emerging from strongly cocooned hosts having less tough pupae chew a large ventral hole (Fig. 70).

Pimplinae: Pimplini

As a subfamily Pimplinae has very wide host associations and a correspondingly wide range of biology overall, but in the tribe Pimplini there are specialist idiobiont parasitoids of Lepidoptera pupae.

In the genera Apechthis and Pimpla, emergence is always through a chewed hole, usually rather irregular but with varying degrees of tidiness (Figs 72–75). Emergence is generally subapical but there is little consistency in position otherwise and ventral
Figure 51–60. 51 Apaeleticus bellicosus Wesmael from Idaea ochrata (Scopoli) 52, 53 Cyclolabus pactor (Wesmael) from 52 Eupithecia pimpinellata (Hübner) (Geometridae) 53 indet. Geometridae 54 Eurylabus torvus Wesmael from Eremobia ochroleuca (Dennis & Schiffermüller) (Noctuidae) 55 Goedartia alboguttata (Gravenhorst) from Calliteara pudibunda (Linnaeus) (Erebidae: Lymantriinae) 56 Cotiberesiarches dirus (Wesmael) from Eriogaster lanestris (Linnaeus) (Lasiocampidae) 57 Aphanistes gliscens (Hartig) from Dryobota labecula (Esper) (Noctuidae) 58 Clypeocampulum barbarae Schnee from Anthocharis euphenoidees Staudinger (Pieridae) 59, 60 Clypeocampulum lubricum (Atanasov) from Zegris eupheme (Esper) (Pieridae).
Emergence behaviour of adult *Trogus lapidator* (Fabricius)...

**Figure 61–70.**

61 *Agrypon polyxenae* (Szépligeti) from *Archon apollinus* (Herbst) (Papilionidae)
62 *Agrypon delarvatum* (Gravenhorst) from *Coenonympha* sp. (Nymphalidae: Satyrinae)
63 *Agrypon anomalas* (Gravenhorst) from *Agrodaetus* sp. (Lycaenidae)
64 *Agrypon polyxenae* from *Zerynthia polyxena* (Dennis & Schiffermüller) (Papilionidae)
65 *Chorineaus funebris* (Gravenhorst) from *Clepsis spectrana* (Treitschke) (Tortricidae)
66 *Triclistus epermeniae* Shaw & Aeschlimann from *Epermenia chaerophyllella* (Goeze) (Epermeniidae)
67 *Triclistus anthophilae* Aeschlimann from *Anthophila fabriciana* (Linnaeus) (Choreutidae)
68 *Scolomus borealis* (Townes) from *Schreckensteinia festaliella* (Hübner) (Schreckensteiniidae)
69 *Synosis parenthesellae* Broad & Shaw from *Ypsolophia parenthesella* (Linnaeus) (Ypsolophidae)
70 *Metopus dentatus* (Fabricius) from *Lasiocampa quercus* (Linnaeus) (Lasiocampidae).
Figure 71–80. 71 *Metopus leiopygus* Foerster from indet. Arctiinae (Erebidae) 72, 73 *Apechthis compunctor* (Linnaeus) from 72 *Aglais urticae* (Linnaeus) (Nymphalidae) 73 *Aporia crataegi* (Linnaeus) (Pieridae) 74, 75 *Pimlea rufipes* (Miller) from 74 *Pieris brassicae* (Linnaeus) (Pieridae) 75 *Charaxes jassius* (Linnaeus) (Nymphalidae) 76 *Zoophthorus bridgmani* (Schmiedeknecht) from ?*Argyresthia* sp. (Argyresthiidae) 77 *Zoophthorus palpator* (Müller) from *Stephensia brunichella* (Linnaeus) (Elachistidae) 78 *Campoplex brevicornis* (Szépligeti) from *Eupithecia venosata* (Fabricius) (Geometridae) 79 *Diadegma scotiae* (Bridgman) from *Phaulernis fulviguttella* (Zeller) (Epermeniidae) 80 *Dusona leptogaster* (Holmgren) from (left) indet. Geometridae and (right) *Alsophila aescularia* (Dennis & Schiffermüller) (Geometridae).
emergence is certainly not especially favoured. Rarely emergence towards the caudal end of the host pupa occurs (Fig. 75). Slight staining is sometimes seen (Fig. 73), and the arguably most specialised genus *Apechthis* often leaves clear signs of strip-cutting around its neater holes (Figs 72, 73).

**Cryptinae**

As a subfamily Cryptinae has a very wide host range, in which Lepidoptera do not play a dominant part. A few of the idiobiont species directly associated with Lepidoptera in Europe, however, do emerge as adults from the host pupa, and in some genera this may be normal.

The only relevant genus for which we have seen host remains is *Zoophthorus* (Phygadeuontini) (Figs 76, 77), in which unspecialised type 2 or type 3 emergence is found, similar to that in Phaeogenini.

**Campopleginae**

A large part of this koinobiont subfamily is associated with Lepidoptera but almost all kill and erupt from the host before it pupates. In some genera species that occasionally kill the host after its pupation are found but it is not usually a consistent strategy and, in general, if pupation takes place within the host pupa it is ruptured during the process of cocoon construction by the parasitoid, as occurs also in a few species of *Ophion* (Ophioninae).

However, odd species in genera such as *Campoplex* (Fig. 78), *Diadegma* (Fig. 79) and *Dusona* (Fig. 80, 81) consistently pupate within the unruptured pupal shell of their hosts, although sometimes within at least a frail cocoon of their own as well (Fig. 78). In all these cases the emergence hole is generally of the least specialised kind (albeit that emergence is generally at the head end), through a subapical chewed hole lacking consistent dorso-ventral orientation and with no tendency for detachment of a cap. Staining is not evident.

**Chalcidoidea**

Several families of Chalcidoidea include idiobiont parasitoids that attack and emerge as adults from Lepidoptera pupae.

In *Brachymeria* (Chalcididae) emergence is through a chewed hole, usually in a subapical position (Fig. 82) or more or less laterally (Fig. 83), but with little evident orientation otherwise. Often there is considerable staining (Fig. 83).

A few species of *Pteromalus* (Pteromalidae) and some related genera that attack Lepidoptera pupae are gregarious, and a succession of adults emerge through one or a few emergence holes (Figs 84, 85). Only rarely is staining seen.
Figure 81–90. 81 *Dusona admontina* (Speiser) from *Herminia grisealis* (Dennis & Schiffermüller) (Er-ebidae) 82 *Brachymeria tibialis* (Walker) from *Aporia crataegi* (Linnaeus) (Pieridae) 83 *Brachymeria albicrus* (Klug) from *Danaus chrysippus* (Linnaeus) (Nymphalidae: Danainae) 84, 85 *Pteromalus puparum* (Linnaeus) 84 from *Aglais urticae* (Linnaeus) (Nymphalidae) 85 emerging from *Papilio machaon* Linnaeus (Papilionidae) 86–88 *Agrypon* sp. from *Copaxa multifenestrata* Herrich-Schäffer (Saturniidae) 88 showing host residue removed from its pupa 89, 90 *Xanthopimpla* sp. from *Attacus atlas* (Linnaeus) (Saturniidae). The figures are not all to the same scale (applies to all plates).
Discussion

Quite apart from the video evidence (Trogus lapidator 1st emerging – 16 September 2013; https://www.youtube.com/watch?v=GrhKhsj00BE and Trogus lapidator 2nd emerging - 16 September 2013; https://www.youtube.com/watch?v=y_p8QcnHgjs) and Figs 1–4, it is seen from the host remains through which Trogus and the closely related Psilomastax have emerged long before (Figs 5–9) that the pupal cuticle has been degraded by a wet substance, not only because of the ‘molten’ appearance around the edges of the emergence hole but also because it is more or less extensively stained. Unsurprisingly, papilionid pupae in BMNH from which Holcojoppa species (another Trogus-subgroup genus) have emerged have the same appearance (G. Broad, pers. comm.). We have observed generally sharper edges to the emergence holes of other ichneumonids, but staining would be expected to be less noticeable in the tough dark brown (subterranean) pupae typical of many large moths. Despite a moderate incidence of staining (see below), especially in Ichneumoninae, we have searched hard to see damage comparable with that caused by the Trogus-subgroup but have found very little sign of it elsewhere, even in the same tribe Heresiarchini (Figs 10–12) or in the relatively tough and often pale-coloured pupae (many of which are exposed and sometimes of long duration) of many butterflies from which a variety of ichneumonids have emerged. Thus we conclude that in general emergence from host pupae depends, as the existing perception has it, essentially on the action of the adult parasitoid’s mandibles, and that the Trogus-subgroup is exceptional. However, elsewhere in Ichneumoninae staining is rather widely seen, especially in some of the lighter-coloured butterfly pupal remains examined, such as Nymphalis polychloros (Linnaeus) parasitized by Hoplistemenus terrificus (Wesmael) (Figs 13, 14), and some (Fig. 15) (but not all; Fig. 16) Coenonympha pamphilus (Linnaeus) parasitized by Hoplistemenus bispinatus (Thunberg), as well as in several Ichneumon species (Figs 18, 21, 23–25) although again without consistency (compare Figs 25 and 26). There are also a few weak examples from Listrodromini (Figs 38, 40). It is hard to be absolutely dismissive of the possibility of help from biochemicals in view of these clear signs of rather even staining around the edges of the emergence hole, especially in cap-cutting taxa. On the other hand, both in Ichneumoninae and in other subfamilies, it is possible that this can arise coincidentally (Fig. 40 shows an unusually stained Celastrina argiolus (Linnaeus) parasitized by Lystrodromus nycthemerus (Gravenhorst), with numerous others being free of staining; and Fig. 59 is the only example seen of staining in Anomaloninae, affecting only one area of the pupa of Zegris eupheme (Esper) from which Clypeocampulum lubricum (Atanasov) has emerged). While staining may result only incidentally, for there are always liable to be fluids accompanying the emergence of parasitoids (see Fig. 85, showing fluid in the hole being chewed by Pteromalus puparum, despite the typically clean appearance of the hole left in the (different) pupal cuticle once dry in Fig. 84), it points to a possibly interesting study awaiting anyone with the necessary patience, or luck, to investigate these phenomena in action. Chalcididae often leave strong stains behind them (Fig. 83) but this may just represent excess fluid consequent on their unusually small size in relation to the size of the pupa parasitized. The state of the host
when arrested by the parasitoid is another factor likely to have a bearing on staining, as it might be expected that histolyzed hosts could potentially leak quite a lot of fluid during parasitoid emergence, and in a rather inconsistent way.

Overall, even from this very sparse survey within a narrow zoogeographical region, there is considerable evidence that the form, toughness, concealment and duration of the host pupa plays a major role in determining how specialist ichneumonids emerge from it, although the trends within and between ichneumonid tribes and subfamilies outlined above might repay a more detailed and wider survey. While the toughness of the host pupa correlates quite strongly with its duration, and the strongest pupae tend to be cap-cut, there is not complete correspondence (for example, *Agrypon polyxenae* ecloses by chewing a hole (Figs 61, 64) in very tough exposed pupae that must persist unprotected for ten or more months in the field). In the subfamily Ichneumoninae both koinobiont and idiobiont taxa are found, but there seems to be no consistent difference in the mode of emergence between them, although we have had fewer examples of the latter category to explore.

It is rather obvious that some correlation between mandibular structure and the kind of exit holes made would be expected, and indeed this is rather easily seen — *Trogorus*, in particular, has quite small coarsely punctate but otherwise unremarkable mandibles; the teeth are blunt and although the upper tooth is the larger the two teeth are in the same plane. But it is well beyond the scope of the present paper to attempt more than cursory notes on variation in mandibular structure in relation to eclosion, especially bearing in mind the minimal extent of the data. Also, the mandibular structure of the Chalcidoidea mentioned is fundamentally different from that of ichneumonids and cannot be compared. Nevertheless it seems clear that the best cap-cutters in the Ichneumonidae (e.g. Listrodromini (Figs 38–40), *Hoplismenus* (Figs 14–16) and *Eurylabus* (Fig 54), as well as some *Ichneumon* and close relatives) have a relatively long and acute upper tooth and a reduced and sometimes inflexed lower tooth; and some of the crudest emergence holes are made by the taxa with untwisted mandibles and the least difference in size of the two teeth, such as *Goedartia* (Fig. 55) and *Pimpla* (Fig. 74). Even within a genus, such as in *Ichneumon* (Figs 17–29), this trend appears to hold up moderately well. However, it is not always the case: for example the phaeogenine *Herpestomus brunnicornis* (Gravenhorst) has the sharp upper tooth substantially the longer and yet exhibits predominantly type 2 emergence (Fig. 42). Another phaeogenine, *Heterischmus*, shows a consistent detaching of an apicoventral cap (Fig. 43), presumably in some way connected with its strongly curved, narrow and unidentate mandibles. Among Anomaloninae, the type 1 cap-cutting taxa seem to have the upper tooth on the whole slightly longer than in the hole-chewing taxa, and perhaps more particularly to have a stronger flange along the lower margin of the mandible. For some taxa, evolutionary pressure on the form of the mandible to enable emergence from the host’s substrate (or cocoon) must trump the barrier of the mere host pupal cuticle. This presumably accounts for the form of the widened blunt mandible of *Chasmias* and the crudity with which it leaves the host (Figs 36, 37) en route to chewing through a tough plant stem. Another case is the mess made of the host pupa (Fig. 56) by *Cotiheresiarches*...
before it tackles the hard cocoon of its host; in this case the whole face is unusual, with a protruding plate-like clypeus as well as blunt, unidentate and curved mandibles, which presumably aid its escape from the host’s tough obovoid cocoon through a small circular hole (much as that made by the moth when it emerges, and possibly exploiting the same prepared facility; the cocoon in Fig. 56 has been artificially torn open). For others, such as Pimplini, not much specialisation of the rather broadly tapering and small toothed subequidentate mandible for eclosion seems to have occurred, although the arguably most specialised genus of those examined, *Apechthis*, which tends to cut strips (visible in both Figs 72 and 73) and emerges from relatively neat holes, does have an out-turned lower rim of the mandible if not quite a flange. An interesting difference is seen in the two species of *Metopius* examined (Figs 70 and 71), which belong to two subgenera with significantly different mandibular structure. Although *Metopius* (*Peltocharus*) *dentatus* (Fabricius) (Fig. 70), which has a tough host cocoon in addition to its pupa to contend with, has a slightly longer upper tooth the two teeth are in the same plane, while in *M.* (*Tylopius*) *leiopygus* Foerster (Fig. 71) the mandible is twisted, with the smaller lower tooth inflexed, so that the mandibles present as scissor-like blades. This brief survey by no means covers the range of mandibular structure seen in Ichneumonidae, and in fact most of the truly bizarre structures known in the family are not included, although probably most have nothing to do with Lepidoptera pupal hosts. Nevertheless, it seems clear that among taxa eclosing from Lepidoptera pupae there are strong links between the form of the mandibles and the kind of egress opening that is made, allowing also for any need to break through whatever additional protection the host pupa may habitually have, with some predictive possibilities. The extent to which these characteristics have evolved to meet (sometimes extreme) autecological requirements, rather than being rooted in phylogeny, is an unresolved question of a familiar kind, but the more that is understood about the basic biology of the host-parasitoid relationships the better such conundrums can be resolved.

The origin of the presumed secretion through which the host pupal cuticle is degraded by the eclosing *Trogus* adult is unknown. Although it would not explain how the necessary agent reaches the site of emergence, it would be worth investigating whether the extremely coarsely sculptured metasomal tergites of the *Trogus*-subgroup (which are unlike those of other ichneumonines, and whose morphology appears not to have been explained) may have a glandular, secretory function, as it is difficult to see what other (externally visible) morphological feature of *Trogus* might be involved, although its mandible is unusually coarsely punctate. Otherwise, the glossa and other submandibular aspects of the mouthparts seem rather enlarged in *Trogus*, but this might be a modification for merely spreading the fluid.

As an extension of this survey, we examined extralimital pupae of very large saturniid moths from which rather smaller solitary ichneumonids had emerged, on the one hand an unidentified koinobiont *Agyron (=Trichionotus)* species (Anomaloninae) from a Mexican saturniid, *Copaxa multifenestrata* Herrich-Schäffer (Figs 86–88), and on the other hand an unidentified idiobiont *Xanthopimpla* sp. (Pimplinae) from *Attacus atlas* (Linnaeus) in Thailand (Figs 89, 90). In the first (*Agyron*) the *Copaxa*
pupa was very malodorous some days after after the parasitoid’s emergence, and when opened (Fig. 88) the now-hard but messy remains of the host (apparently very unstructured; probably fully histolysed) could be seen to have been separated (perhaps within a membrane) from the area that had been occupied by the pupating parasitoid, which did not appear to have been isolated from them by any clear cocoon and may have resided in the ecdysal space of the host pupa. The staining seen in Fig. 87 seems more likely to have been a result of incidental spillage from wet host content than to indicate biochemical degradation of the host pupal chitin. In the case of Xanthopimpla the parasitoid appeared to have scraped its way through considerable distances of accreted material in the Atticus pupa, leaving a more or less smooth tunnel through a very hard substance, without resulting in any clear staining of the cuticle (Fig. 89) that might have indicated biochemical assistance (although most of its arduous journey to the surface did not involve the host cuticle, Xanthopimpla has basally broad but evenly narrowed mandibles which are strongly twisted so that the small ventral tooth is not involved in the powerful pickaxe-like anterior presentation). In contrast to the smelly Copaxa remains, those of the Attacus were set hard and (at least by the time they were received by MRS) inoffensive with a faintly sweet smell, perhaps indicating that some suppression of microorganisms had occurred, possibly in the manner reported for Pimpla by Führer and Willers (1986). But whether or not pimplines such as Pimpla and Xanthopimpla may provide interesting antibacterials, they do not seem to degrade chitin or other impediments to adult emergence biochemically.

It should be noted that no consideration has been given here to emergence of adult Ichneumonidae through the host pupal (or puparial) cuticle of Diptera (e.g. as by Diplazontinae which have specialized mandibles: Rotheray 1981), or the Orthocentrus-group of Orthocentrinae (in which mandibles are severely reduced).

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Intraspecific variations in the venom peptidome of the ant Odontomachus haematodus (Formicidae: Ponerinae) from French Guiana

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Abstract
Ant venoms are complex cocktails of toxins employed to subdue prey and to protect the colony from predators and microbial pathogens. Although the extent of ant venom peptide diversity remains largely unexplored, previous studies have revealed the presence of numerous bioactive peptides in most stinging ant venoms. We investigated the venom peptidome of the ponerine ant Odontomachus haematodus using LC-MS analysis and then verified whether the division of labor in the colonies and their geographical location are correlated with differences in venom composition. Our results reveal that O. haematodus venom is comprised of 105 small linear peptides. The venom composition does not vary between the different castes (i.e., nurses, foragers and queens), but an intraspecific variation in peptide content was observed, particularly when the colonies are separated by large distances. Geographical variation appears to increase the venom peptide repertoire of this ant species, demonstrating its intraspecific venom plasticity.

Keywords
Ant venoms, MALDI-TOF MS, Odontomachus haematodus, peptidome, polyethism

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Introduction

Due to their ubiquity in terrestrial environments, ants are amongst the most abundant venomous animals on Earth; for instance, they represent 15-20% of the animal biomass in tropical forests (Hölldobler and Wilson 1990). Among ants, ca. 9100 species belonging to 16 subfamilies possess a stinging apparatus, while other subfamilies have lost their ability to sting (Hölldobler and Wilson 1990). Ant venoms contain a variety of toxins that paralyze prey, ward off predators and protect the colony against microbial pathogens (Orivel et al. 2001; Schmidt 1982). As they have been little studied, ant venom peptides represent a potentially promising source of bioactive molecules with novel scaffolds and original pharmacological activities. Previous studies (Aili et al. 2014; Touchard et al. 2014; Touchard et al. 2015) have demonstrated that the venoms of stinging ants are mostly comprised of small peptides, similarly to spider, scorpion and cone snail venoms. A limited number of peptidic toxins from several ant subfamilies such as the Ponerinae (Cologna et al. 2013; Johnson et al. 2010; Orivel et al. 2001), Paraponerinae (Piek et al. 1991), Ectatomminae (Arseniev et al. 1994; Pluzhnikov et al. 2000), Myrmicinae (Rifflet et al. 2012), Myrmeciinae (Inagaki et al. 2004; Inagaki et al. 2008) and Pseudomyrmecinae (Pan and Hink 2000) have been characterized.

One of the major issues in the biochemical and pharmacological study of venoms is the reproducibility of studies conducted on field-collected samples, which requires accurate species identification. We have previously demonstrated that, at the species level, the peptidic fingerprints of ant venoms are reliable chemotaxonomic markers for species determination and may possibly allow the discrimination of unresolved species complexes (Touchard et al. 2014). However, intraspecific variations can also occur as shown recently in Dinoponera quadriceps for which only 48 peptides were shared between colonies out of the more than 300 peptides found in total (Cologna et al. 2013). Also, intraspecific variations in venom composition have been observed in snakes, scorpions, tarantulas and cone snails, this variation being linked to geographical distribution (Núñez et al. 2009; Shashidharamurthy et al. 2002), age (Escoubas et al. 2002) or sex (Escoubas et al. 1997; Herzig and Hodgson 2009; Herzig et al. 2002; Herzig et al. 2008). However, it remains unclear whether such variation is a common denominator for all venomous animals or is restricted to some taxa or species. This key point is still a subject of debate amongst specialists, since venom sampling conditions are often limiting and broad species-wide surveys of venom composition have been difficult to conduct.

In ants as in all hymenopterans, only females are venomous, so that sex cannot account for venom variation. Therefore, intraspecific variations in venom composition could be related to geographical distribution, diet, age or division of labor (polyethism). In most ant species, reproduction is carried out by the queen(s), while all other tasks are performed by the workers for whom the division of labor is based on physical caste (there is polymorphism in the worker caste) or, most often, age (Fresneau 1994). Usually, the youngest workers are involved in intranidal activities, whereas older workers are assigned to tasks outside the nest such as defense and foraging (da Silva-Melo and Giannotti 2012; Sendova-Franks and Franks 1999; Wilson 1963).
As venom is mostly used by workers performing extranidal activities, one can hypothesize that polyethism could affect venom composition. To test this hypothesis, we investigated both intracolonial and intercolonial variations in venom composition in the Neotropical ponerine species, *Odontomachus haematodus*. The monomorphic workers of this species possess a peptide-rich venom (Touchard et al. 2014) used in colony defense and prey capture. To assess possible venom variations, we characterized the entire venom peptidome by combining HPLC chromatographic separation with offline MALDI-TOF mass spectrometry analysis and explored the putative differences in venom composition between castes and type of activity (i.e., queens, and worker nurses and foragers) as well as colonies from different geographical locations via the comparison of their venom peptidic fingerprints.

**Materials and methods**

**2.1. Ant collection and taxonomy**

*Odontomachus haematodus* colonies were collected from three different areas in French Guiana: six colonies were collected on the *Campus Agronomique*, Kourou; three in Sinnamary; and one in Angoulême (Fig. 1). In the laboratory, the ant colonies were conserved in artificial nests made of plastic boxes (11 cm x 11 cm x 6 cm) filled with 2 cm of molded plaster to create chambers and covered by a plate of red glass. These boxes were connected to a foraging arena consisting of a second, similar plastic box without plaster. The colonies were kept at 25 °C and provided with dead mealworms and honey twice a week.

Voucher specimens were deposited in the *Laboratorio de Mirmecologia*, Cocoa Research Centre, Ilhéus, Bahia, Brazil.

**2.2. Behavioral observations and ant groups**

To investigate the division of labor, workers were individually marked with different colored dots of paint on their thoraxes and gasters. Worker tasks were determined by scan sampling their behavior (three scans per day at 9:00 am, 2:00 pm and 5:00 pm; 5 days per week over 3 weeks). The percentage of presence in the foraging area for each individual over the 3-week period was calculated to define the behavioral groups. Workers that had either never been seen in the foraging area or were there between 0% to 25% of the time were considered nurses ([group 0%] and [group 25%], respectively). Those observed between 25% to 50% of the time in the foraging area were considered intermediates [group 50%], and those observed between 50% to 75% or between 75% to 100% of the time in the foraging area were considered foragers ([group 75%] and [group 100%], respectively). Moreover, winged females present in the colonies were named “virgin queens” in order to differentiate them from “reproductive queens” devoid of wings (see Fig. 1).
2.3. Venom analysis

Ants were killed by freezing at -20 °C prior to dissecting their venom glands. The dissected venom glands were placed in 10% acetonitrile (ACN)/water (v/v), centrifuged for 5 min at 14,400 rpm and the supernatant was collected and lyophilized prior to storage at -20 °C for subsequent biochemical analysis. To study intra-colonial variations and the influence of the role of individual ants in the colony, five venom glands from each behavioral group (cf. Fig. 1) were dissected and pooled for each colony. Furthermore, 100 venom glands from the workers of one colony (Kou06; 3.55 mg of dry crude venom in total) were dissected to carry out an in-depth exploration of the whole venom by LC-MS.

Figure 1. Sites where the 10 *Odontomachus haematodus* colonies were collected in French Guiana. Table panels show information about each colony, including GPS coordinates, colony code and the different behavioral groups. One hundred dissected workers from colony Kou06 were used for LC-MS investigation.
2.4. Chromatographic separation

In order to fully explore the *Odontomachus haematodus* peptidome, a venom sample pooled from 100 worker workers was fractionated by reversed-phase high performance liquid chromatography (RP-HPLC) on a Waters Xterra-C18 5µm, 2.1 × 100 mm column with an Agilent HP 1100 HPLC system. Fractionation was achieved using a gradient of solvent A (water / 0.1% trifluoroacetic acid TFA) and solvent B (ACN / 0.1% TFA). The percentage of solvent B was modified at a flow rate of 0.3 mL/min as follows: 0% for 5 min, 0-60% for 60 min, 60-90% for 10 min and 90-0% for 15 min. The absorbance of the column effluent was monitored at 215 nm on a diode-array detector. The signal was monitored in real time, and fractions were collected manually for each eluting peak. Individual fractions were then dried and reconstituted in 50µL of water/0.1% TFA for subsequent off-line MALDI-TOF MS analysis and disulfide bond reduction.

2.5. Chemical reduction of disulfide bonds

To map the distribution of disulfide-linked peptides in the venom, 5µL of each fraction were incubated in 10µL of a reducing buffer (100 mM Tris, pH 8, 6M guanidine) with 10 mM dithiothreitol (DTT) for 1h at 56 °C in the dark. The reaction was stopped by the addition of 5µL of water / TFA 0.1%. Prior to mass spectrometry analysis, reduced fractions were desalted using Ziptip® C18 (Millipore) pipette tips. As the chemical reduction of disulfide bonds results in a mass increase of 2 Da for each bond, comparing mass shifts between native and reduced venom fractions allowed the presence of disulfide-linked peptides and the number of disulfide bonds for each to be compared.

2.6. Mass spectrometry analysis

Mass spectrometry analyses were performed on a Voyager DE-Pro MALDI-TOF mass spectrometer (Applied Biosystems; CA, USA) using α-cyano-4-hydroxycinnamic acid (CHCA) matrix dissolved at 5 mg/mL in water/ACN/TFA (50/50/0.1 v/v/v). Prior to MS analysis, crude venoms were desalted using Ziptip® C18 (Millipore) pipette tips. Then, 1µL of each reconstituted HPLC fraction or the desalted crude venom was deposited on the MALDI target plate followed by 1 µL of the matrix. Each spectrum was calibrated externally using a mixture of peptides of known molecular masses in the same m/z range (Peptide calibration Mix 4, LaserBio Labs, Sophia-Antipolis, France). External calibration was performed by depositing, adjacent to each sample, 0.5 µL of the calibration mixture co-crystallised with 0.5 µL of the CHCA matrix. All spectra were acquired in reflector mode to maximize the accuracy of the mass determination. Spectra were collected over the m/z 500–10,000 range in positive ion mode (200 shots per spectrum) and were automatically calibrated using the sequence module of the Voyager® control software (Applied Biosystems, USA).
2.7. Data analysis

The mass spectra were subjected to a baseline correction (0.7 correlation factor) and Gaussian smoothing (5-point filter width) using Data Explorer® 4.11 software. Potential sodium and potassium adducts were manually removed from all mass lists. Masses matching within ± 1.0 Da were defined as identical peptides in this study. Identical masses in adjoining HPLC fractions, which were interpreted as reflecting an incomplete separation, were also removed. Two-dimensional scatter plots, termed “2D venom landscapes”, were constructed using SigmaPlot 12.0 software. All peptide masses detected in the HPLC fraction spectra were plotted as dot graphs with \( m/z \) values on the y-axis and RP-HPLC elution time on the x-axis. A Principal Component Analysis (PCA) of the relative abundance of peptides in the mass spectra was performed using PAST 3.02 software.

Results

3.1. Venom peptidome analysis

The LC-MS analysis of *Odontomachus haematodus* venom revealed the presence of 105 peptides (Table 1). All of the peptides are small, falling within a narrow mass range of \( m/z \) 777.49 to 2978.5 (M+H⁺) (Fig. 2A-B). We estimated that the number of residues varied between 7 and 27 based on a theoretical estimate of MWᵣ of 111.1254 Da for an average amino acid (Averagine). The value is derived from the statistical occurrence of amino acids in proteins (Senko et al. 1995), and calculated with the formula \( C_{4,9384}H_{7,7583}N_{1,3577}S_{0,0417} \). All of the peptides eluted between 15% and 45% ACN (retention time: 20-50 min), with the most abundant peptides in the venom eluting between 35% and 45% ACN (retention time: 40-50 min) (Fig. 2C).

**Table 1.** Mass list of peptides (\( m/z \)) from *O. haematodus* venom collected in Kourou (Kou06).

<table>
<thead>
<tr>
<th>( m/z )</th>
<th>777.49</th>
<th>800.84</th>
<th>809.31</th>
<th>822.3</th>
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<th>888.6</th>
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<tr>
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<td>1044.73</td>
<td>1047.5</td>
<td>1058.75</td>
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<tr>
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<td>1978.03</td>
<td>1979.02</td>
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</tbody>
</table>
Intraspecific variations in the venom peptidome of the ant Odontomachus haematodus...

3.2. Venom peptidome variations

We collected 43 venom samples from the nine Odontomachus haematodus colonies monitored: 18 venoms from nurses; eight from intermediates, seven from foragers, six from fertilized queens and four from virgin queens. The MALDI-TOF MS peptidic mass fingerprinting of these 43 crude venoms resulted in the selection of the 20 characteristic peptides masses (i.e., showing the most abundant signals) which constituted the matrix used for the principal component analysis (PCA) (m/z 1842.6, 1861.1, 1916.91, 1962.89, 2019.91, 2044.69, 2062.27, 2086.03, 2095.89, 2117.03, 2219.16, 2245.32, 2387.28, 2473.22, 2515.32, 2590.18, 2679.79, 2784.34, 2789.25, 2802.39).

The mass analysis of the chemically reduced HPLC fractions did not show any mass shift between native and reduced fractions, demonstrating that Odontomachus haematodus venom is exclusively composed of linear peptides (i.e., devoid of disulfide bonds).

Figure 2. Investigation of the whole Odontomachus haematodus venom peptidome by LC-MS. (A) Two-dimensional landscape of the venom. Dots indicate peptides. (B) Box-and-whisker plot of the peptide mass distribution presented in the 2D venom landscape. The bottom and top ends of the box represent the first and third quartiles, respectively, while the line inside each box represents the median mass. The ends of the whiskers represent the 5-95 percentile range while the dots represent masses outside the 5-95 percentile range. (C) C18 RP-HPLC chromatogram of the venom. The dashed line shows the slope of the ACN gradient.
A PCA based on the relative abundance of the selected peptides revealed that the first two principal components accounted for 68.1% of the variance (Fig. 3). The PCA showed that the venom composition was not related to caste or type of activity (Fig. 3). Indeed, venoms from different behavioral groups showed similar patterns, indicating that polyethism and reproductive status did not affect the peptidic composition of the venoms (Fig. 4). Yet, some qualitative intracolonial differences were noted in the behavioral groups as illustrated by the nurse group (Fig. 5).

The venoms from Angoulême, which contained two specific peptides ($m/z$ 1861.1 and 2062.27), were separated from those from the two other localities and differed only by the relative proportions of the mass 2019.91 $m/z$ (Fig. 3).

**Discussion**

Ant venoms are complex cocktails of peptides which have evolved to act on multiple biological targets. By combining MALDI-TOF MS with chromatographic separation, we have shown that the *Odontomachus haematodus* venom peptidome is composed of more than 100 small and linear peptides in the 700-3000 $m/z$ mass range. This feature is consistent with a previous study on the venoms of five Neotropical *Odontomachus* species (Touchard et al. 2014) as well as wasp and cone snail venoms which are usually comprised of peptides with fewer than 35 residues (Baptista-Saidemberg et al. 2011;
Figure 4. Intracolonial mass spectra variations among the different behavioral groups of colony Kou05. Few qualitative variations can be observed and many dominant peptides were present in all groups, particularly the shaded masses.
Cologna et al. 2013; de Souza et al. 2004; Gomes et al. 2014; Johnson et al. 2010; Lewis et al. 2012; Orivel et al. 2001). In contrast, spider, scorpion and snake venoms may also contain larger peptides with typically from 30 to 100 amino acids (Olivera et al. 1990).

Because the presence of peptides and proteins in venoms is associated with the metabolic cost of venom production, we hypothesized that ants dedicated to tasks within the nest, typically nurses and queens, may possess less complex venoms than foragers, the latter using their venom to subdue prey and deter enemies and therefore needing venoms with a higher level of efficacy. Yet, our results show that the venom composition does not differ between nurses, intermediates, foragers or even queens in *Odontomachus haematodus*. By comparison, the toxicity of *Neoponera commutata* (Ponerinae) worker venoms was not related to age or task specialization, but the workers from different behavioral castes possess different amounts of venom in their reservoir (Schmidt and Overal 2009). Also, callow *Harpegnathos saltator* (Ponerinae) workers have empty venom sacks, and workers dedicated to tasks inside the nest have lower
Intraspecific variations in the venom peptidome of the ant Odontomachus haematodus...

amounts of venom than do older ones (Haight 2012). Therefore, we can hypothesize that, in most Ponerinae, nurses limit the metabolic cost of venom by producing lower amounts, but with the same peptidic composition as the foragers. Interestingly, these results contrast with the case of fire ants whose alkaloid venom composition changes with the size and age of the workers (Deslippe and Guo 2000; Haight and Tschinkel 2003). The venom composition of all fire ant queens is also presumably different from workers which assumes different biological effects (Eliyahu et al. 2011; Fox et al. 2012). Also, Neoponera commutata (Ponerinae) and Pogonomyrmex spp. (Myrmicinae) queens produce less venom than do workers and their venom is significantly less lethal and paralytic than that of the workers, suggesting differences in venom composition (Schmidt and Overal 2009; Schmidt and Schmidt 1985).

In Odontomachus haematodus, differences in venom composition seem rather associated with geographic variations as the venom peptidic fingerprints clearly differed between colonies, particularly if they came from locations separated by large distances. Such inter-colonial variations have previously been reported for Dinoponera quadriceps (Ponerinae) collected from different areas in Brazil (Cologna et al. 2013). Among animal venoms, intraspecific variations related to geography are a common phenomenon and have been reported in snakes (Shashidharamurthy et al. 2002), cone snails (Duda et al. 2009), scorpions (Omran and McVean 2000), spiders (Escoubas et al. 1998) and both social (Dias et al. 2014) and parasitoid wasps (Poirié et al. 2014). In snakes, intraspecific variations have been shown to exhibit a differential venom effectiveness towards different prey (Casewell et al. 2013). This may be the result of allelic variations in the genes coding the peptides as shown for Conus ebraeus venom (Duda et al. 2009), increasing the venom peptidic diversity in this species. This intraspecific diversity is essential for natural selection and ant venom diversification.

Previous studies have demonstrated that toxins (peptide and alkaloids) in ant venoms can be used as chemotaxonomic markers in order to identify species but also to reveal cryptic ant species (Fox et al. 2012; Touchard et al. 2014). Thus, the observed intraspecific variation in the venom composition of Odontomachus haematodus might also result from the presence of cryptic species. It would be interesting in the future to extend this study through further genetic analysis to assess the possible presence of different cryptic species.

Conclusion

The present study constitutes the first exploration of the Odontomachus haematodus venom peptidome, revealing that this venom is comprised of more than 100 small linear peptides. Also, the peptidic diversity in this species is amplified due to intraspecific variations. The present results show that these venom variations are not related to caste or type of activity, but seem to be related to the geographical location of the ant colonies or to a hypothetical complex of cryptic species. It would be interesting in the future to analyze whether such variations can affect the effectiveness of the venom
in prey capture. It will also be necessary to consider such intercolonial variations in peptidic composition to ensure the reproducibility of further biochemical and pharmacological studies on ant venoms.

Acknowledgments

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References


Intraspecific variations in the venom peptidome of the ant Odontomachus haematodus...


The body size of the oil-collecting bee
Tetrapedia diversipes (Apidae)

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Abstract
The body size of bees can affect their fitness in many ways. There is an indirect relationship between the size of the mother and the size of her progeny. This is so because large mothers use larger nests and brood cells and have higher foraging capacity than small mothers, and consequently large mothers supply a larger amount of food to their larvae, which grow larger. We analyzed the relationship between body size of individual oil-collecting bees of the species Tetrapedia diversipes and the size of their brood cells from Boracéia and Ilhabela, southeastern Brazil. In addition, we manipulated 26 brood cells of a population at the campus of Universidade de São Paulo by removing food from 13 brood cells. In this case, we checked the relationship between the body size of these bees and the amount of food consumed. We measured 241 individuals: 135 males and 106 females. No significant size difference was found between males and females. Only a weak relationship between body size and brood cell volume was detected, possibly due to the low variation in both female size and brood cell size. In the food manipulation experiment, the unmanipulated individuals were larger than individuals for whom part of the provisions were removed but no correlation was found between amount of food removed and offspring size.

Keywords
Solitary bee, morphometry, cell provisions, parental investment, trap nest
**Introduction**

The body size of bees strongly affects their fitness. Larger individuals can produce more eggs (Tengo and Baur 1993) and survive diapause better (Tepedino and Torchio 1982); larger males can defend more effectively their territories and have more mating opportunities (Alcock 1995); in social species larger females can become dominant (Buckle 1982). However, the size of bees is a balance between advantages and disadvantages that determine the range of optimal body size (Blanckenhorn 2000, Seidelmann 2014).

The body size of a bee is also correlated to the body size of its parents. However, size is probably not genetically inherited; when it occurs, the inheritance is very weak (Tepedino et al. 1984). The body size of parent bees and the body size of their progeny are correlated due to the parents’ contribution to the amount of food supplied to their larvae (Johnson 1990, Ribeiro 1994). Larger individuals build larger nests with larger brood cells and larger amounts of food (Rooijakkers and Sommeijer 2009, O’Neill et al. 2010). In bee species that nest in pre-existing cavities, the size of the brood cell is related to the diameter of the cavity, and the diameter of the cavity is related to the amount of food supplied (Klostermeyer et al. 1973).

Besides food resources (pollen and nectar), bees of the genus *Tetrapedia* (Tetrapediini, Apidae) collect floral oils and have morphological and behavioral adaptations to exploit this resource (Alves dos Santos et al. 2007). Females of this genus nest in pre-existing cavities such as bamboo hollows, and trap nests of several sizes (Camillo 2000, Garófalo et al. 2004). In general, the nests have between 6 and 15 cells and construction time varies from 3 to 6 weeks (Alves dos Santos et al. 2002). As obvious as it may seem few studies have analyzed the amount of food for the larvae and size of emerged bees (Klostermeyer et al. 1973, Danforth 1990, Johnson 1990). However, in none of them was removed food from brood cells.

This study examined variation in several morphometric measures in individuals of *Tetrapedia diversipes* in relation to population origins, gender, and brood cell size. For this proposal, the study consisted of two parts: In the first part we used data from trap nests from two populations and related the morphometric measurements of bees with their populations of origin, sex and brood cell volume. In the second part we used a third population to manipulate the amount of food that was available to the larvae and related that to measures of these bees.

**Methods**

The specimens of *Tetrapedia diversipes* used in our analysis were reared from trap nests from areas within the Atlantic Forest in the state of Sao Paulo, Southeastern Brazil: (1) Ilhabela State Park (23°45’S – 45°27’W; 7 m a.s.l.); (2) Boracéia Biological Station (23°38’S – 45°52’W; 492 m a.s.l.). The individuals from Ilhabela and Boracéia were obtained in a trap nest inventory carried out by Cordeiro (2009). We obtained 730 individuals of *T. diversipes* from 347 nests (249 individuals from 116 nests in Boracéia...
and 481 individuals from 231 nests in Ilhabela). We used part of this material. The trap nests consisted of were composed of perforated wood blocks filled with black cardboard, each 0.6 cm in diameter and 5.8 cm in length (two nests, one from Ilhabela and other from Boracéia had a diameter of 0.8 cm).

**Morphometry of individuals and cells of two populations**

The measurements taken from the specimens were: head width, the distance from the central ocellus to the fissure of the labrum, and intertegular span (Fig. 1). To estimate the dry mass of bees, we used the following formula: \( ITS = 0.77 \ DM^{0.405} \), where ITS is the intertegular distance and DM is the dry mass (Bullock 1999). The bees were photographed with a digital camera coupled to a stereomicroscope and the structures were measured with Gimp® software. The individuals were separated by place of collection, sex, and nest of origin. In total, 135 males and 106 females from Ilhabela (105 males and 69 females) and Boracéia (30 males and 37 females) were measured. Brood cell length was measured with a digital caliper, and the volume of the cells was estimated (considering the nest diameter, as described in the previous topic, the cells are cylindrical). The body size measures were analyzed by PCA (Principal component analysis) and with the PCA scores we performed ANOVAs to do the comparison of body sizes between populations. We used the estimated dry weight in the analysis. The relationship between PCA scores and brood cell volume was estimated for individuals from Ilhabela and Boracéia using a regression test. The statistical analysis was performed in R (R Development Core Team 2009).

**Food manipulation experiment and morphometry**

We tested the influence of the amount of food available on the size of the individual in the campus at the University of São Paulo (23°33’S, 46°43’W) at the Laboratory of Bees, in the state of Sao Paulo, Southeast Brazil. For this, we used individuals obtained

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**Figure 1.** Measurements taken from individual *Tetrapedia diversipes*. **A** Head width between eyes (dotted arrow) and head length (from the central ocellus to the fissure of the labrum) **B** Intertegular distance.
from trap nests maintained permanently at the Laboratory of Bees. For four months, from November 2010 to February 2011, we monitored trap-nests that were occupied by *Tetrapedia diversipes* females. When the construction of one cell was concluded (after monitoring the behavior of the female for some days), the tube containing the nest was removed from the wooden block and examined. The space occupied by the provisions in each cell was measured with a digital caliper, and the last cell of the series had part of its provisions removed. The pollen removed portions of the provisions were weighed (fresh weights) on a high-precision scale (Explorer OHAUS) and stored in Eppendorf tubes in the freezer. All the provisions from five cells was removed and to obtain the amount of food deposited for one larva (N = 5). The manipulated nests were placed in laboratory tubes and monitored daily until emergence (N = 13), other 13 cells of imatures were unmanipulated. After emergence, individual *T. diversipes* were killed with ethyl acetate, pinned, labeled, and deposited at the Paulo Nogueira Neto Entomological Collection (CEPANN). The same measurements cited in the previous item were taken from these individuals (Fig. 1).

The measures of body sizes used in this work are correlated, thus, the body measures were analyzed by PCA (Principal component analysis). We used the PCA scores we to perform t tests to evaluate the morphometric differences among individuals that had their food manipulated and those that did not. The regression analysis was made to test the influence of the amount of food removed on bee size.

**Results**

**Morphometry of individuals from Ilhabela and Boracéia**

The PCA analysis showed that about 70% of the measures variation was explained for the first axis of the PCA (PC1) (70% in Ilhabela and 69% in Boracéia). In 241 individuals measured (67 from Boracéia Biological Station and 174 from Ilhabela State Park, Table 1) we observed a difference in the head length of bees between populations (F = 69.39; d.f. = 1; p = 0.0001) (Fig. 2). In the Ilhabela and Boracéia populations there were not significant difference between sexes and the PC1 (F = 3.12; d.f. = 1; p = 0.07 and F = 2.53; d.f. = 1; p = 0.11, respectively).

<table>
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<tr>
<th>Locality</th>
<th>Sex</th>
<th>N</th>
<th>Head length (mm)</th>
<th>Head width (mm)</th>
<th>Intertegular distance (mm)</th>
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<td>69</td>
<td>2.11 ± 0.16</td>
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<td>♂️</td>
<td>30</td>
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<td>♂️</td>
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<td>22.99 ± 0.10</td>
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*Table 1.* Mean ± standard deviation of the measurements taken from *Tetrapedia diversipes* males and females from Ilhabela and Boracéia.
Body size of *Tetrapedia diversipes*

The 241 cells of *Tetrapedia diversipes* from Ilhabela (174) and Boracéia (67) populations had similar volumes (Table 2). In both populations, most cells (> 80%) had a volume close to the mean value (between 0.22 cm³ and 0.30 cm³).

No relationship was observed between PC1 and brood cell volume for the Ilhabela population (F = 0.027; d.f = 172; p = 0.86, R² = -0.005). For the Boracéia population, there was a positive relation between PC1 and brood cell volume (F = 5.89; d.f = 65; p = 0.01, R² = 0.06).

Food manipulation experiment and morphometry

Twenty-six individual *T. diversipes* emerged from the monitored cells: 13 from cells that had the food manipulated and 13 from unmanipulated cells (Suppl. material 1). Individuals from unmanipulated cells were significantly larger than individuals from manipulated cells in all measurements analyzed: head width (F = 55.53; d.f. = 25; p = 0.0001), head length (F = 31.03; d.f. = 25; p = 0.0001), and estimated dry mass (F = 59.11; d.f = 25; p = 0.0001) (Fig. 4). The food from unmanipulated cells weighed, on average, 89.28 ± 8.13 mg (max. = 92.04 mg; min. = 70.53 mg).

The PC1 and the second component (PC2) of PCA explained 63% and 20%, respectively, of all variation on the measures manipulated bees and 62% and 25% of all variation on the measures unmanipulated bees. Thus, we used the PC1 and the PC2 to compare manipulated and unmanipulated bees. The scores of the PC1 and the PC2 of

**Table 2.** Measurements of *T. diversipes* brood cells, from which the studied individuals emerged.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Number of cells</th>
<th>Mean length of cells (cm)</th>
<th>Mean volume of cells (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boracéia</td>
<td>64*</td>
<td>0.97 ± 0.07</td>
<td>0.27 ± 0.035</td>
</tr>
<tr>
<td>Ilhabela</td>
<td>171*</td>
<td>0.89 ± 0.09</td>
<td>0.25 ± 0.03</td>
</tr>
</tbody>
</table>

*Four cells from Boracéia and three from Ilhabela, whose nest diameter was 0.8 cm, were removed from this analysis.

The 241 cells of *Tetrapedia diversipes* from Ilhabela (174) and Boracéia (67) populations had similar volumes (Table 2). In both populations, most cells (> 80%) had a volume close to the mean value (between 0.22 cm³ and 0.30 cm³).

No relationship was observed between PC1 and brood cell volume for the Ilhabela population (F = 0.027; d.f = 172; p = 0.86, R² = -0.005). For the Boracéia population, there was a positive relation between PC1 and brood cell volume (F = 5.89; d.f = 65; p = 0.01, R² = 0.06).

**Figure 2.** Head length of emergent individual *Tetrapedia diversipes* from the studied populations. The box plot shows the median, the quartiles, and the maximum and minimum of the values measured.
the unmanipulated bees were significantly larger than the manipulated bees ($t= 7.94$; d.f. = 51; $p = 0.0001$).

No correlation was found between PCI and the amount of food removed from a cell ($R^2 = -0.08$; d.f. = 11; $p = 0.88$).

**Discussion**

There was difference in head length between the two populations of *Tetrapedia diversipes* studied. In general, morphometric differences are expected in bee populations that do not maintain a gene flow and are separated into different subspecies (Amssalu et al. 2004, Borsuk and Olszewski 2010). The studied populations are 52 km apart, Boracéia in the mountains (499 m a.s.l.) and Ilhabela in the coast (7 m a.s.l.), further-
more Ilhabela is an oceanic island. Therefore, there is probably little or no gene flow between them. This could explain the difference in head length between populations. Neves (2012) observed that three populations of *T. diversipes* in Bahia, separated by up to 500 m, differed in wing morphometry. The author suggested that low gene flow between populations may explain the difference found.

We found a positive correlation between bee size and brood cells volume in one of the populations studied (Boracéia), however, there was a poor fit of the data to the straight line correlation. This may be a reflection of both the small size variation of the female bees and lack of variation among the size of the cells built. Although, the trap nests offered have a standardized diameter (0.6 cm), the bees could adjust the available space cells by making cells longer or shorter. Nonetheless, even for bees that emerged from the seven cells (two nests) whose nest diameter was 0.8 cm, we did not find a significant difference. The body sizes of individuals that emerged from these cells were similar to the population mean (Suppl. material 2). The amount of food supplied may vary if the females build longer cells. Nevertheless, we do not have data on the amount of food available for the bees from Ilhabela and Boracéia. This becomes evident if we analyze the volumes of the cells built. In both areas most cells built (approximately 80%) had a volume close to the mean. Hence, the weak relationship found may be a consequence of most cells having similar volumes.

Differences in body sizes are probably not hereditary (Tepedino et al. 1984), but rather are related to the size of the mother through non-genetic mechanisms. There are positive correlations of mother size and brood cell size (Tepedino and Torchio 1989), mother size and amount of food supplied (Boomsm and Eickwort 1993), brood cell size and amount of food supplied (Krombein 1967, Johnson 1990), amount of food supplied and size of emergent bees (Klostermeyer et al. 1973, Danforth 1990, Johnson 1990), and brood cell size and size of emergent bees (Klostermeyer et al. 1973, Kamm 1974, Alcock 1979, 1999, Tepedino and Torchio 1982). In *Lasioglossum zephyrum* bee size is correlated with brood cell size: the larger bees construct larger cells and larger bees emerge from larger cells (Kamm 1974). For this same species, offspring body size appears to result from a combination amount of food and pollen protein concentration (Roulston and Cane 2002). There was a relation between larger brood cells and amount of food supplied and between the amount of food weight of emergent bees in *Megachile rotundata* (Klostermeyer et al. 1973) the relation between amount of food and size of emergent bees was also verified in *Caliopsis persimilis* (Danforth 1990) and in *Ceratina calcarata* (Johnson 1990). Larger female *Centris palida* showed higher supply capacity (Alcock 1979). A higher foraging capacity of larger bees was observed in six Meliponini species, in which larger bees covered larger flight distances (Araújo et al. 2004).

The individuals from the unmanipulated cells were larger than those that had some provisions removed. However, there were no significant differences among individuals from manipulated cells as bees from cells where more food was removed were not significantly smaller than those where less was removed. Those that received more food did not grow larger. Roulston and Cane (2000) suggest that, for bee species that nest in natural cavities, the size of the cavity used is strongly related to the amount of food supplied. Studies that compared resource availability and dry mass showed that bees with
access more food are bigger (Peterson and Roitberg 2006) and there is a positive relation between adult dry mass and food provisioned (Bosch and Vicens 2002). We did not find a correlation between PC1 and food supplied, but when we used just the measures from estimated dry mass there is a correlation between estimated dry mass and food supplied ($R^2 = 0.72$; d.f. = 11; p = 0.0001). Thus, the nonsignificant relation between PC1 and amount of provisions removed may be due to the other two size measures (head width and head height). An influence of the amount of food ingested and the size of emergent bees was already reported for other species. In *Megachile rotundata* there is a strong relationship between the weight of the food supply and the weight and sex of the resulting progeny (Klostermeyer et al. 1973, O’Neill et al. 2010), in this case females emerged from cells with larger provisions. A similar result was found for *Calliopsis persimilis* (Danforth 1990). For *Ceratina calcarata* larger bees were those that received more food when larvae and larger females had larger foraging capacity and built cells with more food (Johnson 1990). Our food manipulation experiment on *T. diversipes* corroborated that larger bees ingested more food in the larval phase.

**Conclusion**

We conclude that in *Tetrapedia diversipes*, removing some of the provisions produces results in smaller offspring than bees from cells with intact provisions. We also found a weak positive correlation between relationship brood cell volume and body size, in one of the populations studied.

**Acknowledgments**

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**References**

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Supplementary material 1

Individual *Tetrapedia diversipes* emerged from trap nests at the Campus of the University of São Paulo

Authors: Carlos Eduardo Pinto, Adriana da Silva, Guaraci Duran Cordeiro, Isabel Alves-dos-Santos

Data type: MS Word doc file

Explanation note: Of 26 emergent individuals, 13 did not have their food manipulated (control) and 13 part of their food removed (manipulated). The bees were measured and the value of pollen weight refers to the amount of pollen mass removed from each cell.

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Supplementary material 2

Bees measures that emerged in seven brood cell with 0.8 cm diameter

Authors: Carlos Eduardo Pinto, Adriana da Silva, Guaraci Duran Cordeiro, Isabel Alves-dos-Santos

Data type: MS Word doc file

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Nests of bees of the anthidiine genus *Ananthidium* Urban (Hymenoptera, Apidae, Megachilinae)

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http://zoobank.org/75402A53-667E-45FD-BEFE-5954C70CA3CF


Abstract

We present data on nests of the two species of the neotropical bee genus *Ananthidium* Urban (Megachilinae, Anthidiini). Five nests of *Ananthidium dilmae* Urban, a species from southern and southeastern Brazil, were found in grassland areas at the Vila Velha State Park, Ponta Grossa, in Paraná State. The aerial nests were made of resin mixed with plant fibers and each contained one or two cells. One female and one male emerged from two of the nests. Notes on the nest of *Ananthidium inerme* (Friese), a species known from Argentina and Paraguay, are provided based on two nests deposited in Berlin's Museum für Naturkunde, Germany. This species also builds aerial resin nests attached to plant stems, with external shape and dimensions similar to those of *Ananthidium dilmae*.

Keywords

Anthidiini, biology, neotropical, resin, solitary bee

Introduction

Most species of Anthidiini are solitary, with females building their nests exposed, in hollows of trees, in cavities abandoned by other insects, or in the soil, such as those constructed by *Trachusa* Panzer and *Paranthidium* Cockerell & Cockerell (Grigarick and Stange 1968, Morato 2001, Gess and Gess 2007, Rozen and Hall 2012). The nests are constructed with a great variety of materials, such as resin, pieces of leaves and
flowers, plant fibers and pebbles or even animal hair. The tribe also possesses obligatory
kleptoparasitic genera that parasitize other species of Megachilinae, with a few taxa at-
tacking unrelated bee groups, such as *Hoplostelis* Dominique that parasitizes nests of
orchid bees (Apinae, Euglossini). Reports on the nest biology of the tribe are relatively
scarce, especially for neotropical taxa (Jörgensen 1912, Claude-Joseph 1926, Janvi-
er 1955, Laroca and Rosado-Neto 1975, Morato and Campos 2000, Morato 2001,
Alves-dos-Santos 2004, Alves-dos-Santos et al. 2004, Camarotti-de-Lima and Martins
species, limited information indicates that *Dicranthidium arenarium* (Ducke, 1907)
(Laroca and Rosado-Neto 1975) and *Anthodioctes manauara* Urban, 1999 (Morato
2001) construct their nests in abandoned mud nest of eumenine wasps.

*Ananthidium* Urban is a neotropical genus with two species, *Ananthidium inerme*
(Friese, 1908) and *Ananthidium dilmae* Urban, 1992. The first species is known from
Argentina and Paraguay while the second is known only from southern and southeastern
Brazil. This small group was given genus status by Urban (1992) and Stange (1995)
whereas Michener (2007) treated it as a subgenus of *Epanthidium* Moure, along with two
other subgenera (*Epanthidium* and *Carloticola* Moure & Urban). A phylogenetic analysis
of the tribe (Parizotto 2011) indicated that *Ananthidium* is not closely related to *Epanthidium*
and is herein treated as a separate genus, according to the classification proposed by Urban
and Moure (2007). Here we provide information on the nest architecture of *Ananthidium*
as a contribution to the knowledge of the biology of the tribe in the Neotropical region.

**Results**

*Ananthidium dilmae*

A total of five nests were collected in the Vila Velha State Park, a reserve in the mu-
icipality of Ponta Grossa, Paraná, Brazil. Vila Velha contains sandstone formations of
significant scientific, cultural and ecological value, located in southern Brazil (25°15’S;
50°00’W) within a broader region known as Campos Gerais (Gonçalves and Melo
2005, Schimandeiro et al. 2008). The park has a total area of 3,122 hectares primarily
covered by grasslands with predominance of Poaceae, Cyperaceae, Asteraceae, Verben-
aceae and Fabaceae. Nests were found on three different dates: the first one was collected
and the last three nests were found in the highest plateau within the park, a site known
as “Fortaleza”, while the second nest was found in a nearby grassland site within the
park. All material studied here are deposited at DZUP (Coleção Entomológica Padre
Jesus Santiago Moure, Universidade Federal do Paraná). Nests were collected in shrub
vegetation at about 0.5 m above the ground and, except for one nest attached to a living
branch, all nests were attached to dead branches of *Baccharis* spp. (Asteraceae) (Fig. 1).
Figures 1–4. Nests of *Ananthidium dilmae*. 1 Nest on a branch of *Baccharis* sp. (Asteraceae), found in the Vila Velha State Park, in southern Brazil (nest 1) 2 Close up view of the two-celled nest in the field 3, 4 Close up views of nest 5 under the stereomicroscope using different lighting to show plant fibers mixed with resin; the tip of the metasoma of the male completely emerged in Fig. 4 can be seen in Fig. 3 exiting through the cell entrance.
Nests consisted of one or two oval-shaped brood cells attached to the branches along its longest axis, with the circular cell openings facing downward (Fig. 2). Cell size was similar between one and two-celled nests (Table 1). Nests were made of resin mixed with plant material, apparently plant trichomes (Fig. 3). Although externally the appearance of the resin ranged from rugose (nest 1) to smooth (other nests) among nests, it was always brown to dark brown in color (Figs 3–4). The cell closure has a vitreous aspect, perhaps due to the presence of more resin and fewer trichomes.

The first nest had two closed cells from which a female of *Ananthidium dilmae* emerged; the second and third nests were empty with their brood cells open; the single-celled fourth nest was opened and only a damaged Diptera puparium was found inside the cell; a male of *Ananthidium dilmae* emerged from the fifth nest (Figs 3–4).

### Table 1. Measurements (in millimeters) of the main structures of the nests of *Ananthidium dilmae* collected at Vila Velha State Park, Ponta Grossa, Paraná, Brazil and *Ananthidium inerme* collected in Mendoza, Argentina.

<table>
<thead>
<tr>
<th>Nest</th>
<th>Number of cells</th>
<th>Branch diameter</th>
<th>Cell length</th>
<th>Cell width</th>
<th>Cell wall thickness</th>
<th>Diameter of cell opening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Ananthidium dilmae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2.4</td>
<td>11.6/12.5</td>
<td>5.4/5.0</td>
<td>1.1</td>
<td>4.5/4.0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1.1</td>
<td>13.1/12.6</td>
<td>6.4/6.5</td>
<td>1.1</td>
<td>3.2/3.6</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>3.0</td>
<td>14.3/15.7</td>
<td>6.8/6.0</td>
<td>1.2/1.1</td>
<td>3.0/3.5</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>2.0</td>
<td>13.4</td>
<td>5.9</td>
<td>1.2</td>
<td>4.5</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>7.9</td>
<td>13.3</td>
<td>6.1</td>
<td>1.2</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Ananthidium inerme</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>3.0</td>
<td>11.0</td>
<td>4.8</td>
<td>-*</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>3.3</td>
<td>9.0</td>
<td>5.0 (4.5)</td>
<td>0.5–0.7</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*Nest entirely closed.

Nests consisted of one or two oval-shaped brood cells attached to the branches along its longest axis, with the circular cell openings facing downward (Fig. 2). Cell size was similar between one and two-celled nests (Table 1). Nests were made of resin mixed with plant material, apparently plant trichomes (Fig. 3). Although externally the appearance of the resin ranged from rugose (nest 1) to smooth (other nests) among nests, it was always brown to dark brown in color (Figs 3–4). The cell closure has a vitreous aspect, perhaps due to the presence of more resin and fewer trichomes.

The first nest had two closed cells from which a female of *Ananthidium dilmae* emerged; the second and third nests were empty with their brood cells open; the single-celled fourth nest was opened and only a damaged Diptera puparium was found inside the cell; a male of *Ananthidium dilmae* emerged from the fifth nest (Figs 3–4).

### Ananthidium inerme

Two nests of this species (Fig. 5) are deposited in the bee collection of the Berlin’s Naturkunde Museum, Germany (ZMB). The label associated with the nests reads “Nest cells of *Anthidium inerme* Fr. made of resin in thorn bushes - Mendoza (Argentina 1912)” [“Nestzelle aus Harz an Dornbüschen von *Anthidium inerme* Fr. - Mendoza (Argentina) 1912” in the original label (see Fig. 5)].

The shape and size of these nests are comparable to those of *Ananthidium dilmae* (Table 1) although apparently no plant fibers were used. Nest 1 (Fig. 6) has a large number of fine soil particles attached to its upper surface, but because no sand or soil was found within the resin matrix this might be the result of soil being spilled over the nest by rain drops or simply contamination during its collection. No soil particles were observed on the surface of the second nest (Fig. 7).
Figures 5–7. Nests of *Ananthidium inerme*. 5 The two nests examined in this study, which are glued to a paper card, are deposited in the insect collection of the Berlin’s Naturkunde Museum, Germany. 6, 7 Close up views of nests 1 and 2 taken under a stereomicroscope.
Nest 1 is entirely closed (Fig. 6), apparently as it was found in the field. Nest 2 is open and it might have been partially dissected soon after it was collected because a piece of its border is turned up, something that could have been done only when the resin was still malleable (Fig. 7). Inside this nest there are remains of an adult bee that had been partially eaten by dermestid beetles. We examined these remains and confirmed that they belong to a female of *Ananthidium inerme* (these remains were glued to a paper rectangle and pinned together with the nests in the drawer). Examination of the inner walls revealed no signs of a cocoon and one can conclude that the dead female might have been the nest owner and was collected with it. No further dissection of the nests was attempted to preserve their integrity.

These two nests might represent some of the nests upon which Jörgensen (1912) briefly reported on the biology of *Ananthidium inerme* (cited as *Anthidium inerme*). They might have been sent to Friese, whose original collection has to a large extent been incorporated to the ZMB (see Rasmussen and Ascher 2008), together with adult bees. None of the pinned specimens of *Ananthidium inerme* in the ZMB collection had any indication that they emerged from collected nests, although two females and two males are from Mendoza, Argentina, and were likely received through Jörgensen.

**Discussion**

The females of *Ananthidium* possess short, robust mandibles, presumably adapted for the manipulation of resin and plant particles. Considering that *Ananthidium* has been recovered as sister group of the clade containing *Allanthidium* Moure, *Anthidianum* Michener, *Chrisanthidium* Urban and *Notanthidium* Isensee (Parizotto 2011, see also Parizotto 2009), it is likely that these genera also make aerial resin nests. Indeed, Rozen (2015) reported recently on an aerial nest of *Anthidianum chilense* Urban, 2003 (as *Notanthidium* (*Allanthidium*) *chilense*) that is quite similar to those of *Ananthidium* described herein in the use of plant resins and in being attached to thin plant stems. Differently from the nests of *Ananthidium* reported here, in *Anthidianum* the nest was larger, with four brood cells, and had a more irregular and thicker outer surface, in which the cell contour could not be seen from the outside.

On the other hand, the available data for *Notanthidium steloides* (Spinola, 1851), the single species in the genus, indicate that it uses pre-existing cavities [wood galleries made by beetles and bamboo hollows; see Claude-Joseph (1926)]. It is possible that *Notanthidium* could be an exception in relation to the nesting biology of the clade, since they are elongate, hoplitiform bees, whose females have greatly modified heads, with a peculiar mandibular and clypeal morphology. Nothing is known of the biology of *Allanthidium* and *Chrisanthidium*, the other two genera in this Neotropical clade of Anthidini. Addressing more general conclusions on nest morphology and on use of substrate will have to wait until further data is obtained on this large and diverse bee tribe.
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