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A preliminary molecular phylogeny of the Sigalphinae (Hymenoptera: Braconidae), including *Pselaphanus* Szépligeti, based on 28S rDNA, with descriptions of new Afrotropical and Madagascan *Minanga* and *Malasigalphus* species

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Minanga phoebea sp. n. Quicke, from Uganda, and *Malasigalphus roa* sp. n. Sharkey, from Madagascar, are described and illustrated. The new species of *Minanga* displays a distinct posterior metasomal carina and thus provides another example of the co-occurrence of this feature with a metasomal carapace within the Braconidae. *Malasigalphus roa* is the second species of the genus recently described by Achterberg and Austin. A preliminary molecular phylogeny of the Sigalphinae is presented including the rare Neotropical genus *Pselaphanus* the placement of which has been debated. Sensitivity analysis to multiple alignment parameters was conducted and a single elided data set, based on the combined 21 separate alignments, was analysed. Strong support was obtained for the following relationships: *Pselaphanus*+(*Malasigalphus*+(other Sigalphinae)), (*Minanga*+(*Sigalphus bicolor*+(*S. irrorator*+*S. gyrodontus*))), and Earinini being basal (as a grade in these analyses) to other Agathidinae. The *Mesocoelus* group of genera (Mesocoelini in part) are shown to be derived agathidines rather than being associated with the Orgilini. The Microtypinae is shown be non-monophyletic and *Plesiotypus* Achterberg is proposed as the sister group to the Agathidinae+Sigalphinae clade. The Acampsohelconinae does not appear as monophyletic; however, the placements of both of the two included genera, *Afrocampsis* and *Canalicephalis*, had less than 100% support in the elised analysis tree, and therefore monophyly of this recently redefined subfamily must be more rigorously tested.

Keywords: Afrotropical; Agathidinae; Minangini; Acampsohelconinae; *Plesiotypus*; elision; functional morphology; sensitivity analysis

Introduction

Minanga Cameron is the sole genus in the tribe Minangini which is known from sub-Saharan Africa including Madagascar and Mexico (Sharkey 2004), the latter possibly as a relict from an earlier wider distribution. The African species of the genus were revised by De Saeger (1948) to accommodate seven species ranging from South Africa to Belgian Congo (=Democratic Republic of the Congo), East Africa

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and Madagascar. Since then the only new species to have been added is the Mexican, *M. achterbergi* Sharkey mentioned above, bringing the total described species to eight. The new species of *Minanga* described here is of interest for two reasons. Firstly, it has enabled DNA sequence data to be obtained for the Minangini for the first time, and secondly, it provides yet another example of the co-occurrence of a metasomal carapace and a distinctly developed postpectal carina on the mesosoma. It is the only sigalphine known with a complete postpectal carina though a trace is present in some other *Minanga* species (see Discussion section). In addition, partly through use of newly designed internal primers we have been able to obtain sequence data for *Pselaphanus* Szépligeti, the type genus of the Pselaphaninae (Achterberg, 1985, as Pselaphanini). Although there has been little doubt that this monotypic group is a member of the Agathidinae+Sigalphinae clade, the affinities of *Pselaphanus* have been debated. Although it was originally included within the Helconinae, Achterberg (1985) placed it in its own tribe, the Pselaphanini which he regarded as a "comparatively archaic group within the Agathidinae". Subsequently, Sharkey (1997) treated the Pselaphaninae as a synonym of the Sigalphinae. The new molecular evidence presented here supports Sharkey's conclusion and shows that it is probably the sister group of the Sigalphinae *sensu stricto*.

In our previous study of Agathidinae relationships (Sharkey et al. 2006), we employed both multiple alignment using Clustal W (Thompson et al. 1994) and direct optimization with POY (Gladstein and Wheeler 1996). Comparison of the two methods showed that Clustal W, with a variety of gap parameters, generally produced more resolved trees and these were more consistent between parameter settings than the trees produced by POY. Direct optimization using POY has been shown to be particularly strongly influenced by large indels even when these display little or no sequence homology (Laurenne et al. 2006), and the sequences of Sigalphinae and Agathidinae are very length-variable, therefore making it likely that the results of direct optimization could be biased by the presence of large indels. Recent simulation studies have shown that multiple alignment generally recovers a larger proportion of true homologies than does direct optimization (Ogden and Rosenberg 2007), and, in another investigation of POY, Kjer et al. (2007) showed that different parameters led to very different results, such that in practice, different investigators were unlikely to recover the same trees unless using exactly the same parameters. Indeed, they showed that even when applying the same sets of parameters, POY did not always produce the same trees. Therefore, in the study presented below we analyzed the sequence data using multiple alignments from Clustal W with a range of gap cost parameters.

We take this opportunity to describe a second species of *Malasigalphus* and the previously unknown male of *Malasigalphus petiolaris* Achterberg and Austin, both of which are included in the molecular analysis.

Molecular methods

Sequence data were obtained for either the D2 or the D2+D3 variable regions of the nuclear 28S rDNA gene that have been extensively used for studies of parasitoid wasp evolutionary relationships (Broad et al. 2004; Quicke et al. 2005; Laurenne et al. 2006). Sequencing protocols follow previously published work (Sharkey et al. 2006; Laurenne et al. 2006) except that in order to obtain 28S D2 sequence

information from *Pselaphanus* for which we had only a leg from an old specimen whose DNA was substantially degraded, we used a novel set of internal primers. Primer design was based on our previously obtained Agathidinae and Sigalphinae sequences as the internal primers used for other ichneumonoids (Quicke et al. 2007) will not work on members of these two subfamilies. The newly designed internal primers, which were used to amplify template DNA directly, were R2PSEL: 5′-TTG CRR YWG GCY RGA CGC A-3′ as a reverse in conjunction with the normal D2 forward primer (28SF: AGA GAG AGA GTT CAA GAG TAC GTG), F2PSEL: (5′-TGC GTC YRG CCW RYY GCA A-3′, the reverse complement of R2PSEL) used with the reverse internal primer R3PSEL (5′-ACT CCT TGG TCC GTG TTT-3′), and F3PSEL (5′-AAA CAC GGA CCA AGG AGT-3′, the reverse complement of R3PSEL) used with the reverse R2newq (5′-CCG TGT TTC AAG ACG GGT-3′) at the beginning of the D3 region.

Materials

Eighteen genera of Agathidinae were included, as adult and immature stage morphology as well as molecular data strongly support the idea that this subfamily constitutes the sister group of the Sigalphinae (Quicke and Achterberg 1990; Shaw and Quicke 2000; Quicke et al. 2002). These were selected to represent each of the four currently recognized formal tribes (Agathidini s.l. (i.e. incl. Microdini Authors), Cremnoptini, Disophrini and Earinini) as well as the grouping revealed by Sharkey et al. (2006) tentatively labelled therein as ''new tribe?''. A further 20 out-group genera were selected to cover the major non-cyclostome subfamilies excluding the ''microgastroid lineage''. Among these we included five genera of Orgilinae, a subfamily not included in Sharkey et al.'s (2006) study, which therefore failed fully to test Achterberg's (1990) conclusion that his tribe Mesocoelini (for *Mesocoelus* and *Aneurobracon*) belonged to the Orgilini rather than to the Agathidinae. Trees were rooted with *Helcon* Nees because morphology and some molecular studies suggest that the Helconini are likely either to be close to the base of the non-cyclostome braconid tree (Belshaw et al. 2000) or at least to belong to a clade outside of the Agathidinae+Sigalphinae lineage (Belshaw and Quicke 2002).

DNA sequences are deposited in the EMBL/GenBank database; accessions numbers, provenances and voucher numbers are given in the Appendix.

The D2–D3 gene fragment is very length variable in the Agathidinae+ Sigalphinae clade and therefore we used Clustal W multiple alignments under a range of gap opening (GO) and gap extension (GE) costs (GO:GE=1:1, 2:1, 2:2, 4:1, 4:2, 4:4, 6:1, 6:2, 6:4, 6:6, 8:1, 8:2, 8:4, 8:6, 8:8, 10:1, 10:2, 10:4, 10:6, 10:8 and 10:10). For Clustal W analyses, areas of missing data (predominantly small terminal parts of the sequences but in some cases all of the D3 region, and for *Aposigalphus*, a small length-conserved part at the 3′ end of the D2 region, were represented by a number of Ns corresponding to the minimum number of bases in the corresponding region of all those taxa for which the sequence was complete. This ensured that extra gaps were not forced into the alignments by overestimating number of bases in the absence of evidence.

Parsimony analyses were carried out using PAUP* (Swofford 2002) with 1000 random additions each followed by tree bisection resection (TBR) branch swapping holding only one tree in memory at any one time. Resulting trees were then used as

starting trees for a further round of TBR branch-swapping with unlimited maxtrees. Additionally, the 21 Clustal W alignments were combined into a single file for simultaneous analysis, i.e. the elision procedure suggested by Wheeler et al. (1995) which effectively increases weight to bases whose alignment is less susceptible to variation resulting from different multiple alignment parameters. The elided data set was bootstrapped using 100 bootstrap replicates each using 100 random additions followed by TBR branch-swapping holding only one tree at a time. Using a relatively inefficient search strategy means that the resulting bootstrap values ought to be quite conservative, but this is compensated for by the repetitive nature of the elided data set.

Results

Parsimony analysis of the 21 Clustal W alignments always recovered a monophyletic Agathidinae as the sister group to a monophyletic *Pselaphanus*+Sigalphinae clade. Within each of the two clades there were, however, considerable differences in recovered relationships though there were also some consistent features among the lower-level relationships. Thus, all analyses recovered as monophyletic groups both species of *Minanga*, both species of *Malasigalphus*, the three species of *Sigalphus* with *S. bicolor* always being sister to the other two, the Agathidini, the genera of Disophrini and Cremnoptini, and three of the genera of Earinini (viz. *Amputoearinus*, *Austroearinus* and *Sesioctonus*). Several other relationships were variable and some of these are summarized in Figure 17. In particular, *Pselaphanus* was recovered within the Sigalphinae (as a result of *Malasigalphus* species comprising the sister to *Pselaphanus*+the remaining Sigalphinae). In a middle zone of our parameter space, *Sigalphus*, *Minanga*, *Aposigalphus* and *Acampsis* formed a monophyletic group in nearly all cases, but almost all possible relationships among them were recovered with various parameter combinations. Within the Agathidinae, the Earinini were never recovered as monophyletic, and across a broad intermediate zone of parameter space they were recovered as derived within the subfamily, the Disophrini+Cremnoptini forming the sister group to all other members of the subfamily.

The elision analysis yielded a single most parsimonious tree (Figure 18) with generally high bootstrap support values.

Descriptions of new species

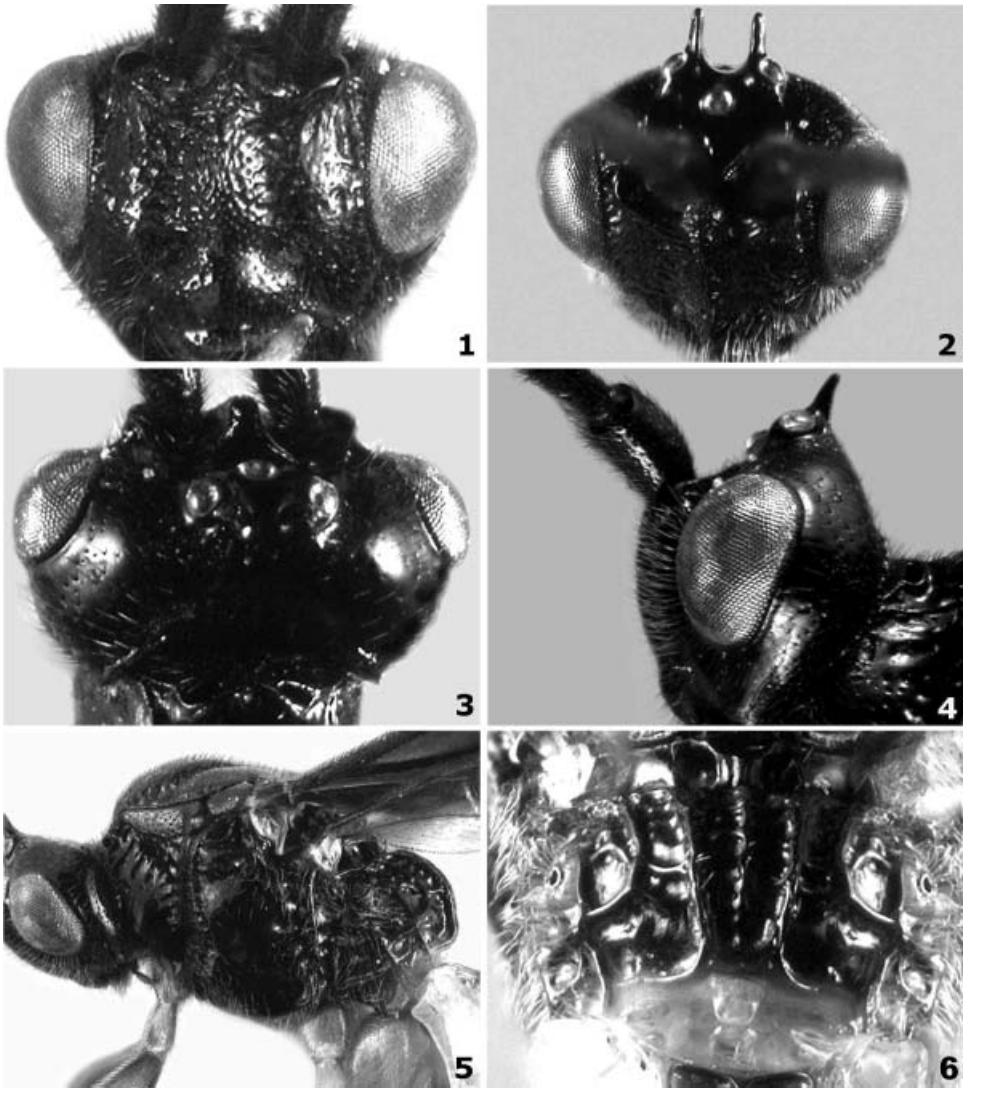
Minanga phoebea Quicke sp. nov.
(Figures 1–11)

Material examined

Holotype: female (Natural History Museum, London): Uganda, Kibale Forest National Park, near Makerere University Biological Field Station at Kanyawara, August 2004, malaise trap.

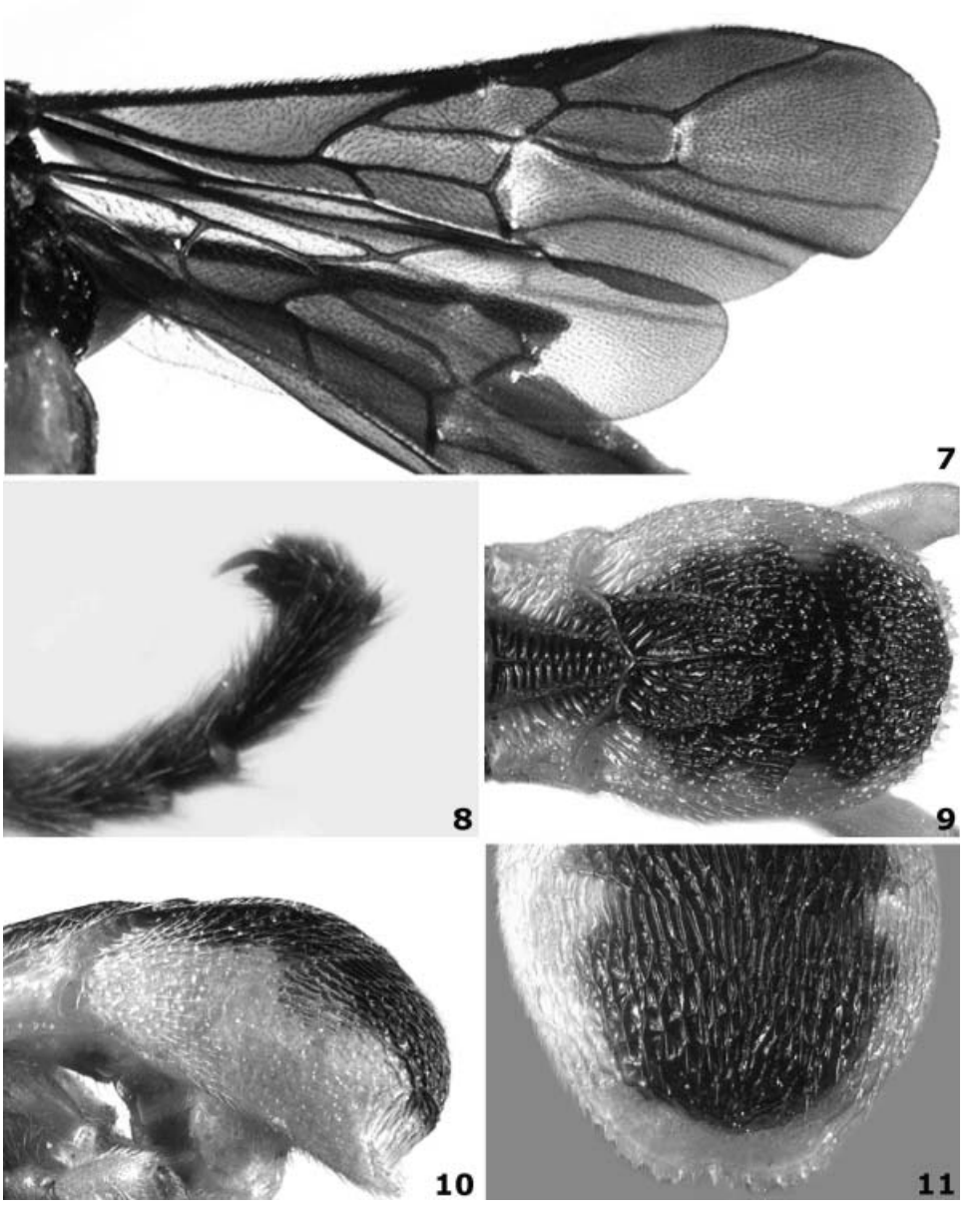
Description

Female: length of body 4.2mm, of fore wing 4.6mm, of antenna 5.1mm.



Figures 1–6. Photomicrographs of *Minanga phoebea* sp.n. features of the head and mesosoma.

Head: antenna with 34 flagellomeres. Terminal flagellomere sub-conical, pointed but not acuminate. First flagellomere 1.3 and 1.4 times longer than second and third respectively, the latter 1.8 times longer than wide. First flagellomere with three ranks of placoid sensillae, remaining flagellomeres with two ranks of sensilla. Clypeus distinctly medio-ventrally emarginate. Clypeus separated from face dorsally indistinctly by a shallow broad depression. Anterior tentorial pits deep. Height of clypeus: inter-tentorial distance: tentorio-ocular distance=1.0:1.25:1.1. Face with median raised area that narrows between antennal sockets to form a double ridge; area bordered laterally by irregular transverse crenulae. Height of eye: width of head: shortest distance between eyes=1.0:2.9: 1.6. Frons with a submedial pair of posteriorly diverging carina and strong sublateral carinae. Horns on stemmaticum



Figures 7–11. Photomicrographs of *Minanga phoebea* sp.n. features of the wings, claw and metasoma.

long and virtually vertical and parallel. Occipital carina strong and lamelliform laterally but absent ventrally and also broadly missing medio-dorsally.

Mesosoma shiny, 1.45 times longer than maximally deep. Notauli strong, deep and crenulate, approaching one another but remaining separate posteriorly. Scutellar sulcus with three strong carinae. Precoxal suture smooth and shallow except posteriorly more strongly impressed. Medial area of metanotum with a pair of well-developed anteriorly diverging carinae and weak midlongitudinal ridge

posteriorly. Propodeum strongly sculptured with pair of submedian carinae, the area between these with ladder-like pattern of ridges that medially form a less distinct mid-longitudinal carina.

Fore wing: vein SR1 reaching wing margin 0.67 distance from apex of pterostigma to wing tip. Veins 1-M and 1-SR approximately equally long. Lengths of veins r: 3-SR=1.0:5.0. Lengths of veins 2-SR:3-SR:r-m=1.2: 2.5: 1.0. Lengths of veins 1-CU:2-CU:3-CU=1.0: 4.4: 1.55. Vein cu-a straight and strongly inclivous. Hind wing: length of vein M+CU 1.55 times 1-M. Without distinct basal hamular bristles, with four distal hamuli.

Claws all with pointed basal lobe. Fore legs robust, the femur swollen and distinctly concave on medial face; fore tarsus wide and distinctly dorso-ventrally compressed. Length of hind femur: tibia: basitarsus=1.95: 2.26:1.0. Hind femur 3.15 times longer than maximally deep. Inner hind tibial spur reaching 0.49 along length of hind basitarsus.

Metasoma strongly sculptured. Submedial carinae on second tergum distinctly curved. Second metasomal suture distinct and crenulate on lateral third, but indicated on medial third largely by change in sculpture. Posterior margin lamelliform and dentate, medially weakly emarginate. Ovipositor down-curved, sharp, 0.67 length of hind tibia.

Colour: head (except largely brown mandible) and mesosoma (except narrowly brown posterior margin of propodeum) black. Metasoma honey yellow, to yellow and white-yellow laterally and narrowly posteriorly, medially black. Wings brown with dark brown venation. Fore legs including coxae yellow, the femur somewhat darker more honey-yellow. Mid and hind legs largely yellow.

Notes

The new species differs from all other Afrotropical species in its colour having the head and mesosoma almost entirely black and the wings uniformly brown (infumate).

Etymology

Named after Miss Phoebe Purvis.

Malasigalphus petiolaris Achterberg male
(Figure 12)

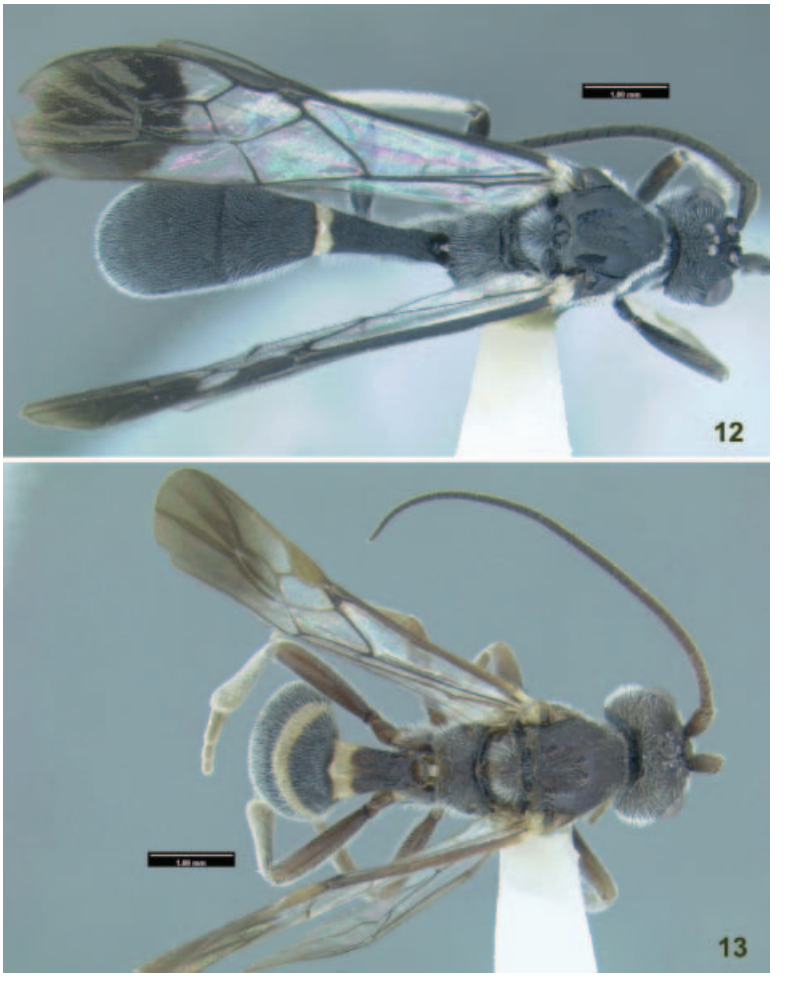
Malasigalphus petiolaris Achterberg

Material examined

Male: Madagascar, Fianarantsoa Province, Parc National d'Isalo, 9.1 km 354°N Ranohira, elev. 725m., 27–31 Jan., 2003. 22°28′54″S, 45°27′42″E, Malaise trap in gallery forest, coll. B. Fisher, T. Griswold et al. BLF7303, California Academy of Sciences.

Description

The species was described based on a single female. This is the first record of a male and it is essentially similar to the female holotype with the following exceptions:



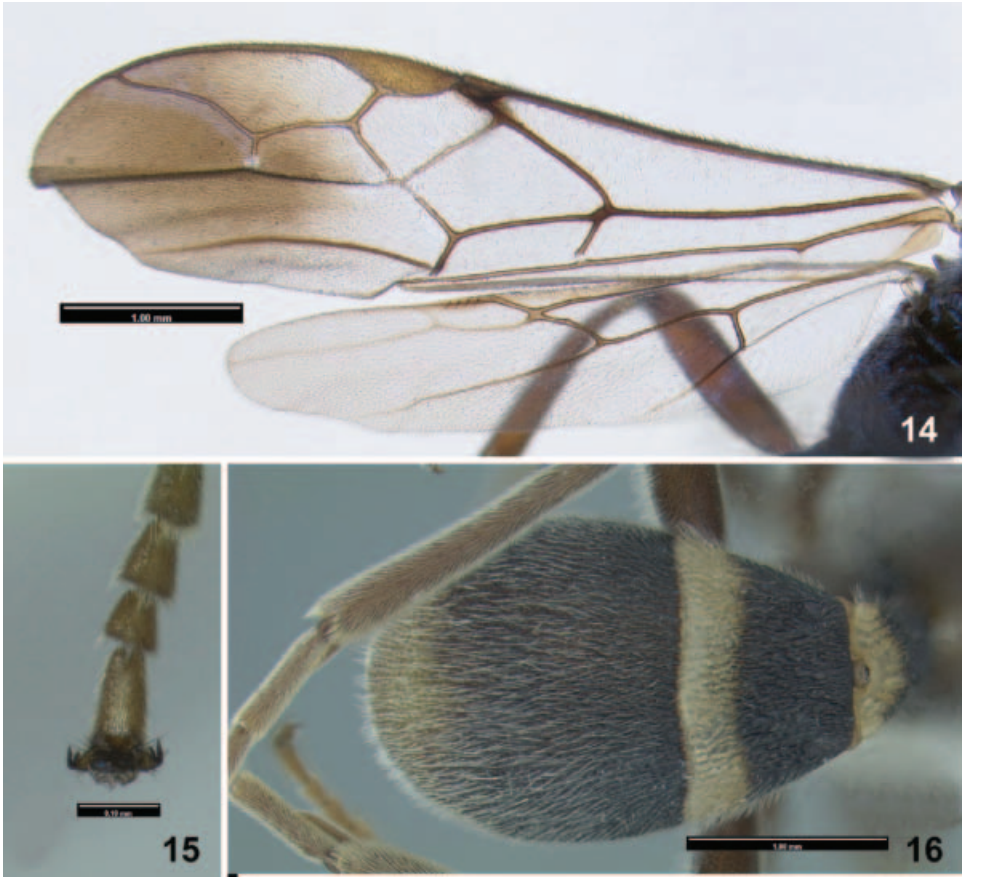
Figures 12–13. Dorsal habitus. (12) Male *M. petiolaris*; (13) female *M. roa* sp. nov.

body length 9.8mm; wing length 8.7mm; antenna with 40 flagellomeres (broken after tenth flagellomere in holotype); occipital carina strong dorsally and evenly rounded; fore femur black, fore and mid tibia yellow laterally, brown medially.

Malasigalphus roa Sharkey sp. nov.
(Figures 13–16)

Material examined

Holotype: female: Madagascar, Tulear Province, Parc National Andohahela, Ihazofotsy Parcelle III, 7–17 December, 2003, elevation 80m., 24°50.09′S, 46°29.21′E, Malaise trap in dry spiny forest, coll. M. Irwin, F. Parker, R. Harin'Hala, MA-02-21-41, California Academy of Sciences.



Figures 14–16. Female *M. roa* sp. nov. (14) wings; (15) foretarsal claws; and (16) carapace, median tergites 2 and 3.

Description

Female: length of body 8.4mm, of fore wing 9.9mm.

Head: antenna with 42 flagellomeres (right antenna broken after scape); occipital carina complete, head rugose and setose except for small, smooth glabrous area anterad median ocellus; ocelli on a raised triangular area (stemmaticum); area between antennae elevated and with two longitudinal carinae; occipital carina straight/transverse dorsally, not rounded as it is laterally; maxillary palpi about equal to head height; second labial palpomere greatly swollen apically, palpomere about as wide apically as it is long.

Mesosoma: entirely rugose or rugose-crenulate, except anterior and lateral areas of mesoscutum smooth with punctures; medial triangular cell of metanotum with strong complete medial longitudinal carina; propodeum with medial area outlined by strong irregular carinae (Figure 13), spiracle large and more than twice as long as wide. Forewing: (Figure 14). Legs: Tarsal claws cleft (Figure 15).

Metasoma: first three metasomal median tergites rugose to minutely areolate rugose and moderately setose, setosity denser distally; first median tergite approximately 1.2× longer than maximum width (1.42mm: 0.89mm; basal

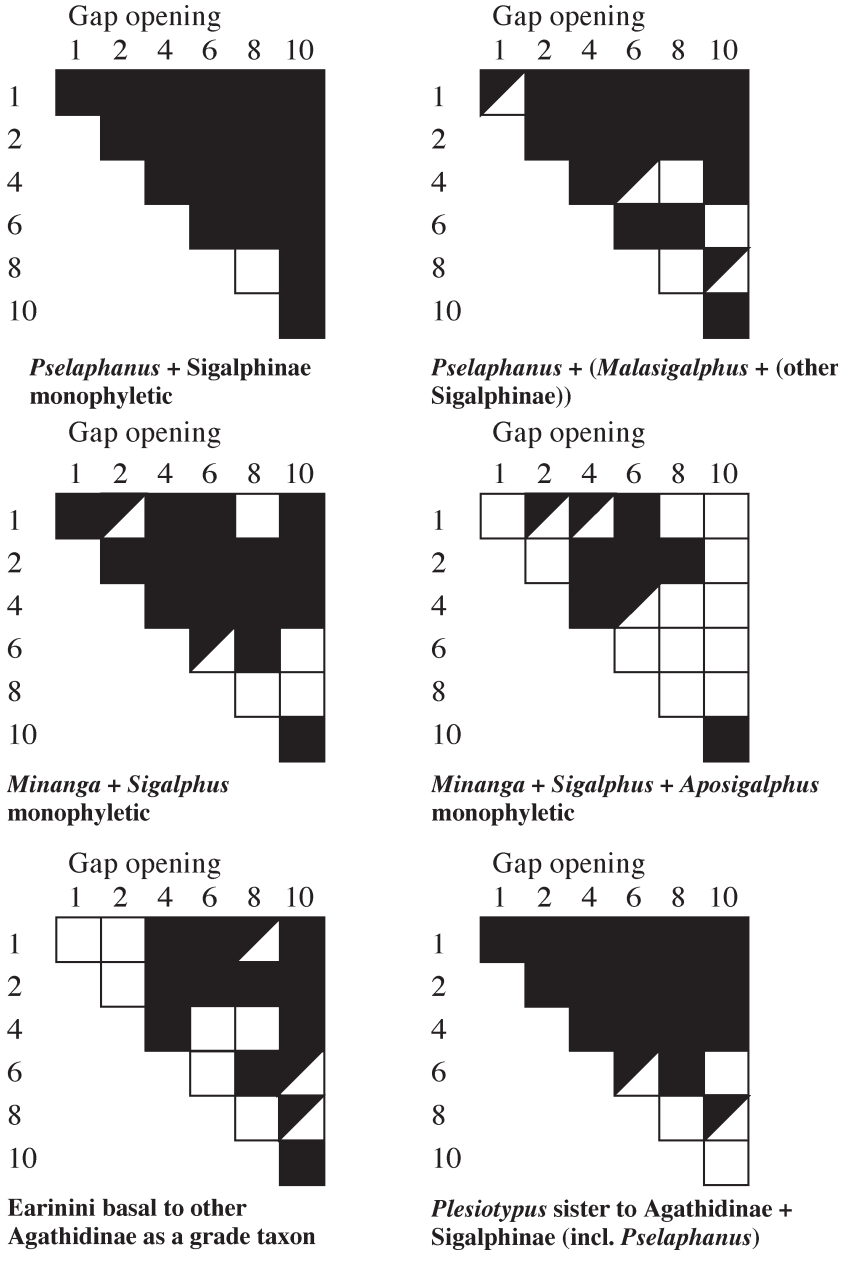


Figure 17. Summaries of selected phylogenetic relationships recovered from parsimony analysis of multiple alignments obtained using different gap opening and gap extension parameter combinations in Clustal W. Half-filled cells indicate that the given relationship was recovered in some but not all of the MPTs obtained with that parameter combination.

measurement starts at apex of tendon); first median tergite with pair of strong percurrent longitudinal carinae, delimiting elevated medial region (Figure 16); lateral and apical margins of third median tergite, except basal 1/4, with a wide lamellate flange apically which is weakly notched medially for reception of the ovipositor.

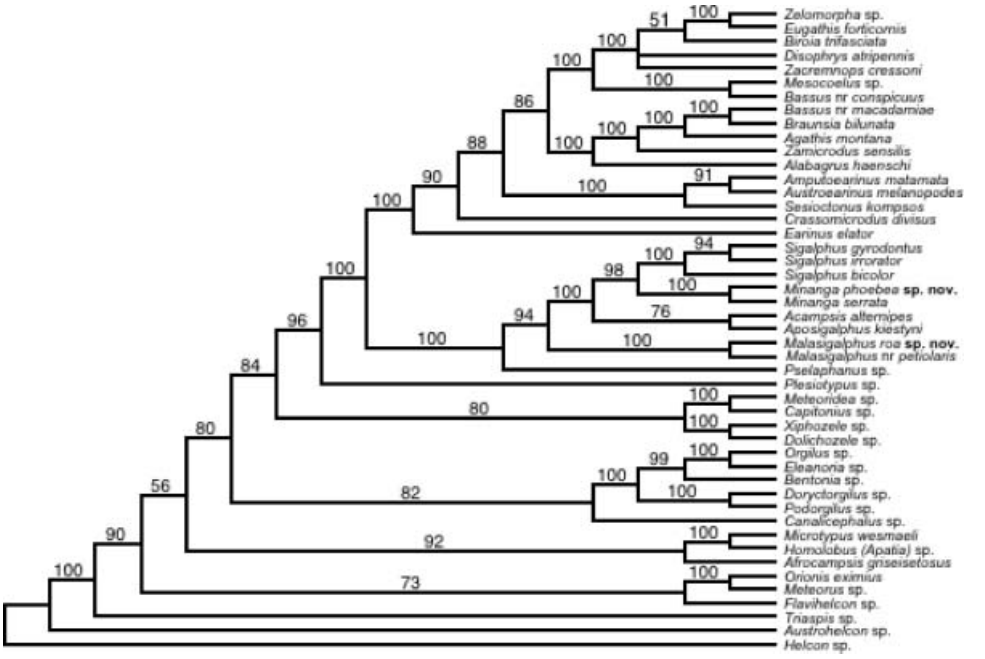


Figure 18. Bootstrap consensus tree derived from analysis of elised matrix comprising all 21 individual multiple alignments.

Colour: mostly red-brown except as follows; palpi, stigma and extreme base of wings, apices of median tergites 1–3 yellow, the colour change is abrupt on median tergites 1 and 2 and gradual towards apex of tergite 3 (Figure 16); anterior margins of meso- and metapleura, scutellar sulcus, and basal 3/4 of metasomal median tergites 1–3 all brownish black; fore wing mostly hyaline basal to the apex of the stigma, infuscate around parastigma and apex of wing (the transition from hyaline to infuscate is gradual not acute as in *M. petiolaris*), stigma and veins immediately apical and posterior to stigma, yellow (since the wings in Figure 14 are illuminated from below, the yellow coloration is not as striking in the image as it is in natural light); hind wing mostly hyaline except costal margin basal to hamuli infuscate and hint of infuscation apically.

Notes

The new species is easily distinguished from *M. petiolaris*, the only other known species, in colour, and dimensions of the first metasomal median tergite. In *M. roa* the apices of the first three metasomal median tergites, those forming the carapace, are all yellow, whereas in *M. petiolaris* only the first tergite is yellow apically and the second and third median tergites are uniformly black. The length–width ratio of the first median tergite of *M. roa* is 1.2 and for *M. petiolaris* it is 2.7.

Etymology

Roa is Malagasy for two and refers to the fact that this is the second species to be discovered in this genus which is restricted to Madagascar.

Discussion

Association of postpectal carina and modified metasoma

The non-independence of morphological characters is a serious problem in phylogeny reconstruction but is perhaps less well-appreciated than it ought to be. Usually it is reasoned that non-independence is minimized by including characters from numerous body regions and systems. However, it is becoming increasingly apparent that adaptations to a single factor can affect multiple parts of the body, often ones that do not, at first sight, appear to have any particular connection with the selective force. These may be due to direct actions of the selective force that are not recognized by investigators. But there can also be indirect effects where changes in one character system due to selection impose strong pressures on other systems which may be in different parts of the body. The presence of a well-developed postpectal carina in *Minanga phoebea* sp. nov. may be another example of such an indirect effect. In previous braconid systematic studies, a postpectal carina has been widely taken to be a synapomorphy, when present in conjunction with a three-segmented, completely fused, metasomal carapace, of the Cheloninae, though it also occurs in the morphologically and biologically distinct Cenocoeliinae. While not having a carapaciform metasoma, it is worth noting that the metasoma of cenocoeliines is highly derived in that it is inserted high on the propodeum, and thus probably requires special adaptations for its manipulation/control. More recently, a postpectal carina has been found to occur also in some Opiinae that also possess a metasomal carapace (e.g. *Bitomus* Szépligeti and *Coleopius* Fischer), and also in some Acampsohelconinae (Quicke, unpublished observations), though the carina is not developed in all cases when a carapace is present. Of the six genera of Sigalphinae, the one with the most developed carapace is *Minanga*, with all three of the carapace tergites immovably fused (see Figures 9 and 10). That at least the new species of *Minanga* described below also has a distinct complete postpectal carina, and *M. achterbergi* has traces of one, suggest strongly that this feature is functionally linked to the possession of a metasoma that requires a specialized mechanism for its control. While the presence of the postpectal carina in *Minanga phoebea* sp. nov. is clearly an autapomorphy and thus useful for diagnosis, more generally such character non-independence means that care should be exercised in their use in phylogeny reconstruction.

Phylogenetic implications

Relationships within Sigalphinae

Recognition of a separate tribe, the Minangini, originally proposed by De Saeger (1948) and accepted by Achterberg and Austin (1992), for *Minanga* is unwarranted as the molecular analyses clearly place this genus as derived within the subfamily in many individual analyses as well as in the elision analysis tree.

The molecular analysis support Sharkey's (1997) synonymy of Pselaphaninae with Sigalphinae in that this group was recovered as monophyletic in nearly all individual analyses; the one in which Pselaphaninae and Sigalphinae were not recovered together was very anomalous with *Helcon* appearing among the Sigalphinae (i.e. the sigalphines were distributed over the bases of the two branches above *Helcon*), and therefore think this a highly improbable set of relationships and do not consider it further here. That *Pselaphanus* was basal to the other sigalphines

in nearly all analyses is consistent with its being the only member of the group that lacks a metasomal carapace. The cladistic placement and the distinct morphological differences lead us to propose tribal status and recognize Pselaphinini *stat. nov.* with the remaining genera of Sigalphinae comprising the tribe Sigalphini.

Sister group of Sigalphinae+Agathidinae and non-monophyly of Microtypinae

Our results never recovered the two included genera of Microtypinae *sensu* Achterberg (1992), *Microtypus* Ratzburg and *Plesiotypus* Achterberg, as a monophyletic group. It is worth noting that despite Achterberg's (1992) inclusion of *Plesiotypus* in the Microtypinae, he presented no unambiguous synapomorphies for the relationship. Both genera have a triangular second submarginal cell but that condition occurs also in many Microgastrinae, most Agathidinae and some Meteorinae. Instead, bootstrapping of the elision data set gave 96% support for a clade comprising *Plesiotypus* and the Agathidinae+Sigalphinae, whereas *Microtypus* was far removed and strongly supported with Homolobinae. Because of the rather sparse sampling outside the Agathidinae+Sigalphinae clade and the use of a single gene fragment, these other results should be treated cautiously, and generally the failure to recover some "traditional" groups should best be regarded as a lack of evidence for, rather than strong evidence against.

Monophyly of the Acampsohelconini

The Acampsohelconinae was originally proposed for the extinct genus, *Acampsohelcon* Tobias from Baltic amber (Tobias 1987). *Afrocampsis* Achterberg and Quicke was originally described in the Sigalphinae (Achterberg and Quicke 1990), but despite having a carapace and hind wing vein 2-CU, molecular evidence from the 28S D2–D3 gene region, and more careful consideration of its morphology, led Quicke et al. (2002) to transfer it to the Helconinae. More recently, two other extant genera, *Urosigalphus* Ashmead and *Canalicephalus* Gibson, as well as *Acampsohelcon*, were tranferred to the Acampsolconinae by Achterberg (2002), all of which share the same two potentially derived characters of wing venation and carapace along with "a more or less developed post-pectal carina… transverse first discal cell of the fore wing… and the far postfurcal, oblique vein cu-a of fore wing". Despite these putative apomorphies, the molecular data analysed here fail to find support for *Canalicephalus+Afrocampsis*, and therefore monophyly of the extant Acampsohelconinae *sensu* Achterberg (2002).

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Appendix. EMBL/Genbank accessions numbers for 28S D2–D3 rDNA sequences obtained and used for molecular phylogenetic analyses.

Taxon	Provenance	Specimen voucher	Sequence accession number
Sigalphinae			
Acampsis alternipes (Nees von Esenbeck)	UK, Berkshire	–	AAZ83609
Aposigalphus kiestyni Achterberg and Austin	Australia, Queensland	JS253	EU427388
Malasigalphus sp.	Madagascar	–	DQ201888
Malasigalphus sp. nr *petiolaris* Achterberg & Austin	Madagascar	JS04	EU427390
Malasipalphus roa sp. n.	Madagascar	JS274	EU427389
Minanga phoebea sp. n.	Uganda, Kibale	NL1149	EU427391
Minanga serrata Cameron	South Africa	JS209	EU427392
Sigalphus bicolor (Cresson)	USA, Kentucky	JS210	EU427394
Sigalphus irrorator (Fabricius)	France	–	Z97942
Sigalphus gyrodontus He and Chen	Vietnam	–	AJ416966
Pselaphaninae			
Pselaphanus sp.	Brazil	JM463	EU427393
Agathidinae			
Earinini			
Amputoearinus matamata Sharkey	Colombia	–	DQ201928
Austroearinus melanopodes Sharkey	Costa Rica	–	DQ201948
Crassomicrodus divisus (Cresson)	Mexico	NL452	DQ201945
Earinus elator (Fabricius)	UK, Silwood	–	Z97944 and DQ201926
Sesioctonus kompsos Briceño and Agathidini	Costa Rica	–	DQ201949
Agathis montana Shestakov	Turkey	–	AJ302786 and DQ201900

Taxon	Provenance	Specimen voucher	Sequence accession number
Alabagrus haenschi (Enderlein)	Neotropics	–	AJ302787 and DQ201891
Bassus nr *macadamiae* Briceño and Sharkey	Costa Rica	NL456	DQ201902
Braunsia bilunata Kriechbaumer	Sao Tome	–	AJ302797 and DQ201903
Zamicrodus sensilis Viereck Cremnoptini	Colombia	NL347	DQ201911
Biroia trifasciata (Cameron)	Tanzania	–	DQ201933
Zacremnops cressoni (Cameron) Disophrini	Costa Rica	NL438	DQ201925
Disophrys atripennis (Szépligeti)	Indonesia, Sulawesi	–	AJ302826 and DQ201924
Eugathis forticarinata (Cameron)	Thailand	NL502	AJ302810 and DQ201920
Zelomorpha sp. new tribe?	Peru		AJ302836
Bassus nr *conspicuus* (Wesmael)	–	NL451	DQ201908/9
Mesocoelus sp.	Costa Rica	NL449	DQ201907
Acampsohelconinae			
Afrocampsis griseisetosus Achterberg & Quicke	–	–	AJ517428
Canalicephalus sp.	Malaysia, Sabah	–	AJ302799
Cenocoeliinae			
Capitonius sp.	Bolivia	JM5	EU427378
Euphorinae			
Meteorus sp.	Taiwan	NL421	EU427379
Orionis eximius (Muesebeck)	–	–	AJ302824
Helconinae s.l.			
Austrohelcon sp.	Australia, Brisbane	–	AJ416970
Helcon sp.	Malaysia, Sabah	–	AJ302815
Flavihelcon sp.	Malawi	JM569	EU427380
Plesiotypus sp.	Madagascar	JM834	EU427381
Triaspis sp.	Turkey	–	AJ302833
Homolobinae			
Homolobus (Apatia) truncator (Say)	USA	JM/RB1	EU427382
Macrocentrinae			
Dolichozele sp.	French Guyana	JM814	AJ302809
Meteorideinae			
Meteoridea sp.	Thailand	–	AJ416974
Microtypinae			
Microtypus wesmaelii Ratzeburg	–	–	AJ302822

Taxon	Provenance	Specimen voucher	Sequence accession number
Orgilinae			
Stantonia (Bentonia) sp.	no data	–	EU427383
Doryctorgilus morvanae Braet and Achterberg	French Guyana	NL733	EU427384
Eleonoria sp.	Madagascar	–	EU427385
Orgilus sp.	Indonesia, Sulawesi	–	EU427386
Podorgilus sp.	French Guyana	–	EU427387
Xiphozelinae			
Xiphozele sp.	Indonesia, Java	–	AJ302931