J. Insect Biodivers. Syst. 10 (4), 965–981
https://jibs.modares.ac.ir

OPEN ACCESS

Tarbiat Modares University Press
Entomological Society of Iran
Research Article
Phylogeny
https://doi.org/10.61186/jibs.10.4.965
ISSN: 2423-8112
https://zoobank.org/urn:lsid:zoobank.org:48340F35-37F7-484C-BC5B-780F5A9766C1

Contribution to the phylogeny of Microgastrinae (Hymenoptera: Braconidae) based on mitochondrial COI and nuclear 28S rDNA genes, with comments on the identity of *Pholetesor circumscriptus* (Nees, 1834)

Parisa Abdoli, Ali Asghar Talebi
Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, I.R. Iran.
✉ *p.abdoli@modares.ac.ir* *https://orcid.org/0000-0001-6866-0337*
✉ *talebia@modares.ac.ir* *https://orcid.org/0000-0001-5749-6391*

Nickolas G. Kavallieratos
Laboratory of Agricultural Zoology and Entomology, Department of Crop Science, Agricultural University of Athens; 75 Iera Odos str., 11855 Athens, Attica, Greece.
✉ *nick_kaval@aua.gr* *https://orcid.org/0000-0001-5851-5013*

Rasoul Khosravi
Department of Natural Resources and Environmental Engineering, College of Agriculture, Shiraz University, Shiraz, Iran.
✉ *r-khosravi@shirazu.ac.ir* *https://orcid.org/0000-0002-7231-563X*

Farzad Bidari
Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, Australia.
✉ *f.bidari@westernsydney.edu.au* *https://orcid.org/0009-0000-6953-7218*

ABSTRACT. Microgastrines are diverse group of endoparasitoid wasps attacking caterpillars (Lepidoptera). Despite their importance in biological control, there is still no consensus concerning the phylogeny relationships among taxa. Although previous phylogenetic analyses have advanced the overall understanding of phylogenetic relationships of Microgastrinae, the small numbers of sampled taxa have led to disagreement in taxonomic assignments. In the present study, we performed a molecular genetic survey using both mitochondrial and nuclear data, increasing the taxons' sampling, to clarify the generic relationships and improve the inferences of the taxonomic status within Microgastrinae. We reconstructed a phylogenomic tree of Microgastrinae with sequences that exist up till now, from fifty-five genera for COI and thirty genera for 28S rDNA, both new and from previous studies. Several species and genera have been sequenced for the first time. In this study, we identified some of the closest phylogenetic relatives of Microgastrinae genera by analyzing DNA sequences from the mitochondrial COI and 28S rDNA. Most clades of the current findings correspond to the latest morphological classification of Microgastrinae. New clades and several well-supported clades, conform to the most previously recorded clades and provide an increased understanding of the Microgastrinae evolution. Based on molecular examination, *Pholetesor psedocircumscriptus* Abdoli, 2019 is synonymized with *Pholetesor circumscriptus* (Nees, 1834).

Keywords: Molecular phylogeny, DNA sequences, Bayesian method, new synonym

Received:
11 July, 2024

Accepted:
23 August, 2024

Published:
01 September, 2024

Subject Editor:
Mohammad Mehrabadi

Citation: Abdoli, P., Talebi, A.A., Kavallieratos, N.G., Khosravi, R. & Bidari, F. (2024) Contribution to the phylogeny of Microgastrinae (Hymenoptera: Braconidae) based on mitochondrial COI and nuclear 28S rDNA genes, with comments on the identity of *Pholetesor circumscriptus* (Nees, 1834). *Journal of Insect Biodiversity and Systematics*, 10 (4), 965–981.

INTRODUCTION

Microgastrinae (Hymenoptera, Braconidae) is one the most species-rich subfamilies of Braconidae consisting of 2999 known species belonging to 81 genera across the world (Fernandez-Triana et al., 2020).

Corresponding author: Talebi, A.A., ✉ *talebia@modares.ac.ir*

Microgastrines play a crucial role as koinobiont endoparasitoids of Lepidopteran larva (Shaw & Huddleston, 1991). Despite their importance in biological control, the phylogenetic relationships within Microgastrinae still remain questionable and controversial while no comprehensive studies have investigated their phylogeny, genetic diversity, and spatial patterns. Existing molecular studies have provided inconclusive results, highlighting the monophyletic position of many microgastrine taxa, while their phylogenetic position varies depending on the utilized molecular marker and the number of sampled taxa (Belshaw et al., 1998; Pitz et al., 2007; Sharanowski et al., 2011; Shi et al., 2005; Whitfield et al., 2002, 2018; Jasso-Martinez et al., 2022).

Numerous hypotheses have been proposed regarding the phylogenetic relationships among taxa and their phylogenetic inconsistencies. Murphy et al. (2008) presented a clade comprising five subfamilies (i.e., Cardiochilinae, Khoikhoinae, Mendesellinae, Microgastrinae, and Miracinae) as a sister lineage in the family Braconidae. Whitfield et al. (2018) synthesized molecular data from various literatures to outline some generic relationships within the subfamily Microgastrinae. According their summary *Microplitis*, *Snellenius*; *Cotesia*, *Glyptapanteles*, *Sathon*, *Venanides*; *Apanteles* s.str., *Alphomelon*, *Rhygoplitis*; *Prasmodon*, *Pseudapanteles*; *Dolichogenidea*, *Pholetesor* (in part); *Promicrogaster* and *Sendaphne* are closely related and form a clade (Banks & Whitfield, 2006; Mardulyn & Whitfield, 1999; Whitfield et al., 2002, 2018). Previous studies have identified conflicts among molecular and morphological data within the genera of Microgastrinae, resolving only some terminal nodes (Mardulyn & Whitfield, 1999; Whitfield et al., 2002, 2018; Fernandez-Triana et al., 2020). Therefore, the investigation of the phylogenetic position of Microgastrinae genera and precisely reconstructing their phylogeny improves the overall knowledge of the phylogeny of this subfamily.

The incongruent phylogenetic relationships within Microgastrinae and the possibility of increasing the sampling of taxa have motivated researchers to conduct more studies to unravel the exact phylogenetic relationships among taxa. It has been noted that the inclusion of additional molecular markers, without a concurrent expansion of taxon sampling, may result in a diminished phylogenetic signal (Banks & Whitfield, 2006; Jantzen et al., 2019; Dong et al., 2022). On the other hand, some studies have suggested that effective taxon sampling plays a crucial role in resolving controversies in phylogenetic inference, highlighting the impact of adding specific taxa on inference performance. As more taxon sequencing data becomes available, the positioning of species within clades offers an avenue to enhance our understanding of these taxa, revealing new relationships. The availability of such new data can significantly influence hypotheses supported by phylogenetic inference, promoting researchers to formulate novel ideas about potential relationships among taxa (Rannala et al., 1998; Nabhan & Sarkar, 2012; Jantzen et al., 2019; Dong et al., 2022). The genera of Microgastrinae exhibit challenges for taxonomists due to convergent morphological characters, and complicating classification. Fernandez-Triana et al. (2020) categorized the 81 genera into three specified groups, i.e., *Cotesia*, *Microplitis*, and *Apanteles*, with distinct morphological diagnosis for each group. They also identified a group with unplaced genera that likely belonged elsewhere, designating it as the unplaced group. They emphasized that these groups do not represent a new phylogeny for the subfamily.

The phylogeny of Microgastrinae is challenging due to the limited genetic available data and the complexity of their evolutionary relationships. Previous studies have primarily relied on morphological data that have not fully resolved the phylogenetic relationships within this subfamily. To address these gaps and provide a comprehensive approach, we conducted an extensive phylogenetic analysis incorporating a broad range of taxa and utilized both nuclear and mitochondrial markers. The results provide a better framework for resolving the phylogenetic relationships among these poorly studied taxa. Furthermore, by comparing our molecular phylogeny with the latest morphological classification of Microgastrinae, we aim to provide new insights and potential revisions to the current understanding of their evolutionary history. Our study represents a novel effort to integrate molecular and morphological data to enhance the phylogenetic resolution of Microgastrinae.

MATERIAL AND METHODS

Sampling and morphological studies. Specimens were collected using Malaise traps from March to November in 2020 and 2021 in the north-central Iran (i.e., Alborz, Guilan, Mazandaran, Qazvin and Tehran provinces). The north-central region of Iran includes both the northern and southern slopes of Alborz Mountains. The northern slope, recognized as the southern part of the Caucasus biodiversity hotspot (Noroozi et al., 2019), comprises Guilan and Mazandaran provinces, where 16 Malaise traps were placed. In contrast, the southern slope, as a part of the Irano-Anatolian biodiversity hotspot (Noroozi et al., 2019), includes Alborz, Tehran and Qazvin provinces, where 15 Malaise traps were placed. Malaise traps were set up in a range of different habitats such as forests, rangelands and orchards to ensure the actual reflection of the biodiversity in these environments. The collected parasitoids were preserved in 70% ethanol, and subsequent identification was conducted at the genus or species level using appropriate identification keys (Telenga, 1955; Nixon, 1965; Mason, 1981; Tobias, 1986). Each specimen was examined under the Olympus™ SZX9 stereomicroscope. The results of the identification of Microgastrinae in the North-central Iran, based on the morphological characters, have been published in recent years (Abdoli et al., 2019a, 2019b, 2019c; 2021a, 2021b; 2022). The examined material is deposited in the Insect Collection of the Department of Entomology, Tarbiat Modares University, Tehran, Iran (TMUC).

Extraction and sequencing. Total genomic DNA was extracted from the legs of the individuals using the Qiagen Dneasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). The quality of the extracted DNA was determined on a 1% agarose gel and the amount of total genomic DNA was quantified using Nanodrop (Allsheng, Nano -200, China). Two molecular markers were used to reconstruct the phylogeny tree including mitochondrial Cytochrome c oxidase subunit I, COI (5′ GGTCAACAAAT CATAAAGATATTGG 3′), HCO2198 (5′ TAAACTTCAGGGTGACCA AAAAATCA 3′) (Folmer et al. 1994) and 28S nuclear ribosomal DNA, 28S rDNA (5′ AAGAGAGAGTTCAAGAGTACGTG 3′), 28S_R (5′ TAGTTCACCATCTTTCGGGTCCC 3′) (Mardulyn & Whitfield, 1999). The PCR was performed in a 25 µL solution containing 12.5 µL Master Mix, 1 µL of each primer (10 pmol µL-1), 1 µL of extracted DNA and 9.5 µL double-distilled water. The PCR was carried out in the following steps: initial denaturation at 95°C for 3 min, followed by 5 cycles of 1 minute at 94ºC, 1 minute at 45ºC, 1 minute at 72ºC, and then 35 cycles of 1 minute at 94ºC, 1 minute at 51ºC, 1 minute at 72ºC, with a final extension step at 72ºC for 5 minutes. All PCR products were directly sequenced with both primers by Bio-Magic-Gene company in Iran. The COI and 28S rDNA sequences of the specimens were deposited in GenBank and other sequences used in comprehensive phylogenetic analyses were downloaded from NCBI and BOLD System (Table 1, and Table 2).

Phylogenetic analyses. Sequence alignment was performed using MAFFT online for COI and Muscle for 28S rDNA with MEGA7 (Tamura et al., 2013). The curation of alignments was performed manually using the MEGA7 method (Castresana, 2000). Sequences of both COI and the 28S rDNA were trimmed to 660 bp. The best-fit nucleotide substitution model was determined using MrModeltest 2 (Nylander, 2004). We employed BEAST v2.7.3 using an uncorrelated lognormal relaxed clock model (Drummond et al., 2012) and the constant rate birth-death process for the prior distribution on node heights (Gernhard, 2008), with default priors. A random coalescent starting tree, using default values for demographic parameters, was used for analyses in which BEAST was allowed to infer the root position. Convergence of likelihoods and model parameters was determined using Tracer. Most runs were terminated once these measures had been stable for at least 10 million generations, with preceding generations discarded as burn-in. Maximum clade credibility trees with mean node depths were calculated in Tree Annotator v2.7.3 (Drummond & Rambaut, 2007). The trees were rooted with Miracinae as outgroup. We visualized the resulting topology using FigTree v1.4.3.

Table 1. Taxa used in the molecular analysis of COI, along with accession numbers and source or locality.

Taxa	Accession number or BIN ID	Source/locality
Miracinae	JN289534	French Guiana
Alloplitis Nixon, 1965	JN659929	Rodriguez et el. (2013)
Alphomelon Mason, 1981	JQ855429	Smith et al. (2013)
Alphomelon xestopyga Deans, 2003	JQ855430	Smith et al. (2013)
Apanteles Foerster, 1863	PQ144870	Present study
Apanteles Foerster, 1863	GU141050	Fernandez-Triana et al. (2011a)
Beyarslania insolens (Wilkinson, 1930)	BOLD:ABV1136	South Africa
Buluka De Saeger, 1948	HM430407	Smith et al. (2013)
Choeras consimilis (Viereck, 1911)	KR802979	Hebert et al. (2016)
Choeras formosus Abdoli & Fernandez-Triana, 2019	PQ144876	Present study
Choeras taftanensis Ghafouri Moghaddam & van Achterberg,	PQ144877	Present study
Choeras tiro (Reinhard, 1880)	PQ145584	Present study
Clarkinella Mason, 1981	MF929335	Canada
Clarkinella Mason, 1981	JQ849626	Smith et al. (2013)
Cotesia ruficrus (Haliday, 1834)	HM397148	Smith et al. (2013)
Cotesia Cameron, 1891	PQ144867	Present study
Cotesia Cameron, 1891	PQ144868	Present study
Dasylagon Muesebeck, 1958	AF102719	Mardulyn & Whitfield (1999)
Deuterixys rimulosa (Niezabitowski, 1910)	DQ538824	Banks & Whitfield (2006)
Deuterixys Mason, 1981	MG439334	Canada
Diolcogaster alvearia (Fabricius, 1798)	PQ144866	Present study
Diolcogaster alvearia (Fabricius, 1798)	KJ459109	-
Diolcogaster mayae (Shestakov, 1932)	PQ152953	Present study
Diolcogaster Ashmead, 1900	MH138685	Australia
Distatrix loretta Grinter, 2009	BOLD:ABA9259	Costa Rica
Distatrix papilionis (Viereck, 1912)	KC867697	Smith et al. (2013)
Distatrix Mason, 1981	JQ854979	Smith et al. (2013)
Dolichogenidea laevigata (Ratzeburg, 1848)	PQ144865	Present study
Dolichogenidea fernandeztrianai Abdoli & Talebi, 2019	PQ144864	Present study
Dolichogenidea Viereck, 1911	JF271346	Papua New Guinea
Exoryza mariabustosae Fernandez-Triana, 2016	KX146409	Fernandez-Triana et al. (2016)
Exoryza rosamatarritae Fernandez-Triana, 2016	KX146408	Fernandez-Triana et al. (2016)
Fornicia Brullé, 1846	JQ854916	Smith et al. (2013)
Fornicia Brullé, 1846	JN282333	Smith et al. (2013)
Glyptapanteles compressiventris (Muesebeck, 1921)	JN282008	Smith et al. (2013)
Glyptapanteles Ashmead, 1904	PQ144863	Present study
Hygroplitis melligaster (Provancher, 1886)	KM897007	Fernandez-Triana et al. (2014)
Hygroplitis Thomson, 1895	JQ855071	Smith et al. (2013)
Hypomicrogaster Ashmead, 1898	KR881266	Hebert et al. (2016)
Hypomicrogaster Ashmead, 1898	KC130370	Smith et al. (2013)
Iconella radiata Abdoli & Talebi, 2020	PQ144875	Present study
Iconella Mason, 1981	KC685309	Fernandez-Triana et al. (2013)
Iconella Mason, 1981	KC685304	Fernandez-Triana et al. (2013)
Illidops Mason, 1981	HM396642	Smith et al. (2013)
Illidops Mason, 1981	HQ925944	Smith et al. (2013)
Janhalacaste winnieae Fernandez-Triana and Boudreault, 2018	BOLD:AAK0117	Fernandez-Triana & Boudreault (2018)
Janhalacaste danieli Fernandez-Triana and Boudreault, 2018	BOLD:ACB2460	Fernandez-Triana & Boudreault (2018)
Jenopappius magyarmuzeum Fernandez-Triana & Boudreault,	BOLD:AAH1374	Fernandez-Triana & Boudreault (2018)
Jimwhitfieldius Fernandez-Triana, 2018	BOLD:AAH1239	Fernandez-Triana & Boudreault (2018)
Kiwigaster variabilis Fernandez-Triana & Ward, 2011	BOLD:ACL7939	New Zealand
Kiwigaster variabilis Fernandez-Triana and Ward, 2011	BOLD:ACL7939	Fernandez-Triana et al. (2011b)
Kotenkosius tricarinatus Fernandez-Triana & Boudreault, 2018	BOLD:AAV2185	Fernandez-Triana & Boudreault (2018)
Larissimus cassander Nixon, 1965	JQ851749	Smith et al. (2013)
Larissimus cassander Nixon, 1965	JQ851749	Smith et al. (2013)
Larissimus Nixon, 1965	JQ854418	Locality unknown
Larissimus Nixon, 1965	JQ854418	Smith et al. (2013)
Lathrapanteles Williams, 1985	JQ854802	Smith et al. (2013)
Lathrapanteles Williams, 1985	HQ550264	Smith et al. (2013)

Taxa	Accession number or BIN ID	Source/locality
Mariapanteles felipei Whitfield, 2012	BOLD:AAE8276	Costa Rica
Mariapanteles Whitfield & Fernandez-Triana, 2012	BOLD:ADE4712	Brazil
Microgaster Latreille, 1804	GU141238	Fernandez-Triana et al. (2011a)
Microgaster Latreille, 1804	JN293671	Fernandez-Triana et al. (2011a)
Microgaster Latreille, 1804	PQ144871	Present study
Microplitis alborziensis Abdoli & Talebi, 2021	MN820452	Present study
Microplitis kaszabi Papp, 1980	PQ144874	Present study
Microplitis Foerster, 1863	HM397413	Smith et al. (2013)
Miropotes Nixon, 1965	BOLD:ABX1530	Australia
Miropotes Nixon, 1965	BOLD:ADM0565	Australia
Miropotes Nixon, 1965	BOLD:ABA6079	Australia
Neoclarkinella Rema & Narendran, 1996	HM430522	Smith et al. (2013)
Neoclarkinella Rema & Narendran, 1996	HM430450	Smith et al. (2013)
Nyereria Mason, 1981	HQ558996	Smith et al. (2013)
Nyereria Mason, 1981	JQ848839	Smith et al. (2013)
Papanteles Mason, 1981	JQ854942	Smith et al. (2013)
Papanteles Mason, 1981	JQ847483	Smith et al. (2013)
Parapanteles eros Gupta, 2014	KT334011	India
Parapanteles Ashmead, 1900	JQ852327	Smith et al. (2013)
Parenion kokodana (Wilkinson, 1936)	BOLD:ABA0055	Papua New Guinea
Parenion Nixon, 1965	BOLD:AAZ8941	New Caledonia
Paroplitis Mason, 1981	BOLD:AAP0533	Germany
Philoplitis Nixon, 1965	JN660042	Rodriguez et al. (2013)
Philoplitis striatus Fernandez-Triana & Goulet, 2009	JQ846716	Smith et al. (2013)
Pholetesor psudocircumscriptus Abdoli, 2019	PQ144872	Present study
Pholetesor Mason, 1981	PQ144872	Present study
Pholetesor Mason, 1981	KR788874	Hebert et al. (2016)
Prasmodon Nixon, 1965	DQ538832	Banks & Whitfield (2006)
Prasmodon Nixon, 1965	JQ854850	Smith et al. (2013)
Promicrogaster liagrantae Fernandez-Triana & Boudreault, 2016	KR808817	Hebert et al. (2016)
Promicrogaster Brues & Richardson, 1913	JN281691	Smith et al. (2013)
Protapanteles Ashmead, 1898	KR808264	Hebert et al. (2016)
Protapanteles Ashmead, 1898	GU141378	Fernandez-Triana et al. (2011a)
Protomicroplitis Ashmead, 1898	HM397594	Smith et al. (2013)
Protomicroplitis Ashmead, 1898	JQ848676	Smith et al. (2013)
Pseudapanteles Ashmead, 1898	JN281754	Smith et al. (2013)
Pseudapanteles Ashmead, 1898	KJ840799	Fernandez-Triana et al. (2014)
Rasivalva Mason, 1981	JQ855198	Smith et al. (2013)
Rasivalva Mason, 1981	JQ852962	Smith et al. (2013)
Rhygoplitis Mason, 1981	JQ854244	Smith et al. (2013)
Rhygoplitis Mason, 1981	KC755365	Fernandez-Flores et al. (2013)
Sathon Mason, 1981	HQ941789	Smith et al. (2013)
Sathon Mason, 1981	JF864698	Canada
Sendaphne Nixon, 1965	HQ550197	Smith et al. (2013)
Sendaphne Nixon, 1965	BOLD:AAA7170	Fernandez-Flores et al. (2013)
Shireplitis Fernandez-Triana & Ward, 2013	JQ850080	Smith et al. (2013)
Shireplitis Fernandez-Triana & Ward, 2013.	BOLD:AAV6352	Smith et al. (2013)
Snellenius Westwood, 1882	HM430408	Smith et al. (2013)
Snellenius Westwood, 1882	JQ846757	Smith et al. (2013)
Venanides caspicus Abdoli, Fernandez-Triana & Talebi, 2019	PQ144869	Present study
Venanides Mason, 1981	JQ848252	Smith et al. (2013)
Venanus Mason, 1981	JQ854847	Smith et al. (2013)
Venanus Mason, 1981	KR925140	Hebert et al. (2016)
Wilkinsonellus Mason, 1981	JN282230	Smith et al. (2013)
Wilkinsonellus Mason, 1981	JN282286	Smith et al. (2013)
Wilkinsonellus Mason, 1981	HM907598	Smith et al. (2013)
Xanthomicrogaster Cameron, 1911	JQ854715	Smith et al. (2013)
Xanthomicrogaster Cameron, 1911	HQ550277	Smith et al. (2013)
Zachterbergius tenuitergum Fernandez-Triana & Boudreault, 2018	BOLD:AAV2126	Smith et al. (2013)

Table 2. Taxa used in the molecular analysis of 28S rDNA, along with accession numbers and source or locality.

Taxa	Accession number or BIN ID	Source/locality
Miracinae	EU106929	Murphy et al. (2008)
Alphomelon Mason, 1981	AF102732	Mardulyn & Whitfield (1999)
Alphomelon Mason, 1981	AF102732	Mardulyn & Whitfield (1999)
Apanteles Foerster, 1863	PP959388	Present study
Apanteles Foerster, 1863	GU141402	Canada
Choeras fulviventris Fernandez-Triana & Abdoli, 2019	PP959383	Present study
Choeras qazviniensis Fernandez-Triana & Talebi, 2019	PP959382	Present study
Choeras taftanensis Ghafouri Moghaddam & van Achterberg, 2018	PP959384	Present study
Cotesia Cameron, 1891	PP959386	Present study
Cotesia Cameron, 1891	PP959385	Present study
Dasylagon Muesebeck, 1958	AF102744	Mardulyn & Whitfield (1999)
Deuterixys rimulosa (Niezabitowski, 1910)	AY044219	Whitfield et al. (2002)
Deuteryxis Mason, 1981	PP959398	Present study
Diolcogaster mayae (Shestakov, 1932)	PP959381	Present study
Dolichogenidea Viereck, 1911	MN645027	Parks et al. (2020)
Exoryza yeimycedenoae Fernandez-Triana, 2016	MN645035	Parks et al. (2020)
Fornicia Brullé, 1846	DQ538984	Banks & Whitfield (2006)
Fornicia Brullé, 1846	Z97959	Belshaw et al. (1998)
Glyptapanteles Ashmead, 1904	FJ396429	Smith et al. (2009)
Glyptapanteles Ashmead, 1904	GU141478	Canada
Hypomicrogaster Ashmead, 1898	AF102737	Mardulyn & Whitfield (1999)
Iconella radiata Abdoli & Talebi, 2021	PP959396	Present study
Iconella Mason, 1981	PP959395	Present study
Illidops Mason, 1981	PP959397	Present study
Microgaster canadensis Muesebeck, 1922	AF102733	Mardulyn & Whitfield (1999)
Microgaster Latreille, 1804	PP959389	Present study
Microplitis alborziensis Abdoli & Talebi 2021	PP959392	Present study
Microplitis kaszabi Papp, 1980	PP959393	Present study
Microplitis matures Weed, 1888	AF102727	Mardulyn & Whitfield (1999)
Miropotes Nixon, 1965	AF379920	Dowton & Austin (2001)
Miropotes Nixon, 1965	AY044225	Whitfield et al., 2002
Parapanteles Ashmead, 1900	MN645374	Parks et al. (2020)
Parapanteles Ashmead, 1900	MN645261	Parks et al. (2020)
Pholetesor circumscriptus (Nees 1834)	PP959390	Present study
Pholetesor ornigis (Weed, 1887)	AF102736	Mardulyn & Whitfield (1999)
Pholetesor Mason, 1981	PP959391	Present study
Prasmodon eminens Nixon, 1965	AF102725	Mardulyn & Whitfield (1999)
Prasmodon Nixon, 1965	DQ538986	Banks & Whitfield (2006)
Promicrogaster Brues & Richardson, 1913	DQ538988	Banks & Whitfield (2006)
Promicrogaster Brues & Richardson, 1913	DQ538987	Banks & Whitfield (2006)
Protapanteles Ashmead, 1898	PP959394	Present study
Protapanteles Ashmead, 1898	GU141564	Canada
Pseudapanteles dignus (Muesebeck, 1938)	DQ538989	Banks & Whitfield (2006)
Pseudapanteles Ashmead, 1898	DQ538990	Banks & Whitfield (2006)
Rhygoplitis Mason, 1981	DQ538992	Banks & Whitfield (2006)
Sathon falcatus (Nees 1834)	AF102746	Mardulyn & Whitfield (1999)
Sathon falcatus (Nees 1834)	AF029130	Dowton & Austin (1998)
Sendaphne Nixon, 1965	DQ538993	Banks & Whitfield (2006)
Snellenius Westwood, 1882	AF102726	Mardulyn & Whitfield (1999)
Snellenius Westwood, 1882	DQ538994	Banks & Whitfield (2006)
Venanides caspicus Abdoli, Fernandez-Triana & Talebi, 2019	PP959387	Present study
Venanus minutalis (Muesebeck, 1958)	AY044226	Whitfield et al. (2002)
Venanus Mason, 1981	DQ538995	Banks & Whitfield (2006)
Xanthomicrogaster Cameron, 1911	DQ538996	Banks & Whitfield (2006)

Phylogenetic relationships. A phylogenetic reconstruction of the subfamily Microgastrinae using DNA sequences from Mitochondrial COI and 28S rDNA molecular markers was explored, combining both newly obtained data and information from previous studies through Bayesian methods. The best-fitting model of nucleotide substitution was determined GTR+G+I for COI and 28S rDNA sequences. Molecular data were collected from 115 specimens belonging to 55 genera for COI (18 specimens determined in this study and 97 specimens from previously published data) (Table 1) and 52 specimens from 30 genera for 28S rDNA (18 specimens identified in this study and 34 specimens from previously published data) (Table 2). The following taxa were sequenced to reconstruct phylogenetic relationships of Microgastrinae: mitochondrial COI gene of ten species, *Choeras formosus* Abdoli & Fernandez-Triana, 2019, *Choeras taftanensis* Ghafouri Moghaddam & van Achterberg, 2018, *Choeras tiro* (Reinhard, 1880), *Iconella radiata* Abdoli & Talebi, 2020, *Microplitis alborziensis* Abdoli & Talebi, 2021, *Microplitis kaszabi* Papp, 1980, *Dolichogenidea Fernandeztrianai* Abdoli & Talebi, 2019, *Diolcogaster mayae* (Shestakov, 1932), *Venanides caspicus* Abdoli, Fernandez-Triana & Talebi, 2019, *Pholetesor pseudocircumscriptus* Abdoli, 2019, two genera (*Venanides*, *Iconella*); the 28S rDNA gene of nine species, *Diolcogaster mayae* (Shestakov, 1932), *Choeras taftanensis* Ghafouri Moghaddam & van Achterberg, 2018, *Choeras qazviniensis* Fernandez-Triana & Talebi, 2019, *Choeras fulviventris* Fernandez-Triana & Abdoli, 2019, *Venanides caspicus* Abdoli, Fernandez-Triana & Talebi, 2019, *Pholetesor psudocircumscriptus* Abdoli, 2019, *Microplitis alborziensis* Abdoli & Talebi, 2021, *Microplitis kaszabi* Papp, 1980, *Iconella radiata* Abdoli & Talebi, 2020 (Table 1, and Table 2).

RESULTS

Taxonomic hierarchy

Order Hymenoptera Linnaeus, 1758

Family Braconidae Nees von Esenbeck, 1811

Subfamily Microgasterinae Foerster, 1863

Phylogenetic analysis. The constructed phylogenetic trees of Microgastrinae based on the COI and 28S rDNA genes are shown in Figure 1, and Figure 2, respectively. In the COI gene tree, some taxa were recovered as well-supported sister, comprising *Hypomicrogaster, Apanteles, Illidops; Iconella, Neoclarkinella; Dolichogenidea, Exoriza, Parapanteles; Alphomelon, Janhalacaste, Pseudapanteles*, but in a well-supported clade with *Rhygoplitis, Hygroplitis, Microgaster, Papanteles, Sendaphne, Dasylagon, Promicrogaster, Paroplitis, Shireplitis, Clarkinella, Glyptapanteles, Cotesia, Protapanteles, Sathon, Lathrapanteles*. Other clades that were recovered together as paraphyletic included *Diolcogaster, Buluka, Protomicroplitis, Larrismus, Parenion, Xanthomicrogaster; Jimwhitfieldius, Kotenkosius, Venanus, Mariapanteles, Miropotes, Venanides; Alloplitis, Philoplitis, Prasmodon, Zachterbergius, Rasivalva, Wilkinsonellus*, and *Microplitis, Snellenius, Choeras, Deuterixyes, Beyarslania*. In the 28S rDNA gene tree, the genera which were recovered as well-supported sister taxa included *Apanteles, Illidops, Alphomelon, Pholetesor, Rhygoplitis, Iconella, Exoryza, Dolichogenidea, Parapanteles; Pseudapanteles, Prasmodon; Glyptapanteles, Cotesia, Protapanteles; Venanides, Miropotes; Deuteryxis, Xanthomicrogaster; Microplitis, Snellenius*, and *Sendaphne, Dasylagon, Promicrogaster*.

Synonymy. The available data in NCBI (National Center for Biotechnology Information: show that *P. circumscriptus* (Fig. 3A) and the recently described species, *P. pseudocircumscriptus* (Fig. 3B), for which DNA barcodes are available, differ by only 0.78% in their nucleotide sequences (a difference of 5 base pairs, resulting 99.22% identity). This minimal genetic divergence, combined with their morphological similarities, indicated that these two taxa are the same species, despite previous differentiation based on certain morphological features ((Abdoli & Pourhaji, 2019). Therefore *P. psedocircumscriptus* is proposed as a new synonym of *P. circumscriptus* (Table 3). Notably, it is mentioned that *Pholetesor circumscriptus* exhibits some variation in colouration, particularly in the legs and metasomal segments, depending on the region, especially in the Old World (Whitfield, 2006).

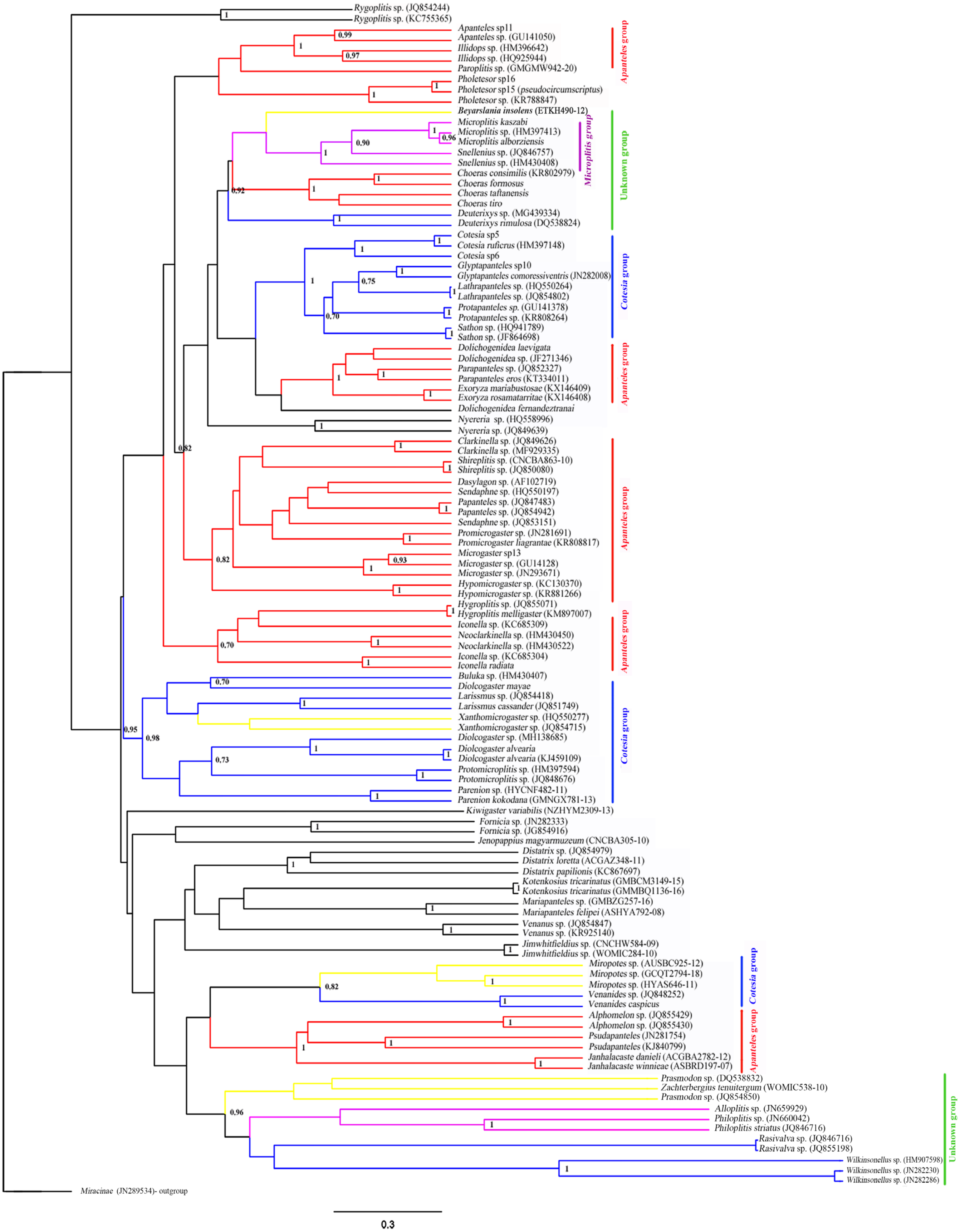

Figure 1. Bayesian tree to reconstruct phylogenetic relationships within Microgastrinae based on COI. Bayesian posterior probabilities greater than 0.70 are shown at the nodes.

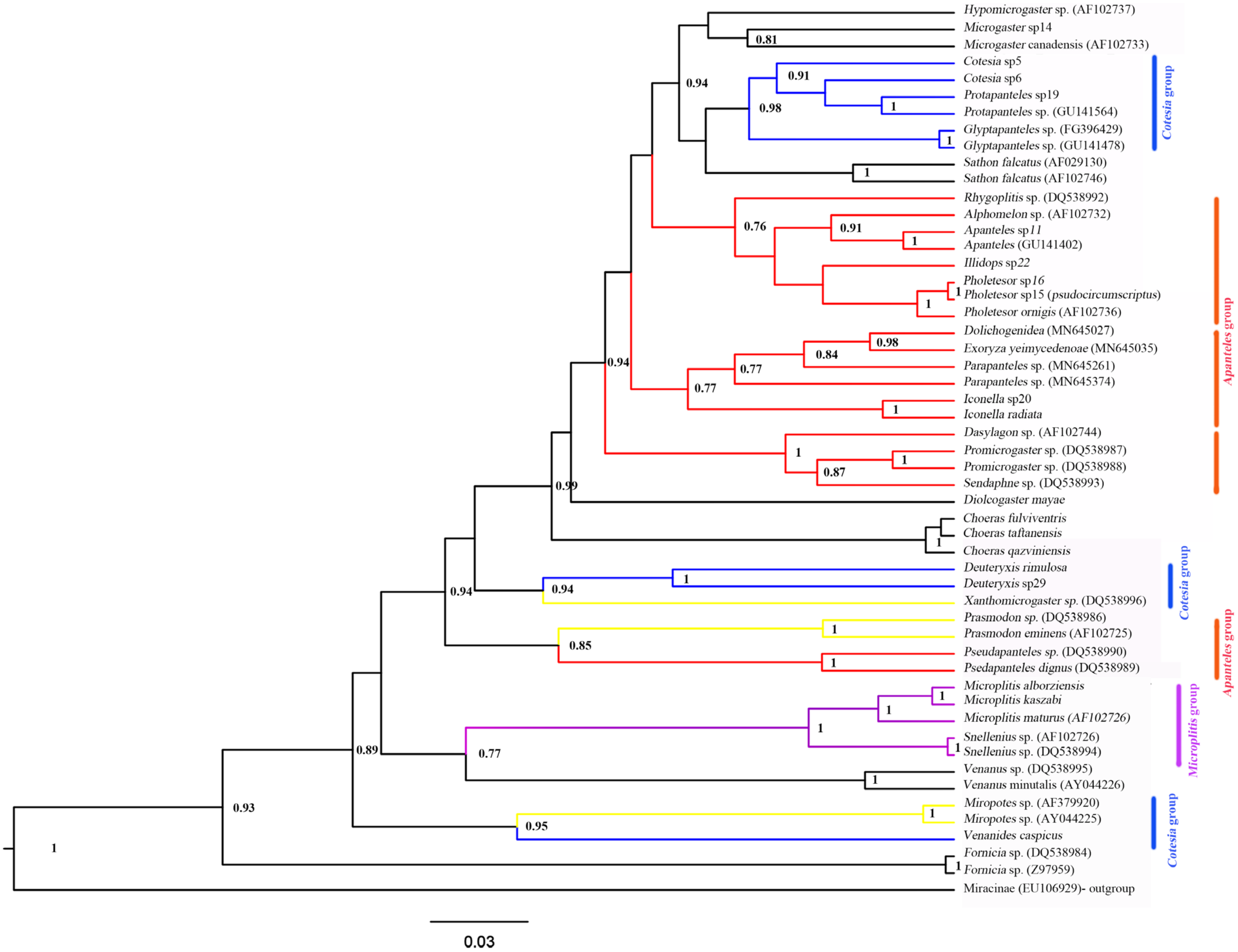

Figure 2. Bayesian tree to reconstruct phylogenetic relationships within Microgastrinae based on 28S rDNA. Bayesian posterior probabilities greater than 0.70 are shown at the nodes.

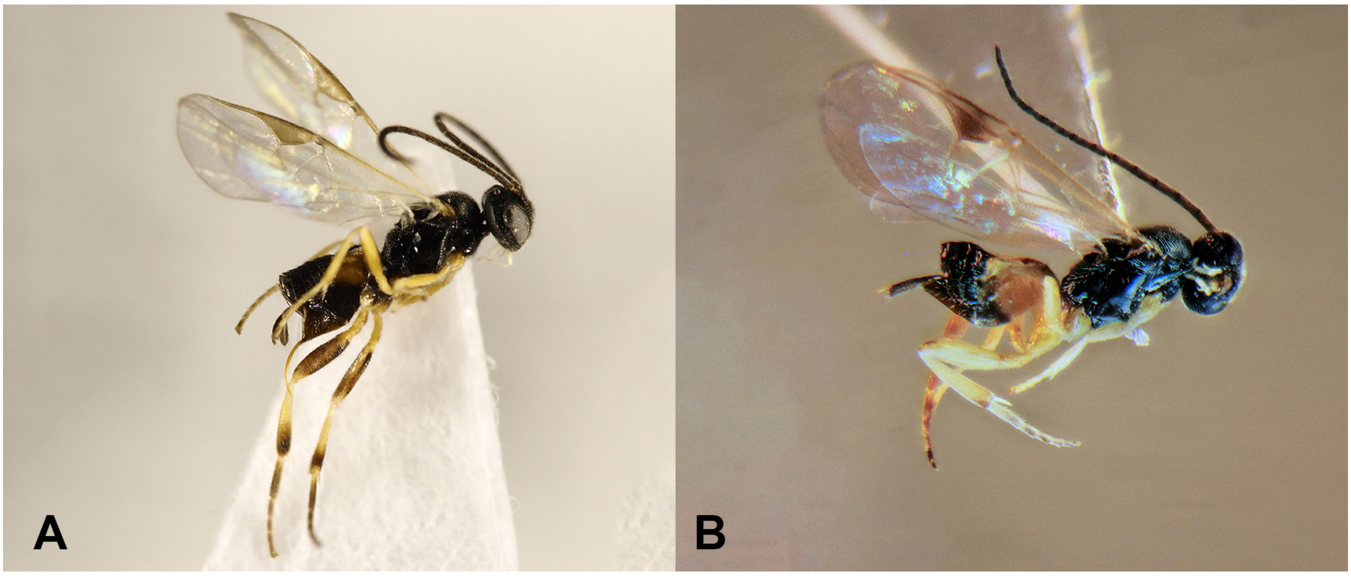

Figure 3. Habitus, lateral view of *Pholetesor* species (females). **A.** *Pholetesor circumscriptus* (Nees, 1834) (Bold Systems); **B.** *Pholetesor pseudocircumscriptus* Abdoli, 2019 **syn. nov.**

Table 3. DNA barcodes of mitochondrial cytochrome c oxidase I (*COI*) of two *Pholetesor* species (Hymenoptera: Braconidae).

Species	COI sequence
Pholetesor circumscriptus (Nees, 1834)	ATTTTTT**A**TTTGGATTATGAGCTGGTATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTGGGAATACCTGG**G**AGTTTAATTATAAATGATCAAATTTATAATAGTATTGTTACATCTCATGCATTTATTATAATTTTTTTATAGTTATACCTGTTATAATTGGAGGATTTGGTAATTGATTAATTCCTTTAATATTAGGTGCTCCAGATATATCATTCCCACGTATAAATAATATAAGATTTTGATTATTAATTCCTTCAATTATTATATTAATTATAAGAAGATTTATTAATGTTGGTGTTGGTACAGGTTGGACAGTTTA**C**CCTCCTTTATCTTTAAT**C**TTAGGTCATGGTGGTATATCAGTAGATTTAGGAATTTTTTCATTACATTTAGCTGGTGCTTCTTCAATTATAGGGGCAGTTAATTTTATTACAACAATTTTAAATATACGAACGAATTTATATAGAATAGATAAAATATCTTTATTTATTTGATCAGTTTTTATTACAGCAATTTTATTATTATTATCTTTACCAGTTTTAGCTGGTGCTATTACTAT**G**TTATTAACTGATCGTAATCTTAATACAAGATTTTTTGATCCTGCAGGAGGTGGTGATCCTATTTTATATCAACATT
Pholetesor pseudocircumscriptus Abdoli, 2019, **syn. nov.**	ATTTTTT**T**TTTGGATTATGAGCTGGTATATTAGGATTTTCAATAAGTTTAATTATTCGTTTAGAATTGGGAATACCTGG**A**AGTTTAATTATAAATGATCAAATTTATAATAGTATTGTTACATCTCATGCATTTATTATAATTTTTTTATAGTTATACCTGTTATAATTGGAGGATTTGGTAATTGATTAATTCCTTTAATATTAGGTGCTCCAGATATATCATTCCCACGTATAAATAATATAAGATTTTGATTATTAATTCCTTCAATTATTATATTAATTATAAGAAGATTTATTAATGTTGGTGTTGGTACAGGTTGGACAGTTTA**T**CCTCCTTTATCTTTAAT**T**TTAGGTCATGGTGGTATATCAGTAGATTTAGGAATTTTTTCATTACATTTAGCTGGTGCTTCTTCAATTATAGGGGCAGTTAATTTTATTACAACAATTTTAAATATACGAACGAATTTATATAGAATAGATAAAATATCTTTATTTATTTGATCAGTTTTTATTACAGCAATTTTATTATTATTATCTTTACCAGTTTTAGCTGGTGCTATTACTAT**A**TTATTAACTGATCGTAATCTTAATACAAGATTTTTTGATCCTGCAGGAGGTGGTGATCCTATTTTATATCAACATT

DISCUSSION

The comparison of our phylogenetic results shows notable congruence with the topologies presented by Whitfield et al. (2002), Mardulyn & Whitfield (1999) and Banks & Whitfield (2006). studies established that *Apanteles* s.str., *Alphomelon* and *Rhygoplitis* form a distinct clade (Banks & Whitfield, 2006; Mardulyn & Whitfield, 1999; Whitfield et al., 2002, 2018). Our 28S rDNA gene tree supports these taxonomic assignments, placing them within a newly identified and broader clade previously consisting of *Apanteles*, *Illidops*, *Alphomelon*, *Pholetesor*, *Rhygoplitis*. However, the COI gene tree suggests these genera as separate entities. The relationship between *Promicrogaster* and *Sendaphne*, highlighted by Whitfield et al. (2002), is confirmed in our analyses, revealing a new and broader clade in the COI gene tree, including *Microgaster*, *Papanteles*, *Sendaphne*, *Dasylagon*, *Promicrogaster*, *Shireplitis*, *Clarkinella*, *Hypomicrogaster*. In the 28S rDNA gene tree *Sendaphne*, *Dasylagon*, *Promicrogaster* form a distinct clade. Similarly, the close association of *Prasmodon*, *Pseudapanteles* into a distinct clade, as observed in prior studies (Banks & Whitfield, 2006; Mardulyn & Whitfield, 1999; Whitfield et al., 2002, 2018), is confirmed by our 28S rDNA gene tree, although COI gene tree fails to recover this phylogenetic relationship. The COI gene tree introduces a novel clade comprising *Glyptapanteles*, *Cotesia*, *Protapanteles*, *Sathon*, *Lathrapanteles*. The 28S rDNA gene tree also recovers *Glyptapanteles*, *Cotesia*, *Protapanteles* as sister taxa. Previous molecular analyses suggested a distinct clade including *Glyptapanteles*, *Cotesia* and *Sathon* (Whitfield et al., 2002; Mardulyn & Whitfield, 1999), but didn't include *Protapanteles* and *Lathrapanteles*. Banks & Whitfield (2006) identified a clade with *Glyptapanteles*, *Cotesia* and *Venanides*, whereas our study incorporates *Protapanteles*, *Sathon* and *Lathrapanteles*. In both the 28S rDNA and COI gene trees, *Microplitis* is consistently recovered as sister to *Snellenius*, aligning with previous studies (Whitfield et al., 2002, 2018; Mardulyn & Whitfield, 1999; Banks & Whitfield, 2006).

The findings of this study reveal a close relationship between the current molecular analysis and the morphological classification by Fernandez-Triana et al. (2020). These results validate several of their morphological classifications and clarify the positions of certain previously unplaced genera (Four genera include *Clarkinella*, *Neoclarkinella*, *Miropotes* and *Xanthomicrogaster*) that were unresolved in the morphological study. For instance, in the COI gene tree, the positions of the genera within the *Apanteles* group as defined by Fernandez-Triana et al. (2020) are as follows: *Apanteles*, *Illidops*; *Iconella*, *Neoclarkinella*, *Hygroplitis*; *Dolichogenidea*, *Exoriza*, *Parapanteles*; *Alphomelon*, *Janhalacaste*, *Pseudapanteles*; *Hypomicrogaster*, *Microgaster*, *Papanteles*, *Sendaphne*, *Dasylagon*, *Promicrogaster*, *Shireplitis*, *Clarkinella*. In the 28S rDNA gene tree, the positions are *Apanteles*, *Illidops*, *Alphomelon*, *Pholetesor*, *Rhygoplitis*; *Iconella*, *Exoryza*, *Dolichogenidea*, *Parapanteles*, *Sendaphne*, *Dasylagon*, *Promicrogaster*. These results indicate a close relationship among the genera within the *Apanteles* group as defined by Fernandez-Triana et al. (2020). Although the analysis did not support the formation of a comprehensive clade, it demonstrates that the members of this group are not associated with genera outside the *Apanteles* group. Additionally, the findings suggest that the genera *Clarkinella* and *Neoclarkinella*, previously unplaced according to Fernandez-Triana et al. (2020) are closely related to the members of this group and likely belong to the *Apanteles* group.

The results from the COI gene tree analysis also reveal specific relationships among the members of *Cotesia* group as defined by Fernandez-Triana et al. (2020). The clades are as follows: *Glyptapanteles*, *Cotesia*, *Protapanteles*, *Sathon*, *Lathrapanteles*; *Diolcogaster*, *Buluka*, *Protomicroplitis*, *Larrismus*, *Parenion*, *Xanthomicrogaster*; and *Miropotes*, *Venanides*. Similarly, the 28S rDNA gene tree supports the associations of *Glyptapanteles*, *Cotesia*, *Protapanteles*; *Deuteryxis*, *Xanthomicrogaster*; and *Venanides*, *Miropotes*. The analysis demonstrates that *Miropotes* and *Xanthomicrogaster*, previously classified as unplaced by Fernandez-Triana et al. (2020), likely belong to the *Cotesia* group based on current genetic evidence, and consequently, they should be considered members of this group. The results of the Bayesian analyses of COI also exposed discrepancies with the classifications proposed by Fernandez-Triana et al. (2020), indicating the need for further investigation. In the present study, the taxa *Microplitis*, *Snellenius*, *Alloplitis*, *Philoplitis*, which were previously grouped together under the *Microplitis* group by Fernandez-Triana et al. (2020), were found to be separated. Specifically, *Microplitis* and *Snellenius* were placed apart from the other two genera. Current Bayesian analyses revealed some strongly supported clades, introducing novel phylogenetic hypotheses within Microgastrinae. In the COI phylogenetic tree, we identified two new clades, *Alloplitis*, *Philoplitis*, *Prasmodon*, *Zachterbergius*, *Rasivalva*, *Wilkinsonellus* and *Microplitis*, *Snellenius*, *Choeras*, *Deuterixyes*, *Beyarslania*. These clades are particularly noteworthy because they group together genera that were previously placed in distinct categories according to the classifications by Fernandez-Triana et al. (2020). This finding suggests a potential need for revising the current taxonomic framework, as the molecular data offer a more detailed understanding of the evolutionary relationships within Microgastrinae.

However, while the branching patterns in our COI and 28S rDNA phylogenetic trees exhibit some similarities, there are notable differences, particularly in the support for certain clades. The COI analyses tend to reveal more strongly supported clades compared to the 28S rDNA analyses, likely due to a more extensive database. The differences in results highlight the impact of missing data and how genera or groups evolved from the ancestors. The COI gene is more divergent compared to 28S rDNA and provides higher resolution at the species and genus levels compared to less variable genes like 28S rDNA (Machida & Tsuda, 2010; Blanco-Bercial et al., 2011; Patwardhan et al., 2014). Therefore, we used the 28S rDNA phylogenetic tree as a complementary source of information to the COI tree. In the COI phylogenetic tree, a clade labelled as the 'unknown group' was identified, comprising genera from the *Microplitis* group, *Cotesia* group, and several genera from the unplaced group. This newly recognized clade, supported by a high posterior probability, highlights the close evolutionary relationships among these taxa.

Molecular phylogenetic studies of Microgastrinae have historically been limited, often involving only a small number of samples, which can compromise the accuracy of phylogenetic inferences. Our study provides new insights into the evolutionary relationships within this subfamily by identifying well-supported clades at shallower taxonomic levels. The inclusion of a larger number of taxa in our analysis has led to a clearer understanding of phylogenetic relationships among the genera of Microgastrinae. However, due to incomplete data from taxa, further fieldwork efforts and the integration of additional molecular markers are necessary to enhance the robustness of our taxonomic conclusions. This study also proposed that *P. psedocircumscriptus* should be considered a new synonym of *P. circumscriptus* (Table 3). Notably, it is mentioned that *Pholetesor circumscriptus* exhibits some variation in colouration, particularly in the legs and metasomal segments, depending on the region, especially in the Old World (Whitfield, 2006). Based on the original description, *P. circumscriptus* is characterized by the following set of characters: vein R1 is long, longer than pterostigma and not less than 3.00–4.00 × longer than the distance from to the apex of the wing; ovipositor valve much less expanded apically; tergite 1 length 1.50× basal width, distinctly narrowed posteriorly, posterior width not more than one-third of basal width, and smooth posteriorly; posterior width of tergite 2, 1.50× (less than 2.00×) its medial length; anterior margin of postscutellum between the forwards-pointing projection and mid-point of postscutellum concave and phragma of scutellum strongly revealed, tergites 1–3 black or blackish, less frequently orange or yellow; body length about 1.80–2.00 mm (Nees, 1834). In *P. psudocircumscriptus* Tergite 1 shallowly rugulose posteriorly, the length of tergite I 1.80× basal width; posterior width of tergite II, 2.00× its medial length; Tergites II-II and basal half of Tergite III yellow; body length 1.40–1.50 mm (Abdoli & Pourhaji, 2019). Previous comprehensive studies on other species of Microgastrinae such as *Microplitis ceratomiae* Riley, 1881 (Ghafouri Moghaddam et al., 2021) and *Microplitis manilae* Ashmead, 1904 (Ghafouri Moghaddam & Butcher, 2023) revealed that the specimens show intraspecific variations in size and/or colour. Ecological factors play an important role in morphological differences which are common among populations of the same species (Pan et al., 2018). Furthermore, in braconid parasitoids with a wide host range, such as *Habrobracon hebetor* (Say, 1836), different hosts with varying sizes affect the size of wasp adults (Abou El-Ela et al., 2021).

Molecular methods are crucial for accurately identifying closely related species and distinguishing morphologically similar but genetically distinct species (Sharanowski et al., 2011). These techniques offer a level of precision that is often unattainable through traditional morphological approaches, effectively resolving taxonomic ambiguities and enhancing our understanding of species diversity (Belshaw & Quicke, 2002; Whitfield, 2002).

AUTHOR'S CONTRIBUTION

The authors confirm their contribution to the paper as follows: P. Abdoli: performed lab work, data curation, computational analyses, compiling the literature, drafting the manuscript; A.A. Talebi: conceived and designed the study, conceptualization, supervising, organizing the collection, editing and proofreading; N.G. Kavallieratos: conceived and designed the study, revised and edited previous and final version of this manuscript; R. Khosravi: computational analyses, revised and edited previous and final version of this manuscript; F. Bidari: performed lab work, data curation and computational analyses. All authors read and approved the final version of the manuscript.

FUNDING

This work has been supported by the center for International Scientific Studies & Collaboration (CISSC), Ministry of Science Research and Technology, Islamic Republic of Iran (Project No: 991215).

AVAILABILITY OF DATA AND MATERIAL

The COI and 28S rDNA sequence data of the specimens that support the findings of this study were deposited in the NCBI (GenBank accession numbers in Tables 1 and 2).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study only included arthropod material, and all required ethical guidelines for the treatment and use of animals were strictly adhered to in accordance with international, national, and institutional regulations. No human participants were involved in any studies conducted by the authors for this article.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

ACKNOWLEDGMENTS

Thanks to Insect Collection of the Department of Entomology, Tarbiat Modares University, Tehran, Iran (TMUC) for providing facilities. Special thanks to Dr Majid Pedram, Dr Mohammad Mehrabadi and Mr Ehsan Baradaran for their guidance on this study. We cordially thank the editor and two anonymous reviewers for their critical reviews and constructive comments which significantly improved the paper.

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مطالعه شجره‌شناسی زنبورهای زیرخانواده Microgastrinae (Hymenoptera: Braconidae) بر اساس ژن‌های COI میتوکندریایی و 28S rDNA هسته‌ای، با نکاتی از هویت *Pholetesor circumscriptus* (Nees, 1834)

پریسا عبدلی[1]، علی اصغر طالبی[1*]، نیکولاس ج. کاوالیراتوس[2]، رسول خسروی[3] و فرزاد بیداری[4]

۱ گروه حشره شناسی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران
۲ آزمایشگاه جانورشناسی کشاورزی و حشره شناسی، گروه علوم زراعی، دانشگاه کشاورزی آتن، یونان
۳ گروه مهندسی منابع طبیعی و محیط زیست، دانشکده کشاورزی، دانشگاه شیراز، شیراز، ایران
۴ موسسه محیط زیست هاکسبری، دانشگاه وسترن سیدنی، استرالیا
* پست الکترونیک نویسنده مسئول مکاتبه: *talebia@modares.ac.ir*

| **تاریخ دریافت:** ۲۱ تیر ۱۴۰۳ | **تاریخ پذیرش:** ۰۲ شهریور ۱۴۰۳ | **تاریخ انتشار:** ۱۱ شهریور ۱۴۰۳ |

چکیده: زنبورهای زیرخانواده Microgastrinae گروه متنوعی از زنبورهای پارازیتویید داخلی هستند که به لاروهای بال‌پولکداران حمله می‌کنند. علیرغم اهمیت آنها در کنترل بیولوژیک، در مورد روابط فیلوژنی بین آن‌ها اختلاف نظر وجود دارد. اگرچه تجزیه و تحلیل‌های شجره‌شناسی گذشته درک کلی روابط این زنبورها را ارتقا داده است اما تعداد کم آرایه‌های منتخب منجر به اختلاف در طبقه‌بندی آن‌ها شده است. در مطالعه حاضر روابط شجره‌شناسی جنس‌های این زیرخانواده با استفاده از داده‌های میتوکندری و هسته‌ای مورد بررسی قرار گرفت و با افزایش تاکسون‌ها سعی شد روابط بین آن‌ها و وضعیت طبقه‌بندی این زنبورها واضح‌تر شود. در این پژوهش، درخت شجره‌شناسی جنس‌های زنبورهای زیرخانواده Microgastrinae را با توالی‌هایی موجود و جدید شامل ۵۵ جنس از COI و ۳۰ جنس از 28S rDNA بازسازی شد. در این مطالعه، چندین گونه و جنس برای اولین بار توالی یابی شدند. برخی روابط نزدیک فیلوژنتیکی در بین جنس‌های زیرخانواده Microgastrinae با تجزیه و تحلیل توالی COI میتوکندری و 28S rDNA شناسایی شد. بیشتر کلادهای بدست آمده فعلی با آخرین طبقه‌بندی مورفولوژیک زنبورهای این زیرخانواده مطابقت داشت. کلادهای جدید با پشتیبانی خوب، با کلادهای ثبت شده قبلی مطابقت داشته و درک بیشتری از تکامل این زنبورها ارایه شد. بر اساس بررسی مولکولی، *Pholetesor psedocircumscriptus* Abdoli, 2019 مترادف با *Pholetesor circumscriptus* (Nees 1834) در نظرگرفته شد.

واژگـان کلیدی: فیلوژنی مولکولی، توالی‌های DNA، روش بیزین، مترادف جدید