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Revision of the Agathidinae (Hymenoptera: Braconidae) with comparisons of static and dynamic alignments

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Abstract

The phylogeny of the Agathidinae (Insecta: Hymenoptera: Braconidae) is investigated based on morphological and sequence data from the D2–3 regions of 28S rDNA. Morphology and molecular data were run simultaneously and separately and the molecular and combined data sets were analyzed using both static, Clustal W, and dynamic, POY, alignments. Both alignments were conducted under a variety of gap costs and results are compared. Sixty-two ingroup exemplars representing 22 genera and six outgroup taxa representing two subfamilies and five genera were included. Numerous taxa at the generic and tribal levels were tested for monophyly and the evolutionary history of several characters is discussed. The tribe Agathidini *s.s.* is found to be a derived member of the Microdini and the two are synonymized under the older name, Agathidini *s.l.* Support is substantial for the tribes Cremnoptini and Disophrini and Earinini but equivocal for the Agathidini *s.l.* At the generic level, *Bassus* is found to be polyphyletic. Numerous new synonymies and combinations are proposed.

The Agathidinae is a moderately large subfamily of Braconidae with about 1000 described species (Yu et al., 2005), though we estimate an additional 2000–3000 species await description. It has a worldwide distribution and members are found in most terrestrial habitats. Though all known species are koinobiont endoparasitoids of lepidopteran larvae, life-history traits vary considerably. Depending on the species, they may be nocturnal or diurnal, gregarious or solitary, attack exposed or concealed hosts, and attack any larval instar. In general they are solitary, attack first-instar Lepidoptera larvae in concealed microhabitats such as leaf-rolls or stems, and emerge from the last larval instar of the host after it has spun its cocoon. Several species of *Zelomorpha* Ashmead are known to be gregarious (Sarmiento et al. 2004, as *Coccygidium*). Detailed studies of life history have been conducted for a few species (e.g., Simmonds, 1947; Dondale, 1954; Odebiyi and Oatman, 1972, 1977; Janzen et al., 1998) and a few have been used in classical biological control efforts. Currently there are about 50 genera recognized (Sharkey, 1992). The history of higher classification of the Agathidinae was summarized by Sharkey (1992) who also proposed a tribal-level classification based on groundplan coding.

This is the first study using both molecular and morphological data sets to examine agathidine relationships. For molecular data sets, selecting appropriate alignment parameters is a well recognized problem (Wheeler, 1995; Giribet, 2003; Terry and Whiting, 2005). Additionally, various alignment programs evaluated under different parameter sets can affect phylogenetic reconstruction (Morrison and Ellis, 1997; Hickson

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et al., 2000). Here we compare the results of five parameter sets employing POY (Wheeler et al., 1996–2003) and Clustal W (Thompson et al., 1994) on topological stability.

The purposes of this paper are: to compare the results from POY and Clustal over five different gap costs; to test the tribal classification proposed by Sharkey (1992); to test the monophyly of numerous genera; to test the monophyly of the Agathidinae and the placement of the genera *Mesocoelus* Schulz and *Aneurobracon* Brues, which have been placed in the Orgilinae; to identify the phylogenetic placement of *Austroearinus* and *Amputoearinus*, both recently described by Sharkey (2006); and to elucidate the evolutionary history of several interesting morphological characters.

Methods

Morphological terminology is based on Sharkey and Wharton (1997).

Outgroup and exemplar selection

Based on morphological data, Quicke and van Achterberg (1990), proposed *Pselaphanus* Szépligeti as the sister group to the Agathidinae, though Sharkey (1997) places it as a basal member of the Sigalphinae. No specimens of this rare genus were available for molecular data therefore it was not included in the analysis. The subfamily Sigalphinae, based on both morphological and molecular evidence (Quicke and van Achterberg, 1990; Belshaw et al., 1998) is thought to be the sister to Agathidinae and therefore four species of this subfamily were included in the analysis. Additional evidence of the close relationship shared by Sigalphinae and Agathidinae comes from observations of their egg placement and first-instar larval morphology. In most studied agathidines and in the one studied sigalphine, *Acampsis alternipes* (Nees), the egg is deposited within one of the thoracic or abdominal ganglia of the early instar host caterpillar (Shaw and Quicke, 2000) where it swells before hatching. Whether or not this strategy is ubiquitous in all species of Sigalphinae is uncertain. This behavior is unlikely in the North American *Sigalphus bicolor*, which produces single-sex broods of up to 30 individuals (Clausen, 1940), and is therefore most likely polyembryonic. Although Simmonds (1947) stated that eggs of *Cremnops vulgaris* (Cresson) (as *Bracon*) are found free-floating in the hemocoel, most other detailed studies of agathidine development note intraganglionic egg placement. A more convincing synapomorphy concerns the first-instar larvae, which in both subfamilies are caudate and polypodiform. The second and third thoracic segments are furnished with paired ventrolateral papilliform processes and each abdominal segment possesses paired ventrolateral processes that terminate in a pair of posteriorly directed claw-like structures (Odebiyi and Oatman, 1972; Shaw and Quicke, 2000). Although polypodiform larvae are found in a few other braconids, the structure of the processes is quite unlike that of the Agathidinae and Sigalphinae.

We included members of two genera of Helconinae and rooted on the genus *Helcon* Nees to help polarize character states. Preliminary results were robust to switching the root between the two helconine genera. We used an exemplar approach that included 62 species of Agathidinae, representing 22 genera and all five tribes. Our aim was to include as many agathidine genera as possible but fresh specimens were a limiting factor. Several analyses were conducted as discussed below.

Molecular protocols

DNA was extracted from ethanol-preserved or freshly collected wasps using a DNeasy® Tissue Kit (Qiagen, Valencia, CA) and protocols therein. Domains 2–3 of the nuclear ribosomal 28S gene region have been heavily sampled within the Hymenoptera and were amplified in this study using the following primer pair: (28SD2F 5′-AGTGGGTGGTAAACTCCATCTAA-3′) with (28SD2R 5′-TGGTCCGTGTTTCAAGAC-3′). Reactions contained 2.5 units Taq DNA polymerase, 1.5 mM $MgCl_2$, 200 M dNTP, 0.5 M of each primer, and 1–5 μL of template DNA. Thermal conditions were 1 min denaturing at 95 °C, 1 min annealing at 50 °C, and 1.5 min extension at 72 °C for 25 cycles, with a 5 min final extension at 72 °C. Samples were then purified using Qiagen QIAquick PCR Purification kit. GenBank numbers are presented in Appendix 3.

Sequence alignment and phylogenetic analyses

For static alignments, sequences were aligned in Clustal W (Thompson et al., 1994). The Clustal parameters employed were: -align -type = dna -gapopen = X-gapext = X-gapdist = 2-nopgap-nohgap -transweight = 1. Gap opening and extension penalties were set at 1, 2, 3, 5 and 10 times substitution costs. The Clustal results were not "corrected" manually. All substitutions were treated equally. The aligned molecular data may be found at http://www.uky.edu/~mjshar0.

The morphological data were analyzed separately, in addition to the combined analyses under the various parameter sets. The morphological characters are in Appendix 1; the corresponding matrix is in Appendix 2 and it is also posted at http://www.uky.edu/~mjshar0. The combined data sets were analyzed with POY and with TNT. Analyses performed in TNT (Goloboff et al.,

2003) were completed with the traditional search function, TBR, random seed set to 0, 1000 replications of random addition sequences, and holding 10 trees per replication. The memory space for trees was increased to 15 000 prior to the search. Strict consensus trees were generated followed by a re-sampling using the standard bootstrap option with 1000 replications. Absolute Bremer support values were obtained by retaining trees suboptimal by 20 steps, re-searching using the same criteria outlined above, calculating values using the Bremer Supports function, and collapsing nodes with support 0.

The five POY analyses (version 3.0.6., Wheeler et al., 1996–2003) on the combined data, with the following gap/substitution ratios 1 : 1, 2 : 1, 3 : 1, 5 : 1 and 10 : 1, were run with the following commands: -parallel -solospawn 15 -random 200 -fitchtrees -norandomizeoutgroup -noleading -seed -1 -multibuild 10 -treefuse -fuselimit 50 -slop 8 -checkslop 2 -buildmaxtrees 2 -maxtrees 2 -impliedalignment -holdmaxtrees 50 -driftspr -drifttbr -numdriftspr 5 -numdrifttbr 5 -fuseafterreplicates -fusemaxtrees 20. The supercomputer used was an IBM eServer Cluster 1600 and consisted of 16 p690 nodes including a total of 512 processors. Strict consensus trees were produced in POY using the following commands: -parallel -solospawn 15 -random 300 -norandomizeoutgroup -noleading -seed -1 -maxtrees 3 -tbr -buildspr -oneasis -repintermediate. We used a quick consensus method for estimating branch support (Goloboff and Farris, 2001). The version of POY we used (3.06) would not perform a quick consensus, perhaps due to a bug. Therefore we imported the strict consensus trees from the separate POY runs into PAUP* 4.0b10 (Swofford, 2002), majority rule consensus trees were generated using 50% as a cut-off value, and the relative support values were manually added to the strict consensus trees.

To obtain the number of shared components for the 45 pair-wise comparisons of the POY and Clustal strict consensus trees, the trees were compared by eye (Table 2). To generate the 45 pruned Nelson trees, a PHP script was used to generate a batch file, which was then run with TNT (Goloboff et al., 2003). While the only command employed to generate the pruned trees was "prunnelsen + ", the majority of the batch file (218 of 263 lines) was comprised of input and logging commands necessary to load the proper data sets and record the trees. Copies of the pruned trees, batch file and generation script are available from the first author (M.J.S.).

Results

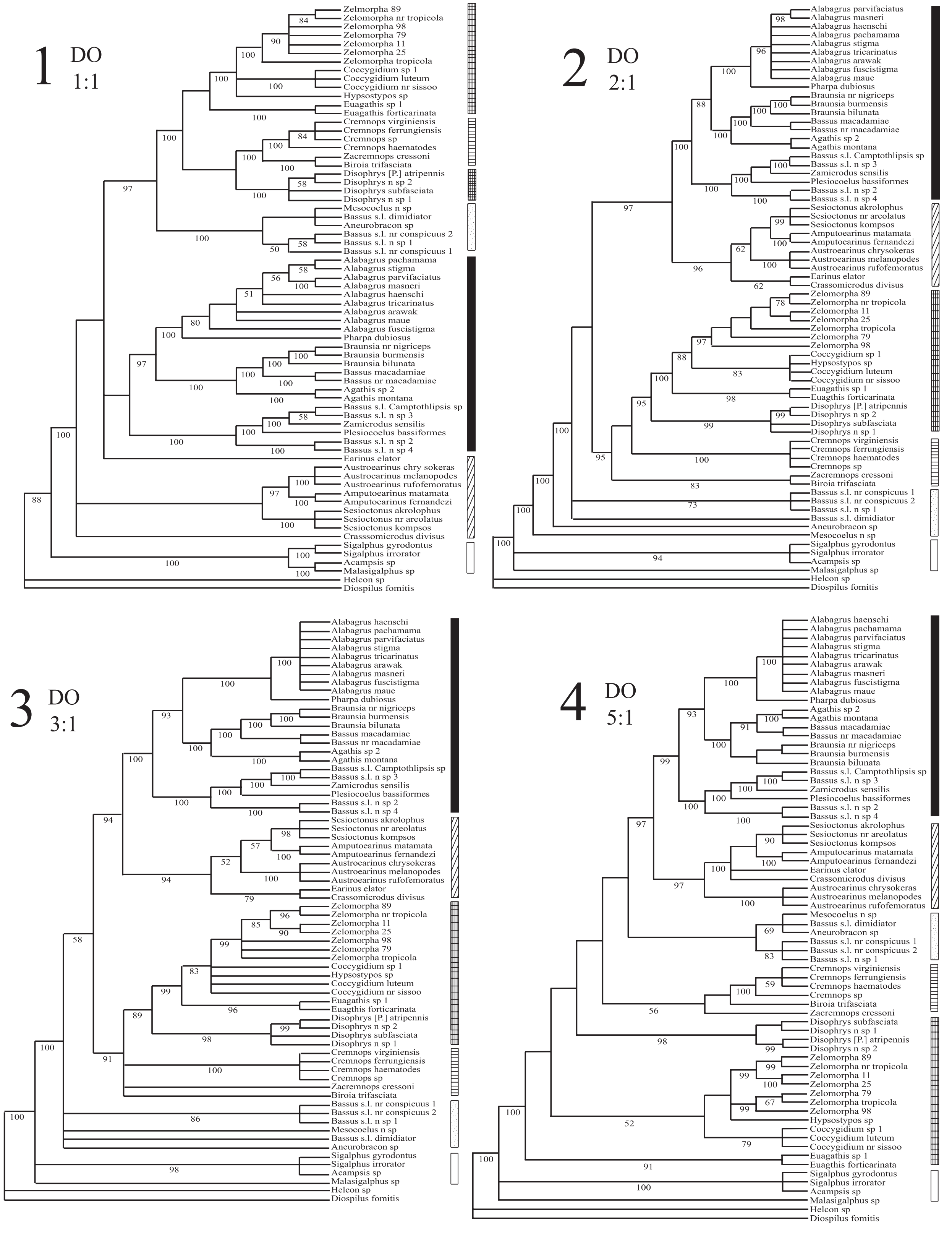
Figures 1–5 are the strict consensus trees resulting from the direct optimization (DO) alignments of the POY analyses of the combined data with the following gap cost parameters 1 : 1, 2 : 1, 3 : 1, 5 : 1 and 10 : 1, respectively. The lengths of these trees are as follows: 1 : 1, 2347; 2 : 1, 2748; 3 : 1, 3083; 5 : 1, 3698; 10 : 1, 5155. Figures 6–10 are the strict consensus trees resulting from the Clustal W alignments (CW) of the TNT analyses of the combined data with the following gap cost parameters 1 : 1, 2 : 1, 3 : 1, 5 : 1 and 10 : 1, respectively. The statistics for each of these trees is as follows: 1 : 1, length 2695, CI 0.441, RI 0.683; 2 : 1, length 2519, CI 0.432, RI 0.691; 3 : 1, length 2508, CI 0.427, RI 0.697; 5 : 1, length 2535, CI 0.409, RI 0.695; 10 : 1, length 2556, CI 0.416, RI 0.703. Figure 11 is a majority rule cladogram, with tribes as terminals. It is the product of the strict consensus trees of the 10 separate analyses (Figs 1–10), and it includes one clade that was found in only four of the 10 trees. Figure 12 is the cladogram obtained from the morphological data, length = 171, CI = 0.31, RI = 0.78; it is somewhat simplified in that when the monophyly of a genus was not contradicted it was represented as one terminal.

Discussion

Comparisons among POY and Clustal alignments

Table 1 gives the number of clades that were recovered in each of the 10 analyses. The average number of clades recovered by DO was 46.8, and an average of 56.8 clades was recovered by CW. Clearly more resolution was obtained by CW. Resolution is a test of character congruence; and one might expect that resolution would increase with the correct alignment parameters. Among the CW trees the most resolution was found in the CW analysis with a gap cost of 3 : 1 (61 nodes) though the alignment based on a gap cost of 1 : 1 was not significantly different (60 nodes). Of the DO analyses, a gap cost of 1 : 1 obtained the most resolution. Interestingly, the DO analysis based on gap costs of 3 : 1 provided the least resolution. Unfortunately, the number of nodes recovered in the DO and CW alignments are not directly comparable because the trees of each CW analysis are based on a single alignment, whereas those of each POY analysis are based on separate alignments.

Table 2 illustrates the number of shared clades (components) in the 45 pair-wise comparisons among all of the DO and CW strict consensus trees. The 10 DO trees shared an average of 29 components. The CW trees had an average of 45 shared components. DO and CW trees shared an average of 32 components. Because the DO trees had less resolution and therefore fewer components, we conducted another comparison in which this bias was corrected by dividing the number of components recovered by the maximum that could be



Figures 1 – 4. Strict consensus trees of all evidence, morphological plus molecular, generated with POY with gap costs set at 1 : 1, 2 : 1, 3 : 1 and 5 : 1, respectively. Numbers below branches are relative support values. BARS: Sigalphinae ▭, Cremnoptini ▥, Disophrini ▦, n. tribe? ░, Earinini ▨, Agathidini ■.

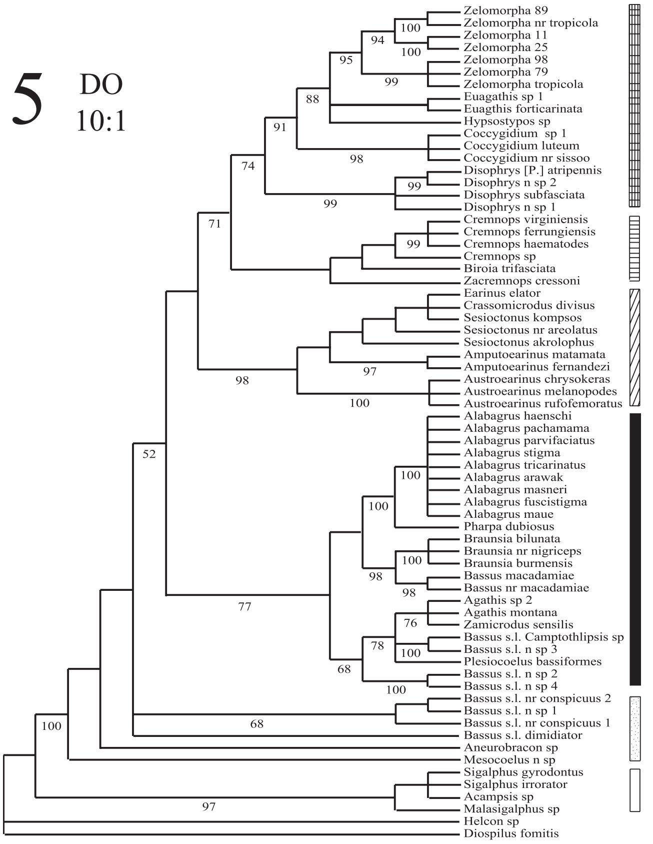


Fig. 5. Strict consensus trees of all evidence, morphological plus molecular, generated with POY with gap costs set at 10 : 1. Numbers below branches are relative support values. BARS: Sigalphinae ▭, Cremnoptini ▥, Disophrini ▦, n. tribe? ▒, Earinini ▨, Agathidini ■.

recovered, i.e., the number found in that tree in each pair-wise comparison with the fewest components. These results are presented in Table 3. Again the DO × DO comparisons were lowest with an average of 63% similarity in shared components, DO × CW had 72% shared component similarity, and CW × CW had an average of 84% shared components. The DO × DO comparisons had less similarity than DO × CW suggesting that DO was more sensitive to changes in parameter space, i.e., small changes in parameter space had a large affect on topology. The tree with the highest average component similarity, 78.1%, over all tree comparisons was CW with a gap cost of 3 : 1.

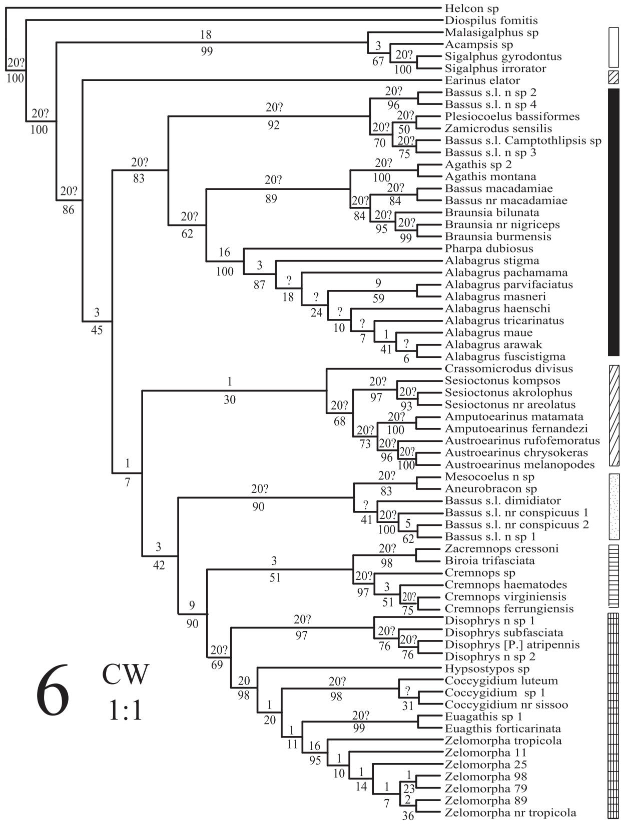
We tested for correlation between identical gap costs between the two alignment methods by comparing the percent component similarity of the DO and CW strict consensus trees with identical gap costs (from Table 3). The average component similarity from these comparisons was 68.6%, lower than the average value, 72%, of all DO × CW comparisons across all gap costs. One interesting datum was the value, 83%, for the comparison of the DO × CW trees with gap costs of 3 : 1. This was the highest percent component similarity of the 25 pair-wise comparisons of DO × CW trees. Nonetheless, for this data set the choice of optimization method appears to be more influential than the gap cost parameter setting. Although correlation in nodes recovered in the comparisons between identical gap costs was low, there are numerous instances where identical gap costs in the CW and DO analyses failed to recover nodes that were supported in all or most other analyses. Some of these are identified in the Taxonomic considerations section.

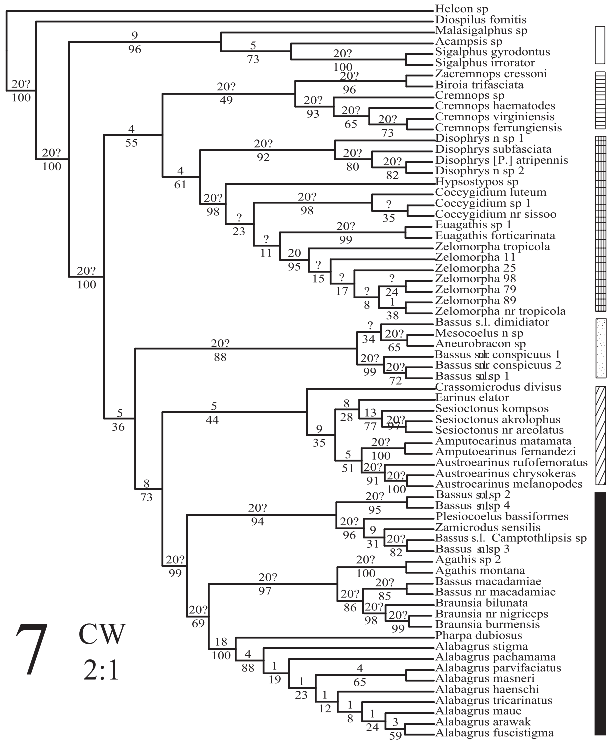
We suspected that the lack of resolution in comparisons among the DO strict consensus trees may have been the result of a few rogue taxa such as *Crassomicrodus* and *Earinus* whose placements were quite variable over all 10 strict consensus trees. We therefore generated “pruned Nelson” trees for the five different parameter settings for both DO and CW. In the pruned Nelson trees, terminal taxa are deleted or “pruned” until the largest set of taxa that are in topological agreement remain. The measure of a pruned tree is the number of taxa that remain. To obtain pruned trees we used the source trees from each of the 10 data sets and pair-wise comparisons were made between each set of source trees. These results are presented in Table 4. Contrary to our suspicion, the results were similar to those presented in Tables 2 and 3, which were based on strict consensus trees. Here too the DO × DO comparisons had the lowest component similarity, the DO × CW comparisons had intermediate similarity values, and the CW × CW comparisons had the highest similarity values. Once again the CW analysis with a gap cost of 3 : 1 had the highest score, with an average of 43.6 shared taxa.

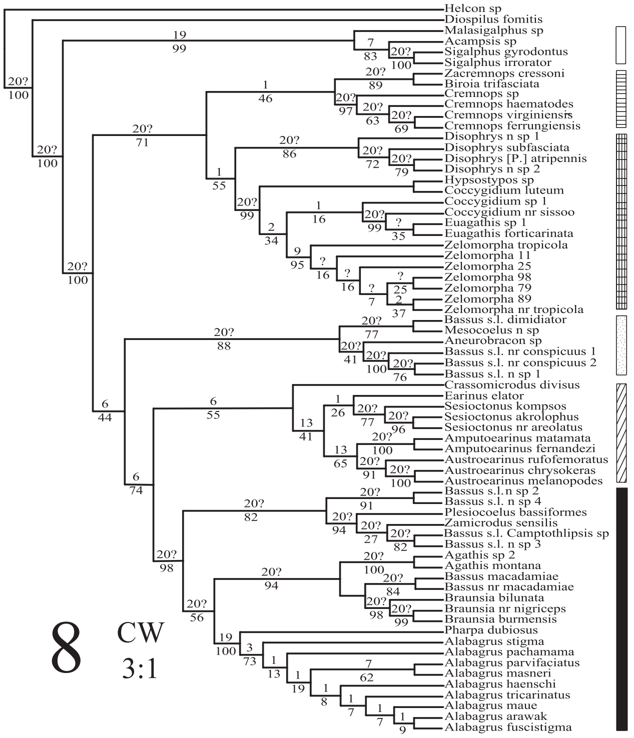
Many more data sets will have to be investigated to understand the behavior of DO and CW. Nonetheless, our data set suggests that DO was more sensitive than CW to changes in gap cost parameters. DO trees generated with different gap costs were more similar to CW trees than they were to each other. CW trees generated with different gap costs were more similar to each other than they were to DO trees. DO results were less resolved, more conservative, than CW results. Recently, both Petersen et al. (2004), Aagesen et al. (2005) and Laurenne et al. (2006) have shown that DO is particularly sensitive over a range of gap cost parameters to the presence of large indels. The considerable length variation in 28S region of the Agathidinae may therefore at least partially explain why alignment method was more influential than gap cost with the current data.

Character evolution

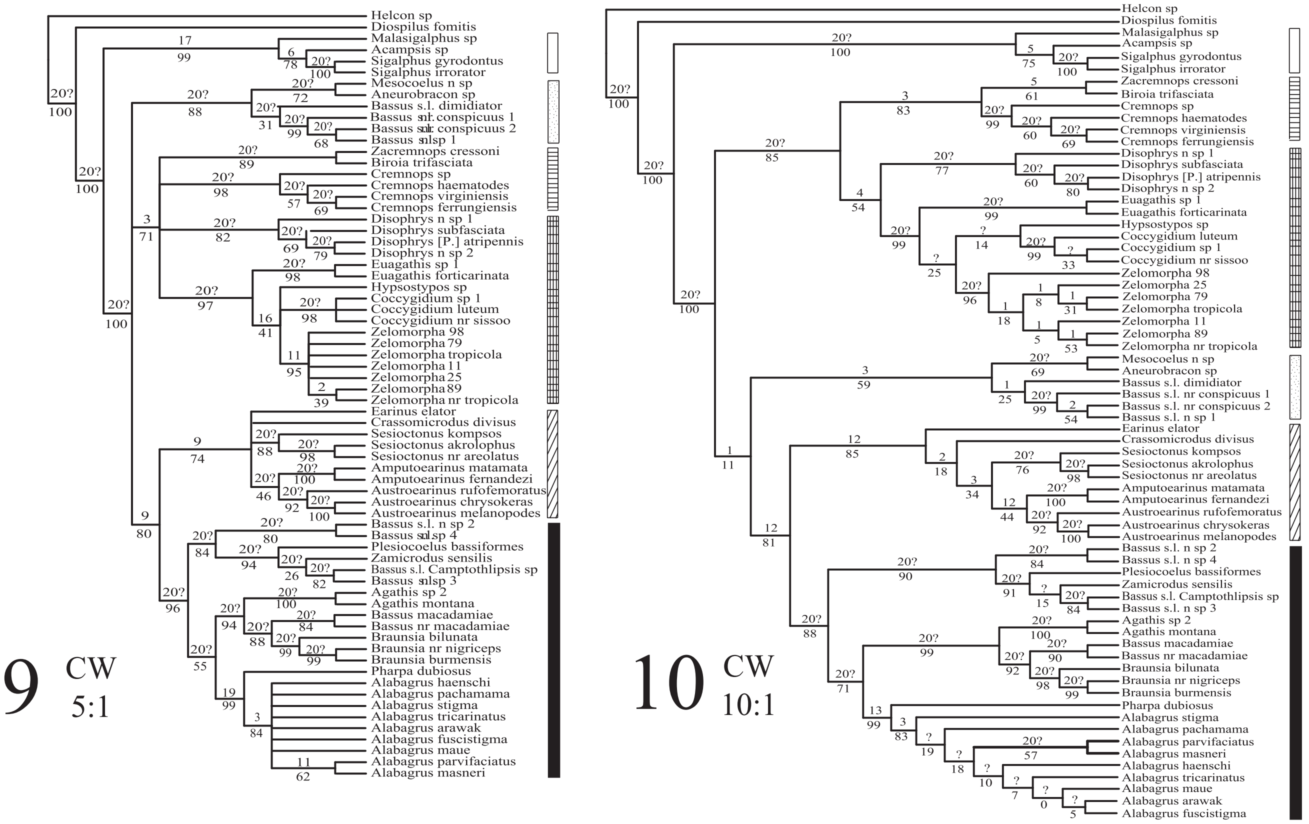
In this section we discuss character evolution and must therefore refer to tree topologies, to this effect a


6 CW 1:1


7 CW 2:1


8 CW 3:1

Figures 6–8. Strict consensus trees of all evidence, morphological plus molecular, based on Clustal W alignment with gap costs set at 1 : 1, 2 : 1, 3 : 1, respectively. Numbers above branches are bootstrap values, those below branches are Bremer support values. BARS: Sigalphinae ▭, Cremnoptini ▥, Disophrini ▦, n. tribe? ░, Earinini ▨, Agathidini ■.



Figures 9 and 10. Strict consensus trees of all evidence, morphological plus molecular, based on Clustal W alignment with gap costs set at 5 : 1 and 10 : 1, respectively. Numbers above branches are bootstrap values, those below branches are Bremer support values. BARS: Sigalphinae ▭, Cremnoptini ▥, Disophrini ▦, n. tribe? ▒, Earinini ▨, Agathidini ■.

simplified majority rule consensus tree with tribes as terminals, which was derived from the 10 DO and CW trees with different gap costs, is presented in Fig. 11. It includes a clade ((n. tribe) (Earinini + Agathidini)) that was found in only four of the 10 strict consensus source trees. The majority rule tree is presented to simplify discussion of the major clades. In the following discussions we note the degree to which the clades discussed were contradicted by the 10 strict consensus trees produced from the different gap cost parameters.

Wing vein reacquisition

Phylogenetic analyses within the Braconidae and across the Hymenoptera repeatedly demonstrate that the presence of tubular wing veins (veins innervated with trachea) is almost invariably the plesiomorphic condition relative to absence of veins. In other words, when veins are present or absent within a clade phylogenetic analyses usually show that absence is the derived condition. Of the taxa included in our analysis, *Earinus elator* Fabricius and two species of *Amputoearinus* have a complete and tubular Rs + Ma vein in the forewing. Mapping of the character onto any of the cladograms (Figs 1–10) shows that Rs + Ma was lost as part of the ground-plan of the Agathidinae and that it has reappeared one or two independent times. Despite the fact that this was not contradicted in any of the 10 strict consensus trees, we find it inconclusive. Considering that a complete Rs + Ma vein is present in many *Sesioctonus* species that are not exemplars in this analysis, that Rs + Ma is almost complete in some other Earinini, and that the basal branches of the Agathidini + Earinini clade have weak support, it is likely that the presence of Rs + Ma is part of the Agathidinae ground-plan and that it has been lost multiple times. Only a more detailed analysis will resolve this issue. The DO trees produced with gap costs of 1 : 2 and 1 : 10 (Figs 1 and 5) both place *Mesocoelus* and *Aneurobracon*, which have lost almost all major wing veins, in a grade at the base of the Agathidinae, making the reacquisition of most wing veins an equally parsimonious, though dubious, explanation for the remainder of the Agathidinae.

Wing venation reduction

The loss of wing venation is a character state that has appeared convergently many times within the Hymenoptera and this analysis shows the Agathidinae to be no

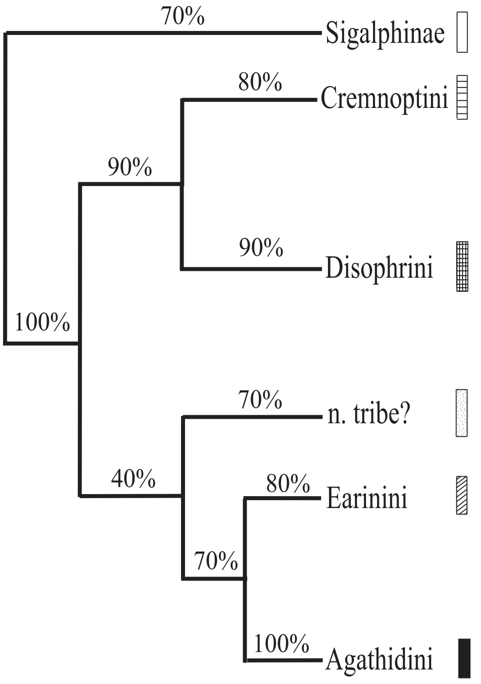


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Fig. 11. Simplified Majority Rule consensus of the 10 analyses (Figs 1–10) to illustrate tribal level phylogeny. The numbers indicate the frequency that the nodes were recovered in the source trees. It includes one branch found in only four of the 10 source trees. BARS: Sigalphinae ▭, Cremnoptini ▥, Disophrini ▦, n. tribe? ▒, Earinini ▨, Agathidini ■.

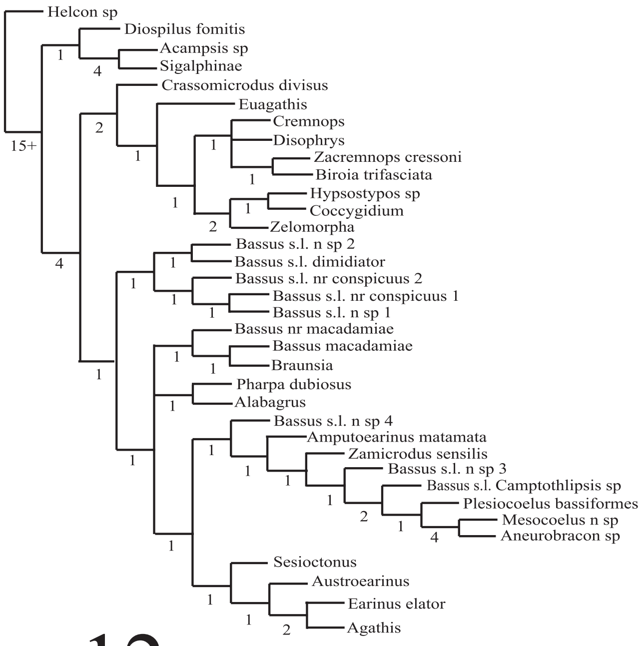

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Fig. 12. Simplified Strict consensus based on morphological data. The number of terminals was reduced with the following rule: if the monophyly of a genus was not contradicted it was represented by one terminal. Numbers below branches are Bremer support. The tribes are not indicated because of the high degree of scattering.

Table 1
The number of clades recovered in each of the DO and CW strict consensus trees

	POY (DO)					Clustal (CW)				
Gap cost	1 : 1	2 : 1	3 : 1	5 : 1	10 : 1	1 : 1	2 : 1	3 : 1	5 : 1	10 : 1
Number of nodes	52	48	40	48	46	60	58	61	48	57

POY (DO) average = 46.8, Clustal (CW) average = 56.8.

Table 2
The number of shared clades (components) in the 45 pair-wise comparisons among all of the DO and CW strict consensus trees

		POY (DO)				Clustal (CW)					
	Gap cost	2 : 1	3 : 1	5 : 1	10 : 1	1 : 1	2 : 1	3 : 1	5 : 1	10 : 1	Average
POY (DO)	1 : 1	33	31	26	23	36	35	37	35	35	32.3
	2 : 1		37	30	26	32	33	34	34	33	31.0
	3 : 1			30	24	30	31	33	32	32	29.8
	5 : 1				24	29	31	31	31	33	28.0
	10 : 1					26	27	27	26	26	28.8
Clustal (CW)	1 : 1						45	44	42	45	37.0
	2 : 1							53	44	46	38.8
	3 : 1								47	45	37.3
	5 : 1									45	37.8
	10 : 1										33.4

POY (DO) × POY (DO) average = 29, Clustal (CW) × POY (DO) average = 32, Clustal (CW) × Clustal (CW) average = 45.

exception. Forewing venation is reduced to the point that the Rs vein is not complete to the wing margin and the second cubital cell is absent in four terminal taxa included in this analysis, *Mesocoelus*, *Aneurobracon*, *Plesiocoelus* van Achterberg, and *Camptothlipsis* Enderlein. The results of most (six) analyses suggest that these

Table 3
Corrected number of shared clades in the 45 pair-wise comparisons among all of the DO and CW strict consensus trees. Corrections were made by dividing the number of components recovered by the maximum that could be recovered, i.e., the number found in that tree in each pair-wise comparison with the fewest components

		POY (DO)				Clustal (CW)					
	Gap cost	2 : 1	3 : 1	5 : 1	10 : 1	1 : 1	2 : 1	3 : 1	5 : 1	10 : 1	Average
POY (DO)	1 : 1	69	78	54	50	69	67	71	73	67	66.4
	2 : 1		93	63	57	67	69	71	71	69	70.9
	3 : 1			75	60	75	65	83	80	80	70.6
	5 : 1				52	60	65	65	65	69	60.3
	10 : 1					57	59	59	57	57	62.2
Clustal (CW)	1 : 1						78	73	88	79	71.4
	2 : 1							91	92	81	76.2
	3 : 1								98	79	78.1
	5 : 1									94	75.0
	10 : 1										70.1

POY (DO) × POY (DO) average = 63, Clustal (CW) × POY (DO) average = 72, Clustal (CW) × Clustal (CW) average = 84.

Table 4
The number of taxa in the pruned Nelson trees generated by using the source trees of each of the 10 analyses in 45 pair-wise comparisons

		POY (DO)				Clustal (CW)					
	Gap cost	2 : 1	3 : 1	5 : 1	10 : 1	1 : 1	2 : 1	3 : 1	5 : 1	10 : 1	Average
POY (DO)	1 : 1	34	34	27	30	41	33	35	34	32	33.3
	2 : 1		52	36	38	34	42	45	41	42	40.4
	3 : 1			36	35	34	42	44	41	41	39.9
	5 : 1				29	25	37	37	32	36	32.8
	10 : 1					41	35	35	30	35	34.2
Clustal (CW)	1 : 1						39	39	32	38	35.9
	2 : 1							30	42	52	42.4
	3 : 1								44	53	43.6
	5 : 1									41	37.4
	10 : 1										41.1

POY (DO) × POY (DO) average = 35, Clustal (CW) × POY (DO) average = 37, Clustal (CW) × Clustal (CW) average = 44.

reductions took place on three separate occasions. In some (four) trees *Mesocoelus* and *Aneurobracon* were not recovered as sister groups, but the members of the two genera are so similar morphologically we doubt these results.

Ovipositor length

The ovipositor of most species of Agathidinae is usually longer than the metasoma, indicating that they attack larvae that are hidden inside plant tissues such as stems or leaf-rolls. Short ovipositors, on the contrary, suggest that exposed hosts are targeted. Short ovipositors are found in the Sigalphinae, which appears in this analysis as the sister group of the Agathidinae. The results of our analyses do not resolve the question of the ground-plan state for the Agathidinae. The short ovipositor state is scattered throughout the tree, being found in the Disophrini, which is monomorphic for the state, in *Crassomicrodus divisus* Cresson, and in the two included species of *Amputoearinus*, but is not clear if these are the result of a series of changes to a long ovipositor, convergent gains, or a combination of both. There are only a few parsimonious solutions on most trees (e.g., Fig 8) but because they rely on the position of a number of clades that are poorly supported, we offer no further conjecture here.

Elongate mouthparts

Our results show that this characteristic, which is found in only a few other braconid subfamilies (see Jervis, 1998; for a review), has evolved multiple times within the Agathidinae, i.e., in *Disophrys* Förster, in Cremnoptini, in *Agathis s.s.*, and in a few North American members of *Bassus s.s.* Members of *Agathirsia* Westwood were not included in this analysis, but based on morphological characteristics they are almost certainly sister to *Crassomicrodus* Ashmead. Not all members of *Agathirsia* have elongate mouthparts; however, the longest mouthparts in the Ichneumonoidea are found in this genus. This manifestation of long mouthparts is also likely to be independently derived in members of *Agathirsia*.

Taxonomic considerations

Subfamily level

The monophyly of the Agathidinae is well supported with a bootstrap or relative support value of 100 in all trees and a Bremer support value of 20+ in all CW trees. There have been no pretenders for inclusion in, or exclusion from, the Agathidinae other than *Mesocoelus* and *Aneurobracon*. The original placement of these genera was in the Agathidinae but van Achterberg (1990) proposed the tribe Mesocoelini for these two genera and transferred them to the Orgilinae. Sharkey (1986) argued against this change citing morphological synapomorphies. These genera are well nested within the Agathidinae in most analyses; however, DO results were ambiguous, sometimes placing them as a basal grade (Figs 2 and 5) or as part of a basal polytomy (Fig. 3) within the Agathidinae. Despite the fact that they are usually recovered as a derived clade within the Agathidinae, the van Achterberg hypothesis has not been refuted as members of the Orgilinae were not included in this study. The four exemplars of the subfamily Sigalphinae are shown to be monophyletic in all trees except three DO trees (Figs 2–4) but in these monophyly is not contradicted.

Tribal level

Sharkey (1992) proposed a tribal phylogeny and classification of the Agathidinae composed of the Agathidini, Cremnoptini, Disophrini, Earinini and Microdini. The monophyly and sister-group relationship of the Cremnoptini, and Disophrini is corroborated by all analyses except the DO analyses with gap costs of 5 : 1 (Fig. 4).

All results refute the monophyly of the Agathidini (*sensu* Sharkey, 1992). *Agathis* Latreille and *Crassomicrodus*, the only two members of the tribe included in the analysis, were not resolved as sister taxa in any of the analyses. The two species of *Agathis* in the analysis were well nested within a group of Microdini (*sensu* Sharkey, 1992) in all analyses (Figs 1–10) and there is little question that tribal rank is not justified. We therefore follow Simbolotti and van Achterberg (1999), and treat the Microdini as a junior synonym of Agathidini, hereafter referred to as Agathidini *s.l.*

The Earinini is recovered in eight of the 10 source trees with the inclusion of *Crassomicrodus*. Both *Crassomicrodus* and *Earinus* are missing large portions of the D3 region of 28S, about 250 base pairs, in this analysis, and the missing data may be the reason that Earinini is not recovered as monophyletic in all. Interestingly, the only two trees that do not recover a monophyletic Earinini were the DO and CW trees with gap costs of 1 : 1. In both of these trees *Earinus* was placed near the base of the tree.

The monophyly of the Agathidini *s.l.* is refuted in all analyses. This is because a clade, labeled as "n. tribe?" in Fig. 11, is consistently placed outside of the Earinini plus the remaining Agathidini (simply referred to as "Agathidini" in Fig. 11). This entire clade, Earinini + Agathidini *s.l.*, was referred to by Simbolotti and van Achterberg (1999) as the Agathidini. However, this clade was only recovered in four of the 10 source trees (Fig. 11). In the three other trees that recovered the clade labeled "n. tribe" it was placed as sister to the Cremnoptini + Disophryini (Figs 1 and 6) or in an unresolved trichotomy with Cremnoptini + Disophryini and the remaining Agathidini (Fig. 9). (Note: again we see a similarity between the DO and CW trees with gap costs of 1 : 1 that is not found in any other trees.) As Agathidini, in the wide sense proposed by Simbolotti and van Achterberg, is poorly supported, we hesitate to recommend its recognition.

The other way to treat the clade, Agathidini, Earinini and "n. tribe?", is to formally propose a new tribe for those members of Agathidini found to be in the clade labeled "n. tribe" (Fig. 11). This group is found in seven of the 10 source trees. The members are part of the Agathidini *s.l.* as it is here defined. Close examination of the included exemplars shows that they share a few distinct but primitive morphological features not found in any of the other exemplars used in this study. The six exemplar species all have a very deep depression on the posterior margin of the scutellum, the postscutellar depression; and vein Cub of the hind wing is tubular in all exemplars, though in *Mesocoelus* and *Aneurobracon*, it is represented by a small stub. In four of the five CW trees *Mesocoelus* + *Aneurobracon* is the sister to the remaining members of "n. tribe?" and it might be that they deserve tribal status as well. Morphologically, members of these two genera are very similar and quite unlike all other agathidines. The ambiguity illustrated above indicates to us that a more thorough analysis should be conducted, with denser taxon sampling in the Agathidini *s.l.*, before formal changes to the tribal classification are proposed.

In summary, our only formal proposal is that the Agathidini *s.s.* (Sharkey, 1992) should be considered part of the Agathidini *s.l.*, even though the latter is clearly polyphyletic.

Generic level

Many genera in this analysis were represented by only one or a few species; therefore, this study is not a strong test of monophyly for many of them. Some of these taxa were included simply to test the monophyly of other genera. In the following paragraphs, the included genera are treated in alphabetical sequence and several new combinations and synonyms are proposed.

Agathis *Latreille*

Included in the analysis were two species, *A. Montana* Shestakov and an undescribed species, *Agathis*. n. sp. The former is from Turkey and the new species was collected in Costa Rica. Interestingly, members of the species from Costa Rica do not have the typical *Agathis* characters of elongate genae and elongate mouthparts. The only indications of an affinity with *Agathis* are the sculpture of the propodeum, the excavated occiput, and the open hind coxal cavities. As these two species are at the extreme end of the morphological and geographic spectrum of *Agathis*, as there are no other nominal genera as candidates for inclusion in *Agathis*, and as they were recovered as sister species in all analyses except for the DO analysis with a gap cost of 10 : 1 (Fig. 10), which is equivocal, we consider the monophyly of *Agathis* to be well corroborated. The most reliable characters to diagnose members of *Agathis* are as follows: the reduction of the propodeal carinae to two or three medial longitudinal carinae and sometimes an anterior transverse carina; penultimate labial palpomere well developed, more than half length of terminal palpomere; occiput moderately to greatly excavated; labrum with convex anterior surface; hind coxal cavities open (contiguous with the metasomal foramen) or closed by a very narrow sclerite.

Alabagrus *Enderlein*

The monophyly of *Alabagrus* and its sister-group relationship with *Pharpa* Sharkey, as proposed by Sharkey (1988), were corroborated in all analyses.

Amputoearinus *Sharkey*

A sister-group relationship with *Austroearinus* was recovered in all CW trees and in one DO tree (Fig. 1). This relationship was not found on the morphological tree but the two genera do share the character state of lacking notauli. Monophyly of the genus is supported in all analyses with high support values. Placement in the Earinini is suggested in all trees except the DO and CW trees with gap costs of 1 : 1 (Figs 1 and 6).

Aneurobracon *Brues*

A sister-group relationship with *Mesocoelus* is suggested in four of the five CW trees but in none of the DO trees. Based on the many morphological synapomorphies (Fig. 12) we have little doubt of this relationship. *Contra* van Achterberg (1990) this clade is distantly related to *Plesiocoelus*. All three genera have reduced wing venation but the reductions are convergent in the two clades, *Aneurobracon* + *Mesocoelus*, and *Plesiocoelus*. The POY analyses always placed *Aneurobracon* and *Mesocoelus* close to each other but never as sister groups. *Plesiocoelus* is placed in distant clades in these analyses as well. Three of the POY analyses placed *Aneurobracon* and *Mesocoelus* at the base of the Agathidinae phylogeny, twice as a grade (Figs 2 and 5) and once as part of a basal polytomy (Fig. 3). Curiously these placements occurred with non-sequential gap costs, i.e., 2 : 1, 3 : 1 and 10 : 1. With gap costs of 1 : 1 and 5 : 1 the placement was similar to that of the results generated by the CW analyses; that is, within the clade labeled “n. tribe?”.

Austroearinus *Sharkey*

Monophyly is strongly supported with high support values in all analyses. See Discussion under *Amputoearinus*.

Bassus *Fabricius*

All results show that the present generic concept of *Bassus* is polyphyletic. On morphological grounds, the type species of *Bassus*, *Bassus calculator* Fabricius, belongs to the clade composed of *Bassus macademiae* Briñceo and Sharkey and *Bassus* nr. *macademiae*. The option of referring to this restricted clade as *Bassus* (hereafter referred to as *Bassus s.s.*) and reassigning the other three clades that contain members of *Bassus* in this analysis would require large-scale nomenclatorial changes and would leave many members of *Bassus* unplaced. *Bassus* should be densely sampled before major taxonomic changes are made.

Members of *Bassus s.s.* have a strong transverse carina between the hind coxal cavities and the metasomal foramen, and many have striate sculpture on the first to third metasomal median tergites. Both character states are shared with *Braunsia* Kriechbaumer and allies. *Camptothlipsis* was synonymized under *Bassus* by Simbolotti and van Achterberg (1992). One species that we included in the analysis, *Bassus s.l.* (*Camptothlipsis*) sp., fits within the concept of *Camptothlipsis* and all of our results show it nesting in a group of *Bassus s.l.*, in agreement with their decision. Of course, just about all nominal genera that are not members of Cremnoptini and Disophrini nest within *Bassus s.l.* so it is possible that there will be justification for the recognition of *Camptothlipsis* with a more complete analysis. We have retained the name *Camtothlipsis* here to simplify viewing the cladograms and writing the results section, but we are agnostic about its proper status.

We take this opportunity to synonymize *Aerophilus* Enderlein (1914) with *Bassus* and transfer *Aerophilus szepligetii* Enderlein to *Bassus*, *Bassus szepligetii* **n. comb.** This species has a strong transverse carina between the hind coxal cavities and the metasomal foramen, a structure unique to *Bassus s.s.* and the Old World *Braunsia* and allies. The genus *Faciliagathis*, recently described by van Achterberg and Chen (2004) also fits within the concept of *Bassus s.s.* with a strong transverse carina between the hind coxal cavities and the metasomal foramen, and it too is synonymized with *Bassus* **n. syn**. The two species included in *Facilagathis*, *F. spinulata* van Achterberg & Chen and *F. macrocentroides* van Achterberg & Chen are transferred to *Bassus*, *Bassus spinulatus* **n. comb**. and *Bassus macrocentroides* **n. comb.**

Biroia *Szépligeti*

The type species of *Biroia*, *B. elegans* Szépligeti, is congeneric with that of *Isoptronotum* Enderlein, *I. taeiocauda* Enderlein. Both nominal genera have rather elongate ovipositors, excluding them from the Disophrini, and their mesoscuta are smooth and lack notauli. *Isoptronotum* Enderlein (1920) is here synonymized under *Biroia* Szépligeti (1900) **n. syn.** The many New World species previously included in *Biroia* are members of *Zelomorpha s.s.*; all have short decurved ovipositors, and their formal synonymies are forthcoming (Sarmiento in prep.). All CW analyses the DO analyses with 1 : 1 and 2 : 1 gap costs recovered a sister-group relationship between the Paleotropical genus *Biroia* and the Neotropical genus *Zacremnops* Sharkey and Wharton. Where it was recovered support values were high, and the alternative placements in DO analyses with high gap costs were not well supported. The sister-group relationship was also recovered in the morphological analysis; therefore, the relationship appears to be well supported.

Braunsia *Kriechbaumer*

Braunsia is the sister group of *Bassus s.s.* This relationship is well supported in all analyses (Figs 1–10), and it is supported on morphological grounds by a synapomorphic character state that is unique within the Agathidinae; the hind coxal cavities are separated from the metasomal foramen by well developed sclerite that has a strong transverse ridge. Several nominal genera, *Metriosoma* Szépligeti, *Lissagathis* Cameron, *Laccagathis* Watanabe, and *Pholeocephala* van Achterberg, which were not included in this analysis, are likely to be derived members of *Braunsia*, at least there is no morphological evidence supporting the monophyly of *Braunsia* without their inclusion. *Metriosoma* and *Lissagathis* are similar to most members of *Braunsia* but lack striate sculpture on the metasoma. *Laccagathis* and *Pholeocephala* appear to have somewhat exaggerated *Braunsia* characters states. None of these four genera has been justified with phylogenetic rationale and in total they constitute five species; all but *Laccagathis* are monotypic. As these nominal genera form a well supported monophyletic group with *Braunsia*, based on morphological characters, we synonymize the four genera here. *Laccagathis* Watanabe, junior synonym of *Braunsia* Kriechbaumer **n. syn.** *Lissagathis* Cameron junior synonym of *Braunsia* Kriechbaumer **n. syn**. *Metriosoma* Szépligeti junior synonym of *Braunsia* Kriechbaumer **n. syn**. *Pholeocephala* van Achterberg junior synonym of *Braunsia* Kriechbaumer **n. syn.**

Coccygidium *Saussure*

The concept of *Coccygidium* has been treated in different ways. Van Achterberg and Maetô, (1990) placed those species with a relatively short fore tibial spur in *Zelomorpha* (hereafter referred to as *Zelomorpha s.l.*), and included in *Coccygidium s.s.* those species with an obvious morphological autapomorphy in the form of an elongate fore tibial spur. Because of the lack of autapomorphies for *Zelomorpha s.l.*, Chou and Sharkey (1989) synonymized *Zelomorpha s.l.* under *Coccygidium*.

The monophyly of *Coccygidium s.l.* (*sensu* Chou and Sharkey, 1989), which includes species here placed in *Zelomorpha s.s.*, *Hypsostypos* Baltazar and *Coccygidium s.s.*, was found in only six of the 10 analyses but few show high support. In the remaining four analyses (DO 10 : 1, CW 1 : 1, 2 : 1, 3 : 1) *Coccygidium s.l.* is paraphyletic with respect to *Euagathis* Szépligeti.

Zelomorpha s.l. includes both New World and Old World forms, and this grouping is not supported by our data. Our analyses support monophyly of two clades within *Zelomorpha s.l.* In the Old World all of the Zelomorpha-like species, those with relatively short tibial spurs, have two apparent synapomorphies; i.e., lack of lateral carinae on the frons, and rugose sculpture on the ventral surface of the hind femur. The Old World members of *Zelomorpha s.l.* are restricted to the East Palearctic, Oriental, and Australian realms. The New World species of *Zelomorpha s.l.* are primarily Neotropical with several species occurring in the southern Nearctic region and there are no apparent morphological autapomorphies. Our analyses support three monophyletic groups within *Coccygidium s.l.* As the monophyly of each of these has a much higher support value than that of *Coccygidium s.l.*, due to the variable placement of *Euagathis*, we choose to recognize each of these at the generic level. *Zelomorpha s.s.* is restricted to all New World members formerly placed in *Coccygidium*, *Zelomorpha* and *Biroia*. *Coccygidium s.s.* is restricted to Old World species with long fore tibial spurs, and the concept of the genus *Hypsostypos* is expanded to include Old World species with relatively short fore tibial spurs and which also lack lateral carinae on the frons. *Hypsostypos* in this wider sense includes

Table 5
Synopsis of genera formerly placed in *Coccygidium s.l.*

Genus	Distinguishing characters	Distribution
Zelomorpha	frons bordered with carinae, short fore tibial spurs	New World, primarily Neotropical
Coccygidium	frons bordered with carinae, long fore tibial spurs	Widely distributed in warm and warm-temperate regions of the Old World
Hypsostypos	frons not bordered with carinae, short fore tibial spurs	East Palearctic, Oriental, and northern Australian realms

the *Sulana* species group of *Zelomorpha* (Bhat and Gupta, 1977).

Coccygidium s.s., is supported in four of the five DO analyses, and in four of the five CW analyses. Interestingly, the two analyses that did not support the relationship both had gap costs of 3 : 1. Table 5 summarizes the distinguishing characteristics and distributions of each genus formerly placed in *Coccygidium s.l.*

Crassomicrodus *Ashmead*

Large portions of the D3 region of 28S are missing for the only exemplar, *C. divisus*. Sharkey (1992) placed *Crassomicrodus* in Agathidini *s.s.*, but none of our analyses placed it close to *Agathis*. Eight of the 10 trees recovered a monophyletic Earinini. The exceptions were the two analyses with gap costs of 1 : 1, which placed *Earinus* near the base of the Agathidinae. In the eight cases where Earinini was recovered *Crassomicrodus* was included. *Crassomicrodus* and its probable sister group, *Agathirsia*, are rather aberrant agathidines. The only obvious morphological character that they share with members of the Earinini is the lack of wide sclerites separating the metasomal foramen and the hind coxal cavity cavities. *Crassomicrodus* and *Agathirsia* have well developed notauli, unlike all other members of Earinini, which lack notauli. In three of the four CW trees (Figs 6–8) *Crassomicrodus* was placed as the sister group to the remaining Earinini, and from a morphological perspective this seems reasonable. Denser sampling of *Crassomicrodus* and its putative sister-genus, *Agathirsia*, is necessary, but both of these genera are tentatively placed in Earinini.

Cremnops *Förster*

Monophyly of the genus is corroborated in all analyses. Two candidates for membership in *Cremnops*, i.e., *Biroia* and *Zacremnops*, were included in the analysis and their addition did not refute monophyly of the genus. To fully test the monophyly of *Cremnops* other putative members of the Cremnoptini, i.e., *Megagathis* Kriechbaumer, *Mesoagathis Cameron*, *Labagathis* Enderlein, *Hyrtanommatium* Enderlein, and *Cremnoptoides* van Achterberg & Chen, must be investigated.

Disophrys *Förster*

Disophrys, with the inclusion of the monotypic genus *Pseudocremnops* Szépligeti, was recovered as monophyletic in all analyses. *Pseudocremnops*, labeled as *Disophrys [P.] atripennis* on the cladograms, is shown to be a derived member of *Disophrys* in all analyses. Therefore, we here synonymize *Pseudocremnops* under *Disophrys* **n. syn.**, *Disophrys atripennis* (Szépligeti) **n. comb**. Eight of the 10 analyses placed *Disophrys* as the sister group of the remaining Disophrini, and of the two that did not show this relationship one was equivocal. The dissenting analyses were the DO and CW analyses with gap costs of 5 : 1.

Earinus *Wesmael*

Only one species was included in the analysis, so monophyly was not tested. The sole exemplar of the genus, *E. elator*, is lacking about 250 base pairs of the D3 region. In future studies it would be interesting to include representatives of species of *Earinus* from austral South America (Berta, 2000). In most analyses *Earinus* nests with other members of the Earinini.

Euagathis *Szépligeti*

Only two exemplars were included so monophyly was only weakly tested, but morphological uniformity and several distinct morphological synapomorphies, such as the shape of the vertex and the lack of lateral carinae on the frons also suggest monophyly of the group. All analyses recovered a monophyletic *Euagathis*. The placement of *Euagathis* within the Disophrini was diverse; in five of the 10 analyses (Figs 1–3, 9 and 10) it was recovered as sister to (*Coccygidium* + *Zelomorpha* + *Hypsostypos*), but other analyses it was found nested within this clade.

Hypsostypos *Baltizar (*Figs 13 and 14)

To date *Hysostypos* has been monotypic, with only the type species, *H. rugifrons* included. It has been recognized by what appears to be a distinct autapomorphy, large paired lamellae between the antennal insertions (Fig. 14b). The senior author (MJS) has three other species in his collection that possess similarly large

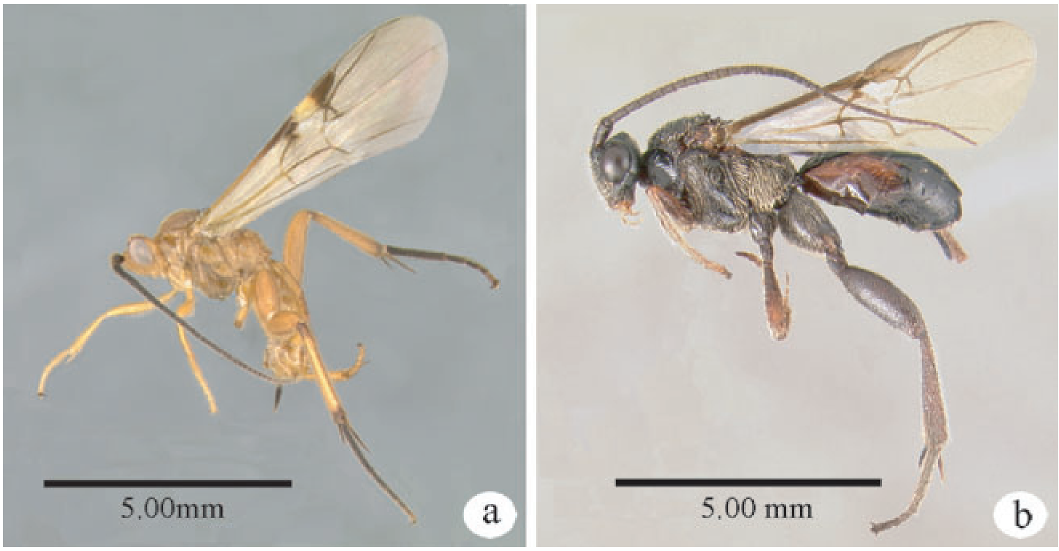

Fig. 13. Lateral habitus photographs. (a) *Hypsostypos concolor*. (b) *Hypsostypos* sp.

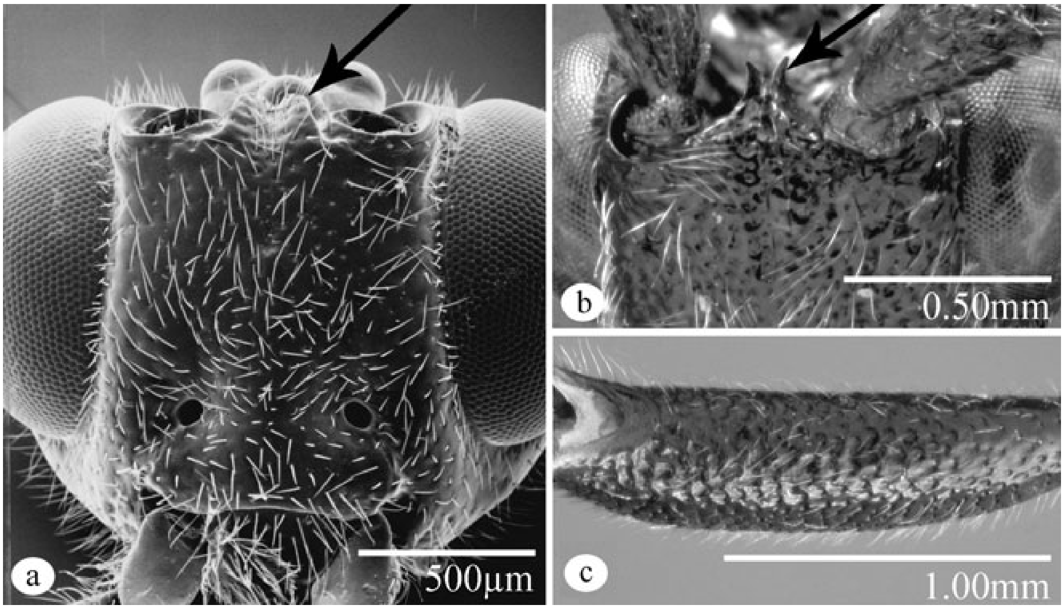

Fig. 14. (a) *Hypsostypos varipes*, anterior head showing weak paired projections between antennal insertions. (b) *Hypsostypos* sp. anterior head showing strong paired projections between antennal insertions. (c) *Hypsostypos varipes*, ventral hind femur, showing rugose sculpture.

lamellae. These species share another rare character state with *H. rugifrons* in that the metapleuron is covered with a dense mat of setae (Fig. 13b). These species, three of which are not described, undoubtedly constitute a monophyletic group. However the concept of *Hypsostypos* is expanded here to include many Old World species formerly included in *Zelomorpha s.l.* This group of *Zelomorpha s.l.* is equivalent to the *Sulana* species group as defined by Bhat and Gupta (1977).

In the following discussion we will refer to *Hysostypos s.s.* for the group with members possessing large lamellae between the antennae and matted metapleura, and the *Sulana* species group for the species formerly placed in *Zelomorpha*. Members of the *Sulana* species group share two synapomorphic character states with those of *Hypsostypos s.s.* The frons is not margined with lateral carinae, and the ventral surface of the hind femur is rugose (Fig. 14c).

These character states are hypothesized to be synapomorphies for the entire clade, i.e., *Hypsostypos s.l.* Though some species of *Coccygidium* and *Zelomorpha* have rugose or rugosopunctate sculpture on the ventral surface of the hind femur, the state is rare in these groups. We predict that more detailed phylogenetic studies will show that this state is independently derived. Members of the *Sulana* species group do not possess strong lamellae between the antennal insertions (Fig. 14a, compare with. Fig. 14b).

Maintaining the concept of *Hypsostypos s.s.* and proposing a new genus for the *Sulana* species group would leave the latter concept devoid of autapomorphic character states. The only phylogenetically consistent solution is to adopt a wide concept for *Hypsostypos*. Because the concept of *Hypsostypos* is new, a complete description is given in the descriptive section of this paper.

Isoptronotum *Enderlein*

Junior synonym of *Biroia* Szépligeti **n. syn**. See Discussion under *Biroia*.

Mesocoelus *Schulz*

See comments under *Aneurobracon*.

Plesiocoelus *van Achterberg*

Contra van Achterberg (1990), members of this group are not closely related to *Aneurobracon* and *Mesocoelus*, but rather are derived within one of the *Bassus s.l.* clades. It is doubtful that this small clade merits generic status but a more detailed investigation of *Bassus s.l.* should precede a taxonomic decision.

Pharpa *Sharkey*

One exemplar was included but due to the limited morphological variation, the genus is likely monophyletic. A sister group relationship with *Alabagrus* was recovered in all analyses.

Pseudocremnops *Szépligeti*

Junior synonym of *Disophrys* Förster. See Discussion under *Disophrys*.

Sesioctonus *Viereck*

Three species were included in the analysis and the genus was recovered as monophyletic in all but one analysis (Fig. 5). The position of *Sesioctonus* within the Earinini was variable.

Zacremnops Sharkey and Wharton

Only one exemplar of this rather monomorphic Neotropical genus was included and a sister group relationship with the Paleotropical genus *Biroia* is strongly supported, see Discussion under *Biroia*.

Zamicrodus *Viereck*

Zamicrodus sensilis Viereck has been the only included species; *Agathis laeta* Brullé is transferred here to *Zamicrodu*s, *Zamicrodus laetus* **n. comb.** These two nominal species are nearly identical morphologically and they are very similar in color; they probably represent one species, but we leave this decision to a future reviser. This very distinctive genus is nested within a well supported clade comprised of a number of exemplars assigned to *Bassus s.l.* as well as *Plesiocoelus* and *Bassus s.l.* (*Camptothlipsis*) sp. There are no obvious morphological synapomorphies for this more inclusive clade suggesting that the eventual splitting of *Bassus s.l.* into monophyletic groups may have to be rather comprehensive.

Zelomorpha *Ashmead*

Zelomorpha s.s. refers to New World members of *Coccygidium s.l.* See Discussion under *Coccygidium*. Sarmiento (in prep.) is currently revising the species of *Zelomorpha*. Recently, a new species was described by Sarmiento et al. (2004), as *Coccygidium gregarium*. This differs from other described New World species in that the larvae are gregarious, the lateral carinae on the frons are weak, and the ventral surface of the hind femur is rugose. Current studies by Sarmiento (in prep.) will determine the phylogenetic placement of this species and several others that have recently been discovered with the same characteristics. Owing to the rugose ventral surface of the hind femur and the weak carinae of the frons, the gregarious species may prove to be sister to *Hypsostypos*. As mentioned in Sarmiento et al. (2004), *Hemichoma* Enderlein is likely to be found to be a junior synonym of *Zelomorpha s.s.* but this is being dealt with by Sarmiento (in prep.).

Descriptive section

Hypsostypos Baltizar 1963 (Figs 13 and 14)

Note: This genus is redescribed because the concept presented here expands the currently accepted limits. Type species: *Agathis rugifrons* Smith.

Diagnosis

Members of *Hypsostypos* may be distinguished from all other agathidine genera with the following combination of characters: tarsal claws cleft; ventral surface of hind femur rugose (Fig. 14c), foretibial spur not elongate, ½ to ¾ length of basitarsus; ovipositor shorter than half length of metasoma (Fig. 13), frons lacking lateral carinae; rostrum not elongate; trochantellus with pair of carinae.

Description

Head

Lateral carina of frons lacking; interantennal space usually with two prominences separated by shallow groove; these can be very elongate or not (Fig. 14a,b); frons not bordered by carinae laterally; antennal insertion bordered anteriorly, medially, and laterally by elevated ridge; gena not extended ventroposteriorly into sharp prominence; labial palp with four segments, third segment not reduced, more than half length of apical segment; apical antennomere acute.

Mesosoma

Mesoscutum with sculptured notauli; posteroscutellar depression absent; median areola of metanotum well defined with sharp carinae laterally and posteriorly; metapleuron with dense mat of setae or not (Fig. 13a,b); propodeum areolate carinate; posterolateral corners of propodeum elongate; propleuron mildly convex to flat; propodeal pseudosternite well developed, separating hind coxal cavities from metasomal foramen.

Legs

Foretibial spur not elongate, from ½ to ¾ length of basitarsus; foretibial spur with setae extending to its apex or nearly so; foretibia lacking pegs; tarsal claws bifid; midtibia with apical pegs but lacking pegs at midlength; hind femur rugose ventrally (Fig. 14c); hind tibia with 2 apical pegs, posterior peg larger than anterior peg.

Wings

Rs + Ma vein incomplete and not tubular throughout; second submarginal cell triangular and sessile; 3RSb straight to slightly sinuate; hind wing crossvein r absent; hind wing crossvein r-m weakly indicated as a short nebulous or spectral thickening, i.e., as depressed line that may or may not be pigmented, near the base of Rs; hind wing Cub present as nebulous or spectral vein.

Metasoma

All terga smooth, lacking sculpture; median tergite 1 lacking pair of longitudinal carinae; median syntergite 2 + 3 lacking transverse depression separating tergites 2 and 3 or with depression barely indicated; ovipositor, decurved, shorter than half the length of the metasoma when fully extended (Figs 13a.b).

Distribution

Oriental, East Palearctic and Northern Australian, from Korea and Japan south to Northern Australia and west to India. Van Achterberg (pers. comm.) also reports this group from North Africa.

Included species

Hypsostypos albipilosellum Cameron **n. comb.**, *Disophrys albipilosellus* Cameron

Hypsostypos chromopterum Roman **n. comb.**, *Euagathis chromoptera* Roman

Hypsostypos concolor (Szépligeti) **n. comb.**, *Disophrys concolor* Szépligeti [= *Coccygidium nihonense* Sharkey, **n. syn**]

Hypsostypos cordatum (Bhat and Gupta) **n. comb.**, *Zelomorpha cordata* Bhat and Gupta

Hypsostypos dilutum (Turner) **n. comb.**, *Disophrys diluta* Turner

Hypsostypos dravidum (Bhat and Gupta) **n. comb.**, *Zelomorpha dravida* Bhat and Gupta

Hypsostypos exornatum (Turner) **n. comb.**, *Disophrys exornata* Turner

Hypsostypos longidorsatum (Bhat and Gupta) **n. comb.**, *Zelomorpha longidorsata* Bhat and Gupta

Hypsostypos nigrum (Bhat and Gupta) **n. comb.**, *Zelomorpha nigra* Bhat and Gupta

Hypsostypos philippinense (Bhat and Gupta) **n. comb.**, *Zelomorpha philippinensis* Bhat and Gupta

Hypsostypos quadrifossulatum (Enderlein) **n. comb.**, *Euagathis quadrifossulata* Enderlein

Hypsostypos ruidum (Sharkey) **n. comb.**, *Coccygidium ruidum* Sharkey

Hypsostypos simile (Bhat and Gupta) **n. comb.**, *Zelomorpha similis* Bhat and Gupta

Hypsostypos sulanum (Enderlein) **n. comb.**, *Euagathis sulana* Enderlein

Hypsostypos varipes (van Achterberg and Maetô) **n. comb.**, *Zelomorpha varipes* van Achterberg and Maetô.

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Appendix 1 Morphological character list

1. Length of third labial palpomere. 1. absent or reduced (less than half as long as both palpomeres 2 and 4). 2. subequal in length relative to palpomeres 2 and 4.

2. Length of labio-maxillary complex. 1. of normal proportions, galea shorter than mandible. 2. elongate, galea longer than mandible.

3. Lateral carinae of frons. 1. present. 2. absent.

4. Sculpture between antennae. 1. two carinae. 2. one or no carinae

5. Posterior area of vertex. 1. excavated. 2. not excavated

6. Apical flagellomere shape. 1. blunt. 2. acute. 3. with apical nipple.

7. Presence of basal lobe on foreclaw. 1. present. 2. absent.

8. Shape of basal lobe of foreclaw. 1. small, sharp but not curved. 2. quadrate. 3. curved and sharp (claws bifid, cleft).

9. Size of basal tooth of hind claw (cleft claws only). 1. small or absent. 2. normal.

10. Long setae at apex of spur of foretibia. 1. present. 2. absent.

11. Non-apical spines on midtibia. 1. present. 2. absent.

12. Hind trochanter. 1. elongate. 2. not elongate.

13. Prominence (swelling) of propleuron. 1. present. 2. absent.

14. Notauli. 1. present. 2. absent.

15. Notauli sculpture. 1. present. 2. absent.

16. Post-scutellar depression. 1. present. 2. absent.

17. Propodeal sculpture. 1. with 1–3 closely aligned median longitudinal carinae, anterior transverse carinae present or absent, posterior transverse carinae always absent. 2. areolate posterior transverse carina present. 3. two median longitudinal carinae bordering a spindle-shaped area. 4. lacking macrosculpture. 5. scattered rugae.

18. Size of medio-posterior areola of propodeum. 1. large. 2. normal

19. Rugose sculpture of propodeum. 1. present. 2. absent.

20. Coriarious sculpture of propodeum. 1. present. 2. absent.

21. Hind coxal cavities. 1. open, sharing common foramen with metasoma. 2. closed, separated from metasoma.

22. Elevated ridge between hind coxal cavities. 1. present. 2. absent

23. Longitudinal ridge of setae of hind basitarsus. 1. present. 2. absent.

24. Longitudinal carinae of hind trochantellus. 1. present. 2. absent.

25. Forewing vein 1Rs + M. 1. complete. 2. incomplete.

26. Last abscissa of RS of forewing. 1. curved towards wing apex. 2. straight. 2. curved towards anterior wing margin.

27. Last abscissa of Rs of forewing. 1. complete. 2. incomplete.

28. Marginal cell. 1. long and narrow. 2. normal.

29. Forewing vein 2Rs2. 1. present. 2. absent.

30. Second cubital cell. 1. wider than long. 2. not wider than long

31. Rs and r-m veins of forewing. 1. converging anteriorly. 21. not converging anteriorly square or rectangular

32. Second cubital cell of forewing. 1. present. 2. absent.

33. Last abscissa of Cu of hind wing. 1. contiguous with penultimate abscissa of Cu. 2. not contiguous with penultimate abscissa of Cu or absent.

34. 2r-m of hind wing. 1. complete (as an unsclerotized vein). 2. incomplete. 3. absent.

35. Pair of longitudinal carinae on first metasomal tergum. 1. present. 2. absent.

36. Median longitudinal swelling of first metasomal tergum. 1. present. 2. absent.

37. Sculpture of first median tergite. 1. coriarious. 2. striate. 3. smooth. 4. granulostriate. 5. rugosostriate. 6. rugose.

38. Sculpture of second median tergite. 1. smooth. 2. coriarious. 3. striate. 4. granulostriate. 5. rugosostriate. 6. rugose.

39. Sculpture of third median tergite. 1. smooth. 2. striate. 3. coriarious. 4. granulostriate. 5. rugosostriate. 6. rugose.

40. Ovipositor shape. 1. short and decurved. 2. long and straight.

Appendix 2 Morphological matrix

	1	1111111112	2222222223	3333333334
	1234567890	1234567890	1234567890	1234567890
Outgroups				
Helconinae				
Helcon sp. (ROOT)	2122212--2	2221112212	2122121221	1123126512
Diospilus fomitis	21222321-2	2221112112	1222121222	2223126112
Sigalphinae				
Sigalphus gyrodontus	21112112-2	2221113212	1222121221	2111126661
Sigalphus irrorator	21122112-2	2221113212	1222121221	2111126661
Acampsis sp.	21222112-2	2221123212	1222121222	2123126551
Malasigalphus sp.	21122?2?-2	2221114?12	1222121221	2123126551
Agathidinae				
Agathidini (formerly Microdini)				
Pharpa dubiosus	11122212-2	1221222222	2222221212	1122123112
Alabagrus haenschi	11122212-2	1221222222	2222221222	1122213112
Alabagrus pachamama	11122212-2	1221222222	2222221222	1122213112
Alabagrus parvifaciatus	11122212-2	1221222222	2222221222	1122aa3112
Alabagrus stigma	11122212-2	1221222222	2222221222	112211de12
Alabagrus tricarinatus	11122212-2	1221222222	2222221222	1122213112
Alabagrus arawak	11122212-2	1221222222	2222221222	1122213112
Alabagrus masneri	11122212-2	1221222222	2222221222	1122213112
Alabagrus fuscistigma	11122212-2	1221222222	2222221222	1122213112
Alabagrus maue	11112212-2	1221222222	2222221212	1122213112
Braunsia bilunata	112a2212-2	1221222212	2122221212	1123122322
Braunsia burmensis	11222212-2	1221122212	2122221212	1123122322
Braunsia nr. *nigriceps*	11222212-2	1221221212	2122221212	1123122322
Plesiocoelus bassiformes	11222212-2	2211124111	22222-22??	?223221212
Mesocoelus n.sp.	1122222?-2	112a211-21	22222-22??	?22312ab12
Aneurobracon sp.	11222211-2	1121111221	22222?222-	-223221112
Zamicrodus sensilis	11222212-2	1211224?22	2222221222	1123223112
Bassus macadamiae	11222212-2	1221212222	2122221222	1123122cf2
Bassus nr. *macadamiae*	11222212-2	1221224122	2122221222	1123123312
Bassus (s.l.) n.sp.2	1a222212-2	1211122211	2222221222	1123122312
Bassus (s.l.) (*Camptothlipsis*) *sp.*	11222212-2	1211124?21	222222122-	-223221112
Bassus (s.l.) n.sp.3	11222212-2	1221124?22	222222122-	-223223112
Bassus (s.l.) *dimidiator*	11222212-2	1211112212	2222221222	1123222312
Bassus (s.l.) n.sp.4	11222212-2	1212-22212	2222221222	112322311?
Bassus (s.l.) nr. *conspicuus* 1	1121a211-2	1221112a12	1222211222	1113222312
Bassus (s.l.) nr. *conspicuus* 2	11222212-2	122121221a	2222211222	1113122112
Bassus (s.l.) n.sp.1	11122212-2	12?1112212	1222221212	1113222112

Appendix 2 *Continued*

	1	1111111112	2222222223	3333333334
	1234567890	1234567890	1234567890	1234567890
Agathidini (s.s.)				
Agathis montana	22221211-2	12211?1212	1222221222	1113125112
Agathis sp.2	11221211-2	1221221211	1222221222	1113125112
Crassomicrodus divisus	21212213-2	?221122212	2222211222	1123223111
Cremnoptini				
Cremnops virginiensis	2211121322	2221222212	22212112a2	212122311a
Cremnops ferrungiensis	2211221322	2221122222	22212112a2	212122311a
Cremnops haematodes	2211221322	2221122212	222a211222	2122223112
Cremnops sp.	2212221322	2221221?12	22222a1222	2122223112
Zacremnops cressoni	2221221322	2221222222	2222211212	2112223112
Biroia trifasciata	2211221322	2222?22222	2222211222	2111223112
Disophrini				
Disophrys subfasciata	2211221312	2221222112	2212221212	2122223111
Disophrys [P]seudocremnops atripennis	2211221322	2221122212	2221221212	2122223111
Disophrys n.sp.2	2211221312	2221122112	2211221112	212b223111
Disophrys n.sp.1	2211221312	2221122112	22212a1222	2122223111
Hypsostypos sp.	2121231322	2221122a12	2211221112	1123223111
Euagathis forticarinata	2121221322	2221222212	2212221212	1122223111
Euagathis sp.1	2121221322	2221222212	2212221212	1122223111
Coccygidium sp.1	2111231321	2221122212	2211221112	1123213111
Coccygidium luteum	2111231321	2221122212	2211221122	1123223111
Coccygidium nr. *sissoo*	2111231321	2221122212	2211221112	1123213111
Zelomorpha 98	2111231322	2221222222	22112211?2	1122223111
Zelomorpha 79	2111231322	2222222222	22112211?2	1122213111
Zelomorpha tropicola	2111231322	2221222222	2211221112	1122213111
Zelomorpha 89	2111231322	2221222222	22112211?2	1122223111
Zelomorpha 11	2111231322	2221222222	22112211?2	1122223111
Zelomorpha 25	2111231322	2221222222	22112211?2	1122223111
Zelomorpha nr. *tropicola*	2111231322	2221222222	2211221112	1123213111
Earinini				
Earinus elator	21212211-2	1212-21212	1222121222	111312?112
Sesioctonus nr. *areolatus*	1122212--2	1222-23222	1222221222	1123123112
Sesioctonus kompsos	1122212--2	1212-21222	1222221222	1123123112
Sesioctonus akrolophus	1122222--2	21222-2222	1222221212	1123123112
Amputoearinus matamata	21212212-2	1212-24?22	2222121222	112322311?
Amputoearinus fernandezi	21212212-2	1212-24?22	2222121222	112322311?
Austroearinus chrysokeras	21222212-2	1222-23222	1222221222	1123123112
Austroearinus melanopodes	21222212-2	1222-21222	1222221222	1123123112
Austroearinus rufofemoratus	21222212-2	1222-21222	1222221222	1123123112

Appendix 3 Voucher data

Voucher specimens are housed in the collection of the Hymenoptera Institute (University of Kentucky). An asterisk (*) indicates a specimen that was destroyed in the process of acquiring sequence data

Higher group	Genus/species	Locality	EMBL/GenBank accession no.
Helconinae	*Diospilus fomitis*	Canada*	DQ201887
	Helcon sp.	Europe*	DQ201886
Sigalphinae	*Acampsis alternipes*	Europe*	AAZ83609
	Malasigalphus sp.	Madagascar	DQ201888
	Sigalphus gyrodontus	China*	AJ416966
	Sigalphus irrorator	France*	Z97942
Agathidinae			
Agathidini (including Microdini)	*Agathis montana*	Turkey*	AJ302786 and DQ201900
	Agathis sp. 2	Costa Rica	DQ201889
	Alabagrus arawak	Neotropics*	DQ201896
	Alabagrus fuscistigma	Neotropics*	DQ201898
	Alabagrus haenschi	Neotropics*	AJ302787 and DQ201891
	Alabagrus masneri	Neotropics*	DQ201897
	Alabagrus maue	Neotropics	AJ302785 and DQ201899
	Alabagrus pachamama	Neotropics*	AJ302788 and DQ201892
	Alabagrus parvifaciatus	Neotropics*	AJ302789 and DQ201893
	Alabagrus stigma	Neotropics*	AJ245683 and DQ201894
	Alabagrus tricarinatus	Neotropics*	DQ201895
	Aneurobracon sp.	Malaysia	DQ201944
	Bassus s.l. dimidiator	Mich., USA	DQ201943
	Bassus macadamiae	Costa Rica	DQ201901
	Bassus s.l. nr *conspicuus* 1	Thailand	DQ201908
	Bassus s.l. nr *conspicuus* 2	Costa Rica	DQ201909
	Bassus nr *macademiae*	Costa Rica	DQ201902
	Bassus s.l. n sp. 3	Madagascar	DQ201935
	Bassus s.l. n sp. 4	Australia	DQ201939
	Bassus s.l. Camptothlipsis sp.	Madagascar	DQ201934
	Bassus s.l. n sp1	Colombia	DQ201910
	Bassus s.l. n sp. 2	Malaysia	DQ201931
	Braunsia bilunata	Sao Tome, Africa	AJ302797 and DQ201903
	Braunsia burmensis	Malaysia	DQ201930
	Braunsia nr. *nigriceps*	Africa	AJ302919 and DQ201904
	Crassomicrodus divisus	Mexico	DQ201945
	Mesocoelus n sp.	Costa Rica	DQ201907
	Pharpa dubiosus	Neotropics*	DQ201890
	Plesiocoelus bassiformes	Costa Rica	DQ201906
	Zamicrodus sensilis	Colombia	DQ201911
Cremnoptini	*Biroia trifasciata*	Tanzania	DQ201933
	Cremnops ferrungiensis	Costa Rica	DQ201922
	Cremnops hematodes	Colorado, USA	DQ201941
	Cremnops virginiensis	Kentucky, USA	DQ201921
	Cremnops sp.	Australia	DQ201942
	Zacremnops cressoni	Costa Rica	DQ201925
Disophrini	*Coccygidium* nr *sissoo*	Australia	DQ201940
	Coccygidium luteum	Kenya	DQ201938
	Coccygidium sp. 1	Kenya	DQ201919
	Disophrys subfasciata	Thailand	DQ201923
	Disophrys n sp. 1	Madagascar	DQ201937
	Disophrys n sp. 2	Madagascar	DQ201936
	Disophrys [P]seudocremnops atripennis	Sulawesi	AJ302826 and DQ201924
	Euagathis forticarinata	Thailand	AJ302810 and DQ201920
	Euagathis sp. 1	Thailand	DQ201905
	Hypsostypos sp.	Malaysia	DQ201932
	Zelomorpha nr tropicola	Guyana	DQ201918
	Zelomorpha tropicola	Colombia	DQ201914
	Zelomorpha 11	Neotropics	DQ201916
	Zelomorpha 25	Colombia	DQ201917
	Zelomorpha 79	Neotropics	DQ201913

Appendix 3 *Continued*

Higher group	Genus/species	Locality	EMBL/GenBank accession no.
Zelomorpha 89	Costa Rica	DQ201915	
Zelomorpha 98	Colombia	DQ201912	
Earinini	*Amputoearinus fernandezi*	Guyana	DQ201946
	Amputoearinus matamata	Colombia	DQ201928
	Austroearinus chrysokeras	Costa Rica	DQ201929
	Austroearinus melanopodes	Costa Rica	DQ201948
	Austroearinus rufofemoratus	Costa Rica	DQ201950
	Earinus elator	UK*	Z97944 and DQ201926
	Sesioctonus akrolophus	Costa Rica	DQ201927
	Sesioctonus kompsos	Costa Rica	DQ201949
	Sesioctonus nr *areolatus*	Costa Rica	DQ201947