

# Phylogenetic affinities of the non-cyclostome subfamilies Amicrocentrinae and Dirrhopinae (Hymenoptera, Braconidae) confirmed by ultraconserved element data

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## Abstract

The subfamilies Amicrocentrinae and Dirrhopinae (Hymenoptera, Braconidae) are two small, monogeneric braconid subfamilies whose species exclusively attack lepidopteran larvae. The phylogenetic placement of Amicrocentrinae as a member of the helconoid complex of subfamilies has been supported by morphological and nuclear Sanger sequence data. The subfamilial status of Dirrhopinae on the other hand has been subject to debate, although it has been suggested as closely related to the microgastroid complex based on morphology only. Here we generated for the first time genomic ultraconserved element data for members of the above subfamilies (*Amicrocentrum seyrigi* van Achterberg and *Dirrhope americana* Muesebeck) to assess their phylogenetic affinities using exhaustive taxon sampling that includes all but one of the currently valid braconid subfamilies. Our results strongly confirm the placement of both taxa within the non-cyclostome helconoid and microgastroid complexes, respectively.

## Keywords

Amicrocentrinae, Braconidae, Dirrhopinae, non-cyclostomes, ultraconserved elements

## Introduction

The parasitoid wasp family Braconidae is an extremely species-rich group within the order Hymenoptera, currently having more than 21,000 described species (Yu et al. 2016). Braconids mainly attack larval stages of other holometabolous insects with only some exceptions, including a few species within the subfamilies Braconinae, Doryctinae and Mesostoinae that are secondarily phytophagous (Infante et al. 1995; Dangerfield and Austin 1998; Ranjith et al. 2016; Zaldívar Riverón et al. 2014). The members of this family are divided into two major monophyletic groups (Dowton et al. 2002; Sharanowski et al. 2011, 2021; Jasso-Martínez et al. 2022a, 2022b), the cyclostomes and the non-cyclostomes, with the respective groups originally proposed based on the presence or absence of an ovoid cavity between the clypeus and mandibles with the labrum visible and concave in cyclostomes (Sharkey 1993; Wharton 1993). Some groups within the cyclostome clade, remarkably Alysini and Opiini, have secondarily lost the cyclostome condition, although this is also observed in some members of other subfamilies such as Rogadinae and Telengaiinae.

As for other megadiverse groups, the phylogenetic affinities of various taxa within Braconidae have been debated extensively due to the lack of morphological synapomorphies, limited taxon sampling and limited molecular information (Quicke and van Achterberg 1990; Dowton et al. 2002; Pitz et al. 2005). In recent past, a consensus has emerged regarding subfamily-level relationships within the family (Zaldívar-Riverón et al. 2006; Sharanowski et al. 2011, 2021; Jasso-Martínez et al. 2022a, 2022b). Recently, the family Braconidae was proposed to comprise 41 subfamilies (Apozyginae, 25 non-cyclostome and 15 cyclostome *s.l.* subfamilies) based on ultraconserved element (UCE) and mitogenome sequence data (Jasso-Martínez et al. 2022a, 2022b). However, the subfamilies Amicrocentrinae, Dirrhopinae and Xiphozelinae were not included in the above study due to a lack of either available specimens or sufficient sequence data.

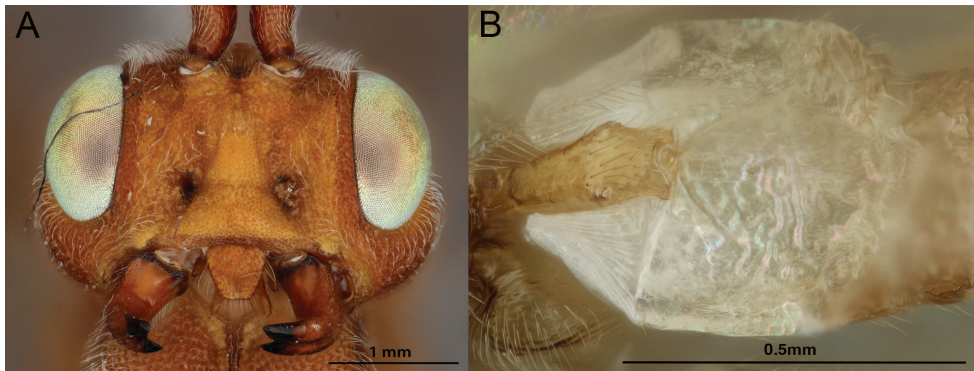
The subfamily Amicrocentrinae was established by van Achterberg (1979) and contains five species, two of which have been reared from stem-boring Lepidoptera larvae (van Achterberg 1979). Its single genus, *Amicrocentrum* Schulz, was placed previously in the non-cyclostome subfamily Macrocentrinae; however, *Amicrocentrum* lacks synapomorphic features of Macrocentrinae such as trochantellus with spines (van Achterberg 1979) (Fig. 1). A molecular phylogeny supported the subfamily status of *Amicrocentrum*, recovering it as more closely related to the subfamily Charmontinae than Macrocentrinae within the helconoid complex in the braconid non-cyclostome clade (Sharanowski et al. 2011).

The placement within Braconidae of the rare, lepidopteran parasitoid genus *Dirrhope* Foerster has changed through time, with it suggested as a member of Microgastrinae (Muesebeck 1935; Tobias 1967; Marsh 1979), Miracinae (Tobias 1986; Belokobylskij 1989) or the previously recognized subfamily Adeliinae (Telenga 1955; Capek 1970), the latter currently regarded as a tribe within Cheloninae (Dowton and Austin 1998; Kittel et al. 2016). However, *Dirrhope* shows autapomorphic features



**Figure 1.** Lateral habitus of *Amicrocentrum seyrigi*.

such as the spiracles of the first metasomal tergum posterior to the middle of the tergum (Fig. 2A) that led to its recognition as a subfamily (van Achterberg 1984) that currently contains five species (van Achterberg 1984; Belokobylskij 1989; Whitfield and Wagner 1991; Belokobylskij et al. 2003). The placement of Dirrhopinae as closely related to the microgastroid complex of subfamilies has been recovered based on morphological data (van Achterberg 1984; Quicke and van Achterberg 1990; Wharton et al. 1992; Whitfield and Mason 1994; Belokobylskij et al. 2003), but it has never been included in a molecular-based study.



**Figure 2.** **A** Head of *Amicrocentrum seyrigi* showing two-segmented palpi **B** *Dirrhope americana* metasomal terga 1–3 showing the location of spiracles on the first metasomal tergum.

In this study we included representatives of Amicrocentrinae and Dirrhopinae for the first time in a phylogenomic dataset that includes all currently extant valid braconid subfamilies except Xiphoselinae. Based on genomic-scale data obtained from UCEs, we evaluated both their proposed subfamilial status and their phylogenetic affinities within Braconidae. Additionally, we assessed previously proposed morphological apomorphic features for these taxa in relation to their recovered phylogenetic relationships.

## Methods

### Taxon sampling

Our taxon sampling comprises a total of 401 terminal taxa, including outgroups. Our braconid ingroup includes 233 species that belong to the cyclostome *s.l.* group, 156 species of the non-cyclostome group (including *Amicrocentrum seyrigi* and *Dirrhope americana*) and *Apozyx penyai* Mason (Apozyginae). All braconid taxa used in this study, except *A. seyrigi* and *D. americana*, are part of the dataset that was included in a recently published phylogenetic study of Braconidae (table S1 in Jasso-Martínez et al. 2022a). As outgroup, we included 10 species belonging to nine subfamilies of Ichneumonidae, representing all major lineages within the family. Both Braconidae and Ichneumonidae are the only two extant families of the superfamily Ichneumonoidea. In a recent study that used UCEs as the data source for phylogenetic reconstruction (Blaimer et al. 2023), the superfamily Ichneumonoidea was recovered as sister to all remaining Apocrita, which includes the superfamily Gasteruptiidae; thus, we used *Gasteruption floridanum* Bradley to root our trees as was done by Jasso-Martínez et al (2022a) for a dataset that included most of the taxa in the present study. Specimens of *A. seyrigi* and *D. americana* (Table 1) are deposited in the Hymenoptera collection of the National Museum of Natural History (NMNH), Smithsonian Institution, Washington, D.C.

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

**Table 1.** Locality and SRA information of *Amicrocentrum seyrigi* and *Dirrhope americana*.

Specimen voucher	Subfamily	Genus	Species	Label information	SRA accession number
USNMMENT1322780	Amicrocentrinae	<i>Amicrocentrum</i>	<i>seyrigi</i>	MADAGASCAR: Antsokay. Latitude: 23°25.40'S, longitude: 43°44.51'E. Collection year: 2012. Collectors: M. Irwin, Rin'ha.	SAMN37185746
USNMMENT1322781	Dirrhopinae	<i>Dirrhope</i>	<i>americana</i>	USA: Virginia, Fauquier Co. Granruth Grove. Latitude: 38°49'9.60"N, longitude: 77°54'35.60"W. Collection year: 2014. Collector: Kula, R., et al.	SAMN37185747

## UCE data and matrices

Genomic DNA extraction of *A. seyrigi* and *D. americana* was performed using a leg and whole specimen, respectively, with the Quiagen DNeasy Blood and Tissue kit (Quiagen Inc., Valencia, California, U.S.A.). DNA quantitation was measured with Qubit 2.0 fluorometer (Life Technologies Inc. Carlsbad, CA). Total DNA of both samples (*A. seyrigi*: 41 ng; *D. americana* 16 ng) was used as input for shearing and library preparation. DNA was sheared for 45 seconds in a Qsonica Q800R sonicator (Qsonica LLC, Newton, CT). Genomic libraries were prepared using the Kapa Hyper Prep Kit (Roche) and the custom Tru-Seq-style dual-indexing adapter (Glenn et al. 2016). We included both libraries in a pool that was enriched with the Hymenoptera bait set 2.5Kv2 (Branstetter et al. 2017), which targets 2,590 UCE loci. Sequencing was conducted on an Illumina HiSeq 2500 instrument (2×150 rapid run; Illumina Inc. San Diego, CA).

Raw reads were cleaned and trimmed using Illumiprocessor v 2.0.7 (Faircloth, 2013), a wrapper around Trimmomatic (Del Fabbro et al. 2013; Bolger et al. 2014) in the phylyce pipeline v 1.7.0 (Faircloth 2016). *Amicrocentrum seyrigi* and *D. americana* cleaned reads assembly was performed in spades v 3.14.0 (Prjibelski et al. 2020). We merged both newly generated and previously published assemblies for UCE loci extraction in phylyce version 1.7.0 (Faircloth 2016). Extracted UCE loci were aligned with MAFFT v 7 (Kato and Standley 2013), and the resulting alignments were filtered with Gblocks version 0.91b (Castresana 2000) using reduced stringency settings for b1–b4: 0.5, 0.5, 12 and 7, respectively. We prepared two matrices containing loci recovered for at least 25% and 50% of the taxa included. Raw data are available in the Sequence Read Archive (SRA) database under the BioProject PRJNA1010366. SRA accession numbers are provided in Table 1.

## Phylogenetic reconstruction

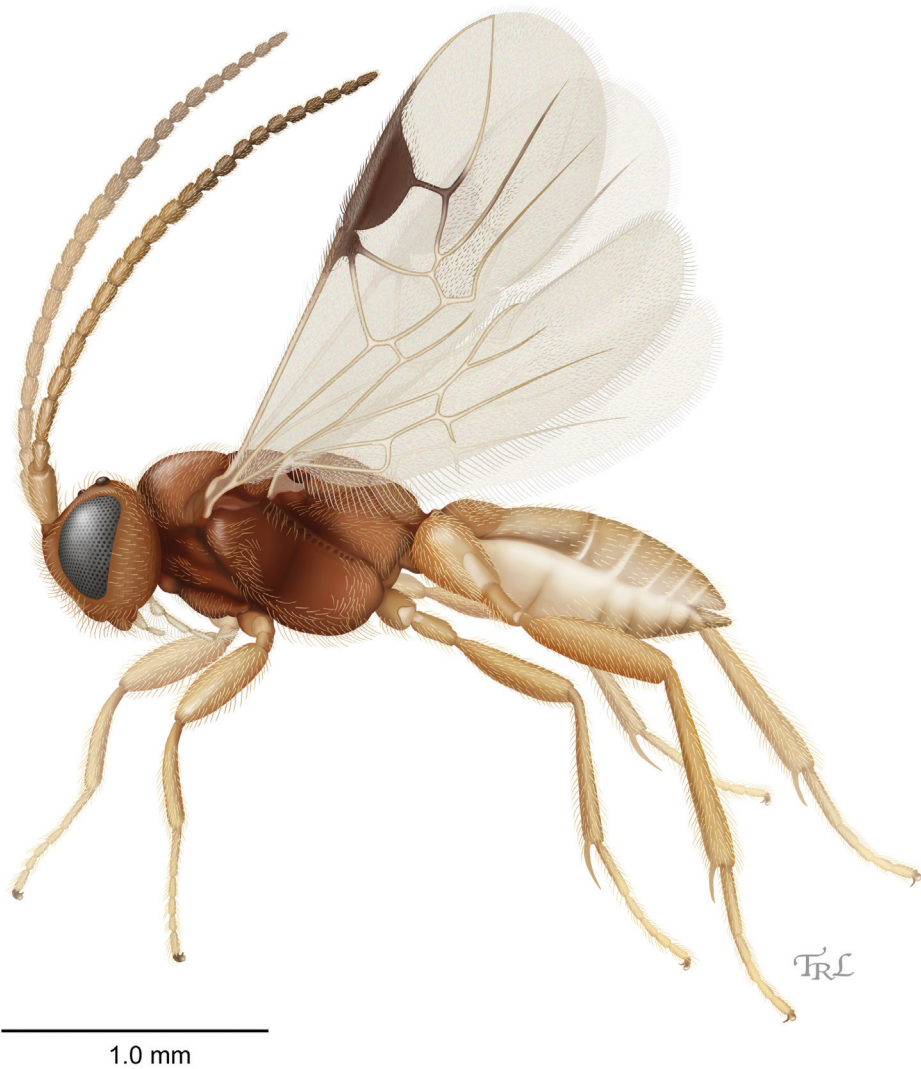
Both aligned matrices were partitioned using the Sliding-Window Site Characteristics Entropy (SWSC-EN) algorithm (Tagliacollo and Lanfear 2018) to define partitions within each UCE locus accounting for rate heterogeneity. We selected the best-fit partitioning scheme and substitution model with ModelFinder (Kalyanamoorthy et al. 2017) in IQ-TREE v2 (Minh et al. 2020) according to the Bayesian information criterion. Finally, we conducted Maximum Likelihood (ML) analyses using IQ-TREE v2 (Minh et al. 2020) with 10,000 bootstrap (BTP) replicates.



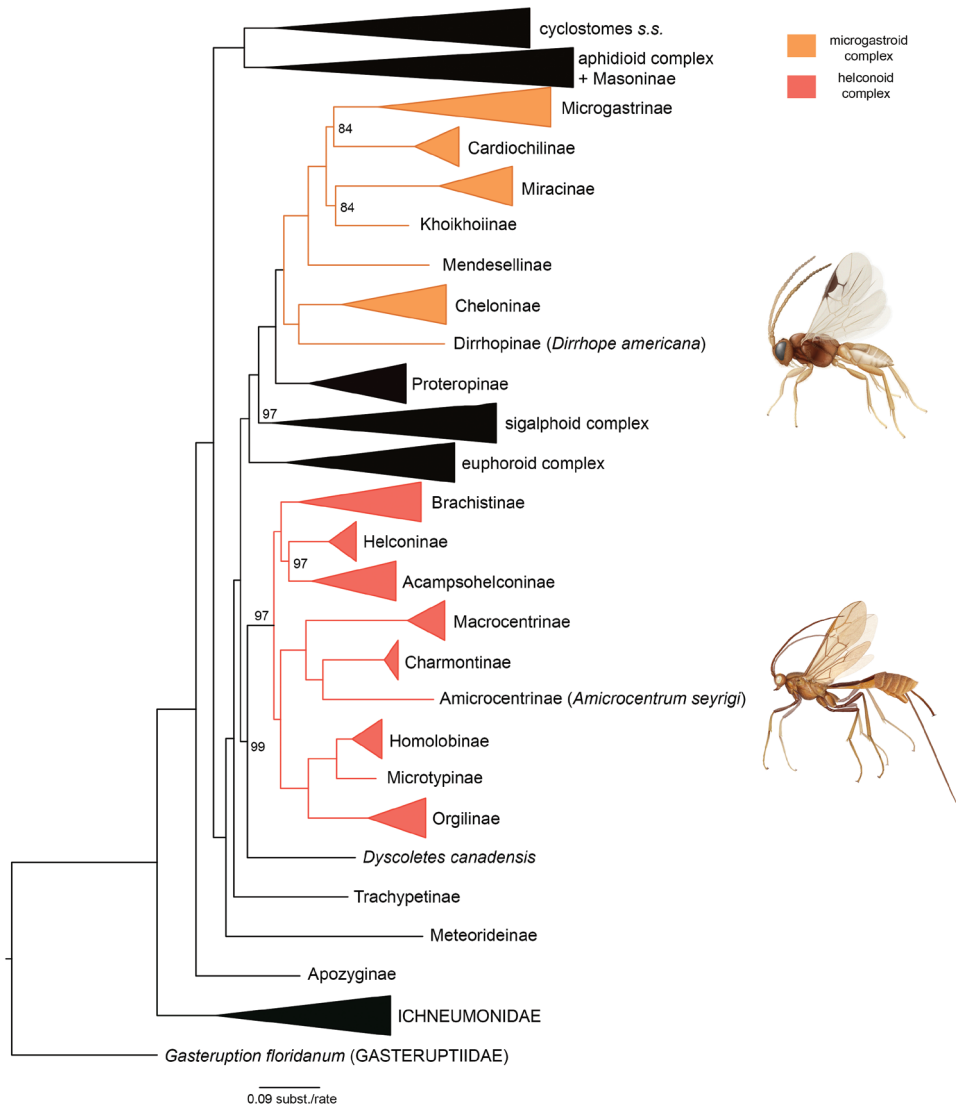
## Results

### UCE data

We obtained a total of 2,526 UCE loci in a complete dataset. We recovered 1,161 UCE loci with a mean length of 762.25 bp for the included species of *A. seyrigi*, whereas for *D. americana* we obtained 828 UCE loci with a mean length of 442.22 bp. A total of 1,578 and 917 UCE loci were retained for the 25% and 50% matrix, resulting in matrices with lengths of 342,464 and 170,549 bp, respectively.



**Figure 3.** Lateral habitus of *Dirrhope americana*.



**Figure 4.** Maximum likelihood phylogram resulting from the 50% complete matrix. Amicrocentrinae was recovered as sister to Charmontinae within the non-cyclostome helconoid complex (red). Dirrhopinae was recovered as sister to Cheloninae in the microgastroid complex (orange). Remaining Braconidae and outgroup taxa are in black. Numbers near nodes are BTP values that were under 100.

## Phylogenetic inference

The ML phylograms derived from the two different datasets analyzed (50% and 25%; Fig. 4, Suppl. materials 1, 2, respectively) were highly similar and strongly supported, recovering as monophyletic the three main clades of the braconoid complex, *i.e.* cyclostomes *s.s.*, aphidioid subfamilies, Masoninae and non-cyclostomes. Similarly, the

phylogenetic relationships among the four non-cyclostome complexes were identical, with the sigalphoid and microgastroid complexes recovered as sister taxa, followed by the euphoroid complex and the helconoid complex as sister to all of them (Fig. 4, Suppl. materials 1, 2).

Within the helconoid complex both topologies recovered Amicrocentrinae as sister to Charmontinae with high support (BTP=100), with both subfamilies being sister to Macrocentrinae. These three subfamilies together were recovered as sister to a clade comprised by the subfamilies Homolobinae, Microtypinae and Orgilinae, followed by a clade containing the subfamilies Brachistinae, Helconinae and Acampsohelconinae (Fig. 4, Suppl. materials 1, 2). Finally, in both topologies Dirrhopinae was recovered as sister to Cheloninae (BTP = 100) within the microgastroid complex and both were sister to all remaining microgastroid subfamilies, *i.e.*, Micrograstinae, Cardiochilinae, Miracinae, Khoikhoinae and Mendesellinae (Fig. 4, Suppl. materials 1, 2).

The only topological difference at the subfamily level between both datasets analyzed was the placement of the cyclostome subfamily Pambolinae, which was recovered in a clade containing “Old World” Doryctinae with weak support (BTP = 60) or as sister to the Avgiini with moderate support (BTP = 86) in the 25% (Suppl. material 2) and 50% datasets (Suppl. material 1), respectively.

## Discussion

In its current status, the non-cyclostome helconoid complex contains eight subfamilies, Acampsohelconinae, Brachistinae, Charmontinae, Helconinae, Homolobinae, Macrocentrinae, Microtypinae and Orgilinae (Sharanowski et al. 2011; Jasso-Martínez et al. 2022a, 2022b). Amicrocentrinae, together with other subfamilies that are now placed within different non-cyclostome complexes, has been also treated as a member of this complex (Wharton 1993). However, Amicrocentrinae was not included in the most recent phylogenetic hypothesis of Braconidae based on genomic-scale data; thus, the authors did not include it in the helconoid complex (Jasso-Martínez et al. 2022a).

*Amicrocentrum*, the only genus of Amicrocentrinae, was previously considered a member of Macrocentrinae; however, species of *Amicrocentrum* lack spines on the hind trochantellus (Fig. 1), which is considered an apomorphy for Macrocentrinae, and lack prepectal and hypostomal carinae, which are also features of Macrocentrinae. Further, in macrocentrines the maxillary palpi are 5–6 segmented, the labial palpi 4 segmented, and forewing vein 2RS is basad vein m-cu; in species of *Amicrocentrum* the palpi are two segmented (Fig. 2A) (van Achterberg 1979: fig. 23) and 2RS is distad m-cu (Fig. 1). A new subfamily was proposed for species of *Amicrocentrum* given those differences with respect to Macrocentrinae and the large number of morphological apomorphies shared among amicrocentrines (van Achterberg 1979). The only molecular study that has included a member of Amicrocentrinae recovered it as sister to Charmontinae with strong support, with both being sister to a clade containing Xiphoselinae and Macrocentrinae (Sharanowski et al. 2011). These four subfamilies plus Homolobinae,



Microtypinae and Orgilinae comprise a subcomplex within the helconoid complex, named 'macrocentroid' (Sharanowski et al. 2011). The macrocentroid subcomplex is biologically characterized as parasitoids of immature stages of Lepidoptera rather than Coleoptera as is the case with the other members of the helconoid complex, *i.e.*, Helconinae and Brachistinae (Sharanowski et al. 2011).

In this study, we confirm with strong support the phylogenetic placement of Amicrocentrinae as sister to Charmontinae, with both being sister to Macrocentrinae within the helconoid complex based on genomic UCE data, as was previously proposed based on nuclear Sanger sequence data (Sharanowski et al. 2011). Among the shared morphological features of Amicrocentrinae and Charmontinae are a well-developed ctenidia of the lower valve egg canal (Rahman et al. 1998), and a long ovipositor with a pre-apical swelling of the rachis of olistheter mechanism (Quicke et al. 1995).

Some lineages of the braconid non-cyclostome group such as Cardiochilinae and Miracinae have been treated as members of Microgastrinae (Nixon 1965). After their establishment as separate subfamilies, and the description of other closely related subfamilies, the name 'microgastroid' group or complex was adopted to refer to those subfamilies collectively. The microgastroid subfamilies in general comprise species with a small metasoma relative to the length of wings and the rest of the body (Quicke 2015). However, among the members of the microgastroid complex (and other braconids), members of *Dirrhope* show the autapomorphy of having the spiracles of the first metasomal tergum posterior to the middle of the tergum (Fig. 2B); thus, it was elevated to subfamily status by van Achterberg (1984).

The limits of the microgastroid complex have remained relatively stable since the publication of a phylogenetic hypothesis based on morphology, together with the recognition of the subfamilies Khoikhoiinae and Mendesellinae (Mason 1983; Whitfield and Mason 1994). The monophyly of this assemblage of subfamilies has been recovered using different sources of data, including molecular (Whitfield 1997a; Belshaw et al. 1998; Dowton et al. 1998; Banks and Whitfield 2006; Murphy et al. 2008; Sharanowski et al. 2011; Jasso-Martínez et al. 2022a, 2022b) and combined molecular and morphological (Dowton et al. 2002). However, none of the phylogenetic hypotheses of this complex based on molecular data included *Dirrhope*, and thus, its subfamily-level status based on morphology was never tested using additional sources of evidence.

Our genomic-scale data recovered Dirrhopinae as sister to Cheloninae within the microgastroid complex of subfamilies with strong support. *Dirrhope* species resemble members of the chelonine tribe Adeliini in body shape; however, in addition to the spiracle synapomorphy for dirrhopines, they have forewing vein RS distinctly curved anteriorly (Fig. 3), while RS is relatively straight in chelonines. Further, tergum 1 articulates with tergum 2 in dirrhopines, while the first and second terga are fused in chelonines (Shaw 1997; Whitfield 1997b). As in previous morphology-based studies (Quicke and van Achterberg 1990; Whitfield and Mason 1994), our UCE data recovered the Dirrhopinae + Cheloninae clade as sister to the remaining microgastroid subfamilies. Thus, based on molecular phylogenetic analysis, we confirm the placement of Dirrhopinae as a member of the non-cyclostome microgastroid complex.

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## Supplementary material I

### Maximum likelihood tree resulting from the 50% complete matrix without collapsed clades and with full names of the terminal taxa

Authors: Jovana M. Jasso-Martínez, Seán G. Brady, Robert R. Kula

Data type: jpg

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## Supplementary material 2

### Maximum likelihood tree resulting from the 25% complete matrix

Authors: Jovana M. Jasso-Martínez, Seán G. Brady, Robert R. Kula

Data type: jpg

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