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Phylogeny of Eulophidae (Hymenoptera: Chalcidoidea), with a reclassification of Eulophinae and the recognition that Elasmidae are derived eulophids

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Abstract. Eulophidae is a large and biologically varied family of parasitoid wasps, traditionally split into four subfamilies; Elasmidae is a uniform (single genus) and morphologically distinct family of wasps that are thought to be related to Eulophidae. The D2 region of the 28S rDNA gene (≈ 560 bp) of eighty-seven species of eulophid, three species of elasmid and sixteen outgroup species in five families was sequenced. Cladograms were constructed, and the results compared with conclusions drawn from morphological studies. The gene was most informative at the level of subfamily and tribe. The monophyly of both Eulophinae and Tetrastichinae is supported; that of Entedoninae and Euderinae is less clear. Results indicate that Eulophinae is a derived group within Eulophidae, rather than an ancestral group as previously thought, and that *Elasmus*, the sole genus of Elasmidae, belongs within this subfamily. The tribes of Eulophinae are reassessed and only three accepted: Eulophini (including Euplectrini and Elachertini), Elasmini and Cirrospilini LaSalle trib.n. for Bouček's Ophelimini with *Ophelimus* and *Australsecodes* excluded. Three small Australian tribes, Anselmellini, Ophelimini and Platytetracampini, are removed from Eulophinae and Entedoninae, respectively, but their exact relationships and subfamily status cannot as yet be decided. Another tribe, Keryini, known from a single Australian genus, is excluded from both Eulophinae and Eulophidae.

Introduction

Eulophidae (Hymenoptera: Chalcidoidea) is one of the largest families of parasitoid wasps with a total of 283 genera and 3977 described species (Noyes, 1998). Most eulophids are small to very small insects; they are abundant in all tropical and temperate regions, and many species have proven to be highly successful biological control agents. Although eulophids are generally parasitoids of holometabolous insects, the overall range of hosts and biologies in Eulophidae is remarkably diverse. A thorough review of the biology of this family is now needed, although limited reviews are available (Clausen, 1940; Bouček & Askew, 1968; LaSalle & Schauff, 1995; Efremova, 1997; Noyes, 1998). Although the majority of species are parasitoids, the family also contains a few phytophagous or 'predatory' species. Parasitoid forms can be endoparasitoids or ectoparasitoids; idiobionts or koinobionts; solitary or gregarious; primary parasitoids, hyperparasitoids or facultative hyperparasitoids; or specialists or generalists. Parasitoid species can attack eggs, larvae, pupae or even adults in a few cases. Predatory eulophids display a specialised form of parasitism where the wasp larva consumes many prey within an enclosed space (such as a gall or an egg sac). Species that develop this way are known to consume spider eggs in silken egg sacs (LaSalle, 1990a), eriophyid mites in galls (Taylor, 1909; Vereshchagina, 1961) or even nematodes (van den Berg *et al*., 1990). Phytophagous species again display a variety of life styles, and may be inquilines within galls (Sheng

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& Zhao, 1995), gall-formers themselves (Somerfield, 1976; Hawkins & Goeden, 1982; Headrick *et al*., 1995) or internal seed feeders (Bouček, 1988; LaSalle, 1994).

Many other aspects of their biology are interesting or unique. Species of *Euplectrus* are some of the only chalcidoids known to spin silken cocoons (Gerling & Limon, 1976; Puttler *et al*., 1980). Several species are parasitoids of aquatic insects, attacking either eggs (Fursov, 1995) or prepupae and pupae (Brown, 1968). Some gregarious species can produce over 200 parasitoid larvae from a single individual (Yang & Xie, 1998). Two species are known to be phoretic (Caroll, 1978; Macedo *et al*., 1990) and one species acts as a pollinator of orchids (Nilsson, 1979).

By contrast, Elasmidae, as traditionally conceived, is a much smaller family with the single rather distinctive genus *Elasmus* and just over 200 described species (Noyes, 1998). Elasmids are cosmopolitan in distribution, although most abundant in the Old World tropics. The majority of species are parasitoids or hyperparasitoids of lepidopteran larvae or pupae living in cases or spinnings, although a few other biologies are known (Noyes, 1998).

Eulophids have provided a series of extremely valuable experimental model systems for investigating a wide variety of questions in ecology and evolution (Godfray, 1994). The diversity of their biology, as well as the wealth of literature on the group, make eulophids a particularly appropriate group for asking questions concerning the evolution of a variety of biological traits. To do this, a sound hypothesis of relationships within the family is essential.

Eulophidae are normally classified into four subfamilies: Eulophinae, Euderinae, Entedoninae and Tetrastichinae. Although these divisions have received general acceptance, there is much less agreement about the phylogenetic relationship between them, or among their constituent tribes and genera, or about the identity of the sister group to Eulophidae (Bouček & Askew, 1968; Bouček, 1988; LaSalle & Schauff, 1995; Schauff *et al*., 1997; Gibson *et al*., 1999). The affinities of Elasmidae and Eulophidae have been recognized in the past and the former have sometimes been treated as a eulophid subfamily (Riek, 1970; Burks, 1979). However, all recent reviews have accorded them family status (e.g. Bouček, 1988; Hanson & Gauld, 1995; Gibson *et al*., 1997; Gibson *et al*., 1999). Reconstructing the phylogeny of taxa of generally small species of parasitoid is difficult because of the relatively small number of informative characters, and because of the typically high levels of homoplasy. Modern molecular methods offer an important tool to overcome these problems.

This paper describes the first phylogenetic study of Eulophidae and Elasmidae using DNA sequence data from the second expansion segment (D2) of the 28S ribosomal subunit. The phylogeny derived from the sequence data is critically assessed using traditional and recently discovered morphological character systems. The goals of the study were two-fold: first, to contribute towards a modern phylogenetic classification of these parasitoid families; second, to begin to provide a phylogenetic framework with which hypotheses about the nature and evolution of host shifts and life history radiation could be tested.

Materials and methods

Taxa examined

DNA sequences were obtained from eighty-seven species of Eulophidae from genera distributed among the four subfamilies. Three species of Elasmidae were sequenced, and also sixteen species from the chalcidoid families Trichogrammatidae, Tetracampidae, Pteromalidae, Aphelinidae and Agaonidae, all of which are possible relatives of Eulophidae. All the species studied are listed in Appendix 1. The identity of all specimens used for sequencing was checked by one of us (J.L.).

DNA amplification and sequencing

Sequences of the 28S rDNA D2 region from single individuals were analysed. Genomic DNA was extracted from specimens stored dry after critical-point drying or in 70–100% ethanol using Chelex (BioRad, Hercules, California) (Belshaw *et al*., 1999). Standard 50 µl PCR reactions were performed using 2.5 U Taq polymerase (Roche, Lewes, U.K.), 5.0 µl Taq buffer (1.5 mM $MgCl_2$), 12.5 nmol dNTPs and 20 pmol primer. Primer sequences were from Campbell *et al*. (1993). Weak PCR products were cleaned with QIAQuick PCR purification kits (Qiagen, Crawley, U.K.) and reamplified using an internal forward primer from Belshaw & Quicke (1997). PCR conditions were 35 cycles of 98 °C denaturation (15 s), 55–57 °C annealing (30 s) and 72 °C extension (40 s), with an initial denaturation of 96 °C (3 min) and a final extension of 72 °C (3 min). Products were purified by using the Sephaglas^{TM} Band Prep Kit (Pharmacia Biotech, St Albans, U.K.). They were then sequenced in both directions with the same primers used in the original PCR amplification (5 pmole), and dye-labelled terminators and thermosequenase (Pharmacia Biotech) on an ABI 373 automated sequencer. All reported sequences are the consensus obtained after sequencing both strands (forward and reverse) from an individual. All sequences used in this study are deposited in EMBL/GenBank/DDBJ databases (for accession numbers see Appendix 1).

Phylogeny reconstruction

The large size of the dataset (111 taxa) required certain modifications to standard methodology to overcome computational difficulties with data analysis. Sequences were aligned manually for the PAUP* analyses, because the large number of taxa involved made use of computer packages either not computationally feasible (e.g. MALIGN, Wheeler & Gladstein, 1994), or produced obviously non-homologous alignments upon visual inspection. However, use of POY (see below) provided an independent method of cladogram construction without reference to pre-aligned sequences.

Phylogenies were reconstructed in five ways. (1) Maximum parsimony analysis of manually aligned sequences using PAUP* (Swofford, 1998), with all substitutions given equal weight and gaps treated as missing data. (2) As in (1) but with

indels (insertion/deletion events) coded as additional binary characters according to the protocol of Barriel (1994). Changes in indel characters were given the same weight as substitutions. (3) Maximum parsimony analysis of manually aligned sequences with implied weighting (Goloboff, 1993) and with all substitutions given equal weight and gaps treated as missing data. For this, the concavity constant was set equal to two (the default option on PAUP*); trials with higher values, e.g. three, gave clearly nonsensical results. (4) As in (3) above but with indels coded as additional binary characters according to the protocol of Barriel (1994). Changes in indel characters were given the same weight as substitutions. (5) Using a dynamic programming method implemented in POY (Wheeler, 1998) that uses optimization alignment during tree building and tree search. POY finds trees that minimise cost by effectively optimising positions of indels and substitutions for internal nodes of the cladograms given *a priori* indel and substitution costs. For this, indels were given a weight of two, and all base substitutions were weighted 1.

Methods 1–4 were implemented in PAUP* (version 4.0b2a) (Swofford, 1998). PAUP* searches for optimum trees by swapping branches using a tree bisection reconnection (TBR) algorithm and storing a maximum number of equally parsimonious trees (MAXTREES) in memory. Because of the large number of taxa in this study, straightforward TBR with unlimited MAXTREES was very inefficient. Instead, initial cladograms were obtained by carrying out TBR on at least fifty 'random addition trees' (trees obtained by adding species selected at random, sequentially in their optimum position) with MAXTREES set at one. This rapid search strategy was efficient at finding cladograms within five steps of the final cladograms. Using these initial cladograms, TBR branch-swapping was then carried out with MAXTREES increased to 3000. The resulting cladograms were then used to estimate the maximum retention indices of each character, a measure of their phylogenetic informativeness. The indices were then employed as weights and a new set of cladograms calculated using the previous most parsimonious cladograms as the starting values (TBR with MAXTREES = 3000). The cladograms from this operation were then used as starting cladograms in another search with the character weights reset to unity. The last two steps were repeated until convergence occurred and no more parsimonious cladograms were found. The validation of this search strategy on several datasets will be described in detail elsewhere (Quicke, Taylor & Purvis, 2000). In no cases in this study were the cladograms found with this new strategy longer than those obtained by traditional branch-swapping without reweighting, and in several cases, they were shorter. Thus, the cladograms presented here are the shortest found.

In the maximum parsimony cladograms (Figs 1, 2), the maximum retention index (RI) was used for character reweighting in preference to the rescaled consistency index advocated by Farris (1989) because the latter results in down-weighting of characters that display intermediate levels of homoplasy. The consistency index also has the undesirable (though conservative) property of giving relatively high weights to characters that display no phylogenetic signal in the input cladograms. The maximum value of RI from all of the initial input cladograms was chosen as the reweighting function so as to be conservative; that is, to give each character the greatest opportunity to contribute to the results of the reweighted search. The alternative of using the minimum value would have given high influence only to those characters that performed well on all input cladograms and so would have down-weighted a character even if it had only performed poorly on one of them.

The level of statistical support for the different clades in the maximum parsimony cladograms (Figs 1, 2) was assessed by bootstrapping (see discussion in Kitching *et al*., 1998). Because of the large size of the data matrix, a modified form of bootstrapping with PAUP* was used. Five hundred bootstrap replicates were performed, each one involving TBR branch-swapping on cladograms produced by random addition with HOLD set to 20 and the MAXTREES parameter set to 2 (HOLD is the optimal number of trees that are examined at every stage during the initial tree-building phase). Searches of this type have a reasonable chance of finding near optimal cladograms in each bootstrap replicate, but because some of the cladograms found may be suboptimal, the bootstrap support values obtained by this method will underestimate the true support for the clade, and, therefore, the values are conservative. Our experience is that much more extensive and time-consuming searches, for example by using a high value of MAXTREES and many random additions for each bootstrap replicate, change bootstrap values by only a few percent (usually upwards), the largest changes occurring at nodes with low support

Results

The manually aligned D2 28S rDNA sequences consist of 567 positions. Of these sites, 223 (38.8%) were phylogenetically informative when gaps were treated as missing values and 262 (45.6%) when gaps were treated as a character. Scoring informative indel events using the system of Barriel (1994) resulted in the addition of a further seventy-seven binary characters. A few indel events were too complex to allow a reasonable assessment of homologies with the present set of taxa and these were not scored.

Figure 1 shows the strict consensus of more than 10 000 equally parsimonious cladograms (length 1862 steps) from the analysis in which gaps were treated as missing data. The equivalent phylogeny in which indels are coded as additional binary characters is shown in Fig. 2, and is also based on more than 10 000 equally parsimonious cladograms (length 2100 steps). The consistency index from analysis 2 is slightly higher (0.652 cf 0.632) than for analysis 1, indicating that the indel events carried slightly more signal than the substitution data. The two analyses are in broad agreement both in terms of their overall topology and which nodes are supported by high bootstrap values. Figures 3 and 4 show the cladograms obtained from implied weighting analysis, with gaps treated as missing data and indels scored as separate characters, respectively. These cladograms, when measured using unweighted parsimony, were markedly longer than the most parsimonious cladograms found from their respective datasets

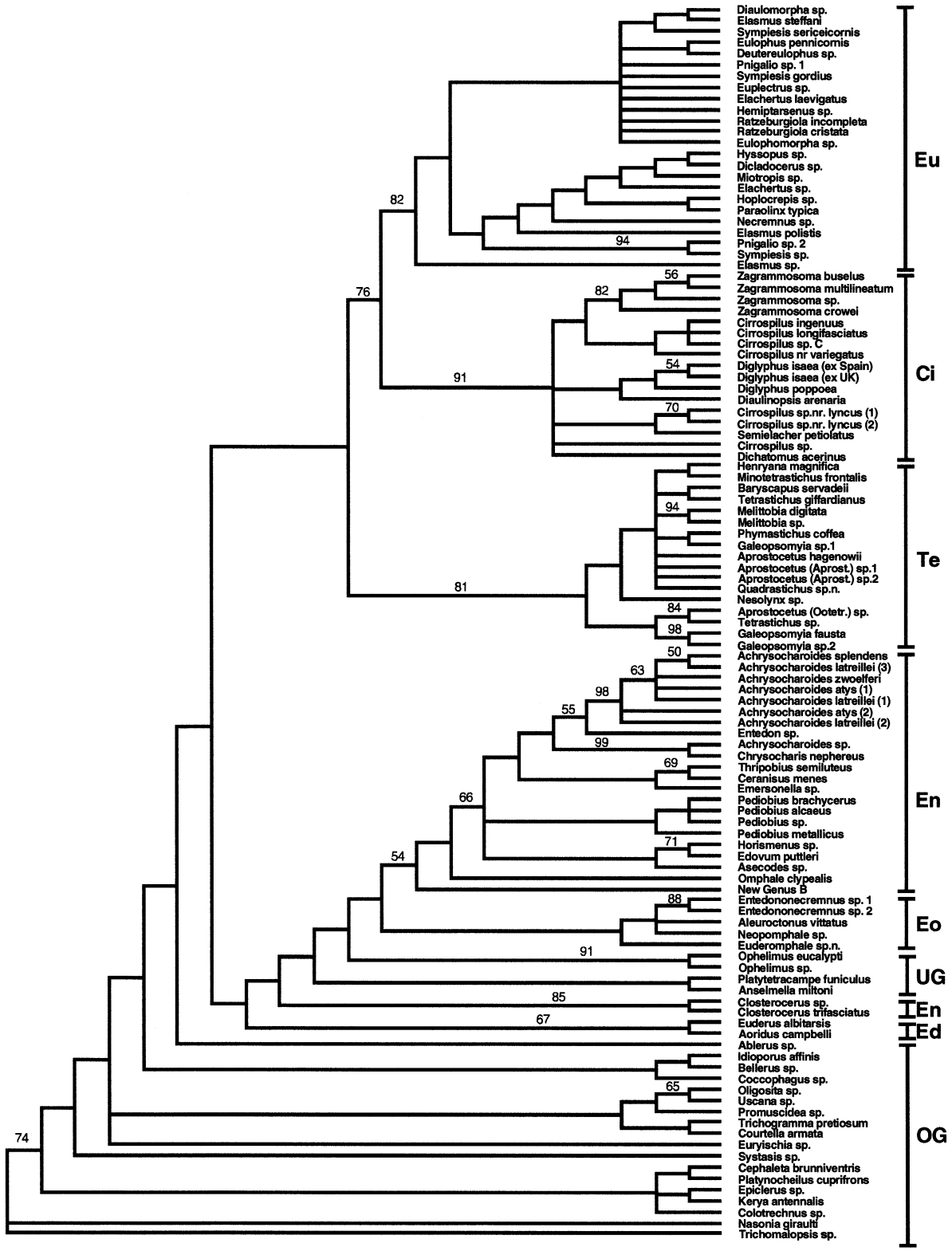

Fig. 1. Strict consensus of more than 10 000 most parsimonious cladograms obtained from analysis of manually aligned sequences, gaps treated as missing data (length = 1862; CI = 0.362; RI = 0.632). Bootstrap values are shown for nodes with ⩾ 50% bootstrap support. Ci = Cirrospilini; Ed = Euderinae; En = Entedoninae; Eo = Euderomphalini; Eu = Eulophini + *Elasmus*; Te = Tetrastichinae; UG = unplaced genera; OG = outgroups + *Bellerus*.

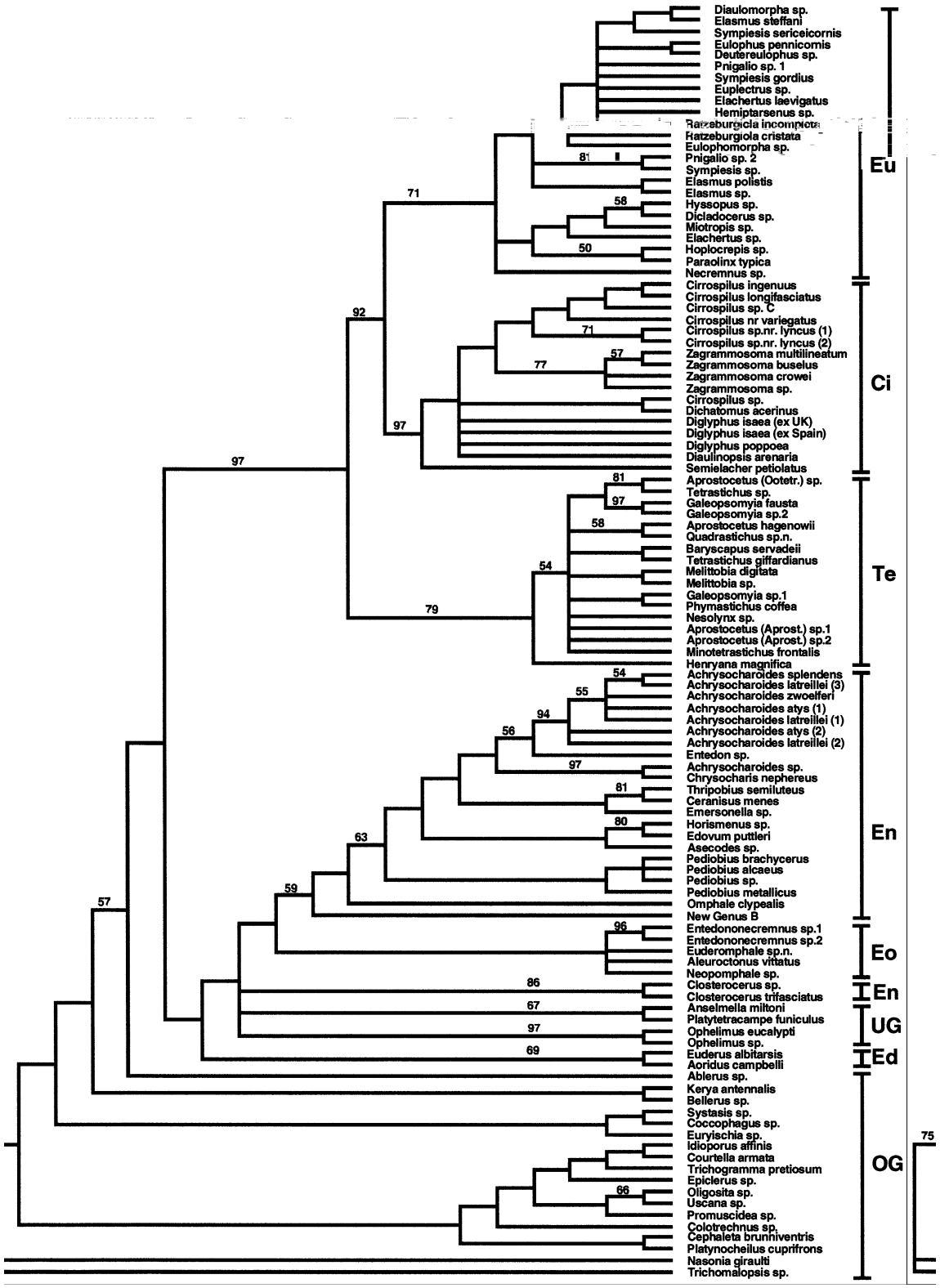

Fig. 2. Strict consensus of more than 10 000 most parsimonious cladograms obtained from analysis of manually aligned sequences, indels scored as separate binary characters (length = 2100; CI = 0.355; RI = 0.652). Bootstrap values are shown for nodes with ⩾ 50% bootstrap support. Ci = Cirrospilini; Ed = Euderinae; En = Entedoninae; Eo = Euderomphalini; Eu = Eulophini + *Elasmus*; Te = Tetrastichinae; UG = unplaced genera; OG = outgroups + *Bellerus*.

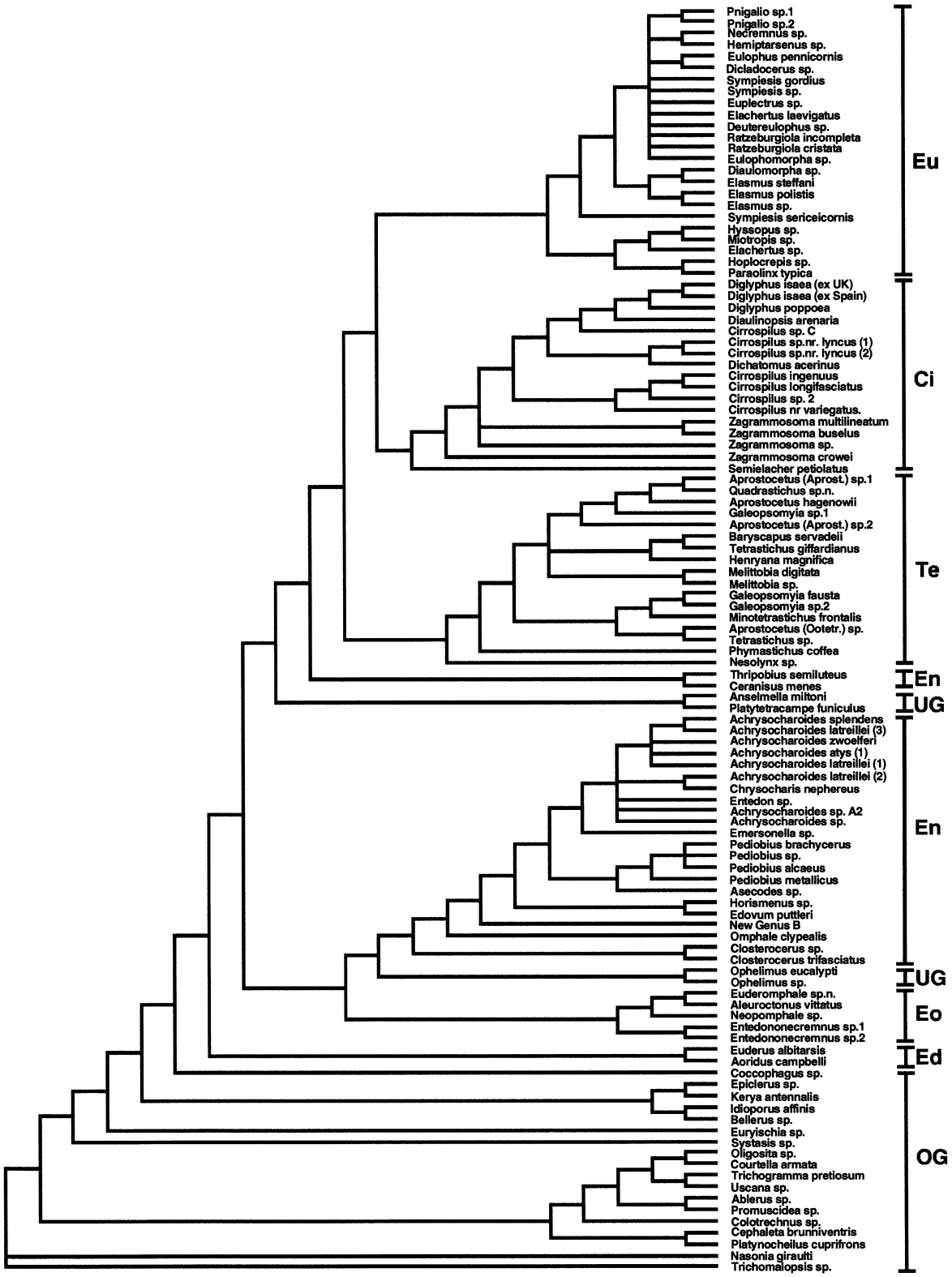

Fig. 3. Strict consensus of 2000 cladograms obtained from analysis of manually aligned sequences using implied weighting as the tree optimality criterion (concavity constant set to 2) and gaps treated as missing data (score = – 125.95656). The shortest cladogram measured with unweighted parsimony had length 1923 steps. Ci = Cirrospilini; Ed = Euderinae; En = Entedoninae; Eo = Euderomphalini; Eu = Eulophini + *Elasmus*; Te = Tetrastichinae; UG = unplaced genera; OG = outgroups + *Bellerus*.

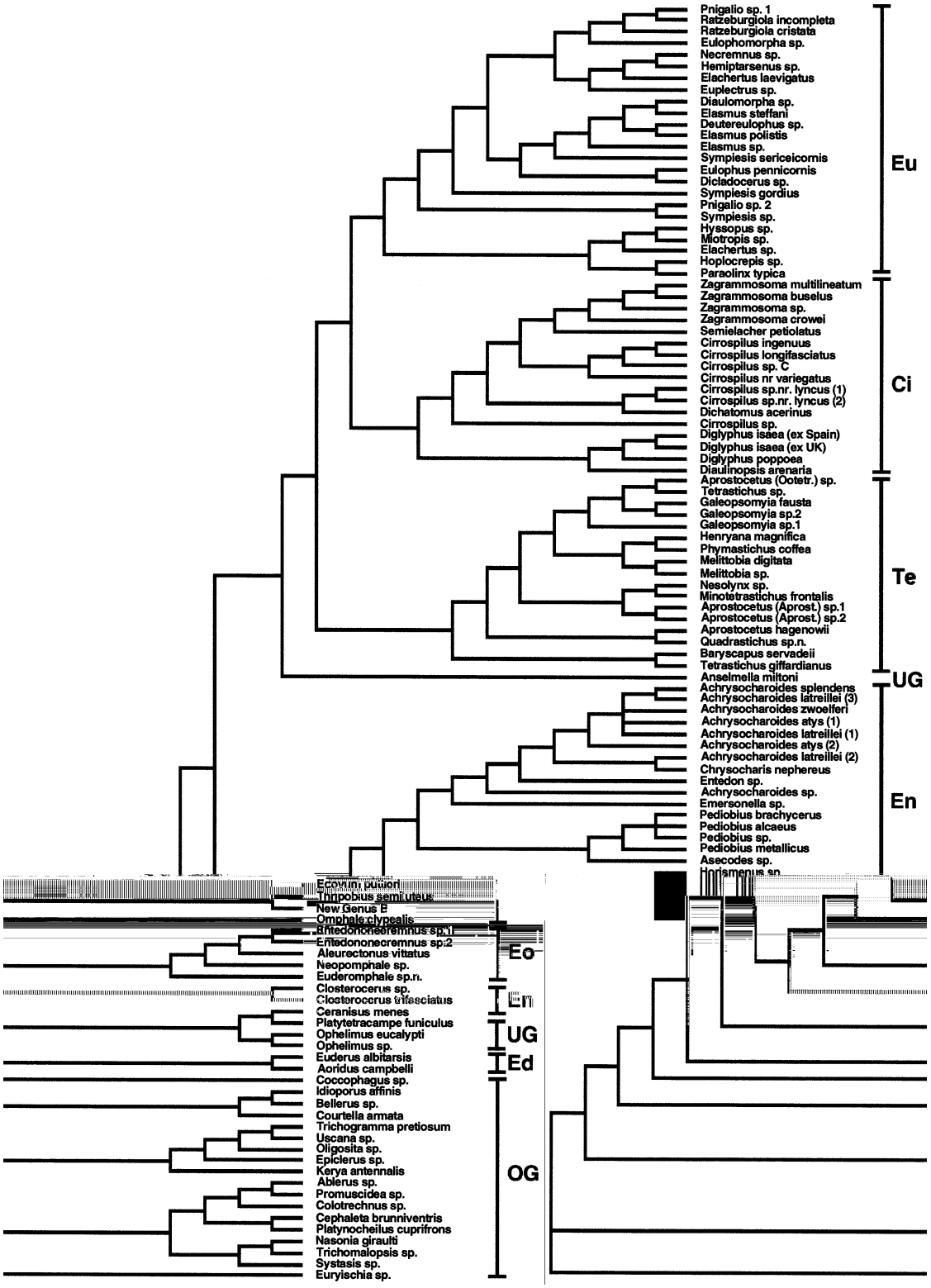

Fig. 4. Strict consensus of 2000 cladograms obtained from analysis of manually aligned sequences using implied weighting as the tree optimality criterion (concavity constant set to 2) and indels scored as separate binary characters (score = – 168.69244). The shortest cladogram measured with unweighted parsimony had length 2163 steps. Ci = Cirrospilini; Ed = Euderinae; En = Entedoninae; Eo = Euderomphalini; Eu = Eulophini + *Elasmus*; Te = Tetrastichinae; UG = unplaced genera; OG = outgroups + *Bellerus*.

(by 62 and 63 steps, respectively). Finally, the results of the POY analysis are shown in Fig. 5.

Phylogenetic considerations

Sequence data were obtained chiefly from Eulophidae, but also on three species of Elasmidae and members of five families as outgroup taxa. As discussed in more detail below, elasmids were found to be derived eulophids in Eulophinae. The gene sequenced was not able to resolve relationships at the family level but in general our data support the monophyly of Eulophidae, although the exact position of a number of basal genera needs further study. Implications of the work are now discussed for the four well recognized subfamilies of eulophids, and the molecular findings are related to the morphology of the insects. Finally, a series of small taxa whose subfamiliar affinities are still obscure, and one tribe excluded from Eulophidae are discussed. Appendix 2 provides a full placement, to tribal level, of the world genera of Eulophidae.

Eulophinae

The major changes that the results suggest to the existing classification of the family involve the treatment of Eulophinae. Eulophinae has generally been considered to be the most primitive of eulophid subfamilies (Graham, 1987; Bouček, 1988; LaSalle & Schauff, 1995; Schauff *et al*., 1997; Gibson *et al*., 1999) and possibly paraphyletic with respect to the other subfamilies. This view is based on their possession of characters that are thought to be primitive, such as weakly advanced axillae, the presence of many (three or more) setae on the submarginal vein, the submarginal vein smoothly joining the parastigma and the presence of two or more pairs of setae on the scutellum. Bouček placed six tribes in Eulophinae: Anselmellini, Keryini, Ophelimini, Eulophini, Elachertini and Euplectrini.

The present study indicates that Eulophinae is a derived rather than a primitive lineage, consisting of three groups referred to here as Eulophini (including Elachertini and Euplectrini), Elasmini and Cirrospilini. Morphological support (synapomorphic characters) is lacking for the monophyly of Eulophinae, but there does seem to be morphological support for the monophyly of each of the tribes (Eulophini, Elasmini, Cirrospilini). The status of the excluded Anselmellini, Keryini and Ophelimini (*sensu stricto*) is discussed below. Moreover, *Elasmus*, the sole genus in the long-recognized family Elasmidae, is a morphologically specialised member of Eulophinae.

The various methods of phylogenetic reconstruction were not in agreement on overall relationships in Eulophidae, particularly concerning the relationships of Euderinae, Entedoninae and the three unplaced tribes (Figs 1–5, see below). However, all methods consistently showed Eulophinae to be a derived lineage within the family, consisting of two major clades, Eulophini (including *Elasmus*) and Cirrospilini, and with a sister-group relationship to Tetrastichinae. Bootstrap values on the maximum parsimony cladograms (Figs 1, 2) are generally high for these clades: Eulophini (+ *Elasmus*) = 82, 71; Cirrospilini = 91, 97; Eulophini + Cirrospilini = 76, 92; Tetrastichinae = 81, 79; Eulophinae + Tetrastichinae = 85, 97. The higher support for the relationship of Eulophinae + Tetrastichinae in Fig. 2 (97) as opposed to Fig. 1 (85) is due, at least in part, to apomorphic deletions in the 254–280 and 507–531 base pair regions (Fig. 6). It is interesting that two thrips-parasitising genera of Entedoninae (*Ceranisus*, *Thripobius*) also share the deletions in the 507–531 base pair region.

Eulophinae: Eulophini. Eulophini as defined here consists of most genera from Bouček's (1988) Eulophini, Elachertini and Euplectrini. The molecular data do not differentiate these three groups, and there is no convincing morphological support to differentiate them. There is one morphological synapomorphy to support the Eulophini as defined here, the propleura meet posteriorly and cover the prosternum (Fig. 7A). This state is found in all taxa which are placed in this group according to the molecular data, with the exception of a few genera in which the propleura are slightly separated posteriorly (*Dicladocerus*, *Colpoclypeus*); it is also found in *Elasmus*. In the ancestral state, found within all other Eulophidae (and Chalcidoidea), the propleura are distinctly separated posteriorly, with the prosternum exposed (Fig. 7B). Other characters which help to define this group are: funicle with three or four segments (with the exception of *Colpoclypeus*), submarginal vein with three or more setae, and submarginal vein smoothly joining the parastigma.

Eulophinae: Elasmini. The placement of *Elasmus* within Eulophinae was unexpected. However, sequence data from three different species place them all unambiguously in the same clade as Eulophini. It is highly unlikely that these sequences arose as laboratory contaminants because they differ from any eulophid sequence obtained, and because one species was sequenced independently in another laboratory (B. Campbell, USDA-ARS, Albany, California and J. M. Heraty, University of California, Riverside). In addition, *Elasmus* possesses the main synapomorphy defining Eulophini, the structure of the propleura (Fig. 7C,D), whereas the number of tarsal segments and the structure of the protibial spur also link them to Eulophidae. The distinctive features of *Elasmus* suggestive of separate family status (see LaSalle *et al*., 1997) are thus probably derived specializations.

The relationship of Elasmini to Eulophini is unclear. Limitations of the D2 region of the 28S rDNA gene meant that it gave little resolution at the generic level within subfamilies and tribes. Although support is strong for a Eulophini + Elasmini relationship, it is not clear whether *Elasmus* is the sister group to Eulophini or a derived member from within Eulophini. In the latter case, the retention of tribal status for *Elasmus* would be inappropriate. However, without more convincing evidence one way or the other, the well known taxon Elasmini is retained.

Eulophinae: Cirrospilini. Bouček (1988) resurrected Ophelimini and included in it genera which had previously been placed in Ophelimini (*Ophelimus*, *Australsecodes*), Elachertini (*Aulogymnus*, *Cirrospilus*, *Cirrospiloidelleus*,

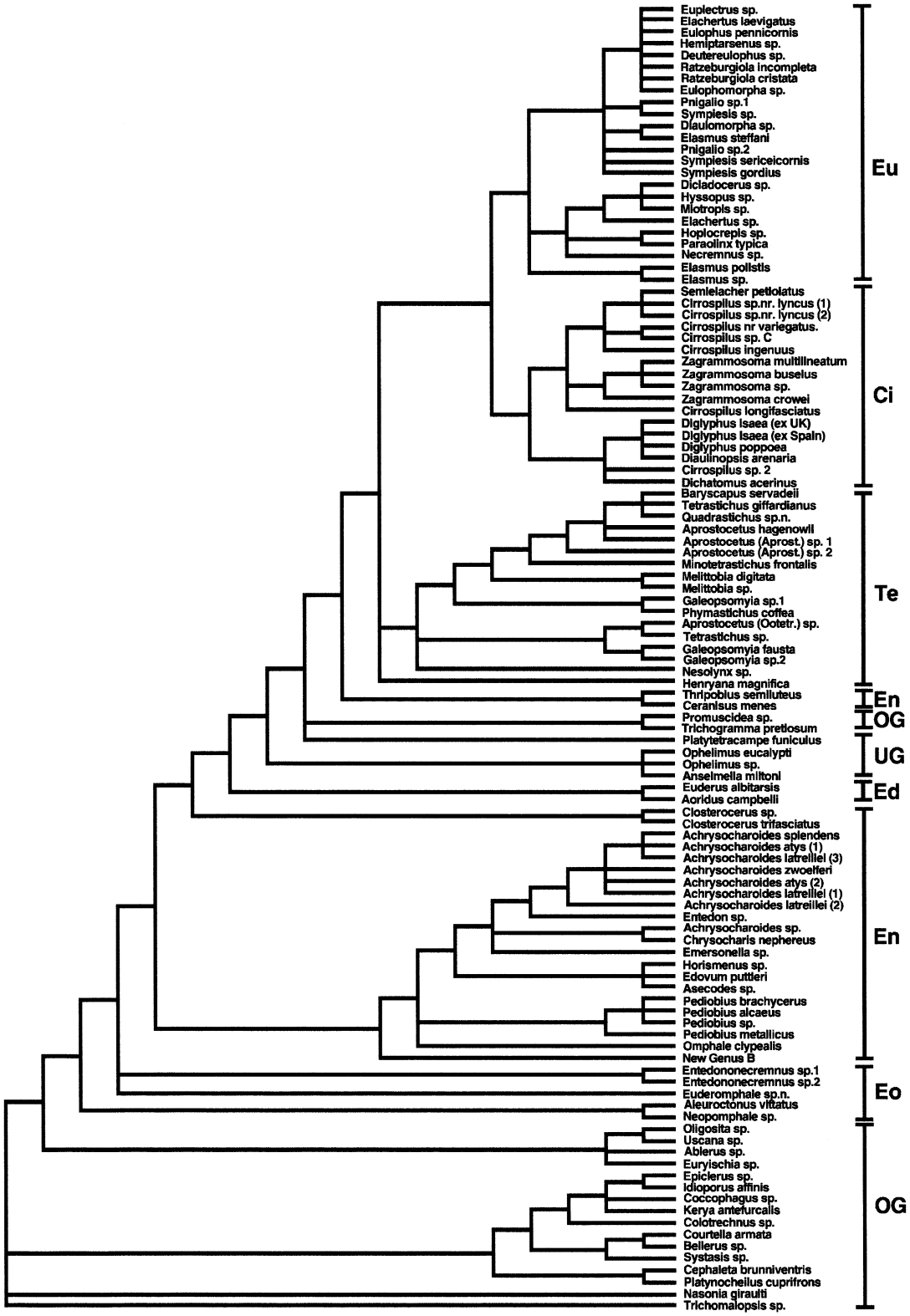

Fig. 5. Strict consensus of twenty-five cladograms obtained from analysis of unaligned data using POY with indels weighted twice as costly as substitutions. Ci = Cirrospilini; Ed = Euderinae; En = Entedoninae; Eo = Euderomphalini; Eu = Eulophini + *Elasmus*; Te = Tetrastichinae; UG = unplaced genera; OG = outgroups + *Bellerus*.

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                      25       26         27         28              50        51         52         53
                    890123456789012345678901234567890123456      1234567890123456789012345678901234567
Outgroups
Epiclerus sp        cg--cgctctt-ac-gagcgtgctgttttgcg-cgacag      tggctccg------ttacattcgaac-gaatataccg
Euryischia sp       cg--cg---tt--c-g--cgcgt-a-----cg-aggcag      tggctct------tttacattcgaaa-gaatataccg
Idioporus affi      aaa-cg---ttt-c-g---gcgt-ttttaacg-ttacaa      cggctctttaataattatattcttac-ggatatacca
Platynocheilus c    cg--cg---tt--c-g--cgcgt-a-----cg-aggcag      tggctc--------tcatattcgaac-ggatataccg
Promuscidea         cg--cg---ttcac-g--cgcgc-a-----cg-aggcag      gggctc-------------ttc--------tttacca
Systasis sp         cg--cg---tttac-g--cgtgc-g-----cg-cggcag      tggctcg-------ttacattcgaac-ggatataccg
Unplaced tribes
Anselmella milt     ct--cg---tgtat-g--cgtt--a-t---cg-tgacag      aggctc-------------ttcgaaa-gttactaccg
Kerya ante f        cg--cg---ttcac-g--cgcgc-a-c---cg-caacag      tggctc--------ttacattcgaac-ggatatacca
Ophelimus sp        ct--cg---tttac-g--cgtgc-a-----cg-gggcag      tggctct-------tgaatttc------ga-ttaccg
Platytetracampe     cg--cg---tttat-t--cgcgt---t---cg-gggcag      tggctc-------------ttc--------tttacct
Euderinae
Aoridus camp        cg--cg---ttcgc-g--cgcgc-a-----cg-ggacag      tggctc--------actccttc-a-cgggagataccg
Bellerus sp         cg--ca---tttat-g--cgcgc--------g-gagcag      aggctc--------a-tccaaaat----gata--ccg
Euderus albi        cg--cg---tttac-g--cgcgtca-----cg-tggcag      gggctctc------tctccttt-t-ggaga-ataccg
Entedoninae: Euderomphalini
Aleurocto vitt      ta--cg---ttaac-g--cgtgc-a-----cg-tggtag      tggctc------aagaca-ttcgaaa-ggatttaccg
Entedononecr sp     ag--cg---ttttc-g--cgttt-t-----cg-ggatag      tggctc---atatattctcatttgaa-ggatttaccg
Euderomphale sp     cg--cg---ctcac-g--cgcgc-a-----cg-tggcag      tggctc--------tacaattcgtaa-ggatttaccg
Neopompale sp       ag--cg---ttcac-g--cgttg-a-----cg-gagcag      tggctccct---gcattcgattttca-ggatttaccg
Entedoninae: Entedonini
Achrysocha sp       cg--cg---ttcac-g--cgtgc-a-----cg-tggcag      tggctc--------taaaattcgaaa-gga-ttaccg
Asecodes sp         cg--cg---ttcac-g--cgtgc-a-----cg-gggcag      tggctc--------taaaattcgaaa-gga-ttaccg
Ceranisus mene      cg--cg---ttcac-g--cgcgc-a-----cg-gggcag      tggctc--------------------------taccg
Chrysocharis nep    cg--cg---ttcac-g--cgtgc-a-----cg-tggcag      tggctc--------taaaattcgaaa-gga-ttaccg
Closterocerus sp    cg--cg---ttcac-g--cgcgc-a-----cg-cggcag      tggctc--------taaaattcgaaa-gga-ttacct
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Emersonella sp      cg--cg---ttcac-g--cgtgc-a-----cg-tggcag      tggctc--------taaaattcgaaa-gga-ttaccg
Entedon sp1         cg--cg---ttcac-g--cgtgt-a-----cg-tggcag      tggctc--------taaaattcgaaa-gga-ttaccg
Horismenus sp       cg--cg---ttcac-g--cgtgc-a-----cg-tggcag      tggctc--------tacaattcgaaa-gga-ttaccg
New genus           cg--cg---ttcgc-g--cgtgc-a-----cg-gggcag      tggctc--------gaacattcgaaa-gga-ttaccg
Omphale clyp        cg--cg---tttac-g--cgcgc-a-----cg-tggcag      tggctc--------tgaaattcgaaa-gga-ttaccg
Pediobius brac      cg--cg---tttac-g--cgtgc-a-----cg-tggcag      tggctc--------tgaaattcgaaa-gga-ttaccg
Thripobius semi     cg--cg---ttcgc-g--cgcgc-a-----cg-gggcag      tggctc-------------------------ttaccg
Tetrastichinae
Aprostocetus (Oot   cgg-cg---------------------------cggcag      tggctc-------------------------ttaccg
Baryscapus serv     cgg-cg------------------a-----cg-cggcag      tggctc-------------------------ttaccg
Galeopsomyia fau    cgg-tg---------------------------cggcag      tggctc-------------------------ttaccg
Henryana magn       cgt-gg------------------a-----cgtcggcag      tggctc-------------------------ttaccg
Melittobia digi     cgg-cg---------------------------cggcag      tggctc-------------------------ttaccg
Minotetrastichus    cgg-cg---------------------------cggcaa      tggctc-------------------------ttaccg
Nesolynx sp         cg--cg------------------------cg-cggcag      tggctc-------------------------ttaccg
Phymastichus cof    cg--cg---------------------------cggcag      tggctc-------------------------ttacct
Quadrastichus sp    cgg-cg--------------------------ttggcag      tggctc-------------------------ttaccc
Tetrastichus giff   cggtcg------------------a-----cg-cggcag      tggctc-------------------------ttaccg
Eulophinae: Eulophini
Deutereulophus s    cg--cg---------------------------cttcag      cggctc-------------------------ttaccc
Diaulomorpha sp     cg--cg------------------------cg-tttcag      tggctc-------------------------ttaccc
Dicladocerus sp     cg--cg---------------------------cttcag      tggctc-------------------------ttaccg
Elachertus laev     cg--cg---------------------------cttcag      tggctc-------------------------ttaccc
Elachertus sp.      cg--cg---------------------------cttcag      tggctc-------------------------ttaccg
Eulophomorpha sp    tg--cg---------------------------cttcag      tggctc-------------------------ttaccc
Eulophus penn       cg--cg---------------------------cttcag      cggctc-------------------------ttaccc
Euplectrus sp       cg--cg---------------------------cttcag      tggctc-------------------------ttacct
Hemiptarsenus sp    cg--cg---------------------------cttcag      tggctc-------------------------ttaccc
Hoplocrepis sp      cg--cg------------------------cg-cttcag      aggctc----------------------ttactaccg
Hyssopus sp         cg--cg---------------------------cttcag      tggctc-------------------------ttaccg
Miotropis sp        cg--cg---------------------------cttcag      tggctc-------------------------ttaccg
Necremnus sp        ca--cg------------------------cg-cttcag      tggctc-------------------------ttaccg
Paraolynx typi      cg--cg------------------------cg-cttcag      tggctc-------------tt----------ctacct
Pnigalio sp         gg--tg---cgctc-g--cgcat-t-----cg-ca-cag      tgg-----------------------------tacca
Ratzeburgiola cr    cg--cg---------------------------cttcag      tggctc-------------------------ttaccc
Sympiesis gordius   cg--cg------------------------cg-cttcag      tggctc-------------------------ttaccc
Eulophinae: Elasmini
Elasmus sp          cg--cg------------------------cg-cttcag      cggctc-------------------------ttaccc
Elasmus stef        cg--cg------------------------cg-tttcag      tggctc-------------------------ttaccc
Eulophinae: Cirrospilini
Cirrospilus inge    cg--cg------------------aga--gct-cgtcag      tggctc-------------------------ttaccg
Cirrospilus long    ag--cg------------------------cg-cggcag      tggctc-------------------------ttaccg
Diaulinopsis are    ca--cg------------------------cg-cttcag      tggctc-------------------------ttaccg
Dichatomus acer     ct--cg------------------------cg-cttcag      tggctc-------------------------ttaccg
Diglyphus isae2     ct--cg------------------------cg-cttcag      tggctc-------------------------ttaccg
Semielacher peti    tc--ct---------c--cgggg-a----gcg-cttcag      tggctc-------------------------ctaccg
Zagrammosoma mul    tg--ct------------ct-gc-a-tt-gcg-cggcag      tggctc-------------------------ttaccg
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Fig. 6. Sections of the D2 28S rDNA gene sequences for selected taxa, showing apomorphic deletions in the 254–280 and 507–531 base pair regions which support the relationship of Eulophinae + Tetrastichinae.

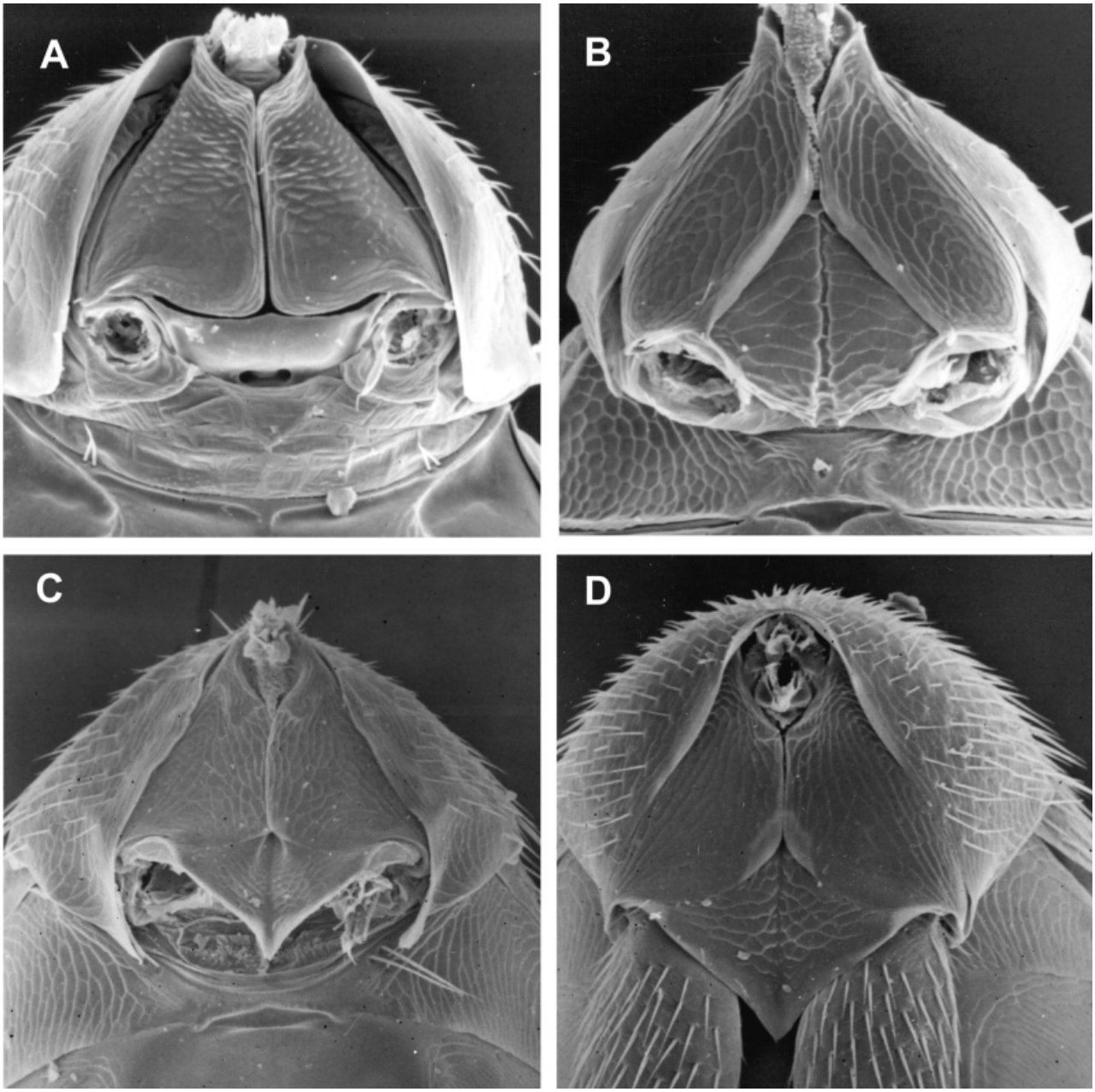

Fig. 7. Propleural plates in Eulophinae. The propleura meeting posteriorly and covering the prosternum is a synapomorphy for Eulophini. A, *Hyssopus* sp. (Eulophini), with propleura meeting posteriorly and covering prosternum; B, *Diglyphus isaea* (Cirrospilini), with propleura separate posteriorly and exposing prosternum; C, *Elasmus steffani*; D, *Elasmus* sp. (Elasmini), with propleura meeting for almost their entire distance.

Diaulinopsis, *Semielacher*, *Zagrammosoma*) and Eulophini (*Diglyphus*, *Meruana*). The molecular results indicate that although most of the genera placed by Bouček in Ophelimini do form a natural group, they are not that closely related to *Ophelimus*. This is supported by morphological characters: Ophelimini (*sensu stricto*) differ in having a larger total number of segments between the pedicel and club, in their slightly swollen marginal vein and in their large, smoothly rounded face, which lacks a transverse sulcus. This necessitates a new tribal group to accommodate the remaining genera and a formal diagnosis is now provided.

Cirrospilini LaSalle, trib.n.

Type genus: *Cirrospilus* Westwood, 1832.

Diagnosis. Funicle with 2 or 3 segments (rarely 4 in some males if *Aulogymnus* is included). Face usually with

transverse sulcus about midway between torulus and anterior ocellus (Fig. 8A–D). Propleura separated posteriorly, exposing prosternum (Fig. 7B). Notauli variable: complete, straight or nearly so, and reaching hind margin of mesoscutum; complete, curved to meet anterior margin of axilla; incomplete. Scutellum with at least 2 pairs of setae, and with sublateral grooves often (usually) present (even if only faintly indicated). Submarginal vein with 3 or more setae on dorsal surface. Postmarginal vein present, from about half length of stigmal vein to twice length of stigmal vein, although most often about equal in length to stigmal vein.

Cirrospilini also includes, on morphological grounds, genera that were placed by Bouček (1988) in Elachertini (e.g. *Ascotolinx*, *Gallowayia*, *Gattonia*, *Naumanniola*, *Pseudiglyphus*) or were considered in other works (Peck *et al*., 1964; Bouček & Askew, 1968) as belonging in Eulophinae (*Danuviella*).

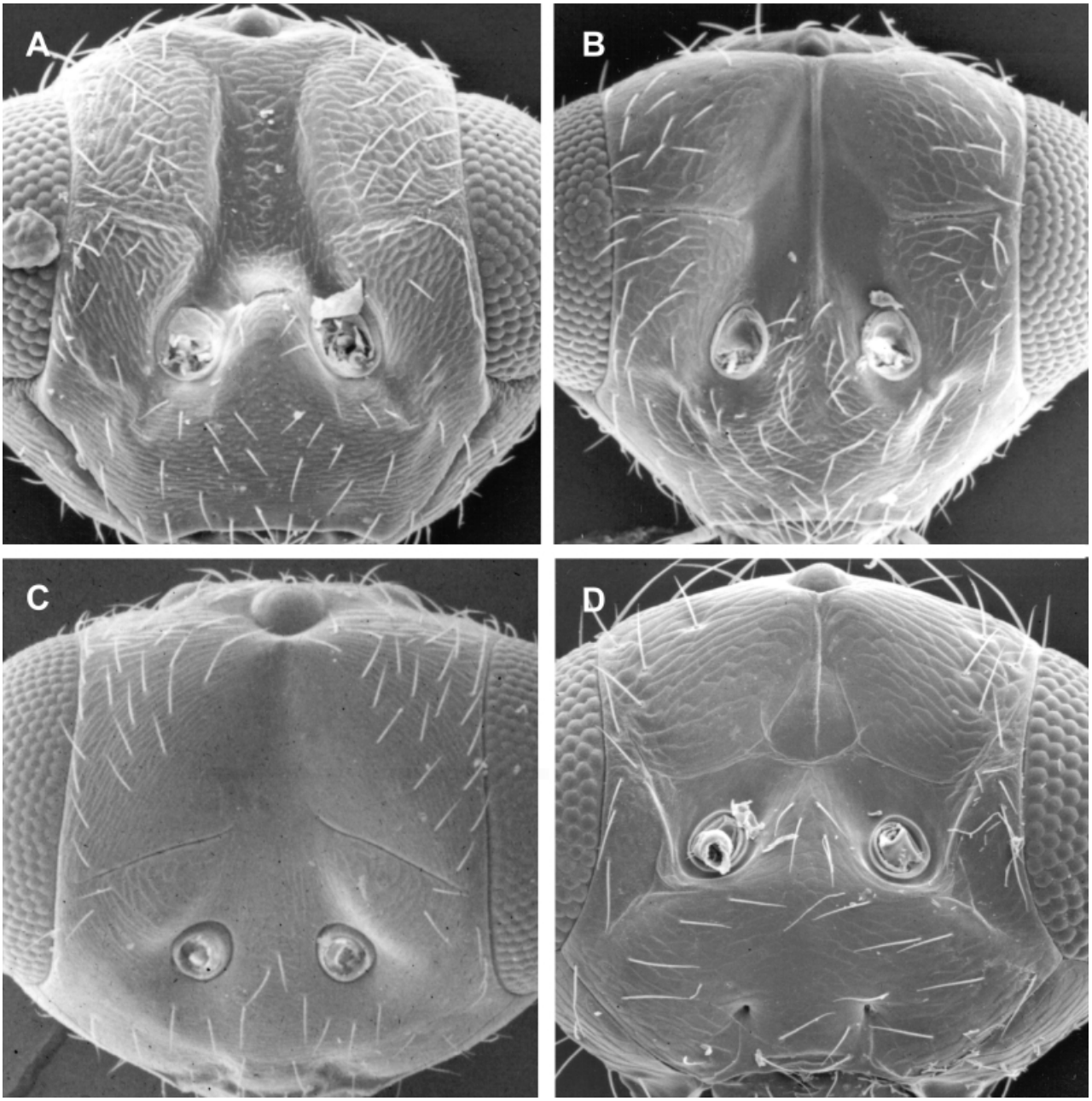

Fig. 8. Frontofacial sutures in Cirrospilini. The presence of a transverse frontofacial suture about halfway between the torulus and the anterior ocellus is a putative synapomorphy for Cirrospilini. A, *Cirrospilus pictus*; B, *Diglyphus isaea*; C, *Ascotolinx funeralis*; D, *Semielacher petiolatus*.

One major problem in the characterization of Cirrospilini is whether it contains *Aulogymnus* (synonym *Olynx*). *Aulogymnus* is provisionally included in Cirrospilini; however, this genus differs from most other Cirrospilini in lacking the transverse sulcus on the frons. Members usually also have three funicular segments, although some species can have two, and some males even have four segments. *Aulogymnus* has generally been considered to be closely related to *Cirrospilus* (Bouček, 1988) and it is possible that this is a primitive member of Cirrospilini lineage; however, clear morphological evidence to support this is lacking. *Dichatomus* is another problematical genus that seems to be more closely related morphologically to *Aulogymnus* than to other cirrospilines. However, sequence data show it belongs in Cirrospilini. Further work is needed to define properly this lineage.

Eulophinae; *uncertain genera.* There are a few genera that do not fit into the tribes as defined here, and for which fresh material could not be obtained for sequencing. The most disturbing of these, *Trichospilus*, has only two funicular segments, the propleura slightly diverging posteriorly, lacks a transverse sulcus on the frons and generally does not seem to have the synapomorphies used to define either of the tribes. Further studies are necessary to determine whether this genus actually belongs to one of the existing tribes or will require separate tribal status. *Colpoclypeus* also has two funicular segments and the propleura slightly diverging posteriorly; however, it is more likely that this is just an aberrant member of Eulophini. Finally, it is noted that one of the characters traditionally used to divide the taxa presently belonging to Eulophinae is the condition of the notauli (complete or incomplete). It appears that this character is generally unreliable, as taxa with either form are found in both of the redefined tribes.

Tetrastichinae

Molecular data provided strong support for the monophyly of the subfamily, although they are not able to resolve relationships at lower levels. The subfamily is monophyletic in four out of five cladograms (Figs 1–4) and with high bootstrap values on the maximum parsimony cladograms (Figs 1, 2). Only in the POY tree was one member, *Henryana magnifica*, questionably excluded from the subfamily. As mentioned above, there is good molecular support for an association between Eulophinae and Tetrastichinae.

Tetrastichinae can be recognized by a combination of characters that include: notauli complete, straight; scutellum with two pairs of longitudinal lines (submedian and sublateral); postmarginal vein absent or highly reduced (less than half the length of the stigmal vein); axillae usually strongly advanced, hind margin of scapula deeply excised; female antenna with three funicular segments, male antenna with four; submarginal vein not smoothly joining parastigma, but tapering to a point that joins the parastigma slightly distal to its base; frequent presence of a median longitudinal line on the mesoscutum; both maxillary and labial palpi reduced to a single segment; presence of a sensory plaque on the ventral edge of the male antennal scape (Graham, 1987; Bouček, 1988; LaSalle, 1994).

Although most authors think of Tetrastichinae as a natural group (Graham, 1987; Bouček, 1988; LaSalle, 1994), morphological evidence is ambiguous. For example, all of the characters listed above are absent in some members of Tetrastichinae, or occur in members of other subfamilies, or both. Probably the best character to support monophyly of Tetrastichinae, as suggested by Graham (1987), is the presence of a sensory plaque on the ventral edge of the male antennal scape. This character is found only in Tetrastichinae and it is present in all Tetrastichinae with the exception of *Phymastichus* (LaSalle, 1990b).

Bouček (1988) recognized two tribes: Gyrolasomyiini containing *Gyrolasomyia* and Tetrastichini containing all other genera. It remains to be seen whether *Gyrolasomyia*, for which sequence data were unobtainable, can be supported as a sister group to the rest of Tetrastichini or is merely a derived member of Tetrastichinae.

Entedoninae

Bouček (1988) recognized two tribes in Entedoninae: Entedonini containing most of the genera and Platytetracampini containing *Platytetracampe* (which Bouček knew from only limited material). LaSalle & Schauff (1994) recognized another small tribe, Euderomphalini, into which they placed genera that had previously been included in both Entedoninae and Eulophinae. Recent examination of fresh specimens of *Platytetracampe* reveal that this genus does not belong in Entedoninae (LaSalle & Burwell, unpublished), and it is discussed further below.

Entedonini is easily recognized by a variety of characters which include: scutellum with a single pair of setae; submarginal vein with two dorsal setae; mesoscutal midlobe with two pairs of setae; male scape with sensory pores restricted to the ventral edge; face with frontal grooves which are distinctly separated from the anterior ocellus; propodeum with a subspiracular tubercle; marginal vein relatively long; stigmal vein relatively short (Bouček, 1988; Schauff, 1991). These characters have been thought to provide strong support for Entedoninae monophyly, although it is not clear whether Euderomphalini renders them paraphyletic (LaSalle & Schauff, 1994).

The molecular data only partially resolve these problems. In two of the cladograms (Figs 1, 2), Entedoninae are part of a clade containing Euderinae, Ophelimini, Anselmellini and Platytetracampini. In other reconstructions, various of these groups, at times even including some entedonine genera, are excluded from the clade containing Entedoninae.

The monophyly of the tribes presents another problem. Euderomphalini is a monophyletic group in four out of five cladograms (Figs 1–4) and is a grade taxon in the fifth (Fig. 5). In none of the reconstructions is it the sister group to the remainder of the entedonines, but this is generally due to a few problematical taxa (such as *Closterocerus*, see below).

Entedonini is monophyletic on only one cladogram (Fig. 3), which also includes Ophelimini, between Euderomphalini and Entedonini. In two of the five cladograms *Closterocerus* falls outside Entedoninae (Figs 1, 5), in another two cladograms it falls outside Entedonini (Figs 2, 4); in two cladograms the two closely related thrips-parasitising genera, *Thripobius* and *Ceranisus*, fall outside Entedoninae (Figs 3, 5), whereas in a third cladogram only *Ceranisus* falls outside Entedoninae (Fig. 4). None of these placements has significant bootstrap support and all three genera are typical entedonines on morphological grounds. The results thus point toward the monophly of Entedoninae and of its constituent tribes, Entedonini and Euderomphalini, but additional data are needed to resolve these relationships.

Euderinae

This is a relatively small subfamily, and sequence data were obtained for only three genera. Two of these (*Euderus*, *Aoridus*) always clustered together; the unusual Chilean euderine *Bellerus* consistently came outside of Eulophidae, usually clustering either with *Idioporus* or *Kerya*. *Kerya* was originally placed in Eulophidae (Bouček, 1988), but is removed in this paper. The placement of *Idioporus* was discussed by LaSalle *et al*. (1997), but it is not considered to be close to Eulophidae.

In the implied weighting cladograms, Euderinae are the most primitive members of the family (Figs 3, 4); in the maximum parsimony cladograms they are the most primitive members of the clade containing euderines, entedonines, Ophelimini, Anselmellini and Platytetracampini (Figs 1, 2). Only in the POY cladogram (Fig. 5) did they have a relatively intermediate placement in the family.

Little can be said about this subfamily from the few genera included in this study. The results suggest that Euderinae, which may or may not be monophyletic as currently conceived, is a relatively primitive member of Eulophidae.

Within Eulophidae, Euderinae can be distinguished because they possess eight gastral segments, rather than seven segments that occur in other eulophids (Bouček, 1988; Coote, 1994). It is equivocal as to whether this character supports the monophyly of Euderinae; if Euderinae is the basal group, the reduction to seven gastral segments could support the monophyly of all Eulophidae minus Euderinae. Another character that might indicate that euderines have a basal position in Eulophidae is the number of funicular segments; in euderines there are always four or even five funicular segments. Bouček (1988) and Coote (1994) give detailed discussions of the morphology of this subfamily.

Anselmellini, Ophelimini and Platytetracampini

The results indicate that these three tribes do not belong in the subfamilies in which they were previously placed (Anselmellini, Ophelimini in Eulophinae; Platytetracampini in Entedoninae); however, their placement from the molecular data is extremely ambiguous. The molecular data place the three taxa variously near (or within) the base of Entedoninae, or as the sister group of Tetrastichinae + Eulophinae, but in no case with strong bootstrap support. It is possible that some or all of them may be related, perhaps as a monophyletic assemblage or as a grade lineage between Euderinae and Entedoninae.

Morphologically, all three groups have more antennal segments than is usual for eulophids, and ophelimines and anselmellines share a widened marginal vein (Bouček, 1988). However, further work is needed to resolve their phylogenetic positions. It is worth noting that all these groups are basically Australasian in distribution. Anselmellini and Ophelimini are phytophagous (Bouček, 1988), whereas Platytetracampini are endoparasitoids in whiteflies (LaSalle & Burwell, unpublished). For additional descriptions of apomorphies defining these groups see Bouček (1988).

Keryini

Bouček (1988) placed the Australian *Kerya* in Eulophinae based mainly on the fact that it had four tarsal segments. However, he noted that certain features, for example the 12-segmented antenna and unusually long wing veins, were uncharacteristic of eulophids. He suggested that these and other morphological features resembled members of Melanosomellini (Pteromalidae: Ormocerinae). The molecular results indicate that this genus does not belong in Eulophidae.

Discussion

This is the first large phylogenetic study of Eulophidae using molecular techniques, which were to some extent combined with morphological studies. The chosen gene, the 2D region of the 28S rDNA, proved most informative at the subfamily and tribal level. Different genes will be required to resolve relationships at higher and lower levels. For example, Cook *et al*. (unpublished observation) have found that the cytochrome b gene was informative in resolving species-level phylogenies in the entedonine genus *Achrysocharoides.*

The 2D 28S rDNA gene fragment has proved to be of some use in resolving relationships between families in Chalcidoidea (Campbell *et al*., 2000). However, in the present study there was no clear indication regarding the sister group to Eulophidae. Although in four out of five cladograms (Figs 1–4) the sister group was a taxon of Aphelinidae, this is not very convincing for the following reasons. In none of these cladograms did the two aphelinid genera (*Ablerus*, *Coccophagus*) cluster together, there was never strong bootstrap support for this relationship and other well established family-level relationships were not recovered using this gene.

The molecular analysis largely supports the existing subfamiliar classification of Eulophidae. Tetrastichinae, Entedoninae (with some caveats, see above) and Euderinae (except *Bellerus*) possess both molecular and morphological synapomorphies. Eulophinae is supported by molecular data, but lacks a good shared derived morphological character;

however, Eulophini and Cirrospilini both have morphological and molecular support. Elasmini is placed in Eulophinae, but the molecular data were unable to resolve whether it merits distinct tribal status or is simply a derived Eulophini. At present, the tribal group Elasmini is retained.

Most commentators have considered Eulophinae to be the most plesiomorphic subfamily, but the molecular data show it to be a derived group and suggest that Euderinae may be the most primitive. The most basal clades of Eulophidae are not well resolved in the cladograms, and additional data will be needed to clarify the relationship of some basal genera of Entedoninae, Euderinae and a series of relatively poorly known Australian groups (Anselmellini, Ophelimini and Platytetracampini). Keryini is shown not be a eulophid.

The tribal classification of Eulophinae has long been considered problematic and a variety of solutions have been proposed (Burks, 1979; Bouček, 1988; Schauff & LaSalle, 1993). The results suggest that at present only three tribes should be recognized: Eulophini, Elasmini and Cirrospilini. The tribal position of a few eulophine genera requires further study.

The single most surprising result from the molecular analysis is the demise of Elasmidae, a taxon that dates back to 1856 (Förster, 1856). The molecular data place the three species of *Elasmus* that were sequenced in this study unambiguously within Eulophinae, and the genus also possesses the major defining morphological synapomorphy of the tribe. The true affinities of *Elasmus* appear to have been masked by a series of highly derived morphological features. Interestingly, many of these characters are also possessed by the eriaporine aphelinid genus *Euryischia* (LaSalle *et al*., 1997), which was also sequenced and is clearly unrelated to *Elasmus* in all cladograms (Figs 1–5). It thus appears that this combination of distinctive characters has evolved twice, presumably through convergent selection pressures, although as *Euryischia* attacks mainly homopteran nymphs (and occasionally their predators) and most elasmids attack lepidopterans living in protected feeding sites, it is unclear what these selection pressures might be.

Finally, this contribution to the higher classification of Eulophidae is a first step toward a more comprehensive phylogenetic hypothesis that will allow ideas about host shifts and life-history radiations to be tested. Eulophidae are very suitable for this type of study as all the major subfamilies are very diverse biologically, with perhaps most (but not all) variation segregating at the generic level. Efremova (1997) recently surveyed the life histories of Palearctic Eulophinae, argued that the family may be derived from parasitoids of leaf-mining Lepidoptera, and also put forward a series of other hypotheses about switches between host taxa, host-feeding niche, and ecto- and endoparasitism. However, these ideas were based on the assumption that Eulophinae formed the most primitive subfamily, and used the older tribal classification. Formal testing of hypotheses of the evolution of various life-history traits absolutely requires a sound phylogenetic hypothesis for Eulophidae.

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Appendix 1. Taxa included in analysis (with GenBank numbers given in parentheses).

Eulophinae: Eulophini
- *Deutereulophus* sp. (Costa Rica) (AJ274410)
- *Dicladocerus* sp. (California) (AJ274411)
- *Diaulomorpha* sp. (Australia, *ex* galls of *Mesostoa kerri* on *Banksia marginata*) (AJ274412)
- *Elachertus laevigatus* (Howard) (Costa Rica) (AJ274414)
- *Elachertus* sp. (Cirrospiloideus) (Costa Rica) (AJ274409)
- *Eulophomorpha* sp. (Indonesia) (AJ274415)
- *Eulophus pennicornis* Nees (U.K.) (AJ274413)
- *Euplectrus* sp. (Costa Rica) (AJ274416)
- *Hemiptarsenus* sp. (U.K.) (AJ274417)
- *Hoplocrepis* sp. (Costa Rica) (AJ274418)
- *Hyssopus* sp. (Washington) (AJ274419)
- *Miotropis* sp. (U.K.) (AJ274420)
- *Necremnus* sp. (U.K.) (AJ274421)
- *Paraolinx typica* (Howard) (California, *ex Paramyelois transitella*) (AJ274422)
- *Pnigalio* sp. 1 (Spain) (AJ274423)
- *Pnigalio* sp. 2 (France) (AJ274424)
- *Ratzeburgiola cristata* Ratzeburg (Tunisia) (AJ274425)
- *Ratzeburgiola incompleta* Bouček (Israel, *ex Phyllocnistis citrella*) (AJ274426)
- *Sympiesis gordius* (Walker) (France) (AJ274427)
- *Sympiesis sericeicornis* (Nees) (U.K.) (AJ274428)
- *Sympiesis* sp. (France) (AJ274429)

Eulophinae: Elasmini
- *Elasmus polistis* Burks (sequence from Bruce Campbell and John Heraty)
- *Elasmus steffani* Viggiani (Italy, *ex Prays oleae*) (AJ274431)
- *Elasmus* sp. (Brazil, ex. *Phyllocnistis citrella*) (AJ274432)

Eulophinae: Cirrospilini
- *Cirrospilus ingenuus* Gahan (Israel, *ex* lab culture *Phyllocnistis citrella*) (AJ274433)
- *Cirrospilus longifasciatus* Ferrière (Yemen) (AJ274434)
- *Cirrospilus* sp. nr *lyncus* Walker (Spain) (AJ274436)
- *Cirrospilus* sp. nr *lyncus* Walker (Spain) (AJ274437)
- *Cirrospilus* sp. nr *variegatus* (Masi) (Malaysia) (AJ274435)
- *Cirrospilus* sp.C (Colombia, *ex Phyllocnistis citrella*) (AJ274438)
- *Cirrospilus* sp. (sequence from Bruce Campbell and John Heraty)
- *Diaulinopsis arenaria* (Erdös) (Syria) (AJ274441)
- *Dichatomus acerinus* Förster (Austria, *ex* galls on Acer) (AJ274442)
- *Diglyphus isaea* (Walker) (Spain) (AJ274443)
- *Diglyphus isaea* (Walker) (U.K., *ex Chromatomyia* sp.) (AJ274444)
- *Diglyphus poppoea* Walker (U.K., *ex Chromatomyia syngenesiae*) (AJ274445)
- *Semielacher petiolatus* (Girault) (Jordan, *ex Phyllocnistis citrella*) (AJ274446)
- *Zagrammosoma buselus* (Walker) (Galapagos Islands) (AJ274447)
- *Zagrammosoma crowei* (Kerrich) (Yemen) (AJ274448)
- *Zagrammosoma multilineatum* (Ashmead) (Florida, *ex Phyllocnistis citrella*) (AJ274449)
- *Zagrammosoma* sp. (Costa Rica) (AJ274450)

Tetrastichinae: Tetrastichini
- *Aprostocetus* (*Aprostocetus*) sp. 1 (Costa Rica) (AJ274451)
- *Aprostocetus* (*Aprostocetus*) sp. 2 (U.K.) (AJ274452)
- *Aprostocetus* (*Ootetrastichus*) sp. (U.K.) (AJ274453)
- *Aprostocetus* (*Tetrastichodes*) *hagenowii* (Ratzeburg) (Hawaii, *ex Periplaneta americana*) (AJ274454)
- *Baryscapus servadeii* (Domenichini) (Spain) (AJ274455)
- *Galeopsomyia fausta* LaSalle (Brazil, *ex Phyllocnistis citrella*) (AJ274456)
- *Galeopsomyia* sp. 1 (Costa Rica) (AJ274457)
- *Galeopsomyia* sp. 2 (Costa Rica) (AJ274458)
- *Henryana magnifica* Yoshimoto (Costa Rica) (AJ274459)
- *Melittobia digitata* Dahms (sequence from EMBL)
- *Melittobia* sp. (Costa Rica) (AJ274460)
- *Minotetrastichus frontalis* (Nees) (U.K.) (AJ274461)
- *Nesolynx* sp. (Kenya) (AJ274462)
- *Phymastichus coffea* LaSalle (Kenya, *ex Hypothenemus hampei*) (AJ274463)
- *Quadrastichus* sp.n. (Spain, *ex* lab culture *Phyllocnistis citrella*) (AJ274464)
- *Tetrastichus giffardianus* Silvestri (Kenya) (AJ274465)
- *Tetrastichus* sp. (unknown) (AJ274466)

Entedoninae: Entedonini
Achrysocharoides atys (Walker) A1 (U.K.) (AJ274469)
Achrysocharoides atys (Walker) A2 (U.K.) (AJ274470)
Achrysocharoides latreillei (Curtis) L1 (U.K.) (AJ274471)
Achrysocharoides latreillei (Curtis) L2 (U.K.) (AJ274472)
Achrysocharoides latreillei (Curtis) L3 (U.K.) (AJ274474)
Achrysocharoides splendens (Delucchi) S (U.K.) (AJ274468)
Achrysocharoides zwoelferi (Delucchi) Z (U.K.) (AJ274467)
Achrysocharoides sp. (sequence from Bruce Campbell and John Heraty)
Asecodes sp. (Australia) (AJ274475)
Ceranisus menes (Walker) (Kenya) (AJ274476)
Chrysocharis nephereus (Walker) (U.K.) (AJ274477)
Closterocerus trifasciatus Westwood (U.K., *ex Phytomyza ilicis*) (AJ274479)
Closterocerus sp. 1 (Florida) (AJ274478)
Edovum puttleri Grissell (Brazil) (AJ274480)
Emersonella sp. (Brazil) (AJ274481)
Entedon sp. (U.K.) (AJ274482)
Horismenus sp. (Colombia) (AJ274483)
Omphale clypealis (U.K.) (AJ274484)
Pediobius metallicus (Nees) (Canary Islands) (AJ274485)
Pediobius alcaeus (Walker) (U.K.) (AJ274486)
Pediobius brachycerus (Thomson) (Spain) (AJ274487)
Pediobius sp. (U.K.) (AJ274488)
Thripobius semiluteus Bouček (California, *ex* lab culture *Heliothrips haemorrhoidalis*) (AJ274489)
New genus B (Brazil, *ex*. galls on *Copaifera langsdorfii*) (sequence to be submitted when named)
Entedoninae: Euderomphalini
Aleuroctonus vittatus (Dozier) (Costa Rica) (AJ274490)
Euderomphale sp.n. (Canary Islands) (AJ274491)
Entedononecremnus sp. 1 (Costa Rica, *ex Ceraleurodicus altissimus*) (AJ274492)
Entedononecremnus sp. 2 (sequence from Bruce Campbell and John Heraty)
Neopomphale sp. (Costa Rica) (AJ274494)
Euderinae
Aoridus campbelli Yoshimoto (Costa Rica) (AJ274495)
Euderus albitarsis (Zetterstedt) (Portugal) (AJ274496)
Bellerus sp. (Chile) (AJ274497)
Anselmellini
Anselmella miltoni Girault (Australia) (AJ274512)
Ophelimini
Ophelimus sp. (Australia) (AJ274498)
Ophelimus eucalypti (Gahan) (Australia) (AJ274499)
Platytetracampini
Platytetracampe funiculus Girault (Australia) (AJ274500)
Keryini
Kerya antennalis Bouček (Australia) (AJ274501)
Outgroup taxa
Agaonidae: Agaoninae
Courtella armata (Wiebes) (sequence from Jean-Yves Rasplus)
Aphelinidae: Aphelininae
Ablerus sp. (Costa Rica) (AJ274502)
Coccophagus sp. (Costa Rica) (AJ274503)
Aphelinidae: Eriaporinae
Euryischia sp. (Australia) (AJ274504)
Promuscidea sp. (India) (AJ274505)
Pteromalidae: Colotrechninae
Colotrechnus sp. (Guatemala) (AJ274507)
Pteromalidae: Eunotinae
Cephaleta brunniventris Motschulsky (Burma) (AJ274506)
Idioporus affinis LaSalle & Polaszek (Mexico) (AJ274508)
Pteromalidae: Ormocerinae: Systasini
Systasis sp. (Canary Islands) (AJ274509)
Pteromalidae: Pteromalinae
Nasonia giraulti Darling (sequence from EMBL)
Trichomalopsis sp. (sequence from Bruce Campbell and John Heraty)

Tetracampidae: Platynocheilinae
Platynocheilus cuprifrons (Nees) (Tunisia) (AJ274511)
Tetracampidae: Tetracampinae
Epiclerus sp. (U.K.) (AJ274510)
Trichogrammatidae
Oligosita sp. (sequence from Bruce Campbell and John Heraty)
Trichogramma pretiosum Riley (sequence from Bruce Campbell and John Heraty)
Uscana sp. (sequence from Richard Stouthamer)

Appendix 2. Genera of Eulophidae (from Noyes, 1998) with their current subfamily and tribal placement.

Eulophinae
Eulophini
Alophomorphella
Alophomyia
Alveoplectrus
Arachnolophus
Aroplectrus
Arunus
Austeulophus
Bryopezus
Cleolophus
Clotildiella
Cobarus
Colpoclypeus
Cristelacher
Dahlbominus
Dasyeulophus
Dermatopelte
Deutereulophus
Diaulomorpha
Dicladocerus
Diglyphomorpha
Diglyphomorphomyia
Dimmockia
Dineulophus
Elachertomorpha
Elachertus
Eulophinusia
Eulophomorpha
Eulophomyia
Eulophus
Euplectromorpha
Euplectrophelinus
Euplectrus
Eupronotius
Eurycephaloplectrus
Ginsiella
Grotiusomyia
Hemiptarsenus
Hoplocrepis
Hyssopus
Metaplectrus
Microlycus
Miotropis
Mohaniella
Necremnoides
Necremnus
Nesympiesis
Notanisomorphella
Noyesius
Paraolinx
Pauahiana
Petiolacus
Platyplectrus
Pnigalio
Ratzeburgiola
Renaniana
Rhicnopelte
Ryhonos
Setelacher
Skoka
Stenomesius
Stenopetius
Sympiesis
Sympiesomorpha
Tooloomius
Trielacher
Tylomischus
Xanthellum
Zasympiesis
Zealachertus
Elasmini
Elasmus
Cirrospilini
Ascotolinx
Aulogymnus
Cirrospiloidelleus
Cirrospilus
Danuviella
Diaulinopsis
Dichatomus
Diglyphus
Gallowayia
Gattonia
Melittobiopsis
Meruana
Naumanniola
Oxycantha
Pseudiglyphus
Semielacher
Zagrammosoma
Unplaced
Trichospilus
Unplaced (not seen)
Alibertia
Hamonia
Parasympiesis
Perinetia
Proelachertus
Tropimus
Tetrastichinae
Gyrolasomyiini
Gyrolasomyia
Tetrastichini
Aceratoneura
Aceratoneuromyia
Agmostigma
Anaprostocetus
Apotetrastichus
Aprostocetus
Apterastichus
Arachnoobius
Aranobroter
Awara
Baryscapus
Benoitius
Careostrix
Ceratoneura
Ceratoneuronella
Ceratoneuropsis
Chaenotetrastichus
Chouioia
Chytrolestes
Cirrospilopsis
Citrostichus
Comastichus
Crataepus
Cucarastichus
Dapsilothrix
Dubiostalon
Dzhanokmenia
Enneastichus
Epichrysocharis
Eriastichus
Euceratoneura
Eulophoscotolinx
Exalarius
Exastichus
Galeopsomyia
Gasterichus
Goethella
Hadranellus
Hadrotrichodes
Henryana
Holcotetrastichus
Kocaagizus
Kocourekia
Kolopterna
Kostjukovius
Lisseurytomella
Megaceratoneura
Melittobia
Mesofrons
Mestocharella
Minotetrastichus
Mischotetrastichus
Neohyperteles
Neotrichoporoides
Nesolynx
Oncastichus
Oomyzus
Oxypracetus
Palmistichus
Parachrysocharis
Paragaleopsomyia
Paraspalangia
Paratetrastichus
Peckelachertus
Pentastichus
Petalidion
Phymastichus
Planotetrastichus
Pracetus
Pronotalia
Puklina
Quadrastichodella
Quadrastichus
Sigmophora
Sphenolepis
Stipecarinata
Styotrichia
Tachinobia
Tamarixia
Tetrasta
Tetrastichomphale
Tetrastichomyia
Tetrastichus
Thripastichus
Thymus
Xenaprostocetus
Entedoninae
Euderomphalini
Aleuroctonus
Baeoentedon
Dasyomphale
Entedononecremnus
Euderomphale
Neopomphale
Pomphale
Entedonini
Achrysocharoides
Alachua
Ametallon
Apleurotropis
Asecodes
Astichomyiia
Atullya
Bridarolliella
Callifrons
Ceranisus
Chrysocharis
Chrysocharodes
Chrysonotomyia
Closterocerus
Clypomphale
Colpixys
Davincia
Derostenoides
Derostenus
Edovum
Emersonella
Encyrtomphale
Entedon
Entedonastichus
Epichrysoatomus
Eprhopalotus
Goetheana
Grahamia
Grassator
Hispinocharis
Holcopelte
Horismenoides
Horismenus
Ionympha
Kokandia
Kratoysma
Ladna
Mangocharis
Mestocharis
Microdonophagus
Monteithius
Myrmokata
Neochrysocharis
Nepediobopsis
Obesulus
Omphale
Omphalentedon
Paphagus
Paracrias
Parahorismenus
Parpholema
Parzaommomyia
Pediobius
Pediobomyia
Pediobopsis
Pediocharis
Pelorotelus
Perditorulus
Piekna
Platocharis
Pleurotropopseus
Pleurotroppopsis
Proacrias
Rhynchentedon
Sanyangia
Sarasvatia
Schizocharis
Tanava
Teleopterus
Thripobius
Uroderostenus
Xiphentedon
Zaommomentedon
Zaommomyiella
Euderinae
Acrias
Allocerastichus
Aoridus
Astichus
Bellerus
Beornia
Boucekastichus
Carlyleia
Euderus
Hubbardiella
Opeuderus
Parasecodella
Parasecodes
Pseudosecodes
Uroentedon
Wichmannia
Zeastichus
Unplaced tribes
Platytetracampini
Platytetracampe
Makarora
Anselmellini
Anselmella
Perthiola
Ophelimini
Ophelimus
Australsecodes