
Morphological, palaeontological and molecular aspects of ichneumonoid phylogeny (Hymenoptera, Insecta)

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Ichneumonoid phylogeny is revised on the basis of morphological, palaeontological and molecular evidence. The only previous formal cladistic study of the phylogeny of the families of the superfamily Ichneumonoidea made many assumptions about what families lower taxa belonged to and was based on a very limited set of characters, nearly all of which were uninformative at family level. We have subdivided both Ichneumonidae and Braconidae into major groups, investigated several new character systems, reinterpreted some characters, scored several character states for extinct taxa by examining impression fossils using environment chamber scanning electron microscopy, and included data for a significant new subfamily of Braconidae from Cretaceous amber of New Jersey. Sixteen different variants of the data set were each subjected to parsimony analysis without weighting and with successive approximations weighting employing both maximum and minimum values of both the retention and rescaled consistency indices. Each analysis resulted in one of seven different strict consensus trees. Consensus trees based on subsets of these trees, selected on the basis of the optimal character compatibility index (OCCI), resulted in an eighth distinct tree. All trees had the Braconidae monophyletic with the Trachypetinae as the basal clade, and also had a clade comprising various arrangements of Apozyginae, the Rhyssalinae group, Aphidiinae and 'other cyclostomes', but relationships among the remaining braconid groups varied between trees. Only one of the consensus trees had the Ichneumonidae (including *Tanychorella*) monophyletic. The Eoichneumonidae + *Tanychora* are the sister group the Braconidae in two of the consensus trees. Paxylommatinae were basal in the clade comprising the Eoichneumonidae + *Tanychora* and the Braconidae. The preferred tree, based on the highest OCCI was used for interpreting character state transitions.

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Introduction

Sharkey & Wahl (1992) presented the first formal phylogenetic analysis of the relationships between the ichneumonoid families based on both extant and extinct taxa and treating the individual genera of the fossil family Eoichneumonidae separately. Their analysis, however, was inadequate and potentially flawed in several respects. Their data set, comprising seven taxa plus an hypothetical

outgroup and 10 characters, is of little value for their stated purpose since 6 of the characters are totally uninformative (autapomorphic for various taxa), and a further two are informative only within the Eoichneumonidae leaving only two characters that are actually informative at family level, and one of these was polymorphic within one of the two families displaying the derived state. They concluded that the monophyly of the Eoichneumonidae was

supported by the reduction or absence of forewing transverse vein 3rs-m, and the clade Eoichneumonidae plus Braconidae was supported by loss or reduction of forewing transverse vein 2 m-cu. However, re-examination of eoichneumonid fossils using environment chamber scanning electron microscopy shows that forewing vein 2m-cu is well developed in these, although having a broad median bulla (Fig. 7A-C), thus casting further doubt on the results of their analyses.

Recent studies of several morphological character systems (for example, Maetô 1987; Quicke & van Achterberg 1990; Quicke *et al.* 1992a-c, 1994; Basibuyuk & Quicke 1995, 1997, 1999) and parallel work on molecular phylogenetics of the extant Ichneumonoidea (Belshaw & Quicke 1997; Belshaw *et al.* 1998; Quicke *et al.* 1999) have thrown new light on relationships within both the Ichneumonidae and Braconidae. These internal relationships are important because both families are extremely large and heterogeneous, with few characters being fixed in all members of either of them. Better understanding of the relationships within the Braconidae and Ichneumonidae, and in particular the recognition of monophyletic groupings within each should facilitate reconstruction of the correct groundplan character assignments in each family and so increase the possibility of resolving relationships between the higher taxa involved.

The heterogeneity of many characters within the Braconidae and Ichneumonidae was undoubtedly a major factor in limiting the number of informative characters available for Sharkey & Wahl's (1992) study. By breaking up both families into a number of more homogeneous groups we attempt to overcome this problem. In particular we have treated separately taxa, such as Paxylommatinae, whose family placement has been disputed in the past (van Achterberg 1976; Mason 1981). Further, we have been able to examine many of the fossil specimens, including those that Sharkey & Wahl referred to, using not only light microscopy but also scanning electron microscopy by means of the environment chamber facility that removes the need to coat specimens. This latter technique has enabled us to assess various character state assignments with greater confidence and so enabled us to score fossils for additional characters. For example, Wharton *et al.* (1992) criticised Quicke & van Achterberg (1990) for interpreting the presence of bullae in the wing veins of various eoichneumonid fossils. Examination using environment chamber electron microscopy, however, supported the reported interpretations of bullae showing that these regions were not areas of poor preservation or damage but had the characteristic cross-striate appearance of bullae found in extant taxa. In addition to bullae, we have been able to come to firmer conclusions about the states of a

number of other characters in the extinct taxa including shape, distribution and internal aperture of multiporous plate sensilla on the antennae (Fig. 8B), the relationship of vein R to the parastigma in the forewing (Fig. 4A), the relationship of forewing vein C + SC to R (Figs 4A, 7B, 8A), and presence or absence of various other wing veins (Table 1a, b). Nevertheless, it should be noted that more than 20% of our characters cannot be scored in the fossil taxa and these missing data may hamper finding fully resolved trees.

There has been extensive debate about higher relationships within the Braconidae over the past dozen or so years. Most studies centred on single character systems, but that of Quicke & van Achterberg (1990) was based on parsimony analysis of 96 characters and all subfamilies recognized at the time. The results, which suggested a paraphyletic cyclostome grade leading to a single origin of endoparasitism among Aphidiinae and other noncyclostomes were surprising to many workers (Wharton *et al.* 1992; van Achterberg & Quicke 1992) but were apparently robust to addition and deletion of many characters and subsampling and finer sampling of taxa (Quicke 1994). However, the prevailing opinion that at least the extant cyclostome braconids form a monophyletic clade as indicated by their derived cyclostome mouthparts has subsequently been supported strongly by analyses which have now been carried out with molecular sequence data from four different gene fragments (16S rDNA, 28S rDNA, cytochrome b, and elongation factor 1- α) (Belshaw & Quicke 1997; Gimeno *et al.* 1997; Belshaw *et al.* 1998; Dowton *et al.* 1998; Quicke & Belshaw in press). These molecular studies also indicate various other groups of subfamilies as monophyletic, though relationships, particularly among the noncyclostomes, were poorly resolved. Within the Ichneumonidae, a comprehensive phylogenetic analysis of subfamilial relationships based on morphology has not been published. Four major groups are recognized (Gauld 1997) though many subfamilies remain 'unplaced'. Molecular studies (Belshaw *et al.* 1998; Quicke *et al.* 1999) provide some indication that the Paxylommatinae, Xoridiinae and Labeninae may be the most basal groups of the extant Ichneumonidae, largely in agreement with opinion based on biology, morphology and biogeography (Gauld 1983; Wahl 1993). Reasons for treating various of these taxa separately are discussed in more detail below.

Material and methods

Selection of taxa

Outgroup. We included the Praeichneumonidae in initial analyses as a relatively underderived, potentially basal member of the Ichneumonoidea. Although Sharkey & Wahl (1992) found no synapomorphies to support this placement they

appear as the sister group in the re-analysis of Rasnitsyn's (1988) data set by Ronquist *et al.* (1999). However, as we were unable to score this poorly preserved fossil group for the great majority of the characters, rooting was more or less meaningless. There is considerable evidence that the sister group of the Ichneumonoidea are the Aculeata (Zessin 1985; Sharkey & Wahl 1992; Quicke *et al.* 1992a; Basibuyuk & Quicke 1995). Unfortunately, aculeates do not make a sensible outgroup for present purposes as they are all highly derived for many of the character systems referred to here. We therefore chose, despite difficulties thereby created, to include an hypothetical ancestor in analyses. Character states for this hypothetical taxon were derived from consideration of Siricidae, Anaxyelidae, Orusidae, Megalynidae and the extinct Karatavidae and Ephialtidae.

Braconidae. We broke down the Braconidae into eight component taxa. These are the Trachypetinae, the Aphidiinae, the Apozyginae, the Rhyssalinae group of subfamilies (Rhyssalinae and Histeromerinae), the remaining extant cyclostome subfamilies (see below), the Agathidinae group of subfamilies (Agathidinae, Meteorideinae, Pselaphaninae and Sigalphinae), the other extant noncyclostome subfamilies (see below) and the Protorhyssalinae (Basibuyuk *et al.* 1999).

The Trachypetinae (= Cercobarconinae; Austin *et al.* 1993) are a problematic group of large braconids known only from Australia, and particularly the more arid parts of that continent. The relatively few available specimens of the group has hampered research on them, and their biology is unknown. In the morphological analyses of Quicke & van Achterberg (1990) they came out consistently among the 'helconoid' group of subfamilies near to the Agathidinae, Sigalphinae and Meteorideinae with which they share the presence of hindwing vein 2-CU emerging from 1-CU and cu-a (character 13; Fig. 3F). That the Trachypetinae are potentially of considerable importance in unravelling relationships within the Ichneumonoidea is indicated by several features. They have a distally open, though narrow costal cell in the forewing with vein R running direct to the parastigma, the tracheal trunk of this vein also not deviating at all (characters 6 and 7), hindwing vein R is separate virtually at the base of the wing and runs distinct from C + SC (character 15; Fig. 2D), a condition very similar to that found in ichneumonids (Figs 2B, 3A-E), but not found elsewhere among the braconids (Figs 2C,E, 3F,J), and the trachypetines are the only non-cyclostome braconids, other than a few Aphidiinae, that share a massively muscularised venom reservoir with a spirally sculptured cuticular lining (character 22). Further, their 28S rDNA sequence is extremely different from those of other braconids. Whilst not particularly more

similar to ichneumonids in terms of identifiably homologous base positions, trachypetines have a GC:AT ratio of nearly 60% which is almost the same as that found in all ichneumonids studied, but very different from all other braconids whose 28S sequence was nearly 60% AT rich (Belshaw *et al.* 1998) (character 23).

The position of the Aphidiinae within the Braconidae rather than as a separate family, has now been generally accepted, though their relationship to either the cyclostomes or non-cyclostomes is still debatable. Molecular studies (Belshaw & Quicke 1997) suggest that they, together with the small, endemic Australian subfamily Mesostoinae (Belshaw *et al.* 1998) which induce galls in *Banksia* spp., are a basal clade of the non-cyclostome lineage, although some aphidiines and mesostoinae are distinctly cyclostome, perhaps indicating that cyclostomicity may be plesiomorphic for the braconids as a whole and secondarily lost in the non-cyclostome group, as it undoubtedly has been in the Opiinae and Alysiinae which belong to the 'cyclostome' group of subfamilies (see Gimeno *et al.* 1997).

While the cyclostome condition of the mouthparts (Fig. 1B) has long been accepted as a probable synapomorphy for the cyclostome group of subfamilies, no synapomorphy was known for the non-cyclostomes. That all the non-cyclostome taxa are koinobiont endoparasitoids provides only weak evidence of monophyly because it is clear that endoparasitism has evolved on several independent occasions in the Braconidae (Whitfield 1992; Belshaw & Quicke 1997). The only morphological character that has been found so far to suggest monophyly of the non-cyclostomes is the presence of highly modified sperm (see character 19; Fig. 10A,B) (Quicke *et al.* 1992b). However, these derived sperm which are very short and dominated by their nucleus and axoneme, do not occur in at least two of the noncyclostome subfamilies (Agathidinae and Sigalphinae) (Fig. 10D) and so at best may be a synapomorphy for a large subset of non-cyclostomes. Molecular studies have not yet provided any clear evidence that the non-cyclostomes, either with or without the Agathidinae group of subfamilies, form a monophyletic group (Belshaw *et al.* 1998). The subfamilies making up our 'other noncyclostome' group are the Adeliinae, Blacinae, Cardiochilinae, Charmontinae, Cenocoeliinae, Cheloninae, Dirrhopinae, Ecnomiinae, Euphorinae, Helconinae, Homolobinae, Ichneutinae, Khoikhoiinae, Macrocentrinae, Mendeselinae, Meteorinae, Microgastrinae, Miracinae, Neoneurinae, and Orgilinae.

The agathidine group of subfamilies (Agathidinae, Meteorideinae, Pselaphaninae and Sigalphinae) was treated separately for two reasons: they do not share derived sperm morphology with other non-cyclostome subfamilies

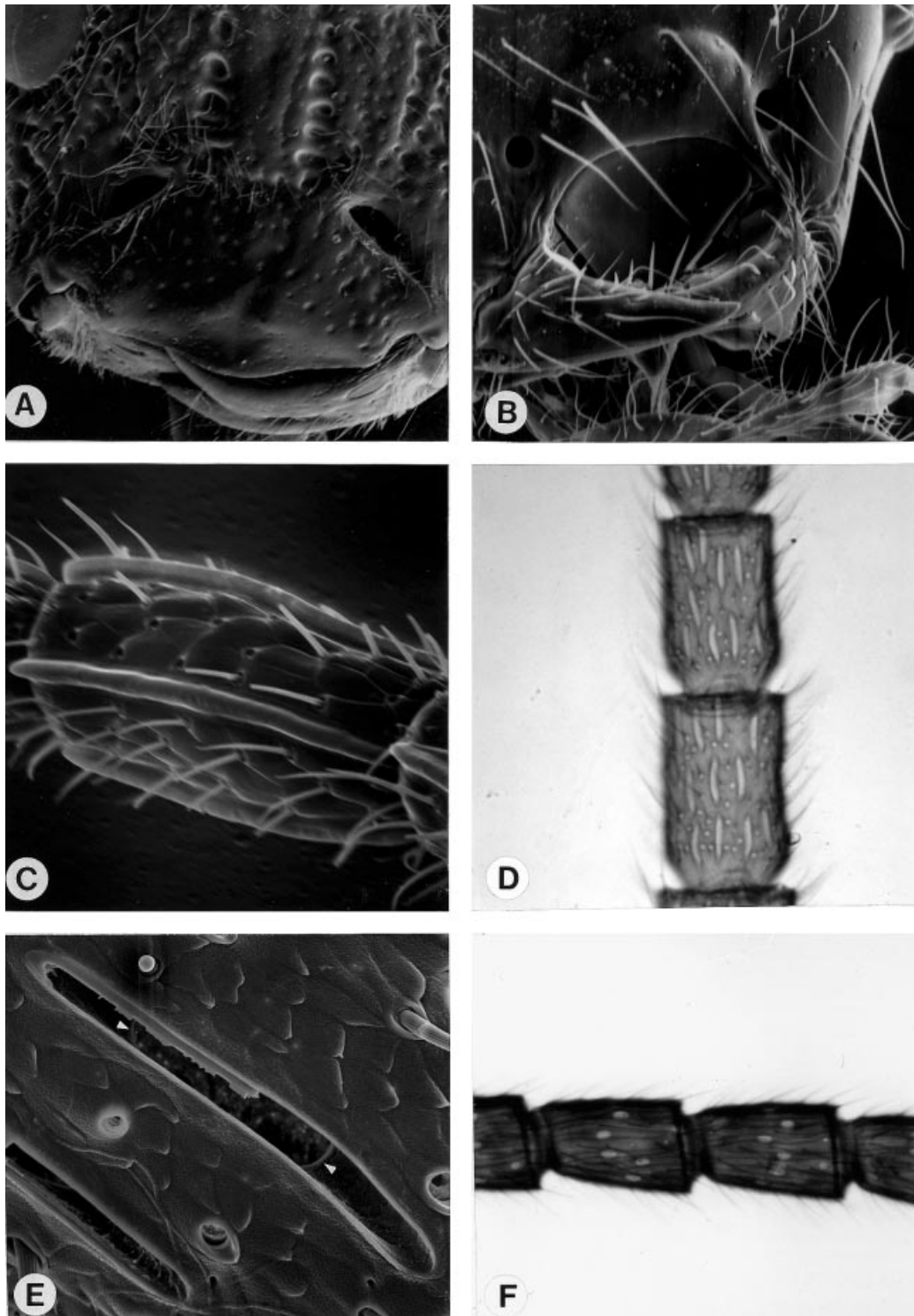


Fig. 1 Various features of mouthparts and antenna in extant Ichneumonoidea. — *A*. Frontal view of *Trachypetetus* sp. (noncyclostome, Trachypetinae).—*B*. *Rhyssalus* sp. (cyclostome, Rhyssalinae). —*C*. Entire multiporous plate sensilla in *Acrisis* sp. (Exothecinae). —*D*. Intermediate length internal aperture of the sensilla in *Labena* sp. (Labeninae) viewed in transmitted light. —*E*. Intermediate length internal aperture of the sensilla in *Megastylus* sp. (Oxytorinae) viewed with SEM from outside in a specimen in which the multiporous plate has been removed; arrow-heads indicate ends of aperture.—*F*. Short internal aperture of the sensilla in *Biosteres* sp. (Opiinae).

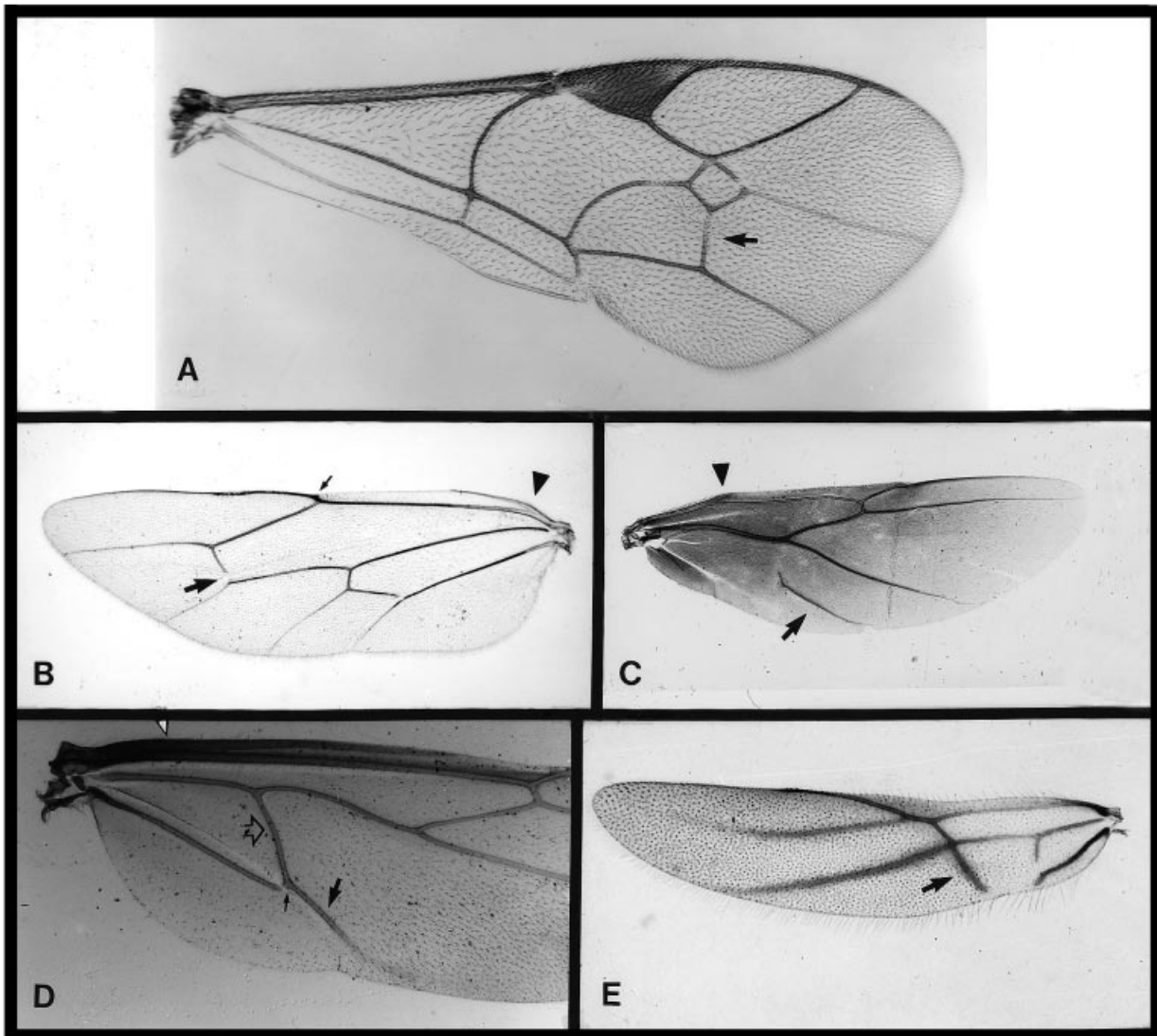


Fig. 2 Wing venation in various extant Ichneumonoidae. — *A*. Forewing of *Mesochorus* sp. (Mesochorinae); arrow showing vein 2 m-cu. — *B*. Hindwing of *Netelia* sp. (Tryphoninae); small arrow showing stub of vein C, large arrow showing bulla in vein r-m, arrow-head indicating the separation of vein SC + R from vein C close to wing base. — *C*. Hindwing of *Calcaribracon rostratus* (Braconinae); arrow showing conspicuous spurious vein in discal cell remote from remaining venation; arrow-head indicating the separation of vein SC + R from vein C well away from wing base. — *D*. Hindwing of *Trachypetius* sp. (Trachypetinae); white arrow-head indicating the separation of vein SC + R from vein C close to wing base, wide, open arrow showing vein 1-CU, solid black arrow showing 2-CU, small black arrow showing short vein cu-a just anterior to flexion line. — *E*. Hindwing of *Histeromerus* sp. (Histeromerinae); arrow showing vein 'm-cu' (see text for discussion).

(although only sperm of Agathidinae and Sigalphinae have been examined; Fig. 10D) and they are the only members of the Braconidae apart from the Trachypetinae (see above), Apozyginae and Protorhyssalinae (see below) that possess hindwing vein 2-CU emerging from 1-CU and cu-a (see Figs 2D, 3F). If possession both of 2-CU in this position and long, unmodified sperm are plesiomorphic, as indicated by outgroup comparison, then it is possible that

the agathidine group may have diverged from the base of the noncyclostome braconid clade before loss of this vein. Monophyly of the Agathidinae + Pselaphaninae + Sigalphinae is indicated by internal ovipositor sculpture features (Rahman *et al.* 1998) and molecular sequence data (Belshaw *et al.* 1998; Dowton *et al.* 1998; Belshaw & Quicke, unpublished observations).

The Apozyginae, known only from a handful of speci-

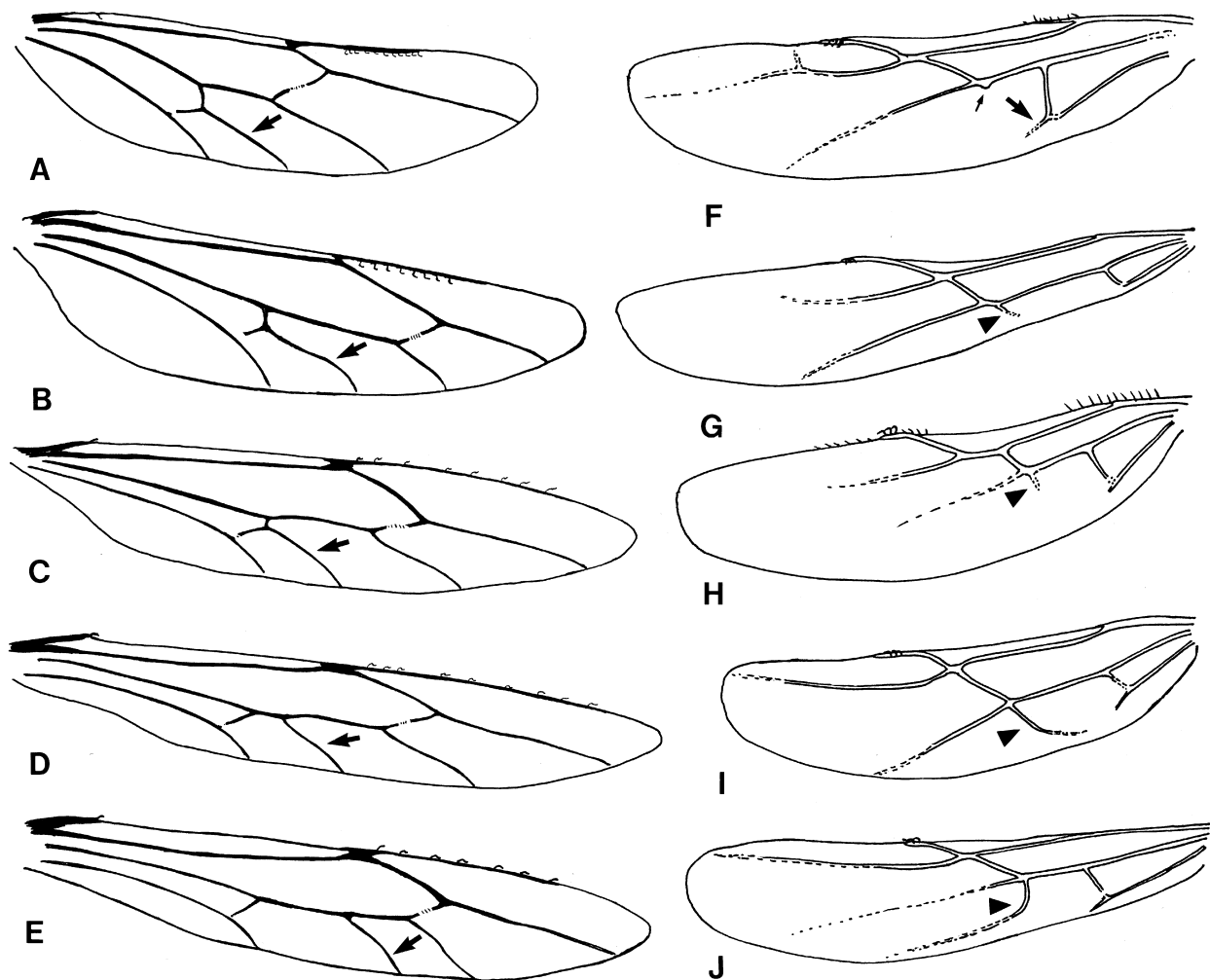


Fig. 3 Hindwing venation in various extant Ichneumonoidea. Arrows (A-F) showing vein 2-CU, arrow-heads showing vein 'm-cu' in Braconidae (see character 13 in character list for further discussion). Note the migration of 2-CU in *Apechoneura* species (C-E). —A. *Xorides* sp. (Xoridinae) —B. *Labena* sp. (Labeninae). —C. *Apechoneura nigritarsis* (Labeninae). —D. *A. longicauda* (Labeninae). —E. *A. minor* (Labeninae). —F. *Acampsis* sp. (Sigalphinae) —G. *Rhyssalus* sp. (Rhyssalinae) —H. *Dirrhope* sp. (Dirrhopinae). —I. *Yelicones* sp. (Rogadinae) —J. *Zombrus fuscipennis* (Doryctinae).

mens of a single species from Chile, were originally treated as a separate family because of their possession of transverse vein 2m-cu in the forewing (Mason 1978). Sharkey & Wahl (1992) regarded them simply as cyclostome braconids, and thus failed to consider the possibility that the presence of 2m-cu in these is actually plesiomorphic rather than derived/atavistic. We did not wish to make such an assumption.

The Rhyssalinae group (Rhyssalinae and Histeromerinae) were treated separately from the other cyclostome subfamilies. The Rhyssalinae group have been considered to display a largely plesiomorphic morphology and biology (but see Shaw 1995). Despite this, monophyly of the Rhyss-

alinae + Histeromerinae is supported by molecular data (Belshaw *et al.* 1998) and by their possession of a unique form of venom apparatus with a very long duct and small weakly sclerotized reservoir (Quicke *et al.* 1992c) (but see also Jimenez *et al.* 1997 who found a similar structure in *Cosmophorus*, a genus which is currently placed in the non-cyclostome subfamily Euphorinae).

The other cyclostome subfamilies; the Alysiinae, Betylobraconinae, Braconinae, Doryctinae, Exothecinae, Gnampodontinae, Hormiinae, Opiinae and Rogadinae are treated as a single entity, the monophyly of which is supported by molecular studies (Belshaw & Quicke 1997; Belshaw *et al.* 1998; Dowton *et al.* 1998). In addition, they all have a

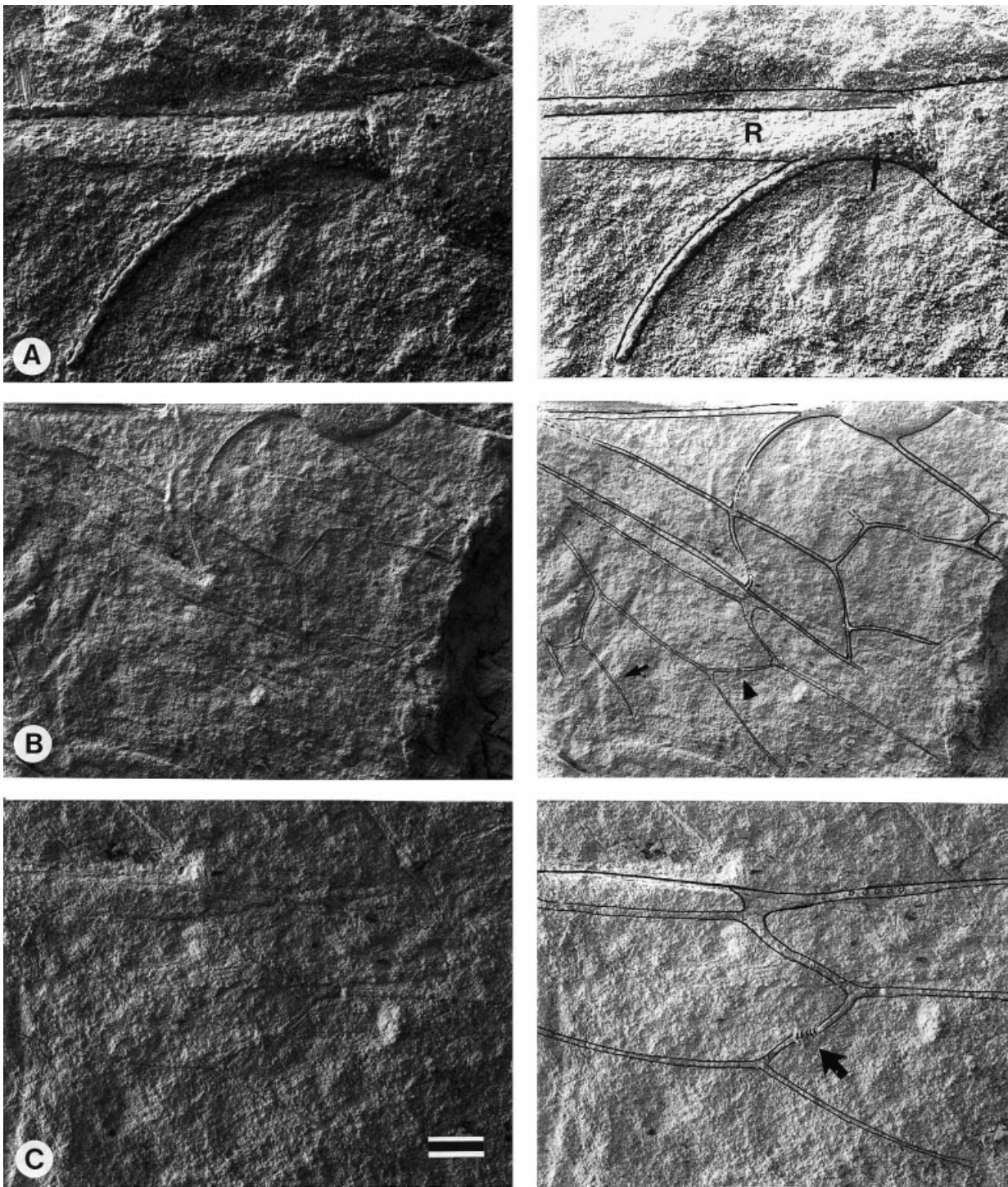


Fig. 4 Various wing features of *Tanychorella* impression fossil (PIN 35594582). Right hand lower contrast copies show our interpretations of features. —*A.* Showing forewing vein R contiguous with parastigma (arrow) (scalebar = 100 μ m). —*B.* Fore- and hindwing venation features, note that forewing vein 1-SR+M is absent (scalebar = 333 μ m), arrow indicates vein 2-CU, arrowhead indicates vein r-m with bulla. —*C.* Hindwing vein venation, vein r-m is far distal to junction of 1-SC+R & SC+R1 and with bulla (arrow) (PIN 35594582) (scalebar = 167 μ m).

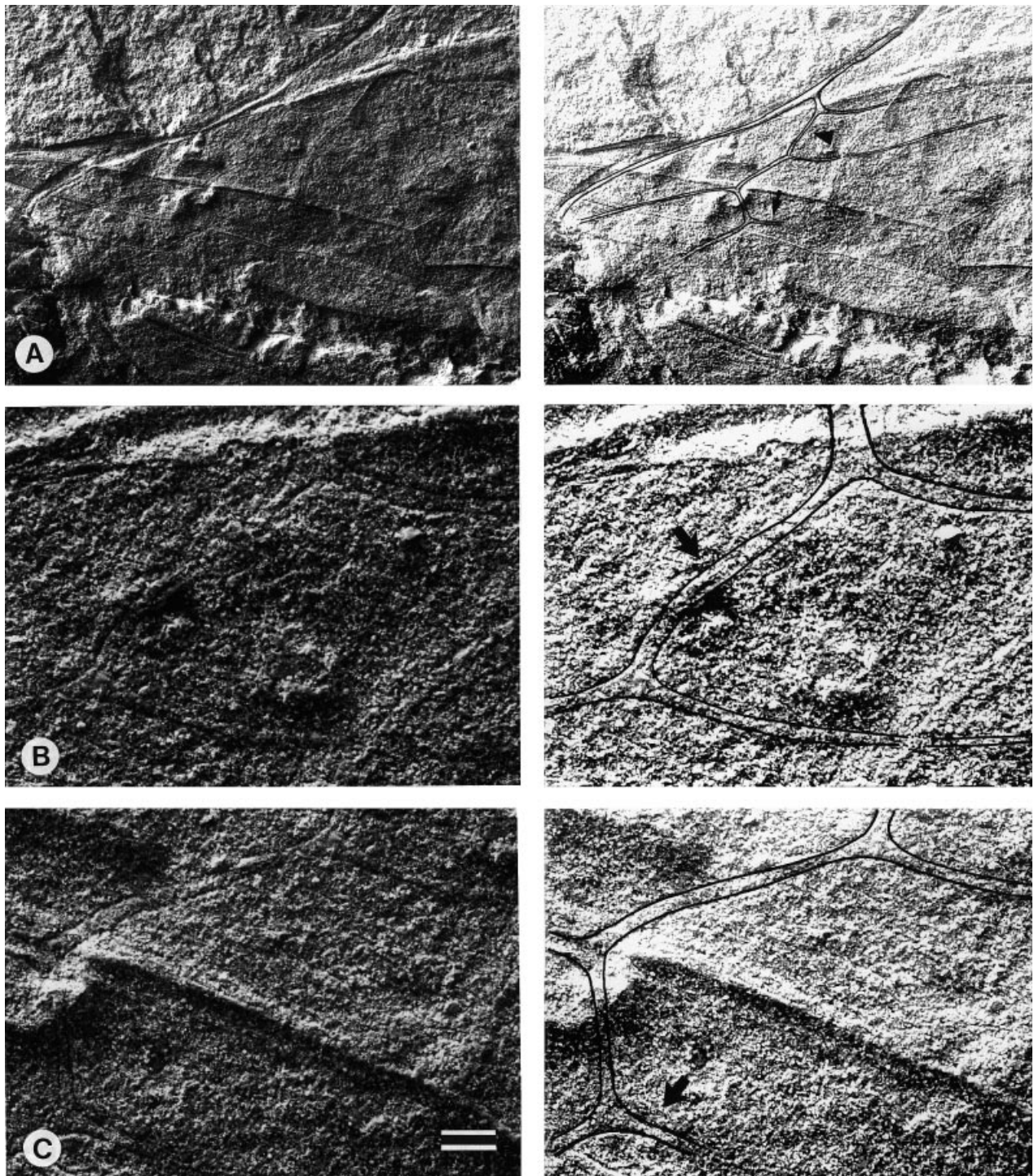


Fig. 5 Hindwing venation of *Cretobraconus mongolensis* (Eoichneumonidae). Right hand lower contrast copies show our interpretations of features — *A*. Hindwing venation, note the position of vein r-m (arrow-head) close to junction of 1-SC+R and SC+R1; arrow indicating vein 2-CU (scalebar = 175 mm).—*B*. Arrow indicating vein r-m without bulla (scalebar = 28.6 μ m). —*C*. Detail of *A*, arrow indicating vein 2-CU confluent with cu-a (scalebar = 32.3 μ m).

Table 1a Informative characters for intra-Ichneumonoidea relationships: character 13 expressing new interpretation of hindwing vein 2-CU (see character list).

Taxa	characters																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
hypothetical ancestor	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0
Praeichneumonidae	?	?	?	?	0	0	0	?	?	0	?	0	?	?	0	?	0	?	?	?	?	?	?
Eoichneumonidae	?	0	0	?	0	0	1	0	?	0	1	1	0	1	0	?	0*	?	?	?	?	?	?
<i>Tanychora</i>	?	0	0	?	0	0	1	0	?	0	1	0	0	?	?	?	?	?	?	?	?	?	?
<i>Tanychorella</i>	?	0	0	?	1	0	1	1	1	0	0	0	0	?	0	?	0	?	?	?	?	?	?
Paxylommatinae	0	0	2	1	1	0	2	1	1	0	0	1	?	1	1	1	0	0	?	1	1	1	0
Xoridinae	0	0	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	?	0	0	0	0
other Ichneumonidae	0	0	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0&1	1	0
Apozyginae	1	0	0	0	0	1	2	1	1	0	1	2	0	1	1	1	1	0	?	?	0	?	?
Protorhyssalinae	1*	1	?	0	0	0	2	0	0	1	1	2	0	1	1	1	1	?	?	?	?	?	?
Rhyssalinae group	1	1	1	0	0	1	2	1	1	1	1	2	1	1	1	1	1	0	0	2	0	1	1
other cyclostomes	1	1	1	0	0	1	2	1	1	1	1	2	1	1	1	1	1	0	0	1	0	0	1
Aphidiinae	0&1	1	2	1	0&1	1	2	1	1	1	1	2	?	1	1	1	1	1	1	2	1	0&1	1
Trachypetinae	0	0	0	0	0&1	0	1	1	0	1	1	1	0	1	0	1	1	1	?	1	1	0	0
Agathidinae group	0	0	0	0	0&1	1	2	1	0	1	1	2	0	1	1	1	1	1	0	1	1	1	1
other non-cyclostomes	0	0	0	0	0	0	1&2	0	0	1	1	2	?	1	1	1	1	1	2	1	1	1	1

*character state assignments marked with an asterisk are not absolutely certain and analyses were also performed with the alternative coding.

Table 1b Informative characters for intra-Ichneumonoidea relationships: characters 13A and 13B expressing new interpretation of hindwing vein 2-CU (see character list).

Taxa	character																							
	1	2	3	4	5	6	7	8	9	10	11	12	13A	13B	14	15	16	17	18	19	20	21	22	23
hypothetical ancestor	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	
Praeichneumonidae	?	?	?	?	0	0	0	?	?	0	?	0	?	1	?	0	?	0	?	?	?	?	?	?
Eoichneumonidae	?	0	0	?	0	0	1	0	?	0	1	1	0	1	1	0	?	0*	?	?	?	?	?	?
<i>Tanychora</i>	?	0	0	?	0	0	1	0	?	0	1	0	0	1	?	?	?	?	?	?	?	?	?	?
<i>Tanychorella</i>	?	0	0	?	1	0	1	1	1	0	0	0	0	1	?	0	?	0	?	?	?	?	?	?
Paxylommatinae	0	0	2	1	1	0	2	1	1	0	0	1	0	1	1	1	1	0	0	?	1	1	1	0
Xoridinae	0	0	0	1	1	0	1	1	1	0	0	0	0	1	0	0	0	0	0	?	0	0	0	0
other Ichneumonidae	0	0	0	1	1	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0&1	1	0
Apozyginae	1	0	0	0	0	1	2	1	1	0	1	2	0	1	1	1	1	1	0	?	?	0	?	?
Protorhyssalinae	1*	1	?	0	0	0	2	0	0	1	1	2	0	1	1	1	1	1	?	?	?	?	?	?
Rhyssalinae group	1	1	1	0	0	1	2	1	1	1	1	2	1	0	1	1	1	1	0	0	2	0	1	1
other cyclostomes	1	1	1	0	0	1	2	1	1	1	1	2	1	0	1	1	1	1	0	0	1	0	0	1
Aphidiinae	0&1	1	2	1	0&1	1	2	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1	0&1	1
Trachypetinae	0	0	0	0	0&1	0	1	1	0	1	1	1	0	1	1	0	1	1	1	?	1	1	0	0
Agathidinae group	0	0	0	0	0&1	1	2	1	0	1	1	2	0	0	1	1	1	1	1	0	1	1	1	1
other non-cyclostomes	0	0	0	0	0	0	1&2	0	0	1	1	2	1	1	1	1	1	1	1	2	1	1	1	1

*character state assignments marked with an asterisk are not absolutely certain and analyses were also performed with the alternative coding.

highly muscularised venom gland reservoir as found elsewhere only in some Aphidiinae and Trachypetinae among the Braconidae.

The Protorhyssalinae (Basibuyuk *et al.* 1999) differs from all other braconid subfamilies apart from the Apozyginae in both being cyclostome and in possessing hindwing vein 2-CU. The forewing venation with a large five sided second submarginal cell distinguishes it from all other non-cyclostome braconids that possess hindwing vein 2-CU apart from the Trachypetinae. The Trachypetinae

differ from the Protorhyssalinae in having veins C and SC + R of the hind wing separate from near the base of the wing, and in having a distinctly apically open forewing costal cell.

Ichneumonidae. We broke down the Ichneumonidae into only four component taxa. Within the Ichneumonidae, there has been far less work in terms of comprehensive phylogenetic analyses of large numbers of taxa, though a number of studies have been presented on subsets of subfamilies, and a general informal classifica-

Table 2 Summary of data set permutations subjected to analysis.

Analysis	Protorhyssalus	T2 + T3 articulation type scored for eioichneumonids	HW vein 2-CU scoring for noncyclostomes	HW vein m-cu scoring for non-cyclostomes
1	cyclostome	ichneumonid	N/A	—
2	cyclostome	ichneumonid	present	—
3	cyclostome	ichneumonid	—	absent
4	cyclostome	ichneumonid	—	present
5	cyclostome	braconid	N/A	—
6	cyclostome	braconid	present	—
7	cyclostome	braconid	—	absent
8	cyclostome	braconid	—	present
9	non-cyclostome	ichneumonid	N/A	—
10	non-cyclostome	ichneumonid	present	—
11	non-cyclostome	ichneumonid	—	absent
12	non-cyclostome	ichneumonid	—	present
13	non-cyclostome	braconid	N/A	—
14	non-cyclostome	braconid	present	—
15	non-cyclostome	braconid	—	absent
16	non-cyclostome	braconid	—	present

tion has been developing (see Gauld 1997), though there are many problems remaining in recognizing monophyletic groups of ichneumonid subfamilies. The studies, together with molecular data (Belshaw *et al.* 1998) have revealed the existence of a major grouping (the Pimpliformes) which includes among others many of the subfamilies with putatively underived biologies (ectoparasitic idiobionts of xylophagous hosts). However, the placement of the Xoridinae, a group with similarly underived biology, was not clear, although molecular evidence suggests that it does not belong to any of the previously recognized groupings of subfamilies and may be one of the most basal groups along with the Paxylommatinae (Belshaw *et al.* 1998; Quicke *et al.* 1999). We have therefore treated the Xoridinae separately.

The Paxylommatinae (= Hybrizontinae) are a peculiar group associated with ants, though their biology is unknown. Their placement with respect to the Ichneumonidae and the Braconidae has been discussed by several authors (Rasnitsyn 1980; Mason 1981; van Achterberg 1984; Gauld 1984; Sharkey & Wahl 1992). Because of the lack of forewing transverse vein 2 m-cu in extant genera they have at times been regarded as belonging to the Braconidae rather than the Ichneumonidae, though their placement in the latter is indicated by several characters including the presence of vein 2m-cu in fossil representatives (Kasparyan 1988). Nevertheless, other features of wing venation are not typical of ichneumonids (e.g. position of hind wing vein r-m: see character 12 below), and sequence data from the D2 region of the nuclear 28S rDNA gene (Belshaw *et al.* 1998; Quicke *et al.* 1999) is very different from that of other extant ichneumonids. Analysis of the paxylommatine 28S sequence with those of

a limited subset of other ichneumonids and braconids suggests that they may be the most basal ichneumonid or even a braconid, but caution should be exercised in interpreting these results given the long branch length leading to *Hybrizon* (Paxylommatinae) compared with those of the other ichneumonids included, i.e. there is a possibility of long branch attraction/distortion in the analyses. We therefore treated Paxylommatinae separately.

It has also been suggested that the Labeninae (and Brachycyrtinae) are potentially basal with a Gondwanan distribution and a presumed plesiomorphic biology. (Gould 1985) However, molecular data provide little support for this (Quicke *et al.*, in press) and therefore we have not treated them separately here.

The fossil genus *Tanychorella* cannot be assigned to any extant subfamily of Ichneumonidae and we have treated it separately. It differs principally from extant ichneumonids in having a particularly large second submarginal cell.

Apart from the above three taxa (Xoridinae, Paxylommatinae and *Tanychorella*) we have therefore treated the remaining Ichneumonidae as a single taxon 'other Ichneumonidae', although there is not unambiguous evidence that this is a monophyletic group, and the possibility that both Xoridinae and Paxylommatinae may belong within that group cannot be excluded. Despite the fact that the Agriotypinae has often been afforded family status, this subfamily appears to be just a highly derived ichneumonid, specialized for parasitising caddis fly larvae and possibly closely related to the Cryptinae, and is not therefore treated separately in our analyses.

Eioichneumonidae and Tanychora. The Eioichneumonidae and *Tanychora* both have wing venation features absent from extant braconids and ichneumonids (Figs 5, 8A,

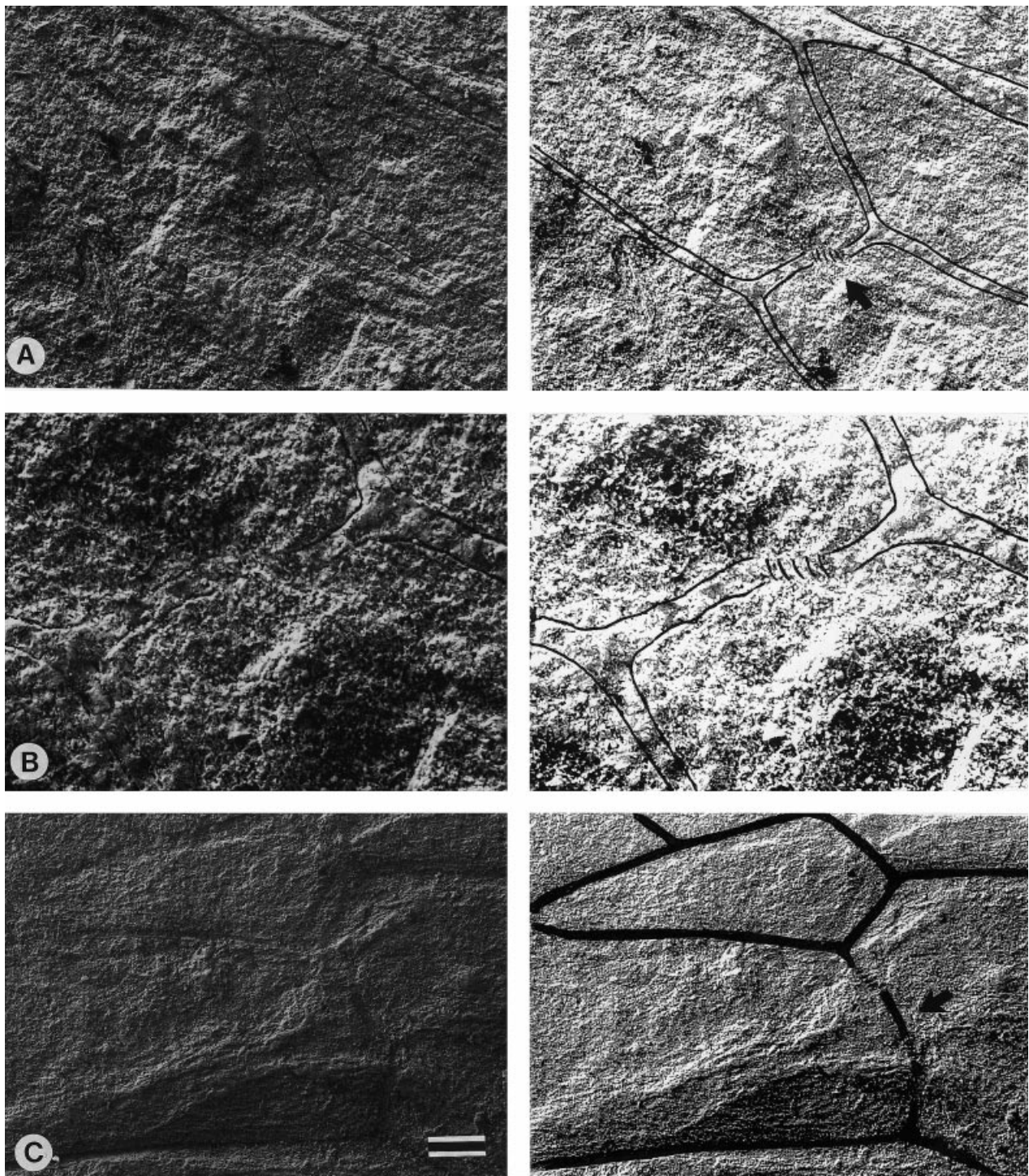


Fig. 6 Some features of hind and fore wing of *Tanychora petiolata*. Right hand lower contrast copies show our interpretations of features —*A*. Showing the position of hindwing vein r-m and bulla (arrow) (scalebar = 71.4 μ m). —*B*. Detail of hindwing vein r-m, note the distinctive cross-striation of the bulla (scalebar = 30.3 μ m). —*C*. Forewing vein 2m-cu with two bullae separated by tubular vein (arrow); note folds of flexion lines are also visible (scalebar = 125 μ m).

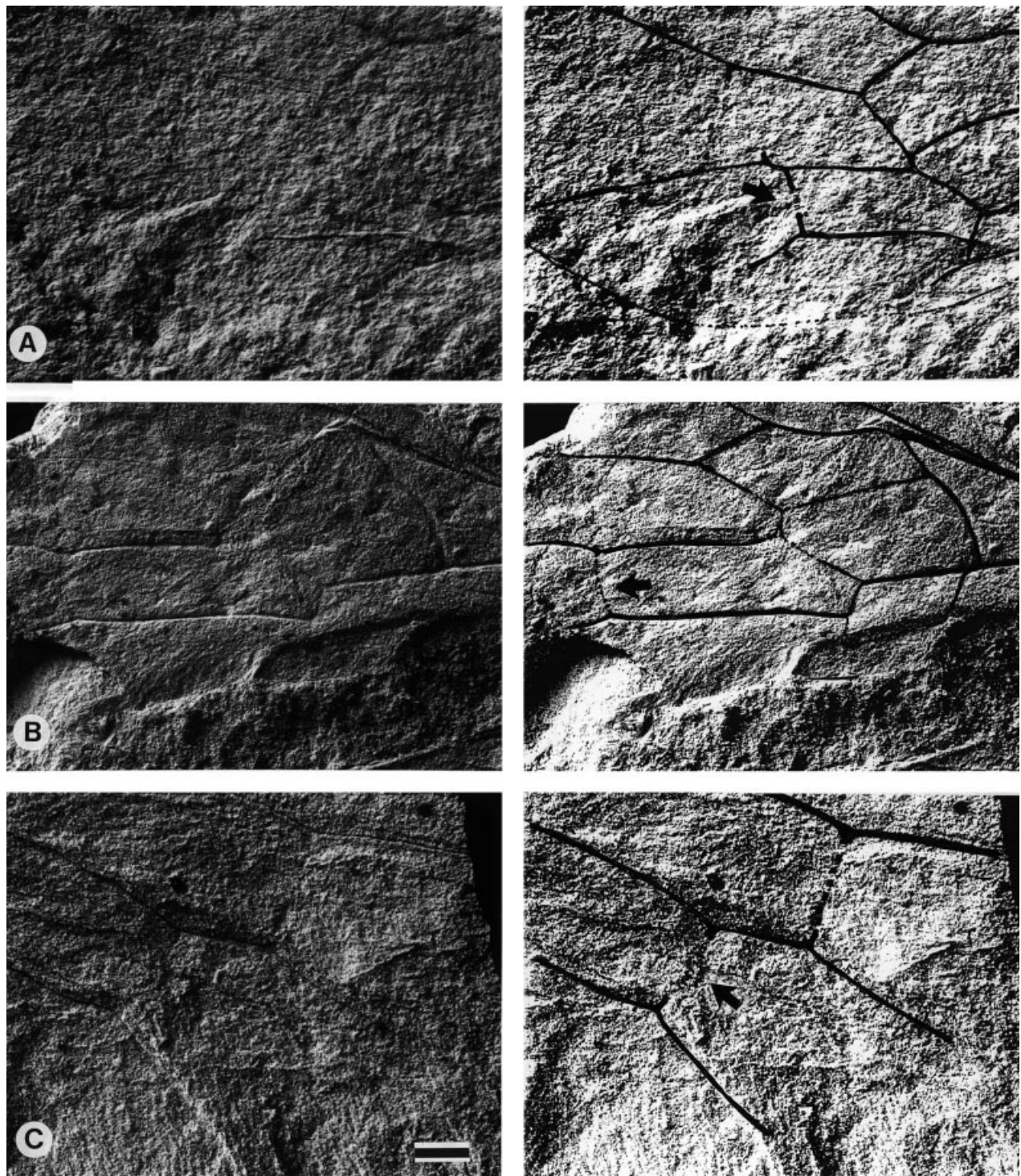


Fig. 7 Forewing vein 2m-cu of Eoichneumonidae. Right hand lower contrast copies show our interpretations of features. —*A. Archobraconus pallidus* showing a relatively short bulla in vein 2 m-cu (arrow) (scalebar = 167 μ m). —*B*, —*C. Cretobraconus mongolensis* showing a broad median bulla (arrows) (scalebars = 192 and 90.9 μ m, respectively).

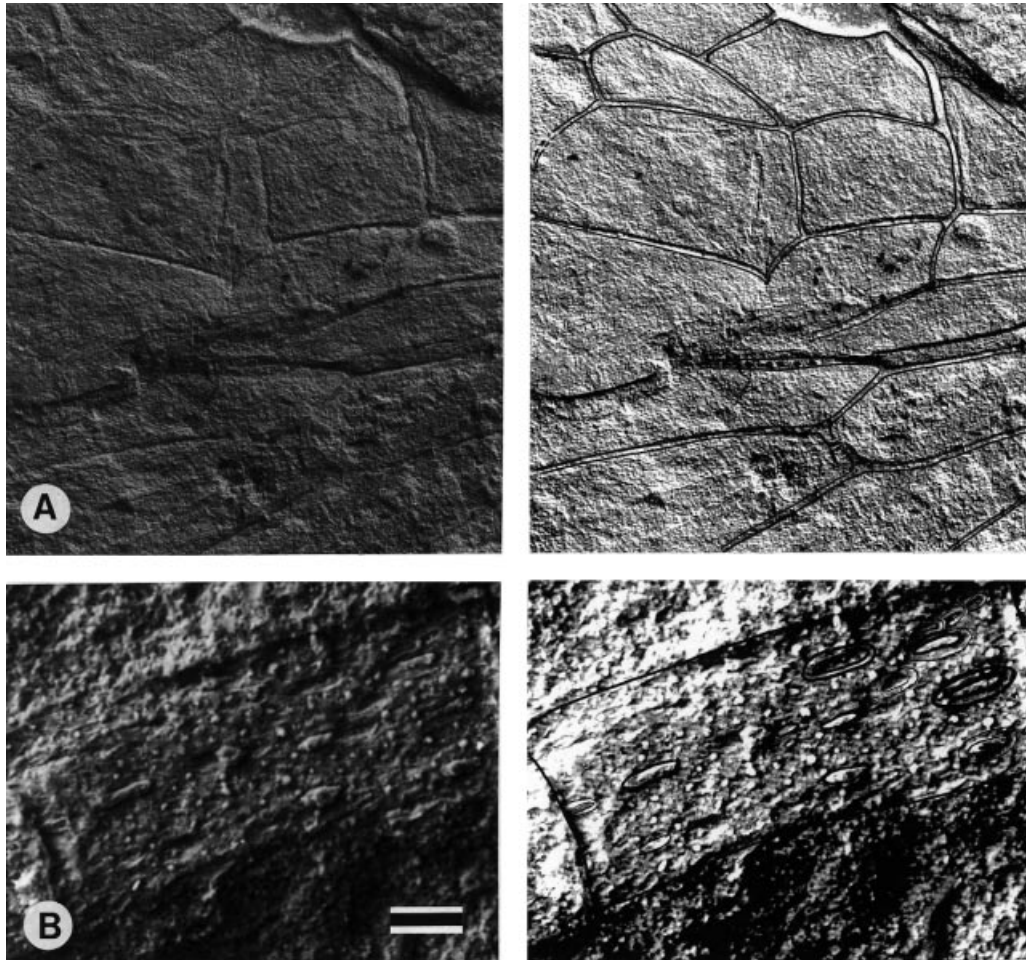


Fig. 8 Wing venation and multiporous plate sensilla in *Tanychora petiolata*. Right hand lower contrast copies show our interpretations of features. —A. Details of fore- and hindwing venation (scalebar = 303 μm). —B. Multiporous plate sensilla with intermediate length internal aperture (scalebar = 33.3 μm).

respectively). Eoichneumonids have venation very similar to that of Apozyginae but have hind wing vein r-m more or less level with the separation of 1-SC + R and SC + R1. *Tanychora* has a large and oblique 2nd submarginal cell and retains a complete vein 1-SR + M. The four known genera of Eoichneumonidae (Jell & Duncan 1986; Rasnitsyn & Sharkey 1988) were treated separately and monophyly of the family was supported in the analysis of Sharkey & Wahl (1992) albeit on the basis of one character. As the four genera of Eoichneumonidae do not vary for the characters used here and appear relatively homogeneous in terms of wing venation and habitus we treated this family as a single taxon.

Terminology

Terminology follows van Achterberg (1979).

Material examined

The present study included evidence from both extant and extinct taxa, the latter represented by both amber and impression fossils including some that are newly described (Basibuyuk *et al.* 1999). Results are chiefly gathered by examination of the material in The Natural History Museum, London. Lists of examined material are not presented for the extant groups, although efforts have been made to examine characters in a full range of the component taxa in each case. Fossil material is referred to by accession number: Palaeontological Institute, Moscow (PIN number) and American Museum of Natural History, New York (AMNH number). The fossil material comes from Lower Cretaceous strata, Baissa, Transbaikalia, unless otherwise stated.

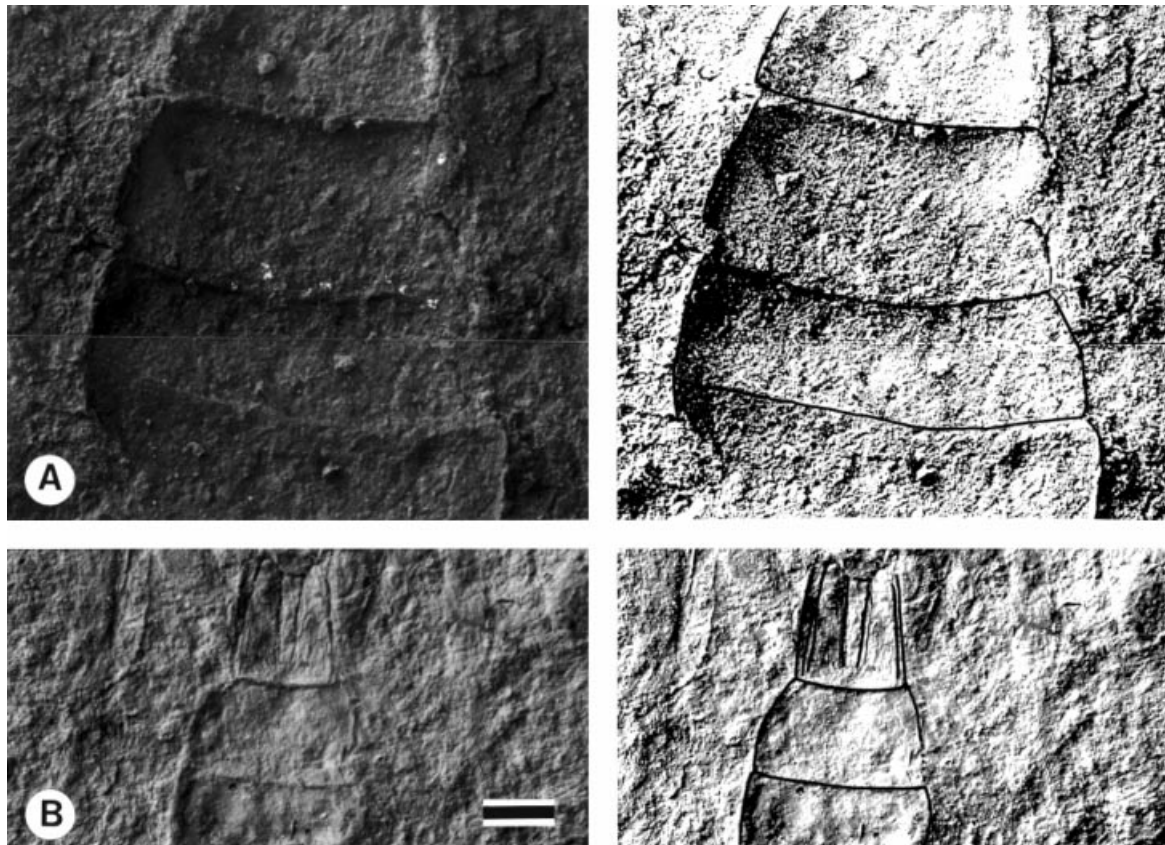


Fig. 9 Articulation of metasomal tergites 2 and 3 in *Eoichneumonidae*. Right hand lower contrast copies show our interpretations of features. —A. *Cretobraconus maculatus* (scalebar = 172 µm). —B. *Archobraconus oculatus*, note well developed dorsal carinae on 1st metasomal tergite (scalebar = 166.7 µm).

Fossil material

Praeichneumonidae: *Praeichneumon townesi* Rasnitsyn, F, Khutel-Khara, Mongolia, Lower Cretaceous (PIN 3965/416); undet. gen. sp., Tuzga, Transbaicalia, Lower Cretaceous (PIN 1742/138).

Eoichneumonidae: *Archobraconus* sp. (PIN 3064/1988); *Archobraconus* sp. (PIN 4210/1153); *A. caudatus* Rasnitsyn & Sharkey (PIN 3064/1193); *A. imperfectus* Rasnitsyn & Sharkey (PIN 3064/1190); *A. oculatus* Rasnitsyn & Sharkey (PIN 3064/1192); *A. pallidus* Rasnitsyn & Sharkey (PIN 4210/1157); *A. microphthalmus* Rasnitsyn & Sharkey (PIN 3064/1194); *Bassiobracon striatus* Rasnitsyn & Sharkey (PIN 4210/1150); *Cretobraconus* sp., Khotont, Mongolia (PIN 4307/14); *C. antennatus* Rasnitsyn & Sharkey (PIN 4210/1151); *C. brachyurus* Rasnitsyn & Sharkey (PIN 4210/1148); *C. maculatus* Rasnitsyn & Sharkey, Khutulii-Khira, Mongolia, Lower Cretaceous (PIN 4288/261); *C. micron* Rasnitsyn & Sharkey (PIN 4210/1149); *C. mongolensis* Rasnitsyn & Sharkey, Khotont, Mongolia (PIN 4307/2); *C. pusillus* Rasnitsyn

& Sharkey (PIN 3064/1991); *C. robustus* Rasnitsyn & Sharkey (PIN 3064/1989); gen. sp. (PIN 3064/1983); gen. sp., Mongolia (4306/95).

Genus Tanychora: *Tanychora* sp. (PIN 4210/5248); *T. petiolata* Townes, Zaza, Transbaicalia (PIN 292/8); *T. sessilis* Townes (PIN 1989/2594).

Genus Tanychorella: *Tanychorella parvula* Rasnitsyn, Romanovca, Transbaicalia (PIN 3002/6); *Tanychorella* sp., Bon Tsagan, Mongolia (PIN 3559/663 = 680); *Tanychorella* sp., Bon Tsagan, Mongolia (PIN 3559/4488); *Tanychorella* sp., Bon Tsagan, Mongolia (PIN 3559/4489); *Tanychorella* sp., Bon Tsagan, Mongolia (PIN 3559/4490); *Tanychorella* sp., Bon Tsagan, Mongolia (PIN 3559/4582); *Tanychorella* sp., Bon Tsagan, Mongolia (PIN 3559/4497); *Tanychorella* sp., Bon Tsagan, Mongolia (PIN 3559/4492).

Braconidae: *Protorhyssalinae*: *Protorhyssalus goldmani* Basibuyuk & Quicke, 7 specimens from New Jersey amber, Middlesex Co. Sayreville, White Oaks Pit, Late Cretaceous (AMNH NJ-243, NJ-269, NJ-427 A, NJ-427B, NJ-427E).

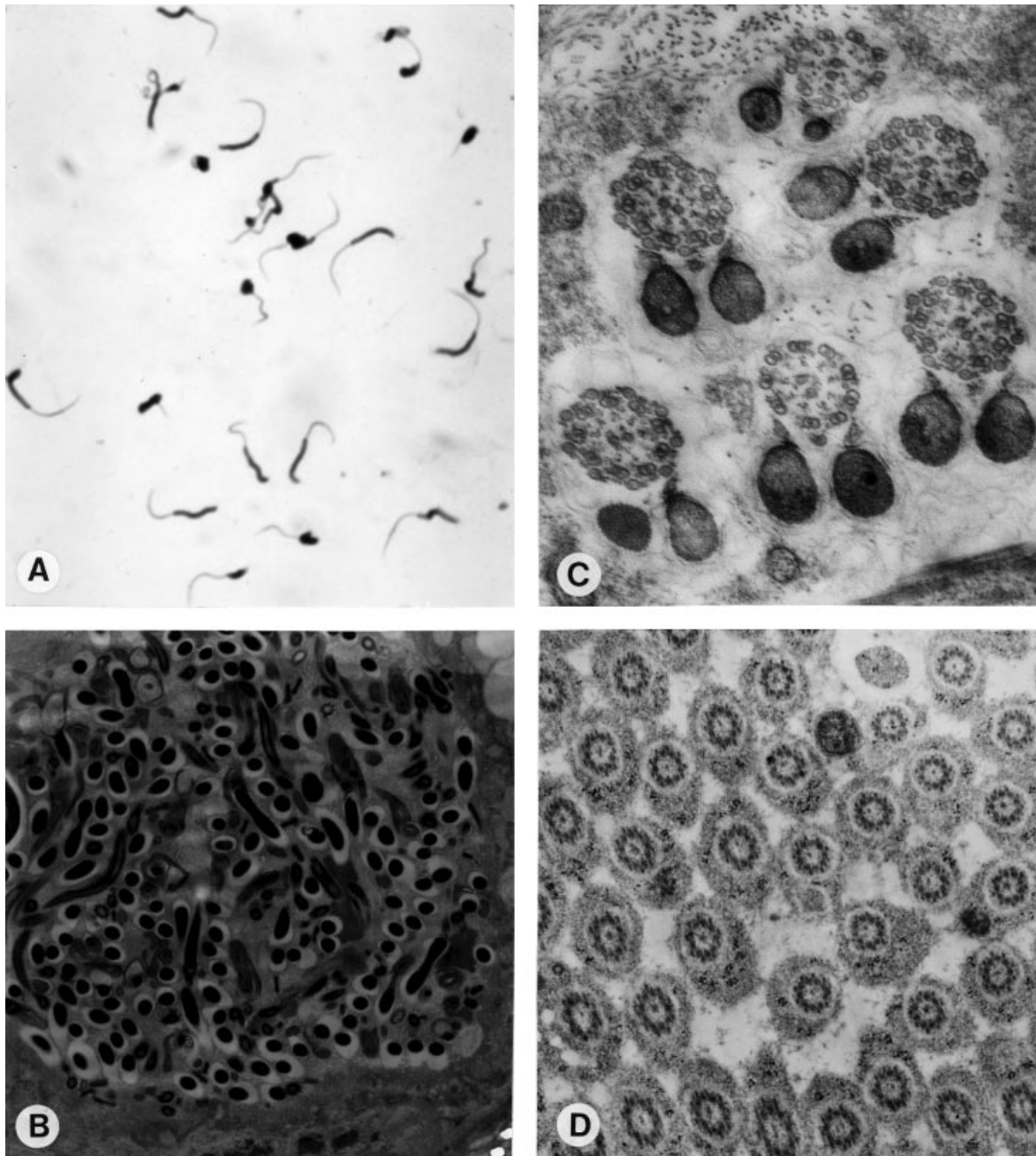


Fig. 10 Sperm structure and ultrastructure in various Braconidae. —*A.* Microgastrinae gen. sp., toluidine blue-stained derived sperm with reduced tails. —*B.* Microgastrinae gen. sp., transmission electron micrograph of section through vas deferens showing high proportion of nucleus-level sections resulting from the large proportion of sperm length occupied by this organelle. —*C.* *Aleiodes* sp. (Rogadinae) transmission electron micrograph of section through sperm in vas deferens showing underderived axoneme and mitochondrial derivatives. —*D.* *Bassus* sp. (Agathidinae) transmission electron micrograph of section through sperm in testes showing derived axonemes with predominantly a single central microtubule.

Analysis

Two basic data matrices are presented (Table 1a, b), which differ in the interpretation of hindwing veins 2-CU and m-cu. Because of uncertainty about the scoring of some other

characters, either because of difficulty of being certain about the condition present in fossil taxa (cyclostome or non-cyclostome and the type of articulation between the 2nd and 3rd metasomal tergites) or because of alternative interpreta-

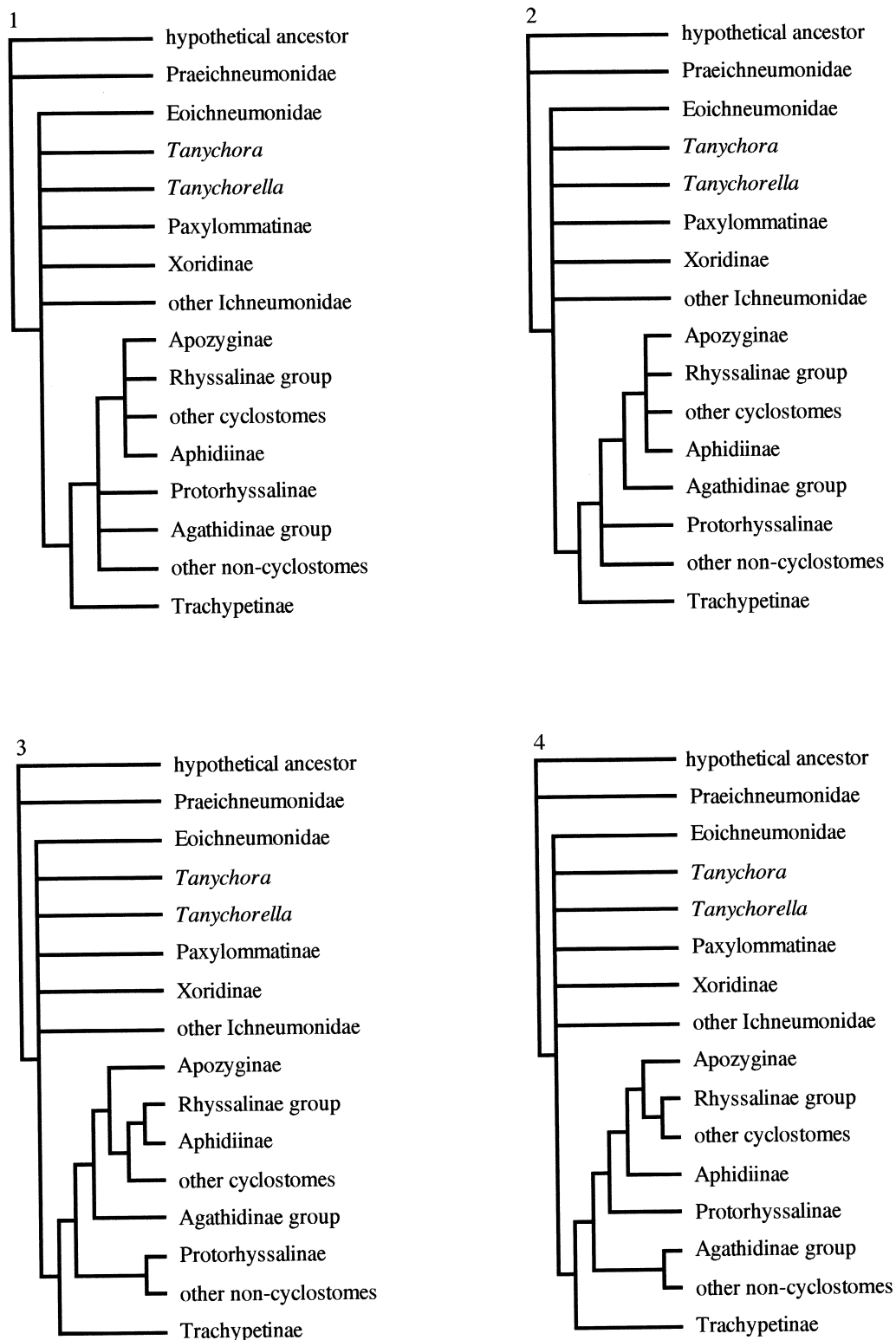


Fig. 11 Strict consensus trees 1–4 obtained from various analyses (see Tables 2–4).

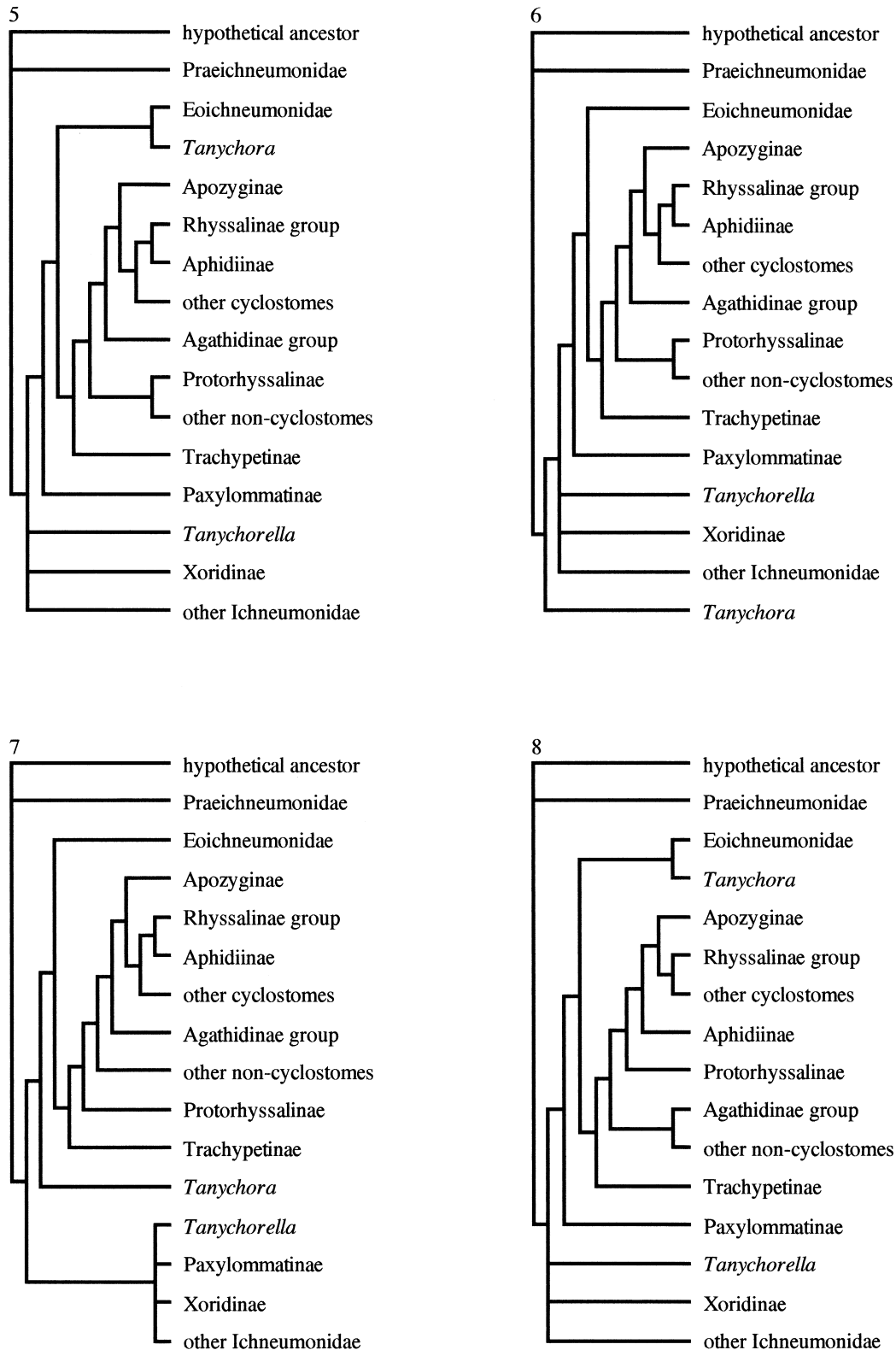


Fig. 12 Strict consensus trees 5–8 obtained from various analyses (see Tables 2–4).

Table 3 Results of analyses of dataset permutations before and after successive approximations weighting.

	Length of unweighted: MP trees	No. MP unweighted trees	Unweighted: MP tree set no. (RI/RC)	Rewighted RC-max: Tree set no. (RI/RC)	Rewighted RC-min: Tree set no. (RI/RC)	Rewighted RI-max: Tree set no. (RI/RC)	Rewighted RI-min: Tree set no. (RI/RC)
Analysis 1	56	95	1 (747/467)	3 (9008/6979)	6 (9097/7217)	3 (8389/5576)	6 (8404/5812)
Analysis 2	57	95	1 (7381/4532)	3 (8941/6758)	6 (9025/6987)	3 (8306/5586)	6 (8319/5618)
Analysis 3	60	120	1 (7241/4345)	3 (8934/6734)	6 (9019/6955)	3 (8306/5571)	6 (8320/5603)
Analysis 4	61	20	1 (7191/4244)	3 (8934/6734)	6 (9019/6955)	4 (8050/5157)	6 (8320/5603)
Analysis 5	56	95	1 (7439/4649)	3 (8986/6962)	6 (9079/7199)	3 (8361/5757)	6 (8376/5793)
Analysis 6	57	95	1 (7349/4513)	3 (8916/6740)	6 (9003/6965)	3 (8227/5564)	6 (8290/5599)
Analysis 7	60	120	1 (7209/4326)	3 (8910/6715)	6 (8995/6936)	3 (8278/5552)	6 (8291/5584)
Analysis 8	61	20	1 (7159/4225)	3 (8910/6715)	6 (8995/6936)	4 (8018/5317)	6 (8291/5584)
Analysis 9	55	50	2 (7561/4812)	3 (9049/7103)	6 (9140/7349)	3 (8467/5952)	6 (8484/5992)
Analysis 10	59	75	2 (7470/4669)	3 (8982/6886)	6 (9070/7119)	3 (8384/5759)	6 (8399/5795)
Analysis 11	59	75	2 (7326/4470)	3 (8975/6860)	6 (9062/7089)	3 (8383/5742)	6 (8398/5777)
Analysis 12	60	5	7 (7273/4364)	5 (9004/6943)	5 (9004/6943)	7 (8091/5219)	7 (8091/5219)
Analysis 13	55	50	2 (7531/4792)	3 (9027/7086)	6 (9119/7332)	3 (8441/5934)	6 (8458/5974)
Analysis 14	56	50	2 (7439/4649)	3 (8959/6866)	6 (9048/7102)	3 (8357/5740)	6 (8372/5776)
Analysis 15	59	75	2 (7294/4451)	3 (8952/6842)	6 (9039/7071)	3 (8356/5723)	6 (8371/5758)
Analysis 16	60	5	7 (7241/4345)	5 (8979/6925)	5 (8979/6925)	7 (8059/5198)	7 (8059/5198)

Table 4 Results of application of largest number of characters with CI = 1 for arbitrarily selecting preferred subset of equally parsimonious trees.

	No. trees	Max no. chars with CI = 1	No. of trees with max no. chars with CI = 1	Tree set no.
Analysis 1	56	8	10	3
Analysis 2	57	7	10	3
Analysis 3	60	7	10	3
Analysis 4	61	6	5	4
Analysis 5	56	8	10	3
Analysis 6	57	7	10	3
Analysis 7	60	7	10	3
Analysis 8	61	7	10	8
Analysis 9	55	9	10	3
Analysis 10	59	8	10	3
Analysis 11	59	8	10	3
Analysis 12	60	6	5	7
Analysis 13	55	9	10	3
Analysis 14	56	8	10	3
Analysis 15	59	8	10	3
Analysis 16	60	6	5	7

tions of the significance of hindwing vein m-cu (see character 13 below), 16 different matrices were analysed; differences are summarised in Table 2. Each matrix was analysed using maximum parsimony with the branch and bound option of paup 3.1 (Swofford 1993), which guarantees finding all or most parsimonious trees. Strict consensus trees were constructed from each of the sets of most parsimonious trees obtained. A hypothetical ancestor was assigned as the outgroup for rooting purposes as well as including the Praeichneumonidae (see Selection of taxa, above).

Each set of trees obtained from the unweighted data in these 16 initial analyses was then subjected to four different successive approximations weighting procedures, using the maximum and minimum values of both the retention index (RI) and the rescaled consistency index (RC), giving a total of 80 separate analyses. It is expected that relationships suggested by use of the minimum values of these indices would be most well supported as those characters contributing most to the reweighted analyses would have performed 'well' in all of the input trees. We generally prefer the RI as the weighting function, as the RC is the RI multiplied by the consistency index and the latter can be a non-zero for characters that are completely homoplastic whilst intermediately homoplastic characters can be given very low weights — results of using RC are included, however, because that is the most widely used reweighting function.

The optimal character compatibility index (OCCI) (Rodrigo 1992) was used as an objective criterion for the selection of a preferred subset of trees from each of the different consensus groups obtained. This criterion prefers those trees with the largest proportion of characters that have a consistency index of 1. Strict consensus trees were also constructed from these preferred subsets.

Character coding

Although some of our characters are unambiguously scorable for all the taxa in which they can be observed, some are polymorphic within some groups. We therefore used, where possible, an interpretation of the groundplan condition for each group. In the case of wing veins, we generally took the presence of at least one member to indicate that the vein has been subsequently lost in others. In some cases, existing phylogenies enabled us to identify some conditions as derived within a particular group, e.g. some members of the Opiinae, Betylobraconinae and all Alysiinae are non-cyclostome morphologically, but as these braconid subfamilies are believed to be independently derived within the cyclostome clade (see Quicke & Belshaw in press), we considered the absence of cyclostome mouthparts in these to be reversal or loss.

Character list

1. Mouthparts: 0 = not cyclostome (Fig. 1A); 1 = cyclostome (having lower part of clypeus recessed and labrum largely exposed, usually glabrous and concave) (Fig. 1B). Some species of Aphidiinae appear distinctly cyclostome but whether this is the same character state as in the other cyclostome taxa is questionable — for the present study we scored them as polymorphic. Preservation of the fossil Protorhyssalinae (Basibuyuk *et al.* 1999) is not sufficiently good for us to be unambiguously certain about its character state, and although we tend towards concluding they are cyclostome; we have therefore performed separate analyses with them scored either as cyclostome or not.
2. Multiporous plate sensilla of antenna: 0 = not entire, occupying less than 0.6 length of flagellomere (Fig. 1D-E); 1 = entire, occupying more than 0.7 length of flagellomere (Fig. 1C,F).
3. Internal aperture of multiporous plate sensilla: 0 = intermediate, up to $0.67 \times$ length of external multiporous plate (Fig. 1D,E); 1 = very short, up to 0.2 length of external multiporous plate (Fig. 1F); 2 = large, occupying almost entire length of external multiporous plate. Barlin & Vinson (1981) first reported this character, suggesting its potential for braconid systematics, and it was subsequently included in Quicke's 1994 phylogenetic analysis. We treated this character as unordered because although the states may seem to form a logical progression, lack of intermediates and almost total lack of a septum (internal floor) in some taxa (see Borden *et al.* 1978) displaying state 2 could indicate that loss of the septum might not simply be a matter of increasing the length of the aperture.
4. Number of maxillary palp segments (in groundplan): 0 = six; 1 = five.
5. Forewing vein 1-SR + M: 0 = present (Figs 7A,B, 8A); 1 = absent (Figs 2A, 4B).
6. Forewing vein R and parastigma: 0 = contiguous with trachea not diverted (Figs 2A, 4A); 1 = separate, or if still with weak sclerotized connection, trachea often diverted and entering parastigma more distally. State 1 was suggested as a synapomorphy of the Agathidiinae by Sharkey (1986); however, it is also displayed by cyclostome braconids as well as the other members of the Agathidiinae subfamily group (Sigalphinae and Pselaphaninae).
7. Forewing veins C + SC and R: 0 = separate, a costal cell present along whole length; 1 = merged (a narrow costal cell may be present apically only) but a distinct groove between C + SC and R is present in dorsal profile (Fig. 2A); 2 = completely fused.

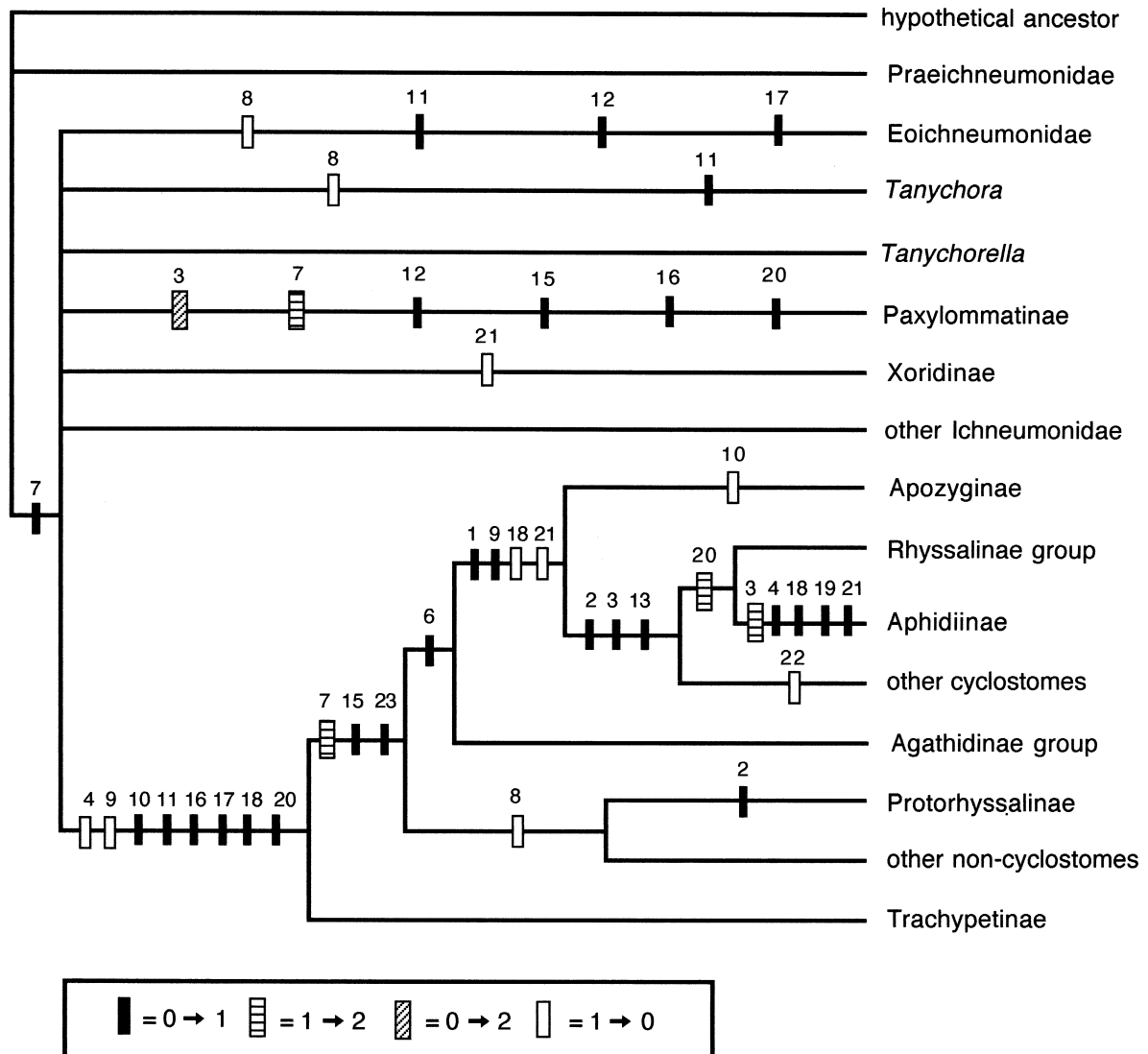


Fig. 13 Preferred tree on the basis of OCCI obtained from analyses 9 and 13 (see Tables 2 and 4). Unambiguous character state changes are indicated. Their distribution is the same in the trees obtained from both analyses except for character 17 which does not change on that tree obtained from analysis 9 since in that case, the articulation of metasomal tergites 2 and 3 in the Eoichneumonidae was scored as the ichneumonoid type (Table 2).

8. Forewing vein a: 0 = present; 1 = absent.
9. Forewing vein 2 A: 0 = present; 1 = absent.
10. Forewing vein 2 m-cu: 0 = present (Fig. 2A, arrow); 1 = absent.
11. Hindwing vein r-m: 0 = with a bulla (Figs 3A-E, 4C, 6A-B); 1 = without a bulla (Fig. 3F-J, 5B).
12. Hindwing vein r-m: 0 = far distal to junction of 1-SC + R & SC + R1 (Figs 2B, 3A-E, 4C, 6A); 1 = at or just distal to junction of 1-SC + R & SC + R1 (Figs 2D, 5A); 2 = basal to junction of 1-SC + R & SC + R1 (Figs 2C,E, 3F-J).
13. Hindwing with vein 2-CU (when present): 0 = confluent with cu-a (Figs 2B,D, 3A,B,F, 4B, 5C); 1 = arising independently beyond origin of vein cu-a (Figs 2C,E, 3C-E,G,F). State two has often been regarded as indicating the presence of vein m-cu; however, the alternative possibility, that the vein has shifted, is considered here. In order to test the traditional hypothesis, we also ran a set of analyses by breaking down this character in to two characters: 13 A. Hindwing vein 2-CU: 0 = present; 1 = absent. 13B. Hindwing m-cu: 0 = present; 1 = absent.

14. Hindwing distal spur of vein C joining SC + R1 near the distal hamuli: 0 = present (Fig. 2B); 1 = absent (Fig. 2C-E). Reference: Sharkey & Wahl (1992).
15. Hindwing vein SC + R: 0 = separating from C very close to the wing base, the two either diverging or if parallel, then still distinctly formed by two veins (Fig. 2B,D); 1 = fused completely for a considerable distance from base (Fig. 2C).
16. Secondary hamuli: 0 = hook-shaped; 1 = more or less simple and setiform. References: Sharkey & Wahl (1992); Basibuyuk & Quicke (1997).
17. Metasomal tergites 2 and 3: 0 = with lateral hinged connection; 1 = without lateral hinged connection. The absence of a lateral hinge has often been considered as a putative synapomorphy for the Braconidae. However, examination of fossil Eoichneumonidae indicates the possibility that these may also have had the 2nd and 3rd tergites immovably connected (Fig. 9A,B). Because we cannot be absolutely certain about this, we carried out separate analyses with both codings for this family.
18. Testes: 0 = fused above the gut; 1 = separate. Maetô (1987) drew attention to this character for revealing relationships within the Braconidae, and further taxa were examined by Quicke & van Achterberg (1990).
19. Spermatozoa: 0 = elongate, 60–150 μm long, of typical structure; 1 = intermediate length, 20–35 μm long, with normal structure; 2 = short, 10–20 μm long (Fig. 10A), with highly modified structure (Fig. 10B). This character was treated as ordered. Data come from Quicke *et al.* (1992b) and Shaw & Quicke (in press).
20. Number of rectal pads in groundplan: 0 = six; 1 = four; 2 = two. We have surveyed this character in a wide range of ichneumonoid genera (Appendix I), and data for many other families of Hymenoptera are presented in Quicke (1997). Rectal pads are involved in water and ionic regulation in insects; they are recognizable even in dry specimens after KOH treatment as their margins are distinctly indicated by thicker rectal cuticle. For Ichneumonidae, those of several taxa are illustrated by Pampel (1914).
21. Ovipositor dorsal valve: 0 = lumen with a complete mid-longitudinal division; 1 = lumen entire.
22. Venom reservoir: 0 = very strongly muscularised and with spiral cuticular lining; 1 = weakly muscularised and without spiral cuticular lining. Data come from Togashi (1963), Edson & Vinson (1979) and Quicke & van Achterberg (1990).
23. G + C content of 28S rDNA (D2 region): 0 = more than 55% (59–67); 1 = less than 55% (40–52). Data on GC content are presented in Belshaw *et al.* (1998).

Results and Discussion

Monophyly of the Ichneumonoidea

Monophyly of the Ichneumonoidea excluding the Praeichneumonidae has been generally accepted by all recent workers on the group and has been discussed by Sharkey & Wahl (1992) who noted several putative synapomorphies for them, including mandibles with two teeth, prepectus fused to posterolateral margin of pronotum, 1st metasomal sternite divided into two, 1st and 2nd metasomal tergites articulating by a pair of dorso-lateral condyles, loss of forewing costal cell (Fig. 13: character 7), and absence of vein 2rs-m of forewing. In addition, monophyly of the group is supported by the utilization of manganese as hardening material in the cuticle of their ovipositors, though the ovipositors of cynipoids and megalyroids also possess manganese (Quicke *et al.* 1998).

Interpretations of hindwing veins 2-CU and m-cu

Abscissa 2-CU of vein CU of the hindwing is present in almost all ichneumonids, in the Eoichneumonidae and in the subfamilies Trachypetinae, Apozyginae, Protorhyssalinae and Agathidinae subfamily group of the Braconidae. While vein 2-CU is not present in cyclostome braconids apart from *Apozyx* and *Protorhyssalus*, many (indeed some members of all subfamilies except for the Braconinae) have another vein arising more distally from the presumed 1-M, which has always been considered to represent transverse vein m-cu. However, whereas hindwing vein 2-CU is found in Ichneumonidae and many other Apocrita, hindwing vein m-cu is not. Indeed, m-cu is found among the Apocrita only in the extinct and putatively very basal Karatavidae (see Rasnitsyn 1980). Thus for various braconids to have vein m-cu this would require either that this vein has been independently lost in many other lineages within the Apocrita or that this vein is not in fact homologous with m-cu of the sawflies and Karatavidae. If it is not m-cu then there are two possibilities: (1) it could be a new vein; (2) it could be an existing vein in an unexpected position. The latter of these seems possible for several reasons. Firstly, '2-CU' has migrated distally so that in some species '2-CU' arises far beyond the separation of cu-a from M+CU, in many members of the Apoidea and Vespoidea and also in some ichneumonids. A clear transition series is well illustrated by members of the labenine ichneumonid genus *Apechoneura* in which different species have the distal CU abscissa in the normal ichneumonid position whilst others have it far distal to the separation of cu-a (Townes 1969; Fig. 3C-E). Members of a few other ichneumonid genera also have 2-CU emerging from the point where M+CU and cu-a meet, e.g. *Poemenia* (Poemeniinae), *Echthromorpha* (Pimplinae) and *Banchus* (Banchinae). Further, with the exception of two members of the

Sigalphinae, *Acampsis* and *Sigalphus romeroi* (Sharkey & Janzen 1995) (see below), no braconids are known that possess both 2-CU and 'm-cu', and the same is true of all ichneumonids. In other words, the possibility needs to be considered that in cyclostomes other than *Protorhyssalus* and *Apozyx*, what has been called m-cu is in fact CU which would therefore be required to have migrated to a more distal separation from M + CU than cu-a. This new interpretation of braconid vein 'm-cu' as a distally migrated CU would have several implications for braconid phylogeny.

In the noncyclostomes only four taxa are known to possess 'm-cu', and in none of these is it well developed. The most conspicuous manifestation is in *Dirrhope* (Dirrhopinae) an uncommon, monotypic subfamily which has variously been associated with the Microgastrinae or more generally with the 'microgastroid' group of subfamilies (Quicke & van Achterberg 1990), in which a spectral vein is present (Fig. 3H). The other noncyclostome candidates are the helconine, *Calobelcon* Turner from Australia (Quicke & Holloway 1991), and the sigalphines, *Acampsis alternipes* and *Sigalphus romeroi* (Agathidinae subfamily group). In the first of these, the putative m-cu vein is represented by only a small spur close to the separation of 1r-m and 1-M (Fig. 3F, small arrow), though it is better developed in the *Sigalphus* sp., it is not a tubular vein. Whether these spurs and spectral veins are homologous with the obviously complete tubular vein found in many cyclostome braconids is uncertain, and in this respect it is worth noting that several braconids are known to have spurious veins of no obvious ancestry (Riegel 1948; Tobias & Belokobylskij 1983).

In the cyclostome group, the form of vein 'm-cu' varies considerably. The majority of taxa have a more or less straight vein, but it can curve gradually or sharply towards the wing tip as in the Odontobraconini (Doryctinae; Fig. 3J) and some *Yelicones* (Rogadinae) species (Marsh 1970; Quicke *et al.* 1997), or it can curve strongly towards the wing base as in other *Yelicones* species (Quicke *et al.* 1997; Fig. 3I). Within the subfamily Braconinae, a spurious vein remote from both cu-a and 1-M is found in two genera, *Archibracon* and *Calcaribracon* (Fig. 2C), which do not appear to be closely related to one another (Quicke 1986, 1988). In *Calcaribracon*, the basal end of this spurious vein sometimes turns slightly anteriorly suggesting (according to Quicke 1986) a small remnant of 'm-cu'.

Phylogenetic consideration of the above suggests several possible relationships depending on whether the traces of a 'm-cu' in the three non-cyclostomes are homologous with the often tubular vein 'm-cu' found in many members of the cyclostome group of subfamilies. If they are, then it implies that the non-agathidine group of non-cyclostomes may be the sister group of the extant cyclostomes exclud-

ing *Apozyx*, otherwise there would need to be two independent migrations of 2-CU to a distal position giving rise to an m-cu like vein. If the traces in *Calobelcon* and *Dirrhope* represent either a new development or a teratological feature, as the asymmetry observed in some *Calobelcon* might suggest, then the non-agathidine group of non-cyclostomes may be derived simply by loss of 2-CU. Evidence for monophyly of the noncyclostomes is sparse, the most convincing character being the very short sperm with atypical axoneme structure (largely with no or only a single central microtubule rather than a pair as in other Hymenoptera) (Quicke *et al.* 1992b). These derived sperm are found in both the 'microgastroid' and 'helconoid' groups of subfamilies (Fig. 10A). Unfortunately, sperm ultrastructure has only been investigated in the Agathidinae and Sigalphinae out of those noncyclostome subfamilies that possess a well developed basally positioned vein 2-CU. Sigalphine sperm are of normal morphology (Shaw & Quicke in press) whilst agathidine sperm, although appearing to have a derived microtubule complement in the axoneme, are far from short, instead being extremely long and forming a tangle in the vas deferens that is extremely difficult to investigate (Fig. 10D). Thus sperm ultrastructure does not provide any evidence for a monophyletic noncyclostome group of subfamilies including the Agathidinae group. Another putative synapomorphy for non-cyclostomes is the separation of the testes (Maetô 1987) from their plesiomorphic state, i.e. fused dorsal to gut. This is a fairly homoplastic character and it is known to vary even within some genera (Wharton *et al.* 1989) including within the Agathidinae itself (Maetô 1987).

The outcome, from the present analyses, of treating m-cu as a separate character from 2-CU (Analyses 3, 4, 7, 8, 11, 12, 15 and 16) had no effect on the strict consensus of most parsimonious trees obtained nor on any of the trees resulting from successive approximations weighting (Figs 11,12). This is surprising but suggests that the strength of phylogenetic signal among the other independent characters is great enough not to be influenced by this single character.

Relationships within Braconidae

All analyses placed the rare endemic Australian subfamily Trachypetinae as the sister group to the remaining braconids including Protorhyssalinae (Figs 11,12). The high GC content of the 28S rDNA gene, together with large unique insertions (Belshaw *et al.* 1998), made alignment of this gene in Trachypetinae (*Megalobelcon ichneumonoides*) with other braconids difficult, and although in sequence analysis trees this subfamily was also placed as the basal clade within the Braconidae, this might have been due to compositional bias (ichneumonids also having high GC

content). However, Dowton & Austin (1997) have also found that there is a smaller but clear difference in base composition between Ichneumonidae and Braconidae in the mitochondrial 16S rDNA gene, with the trend in the same direction, and as with the 28S gene, the GC:AT ratio of the trachypetinae agrees more closely with those of ichneumonids and not with that of the other Braconidae (M. Dowton, pers. comm.).

All the strict consensus trees also contained a cyclostome plus aphidiine clade (Apozyginae, Rhyssalinae group, Aphidiinae and other cyclostomes), though the relationships among these taxa varied considerably. The inclusion of the aphidiines among the cyclostomes is in agreement with the fact that several aphidiines are distinctly cyclostome (note, they were scored as polymorphic for this character in all analyses) and that some have an highly muscularised venom reservoir (character 22), but disagrees with recent molecular studies (Belshaw & Quicke 1997; Belshaw *et al.* 1998) that have provided strong evidence that the Aphidiinae (+ Mesostoinae) are more closely related to the non-cyclostome though they are placed near the base of that group. It should be noted that the large difference in GC content, the paucity of regions that can be unambiguously aligned between Braconidae and Ichneumonidae, and the presence of long branches in the 28S gene makes interpretation of basal relationships within the Braconidae using this gene difficult (see Quicke *et al.* 1999).

Although the Protorhyssalinae were scored as cyclostome in analyses 1–8 (see Table 2), they only formed a monophyletic group with the other cyclostome braconids in trees obtained from two of the 80 analyses that we performed (Fig. 11: tree 4), both of which were after successive approximations weighting using the maximum value of the retention index (Table 3). They also appeared with the other cyclostomes in tree 8 (Fig. 12) which was a consensus of a subset of the trees that gave tree 1 (Fig. 11) selected on the basis of OCCI. Thus, it will be interesting to see whether they are truly cyclostome when either techniques for studying these old amber inclusions are improved, or new and better preserved specimens become available.

Relationships within Ichneumonidae

Our study treated separately four taxa that may be considered as ichneumonids in the strict sense, viz. *Tanychorella*, Paxylommatinae, Xoridinae and other extant ichneumonids. Surprisingly, these only came out as a monophyletic group in one of the sets of consensus trees (Fig. 12: tree 7), and in that case, there was no resolution among the four included taxa. Monophyly of the Ichneumonidae is potentially supported by the veins comprising the 2nd submarginal cell (areolet) of the forewing but the exact homologies of the

veins involved are far from certain and interpretations have been based on the relative positions of flexion lines, transverse vein 2 m-cu, and also of teratological specimens. Further, the accessory longitudinal tracheal commissure found in larval ichneumonids may be a synapomorphy for the Ichneumonidae (see, e.g. van Achterberg 1984). However, this character has not been investigated in all relevant taxa and it should be noted that whilst van Achterberg (1984: Fig. 12) indicated that it was an autapomorphy for Ichneumonidae excluding Paxylommatidae, the larvae of the latter are unknown.

The Paxylommatinae appeared basal to a clade comprising Braconidae plus either Eoichneumonidae or Eoichneumonidae + *Tanychora* in three of the strict consensus trees (Fig. 12: trees 5, 6 and 8). That this subfamily may be the most basal of the extant ichneumonids may at first sight seem surprising since they are clearly highly morphologically and probably biologically specialized. The morphological characters supporting the position of the Paxylommatinae closer to the braconids than to other ichneumonids (including *Tanychorella*) are mainly connected to wing venation, though braconids and paxylommatines also share a rectum with four rectal pads arranged in a ring. As regards wing venation, the extant paxylommatine, *Hybrizon*, is capable of very precise hovering flight either over lines of foraging ants or within a small glass tube, similar to that of many braconids but rather unlike most ichneumonids. Thus, for *Hybrizon* the need to be able to hover could be adaptive and consequently have led to convergent wing morphologies. That sequence data analyses for the 28S rDNA D2 gene fragment similarly support this relationship (Belshaw *et al.* 1998; Quicke *et al.* 1999) means that this possibility needs further consideration; sequence data for other genes will, however, be needed to resolve this matter as the *Hybrizon* sequence constitutes a long branch that could distort the analyses conducted to date.

Relationships of *Tanychora* and *Eoichneumonidae*

Our analyses did not provide consistent evidence for the placement of *Tanychora*. Following successive approximations weighting with either maximum or minimum value of RC in analysis 12 and 16 (Fig. 12: tree 5) and in a strict consensus of the subset trees from analysis 8, selected on the basis of the OCCI (Table 4; Fig. 12: tree 8) *Tanychora* appeared as the sister group to Eoichneumonidae. *Tanychora* differs from Eoichneumonidae only in the position of hindwing vein r-m, and therefore may be best regarded as a relatively basal eoichneumonid. None of our analyses placed *Tanychora* among the Ichneumonidae where it had originally been assigned,

but until more material is available, assessment of its affinities will remain uncertain.

A sister group relationship between Eoichneumonidae (or Eoichneumonidae + *Tanychora*) and the Braconidae appeared in nearly all analyses when successive approximations weighting using the minimum values of either RC or RI was applied (Fig. 12: tree 6; Table 3). This weighting may be viewed as conservative in that only those characters with high RC or RI in all initial trees are given high weight. However, the relationships of Eoichneumonidae and *Tanychora* were unresolved in tree 3 (Fig. 11: tree 3) which was the one preferred on the basis of OCCI (see Table 4).

Sharkey & Wahl (1992) also suggested a sister group relationship between Eoichneumonidae and Braconidae from their unweighted analysis but this resulted from a single character (loss or reduction of forewing vein 2 m-cu in both). We have shown that this relationship is sensitive to certain character decisions for fossil taxa. It should be noted that we scored vein 2m-cu of the forewing as present in the Eoichneumonidae, whereas Sharkey & Wahl (1992) scored it as spectral or absent in both Braconidae and Eoichneumonidae. Figure 7(A-C) show that in the Eoichneumonidae, the anterior and posterior portions of 2m-cu are clearly tubular and the vein simply has a broad median bulla, as is also seen in numerous extant ichneumonids.

Effects of ambiguous character scoring decisions and successive approximations analysis

Although we carried out parsimony analyses on 16 variations of the data matrix (see Table 2) only three different strict consensus trees (Fig. 11: trees 1, 2; Fig. 12: tree 7) were obtained and these were dependent only on whether the Protorhyssalinae were scored as cyclostome or non-cyclostome and whether hindwing vein 'm-cu' was treated as an independent character from vein 2-CU in those analyses in which the Protorhyssalinae were regarded as noncyclostome. Even after successive approximations character weighting, whether the Eoichneumonidae were scored as having the ichneumonid or the braconid type of connection between the 2nd and 3rd metasomal tergites (character 17) had no effect on the trees obtained.

Successive approximations character weighting did have a major effect on the set of shortest trees found, though, in general, using RC or RI had a far lesser effect than whether reweighting was carried out with the maximum or minimum values of these indices (see Materials and methods — *Analysis*). Using the minimum value, the resulting consensus trees (Fig. 12: trees 5, 6 and 7) all had the Eoichneumonidae (or Eoichneumonidae + *Tanychora*) as the sister group of the Braconidae whereas with the maximum values of RC and RI, most analyses failed to resolve eoichneumonid relationships (Fig. 11: trees 3 and 4).

Preferred tree and character evolution

On the basis of the optimum character compatibility index (Rodrigo 1992), tree 3 is preferred as our working hypothesis for ichneumonoid relationships (Fig. 13). This tree had a maximum of 9 out of 23 characters with a consistency index of 1.0. Unfortunately it is entirely unresolved outside of the Braconidae, making further research on ichneumonoid phylogeny essential.

Examining character state changes on this tree (Fig. 13), monophyly of the Braconidae is supported by eight characters but none of these is without homoplasy on the tree. Thus, although we have no doubts about the monophyly of the Braconidae, or the Ichneumonidae for that matter, finding unambiguous characters that support this for such large and morphologically diverse families is no easy business. All analyses show the Aphidiinae as a derived group of braconids in agreement with most current authors and a growing and convincing body of molecular evidence (Belshaw & Quicke 1997).

Within the Braconidae, cyclostomicity (character 1) is seen as a synapomorphy uniting the traditional cyclostome subfamilies with the Apozyginae and Aphidiinae in agreement with traditional views (Wharton *et al.* 1992). However, it should be noted that character coding is based on groundplan interpretation, and in particular, all of the Alysiinae, together with many Opiinae and Betylobraconinae are generally accepted to have lost this condition, and the Aphidiinae were scored as polymorphic for this feature. The presence of forewing vein 2m-cu in the Apozyginae (character 10) appears to be derived in agreement with Sharkey & Wahl (1992), rather than indicating that this subfamily is the most basal extant braconid. That the Apozyginae are members of the cyclostome clade is also supported by character 9, the loss (in the groundplan) of forewing vein 2 A, though this may be viewed as a relatively weak character given the number of occasions that it appears to have been lost independently in various braconid subfamilies. Monophyly of the remaining cyclostome braconids plus the Aphidiinae is supported by three derived characters but two of these are homoplastic (characters 2 and 3) while character 13, the presence of hindwing vein 'm-cu' in the groundplan, is interpreted in the sense that the similar vein stub seen in a few noncyclostomes (e.g. *Dirrhope*, *Acampsis*, some *Sigalphus* and *Calobelcon*; see above) is not homologous, and are either teratological or independent acquisitions of a novel venational feature.

As all analyses placed the non-cyclostome Trachypetinae as the most basal braconid, the ground plan state for the Braconidae is therefore non-cyclostome. The basal position of the Trachypetinae is in marked disagreement with the morphological findings of Quicke & van Achterberg (1990). However, as pointed out by Austin *et al.* (1993), few charac-

ters provided convincing support for that placement. Austin *et al.* (1993) did not explicitly suggest any relationships for the Trachypetinae though they mostly discussed their characters with reference to other noncyclostome taxa. Our discovery of several putatively plesiomorphic characters together with a growing body of molecular evidence seems now to strongly support the notion that Trachypetinae are the most basal extant braconids. Given that they are also among the largest braconids, it could indicate that the family was originally comprised of relatively large insects.

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Appendix I. Numbers of rectal pads in Ichneumonoidea

Ichneumonidae		<i>Oetophorus</i> sp.	6	<i>Proclitus</i> sp.	4
Acaenitinae		<i>Onarion ploughmani</i> sp.	6	<i>Stenomacrus</i> sp.	6
<i>Arotes amoensis</i>	6	<i>Opheltes glaucopterus</i>	6	Orthopelmatinae	
<i>Coleocentrus excitator</i>	17	<i>Perilissus albitarsus</i>	9/10	<i>Orthopelma mediator</i>	2
<i>Coleocentrus occidentalis</i>	18	<i>Pion fortipes</i>	6	<i>Orthopelma occidentale</i>	2
Adelognathinae		<i>Westwoodia</i> sp.	6	<i>Oxytorinae</i>	
<i>Adelognathus</i> sp.	6	<i>Diplazontinae</i>	0.	<i>Oxytorus</i> sp.	6
Alomyinae		<i>Diplazon laetatorius</i>	6	Paxylommatinae	
<i>Alomya</i> sp. A	6	<i>Diplazon pectoratorius</i>	6	<i>Hybrizon</i> sp.	4
<i>Alomya</i> sp. B	4	<i>Diplazon</i> sp.	6	Phrudinae	
Anomaloninae		<i>Enizemum</i> sp.	6	<i>Phrudus monilicornis</i>	2
<i>Agrypon</i> sp.	8	<i>Promethes sulcator</i>	6	<i>Erythrodolius</i> sp.	6
<i>Anomalon</i> sp.	6	Eucerotinae		<i>Apechthis</i> sp.	6
<i>Anomalon</i> sp.	7	<i>Euceros</i> 2 sp.	8	<i>Clistopyga</i> sp.	6
<i>Barylypa sulcata</i>	12	Ichneumoninae		<i>Dolichomitus</i> sp.	15
<i>Barylypa sulcata</i>	10	<i>Amblyteles armatoria</i>	6	<i>Eriostethus pulcherrimus</i>	6
<i>Gravenhorstia picta</i>	10	<i>Chasmias motatorius</i>	6	<i>Exeristes</i> sp.	6
<i>Habronyx</i> sp.	6	<i>Cratichneumon fabricator</i>	6	<i>Hymenoepepimecis</i> sp. A	6
<i>Heteropelma amictum</i>	4	<i>Ichneumon sarcitorius</i>	6	<i>Hymenoepepimecis</i> sp. B	4
<i>Therion circumflexum</i>	4	<i>Ischnojoppa</i> sp.	6	<i>Lissopimpla excelsa</i>	6
Banchinae		<i>Limerodops elongatus</i>	6	<i>Pimpla hypochondriaca</i>	6
<i>Apophua</i> sp.	6	<i>Phaeogenes ophthalmicus</i>	6	<i>Pimpla pedalis</i>	6
<i>Banchus canadensis</i>	7	<i>Probolus culpatorius</i>	6	<i>Pimpla flavicoxis</i>	6
<i>Banchus flavescens</i>	6	Labeninae		<i>Schizopyga</i> sp.	6
<i>Cryptopimpla blanda</i>	6	<i>Brachycyrtus</i> sp.	6	<i>Theronia</i> sp.	6
<i>Exetastes</i> sp.	6	<i>Certonotus</i> sp.	many	<i>Tromatobia notator</i>	6
<i>Glypta</i> 3 spp.	6	<i>Grotea</i> sp.	6	Poemeniinae	
<i>Lissonota</i> sp.	6	<i>Labena grallator</i>	6	<i>Deuteroxorides albitarsus</i>	6
<i>Panteles</i> sp.	6	<i>Labena</i> sp.	6	<i>Ganodes</i> sp. A	12–16
Campopleginae		<i>Labium longiceps</i>	6	<i>Ganodes</i> sp. B	≈20
<i>Campoletis</i> sp.	6	<i>Macrogrotea</i> sp.	6	<i>Podoschista vittifrons</i>	13
<i>Campoplex geniculatus</i>	6	<i>Lycorininae</i>		<i>Poemenia hectica</i>	6
<i>Casinaria miora</i>	6	<i>Lycorina</i> sp.	4	<i>Poemenia</i> sp.	6
<i>Dusona falcator</i>	4	Mesochorinae		<i>Pseudorhyssa</i> sp.	6
<i>Dusona erythrogaster</i>	5	<i>Astiphromma strenuum</i>	6	Rhyssinae	
<i>Nonnus</i> sp. A	6	<i>Mesochorus</i> sp.	4	<i>Megarhyssa</i> sp.	120
<i>Nonnus</i> sp. B	5/6	<i>Mesochorus</i>	3	<i>Rhyssa persuasoria</i>	96
<i>Skiapus</i> sp.	4	<i>Cidaphus</i> sp.	12	<i>Rhyssa</i> sp. many	
<i>Venturia canescens</i>	5	Metopiinae		Stilbopinae	
Collyriinae		<i>Colpotrochia</i> sp.	6	<i>Stilbops vetula</i>	4
<i>Collyria coxator</i>	6	<i>Colpotrochia</i> sp.	6	Tersilochinae	
Cremastinae		<i>Exochus</i> sp.	?6	gen. sp. 2	
<i>Eiphosoma</i> sp.	6	<i>Metopius</i> sp. A	many	gen. sp. 2	
<i>Pristomerus</i> sp. A	4	<i>Metopius</i> sp. B	many	Tryphoninae	
<i>Pristomerus</i> sp. B	4	<i>Tricetes bicalcaratus</i>	6	<i>Boethus</i> sp.	6
<i>Temelucha pictus</i>	4	gen. sp.	6	<i>Cosmoconus elongator</i>	6
<i>Temelucha</i> sp.	4	gen. sp.	6	<i>Exyston pratorum</i>	6
Cryptinae		Ophioninae		<i>Hercus frontalis</i>	6
<i>Osprychotus</i> sp.	6	<i>Alophophion</i>	c17	<i>Idiogramma</i> sp.	?6
<i>Sphecephaga vesparum</i>	6	<i>Enicospilus</i> sp.	c20	<i>Netelia</i> sp.	6
<i>Xanthocryptus</i>	6	<i>Enicospilus</i> sp.	c10	<i>Phytodietus</i> sp.	6
Ctenopelmatinae		<i>Ophion luteus</i>	12	<i>Tryphon</i> sp. A	6
<i>Alexeter</i> sp.	6	<i>Ophion obscuratus</i>	12	<i>Tryphon</i> sp. B	6
<i>Euryproctus</i> sp.	6	<i>Ophion</i> sp.	c20	Xoridinae	
<i>Hadrodactylus</i> sp.	6	<i>Thyreodon</i> sp.	c12	<i>Ischnocerus rusticus</i>	6
<i>Labrossyta</i> sp.	4	Orthocentrinae		<i>Odontocolon</i> sp.	6
<i>Mesoleius</i> sp.	?6	<i>Eusterinx</i> sp.	4		
<i>Occapes</i> sp.	12	<i>Megastylus</i> sp.	6		

Appendix 1—Continued.

Braconidae		<i>Iphiaulax</i> sp.	4	<i>Diachasma wichmanni</i>	4
Adeliinae		<i>Stenobracon deesae</i>	4	<i>Nipponopius incisus</i>	4
<i>Adelius</i> sp.	4	Cardiochilinae		<i>Opius truncatus</i>	4
Agathidinae		<i>Cardiochiles nigriceps</i>	4	<i>Psytalia fletcheri</i>	4
<i>Agathis</i> sp.	4	Cheloninae		Pambolinae	
Alysiinae		<i>Chelonus</i> sp.	4	<i>Notiopambolus depressicauda</i>	4
<i>Adeluroloa florimela</i>	4	Doryctinae		<i>Pambolus</i> sp.	4
<i>Alloea</i> sp.	4	<i>Doryctes erythromelas</i>	4	Rhysipolinae	
<i>Alysia manducator</i>	4	Exothecinae		<i>Rhysipolis</i> sp.	4
<i>Aphaerta minuta</i>	2	<i>Phanomeris catenator</i>	4	Hormiinae	
<i>Dacnusa laevipectus</i>	4	Helconinae		<i>Monitoriella elongata</i>	2
<i>Phaenocarpa conspurcator</i>	4	<i>Helcon tardator</i>	4	<i>Monitoriella rufithorax</i>	2
Betylobraconinae		Histeromerinae		Rogadinae s.l.	
<i>Mesocentrus</i> sp.	4	<i>Histeromerus canadensis</i>	2	<i>Aleiodes</i> 2 spp.	4
Braconinae		Macrocentrinae		<i>Aulosaphes</i> sp.	?2
<i>Archibracon</i> spp.	4	<i>Macrocentrus</i> sp.	4	Trachypetinae	
<i>Euurobracon</i> spp.	> 12	Opiinae		<i>Cercobarcon riei</i>	4
<i>Euurobraconoides</i> sp.	4	<i>Adeluroloa florimela</i>	4	<i>Rhamphagathis</i> sp.	4
<i>Craspedolcus</i> sp.	4	<i>Biosteres arisanus</i>	4		