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Phylogenetic relationships among microgastrine braconid wasp genera based on data from the 16S, COI and 28S genes and morphology

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Abstract. Phylogenetic relationships among the genera of the large braconid wasp subfamily Microgastrinae were explored using DNA sequence data from the mitochondrial large ribosomal subunit (16S), nuclear large ribosomal subunit (28S) and mitochondrial cytochrome oxidase (COI) genes, along with morphological characters, both new and from previous studies. The taxonomic history of this group of wasps is reviewed, along with a critique of previous phylogenetic studies on the group. Molecular data were sampled from forty-six species representing twenty-six genera of microgastrines, plus three species representing the close outgroup taxa Cardiochilinae and Miracinae. Some 2300 base pairs of aligned sequence were obtained per taxon from the three genes. In addition, fifty-three morphological characters were coded for all known genera, including two undescribed genera, except *Semionis* Nixon (known from only a single male type specimen). Relationships among several groups of genera are clarified and challenge some major assumptions made in earlier classifications. In particular, it is clear that dependence on one or a few major morphological character systems oversimplifies relationships, and can lead to misleading results. Despite the large amount of data analysed, basal divergences within the subfamily remain poorly resolved and essentially unsupported in any rigorous statistical sense.

Introduction

Within Braconidae, subfamily Microgastrinae is the most conspicuous single group of parasitoids of Lepidoptera in the world, both in species richness and in economic importance. Over 1500 species have been described, and Mason (1981) has estimated that the actual world total may reach 5000–10 000 species when the rich tropical and south temperate faunas are fully known. Microgastrines are found world-wide from tropical to arctic climates, and attack virtually the entire taxonomic and biological spectrum of Lepidoptera. More than 100 species in this group have been used in the biological control of lepidopteran pests, and this total is likely to rise.

Microgastrinae have become the focus of recent genetic interest as carriers of polydnaviruses (Fleming, 1992; Stoltz & Whitfield, 1992; Whitfield, 1997b). There is strong evidence that these remarkable viruslike entities are hereditary (Fleming & Summers, 1987, 1991; Stoltz, 1990; Xu & Stoltz, 1991) and play essential roles in the process of parasitism by the wasps. They are known to affect various aspects of the host's immune response, nutritional chemistry and development (for a review see Fleming, 1992). To our knowledge, this is the only known case of obligate mutualism between eukaryotes and viruses (Whitfield, 1990; Fleming, 1992). Current phylogenetic and survey studies indicate that the polydnaviruses found in braconids are restricted to a single monophyletic lineage of the wasps, including Microgastrinae (Whitfield, 1997b). As the viruses are inherited as integrated proviruses in the wasp chromosomal DNA,

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it is likely that knowledge of the wasp relationships will also be informative about viral relationships (Stoltz & Whitfield, 1992).

Preliminary phylogenetic tests of wasp vs polydnaviral DNA sequences suggest that they may have strictly co-evolved in a phylogenetic sense (Whitfield, 2000).

The classification of Microgastrinae (outlined later in this paper) has been notably unstable, with major discrepancies between the classifications used by taxonomic specialists and field biologists on different continents. The recognition of several large genera (affecting hundreds of species) and the most recent classification of the tribes (Mason, 1981) have recently been questioned. To a large degree, these classificatory problems stem from the lack of a sound phylogenetic understanding of microgastrine relationships. Only one comprehensive phylogenetic study of the entire group has been published to date (Mason, 1981). This study did not employ modern computer analytical methods, and explicit matrices showing character codings for all taxa were not presented. Subsequent reassessments of both character state trees and taxon relationships in the group (Williams, 1988; Austin, 1990; Walker *et al*., 1990) have been unable to recover many of the results of the original study. Currently, there are a large number of nominal genera without a higher classificatory framework.

The history of microgastrine classification is provided in more detail later in this paper. Following this discussion, we present a multistage analysis with the following components: (1) re-analysis of microgastrine generic relationships based on a new and expanded morphological dataset; (2) review and analysis of new DNA sequence data for phylogenetic analysis; (3) a detailed comparison of the results from the morphological and molecular datasets; and (4) a series of analyses that combine molecular and morphological data.

Our use of both morphological and multiple molecular datasets for the estimation of phylogeny is among the most comprehensive incorporation of characters for a study of relationships among genera in an insect subfamily. The results of this expanded phylogenetic analysis will ultimately contribute to a more robust classification of Microgastrinae. Such a classification will be of significant practical benefit to applied entomologists, and will also provide a clear framework for handling the extensive and largely undescribed tropical faunas. The phylogeny of this group will also be of great interest for testing evolutionary trends among the polydnaviruses carried by these wasps, and among host relationships.

History of research on microgastrine classification (see also Fig. 1)

Until 1862, all the species described in Microgastrinae were assigned to genus *Microgaster* Latreille, 1804. Förster (1862), in a sweeping but superficial treatment of Braconidae, erected two additional genera, *Microplitis* and *Apanteles*. During the late nineteenth and early twentieth centuries, a massive number of new species were described in the subfamily, eventually leading to the proposal of a number of new genera by Ashmead (1898, 1900, 1904), Cameron (1891, 1906, 1910), Viereck (1910, 1911, 1913) and others (e.g. Thomson, 1895; Bréthes, 1915). These genera were proposed from limited knowledge of the world fauna, and hence subsequent workers found them difficult to interpret and mostly ignored them. To resolve the confusion, Muesebeck (1920, 1922), in his comprehensive treatment of the Nearctic Microgastrinae, synonymized most of these names (at least for temperate faunas) under *Apanteles*, *Microgaster* and *Microplitis*. His action stabilized the nomenclature for microgastrine species for many years.

The first half of the twentieth century saw great activity in revisions of microgastrine faunas. These revisions included Australasia (Wilkinson, 1928, 1929), Japan (Watanabe, 1937), Africa and Madagascar (Wilkinson, 1932; de Saeger, 1944; Granger, 1949) and the more intensively studied Palaearctic (Wilkinson, 1945; Telenga, 1955) and Nearctic (Muesebeck, 1920, 1922) Regions. Aware of the huge mass of species accumulating in the subfamily (especially within genus *Apanteles* with close to 1000 species at that time), D. S. Wilkinson began to build upon earlier attempts by Marshall (1885) and devised a system of letter-designated groups within *Apanteles* (Wilkinson, 1932). Unfortunately, Wilkinson was lost at sea during World War II and his vast knowledge of the group was not consolidated into a world classification.

It fell to Wilkinson's successor at the British Museum of Natural History (now The Natural History Museum, London), Gilbert Nixon, to organize the pieces of Wilkinson's life work on Microgastrinae. After years of study, Nixon (1965; successive, more regional treatments of Old World faunas in 1967, 1968, 1970, 1972, 1973, 1974, 1976) produced a major reclassification of the entire subfamily, recognizing several new genera (see Fig. 1). His treatment retained a broad definition of genus *Apanteles,* which he divided into forty-four species groups. It was his opinion (Nixon, 1972) that *Apanteles* would be shown to be polyphyletic and would eventually require splitting into more natural genera. Nixon's classification was more or less universally followed for a number of years, and served as the basis for further treatments of Old World faunas (Tobias, 1975, 1986; Papp, 1976, 1978, 1979, 1980, 1981, 1982, 1983, 1984a,b, 1986a,b, 1987).

After about 20 years of study of Microgastrinae, Mason (1981) finally succeeded in splitting *Apanteles* and providing a more radical generic reclassification of the subfamily. Mason's reclassification, which recognized fifty-one genera, twenty-six of which contained species previously placed in *Apanteles*, brought the first application of phylogenetic reasoning to Microgastrinae (Fig. 2A). He used a number of new character systems, including larval features introduced by Short (1953) and Čapek (1970), and a detailed treatment of the ovipositor system and antennal sensilla.

In many ways, Mason's (1981) new microgastrine classification was a great improvement and was adopted, at least

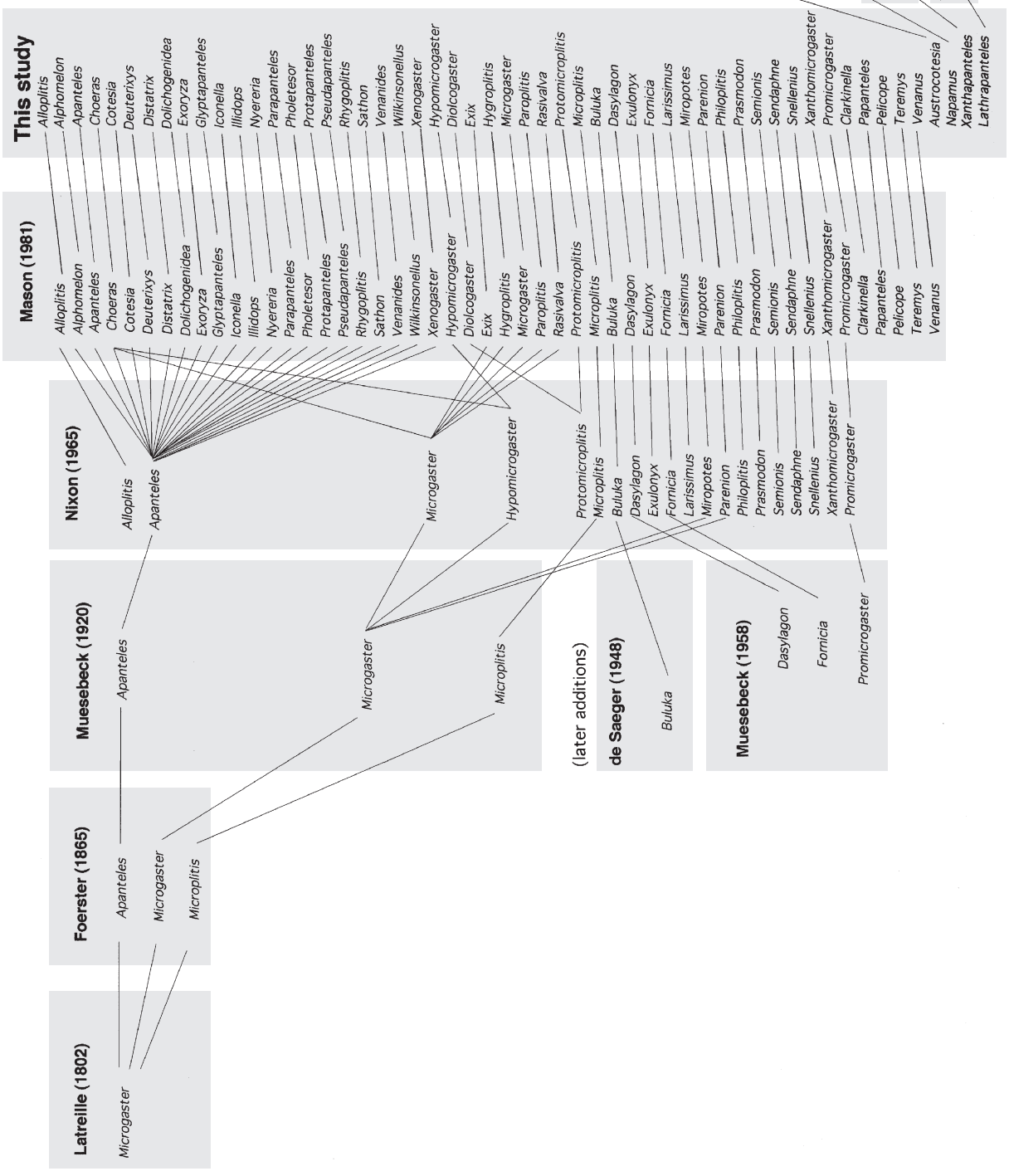

Fig. 1. Major changes in the classification of Microgastrinae during the last 100 years. Listing of a genus under an author is not necessarily meant to imply that the genus was originally proposed by that author, rather that the genus was recognized by that author. Authorship for each genus is given in Table 1.

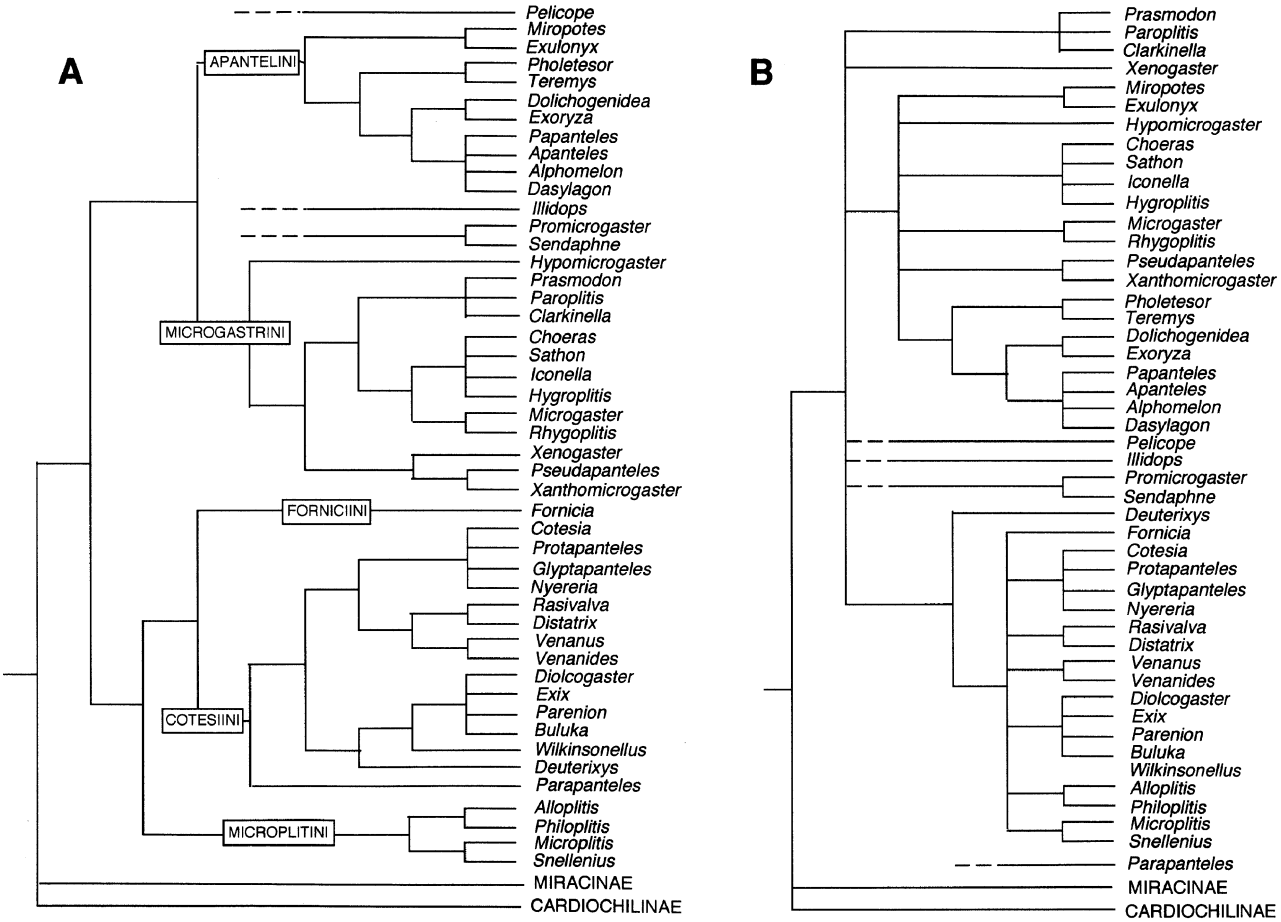

Fig. 2. Previous phylogenies proposed for Microgastrinae. A, Phylogenetic relationships among the genera of Microgastrinae, according to the intuitive analysis of Mason (1981). Genera within his 'genus-groups' (which he treated as terminal taxa) have been indicated as unresolved terminal polytomies, so that the placement of individual genera can be compared to the results of the present study. *Semionis* is omitted from the tree because it is known from only a unique male type specimen and has not been treated in subsequent studies. B, Phylogenetic relationships among microgastrine genera according to the cladistic reanalysis of Mason's data by Walker *et al.* (1990). In their study, the taxa showing dashed lines connecting them to the tree were omitted; they are shown in unresolved positions roughly where Mason (1981) placed them.

in outline, by many subsequent workers (Whitfield, 1985, 1995a,b, 1997a,b; Williams, 1985, 1988; Marsh *et al.*, 1987; Papp, 1988; Austin, 1989, 1990; Whitfield & Wagner, 1991; Austin & Dangerfield, 1992, 1993). However, several weaknesses of Mason's classification gradually became more evident, especially as additional new taxa were discovered and attempts were made to incorporate these into his system. First, many Old World taxa were not treated in sufficient detail by Mason (1981), leaving large, unresolved problematic genera such as *Diolcogaster* Ashmead and *Glyptapanteles* Ashmead (Walker *et al.*, 1990; Austin & Dangerfield, 1992). Mason also neglected to assign most of the Old World species (and some from the New World) to his new genera, leaving subsequent workers to place these species according to his generic concepts. Secondly, some of Mason's assumptions about several character systems, notably the ovipositor mechanism that featured so prominently in his analysis, were shown to be flawed (Williams, 1985; Austin, 1990), as he later realized. In particular, he linked together sets of structural features as functional complexes that were later found to vary independently of one another (Williams, 1985; Austin, 1990), and contradicted his own outgroup perspectives (later outlined in Mason, 1983) in his assignments of polarity to several of these characters. Thirdly, Mason did not provide an explicit treatment of his character codings, nor fully explain how they were used in his phylogenetic analysis. During the late 1970s, when most of his work was being completed, computer phylogenetic analysis of datasets as large as those that he had compiled (fifty-one genera, approximately fifty characters) was problematic, if not impossible. His analysis was apparently intuitive, fitting characters to a larger pattern he had already constructed. It was not evident which characters were used to infer the phylogeny and which were accommodated later.

Criticism of Mason's (1981) phylogeny of Microgastrinae culminated in the reassessment by Walker *et al.* (1990). They attempted to replicate Mason's analysis using the characters

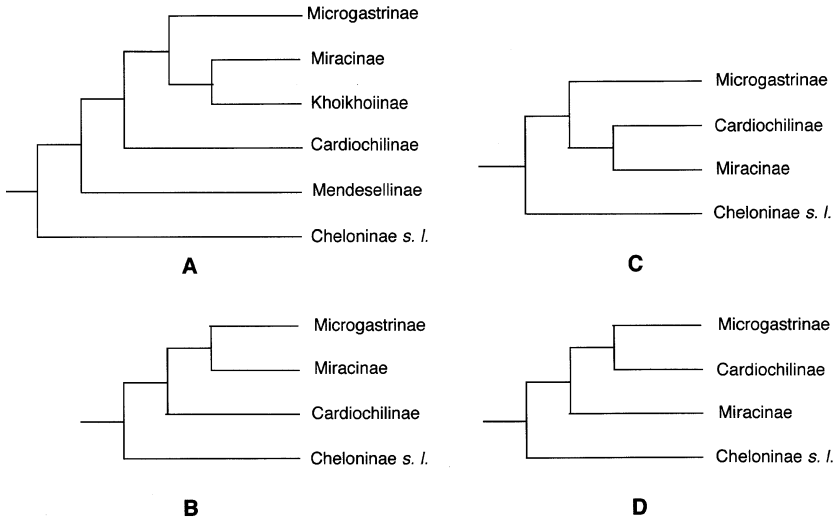

Fig. 3. Relationships among the closest outgroups to Microgastrinae: A, according to the analyses of Whitfield & Mason (1994); B, Whitfield (1997b); C, Belshaw *et al.* (1998); D, Dowton & Austin (1998). The subfamilies Khoikhoiinae and Mendesellinae were omitted from all studies subsequent to Whitfield & Mason (1994) because no suitable material for extracting molecular data was available; their placement in this complex of subfamilies is still considered probable. Cheloninae *s.l.* includes the traditional subfamily Adeliinae, shown by all of these studies to be either the sister group to Cheloninae or actually a phylogenetic subgroup of Cheloninae.

plotted on his phylogenetic diagram and computer-assisted parsimony analysis. Using various assumptions and weighting schemes, Walker *et al.* were unable to recover anything resembling the tribal relationships suggested by Mason. In fact, their cladograms were largely unresolved in critical parts (Fig. 2B), and although the authors attempted to apply rigorous character-coding schemes and phylogenetic methods to Mason's data, they were unfamiliar with some of the taxa and characters. Hence, their reassessment omitted a substantial proportion of Mason's characters, and left the placement of some genera unanalysed. The study by Walker *et al.* highlighted many problems with Mason's system, but failed to produce an improved alternative.

In response to Walker *et al.* (1990), Mason began to compile a more comprehensive morphological dataset for Microgastrinae. He filled in many of the missing characters from Walker *et al.* and added several new ones. He also expanded the list of taxa to include genera described after his 1981 paper in a more comprehensive study in collaboration with the first author (J.B.W.). Tragically, Mason died in the later stages of compiling this dataset (late 1991). The morphological dataset used in this study is largely a result of that earlier collaboration.

Also since the early 1990s, the outgroup relationships within the lineage of braconid subfamilies related to Microgastrinae have been analysed further using both morphological (Whitfield & Mason, 1994) and molecular (Whitfield, 1997b; Belshaw *et al.*, 1998; Dowton & Austin, 1998; Dowton *et al.*, 1998) data. In all studies to date, the monophyly of Microgastrinae has been supported, as well as that of a 'microgastroid assemblage' that also includes Cheloninae in addition to groups traditionally associated with Microgastrinae. The closest outgroup taxa in all analyses include subfamilies Miracinae and Cardiochilinae; the actual relationships found in these studies are summarized in Fig. 3. The distribution of many character states within these outgroup subfamilies has also become better understood as a result of more recent phylogenetic studies (Saeed, 1996, for character distributions in some Miracinae; Dangerfield *et al.*, 1999, for Cardiochilinae). With a strong phylogenetic framework surrounding the study, we are now in a better position to fully analyse relationships among microgastrine genera using both morphological and molecular data.

Materials and methods

Morphological data

Representatives of all genera of Microgastrinae except *Semionis* Nixon (known only from a single male type specimen) were assembled for the morphological analysis (see Acknowledgements for instititutions and individuals who loaned taxa that we did not have in our own collections). In many genera, a number of species were available for determining character variability, and in a few cases taxa (the diverse and possibly polyphyletic genera *Diolcogaster* and *Glyptapanteles*) were treated as polymorphic for some characters when the variation seemed significant. The primary morphological character coding was based, however, on the taxa listed in Table 1. In a large number of cases the type species was used, so that the phylogenetic placements we infer will apply to the species with which each genus name is most closely associated. In some large, possibly polyphyletic, genera such as *Diolcogaster* Ashmead, character coding would have been highly ambiguous if such an approach had not been taken.

Table 1. Microgastrine genera and the species used for primary character coding in the morphological analysis.

Genus	Type species	Taxa used
Alloplitis	*guapo* Nixon	*completus* Mason
Alphomelon	*U. nigriceps* Ashmead	undet. (Costa Rica)
Apanteles	*M. obscurus* Nees	type species
Austrocotesia	*exigua* A. & D.	type species
Buluka	*straeleni* De Saeger	type species
Choeras	*A.* (*P.*) *consimilis* Viereck	*C. parasitellae* Bouché
Clarkinella	*canadensis* Mason	type species
Cotesia	*flavipes* Cameron	type species *C. glomerata* (L.)
Dasylagon	*aegeriae* Muesebeck	sp.n. (Costa Rica)
Deuterixys	*M. carbonarius* Wesmael	*pacifica* Whitfield
Diolcogaster	*M. brevicauda* Provancher	*facetosa* (Weed)
Distatrix	*A. papilionis* Viereck	type species
Dolichogenidea	*A.* (*D.*) *banksi* Viereck	*absona* (Muesebeck)
Exix	*mexicana* Mason	type species
Exoryza	*A. schoenobii* Wilkinson	*minnesota* Mason
Exulonyx	*A. camma* Nixon	type species
Fornicia	*clathra* Brullé	undet. (Costa Rica)
Glyptapanteles	*A. ashmeadi* Wilkinson	*flavicoxis* (Marsh) (vit.-grp), *militaris* (Walsh) (oct.-grp)
Hygroplitis	*M. russatus* Haliday	*melligaster* Provancher
Hypomicrogaster	*M. zonaria* Say	type species
Iconella	*A. etiellae* Viereck	undet. (Nearctic)
Illidops	*A. butalidis* Marshall	undet. (Iran)
Larissimus	*cassander* Nixon	type species
Lathrapanteles	*A. papaipemae* Muesebeck	type species
Microgaster	*australis* Thomson	*tibialis* Nees
Microplitis	*M. sordipes* Nees	*spinolae* (Nees) (Pal.)
Miropotes	*creon* Nixon	*chookolis* Austin
Napamus	*A. vipio* Reinhard	undet. (Iran)
Nyereria	*A. mlange* Wilkinson	type species
Papanteles	*peckorum* Mason	type species
Parapanteles	*A. aletiae* Riley	type species
Parenion	*kokodana* Nixon	*beelaronga* A. & D.
Paroplitis	*beringianus* Mason	type species
Pelicope	*yuccamica* Mason	type species
Philoplitis	*coniferens* Nixon	type species
Pholetesor	*A. ornigis* Weed	type species
Prasmodon	*eminens* Nixon	type species
Promicrogaster	*terebrator* B. & R.	*munda* Muesebeck
Protapanteles	*A. paleacritae* Riley	*alaskensis* Ashmead
Protomicroplitis	*M. mediatus* Cresson	undet. (Costa Rica)
Pseudapanteles	*annulicornis* Ashmead	*sesiae* Viereck
Rasivalva	*M. stigmaticus* Muesebeck	undet. (Nearctic)
Rhygoplitis	A. (*P.*) *terminalis* Gahan	type species
Sathon	*A. neomexicanus* Muesebeck	type species
Semionis	*rarus* Nixon	–
Sendaphne	*olearus* Nixon	undet. (Neotropical)
Snellenius	*vollenhovii* Westwood	sp.n. (Brazil)
Teremys	*masneri* Mason	type species
Venanides	*xeste* Mason	type species
Venanus	*pinicola* Mason	undet. (Neotropical)
Wilkinsonellus	*A. iphitus* Nixon	undet. (Sulawesi)
Xanthapanteles	*cameronae* Whitfield	type species
Xanthomicrogaster	*fortipes* Cameron	undet. (Neotropical)
Xenogaster	*A. insolens* Wilkinson	type species

Morphological characters were originally assembled directly from Mason (1981), although considerable recoding was required before the data were appropriate for phylogenetic analysis. In most cases the recoding consisted of forming unordered multistate characters from either putatively ordered characters, or from multiple non-independent characters redundantly describing the same structure. In a few cases, new character states were added to better reflect the observed variation. For the propodeal carination patterns, the codings were influenced by the recodings of Saeed (1996). The complex ovipositor mechanism characters that formed so much a part of Mason's 'Macrolepidoptera suite' of characters were, following Williams (1985) and Austin (1990), coded as multiple independently varying characters. Two male genitalic characters were included from Maetô (1996), and a new character (for this group), tarsal claw pectination, was added. Larval characters included from two genera, *Fornicia* Brulle and *Miropotes* Mason, were not previously known. New data are included in the matrix based on dissections by the senior author of an undescribed species of *Alphomelon* reared from Hesperiidae in Costa Rica, *Fornicia* sp. reared from Limacodidae in Costa Rica and *Miropotes chookolis* Austin reared from *Samia multiplicalis* in Australia. Larval slides and voucher specimens of these taxa are available from the authors. After completing these dissections, we discovered that Penteado-Dias (1985) had, unknown to us at that time, already described the larva of *Alphomelon*; our data correspond well with what she found. Additional data on larval characters for outgroup taxa were obtained from Čapek (1970), and verified with material we had at hand.

Two undescribed putative genera (identified in an unpublished study of *Diolcogaster* Ashmead by Saeed) are included, as are several new species of *Microplitis* (D. H. Janzen *et al*., unpublished data). Two outgroup species from the closely related Cardiochilinae were included. Analyses were also run using *Mirax* (Miracinae) as an additional outgroup. Alternative analyses explored the effect of including the large number of reductional autapomorphies in adult and larval data in this subfamily. The complete set of morphological characters and states is provided in Appendix 1, and the data matrix is shown in Appendix 2.

Molecular data

Specimens for molecular study were collected fresh from the field and placed into an ultra-cold freezer when possible, but many of the genera were available only as ethanol-preserved specimens or as dried, pinned specimens. Dried specimens typically produced only shorter DNA seqments from extraction and PCR amplification, but in some cases we were able to obtain quality data for multiple genes, especially if the specimens were pinned from ethanol relatively recently. Some genera available as dried specimens (e.g. *Illidops* Mason, *Larissimus* Nixon, *Protapanteles* Ashmead and *Rasivalva* Mason) yielded no data for analysis, whereas others yielded data for one or more but not all three genes. The taxa used in the molecular analyses are listed, along with their source localities, collection dates and success in obtaining molecular data, in Table 2.

Phylogenetic analyses

DNA fragments from the mitochondrial cytochrome oxidase I (COI, 1235 nucleotides), the mitochondrial large subunit (16S) rDNA (442 aligned nucleotides) and the nuclear large subunit (28S) rDNA (D2 + D3 expansion regions, 627 aligned nucleotides) were sequenced as described by Mardulyn & Whitfield (1999). The COI sequences were easily aligned by eye. The 16S sequences were aligned to secondary structure following initial general alignment using several sets of starting parameters in Clustal X (Thompson *et al*., 1997; Jeanmougin *et al*., 1998), as described by Whitfield & Cameron (1998). This procedure ultimately resulted in a single alignment, as the different starting parameters resulted in alignments that differed only in respects that were corrected by alignment to secondary structure. The 16S sequences were also aligned using a series of alternative parameters in Malign version 2.5 (Mardulyn & Whitfield, 1999), but these alignments proved to be more strongly incongruent with the other datasets, probably because the secondary structure was being ignored. The 28S sequences were aligned based on the criterion of maximum parsimony using Malign 2.5 (command 'build', randorderns 10, alignswap) (Wheeler & Gladstein, 1995), specifying a gap : change cost ratio of 2.

All phylogenetic analyses were performed using PAUP*4.0 b4a (Swofford, 1998). Unweighted parsimony analyses were performed on the morphology and the DNA datasets separately (heuristic search, 100 random addition sequences, TBR swapping). Each of the DNA datasets was also subjected to maximum likelihood (ML) analysis, after 'successive approximation' estimation of model parameters. The first round of approximation used the maximum parsimony tree as a guide for estimation of parameters. The estimated parameters were then used to estimate a new ML tree, which in turn was used to re-estimate parameters, etc. Typically, one additional round of ML estimation was sufficient to obtain relatively stable parameter estimates. For the AT-rich mtDNA datasets (16S and COI, the general time-reversible (GTR) model (Rodriguez *et al*., 1990) was used with site-to-site rate variation modelled using a gamma-distribution with four rate categories and the shape parameter estimated from the data (Yang, 1994). The 28S dataset was analysed similarly (with a gamma distribution), except that an HKY-85 model (Hasegawa *et al*., 1985) was adopted rather than the GTR, as the absence of a strong AT bias in the 28S data eliminated the need to treat the transversion categories separately.

Following application of pairwise incongruence length difference (ILD) tests among datasets (Farris *et al*., 1995), two combined maximum parsimony analyses (morphology + DNA) were conducted in a 'total evidence' (simultaneous analysis) approach (Kluge & Wolf, 1993; Wiens & Reeder, 1995), i.e. one analysis on all taxa for which some data

Table 2. Taxa used in the molecular analysis, along with their source localities and other data.

Taxon	Locality/source	Preservation	Collected	COI	16S	28S
Ingroup taxa						
Alphomelon sp.n. 1	Santa Rosa, Costa Rica	ethanol	1994	AY044205	AY044190	–
Alphomelon talidicida (Wilkinson)	Santa Rosa, Costa Rica	ethanol	1996	AY044206	AY044191	AY044217
Apanteles nephoptericis (Packard)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102720	AF102763	AF102745
Apanteles canarsiae Ashmead	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102703	AF102750	AF102728
Choeras consimilis (Viereck)	Haw Creek Falls, Arkansas	ethanol	1994	–	–	AY044218
Cotesia autographae (Muesebeck)	Whitfield (1997)	see ref.	see ref.	–	U68156	–
Cotesia chilonis (Munakata)	Smith & Kambhampati (1999)	see ref.	see ref.	–	AF110825	–
Cotesia congregata (Say)	Whitfield (1997)	see ref.	see ref.	–	U68157	–
Cotesia flavipes Cameron	Derr *et al.* 1992a,b	see ref.	see ref.	–	see ref.	–
Cotesia glomerata (Linnaeus)	Dowton & Austin (1994)	see ref.	see ref.	–	U06958	–
Cotesia griffini (Viereck)	Mardulyn & Whifield 1999	see ref.	see ref.	AF102704	AY044192	AF102729
Cotesia marginiventris (Cresson)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102705	–	AF102730
Cotesia rubecula (Marshall)	Dowton & Austin (1994)	see ref.	see ref.	–	U06959	–
Cotesia ruficrus (Haliday)	Smith & Kambhampati (1999)	see ref.	see ref.	–	AF110826	–
Cotesia sesamiae (Cameron)	Smith & Kambhampati (1999)	see ref.	see ref.	–	AF110827	–
Dasylagon sp.n.	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102719	AF102762	AF102744
Deuteryxis rimulosa (Niez.)	Villena, Spain	ethanol	1997	–	–	AY044219
Diolcogaster (*basimacula*-group)	Santa Rosa, Costa Rica	ethanol	1996	AY044208	AY044193	AY044220
Diolcogaster schizurae (Muesebeck)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102716	AF102759	AF102741
Diolcogaster (*xanthaspis*-group)	Santa Rosa, Costa Rica	ethanol	1996	AY044209	AY044194	AY044221
Dolichogenidea lacteicolor (Viereck)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102717	AF102760	AF102742
Dolichogenidea sp.n.	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102718	AF102761	AF102743
Fornicia sp.	Santa Rosa, Costa Rica	ethanol	1986	AY044210	AY044195	–
Glyptapanteles indiensis (Marsh)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102713	AF102757	AF102738
Glyptapanteles porthetriae (Mues.)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102714	AF102758	AF102739
Hypomicrogaster ecdytolophae (Mues.)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102712	AF102756	AF102737
Microgaster canadensis Muesebeck	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102708	U68154	AF102733
Microplitis demolitor Wilkinson	Laboratory culture, M. Strand, Wisconsin	−80 °C	1997	–	AY044196	–
Microplitis sp.n. 1	Santa Rosa, Costa Rica	ethanol	1992	AY044211	AY044197	AY044222
Microplitis sp.n. 2	Santa Rosa, Costa Rica	ethanol	1992	AY044212	AY044198	AY044223
Microplitis sp.n. 3	Santa Rosa, Costa Rica	ethanol	1994	AY044213	AY044199	AY044224
Microplitis maturus Weed	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102702	U68155	AF102727
Miropotes sp.	Australia	ethanol	1998	AY044214	AY044200	AY044225
Parapanteles paradoxus (Muesebeck)	Santa Rosa, Costa Rica	see ref.	see ref.	AF102709	AF102753	AF102734
Pholetesor bedelliae (Viereck)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102715	U68153	AF102740
Pholetesor ornigis (Weed)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102711	AF102755	AF102736
Prasmodon eminens Nixon	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102700	AF102748	AF102725
Promicrogaster munda Muesebeck	Santa Rosa, Costa Rica	ethanol	1985	AY044215	–	–
Protomicroplitis calliptera (Say)	Ouachita Mtns, Arkansas	dried, pinned	1994	–	AY044201	–
Pseudapanteles dignus (Mues.)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102706	AF102751	AF102731

Table 2. Continued.

Taxon	Locality/source	Preservation	Collected	COI	16S	28S
Rhygoplitis terminalis (Gahan)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102710	AF102754	AF102735
Sathon falcatus (Nees)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102721	AF102764	AF102746
Sendaphne paranaensis S. & P.-D.	Telemaco Borba, Brasil	ethanol	1986	–	AY044202	–
Snellenius sp.n.	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102701	AF102749	AF102726
Venanus minutalis (Muesebeck)	Campanas Nat. Park, Chile	ethanol	1998	AY044216	AY044203	AY044226
Wilkinsonellus sp.	Sulawesi, Indonesia	ethanol	1985	–	AY044204	–
Outgroup taxa						
Cardiochiles fuscipennis Szepligeti	Dowton & Austin (1998)	see ref.	see ref.	AY044207	AF029112	AF029118
Mirax lithocolletidis Ashmead	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102722	AF102765	AF102747
Toxoneurone nigriceps (Viereck)	Mardulyn & Whifield (1999)	see ref.	see ref.	AF102724	U68151	AF029120

were available (more details below), and one analysis on a restricted set of taxa, excluding taxa for which only morphological data were available. Support for individual clades in each analysis was estimated by performing bootstrap analyses (400 replicates, heuristic search, TBR swapping, simple addition sequence, MAXTREES not limited), and by evaluating Bremer support (Bremer, 1988) using AutoDecay version 4 (Eriksson, 1999) followed by TreeView (Page, 1996) display. For a more thorough exploration and analysis of the phylogenetic signal in each of the molecular datasets, see Mardulyn & Whitfield (1999). In addition to the two DNA + morphology analyses, an analysis combining the three genes was conducted for comparison with the morphological results.

The number of genera included in the molecular analyses was fewer than in the morphological analyses, and the molecular datasets represented several genera (*Alphomelon*, *Apanteles*, *Cotesia*, *Diolcogaster*, *Dolichogenidea*, *Glyptapanteles*, *Microplitis*, *Pholetesor*) with more than one species. As a result, analysis of the larger combined dataset involved several steps. As with the more taxonomically limited combined analyses discussed above, all species used in the molecular analysis were checked and coded for all morphological characters (usually this coding corresponded to that used for the genus in the morphological analyses but with some variation). The taxa (genera) for which no molecular data were available were then added to the matrix, with all molecular characters (sites) coded as uncertain (missing).

Results and discussion

Morphology only

Fifty-three morphological characters were delineated and coded; the list of characters and their states (Appendix 1) and the complete data matrix (Appendix 2) are provided here and also are available in TreeBASE (Morell, 1996; Sanderson *et al*., 1996). A summary of analyses run on the datasets is shown in Table 3. Inclusion or exclusion of the third outgroup *Mirax* had no effect on the ingroup topology, other than very slight alterations in bootstrap values; tree statistics presented below for morphology only are for analyses with two outgroup taxa. Maximum parsimony analysis of the matrix resulted in a total of 5740 equally most parsimonious trees (length 325, CI excluding uninformative characters = 0.24, RI = 0.64), the strict consensus of which is shown in Fig. 4A. For the most part, a large clade representing (Mason, 1981) Cotesiini + Forniciini + Microplitini is evident in the upper part of the consensus tree, with fairly complete resolution of relationships within this clade, although most of the nodes are not strongly supported. Other than Forniciini, which contains a single genus, Mason's tribes within this group are not resolved as monophyletic. Mason's two other tribes, Apantelini and Microgastrini, appear as a nearly completely unresolved subbasal polytomy other than a few basal taxa (*Austrocotesia*, *Miropotes*, *Pelicope*). In all, the phylogenetic results based on

Table 3. Analyses conducted on microgastrine genera, with methods employed and brief summary of results.

	Ingroup taxa	Informative characters	Sites excluded	% clades with BV > 70
16S				
Maximum likelihood GTR + gamma	43	159	26 hypervariable	
Maximum parsimony TBR 100 random additions	43	159	26 hypervariable	9.3
COI				
Maximum likelihood GTR + gamma	32	423	none	
Maximum parsimony TBR 100 random additions	32	423	none	13.3
28S				
Maximum likelihood GTR + gamma	31	155	none	
Maximum parsimony TBR 100 random addtions	31	155	none	41.4
Morphology				
Maximum parsimony TBR 100 random additions	55	53	not applicable	3.8
Combined DNA				
Maximum parsimony TBR 100 random additions	38	726	26 from 16S	16.7
Morphology + DNA				
Maximum parsimony TBR 100 random additions	38	779	26 from 16S	25
Maximum parsimony TBR 100 random additions	67	752	26 from 16S	10.8

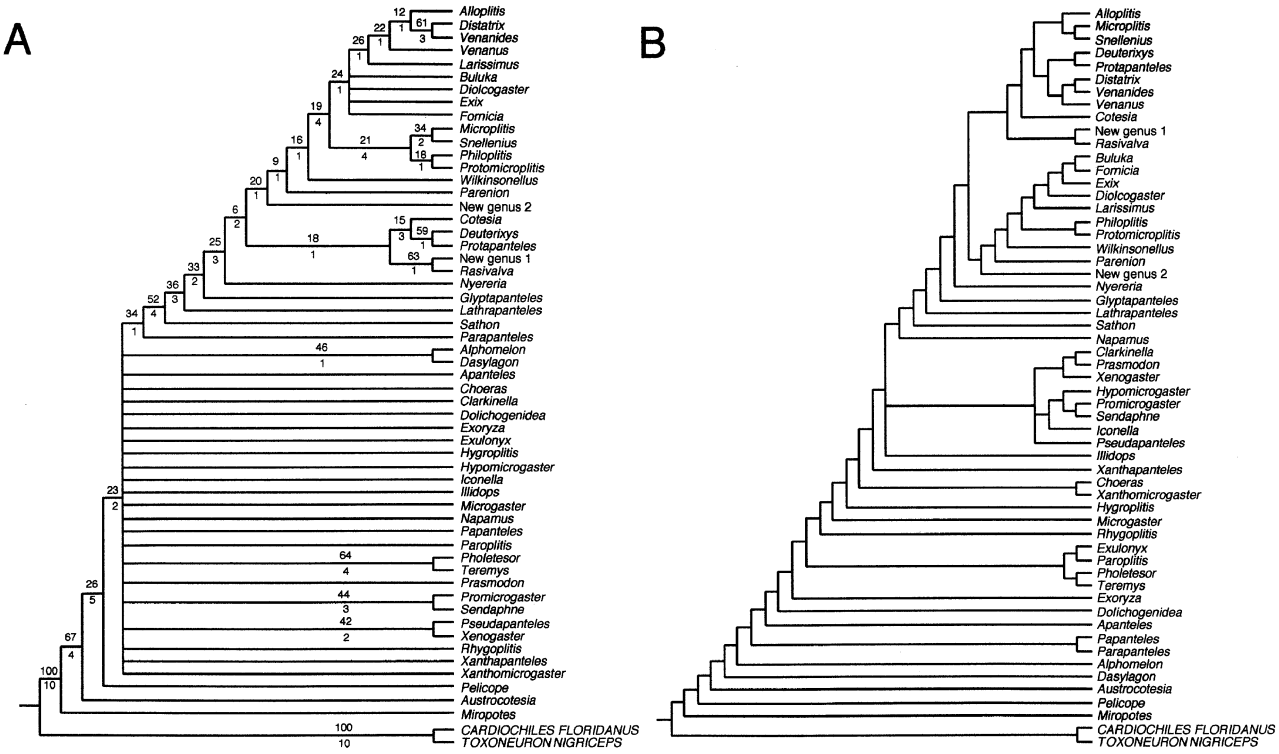

Fig. 4. Results of analyses of the morphological dataset (Table 2). A, Maximum parsimony strict consensus tree (from 5740 trees of length 326, CI excluding uninformative characters = 0.24, RI = 0.64) from heuristic analysis (TBR branch swapping, 100 random taxon addition sequences). Values above branches refer to bootstrap value (from 400 replicates). Values below branches refer to Bremer support, as calculated using AutoDecay 4.0. B, Tree shown in A followed by three rounds of successive approximations character weighting (Farris, 1969; Carpenter, 1988) using the rescaled consistency index (after three rounds the tree and weights stabilized).

morphology alone appear to strongly support Mason's division into groups principally associated with macrolepidoptera and microlepidopteran hosts, respectively. Two exceptions (previously pointed out as potential problems with Mason's phylogeny by Williams, 1985), are the placements of *Parapanteles* Ashmead and *Sathon* Mason. Mason (1981) treated *Parapanteles* as the most basal member of his Cotesiini based on its putatively plesiomorphic propodeal

areola, and placed *Sathon* in Microgastrini based on its (presumably plesiomorphic) long ovipositor and sheaths. Our re-analysis of the morphological data indicates that *Parapanteles* belongs with other genera in the *Apanteles* group of genera (as was assumed by Wilkinson & Nixon before Mason's reclassification) and *Sathon* belongs near *Glyptapanteles* (where Wilkinson & Nixon usually placed the species now belonging to *Sathon* in their series of studies). Thus, in these taxa, Mason's 'macrolepidoptera' and 'microlepidoptera' suites of ovipositor system characters appear to break down as higher-level phylogenetic indicators. (See '*Phylogenetic Performance of Morphological Character Complexes*', for more detail on the performance of these and other characters in the combined analyses.)

A more fully resolved tree was obtained based on the 'successive approximations' approach, reweighting by the maximum rescaled character consistency indices (Farris, 1969; Carpenter, 1988). Although this tree (Fig. 4B) shows some increased resolution that matches resolution in the combined-data tree (see below), it also shows some apparent anomalous phylogenetic relationships, especially among the *Apanteles* group of genera. This is probably due to the principal weakness of the 'successive approximations' approach as originally formulated, that it is overly dependent upon the original MP trees obtained (which may not be the optimum eventual solution), for reweighting the characters. It can thus converge on a locally optimal, but generally suboptimal, solution (Neff, 1986; Goloboff, 1993; Swofford *et al.*, 1996). The effectiveness of the method would, perhaps, be enhanced if broader sets of trees are taken to evaluating trees before the characters are weighted.

Molecular data

DNA sequences are available from GenBank, under accession numbers listed in Table 2, and both morphological and molecular (aligned sequence) matrices and resulting trees are deposited in TreeBASE. A total of 2300 base pairs of aligned sequence (16S, 442; 28S, 627; COI, 1235) was obtained. A complete account of the molecular datasets available for each taxon is available in Table 2.

16S sequence data

For all analyses, a small set of length-variable and unalignable sites was excluded. These correspond to characters 245–270 in all datasets. Maximum parsimony analysis resulted in nine equally parsimonious trees, with a length of 1159 (CI excluding uninformative characters = 0.25, RI = 0.45). Bootstrap analyses indicated, however, only a few clades with high support (Fig. 5A). The reason for this pattern is easily seen in the phylogram from the maximum

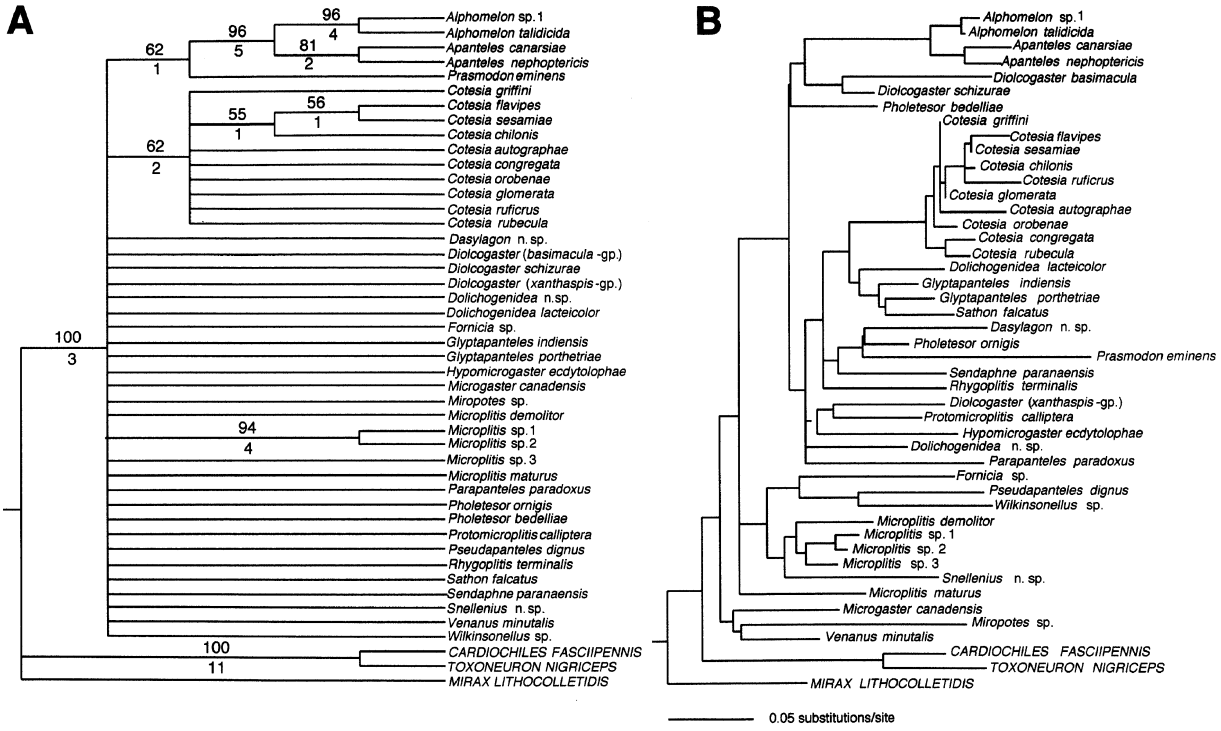

Fig. 5. Results from analysis of the 16S dataset, with hypervariable region (aligned sites 245–270) removed. A, Maximum parsimony bootstrap consensus tree (400 reps), with only branches with bootstrap values over 50 shown as resolved; values below branches are Bremer support. B, Maximum likelihood tree, using general time-reversible models with gamma-distributed sites and four rate categories (parameters estimated as described in the text).

likelihood analysis (Fig. 5B), where most internal branches are extremely short relative to terminal ones (see also Mardulyn & Whitfield, 1999, for further exploration of the relationship of short branches with low bootstrap values). The exceptional long internal branches are those with high bootstrap support in the MP analysis (*Apanteles* + *Alphomelon*, monophyly of *Cotesia*, etc.). Clearly, 16S by itself is insufficient for resolution of generic relationships.

28S sequence data

Maximum parsimony analysis resulted in 503 equally parsimonious trees, with a length of 654 (CI = 0.40, RI = 0.53). Bootstrap analyses indicated more strongly supported clades than 16S, although still not pervasive enough to be considered a strong result (Fig. 6A). Those clades well supported by 16S data (*Apanteles* + *Alphomelon,* monophyly of *Cotesia*) are also strongly supported in the 28S analysis, with the addition of *Microplitis* + *Snellenius* and *Glyptapanteles* + *Sathon*, which are also consistent with morphological results. A phylogram from the ML analysis (Fig. 6B) shows more or less the same problematical pattern of short internal branches as 16S, although not as severely so at the intergeneric level.

COI sequence data

Maximum parsimony analysis resulted in seventeen equally parsimonious trees, with a length of 2386 (CI = 0.31, RI = 0.35). Bootstrap analyses revealed even fewer clades than 16S with high support (Fig. 7A). Again, the ML analysis (Fig. 7B) revealed that most internal branches (especially those among genera) were short. The ML results showed some groupings that were consistent with those from 16S, 28S and morphology, but without strong enough support to appear in the MP bootstrap tree.

Incongruence among datasets

The ILD tests, consistent with the results from somewhat smaller datasets reported by Mardulyn & Whitfield (1999), indicated that the separate gene partitions were not significantly incongruent with one another or with morphology, with the (marginal, $P = 0.010$–0.21) exception of 16S (with all other datasets). Using the recommended significance level of 0.01 suggested by Cunningham (1997a,b), we have considered the datasets sufficiently congruent to combine in simultaneous parsimony analyses. Although such a combined MP analysis is clearly not as efficient in extracting the phylogenetic information from each of the three genes as the ML analyses, it is currently not yet feasible to apply the appropriate model parameters to each of the three datasets in a combined analysis. The combined MP analysis does have the advantage of allowing datasets that may provide resolution in different portions of the phylogeny to contribute to an overall phylogenetic estimate.

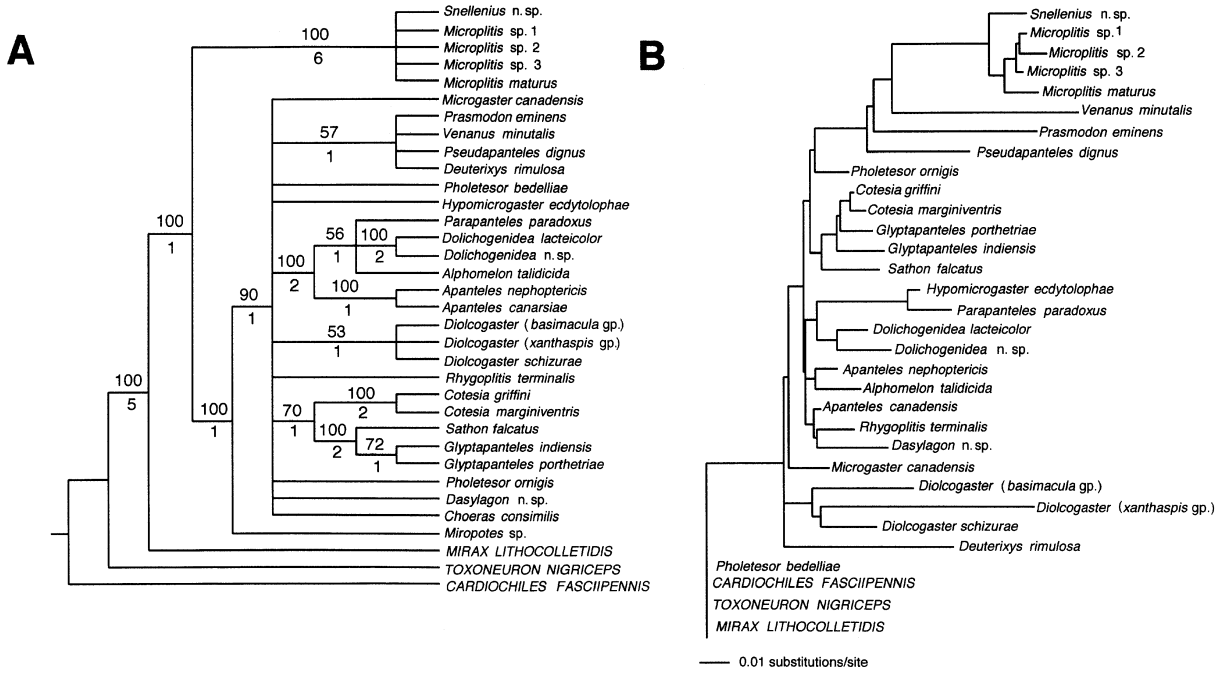

Fig. 6. Results from analysis of the 28S dataset. A, Maximum parsimony bootstrap tree, with branches over BV = 50 shown as resolved. Values below branches are Bremer support. B, Maximum likelihood tree, using HKY85 model (Hasegawa *et al.*, 1985) with gamma-distributed sites. All model parameters estimated from the data as described in text.

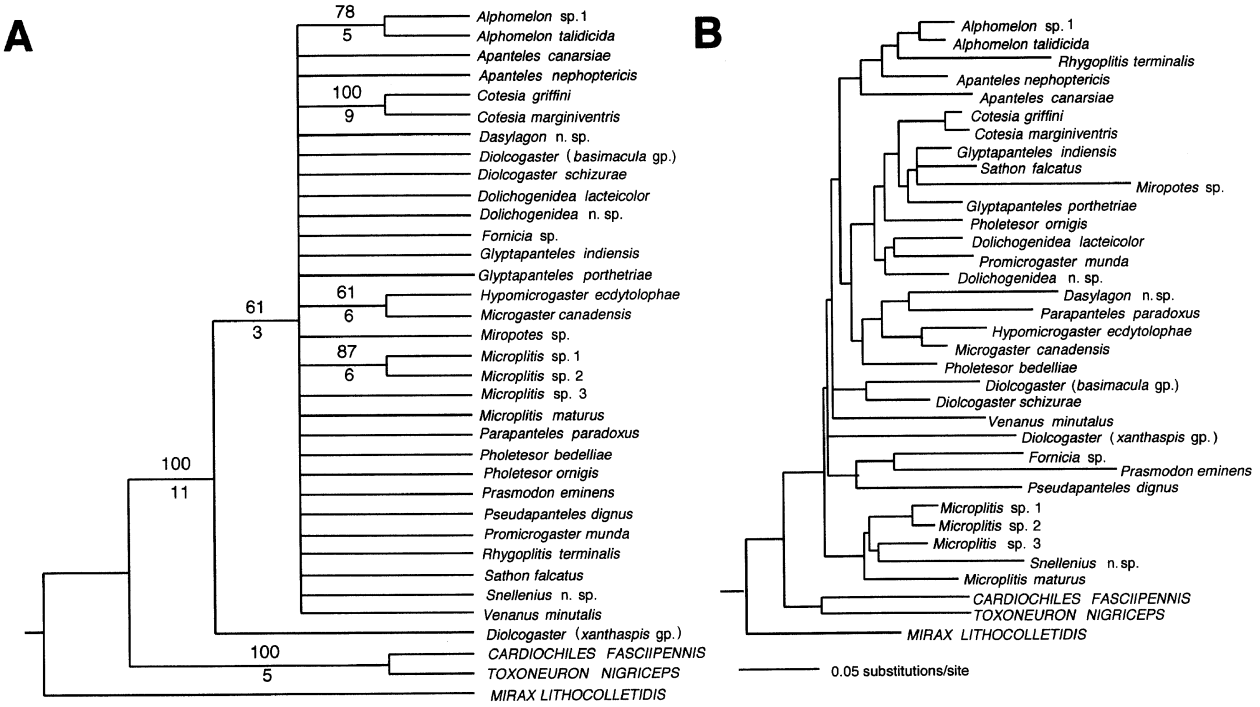

Fig. 7. Results from analysis of the COI dataset. A, Maximum parsimony bootstrap tree, with branches over BV = 50 shown as resolved. Values subtending branches are Bremer support. B, Maximum likelihood tree, using general time-reversible model with gamma-distributed sites. All ML parameters were estimated from the data as described in text.

Combined DNA analyses

Several of *Cotesia* species from the 16S analysis were omitted from the combined analyses because only 16S data are available for them and the genus was always shown to be monophyletic. Maximum parsimony analysis of the combined 16S, 28S and COI data resulted in six equally parsimonious trees, with a length of 3887 (CI = 0.30, RI = 0.37). Resolution within the resulting consensus tree (Fig. 8A) is relatively high, although bootstrap analyses still revealed few clades with high support. The MP consensus tree appears to be in fairly general agreement with results from morphology at the tips (Fig. 4), but differs dramatically at the base of the tree, where support is extremely weak.

Combined DNA + morphology analyses

When the above combined DNA dataset was merged with morphological data, maximum parsimony analysis resulted in three equally parsimonious trees, with a length of 4152 (CI = 0.30, RI = 0.40). Again, resolution within the resulting consensus tree (Fig. 8B) is relatively high, with some improvement in the number of clades showing high bootstrap support. The MP consensus tree still appears to incorporate the phylogenetic signal of the morphological data, effectively. Nonetheless, support towards the base of the tree is extremely weak.

In an effort to provide the best current estimate of relationships among all microgastrine genera, a final analysis was included that incorporated the genera for which only morphological data were available. It was expected that this combined analysis, with its high proportion of missing data, might produce highly unresolved results. As expected, the results of MP analysis on this large dataset (Fig. 9) do show a lack of basal resolution, but resolution and branch support towards the terminal branches is not seriously weakened by the inclusion of the additional taxa with large amounts of missing data. A conspicuous element of this consensus tree is the appearance of a clade essentially consisting of Mason's Microplitini + Cotesiini + Forniciini, which was present in the morphological tree (Fig. 4A) but absent in all of the molecular trees (although not contradicted by any of them via clades with high bootstrap support). This clade, in our results, shows a split into two subclades rather than three, and neither of these two corresponds to any of Mason's original tribes.

Phylogenetic performance of morphological character complexes

As the number of taxa available for morphological analysis is greater than that available for molecular analysis, we attempted to gain some perspective, from our analyses, on which morphological character systems seemed to be supplying the most reliable phylogenetic signal (or at least the signal most broadly corroborated by other data).

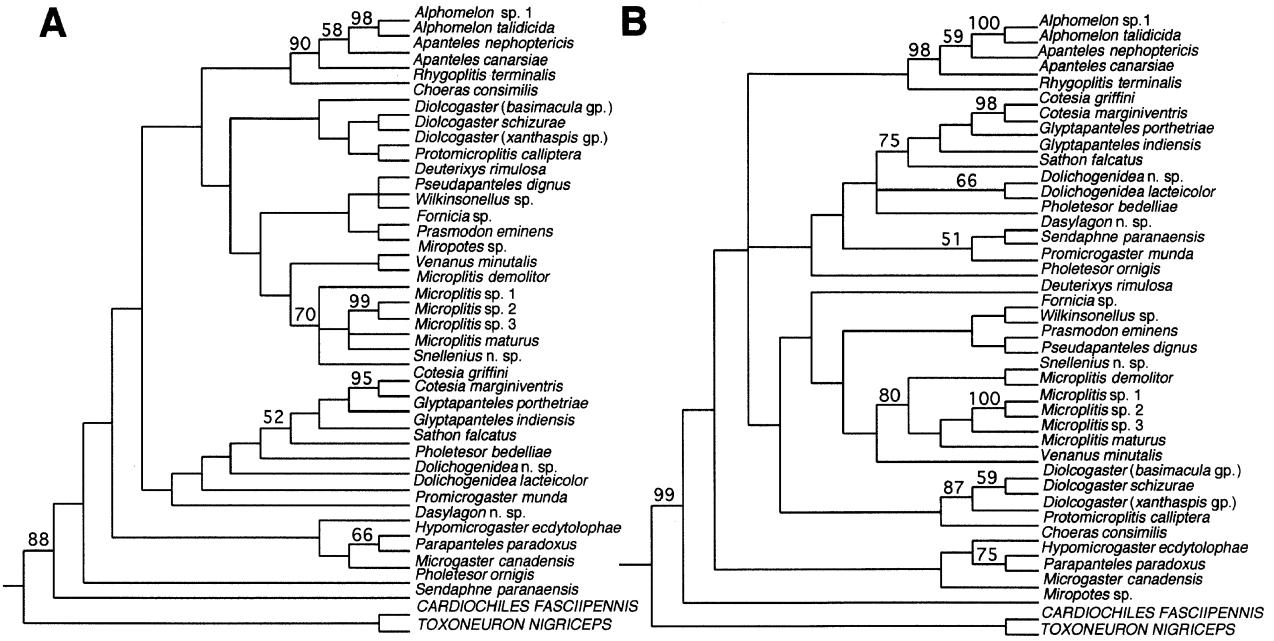

Fig. 8. Combined analyses for taxa with molecular data available. A, MP consensus tree (from six equally parsimonious trees of length 3887) from combined data from 16S, 28S and COI genes; 16S dataset treated as in Fig. 5. Bootstrap values over 50 shown. B, MP strict consensus tree (of three equally most parsimonious trees with length 4152) from analysis of the combined genes plus the morphological data. Bootstrap values over 50 shown.

The results of our comparison of performance of some of the character systems emphasized by Mason (1981) and others are summarized in Table 4.

The ovipositor mechanism heavily emphasized by Mason (1981) as part of his 'microlepidoptera' and 'macrolepidoptera' suites of characters showed a much higher mean character consistency index on the morphology tree than other characters as a whole. In fact, all four heavily used complexes of characters (ovipositor mechanism, patterns of propodeal carinae, shapes of anterior metasomal tergites and final instar larval head features), appeared to perform better than the mean of morphological characters on the morphology based tree. This superior performance of previously emphasized character systems is not surprising, as these were originally selected by Mason and others based on their correspondence with apparent general morphological patterns of relationship.

However, when these morphological characters are placed on the combined-DNA tree, two of the four character sets, specifically the ovipositor mechanism and anterior metasomal tergite characters, performed worse than the other morphological characters. Only the larval features performed markedly better than the mean. The poor correspondence with molecular phylogenetic signal results in these character systems generally performing worse than the average morphological character when mapped on to the combined (DNA + morphology) tree, with the conspicuous exception, again, of the final instar larval features.

Of the four character systems, the ovipositor mechanism and the final instar larval features would appear, initially, to be the most susceptible to convergence due to their direct relationship to the hosts utilized by the wasps. Yet one of these (ovipositor mechanism) seems to conflict with phylogeny, whereas the other (larval features) is more strongly congruent. Although this result cannot currently be explained easily, it does provide a window into possible future avenues of research upon these morphological characters and their functional and phylogenetic significance. For instance, would the ovipositor features show greater congruence with phylogeny if each feature were individually studied in greater detail? Are some of the features directly selected upon during host finding and parasitization, and thus more closely match host biology rather than phylogenetic history, whereas others are more strongly conserved phylogenetically despite host differences? The answers to these questions would not only improve estimates of wasp phylogeny, but also contribute directly to our understanding of wasp evolution.

Conclusions

The lack of basal resolution and branch support in the trees, despite the large amount of data collected and analysed (2300 bp from three genes, plus fifty-three morphological characters), precludes our proposing a mature classification for Microgastrinae. It is clear that a tribal classification

along the lines of that proposed by Mason (1981) is not yet possible. For the time being we would advocate an approach based on clarifying and recognizing informal genus groups, rather than more formal tribal groupings. The results of this study corroborate those of Mardulyn & Whitfield (1999), in suggesting that a rapid early radiation of microgastrine genera occurred, with a resulting basal phylogenetic pattern of short internal branches that is difficult to resolve. However, the relationships of some of the genera have been greatly clarified by our study. As mentioned above, *Sathon* (and probably also *Lathrapanteles*) clearly belongs with *Glyptapanteles*, which it resembles in all features except its elongate ovipositor and sheaths. Its concealed hosts would appear to have led to convergence

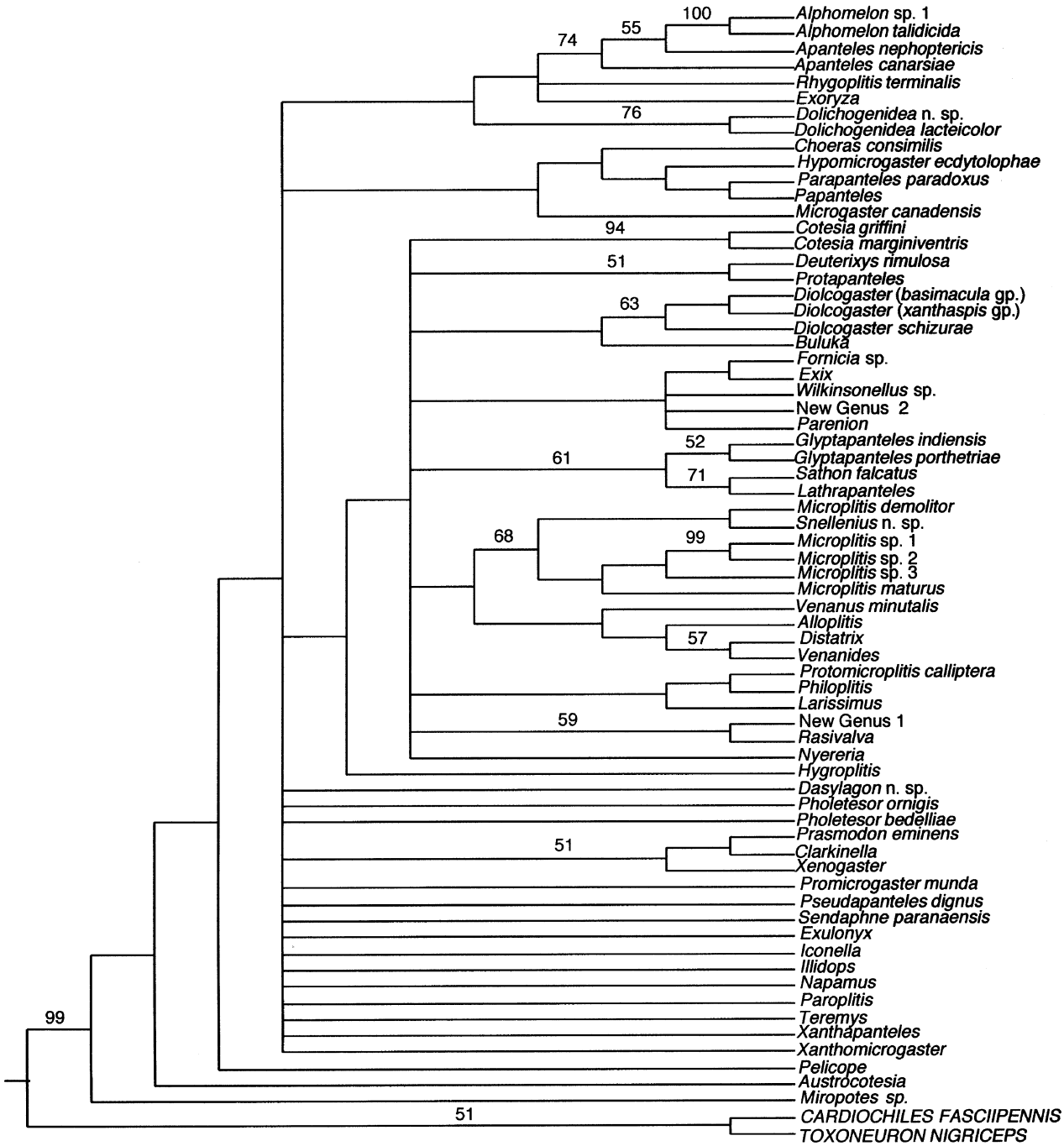

Fig. 9. MP consensus tree (from 2124 trees of length 4947) from analysis of the three genes plus morphology (as in Fig. 8) with the addition of genera for which only morphological data are available (these can be identified as being labelled with only the genus name). BV $\geq$ 50 shown.

Table 4. Relative conflict in morphological character systems, as measured by the consistency index when characters are fitted to the optimum topologies based on morphology, on DNA sequences from the three genes, and on all data combined. Autapomorphies have been removed from molecular and combined trees in which fewer taxa are represented. Character systems featured include those traditionally used in microgastrine taxonomy and classification.

	Mean character consistency index			
Character system	Characters	On morphology tree	On DNA tree	On combined tree
Ovipositor mechanism	26–32	0.549	0.15	0.229
Propodeal carinae	11, 12, 13, 14	0.374	0.24	0.244
Anterior metasomal tergites	18–24	0.428	0.205	0.253
Final instar larval features	45–51	0.5	0.33	0.445
All characters	all 53	0.238	0.225	0.321

in ovipositor length with the *Microgaster* and *Apanteles* groups of genera. Secondly, *Parapanteles* does not belong with Cotesiini, but instead with some other genera with areolate propodea. In this case, the reverse pattern of convergence appears to have taken place, towards short ovipositors in unrelated taxa attacking more or less exposed large hosts. Both of these rearrangements illustrate the problems that can be generated by overemphasizing a single character system in classification.

The monophyly of several genera which were represented by multiple exemplars in our study (especially *Cotesia* Cameron, *Microplitis* Foerster *s.l.*) seems to be supported. This includes the morphologically diverse genus *Diolcogaster* Ashmead. It must be pointed out, however, that too scanty a representation of the species group diversity in this genus has been included to conclude very much.

Several groupings that would appear to be natural based on morphology (for example, the Cotesiini + Microplitini + Forniciini clade, and the *Apanteles* group of genera) are weakly supported, or at least not strongly contradicted, by molecular data. Within these clades, however, the phylogenetic structure differs from previous classifications. It would thus seem useful to sample additional morphological character systems, and to focus on finding additional genes that show suitable variation at the level of relatively closely related genera.

The results provide a first approximation of the phylogenetic position of the two undescribed genera, while confirming the close relationships among some genera that could constitute informal, but relatively well supported genus groups. These include *Glyptapanteles* + *Sathon* + *Lathrapanteles*, *Microplitis* + *Snellenius*, *Apanteles* + *Alphomelon*, and *Prasmodon* + *Clarkinella* + *Xenogaster*. It is clear that two Australasian genera, *Miropotes* and *Austrocotesia*, occupy basal positions in the phylogeny, along with a genus currently known only from California, *Pelicope*. The implications of these positions for the biogeographical history of the group, although not yet clear, would be well worth pursuing.

It is apparent that a very large amount of data will be required to fully resolve the relationships among the microgastrine genera. At this point, the prospects are good for additional data providing a clearer view of the relationships among closely related genera. It is not yet obvious that the basal divergences within this group of wasps can be resolved unambiguously using practically obtainable quantities of molecular and morphological data.

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Appendix 1.

List of morphological characters used in the phylogenetic analysis of microgastrine relationships. Character state descriptions are abbreviated for brevity. Characters compiled or modified, or both, from Mason (1981, 1983), Williams (1985), Whitfield (1985), Walker *et al*. (1990), Austin (1990), Austin & Dangerfield (1992), Whitfield & Mason (1994) and Maetô (1996). State 0 is not necessarily assumed to be plesiomorphic; multiple outgroup taxa are used in analyses. All characters treated as unordered.

Head

1. *Antennal placodes on basal half of flagellum (females only)*: (0) single-ranked; (1) 2-ranked; (2) in irregular ranks.
2. *Antennal placodes on medioapical flagellomeres* (females only): (0) ventrally same as dorsally; (1) ventrally absent on some segments.
3. *Glossa*: (0) with truncate glossa; (1) with bilobed but short glossa; (2) with divaricate glossa.

Thorax/propodeum

4. *Number of lateral pronotal grooves present*: (0) 2 (dorsal and ventral); (1) one (ventral); (2) none; (3) lower margin of pronotum excavated.
5. *Lower outer corner of propleuron*: (0) simple; (1) with upwardly projecting flange.
6. *Epicnemial carina*: (0) present; (1) absent.
7. *Scutellar 'lunules'*: (0) of nearly uniform width; (1) lunate in outline; (2) strongly triangular.
8. *Medioposterior band of scutellum*: (0) smooth; (1) sculptured.
9. *Sublateral hairs on metanotum*: (0) present throughout; (1) developed as a tuft; (2) absent.
10. *Anterior margin of metanotum*: (0) appressed to scutellum; (1) excavated sublaterally, sublaterally exposing phragma; (2) sloping away laterally, phragma well exposed.
11. *Dorsal, anterior, horizontal portion of propodeum*: (0) angled relative to posterior declivous portion at about mid-length of propodeum; (1) dorsal part greatly shortened; (2) dorsal part evenly and gently curved relative to posterior portion of propodeum.
12. *Medial longitudinal carina of propodeum*: (0) present and complete; (1) present only anteriorly; (2) present anteriorly as divided (parallel) short carinae; (3) present only posteriorly; (4) absent.
13. *Areola of propodeum*: (0) present and complete; (1) incomplete (broadly open anteriorly); (2) absent.
14. *Transverse carinae of propodeum*: (0) transverse carinae present; (1) transverse carinae absent.
15. *Hind coxae*: (0) distinctly shorter than metasomal T1; (1) normal; (2) much longer than T1, enlarged.
16. *Tarsal claws*: (0) simple; (1) pectinate; (2) single basal tooth.
17. *Female distal fore tarsomere*: (0) normal; (1) excavated ventrally with curved seta.

Metasoma

18. *Metasomal tergite 1 basal excavation*: (0) present; (1) absent.
19. *Metasomal tergite 1 mediobasal sharp longitudinal groove*: (0) present; (1) absent.
20. *Apical half of T1*: (0) flat to gently arched and without excavation; (1) with shallow medial longitudinal excavation.
21. *Metasomal tergite 1 shape*: (0) broad, covering most of tergum, sometimes exposing laterotergites; (1) more or less evenly narrowing towards apex; (2) strongly narrowed, elongate and nearly parallel-sided; (3) more or less strongly narrowed basally, broadly exposing laterotergites.
22. *Junction of metasomal tergites 1 and 2*: (0) not fused, movably articulated; (1) fused.
23. *Metasomal tergite 2 shape*: (0) rectangular and covering most of dorsal surface; (1) broadly triangular or transverse, much broader than medially long; (2) narrowly triangular; (3) desclerotized anterolaterally and medioapically to form slender inverted Y-shape.
24. *Delineation between metasomal T2 and T3*: (0) by a fine distinct suture; (1) weak or absent; (2) by a broad crenulate or transcostate groove.
25. *Metasomal T3*: (0) not contributing to carapacelike structure; (1) carapacelike.
26. *Hypopygium*: (0) evenly sclerotized, not medially folded; (1) more or less evenly sclerotized but sharply folded medially; (2) medially strongly desclerotized and longitudinally folded into pleats.
27. *Metasomal tergite 8 of female*: (0) 2–4X taller than long with apodeme shorter than wide; (1) little taller than long, with apodeme as long as or longer than wide.
28. *Second valvifer*: (0) tall, lorate, not expanded apically; (1) short, expanded apically (at least dorsally).
29. *Ovipositor sheath (third valvulae)*: (0) attached to second valvifer near apex on ventral side; (1) attached proximally between base and middle.
30. *Ovipositor sheath length*: (0) very short, mostly hidden by hypopygium; (1) elongate, exserted and exposed for large part of length.
31. *Ovipositor sheath distribution of hairs*: (0) hairs over apical half or more of length; (1) hairs concentrated at extreme apex.
32. *Ovipositor (first and second valvulae)*: (0) tapered throughout length; (1) abruptly narrowing at or just beyond midlength; (2) strongly decurved apically.

Wings

33. *Radius (3Rs) of forewing*: (0) proximally strongly convex towards costal margin; (1) straight or only weakly curved.

34. *Basal vein (1M + 1Rs) of forewing*: (0) conspicuously angled (approximately 90°) at junction of M and Rs; (1) weakly angled (20–60°); (2) straight or nearly so.
35. *R-m of forewing (form of areolet)*: (0) r-m meeting 3Rsa; (1) r-m meeting 2Rs or junction; (2) r-m absent.
36. *Anal cross vein (2A) of forewing*: (0) present (often spectral); (1) absent.
37. *Radial cross vein of hind wing (r)*: (0) present, nearly always spectral; (1) completely absent.
38. *Cells 1R and 2R of hindwing*: (0) of approximately same width; (1) cell 1 R1 much wider than cell 2 R1 due to curvature in 1Rs.
39. *Vein 2r-m of hind wing*: (0) present; (1) absent.
40. *2A vein in hindwing*: (0) present as stump; (1) absent.
41. *2Cu and cu-a of hindwing*: (0) straight or concave; (1) concave anteriorly (sinuate overall).
42. *Clavum (vannal lobe) of hind wing*: (0) separated from remainder of wing margin by a sharp notch; (1) notch absent or indistinct.
43. *Claval (vannal) margin of hindwing*: (0) distinctly convex beyond widest point; (1) distally flattened to concave beyond widest point.
44. *Claval (vannal) fringe*: (0) long, even and dense beyond broadest point of clavum; (1) short, much sparser beyond broadest point; (2) absent beyond broadest point.

Larva

45. *Larval antenna:* (0) present; (1) absent.
46. *Blade of larval mandible*: (0) with approximately 15–25 large teeth; (1) with 8–14 teeth, concentrated apically; (2) with less than 5 teeth, at extreme apex; (3) toothless; (4) abruptly bent outward and with many teeth of varying sizes near apex.
47. *Apex of larval mandible:* (0) bifid; (1) simple.
48. *Larval palpi*: (0) with one sclerotized article; (1) soft and unsclerotized.
49. *Larval maxilla*: (0) with 2 setae; (1) with one short seta; (2) with greatly enlarged seta (over half as long as mandible).
50. *Larval labium*: (0) with 4 setae; (1) with 2 setae; (2) with over 10 setae.
51. *Larval skin*: (0) densely covered in papulae, each bearing a long central spine; (1) as in (0) but spines shorter than length of papulae; (2) papulae without spines and sometimes inconspicuous.

Male genitalia

52. *Apex of digitus of male genitalia*: (0) round, broadly truncate; (1) acute, directed dorsally; (2) obtuse, directed dorsally; (3) narrowly truncate, directed dorsally; (4) tubiform, curved dorsally.
53. *Ventral edge of digitus of male genitalia*: (0) straight or nearly so; (1) strongly convex.

Appendix 2. Matrix of coded characters for the morphological analyses. Character numbers correspond to those used in Appendix 1. Polymorphic taxa are indicated by entry of the second state immediately under the first (especially characters 16 and 17).

Taxon	1 1234567890	1111111112 1234567890	2222222223 1234567890	3333333334 1234567890	4444444445 1234567890	555 123
Alloplitis	0001011111	1100100010	0000001110	1?11010000	1012??????	?00
Alphomelon	1000012020	1200110011	0010000001	0011200001	0012100011	111
Apanteles	1000011010	1400100011	0010020001	0010200001	0012100011	011
Austrocotesia	1001010020	2100100010	1020000001	0011010001	0010??????	?11
Buluka	1110010110	2021200000	0002101110	1111010001	0000??????	?01
Choeras	1000011020	2021200010	0012020001	0011010001	0011100010	011
Clarkinella	1000011011	1020100010	1000010001	0011100001	0011??????	?11
Cotesia	1000011020	2021100010	0002001110	1111210001	0000131000	200
Dasylagon	1000012020	1100110010	0010020001	0011100000	1012??????	?11
Deuterixys	1000011011	1021100000	0002001110	1211210001	0000110000	000
Diolcogaster	1001010110	2021200000 1	0002001110	1111010001	0000031000	200
Distatrix	1001011012	2421100010 1	0020001110	1011200001	0011121000	100
Dolichogenidea	1000011011	1411100011	0010020001	0011200001	0000100011	011
Exix	1100011110	2021200000	0002001110	1111110001	101213?100	100
Exoryza	1000011010	2411100011	0002020001	0010200001	0000??????	?11
Exulonyx	1001011020	0110100010	0002010001	0011210101	0000??????	?11
Fornicia	1101100120	1020200000	0102101110	1111210001	1012131010	101
Glyptapanteles	1000011022	2421100010 1	1020001110	1111210001	0000131011	100
Hygroplitis	0000011020	2021100010	0002000001	0011010001	0000??????	?21
Hypomicrogaster	1000112010	1001100010	0010020001	0011110001	0011100010	011
Iconella	1000012011	2021110010	1010020001	0011210001	10121000??	011
Illidops	1000011120	1421100011	1010020001	0011210001	0012100010	041
Larissimus	0000011010	2021200000	1022001110	1111010001	0110??????	???
Lathrapanteles	1000011022	2021100010	1010001111	0011210001	0000131011	230
Microgaster	1000011010	1021100010 1	0002020001	0011010001	0000000010	011
Microplitis	1001011110	1021000010	0000001110	1111010001	0000121100	200
Miropotes	1000010020	0100000110	3020020001	0201010111	0000??????	?11
Napamus	1020011020	1021100010	1010020001	0010210001	0001??????	?11
New Genus 1	1101011020	202110?010	102200???0	1111010101	0000??????	???
New Genus 2	1001011020	2021200010	1000001110	1111210001	0012??????	?00
Nyereria	1001011022	2021100010	0000001110	1111210001	0000??????	?00
Papanteles	1000011010	1100220011	1020020001	0011110001	0012??????	?11
Parapanteles	1000011012	1100100011 1	0010000010	1111210001	0000??????	?11
Parenion	1001011020	2021200000 1	3021001110	1210110001	0000??????	?01
Paroplitis	0000011010	1100000010	0001010001	0011010001	0000??????	?11
Pelicope	1000010020	1421100010	1010020001	0002011011	0011??????	?11
Philoplitis	1011110111	1021100000	1021001110	1111010001	0011??????	?00
Pholetesor	1000011011	1411100010	1022010001	0011210001	0000100002	011
Prasmodon	1011010010	1020110010	1010000001	0011110000	0111??????	?11
Promicrogaster	1020012011	1021200010	1020020001	0011110001	1012??????	?11
Protapanteles	1000011021	1021101010	0022001110	1111210001	0000110000	100
Protomicroplitis	0000011110	1021100000	2001001110	1111100001	0011131000	201
Pseudapanteles	1020011010	1021100000	1010020001	0011210001	0011??????	?11
Rasivalva	1101011010	2021100010	1022001110	1111010001	0000131000	200
Rhygoplitis	1010011010	1021100010	0002020001	0010210001	0000100012	011
Sathon	1000011022	1021100010	1020000001	0011210001	00001?1012	200
Sendaphne	1020012020	1421200010	1020020001	0011100001	0012??????	?11
Snellenius	1001000110	0021000000	2001001110	1111000001	0000121100	200
Teremys	1000011011	1411100010	0002110001	0011210001	0000??????	?11
Venanides	0001011012	2421101010	1021001110	1111210001	0012121000	100

Appendix 2. Continued.

Taxon	1 1234567890	1111111112 1234567890	2222222223 1234567890	3333333334 1234567890	4444444445 1234567890	555 123
Venanus	0001011010	2421100010	0002001110	1111010001	0000121000	100
Wilkinsonellus	1001111120	2011200000	2001001110	1111210001	0001??????	?00
Xanthapanteles	0000011020	1200100010	0102120001	0010210001	0011??????	???
Xanthomicrogaster	1010011020	1021200000	0002000001	0011110001	1012??????	?11
Xenogaster	10?0011010	1020100000	1011010001	0011210001	0011??????	???
Outgroup						
Card. floridanus	0020010000	1400110000	3000020001	0001000010	0100000000	000
Toxo. nigriceps	0020000100	1100110000	300000???0	0001000010	0100000011	000
Mirax	0003000000	1021120010	203100??01	0002211011	0100000011	000