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Phylogenomics of braconid wasps (Hymenoptera, Braconidae) sheds light on classification and the evolution of parasitoid life history traits

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ABSTRACT

The parasitoid lifestyle is largely regarded as a key innovation that contributed to the evolutionary success and extreme species richness of the order Hymenoptera. Understanding the phylogenetic history of hyperdiverse parasitoid groups is a fundamental step in elucidating the evolution of biological traits linked to parasitoidism. We used a genomic-scale dataset based on ultra-conserved elements and the most comprehensive taxon sampling to date to estimate the evolutionary relationships of Braconidae, the second largest family of Hymenoptera. Based on our results, we propose Braconidae to comprise 41 extant subfamilies, confirmed a number of subfamilial placements and proposed subfamily-level taxonomic changes, notably the restoration of Trachypetinae **stat. rev.** and Masoninae **stat. rev.** as subfamilies of Braconidae, confirmation that *Apozyx penyai* Mason belongs in Braconidae placed in the subfamily Apozyginae and the recognition of Ichneutinae *sensu stricto* and Proteropinae as non-cyclostome subfamilies robustly supported in a phylogenetic context. The correlation between koinobiosis with endoparasitoidism and idiobiosis with ectoparasitoidism, long thought to be an important aspect in parasitoid life history, was formally tested and confirmed in a phylogenetic framework. Using ancestral reconstruction methods based on both parsimony and maximum likelihood, we suggest that the ancestor of the braconoid complex was a koinobiont endoparasitoid, as was that of the cyclostome *sensu lato* clade. Our results also provide strong evidence for one transition from endo- to ectoparasitoidism and three reversals back to endoparasitoidism within the cyclostome *sensu stricto* lineage. Transitions of koino- and idiobiosis were identical to those inferred for endo- *versus* ectoparasitoidism, except with one additional reversal back to koinobiosis in the small subfamily Rhysipolinae.

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1. Introduction

It is widely assumed that different attributes such as physiological, behavioral and life history traits may have major impacts on the evolutionary history of organisms and influence their adaptive success and diversification (Mayhew, 2007; Ebel et al., 2015; Condamine et al., 2016). Within insects, the Hymenoptera (ants, bees, wasps, sawflies, parasitoid wasps and woodwasps) are one of the most successful lineages and have recently been suggested as the insect order with the highest species richness, largely due to the extraordinary diversity found among parasitoid wasps (Forbes et al., 2018). The adoption of a parasitoid lifestyle is widely regarded as a key innovation in the evolutionary history of Hymenoptera (Hanson and Gauld, 1995; Grimaldi and Engel, 2005), leading not only to extreme diversity but to ubiquitous niche occupation: almost every insect species is attacked by at least one parasitoid species (Schoenly, 1990; Memmott and Godfray, 1993). As a consequence, parasitoids exert key functional roles in ecosystems and have enormous economic impact by controlling the populations of insect pests (Godfray, 1994).

In light of their extreme diversity and ecological importance, understanding the phylogenetic history of hyperdiverse parasitoid families is a fundamental step in elucidating the evolution of biological traits linked to parasitoidism. Obtaining comprehensive and reliable phylogenies for such broad lineages, however, has been challenging due to

Fig. 1. Morphological variation in Braconidae. (A–C) Live specimens in the field; photos taken by Steve Marshall in Canada (University of Guelph), used with permission. (A) *Bracon* sp. (Braconinae); (B) *Meteorus* sp. (Euphorinae); (C) *Spathius* sp. (Doryctinae). (D–L) Habitus images of pinned specimens. (D) *Aphaereta genevensis* (Alysiinae); (E) *Aphidius ohioensis* (Aphidiinae); (F) *Rhaconotus fasciatus* (Doryctinae); (G) *Chelonus* sp. (Cheloninae); (H) *Toxoneuron viator* (Cardiochilinae); (I) *Ontsira mellipes* (Rhyssalinae); (J) *Alabagrus texanus* (Agathidinae); (K) *Proterops abdominalis* (Proteropinae); (L) *Paroligoneurus newharti* (Ichneutinae). Scale bars = 1 mm.

their extreme diversity and historical taxonomic neglect. One such highly speciose group is the Braconidae (Fig. 1), the second largest family of Hymenoptera with more than 21,000 described species worldwide (Yu et al., 2016) currently distributed in 41 extant subfamilies. Nearly all braconids are parasitoids, with a few species reported or being suspected as secondarily phytophagous (Maqbool et al., 2018; Ranjith et al., 2016; Wharton and Hanson, 2005; Zaldívar-Riverón et al., 2014). Braconids exhibit an impressive array of biological strategies, and their hosts collectively span 12 insect orders (Quicke, 2015; Yu et al., 2016), making them an excellent study system to investigate the evolution of biological traits related to parasitoidism.

1.1. Taxonomic history and phylogeny of Braconidae

Van Achterberg (1984a) provided the first hypothesis of relationships among braconids based on a Hennigian cladistics approach using morphological characters from adults and larvae, as well as biological data. The first quantitative cladistic analyses for Braconidae (Quicke and van Achterberg 1990), also based on morphological and biological data, shed light on the difficulties of interpreting morphological characters and resulted in subsequent reassessments (Wharton et al., 1992; van Achterberg and Quicke, 1992). These early quantitative cladistic studies (Quicke and van Achterberg, 1990; Wharton et al., 1992; van Achterberg and Quicke, 1992) significantly advanced our understanding of evolutionary relationships among braconids but were also limited due to the use of a relatively small number of characters to infer relationships within a hyperdiverse group.

The advent of DNA sequencing and increased computational capabilities resulted in more extensive and robust phylogenies, and the results of those efforts over the last ~30 years serve as the basis for contemporary classifications of Braconidae (Chen and van Achterberg, 2019). These phylogenetic studies have revealed that some of the morphological traits traditionally employed to define higher-level taxa are actually homoplastic (Zaldívar-Riverón et al., 2007; Quicke, 2015).

Braconidae are considered the sister group to Ichneumonidae (Belshaw et al., 1998; Quicke et al., 1999a; Dowton et al., 2002; Wei et al., 2010; Sharanowski et al., 2011; Li et al., 2016; Sharanowski et al., 2021), although familial placement is historically uncertain for a few taxa in Braconidae (e.g., Apozyginae, Mason, 1978; Masoninae, Quicke et al., 2020a; Trachypetinae, Quicke et al., 2020b). Aphidiinae has been treated as a family previously (Mackauer, 1961; Tobias and Kirijak, 1986; Finlayson, 1990; Chen and Shi, 2001; Davidyan, 2007) but clearly belongs in Braconidae (Belshaw et al., 1998; Dowton et al., 1998; Belshaw et al., 2000; Dowton et al., 2002; Belshaw and Quicke, 2002; Pitz et al., 2007; Sharanowski et al., 2011; Li et al., 2016; Sharanowski et al., 2021).

Braconids are frequently divided into two informal groups: the cyclostomes, characterized by having the lower part of the clypeus sharply recessed exposing a concave labrum and the non-cyclostomes, which have a clypeus that conceals the labrum, or if the labrum is exposed it is flat or convex (Sharkey, 1993; Wharton, 1993a; Wharton et al., 1997) (Fig. 2). Beyond the cyclostome and non-cyclostome groupings, braconids have been arranged into subfamily complexes.

The cyclostome subfamilies have been named as the cyclostome complex *sensu stricto* (Sharanowski et al., 2011). Within this complex, the subfamilies Alysiinae, Opiinae, Exothecinae, Telengaiinae, Gnamptodontinae and Braconinae are grouped within the alysioid subcomplex (Sharanowski et al., 2011; Quicke, 2015). The cyclostome complex *s.s.* has been recovered as sister to the aphidioid complex, a clade that includes the subfamilies Aphidiinae, Maxfischeriinae and Mesostoinae (Dowton et al., 2002; Zaldívar-Riverón et al., 2006; Wei et al., 2010; Sharanowski et al., 2011; Li et al., 2016; Sharanowski et al., 2021). A clade with the Aphidiinae and the members of the cyclostome complex *s.s.* was named in a previous molecular phylogenetic study as the braconoid complex (Dowton et al., 1998). Some members of the aphidioid complex—*Maxfischeria* (Maxfischeriinae), *Mesostoa* (Mesostoinae) and most aphidiines—lack the cyclostome condition (Quicke, 2015), but the remaining aphidioids have this condition. It has been suggested that the cyclostome feature was secondarily lost in members of the aphidioid complex (Dowton et al., 2002), which is the case for some taxa within the cyclostomes *s.s.*, such as Alysiinae, Opiinae and Betylobraconini (Rogadinae).

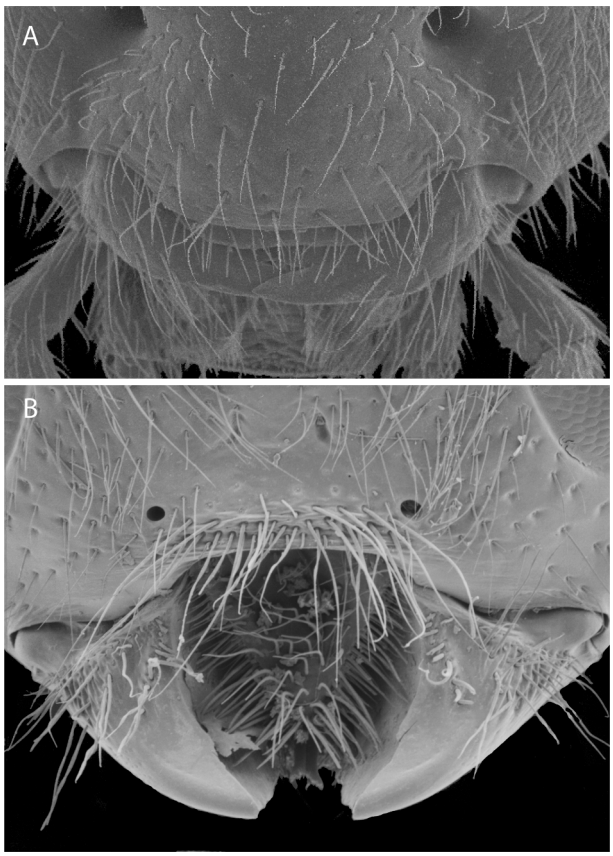

Fig. 2. Anterior view of head. (A) *Orgilius* sp. (Orgilinae), a non-cyclostome braconid with a convex clypeus that mostly conceals the labrum. (B) *Doryctes erythromelas* (Doryctine), a cyclostome braconid with a hypoclypeal depression exposing a concave labrum.

Non-cyclostome braconids have been further grouped into the euphoroid, microgastroid, sigalphoid and helconoid complexes (Quicke and van Achterberg, 1990; Wharton, 1993a; Belshaw et al., 2002; Sharanowski et al., 2011), the latter containing the macrocentroid subcomplex with Amicrocentrinae, Charmontinae, Homolobinae, Macrocentrinae, Microtypinae, Orgilinae and Xiphozelinae (Sharanowski et al., 2011).

These past efforts have provided strong support for several hypothesized relationships among braconids, but consistent, well-supported definitions remain elusive for certain subfamilies (e.g., Doryctinae, Hormiinae, Ichneutinae, Mesostoinae). In addition, it is still unclear whether some enigmatic taxa belong to Braconidae (e.g., Apozyginae, Masoninae and Trachypetinae) (Schulz, 1911; Tobias, 1979; Mason, 1978; Quicke et al., 2020a; Quicke et al., 2020b), as well as their phylogenetic relationships to other braconids.

1.2. Evolution of parasitoidism strategies in Braconidae

The Braconidae undoubtedly represent one of the most diverse of all parasitoid lineages in terms of biological traits and strategies for host use. The majority of species parasitize the immature stages of holometabolous insects, but there are remarkable exceptions, including the use of adult holometabolous and hemimetabolous insects (e.g., Euphorinae

and Aphidiinae; Mackauer et al., 1996; Stigenberg et al., 2015), as well as secondary phytophagy (in Doryctinae, Braconinae and Mesostoinae; Dangerfield and Austin, 1998; Infante et al., 1995; Ranjith et al., 2016; Zaldívar-Riverón et al., 2014).

The family includes ecto- and endoparasitoids (i.e. eggs are laid on or into the host, respectively) and both idiobionts and koinobionts (i.e. the host is either paralyzed permanently or continues to develop, respectively). Non-cyclostome braconids are koinobiont endoparasitoids, whereas an array of life history strategies is found among cyclostome braconids (koinobiont endo- and ectoparasitoids, idiobiont endo- and ectoparasitoids, herbivores). Other remarkable specializations include the use of endogenous viruses to disable the immune system of the host (in the microgastroid complex and opiines; Whitfield, 2002; Burke and Strand, 2012; Whitfield et al., 2018; Burke et al., 2018), host mummification (in Rogadinae and Aphidiinae; Hagvar and Hofsvang, 1991; van Achterberg, 1995; Zaldívar-Riverón et al., 2008a), gregarious parasitoidism (e.g., Microgastrinae; Michel-Salzat and Whitfield, 2004) and polyembryony (*Macrocentrus*, Macrocentrinae; Krugner et al., 2005).

Understanding the evolutionary history of biological traits related to host use has been a common theme in braconid-related research (e.g., Whitfield, 2002; Zaldívar-Riverón et al., 2006; Zaldívar-Riverón et al., 2008a; Stigenberg et al., 2015; Sharanowski et al., 2021; Samacá-Saenz et al., 2022). It has been proposed that parasitoidism in Ichneumonoidea has evolved from idiobiont ectoparasitoid wasps that attacked weakly concealed hosts (Gauld, 1988; Whitfield, 1992; Vilhelmsen, 1997) to koinobiont endoparasitoids of deeply concealed, as well as exposed hosts (Quicke et al., 1999b). More recently, using a phylogeny based on genomic-scale data, Sharanowski et al. (2021) tested this hypothesis and found evidence that the ancestor of Ichneumonoidea was indeed an idiobiont ectoparasitoid, with multiple transitions in mode of parasitoidism occurring within the superfamily. In the case of Braconidae, the results suggested that its ancestor may have been a koinobiont endoparasitoid; however, inferred ancestral states varied widely, leading the authors to refrain from making strong conclusions about the evolution of parasitoidism in the group (Sharanowski et al., 2021).

The use of next-generation sequencing techniques (NGS) is rapidly increasing as an approach for exploring evolutionary questions in entomology (Paula, 2021). In particular, data obtained from targeted enrichment methods such as the capture of ultra-conserved elements (UCEs) (Faircloth et al., 2015) and anchored hybrid enrichment (AHE) (Lemmon et al., 2012) have been used to generate a reliable phylogenetic framework for many groups of Hymenoptera, including Braconidae (Sharanowski et al., 2021 for AHE; Samacá-Sáenz et al., 2019; Samacá-Sáenz et al., 2022; Jasso-Martínez et al., 2021 for UCEs). As noted by Zhang et al. (2019), AHE and UCE approaches target different types of loci; AHE recovers fewer loci (300–600) that are longer and exclusively exonic, while UCEs target a larger number of shorter loci (greater than 1,000) that include both coding and non-coding regions. Among the advantages of UCEs in phylogenetics are their performance for obtaining hundreds or thousands of loci even from low-quality and degraded samples, as well as the availability of protocols, bioinformatic pipelines and baits that aid in their reproducibility.

1.3. Study aims

This study aims to reconstruct the phylogenetic relationships of Braconidae based on genomic data from ultra-conserved elements (UCEs) and on the most comprehensive taxonomic sampling carried out to date for the family, affording an unprecedented phylogenetic analysis in terms of data volume and robustness of the results. We then use these phylogenies to develop a revised classification for Braconidae, as well as to explore long pursued questions regarding the evolution of life history strategies within the family.

2. Methods

2.1. Taxon sampling

We sequenced UCE data for a total of 393 braconid species from 276 genera (Supplementary Table S1), including members of all subfamilies except for three small subfamilies: Amicrocentrinae (5 spp.), Dirrhophinae (5 spp.) and Xiphozelinae (16 spp.). Within our braconid ingroup, 236 species belong to the cyclostomes *s.s.* and aphidioid complex and 156 species to the non-cyclostome group; for the latter, all subfamily complexes are represented (i.e. the helconoid, euphoroid, sigalphoid and micrograstroid complexes; Sharanowski et al., 2011).

We included species of three taxa for which familial placement is unclear. *Apozyx penyai* Mason is currently considered part of Braconidae within the monotypic subfamily Apozyginae (Quicke and van Achterberg, 1990; Perrichot et al., 2009; Belokobylskij and Jouault, 2021), although it was originally placed in its own family (Apozygidae; Mason, 1978). Also, we included a specimen of *Trachypetus clavatus* Guérin-Meneville for Trachypetinae, a group traditionally treated as a subfamily of Braconidae (Schulz, 1911; Tobias, 1979) that was recently raised to family status based on molecular and morphological evidence (Trachypetidae: Quicke et al., 2020b). Similarly, we included a specimen of *Masona* for Masoninae, a subfamily previously considered within Braconidae (van Achterberg, 1995) that was recently transferred to Ichneumonidae based on molecular evidence (Quicke et al., 2020a). Phenotypic vouchers for all of the sampled species are housed at the Smithsonian Institution National Museum of Natural History, Washington, DC; in the Colección Nacional de Insectos at the Instituto de Biología, Universidad Nacional Autónoma de México (CNIN IB-UNAM); in the Zoological Institute, Russian Academy of Sciences, St Petersburg, Russia (ZISP) and at the Canadian National Collection of Insects (CNC), Ottawa, Canada.

Subfamily and species names are as in Yu et al. (2016). Genus and subgenus names are as in Wharton et al. (1997) except we used Yu et al. (2016) for exclusively Old World taxa. Exceptions to the aforementioned use of names are as follows: Histeromerinae as a junior synonym of Rhyssalinae (Zaldívar-Riverón et al., 2006); Lysiterminae as a junior synonym of Hormiinae, *Parahormius* and *Pseudohormius* as Hormiinae and *Allobracon* and *Parachremylus* as Rhysipolinae (Jasso-Martínez et al., 2021); *Chremylus* as Hormiinae (Gadallah et al., 2021); *Monitoriella* as Doryctinae (Zaldívar-Riverón et al., 2006); *Avga dorsomaculata sensu* Belokobylskij and Tobias (1986); *Tetrasphaeropyx* and *Xenolobus* as junior synonyms of *Aleiodes* (Fortier, 2006; Jasso-Martínez et al., 2021); *Triraphis sensu* van Achterberg (1991) and Valerio and Shaw (2015); Blacinae as a junior synonym of Brachistinae (Sharanowski et al., 2011); *Vadumasonium* for the primary homonym *Vadum* Mason (Kammerer, 2006); *Euphoriella* as a junior synonym of *Leiophron* (Zhang et al., 2018) and Microgastrinae species and genus names as in Fernández-Triana et al. (2020).

The outgroup includes 11 species of Ichneumonidae from the subfamilies Cremastinae, Ctenopelmatinae, Cryptinae, Ichneumoninae, Labeninae, Orthocentrinae, Pimplinae, Tryphoninae and Xoridinae, representing all major lineages in the family. In order to provide further clarity on the placement of taxa of uncertain familial status (i.e. whether they are closer to Braconidae or Ichneumonidae) and following the observation by Sharanowski et al. (2021) that it may not be appropriate to root braconid or ichneumonid phylogenies with their sister family, we used *Gasteruption floridanum* Bradley (Evanioidea, Gasteruptiidae) to root the trees. A list with details of the taxa examined in this study, subfamily classification and NCBI accession numbers of the UCE raw data analyzed is provided in Supplementary Table S1.

Terminology of external morphology, including wing venation, follows Sharkey and Wharton (1997).

2.2. Library preparation, target enrichment and sequencing

Library preparation and enrichment was conducted in three different facilities (the Laboratories of Analytical Biology at the Smithsonian Institution National Museum of Natural History, the Laboratorio de Biología Molecular de Zoología at the Instituto de Biología, Universidad Nacional Autónoma de Mexico and the Ottawa Research and Development Centre, Agriculture and Agri-Food Canada). Protocols varied slightly across institutions; a detailed overview of methods is provided in Supplementary File S2 but can be summarized as follows.

Genomic DNA was extracted using commercial spin-column based kits by Qiagen (Hilden, Germany). The DNA yield was quantified using a Qubit fluorometer (High sensitivity kit, Life Technologies, Inc., Carlsbad, CA), and an aliquot of < 2 to 150 ng was used as input for library preparation. Samples with high molecular weight DNA were sheared either enzymatically or using a sonicator (Q800, Qsonica Inc., Newtown, CT) to obtain fragments with a size range of approximately 200–600 bp. Library preparation used commercially available kits targeted for Illumina libraries (Kapa Hyper Prep Kit and NEBNext Ultra II FS), with dual-indexing adapter-primers adopted to allow for *in silico* de-multiplexing of each sample. Stub and adaption ligation and PCR were followed by a purification step using SPRI magnetic beads, and the DNA of the resulting libraries was again quantified using a Qubit fluorometer. Samples were pooled at equimolar concentrations in groups of 8–12 libraries for enrichment, with 500 ng of DNA input at each enrichment reaction. UCE enrichment was performed using custom probe libraries for Hymenoptera UCE loci. For the vast majority of samples, we used a probe set targeting 2,590 loci (Hymenoptera v2; Branstetter et al., 2017), but some samples were also enriched using a previous probe set (Hymenoptera v1; Faircloth et al., 2015) that targets 1,510 loci, most of them compatible with the latter probe set (see Supplementary File S2 for details). Enrichment protocols followed the standard MYBaits kit procedure (Blumenstiel et al., 2010) except using a lower concentration for the biotinylated RNA probes. Incubation of 24 h at 65 °C was followed by a series of cleanups with Streptavidin beads (Thermo Fisher Scientific Inc., Waltham, MA) and a final PCR step using KAPA Hifi HotStart ReadyMix (Roche). Post-enrichment DNA pools were quantified and combined at equimolar ratios. Fragment size distribution and final molarity were checked prior to sequencing with a 4200 TapeStation system (Agilent Technologies, Santa Clara, CA) using a High Sensitivity D1000 ScreenTape Assay. Size-selected pools were sequenced at 4 nM as single lanes on Illumina MiSeq, HiSeq 2500, HiSeq 4000 or HiSeq X Ten platforms. Raw sequence reads for all samples are available from the NCBI Sequence Read Archive (SRA) under BioProject accession numbers PRJNA813697, PRJNA814466 and PRJNA818661 (Supplementary Table S1).

2.3. UCE data processing

All informatic processing and analyses were conducted using the Smithsonian's High-Performance Computing cluster (Smithsonian Institution, 2020). Sequencing reads were filtered and trimmed using Illumiprocessor (Faircloth, 2013; Bolger et al., 2014) and assembled using either Trinity v. r2013-02-25 (Grabherr et al., 2011) or SPAdes (Bankevich et al., 2012). The resulting contigs were then processed following the Phyluce v1.5 pipeline (Faircloth, 2016). First, contigs were queried against a FASTA file of all enrichment baits, creating a relational database with the location of the UCE loci. Samples that recovered <100 UCE loci were discarded from the pipeline and not used in downstream analyses. Individual loci were then extracted to separate FASTA files, and each locus was aligned using MAFFT v. 7.130b (Katoh et al., 2002) and trimmed with GBLOCKS v. 0.91b (Castresana, 2000; Talavera and Castresana, 2007) with reduced stringency settings (0.5, 0.5, 12, and 7 for b1–b4 settings, respectively). Alignments were filtered with different settings to produce two matrices with different levels of completeness: one including only loci available for at least 50% of the taxa and one with loci available for at least 25% of the taxa.

2.4. Phylogenetic reconstruction

We used the SWSC-EN algorithm (Tagliacollo and Lanfear, 2018) to define partitions within each UCE locus that account for rate heterogeneity and patterns of molecular evolution. The resulting concatenated alignments were then partitioned by schemes defined by PartitionFinder2 (Lanfear et al., 2016). Maximum-likelihood (ML) analyses were run with IQTREE v1.6.12 (Nguyen et al., 2015), with 10,000 rounds of ultra-fast bootstrapping (Hoang et al., 2018) to assess clade support and using ModelFinder (Kalyaanamoorthy et al., 2017) to choose the best model for each partition via the option -MFP. Analyses were run with the safe numerical mode (option -safe) to avoid numerical underflow that can result from large datasets.

2.5. Ancestral state reconstruction

Ancestral state reconstruction methods were used to investigate the evolutionary history of two key biological traits in Braconidae: ectoparasitoidism (0) *versus* endoparasitoidism (1) and idiobiosis (0) *versus* koinobiosis (1). Character coding was performed collectively by most authors (RRK, JMJM, AZR, JFT, BJS) based on a comprehensive review of original and compiled literature, notably Wharton et al. (1997) and Yu et al. (2016) (Supplementary Table S1). For *Masona* and *Trachypetus*, biological traits were inferred following the reasoning and morphological evidence provided by Quicke et al. (2020a) and Belshaw et al. (2003), but coding either as unknown (missing data) had negligible impact in the results (unpublished data). Natural history data are scarce for most parasitoid groups, and host records are missing for many braconid species, but in many cases such records are available for closely related species in Yu et al. (2016), the most complete summary of host use for the family. The examination of the above resource shows that host use is almost always conserved within genera, which means that almost no genera have records of both ectoparasitoids and endoparasitoids or koinobionts and idiobionts. For the purposes of our analyses, biological traits were extrapolated from congeneric species when known for at least one member of the genus and with no conflicting evidence. We did not include *A. penyai* in the ancestral reconstruction analyses since it represents a monotypic taxon, which makes extrapolating its biological traits difficult.

The association between koinobiosis/endoparasitoidism and idiobiosis/ectoparasitoidism is well known based on general observation (Hanson and Gauld, 2006); in order to explicitly test this correlation while accounting for phylogenetic history, we used the 'fitPagel' function in the *phytools* package (Revell, 2012) in R (R Core Team 2021), which fits Pagel's (1994) model for correlated evolution of binary characters.

Reconstructions were performed first by optimizing the characters under parsimony onto the reference tree using the 'change' command in TNT (Goloboff et al., 2008) and obtaining a visual representation of state switches in Winclada (Nixon, 1999). In addition, a maximum-likelihood approach was used to estimate relative probabilities for each state using the 'ray.disc' function of the *corHMM* package (Beaulieu et al., 2013) in R (R Core Team 2021). To that end, phylogenetic trees were ultrametricized using the penalized likelihood criterion under a relaxed clock model as implemented in the function 'chronos' of the *ape* package (Paradis et al., 2004). Both equal (ER, "equal rates") and unequal (ARD, "all rates different") transition rate matrices were tested, and the difference in log likelihoods obtained under the two models were compared against a chi-square distribution to determine whether the gain in likelihood justified the adoption of the more parameterized model.

3. Results

3.1. UCE performance and alignment statistics

We recovered a total of 1,829 UCE loci (mean length prior to trimming = 464.86 bp), with the braconids *Parachremylus* sp. and *Proterops* sp. having the lowest (101) and highest (1809) number of loci, respectively. Matrices with higher levels of locus completeness resulted in a rapid drop in the number of loci (e.g., 0 loci recovered for 90% of the taxa) and in preliminary analyses indicated some clearly artifactual results. The 25% completeness matrix had 1,299 UCE loci with a mean length of 146.16 bp; the 50% matrix, included 780 UCE loci with a mean length of 134.26 (Supplementary Files S1, S3–S5).

3.2. Phylogenetic relationships

We recovered highly similar topologies from the two analyzed datasets (matrix completeness of 25% and 50%; Supplementary File S5A–B, Figs. 3–8). Most relationships were strongly supported with bootstrap (BTP) values of 100 (average BTP was 98.85 for the 50% completeness tree and 99.01 for the 25% completeness tree). We only found differences in the BTP values of some nodes and in various generic-level relationships. The only topological change at the subfamily level was the placement of *Avga* + *Xenosternum orginis*. This group was recovered as sister to the alysioid subcomplex (Fig. 8) in the 50% matrix but sister to the subfamilies Rogadinae, Hormiinae and Rhysipolinae in the 25% matrix (Supplementary File S5A).

Hereafter, we only describe the relationships obtained in the phylogram derived from the 50% completeness matrix (Figs. 4–8) and only mention BTP values < 100. Braconidae was recovered as monophyletic as were all but four subfamilies: Doryctinae, Brachistinae, Ichneutinae and Mesostoinae. Of particular interest was the consistent recovery of several subfamilies of previously contested placement. For instance, Apozyginae, represented by *A. penyai*, was recovered as sister to all remaining braconid subfamilies (Fig. 3). *Meteoridea hutsoni* Nixon, the single representative of Meteorideinae, was sister to all other non-cyclostomes followed by *Trachypetus clavatus* (Trachypetinae). *Masona* sp. was recovered as sister to the aphidioid complex, representing a relationship not previously recovered (Figs. 3, 6). Both cyclostomes and non-cyclostomes were recovered as monophyletic and are discussed in detail below along with the aphidioid complex.

3.3. Non-cyclostomes

The subfamily Meteorideinae, represented by *Meteoridea hutsoni*, was recovered as sister to the remaining non-cyclostomes. *Trachypetus clavatus* (Trachypetidae *sensu* Quicke et al., 2020b) was sister to all non-cyclostomes other than *M. hutsoni*. The helconoid complex *sensu* Sharanowski et al. (2011) was monophyletic (Fig. 4). Within the helconoid complex, Acampsohelconinae was sister to the remaining helconoid complex *sensu* Sharanowski et al. (2011). Within Brachistinae (excluding *Dyscoletes canadensis* Mason), *Vadumasonium* sp. (Diospilini) was sister to the remaining Brachistinae, recovered as Blacini (Diospilini + Brachistini); thus, Diospilini was paraphyletic. Helconinae was sister to the macrocentroid subcomplex *sensu* Sharanowski et al. (2011), recovered as the (Microtypinae + Homolobinae) Orgilinae + (Charmontinae + Macrocentrinae) clade (note that Xiphozeliinae and Amicrocentrinae were not represented in the present study).

The euphoroid complex *sensu* Sharanowski et al. (2011) (i.e. Cenocoeliinae + Euphorinae) was monophyletic (Fig. 4). Within Euphorinae (*sensu* Stigenberg et al., 2015), Centistini (*Centistes* sp.) and Meteorini (*Zele* sp., *Meteorus*) were recovered as sister tribes but with low support (BTP = 81). The *Elasmosona* sp. (*Syntretus* sp. + *Myiocephalus* sp.) clade, representing the tribes Neoneurini, Syntretini and Myiocephalini, respectively, was recovered as sister to Centistini + Meteorini clade. Cosmophorini, represented by *Cosmophorus* sp., was sister to the clade consisting of all the aforementioned euphorine tribes. Pygostolini (*Pygostolus falcatus*) and Perilitini (*Perilitus rutilus*, *Microctonus*) were recovered as sister tribes, whereas Euphorini (*Peristenus* and *Leiophron sensu* Zhang et al., 2018) was sister to Ecnomiini + Helorimorphini (i.e.

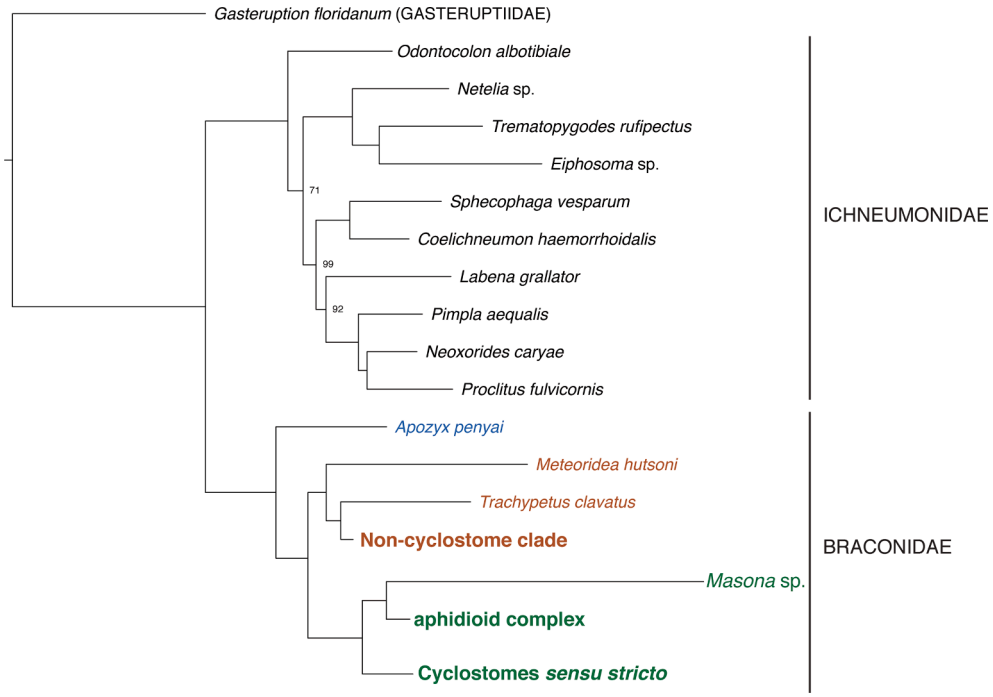

Fig. 3. Summary of phylogenetic relationships recovered in this study. Within Braconidae *Apozyx penyai* (Apozyginae) is in blue text; non-cyclostome braconids, including *Meteoridea hutsoni* and *Trachypetus clavatus*, are in orange text and cyclostome braconids *sensu lato*, including *Masona* sp., are in green text.

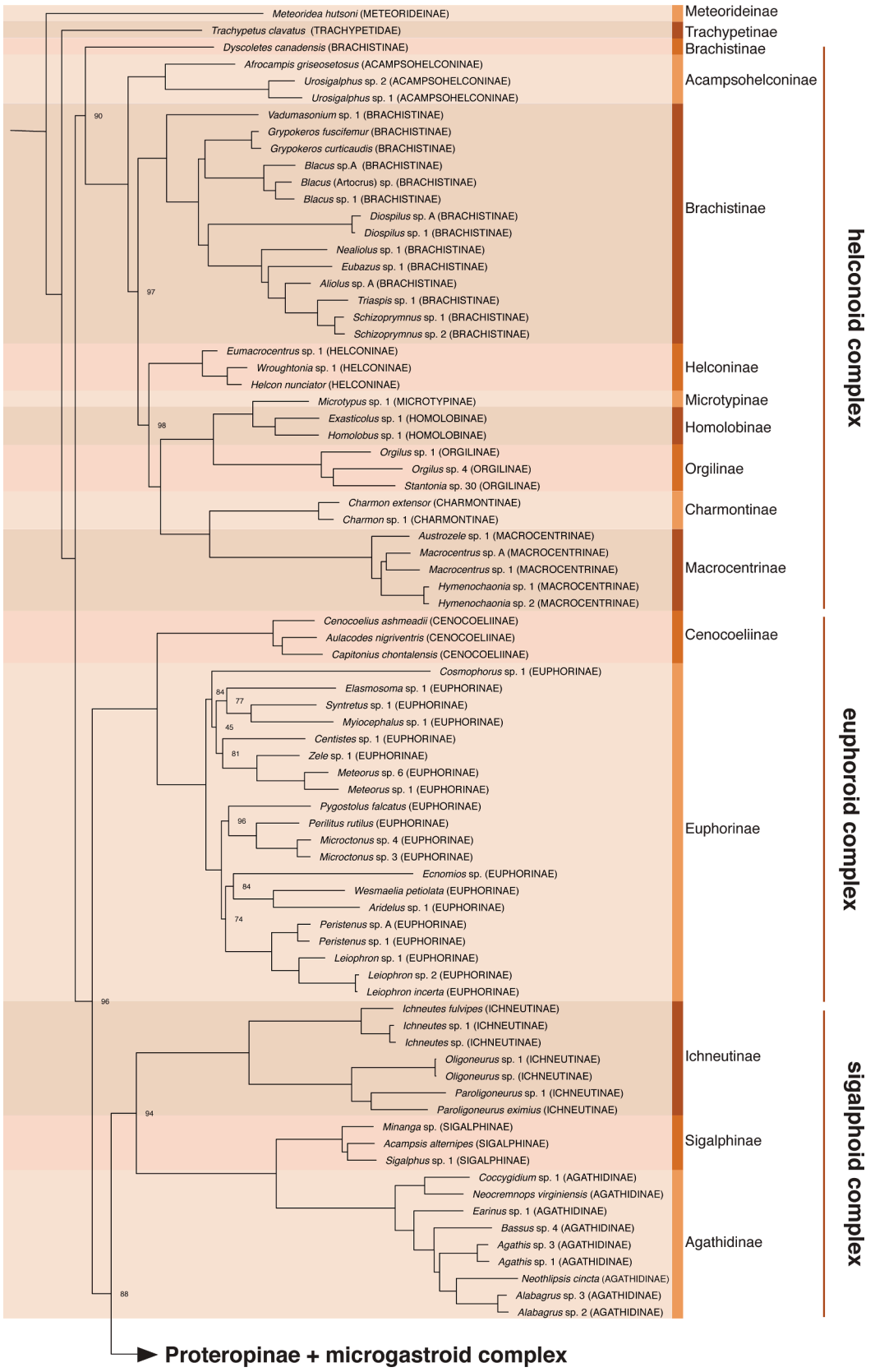

Fig. 4. Section of the ML phylogram derived from the 50% completeness matrix showing the non-cyclostome complexes helconoid, euphoroid and sigalphoid. Family and subfamily names in parentheses correspond to the classification we followed prior to this study. Numbers near nodes are bootstrap (BTP) values < 100. Nodes without a number are supported with BTP = 100. Each subfamily within each complex is highlighted in different shades of orange. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

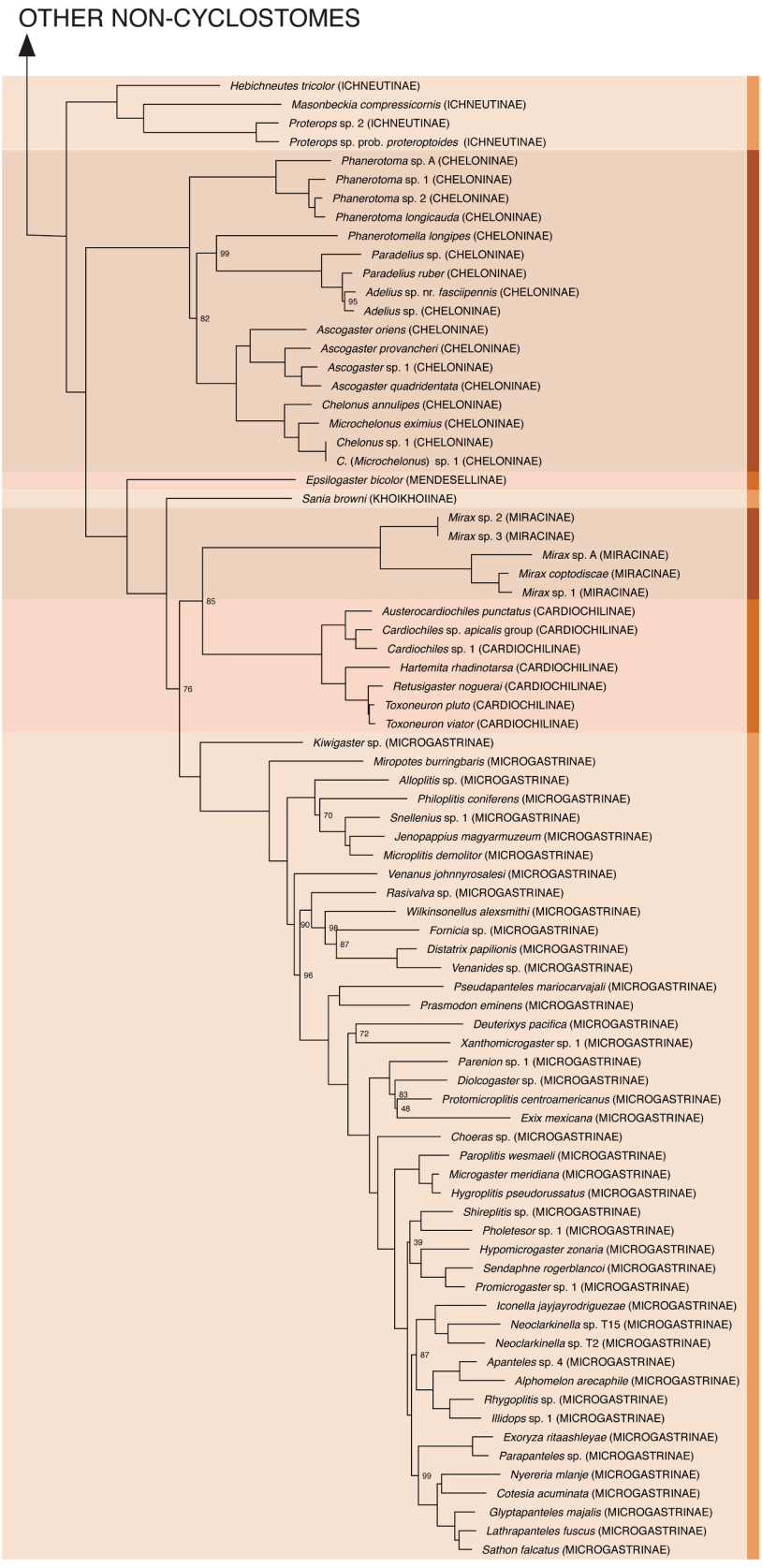

Fig. 5. Section of the ML phylogram derived from the 50% completeness matrix showing the non-cyclostome microgastroid complex and Proteropinae. Subfamily names in parentheses correspond to the classification we followed prior to this study. Numbers near nodes are bootstrap (BTP) values $<$ 100. Nodes without a number are supported with BTP $=$ 100. Each subfamily within the complex is highlighted in different shades of orange. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

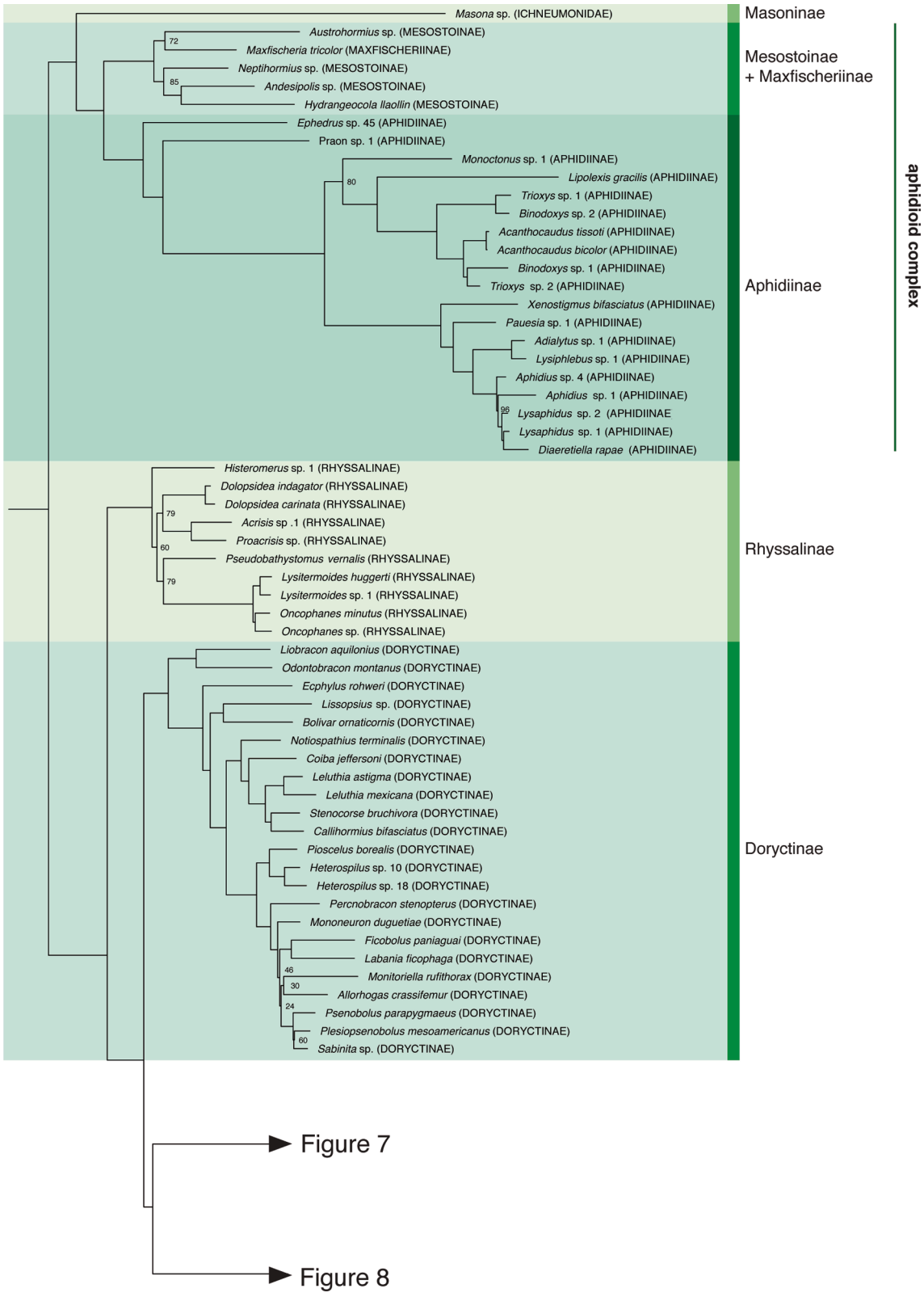

Fig. 6. Section of the ML phylogram derived from the 50% completeness matrix showing Masoninae, the three aphidioid subfamilies, Rhyssalinae and the "South American" doryctine clade. Family and subfamily names in parentheses correspond to the classification we followed prior to this study. Numbers near nodes are bootstrap (BTP) values < 100. Nodes without a number are supported with BTP = 100. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

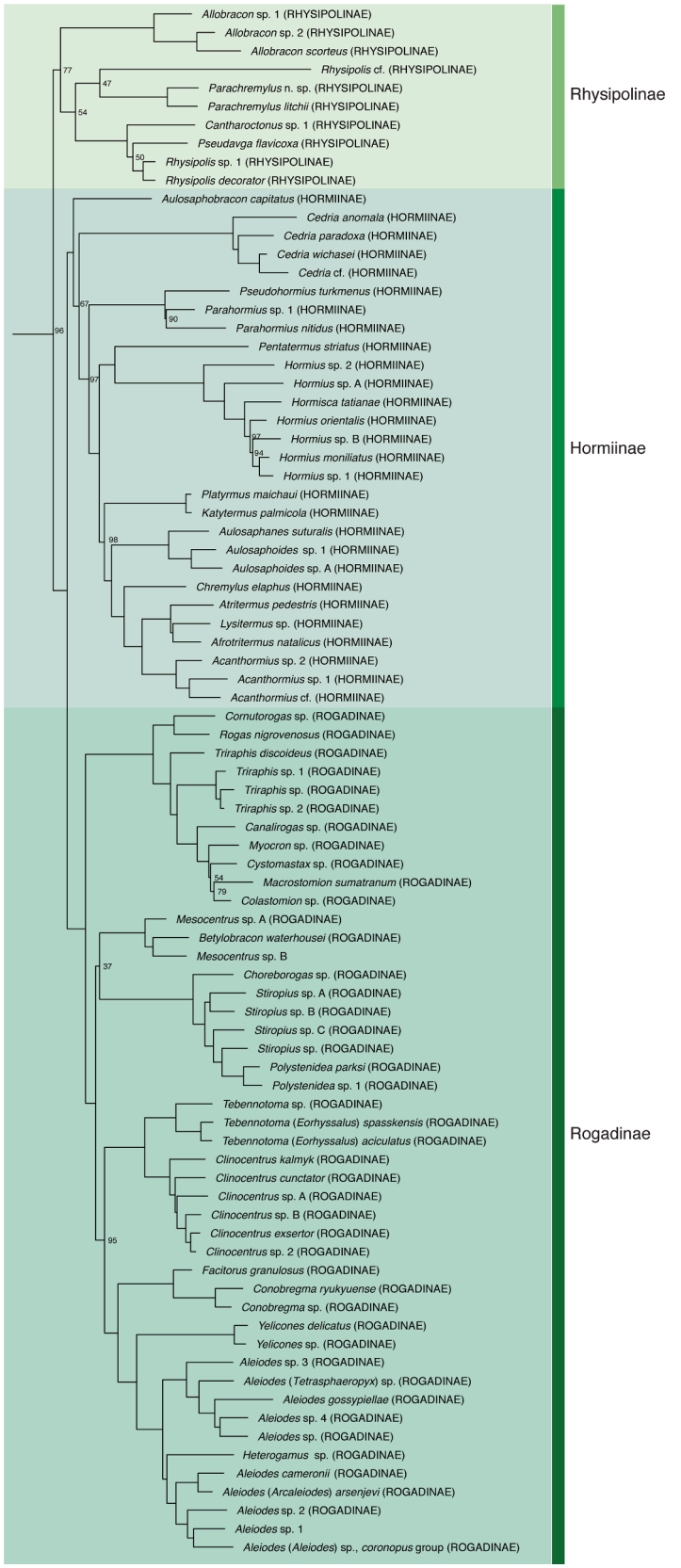

Fig. 7. Section of the ML phylogram derived from the 50% completeness matrix showing the subfamilies Rhysipolinae, Hormiinae and Rogadinae. Subfamily names in parentheses correspond to the classification we followed prior to this study. Numbers near nodes are bootstrap (BTP) values < 100. Nodes without number are supported with BTP $= 100$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 8. Section of the ML phylogram derived from the 50% completeness matrix showing the African-Holarctic-Madagascan doryctines + Pambolinae, *Xenosternum* + *Avga* and the subfamilies that comprise the braconoid subcomplex. Subfamily names in parentheses correspond to the classification we followed prior to this study. Numbers near nodes are bootstrap (BTP) values < 100. Nodes without number are supported with BTP = 100. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

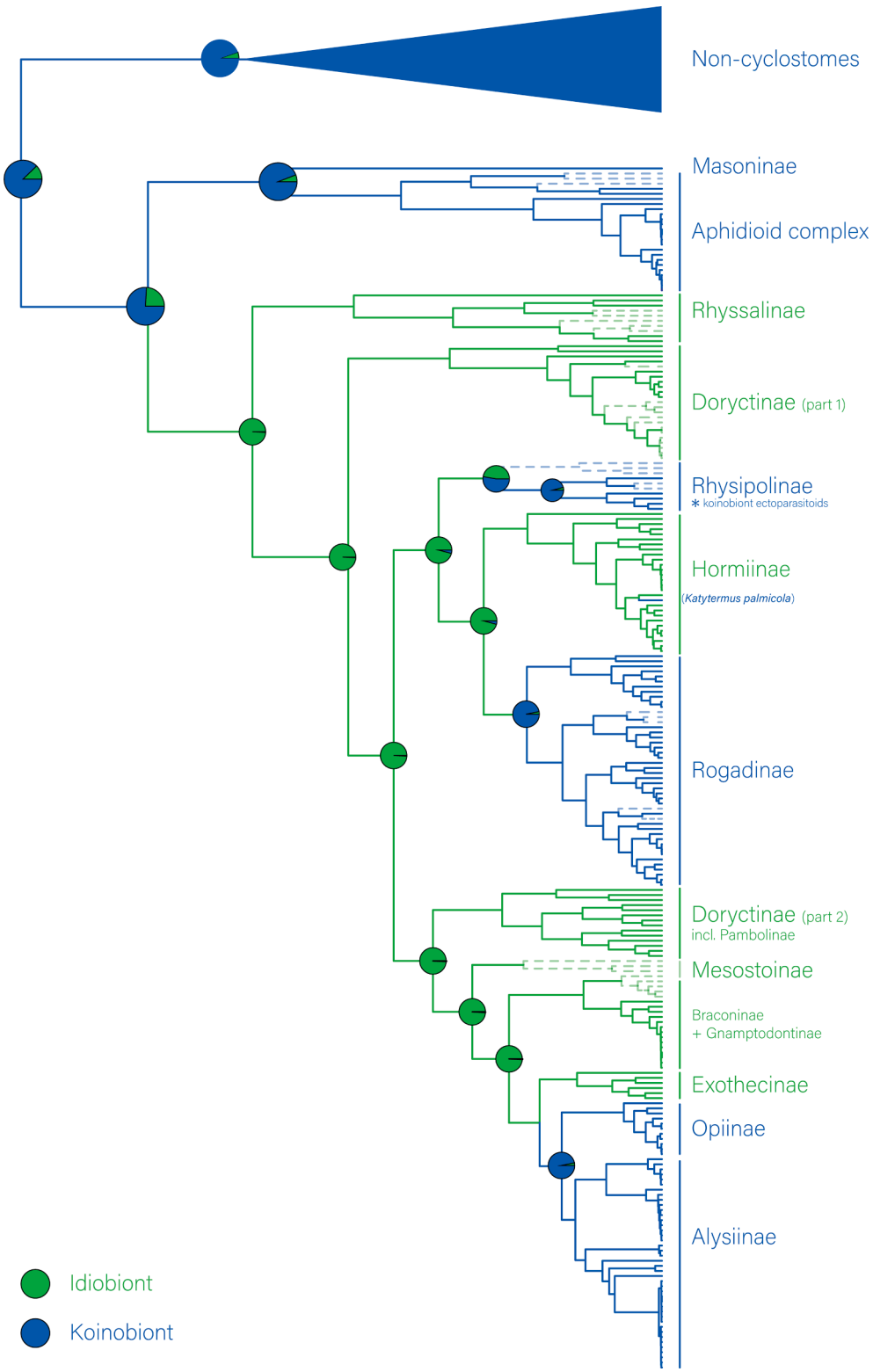

Fig. 9. Ancestral state reconstruction for idiobiosis (green) *vs*. koinobiosis (blue). Pie charts show proportional likelihoods as inferred under an unequal rate transition model; branch colors represent state transitions as inferred from ACCTRAN parsimony optimization. Branches in lighter shades and dashed lines represent terminals for which biological traits are unknown, colored according to the inferred state suggested by the reconstruction analyses. Reconstructed states for ectoparasitoidism and endoparasitoidism match those of idiobiosis and koinobiosis except for Rhysipolinae, which are koinobiont ectoparasitoids.

Ecnomius sp. [*Aridelius* sp. + *Wesmaelia petiolata* Wollaston]; BTP = 84).

Ichneutinae was non-monophyletic and divided into two separate clades. One clade consisted of *Ichneutes*, *Oligoneurus* and *Paroligoneurus* and was sister to the sigalphioid complex (i.e. Sigalphinae and Agathidinae, *sensu* Sharanowski et al., 2011) (Fig. 4). The second clade of ichneutines contained *Hebichneutes*, *Masonbeckia* and *Proterops* and was sister to the remaining represented microgastroid subfamilies (Microgastrinae, Cardiochilinae, Miracinae, Khoikhoiinae, Mendesellinae, Cheloninae) (Fig. 5). Mendesellinae, represented by *Epsilogaster bicolor* Whitfield and Mason, was sister to the Khoikhoiinae + Microgastrinae + Miracinae + Cardiochilinae clade; Khoikhoiinae, represented by *Sania browni* Sharkey, was sister to all other taxa in that clade. Within Cheloninae, *Phanerotomella longipes* Szépligeti was sister to Adeliini (*Paradelius* and *Adelius*), rendering Phanerotomini non-monophyletic (Fig. 5). Within Cardiochilinae, *Toxoneuron* was sister to *Retusigaster noguerai* Mercado. In Microgastrinae, the New Zealand genus *Kiwigaster*

was sister to the remaining microgastrine taxa, which formed a large clade with intermingled members of the *Microplitis, Cotesia* and *Parapanteles* genus groups, together with five unplaced genera *sensu* Fernández-Triana et al. (2020)—*Miropotes*, *Prasmodon*, *Xanthomicrogaster*, *Neoclarkinella* and *Fornicia*.

3.4. Aphidioid complex and cyclostomes sensu stricto

Members of the aphidioid complex (*sensu* Sharanowski et al., 2011), Aphidiinae, Mesostoinae and Maxfischeriinae, formed a clade with *Masona* sp. as its sister-group (Fig. 6). Mesostoinae was recovered as non-monophyletic due to the inclusion of *Maxfischeria tricolor* Papp as sister to *Austrohormius* sp. (Fig. 6) and the placement of *Avga* + *Xenosternum* as sister to the alysioid subcomplex (Fig. 8). The clade of mesostoines including *M. tricolor* was recovered as sister to Aphidiinae. Within Aphidiinae, the only included member of Ephedrini, a species of *Ephedrus*, was sister to the representatives of the remaining aphiidine tribes (Praiini + Aphidiini), although Praiini was represented only by *Praon*. The aphidioid complex + *Masona* sp. were sister to the cyclostomes *s.s.*

Rhyssalinae was sister to the remaining cyclostomes *s.s.*, being composed of two main clades with *Histeromerus* as sister to both (Fig. 6). One of those clades had *Pseudobathystomus* (*Pseudobathystomus*) *vernalis* Belokobylskij sister to *Lysitermoides* + *Oncophanes*; the other clade had species of *Dolopsidea* as sister to *Acrisis* sp. + *Proacrisis* sp. Doryctinae was polyphyletic and recovered in two main clades. One of the clades was composed of Neotropical genera ("South American" major clade *sensu* Zaldívar-Riverón et al., 2008b), and the other clade mostly consisted of Old World genera ("Holarctic-African-Madagascan" major clade *sensu* Zaldívar-Riverón et al., 2008b) (Figs. 6, 8). The latter also contained Pambolinae deeply nested within the clade and sister to *Spathius*, although with low support (BTP = 77) (Fig. 8).

Rhysipolinae (with the inclusion of *Allobracon* and *Parachremylus sensu* Jasso-Martínez et al., 2021) was recovered as sister to Hormiinae + Rogadinae (Fig. 7). Hormiinae (*sensu* Jasso-Martínez et al., 2021) had *Aulosaphobracon capitatus* Belokobylskij and Long, of the tribe Aulosaphobraconini, as sister to the remaining hormiines followed in a nested configuration by the representatives of Cedriini, Hormiini (including *Pentatermus striatus* Szépligeti of Pentatermini) and Lysitermini + Tetratermini, in that order (Fig. 7), although Hormiini was paraphyletic. Within Rogadinae, a clade with the included members of Rogadini was sister to the remaining rogadine tribes. The monotypic Telengaiinae, represented by *Telengaia ventralis* Tobias, was sister to Gnamptodontinae and they in turn were sister to Braconinae, albeit with lower support (BTP = 72) (Fig. 8). The latter subfamily had a species of *Tropobracon* as sister to the remaining genera, and *Digonogastra* was non-monophyletic.

The clade Braconinae (Telengaiinae + Gnamptodontinae) was sister to the clade Exothecinae (Opiinae + Alysiinae), and thus, the alysioid subcomplex *sensu* Sharanowski et al. (2011) was not recovered as monophyletic (Fig. 8). Two main clades were recovered within Alysiinae—one with most members of the tribe Alysiini, and the other clade containing species of Dacnusini along with four taxa placed historically in Alysiini (Fig. 8). One species of Alysiini in *Glyphogaster* was recovered as sister to Dacnusini; the other three species of Alysiini formed the clade *Oenonogastra* (*Dapsilarthra* sp. + *Pseudopezomachus masii* Nixon) that was sister to all other dacnusines + *Glyphogaster* sp. Within Opiinae, members of the tribes Opiini and Biosterini were not sister taxa, *Diachasma muliebris* (Muesebeck) was sister to *Diachasmimorpha* (*Diachasmimorpha*) *longicaudata* (Ashmead) within the Opiini clade, and *Biosteres* (*Biosteres*) *spinaciae* (Thomson) was sister to the remaining opiines (Fig. 8). Within the Exothecinae, the genera *Colastes* and *Xenarcha* were non-monophyletic.

3.5. Ancestral states reconstructions

Explicit testing for correlation between the koinobiosis/endoparasitoidism and idiobiosis/ectoparasitoidism characters showed that a model of dependent trait evolution was a significantly better fit (AIC = 90.86) than a model of independent evolution (AIC = 119.90) ($P < 0.0001$; Supplementary Fig. S6). The only two cases in which both traits are decoupled in Braconidae occurs in Rhysipolinae and Aspidobraconina (Braconinae), which are koinobiont ectoparasitoids and idiobiont endoparasitoids, respectively.

The ARD model was significantly better for endoparasitoidism *vs.* ectoparasitoidism ($P = 0.0099$) with an estimated rate of change from idiobiont to koinobiont about 17 times higher than the opposite. For idiobiosis *vs.* koinobiosis the difference in rate was non-significant ($P = 0.0654$), hence the ER model was marginally better. Both ER and ARD models suggest that the ancestor of all braconids except *A. penyai* was a koinobiont endoparasitoid (Fig. 9; Supplementary Fig. S6). Inferred proportional likelihoods (PL) for koinobiosis were 0.877 and for endoparasitoidism 0.961, using the best-fit model for each trait. The inferred biology at the most ancestral node of the non-cyclostome clade was also koinobiont (PL = 0.961) endoparasitoid (PL = 0.994). Cyclostomes *s.s.* + aphidioid complex + Masoninae were also inferred as most likely to have been ancestrally koinobionts but with much lower proportional likelihood (PL = 0.911 and 0.759 for endoparasitoidism and koinobiosis, respectively). Within this clade, many transitions in biological traits were inferred both by the proportional likelihoods observed at the nodes and by parsimony optimization. An ACCTRAN optimization (*sensu* Farris, 1970) is most consistent with the results of the ML analyses and suggests one transition from endo- to ectoparasitoidism in the cyclostomes *s.s.* Within this broad clade, three reversals back to endoparasitoidism were identified: one in *Katytermus palmicola* van Achterberg (Hormiinae), one at the node leading to Rogadinae and one in the Alysiinae + Opiinae clade. For idiobiosis *vs.* koinobiosis, inferred transitions were identical except for one additional switch from idio- to koinobiosis in Rhysipolinae. Most changes in biological traits were largely unambiguous across the braconid tree, with over 90% of the internal nodes showing over 0.99 proportional likelihood towards one state or another.

4. Discussion

4.1. Family-level classification in Ichneumonoidea

The superfamily Ichneumonoidea currently includes the extant families Braconidae and Ichneumonidae, as well as the extinct families Eoichneumonidae (Jell and Duncan, 1986) and Praeichneumonidae (Rasnitsyn, 1983). Species of the ichneumonid subfamily Tanychorinae, known only from fossils, clearly belong in Ichneumonoidea. While tanychorines have been placed within Ichneumonidae (Quicke, 2015; Yu et al., 2016), their phylogenetic affinities with other ichneumonoids remain uncertain (Spasojevic et al., 2021). More recently, Trachypetidae, which contains extant species, have been treated as a family in Ichneumonoidea (Quicke et al., 2020b). The placement of Trachypetidae has historically been uncertain. One of its three recognized genera, *Megalohelcon*, has been included in Helconinae (Turner, 1918). Decades later, its three genera were split into two separate subfamilies, one containing *Megalohelcon* and *Cercobarcon* (Cercobarconinae; Tobias, 1979) and the other containing *Trachypetus* (Trachypetinae) (Schulz, 1911; Tobias, 1979). The monophyly of the aforementioned three genera was first proposed on the basis of one morphological trait—the presence of a glandular structure at the base of the mandible (Austin et al., 1993). More recently, a phylogenetic analysis of molecular and morphological data including members of the three aforementioned genera (Quicke et al., 2020b) recovered the group as robustly monophyletic and sister to all other braconids, although cyclostomes and non-cyclostomes were not recovered as sister taxa in that study, unlike almost all previous studies. Based on multiple morphological character states found in Trachypetinae that are atypical for Braconidae, as well as a number of molecular diagnostic features such as specific indels in the 18S and 16S rRNA loci, those authors decided to raise Trachypetinae to

<table>
<tr><th colspan="3">CLASSIFICATION PRIOR TO THIS STUDY</th><th colspan="5">UPDATED CLASSIFICATION</th></tr>
<tr><td colspan="2">ICHNEUMONIDAE</td><td>Masoninae</td><td colspan="5" rowspan="3">BRACONIDAE</td></tr>
<tr><td colspan="2">TRACHYPETIDAE</td><td>Trachypetus Guérin de Meneville, Megalohelcon Turner, Cercobracon Tobias (Quicke et al., 2020b)</td></tr>
<tr><td colspan="3">BRACONIDAE</td></tr>
<tr><td colspan="2" rowspan="2">Unplaced</td><td>Apozyginae</td><td colspan="3" rowspan="3">Not sampled</td><td>Amicrocentrinae</td></tr>
<tr><td>Meteorideinae</td><td>Dirrhophinae</td></tr>
<tr><td rowspan="13">Cyclostomes sensu stricto</td><td rowspan="6">alysioid subcomplex</td><td>Alysiinae</td><td>Xiphozelinae</td></tr>
<tr><td>Braconinae</td><td rowspan="12">braconoid complex</td><td rowspan="7">Cyclostomes sensu lato</td><td rowspan="5">Cyclostomes sensu stricto</td><td rowspan="5">braconoid subcomplex</td><td>Alysiinae</td></tr>
<tr><td>Exothecinae</td><td>Braconinae</td></tr>
<tr><td>Gnamptodontinae</td><td>Exothecinae</td></tr>
<tr><td>Opiinae</td><td>Opiinae</td></tr>
<tr><td>Telengaiinae</td><td>Telengaiinae</td></tr>
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<tr><td>Hormiinae</td><td>Hormiinae</td></tr>
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<tr><td>Maxfischeriinae</td><td colspan="2" rowspan="2">aphidioid complex</td><td>Aphidiinae</td></tr>
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<tr><td>Amicrocentrinae</td><td>Euphorinae</td></tr>
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<tr><td>Sigalphinae</td><td>Sigalphinae</td></tr>
<tr><td colspan="5">Apozyginae (Apozyx penyai Mason)</td></tr>
</table>

Fig. 10. List of braconid subfamilies prior to and after this study. Former classification of subfamilies and complex composition mainly follow Zaldívar-Riverón et al. (2006), Sharanowski et al. (2011), Yu et al. (2016), Quicke (2015) and Jasso-Martínez et al. (2021). *Avga*, *Xenosternum* and *Dyscoletes* are treated here as *incertae sedis*.

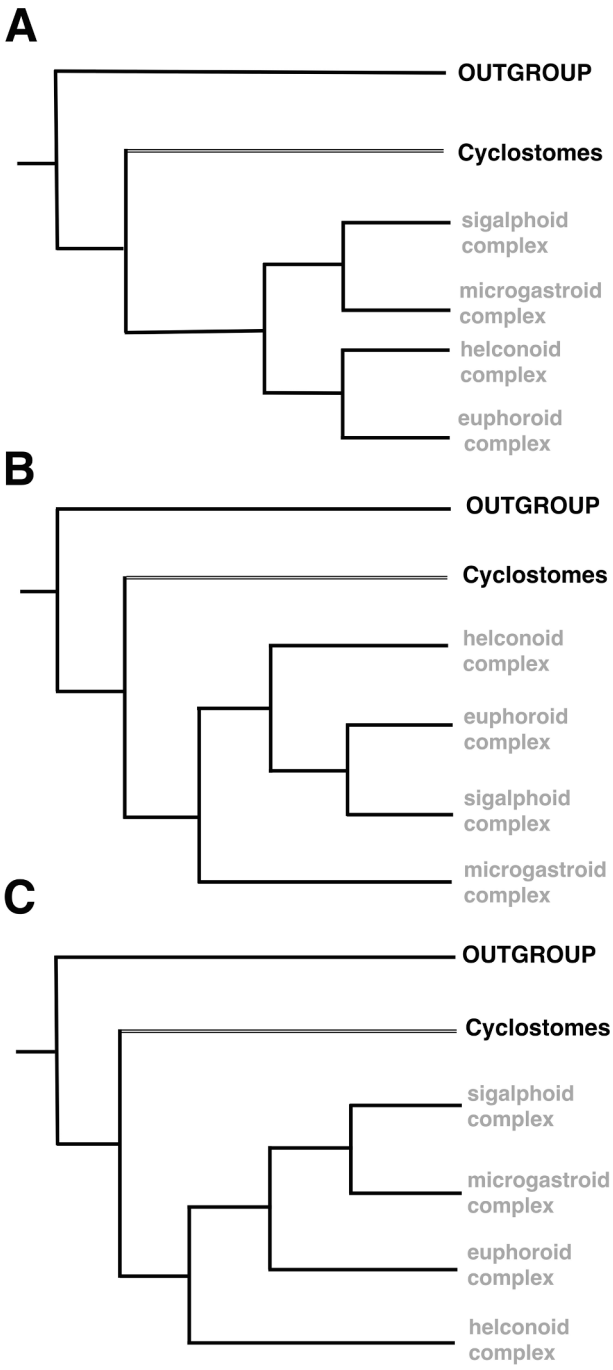

Fig. 11. Summary of relationships among braconids in the non-cyclostome subfamily complexes from this and other studies. (A) Sharanowski et al. (2011); (B) Jasso-Martínez et al. (in press); (C) This study.

family level.

Our analyses consistently recovered the monophyly of Braconidae with the inclusion of *T. clavatus* as sister to all non-cyclostome subfamilies except Meteorideinae and not as a separate family as proposed recently (Quicke et al., 2020b). The placement of *T. clavatus* within Braconidae was also obtained in a phylogenetic study based on mitochondrial genome sequence data but in that case as sister to the euphoroid complex (Jasso-Martínez et al., in press). Trachypetines possess a well-developed hind wing vein 2-CU and a distinctly small open fore wing costal cell, both present in some non-cyclostome lineages such as Agathidinae, Sigalphinae, Acampsohelconinae and Meteorideinae (Sharkey and Wahl, 1992; Quicke et al., 2020b). Given our results based on nuclear genome-scale and mitogenomic data (Jasso-Martínez et al., in press), as well as the above morphological information, we restore Trachypetinae **stat. rev**. as a non-cyclostome braconid subfamily.

The monotypic Apozyginae, with its single species *A. penyai*, was originally described as a separate family (Apozygidae) within Ichneumonoidea (Mason, 1978). Subsequent studies based on morphological characters placed this taxon as a cyclostome subfamily within Braconidae (Quicke and van Achterberg, 1990; Sharkey and Wahl, 1992; Quicke et al., 1999a). Our study represents the first phylogenetic analysis based on nuclear DNA sequence data that includes *A. penyai*. All our analyses consistently place this species as sister to all extant braconid subfamilies, and the same relationship was found with mitochondrial genome sequence data (Jasso-Martínez et al., in press). *Apozyx penyai* shares

with Ichneumonidae the presence of fore wing vein 2m-cu, which is absent in all braconids with the rare exception of some rhyssalines and doryctines (van Achterberg, 1993; Sharkey, 1993; Quicke et al., 2020c). Nevertheless, *Apozyx* shares morphological features with Braconidae, including fusion of second and third metasomal terga, hind wing vein 1r-m basal to the separation of veins R1 and Rs, and the presence of a hypoclypeal depression that characterizes the members of most cyclostome *s.s.* subfamilies (Sharkey and Wahl, 1992). We thus confirm for the first time based on molecular data a clade consisting of *A. penyai* + Braconidae and consider *A. penyai* a braconid in the monotypic subfamily Apozyginae.

4.2. Relationships and taxonomic inferences within Braconidae

Based on our estimate of phylogeny, previous classifications and the diagnostic morphological features of the included taxa, we propose a total of 41 extant subfamilies within Braconidae, of which 25 are included within the non-cyclostome group, three within the aphidioid complex with Masoninae as its sister taxon, and 11 within the cyclostomes *s.s.* We consider the cyclostomes *s.l.* as a lineage consisting of the cyclostomes *s.s.* + aphidioid complex + Masoninae, and we refer to the cyclostome *s.l.* + non-cyclostome lineage as the braconoid complex, with Apozyginae as its sister subfamily (Fig. 10). Below we discuss the most relevant relationships obtained and the main taxonomic implications derived from this study.

4.3. Non-cyclostome braconids

We recovered the four monophyletic non-cyclostome complexes mentioned by Sharanowski et al. (2011), although the relationships among them were different than those recovered in the latter work and also in Jasso-Martínez et al. (in press) (Fig. 11). Meteorideinae was previously found closely related to the sigalphioid complex based on both morphological and molecular data (e.g., Quicke and van Achterberg, 1990; Belshaw et al., 2003; Belshaw et al., 2002) and also as sister to the sigalphoid + microgastroid complexes but with low support (Sharanowski et al., 2011). Members of Meteorideinae have the Cub vein present in the hind wing as in some agathidines and sigalphines (Sharkey, 1997; Sharkey et al., 2021), although this trait is also present in *A. penyai* (Apozyginae). Therefore, this trait is likely a symplesiomorphy, as indicated by the position of Meteorideinae in our tree.

The helconoid complex was recovered here as sister to the remaining non-cyclostomes. *Dyscoletes canadensis* (Brachistinae) was sister to the rest of the helconoid complex, and Acampsohelconinae was sister to the clade containing all the helconoid subfamilies as recovered in Sharanowski et al. (2011). The three genera that comprise Acampsohelconinae have been recovered both as monophyletic (Quicke et al., 2002) and non-monophyletic (Quicke et al., 2008). We did not include species of *Canalicephalus*, but we consistently recovered *Urosigalphus* and *Afrocampsis* as monophyletic. All the helconoid complex subfamilies were recovered as monophyletic except Brachistinae due to the position of *D. canadensis* as sister to all helconoid subfamilies. Species of *Dyscoletes* have been placed in Diospilini of Helconinae (Mason, 1976) and in Blacinae within the tribe Dyscoletini (van Achterberg, 1988) but were further moved to Brachistinae with other blacines (Sharanowski et al., 2011). Here our results support a basal placement of *Dyscoletes* relative to other members of the helconoid complex. Given that it is not recovered near any other Brachistinae, it may warrant its own subfamily status. This is further supported by its unique biology as parasitoids of larval Mecoptera (Mason, 1976). However, because we did not include either the type species, *Dyscoletes lancifer* (Haliday), or species of other putative closely related taxa (e.g., *Hellenius*, also placed in Dyscoletini), we consider the genus *Dyscoletes* as *incertae sedis* within Braconidae pending further studies to confirm its taxonomic status.

A close relationship between Cenocoeliinae and Euphorinae has been obtained in previous phylogenetic studies using molecular data (Belshaw and Quicke, 2002; Sharanowski et al., 2011). The limits of Euphorinae with respect to other closely related groups (i.e. Neoneurinae, Ecnomiinae and Meteorinae) have been unclear (Sharanowski et al., 2011). Stigenberg et al. (2015) recently recovered the latter three groups within Euphorinae and proposed to treat them as its tribes, and our results support that classification. Thus, we consider the euphoroid complex to contain only two subfamilies, Cenocoeliinae and Euphorinae, with the latter including the tribes Neoneurini, Ecnomiini and Meteorini. Meteorini was not sister to all other euphorines as was recovered in Stigenberg et al. (2015). Rather, euphorines consisted of two major lineages—one with Cosmophorini, Neoneurini, Syntretini, Myiocephalini, Centistini and Meteorini, and the other with Pygostolini, Perilitini, Ecnomiini, Helorimorphini and Euphorini. Whether meteorines are a derived group within Euphorinae could change the interpretation regarding the evolution of host use in Euphorinae, suggesting a potential reversion in the Meteorini clade from attacking adults to attack larvae of Coleoptera. The sister relationship between Agathidinae and Sigalphinae has also been consistently recovered in several studies (Belshaw et al., 1998; Belshaw et al., 2002; Dowton et al., 2002; Pitz et al., 2007; Sharanowski et al., 2011; Jasso Martínez et al., in press). Both subfamilies (and Ichneutinae *s.s.*, see below) comprise the sigalphoid complex (Belshaw et al., 2002; Sharanowski et al., 2011), which has been recovered as sister to either the euphoroid complex (Sharanowski et al., 2011; Jasso-Martínez et al., in press) or the microgastroid complex (Sharanowski et al., 2011) depending on analysis used.

The Ichneutinae *s. l.* has been proposed as closely related to either the sigalphoid complex (Sharkey and Wharton, 1994) or the microgastroid complex (e.g., Quicke and van Achterberg, 1990; Dowton et al., 2002; Shi et al., 2005; Pitz et al., 2007; Sharanowski et al., 2011). Similar to Quicke and van Achterberg (1990) and Jasso-Martínez et al. (in press), in this work we recovered a non-monophyletic Ichneutinae, with *Ichneutes*, *Oligoneurus* and *Paroligoneurus* sister to the sigalphoid complex, whereas *Hebichneutes*, *Masonbeckia* and *Proterops* were sister to the microgastroid subfamilies.

Ichneutinae and Agathidinae share the presence of spines on the fore tibia, although in the latter the spines are not restricted to the apex; ichneutines have subpronopes as in Agathidinae and Sigalphinae, although these are absent in the ichneutine genera *Oligoneurus*, *Paroligoneurus* and *Lispixys*; both ichneutines and sigalphines share short ovipositors and Ichneutinae, Sigalphinae, Agathidinae and Cheloninae (the last belonging to the microgastroid complex) have a derived position of the last abscissa of Rs of the fore wing (Sharkey and Wharton, 1994). Given that we recovered *Ichneutes* (*Oligoneurus* + *Paroligoneurus*) as sister to Sigalphinae + Agathidinae, along with the morphological evidence described above, we propose to expand the sigalphoid complex to include Ichneutinae *s.s.* Five genera that were previously in Ichneutinae *s.l.* are now placed in a different subfamily (Sharkey et al., 2021, see further discussion below); thus, Ichneutinae *s.s.* currently consists of *Ichneutes*, *Lispixys*, *Oligoneurus*, *Paroligoneurus* and *Pseudichneutes*. On the other hand, *Hebichneutes*, *Masonbeckia* and *Proterops* were recovered as sister to the microgastroid complex. These genera were previously within Ichneutinae *s.l.* but were recognized recently as members of the subfamily Proteropinae (Chen and van Achterberg, 2019; Sharkey et al., 2021) given that previous phylogenetic analyses did not recover Ichneutinae *s.l.* as monophyletic (e.g., Sharanowski et al., 2011). Sharkey et al. (2021) provided a diagnosis for Proteropinae, with the subfamily consisting of *Hebichneutes*, *Helconichia*, *Masonbeckia*, *Michener*, *Muesonia* and *Proterops.* This is the first phylogenetic study that recovered, with strong support, Proteropinae and Ichneutinae as separate lineages. Therefore, we support the recognition of Proteropinae within the non-cyclostomes as sister to the microgastroid complex.

The relationships within the microgastroid complex are mostly in agreement with previous works. We do not consider Proteropinae as part of the microgastroid complex, as members of that subfamily utilize sawfly larvae as hosts (van Achterberg, 1976; Sharkey et al., 2021). Rather, we regard Proteropinae as sister to the microgastroid complex,

as members of the latter utilize, or in the case of Khoikhoiinae likely utilize (Sharkey et al., 2009), Lepidoptera larvae as hosts (Quicke and van Achterberg, 1990; Whitfield, 1997; Murphy et al., 2008; Whitfield et al., 2018; Fernandez-Triana et al., 2020). The relationships between Mendesellinae and Khoikhoiinae with the rest of the complex have varied slightly among authors (Mason, 1983; Whitfield and Mason, 1994; Whitfield, 1997; Belshaw et al., 1998; Banks and Whitfield, 2006; Murphy et al., 2008; Sharanowski et al., 2011) but in all cases, including our present work, Cheloninae has been recovered as sister to all other microgastroids and Microgastrinae as sister to Cardiochilinae + Miracinae.

4.4. Cyclostomes sensu lato

The aphidioid complex, which currently comprises the subfamilies Aphidiinae, Mesostoinae and Maxfischeriinae, has been consistently recovered as sister to the cyclostomes *s.s.* in the latest molecular phylogenetic studies (Zaldívar-Riverón et al., 2006, Wei et al., 2010, Sharanowski et al., 2011; Sharanowski et al., 2021; Jasso-Martínez et al., submitted). The composition of the aphidioid complex was again supported in our study but with *Maxfischeria* nested within Mesostoinae and *Masona* as sister to all aphidioids.

The composition of Mesostoinae is still unclear, with various genera being recently transferred to either this subfamily (e.g., *Metaspathius*, Quicke et al., 2018; *Austrohormius* and *Neptihormius,* Shimbori et al., 2017) or from Mesostoinae to other groups (e.g., *Parachremylus* and *Allobracon* to Rhysipolinae, Jasso-Martínez et al., 2021). However, the type genus of Mesostoinae, *Mesostoa*, has been recovered previously as part of the aphidioid complex in a clade with *Andesipolis*, *Aspilodemon* and *Hydrangeocola* (Zaldívar-Riverón et al., 2006) thus supporting our treatment of *Andesipolis, Hydrangeocola, Austrohormius* and *Neptihormius* as Mesostoinae. We also recovered the *Avga* + *Xenosternum* clade as sister to the alysioid subcomplex, whereas in Jasso-Martínez et al. (in press) *Avga* was sister to a large clade comprising Rogadinae, Hormiinae, Rhysipolinae, the alysioid subcomplex and the Holarctic-African-Madagascan doryctines + Pambolinae. *Avga* and *Xenosternum* were proposed to comprise the tribe Avgini together with *Parachremylus, Pseudohormius* and *Parahormius* (Ranjith et al., 2017). The placement of these genera, however, has varied considerably, as they have been placed either within Exothecinae, Mesostoinae or Hormiinae (Nixon, 1940; Belokobylskij, 1993a; Belokobylskij, 1993b; Wharton, 1993b; Ranjith et al., 2017; Quicke et al., 2018; Quicke et al., 2020c). Our best phylogenetic estimate confirms the placement of *Parachremylus* within Rhysipolinae and *Pseudohormius* and *Parahormius* within Hormiinae. Moreover, we confirm that *Avga* and *Xenosternum* do not belong to Mesostoinae, although given their poorly supported relationships, we suggest maintaining them as *incertae sedis* within Braconidae pending further studies to definitively discern their phylogenetic affinities.

In our study, *Maxfischeria* was found nested within Mesostoinae and not as sister to Aphidiinae as found by Sharanowski et al. (2011). Our results are congruent with those recently obtained with mitogenome sequence data (Jasso-Martínez et al., in press) suggesting that *Maxfischeria* actually belongs to the Mesostoinae. However, we recommend the continued treatment of Maxfischeriinae as a subfamily within the aphidioid complex pending analyses that include *Mesostoa* and more extensive sampling of taxa historically placed in Mesostoinae.

We recovered *Masona* as sister to the aphidioid complex, indicating its clear placement in Braconidae. *Masona* was originally placed in its own subfamily within Braconidae (Masoninae) based on fusion of the second and third metasomal terga and reduced fore wing venation of males (van Achterberg, 1995), although Quicke et al. (2020a) interpreted a small separation of the second and third terga laterally in two species of *Masona* (cf. Quicke et al., 2020a: Fig. 1e—f). Belshaw et al. (2002) recovered this genus within Braconidae based on molecular data, although they could not confirm its phylogenetic affinity since the relationships obtained were sensitive to the phylogenetic method employed. More recently, Quicke et al. (2020a) transferred Masoninae within Ichneumonidae based on a phylogenetic analysis with Sanger sequencing markers, as well as on the absence of fore wing vein RS + M and interpretation that the second and third terga are separated laterally in *M. popeye* and *M. similis*. However, more extensive examination of *Masona* species via scanning electron microscopy would help facilitate interpretation of the latter morphological feature. Metasomal terga 2 and 3 are fused in all braconids, although in aphidiines terga 2 and 3 are flexible at the groove between them (van Achterberg, 1997). Furthermore, the absence of RS + M occurs not only in multiple genera of Aphidiinae but is also observed in a broad phylogenetic spectrum of Braconidae (Wharton et al., 1997). Given the placement of *Masona* as sister to the aphidioid complex with the highest support and the uncertain morphological support for placing masonines within Ichneumonidae, we restore Masoninae **stat. rev**. as a subfamily of Braconidae. It is worth noting, however, that the reduction of anatomical features in masonines, due to allometry given their diminutive size, hinders the discovery and interpretation of morphological synapomorphies that support their phylogenetic placement and whether it represents a monophyletic group. Thus, like other subfamilies of Braconidae, morphological support for Masoninae may rely on the absence of features present in other braconids.

We recovered Rhyssalinae as sister to the remaining cyclostome subfamilies and confirm the inclusion of *Histeromerus* as a tribe within Rhyssalinae (Histeromerini) as proposed by Zaldívar-Riverón et al. (2006). The highly diverse, morphologically heterogeneous subfamily Doryctinae, on the other hand, was non-monophyletic, being divided into two clades that are similar in composition to the "South American" and the "Holarctic-African-Madagascan" major clades recovered in Zaldívar-Riverón et al. (2007; 2008b). This division of Doryctinae in two separate clades was not strongly supported in any of these previous studies, but it emerges very clearly from our trees. Doryctinae also fell into two separate clades in Sharanowski et al. (2011), but the composition of those clades as "South American" and the "Holarctic-African-Madagascan" was uncertain due to limited taxon sampling. Among the morphological synapomorphies that have been proposed for Doryctinae are the presence of two secondary ducts in the venom apparatus, the presence of a series of pegs on the fore tibia, ovipositor structure and microsculpture of the egg canal (Quicke et al., 1992a; Quicke et al., 1992b; Belokobylskij et al., 2004). However, none of these features are shared by all members included in this group. Further assessment of the taxonomic status of Doryctinae is necessary and requires more extensive taxon sampling, particularly pantropical taxa.

The relationship of Pambolinae with the members of Doryctinae needs to be further assessed, as the former taxon was recovered deeply nested within the clade comprising Holarctic-African-Madagascan doryctine genera. Pambolinae is a small subfamily with species distributed on all continents, with some of them being reported as ectoparasitoids of coleopteran and lepidopteran larvae (Belokobylskij, 1986; Quicke, 2015). The main diagnostic morphological feature for this group is the presence of a pair of lateral spines on the propodeum; however, this condition also occurs in doryctines of the Neotropical and Australasian genera *Doryctopambolus* and *Equinodoryctes,* respectively (Belokobylskij et al., 2004).

Our results confirmed the placement of *Allobracon* and *Parachremylus* within Rhysipolinae as in Jasso-Martínez et al. (2021; in press). Both *Parachremylus* and *Allobracon* share various morphological features, including the first metasomal tergum with membranous postero-lateral parts (Ranjith et al., 2017), dorsope absent and a median carina of the petiole present (Wharton, 1993b), which support their close relationship within Rhysipolinae. Rhysipolines are the only known members of Braconidae that display the unusual combination of ectoparasitoid koinobiosis (Shaw, 1983; Shaw, 2017). The biology of *Allobracon* and *Parachremylus* is unknown; thus, additional studies are needed to confirm whether both genera are also ectoparasitoid koinobionts.

Hormiinae was for a long time a heterogeneous assemblage of taxa,

although phylogenetic studies carried out in the last 15 years have transferred a number of its genera to other subfamilies (e.g., *Monitoriella*: Zaldívar-Riverón et al., 2006). The main diagnostic morphological feature used to characterize Hormiinae was their moderately to strongly desclerotized metasomal terga (Wharton, 1993b; van Achterberg, 1995). However, Wharton (1993b) suggested that the subfamily also could include genera with a carapacelike metasoma, such as those placed historically in Lysiterminae, given their similarity in various wing venation, leg and body sculpture features. This latter suggestion was confirmed by Jasso-Martínez et al. (2021) in a phylogenetic study based on UCE data, where they formally synonymized Lysiterminae with Hormiinae. This synonymy was supported in Quicke et al.'s (2021) phylogenetic study of Rogadinae and related subfamilies using a vast taxon sampling. Our results also confirm this concept of Hormiinae, although here with Aulosaphobraconini as sister to the remaining hormiines rather than Cedriini, as recovered by Jasso-Martínez et al. (2021; in press). Both Aulosaphobraconini and Cedriini have strongly sclerotized metasomal terga, and whether any of them are sister to the remaining hormiines, they support an early appearance of the sclerotized metasoma within the Hormiinae lineage with subsequent transitions to desclerotization.

Rogadinae is a subfamily exclusively composed of koinobiont endoparasitoids of lepidopteran larvae, whose diagnostic synapomorphy is the mummification of the host within which the parasitoid larva pupates and then emerges as an adult (Quicke and Shaw, 2005). Previous concepts of Rogadinae had been generally broader, including genera currently placed within other subfamilies such as Rhysipolinae (Shaw and Huddleston, 1991) and Hormiinae (van Achterberg, 1991). This subfamily has been recently confirmed as monophyletic with the inclusion of Betylobraconini using different nuclear and mitogenomic data (Jasso-Martínez et al., 2021; Quicke et al., 2021; Jasso-Martínez et al., in press). Our results confirm this composition, with Rogadini being sister to the remaining tribes, and also support the close relationship between Rogadinae and Hormiinae, with the latter also attacking concealed lepidopterans, although its species mostly are ectoparasitoid idiobionts. This reinforces Jasso-Martínez et al.'s (2021) hypothesis that the ancestral host preference of Rogadinae was attacking weakly concealed lepidopterans with subsequent transitions to concealed and exposed host larvae. Further discovery of host preferences for members of Betylobraconini and some of Hormiinae will help to confirm this hypothesis.

The alysioid subcomplex (Sharanowski et al., 2011) was proposed to include the subfamilies Alysiinae, Opiinae, Exothecinae, Gnamptodontinae and Telengaiinae, with Braconinae as its sister group. The close relationship of braconines to the members of the alysioid subcomplex has been recovered in other studies (Belshaw et al., 1998; Dowton et al., 2002; Zaldívar-Riverón et al., 2006); based on that Quicke (2015) proposed to expand this complex to include braconines. Here we did not recover Braconinae as sister to the remaining alysioid subfamilies but as sister to Telengaiinae + Gnamptodontinae. In Jasso-Martínez et al. (in press), braconines were also recovered as part of the alysioid subcomplex but as sister to Exothecinae (Opiinae + Alysiinae). Braconines share various morphological features with some members of the alysioid subfamilies, including a distinct pair of diagonal grooves near the anterior corners of the third metasomal tergum (shared with telengaiines and gnamptodontines) and a complete loss of both occipital and epicnemial carinae (as in most alysiines, opiines, telengaiines and gnamptodontines) (Wharton et al., 2006; Quicke, 2015). We therefore confirm the expansion of this subcomplex to include braconines, and we update its name to the "braconoid subcomplex" (Braconinae Nees, 1811; Alysiinae Leach, 1815). The monotypic genus *Vaepellis* is currently placed within Braconinae (Tobias, 1988), although it was originally described in its own subfamily, Vaepellinae (Quicke, 1987). The known species, *Vaepellis varica* Quicke, has not been assessed in a phylogenetic context, in part due to its rarity in insect collections. Thus, further studies are needed to elucidate the placement of this taxon within Braconidae.

A close relationship between the monotypic Telengaiinae and the Gnamptodontinae has been recovered by our analyses, as well as in other studies using both Sanger markers and mitogenome sequence data (Zaldívar-Riverón et al., 2006; Jasso-Martínez et al., in press). Species of *Telengaia* do not possess the transverse rectangular area at the base of the second metasomal tergum as in gnamptodontines (Quicke, 2015). However, the former taxon shares with the gnamptodontines a distinct pair of diagonal grooves near the anterior corners of the third metasomal tergum (Quicke, 2015), as well as similarities in the venom apparatus (Zaldívar-Riverón et al., 2004). Considering the close relationship between both taxa recovered in previous studies (i.e. Zaldívar-Riverón et al., 2006), Chen and van Achterberg (2019) treated Telengaiinae as a tribe within Gnamptodontinae. Given our results and considering morphological similarities between *Telengaia* and gnamptodontines, we agree in treating them as a single subfamily, although following the principle of priority, gnamptodontines should treated as a tribe (Gnamptodontini **stat. rev.**) within Telengaiinae (Telengaiinae: Tobias, 1962; Gnamptodontini: Fischer, 1970, the latter elevated to subfamily in van Achterberg, 1983). The tribe Exodontiellini, comprising the exodont genus *Exodontiella*, was transferred from Opiinae to the Gnamptodontinae based on both molecular and morphological data (Wharton et al., 2006). In this study we did not include members of *Exodontiella*, and thus, we have decided to maintain it as the tribe Exodontiellini following Wharton et al.'s (2006) study. The subfamily Telegaiinae, therefore, is regarded as consisting of the tribes Telengaiini, Exodontiellini and Gnamptodontini pending further assessment to discern the placement of *Exodontiella* within Braconidae.

Alysiinae and Opiinae were recovered as sister taxa, as has been the case in previous analyses based on morphological characters and Sanger sequencing (Quicke and van Achterberg, 1990; Gimeno et al., 1997; Belshaw et al., 1998; Dowton et al., 1998; Zaldívar-Riverón et al., 2006; Sharanowski et al., 2011). However, within Alysiinae, the tribes Alysiini and Dacnusini were not monophyletic. One species of Alysiini was recovered as sister to Dacnusini, and a clade of three other species of Alysiini was sister to that clade. Dacnusines are parasitoids of plant-feeding flies, almost exclusively Agromyzidae, Chloropidae, and Ephydridae, with most species parasitic on agromyzids. Nearly all species of Alysiini are parasitoids of saprophagous flies (Shaw and Huddleston, 1991; Wharton, 1997; Yu et al., 2016). The four species of Alysiini that formed a clade with the dacnusines in this study are all parasitoids of leaf-mining Agromyzidae (Wharton, 1997; Yu et al., 2016). These four species belong to *Glyphogaster*, *Dapsilarthra*, *Pseudopezomachus* and *Oenonogastra*; Quicke et al. (1997) found that like most or perhaps all dacnusines, species of those four genera have an unsculptured anterior bulbous swelling on the venom reservoir. Thus, the monophyly of Dacnusini as defined currently, based on the absence of fore wing vein r-m, is questionable, but host use and morphology of the venom apparatus might give biological and morphological character support for a reconfigured Dacnusini that includes parasitoids of plant-feeding flies currently in Alysiini. More extensive taxon sampling, as well as more complete data on host use and morphology of the venom apparatus, are necessary for determining the utility of those features for establishing monophyletic tribes within Alysiinae.

The subfamily Opiinae is a group for which limited molecular-based phylogenetic studies have been carried out (i.e. Gimeno et al., 1997; Li et al., 2013). We did not recover the tribes Opiini and Biosterini as monophyletic, similar to Li et al. (2013; when using both nuclear and mitochondrial data and Bayesian phylogenetic reconstruction), with *Biosteres* being sister to all other opiines, as well as *Diachasma* as sister to *Diachasmimorpha* and nested within the clade containing all Opiini species included in this study. *Biosteres* (Biosterini) has been characterized by the presence of a short second submarginal cell of the fore wing (Fischer, 1972); however, this is not exclusive of this genus but also present in other opiines, including *Fopius* (Opiini). Given the extraordinary species richness of this subfamily, further phylogenetic studies

are needed to delimit tribes within Opiinae.

4.5. Transitions in the mode of parasitoidism

The koinobiont-idiobiont distinction has long been thought to be one of the most important in the evolution of parasitoid wasps. Whether or not wasps interrupt the development of the host during/after oviposition is thought to be linked to a number of other important biological distinctions, from the degree of host specificity to the size of the eggs laid (Gauld, 2006; Quicke, 2015). Although much sensible reasoning has been used to draw conclusions regarding the biological and evolutionary implications of this single trait, few studies have explicitly tested for correlation between idiobiosis/koinobiosis and other biological traits, particularly using a phylogenetic framework. Mayhew and Blackburn (1999) attempted such an investigation, but in that study taxonomy was used as a proxy for phylogeny across parasitoid wasps as a whole. In that sense, our study helps formally establish the link between koinobiosis-endoparasitoidism and idiobiosis-ectoparasitoidism in Braconidae.

As expected in traits subject to interdependent evolution, we recovered an almost identical character history for both idiobiosis-ectoparasitoidism and koinobiosis-endoparasitoidism. In fact, most of the differences in proportional likelihoods observed between the two traits arise from the difference in the evolutionary model adopted for each trait (ER for ectoparasitoidism/endoparasitoidism, ARD for idiobiosis/koinobiosis). Rhysipolines are braconids in which the association between the respective states at each trait is broken and whose species for which biological data are known are koinobiont ectoparasitoids. For instance, species of *Rhysipolis* attack leaf-mining caterpillars and lay their eggs onto the host's intersegmental membranes (Shaw, 1983). While the host continues feeding, it ceases molting (thus preventing the dislodging of the parasitoid from its external surface) and usually enters a prepupal state prematurely. This interesting interaction with host development led Shaw (1983) to hypothesize that the biology of *Rhysipolis* represented an intermediate state towards "true" koinobiont endoparasitoidism, but our topology suggests it is better understood as an independent offshoot from a clade with ancestrally idiobiont ectoparasitoid biology. Species of Aspidobraconina, a subtribe of Braconini (van Achterberg, 1984b), are another example in which both traits are decoupled, being idiobiont endoparasitoids (Quicke, 1989; Quicke, 1997; Quicke, 2015). We did not include any member of this group in our analyses; however, their biology could potentially represent another independent origin of endoparasitism, as it was recovered by Zaldívar-Riverón et al. (2006) in a clade composed of idiobiont ectoparasitoids.

Although there have been many studies investigating the phylogeny of braconid wasps at many levels, there have been relatively few efforts to reconstruct ancestral states for biological characters across the whole family. Former evolutionary studies have sought to understand a diverse array of biological traits but focused on more limited subgroups; for example, Belshaw and Quicke (2002) analyzed the transition between the use of exposed *versus* concealed hosts among braconid koinobiont lineages; Zaldívar-Riverón et al. (2008a) reconstructed the evolution of lepidopteran host ranges and mummy characteristics in Rogadinae; and Samacá-Saenz et al. (2022) investigated the evolution of gall-association in Doryctinae.

Meanwhile, Sharanowski et al. (2021) performed an ancestral state reconstruction for ecto- *versus* endoparasitoidism and idio- *versus* koinobiosis across the whole of Ichneumonoidea using a phylogeny based on anchored hybrid enrichment (Lemmon et al., 2012). Their results were variable according to the analytical framework and whether traits were reconstructed individually for Braconidae and Ichneumonidae or using both families together. Specifically, analyses including all Ichneumonoidea recovered the ancestor of all Braconidae as likely to be an idiobiont ectoparasitoid, whereas the analysis of the family on its own suggested a koinobiont endoparasitoid state. Our study with a much deeper taxon sampling within Braconidae strongly suggests that the ancestral states for the braconoid complex are koinobiosis and endoparasitoidism. Note, however, that while our selected outgroup taxa represented all major lineages of Ichneumonidae, a more thorough sampling may be necessary to draw stronger conclusions regarding these biological traits for the braconoid complex. Also, unraveling biological information for *Apozyx* has the potential to change our interpretations or to greatly improve confidence in the current results.

Regardless of the specific ancestral state, our tree topology implies that multiple transitions across states must have happened across the evolution of Braconidae. Our topology suggests that the most parsimonious character history include either one transition from koinobiosis to idiobiosis and four reversals back to koinobiosis (under ACCTRAN optimization) or two transitions from koinobiosis to idiobiosis and three reversals back to koinobiosis (under DELTRAN). It is noteworthy that these particular reconstructions are recovered in parsimony when state changes are defined *a priori* as symmetrical, and the inferred character histories could change under different cost regimes. However, the results from ACCTRAN are supported by the probabilities inferred by ML at each node where the state transitions were inferred to have occurred (Fig. 8).

One important caveat to these results is that there are no empirical data to suggest the comparative probability of changing from one state to another or *vice versa;* therefore, cost matrices used in any set of analyses can be seen as arbitrary. It has been suggested that transitions in host use would logically occur from a supposedly less specialized state–idiobiosis–to a more specialized strategy–koinobiosis (Gauld, 1988; Bennett et al., 2019). The reasoning is that koinobiosis, and especially the endoparasitoidism that seems to accompany it, requires deep changes to adult and larval morphology, venom properties and oviposition behavior; hence a reversion back to idiobiosis/ectoparasitoidism would be unlikely. However, one could argue that the repeated evolution of such specialized characters is also unlikely and that reversals back to a less specialized state are also plausible; for example, a recent phylogeny of Ichneumonidae, the sister group to Braconidae, recovered more transitions from koinobiosis to idiobiosis than the opposite (Bennett et al., 2019). It is important to note, however, that while koinobiont endoparasitism has been considered historically a more specialized life history strategy than idiobiont ectoparasitism, koinobiosis/idiobiosis and endoparasitoidism/ectoparasitoidism involve the evolution of an array of associated traits that could be misinterpreted as more or less specialized relative to one another.

Recent research has shown that a number of apparently complex biological traits can undergo multiple transitions in parasitoid lineages. For instance, switching between larval and pupal hosts seems to be common in the evolution of Ichneumoninae (Santos et al., 2021), transitioning into the use of deeply concealed hosts has happened many times in Cryptinae (Santos and Perrard, 2018) and the use of endogenous viral elements to overcome the immune defenses of lepidopteran hosts has occurred multiple times in Ichneumonoidea (Sharanowski et al., 2021; Santos et al., 2022). We are clearly only beginning to understand the complex evolutionary pathways for host use in parasitoid wasps, and building robust phylogenies with deep taxonomic sampling will be an essential step to investigate these complex interactions.

Credit authorship contribution statement

Jovana M. Jasso-Martínez: Conceptualization, Investigation, Formal analysis, Methodology, Software, Writing – original draft. **Bernardo F. Santos:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft. **Alejandro Zaldívar-Riverón:** Investigation, Resources, Writing – review & editing. **José L. Fernández-Triana:** Investigation, Resources, Writing – review & editing. **Barbara J. Sharanowski:** Resources, Writing – review & editing. **Robin Richter:** Methodology. **Jeremy R. Dettman:** Methodology. **Bonnie B. Blaimer:** Resources, Writing – review & editing. **Seán G. Brady:** Funding acquisition, Resources, Writing – review & editing. **Robert R. Kula:** Conceptualization, Funding acquisition,

Investigation, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2022.107452.

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