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Combined molecular and morphological phylogeny of Eulophidae (Hymenoptera: Chalcidoidea), with focus on the subfamily Entedoninae

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Abstract

A new combined molecular and morphological phylogeny of the Eulophidae is presented with special reference to the subfamily Entedoninae. We examined 28S D2–D5 and CO1 gene regions with parsimony and partitioned Bayesian analyses, and examined the impact of a small set of historically recognized morphological characters on combined analyses. Eulophidae was strongly supported as monophyletic only after exclusion of the enigmatic genus *Trisecodes*. The subfamilies Eulophinae, Entiinae (= Euderinae) and Tetrastichinae were consistently supported as monophyletic, but Entedoninae was monophyletic only in combined analyses. Six contiguous bases in the 3e′ subregion of the 28S D2 rDNA contributed to placement of nominal subgenus of *Closterocerus* outside Entedoninae. In all cases, Euderomphalini was excluded from Entiinae, and we suggest that it be retained in Entedoninae. Opheliminae **n. stat.** is raised from tribe to subfamily status. *Trisecodes* is removed from Entedoninae but retained as incertae sedis in Eulophidae until its family placement can be determined **new placement**. The genera *Neochrysocharis* **stat. rev.** and *Asecodes* **stat. rev.** are removed from synonymy with *Closterocerus* because strong molecular differences corroborate their morphological differences. *Closterocerus* (*Achrysocharis*) *germanicus* is transferred to the genus *Chrysonotomyia* **n. comb.** based on molecular and morphological characters.

Eulophidae is one of the largest families of Chalcidoidea, with over 4400 described species in four subfamilies (Noyes, 2003). It is also one of the most diverse and economically important chalcidoid families, with many species important for biological control of agricultural pests, especially of leaf-mining Diptera (Clausen, 1978). Other species are gall-formers on a variety of plants, including *Eucalyptus* (Bouček, 1988; La Salle, 2005), but the range of diversity in life-history traits of eulophids is comparable with that of Chalcidoidea itself, with several unique examples. Hosts include species from most insect orders, and some eulophids are even predators in spider egg sacs or in galls of mites or nematodes (LaSalle, 1994). Most subgroups of eulophids, including many genera, have very broad host ranges that may include several orders of insects or other taxa as well. A notable exception is the tribe Euderomphalini, which apparently contains only parasitoids of whiteflies (Hemiptera: Aleyrodidae).

Geographic distribution is similarly broad for most eulophid groups, with new continental distribution records of genera being discovered on a regular basis (Bouček, 1988; LaSalle, 1994; Schauff et al., 1997; Burks, 2003). It seems likely that most genera with a significant number of species occur in most continents. However, there is another notable exception in this case as well, as the unplaced tribe Anselmellini is strictly Australasian (Bouček, 1988).

Although Eulophidae and most of its major subgroups cannot be characterized succinctly in terms of life history or distribution, the family can be defined by a

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combination of morphological characters that is itself not unique within Chalcidoidea. All eulophids have 12 or fewer antennal segments, a small and straight protibial spur, and four or fewer tarsal segments (Gibson et al., 1999). The unplaced chalcidoid genus *Cales* Howard and the family Trichogrammatidae possess all the above features, but both these groups are distinguished from Eulophidae in having a broad petiole allowing the mesophragma to extend through the petiole into the gaster (Burks, 2003). The petiole in all Eulophidae, as in most other chalcidoids, is very narrow and does not permit the mesophragma to extend through it. Many other chalcidoids have a reduced antenna and four or fewer tarsomeres, but differ in having a more strongly developed protibial spur (Gibson et al., 1999). However, the protibial spur is apparently not uniform in either Eulophidae or other chalcidoids (LaSalle et al., 1997).

Because the defining morphological characters of Eulophidae are shared with so many other chalcidoids and are reductions from the usual chalcidoid states, they have come under suspicion of being potentially convergent (LaSalle et al., 1997; Gauthier et al., 2000). However, a core monophyletic group of eulophids is present in recent molecular analyses (Campbell et al., 2000; J.B. Munro, J.M. Heraty, R.A. Burks, unpublished). The more controversial issues remaining in eulophid taxonomy involve definition of its subfamilies and genera.

The subfamily Eulophinae has historically contained a diverse set of smaller tribes in addition to the more characteristic genera near *Eulophus* Geoffroy (Bouček, 1988). Gauthier et al. (2000) removed the primarily Australasian tribes Anselmellini, Keryini, and Ophelimini from Eulophinae, based on 28S D2 molecular data and morphological differences. They transferred Keryini to Pteromalidae because of its gestalt morphological similarity to the subfamily Ormocerinae. Anselmellini and Ophelimini were left as incertae sedis in Eulophidae because no clear indication of their relationships was supported by the molecular data. They also transferred *Elasmus* Westwood into Eulophinae as the sole member of the tribe Elasmini. Finally, Gauthier et al. (2000) proposed the new tribe Cirrospilini, and synonymized the previously recognized tribes Elachertini and Euplectrini under Eulophini.

Tetrastichinae is potentially the most diverse subfamily of Eulophidae in terms of species and life history traits (LaSalle, 1994), but it contains a large number of morphologically similar genera (Schauff et al., 1997). Tetrastichinae cannot be succinctly defined morphologically (LaSalle, 1994), but it has been strongly supported molecularly (Gauthier et al., 2000; J.B. Munro, J.M. Heraty, R.A. Borks, unpublished data).

Entiinae (formerly Euderinae, see Hansson and Straka, 2009) has been considered primitive because most of its species retain putatively plesiomorphic characters, such as a separate 9th metasomal tergite and complete notauli (Bouček, 1988; Coote, 1994). Gumovsky (2002) transferred Euderomphalini from Entedoninae to Entiinae, based in part on a reinterpretation of the morphology of some Euderomphalini, stating that the grooves previously considered to be axillar grooves were actually notauli.

Entedoninae, prior to Gumovsky's (2002) new interpretation, contained two tribes, Entedonini and Euderomphalini. Entedonini was revised and redefined by Schauff (1991), while Euderomphalini was revised by LaSalle and Schauff (1994) and Hansson and LaSalle (2002). Although entedonines have highly diverse life histories, all host records of Euderomphalini indicate that they are parasitoids of whiteflies (LaSalle and Schauff, 1994; Hansson and LaSalle, 2002). Platytetracampini was described in Entedoninae by Bouček (1988), but was changed to incertae sedis within Eulophidae by Gauthier et al. (2000) because 28S D2 data placed it near Anselmellini.

While Entedonini is usually characterized as having only one pair of scutellar setae and a single dorsal submarginal vein seta (Schauff, 1991; Schauff et al., 1997), this definition does not hold true for all members. Some species in several different genera that clearly belong to Entedonini have several setae in one of these locations, and a few even have several setae in both locations. Additional characters provided by LaSalle and Schauff (1994) and further discussed by Gibson et al. (1999), such as pores on the male scape restricted to a ridge along the ventral edge, mesoscutal midlobe with two pairs of bristles, transverse facial sutures separated from the median ocellus, and tubercle present behind the propodeal spiracle, also do not occur in all Entedonini. Gumovsky (2002) proposed a new character for the definition of Entedonini, mentioning that the mesothoracic spiracle is hidden in all species of that tribe, but the spiracle is exposed in the controversially placed Euderomphalini. A hidden spiracle also occurs in various other families of chalcidoids, but it may be locally informative within Eulophidae.

The problems of ambiguous morphological data and lack of other definitive grouping evidence apply to all four currently recognized subfamilies of Eulophidae and to most current tribes within these subfamilies (Burks, 2003). It has been difficult to decide in which subfamily the more problematic groups, such as Anselmellini, Euderomphalini, Ophelimini, and Platytetracampini, could belong. Uncertain homology in morphological characters presents a situation where molecular data could be helpful in determining the position of these groups and in addressing these questions of homology. For some of these groups, the broader context of a combined morphological and molecular phylogeny of Chalcidoidea will be needed (J.B. Munro, J.M. Heraty, R.A. Burks, unpublished data; J.M.H. et al., unpublished data).

Further controversy exists in generic classification within Entedonini. There is no agreement among specialists upon generic classification within the tribe. This problem is most pronounced in the classification of small-bodied genera, such as *Closterocerus* Westwood, *Neochrysocharis* Kurdjumov, and similar genera, with every expert in recent literature either using a different classification or expressing reservations about the one being used (Hansson, 1990, 1994, 2002; Gumovsky, 2001, 2002; Fisher and LaSalle, 2005).

Disagreement over classification of entedonine genera focuses upon debate over the reliability of certain morphological characters. Hansson (1990, 1994) discovered that the shape of basiconic peg sensilla of the antennal flagellum differed between species that he reclassified from *Chrysonotomyia* Ashmead to *Closterocerus* and *Neochrysocharis*. Most of the species transferred to *Closterocerus* were placed in the subgenus *Achrysocharis* Girault, while those with a carinate pedicel were retained in the nominal subgenus (discussed from here on as *Closterocerus* sensu strictu). Gumovsky (2001) suggested a different classification based on delimitation of the clypeus and the presence of subtorular grooves, synonymizing *Neochrysocharis*, *Asecodes* Förster, and a number of other genera under *Closterocerus*. Gumovsky did not make reference to Hansson's antennal characters. Hansson (2004) later suggested a different definition of *Chrysonotomyia*, combining all species with a single set of volsellar spines on the male genitalia into that genus, but acknowledging subtorular grooves as a valid character. Some species of *Closterocerus* (*Achrysocharis*) were reclassified into *Chrysonotomyia* based on this new interpretation (Hansson, 2004).

A common thread in this controversy is that groups have been defined largely without a phylogenetic context, using only a small number of characters without complete comparison across other potentially related genera. This approach especially ignores the concept of evolutionary polarity. While certain species may be similar in having a delimited clypeus, for instance, it is quite possible that primitive entedonines also had a well defined clypeus, such that its lack could instead be an informative character at some level. This approach also ignores the possibility of reversals or parallel evolution. There are no indications that every character has been derived only once within the lineage, and this possibility can be adequately addressed only in a phylogenetic context.

The most recent published morphological phylogeny of Entedoninae was by Schauff (1991), but that study did not include Euderomphalini or several other genera of Entedonini from outside the Holarctic region. The molecular phylogenies of Eulophidae (Gauthier et al., 2000) and Entedoninae (Gumovsky, 2002) did not, by the authors' own admission, make convincing statements on the classification of genera within Entedonini. Gumovsky's (2002) molecular analysis focused more on Euderomphalini. He concluded that euderomphalines should be transferred from Entedoninae to Entiinae, based on 28S D2 ribosomal sequences, distinctness of the clypeus, and presence of apparently complete notauli. Again, this approach to morphological interpretation ignores the concept of character polarity. The presence of distinct notauli has a strong possibility of being plesiomorphic for all subfamilies of Eulophidae, again meaning that this character may not be informative among tribes and subfamilies.

The taxonomic instability of Entedoninae has led to the problem that it is not always clear which classification to follow, because there has been no clear reason to prefer one interpretation of generic characters over the other. Few studies have provided formal analyses with indications of character support. One of these was by Gauthier et al. (2000), who found strong parsimony bootstrap support for only Ophelimini, and weaker bootstrap support for Eulophinae and Tetrastichinae, in their phylogeny of the family. Although they sampled a relatively large number of Entedoninae, and Entedonini + Euderomphalini formed a monophyletic group in their analysis, they found no bootstrap support for monophyly of the subfamily. There was also no strong bootstrap support for monophyly of any entedonine genus.

A more definitive classification of Eulophidae addressing available information would provide not only a more stable and informative classification, but also a better framework for all aspects of research involving eulophids. The focus of this study is to analyse additional molecular data, with the goal of providing a stronger and less equivocal statement regarding the controversies of eulophid classification.

Materials and methods

A broad range of eulophids was chosen for this analysis, based on morphological diversity and specimen availability (Table 1). Outgroups from three different families were chosen, based on morphological similarity to Eulophidae. Tetracampinae has the advantage of possible molecular proximity to Eulophidae as well (Campbell et al., 2000; J.B. Munro, J.M. Heraty, R.A. Burks, unpublished data). While Rotoitidae, *Cales* Howard (Calesinae; family unplaced), and the pteromalid species *Idioporus affinis* LaSalle & Polaszek also possess four tarsomeres and a small protibial spur, they were excluded from this analysis because 28S data indicated in a larger analysis of Chalcidoidea that *Chiloe micropteron* Gibson & Huber, *Cales*, and *I. affinis* are not closely related to Eulophidae (J.B. Munro, J.M. Heraty, R.A. Burks, unpublished).

Most specimens were killed in 95% EtOH and stored at −80 °F until extraction. The entire body was used for extraction using the chelex method (Walsh et al., 1991),

Table 1
Specimens used in this study

Taxon	Classification	Locality	Voucher no.	GenBank accession numbers 28S D2	28S D3-D5	CO1
Achrysocharoides sp.	Entedoninae: Entedonini	Italy: Campania	161085	HM364928	HM364998	HM365040
Aprostocetus sp. 1	Tetrastichinae	USA: CA	49012	AY599265	HM364966	–
Aprostocetus sp. 2	Tetrastichinae	USA: CA	00251714	HM364958	HM364967	–
Asecodes sp.	Entedoninae: Entedonini	Italy: Campania	161135	HM364910	HM364980	HM365029
Astichomyiia latiscapus	Entedoninae: Entedonini	Costa Rica	161116	HM364935	HM365005	–
Astichus mirissimus	Entiinae	Australia: Qld	92142	AY599261	HM364971	–
Astichus n. sp.	Entiinae	Australia: Qld	92141	AY599260	HM364970	HM365048
Aulogymnus n. sp.	Eulophinae: Cirrospilini	USA: CA	161048	HM364963	HM365024	HM365054
Bellerus sp.	Entiinae	Chile: R. IX	161250	HM364949	HM365014	HM365049
Beornia n. sp.	Entiinae	Australia: Qld	161042	HM364945	HM365013	–
Cabeza n. sp.	Entedoninae: Euderomphalini	Argentina: Miss.	161082	HM364940	HM365010	–
Ceranisus menes	Entedoninae: Entedonini	India: Uttar P.	161120	HM364921	HM364991	HM365037
Ceratogramma sp.	Trichogrammatidae	Guadeloupe	251716	HM364942	HM364972	–
Chrysocharis sp.	Entedoninae: Entedonini	USA: CA	161050	HM364911	HM364981	HM365030
Chrysonotomyia sp.	Entedoninae: Entedonini	Thailand: Trang	161076	HM364912	HM364982	HM365031
Chrysonotomyia germanica	Entedoninae: Entedonini	Ukraine: Kiev	161156	HM364920	HM364990	HM365036
Chrysonotomyia maculata	Entedoninae: Entedonini	USA: CA	161130	HM364914	HM364984	–
Cirrospilus coachellae	Eulophinae: Cirrospilini	USA: CA	00000776	AY599268	HM365015	–
Closterocerus tau	Entedoninae: Entedonini	USA: CA	161070	HM364919	HM364989	HM365035
Closterocerus trifasciatus	Entedoninae: Entedonini	Germany: Stutt.	161090	HM364918	HM364988	HM365034
Colotrechnus ignotus	Pteromalidae: Colotrechninae	USA: CA	161379	HM364904	HM364976	HM365026
Crataepus marbis	Tetrastichinae	France: Hérault	175179	AY599262	HM365021	–
Dasyomphale chilensis	Entedoninae: Euderomphalini	Chile: R. V	161065	HM364938	HM365008	HM365044
Dicladocerus westwoodi	Eulophinae: Eulophini	Italy: Campania	174915	HM364952	HM365016	–
Elachertus sp. 1	Eulophinae: Eulophini	Italy: Lazio	161043	HM364954	HM365018	–
Elachertus sp. 2	Eulophinae: Eulophini	Thailand: Trang	161036	HM364962	HM365023	–
Emersonella planiceps	Entedoninae: Entedonini	Costa Rica	161149	HM364936	HM365006	–
Entedon diotimus	Entedoninae: Entedonini	Sweden: Skane	161141	HM364929	HM364999	HM365041
Entedononecremnus sp.	Entedoninae: Euderomphalini	USA: CA	175196	HM364939	HM365009	HM365045
Epiclerus sp. 1	Tetracampidae	Italy: Lazio	161340	HM364907	HM364974	–
Epiclerus sp. 2	Tetracampidae	USA: CA	174775	HM364908	HM364975	–
Euderomphale sp.	Entedoninae: Euderomphalini	USA: CA	161523	HM364961	HM365022	–
Euderus sp.	Entiinae	Italy: Campania	174911	AY599259	HM364969	HM365047
Eulophus sp.	Eulophinae: Eulophini	Italy: Campania	174914	HM364955	HM365019	HM365051
Euplectrus sp.	Eulophinae: Eulophini	Italy: Campania	161110	HM364953	HM365017	HM365050
Foersterella reptans	Tetracampidae	Italy: Lazio	174913	HM364906	HM364978	–
Hadrotrichodes waukheon	Tetrastichinae	USA: CA	161071	HM364959	HM365020	HM365053
Horismenus floridensis	Entedoninae: Entedonini	Costa Rica	161101	HM364930	HM365000	–
Horismenus longicornis	Entedoninae: Entedonini	Costa Rica	161096	HM364926	HM364996	–
Horismenus n. sp.	Entedoninae: Entedonini	Costa Rica	161122	HM364927	HM364997	–
Horismenus petiolatus	Entedoninae: Entedonini	Costa Rica	161169	HM364925	HM364995	HM365039
Hubbardiella n. sp.	Entiinae	Honduras	174912	AY599258	HM364973	–
Neochrysocharis formosa	Entedoninae: Entedonini	Italy: Lazio	161075	HM364909	HM364979	HM365028
Neochrysocharis clinias	Entedoninae: Entedonini	Italy: Campania	161184	HM364924	HM364994	HM365038
Neopomphale sp.	Entedoninae: Euderomphalini	Chile: R.V	161381	HM364941	HM365011	–
Omphale chryseis	Entedoninae: Entedonini	Sweden: Skane	161161	HM364916	HM364986	–
Omphale radialis	Entedoninae: Entedonini	Italy: Lazio	161095	HM364915	HM364985	HM365033
Ophelimus maskelli	Opheliminae	Italy: Lazio	161366	HM364944	HM365012	HM365046
Paracrias pubicornis	Entedoninae: Entedonini	Costa Rica	161187	HM364922	HM364992	–
Parzaommomyia sp.	Entedoninae: Entedonini	Australia: Qld.	161113	HM364917	HM364987	–
Pediobomyia canaliculata	Entedoninae: Entedonini	Costa Rica	161073	HM364931	HM365001	HM365042
Pediobius sp.	Entedoninae: Entedonini	Kenya: Kakam.	161212	HM364934	HM365004	HM365043
Pediobius alaspharus	Entedoninae: Entedonini	Sweden: Skane	161117	HM364933	HM365003	–
Pediobius pullipes	Entedoninae: Entedonini	Costa Rica	161126	HM364932	HM365002	–
Pleurotroppopsis sp.	Entedoninae: Entedonini	Thailand: Trang	161038	HM364923	HM364993	–
Pnigalio sp.	Eulophinae: Eulophini	USA: CA	49088	AY599279	HM364965	HM365052
Rhynchentedon maximus	Entedoninae: Entedonini	Thailand: Trang	161178	HM364913	HM364983	HM365032
Tetracampe sp.	Tetracampidae	Russia: Pr. Krai	174910	HM364905	HM364977	HM365027
Trisecodes agromyzae	Eulophidae: *incertae sedis*	Honduras	161204	HM364964	HM365025	HM365055
Tropicharis cecivora	Entedoninae: Entedonini	Costa Rica	161194	HM364937	HM365007	–
Zagrammosoma sp.	Eulophinae: Cirrospilini	USA: CA	49013	AY599263	HM364968	–

by a non-destructive means in which the body was not macerated but removed from the proteinase-K after a short time and cleaned for use as a primary voucher. DNA extracts were stored at −80 °F until needed. All vouchers are stored at the University of California, Riverside Entomology Research Museum (UCRC). Table 1 lists the specimens used, their classification, general locality, UCRC voucher numbers, and Genbank accession numbers of all sequences.

Polymerase chain reactions were carried out in 20-μL reactions using Promega Taq DNA polymerase (Madison, WI, USA), Qiagen 10 × PCR buffer (15 mM $MgCl_2$) and Qiagen 5 × Q-solution (Valencia, CA, USA). All genes were sequenced in the forward and reverse directions, and the resulting pair of chromatograms were compared to find PCR or reading errors. PCR products were gene-cleaned using the Bio 101 Geneclean kit (Carlsbad, CA, USA) with NaI and glassmilk. Cleaned samples were directly sequenced at either the San Diego State Microchemical Core Facility or the UC Riverside Genomics Center.

Ribosomal sequences were aligned using the secondary structure model from Gillespie et al. (2005) with regions of ambiguous alignment (RAA) aligned by eye. RAAs were retained in the analysis because they improved resolution of the ingroup. Mitochondrial sequences translated to valid amino acids, and did not possess gaps. Molecular data were partitioned by gene region, with 28S D2 and D3–D5 as separate partitions, and CO1 partitioned by codon position. Maximum percent divergence values (uncorrected *p*) were calculated using PAUP* 4.0b10 (Swofford, 2002).

Molecular and combined parsimony analyses were conducted using TNT version 1.1 (Goloboff et al., 2000). New technology searches were used in all analyses, including the bootstrap, with default settings except: ratchet weighting probability of 5% and with 200 iterations, drift of 50 cycles, tree fusing of five rounds, and find minimum length 25 times. Standard bootstrap resampling was conducted with 1000 replicates with absolute frequencies reported.

Bayesian analysis was performed using MrBayes 3.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). For each molecular partition, a six-parameter model with rates subject to a gamma distribution with a proportion of invariant sites (nst = 6 rates = invgamma) was used as suggested by hierarchical likelihood ratio tests performed using MrModeltest (Nylander, 2004). The morphological partition in the combined analysis was analysed as unordered using the Mk model (Lewis, 2001) with coding = variable and rates = gamma. In each analysis, two independent simulations of four simultaneous MCMC chains were used, sampling every 1000 generations. Convergence was reached at 5 million generations in each analysis with <0.01 standard deviation of split frequencies. The burn-in was 1.25 million generations for each analysis.

Morphological characters

The morphological component of this study includes 31 characters (Table 2). Terms follow Gibson (1997). Montage photographs were taken using either Auto-Montage software (Synoptics Ltd, UK) or the EntoVision Mobile Imaging System (GT Vision LLC). Electron micrographs were taken from uncoated specimens with a JEOL JSM 5600LV scanning electron microscope.

1. Number of flagellomeres: coded using actual number or a letter substitute from 6 to C (= 12).

The small terminal flagellomere found in some families of Chalcidoidea, including Pteromalidae, is interpreted as a segment (Onagbola and Fadamiro, 2008). The apparent maximum number of flagellomeres for Chalcidoidea is therefore 14, based on the number found in Rotoitidae (Bouček and Noyes, 1987) and in many other chalcidoid taxa, including solely *Colotrechnus* in this study. Eulophids have at most 10 flagellomeres, with a variable number in all subfamilies except Entiinae, which have a constant number of 8. This character can become problematic when claval segments are fused or when there are several basal anelliform segments that are difficult to distinguish. In the case of the club, partially fused segments are counted as separate segments. Anelliform segments were counted using slide-mounted specimens in species where the count could be problematic.

2. Number of separate claval segments in females: coded using actual number, from 1 to 4.

The antennal club is interpreted as the apical set of approximated flagellomeres, consisting of at least one segment. This character was chosen to best represent the difference in flagellar form between taxa such as *Closterocerus*, which have two funicular segments and three claval segments, from that of some other Entedonini, with three or more funicular segments and a correspondingly reduced number of claval segments. Only females were used for this count because the number can vary between sexes in a pattern that is sometimes valuable for species distinction, but is not informative across genera.

3. Shape of flagellomeres in males: 0 = cylindrical and without branches (Fig. 5: flg); 1 = nodose, with a rounded expanded section bearing elongate setae (Fig. 2); 2 = bearing two or three branches (Figs 6 and 7); 3 = cylindrical apically but with a slight basal expansion (Fig. 8).

The form of the flagellum in males is variously modified in many groups of eulophids, although there are exceptional species with a cylindrical flagellum (3:0) in each of these groups (Bouček, 1988; Burks, 2003). Strongly nodose flagellomeres (3:1), with the expanded section bearing elongate setae, are found in males of most species of Entiinae (Fig. 2). Males of many species of Tetrastichinae

Table 2
Morphological codings

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Pteromalidae: Colotrechninae																															
Colotrechnus ignotus	C	4	0	0	0	0	0	0	0	1	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	5	0	3	0	1	2
Trichogrammatidae																															
Ceratogramma sp.	8	3	0	0	0	0	0	1	2	1	0	0	0	0	2	1	2	0	0	0	0	0	0	0	1	3	0	2	0	1	2
Tetracampidae: Tetracampinae																															
Epiclerus sp. 1	A	3	0	0	0	0	0	1	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	1	1	5	1	3	0	1	2
Epiclerus sp. 2	A	3	0	0	0	0	0	1	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	1	1	5	1	3	0	1	2
Foersterella reptans	8	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	1	1	5	1	3	0	1	2
Tetracampe sp.	8	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	1	1	5	1	3	0	1	2
Eulophidae																															
Entedoninae: Entedonini																															
Achrysocharoides sp.	6	1	0	0	0	0	0	1	0	1	0	0	1	0	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Asecodes sp.	6	1	0	0	0	0	0	1	1	1	0	0	0	0	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Astichomyiia latiscapus	6	3	0	0	0	0	0	1	0	1	0	1	0	1	1	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Ceranisus menes	6	3	0	0	0	0	0	1	0	1	0	0	0	1	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Chrysocharis sp.	8	2	0	1	0	0	0	1	0	1	0	0	0	1	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Chrysonotomyia sp.	6	3	0	1	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	1
Chrysonotomyia maculata	6	3	0	1	0	0	0	1	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	1
Closterocerus germanicus	6	3	0	1	0	0	0	1	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	1
Closterocerus tau	6	3	0	1	1	0	0	1	1	1	0	0	0	1	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Closterocerus trifasciatus	6	3	0	1	1	0	0	1	1	1	0	1	0	0	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Emersonella planiceps	6	1	0	0	0	0	0	1	0	1	0	1	0	1	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Entedon ?diotimus	6	2	0	0	0	0	0	1	0	1	0	1	1	0	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Horismenus floridensis	6	2	0	0	0	0	0	1	0	1	0	1	0	1	2	0	1	0	1	0	1	0	1	0	1	4	0	2	0	1	2
Horismenus longicornis	6	2	0	0	0	0	0	1	0	1	0	1	0	0	2	0	1	0	1	0	1	0	1	0	1	4	0	2	0	1	2
Horismenus n. sp.	6	2	0	0	0	0	0	1	0	1	0	1	0	0	2	0	1	2	1	0	1	0	1	0	1	4	0	2	0	1	2
Horismenus petiolatus	6	1	0	0	0	0	0	1	0	1	0	1	0	0	2	0	1	0	1	0	1	0	1	0	1	4	0	2	0	1	2
Neochrysocharis clinias	6	3	0	0	0	0	0	1	1	1	0	0	0	0	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Neochrysocharis formosa	6	3	0	0	0	0	0	1	1	1	0	0	0	0	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Omphale chryseis	6	1	0	2	0	0	0	1	0	0	1	0	0	0	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Omphale radialis	6	1	0	2	0	0	0	1	0	1	1	0	0	0	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Paracrias pubicornis	6	3	0	0	0	0	1	1	0	1	0	0	0	1	2	0	1	0	0	0	0	0	1	0	1	4	0	2	0	1	2
Parzaommomyia sp.	6	2	0	2	0	0	0	1	1	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Pediobomyia canaliculata	6	2	0	0	0	0	1	1	0	1	0	0	0	0	2	0	1	0	0	0	1	0	0	0	1	4	0	2	0	1	2
Pediobius alaspharus	6	2	0	0	0	0	1	1	0	1	0	1	0	1	2	0	1	0	0	0	1	0	0	0	1	4	0	2	0	1	2
Pediobius pullipes	6	2	0	0	0	0	1	1	0	1	0	1	0	0	2	0	1	0	0	0	1	0	0	0	1	4	0	2	0	1	2
Pediobius sp.	6	2	0	0	0	0	1	1	0	1	0	1	0	1	2	0	1	0	0	0	1	0	0	0	1	4	0	2	0	1	2
Pleurotroppopsis sp.	6	2	0	0	0	0	1	1	0	1	0	1	1	0	2	0	1	2	0	0	0	0	0	0	1	4	0	2	0	1	2
Rhynchentedon maximus	6	2	0	0	0	0	1	1	0	1	0	1	0	0	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Trisecodes agromyzae	7	3	0	0	0	0	0	1	0	1	0	0	0	0	3	0	3	0	0	0	0	0	0	0	1	3	0	1	1	1	2
Tropicharis cecivora	6	3	0	2	0	0	1	1	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Entedoninae: Euderomphalini																															
Cabeza n sp.	6	3	0	0	0	1	0	1	0	0	0	0	0	1	0	1	2	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Dasyomphale chilensis	6	3	0	0	0	0	0	2	0	0	0	0	0	1	3	0	2	0	0	0	0	0	0	0	1	4	0	3	0	1	2
Entedononecremnus sp.	6	3	0	0	0	0	0	0	0	0	0	0	0	1	3	0	2	0	0	0	0	0	0	0	1	4	0	3	0	1	2
Euderomphale sp.	6	3	0	0	0	1	0	0	0	0	0	0	0	1	2	1	2	0	0	0	0	0	0	0	1	4	0	3	0	1	2
Neopomphale sp.	6	3	0	0	0	1	0	2	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	0	1	4	0	2	0	1	2
Entiinae																															
Astichus mirissimus	8	3	2	0	0	0	0	2	0	1	0	0	0	0	3	0	2	0	0	0	0	1	0	0	1	4	0	3	0	0	2
Astichus n. sp.	8	3	2	0	0	0	0	2	0	1	0	0	0	0	2	0	2	0	0	0	0	1	0	0	1	4	0	3	0	0	2
Bellerus sp.	8	3	2	0	0	0	0	0	0	1	0	0	0	0	1	0	2	0	0	0	0	0	0	0	1	4	0	3	0	0	2
Beornia sp.	8	2	0	0	0	0	0	0	0	0	0	0	0	0	3	0	2	0	0	0	0	0	0	0	1	4	0	3	0	1	2
Euderus sp.	8	3	2	0	0	0	0	2	0	0	0	0	0	0	3	0	2	0	0	0	0	0	0	0	1	4	0	3	0	0	2
Hubbardiella sp.	8	3	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	0	0	0	0	0	0	0	1	4	0	3	0	1	2
Eulophinae: Cirrospilini																															
Aulogymnus sp.	7	3	0	0	0	0	0	1	0	1	0	0	0	0	3	0	2	1	0	0	0	0	0	0	1	4	0	3	0	1	2
Cirrospilus sp.	6	3	0	0	0	0	0	1	0	1	0	0	0	0	3	0	2	1	0	0	0	0	0	0	1	4	0	3	0	1	2
Zagrammosoma sp.	6	3	0	0	0	0	0	1	0	1	0	0	0	0	3	0	2	1	0	0	0	0	0	0	1	4	0	3	0	1	2
Eulophinae: Eulophini																															
Dicladocerus westwoodi	8	3	1	0	0	0	0	0	0	1	0	0	0	1	3	0	2	1	0	0	0	0	0	0	1	4	0	3	0	1	2
Elachertus sp. 1	7	2	0	0	0	0	0	0	0	1	0	0	0	0	3	0	2	2	0	1	0	0	0	0	1	4	0	3	0	1	2
Elachertus sp. 2	7	2	0	0	0	0	0	0	0	1	0	0	0	0	3	0	2	2	0	1	0	0	0	0	1	4	0	3	0	1	2

Table 2 (*Continued*)

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Eulophus sp.	7	3	1	0	0	0	0	0	0	1	0	0	0	1	3	0	2	0	0	1	0	0	0	0	1	4	0	3	0	1	2
Euplectrus sp.	7	2	0	0	0	0	0	0	0	1	0	1	0	0	3	0	2	0	0	1	0	0	0	0	1	4	0	3	0	1	2
Pnigalio sp.	7	2	1	0	0	0	0	0	0	1	0	0	0	1	3	0	2	0	0	1	0	0	0	0	1	4	0	3	0	1	2
Ophelimini																															
Ophelimus maskelli	8	3	0	0	0	0	0	0	0	1	0	0	0	0	1	0	2	0	0	0	0	0	0	0	1	4	0	1	0	1	2
Tetrastichinae: Tetrastichini																															
Aprostocetus sp. 1	8	3	3	0	0	0	0	0	2	1	0	0	0	0	3	0	2	1	0	0	0	0	0	0	1	4	0	3	1	1	2
Aprostocetus sp. 2	8	3	3	0	0	0	0	1	2	1	0	0	0	0	3	0	2	1	0	0	0	0	0	0	1	4	0	3	1	1	2
Crataepus marbis	8	3	0	0	0	0	0	1	0	1	0	0	0	0	3	0	2	1	0	0	0	0	0	0	1	4	0	3	1	1	2
Hadrotrichodes waukheon	8	3	0	0	0	0	0	0	2	1	0	0	0	0	3	0	2	1	0	0	0	0	0	0	1	4	0	3	1	1	2

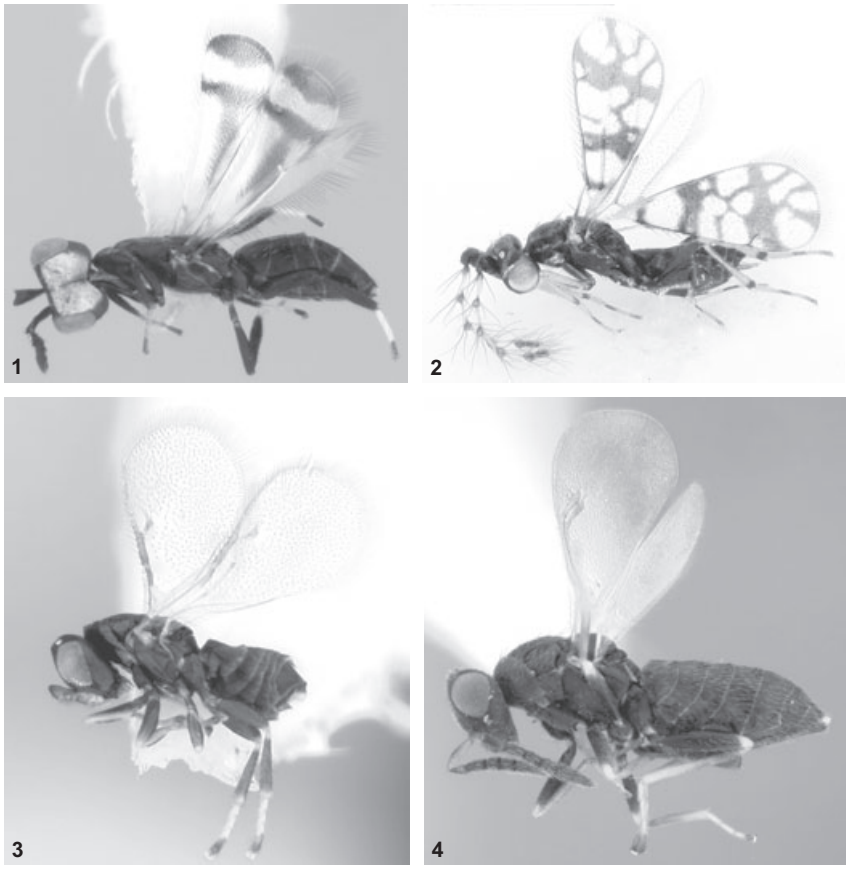

Fig. 1–4. Habitus of selected eulophids. 1. *Closterocerus tau* (Entedoninae: Entedonini). 2. *Astichus* sp. (Entiinae). 3. *Ophelimus maskelli* (Opheliminae). 4. *Aprostocetus* sp. (Tetrastichinae).

have flagellomeres with a similar, always basal, expansion bearing elongate setae (3:3, Fig. 8). However, in these species the flagellomeres are more cylindrical, with a longer apical section and less distinct expansion. These two states occur across different subfamilies that do not form a monophyletic unit in the molecular analyses. Rather than lump them into the same state, it seems best to recognize the subtle differences between them as potentially phylogenetically significant (3:1, 3:3).

Flagellar branches (3:3) are found in males of *Elasmus* Westwood and in many species of Eulophini. Three branches is the usual state in eulophines with branched flagellomeres (Fig. 6), but males of *Dicladocerus* Westwood have only two (Fig. 7). These conditions were lumped together as a single state (3:2) because separating *Dicladocerus* into a different state would needlessly create an autapomorphy.

4. Shape of basiconic peg sensilla of flagellum: 0 = symmetrical (Fig. 9); 1 = slightly asymmetrical, angular (Fig. 10); 2 = strongly asymmetrical, spear-shaped (Fig. 11).

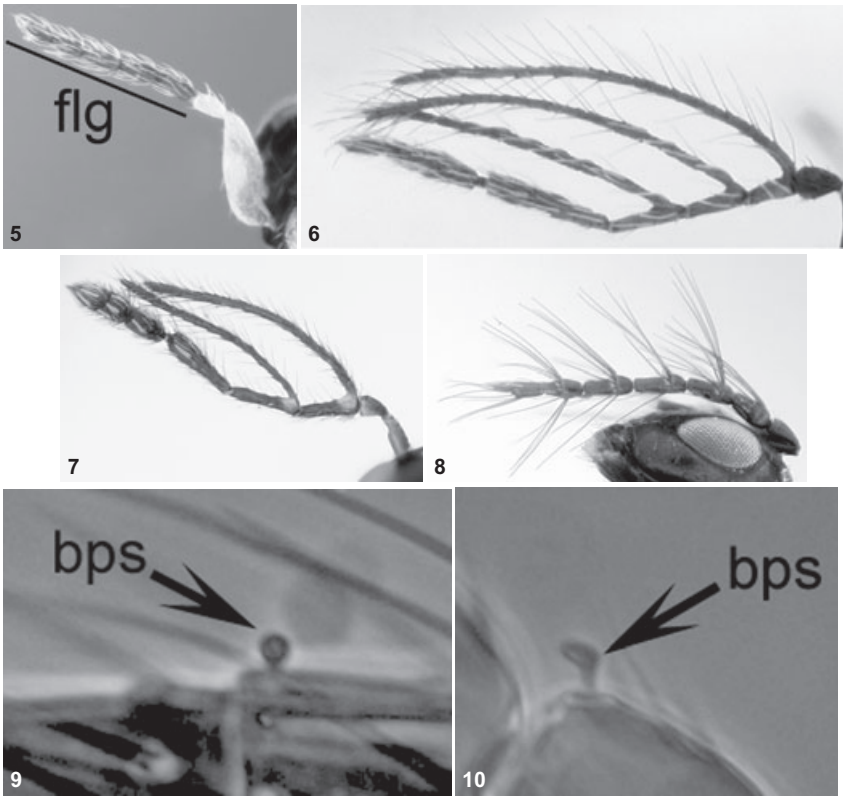

Fig. 5–10. Characters of the antennal flagellum. 5–8. Antennae of Eulophidae. 5. *Achrysocharoides* sp. 6. *Pnigalio* sp. 7. *Dicladocerus westwoodi*. 8. *Aprostocetus* sp. 9–10. Basiconic peg sensilla variation. 9. *Neochrysocharis* sp. 10. *Closterocerus* sp.

Hansson (1990, 1994, 1996) described variation in shape of the socketed, typically mushroom-shaped, basiconic peg sensilla between genera of Entedoninae and between species groups of *Omphale* Haliday. Variation in this character is one principal reason for recognition of *Closterocerus* and *Neochrysocharis* as separate genera.

5. Carinae of pedicel: 0 = absent (Fig. 12: pdl); 1 = present (Fig. 13: carina).

The pedicel in all species of the nominal subgenus of *Closterocerus* is carinate (Fig. 13) along its dorsal and ventral edges (Hansson, 1994). This state does not occur in the other subgenus, *Closterocerus* (*Achrysocharis*) Girault.

6. Sulcus across vertex between median and lateral ocelli: 0 = absent (Fig. 14); 1 = present (Fig. 15: sulcus).

Some genera of Euderomphalini possess a sulcus extending across the occipital triangle between the median and lateral ocelli (LaSalle and Schauff, 1994; Hansson and LaSalle, 2002). This sulcus is interpreted as different from the transverse facial sulci below the ocellar triangle of most Entedonini, based on positional homology. There is a different sulcus crossing the vertex behind the ocellar triangle in *Ceranisus* Walker and other thrips parasitoids in Entedonini (Schauff, 1991). The vertex sulcus in *Ceranisus* was not coded in this analysis because it would be autapomorphic.

7. Vertex posterior carina: 0 = absent (Fig. 14); 1 = present (Fig. 16: carina).

A carina separating the vertex and occiput is found in many species across Eulophidae, but is locally informative in separating the genus *Horismenus* Walker (absent) from *Pediobius* Walker (present) (Hansson, 2002).

8. Transverse facial sulcus: 0 = absent; 1 = present and adjacent to the median ocellus (Fig. 17: tfs); 2 = separated from the median ocellus by at least the diameter of the median ocellus (Fig. 18: tfs).

This character is a modified version of a previous interpretation of the transverse facial sulcus in Entedonini by LaSalle and Schauff (1994), which used the distance between the median ocellus and toruli as a point of comparison. It also incorporates (state 1) a character introduced by Gauthier et al. (2000) as a potential synapomorphy of Entiinae (Fig. 17), but which is also found in some Euderomphalini. The previous interpretation of the entedonine state is problematic because entedonine species in *Chrysonotomyia* and *Emersonella* Girault, among many others, have a transverse facial sulcus near the median ocellus that is

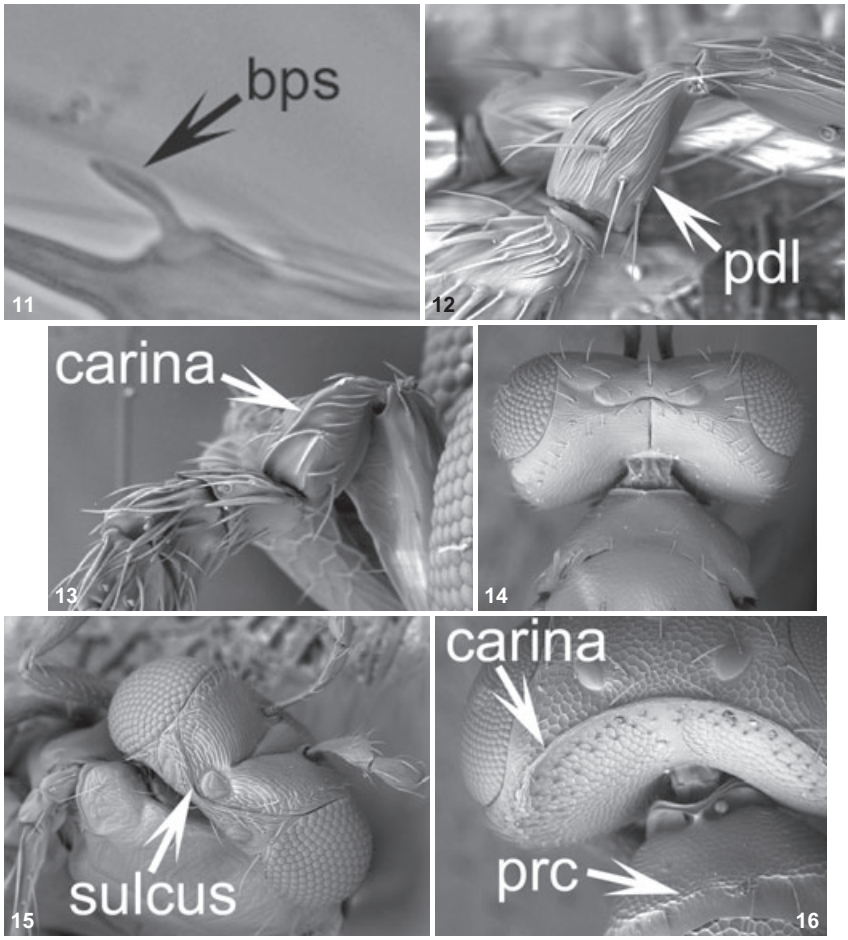

Fig. 11–16. Characters of antenna, head and pronotum. 11. *Omphale* sp. basiconic peg sensilla. 12. *Pediobius* sp. antenna, pdl = pedicel. 13. *Closterocerus tau* antenna: pdl = pedicel. 14. *Asecodes* sp. vertex. 15. *Neopomphale* sp. vertex. 16. *Pediobius* sp. vertex and pronotum, prc = pronotal collar carina.

apparently homologous to the more V-shaped sulcus found in most Entedonini (Fig. 18). The entedonine sulcus is separated from the median ocellus by a greater amount than found in most Entiinae, and therefore is interpreted as being different (8:2).

9. Subtorular grooves: 0 = absent; 1 = present, extending from ventral edge of torulus (Fig. 18: stg); 2 = present, extending from lateral edge of torulus (Fig. 19: stg).

The presence of subtorular grooves (9:1) was used by Gumovsky (2001) as a reason for synonymizing *Neochrysocharis*, *Asecodes* and a number of other genera under *Closterocerus*. The resulting genus was then interpreted as different from *Chrysonotomyia*, which also possesses subtorular grooves, because the latter has a distinctly defined clypeus. It was later acknowledged by Hansson (2004) as a valid means of defining *Chrysonotomyia*, in combination with several other characters. The grooves found in Tetrastichinae (Fig. 19: stg) and Trichogrammatidae (9:2) are here interpreted as different because they contact the torulus near its lateral edge and expand dorsally, instead of ending as simple grooves.

10. Delimitation of clypeus: 0 = delimited at least by lateral grooves (Figs 20 and 21: cly); 1 = not delimited (Fig. 18).

Delimitation of the clypeus has historically been used to separate *Chrysonotomyia*, *Omphale*, *Parzaommomyia*, and some other genera from the rest of Entedonini (Graham, 1959; Bouček, 1988; Hansson, 1990, 1994, 2004; Gumovsky, 2001). However, this character is problematic because the clypeus is distinct in some species not included in these genera and indistinct in some species found within these genera (Hansson, 1996; Burks, 2003). This discrepancy may be due to its being interpreted inconsistently with respect to preconceived notions regarding each of these genera. It seems likely that facial shape and proportions play a role in this inconsistent evaluation, as communicated by the novel wording of character 11.

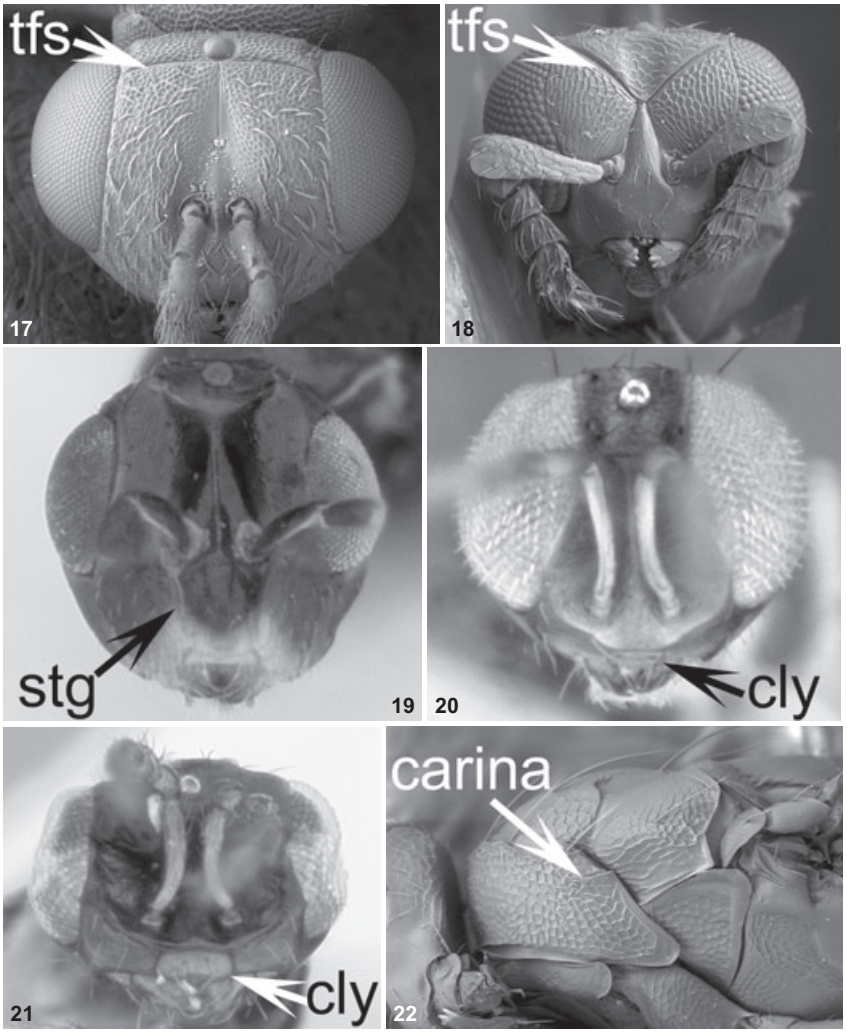

Fig. 17–22. Characters of the head and pronotum. 17. *Euderus* sp., tfs = transverse facial suture. 18. *Closterocerus trifasciatus*, stg = subtorular groove. 19. *Aprostocetus* sp. 20. *Chrysonotomyia germanica* **n. comb.**, cly = clypeus. 21. *Omphale* sp. 22. *Achrysocharoides* sp.

11. Clypeus width: 0 = not enlarged, width less than malar space (Fig. 20: cly); 1 enlarged, width greater than or equal to malar space (Fig. 21: cly).

Most species of *Omphale* have a broadened clypeus (Fig. 21: cly), but in some species the clypeus is either not indicated or not broadened. While state 1 is not present in all species of *Omphale* (Hansson, 1996), it does provide a link between some species groups. Variation in clypeal form is rare among other genera of Entedonini, but an unusual clypeus does also occur in the genus *Clypecharis* Gumovsky.

12. Pronotal collar carina: 0 = absent (Figs 23–27 and 33); 1 = present (Fig. 16: prc).

A carina extending transversely across the anterior edge of the pronotal collar is present in many different eulophids. This character is locally informative for distinguishing some genera, such as *Pediobius* versus *Paracrias* Ashmead (Hansson, 2002).

13. Semicircular ridge of pronotum laterally: 0 = absent; 1 = present (Fig. 22: carina).

State 1 (Fig. 22) was described by Gumovsky (2001) as a possible synapomorphy of *Achrysocharoides* Girault and *Entedon* Dalman. Although a similar ridge is found in some species of *Chrysocharis* (Burks, 2003), this character is coded as specified by Gumovsky.

14. External completeness of notauli posteriorly: 0 = reaching trans-scutal articulation (Figs 23 and 26); 1 = not reaching trans-scutal articulation, essentially absent (Figs 24, 25, 28 and 33).

This character varies across all subfamilies of Eulophidae except Entiinae and Tetrastichinae. While Krogmann and Vilhelmsen (2006) have shown that external incompleteness does not necessarily indicate internal incompleteness, the character is interpreted here in keeping with previous literature (Graham, 1959; Bouček, 1988). It can be problematic in cases where

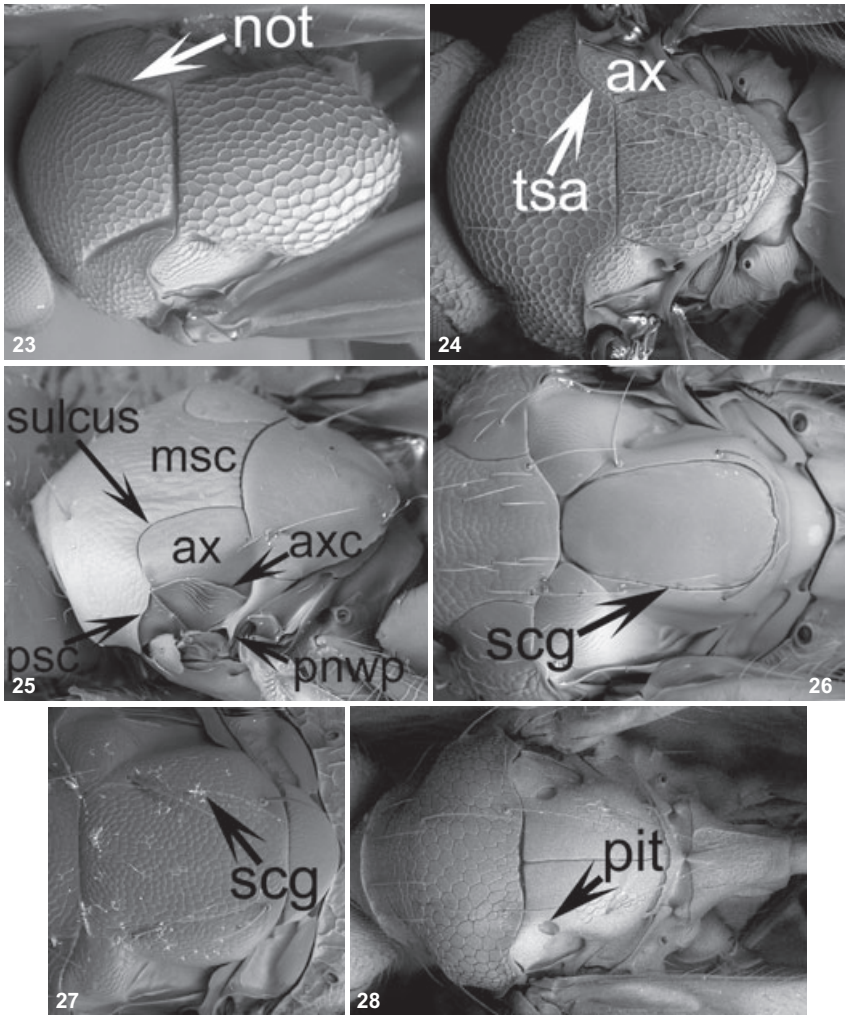

Fig. 23–28. Characters of the mesosoma. 23. *Hubbardiella* n. sp., not = notaulus. 24. *Entedononecremnus* sp., ax = axilla, tsa = trans-scutal articulation. 25. *Euderomphale* sp., axc = axillar carina, msc = mesoscutum, pnwp = posterior notal wing process, psc = parascutal carina, tgl = tegula. 26. *Elachertus* sp., scg = scutellar groove. 27. *Cirrospilus* sp. 28. *Horismenus* sp.

the external indication of the notauli ends as a gradually less-defined groove. In those cases, if the groove could at all be traced to the trans-scutal articulation, it is interpreted as complete. Cases where the notauli end at a strongly advanced axilla instead of extending to the scutellar disc are not distinguished here, because there is a smooth continuum between those two conditions among eulophids. The notauli are incomplete anteriorly (Fig. 23) only in *Hubbardiella* Ashmead (Coote, 1994) among examined eulophids.

15. Pairs of mesoscutal midlobe bristles: coded using the actual number, from 0 to 3 except that 3 includes counts of 3 or greater.

This character was used by Schauff (1991) as a potential means of defining Entedonini. However, it varies within Entedonini in a way that is often useful in distinguishing genera and species groups. The distinction between bristles and setae can sometimes be problematic (cf. Fig. 26, state 3), but in this analysis no distinction is made between them.

16. Advancement of axillae: 0 = dorsal axillar surface not completely advanced beyond anterior margin of scutellar disc (Figs 23, 24, 26–28 and 33: ax); 1 = dorsal axillar surface completely advanced beyond anterior margin of scutellar disc (Fig. 25: ax).

The axillae are advanced entirely beyond the scutellar disc (Fig. 24) in some genera of Euderomphalini (LaSalle and Schauff, 1994), causing them to be interpreted as the side lobes of the mesoscutum (Gumovsky, 2002). They are similarly advanced in the outgroup taxa *Ceratogramma* and *Colotrechnus*. In other eulophids, the dorsal surface of the axilla extends alongside the scutellar disc.

17. Pairs of scutellar disc setae: coded using the actual number, from 1 to 3 except that 3 includes counts of 3 or greater.

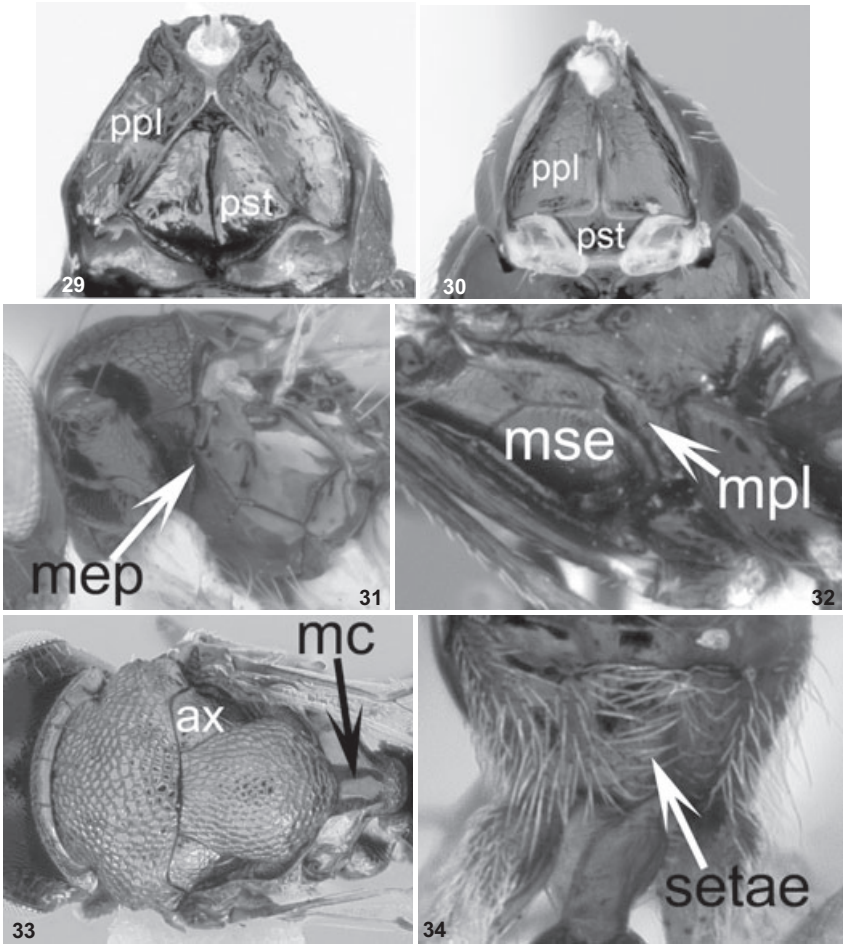

Fig. 29–34. Characters of the mesosoma. 29. *Dicladocerus* sp. prothorax, ventral. 30. *Necremnus* sp. prothorax, ventral. 31. *Horismenus petiolatus*, mep = mesepisternal projection. 32. *Astichus* sp., mse = mesepimeron, mpl = metapleuron. 33. *Paracrias arizonensis*, ax = axilla, mc = median carina. 34. *Epiclerus* sp. propodeum.

This presence of only one pair of setae on the scutellar disc has been used to help define Entedonini (Schauff, 1991), although some species have additional setae. The character remains useful, because there are very few exceptions within each tribe of eulophids. As in character 15, no distinction is made between bristles and setae.

18. Scutellar grooves: 0 = absent (Figs 23, 24, 28 and 33); 1 = present as a U-shaped groove open anteriorly (Fig. 26: scg); 2 = present as parallel grooves open both anteriorly and posteriorly (Fig. 27: scg).

No eulophid subfamily or tribe is constant for either state 1 or 2, but Entiinae and Euderomphalini all lack scutellar grooves. While some Eulophini characteristically have a U-shaped groove (Peck et al., 1964), this state also occurs in some Entedonini (Schauff, 1991). Other Eulophini, a few Entedonini, and many Tetrastichinae have parallel grooves instead. These are not the same as axillular grooves, which co-occur with scutellar grooves.

19. Pit along scuto-scutellar sulcus between axilla and scutellar disc: 0 = absent (Figs 24, 26 and 33); 1 = present (Fig. 28: pit).

This characteristic pit is apparently unique to *Horismenus* among eulophids (Hansson, 2002, 2009).

20. Propleura: 0 = posterior margins diverging angularly along prosternum (Fig. 29: ppl); 1 = posterior margins transverse, diverging at right angles at prosternum (Fig. 30: ppl).

State 1 was used by Gauthier et al. (2000) as a potential synapomorphy of Eulophini, with a reversal occurring in *Dicladocerus*. It also occurs in some species of *Elasmus*, which have a continuous grade of variation between the two states. State 1 is not found in any other eulophids, but does occur in other families of Chalcidoidea, such the pteromalid subfamilies Spalangiinae and Cerocephalinae.

21. Mesepisternal projection over posterior margin of prepectus: 0 = absent; 1 = present (Fig. 31: mep).

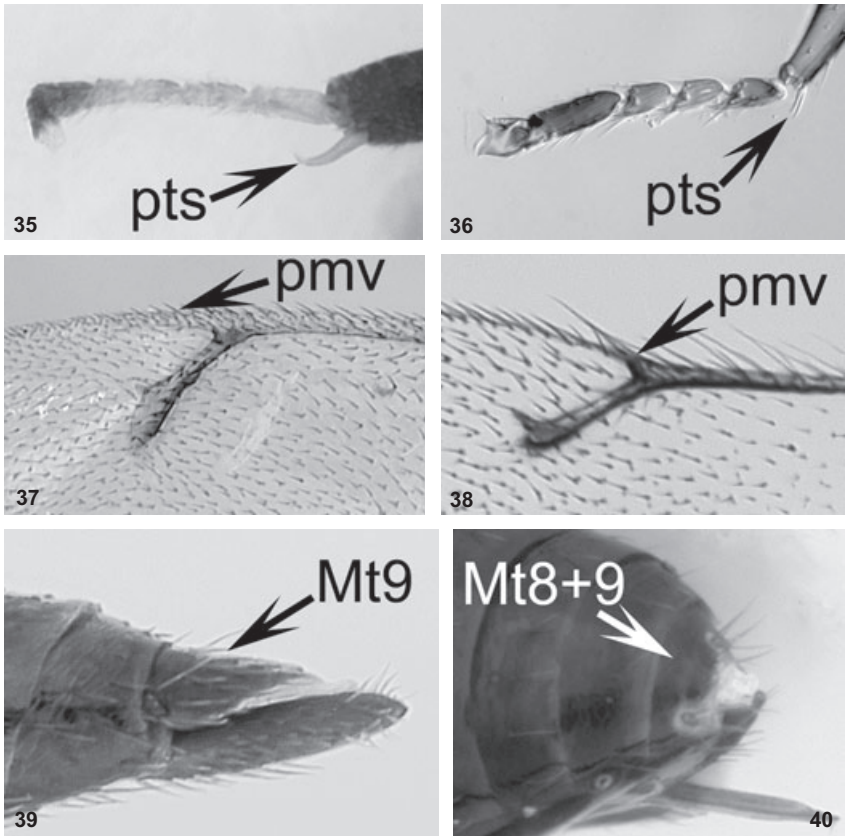

Fig. 35–40. Characters of the legs, wings and metasoma. 35. *Colotrechnus ignotus* fore tarsus (pts = protibial spur). 36. *Euderomphale* sp. fore tarsus. 37. *Aulogymnus* sp. forewing venation (pmv = postmarginal vein, stv = stigmal vein). 38. *Aprostocetus* sp. forewing venation. 39. *Euderus* sp. gastral apex (Mt_9 = metasomal tergite 9). 40. *Beornia* sp. gastral apex (Mt_{8+9} = metasomal tergite 8 + 9).

A narrow, lobe-like projection from the mesepisternum extending anteriorly to slightly overlap the posterior margin of the prepectus was described by Schauff (1991) as a synapomorphy of *Horismenus* (Fig. 31). It has since been found in some other entedonine genera, including *Pediobius* (Hansson, 2002).

22. Expansion of mesepimeron over metapleuron: 0 = mesepimeron not expanded over metapleuron (Fig. 31); 1 = mesepimeron expanded, overlapping metapleuron (Fig. 32: mse).

The mesepimeron is strongly expanded in some genera of Entiinae, becoming convex laterally and overlapping the metapleuron, hiding its anterior edge (Fig. 32). In other eulophids the mesepimeron is flat and either abuts or only slightly overlaps the edge of the metapleuron (Fig. 31).

23. Median carina of propodeum: 0 = not flattened dorsally (Fig. 26); 1 = flattened dorsally (Figs 28 and 33: mc).

The median propodeal carina of most species of *Horismenus* (Fig. 28) and *Paracrias* (Fig. 33: mc) is broadly flattened along its length and may also project to the metapleuron (Schauff, 1991). This character also occurs in some species of *Pediobius* (Hansson, 2002).

24. Setae of propodeal disc: 0 = not curving mesad; 1 = curving mesad (Fig. 34).

The setae along the submedian surface of the propodeal disc, not including the propodeal callus setae, curve mesad in all Tetracampinae (Peck et al., 1964; Bouček, 1988) and is a likely synapomorphy of the subfamily. This character does not occur in other chalcidoids, where most species have an entirely bare propodeal disc (Figs 26, 28 and 33), with rare exceptions.

25. Protibial spur (=calcar): 0 = stout and curved (Fig. 35: pts); 1 = slender and straight (Fig. 36: pts).

The presence of a reduced protibial spur has historically been used to help define Eulophidae (Peck et al., 1964; Bouček, 1988), but also occurs in Tetracampidae, Trichogrammatidae, and arguably in some other families of Chalcidoidea (LaSalle et al., 1997). Although variation exists in this character within Eulophidae and other families, it does not vary among the taxa included in this analysis and is here interpreted in its more conventional sense.

26. Number of tarsomeres in fore leg: coded using the actual number, from 3 to 5.

Rotoitidae, almost all Eulophidae, and a few species of Aphelinidae, Pteromalidae and male Agaonidae have

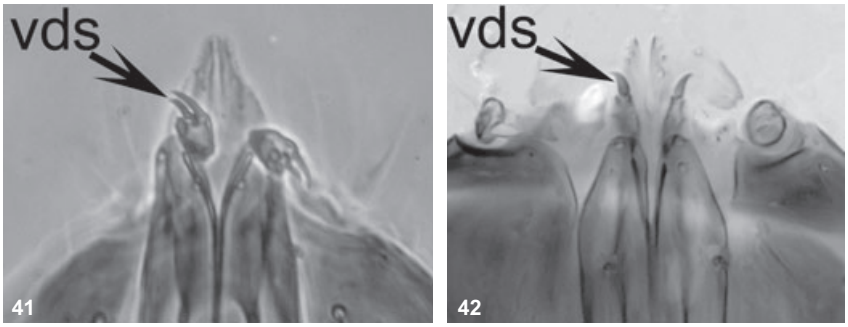

Fig. 41–42. Characters of the male genitalia. 41. *Closterocerus* sp., vds = vosellar digitus spine. 42. *Chrysonotomyia* sp.

four tarsomeres for all legs. *Trisecodes agromyzae* Delvare & LaSalle has been the only exceptional eulophid, having only three. Among the outgroup taxa, *Ceratogramma* has three, as in all other Trichogrammatidae and a few other chalcidoids. *Colotrechnus* has five tarsomeres, as in most Chalcidoidea.

27. Number of tarsomeres in mid leg compared with fore leg in males: 0 = same; 1 = one less.

Male Tetracampinae have four tarsomeres for the mid leg in males, but five for the other legs (Peck et al., 1964; Bouček, 1988). This may be a synapomorphy for the subfamily.

28. Submarginal vein setae: coded using the actual number, where 3 includes counts of 3 or greater.

Most Entedonini have only two submarginal vein setae, and this character has been used as a potential synapomorphy of Entedonini (Schauff, 1991). Although it varies in other eulophids, most notably in Tetrastichinae, it remains a useful character.

29. Postmarginal vein length: 0 = more than one-third stigmal vein length (Fig. 37: pmv); 1 = less than or equal to one-third stigmal vein length (Fig. 38: pmv).

The postmarginal vein in most species of Tetrastichinae is extremely short or absent (LaSalle, 1994). While this character also occurs in some species of Entedoninae and varies within Tetrastichinae, it remains a convenient character for Tetrastichinae in the absence of any known universal diagnostic characters for the subfamily (Gibson et al., 1999).

30. Epipygium (Mt_9) in females: 0 = separate from Mt_8 (Fig. 39: Mt_9); 1 = fused with Mt_8, forming a syntergum (Fig. 40: Mt_{8+9}).

All Entiinae except *Beornia* Hedqvist and *Hubbardiella* have a separate Mt_9 in females (Coote, 1994). This character does not occur in any other eulophids, although it is present in some other Chalcidoidea. A separate Mt_8 (30:0) is generally considered a plesiomorphic trait (Bouček, 1988), but varies throughout Chalcidoidea in ways that suggest rather that it is locally informative.

31. Number of volsellar digital spines: 1 = 1 volsellar spine; 2 = 2 or more volsellar spines (Figs 41 and 42: vds).

Most eulophids have a pair of spines on each digitus (Fig. 41: vds). A single spine is present on each volsellar digitus (Fig. 42: vds) in *Chrysonotomyia* Hansson (2004).

Results and discussion

Monophyly of Eulophidae

Parsimony and Bayesian analyses agree on the higher classification of Eulophidae in all respects except on Entedoninae and *Ophelimus* (Figs 43–46). Monophyly of Eulophidae, excluding *Trisecodes agromyzae*, is supported in all analyses. Monophyly of Eulophidae including *T. agromyzae* was attained in all analyses but supported only in the Bayesian analyses, and then only weakly (58–59%). *Trisecodes* has only three tarsomeres instead of four, and while described as an entedonine, it was placed there with some doubt because it bears no strong similarity to particular known entedonines (Delvare and LaSalle, 2000). *Trisecodes* is also unusual among entedonines in having three pairs of mesoscutal midlobe setae (15:3), three pairs of scutellar setae (17:3), and only one submarginal vein seta instead of two (28:1). It shares a V-shaped transverse facial sulcus (8:2) with other entedonines (as in Fig. 18). Results from an analysis across all chalcidoid families using 28S D2–D5 and 18S rDNA place *T. agromyzae* far outside an otherwise monophyletic Eulophidae (J.B. Munro, J.M. Heraty, R.A. Burks, unpublished), but do not consistently associate it with any other family. These results put family placement of *T. agromyzae* in doubt, but do not indicate a better placement for this monotypic genus. Regardless of family classification, we find no justification for transferring *T. agromyzae* to any current subfamily of Eulophidae, and it seems best to consider it as incertae sedis within Eulophidae **new placement** until new information is available.

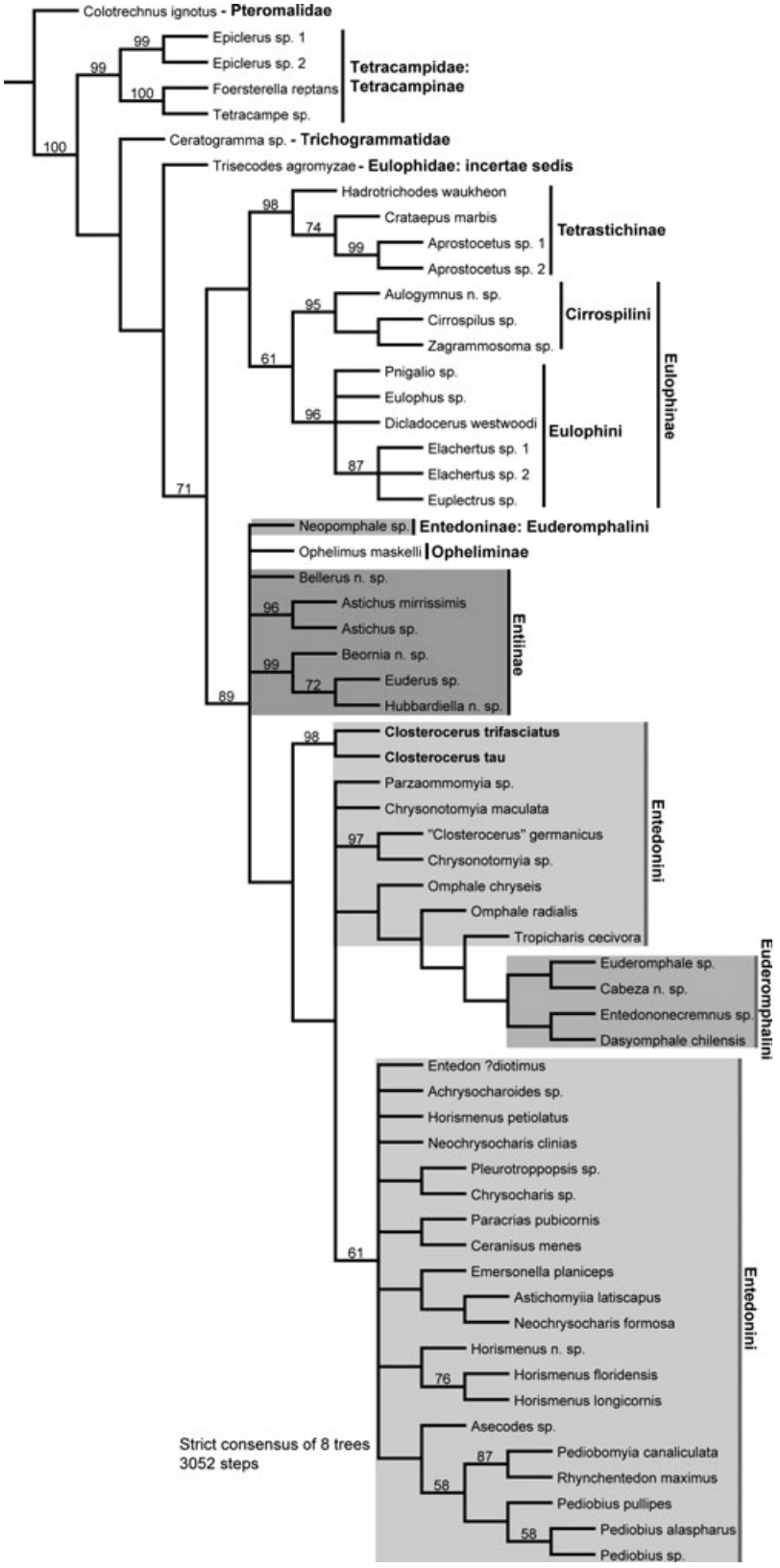

Fig. 43. Strict consensus of 8 trees of 3052 steps, unweighted parsimony analysis of molecular data. Numbers above branches indicate bootstrap support of 55% or above. Suprageneric taxa indicated by a vertical bar. *Closterocerus* s.s. indicated in bold.

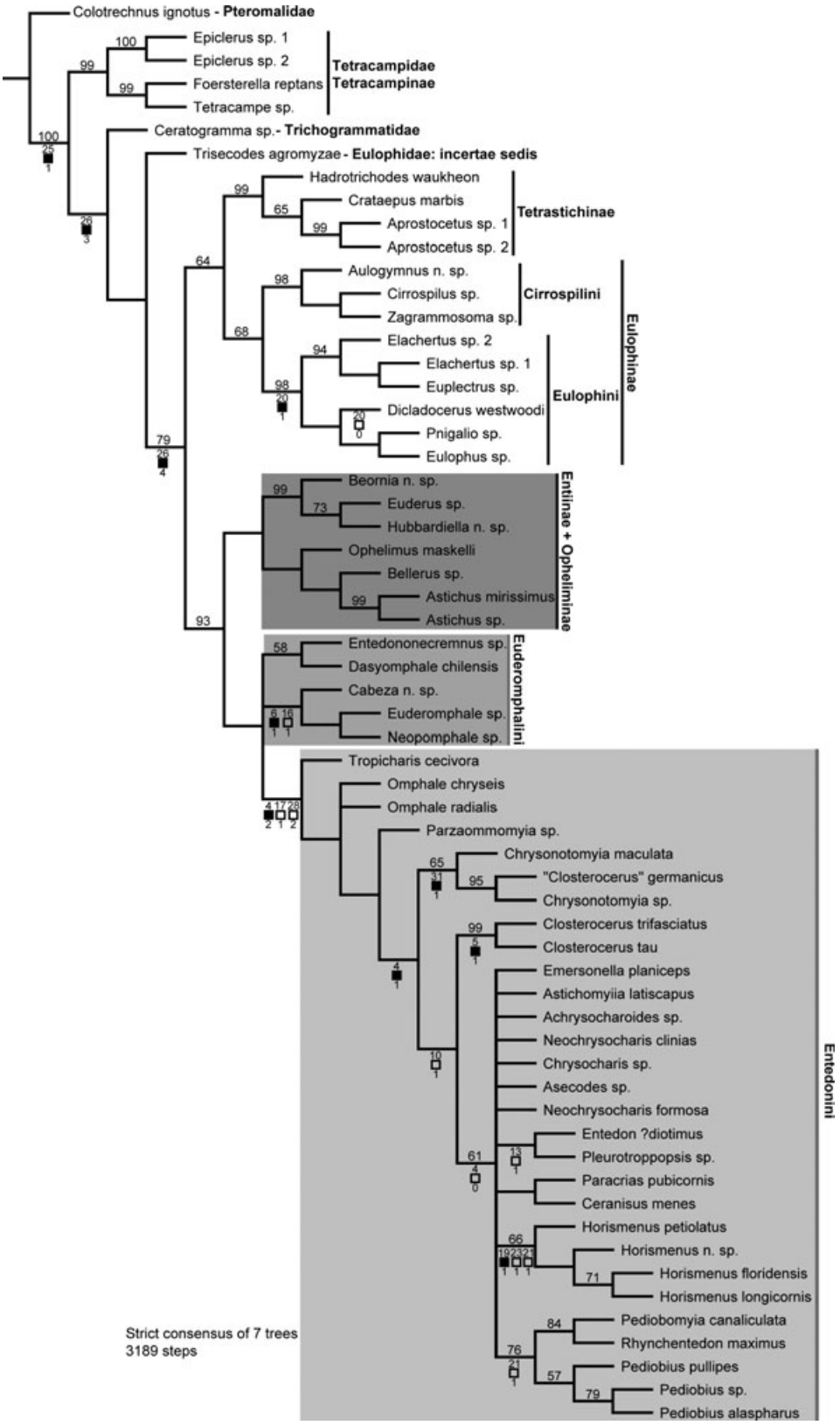

Fig. 44. Strict consensus of 7 trees of 3189 steps, unweighted parsimony analysis of all molecular and morphological data. Numbers above branches indicate bootstrap support of 55% or above. Suprageneric taxa indicated by a vertical bar. *Closterocerus* s.s. indicated in bold.

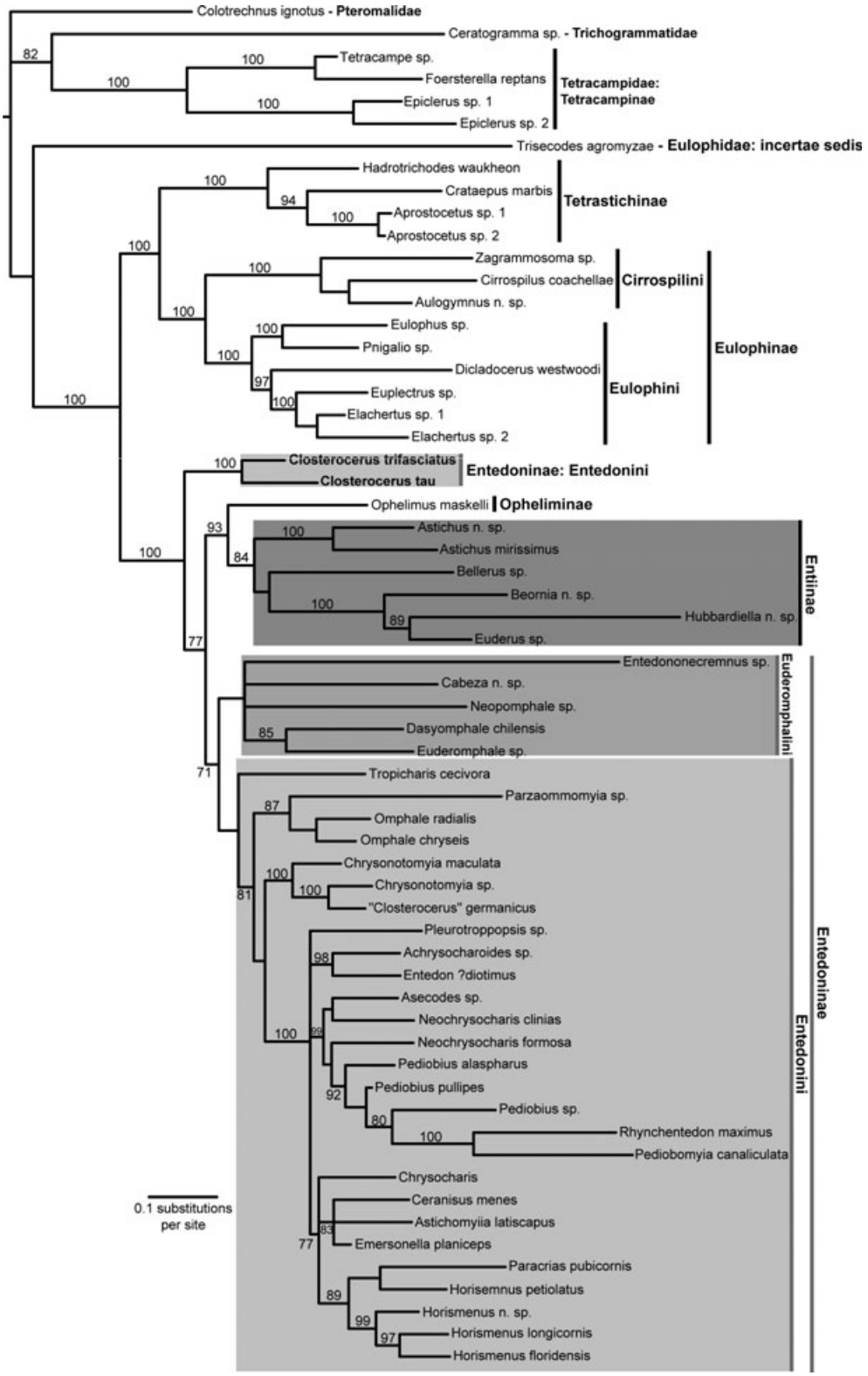

Fig. 45. Bayesian molecular-only results summarized on a 50% majority rule tree with branch lengths included. Posterior probability values higher than 70% indicated on branches. Suprageneric taxa indicated by a vertical bar. Black bars indicate monophyletic groups, gray bars indicate non-monophyletic groups. *Closterocerus* s.s. indicated in bold.

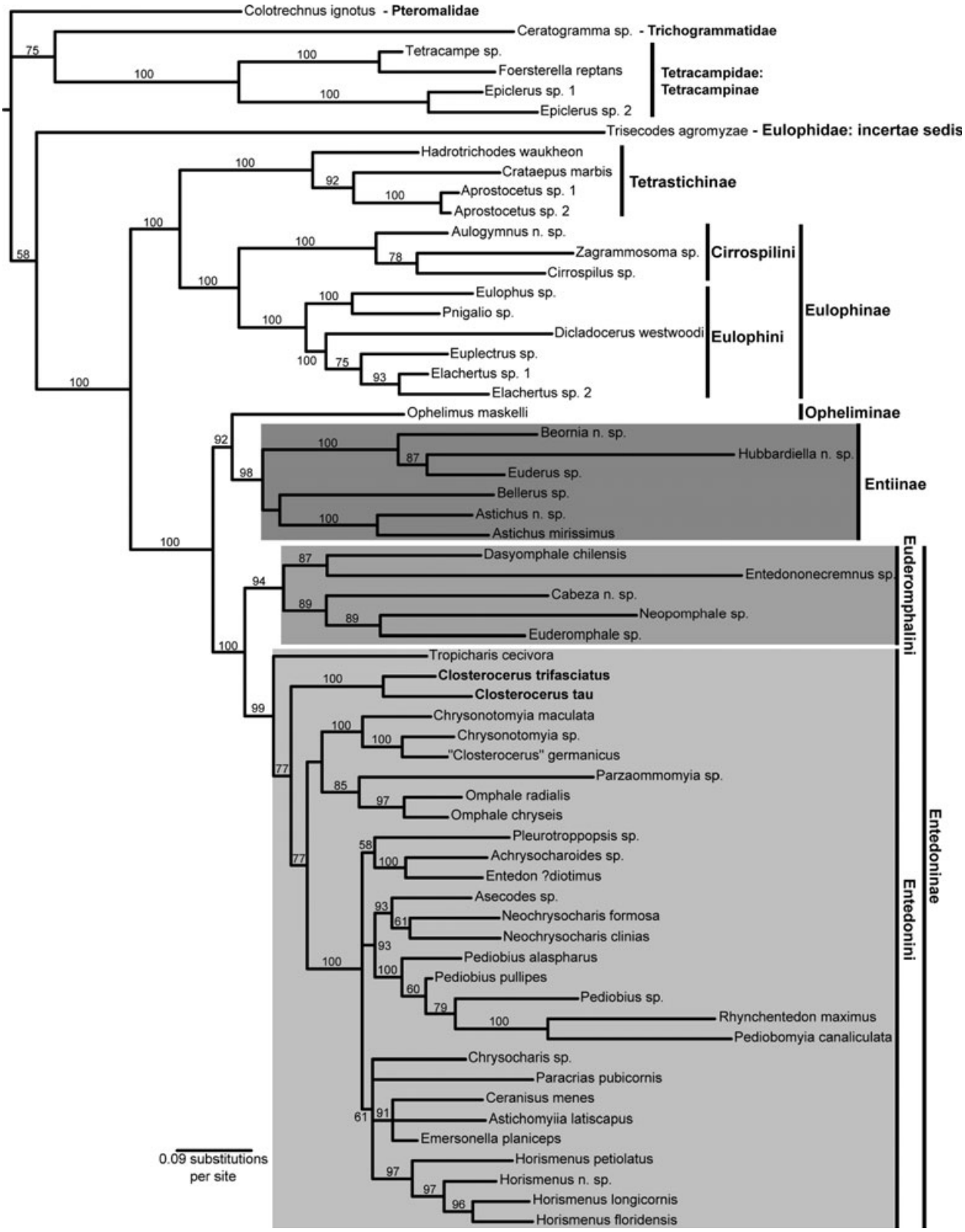

Fig. 46. Bayesian combined morphological and molecular results, 3e′ subregion included, summarized on a 50% majority rule tree with branch lengths included. Posterior probability values higher than 70% indicated on branches. Suprageneric taxa indicated by a vertical bar. *Closterocerus* s.s. indicated in bold.

Ophelimus and monophyly of Entiinae

Bayesian results (Figs 45 and 46) support a monophyletic Entiinae with *Ophelimus* as its sister group. Parsimony (Figs 43 and 44) instead indicates either a clade of *Ophelimus* + Entiinae with *Ophelimus* arising within Entiinae, or an unresolved grouping of *Ophelimus* and Entiinae with parts of Entedoninae. The clade of *Ophelimus* plus or within Entiinae has not been recognized by any previous author.

Ophelimus had previously been placed in Eulophinae along with several other genera in the tribe Ophelimini (Bouček, 1988). Gauthier et al. (2000) removed most of the other genera to form the tribe Cirrospilini. This left only *Ophelimus* and *Australsecodes* Girault in a reduced Ophelimini. This group was then moved to incertae sedis within Eulophidae, outside Eulophinae, because it differed strongly from Eulophinae in 28S D2 data.

While it is tempting to transfer ophelimines to Entiinae (the name Opheliminae would have priority), this ignores the lack of known, explicitly definable morphological characters shared by entiines and ophelimines. Most importantly, ophelimines lack all three of the characters specified by Coote (1994) as helpful in recognizing Entiinae: a bare area under the forewing marginal vein exposing ventral admarginal setae, scutellum overhanging the reduced and concave axillulae, and the separated Mt_9 (character 30:0, Fig. 39). These characterize all Entiinae except *Beornia* and *Hubbardiella* (Fig. 40). While these three characters are neither unique to, nor universally found in, Entiinae, there have been no shared characters found for ophelimines and entiines that are not also found in all other eulophid subfamilies. Combining the two would therefore result in a group that is more difficult to characterize morphologically than either of the currently separate groups. The only benefit of combining ophelimines and entiines into a single subfamily would be the ability to refer to this clade as a subfamily in the current analyses. This seems to be a very minor gain compared with the drawbacks of proposing a newly delimited subfamily that cannot currently be defined morphologically. For these reasons, it seems best to acknowledge the molecularly supported sister-group relationship between ophelimines and entiines by recognizing them as equal in taxonomic rank—elevating Ophelimini to subfamily rank as Opheliminae **new status**, and retaining *Australsecodes* in Opheliminae until it can also be analysed molecularly. Although some other unplaced tribes in Eulophidae, such as Anselmellini and Platytetracampini, have not been analysed in this study, their eventual position should not affect recognition of Opheliminae. While one or both of these taxa, and possibly even Entiinae itself, may eventually become synonyms of Opheliminae, they would be junior synonyms.

In the analyses by Gauthier et al. (2000), the Neotropical entiine genus *Bellerus* Walker placed outside Eulophidae, with either *Idioporus affinis* or *Kerya* Bouček. This raised doubts over its family and subfamily affinities. All molecular and combined analyses (Figs 43–46) place it with Entiinae.

The only consistently supported clade within Entiinae was the grouping of *Beornia* + *Euderus* + *Hubbardiella*. Although *Beornia* and *Hubbardiella* are the only entiines with a fused Mt_{8+9} (character 30:1, Fig. 40), *Hubbardiella* was consistently the sister group to *Euderus* in molecular and combined analyses.

Monophyly of Eulophinae

The reduced version of Eulophinae as defined by Gauthier et al. (2000), and its two sampled tribes Cirrospilini and Eulophini, were at least weakly supported as monophyletic in all molecular and combined analyses. Gauthier et al. (2000) pointed out that in all Eulophini except *Colpoclypeus* Lucchese and *Dicladocerus*, the propleura diverge at right angles upon reaching the prosternum (character 20:1, Fig. 30). This raised some doubts concerning placement of the two exceptions. *Colpoclypeus* was not available for sequencing, but *Dicladocerus westwoodi* was consistently part of a monophyletic Eulophini in all molecular and combined analyses, between the clades *Eulophus* + *Pnigalio* and *Elachertus* + *Euplectrus* (Figs 43–46). This suggests that a reversal in propleural form has occurred at least once within Eulophini.

Monophyly of Entedoninae

Aside from Entiinae, the only other eulophid subfamily that was paraphyletic in any molecular analysis was Entedoninae. Likewise, its tribe Euderomphalini was paraphyletic in parsimony analyses (Figs 43 and 44). Entedonini was paraphyletic in all but the two combined analyses (Figs 44 and 46). While the placement of *Trisecodes agromyzae* had admittedly been controversial (Delvare and LaSalle, 2000), there had never been any doubt concerning the subfamily placement of *Closterocerus* Westwood. The molecular Bayesian analysis (Fig. 45) placed *Closterocerus* sensu stricto as the sister group to other Entedoninae + Opheliminae + Entiinae. This is in agreement with the previous analysis by Gauthier et al. (2000), suggesting that these findings are unlikely to be due to sequencing error. Combined analyses (Figs 44 and 46) instead indicated a monophyletic Entedonini with *Closterocerus* s.s. arising within it. The molecular-only parsimony analysis differed in that *Closterocerus* s.s. was sister group to all other Entedoninae except *Neopomphale* (Fig. 43).

However, removal of six contiguous bases, the 3e′ subregion in the 28S D2 rDNA, from all sequences in

Table 3
Support values for selected clades. Reported as bootstrap and posterior probability values above 55

Group	Parsimony (TNT)			Bayesian (MrBayes)		
	Mol(+3e′)	Mol(–3e′)	Comb	Mol(+3e′)	Mol(–3e′)	Comb
Eulophidae minus *Trisecodes*	71	73	79	100	100	100
Eulophidae + *Trisecodes*	y	y	y	59	n	58
Entiinae + Entedoninae + Opheliminae	89	90	93	100	100	100
Entiinae minus Euderomphalini	n	n	n	84	76	98
Entiinae + Euderomaphlini	n	n	n	n	n	n
Entedonini + Euderomphalini	n	n	y	71	n	100
Euderomphalini	n	n	n	63	n	94
Entedonini + *Closterocerus* s.s.	n	n	y	n	n	99
Entedonini minus *Closterocerus* s.s.	n	n	n	68	n	n
Closterocerus s.s. + *Chrysonotomyia*	n	n	n	n	71	n

Mol = Molecular only (=/–, with and without 3e′ region); Comb = combined analysis; y = clade present but without support; n = clade absent.

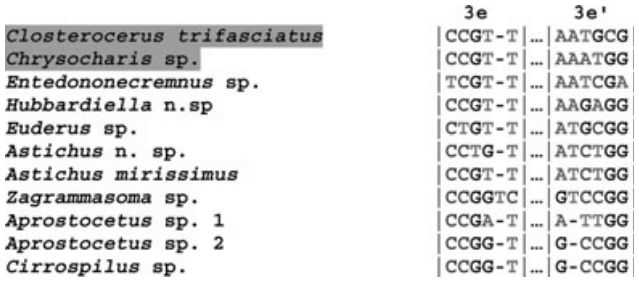

Fig. 47. The 3e and 3e′ subregions for *Closterocerus* compared with that of other selected eulophids as aligned by the secondary structure model provided by Gillespie et al. (2005). Species Other Entedonini have the same sequence as *Chrysocharis* sp. in these subregions. Intervening bases between the two subregions omitted.

the analysis resulted in the placement of *Closterocerus s.s.* within Entedonini as the sister group to *Chrysonotomyia* in both molecular-only analyses (Table 3). The 3e′ subregion in both sampled species of *Closterocerus s.s.* is very different from that of other entedonines (Fig. 47) and could be both the defining trait of the subgenus and the reason why molecular results consistently place it far from morphologically similar genera.

Subfamily placement of Euderomphalini

Gumovsky (2002) transferred Euderomphalini to Entiinae, based on 28S D2 data, the apparently posteriorly complete notauli in all members of each group (character 14:0, Fig. 23), and the distinctness of the clypeus (character 10:0) in at least some members. Each of these criteria appears to be problematic.

The reinterpretation of the notauli as complete in euderomphalines was a novel conclusion based on the state in *Euderomphale* Girault (Fig. 25), where LaSalle and Schauff (1994) had previously considered the notauli not to be indicated externally. If the notauli were complete, this state would be shared with Entiinae. The disagreement is based on differing interpretations of a pair of dorsal thoracic sulci in *Euderomphale* (Fig. 25: sulcus). Positional homology suggests that these sulci are part of the trans-scutal articulation, which separates the axillae and scutellum from the mesoscutum (Gibson, 1997), meaning that they cannot be the notaular grooves. More specifically, the tegula and the posterior notal wing process are landmarks that can be used to recognize the lateral surfaces of the mesoscutum and axilla. The tegula (Fig. 25: tgl) abuts the lateral aspect of the mesoscutum mesally. The posterior notal wing process (Fig. 25: pnwp) extends between the forewing and hind wing bases, connecting with the dorsal sclerites of the mesosoma with two arm-like processes. The anterior arm ends at the anterior edge of the lateral surface of the axilla at the forewing base. The posterior arm reaches the scutellum behind the axilla, separating the axilla from the metanotum. Because the posterior notal wing process occurs alongside the axilla for its entire length, it and the wing bases themselves are reliable indicators of the location of the axilla. In *Euderomphale* (Fig. 25: ax) the axilla is advanced almost entirely anterior to the scutellum, and the mesoscutum is left with only a small side lobe that is not delimited by a notaular groove. The axilla is almost entirely expressed as a flat dorsal surface, with only a very short and steep posterior slope. Because the notauli are best interpreted as incomplete or externally absent in *Euderomphale*, this condition cannot validly be used as a state shared with Entiinae.

Even if one is not convinced by the condition in *Euderomphale*, it is even clearer that *Entedononecremnus* (Fig. 24), another euderomphaline genus, has no externally indicated notauli. Its more typically-shaped axillae are only weakly advanced anteriorly and extend posteriorly as a long slope towards the metanotum, as in most other chalcidoids. Gumovsky (2002) acknowledged this,

but maintained that the state in *Euderomphale* was different. Our interpretation is that the notauli are not externally indicated in *Euderomphale*, and that its axillae simply differ in shape and degree of anterior advancement from those of *Entedononecremnus*.

This change in interpretation leaves only clypeal form and 28S D2 data supporting a grouping of Euderomphalini + Entiinae. In our analyses, this grouping does not occur. Instead, Euderomphalini is either sister group to Entedonini (Figs 44 and 46), sister group to Entedonini minus *Closterocerus* s.s. (Fig. 45), or part of an unresolved clade including Entedonini, Ophliminae, and Entiinae (Fig. 43). While clypeal form in Entiinae and Euderomphalini may be similar in some taxa, the clypeus is not indicated in some species of both groups. This leaves no unambiguous support for Euderomphalini + Entiinae, and therefore it seems best to return Euderomphalini to Entedoninae **stat. rev.**

Monophyly of Entedonini and Euderomphalini

Both tribes of Entedoninae were monophyletic in the combined analyses (Figs 44 and 46). In the molecular parsimony analysis (Fig. 43), *Neopomphale* was part of an unresolved clade with Entiinae and Opheliminae, and the rest of Euderomphalini rendered Entedonini paraphyletic. The molecular-only Bayesian analysis (Fig. 45) indicated a poorly supported but monophyletic Euderomphalini, but Entedonini was paraphyletic because *Closterocerus* s.s. was sister group to other Entedonini + Entiinae + Opheliminae.

The combined parsimony and Bayesian analyses (Figs 44 and 46) contained a monophyletic group of *Cabeza*, *Euderomphale* and *Neopomphale*, all of which have a transverse sulcus or sharp carina extending across the vertex between the median and lateral ocelli (character 6:1, Fig. 15). Molecular-only analyses did not reflect this group (Figs 43 and 45).

Generic relationships within Entedonini

The combined analyses mostly suggest groupings that are consistent with morphology. However, some potentially valid alternative relationships occur in the molecular-only analyses. This allows some interpretation of the results in light of morphological variation.

Closterocerus sensu lato

The unexpected placement of *Closterocerus s.s.* outside Entedoninae in 28S D2 results by Gauthier et al. (2000), or as sister group to all remaining Entedonini by Gumovsky (2002), cast strong doubt upon Gumovsky's (2001) synonymy of *Asecodes* and *Neochrysocharis* under *Closterocerus*. These results are here confirmed by independent sequencing of species in the affected taxa and the addition of 28S D3–D5 and CO1 data. However, the anomalous 3e′ subregion of 28S D2 in *Closterocerus* s.s. appears to contribute to this placement, even though it is only six bases long. Removal of this subregion from the analysis results in a sister-group relationship of *Closterocerus* s.s. + *Chrysonotomyia* within Entedonini (Table 3). The combined analyses (Figs 44–46) also bring *Closterocerus* s.s. back within Entedonini, but not as sister group to *Chrysonotomyia*. No analysis supports inclusion of *Asecodes* and *Neochrysocharis* with *Closterocerus*. Instead, combined Bayesian results (Fig. 46) suggest that they are closely related to a clade of *Pediobomyia* + *Pediobius* + *Rhynchentedon*. A similar relationship between *Asecodes*, *Neochrysocharis*, and *Pediobius* was found independently by Gumovsky (2002) using 28S D2 data.

While some of these results could easily be dismissed as morphologically implausible, there is no known restriction on eulophid evolution that could support such a dismissal. If valid, these results suggest that the form of the basiconic peg sensilla (character 4, Figs 9 and 10), in this particular case, may be a more reliable indicator of phylogenetic relationship than the presence of subtorular grooves (character 9, Fig 18). In summary, there is no molecular evidence supporting the synonymy of *Asecodes* and *Neochrysocharis* under *Closterocerus*. In light of the conflict from both molecular and morphological data, we propose that *Asecodes* and *Neochrysocharis* be reinstated as valid genera, **stat. rev.**

Neochrysocharis is paraphyletic with respect to *Asecodes* and some species of *Pediobius* or other "core" entedonines in the molecular-only analyses and the combined parsimony analysis (Figs 43–45); it is monophyletic in the combined Bayesian analysis (Fig. 46). There is no known morphological reason to expect *Neochrysocharis* to be paraphyletic with respect to *Pediobius*. While *Neochrysocharis* is morphologically similar to *Asecodes*, they differ in some characters and do not consistently form a clade in molecular analyses. While *Neochrysocharis* and *Asecodes* may eventually be combined, they should not be combined on the strength of our data. A proper investigation of the monophyly of *Neochrysocharis* and *Asecodes* will require investigation of additional species.

Closterocerus and Chrysonotomyia

Hansson (2004) suggested a novel set of characters defining *Chrysonotomyia*, most importantly the presence of a single spine on the volsellar digitus (character 31:1, Fig. 42) and an at least partially delimited clypeus (character 10:0, Fig. 20). He reclassified some Neotropical and Nearctic species from the subgenus

Closterocerus (*Achrysocharis*) into *Chrysonotomyia*, based on this character, but other members of the subgenus were not discussed. The European species *Closterocerus* (*Achrysocharis*) *germanicus* (Erdös) included in our analysis renders an otherwise monophyletic *Chrysonotomyia* paraphyletic in all analyses and has both a defined clypeus and single volsellar spine. We therefore transfer it to *Chrysonotomyia* as *Chrysonotomyia germanica* (Erdös) **n. comb**. These results suggest that all other members of *Closterocerus* (*Achrysocharis*) should be examined as potential members of the genus *Chrysonotomyia*.

A close relationship between *Chrysonotomyia* and *Closterocerus* is supported by the shared presence of slightly asymmetrical basiconic peg sensilla (character 4:1, Fig. 10) and subtorular grooves extending from the ventral edge of the torulus (character 9:1, Fig. 18). This relationship is presented as an alternative to placement of *Chrysonotomyia* near *Omphale*, but there is currently not enough data to decide between these alternatives. A sister-group relationship between *Chrysonotomyia* and *Closterocerus* was supported in the Bayesian molecular-only analysis only with the 3e′ subregion removed (Table 3). Therefore, while it may be tempting to combine *Chrysonotomyia* and *Closterocerus* because of their incomplete separation morphologically, our data do not provide any strong support for this act. Instead, our data suggest that analyses of more species of *Closterocerus* in particular should be conducted before carrying out any more synonymies.

Omphale and other Entedonini with delimited clypeus

Gumovsky and Ubaidillah (2002) and Hansson (2004) listed a number of genera that are similar to *Omphale* in possessing a delimited clypeus (character 10:0, Figs 20 and 21). The combined set of genera from these two lists included in this study are *Astichomyiia*, *Chrysonotomyia*, *Parzaommomyia*, and *Tropicharis*. None of the analyses in the current study produced a monophyletic group of these genera, but the combined Bayesian analysis (Fig. 46) presents an unsupported clade of *Chrysonotomyia* + *Omphale* + *Parzaommomyia*. The only supported monophyletic relationship between any genera with a delimited clypeus was *Omphale* + *Parzaommomyia*, in Bayesian analyses (Figs 45 and 46). Parsimony results (Figs 43 and 44) do not support any monophyletic grouping of entedonine genera with an indicated clypeus.

Astichomyiia was consistently placed near the genus *Emersonella*, forming a clade with it and *Ceranisus* in the combined Bayesian analysis (Fig. 46) or with *Neochrysocharis formosa* in the molecular-only parsimony analysis (Fig. 44). It does not actually have a delimited clypeus (Hansson, 2002: Fig. 404). Given that *Astichomyiia* also possesses a pronotal collar carina (character 12:1), which is absent in *Omphale*, we suggest that there is no evidence of any close relationship between it and *Omphale*.

Tropicharis was sister group either to most other Entedonini (Figs 44–46), or to most Euderomphalini (Fig. 43). Combined analyses (Figs 44 and 46) suggest it is at the base of a grade including *Chrysonotomyia*, *Closterocerus* s.s., *Omphale*, and *Parzaommomyia*. This seems plausible if one includes a delimited clypeus in the groundplan state for Entedonini, the character being lost multiple times in entedonine evolution. This scenario is supported by the relatively weakly, only laterally indicated clypeus in some species of *Chrysonotomyia* (Hansson, 2004) and the loss of clypeal delimitation in some species of *Omphale* itself (Hansson, 1996). Given that clypeal delimitation is an apparently vaguely determined and easily lost character, it seems plausible that it has been lost independently multiple times over the course of entedonine evolution.

Horismenus and similar genera

Hansson (2002) suggested a close relationship between *Alachua* Schauff & Bouček, *Edovum* Grissell, *Horismenus*, and *Paracrias*, based on propodeal sculpture and the form of the median carina (character 23:1, Figs 28 and 33). Later, Hansson (2009) synonymized *Edovum* and *Alachua* under *Horismenus*. No species from the former genus *Edovum* were included in this analysis, but the others formed a monophyletic and supported group in the Bayesian molecular-only analysis (Fig. 45). They did not form a monophyletic group in parsimony results or in combined Bayesian analysis (Figs 43, 44 and 46). Parsimony consistently indicated a sister-group relationship between *Paracrias* and *Ceranisus*, while *Paracrias* was in an unresolved clade in combined Bayesian results. *Horismenus* was monophyletic in both combined analyses, paraphyletic in molecular-only analyses.

Paracrias differs from *Horismenus* in a number of morphological characters (Hansson, 2004), most importantly in lacking the scutoscutellar pit. Because of these differences and ambiguous molecular results, it seems best to retain *Paracrias* as a separate genus.

Pediobius and similar genera

Morphological similarity between *Pediobius*, *Pediobomyia*, and *Rhynchentedon* was recognized by Bouček (1988). In all analyses, *Pediobomyia* and *Rhynchentedon* were sister groups forming a clade with at least one species of *Pediobius*. The three genera formed a monophyletic group in all analyses (Figs 43–46). Both parsimony analyses indicated a monophyletic *Pediobius* as

sister group to *Pediobomyia* + *Rhynchentedon*, but *Pediobius* was paraphyletic in both Bayesian analyses. Because of the lack of agreement, there does not seem to be any clear reason for synonymizing any other analysed genera with *Pediobius*.

Entedon and similar genera

Gumovsky (2007) listed a set of genera possessing a longitudinal carina on the lateral surface of the pronotum (character 13:1, Fig. 22). Three of these genera were included in this analysis: *Achrysocharoides*, *Entedon*, and *Pleurotroppopsis*. These genera formed a monophyletic group in the combined Bayesian analysis (Fig. 46), but not in any other analyses.

Astichomyiia, Ceranisus, and Emersonella

In Bayesian results (Figs 45 and 46), these three genera formed a moderately supported clade. With parsimony, *Emersonella* and *Astichomyiia* formed a clade in molecular-only results (Fig. 43), but not in combined results (Fig. 44). *Ceranisus* was consistently the sister group of *Paracrias* in parsimony results (Figs 43 and 44). Hansson (2002) recognized the morphological similarity between *Astichomyiia* and *Emersonella*, but also listed some similarities between *Astichomyiia* and *Closterocerus*, a grouping that is not supported by molecular data.

Ceranisus is part of an assemblage of entedonine parasitoids of thrips united by the presence of a transverse groove across the vertex (Triapitsyn and Morse, 2005). No morphological data have suggested any relationship between this group and either *Astichomyiia*, *Emersonella*, or *Paracrias*, but Gauthier et al. (2000) found that *Ceranisus* and *Thripobius* Ferrière formed an unsupported clade with *Emersonella*.

Conclusions

Our results present the first published phylogenetic analysis of Eulophidae where Entedoninae has been supported as monophyletic. The phylogenetic hypothesis presented in the combined Bayesian analysis (Fig. 46) presents strongly supported nodes, suggesting answers to some controversies concerning eulophid morphology. The combined parsimony analysis (Fig. 43) suggests an alternative hypothesis differing in important ways that call for further examination.

The initial impetus for this study was to determine if new molecular data could be used to address conflicting hypotheses concerning placement of Euderomphalini, *Asecodes*, and *Neochrysocharis* based on morphology—and, in the case of Euderomphalini, 28S D2 data. The addition of 28S D3–D5 and CO1 data provided clarity in that they found no support for the transfer of Euderomphalini to Entiinae, nor for the synonymy of *Asecodes* and *Neochrysocharis* under *Closterocerus*. The addition of morphological characters led to much stronger answers that provided well supported nodes with alternative placements for the taxa involved in both controversies.

Investigation of sequence alignments revealed that the unexpected placement of *Closterocerus* s.s. in previous molecular analyses (Gauthier et al., 2000; Gumovsky, 2002) could be explained by a block of six contiguous bases in 28S D2 rDNA, the 3e′ subregion. Morphological data overrode the signal from the 3e′ subregion in combined analyses, resulting in a more traditional placement of this genus. This suggests that additional molecular data could similarly override the signal from this subregion. This prediction is by no means a certainty, but it seems unwise to adjust the subfamily or tribe classification of *Closterocerus* based on current data.

The presented hypotheses (Figs 43–46) of eulophid relationships make strong alternative statements about entedonine phylogenetics, but they agree in several important ways. A core group of entedonines was supported in all analyses, excluding *Closterocerus* s.s. and genera previously considered close to *Omphale* by Hansson (2004) and Gumovsky and Ubaidillah (2002). While this clade could be characterized by a lack of clypeal delimitation, this varies within the excluded taxa as well, and some disagreement exists over interpretation of the character itself (such as in the case of *Astichomyiia*). Supported placements for most eulophid genera should provide a strong context for future analyses of eulophid phylogenetics at subfamily, genus, and species levels. It seems likely that the addition of more species to the analysis will provide more clarity for those genera without a strongly supported placement.

Our results put family placement of *Trisecodes agromyzae* into question. This species differs from all Eulophidae in having three tarsomeres instead of four. An analysis across chalcidoid families (J.B. Munro, J.M. Heraty, R.A. Burks, unpublished) indicates that this species does not belong in Eulophidae. However, there is no clear indication of its family placement using either molecular or morphological data.

Strong disagreement between the combined analyses (Figs 44 and 46) and molecular-only analyses (Figs 43 and 45) indicates that some controversy yet remains in eulophid phylogenetics. The addition of more gene regions should provide greater clarity in future molecular analyses. Morphological analyses would be improved through more thorough investigation of variation between the species and species groups within the involved genera. While it is possible succinctly to characterize many eulophid genera morphologically, such characterizations often fall apart when all known

species are examined (Burks, 2003). Rather than providing confusion, such variation could be used to provide greater clarity in morphological hypotheses if the variation is analysed in a phylogenetic context.

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