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ORIGINAL RESEARCH ARTICLE

Evolutionary history of microgastrine wasps (hymenoptera: braconidae: microgastrinae) in Sundaland: insights from Peninsular Malaysia

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Abstract

Microgastrinae is a subfamily of lepidopteran endoparasitoids that consume angiosperm nectar as their primary food source. Despite several studies on their biology and phylogeny, the evolutionary history of Microgastrinae remains poorly resolved. A total of 146 sequences were obtained from three markers—28S, 16S, and COI—from 55 Microgastrinae individuals collected from oil palm plantations and forests in Peninsular Malaysia. The objective of this study was to investigate the evolutionary history of this subfamily, using organisms from Peninsular Malaysia as a model. Thirteen genera were included in the analysis. The molecular clock analysis estimated that the Sundaland Microgastrinae species diverged around 51.93 million years ago (mya) in the Eocene, which is consistent with the crown age of this family. The *Fornicia* genus was estimated to be the earliest genus, evolving approximately 51 mya. The *Apanteles* group radiated from 45.83 mya. The *Microplitis* group diverged at 29.74 mya, while the *Neoclarkinella* genus evolved at 24.16 mya. The paraphyletic group of *Cotesia* diverged multiple times, between 32.39 and 13.88 mya. Angiosperms radiated during the Eocene to Oligocene, around 55–23 mya, while lepidopteran species evolved at the beginning of the Miocene, from 23.0 to 15.0 mya; thus, both groups overlapped with the radiation of Microgastrinae. Interestingly, the coevolution between microgastrines, angiosperms, and lepidopteran species from Sundaland was found to be congruent and supported by global data. These findings may contribute new information on the systematics, biology, and evolution of Microgastrinae by providing samples collected from Peninsular Malaysia with a particular focus on Sundaland coevolution with angiosperms and Lepidoptera.

Keywords Endoparasitoid wasp · Mitochondria · Nuclear · Sundaland · Malaysia

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Introduction

Microgastrinae is a taxonomic group of highly diverse, cosmopolitan endoparasitoid wasps that parasitize a wide range of Lepidopteran larvae. This subfamily encompasses over 2,999 recognized species, with estimates suggesting a total of between 17,000 and 46,000 species worldwide (Whitfield et al. 2018). According to Fernandez-Triana et al. (2020), a total of 46 genera and 752 species have been documented in the Oriental region. In Malaysia, research on this subfamily has been primarily limited to the systematics and diversity of the taxa (Aman-Zuki et al. 2019; Rabibah et al. 2018), with nearly all studies focusing on species that serve as biological control agents for Lepidopteran pests in oil palm plantations (Cheong et al. 2010; Halim et al. 2017; 2018; Yusdayati et al. 2014).

Members of Microgastrinae select Lepidopteran larvae as hosts or substrates for oviposition and derive nourishment from nectar obtained from shallow flowers, which is typical behavior among wasp species (Jervis et al. 1993; Shaw 1997). Several records document the presence of various microgastrine species inhabiting oil palm plantations, where they exhibit considerable diversity due to the presence of beneficial plants that attract these parasitoids (Bianchi and Wankers 2008; Fuat et al. 2022b). Their presence is significant in agricultural ecosystems, such as oil palm plantations. Halim et al. (2017; 2018) noted that several braconid species act as parasitoids of *Metisa plana*, a predominant pest of oil palm, including *Dolichogenidea metesae*, *Apanteles aluella*, and *Apanteles* sp.1, all of which belong to the Microgastrinae family. These wasps also function as parasitoids of forestry pests in forest ecosystems; for example, *Apanteles ruidus* infests *Hyblaea puera*, a defoliator of teak (Yousuf and Ikram 2019). Salmah et al. (2012) highlighted that *Apanteles metesae* (now classified as *Dolichogenidea metese*) feeds on nectar from wild plants and flowers surrounding oil palm areas. The presence of these parasitoids is vital in oil palm plantations due to their functional role as parasitoids of the principal pest, *Metisa plana*, in Malaysia and other tropical regions (Fuat et al. 2022a).

Murphy et al. (2008) conducted the most recent study on the evolutionary history and phylogeny of this group utilizing seven molecular markers; however, their discussion was limited to the phylogeny and evolution of the subfamilies that are sister groups to Microgastrinae, namely Cardiochilinae + Miracinae and Khoikhoiinae, due to a restricted sample size. Furthermore, study from Murphy et al. (2008) was limited to 11 Microgastrinae samples compared to this study's multi-marker approach with 55 Microgastrinae samples. Understanding the evolutionary biology and phylogeny of Microgastrinae is crucial for studying the associations and correlations between parasitoid wasps and their coevolved species, such as Lepidopteran angiosperms, for instance, in strategies to echolocate predatory bats as anti-bat adaptations (Kawahara et al. 2019).

The gradual yet consistent transformation of geographical features and the conversion of forests into oil palm plantations in recent years have had a significant impact on the divergences in the ancestral lineages of rapidly evolving organisms, such as insect species. Peninsular Malaysia was formed within the Sundaland region following the Last Glacial Maximum period, approximately 110 to 15 million years ago (Cannon et al. 2009). The rise in sea levels resulted in the current configuration of Peninsular Malaysia, Borneo Island, and the Indonesian archipelago. Notably, Peninsular Malaysia represents a critical biogeographic zone that served as a refugial area for biodiversity during and after the Last Glacial Maximum, preserving lineages that may have gone extinct (Wurster et al. 2010). Thus, the historical context of the geographical zone substantially influences species distribution and evolution, warranting comprehensive investigation. Therefore, the objective of this study is to reconstruct the divergence times of Microgastrinae in Sundaland and test for temporal congruence with angiosperm/Lepidoptera radiations.

Materials and methods

Sampling collection

Specimens were collected from nine distinct localities within Peninsular Malaysia. The selected sampling sites comprised primary, secondary, and fragmented forests, as well as oil palm plantations (see Fig. 1). Peninsular Malaysia has approximately 5.74 million hectares of forested areas including permanent reserve forest (Forestry Department of Peninsular Malaysia 2023). The oil palm plantation was specifically chosen due to its status as the largest plantation in Malaysia, covering an area of approximately 4.49 million hectares (MPOC 2019). Among the efforts to avoid potential bias in sampling was making sure the number of sampling sites between forest and plantation is balanced. Additionally, samples from Aman-Zuki et al. (2015; 2016; 2019) were incorporated to enhance the analytical framework of this study. The sampling methodology involved the use of malaise traps, which were deployed for a duration of one month at each locality before the specimens were transported to the laboratory for sorting and identification. Throughout the sampling period, specimens were preserved in 70% ethanol, which was subsequently replaced with 99% ethanol after sorting and identification to prevent the degradation of DNA samples.

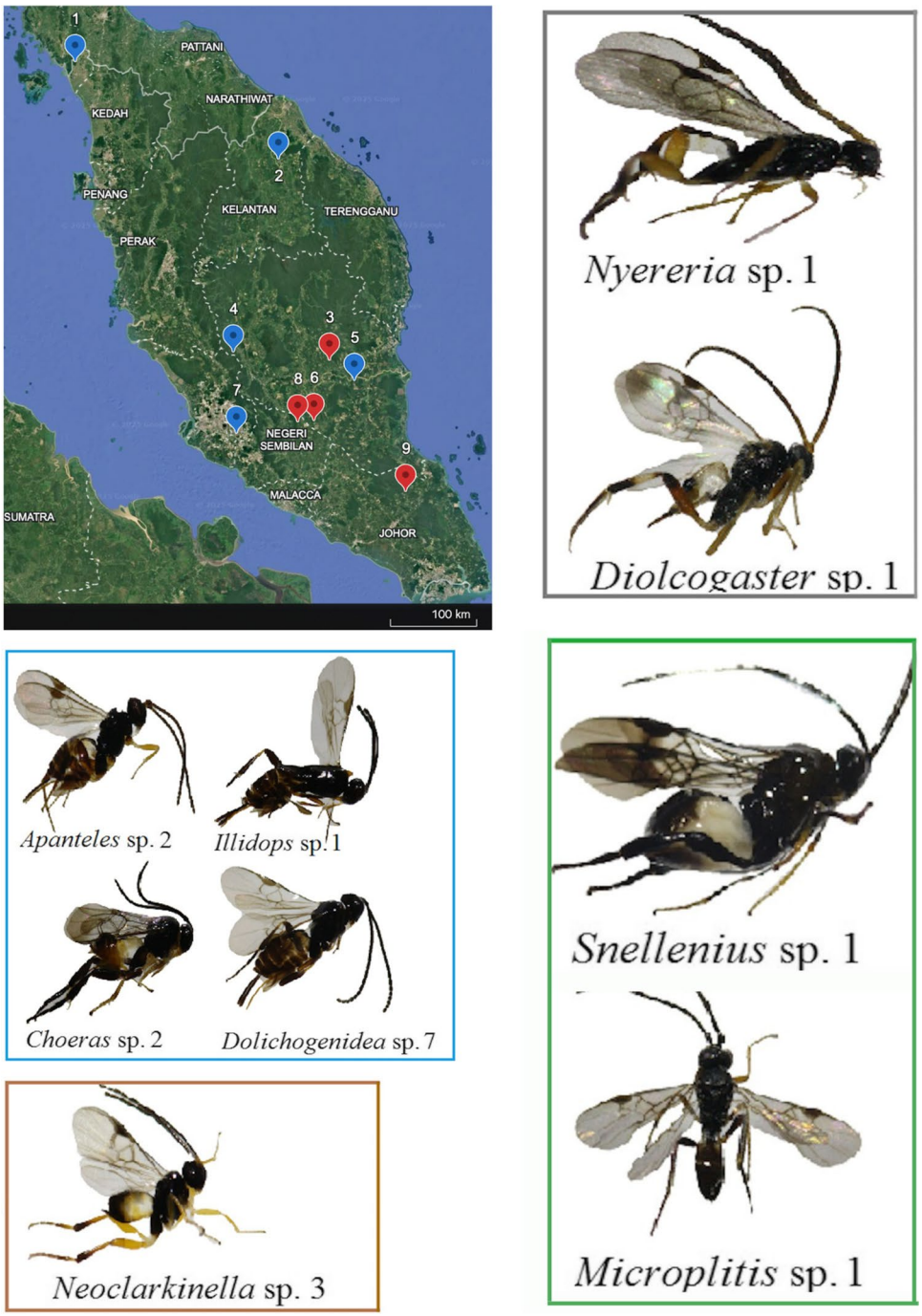

Fig. 1 Sampling localities of microgastrine throughout Peninsular Malaysia with blue placemark icon refers to forest area and red refers to plantation sites. 1. Hutan Simpan Kekal Bukit Bintang, Perlis. 2. Kuala Krai, Kelantan. 3. Jengka, Pahang. 4. Bukit Fraser, Pahang. 5. Tasik Chini, Pahang. 6. Felda Tembangau, Pahang. 7. Hutan Simpan Bangi, Selangor. 8. Felda Lui Muda Serting, Negeri Sembilan. 9. Ladang Zamrud, Kluang, Johor. Photograph of specimens in colour boxes are provided to represent each group of Microgastrinae. Google Earth was used to construct the map

Morphological identification

A total of 55 specimens were sorted and identified to the genus and species level utilizing the identification keys developed by Austin and Dangerfield (1992), Gupta and Fernandez-Triana (2015), Liu et al. (2019), Long and van Achterberg (2013), Song et al. (2014) and Veena et al. (2014). The identification process was facilitated by a Stemi DV4 microscope (Carl Zeiss, Germany), and photographs of the specimens were captured using a Canon DS1000 camera attached to the microscope. All identified specimens were preserved in 99% ethanol at −20 °C to ensure sample integrity.

DNA extraction and polymerase chain reaction (PCR)

Specimens were rinsed with distilled water and 99% ethanol to eliminate any dust or mites that may have remained on the samples, thereby preventing contamination. DNA

extractions were conducted utilizing the DNeasy Blood and Tissue Kit (Qiagen, Germany). The protocol was modified in the initial step of DNA extraction to preserve morphological characteristics, in accordance with the methodology outlined by Yaakop et al. (2013). The extracted DNA samples were stored at −20 °C.

Polymerase chain reaction (PCR) was performed using three molecular markers: 28S, 16S, and cytochrome c oxidase subunit I (COI). Table 1 presents the primer sequences utilized for PCR and sequencing in this study. Each PCR reaction was conducted using Green MasterMix (Promega, USA), with the following volume and concentration: 12.5 µl of 1X MasterMix, 1 µl of 0.4 µM of each reverse and forward primer, 3 µl of 10 ng/µl template DNA, and 7.5 µl of double-distilled water (ddH2O) to achieve a total reaction volume of 25 µl. The PCR conditions adhered to those established by Aman-Zuki et al. (2016; 2019), with slight modifications to the annealing temperature, which ranged between 45–48 °C.

Sequencing and sequence editing

Successful PCR products were outsourced for sequencing analysis at Apical Scientific Sdn. Bhd. (Serdang, Selangor). The results from the sequencing analyses were provided in.ab1 and.seq file formats. Both forward and reverse sequences were contiguated using BioEdit version 7.0.5.3 (Hall 1999) to generate a consensus sequence for each sample.

BLAST and BOLD analyses

Each sequence was subjected to analyses using the Basic Local Alignment Search Tool (BLAST) (McGinnis and Madden 2004) and The Barcode of Life Data System (BOLD) to verify the absence of cross-contamination (Ratnasingham and Hebert 2007).

Sequences alignment

A total of 1800 bp of concatenated data from all sequences were aligned by referencing outgroup sequences (095 Cheloninae and 063 Braconinae) using ClustalW in MEGA7 (Kumar et al. 2016). Cheloninae was selected as the closest subfamily (microgastroid complex) and the most recent ancestor to Microgastrinae, while Braconinae was chosen as a representative of ancestral traits within Braconidae (cyclostome ectoparasitoids) (Austin and Dowton 2000). The alignment of COI, 16S, and 28S sequences was performed using Clustal W with default parameters, prior to the application of McClade 4.08. Subsequently, the 16S and 28S sequences were aligned manually. The sequences of the secondary structure model served as a guide for the manual alignment: the model of chalcidoid wasps (Gillespie et al. 2005) was utilized for the 28S D2 region, while the model for evaniid wasps (Deans et al. 2006) was employed for the 16S sequences; the alignment of the COI region was conducted according to its protein codon positions.

Molecular time divergence

The divergence time of Microgastrinae was formulated using BEAUTi v1.7 and estimated with BEAST v1.7 (Drummond et al. 2012). The parameters established for the analysis included an uncorrelated lognormal relaxed clock for the clock model, a Speciation: Birth–Death Process for the tree prior, an HKY nucleotide substitution model, an estimated base frequency, and a random starting tree with a mean of 0 and standard deviation of 10 defined as uniform.

Table 1 List of primer sequences used in PCR with their references and information on the PCR product and anneal temperature

Primer sequences 5'−3'	PCR product (bp)	Annealing temperature (°C)	References
COI	750	47	
LCO1490 GGTCAACAAATCATAAAGATATTGG			Folmer et al. (1994)
HC02198 TAAACTTCAGGGTGACCAAAAAATCA			Folmer et al. (1994)
28S	550	45	
28S D2 forward AGAGAGAGTTCAAGAGTACGTG			Belshaw and Quicke (1997)
28S D2 reverse TTGGTCCGTGTTTCAAGACGGG			Campbell et al. (1994)
16S	500	48	
16S Wb CACCTGTTTATCAAAAACAT			Dowton and Austin (1998)
16S outer CTTTAATTCAACATCGAGGTC			Whitfield (1997)

The analysis was conducted in accordance with the methodologies outlined by Aman-Zuki et al. (2021) and Yaakop et al. (2015). The fossil data utilized in this analysis were sourced from Murphy et al. (2008) and Whitfield (1997). Despite many fossil specimens of Microgastrinae have been found, revised and recorded over the years, the fossil data for Microgastrinae date back to 20–44 mya during the Eocene to Miocene epochs (Fernandez-Traina et al. 2020). Furthermore, the fossil taxa used for calibration in Murphy et al. (2008) were described at the species level, while some newly recorded fossils represented higher taxa (Antropov et al. 2013). Fossil taxa from Braconidae and Cheloninae were designated as stem ages, while Microgastrinae was assigned as the crown age. The Markov Chain Monte Carlo (MCMC) analysis was executed for 10 million generations, with trees sampled every 1,000 generations. Tracer 1.5 (Rambaut and Drummond 2009) was employed to verify the adequacy of all parameters by confirming that the estimated sample size (ESS) exceeded 200. TreeAnnotator v1.7 was utilized to generate the molecular clock tree, discarding 25% of the trees as burn-in. The resultant molecular clock tree was visualized using FigTree v1.4.

Results

Morphological identification

The morphological identification of 55 microgastrine individuals has successfully delineated 29 species across 13 genera, namely *Apanteles* Foerster, *Choeras* Mason, *Cotesia* Cameron, *Diolcogaster* Ashmead, *Dolichogenidae* Viereck, *Fornicia* Brulle, *Glyptapanteles* Ashmead, *Illidops* Mason, *Microplitis* Foerster, *Nyereria* Mason, *Parapanteles* Ashmead, *Snellenius* Westwood, and *Neoclarkinella* Rema and Narendran. These species were classified into four groups: Apanteles, Microplitis, Cotesia, and an Unplaced Genera group following Fernandez-Triana et al. (2020). Two specimens (074, 102) that exhibited incomplete morphological features were excluded from the identification process. This morphological identification was conducted prior to the molecular analysis.

Molecular identification

Three molecular regions were amplified in this study, i.e., COI, 16S and 28S. COI amplified in 100% of samples while 16S and 28S amplified in 98% of samples, respectively. BLAST and BOLD analyses led to the identification of 13 genera, exhibiting similarity values ranging from 89 to 100%. Notably, the BOLD analysis also identified 13 genera with similarity values between 89.04% and 100%. Furthermore, the BLAST analysis successfully identified 42 individuals at the genus level (see Table 2).

Molecular time divergence

The molecular time divergence tree was constructed using concatenated data from 28S, 16S, and COI genes. As illustrated in Fig. 2**,** the estimated divergence time for the Microgastrinae during the Eocene epoch was approximately 51.93 million years ago (mya). The genus *Fornicia*, identified as the earliest genus, is estimated to have radiated around 51 mya, followed by subsequent diversification of other groups. The *Apanteles* group is believed to have diverged around 45.83 mya. The *Microplitis* group, which includes the genera *Microplitis* and *Snellenius*, diverged approximately 29.74 mya, while the genus *Neoclarkinella*, categorized under the unplaced genera group, radiated around 24.16 mya. The paraphyletic group of *Cotesia* exhibited multiple divergence events occurring between 32.39 and 13.88 mya. Paraphyletic refers to a taxonomic group of organisms where it includes one or some of the most recent common ancestors but not all of its descendants (Horandl and Stuessy 2010). The evolutionary history of the microgastrines presented in this study has been calibrated using fossil records of lepidopteran species and angiosperms documented from the Sundaland region. The angiosperms underwent significant radiation during the Eocene to Oligocene epochs, estimated to have occurred between 55 and 23 mya, whereas lepidopteran species are thought to have evolved at the onset of the Miocene, approximately between 23.0 and 15.0 mya. Both outgroup species, Cheloninae and Braconinae, are hypothesized to have diverged prior to the Microgastrinae, specifically before 52.0 mya.

Discussion

Divergence time analysis in this study was conducted to visualize and enhance the understanding of the evolutionary history of microgastrines. Peninsular Malaysia served as the model location for the Sundaland or Sunda Shelf in this study due to the close evolutionary relationships among species across all islands and the mainland of the Sunda Shelf, such as those from the Malay Peninsula and Sumatra, which are sister to those from Borneo. In contrast, bird species from Java were found to be the most divergent and more closely related to other locations on the Sunda Shelf (Leonard et al. 2015). Our sampling however, lacks specimens from key areas like Java and Borneo. These gaps limit our understanding of Microgastrinae diversity in Sundaland and may overlook unique or endemic lineages. Broader geographic sampling will be essential to refine these biogeographic patterns. In the present study, three DNA data

Table 2 List of microgastrine specimens used in this study with sampling site, date of collection and GenBank Accession Number. Samples with citation were from previous studies and without citation are from this study

Sample Code	Genus/Species	GenBank Acession No		
		COI	*28S*	*16S*
Bukit Fraser, Pahang, Malaysia 3°43′20.6"N 101°43′31.5"E 17–20/X/2014				
051	*Apanteles* sp.	KT699082 (Aman-Zuki et al. 2016)	KY441862 (Aman-Zuki et al. 2019)	KX354731 (Aman-Zuki et al. 2016)
053	*Apanteles* sp.	KT699084 (Aman-Zuki et al. 2016)	KY441865 (Aman-Zuki et al. 2019)	KX354733 (Aman-Zuki et al. 2016)
054	*Apanteles* sp.	KT699085 (Aman-Zuki et al. 2016)	KY441850 (Aman-Zuki et al. 2019)	KX354734 (Aman-Zuki et al. 2016)
048	*Apanteles* sp.	KT699080 (Aman-Zuki et al. 2016)	KY441849 (Aman-Zuki et al. 2019)	KX354729 (Aman-Zuki et al. 2016)
052	*Dolichogenidea* sp. 7	KT699083 (Aman-Zuki et al. 2016)	KY441871	KX354732 (Aman-Zuki et al. 2016)
046	*Dolichogenidea* sp. 7	KT699078 (Aman-Zuki et al. 2016)	KY441870	KX354727 (Aman-Zuki et al. 2016)
045	*Choeras* sp. 2	MK568910	MK568818	MK568866
040	*Cotesia* sp. 2	KT699076 (Aman-Zuki et al. 2016)	MG198615 (Aman-Zuki et al. 2019)	KX354725 (Aman-Zuki et al. 2016)
044	*Cotesia* sp. 2	MK568916	MK568838	MK568886
029	*Parapanteles* sp. 1	MK568923	MK568822	MK568870
Felda Lui Muda, Serting, Negeri Sembilan, Malaysia 3°01′41.7"N 102°22′05.1"E 19/V/2015				
110	*Dolichogenidea* sp. 5	MK568904	MK568811	MK568859
089	*Dolichogenidea* sp. 4	MK568905	MK568808	MK568856
091	*Dolichogenidea* sp. 3	MK568908	MK568809	MK568857
111	*Diolcogaster andamanensis*	MK568930	MK568846	MK568894
112	*Diolcogaster andamanensis*	MK568931	MK568847	MK568895
113	*Diolcogaster andamanensis*	MK568932	MK568848	MK568896
114	*Diolcogaster andamanensis*	MK568933	MK568849	MK568897
086	*Diolcogaster andamanensis*	MK568934	MK568843	MK568891
093	*Diolcogaster andamanensis*	MK568935	MK568844	MK568892
115	*Diolcogaster andamanensis*	MK568937	MK568850	MK568898
Bukit Rupa, Hutan Simpan Bangi, Selangor, Malaysia 2°55′10.9"N 101°46′01.2"E 01/XII/2015				
105	*Dolichogenidea* sp. 1	MK568906	MK568810	MK568858
103	*Choeras* sp. 1	MK568912	MK568820	MK568868
104	*Parapanteles* sp. 1	MK568918	MK568825	MK568873
076	*Parapanteles* sp. 1	MK568921	MK568824	MK568872
106	*Glyptapanteles* sp. 1	MK568925	MK568840	MK568888
108	*Glyptapanteles* sp. 1	MK568926	MK568841	MK568889
102	*Nyereria* sp. 1	MK568939	MK568829	MK568877
075	*Neoclarkinella* sp. 4	MK568948	MK568832	MK568880
Ladang Zamrud, Kluang, Johor, Malaysia 2°20′53.4"N 103°23′09.9"E 12/IV/2014				
119	*Dolichogenidea* sp. 2	MK568907	MK568812	MK568860
071	*Illidops* sp. 1	MK568914	MK568816	MK568864
118	*Illidops* sp. 2	MK568915	MK568817	MK568865

Table 2 (continued)

Sample Code	Genus/Species	GenBank Acession No		
		COI	*28S*	*16S*
073	*Parapanteles* sp. 1	MK568919	MK568823	MK568871
074	*Fornicia* sp. 1	MK568940	MK568830	MK568878
069	*Microplitis* sp. 2	MK568944	MK568853	MK568901
Tekam-Jengka, Pahang, Malaysia 3°50′11.4"N 102°31′44.7"E 19/X/2014				
126	*Dolichogenidea* sp. 7	MK568909	MK568813	MK568861
125	*Cotesia* sp. 1	MK568917	MK568839	MK568887
123	*Parapanteles* sp. 1	MK568920	MK568827	MK568875
121	*Parapanteles* sp. 1	MK568922	MK568826	MK568874
124	*Neoclarkinella* sp. 1	MK568946	MK568834	MK568882
127	*Neoclarkinella* sp. 1	MK568949	MK568835	MK568883
129	*Neoclarkinella* sp. 2	MK568950	MK568836	MK568884
131	*Neoclarkinella* sp. 2	MK568951	MK568837	MK568885
Tasik Chini, Pahang, Malaysia 3°25′17.1"N 102°54′55.1"E 23/III/2015				
096	*Choeras* sp. 2	MK568911	MK568819	MK568867
138	*Choeras* sp. 2	MK568913	MK568821	MK568869
142	*Parapanteles* sp. 2	MK568924	MK568828	MK568876
139	*Snellenius* sp. 1	MK568942	-	-
143	*Microplitis* sp. 1	MK568943	MK568854	MK568902
095	*Microchelonus* sp.	MK568952	MK568855	MK568903
Hutan Simpan Kekal Bukit Bintang Perlis, Malaysia 6°32′09.3"N 100°11′36.0"E 10–16/II/2015				
133	*Glyptapanteles* sp. 2	MK568927	MK568842	MK568890
100	*Diolcogaster andamanensis*	MK568936	MK568845	MK568893
134	*Diolcogaster andamanensis*	MK568938	MK568851	MK568899
099	*Neoclarkinella* sp. 4	MK568947	MK568833	MK568881
Kuala Krai, Kelantan, Malaysia 5°36′30.6"N 102°12′44.3"E 07/X/2015				
144	*Dolichogenidea* sp. 6	MK568928	MK568814	MK568862
146	*Dolichogenidea* sp. 6	MK568929	MK568815	MK568863
Hutan Pendidikan Alam, Bangi, Selangor, Malaysia 2°54′45.1"N 101°47′12.2"E 19/XII/2013				
003	*Snellenius* sp. 1	MK568941	MK568852	MK568900
004	*Neoclarkinella* sp. 3	MK568945	MK568831	MK568879
Felda Tembangau, Pahang, Malaysia 3°06′48.8"N 102°31′48.1"E 15/X/2014				
063	Braconinae	KY441903 (Aman-Zuki et al. 2019)	KY441874 (Aman-Zuki et al. 2019)	KY441840 (Aman-Zuki et al. 2019)

sets (mitochondrial and nuclear regions) were combined for molecular time divergence analysis to mitigate bias in the time divergence results (Zheng et al. 2012). To reduce bias from mitochondrial DNA, which can suffer from saturation and misleading signals at deeper nodes, we used nuclear data to improve phylogenetic accuracy. Nuclear markers evolve more slowly and offer independent inheritance, helping to clarify both recent and older evolutionary splits

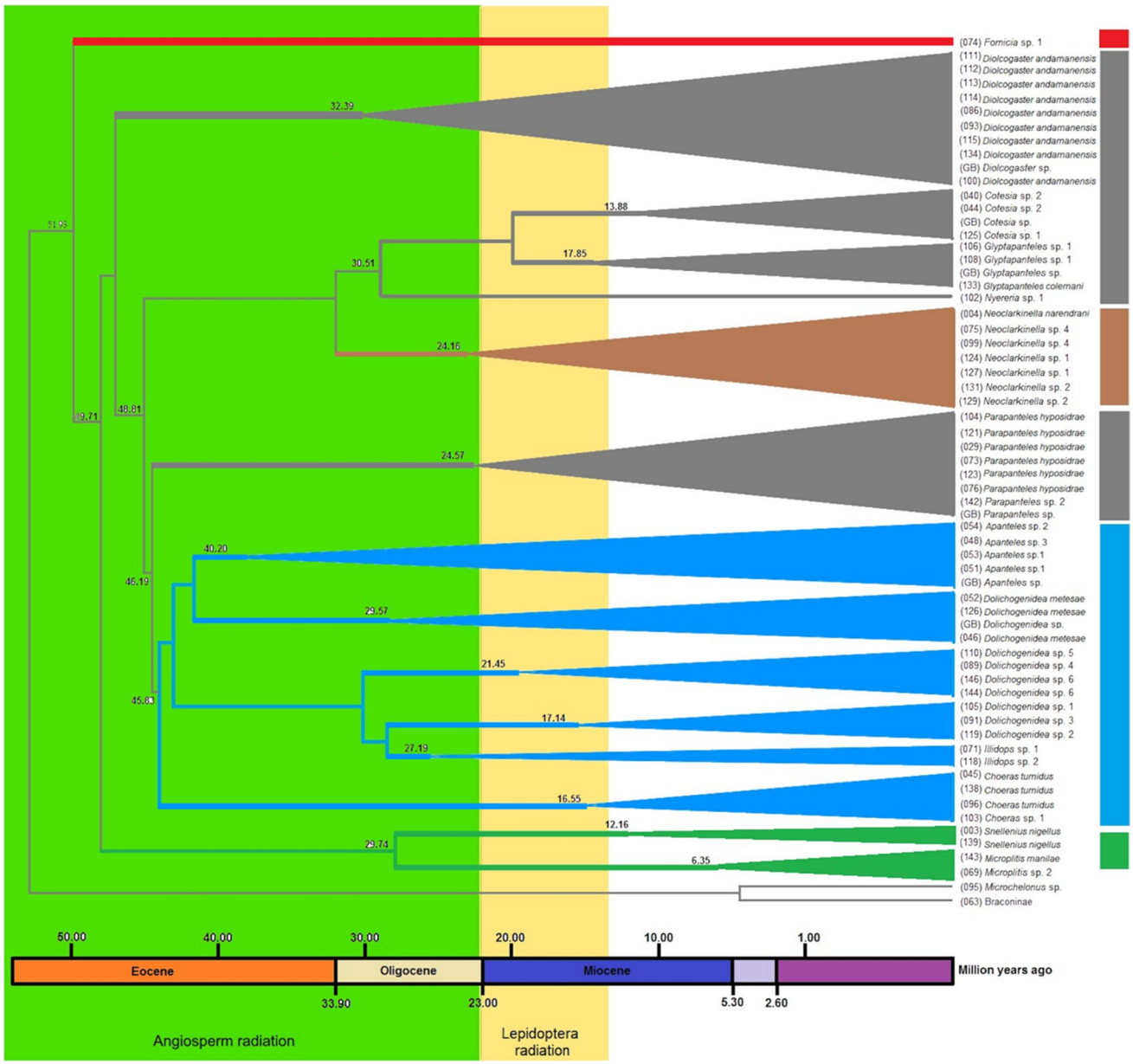

Fig. 2 Molecular time divergence of microgastrine generated using BEAST software by concatenated *16S*, *28S* and *COI* molecular markers. The estimated time divergence was shown above the branches. Colours represent the group. Blue: *Apanteles* group. Brown: unplaced genera group. Red: *Fornicia* genus. Gray: *Cotesia* group. Green: *Microplitis* group. The bar shows the geological time scale

(Kaur and Singh 2020). The molecular clock constructed solely from mitochondrial data yielded older time estimates, which were inordinately biased towards calibration points for the ingroup. This bias is attributed to the saturation of mitochondrial data concerning older divergences and the loss of calibration points for recent taxa. Furthermore, the maternal inheritance of mitochondrial DNA contributes to demographic asymmetry (Toews and Brelsford 2012). The inclusion of nuclear data facilitates stabilization in the estimation of divergence time due to its slower mutational rate, thereby preventing potential bias in molecular clock analyses (Chippindale et al. 2004; San Mauro 2010). Nuclear data are also capable of providing calibration points for basal and internal branches (Wilson et al. 2011).

The divergence time of Microgastrinae in this study is presented according to its group classification (Fernandez-Triana et al. 2020). Our estimate for Microgastrinae crown age (51.93 mya) aligns with Murphy et al. (2008) (~ 54 mya). Four groups, namely *Apanteles*, *Microplitis*, *Cotesia*, and the unplaced genera group, successfully had their divergence times calculated. The *Apanteles* group diverged at approximately 45.83 million years ago, the *Microplitis*

group at approximately 29.74 million years ago, the genus *Fornicia* at approximately 51.93 million years ago, the genus *Neoclarkinella* at approximately 24.16 million years ago, and the divergence time of the *Cotesia* group appeared ambiguous, with the formation of the clades estimated to have occurred around approximately 32.32 to 24.57 million years ago; however, the divergence time for its genera was successfully estimated. The *Cotesia* group exhibited multiple divergence events between 32.39 and 13.88 million years ago (mya). These events are believed to have resulted from a rapid radiation of Microgastrinae, as indicated by previous studies (Banks and Whitfield 2006). Additionally, the presence of short inner branches within the phylogenetic trees of Microgastrinae supports the rapid radiation hypothesis (Abdoli et al. 2024; Whitfield and Lockhart 2007).

This study highlights the close coevolution between Microgastrinae wasps and angiosperms, emphasizing their shared history with Lepidoptera hosts. As angiosperms and Lepidoptera diversified together, so too did Microgastrinae, which parasitize Lepidopteran larvae. This three-way coevolution likely drove major diversification events in the subfamily. The current formation of Sundaland was preceded by the Last Glacial Maximum period (110–15 mya) (Cannon et al. 2009). The rising sea levels resulted in a reduction and contraction of floral distribution (Woodruff 2010). Angiosperm radiation in the Sundaland region is estimated to have occurred around the Eocene/Oligocene boundary (Clark et al. 2009; Grudinski et al. 2014; Su and Saunders 2009), while the radiation of its associated group, Papilionidae (Lepidoptera), occurred in the early to middle Miocene (Kondo et al. 2003). Microgastrines, as endoparasitoid wasps, parasitize lepidopteran larvae. The divergence of microgastrines in the early Eocene and the subsequent radiation of the tribes from the middle of the Oligocene to the Miocene support the rapid radiation proposed by Murphy et al. (2008). The overlapping divergence times for Lepidoptera and microgastrines in Sundaland indirectly provide evidence for a rapid radiation event within the lineage. The short branches in the phylogenetic tree forming the paraphyletic group of *Cotesia* are indicative of the loss of signal during rapid adaptation and mutation within the tribe in response to its host.

Conclusion

In the molecular divergence study, samples from Sundaland were utilized to estimate the divergence times of microgastrines during the Eocene epoch, taking into account the coevolution of lepidopteran hosts and angiosperm food sources. The genus *Fornicia*, identified as the earliest genus, is estimated to have undergone radiation approximately 51 million years ago (mya), followed by the radiation of other genera beginning around 49.71 mya. The *Apanteles* group exhibited radiation from 45.83 mya, whereas the *Microplitis* group diverged at 29.74 mya, and the genus *Neoclarkinella* evolved at 24.16 mya. The paraphyletic group of *Cotesia* diverged multiple times, within the timeframe of 32.39 to 13.88 mya. The evolutionary history of microgastrines delineated in this study has been calibrated against the fossil records of lepidopterans and angiosperms documented from Sundaland. Angiosperms are believed to have radiated during the Eocene to Oligocene epochs, approximately 55 to 23 mya, while lepidopteran species are thought to have evolved at the onset of the Miocene, between 23.0 and 15.0 mya. Both outgroup species, Cheloninae and Braconinae, are posited to have diverged prior to 52.0 mya, predating the Microgastrinae. These findings are congruent and provide support for the estimation of phylogeny and molecular divergence times for this group, based on worldwide species. The results contribute to a deeper understanding of the systematics, biology, and evolution of Microgastrinae by incorporating samples collected from Peninsular Malaysia.

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Author contributions A.A.Z designed and performed the experiments, data analysis, identification, interpretation, drafted and wrote the manuscript. M.A.M and A.S.B interpreted and revised the manuscript. M.I.I and M.F.S revised the manuscript and format preparation. S.Y designed the experiments, revised the manuscript and oversaw the whole project.

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Data availability All data generated in this study are available in manuscript.

Declarations

Conflict of interest All the authors declare that they have no conflict of interest.

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