Blackwell Publishing

Cladistics 23 (2007) 1–29

Cladistics

10.1111/j.1096-0031.2007.00153.x

The phylogeny and evolution of Figitidae (Hymenoptera: Cynipoidea)

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Accepted 23 January 2007

Abstract

A phylogeny of the Figitidae (Hymenoptera: Cynipoidea) is presented based on combined analysis of molecular (28S-D2 and D3, COI and 18S-E17-35), morphological and life-history data. Data are analyzed by parsimony and Bayesian inference methods. Taxon sampling was held at a premium, and the resulting matrix contained 168 terminal taxa representing eight of nine subfamilies (Pycnostigminae not included) and all major subgroups of each subfamily. Alignment of the 28S D2 + D3 gene fragment based on a structural model resulted in the most defendable and least conflicting alignment tested. *Melanips*, previously classified in Figitinae, was consistently found to be the sister group of the Aspicerinae; *Euceroptres*, historically classified in Thrasorinae, frequently rendered that subfamily paraphyletic in these analyses. The general evolutionary trend is for early figitids to be parasitoids of gall inducing insects, with later host shifts occurring to exposed hosts associated with aphids.

Cynipoid wasps (Hymenoptera: Apocrita) form a fairly large assemblage of species (≈ 223 genera, 3000 species) with a world-wide distribution (Ronquist, 1999). Cynipoids can be divided in the two major groups, the so-called macro- and microcynipoids. Macrocynipoids are comprised of the rarely encountered families Austrocynipidae, Ibaliidae and Liopteridae. Species in these groups, when known, are koinobiont endoparasites of wood-boring or cone-boring insect larvae (Ronquist, 1999). The microcynipoids are comprised of Cynipidae and Figitidae. Most researchers and amateur naturalists are familiar with the phytophagous Cynipidae, or gall wasps, which induce often spectacular plant tissue swellings on a variety of host plants. Less attention has been paid to the Figitidae, a group comprised almost exclusively of koinobiont endoparasitoids of endopterygote insect larvae. Figitids are arguably the most species diverse family within Cynipoidea (Nordlander, 1984; Ronquist, 1999; Fontal-Cazalla et al., 2002) and until recently (Ronquist, 1999), the classification of the group was chaotic.

Figitid wasps are a uniquely derived group within the parasitic Hymenoptera in that they possess a distinctive marginal cell in the forewing while all other wing venation is either reduced or absent (Fig. 1A,B,F,I). Presently, there are nearly 1400 described species in 132 genera (Buffington et al., 2005). Nordlander (1984) estimates the global diversity to be nearly 24 000 species. Tropical regions are extremely species rich (Fergusson and Hanson, 1995; Nieves-Aldrey and Fontal-Cazalla, 1997; Fontal-Cazalla and Nieves-Aldrey, 1999), though the majority of described species are Holarctic (Dalla Torre and Kieffer, 1910; Weld, 1952).

The few substantiated host records gathered thus far for Figitidae indicate that most are primary parasitoids of the "higher" flies (Diptera: Schizophora) in habitats ranging from leaf-mines to algae to dung and carrion (Ronquist, 1999; Buffington, 2002; Fontal-Cazalla et al.,

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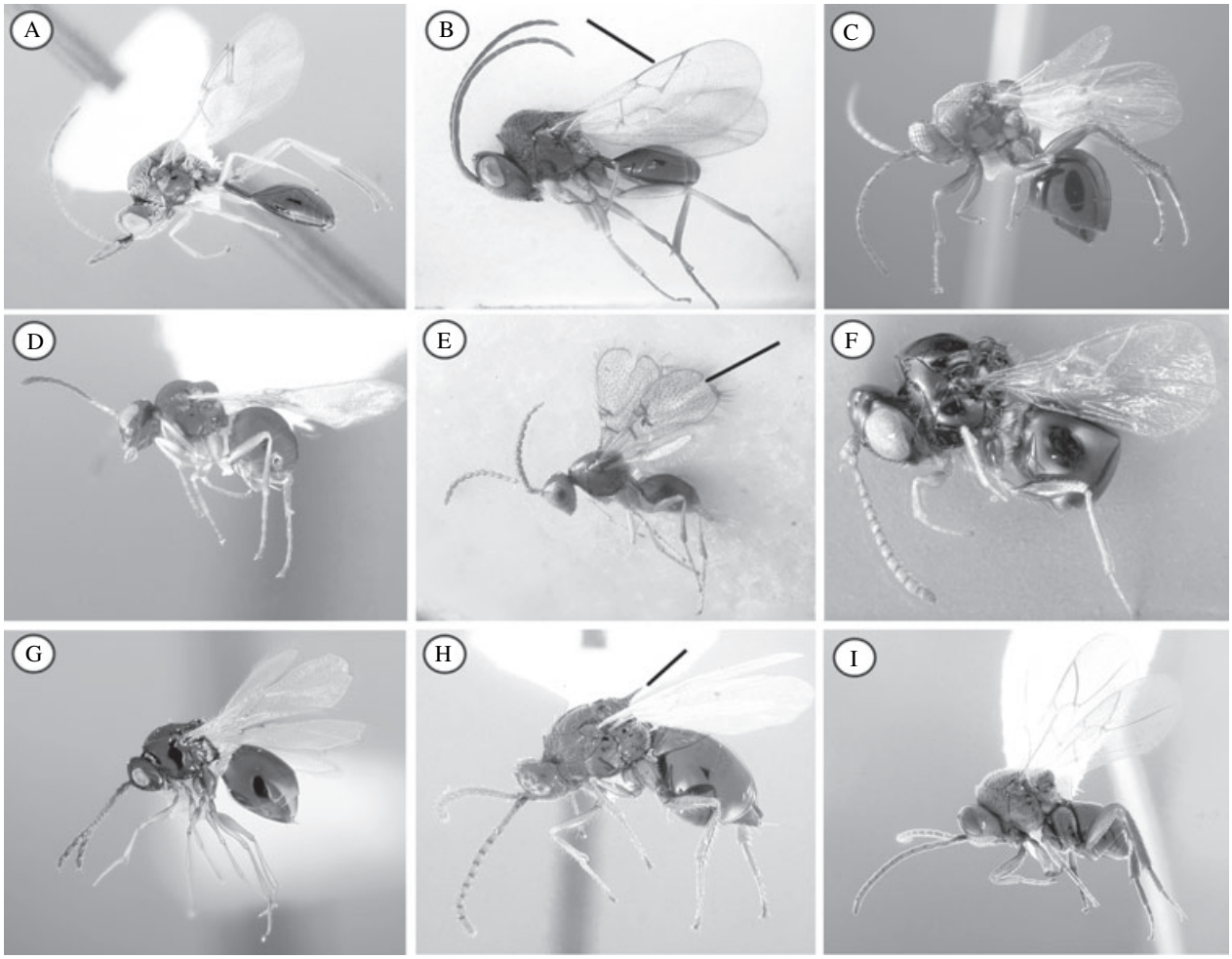

Fig. 1. Figitidae. (A) *Anacharis* (Anacharitinae); (B) *Xyalaspis* (Anacharitinae), arrow indicates marginal cell; (C) *Callaspidea* (Aspicerinae); (D) *Alloxysta* (Charipinae); (E) *Thoreauella* (Emargininae); (F) *Aegeseucoela* (Eucoilinae); (G) *Kleidotoma* (Eucoilinae); (H) *Neralsia* (Figitinae); (I) *Euceroptres* (Thrasorinae).

2002). The subfamilies Aspicerinae, Eucoilinae and Figitinae can all be found attacking these flies (Weld, 1952; Ros-Farré et al., 2000; Buffington, 2002). Some smaller subfamilies have specialized on other groups, such as: Anacharitinae, which are primary parasitoids of lacewings (Neuroptera: Chrysopidae; Weld, 1952; Miller and Lambdin, 1985); the Charipinae, which are hyperparasitoids of braconid and chalcidoid Hymenoptera (Clausen, 1940; Ronquist, 1999); and the Parnipinae and Thrasorinae, which are parasitoids of gall-forming Hymenoptera (Ronquist, 1999; Ronquist and Nieves-Aldrey, 2001). The host preferences of two subfamilies of Figitidae, the Pycnostigminae and Emargininae, are currently unknown.

Following Ronquist's (1999) phylogenetic-based re-classification of Cynipoidea, there has been a renewed focus on the phylogenetic relationships of Figitidae. Ros-Farré et al. (2000) examined anacharitine, aspicerine and figitine relationships. Though lacking thorough taxon and character sampling (20 ingroup taxa, 21 morphological characters), some relationships clearly emerged in this study. The aspicerines rendered the figitines paraphyletic, suggesting that members of these groups were somehow closely related. Further, charipines were not found to be the sister group to the anacharitines; cf. Ronquist (1999) and Vårdal et al. (2003). Fontal-Cazalla et al. (2002) produced the most comprehensive taxon-focused phylogenetic analysis of the figitids to date. This study was primarily concerned with testing (Nordlander, 1982) genus groups of Eucoilinae. With respect to sister groups of eucoilines, Fontal-Cazalla et al. (2002) found that the Emargininae, Eucoilinae and Pycnostigminae formed an apical trichotomy within the core figitids.

Two morphological synapomorphies support the monophyly of the Figitidae: the forewing vein Rs + M issuing from the posterior end of the basal vein (Ronquist, 1995), and the ninth tergum of the

female with a distinct line of weakness at the base of the ovipositor (Ronquist, 1999). The latter character has been linked to oviposition behavior and increasing the flexibility of the ovipositor apparatus (Ronquist, 1999). The phylogeny of the Figitidae proposed by Ronquist (1999) suggests a number of important relationships that are tested here. The recently described subfamily Parnipinae (Ronquist and Nieves-Aldrey, 2001) was found to be the sister group of all other figitid subfamilies. The newly circumscribed Thrasorinae were recovered as derived relative to Parnipinae and sister group to the remaining Figitidae. The newly circumscribed Emargininae were recovered deeply nested within the core Figitidae, as a possible sister group to the Eucoilinae. The Anacharitinae were proposed as the sister group to Charipinae (supported by two synapomorphies). Vårdal et al. (2003) found the same sister group relationship between charipines and anacharitines based on egg structure.

The purpose of this study is to expand on previous studies of figitid phylogenetic relationships through the use of additional taxa and additional characters. Molecular sequence data from the length variable ribosomal 28S D2 and D3 gene fragments were aligned using a novel secondary structure model following the model developed for Chalcidoidea (Hymenoptera: Apocrita) (Gillespie et al., 2005). Parsimony and Bayesian inference were used to analyze a combined molecular and morphological data set, the results of which are presented here.

Materials and methods

Taxonomic sampling

The taxa sampled are listed in Appendix A. Every figitid subfamily is represented in the matrix except the rare Pycnostigminae. Outgroups consisted of four liopterids (representing three of four subfamilies) and 17 cynipids (representing all tribes). For the ingroup, the diversity of each taxonomic group was reflected in the taxon sampling. In total, 168 terminal taxa were present in the final matrix.

All primary (where a body part was removed for sequencing) and secondary (compared specimen) vouchers are housed within the Entomology Research Museum (UCRC), UC Riverside (complete collection data available upon request from MLB). Appendix A summarizes the voucher and sequence data generated from each specimen. Images of all primary and/or secondary vouchers were captured using techniques summarized in Buffington et al. (2005) and are available from the MorphBank image database (http://morphbank.net/Show/?id = 111609). Specimens have been obtained from around the world by a consortium of biodiversity projects and strategic collecting (summarized in Appendix A and acknowledgments). All sorted material was stored in 95% EtOH at −80 °C.

DNA extraction and amplification

Molecular data presented here are based on the sequencing of four gene regions, the ribosomal 28S D2 expansion region (all taxa, cf. Gillespie et al., 2005), the ribosomal 28S D3 expansion region (all taxa), the ribosomal 18S E17–35 expansion region (Ouvrard et al., 2000; taxon sampling restricted to major clades only), and partial mitochondrial COI (nearly all taxa). These specific gene fragments and/or the combination of these gene fragments has been shown to be reliable and informative for the level of this study (Cameron et al., 1992; Simon et al., 1994; Campbell et al., 2000; Wiegmann et al., 2000; Babcock et al., 2001; Dowton and Austin, 2001; Rokas et al., 2002; Schulmeister et al., 2002; Schulmeister, 2003; Heraty et al., 2004; Heraty, 2004; Lin and Danforth, 2004; Nylander et al., 2004; Nylander et al., in prep.).

The metasoma was removed from each specimen and allowed to dry in a 32 °C water bath. DNA was extracted using the phenol–chloroform techniques outlined in Babcock and Heraty (2000) or the Chelex–proteinase K technique (Cano and Poinar, 1993). The Chelex technique was better at extracting DNA from poorly preserved specimens. Metasomas were ground in 5 μL proteinase K using sterile pestils; 80 μL 5% chelex was then added, and the entire sample incubated for 1 h at 55 °C, followed by 10 min at 98 °C. Samples were then centrifuged at 13200 r.p.m. (16110 ***g***) for 3 min; 75 μL of supernatant was then pulled off the Chelex beads, and stored at −80 °C until needed.

Polymerase chain reaction (PCR) amplification primers and protocols for the ribosomal 28S D2 and D3 and 18S partitions follow that of Heraty et al. (2004); primers and protocols for the mitochondrial COI partition follows that of Schulmeister et al. (2002). PCR products were all directly sequenced at either the Microchemical Core Facility, San Diego State University (San Diego, CA) or at the Genomics Institute (UC Riverside). All sequences are deposited in GenBank; accession numbers are listed in Appendix A.

Morphological data

Morphological characters and character states were taken from Ronquist (1995, 1999), Ros-Farré et al. (2000) and Fontal-Cazalla et al. (2002). In addition, some characters are newly described here. Characters and character states that were either altered or corrected from the original source are listed in Appendix B. Characters were coded from scanning electron micrographs (SEMs) of species deposited in MorphBank (http://www.morphbank.com) and/or from whole

(undissected) specimens using a Leica MZ8 stereoscope and fluorescent lighting. Ovipositor features were examined using a Zeiss Axioscop 2 after being dissected and mounted in Hoyer's mounting medium.

Life-history data

Characters used here were selected from previously published matrices on cynipoid phylogenetics (Ronquist, 1999; Ros-Farré et al., 2000; Nylander et al., in prep.) with additional character states added to encompass the diversity of traits found within Figitidae. Terminal taxa were coded from published records of biological attributes (host order, host microhabitat, host biology).

Sequence analysis—alignment

Ribosomal 28S and 18S are often length variable across distantly related taxa, and consequently, sequences need to be aligned prior to a cladistic analysis (DePinna, 1991; Brower and Schawaroch, 1996). Methods exist that handle length-variable data that are "alignment-free" (e.g., Wheeler, 1996; Stuart et al., 2002), but they are not pursued here. DNA sequence data are also typically aligned prior to a Bayesian Markov chain Monte Carlo (MCMC) inference of phylogeny (but see, e.g., Redelings and Suchard, 2005). One way to handle the problem of length-variable sequence data in alignments is to build a structural model of the gene in question. Kjer (1995) proposed a notation system that identified regions of the alignment that were more reliable than others. Gillespie (2004) utilized Kjer's (1995) system to refine the techniques for identifying and analyzing the various regions of the 28S gene. Gillespie et al. (2005) has proposed the first secondary structural model of 28S for Chalcidoidea (Hymenoptera), which is relatively closely related to Cynipoidea within Hymenoptera (Ronquist et al., 1999; Dowton and Austin, 2001). Sequence data within regions of ambiguous alignment (RAA) and regions of expansion and contraction (REC), as defined in our structural model (Appendix C), were aligned manually and included in all analyses. The 18S fragment varied only slightly across this range of taxa, and was easily aligned by eye.

Manual alignment of the 28S D2 + D3 data partition was achieved by arranging taxa into a rough phylogenetic order based on previous studies by Ronquist (1995, 1999), Buffington (2000), Ros-Farré et al. (2000)) and Fontal-Cazalla et al. (2002). The data were aligned by first diagnosing the conserved regions, then focusing on the variable regions. Following the 28S D2 + D3 alignment (whether manually or structurally), the 28S D2 + D3 data partitions were combined with the 18S, COI and morphology data partitions prior to analysis.

Data analysis

Sensitivity

Alternate alignments of the 28S D2 + D3 data blocks were achieved using ProAlign (Löytynoja and Milinkovitch, 2002) to examine topological sensitivity to alignment parameter perturbations of hypervariable regions (Gatesy et al., 1993; Wheeler, 1995). A series of 28S D2 + D3 alignments were produced wherein the gap opening penalty was varied across 0.5, 1, 2, 4, 8 and 16 and the gap extension penalty was varied across 0.25, 0.5, 1, 2, 4 and 8 for a total of 36 alignments. These 36 alignments were then combined with the 18S, COI and morphology data partitions before being analyzed.

Partitions

Analyzing partitions separately helps reveal the phylogenetic signal of each partition. Hence a partition's overall contribution to a given tree can be evaluated (Baker and DeSalle, 1997; Gatesy et al., 1999; Winterton et al., 2001; Lambkin et al., 2002; Lambkin and Yeates, 2003; Lin and Danforth, 2004). Partitioned Bremer support (PBS) (Baker and DeSalle, 1997) is the primary criterion for measuring data partition signal in this study. PBS was calculated using TreeRot ver. 2c (Sorenson, 1999) and PAUP* ver. 4.0b10 (Swofford, 2002). PBS was run on the manually and structurally aligned data sets, as well as one of the 36 ProAligned matrices (gap cost/extension cost matrix of 1 : 2). Five partitions were analyzed: 28S-D2, 28S-D3, 18S, COI, and morphology. To reduce analytical time, analyses were run with nchuck = 100 and chuckscore = 1000. Trees used for creating TreeRot batch files were found under the same criteria as the PBS analyses. A caveat to the use of PBS on a large matrix such as these presented here is that with each round of PBS, the potential exists for multiple trees to be discovered (Lambkin et al., 2002). The result is an average PBS value for each node in the tree, which may mask actual conflict between data sets (see Lambkin et al., 2002, for an example). To determine the presence of hidden partitioned Bremer support (HBS) and hidden conflict (Gatesy et al., 1999), standard Bremer support (BS) was calculated (using TreeRot) for all nodes across the three matrices in which PBS was calculated. The BS values were then compared with the ΣPBS values across all partitions. If the BS score was less than the ΣPBS score, hidden character support was revealed; if the BS score was higher than the ΣPBS score, then hidden character conflict was revealed.

Parsimony

The manually aligned combined matrix, the structurally aligned combined matrix and the 36 ProAlign derived combined matrices were each analyzed with heuristic search methods using PAUP*. Heuristic

searches of both the combined analyses and partitioned analyses (molecules only, morphology only) were done using 10 000 random addition sequence (RAS) searches using TBR branch swapping. Bootstrapping of the data (Felsenstein, 1985) was done using PAUP* across all matrices using the following search criteria (DeBry and Olmstead, 2000): addseq = random, nreps = 10, swap = tbr, maxtrees = 10, bootstrap nreps = 1000, search = heuristic, grpfreq = no, addseq = random, swap = tbr, nreps = 5, nchuck = 10, chuckscore = 1. The morphological data partition was analyzed using the implied weights method (Goloboff, 1993) of tree searching with k = 2. This type of parsimony was also used in Buffington (2000) and Fontal-Cazalla et al. (2002) for a similar taxon and character set. Compared with successive weighting (Farris, 1969), implied weights analysis is more self-consistent and typically produces more resolved, better supported trees than standard unweighted parsimony (Goloboff, 1993; Fontal-Cazalla et al., 2002).

Bayesian inference

Bayesian inference of phylogeny using MCMC (reviewed in Holder and Lewis, 2003) provides a powerful alternative to parsimony for phylogenetic and evolutionary analysis. The advantages of a Bayesian analysis are the straightforward (theoretical) interpretation of probabilities (Huelsenbeck and Rannala, 2004) and the way uncertainties in trees and parameter values can be accommodated: instead of basing conclusions on point estimates, uncertainty in tree topology can be integrated out (Huelsenbeck et al., 2000). The cost of using a Bayesian framework is the need for specifying prior assumptions (priors) about probabilities of trees and other parameters in a model of character evolution. Priors are, in general, controversial (e.g., Efron, 1986) and the application to phylogenetics is no exception (Lewis et al., 2005; Yang and Rannala, 2005; Brandley et al., 2006; Randle and Pickett, 2006). Controversial in phylogenetics is also the use of parametric methods—methods that rely on an explicit model of character evolution—(see Felsenstein, 2001 for a historical account), and recent discussions have centered around the robustness of inference methods to violations of the underlying model of character evolution (see Steel, 2005; for a brief review). We concur, however, with other authors (e.g., Thornton and Kolaczkowski, 2005) that using both a non-parametric (parsimony) and a parametric method can serve as a heuristic (*sensu* Grant and Kluge, 2003) in data exploration. Parametric phylogenetic estimation is generally a computer intensive task (Sanderson and Kim, 2000) but the Bayesian framework, and especially the use of the MCMC technique, has opened up the possibility for the use of more parameter-rich models. For example, combined analysis of morphological and molecular data using Bayesian MCMC is straightforward (Ronquist and Huelsenbeck, 2003; Nylander et al., 2004).

In the Bayesian analyses, both the manual and structural based alignments described above were divided into the same partitions as the parsimony analyses. The structural model based alignment was further divided into the following partitions: stems, RAAs, RECs, 18S, COI and morphology. The COI partition was further divided into three partitions reflecting each codon position (Nylander et al., 2004). The likelihood ratio test as performed using Modeltest 3.06 (Posada and Crandall, 1998) was used to select a model of molecular evolution for each data partition. The GTR model (Lanave et al., 1984; Tavaré, 1986), under the assumption that rates varied across sites according to a discrete gamma distribution with four rate categories (Γ; Yang, 1994), with a proportion of the sites invariable (I; Gu et al., 1995) was determined to be the best-fit model for each of the molecular partitions. The Markov k model (Lewis, 2001), under the assumption that variable characters where sampled, was applied to the morphological and life history partitions and with rate variation modeled using the Γ distribution. All model parameters were allowed to be partition-specific using a rate multiplier (Nylander et al., 2004). Analyses of MCMC were carried out using MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck, 2003). Each analysis was continued for 6 million generations using four chains with default settings for prior distributions, proposal rates and proposal distributions. The chains were thinned by sampling every 1000th generation and two separate runs starting with random trees were completed for each data set to help ensure stability was reached in the analyses (Huelsenbeck et al., 2002). Burn-in was set to 500 trees, and the posterior distribution of trees was summarized as a majority-rule consensus.

Results

Parsimony based tree statistics are summarized in Table 1 for all alignments analyzed. The structural alignment was favored over the manual and automated alignments for phylogeny reconstruction since it was the only alignment based on explicit criteria and had the least amount of hidden conflict (Tables 2 and 3). Excluding regions of ambiguous alignment resulted in branch collapse within smaller groups at the tips of the tree, but did not alter deeper relationships. Hence, results reported here are based on the inclusion of all data. The model for the structural alignment can be found in Appendix C, represented by five figitid taxa sampled from across the family.

Percent divergence of the 28S D2 + D3 combined data partition is presented in Table 1; the 18S partition showed a divergence of 10.9%; the COI partition

Table 1
Results of 28S D2 + D3 alignment sensitivity analysis. Alignments were generated by ProAlign. Gap opening and gap extension penalty values are listed in the left-hand columns, and pertinent tree statistics are in the remaining columns. All values reported are for the combined data set (28S D2 + D3, 18S E17–35, COI and morphology) after the 28S D2 + D3 partition was aligned using ProAlign. For comparative purposes, both the manually and structurally aligned data sets are at the bottom. The values in **bold** represent the optimal ProAlign alignment. Percent divergence (%Division) is given for all 28S D2 + D3 alignments

Gap opening	Gap extension	% Division	CI	RI	No. of trees	Tree length	No. of informative sites	Total no. of characters	Proportion informative sites	Information index[1]
0.5	0.25	20.7	0.18	0.67	384	8793	818	1791	0.458	0.0012
1	0.25	21.8	0.17	0.67	16	8628	800	1894	0.422	0.0264
2	0.25	20.4	0.17	0.66	54	8594	798	1899	0.421	0.0078
4	0.25	20.6	0.17	0.66	170	8608	802	1893	0.424	0.0025
8	0.25	20.4	0.17	0.67	107	8613	803	1894	0.424	0.0039
16	0.25	20.3	0.18	0.67	18	8634	815	1896	0.430	0.0240
0.5	0.5	20.9	0.18	0.67	48	8818	823	1847	0.446	0.0093
1	0.5	21.0	0.17	0.67	26	8637	807	1889	0.427	0.0164
2	0.5	21.3	0.17	0.67	428	8613	799	1888	0.423	0.0098
4	0.5	20.6	0.17	0.67	44	8625	806	1891	0.428	0.0097
8	0.5	20.4	0.17	0.67	141	8613	804	1897	0.424	0.0030
16	0.5	20.3	0.18	0.67	22	8575	805	1889	0.426	0.0193
0.5	1	20.3	0.17	0.67	14	8611	808	1883	0.429	0.0306
1	1	20.8	0.17	0.67	22	8635	811	1888	0.430	0.0195
2	1	20.9	0.18	0.67	152	8575	812	1881	0.432	0.0028
4	1	20.0	0.18	0.67	24	8620	807	1905	0.424	0.0176
8	1	21.1	0.17	0.67	516	8614	803	1884	0.426	0.0008
16	1	20.9	0.18	0.67	66	8616	811	1884	0.430	0.0065
0.5	2	20.6	0.17	0.67	10	8598	800	1904	0.420	0.042
1	**2**	**20.8**	**0.17**	**0.66**	**5**	**8564**	**800**	**1907**	**0.420**	**0.084**
2	2	20.9	0.17	0.66	610	8621	810	1894	0.428	0.0007
4	2	20.4	0.17	0.67	18	8642	803	1887	0.426	0.0237
8	2	20.0	0.17	0.67	96	8621	805	1889	0.426	0.0044
16	2	20.4	0.17	0.67	8	8605	806	1873	0.430	0.054
0.5	4	20.8	0.17	0.67	8	8573	793	1883	0.421	0.053
1	4	20.5	0.17	0.67	90	8599	802	1886	0.425	0.0047
2	4	20.3	0.17	0.67	6	8597	801	1884	0.425	0.0708
4	4	20.2	0.17	0.67	28	8633	808	1892	0.427	0.0152
8	4	21.1	0.17	0.67	28	8638	802	1881	0.426	0.0152
16	4	20.6	0.17	0.67	18	8602	800	1867	0.428	0.0238
0.5	8	21.1	0.17	0.67	24	8590	799	1892	0.422	0.0176
1	8	20.8	0.17	0.67	21	8595	798	1881	0.424	0.0202
2	8	20.4	0.17	0.67	713	8576	800	1888	0.423	0.0006
4	8	20.8	0.17	0.67	48	8600	797	1884	0.423	0.0088
8	8	19.9	0.18	0.67	8	8643	808	1883	0.429	0.0536
16	8	21.2	0.17	0.67	11	8590	803	1877	0.428	0.0389
Manual alignment		19.4	0.19	0.67	64	8693	779	1894	0.411	0.0064
Structural alignment		19.4	0.17	0.67	80	8741	782	1875	0.417	0.0053

[1]Information index = proportion of informative sites/number of best trees found.

showed a divergence of 30.4%. Results of PBS analyses are presented in Tables 2 and 3. Hidden character conflict was detected in the manual and most optimal ProAlign alignments at several nodes, but not in the structural alignment (Tables 2,3). Several major nodes are supported mostly by the morphological data partition (nodes 1, 2, 10, 20, 21 and 22; Table 2). Within the Eucoilinae (Table 3), the contribution of the morphological data partition declines while the contribution of the 28S D2 partition increases.

Figure 2(A,B) summarizes the results of the parsimony and Bayesian analyses, respectively, of the combined data set where 28S D2 + D3 were structurally aligned. The length of the parsimony tree was 8741 steps (ci = 0.18, ri = 0.68) (Fig. 2a). The same combined data sets, with the 28S D2 + D3 data partition manually aligned or aligned via ProAlign, resulted in the same summary tree for each analytical method. Figures 3–6 show the full phylogram generated by parsimony analysis of the combined matrix in which the 28S D2 + D3 data block were structurally aligned. Nodes 1, 5, 9 and 21 conflicted between the parsimony and Bayesian trees, and were further characterized by possessing little to no bootstrap/posterior probability values. These results indicate not only clade robustness (*sensu* Grant and Kluge, 2003),

Table 2
Partitioned Bremer support (PBS) and non-partitioned Bremer support (BS) of higher figitid relationships. Support values were calculated using TreeRot and PAUP*. "Taxon" and "node" refer to Figs 2(a), and 3–6. "A" refers to alignment method of 28S D2 + D3 data; M = manual; *P* = ProAlign (gap cost 1, extension cost 2); S = structural model. "ΣPBS" is the additive Partitioned Bremer Support value; **bold values** indicate that both molecular and morphological partitions return positive BS values. "Not found" indicates the relationship being measured was not obtained in the unconstrained analysis

Taxon	Node	A	28S D2	28S D3	COI	18S	Morph	ΣPBS	BS
Figitidae	**1**	M	−0.44	−2.18	−7.87	−0.52	13	2	2
		P	−5.61	−0.94	−1.58	−1	11.12	2	1
		S	−2.3	−1.59	−8.92	0	13.81	1	1
Thrasorinae	**2**	M	−16.68	−0.47	0.28	0.87	22	6	4
		P	−12.93	0	8.83	0	6.1	2	1
		S	−3.58	−0.34	1.43	−0.96	8.45	5	5
Anacharitinae	**3**	M	18.62	−2.47	−22	0	10.85	5	5
		P	7.36	−1.46	1.09	0	0	7	6
		S	5.42	0.12	−10.87	−0.94	11.25	5	5
Lonchidia + (Aspicerinae + Figitinae)	**4**	M	10.61	0.19	3.4	−11	7.81	**12**	11
		P	11.27	−1.8	−8.07	0.2	6.4	**8**	7
		S	9.83	−0.37	8.25	−11	4.3	**11**	11
Figitinae[1]	**5**	M	1.27	−1.9	−0.09	−0.21	8.93	8	8
		P	15.94	−2.95	11.09	0.75	4.85	**6**	6
		S	11.07	−2.19	0.53	1.25	−3.66	7	7
Aspicerinae[2]	**8**	M	3.95	1.96	3	1	0.1	**10**	10
		P	11.57	−0.5	−6.17	−1	6.1	**10**	10
		S	3.16	2.21	1.07	0.8	2.77	**10**	10
Aspicerinae + Figitinae	**6**	M	−0.94	−0.04	4	0	−1.03	2	2
		P	not found	not found	not found	not found	not found	–	–
		S	13.97	0.46	1.49	−0.12	−11.8	4	4
Charipinae + (Emargininae + Eucoilinae)	**9**	M	−1.44	0.86	−5.74	−1.07	11.4	6	6
		P	29.27	−1.5	−9.77	−1	−15	2	2
		S	0.37	−0.3	−1.63	−1.12	6.69	4	4
Emargininae + Eucoilinae	**10**	M	8.43	−0.52	−9.56	−0.66	10.31	8	8
		P	16.73	0.13	−4.2	1.96	5.7	**5**	4
		S	6.57	0.18	−10.53	0	10.78	7	7
Eucoilinae	**11**	M	29.66	2.05	2.03	0	7.26	**41**	42
		P	30.7	0.7	−5.87	−1.5	6.98	**31**	29
		S	28.9	1.55	4.63	0	5.9	**41**	41
Thrasorinae sister to remaining figitids	**20**	M	−1.88	−1.47	−4.5	−1	10.85	2	2
		P	−2.21	−1.68	3.34	−0.65	4.19	3	3
		S	−2.74	−0.92	−2.7	−1	9.37	2	2
Anacharitinae sister to remaining figitids	**21**	M	−1.21	−0.08	−11.26	−1	15.55	2	2
		P	10.97	0.6	0.8	−1	−6.18	4	4
		S	1.76	−0.59	−12.53	−1	13.36	1	1
Figitine–Aspicerine clade sister to ((Emargininae + Eucoilinae) Charipinae)	**22**	M	2.44	−0.2	−8.6	−1	11.35	6	6
		P	3.77	−1	2.23	0	−4	1	1
		S	5.12	0.99	−7.76	−1.31	8.96	6	6

[1]Not including *Lonchidia*.
[2]Including *Melanips*.

but also that unstable clades are sensitive to hypotheses of positional homology. Figure 7 summarizes relationships inferred from the molecular data partition (Fig. 7A,B) and the morphological/life-history data partition (Fig. 7C,D).

Discussion

Data alignment

Though automated multiple alignment programs have been postulated as being superior to other alignment methods due to their objectivity and rigidness (e.g., Sanchis et al., 2000), structural alignments are desirable because they are based on a strict positional homology criteria (Kjer, 1995; Gillespie, 2004; but see Ogden et al., 2005). The goal behind the generation of the automated alignments presented here was to test how sensitive phylogeny reconstruction is to variation in interpretation of the hypervariable regions in the 28S D2 + D3 data partition (Wheeler, 1995). For the major nodes, 67% were consistently recovered across all alignments, suggesting that these nodes are robust to competing alignment hypotheses.

Table 3
Partitioned Bremer support (PBS) and non-partitioned Bremer support (BS) of eucoiline relationships. Support values were calculated using TreeRot and PAUP*. "Taxon" and "node" refer to Figs 2(a) and 3,4–6. "A" refers to alignment method of 28S D2 + D3 data; M = manual; *P* = ProAlign (gap cost 1, extension cost 2); S = structural. "ΣPBS" is the additive Partitioned Bremer Support value; **bold values** indicate both molecular and morphological partitions return positive BS values. "Not found" indicates the relationship being measured was not obtained in the unconstrained analysis

Taxon	Node	A	28S D2	28S D3	COI	18S	Morph	ΣPBS	BS
Gronotoma group	13	M	10.79	−0.32	−8	1.02	0.51	**4**	4
		P	19.27	0.67	−2.27	−2	−11.67	4	3
		S	13.79	−1.47	−7.37	1.47	−2.42	4	4
Zaeucoila group	14	M	14.82	1.36	−6.85	0.22	−6.54	3	3
		P	25.13	−0.1	6.18	−0.24	−28.98	2	0
		S	11.09	1.12	−2.85	0	−8.36	1	1
Gronotoma + *Zaeucoila* groups	12	M	25.24	2.12	−2.56	0	−21.8	3	3
		P	18.19	−1.03	3.28	−1.21	−14.22	4	3
		S	25.65	0.63	−0.58	0	−22.7	3	3
Core Eucoilinae	16	M	20.76	−0.73	−13.6	1	−5.43	2	2
		P	8.3	−0.93	−4.34	−1.44	−0.6	1	−1
		S	16.32	−0.23	−7.73	1.42	−7.78	2	2
Kleidotoma group	18	M	27.57	−1.0	−15.71	2.32	−6.18	7	7
		P	8.71	−1.85	2.9	0	−1.76	8	7
		S	22.22	−1.14	−9.64	3	−8.44	6	6
Kleidotoma group sister to *Zamischus* group	17	M	3.12	−0.47	−1.5	1	−1.15	1	1
		P	not found	not found	not found	not found	not found	–	–
		S	not found	not found	not found	not found	not found	–	–
Zamischus group	19	M	4.48	1.46	−8.79	0	3.85	5	1
		P	33.5	−0.24	−12.14	0.18	−17.93	3	2
		S	not found	not found	not found	not found	not found	–	–
Core Eucoilinae + *Zamischus* group + *Kleidotoma* group	15	M	18.45	2.97	−12.56	−2.17	−3.7	5	5
		P	−1.51	1.56	−2.16	4.33	9.44	**3**	2
		S	18.06	2.35	−10.52	−3	−2.89	4	4

Data partitions

The combined analyses, regardless of alignment technique for the 28S D2 + D3 partition, consistently produced more resolved and more strongly supported clades than any of the partitioned analyses. These results are consistent with other studies that support the inclusion of a maximum amount of informative data for phylogenetic analyses (Nixon and Carpenter, 1996; Babcock et al., 2001; Dowton and Austin, 2001; Winterton et al., 2001; Schulmeister et al., 2002; Lambkin and Yeates, 2003; Schulmeister, 2003; Heraty et al., 2004; Heraty, 2004; Nylander et al., 2004; Nylander et al., in prep.). Although the morphology/life-history data account for only about 8% of the total data set, these data play a significant role of determining a final tree topology, regardless of phylogenetic technique.

An exception to this pattern was the *Zamischus* group (Eucoilinae, node 19, Fig. 6), which showed an increase in bootstrap support in the absence of the morphological data. Fontal-Cazalla et al. (2002) never recovered this group as a clade using morphological data alone, and the morphological data partition analyses (Fig. 7A,B) similarly do not recover the clade, regardless of analysis type. These results suggest the current morphological data partition discourages the monophyly of the *Zamischus* group and is in conflict with the combined molecular data (dashed line, Fig. 7A,B). This observation is consistent with the PBS results (Table 3) in which the strongest character support for this clade is associated with the 28S D2 partition.

Bootstrap support indices suggest that the backbones of the molecular-data only trees are unstable (Fig. 7a,b). This was also observed in the Bayesian trees in which many branches are collapsed (< 0.50 posterior probability) along the backbone. There are only a few morphological characters coded that unequivocally indicate relationships at the subfamily level (characters 29 : 0, 33 : 0, 70 : 0, 77 : 2, 92: all states informative, 142: all states informative, 151 : 0, 159: all states informative, 161–167: all states informative), but these characters have a significant impact in determining tie-breaks between molecular characters and ultimately "drive" the analysis within a range of tree topologies. By contrast, the morphological/life-history data partition, when analyzed alone, provides little support along the backbone of the tree (note thin lines, Fig. 7C,D).

Partitioned Bremer support

PBS values (Tables 2 and 3) indicate that only a few clades show positive PBS values across all of the data partitions. These include the Aspicerinae (node 8,

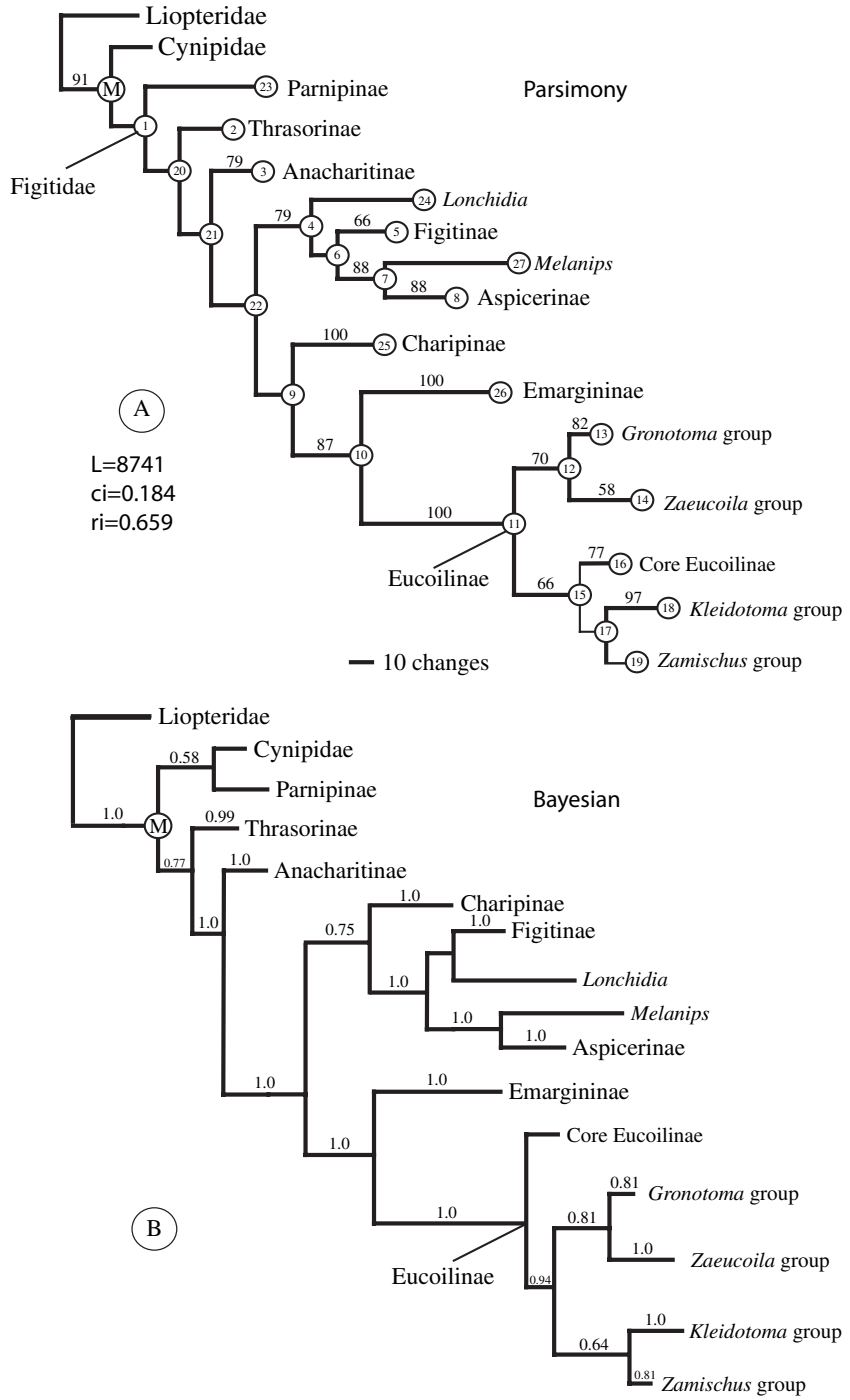

Fig. 2. Summary phylograms of higher Figitidae relationships based on combined analysis (28S D2 + D3, 18S, COI and morphology), where the 28S D2 + D3 data partition was structurally aligned. (A) parsimony result. (B) Bayesian inference result. Numbers above and or pointing to branches indicate bootstrap suppport (parsimony, > 50% shown) or posterior probability (Bayesian, > 0.5 shown). Thin branches in (A) collapse in the strict consensus of trees. Node numbers in (A) refer to Figs 3–6 and are referenced in Tables 2 and 3. Node "M" in both trees refers to the Microcynipoidea clade.

Table 2) (*Lonchidia* (Aspicerinae + Figitinae)) (node 4, Table 2), Eucoilinae (node 11, Table 2) and the *Gronotoma* group (node 13, Table 3). The majority of nodes summarized in Table 2 have high PBS scores for the morphology data partition, indicating that this partition is largely responsible for the character support of these clades. This is consistent with the results of the trees based only on molecular data in that clades strongly

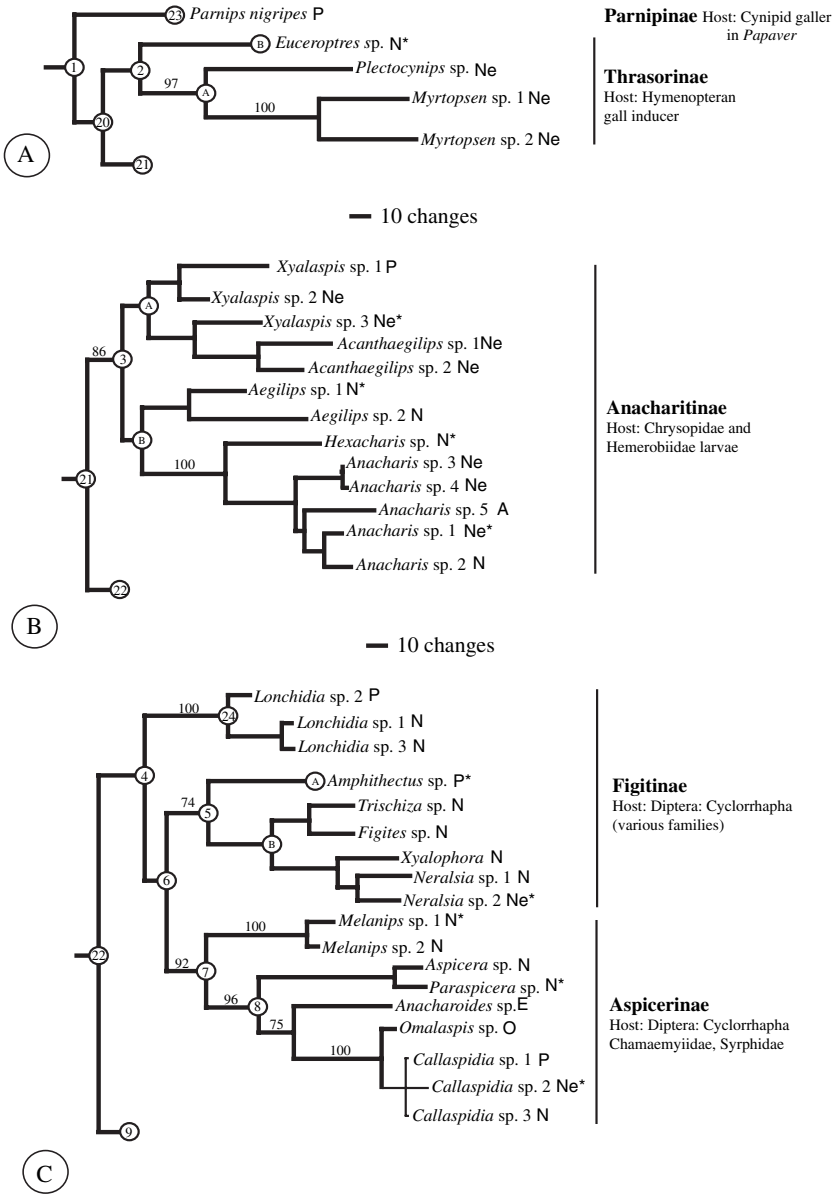

Fig. 3. Phylograms of subfamilies of Figitidae expanded from the parsimony analysis in Fig. 2(A). (A) Parnipinae and Thrasorinae. (B) Anacharitinae. (C) Figitinae and Aspicerinae. Numbers above branches indicate bootstrap support. Circled node numbers in all trees refer to Fig. 2(A) and are referenced in Tables 2 and 3. Letters after taxon names refer to biogeographical region in which specific terminal taxon was collected: A, Australian; E, Ethiopian; N, Nearctic; Ne, Neotropical; O, Oriental; P, Palearctic. Thin branches indicate collapse in the strict consensus of trees.

supported by the morphological data partition in the PBS analyses were not recovered as monophyletic: Thrasorinae, Anacharitinae (Charipinae (Emargininae + Eucoilinae)) and (Emargininae + Eucoilinae) (Fig. 7a,b). Within eucoilines, the influence of the morphological data partition is less, and instead, the 28S D2 partition dominates (Table 3). This is consistent with analytical results of the morphological data partition alone where resolution within the Eucoilinae is diminished in the absence of the molecular data.

Hidden character conflict (Gatesy et al., 1999) was observed at nodes 1–4, 10–11, 12–14, 15–16 and 18–19 for the manual and automated alignments of the 28S D2 + D3 data partitions (Tables 2,3). This result is interpreted as the presence of internal conflict among data sets as to how groups are to be resolved. The structural alignment of the 28S D2 + D3 data partitions did not possess any hidden conflict (PBS value greater than standard BS value in the last two columns of Tables 2 and 3), thereby minimizing the conflict

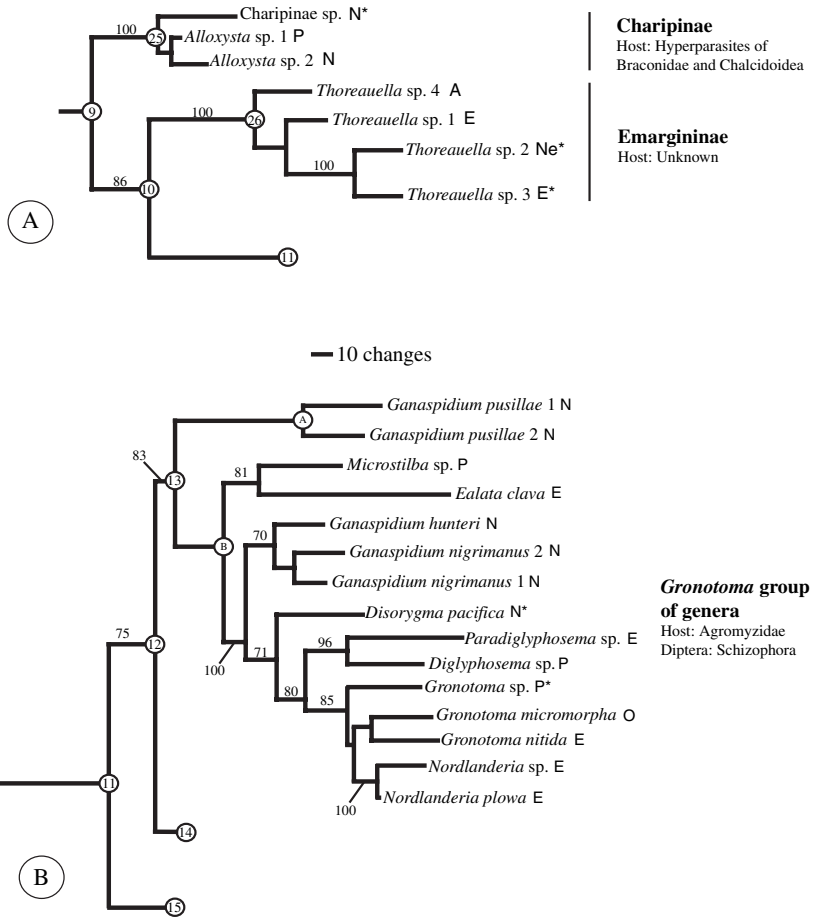

Fig. 4. Phylograms of subfamilies of Figitidae expanded from the parsimony analysis in Fig. 2(A). (A) Charipinae and Emargininae. (B) Eucoilinae: *Gronotoma* group. Numbers above branches indicate bootstrap support. Circled node numbers refer to (A) and are referenced in Tables 2 and 3. Letters after taxon names refer to biogeographical region in which specific terminal taxon was collected: A, Australian; E, Ethiopian; N, Nearctic; Ne, Neotropical; O, Oriental; P, Palearctic.

among data sets. As the power of combined analyses rely on the synergistic effects of multiple data sets (Nixon and Carpenter, 1996), it seems reasonable to choose the data set that also possesses the least amount of internal conflict. Hence, the structural alignment of the 28S D2 + D3 data partition was preferred.

Monophyly of the Figitidae

All parsimony-based combined analyses resulted in a monophyletic Figitidae (Fig. 2A). Owing to the placement of Parnipinae as sister group to the Cynipidae (Fig. 2B), the Figitidae were never recovered as being monophyletic in any of the Bayesian combined analyses. Figitid monophyly in the parsimony analyses is strongly dependent upon the morphological data partition, especially character states 161 : 1 (position of Rs + M forewing vein) and 162 : 1,2 (ovipositor with distinct point of weakness), both of which unambiguously support figitid monophyly (Ronquist, 1999). This observation is underscored by the PBS scores for this node (Table 2, node 1), which clearly indicate that the morphology data block has the only positive PBS score. However, the terminal taxon representing Parnipinae, *Parnips nigripes* (Barbotin), is lacking data for the 18S partition as well as several morphological characters. Both of these data partitions are important; the 18S fragment has a low rate of sequence divergence (Heraty, 2004) and is informative at the family/subfamily level within cynipoids (Rokas et al., 2002). Further, the morphological data partition is relatively important in the Bayesian analysis in resolving basal nodes within Figitidae. Perhaps the Bayesian analysis is sensitive to the missing data for *Parnips*, thus erroneously placing the taxon as sister group to Cynipidae.

Parnipinae is a key group for understanding figitid and cynipid evolution. It was not until just recently that *Parnips nigripes* was determined not to be a cynipid (where it was originally placed in *Aulacidia*) but rather as a new lineage of Figitidae (Ronquist and

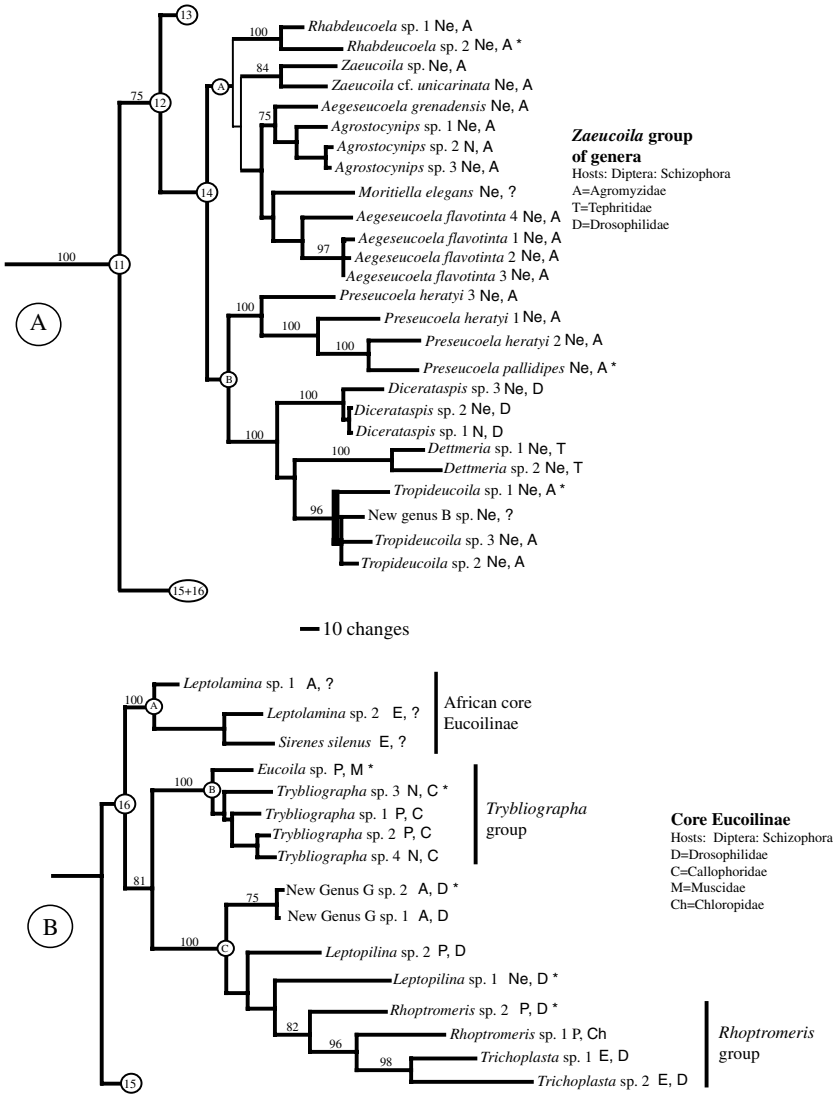

Fig. 5. Phylograms of subfamilies of Figitidae expanded from the parsimony analysis in Fig. 2(A). (A) Eucoilinae: *Zaeucoila* group. (B) Eucoilinae: Core eucoilines. Numbers above branches indicate bootstrap support. Circled node numbers in all trees refer to Fig. 2(A) and are referenced in Tables 2 and 3. Letters after taxon names refer to biogeographical region in which specific terminal taxon was collected: A, Australian; E, Ethiopian; N, Nearctic; Ne, Neotropical; O, Oriental; P, Palearctic.

Nieves-Aldrey, 2001). External morphology and life-history traits suggest a close relationship between Parnipinae and Cynipidae (Ronquist, 1999; Ronquist and Nieves-Aldrey, 2001; Nylander et al., in prep.). Ronquist (1999) showed through parsimony mapping of biological traits that Parnipinae possess the same biological traits as the last figitid + cynipid ancestor.

Figitid subfamily monophyly and relationships

Thrasorinae

The monophyly of this subfamily depends strongly on the inclusion of the morphology data within the combined analyses (Fig. 2). The placement of the thrasorine clade as sister group to all other figitids, excluding Parnipinae, is consistent with Ronquist (1999), and supports the hypothesis that older lineages of Figitidae are associated with hymenopterous gall inducers (Ronquist, 1999; Ronquist and Nieves-Aldrey, 2001). With the exclusion of the morphology data block, *Euceroptres* no longer groups with the remaining thrasorines (*Plectocynips* and *Myrtopsen*). Further, the inclusion of *Euceroptres* within Thrasorinae was not supported in the bootstrap analysis (Fig. 3A); the placement of *Euceroptres* is the focus of a pending study (Buffington and Liljeblad, in prep.) The morphology-only analyses consistently recovered the thrasorines as monophyletic and nested within the Figitidae (Fig. 7C,D).

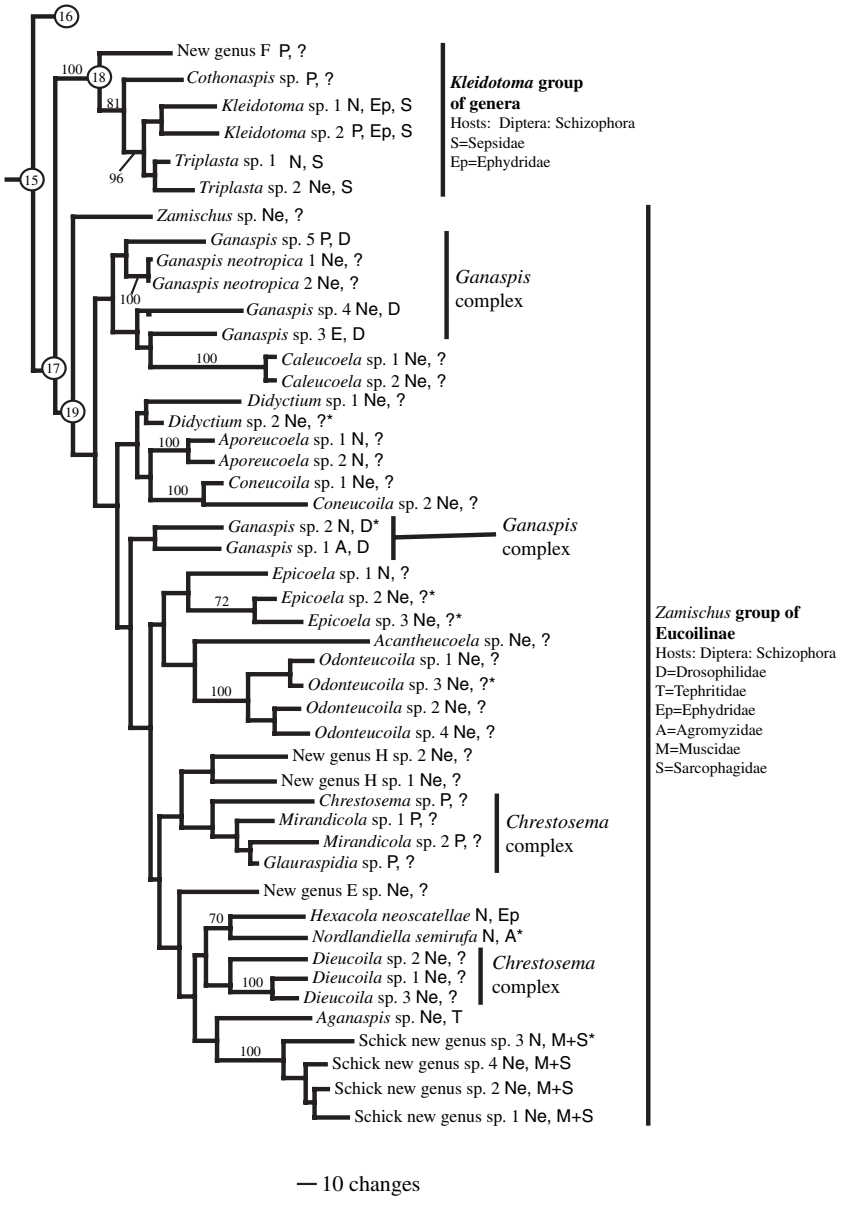

Fig. 6. Phylograms of subfamilies of Figitidae expanded from the parsimony analysis in Fig. 2(A). Eucoilinae: *Kleidotoma* group and *Zamischus* group eucoilines. Numbers above branches indicate bootstrap support; thin branches indicate collapse in the strict consensus of trees. Circled node numbers in all trees refer to Fig. 2(A) and are referenced in Tables 2 and 3. Letters after taxon names refer to biogeographical region in which specific terminal taxon was collected: A, Australian; E, Ethiopian; N, Nearctic; Ne, Neotropical; O, Oriental; P, Palearctic.

Anacharitinae

The Anacharitinae were consistently recovered as monophyletic in the combined analyses and as sister group to all figitids, excluding Parnipinae and Thrasorinae (Fig. 2). This agrees with Ronquist (1999), except that Charipinae were never recovered as the sister group to Anacharitinae. The monophyly of the anacharitines depends strongly on the inclusion of the morphological data partition (Table 2). Exclusion of the morphological and biological data partitions results in a split of anacharitines into two distinct clades (Fig. 7A,B). The members of these two clades share the same biogeographical distribution patterns, with the *Aegilips–Anacharis–Hexacharis* clade being mostly Holarctic and an *Acanthaegilips–Xyalaspis* clade being mostly Neotropical. All morphological analyses recovered anacharitine monophyly (Fig. 7C,D).

Charipinae

Charipinae monophyly was recovered in all combined analyses (Fig. 2), molecular-data-only analyses (Fig. 7A,B), and morphology-only analyses (Fig. 7C,D),

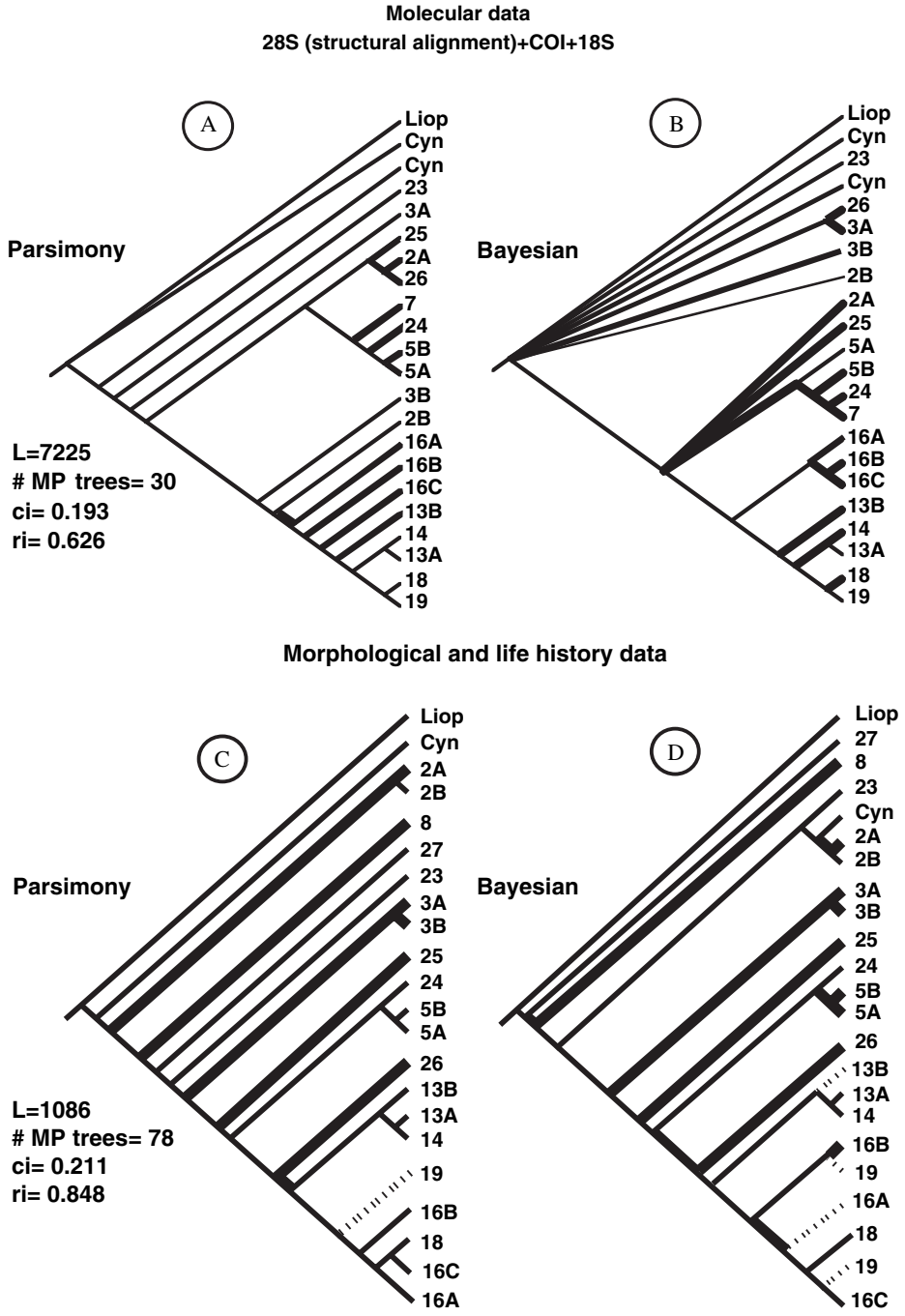

Fig. 7. Summarized tree topologies of figitid relationships inferred from parsimony (A) and Bayesian analyses (B) of the molecular data partitions (28S, COI, 18S), and parsimony (C) and Bayesian analyses (D) of the morphology/biology data partition. Thick branches in parsimony trees and Bayesian trees indicate > 75% bootstrap support or > 75% posterior probability,respectively; dashed branches indicate paraphyly in the terminal group that branch leads to. Terminal names refer to clades and subclades in Figs 4–7. Liop = liopterid outgroup; Cyn = cynipid outgroup; 2A = Thrasorinae: *Plectocynips*, *Myrtopsen*; 2B = Thrasorinae: *Euceroptres*; 3A = Anacharitinae subclade A; 3B = Anacharitinae subclade B; 5A = Figitinae: *Amphithectus*; 5B = Figitinae: all other genera; 7 = Aspicerinae (including *Melanips*); 8 = Aspicerinae without *Melanips*; 13A = *Gronotoma* group: *Ganaspidium pussilae*; 13B = *Gronotoma* group: all other genera; 14 = *Zaeucoila* group; 16A = core eucoilines subclade A; 16B = *Trybliographa* group; 16C = *Rhoptromeris* group C; 18 = *Kleidotoma* group; 19 = *Zamischus* group; 23 = Parnipinae; 24 = *Lonchidia* (Figitinae); 25 = Charipinae; 26 = Emargininae; 27 = *Melanips*.

regardless of analytical method. Sister group relationships between charipines and other figitids, however, were unstable. Ronquist (1999) placed Charipinae as sister group to the Anacharitinae. This relationship was not recovered in any analysis performed here, regardless of data type (molecules, morphology, or both) or analytical method (Fig. 2). Further, in none of the Bayesian trees was a Charipinae + Anacharitinae sister group relationship recovered. Instead, combined analyses placed Charipinae as sister group to either Emargininae + Eucoilinae (parsimony, Fig. 2A) or sister group to Figitinae + Aspicerinae (Bayesian, Fig. 2B). PBS scores supporting (Charipinae (Emargininae + Eucoilinae)) are high (Table 2), with the majority of support coming from the morphology data partition.

Aspicerinae

Aspicerinae + *Melanips* was supported in all combined analyses (node 8, Fig. 2; Table 2) and molecular-data-only analyses (Fig. 7A,B) regardless of analytical method. This result contrasts strongly with previous hypotheses regarding the placement of *Melanips*. Ronquist (1999) and Ros-Farré et al. (2000) recovered *Melanips* as sister group to Aspicerinae (Figitinae, Emargininae (Eucoilinae)). *Melanips* has traditionally been a difficult taxon to place phylogenetically (Ronquist, 1994, 1995, 1999; Ros-Farré et al., 2000). In the morphology-only analyses presented here, *Melanips* is never recovered with Aspicerinae (terminal 27, Fig. 7C,D). This is likely the result of *Melanips* lacking several morphological synapomorphies of all other Aspicerinae (lack of median sculpture on scutellum, 76 : 1; wings glabrous, 127 : 1; rounded posterior margin of tergum 3, 159 : 0). *Melanips* does possess a similar host range as other aspicerines (Ros-Farré et al., 2000) and has been recorded from the aphidophagous Chamaemyiidae (Diptera) (Buffington, pers. obs.) and Syrphidae (Narayanan, 1941). Sensitivity analyses indicate the clade is not influenced by alignment issues. Based on the results of the combined and molecular-only analyses, species of *Melanips* are here placed in the Aspicerinae.

Figitinae

Bayesian combined analysis (Fig. 2B) recovered a monophyletic Figitinae, albeit with low posterior probability. In the parsimony based combined analysis, Figitinae is rendered paraphyletic by *Lonchidia* (Fig. 2A). In some of the molecular-data-only analyses, *Lonchidia* was found to be sister group to the remaining figitine taxa (Fig. 7A,B). In both parsimony and Bayesian analysis of the morphological data partition, the same sister group relationship was also recovered (Fig. 7C,D). With *Lonchidia* excluded from Figitinae, PBS values increase substantially for the remaining figitine taxa (Table 2), but this clade is not well supported when the morphology data partition is excluded. Ronquist (1999) did not hesitate to include *Lonchidia* in the Figitinae, though Hellèn (1937) suggested that it belonged within its own tribe. Data herein support the conclusion of Hellèn (1937), but it seems premature at the present to erect a new subfamily to accommodate this genus.

Emargininae

Emargininae are strongly supported as monophyletic and as the sister group of Eucoilinae by all combined analyses (Fig. 2) and any reduced data partition analysis that included the morphological data partition (Table 2). This sister group relationship is not supported by the molecular-data-only analyses (Fig. 7A,B), nor by the morphology-only analyses (Fig. 7C,D). The emarginines were considered to be one of two possible sister groups to Eucoilinae by Fontal-Cazalla et al. (2002), the other being Pycnostigminae.

Eucoilinae

As in Fontal-Cazalla et al. (2002) and Ronquist (1999), the monophyly of the eucoilines is strongly supported in all combined and reduced data partition analyses, regardless of analytical method (Figs 2 and 7).

The Gronotoma *and* Zaeucoila *genus groups*

In all combined analyses presented here, these two genus groups were recovered as monophyletic and sister groups of each other (Fig. 2). The majority of the sister group branch support came from the 28S D2 partition (Table 3). All combined analyses recovered a monophyletic *Gronotoma* group (Figs 2 and 4B) though the clade was not recovered in the sensitivity analyses (not shown). The *Gronotoma* group was frequently rendered paraphyletic by *Ganaspidium pussilae* Weld when the morphological data partition was excluded (Fig. 7A,B). These data also suggest *Nordlanderia* is a subgroup of *Gronotoma* (Fig. 4B).

The *Zaeucoila* group was consistently recovered as monophyletic in all partitioned data analyses, and PBS scores (Table 3) indicate the 28S D2 data partition was the strongest supporter of this monophyly. Two groups were recovered within the *Zaeucoila* group of genera (Fig. 5A), a *Zaeucoila* clade (comprised of *Aegeseucoela*, *Agrostocynips*, *Moritiella*, *Rhabdeucoela* and *Zaeucoila*) and a *Tropideucoila* clade (composed of *Dettmeria*, *Diceratapsis*, *Preseucoela* and *Tropideucoila*). The *Zaeucoila* clade collapses under the strict consensus of the combined-parsimony tree. *Aegeseucoela* was not recovered as monophyletic, which was speculated upon by Buffington (2002).

Core Eucoilinae

A ''Core Eucoilinae'' clade, composed of the *Trybliographa* and *Rhoptromeris* genus groups, a grade of *Leptopilina*, an undescribed New Genus G, and the African endemic genera *Leptolamina* and *Serenes*

(referred to here as the African core Eucoilinae clade), was recovered in all combined analyses (Figs 2 and 5B). Unambiguous morphological evidence supports the inclusion of *Leptopilina* within the *Trybliographa* group (characters 17 : 1, 107 : 1, 116 : 2), but the molecular data partitions counter this. The ''Core Eucoilinae'' were not recovered as monophyletic in some of the reduced data partition analyses and never in the morphology-only analyses (Fig. 7C,D). In the molecular-data-only analyses, each of the three clades composing the ''Core Eucoilinae'' were recovered as monophyletic (Fig. 7A,B). PBS scores were low for the ''Core Eucoilinae'' (Table 3) and indicate the 28S D2 data partition provided the strongest character support for the clade.

The Zamischus *group*

A clade here named the *Zamischus* group of genera was recovered as monophyletic in five of six combined analyses and molecular-data-only analyses (Figs 2, 6 and 7A,B). The strict consensus of the structural alignment analyzed via parsimony resulted in a polytomy between the *Zamischus* and *Kleidotoma* groups and the ''Core Eucoilinae'' (thin lines, Fig. 2A). All of the taxa considered members of the Neotropical Grade in Fontal-Cazalla et al. (2002) were recovered within the *Zamischus* group. Paraphyly of the ''Neotropical Grade'' of Fontal-Cazalla et al. (2002) was not recovered in any of the combined analyses presented here. The results of the sensitivity analyses indicate branch support for the *Zamischus* group is higher when the morphological data partition is excluded. PBS scores for this node confirm this observation (Table 3). The *Chrestosema* complex (comprised of *Chrestosema*, *Mirandicola*, and *Glauraspidia*) was recovered nested deeply within the *Zamischus* group in all combined analyses (Fig. 6) as well as the reduced data partition analyses (not shown). This result is in direct opposition to the results of Fontal-Cazalla et al. (2002), which recovered a monophyletic *Chrestosema* group of genera as part of the ''Core Eucoilinae''.

The *Zamischus* group represents one of the biggest challenges to eucoiline systematics. Though this group was routinely recovered as monophyletic in the absence of morphological data, the branch lengths along the backbone of this clade are exceedingly short (Fig. 6). These short branch lengths contribute to low bootstrap (Fig. 6) and posterior probability values (not shown). The molecular data demonstrate what has already been observed for the morphological data of this group: a great deal of autapomorphic data and little synapomorphic data (Fontal-Cazalla et al., 2002). Representatives of at least 60% of the described *Zamischus* group genera were included in this analysis. However, future studies on this group may require even richer taxon sampling as well as exploration of more informative character systems.

The Kleidotoma *group*

Support for the monophyly of the *Kleidotoma* group was strong (100% bootstrap and posterior probability of 1.0, Fig. 2; PBS scores between 6 and 8, Table 3). Based on combined analyses, the sister group to the *Zamischus* group is the *Kleidotoma* group of genera (Figs 2 and 6); however, these branches collapse in the strict consensus of the structural alignment combined parsimony tree (note thin lines, Fig. 2a). This *Zamischus* + *Kleidotoma* group relationship was found across most molecular-data-only analyses. Fontal-Cazalla et al. (2002) found this group to be monophyletic and part of an unresolved polytomy of higher Eucoilinae.

Conclusions

The structural alignment of the 28S D2 + D3 gene fragment resulted in the most defendable (due the explicit nature of the alignment procedure) and least conflicting alignment (based on the lack of hidden character conflict) tested here. Groups that were poorly supported in the combined analyses and reduced data partition analyses were typically alignment sensitive and were not recovered across all tested alignments. Though the combined analyses resulted in the most resolved phylogenetic trees (in both parsimony and Bayesian analyses), the trees produced by reduced data partition analyses and PBS results indicated that groups such as Parnipinae, Thrasorinae and Anacharitinae strongly rely on the morphological data partition to support their monophyly.

The general evolutionary pattern emerging from these analyses matches much of what was proposed by Ronquist (1999). At the base of the figitid tree are groups associated with the gall community (Parnipinae, Thrasorinae), a possibly relictual life-history strategy shared with some species of Cynipidae. A shift was later made to primary parasitization of exposed hosts associated with the aphid community, the extant example of this lineage being Anacharitinae. A later shift to parasitism of Diptera associated with aphids occurred within Aspicerinae. Similar shifts occurred in two separate lineages to schizophoran Diptera in exposed habitats (Figitinae, some Eucoilinae) and schizophoran Diptera in concealed habitats (some Eucoilinae and possibly Emargininae). A shift into this incredibly diverse fauna of hosts is likely responsible for the extreme diversification within the Eucoilinae.

Future research on this family needs to focus on deep level divergence patterns within Figitidae. Understanding the precise placement of Parnipinae, and to a lesser extent, Thrasorinae, is essential in understanding

the relationship between the phytophagous Cynipidae and entomophagous Figitidae. The Pycnostigminae (material not available for this study) may also prove to be critical to understanding the evolution of this family; future work on this system needs to include this taxon. Finally, a combination of the data presented here on figitid relationships, coupled with data on cynipid relationships (Nylander et al., in prep.) will allow for a comprehensive phylogenetic study of all Cynipoidea, bringing a new understanding to this ubiquitous, hyper-diverse, yet poorly understood group of parasitoids.

Acknowledgments

Many early ideas and drafts of this manuscript were reviewed by J. Pinto and M. Springer. D. Hawks assisted MLB considerably with sequencing. F. Ronquist and M. Springer helped with Bayesian analyses; M. Sorenson helped with the use of TreeRot. M. Forshage made some critical eucoiline identifications. Specimens were donated by the following researchers: C. Deitrich, T. Erwin, M. Irwin, J. Noyes and M. Sharkey (further acknowledgments can be found at the bottom of Appendix A). These researchers, and the taxonomists they employ for sorting material (e.g., B. Zuparko, California Academy of Science), deserve special recognition. S. Winterton and S. Morita reviewed earlier drafts of this manuscript. Funding to MLB for much of this research comes from NSF PEET BSR 9978150 (awarded to J. Heraty and J. Pinto).

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Appendix A

Listing of cynipoid taxa included in this analysis. Included is UCR DNA code (MB#; Ronquist Lab codes begin with "FR"), a brief description of the collection locality, the collector(s) of specimens ('Source'; detailed list at bottom of table), the UCR Entomology Research Museum specimen accession number (UCR#; * in this column indicates no voucher exists; - indicates the vouchers are associated with the Ronquist Lab) and the GenBank accession number for a given gene ('Nylander' are sequences not deposited in GenBank).

Taxon	MB Code#	Locality	Source**	UCR#	28S	COI	18S	Morphology
Liopteridae								
***Dallatorella* sp.**	341	New Guinea	UCDC	56733	AY675667	AY675816	–	✔
Liopteron sp.	398	Colombia	S	56734	AY675668	–	–	✔
Paramblynotus sp.	321	Colombia	S	56735	AY675666	AY675815	–	✔
Pseudibalia sp.	400	Colombia	S	56736	AY675669	–	AY675631	✔
Cynipidae								
***Andricus kollari* (Hartig)**	–	Hungary	R	–	AF395156	AF395176	–	✔
Andricus curvator Hartig	FRj007	Hungary	R	–	AF395155	DQ012621	–	✔
Andricus sp.	438	USA:AZ	UCR	56795	AY833724	AY833732	AY833728	✔
Barbotinia oraniensis (Barbotin)	–	Spain	R	–	AF395150	AF395179	–	✔
Ceroptres sp.	440	USA:ND	N	56797	AY833726	AY833734	AY833730	✔
***Cynips quercus* (Fourcroy)**	FRf258	Spain	R		DQ012596	DQ012638	–	✔
***Diplolepis rosae* (L.)**	–	Sweden	R	–	AF395157	AF395174	–	✔
Libelia fukudae Shinji	FRf254	Japan	R	–	DQ012601	DQ012645	–	✔
Neuroteras sp.	439	USA:AZ	UCR	56796	AY833725	AY833733	AY833729	✔
Panteliella bicolor Ionescu & Roman	–	Hungary	R	–	AF395153	AF395180	–	✔
Periclistus brandtii (Ratzeburg)	–	Sweden	R	–	AF395152	AF395181	–	✔
Pediaspis aceris (Gmelin)	–	Hungary	R	–	AY368955	AY368929	–	✔
Plagiotrochus quercusilicis (F.)	–	Spain	R	–	AF395154	AF395178	–	✔
Saphronecrus lusitanica Tavares	FR j013	Spain	R	–	DQ012608	DQ012651	–	✔
Synergus crassicornis Curtis	–	Spain	R	–	AY368936	AY368909	–	✔
Synophrus pilulae Hartig	FRf248	Hungary	R	–	DQ012656	–	–	✔
Xanthoteras sp.	437	USA:AZ	UCR	56794	AY833723	AY833731	AY833727	✔
Figitidae								
Parnipinae								
***Parnips nigripes* (Barbotin)**	–	Spain	R	–	AY368958	AY368932	–	✔
Thrasorinae								
Euceroptres sp.	425	USA:CA	UCR	56737	AY675673	AY675820	AY675632	✔
Myrtopsin sp.1	077	Colombia	S	56738	AY675671	AY675818	–	✔
Myrtopsin sp.2	422	Colombia	S	56740	AY675672	AY675819	–	✔
Plectocynips sp.	264	Chile	EMEC	56739	AY675670	AY675817	–	✔
Anacharitinae								
Acanthaegilips sp.1	271	Honduras	UCR	56758	AY675679	AY675826	–	✔
Acanthaegilips sp.2	029	Colombia	S	*	AY675680	AY675827	–	✔
Aeglips sp.1	246	USA:NM	W	56755	AY675681	AY675828	AY675634	✔
Aeglips sp.2	382	Canada	Y	56757	AY675683	AY675830	–	✔
Anacharis sp.1	274	USA:GA	UCR	56744	AY675674	AY675821	AY675633	✔
Anacharis sp.2	310	Canada	Y	56760	AY675675	AY675822	–	✔
Anacharis sp.3	412	Colombia	S	56741	AY675676	AY675823	–	✔
Anacharis sp.4	421	Colombia	S	56742	AY675677	AY675824	–	✔
Anacharis sp.5	370	Australia	UCR	56743	AY675678	AY675825	–	✔

Taxon	MB Code#	Locality	Source**	UCR#	28S	COI	18S	Morphology
Hexacharis sp.1	301	USA:CA	UCR	56746	AY675682	AY675829	AY675635	✔
Xyalaspis sp.1	218	Kyrgyzstan	D	56761	AY675684	AY675831	–	✔
Xyalaspis sp.2	141	Guatemala	UCR	56759	AY675685	–	–	✔
Xyalaspis sp.3	413	Colombia	S	56745	AY675686	AY675832	AY675636	✔
Aspicerinae								
Anacharoides sp.	319	Madagascar	I&Z	56774	AY675696	AY675840	–	✔
Aspicera sp.	424	USA:CA	UCR	56769	AY675695	AY675839	–	✔
Callaspidea sp.1	059	Russia	UCR	56770	AY675692	AY675837	–	✔
Callaspidea sp.2	031	Colombia	S	56768	AY675693	AY675838	AY675638	✔
Callaspidea sp.3	226	USA:CA	UCR	56773	AY675694	–	–	✔
Melanips sp.1	281	USA:CA	UCR	56772	AY675690	AY675835	AY675637	✔
Melanips sp.2	302	USA:CA	UCR	56771	AY675691	AY675836	–	✔
Omalaspis sp.	381	India	UCR	56775	AY675697	AY675841	–	✔
Paraspicera sp.	387	USA:CA	UCR	56767	AY675698	AY675842	AY675639	✔
Figitinae								
Amphithectus sp.	216	Kyrgyzstan	D	56747	AY675701	AY675845	AY675640	✔
Figites sp.	305	Canada	Y	56753	AY675700	AY675844	–	✔
Lonchidia sp.1	284	USA:NM	G&G	56750	AY675687	AY675833	–	✔
Lonchidia sp.2	224	Kyrgyzstan	D	*	AY675688	–	–	✔
Lonchidia sp.3	286	USA:NM	G&G	56749	AY675689	AY675834	–	✔
Neralsia sp.1	233	USA:TX	Y	56751	AY675702	AY675846	–	✔
Neralsia sp.2	245	Mexico	UCR	56748	AY675703	AY675847	AY675641	✔
Trischiza sp.	049	USA:MT	M	56754	AY675699	AY675843	–	✔
Xyalophora sp.	289	USA:NM	G&G	56752	AY675704	AY675848	–	✔
Charipinae								
Alloxysta sp.1	FRj015	Sweden	R	–	DQ012577	DQ012618	–	✔
Alloxysta sp.2	300	USA:CA	UCR	56766	AY675705	AY675849	–	✔
unknown genus	239	USA:CA	UCR	56756	AY675706	AY675850	AY675642	✔
Emargininae								
Thoreauella sp.1	255	Kenya	Sn	56762	AY675707	–	AY675643	✔
Thoreauella sp.2	311	Nicaragua	UCR	56764	AY675708	AY675851	AY675644	✔
Thoreauella sp.3	322	Madagascar	I&Z	56763	AY675709	AY675852	AY675645	✔
Thoreauella sp.4	392	Australia	UCR	56765	AY675710	–	–	✔
Eucoilinae								
Acantheucoila sp.	409	Colombia	S	56821	AY675748	AY675885	AY675654	✔
Aegeseucoela flavotincta (Kieffer) 1	054	Ecuador	E/UCR	56851	AY675795	AY675924	–	✔
Aegeseucoela flavotincta (Kieffer) 2	083	Colombia	S	56852	AY675792	AY675922	–	✔
Aegeseucoela flavotincta (Kieffer) 3	396	Colombia	S	56802	AY675793	AY675923	–	✔
Aegeseucoela flavotincta (Kieffer) 4	414	Colombia	S	56824	AY675794	–	–	✔
***Aegeseucoela grenadensis* (Ashmead)**	084	Colombia	S	56853	AY675799	AY675928	–	✔
***Aganaspis* sp.**	121	Colombia	S	56812	AY675777	AY675909	–	✔
Agrostocynips sp.1	069	Colombia	S	56809	AY675797	AY675926	–	✔
Agrostocynips sp.2	187	USA:FL	UCR	56777	AY675796	AY675925	–	✔
Agrostocynips sp.3	294	Mexico	UCR	56776	AY675798	AY675927	–	✔
Aporeucoela sp.1	282	US:CA	UCR	56786	AY675771	AY675906	–	✔
Aporeucoela sp.2	287	US:NM	G&G	56835	AY675753	AY675890	–	✔
Caleucoela sp. 1	027	Ecuador	E/UCR	56828	AY675751	AY675888	–	✔
Caleucoela sp. 1	402	Colombia	S	56829	AY675752	AY675889	–	✔
Coneucoila sp.1	411	Colombia	S	56833	AY675755	–	–	✔
Coneucoila sp.2	417	Colombia	S	56825	AY675756	AY675892	–	✔
Chrestosema sp.	173	Russia	UCR	56863	AY675723	AY675862	–	✔
Cothonaspis sp.	275	S. Africa	UCR	56803	AY675786	AY675916	–	✔
Dettmeria sp.1	135	Colombia	S	56856	AY675813	AY675938	–	✔
Dettmeria sp.2	214	Colombia	S	56810	AY675814	AY675939	–	✔
Diceratapsis sp.1	229	USA:AZ	W	56807	AY675810	–	–	✔
Diceratapsis sp.2	420	Colombia	S	56808	AY675812	AY675937	–	✔
Diceratapsis sp.3	140	Guatemala	UCR	*	AY675811	–	–	✔
Didyctium sp.1	348	Costa Rica	N	56798	AY675772	AY675907	–	✔
Didyctium sp.2	349	Costa Rica	N	56789	AY675773	–	AY675659	✔
Dieucoila sp.1	248	Costa Rica	N	56783	AY675758	AY675894	–	✔
Dieucoila sp.2	342	Mexico	UCR	56815	AY675759	AY675895	–	✔
Dieucoila sp.3	399	Colombia	S	56832	AY675760	AY675896	–	✔
Diglyphosema sp.	057	Russia	UCR	56864	AY675741	AY675880	–	✔

Taxon	MB Code#	Locality	Source**	UCR#	28S	COI	18S	Morphology
***Disorygma pacifica* (Yoshimoto)**	060	USA:CA	UCR	*	AY675734	AY675873	AY675653	✔
***Ealata clava* Quinlan**	320	Madagascar	I&Z	56871	AY675736	AY675875	–	✔
Epicoela sp.1	283	USA:NM	G&G	56785	AY675749	AY675886	–	✔
Epicoela sp.2	407	Colombia	S	56834	AY675750	AY675887	AY675655	✔
Epicoela sp.3	106	Colombia	S	56793	AY675754	AY675891	AY675656	✔
Eucoila sp.	222	Kyrgyzstan	D	56778	AY675716	AY675858	AY675647	✔
Ganaspis neotropica Diaz 1	350	Costa Rica	N	56847	AY675775	–	–	✔
Ganaspis neotropica Diaz 2	364	Costa Rica	N	56848	AY675776	–	–	✔
Ganaspis sp.1	394	Australia	UCR	56841	AY675770	AY675905	–	✔
Ganaspis sp.2	389	Colombia	S	56826	AY675769	AY675904	AY675658	✔
Ganaspis sp.3	367	Costa Rica	N	56792	AY675768	–	–	✔
Ganaspis sp.4	254	Kenya	Sn	56787	AY675767	AY675903	–	✔
Ganaspis sp.5	161	Russia	UCR	56840	AY675766	AY675902	–	✔
Ganaspidium hunteri (Crawford)	259	USA:NV	UCR	56822	AY675737	AY675876	–	✔
Ganaspidium nigrimanus (Kieffer) 1	269	USA:CA	UCR	56868	AY675739	AY675878	–	✔
Ganaspidium nigrimanus (Kieffer) 2	232	USA:NV	UCR	*	AY675738	AY675877	–	✔
Ganaspidium pussilae Weld 1	148	USA:NV	UCR	*	AY675800	AY675929	–	✔
Ganaspidium pussilae Weld 2	236	USA:CA	UCR	56867	AY675801	AY675930	–	✔
Glauraspidia sp.1	165	Russia	UCR	56858	AY675721	–	–	✔
Gronotoma micromorpha (Perkins)	258	U.S. Samoa	UCR	56870	AY675742	AY675881	–	✔
Gronotoma nitida (Benoit)	265	Kenya	S	56869	AY675743	AY675882	–	✔
Gronotoma sp.	153	Russia	UCR	56866	AY675745	AY675884	–	✔
Hexacola neoscatellae Beardsley	062	USA:CA	UCR	56839	AY675765	AY675901	–	✔
Kleidotoma sp.1	273	USA:GA	UCR	56814	AY675782	AY675914	–	✔
Kleidotoma sp.2	171	Russia	UCR	56838	AY675783	AY675915	AY675662	✔
Leptolamina sp.1	393	Australia	UCR	56857	AY675720	–	AY675649	✔
Leptolamina sp.2	318	Madagascar	I&Z	56799	AY675719	AY675860	–	✔
Leptopilina sp.1	117	Colombia	S	56837	AY675729	AY675868	AY675652	✔
Leptopilina sp.2	170	Russia	UCR	56875	AY675727	AY675866	–	✔
Microstilba sp.	150	Kyrgyzstan	D	56865	AY675735	AY675874	–	✔
Mirandicola sp.1	058	Russia	UCR	56860	AY675722	AY675861	AY675648	✔
Mirandicola sp.2	152	Russia	UCR	56859	AY675718	–	–	✔
Moritiella elegans Buffington	085	Colombia	S	56842	AY675711	AY675853	–	✔
Nordlanderia plowa Quinlan	235	S. Africa	UCR	56873	AY675746	–	–	✔
Nordlanderia sp.	266	Kenya	Sn	56872	AY675744	AY675883	–	✔
Nordlandiella semirufa (Kieffer)	279	Hawaii	T	56784	AY675774	AY675908	AY675660	✔
Odonteucoila sp.1	192	Colombia	S	56844	AY675761	AY675897	–	✔
Odonteucoila sp.2	212	Colombia	S	56846	AY675762	AY675898	–	✔
Odonteucoila sp.3	355	Costa Rica	N	56876	AY675763	AY675899	–	✔
Odonteucoila sp.4	369	Costa Rica	N	56813	AY675764	AY675900	AY675657	✔
Paradiglyphosema sp.	288	Kenya	Sn	56874	AY675740	AY675879	–	✔
Preseucoela heratyi Buffington 1	385	Argentina	UCR	56804	AY675805	AY675934	–	✔
Preseucoela heratyi Buffington 2	30	Colombia	S	56849	AY675803	AY675932	–	✔
***Preseucoela heratyi* Buffington 3**	38	Colombia	S	56850	AY675804	AY675933	–	✔
***Preseucoela pallidipes* (Ashmead)**	102	Colombia	S	*	AY675802	AY675931	AY675664	✔
Rhabdeucoela sp.1	067	Colombia	S	56806	AY675788	AY675918	–	✔
Rhabdeucoela sp.2	243	Mexico	UCR	56805	AY675789	AY675859	AY675663	✔
Rhoptromeris sp.1	345	Kyrgyzstan	D	*	AY675717	AY675859	–	✔
Rhoptromeris sp.2	162	Russia	UCR	56791	AY675726	AY675865	AY675651	✔
***Sirenes silenus* Quinlan**	376	Madagascar	I&Z	56862	AY675731	AY675870	–	✔
Trichoplasta sp.1	253	Kenya	Sn	56861	AY675732	AY675871	–	✔
Trichoplasta sp.2	327	Madagascar	I&Z	56830	AY675733	AY675872	–	✔
***Triplasta* sp.1**	234	USA:TX	Y	56845	AY675784	–	–	✔
Triplasta sp.2	361	Costa Rica	N	56843	AY675785	–	–	✔
Tropideucoila sp.1	397	Colombia	S	56823	AY675809	AY675936	AY675665	✔
Tropideucoila sp.2	247	Costa Rica	N	56855	AY675808	–	–	✔
Tropideucoila sp.3	011	Ecuador	E/UCR	56811	AY675807	AY675935	–	✔
Trybliographa sp.1	163	Russia	UCR	56780	AY675712	AY675854	–	✔
Trybliographa sp.2	181	Russia	UCR	56779	AY675714	AY675856	–	✔
Trybliographa sp.3	185	USA:NY	UCR	56781	AY675713	AY675855	AY675646	✔
Trybliographa sp.4	186	USA:NY	UCR	56782	AY675715	AY675857	–	✔
***Zaeucoila* nr. *unicarinata* Ashmead**	386	Argentina	UCR	56820	AY675791	AY675921	–	✔
Zaeucoila sp.	006	Ecuador	E/UCR	*	AY675790	–	–	✔

Taxon	MB Code#	Locality	Source**	UCR#	28S	COI	18S	Morphology
Zamischus sp.	419	Colombia	S	56831	AY675747	AY675920	–	✔
Undescribed genera							–	✔
New Genus B sp.	415	Colombia	S	56854	AY675806	–	–	✔
New Genus E sp.	405	Colombia	S	56827	AY675757	AY675893	–	✔
New Genus F sp.	169	Russia	UCR	56836	AY675787	AY675917	–	✔
New Genus G sp.1	240	Australia	UCR	56788	AY675725	AY675864	–	✔
New Genus G sp.2	242	Australia	UCR	56790	AY675724	AY675863	AY675650	✔
New Genus H sp.1	404	Colombia	S	56801	AY675730	AY675869	–	✔
New Genus H sp.2	353	Costa Rica	N	56800	AY675728	AY675867	–	✔
Schick new genus sp.1	099	Colombia	S	56819	AY675779	AY675911	–	✔
Schick new genus sp.2	201	Colombia	S	56816	AY675780	AY675912	–	✔
Schick new genus sp.3	340	Puerto Rico	G	56817	AY675778	AY675910	AY675661	✔
Schick new genus sp.4	390	Colombia	S	56818	AY675781	AY675913	–	✔

** Codes for 'source' (in bold) are as follows: UCDC, Bohart Collection, UC Davis; S, M. Sharkey, University of Kentucky; R, F. Ronquist and J. Nylander; EMEC, Essig Museum, UC Berkeley; UCR, Entomology Research Collection, UC Riverside, and Heraty/Pinto lab researchers (also see Acknowledgements); Y, M. Yoder, Texas A&M; G&G, M. Gates (SEL, Washington D.C.) & J. George (UCR); D, C. Deitrich, Illinois Natural History Survey; I&Z, M. Irwin (Illinois Natural History Survey) and B. Zuparko (California Academy of Arts and Science); Sn, R. Snelling (LA County Museum), E/UCR, T. Erwin (United States National Museum, Washington D.C., collecting) and Heraty/Pinto Labs (UCR, sorting); G. M. Gates (SEL, Washington D.C.); N, J. Noyes (British Musuem of Natural History).

Appendix B

List of morphological and biological characters used in analysis. All characters were analysed unordered. No transformation series are implied or intended as based on character state number. Characters were taken from the following sources:
1–148, from Fontal-Cazalla et al. (2002); characters modified and complimented as noted.
149–160 and 164 from Ros-Farre et al. (2000) (corresponding, respectively to, characters 2, 4, 6–9, 11–12, 16–17, 19 and 21–22). Characters were modified and complimented as noted.
161 from Ronquist 1995, character 46. Coding conserved.
162, 164–167 from Ronquist (1999) (corresponding, respectively, to characters 7, 9, 10, 12 and 11). Characters were modified and complimented as noted.
165 from Nylander et al. (in preparation).
163 previously unpublished.

Morphological characters

1 Microsculpture on vertex, lateral surface of pronotum and mesoscutum: (0) absent, surface not dull; (1) present, linear, making the surface dull.
2 Shape of head in anterior view: (0) rounded, approximately as high as broad; (1) elongate, higher than broad; (2) triangular. Modified from Fontal-Cazalla et al. (2002).
3 Relative position of eye: (0) close to ocelli, ratio of distance between compound eye and posterior mandibular articulation to distance between posterior ocellus and compound eye 1.2; (1) removed from ocelli, ratio <1.2.
4 Size of ocelli: (0) small, ratio of maximum diameter of a lateral ocellus to shortest distance between lateral ocelli 0.2-0.4; (1) large, ratio > 0.4.
5 Relative position of anterior ocellus: (0) placed close to posterior ocelli, posterior margin of anterior ocellus behind or subcontiguous with a transverse line running through anterior margins of posterior ocelli; (1) placed farther from posterior ocelli, clearly anterior to the anterior margins of posterior ocelli.
6 Pubescence on compound eyes: (0) short or absent; (1) long.
7 Shape of compound eyes in dorsal view: (0) rounded, distinctly protruding from the surface of the head, particularly anteriorly; (1) less rounded, not distinctly protruding from the surface of the head.
8 Lateral frontal carina: (0) absent; (1) present.
9 Hair punctures on lateral part of vertex: (0) indistinct or absent; (1) present, distinctly enlarged.
10 Sculpture on posterior part of vertex: (0) smooth or punctate, without linear component; (1) with parallel or slightly radiating, transverse strigae.
11 Relative position of antennal sockets: (0) close to ocelli; ratio of vertical distance between inner margin of antennal foramen and ventral margin of clypeus to vertical distance between anterior ocellus and antennal rim < 2.0; (1) intermediate, ratio 2.0-4.0; (2) far from ocelli, ratio > 4.0.
12 Vertical carina adjacent to ventral margin of antennal socket: (0) absent; (1) present.
13 Vertical delineations on lower face: (0) absent; (1) single carina or ledge; (2) several parallel or subparallel carinae.
14 (Subdivision of 13:1) Shape of single vertical delineation of lower face: (0) rounded divergent ledges running from antennal sockets to dorsal end of malar sulcus; (1) sharp divergent carinae running from antennal sockets to dorsal end of malar sulcus; (2) sharp convergent carinae running from antennal sockets to clypeus.
15 Size of anterior tentorial pits: (0) large; (1) small.
16 Shape of ventral clypeal margin medially: (0) emarginate or straight; (1) triangularly projecting.
17 Shape of ventral clypeal margin laterally, close to anterior mandibular articulation: (0) straight; (1) distinctly angled.
18 Malar sulcus: (0) absent; (1) present.
19 Small submarginal pyramidal prominence of malar space, adjacent to anterior articulation of mandible: (0) absent; (1) present.
20 Sculpture of malar space posterior to anterior mandibular articulation: (0) without linear sculpture; (1) with a series of parallel strigae.
21 Length of gena (from compound eye to posterolateral margin of head): (0) short, ratio of length of gena to length of compound eye in dorsal view <0.3; (1) long, ratio >0.3.
22 Shape of posterior surface of head: (0) deeply impressed around postocciput; (1) almost flat, not deeply impressed.
23 Lateral margin of occiput: (0) not well defined; (1) defined by a raised, blunt carina; (2) defined by a raised, sharp carina.
24 Sculpture along lateral margin of occiput (except for raised carina, if present): (0) linear sculpture absent; (1) one costula; (2) many costulae.
25 Sculpture on occiput (except extreme lateral margin): (0) without linear sculpture, at most with a few weak strigae along the peripheral

margin; (1) with some weak subvertical, irregular strigae; (2) with distinct subvertical, slightly and evenly curved costulae.
26 Carina issuing from lateral margin of postocciput, proximally horizontal but distally bending ventrally: (0) absent, at most vaguely indicated basally; (1) present, distinct.
27 Direction of longitudinal axis of posterior tentorial pits: (0) vertical; (1) oblique.
28 Length of gula: (0) short; (1) long.
29 Median hairy strip of gula: (0) present; (1) absent.
30 Shape of hypostomal carina medially: (0) straight; (1) distinctly angled laterally about 1/3 from proximal end.
31 Shape of hypostomal carina ventrally: (0) ends at ventral head margin close to posterior mandibular articulation, not projecting beyond head margin; (1) ending in a distinct process some distance posterior to the mandibular articulation.
32 Shape of female antenna: (0) cylindrical, not widened towards apex; (1) cylindrical, distinctly widened towards apex; (2) distinctly widened and laterally compressed towards apex.
33 Articulation between flagellomeres in female antenna: (0) connate with the segments broadly joined; (1) at least distally moniliform with the segments distinctly separated by a narrow neck-like articulation.
34 Shape of the three last antennal flagellomeres of female antenna: (0) of normal width or widened but not conspicuously enlarged; (1) conspicuously enlarged compared to adjacent flagellomeres.
35 Number of articles of male antenna: (0) fourteen; (1) fifteen; (2) more than fifteen.
36 Shape of second flagellomere of male antenna: (0) not modified, cylindrical; (1) slightly asymmetric basally; (2) strongly asymmetric, excavated laterally.
37 Length of second flagellomere of male antenna: (0) shorter than first flagellomere; (1) longer than first flagellomere.
38 Shape of right mandible: (0) subquadratic, first and second tooth not conspicuously long; (1) elongate to triangular, first and second tooth conspicuously long.
39 Basal height of right mandible: (0) short; (1) long.
40 Sculpture on basal third of right mandible: (0) smooth or punctate; (1) weakly irregularly striate.
41 Third tooth of right mandible: (0) present; (1) absent.
42 Submarginal ridge interiorly along dorsal margin of left mandible: (0) absent; (1) present.
43 Number of setae on interior side of left mandible, close to dorsal margin: (0) five or more; (1) two or three, occasionally up to four.
44 Shape of posterior mandibular process of left mandible in posterior view: (0) evenly rounded, not conspicuously projecting; (1) forming a small, distinctly set off, rounded projection; (2) forming a large semi-triangular projection.
45 Number of segments of maxillary palp: (0) five; (1) four (basal two segments fused); (2) three or less. *Last state not in Fontal-Cazalla et al. (2002)*.
46 Relative position of the two last segments of maxillary palp in normal repose: (0) curved inwards; (1) straight.
47 Angle of the distal margin of the subapical segment of the maxillary palp and movement of apical segment: (0) margin slants inwards, apical segment bends inwards; (1) margin is straight or slants distinctly outwards, apical segment bends outwards.
48 Relative length of the apical segment of maxillary palp: (0) more than 1.5 times as long as the preceding segment; (1) 1-1.5 times as long; (2) shorter than the preceding segment.
49 Pubescence on apical segment of maxillary palp: (0) consisting of a small number of erect setae and more appressed setae; (1) only consisting of erect setae.
50 Apical seta on apical segment of maxillary palp: (0) relatively short, much shorter than twice the length of the second longest apical seta; (1) long, only slightly shorter than twice the length of the second longest apical seta; (2) conspicuously long, longer or much longer than twice the length of the second longest apical seta.
51 Erect setae medially on apical segment of maxillary palp: (0) present; (1) absent.
52 Number of segments of labial palp: (0) three; (1) two; (2) one.
53 Shape of first segment of labial palp: (0) short, shorter than or equal to apical segment; (1) long, longer than apical segment.
54 Shape of anterior flange of pronotal plate: (0) subvertical, not protruding; (1) distinctly protruding anteriorly.
55 Sculpture of anterior flange of pronotal plate: (0) smooth or punctate; (1) transversely strigate; (2) linearly striate. *Last state not in Fontal-Cazalla et al. (2002)*.
56 Shape of submedian pronotal depressions laterally: (0) open; (1) closed.
57 Depth of submedian pronotal depressions medially: (0) deep; (1) shallow.
58 Width of pronotal plate: (0) narrow; (1) wide, almost as wide as mesonotum.
59 Lateral margin of pronotal plate: (0) defined only anteriorly; (1) defined all the way to the dorsal margin of the pronotum.
60 Shape of dorsal margin of pronotal plate in anterior view: (0) rounded, straight, or occasionally slightly emarginate; (1) distinctly emarginate.
61 Lateral part of dorsal margin of pronotal plate: (0) not raised into a crest; (1) raised into a distinct crest but not projecting above the dorsoposterior margin of pronotum; (2) raised into a distinct process projecting above the dorsoposterior margin of the pronotum.
62 Ridges extending posteriorly from lateral margin of pronotal plate: (0) absent or merely indicated; (1) distinct but short, not extending to the dorsal margin of pronotum; (2) long, extending to the dorsal margin of pronotum.
63 Macrosculpture on lateral surface of pronotum: (0) present; (1) absent.
64 Pubescence on lateral surface of pronotum: (0) short and dense; (1) consisting of a few long hairs or absent.
65 Lateral pronotal carina: (0) present; (1) absent.
66 Anteroventral inflection of pronotum: (0) narrow; (1) broad, particularly adjacent to anterior part of pronotal plate.
67 Ventral margin of pronotum: (0) not distinctly raised midlaterally (occasionally raised adjacent to pronotal plate); (1) distinctly raised midlaterally.
68 Curvature of mesoscutal surface: (0) scutum convex and evenly curved (except for notauli, if present); (1) lateral thirds of scutum flat, depressed mesally, median third raised.
69 Median mesoscutal carina: (0) absent; (1) present as a narrow, distinctly defined carina; (2) present as an anteriorly broad elevation narrowing posteriorly.
70 Notauli: (0) present as a deep furrow or series of deep subcontiguous pits; (1) completely absent or merely indicated by a series of isolated, small punctures.
71 Parascutal carina: (0) distinctly sinuate, posteriorly ends in a posteroventrally directed slight projection; (1) less sinuate, posteriorly curved mesally, not drawn out to a posteroventrally directed projection.
72 Length of longitudinal carina or septum separating scutellar foveae and continuing posteriorly in scutellar plate, if present: (0) short; (1) medium; (2) long.
73 Scutellar fovea: (0) not margined posteriorly; (1) distinctly margined posteriorly.
74 Shape of lateral bar: (0) relatively narrow, not conspicuously widened ventrally; (1) conspicuously widened by a large ventral lobe.
75 Strigate sculpture on lateral bar: (0) absent; (1) weak; (2) strong.
76 Structure of the dorsal part of the scutellum: (0) without any clearly differentiated median part; (1) with a differentiated median part.
77 (Subdivision of 76:1) Structure of the modified median part of the scutellum: (0) separated by different sculpture; (1) separated by marginal carinae; (2) raised to form an elevated scutellar plate.
78 (Subdivision of 77:2) Size of scutellar plate: (0) very large and rounded, almost circular, covering most of scutellum; (1) medium-

sized, exposing a large part of scutellum; (2) small and narrow, exposing most of scutellum.
79 (Subdivision of 77:2) Position of the glandular release pit of the scutellar plate: (0) distinctly removed from the posterior margin of the plate; (1) close to the posterior margin of the plate.
80 (Subdivision of 77:2) Shape of rim of scutellar plate in lateral view: (0) flat; (1) only very slightly convex; (2) distinctly convex.
81 (Subdivision of 77:2) Shape of dorsal surface of scutellar plate: (0) almost entire surface concave, with a clear border along the concavity; (1) smaller part of the surface concave, clear border present only anteriorly or absent; (2) flat or slightly convex.
82 (Subdivision of 77:2) Transverse median carina on scutellar plate: (0) absent; (1) present.
83 Projection from the dorsal surface of scutellar plate or homologous region of the scutellum: (0) absent; (1) present as a blunt, tooth-like projection; (2) present as a distinct spine.
84 Longitudinally strigate sculpture on dorsal surface of scutellum: (0) irregularly rugulose; (1) smooth and striate. *Modified from Fontal-Cazalla et al. (2002)*.
85 Carina along scutellar margin, separating the dorsal and ventral scutellar surfaces: (0) absent; (1) present, at least anteriorly.
86 Shape of dorsoposterior part of scutellum in dorsal view: (0) broadly and distinctly emarginate; (1) rounded or truncate, occasionally slightly incised medially; (2) produced posteriorly; (3) with delimited projection (spine) posteriorly.
87 Posterior margin of axillula: (0) marked by a distinct ledge, axillula distinctly impressed adjacent to ledge; (1) axillula only superficially impressed posteriorly or continuous with scutellum.
88 Subalar area: (0) abruptly broadened anteriorly, with an indicated longitudinal division; (1) only slightly broadened anteriorly, without longitudinal division indicated.
89 Sculpture of mesopleuron: (0) smooth, occasionally partly punctate; (1) horizontally strigulate.
90 Mesopleural triangle: (0) distinctly impressed with a marked ventral border continuing into subalar pit; (1) absent or slightly impressed but without a distinct ventral border. *Coding corrected*: Fontal-Cazalla et al. (2002) erroneously coded '*Tropideucoila* sp.' as having state (1). The MorphBank image series for this taxon was corrupted by images belonging to the then undescribed *Aegesecoela flavotincta* (Kieffer) which superficially looks like a *Tropideucoila* (Buffington, 2002). Coding has been corrected to (0) in this analysis for *Tropideucoila*.
91 Subalar pit: (0) large and well defined, lying in the posterior end of the mesopleural triangle; (1) somewhat smaller but well defined, lying in a distinct, more or less narrow subalar groove; (2) reduced in size, usually elongate, subalar groove narrow or absent; (3) absent, subalar groove indistinct or absent.
92 Mesopleural carina/carinae: (0) several long, irregular, curved carinae; (1) several long, parallel, straight carinae; (2) one complete, straight main carina, occasionally one or a few weak or short subordinate carinae; (3) one straight carina indicated anteriorly, otherwise smooth; (4) series of deep pits. *Last state not included in Fontal-Cazalla et al. (2002)*.
93 (Subdivision of 92:2-3) Position of anterior end of single mesopleural carina: (0) high, above notch in anterior margin of mesopleuron; (1) low, at or below notch in anterior margin of mesopleuron.
94 Lateroventral mesopleural carina (extending along entire mesopleuron): (0) absent; (1) present but not marking an abrupt change of slope of the mesopectus; (2) present and marking abrupt change of slope.
95 Posterior part of mesocoxal rim: (0) rounded or only slightly extended posterolaterally; (1) conspicuously extended laterally and posterolaterally, projecting beyond margin of mesopectus.
96 Pubescence on lateral surface of metapectal-propodeal complex (excluding possible presence of felt-like or woolly pubescence posteriorly): (0) evenly covering surface; (1) only present in posterior half; (2) consisting of a few scattered hairs posteriorly.
97 Pubescence on posterior part of metapectal-propodeal complex: (0) not extremely dense; (1) extremely dense and felt-like on posterior part of metapleuron and lateral part of propodeum; (2) extremely dense and long on entire propodeum but not on metapleuron, only slightly curled and not felt-like in appearance.
98 Anterior impression of metepimeron: (0) triangular with the broadest part ventrally; (1) absent or present as a narrow, linear impression which is not broadened ventrally.
99 Posterior margin of metepimeron: (0) distinctly marked; (1) not marked, metepimeron continuous posteriorly with propodeum.
100 Anterior impression of metepisternum (immediately beneath anterior end of metapleural carina): (0) absent or small and narrow; (1) large and wide.
101 Anterior margin of metapectal-propodeal complex: (0) meeting or almost meeting the mesopleuron at the same level at least at a point corresponding to the anterior end of the metapleural carina; (1) separated from the mesopleuron by a deep and broad, uninterrupted marginal impression.
102 Structure of metapectus anterodorsal to metacoxal base: (0) with an ill-defined cavity; (1) with a well-defined cavity; (2) without a cavity.
103 Depression anterolaterally on metasubpleuron, anterior to metacoxal foramen: (0) narrow or absent; (1) broad.
104 Shape of posteroventral corner of metapleuron in lateral view: (0) rounded, not drawn out posteriorly; (1) extended posteriorly.
105 Anterior part of metacoxal rim: (0) rounded, not extended anteriorly; (1) extended anteriorly into a distinct process.
106 Shape of metacoxal foramen: (0) round; (1) elongate.
107 Lateral part of metacoxal rim: (0) narrow and not projecting, or forming a posteriorly or posterolaterally facing, rounded projection which is usually pubescent; (1) forming a smooth and nude triangular projection having its surface directed more laterally.
108 Posterior part of metacoxal rim: (0) rounded or extended into a small posterolateral process; (1) extended into a large posterior process.
109 Shape of propodeum: (0) normal shape, not drawn out posteriorly; (1) drawn out posteriorly.
110 Shape of calyptra in lateral view: (0) rounded; (1) elongate.
111 Shape of calyptra in posterior view: (0) rounded; (1) dorsoventrally elongate.
112 Horizontal carina running anteriorly from lateral propodeal carina: (0) absent; (1) present.
113 Shape of lateral propodeal carina: (0) straight; (1) distinctly angled.
114 Dorsal extent of lateral propodeal carinae: (0) not reaching scutellum; (1) projecting beyond metanotum to reach scutellum. *Coding corrected*: Fontal-Cazalla et al. (2002) erroneously coded '*Tropideucoila* sp.' as having state (1). The MorphBank image series for this taxon was corrupted by images belonging to the then undescribed *Aegesecoela flavotincta* (Kieffer) which superficially looks like a *Tropideucoila* (Buffington, 2002). Coding has been corrected to (0) in this analysis for *Tropideucoila*.
115 Shape of ventral end of lateral propodeal carina and dorsal part of nucha: (0) lateral propodeal carinae ending before reaching nucha; (1) lateral propodeal carinae reaching nucha but separated from each other; (2) lateral propodeal carinae reaching nucha and joined with each other along the dorsal margin of the nucha.
116 Structure of petiolar foramen: (0) anteriorly situated, close to metacoxae, foramen directed ventrally; (1) removed from metacoxae, foramen directed ventrally; (2) removed from metacoxae, foramen directed posteriorly.
117 Dorsal part of petiolar rim: (0) narrow, about the same width as the rest of the petiolar rim; (1) wide, distinctly wider than the rest of the rim.
118 Ventral and lateral parts of petiolar rim: (0) narrow; (1) broad.

119 Pubescence posterolaterally on metacoxa: (0) sparse to moderately dense but never with confined dense hair patch basally; (1) with a confined, dense hair patch or hair band originating basally, other pubescence usually lacking; (2) metacoxa completely nude. *Coding corrected*: Fontal-Cazalla et al. (2002) erroneously coded '*Tropideucoila* sp.' as having state (0). The MorphBank image series for this taxon was corrupted by images belonging to the then undescribed *Aegesecoela flavotincta* (Kieffer) which superficially looks like a *Tropideucoila* (Buffington, 2002). Coding has been corrected to (1) in this analysis for *Tropideucoila*.

120 (Subdivision of 119:1) Shape of dense hair patch basally on the posterolateral surface of the metacoxa: (0) small, circular; (1) elongate. *Coding corrected*: Fontal-Cazalla et al. (2002) erroneously coded '*Tropideucoila* sp.' as having state (0). The MorphBank image series for this taxon was corrupted by images belonging to the then undescribed *Aegesecoela flavotincta* (Kieffer) which superficially looks like a *Tropideucoila* (Buffington, 2002). Coding has been corrected to (1) in this analysis for *Tropideucoila*.

121 Microsculpture on hind coxa: (0) absent; (1) present.

122 Shape of metatarsal claw: (0) base strongly expanded and apex strongly bent, ratio width of base to length of apex > 0.6; (1) base weakly expanded and apex slightly bent, ratio < 0.6.

123 Microsculpture on outer surface of metatarsal claw: (0) microcarinate; (1) entirely or almost entirely smooth.

124 Pubescence on outer surface of metatarsal claw: (0) more dense, consisting of a considerable number of setae; (1) more sparse, consisting of only a few setae.

125 Position of the apical seta of the metatarsal claw: (0) situated on outer surface of claw below dorsal margin; (1) situated on dorsal margin of claw.

126 Shape of the apical margin of female fore wing: (0) rounded; (1) emarginate.

127 Pubescence of fore wing: (0) long and dense on most of the surface; (1) shorter, scattered or absent on basal half of wing.

128 Hair fringe along apical margin of fore wing: (0) very short or absence; (1) present, long or very long.

129 Marginal cell of fore wing: (0) membranous, similar to other wing cells; (1) sclerotised to form a pseudopterostigma.

130 Extent of R_1: (0) tubular along at least basal part of anterior margin of marginal cell; (1) ending at anterior margin (marginal cell open anteriorly); (2) not reaching anterior margin (marginal cell open anteriorly and basally) but at least partly present beyond 2r; (3) completely absent beyond 2r.

131 Basal abscissa of R_1 (the abscissa between 2r and the wing margin) of fore wing: (0) as broad as adjacent wing veins; (1) clearly broader than adjacent wing veins.

132 Areolet of fore wing: (0) present; (1) absent.

133 Submedian dorsal depressions of the articular bulb of female petiole: (0) present, large and distinct, articular bulb raised to a median ridge or keel between them; (1) absent or merely indicated, articular bulb not raised into a median keel.

134 Position of patches of mechanosensory hairs on articular bulb of female petiole (only seen in high magnification): (0) situated more laterally, not delimited by a raised central area; (1) situated more ventromedially, delimited by a slightly raised smooth central area.

135 Shape of the posterior part of female petiole: (0) abruptly widened; (1) not abruptly widened.

136 Ventral flange of annulus of female petiole: (0) large and broad, its anterior margin projecting and partially covering the articular bulb of the petiole; (1) absent or small and narrow, only slightly projecting anteriorly.

137 Fusion of terga in female metasoma: (0) all postpetiolar terga free; (1) terga 3 to 5 fused to a large syntergum; (2) terga 3-4 fused to form a syntergum. *Last state not included in Fontal-Cazalla et al. (2002)*.

138 (Subdivision of 137:1) Shape of syntergum (or tergum 3 to 5) of female metasoma: (0) not extending ventrally beneath sterna, not folded inwards, ventral margin rounded; (1) extending ventrally and folded inwards beneath sterna, forming a straight ventral margin.

139 Size of third abdominal tergum of female metasoma: (0) about as large as fourth tergum; (1) distinctly smaller than fourth tergum; (2) larger than fourth. *Last state not included in Fontal-Cazalla et al. (2002)*.

140 Pubescence on third abdominal tergum of female metasoma: (0) not extending ventrally; (1) extending ventrally to ventral margin of third tergum, beneath the petiole.

141 Relation between fifth and sixth abdominal tergum of female metasoma: (0) telescoping into each other; (1) anterior margin of sixth tergum abutting and articulating with posterior margin of syntergum (tergum 5).

142 Fusion of terga in male metasoma: (0) all terga free; (1) terga 3 and 4 fused; (2) terga 3-5 fused.

143 Length of terebra: (0) short, basal part of ovipositor not bent posteriorly, basal articulation of terebra curved less than 180°; (1) long, basal part of ovipositor distinctly curved posteriorly, basal articulation of terebra curved 180° or more.

144 Shape of apical part of aedeagus: (0) only slightly expanded subapically; (1) distinctly and abruptly expanded subapically.

145 Dorsal suture of apical part of aedeagus: (0) long, reaching at least 0.5 the length of the expanded part of the aedeagus; (1) short, not reaching 0.5 the length of the expanded aedeagus.

146 Length of paramere: (0) short, the paramere never reaches the apex of the penis valve; (1) long, the paramere almost reaches the apex of the penis valve.

147 Lateroapical setae of paramere (best seen in lateral or ventral view): (0) present; (1) absent.

148 Erect ventroapical seta or pair of setae of paramere: (0) present; (1) absent.

149 Facial impression: (0) absent; (1) present.

150 Shape of occipital carina and posterior part of head: (0) occipital carina lacking, posterior surface of head concave (not flat); (1) occipital carina present, posterior surface of head flat. *Note*. In the discussion of this character in Ros-Farre et al. (2000), the authors comment on the uniqueness of this character to aspicerines and *Neralsia*. Fontal-Cazalla et al. (2002) found this character to also be present in the *Zaeucoila* genus group within the Eucoilinae.

151 Anterior pronotal plate: (0) completely defined, delimited laterally by the lateral pronotal carinae running completely to the ventral margin of the plate; ventral margin of plate not raised; (1) incomplete, lateral pronotal present but not running completely to the ventral margin of pronotal plate; ventral margin of plate not raised; (2) incomplete, lateral pronotal present but not running completely to the ventral margin of pronotal plate; ventral margin of plate raised slightly and seperated from dorsal portion; (3) incomplete, lateral pronotal present but not running completely to the ventral margin of pronotal plate; ventral margin of plate raised distinctly and seperated from dorsal portion; (4) incomplete, lateral pronotal present but not running completely to the ventral margin of pronotal plate; ventral margin of plate raised distinctly and seperated from dorsal portion and joined to the dorsal portion of the plate (no gap present between ventral and dorsal projections).

152 Sculpture on mesoscutum: (0) lacking, entire surface smooth and shiny; (1) present, microsculpture gives the mesoscutum a dull appearance (2) present, shiny macrosculpture (no microsculpture present).

153 Circumscutellar carina: (0) absent; (1) present.

154 Longitudinal scutellar carinae: (0) absent; (1) present as a series of small parallel carinae; (2) present as a pair of distinct carinae.

155 Lateral propodeal carinae: (0) present; (1) absent.

156 Longitudinal ridge or furrow on the posterior surface of metatibia: (0) absent (possibly present but poorly developed; (1) present, well developed.

157 Length of petiole: (0) 1.5-2x longer than broad (slightly longer than broad); (1) 0.5-0.75x than broad (shorter than broad); (2) > 5-6x longer than broad (distinctly elongate).
158 Annulus: (0) present as a continuous ring; (1) seperated into distinct tergal and sternal parts (best viewed ventro-laterally); (2) present with sternal part absent (only tergal segment remains; (3) absent, both sternal and tergal segments lost.
159 Shape of posterior margin of third abdominal tergum: (0) smoothly rounded; (1) slightly but distinctly concave; (2) stongly sinuate, resulting in a 'saddle-shaped' third tergum.
160 Shape of terebrum and hypopygium (as seen in lateral view): (0) curved, pointing upwards; (1) straight, pointing posteriorly.
161 Position of Rs + M forewing vein, particularly the mesal end: (0) situated closer to anterior margin of wing, its mesal end directed towards middle or anterior half of basalis; (1) situated closer to posterior margin of wing, its mesal end directed towards posterior end of basalis. *Note*. Character =46 in Ronquist (1995).
162 Structure of ovipositor: (0) coiled in a spiral without any points of weakness; (1) coiled in a spiral with a distinct point of weakness in the ninth tergum providing a flexion point at the base of the third valvula; (2) distinctly angled or elbowed at base of third valvula separating a large, swinging part of the ovipositor from an apical part attached to the eighth tergum. *Note*. Character=7 in Ronquist (1999).
163 Ovipositor clip (*sensu* van Lenteren et al., 1998): (0) absent; (1) present. *Note*. Previously unpublished character.
164 Basal part of terebra: (0) twisted 180° so first valvulae are in dorsal position at apex of ovipositor; (1) straight, not twisted 180°. *Note*. Character=9 in Ronquist (1999).
165 Shape of first valvulae: (0) narrowing gradually, not broadened apically; (1) distinctly broadened towards apex.

Life History characters

1 Hosts: (0) Syrphid or chamaemyiid larvae (Diptera: Shizophora); (1) chrysopid or hemerobiid larvae (Neuroptera); (2) Other schizophoran Diptera, usually in dung, carrion, fruit or leaf-mines; (3) hyperparasitic of braconids or chalcidoids; (4) hymenopteran gall inducer (not a cynipid); (5) cynipid gall inducer. *Note*: States 4 and 5 are not present in Ros-Farre et al. (2000).
2 Feeding niche of larva: (0) insect parasitoid; (1) inquiline; (2) gall inducer.
3 Microhabitat of larva: (0) chamber in wood/log; (1) gall; (2) aphid community; (3) decomposing organic material; (4) living plant material (including non-rotting fruit). *Lonchidia* coded as (4) due to collecting records of this genus in open meadow/grasses with no dung observed (Buffington, unpublished data). *Note*. Modified from character 12 in Ronquist (1999).
4 Host insect order (0) Hymenoptera; (1) Coleoptera; (2) Neuroptera; (3) Diptera. *Note*. Modified from character 11 in Ronquist (1999).

Appendix C

Structural model of 28S D2+D3 gene fragment. The model was applied to all taxa in the matrix, though only 5 taxa are shown here. The taxa are, in order of appearance, *Parnips nigripes*, *Anacharis* sp. 3, *Aspicera* sp., Charipinae (unknown genus), *Aegeseucoela grenadensis*. See Gillespie et al. (2005) for a description of terminology.

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              H375               H375'             H406        H413     H406'      H265'

            (((((((  ........  )))))))   .....   (((((  ..  ......   )))))   ..  ....  ..
Prnj004.0.1                          --|| ----- ||-----| -- |------| |-----|| -- |----| --
AnachCO.412                          UC|| UAAGU ||GGGUG| GU |AAACUC| |CAUCU|| AA |GGCU| AA
Aspicera.424                         UC|| UAAGU ||GGGUG| GU |AAACUC| |CAUCU|| AA |GGCU| AA
CharpSF.239                          UC|| UAAGU ||GGGUG| GU |AAACUC| |CAUCU|| AA |GGCU| AA
Aegren.084                           UC|| UAAGU ||GGGUG| GU |AAACUC| |CAUCU|| AA |GGCU| AA

H234'         H35'                      H461       H461'      H31'            H484        H484'

 .  ...  ............  ................   ((  ....  ))   ....  ..  .........   (((( ....   ))))   ............
|-| --- |----------AU| ---AGCGAACAAGUA ||CC| GUGA |GG|| GAAA |GU| UGAAAAGAA ||CUUU| GAAG |AGAG|| AGUUCAAGAGUAC
|A| UAC |AACCACGAGACC| GAUAGCGAACAAGUA ||CC| GUGA |GG|| GAAA |GU| UGAAAAGAA ||CUUU| GAAG |AGAG|| AGUUCAAGAGUAC
|A| UAU |AACCACGAGACC| GAUAGCGAACAAGUA ||CC| GUGA |GG|| GAAA |GU| UGAAAAGAA ||CUUU| GAAG |AGAG|| AGUUCAAGAGUAC
|A| UAU |AACCACGAGACC| GAUAGCGAACAAGUA ||CC| GUGA |GG|| GAAA |GU| UGAAAAGAA ||CUUU| GAAG |AGAG|| AGUUCAAGAGUAC
|A| UAU |AACCACGAGACC| GAUAGCGAACAAGUA ||CC| GUGA |GG|| GAAA |GU| UGAAAAGAA ||CUUU| GAAG |AGAG|| AGUUCAAGAGUAC

        H15'              D2-1a       D2-1b                    2               2a       RAA       2b
                        -  ---                                 *                *     *     (1)     * *
 .................  .....  (((((((  ..  ((((((  ..........  (.((((((  .....  (((.((((((.(           (.(.  ((((
|GUGAAACCGUUCAGGG| GUAAA |CCUGAGA| AA |CCCAAA| AGAUCGAAUG |G-GGAGA| UUCAU |CGU-CAGCG-G| [-----] |C-U---UUGG|
|GUGAAACCGUUCAGGG| GUAAA |CCUGAGA| AA |CCCAAA| AGAUCGAAUG |A-GGAGA| UUCAU |CGU-CAGCG-G| [-----] |C-U---UUGG|
|GUGAAACCGUUCAGGG| GUAAA |CCUGAGA| AA |CCCAAA| AGUUCGAAUG |G-GGAGA| UUCAU |CGU-CAGCG-A| [-----] |C-U---UUGG|
|GUGAAACCGUUCAGGG| GUAAA |CCUGAGA| AA |CCCAAA| AGAUCGAAUG |G-GGAGA| UUCAU |CGU-CAGCG-G| [-----] |C-U---UUGG|
|GUGAAACCGUUCAGGG| GUAAA |CCUGAGA| AA |CCCAAA| AGAUCGAAUG |G-GGAGA| UUCAU |CGU-CAGCG-A| [-----] |C-A---CUGG|

  2c     2d          2e          RAA       2f       REC       2g       REC     RAA      REC    2g'              REC
          -   *    * * *       (2)         *       (1)      * --^    (2)     (3)      (2') ^ * -*            (1')
 (-((   ((-(((  .  ((.(.((             ((-(((-(            (-(((((                          )-----))))

|C-UU| |UC-GUG| - |UG-G-UUC-| [-----] |GC-GAUGC| [UC----] |G-UGAC| [-----] [---UUCUG] [-] |G-----UCAC| [---] [GUG]
|C-UU| |UC-GUG| - |UG-G-UUAU| [-----] |GU-GAUGU| [CA----] |G-AGAU| [-----] [---UUC-G] [-] |G-----UCUC| [---] [ACG]
|C-UU| |UC-GUG| - |UG-U-CAU-| [-----] |GUG-AAGU| [UC----] |U-UAAC| [-----] [---UUU-G] [-] |G-----UUAU| [---] [GCG]
|C-UU| |UC-GUG| - |UG-G-UUUU| [-----] |GU-GAUGU| [UC----] |G-GGAU| [-----] [---UUC-G] [-] |A-----UCUU| [---] [GUA]
|C-UU| |UU-GUG| - |UG-G-UUUU| [U----] |GUCACUGU| [UC----] |G-GGAC| [-----] [---UUC-G] [-] |G-----UCUC| [---] [GCA]


    2f'       2e'        2d'    RAA   2c'    RAA      2b'    RAA       2a'               2'
 *   *    *  -*--    -**  -  -  (4)    -     (5)     -   -   (6)    *
 )-)))-))   )-))))      )))))         )--))          ))-))))         )--)))))))  ......  ))))))
|G-CAC-AC|-|G-UCCA| C--|UGCGG| [-U] |A--UG| [---C] |CCGGAGU| [---] |C--GUCGGCG| UGCACU |UCUCCC|
|G-CAC-NC|-|U-AUCA| C--|UGCGA| [-U] |A--UG| [---U] |CUGAUGU| [---] |C--GUCGGCG| UGCACU |UCUCCU|
|A-CUG-AC|-|U-GGCA| C--|UGCGU| [-U] |A--UG| [---U] |CUGAGGU| [---] |U--GUCGGCG| UGCACU |UCUCCU|
|G-CAC-GC|-|A-ACCA| C--|UGCGG| [-U] |A--UG| [---U] |CUGAGGU| [---] |C--GUCGGCG| UGCACU |UCUCCC|
|A-UGU-GC|-|A-UAAC| U--|AUUGC| [-A] |A--UA| [---U] |GCUGGGU| [---] |U--GUCGGCG| UGCACU |UCUCCC|

              3            3a      3b            3c           RAA          3d         3e      RAA    3f-1
                                   **     ****       -           (7)           -  ?   **   *    (8)     * -
.........  ((((((  ...  ((((((   (..(((  ....  ((((((                    (((  .    (..(((.(         ((.((  .....
CUAGUAGGA |CGUCGC| GAC |CCGUUG| |G--GUG| ---- |UCGGUU| [UAC-------------] |GAA| C-- |U--GGG-U| [---] |GG-UU| --GCC
CUAGUAGGA |CGUCGC| GAC |CCGUUG| |U--GUG| ---- |UCGGUU| [UAC-------------] |GGU| A-- |U--GGG-C| [---] |GA-UU| --GCC
CUAGUAGGA |CGUCGC| GAC |CCGUUG| |C--GUG| ---- |UCGGUC| [UAC-------------] |GAU| C-- |UCUGGA-U| [---] |GG-UU| --GCC
CUAGUAGGA |CGUCGC| GAC |CCGUUG| |G--GUG| ---- |UUGGUU| [UAC-------------] |GAU| C-- |U--AGG-U| [---] |UA-AU| --GUU
CUAGUAGGA |CGUCGC| GAC |CCGUUA| |A--GUG| ---- |UCGGUU| [UAC-------------] |GGG| C-- |U--AGG-U| [---] |GG-UU| --GCC

    3f-2        REC      3f-3     REC     RAA     REC    3f-3'     REC          3f-2'          3f-1'  RAA    3e'
 --  * ** ^*^    (3)     ^^*^^^    (4)     (9)     (4')  ^^^*^^     (3')    ^  *- ** **-*-   -      (10)   *
 ((((.(..((.(            (( ((((                        ))))))             ))).))..)..).)    ))))        ).))))
|UGUU-C--CG-U| [------] |CG--CAU| [---] [G-AAUAA] [---] |UGU-GU| [-------] |AUG-GU--G--C-A| |-GACC| [--] |C-CUGG|
|UGUU-C--CG-U| [------] |CG--CAU| [---] [U-CAUU-] [---] |UGU-GU| [-------] |ACG-GG--G--C-A| |-GAUC| [--] |C-CCAU|
|UGUU-U--UG-U| [------] |CA--CAU| [---] [U-UAUU-] [---] |UGU-GU| [-------] |AUA-GA--G--C-A| |-GACC| [--] |U-CUGA|
|UGCU-C--UG-U| [------] |CG--CAU| [---] [U-UCGG-] [---] |UGU-GU| [-------] |ACA-GG--G--C-A| |-UAUU| [--] |C-CUGG|
|UGCU-U--UA-U| [------] |CA--CAC| [---] [U-UGCU-] [---] |-GU-GC| [-------] |AUG-AG--G--C-A| |-GACC| [--] |C-CUGG|
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RAA         3d'     RAA           3c'        3b'    3a'                  RAA              3g      RAA     3h
 (11)       --     (12)           *     -                               (13)              *       (14)
             )))                ))).)))  .  ))))   ))))))     ..                          (.((((          (((((
[A----------] |UAU| [--------CU] |GAC-CGA| C |UGCC| |UGGCGG| UA [CUC-G----CAC-----------] |G-GUA-| [UC--] |GAGCC|
[U----------] |UAC| [--------CU] |AAC-CGA| C |UGCU| |CGGCGG| UA [CUC-G----CAA-----------] |G-GUA-| [UA--] |GAGUC|
[A----------] |UAU| [--------CU] |GAC-CGA| C |UGCA| |CGGCGG| UA [GAC-C----AAU-----------] |G-GUA-| [UA--] |GAGCC|
[-----------] |UAU| [--------CU] |GAC-CAA| C |UGCC| |CGGCGG| UA [CAC-G----UAC-----------] |G-GUA-| [UA--] |GGGCC|
[U----------] |UUU| [AA------CU] |AAU-CGA| C |UGCU| |UGACGG| UA [CUC-G----CAA-----------] |G-GUA-| [UU--] |GGGCC|

 3i     REC      RAA      REC                         3i'    3j    RAA     3k-1     3k-2
    ^   (5)      (15)     (5')                         ^      --     (16)     - -       -
 ((((                                                  ))))   ((((          ((((((    ((((
|GCAC| [-----] [UAC----] [-------------------------] |GUGC| |GUCG| [----] |GGCCCG-| |CCGC| A
|GCAC| [-----] [UUU----] [-------------------------] |GUGC| |GUCA| [----] |GGCCCG-| |UCGC| A
|GCAC| [-----] [UUAAUUG] [U------------------------] |GUGC| |GUCG| [----] |GGCCCG-| |UUGC| A
|GCAC| [-----] [UUU----] [-------------------------] |GUGC| |GUCG| [----] |GGCCCG-| |CUGC| A
|GCAC| [-----] [UUUAU--] [-------------------------] |GUGC| |GUCA| [----] |GGCUCA-| |CCGC| A

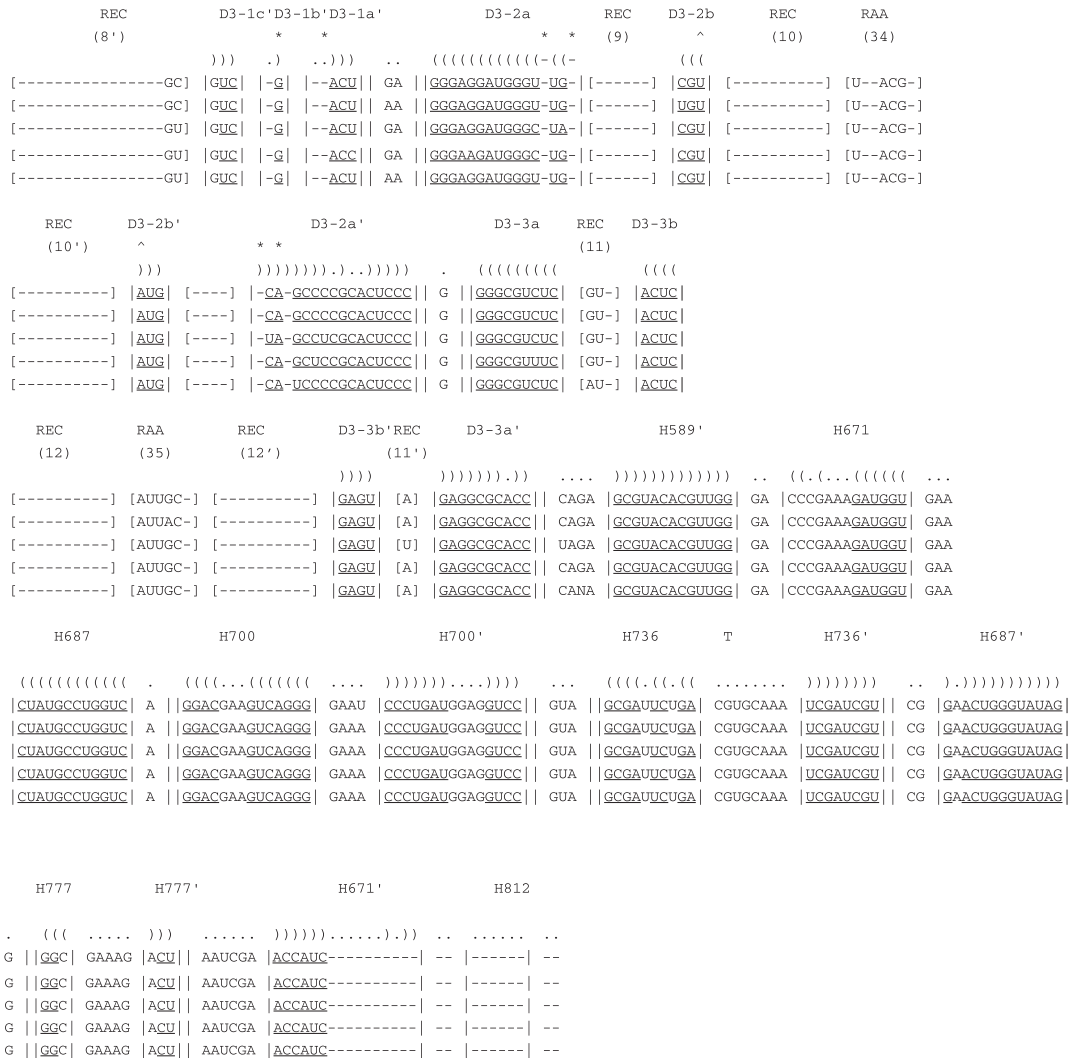
    3l       3m        RAA           3n         RAA    3o    3p   RAA    3p' RAA    3o'  RAA      3n'
     *                 (17)      *  * -         (18)              (19)       (20)        (21)   * **  *
 (((.  ((  .  (((  .             ((. ((((  (         ( (((   ((          ))        ))))        )))..)).))
|AGC---GA| - |GAU| C [AGUGAUA-C] |CC---GGAG--G| [U] |G-CGG| |AC| [CUA] |GU| [-G] |CCGU| [CC] |CCG--GG-CC|
|AGC---AU| - |UAU| C [AGUGUU--C] |CC---GGAG--G| [U] |G-CGG| |AC| [CUA] |GU| [-G] |CCGU| [CC] |CCA--GG-CC|
|AGC---GA| - |GAU| C [AGUGAUA-C] |CU---GGAU--G| [U] |A-UGG| |AC| [CUA] |GU| [--] |CCGU| [CG] |CCA--GG-CC|
|AGC---AA| - |GAU| C [AGUGUAA-C] |CU---GGAU--G| [U] |G-CGG| |AC| [CUA] |GU| [-G] |CCGU| [CA] |CCA--GG-CC|
|AGC---UU| - |UAU| U [AAUCUUU--] |-----GGAG--G| [U] |U-CGG| |AC| [CUA] |GU| [-G] |CCGU| [CC] |CCG--AG-CC|

 RAA   3m'   RAA     3l'  RAA     3k-2' RAA   3k-1'         3j' RAA    3h'     RAA     REC  3q        RAA
 (22)        (23)      *  (24)      *   (25)    * -  *            (26)    ---     (27)        (6)          (28)
       )))         ).)))          ) )))   ))) )))) )  ...  ))))       )))))
[UGG] |UCA|[-------] - [GCUGUUG] |G-UCG| |GCG-GUGU-U| CUC |GGAC| [U] |GGCUC| [AUCUC] [-AAUU---A] [UU---------]
[UGU] |UGA|[-------] - [GCUGUUG] |G-UCG| |ACG-GUGU-U| CUC |UGAC| [U] |GGCUC| [GUCUC] [-AUUUA--U] [UU---------]
[UGC] |UCA|[-------] - [GCUGUUA] |G-UUG| |ACG-GUGU-U| CUC |GGAC| [U] |GGCUC| [AUUU-] [-AG------] [-----------]
[UGG] |UCU|[-------] - [GCUGUUG] |G-UCG| |GCG-GUGU-U| CUC |GGAC| [U] |GGCUC| [AUUUC] [-AAUUA--U] [UU---------]
[UGU] |UCA|[-------] - [GCUGUUG] |G-UCG| |GUG-AUGU-U| CUC |UGAC| [U] |GGCUA| [-----] [--------A] [UU---------]

  REC3q'     RAA      3g'            3'              1b'           1a'        H563            H563'    H579
  (6')       (29)    -  -    -            -         *
                     )))))  )))))  ))))))  ........  ))))))  ...  )))))))  ..   ((((  ........  ))))    ......
[------] [GAA------]|-UACC| GGUCG |GCGACG| CUACUGC- |UUUGGG| UAC |UUUCAGG| AC ||CCGU| CUUGAAAC |ACGG|| |ACCAAG|
[------] [GAA------]|-UACC| GGUCG |GCGACG| CUACUGC- |UUUGGG| UAC |UUUCAGG| AC ||CCGU| CUUGAAAC |ACGG|| |ACCAAG|
[------] [---------]|-UACU| GGUCA |GCGACN| CNUUUGC- |UUUGGG| UAC |UUUCAGA| AC ||CCGU| CUUGAAAC |ACGG|| |ACCAAG|
[------] [GAA------]|-UACC| GGUCA |GCGACG| CUACUGC- |UUUGGG| UAC |UUUCAGG| AC ||CCGU| CUUGAAAC |ACGG|| |ACCAAG|
[------] [GAAAGAA--]|-UACC| UGUCA |GCGACG| CUACUGC- |UUUGGG| UAC |UUUCAGG| AC ||CCGU| CUUGAAAC |ACGG|| |ACCAAG|

          H589             H604           REC                 RAA                REC
                                          (7)                 (30)               (7')
 ....  ((((((((((((  ..   ((((((((                                                                   ...
 GAGU |CUAACAUGUGCGC| GA ||GUCAUUGGG| [ACU-----------] [GC-----------------] [-------------A] AAA
 GAGU |CUAACAUGUACGC| GA ||GUCAUUGGG| [ACC-----------] [GC-----------------] [-------------A] AAA
 GAGU |CUAACAUGUACGC| GA ||GUCAUUGGG| [AAU-----------] [AA-----------------] [-------------A] AAA
 GAGU |CUAACAUGUGCGC| GA ||GUCAUUGGG| [ACC-----------] [GC-----------------] [-------------A] AAA
 GAGU |CUAACAUGUGCGC| GA ||GUCAUUGGG| [ACC-----------] [GC-----------------] [-------------A] AAA

    H604'        H628      H628'      D3-1a RAA D3-1b RAA   D3-1c          REC             RAA
                                            (31)     (32)   *              (8)             (33)
 ))))))))    ...   ((  ....  ))   ...   (((        (          (.((
|CCUAAAGGC|| GUA ||AU| GAAA |GU|| GAA ||GGU| [--]|C| [---] |-GA-| [CC----------------] [UU------]
|CCUAAAGGC|| AUA ||AU| GAAA |GU|| GAA ||GGU| [--]|C| [---] |-GA-| [CC----------------] [UU------]
|CCUAAAGGC|| GUA ||AU| GAAA |GU|| AAA ||GGU| [--]|C| [---] |-GA-| [CC----------------] [UU------]
|CCUAAAGGC|| GUA ||AU| GAAA |GU|| GAA ||AGU| [--]|C| [---] |----| [CC----------------] [UU------]
|CCUAAAGGC|| GUA ||AU| GAAA |GU|| GAA ||GGU| [--]|C| [---] |-GG-| [CC----------------] [UU------]
```


REC (8') D3-1c'D3-1b'D3-1a' D3-2a REC (9) D3-2b REC (10) RAA (34)
REC (10') D3-2b' D3-2a' D3-3a REC (11) D3-3b
REC (12) RAA (35) REC (12') D3-3b'REC (11') D3-3a' H589' H671
H687 H700 H700' H736 T H736' H687'
H777 H777' H671' H812