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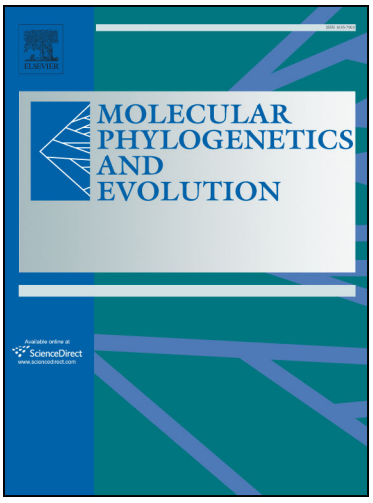

A five-gene molecular phylogeny reveals *Parapanteles* Ashmead (Hymenoptera: Braconidae) to be polyphyletic as currently composed

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TITLE

A five-gene molecular phylogeny reveals *Parapanteles* Ashmead (Hymenoptera: Braconidae) to be polyphyletic as currently composed.

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KEYWORDS

Parapanteles; Microgastrinae; Braconidae; molecular phylogenetics; host use; DNA barcoding

ABSTRACT

Parapanteles Ashmead (Braconidae: Microgastrinae) is a medium-sized genus of microgastrine wasps that was erected over a century ago and lacks a unique synapomorphic character, and its monophyly has not been tested by any means. *Parapanteles* usually are parasitoids of large, unconcealed caterpillars (macrolepidoptera) and have been reared from an unusually large diversity of hosts for a relatively small microgastrine genus. We used Cytochrome Oxidase I sequences ("DNA barcodes") available for *Parapanteles* and other microgastrines to sample the generic diversity of described and undescribed species currently placed in *Parapanteles,* and then sequenced four additional genes for this subsample (*wingless, elongation factor 1-alpha, ribosomal subunit 28s,* and *NADH dehydrogenase subunit 1*). We constructed individual gene trees and concatenated Bayesian and maximum-likelihood phylogenies for this 5-gene subsample. In these phylogenies, most *Parapanteles* species formed a monophyletic clade within another genus, *Dolichogenidea*, while the remaining *Parapanteles* species were recovered polyphyletically within several other genera. The latter likely represent misidentified members of other morphologically similar genera. Species in the monophyletic clade containing most *Parapanteles* parasitized caterpillars from only five families - Erebidae (Arctiinae), Geometridae, Saturniidae, Notodontidae, and Crambidae. We do not make any formal taxonomic decisions here because we were not able to include representatives of type species for *Parapanteles* or other relevant genera, and because we feel such decisions should be reserved until a comprehensive morphological analysis of the boundaries of these genera is accomplished.

1.1 INTRODUCTION

Parapanteles Ashmead is a genus of parasitoid wasps that exhibits many of the taxonomic and systematic challenges of the subfamily Microgastrinae (Hymenoptera: Braconidae), one of the most species-rich groups of parasitoid wasps (Whitfield 1995; Smith *et al.* 2008; Rodriguez *et al.* 2012). The number of species attributed to *Parapanteles*, the diversity of host use records of this genus, and the number of countries where it has been collected have all sharply increased in the last two decades (Whitfield *et al.* 2018). Despite this, *Parapanteles* does not have a strong morphological synapomorphy and its generic cohesiveness has not been scrutinized via molecular evidence.

Parapanteles was erected in 1900 as a monotypic genus containing one species (*P. aletiae* (Riley)) from the southeastern US (Ashmead 1900). Only three species, each from a different continent, were added by 2005: one from Australia (*P. masoni* Austin & Dangerfield (1992)), one from Costa Rica (*P. paradoxus* (Muesebeck) (Mason 1981), and one from South Africa (*P. rooibos* Valerio, Whitfield, & Kole (Valerio *et al.* 2005)). Fourteen species, primarily from Costa Rica, were added in 2009 (Valerio *et al.*), and eight more species have since been described from India (Rousse & Gupta 2013, Gupta *et al.* 2014a, Gupta *et al.* 2014b). These 26 species are recorded from 12 Lepidoptera host families (Supplemental Materials 1). About twice as many more undescribed species (including six more unique host family records) have been attributed to *Parapanteles*, either by morphological diagnosis, genetic similarity of the "DNA barcoding" region of *Cytochrome oxidase I* (COI), or a combination of the two (Janzen & Hallwachs 2009, 2016, Smith *et al.* 2013).

Parapanteles is defined by a combination of three continuously varying characters which are individually present in many other microgastrine genera: a short ovipositor, an inflexible hypopygium, and a propodeal areola (Mason 1981, Valerio *et al.* 2009). Ovipositor length is prone to convergent evolution since it has immediate fitness consequences for female wasps: species that attack larger, unconcealed hosts tend to have shorter ovipositors, while species that attack concealed hosts (e.g., leaf rollers, leaf miners) tend to have longer ovipositors, regardless of genus (Mason 1981). Ovipositor characters (especially length) have low phylogenetic value in other groups of Braconidae (Wild *et al.* 2013) and the accuracy of ovipositor length for assigning new species to genus in Microgastrinae is untested. The relatively inflexible hypopygium, while fixed in some genera, is highly correlated with shortness of ovipositor in other genera within Microgastrinae such as *Apanteles* Förster. The third character, presence of a propodeal areola, is of unclear function but is highly variable across genera. In *Parapanteles* and several other genera (e.g. *Dolichogenidea* Viereck), a ring-shaped or pentagonal pattern of ridges forms an areola on the propodeum; these ridges are sometimes very faint, obscured by setae, or obscured by additional propodeal ridges and/or sculpturing (Mason 1981). Each of these characters is difficult to interpret in specimens with intermediate morphology and alternative interpretations can easily place a specimen in a distantly related genus. For example, specimens with areolate propodea and short-to-intermediate ovipositors can be interpreted as *Parapanteles* or *Dolichogenidea*, while specimens with unambiguously short ovipositors and weak-to-faint propodeal areolae could be placed in either *Parapanteles* or *Glyptapanteles* Ashmead (Whitfield 1997). In addition to the problem of ambiguous *Parapanteles* specimens, some described species (e.g. *P. scotti* Valerio &

Whitfield*, P. mariae* Valerio & Whitfield) resemble species of *Cotesia* Cameron in overall appearance (Valerio *et al.* 2009; de Freitas *et al.*, 2019).

Previous molecular phylogenies have not confidently placed *Parapanteles* in relation to other microgastrine genera, nor sampled it broadly. Microgastrine generic diversity increased during an ancient rapid radiation (Banks & Whitfield 2006) and previous genus-level molecular phylogenies of Microgastrinae typically have many short and poorly-supported internal branches, especially near the bases of the trees (Mardulyn & Whitfield 1999, Whitfield *et al.* 2002, Banks & Whitfield 2006). *Parapanteles* has been recovered, generally with poor support, in several different places in these phylogenies: sister to or within *Hypomicrogaster* Ashmead, near *Dolichogenidea*, or sister to various smaller and rarer genera (Mardulyn & Whitfield 1999, Whitfield *et al.* 2002, Banks & Whitfield 2006). These phylogenies each include a single *Parapanteles* specimen: an unidentified *Parapanteles* species in Mardulyn & Whitfield (1999) and Banks & Whitfield (2006), and *P. paradoxus* in Whitfield *et al.* (2002).

Examining the phylogenetic patterns of host specialization in parasitoid wasps is of significant ecological and evolutionary interest, especially those with extensive host data such as Microgastrinae. In this context, it is essential that phylogenies be focused upon groups whose monophyly can be established. *Parapanteles'* ambiguous diagnostic characters, diverse host use, cosmopolitan distribution, and lack of molecular evidence suggest that it may be a catch-all genus for morphologically difficult species rather than a monophyletic group. To test this, we constructed a large phylogeny of all available COI sequences for *Parapanteles* and 16 other microgastrine genera, then used this phylogeny to select a subsample of available *Parapanteles* specimens that represents the breadth of the molecular diversity of described and putative species currently attributed to *Parapanteles.* We sequenced four additional genes from that subsample and constructed a 5-gene molecular phylogeny to test the monophyly of this genus and to examine how it relates to other microgastrine genera.

2.1 METHODS

2.2 Taxon & gene selection

2.2.1 *COI-tree based within-group taxon sampling*

To approximate the diversity of *Parapanteles* under its current morphological definition, we accessed all available COI sequences for *Parapanteles* and 16 other microgastrine genera: *Alphomelon* Mason*, Apanteles, Clarkinella* Mason*, Cotesia, Diolcogaster* Ashmead*, Dolichogenidea, Exoryza* Mason*, Glypapanteles, Hypomicrogaster, Microplitis* Förster*, Pholetesor* Mason*, Prasmodon* Nixon*, Promicrogaster* Bues & Richardson*, Protapanteles* Ashmead*, Rhygoplitis* Mason*,* and *Xanthomicrogaster* Cameron from the Barcode of Life Database (BOLD) (Ratnasingham & Hebert 2007; http://v3.boldsystems.org/) and GenBank (http://www.ncbi.nlm.nih.gov/genbank/) on 01/11/2017. The long-term caterpillar rearing project at Área de Conservación Guanacaste (ACG), Costa Rica (Janzen & Hallwachs 2009, 2016) has provided the majority of known yet undescribed *Parapanteles* specimens, which have been morphologically identified to genus by professional braconid taxonomists and DNA barcoded (Janzen & Hallwachs 2009, 2016), and is the source of approximately 43% of the DNA barcode sequences and putative species used in this study (Supplemental Materials 17). This dataset includes the

most species-rich microgastrine genera, genera which *Parapanteles* has been recovered sister to in previous phylogenies, genera with morphological similarities to *Parapanteles,* and several small microgastrine genera. In addition, we sequenced and included *COI* for 110 more *Parapanteles* specimens reared at Yanayacu Biological Station in Ecuador (Dyer *et al.* 2017). We aligned this dataset with PASTA v1.6.3 (Mirarab *et al.* 2014) and edited it manually in Geneious v9.1.5 (http://www.geneious.com, Kearse *et al.* 2012), discarding sequences that were missing approximately 200 or more base pairs (bp) of the 658 bp *COI* barcoding region. Our dataset then contained 24,611 aligned sequences from at least 1,713 described or putative species (the number of species may be higher because many sequences were only identified to genus) (Supplemental Materials 2 & 3), which we used to make a phylogenetic tree with FastTree 2.1.8 (Price & Arkin 2010) using the GTR+I+G substitution model (Supplemental Materials 4). We used this tree to selected 56 *Parapanteles* specimens recovered from disparate clades in this tree to sequence additional genes. This subsample included representatives of *Parapanteles* specimens recovered in 12 of 15 major clades throughout our initial COI tree. *Dolichogenidea* specimens were frequently intermixed with *Parapanteles* specimens in this tree, so we also included 13 *Dolichogenidea* species in our subset.

2.2.2 *Gene selection and outgroups*

We sequenced portions of two mitochondrial genes [655 bp of *cytochrome oxidase I* (COI) (the "DNA barcoding" region) and 447 bp of *NADH dehydrogenase subunit 1* (ND1)] and three nuclear genes [451 bp of *wingless* (WG), 418 bp of *elongation factor 1-alpha* (EF1a) and 666 bp of *ribosomal subunit 28s* (28S)] (Supplemental Materials 5) to construct a molecular phylogeny. We used the "DNA barcoding" region of *cytochrome oxidase I* because of the availability of other microgastrine *COI* sequences through the Barcode of Life Database (BOLD, Ratnasingham & Herbert 2007) because it is commonly used for species delimitation in this group (Whitfield *et al.* 2002, Banks & Whitfield 2006, Smith *et al.* 2008, 2013, Janzen & Hallwachs 2009, Fernández-Triana *et al.* 2014). *Wingless* has been used in generic-level phylogenies of microgastrines (Banks & Whitfield 2006; Murphy et al 2008), and sequences are available for all *Parapanteles* that attack *Eois* Hübner (Geometridae: Larentiinae) caterpillars to date (Wilson *et al.* 2012) and all outgroup taxa with pre-existing sequences (Banks & Whitfield 2006). Similarly, EF1a has been used extensively in insect systematics and sequences were available from the *Parapanteles* attacking *Eois* (Wilson *et al.* 2012). Previous microgastrine phylogenies have used ND1 and 28s (Dowton & Austin 1998, Michel-Salzat & Whitfield 2004, Kankare & Shaw 2004, Rodriguez 2009, O'Connor 2011), and we were able to incorporate existing outgroup sequences by including these genes in our dataset.

We included all available non-*Parapanteles* microgastrine specimens that have sequences available in GenBank for at least 3 of the 5 genes used in this study. This outgroup set includes 11 species from 8 other microgastrine genera: *Apanteles, Cotesia, Dolichogenidea, Glyptapanteles, Hypomicrogaster, Microplitis, Pholetesor, Prasmodon, Promicrogaster* and *Rhygoplitis* (Supplemental Materials 6). We used one Cheloninae species (*Phanerotoma* Wesmael), the earliest-diverging group in the microgastroid complex (including Microgastrinae and four other smaller Braconidae subfamilies: Cardiochilinae, Cheloninae, Mendesellinae and Miracinae) (Dowton & Austin 1998, Murphy *et al.* 2008), to root trees. Preliminary results placed many *Parapanteles* among several other genera, so we included additional specimens from *Glyptapanteles* and *Apanteles*, including sequences from two unpublished molecular

phylogenies of these genera (Rodriguez 2009, Arias-Penna 2015). We used *COI* and *WG* sequences from 28 undescribed species of *Glyptapanteles*, to which we added sequences of *EF1a, 28s,* and *ND1.* We sequenced all 5 genes in our dataset for 17 additional *Glyptapanteles* specimens from Yanayacu, Ecuador (Arias-Penna 2015). We used existing sequences of all five genes for 19 *Apanteles* species (Rodriguez 2009).

2.3 Sequencing

Genomic DNA was extracted from adult microgastrines using Qiagen DNEasy Blood and Tissue kits (Qiagen, Valencia, CA, USA) following the manufacturer's directions. For gregarious species (multiple conspecific larvae developing in the same host), we extracted DNA from whole specimens. For solitary species, we extracted DNA from one hind leg, removed above the coxa, or one mid- and/or foreleg if one or more hind legs were missing. We used New England Biolabs *Taq* DNA Polymerase with Standard *Taq* buffer and the primers and thermocycler protocols listed in Supplemental Materials 5. We used the following primer pairs: 28S: 28SF (5'-AAGAGAGAGTTCAAGAGTACGTG-3') & 28S-PM (5'-TAGTTCACCATCTTTCGGGTCCC-3') (Mardulyn &Whitfield 1999), COI: LepF (5'-ATTCAACCAATCATAAAGATATTGG-3') & LepR (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') (Hajibabaei et al. 2006), EF1A: EF1A1F (5'-AGATGGGYAARGGTTCCTTCAA-3') & EF1A1R (5'-AACATGTTGTCDCCGTGCCATCC-3') (Belshaw & Quicke 1997), ND1: ND1F (5'-ACTAATTCAGATTCTCCTTCT-3') & ND1R (5'-CAACCTTTTAGTGATGC-3') (Smith et al. 1999), WG: Wg550F (5'-ATGCGTCAGGARTGYAARTGYCAYGGYATGTC-3') & WgAbRZ (5'-CACTTNACYTCRCARCACCARTG-3') (Brower & DeSalle 1998). We purified PCR products with EXO SAP and performed sequencing reactions with ABI Prism BigDye Terminator v3.1 Cycle Sequencing Kits, typically using $1/8^{th}$-$1/16^{th}$ of the recommended amount of BigDye Terminator 3.1 Ready Reaction Mix (1µl-0.5µl) but otherwise following the manufacturer's instructions. PCR products were sequenced at the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois. We edited sequences with Geneious v9.1.5 (http://www.geneious.com, Kearse *et al.* 2012). All novel sequences are deposited in GenBank (Supplemental Materials 6).

2.4 Alignment and Phylogenetic Analysis

We excluded from concatenated analyses any taxon for which we were unable to sequence at least three genes, but still included all available sequences in individual gene trees. We therefore included 142 species in our concatenated alignment, with the following numbers of species missing for each gene followed by the number of species included in each individual gene tree in parentheses: *COI*: 0/142 (295), *WG*: 4/142 (160), *ND1*: 50/142 (126), *EF1a*: 27/142 (135), *28s*: 18/142 (139). We aligned sequences with MUSCLE v3.8.31 (Edgar 2004). Our concatenated alignment had 2626 characters total, with 169 invariable sites across all taxa. We used Partitionfinder v1.1.1 (Lanfear *et al.* 2012) to select appropriate models for phylogenetic analysis based on their Bayesian Information Criterion (BIC) score. In all analyses we partitioned *COI* and *ND1* alignments into three partitions by 1^{st}, 2^{nd}, and 3^{rd} codon positions, *WG* and *EF1a* into two partitions by 1^{st}+2^{nd} and 3^{rd} codon positions, and *28s* into two partitions, with the conserved regions flanking the D2 variable region in one partition and the variable region in the other, for a total of 12 partitions (Supplemental Materials 7). We constructed Maximum

Likelihood (ML) trees in RAxML v8.1.15 (Stamatakis 2014) with 1000 bootstrap replicates for each gene independently and for all genes concatenated. For each analysis we selected either GTR+G or GTR+I+G depending on which model was favored by the majority of partitions. We constructed an additional tree for each analysis with MrBayes v.3.2.2 (Ronquist *et al.* 2012) using mixed models. We ran each Bayesian analysis for 10 million generations with 4 MCMC chains, and sampled trees every 1000th generation. Appropriate burn-in values were estimated in Tracer v.1.5 (Rambaut & Drummond 2007). All trees except ND1 trees were rooted with the outgroup *Phanerotoma*, representing Cheloninae, the most closely related subfamily to Microgastrinae, as the most distant outgroup. ND1 trees were rooted with Microplitis demolitor, as Microplitis has been shown to be the most early-diverging genus of microgastrines in our sample (Whitfield et al. 2002, Banks and Whitfield 2006). We graphically edited all trees in FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) and poorly supported branches were manually collapsed in Adobe Illustrator CC 2015.3. All alignments and unedited tree files are deposited in Dryad (DOI: 10.5061/dryad.3xsj3txb2).

2.5 Specimen and provisional species naming conventions

Specimens collected by the ACG rearing project in Costa Rica are either assigned to described species or assigned interim provisional species epithets based on COI sequence similarity as displayed in a Nearest Neighbor Joining tree and host associations (Janzen *et al.* 2009, Janzen & Hallwachs 2009, 2016). Interim species names follow the convention of the last name of an ACG taxonomic collaborator and a number (e.g., *Apanteles* Rodriguez01, *Parapanteles* Whitfield113). Specimens of undescribed species from ACG used in this study are identified by these interim species epithets. The voucher codes for them, in the form DHJPAR#######, refer to the specific wasp specimen, while the individual caterpillar from which it was reared has its own voucher code in the form of yy-SRNP-#####. The voucher specimens are deposited in the Canadian National Insect Collection (CNC) in Ottawa, Canada and at the University of Illinois Urbana-Champaign to eventually be transferred to the CNC.

Specimens from the Yanayacu rearing project in Ecuador are not routinely sequenced or grouped into interim species as they are in the ACG inventory, so specimens of undescribed species from Yanayacu used in this study are identified by “yy” and their individual sample number (e.g. *Parapanteles* yy3653). This identifies the specific specimen used, not an informal species name. We grouped specimens from the Yanayacu rearing project into interim species based on COI sequence similarity, natural history, and then morphological similarity. We calculated the pair-wise distances of COI sequences with MEGA v7.0.26 (Kumar *et al.* 2016).

3.1 RESULTS

We recovered *Parapanteles* as polyphyletic in all analyses, with both described species and undescribed putative species morphologically identified as *Parapanteles* appearing within clades dominated by *Apanteles, Cotesia, Dolichogenidea*, or *Glyptapanteles.* In our concatenated analyses, the majority of *Parapanteles* taxa were recovered as a monophyletic clade (Fig. 1 clade A) within *Dolichogenidea* (Fig. 1 clade B), followed by eleven *Parapanteles* taxa recovered throughout the predominantly *Glyptapanteles*

clade (Fig. 1 clade E & F), four within the *Cotesia* clade (Fig. 1 clade G), and one within the predominantly *Apanteles* clade (Fig. 1 clade C).

The topologies of COI and EF1a gene trees were least similar to the topologies of our concatenated analyses, while the topologies of our WG trees were the most similar to the topology of our concatenated analyses, followed by our ND1 trees (Supplemental Materials 15 & 16). While these individual gene trees differ from each other and no individual gene tree reflects all of the relationships that we recovered in concatenated analyses, clades that strongly contradict the relationships recovered in our concatenated analysis are rare or absent in most individual gene trees. The largest source of conflicting relationships are basal relationships in our EF1a gene trees. The majority of differences between our concatenated analysis and individual gene trees are clades in the concatenated analysis that are partial or complete polytomies in one or more individual gene trees (Table 1).

We identified 10 provisional undescribed species from the Yanayacu Rearing Project in Ecuador based on COI sequence similarity, natural history, and then morphological similarity (Supplemental Materials 8).

4.1 DISCUSSION

We found *Parapanteles* to be polyphyletic (Fig. 1). The diversity of hosts parasitized by species of what have been called *Parapanteles* is inflated due to the polyphyly of this genus, and most of its host family diversity is accounted for by species placed in *Parapanteles* that belong elsewhere, especially those that belong in *Cotesia* or *Glyptapanteles* (Fig. 1 clades D-H). We recovered one strongly-supported clade containing the majority of named and unnamed *Parapanteles* species included in our dataset (Fig. 1, clade A). Species in this clade parasitize Erebidae (Arctiinae), Geometridae, Notodontiade, and Saturniidae, all relatively large and unconcealed hosts. This *Parapanteles* clade rendered *Dolichogenidea* paraphyletic, although the branch defining it has low support. *Dolichogenidea* is, to date, a much larger genus than *Parapanteles* and usually parasitizes leaf miners, leaf tiers and other concealed microlepidotera. COI barcode data suggest *Dolichogenidea* may also be polyphyletic (Mason 1981, Smith *et al.* 2013, Supplemental Materials 4). Therefore, *Parapanteles s. s.* may be interpretable as a clade of *Dolichogenidea* that shifted to parasitizing macrolepidoptera. An appropriate taxonomic revision of *Parapanteles* will require a revision of *Dolichogenidea* that should include much broader phylogenetic sampling of species of *Dolichogenidea*, and which includes the type species of both genera. These were not available for this study in a form suitable for molecular sampling.

Several previous microgastrine phylogenies placed *Parapanteles* as close to or as a sister group to *Hypomicrogaster* but we did not find this relationship in any of our analyses. These studies included representatives of many microgastrine genera, but few species within each (Whitfield *et al.* 2002, Banks & Whitfield 2006). Whitfield *et al.* (2002) included one unidentified *Parapanteles* species, which may have been from any of the disparate taxa currently considered *Parapanteles*. Banks & Whitfield (2006) used *Parapanteles paradoxus,* a Costa Rican species included in this study. In some of their analyses they recovered *P. paradoxus* near, sister to, and/or within *Hypomicrogaster,* but with poor support. We were unable to locate the vouchers for these specimens for additional sequencing, but we recovered their

existing COI sequences with the other *P. paradoxus* sequences recovered within the main *Parapanteles* clade in our 26k COI phylogeny (Supplemental Materials 4). Our concatenated analyses (Fig. 1) and our broad COI survey of microgastrine genera (Supplemental Materials 4) supports *Hypomicrogaster* as a distinctive monophyletic genus that is not closely related to the majority of *Parapanteles* clades. Although logistically prohibitive at the time, had these previous studies included broad sampling both within and across genera, they would not likely have recovered *Parapanteles* as being closely related to *Hypomicrogaster*.

The polyphyly of *Parapanteles* reflects the difficulty of assigning this group of microgastrines to genus via diagnostic morphological traits only, especially within the hugely diverse neotropical taxa. As genera are currently described, the presence of a propodeal areola and possession of a relatively short ovipositor are critical characters for separating *Parapanteles* from *Glyptapanteles* and *Dolichogenidea* respectively (Whitfield 1997). Accurate morphological diagnosis of main clade (Fig. 1 clade A) *Parapanteles* from *Dolichogenidea* is a complex problem. It relies largely on interpretation of ovipositor length, which may be prone to convergent evolution when unrelated species attack similar host species (e.g. leafminers vs. macrolepidoptera). As previously stated, we also recovered *Dolichogenidea* to be paraphyletic in our 5-gene analysis (Fig. 1 clade B), containing *Parapanteles, Exoryza*, and *Pholetesor* species, and polyphyletic in our 24k COI analysis (Supplemental Materials 4), containing *Exoryza, Parapanteles*, *Protopanteles,* and *Apanteles* species while also being recovered within *Pholetesor* and *Apanteles*. Identification of useful morphological diagnoses for *Parapanteles* vs. *Dolichogenidea* species, and the status of *Parapanteles* as a distinct genus, should be included in a broader reassessment and revision of *Dolichogenidea* and *Pholetesor.* Sculpturing on the propodium varies within the *Parapanteles* species we recovered in clade A (Fig. 1). For example, *P. paradoxus, P. em,* and *P. tlinea* have heavily sculpted propodea to the point that it obscures the areolar ridges, while *P. continua* and *P. tessares* have little sculpting with very clear areolar ridges, and *P.* sp. J and *P.* sp. K have almost no sculpting and very faint areolar ridges. Many of the *Parapanteles* that grouped within *Glyptapanteles* (and *vice versa*) have what was considered a faint propodeal areola rather than the complete absence of this character, while many that grouped with *Cotesia* have heavily sculpted propodea that obscure the state of areolar ridges. Our results suggest that interpretation of this character, especially when it is weakly expressed or heavily sculptured, is subjective and unreliable. The shape of the 1st metasomal tergite is variable across *Parapanteles* species, distally increasing in width in most species, roughly the same width throughout in some, and narrowing sharply distally in a few (Valerio *et al.* 2009). All but two of the species in clade A of our analysis (Fig. 1) have 1st metasomal tergites that are wider distally or with roughly equal width throughout. The two exceptions are solitary geometrid-attacking species whose 1st metasomal tergites are longer, thinner and narrow sharply distally. These two species morphologically resemble *Glyptapanteles* species that attack geometrids in the same genus, *Eois*, which reflects many of the misdiagnosed *Parapanteles* species we recovered within *Glyptapanteles* and vice versa (Fig. 1 clades A & D). Correct generic identification of *Parapanteles*, *Glyptapanteles*, and *Dolichogenidea* species with intermediate phenotypes for these traits is extremely difficult via morphology alone, especially for males, which lack ovipositors and may be impossible to determine unless molecular or biological data are available. Whenever possible, generic placement should be corroborated with COI data.

Eight new *Parapanteles* species have been described recently from India (Rousse & Gupta 2013, Gupta *et al.* 2014a, b). The majority of these species were reared from butterfly caterpillars: four from species of Lycaenidae, one from a species of Riodinidae, and one from a species of Nymphalidae. Of the butterfly-attacking *Parapanteles* species we included in our analysis, none were recovered in the largest monophyletic clade of *Parapanteles* (Fig. 1 clade A). Most grouped within *Cotesia,* followed by *Glyptapanteles,* and one riodinid-attacking species within *Apanteles*. Therefore, we predict that molecular analysis of these Indian species may place them in *Cotesia* or *Glyptapanteles*.

Investigations into the coevolution and ecology of two hyperdiverse neotropical taxa, *Piper* (Piperales: Piperaceae) and one of its specialist herbivores *Eois* (Lepidoptera: Geometridae), have identified *Parapanteles* wasps as the most numerous and diverse parasitoids of *Eois* caterpillars (Bodner *et al.* 2010, Brehm *et al.* 2011, Wilson *et al.* 2012). Wilson *et al.* (2012) identified at least six putative *Eois*-attacking *Parapanteles* species based on adult and cocoon morphology and molecular results. We included many of the same samples that were used by Wilson *et al.* 2012 in our own analyses. Our results suggest that these *Eois*-attacking *Parapanteles* are in fact two sister species within the main *Parapanteles* clade we recovered (Supplemental Materials 8, provisional spp. J & K), along with three or more *Glyptapanteles* species. The COI "barcoding" region of provisional species J & K are each almost identical within species (0-0.7%) and about 2.3% different from each other (Supplemental Materials 8). Both species have rearing records from *Eois olivacea* Felder, Felder, & Rogenhofer, while one has additionally been reared from *E. pallidicosta* Warren (Dyer *et al.* 2017). These two species are the most morphologically similar to *Glyptapanteles* of any of the *Parapanteles* species we recovered in clade A (Fig. 1), and the only species with long, narrow first metasomal tergites that narrow distally.

In summary, our study strongly corroborates the notion that *Parapanteles*, as currently defined, is polyphyletic, consisting of a core clade embedded within *Dolichogenidea* as currently defined, and containing several species of *Apanteles, Cotesia*, and *Glyptapanteles* that are difficult to diagnose morphologically. Should *Parapanteles* be retained as a valid genus upon revision and possible division of *Dolichogenidea*, it needs to be diagnosed using a more distinguishable set of morphological or genetic features. In the meantime, reassignment of the obviously misdiagnosed members of other genera is clearly called for (Supplemental Materials 18).

5.1 ACKNOWLEDGMENTS

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7.1 Table and Figure legends

Figure 1: Consensus tree of RAxML and MrBayes analyses of concatenated 5-gene dataset. Bootstrap supports/posterior probabilities are reported on each branch. Nodes with poor support from both bootstrapping and posterior probability (i.e. >50 bootstrap support & >0.9 posterior probability) were collapsed. Branches are colored by genus, with purple corresponding to *Parapanteles,* green to *Dolichogenidea*, red to *Apanteles*, blue to *Glyptapanteles*, and yellow to *Cotesia*. Branches of all other genera are black. Branches of *Parapanteles* specimens are shaded by host family.

Table 1: Comparison of 9 clades (A-I) recovered in our concatenated analysis to their status in individual gene trees. "yes" indicates the clade was recovered. "polytomy" indicates that the clade was not recovered due to polytomy, but not otherwise contradicted by a relationship not recovered in the concatenated analysis. If a clade was recovered within a clade recovered as separate in the concatenated analysis, we listed the most common genus of the species within that clade.

Clade	COI	WG	*ND1*	*EF1a*	28s
A	*polytomy*	*yes*	*yes*	*no*	*yes*
B	*polytomy*	*yes*	*yes*	*no*	*polytomy*
C	*yes*	*yes*	*yes*	*no*	*yes*
D	*yes*	*yes*	*yes*	*no*	*yes*
E	*yes*	*polytomy*	*yes*	*no*	*yes*
F	*polytomy*	*yes*	*yes*	*no*	*polytomy*
G	*polytomy*	*yes*	*yes*	*polytomy*	*yes*
H	*polytomy*	*Glyptapanteles*	*Apanteles*	*polytomy*	*polytomy*
I	*yes*	*yes*	*no data*	*yes*	*yes*

Supplemental Materials 1: Rearing/collection records and host associations of described and provisional *Parapanteles* species as currently morphologically defined.

Supplemental Materials 2: GenBank and/or BOLD accession numbers of sequences used in 24611 sample COI tree of microgastrine genera.

Supplemental Materials 3: Pasta Alignment of sequences used in 24611 sample COI tree of microgastrine genera.

Supplemental Materials 4: Fasttree approximated maximum-likelihood phylogeny of 24611 microgastrine COI sequences. Taxon labels are colored by genus, with purple corresponding to *Parapanteles,* green to *Dolichogenidea*, teal to *Pholetesor,* red to *Apanteles*, blue to *Glyptapanteles*, and yellow to *Cotesia*. Taxa labels of all other genera are grey. Subsamples selected for 5-gene concatenated analysis are indicated by extended taxon labels.

Supplemental Materials 5: List of primers and annealing temperatures used in this study.

Supplemental Materials 6: GenBank and/or BOLD accession numbers of sequences used in 5-gene concatenated analysis and individual gene trees.

Supplemental Materials 7: Partitionfinder model scheme used in Bayesian analysis.

Supplemental Materials 8: Rearing/collection records and host associations of 10 new provisional species from the Yanayacu Rearing Project in Ecuador.

Supplemental Materials 9: Alignment of sequences used in COI trees.

Supplemental Materials 10: Alignment of sequences used in WG trees.

Supplemental Materials 11: Alignment of sequences used in ND1 trees.

Supplemental Materials 12: Alignment of sequences used in 28s trees.

Supplemental Materials 13: Alignment of sequences used in EF1a trees.

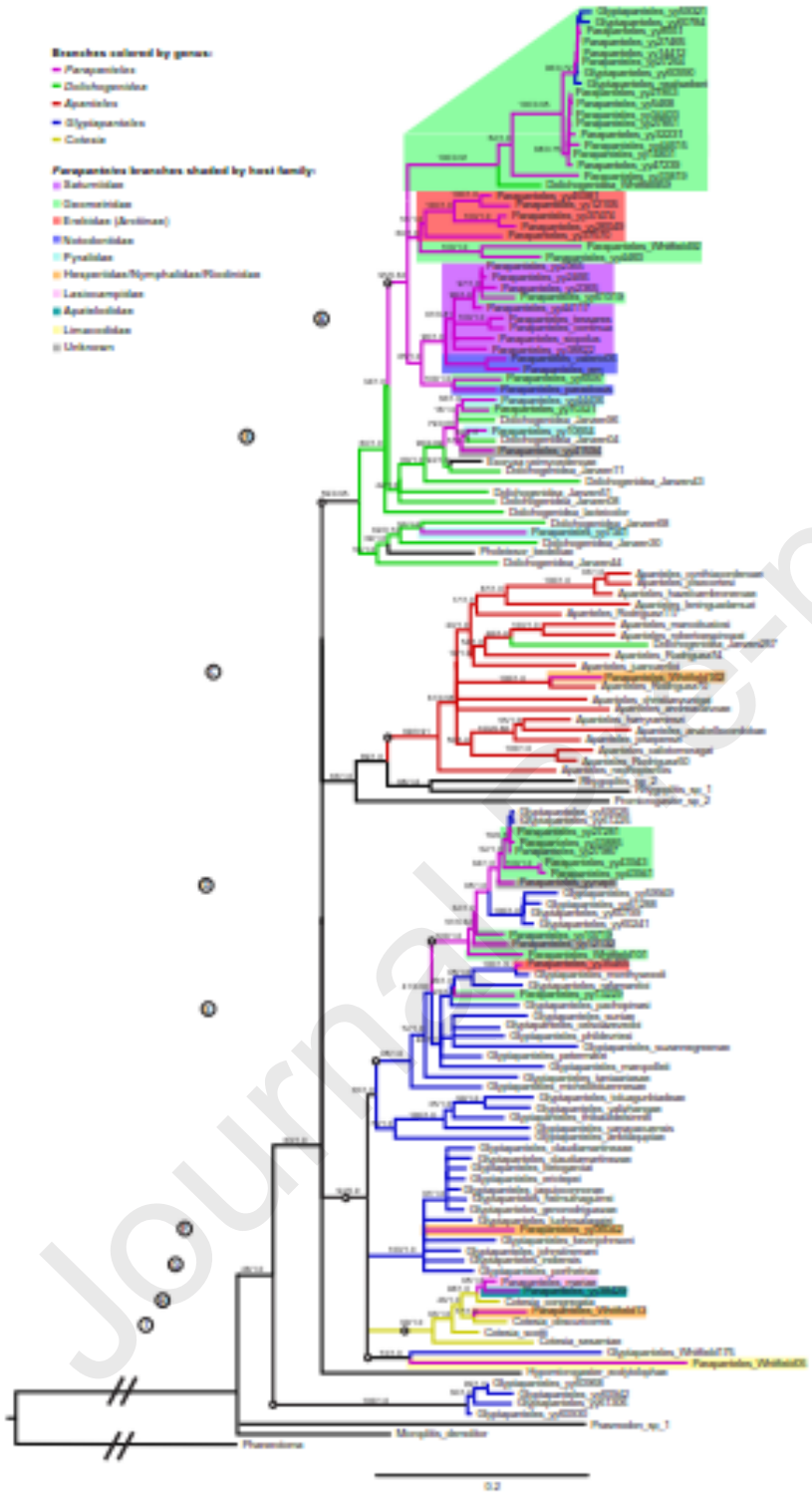
Supplemental Materials 14: Alignment of sequences used in 5-gene concatenated trees.

Supplemental Materials 15: RAxML maximum likelihood COI (a), WG (b), ND1 (c), 28s (d), EF1a (e), & Concatenated (f) phylogenies. Bootstrap supports are reported on each branch. Branches are colored by genus, with purple corresponding to Parapanteles, green to Dolichogenidea, red to Apanteles, blue to Glyptapanteles, and yellow to Cotesia. Branches of all other genera are black. Branches of Parapanteles specimens are shaded by host family.

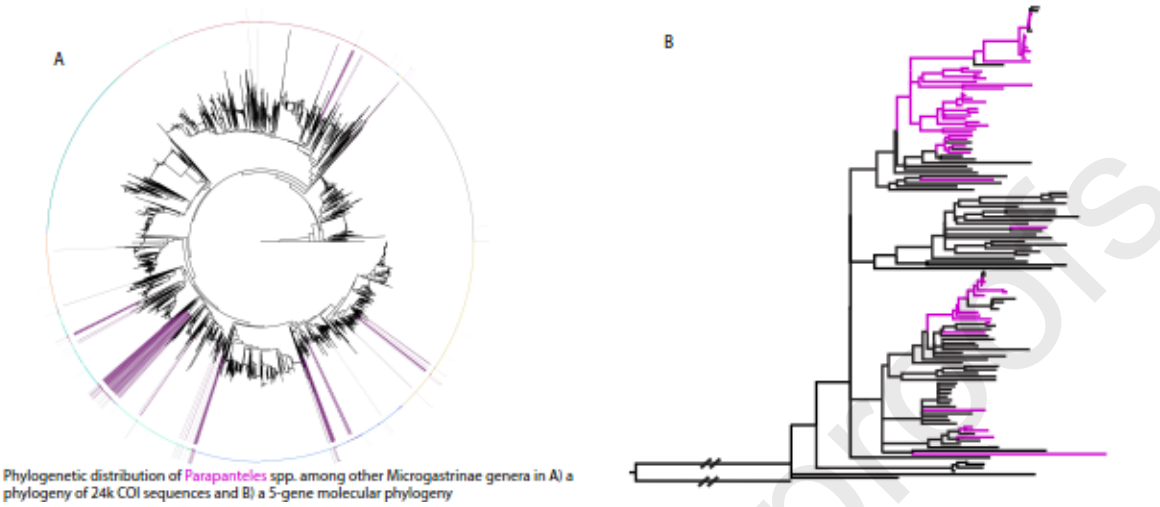
Supplemental Materials 16: Mr. Bayes Bayesian analysis COI (a), WG (b), ND1 (c), 28s (d), EF1a (e), & Concatenated (f) phylogenies. Posterior probabilities are reported on each branch. Branches are colored by genus, with purple corresponding to Parapanteles, green to Dolichogenidea, red to Apanteles, blue to Glyptapanteles, and yellow to Cotesia. Branches of all other genera are black. Branches of Parapanteles specimens are shaded by host family.

Supplemental Materials 17: Table of the most common sources of COI sequences used in Supplemental Materials 2-4 by country of origin and institution.

Supplemental Materials 18: Table of current species assignment and suggested genus of informal *Parapanteles* species.



Graphical abstract



Phylogenetic distribution of Parapanteles spp. among other Microgastrinae genera in A) a phylogeny of 24k COI sequences and B) a 5-gene molecular phylogeny

Highlights:

Parapanteles is polyphyletic.

Most *Parapanteles* species were recovered as a monophyletic clade within *Dolichogenidea.*

Most other *Parapanteles* species were recovered in *Glyptapanteles* or *Cotesia*.

Kyle S Parks: Conceptualization, methodology, formal analysis, investigation, data curation, writing – original draft, writing – review & editing, visualization, project administration

Daniel H Janzen: Resources, data curation, writing – review & editing, funding acquisition

Winnie Hallwachs: Resources, data curation, writing – review & editing, funding acquisition

José Fernández-Triana: Data curation, writing – review & editing

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Diana C Arias-Penna: Resources, data curation, writing - review & editing

James B Whitfield: Conceptualization, methodology, resources, writing – review & editing, supervision, funding acquisition