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Phylogeny of hornets: a total evidence approach (Hymenoptera, Vespidae, Vespinae, *Vespa*)

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Abstract

The only previous comprehensive phylogenetic analysis of the 22 species of the genus *Vespa* was based on just 11 morphological characters and resulted in only limited resolution. In order to improve the phylogenetic inference, we carried out a simultaneous analysis with 45 morphological characters and data from four mitochondrial and two nuclear genes. The results support a number of the previously found relationships. The monophyly of the genus *Vespa* and the existence of a main clade excluding *V. basalis* and *V. binghami* are confirmed. The *tropica* group is supported. The *affinis* group is not supported; molecular data relate the previously unresolved *V. orientalis* to *V. affinis* + *V. mocsaryana*.

Keywords

Hornet, Phylogeny, Total Evidence, Vespidae

Introduction

The genus *Vespa* is one of the four genera of the subfamily Vespinae; it is composed of 22 extant species of hornets (Archer 1991, Nguyen et al. 2006). Most species have a distribution restricted to Asia, with the highest diversity found in northern Indo-

Malaya (Matsuura and Yamane 1990, Carpenter and Kojima 1997). Two species are also naturally distributed outside of Asia: *Vespa crabro* is found in Europe and around the Black Sea and the Caspian Sea and *V. orientalis* in the north of Africa, Mediterranean regions and across the Middle-East. In the mid-19th century *V. crabro* was introduced into North America where it is now established (de Saussure 1898), while more recently, *V. velutina* was accidentally introduced into Europe, where it became invasive (Villemant et al. 2011). Several hornet species have been the subject of various biological studies, either because of their social habits (Matsuura 1991, Foster et al. 2000, Ishay et al. 2008), their threat to human health (Vetter et al. 1999), their impact on apiculture (Abrol 1994, Ranhabat et al. 2009) or even their interest as edible insects (Ying et al. 2010). However, the phylogeny of this genus is not yet well resolved.

The first cladistic study of *Vespa* was that of Archer (1994a), based on 11 morphological characters (10 binary and one multistate). It distinguished a main clade comprising most *Vespa* species, and two species unresolved at the base of the entire tree (Fig. 1). One of them, *V. binghami*, is the only *Vespa* species presenting morphological adaptation to nocturnal habits (van der Vecht 1959). The other unplaced species of Archer's cladogram, *V. basalis*, is a small hornet readily distinguishable by its reduced punctation, especially on the clypeus. The main clade found by Archer (1994a) presented an unresolved basal node with four lineages, one comprising a single species (*V. orientalis*) and a second he termed the *crabro* group (*V. crabro* and *V. dybowskii*). The third lineage, or *tropica* group, included five species in two clades: one with *V. mandarinia* + *V. soror*, and the other with three species (*V. ducalis, V. philippinensis* and *V. tropica*). Archer's last lineage, called the *affinis* group (Fig. 1), was composed of the 12 remaining species including an unresolved clade of four species which have very similar morphology (*V. bicolor*, *V. simillima*, *V. velutina* and *V. vivax*). This unresolved clade was termed the *bicolor* group by Archer in another paper (1994b) and was sister-group of two unresolved species from so-called Sundaland: *V. bellicosa* and *V. multimaculata*.

Archer's study was based mostly on male characters, which are known to be reliable phylogenetic characters (e. g. Song and Bucheli 2010), but his cladogram has many unresolved relationships. Furthermore, some of the lineages are only supported by single characters from female morphology, such as the *tropica* group, which is characterized by the female clypeus shape. Corroborating such groups thus requires further analyses.

This study presents results from an ongoing project on the evolution of vespine wasps, focusing on the genus *Vespa*. Our aims are to confirm the monophyly of the genus *Vespa*, a question not addressed by Archer (1994a), and to clarify relationships among the species based on a more extensive morphological matrix and combining molecular data.

Methods

Phylogenetic relationships were inferred for the 22 species of the genus *Vespa* and five other species of Vespidae as outgroup: *Dolichovespula media* (Retzius, 1783), *Provespa anomala* (de Saussure, 1854), *Provespa barthelemyi* (du Buysson, 1905) and *Vespula ger-*

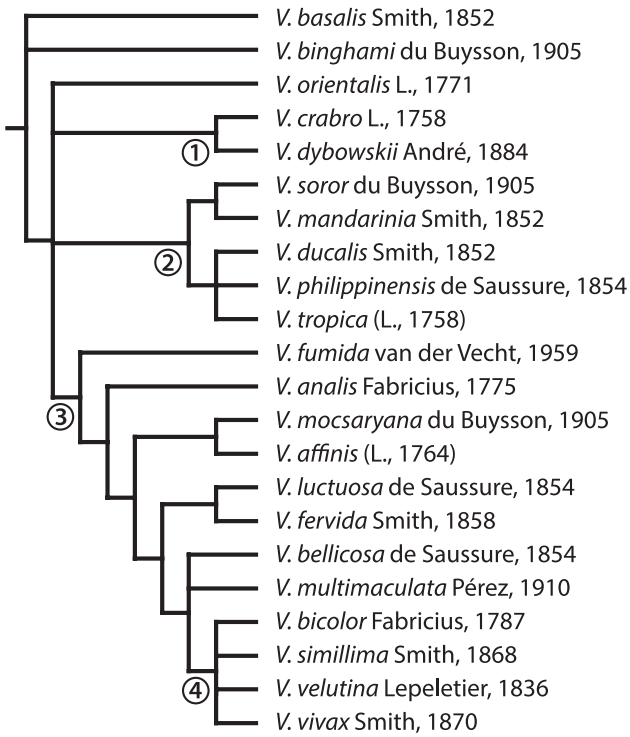

Figure 1. Phylogeny of the genus *Vespa* after Archer (1994a: figure 8). Tree updated for the current classification (Nguyen et al. 2006). The species groups discussed in this paper are as follows: **1** *crabro* **2** *tropica* **3** *affinis* **4** *bicolor sensu* Archer (1994b).

manica (Fabricius, 1793) from the subfamily Vespinae and *Polistes dominula* (Christ, 1791) from the Polistinae.

The morphological matrix was scored from specimens in the following natural history collections: American Museum of Natural History, Muséum National d'Histoire Naturelle, Nationaal Natuurhistorisch Museum and United States National Museum of Natural History. Specimens for molecular study were collected by J.K. and J.M.C. and preserved in 95 - 99% ethanol.

DNA was extracted from one leg and one antenna per specimen using QIAGEN "DNeasy tissue Kit". Genes were amplified using PCR with PuReTaq Ready-To-Go beads in a total volume of 25μL including primers (Appendix 1) and DNA. Amplification cycles were specific to genes (Appendix 2). AMPures and CleanSEQ procedures were used for DNA purification and sequencing was performed on ABI PRISM 3730xl machines by Agencourt Biosciences (Beverly, USA). One missing gene fragment of *V. basalis* was obtained from Genbank database (accession number: AB585949).

The analyses are based on 45 morphological characters and multiple nuclear and mitochondrial loci, comprising: 374 sites of 12S, 528 sites of 16S, 2231 sites of 28S (sequenced in 4 fragments), 1442 sites of CO1 (sequenced in 2 fragments), 880 sites of elongation factor 1α (EF1α), and 328 sites of H3. Each of these genes was aligned

separately using the MAFFT software with the L-INS-i algorithm (Katoh et al. 2005). Morphological characters were coded with Winclada V. 1.00.08 (Nixon 2002).

Phylogenetic analyses were performed in a parsimony framework using TNT (Goloboff et al. 2008). Analyses were first conducted on the morphological matrix and on the different molecular datasets separately, then on a matrix of all the data combined. Sequence alignments were merged with Winclada, and the final composite molecular matrix contained 5783 aligned nucleotides. Each analysis was performed with the new technology search algorithms including sectorial searches, the parsimony ratchet, tree drifting and tree fusing, default parameters except: 200 ratchet iterations, upweighting percentage 8, downweighting 4; 50 cycles of drift; minimum length hit 25 times. Molecular gaps were treated as missing data. Node supports were computed as GC-values of a symmetric resampling of 1000 replicates (group supported/contradicted values; Goloboff et al. 2003). GC values range from -1 to 1 and are the differences in group frequency between the group found in most parsimonious trees and the most frequent contradictory group. Only supported groups (support value above zero, scaled by TNT 0-100) are shown.

Results

Morphological analysis

Analysis of the 45 morphological characters (Appendix 3) resulted in a single most parsimonious tree (not shown; Length: 107, Consistency Index (CI): 0.617, Retention Index (RI): 0.784). The support tree is shown in Fig. 2. The only difference is that the most parsimonious cladogram resolves *V. orientalis* as the sister species to the clade composed of *mandarinia* + *soror* and the *affinis* group; the support tree (Fig. 2) does not include this node. On both trees the genus *Vespa* appears as monophyletic, and *V. basalis* the sister species of the rest of the genus, followed by *V. binghami*. The 20 remaining species are grouped in three clades, relationships among which are not resolved (Fig. 2): a clade with only *V. orientalis*, the second comprising *crabro* + *dybowskii* together with *V. tropica* and its two closely related species, and the third clade with the remaining 14 species. The *tropica* group *sensu* Archer (1994a) is thus not monophyletic according to these morphological data: *V. mandarinia* + *V. soror* are not closely related to *V. tropica*, rather they form the sister group of what corresponds to the *affinis* group of Archer. Finally, *V. bellicosa* and *V. multimaculata* are internal components of a clade including the four species of the *bicolor* group *sensu* Archer (1994b).

Molecular analyses

Of the 27 studied species, specimens relatively recently collected were available for 17 species (including the outgroups). Due to low quality DNA templates and the use

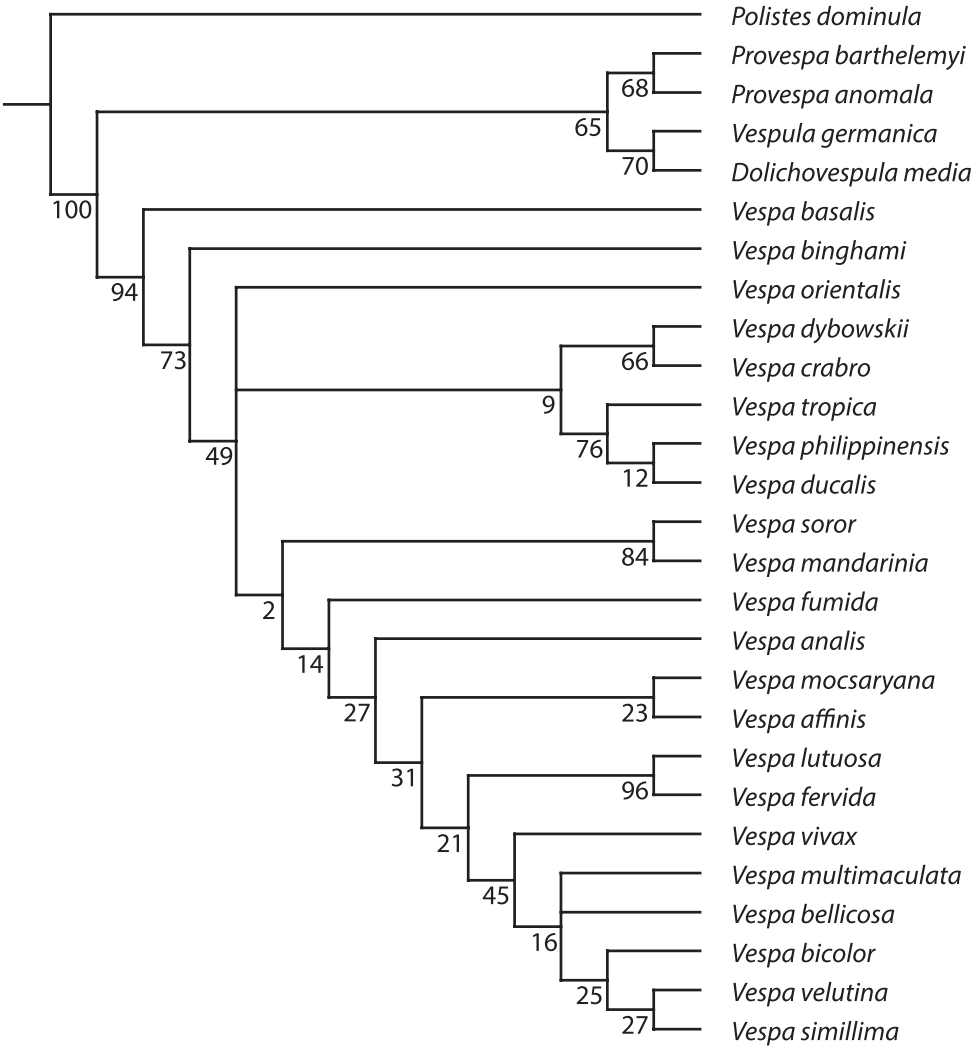

Figure 2. Support tree for relationships among the 22 *Vespa* species based on 45 morphological characters. Supports for nodes are given in GC-values (see text for explanation) when they are greater than zero.

of non-specific primers, molecular data are not homogeneous across the genus. The monophyly of the genus *Vespa* was found in most analyses based on single genes and in the analysis of merged alignments (Table 1, Fig. 3). The main species-groups appear monophyletic over most analyses except for the low variation 28S. *Vespa basalis* remains the basal species in the genus. Two main clades diverge in the remaining species: the monophyletic *tropica* group is the first clade, while the *bicolor* group and a clade of *V. crabro*, *V. mocsaryana* and *V. affinis* form the second. The *bicolor* group *sensu* Archer (1994b) is supported in all molecular analyses, with *V. vivax* being resolved as the sister species of *V. velutina*.

Table 1. Results of phylogenetic analyses of molecular data for the genus *Vespa*. Marker: gene used in the analysis. "Multi-locus" marker is a combination of all genes used. N tree: number of most parsimonious trees. Length: length of the most parsimonious trees. *Vespa*, *bicolor*, *mandarinia*, *tropica*: monophyly of the considered clade when it is tested.

marker	N tree	length	CI	RI	*Vespa*	*bicolor*	*mandarinia*	*tropica*
12S	5	261	0.709	0.651	yes	yes	yes	-
16S	2	332	0.654	0.545	yes	yes	yes	yes
28S	>1000	145	0.909	0.750	no	yes	no	no
CO1	1	1431	0.508	0.369	yes	yes	yes	-
EF1a	1	219	0.886	0.819	yes	yes	yes	-
H3	1	81	0.889	0.690	no	yes	-	-
Multi-locus	207	2494	0.620	0.468	yes	yes	yes	yes

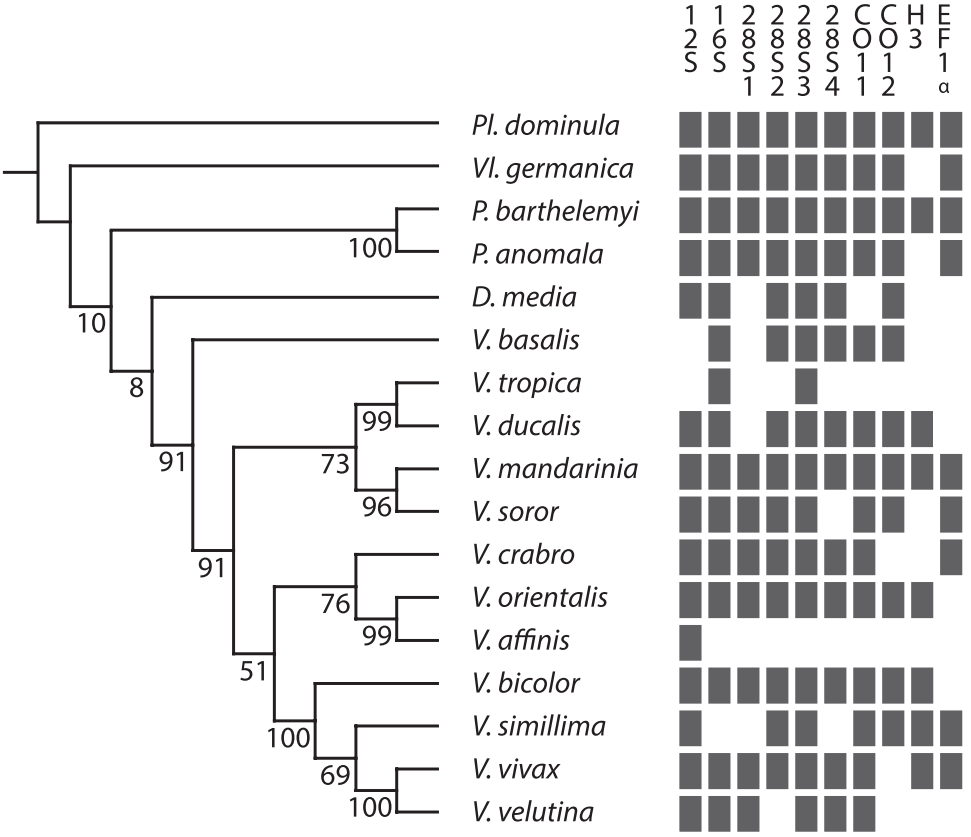

Figure 3. Support tree for the relationships among 13 *Vespa* species based on the six genes. Supports for nodes are given in GC-values. Grey rectangles show the molecular markers available for each species.

Total evidence analysis

The combined analysis of morphological and molecular data returned eight equally parsimonious trees all showing *Vespa* monophyletic with *V. basalis* as the sister species of the rest of the genus (not shown; Length: 2665, CI: 0.608, RI: 0.488). The consensus tree (not shown) is completely resolved except for a node including the *bicolor* group, *V. bellicosa* and *V. multimaculata*. In the support tree (Fig. 4), two internal nodes are collapsed, because of a different position of *V. fumida*.

The monophyly of the genus is supported by five synapomorphies: the long prestigma, the developed vertex, the strongly elevated interantennal space, the presence of a carina on the hindcoxa, and the projection at the apex of the digitus in males.

The addition of molecular data to the morphological matrix resulted in stronger support of the *tropica* group and resolved the position of *V. orientalis*, as part of a clade with two morphologically different species, *V. affinis* and *V. mocsaryana*, close to *V. crabro*. The *affinis* group *sensu* Archer (1994a) thus did not appear monophyletic. These changes resulted in fewer steps for the morphological character of the clypeal apical margin, a synapomorphy of the *tropica* group, while the CIs of eight other morphological characters (five pronotal and head characters and three male characters) diminished.

In the combined analysis tree, most of the clades of *Vespa* species are supported by morphological characters, five of which are uncontroverted synapomorphies. *Vespa basalis* is distinguished from the other species on the basis of the aedeagal apical lobes in males and the edges of the interantennal space. The main clade excluding *V. basalis* and *V. binghami* is supported by the clypeal punctures dense mesally in females and the emarginated apical margins of the metasomal sterna VI and VII in males.

Within the main *Vespa* clade, the *tropica* group is morphologically supported by the triangular apico-lateral angles of female clypeus only, but molecular data confirms this homology. Within this group, *V. mandarinia* + *V. soror* is defined by two uncontroverted characters: the spade-shape of the aedeagus apex in males and the expansion of the gena behind the eyes. The three other species of the *tropica* group share the presence of pronotal striae, long first metasomal segment and marked scutal and metapleural punctures.

The two species of the *crabro* group share two secondary reversions: the straight apical margin of the male metasomal sternum VI and the loss of digital apical process. *Vespa affinis* and *V. mocsaryana* share the posteromedially deeply emarginated male metasomal sterna VI and VII and long first metasomal segment. The clade consisting of *V. orientalis* and *V. affinis* + *V. mocsaryana* is supported by the short malar space, a homoplastic synapomorphy found also in the clade of *V. analis*, *V. luctuosa* + *V. fervida*, *V. multimaculata*, *V. bellicosa* and the *bicolor* group. This latter clade is also supported by the bulbous aedeagal shaft in males. The clade of these species but *V. analis* is morphologically supported by a pretegular carina ventrally effaced, the presence of a few ventral striae on the female pronotum and the apical margin of the male metasomal sternum VII deeply emarginate. *Vespa fervida* + *V. luctuosa* is supported by the well

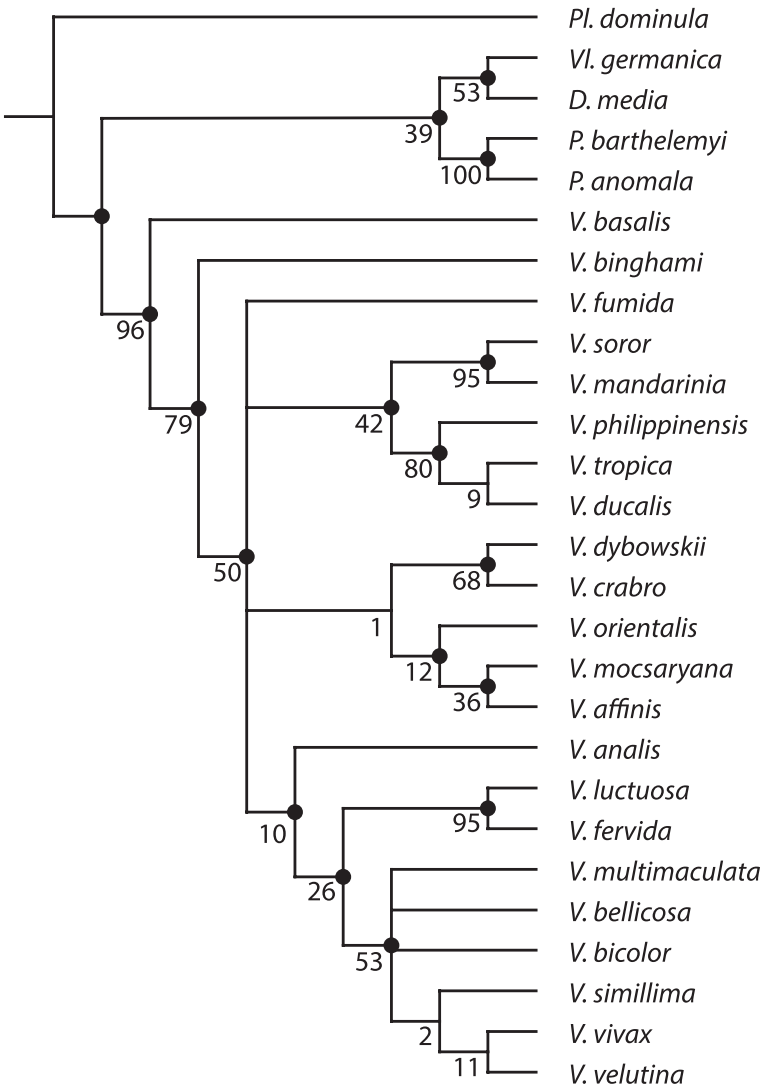

Figure 4. Support tree for the relationships within the genus *Vespa* based on a combined analysis. Support tree based on 45 morphological characters and six genes. Black nodes indicate clades supported by morphological characters. Absence of mark on nodes indicates clades diagnosed by molecular data only. Supports for nodes are given in GC-values.

defined punctures on metapleura and lateral faces of the metasomal tergum II as well as the uncontroverted synapomorphy of the median process in the apical margin of the metasomal sternum VII. Finally, the clade of the *bicolor* group and *V. multimaculata* and *V. bellicosa* is supported by the distinct interruption of the pronotal carina by the pronotal pit. The four remaining clades within the genus *Vespa* are not diagnosed by morphological characters. These latter clades also have low support under symmetric resampling (Fig. 4).

Discussion

Our analyses of both morphological and molecular characters confirm the monophyly of the genus *Vespa*. This genus was first diagnosed from other Vespidae on the basis of the shape of the head (Thomson 1869) and especially the vertex length, which is congruent with the other synapomorphies of the genus. In our morphological and total evidence analyses, the genus *Vespa* is the sister-group to the other vespine genera with similar relationships to those described by Carpenter (1987). The results of Carpenter (1987) based on morphology and ours based on combined analysis contradict Pickett and Carpenter (2010).

While our molecular sample is incomplete, it nonetheless confirms the monophyly of two of Archer's species groups within *Vespa* based on the morphology (*crabro* and *tropica* groups). Molecular data help to place *V. orientalis,* which both Archer's and our morphological analyses failed to resolve well. *Vespa orientalis* is the only *Vespa* species distributed in arid areas in central Asia and the Middle-East. A close relationship of this species to *V. affinis* + *V. mocsaryana* despite obvious morphological differences begs the question of morphological adaptations to arid climates in *V. orientalis* that may have blurred the morphological phylogenetic signal. However, the close relationship of *V. orientalis* and *V. affinis* is suggested only by the 12S gene in the molecular data. Further gene sequences for this last species and for *V. mocsaryana* are necessary to clarify whether the clade consisting of *V. orientalis* and *V. affinis* + *V. mocsaryana* is definitively supported.

Our results are also consistent with previous authors regarding the close relationships of *V. luctuosa* and *V. fervida*, which are very similar in their morphology (van der Vecht 1957, Archer 1994a, 1999). On the other hand, *V. bellicosa* and *V. multimaculata*, for which close relationships to *V. luctuosa* were suggested based on their distribution and morphological similarities (Bequaert 1934), are not closely related to *V. luctuosa*. They appear to form a clade together with Archer's *bicolor* group. Relationships within this last clade are still poorly resolved with low node supports. Molecular data showed a closer relationship between *V. vivax* and *V. velutina* (Fig. 3), while the morphological characters placed *V. simillima* and *V. velutina* as sister species. Such a discrepancy may have resulted from the fact that no molecular data were available for *V. multimaculata* and *V. bellicosa*.

Archer's finding of a main clade of *Vespa* excluding *V. basalis* and *V. binghami* has been confirmed both by morphological and molecular data. Our results also suggest that the nocturnal species *V. binghami* is closer to the main clade of *Vespa* than is *V. basalis*. Morphological adaptations to nocturnal habits in *V. binghami* such as enlarged ocelli are thus autapomorphies. Recognition of the subgenus *Nyctovespa*, with *V. binghami* as sole included species (van der Vecht 1959), would thus render the subgenus *Vespa* paraphyletic, and the synonymy of *Nyctovespa* (Carpenter 1987) is justified on that basis.

Our extended morphological matrix and the molecular sequences partly support Archer's results, and this analysis confirms that male characters such as the shape of the last metasomal sterna and the genitalia are reliable phylogenetic characters in Vespidae.

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Appendix 1

Primers used for sequencing the six genes.

Marker	Primer	Name	Sequence (5'-3')
12S	F	12S AI	AAACTAGGATTAGATACCCTATTAT
12S	R	12S BI	AAGAGCGACGGGCGATGTGT
16S	F	16S A	CGCCTGTTTATCAAAAACAT
16S	R	16S B	CTCCGGTTTGAACTCAGATCA
28S-1	F	28S 1A	CCCSCGTAAYTTAGGCATAT
28S-1	R	28S 4 BR	CCTTGGTCCGTGTTTCAAGAC
28S-2	F	28S 3.2a	AGTACGTGAAACCGTTCASGGGT
28S-2	R	28S Br	TCGGAAGGAACCAGCTACTA
28S-3	F	28S 4a	GGAGTCTAGCATGTGYGCAAGTC
28S-3	R	28S 5b	CCACAGCGCCGATTCTGCTTACC
28S-4	F	28S 4.8a	ACCTATTCTCAAACTTTAAATGG
28S-4	R	28S 7b1	GACTTCCCTTACCTACAT
CO1-1	F	LCO	GGTCAACAAATCATAAAGATATTGG
CO1-1	R	HCO out out	GTAAATATATGRTGDGCTC
CO1-2	F	Jerry	CAACATTTATTTTGATTTTTTGG
CO1-2	R	Pat	TCCAATGCACTAATCTGCCATATTA
EF1α	F	HaF2For	GGGYAAAGGWTCCTTCAARTATGC
EF1α	R	F2Rev1	AATCAGCAGCACCTTTAGGTGG
H3	F	H3-AF	ATGGCTCGTACCAAGCAGACVGC
H3	R	H3-AR	GTCACYATYATGCCYAAGGATAT

Appendix 2

PCR program of each marker with temperature and time (°C – minute). Den. = Denaturing phase. Anneal. = Annealing phase. Elong. = Elongation phase. N = number of cycles of Denaturing + Annealing + Elongation phases.

Marker	Den.	Anneal.	Elong.	N
12S	97 – 0.5	42 – 0.75	68 – 0.5	40
16S	94 – 0.5	42 – 0.5	72 – 0.5	40
28S-1	94 – 1	43.5 – 0.5	72 – 1	40
28S-2	94 – 1	43.5 – 0.5	72 – 1	40
28S-3	94 – 1	43.5 – 0.5	72 – 1	40
28S-4	94 – 1	40 – 0.5	72 – 1	40
CO1-1	94 – 0.5	36 / 51 – 0.5	72 – 0.5	5 / 35
CO1-2	94 – 0.5	36 / 48 – 1	72 – 1	5 / 35
EF1α	94 – 1	54 – 1	72 – 1.5	35
H3	94 – 0.4	51 – 0.5	72 – 0.75	40

Appendix 3

Matrix of morphological characters. Outgroup species are in grey.

Character	0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 4 4 4 4 4 4
	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5
P. dominula	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0
D. media	1 1 1 1 1 1 0 0 0 0 1 1 2 0 0 0 1 1 1 0 0 2 2 1 1 1 1 1 1 1 1 0 1 0 1 0 2 0 5 0 2 0 0 0
V. germanica	1 1 1 1 1 1 2 0 0 0 1 1 2 0 0 0 1 1 3 - - 2 0 1 1 1 1 1 1 1 1 1 1 0 0 0 1 0 6 0 1 0 0 0
P. anomala	1 1 1 1 1 1 2 0 0 1 1 1 1 0 0 0 1 0 2 - 0 2 0 0 1 1 1 1 1 1 1 1 0 0 1 0 0 0 0 7 1 1 0 0 0
P. barthelemyi	1 1 1 1 1 1 0 0 0 1 1 1 1 0 0 0 1 0 2 - 0 2 0 0 1 1 1 1 1 1 1 1 0 0 1 0 0 0 0 4 0 1 0 0 0
V. affinis	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 0 0 1 0 0 0 0 0 1 0 1 1 1 1 1 1 2 1 1 1 0 2 2 0 1 1 1 1 1 0
V. analis	2 0 1 0 1 1 0 1 0 0 3 1 1 1 1 0 / 1 0 1 0 0 1 0 0 0 0 2 1 1 1 1 1 1 1 1 1 0 0 1 2 0 1 1 1 1 1 0
V. basalis	2 0 1 0 1 1 0 1 1 0 2 1 1 0 0 0 1 1 0 0 1 0 1 0 0 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 2 0 1 0 1 0
V. bellicosa	2 0 1 0 1 1 0 1 0 0 2 1 1 0 1 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 0 2 2 0 1 1 1 1 1 0
V. bicolor	2 0 1 0 1 1 0 1 0 0 2 1 1 0 0 0 0 1 0 0 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 0 2 2 0 1 1 1 1 1 0
V. binghami	2 0 1 0 1 1 1 1 1 0 1 3 1 1 0 0 0 0 1 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 1 1 1 0 1 0
V. crabro	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 1 0 / 1 1 0 0 0 0 0 1 0 1 1 1 1 1 1 1 1 1 1 1 0 0 0 1 0 1 1 1 0 0 0
V. ducalis	2 0 1 0 1 1 0 1 0 0 3 1 2 0 1 1 0 0 0 0 1 0 2 1 1 1 1 1 2 1 1 1 0 1 1 0 1 0 1 1 1 0 1 0
V. dybowskii	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 1 0 0 0 0 1 0 1 1 1 1 1 1 1 1 1 1 1 0 0 0 1 0 1 1 1 0 0 0
V. fervida	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 0 0 1 0 0 1 0 1 1 0 2 1 1 1 1 1 2 1 1 0 1 2 1 1 1 1 1 1 1 0
V. fumida	2 0 1 0 1 1 0 1 0 0 3 1 1 0 0 1 1 0 0 1 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 0 0 1 2 0 1 1 1 0 1 1
V. luctuosa	2 0 1 0 1 1 0 1 0 0 2 1 1 0 1 0 0 1 0 0 1 0 1 1 0 2 1 1 1 1 1 2 1 1 0 1 2 1 1 1 1 1 1 1 0
V. mandarinia	2 0 1 0 1 1 0 2 0 0 3 1 2 0 1 1 0 0 1 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 0 0 1 1 0 3 1 1 0 1 0
V. mocsaryana	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 0 0 1 0 0 1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 1 0 2 2 0 1 1 1 0 1 0
V. multimaculata	2 0 1 0 1 1 0 1 0 0 2 1 1 0 1 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 0 2 2 0 1 1 1 1 1 0
V. orientalis	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 0 / 1 0 1 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 0 0 1 1 0 1 1 1 0 1 0
V. philippinensis	2 0 1 0 1 1 0 1 0 0 3 1 2 0 1 0 0 1 0 0 0 0 1 0 2 1 1 1 1 2 1 1 1 0 1 1 0 1 1 1 0 1 0
V. simillima	2 0 1 0 1 1 0 1 0 0 3 1 1 0 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 0 2 2 0 1 1 1 1 1 0
V. soror	2 0 1 0 1 1 0 2 0 0 3 1 2 0 1 1 0 0 1 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 0 0 0 1 0 3 1 1 0 1 0
V. tropica	2 0 1 0 1 1 0 1 0 0 3 1 2 0 1 1 0 0 0 0 0 1 0 2 1 1 1 1 1 2 1 1 1 0 0 1 0 1 1 1 0 1 0
V. velutina	2 0 1 0 1 1 0 1 0 0 2 1 1 0 0 1 0 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 0 2 2 0 1 1 1 1 1 0
V. vivax	2 0 1 0 1 1 0 1 0 0 3 1 1 0 1 0 0 1 0 0 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 0 0 2 2 0 1 1 1 1 1 0

Morphological codes
(F / M): character restricted to Females / Males

1. Prestigma length: (0) prestigma shorter than pterostigma, (1) prestigma longer than pterostigma, (2) prestigma length 3X pterostigma length.
2. Base of second submarginal cell: (0) M obliquely oriented with respect to m-cu1, (1) M vertically oriented (angle at M noticeable).
3. Placement of forewing m-cu2: (0) close to r-m2, (1) far from r-m2.
4. Hamuli placement: (0) beginning basad of fork of R1&Rs, (1) beginning at fork R1&Rs.
5. Hindwing jugal lobe: (0) present, (1) absent.
6. Hindwing axillary incision: (0) present, (1) absent.

7. (M) Tyloides: (0) two on apical flagellomeres, (1) one on apical flagellomeres, (2) absent.
8. Vertex length: (0) ocelloccipital distance short, < length of ocellar triangle, (1) ocelloccipital distance long, > length of ocellar triangle, (2) ocelloccipital distance long, gena produced behind eye.
9. Vertex indentation: (0) absent, (1) present.
10. Ocelli diameter: (0) less than distance between posterior ocellus and eye, (1) greater than this distance.
11. Interantennal space: (0) broad, rounded, (1) defined triangular area, (2) strongly elevated, with rounded edges, (3) defined triangular area strongly elevated, with sharp edges.
12. Clypeus dorsum: (0) straight, (1) bisinuate.
13. (F) Apex of clypeus: (0) pointed, (1) shallowly emarginated, anterior angles blunt, broad, (2) shallowly emarginated, anterior angles triangular.
14. (F) Mesal clypeal tooth: (0) absent, (1) present.
15. (F) Clypeal punctures: (0) sparse, superficial mesally, (1) dense mesally.
16. (M) Clypeal-eye contact: (0) touching, (1) gap.
17. (F) Malar space: (0) short, (1) long, > length of penultimate flagellomere.
18. Mandibular teeth: (0) pointed, (1) with elongate cutting edge, twice length of apical part.
19. Labial palpus third segment: (0) with strong seta, (1) without this seta, but with hairs.
20. Pronotal carina: (0) present, (1) dorsally reduced, (2) lateral remnants, (3) absent.
21. Pronotal carina dorsally: (0) largely transverse before scutum, (1) deeply U-shaped before scutum.
22. Pronotal carina laterally: (0) little interrupted by the pronotal fovea, (1) widely interrupted by the pronotal fovea.
23. Pretegular carina: (0) complete, (1) ventrally effaced, (2) absent.
24. (F) Pronotal striae: (0) absent, (1) few ventral striae, (2) dense ventral and dorsal striae.
25. Scutal lamella: (0) present, (1) absent.
26. Scutal punctures: (0) dense micropunctures, (1) superficial, (2) dense and deep micropunctures.
27. Epicnemial carina: (0) present, (1) absent.
28. Dorsal groove: (0) present, (1) absent.
29. Scutellum profile in lateral view: (0) bulging, (1) largely flat.
30. Metanotal orientation: (0) partly vertical, (1) largely vertical (dorsal surface reduced).
31. Metanotal lobe: (0) absent, (1) posteromedial lobe present.
32. Metapleural sculpture: (0) striae, (1) superficial punctures ventrally, (2) well-defined punctures ventrally.
33. Hind coxa carina: (0) absent, (1) present.
34. Metasomal segment I: (0) rounded in lateral view, (1) sharply angled between anterior and dorsal faces.
35. Metasomal segment I length: (0) short, (1) long.

36. Metasomal tergum II lateral macropunctures: (0) superficial to sparse, (1) dense, well defined.
37. (M) Apical margin of metasomal sternum VI: (0) almost straight, (1) shallowly emarginated, (2) deeply emarginated.
38. (M) Apical margin of metqasomal sternum VII: (0) convex, (1) shallowly emarginated, (2) deeply emarginated.
39. (M) Median process of metasomal sternum VII: (0) absent, (1) present.
40. (M) Aedeagal apex: (0) little differentiated, (1) transverse, projecting laterally, (2) rounded with subapical processes, (3) spade-shaped, (4) elongate, (5) narrow, (6) subcircular, (7) triangular.
41. (M) Aedeagal apical lobes: (0) absent, (1) apex forming expanded lobes.
42. (M) Aedeagus width: (0) narrow throughout, (1) as wide or wider apically as medially, (2) narrower apically than medially.
43. (M) Aedeagal shaft: (0) non-bulbous, (1) bulbous.
44. (M) Digital apical processes: (0) absent, (1) present.
45. (M) Inner apical margin of paramere: (0) obtusely angled with aedeagus, (1) forming right angle to aedeagus.

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RESEARCH ARTICLE

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A new genus and species of fairyfly, *Tinkerbella nana* (Hymenoptera, Mymaridae), with comments on its sister genus *Kikiki*, and discussion on small size limits in arthropods

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Abstract

A new genus and species of fairyfly, *Tinkerbella nana* (Hymenoptera: Mymaridae) **gen. n.** and **sp. n.**, is described from Costa Rica. It is compared with the related genus *Kikiki* Huber and Beardsley from the Hawaiian Islands, Costa Rica and Trinidad. A specimen of *Kikiki huna* Huber measured 158 µm long, thus holding the record for the smallest winged insect. The smallest size possible, as measured by body length, for flying insects and wingless arthropods is discussed.

Keywords

Mymaridae, *Tinkerbella*, *Kikiki*, *Dicopomorpha*, *Alaptus*, smallest insect, smallest arthropods

Introduction

The family Mymaridae (Hymenoptera) includes the smallest known insects. Enock (1895) observed a species of *Alaptus* Westwood searching for and parasitizing eggs of *Psocus fasciatus* Fabricius [now *Loensia fasciata*] (Psocoptera: Liposcelididae) and aptly gave it the English common name fairy-fly, presumably because of its small size and delicate wings with long fringes, resembling the mythical fairies. A specimen of *Alaptus borinquensis* Dozier was reported as being as small as 186 μm (Dozier 1932). A male of *Dicopomorpha echmepterygis* Mockford measuring 139 μm in length is the smallest insect ever recorded but males are greatly modified though losses of various body parts (Mockford 1997, Huber and Landry 2000, Huber 2009a, fig. 12.2). *Megaphragma caribea* Delvare (Trichogrammatidae) has been recorded at 170 μm (Delvare 1993) and was, until now, the smallest winged insect known. Almost 2000 years ago, Pliny the Elder (ca. 23–79 A.D.) stated "*Rerum natura nusquam magis quam in minimis tota est*" loosely translated as "nature is nowhere as great as in its smallest." In the absence of any means of magnification he could not possibly have seen the intricate structure and beauty of fairyflies or other minute organisms. But his statement certainly holds true.

Here we describe a new genus of Mymaridae from Costa Rica with specimens below 250 μm in length. We compare it with specimens of *Kikiki*, a genus described first from the Hawaiian Islands (Huber and Beardsley 2000b), then recorded from Australia (Lin et al. 2007), Argentina (Luft Albarracin et al. 2009), Costa Rica, and Trinidad and Tobago (this paper). Modified couplets for the key to *Alaptus*-group genera (Huber 2009b) are provided to account for the new genus but both it and *Kikiki* are shown to be misplaced there. Small body size in insects and other arthropods is discussed.

Methods

Specimens were collected in Costa Rica by JSN using a heavy triangular sweep net fitted with a galvanized metal screen with 4 mm mesh over the opening. Sweeping was done by gently dragging the net through vegetation and dumping the net contents at five minute intervals into 80% ethanol in a sturdy polythene bag to reduce damage to insects in the debris. Sweeping was done for two hours periods (barring rain) so different samples could be compared, if necessary. The samples were sorted later in a laboratory using the method described here to ensure that all the smallest Hymenoptera were found. The procedure is:

1 Stir the entire sample in 80% ethanol very gently to cause the smaller insects to float up from the bottom.
2 Using a 3 ml teaspoon, take enough of the floating material to fill it (a slight stir allows one to gently place the teaspoon into the sample to allow material to settle on to it).
3 Add ethanol from the sample jar to a sorting dish to a depth of 2–3 mm. The sorting dish used in this case consisted of a 9 cm plastic Petri dish with grooves

scored at 1 cm intervals on the outside and made visible by drawing black lines in India ink.

4 Examine the sorting dish contents under a binocular microscope by gently moving material across with a pair of forceps and extracting the insects desired. When finished, pour the sorted waste into a 500 g jar.

5 Repeat steps 1–4.

6 After a few repetitions gently stir the jar containing the waste ethanol, allow it to settle for 10 seconds, then pour the supernatant gently into the sorting dish as in step 3 and scan for any remaining small insects.

7 When the unsorted sample has too little ethanol to stir it properly, gently stir the waste jar, allow it to settle for 10 seconds, and use the supernatant ethanol to top up the unsorted sample.

8 If there is not enough ethanol for step 8, add fresh ethanol to the waste jar, stir it slightly, and repeat step 6.

Specimens retrieved were critical point dried and card mounted. Photographs were taken with a digital scanning camera attached to a microscope, and the resulting layers combined electronically using Syncroscopy Auto-Montage™ and, except for primary types, retouched as needed with Adobe™ Photoshop. Micrographs were taken with a Philips environmental scanning electron microscope (ESEM) from uncoated specimens on their cards. A few specimens were slide mounted, either with prior clearing in 10% KOH or without clearing.

Morphological terms are according to Gibson (1997) and Huber (2012). Abbreviations used are: fl_x = funicle segment x; gt_x = gastral tergum x. Measurements in micrometres (μm) were taken from card mounted specimens at 200× using an ocular micrometer on a Zeiss microscope, and the two smallest specimens of *Kikiki* were also measured with the ESEM measurement tool. Slide-mounted specimens were measured at 200× or 400× magnification using a filar micrometer on a compound microscope. Specimens are deposited in the following institutions:

BMNH Department of Entomology, The Natural History Museum, London, UK.
CNC Canadian National Collection of Insects, AAFC, Ottawa, ON, Canada
INBio Instituto National de Biodiversidad, San José, Costa Rica.
UCRC University of California, Riverside, CA, USA.

Taxonomy

***Tinkerbella* Huber & Noyes, gen. n.**
urn:lsid:zoobank.org:act:E3F95FC3-C247-41D3-90F9-47C7724EA7E3
http://species-id.net/wiki/Tinkerbella

Type species: *Tinkerbella nana* Huber and Noyes.

Derivation of genus name. After the fairy Tinker Bell in the 1904 play "Peter Pan" by J.M. Barrie. Gender: feminine.

Diagnosis. *Tinkerbella* is defined by the following combination of features: body length at most about 250 μm; female antenna with funicle 5-segmented and clava entire (Figs 1, 3, 5, 13), compound eye with about 50 ommatidia (Figs 11, 13), and tarsi 4-segmented (Fig. 22).

Kikiki is the most closely related genus to *Tinkerbella*. It is distinguished from *Tinkerbella* by the following combination of features: female with funicle 4-segmented and clava 2-segmented (Figs 24, 37, 38), compound eye with about 20–25 ommatidia (Figs 24, 38, 39, 40), and tarsi 3-segmented (Fig. 47, 48).

Description. Female. Body minute, at most about 250 μm long. **Head** in dorsal view (Fig. 12) about 1.6× as wide as long, in lateral view (Fig. 11) about 1.6× as high as long, and in anterior view (Figs 5, 9) about 1.3× as wide as high. Face slightly longer than wide and ventrally separated from clypeus by curved epistomal sulcus. Toruli about their own diameter from transverse trabecula. Clypeus apparently extending entire width of mouth opening, with anterior tentorial pits visible, slit-like (Fig. 10). Mandible with 4 teeth, the lower one more distinct than the upper three (Fig. 10). Eye with about 50 facets, in lateral view about 1.4× as high as long and not extending to back of head dorsally. Malar distance about 0.4× eye height, with straight malar sulcus. Vertex at about right angle to face above toruli, with supraorbital trabecula divided medially into two parts. Ocelli in normal triangle with POL: LOL: OOL about 1.1: 0.5: 0.5, enclosed by conspicuous rectangular stemmaticum and with sulci extending laterally to endpoints of posterior section of supraorbital trabecula (Fig. 12); occiput divided by transverse sulcus just dorsal to foramen medially and ventral to eyes laterally (Fig. 17). **Antenna**. Scape about 3.9× as long as wide, with faint longitudinal reticulations on outer surface and distinct transverse ridges on inner surface (Figs 5, 13). Pedicel about 0.2× as long as scape. Funicle 5-segmented. clava entire (a faint, partial division visible, however) (Figs 5, 13). **Mesosoma**. About 1.34× as long as high and about 1.3× as long as wide. Pronotum thin, in dorsal view scarcely visible (Fig. 7), in lateral view with large lateral panel extending posteriorly to level of wing base (Fig. 11). Mesoscutum (Figs 16, 17) slightly longer than scutellum, with deep notauli. Anterior scutellum and axilla completely separated by deep straight transverse sulcus from slightly longer, longitudinally divided frenum. Second phragma projecting to apex of propodeum. Metanotum with dorsellum distinct, about 0.5× as long as frenum, apparently divided by a median longitudinal groove (Figs 16, 17), and much wider than very narrow lateral panel of metanotum (Figs 16, 17). Mesopleuron (Fig. 17) divided by fairly straight, faint transepimeral sulcus into narrower mesepisternum and wider mesepimeron. Metapleuron triangular. Propodeum in dorsal view (Fig. 16) with anterodorsal area mostly smooth, posterodorsal area with minute rounded spicules medially, with propodeal seta about midway between spiracle and posterior margin of propodeum; in lateral view (Fig. 18) strongly sloping. Propodeal spiracle circular, smaller than pronotal spiracle and distinctly separated from anterior margin of propodeum. **Wings**. Fore wing (Figs 6, 8, 20) narrow, almost parallel sided, with posterior margin distinctly concave beyond pronounced lobe (Fig. 21) at level of parastigma;

Figures 1, 2. Habitus, lateral. **1** *Tinkerbella nana* female **2** *Kikiki huna*, female. Scale line = 100 μm.

wing surface bare except for fewer than about 10 scattered microtrichia on dorsal and ventral surfaces; fringe setae much longer than wing width. Venation extending almost 0.7× fore wing length; submarginal vein about 1.6× parastigma length, and parastigma about 0.5× marginal + stigmal vein lengths (their relative proportions about 24/15/29); hypochaeta close to proximal macrochaeta (Fig. 21); parastigma with distal macrochaeta

about 3× as long as proximal macrochaeta (Fig. 21). Hind wing (Figs 6, 8) curved, with anterior margin concave, paralleling convex posterior margin, apex bluntly rounded, with long fringe setae at apex and along posterior margin to just beyond level of relatively large hamuli; wing surface bare except for row of fewer than about 8 microtrichia along anterior margin except toward hamuli where they are located more posteriorly. **Legs**. Tarsi 4-segmented, tarsomere 1 slightly shorter than remaining tarsomeres, and tarsomere 4 distinct, as long as tarsomere 3 (Fig. 22). **Metasoma**. Slightly shorter than mesosoma (critical point dried specimens) (Fig. 7); petiole very short (scarcely visible), about 4.3× as wide as long, but clearly narrower than gt_1; gaster in dorsal view distinctly narrower anteriorly than medially (Fig. 7) with gt_6 the longest tergite and apparently without a spiracle. Ovipositor (Figs 7, 18) as long as gaster, slightly exserted beyond gastral apex. Cerci with 3 long setae extending just beyond ovipositor apex (Fig. 19).

Male. Antenna 11-segmented (Fig. 14), with 2 mps each, except fl_3 and fl_5 much shorter and without mps (Fig. 15), the mps decreasing in width on each segment, from almost circular on fl_1 to linear on fl_{11}.

Tinkerbella nana Huber & Noyes, sp. n.

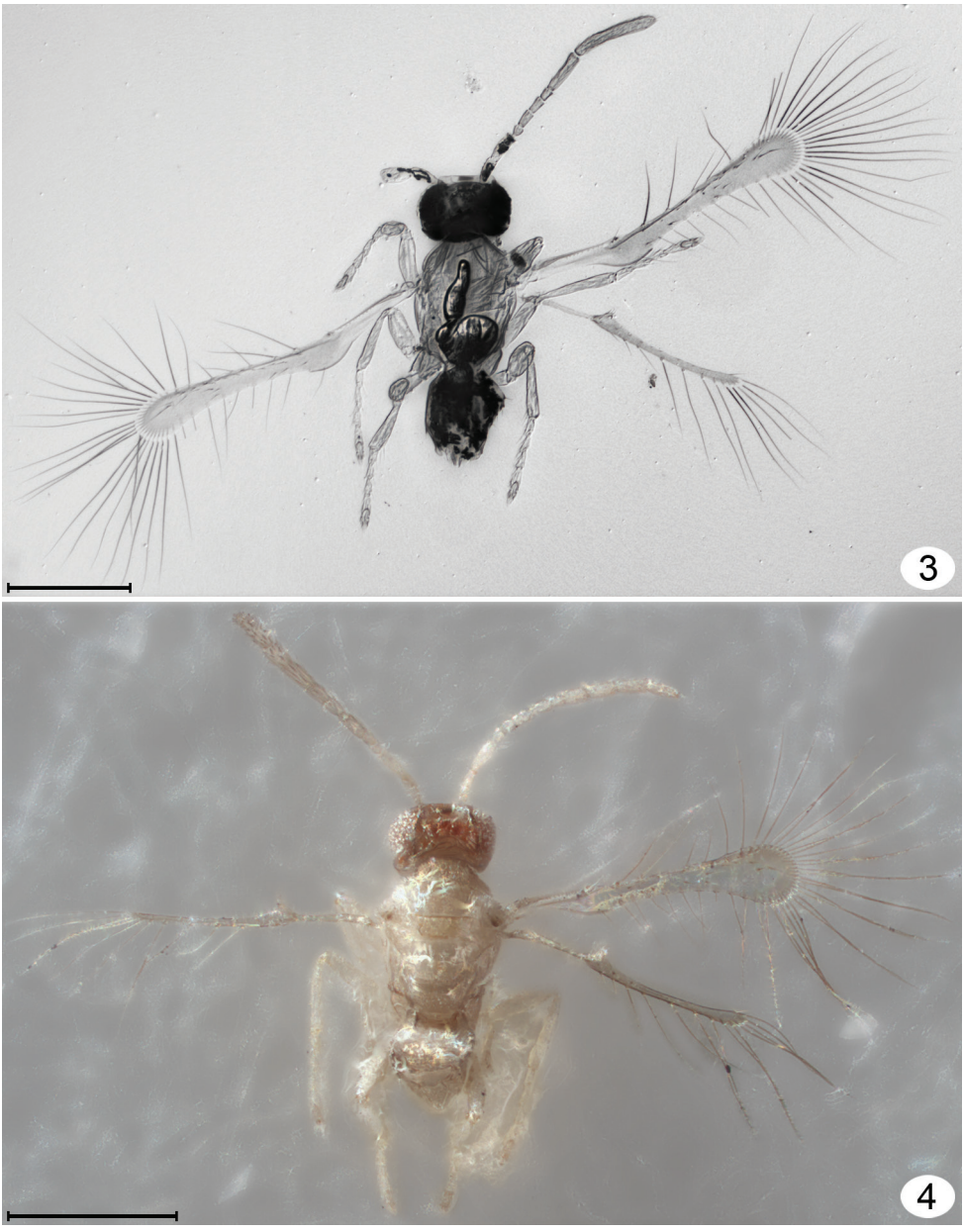
urn:lsid:zoobank.org:act:EFCB279C-935F-4098-873F-4B7C5C935E8F
http://species-id.net/wiki/Tinkerbella_nana
Figs 1, 3–22

Holotype ♀ (INBio) on slide labelled, 1. "COSTA RICA: Heredia, La Selva, 75m, 10°26'N, 84°01'W, 27–28.ii.2003, J.S. Noyes, sweeping, cleared in clove oil, mounted in Canada balsam". 2. "Tinkerbella nana Huber and Noyes ♀dorsal Holotype".

Paratypes. 7♀ and 2♂. **COSTA RICA**: **Alajuela**. Reserva Rincón Forestal, Estación Caribe, 400m, 10°53'N, 85°18'W, 400m, 19–22.ii.2003, J.S. Noyes (1♀, 1♂, CNC); Arenal National Park, sendero Pilón 10°27'N, 84°43'W, 600m, 26.ii.2003, J.S. Noyes (1♀, CNC). **Heredia**. Same data as holotype (5♀, 1♂, CNC, BMNH, InBio).

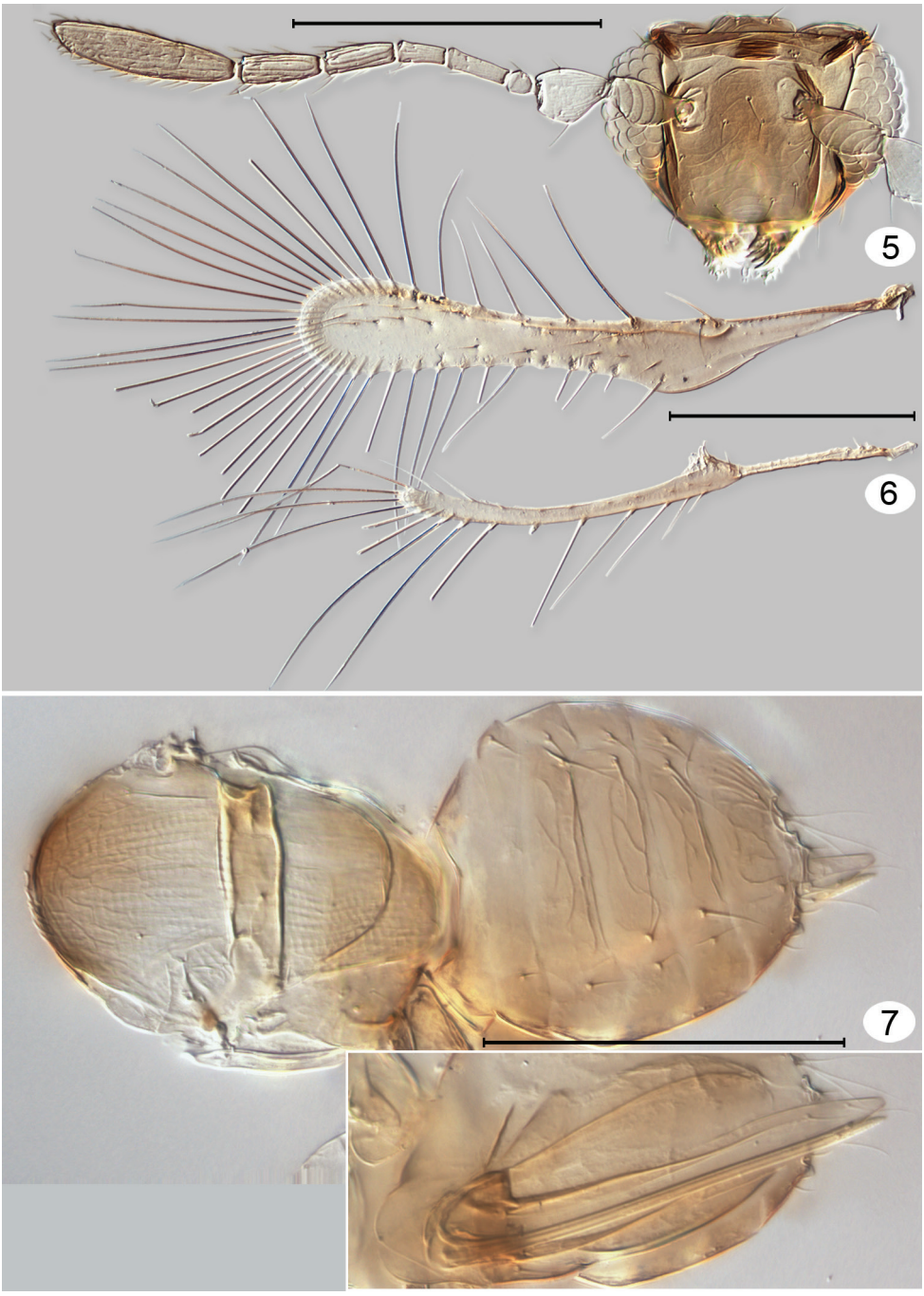
Derivation of species name. After the dog Nana in Peter Pan and coincidentally from *nanos*, the Greek word for dwarf. Treated as a (feminized) noun in apposition.

Description. Female. Body length 225–250 μm (n=6). **Colour.** Very pale, the scape, pedicel, pronotum laterally, gaster laterally, and legs except apical tarsomere sometimes lighter, almost white; head, mandibles, mesoscutum, anterior scutellum and propodeum with a pale yellow or pale brown tinge, occasionally head and mesosoma, especially mesoscutum, more uniformly and extensively brown; trabeculae and a minute spot next to fore wing base dark brown; eyes and ocelli distinctly reddish (Fig. 3). Fore wing with brown tinge behind most of venation except its apex (Fig. 6). Hind wing fairly uniformly light brown from just before hamuli to slightly lighter apex. **Head**. 65–99 wide, with transverse reticulate sculpture on face (Fig. 9), vertex (Fig. 12), and occiput (Fig. 12). **Antenna**. Flagellum (Figs 5, 13) with 1 mps on fl_4, 1 mps on fl_5, and 4 mps on clava. Fl_1–clava length/width (n=2–width or 4–length): scape, 25–30/13–14, pedicel 19–27/17–18, fl_1 8–10/7–8, fl_2 12–21/6–7, fl_3 11–16/7–

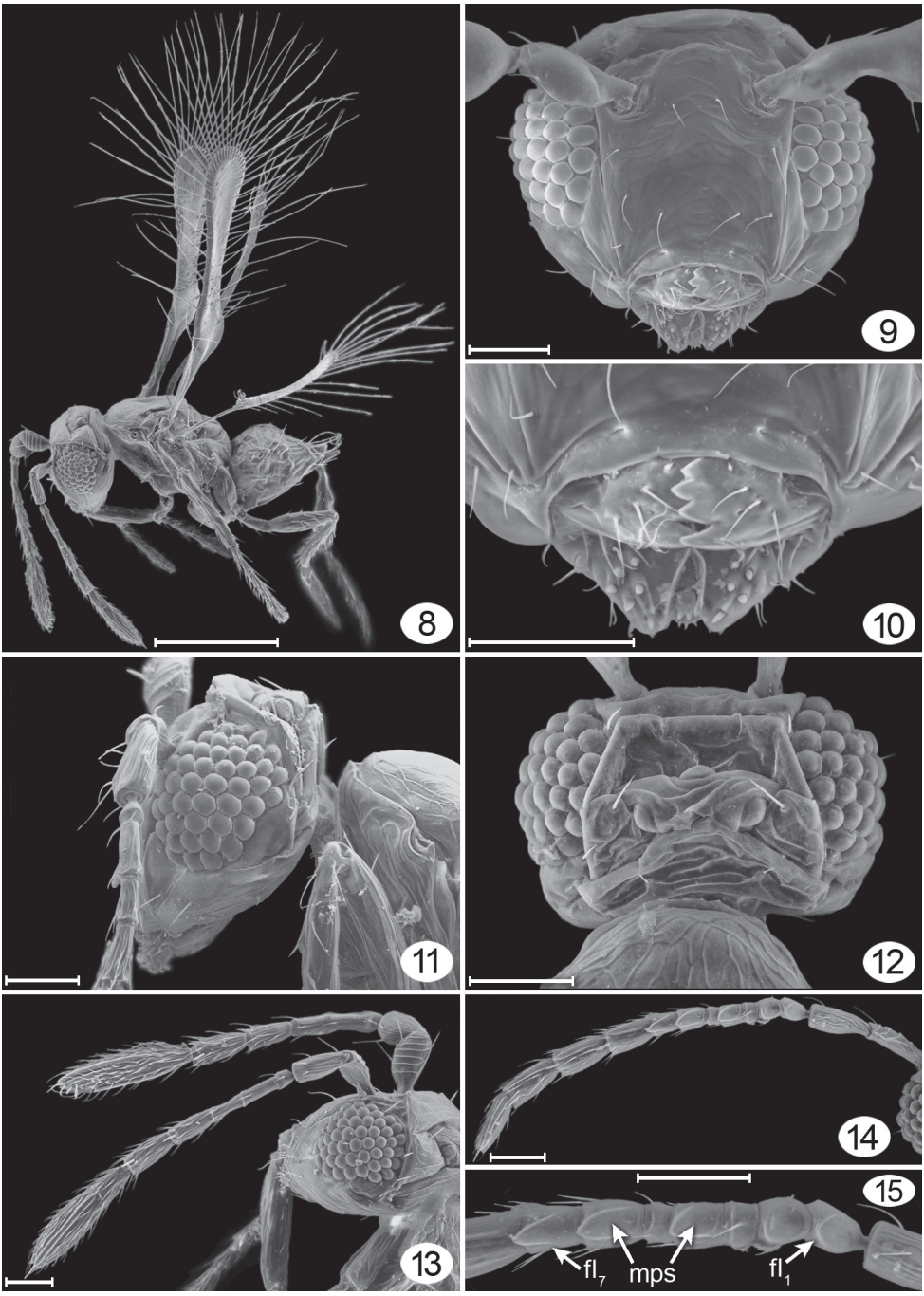


Figures 3, 4. *Tinkerbella nana* female. **3** holotype, uncleared on slide **4** paratype, critical point dried on card. Scale line = 100 μm.

8, fl_4 8–24/9, fl_5 23–31/10–11, clava 59–65/15–17. **Mesosoma**. Mesoscutum (Figs 7, 16, 17) with slightly longitudinal, reticulate sculpture and raised meshes. Scutellum smooth anteriorly, frenum slightly wrinkled. Dorsellum smooth to slightly wrinkled. **Wings**. Fore wing with a few scattered microtrichae behind venation from proximal



Figures 5–7. *Tinkerbella nana* female paratype, cleared on slide. **5** head + right antenna, anterior **6** wings **7** mesosoma + metasoma dorsal, and ovipositor ventral (inset). Scale line = 100 μm.



Figures 8–15. *Tinkerbella nana* paratype female (except 14, 15), micrographs. **8** habitus, lateral **9** head, anterior **10** mouthparts, anterior **11** head + pronotum, lateral **12** head, dorsal **13** head + antennae, lateral **14** male antenna, dorsal **15** male antenna, pedicel–fl_8, dorsal. Scale line = 20 μm, except Fig. **8** = 100 μm.

macrochaeta on, and 3 or 4 just beyond venation (Fig. 6). Fore wing length 195–240 (n=5, card mounts at 200×), width 30–50, longest marginal setae 100–155; hind wing length 145–200, width 5–10, longest marginal setae 105–130. **Metasoma**. Petiole 34 wide, 7 long (n=1). Gaster with segments somewhat wrinkled and sometimes transversely creased posteriorly (Figs 7, 18); gt_2–gt_6 each with a few fairly long, suberect setae dorsally and laterally.

Male. Body length 210–230 μm (n=2). Colour light brown (Fig. 4). Fl_3 and fl_5 the shortest segments, less than half length of the segment following and without mps (Fig. 15).

Hosts and habitat. Unknown. The specimens from Alajuela were collected by sweeping in fairly young secondary forest (20 years old maximum) mixed with a small amount (ca. 1 ha) of primary forest.

Distribution. Costa Rica.

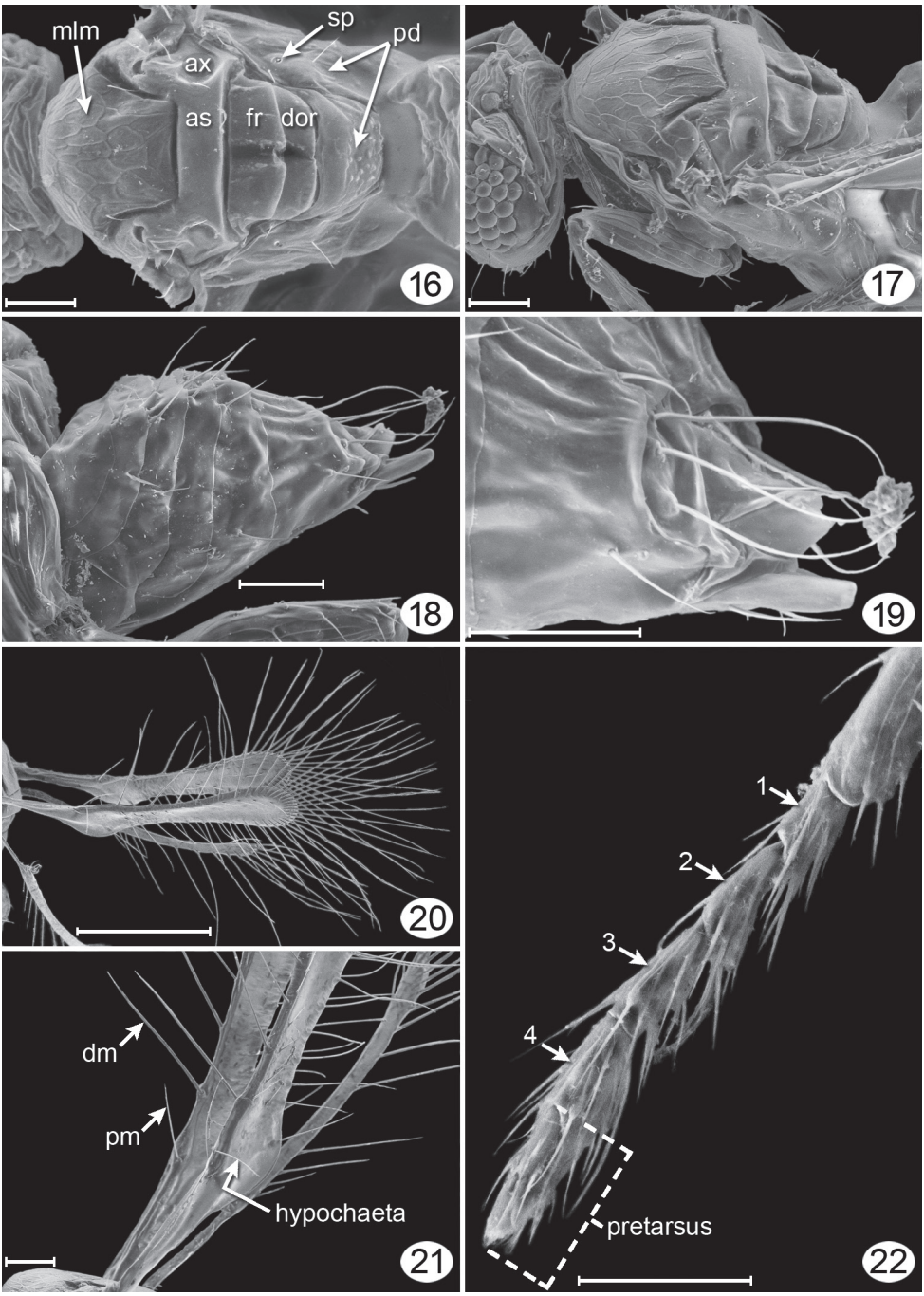
Kikiki Huber & Beardsley

http://species-id.net/wiki/Kikiki

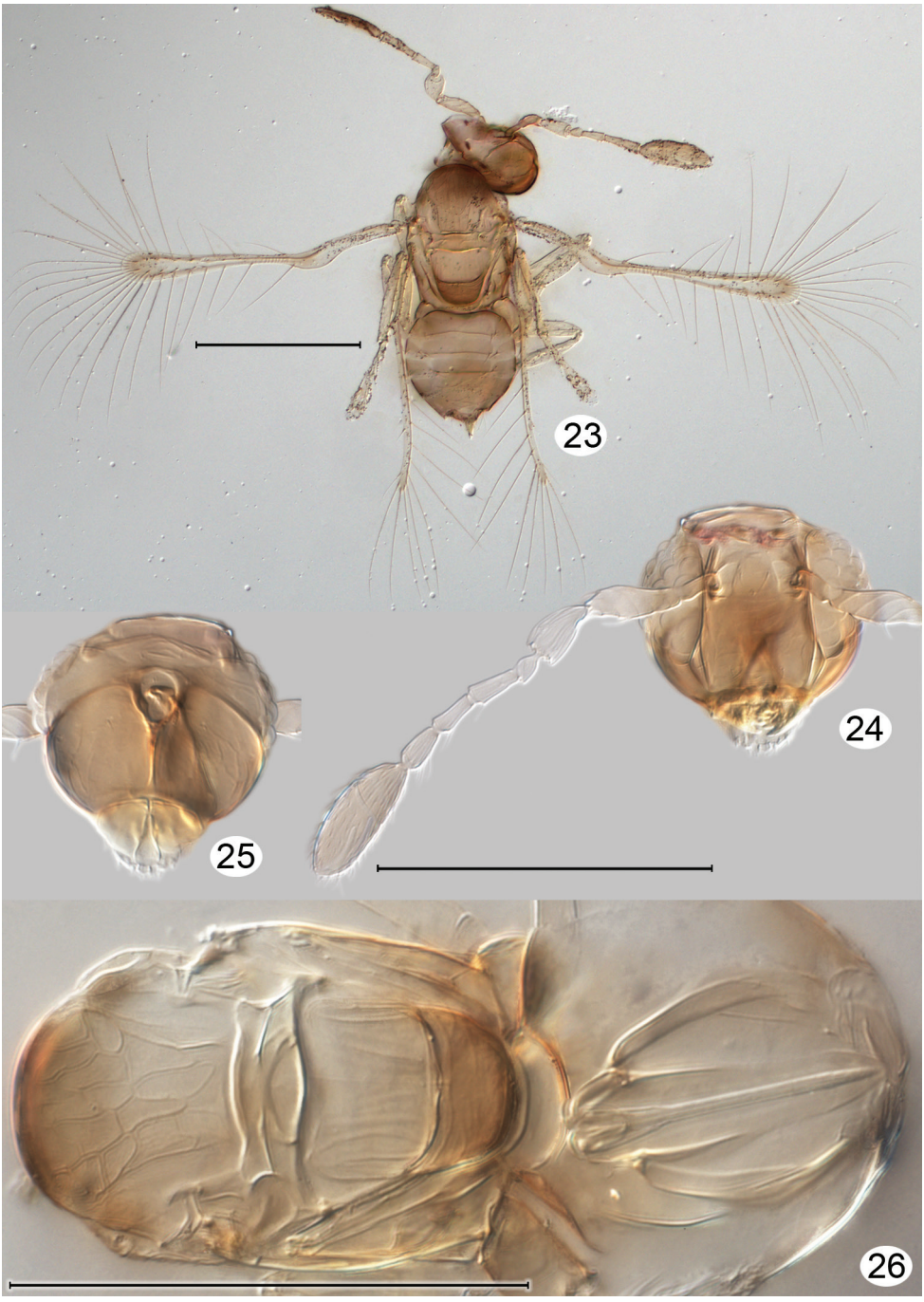
Kikiki: Huber and Beardsley 2000b: 66 (original description); Huber 2009b: 235 (key); Luft Albarracin et al. 2009: 12 (key); Lin et al. 2007: 16 (key [male]), 37 (diagnosis).

Remarks. Specimens of *Kikiki* from Costa Rica were collected and examined since the original generic description based on eight specimens from the Hawaiian Islands (Huber and Beardsley 2000b). Additional features not seen clearly or at all on the slide-mounted type series can therefore be described. Based on the images and better slide mounts, several corrections to the original description are made: the axilla is distinctly advanced (Figs 26, 44), the lateral lobe of the mesoscutum has 1 seta (Fig. 26), and the second phragma (mesophragma) does not project into the gaster. A cleared and slide mounted Costa Rica specimen has 3 mps on the apical claval segment but none elsewhere, and JTH checked one paratype (CNC) and found that it also lacks mps on fl_4 and on the basal segment of the clava—the apparent mps (Huber and Beardsley 2000b, fig. 3) are actually not mps but an artifact of lighting.

Description. Female. **Head**. Face about 1.7× as high as wide, slightly depressed medially (Fig. 24), separated ventrally from oral cavity by distinct epistomal suture. Clypeus transverse, in same plane as face, narrowly oval and extending entire width of oral cavity, with anterior tentorial pits visible sublaterally (Fig. 37). Mandible with lower tooth separated from remaining teeth by deeper notch than notches separating the teeth above it. Vertex (Figs 30, 40) laterally with well defined supraorbital trabecula divided medially; ocelli enclosed by a distinct, rectangular stemmaticum, the vertex thus divided into anterior (smooth) and posterior (faintly sculptured) areas by a transverse groove extending between eyes from just anterior to each supraorbital trabecula and along anterior margin of slightly triangular mid ocellus; lateral ocelli almost vertical, facing away from each



Figures 16–22. *Tinkerbella nana* female paratype, micrographs. **16** mesosoma, dorsal **17** mesosoma + back of head, dorsolateral **18** metasoma, lateral **19** apex of gaster, dorsolateral **20** wings (left wing ventral surface, right wing dorsal surface) **21** wing, basal half **22** left mesotarsus. See appendix for abbreviations. Scale line = 20 μm, except Fig. **20** = 100 μm.



Figures 23–26. *Kikiki huna* female, on slide (cleared, except Fig. 23). **23** habitus, dorsal **24** head + right antenna, anterior **25** head, posterior **26** mesosoma, dorsal + metasoma, dorsal but focus at lower plane to show ovipositor. Scale line = 100 μm.

Figures 27–29. *Kikiki huna* female, cleared on slide. **27** wings **28** metasoma, dorsal surface **29** metasoma, ventral surface (seen dorsally through cleared metasoma). Scale line = 100 μm.

other. Occiput separated from vertex by slightly curved groove extending between posterior apex of each supraorbital trabecula and almost touching lateral ocelli (Figs 40, 41), and occiput divided into dorsal and ventral areas by curved groove extending between lower margin of eyes and above foramen (Figs 25, 42). Head, except vertex anteriorly, with faint, reticulate sculpture. **Mesosoma**. Pronotum with a slightly crenulated dorsal margin (Figs 40, 42). Mesoscutum (Figs 26, 42, 44) with notauli narrow near anterior apex, distinctly widening (narrowly triangular) posteriorly to the medially deep and wide transscutal articulation. Scutellum with axilla strongly advanced anteriorly into lateral lobe of mesoscutum. Frenum apparently divided mediolongitudinally by a wide depression (Figs 44, 45). Mesosoma except anterior scutellum with faint, reticulate sculpture. **Legs**. Fore leg with bifurcate strigil and calcar with 4 rounded teeth, separated from one another by almost their own diameter (Fig. 34-male). **Metasoma**. Petiole (Fig. 26) extremely short and distinctly narrower that gt_1. Terga each with a median transverse fold extending across tergum medially or nearer posterior margin (Figs 28, 45, 46).

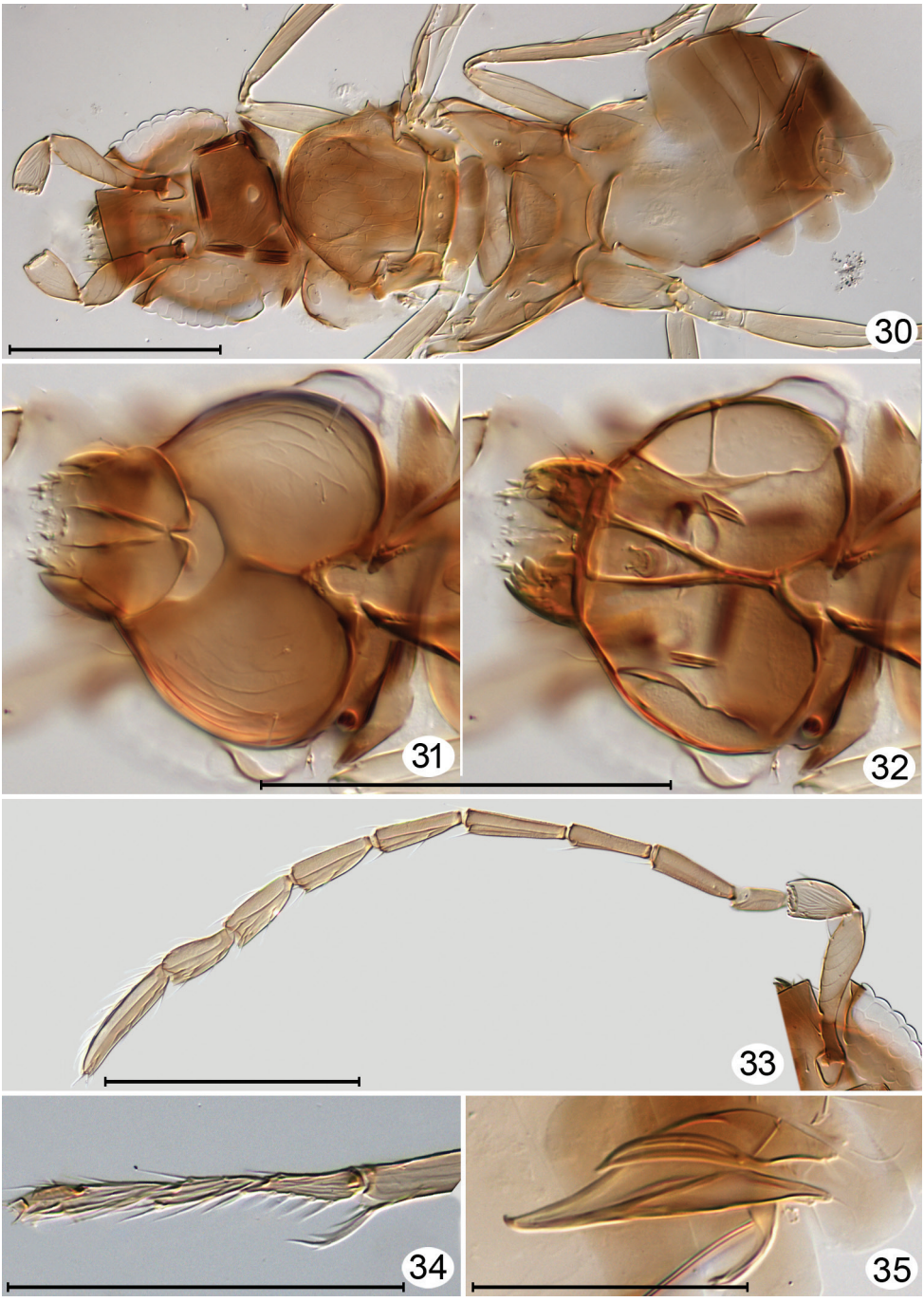
Male. Body 235 μm from transverse trabecula to gastral apex (Fig. 30). Antenna with 9-segmented funicle (Fig. 33; Lin et al. 2007, fig. 154), with fl_1 the shortest and fl_9 the longest, and each segment with 2 mps. Eye large (Fig. 30) with many ommatidia (about 40?); ocular apodeme long, narrow and parallel side for most of its length, slightly widened apically (Fig. 32). Mandibles with 4 teeth. Occiput with strongly curved groove (Fig. 31) different in shape from female, apparently extending from mouthparts, along posterior eye margin, and inwards to lateral margins of foramen. Petiole short but distinct, about 3.7× as wide as long. Spiracle absent. Cerci with 4 setae. Genitalia encapsulated; aedeagus almost as long as aedeagal apodemes (Fig. 35).

The male is described from a single slide-mounted specimen from Australia. No males have yet been collected in the New World. Whether the larger body and eyes of the Australian specimen are sex differences or indicative of a species different from *K. huna* cannot be determined until females are obtained from the same locality in Western Australia and compared with the Hawaiian and Neotropical specimens.

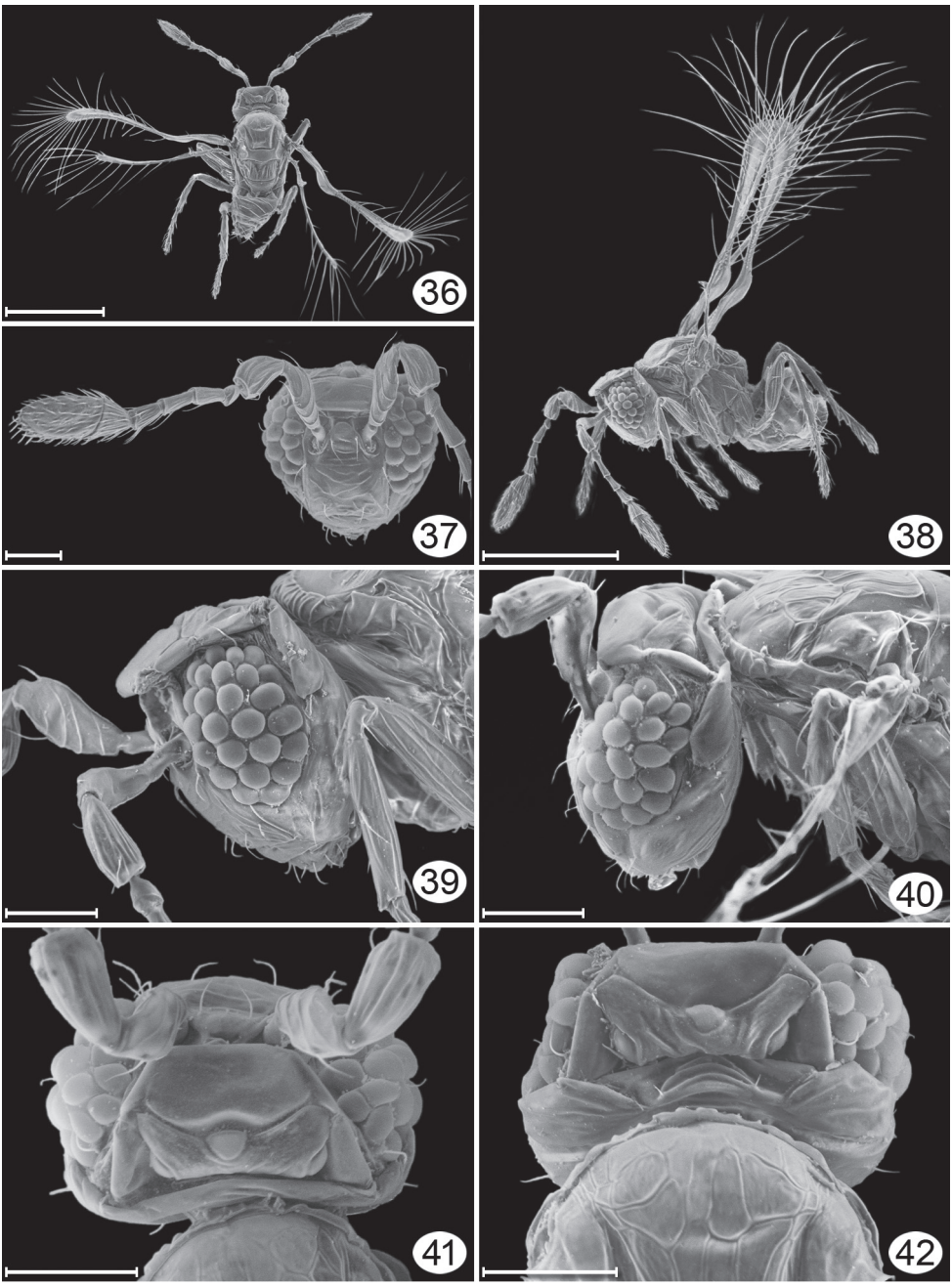
***Kikiki huna* Huber & Beardsley**
http://species-id.net/wiki/Kikiki_huna
Figs 2, 23–48

Description. Female. Body length (critical point dried specimens) 158–190 μm (n=10). **Antenna**. Funicle segments and basal claval segment without mps, apical claval segment with 3 mps (Figs 24, 43). Antennal length/width measurements (n=4, Costa Rica specimens): scape, 36–47/10–11, pedicel 19–25/12–13, fl_1 8–9/5–6, fl_2 16–18/6, fl_3 14–17/6–7, fl_4 12–18/7–8, clava 46–54/15. **Wings.** Fore wing (Fig. 27) length 182–226, width 20–24, length/width 9.10–9.24, longest marginal setae 102–123 (n=3, slide mounts), hind wing (Figs 23, 27) length 162–198, width 4–5, longest marginal setae 96 (n=1–3).

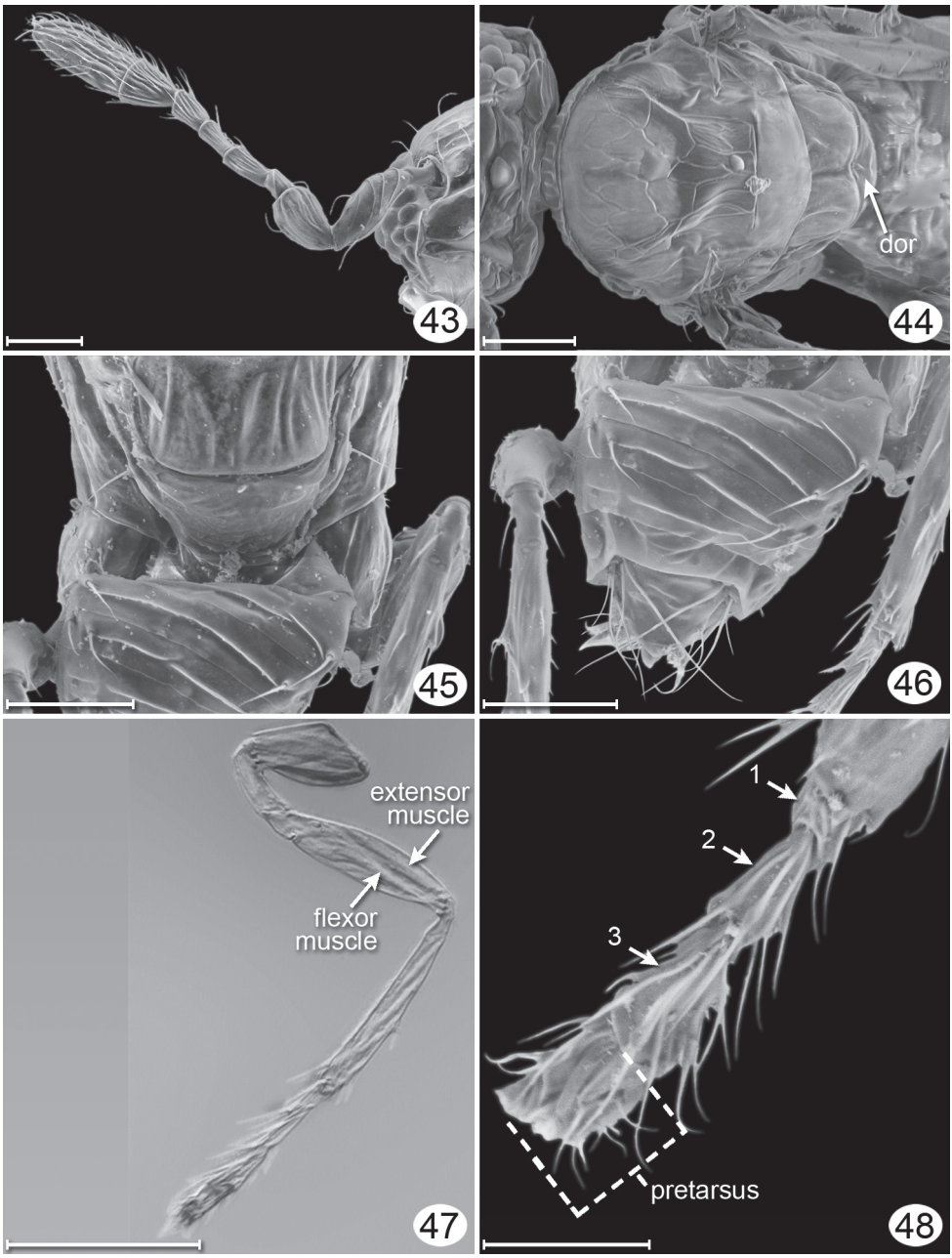
Male. Unknown for Neotropical region.



Figures 30–35. *Kikiki* sp. male, cleared on slide. **30** head anterodorsal + mesosoma and metasoma, dorsal **31** head, posterior **32** head from anterior, focused at different plane to show internal skeleton and mandibles **33** antenna **34** protarsus **35** genitalia, dorsolateral. Scale line = 100 μm, except Figures **34** and **35** = 50 μm.



Figures 36–42. *Kikiki huna* female, micrographs. **36** habitus, dorsal **37**, head + antennae, anterior **38** habitus, lateral **39** head, lateral **40** head + anterior mesosoma, dorsolateral **41** head + base of antenna, dorsal **42** head, posterodorsal + anterior mesosoma, dorsal. Scale line = 20 μm, except 36, 38 = 100 μm.



Figures 43–48. *Kikiki huna,* female, micrographs except Fig. 47. **43** antenna, lateral **44** mesosoma, dorsal **45** frenum – anterior half of metasoma, dorsal **46** metasoma, dorsal **47** hind leg, uncleared lateral, showing muscles; 48, right metatarsus, dorsal. Scale line = 20 μm, except Fig. 47 = 50 μm and **48** = 10 μm.

Material examined. 20♀. **COSTA RICA**. **Heredia**. La Selva Biological Station, 10°26'N, 84°01'W, 75m, 27–28.ii.2003, J.S. Noyes (1♀, CNC). **Puntarenas**. La Gamba Biological Station, 8°42'N, 83°12'W, 150m, 13–14.ii.2006, J.S. Noyes (1♀, BMNH); Reserva Absoluta Cabo Blanco, 9°35'N, 85°36'W, 30m, 16–17.ii.2009, J.S. Noyes, sweeping (9♀, CNC, INBio, UCRC); Reserva Privada Karen Morgensen, 9°52'N, 85°03'W, 305m, 23–24.ii.2007, J.S. Noyes, sweeping (6♀, BMNH). **HAWAIIAN ISLANDS**. **Molokai I.**: Mapulehu (1♀ paratype, CNC). **TRINIDAD & TOBAGO**. **Trinidad**. Curepe, Santa Margarita Circular Road, 8.xii.1974–2.ii.1975, F.D. Bennett (2♀, CNC).

Discussion. We cannot find any morphological differences suggesting that the specimens from Costa Rica and Trinidad are different from the Hawaiian specimens. The number and distribution of mps on the antennal segments as reported by Huber and Beardsley (2000b) are incorrect, as mentioned above. Both the Hawaiian and the Neotropical American specimens have the same mps distribution. The body length of the former averages slightly larger, from 190–ca 330 μm long (Huber and Beardsley 2000b) but this is insufficient evidence for species separation.

At the genus level, and even the species level, the Hawaiian fauna at low elevation appears to be almost entirely represented by exotic species (Huber and Beardsley 2000a, Beardsley and Huber 2000, Triapitsyn and Beardsley 2000), except for one genus (*Polynema*) that has numerous, native species at higher elevations. Although *K. huna* appeared to be endemic (Huber and Beardsley 2000b), this is simply because specimens of the genus had not yet been collected elsewhere—not surprising given their minute size. Specimens have since been found in Argentina (Luft Albarracin et al. 2009), Australia (Lin et al. 2007) and Costa Rica (this paper) indicating that the genus is widespread. Given its mostly low elevation range in the Hawaiian Islands it was almost certainly accidentally introduced from elsewhere. We therefore treat all the specimens as the same species and suggest *K. huna* in the Hawaiian Islands came originally from Central America.

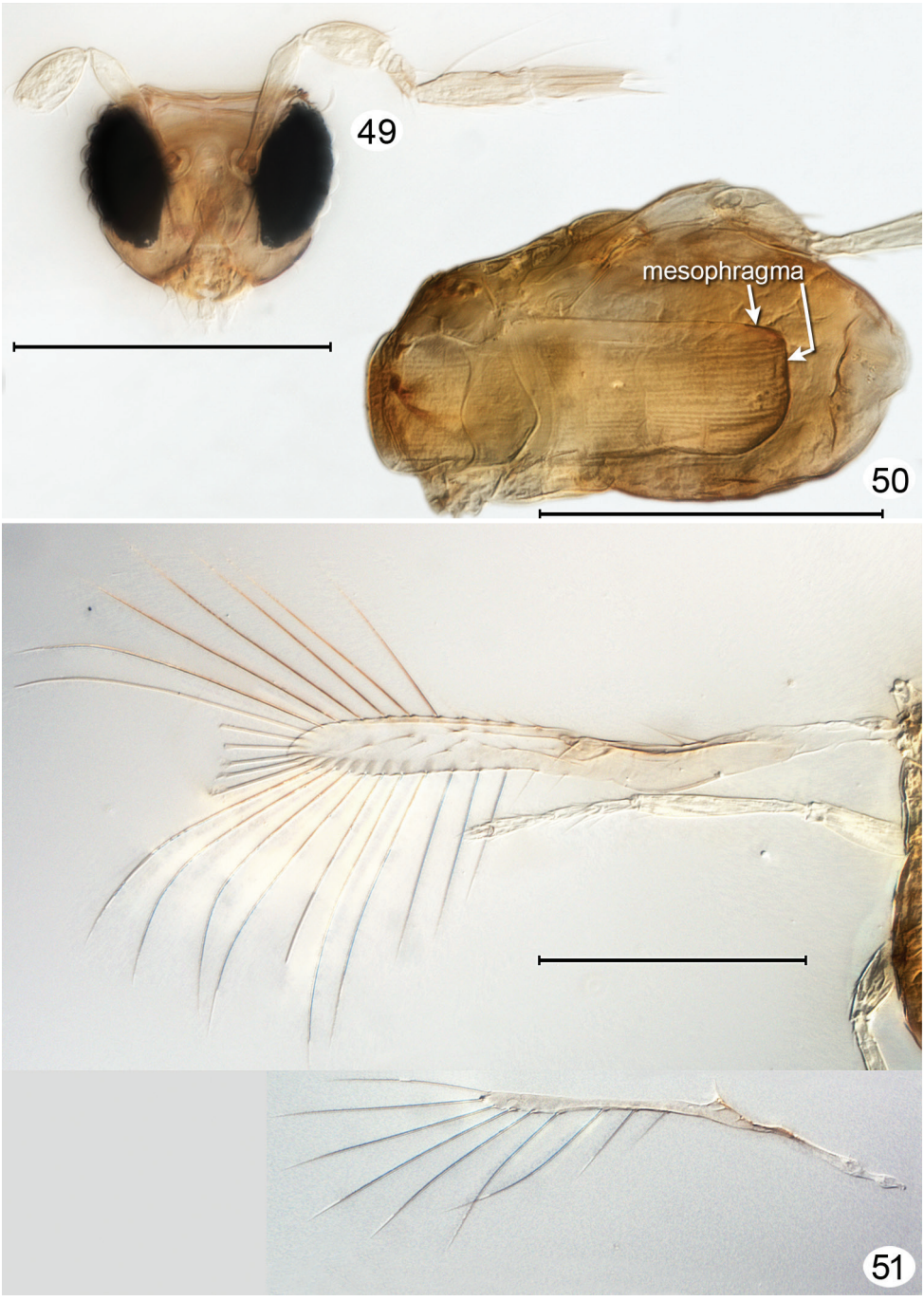
Tinkerbella would key to *Kikiki* in Huber (2009b) because at the time he thought that the genus included species with a variable number of tarsomeres (3 or 4) and, in females, funicle segments (4 or 5) and clava segments (1 or 2). Because *Tinkerbella* is distinct on other features as well it is described here as a new genus. Huber (2009b) and Lin et al. (2007) included *Kikiki* in the *Alaptus* group of genera, mainly because of its minute size. If the key in Huber (2009b) is used, couplet 1 should be deleted and replaced with the following two couplets at the beginning of the key to separate *Tinkerbella* and *Kikiki* from the remaining *Alaptus*-group genera, and to distinguish them from each other.

1 Venation long, clearly more than half wing length; tarsi 3- or 4-segmented.... **1a**

– Venation short, clearly less than half wing length; tarsi 5-segmented..... ..... **couplet 2 in Huber (2009b)**

1a Clava 2-segmented, funicle 4-segmented; tarsi 3-segmented .... ***Kikiki* Huber and Beardsley**

– Clava entire; funicle 5-segmented, tarsi 4-segmented .... ***Tinkerbella* Huber and Noyes**



Figures 49–51. *Megaphragma* sp., uncleared on slide. **49** head + antenna, anterior (note black eyes) **50** mesosoma + metasoma, dorsal (note huge mesophragma, hence the genus name) **51** wings + middle leg (note 3-segmented tarsi, diagnostic for Trichogrammatidae). Scale line = 100 μm.

However, several features of both genera show that their placement in the *Alaptus* group of genera is wrong and that they are best placed in the *Anagrus*-group because they share at least seven features with some or all of those genera: frenum apparently longitudinally divided by a groove, petiole and base of gaster distinctly narrower than propodeum and middle of gaster, tarsi with at most 4 tarsomeres (3 in *Kikiki*), stemmaticum present, mandibles with 4 teeth, fore wing venation with proximal macrochaeta much shorter than distal macrochaeta, and second phragma not projecting past posterior margin of propodeum.

Both *Kikiki* and *Tinkerbella* are distinguished from other *Anagrus*-group genera by the venation clearly longer than half the wing length and hind wing essentially without fringe setae on the anterior margin. The other genera in the group have the venation clearly less than half wing length and the hind wing fringed with fairly long setae on the anterior margin. No member of the *Anagrus* group of genera is anywhere near as small as *Tinkerbella* or *Kikiki* so these genera can be distinguished on body size alone.

Some members of the *Alaptus* or *Camptoptera* groups of genera are also extremely small but except for *A. borinquensis* none has been found that equal minute size of *Tinkerbella* or *Kikiki,* i.e., less that about 250 μm. One specimen of *Alaptus* from the same collecting event in Costa Rica as *K. huna* measured 272 μm and species of *Eofoersteria* (also with 4-segmented tarsi, despite being in the *Camptoptera* group) are about 320 μm. The six specimens (4 females, 2 males, on 4 slides, USNM) of *A. borinquensis* are uncleared and mounted mostly in lateral view in Canada balsam, and some are slightly shrivelled. The smallest specimen, a male, measured 203 μm in length, not 186 μm as reported by Dozier (1932). The ranges for females and males, respectively, are 215–411 μm (n=4) and 203–311 μm (n=2), measured with a filar micrometer at 400× by JTH.

Body size limits in arthropods

What is the smallest size that an adult insect can attain, as measured by body length, and/or fore wing length for flying insects? If something is physically possible in living things some individuals of at least one species, extinct or extant, will likely have achieved it. So the lower size limit, by whatever measure of size is chosen, was almost certainly already evolved—somewhere, sometime. If we have not already found them, we must surely be close to discovering the smallest insects and other arthropods.

The minimum size possible for invertebrates with articulated appendages of locomotion (superphylum Arthropoda) that would allow crawling, walking, or active flight (wing flapping, not gliding) is determined by two types of constraint: internal (physiological and structural) and external (physical). For multicellular animals, the size and structure of cells cannot go below a certain minimum before they cannot function for the purpose intended. Therefore, once any type of cell has attained its minimum size, the number of cells making up a particular tissue or organ must decrease as the animal becomes smaller. But there evidently is a lower limit to cell number in any particular

organ for it to function as intended, perhaps as low as one, e.g., a single muscle fiber or ommatidium. Or the tissue or organ may be dispensed with entirely, usually because it is no longer necessary, e.g., eye loss in obligate cave-dwelling species (troglobites). Once these internal constraints are reached the arthropod cannot become any smaller. Nuzzaci and Alberti (1996) showed that Eriophyidae (Acari) have no respiratory system and no striated muscle [in contrast to insects and other Acari, which have striated muscle exclusively (Beinbrech 1998, Alberti and Coons 1999)]. Polilov (2007) discussed miniaturization related features in Mymaridae, and Polilov (2012) showed that neurons of *Megaphragma mymaripenne* Timberlake are anucleate. Fischer et al. (2011) discussed size limits in ommatidia in a small parasitic wasp, *Trichogramma evanescens* Westwood. Grebennikov (2008) reviewed the limiting factors for small size in arthropods.

As body size decreases, external constraints, e.g., desiccation (Neville 1998), surface tension, and fluid viscosity become relatively more important. Thus, in minute organisms the muscle strength needed to power an articulated appendage for active locomotion is determined not only by internal factors such as the minimum muscle cell size and cell number but also by external factors such as viscosity of the fluid (air or water) in which active movement occurs. Although surface area to volume ratio increases with decreasing size allowing small organisms to be relatively stronger than larger organisms, there still comes a point at which muscles are so small that they cannot power an articulated appendage in a medium that, for their size, must be quite viscous. Yet, external physical factors such as air viscosity are likely still not a constraining factor because even the smallest walking or flying insects appear to be large enough to overcome them. Their problem is to overcome their own inertia, not viscosity of the fluid in which they move. For even smaller organisms than arthropods, viscosity and surface tension may finally become the limiting factor. These organisms do not (and cannot) have articulated appendages of locomotion, particularly if the appendages have intrinsic muscles that move the various segments independently.

Even with the increased mechanical efficiency resulting from smaller body size and energy conservation efficiencies conferred by Weis-Fogh clap-and-fling flapping (Weis-Fogh 1973, Miller and Peskin 2005), the elastomeric protein resilin (Neville 1998, Elvin et al. 2005), the natural elasticity of muscle and cuticle itself, and light cuticular wings (Neville 1998) it is difficult to believe for winged insects that such a small size as occurs in *Kikiki huna* is possible. The fact that some specimens of *Megaphragma* are about the same minimum size as some specimens of *Kikiki* suggests that winged insects have indeed reached their limit for size reduction.

A diversity of other very small insects capable of active flight have the wing surface (membrane) reduced and wing fringes, especially of the hind wing, greatly lengthened, e.g., species of Thysanoptera, various families of small Lepidoptera and parasitic Hymenoptera, and Ptiliidae (Coleoptera). While this may slightly reduce wing weight the reduced wing surface and relatively long setae are more likely to have an aerodynamic function, perhaps to reduce turbulence and hence drag on a wing flapping at several hundred beats per second. Interestingly, in *Kikiki* (Fig. 27), *Tinkerbella* (Fig. 6) and *Megaphragma* (Fig. 51) the fringe setae along the leading edge of the hind wing are

absent or almost so, whereas in other genera of Trichogrammatidae and Mymaridae they are present, albeit short. The separation of muscles that power flapping, i.e., the indirect flight muscles that cause thoracic distortion, resulting in wing flapping, from the direct flight muscles that control wing pitch may also be important in allowing insects capable of active flight to attain a minute body size.

Relatively long legs powered by sufficiently strong muscles to elevate the body may be linked to and necessary for active flight. The muscles required to move articulated legs to enable walking by lifting the entire body off the ground and moving forward must have a lower size limit or the legs could not be used for that purpose. Two sets of opposing intrinsic muscles are needed: extensor/elevator muscles strong enough to lift the entire body sufficiently above the substrate to initiate flight, perhaps by allowing a wing stroke of more than 90°, and flexor/depressor muscles to allow walking, and perhaps secondarily to draw the legs against the body during flight to reduce drag. High speed photography of *Trichogramma* lifting off a substrate (Lentink and Voesenek 2012) suggests that the first wing stroke is greater than 90° and, once airborne, that individuals in flight move forward by pushing air forcefully backwards, especially on the up stroke. With about 350 wing beats per second *Trichogramma* individuals keep themselves in a reasonably high Reynolds number range. But the video clip does not seem to show that the legs are used to leap vertically off the substrate prior to initiating a wing flap. In these wasps the legs need only be long and strong enough to lift the entire body off the ground for normal walking and perhaps to allow the first wing flap to be greater than 90°, sufficient to allow lift off. *Trichogramma* spp, have relatively wide wings, (200–300 μm), with a fore wing width to length ratio about 0.5 and wing fringes that are about 0.2× wing width. These are quite unlike *Kikiki*, with a forewing width of 20–25 μm, a width to length ratio of 0.11 and fringes about 5.0× the wing width, so flight aerodynamics between the two genera may be very different.

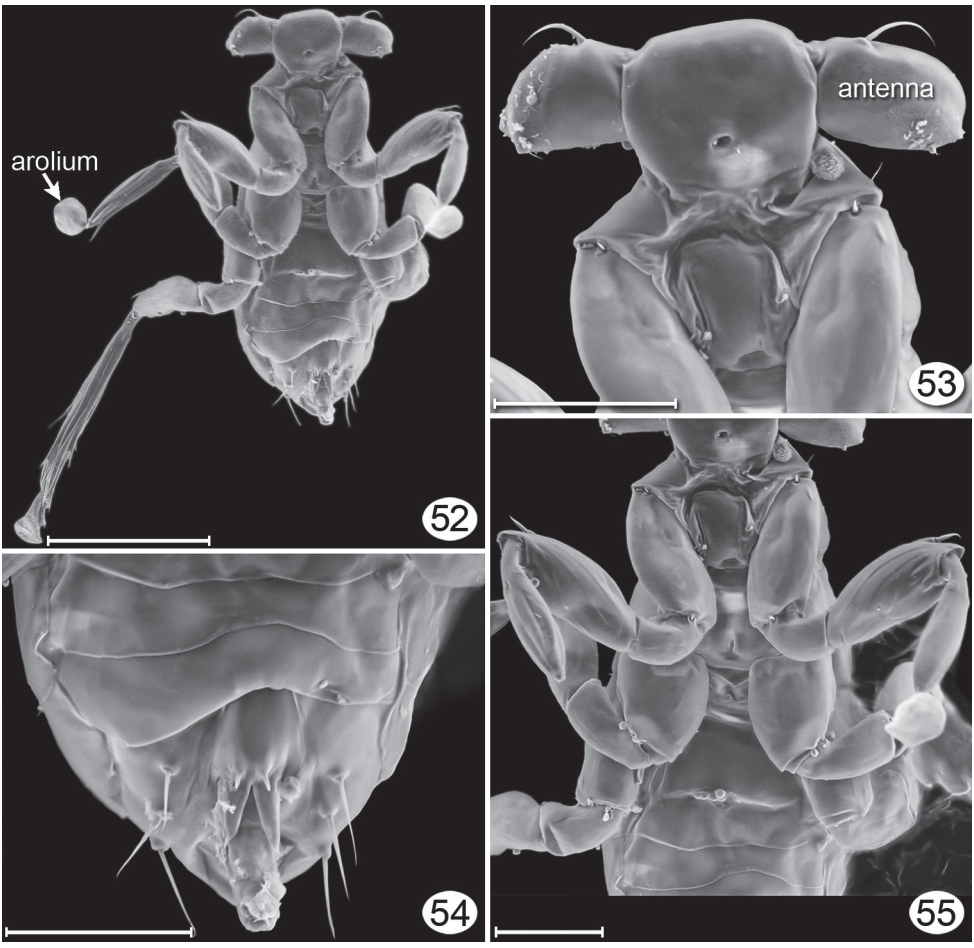
In insects with free living adults and larvae the lower body length limit seems to be about 400 μm (Grebennikov 2008), imposed by the need to have a sufficiently large egg to nourish the developing larva sufficiently so it can be free-living, i.e., it must have the necessary initial body resources to move around actively upon hatching, perhaps to search for food and avoid potential predators. Females of the smallest oribatid mites (Acari) also lay only a single, relatively enormous egg at a time, e.g., a species of *Brachychthonius* (Brachychthoniidae) with a female 180 μm long (distorted by compression of the coverslip) has an egg of 100 μm (D. Walter, pers. comm.). The smallest known fly is also 0.4 mm long (Brown 2012).

The constraint of minimum egg size as a determinant of minimum body size does not apply to parasitic insects. Eggs in these can be much smaller because the larvae hatch inside the host. They do not have to search actively for food because it completely envelops them. The limiting factor to small size in adult parasitic insects must therefore be minimum cell size and sufficient cells of each type of tissue, as discussed above. Females of *Tinkerbella*, *Kikiki,* and *A. borinquensis* are the smallest Mymaridae, and some specimens of *Kikiki* are the smallest recorded winged insects. The body length of five specimens from Costa Rica is 158 μm (1 female), 160 μm (2 females), and 170

μm (2 females). Three specimens are thus smaller than the smallest recorded females of *Megaphragma caribea*, the previous record holder at 170 μm (Delvare 1993).

The next step in body length reduction in insects is in *D. echmepterygis*. Females are winged and relatively long (one measured 386 μm dry but not shivelled and 550 μm on a slide mount), but the males slide-mounted males were 139–240 μm long (n=8) (Mockford 1997). One critical point dried female (CNC) measured by JTH is 340 μm long and two critical point dried males (BMNH, CNC) from the remaining 10 male paratypes, measured independently by JTH and JSN are 126.17 μm and 130 μm, respectively (ESEM measurement). Males have significant morphological reductions or losses. They lack eyes and ocelli, and the appendages are greatly reduced (antenna, tarsi) or absent (wings, mouthparts) (Figs 52–55). Because they cannot feed their only energy source is what has been stored as larvae, which would have obtained all their nutrients from their psocopteran host egg. The leg segments of males are strangely disproportionate, with huge coxae (Figs 52, 53, 55) as long as the femora, protibiae shorter than the femora, and the tarsi absent except for the large, bell-shaped arolium on each (Figs 52, 55) that presumably acts as suction cups to attach to females. Because the legs, especially the hind legs, are long relative to the body length, males clearly can raise their entire body above the substrate in order to walk more or less normally over the short distance necessary to find a female. The tarsal structure, and the fact that some males were found attached to females (Mockford 1997), show that males are phoretic and need only walk only far enough to crawl onto a female to copulate, almost certainly while the female is still in the egg (females presumably emerge from the same host egg or egg cluster so the distance traversed by a male is very short). Males evidently have enough energy to do this and probably nothing else.

Further reductions in body length occur in terrestrial Arthropoda other than insects. In mites, the smallest adult individuals of several species in three families are less than 95 μm in length: *Cochlodispus minimus* Mahunka at 79 μm (Mahunka 1976) and *Microdispus australis* Mahunka at 82 μm (Mahunka 1969) (both Microdispidae), female of *Indosetacus rhinacanthi* Ghosh and Chakrabarti at 86 μm (Ghosh and Chakrabarti 1987), male of *Eriophyes parvulus* (Nalepa) at 90 μm (Nalepa 1892), both sexes of *Achaetocoptes quercifolii* Farkas at 90 μm (Farkas 1961) (all Eriophyidae), and females of *Iponemus truncatus eurus* Lindquist at 93 μm and *I. confusus oriens* (Lindquist and Bedard) at 94 μm (both Tarsonemidae) (Lindquist 1969). That small size in arthropods is not a recent evolutionary phenomenon is shown by a Triassic mite, *Ampezzoa triassica* Lindquist and Grimaldi (Eriophyidae), 124 μm in length (Schmidt et al. 2012). Adults of Eriophyidae have only two pairs of usually 5-segmented legs yet they are evidently capable of locomotion but the legs are so reduced that they cannot lift the entire body off the substrate. "Walking" in Eriophyidae is an inching or looping motion. The more or less worm-like body is arched between the two pairs of forelegs anteriorly and the terminal sucker posteriorly, and alternate gripping and releasing by the legs and sucker allow the mite to inch along. True walking using the legs only presumably does not occur and they probably do not move much by this method during their life time. Instead, dispersal is by aerial drifting. However, Microdispidae and Tarsonemidae have 4 pairs



Figures 52–55. *Dicopomorpha echmepterygis* male, paratype, ventral. **52** habitus **53** head + prothorax + procoxa **54** apex of gaster **55** mesosoma + base of most legs and metasoma. Scale line = 20 μm, except Fig. **52** = 50 μm.

of legs and are capable of normal walking. The smallest adult of *Neoliochthonius piluliferus* (Forsslund) (Brachychthoniidae) is 123 μm (Forsslund 1942). If a mite doubles in size with each moult or increases in body length by a factor of about 1.3 (Hutchinson's Ratio—applied to comparison of different life stages within a species instead of comparing competing species in the same habitat) a larva or protonymph would be as small as about 50 μm in length. A larva of this length was found by D. Walter (pers. comm.). Whereas Insecta have two sets of opposing intrinsic muscles in their leg segments (Fig. 47) as indicated above, Acari only have flexors (except for those moving the apotele, at the apex of the tarsus). In all Acari the distal segments flex due to muscular contraction and extend due to hydrostatic pressure (Alberti and Coons 1999).

For comparison with terrestrial arthropods, larvae of the marine parasites of Copepoda *Stygotantulus stocki* Boxhall and Huys at 94 μm (Boxhall and Huys 1989) and *Tan-*

tulus dieteri Mohrbeck, Arbizu and Glatzel (Crustacea: Tantulocarida, Basipodellidae) (Mohrbeck et al. 2010, Martin and Davis 2001) at ca. 85 μm are the shortest. Notably, all members of the subclass lack recognizable cephalic limbs, other than paired antennules in one known stage (Martin and Davis 2001). The loss of appendages and the parasitic life style of adults means that the much higher viscosity of water compared to air is irrelevant in impeding locomotion, because the immature stages evidently disperse by passive drifting (as do Eriophyidae), and adults are parasitic so evidently do not move.

Below a certain body length it is useless to have articulated appendages because the segments could not be moved relative to one another, or the entire appendage relative to the body, by intrinsic muscle power alone. Instead, if appendages of locomotion exist at all (e.g., pseudopods), they would be short and wide, would not be articulated, and would be moved instead by body muscles causing hydrostatic changes in pressure, combined perhaps with flexor muscles originating within the body but attached near or at the appendage apex. The length of the larva of *N. piluliferus* rivals some Rotifera, also as short as 50 μm, suggesting that at about this size the changeover from locomotion by partial muscle power intrinsic to leg segments (Acari) to hydrostatic power alone (Rotifera, other non-Arthropoda) may occur.

We suggest that the smallest winged insects capable of flapping flight could not be less than about150 μm in length, and the smallest capable of normal walking (body lifted entirely of substrate) not below about 125 μm. Among insects, *Kikiki huna* may well have attained the lower limit for active flight and *Dicopomorpha echmepterygis* the lower limit for normal walking. Among other arthropods capable of walking, 80 μm is suggested as the lower limit for adults and ca. 50 μm for immatures.

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Appendix

Abbreviations used in the figures: as = anterior scutellum, ax = axilla, dm = distal macrochaeta, dor = dorsellum, fl = funicle segment, fr = frenum, mlm = mid lobe of mesoscutum, mps = multiporous plate sensillum, pd = propodeum, pm = proximal macrochaeta, sp = spiracle.

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RESEARCH ARTICLE

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Two new species of the genus *Discoelius* Latreille (Hymenoptera, Vespidae, Eumeninae) from China, with a key to the Chinese species

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Abstract

Two new species, namely *Discoelius nigriclypeus* Zhou & Li, **sp. n.** from Hunan, China, and *Discoelius emeishanensus* Zhou & Li, **sp. n.** from Sichuan, China, are described. A key to the Chinese species of the genus is provided, along with illustrations of the new species. Type specimens of these two new species are deposited in the Institute of Entomology and Molecular Biology, Chongqing Normal University.

Keywords

Hymenoptera, Vespidae, Eumeninae, *Discoelius*, new species, China

Introduction

The eumenine wasp genus *Discoelius* was established by Latreille (1809). The members of the genus are solitary wasps that are easily distinguished from other genera occurring in China by the presence of two spurs on the mid tibia and the petiolate metasomal

segments 1 and 2. *Discoelius* is similar to *Zethus* Fabricius (just one species, *Zethus dolosus* Bingham, 1897 recorded from China according to Li 1985), but the latter has a longer petiolate metasomal segment 2 and the valvula prominent beneath the submarginal carina, usually quadrate in shape. Presently, seven species and one subspecies are recongnized worldwide, of which *D. dufourii dufourii* Lepeletier, *D. d. manchurianus* Yasumatsu and *D. pictus* Kostylev are from the Palearctic Region, *D. esakii* Yasumatsu, *D. longinodus* Yamane, *D. turneri* (Meade-Waldo) and *D. wangi* Yamane from the Oriental Region, while *D. zonalis* (Panzer) is known from both the Palearctic and Oriental Regions (Meade-Waldo 1910; Yasumatsu 1934; Kostylev 1940; Giordani Soika 1971; van der Vecht and Fischer 1972; Li 1982, 1985; van der Vecht and Carpenter 1990; Yamane 1990, 1996; Gusenleitner 1999; Kim 2005). Five species and one subspecies have been recorded from China, of which *D. dufourii dufourii* Lepeletier and *D. d. manchurianus* Yasumatsu occur in the north of China (Kim 2005); *D. zonalis* (Panzer) occurs in the south of China (Li 1985; Kim 2005), and *D. esakii* Yasumatsu, *D. longinodus* Yamane and *D. wangi* Yamane in Taiwan (Yamane 1996). In the study of Chinese *Discoelius*, we found two species that are new to science. In the present paper, we describe these two new species, and provide a key to the Chinese *Discoelius* species.

Materials and methods

The examined specimens are deposited in the Institute of Entomology and Molecular Biology, Chongqing Normal University, Chongqing, China (CQNU), the Entomological Museum, China Agricultural University, Beijing, China (CAU), Yunnan Agricultural University, Kunming, Yunnan (YNAU), Central South Forestry University, ChangSha, Hunan (CSCS) and Guangxi Normal University, Guilin, Gungxi (GXNU). Morphological terminology follows Bohart and Stange (1965), Carpenter and Cumming (1985) and Yamane (1990). All measurements were taken as the maximal length of body parts measured under an image analyzer (Nikon SMZ1500), figured with the aid of a stereomicroscope (Olympus SZX7). Body length was measured from the anterior margin of head to the posterior margin of metasomal tergum 2.

Taxonomy

Genus *Discoelius* Latreille
http://species-id.net/wiki/Discoelius

Discoelius Latreille, 1809: 140; Saussure 1852: 24; Yasumatsu 1934: 3; Vecht and Fischer 1972: 2; Li 1985: 80; Carpenter 1986: 68; Vecht and Carpenter 1990: 19; Yamane 1990: 22; Yamane 1996: 111; Kim 2005: 111.

Type species. *Vespa zonalis* Panzer, 1801.

Diagnosis. Mandible short: not distinctly crossing each other, just a small apical portion overlapping. Clypeus transverse, with widely rounded or somewhat truncate apical margin. Antennal scape somewhat depressed in both sexes. Vertex and gena more or less convex. Mesoscutum with notaulices for its whole length. Metasomal segment 1 less than half as broad as segment 2; both terga 1 and 2 petiolate. Mid tibia with two apical spurs (Kim 2005).

Distribution. China (Hebei, Zhejiang, Jiangxi, Sichuan, Chongqing, Fujian, Guangxi, Yunnan, Hunan, Liaoning, Shaanxi, Guangdong, Beijing, Inner Mongolia, Taiwan); Palaearctic and Oriental regions.

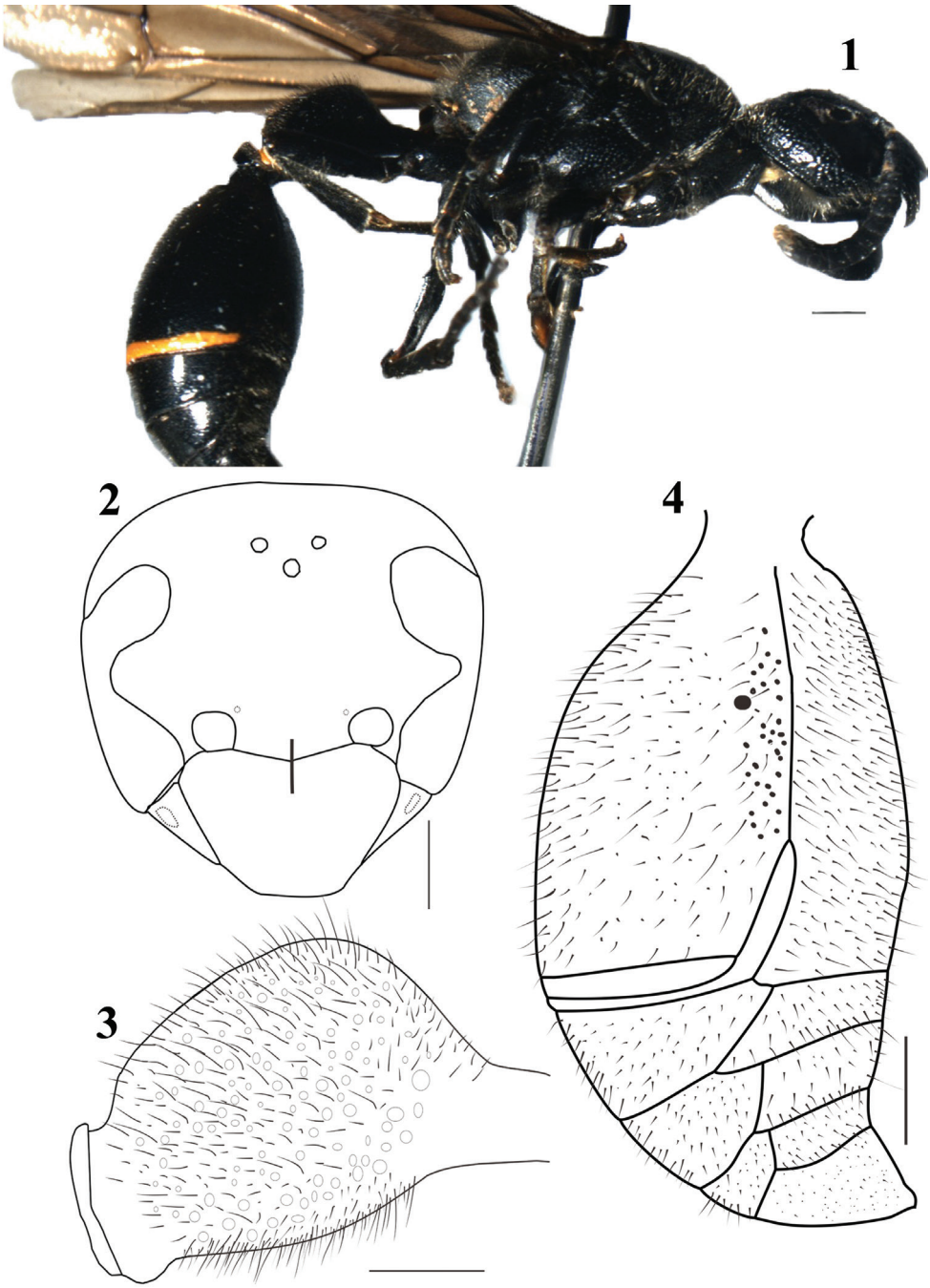
***Discoelius nigriclypeus* Zhou & Li, sp. n.**
urn:lsid:zoobank.org:act:8AB3F0A2-C506-45CD-82EF-4B1CD47472B4
http://species-id.net/wiki/Discoelius_nigriclypeus
Figs 1–4

Material examined. Holotype, ♀, China, Hunan Prov., Changde City, Shimen County, Fuping mountain, 29°42'51"N, 110°45'21"E, 1200–1600 m, 9.VI.2004, Yang Jiang (CQNU).

Description. Female, body length 18.0 mm, forewing length 15.0 mm. Black, with the following parts yellow or orange yellow: a spot on mandibular base, a very small spot just above antennal socket (Fig. 2), narrow apical bands on metasomal terga 1-2 (Figs 1, 4); the base of mid femur with a very small deep yellow line.

Head: flat and subcircular, covered densely with punctures and sparsely with brownish setae; mandible stout, not long, outer surface with two longitudinal carinae developed, between them with a short one, inner margin with two wide teeth; width of clypeus as long as length, not distinctly convex, irregularly punctuate and with strong punctures, emarginate basally and apical margin truncate; frons sparsely with short brownish setae and a strong vertical carina just above the clypeus, the carina distinct on the upper about one-fourth of clypeus (Fig. 2); antenna very short, scape and pedicel of antenna shining, densely with minute punctures, flagellum dull and with microscopic punctures, the length of flagellum 1 about 1.5 times flagellum 2, flagellums 2-8 wider than length, respectively, and the width about 1.2 times the length; in profile, gena very broad and sparsely with punctures and short erect setae; postgena densely with long setae.

Mesosoma: From above, length about 2–3 × width; mesoscutum and scutellum flat and in single plane; mesoscutum and scutellum densely with longitudinal carinae, the interspaces between the carinae with very minute punctures; mesoscutum with developed notaulices, between the notaulices shining apically, not punctate and without carina; scutellum and metanotum sparsely punctate, with strong punctures; propodeum reticulate, not shining, with lateral carina and dense long brownish erct setae; the setae on mid tibia denser than those on femur and the tibia widening from base, the apical width about 4 times basal width; the length of mid tarsal 1:2:3:4 ≈ 10:2:1:1, and ≈ 16:4:3:2 in hind tarsus.



Figures 1–4. *Discoelius nigriclypeus* Zhou and Li, sp. n. ♀. **1** general habitus (lateral view) **2** frons and clypeus **3** metasomal segment 1 (lateral view) **4** metasomal segments 2-6 (lateral view). Scale bar: 1mm.

Metasoma: In profile, anterior slope of metasomal tergum 1 steep, and tergum covered wholly with brownish erect setae and punctures, the setae on ventral-lateral part denser and longer than those on dorsum, the interspaces between punctures as large as punctures, the preapical furrow deep (Fig. 3); segment 2 with more sparser, smaller and shallower punctures, the interspaces between the punctures 3-4 times punctures, basally with one smooth area and apical lamella very narrow (Fig. 4); the punctures on metasomal terga and sterna 3–6 smaller and denser than those on tergum 2, setae shorter than those on tergum 2; the apical margin almost truncated in terminal metasoma.

Male. Unknown.

Remarks. This species can be distinguished from the similar *D. zonalis* (Panzer, 1801) and other members of the genus by the combination of the following characters: more strongly punctate body, length of mesosoma about 2–3 times width, mesoscutum and scutellum flat, clypeus entirely black and emarginate basally, not distinct convex, postgena densely with long setae, mesoscutum apically between developed notaulices shining and not punctate, metasomal tergum 2 basally with one smooth area and apically with narrow lamella.

Distribution. China (Hunan).

Etymology. The specific name *nigriclypeus* is the Latin *nigr-* (= black) + *clypeus* (= clypeus), which refers to the clypeus of the species black.

***Discoelius emeishanensus* Zhou & Li, sp. n.**

urn:lsid:zoobank.org:act:D74C8DEC-5373-4DA4-B017-3ED1B3DE9F9B

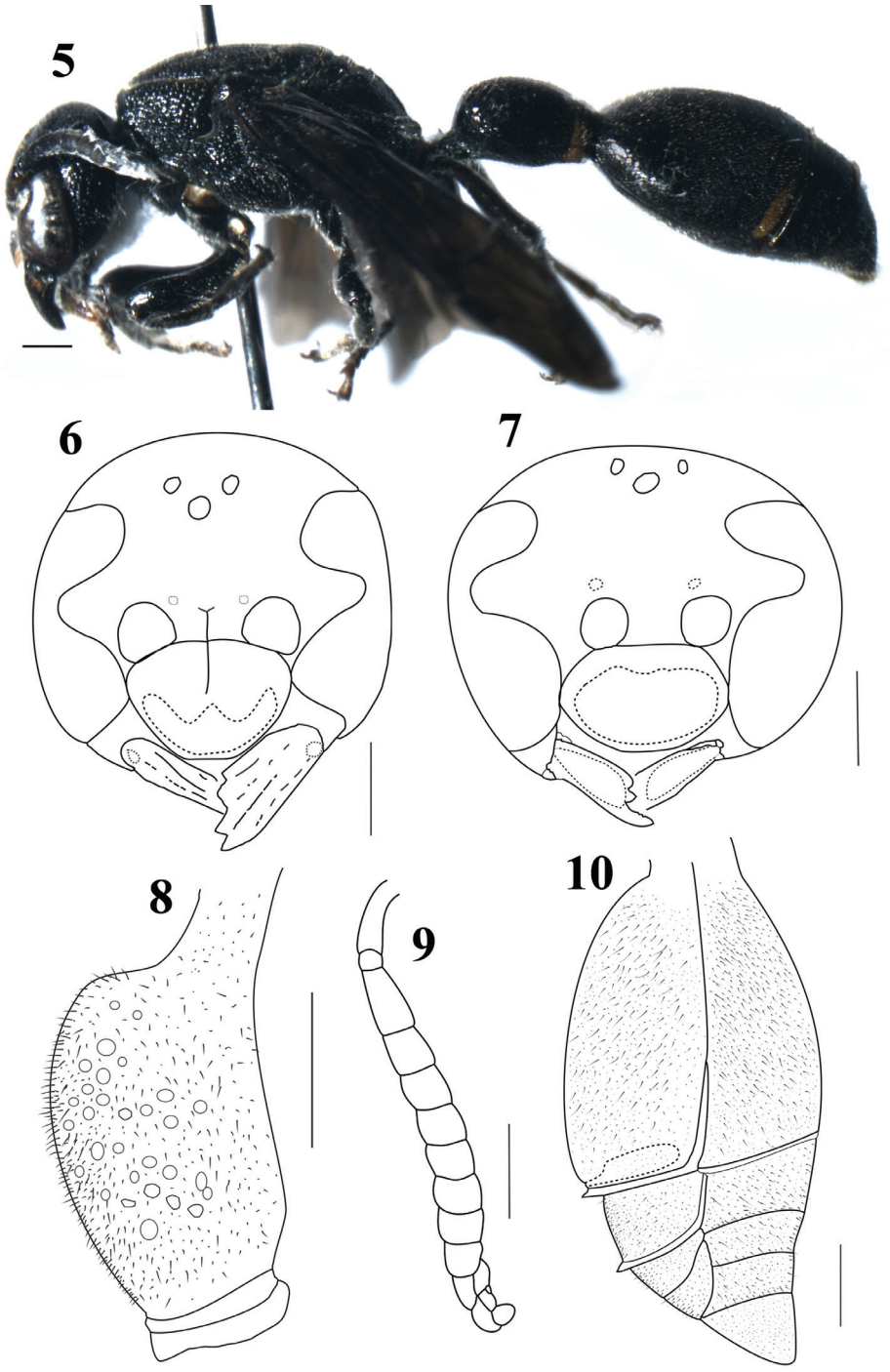
http://species-id.net/wiki/Discoelius_emeishanensus

Figs 5–10

Material examined. Holotype, ♀: China, Sichuan Prov., Leshan City, Emeishan Country, Gaoqiao Town, Yanshi Village, 29°30'14"N, 103°25'35"E, 551 m, 11. VIII. 2011, Tingjing Li (CQNU). Paratype: 1♂, the same as holotype (CQNU).

Description. Female body length 15.0 mm, forewing length 12.0 mm. Black; with the following parts yellow or orange yellow: a marking on mandibular base, apex of clypeus, a small spot just above antennal socket (Figs 6, 7), a line on the anterior face of fore tibia, narrow apical bands on terga 1–2 (Figs 5, 10); anterior face of antennal scape with a deep brownish line.

Head: In frontal view, head subcircular; punctures mostly dense and coarse; mandible stout, not long, its apex sharply pointed and slightly curved inward, inner margin with four wide teeth, outer surface with four longitudinal carinae; clypeus wider than long, with short white setae and irregular strong punctures (somewhat reticulate), its apical margin truncate (Fig. 6), in profile, not concave; frons weakly concave in the middle, one strong vertical carina just above the clypeus and the carina distinct in the upper about one-half of clypeus (Fig. 6); scape of antenna somewhat shining, densely covered with minute punctures, flagellum not shining and with microscopic punc-



Figures 5–10. *Discoelius emeishanensus* Zhou and Li, sp. n. **5** general habitus (lateral view), ♀ **6** frons and clypeus, ♀ **7** frons and clypeus, ♂ **8** metasomal segment 1 (lateral view), ♀ **9** antenna (lateral view), ♂ **10** metasomal segments 2-6 (lateral view), ♀. Scale bar: 1mm.

tures, antennal article 3 longer than article 4, articles 4-10 wider than long and apical width about 1.2 times basal width, respectively; in profile, gena very wide, gradually narrowing towards the base of mandible and the narrowest near the base of mandible.

Mesosoma: From above, length of mesosoma about 2 times its width, and the whole mesosoma with white short appressed pubescenses and coarse punctures; pronotum with one continuous ridge extending laterally to fore coxa; one weak carina between the notaulices; scutellum and metanotum strongly punctate, with longitudinal, elongate wrinkles and without median furrow; propodeum shining, with lateral carina and sparsely short setae, dorsal and posterior faces rugosely striate to reticulate, lateral face shining, without punctures and setae; the setae on the mid tibia denser than the femur and the apical width about 2 times basal ones; the mid tarsal 1 dumpy and the hind tarsal 1 slender; the length of the mid tarsal 1:2:3:4 ≈ 4:1:1:1, and ≈ 20:4:2:1 in hind tarsus.

Metasoma: Length of metasomal segment 1 more than 2 times its apical width; tergum 1 with big and sparse punctures and white short setae, in profile, the anterior slope rather steep (Fig. 8); the length of tergum 2 about 1.1 times apical width, terga 2-3 apically with developed reflexed lamellae, respectively; the other terga without apical lamellae, with weaker and sparser punctures (Fig. 10); the apical margin of terminal metasoma triangle.

Male (Figs 7, 9). Differs from the female as follows: body length 12.0 mm, forewing length 8.0 mm; mandible with three teeth, of which the apical one very long and the yellow marking bigger, almost covering the wholly outer surface of mandible; clypeus almost wholly yellow (Fig. 7); the length of antennal flagellum 1 about 1.8 times apical width and gradually widening from base; flagellum 2 about 1.2 times width and not widening from base; flagellums 3–7 as long as apical width; antennal article 13 almost black, dull and small, folded beneath (Fig. 9); outer face of fore femur with a very broad yellow line, and mid femur apically with a small yellow markings; mid tibia sparsely with short white setae, and hind tibia with long goldenish denser setae; the length of mid tarsal 1:2:3:4 ≈ 5:1:1:1, and ≈ 24:6:2:1 in hind tarsus, the length of hind tibia about 1.5 times mid tibia; in dorsal view, the length of metasomal tergum 1 about 1.2 times width and the length of tergum 2 about 0.8 times width, the setae on sterum 2 denser than those on tergum 2; terminal metasoma subcircular, punctures bigger and denser than those on tergum 2, setae shorter and sparser and apical margin brownish.

Remarks. This species can be distinguished from the similar *D. zonalis* (Panzer, 1801) and other members of the genus by the combination of the following characters: propodeum shining, sparsely with short setae; in male mandible with three teeth, of which the apical one very long, in female mandible with four teeth; scutellum and metanotum without obviously median furrow; terga 2-3 with a developed reflexed apical lamella, respectively (Fig. 10); tegula entirely black; in male, outer face of fore femur with a broad yellow line; metasomal tergum 1 with big punctures.

Distribution. China (Sichuan).

Etymology. The specific name *emeishanensus* is the Neolatin adjective, which refers to the region from which the type specimens were collected.

Key to the Chinese species of the genus *Discoelius*

1 Large species: body length more than 17.5 mm and forewing length more than 15.0 mm in female; clypeus basally distinctly emarginate; between the notaulices shining apically, not punctate and without carina; metasomal tergum 2 basally with one smooth area ..... ***D. nigriclypeus*** **Zhou & Li, sp. n.**

– Small species: body length less than 15.0 mm and forewing length less than 14.0 mm in female; clypeus basally indistinctly emarginate; between the notaulices usually not shining, with punctures or carina; metasomal tergum 2 basally without one smooth area **2**

2 Clypeus slightly emarginate apically; anterior slop of metasoma tergum 1 rather gentle; tergum 2 with a very narrow "neck" and entirely black (Yamane 1996)..... ***D. longinodus*** **Yamane**

– Clypeus usually widely rounded or truncated apically; anterior slop of metasoma tergum 1 steep; tergum 2 without narrow "neck" and usually with yellow apical band..... **3**

3 Metasomal terga 2-4 with a reflex apical lamella, respectively..... **4**

– Metasomal terga 2-3 usually with a lamella, respectively (sometimes just tergum 2 with lamella)..... **5**

4 Propodeum with lateral ridges and lateral face with superficial and irregular large punctures in the upper portion; clypeus and mesosoma usually entirely black; stigma of forewing brown to dark brown (Yamane 1996)..... ***D. wangi*** **Yamane**

– Propodeum without lateral ridges and lateral face weakly reticulated; usually two-third of clypeus apicaly yellow and mesosoma with rich yellow markings; stigma of forewing amber yellow (Yasumatsu 1934; Yamane 1996) ..... ***D. esakii*** **Yasumatsu**

5 In female, mandible with four teeth; in male, mandible with three teeth and the apical teeth very long; scutellum and metanotum without obviously median furrow; tegula usually entirely black; the punctures on the metasoma tergum 1 bigger and sparser, sternum 2 without yellow apical band; in male, outer face of fore femur with a broad yellow line ..... ***D. emeishanensus*** **Zhou & Li, sp. n.**

– Mandible with two or three teeth in female and three or four teeth in male, the apical teeth not long in both sexes; scutellum and metanotum with median furrow; tegula usually with yellow or brownish colour; the punctures on the metasoma tergum 1 smaller and denser, sternum 2 usually with yellow apical band; in male, outer face of fore femur usually entirely black..... **6**

6 In frontal view, face including clypeus flattened in female; frons, clypeus, mesoscutum and scutellum densely with longitudinal carinae in the female; male antennal articles 8-10 distinctly with tyloids covering their lower faces extensively; terminal article stumpy, in profile slightly longer than breadth, and obliquely truncated on apical margin (Kim, 2005)..... **7**

– In frontal view, face including clypeus not flattened in female; frons, clypeus, mesoscutum and scutellum densely with punctures, which only partially form

carinae and reticulae, in the female; in male antennal articles 10 ventrally and apical part of article 9 with weak and small spot-like tyloids; terminal antennomere slender, in profile distinctly longer than breadth, with apical margin somewhat rounded (Kim, 2005) ..... ***D. zonalis* (Panzer)**

7 Vertex without yellow line; mesosoma black, except pronotum with a pair of yellow dots..... ***D. dufourii dufourii* Lepeletier**

– Vertex with a pair of short oblique yellow line; pronotum, tegula, mesepisternum, scutellum and metanotum with yellow markings, respectively .... ***D. d. manchurianus* Yasumatsu**

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Nesting biology and phenology of a population of *Halictus farinosus* Smith (Hymenoptera, Halictidae) in northern Utah

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Abstract

Nesting biology and phenology in an aggregation of the primitively eusocial ground-nesting bee *Halictus farinosus* were studied at Green Canyon, Utah from May to August, 2010. Nest architecture was typical of the genus. Nests were small with an average of 3.5 worker and 13.5 reproductive brood per colony. Most workers were mated (77.5%) and had ovarian development (73.4%). The queen-worker size differential was moderate (8.8% for head width and 6.2% for wing length), indicating that sociality in this species is of intermediate strength compared to other social *Halictus* species. Results from 2010 were compared with those from 1977/1978 and 2002. Varying weather patterns in the years of study led to changes in phenological milestones: in the colder and wetter spring of 2010, nesting behavior was delayed by up to two weeks compared to the other years. While nest productivity was comparable among years, in 2010 the size difference between queens and workers was significantly larger than in 2002, indicating an effect of annual variation in weather conditions on social parameters in this species.

Keywords

Sweat bee, phenology, nesting biology, natural history, nest architecture, primitive eusociality, social variability, degree days

Introduction

The development of eusociality from solitary ancestors is considered one of the major transitions in evolution (Maynard Smith and Szathmary 1995). In order to understand how this transition took place, it is necessary to study organisms exhibiting primitive or weak eusociality, as is found in the subfamily Halictinae (Packer 1986; Packer 1992; Richards and Packer 1995; Mueller 1996; Richards 2001; Soucy 2002; Richards et al. 2010; and see Schwarz et al. 2007). Indeed, primitively eusocial halictines share many similarities with the predicted ancestors of the highly eusocial corbiculate apids (Cardinal and Danforth 2011). Their potentially pivotal role in aiding out understanding of the evolution of eusociality requires the comparison of critical sociobiological parameters among populations both within and between species. The most frequently studied variables include the number of workers and reproductives produced per colony, the sex ratio in the worker and reproductive brood, queen-worker size differential, queen-worker ovarian development differences and the proportion of workers that mate (Packer 1986; Packer 1992; Richards et al. 2010).

The size of an adult bee depends on the quality (Roulston and Cane 2002) and size (Plateaux-Quénu 1983; Richards and Packer 1994) of the food mass provided for it. In ground nesting bees the size of the food mass and the number that can be produced will depend on multiple environmental and biological factors including the size and activity levels of active foragers in the nest (Gathmann and Tscharntke 2002; Pereboom and Biesmeijer 2003) and the amount of available resources which, in turn, are highly dependent on weather and other environmental factors (Packer 1990; Minckley et al. 1994; Richards and Packer 1996; Richards 2004). Repeat field studies of the same population can allow us to make comparisons between years to determine how weather patterns might affect phenology, productivity and sociobiology. This will also help us to better predict how pollinators might respond to changing ecological conditions (Richards and Packer 1995; Bartomeus et al. 2011).

Halictus farinosus Smith is found in western North America ranging from British Columbia, south to California and east to Nebraska (Ascher and Pickering 2011). It is polylectic and has been reported foraging on 43 plant species from 14 different families (Nye 1980) and is thought to be an important pollinator of carrot (Nye 1980), onion (Parker 1982), and sunflower (Parker 1981). In Utah, *H. farinosus* nests in dry, sandy soil in areas of sparse vegetation. Its nests are usually unbranched and are up to 65 cm deep (Nye 1980; Sellars 2004).

Halictus farinosus has a two-phase life cycle and exhibits colony social organization similar to other primitively eusocial halictines (reviewed by Schwarz et al. 2007; Richards et al. 2010). In northern Utah, queens found nests independently from April to May (Nye 1980; Sellars 2004), and produce a first worker brood. In turn the workers help to produce a second, reproductive brood (gynes and males) which begins to emerge in mid August. The species has also been studied in California (Eickwort 1985) where its biology is similar except that the nests extend up to 80 cm in depth and a

larger worker brood is produced (Eickwort 1985). The nesting season is also longer in California, lasting from mid February to October (Eickwort 1985).

In this study we present phenological, sociobiological and nest architecture data from field studies on *Halictus farinosus* in North Logan, Utah. As this population has been studied previously (Nye 1980; Sellars 2004) we compare phenological and nest productivity data among years of study. We also make comparisons between *H. farinosus* and other closely related *Halictus* species.

Methods

Study sites and nest excavations

Nests were excavated in July and August 2010 at Green Canyon, North Logan, Utah (N41°46.15, W111°46.39, elevation 1590 m) where *H. farinosus* had been previously studied (Nye 1980; Sellars 2004). The site was a 0.25 km^2 field near the mouth of the canyon. Six 2 × 2 m study sites with high levels of *H. farinosus* nesting activity were sectioned off and marked B to G. These sites were further divided into quadrats of 1 m^2 each. Sites varied in nest density from ten to over fourty nests per site in early June. The majority of nests excavated came from these sites and those that were from outside of these quadrats were given a code beginning with N. Each site was monitored every second day for at least two hours during daylight from 13 June until 30 July and on 5 and 6 August excepting days with exceptionally bad weather (ie. heavy rain throughout most of the day). Monitoring consisted of observing and recording nests at each site for any activity around nest entrances, including: foundresses or workers leaving or returning to the nest, conspecific interactions near the nest entrance, predation of *H. farinosus* in the nest area and the entry of natural enemies into the nest. Nest mortality was calculated by comparing the number of nests with activity in the first round of monitoring, while foundresses are foraging to produce the first brood (mid-June), compared to those active in the last round during the worker foraging period just before the reproductives were ready to emerge (August 5th and 6th). Before the emergence of workers, foundresses from sites C-H were marked with unique 3-colour combinations of Testors™ enamel paint on their thoracic dorsum so they could be identified later in the season. Marked foundresses were confirmed and unmarked queens were identified as the mother of the worker offspring by genotyping at six microsatellite loci (see Albert 2012).

Nests referred to as first brood nests are those excavated on or before 17 July which was the date when the first workers emerged. Second brood nests were any nests excavated after 17 July. Most second brood nests, referred to as late summer nests, were excavated during a short period in the week following 7 August, just before the second brood began to emerge (see Table S1 for excavation dates). This was done to maximize the amount of the second brood that was present during excavation.

Table 1. Summary of phenological data for all *H. farinosus* studies in Green Canyon. N/A indicates that the information in the column was not given in the study. In Sellars (2004) female and male reproductive emergence dates were not differentiated so the same date was given for each.

	1976	**1977**	**2002**	**2010**
Queen foraging slows	N/A	N/A	28 June	12 July
First adult worker	"Late June"	8 July	2 July	15 July
First reproductive brood cells	N/A	N/A	3 July	17 July
First reproductive adult male	"Late July"	"Early August"	30 July	11 August
First reproductive adult female	"Early August"	"Mid August"	30 July	12 August
Source	Nye, 1980	Nye, 1980	Sellars, 2004	Present study

Nest entrances were blocked before dawn on the morning of excavation to ensure that all nest members would be captured during excavation. Nest excavation methods were as described by Packer and Knerer (1986). All nest occupants including juveniles in various developmental stages were collected in ethanol and stored at -20°C.

Dissections and measurements

Adult females were dissected and measured using a Leica *WILD M3B* light microscope equipped with an ocular micrometer at 16 x magnification following the general methods of Ordway (1965). Wing and mandible wear were each ranked with a score ranging from 0 to 5 where 0 was no wear and 5 was extremely worn. Wear scores, head width and wing length measurements followed the methods of Richards et al. (2010). Ovarian development was assessed based on the size of developing oocytes following Packer (1986): 0 when no oocytes were developed and 1 for each fully developed oocyte. Intermediate conditions were scored to the nearest quarter based on size relative to a complete egg. These data were summed to give an overall development score (see Packer 1986). Adults said here to have "ovarian development" had a score of 0.25 or greater. Matedness was determined by inspection of the spermatheca. Sexing of juvenile brood was accomplished by genotyping at six microsatellite loci (see Albert 2012) and confirmed in pupae by morphological inspection. Sex ratio was calculated in nests where the sexes of the full complement of brood were determined.

Size differences between castes were calculated following Packer (1992) as

$$D = \frac{S_Q - S_W}{S_Q} \quad (1)$$

where S_Q and S_W are the sizes (head width or wing length in mm) of the queen and worker respectively.

Weather

Weather data were gathered from the Utah State University climate centre website (2011) from a weather station 5.5 km away from the study site for all years this population had been studied (1976, 1977, 2002, 2010). The 65 year average temperature, used to cover all years of study and the 30 years before the initial year of study, was also obtained from this website. Degree days (base 10) were calculated for each day by the basic equation:

$$DD = \frac{T_{min} - T_{max}}{2} - 10 \quad (2)$$

Where T_{min} and T_{max} are the minimum and maximum temperatures reached that day. These data were used to calculate cumulative degree days (Zalom et al. 1983).

Calculations and statistical analyses

Foundress productivity was measured as the average number of offspring (including all eggs, larvae, pupae and callow adults) in first brood nests after the foundress had stopped foraging. Productivity in second brood nests was calculated as brood per working female. Because worker mortality was high, minimum productivity was also calculated as the number of second brood offspring present divided by the average number of first brood individuals found in nests multiplied by the proportion of first brood that was female. The sex ratio presented is the number of males divided by all brood.

A paired T-test was used to compare the size of the queen with that of her largest worker. Because most parameters measured were not normally distributed, all other tests of significance were Wilcoxon Mann-Whitney rank sum tests performed in R (version 2.15.2, R core team 2012).

Results

Phenology

All colonies studied were singly founded and foraging had commenced in some colonies by the initiation of the study on 13 June 2010. Only one *H. farinosus* female was witnessed to initiate excavation of her nest after this point. The last foraging trip taken by a foundress was observed on 12 July (Table 1). From early July, foundresses were frequently seen guarding nest entrances and rarely left the nest. Nest excavations began on 28 June, the first fully developed adult worker found in a cell was excavated on 15 July and the first adult first brood individual, a female, was observed leaving a nest on 17 July (Table 1). No new nests were founded after first brood emergence in the area of

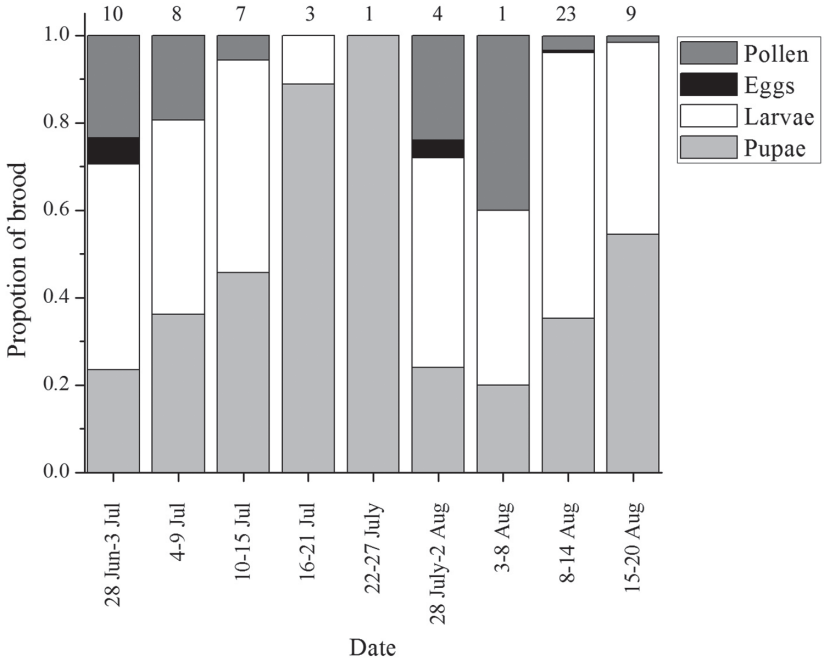

Figure 1. Percentage of brood in different developmental stages throughout the season, taken every six days. The rate of nest excavation throughout the season was not consistent. Numbers above each column represents the number of nests excavated during that time period.

observation. Although detailed data were not collected, daily observations at the nest site indicated that worker foraging slowed noticeably after the first week of August. The first fully developed, second brood male was found on 11 August and the first adult female in the second brood was excavated on 12 August. Figure 1 shows nest contents by developmental stage over 6 day periods throughout the season.

Surveys of all study plots conducted in mid-June and repeated in early August indicated a nest mortality rate between these times of 59.5%. Colonies that were particularly inactive may have been missed in either survey.

Nest architecture

Halictus farinosus nests in Green Canyon were easily identified by their large tumuli (see Nye, 1980). Bees entered the nest through a tunnel that ran horizontally through to the middle of the tumulus to the main burrow which was typically vertical. Two nests had one brood-containing branch off the side of the main tunnel and one had two brood-containing branches (as in Packer and Knerer 1986). All cells were constructed directly off the side of the main tunnel (see Nye 1980) or off of a side branch and measured approximately 1.4 cm long ($\bar{x}$ = 1.40, SD = 0.29, N = 7) and 0.7 cm wide ($\bar{x}$ = 0.66, SD = 0.14, N = 7) with a slightly constricted neck.

Nest depth for first brood nests ranged from 11 to 41 cm ($\bar{x}$ = 20.4 cm, SD = 7.3, N = 29), and shallowest and deepest brood cells were 7 cm and 20 cm respectively ($\bar{x}$ = 14.4 cm, SD = 2.8, N = 29). Second brood nests ranged from 12 cm to 44.5 cm in depth ($\bar{x}$ = 30.3 cm, SD = 6.1, N = 37) and as a result were significantly deeper (W = 153, $p < 0.001$) than first brood nests. Second brood nests had cells reaching to or very near to the end of the burrow.

The average number of cells in first brood nests was 4.4 (SD = 2.0, N = 29) including brood cells, empty cells and those containing only pollen. There were significantly more cells in second brood nests with an average of 20.1 in nests excavated late in summer (after 7 August) (SD = 10.1, N = 32, W = 79, $p < 0.0001$) which were expected to approach maximum brood sizes. This included empty cells that were previously occupied by first or second brood individuals and cells with mouldy contents. The percentage of cells containing mouldy contents (mostly pollen) over all nests was 6.7%.

In both broods, individuals at later developmental stages tended to be relatively closer to the nest entrance and cells containing only fresh pollen were always among the deepest excavated indicating that shallower cells were completed first.

Spring and summer brood productivity

Sixty-six nests were excavated, 29 containing juvenile first brood and 37 containing mostly the second, reproductive brood. The first brood nests contained between 0 and 7 first brood individuals with an average of 3.5 (SD = 2.0, N = 29). There were fewer brood than brood cells because in some cases cells were unfinished or contained mouldy pollen.

At the time of excavation an average of only 1.7 adult workers (range 0 – 7) were present within second brood nests. Active second brood nests produced between 1 and 46 brood with an average of 13.5 per nest (SD = 9.9, N = 37).

A breakdown of all nest contents in the first and second brood nests is presented in Table S1. The nest foundress was present in 22 of 29 first brood nests and 13 of 32 second brood nests (Figure 2). It could not be determined if the foundress was present in the remaining five second brood nests because adults in those nests had not been marked and genotype data were not available. Mean productivity of the foundress alone (first brood nests) was between 0 and 7 with an average of 3.5 brood, comprising 3.0 females and 0.5 males per nest. The sex ratio in the first brood was 0.149 weighting nests equally and 0.159 with individuals weighted equally. Mean productivity of first brood females was calculated using an estimate of the number of first brood females over the lifetime of the colony. As the mean number of second brood individuals produced per nest was 13.5 this gives 4.5 reproductive brood per worker female. Mean maximum second brood productivity per worker using the actual number of workers found in the nest was 7.0. The sex ratio in the second brood was not significantly different from 1:1 at 0.464 averaged over nests and 0.445 in the population as a whole.

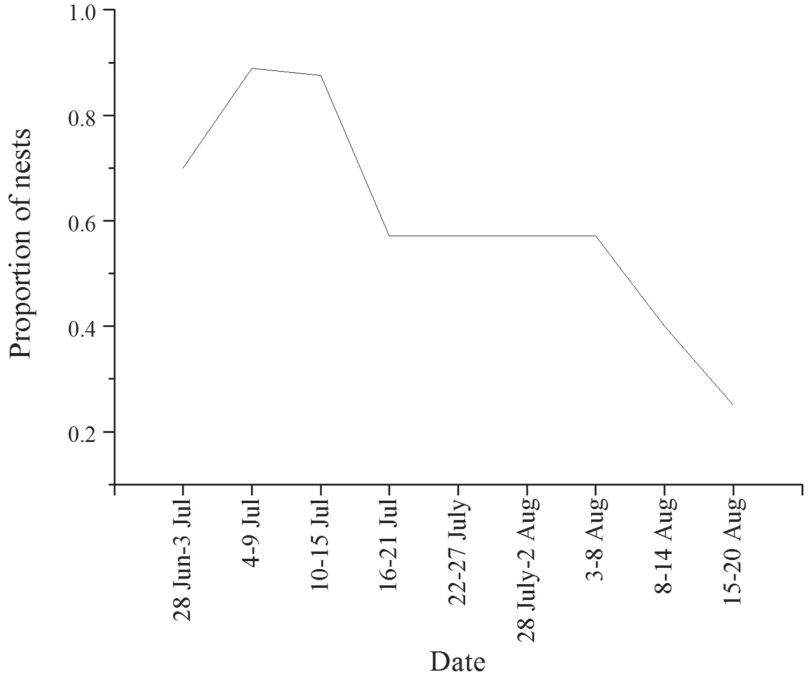

Figure 2. Proportion of nests excavated with the queen present, organized in 6 day intervals. Very few excavations took place between 16 July and 8 August so data are pooled together to show overall trend during this period. See Figure 1 for the number of nests excavated during each period.

Caste dimorphism

Dissections of 49 workers revealed that 36 (73.5%) had some level of ovarian development and 31 of 40 (77.5%) were mated (the spermatheca was not found in 9 of the workers dissected)(Table 2). All queens dissected were mated (N = 31) and all but one that was found dead in the nest with desiccated ovaries had some level of ovarian development (N = 31). The level of ovarian development in queens ($\overline{x}$ = 1.185, SD = 0.668, N = 31, Table 2) was significantly greater than in workers ($\overline{x}$ = 0.648, SD = 0.673, N = 49, W = 884.5, p = 0.001, Table 2). Workers in nests where the queen was present had lower but not significantly less ovarian development than workers in orphaned colonies (Table 3).

Queens averaged significantly larger than workers in both head width and wing length (Table 2). This gives a population-wide caste size difference of 6.25% for head width and 6.37% for wing length. Queens were also generally larger in both measures than their own workers with a mean percent difference of 8.79% for head width and 6.19% for wing length. Queens were found to be significantly larger than their largest worker in a paired T-Test for both head width (t = 3.34, p = 0.007) and wing length (t = 2.77, p = 0.018).

Table 2. Comparisons of ovarian development, matedness, head width, wing length, mandible and wing wear between queens and workers throughout the 2010 season. Means are presented with their standard deviations.

	Queen	Worker	Statistical comparison
Amount of ovarian development	1.185 +/- 0.668, 31	0.648 +/- 0.673, 49	W = 884.5, p = 0.001
Percentage with developed ovaries	96.8, N = 31	73.5, N = 49	
Percentage mated	100, N = 31	77.5, N = 40	
Head width (mm)	3.52 +/- 0.15, N = 33	3.30 +/- 0.15, N = 55	W = 1528, p < 0.0001
Wing length (mm)	3.14 +/- 0.15, N = 27	2.94 +/- 0.13, N = 45	W = 1017, p < 0.0001
Mandible wear	3.75 +/- 1.21, N = 28	3.57 +/- 1.53, N = 45	W = 749, p = 0.61
Wing wear	3.13 +/- 1.36, N = 30	2.81 +/- 1.64, N = 43	W = 655, p = 0.232

Queens were not generally more worn than workers in either mandible or wing wear (Table 2). Both wing and mandible wear increased throughout the season in queens, and more steeply in workers (Figure 3).

Temperature and rainfall

Minimum and maximum temperatures were low in the spring of 2010 compared to the 65 year average but were comparable to the average throughout the summer (Figure 4). Spring rainfall was high in 2010 compared to average, but was close to average throughout the rest of the season (Figure 5). Degree days accumulated slowly in 2010 compared to the other years of study (Figure 6) due to colder maximum and minimum temperatures in the spring (Figure 4). Degree days in 1976 and 1977 accumulated more quickly than in the more recent years of study (Figure 6) due to high minimum temperatures in these years (Figure 4).

Comparisons between years of study

Nests were shallower in both brood producing periods in 2010 (Brood 1: $\overline{x}$ = 22.1, SD = 7.4, N = 21, W = 434, p = 0.022, Brood 2: $\overline{x}$ = 30.3, SD = 6.1, N = 37, W = 2694, p < 0.0001) compared to 2002 (Sellars, 2004; Brood 1: $\overline{x}$ = 26.7, SD = 6.8, N = 30, Brood 2: $\overline{x}$ = 42.7, SD = 10.6, N = 86, Table 3). However, the number of brood produced per nest in both worker and reproductive phases was comparable. The number of first brood individuals produced in 2010 ($\overline{x}$ = 3.52, SD = 1.96, N = 29) and 2002 ($\overline{x}$ = 3.20 SD = 2.64, N = 30) did not differ significantly (W = 488, p = 0.420, Table 3). Second brood productivity also did not differ significantly (2010: $\overline{x}$ = 13.49, SD = 9.93, N =

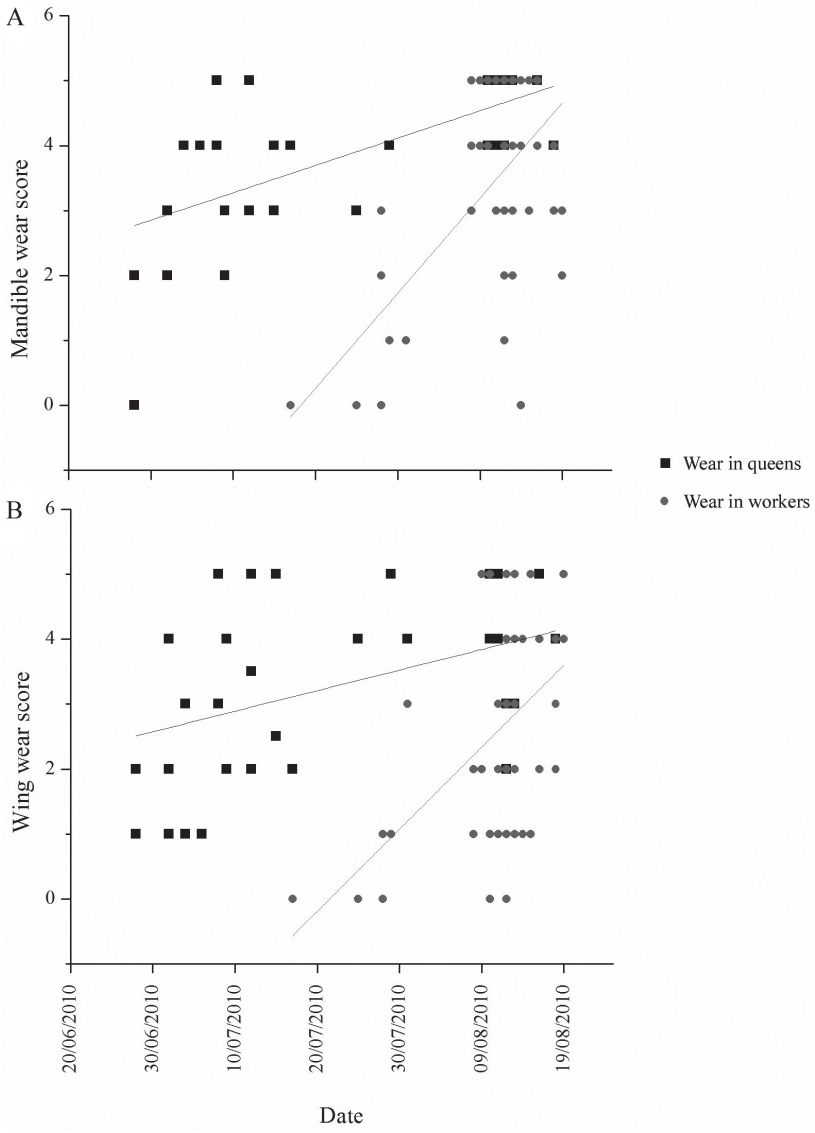

Figure 3. Wear of mandibles (**a**) and wings (**b**) of queens (black squares) and workers (grey circles) over time.

Table 3. Comparison of *H. farinosus* nest size, number of cells and mean offspring number in 2002 and 2010.

		2002	2010	Statistical comparison
First Brood	Depth (cm)	26.7 +/- 6.8	22.1 +/- 7.4	W = 434, p = 0.022
	Cells per nest	1 – 12	1 – 9	
	Mean offspring +/- SD	3.2 +/- 2.64	3.5 +/- 2.0	W = 488, p = 0.420
Second Brood	Depth (cm)	42.7 +/- 10.6	30.3 +/- 6.1	W = 2694, p < 0.0001
	Cells per nest	3 – 56	3 – 45	
	Mean offspring +/- SD	13.1 +/- 13.4	13.5 +/- 9.9	W = 1727.5, p = 0.184

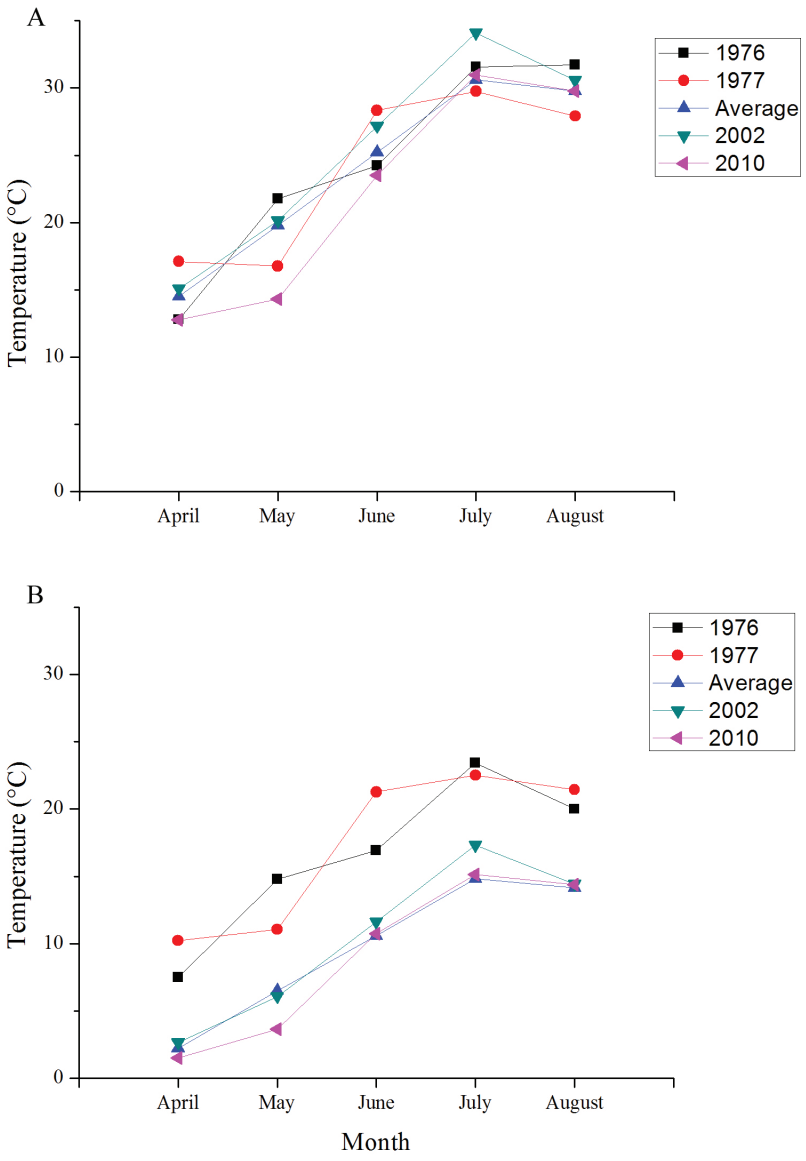

Figure 4. Average daily maximum (**a**) and minimum (**b**) temperatures for the four years of study on *H. farinosus* in Green Canyon and the seasonal average over the previous 65 years.

37, 2002: $\bar{x}$ = 13.06, SD = 13.36, N = 81, W = 1727.5, p = 0.184) (Table 3). Brood per working female (as estimated based on workers present) did differ significantly between years with a greater amount of productivity per female occurring in 2010 (2010, $\bar{x}$ = 6.99, SD = 4.21, N = 35)(2002, $\bar{x}$ = 6.09 , SD = 7.11, N = 70) (W = 914, p = 0.021). The percent difference in head width between queens and workers was significantly greater in 2010 than in 2002 (8.79% cf 5.79%; W = 424, p = 0.027). The proportion

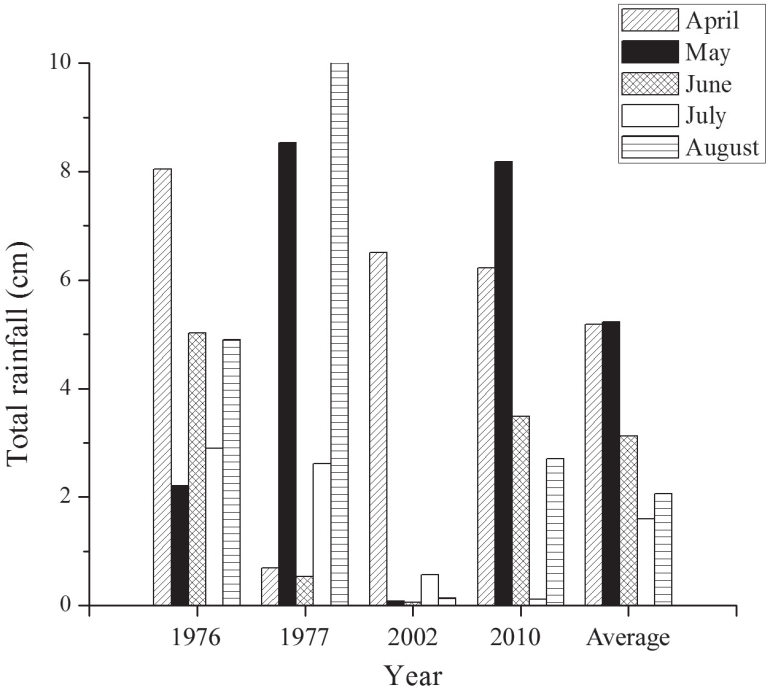

Figure 5. Total rainfall per month for the four years of study on *H. farinosus* in Green Canyon and the monthly average over the previous 65 years.

Table 4. Summary of egg development and matedness in queens and workers in 2002 and 2010. Averages are presented with standard deviations. Females are grouped based on their development score (ds).

	2002		**2010**			
	Queens	Workers	Queens	Workers	Workers: Queen present	Workers: Queen not present
N	46	91	31	49	26	23
Number of females with ds < 0.5	4	40	2	21	12	9
Number of females 0.5 < ds < 1	4	15	9	13	7	6
Number of females with ds > 1	38	36	20	15	7	8
% with developed ovaries	93.4	60.4	96.8	73.4	73.1	69.6
% mated	91.3	46.1	100	77.5	75.0	80.0
Average amount of ovarian development	1.429 +/- 0.765	0.913 +/- 1.070	1.185 +/- 0.668	0.648 +/- 0.673	0.625 +/- 0.458	0.793 +/- 0.842

of workers that were mated also differed greatly between years with 77.5% of workers mated in 2010 and only 46.1% mated in 2002 (Table 4). A greater percentage of workers in 2010 had developed ovaries but the average level of egg development in workers was not significantly different between years (W = 2114.5, p = 0.607, Table 4).

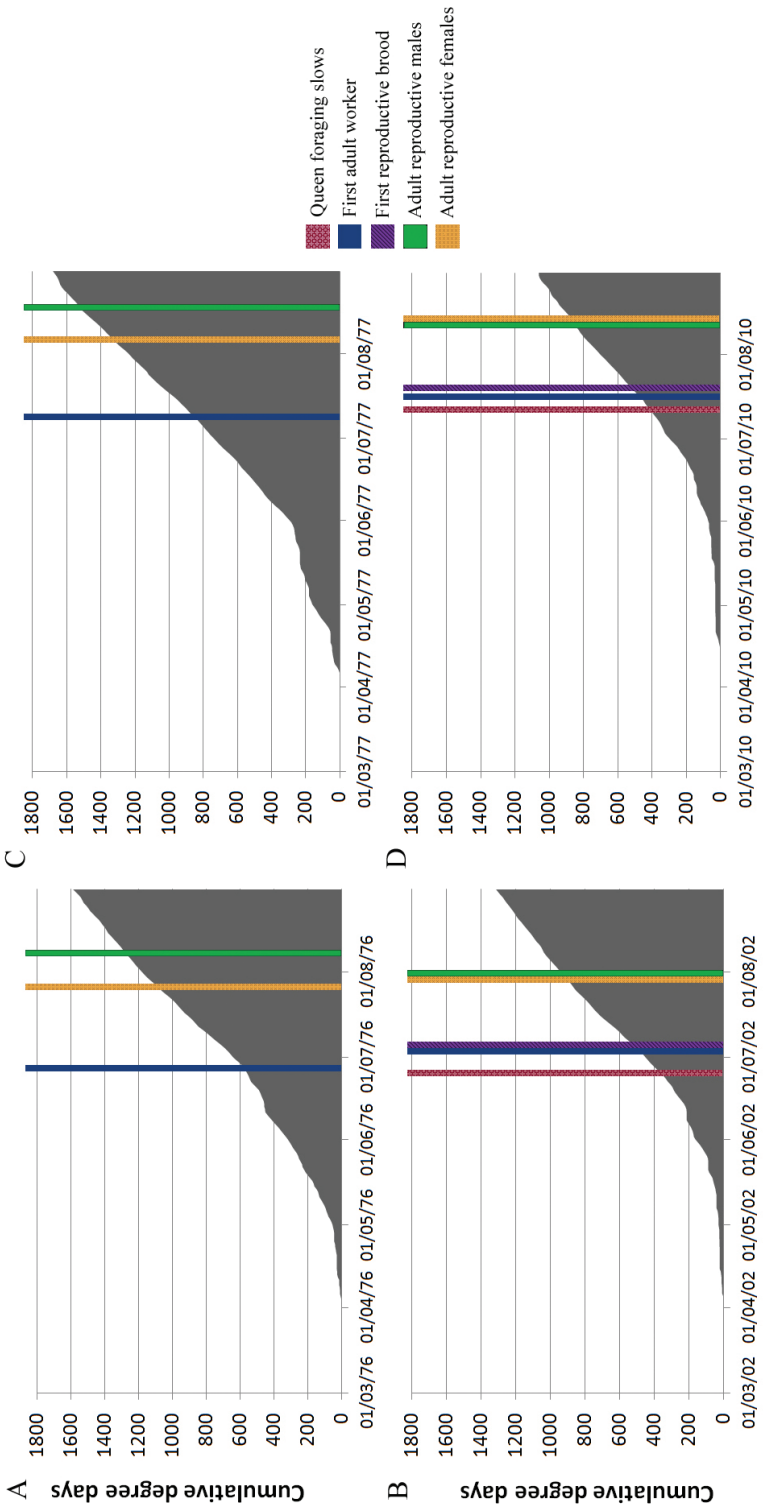

Figure 6. Cumulative degree days between March and August in 1977, 1978, 2002 and 2010. Phenological markers (queen foraging slows, first adult worker, first reproductive brood cell, first adult male reproductive, first adult female reproductive) have been included as coloured bars to note the dates of the events. Males and females from the second brood were not differentiated in the 2002 study, and some events were not recorded in 1977 and 1978.

Discussion

Comparisons of nesting biology of *Halictus farinosus* among years

Halictus farinosus was studied at Green Canyon in the 1970s by Nye (1980) and in 2001 and 2002 by Sellars (2004). In this section we compare the results obtained among years.

A combination of temperature and rainfall patterns explain variation in phenological events in *H. farinosus* between 2002 and 2010: major events in the colony cycle coincide well with the number of degree days accumulated, with the first adult brood emerging at around 500 degree days (by equation (2), Figure 6). However, in 1976 and 1977 minimum temperatures were much higher and a larger number of degree days had accumulated before workers emerged (800 and 600 days respectively, Figure 6).

The amount of rainfall may have been responsible for the differences in emergence dates between years and the poor correlation with degree day accumulation. Brood development and emergence dates (Table 1) were delayed about two weeks in 2010 compared to 2002 (Sellars 2004) but were similar to those reported by Nye (1980) for 1977. Timing in 1976 was intermediate (Table 1). The amount of spring rainfall followed the same pattern: high in May of 1977 and 2010 low in 2002 and intermediate in 1976. The drier weather in May of 1976 and 2002 likely allowed queens to initiate foraging earlier, to forage more often than in 1977 and 2010 and to produce brood sooner.

Despite the differences in phenology and weather patterns between 2002 and 2010, there were no significant differences in brood size in either brood producing period. More brood were produced per working female in 2010 than in 2002 but this likely reflects differences in the excavation schedule between the two years given that a greater proportion of nests were excavated later in the season in 2010 when more brood and fewer workers were present.

Differences in weather patterns may account for the disparity in caste size dimorphism between years. Weather was much harsher in the spring of 2010 with more rain and colder temperatures compared to 2002 (Figures 4, 5, 6). Poor spring weather conditions may have led to smaller workers being produced in 2010 compared to 2002, as found for a congeneric species by Richards and Packer (1996). It is generally thought that smaller individuals are less effective at foraging than larger ones because they cannot forage as far or carry as much pollen (Gathmann and Tscharntke 2002; Richards 2004). Floral resources in Green Canyon are generally very sparse (Nye 1980; Sellars 2004; personal observations) and foraging trips in the *Halictus farinosus* population there are typically very long, often well over an hour (Nye 1980; Sellars 2004; personal observations), though it is not known if this is a local phenomenon or if it is typical of the species. Despite this worker size difference, reproductive nest productivity between the two years was not significantly different.

A much greater percentage of workers in 2010 were found to be mated compared to 2002 and ovarian development in workers was comparable between years (Table

4). This result is unexpected as the workers would be predicted to be less fertile when the queen-worker size differential is larger and queens presumably have more control (Richards et al. 1995; Richards and Packer 1996). A smaller proportion of the first brood in 2002 were male (5.1%), compared to 2010 (15.9%) indicating that perhaps fewer males were available to mate with first brood females in 2002. In addition, excavations in 2002 were performed at regular intervals throughout the season as opposed to 2010 where many were excavated in a short period of time late in the season. The difference in excavation dates between years could account for the greater number of mated workers and greater than expected ovarian development in workers in 2010.

Comparisons to other *Halictus* species

Phylogenetic data, both morphological (Pesenko 1984) and molecular (Danforth et al. 1999) have been used to understand the systematics of *Halictus*. *Halictus farinosus* forms a sibling species pair with *H. parallelus* Say which, although stated by Knerer (1980) to be solitary, is a social species (Packard 1868; Taylor and Packer unpublished observations). These two are closely related to the socially polymorphic *H. rubicundus* Christ (Soucy 2002; Soro et al. 2010) and the solitary, occasionally communal, *H. quadricinctus* Fabricius (Knerer 1980; Sitdikov 1987). It is more distantly related to the other *Halictus* species that are known to be eusocial such as *H. ligatus* Say and *H. sexcinctus* Fabricius (Packer 1986; Richards 2001), though the latter exhibits some additional social complexities (Richards et al. 2003).

Halictus farinosus produces a small number of workers and has low to moderate worker-queen size dimorphism compared to other social *Halictus* species (Table 5). It also has a comparatively large number of worker females that are mated and/or have ovarian development, but this varies widely between years. Small size dimorphism, the small number of workers and high degree of mating and ovarian development in workers indicates that the level of sociality in *H. farinosus* is relatively weak compared to that in other social *Halictus* species (Packer 1992). The nesting biology and behav-

Table 5. Comparison of aspects of sociality and natural history in social species of the subgenus *Halictus*.

	H. farinosus	***H. rubicundus***	***H. ligatus***	***H. sexcinctus***
First brood size	3.5–3.6	4.4	5.8–> 9	7–8.5
Second brood size	13.0–13.5	10.5	10–16	4.9–5.8
First brood sex ratio	5–15%	10.3%	5.5–14%	23%
% workers with ovarian development	60.4–77.4%	N/A	57.16%	73.70%
% workers mated	46.1–77.5%	N/A	42%	56%
Queen-worker size dimorphism	6.4–8.6%	9.6%	12.7%	6.9–11.8%
Source	Sellars, 2004; Present study	Soucy, 2002 (social populations)	Packer, 1986; Richards and Packer, 1995	Richards, 2001

ior of social populations of *H. rubicundus* are similar to those of *H. farinosus* in brood size, nest architecture and the level of caste size dimorphism (Table 5; Soucy 2002). However, unlike *H. farinosus*, *H. rubicundus* is socially polymorphic (Eickwort et al. 1996; Soucy and Danforth 2002; Soro et al. 2010). While solitary and social populations of *H. rubicundus* may form phylogenetically separate lineages (Soucy and Danforth 2002) some halictines exhibit social polymorphism even within the same population (Packer 1990; Richards et al. 2003; Hirata and Higashi 2008). While there are no known solitary *H. farinosus* populations, seasonal climatic differences might affect its sociality through the queen-worker size differential (Richards and Packer 1996). The biology of *H. farinosus* should be studied in locations throughout its geographic distribution, including populations at high altitude and latitude where the species is more likely to be solitary, to fully understand the effect of climate conditions on life history and sociality in this species and determine whether solitary behavior is within the species' repertoire.

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Appendix

Table S1: Excavation details for all nests. (doi: 10.3897/JHR.32.4646.app) File format: Adobe PDF file (pdf).

Explanation note: Table listing all excavated nests, the date of excavation and a breakdown of their brood contents by developmental stage.

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SHORT COMMUNICATION

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First detection of *Agrilus planipennis* in Connecticut made by monitoring *Cerceris fumipennis* (Crabronidae) colonies

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Abstract

Smoky winged beetle bandits, *Cerceris fumipennis* Say, digger wasps in the family Hymenoptera: Crabronidae: Cercerini, provision their underground nests with adult metallic wood-boring beetles (Coleoptera: Buprestidae). Researchers, as well as engaged community volunteers, in several states have monitored female wasps returning to their nests as a means to detect invasive buprestid species. In this paper, we report the first detection of emerald ash borer (*Agrilus planipennis* Fairemore), an invasive beetle responsible for killing millions of ash trees in North America, in Connecticut by *C. fumipennis* and discuss its relationship to *A. planipennis* survey efforts by other modalities in the state. We also report detections of *A. planipennis* by *C. fumipennis* in Illinois, New York and Ontario; all of which were made after it was known the beetle was in the area. These findings support the use of *C. fumipennis* as a biomonitoring tool and bolster the use of engaged volunteers.

Keywords

Biosurveillance, *Cerceris fumipennis*, *Agrilus planipennis*, survey technique

Introduction

Emerald ash borer (*Agrilus planipennis* Fairemore) (EAB) is native to far eastern Asia, and was accidentally introduced to North America in the mid 1990's. It was first detected in 2002 in Detroit MI, and has spread to 18 US states and 2 Canadian provinces. North American *Fraxinus* spp. show little to no resistance to the beetle, allowing it to attack and kill trees regardless of tree condition (Rebek et al. 2008). One major concern has been locating new infestations of EAB. The average time from infestation to detection is 6-12 years (McCullough et al. 2011; MFK unpublished data), ample time for the beetles to become firmly established in a new location. Much research and resources have been devoted to developing survey techniques to shorten the time between infestation and detection (Francese et al. 2006; Poland et al. 2006; Crook et al. 2009; Francese et al. 2011; Grant et al. 2011; McCullough et al. 2011; Ryall et al. 2012).

A novel survey technique called biosurveillance has been developed as part of that effort (Marshall et al. 2005; Careless 2009). This technique takes advantage of the prey specialization of *Cerceris fumipennis* Say, 1837 (Hymenoptera: Crabronidae: Cercerini), a native solitary digger wasp. As is typical for digger wasps, females provision larval cells with paralyzed prey. A wide range of adult beetles in the family Buprestidae are utilized by *C. fumipennis* females (Marshall et al. 2005; Careless and Marshall 2010; Rutledge et al. 2011) to provision their nests. Included in that range are beetles in the genus *Agrilus*, the genus of the EAB. By monitoring the prey returning wasps bring to their nests, surveyors can sample the buprestid beetles in the area surrounding the colony, including EAB. Studies by Careless (2009) suggested that the majority of species in an area are sampled after collecting 50 beetles from a colony, and that further collecting yields diminishing returns. Thus, the goal of an EAB biosurveillance program is to collect at least 50 beetles from each colony (Carrier and Jackson 2012).

Biosurveillance has been undertaken in several states and provinces where EAB is already known to occur and in areas, like New England, where the beetle had not yet been detected (Table 1). In Connecticut, a biosurveillance program was begun in 2008 by The Connecticut Agricultural Experiment Station (CAES) with surveys for *Cerceris fumipennis* colonies and the monitoring of nest sites, collecting 129 beetles. The program was expanded in 2009 the discovery of 40 additional colonies suitable for monitoring for EAB, biosurveillance of 36 sites and the capture of 315 beetles. To enhance colony survey efforts in Connecticut, a citizen-scientist program was initiated in 2010 modeled on a volunteer program in Maine (Rosenholm 2012; Teerling 2012) called 'Wasp Watchers' that assigns volunteers to specific colonies and trains them to monitor the wasps' prey for EAB. The 'Wasp Watcher' program in Connecticut has grown steadily, with 23 watchers in 2010, 29 in 2011 (12 returning) and 52 watchers in 2012 (19 returning), increasing the number of colonies monitored and beetles collected.

In 2010 and 2011, 1,605 beetles were collected by CAES volunteers and researchers. Maine, Vermont and Massachusetts also have flourishing 'Wasp Watcher' pro-

Table 1. Sites with known *Cerceris fumipennis* captures of emerald ash borer.

Site[1]	Collector[2]	Year	#EAB collected[3]	EAB known in State/ Province?
Windsor, ON	University of Guelph	2006	48	Yes
LaSalle, ON	University of Guelph	2006	1	Yes
Walpole Is., ON	University of Guelph	2006	8	Yes
Wheatley, ON	University of Guelph	2007	1	Yes
Toronto, ON	University of Guelph	2008	31	Yes
Turkey Point, ON	University of Guelph	2009	107	Yes
Ottawa, ON	University of Guelph	2010	1	Yes
Lancaster, NY	SUNY ESF	2011	3	Yes
Cook County, IL	USDA APHIS PPQ	2011	present	Yes
DuPage County, IL	USDA APHIS PPQ	2011	present	Yes
Kane County, IL	USDA APHIS PPQ	2011	present	Yes
Lake County, IL	USDA APHIS PPQ	2011	present	Yes
Prospect, CT	CAES	2012	36	No
Beacon Falls, CT	CAES	2012	1	No

[1] Data obtained from APHIS PPQ surveys cannot not be identified below county level

[2] Collector refers to the institution for which the collector was working at the time of the find

[3] Data obtained from APHIS-PPQ surveys only noted presence/ absence of emerald ash borer

grams (Rosenholm 2012). With the detection of EAB in Saugerties, NY in 2010, a collaborative effort between the USDA Animal Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) and CAES, with support of the U.S. Forest Service, was made to monitor as many colonies in Connecticut as possible. In 2011 and 2012, USDA-APHIS-PPQ personnel monitored colonies in the four western counties, while personnel from CAES monitored colonies in the four eastern counties, in addition to administering the 'Wasp Watcher' program. In the 2012 season, 2,139 buprestids were collected from 81 *C. fumipennis* colonies.

Emerald ash borers have been recovered from wasps in several areas with known EAB populations (Table 1), but this approach had not yet made the initial detection of emerald ash borer in a state or province where it had previously been undetected. However, on the morning of July 16, 2012, CAES personnel captured a wasp carrying a female emerald ash borer in the Town of Prospect in New Haven County, CT. A further six emerald ash borers were captured at that colony that same afternoon. Since EAB is a federal and state regulated pest, the USDA-APHIS-PPQ State Plant Health Director submitted the initial specimen to the PPQ identifier, James Zablotny, for identification. James Zablotny confirmed the state identification on July 17, 2012 and forwarded the specimen onto the Systematic Entomology Laboratory (SEL) in Beltsville, MD for confirmation as a new state record. Alexander Konstantinov, SEL, confirmed the identification on July 18, 2012. This represented the first new state detection using *Cerceris* biosurveillance for the detection of EAB. In subsequent days, an additional 26 EAB adults were captured, including EAB found on the ground near the entrance to nest holes (Fig. 1). As fewer *C. fumipennis* colonies were

Figure 1. Abandoned emerald ash borer next to the nest entrance of *Cerceris fumipennis* at a colony in Prospect CT.

being monitored in this area than in other parts of the state, additional colonies were located in Prospect and towns immediately adjacent to it (Beacon Falls, Cheshire, Naugatuck, Bethany, Seymour). Surveys of these colonies yielded two more EAB positive colonies. On July 17th, three EAB were captured at a Prospect site approximately one km due south of the first find. One additional EAB was caught on July 24th in the Town of Beacon Falls, about 11 km southwest of the colonies in Prospect. A 'Wasp Watcher' volunteer had caught nine EAB at the original Prospect colony in the first week of July, but did not recognize the beetles and so the finds were not reported until July 19th.

In addition to the three colonies at which EAB were collected, 25 other *Cerceris fumipennis* colonies had 50+ beetles collected (Table 2A, B). By USDA-APHIS-PPQ criteria, collecting 50 beetles at a site is not an official 'negative' for EAB (Carrier and Jackson 2012), but it does suggest EAB is likely not present, or is present at very low densities. The minimum EAB density for *C. fumipennis* detection is not known, however, we do know that EAB was found at the first Prospect colony after only 15 beetles were collected in 2012. However, that same colony was monitored in 2011, with 68 beetles representing 11 species captured (and no EAB).

As part of Connecticut's statewide EAB surveillance efforts, USDA-APHIS-PPQ purple-prism traps were deployed around the state in 2011 (940 traps) and 2012 (544

Table 2A. Colonies within New Haven County with 50+ beetles collected and/or colonies at which emerald ash borer was collected.

City	Date	Holes	Beetles	# EAB
Beacon Falls	24-Jul	70	25	1
Cheshire	18-Jul	100+	76	0
Middlebury	25-Jul	75	66	0
North Branford	29-Jun	100+	66	0
Orange	6-Jul	50	59	0
Prospect 1	16-Jul	100+	124	33
Prospect 2	17-Jul	50	42	3

Table 2B. Number of *Cerceris fumipennis* colonies per county in Connecticut at which 50+ buprestids were collected in 2012, but at which no emerald ash borer was found.

County	# Colonies
Fairfield	1
Hartford	3
Litchfield	11
Middlesex	2
New London	1
Tolland	2
Windham	1

traps) by the University of Connecticut with support from APHIS-PPQ. During routine trap monitoring, suspect specimens were collected from traps in Prospect on June 29th, 2012 and Naugatuck on July 9th, 2012 but had not yet been processed when EAB was first collected from *Cerceris fumipennis*. After confirmation of EAB was received from SEL, the EAB from the Prospect trap was identified by Claire Rutledge, the designated state-identifier, on July 18th. During the week following the initial find in Prospect, the purple-prism traps in the area were re-checked and additional suspect specimens were collected in Prospect, Naugatuck and Bethany (all New Haven County). Because Bethany and Naugatuck would represent new towns for EAB, the specimens collected from those traps were sent to PPQ identifier Bobby Brown for confirmation of identity, which was received on July 20th. An additional 29 traps were deployed in Prospect, Cheshire, Naugatuck and Bethany to help further define the extant of the infestation. One of these subsequently deployed traps, across the road from the second positive *C. fumipennis* colony in Prospect, also captured an EAB (Table 2A, B).

No purple-prism traps outside of the four towns mentioned above captured an emerald ash borer (Table 3). Ash trees with heavy EAB infestations were subsequently found in the Town of Prospect and a state quarantine for New Haven County was established on August 9, 2012 followed by a parallel federal quarantine on September 12, 2012.

Together, biosurveillance with *Cerceris fumipennis* and use of purple-prism traps provided the first detection of an EAB infestation in Connecticut. Subsequent *C.*

Table 3. Summary of purple prism traps in Connecticut in2012.

County	# traps	# traps with EAB	# EAB captured
Fairfield	57	0	0
Hartford	74	0	0
Litchfield	171	0	0
Middlesex	55	0	0
New Haven	49	4	38
New London	43	0	0
Tolland	83	0	0
Windham	43	0	0

fumipennis colony identification and biosurveillance also provided an initial estimate of its extent. Delimitation surveys for infested trees will continue over the winter by peeling bolts. In summer 2013, multiple *C. fumipennis* colonies at the known limits of the infestation will be surveyed to further delimit the infestation.

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