Systematic Entomology

Systematic Entomology (2011), **36**, 549–572

Molecular phylogenetics of Braconidae (Hymenoptera: Ichneumonoidea), based on multiple nuclear genes, and implications for classification

BARBARA J. SHARANOWSKI[1], ASHLEY P. G. DOWLING[2] and MICHAEL J. SHARKEY[3]

[1]Department of Entomology, University of Manitoba, Winnipeg, Canada, [2]Department of Entomology, University of Arkansas, Fayetteville, AR, U.S.A. and [3]Department of Entomology, University of Kentucky, Lexington, KY, U.S.A.

Abstract. This study examined subfamilial relationships within Braconidae, using 4 kb of sequence data for 139 taxa. Genetic sampling included previously used markers for phylogenetic studies of Braconidae (*28S* and *18S* rDNA) as well as new nuclear protein-coding genes (*CAD* and *ACC*). Maximum likelihood and Bayesian inference of the concatenated dataset recovered a robust phylogeny, particularly for early divergences within the family. This study focused primarily on non-cyclostome subfamilies, but the monophyly of the cyclostome complex was strongly supported. There was evidence supporting an independent clade, termed the aphidioid complex, as sister to the cyclostome complex of subfamilies. *Maxfischeria* was removed from Helconinae and placed within its own subfamily within the aphidioid complex. Most relationships within the cyclostome complex were poorly supported, probably because of lower taxonomic sampling within this group. Similar to other studies, there was strong support for the alysioid subcomplex containing Gnamptodontinae, Alysiinae, Opiinae and Exothecinae. Cenocoeliinae was recovered as sister to all other subfamilies within the euphoroid complex. *Planitorus* and *Mannokeraia*, previously placed in Betylobraconinae and Masoninae, respectively, were moved to the Euphorinae, and may share a close affiliation with Neoneurinae. Neoneurinae and Ecnomiinae were placed as tribes within Euphorinae. A sister relationship between the microgastroid and sigalphoid complexes was also recovered. The helconoid complex included a well-supported lineage that is parasitic on lepidopteran larvae (macrocentroid subcomplex). Helconini was raised to subfamily status, and was recovered as sister to the macrocentroid subcomplex. Blacinae was demoted to tribal status and placed within the newly circumscribed subfamily Brachistinae, which also contains the tribes Diospilini, Brulleiini and Brachistini, all formerly in Helconinae.

Introduction

Reconstructing the phylogenetic history of Braconidae has long been of interest to biologists in many fields. Aside from their valuable use in biological control (Austin & Dowton, 2000), Braconidae provide an excellent system for studies on biodiversity and conservation (Lewis & Whitfield, 1999), as well as evolutionary studies on the development of parasitism (Shaw, 1983; Gauld, 1988; Whitfield, 1992; Quicke & Belshaw, 1999; Jervis *et al*., 2001; Belshaw & Quicke, 2002; Zaldivar-Riverón *et al*., 2008), host–parasite interactions (Clausen, 1954; Strand & Obrycki, 1996; Whitfield, 1998; Strand, 2000), morphological adaptation and convergence (Quicke & Belshaw, 1999; Belshaw *et al*., 2003), and the evolution of polydnaviruses (Whitfield, 1997; Bezier *et al*., 2009). Braconidae are one of the most diverse families of Hymenoptera, with over 17 000 described species (Yu *et al*., 2004), and at least 25 000 species yet to be described, although

Correspondence: Barbara J. Sharanowski, Department of Entomology, University of Manitoba, 223 Animal Science and Entomology Bldg, Winnipeg, Manitoba, R3T 2N2, Canada. E-mail: Barb.Sharanowski@gmail.com

this estimate is probably extremely conservative (Marsh & Carlson, 1979; Jones *et al*., 2009).

One of the greatest challenges for studies on braconid systematics has been the inability to resolve relationships among subfamilies, thus hindering the testing of evolutionary theories on the development of modes of parasitism (Whitfield, 1992; Zaldivar-Riverón *et al*., 2006). Part of the difficulty stems from the immense diversity within Braconidae, as comprehensive taxonomic sampling for large morphological and molecular datasets is often impractical. Useful morphological characters are also difficult to ascertain because of the high level of convergence among phenotypes adapted for a particular host group (Quicke & van Achterberg, 1990; Shaw & Huddleston, 1991; Quicke & Belshaw, 1999). Additionally, molecular datasets have typically focused on a small handful of rDNA or mtDNA genes (Belshaw *et al*., 1998; Dowton *et al*., 1998; Belshaw *et al*., 2000; Belshaw & Quicke, 2002; Dowton *et al*., 2002; Chen *et al*., 2003; Shi *et al*., 2005; Zaldivar-Riverón *et al*., 2006; Pitz *et al*., 2007). Although these genes are relatively easy to amplify across a broad spectrum of taxa, their utility has been limited for resolving higher-level relationships within Braconidae.

Another handicap for braconid systematists is variability in classification schemes. The number of recognized subfamilies has ranged from 17 to 50, with no universally accepted classification (Wharton, 2000; Wharton & van Achterberg, 2000). Currently, there are 47 subfamilies listed in the Ichneumonoidea database Taxapad (Yu *et al*., 2004), although several other subfamilies have been proposed, and are variably recognized by different authors (Tobias, 1967; Wharton *et al*., 1997; Wharton & van Achterberg, 2000). Informal complexes based on putative phylogenetic relationships have also been proposed, although the membership of several complexes has varied in different analyses and classification schemes, particularly the membership of the helconoid complex (Tobias, 1967; van Achterberg, 1984; Maetô, 1987; Wharton, 1993; Belshaw *et al*., 1998; Belshaw & Quicke, 2002).

In this study, we examined relationships among subfamilies of Braconidae using both nuclear rDNA and nuclear protein-coding genes. This study represents the largest genetic sampling of the family to date. Approximately 4 kb of sequence data were gathered for 139 taxa, with several subfamilies sequenced for the first time. Additionally, the use of new genes allowed for independent testing of hypotheses on braconid evolution. We present a robust phylogeny of higher-level relationships based on phylogenetic inferences of the concatenated dataset, and investigate the phylogenetic signal present across individual gene trees. Based on the phylogenetic analyses, we propose some changes to the classification of the Braconidae.

Taxonomic background

Members of Braconidae have typically been separated into two informal groups based primarily on mouthpart morphology: the non-cyclostomes, with a flat or convex clypeus and flat, setose labrum, and the cyclostomes, with a depressed clypeus and concave, glabrous labrum (Tobias, 1967; van Achterberg, 1984; Quicke & van Achterberg, 1990; Wharton, 1993). All members of the non-cyclostome lineage are koinobiont endoparasitoids, whereas members of the cyclostome lineage demonstrate a wider range of biologies (Tobias, 1967; van Achterberg, 1984; Maetô, 1987; Quicke & van Achterberg, 1990). Although most previous analyses suggest that these two groups form natural lineages, the membership of each group and relationships between the two groups has differed across analyses based on both morphological (van Achterberg, 1984; Quicke & van Achterberg, 1990) and molecular datasets (Belshaw *et al*., 1998; Dowton *et al*., 1998; Belshaw *et al*., 2000; Belshaw & Quicke, 2002), and also in combined analyses (Dowton *et al*., 2002; Shi *et al*., 2005; Zaldivar-Riverón *et al*., 2006; Pitz *et al*., 2007).

There have been three competing hypotheses on the phylogeny of Braconidae. First, the non-cyclostomes have been proposed as a lineage derived from cyclostome ancestors (Čapek, 1970; Quicke & van Achterberg, 1990). Second, the non-cyclostomes have been proposed as sister to the cyclostomes, both having evolved from an unknown ancestor, presumably ectoparasitic on concealed xylophagous coleopteran larvae (Tobias, 1967; van Achterberg, 1984; Gauld, 1988; Wharton *et al*., 1992; Belshaw *et al*., 1998; Shi *et al*., 2005; Pitz *et al*., 2007). Third, the non-cyclostomes have been proposed as a basal grade leading to the cyclostomes (Dowton *et al*., 1998). Several recent phylogenetic analyses (Belshaw *et al*., 2000; Dowton *et al*., 2002; Pitz *et al*., 2007) suggest that the cyclostome lineage is sister to the endoparasitic non-cyclostomes (excluding Trachypetinae). To date, however, the evidence for this hypothesis, or any of the competing ideas, has not been conclusive.

The non-cyclostome lineage has previously been divided into two main complexes: the helconoid and microgastroid complexes (Wharton, 1993). Of these two lineages, only the microgastroid complex has been well supported in most molecular analyses (Whitfield, 1997; Belshaw *et al*., 1998, 2000; Dowton & Austin, 1998; Dowton *et al*., 1998; Banks & Whitfield, 2006; Murphy *et al*., 2008), although the branching order among the representative subfamilies has fluctuated. Based on these analyses, the following subfamilies are recognized as part of the microgastroid complex sensu stricto: Microgastrinae, Cardiochilinae, Cheloninae, Adeliinae, Khoikhoiinae, Mendesellinae and Miracinae (Murphy *et al*., 2008). The enigmatic genus *Dirrhope* Foerster has also been suggested to be closely related to the microgastroid complex (van Achterberg, 1984; Quicke & van Achterberg, 1990; Wharton *et al*., 1992; Whitfield & Mason, 1994), but still remains to be incorporated in molecular analyses (for a detailed discussion, see Belokobylskij *et al*., 2003). Additionally, the Ichneutinae have been suggested as the sister group to the microgastroid complex (Quicke & van Achterberg, 1990; Belshaw *et al*., 2000; Belshaw & Quicke, 2002; Dowton *et al*., 2002), and their inclusion within this lineage might be referred to as the microgastroid complex

sensu lato, as members of this subfamily are not known to have polydnaviruses.

Wharton (1993) placed 14 subfamilies within the poorly understood helconoid complex, including: Amicrocentrinae, Agathidinae, Blacinae, Cenocoeliinae, Euphorinae, Helconinae, Homolobinae, Macrocentrinae, Meteorideinae, Meteorinae, Orgilinae, Sigalphinae, Trachypetinae and Xiphozelinae. More recently, Belshaw & Quicke (2002) suggested that Euphorinae, Meteorinae and Neoneurinae could be separated into another lineage, referred to as the euphoroid complex. Additionally, they suggested that Cenocoeliinae may be affiliated with the euphoroid complex, as opposed to being closely related to Helconinae, as has been suggested in the past (Muesebeck & Walkley, 1951; Tobias, 1967; van Achterberg, 1994). A close relationship between Cenocoeliinae and the euphoroid complex was also recovered by Shi *et al*. (2005) and Belshaw *et al*. (2000).

The helconoid complex has probably been the least understood lineage, partially because of the retention of primitive characters among several subfamilies contained within this putative complex (Tobias, 1967; van Achterberg, 1984). This ancestral morphology is most prominent among members of the Helconinae; thus, several authors have suggested that Helconinae is potentially one of the most ancestral lineages among the non-cyclostomes (Tobias, 1967; van Achterberg, 1984; Shaw & Huddleston, 1991). However, the branching order among the subfamilies typically placed within the helconoid complex has been extremely variable across different analyses, leaving no currently accepted phylogenetic hypothesis. The position of Trachypetinae has also been highly variable across different molecular and morphological analyses (Quicke & van Achterberg, 1990; Wharton *et al*., 1992; Belshaw *et al*., 2000; Dowton *et al*., 2002; Shi *et al*., 2005; Pitz *et al*., 2007), and it still remains uncertain as to whether this taxon diverges early in braconid evolution or not.

The placement of Aphidiinae has also differed across analyses, being variably placed as sister to the cyclostomes (van Achterberg, 1984; Dowton *et al*., 2002), within the cyclostomes (Dowton *et al*., 1998; Belshaw & Quicke, 2002) or within the non-cyclostomes (Čapek, 1970; Shi *et al*., 2005; Pitz *et al*., 2007). Recently, Zaldivar-Riverón *et al*. (2006) recovered Aphidiinae + Mesostoinae as sister to the cyclostomes with relatively high support, consistent with some previous analyses (Belshaw *et al*., 2000; Dowton *et al*., 2002).

Relationships among the cyclostome subfamilies have differed across analyses, and the monophyly of several large subfamilies remains in doubt, including Doryctinae, Rogadinae and Hormiinae. Not considering Aphidiinae + Mesostoinae, several analyses have recovered Rhyssalinae (including *Histeromerus* Wesmael) as sister to the remaining cyclostomes (Quicke & van Achterberg, 1990; Belshaw *et al*., 1998; Dowton *et al*., 2002; Zaldivar-Riverón *et al*., 2006; Pitz *et al*., 2007). Doryctinae and Rogadinae have often been recovered as paraphyletic in molecular analyses (Dowton *et al*., 1998; Belshaw *et al*., 2000; Pitz *et al*., 2007; Zaldivar-Riverón *et al*., 2008), or if monophyletic, with very little nodal support (Belshaw *et al*., 1998). Interestingly, phylogenetic inferences that have included morphological data have invariably recovered Doryctinae as monophyletic (Quicke & van Achterberg, 1990; Dowton *et al*., 2002; Zaldivar-Riverón *et al*., 2006), but not necessarily Rogadinae (although see Chen *et al*., 2003; Zaldivar-Riverón *et al*., 2008).

Betylobraconinae has been suggested to have a close affiliation with Rogadinae (van Achterberg, 1995; Chen & He, 1997; Dowton *et al*., 2002; Zaldivar-Riverón *et al*., 2008), but until recently taxon sampling of the subfamily has been limited, and the subfamily as currently comprised has been demonstrated to be polyphyletic (Belokobylskij *et al*., 2008). Belokobylskij *et al*. (2008) demonstrated that the tribe Facitorini belongs within Rogadinae, and there is some evidence that Aulosaphobraconini and Betylobraconini may also be members of Rogadinae (members of Planitorini have never be analysed in a phylogenetic study prior to this paper). The monophyly of Doryctinae in morphological analyses is supported by the robust autapomorphies for this subfamily (including Ypistocerinae), such as an ovipositor with a heavily sclerotized apex, a double nodus on the dorsal valve of the ovipositor and two separate venom gland insertions on the non-sculptured region of the reservoir (Quicke *et al*., 1992a, b).

Several analyses have also recovered a relatively well-supported clade consisting of Gnamptodontinae, Exothecinae, Opiinae and Alysiinae, which has been further corroborated with increased taxonomic sampling (Wharton *et al*., 2006; Zaldivar-Riverón *et al*., 2006). These studies also recovered Braconinae as the sister group to this clade. According to Tobias (1988), *Vaepellis* Quicke (originally described as a monotypic subfamily, Vaepellinae; Quicke, 1987) should be considered as an aberrant braconine, although this relationship was not recovered under a morphological analysis (Quicke & van Achterberg, 1990). The aberrant genus *Telengaia* Tobias may have a close affinity with Exothecinae or Gnamptodontinae according to similarities in the venom apparatus (Zaldivar-Riverón *et al*., 2004) and results of recent molecular phylogenetic analyses (Zaldivar-Riverón *et al*., 2006).

Thus, the phylogenetic hypotheses for Braconidae have been quite variable, regardless of whether morphological or molecular characters were used. One problem that plagues braconid phylogenetic scholarship is the use of morphological matrices coded at the level of subfamily (Dowton *et al*., 2002; Shi *et al*., 2005; Pitz *et al*., 2007), whereby subfamilial monophyly is not tested (Wharton *et al*., 1992). Sampling error is another, and somewhat unavoidable, problem. Given the vast number of species within Braconidae, it is challenging to have both comprehensive taxonomic and character sampling.

Figure 1 depicts a summary tree of highly corroborated relationships among subfamilies of Braconidae. It is based on previous molecular and morphological analyses (Quicke & van Achterberg, 1990; Wharton *et al*., 1992, 2006; Belshaw *et al*., 1998, 2000, 2003; Dowton *et al*., 1998, 2002; Quicke & Belshaw, 1999; Belshaw & Quicke, 2002; Zaldivar-Riverón *et al*., 2006; Pitz *et al*., 2007). Only relationships that have been well supported across multiple analyses are included, whereas relationships that are in conflict across analyses are

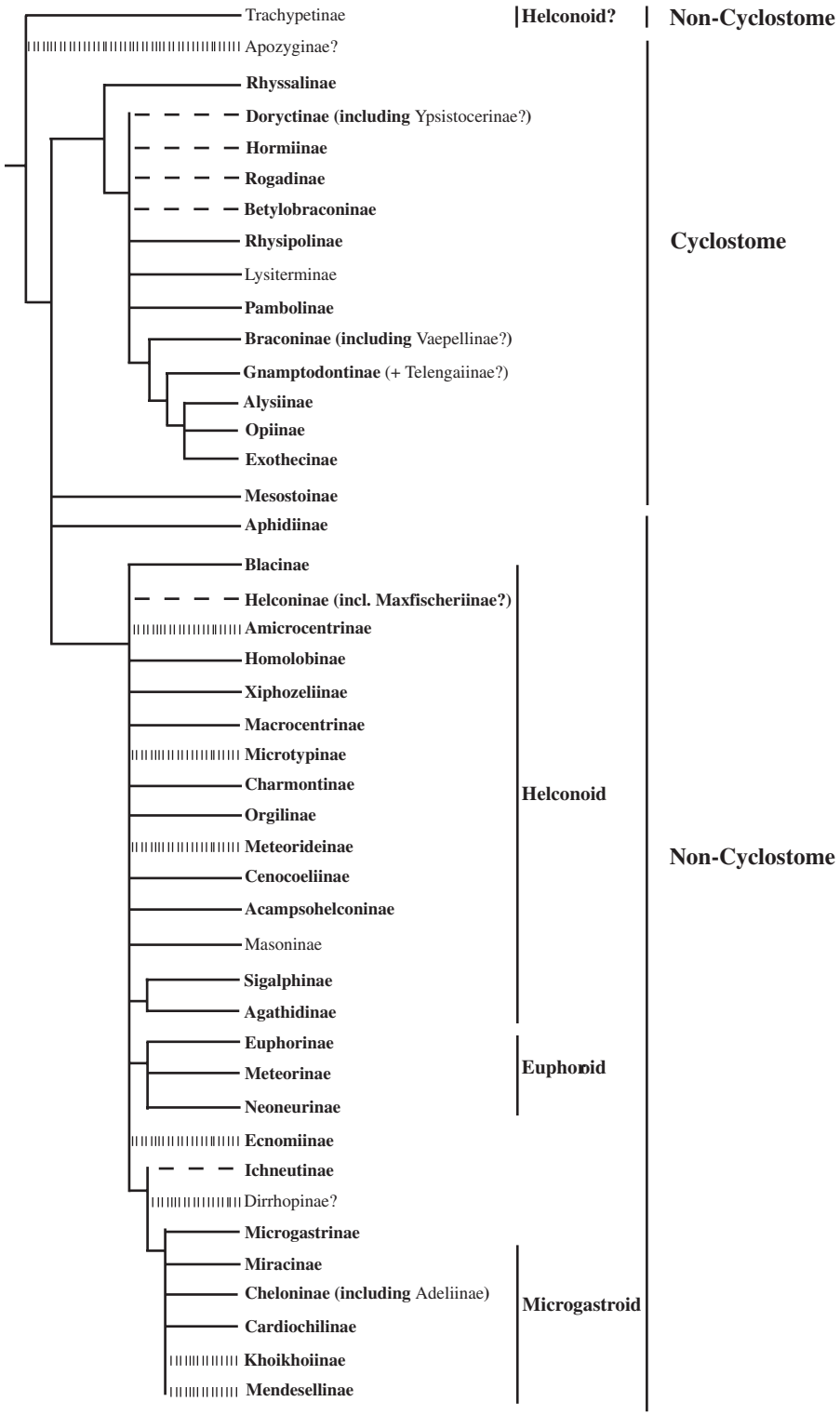

Fig. 1. General consensus tree summarizing current knowledge of braconid subfamilial relationships, based on previous molecular and morphological analyses. Subfamily names in bold are represented in the current dataset, whereas subfamily names in regular type are not represented. Dashed lines indicate likely paraphyly. Vertical lines indicate subfamilies that have not previously been analysed in molecular datasets for subfamilial relationships across Braconidae. The placement of subfamilies with a question mark after the name are based on limited morphological or molecular data, and thus their placement represents the current opinion in the field, rather than the results of phylogenetic testing.

collapsed to polytomies. Thus the figure is quite conservative. Subfamilies that have been variably recovered as paraphyletic are depicted with dashed lines, subfamilies that have never been analysed in molecular datasets are depicted with vertically lined branches, and subfamilies that are represented in this dataset are set in boldface type (see Fig. 1 legend).

Material and methods

Taxon sampling

Exemplars were obtained for 134 species of Braconidae and five species of Ichneumonidae that were employed as out-groups (Table 1). As mentioned previously, the number of recognized subfamilies within Braconidae is constantly in flux, and differs depending on the author. The subfamily classification employed here is an attempt to use the most current phylogenetic information available. Thus, the placement of the exemplars within subfamilies follows that of Belshaw *et al*. (1998) with modifications to the cyclostome subfamilies, based on the results of Zaldivar-Riverón *et al*. (2006) (Table 1). Employing this classification, and recognizing Maxfischeriinae (see results and Boring *et al*., 2011, this issue) and Betylobraconinae (sensu Belokobylskij *et al*., 2008), there are a total of 47 braconid subfamilies.

Thus, 39 subfamilies were represented in the dataset with at least one exemplar (Table 1). Subfamilies without representation include Adeliinae, Apozyginae, Betylobraconinae (see discussion regarding placement of *Planitorus* van Achterberg), Dirrhopinae, Lysiterminae, Masoninae (see discussion regarding placement of *Mannokeraia* van Achterberg), Telengainae and Trachypetinae. The data matrix includes seven subfamilies that have not been previously incorporated into a molecular family-level analysis, including Amicrocentrinae, Ecnomiinae, Khoikhoiinae, Maxfischeriinae and Mendesellinae. Table 1 lists subfamilies under the complexes discussed previously.

There is an emphasis on helconoid subfamilies, particularly Helconinae, because of the very contradictory and ambiguous placements in previous analyses (Belshaw *et al*., 1998; Belshaw & Quicke, 2002; Dowton *et al*., 2002; Pitz *et al*., 2007). Additionally, all previous phylogenetic studies recovered a polyphyletic Helconinae with varying placement of some members at the base of the braconid phylogeny (Quicke & van Achterberg, 1990; Belshaw *et al*., 1998, 2000; Dowton *et al*., 1998, 2002; Belshaw & Quicke, 2002; Shi *et al*., 2005; Pitz *et al*., 2007). Thus, Helconinae is a potentially important basal lineage, and was therefore heavily sampled. Five out-group taxa were selected from Ichneumonidae, well established to be the sister group to Braconidae (Sharkey & Wahl, 1992; Belshaw *et al*., 1998; Dowton *et al*., 2002).

DNA protocols

Genomic DNA was extracted from ethanol-preserved or previously mounted specimens following Qiagen protocols in conjunction with the DNeasy™ Tissue Kit (Qiagen, Valencia, CA, U.S.A.). Voucher specimens were deposited in the Hymenoptera Insect Collection, University of Kentucky. However, specimens of *Maxfischeria* spp. were deposited in the Australian National Insect Collection (see Boring *et al*., 2011, this issue). Several previous studies (Belshaw *et al*., 1998, 2000; Mardulyn & Whitfield, 1999; Chen *et al*., 2003; Shi *et al*., 2005; Zaldivar-Riverón *et al*., 2006; Pitz *et al*., 2007) have used *28S* and/or *18S* rDNA to infer braconid relationships. However, the recent development of primers for protein-coding genes in other insects offers a new source of genetic information that may be useful for relationships among Braconidae. The phylogenetic utility of carbamoyl-phosphate synthetase-aspartate transcarbamoylase-dihydroorotase (*CAD*, also called rudimentary) has been demonstrated for several insects, including flies (Moulton & Wiegmann, 2004), beetles (Wild & Maddison, 2008), green lacewings (Winterton & de Freitas, 2006) and more recently for hymenopterans, including pteromalids (Desjardins *et al*., 2007) and megachilids (Praz *et al*., 2008). Acetyl-coenzyme A carboxylase, or *ACC*, has been suggested as a useful marker for Lepidoptera (Regier, 2007; Regier *et al*., 2008). Here, four genes were targeted for amplification, including: *28S* rDNA (expansion regions D1–D5, sequenced in two fragments); *18S* rDNA (domains 1–3); two non-contiguous segments of the CPSase (carbamoylphosphate synthetase) region of *CAD*; and one region of *ACC*. All primer pairs and associated references are listed in Table S1.

The CPSase small chain of *CAD* (54F/405R) was amplified using primers developed by Moulton & Wiegmann (2004) (hereafter referred to as *CAD54*). Cycling conditions were slightly modified from the published protocols, and included an initial denaturation at 94°C for 4 min, three cycles of 94°C for 30 s, 59°C for 30 s and 72°C for 90 s, followed by five cycles of 94°C for 30 s, 57.5°C for 30 s and 72°C for 90 s, then 28 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 90 s, with a final extension for 3 min at 72°C. A region of the CPSase large chain of *CAD* (primer pair ap*CAD*for1/ap*CAD*rev1mod) was amplified using the primers and protocols developed by Danforth *et al*. (2004) (hereafter referred to as *CADap*). An approximately 500-bp region of *ACC* was amplified using the primers of Regier (2007). A touchdown protocol was used to amplify *ACC*, which included an initial denaturation at 95°C for 4 min, followed by 29 cycles of a 30-s denaturation at 95°C, a 30-s annealing step, starting at 60°C and decreasing 0.5°C every cycle, and an elongation step at 72°C for 40 s. This touchdown protocol was followed by eight cycles of 95°C for 30 s, 45°C for 30 s and 72°C for 40 s, with a final elongation step for 7 min at 72°C. Both regions (D1–D3 and D3–D5) of *28S* rDNA were amplified with an initial denaturation of 3 min at 94°C, followed by 35 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 70 s, and a final elongation for 7 min at 72°C. The *18S* rDNA fragment was amplified using the same protocol for *28S* rDNA except the denaturation and annealing steps were lengthened to 45 s each.

All polymerase chain reactions (PCRs) were performed on a Bio-Rad PTC-0200 DNA Engine thermal cycler, using

Table 1. Exemplars used in this study, including location of collection and the genes that were amplified for each taxon. Exemplars are divided by the putative containing lineages as hypothesized prior to the study.

Exemplar	Internal voucher number	*28S* rDNA			*CAD* CPSase			Country collected from
		D1D3	D3D5	18S	54	apmod	acc	
ICHNEUMONIDAE – *out-groups*								
Odontocolon albotibiale (Bradley) (XORIDINAE)	DM054	×	×	×	×	×	×	U.S.A.
Baryceros texanus (Ashmead) (CRYPTINAE)	DM057	×	×	×	–	–	×	U.S.A.
Zagryphus nasutus (Cresson) (TRYPHONINAE)	DM059	×	×	×	–	×	–	U.S.A.
Pimpla sp. (PIMPLINAE)	DM094	×	×	×	–	×	×	U.S.A.
Dusona sp. Cameron (CAMPOPLEGINAE)	DM095	×	×	×	×	×	×	U.S.A.
BRACONIDAE – Helconoid complex								
HELCONINAE – Helconini								
Wroughtonia sp.1	BJS001	×	×	×	×	×	×	U.S.A.
Wroughtonia ferruginea (Brues)	BJS013	×	×	×	×	×	–	U.S.A.
Wroughtonia ligator (Say)	BJS017	×	×	×	×	×	×	U.S.A.
Wroughtonia sp.4	BJS022	×	×	×	×	×	×	U.S.A.
Eumacrocentrus americanus	BJS012	×	×	×	×	×	×	U.S.A.
Helcon texanus	BJS015	×	×	×	–	–	–	U.S.A.
Helcon tardator	BJS095	×	×	×	×	×	×	France
Helcon sp.3	BJS108	×	×	×	×	×	–	Chile
Helcon sp.4	BJS110	×	×	×	×	×	×	Chile
Helcon sp.5	BJS043	×	×	×	×	×	×	Australia
Helcon sp.6	BJS045	×	×	×	×	×	–	Australia
Helcon sp.7	BJS102	×	×	×	×	×	×	Australia
Austrohelcon inornatus	BJS103	×	×	×	×	×	–	Australia
Topaldios sp.	BJS040	×	×	×	×	–	×	Chile
Helconini gen. sp.1	BJS098	×	×	×	×	×	×	Australia
Calohelcon sp.	BJS093	×	×	×	×	×	×	Australia
Ussurohelcon nigricornis	BJS044	×	×	×	×	–	×	Thailand
HELCONINAE – Diospilini								
Diospilini gen. sp.1	BJS099	×	×	×	–	×	×	Australia
Taphaeus sp.1	BJS018	×	×	×	×	×	×	France
Diospilus sp.2	BJS020	×	×	×	×	×	×	France
Diospilus sp.3	JS059	×	×	×	–	–	×	Colombia
Diospilus sp.4	JS093	×	×	×	×	×	×	Panama
Diospilus sp. 5	BJS014	×	×	×	–	–	×	Madagascar
Baeacis sp.1	JS091	×	×	×	×	×	×	Panama
Baeacis sp.2	BJS007	×	–	×	–	–	×	Madagascar
Schauinslandia sp.2	BJS046	×	×	×	–	–	–	Australia
Vadumasonium sp.1	BJS087	×	×	×	×	×	×	U.S.A.
Diospilini gen. sp.3	BJS048	×	×	×	×	×	×	Mexico
HELCONINAE – Brachistini								
Eubazus sp.1	BJS003	×	×	×	×	–	×	Colombia
Eubazus (*Calyptus*) sp.2	BJS029	×	×	×	×	×	×	Colombia
Eubazus (*Aliolus*) sp.3	BJS011	×	×	×	×	×	×	Japan
Eubazus (*Allodorus*) sp.4	BJS024	×	×	×	×	×	×	U.S.A.
Eubazus (*Brachistes*) sp.5	BJS026	×	×	×	×	×	×	Costa Rica
Eubazus (*Brachistes*) sp.6	BJS034	×	–	×	×	×	–	France
Eubazus (*Aliolus*) sp.7	BJS010	×	×	×	×	×	×	U.S.A.
Eubazus (*Aliolus*) sp.8	BJS019	×	×	×	×	×	×	U.S.A.
Eubazus (*Aliolus*) sp.9	BJS037	×	×	×	×	×	–	Costa Rica
Schizoprymnus sp.1	BJS008	×	×	×	×	×	×	South Africa
Schizoprymnus sp.2	BJS021	×	×	×	×	×	×	China
Schizoprymnus sp.3	BJS023	×	×	×	–	×	×	U.S.A.
Nealiolus sp.	BJS025	×	×	×	×	×	×	U.S.A.
Triaspis sp.1	BJS027	×	×	–	–	×	×	South Africa
Triaspis sp.2	BJS036	×	–	×	×	×	×	U.S.A.
HELCONINAE – Brulleiini								
Flavihelcon distanti (Turner)	BJS085	×	×	×	×	×	×	Malawi
Brulleia sp.	BJS113	×	×	×	×	×	×	Thailand

Table 1. Continued

Exemplar	Internal voucher number	*28S* rDNA			*CAD* CPSase			Country collected from
		D1D3	D3D5	18S	54	apmod	acc	
ACAMPSOHELCONINAE								
Urosigalphus sp.1	BJS030	×	×	×	×	×	–	U.S.A.
Urosigalphus sp.2	BJS086	×	×	×	–	×	×	Mexico
Urosigalphus sp.3	DM084	×	×	×	×	×	×	U.S.A.
BLACINAE								
Grypokeros sp.1	BJS112	×	×	×	×	×	×	Chile
Grypokeros sp.2	JS214	×	×	×	–	×	–	Chile
Apoblacus sp.	JS211	×	×	×	×	×	–	Chile
Blacus sp.1	DM011	×	×	×	×	×	×	U.S.A.
Blacus sp.2	JS102	×	×	×	×	×	–	Panama
METEORIDEINAE								
Meteoridea sp.1	DM087	×	×	×	×	×	×	Thailand
Meteoridea sp.2	JS228	×	×	×	×	×	–	Thailand
XIPHOZELINAE								
Xiphozele sp.	ZOO35	×	×	×	×	×	×	Thailand
MACROCENTRINAE								
Macrocentrus sp.	DM089	×	×	×	×	×	×	U.S.A.
Hymenochaonia sp.	JS008	×	×	×	×	×	×	U.S.A.
HOMOLOBINAE								
Homolobus sp.	JS027	×	×	×	×	–	×	U.S.A.
CHARMONTINAE								
Charmon cruentatus Haliday	JS012	×	×	×	×	×	×	U.S.A.
ORGILINAE								
Stantonia sp.	JS017	×	×	×	×	×	×	U.S.A.
Orgilus sp.	JS147	×	×	×	×	×	×	South Africa
AMICROCENTRINAE								
Amicrocentrum concolor (Szépligeti)	JS276	×	×	×	×	×	×	Malawi
MICROTYPINAE								
Microtypus wesmaelii Ratzeberg	JS261	×	×	×	×	×	×	England
CENOCOELIINAE								
Capitonius chontalensis (Cameron)	KP011	×	×	×	×	×	×	Costa Rica
MAXFISCHERIINAE								
Maxfischeria anic Boring	BJS114	×	×	×	×	–	×	Australia
Maxfischeria folkertsorum Boring	BJS115	×	×	×	×	×	×	Australia
Maxfischeria ameliae Boring	BJS116	×	×	×	×	×	×	Australia
Maxfischeria tricolor Papp	BJS117	×	×	×	–	–	×	Australia
Maxfischeria ovamancora Boring	BJS089	×	×	×	×	–	×	Australia
BRACONIDAE – Sigalphoid complex								
AGATHIDINAE								
Cremnops montrealensis (Morrison)	JS031	×	×	×	×	×	–	U.S.A.
Bassus annulipes (Cresson)	JS046	×	×	×	×	×	×	U.S.A.
Earinus limitaris (Say)	JS106	×	×	×	×	×	×	Canada
SIGALPHINAE								
Minanga serrata Cameron	JS209	×	×	×	×	–	×	South Africa
BRACONIDAE – Euphoroid Complex								
EUPHORINAE								
Euphorinae gen. sp.1	BJS035	×	×	×	×	×	×	U.S.A.
Planitorus sp.	BJS101	×	×	×	×	×	–	Australia
Mannokeraia sp.1	BJS100	×	×	×	×	×	×	Australia
Mannokeraia sp.2	BJS104	×	×	×	×	×	×	Australia
Mannokeraia sp.3	BJS105	×	×	×	×	×	×	Australia
Leiophron sp.	JS068	×	×	×	×	×	×	Colombia
Perilitus sp.	JS124	×	×	×	×	–	–	Madagascar
METEORINAE								
Meteorus sp.3	BJS111	×	×	×	×	×	×	Chile
Meteorus sp.1	BJS107	×	×	×	×	×	×	Thailand
Meteorus sp.2	JS010	×	×	×	×	–	×	U.S.A.

Table 1. Continued

Exemplar	Internal voucher number	*28S* rDNA			*CAD* CPSase			Country collected from
		D1D3	D3D5	18S	54	apmod	acc	
ECNOMIINAE								
Ecnomios sp.	JS001	×	×	×	×	×	×	Madagascar
NEONEURIINAE								
Kollasmosoma sp.	JS220	×	×	×	–	–	–	Spain
BRACONIDAE – Microgastroid complex sensu lato								
MENDESELLINAE								
Epsilogaster sp.	JS252	×	×	×	×	×	×	Guyana
CHELONINAE								
Phanerotoma sp.	DM072	×	×	×	–	×	×	Colombia
CARDIOCHILINAE								
Cardiochiles sp.	JS034	×	×	×	×	×	×	Colombia
KHOIKHOIINAE								
Khoikhoia sp.	JS165	×	×	×	–	×	×	South Africa
MICROGASTRINAE								
Snellenius sp.	JS078	×	×	×	×	×	–	Colombia
Microplitis sp.	DM037	×	×	×	×	–	×	
Fornicia sp.	JS222	–	–	×	×	×	×	Thailand
MIRACINAE								
Miracinae gen. sp.	JS272	×	×	×	×	×	×	
ICHNEUTINAE								
Ichneutes sp.	DM090	×	×	×	×	×	×	U.S.A.
Proterops nigripennis Wesmael	JS003	×	×	×	×	×	×	U.S.A.
Muesonia straminea Sharkey & Wharton	JS042	×	×	×	×	×	×	Colombia
BRACONIDAE – Cyclostome lineage								
APHIDIINAE								
Ephedrus sp.	JS207	×	×	×	×	–	×	France
Pseudopraon sp.	JS208	×	×	×	×	×	×	France
MESOSTOINAE								
Andesipolis sp.	JS225	×	×	×	×	×	×	Chile
Aspilodemon sp.	JS007	×	×	×	×	–	–	Colombia
Hydrangeocola sp.	JS054	×	×	×	×	×	×	Colombia
ROGADINAE								
Aleiodes sp.	DM070	×	×	×	×	×	×	Colombia
Clinocentrus sp.1	JS058	×	×	×	×	–	×	Colombia
Clinocentrus sp.2	ZOO8	×	×	×	×	×	×	U.S.A.
Macrostomion sp.	JS079	×	×	×	×	×	×	Colombia
Cystomastax sp.	JS069	×	×	×	×	×	×	Colombia
Polystenidea sp.	JS024	×	×	×	×	×	×	U.S.A.
Conobregma sp.	ZOO27	×	×	×	×	×	×	Dominican Republic
DORYCTINAE								
Doryctes anatolikus Marsh	DM086	×	×	×	×	×	×	U.S.A.
Doryctes sp.	ZOO12	×	×	×	×	×	×	U.S.A.
Leluthia sp.	ZOO18	×	×	×	×	×	×	Colombia
Liobracon sp.	ZOO20	×	×	×	×	×	×	Kenya
Heterospilus sp.1	ZOO11	×	×	×	×	–	–	U.S.A.
Heterospilus sp.2	DM103	×	×	×	×	–	–	U.S.A.
Notiospathius sp.	DM071	×	×	×	×	×	×	Colombia
RHYSIPOLINAE								
Rhysipolis sp.1	DM081	×	×	×	×	×	×	U.S.A.
Rhysipolis sp.2	JS243	×	×	×	×	×	×	Hungary
BRACONINAE								
Bracon sp.	DM073	×	×	×	×	×	×	Colombia
Cyanopterus sp.	ZOO23	×	×	×	–	–	×	U.S.A.
Vipio texanus (Cresson)	JS005	×	×	×	–	–	–	U.S.A.
Hemibracon sp.	JS086	×	×	×	–	–	×	Panama
EXOTHECINAE								
Colastes sp.	JS081	×	×	×	×	×	×	Panama
Shawiana sp.	JS195	×	×	×	×	–	×	U.S.A.

Table 1. Continued

Exemplar	Internal voucher number	*28S* rDNA			*CAD* CPSase			Country collected from
		D1D3	D3D5	18S	54	apmod	acc	
OPIINAE								
Opius sp.	JS025	×	×	×	×	–	×	Colombia
ALYSIINAE								
Hoplitalysia slossonae Ashmead	JS029	×	×	×	×	×	×	U.S.A.
HORMIINAE								
Hormius sp.	JS094	×	×	×	–	×	×	Panama
RHYSSALINAE								
Histeromerus canadensis	JS202	×	×	×	–	×	×	U.S.A.
Oncophanes sp.	JS023	×	×	×	×	×	×	U.S.A.
Acrisis sp.	DM100	×	×	×	–	×	×	U.S.A.
Dolopsidea sp.	JS223	×	×	×	–	–	×	U.S.A.
PAMBOLINAE								
Pambolus sp.	DM074	×	×	×	×	×	×	U.S.A.
Pseudorhysipolis sp.	JS082	×	×	×	×	–	×	Colombia
GNAMPTODONTINAE								
Pseudognaptodon sp.	JS020	×	×	×	–	×	×	U.S.A.
Total taxa amplified		138	135	138	114	109	116	

An x indicates that the gene region was amplified, whereas a dash indicates that the gene was not amplified.

0.2–1 μg DNA extract, 1 X Standard *Taq* Buffer (New England Biolabs, Ipswich, MA, U.S.A.) (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$), 200 μM dNTP, 4 mM $MgSO_4$, 400 nM of each primer, 1 unit of *Taq* DNA polymerase (New England Biolabs) and purified water to a final volume of 25 μL. Product purification was performed using Agencourt CleanSEQ magnetic beads, and sequencing was carried out using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, U.S.A.), with reaction products analysed on an Applied Biosystems 3730xl DNA Analyzer. Contigs were assembled and edited using Contig Express (Vector NTI Advance10™; Invitrogen, Carlsbad, CA, U.S.A.). Sequences were deposited in GenBank under accession numbers JF979544–JF980292.

Multiple sequence alignment

The rDNA genes were aligned based on a secondary structure model for Ichneumonoidea developed by Yoder & Gillespie (2004) and Gillespie *et al.* (2005). Some modifications were made to the model to adjust for taxon-specific indels. A model was also determined for the D1 expansion region of *28S* rDNA using the Mfold web server (Zuker, 2003) and the methods outlined in Gillespie (2004). The alignment model delimits regions of alignment ambiguity (RAAs), regions of slipped-strand compensation (RSCs), and regions of expansion and contraction (RECs) (see Gillespie, 2004 for details on the determination of ambiguous regions), which are typically excluded from analyses as the homology statements might not be valid (Kjer, 1995; Hickson *et al*., 2000). For simplicity, all of these regions are hereafter referred to as RAAs. A significant level of phylogenetic information is potentially lost with the exclusion of these often variable, but informative regions (Gatesy *et al*., 1993). Automated multiple sequence alignment programs can have difficulty aligning highly variable regions because of the extreme length variability across taxa (Thompson *et al*., 1999; Hickson *et al*., 2000). However, several regions of alignment ambiguity in Ichneumonoidea have a similar distribution of sequence length across all taxa (Table S2), and thus may be more amenable to accurate automated alignment (Thompson *et al*., 1999). Therefore, we included RAAs (as determined in the secondary structure model) if the sequence length across all taxa had a standard deviation of less than one. This means that most sequences had a length within one base pair of the mean, and thus, were relatively similar in length across all taxa. These regions were then aligned using MAFFT (Katoh *et al*., 2002) under default settings on the European Bioinformatics Institute (EBI) server. The cut-off is arbitrary in that the alignment accuracy was not tested using different cut-off levels of sequence length variability. However, it provides a distinct criterion and repeatable method for including some RAAs, and a compromise between accuracy and resolution reduction. For the protein-coding genes, alignment was performed using MAFFT (Katoh *et al*., 2002) on the EBI server, and hand corrected in BioEdit (Hall, 1999) for reading frame accuracy. Protein-coding alignments were run through GBLOCKS 0.91b (Castresana, 2000) under default settings to remove regions with low sequence conservation. This treatment effectively removed all introns and uninformative indels.

Phylogenetic analyses

Tests for base composition homogeneity were performed in PAUP* 4.0b10 (Swofford, 2000) using the PAUPUP graphical interface (Calendini & Martin, 2005). Base composition of different gene partitions were calculated in MEGA 4.0 (Tamura *et al*., 2007). To determine the best-fitting model of nucleotide substitution for each gene and partition, MODELTEST 3.8 on

the ModelTest Server (Posada & Crandall, 1998; Posada, 2006) was used with PAUP* (Swofford, 2000) to determine the best-fitting model of nucleotide substitution using hierarchical likelihood ratio tests (hLRTs). To determine the most appropriate partitioning strategy, Bayes factors were calculated as $2\ln(H_1 - H_0)$, where H_1 is the harmonic mean likelihood of the strategy with the greater number of partitions (after Nylander *et al*., 2004). The more complex partitioning strategy was considered in favour of the alternative when the Bayes factors were >10 (following Kass & Raftery, 1995). The different strategies and model selection for each partition are listed in Tables S3 and S4, respectively. The Bayes factors comparing the harmonic mean likelihoods of each partitioning strategy is listed in Table S5. Bayesian inference was performed using MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001). All Bayesian analyses were performed with two independent searches and four chains. Convergence diagnostics, parameter values, appropriate mixing and suitable burn-in values were examined using TRACER v.15 (Rambaut & Drummond, 2009). Among partition rate variation was modelled and parameters were unlinked across all partitions. In more partitioned strategies, a failure to reach convergence was always associated with distorted rate multipliers in certain partitions. In these cases the analyses were rerun with an exponential branch length prior of 100 (following Marshall, 2010). Using the best-fit partitioning strategy (strategy E; Table S5), a final Bayesian analysis of the concatenated dataset was run for 10 000 000 generations, with the priors set as previously described. The same was completed for the dataset with the third position removed for protein-coding genes because of base compositional heterogeneity in these partitions (strategy I; Table S5). For comparison, an additional analysis was run with all RAAs excluded, in addition to third codon positions (strategy J; Table S5). After discarding trees for the burn-in phase, trees and branch lengths were summarized from the two independent searches with a majority rule consensus. Bayesian inference of individual genes was also completed using the same methods. The maximum likelihood analysis was performed on the concatenated dataset with all data included under the general time-reversible model, with a parameter for invariant sites and rate heterogeneity modelled under a gamma distribution (GTR + I + G), using GARLI 1.0 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl, 2006a, b) with default parameters on the CIPRES Science Gateway 3.0 (Miller *et al*., 2009). Twenty separate runs were performed and the tree with the highest log likelihood was chosen. A bootstrap analysis with 100 pseudoreplications was also performed using GARLI on the CIPRES Science Gateway. Data files are available at the Dryad digital repository (http://datadryad.org) under DOI: 10.5061/dryad.1688p.

Results

Gene statistics

Of the 139 taxa examined in this study, 58% were amplified for all six gene regions, 85% for at least five regions, and 96% for at least four regions (Table 1). The final concatenated dataset had an aligned length of 3982 bp (with included regions of ambiguity) of which 42% of the sites were parsimony informative (PI) (Table S6). Among the individual gene datasets, *CAD54* had the greatest number of PI sites, even with the third position removed. Generally, the other two protein-coding gene regions (*ACC* and *CADap*) were more conserved. Both regions lost a significant portion of informative sites when the third position was removed (Table S6). Of the rDNA genes, *28S* had the greatest percentage of PI sites. Most of the variability was in the D2–D3 regions, which has been the most widely used amplicon for braconid systematics (Belshaw *et al*., 1998; Belshaw & Quicke, 2002; Zaldivar-Riverón *et al*., 2006, 2008). When all of the regions of ambiguity were excluded, 145 parsimony informative sites were lost, although the percentage of PI sites increased, suggesting that at least some of the included regions of ambiguity were relatively conserved.

All protein-coding genes demonstrated heterogeneity in base composition when all data were included (Table S7). However, the null hypothesis of base composition homogeneity was accepted when the third position was removed, suggesting saturation in third position sites for all protein-coding genes. Comparing the stem and ambiguous regions, only the stems of *28S* exhibited heterogeneity in base composition (Table S7). Non-stationarity in the stems affected the chi-square tests for all sites, which also exhibited heterogeneity across taxa. Given that G–T interactions are common in rDNA, some taxa may have exhibited higher G–T content among paired sites in stem regions, whereas other taxa retained higher A–T content at these same sites, potentially causing the test for homogeneity to fail. Although the doublet model accounts for some heterogeneity in stem regions of the secondary structure, it was not used here because of the higher computational times associated with this model. All regions of *18S* exhibited homogeneity in base composition across taxa (Table S7). Interestingly, the regions of ambiguity included (Table S2) demonstrated base composition stationarity (Table S7), suggesting the higher A–T composition in these regions was relatively consistent across all taxa.

Concatenated analysis

The best partitioning strategy with the third codon positions removed was a ten-partition analysis (strategy I; Tables S3 and S5). Bayesian inference of this concatenated dataset recovered a fairly resolved and well-supported topology with a monophyletic Braconidae (Fig. 2), demonstrating the following higher-level relationships: [non-cyclostomes (Aphidioid complex + cyclostomes sensu stricto)]. All three lineages were strongly supported in the Bayesian analysis (posterior probability, PP > 0.95), although the non-cyclostomes were not supported in the bootstrap analysis. The aphidioid complex consisted of Mesostoinae (Aphidiinae + *Maxfischeria* Papp). The sister relationships between the aphidioid complex and the cyclostomes were consistent with the findings

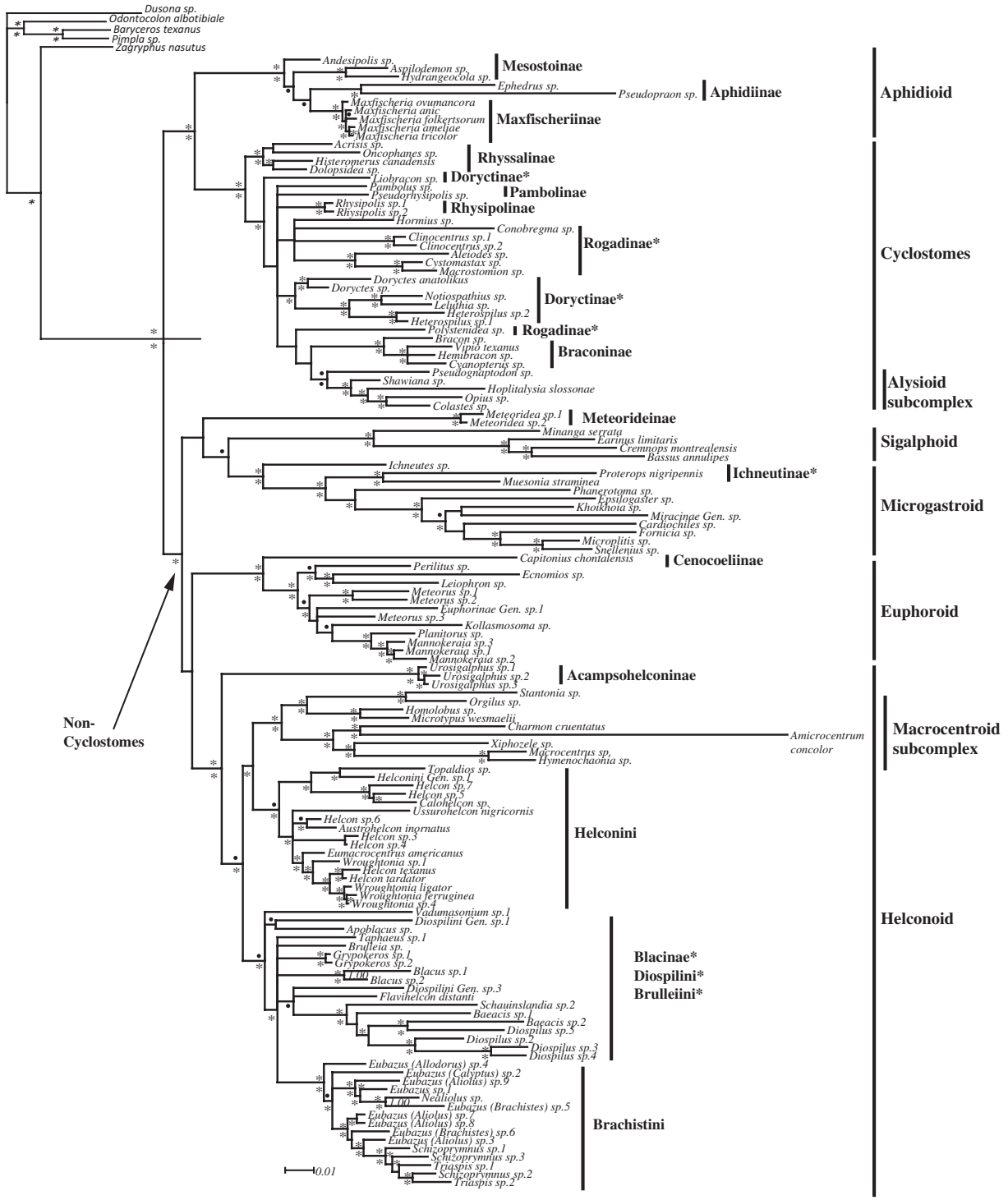

Fig. 2. Inferred topology from the Bayesian analysis of all genes concatenated [with the third position removed, and with regions of ambiguity (RAAs) of the rDNA genes excluded if the standard deviation of sequence length across all taxa was greater than 1; strategy I, Table S4]. Posterior probabilities are indicated below by an asterisk if the posterior probabilities were $\geq$0.95, or by a black dot if the posterior probabilities were between 0.90 and 0.94. Bootstrap values from the likelihood analysis are indicated above the node, with an asterisk if the value was over 70 and a black dot if the value was between 50 and 69. The out-group branches were moved to the right because of space limitations; however, the proper scale is retained.

of Zaldivar-Riverón (2006) and Dowton *et al*. (2002), who also recovered Mesostoinae + Aphidiinae as sister to the cyclostomes (*Maxfischeria* was not included in either analysis).

Within the aphidioid complex, only *Maxfischeria* and Aphidiinae were recovered as monophyletic (Fig. 2). The likelihood analysis recovered Mesostoinae as sister to *Maxfischeria* (Figure S1), whereas all Bayesian analyses recovered Aphidiinae + *Maxfischeria* (Fig. 2; Figs S2, S3). Among the cyclostome subfamilies with multiple representatives (further referred to as the cyclostome complex), only Braconinae, Rhyssalinae and Rhysipolinae were recovered as monophyletic with robust support (PP $\geq$ 0.95). Additionally, there was strong evidence suggesting Opiinae, Alysiinae and Exothecinae are closely related, with Gnamptodontinae as sister to these subfamilies. These four subfamilies are further referred to as the alysioid subcomplex. Braconinae was recovered as the sister group to the alysioid complex, similar to the findings of Dowton *et al*. (2002) and Zaldivar-Riverón (2006), although this relationship was weakly supported. Generally, there was a lack of resolution between subfamilies within the cyclostomes, which may be due to the lower taxonomic sampling of cyclostome subfamilies. Rogadinae (including *Conobregma* van Achterberg) was recovered as a distinct clade, but did not include *Polystenidea* Viereck (Fig. 2). With the exception of *Liobracon* Nason, the rest of the doryctine exemplars were recovered in a strongly supported clade.

Among the non-cyclostome subfamilies, a clade containing Meteorideinae (sigalphoid + microgastroid complexes) was recovered as sister to the remaining non-cyclostomes (Fig. 2). However, the position of Meteorideinae was variable across the various concatenated and individual gene analyses (Figs S1–S13), probably leading to the low support for this clade. The microgastroid complex was recovered as monophyletic, including all Ichneutinae. However, Ichneutinae was recovered as paraphyletic, but sister to all remaining microgastroid subfamilies (Fig. 2). Among the remaining microgastroid subfamilies, the branching order was variably supported, although there was strong evidence indicating Cheloninae as the sister lineage to the other subfamilies, similar to several other studies (Whitfield & Mason, 1994; Whitfield, 1997; Dowton & Austin, 1998; Dowton *et al*., 1998; Belshaw *et al*., 2000; Banks & Whitfield, 2006; Murphy *et al*., 2008). Sigalphinae + Agathidinae (referred to as the sigalphoid complex by Belshaw & Quicke 2002) was recovered as the sister group to the microgastroid complex, suggesting the sigalphoid subfamilies do not belong within the helconoid complex. The sister relationship between the sigalphoid and microgastroid complexes was consistent across all concatenated analyses (Fig. 2; Figs S1–S3), although the support for this clade varied widely. Both the sigalphoid and microgastroid complexes were strongly supported (PP $\geq$ 0.95; bootstrap $>$ 70).

Of the remaining non-cyclostomes, the euphoroid complex was recovered as sister to the helconoid complex, albeit with low nodal support (Fig. 2). The phylogenetic placement of the euphoroid complex demonstrated the greatest variability across the different concatenated analyses, occasionally recovered in a polytomy with Meteorideinae and the sigalphoid and microgastroid complexes (Figs S2, S3). The euphoroid complex was recovered with Cenocoeliinae as sister to all remaining euphoroid subfamilies with strong support (PP = 1.0; bootstrap = 100). However, neither Euphorinae nor Meteorinae (the only subfamilies with multiple representatives) were recovered as monophyletic. Within the euphoroid complex, *Ecnomios* Mason (Ecnomiinae) was recovered with other euphorine exemplars with strong support, suggesting that this subfamily should be included within Euphorinae (Fig. 2). This was also true of Neoneurinae. Neoneurinae was recovered as the sister taxon to a strongly supported clade consisting of *Planitorus* + *Mannokeraia*. Although the unusual genera *Planitorus* and *Mannokeraia* have previously been considered as part of the Betylobraconinae and Masoninae, respectively (van Achterberg, 1995), a recent analysis suggested that *Mannokeraia* was more closely related to the Euphorinae (Belshaw & Quicke, 2002). The genus *Planitorus* is also suspected of having a close relationship to Euphorinae (D.L.J. Quicke, personal communication). These suggestions are confirmed here, as both genera are consistently recovered as sister taxa within the Euphorinae sensu lato (including Meteorinae, Ecnomiinae and Neoneurinae).

The helconoid complex was consistently recovered and strongly supported (PP $\geq$ 0.95) in all concatenated analyses (Fig. 2; Figs S1–S3). Acampsohelconinae was recovered as the sister group to all other members of the helconoid complex. Of the remaining subfamilies, three distinct clades were recovered, all with strong support in both the Bayesian and likelihood analyses. One clade included the subfamilies Orgilinae, Homolobinae, Microtypinae, Charmontinae, Amicrocentrinae, Xiphozelinae and Macrocentrinae. These subfamilies are similar morphologically and biologically (all endoparasitoids of Lepidoptera), and have variably been placed together in different classification schemes for Braconidae (van Achterberg, 1984). For ease of discussion, this clade is hereafter referred to as the macrocentroid subcomplex. There was also strong evidence indicating that the latter four subfamilies (Charmontinae, Amicrocentrinae, Xiphozelinae and Macrocentrinae) are closely related (PP = 1.0; bootstrap = 93). Additionally, Orgilinae was recovered as sister to Homolobinae + Microtypinae with robust support (PP = 1.0; bootstrap = 99).

The second clade consisted of members of Helconini (Helconinae). Helconini (including *Ussurohelcon* Belokobylskij and *Topaldios* Papp) was consistently recovered in a strongly supported clade in all concatenated and numerous individual gene analyses (Figs S1–S13). Helconini was also consistently recovered as the sister group to the macrocentroid complex with strong support in all concatenated Bayesian analyses (Fig. 2; Figs S2, S3). This relationship was also recovered in the maximum likelihood analysis (Figure S1); however, it was not supported in the bootstrap analysis. The third clade contained members of Blacinae and the remaining helconine tribes (Brachistini, Brulleiini and Diospilini). Within this clade, Brachistini (Helconinae) was recovered as monophyletic with strong support across concatenated and individual gene analyses (PP $\geq$ 0.95; bootstrap = 100).

However, the other tribes and subfamilies were consistently polyphyletic.

Individual gene analyses

All four genes were analysed individually using Bayesian inference, with the two gene fragments of *CAD* analysed separately as they are non-contiguous fragments. Both *18S* and *28S* genes were analysed once with regions of ambiguity included ($\sigma < 1$), and once with these regions excluded from the analysis. Additionally, each protein-coding gene was analysed with all data included, and a second time with the third position excluded given the heterogeneity in base composition for this codon position (Table S7). All gene trees are provided in Figs S4–S13. Recovered clades across the individual gene trees and for the concatenated analyses are summarized in Table 2. The exclusion of all ambiguous regions resulted in less resolution among the non-cyclostomes, whereas resolution was reduced within the cyclostomes when the ambiguous regions were included.

Discussion

Utility of protein-coding markers

The phylogenetic utility of *CAD*, or rudimentary, has been well-documented in other insects (Moulton & Wiegmann, 2004; Winterton & de Freitas, 2006; Desjardins *et al*., 2007; Praz *et al*., 2008; Wild & Maddison, 2008). Of the two regions of *CAD* used here, the small chain fragment of the CPSase region (*CAD54*) is considerably more informative for resolving relationships among braconid subfamilies (Table 2). Both regions demonstrate heterogeneity in base composition in the third position, potentially indicating saturation. The large chain of the CPSase region of *CAD* (*CADap*) may have greater phylogenetic utility for higher-level relationships than those analysed here. For subfamilial relationships within the Braconidae, *ACC* seems to be too conserved to have any significant resolving power, particularly when the third position is removed. However, the slow rate of evolution and ease of amplification and alignment of this gene fragment may be advantageous for higher-level phylogenetic studies of the Hymenoptera and other insect orders. Given the extensive diversity within Braconidae, future studies should employ greater taxonomic sampling with additional nuclear protein-coding markers to test the relationships recovered in this dataset.

Phylogenetic implications

The position of the aphidioid complex within the braconid phylogeny obfuscates the meaning of the informal terms ‘cyclostome’ and ‘non-cyclostome’. Within the aphidioid complex, some members of Mesostoinae appear to possess the cyclostome condition, but in other genera the condition is less obvious. Members of Aphidiinae do not have a depressed labrum, although previous researchers have suggested that members of Aphidiinae must have secondarily lost the cyclostome condition, given their apparent sister relationship with Mesostoinae recovered in previous analyses (Belshaw *et al*., 1998, 2000; Dowton *et al*., 2002; Zaldivar-Riverón *et al*., 2006). Sharkey (1993) was the first to suggest that Aphidiinae was related to the cyclostomes by the presence of a smooth, glabrous labrum, similar features in the hindwing, and a biology that includes host mummification, similar to Rogadinae. However, the inclusion of *Maxfischeria*, which is also non-cyclostome, highlights the need for further study on labrum morphology within the aphidioid complex, and within Braconidae in general.

The question remains as to whether we can consider the aphidioid complex + cyclostomes as having a cyclostome ancestor (perhaps referred to as cyclostomes sensu lato), or whether the ancestor to all braconids was non-cyclostome. If the latter is true, then the evolution of several morphological and biological features within Braconidae need to be re-examined without typical a priori assumptions of character polarity. Additionally, the recognition of cyclostomes sensu stricto would render the non-cyclostomes paraphyletic. It is clear that the classification of Braconidae, in terms of informal group names between subfamily and family, needs to be revised to better reflect phylogenetic relationships. Such revisions are likely to be best if based on combined molecular and morphological data. However, as this work is based strictly on molecular data, we retain the usage of the commonly used names based on mouthpart morphology, and refer to the cyclostomes sensu stricto (not including members of the aphidioid complex) as the cyclostome complex. Based on the individual gene analyses, and on the concatenated dataset, there is increased support for several previously proposed relationships and strong evidence for several new relationships among braconid subfamilies. These relationships are summarized below and in Table 3.

Non-cyclostomes

The non-cyclostomes include the following complexes: microgastroid, sigalphoid, euphoroid, helconoid and, within the helconoid complex, the macrocentroid subcomplex (Table 3). Based on a lack of evidence, the subfamilies Meteorideinae and Masoninae (the latter not analysed in this study) are not placed within any complex. Previous molecular studies have demonstrated members of Trachypetinae to be sister to all remaining braconids (Belshaw *et al*., 1998, 2000; Belshaw & Quicke, 2002; Dowton *et al*., 2002; Pitz *et al*., 2007). However, long, hypervariable indels and base compositional biases have been suspected to contribute to this placement (Dowton & Austin, 1997; Belshaw *et al*., 1998; Belshaw & Quicke, 2002). Upon future sequencing and morphological efforts it is possible that Trachypetinae will also be included within the non-cyclostomes, but is currently designated as unplaced (Table 3).

Table 2. Summary of recovered nodes for individual gene trees and the concatenated analyses for major clades of interest.

Associated figure	Gene region	CYAP	CYCL	APHI	ALYS	NOCY	MSM	SIMI	SIGA	MISL	EUPH	EUHE	HELC	MACR	OHM	CAXM	MAHE	HELI	BRAC	BBBD
2	Concatenated: RAA-IN; 3-OUT	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
S1	Concatenated RAA-IN; Likelihood	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
S2	Concatenated: RAA-OUT; 3-OUT	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
S3	Concatenated: RAA-IN;	×	×	×	×	×		×	×	×	×		×	×	×	×	×	×	×	×
S4	*28S*: RAA-IN	×	×		×	×			×	×	×				×			×	×	×
S5	*28S*: RAA-OUT	×	×	×	×				×	×	×				×			×		
S6	*18S*: RAA-IN																	×		
S7	*18S*: RAA-OUT																	×		
S8	*CAD54*	×		×				×	×		×		×					×	×	
S9	*CAD54*: 3-OUT	×		×	×			×	×		×		×	×					×	×
S10	*CADap*								×									×	×	
S11	*CADap*: 3-OUT								×										×	
S12	*ACC*			×												×		×	×	
S13	*ACC*: 3-OUT			×												×				

Recovery of a node is indicated with an x. Clades with a posterior probability ≥0.95 are indicated with a shaded box.

Clades of interest: ALYS, alysioid subcomplex; APHI, aphidioid complex; BBBD, brachistini + blacinae + brulleiini + diospilini; BRAC, Brachistini; CAXM, charmontinae + amicrocentrinae + xiphozelinae + macrocentrinae; CYAP, cyclostome + aphidioid complexes; CYCL, cyclostome complex; EUHE, euphoroid + helconoid complex; EUPH, euphoroid complex; HELC, helconoid complex; HELI, helconini; MACR, macrocentroid subcomplex; MAHE, macrocentroid subcomplex + helconini; MISL, microgastroid complex (including Ichneutinae); MSM, Meteorideinae + sigalphoid complex + microgastroid complex; NOCY, non-cyclostomes; OHM, orgilinae + (Homolobinae + Microtypinae); 3-OUT, third codon position excluded; RAA-IN, regions of ambiguity included if standard deviation of sequence length across all taxa was less than 1; RAA-OUT, all regions of ambiguity excluded; SIGA, sigalphoid complex; SIMI, sigalphoid + microgastroid complexes.

Table 3. Proposed classification for Braconidae.

Family Braconidae
Unplaced subfamilies
Apozyginae
Trachypetinae
Aphidioid complex
Maxfischeriinae
Aphidiinae
Mesostoinae (including *Andesipolis)*
Cyclostome complex
Betylobraconinae
Braconinae (including Vaepellinae)
Doryctinae (including Ypistocerinae)
Hormiinae
Lysiterminae
Pambolinae
Rhyssalinae
Rogadinae including Facitorini
Alysioid subcomplex
Alysiinae
Exothecinae
Gnamptodontinae
Opiinae
Telengainae
Noncyclostomes
Unplaced subfamilies
Meteorideinae
Masoninae
Helconoid complex
Acampsohelconinae
Helconinae (including *Ussurohelcon* and *Topaldios*)
Brachistinae (including Brachistini, Blacini, Brulleiini, Diospilini)
Macrocentroid subcomplex
Amicrocentrinae
Charmontinae
Macrocentrinae
Xiphozelinae
Orgilinae
Homolobinae
Microtypinae
Euphoroid complex
Cenocoeliinae
Euphorinae (including *Planitorus, Mannokeraia,* Ecnomiini, and Neoneurini)
Meteorinae
Sigalphoid complex
Agathidinae
Sigalphinae
Microgastroid complex
Cardiochilinae
Cheloninae (including Adeliinae)
Dirrhopinae?
Ichneutinae
Khoikhoiinae
Microgastrinae
Miracinae

Microgastroid complex

The microgastroid complex has been the subject of numerous studies because of the extensive utility of its members as biological control agents and as a model group to understand the evolution of polydnaviruses (Whitfield, 1997, 2002; Banks & Whitfield, 2006; Murphy *et al*., 2008; Whitfield & Kjer, 2008). The monophyly of the complex has been well supported in numerous molecular analyses (Whitfield, 1997; Belshaw *et al*., 1998; Dowton & Austin, 1998; Dowton *et al*., 1998; Banks & Whitfield, 2006; Murphy *et al*., 2008), although the branching order of the included subfamilies has differed across analyses. Here, the monophyly of the microgastroid complex, including Ichneutinae, is robustly demonstrated across all concatenated analyses and in the *28S* rDNA gene trees (Table 2). However, similar to other molecular analyses (Belshaw *et al*., 2000; Belshaw & Quicke, 2002; Pitz *et al*., 2007), a monophyletic Ichneutinae is never recovered, partially because of the volatile placement of *Ichneutes* across the individual gene trees. The subfamily Dirrhopinae, which has never been analysed with molecular data, is also included in the Microgastroid complex (Table 3) (see Whitfield & Mason, 1994; and Belokobylskij *et al*., 2003 for detailed discussion).

Some of the internal branches within the microgastroid complex are not strongly supported (Fig. 2), a common issue with phylogenetic studies of microgastroid subfamilies (for a detailed discussion, see Murphy *et al*., 2008). However, consistent with previous studies (Belshaw *et al*., 1998, 2000; Dowton *et al*., 1998, 2002; Banks & Whitfield, 2006; Murphy *et al*., 2008), Cheloninae is robustly recovered as a basal lineage within the complex. Additionally, Mendesellinae is consistently recovered as the sister group to a strongly supported clade consisting of Khoikhoiinae, Miracinae, Cardiochilinae and Microgastrinae. The branching order of the latter four subfamilies varies across the concatenated analyses (Fig. 2; Figs S1–S3).

Sigalphoid complex

The robustly recovered sister relationship between Sigalphinae and Agathidinae, called the sigalphoid complex by Belshaw & Quicke (2002), confirms the findings of several recent analyses (Belshaw *et al*., 1998, 2000; Belshaw & Quicke, 2002; Dowton *et al*., 2002; Shi *et al*., 2005; Pitz *et al*., 2007). However, the sister group to this complex has never been robustly recovered and is somewhat under debate (for a detailed discussion, see Sharkey, 1992). In all concatenated analyses and the *CAD54* gene tree, the sigalphoid complex is recovered as sister to the microgastroid complex, although levels of support vary across analyses. There is some morphological evidence to suggest a close affinity between sigalphoid members and Ichneutinae. Sharkey & Wharton (1994) hypothesized that the Agathidinae + Sigalphinae (including *Pselephanus* Szépligeti) were sister to Ichneutinae. However, Ichneutinae has more recently been placed as sister to the microgastroid complex (Belshaw *et al*., 1998, 2000; Dowton *et al*., 2002; Shi *et al*., 2005; Pitz *et al*., 2007; Murphy *et al*., 2008), and this relationship was also recovered here with robust support (Fig. 2).

As an alternative hypothesis, Meteorideinae is recovered as the sister group to the sigalphoid complex in the *CADap* gene

trees (Figs S10, S11), which has stronger support when the third position is removed. A close relationship between the sigalphoid complex and Meteorideinae has been recovered in some morphological (Quicke & van Achterberg, 1990; Wharton *et al*., 1992; Belshaw *et al*., 2003) and molecular analyses (Belshaw & Quicke, 2002), but not consistently. Alternatively, Quicke *et al*. (2008) recovered a paraphyletic Microtypinae as sister to Agathidinae + Sigalphinae (including *Pselephanus*). However, this study was based on only one gene (*28S*), and did not include any representatives from the microgastroid complex. Thus, the sister-group relationship between the sigalphoid and microgastroid complexes remains tentative. However, the majority of the evidence suggests that Meteorideinae is sister to sigalphoid + microgastroid complexes (Fig. 2).

Euphoroid complex

The euphoroid complex, first named by Belshaw & Quicke (2002), is robustly supported in all concatenated analyses (Fig. 2; Figs S1–S3), and in the *28S* and *CAD54* gene trees Figs S4, S5, S8, S9). Based on the analyses performed herein, the following subfamilies are contained within the euphoroid complex: Cenocoeliinae, Ecnomiinae, Neoneurinae, Euphorinae and Meteorinae (Table 3). Although there is only one member of Cenocoeliinae included in the dataset, it was convincingly recovered as the sister group to all remaining subfamilies within the complex (Fig. 2). The relationships among the remaining subfamilies are less clear, in part because of limited taxonomic sampling. The limits of Euphorinae and Meteorinae are not clear from these analyses, and certainly require greater sampling to resolve the question of monophyly of these two subfamilies. The evidence from the current analyses suggests that Meteorinae may need to be included within Euphorinae, supporting the findings of Li *et al*. (2003). Building on the work of Shaw (1985), Maetô (1990) and Chen & van Achterberg (1997), a re-examination of these subfamilies using both morphological and molecular data is desperately needed (a revision of Euphorinae is currently underway; A. Boring, unpublished data). Neoneurinae, represented by only one exemplar in this dataset, clearly falls within Euphorinae, similar to the findings of Belshaw *et al*. (2000), and thus are placed as a tribe within Euphorinae in Table 3. Likewise, Ecnomiinae is consistently recovered with other Euphorine taxa, and is also placed as a tribe within Euphorinae (Table 3).

Van Achterberg (1995) originally placed *Mannokeraia* within Masoninae because of the apterous nature of the originally described female specimens. In the same comprehensive monograph illustrating dozens of unique taxa with modified foretarsi, van Achterberg (1995) described *Planitorus* as a member of Betylobraconinae. However, the strongly supported clade of *Mannokeraia* + *Planitorus* within the euphoroid complex suggests that these interesting genera are members of Euphorinae. The exemplar species of *Planitorus* and *Mannokeraia* used in this dataset (all winged forms) are currently being described by van Achterberg (personal communication). Until the formal revision of these genera is complete, these genera are placed within Euphorinae (Table 3). Masoninae is not included in Euphorinae as *Masona* van Achterberg, the only other genus placed in this subfamily, differs significantly from *Mannokeraia*, as the former lacks pegs on the forelegs and a complete occipital carina (van Achterberg, 1995).

Helconoid complex

The ten remaining subfamilies within the non-cyclostomes (traditionally placed in the helconoid complex) are recovered in a strongly supported clade (Fig. 2) and include: Acampsohelconinae, Blacinae, Helconinae, Amicrocentrinae, Charmontinae, Homolobinae, Macrocentrinae, Microtypinae, Orgilinae and Xiphozelinae (Fig. 2). Acampsohelconinae is recovered as the sister group to the remaining members of the helconoid complex. Thus, there is evidence supporting *Urosigalphus* Ashmead (the only member of Acampsohelconinae analysed here) as being separate from Helconinae or Blacinae, as proposed by van Achterberg (2002). However, the phylogenetic placement of *Urosigalphus* is often unresolved or contradictory across the individual gene trees. The *CAD54* gene tree supports Acampsohelconinae as a basal member of the helconoid complex (Figs S8, S9), but the *28S* gene tree suggests a sister relationship with Meteorideinae (Figs S4, S5). Clearly more taxonomic sampling is needed to resolve the placement of Acampsohelconinae within the non-cyclostomes, particularly as there is some evidence to suggest that Acampsohelconinae is not monophyletic (Quicke *et al*., 2008).

Macrocentroid subcomplex

Unlike all other members within the helconoid complex that parasitize immature Coleoptera, members of the macrocentroid subcomplex utilize lepidopteran hosts (Fig. 2). This distinct lineage is robustly supported in all concatenated analyses, and includes seven subfamilies: Orgilinae, Homolobinae, Microtypinae, Macrocentrinae, Charmontinae, Amicrocentrinae and Xiphozelinae. The latter four subfamilies are recovered in a strongly supported clade in the concatenated analyses (Fig. 2; Figs S1–S3) and in the *ACC* gene trees (Figs S12, S13). Amicrocentrinae is recovered as the sister group to Charmontinae, and Xiphozelinae is recovered as the sister group to Macrocentrinae. Charmontinae, which has variably been placed within Homolobinae (van Achterberg, 1979), Macrocentrinae (Čapek, 1970), Orgilinae (Čapek, 1973) or as its own subfamily (Quicke & van Achterberg, 1990), is never recovered as sister to Homolobinae or Orgilinae. Rather, there is strong evidence placing Charmontinae closer to Macrocentrinae and related subfamilies, as has been suggested by van Achterberg & Quicke (1992), based on ovipositor morphology, and Čapek (1970), based on larval cephalic structures. For simplicity, it may be prudent in the future to demote these four subfamilies (Amicrocentrinae, Charmontinae, Macrocentrinae and Xiphozelinae) to tribes contained within Macrocentrinae,

if future morphological evidence warrants this classification. The relationship Orgilinae (Homolobinae + Microtypinae) is also robustly supported in the *28S* gene trees and the concatenated analyses (Table 2). This sister relationship has also been proposed by a number of authors based on larval and adult morphology and biology (Čapek, 1970; van Achterberg, 1984, 1992). Again, future morphological studies may demonstrate that these subfamilies are a monophyletic clade that might best be considered as tribes within Orgilinae.

Helconinae and Blacinae

The limits of these two subfamilies have never been well defined, with genera from each group variably included within the two different subfamilies (Martin, 1956; van Achterberg, 1988), and with Blacinae often considered as a tribe of Helconinae (van Achterberg, 1975; Sharkey, 1993). In this study, Helconini was recovered as sister to the Macrocentroid subcomplex. However, the validity of this relationship is questionable, as it was not recovered in any of the individual gene trees and lacked bootstrap support in the likelihood analysis. However, Helconini was consistently well supported across several gene trees and was never recovered in a clade with the other Helconine tribes.

The limits of most genera within Helconini are poorly defined or are diagnosed by characters that show continuous variation across the putative genera (Watanabe, 1972). For example, *Wroughtonia* Cameron is diagnosed by the presence of a single blunt tooth on the ventral side of the hind femur, whereas species of *Helcon* Nees typically have a rugulose hind femur. However, there are species with intermediate character states ranging from multiple teeth to heavily rugulose, suggesting a need for a revision of the genera within this tribe (currently underway; B.J. Sharanowski & M.J. Sharkey, unpublished data). From this study, *Helcon* and *Wroughtonia* are paraphyletic with respect to each other. Additionally, the limits of other genera need to be examined in relation to *Helcon*, including *Austrohelcon* Turner and *Eumacrocentrus* Ashmead. Interestingly, *Ussurohelcon* is firmly recovered within Helconini, similar to the findings of Belshaw & Quicke (2002), and thus should be replaced within Helconini, contrary to its placement within Cenocoeliinae as suggested by van Achterberg (1994). *Topaldios* was originally placed within Diospilini (Papp, 1995), but was strongly recovered within Helconini in all concatenated analyses and most individual gene analyses. Species of *Topaldios* have the forewing with 1RS present and a trapezoidal second submarginal cell, which also suggest a placement within Helconini and not Diospilini.

Members of Blacinae were consistently recovered in clades with members of the helconine tribes Diospilini and Brulleiini. These three groups were never recovered as monophyletic in any of the individual gene trees or the concatenated analyses. The two members of Brulleiini analysed here, *Flavihelcon* van Achterberg and *Brulleia* Szépligeti, were never recovered together, and *Flavihelcon* was often recovered with members of Diospilini. Although van Achterberg (1990) suggested that perhaps *Flavihelcon* belongs within Diospilini, he originally placed it and other similar African genera in Pseudohelconina within Brulleiini.

Brachistini was monophyletic and well supported (Fig. 2), but was recovered as a derived lineage from both diospiline and blacine members. With the current understanding of these groups, and based on this study, only Brachistini is monophyletic. Determination of the limits of the remaining three tribes will require further phylogenetic testing and greater sampling of diospiline, brulleiine and blacine taxa (currently underway; B.J. Sharanowski & M.J. Sharkey, unpublished data), as well as *Eadya* Huddleston & Short (see van Achterberg *et al*., 2000 and Belshaw & Quicke, 2002). To rectify the classification of the Helconinae and Blacinae, we propose that Helconini and Brachistini be elevated to subfamily status, with the latter subfamily containing the following four tribes: Brachistini, Diospilini, Blacini and Brulleiini (Table 3). Some taxonomists have used Brachistinae as a subfamily name, although it has never been formally elevated to subfamily subsequent to its placement as a tribe within Helconinae by Mason (1974, also suggested by Čapek, 1970).

Aphidioid complex

Based on the analyses performed herein, the aphidioid complex is established to include Aphidiinae, Mesostoinae and Maxfischeriinae. This lineage is recovered in three individual gene datasets and in all concatenated analyses (Table 2). The monotypic genus *Maxfischeria* Papp is here elevated to the rank of subfamily (see also Boring *et al*., 2011, this issue) and excluded from Helconinae, where it was originally placed (Papp, 1994). The sister group to Maxfischeriinae is not clear. Both Aphidiinae and Mesostoinae are recovered as the sister group in different concatenated analyses and individual gene trees. The aphidiine *Pseudopraon* Starý demonstrates volatility in its placement across the individual gene trees, and may have contributed to the differential branching order recovered across the different analyses. Belshaw *et al*. recovered a monophyletic Aphidiinae with *Pseudopraon* in a well-supported clade with other members of Praini.

The enigmatic genus *Andesipolis* Whitfield and Choi was originally described without placement in a specific subfamily (Whitfield *et al*., 2004). However, recent molecular results suggested placement within Mesostoinae (Zaldivar-Riverón *et al*., 2006). Townsend & Shaw (2009) refuted this placement and suggested *Andesipolis* belonged within Rhysipolinae. Similar to Zaldivar-Riverón *et al*. (2006), we recovered *Andesipolis* with other members of Mesostoinae in the concatenated analysis (Fig. 2), and never with members of Rhysipolinae. However, the monophyly of Mesostoinae including *Andesipolis* remains uncertain, as the placement of *Andesipolis* varied across individual gene trees, occasionally recovered as sister to *Maxfischeria* (Figs S8, S9). The aphidioid complex remains an exciting area of research within Braconidae, and further biological and phylogenetic understanding of this group will have immense implications

for braconid systematics and reconstructions of evolutionary history.

Cyclostome complex

The cyclostome complex is strongly supported in all concatenated analyses (Table 2). However, it is only recovered as monophyletic in the *28S* gene trees (Figs S4, S5). There is also some support for this lineage in the *CAD54* (3-OUT) gene tree (Figure S9), but the position of *Pambolus* Halliday renders this clade paraphyletic. Within the cyclostomes, most of the relationships among the subfamilies lack convincing nodal support. Furthermore, the branching order varies across the different concatenated analyses. These results are probably related to limited taxon sampling among the cyclostome subfamilies (only 32 of the 135 braconid taxa were members of the cyclostomes), and the less successful amplification of the protein coding genes (Table 1).

Alysioid subcomplex

An exception to the instability within the cyclostome complex is a clade consisting of Gnamptodontinae, Exothecinae, Opiinae and Alysiinae, which is robustly recovered in most concatenated analyses (Table 2). This clade is also recovered in the *28S* and *CAD54* (3-OUT) gene trees (Figs S4, S5, S9). This corroborates the findings of several previous analyses (Belshaw *et al*., 1998, 2000; Dowton *et al*., 2002; Wharton *et al*., 2006; Zaldivar-Riverón *et al*., 2006). Contrary to some previous studies (Whitfield, 1992; Quicke, 1993), there is additional evidence suggesting a close relationship between Alysiinae, Opiinae and Exothecinae, to the exclusion of Gnamptodontinae, which are recovered together in several individual gene analyses and in the concatenated analyses (Fig. 2; Figs S1, S5, S9, S12, S13). This also confirms the findings of Wharton *et al*. (2006) who recovered Gnamptodontinae [Exothecinae (Opiinae + Alysiinae)]. In this study, however, the branching order among the latter three subfamilies varied across the different analyses, with Exothecinae often recovered as paraphyletic. Cleary there was not enough taxonomic sampling to fully resolve the relationships within this complex. However, the current composition of the alysioid subcomplex includes Gnamptodontinae, Alysiinae, Exothecinae, Telengaiinae (not analysed here) and Opiinae (Table 3), with Gnamptodontinae (+ Telengaiinae, see Zaldivar-Riverón *et al*., 2006) as the likely sister group to the latter three subfamilies. Based on this research and previous studies (Belshaw *et al*., 1998, 2000; Dowton *et al*., 2002; Wharton *et al*., 2006; Zaldivar-Riverón *et al*., 2006), Braconinae is the sister group to the alysioid subcomplex. However, Braconinae is not included within this subcomplex as there are several morphological features uniting members of the alysioid subcomplex to the exclusion of Braconinae (for a detailed discussion of these features see Quicke, 1993; Wharton *et al*., 2006; and Whitfield, 1992b).

All other subfamilies within the cyclostomes are left as unplaced (Table 3) because of the lack of evidence across the multiple genes used here and in previous studies. A recent study of cyclostome relationships based on *28S* rDNA, morphological data and comprehensive taxonomic sampling recovered Rhyssalinae as the ancestral lineage of the cyclostomes (Zaldivar-Riverón *et al*., 2006). Additionally, this basal placement of Rhyssalinae was indicated using *16S* rDNA (Dowton *et al*., 1998), and was robustly recovered using a combination of *16S* and *28S* rDNA gene fragments (Belshaw *et al*., 2000). This study supports these findings, as two of the four concatenated analyses recovered this lineage as sister to all remaining subfamilies of cyclostomes (Fig. 2; Fig. S2). The two concatenated analyses that recovered Hormiinae as the basal taxon included the third codon position, which probably gave rise to the different result (Figs S1, S3).

Rogadinae, excluding *Polystenidea* sp., is recovered with strong support (Fig. 2). These results are somewhat consistent with previous analyses (Zaldivar-Riverón *et al*., 2006, 2008) that have found weak support for a monophyletic Rogadinae and variable placement of the Stiropiini (which includes *Polystenidea*, the only representative of the tribe in this analysis). However, based on biology (Whitfield, 1988), morphology (Whitfield, 1990) and other recent phylogenetic studies (Chen *et al*., 2003), the placement of *Polystenidea* in this analysis is probably incorrect. The monophyly of Doryctinae has rarely been recovered in molecular analyses, and this study is no exception. Several morphological synapomorphies have been identified for Doryctinae (Quicke & van Achterberg, 1990; Quicke *et al*., 1992a, b; Belokobylskij *et al*., 2004), and thus the inclusion of morphological data into phylogenetic analyses has typically recovered this group as monophyletic (Dowton *et al*., 2002; Zaldivar-Riverón *et al*., 2006). An in-depth phylogenetic examination of Doryctinae, using both morphological and molecular data, remains a fertile area of research. The phylogenetic placement of Apozyginae (not analysed here) remains uncertain, although the retention of the second recurrent vein suggests a basal phylogenetic position within Braconidae (Sharkey & Wahl, 1992). However, future studies may indicate that Apozyginae belongs within the cyclostomes, as members possess the hypoclypeal depression and share many similarities with members of Doryctinae (Sharkey, 1993; Perrichot *et al*., 2009). Currently Apozyginae is left as unplaced within Braconidae (Table 3).

Conclusions

The Braconidae provide an excellent system to study evolutionary processes, such as transitional patterns of host use, the evolution of host-finding mechanisms, phenotypic convergence and the evolution of parasitic life strategies. However, robust phylogenies based on multiple lines of evidence are necessary to understand patterns of evolutionary change through time. In this study, a relatively robust phylogeny of Braconidae was generated using several molecular markers.

Several higher-level relationships were recovered with significant support across multiple genes, providing independent lines of evidence to support the phylogenetic hypotheses. This study focused primarily on the non-cyclostome subfamilies, particularly the Helconinae, but the monophyly of the cyclostome complex (with a sister relationship to the aphidioid complex) was strongly supported. However, relationships within the cyclostomes were poorly supported, probably because of the weaker taxonomic sampling of exemplars from this group. Several taxonomic changes were proposed based on the findings of this study (summarized in Table 3).

These results support the suspicions espoused by Wharton (1993) and alluded to by others (Tobias, 1967; Shaw & Huddleston, 1991), that the non-cyclostomes probably had a separate evolutionary history with respect to the cyclostome + aphidioid complexes. Given this phylogeny, notions of the ancestral condition of several characters may need to be re-examined and critically tested, in addition to the presumption of an ectoparasitic ancestor for Braconidae. Future work should focus on the aphidioid complex, particularly on understanding the biology of its members, as this clade will have enormous significant influence on ancestral state reconstructions and our understanding of evolution within Braconidae. Additionally, identifying the sister group to Ichneumonoidea is still desperately needed for a better understanding of braconid evolution. Aculeata has often been suggested as the sister group to Ichneumonoidea (Rasnitsyn, 1988; Dowton & Austin, 1994; Vilhelmsen *et al*., 2010). However, recent studies suggest that Ichneumonoidea may be sister to Proctotrupomorpha + Aculeata (Sharanowski *et al*., 2010) or a basal lineage within Apocrita (Castro & Dowton, 2006). The current study should provide a scaffold for the testing of evolutionary theories within Braconidae, as well as provide a basis for the determination of appropriate out-groups for future revisions of braconid subfamilies. However, much work still needs to be completed to fully understand the evolutionary relationships within Braconidae. Of particular interest is clarifying the relationships within the cyclostome complex, and this will probably require dense taxonomic, morphological and genetic sampling. Within the non-cyclostomes, understanding the limits of the subfamilies within the euphoroid complex and the tribes newly placed within Brachistinae remain fertile areas of research. Additionally, the relationship of the euphoroid complex with the other complexes within the non-cyclostomes needs to be clarified, as well as the phylogenetic placement of Meteorideinae and Trachypetinae.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-3113.2011.00580.x

Figure S1. Maximum-likelihood analysis under a GTR + I + G model using GARLI (lnL = −107487.57). Bootstrap values are listed above the nodes. The out-group branches were moved to the right because of space limitations; however, the proper scale is retained.

Figure S2. Bayesian inference of the concatenated dataset, partitioning strategy J (see also Table S4). This strategy excludes all regions of ambiguity and all third codon positions. Posterior probabilities are listed below or beside the nodes, depending on space.

Figure S3. Bayesian inference of the concatenated dataset, partitioning strategy E (see also Table S4). This stratgey includes all third codon positions and regions of ambiguity (RAAs) in the rDNA that have sequence lengths across all taxa with a standard deviation of less than 1. Posterior probabilities are listed below the nodes or beside the nodes, depending on space.

Figure S4. Bayesian inference of *28S* rDNA, with regions of ambiguity (RAAs) included if the sequence length across all taxa had a standard deviation of less than 1. Posterior probabilities are listed below or beside the nodes, depending on space.

Figure S5. Bayesian inference of *28S* rDNA, with all regions of ambiguity (RAAs) excluded. Posterior probabilities are listed below or beside the nodes, depending on space.

Figure S6. Bayesian inference of *18S* rDNA with regions of ambiguity (RAAs) included if the sequence length across all taxa had a standard devaition of less than 1. Posterior probabilities are listed below or beside the nodes, depending on space.

Figure S7. Bayesian inference of *18S* rDNA, with all regions of ambiguity (RAAs) excluded. Posterior probabilities are listed below or beside the nodes, depending on space.

Figure S8. Bayesian inference of *CAD54* with all data included. Posterior probabilities are listed below or beside the nodes, depending on space.

Figure S9. Bayesian inference of *CAD54* with the third codon position removed. Posterior probabilities are listed below or beside the nodes, depending on space.

Figure S10. Bayesian inference of *CADap* with all data included. Posterior probabilities are listed below or beside the nodes, depending on space.

Figure S11. Bayesian inference of *CADap* with the third codon position excluded. Posterior probabilities are listed below or beside the nodes, depending on space.

Figure S12. Bayesian inference of *ACC* with all data included. Posterior probabilities are listed below or beside the nodes, depending on space.

Figure S13. Bayesian inference of *ACC* with the third codon position excluded. Posterior probabilities are listed below or beside the nodes, depending on space.

Table S1. Primer pairs used to amplify gene regions employed in this study, and associated references.

Table S2. Standard deviation (σ) of sequence length of all regions of alignment ambiguity (RAAs) and regions of expansion and contraction (RECs) for *28S* and *18S* rDNA. Regions were determined according to the model of secondary structure developed by Yoder & Gillespie (2004) and Gillespie *et al*. (2005) for rDNA in Ichneumonoidea.

Table S3. Different partitioning strategies for concatenated datasets. The number of partitions range from 1 to 13. The different strategies have the regions of ambiguity (RAAs) excluded if the standard deviation of sequence length was greater than 1 (see Table S2) or all RAAs were excluded. Additionally, some strategies excluded the third codon position of all protein-coding genes.

Table S4. Model selection for various genes and gene partitions. Model selection was determined using hierarchical likelihood ratio tests (hLRTs) in MODELTEST 3.8, run on the ModelTest Server (Posada & Crandall, 1998; Posada, 2006).

Table S5. Comparison of harmonic mean likelihoods for different partitioning strategies using Bayes factors (following Kass & Raftery, 1995; Nylander *et al*., 2004).

Table S6. Statistics regarding the gene regions used for individual and concatenated analyses, with the number of included taxa, aligned length, and number and percentage of parsimony informative sites. *For protein coding genes, the aligned length is reported after treatment with GBLOCKS.

Table S7. Chi-square tests for base composition homogeneity for genes and certain gene partitions.

Please note: Neither the Editors nor Wiley-Blackwell are responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Acknowledgements

We would like to thank all of the collectors and colleagues that provided specimens for this study. Specimens were kindly received from several collecting efforts around the world, including: Madagascar, through B.L. Fisher and C.E. Griswold, supported by the National Science Foundation (NSF) under grant no. DEB-0072713 and through B.L. Fisher and P.S. Ward under NSF grant no. DEB-0344731; Thailand and Colombia, through author M.J.S. under NSF grant no. DEB-0205982 and DEB-0542864; Costa Rica, through Arthropods of La Selva project (ALAS) under NSF grant no. DEB-0072702 (to P.I. Hartshorn); and personal donations from a variety of researchers, including Donald Quicke, Bob Wharton, David Smith, Mike Irwin, Bob Kula and Kevin Pitz. We extend our gratitude to the three anonymous reviewers for extremely insightful comments that inspired a better article. We also gratefully thank Dicky Yu, Eric Chapman, Andy Boring, Kacie Johansen and Stephanie Clutts for helpful comments on the article. We would like to specially thank Bob Wharton for providing a wonderfully insightful critique of the article, which helped to vastly improve the final version. We would like to kindly acknowledge Debra Murray and Jessie Sherman for help with molecular bench work, and Cheryl Lindsay, Jonathan Roden, Khartik and Jennie Edelen for help with sorting malaise trap samples. We also extend sincere gratitude to all of the organizations that have provided funding for research and travel for this study, including the University of Kentucky Graduate School (to B.J.S.), The University of Kentucky Women's Club (to B.J.S.) and the National Science Foundation (to M.J.S., grant EF-0337220 and to B.J.S., travel award).

References

van Achterberg, C. (1975) A revision of the tribus Blacini (Hymenoptera: Braconidae: Helconinae). *Tijdschrift voor Entomologie*, **118**, 159–322.

van Achterberg, C. (1979) A revision of the subfamily Zelinae Auct. (Hymenoptera: Braconidae). *Tijdschrift voor Entomologie*, **122**, 241–479.

van Achterberg, C. (1984) Essay on the phylogeny of Braconidae (Hymenoptera: Ichneumonoidea). *Entomologisk Tidskrift*, **105**, 41–58.

van Achterberg, C. (1988) Revision of the subfamily Blacinae Foerster (Hymenoptera: Braconidae). *Zoologische Verhandelingen Leiden*, **249**, 1–324.

van Achterberg, C. (1990) Revision of the subtribe Pseudohelconina nov. (Hymenoptera: Braconidae). *Zoologische Mededelingen Leiden*, **63**, 283–308.

van Achterberg, C. (1992) Revision of the genera of the subfamily Microtypinae (Hymenoptera: Braconidae). *Zoologische Mededelingen*, **66**, 369–380.

van Achterberg, C. (1994) Revision of the subfamily Cenocoeliinae Szépligeti (Hymenoptera: Braconidae). *Zoologische Verhandelingen Leiden*, **292**, 1–52.

van Achterberg, C. (1995) Generic revision of the subfamily Betylobraconinae (Hymenoptera: Braconidae) and other groups with modified fore tarsus. *Zoologische Verhandelingen Leiden*, **298**, 1–242.

van Achterberg, C. (2002) Revision of the genus *Canalicephalus* Gibson and the recognition of the Acampsohelconinae (Hymenoptera: Braconidae) as extant. *Zoologische Mededelingen*, **76**, 247–270.

van Achterberg, C. & Quicke, D.L.J. (1992) Phylogeny of the subfamilies of the family Braconidae: a reassessment assessed. *Cladistics*, **8**, 237–264.

van Achterberg, C., Kenis, M. & Sileshi, G. (2000) *Perilitus* (*Microctonus*) *larvicida* spec. nov. (Hymenoptera: Braconidae: Euphorinae) from Zambia. *Zoologische Mededelingen Leiden*, **74**, 57–62.

Austin, A. & Dowton, M. (2000) *Hymenoptera: Evolution, biodiversity, and biological control.* CSIRO, Canberra.

Banks, J.C. & Whitfield, J.B. (2006) Dissecting the ancient rapid radiation of microgastrine wasp genera using additional nuclear genes. *Molecular Phylogenetics and Evolution*, **41**, 690–703.

Belokobylskij, S.A., Iqbal, M. & Austin, A.D. (2003) First record of the subfamily Dirrhopinae (Hymenoptera: Braconidae) from the

Australian region, with a discussion of relationships and biology. *Australian Journal of Entomology*, **42**, 260–265.

Belokobylskij, S.A., Zaldivar-Riverón, A. & Quicke, D.L.J. (2004) Phylogeny of the genera of the parasitic wasps subfamily Doryctinae (Hymenoptera: Braconidae) based on morphological evidence. *Zoological Journal of the Linnean Society*, **142**, 369–404.

Belokobylskij, S.A., Zaldivar-Riverón, A., Maetô, K. & Sáez, A.G. (2008) Asian Betylobraconinae (Hymenoptera, Braconidae), with description of a new genus and phylogenetic affinities of the tribe Facitorini. *Insect Systematics and Evolution*, **39**, 133–154.

Belshaw, R. & Quicke, D.L.J. (2002) Robustness of ancestral state estimates: evolution of life history strategy in ichneumonoid parasitoids. *Systematic Biology*, **51**, 450–477.

Belshaw, R., Fitton, M., Herniou, E., Gimeno, C. & Quicke, D.L.J. (1998) A phylogenetic reconstruction of the Ichneumonoidea (Hymenoptera) based on the D2 variable region of 28S ribosomal RNA. *Systematic Entomology*, **23**, 109–123.

Belshaw, R., Dowton, M., Quicke, D.L.J. & Austin, A. (2000) Estimating ancestral geographical distributions: a gondwanan origin for aphid parasitoids? *Proceedings of the Royal Society of London B*, **267**, 491–496.

Belshaw, R., Grafen, A. & Quicke, D.L.J. (2003) Inferring life history from ovipositor morphology in parasitoid wasps using phylogenetic regression and discriminant analysis. *Zoological Journal of the Linnean Society*, **139**, 213–228.

Bezier, A., Annaheim, M., Herbiniere, J. *et al*. (2009) Polydnaviruses of braconid wasps derive from an ancestral nudivirus. *Science*, **323**, 926–930.

Boring, C.A.B. (2010) *Morphology and systematics of braconid wasps*. PhD Dissertation, University of Kentucky, Lexington, Kentucky.

Boring, C.A.B., Sharanowski, B.J. & Sharkey, M.J. (2011) Maxfischeriinae: a new braconid subfamily (Hymenoptera) with highly specialized egg morphology. *Systematic Entomology*, in press.

Calendini, F. & Martin, J.-F. (2005) *PaupUP v1.0.3.1: A Free Graphical Frontend for Paup* Dos Software*. Published by the authors, Montpellier, France [WWW document]. URL http://www.agro-montpellier.fr/sppe/Recherche/JFM/PaupUp/main.htm [accessed on 10 September 2008].

Čapek, M. (1970) A new classification of the Braconidae (Hymenoptera) based on the cephalic structures of the final instar larva and biological evidence. *The Canadian Entomologist*, **102**, 846–875.

Čapek, M. (1973) Key to the final instar larvae of the Braconidae (Hymenoptera). *Acta Instituti Forestalis Zvolenensis*, **4**, 259–268.

Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, **17**, 540–552.

Castro, L. & Dowton, M. (2006) Molecular analyses of the Apocrita (Insecta: Hymenoptera) suggest that the Chalcidoidea are sister to the diaprioid complex. *Invertebrate Systematics*, **20**, 603–614.

Chen, X.X. & van Achterberg, C. (1997) Revision of the subfamily Euphorinae (Hymenoptera: Braconidae) from China. *Zoologische Verhandelingen Leiden*, **313**, 1–217.

Chen, X.X. & He, J.H. (1997) Revision of the subfamily Rogadinae (Hymenoptera: Braconidae) from China. *Zoologische Verhandelingen Leiden*, **308**, 1–187.

Chen, X.X., Piao, M.H., Whitfield, J.B. & He, J.H. (2003) Phylogenetic relationships within the Rogadinae (Hymenoptera: Braconidae) based on the D2 variable region of 28S ribosomal RNA. *Acta Entomologia Sinica*, **46**, 209–217.

Clausen, C.P. (1954) The egg-larval host relationship among the parasitic Hymenoptera. *Bollettino del Laboratorio di Zoologia Generale e Agraria della Facoltá Agraria in Portici*, **33**, 119–133.

Danforth, B.N., Fang, J., Sipes, S. & Brady, S.G. (2004) *Phylogeny and Molecular Systematics of Bees (Hymenoptera: Apoidea)*. Cornell University, Ithaca, New York [WWW document]. URL http://www.entomology.cornell.edu/BeePhylogeny/ [accessed on January 2007].

Desjardins, C.A., Regier, J.C. & Mitter, C. (2007) Phylogeny of pteromalid parasitic wasps (Hymenoptera: Pteromalidae): initial evidence from four protein-coding nuclear genes. *Molecular Phylogenetics and Evolution*, **45**, 454–469.

Dowton, M. & Austin, A. (1994) Molecular phylogeny of the insect order Hymenoptera: apocritan relationships. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 9911–9915.

Dowton, M. & Austin, A.D. (1997) The evolution of strand-specific compositional bias. A case study in the hymenopteran mitochondrial 16S rRNA gene. *Molecular Biology and Evolution*, **14**, 109–112.

Dowton, M. & Austin, A.D. (1998) Phylogenetic relationships among the microgastroid wasps (Hymenoptera: Braconidae): combined analysis of 16S and 28S rDNA genes and morphological data. *Molecular Phylogenetics and Evolution*, **10**, 354–366.

Dowton, M., Austin, A.D. & Antolin, M.F. (1998) Evolutionary relationships among the Braconidae (Hymenoptera: Ichneumonoidea) inferred from partial 16S rDNA gene sequences. *Insect Molecular Biology*, **7**, 129–150.

Dowton, M., Belshaw, R., Austin, A.D. & Quicke, D.L.J. (2002) Simultaneous molecular and morphological analysis of braconid relationships (Insecta: Hymenoptera: Braconidae) indicates independent mt-tRNA gene inversions within a single wasp family. *Journal of Molecular Evolution*, **54**, 210–226.

Gatesy, J., DeSalle, R. & Wheeler, W. (1993) Alignment-ambiguous nucleotide sites and the exclusion of systematic data. *Molecular Phylogenetics and Evolution*, **2**, 152–157.

Gauld, I.D. (1988) Evolutionary patterns of host utilization by ichneumonoid parasitoids (Hymenoptera: Ichneumonidae and Braconidae). *Biological Journal of the Linnean Society*, **35**, 351–377.

Gillespie, J.J. (2004) Characterizing regions of ambiguous alignment caused by the expansion and contraction of hairpin-stem loops in ribosomal RNA molecules. *Molecular Phylogenetics and Evolution*, **33**, 936–943.

Gillespie, J.J., Yoder, M.J. & Wharton, R.A. (2005) Predicted secondary structure for 28S and 18S rRNA from Ichneumonoidea (Insecta: Hymenoptera: Apocrita): impact on sequence alignment and phylogeny estimation. *Journal of Molecular Evolution*, **61**, 114–137.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Hickson, R.E., Simon, C. & Perrey, S.W. (2000) The performance of several multiple-sequence alignment programs in relation to secondary-structure features for an rRNA sequence. *Molecular Biology and Evolution*, **17**, 530–539.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: bayesian inference of phylogeny. *Bioinformatics*, **17**, 754–755.

Jervis, M.A., Heimpel, G.E., Ferns, P.N., Harvey, J.A. & Kidd, N.A.C. (2001) Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. *Journal of Animal Ecology*, **70**, 442–458.

Jones, O.R., Purvis, A., Baumgart, E. & Quicke, D.L.J. (2009) Using taxonomic revision data to estimate the geographic and taxonomic distribution of undescribed species richness in the Braconidae (Hymenoptera: Ichneumonoidea). *Insect Conservation and Diversity*, **2**, 204–212.

Kass, R.E. & Raftery, A.E. (1995) Bayes factors. *Journal of the American Statistical Association*, **90**, 773–795.

Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**, 3059–3066.

Kjer, K.M. (1995) Use of rRNA secondary structure in phylogenetic studies to identify homologous positions: an example of alignment and data presentation from the frogs. *Molecular Phylogenetics and Evolution*, **4**, 314–330.

Lewis, C.N. & Whitfield, J.B. (1999) Braconid wasp (Hymenoptera: Braconidae) diversity in forest plots under different silvicultural methods. *Environmental Entomology*, **28**, 986–997.

Li, F.F., Chen, X.X., Piao, M.H., He, J.H. & Ma, Y. (2003) Phylogenetic relationships of the Euphorinae (Hymenoptera: Braconidae) based on the D2 variable region of 28S ribosomal RNA. *Entomotaxonomia*, **25**, 217–226.

Maetô, K. (1987) A comparative morphology of the male internal reproductive organs of the family Braconidae (Hymenoptera, Ichneumonoidea). *Kontyu*, **55**, 32–42.

Maetô, K. (1990) Phylogenetic relationships and host associations of the subfamily Meteorinae Cresson (Hymenoptera, Braconidae). *Japanese Journal of Entomology*, **58**, 383–396.

Mardulyn, P. & Whitfield, J.B. (1999) Phylogenetic signal in the COI, 16S and 28S genes for inferring relationships among genera of Microgastrinae (Hymenoptera: Braconidae): evidence of a high diversification rate in this group of parasitoids. *Molecular Phylogenetics and Evolution*, **12**, 282–294.

Marsh, P.M. & Carlson, R.W. (1979) Superfamily Ichneumonoidea. *Catalog of Hymenoptera in America North of Mexico*, Vol. I (ed. by K.V. Krombein, P.D. Hurd, D.R. Smith and B.D. Burks), PP. 143–144. Smithsonian Institution Press, Washington, District of Columbia.

Marshall, D.C. (2010) Cryptic failure of partitioned bayesian phylogenetic analyses: lost in the land of long trees. *Systematic Biology*, **59**, 108–117.

Martin, J.C. (1956) *A taxonomic revision of the triaspidine braconid wasps of Nearctic America (Hymenoptera).* Canadian Department of Agriculture, Ottawa.

Mason, W.R.M. (1974) A generic synopsis of Brachistini (Hymenoptera: Braconidae) and recognition of the name *Charmon* Haliday. *Proceedings of the Entomological Society of Washington*, **76**, 235–246.

Miller, M.A., Holder, M.T., Vos, R. *et al*. (2009) *The CIPRES Portals* [WWW document]. URL http://www.webcitation.org/5imQlJeQa [accessed on 4 August 2009].

Moulton, J.K. & Wiegmann, B.M. (2004) Evolution and phylogenetic utility of CAD (rudimentary) among Mesozoic-aged eremoneuran Diptera (Insecta). *Molecular Phylogenetics and Evolution*, **31**, 363–378.

Muesebeck, C.F.W. & Walkley, L.M. (1951) *Hymenoptera of America North of Mexico, synoptic catalog*, *agriculture monographs* (ed. by U. D. o. Agriculture). United States Department of Agriculture, PP. 90–184. Washington, D.C.

Murphy, N., Banks, J., Whitfield, J.B. & Austin, A. (2008) Phylogeny of the parasitic microgastroid subfamilies (Hymenoptera: Braconidae) based on sequence data from seven genes, with an improved time estimate of the origin of the lineage. *Molecular Phylogenetics and Evolution*, **47**, 378–395.

Nylander, J., Ronquist, F., Huelsenbeck, J.P. & Nieves-Aldrey, J.L. (2004) Bayesian phylogenetic analysis of combined data. *Systematic Biology*, **53**, 47–67.

Papp, J. (1994) *Maxfischeria tricolor* gen.n. et sp.n. from Australia (Insecta: Hymenoptera: Braconidae). *Annalen des Naturhistorischen Museums Wien*, **96**, 143–147.

Papp, J. (1995) New diospiline taxa from the neotropical region (Hymenoptera: Braconidae: Helconinae). *Acta Zoologica Academiae Scientiarum Hungaricae*, **41**, 103–114.

Perrichot, V., Nel, A. & Quicke, D.L.J. (2009) New braconid wasps from French Cretaceous amber (Hymenoptera, Braconidae): synonymisation with Eoichneumonidae and implications for the phylogeny of Ichneumonoidea. *Zoologica Scripta*, **38**, 79–88.

Pitz, K., Dowling, A.P.G., Sharanowski, B.J., Boring, C.A.B., Seltmann, K.C. & Sharkey, M.J. (2007) Phylogenetic relationships among the Braconidae (Hymenoptera: Ichneumonoidea) as proposed by Shi *et al*.: a reassessment. *Molecular Phylogenetics and Evolution*, **43**, 338–343.

Posada, D. (2006) ModelTest server: a web-based tool for the statistical selection of models of nucleotide substitution. *Nucleic Acids Research*, **34**, W700–W703.

Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Praz, C.J., Muller, A., Danforth, B.N., Griswold, T.L., Widmer, A. & Dorn, S. (2008) Phylogeny and biogeography of the tribe Osmiini (Hymenoptera: Megachilidae). *Molecular Phylogenetics and Evolution*, **49**, 185–197.

Quicke, D.L.J. (1987) A new subfamily of Braconidae, the Vaepellinae, based on a new genus and species from Ghana (Insecta, Hymenoptera). *Zoologica Scripta*, **16**, 73–77.

Quicke, D.L.J. (1993) The polyphyletic origin of endoparasitism in the cyclostome lineages of Braconidae (Hymenoptera): a reassessment. *Zoologische Mededelingen Leiden*, **67**, 159–177.

Quicke, D.L.J. & van Achterberg, C. (1990) Phylogeny of the subfamilies of the family Braconidae (Hymenoptera: Ichneumonoidea). *Zoologische Verhandelingen Leiden*, **258**, 1–180.

Quicke, D.L.J. & Belshaw, R. (1999) Incongruence between morphological data sets: an example from the evolution of endoparasitism among parasitic wasps (Hymenoptera: Braconidae). *Systematic Biology*, **48**, 436–454.

Quicke, D.L.J., Ficken, L.C. & Fitton, M.G. (1992a) New diagnostic characters for doryctine wasps (Hymenoptera, Braconidae). *Journal of Natural History*, **26**, 1035–1046.

Quicke, D.L.J., Tunstead, J., Falco, J.V. & Marsh, P.M. (1992b) Venom gland and venom reservoir morphology in cyclostome braconid wasps with special reference to the subfamily Doryctinae (Hymenoptera, Braconidae). *Zoologica Scripta*, **21**, 403–416.

Quicke, D.L.J., Sharkey, M.J., Laurenne, N.M. & Dowling, A.P.G. (2008) A preliminary molecular phylogeny of the Sigalphinae (Hymenoptera: Braconidae), including *Pselaphanus* Szépligeti, based on 28S rDNA, with descriptions of new Afrotropical and Madagascan *Minanga* and *Malasigalphus* species. *Journal of Natural History*, **42**, 2703–2719.

Rambaut, A. & Drummond, A.J. (2009) *Tracer v1.5*. University of Edinburgh, Edinburgh, UK. [WWW document]. URL http://tree.bio.ed.ac.uk/software/tracer/ [accessed on February 2010].

Rasnitsyn, A.P. (1988) An outline of evolution of hymenopterous insects (order Vespida). *Oriental Insects*, **22**, 115–145.

Regier, J.C. (2007) *Protocols, Concepts, and Reagents for Preparing DNA Sequence Templates Version* 9/19/07 [WWW document] URL www.umbi.umd.edu/users/jcrlab/PCR_primers.pdf [accessed on January 2008], Vol. 2008.

Regier, J.C., Shultz, J.W., Ganley, A.R.D. *et al*. (2008) Resolving arthropod phylogeny: exploring phylogenetic signal within 41 kb of protein-coding nuclear gene sequence. *Systematic Biology*, **57**, 920–938.

Sharanowski, B.J., Robbertse, B., Walker, J., Voss, S.R., Yoder, R., Spatfora, J. & Sharkey, M.J. (2010) Expressed sequence tags reveal Proctotrupomorpha (minus Chalcidoidea) as sister to Aculeata

(Hymenoptera: Insecta). *Molecular Phylogenetics and Evolution*, **57**, 101–112.

Sharkey, M.J. (1992) Cladistics and tribal classification of the Agathidinae (Hymenoptera: Braconidae). *Journal of Natural History*, **26**, 425–447.

Sharkey, M.J. (1993) Family Braconidae. *Hymenoptera of the World: An identification guide to families* (ed. by H. Goulet and J.T. Huber), PP. 362–395. Research Branch, Agriculture Canada and Canada Communications Group, Ottawa.

Sharkey, M.J. & Wahl, D.B. (1992) Cladistics of the Ichneumonoidea (Hymenoptera). *Journal of Hymenoptera Research*, **1**, 15–24.

Sharkey, M.J. & Wharton, R.A. (1994) A revision of the genera of the world Ichneutinae (Hymenoptera: Braconidae). *Journal of Natural History*, **28**, 873–912.

Shaw, M.R. (1983) One evolution of endoparasitism: the biology of some genera of Rogadinae (Braconidae). *Contributions to the American Entomological Institute*, **20**, 307–328.

Shaw, S.R. (1985) A phylogenetic study of the subfamilies Meteorinae and Euphorinae (Hymenoptera: Braconidae). *Entomography*, **3**, 277–370.

Shaw, M.R. & Huddleston, T. (1991) Classification and biology of braconid wasps (Hymenoptera: Braconidae). *Handbooks for the Identification of British Insects*, **7**, 1–126.

Shi, M., Chen, X.X. & van Achterberg, C. (2005) Phylogenetic relationships among the Braconidae (Hymenoptera: Ichneumonoidea) inferred from partial 16S rDNA, 28S rDNA D2, 18S rDNA gene sequences and morphological characters. *Molecular Phylogenetics and Evolution*, **37**, 104–116.

Strand, M.R. (2000) Developmental traits and life-history evolution in parasitoids. *Parasitoid Population Biology* (ed. by M.E. Hochberg and A.R. Ives), PP. 139–160. Princeton University Press, Princeton, New Jersey.

Strand, M.R. & Obrycki, J.J. (1996) Host specificity of insect parasitoids and predators. *Bioscience*, **46**, 422–429.

Swofford, D.L. (2000) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods) Version 4.* Sinauer Associates, Sunderland, Massachusetts.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Moflecular Biology and Evolution*, **24**, 1596–1599.

Thompson, J.D., Plewniak, F. & Poch, O. (1999) A comprehensive comparison of multiple sequence alignment programs. *Nucleic Acids Research*, **27**, 2682–2690.

Tobias, V.I. (1967) A review of the classification, phylogeny and evolution of the family Braconidae (Hymenoptera). *Entomologicheskoye Obozreniye*, **46**, 645–669.

Tobias, V.I. (1988) A new subfamily of braconids (Hymenoptera, Braconidae) from Australia. *Entomologicheskoye Obozreniye*, **67**, 644–648.

Townsend, A.C. & Shaw, S.R. (2009) A new species of *Andesipolis* Whitfield & Choi from the eastern Andes of Ecuador with notes on biology and classification (Hymenoptera: Braconidae: Rhysipolinae). *Journal of Insect Science*, **9**, 1–7.

Vilhelmsen, L., Mikó, I. & Krogmann, L. (2010) Beyond the wasp-waist: structural diversity and phylogenetic significance of the mesosoma in apocritan wasps (Insecta: Hymenoptera). *Zoological Journal of the Linnean Society*, **159**, 22–194.

Watanabe, C. (1972) A revision of the Helconini of Japan and a review of Helconine genera of the world. *Insecta Matsumurana*, **35**, 1–18.

Wharton, R.A. (1993) Bionomics of the Braconidae. *Annual Review of Entomology*, **38**, 121–143.

Wharton, R. (2000) Can braconid classification be reconstructed to facilitate portrayal of relationships? *Hymenoptera: Evolution, biodiversity, and biological control* (ed. by A.D. Austin and M. Dowton), PP. 143–153. CSIRO, Canberra.

Wharton, R.A. & van Achterberg, C. (2000) Family group names in Braconidae (Hymenoptera: Ichneumonoidea). *Journal of Hymenoptera Research*, **9**, 254–270.

Wharton, R.A., Shaw, M.R., Sharkey, M.J. *et al*. (1992) Phylogeny of the subfamilies of the family Braconidae (Hymenoptera: Ichneumonoidea): a reassessment. *Cladistics*, **8**, 199–235.

Wharton, R.A., Marsh, P.M. & Sharkey, M.J. (eds) (1997) *Manual of the New World genera of the family Braconidae.* The International Society of Hymenopterists, Washington, District of Columbia.

Wharton, R.A., Yoder, M.J., Gillespie, J.J., Patton, J.C. & Honeycutt, R.L. (2006) Relationships of *Exodontiella*, a non-alysiine, exodont member of the family Braconidae (Insecta, Hymenoptera). *Zoologica Scripta*, **35**, 323–340.

Whitfield, J.B. (1988) Revision of the Nearctic species of the genus *Stiropius* Cameron (=*Bucculatriplex* Auct.) with the description of a new related genus (Hymenoptera: Braconidae). *Systematic Entomology*, **13**, 373–385.

Whitfield, J.B. (1990) Phylogenetic review of the *Stiropius* group of genera (Hymenoptera: Braconidae, Rogadinae) with description of a new Neotropical genus. *Proceedings of the Entomological Society of Washington*, **92**, 36–43.

Whitfield, J.B. (1992) The polyphyletic origin of endoparasitism in the cyclostome lineages of Braconidae (Hymenoptera). *Systematic Entomology*, **17**, 273–286.

Whitfield, J.B. (1997) Molecular and morphological data suggest a common origin for the polydnaviruses among braconid wasps. *Naturwissenschaften*, **34**, 502–507.

Whitfield, J.B. (1998) Phylogeny and evolution of host-parasitoid interactions in Hymenoptera. *Annual Review of Entomology*, **43**, 129–151.

Whitfield, J.B. (2002) Estimating the age of the polydnavirus/braconid wasp symbiosis. *Proceedings of the National Academy of Sciences, of the United States of America*, **99**, 7508–7513.

Whitfield, J.B. & Kjer, K.M. (2008) Ancient rapid radiations of insects: challenges for phylogenetic analysis. *Annual Review of Entomology*, **53**, 449–472.

Whitfield, J.B. & Mason, W.R.M. (1994) Mendesellinae, a new subfamily of braconid wasps (Hymenoptera, Braconidae) with a review of relationships within the microgastroid assemblage. *Systematic Entomology*, **19**, 61–76.

Whitfield, J.B., Choi, W.-Y. & Suh, K.-I. (2004) *Andesipolis*, a puzzling new genus of cyclostomes Braconidae (Hymenoptera) from the Chilean andes, with descriptions of three new species. *Zootaxa*, **438**, 1–15.

Wild, A.L. & Maddison, D.R. (2008) Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. *Molecular Phylogenetics and Evolution*, **48**, 877–891.

Winterton, S. & de Freitas, S. (2006) Molecular phylogeny of the green lacewings (Neuroptera: Chrysopidae). *Australian Journal of Entomology*, **45**, 235–243.

Yoder, M. & Gillespie, J. (2004) *jRNA. Exploring insect phylogeny using RNA secondary structure* [WWW document]. URL http://hymenoptera.tamu.edu/rna [accessed on 6 November 2008].

Yu, D.S., Horstmann, K. & van Achterberg, C. (2004) Taxapad 2004: scientific names for information management. *Biological and Taxonomical Information: Ichneumonoidea 2004, CD.* Taxapad, Vancouver.

Zaldivar-Riverón, A., Areekul, B., Shaw, M.R. & Quicke, D.L.J. (2004) Comparative morphology of the venom apparatus in the braconid wasp subfamily Rogadinae (Insecta, Hymenoptera, Braconidae) and related taxa. *Zoologica Scripta*, **33**, 223–237.

Zaldivar-Riverón, A., Mori, M. & Quicke, D.L.J. (2006) Systematics of the cyclostome subfamilies of braconid parasitic wasps (Hymenoptera: Ichneumonoidea): a simultaneous molecular and morphological Bayesian approach. *Molecular Phylogenetics and Evolution*, **38**, 130–145.

Zaldivar-Riverón, A., Shaw, M.R., Sáez, A.G., Mori, M., Belokobylskij, S.A., Shaw, S.R. & Quicke, D.L.J. (2008) Evolution of the parasitic wasp subfamily Rogadinae (Braconidae): phylogeny and evolution of lepidopteran host ranges and mummy characteristics. *BMC Evolutionary Biology*, **8**, 329.

Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research*, **31**, 3406–3415.

Zwickl, D.J. (2006a) *GARLI: Genetic algorithm for rapid likelihood inference*. Program distributed by the author at [WWW document]. URL http://www.nescent.org/informatics/download.php?software_id=4

Zwickl, D.J. (2006b) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD Dissertation, The University of Texas, Austin, Texas.

Accepted 7 April 2011