EDITORIAL

JOURNAL OF
Hymenoptera
RESEARCH
The International Society of Hymenopterists
A peer-reviewed open-access journal

The move to open access and growth: experience from Journal of Hymenoptera Research

Stefan Schmidt[1], Gavin R. Broad[2], Pavel Stoev[3],
Daniel Mietchen[4], Lyubomir Penev[3]

1 *Zoologische Staatssammlung, Münchhausenstr. 21, 81247 Munich, Germany* **2** *Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK* **3** *Bulgarian Academy of Sciences & Pensoft Publishers, Sofia, Bulgaria* **4** *EvoMRI Communications, Zwätzengasse 10, 07743 Jena, Germany*

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

The Journal of Hymenoptera Research (JHR) is published by the International Society of Hymenopterists. It is devoted to the study of all aspects of Hymenoptera and covers a broad range of research disciplines, including biology, morphology, behaviour, ecology, genetics, systematics and taxonomy. The journal was launched in 1992 and for most of its existence was published as two issues per annual volume. In 2011, after publication of 19 volumes, the Society decided to move from restricted to open access, with Pensoft as a publisher. This move had several important consequences for the publication and dissemination of information about Hymenoptera.

The new publication model has replaced the traditional schedule of two issues per year with a more flexible one allowing publication of unlimited numbers of issues, depending only on the availability of publishable content. The unrestricted number of issues speeded up the publication process and increased the number of published pages as a whole. For instance, from 2000 to 2010 (vol. 10–19), JHR published an annual average of 306 pages (Fig. 1), while shortly after changing the publication model, the number of published pages increased to 366 in 2011 and 712 pages in 2012. Likewise, there has been growth in submissions to JHR and published articles, reaching 51 and 38 articles, respectively, in 2012. The rejection rate, at about 20%, remained about the same, with 10 rejected manuscripts in 2011 and 11 rejections in 2012.
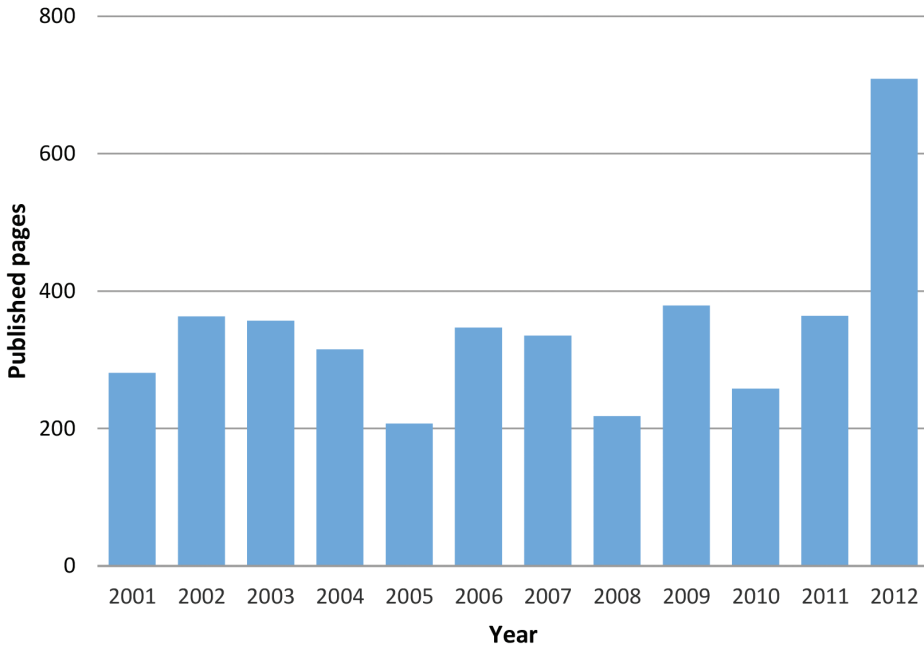
Figure 1. Annual number of published pages of Journal of Hymenoptera Research. The most recent two volumes (2011 and 2012) were published open access.

Apart from a significant increase in the volume of published content, the move to open access was accompanied by several other changes. Most importantly, published information is immediately disseminated, through XML markup, to global aggregators and scientific databases, including Encyclopedia of Life (EOL), the Global Biodiversity Information Facility (GBIF), Plazi, and Species-ID (see, e.g., Penev et al. 2010, 2011). Similarly, publication records are distributed to scientific databases, indices and search engines, such as Zoological Record, Web of Science, Google Scholar, CAB Abstracts, DOAJ Content and others. The publisher provides an alert service through email, RSS, and social networks, including Facebook, Twitter and Mendeley to inform readers of the publication of new issues.

JHR and the International Society of Hymenopterists have benefited also from Pensoft's dissemination services. Since May 2011, Pensoft's PR team has been supporting authors actively in "translating" the technical texts into more popular language. Although still not a routine practice, the press release by Shaw et al. (2011), posted at Eurekalert, received around 800 page views during a short time. The accelerated dissemination workflow led to a increase of the visits to the JHR website. While web traffic statistics are not available from before the switch to open access, the number of page loads rose from about 25,000 in 2011 to more than 40,000 in 2012. During the same period, the number of unique visitors increased from ca. 8,200 to almost 14,350 (Fig. 2), indicating an improved visibility of the journal. The list of most viewed articles is a quite accurate representation of the topics and taxa that qualify as the most prominent research topics of articles published in JHR.
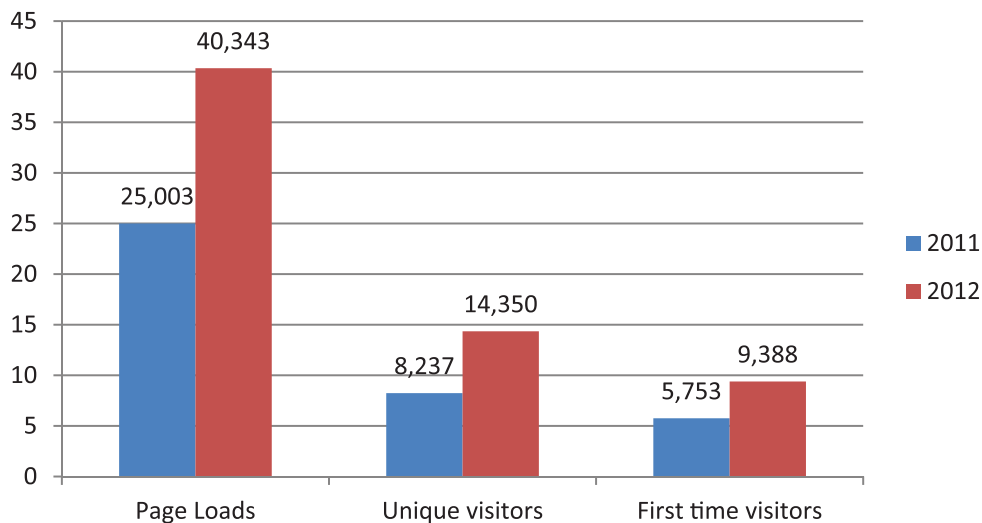
Figure 2. JHR Statcounter statistics, showing the number of visits to the website.

Two groups of Hymenoptera stand out as receiving considerable attention in terms of published articles: Braconidae and aculeate wasps. In 2011 and 2012, a total of a dozen articles were published about Braconidae and they make up for over half of the top ten most viewed articles (Table 1). The majority of the most accessed articles comprise revisionary studies, including the large-scale revision of the genus *Quartinia* in Southern Africa (Gess 2011a, b; 2012a, b), with part IV being the most viewed article (Table 1). Eleven, or 19%, of all published articles in 2011/2012 had behavioural ecology as their main subject, with a prevalence of articles concerning aculeate wasps (Alcock 2011, Buys 2012, Nalepa 2012, Dalmazzo and Roig Alsina 2012, Barthelemy and Pitts 2012, Sarzetti et al. 2012, Wcislo et al. 2012). Although there is a predominance of articles that have systematics, phylogeny, and ecology as their prime subjects, there have been several articles dealing with important, more general subjects. Gates and Buffington (2011) describe innovative techniques for processing the increasing number of parasitoid specimens that are obtained through biodiversity studies. The tools developed and provided by Seltmann et al. (2012) in their article describing the Hymenoptera Anatomy Ontology represent an important milestone for the future of sustainable digital publication, allowing authors to reference their hymenopteran anatomical terminology to well-defined and illustrated anatomical concepts.

Authors benefit from the move to the Pensoft online editorial platform in that they can expect a faster turnaround time for their manuscripts, usually ranging from one to a few months from submission to publication. The open access model guarantees a better dissemination and visibility of their articles through the simple but important fact of accessibility, augmented by tailored use of cutting-edge Web 2.0 technologies. Manuscripts are submitted through a highly automated online editorial management system that provides authors and editors with a more streamlined editorial pathway. The move to electronic publication also means that there is no page limit for manuscript length,

Table 1. The top ten most viewed articles of JHR published in 2011 and 2012 (according to the JHR website counter, accessed on 29 January 2013).

Rank	Article	Author (Year)	Views
1	The genus *Quartinia* Ed. André, 1884 (Hymenoptera, Vespidae, Masarinae) in Southern Africa. Part IV.	Gess (2011a)	2,706
2	A review of New World *Eurytenes* s. str. (Hymenoptera, Braconidae, Opiinae)	Walker and Wharton (2011)	2,567
3	Sting microsculpture in the digger wasp *Bembix rostrata* (Hymenoptera, Crabronidae)	Matushkina (2011)	2,563
4	Characteristics of the cocoon and natural history of the gregarious *Meteorus restionis* sp. n. (Hymenoptera, Braconidae, Meteorinae) from Costa Rica	Barrantes et al. (2011)	2,282
5	Two new brachypterous species of *Heterospilus* Haliday (Hymenoptera, Braconidae, Doryctinae) from the Nearctic region	Kula (2011)	2,265
6	A revision of Thai Agathidinae (Hymenoptera, Braconidae), with descriptions of six new species.	Stoelb and Sharkey (2011)	2,251
7	Two new synonyms in Oriental Crabronidae (Hymenoptera)	Pulawski (2011)	2,095
8	Discovery of *Rodrigama* Gauld in the Old World, with description of two new species (Hymenoptera, Ichneumonidae, Poemeniinae)	Matsumoto and Broad (2011)	2,068
9	A hymenopterists' guide to the Hymenoptera Anatomy Ontology: utility, clarification, and future directions	Seltmann et al. (2012)	2,052
10	Unusual host carrying by a parasitoid wasp (Braconidae, Braconinae, Pycnobraconoides)	Quicke and Marshall (2011)	2,014

although for large revisionary works, checklists, catalogues, monographs, etc., there is the option of separate publication as a supplement. Nor is there a limit (or extra charge) for colour illustrations or the integration with multimedia (e.g. as in Wcislo et al. 2012), and authors are encouraged to submit non-printable matter like movie clips, sound recordings, or 3D animations. At the same time, we continue to produce a high resolution full-color printed version with a subscription option for individuals and institutions, and Society members benefit from a discount on printed copies and open access fees.

The technological development of JHR continues and the next step will not delay too long. JHR will be transferred in 2013 to the brand new Pensoft Journal System (PJS 2.0), a novel editorial management system that will greatly facilitate the editorial process for our authors, reviewers and editors through improving usability and navigation. PJS 2.0 will offer a number of additional tools, that can be used optionally by the JHR authors, such as community and public peer review, and an online, collaborative, article-authoring tool (Pensoft Writing Tool, PWT) that provides a large set of predefined, but flexible, templates of different types of article. In the PWT environment, authors can work collaboratively on their manuscripts online and may also invite external contributors, such as mentors, potential reviewers, linguistic and copy editors, colleagues, etc., who may watch and comment on the text during the manuscript preparation. The PWT will also facilitate upload of occurrence data in DarwinCore, provide automated registration of new taxa in ZooBank, import of references, embed multimedia, compose plates from single images, and many other features.

References

Alcock J (2011) Hilltopping behavior by males of *Tachysphex menkei* Pulawski (Hymenoptera, Crabronidae). Journal of Hymenoptera Research 21: 65–70. doi: 10.3897/jhr.21.876

Barrantes G, Triana ET, Shaw SR, Jones GZ (2011) Characteristics of the cocoon and natural history of the gregarious *Meteorus restionis* sp. n. (Hymenoptera, Braconidae, Meteorinae) from Costa Rica. Journal of Hymenoptera Research 20: 9–21. doi: 10.3897/jhr.29.867

Barthelemy C, Pitts J (2012) Observations on the nesting behavior of two agenielline spider wasps (Hymenoptera, Pompilidae) in Hong Kong, China: *Macromerella honesta* (Smith) and an *Auplopus* species. Journal of Hymenoptera Research 28: 13–35. doi: 10.3897/jhr.28.3010

Buys SC (2012) Nesting habits, alternative nesting strategies and female territoriality of the cockroach-hunting solitary wasp *Penepodium luteipenne* (Hymenoptera: Sphecidae). Journal of Hymenoptera Research 24: 27–41. doi: 10.3897/jhr.24.1828

Dalmazzo M, Roig Alsina A (2012) Nest structure and notes on the social behavior of *Augochlora amphitrite* (Schrottky) (Hymenoptera, Halictidae). Journal of Hymenoptera Research 26: 17–29. doi: 10.3897/jhr.26.2440

Gates MW, Buffington ML (2011) Description of two techniques to increase efficiency in processing and curating minute arthropods, with special reference to parasitic Hymenoptera. Journal of Hymenoptera Research 22: 133–140. doi: 10.3897/jhr.22.2099

Gess FW (2011a) The genus *Quartinia* Ed. André, 1884 (Hymenoptera, Vespidae, Masarinae) in Southern Africa. Part IV. New and little known species with complete venation. Journal of Hymenoptera Research 21: 1–39. doi: 10.3897/JHR.21.870

Gess F (2011b) The genus *Quartinia* Ed. André, 1884 (Hymenoptera, Vespidae, Masarinae) in Southern Africa. Part V. New and little known species with incomplete venation. Journal of Hymenoptera Research 22: 29–43. doi: 10.3897/jhr.22.871

Gess FW (2012a) The genus *Quartinia* Ed. Andre, 1884 (Hymenoptera, Vespidae, Masarinae) in Southern Africa. Part VI. New and little known species both with complete and incomplete venation. Journal of Hymenoptera Research 24: 95–115. doi: 10.3897/JHR.24.2155

Gess FW (2012b) A new species of *Masarina* Richards 1962 from southern Africa, description of the female of *M. ceres* Gess 1997 and supplementary data on three other species of the genus (Hymenoptera, Vespidae, Masarinae). Journal of Hymenoptera Research 25: 83–92. doi: 10.3897/JHR.25.2442

Kula RR (2011) Two new brachypterous species of *Heterospilus* Haliday (Hymenoptera, Braconidae, Doryctinae) from the Nearctic Region. Journal of Hymenoptera Research 21: 53–64. doi: 10.3897/JHR.21.875

Matsumoto R, Broad GR (2011) Discovery of *Rodrigama* Gauld in the Old World, with description of two new species (Hymenoptera, Ichneumonidae, Poemeniinae). Journal of Hymenoptera Research 20: 65–75. doi: 10.3897/jhr.29.872

Matushkina NA (2011) Sting microsculpture in the digger wasp *Bembix rostrata* (Hymenoptera, Crabronidae). Journal of Hymenoptera Research 21: 41–52. doi: 10.3897/JHR.21.873

Nalepa CA (2012) Wing wear is a poor estimate of age in *Cerceris fumipennis* (Hymenoptera, Crabronidae). Journal of Hymenoptera Research 24: 43–46. doi: 10.3897/jhr.24.2091

Penev L, Agosti D, Georgiev T, Catapano T, Miller J, Blagoderov V, Roberts D, Smith VS, Brake I, Ryrcroft S, Scott B, Johnson NF, Morris RA, Sautter G, Chavan V, Robertson T, Remsen D, Stoev P, Parr C, Knapp S, Kress WJ, Thompson FC, Erwin T (2010) Semantic tagging of and semantic enhancements to systematics papers: ZooKeys working examples. ZooKeys 50: 1–16. doi: 10.3897/zookeys.50.538

Penev L, Hagedorn G, Mietchen D, Georgiev T, Stoev P, Sautter G, Agosti D, Plank A, Balke M, Hendrich L, Erwin T (2011) Interlinking journal and wiki publications through joint citation: Working examples from ZooKeys and Plazi on Species-ID. ZooKeys 90: 1–12. doi: 10.3897/zookeys.90.1369

Pulawski WJ (2011) Two new synonyms in Oriental Crabronidae (Hymenoptera). Journal of Hymenoptera Research 20: 1–7. doi: 10.3897/jhr.29.869

Quicke DLJ, Marshall S (2011) Unusual host carrying by a parasitoid wasp (Braconidae, Braconinae, Pycnobraconoides). Journal of Hymenoptera Research 20: 77–79. doi: 10.3897/jhr.29.868

Sarzetti L, Genise J, Sanchez MV (2012) *Trichothurgus bolithophilus* sp. n. (Hymenoptera, Megachilidae) a bee nesting in horse manure pads in Patagonia, Argentina. Journal of Hymenoptera Research 29: 1–14. doi: 10.3897/jhr.29.3529

Seltmann KC, Yoder MY, Miko I, Forshage M, Bertone MA, Agosti D, Austin AD, Balhoff JP, Borowiec ML, Brady SG, Broad GR, Brothers DJ, Burks RA, Buffington ML, Campbell HM, Dew KJ, Ernst AF, Fernandez-Triana JL, Gates MW, Gibson GAP, Jennings JT, Johnson NF, Karlsson D, Kawada R, Krogmann L, Kula RR, Mullins PL, Ohl M, Rasmussen C, Ronquist F, Schulmeister S, Sharkey MJ, Talamas E, Tucker E, Vilhelmsen L, Ward PS, Wharton RA, Deans AR (2012) A hymenopterists' guide to the Hymenoptera Anatomy Ontology: utility, clarification, and future directions. Journal of Hymenoptera Research 27: 67–88. doi: 10.3897/jhr.27.2961

Shaw MR, Jennings MT, Quicke DLJ, and Pensoft Publishers (2011) Misleading morphology: 3 European parasitoid wasp ‚species' are seasonal forms of just 1. EurekAlert!, October 26, 2011. URL: http://www.eurekalert.org/pub_releases/2011-10/pp-mmt102611.php . Archived at http://www.webcitation.org/6E0O27iPA

Stoelb S, Sharkey MJ (2011) A revision of Thai Agathidinae (Hymenoptera, Braconidae), with descriptions of six new species. Journal of Hymenoptera Research 22: 69–132. doi: 10.3897/jhr.22.1299

Walker AK, Wharton RA (2011) A review of New World *Eurytenes* s. str. (Hymenoptera, Braconidae, Opiinae). Journal of Hymenoptera Research 20: 23–46. doi: 10.3897/jhr.29.877

Wcislo DO, Vargas G, Ihle KE, Wcislo WT (2012) Nest construction behavior by the orchid bee *Euglossa hyacinthina*. Journal of Hymenoptera Research 29: 15–20. doi: 10.3897/jhr.29.4067

SHORT COMMUNICATION

JOURNAL OF
Hymenoptera
RESEARCH
The International Society of Hymenopterists

Characterization of microsatellite loci for the stingless bee *Scaura latitarsis* (Hymenoptera, Apidae, Meliponini)

Flávio O. Francisco[1], Alayne M. T. Domingues-Yamada[1],
Paulo Henrique P. Gonçalves[1], Rute M. Brito[1,2],
Fabiana C. Pioker[3], Sidnei Mateus[4], Maria C. Arias[1]

1 *Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, Rua do Matão 277 – sala 320, CEP 05508-090, São Paulo, SP, Brazil* **2** *Instituto de Genética e Bioquímica, Universidade Federal de Uberlândia, Av. Pará 1720 – 2E, sala 246, CEP 38400-902, Uberlândia, MG, Brazil* **3** *Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, travessa 14, nº 321, CEP 05508-900, São Paulo, SP, Brazil* **4** *Departamento de Biologia, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes 3900 - Bloco 7, CEP 14040-901, Ribeirão Preto, SP, Brazil*

Corresponding author: *Maria C. Arias* (mcarias@ib.usp.br)

Abstract

Seven microsatellite loci were isolated and characterized from a microsatellite enriched genomic library for the stingless bee *Scaura latitarsis*. Primers were tested in 12 individuals. The number of alleles per locus ranged from 3 to 6 (mean = 4.29) and observed heterozygosity ranged from 0.00 to 0.75 (mean = 0.40). Cross-species tests showed successful amplification for *Scaura atlantica*, *Scaura longula*, *Scaura tenuis*, *Schwarzula timida*, *Schwarziana quadripunctata*, *Plebeia droryana*, and *Plebeia remota*.

Keywords

Microsatellites, *Scaura latitarsis*, genetic variability, Meliponini

Introduction

Scaura latitarsis is small (4.0 mm) and black stingless bee which nidifies in arboreal termite nests of *Nasutitermes* (Michener 2007). The species is widely distributed in tropical South America, with records in the following countries: Colombia, Venezuela,

Guyana, Suriname, Peru, Bolivia, and Brazil (Camargo and Pedro 2012). The large geographic distribution and the well reported female philopatric behavior in stingless bees (Nogueira-Neto 1954, Brito and Arias 2010, Francisco and Arias 2010) suggest that *S. latitarsis* populations should be highly differentiated. In general, population studies apply combined molecular markers of nuclear and cytoplasm origins. Mitochondrial polymorphism is easily assessed due to universal primers already described (Simon et al. 1994). However, the same is not true for nuclear markers. Microsatellites have been the nuclear marker most used in population genetic studies. As they present Mendelian and biparental inheritance, it is possible to evaluate the parental contribution, especially from males when mitochondrial data are also available (e.g. Estoup et al. 1996, Franck et al. 2001, Quezada-Euán et al. 2011). For future population studies, our first goal was to construct a microsatellite-enriched genomic library to isolate and characterize microsatellite loci for *S. latitarsis*.

Total DNA of 12 workers was extracted using a phenol/chloroform protocol. The microsatellite-enriched genomic library was constructed according to Billotte et al. (1999) with some modifications (Brito et al. 2009). From 96 selected colonies of transformed *Escherichia coli* DH5α lineage, 48 were sequenced and 25 contained microsatellite sequences. Fifteen of these were chosen for primers design based on sequence quality. The software Primer3 was used (Rozen and Skaletsky 2000) to indicate the best primer sequences. Primers were tested on 12 workers of *S. latitarsis* from seven different locations in Brazil: Ribeirão Preto, SP (n = 2), Cajuru, SP (n = 2), Pedregulho, SP (n = 2), Ribeirão Cascalheira, MT (n = 2), Nova Xavantina, MT (n = 1), Manaus, AM (n = 2), and Londrina, PR (n = 1). Amplification reactions were conducted in a volume of 20 μL containing 2 μL of the PCR reaction buffer (Invitrogen), 1 μL of dNTP 2 mM each, 0.6 μL of $MgCl_2$ 50 mM, 0.4 μL of each primer 20 μM, 15 ng of template DNA and 0.3 μL of *Taq* DNA polymerase 5U/μL (Invitrogen). PCR conditions were the following: 93 °C for 4 min, 35 cycles of denaturation at 93 °C for 40 s, annealing at 60 °C for 50 s and elongation at 72 °C for 40 s. A final extension step of 72 °C for 5 min was performed. PCR products were separated on 5.6% polyacrylamide gels and stained with silver nitrate. Allelic richness (k), observed (H_O) and expected (H_E) heterozygosity were calculated in FOFPOP 2.0 (Francisco 2009). GENEPOP 4.1.4 (Rousset 2008) was used for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium tests. Bonferroni corrections were used for multiple comparisons.

Seven out of 15 primer pairs generated fragments of expected size, and they were polymorphic (Table 1). The number of alleles ranged from 3 to 6, with an average of 4.29. Values of H_O ranged from 0.000 to 0.750 (mean = 0.405) and H_E from 0.531 to 0.736 (mean = 0.664). After Bonferroni correction, only the loci SLAT 16 and SLAT 40 showed significant deviation from Hardy-Weinberg-expectations. Linkage disequilibrium was detected after Bonferroni correction between SLAT 16/SLAT 24, SLAT 24/SLAT 43, and SLAT 40/SLAT 43. Deviations from HWE and linkage disequilibrium are expected when individuals are sampled from genetically differentiated populations (Brito et al. 2009).

Using the same conditions aforementioned we tested the primers for the polymorphic loci in seven stingless bee species: *Scaura atlantica*, *Scaura longula*, *Scaura tenuis*, *Schwarzula timida*, *Schwarziana quadripunctata*, *Plebeia droryana*, and *Plebeia remota*. Cross-species amplification was 100% efficient for SLAT 10 and SLAT 44 (Table 2). However those loci were the only ones amplified in *S. longula* and *S. tenuis*. At least five out of seven primer pairs were successfully amplified in species from three other genera.

The microsatellite loci described here will be very helpful to gather molecular data of *S. latitarsis* to understand the population structure of this widely distributed species. If queen philopatry is confirmed for this species, these microsatellite loci will allow an evaluation of male's contributions as gene flow mediators.

Table 1. Summary of general information for the microsatellite loci isolated from *Scaura latitarsis*. *k*: number of alleles; ASR: allele size range (bp); H_O: observed heterozygosity; H_E: expected heterozygosity.

Locus	Repeat motif	Genbank accession number	Primer sequences (5'-3')	k	ASR	H_O	H_E
SLAT 10	$(AG)_{12}TA(AG)_3$	JQ001766	F: TGAATTAACAGGCCGGAATC R: TTCGTTGCCTCGCTCTTTAT	5	183–197	0.417	0.694
SLAT 16	$(AG)_{21}$	JQ001767	F: AAACGAAGGACAGACGTTGG R: AATATGTGGACCGCGTGTTA	5	145–179	0.000	0.736
SLAT 18	$(AG)_{24}$	JQ001768	F: GGATCGTCGAACGGAATATC R: TCGTCAACATTCCTCACTGG	3	232–240	0.250	0.531
SLAT 24	$(GAA)_{16}$	JQ001769	F: CGAACCAGTGTCTGAATCGT R: GCCTCGAACTCTGGCGTAT	6	184–202	0.500	0.715
SLAT 40	$(GA)_3AA(GA)_{18}$	JQ001771	F: GTTCCACACCGGAAGACC R: GTGAACGAGCCTCTGCAATC	4	200–214	0.333	0.653
SLAT 43	$(AG)_{24}$	JQ001772	F: GCGGAATTAACAACGGACAT R: GCATACCGGCGAATATAACC	4	119–135	0.583	0.719
SLAT 44	$(GA)_{19}(GAA)_9$	JQ001773	F: GGCATAGGTTACGATTTGGAG R: GTGGCAGTCAACGTGTTAGG	3	222–230	0.750	0.601

Table 2. Cross-species amplification of 7 microsatellite loci from *Scaura latitarsis* in *S. atlantica*, *S. longula*, *S. tenuis*, *Schwarzula timida*, *Schwarziana quadripunctata*, *Plebeia droryana*, and *Plebeia remota*. (+): successful amplification; (-): no product or multiple bands.

Locus	*Scaura atlantica*	*Scaura longula*	*Scaura tenuis*	*Schwarzula timida*	*Schwarziana quadripunctata*	*Plebeia droryana*	*Plebeia remota*
SLAT 10	+	+	+	+	+	+	+
SLAT 16	+	-	-	-	-	-	-
SLAT 18	-	-	-	+	+	+	+
SLAT 24	+	-	-	+	+	+	+
SLAT 40	-	-	-	+	+	+	+
SLAT 43	+	-	-	-	-	+	-
SLAT 44	+	+	+	+	+	+	+

Acknowledgements

References

Billotte N, Lagoda PJR, Risterucci AM, Baurens FC (1999) Microsatellite-enriched libraries: applied methodology for the development of SSR markers in tropical crops. Fruits 54: 277–288.

Brito RM, Arias MC (2010) Genetic structure of *Partamona helleri* (Apidae, Meliponini) from Neotropical Atlantic rainforest. Insectes Sociaux 57: 413–419. doi: 10.1007/s00040-010-0098-x

Brito RM, Francisco FO, Domingues-Yamada AMT, Gonçalves PHP, Pioker FC, Soares AEE, Arias MC (2009) Characterization of microsatellite loci of *Tetragonisca angustula* (Hymenoptera, Apidae, Meliponini). Conservation Genetics Resources 1: 183–187. doi: 10.1007/s12686-009-9045-4

Camargo JMF, Pedro SM (2012) Meliponini Lepeletier, 1836. In: Moure JS, Urban D, Melo GAR (Eds) Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region. http://moure.cria.org.br

Estoup A, Solignac M, Cornuet J-M, Goudet J, Scholl A (1996) Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. Molecular Ecology 5: 19–31. doi: 10.1111/j.1365-294X.1996.tb00288.x

Francisco FO (2009) FOFpop v.2.0, a pool of Microsoft Excel sheets to analyze genotypic data. http://www.ib.usp.br/~lgea/fofpop

Francisco FO, Arias MC (2010) Inferences of evolutionary and ecological events that influenced the population structure of *Plebeia remota*, a stingless bee from Brazil. Apidologie 41: 216–224. doi: 10.1051/apido/2009079

Franck P, Garnery L, Loiseau A, Oldroyd BP, Hepburn HR, Solignac M, Cornuet JM (2001) Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data. Heredity 86: 420–430. doi: 10.1046/j.1365-2540.2001.00842.x

Michener CD (2007) The Bees of the World, 2nd ed. Johns Hopkins University Press, Baltimore, 992 pp.

Nogueira-Neto P (1954) Notas bionômicas sobre meliponíneos: III – Sobre a enxameagem. Arquivos do Museu Nacional 42: 419–451.

Quezada-Euán JJG, May-Itzá WJ, Rincón M, De La Rúa P, Paxton RJ (2011) Genetic and phenotypic differentiation in endemic *Scaptotrigona hellwegeri* (Apidae: Meliponini): im-

plications for the conservation of stingless bee populations in contrasting environments. Insect Conservation and Diversity. doi: 10.1111/j.1752-4598.2011.00179.x

Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources 8: 103–106. doi: 10.1111/j.1471-8286.2007.01931.x

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (Eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, 365–386.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. Annals of the Entomological Society of America 87: 651–701.

RESEARCH ARTICLE

Species status and new distribution records for *Lithurgus huberi* Ducke (Hymenoptera, Megachilidae, Lithurginae)

Victor H. Gonzalez[1], Michael S. Engel[2], Mariano Lucia[3], Leopoldo J. Alvarez[3]

1 *Southwestern Oklahoma State University, Biological Sciences, 100 Campus Drive, Weatherford, Oklahoma, 73096, USA* **2** *Division of Entomology, Natural History Museum, and Department of Ecology & Evolutionary Biology, 1501 Crestline Drive – Suite 140, University of Kansas, Lawrence, Kansas, 66045, USA* **3** *División Entomología, Museo de La Plata, Universidad Nacional de La Plata, Paseo del Bosque s/n, 1900FWA, La Plata, Argentina. CONICET*

Corresponding author: *Victor H. Gonzalez* (victorgonzab@gmail.com)

Citation: Gonzalez VH, Engel MS, Lucia M, Alvarez LJ (2013) Species status and new distribution records for *Lithurgus huberi* Ducke (Hymenoptera, Megachilidae, Lithurginae). Journal of Hymenoptera Research 30: 13–18. doi: 10.3897/JHR.30.4018

Abstract

Notes are provided on the morphology of males and females of the enigmatic *Lithurgus huberi* Ducke (Lithurginae: Lithurgini), a species historically believed to have been introduced into South America from Asia and to be a possible synonym of the more widespread *L. atratus* Smith. Distinctive differences are documented between *L. huberi* and *L. atratus*, perhaps indicative of separate species. In addition, we provide new records of *L. huberi* in Argentina and Paraguay.

Keywords

Anthophila, bees, Apoidea, wood-boring bees

Introduction

Megachilid bees of the subfamily Lithurginae Newman are commonly known as wood-boring bees because they usually excavate burrows in dead, dry, often decayed wood (Michener 2007). The subfamily is unquestionable monophyletic and the sis-

ter group of Megachilinae (Roig-Alsina and Michener 1993, Engel 2001, Michener 2007, Gonzalez et al. 2012). Lithurginae consists of two tribes, Protolithurgini Engel, an extinct lineage preserved in mid-Eocene Baltic amber, and Lithurgini Newman, an extant taxon containing about 60 species in three genera: *Lithurgus* Berthold (currently with two subgenera), *Microthurge* Michener, and *Trichothurgus* Moure. Although the subfamily is relatively small in number of species, it is found on all continents except Antarctica. Only species of *Lithurgus* s.str. are found in the Eastern Hemisphere except for *Lithurgus huberi* Ducke, which occurs in Brazil and Argentina (Roig-Alsina 2006, Moure and Melo 2007). Snelling (1983) suggested that *L. huberi* was adventive to South America and probably a synonym of the Indo-Australian species *L. atratus* Smith given the morphological similarity between the two. Such a hypothesis is also supported by the wood-nesting habits of *Lithurgus*, which facilitates dispersion across great distances.

We confirmed the close morphological similarity between *L. atratus* and *L. huberi*, as noted by Snelling (1983). However, we also noted some morphological features in both sexes of *L. huberi* that seem to be consistent and that may prove to be useful in species recognition. Accordingly, the purpose of this note is to document and illustrate those characters, as well as record *L. huberi* for the first time for Paraguay and supplementing this with new distribution records for Argentina (*vide infra*).

Materials and methods

We examined (V.H.G. & M.S.E) the type series of *L. atratus* as well as of *L. dentipes* Smith, a species that has been considered a synonym of *L. atratus*, deposited in The Natural History Museum, London (NHML). Additionally, we examined 42 specimens of *L. atratus* from India, Celebes, Borneo, Solomon Islands, and Australia deposited in the Snow Entomological Collection (SEMC), Division of Entomology, University of Kansas Natural History Museum, Lawrence, Kansas, USA, and the U.S. National Pollinating Insects Collection, Bee Biology and Systematics Laboratory (BBSL), Utah State University, Logan, Utah.

Photomicrographs were prepared using a Canon 7D digital camera attached to an Infinity K-2 long-distance microscope lens, and were assembled with the CombineZM™ software package. Specimens are deposited in SEMC and the entomological collection of the Museo de La Plata, Argentina (MLP). Occurrence data for *L. huberi* were plotted using CorelDRAW® X5 and were taken from the literature (*i.e.*, Ducke 1907, 1908, 1910, Camillo et al. 1983, 1994, Wittmann and Hoffmann 1990, Roig-Alsina 2006, Pick and Schlindwein 2011) as well as from specimen data retrieved from the Inter-American Biodiversity Information Network (IABIN) provided by the USDA, Agricultural Research Service Pollinating Insect Research Unit, Logan, USA, and the Coleção de Entomologia do Laboratório de Biologia Vegetal, Universidade Federal de Pernambuco, Recife, Brazil.

Results

Females of *L. huberi* primarily differ from those of *L. atratus* by the facial prominence. In *L. huberi* it is more strongly punctate and more depressed along the epistomal sulcus than in *L. atratus* (*cf.* Figs 1, 3 vs. 2, 4). Likewise, males of *L. huberi* differ from those of *L. atratus* in the inner glabrous surface of the metabasitarsus, which is broader and more projected (*cf.* Figs 5 vs. 6). Although subtle, such morphological differences appear to be consistent across the specimens examined. However, we have had access to a limited number of individuals from a restricted set of localities and cannot rule out that such differences represent mere geographic variations.

Despite the small number of species in *Lithurgus* s.str., the status of most of them remains questionable, particularly those from Southeast Asia. For example, at least eight 'species' that are closely related to *L. atratus* have been suggested to represent a single taxonomic unit (Michener 1965), although they could be a complex of rather cryptic species. Some of these are practically indistinguishable from *L. atratus*, differing mostly in body size (*e.g.*, *L. atratiformis* Cockerell). Undoubtedly, a revision of the group that includes a great number of specimens from multiple locations (thereby necessitating extensive fieldwork as existing collections have significant gaps for *Lithurgus* across its distribution) is needed before any taxonomic action can be taken with certainty. Such a work would ideally be accompanied by molecular data to further test putative species boundaries as well as to elucidate possible routes and times of dispersion.

In conclusion, we support the view that *L. huberi* is closely related to the Indo-Australian *L. atratus* species or species group and, if dispersed by human activity, it might have reached South America at least 100 years ago when it was described (Ducke 1907). However, we show here consistent, albeit somewhat subtle, morphological features to distinguish *L. huberi* from *L. atratus*. For the time being we recommend that they should be treated as separate species.

Taxonomy

Lithurgus (*Lithurgus*) *huberi* Ducke
http://species-id.net/wiki/Lithurgus_huberi
Figs 1, 3, 5, 7

New records. Argentina: 1♂, Buenos Aires, Berazategui (Parque Pereyra Iraola), 24-1-2011, Col. Alvarez. L-Lucia. M (MLP) (foraging on *Ipomoea purpurea* (L.) Roth); 1♀, Misiones, Loreto, February 1954 (SEMC). Paraguay: 2♀♀, 4♂♂, Itapua Vega, Dec 1955, Juan Foerster (SEMC).

Additional material examined. Brazil: 3♀♀, 3♂♂, B. Horizonte, Minas, Brasil. A. Costa Jr. 20-4-49; 1♂, Para, Conceicão do Araguaia, July 1959, M. Alvarenga; 1♂, Paraíba, Santa Luzia, Mun. Serra do Brandão dos Chandoca, 4/8 December 1955, Sebastidos Madeiros (SEMC).
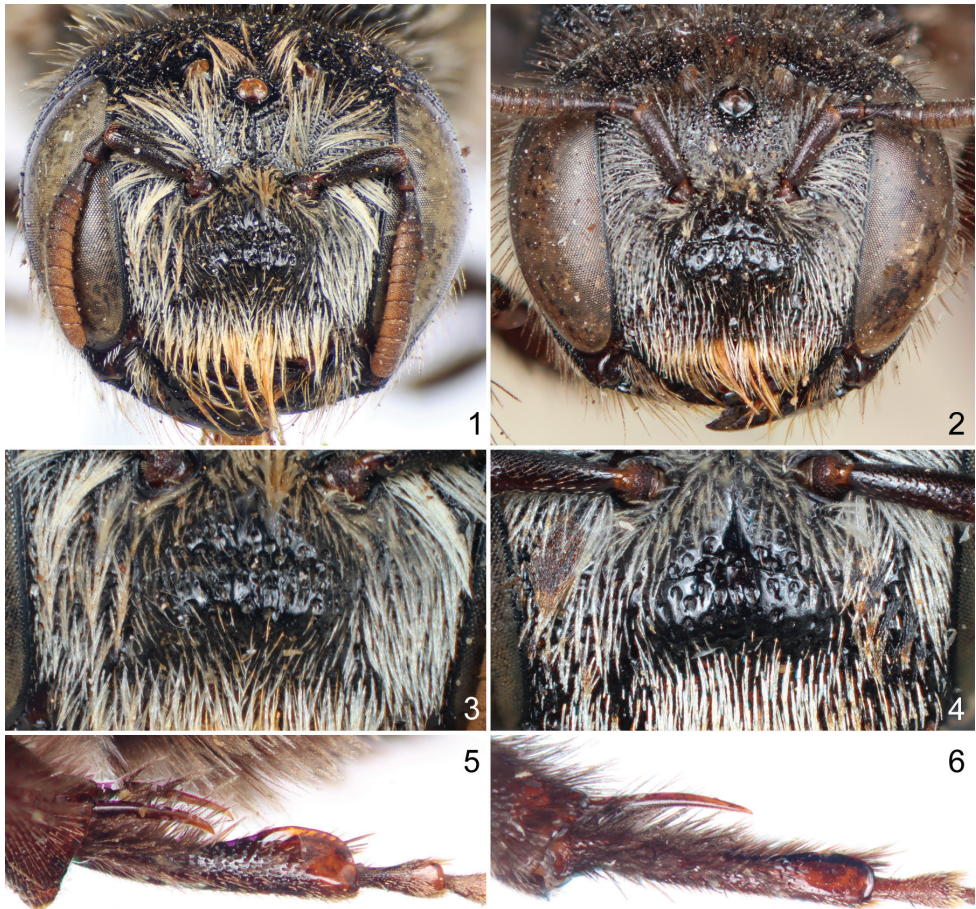
Figures 1–6. Details of *Lithurgus huberi* Ducke from Minas Gerais, Brazil (left column: **1, 3, 5**) and *L. atratus* Smith (NHML syntypes) (right column: **2, 4, 6**). Facial views (**1, 2**), detail of facial prominence (**3, 4**), and inner surface of metabasitarsus emphasizing the apical expansion and glabrous surface (**5, 6**).

Acknowledgements

Figure 7. Distribution records of *Lithurgus huberi* Ducke, including the new records discussed herein.

References

Camillo E, Garófalo CA, Campos MJO, Serrano JC (1983) Preliminary notes on the biology of *Lithurgus huberi* (Hymenoptera, Megachilidae). Revista Basileira de Biologia 43(2): 151–156.

Camillo E, Garófalo CA, Serrano JC (1994) Nesting activities and nest reuse of *Lithurgus huberi* (Hymenoptera, Megachilidae). Revista Basileira de Biologia 54(2): 183–194.

Ducke A (1907) Contribution a la connaissance de la faune hyménoptérologique du Nord-Est du Brésil. Revue d'Entomologie 26: 73–96.

Ducke A (1908) Contribution à la connaissance de la faune hyménoptérologique du Nord-Est du Brésil. Revue d'Entomologie 27: 57–87.

Ducke A (1910) Explorações botânicas e entomológicas no Estado do Ceará. Revista Trimestral do Instituto do Ceará 24: 3–61.

Engel MS (2001) A monograph of the Baltic amber bees and evolution of the Apoidea (Hymenoptera). Bulletin of the American Museum of Natural History 259: 1–192. doi: 10.1206/0003-0090(2001)259<0001:AMOTBA>2.0.CO;2

Gonzalez VH, Griswold T, Praz CJ, Danforth B (2012) Phylogeny of the bee family Megachilidae (Hymenoptera: Apoidea) based on adult morphology. Systematic Entomology 37(2): 261–286. doi: 10.1111/j.1365-3113.2012.00620.x

IABIN (2012) Inter-American Biodiversity Information Network. Avalible at: http://www.IABIN.net [accessed on 19 September 2012]

Michener CD (1965) A classification of the bees of the Australian and South Pacific regions. Bulletin of the American Museum of Natural History 130: 1–362.

Michener CD (2007) The Bees of the World [2nd Edition]. Johns Hopkins University Press, Baltimore, MD, 953 pp.

Moure JS, Melo GAR (2007) Lithurgini Newman, 1834. In: Moure JS, Urban D, Melo GAR (Eds) Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region. Sociedade Brasileira de Entomologia, Curitiba, Brazil, 914–917. [total volume pages xiv+1058 pp.] [updated at URL: http://www.moure.cria.org.br/catalogue (last accessed 20 September 2012)]

Pick RA, Schlindwein C (2011) Pollen partitioning of three species of Convolvulaceae among oligolectic bees in the Caatinga of Brazil. Plant Systematics and Evolution 293(1/4): 147–159. doi: 10.1007/s00606-011-0432-4

Roig-Alsina A, Michener CD (1993) Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). University of Kansas Science Bulletin 55(4): 124–162.

Roig-Alsina A (2006) *Hylaeus punctatus* (Brullé) (Colletidae), a Palaearctic bee long established in South America. Journal of Hymenoptera Research 15(2): 286–289.

Snelling RR (1983) The North American species of the bee genus *Lithurge* (Hymenoptera: Megachilidae). Contributions in Science, Natural History Museum of Los Angeles County 343: 1–11.

Wittmann D, Hoffman M (1990) Bees of Rio Grande do Sul, southern Brazil (Insecta, Hymenoptera, Apoidea). Iheringia, Série Zoologia 70: 17–43.

RESEARCH ARTICLE

JOURNAL OF
Hymenoptera
RESEARCH
The International Society of Hymenopterists

New species of egg parasites from the Oil Palm Stick Insect (*Eurycantha insularis*) in Papua New Guinea (Hymenoptera, Chrysididae, Phasmatodea, Phasmatidae)

Lynn S. Kimsey[1,†], Charles F. Dewhurst[2,‡], Seno Nyaure[2,§]

1 *Bohart Museum of Entomology, Department of Entomology, University of California, One Shields Ave., Davis, California 95616, USA* **2** *Papua New Guinea Oil Palm Research Association Inc., Dami Research Station, Kimbe, West New Britain, Papua New Guinea*

† *urn:lsid:zoobank.org:author:ADC91A84-7D87-41E4-A4B3-87BA1041D7DD*
‡ *urn:lsid:zoobank.org:author:579B5A2F-7745-413F-B647-CA93B27A206F*
§ *urn:lsid:zoobank.org:author:335C5132-FC9A-468C-AED9-E71D0E780A37*

Corresponding author: *Lynn S. Kimsey* (lskimsey@ucdavis.edu)

Abstract

Two new species of amisegine chrysidids, *Cladobethylus insularis* and *Exova tunana,* are described. Both species were reared from eggs of the stick insect, *Eurycantha insularis* Lucas (Phasmatidae), collected from infestations of oil palm pests in Papua New Guinea. This is the first host record for either genus.

Introduction

Outbreaks of the oil palm stick insect, *Eurycantha insularis* Lucas, 1869 (Figs 1, 2), occurred in 2007 and 2008 in smallholder oil palm plantations in the Tunana smallholder blocks in Northern (Oro) Province, Papua New Guinea. Damage caused by these stick

Figures 1–3. *Eurycantha insularis* Lucas **1** feeding damage in Tunana small holding oil palm plantation in Papua New Guinea **2** Male **3** Female with parasitized egg; egg not to scale.

insects can be seen in Fig. 3. Eggs from this stick insect were regularly collected during these infestations and two new species of chrysidid egg parasites, *Exova tunana* sp. n. and *Cladobethylus insularis* sp. n. described below were reared from the eggs. There is no information on the ecology of these parasitoids except for the rearing information.

There are seven named species of *Cladobethylus* and two species of *Exova* described prior to those described below (Kimsey and Bohart 1991, Kimsey 2011). The new species of *Cladobethylus* described below is the second one recorded from New Guinea. *Exova* is known from northern Australia and Fiji, so it is not surprising to find a species in Papua New Guinea.

Materials and methods

Specimens were all reared by the second and third authors and their staff at PNGO-PRA, Higaturu Centre, Northern Province, Papua New Guinea.

Types are deposited in the following museums and institutions: CANBERRA – Australian National Insect Collection, CSIRO, Canberra, ACT, Australia; DAVIS – Bohart Museum of Entomology, University of California, Davis, USA; KIMBE - Papua new Guinea, PNG Oil Palm Research Association Dami Research Station, Kimbe, West New Britain Province; LONDON – The Natural History Museum, London, England, and PORT MORESBY – the National Insect Collection, Port Moresby, Papua New Guinea.

Cladobethylus insularis Kimsey & Dewhurst, sp. n.
urn:lsid:zoobank.org:act:B4B4A207-A0D8-43E8-87D2-1E76C0F155E0
http://species-id.net/wiki/Cladobethylus_insularis
Figs 4–10

Holotype male. Papua New Guinea, Northern (Oro) Province, Higaturu, ex *Eurycantha insularis* egg, second generation coll. 23/ii/2009, died 23/ii/2009 C. F. Dewhurst, No. 888 (LONDON).

Paratypes (17 males, 3 females): same data as holotype except: 1 male, 1st generation, em. (emerged) 28/xii/2008, died 5/i/2009, C. F. Dewhurst , No. 855; 1 male, 1st generation, emerged 28/xii/2008, died 5/i/2009, S. Nyaure, No. 855; 1 female, 1st generation, emerged 8/ii/2008, died 14/ii/2008, C. F. Dewhurst, No. 833; 1 male, 1st generation, emerged 6/i/2009, died 17/i/2009, S. Nyaure, No. 869; 1 male, 1st generation, emerged 2/i/2009, died 9/i/2009, S. Nyaure, No. 862; 1 male, 1st generation, emerged& dead 22, 23, 25/i/2009, S. Nyaure, No. 875; 1 male, 2nd generation, coll. 3/ii/2009, died 16/ii/2009, C. F. Dewhurst, No. 887; 1 male, Higaturu Oil Palms, coll. 8/v/2007, H9/vi/2007, C. F. Dewhurst, Nos. 823; 3 males, 2nd generation, coll. 25/vii/2009, died 27/vii/2009, C. F. Dewhurst, No. 893, 894, 895; 1 male, 2nd generation, coll. 5/ii/2009, died 20/ii/2009, C. F. Dewhurst, No. 890; 1 male, 1 female,

Figures 4–5. *Cladobethylus insularis*, side views **4** Male **5** Female.

2nd generation, coll. 26/i/2009, died 16/ii/2009, C. F. Dewhurst, No. 881, 882; 1 male; 2nd generation, coll. 8/ii/2009, died 16/ii/2009, C. F. Dewhurst, No. 8923; 1 male, 2nd generation, coll. 2/ii/2009, died 15/ii/2009, C. F. Dewhurst, No. 886; 1 male, emerged 12/ii/2008, died 17/ii/2008, C. F. Dewhurst, No. 837; 1 female, 2nd generation, coll. 27/i/2009, died 21/ii/2009, C. F. Dewhurst, No. 883 (CANBERRA, LONDON, DAVIS, KIMBE, PORT MORESBY).

Diagnosis. This is the largest bodied species of *Cladobethylus*, with males averaging twice the length of the known other species. Male *insularis* can be distinguished from *aquilus*, the only other species known from New Guinea by the longer malar
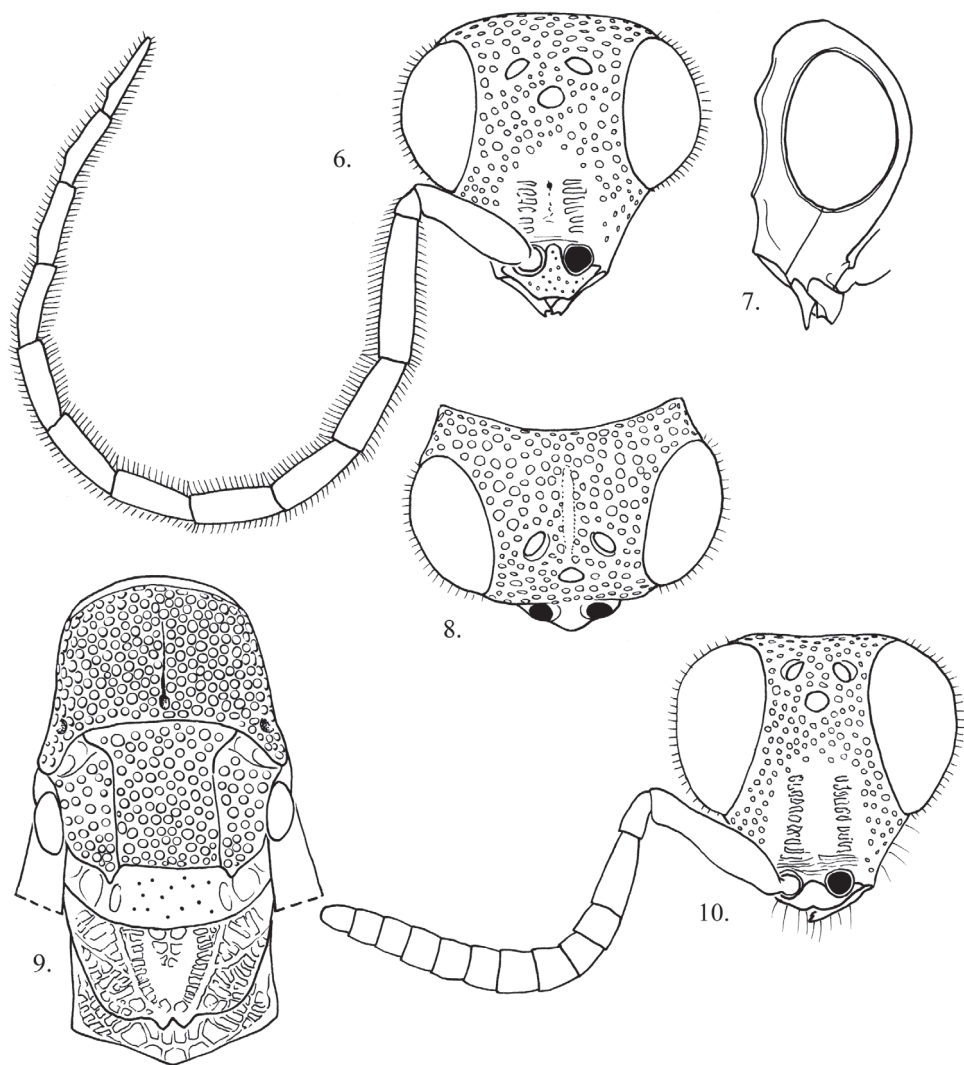
Figures 6–10. *Cladobethylus insularis* **6–8** Male head **6** Front view **7** Lateral view **8** Dorsal view **9** Dorsal view of male mesosoma, wings removed **10** Front view of female face.

space (4 midocellus diameters versus 3.5 in *aquilus*), pronotum not blue-tinted, much broader zone of cross-ridging in the scapal basin, legs brown instead of yellow (*aquilus*) flagellomere I shorter (4.5× as long as broad versus 5× in *aquilus*) and flagellomere XI shorter (5× as long as broad versus 6× in *aquilus*). Features of female *insularis* not shared with other *Cladobethylus* include the bicolored antenna and legs, long clypeus (0.8 midocellus diameter long versus 0.3–0.6 midocellus diameters in other species), and narrow distance between the midocellus and nearest eye margin (up to 2 midocellus diameters versus 2.6–2.7 midocellus diameters in the others).

Description. Male (Fig. 4). *Body length*. 5–7 mm.

Head (Figs 6–8). Face about as long as broad across the eyes; genal area without foveae; midocellus 1.8 diameters from ocular margin; hindocelli 4 diameters from posterior margin of vertex; scapal basin with narrow, longitudinal submedial band of cross-ridges; malar space 3.5–3.8 midocellus diameters; subantennal distance 1 midocellus diameter long; flagellomere I 4× as long as broad; flagellomere II 2.8–3.0× as long as broad; flagellomere IX 5× as long as broad, flagellar setae 0.7 midocellar diameter long; ocular setulae 0.4 midocellus diameter long.

Mesosoma (Fig. 9). Pronotum about as long as scutum; punctation on pronotum, scutum and mesopleuron large, deep and nearly contiguous punctuation, without scrobal sulcus or omaulus; scutellum polished with scattered tiny punctures; metapleuron polished, impunctate.

Metasoma. Tergum I polished with few tiny highly scattered punctures; tergum II with two large ovoid patches of small punctures, 1 puncture diameter apart, separated medially by impunctate band and with broad apical impunctate band; terga III–IV with tiny punctures 1 puncture diameter apart, becoming sparser toward apical margin.

Color. Head, meso- and metasoma black with metallic bluish green highlights dorsally on head, pronotum and scutellum, sometimes faint on metasomal terga; mandible and antenna dark brown; legs light reddish brown, except coxae dark brown to black basally; wing membrane brown-tinted, darkest in vicinity of stigma; wing veins dark brown.

Female (Fig. 5). *Body length*. 4.5–5.5 mm. As in the male, except face (Fig. 10) about as long as broad across the eyes; malar space 3.6-3.8midocellus diameters; subantennal distance 0.8 midocellus diameter; flagellomere I 2.4× as long as broad; flagellomere II long as broad; flagellomere IX 1.3× as long as broad; scape, pedicel black, flagellomeres I–III white, remaining flagellomeres black; coxae, femora, mid and hindtibiae black basally, whitish apically; foretibia and tarsi brown; wing membrane brown-tinted with dark brown veins.

Etymology. The species is named after the host species.

Exova tunana **Kimsey & Dewhurst, sp. n.**
urn:lsid:zoobank.org:act:74C249EB-0312-4710-A9E1-178BEA990A75
http://species-id.net/wiki/Exova_tunana
Figs 11–18

Holotype male. Papua New Guinea, Northern (Oro) Province, Saiho Division (Tunana), ex *Eurycantha insularis* egg, emerged 27/vii/2007, died 30/vii/2007, C. F. Dewhurst, No. 828 (LONDON).

Paratypes: 1 male, emerged 5/ii/2008, died 13/ii/2008, C. F. Dewhurst, No. 831; 1 female, emerged 10/ii/2008, died 15/ii/2008, C. F. Dewhurst, No. 835; 1 female, Higaturu, 1st generation, coll. 2/vi/2008, died 12/vi/2008, C. F. Dewhurst, No. 841 (CANBERRA, DAVIS, KIMBE, PORT MORESBY).

Diagnosis. This is the largest bodied species of *Exova*. Female *tunana* can be distinguished from *fijiensis* Kimsey by the long spine-like propodeal angle (a feature shared

Figures 11–13. *Exova tunana*, side views **11** Male **12** Female **13** Dorsal view of female Mesosoma.
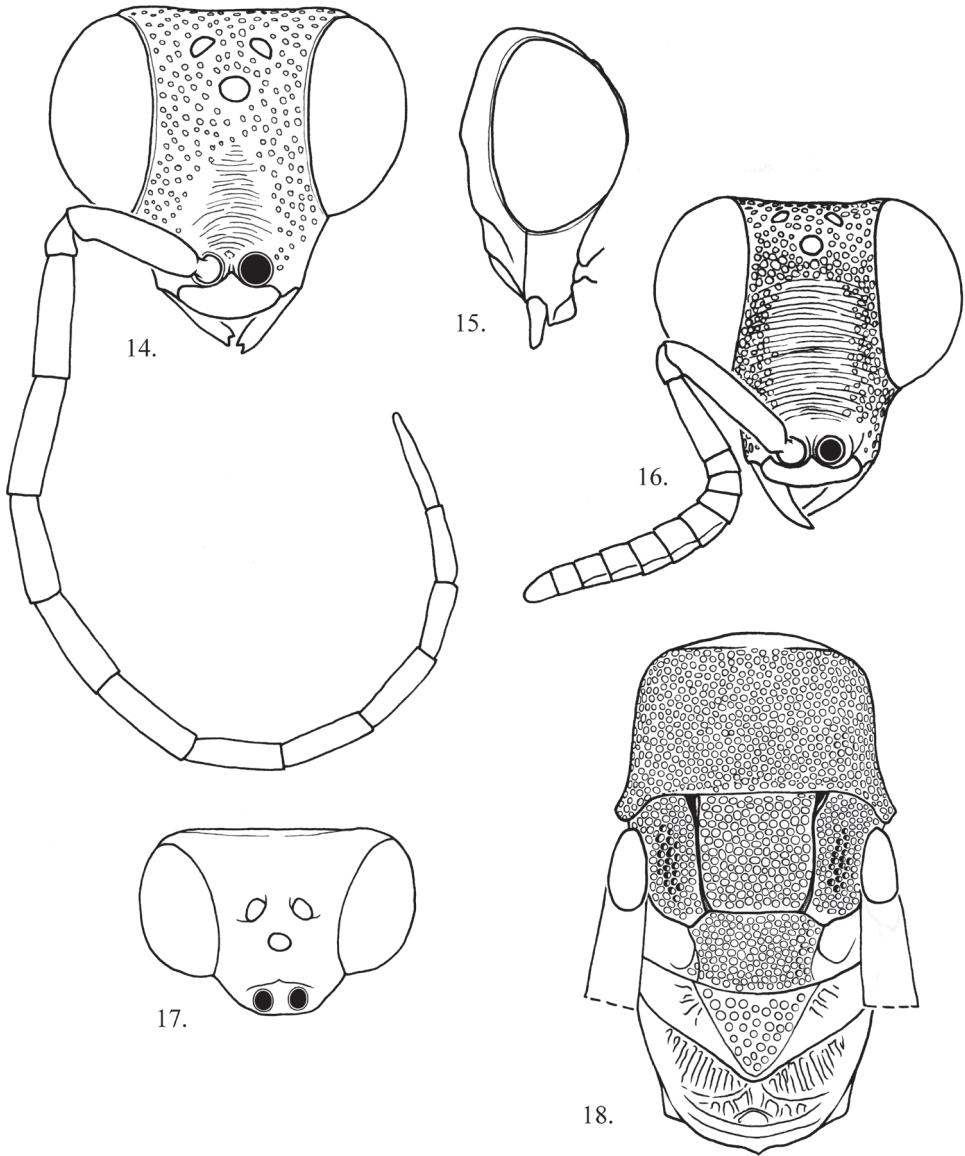
Figures 14–18. *Exova tunana* **14, 15, 18** Male head **14** Front view **15** Lateral view **18** Dorsal view **16** Female head front view. **17** Dorsal view of male mesosoma, wings removed.

with *tetraspina*), which is conical in *fijiensis*. Female *fijiensis* can be distinguished from *tetraspina* by the more weakly produced medial propodeal projections and the highly polished and smooth metapleuron and propodeum. Male *tunana* can be distinguished from *fijiensis* by the shorter distance from the midocellus to ocular margin (2 midocellus diameters or less in *tunana* versus 2.5 midocellus diameters in *fijiensis*), flagel-

lomeres I and II equivalent versus I much longer than broad than II in *fijiensis* and the body with bluish green tints versus brassy in *fijiensis*.

Description. Male (Fig. 11). *Body length*. 5.0–5.5 mm.

Head (Figs 14, 15, 17). Face 1.0–1.2 × as long as broad across the eyes; face between scapal basin and vertex with punctures separated by about 1 puncture diameter; scapal basin with cross-ridged medial zone occupying one-third of area between inner eye margins; midocellus 1.9 diameter from ocular margin; malar space 2.5 midocellus diameters; subantennal distance 1 midocellus diameter long; flagellomeres I-II 3.5–3.6× as long as broad; flagellomere IX 6x as long as broad; flagellar setae short, 0.1 midocellus diameter long; ocular setulae minute or absent; postocular distance 0.4 midocellus diameter wide.

Mesosoma (Fig. 18). Pronotum about as long as scutum; mesopleural punctures nearly contiguous, without scrobal sulcus or omaulus; metapleuron horizontally cross-ridged.

Metasoma. Tergum I polished, nearly impunctate with scattered tiny punctures; terga II–IV with small punctures separated by 2–3 puncture diameters.

Color. Head, meso- and metasoma black with metallic bluish green tints dorsally; antenna black, legs pale yellow; wing membrane brown-tinted, veins dark brown.

Female (Fig. 12). *Body length*. 6–7 mm.

Head (Fig. 16). Face 0.9× as long as broad across the eyes in front view; scapal basin with medial one-half to one-third coarsely transversely striate, laterally with dense contiguous punctures; malar space 4.3 midocellus diameters; subantennal distance 1 midocellus diameter; flagellum fusiform, broadest medially, flattened ventrally; flagellomere I 2.8× as long as broad; flagellomere II 0.7× as long as broad; flagellomere IX 1.2× as long as broad.

Mesosoma (Fig. 13). Apterous; pronotum and scutum densely longitudinally striate, pronotum 0.7× as broad as long, 1.4× as long as length of scutum plus scutellum; scutellum transversely striate.

Metasoma. Polished; tergum I with fine dense scratches in two touching posteromedial patches, scratches parallel and curving laterally; tergum II with two anteromedial patches of dense curved scratches subtended by large medial patch of dense posteriorly curved fine scratches; terga III and IV smooth with scattered tiny punctures.

Color. Body dark reddish brown, paler on side of scapal basin, mesopleuron medially, propodeal spines, legs and metasomal apex; apical flagellar segments yellowish beneath; coxae whitish.

Etymology. The species is named after the collection site in Northern Province, PNG.

Acknowledgements

References

Kimsey LS (2011) Re-examination of the genus *Exova* Riek, with the first chrysidid recorded from Fiji (Hymenoptera: Chrysididae: Amiseginae). Proceedings of the Entomological Society of Washington 113(4):436–441. doi: 10.4289/0013-8797.113.4.436

Kimsey LS, Bohart RM (1991 [1990]) Chrysidid Wasps of the World. Oxford University Press 651 pp.

RESEARCH ARTICLE

JOURNAL OF
Hymenoptera
RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

The male genitalia of ants: musculature, homology, and functional morphology (Hymenoptera, Aculeata, Formicidae)

Brendon E. Boudinot[1]

1 *The Evergreen State College, 2700 Evergreen Parkway NW, Olympia, WA 98505, USA*

Corresponding author: Brendon E. Boudinot (boudinotb@gmail.com)

Abstract

The genitalia of male ants are interpreted in the context of the broader Hymenoptera. For the first time muscle homologies are established for twenty six species of ants in nine subfamilies: Amblyoponinae, Cerapachyinae, Dolichoderinae, Ecitoninae, Ectatomminae, Formicinae, Leptanilloidinae, Myrmicinae and Ponerinae. Fifteen muscles in total are found in the external genitalia of male ants and all are homologous with the musculature of basal Hymenoptera (Schulmeister 2001). Secondary fusion, reduction or losses of muscles have occurred in different lineages. From generalized to derived, the functional morphology and qualitative mechanics of three taxa, *Formica obscuripes* Forel 1886, *Messor andrei* Mayr (1886) and *Labidus coecus* Latreille (1802) are described and compared. Special reference is made to the Ecitoninae, where the work of Birket-Smith (1981) is reinterpreted and volsellar homology is clarified. The true digitus of *Labidus* is revealed to be a small sclerite at the base of the distal volsellar element which is re-identified as the cuspis.

Keywords

Anatomy, army ants, kinesiology, morphology, reproductive biology

Introduction

Insect male genitalia are spectacular mechanical adaptations with diverse morphology and function. Examples of this diversity include the twin hypodermic intromittent organs of the *Drosophila bipectinata* complex (Kamimura 2007), the sperm scoopulas of Odonata (Córdoba-Aguilar et al. 2003), 'Symphytan' suction cups (Schulmeister 2001), the copu-

latory hooks of *Metrioidea* (Chrysomeloidea; Flowers and Eberhard 2006), and genitalia modified as stridulatory instruments for courtship (*Micronecta*, Corixidae; Sueur et al. 2011) or for stridulation in copulo (*Tipula*, Tipulidae; Eberhard and Gelhaus 2009). The diversity of male genitalia may also be cryptic and in the realm of functional morphology. For example, male *Drosophila melanogaster* with ablated genital clasper mechanosensory sensillae were found to copulate less frequently, and unilaterally ablated males mounted females skewed contralaterally from the typical position (Acebes et al. 2003).

The morphology and mechanics of male ant genitalic musculature remain undescribed for all ~12,000 species of ants (Bolton 1995), with the exception of *Labidus coecus*, *Dorylus* (*Anomma*) *funereus* (Birket-Smith 1981), and *Myrmica kotokui* Forel 1911 (Ogata 1991). The function of ant genitalic valves has been described from primary observations for few taxa (Allard et al. 2002, 2006; Baer and Boomsma 2006), while the entire sequence of copulation has never been reported; a composite description is as follows. Copulation of all non-'Symphyta' Hymenoptera is orthandrous (i.e. male atop female, facing same direction; Schulmeister 2001), which holds true for ants. Males of *Carebara vidua* F. Smith 1858 and *Plectroctena mandibularis* F. Smith 1858 were observed to use their genitalia to probe the apex of the female's gaster while grasping her with his legs (Robertson and Villet 1989; Villet 1999). Probing is likely a general requisite of ant copulatory behavior (Ross 1982; Schulmeister 2001) followed by penetration which may be via the penisvalvae apices, the digiti or both. The penisvalvae of the aedeagus anchor the male inside of the female's bursa copulatrix or the mussel organ of *Atta* (Baer and Boomsma 2006), sometimes causing female scarring (Kamimura 2008) while the endophallus is putatively everted (Allard et al. 2002; although see Schulmeister 2001). The typically opposable digitus and cuspis have been observed to clasp the terminal female sternite in a ponerine *Diacamma* sp. (Allard et al. 2002). The parameres serve as a clamp-type attachment mechanism (Gorb 2008; Ogata 1991; Schulmeister 2001), potentially enhanced by wet adhesion from a putative telomeral gland (Francoeur 1988). The male will often release grasp the female and hang on to her by his genitals only (Robertson and Villet 1989) and will remain anchored within the female in this position during a variable period of time until copulation is terminated (Hölldobler and Wilson 1990; Foitzik et al. 2002).

Knowledge of genitalic functional morphology contributes to our understanding of the sexual selection pressures which act on these organs (Eberhard 2010) and may possibly be used to make predictions about the reproductive biology of ants. This is of significance given the effects of mating systems (Boomsma et al. 2005), paternity assurance (Baer 2011; Baer and Boomsma 2006) and polyandry (Hughes and Boomsma 2007; Robinson and Page Jr. 1989) on social dynamics. Furthermore, understanding male ant morphology is important for taxonomy (Fisher 2009; LaPolla et al. 2012; Yoshimura and Fisher 2012) and systematics (LaPolla 2004; Brady and Ward 2005). Thus the musculature and gross morphology of male ant genitalia represents an appealing and untapped source for new, potentially useful morphological characters (Keller 2011; Schulmeister 2003; Song and Bucheli 2010), which may also serve as synapomorphies for clades identified via molecular techniques.

The goal of this paper is to discuss the homologies of male ant genitalic musculature in the context of the broader Hymenoptera and to provide descriptions of the functional mechanics of three distinct forms. The work of Schulmeister (2001, 2003) has identified and clarified the muscular homologies within the basal Hymenoptera, or the paraphyletic 'Symphyta' (Sharkey 2007). While the external genitalia of male ants have been characterized for several subfamilies (Ogata 1991; Yoshimura and Fisher 2009, 2011, 2012; Yoshimura and Onoyama 2002), musculature has been completely overlooked excepting Birket-Smith (1981) and Ogata (1991), the former of which is here reassessed.

Materials and methods

All specimens examined in this study were from the author's personal collection (BEBC) or the Longino collection (JTLC, University of Utah). Collections in 95% ethanol were made in Central America, and in the states of Arizona, California, Tennessee and Washington. Specimens were softened by boiling in water for 2–5 minutes. Dissections were carried out in petri dishes, with or without wax and minuten pins. A Leica MZ16 microscope with maximum magnification of 115× and mounted with a JVC Digital Camera KY-F708 3-CCD was used for dissections. Micrographs were generated for illustration using Auto-Montage Pro Syncroscopy (Synoptics Ltd., Frederick, MD USA) software. For especially small males, temporary mounts of minute sclerites and musculature were prepared for observation with a Leica DME compound microscope using glycerin as the mounting medium.

General hymenopteran terms preferred by the Hymenoptera Anatomy Ontology (Yoder et al. 2010) are used when homology is clear (Table 1). For an overview of terms applied to the genitalia of ants and other Hymenoptera, refer to Yoshimura and Fisher (2011) and Schulmeister (2001). Walker Jr. and Liem's (1994) concepts of origin and insertion are used when discussing musculature: origins are broad anchoring attachments; while insertions are the narrow, usually distal or posterior, ends of muscles which transfer force to a movable element. The muscles of hymenopteran male genitalia are named alphabetically (Boulangé 1924; modified by Schulmeister 2001): anterior to posterior, ventral to dorsal, medial to lateral; starting with sternum IX and ending with the muscles of the parameres. Thus lettering begins on the anteroventral apex of sternum IX with muscle *a* and ends, in the Formicidae, with the intrinsic muscle *t* of the parameres.

The mechanics of sclerite movement were assessed qualitatively by examining the angles of muscular origin and insertion, and the attachment or position of the sclerite relative to the local components of the genital capsule. Simple physical models were used to predict the mechanics of muscular contraction and element movement. *Formica obscuripes* Forel 1886, *Messor andrei* Mayr (1886) and *Labidus coecus* Latreille (1802) were chosen as models for functional morphology because their genitalia are well developed, represent cases of relatively generalized to specialized morphology, and

Table 1. Anatomical terms of the external genitalia used herein, excluding specific membrane and apodeme names. Hymenoptera Anatomy Ontology (HAO) Uniform Resource Identifiers (URIs) link to concept definitions. Synonyms discussed or used are indicated.

Term	HAO URI	Synonyms
Aedeagus	http://purl.obolibrary.org/obo/HAO_0000091	
Apex gonostipitis	http://purl.obolibrary.org/obo/HAO_0000134	
Apodeme	http://purl.obolibrary.org/obo/HAO_0000142	
Apophysis	http://purl.obolibrary.org/obo/HAO_0000143	
Cupula	http://purl.obolibrary.org/obo/HAO_0000238	Basal ring
Basivolsella	http://purl.obolibrary.org/obo/HAO_0001085	
Digitus	http://purl.obolibrary.org/obo/HAO_0000385	
Cuspis	http://purl.obolibrary.org/obo/HAO_0000239	Distivolsella
Endophallus	http://purl.obolibrary.org/obo/HAO_0000291	
Foramen genitale	http://purl.obolibrary.org/obo/HAO_0000346	
Genital capsule	http://purl.obolibrary.org/obo/HAO_0000312	
Gonocondyle	http://purl.obolibrary.org/obo/HAO_0000380	
Gonopore	http://purl.obolibrary.org/obo/HAO_0000821	
Basimere	http://purl.obolibrary.org/obo/HAO_0000386	Gonostipes
Gonostipital arm	http://purl.obolibrary.org/obo/HAO_0000387	
Telomere	http://purl.obolibrary.org/obo/HAO_0000395	Harpe
Paramere	http://purl.obolibrary.org/obo/HAO_0000389	
Parossiculus	http://purl.obolibrary.org/obo/HAO_0000703	
Penisvalva	http://purl.obolibrary.org/obo/HAO_0000707	
Phallotrema	http://purl.obolibrary.org/obo/HAO_0000714	
Spiculum	http://purl.obolibrary.org/obo/HAO_0000946	
Valviceps	http://purl.obolibrary.org/obo/HAO_0001047	
Valvura	http://purl.obolibrary.org/obo/HAO_0001050	
Volsella	http://purl.obolibrary.org/obo/HAO_0001084	

among these three taxa all the observed genitalic muscles of male ants are represented. The more complicated movements described here should be treated as predictions to be tested either via direct observation of copulation or via neuroethological studies.

Results and discussion

Genitalic morphology (Figs 1–17)

External genitalia (Figs 1–16)

The external genitalia of ants are composed of five elements: sternum IX, the cupula, and the paired valves of the penisvalvae, volsellae, and parameres (Figs 1–4). Sternum IX (Fig. 4) is produced anteromedially into a process called the spiculum (Schulmeister 2001) and has a cranial apodeme traversing the mesal face (Forbes 1967; Forbes and
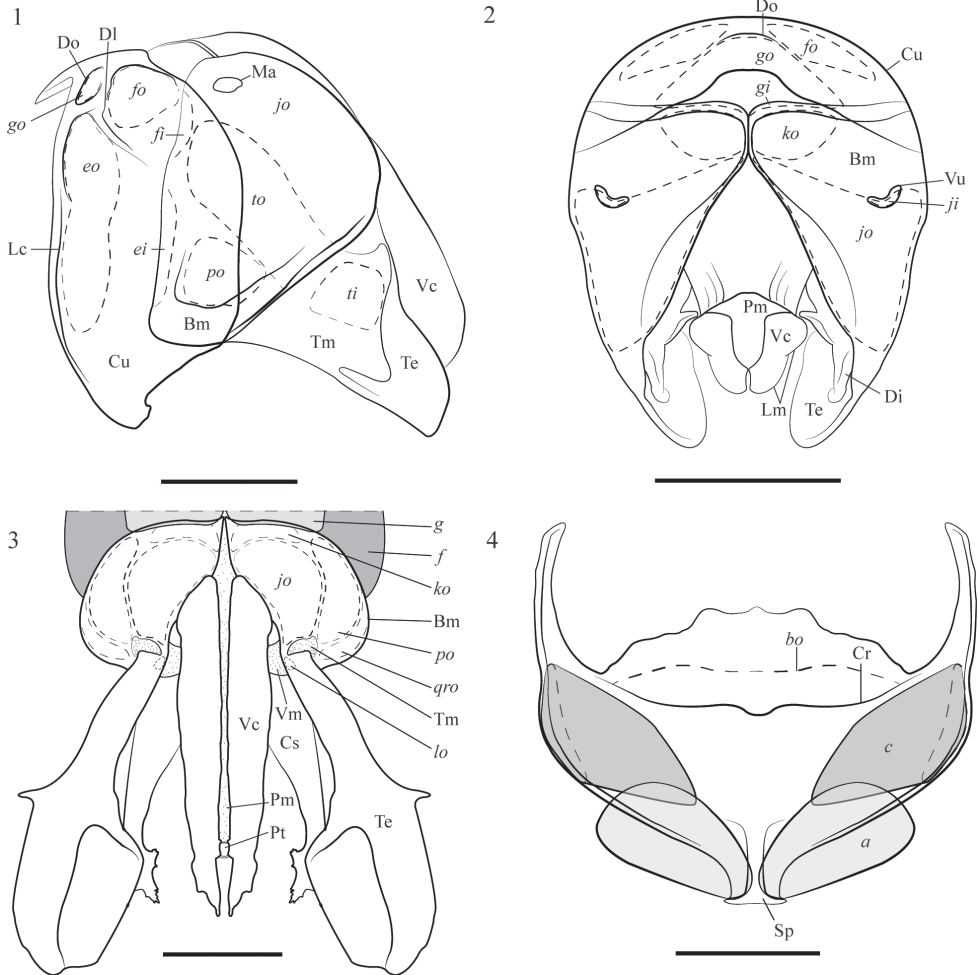
Figures 1–4. Genital capsule and sternum IX habitus and musculature. **1** *Formica obscuripes* lateral oblique view, 0.5 mm **2** *Messor andrei* dorsal view, 0.5 mm **3** *Labidus praedator* dorsal view, 1.0 mm **4** *F. obscuripes* sternum IX anterior-to-posterior oblique mesal view, 0.5 mm. Abbreviations: **Bm** basimere; **Cr** cranial apodeme of sternum IX; **Cs** cuspis; **Cu** cupula; **Di** digitus; **Dl** dorsolateral apodeme of cupula; **Do** dorsal apodeme of cupula; **Lc** lateral apodeme of cupula; **Lm** valviceps lamina; **Ma** maculation; **Pm** penisvalva membrane; **Pt** phallotrema; **Sp** spiculum; **Te** telomere; **Tm** telomeral membrane; **Vm** volsellar membrane; **Vu** valvura. Muscles: *a b c d e f g h i j k l p qr t*; insertion of muscle '*x*': *xi*; origin of muscle '*x*': *xo*.

Hagopian 1965), which may be a modified sternal antecosta. The term cupula is preferred over basal ring, as the latter refers to non-homologous structures in the Protura and Diptera (Tuxen 1970). The cupula (Figs 1, 2, 5–7) is a hymenopteran synapomorphy (Sharkey 2007) and is variable in form for ants. The anterior opening of the cupula is the foramen genitale (Fg, Figs 5–7; Schulmeister 2001), and is constricted to varying degrees across different lineages. In the ecitonines, the foramen genitale is reduced to
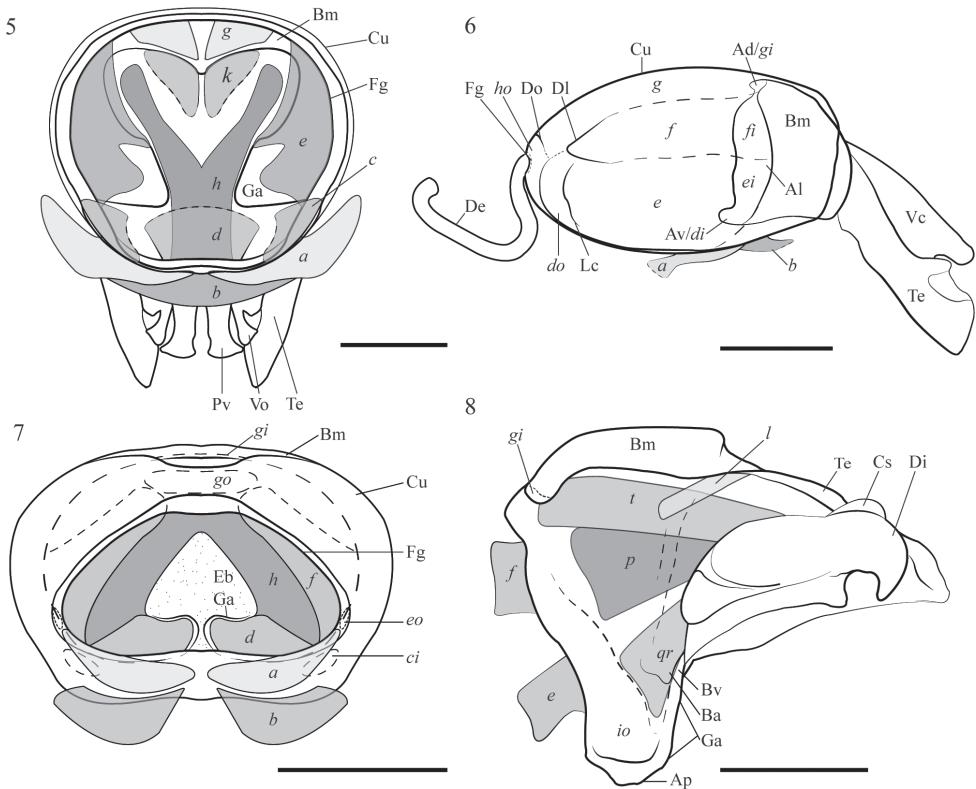
Figures 5–8. Muscles of the genital capsule and sternum IX. **5** *Formica obscuripes* genital capsule anterior-to-posterior view, 0.5 mm **6** *Neivamyrmex longiscapus* lateral view, 1.0 mm **7** *Messor andrei* genital capsule anterior-to-posterior view, 0.5 mm **8** *F. obscuripes* volsella and paramere mesal view, with all penisvalvar muscles removed except *l*, 0.5 mm. Abbreviations: **Ad** anterodorsal apodeme of basimere; **Al** anterolateral apodeme of basimere; **Ap** apex gonostipitis; **Av** anteroventral apodeme of basimere; **Ba** basivolsellar apodeme; **Bm** basimere; **Bv** basivolsella; **Cs** cuspis; **Cu** cupula; **Dl** dorsolateral apodeme of cupula; **Do** dorsal apodeme of cupula; **Eb** endophallic bladder; **Fg** foramen genitale; **Ga** gonostipital arm; **Lc** lateral apodeme of cupula; **Pv** penisvalva; **Te** telomere; **Vc** valviceps; **Vo** volsella. Muscles: *a b c d e f g h i j k l p qr t*; insertion of muscle '*x*': *xi*; origin of muscle '*x*': *xo*.

a miniscule aperture through which only the ductus ejaculatorius and tracheae pass (Fig. 6). In all ant taxa I examined, the ventral gonocondyle (Schulmeister 2003) of the cupula was absent.

Lateral to medial, the three paired valves are the parameres, volsellae, and penisvalvae (Figs 2 and 3). Homology of the parameres of the Formicidae with those of other Hymenoptera is reasonable, as the following complex characters are shared: 1) at least four of the five penisvalvae muscles attach to the basimeres; 2) an intrinsic muscle has an origin in the basimere and inserts in the telomere; and 3) a membrane is present at the telomere articulation with the basimere. It should be noted that this telomeral membrane is variable in development for the Formicidae, from well-developed to ab-
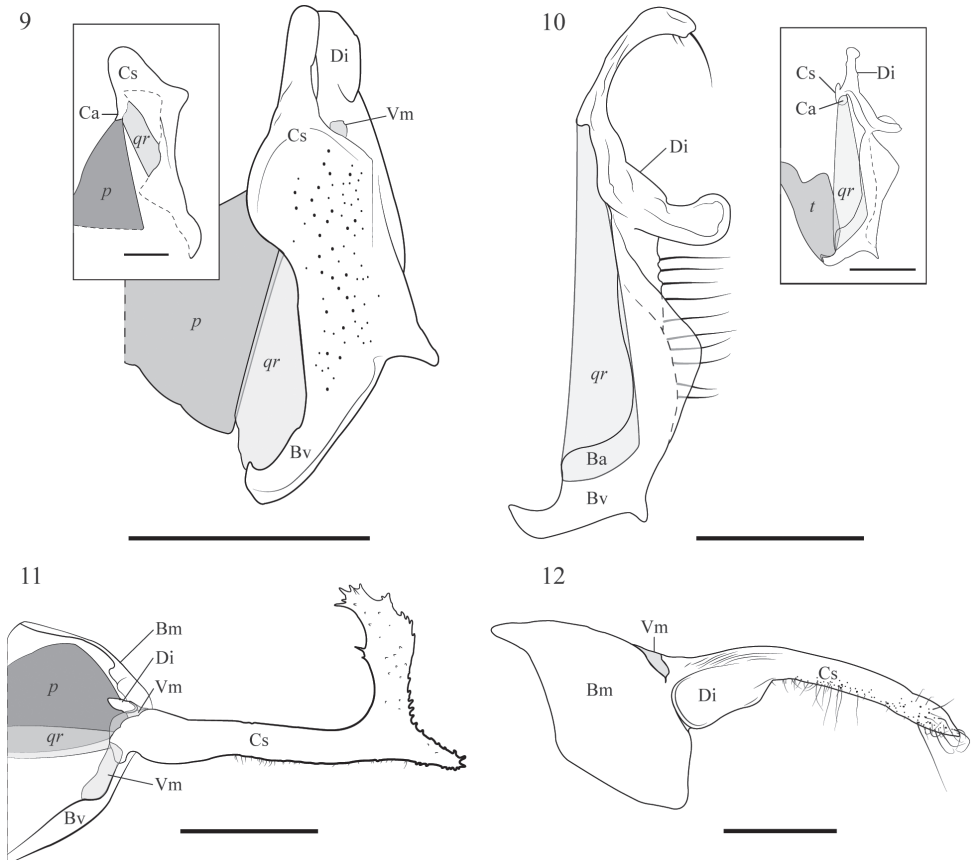
Figures 9–12. Volsella muscles and morphology; the parossiculus is composed of the basivolsella and distivolsella. **9** *Formica obscuripes* ventral view; inset mesal with digitus removed, dorsal left; both 0.5 mm **10** *Messor andrei* mesal view, dash indicates base of twisted lamina and part of setose ridge; inset dorsal; both 0.2 mm **11** *Labidus coecus* mesal view, 1.0 mm **12** *Neivamyrmex longiscapus* lateral view, 0.5 mm. Stippling on 9 and 12 indicate setal bases. Abbreviations: **Bm** basimere; **Bv** basivolsella; **Ca** cuspal apodeme; **Cs** cuspis; **Di** digitus; **Vm** volsellar membrane. Muscles: *p qr t*; insertion of muscle '*x*': *xi*; origin of muscle '*x*': *xo*.

sent across numerous clades (Yoshimura and Fisher 2011). Thus the specific homology of the telomere with the basal hymenopteran harpe is dubious; therefore the application of "basimere" and "telomere" is preferable over "gonostipes" and "harpe" for ant genitalia. The parameres of ants are thus composed of a dish-like basimere and distal telomere (Figs 1 and 3). A ventromedial extension of the basimere is the origin site of several volsellar and penisvalvar muscles, and is termed the gonostipital arm (Ga, Fig. 8); this structure is not a synapomorphy of the Myrmicinae as claimed by Ogata (1991) because the gonostipital arm is present in other ant subfamilies (this study) and in many other hymenopteran lineages (Schulmeister 2001, 2003). The volsella, another hymenopteran synapomorphy along with the cupula (Sharkey 2007), is composed
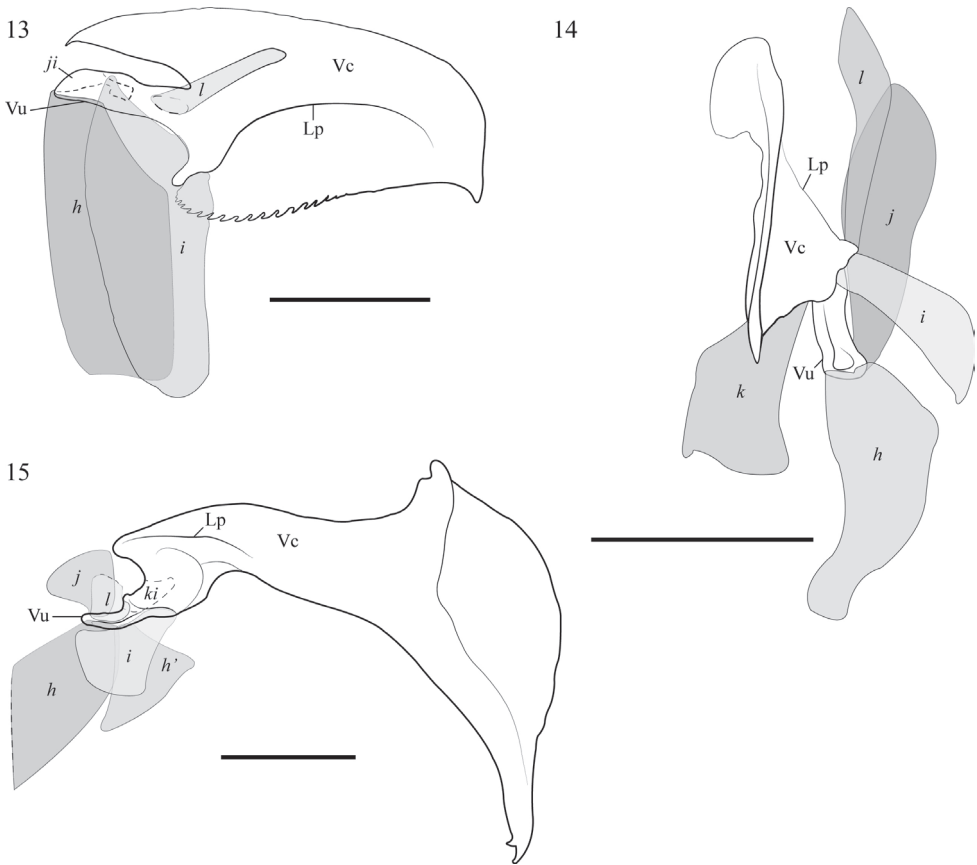
Figures 13–15. Penisvalva muscles and morphology. **13** *Formica obscuripes* ectal view, 0.5 mm **14** *Messor andrei* ventral, 0.2 mm **15** *Labidus coecus* ectal view, *ki* is on mesal face of valvura, 1.0 mm. Abbreviations: **Lp** lateral apodeme of penisvalva; **Vc** valviceps; **Vu** valvura. Muscles: *h h' i j k l*; insertion of muscle '*x*': *xi*; origin of muscle '*x*': *xo*.

of the digitus which is basolaterally articulated with the parossiculus (Figs 8–12). The parossiculus is generally divided into the basivolsella and cuspis (= distivolsella, Figs 9–11). As the volsella is unique to the Hymenoptera, term preference is ambiguous; however, given that distivolsella has never been applied to Formicidae, I propose that "cuspis" be maintained. The cuspis is usually setose (Schulmeister 2001); this is indeed the case for all ant taxa examined (Cs, Figs 9–12).

Although the term aedeagus has a long history and is frequently used in the myrmecological literature to describe the middle-most valves (Brown Jr. 1960; Serna and Mackay 2009; Smith 1942; Taylor 1965; Yoshimura and Fisher 2012), this term is correctly applied to the whole intromittent apparatus which includes the sclerites, or penisvalvae, and the endophallus. The term "penisvalva" is preferred over "aedeagal plate" as the former term is virtually restricted to and broadly used for the Hymenoptera and represents the homology of the sclerite in question between ants and other

hymenopterans, while the latter term is used for non-homologous structures among several insect orders ("penisvalva" e.g. Apoidea, Pesenko and Astafurova 2006; "Parasitica", Karlsson and Ronquist 2012; Vespoidea and 'Symphyta', Schulmeister 2003; "aedeagal plate" e.g. Hemiptera: Rivers 1948; Diptera: Amorim 2007; Hymenoptera: Ogata 1987). The homologies of the cranially-directed apophysis and the distal component of the ant penisvalva with the valvura and valviceps are highly likely; the location and musculature of the apophysis are equivalent to those in the basal Hymenoptera and Aculeata (Schulmeister 2003). I thus adopt the terms valvura for the arm-like apophysis and valviceps for the distal component of the penisvalva (Figs 13–15; Schulmeister 2001). The penisvalvae are joined mediodorsally via a tough membrane (Pm, Figs 2 and 3), and an aedeagal gland is present in various forms between the valves (not figured; Hölldobler and Engel-Siegel 1982). A lower oblique carina (Ward 2001) is present in some taxa along the ectal face of the valviceps (Lp, Figs 13–15), forming a channel-like groove in formicines. Forbes (1952) hypothesized that the channel functions as a "sperm gutter"; this seems unlikely as the channel is separated from the phallotrema by the blade of the valviceps. I hypothesize that the lower oblique carina functions as an arch, transferring the force of the pensivalvar muscles to the valviceps apex.

Internal genitalia (Figs 16 and 17)

As the aedeagus is the interface between the internal and external genitalia, a general description of the internal genitalia is warranted (Fig. 16). Spermatozoa are produced in the follicles of the paired testes and pass through the vas deferens (Snodgrass 1935; Schulmeister 2001) into the vasicula seminalis where they are kept until copulation. The two vasiculae seminalae join together into the ductus ejaculatorius which opens into the endophallic bladder via the gonopore (Fig. 17; Snodgrass 1935). The endophallic bladder was formerly known as the aedeagal bladder, but it is definitely a component of the endophallus (Snodgrass 1935); its function remains unknown (Ball and Vinson 1984; Ball et al. 1984). A sclerite is present in the ductus ejaculatorius which has been identified as the fibula ducti in 'Symphyta' (Schulmeister 2003), the wedge in *Neivamyrmex* (We, Fig. 16; Forbes and Do-Van-Quy 1965) and *sperrkeil* in *Formica rufa* (translates contextually to keel-shaped valve, Clausen 1938). Although this sclerite was found in most taxa examined, it was not homologized across the Hymenoptera or among ant subfamilies as its morphology was not explicitly examined. The endophallus opens externally via the phallotrema at the bases or the dorsal apices of the penisvalvae (Pt, Fig. 3).

Musculature

The homology of genitalic musculature for each species examined is summarized in Table 2. Fifteen total muscles were observed among the examined ant taxa. Considerable variation exists in the morphology of tergites IX and X, but as these are not primary components of the genitalia these were excluded from this investigation. Three muscles,
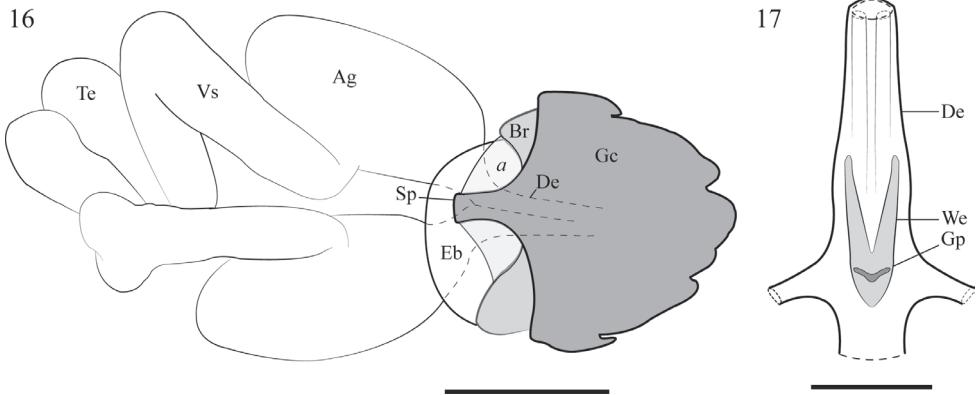
Figures 16–17. Internal genitalia. **16** *Formica obscuripes* ventral oblique view, 1.0 mm **17** *Labidus coecus* ductus ejaculatorius with wedge sclerite ventral view, 0.5 mm. Abbreviations: **Vs** vasicula seminalis; **Ag** accessory gland; **De** ductus ejaculatorius; **Eb** endophallic bladder; **Gc** genital capsule; **Gp** gonopore; **Sp** spiculum; **Te** testis; **We** wedge sclerite. Muscle: *a*.

a, *b* and *c*, have their origins on sternum IX and insert on the cupula (Figs 4, 5–7). Muscle *a* is paired and originates on the spiculum from which it diverges to the ventrolateral margin of the cupula, except in the ecitonines where *a* is inserted in a median groove ventrad to the reduced foramen genitale. The origin of the unpaired muscle *b* is along the cranial apodeme; the muscle converges anteriorly to its ectal insertion on the medioventral margin of the cupula. Each element of the paired muscle *c* originates on an anterolateral lobe of the sternum and inserts on the ventrolateral margin of the cupula.

Four muscles, *d*, *e*, *f*, and *g*, have their origins on the cupula (Figs 1–3, 5–8). Muscles *e*, *f*, and *g* are paired, while *d* is unpaired. The origins of the cupula muscles are usually associated with an apodeme: muscle *d* with the ventral, *e* with the lateral, *f* with the dorsolateral and *g* with the dorsal apodeme (Figs 1, 2, 6). In general the gonostipital arms, or anteromedian projections of the basimeres, receive the insertions of *d* on the ventral margin and *e* ventrolaterally (Figs 1, 5, 7, 8). Both *f* and *g* insert ectally on the basimere, *f* on the lateral margin and *g* on the dorsal margin (Figs 1–3, 5–8). Loss of cupula muscles was observed for three species: *Atta cephalotes* L. (1758), *Crematogaster nigropilosa* Mayr 1870, and *Leptanilloides* sp. in which the cupula is reduced to a thin strip with only muscle *e* present. Muscles *f* and *g* are fused in *Pheidole californica* Mayr 1870, and muscle *d* could not be confirmed for *Hypoponera opacior* Forel (1893), *Odontomachus chelifer* Latreille (1802), and *Cerapachys* nr. *augustae*.

In total, five penisvalva muscles (Figs 13–15) were found; *h*, *i*, *j*, and *k* were consistently present except in the reduced genitalia of *Leptanilloides* sp. (Table 2), while *l* was lost in disparate taxa. These muscles are paired, with one element of each associated with a left or right penisvalva. Three of the penisvalva muscle origins are on the mesal face of the basimere: *i* on the ventral, *j* on the dorsolateral, and *k* on the dorsomedian face (*j, k* Figs 1–3; *i* Fig. 8). The origin of muscle *h* is variable, in some cases spanning the apices gonostipitum to the ventral apodeme of the cupula. Although the dorsome-

Table 2. Muscle presence or absence per taxon. Only muscles recorded from the Aculeata are included. 0 absent; 1 present; 2 fused; ? presence/absence unconfirmed; ‡ Amblyoponinae; § Cerapachyinae; | Dolichoderinae; ¶ Ecitoninae; # Ectatomminae; †† Formicinae; ‡‡ Leptanilloidinae; §§ Myrmicinae; || Ponerinae; ¶¶ Sphecidae and ## Vespidae (final two from Schulmeister 2003). Note: *Myrmica kotokui* data coded from Ogata (1991) in which the following muscles were mislabeled: *f, i, j, k, qr, t*.

Species	Muscles															
	a	b	c	d	e	f	g	h	i	j	k	l	o	p	qr	t
Prionopelta nr. *modesta*‡	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1
Cerapachys nr. *augustae*§	1	1	1	?	1	1	1	1	1	1	1	1	0	1	?	1
Cylindromyrmex brevitarsus§	1	1	1	0	1	1	1	1	1	1	1	1	0	1	0	1
Dolichoderus bispinosus|	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
Eciton lucanoides¶	1	?	1	1	1	1	1	1	1	1	1	1	0	1	1	0
Labidus coecus¶	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0
Labidus praedator¶	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0
Neivamyrmex longiscapus¶	1	1	1	1	1	1	1	1	1	1	1	?	0	1	1	0
Nomamyrmex eisenbeckii¶	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
Gnamptogenys mordax#	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
Camponotus sansabeanus††	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
Camponotus atriceps††	1	1	?	1	1	1	1	1	1	1	1	0	0	1	1	1
Formica obscuripes††	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
Prenolepis imparis††	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
Leptanilloides sp. ‡‡	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	1
Aphaenogaster nr. *rudis*§§	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0
Atta cephalotes§§	1	1	1	0	1	1	1	1	1	1	1	1	0	0	1	0
Crematogaster nigropilosa§§	1	1	1	1	0	1	1	1	1	1	1	0	0	1	1	1
Messor andrei§§	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1
Myrmica kotokui§§	1	?	1	1	1	1	1	1	1	1	1	?	0	1	1	1
Pheidole californica§§	1	1	1	1	1	2	2	1	1	1	1	0	0	0	1	0
Hypoponera opacior|	1	1	1	?	1	1	1	1	1	1	1	0	0	0	1	0
Leptogenys donisthorpei|	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
Odontomachus chelifer|	1	1	1	?	1	1	1	1	1	1	1	1	0	1	1	0
Platythyrea prizo|	1	1	0	?	1	1	1	1	1	1	1	1	0	1	1	0
Sceliphron caementarum¶¶	0	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0
Dolichovespula adulterina##	0	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0

dial basimeral area where *k* originates has been termed the parapenis in basal Hymenoptera (Schulmeister 2001), this term is not used for the Formicidae due to uncertain homology. Muscle *l* has its origin on the basal dorsomedian area of the telomere (Figs 3 and 8). The insertions of *h* and *j* are on the ventral and dorsal faces of the valvura apices, respectively. Muscles *i* and *l* are inserted on the penisvalva lateral apodeme (Figs 13–15; Ward 2001), an equivalent structure to the ergot of basal hymenoptera (Schulmeister 2001). Finally, *k* is mesally inserted on the base of the valvura or the valviceps (Fig. 15).

Across the taxa examined there was great variation in the form of the volsellae, with concomitant variation in the presence and form of the two muscles, *p* and *qr* (Figs 9–11) which both insert on the cuspal apodeme. Similar to the penisvalvar muscles, the volsellar muscles are paired with one element of each associated with a left or right volsella. Muscles *m, n, o, s,* and *si* (Schulmeister 2001) were not present in the examined taxa, and were presumably lost in the ancestral ant lineage or earlier. Muscle *p* has its origin on the internal lateral face of the basimere. Reduction of the cuspis and loss of *p* was observed in *Aphaenogaster* nr. *rudis, Messor andrei, Pheidole californica* and *Hypoponera opacior*. This contrasts with the intrinsic volsellar muscle *qr* which was present in all but two taxa examined, and could not be confirmed for a third. The origin on *qr* is on the basivolsellar apodeme.

A single intrinsic muscle of the paramere, *t*, was found in several of the taxa examined. Muscles *u* and *v* (Schulmeister 2001) were never present. Muscle *t* is paired, with the left or right element originating anterolaterally on the respective basimere (Fig. 8) and inserting on the telomeral corium (Fig. 1). Presence of *t* was inconsistent; in the present dataset, loss is associated with telomere fusion to the basimere, in reduced genitalia, and with change in telomere function. In these circumstances, other muscles including the volsellar *p* and penisvalvar *j* are hypothesized to affect the grasping capacity of the parameres via elastic forces.

Functional morphology

Formica obscuripes Forel, 1886

Among the taxa examined this species has relatively generalized genitalia, with all fifteen muscles present and without extreme modifications of the sclerites. The three muscles of sternum IX, *a, b,* and *c*, are present in this species (Figs 4 and 5). Simultaneous contraction of both elements of *a* contracts the genital capsule into the gaster to varying degrees. Antagonistic contraction of either the left or right element of *a* adducts the genital capsule towards the respective side, an action which is synergistic with the genital capsule protractor *b*. Muscle *c* inserts ventrolaterally on the ventral apodeme of the cupula, above the insertions of *a* and *b* (Fig. 5), and torques the genital capsule via elevation of the basal margin of the cupula toward the muscle's origin.

Muscles of the cupula rotate the genitalic valves within the cupula similarly to a ball-in-socket mechanism. Each muscle is inserted on the anterior margin of the basimere except for *d*, which inserts on the anteroventral face of the endophallic bladder (Figs 5 and 8). Muscle *e* adducts the valves ventrally, while *g* acts antagonistically to elevate the apices while contracting the valves. Muscle *e* also adducts the telomere to the mediosagittal plane by drawing the medioventral apices of the basimeres dorsolaterally. Contraction of *f* may affect torsion of the valves.

All five muscles of the penisvalvae are present in this species (Fig. 13). The penisvalvae can move independently along the mediosagittal plane and are capable of rotation,

pivoting on their lateral apodemes. Elevation of the valviceps is caused by contraction of *h*, while depression is caused by *j*. Muscle *k* is inserted basally on the valviceps and may dig the basal teeth into the female's bursa copulatrix (Kamimura 2008) by drawing the valviceps anterad and adducting the apices ventrally. Rhythmic contraction of *i* may enhance this hypothetical function in order to gain greater purchase in the female via a motion analogous to mastication. Muscle *l* abducts the valviceps ventrolaterally swinging the apices apart, potentially to allow passage of ejaculate during copulation or to remove the sharply curved valviceps teeth from the female.

Two muscles, *p* and *qr*, are attached ectally and mesally to the cuspal apodeme of the volsellae, respectively, and act antagonistically to open or close the cuspides (Fig. 9). Adduction of the cuspis to the digitus is caused by contraction of *qr*, which bends the volsellar plate toward the penisvalvae while drawing the cuspal apex toward the digitus. The antagonistic abduction of the cuspis is induced by *p*, which extends laterally to the basimere from the ectal face of the cuspal apodeme (Figs 8 and 9).

The single intrinsic muscle of the paramere, *t*, is relatively well developed in this species. Contraction of *t* flexes the telomere ventrally via folding of the telomere corium. Upon relaxation of *t*, the elastic force of the sclerotic bridge between the basimere and telomere sclerite erects the telomere. Flexion of the telomere may also be affected by the penisvalvar muscle *j* which has a major portion of its origin along a mediodorsal inflection of the basimere. Intense contraction of *j* may roll the telomere ventrally via torsion of the inflection ectally, while also flexing the penisvalvae ventrally; this may function to strengthen the clamp-hold of the female.

Messor andrei Mayr, (1886)

With the loss of *p*, fourteen of the fifteen genitalic muscles are found in this species. Although the foramen genitale is somewhat constricted (Fg, Fig. 7), the muscular function of the cupula and sternum IX are not distinct enough from *Formica obscuripes* to warrant separate discussion. The clasping function of the paramere is maintained despite the replacement of most of the telomere corium by sclerite. In this case, there is very little telomere flexion. Rather, *t* adducts the apices of the telomere toward the mediosagittal plane while pulling the medioventral faces dorsally. The mesal face of the telomere has a cup-like lamina which, coupled with hypothetical secretions of the telomeral gland, may enhance the male's grasp on the female.

The valviceps is produced dorsally with a laterally-produced lamina (Lm, Fig. 2) which is divided into anterior and posterior lobes by a constriction, making the valviceps similar in shape to an anvil. The dorsal laminae may function as a passive anchor in the female's bursa copulatrix while the aedeagus is intromittent. Between the two valviceps, an ellipsoid chamber is formed with the penisvalvar membrane closing it dorsally. I hypothesize that this chamber acts as a mechanical sperm pump, forcefully pulsing ejaculate out of the anterior-directed phallotrema via contraction of *l*, which spreads the valviceps bases. Contraction of *j* may synergistically contribute to this hypothetical action via rotation around a vertical axis placed at the dorsal constriction,

drawing the dorsal posterior lobes together while spreading the anterior lobes and valviceps apices ectally. Muscle *j* also adducts the valviceps ventrally. The basal wall of the valviceps, where *k* is inserted, is inflected dorsally and ventrally into a rough parabola with its vertex pointed anteriorly. This inflection divides *k* into dorsal levator and ventral contractor halves. Motion of *l* and *k* together may pull the valviceps anteroventrally, potentially digging the valviceps teeth into the female. Muscle *h* has most, if not all, of its origin along the endophallic bladder.

Clasping is not the function of this species' modified volsella (Fig. 10), since the digitus is completely fused with the setose cuspal shaft, and is in the form of a crescent with a basal flange and apical process. The apical component of the cuspis is reduced to a small ellipsoid plate that articulates with the digitus laterally (Cs, Fig. 10, inset). Muscle *p* has been functionally replaced by *qr* which is divided into dorsal and ventral halves by the flange-like basivolsellar apodeme (Ba, Fig. 10). The dorsal margin of the cuspis is produced into a lamina which twists ~180° around the basal-distal axis. The reduced cuspis apex acts as a pivot for rotation of the digital crescent, while the twisted lamina provides strength and elastic flexibility for torsion of the volsella. Ventral contraction of *qr* in the left volsella rotates the apex of the digitus medioventrally clockwise, while dorsal contraction rotates the digital flange dorsolaterally counterclockwise. Thus *qr* has a fine-tuned forward and reverse function via differential contraction of the dorsal or ventral sides coupled with the elastic torsion of the cork-screw cuspis. The copulatory function is hypothesized to be prying the terminal plates of the female open, akin to a lock-pick, via insertion of either the digitus or digital flange into the female followed by lifting or depressing torsion.

Labidus coecus Latreille, (1802)

The genitalia of *Labidus* have been greatly modified in form relative to the non-ecitonine taxa examined. Sternum IX is produced apically into a bifid lobe and muscle *c* is absent. An external longitudinal groove extends along the ventral face of the egg-shaped cupula (Cu, Fig. 8) and receives the insertions of muscles *a* and *b*. The origin of *b* has migrated up the anterolateral lobes of the sternum; the muscle extends mesally to its insertion in the ventral groove just posterad the insertion of *a*, which is ventrad the foramen and is longitudinally elongated. This elongation of *a* along the curvature of the cupula prevents pronounced dorsal or ventral pitch during contraction of *a*. Muscle *b* may either torque the capsule contralaterally with contraction of the left or right half, or both halves together may adduct the apices of the genital valves ventrally.

Contrary to the arguments of Birket-Smith (1981), I found the cupula muscles of *Labidus* to have clear homology with the pleisiomorphic muscles *d, e, f,* and *g* (Fig. 4). Birket-Smith (1981) suggests that muscles *e, f* and *g* have been lost during the evolution of *Labidus* to be replaced by a "vaguely differentiated muscle, *d'.*" I disagree with this interpretation: the muscular origins are identical with respect to association with cupula apodemes (Do, Dl, Lb, Fig. 4) while the muscles themselves are distinguish-

able by seams between the bundles. Moreover, the insertions on the apodemes of the basimeres are relatively distinct (Ad, Ac, Av, Fig. 6), especially that of *g* which is broadest medially and tapers laterally. The lateral basimere apodeme is emarginate between the insertion of *f* on the dorsal and *e* on the ventral half. Muscle *d* is inserted on a cranially-directed anteroventral process of the gonostipital arm.

Contraction of the cupula muscles may cause splaying and movement in the mediosagittal plane. The cupula of all ecitonine species investigated is extremely strong and well-constructed to withstand the forces applied to it internally. A tall longitudinal apodeme is present along most of the length of the ring, from the foramen genitale to the apices gonostipitum. This apodeme may function as a strut, strengthening the cupula from the antero-posterior compression forces exerted by the cupular muscles. The anteroventral processes of the basimeres are extended anteriorly to a greater magnitude than the dorsal margins of the basimeres. Thus the dorsal margins of the basimeres act as a fulcrum for a third-order lever: synergistic contraction of *d* and *e* act as in-forces and cause the apices of the genitalic valves to swing ventrally. As muscle *e* converges mesally from its ventrolateral origin, it may also be able to fine-tune the yaw of the adduction motion. Muscle *f* contributes primarily to the spreading of the genitalic valves around a vertical axis of rotation between the apices gonostipitum and the bases of the valviceps.

With respect to the penisvalva muscles, Birket-Smith (1981) discussed a muscle he termed *h'*. This is not a novel muscle; rather it is the well-developed posterior portion of muscle *h* which extends into the basimere. This portion may be seen in several other taxa, including *Formica obscuripes* in which *h* is not fully divided. Muscle *k* has the typical origins and insertions on the dorsal anteromedian faces of the basimeres (Fig. 3) and mesal face of the valvurae (Fig. 15). Muscle *h* has its origin across the length of the cupula on the cupula's anterior wall, opposite the insertion of *a*. The origin of *i* extends from the ventromedial to the anterolateral face of the basimere. This lateral projection was interpreted by Birket-Smith (1981) to be the only component of *i*. Comparison with *Formica obscuripes* again clarifies the homology, as muscle *i* of the formicine may be seen to extend to the lateral face of the basimere as well.

Four of the five penisvalva muscles seem to act synergistically by contributing forces to the ventral adduction of the penisvalva apex, while only *h* acts antagonistically. The muscles all act as in-forces for moving the penisvalva as a lever and may be conveniently grouped by the order of their lever mechanism: muscle *h* as first-order dorsal adductor; muscles *k* and *j* as first-order and *i* as a third-order ventral adductor. Besides contributing to ventral or dorsal adduction, *h* antagonistically closes or spreads the ventral apices of the penisvalvae.

The volsellae of *Labidus* are highly modified, which has led to confusion of their homology (Birket-Smith 1981; Brady and Ward 2005; Watkins II 1982). The entire organ has migrated to a ventral position beneath the penisvalvae (Fig. 3), and the stalked distal element hinges ventrally on the reduced basivolsella which is completely fused with the basimere. In the literature, the distal element of the volsella has been termed the digitus: it is clear that this is not the case. The true digitus is a small ellipsoid plate, hitherto unreported, attached to the volsellar corium in a dorsolateral

position at the base of the distal element (Di, Fig. 11). Moreover the distal volsellar element is setose, a character state associated solely with the cuspis and potentially a deep autapomorphy for the Hymenoptera (Schulmeister 2001; Sharkey 2007). As a corollary, the digitus of *Neivamyrmex longiscapus* Borgmeier 1953 is retained as a rounded quadrangular plate fused with the shaft of the cuspis (Di, Fig. 12), while that of *Nomamyrmex eisenbeckii* Borgmeier 1936 is reduced and completely fused with the cuspal shaft.

Both muscles of the volsellae are present (Fig. 11), with their insertions on the cuspal apodeme. Birket-Smith (1981) identified the cuspal muscle*r* and *s*. As neither *r* nor *s* are present in any of the other taxa examined, it is most parsimonious at present to recognize them as *p* and *qr*, respectively. The origin of muscle *p* covers a large area within the basimere laterally to dorsolaterally, while the origin of *qr* has migrated from its position on the basivolsellar apodeme to the basimeral anterolateral region. The movements of the cuspis were difficult to ascertain. However, the muscles may act antagonistically to elevate or depress the cuspis. During depression of the cuspis, the cuspal condyle slides ventrally along a vertical shelf of the basimere until it strikes a medially-projected shelf. Because this shelf has a margin contour complementary to the basal swelling of the cuspis, the basal swelling may be pulled into the volsellar corium, potentially providing torque to the cuspis. The copulatory function of these modified volsellae is not known; nor is it known whether they are intromittent.

The final point of contention with Birket-Smith's (1981) description of the musculature of *Labidus* is the muscle of the telomere. From my dissections of *Labidus*, I found that when a telomere was not carefully disarticulated, a small muscle fragment could be found with its insertion on the medial face of its base, suggesting the presence of a unique muscle specific to the telomere. It became clear, however, after more care was taken that this was actually *l*, and that its insertion on the valvura could be observed. I thus conclude that Birket-Smith's putative muscle *y* is actually muscle *l*, and that no novel muscle has evolved in the telomeres of *Labidus*. The telomere is restricted to lateral movement as the medial base is fused to the basimere via sclerotization, while the rest of the telomere base is surrounded by membrane (Tm, Fig. 3). The differential flexibility of the sclerotic bridge and the corium swing the telomeres laterally when *j* is contracted, with the bridge acting as a pivot. Thus the grasping function of the telomeres of *Labidus* and other ecitonines has been lost, excepting *Nomamyrmex*.

Conclusion

The intersubfamilial homology of male ant genitalic musculature is described for the Amblyoponinae, Cerapachyinae, Dolichoderinae, Ectatomminae, Formicinae, Leptanilloidinae, Myrmicinae and Ponerinae for the first time, while the musculature and sclerite homology of the ecitonines *Labidus* and *Neivamyrmex* are clarified. Overall, the generalized male ant has fifteen muscles: three on sternum IX, four on the cupula,

five penisvalvar, two volsellar and one paramere muscle. Muscles of sternum IX act to protract, contract or torque the genitalic capsule, which may aide in probing and intromittent behavior. The cupula muscles attach to the basimeres, and are most variable in presence or absence among the myrmicine taxa examined. Muscles of the cupula exert greater control over the genitalic valves relative to the muscles of sternum IX. The penisvalva muscles have functions which vary with the lineage, and muscle *l* is lost in some. In general, the penisvalva muscles may torque the penisvalvae apices, while adducting the individual penisvalvae in the mediosagittal plane. Volsellar mechanics varies greatly with the morphology of the volsellar sclerites. In species with opposable diguti and cuspides, the muscles may spread or close the apices. In other species, the diguti may torque clockwise or counter-clockwise, while movement in *Labidus* is restricted to the mediosagittal plane. Finally, the parameres have a single muscle which is lost in some lineages to be replaced by any of the proximal muscles.

The mechanics of the sclerites and their muscles are labile over evolutionary time: the axes of rotation, the pivots, and the muscular origins and insertions shift across the sclerites. Detecting the patterns of morphological and mechanical evolution of male ant genitalia may provide useful phylogenetic characters, especially for clades currently with few or without morphological synapomorphies (e.g. the Formicoid clade, Ward 2011). Future studies should include representative taxa of each subfamily where males are known, emphasizing the differences among lineages. The hypertrophic genitalia of the Leptanillinae (Bolton 1990) provide an especially enticing opportunity, while the volsellar morphology of dorylomorphs (Brady and Ward 2005) may be valuable to reconsider, especially with musculature characters. A context and critical comparison of genitalic sclerites and muscles with the Vespoidea would be valuable and may potentially clarify the phylogenetic position of the Formicidae. Investigations of the mechanical processes involved in ant copulation will aid in understanding of the adaptations of the musculature and valves. Furthermore, we may use male genitalic musculature to predict evolutionary trends in behavior and reproductive biology. A foundation for interpreting genitalic musculature allows for neuroethological investigations with which comparative copulatory procedures may be experimentally observed (Kumashiro and Sakai 2001). The ground is set for describing the sequence and fine-scale interaction of the genitalic muscles on the male sclerites.

Acknowledgments

References

Acebes A, Cobb M, Ferveur JF (2003) Species-specific effects of single sensillum ablation on mating position in *Drosophila*. The Journal of Experimental Biology 206: 3095–3100. doi: 10.1242/jeb.00522

Allard D, Gobin B, Ito F, Tsuji K, Billen J (2002) Sperm transfer in the Japanese queenless ant *Diacamma* sp. (Hymenoptera: Formicidae). Netherlands Journal of Zoology 52: 77–86. doi: 10.1163/156854202760405203

Allard D, Børgesen L, Van Hulle M, Bobbaers A, Billen J, Gobin B (2006) Sperm transfer during mating in the pharaoh's ant, *Monomorium pharaonis*. Physiological entomology 31: 294–298. doi: 10.1111/j.1365-3032.2006.00519.x

Amorim DDS (2007) Two new genera of Swammerdamellini (Diptera, Scatopsidae), with a discussion of the position of the species of *Rhexoza*. Zootaxa 1640: 41–53.

Ball DE, Williams HJ, Vinson SB (1984) Chemical analysis of the male aedeagal bladder in the fire ant, *Solenopsis invicta* Buren. Journal of the New York Entomological Society 92: 365–370. doi: 10.1016/0020-7322(84)90003-5

Ball DE, Vinson SB (1984) Anatomy and histology of the male reproductive system of the fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae). International Journal of Insect Morphology and Embryology 13: 283–294.

Baer B (2011) The copulation biology of ants (Hymenoptera: Formicidae). Myrmecological News 14: 55–68.

Baer B, Boomsma JJ (2006) Mating biology of the leaf-cutting ants *Atta columbica* and *A. cephalotes*. Journal of Morphology 267: 1165–1171. doi: 10.1002/jmor.10467

Birket-Smith SJR (1981) The male genitalia of Hymenoptera–a review based on morphology in Dorylidae (Formicoidea). Entomologica Scandinavica 15: 377–397.

Bolton B (1990) The higher classification of the ant subfamily Leptanillinae (Hymenoptera: Formicidae). Systematic Entomology 15: 267–282. doi: 10.1111/j.1365-3113.1990.tb00063.x

Bolton B (1995) A New General Catalog of the Ants of the World. Harvard University Press, Cambridge, 504 pp.

Boomsma JJ, Baer B, Heinze J (2005) The evolution of male traits in social insects. Annual Review of Entomology 50: 395–420. doi: 10.1146/annurev.ento.50.071803.130416

Boulangé H (1924) Recherches sur l'appareil copulateur des Hyménoptères et spécialement des Chalastogastres. Mémoires et Travaux de la Faculté Catholique de Lille 28: 1–444.

Brady SG, Ward PS (2005) Morphological phylogeny of army ants and other dorylomorphs (Hymenoptera: Formicidae). Systematic Entomology 30: 593–618. doi: 10.1111/j.1365-3113.2005.00290.x

Brown Jr W (1960) Contributions toward a reclassification of the Formicidae. III. Tribe Amblyopini (Hymenoptera). Bulletin of the Museum of Comparative Zoology 122: 145–230.

Clausen R (1938) Untersuchungen über den männlichen Copulationsapparat der Ameisen, speziell der Formicinae. Mitteilungen der schweizerischen entomologischen Gesellschaft 17: 233–246.

Córdoba-Aguilar A, Uhía E, Rivera AC (2003) Sperm competition in Odonata (Insecta): the evolution of female sperm storage and rivals' sperm displacement. Journal of Zoology 261: 381–398. doi: 10.1017/S0952836903004357

Eberhard WG (2010) Rapid divergent evolution in genitalia: theory and data updated. In: Leonard JL, Córdoba-Aguilar A (Eds) The evolution of primary sexual characters in animals. Oxford University Press (New York): 40–79.

Eberhard WG, Gelhaus J (2009) Genitalic stridulation in a male tipulid fly. Revista de Biología Tropical 57: 251–256.

Fisher BL (2009) Two new dolichoderine ant genera from Madagascar: *Aptinoma* gen. n. and *Ravavy* gen. n. (Hymenoptera: Formicidae). Zootaxa 2118: 37–52.

Flowers RW, Eberhard WG (2006) Fitting together: copulatory linking in some Neotropical Chrysomeloidea. Revista de Biología Tropical 54: 829–842.

Foitzik S, Heinze J, Oberstadt B, Herbers JM (2002) Mate guarding and alternative reproductive tactics in the ant *Hypoponera opacior*. Animal Behaviour 63: 597–604. doi: 10.1006/anbe.2001.1945

Forbes J (1952) The genitalia and terminal segments of the male carpenter ant, *Camponotus pennsylvanicus* Degeer (Formicidae, Hymenoptera). Journal of the New York Entomological Society 60: 157–171.

Forbes J, Hagopian M (1965) The male genitalia and terminal segments of the ponerine ant *Rhytidoponera metallica* F. Smith (Hymenoptera: Formicidae). Journal of the New York Entomological Society 73: 190–194.

Forbes J (1967) The male genitalia and terminal gastral segments of two species of the primitive ant genus *Myrmecia* (Hymenoptera: Formicidae). Journal of the New York Entomological Society 75: 35–42.

Forbes J, Do-Van-Quy D (1965) The anatomy and histology of the male reproductive system of the legionary ant, *Neivamyrmex harrisi* (Haldeman) (Hymenoptera: Formicidae). Journal of the New York Entomological Society 73: 95–111.

Francoeur A, Loiselle R (1988) The male of *Leptothorax wilda* with notes on the subgenus *Nesomyrmex* (Formicidae, Hymenoptera). In: Arnett D (Ed.) Advances in Myrmecology. Brill, New York, 43–54.

Gorb SN (2008) Biological attachment devices: exploring nature's diversity for biomimetics. Philosophical transactions: Material, Physical and Engineering Sciences 336: 1557–1574. doi: 10.1098/rsta.2007.2172

Hölldobler B, Engel-Siegel H (1982) Tergal and sternal glands in male ants. Psyche 89: 113–132.

Hölldobler B, Wilson EO (1990) The Ants. The Harvard University Press, Oxford, 732 pp.

Hughes WOH, Boomsma JJ (2007) Genetic polymorphism in leaf-cutting ants is phenotypically plastic. Proceedings of the Royal Society B 274: 1625–1630. doi: 10.1098/rspb.2007.0347

Kamimura Y (2007) Twin intromittant organs of *Drosophila* for traumatic insemination. Biology Letters 3: 401–404. doi: 10.1098/rsbl.2007.0192

Kamimura Y (2008) Copulatory wounds in the monandrous ant species *Formica japonica* (Hymenoptera, Formicidae). Insectes Sociaux 55: 51–53. doi: 10.1007/s00040-007-0968-z

Keller RA (2011) A phylogenetic analysis of ant morphology (Hymenoptera: Formicidae) with special reference to the Poneromorph subfamilies. Bulletin of the American Museum of Natural History 335: 1–90. doi: 10.1206/355.1

Kumashiro M, Sakai M (2001) Reproductive behaviour in the male cricket *Gryllus bimaculatus* DeGeer. The Journal of Experimental Biology 204: 1123–1137.

LaPolla JS (2004) *Acropyga* (Hymenoptera: Formicidae) of the world. Contributions of the American Entomological Institute 33: 1–130. doi: 10.1111/j.1365-3113.2011.00605.x

LaPolla JS, Kallal RJ, Brady SG (2012) A new ant genus from the Greater Antilles and Central America, *Zatania* (Hymenoptera: Formicidae) exemplified the utility of male and molecular systems. Systematic Entomology 37: 200–214.

Ogata K (1987) A generic synopsis of the Poneroid complex of the family Formicidae in Japan (Hymenoptera). Part I. Subfamilies Ponerinae and Cerapachyinae. Esakia 25: 97–132.

Ogata K (1991) A generic synopsis of the Poneroid complex of the family Formicidae in Japan (Hymenoptera). Part II. Subfamily Myrmicinae. Bulletin of the Institute of Tropical Agriculture Kyushu University 14: 61–149.

Pesenko YA, Astafurova YV (2006) Contributions to the halictid fauna of the eastern Palearctic region: subfamily Rophitinae (Hymenoptera: Halictidae). Entomofauna Zeitschrift für Entomologie 27: 317–356.

Rivers IL (1948) A new species of *Pelocoris* from Nevada, with notes on the genus in the United States (Hemiptera: Naucoridae). Annals of the Entomological Society of America 41: 371–376.

Robertson HG, Villet M (1989) Mating behaviour in three species of myrmicine ants (Hymenoptera: Formicidae). Journal of Natural History 23: 767–773. doi: 10.1080/00222938900770401

Robinson GE, Page Jr RE (1989) Genetic determination of nectar foraging, pollen foraging, and nest-site scouting in honey bee colonies. Behavioral Ecology and Sociobiology 24: 317–323. doi: 10.1007/BF00290908

Ross KG (1982) Laboratory studies of the mating biology of the Eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Formicidae). Journal of the Kansas Entomological Society 56: 523–537.

Schulmeister S (2001) Functional morphology of the male genitalia and copulation in lower Hymenoptera, with special emphasis on the Tenthredinoidea s. str. (Insecta, Hymenoptera, 'Symphyta'). Acta Zoologica 82: 331–349. doi: 10.1046/j.1463-6395.2001.00094.x

Schulmeister S (2003) Genitalia and terminal abdominal segments of male basal Hymenoptera (Insecta): morphology and evolution. Organisms Diversity and Evolution 3: 253–279. doi: 10.1078/1439-6092-00078

Serna F, Mackay W (2010) A descriptive morphology of the ant genus *Procryptocerus* (Hymenoptera: Formicidae). Journal of Insect Science 10: 1–36. doi: 10.1673/031.010.11101

Sharkey MJ (2007) Phylogeny and classification of Hymenoptera. Zootaxa 1668: 1–766.

Smith MR (1942) The legionary ants of the United States belonging to *Eciton* subgenus *Neivamyrmex* Borgmeier. American Midland Naturalist 27: 537–590. doi: 10.2307/2420913

Sueur J, Mackie D, Windmill JFC (2011) So small, so loud: extremely high sound pressure level from a pygmy aquatic insect (Corixidae, Micronectinae). PLoS ONE 6: e21089. doi: 10.1371/journal.pone.0021089

Snodgrass RE (1935) Principles of Insect Morphology. Comstock Publishing Associates, London, 667 pp.

Song H, Bucheli SR (2010) Comparison of phylogenetic signal between male genitalia and non-genital characters in insect systematics. Cladistics 26: 23–35. doi: 10.1111/j.1096-0031.2009.00273.x

Taylor RW (1965) A monographic revision of the rare tropicopolitan ant genus *Probolomyrmex* Mayr (Hymenoptera: Formicidae). Transactions of the Royal Entomological Society of London 117: 345–365. doi: 10.1111/j.1365-2311.1965.tb00044.x

Tuxen SL (1970) Taxonomist's glossary of genitalia in insects. Munksgaard, Copenhagen, 359 pp.

Villet MH (1999) Reproductive behaviour of *Plectroctena mandibularis* F. Smith (Hymenoptera: Formicidae), a ponerine ant with ergatoid queens. African Entomology 7: 289–291.

Walker Jr WF, Liem KF (1994) Functional anatomy of the vertebrates: an evolutionary perspective, 2nd ed. Saunders College Publishing, Fort Worth, 778 pp.

Ward PS (2001) Taxonomy, phylogeny and biogeography of the ant genus *Tetraponera* (Hymenoptera: Formicidae) in the Oriental and Australian regions. Invertebrate Taxonomy 15: 589–665. doi: 10.1071/IT01001

Ward PS (2011) Integrating molecular phylogenetic results into ant taxonomy (Hymenoptera: Formicidae). Myrmecological News 15: 21–29.

Watkins II JF (1982) The army ants of Mexico (Hymenoptera: Formicidae: Ecitoninae). Journal of the Kansas Entomological Society 55: 197–247.

Yoder MJ, Mikó I, Seltmann KC, Bertone MA, Deans AR (2010) A gross anatomy ontology for Hymenoptera. PLoS ONE 5: e15991. doi: 10.1371/journal.pone.0015991

Yoshimura M, Fisher BL (2009) A revision of male ants of the Malagasy region (Hymenoptera: Formicidae): Key to genera of the subfamily Proceratiinae. Zootaxa 2216: 1–21.

Yoshimura M, Fisher BL (2011) A revision of male ants of the Malagasy region (Hymenoptera: Formicidae): key to genera of the subfamily Dolichoderinae. Zootaxa 2794: 1–34.

Yoshimura M, Fisher BL (2012) A revision of male ants of the Malagasy Amblyoponinae (Hymenoptera: Formicidae) with resurrections of the genera *Stigmatomma* and *Xymmer*. PLoS ONE 7: e33325. doi: 10.1371/journal.pone.0033325

Yoshimura M, Onoyama K (2002) Male-based keys to the subfamilies and genera of Japanese ants (Hymenoptera: Formicidae). Entomological Science 5: 421–443**.**

RESEARCH ARTICLE

Three cryptic species in *Asecodes* (Förster) (Hymenoptera, Eulophidae) parasitizing larvae of *Galerucella* spp. (Coleoptera, Chrysomelidae), including a new species

Christer Hansson[1,†], Peter A. Hambäck[2,‡]

1 *Zoological Museum, Department of Biology, Sölvegatan 37, SE-22362 Lund, Sweden* **2** *Department of Botany, Stockholm University, SE-106 91 Stockholm, Sweden*

† *urn:lsid:zoobank.org:author:EC91EABD-7115-4B05-BC80-9195C86FA55D*
‡ *urn:lsid:zoobank.org:author:C1A8AB9C-3814-459F-AE78-3981CCFA417C*

Corresponding author: *Christer Hansson* (Christer.Hansson@biol.lu.se)

Abstract

Three morphologically very similar species of *Asecodes* Förster (Hymenoptera: Eulophidae) are reviewed. *Asecodes parviclava* (Thomson) is removed from synonymy under *A. lucens* **stat. rev.**, and differentiated from *A. lucens* (Nees) and *A. lineophagum* **sp. n.** All three species develop as gregarious endoparasitoids in larvae of *Galerucella* spp. (Coleoptera: Chrysomelidae), but each species has its own unique host range. *Asecodes lineophagum* attacks only *Galerucella lineola* (Fabr.) and *A. lucens* only *G. sagittariae* (Gyllenhal), whereas *A. parviclava* parasitizes *G. tenella* (L.), *G. calmariensis* (L.) and *G. pusilla* (Duftschmid). The *Asecodes* species are similar but display small though distinct morphological differences, and are distinguished also through molecular differences. The genetic distance in mitochondrial CO1 ranged from 2.3% to 7.3% between the species. Five names, one valid and four synonyms, were available for this group of species, but none of them was linked to a primary type. To promote stability of nomenclature, primary types are designated for all five names, neotypes for *Eulophus lucens* Nees, *Entedon mento* Walker and *Derostenus parviclava* Thomson, and lectotypes for *Entedon chthonia* Walker and *Entedon metagenes* Walker. *Entedon mento, E. chthonia* and *E. metagenes* remain synonymized under *A. lucens*.

Introduction

Species of *Asecodes* Förster are described in the literature as parasitoids of chrysomelid beetles (Askew and Viggiani 1978, Kamijo 1986, Schauff 1991). *Asecodes lucens* (Nees) (as *A. mento* (Walker)), was reared from *Galerucella nymphaeae* (L.) (Hippa and Koponen 1984) and *Lochmaea suturalis* (Thomson) (Golightly 1962) (Coleoptera: Chrysomelidae). In an unpublished study in Sweden the junior author and associates also reared *A. lucens* from five additional species of *Galerucella*, *G. calmariensis* (L.), *G. lineola* (Fabr.), *G. pusilla* (Duftschmid), *G. sagittariae* (Gyllenhal) and *G. tenella* (L.). However, parasitism rates differed considerably in different *Galerucella* species collected from the same locality. These patterns caused us to wonder whether a single species, *A. lucens*, was using multiple hosts or if there were populational differences in host range. To examine the possibility that population differentiation occurs within *A. lucens* based on host use we sequenced both mitochondrial and nuclear genes (Hambäck et al. unpubl data). This analysis indicated the occurrence of multiple species.

In this paper, we identify morphological traits that separate three species of *Asecodes,* corresponding also to molecular and biological data. We compared the morphotypes with previous descriptions and found that two correspond to described species, *A. lucens* (Nees) and *A. parviclava* (Thomson), whereas the third was not described. The new species is described under the name *A. lineophagum*.

Material and methods

The colour photos were made with a Nikon SMZ 1000 stereomicroscope and a Nikon DS-5M camera. To eliminate reflections from the metallic and shiny body, a dome-light manufactured as described by Kerr et al. (2008), was used as the light source for photography. Photos were taken at different focus levels and Helicon Focus Pro version 4.75 was used to merge them into a single image. The photography of wing interference patterns is described in detail by Shevtsova and Hansson (2011). The SEM photos were made from uncoated specimens on their original cardboard mounts. These were taken in low vacuum mode on a JEOL˚ JSM 5600LV SEM microscope.

Morphological terms follow Gibson (1997). For illustrations of the morphological terms see www.neotropicaleulophidae.com.

The genetic distances were estimated from pairwise comparisons of 784 base pairs corresponding to the three prime end of CO1 (as used in Hambäck et al., unpublished). In the analysis, 7 individuals of *A. lucens*, 23 individuals of *A. parviclava* and 3 individuals of *A. lineophagum* were used. The analysis was done in PAUP* ver. 4.0.a125 (Swofford 2002), and genetic distances were calculated under the Kimura-2-parameter

model (K2P) with pairwise deletion of missing data. Additional mitochondrial and nuclear genes data were used for reconstructing the phylogeny (Hambäck et al., unpublished), but were not included in the estimate of genetic distances due to a less extensive data set than for CO1.

Museum acronyms

BMNH   the Natural History Museum, London, United Kingdom (N. Dale-Skey Papilloud)
CH   private collection of Christer Hansson
GNM   the Natural History Museum, Gothenburg, Sweden (C.G. Jonsson)
LUZM   Lund University Zoology Museum, Sweden (R. Danielsson)
NHRS   the Natural History Museum, Stockholm, Sweden (H. Vårdal)
ZSM   Zoologische Staatssammlung, München, Germany (S. Schmidt)

Designation of primary types

Five names were available for this complex of species prior to this investigation, with one name, *A. lucens*, considered as valid and four other names as synonyms under the latter name. However, none of the names were fixed to a primary type and three of the names lacked type material altogether. For nomenclatural stability names must be fixed to a type specimen, and we therefore designate primary types, neotypes or lectotypes, for all five available names.

Neotypes

Eulophus lucens was described by Nees from a single female caught on *Robinia pseudoacacia* in Sickershausen [Bavaria, Germany] June 22, 1812, which was placed in his own collection. Specimens in the collection of Nees no longer exist apart from specimens sent to Westwood, now in the Oxford University Museum of Natural History (Graham 1988). No material of *E. lucens* exists in the Oxford collection (Graham 1988) and it is thus safe to assume that the holotype of *E. lucens* was destroyed. A female from Hungary, Vas County, Szakonyfalu, collected 23.vi.1960, agrees well with the description of *E. lucens* and is here designated as neotype. The locality in Hungary is the closest to the type locality where it has been possible to find material of this species.

    Entedon mento was described by Walker from an unspecified number of males from near London and from Belfast. There is no material that agrees with the original description, either in the general collection or in the type collection of the Natural History Museum, London, where the material Walker based his descriptions is kept. In the type collection, the box supposed to contain some type material of *E. mento* is emp-

ty, but with a note "mento??". Thus, it appears the material on which Walker based his description of *E. mento* is now lost. A female from England, Middlesex, Southgate, collected 6.vi.1972, fits the description of *E. mento* and is here designated as neotype for *Entedon mento*. The species was allegedly described from males, but males and females of this species are very similar. Further, Walker frequently misidentified the sex (Graham 1963), and a female is selected for neotype to make this species comparable to the other species of this complex, all of which are represented by females.

Derostenus parviclava was described by Thomson from an unspecified number of females he collected on Öland, an island in the Baltic Sea, and by G.F. Möller in Holmeja, a locality in Skåne, the southernmost province in Sweden. The collection of C.G. Thomson is in the Lund museum, and the collection of G.F. Möller is in the Natural History Museum in Gothenburg, both in Sweden. However, neither collection has any specimens from the type localities of *D. parviclava*. There is a female under the name *D. parviclava* in the G.F. Möller collection from Bökeberg (labeled "Bök"), which is a locality very close to Holmeja, one of the type localities. Because Thomson was very specific concerning localities from the province Skåne, where he lived and worked, it seems unlikely that he interchanged "Holmeja" with "Bökeberg". However, the female from Bökeberg agrees well with the original description of *D. parviclava* and it is from a locality very close to one of the original type localities. This specimen fulfills the criteria for a neotype for *Derostenus parviclava* and is designated as such here.

Lectotypes

The descriptions of *Entedon chthonia* and *E. metagenes* do not have information on the number of specimens used, and neither has been fixed to a primary type. The type collection of the Natural History Museum, London, has a specimen each of *E. chthonia* (type no. 5.2603) and *E. metagenes* (type no. 5.2604). These specimens fit the original descriptions and are here designated as lectotypes.

Biology of the parasitoids

The *Asecodes* species included here are gregarious koinobiont endoparasitoids of beetle larvae (Stenberg and Hambäck 2010). Females lay their eggs in the early larval stage and successful parasitoid development leads to a mummification of the host larva. The host larval mummies are black and morphologically resemble larvae, whereas unparasitized larvae form soft yellow pupae typical of chrysomelid beetles (photos in Hambäck 2004). After successful development the parasitoid larvae pupate inside the mummified host larva. The number of parasitoid pupae in the mummies is highly variable, from 1–14 within one mummy. The emergence of adult parasitoids typically occurs in intervals, which probably reflects separate egg-laying events and thus indicates superparasitism as a common trait. The number of parasitoids in each host affects both the sex ratio

and the adult body size of emerging offspring. Single emerging parasitoids are invariably females, but at high parasitoid densities the sex ratio is male biased (up to 80%) (Stenberg and Hambäck 2010). It also seems that density dependence in sex ratio and adult body size are correlated with host species, as indicated by a comparison of parasitoids emerging from *Galerucella tenella* and *G. calmariensis* (Stenberg and Hambäck 2010).

Parasitism rates may at times be very high, close to 100%, but may at other times be very low. In Sweden, where this study was performed, there seems to be a latitudinal shift in parasitism rates, at least for some hosts. The parasitism rates for *G. calmariensis* and *G. tenella* in northern localities, close to Umeå, are typically very high, between 50% and 100%, but less than 10% in more southern localities. The genus contains both strictly monophagous species and oligophagous species and the different *Galerucella* species often occur in the same localities, but on different wetland plants. There are often large differences in the parasitism rates between *Galerucella* species within the same locality. For instance, parasitism rates may be very high on *G. lineola* and very low on other *Galerucella* species in one locality, whereas parasitism rates are high in another species in another locality. The different parasitism rates are not likely to be due to phenological differences or spatial distributions within localities because host plants colonised by different larval species may occur on neighboring plant individuals.

Genetic analysis

Genetic distances of *A. lucens* vs *A. lineophagum* calculated from mitochondrial gene data varied between 4.8–6.0 % (mean = 5.3%), *A. lucens* and *A. parviclava* 5.3–7.3 % (mean = 6.4%) and *A. lineophagum* vs *A. parviclava* 2.3–3.8% (mean = 3.0%). Variation was estimated as 2.6% for *A. parviclava*, whereas no variation was found within *A. lucens* and *A. lineophagum* for the sampled individuals.

Identification

For identification of the species treated here the following additions can be made to the latest key to European species of *Asecodes* in Askew and Viggiani (1978).

Couplet 9, replace "mento" with "11", and include the following:

11	Forewing speculum open (Fig. 2); propodeal callus with 3–5 setae (Fig. 22). ....***A. lineophagum* sp. n.**
–	Forewing speculum closed posteriorly by costal setal line (Fig. 6); propodeal callus with 2 setae (Fig. 23)..**12**
12	Forewing bare just behind marginal vein, and relatively sparsely setose (Figs 4, 9, see also Fig. 2) ....***A. parviclava* (Thomson)**
–	Forewing setose just behind marginal vein setose, and relatively densely setose (Figs 6, 8) ....***A. lucens* (Nees)**

Species treatments

Asecodes lineophagum **sp. n.**
urn:lsid:zoobank.org:act:3B302B7C-F323-45E3-8E78-19C080201E1A
http://species-id.net/wiki/Asecodes_lineophagum
Figures 2, 3, 10, 13, 16, 17, 22, 26, 27

Diagnosis. Forewing (Fig. 2) with speculum open posteriorly (i.e. setal line absent), bare just behind marginal vein and otherwise relatively sparsely setose; propodeal callus with 3–5 setae (Fig. 22).

Description. FEMALE. Length 1.0–1.8 mm.

Antenna dark brown (Fig. 10). Frons below frontal suture metallic purple with upper-lateral corners close to eyes and frontal suture golden-green (Fig. 26), above suture golden green. Vertex metallic purple inside ocellar triangle, golden-green outside triangle. Mesoscutum black with metallic purple tinges (Fig. 16). Scutellum metallic bluish-green (Fig. 16). Axillae black with metallic purple tinges (Fig. 16). Dorsellum metallic bluish-green (Fig. 16). Propodeum metallic bluish-green (Fig. 16). Coxae, femora and tibiae dark brown to black, and shiny (as in Fig. 1); fore tarsus dark brown, mid and hind tarsi with tarsomeres 1–3 yellowish-white, tarsomere 4 dark brown. Forewing hyaline (Fig. 2), wing interference pattern as in Fig. 3. Petiole dark brown to black. Gaster with 1st tergite metallic bluish-green, remaining tergites dark brown to black with metallic purple tinges.

Antenna as in Fig. 10. Frons below frontal suture with weak reticulation, above suture smooth; antennal scrobes join on frontal suture. Vertex with very weak reticulation inside ocellar triangle, smooth outside triangle.

Mesoscutum with weak reticulation (Fig. 17). Scutellum with very weak reticulation in anterior 2/3 (Fig. 17), posterior 1/3 smooth. Axillae with weak reticulation (Fig. 17). Dorsellum slightly convex and smooth (Fig. 17). Propodeum with a wide groove along anterior margin (Fig. 17), with weak reticulation; propodeal callus with 3–5 setae. Forewing (Fig. 2) bare just behind marginal vein, speculum open, setation relatively sparse, and with 5–9 admarginal setae.

Petiole as a short, transverse, narrow stripe. Gaster circular.

Ratios. Height of eye/malar space/width of mouth = 2.6/1.0/1.8; shortest distance between posterior ocelli/posterior ocellus and eye/posterior ocellus and occipital margin = 10.4/5.4/1.0; width of head/width of mesosoma = 1.1; length of forewing/length of marginal vein/height of forewing = 2.4/1.0/1.0; length of postmarginal vein/length of stigmal vein= 0.6; length of mesosoma/length of gaster = 1.0.

MALE. Length 0.9–1.4 mm.

Very similar to female except antenna (Fig. 13) with scape wider, flagellomeres longer and more slender, and apical two flagellomeres distinctly separated.

Ratios. Height of eye/malar space/width of mouth = 2.1/1.0/1.6; length of mesosoma/length of gaster = 1.0–1.2.

Hosts and sex ratio. All Swedish specimens were reared from *Galerucella lineola* (Coleoptera: Chrysomelidae) on *Salix* spp., mainly *S. cinerea*. The sex ratio of each clutch on average is closer to one than for *A. lucens*, and the standard deviation is distinctly higher in *A. lineophagum*. Ratio female/male (n = 48): 3.10±2.68/2.27±2.17

Material examined. HOLOTYPE female (BMNH) labelled "SWEDEN: Uppland, Ludden, 59°46'18"N, 18°40'19"E, 21.vi.2011, ex *Galerucella lineola* on *Salix cinerea*". PARATYPES. 45♀ 49♂(BMNH, CH, LUZM, NHRS, ZSM): 25♀ 19♂with same label data as holotype; 3♀ 1♂"Sweden: Uppland, Fläktan, 59°46'54"N, 17°44'30"E, 24.vii.2011, ex *Galerucella lineola* on *Salix cinerea*."; 3♀ 5♂"SWEDEN: Uppland, Liljekonvaljholmen, 59°48'18''N, 17°39'51''E, 18.vi.2011, ex *Galerucella lineola* on *Salix* sp."; 6♀ 4♂"SWEDEN: Uppland, Mörtsjön, 59°38'39'' N 18°09'58'' E, 5.vii.2011, ex *Galerucella lineola* on *Salix cinerea*"; 1♂"SWEDEN: Uppland, Sundängen, 59°33.954'N, 16°51.292'E, 25.vi.2011, ex *Galerucella lineola* on *Salix cinerea*"; 3♀ 6♂"SWEDEN: Uppland, Haknäs, 59°43'05"N, 17°41'57"E, 25.vi.2011, ex *Galerucella lineola* on Salix *cinerea*"; 1♀ "SWEDEN: Skåne, Skäralid, 6–17.viii.1994, M . Sporrong"; 3♀ (on two pins) labelled "Småland" [which is: SWEDEN: Småland, without further information]; 1♀ 1♂"SWEDEN: Skåne, Sövde, 5.vii.1985, C. Hansson"; 1♂"SWEDEN: Småland, Hyltebruk, 7–14.ix.1986, J. Ardö"; 1♂"NORWAY: Jostedalen, Gupne, 19.vii.1979, Hull University Expedition".

Asecodes lucens (Nees)
http://species-id.net/wiki/Asecodes_lucens
Figures 6–8, 12, 15, 20, 21, 23, 24

Eulophus lucens Nees, 1834: 175. Neotype female in ZSM, designated here.
Entedon mento Walker, 1839: 28. Neotype female in BMNH, designated here. Synonymized by Graham (1993: 227).
Entedon chthonia Walker, 1839: 122. Lectotype female in BMNH, designated here. Synonymized by Graham (1993: 227).
Entedon metagenes Walker, 1848: 230. Lectotype female in BMNH, designated here. Synonymized by Graham (1993: 227).
Asecodes lucens (Nees), Graham (1993: 227).

Diagnosis. Forewing (Figs 6, 8) with speculum closed posteriorly by a setal line, setose just behind marginal vein and otherwise relatively densely setose; propodeal callus with 2 setae (Fig. 23).

Hosts and sex ratio. All Swedish specimens were reared from *Galerucella sagittareae* (Coleoptera: Chrysomelidae) on *Lysimachia thyrsiflora, L. vulgaris*, and *Potentilla palustris*. The sex ratio of each clutch is female biased. Ratio female/male (n = 48): 4.27±1.83/0.92±0.94.

Material examined. TYPE MATERIAL: Neotypes of *E. lucens* (ZSM) and *E. mento* (BMNH), lectotypes of *E. chthonia* (BMNH) and *E. metagenes*

(BMNH), all types are females. ADDITIONAL MATERIAL: DENMARK: 4♀ 2♂(CH, LUZM). HUNGARY: Vas Co. 5♀ 2♂(ZSM). SWEDEN: Skåne 106♀ 13♂(swept) (BMNH, CH, LUZM); Uppland 205♀ 44♂(91♀ 25♂from *Lysimachia*, 114♀ 19♂from *Potentilla palustris*) (BMNH, CH, NHRS, ZSM); Öland 1♂(swept) (CH).

Asecodes parviclava (Thomson), stat. rev.
Figures 1, 4, 5, 9, 11, 14, 18, 19, 25

Derostenus parviclava Thomson, 1878:272–273. Neotype female in GNM, designated here.
Asecodes parviclava (Thomson), Bouček and Askew (1968: 131).
Synonym of *Asecodes lucens* (Nees) (Graham 1993: 227).

Diagnosis. Forewing (Figs 4, 9) with speculum closed posteriorly by a setal line, bare just behind marginal vein and otherwise relatively sparsely setatose; propodeal callus with 2 setae (as in Fig. 23).

Hosts and sex ratio. The Swedish specimens were reared from *Galerucella calmariensis and G. pusilla* (Coleoptera: Chrysomelidae) on *Lythrum salicaria*, and *G. tenella* on *Filipendula ulmaria*. The number of samples is smaller than for the other two species (n = 23, 10 from *G. calmariensis*, 6 from *G. pusilla*, 7 from *G. tenella*). Ratio female/male: 2.09±1.16/0.91±0.95.

Material examined. TYPE MATERIAL: Neotype female of *D. parviclava* (GNM). ADDITIONAL MATERIAL: HUNGARY: Vas Co. 1♀ (BMNH); SWEDEN: Skåne 21♀ 16♂(CH, LUZM); Uppland 48♀ 21♂(24♀ 8♂from *G. calmariensis*, 10♀ 8♂from *G. pusilla*, 14♀ 5♂from *G. tenella*) (BMNH, CH, NHRS); Västergötland 14♀ 5♂(CH, LUZM).

Discussion

The separation of *A. lucens* into three species based on molecular and morphological evidence is supported by biological data. *Asecodes lucens* and *A. lineophagum* were reared from only one host species, *Galerucella sagittariae* and *G. lineola*, respectively, whereas *A. parviclava* was reared from three host species, *G. tenella*, *G. calmariensis* and *G. pusilla*. These observations have also been confirmed with independent observations of parasitoid behaviour in the laboratory, where females were found to attack the respective host species, but not other species (L. Fors, unpubl. data). The delimitation of three species is also supported by observations in the field, where one species of *Galerucella* larvae may be heavily parasitized and another is not attacked in the same locality. Such parasitism patterns have, however, not been observed for *G. tenella*, *G. calmariensis* and *G. pusilla*. In fact, earlier studies show

Figure 1. *Asecodes parviclava* (Thomson), female habitus (length = 1.5 mm).

strong correlations in parasitism rates between *G. tenella* and *G. calmariensis* among localities (Hambäck et al. 2006). Moreover, field observations suggested that parasitism rates on *G. tenella* were consistently higher when this species was sympatric with *G. calmariensis*. The reason for this pattern was not resolved (Hambäck et al. 2006), but the pattern suggest that the parasitoid population may mediate indirect interactions between its hosts, as is known for other host-parasitoid systems. The current information on the species delimitation within *Asecodes* was important to identify pairs of host species where such effects would be likely. Based on previous information, we could have expected similar indirect effects also for other species pairs but the novel information on population differentiation among parasitoid individuals suggest this not to be the case.
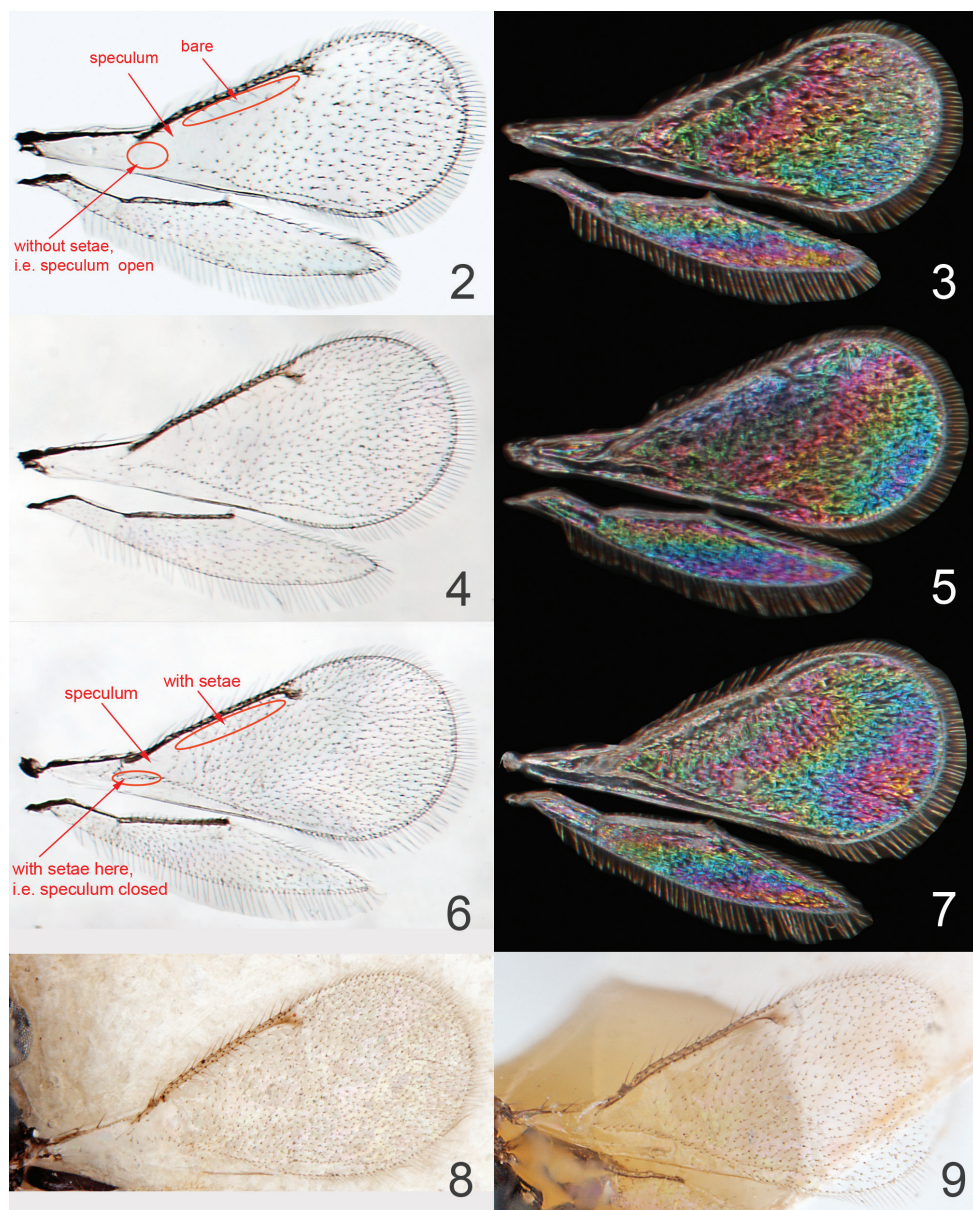
Figures 2–9. *Asecodes* spp.,wings, females **2–3** *A. lineophagum* sp. n. **2** transparent wings **3** wing interference pattern **4–5** *A. parviclava* (Thomson) **4** transparent wings **5** wing interference pattern **6–7** *A. lucens* (Nees) **6** transparent wings **7** wing interference pattern **8** *A. lucens*, forewing, neotype **9** *A. parviclava*, forewing, neotype.

In view of our findings, the previous host records of *Galerucella nymphaeae* and *Lochmaea suturalis* for *A. lucens* need confirmation. Investigation of *Asecodes* specimens reared from these hosts might quite possibly reveal additional cryptic species in this group.

Figures 10–15. *Asecodes* spp., antennae **10–12** females **10** *A. lineophagum* sp. n. **11** *A. parviclava* (Thomson) **12** *A. lucens* (Nees) **13–15** males **13** *A. lineophagum* **14** *A. parviclava* **15** *A. lucens.*
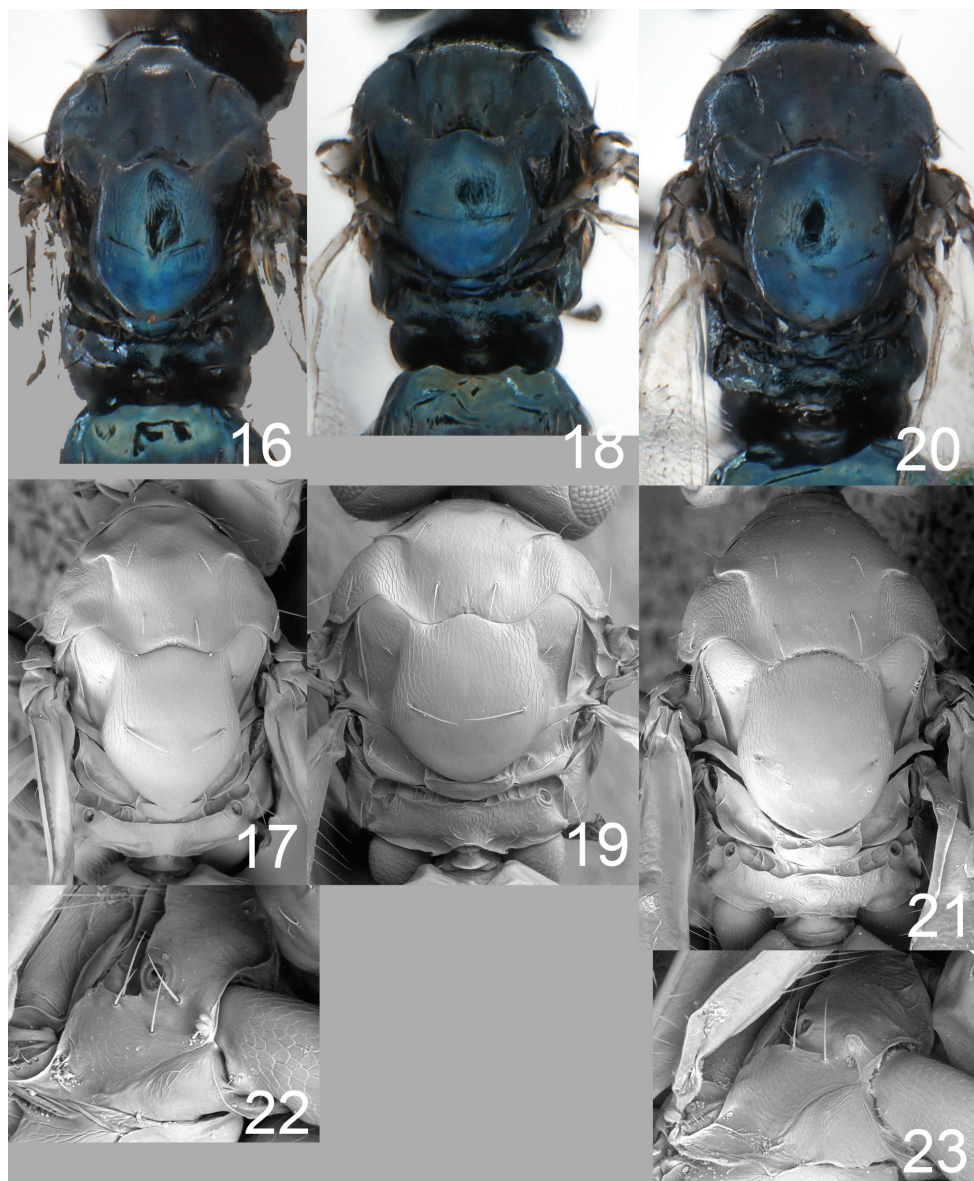
Figures 16–23. *Asecodes* spp. **16–21** thoracic dorsum, females **16–17** *A. lineophagum* sp. n. **18–19** *A. parviclava* (Thomson) **20–21** *A. lucens* (Nees) **22–23** lateral propodeum in side view (anterior part to the left), female **22** *A. lineophagum* **23** *A. lucens*.

Figures 24–27. 24–25 *Asecodes* spp., neotypes, females **24** *A. lucens* (Nees) **25** *A. parviclava* (Thomson) **26–27** *A. lineophagum* sp. n., head in frontal view **26** female **27** male.

Acknowledgements

References

Askew RR, Viggiani G (1978) Two new species of *Asecodes* (Foerster) (Hym., Eulophidae) parasitic upon *Galeruca* spp. (Col.,Chrysomelidae) in Italy. Bollettino del Laboratorio di Entomologia Agraria Filippo Silvestri 35: 43–58.

Bouček Z, Askew RR (1968) Palearctic Eulophidae, excl. Tetrastichinae. Index of Entomophagous insects. Le Francois, Paris, 254 pp.

Gibson GAP (1997) Morphology and terminology. In: Gibson GAP, Huber JT, Woolley JB (Eds.) Annotated Keys to the Genera of Nearctic Chalcidoidea (Hymenoptera). National Research Council Research Press, Ottawa, 16–44.

Golightly WH (1962) Biological control of *Lochmaea suturalis* (Thomson) (Col., Chrysomelidae). Entomologist's Monthly Magazine 98: 1–196.

Graham MWRdeV (1959) Keys to the British genera and species of Elachertinae, Eulophinae and Euderinae (Hym., Chalcidoidea). Transactions of the Society for British Entomology 13: 169–204.

Graham MWRdeV (1963) Additions and corrections to the British list of Eulophidae (Hym., Chalcidoidea), with descriptions of some new species. Transactions of the Society for British Entomology 15: 167–275.

Graham MWRdeV (1988) The remains of Nees von Esenbeck's collection of Hymenoptera in the University Museum, Oxford. Entomologist's Monthly Magazine 124: 19–35.

Graham MWRdeV (1993) The identity of some species of Chalcidoidea (Hym.) described by Nees von Esenbeck (1834), with new synonymy. Entomologist's Monthly Magazine 129: 221–230.

Hambäck PA (2004) Varför fackelblomster i porsbuskar undgår skalbaggsangrepp. Entomologisk Tidskrift 125: 93–102. [in Swedish: "Why purple loosestrife in sweet gale shrubs are less attacked by herbivorous beetles"]

Hambäck PA, Stenberg JA, Ericson, L (2006) Asymmetric indirect interactions mediated by a shared parasitoid: connecting species traits and local distribution patterns for two chrysomelid beetles. Oecologia 148: 475–481. doi: 10.1007/s00442-006-0387-2

Hippa H, Koponen S (1984) Parasitism of larvae of Galerucini (Coleoptera, Chrysomelidae) by larvae of *Asecodes mento* (Hymenoptera, Eulophidae). Annales Universitasis Turku, Series A II 68: 63–66.

Kamijo K (1986) Two new species of *Asecodes* (Hymenoptera, Eulophidae) from Japan. Kontyû 54: 596–599.

Kerr PH, Fisher EM, Buffington M (2008) Dome lighting for insect imaging under a microscope. American Entomologist 54: 198–200.

Nees ab Esenbeck CG (1834) Hymenopterorum Ichneumonibus affinium monographiae, genera europaea et species illustrantes. Stuttgart et Tubingae, 448 pp.

Schauff ME (1991) The Holarctic genera of Entedoninae (Hymenoptera: Eulophidae). Contributions of the American Entomological Institute 26(4): 1–109.

Shevtsova E, Hansson C (2011) Species recognition through wing interference patterns (WIPs) in *Achrysocharoides* Girault (Hymenoptera: Eulophidae) including two new species. Zookeys 154: 9–30. doi: 10.3897/zookeys.154.2158

Stenberg JA, Hambäck PA (2010) Host species critical for offspring fitness and sex ratio for an oligophagous parasitoid: Implications for host coexistence. Bulletin of Entomological Research 100: 735–740. doi: 10.1017/S0007485310000143

Swofford DL (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Ass., Sunderland, Massachusetts.

Thomson CG (1878) Hymenoptera Scandinaviae. Lundae 5: 1–307.

Walker F (1839) Monographia Chalciditum I. London, 330 pp.

Walker F (1848) List of the specimens of Hymenopterous Insects in the collection of the British Museum. Part II Chalcidites. Additional species. London, 99–237.

RESEARCH ARTICLE

Hybrizon Fallén (Hymenoptera, Ichneumonidae, Hybrizoninae) found in Hunan (China)

Cornelis van Achterberg[1], Lan-Shao You[2], Xi-Ying Li[2]

1 *Department of Terrestrial Zoology, NCB Naturalis, Postbus 9517, 2300 RA Leiden, The Netherlands*
2 *College of Bio-Safety Science and Technology, Hunan Agriculture University, Changsha 410128, China*

Corresponding author: *Cornelis van Achterberg* (Cees.vanAchterberg@ncbnaturalis.nl)

Abstract

The species of the genus *Hybrizon* Fallén (Hymenoptera: Ichneumonidae: Hybrizoninae) from China are reviewed, with special reference to Hunan (South China). The genus *Hybrizon* and two species (*H. flavofacialis* Tobias, 1988, and *H. ghilarovi* Tobias, 1988) are reported for the first time from the Oriental region. The species known from the Palaearctic and Oriental regions are keyed.

Introduction

The small subfamily Hybrizoninae Blanchard, 1845 (= Paxylommatinae Foerster, 1862, Hybrizontinae of authors, "Hybrizonites" of Blanchard, 1845; Wharton and van Achterberg 2000) is associated with ants and most likely belongs to the family Ichneumonidae, but was often associated with Braconidae (van Achterberg 1976) or considered to be a separate family (He 1981, Tobias 1988). The group is treated as a subfamily of the family Ichneumonidae Latreille, 1802, by Rasnitsyn (1980)

and Yu and Horstmann (1997) because of the structure of the connection of the second and third metasomal tergites and the venation of the hind wing, both indicate a closer relationship with the family Ichneumonidae (Sharkey and Wahl 1987; Wahl and Sharkey 1988) than with the Braconidae. From analysis of the 28S ribosomal RNA from the genus *Hybrizon* Fallén, 1813, it may be concluded that the Hybrizoninae are at a basal position of the Ichneumonidae-lineage (Belshaw et al. 1998; Quicke et al. 2000; Belshaw and Quicke 2002), but Gillespie et al. (2005) documented the unusual structure of 28S in *Hybrizon*, which makes alignment difficult. Quicke et al. (2009) found that *Hybrizon* likely is a derived subfamily within the ophioniformes-group of the Ichneumonidae, which agrees with the derived morphology of the Hybrizoninae.

The subfamily is known only from the Holarctic region and we report for the first time two species of the genus from the Oriental part of China. There are only two reports of the genus *Hybrizon* from China (He 1981, Konishi et al. 2012) but only from Palaearctic northern China (*H. buccatus* (de Brébisson, 1825) from Jilin and Heilongjiang and *H. ghilarovi* Tobias, 1988, from Jilin). The second author collected in Hunan province two species of the genus, resulting in an enormous extension of the known distribution by 2200+ km southwards.

The biology of the Hybrizoninae has been for long time uncertain, but recently oviposition has been documented by photographing and filming two different genera (Komatsu and Konishi 2010; Gómez Durán and van Achterberg 2011). It shows that the final instar ant larva is used for oviposition when the worker ants transport the larvae outside the nest. Of one species (*H. buccatus*) we have some host records indicating that predominantly ant larvae from the subfamily Formicinae (Formicidae) are selected, but also larvae from non-Formicinae may be used (Gómez Durán and van Achterberg 2011). It is too early to conclude a lack of specialisation, because in most cases the true nature of the associations has not really been established and the host associations are largely unknown for the other species.

Material and methods

The collecting site is at the border of the Southeast Lake near Yuanjiang (N. Hunan) in the common reed (*Phragmites australis* (Cav.)) zone, with Oriental "*Lasius fuliginosus*" (= *Lasius fuyi* Radchenko, 2005; see Radchenko 2005) as possible host. The collecting in this wetland habitat along the lake was rather cumbersome and done by hand netting among the common reed.

For references to genera and species of Hybrizoninae, see Yu et al. (2009) and updates, for the East Palaearctic species, see Konishi et al. (2012) and for morphological terminology, see van Achterberg (1988). The specimens are deposited in the College of Bio-Safety Science and Technology, Hunan Agriculture University (HUNAU) at Changsha and in the NCB Naturalis collection (RMNH) at Leiden.

Systematics

Genus *Hybrizon* Fallén, 1813
Figs 1–15

Hybrizon Fallén, 1813: 19 (no species); Shenefelt 1969: 2; Marsh 1979: 313; Tobias 1988: 133–134 (key to Palaearctic species); Marsh 1988: 30–31 (key to Nearctic species); van Achterberg 1999: 17–18 (key to Palaearctic species); Gómez Durán and van Achterberg 2011: 94–99 (biology); Konishi et al. 2012: 20 (key to East Palaearctic species). Type species (by subsequent monotypy): *Hybrizon latebricola* Nees, 1834 (= *Hybrizon buccatus* (de Brébisson, 1825)).

Paxylomma de Brébisson, 1817: 66 (no species); Shenefelt, 1969: 2 (as synonym of *Hybrizon* Fallén, 1813); Marsh 1979: 313 (id.), 1988: 30 (id.); Tobias 1988: 133 (id.). Type species (by subsequent monotypy): *Paxylomma buccata* de Brébisson, 1825.

Paxyloma Stephens, 1835: 119; Shenefelt 1969: 2. Misspelling for *Paxylomma* de Brébisson, 1817.

Paxylomme Wesmael, 1835: 88; Shenefelt 1969: 2. Misspelling for *Paxylomma* de Brébisson, 1817.

Paxyllomma Curtis, 1837: 115; Shenefelt 1969: 2. Misspelling for *Paxylomma* de Brébisson, 1817.

Paxylloma Blanchard, 1840: 335; Shenefelt 1969: 2. Misspelling for *Paxylomma* de Brébisson, 1817.

Pachylomma Ratzeburg, 1848: 53; Shenefelt 1969: 2. Invalid emendation of *Paxylomma* de Brébisson, 1817.

Plancus Curtis, 1833: 188; Shenefelt 1969: 2 (as synonym of *Hybrizon* Fallén, 1813); Marsh 1979: 313 (id.), 1988: 30 (id.); Tobias 1988: 133 (id.). Type species (by monotypy): *Plancus apicalis* Curtis, 1833 [examined; = *Hybrizon buccatus* (de Brébisson, 1825)].

Eupachylomma Ashmead, 1894: 58; Shenefelt 1969: 1 (as valid genus); Marsh 1979: 313 (as synonym of *Hybrizon* Fallén, 1813), 1988: 30 (id.). Type species (by original designation): *Wesmaelia rileyi* Ashmead, 1889.

Species occurring in China

### *Hybrizon buccatus* (de Brébisson, 1825)
http://species-id.net/wiki/Hybrizon_buccatus
Figs 9–13

Material. Reported from North China by He (1981: Heilongjiang, Jilin) and by Konishi et al. (2012: Jilin). Unknown from Oriental China.

Diagnosis. Basal cell of fore wing largely glabrous, with at most 15 setae (Fig. 10); scapus somewhat smaller than pedicellus (Fig. 11); third antennal segment comparatively
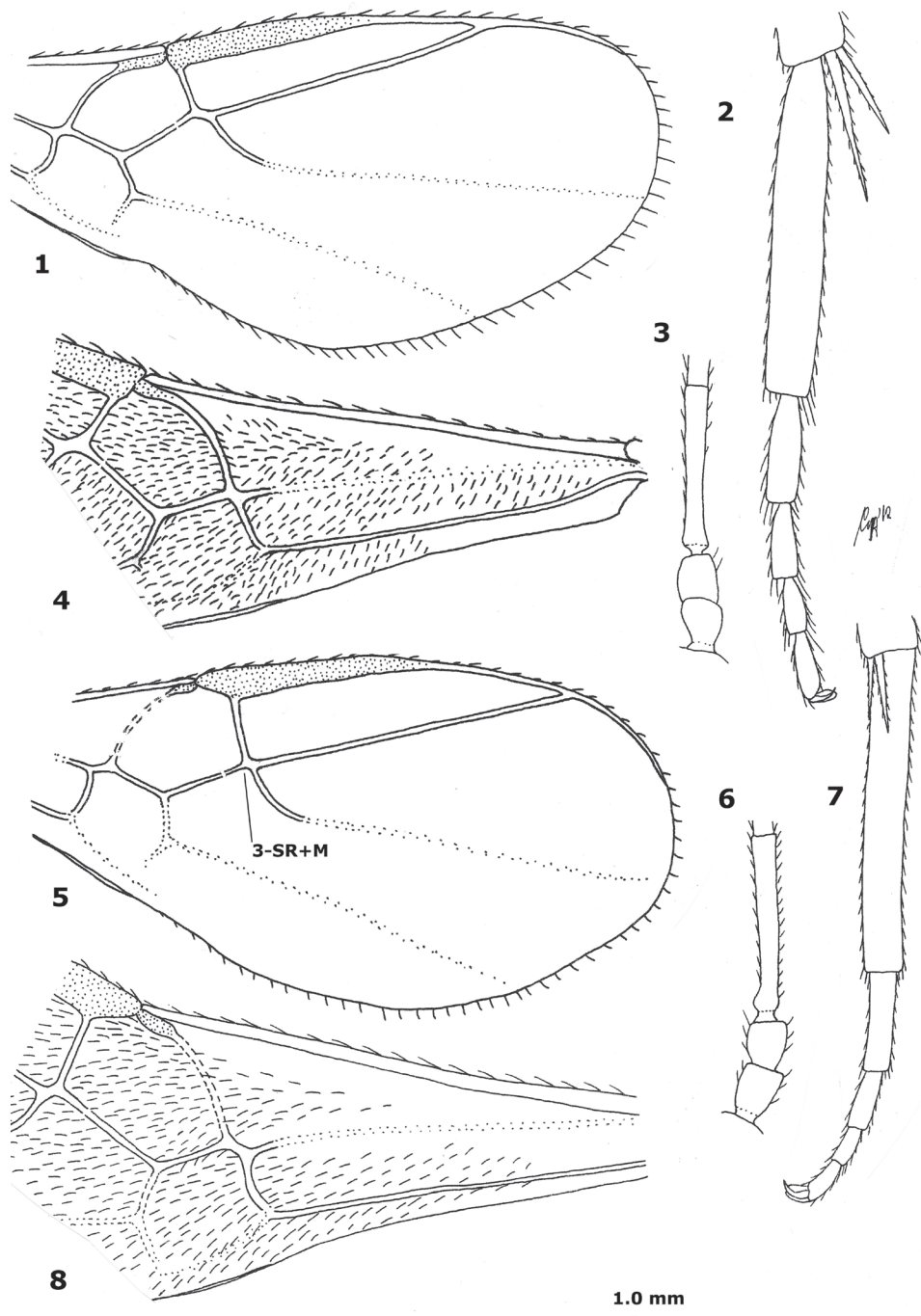
Figures 1–8. 1–4 *Hybrizon flavofacialis* Tobias, female, China, Hunan, Yuanjiang 5–8 *H. ghilarovi* Tobias, female, China, Hunan, Yuanjiang **1, 4** apical half of fore wing **2, 6** three basal antennal segments **3, 7** hind basitarsus lateral **4, 8** basal half of fore wing. **1** scale-line (= 1.0×); **2**=1.7×; **3, 6–8**=1.4×; **4, 5** =1.1×.

stout (Fig. 11); ventral half of face and scutellum largely smooth; maximum width of face 1.4–1.5 times its minimum width; eyes glabrous; mesoscutum with pair of bands of distinct punctures, rarely punctures absent or obsolescent; scutellum (except sometimes laterally) and notaulic area of mesoscutum usually dark brown; propodeum largely smooth or granulate, except for medial carinae and posteriorly with weak or obsolescent curved carinae; vein 1-M of fore wing distinctly curved anteriorly (Figs 9, 10); vein r of fore wing issued comparatively close to base of pterostigma (Fig. 9); vein 1-M of fore wing paler than vein 2-CU1 of fore wing; in lateral view length of hind basitarsus 4–5 times its maximum width (Figs 12, 13); ventral half of metapleuron coriaceous; sparsely setose part of ovipositor sheath 0.2–0.3 times as long as second tergite; length of fore wing 2–3 mm.

Hybrizon flavofacialis Tobias, 1988
http://species-id.net/wiki/Hybrizon_flavofacialis
Figs 1–4

Material. 3 ♀ + 22 ♂ (HUNAU, RMNH), S. China: Hunan, Yuanjiang, Southeast Lakeside, Ben-Zhu Dai, together with *Lasius "fuliginosus"* (= *L. fuyi* Radchenko): 2 ♂, 3.VI.1989; 1 ♀ + 1 ♂, 4.VI.1989; 2 ♂, 8.X.1989; 11 ♂, 9.X.1989; 1 ♀ + 7 ♂, 10.X.1989; 1 ♀, 12.X.1989.
 Diagnosis. Face yellow; eyes glabrous; pedicellus wider and slightly longer than scapus (Fig. 3) and dark brown, contrasting with yellowish scapus; third antennal segment comparatively slender (Fig. 3); maximum width of face 1.2–1.3 times its minimum width; ventral half of face and scutellum more or less granulate; distance between posterior ocelli of female about 1.5 times diameter of ocellus (about twice in male); mesoscutum antero-laterally smooth; ventral half of metapleuron rugose or densely rugulose; posteriorly propodeum with strong curved carinae (but sometimes disappearing in rugosity); basal cell of fore wing (except basally) with 50–70 setae (Fig. 4); vein r issued at base of pterostigma (Fig. 1); vein 3-SR+M of fore wing medium-sized (Fig. 1); vein 1-M of fore wing weakly and gradually curved anteriorly or straight (Fig. 4); in lateral view length of hind basitarsus 6–7 times its maximum width (Fig. 2); sparsely setose part of ovipositor sheath 0.2–0.4 times as long as second tergite.
 **Notes.** Up to now only known from the holotype from Far East Russia (Khabarovsk kray). The holotype is illustrated by Konishi et al. (2012). New for China and for the Oriental region.

Hybrizon ghilarovi Tobias, 1988
http://species-id.net/wiki/Hybrizon_ghilarovi
Figs 5–8

Material. 10 ♂ + 5 ♀ (HUNAU, RMNH), S. China: Hunan, Yuanjiang, Southeast Lakeside, together with *Lasius "fuliginosus"* (= *L. fuyi* Radchenko), Ben-Zhu Dai: 2 ♀
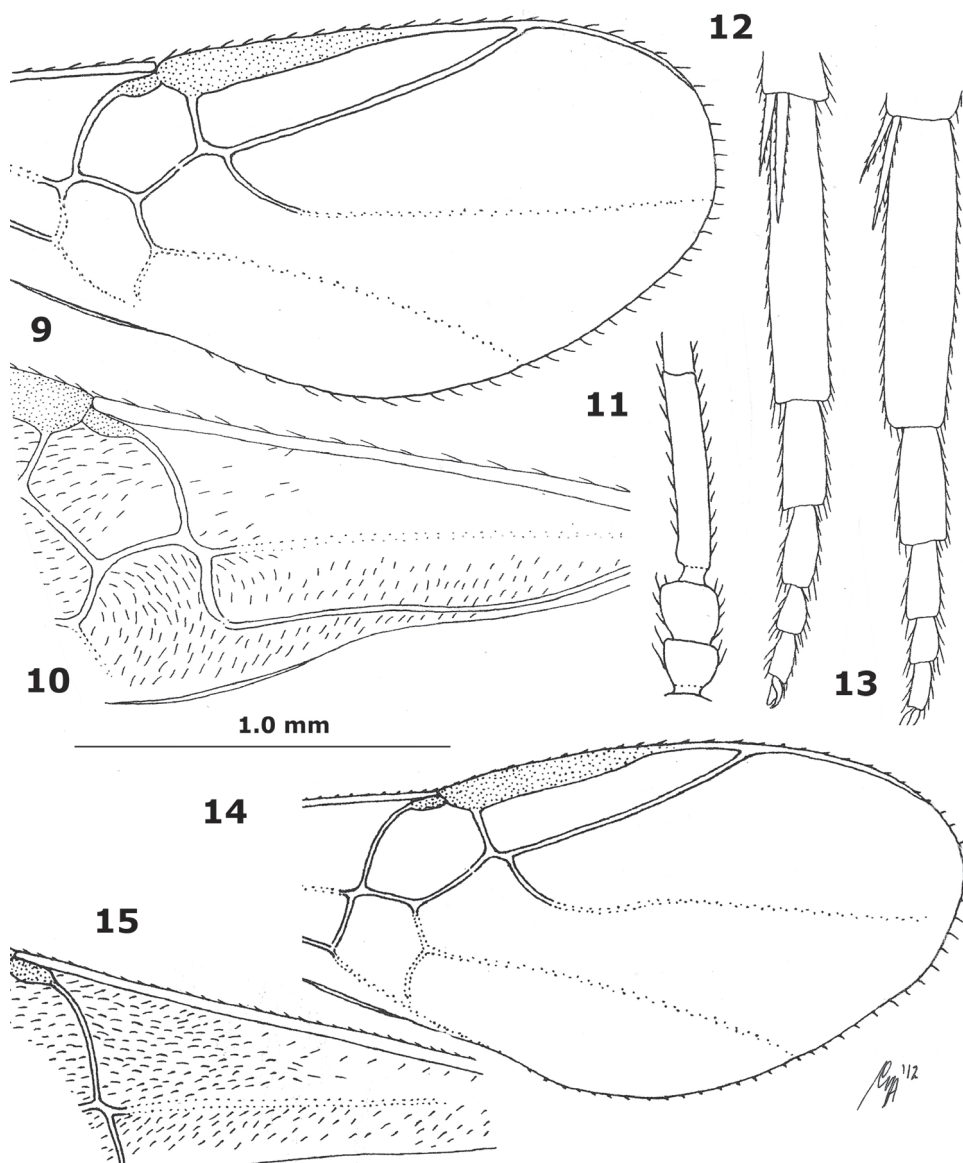
Figures 9–15. 9–13 *Hybrizon buccatus* (de Brébisson), female, Bulgaria, Brodilovo, but **13** of female from Netherlands, Nunspeet **14–15** *H. pilialatus* Tobias, female, Italy, Funes **9, 14** apical half of fore wing **10, 15** basal half of fore wing **11** three basal antennal segments **12, 13** hind basitarsus lateral. **9** scale-line (=1.0×); **10–13**=1.3×; 1**4, 15** from van Achterberg (1999).

+ 2 ♂, 10.X.1989; 1 ♂, 14.V.1989; 3 ♂, 25.V.1989, Lan-Shao You; 1 ♀, 4. VI.1989; 2 ♂, 8.X.1989; 2 ♀, 9.X.1989; 1 ♂, 11.X.1989; 1 ♂, 3.VI.1989.

Diagnosis. Eyes distinctly setose; face dark brown, except near its tentorial pits; distance between posterior ocelli of female about 1.6 times diameter of ocellus; pedicellus

about as wide as scapus and slightly shorter than scapus (Fig. 6), ventrally similarly yellowish coloured as scapus; third antennal segment comparatively slender (Fig. 6); maximum width of face 1.2–1.3 times its minimum width; ventral half of face and scutellum more or less granulate; area behind malar space flat or nearly so and rugose; scutellum granulate; propodeum areolate; ventral half of metapleuron largely rugose or rugulose; length of hind basitarsus about 7 times its maximum width (Fig. 7); mesoscutum antero-laterally rugulose; ventral half of metapleuron rugose or densely rugulose; vein r issued after base of pterostigma (Fig. 8); vein 3-SR+M of fore wing often short (Fig. 5); vein 1-M of fore wing weakly developed, straight anteriorly or nearly so (Fig. 5); basal cell of fore wing with 30–40 setae (Fig. 8); marginal cell of fore wing 4.0–5.5 times longer than its maximum width (Fig. 5); vein SR1 of fore wing straight (Oriental China) or sinuate (typical); posteriorly propodeum with strong curved carinae (but sometimes disappearing in rugosity); sparsely setose part of ovipositor sheath 0.6–0.7 times as long as second metasomal tergite.

Notes. A female paratype is illustrated by Konishi et al. (2012). Up to recently only known from Far East Russia and Bulgaria, but Konishi et al. (2012) report this species from NE China (Jilin), Korea and Japan. New for the Oriental region.

The Old World species can be separated as follows:

Key to Old World species of the genus *Hybrizon* Fallén

1	Basal cell of fore wing largely glabrous, with at most 15 setae (Fig. 10); posteriorly propodeum with weak or obsolescent curved carinae; in lateral view length of hind basitarsus 4–5 times its maximum width (Figs 12, 13); vein 1-M of fore wing distinctly curved anteriorly (Figs 9, 10); third antennal segment less slender (Fig. 11); ventral half of face and scutellum largely smooth; ventral half of metapleuron coriaceous; maximum width of face 1.4–1.5 times its minimum width..**2**
–	Basal cell of fore wing (except basally) more or less setose (Figs 4, 8, 15); posteriorly propodeum with strong curved carinae (but sometimes disappearing in rugosity); in lateral view length of hind basitarsus 6–7 times its maximum width (Figs 2, 7); vein 1-M of fore wing weakly and gradually curved anteriorly or straight (Figs 4, 8, 15); third antennal segment comparatively slender (Figs 3, 6); ventral half of face and scutellum more or less granulate; ventral half of metapleuron rugose or densely rugulose; maximum width of face 1.2–1.3 times its minimum width **3**
2	Vein r of fore wing issued comparatively far removed from base of pterostigma; mesoscutum without bands of punctures, at most with some punctures; vein 1-M of fore wing as dark as vein 2-CU1 of fore wing; scapus about as large as pedicellus; scutellum (except medio-anteriorly) and more or less notaulic area of mesoscutum ivory; length of fore wing 3.0–3.6 mm; propodeum distinctly rugose-granulate; Spain, South Korea ....**H. juncoi** (Ceballos, 1957)

–   Vein r of fore wing issued comparatively close to base of pterostigma (Fig. 9); mesoscutum with pair of bands of distinct punctures, rarely punctures largely absent or obsolescent; vein 1-M of fore wing paler than vein 2-CU1 of fore wing; scapus somewhat smaller than pedicellus (Fig. 11); scutellum (except sometimes laterally) and notaulic area of mesoscutum usually dark brown; length of fore wing 2–3 mm; propodeum largely smooth or granulate, except for medial carinae; Northwest and East Palaearctic ....
....*H. buccatus* **(de Brébisson, 1825)**

3   Eyes distinctly setose; pedicellus about as wide as scapus and about as long scapus (Fig. 6), ventrally similarly yellowish coloured as scapus; vein 1-M of fore wing straight anteriorly or nearly so (Fig. 8); sparsely setose part of ovipositor sheath 0.6–0.7 times as long as second metasomal tergite; vein 3-SR+M of fore wing often short (Fig. 5); East Palaearctic (Far East Russia); China (*Hunan, Jilin), South Korea, Japan (Hokkaido); Southeast Europe (Bulgaria) .... *H. ghilarovi* **Tobias, 1988**

–   Eyes glabrous; pedicellus wider and slightly longer than scapus and dark brown, contrasting with yellowish scapus; vein 1-M of fore wing weakly curved anteriorly (Figs 1, 14); sparsely setose part of ovipositor sheath 0.2–0.4 times as long as second tergite; vein 3-SR+M of fore wing medium-sized (Fig. 1) **4**

4.   Face yellow; vein r of fore wing issued at base of pterostigma (Fig. 1); distance between posterior ocelli of female about 1.5 times diameter of ocellus (but about twice in male); East Palaearctic (Far East Russia); *China (Hunan).....
....*H. flavofacialis* **Tobias, 1988**

–   Face dark brown, except near its tentorial pits; vein r of fore wing issued distinctly removed from base of pterostigma (Fig. 14); distance between posterior ocelli of female usually about twice diameter of ocellus; West Palaearctic .... *H. pilialatus* **Tobias, 1988**

References

Achterberg C van (1976) Hybrizontinae or Hybrizontidae? (Hymenoptera, Ichneumonoidea). Entomologische Berichten Amsterdam 36: 61–64.

Achterberg C van (1988) Revision of the subfamily Blacinae Foerster (Hymenoptera, Braconidae). Zoologische Verhandelingen Leiden 249: 1–324.

Achterberg C van (1999) The West Palaearctic species of the subfamily Paxylommatinae (Hymenoptera: Ichneumonidae), with special reference to the genus *Hybrizon* Fallén. Zoologische Mededelingen Leiden 73: 11–26.

Ashmead WH (1889) Descriptions of new Braconidae in the collection of the U.S. National Museum. Proceedings of the United States National Museum 11(1888): 611–671.

Ashmead WH (1894) Notes on the family Pachylommatoidae. Proceedings of the entomological Society of Washington 3: 55–60.

Belshaw R, Fitton M, Herniou E, Gimeno C, Quicke DLJ (1998) A phylogenetic reconstruction of the Ichneumonoidea (Hymenoptera) based on the D2 variable region of 28S ribosomal RNA. Systematic Entomology 23: 109–123. doi: 10.1046/j.1365-3113.1998.00046.x

Belshaw R, Quicke DLJ (2002) Robustness of ancestral state estimates: evolution of life history strategy in ichneumonoid parasitoids. Systematic Biology 51(3): 450–477. doi: 10.1080/10635150290069896

Blanchard CE (1840) Histoire naturelle des animaux articulés. 3. Hyménoptères. Paris, 672 pp.

Blanchard CE (1845) Histoire naturelle des insects traitant de leurs moeurs et de leurs metamorphoses en général et comprenant une nouvelle classification fondée sur leurs rapports naturels. Paris, 398 pp.

Brébisson LA de (1817) Paxylomme, *Paxylomma*: 66. *Paxylomma*. In: Latreille PA (Ed.) Nouveau Dictionnaire d'Histoire naturelle appliquée aux arts, ... (second ed.) 25, 610 pp.

Brébisson LA de (1825) *Paxylomma*: 23. In: Lepeletier de St Fargeau ALM, Audinet-Serville JG, Olivier AG (Eds) Encyclopédie Méthodique. Dictionnaire des Insectes 10(1825–1828). 833 pp.

Curtis J (1833) Characters of some undescribed genera and species, indicated in the "Guide to an arrangement of British insects". Entomological Magazine 1: 186–199.

Curtis J (1837) A guide to an arrangement of British insects. London, 294 pp.

Fallén CF (1813) Specimen novam Hymenoptera disponendi methodum exhibens. Thesis, Lund, 41 pp.

Foerster A (1862) Synopsis der Familien und Gattungen der Braconen. Verhandlungen des Naturhistorischen Vereins der Preussischen Rheinlande und Westfalens 19: 225–288.

Gillespie JJ, Yoder MJ, Wharton RA (2005) Predicted secondary structure for 28S and 18S rRNA from Ichneumonoidea (Insecta: Hymenoptera: Apocrita): impact on sequence alignment and phylogeny estimation. Journal of Molecular Evolution 61(1): 114–137. doi: 10.1007/s00239-004-0246-x

Gómez Durán JM, Achterberg C van (2011) Oviposition behaviour of four ant parasitoids (Hymenoptera, Braconidae, Euphorinae, Neoneurini and Ichneumonidae, Hybrizontinae), with the description of three new European species. ZooKeys 125: 59–106. doi: 10.3897/zookeys.125.1754.

He JH (1981) A new record of Hybrizonidae (Hymenoptera) from China. Journal of Zhejiang Agricultural University 7(3): 89.

Komatsu T, Konishi K (2010) Parasitic behaviors of two ant parasitoid wasps (Ichneumonidae: Hybrizontinae). Sociobiology 56(3): 575–584.

Konishi K, Choi M-B, Lee J-W (2012). Review of the East Asian species of the genera *Hybrizon* Fallén and *Ghilaromma* Tobias (Hymenoptera: Ichneumonidae: Hybrizontinae). Entomological Research 42: 19–27. doi: 10.1111/j.1748-5967.2011.00352.x

Marsh PM (1979) Hybrizontidae. In: Krombein KV, Hurd PD, Smith DR, Burks BD (Eds) Catalog of Hymenoptera in America north of Mexico. Washington, 313 p.

Marsh PM (1989) Notes on the genus *Hybrizon* in North America (Hymenoptera: Paxylommatidae). Proceedings of the Entomological Society of Washington 91: 29–34.

Nees von Esenbeck CG (1834) Hymenopterorum Ichneumonibus affinium monographiae, genera Europaea et species illustrantes 1. Stuttgartiae & Tubingae, 320 pp.

Quicke DLJ, Fitton MG, Notton DG, Broad GR, Dolphin K (2000) Phylogeny of the sub-families of Ichneumonidae (Hymenoptera): a simultaneous molecular and morphological analysis. In: Austin AD, Dowton M (Eds) Hymenoptera: evolution, biodiversity and biological control. CSIRO Publishing, Collingwood, 74–83.

Quicke DLJ, Laurenne, NM, Fitton MG, Broad GR (2009) A thousand and one wasps: a 28S rDNA and morphological phylogeny of the Ichneumonidae (Insecta: Hymenoptera) with an investigation into alignment parameter space and elision. Journal of Natural History 43(23/24): 1305–1421. doi: 10.1080/00222930902807783

Radchenko A (2005) A review of the ants of the genus *Lasius* Fabricius, 1804, subgenus *Dendrolasius* Ruzsky, 1912 (Hymenoptera: Formicidae) from East Palaearctic. Annales Zoologici (Warszawa) 55(1): 83–94.

Rasnitsyn A (1980) The origin and evolution of Hymenoptera. Trudy Paleontologicheskogo Instituta 174: 1–190. [in Russian]

Ratzeburg JTC (1848) Die Ichneumonen der Forstinsecten in forstlicher und entomologischer Beziehung. Zweiter Band, Berlin, 238 pp.

Sharkey M, Wahl D (1987) Phylogeny of the Ichneumonoidea. Ichnews 10: 4–10.

Shenefelt RD (1969) Braconidae 1. Hymenopterorum Catalogus 4: 1–176.

Stephens JF (1835) Illustrations of British Entomology. Mandibulata 7: 1–312.

Tobias VI (1988) [The family Paxylommatidae (Hymenoptera) in the fauna of the USSR.] Trudy Vsesoyuznogo Entomologicheskogo Obshchestva 70: 131–143. [in Russian]

Wahl D, Sharkey M (1988) Rejoinders at various quarters. Ichnews 11: 12–17.

Wesmael C (1835) Monographie des Braconides de Belgique. Nouveaux Mémoires de l'Academie Royale des Sciences et Belles-lettres Bruxelles 9: 1–252.

Wharton RA, Achterberg C van (2000) Family group names in Braconidae (Hymenoptera: Ichneumonoidea). Journal of Hymenoptera Research 9: 254–270.

Yu DS, Horstmann K (1997) A catalogue of world Ichneumonidae (Hymenoptera). Memoirs of the American Entomological Institute 58: 1–1558.

Yu DS, Achterberg K van, Horstmann K (2009). Biological and taxonomical information: Ichneumonoidea 2006 (updated version). Taxapad Interactive Catalogue, Lexington.

RESEARCH ARTICLE

A new species of *Triclistus* Förster (Hymenoptera, Ichneumonidae, Metopiinae) parasitizing cone-borers from the Eastern Palaearctic part of China

Shu-Ping Sun[1,†], Qing-Shu Luan[2,‡], Mao-Ling Sheng[1,§]

1 *General Station of Forest Pest Management, State Forestry Administration, 58 Huanghe North Street, Shenyang 110034, P. R. China* **2** *Liaoning Academy of Forestry Science, Shenyang 110032, P. R. China*

† *urn:lsid:zoobank.org:author:974C0354-6118-4EA9-890F-EF5ECE8F257A*
‡ *urn:lsid:zoobank.org:author:D28E3571-EF1D-4629-A27B-1CC0B0CA628D*
§ *urn:lsid:zoobank.org:author:3C0EBDB7-26F7-469B-8DB1-5C7B1C6D9B89*

Corresponding author: *Mao-Ling Sheng* (shengmaoling@163.com)

Citation: Sun S-P, Luan Q-S, Sheng M-L (2013) A new species of *Triclistus* Förster (Hymenoptera, Ichneumonidae, Metopiinae) parasitizing cone-borers from the Eastern Palaearctic part of China. Journal of Hymenoptera Research 30: 75–82. doi: 10.3897/JHR.30.4121

Abstract

A new species, *Triclistus strobilius* **sp. n.**, belonging to the subfamily Metopiinae (Hymenoptera, Ichneumonidae), reared from *Dioryctria pryeri* Ragonot, *D. rubella* Hampson and *Gravitarmata margarotana* (Hein) in Liaoning, Hunan provinces and Beijing, is reported and described. Illustrations of the new species are provided.

Keywords

Triclistus, new species, host, cone borer, Lepidoptera, *Dioryctria pryeri*, *Dioryctria rubella*, *Gravitarmata margarotana*, host plant

Introduction

The genus *Triclistus* Förster 1869, belonging to the subfamily Metopiinae (Hymenoptera, Ichneumonidae), comprises 82 described species (Yu et al. 2012), of which 15 are from the Oriental Region (Cameron 1897, Morley 1913, Uchida 1932, Chiu 1962, Kusigemati 1985, He et al. 1996), 26 from the Eastern Palaearctic Region (Kusigemati 1971, 1980, 1987, Momoi and Kusigemati 1970, Tolkanitz 2007, Uchida 1932), 25 from the western Palaearctic Region (Aubert 1984, Cresson 1864, Tolkanitz 1981, 1983, 1987, 2007), 16 from the Nearctic Region (Barron and Bisdee 1984, Townes and Townes 1959), 8 from the Neotropical Region (Gauld et al. 2002). The diagnostic characters of the genus were elucidated by Chiu (1962), Tolkanitz (1987) and Townes (1971).

The Palaearctic species were mainly keyed by Tolkanitz (1981, 1983, 1985 1987, 2007) and Aeschlimann (1983). The Japanese species were mainly reported by Kusigemati (1971, 1980, 1987), Momoi and Kusigemati (1970) and Uchida (1932). Ten species have been known in China (Chiu 1962, He et al. 1996, Yu et al. 2012).

In the study of parasitoids that attack tree borers in three forest areas of China, a new species of *Triclistus* has been found. The aim of this contribution is to describe and illustrate this species and provide biological data on its habitat and hosts.

Materials and methods

In the last five years the authors have been researching the parasitoids of borers of tree branches and cones in Heilongjiang, Jilin, Liaoning, Hebei, Henan, Shanxi and Qinghai provinces, situated in the Eastern Palaearctic part of China and in Hunan and Jiangxi Provinces, situated in the northern border of the Oriental part of China.

To rear parasitoids, cones and twigs of naturally heavily infested trees of *Pinus armandi* Franch, *P. massoniana* Lamb., *P. sylvestris* L. var. *mongolica* Litv., and *P. tabulaeformis* Carr. were brought to the laboratory and maintained in large nylon cages at room-temperature. Water was sprayed over the cones and twigs twice a week and emerged insects collected daily.

The hosts were identified by Professor Hou-Hun Li, Nankai University, Tianjing, China.

Images of whole bodies were taken using a CANON Power Shot A650 IS. Other images were taken using a Cool SNAP 3CCD attached to a Zeiss Discovery V8 Stereomicroscope and captured with QCapture Pro version 5.1.

The morphological terminology is mostly that of Gauld (1991). Wing vein nomenclature is based on Ross (1936).

Type specimens and hosts are deposited in the Insect Museum, General Station of Forest Pest Management, State Forestry Administration, P. R. China.

Systematic

Triclistus **Förster, 1869**
http://species-id.net/wiki/Triclistus

Triclistus Förster, 1869. Verhandlungen des Naturhistorischen Vereins der Preussis-
chen Rheinlande und Westfalens, 25(1868):161. Type-species: *Exochus podagricus*
Gravenhorst.

Diagnosis. Head in lateral view very thick. Face and clypeus evenly, roundly con-
vex. Face continued dorso-medially between antennal sockets as a strong, compressed,
semicircular projection, its upper end close to median ocellus, its dorso-posterior part
with median concavity. Gena long, straightly convergent or very slightly incurvate
backwards. Lower tooth of mandible shorter than upper tooth. Occipital carina strong
and complete. Scutellum weakly convex, without lateral carina except basolateral cor-
ner. Fore wing 1cu-a distal of 1/M by 0.2–0.7 its length. Areolet usually present. Hind
wing 1-cu longer than cu-a. Pterostigma broad. Epicnemial carina strong, upper end
reaching anterior end of subalar prominence. Metapleuron smooth, polished. Front
and mid femora rather thick. Mid tibia with two spurs. Fifth tarsomere of female
with or without a ventral, subapical projection. Tarsal claw simple. Propodeal spiracle
subcircular to elliptic. Metasoma short, robust. First tergite usually broad basally, its
spiracle approximately at basal 0.25, its sternite reaching approximately to 0.2 length
of tergum. Subgenital plate of female with or without apical-median notch. Ovipositor
not projecting beyond tip of metasoma.

  Host. According to the most recent catalogue of Ichneumonoidea (Yu et al. 2012),
123 hosts, belonging to 16 families of Lepidoptera, have been recorded, mainly species
of Tortricidae, Pyralidae, Geometridae and Yponomeutidae.

Triclistus strobilius **sp. n.**
urn:lsid:zoobank.org:act:6EF70B06-238D-4089-827B-6D421574D72D
http://species-id.net/wiki/Triclistus_strobilius
Figures 1–8

Material. Holotype, Female, CHINA: Beiling Park, Shenyang, Liaoning Province,
reared from pupa of *Dioryctria rubella* Hampson, collected from cone of *Pinus tabu-
laeformis* Carr., 25 September 2009, Mao-Ling Sheng, Shu-Ping Sun. Paratypes: 1
female, same data as holotype; 1 female, CHINA: Beiling Park, Shenyang, Liaoning
Province, reared from pupa of *Dioryctria rubella* Hampson, collected from cone of
Pinus armandi Franch., 15 October 2010, Mao-Ling Sheng, Shu-Ping Sun; 1 male,
CHINA: Fuxin, Liaoning Province, reared from pupa of *Gravitarmata margarotana*
(Hein), collected from cone of *P. tabulaeformis* Carr., 20 October 2010, Qing-Shu
Luan; 1 male, CHINA: Yanqing, Beijing, reared from pupa of *D. pryeri* Ragonot,

Figures 1–8. *Triclistus strobilius*, sp. n. Holotype, female. **1** Habitus, lateral view **2** Head, anterior view **3** Frons **4** Mesopleuron **5** Fore femur **6** Propodeum **7** Tergites 1–4 **8** Posterior part of metasoma, lateral view.

collected from cone of *P. tabulaeformis* Carr., 9 July 2012, Tao Li; 1 female, CHINA: Jingzhou, Hunan Province, reared from pupa of *Dioryctria rubella* Hampson, collected from twig of *P. massoniana* Lamb., 1 June 2011, Mao-Ling Sheng.

Diagnosis. Fore wing with areolet open. Tergites 2–4 (Figure 7) with approximately identical punctures. Head except whitish yellow projection between antennal sockets, mesosoma, fore and mid legs, all coxae and trochanters and metasoma, entirely black. Fore femora red.

Description. Female. Body length 8.5–9.5 mm. Fore wing length 6.0–7.0 mm.

Head. Face (Figure 2) evenly, strongly convex, with large, dense punctures; upper median part with reddish brown hairs. Dorso-posterior part of upper-median projection with shallow median concavity. Clypeal suture absent. Median part of mandible convex, with distinct dense punctures, lower tooth very short and small; upper tooth long and sharp, approximately 3.0–3.5 times as long as lower tooth. Cheek with indistinct punctures. Malar space 0.5–0.6 times as long as basal width of mandible. Gena strongly and straightly convergent backwards, with dense punctures, distance between punctures 0.5–1.0 times diameter of puncture. Postocellar line 2.0–2.1 times as long as ocular-ocellar line. Frons (Figure 3) with dense punctures; lower part strongly concave; dorso-posterior part of projection between antennal sockets with shallow median concavity. Antenna with 31–32 flagellomeres; first flagellomere approximately 1.5 times as long as second flagellomere. Occipital carina complete and strong.

Mesosoma. Anterior margin of pronotum with fine, short longitudinal wrinkles and fine, indistinct punctures; lateral concavity wide, deep, smooth, shiny; upper-posterior margin with fine, distinct punctures. Epomia strong. Mesoscutum with fine punctures, distance between punctures 0.5–3.5 times diameter of puncture. Notaulus indistinct. Scutellum weakly convex, with texture as that of mesoscutum. Postscutellum transverse, smooth and shiny, with fine, indistinct punctures; anterior part transversely concave. Mesopleuron (Figure 4) strongly convex, with texture as that of mesoscutum; posterior part longitudinally smooth and shiny. Metapleuron almost flat, smooth and shiny, lower part with 15–20 hairs. Juxtacoxal carina absent. Submetapleural carina complete, triangularly convex anteriorly. Wings brownish hyaline. Fore wing vein 1*cu-a* distal of 1/*M*, distance between them 0.2–0.3 times length of 1*cu-a*. Areolet open externally. 2*m-cu* distal of 2*rs-m*, distance between them 1.25–1.3 times length of 2*rs-m*. Vein 2-*Cu* 3.0–4.0 times as long as 2*cu-a*. Hind wing vein 1/*cu* 4.0–5.0 times as long as *cu-a*. Front femur (Figure 5) particularly swollen, with fine, sparse punctures. Ratio of length of hind tarsomeres 1:2:3:4:5 is 10.0:4.3:3.1:1.5:2.3. Hind fifth tarsomere without subapical hook-shaped thorn. Propodeum (Figure 6) almost evenly convex, smooth and shiny, lateral sides and area petiolaris with fine, indistinct punctures, carinae complete and strong. Area superomedia separated from area basalis and from area petiolaris by strong carina, costula located slightly before its middle. Propodeal spiracle obliquely elongate, approximately 2.5–3.0 times as long as maximum width, almost touching pleural carina.

Metasoma. First tergite with even punctures between median dorsal and dorsolateral carinae, smooth and shiny between median dorsal carinae; slightly longer than apical width, weakly convergent towards sub-base, combined points of median dorsal and dorsolateral carinae projecting as lower horns, abruptly narrowed towards base

of tergite. Median dorsal carinae long and strong, reaching 0.7–0.8 length of tergite. Dorsolateral and ventrolateral carinae complete. Tergites 2–4 (Figure 7) and anterior half of tergite 5 with symmetrical punctures, lateral sides almost parallel. Tergite 2 approximately 0.78–0.8 times as long as apical width. Tergite 3 approximately 0.67–0.7 times as long as apical width. Posterior half of tergite 5 and following tergites (Figure 8) with indistinct fine punctures. Posterior part of tergite 8 smooth and shiny. Apical margin of subgenital plate obtuse, without distinct apical-median concavity.

Color. Body almost entirely black (Figure 1). Upper median projection of face between antennal sockets whitish yellow. Upper margin of face faintly brown. Vertex above eye with small yellowish brown spot. Median part of mandible dark-reddish-brown. Front femora red. Apical part of front tibiae reddish brown. Front first tarsomere, apices of mid and hind tibiae, fourth and fifth tarsomeres dark red to blackish brown. Pterostigma and veins brownish black.

Male. Body length 9.0–9.5 mm. Fore wing length 6.5–7.0 mm. Upper-posterior margin of pronotum more or less yellow.

Hosts. *Dioryctria rubella* Hampson, *D. pryeri* Ragonot (Lepidoptera, Pyralidae); *Gravitarmata margarotana* (Hein) (Lepidoptera, Tortricidae).

Host plants. *Pinus massoniana* Lamb., *P. armandi* Franch., *P. tabulaeformis* Carr. (Pinaceae).

Etymology. The name of the new species is based on the host food.

Remarks. Similar to *T. nigrifemoralis* Kusigemati 1971, but can be easily distinguished from the latter in having the areolet open externally; area superomedia (Figure 6) smooth and shiny, anterior side wide, separated from area basalis by distinct carina; third tergite without bare part, entirely with symmetrical hairs; hind tibia entirely black. *Triclistus nigrifemoralis* Kusigemati 1971 has the areolet closed externally; area superomedia rugose, strongly narrowed anteriorly, confluent with area basalis; third tergite bare medially, sparsely hairy laterally and basal 1/3 of hind tibia yellowish brown.

Acknowledgements

References

Aubert JF (1984) Douze Ichneumonides non pétiolées mal connues ou inédites. Bulletin de la Société Entomologique de Mulhouse 1984: 17–23.

Barron JR, Bisdee HE (1984) Hymenopterous parasites with Lepidopterous and sawfly hosts on Lonicera (Honeysuckle) in the Ottawa area. Canadian Entomologist 116(10): 1345–1356. doi: 10.4039/Ent1161345-10

Cameron P (1897) Hymenoptera Orientalia, or contribution to a knowledge of the Hymenoptera of the Oriental Zoological Region. Part V. Memoirs and Proceedings of the Manchester Literary and Philosophical Society 41(4): 1–144.

Chiu S-C (1962) The Taiwan Metopiinae (Hymenoptera: Ichneumonidae). Bulletin of the Taiwan Agricultural Research Institute 20: 1–37.

Cresson ET (1864) Descriptions of North American Hymenoptera in the collection of the Entomological Society of Philadelphia. Proceedings of the Entomological Society of Philadelphia 3: 257–321.

Förster A (1869) Synopsis der Familien und Gattungen der Ichneumonen. Verhandlungen des Naturhistorischen Vereins der Preussischen Rheinlande und Westfalens 25(1868): 135–221.

Gauld ID (1991) The Ichneumonidae of Costa Rica, 1. Introduction, keys to subfamilies, and keys to the species of the lower Pimpliform subfamilies Rhyssinae, Poemeniinae, Acaenitinae and Cylloceriinae. Memoirs of the American Entomological Institute 47: 1–589.

Gauld ID, Sithole R, Gómez JU, Godoy C (2002) The Ichneumonidae of Costa Rica. 4. Memoirs of the American Entomological Institute 66: 1–768.

He J-H, Chen X-X, Ma Y (1996) Hymenoptera: Ichneumonidae. Economic Insect Fauna of China. Beijing, 697 pp.

Kusigemati K (1971) Taxonomic studies on the subfamiliy Metopiinae of Japan (Hymenoptera: Ichneumonidae). Memoirs of the Faculty of Agriculture, Kagoshima University 8(1): 205–298.

Kusigemati K (1980) Notes on some species of Ichneumonidae of Japan with description of a new species (Hymenoptera). Akitu 32: 1–6.

Kusigemati K (1985) Metopiinae collected by the Hokkaido University Expedition to Nepal Himalaya, 1968 (Hymenoptera, Ichneumonidae). Kontyu 53(3): 398–405.

Kusigemati K (1987) Seven new Metopiine Ichneumonids of Japan, with notes on three known species (Hymenoptera). Kontyu 55(2): 220–231.

Momoi S, Kusigemati K (1970) Metopiinae (Hymenoptera: Ichneumonidae) of the Ryukyu Archipelago. Pacific Insects 12(2): 401–415.

Morley C (1913) The fauna of British India including Ceylon and Burma, Hymenoptera, Vol.3. Ichneumonidae. British Museum, London, 531 pp.

Ross HH (1936) The ancestry and wing venation of the Hymenoptera. Annals of the Entomological Society of America 29: 99–111.

Sheng M-L, Sun S-P (2009) Insect fauna of Henan, Hymenoptera: Ichneumonidae. Science Press, Beijing, China, 340 pp.

Tolkanitz VI (1981) [A guide to the insects of the European part of the USSR. Hymenoptera, Ichneumonidae. Subfamily Metopiinae.] Opredeliteli Faune SSSR 129: 451–476.

Tolkanitz VI (1983) New species of Ichneumon-flies of the genus Triclistus (Hymenoptera, Ichneumonidae) from Moldavia. Zoologicheskii Zhurnal 62(6): 954–956.

Tolkanitz VI (1987) Parasitic Hymenoptera. Ichneumonidae – Metopiinae. Fauna Ukraina 11(2): 1–212.

Tolkanitz VI (2007) Metopiinae. In: Lelej AS (Ed.) Key to the insects of Russia Far East, 4. Neuropteroidea, Mecoptera, Hymenoptera. Pt 5. Dalnauka, Vladivostok, 638–667.

Townes HK (1971) The genera of Ichneumonidae, Part 4. Memoirs of the American Entomological Institute 17: 1–372.

Townes HK, Townes M (1959) Ichneumon-flies of American north of Mexico: 1 Subfamily Metopiinae. United States National Museum Bulletin 216(1): 1–318. doi: 10.5479/si.03629236.216.1

Uchida (1932) H. Sauter's Formosa-Ausbeute. Ichneumonidae (Hym.). Journal of the Faculty of Agriculture, Hokkaido University 33: 133–222.

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