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Wallaceaphytis: an unusual new genus of parasitoid wasp (Hymenoptera: Aphelinidae) from Borneo

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Wallaceaphytis Polaszek and Fusu gen. nov. (type species *Wallaceaphytis kikiae* Ayshford and Polaszek sp. nov.) is described from Danum Valley, Sabah, in Malaysian Borneo. Although known from just a single female individual, the genus is extremely unusual morphologically, being the only member of the large subfamily Aphelininae with four-segmented tarsi. The form of the fore wings and head are also unique in the subfamily, and its status as a new genus is confirmed by analysis of nuclear ribosomal DNA. DNA sequence analysis was undertaken by comparison with more than 60 aphelinid sequences from GenBank. The sequence for the standard DNA barcode region (cytochrome oxidase c subunit I; COI) is provided. The new genus is named in honour of Alfred Russel Wallace, co-discoverer of the theory of evolution by natural selection. The new genus and species are published on the exact date of the centenary of his death.

http://www.zoobank.org/urn:lsid:zoobank.org:pub:700D2B2A-1586-4100-85D2-24844EFE3F90

Keywords: Aphelininae; Chalcidoidea; chalcids; phylogeny; Sabah; Malaysia; Alfred Russel Wallace; DNA barcode; non-destructive DNA extraction

Introduction

Within the large and megadiverse parasitoid superfamily Chalcidoidea ("chalcid wasps"), Aphelinidae is one of the smaller families, containing 1300 species belonging to 36 genera (Noyes 2013). Species of Aphelinidae are mostly primary parasitoids or hyperparasitoids of Hemiptera, mainly Sternorrhyncha (Aleyrodidae, Aphididae, Coccidae, Diaspididae & Pseudococcidae, among others), although several genera are known to include species that are parasitoids of insect eggs (Polaszek 1991).

The large subfamily Aphelininae was recently the subject of a major phylogenetic analysis based on morphological characters, which resulted in the description of four new genera (Kim & Heraty 2012). This analysis includes a key to all of the currently recognized 16 genera within the subfamily. The new genus described below differs radically in several characters from any of the currently valid genera of Aphelininae, and these differences are discussed in detail below.

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Material and methods

Collecting

In September and October 2012 a major multidisciplinary joint expedition from the Natural History Museum, London, UK, and Universiti Malaysia Sabah, Kota Kinabalu, Malaysia, undertook extensive sampling and surveying of arthropods and other invertebrates in the Danum Valley and Maliau Basin Conservation Areas. A wide range of collecting techniques was employed, including the use of Winkler bags for soil samples, Malaise traps and yellow pan traps for day-flying insects, and a specially modified sweep net for very small insects found in undergrowth and foliage. The "Noyes-net" (Noyes 1982) is a heavy-duty, long-handled sweep net with 4-mm wire mesh screening the collecting bag, ensuring that only specimens with a maximum length of 4 mm or thereabouts are collected. Sweeping the undergrowth and foliage for several minutes results in an accumulation of usually several hundred microarthropods in the collecting bag, as well as associated debris, seeds etc. These can either be collected directly into a container of 80–100% ethanol for sorting under a microscope later, or the emergent insects can be aspirated individually. In the latter case, less sorting is needed subsequently, but a large proportion of the catch can be lost or overlooked. The latter technique was used in the present case, with the sample sorted back in London several weeks later. Immediate recognition by the second author (TA) that a particular specimen was clearly something extremely unusual, prompted a special study of this individual, which was then subjected to the non-destructive DNA extraction protocol described below.

DNA extraction and slide-mounting

Genomic DNA was extracted using the DNeasy® blood and tissue kit (Qiagen, Hilden, Germany) from the whole specimen, using a non-destructive method slightly modified from the manufacturer's protocol (Noyes 2010). The specimen was removed from ethanol, dried briefly on absorbent paper to remove any visible traces of liquid, and immersed in ATL lysis buffer containing proteinase K in a 1.5 ml microtube ("Eppendorf®"). Specimen lysis was achieved by overnight incubation at 55°C (or for at least 8 hours) after which all internal tissues have been digested, while the exoskeleton remains intact in the enzyme–buffer mix (throughout this time vortexing should be avoided to reduce the risk of damaging the specimen). The lysis buffer containing DNA was transferred by pipetting to a new 1.5 ml microtube, and processed as described in the kit, except that the final DNA elution was into 100 µl. The extracted specimen was immediately washed by pipetting 1 ml distilled water into the microtube, changing the water after 30 min, and finally transferring the specimen into 80% ethanol. If the specimen is not thoroughly washed, crystals can form upon its surface on contact with ethanol. In the unlikely case of crystal formation, these can be dissolved by placing the specimen for a few minutes in warm distilled water. Specimens extracted with this method can be directly mounted without a previous alkaline treatment (e.g. maceration in 10% KOH), or critical-point dried or chemically dried using hexamethyldisilazane, but air drying is likely to result in the specimen collapsing. For slide-mounting, the specimen can be dehydrated through graded alcohols up to 100% and permanently slide-mounted in Canada balsam after clearing with clove oil. In the present case, the specimen was dissected and mounted following the protocol described by Noyes (1982), from the 100% ethanol/clove oil stage

onward, with the modification that dissection takes place in Canada balsam to reduce specimen movement and the consequent risk of losing the dissected parts.

Polymerase chain reaction (PCR) and sequencing

The standard barcode region (Hebert et al. 2003) and the 28S rDNA D2 and D3 expansion regions were amplified by PCR using the LCO1490/HCO2198 primer pair of Folmer et al. (1994) and D23F (5′-GAG AGT TCA AGA GTA CGT G-3′; Park & Foighil 2000)/28Sb (5′-TCG GAA GGA ACC AGC TAC TA-3′, aca D3B; Nunn et al. 1996), respectively. We performed standard 25-µl PCRs containing 2.5 µl of 10× PCR buffer, 0.75 µl of 50 mM $MgCl_2$, 0.2 µl dNTPs solution (25 mM each), 1.25 µl of each primer (10 µM), 0.3 µl *Taq* polymerase (5u/µl Biotaq, Bioline), 6 µl DNA extract, and PCR grade water to final volume. PCR conditions for cytochrome oxidase c subunit I (COI) were 94°C for 2 min, followed by 40 repeated cycles of 94°C for 30 s, 42°C for 50 s and 72°C for 35 s, a final extension at 72°C for 10 min and incubation at 10°C. The same conditions were used for the amplification of 28S rDNA except annealing for 30 s at 55°C. The PCR products were visualized on a 1% agarose gel.

Both DNA strands were sequenced at the Natural History Museum Life Sciences DNA Sequencing Facility using the same primers used for the PCR. Sequences were edited using Pregap4 v1.5 and Gap v4.10 in Staden Package (Bonfield et al. 1995) and sequence verification was conducted by comparing forward and reverse sequences. All sequences are deposited on GenBank (Accession numbers BankIt1665361 Seq1 KF718961 [28S ribosomal]; BankIt1665361 Seq1 KF718962 [CO1]).

Additional sequences for the phylogenetic analysis obtained mostly by J.-W. Kim and J. Heraty (unpublished data) were retrieved from GenBank. Sequences were first aligned using MEGA 5.05 (Tamura et al. 2011) and the ClustalW algorithm for the ML analysis, or were manually aligned using the secondary structure models following Gillespie et al. (2005) and the alignment of Munro et al. (2011) for the Bayesian analysis. Phylogenies were estimated using maximum likelihood in MEGA 5.05 and Bayesian methods in MrBayes version 3.2 (Ronquist & Huelsenbeck 2003). Analyses were run using a GTR + G + I model of nucleotide substitution as this was determined as the most appropriate model with MEGA 5.05. In MrBayes the analysis was run for 10,000,000 Markov Chain Monte Carlo generations, with trees and lnLs sampled every 100 generations. Likelihood stationarity occurred after 15,000 generations that were discarded as "burn in".

Results

Sequence analysis

The aligned sequences of the 28S rDNA D2 and D3 expansion region produced a sequence matrix 1121 base pairs long when automatically aligned using the ClustalW algorithm or 1074 base pairs long in the case of the secondary structure alignment. Maximum likelihood analysis resulted in one best-scoring tree with *Wallaceaphytis* placed as sister group to a clade formed by all *Centrodora* species used in the analysis (Figure 1), but this relation is only weakly supported (54% bootstrap support). In the bootstrap consensus tree *Wallaceaphytis*, *Centrodora*, *Aphytis*, and *Aphelinus* are all part of an unresolved polytomy (tree not shown). In the Bayesian analysis, the exact position of *Wallaceaphytis* was not resolved either, as it was placed in a polytomy with *Marietta*, *Aphytis* and *Aphelinus* (Figure 2).

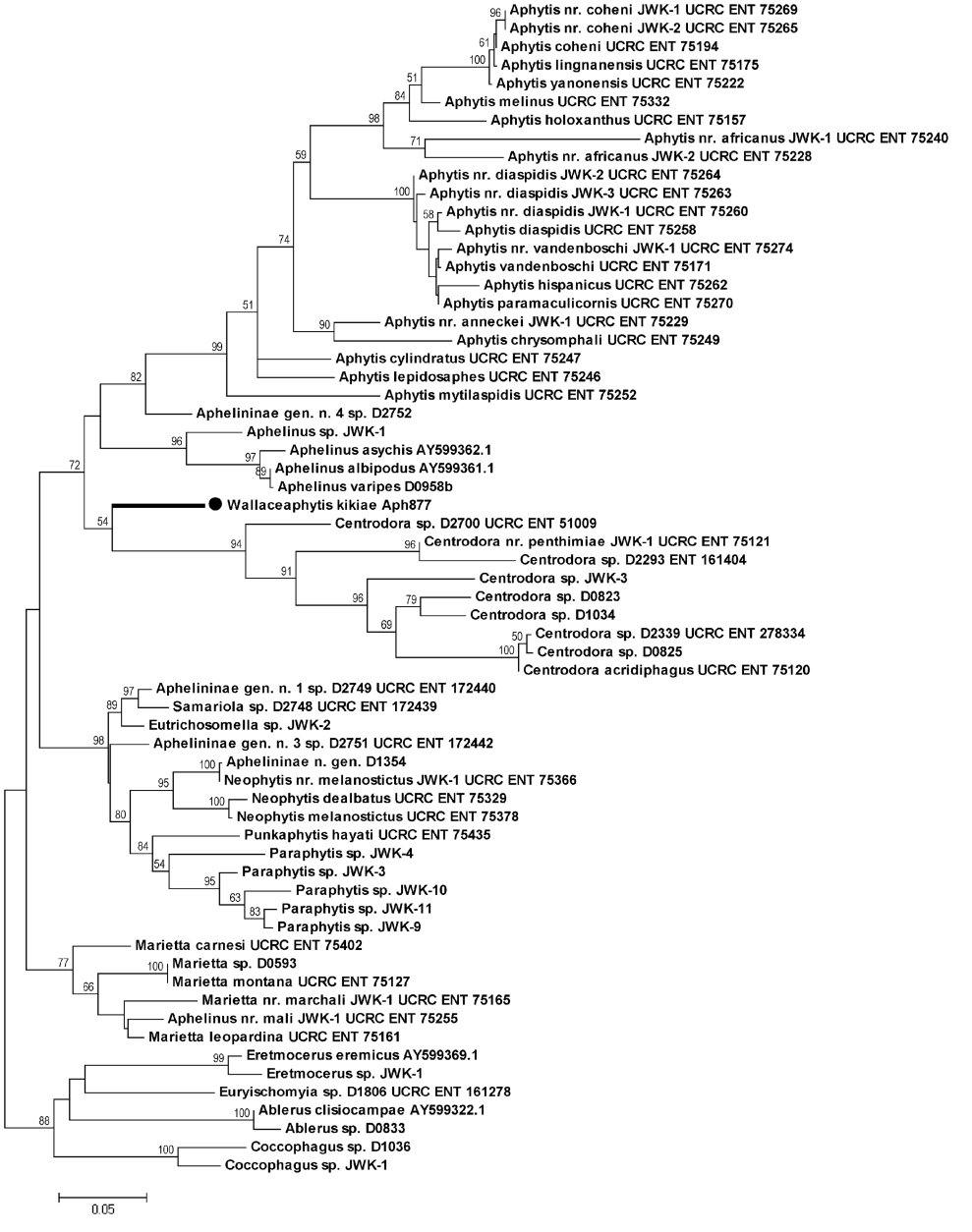

Figure 1. Maximum likelihood tree based on 28S-D2 and D3 sequences. Bootstrap values based on 1000 replications are shown for nodes with more than 50% bootstrap support.

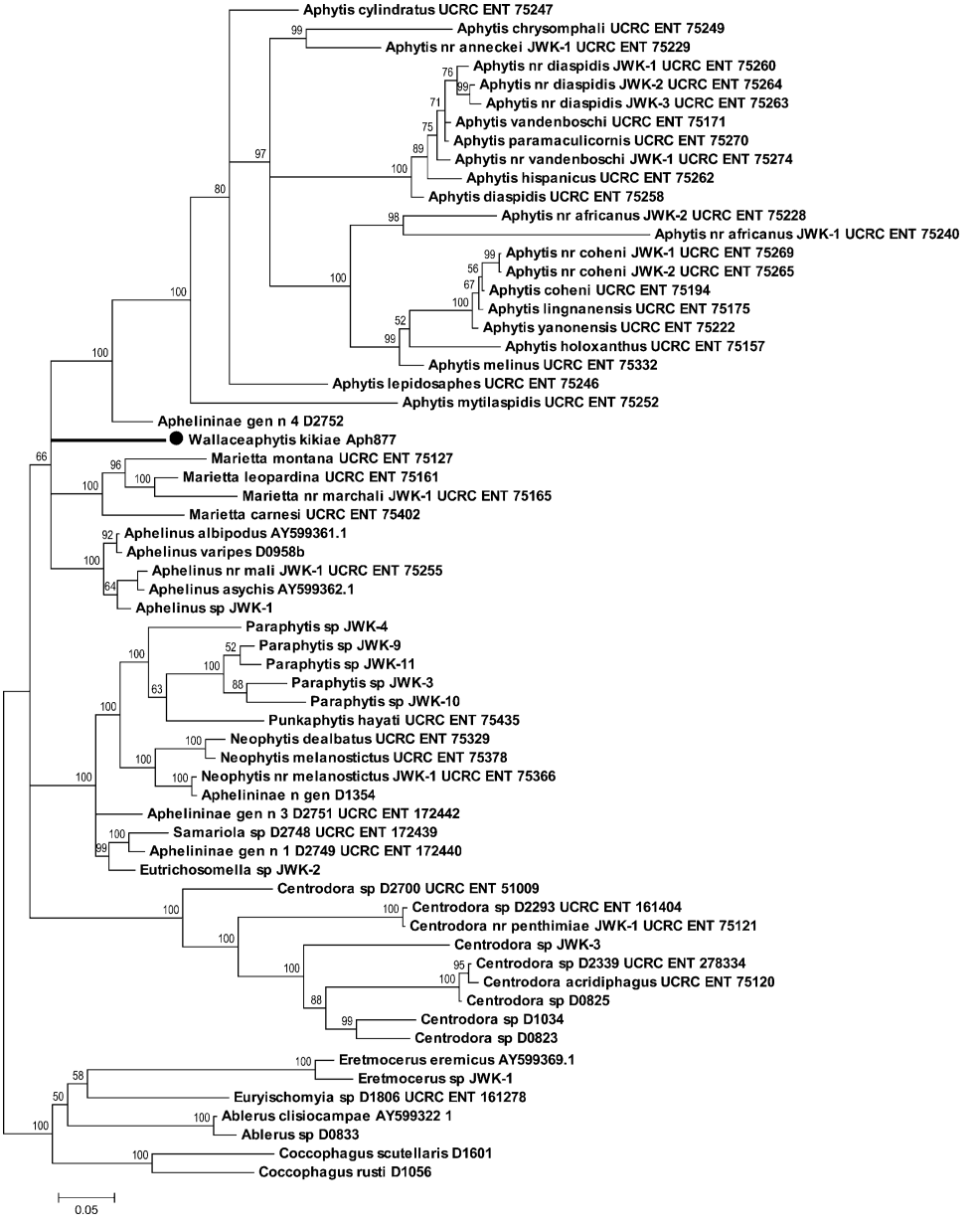

Figure 2. Majority rule consensus Bayesian tree based on 28S-D2 and D3 sequences. Posterior probability values are indicated in bold above nodes.

Figure 3. *Wallaceaphytis kikiae* holotype female; mesosoma and metasoma.

Taxonomy

Wallaceaphytis Polaszek and Fusu gen. nov.
Type species ***Wallaceaphytis kikiae*** Ayshford and Polaszek sp. nov.
(Figures 3–11)

Description/generic diagnosis

Morphology. Antenna with three segments (Figure 5), scape, pedicel and a single flagellar segment. Anellus present, narrower on its internal side. Scape narrow, length 5× maximum width. Maximum width of pedicel 1.6× maximum width of scape. Flagellum length 2.9× maximum width, and 1.4× scape. Head strongly transverse (Figures 4 and 8) 2.9× as wide as long in dorsal view (unmounted specimen Figure 8); 1.3× as wide as maximum width of mesosoma in dorsal view (Figure 8). Mandible very small, with two teeth and a small truncation; mandibular glands

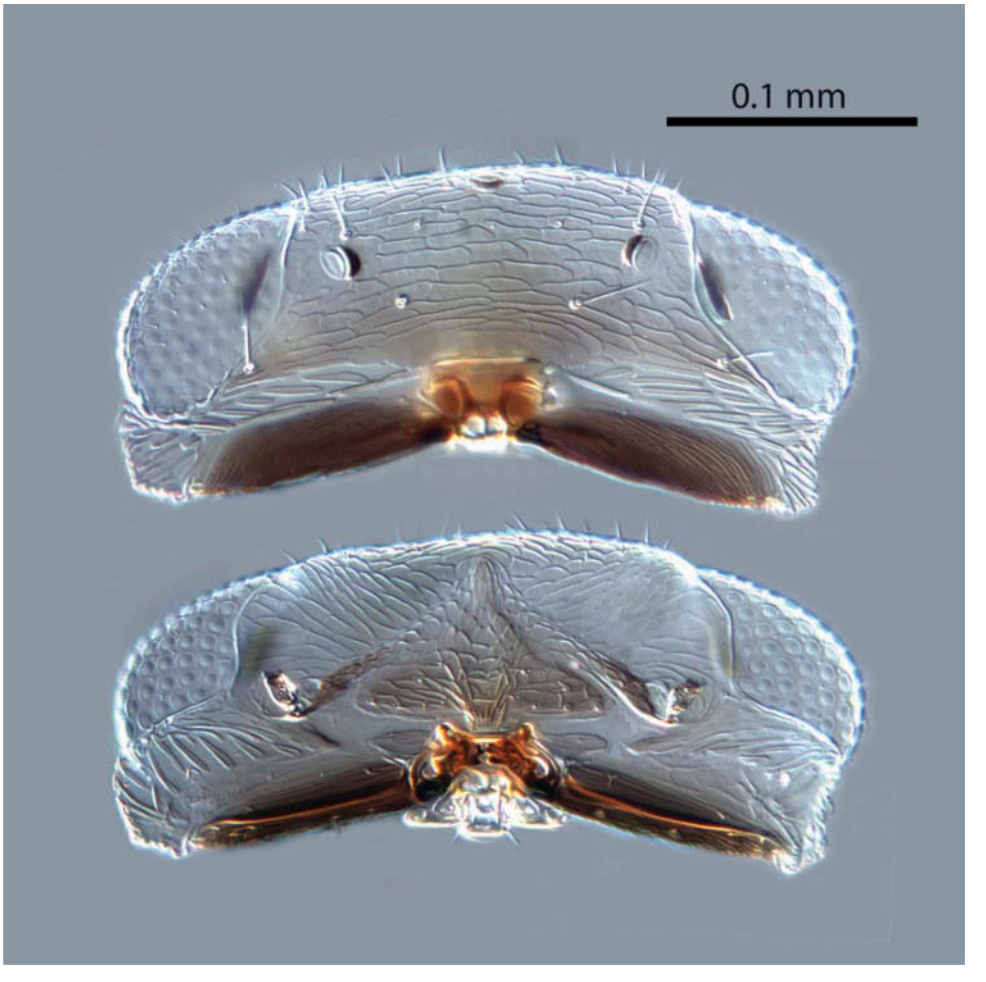

Figure 4. *Wallaceaphytis kikiae* holotype female; head: dorsal (above), ventral (below).

elongate, parallel-sided. Maxillary palp two-segmented, labial palp one-segmented. Lateral ocellus separated from eye margin by slightly more than the maximum width of ocellus. Pronotum centrally membranous, each side with a robust seta at the lateral edge, a fine seta adjacent to it, and two fine setae further towards the centre. Mid-lobe of mesoscutum with two setae laterally. Each side lobe of mesoscutum with two setae; tegula with a robust seta; axilla without setae; scutellum transverse, with two pairs of setae. Propodeum elongate centrally, projecting posteriorly, with a central process, and without crenulae (Figure 3). Propodeal spiracle without anterior groove. Mesofurca of typical Aphytini form (Figure 7; see Heraty et al. 1997). All tarsi four-segmented. Fore wing 3.8× as long as maximum width of disc (excluding marginal fringe); submarginal vein with a single seta; stigmal vein well-developed (Figure 6). Fore wing without setae below marginal vein, remainder of wing very sparsely setose. Anterior gastral sterna without projections (see Woolley 1988, p.469). T1–T6 of gaster each with a pair of setae, T7 (syntergum) with two pairs of setae, and in the form of a single sclerite, undivided and without epiproct.

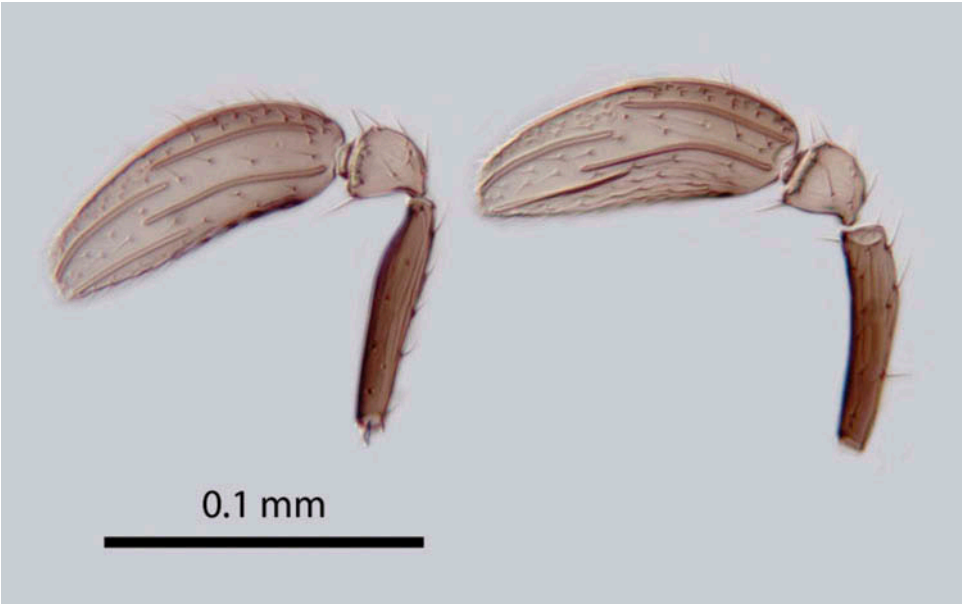

Figure 5. *Wallaceaphytis kikiae* holotype female; antennae.

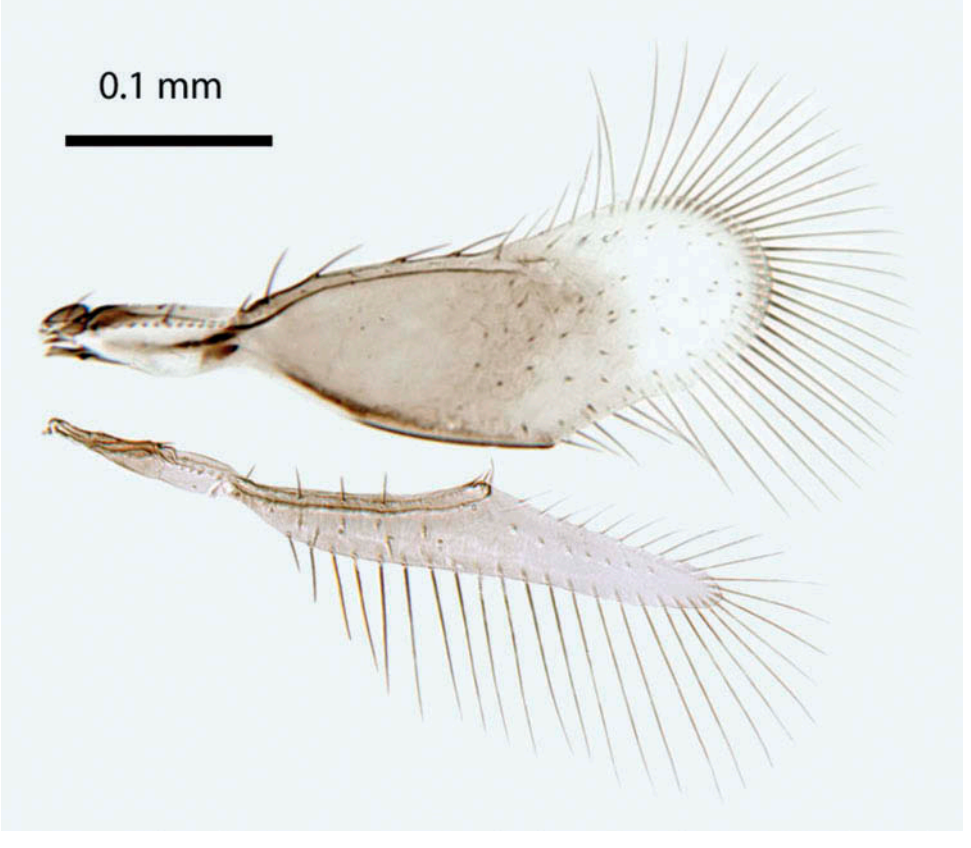

Figure 6. *Wallaceaphytis kikiae* holotype female; fore wing.

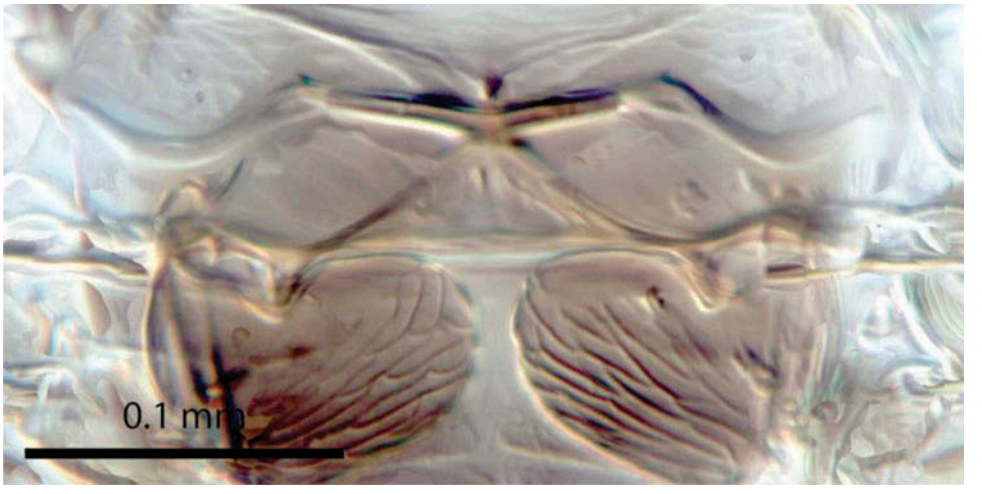

Figure 7. *Wallaceaphytis kikiae* holotype female; mesofurca.

Comments

Wallaceaphytis presents a combination of characters that is so far unique among the family Aphelinidae. Within the family, *Wallaceaphytis* is clearly a member of the subfamily Aphelininae, as shown above based on DNA sequence data, and as follows based on morphological data. The reduced number of antennal segments (three in the present case), elongate and parallel-sided mandibular glands, medially membranous pronotum, propodeal spiracles without anterior grooves, sterna without anterior apodemes, *Aphytis*-like mesofurca and presence of a syntergum, exclude all other subfamilies. *Wallaceaphytis* superficially resembles *Ablerus*, especially the unusual fore wing, but can be easily excluded from that genus and from the subfamily Azotinae (family Azotidae) by the above combination of characters. *Eretmocerus* has been included in Aphelininae by some authors, and also has four-segmented tarsi, while the antennae are five-segmented in females and three-segmented in males. However, in virtually all other respects the two genera are very distinct, and *Eretmocerus* appears to be only distantly related to Aphelininae. *Marlatiella* is the only known genus that also has three-segmented female antennae, but has five-segmented tarsi and very different wing characters.

Molecular DNA analysis supports the status of *Wallaceaphytis* as a distinct genus with unresolved affiliations or allied more closely with *Centrodora*, but this relationship is weakly supported. Despite the Bayesian analysis placing *Wallaceaphytis* in a polytomy with *Marietta*, *Aphytis* and *Aphelinus*, morphology, and the maximum likelihood analysis, suggest a closer relationship with *Centrodora*. In the key to genera of Aphelininae by Kim and Heraty (2012) *Wallaceaphytis* keys immediately with *Eretmocerus* because of the four-segmented tarsi, but is easily separated from this genus by the three-segmented female antenna, and long marginal vein. Although *Wallaceaphytis* is superficially similar to *Ablerus*, the molecular analysis (as with the comparison of morphology discussed above) clearly shows that this genus belongs to Aphelininae and not to Azotinae.

Figure 8. *Wallaceaphytis kikiae* holotype female; habitus.

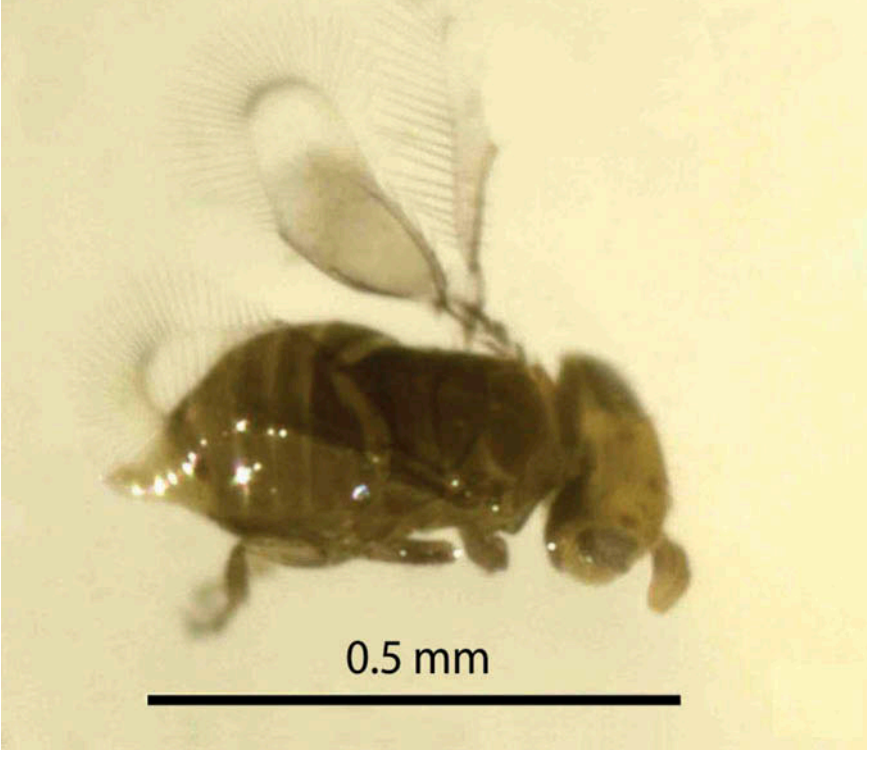

Figure 9. *Wallaceaphytis kikiae* holotype female; habitus.

Figure 10. *Wallaceaphytis kikiae* holotype female; habitus.

Wallaceaphytis kikiae Ayshford and Polaszek sp. nov.

Description

In addition to the genus-level characters above, the following characters are likely to be of species-level significance if additional species of *Wallaceaphytis* are discovered:

Colour. Head dorsally orange-yellow, brown on lower half of occiput (Figure 8). Scape dark brown, pedicel and flagellum orange-yellow, flagellum darker basally. Mesosoma and metasoma brown-black (Figures 9 and 10), a pale intersegmental area behind propodeum laterally. Fore wing infuscate from base to slightly beyond stigma vein (Figures 6 and 9). Legs brown, femora and tibiae paler at their bases; distal tarsal segments darker than proximal segments (Figure 3).

Sculpture. Frons and face with transverse/reticulate sculpture. Mesoscutum with reticulate sculpture in the form of large irregular cells, Fore wing marginal vein with four robust setae and a smaller one at the junction with the submarginal vein.

Additional characters

Ovipositor projecting beyond metasoma; 2.3× mid tibia. Second valvifers 3.3 × third valvulae.

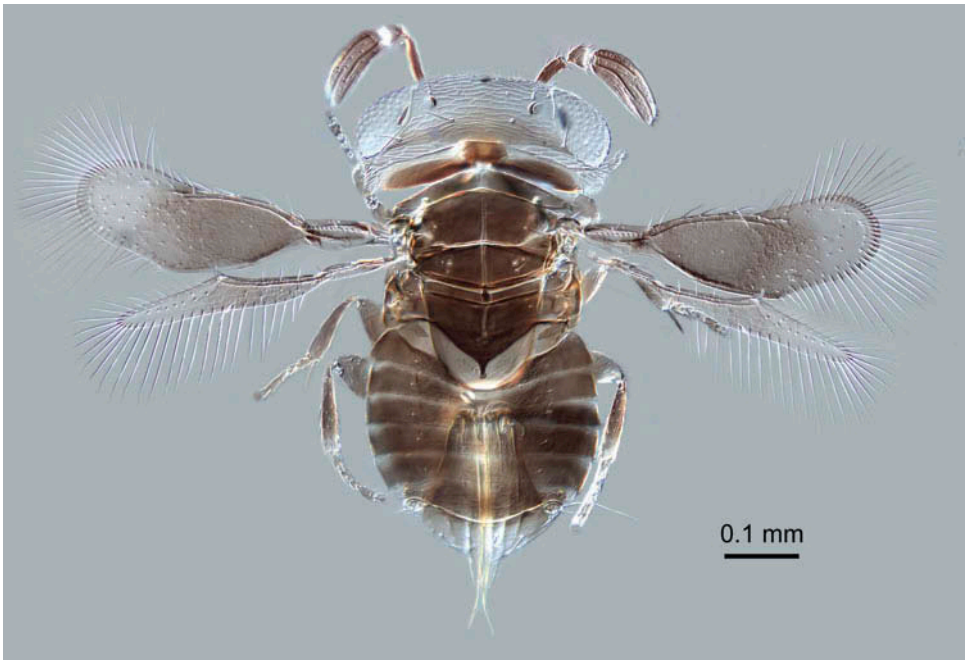

Figure 11. *Wallaceaphytis kikiae* holotype female; habitus.

Holotype female. MALAYSIA: Borneo, Sabah, Danum Valley Field Study Centre, Beach. 5°01' N, 117°48.75' E. 14 September 2012 screen-sweep (A. Polaszek). Holotype dissected and slide-mounted in Canada balsam, deposited permanently in Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Kota Kinabalu, MALAYSIA.

Etymology

The genus name *Wallaceaphytis* is derived from the family name of Alfred Russel Wallace, and the generic name *Aphytis*, to which it is related. Other genera within the subfamily Aphelininae, to which *Wallaceaphytis* belongs, include *Neophytis*, *Paraphytis* and *Punkaphytis*. The genus is described in honour of Wallace, co-discoverer with Charles Darwin of the theory of evolution by natural selection. Wallace's collections and observations on the fauna and flora during his extensive travels in South East Asia, including the island of Borneo, led to his formulation of the theory. The genus and species are described on the exact date of the centenary of his death.

The specific epithet *kikiae* is based on, and in honour of, "Kiki", the second author's mother, Mrs Christian Duke.

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Sabah Biodiversity Council, for the granting of Access Licence JKM/MBS.1000-2/2(77) under which the field work in Sabah was carried out, and Glen Reynolds (Manager, Danum Valley Field Studies Centre) for logistical support, and help with many other aspects of our work in Sabah. LF's research is currently supported by a grant from the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-RU-TE-2012-3-0057. We thank John Heraty, Jung-Wook Kim and Jason Mottern for making their aphelinid sequence data available through GenBank.

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