

The systematic placement of *Afrocampsis* van Achterberg & Quicke (Hymenoptera: Braconidae): molecular and morphological evidence indicate that it belongs to Helconinae s.l. not Sigalphinae

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Key words: Braconidae; Sigalphinae; Helconinae; Diospilini; *Afrocampsis*; *Canalicephalus*; *Urosigalphus*; phylogeny; biology; morphology; convergence; 28S rDNA.

The D2 expansion region of 28S rDNA and the smaller D3 region of *Afrocampsis griseosetosus* van Achterberg & Quicke, 1990, were analysed. Molecular and morphological evidence indicate that *Afrocampsis* is the sister group of *Urosigalphus* + *Canalicephalus* (forming a separate clade close to the tribe Diospilini) or that it is associated with a group of the subfamily Helconinae s.s.

Introduction

Afrocampsis van Achterberg & Quicke, 1990, was described on the basis of one quite frequently collected Afrotropical species and included in the subfamily Sigalphinae Haliday, 1833. At the time of its recognition as a new genus, van Achterberg & Quicke (1990), its authors were unaware that, within the Braconidae, vein 2CU of the hind wing could occur outside of the subfamilies Sigalphinae, Agathidinae Haliday, 1833, Pselaphaninae van Achterberg, 1985, Meteorideinae Čapek, 1965, and Trachypetinae Schulz, 1911 (= Cercobarconinae Tobias, 1979). Given this, and the fact that its metasoma is carapace-like as in all other sigalphines, it seemed that *Afrocampsis* was best placed in the Sigalphinae. However, *Afrocampsis* does not fit well in the Sigalphinae because of the absence of fore wing vein 1SR, the slender marginal cell of the fore wing, the posteriorly converging veins mcu and 1M of fore wing, its enlarged hind claws, the widened hind femur and tibia, the enlarged vein r-m of fore wing, the absence of vein r of hind wing, the slender ovipositor sheath, the presence of a malar suture, and the lamelliform frontal carina. It is now apparent that because of the presence of vein 2CU of the hind wing and the enlarged hind claws, *Afrocampsis* may be more closely related to the *Urosigalphus* group of genera, and putative synapomorphies supporting this relationship are the enlarged tarsal claws, absence of fore wing vein 1SR, vein r-m of fore wing unsclerotized, and the metapleuron without distinct

oblique groove. It is not clear whether the short vein 2CU of the hind wing is also a synapomorphy or a symplesiomorphy, but the former is a valid possibility given its position and the sporadic distribution through the family (viz., in Trachypetinae, Meteorideinae, Agathidinae+ Sigalphinae+ Pselaphaninae, the *Urosigalphus* group, and also in the Apozyginae if indeed these are Braconidae) (Quicke *et al.*, 1999a).

Materials and Methods

We recently had the opportunity to attempt to obtain DNA sequence data from *Afrocampsis griseosetosus* van Achterberg & Quicke, when one of us (DLJQ) discovered several specimens that were preserved in 70% ethanol and had been maintained in a cold room in Ottawa at <5°C for 12 years. Although old parasitic wasp specimens have often proved very recalcitrant to sequencing attempts (Quicke *et al.*, 1999b), that these specimens had been kept cool might have increased the survival of the DNA. A weak amplification product was obtained and sequenced.

Taxa included in the analysis

Table 1. List of taxa of the family Braconidae Nees, 1812, used for the molecular phylogenetic analysis with the EMBL/GenBank accession numbers for 28S D2-D3 rDNA sequences, and collection data where not published previously. Cenocoeliinae were included to represent the 'euphoroid clade' (see Belshaw & Quicke, 2002).

Subfamily Agathidinae Haliday, 1833	
<i>Alabagrus haenschii</i> (Enderlein, 1920), AJ302787	<i>Schizoprymnus</i> spec. 1 UK, Z97947
<i>Earinus elator</i> (Fabricius, 1804), Z97944	<i>Schizoprymnus</i> spec. 2 Israel, AJ302934
<i>Euagathis</i> spec., AJ302810	<i>Brulleia</i> spec., AJ302798
Subfamily Blacinae Foerster, 1862	<i>Diospilus</i> spec., AF029134
<i>Blacus</i> spec. 1, Z79750	<i>Eubazus semirugosus</i> (Nees, 1816), Z83608
<i>Blacus</i> spec. 2, AJ302796	<i>Taphaeus</i> spec., AJ302832
<i>Blacus</i> (<i>Tarpeion</i>) spec., AJ517427	<i>Ussurohelcon nigricornis</i> van Achterberg, 1994, AJ302912
Subfamily Cenocoeliinae Szépligeti, 1901	Subfamily Homolobinae van Achterberg, 1979
<i>Cenocoelius analis</i> (Nees, 1834), Z83605	<i>Exasticolus</i> spec., Panama, Chiriqui, AJ517426
<i>Capitonius</i> spec., AJ245687	Subfamily Macrocentrinae Foerster, 1862
Subfamily Helconinae Foerster, 1862 s.l.	<i>Macrocentrus</i> spec., Z97948
gen. nr <i>Eadya</i> spec., AJ302814	Subfamily Meteorideinae Capek, 1965
<i>Foersteria</i> spec., AJ302813	<i>Meteoridea</i> spec., AJ416976
<i>Triaspis</i> spec., AJ302833	Subfamily Microtypinae Szépligeti, 1908
<i>Vadum</i> spec., AJ302834	<i>Microtypus wesmaelii</i> Ratzeburg, 1848, AJ302822
<i>Austrohelcon</i> spec., AJ416970	Subfamily Sigalphinae Haliday, 1833
<i>Helcon</i> spec. 1 France, Z97946	<i>Sigalphus gyrodontus</i> He & Chen, 1994, AJ416966
<i>Helcon</i> spec. 2 Turkey, AJ517425	<i>Sigalphus irradiator</i> (Fabricius, 1775), Z97942
<i>Wroughtonia</i> spec., AJ416792	<i>Acampsis alternipes</i> (Nees, 1816), Z83609
<i>Eubazus</i> (<i>Aliolus</i>) <i>lepidus</i> (Haliday, 1835), AJ517424	Unplaced taxa
<i>Nealiolus</i> spec., AJ517422	<i>Afrocampsis griseosetosus</i> van Achterberg & Quicke, 1990, AJ517428
<i>Aspigonus diversicornis</i> Wesmael, 1835, Russian Far East, AJ517421	<i>Canalicephalus</i> spec., AJ302799
<i>Aspicolpus</i> spec., Russian Far East, AJ517423	<i>Urosigalphus</i> spec., AJ302923

DNA extraction, amplification and sequencing

Genomic DNA was extracted from single legs using the DNeasy Tissue Kit (Qia-gen) by crushing and incubating at 55°C for 12–36 hours in Proteinase K, with elution into 30 µl distilled water. Standard 50 µl PCR reactions were then carried out in a GeneAmp 9600 thermal cycler using 1.0 µl DNA extract, 5 µl *Taq* buffer (1.5 mM MgCl₂), 1.5 U *Taq* polymerase (Roche), 10 nmol dNTPs (Amersham Pharmacia Biotech; APB) and 20 pmol of each primer. Primer sequences for PCR amplification of the D2 expansion region of 28S rDNA (and the smaller D3 region) were: forward primer 5' GCG AAC AAG TAC CGT GAG GG3'; reverse primer 5'TAG TTC ACC ATC TTT CGG GTC3'. The GFX gel band purification kit (APB) was used in order to clean PCR products, which were then sequenced in both directions with the same primers using Big Dye terminator at half recommended volumes on an ABI 3700 automated sequencer. PCR condition were 35 cycles of 95°C denaturation (30 s), 45°C annealing (30 s) and 72°C extension (1 min) with an initial denaturation for 2 mins and a final extension for 4 mins.

Phylogenetic analysis of molecular data

We initially aligned the *Afrocampsis* sequence by eye next to the previously obtained sigalphine sequences (three species in two genera); our alignment also included 33 other noncyclostome braconid sequences representing a wide range of subfamilies, and in particular, a range of helconine s.l. sequences. Many differences were noted including the absence of several putatively apomorphic features that are present in all previously obtained agathidine+sigalphine sequences.

Additional alignments were performed using Clustal X. We investigated a range of gap opening: gap extension: transversion: transition cost ratios in order to test the sensitivity of our results to these alignment parameters. We used the Clustal X default transversion: transition cost ratio. However, the highly length variable parts of the sequences meant that for many of these sets of costs, Clustal X could not create an alignment. The four cost sets that worked were 10.0: 6.7: 1.0: 0.5, 15.0: 6.7: 1.0: 0.5, 10.0: 5.0: 1.0: 0.5 and 15.0: 5.0: 1.0: 0.5. Heuristic searches on manually aligned data and on Clustal-generated alignments were carried out using PAUP* version 4.06b (Swofford, 1998) by carrying out 10,000 random additions followed by TBR branch-swapping but keeping only one tree in memory at a time (Quicke *et al.*, 2001). Gaps were treated as missing data.

Results and discussion

In addition to adult and larval features (Shaw & Quicke, 2000), the 28S D2 rDNA gene fragment of the Agathidinae+Sigalphinae clade displays many autapomorphies including indels and substitutions (figs 1, 2). More than 300 taxa in all but three braconid subfamilies have been sequenced to date; see also Belshaw *et al.* (1998, 2000); Belshaw & Quicke (2002) and Quicke *et al.* (in preparation). None of these molecular features are displayed by *Afrocampsis*. Instead, parsimony analyses of the aligned sequence data either place it as the sister group of *Urosigalphus* Ashmead, 1889 + *Canalicephalus* Gibson, 1977, and forming together a clade close to the tribe Diospilini Foerster, 1862 (fig. 3a, b), in a grade next to *Urosigalphus* + *Canalicephalus* which leads to the Meteorideinae+Agathidinae+Sigalphinae (all taxa with vein 2CU of hind wing developed)

(fig. 3c), or separate from *Urosigalphus* + *Canalicephalus* and instead associated with either the helconine genus *Foersteria* Szépligeti, 1896 (figs 3e, f) or a larger clade (fig. 3d). Three of these analyses, plus analysis of the manually-aligned data (not shown) associate *Afrocampsis* with *Urosigalphus* + *Canalicephalus*, either forming a clade or a grade. All three of these genera display a short but distinct vein 2CU of the hind wing (Quicke *et al.*, 1999a) and have a carapace-like metasoma. Neither in the above analyses nor in previously published ones which were based additionally on several computer generated sequence alignments using POY (Wheeler, 1996) and which also included *Urosigalphus* and *Canalicephalus* as well as many additional taxa, did these latter two genera appear

Taxon	Sequence fragments
<i>Afrocampsis</i> <i>griseosetosus</i>	TTACGATGT-----GA----NTT-----GTCC
<i>Canalicephalus</i> spec.	TTGCGATGT-----GA----ATT-----ATCC
<i>Urosigalphus</i> spec.	MISSING DATA
<i>Sigalphus</i> <i>gyrodontus</i>	TAGTGATGT-----GA----ATT-----GTC-GA-TAAAGTTTCTGAC
<i>Sigalphus</i> <i>irrorator</i>	TAGTGATGT-----GA----TTT-----GTTTGAAGAATGTTCTGAC
<i>Acampsis</i> <i>alternipes</i>	TAGTGATGC-----GA----GTT-----GTTTAACAGGATTTTAATC
<i>Alabagrus</i> <i>haenschi</i>	TAGTGATGC-----AA----GTT-----TTTGGTAAGAGGGTCAAATC
<i>Earinus</i> <i>elator</i>	TAATGATGT-----GA----GTT-----TTTGGTAAGAGGGTTRAACC
<i>Euagathis</i> spec.	TAGTGATGT-----TG----GTT-----TTGTTGGTC-GGGTCATTCC
<i>Blacus</i> spec. 1	TTATGATGT-----AA----GTT-----ATCC
<i>Blacus</i> spec. 2 (341)	TTATGATAT-----GA----GTT-----GTCC
<i>Blacus</i> (<i>Tarpheion</i>) spec.	TTGTGATGT-----GA----TTT-----ATCC
<i>Cenocoelius</i> <i>analisis</i>	TCATGATGC-----AT----GTT-----GTTT
<i>Capitonus</i> spec.	TTTTGATAT-----T----GTA-----GTCC
gen. nr <i>Eadya</i>	TTGCGATAC-----GT----TTGAT-----CTCC
<i>Triaspis</i> spec.	TTGCGATGT-----GA----GTT-----ATCC
<i>Diospilus</i> spec.	TTGCGATAT-----AATGT-ATT-----TTCT
<i>Vadum</i> spec.	TGGTGATGC-----GA----TGT-----ATAT
<i>Austrohelcon</i> spec.	TTGCGATGT-----AA----GTT-----ATCC
<i>Helcon</i> spec. France	TTATGATGT-----TA----GTT-----GTCC
<i>Helcon</i> spec. Turkey	TTATGATGT-----TA----GTT-----GTCC
<i>Wroughtonia</i> spec.	TTATGATGTTAGTGTGTGTGTGTT-----ATCC
<i>Ussurohelcon</i> <i>nigricornis</i>	TTGCGATGT-----GAGA--GAT-----ATCC
<i>Eubazus</i> <i>lepidus</i>	MISSING DATA
<i>Nealiolus</i> spec.	TTGCGATGT-----GA----GTT-----ATCC
<i>Foersteria</i> spec.	TTGYGATGT-----AG----GTT-----ATCC
<i>Aspigonus</i> <i>diversicornis</i>	TTGCGATAT-----AA----TAT-----ACGT
<i>Aspicolpus</i> spec.	TTGCGATGT-----AR----GATT-----GTCC
<i>Schizoprymnus</i> spec. UK	TTGCGATGT-----GA----GTT-----ATCC
<i>Schizoprymnus</i> spec. Israel	TTGCGATGT-----GA----GTT-----ATCC
<i>Brulleia</i> spec.	TTATGATGT-----AA----GTT-----ATCC
<i>Eubazus</i> <i>semirugosus</i>	TTGCGATGT-----GA----GTT-----ATCC
<i>Taphaeus</i> spec.	TTGCGATAT-----AA----ATT-----TTCT
<i>Exasticolus</i> spec.	TTGCGATGTTAA--GA----AATT-----TTTC
<i>Macrocentrus</i> spec.	TTGTGATAA-----AA----ATT-----TTTC
<i>Meteoridea</i> spec.	TTATGATAT-----GG----GTT-----ATTT
<i>Microtypus</i> <i>wesmaelii</i>	TTGCGATGTTGA--GA----ATTTTTTT-----TTTC

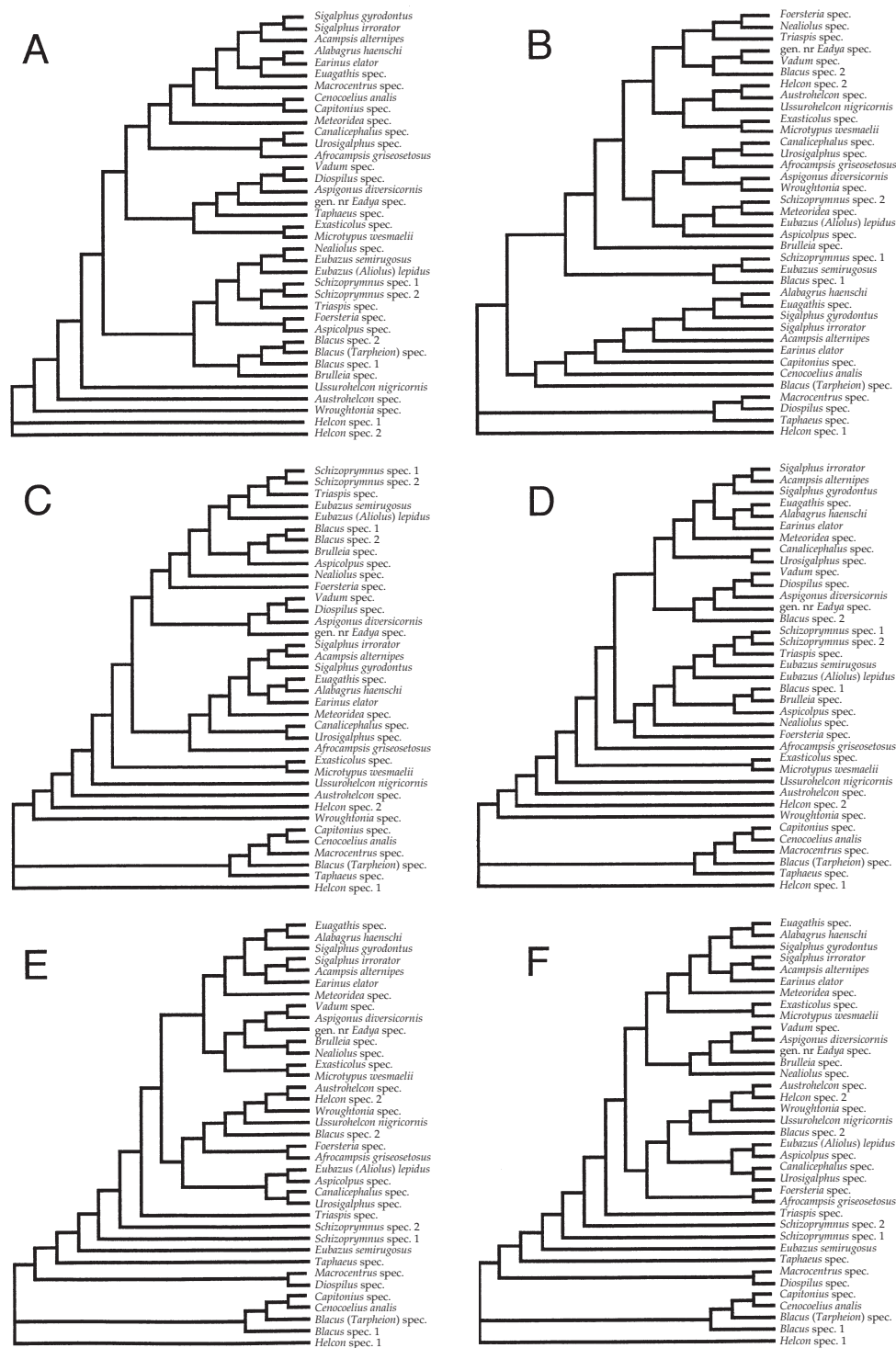
Fig. 1. Manually aligned part of D2 region corresponding to bases starting at position 67 in Fig. 1 in Belshaw *et al.* (1998) and running into box.

within a monophyletic Helconinae. They do not even associate with them (Belshaw & Quicke, 2002), again suggesting that this group might represent a distinct clade (or clades).

In *Afrocampsis*, the mandibles are not twisted as they are in all Agathidinae + Sigalphinae, i.e., they are not modified to cut through a tough silken cocoon and may therefore have a more primitive function such as chewing through wood. Similarly, the deep sulcus of the frons along with the raised medial projection are particular to (at least in the Ichneumonoidea) species that attack wood-boring insects (protecting the antennal bases as they exit woody tissue – see Vilhelmsen, 1997). These features

Taxon	Sequence fragment
<i>Afrocampsis griseosetosus</i>	CTTTAATGTCAT---CGC---AAGATG---TTA-----CTATTAAAGACC
<i>Canalicephalus</i> spec.	CTTTGATGTCAT---TGC---AAAATG---WT-----ACATTGGAGACC
<i>Urosigalphus</i> spec.	CTTTAGTATCAT---TGC---AAAATG---TT-----ATATTGAAGGCC
<i>Sigalphus gyrodontus</i>	CTTCGATACATTCA-----TTT-----GTATTGGAGACC
<i>Sigalphus irrorator</i>	CTTCGATACATTCA-----TTT-----GTATTGAAGACC
<i>Acampsis alternipes</i>	CTTTGATACATTAA-----TTT-----ATATTGAAGACC
<i>Alabagrus haenschi</i>	CTTTAATATTTT-----ATATTAAAGTCC
<i>Earinus elator</i>	CTTTGATATTT-----TT-----ATATTAAAGACC
<i>Euagathis</i> spec.	CTTCATATTT-CA-----TT-----ATATTGAAGACC
<i>Blacus</i> spec. 1	CTTCGAAGTC--AC--CGC---AAGGTG---TT-----ATTTTGAAGACC
<i>Blacus</i> spec. 2 (341)	CTTTGAAGTCA-TC--GT--AAGATG---TT-----ACTTTAGAGGCC
<i>Blacus</i> (<i>Tarpeion</i>) spec.	CTTTGACATT-----ATGTTGAAGGCC
<i>Cenocoelius analis</i>	TTCTAATTTT-----AAATTGGAGACC
<i>Capitonus</i> spec.	CTTTAATTYATT-----TT-----GAATTGAAGACC
gen. nr <i>Eadya</i> spec.	CTTTAATATCAT---AGC---AATATG---TT-----ATATTGAAGACC
<i>Triaspis</i> spec.	CTTTGATGTTT-GC--CGC---AAGGTA---TA-----ATATTGAAGACC
<i>Diospilus</i> spec.	CTTTAATGTCA-TC--TTT--TAGATG---TT-----ACATTGAAGACC
<i>Vadum</i> spec.	CTTTAATGTCA-TC--TTA--TAGATG---TT-----ACATTGAAGACC
<i>Austrohelcon</i> spec.	CTTTAATGTC--GC--CGC--GAGGTG---TT-----ATATTGAAGACC
<i>Helcon</i> spec. France	CTTCGATGTTT-AC--CGTTAAAACGTG--TA-----ATATTGAAGACC
<i>Helcon</i> spec. Turkey	CTTCGATGTTT-AC--CGTTAAAACGTG--TA-----ATATTGAAGACC
<i>Wroughtonia</i> spec.	CTTTAATGTTGTA--AGG--GTTACC--TTT---AAAATATTAAAGACC
<i>Ussurohelcon nigricornis</i>	CTTTGATGTCACTACATAC---TGGTG---TT-----ACATTGAAGACC
<i>Eubazus lepidus</i>	CTTTGATGTTA-GC--CGC---AAGGTA---TT-----ATATTGAAGACC
<i>Nealiolus</i> spec.	CTTTAATGTTTTCG--CGT--AAGGTA---TT-----ATATTGAAGACC
<i>Foersteria</i> spec.	CTTTGATGTT--AC--NGT--AAGGTG---TT-----ATATTGAAGACC
<i>Aspigonus diversicornis</i>	CTTTGATGTCG-TC--TTA--TAGACG---TT-----ACATTGAAGATC
<i>Aspicolpus</i> spec.	CTTTGATGTC--AC--CGC---AAGGTG---TA-----ATGTTGAAGACC
<i>Schizoprymnus</i> spec. UK	CTTTGATGTTT-GC--CGC---AAGGTA---TT-----GATGTTGAAGACC
<i>Schizoprymnus</i> spec. Israel	CTTTGATGTTT-GC--CGC---AAGGTA---TT-----GATGTTGAAGACC
<i>Brulleia</i> spec.	CTTTGATGTC--AC--CTA--TGTGTG--CT-----ATGTTGAAGACC
<i>Eubazus semirugosus</i>	CTTTGATGTTT-GC--CGC---AAGGTA---TT-----ATTTTGAAGACC
<i>Taphaeus</i> spec.	CTTTAATGTC--AT--TGT--AAAATG---TT-----ATATTGAAGATC
<i>Exasticolus</i> spec.	CTTTAATGTAC-AC--TTT-AATCGGTG---TT-----ATTGTTAAAGACC
<i>Macrocentrus</i> spec.	CTTTAGTAAATG-TT--TTT--ATTAATA-----TTATTGAAGACC
<i>Meteoridea</i> spec.	CTTTAATATCA-TC--TTA--CGATG--TTTT-----ATATTAAAATCC
<i>Microtypus wesmaelii</i>	CTTTAATGTAC-AC--TTT-AATCGGTG---TT-----ATTGTTGAAGACC

Fig. 2. Manually aligned part of D2 region corresponding to bases starting at position 200 in Fig. 1 in Belshaw *et al.* (1998) and running into box.



are consistent with the placement of *Afrocampsis* near to *Urosigalphus* and not with the Sigalphinae (+Agathidinae) because the latter are all parasitoids of Lepidoptera larvae from which they exit to form cocoons in situations that are relatively easy to escape from. In contrast, *Urosigalphus* species are parasitoids of bruchid beetle larvae in beans and other seeds (Gibson, 1972; Sharkey, 1997) and egress from the host's feeding chamber requires chewing through hard plant substrate.

Removal of *Afrocampsis* from the Sigalphinae makes the latter subfamily much better defined, viz. fore wing vein m-cu more or less diverging from 1M posteriorly, claws normal, mostly with a large lobe, metapleuron with a distinct oblique groove, no distinct lamelliform frontal carina, no malar suture, ovipositor sheath widened, vein r-m of fore wing normal and vein r of hind wing present. We believe that much of the confusion that exists at present about the composition of various braconid subfamilies, and the changes that molecular data are forcing (see e.g., Belshaw & Quicke, 2002) are due to the very high levels of homoplasy in many morphological characters as a direct result of rampant parallelism (van Achterberg, 1988; Gauld & Mound, 1982). Combining molecular and morphological data seem to provide the best solution at present and hopefully will lead to the discovery of monophyletic groups that can then be used better to interpret the evolution of life history features. Sadly, as the taxa concerned in this study illustrate, we are also lacking a lot of fundamental information about hosts among the Braconidae, as the biologies of neither *Afrocampsis* nor *Canalicephalus* are currently known. Much more careful basic biological work is needed, especially in tropical regions.

Acknowledgements

Mike Sharkey (Lexington) kindly pointed out to us the morphological features that suggest a close association of *Afrocampsis* with the Helconinae (s.l.) and that it is probably associated with a wood or seed feeding host and did not appear to be a sigalphine. He initiated this study, and equally importantly, made a major contribution through his involvement with building the CNCO cold alcohol collection of parasitoids. We would like to thank Henri Goulet (Ottawa) for permission to sequence some material. SM was supported by a grant from the Government of Iran. Miharuru Mori (Silwood Park) kindly provided the *Exasticolus* sequence data. Specimens of some taxa sequenced for this study were provided by Sergey Belokobylskij (St Petersburg) and Mike Sharkey.

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◀ Fig. 3. Most parsimonious trees resulting from analysis of clustal alignments of sequence data. A, B, single trees from 10.0: 6.7: 1.0: 0.5 (see text for details) and 15.0: 6.7: 1.0: 0.5 alignments, respectively; C, D, two trees from 10.0: 5.0: 1.0: 0.5 analysis; E, F two trees from 15.0: 5.0: 1.0: 0.5 analysis.

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