

Synonymy of *Idiococcobius* Hayat with *Coccobius* Ratzeburg (Hymenoptera, Aphelinidae): evidenced by a new species from Malaysian Borneo

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Abstract

Idiococcobius Hayat, **syn. nov.**, is synonymised with *Coccobius* Ratzeburg, based on morphological and molecular data from a new species of *Coccobius* from Malaysian Borneo. The new species is sufficiently similar morphologically to the type species of *Idiococcobius* to place it unambiguously within that genus, but molecular data from the new species, and a reassessment of the morphology of *Idiococcobius*, indicate synonymy of the two genera. *Idiococcobius encarsoides* Hayat is therefore transferred to *Coccobius*; resulting in the new combination: *Coccobius encarsoides* (Hayat), **comb. nov.** *Coccobius islandicus* Geng & Polaszek, **sp. nov.**, is described from morphology and DNA sequence data.

Keywords

chalcid wasps, Chalcidoidea, parasitoids, India, Malaysia, Sabah, taxonomy

Introduction

The genus *Idiococcobius* Hayat, 2010 was described for an unusual species of aphelinid considered to be “extremely close to *Coccobius*...” (Hayat and Khan 2010), but to have affinity with some *Encarsia* species, hence the species name *I. encarsoides* Hayat, 2010.

The morphological characters that purport to establish *Idiococcobius* as a valid genus also occur in a recently discovered new species from Malaysian Borneo. Molecular analysis of the latter species places it firmly within *Coccobius*. The affinities (and differences) between *Coccobius encarsoides* (Hayat) and *Coccobius islandicus* Geng & Polaszek, sp. nov., are detailed and discussed below.

Materials and methods

Morphological study

The single specimen of the new *Coccobius* species was collected in Sabah, Malaysian Borneo using a yellow pan trap. After “non-destructive” DNA extraction (see below), the specimen was dissected and mounted dorsally in Canada balsam on a slide following the method of Noyes (1982) with modifications for dealing with ProteinaseK processed specimens (AP, unpublished). Morphological terminology follows Hayat and Khan (2010).

Photographs were taken with a digital CCD camera attached to an Olympus BX63 compound microscope equipped with differential interference contrast. All measurements were taken from slide-mounted specimens. The holotype is deposited in the Natural History Museum, London, UK

The following abbreviation is used:

NHMUK Natural History Museum, SW7 5BD, London, UK

DNA sequencing

Genomic DNA extraction was undertaken using the protocol in Polaszek et al. (2013), which leaves the sclerotized parts of the specimen intact. The specimen was then mounted in Canada balsam on a microscope slide (see above). Primer sequences are given in Table 1. A total of 25 µl per reaction with 2.5 µl 10× NH₄ Reaction Buffer, 1.5 µl 50mM MgCl₂ Solution, 0.5 µl dNTP's, 1 µl of each primer, 0.5 µl BIOTAQ DNA Polymerase, and 6 µl DNA template. PCR amplifications included a pre-cycle denaturation step for 5 min at 94 °C and a final post-cycle extension step at 72 °C for 5 min (Table 1).

DNA was sequenced at the Natural History Museum Life Sciences DNA Sequencing Facility (London) using the same primers used for the PCR. Forward and reverse sequences were assembled and corrected using Sequencher version 4.8. The 28S sequence of *Coccobius islandicus* Geng & Polaszek, sp.nov., has been deposited in GenBank under accession no MT350291. The resulting COI sequence was short, and not useful for comparative analysis. However, a 28S D2 sequence of 744bp was obtained and sent (including the trace file) to the 3rd author (JM) for comparison and analysis with his substantial database of 147 *Coccobius* DNA sequences.

Table 1. Primer sequences and cycling conditions.

Primer sequence		Cycling conditions			
		Denaturation	Annealing	Extension	Cycles
COI (Hajibabaei et al. 2006)					
MLepF1	5' GCTTTCCCACGAATAAATAATA3'	94 °C	50 °C	72 °C	40
LepR1	5' TAAACTTCTGGATGTCCAAAAAATCA 3'	(30 sec)	(30 sec)	(1 min)	
28S-D2 (Park and O'Foighil 2000)					
D23f	5' GAGAGTTCAAGAGTACGTG 3'	94 °C	50°C	72 °C	40
28Sb	5' TCGGAAGGAACCAGCTACTA 3'	(30 sec)	(30 sec)	(1 min)	

The 28S sequence from *C. islandicus* was incorporated into a larger molecular dataset (JM in-prep; data not shown) that included an encyrtid outgroup (four terminals, from the genera *Anagyryus*, *Encyrtus*, *Metaphycus*, and *Oobius*), non-*Coccobius* coccophagine aphelinids (20 terminals, from the genera *Bardylis*, *Coccophagoides*, *Coccophagus*, *Dirphys*, *Encarsia*, *Euxanthellus*, and *Pteroptrix*), and 147 *Coccobius* terminals representing specimens from all biogeographic regions where *Coccobius* are known. Ribosomal DNA sequences were aligned using the E-INS-I algorithm in Mafft v6 (Kato et al. 2009) with default settings. Ribosomal and mitochondrial genes were then concatenated using SequenceMatrix v.100.0 (Vaidya et al. 2010) for a final alignment length of 3153 base pairs. Maximum likelihood (ML) analysis of the concatenated alignment was conducted using RAxML v8.2.12 (Stamatakis 2014) under a GTR+ Γ substitution model as implemented through the CIPRES Web Portal (Miller et al. 2010) accessed at <http://www.phylo.org/>. The data were analyzed with 1000 rapid bootstraps using five gene partitions: *18S*, *28S-D2*, *28SD3-5*, *COI* codon positions 1 and 2, and *COI* codon position 3. Only the 28S sequence from *C. islandicus* aligned with the existing dataset, so only its 28S sequence is included in the analysis. The resulting best tree (highest likelihood) was visualized using FigTree v1.4.3 (Rambaut 2009).

Results

Morphology

C. islandicus Geng & Polaszek, sp. nov., shares a number of apparent synapomorphies with *C. encarsoides* (Hayat) as follows: pronotum medially membranous; mid lobe of mesoscutum with a reduced number of setae arranged bilaterally; fore wing narrow with very long marginal fringe (more than 2/3 the maximum wing width); submarginal vein with a single seta. These characters, not previously encountered in described *Coccobius* species, clearly indicate that the two species are closely related.

The morphological differences between the two species, tabulated below, strongly support recognition of *C. islandicus* as a new species.

Table 2. Differences between *C. islandicus* (Geng & Polaszek) and *C. encarsoides* (Hayat).

	<i>C. islandicus</i> (Malaysia)	<i>C. encarsoides</i> (India)
colour of body	pronotum brown, lateral metasoma with brown patches; remainder pale.	body largely pale yellow, appearing translucent white after cleaning and mounting except pronotum in anterior half pale brown
colour of antenna	F1 and club brown; remainder white.	entirely white
colour of legs	fore and mid tibiae pale brown, hind femora and tibiae brown; remainder white.	white
ovipositor/mid tibia	1.21	1.49
third valvula/ovipositor	0.27	0.23
mid tibial spur/ mid basitarsus	1.29	1.12
fore wing length/ width	3.22	4.4
fore wing marginal fringe/width	0.68	slightly shorter
setae on mid lobe of mesoscutum	8	12
setae on TII of metasoma	1+1	2+2
sensilla on stigmal vein	in a close group of 4	in 2 separated groups of 2

Molecular analysis

The analysis of the 28S D2 sequence of *C. islandicus* together with 147 *Coccobius* sequences representing at least 125 species unequivocally demonstrates that this species, and by extrapolation therefore *Idiococcobius*, are nested deeply within *Coccobius* and do not represent a distinct genus. *Idiococcobius* Hayat is therefore here synonymized with *Coccobius* Ratzeburg, syn. nov. *Idiococcobius encarsoides* Hayat is here transferred to *Coccobius encarsoides* (Hayat), comb. nov. *C. islandicus* clusters with other Australian / SE Asian species. Its sister species (with low bootstrap support) is from Singapore.

Taxonomy

Coccobius islandicus Geng & Polaszek, sp. nov.

<http://zoobank.org/EE1EE627-07A5-4DFD-B7C1-CB70AD20A853>

Figs 1 A–C, 2 A, B

Material examined. Holotype ♀ (on slide): 21–25 Aug 2016, MALAYSIA: Sabah (N. Borneo), Keningau, Jungle Girl Camp, 5°26'55.7"N, 116°27'08.6"E, rainforest. H. Geng; NHMUK; specimen ID: GH025-92

Diagnosis. Antennal F1 and clava brown (Fig. 1B). Pronotum medially membranous; mid lobe of mesoscutum with 8 setae (Fig. 2A); maximum length of marginal fringe of fore wing 0.68× wing width.

Description (female holotype). Length 0.60 mm.

Colour. Head brown, frons paler. Mandibles very dark, especially toward apices (Fig. 1A). Antenna pale, F1 and clava brown (Fig. 1B). Pronotum and anterior margin

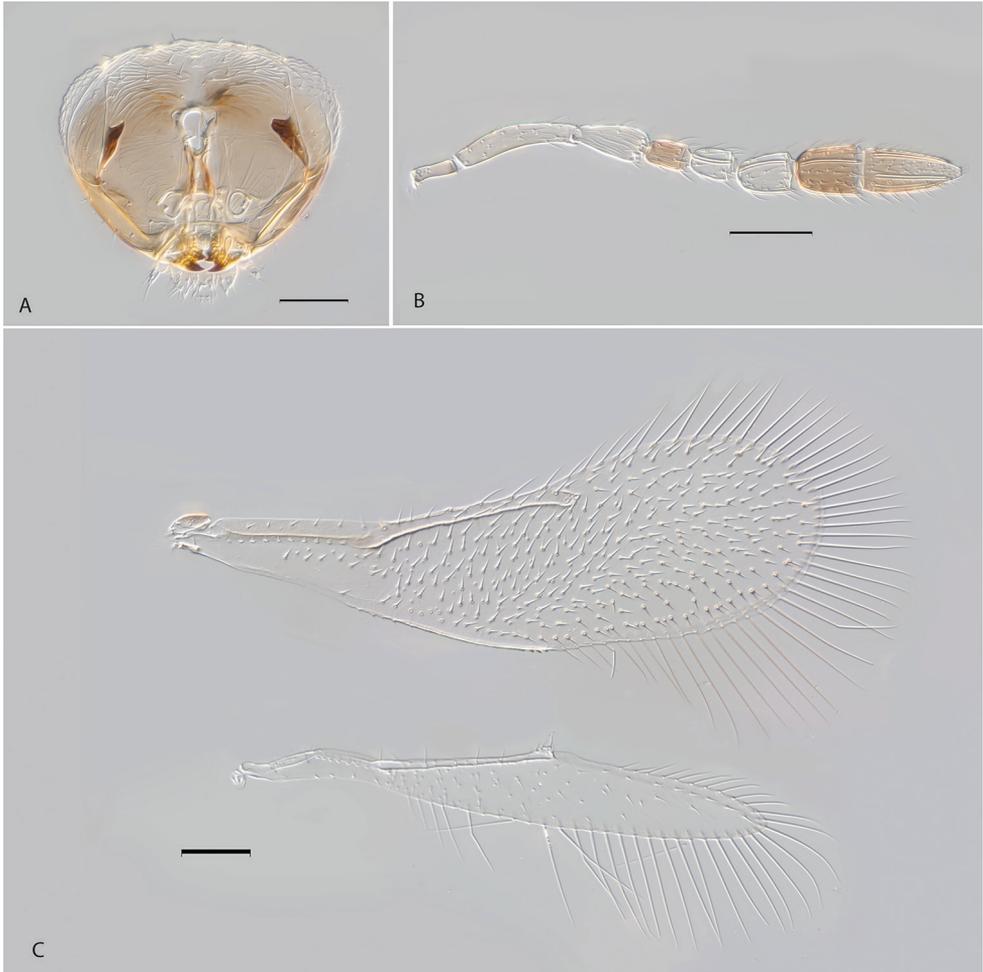


Figure 1. *Coccobius islandicus* Geng & Polaszek, sp. nov. **A** head **B** antenna **C** wings. Scale bars: 50 μ m.

of mesoscutal mid lobe brown, remainder of body largely pale, but brown laterally. Fore and mid tibiae pale brown, hind femora and tibiae brown (Fig. 2B). Wings hyaline except tegula pale brown (Fig. 1C).

Morphology. Head 1.5 times as broad as frontoververtex width (Fig. 1A), frontoververtex width less than length of clava (0.74). Upper margins of antennal toruli slightly below lower eye margins. Eye setose. Antennal radicle long, $0.37\times$ scape length. Pedicel just over $0.5\times$ scape length, $1.56\times$ length F1. F1 as long as F2, $0.78\times$ F3. Clava slightly longer than funicle. Antennomeres with the following numbers of sensilla: F1:0; F2:1; F3:2; C1:3; C2:5.

Mid lobe of mesoscutum with 8 setae arranged in approximate bilateral symmetry. Sculpture of mesoscutal mid lobe consisting of large irregular reticulations, that of scutellum with longitudinal elements. Scutellum with 2+2 setae, the front pair placed

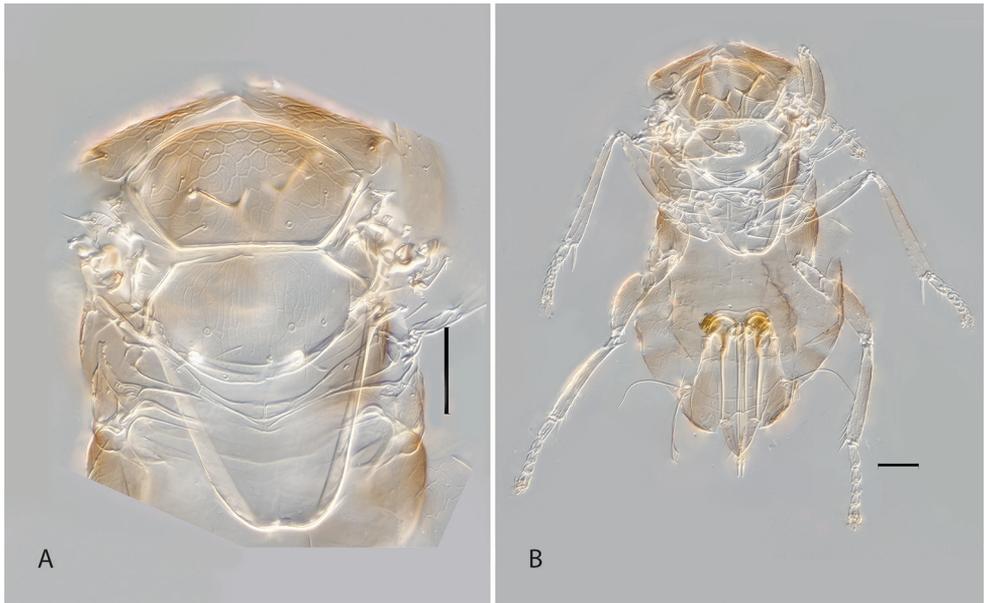


Figure 2. *Coccobius islandicus* Geng & Polaszek, sp. nov. **A** mesosoma **B** mesosoma- and metasoma. Scale bars: 50 μ m.

slightly behind the level of the scutellar sensilla (Fig. 2A). Wings elongate. Fore wing 3.22 \times as long as wide, marginal fringe 0.68 \times as long as maximum wing width, sub-marginal vein with 1 seta, stigmal vein sensilla in a cluster of 4 (Fig. 1C). Hind wing 7 \times as long as wide, marginal fringe 1.6 \times as long as maximum wing width. Mid tibial spur 1.29 \times mid basitarsus.

Metasomal setation: T1: 0+0; T2:1+1; T3-T5: 2+2; T6:4; T7:6. Ovipositor length 1.21 \times mid tibia; 3rd valvula 0.27 \times ovipositor (Fig. 2B).

Male. Unknown.

Host. Unknown.

Distribution. Sabah, Malaysian Borneo.

Etymology. "island"-(*icus*) is the latinised form of the English word "Iceland", in this case referring to a British food retailer that has banned the use of palm oil in its products. The species name does not imply any endorsement by the authors of this retailer's products.

Discussion

Hayat (in Hayat and Khan 2010) described *Idiococcobius* as a genus he considered to be close to, but distinct from, *Coccobius*, based on a combination of characters that had not previously been encountered in any *Coccobius* species known to him at that time. The medially membranous pronotum, reduced and bilaterally symmetrical meso-scutal setae, and narrow fore wing with long marginal fringe, all suggested possible affinity with *Encarsia*, a genus not particularly close to *Coccobius*. These characters

clearly merited the description of a new genus, with the *Encarsia* similarities conveyed in the species name “*encarsoides*”. These kinds of reduction characters appear to be common not only across the Aphelinidae, but among many chalcids and beyond. Setal reduction and wing narrowing (as seen here), reduction in tarsal segment number and antennomeres are all processes known to have occurred independently in multiple lineages, and have confounded traditional morphology-based taxonomy for so long. The development of relatively comprehensive molecular databases, even those (as herein) relying on a single gene, are extremely valuable for the unequivocal elucidation of an organism’s true relationships. While not always perfect, when sample size is adequate and gene variability sufficient, their predictive power is considerable. Studies such as that described above are able both to contribute towards more stable classification as well as reveal widespread morphological convergence, especially in organisms with similar biology.

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