PHYLOGENETICS OF *PARAPANTELES* (BRACONIDAE: MICROGASTRINAE) WASPS, AN UNDERUSED TOOL FOR THEIR IDENTIFICATION, AND AN EXPLORATION OF THE EVOLUTION OF THEIR SYMBIOTIC VIRUSES

BY

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DISSERTATION

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ABSTRACT

Microgastrinae is the most diverse subfamily of Braconidae, one of the largest families of parasitoid wasps. Microgastrines parasitize nearly all families of Lepidoptera, but the majority of species are only known to attack one or two Lepidoptera species. Microgastrinae is diverse and much of this diversity arose during a still poorly-understood ancient rapid radiation, causing many short branches deep in the microgastrine phylogeny that are difficult to reconstruct. Due to these difficulties, many microgastrine genera, especially the more specious genera, may not be monophyletic and their placements within the microgastrine phylogeny are ambiguous.

In Chapter 2, I constructed a 5-gene molecular phylogeny to assess the monophyly of the genus *Parapanteles* Ashmead (Braconidae: Microgastrinae), a medium-sized genus of microgastrine wasps that was first defined over a century ago, lacks a unique synapomorphic character, and its monophyly has not been adequately tested. *Parapanteles* larvae parasitize large, unconcealed caterpillars (macrolepidoptera) and have been reared from an unusually large diversity of hosts for a relatively small parasitoid genus. I used the extensive existing Cytochrome Oxidase I sequences plus four additional genes (*wingless, elongation factor 1-alpha, ribosomal subunit 28s,* and *NADH dehydrogenase subunit 1*) to construct individual gene trees and concatenated Bayesian and maximum-likelihood phylogenies of *Parapanteles* species and several species from other microgastrine genera. In these phylogenies, a plurality of *Parapanteles* species were recovered as a monophyletic group within another genus, *Dolichogenidea*, while the remaining *Parapanteles* species were highly polyphyletic.

In Chapter 3, I describe and assess the usefulness of the wing interference patterns of a monophyletic clade of *Parapanteles* wasps discovered in Chapter 2 for aiding in species identification. Wing interference patterns (WIPs) are color patterns of insect wings caused by thin film interference. We were able to detect consistent WIP differences between *Parapanteles* species. In some cases, WIPs can be used to diagnose sibling species that would otherwise require SEM images. The species-specific patterns of WIPs are diagnostically valuable but of uncertain evolutionary significance.

In Chapter 4 I used an anchored phylogenomics approach to address intergeneric relationships in Microgastrinae more broadly. Previous molecular phylogenies of this taxon have consistently recovered many short and poorly supported basal internal nodes, supporting the hypothesis that Microgastrinae coevolved with their hosts in an ancient rapid speciation event. The systematics of the 64 currently recognized extant genera are still poorly resolved and the monophyly of many of these genera is questionable. To address these challenges, I selected 89 species, broadly from within and across several microgastrine genera, and Drs. Emily and Alan Lemmon at Florida State University performed anchored hybrid enrichment to generate 370 gene fragment sequences for each. Drs. Emily and Alan Lemmon made a concatenated maximum-likelihood analysis of this dataset with RAxML which resolved nearly all nodes with high bootstrap support. This phylogeny supports several larger genera (*Apanteles, Cotesia, Dolihcogenidea,* and *Glyptapanteles*) as mostly monophyletic, although taxa from smaller, rarer genera are recovered within each. It also corroborates previous results that *Parapanteles* is a polyphyletic genus composed of several subclades of disparate genera, although most are within *Dolichogenidea*.

Microgastrinae wasps have symbiotic viruses, known as polydnaviruses, encoded within their nuclear genomes that females produce and inject, along with eggs, into their host caterpillars. In Chapter 5 I sequenced the genomes of 16 microgastrine species from a monophyletic clade of *Parapanteles* Ashmead with extensive host-use records, and annotated polydnavirus genes in each genome. I found that probable duplications, pseudogenes, and rearrangements are common, especially in the protein-tyrosine-phosphatase polydnavirus gene family. These results support the model that frequent gene births and deaths are a major factor in polydnavirus genome evolution, and extend our knowledge of polydnaviruses to a major previously unexplored segment of the microgastrine phylogeny.

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CHAPTER 1: INTRODUCTION TO MICROGASTRINAE

DIVERSITY AND HOST USE

Microgastrinae is the most diverse subfamily of Braconidae, one of the largest families of parasitoid wasps (Hymenoptera) (Whitfield 1995). Over two thousand microgastrine species have been described to date, and another 20-40k undescribed species are predicted to exist (Rodriguez *et al.* 2013). Adult microgastrines are minute, generally 2-4mm in length. Despite their species diversity, their natural history is remarkably consistent: all known microgastrine species are koinobiont (parasitoids that do not completely halt the development of their host) endoparasitoids (parasitoids that develop inside the body of their host) of larval Lepidoptera (Shaw & Huddleston 1991). However, the number of larvae per host is variable. Solitary species inject a single egg into a host, while gregarious species inject two or more eggs, which hatch, develop, and emerge together. The number of eggs injected per host varies among gregarious species, from two to several hundred, and correlates to host size (Le Masurier 1987). Both host use strategies have evolved repeatedly throughout Microgastrinae (Shaw & Huddleston 1991, Janzen *et al.* 2009).

Historically, most microgastrines have been collected as adults, via net, yellow pan, or Malaise trap. While these collecting methods are efficient, wasps collected these ways have virtually no natural history data besides collection locale and date. The only reliable way to identify which host species a microgastrine parasitized as a larva is to rear it from a parasitized caterpillar (Whitfield & Wagner 1988, Smith *et al.* 2008, Rodriguez *et al.* 2013). Several long-term rearing projects, most notably Area de Conservación Guanacaste (ACG) in Costa Rica, and Yanayacu in Ecuador have been rearing a large diversity of caterpillars from Neotropical forests for over a decade (Janzen *et al.* 2009, Dyer *et al.* 2015). These projects have contributed a huge amount of natural history data by associating caterpillars with host plants, adult moths or butterflies, and parasitoids. The parasitoid data has yielded important results for microgastrine wasps especially. While some polyphagous species exist, microgastrines from ACG are highly host-specific, typically attacking 1-3 host species (Smith *et al.* 2008).

Microgastrines parasitize nearly all families of Lepidoptera (Whitfield 1995), but the majority of species are only known to attack one or two Lepidoptera species (Smith *et al.* 2008). Therefore, despite high host specificity within species, switching between hosts of different families has happened frequently in the evolution of this group. Several recent genus-level microgastrine phylogenies corroborate this and show a trend in host-use evolution (Rodriguez 2009, O'Connor 2011, Arias-Penna 2015). Each of these phylogenies can be divided into subclades that largely attack caterpillars of the same host family, although each subclade contains one or more species that have switched to a new host family. Furthermore, the most common host family attacked differs between these subclades. This supports a model in which many microgastrine species radiate on closely-related host species, possibly via co-evolution as host species diversify, but family-level host switching has been common throughout the evolution of microgastrines, both early in microgastrine evolution, when subclades and deeper nodes diversified, and more recently, when single species have switched to new host families.

SYSTEMATIC & TAXONOMIC PROBLEMS

The phylogenetics and classification of Microgastrinae is difficult for several reasons. Microgastrinae is diverse, and much of this diversity arose during a still poorly-understood ancient rapid radiation (Mardulyn & Whitfield 1999). This diversification event underlies many short branches deep in the microgastrine phylogeny that are difficult to reconstruct (Mardulyn & Whitfield 1999, Whitfield *et al.* 2002, Banks & Whitfield 2006). Morphological systematics and classification have also been challenging. Microgastrinae wasps are small (generally 2-4mm in length) and many of their most accessible morphological characters are prone to convergence (Mason 1981, Whitfield *et al.* 2002, Wild *et al.* 2013). For example, female microgastrines use their ovipositors to inject their eggs into host caterpillars. Ovipositors are therefore under selection and prone to convergence. Microgastrine species that attack larger, unconcealed hosts tend to have short ovipositors, while species that attack concealed hosts (e.g., leaf rollers, leaf miners) tend to have longer ovipositors (Mason 1981). Despite this, structures of or related to the ovipositor have frequently been used in the classification and identification of microgastrine genera. Ridges on the propodium are also heavily relied on,

especially the presence or absence of a ring-shaped areola and especially for generic divisions. However, this structure, especially the proximal half, is heavily sculptured and difficult to interpret in many species, while in other species it is not clear if the areola is absent or just greatly reduced, contributing to many uncertain generic identifications (Mason 1981). Due to these morphological and molecular difficulties, many microgastrine genera, especially more specious genera, may not be monophyletic and their placements within the microgastrine phylogeny are ambiguous. I used molecular phylogenetics techniques to address the systematic of microgastrines here: in Chapter 2, I constructed a 5-gene molecular phylogeny to assess the monophyly of a particularly contentious genus, *Parapanteles,* and in Chapter 4 I used an anchored phylogenomics approach to address intergeneric relationships in Microgastrinae more broadly. Wing interference patterns were first identified as stable and often species-specific structures of small insect wings in 2011 (Shevtsova *et al.* 2011), but have not been widely explored by taxonomists since then and have never been explored in Microgastrinae. In Chapter 3, I describe and assess the usefulness of the wing interference patterns of a monophyletic clade of *Parapanteles* wasps discovered in Chapter 2 for aiding in species identification.

POLYDNAVIRUS

Polydnaviruses are mutualistic viruses used by parasitoid wasps to manipulate their hosts (Strand & Burke 2014). Polydnaviridae encompasses two convergent but unrelated symbiotic viruses, the Bracoviruses in braconid wasps and the Ichnoviruses in ichneumonid wasps, both within the wasp superfamily Ichneumoidea (Stoltz *et al.* 1984, Kroemer & Webb 2004). Both polydnaviruses are integrated into their host wasps' nuclear genome rather than existing as free living viruses, they do not reproduce in their wasp's host caterpillar and are dependent on vertical transmission via wasp reproduction for their own reproduction (Theilmann & Summers 1986, Stoltz 1990, Fleming & Summers 1991). Polydnaviruses are produced by female wasps in specialized calyx cells at the base of the oviduct, and are injected with the wasps' eggs into the parasitized host (Stoltz *et al.* 1976). Polydnaviruses infect host tissues, especially hematocytes, where their genes are expressed and cause pathology for the

caterpillar (Strand & Burke 2014). Bracoviruses are found in a single monophyletic group, the Microgastroid complex, containing Microgastrinae and five smaller subfamilies, while Ichnoviruses are only found in Banchinae and Campopleginae (Hymenoptera: Ichneumonidae) (Strand & Burke 2014). Bracoviruses are descended from a free-living nudivirus, an entomopathic virus related to baculoviruses (Bézier *et al.* 2009), that was incorporated into the nuclear genome of an ancestral microgastroid wasp approximately 100 million years ago (Murphy *et al.* 2008). The origin of ichnoviruses is poorly understood, but they arose independently from bracoviruses through a separate but similar endosymbiotic event in which an unknown virus was similarly incorporated into an ichneumonid wasp's nuclear genome (Béliveau *et al.* 2015, Herniou *et al.* 2015, Pichon *et al.* 2015).

The role of polydnaviruses in microgastrine host-specificity or host switching has not previously been studied on a phylogenetic scale. The diversity in the mode of action of bracoviruses is hypothesized to be due to a co-evolutionary arms race between parasitoids and their hosts, in which bracovirus virulence genes rapidly evolve in response to adaptations in host caterpillar immune systems (Huguet *et al.* 2012, Strand & Burke 2014). Supporting this hypothesis, virulence gene families are prone to duplication and evolve rapidly (Desjardins *et al.* 2008, Serbielle *et al.* 2008, Chen *et al.* 2011, Serbielle *et al.* 2012, Burke *et al.* 2014). In Chapter 5, I explore the evolution of polydnaviruses in a monophyletic clade of *Parapapteles* with extensive host records, identified in Chapter 2, by sequencing the genomes of 16 *Parapanteles* species and identifying and comparing, qualitatively and phylogenetically, homologous regions containing polydnavirus genes.

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CHAPTER 2: A FIVE-GENE MOLECULAR PHYLOGENY DOES NOT SUPPORT THE MONOPHYLY OF *PARAPANTELES* ASHMEAD (HYMENOPTERA: BRACONIDAE) AS CURRENTLY DEFINED

ABSTRACT

Parapanteles Ashmead (Braconidae: Microgastrinae) is a medium-sized genus of microgastrine wasps that was first defined over a century ago, lacks a unique synapomorphic character, and its monophyly has not been adequately tested. *Parapanteles* larvae parasitize large, unconcealed caterpillars (macrolepidoptera) and have been reared from an unusually large diversity of hosts for a relatively small parasitoid genus. I used the extensive existing Cytochrome Oxidase I sequences available for *Parapanteles* and other microgastrines to sample the diversity of described and undescribed species currently considered to belong to *Parapanteles,* and sequenced four additional genes for this subsample (*wingless, elongation factor 1-alpha, ribosomal subunit 28s,* and *NADH dehydrogenase subunit 1*). I constructed individual gene and concatenated Bayesian and maximum-likelihood phylogenies for this 5-gene subsample. In these phylogenies, a plurality of *Parapanteles* species were recovered as a monophyletic group within another genus, *Dolichogenidea*, while the remaining *Parapanteles* species were highly polyphyletic, likely representing misplaced members of other morphologically similar genera. When these misplaced members are removed from *Parapanteles*, the diversity of hosts known to be attacked by the genus reduces to arctiine erebids, geometrids, saturniids, and notodontids.

INTRODUCTION

Parapanteles Ashmead is a genus of parasitoid wasps that exemplifies many of the taxonomic and systematic challenges of the subfamily Microgastrinae, the most diverse subfamily of Braconidae (Hymenoptera) and one of the largest groups of parasitoid wasps in general (Whitfield 1995; Smith et al. 2008; Rodriguez et al. 2013). As with many other genera, the diagnostic characters of *Parapanteles* are often difficult to interpret and the majority of species belonging to this genus are undescribed. Unlike most microgastrine genera, *Parapanteles* species attack an unusually high diversity of hosts for a genus of its size. In

Microgastrinae, where 80-95% of species are undescribed (Rodriguez *et al.* 2013, Smith *et al.* 2013), ambiguous genera such as *Parapanteles* are major practical problems from the task of sorting specimens to genus to solving long-standing generic-level phylogenetic relationships.

Parapanteles is primarily defined by a combination of two variable and continuous characters: a short ovipositor and a propodeal areola (Mason 1981, Valerio *et al.* 2009). Characters of or related to the ovipositor are some of the most heavily used for microgastrine generic divisions and morphological phylogenies (Mason 1981, Whitfield *et al.* 2002). Ovipositor morphology has immediate fitness consequences, and, as a result, ovipositor characters are particularly prone to convergence (Wild *et al.* 2013): species that attack larger, unconcealed hosts tend to have relatively short ovipositors, while species that attack concealed hosts (e.g., leaf rollers, leaf miners) tend to have relatively longer ovipositors (Mason 1981). Ridges on the propodeum are highly variable across genera and are also heavily used in microgastrine classification. *Parapanteles* and several other genera have a ring-shaped or pentagonal pattern of ridges, forming an areola on their propodea; these ridges are sometimes very faint, obscured by setae, or obscured by additional propodeal ridges. Sorting undescribed neotropical specimens to genus has been particularly challenging: many specimens with short-to-intermediate ovipositors can be difficult to place between *Parapanteles* and *Dolichogenidea* (propodeal areola but generally longer ovipositor), while many with unambiguously short ovipositors have weak-to-faint propodeal areolas and could be placed in both *Parapanteles* and *Glyptapanteles* (short ovipositor, smooth propodeum). Character state decisions for these specimens are essentially subjective. In addition to the problem of ambiguous *Parapanteles* specimens, some described species (e.g. *Parapanteles scotti, Parapanteles mariae*) resemble *Cotesia* (Valerio *et al.* 2009) in overall appearance. The variation found within *Parapanteles* is unusual in that it causes some specimens to be confused with species in genera that are distantly related to each other.

To date, 2689 microgastrine species have been described, and 20,000-40,000 more species are predicted to exist (Rodriguez et al. 2013, Fernández-Triana & Ward 2015). In this context, *Parapanteles* is a relatively small genus, with 27 described and at least 55 putative undescribed species. Fifteen of the described and nearly all known undescribed *Parapanteles*

species are neotropical, most of which were discovered by two long-term caterpillar rearing projects: Area de Conservación Guanacaste (ACG) in Costa Rica and Yanayacu Biological Station in Ecuador (Janzen & Hallwachs 2009, Valerio *et al.* 2009, Dyer *et al.* 2017). The remaining described species are from Africa (n=1), Australia (n=1), India (n=6), and North America (n=2) (Valerio *et al.* 2009, Janzen & Hallwachs 2009, Rousse & Gupta 2013, Gupta *et al.* 2014a, Gupta *et al.* 2014b, Dyer *et al.* 2017).

All known microgastrine species are endoparasitoids of larval Lepidoptera (Shaw & Huddleston 1991), and all known *Parapanteles* species attack unconcealed macrolepidoptera larvae (Valerio 2005, Valerio *et al.* 2009). Host specificity is high within most *Parapanteles* species, but host use is highly diverse between species (Valerio 2005). The 27 described species attack caterpillars from at least 12 families of Lepidoptera, and putative *Parapanteles* species have been reared from an additional 6 families of host caterpillar (Table 2.1), which is unusually high for a relatively small genus (Valerio *et al.* 2009, Janzen & Hallwachs 2009, Rousse & Gupta 2013, Gupta *et al.* 2014a, Gupta *et al.* 2014b, Dyer *et al.* 2017). In comparison, large microgastrine genera, such as *Glyptapanteles* and *Apanteles,* which both contain an order of magnitude more species than *Parapanteles,* attacked roughly the same number of host families (Janzen & Hallwachs 2009 (summarized in Table 2.2), Dyer et al. 2017).

Microgastrine species diversity appears to have greatly increased during an ancient rapid radiation, and resolving generic relationships within this group is difficult (Banks & Whitfield 2006). Previous generic-level molecular phylogenies of this subfamily 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). This is a particular problem for *Parapanteles,* which has been recovered in several different places in previous molecular and morphological phylogenies, generally with poor support. *Parapanteles* has been recovered sister to or within *Hypomicrogaster*, 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: either an unidentified *Parapanteles* species, or *Parapanteles paradoxus*. Most *Parapanteles* species have not been included in a published phylogeny.

Parapanteles' sometimes ambiguous synapomorphies and the unusually high diversity of host caterpillars it attacks suggests that *Parapanteles* may be a "catch-all" genus for ambiguous species and not a monophyletic group. It has not been well represented in previous molecular phylogenies, in which it has been recovered in conflicting and often poorly-resolved areas. Here, I constructed a 5-gene molecular phylogeny of a representative subsample of described and undescribed *Parapanteles* species, putatively related genera, and several microgastrine outgroups to test the monophyly of this genus and to recover how it relates to other major microgastrine genera.

METHODS

Taxon sampling

The long-term caterpillar rearing project at Area de Conservation Guanacaste (ACG), Costa Rica (Janzen & Hallwachs 2009) has discovered the majority of known undescribed *Parapanteles* specimens, which are morphologically identified to genus and DNA barcoded (Janzen & Hallwachs 2009). To approximate the diversity of *Parapanteles* under its current morphological definition, I accessed all available *COI* sequences for *Parapanteles* and several other microgastrine genera (*Apanteles, Cotesia, Dolichogenidea, Rhygoplitis, Glyptapanteles, Pholetesor, Hypomicrogaster, Diolcogaster,* and *Microplitis*) from the Barcode of Life Database (BOLD) (Ratnasingham & Hebert 2007) and GenBank (http://www.ncbi.nlm.nih.gov/genbank/). The majority of these sequences are from ACG. I sequenced *COI* for 110 additional *Parapanteles* specimens reared at Yanayacu Biological Station in Ecuador (Dyer *et al.* 2017) and included them in our dataset. I aligned this dataset with PASTA v1.6.3 (Mirarab *et al.* 2014) and edited it in Geneious v9.1.5 (http://www.geneious.com, Kearse *et al.* 2012). I discarded sequences from this alignment that were missing approximately 200bp or more of the 658bp *COI* barcoding region, leaving 14,247 aligned sequences (Supplemental Materials 2.2). I made a phylogenetic tree from this alignment with FastTree 2.1.8 (Price & Arkin 2009) using the GTR+I+G substitution model (Supplemental Materials 2.2). I selected 56 *Parapanteles* specimens for our subsample from disparate clades in this tree to sequence additional genes for (Supplemental

Materials 2.3). *Dolichogenidea* specimens were particularly intermixed with *Parapanteles* specimens in this tree, so I also included 13 *Dolichogenidea* samples in our subset.

I included all available non-*Parapanteles* microgastrine specimens that have sequences available on Genbank for at least 3 of the 5 genes used in this study. This outgroup set includes 10 taxa from 8 other microgastrine genera, and one representative from Cheloninae, the sister group to other microgastroids (Whitfield & Mason 1994, Belshaw *et al.* 1998, Dowton & Austin 1998, Whitfield *et al.* 2002, Banks & Whitfield 2006, Murphy et al. 2008, Kittel *et al.* 2016), which was used to root trees.

Specimen naming conventions

Specimens collected by the ACG rearing project in Costa Rica are either assigned to described species or assigned provisional informal species names based on COI sequence similarity and host associations (Janzen *et al.* 2009). Informal species names follow the convention of the last name of an ACG collaborator and a number (e.g. *Apanteles* Rodriguez01, *Parapanteles* Whitfield113). Specimens of undescribed species from ACG used in this study are identified by these provisional species names.

Specimens from the Yanayacu Rearing Project in Ecuador are not automatically barcoded or grouped into provisional species as they are at ACG, so specimens of undescribed species from Yanayacu used in this study are identified by "yy" and their individual sample number (e.g. *Parapanteles* yy3653).

Gene selection

I sequenced portions of two mitochondrial genes (655 bp of *cytochrome oxidase I* (COI) (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)) (primers listed in Table 2.3) to construct a molecular phylogeny. The DNA barcoding region of *Cytochrome oxidase I* is reliable for species delimitation of microgastrines (Whitfield *et al.* 2002, Banks & Whitfield 2006, Smith *et al.* 2008) and sequence data were available on the Barcode of Life Database (BOLD) (Ratnasingham & Hebert 2007) for

almost all species of *Parapanteles* and most species of *Dolichogenidea, Rhygoplitis, Hypomicrogaster, Promicrogaster* and *Pholetesor* that have been reared in the Neotropics (Janzen & Hallwachs 2009). *Wingless* has been useful in generic-level phylogenies of microgastrines (Banks & Whitfield 2006; Murphy et al 2008), and sequences were available for all *Eois*-attacking *Parapanteles* (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 all *Eois*-attacking *Parapanteles* (Wilson et al. 2012). Several microgastrine phylogenies have used ND1 and 28s (Dowton & Austin 1998, Michel-Salzat & Whitfield 2004, Kankare & Shaw 2004, Rodriguez 2009, O'Connor 2011), and I was able to incorporate existing outgroup sequences by including these genes in our dataset. Preliminary results placed many *Parapanteles* among several other genera, so I included additional specimens from *Glyptapanteles* and *Apanteles.* With permission from the authors (Rodriguez 2009, Arias-Penna 2015), I used sequences from two unpublished molecular phylogenies of these genera. I used *COI* and *WG* sequences from 28 *Glyptapanteles* species, to which I added sequences of *EF1a, 28s,* and *ND1.* I sequenced all 5 genes in our dataset for 17 additional *Glyptapanteles* specimens from Yanayacu, Ecuador (Arias-Penna 2015). I used sequences of all five genes for 19 *Apanteles* species (Rodriguez 2009).

Sequencing

Genomic DNA was extracted from adult microgastrines using Qiagen DNEasy Blood and Tissue kits following the manufacturer's directions. For gregarious species (multiple larvae developing in the same host), I extracted DNA from whole specimens. For solitary species, I extracted DNA from one hind leg, removed above the coxa, or one mid- and/or foreleg if one or more hind legs were missing. I used New England Biolabs *Taq* DNA Polymerase with Standard *Taq* buffer and the primers and thermocycler protocols listed in Table 2.3. I 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. I edited sequences with Geneious v9.1.5 (http://www.geneious.com, Kearse *et al.* 2012).

Alignment and Phylogenetic Analysis

I excluded from the concatenated analyses any taxa for which I was unable to sequence at least 3 genes, but still included all available sequences in individual gene trees. I therefore included 142 taxa in our concatenated alignment, with the following numbers of taxa missing for each gene followed by the number of taxa 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). I aligned sequences with MUSCLE v3.8.31 (Edgar 2004). Our concatenated alignment had 2626 characters total, with 169 invariable across all taxa. I 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 I partitioned *COI* and *ND1* alignments into three partitions by 1st, 2nd, and 3rd codon positions, *WG* and *EF1a* into two partitions by 1st+2nd and 3rd 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 2.6). I constructed Maximum Likelihood (ML) trees in RAxML v8.1.15 (Stamatakis 2014) with 1000 bootstrap replicates for each gene independently and all genes concatenated. For each analysis I selected either GTR+G or GTR+I+G depending on which model was favored by the majority of partitions. I constructed an additional tree for each analysis with MrBayes v.3.2.2 (Ronquist *et al.* 2012) using mixed models. I 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 were rooted with the closer outgroup *Phanerotoma* as the most distant outgroup, except *ND1* trees, which were rooted with *Microplitis demolitor*. I 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.

Provisional species from Ecuador

I grouped specimens from the Yanayacu Rearing Project in Ecuador into provisional species based on COI sequence similarity, natural history, and then morphological similarity. I calculated the pair-wise distances of COI sequences with MEGA v7.0.26 (Kumar *et al.* 2016).

RESULTS

Individual gene trees differ significantly from each other, and none reflects all of the relationships I recovered in concatenated analyses. Clades that 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 2.3). 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.

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

I identified 10 provisional species from the Yanayacu Rearing Project in Ecuador based on COI sequence similarity, natural history, and then morphological similarity (Table 2.4).

DISCUSSION

I found *Parapanteles* to be clearly polyphyletic (Fig. 2.1). The diversity of hosts parasitized by *Parapanteles* species is inflated due to the polyphyly of this genus, and most of its host diversity is accounted for by misdiagnosed species, especially those that belong to

Cotesia or *Glyptapanteles* (Fig. 2.1). I recovered one strongly-supported clade containing the majority of named and unnamed *Parapanteles* species included in our dataset (Fig. 2.1, clade A). Species in this clade parasitize arctiine erebids, geometrids, saturniids, and notodontids (Fig. 2.2), all relatively large and unconcealed hosts. This *Parapanteles* clade rendered *Dolichogenidea* paraphyletic, although the branch defining it has low support. *Dolichogenidea* is a larger genus than *Parapanteles* that typically parasitizes leaf miners, leaf tiers and other concealed microlepidotera, and COI barcode data suggest it may also be polyphyletic (Mason 1981, Smith *et al.* 2013). Therefore, *Parapanteles s. s.* may be a clade of *Dolichogenidea* that shifted to a macrolepidopteran host, but appropriate taxonomic revision of *Parapanteles* will require a revision of *Dolichogenidea* that should include much broader phylogenetic sampling of *Dolichogenidea* species, and which includes the type species of both genera, which were not available for this study in a form suitable for molecular sampling.

Several previous microgastrine phylogenies placed *Parapanteles* as close or sister to *Hypomicrogaster*, which I did not find in our results. These studies included representatives of many microgastrine genera, but few representatives of 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,* and with poor support. Our concatenated analyses (Fig. 2.1) and our broad COI survey of microgastrine genera (Supplemental Materials 2.3) 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 likely would not have recovered *Parapanteles* as closely related to *Hypomicrogaster*.

The polyphyly of *Parapanteles* reflects the difficulty of assigning microgastrines to genus via morphology only, especially the hugely diverse neotropical taxa. As genera are currently defined, 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). 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. Our results suggest that interpretation of this character, especially when it is weakly expressed, is somewhat 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. 2.1) 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 and 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 I recovered within *Glyptapanteles* and vice versa (Fig. 2.1 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. Whenever possible, generic placement should be corroborated with COI data.

Eight new *Parapanteles* species have been recently described from India (Rousse & Gupta 2013, Gupta *et al.* 2014a, Gupta *et al.* 2014b). The majority of these species were reared from butterflies: four from Lycaenidae species, one from a Riodinidae species, and one from a Nymphalidae species. Of the butterfly-attacking *Parapanteles* species I included in our analysis, most grouped within *Cotesia,* followed by *Glyptapanteles,* then one riodinid-attacking species within *Apanteles* (Fig. 2.2). Therefore, I expect that these Indian species are misdiagnosed, most likely *Cotesia* species.

Investigations into the coevolution and ecology of two hyperdiverse neotropical taxa, *Piper* (Piperales: Piperaceae) and its specialist herbivore *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). I included many of the same samples that appear in a phylogeny of *Eois*-attacking *Parapanteles* in Wilson *et al.* 2012 in our analysis. Our results suggest that these *Eois*-attacking *Parapanteles* are in fact two sister species

within the main *Parapanteles* clade I recovered (Table 2.4, provisional spp. J & K), along with three or more *Glyptapanteles* species. The COI barcoding region of provisional species J & K are virtually identical within species and about 2.3% different from each other (Table 2.4). Both species have rearing records from *Eois olivacea* Felder & Rogenhofer, while one has additionally been reared from *Eois pallidicosta* (Dyer *et al.* 2017). These two species are the most morphologically similar to *Glyptapanteles* of any of the *Parapanteles* species I recovered in clade A (Fig. 2.1), and the only species with longer narrower 1st metasomal tergites that narrow distally.

In summary, this studies strongly corroborate the notion that *Parapanteles*, as currently defined, is polyphyletic, consisting of a core clade embedded within *Dolichogenidea* as currently defined, and several groups of *Cotesia*, *Glyptapanteles* and *Apanteles* 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 morphologically using a more carefully defined set of features. The genus will thus assume a more clearly cohesive definition both morphologically and in terms of host relationships. In the meantime, reassignment of the obviously misdiagnosed members of other genera is clearly called for, as discussed above.

TABLES & FIGURES

Table 2.1: Rearing/collection records and host associations of described and provisional *Parapanteles* species as currently morphologically defined.

Described Species	Host Families	Host Subfamilies	Host Spp.	Range	Description Reference	Host-use references
Parapantles aletiae	Noctuidae	Scoliopteryginae	*Alabama argillacea*	SE USA	Riley 1869	Riley 1869, Valerio *et al.* 2009
	Notodontidae	nr	nr			
Parapanteles complexus	Gelechiidae	Dichomeridinae	"gelJanzen01 Janzen181"	Costa Rica	Valerio *et al.* 2009	Valerio *et al.* 2009, Janzen *& Hallwachs* 2009
Parapanteles continua	Saturniidae	Hemileucinae	*Hylesia continua*	Costa Rica	Valerio *et al.* 2009	Valerio *et al.* 2009, Janzen & Hallwachs 2009
Parapanteles em	Notodontidae	Dioptinae	*Hemiceras conspirata*	Costa Rica	Valerio *et al.* 2009	Valerio *et al.* 2009, Janzen & Hallwachs 2009
			Hemiceras nigrescens			
			Hemiceras sabis			
		Phalerinae	*Rhogalia epigena*			
			Rosema deolis			
Parapantles lincolnii	Gelechiidae	Gelechiinae	*Chionodes fuscomaculella*	Missouri, USA	Valerio *et al.* 2009	Valerio *et al.* 2009
Parapanteles mariae	Lasiocampidae	Macromphaliinae	*Euglyphis* mariaDHJ03	Costa Rica	Valerio *et al.* 2009	Valerio *et al.* 2009, Janzen & Hallwachs 2009
			Euglyphis maria			
Parapantles masoni	nr	nr	nr	Australia	Austin & Dangerfield 1992	NA
Parapantles nephos	Erebidae	Arctiinae	*Carales astur*	Peru	Valerio *et al.* 2009	Valerio *et al.* 2009
Parapantles noae	nr	nr	nr	Costa Rica	Valerio *et al.* 2009	NA
Parapantles paradoxus	Elachistidae	nr	nr	Costa Rica, El Salvador	Muesebeck 1958	Muesebeck 1958, Valerio *et al.* 2009, Janzen & Hallwachs 2009
	Notodontidae	Dioptinae	*Dioptis longipennis*			
			Tithraustes lambertae			
			Tithraustes noctiluces			
			Tithraustes seminigrata			
			Tithraustes Miller02			
		Hemiceratinae	*Hemiceras conspirata*			
			Hemiceras sp.			
		Nystaleinae	*Dunama mexicana*			
			Dunama janecoxae			
		Phalerinae	*Rosema* sp.			
Parapanteles polus	Erebidae	Arctiinae	*Napata lelex*	Costa Rica	Valerio *et al.* 2009	Valerio *et al.* 2009
			Cyanopepla arrogans			
Parapantles rarus	nr	nr	nr	Costa Rica	Valerio *et al.* 2009	NA
Parapantles rooibos	Geometridae	Ennominae	*Isturgia exerraria*	Africa	Valerio *et al.* 2005	Valerio *et al.* 2005

Table 2.1 continued

Parapanteles scotti	Erebidae Noctuidae	nr Condicinae Noctuinae Plusiinae	"noctuid 92-SRNP-3601" *Condica* sutorDHJ01 *Spodoptera androgea* *Spodoptera latifascia* *Ctenopulsia oxygramma*	Costa Rica	Valerio *et al.* 2009	Valerio *et al.* 2009, Janzen & Hallwachs 2009
Parapanteles sicpolus	Saturniidae	Hemileucinae	*Gamelia* mustaDHJ01 *Lonomia electra*	Costa Rica	Valerio *et al.* 2009	Valerio *et al.* 2009, Janzen & Hallwachs 2009
Parapanteles tessares	Saturniidae	Hemileucinae	*Hylesia continua*	Costa Rica	Valerio *et al.* 2009	Valerio *et al.* 2009
Parapantles thrix	Nolidae	Nolinae	*Meganola minuscula*	Missouri, USA	Valerio *et al.* 2009	Valerio *et al.* 2009
Parapantles tlinea	Erebidae Riodinidae	Arctiinae nr	"artine 03-SRNP-22291" *Clemensia* BioLep03 nr	Costa Rica	Valerio *et al.* 2009	Valerio *et al.* 2009, Janzen & Hallwachs 2009
Parapanteles eros	Lycaenidae	Polyommatinae	*Chilades pandava*	India	Gupta *et al.* 2014	Gupta *et al.* 2014
Parapanteles arka	Lycaenidae	Curetinae	*Curetis thetis*	India	Gupta *et al.* 2014	Gupta *et al.* 2014
Parapanteles esha	Lycaenidae	Polyommatinae	Prosotas dubiosa	India	Gupta *et al.* 2014	Gupta *et al.* 2014
Parapanteles regale	Lycaenidae	Theclinae	*Tajuria cippus*	India	Gupta *et al.* 2014	Gupta *et al.* 2014
Parapanteles echeriae	Riodinidae	Nemeobiinae	*Abisara echeria*	India	Gupta *et al.* 2013a	Gupta *et al.* 2013a
Parapanteles shivranginii	nr	nr	nr	India	Sathe & Ingawale 1989	Sathe & Ingawale 1989
Parapanteles sireeshaae	Geometridae	Ennominae	*Hyposidra successaria*	India	Ahmad & Akhtar 2010	Ahmad & Akhtar 2010
Parapanteles athamasae	Nymphalidae	Charaxinae	*Charaxes athamas*	India	Gupta *et al.* 2013b	Gupta *et al.* 2013b
Described species n=27	**Host families n=12**					

Undescribed Species	**Host Families**		**Host Subfamilies**	**Host Spp.**	**Host-use references**
Parapanteles Fernandez02	Depressariidae	nr	"elachJanzen01 Janzen252"	Janzen & Hallwachs 2009	
Parapanteles Janzen01	Erebidae Noctuidae	Hypocalinae Agaristinae	*Ipnista marina* *Erocha leucotelus*	Janzen & Hallwachs 2009	
Parapanteles Janzen50	Geometridae	Sterrhinae	*Leptostales* Janzen01	Janzen & Hallwachs 2009	
Parapanteles Janzen51	Geometridae	Ennominae	*Macaria pernicata*	Janzen & Hallwachs 2009	
Parapanteles Janzen53	Gelechiidae	Dichomeridinae	*Dichomeris* Janzen822	Janzen & Hallwachs 2009	
Parapanteles Rodriguez02	Riodinidae	Riodininae	*Emesis mandana*	Janzen & Hallwachs 2009	
Parapanteles Rodriguez209	nr	nr	nr	Janzen & Hallwachs 2009	
Parapanteles Valerio05	Notodontidae	Hemiceratinae	*Hemiceras nigrescens*	Janzen & Hallwachs 2009	

Table 2.1 continued

Parapanteles Valerio06	Geometridae	Larentiinae	*Eupithecia* Janzen12	Janzen & Hallwachs 2009
Parapanteles Valerio07	Notodontidae	Hemiceratinae	*Hemiceras clarki*	Janzen & Hallwachs 2009
Parapanteles Valerio08	Erebidae	Erebinae	*Ramphia albizona*	Janzen & Hallwachs 2009
Parapanteles Whitfield05	Limacodidae	Limacodinae	*Euprosterna elea*	Janzen & Hallwachs 2009
Parapanteles Whitfield07	Crambidae	Spilomelinae	"spiloJanzen01 Janzen52"	Janzen & Hallwachs 2009
Parapanteles Whitfield08	Geometridae	nr	"geometrid 09-36194"	Janzen & Hallwachs 2009
Parapanteles Whitfield09	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield10	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield100	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield101	Geometridae	nr	"geoJanzen01 Janzen01"	Janzen & Hallwachs 2009
Parapanteles Whitfield102	Riodinidae	Riodininae	*Napaea eucharila*	Janzen & Hallwachs 2009
Parapanteles Whitfield11	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield12	Riodinidae	Riodininae	*Emesis ocypore* *Emesis cypria*	Janzen & Hallwachs 2009
Parapanteles Whitfield13	Hesperiidae	Eudaminae	*Cephise nuspesez*	Janzen & Hallwachs 2009
Parapanteles Whitfield133	Riodinidae	Riodininae	*Symmachia rubina*	Janzen & Hallwachs 2009
Parapanteles Whitfield14	Geometridae	Geometrinae	*Oospila* dicraspedaDHJ02	Janzen & Hallwachs 2009
Parapanteles Whitfield16	Riodinidae	Riodininae	*Caria rhacotis*	Janzen & Hallwachs 2009
Parapanteles Whitfield17	Riodinidae	Riodininae	*Necyria beltiana*	Janzen & Hallwachs 2009
Parapanteles Whitfield18	Hedylidae		*Macrosoma* rubedinariaDHJ02	Janzen & Hallwachs 2009
Parapanteles Whitfield184	Lycaenidae	Theclinae	*Ocaria ocrisia* *Ocaria* ocrisiaDHJ02	Janzen & Hallwachs 2009
Parapanteles Whitfield19	Erebidae	Arctiinae	*Scaptius obscurata*	Janzen & Hallwachs 2009
Parapanteles Whitfield199	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield21	Geometridae	Ennominae	*Pyrinia* Janzen02	Janzen & Hallwachs 2009
Parapanteles Whitfield213	Riodinidae	Riodininae	*Napaea beltiana*	Janzen & Hallwachs 2009

Table 2.1 continued

Parapanteles Whitfield234	Riodinidae	Riodininae	*Ithomiola calculosa*	Janzen & Hallwachs 2009
Parapanteles Whitfield249	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield300	Lycaenidae	Theclinae	*Arawacus togarna* *Cyanophrys fusius*	Janzen & Hallwachs 2009
Parapanteles Whitfield301	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield302	Riodinidae	Riodininae	*Metacharis victrix*	Janzen & Hallwachs 2009
Parapanteles Whitfield303	Riodinidae	Riodininae	*Mesosemia carissima*	Janzen & Hallwachs 2009
Parapanteles Whitfield304	Riodinidae	Riodininae	*Napaea eucharila*	Janzen & Hallwachs 2009
Parapanteles Whitfield305	Riodinidae	Riodininae	*Juditha caucana*	Janzen & Hallwachs 2009
Parapanteles Whitfield44	Riodinidae	Riodininae	*Symmachia tricolor*	Janzen & Hallwachs 2009
Parapanteles Whitfield45	Riodinidae	Riodininae	*Symmachia tricolor*	Janzen & Hallwachs 2009
Parapanteles Whitfield70	Riodinidae	Riodininae	Thisbe irenea	Janzen & Hallwachs 2009
Parapanteles Whitfield83	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield86	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield87	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield88	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield89	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield90	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield92	Geometridae	Geometrinae	*Nemoria* "same as 02-9055"	Janzen & Hallwachs 2009
Parapanteles Whitfield93	Geometridae	Sterrhinae	Pleuroprucha rudimentaria	Janzen & Hallwachs 2009
Parapanteles Whitfield94	Geometridae	Larentiinae	*Dyspteris* Janzen05	Janzen & Hallwachs 2009
Parapanteles Whitfield98	nr	nr	nr	Janzen & Hallwachs 2009
Parapanteles Whitfield99	Tortricidae	nr	"tortricid 07-SRNP-22617"	Janzen & Hallwachs 2009
Putative species n=55	**Described and putative species n=82**	**Putative species host familiesn=13**	**Unique host families from putative and described species n=18**	

Table 2.2: Family-level diversity of host use by microgastrine genera reared from the Area de Concervación Guanacaste. Records access November 2015.

Genus	# of collection records	# of COI barcode species	# of unique host family records	# of unique host species rearing records
Apanteles	4184	328	21	448
Glyptapanteles	2771	223	21	210
Parapanteles	**903**	**70**	**16**	**67**
Hypomicrogaster	1987	128	16	227
Cotesia	815	63	14	113
Dolichogenidea	1351	100	12	117
Diolcogaster	2177	92	12	143
Pseudapanteles	410	39	6	21
Distatrix	22	9	5	12
Papanteles	67	5	5	19
Xanthomicrogaster	98	19	5	21
Alphomelon	1141	34	4	87
Snellenius	201	23	4	31
Iconella	19	4	3	4
Prasmodon	228	13	3	57
Clarkinella	2	2	2	2
Promicrogaster	113	21	2	2
Fornicia	22	5	2	6
Rhygoplitis	32	2	1	2
Venanides	1	1	1	1
Wilkinsonellus	6	1	1	2
Lathrapanteles	29	3	1	2
Microplitis	563	10	1	26
Rasivalva	1	1	1	1

Table 2.3: Comparison of 9 clades (A-I) recovered in our concatenated analysis to their status in individual gene trees. “y” 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 anaylsis, the genus of the majority of species of that clade was listed.

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

Table 2.4: Rearing/collection records and host associations of ten new provisional species from the Yanayacu Rearing Project in Ecuador.

Provisional Species name	Records	Host Family	Host Species	Host Plant Family	Host Place Species	COI variation within species	Sister species/clade	COI Distance to sister	Notes	Reference
Parapanteles sp. B	2525	Arctiidae	no record	Fabaceae	*Erythrina smithiana*	0.000	*Parapanteles* sp. C	0.063-0.068	*Record is gregarious, but this caterpillar is very small and unlikely to support more than one or two parasitoids. Therefore, this record is likely incorrect.	Dyer et al. *2017*
	20919	Arctiidae	no record	Fabaceae	*Erythrina edulis*					
	26049	Arctiidae	no record	Rosaceae	*Rubus* sp.					
	34670	Geometridae	*Eois olivacea**	Piperaceae	*Piper lanceifolium*					
	37474	Arctiidae	no record	Rosaceae	*Rubus* sp.					
	45714	Arctiidae	no record	Melastomataceae	*Monochaetum lineatum*					
Parapanteles sp. C	12105	Arctiidae	no record	Asteraceae	*Dendrophorbium lloense*	0.000-0.002	*Parapanteles* sp. B	0.063-0.068		Dyer et al. 2017
	45981	Arctiidae	no record	Melastomataceae	*Monochaetum lineatum*					
	48054	Arctiidae	no record	Asteraceae	*Dendrophorbium lloense*					
Parapanteles sp. D	13275	Nymphalidae	no record	Poaceae	*Chusquea scandens*	0.000	*Parapanteles* sp. H	0.016		Dyer et al. 2017
	35934	Saturniidae	*Pseudautomeris yourii*	Poaceae	*Chusquea scandens*					
	37263	Saturniidae	*Pseudautomeris yourii*	Onagraceae	*Fucshia scabriuscula*					
	37275	Saturniidae	*Pseudautomeris yourii*	Poaceae	*Chusquea scandens*					
	37791	Saturniidae	no record	Poaceae	*Chusquea scandens*					
	44117	Saturniidae	*Pseudautomeris yourii*	Poaceae	*Chusquea scandens*					
Parapanteles sp. E*	4460	Rubiaceae	*Palicourea* sp.	Geometridae	no record	n/a	((*Parapanteles* sp. B, *Parapanteles* sp. C), *Parapanteles* sp. F)	(0.085, 0.082-0.087), 0.113	*COI match to *Parapanteles* Whitfield08 from Janzen & Hallwachs 2009	Dyer et al. 2017
Parapanteles sp. F	37570	Arctiidae	no record	Piperaceae	*Piper* sp. 'nov1'	n/a	(*Parapanteles* sp. B, *Parapanteles* sp. C)	0.107, 0.085-0.090		Dyer et al. 2017

Table 2.4 continued

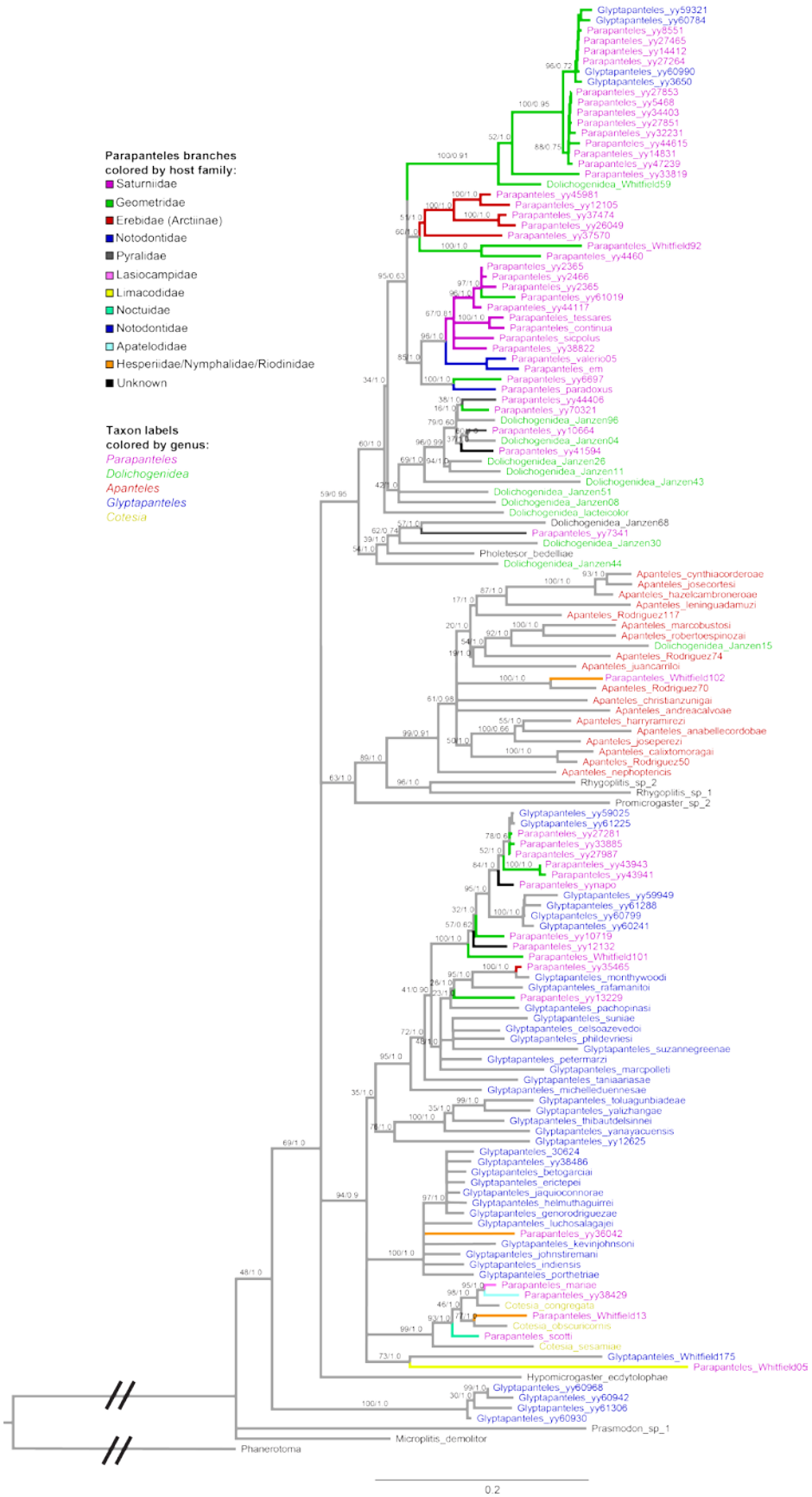
Parapanteles sp. G	38822	Saturniidae	*Gamelia* sp.	Urticaceae	*Boehmeria bullata*	n/a	((*Parapanteles continua*, *Parapanteles tessares*), *Parapanteles sicpolus*)	(0.055, 0.057), 0.035		Dyer et al. 2017
Parapanteles sp. H	2365	Saturniidae	*Leucanella* sp.	Fabaceae	*Erythrina smithiana*	0.000	*Parapanteles* sp. D	0.016	[1]Note in database	Dyer et al. 2017
	2366	Saturniidae	no record	Melastomataceae	*Tibouchina lepidota*					
	4503	Apatelodidae	no record	Melastomataceae	*Monochaetum* sp.					
	24661	no record	no record	no record	no record					
	61019	Geometridae	no record	Piperaceae	*Piper* sp. 'nov1'					
Parapanteles sp. I	6697	Geometridae	no record	no record	no record	0.000-0.002	*Parapanteles paradoxus*	0.088		Dyer et al. 2017
	42069	Arctiidae	no record	Poaceae	*Chusquea scandens*					
	43211	Geometridae	no record	Poaceae	*Chusquea scandens*					
	46466	Geometridae	no record	Poaceae	*Chusquea scandens*					
	46620	Geometridae	no record	Poaceae	*Chusquea scandens*					
Parapanteles sp. J	3071	Geometridae	*Eois olivacea*	Piperaceae	*Piper lanceifolium*	0.000	*Parapanteles* sp. K	0.021-0.030		Dyer et al. 2017
	5468	Geometridae	*Eois olivacea*	Piperaceae	*Piper lanceifolium*					
	14831	Geometridae	*Eois* nr *olivacea*	Piperaceae	*Piper baezanum*					
	27850	Geometridae	*Eois* sp.	Piperaceae	*Piper baezanum*					
	27851	Geometridae	*Eois* sp.	Piperaceae	*Piper baezanum*					
	27852	Geometridae	*Eois* sp.	Piperaceae	*Piper baezanum*					
	27853	Geometridae	*Eois* sp.	Piperaceae	*Piper baezanum*					
	32231	Geometridae	*Eois* nr *fucosa*	Piperaceae	*Piper stiliferum*					
	32568	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					
	32642	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					
	33819	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					
	34115	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					
	34142	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					
	34164	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					
	34403	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					

Table 2.4 continued

	34413	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					
	36533	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					
	44615	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					
	47239	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					
Parapanteles sp. K*	8551	Geometridae	*Eois pallidicosta*	Piperaceae	*Piper* sp.	0.000-0.007	*Parapanteles* sp. J	0.000	*COI match to *Dolichogenidea* Whitfield59 from Janzen & Hallwachs 2009	Dyer et al. 2017
	12546	Geometridae	*Eois* sp.	Piperaceae	*Piper* sp.					
	14412	Geometridae	*Eois pallidicosta*	Piperaceae	*Piper* sp.					
	23541	Geometridae	*Eois olivacea*	Piperaceae	*Piper lanceifolium*					
	27264	Geometridae	*Eois pallidicosta*	Piperaceae	*Piper* sp.					
	27465	Geometridae	*Eois pallidicosta*	Piperaceae	*Piper* sp.					
	27466	Geometridae	*Eois pallidicosta*	Piperaceae	*Piper* sp.					
	28620	Geometridae	*Eois olivacea*	Piperaceae	*Piper lanceifolium*					
	32117	Geometridae	*Eois* sp.	Piperaceae	*Piper* sp.					
	32234	Geometridae	*Eois* sp.	Piperaceae	*Piper immutatum*					
	33815	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					
	36406	Geometridae	*Eois pallidicosta*	Piperaceae	*Piper* sp. 'nov1'					
	36534	Geometridae	*Eois olivacea*	Piperaceae	*Piper baezanum*					
	38844	Geometridae	*Eois* sp.	Piperaceae	*Piper* sp. 'nov1'					
	38845	Geometridae	*Eois* sp.	Piperaceae	*Piper* sp. 'nov1'					
	60786	Geometridae	no record	Piperaceae	*Piper* sp. 'nov1'					
	67394	Geometridae	*Eois* sp.	Piperaceae	*Piper* sp. 'nov1'					
	RPIIPSITII*	no record	no record	no record	no record					

Figure 2.1: 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.

Figure 2.2: 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. Taxon labels are colored by genus, with purple corresponding to *Parapanteles,* green to *Dolichogenidea*, red to *Apanteles*, blue to *Glyptapanteles*, and yellow to *Cotesia*. Ranches of all other genera are black. Branches of *Parapanteles* specimens are colored by host family.



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CHAPTER 3: THE WING INTERFERENCE PATTERNS (WIPS) OF *PARAPANTELES* (BRACONIDAE: MICROGASTRINAE) WASPS: A POWERFUL AND ACCESSIBLE TOOL FOR SPECIES-LEVEL IDENTIFICATION OF SMALL WINGED INSECTS

ABSTRACT

Wing interference patterns (WIPs) are color patterns of insect wings caused by thin film interference. Thin film interference is the same phenomenon responsible for the iridescent colors sometimes visible on soap bubbles, although in insects WIPs are static patterns due to the variable thickness of the transparent portions of wings, rather than shifting patterns due to a thin oil layer as in soap bubbles. While WIPs have been studied in several taxa of small insects, they have not been broadly adopted by insect taxonomists. We surveyed WIPs in one small genus of parasitoid wasps, *Parapanteles* (Braconidae: Microgastrinae). Using an inexpensive microscope camera set-up and free software (ImageJ & R), we were able to detect consistent WIP differences between *Parapanteles* species. In some cases, WIPs can be used to diagnose sibling species that would otherwise require SEM images. We believe WIPs are a largely underemployed character that can be surveyed inexpensively and will likely be similarly useful in other taxa of small insects. The species-specific patterns of WIPs are diagnostically valuable but of uncertain evolutionary significance.

INTRODUCTION

The rainbow colors that can appear on clear insect wings against dark backgrounds are familiar to most entomologists. Shevtsova *et al.* (2011) comprehensively investigated and described these wing colors and discovered that they are actually stable color patterns produced by thin film interference, where light reflected off of the upper or lower surface of a clear membrane constructively or destructively interferes with light approaching the membrane. The perceived colors occur in stable patterns that are primarily caused by the varying thickness of the wing itself, and are, unlike iridescent colors, static at nearly all viewing angles (Shevtsova *et al.* 2011).

These color patterns, “wing interference patterns” (WIPs), are a promising but under-used tool for species-level identification of small insects. Wing interference patterns were entirely ignored before 2011, are still rarely reported in taxonomic works, and are even less frequently used in species diagnoses or identification keys. In addition to discovering them, Shevtsova *et al.* (2011) comprehensively described the physical phenomenon that causes them, and documented examples of WIPs in several Diptera and Hymenoptera taxa. Since then, WIPs have been documented in just 13 taxonomic works (Hansson 2011, Shevtsova & Hansson 2011, Hansson 2012, Hansson & Shevtsova 2012, Hernández-López et al. 2012, Simon 2012, Stigenberg 2012, Buffington & Condon 2013, Mitroiu 2013, Buffington & Forshage 2014, Zhang et al. 2014a, Zhang et al. 2014b, Drohojowska & Szwedo 2015, Zhang et al. 2016), one phylogenetic survey (Buffington & Sandler 2012), and a single experimental study (Katayama *et al.* 2014). Most of these studies focus on Hymenoptera (146 species), followed by Diptera (15 species) and then Hemiptera (8 species) (Table 3.1). Because WIPs are a function of the varying thickness of wings, some authors have speculated that color may vary intraspecifically because overall wing thickness may be correlated to individual size (Shevtsova & Hansson 2011, Hernández-López *et al.* 2012). Therefore, they conclude that the colors of WIPs are less important than the patterns they form. Despite this, the majority of taxonomic works that document WIPs describe them in terms of qualitative colors and the relative portion of the wing those colors occupy (e.g. distal 1/3 magenta). Wing interference patterns have been used as characters in species diagnoses in only three publications to date (Hansson 2011, Shevtsova & Hansson 2011, Hansson & Shevtsova 2012), and have been used in a taxonomic key only twice (Mitroiu 2013, Zhang *et al.* 2014b). Buffinton & Sandler (2012) surveyed the WIPs of 66 species across the phylogeny of Cynipoidea (Hymenoptera) and divided WIPs into four general categories: radiform, campiform, striatiform, and galactiform. To-date, WIPs have not been broadly adopted by insect taxonomists and they have almost exclusively been described qualitatively.

Microgastrinae (Hymenoptera: Braconidae) is a hyper-diverse subfamily of small parasitoid wasps that attack Lepidoptera (Mardulyn and Whitfield. 1999). Microgastrinae currently has 2689 described species, representing roughly 5-10% of the estimated worldwide

diversity of this group (Rodriguez *et al.* 2012, Fernández-Triana & Ward 2015). Their diminutive adult size and small number of morphological characters has made the generic-level taxonomy of this group difficult, and species-level diagnoses, absent DNA barcoding, often rely on subtly variable characters or minute characters that require SEM imaging to observe (e.g. Valerio *et al.* 2009). Wing interference patterns have never been reported for microgastrines.

Parapanteles Ashmead is a small genus of Microgastrinae with several species that are morphologically very similar to other genera (*Dolichogenidea* and *Glyptapanteles*) and frequently misdiagnosed (Valerio *et al.* 2009, Chapter 1). Here, we document the WIPs of 7 described and 12 putative undescribed *Parapanteles* species from Costa Rica and Ecuador and present a simple and inexpensive method for quantifying and comparing WIPs that can contribute to identification keys or rapid species diagnosis.

To our knowledge, no authors to date have attempted to quantitatively describe WIPs in any taxa. Here we present the first study of WIPs in Microgastrinae (Hymenoptera: Braconidae), and the first attempt to quantitatively compare the WIPs of closely related species.

METHODS

One set of fore and hind wings were removed from each adult wasp from samples stored in ethanol. Where available, wings from one male and one female per brood were removed and slide mounted on temporary slides. All species sampled were gregarious (i.e. the female lays multiple eggs in a single host) except *P.* sp. J & *P.* sp. K, which are solitary (i.e. females lay a single egg per host). Wings were sandwiched between two microscope slides which were taped together at the ends. This flattens wings more reliably than using a standard slide cover. As in Shevtsova & Hansson 2011, a drop of India ink was spread on one slide to create a uniform black background behind the wings.

Wings were photographed at 50x magnification using a Cannon Rebel Xsi camera attached to a Leica ## dissecting scope via [connectors] and an Amscope LED-144A-YK 144 LED ring light at maximum brightness. Wing images were not visually adjusted. The majority of wing slides were prepared and photographed by an undergraduate research assistant, Shuyang Jin.

The average RGB (red, green, and blue) values of pixels in each fore wing image were measured using the "RGB Measure" feature in ImageJ v1.49 (Schneider *et al.* 2012). The value for each color component was divided by the average of all three average color values to calculate the relative "redness," "greenness," and "blueness" of each fore wing image (e.g., redness=R/((R+G+B)/3)). This averages out the contribution of black (R/G/B=0/0/0), white (R/G/B=255/255/255), and grey (R/G/B are all equal) pixels.

Arrays of relative redness, greenness, and blueness for each species were testing for normality in R v3.4 (R Core Team 2017) via the Shapiro-Wilk test and for skewness, and then compared across species via ANOVA and Tukey's HSD test. Species with sample size lower than 3 were excluded from our statistical analysis.

Several metrics of forewing size were measured to test whether it they correlated with WIP patterns, because if they do then species-level differences in WIPs may simply be caused by some species being larger than others. Forewing length (measured from the junction of C+Sc+R & M+Cu to the distal end of 3/M) and area were compared to each color array. In additiona, overall forewing shape was measured by dividing length by width (measured from the junction of r-rs and the stigma to the distal end of the anal lobe) to test narrowness has any effect on wing thickness. Measurements were done in ImageJ v1.49 (Schneider *et al.* 2012) and tested for correlation via the Pearson Correlation test in R v3.4 (R Core Team 2017).

RESULTS

Inter- & Intraspecific variation in WIPs

The wing interference patterns of the species surveyed are generally consistent within species, although intraspecific consistency is variable. Both qualitatively (Figs. 3.1-3.15) and in terms of relative redness, greenness, and blueness (R.RGBs) (Tables 3.2 & 3.3, Fig. 3.16), the species with purplish WIPs (*Parapanteles tessares, P. continua, P. sicpolus,* & *P.* sp. H) tended to have the most consistent WIPs, while species with reddish or yellowish WIPs tended to be more variable, especially *Parapanteles* sp. J & *P.* sp. K.

All R.RGB arrays were normally distributed except two *Parapanteles continua* arrays, one *Parapanteles* sp. E, one *Parapanteles* paradoxus, one *Parapanteles sicpolus,* and four

Parapanteles tessares arrays (Tables 3.2 & 3.3). The distribution of forewing and hindwing R.RGBs among closely related species are often similar with one or two parameters significantly different (Fig. 3.16). For example, the R.RGBs of the sister species *P. tessares* and *P. continua* are not significantly different except for forewing relative redness (higher in *P. continua*) and relative blueness (higher in *P. tessares*), which corroborates the more uniformly purple appearance of *P. tessares's* WIP.

Males and females of most species have similar WIPs, although in *P.* sp. D and *P. em* male WIPs are slightly more yellowish (Figs. 3.5 & 3.6). Sexual dimorphism could not be assessed for 6 species: only females were available for *P.* sp. C, *P.* sp. J, *P.* sp. K, and *P.* sp. Valerio05, and only males were available for *P.* sp. I and *P.* sp. E.

Relative redness, greenness, and blueness and wing size

The majority of R.RGB arrays were not significantly correlated with wing length, area, or shape. Eleven of the 33 R.RGB tested were significantly correlated with wing length and 8 of 33 were significantly correlated with wing area. In each case the slope of the line of regression was slight and no R.RGB arrays were correlated with wing shape (Table 3.4).

Descriptions of WIPs of Parapanteles spp. from Ecuador & Costa Rica

***Parapanteles tessares*:**

(Figure 3.1)

Forewing: Proximal 1/2 of 2R1 boardering stigma clear, remainded and 3Rs, 2+3M, 3Cu predominantly purple. Thin irregular green band proximally (thickest in 3Cu), narrow band along distal fringe reddish yellow (yellow band sometimes absent). Otherwise clear.

Hindwing: R1, Rs, & M proximally to distally 1/4th green to 1/2 purple to 1/4th yellow. Otherwise clear.

Materials examined: 14 femals and 11 males from 13 reared broods (ACG ID #s: 07-SRNP-31974, 07-SRNP-31983, 07-SRNP-31995, 07-SRNP-32001, 07-SRNP-32013, 07-SRNP-32056, 07-SRNP-32059, 07-SRNP-32068, 07-SRNP-32069, 07-SRNP-32070, 07-SRNP-32073, 07-SRNP-32074, 08-SRNP-2270)

***Parapanteles continua*:**

(Figure 3.2 a & b)

Forewing: Proximal 1/2 of 2R1 bordering stigma clear, remainder and 3Rs, 2+3M, 3Cu predominantly purple. proximal 1/8th irregular green band, 1/8th-1/4th distal edge reddish yellow. Otherwise clear.

Hindwing: R1, Rs, & M proximally to distally 1/3rd green to 1/3rd purple to 1/3rd yellow. Otherwise clear.

Materials examined: 21 females and 20 males from 21 reared broods (ACG ID #s: 97-SRNP-1677, 97-SRNP-1677, 97-SRNP-1679, 97-SRNP-1685, 97-SRNP-1691, 97-SRNP-1694, 97-SRNP-1696, 97-SRNP-1703, 97-SRNP-1708, 97-SRNP-1715, 97-SRNP-1739, 97-SRNP-2099, 02-SRNP-2213, 02-SRNP-2844, 02-SRNP-3255, 04-SRNP-3010, 05-SRNP-33563, 06-SRNP-1252, 06-SRNP-3375, 07-SRNP-31996, 07-SRNP-32110, 08-SRNP-2322)

***Parapanteles sicpolus*:**

(Figure 3.3)

Forewing: Proximal 1/2 of 2R1 bordering stigma clear, remainder and 3Rs, 2+3M, 3Cu predominantly green. proximal 1/2 irregular green band, 1/4th purple, 1/8th-1/4th distal edge reddish yellow. Otherwise clear.

Hindwing: R1, Rs, & M proximally with thin purple band, then 1/3rd green to 1/3rd purple to red to 1/3rd yellow.

Materials examined: 8 females and 6 males from 8 reared broods (ACG ID #s: 03-SRNP-3418, 03-SRNP-3419, 03-SRNP-3687, 96-SRNP-373.1, 96-SRNP-373.2, 99-SRNP-1416, 99-SRNP-1418, 03-SRNP-22531)

***Parapanteles* sp. H:**

(Figure 3.4)

Forewing: Proximal 1/2 of 2R1 bordering stigma clear, remainder and 3Rs, 2+3M, 3Cu thin purple band proximally & purple along 3/Rs, 3/M, & 3/Cu veins, remainder reddish yellow. Otherwise clear.

Hindwing: R1, Rs, & M proximally to distally narrow green band (sometimes absent) then sub-1/2 purple to 1/2 yellow. Otherwise clear.

Materials examined: 3 females and 6 males from 5 reared broods (YY ID #s: 2365, 2366, 2466, 4503).

***Parapanteles* sp. D:**

(Figure 3.5)

Forewing: Proximal 1/2 of 2R1 bordering stigma clear, remainder and 3Rs, 2+3M, 3Cu thin purple band proximally & purple along 3/Rs (sometimes very reduced so majority of WIP is yellow), 3/M, & 3/Cu veins, remainder reddish yellow. Otherwise clear.

Hindwing: R1, Rs, & M proximally to distally 1/2-1/3rd purple to 1/2-2/3rds yellow. Otherwise clear.

Males: yellow bands/areas tend to be larger than in females.

Materials examined: 6 females and 4 males from 6 reared broods (YY ID #s: 8275, 35934, 37263, 37275, 37791, 44117).

***Parapanteles em*:**

(Figure 3.6)

Forewing: Proximal 1/2 of 2R1 bordering stigma clear, remainder and 3Rs, 2+3M, 3Cu thin purple band proximally & purple along 3/Rs (sometimes very reduced so majority of WIP is yellow), 3/M, & 3/Cu veins, remainder reddish yellow. Otherwise clear.

Hindwing: R1, Rs, & M proximally to distally 1/2-1/3rd purple to 1/2-2/3rds yellow. Otherwise clear.

Males: yellow bands/areas tend to be larger than in females. Very similar to *Parapanteles* sp. D.

Materials examined: 11 females and 5 males from 11 reared broods (ACG ID #s: 00-SRNP-8625, 00-SRNP-8626, 00-SRNP-8627, 00-SRNP-8628, 05-SRNP-2524, 05-SRNP-2568, 07-SRNP-24390, 07-SRNP-33320, 07-SRNP-33764, 07-SRNP-3868, 07-SRNP-58627).

***Parapanteles* sp. valerio05:**

(Figure 3.7)

Forewing: Proximal 1/2 of 2R1 bordering stigma clear, remainder and 3Rs, 2+3M, 3Cu predominantly yellow, sometimes orange to red and/or slightly purple around veins. Otherwise clear.

Hindwing: Rs predominantly yellow, R1 proximally 1/8th purple then predominantly yellow, M 1/3 purple then predominantly yellow. Otherwise clear.

Materials examined: 7 females from 7 reared broods (ACG ID #s: 07-SRNP-5195, 06-SRNP-21433, 07-SRNP-24742, 07-SRNP-24803, 07-SRNP-24804, 07-SRNP-24807, 07-SRNP-24821).

***Parapanteles paradoxus*:**

(Figure 3.8)

Forewing: Proximal 1/2 of 2R1 bordering stigma proximally green to purple, remainder and 3Rs yellow except purple along 3/Rs vein. 2+3M & 3Cu predominantly yellow. Otherwise clear.

Hindwing: R1, Rs, & M proximally to distally 1/2-1/3rd purple to 1/2-2/3rds yellow. Otherwise clear.

Materials examined: 12 females and 4 males from 12 reared broods (ACG ID #s: 02-SRNP-68, 06-SRNP-33973, 07-SRNP-32380, 00-SRNP-11175, 02-SRNP-431, 02-SRNP-434, 02-SRNP-436, 02-SRNP-437, 03-SRNP-7519, 06-SRNP-429, 06-SRNP-430, 06-SRNP-1054).

***Parapanteles* sp. I:**

(Figure 3.9)

Forewing: Proximal 1/2 of 2R1 bordering stigma proximally green to purple, remainder and 3Rs yellow except purple along 3/Rs vein (thinner than as in paradoxus). 2+3M & 3Cu predominantly yellow. Otherwise clear.

Hindwing: R1, Rs, & M proximally to distally 1/2-1/3rd purple to 1/2-2/3rds yellow. Otherwise clear.

Materials examined: 4 females from 4 reared broods (YY ID #s: 42069, 43211, 46466, 66971).

***Parapanteles* sp. J:**

(Figure 3.10)

WIPs somewhat irregular, but:

Forewing: Proximal 1/2 of 2R1 bordering stigma proximally green to purple, remainder and 3Rs yellow. 2+3M & 3Cu predominantly yellow. Distally reddish. Otherwise clear.

Hindwing: R1, Rs, & M proximally to distally 1/4-1/3rd purple to 3/4-2/3rds yellow. Otherwise clear.

Materials examined: 5 solitary females (YY ID #s: 27850, 27851, 34403, 34413, 36533).

***Parapanteles* sp. K:**

(Figure 3.11)

WIPs highly variable, generally similar to J, but some predominantly yellow, some predominantly blue, some with thicker reddish areas.

Materials examined: 5 solitary females (YY ID #s: 28620, 32234, 36406, 36534, 38844).

***Parapanteles* sp. E:**

(Figure 3.12)

Forewing: Proximal 1/2 of 2R1 bordering stigma clear, remainder and 3Rs, 2+3M, 3Cu predominantly yellow. Otherwise clear.

Hindwing: R12 & Rs predominantly yellow, M 1/3 purple then predominantly yellow. Otherwise clear.

Materials examined: 3 males from 3 reared broods (YY ID #s: 36197, 36198, 36520).

***Parapanteles tlinea*:**

(Figure 3.13)

Forewing: 2R1, 3Rs, 2+3M, & 3Cu predominantly yellow with very narrow purple bands along veins. Otherwise clear.

Hindwing: R1, Rs, & M predominantly yellow with narrow purple bands along veins. M proximally 1/4th-1/2 purple. Otherwise clear.

Materials examined: 2 females and 1 male from 1 reared broods (ACG ID #: 04-SRNP-669).

***Parapanteles* sp. B:**

(Figure 3.14)

Forewing: Proximal 1/2 of 2R1 bordering stigma variable but predominantly green, remainder and 3Rs, 2+3M, 3Cu predominantly yellow, although 3Rs & 2+3M . Otherwise clear.

Hindwing: R1 & Rs predominantly yellow, M 1/3 purple then predominantly yellow. Otherwise clear.

Materials examined: 6 females and 2 males from 5 reared broods (YY ID #s: 45714, 26049, 37474, 20919, 24670).

***Parapanteles* sp. C:**

(Figure 3.15)

Forewing: Proximal 1/2 of 2R1 bordering stigma clear, remainder and 3Rs, 2+3M, 3Cu proximally to distally 1/8th green to 1/2 purple to 3/8ths yellow. 2_3M & 3Cu sometimes predominantly yellow. Otherwise clear.

Hindwing: R1, Rs, & M with thin green band proximally, then 1/2 purple to 1/2 yellow.

Materials examined: 3 females from 3 reared broods (YY ID #s: 12105, 45981, 48054).

DISCUSSION

The wing interference patterns of *Parapanteles* are consistent within species and distinct between species, often enough to be diagnostic by themselves. Among the species surveyed, the WIPs of *Parapanteles tessraes, P. continua, P. sicpolus*, *P.* sp. H, and *P.* sp. C were the most distinct. These species tended to have more green and purple in their WIPs, while the remaining species' WIPs were predominantly red and/or yellow.

Wing interference patterns are directly related to the thickness of wing membranes, and previous publications have speculated that WIP colors should change as individuals get larger because cuticle thickness may increase with body size (Shevtsova & Hansson 2011, Hernández-López *et al.* 2012). We are not aware of any studies investigating the allometry of body or wing

cuticle thickness. Among the species we surveyed, some relative redness, greenness, and/or blueness arrays were significantly correlated with wing size and/or area in some species, but in each of these cases the slope of the corresponding linear regression was very slight (Table 3.4). Correlation with wing size (as a proxy for body size) alone does not account for the differences between the WIPs of closely related *Parapanteles* species.

Wing interference patterns are also directly related to the wavelength of the light passing through the wing membrane, which is a major weakness for using any measurement derived from RGB values for diagnostic purposes. The relative RGB values we measured in this study were not consistent if the wing was illuminated with a different light source. This limitation can be solved by using a consistent light source, and the light source which we used for all WIP photographs in this study, an Amscope LED-144A-YK 144 LED ring light, is widely available and relatively inexpensive. Wing interference patterns can be observed *in situ* on pinned specimens, but these are of little use compared to WIPs observed on slide-mounted wings. Including WIP slides (wing slides with India Ink painted on the back) of at least a few paratype individuals with the type series of small winged insects would ameliorate most of the problem posed by variations between light sources, and expand the usefulness of WIPs for future studies.

The evolutionary significance of WIPs is unknown, this and other studies have found that they are frequently species-specific (Shevtsova *et al.* 2011). The colors of WIPs are visible *in situ* and in natural settings whenever insect wings are displayed in front of a dark background (e.g. green leaves), and the colors that compose them occur in spectra visible to most insects (Shevtsova *et al*. *2011*). Anecdotally, we found that closely related sympatric species tended to be more subjectively different (i.e. (*Parapanteles tessares, P. continua*), *P. sicpolus*) and (*P. em, P.* valerio05) from Costa Rica and (*P.* sp. B, *P.* sp. C) from Ecuador), while closely related allopatric species tended to be less distinct (i.e. (*P. paradoxus, P.* sp. I) and (*P.* sp. E, *P. tlinea*). This suggests that WIPs may be used by microgastrines for conspecific recognition, although a much broader survey of microgastrine WIPs is required to determine if our qualitative observations correspond to a real correlation. However, this is entirely speculative and has not been experimentally tested. The only experimental study of WIPs to date found that male WIP

brightness was correlated with female mate choice preference in isogenic *Drosophila melanogaster* lines, but was unable to separate the effect of WIPs from other traits that may be correlated with them (Katayama *et al.* 2014). The evolutionary significance of WIPs and any role they have in conspecific recognition or mate preference merit future study, but any manipulative experiments exploring these subjects will be difficult due to the nature of the phenomenon and the small size of the subject species.

In general, WIPs can be observed and documented with very little additional effort for most taxonomists who work on small winged insects. We predict that they can be a large source of new morphological characters for the taxonomy and systematics of these tiny animals. The only materials required are a dissecting microscope with a camera attachment, a ring light, glass slides, and India Ink. Wing interference patterns are often species-specific and useful for *Parapanteles* wasps, and will likely be for most other microgastrine wasps.

TABLES & FIGURES

Table 3.1: Taxonomic summary of published wing interference pattern images and/or descriptions.

Order	Family	Genus	Number of Species	Reference
Hymenoptera	Austrocynipidae	*Austrocynips*	1	Buffintong & Sandler 2012
	Braconidae	*Spathicopis*	1	Stigenberg 2012
	Cynipidae	*Andricus*	1	Buffintong & Sandler 2012
		Aulicidea	1	Buffintong & Sandler 2012
		Bassettia	1	Buffintong & Sandler 2012
		Biorhiza	1	Buffintong & Sandler 2012
		Callirhytis	1	Buffintong & Sandler 2012
		Cerroneuroterus	1	Buffintong & Sandler 2012
		Diastrophus	1	Buffintong & Sandler 2012
		Diplolepis	1	Buffintong & Sandler 2012
		Disholcaspsis	1	Buffintong & Sandler 2012
		Dryocosmus	1	Buffintong & Sandler 2012
		Loxaulus	1	Buffintong & Sandler 2012
		Neuroterus	1	Buffintong & Sandler 2012
		Odontocynips	1	Buffintong & Sandler 2012
		Periclistus	1	Buffintong & Sandler 2012
		Phanacis	1	Buffintong & Sandler 2012
		Plagiotrochus	1	Buffintong & Sandler 2012
		Pseudoneroterus	1	Buffintong & Sandler 2012
		Rhoophilus	1	Buffintong & Sandler 2012
		Saphonecrus	1	Buffintong & Sandler 2012
		Synergus	1	Buffintong & Sandler 2012
		Synophromorpha	1	Buffintong & Sandler 2012
		Synophrus	1	Buffintong & Sandler 2012
		Trichogalma	1	Buffintong & Sandler 2012
	Eulophidae	*Achrysocharoides*	9	Shevtsova & Hansson 2011
		Achrysocharoides	8	Hansson 2012
		Cornugon	19	Hansson 2011
		Omphale	36	Hansson & Shevtsova 2012
	Figitidae	*Aganaspis*	1	Buffintong & Sandler 2012
		Agrostocynips	1	Buffintong & Sandler 2012
		Alloxysta	1	Buffintong & Sandler 2012
		Anacharis	1	Buffintong & Sandler 2012
		Araucocynips	1	Buffintong & Sandler 2012
		Banancuniculus	1	Buffintong & Sandler 2012
		Chrestosema	1	Buffintong & Sandler 2012
		Cothonaspis	1	Buffintong & Sandler 2012
		Dettmeria	1	Buffintong & Sandler 2012
		Dieucoila	1	Buffintong & Sandler 2012
		Emargo	1	Buffintong & Sandler 2012

Table 3.1 continued

		Euceroptres	1	Buffintong & Sandler 2012
		Eucoila	1	Buffintong & Sandler 2012
		Figites	1	Buffintong & Sandler 2012
		Garudella	4	Buffingtong & Forshage 2014
		Ganaspidium	1	Buffintong & Sandler 2012
		Gronotoma	1	Buffintong & Sandler 2012
		Hexacola	1	Buffintong & Sandler 2012
		Kleidotoma	1	Buffintong & Sandler 2012
		Leptopilina	1	Buffintong & Sandler 2012
		Lonchidia	1	Buffintong & Sandler 2012
		Melanips	1	Buffintong & Sandler 2012
		Myrtopsen	1	Buffintong & Sandler 2012
		Neralsia	1	Buffintong & Sandler 2012
		Nordlanderia	1	Buffintong & Sandler 2012
		Odonteucoila	1	Buffintong & Sandler 2012
		Odontosema	1	Buffintong & Sandler 2012
		Paraspicera	1	Buffintong & Sandler 2012
		Parnips	1	Buffintong & Sandler 2012
		Plectocynips	1	Buffintong & Sandler 2012
		Rhabducoila	1	Buffintong & Sandler 2012
		Rophtromeris	1	Buffintong & Sandler 2012
		Sarothrus	1	Buffintong & Sandler 2012
		Striatovertex	1	Buffintong & Sandler 2012
		Triplasta	1	Buffintong & Sandler 2012
		Trjpystiniola	1	Buffintong & Sandler 2012
		Tropideucoila	1	Buffington & Condon 2013
		Trybliographa	1	Buffintong & Sandler 2012
		Xyalaphora	1	Buffintong & Sandler 2012
		Xyalaspis	1	Buffintong & Sandler 2012
	Ibaliiae	*Ibalia*	1	Buffintong & Sandler 2012
		Eileenella	1	Buffintong & Sandler 2012
	Liopteridae	*Paramblynotus*	1	Buffintong & Sandler 2012
	Pteromalidae	*Watshamia*	3	Mitroiu 2013
Subtotal:	**9**	**74**	**146**	**10**
Diptera	Drosophilidae	*Drosophila*	1	Ktayama *et al.* 2014
	Muscidae	*Lispe*	10	Zhang *et al.* 2016
	Sarcophagidae	*Sarcophage*	1	Zhang *et al.* 2014a
		Sphecapatodes	3	Zhang *et al.* 2014b
Subtotal:	**4**	**4**	**15**	**4**

Table 3.1 continued

Hemiptera	Aleyrodidae	*Aretsaya*	1	Drohojowska & Szwedo 2015
	Coccidae	*Eriopletis*	1	Simon 2012
		Eulecanium	1	Simon 2012
		Luzulaspis	2	Simon 2012
		Parthenolecanium	1	Simon 2012
		Pulvinaria	1	Simon 2012
		Sphaerolecanium	1	Simon 2012
Subtotal:	**2**	**8**	**8**	**2**
Total:	**15**	**86**	**169**	**16**

Table 3.2: Average relative redness (RR), greenness (RG), and blueness (RB) of the forewings of fifteen *Parapanteles* species plus or minus one standard deviation, with minimum and maximum values, results of Tukey's HSD test, skewness, and Shapiro-Wilks' test for normality.

Species	n	Ave. Forewing RR	Min - Max	Tukey's HSD	Skewness	Shapiro-Wilks P-value	Ave. Forewing RG	Min - Max	Tukey's HSD	Skewness	Shapiro-Wilks P-value	Ave. Forewing RB	Min - Max	Tukey's HSD	Skewness	Shapiro-Wilks P-value
Parapanteles tessares	25	1.012 ± 0.025	0.97 - 1.066	f	0.43	0.38	0.827 ± 0.025	0.798 - 0.889	cd	1.17	**0.01**	1.161 ± 0.036	1.045 - 1.198	a	-2.11	**0.00**
Parapanteles continua	41	1.05 ± 0.021	1.009 - 1.094	e	0.22	0.44	0.864 ± 0.018	0.8 - 0.891	bc	-1.19	**0.01**	1.086 ± 0.025	1.042 - 1.148	b	0.22	0.75
Parapanteles sicpulus	14	1.042 ± 0.013	1.016 - 1.057	ef	-0.78	0.25	0.884 ± 0.009	0.871 - 0.9	ab	0.59	0.55	1.074 ± 0.017	1.055 - 1.111	b	0.90	0.13
Parapanteles sp. H	9	1.088 ± 0.022	1.065 - 1.131	d	1.13	0.19	0.813 ± 0.026	0.773 - 0.849	d	0.09	0.73	1.099 ± 0.039	1.021 - 1.15	b	-1.04	0.35
Parapanteles sp. D	10	1.169 ± 0.029	1.125 - 1.213	bc	-0.15	0.86	0.834 ± 0.044	0.774 - 0.909	cd	0.57	0.54	0.997 ± 0.051	0.923 - 1.101	cd	0.59	0.50
Parapanteles em	16	1.149 ± 0.025	1.094 - 1.189	c	-0.52	0.60	0.836 ± 0.038	0.787 - 0.937	cd	1.21	0.09	1.016 ± 0.031	0.954 - 1.069	c	-0.22	0.96
Parapanteles sp. valerio05	7	1.178 ± 0.032	1.122 - 1.213	abc	-0.98	0.44	0.868 ± 0.041	0.816 - 0.921	bc	0.33	0.41	0.954 ± 0.06	0.885 - 1.062	de	0.82	0.58
Parapanteles paradoxus	16	1.199 ± 0.023	1.152 - 1.233	ab	-0.29	0.91	0.912 ± 0.022	0.874 - 0.94	ab	-0.38	0.25	0.89 ± 0.033	0.829 - 0.947	f	0.43	**0.05**
Parapanteles sp. I	4	1.214 ± 0.05	1.149 - 1.267	ab	-0.71	0.87	0.929 ± 0.026	0.907 - 0.96	a	0.47	0.28	0.857 ± 0.037	0.824 - 0.891	f	0.01	0.06
Parapanteles sp. J	5	1.212 ± 0.015	1.195 - 1.237	ab	1.07	0.50	0.887 ± 0.029	0.856 - 0.917	ab	0.03	0.25	0.9 ± 0.04	0.846 - 0.936	ef	-0.70	0.25
Parapanteles sp. K	5	1.142 ± 0.078	1.025 - 1.239	c	-0.58	0.90	0.889 ± 0.026	0.858 - 0.915	ab	-0.17	0.42	0.968 ± 0.069	0.902 - 1.084	cde	1.52	0.25
Parapanteles sp. E	3	1.183 ± 0.017	1.167 - 1.201	abc	0.51	0.81	0.915 ± 0.028	0.883 - 0.933	ab	-1.71	0.11	0.902 ± 0.013	0.889 - 0.916	ef	0.54	0.80
Parapanteles tlinea	3	1.163 ± 0.026	1.141 - 1.192	bc	1.19	0.52	0.915 ± 0.026	0.886 - 0.934	ab	-1.57	0.28	0.922 ± 0.043	0.874 - 0.959	def	-1.05	0.58
Parapanteles sp. B	8	1.216 ± 0.029	1.172 - 1.25	a	-0.55	0.45	0.897 ± 0.051	0.814 - 0.944	ab	-0.75	0.10	0.887 ± 0.063	0.806 - 1.015	f	1.13	0.52
Parapanteles sp. C	3	1.119 ± 0.018	1.102 - 1.137	cd	0.21	0.92	0.832 ± 0.018	0.812 - 0.849	cd	-0.72	0.73	1.049 ± 0.03	1.014 - 1.069	bc	-1.64	0.21

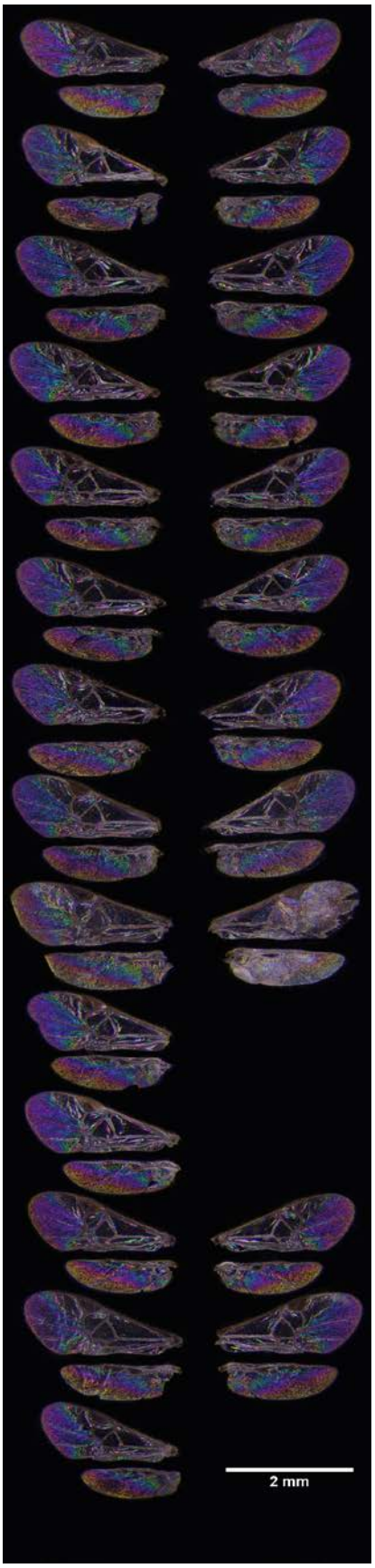
Table 3.3: Average relative redness (RR), greenness (RG), and blueness (RB) of the hindwings of fifteen *Parapanteles* species plus or minus one standard deviation, with minimum and maximum values, results of Tukey's HSD test, skewness, and Shapiro-Wilks' test for normality.

Species	n	Ave. Hindwing RR	Min - Max	Tukey's HSD	Skewness	Shapiro-Wilks P-value	Ave. Hindwing RG	Min - Max	Tukey's HSD	Skewness	Shapiro-Wilks P-value	Ave. Hindwing RB	Min - Max	Tukey's HSD	Skewness	Shapiro-Wilks P-value
Parapanteles tessares	25	1.067 ± 0.019	1.041 - 1.112	ef	-0.58	**0.02**	0.853 ± 0.022	0.827 - 0.918	ab	1.46	**0.00**	1.081 ± 0.026	1.031 - 1.122	a	0.94	0.06
Parapanteles continua	41	1.049 ± 0.026	1.007 - 1.126	f	0.03	0.84	0.89 ± 0.012	0.858 - 0.916	a	-0.63	0.31	1.062 ± 0.025	1.004 - 1.127	ab	1.02	**0.02**
Parapanteles sicpulus	14	1.045 ± 0.012	1.016 - 1.064	f	2.16	**0.00**	0.894 ± 0.009	0.873 - 0.909	a	-0.64	0.47	1.062 ± 0.016	1.04 - 1.111	ab	-0.73	0.68
Parapanteles sp. H	9	1.07 ± 0.014	1.057 - 1.095	ef	0.11	0.18	0.839 ± 0.018	0.81 - 0.87	b	0.09	1.00	1.091 ± 0.015	1.073 - 1.109	a	0.89	0.22
Parapanteles sp. D	10	1.151 ± 0.042	1.095 - 1.231	bcd	0.14	0.87	0.835 ± 0.029	0.793 - 0.874	b	-0.09	0.35	1.014 ± 0.046	0.934 - 1.094	bcd	0.61	0.74
Parapanteles em	16	1.129 ± 0.023	1.085 - 1.159	cd	-0.60	0.29	0.84 ± 0.034	0.795 - 0.931	b	1.09	0.10	1.031 ± 0.046	0.914 - 1.118	bc	-0.54	0.39
Parapanteles sp. valerio05	7	1.17 ± 0.033	1.112 - 1.222	abcd	0.22	0.75	0.891 ± 0.034	0.847 - 0.934	a	0.20	0.46	0.939 ± 0.06	0.846 - 1.042	ef	-0.34	0.73
Parapanteles paradoxus	16	1.168 ± 0.026	1.112 - 1.213	bcd	1.06	0.26	0.863 ± 0.023	0.831 - 0.913	ab	0.53	0.71	0.969 ± 0.034	0.922 - 1.057	def	-0.14	0.70
Parapanteles sp. I	4	1.222 ± 0.043	1.171 - 1.26	a	1.23	0.55	0.875 ± 0.029	0.835 - 0.898	ab	-1.14	0.29	0.904 ± 0.065	0.844 - 0.994	f	-0.34	0.30
Parapanteles sp. J	5	1.191 ± 0.039	1.125 - 1.227	ab	0.54	0.93	0.854 ± 0.043	0.806 - 0.909	ab	0.20	0.71	0.955 ± 0.078	0.864 - 1.069	def	-1.63	0.18
Parapanteles sp. K	5	1.142 ± 0.041	1.101 - 1.188	bcd	0.72	0.81	0.867 ± 0.038	0.829 - 0.915	ab	0.40	0.41	0.99 ± 0.03	0.955 - 1.035	cde	0.19	0.27
Parapanteles sp. E	3	1.172 ± 0.052	1.112 - 1.204	abcd	1.73	**0.03**	0.895 ± 0.014	0.878 - 0.906	a	-1.48	0.35	0.933 ± 0.066	0.894 - 1.01	ef	-1.72	0.06
Parapanteles tlinea	3	1.186 ± 0.039	1.148 - 1.226	abc	0.45	0.83	0.874 ± 0.033	0.837 - 0.902	ab	-1.12	0.55	0.94 ± 0.072	0.872 - 1.015	def	0.20	0.93
Parapanteles sp. B	8	1.169 ± 0.032	1.121 - 1.201	abcd	0.04	1.00	0.872 ± 0.031	0.837 - 0.912	ab	0.24	0.23	0.959 ± 0.047	0.887 - 1.031	def	-0.82	0.11
Parapanteles sp. C	3	1.106 ± 0.018	1.093 - 1.127	de	-0.69	0.74	0.839 ± 0.005	0.835 - 0.844	b	1.62	0.23	1.055 ± 0.016	1.038 - 1.07	abc	1.60	0.25

Table 3.4: Average length, area, and shape (length/height) or the forewings of fifteen *Parapanteles* species plus or minus one standard deviation, with coefficient of determination and the p-value of Pearson correlation tests of each measurement for each forewing color array (relative redness, greenness, and blueness).

Species	n	Forewing Length (mm)	Length/RR r^2	P-value	Length/RG r^2	P-value	Length/RB r^2	P-value	Forewing Area (mm^2)	Area/RR r^2	P-value	Area/RG r^2	P-value	Area/RB r^2	P-value	Forewing Height (mm)	Shape (L/H)	Shape/RR r^2	P-value	Shape/RG r^2	P-value	Shape/RB r^2	P-value
Parapanteles tessares	25	2.33 ± 0.09	0.04	0.37	0.18	**0.03**	0.18	**0.03**	0.61 ± 0.04	0.00	0.89	0.13	0.08	0.07	0.19	0.61 ± 0.04	3.83 ± 0.15	0.00	0.91	0.00	0.73	0.00	0.78
Parapanteles continua	41	2.5 ± 0.18	0.09	0.06	0.16	**0.01**	0.29	**0.00**	0.67 ± 0.05	0.06	0.13	0.15	**0.01**	0.24	**0.00**	0.67 ± 0.05	3.76 ± 0.13	0.01	0.56	0.01	0.51	0.03	0.33
Parapanteles sicpulus	14	2.74 ± 0.13	0.24	0.08	0.07	0.34	0.24	0.07	0.74 ± 0.04	0.25	0.07	0.04	0.47	0.23	0.08	0.74 ± 0.04	3.7 ± 0.18	0.03	0.53	0.03	0.53	0.04	0.47
Parapanteles sp. H	9	3.11 ± 0.47	0.06	0.53	0.00	0.88	0.01	0.82	0.82 ± 0.13	0.06	0.51	0.00	0.00	0.02	0.72	0.82 ± 0.13	3.81 ± 0.08	0.10	0.42	0.37	0.08	0.34	0.10
Parapanteles sp. D	10	3.59 ± 0.15	0.00	0.95	0.66	**0.00**	0.48	**0.03**	0.93 ± 0.06	0.01	0.79	0.76	**0.00**	0.49	**0.02**	0.93 ± 0.06	3.88 ± 0.14	0.00	0.98	0.13	0.30	0.10	0.36
Parapanteles em	16	2.36 ± 0.21	0.45	**0.00**	0.79	**0.00**	0.29	**0.03**	0.64 ± 0.06	0.42	**0.01**	0.85	**0.00**	0.35	**0.02**	0.64 ± 0.06	3.71 ± 0.14	0.00	0.84	0.01	0.71	0.03	0.55
Parapanteles sp. valerio05	7	2.4 ± 0.13	0.07	0.58	0.48	0.09	0.10	0.48	0.62 ± 0.04	0.02	0.77	0.55	0.06	0.18	0.35	0.62 ± 0.04	3.87 ± 0.2	0.30	0.20	0.10	0.49	0.25	0.25
Parapanteles paradoxus	16	2.36 ± 0.21	0.08	0.28	0.04	0.49	0.01	0.76	0.62 ± 0.05	0.08	0.28	0.12	0.19	0.00	0.92	0.62 ± 0.05	3.81 ± 0.23	0.00	0.88	0.01	0.73	0.01	0.73
Parapanteles sp. J	5	2.96 ± 0.21	0.02	0.82	0.02	0.83	0.00	0.95	0.78 ± 0.07	0.03	0.77	0.03	0.77	0.00	0.93	0.78 ± 0.07	3.81 ± 0.12	0.62	0.11	0.01	0.85	0.16	0.50
Parapanteles sp. K	5	2.66 ± 0.47	0.62	0.11	0.02	0.83	0.74	0.06	0.7 ± 0.12	0.09	0.16	0.02	0.82	0.61	0.12	0.7 ± 0.12	3.81 ± 0.17	0.16	0.51	0.05	0.71	0.29	0.35
Parapanteles sp. B	8	2.11 ± 0.1	0.00	0.87	0.86	**0.00**	0.49	**0.05**	0.51 ± 0.03	0.08	0.49	0.69	**0.01**	0.28	0.18	0.51 ± 0.03	4.16 ± 0.15	0.37	0.11	0.04	0.65	0.18	0.30

Figure 3.1: Wing interference patterns of *Parapanteles tessares*. Female wings are shown to the left and males to the right. Horizontal pairs of wing images are of sibling wasps from the same reared brood while each vertical set is from a distinct brood.



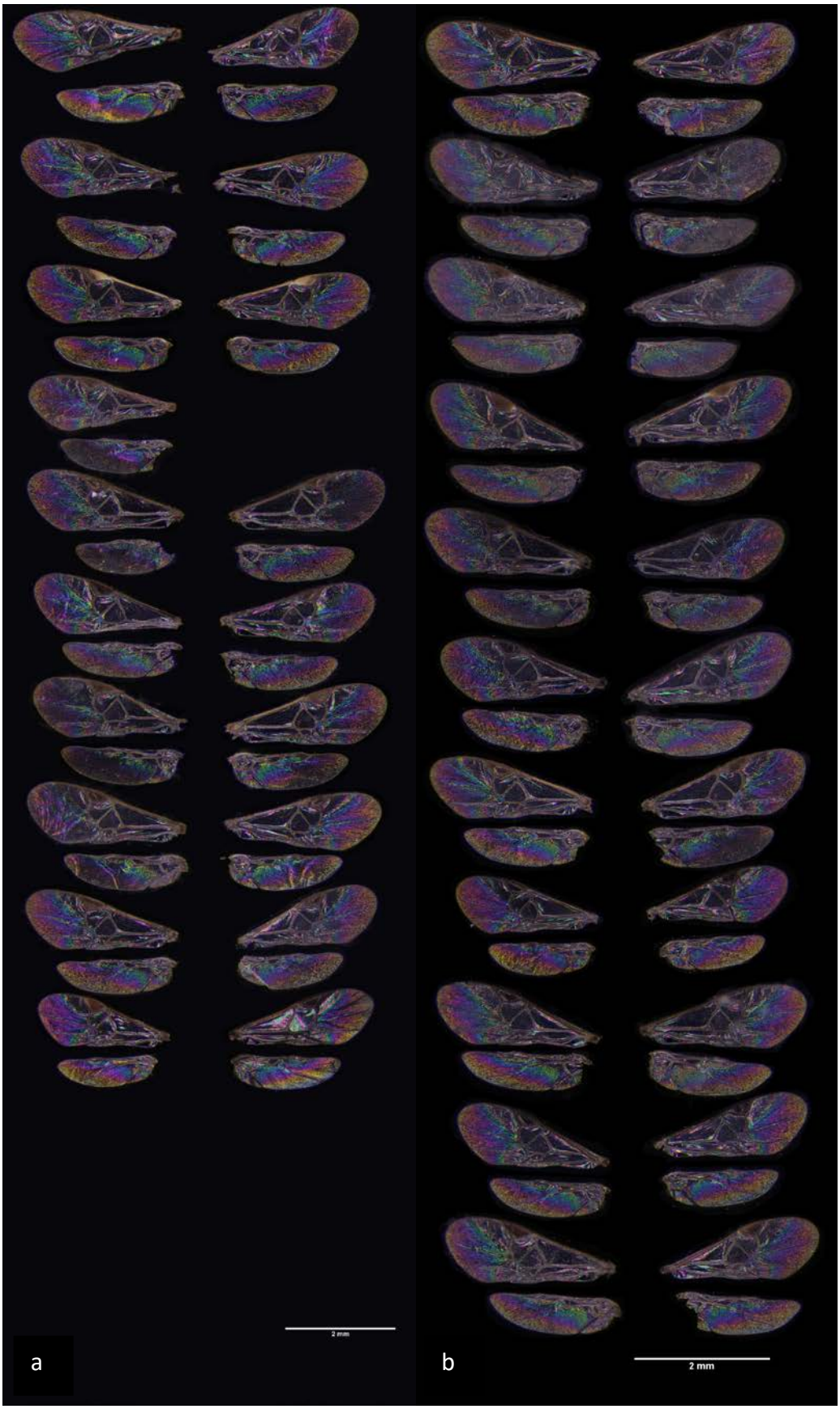


Figure 3.2 a&b: Wing interference patterns of *Parapanteles continua*. Female wings are shown to the left and males to the right. Horizontal pairs of wing images are of sibling wasps from the same reared brood while each vertical set is from a distinct brood.

Figure 3.3: Wing interference patterns of *Parapanteles sicpolus*. Female wings are shown to the left and males to the right. Horizontal pairs of wing images are of sibling wasps from the same reared brood while each vertical set is from a distinct brood.

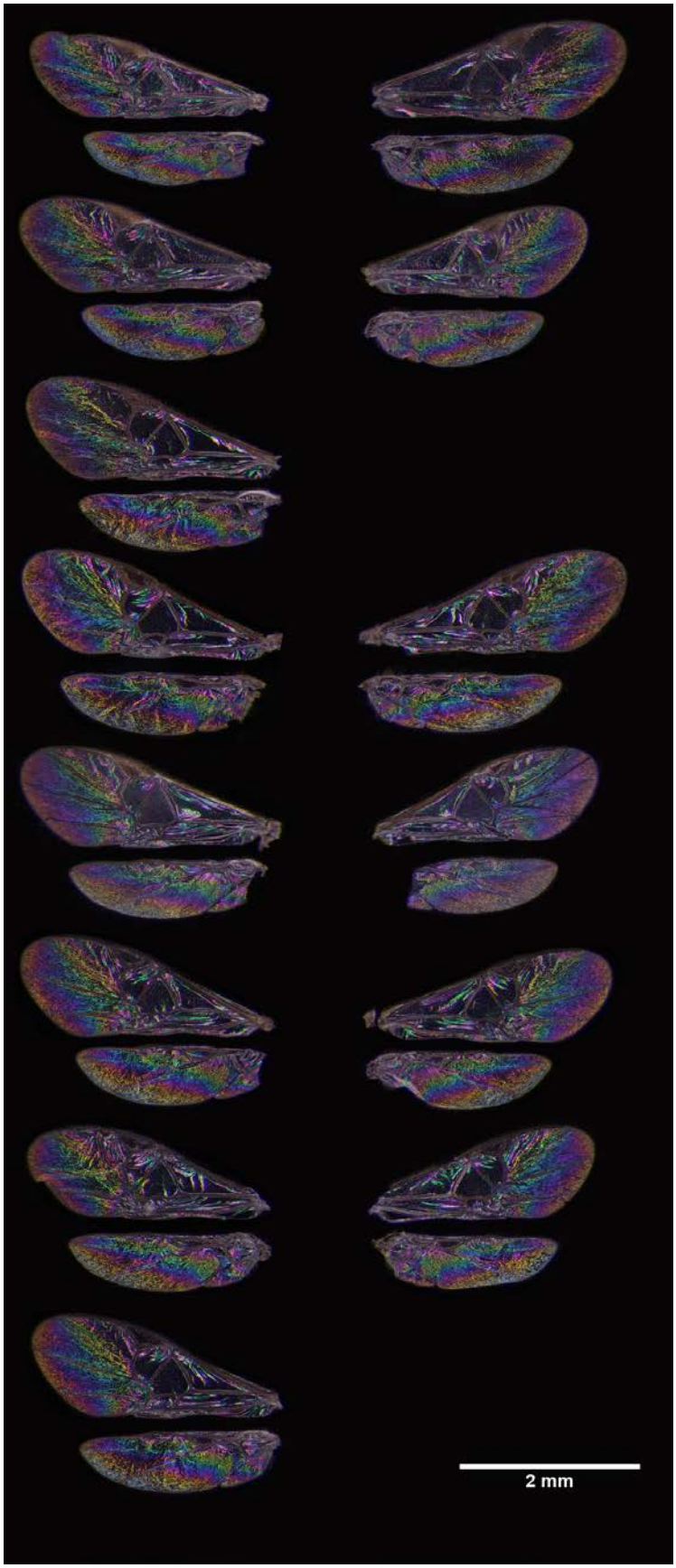


Figure 3.4: Wing interference patterns of *Parapanteles* sp. H. Female wings are shown to the left and males to the right. Horizontal pairs of wing images are of sibling wasps from the same reared brood while each vertical set is from a distinct brood.

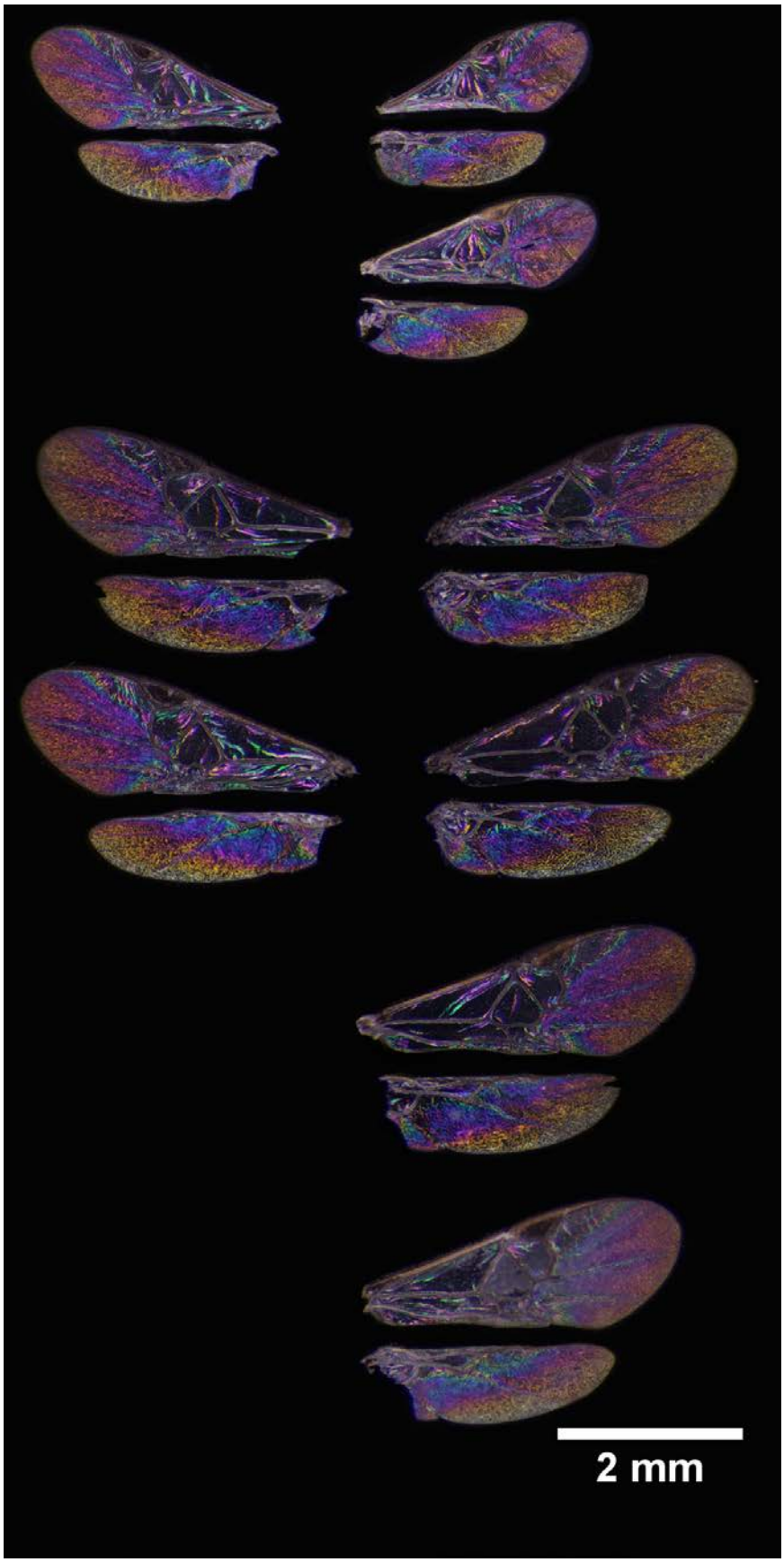


Figure 3.5: Wing interference patterns of *Parapanteles* sp. D. Female wings are shown to the left and males to the right. Horizontal pairs of wing images are of sibling wasps from the same reared brood while each vertical set is from a distinct brood.

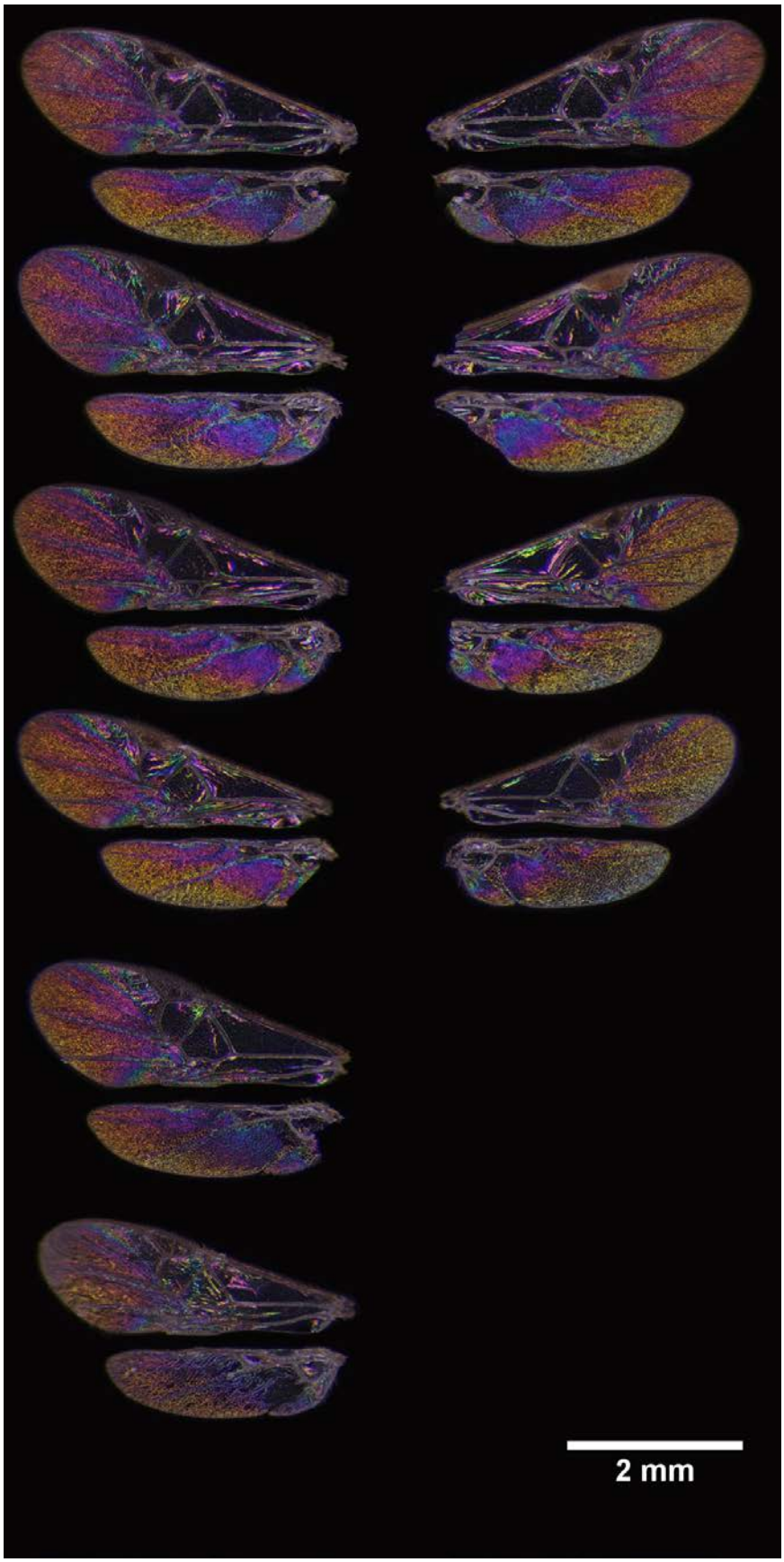


Figure 3.6: Wing interference patterns of *Parapanteles em*. Female wings are shown to the left and males to the right. Horizontal pairs of wing images are of sibling wasps from the same reared brood while each vertical set is from a distinct brood.

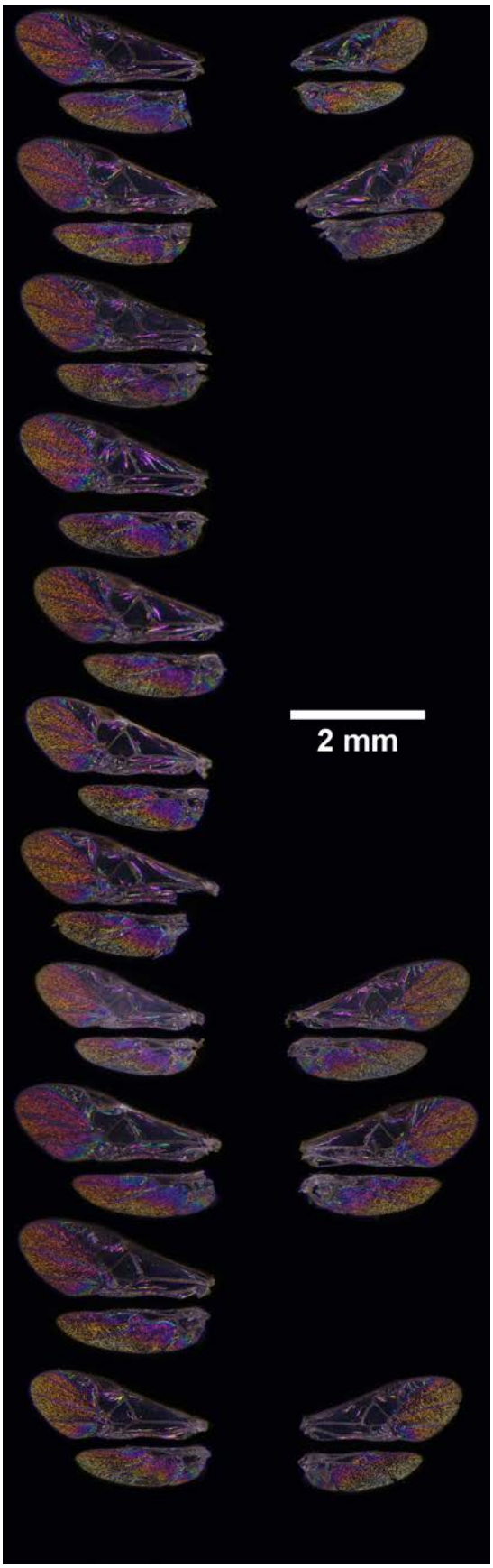


Figure 3.7: Wing interference patterns of *Parapanteles* sp. valerio05. Female wings are shown to the left and males to the right. Horizontal pairs of wing images are of sibling wasps from the same reared brood while each vertical set is from a distinct brood.

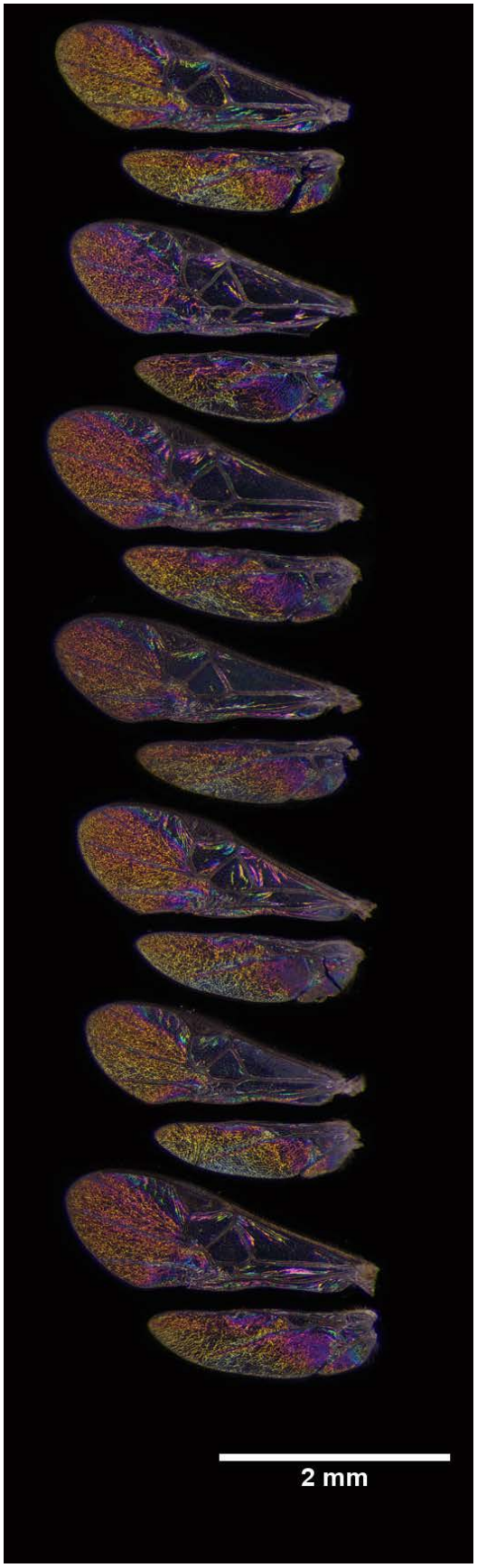


Figure 3.8: Wing interference patterns of *Parapanteles paradoxus*. Female wings are shown to the left and males to the right. Horizontal pairs of wing images are of sibling wasps from the same reared brood while each vertical set is from a distinct brood.

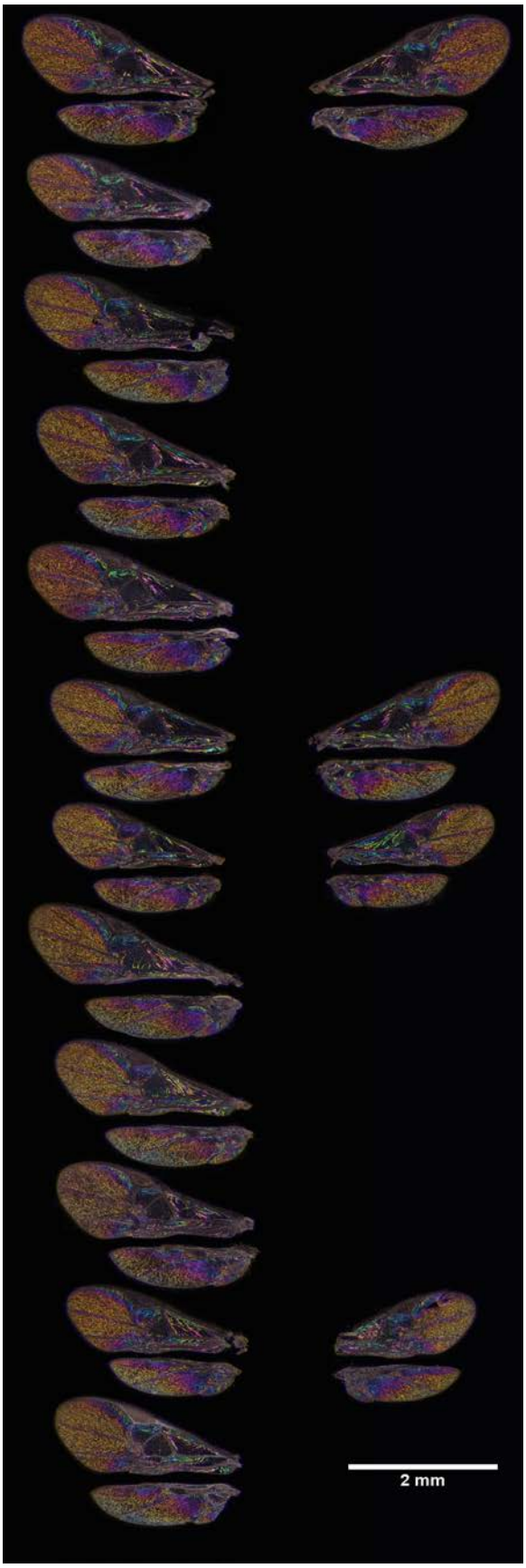


Figure 3.9: Wing interference patterns of *Parapanteles* sp. I. All wings shown are from males, and each vertical set is from a distinct brood.

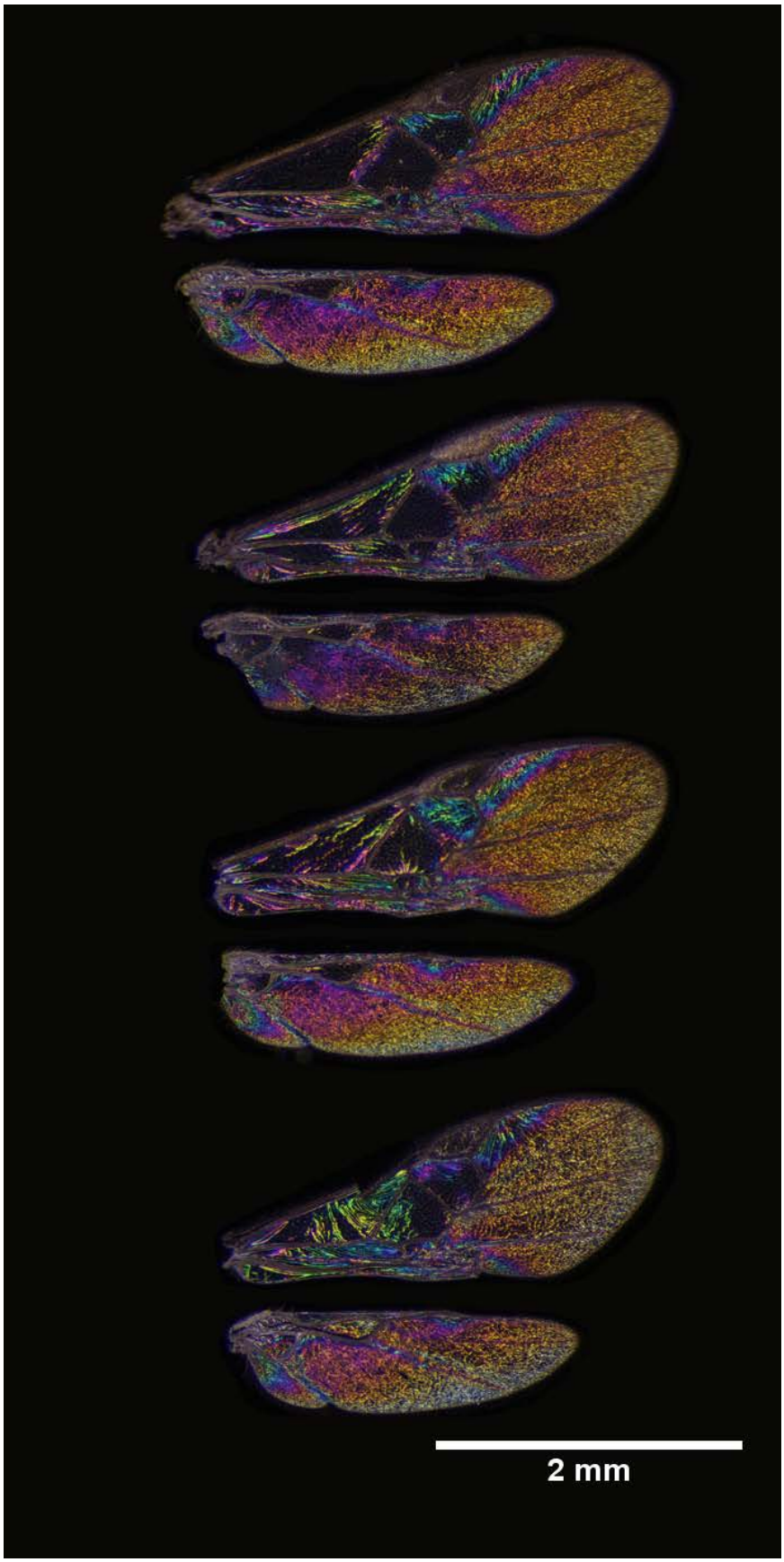


Figure 3.10: Wing interference patterns of *Parapanteles* sp. J. All wings shown are from females, and each vertical set is from a distinct brood.

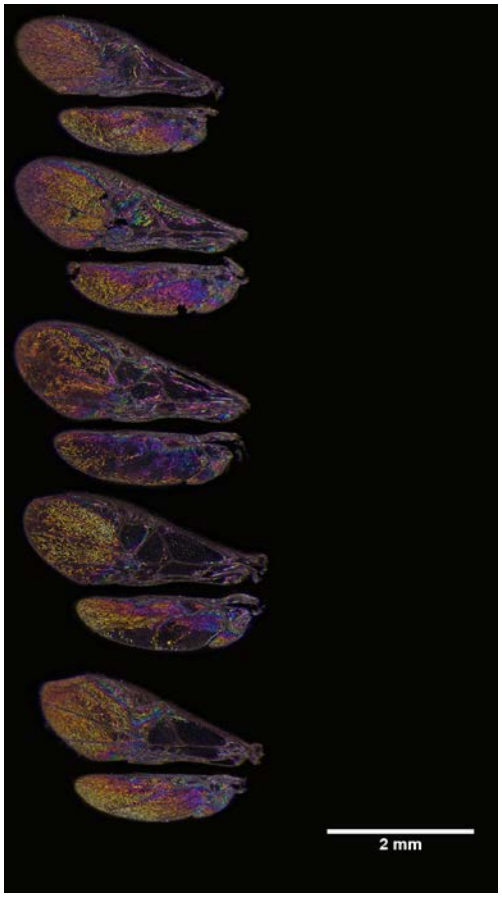


Figure 3.11: Wing interference patterns of *Parapanteles* sp. K. All wings shown are from females, and each vertical set is from a distinct brood.

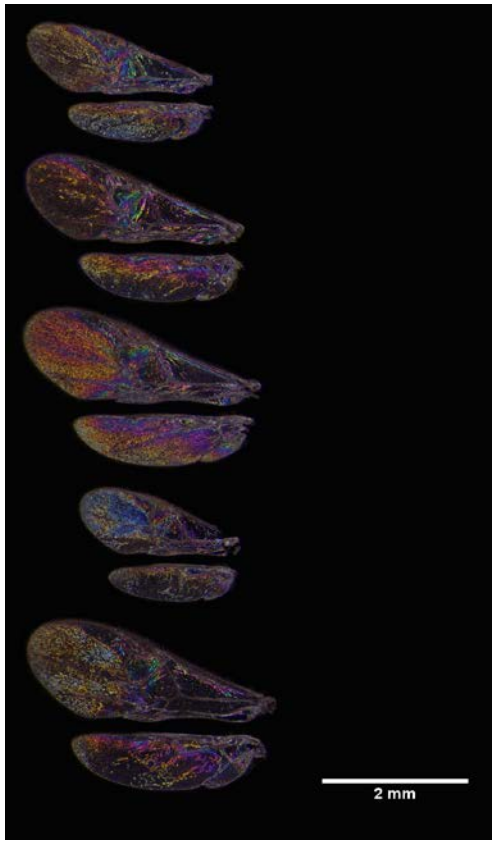


Figure 3.12: Wing interference patterns of *Parapanteles* sp. E. All wings shown are from males, and each vertical set is from a distinct brood.

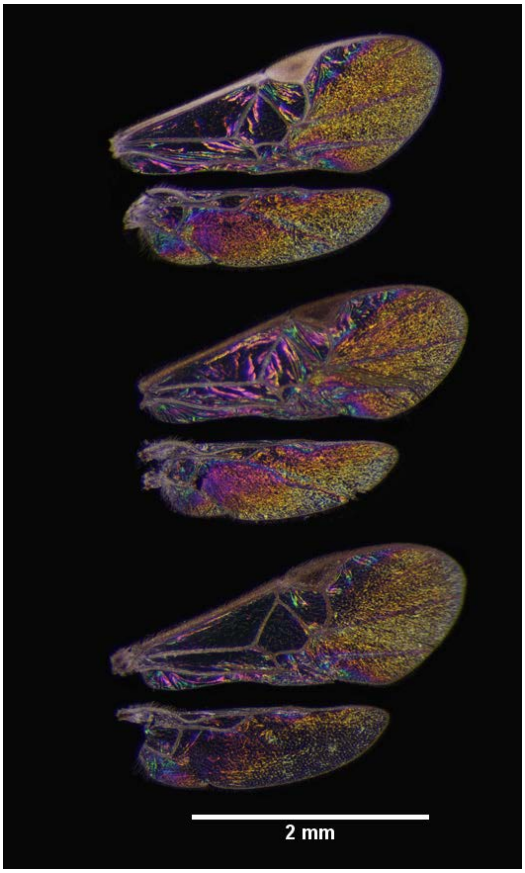


Figure 3.13: Wing interference patterns of *Parapanteles tlinea*. Female wings are shown to the left and males to the right. Horizontal pairs of wing images are of sibling wasps from the same reared brood while each vertical set is from a distinct brood.

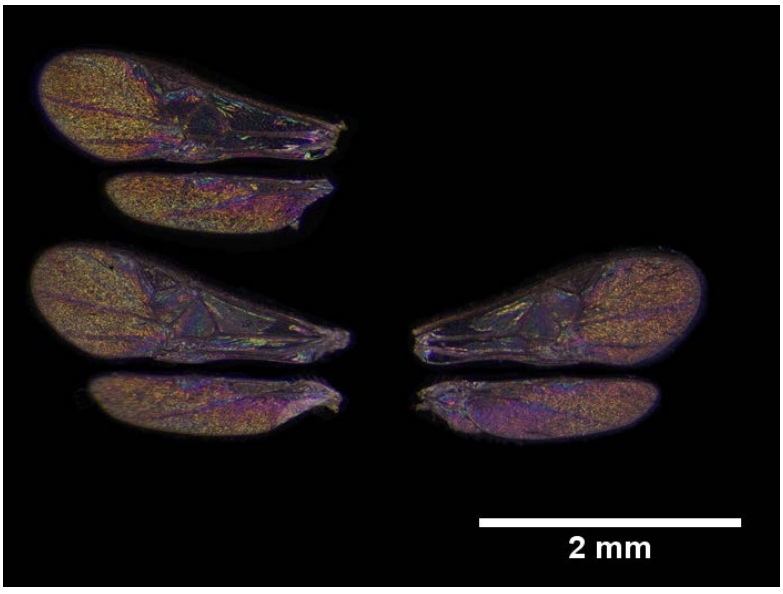


Figure 3.14: Wing interference patterns of *Parapanteles* sp. B. Female wings are shown to the left and males to the right. Horizontal pairs of wing images are of sibling wasps from the same reared brood while each vertical set is from a distinct brood.

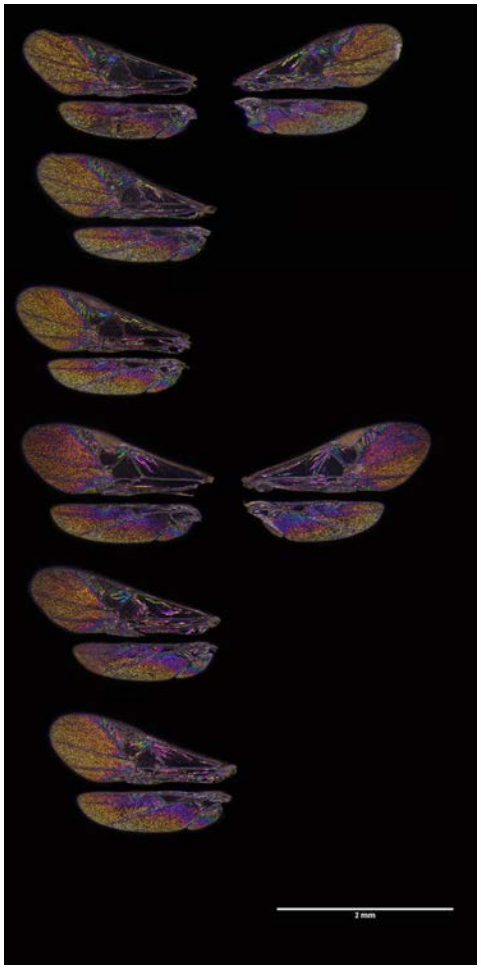
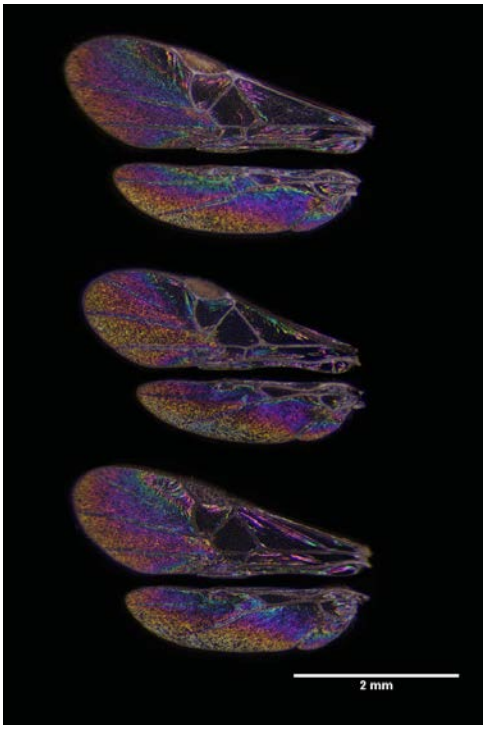


Figure 3.15: Wing interference patterns of *Parapanteles* sp. C. All wings shown are from females, and each vertical set is from a distinct brood.



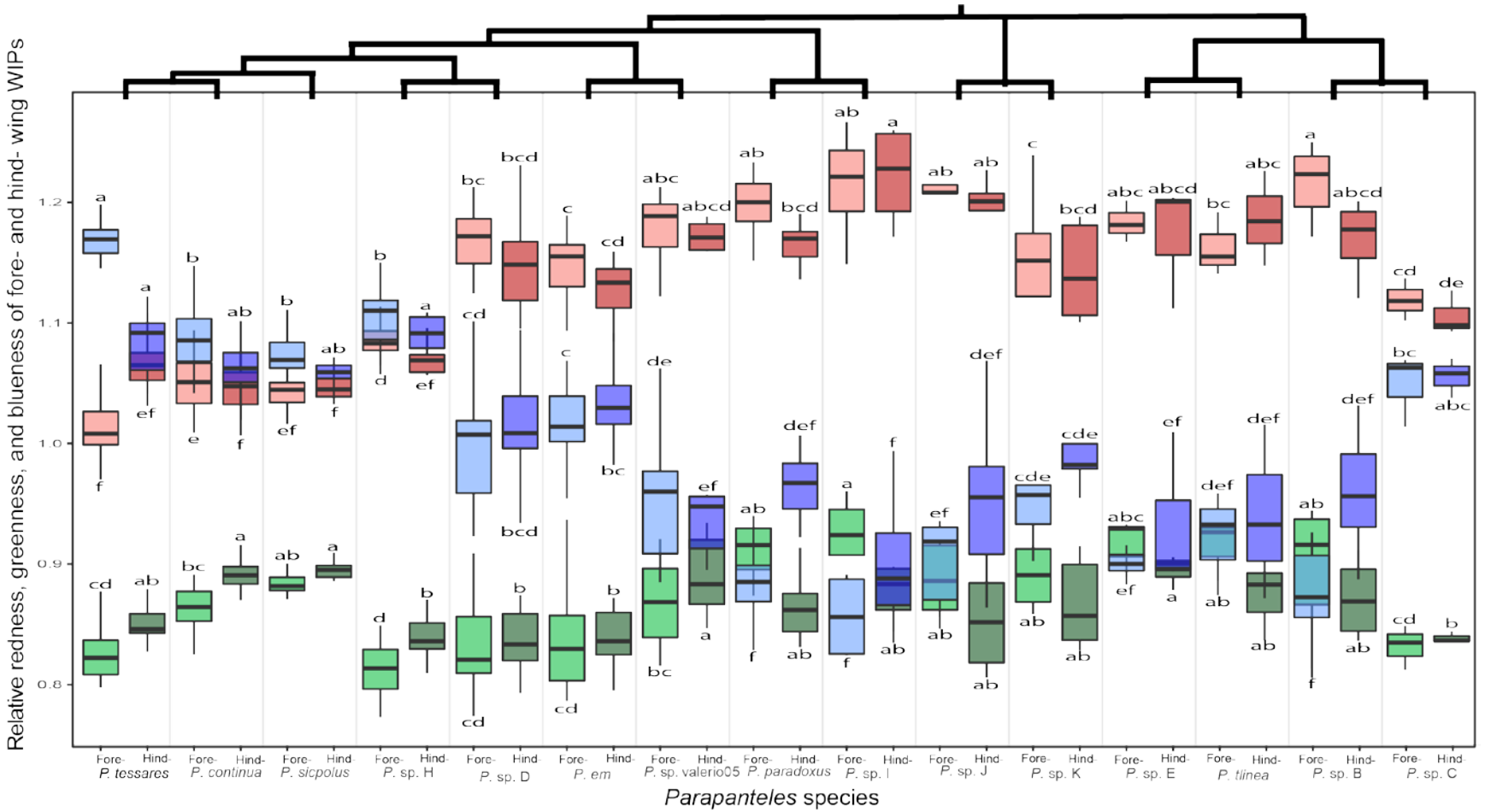


Figure 3.16: Box-and-whiskers plots of forewing and hindwing wing interference pattern relative rednesses (RR), greennesses (RG), and bluenesses (RB) shown in phylogenetic order. The cladogram above the figure is based on results from Chapter 1. RR box-and-whiskers are shown in red, RB in blue, and RG in green. Per-species, forewings are shown to the left of hindwings and shaded a slightly lighter color (for colorblind: all RR values are greater than their corresponding RG values, so all red box-and-whisker plots are above green box-and-whisker plots in the figure). Results of Tukey's HSD test are displayed above or below each box-and-whisker.

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CHAPTER 4: A GENUS-LEVEL MICROGASTRINAE (HYMENOPTERA: BRACONIDAE) PHYLOGENY USING ANCHORED HYBRID ENRICHMENT METHODS

ABSTRACT

Microgastrinae is a hyper-diverse subfamily of Braconidae (Hymenoptera) that parasitizes the larvae of most, if not all, ditrysian Lepidoptera. Previous molecular phylogenies of this taxon have consistently recovered many short and poorly supported basal internal nodes, supporting the hypothesis that Microgastrinae coevolved with their hosts in an ancient rapid speciation event. Microgastrines are also small, about 2-4mm long, and are disproportionately species rich for their morphological diversity, which is estimated to be between 10,000-40,000 species worldwide. Due to these challenges, the systematics of the 64 currently recognized extant genera are still poorly resolved and the monophyly of many of these genera is questionable. To address these challenges, I selected 89 species, broadly from within and across several microgastrine genera, and Drs. Emily and Alan Lemmon at Florida State University performed anchored hybrid enrichment to generate 370 gene fragment sequences for each. Drs. Emily and Alan Lemmon made a concatenated maximum-likelihood analysis of this dataset with RAxML which resolved nearly all nodes with high bootstrap support. This phylogeny supports several larger genera (*Apanteles, Cotesia, Dolihcogenidea,* and *Glyptapanteles*) as mostly monophyletic, although taxa from smaller, rarer genera are recovered within each. It also corroborates previous results that *Parapanteles* is a polyphyletic genus composed of several subclades of disparate genera, although most are within *Dolichogenidea*.

INTRODUCTION

Microgastrinae, one of the most diverse taxa of parasitoid wasps. Many challenges have made the taxonomy and phylogenetics of this group difficult, both of which have changed frequently over the course of the subfamily's history (Nixon 1965, Mason 1981, Whitfield *et al.* 2002, Banks & Whitfield 2006). The subfamily is cosmopolitan, extremely diverse (20,000-40,000 species, with 2689 described to date (Rodriguez *et al.* 2013, Fernández-Triana & Ward

2015)), and both economically and ecologically important as parasitoids of lepidopteran larvae (Shaw & Huddleston 1991). Resolving the generic-level phylogeny of this group is therefore important and, due to a wealth of sampling and advances in sequencing technology, is now more achievable than ever.

The phylogenetics and classification of Microgastrinae have been impeded by their extreme diversity, paucity of easily observed characters due to their small size (generally 2-4mm in body length), and their hypothesized ancient rapid radiation. Microgastrinae is diverse and long-term caterpillar rearing projects coupled with intensive DNA barcoding surveys have hugely expanded species count estimates for this taxon (Smith *et al.* 2008, Janzen *et al.* 2009, Janzen & Hallwachs 2009, Rodriguez *et al.* 2013, Smith *et al.* 2013, Dyer *et al.* 2017). These projects have collected and identified thousands of provisional species but have been predominantly restricted to the Americas, which highlights the amount of entirely undiscovered species that probably exist elsewhere, such as in Old World tropical environments. Much of Microgastrinae's diversity arose during a poorly-understood ancient rapid radiation which corresponds with the diversification of their lepidopteran host species around the same time (Mardulyn & Whitfield 1999, Bands & Whitfield 2006). This diversification event has increased the frequency of poorly supported branches in previous phylogenies and contributed to the inconsistency of generic relationships between phylogenies constructed by previous molecular studies (Mardulyn & Whitfield 1999, Whitfield *et al.* 2002, Banks & Whitfield 2006).

Microgastrinae currently contains 64 genera which have predominantly been defined using morphological methods alone. Many of their most accessible morphological characters are prone to convergence (Mason 1981, Whitfield *et al.* 2002, Wild *et al.* 2013). For example, female microgastrines' ovipositors are used to inject eggs into the body cavity of host caterpillars are therefore under selection. Microgastrine species that attack larger, unconcealed hosts tend to have short ovipositors, while species that attack concealed hosts (e.g., leaf rollers, leaf miners) tend to have longer ovipositors. The suites of characters associated with shorter or longer ovispositors were heavily relied on in the last major revision of Microgastrinae (Mason 1981), which has undoubtedly led to some unnatural taxa due to convergent evolution. Most recent taxonomic publications deal with samples that have been DNA barcoded and contain a

COI tree (e.g. Whitfield *et al.* 2012, Fernández-Triana *et al.* 2013, Fernández-Triana *et al.* 2014), but very few phylogenetic studies of microgastrines have used additional genes. Therefore, beyond the uncertainty surrounding intergeneric phylogenetic relationships, most generic concepts of Microgastrinae have not been adequately scrutinized with molecular evidence. Many larger genera are probably polyphyletic to some degree, and many smaller genera, although often morphologically distinctive, are likely subclades of larger genera.

To address these issues, a good microgastrine phylogeny needs to include multiple species per genus where possible to test the monophlyly of included genera, include as many genera as possible to meaningfully represent the diversity of Microgastrinae itself, and include as much data per species as possible to ameliorate the difficulty of ancient short branches. Thousands of ethanol-preserved samples suitable for phyologenomic sequencing methods are readily available from the aforementioned rearing and DNA barcoding projects (Janzen & Hallwachs 2009, Smith *et al.* 2013, Dyer *et al.* 2017) so assessing whether a set of samples is a good representation of a current generic concept is relatively simple for New World taxa. Anchored hybrid enrichment (AHE) is now relatively inexpensive compared to the costs of traditional Sanger sequencing for multi-gene phylogenies and produces orders of magnitude more data per sample (Lemmon *et al.* 2012). Anchored hybrid enrichment has also been used successfully to produce robust phylogenies of other taxa with similar problems due to ancient rapid radiations (e.g. Etyan *et al.* 2015, Prum *et al.* 2015, Stout *et al.* 2016, Dornburg *et al.* 2017, Léveillé-Bourret *et al.* 2017). The goal of this study is to use DNA barcode sequence data to inform taxon selection for AHE to represent a broad diversity within and between microgastrine genera while being limited to a small number of samples compared to the overall diversity of the taxa included.

METHODS

Taxon Selection, DNA extraction, and DNA quantification

My goals for taxon selection in this study were, in order of priority: to focus on persistent questions in microgastrine systematics, to including multiple disparate species per genus to approximate the breadth of species each genus currently contains, and to include

representative species from as many genera as possible. The genera I prioritized where *Cotesia, Glyptapanteles, Apanteles, Dolichogenidea,* and *Parapanteles.* I used the large COI tree generated for Chapter 2 (Chapter 2 Supplemental Materials 2.3) to select taxa from as many different clades within each genus as possible. I then added or removed taxa to the dataset based on the per-species availability of ethanol-preserved adult wasps or the availability of existing DNA extractions from previous studies, and then ultimately the quality of each DNA extraction. All specimens used in the final dataset that have been sequenced for COI are indicated in Figure 4.1. I included one *Chelonus* species (Braconidae: Cheloninae) as an outgroup.

Where available, I used existing high-quality DNA extractions generated by myself (8 *Dolichogenidea* and 8 *Parapanteles* extractions from Chapter 1) or previous Whitfield lab members (25 Costa Rican *Cotesia* species (e.g. *Cotesia* Whitfield###) were extracted by Jaquiline O'Connor, 13 *Glyptapanteles* species were extracted by Diana Arias-Penna, one *Xanthomicrogaster* species extracted by Andrew Debevec, and I used 11 extractions from several genera that were generated by Mardulyn & Whitfield 1999, Whitfield *et al.* 2002, or Bands & Whitfield 2006). Except for samples from Mardulyn & Whitfield 1999 and Whitfield *et al.* 2002 (DNA extraction method not specified, but probably Phenol-Chloroform), existing DNA extractions and the 23 additional samples I extracted for this study were performed with Qiagen DNEasy Blood and Tissue kits following the manufacturer's protocol. I screened all DNA extractions for quality via gel electrophoresis and quantified each extraction via Qubit dsDNA HS Assay Kits on a Qubit 2.0 Fluorometer at the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois.

Anchored hybrid enrichment and phylogenetic analysis

Library preparation, enrichment, and anchored hybrid enrichment of each genomic DNA samples was performed by Drs. Alan and Emily Lemmon and/or their laboratory technicians at the Center for Anchored Phylogenomics at Florida State University using 57066 probes designed to target 541 loci. Probes were designed based on exon sequences of conserved genes in ant, bee, and ichneumonid genomes. Libraries were sequenced in two Illumina MiSeq lanes.

Reads were screened, assembled, and matched to homologous sequences using the pipeline outlined by Lemmon *et al.* 2012. Loci were individually aligned using MUSCLE (Edgar 2004) and then concatenated. Loci missing for two or more species were discarded. Maximum-likelihood phylogenetic analysis was performed on the concatenated dataset using RAxML v. 8.1.21 (Stamatakis 2014) using the GTR+G substitution model with one partition per locus and 1000 bootstrap replicates.

RESULTS

After elimination of loci missing from two or more species, anchored hybrid enrichment generated sequences for 14973983 base pairs from 168247 sites across 370 loci from 89 species. Of these, 64725 were variable and 52862 were phylogenetically informative. The concatenated alignment contained about 11.5% missing characters.

The RAxML phylogeny (Figure 4.2) had high bootstrap support for most nodes: 77% of nodes were supported with bootstrap values of 100, 11.5% were between 75-99, and 11.5% were below 75. Of the larger genera included, *Cotesia* was recovered within *Glyptapanteles* but with poor support and two major clades of *Glyptapanteles* were recovered. The majority of *Parapanteles* species were recovered within *Dolichogenidea*, with two within *Cotesia* and one within *Apanteles*. *Cotesia* and *Glyptapanteles* were recovered sister to *Dolichogenidea* and *Parapanteles, Apanteles* was recovered sister to *Hypomicrogaster,* but with low support (bootstrap=75), and *Microplitis* was recovered in the most basal clade. Of the smaller genera included, *Alphomelon* was recovered within *Apanteles*; *Exoryza* and *Pholetesor* were within *Dolichogenidea*; and *Prasmodon, Xanthomicrogaster,* and *Diolcogaster* were recovered in the basal clade that contained *Microplitis*. No confidently identified *Protopanteles* were available for this study, but one representative of several tropical species that resemble both *Protopanteles* and *Glyptapanteles* was included in this study, and was recovered within *Glyptapanteles*.

DISCUSSION

Mason's 1981 reclassification of microgastrine genera separated the subfamily into five tribes: Apantelini, of which *Apanteles, Exoryza, Pholetesor, Dolichogenidea,* and *Alphomelon* were included in this study; Microgastrini, of which *Prasmodon, Hypomicrogaster,* and *Xanthomicrogaster* were included; Forniciini, which was not represented in this study; Cotesiini, of which *Glyptapanteles, Protapanteles, Cotesia,* and *Diolcogaster* were included; and Microplitini, of which *Microplitis* was included. Previous molecular phylogenies have not broadly supported these tribes, but the results of different analyses within and between those studies were both too variable to draw strong conclusions (Mardulyn & Whitfield 1999, Whitfield *et al.* 2002, Bands & Whitfield 2006). Here, Apantelini is not recovered as a natural group: *Apanteles* and *Alphomelon* are grouped together, although *Apanteles* contains *Alphomelon*, the clade containing both is sister to *Hypomicrogaster* of Microgastrini, and *Dolichogenidea, Pholetesor,* and *Exoryza* are sister to most members of Cotesiini, although *Dolichogenidea* contains *Exoryza,* and *Pholetesor.* Cotesiini is not generally supported either: while *Glyptapanteles* and *Cotesia,* two major genera of Cotesiini are grouped together, *Cotesia* is recovered within *Glypapanteles* (with poor support). *Parapanteles* is recovered within *Dolichogenidea* of Apantelini (corroborating the results of Chapter 1), and *Diolcogaster* is recovered in the basal clade containing the remaining gerena from Microplitini (*Microplitis*) and Microgastrini (*Prasmodon* and *Xanthomicrogaster*).

All previous Microgastrinae molecular phylogenies have had numerous poorly supported internal nodes, especially between representatives of different genera (Mardulyn & Whitfield 1999, Whitfield *et al.* 2002, Bands & Whitfield 2006). The phylogeny produced by this study is much less ambiguous and support for most nodes is robust. Although many genera are missing from this study, these results place genera that have been recovered in variable positions in previous phylogenies, such as *Dolichogenidea, Parapanteles,* and *Hypomicrogaster* (Mardulyn & Whitfield 1999, Whitfield *et al.* 2002, Bands & Whitfield 2006), more confidently than ever before. Expansion of this method to Microgastrinae more comprehensively and will likely solve most of the lingering phylogenetic problems of this group. Unfortunately most long-term rearing and DNA barcoding projects of Microgastrinae have been restricted to the New

World, and adding Old World taxa to this approach in a meaningful way will require a massive collection effort.

FIGURES

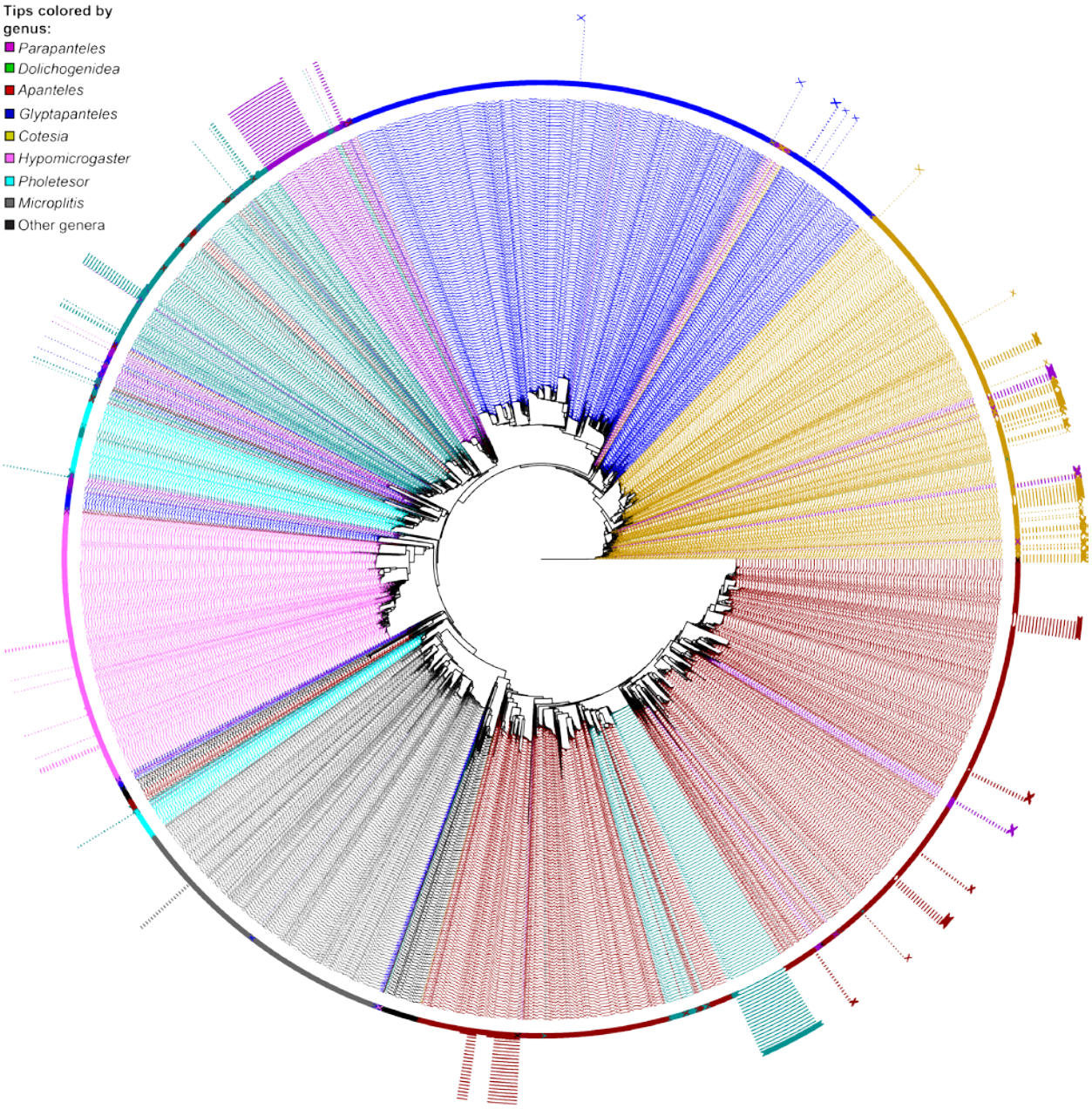
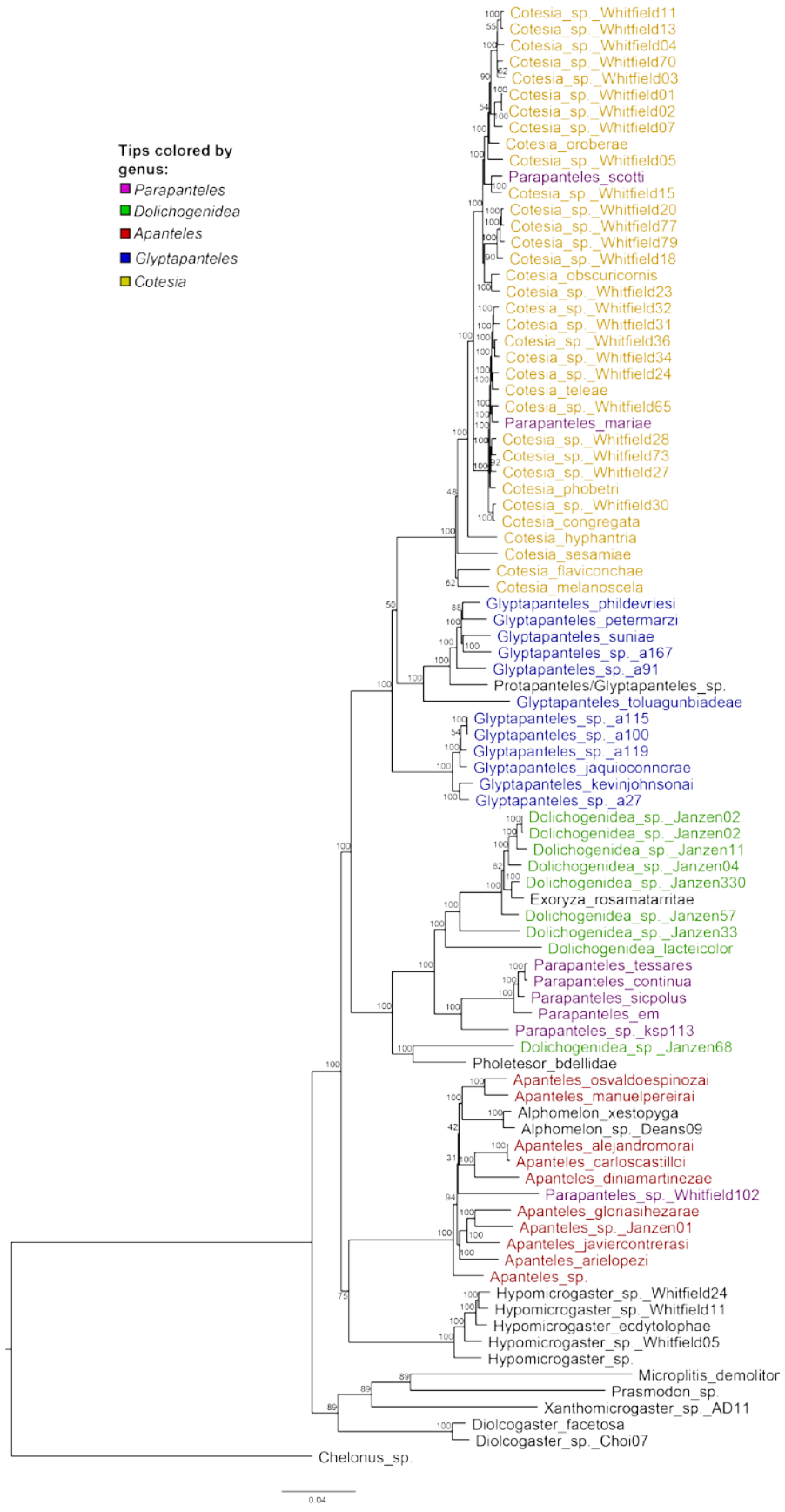


Figure 4.1: Fasttree approximated maximum-likelihood phylogeny of 14247 microgastrine COI samples. Taxon labels are colored by genus, with purple corresponding to *Parapanteles,* green to *Dolichogenidea*, teal to *Pholetesor,* red to *Apanteles*, blue to *Glyptapanteles*, yellow to *Cotesia,* pink to *Hypomicrogaster*, and grey to *Microplitis*. Taxa labels of all other genera are black. Subsamples selected for anchored hybrid enrichment analysis are indicated by extended taxon labels.

Figure 4.2: RAxML maximum likelihood phylogeny of 370 concatenated anchored hybrid enrichment loci of microgastrine wasps. Bootstrap values are shown above or below corresponding branches. Tip labels are colored by genus, with purple corresponding to *Parapanteles*, green to *Dolichogenidea*, red to *Apanteles*, blue to *Glyptapanteles*, yellow to *Cotesia,* and black to all other genera.



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CHAPTER 5: POLYDNAVIRUS VIRULENCE GENE DUPLICATION AND EVOLUTION IN *PARAPANTELES* WASPS (BRACONIDAE: MICROGASTRINAE)

ABSTRACT

Microgastrinae wasps have symbiotic viruses, known as polydnaviruses, encoded within their nuclear genomes, that females produce and inject, along with eggs, into their host caterpillars. Larval microgastrines cannot successfully parasitize their hosts without polydnaviruses. Virtually all that is known about microgastrine polydnaviruses are known from five species from three genera. Here I sequenced the genomes of 16 microgastrine species from a monophyletic clade of *Parapanteles* Ashmead with extensive host-use records, and annotated polydnavirus genes in each genome. I found that probable duplications, pseudogenes, and rearrangements are common, especially in the protein-tyrosine-phosphatase polydnavirus gene family. These results support the model that frequent gene births and deaths are a major factor in polydnavirus genome evolution, and extend our knowledge of polydnaviruses to a major previously unexplored segment of the microgastrine phylogeny.

INTRODUCTION

Polydnaviruses are mutualistic viruses used by two groups of parasitoid wasps to manipulate the immune response and physiology of those wasps' larval hosts. Banchine and campoplegine ichneumonids (Hymenoptera: Ichneumonoidea) have ichnovirus polydnaviruses (a few have virus-like particles derived from alpha-nudiviruses), while bracoviruses are similar but unrelated polydnaviruses, derived from beta-nudiviruses, found in the braconid microgastroid complex (Stoltz *et al.* 1984, Kroemer & Webb 2004, Drezen *et al.* 2017). Microgastrine wasps rely on bracoviruses to successfully parasitize their lepidopteran hosts by altering physiology and suppressing the immune system of these hosts (Strand & Burke 2014). The microgastroid complex (microgastrines and 5 other subfamilies) acquired bracoviruses approximately 100 million years ago, shortly before undergoing a rapid diversification of genera and species, and bracoviruses continue to evolve with host immune adaptations (Banks & Whitfield 2006, Murphy *et al.* 2008, Strand & Burke 2014).

Bracovirus genomes have been studied in five species from three highly diverse and cosmopolitan genera: *Cotesia congregata, C. vestalis, Glyptapanteles flavicoxis, G. indiensis* and *Microplitis demolitor* (Espagne *et al.* 2004, Desjardins *et al.* 2008, Chen *et al.* 2011, Burke & Strand 2012, Burke 2016). *Cotesia* and *Glyptapanteles* are closely related and diverged from *Microplitis* approximately 53 million years ago (Murphy *et al.* 2008). *Cotesia* and *Glyptapanteles* are among the most species-rich microgastrine genera and none have well resolved species phylogenies. The extent to which bracoviruses have influenced host use and speciation in Microgastrinae is therefore not well known.

Bracovirus genomes consist of genes that produce nudivirus-like viral particles, and genes that are packaged into these viral particles as circular DNA segments (Strand & Burke 2014). The genes that produce the nudivirus-like viral particles, generally viral capsid and envelope proteins and various RNA and DNA polymerases, originate from an ancestral nudivirus (Bézier *et al.* 2009). The genes that are packaged into the viral particles, referred to as the "encapsidated genome", are of diverse origin, including orthologs of wasp genes, but generally do not include nudivirus-origin genes (Bézier *et al.* 2009, Huguet *et al.* 2012). In the calyx cells of female wasps, disparate regions of the nuclear genome known as "proviral loci" contain pieces of the encapsidated genome and are copied in varying abundance to produce 15-30 unique circular DNA fragments ranging from 2.5-50kb long each containing virulence genes (Webb *et al.* 2006, Chen *et al.* 2011, Burke *et al.* 2014). Bracoviruses are therefore amalgamations of nudivirus-origin genes that produce the protein structure of viral particles and wasp- and unknown-origin genes that are copied and packaged into those viral particles to cause pathology in the host caterpillar.

The most well-known function of bracoviruses is reduction or disablement of the immune response of their wasps' hosts, preventing those hosts from killing wasp eggs and larvae (Strand & Burke 2014). The genes in the encaspidated genome that contribute to this effect are referred to as "virulence genes". These virulence genes are expressed in the tissues of microgastrines' hosts, often through integration into host cell DNA (Beck *et al.* 2011). The specific mechanism of immunosuppression varies between wasp species and includes disruption of host haemocyte function, destruction of host haemocytes, and disruption of

major insect immunity pathways, such as the phenoloxidase (PO) cascade and the Toll and immune deficiency (IMD) pathways (Kroemer & Webb 2004, Strand & Burke 2014).

The diversity in the mode of action of bracoviruses is hypothesized to be due to a co-evolutionary arms race between parasitoids and their hosts, in which bracovirus virulence genes rapidly evolve in response to adaptations in host caterpillar immune systems (Huguet *et al.* 2012, Strand & Burke 2014). In contrast, the bracovirus viral-origin genes responsible for producing and packaging viral particles in the calyx cells of female wasps are hypothesized to be conserved and to evolve at constant rates. The results of studies analyzing signatures of selection in bracovirus virulence genes conflict: one study of four *Cotesia* species bracoviruses identified several virulence genes under positive selection (Jancek *et al.* 2013), while another study of two *Microplitis* bracoviruses found most virulence genes were under purifying selection (Burke 2016). Further supporting the arms-race hypothesis, virulence gene families are prone to duplication and evolve rapidly (Desjardins *et al.* 2008, Serbielle *et al.* 2008, Chen *et al.* 2011, Serbielle *et al.* 2012, Burke *et al.* 2014). Furthermore, virulence gene families may be acquired or lost frequently throughout microgastrine evolution (e.g. *egf*-motif genes in *Microplitis*, *cys*-motif genes in *Cotesia* and *Glyptapanteles*) (Huguet *et al.* 2012).

Despite these acquisitions, losses, and duplications, two virulence gene families are present in all known bracoviruses: ankyrin repeat (ANK) genes and protein tyrosine phosphatase (PTP) genes (Huguet *et al.* 2012). Some ankyrin repeat genes are known to disrupt Toll and IMD pathways (Thoetkiattikul *et al.* 2005, Bitra *et al.* 2012). The PTP gene family is diverse and highly duplicated in bracovirus genomes and is associated with manipulations of host physiology, including immunosuppression (Provost *et al.* 2004, Falabella *et al.* 2006, Ibrahim & Kim 2008, Serbielle *et al.* 2012). In addition to immunosuppression, bracoviruses also manipulate hosts' development and growth in ways that favor the development of parasitoid larvae. Bracoviruses do this by altering developmental hormone titers, retarding host development and preventing metamorphosis, and by manipulating host metabolic pathways to increase investment in tissues consumed by developing parasitoid larvae (Gundersen-Rindal 2012, Strand & Burke 2014). The specific genes responsible for these hormonal and metabolic alterations are not known, although some PTP genes are involved in these physiological

alterations (Falabella *et al.* 2006, Kim *et al.* 2013). Copy numbers of both gene families vary between all known microgastrine bracovirus genomes: *M. demolitor* has 12 ANK and 13 PTP genes, *G. indiensis* has 9 ANKs and 42 PTPs*, G. flavicoxis* has 8 ANKs and 31 PTPs*, C. vestalis* has 6 ANKs and 33 PTPs*,* and *C. congregata* has 6 ANKs and 27 PTPs (Espagne *et al.* 2004, Desjardins *et al.* 2008, Chen *et al.* 2011, Burke *et al.* 2014). Phylogenetic analyses of both gene families suggests that many conspecific paralogues of both genes are more closely related to each other than to potential orthologues in other species, further suggesting that duplications are common in these gene families (Chen *et al.* 2011).

In order to fully understand polydnavirus evolution, polydnavirus genomes must be sequenced from many more species of microgastrines. However, this poses several challenges. Microgastrines are small animals that contain small amounts of DNA, few species are kept in laboratory colonies, most microgastrine species are difficult to identify morphologically, and most existing specimens are either dried and pinned or preserved in ethanol. In female wasps, calyx cells produce viral particles which contain the encapsidated genome of the wasp's bracovirus (Stoltz *et al.* 1976, Webb *et al.* 2006, Bézier *et al.* 2009). Several previous studies have isolated and sequenced the complete encapsidated bracovirus genomes from the calyx cells (Espagne *et al.* 2004, Desjardins *et al.* 2008, Chen *et al.* 2011, Burke & Strand 2012, Burke 2016). The small size of these tissues necessitates pooling of multiple females' calyces before sequencing. Due to the small amount of tissue per wasp and the non-uniform copy number of each fragment, previous studies have used deep-sequencing approaches of pooled calyx extractions to sequence bracovirus encapsidated genomes (Chen *et al.*, Burke *et al.* 2014, Burke 2016). This approach is impractical for surveying bracoviruses from a broad diversity of microgastrine species. While many microgastrines are gregarious, meaning multiple sibling wasps emerge from a single host, and, if well preserved, calyces from multiple females from a brood can be pooled, the number of individuals per brood varies widely between species and many other species are solitary (Shaw & Huddleston 1991, Janzen *et al.* 2009).

The entire bracovirus genome, including the nudivirus-origin genes that are not packaged into viral particles, are dispersed throughout the nuclear genome of microgastroids (Webb *et al.* 2006, Bézier *et al.* 2009, Burke *et al.* 2014). Nuclear DNA from female or male

wasps can therefore be sequenced to identify bracovirus genes in previously unstudied species. In Hymenoptera, males are haploid, and their lack of heterozygosity makes them ideal for next-generation DNA sequencing methods. However, bracovirus gene families evolve at different rates, are acquired and lost across genera, and are prone to gene loss and duplication (Strand & Burke 2014).

The monophyletic clade containing most *Parapanteles* species identified in Chapter 2 is ideal for studying polydnaviruses because is located within an extremely diverse portion of the broader microgastrine phylogeny, distant from *Cotesia, Glyptapanteles,* and *Microplitis* (Chapter 4 Figure 4.1) (whose bracoviruses have been virtually completely unstudied), it contains several examples of closely related species that parasitize relatively closely related hosts, and it also contains several family-level host-shifts (Chapter 2 Figure 2.2). In this chapter, I sequenced 16 *Parapanteles* species from the core monophyletic clade of *Parapanteles* identified in Chapter 2. and identified and annotated polydnavirus virulence genes in each. My main goals were to determine the number and relative genomic locations of genes from major polydnavirus gene families in each species and to broadly identify trends in their evolution.

METHODS

DNA Extractions, Illumina sequencing & Assembly

I extracted genomic DNA from the whole body of a single male from each of 16 *Parapanteles* species using Qiagen QIAamp Micro DNA Kits. Extractions were quantified using Qubit dsDNA HS Assay Kits on a Qubit 2.0 Fluorometer at the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois Urbana-Champaign.

Library preparation and sequencing for each DNA extraction was performed at the W.M. Keck Center for Comparative and Functional Genomics. One 400 bp-insert shotgun genomic library was prepared from each extraction using the Hyper Library construction kit from Kapa Biosystems. Libraries were sequenced with other samples on an Illumina HiSeq 4000 lane for 151 cycles using HiSeq 4000 sequencing kit version 1. *Parapanteles continua* was titrated to be about 15% of the DNA loaded into a HiSeq 4000 lane containing samples from other research projects. *Parapanteles em, P.* sp. C, *P.* sp. G, *P.* sp. J, *P.* sp. whitfield08, *P. tessares,* and *P. tlinea*

were sequenced together on a second lane, and *P. paradoxus, P. sicpolus, P.* sp. B, *P.* sp. D, *P.* sp. F, *P.* sp. I, *P.* sp. K, and *P.* sp. valerio05 were sequenced together on a third lane. FASTQ files were generated and demultiplexed with bcl2fastq v.2.17.1.14 Conversion Software (Illumina).

I trimmed raw reads using Trimmomatic v0.32 (Bolger *et al.* 2014) and assembled each genome using SOAPdenovo v.2.04 (Luo *et al.* 2012) with k=49, except for *Parapanteles continua* which was assembled using the same programs and paramaters by Kim Walden at the University of Illinois Urbana-Champaign. I assessed genome completeness using BUSCO v3.0.1 with the *odb9* Hymenoptera input dataset containing 4415 "universal sing-copy orthologs" available via the author's website (Simão *et al.* 2015, busco.ezlab.org). The program BUSCO (Benchmarking Universal Single-Copy Orthologs) assesses the completeness of a genome assembly by searching within it for a set of genes with a single ortholog through a specific taxon, producing gene models for each gene found and a summary of the number of complete, duplicated, fragmented, and missing genes it found. I included the *M. demolitor* genome assembly (Mdem2) available on Genbank (Genome ID: 12766) from Burke *et al.* (2014, 2018) for comparison.

BUSCO & species tree

I imported BUSCO gene assemblies for each *Parapanteles* genome into Geneious v.10.1.3 and selected the first 101 genes which had relatively complete gene sequences for all 16 species. I aligned each gene via MUSCLE v.3.8.31 (Edgar 2004). This resulted in over 1 million aligned base pair total, so I trimmed each alignment down to well-aligned (presumably exonic) regions, concatenated all alignments, and constructed a phylogenetic tree using RAxML v.8.2.7 with 1000 bootstrap replicates using the GTR+I+G substation model and all genes partitioned (Stamatakis 2014).

Gene searches & annotations

I converted the FASTA file of each *Parapantles* genome assembly into a searchable BLAST database with BLAST+ v.2.5.0 in Geneious v.10.1.3 (Kearse *et al.* 2012). I accessed 474 translated sequences from bracovirus virulence genes, including redundant orthologs of genes

from several species, from GenBank (SM 5.19). I used the Query-centric alignment only option of BLAST in Geneious to BLAST search the sets of 474 virulence genes against each genome assembly, with the maximum number of hits per query set to 15. I then organized all hits by scaffold/contig, reverse transcribed them, and mapped them to the reference of each individual scaffold with hits.

In all previously sequenced polydnaviruses most viral segments contain multiple virulence genes and in *Microplitis demolitor* most of the proviral loci that produce those segments are adjacent to each other in the genome (Desjardin *et al.* 2008, Chen *et al.* 2011, Burke *et al.* 2014). Consequently, I predicted that search hits for polydnavirus virulence genes that were near other distinct search hits for polydnavirus virulence genes were more likely actual polydnavirus virulence genes than lone search hits on scaffolds with no other apparent polydnavirus genes. Therefore, I initially limited gene annotations to scaffolds from each genome that had two or more distinct search hits and annotated the gene or gene fragments on each of these scaffolds. This resulted in 890 gene annotations. I created a BLAST database of all 16 *Parapanteles* genome assemblies together and BLAST searched my initial 890 gene models against this database using a standard hit table limited to the top 50 BLAST hits. I then created a list of the scaffold or contig containing the top BLAST hit for each *Parapanteles* species, excluding those searches that only produced conspecific hits (e.g. a *Parapanteles* sp. B gene that returned 50 *Parapanteles* sp. B hits) (SM 5.20). I used this list to identify 27 sets of scaffolds related to each other by gene content across *Parapanteles* species (SM 5.21). I limited further annotations to scaffold sets that contained multiple distinct viral ankyrin and/or protein tyrosine phosphatase genes (SM 5.22, sets 10, 11, 15, 16, 18, 19, 20) and annotated all polydnavirus gene BLAST search hits on the scaffolds in these sets that I had not previously annotated. I labeled genes with unexpected stop codons as potential pseudogenes and genes with incomplete sequences due to incomplete genome assembly as fragments.

Gene trees and rough alignment potentially homologous scaffolds

I extracted the nucleotide sequences of all viral ankyrin and protein-tyrosine phosophatase gene and gene fragment annotations from their corresponding scaffolds. I

combined these with available PTP and ANK nucleotide sequences of *Microplitis demolitor* and *Cotesia congregata* accessed from Genbank (SM 5.23), aligned each gene family with MAFFT v. 7.388 (Katoh *et al.* 2002, Katoh & Standley 2014), and constructed phylogenies for each with RAxML v.8.2.7 with 1000 bootstrap replicates using the GTR+G substitution model and no internal partitions. To visualize patterns of PTP gene duplications, I color-coded homologous genes and roughly aligned scaffold sets by lining up those genes in Geneious v10.1.3. I created this "rough alignment" entirely by hand: it is not a base-pair or codon alignment and is only intended to help visualize the orientation and arrangement of PTP and ANK genes across *Parapanteles* genomes, not to infer homology at the sequence level more broadly.

RESULTS

Genome sequencing and assemblies

Each genomic DNA shotgun library produced on average 41.8 million reads with an average assembled genome size of 123.7 megabases. The average N=50 for all species was 146.5kb; the average coverage was 102x. The *P. tlinea* library produced the most raw reads (61.8 million) while *P. tessares* produced the biggest genome assembly (140.5 megabases) and *P.* sp. whitfield08 had the highest N=50 (416.6kb). The *P. tessares* library produced the fewest raw reads (33.1 million), *P.* sp. whitfield08 had the smallest assembled genome (110 megabases), and *P.* sp. J had the lowest N=50 (68.6kb) (Table 5.1).

BUSCO

Of 4415 Hymenoptera-specific BUSCOs searched, the current *Microplitis demolitor* genome assembly returned 4014 complete single-copy BUSCOs (91%), 38 complete duplicated BUSCOs (<1%), 184 fragments (4%), and 179 missing BUSCOs (4%). *Parapanteles* sp. assemblies from this study had on average 3555 complete single-copy BUSCOs (81%), 19 duplicate BUSCOs (<1%), 471 fragmented BUSCOs (11%), and 355 missing BUSCOs (8%) (Table 5.2). The *P. continua* assembly had the fewest complete (68%) and the most missing (17%) BUSCOs, while *P.* sp. G, I, & K had high numbers of complete BUSCOs (85% on average) and the lowest

numbers of missing BUSCOs (6% on average). 143 BUSCOs were missing from all *Parapanteles* sp., of which 125 were also missing from *M. demolitor* (SM 5.19).

Refined species tree

I combined well-aligned fragments, ranging from 575bp to 13.6kb, from 101 orthologous gene models retrieved by BUSCO into a 306.5kb concatenated alignment. The RAxML tree constructed from this alignment fully corroborates the species relationships recovered in Chapter 2 with uniformly improved bootstrap support (Figure 5.1).

Gene annotations

I initially limited gene annotations to scaffolds with multiple distinct polydnavirus gene BLAST search hits. From these, I annotated 890 gene or gene fragments across 336 scaffolds or contigs (SM 5.20). I identified an additional 765 scaffolds containing potential polydnavirus virulence genes by BLAST searching the original 890 gene annotations against all *Parapanteles* genomes together, although most of these additional scaffolds or contigs contain just one polydnavirus gene or gene fragment (SM 5.21). I annotated the remaining BLAST search results on seven sets of scaffolds/contigs containing multiple PTP and/or ANK genes (SM 5.22, sets 10, 11, 15, 16, 18, 19, & 20). I found 170 ANK genes or gene fragments and 435 PTP genes or gene fragments on these scaffold sets (Tables 5.3 & 5.4). Among these many were incomplete due to gaps in genome assembly and I labeled them fragments. Forty seven of the 170 ANK genes and 124 of the 435 PTP genes contain unexpected in-frame stop codons that I could not explain with introns and I labeled them as potential pseudogenes. None of the PTP nor ANK genes from these scaffold sets have obvious introns. Most PTP genes were approximately 800-900bp long and most ANK genes were approximately 500bp long (SM 5.23-5.26).

Gene trees

The RAxML PTP gene tree (Figure 5.22) recovered 27 distinct clades of *Parapanteles* PTP genes, of which the basal nodes of four were supported by a bootstrap value below 95 (Figure 5.2, PTP 1.1 (bootstrap of 88), PTP 5 (bootstrap of 93), PTP 6 (bootstrap of 10), and PTP 7

(bootstrap of 66). Thirteen *Parapanteles* PTP clades are more immediate sisters to a *C. congregata* or *M. demolitor* PTP than to another *Parapanteles* PTP clade (Figure 5.2, PTP clades 5, 9, 10, 11, 14, 15, 18, 19, 20, 23, 24, 25, & 26). The PTP gene tree contains at least 116 gene duplications within *Parapanteles* PTP clades (Figure 5.2, yellow stars) and nine that predate these clades (Figure 5.2, white stars). I divided PTP gene clade 1 into two subsets (1.1 and 1.2) because it is duplicated in all but *P.* sp. B, *P.* sp. C, and *P.* sp. J. Each of the 27 PTP clades contain at least one gene duplication in at least one *Parapanteles* and at least one potential pseudogene.

The RAxML ANK gene tree (Figure 5.3) recovered 11 distinct clades of *Parapantles* ANK genes. All but *Parapantles* ANK clade 1 are supported by a bootstrap value below 95. Four *Parapanteles* ANK clades are more immediate sisters to a *C. congregata* or *M. demolitor* ANK gene than to another *Parapanteles* ANK clade (Figure 5.3, ANK clades 2, 5, 10, & 11). The ANK gene tree contains at least 40 gene duplications within *Parapanteles* ANK clades (Figure 5.3, yellow stars). *Parapanteles* ANK clades 4, 7, and 8 have no gene duplications but are incomplete and missing orthologs from several species. *Parapanteles* ANK clades 4, 8 and 11 have no apparent pseudogenes: clades 4 and 8 are incomplete, but clade 11 contains 9 gene duplications.

The *Parapanteles* sp. B virulence genome appears heavily duplicated, containing 2-3 more copies of most PTP and ANK orthologs than other species and accounting for about a third of all potential pseudogenes I identified (Figures 5.2-5.6 and Tables 5.3 & 5.4). Gene counts by species of PTP and ANK genes are summarized in Tables 5.3 & 5.4.

Rough alignment of PTP*-containing scaffold sets*

Among the PTPs genes that I annotated I found five clusters located near each other in the genome. The first PTP cluster consists of PTPs 1.1, 1.2, 2, 3, 4, 6, 7, 9, 12, 13, 18, and 26 (Figure 5.4), the second consists of PTPs 5, 8, 14, 17, 21, 23, and 24 (Figure 5.5), the third consists of PTPs 15 and 25, the fourth PTPs 10, 21, and 22, and the fifth PTPs 16, 19, and 20 (Figure 5.6). In most cases PTPs which I recovered as sisters in the PTP gene tree are not adjacent to each other. Adjacent PTPs tend to be from disparate parts of the gene tree with

two exceptions: PTPs 1.1/1.2 and 2 have adjacent copies in some species (Figure 5.4) and PTPs 21 and 22 are adjacent to each other in most species (Figure 5.6).

Parapanteles sp. B contains the most gene duplications for both ANKs and PTPs, having 5 or more paralogs of 8 PTPs and 4 ANKs. This species accounts for 48 of the 124 potential PTP pseudogenes and 10 of the 26 potential ANK pseudogenes I identified (Figures 5.2 & 5.3, Tables 5.3 & 5.4). Many of the individual duplicates are located on often very long scaffolds which contain few or no other polydnavirus virulence genes (especially prevalent in Figures 5.4 & 5.6).

DISCUSSION

Microgastrine polydnavirus virulence genomes are available from just five species to date: *Cotesia congregata, Cotesia vestalis, Glyptapanteles flavicoxis, Glyptapanteles indiensis,* and *Microplitis demolitor* (Desjardins *et al.* 2008, Chen *et al.* 2011, Burke *et al.* 2014). *Cotesia* and *Glyptapanteles* are large and diverse genera that are either sisters or paraphyletically related; *Microplitis* is moderately large by comparison and is one of the earliest genera to diverge from the rest of Microgastrinae. The *Parapanteles* species I included in this study are not closely related to any of these three genera, but are somewhat more closely related to *Cotesia/Glyptapanteles* than they are to *Microplitis* (Whitfield *et al.* 2002, also Chapters 2 & 4). Phylogenetically and in gross numbers of PTP and ANK genes, *Parapanteles* are more similar to *Cotesia* and *Glyptapanteles* polydnaviruses than they are to *Microplitis* polydnaviruses. Ten of 13 PTPs and 2 of 4 ANKs with sisters from non-*Parapanteles* genera are sister to *Cotesia congregata* rather than *Microplitis demolitor* (Figs. 5.2 & 5.3). The most thoroughly studied *Cotesia* and *Glyptapanteles* polydnaviruses have 27 or more PTPs and under 10 ANKs (Desjardins *et al.* 2008, Chen *et al.* 2011) while *Microplitis demolitor* has just 16 PTPs and 14 ANKs (Burke *et al.* 2014). The number of PTPs I found in each *Parapanteles* species ranges from 7-71 but the average was 27, and 11 of the 16 species included in this study have at least 20 (Table 5.3). The average number of ANKS per species was 11 (Table 5.4).

I found numerous duplications and potential pseudogenes in both the PTP and ANK gene families and that PTPs arranged closely together in the genome are often not closely related (Figs. 5.4-5.6). *Parapanteles* polydnavirus gene duplications and arrangements follow

similar patterns to those found in other species where duplications of clusters of virulence genes are more common than tandem duplications of individual genes (Friedman & Hughes 2006, Desjardins *et al.* 2008, Chen *et al.* 2011, Burke *et al.* 2014). Of the five polydnavirus virulence genomes that have been sequenced, each have different numbers of genes and different numbers of viral segments (Desjardins *et al.* 2008, Chen *et al.* 2011, Burke *et al.* 2014, 2018). At least one viral segment in *Microplitis demolitor* is a recent duplicate of another segment (Burke *et al.* 2014, segments K & K1). Viral segments have little or no synteny across genera besides flanking regions containing integration motifs (Burke *et al.* 2014, 2018). In addition, chromosomal recombination has been identified in *Cotesia* species that caused a rearrangement in virulence gene orientation (Bézier *et al.* 2013). Based on the results and analysis presented here, I predict that, as in *M. demolitor,* most proviral loci are adjacent to each other in *Parapanteles* species and the patterns of duplication and pseudogenation in *Parapanteles* species is due to duplications of regions containing multiple genes, possibly of whole viral segments, and rearrangements in this genomic region.

Broadly, these results support the gene-birth-and-death model of polydnavirus gene family evolution proposed by previous studies and observed in other species (Friedman & Hughes 2006, Desjardins *et al.* 2008, Chen *et al.* 2011, Burke *et al.* 2014). However, previous comparative studies of polydnavirus evolution only compared viral genomes of up to five microgastrine species at a time (Desjardins *et al.* 2008, Chen *et al.* 2011, Jancek *et al.* 2013, Burke *et al.* 2014, Burke 2016). Besides one study which looked at polydnavirus evolution in *Microplitis* sister species (Burke 2016) and one that looked at polydnavirus evolution in two populations of *C. sesamiae* (Jancek *et al.* 2013), previous studies have compared polydnaviruses of relatively distantly related species from three different genera, *Microplitis, Cotesia,* and *Glyptapanteles* (Desjardins *et al.* 2008, Chen *et al.* 2011, Burke *et al.* 2014, 2018). This study is the first to investigate polydnavirus structure and evolution across a clade of closely related species with a well-defined species tree. Furthermore it adds 16 more microgastrine genome sequences, where previously the only microgastrine genome sequence available was *M. demolitor* (Burke *et al.* 2014, 2018).

Many questions about polydnavirus evolution in *Parpaanteles* wasps remain and will be addressed with this dataset. My immediate next goals are to identify the boundaries of proviral loci in *Parapanteles* species, assess whether genomic rearrangements are more frequent in scaffolds carrying polydnavirus virulence genes than they are more broadly, and to identify a mechanism causing the frequent duplications of these genes. Many of the putative PTPs and ANKs I identified here, especially the potential pseudogenes, may not be located on functional proviral segments. Proviral loci have two types of integration motifs: Wasp Integration Motifs (WIMs) are conserved sequences that flank proviral loci and are recognized by nudivurs-origin polydnavirus genes to copy proviral loci into circular viral segments. Host Integration Motifs (HIMs) are conserved sequences located within viral segments and play a role in integrating the viral segment into host hemocytes (Beck *et al.* 2011). While extremely useful in identifying the ends of proviral loci, WIMs are short and discerning between them and convergently similar loci throughout the genome is not trivial. Female microgastrines produce high concentrations of polydnavirus segments in the calyx cells of their ovaries and incorporating genome sequences of pooled female calyx cells from several *Parapanteles* species to this study, either by assembling and annotating those genomes or by mapping raw reads onto the genome assemblies I produced for this study, will also be extremely useful in identifying proviral segments and their boundaries.

Another important research direction is to explore the specific causes of *Parapanteles* polydnavirus gene duplications. Diverse transposable elements, including p-elements and polintons, have been found in other microgastrine polydnaviruses (Webb *et al.* 2006, Desjardins *et al.* 2008, Dupuy *et al.* 2011) and are probably involved in some of the duplication events observed here. The virulence genes of *P.* sp. B are both the most heavily duplicated and heavily pseudogenized of any species in this study, but duplications and pseudogenizations are common in all species and both PTP and ANK gene families (Figures 5.2-5.6, Tables 5.3 & 5.4). Important future steps include investigating the cause of the duplications in *P.* sp. B and whether other *Parapanteles* virulence gene duplications were caused by similar mechanisms.

In the current study my main goal was to broadly characterize PTP and ANK gene duplicates and pseudogenes. Looking at patterns of selection across polydnavirus genes across

Parapanteles species is another important area of future research. Jancek *et al.* (2013) found different polydnavirus virulence genes under positive selection across three species of *Cotesia* and between two populations of one of those species that attack different hosts. In contrast to these results, Burke (2016) found the majority of virulence gene in *M. demolitor* under purifying selection when compared to its sister species, *M. mediator*. This study shows some indications that the former may be more likely in *Parapanteles* in that different species seem to have different functional and different pseudogenized sets of PTPs and ANKs. For example, PTPs 5, 8, 17, & 24 are complete and appear functional in *P.* sp. J & *P.* sp. K but not in most other species (Figure 5.6).

TABLES & FIGURES

Table 5.1: Sequencing and assembly summaries for shotgun-sequencing genome summaries prepared from single males from 16 *Parapanteles* species.

Species	Sample ID #	Host family	Extraction DNA concentration (ng/ul)	Total Extraction DNA (ng)	Raw reads (bp)	Assembly size (bp)	N50 (bp)	Average coverage	Number of scaffolds	Longest scaffold (bp)
Parapanteles continua	08-srnp-1895	Saturniidae	n/a	n/a	59,375,923	119,429,209	156,077	149	27,521	883,829
Parapanteles tessares	07-srnp-31983	Saturniidae	0.971	92.245	33,107,695	140,495,598	119,018	71	64,304	1,215,459
Parapanteles sicpolus	03-srnp-3418	Saturniidae	1.94	184.3	40,243,688	121,184,736	170,933	100	21,823	1,993,319
Parapanteles sp. G	yy38822	Saturniidae	2.12	201.4	38,368,866	124,624,894	137,255	92	34,083	1,278,449
Parapanteles sp. D	yy44117	Saturniidae	2.14	203.3	42,954,221	116,790,194	117,898	110	31,612	1,010,306
Parapanteles sp. valerio05	06-srnp-21433	Notodontidae	0.93	88.35	44,671,538	133,812,320	114,323	100	42,704	1,331,134
Parapanteles em	07-srnp-33300	Notodontidae	1.13	107.35	44,967,128	130,144,387	107,957	104	49,782	1,253,865
Parapanteles paradoxus	06-srnp-429	Notodontidae	n/a	n/a	38,471,170	122,717,495	83,654	94	39,057	788,596
Parapanteles sp. I	yy43211	Geometridae	0.855	81.225	35,844,532	123,887,601	87,214	87	33,252	958,379
Parapanteles tlinea	08-srnp-31404	Erebidae	2.11	200.45	61,842,437	125,029,492	71,794	148	65,682	622,957
Parapanteles sp. B	yy37474	Erebidae	1.71	162.45	38,354,042	122,084,715	157,929	94	25,042	1,334,768
Parapanteles sp. C	yy48054	Erebidae	1.98	188.1	41,973,472	119,227,408	131,566	106	36,982	1,094,935
Parapanteles sp. F	yy37570	Erebidae	1.2	114	39,403,814	112,460,424	290,153	105	25,632	1,799,439
Parapanteles sp. whitfield08	09-srnp-36520	Geometridae	0.776	73.72	37,408,045	109,979,891	416,607	102	26,939	2,272,552
Parapanteles sp. J	yy33819	Geometridae	1.22	115.9	38,408,004	128,083,191	68,560	90	46,122	1,058,342
Parapanteles sp. K	yy14412	Geometridae	0.716	68.02	34,083,436	129,001,494	112,390	79	39,821	2,381,266
Average	-	-	-	-	41,842,376	123,684,566	146,458	102	38,147	1,329,850

Table 5.2: Output summaries of BUSCO analysis of 16 *Parapanteles* species and *Microplitis demolitor.* Adjusted missing excludes BUSCOs which were missing from both *Microplitis demolitor* and all *Parapanteles* species.

Species	Complete single-copy	Complete dupilcated	Fragmented	Missing	% Missing	Adjusted missing	% Adjusted Missing	Total Searched
Parapanteles continua	2993	15	662	745	17%	634	15%	4415
Parapanteles tessares	3701	24	406	284	6%	173	4%	4415
Parapanteles sicpolus	3742	21	371	281	6%	170	4%	4415
Parapanteles sp. G	3813	19	340	253	6%	142	3%	4415
Parapanteles sp. D	3336	16	642	421	10%	310	7%	4415
Parapanteles sp. valerio05	3653	19	424	319	7%	208	5%	4415
Parapanteles em	3477	17	548	373	8%	262	6%	4415
Parapanteles paradoxus	3663	24	425	303	7%	192	4%	4415
Parapanteles sp. I	3796	22	341	256	6%	145	3%	4415
Parapanteles tlinea	3021	13	762	619	14%	508	12%	4415
Parapanteles sp. B	3727	18	339	271	6%	160	4%	4415
Parapanteles sp. C	3557	17	504	337	8%	226	5%	4415
Parapanteles sp. F	3685	16	432	282	6%	171	4%	4415
Parapanteles sp. whitfield08	3484	17	546	368	8%	257	6%	4415
Parapanteles sp. J	3617	22	464	312	7%	201	5%	4415
Parapanteles sp. K	3611	21	325	258	6%	147	3%	4415
Average	3554.75	18.8125	470.6875	355.125	8%	244.125	6%	4415
Microplitis demolitor	4014	38	184	179	4%	68	2%	4415

Table 5.3: Number of complete (C), potential pseudogene (P), and fragmented (F) copies of each of 26 distinct protein tyrosine phosphatase (PTP) genes by *Parapanteles* species.

Species	PTP 1.1			PTP 1.2			PTP 2			PTP 3			PTP 4			PTP 5			PTP 6			PTP 7			PTP 8			PTP 9			PTP 10			PTP 11			PTP 12			PTP 13			PTP 14		
	n			n			n			n			n			n			n			n			n			n			n			n			n			n			n		
	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F
Parapanteles continua		5			1			0			2			2			1			2			5			1			1			1			1			2			4			1		
	1	1	3	0	1	0	0	0	0	1	0	1	1	1	0	0	0	1	0	2	0	1	0	4	0	0	1	1	0	0	1	0	0	0	0	1	1	0	1	1	1	2	0	0	1
Parapanteles tessares		2			1			1			2			2			0			1			4			0			1			1			0			2			3			0		
	1	0	1	0	1	0	1	0	0	0	1	1	1	0	1	0	0	0	0	1	0	0	2	2	0	0	0	1	0	0	1	0	0	0	0	0	0	0	2	1	2	0	0	0	0
Parapanteles sicpolus		1			0			1			2			0			1			1			2			2			1			1			2			2			1			1		
	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	1	0	0	2	1	0	1	1	0	0	1	0	0	2	0	0	0	0	2	1	0	0	1	0	0
Parapanteles sp. G		1			0			0			1			0			0			1			2			1			2			1			0			1			2			0		
	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	2	1	0	0	0	0	2	1	0	0	0	0	0	0	0	1	0	0	2	0	0	0
Parapanteles sp. D		0			1			1			0			0			2			1			1			2			0			1			1			1			0			1		
	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0
Parapanteles sp. valerio05		2			1			1			0			1			0			2			3			0			2			0			0			1			0			0		
	2	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	3	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Parapanteles em		2			1			1			0			1			1			0			0			0			1			0			1			0			0			0		
	2	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Parapanteles paradoxus		1			2			1			3			1			0			1			1			1			0			1			2			2			0			0		
	1	0	0	2	0	0	1	0	0	1	0	2	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	2	0	0	0	0	0	0
Parapanteles sp. I		1			1			1			1			1			1			1			1			1			1			1			2			2			2			0		
	1	0	0	1	0	0	1	0	0	0	0	1	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	1	1	0	0	1	1	0	0	0	2	0	1	1	0	0	0
Parapanteles tlinea		2			2			1			3			1			2			1			2			1			3			1			1			3			2			1		
	2	0	0	1	1	0	1	0	0	2	0	1	1	0	0	2	0	0	1	0	0	1	0	1	1	0	0	1	0	2	1	0	0	1	0	0	2	0	1	0	0	2	1	0	0
Parapanteles sp. F		0			0			0			0			1			1			0			0			2			0			0			0			0			1			2		
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0
Parapanteles sp. whitfield08		0			0			0			0			0			1			1			0			1			0			0			0			0			1			1		
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Parapanteles sp. B		1			0			2			0			1			5			0			0			8			0			7			1			0			0			6		
	1	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	3	2	0	0	0	0	0	0	0	4	4	0	0	0	1	5	1	0	1	0	0	0	0	0	0	0	0	5	1
Parapanteles sp. C		1			0			1			1			1			2			1			0			1			1			2			0			0			0			1		
	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0
Parapanteles sp. J		1			0			0			2			1			0			4			0			1			2			0			2			2			1			0		
	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1	2	1	0	0	0	1	0	0	1	1	0	0	0	0	1	1	0	1	0	1	1	0	0	0	0	0
Parapanteles sp. K		0			0			0			0			1			0			5			0			1			2			0			1			2			1			0		
	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	2	2	0	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0
Counts	20			10			11			17			14			17			22			21			23			17			17			14			20			18			14		
	14	2	4	6	3	1	9	2	0	7	2	8	9	2	3	7	5	5	7	9	6	4	2	15	7	9	7	9	2	6	8	6	3	9	4	1	6	1	13	6	4	8	5	7	2

Table 5.3 continued

	PTP 13	PTP 14	PTP 15	PTP 16	PTP 17	PTP 18	PTP 19	PTP 20	PTP 21	PTP 22	PTP 23	PTP 24	PTP 25	PTP 26	Tallies	Assembly size	N50	Average coverage	Missing BUSCOs
	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n				
F	C P F	C P F	C P F	C P F	C P F	C P F	C P F	C P F	C P F	C P F	C P F	C P F	C P F	C P F	C P F				
	4	1	1	1	0	2	2	1	1	1	1	1	1	0	41				
1	1 1 2	0 0 1	0 1 0	0 0 1	0 0 0	0 2 0	0 1 1	0 1 0	0 1 0	1 0 0	0 0 1	0 0 1	1 0 0	0 0 0	10 12 19	119,429,209	156,077	149	15%
	3	0	1	1	0	2	2	0	1	1	0	0	0	0	28				
2	1 2 0	0 0 0	0 0 1	1 0 0	0 0 0	1 1 0	0 2 0	0 0 0	0 1 0	1 0 0	0 0 0	0 0 0	0 0 0	0 0 0	9 11 8	140,495,598	119,018	71	4%
	1	1	1	0	0	2	1	1	1	1	1	1	0	0	27				
2	1 0 0	1 0 0	0 1 0	0 0 0	0 0 0	2 0 0	1 0 0	1 0 0	1 0 0	1 0 0	0 0 1	0 0 1	0 0 0	0 0 0	15 3 9	121,184,736	170,933	100	4%
	2	0	1	0	0	0	0	0	1	0	0	0	0	0	14				
1	0 0 2	0 0 0	0 1 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	1 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	5 2 7	124,624,894	137,255	92	3%
	0	1	1	0	1	1	1	1	1	1	1	1	1	0	22				
1	0 0 0	1 0 0	0 1 0	0 0 0	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	0 1 0	1 0 0	1 0 0	0 0 0	15 4 3	116,790,194	117,898	110	7%
	0	0	0	2	1	1	0	0	0	0	0	1	1	0	19				
0	0 0 0	0 0 0	0 0 0	0 0 2	0 0 1	1 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 1	0 0 1	0 0 0	8 1 10	133,812,320	114,323	100	5%
	0	0	0	1	0	0	1	1	1	1	2	0	0	0	15				
0	0 0 0	0 0 0	0 0 0	1 0 0	0 0 0	0 0 0	1 0 0	1 0 0	1 0 0	1 0 0	0 0 2	0 0 0	0 0 0	0 0 0	13 0 2	130,144,387	107,957	104	6%
	0	0	0	1	0	1	1	1	1	1	1	0	0	0	23				
2	0 0 0	0 0 0	0 0 0	1 0 0	0 0 0	1 0 0	1 0 0	1 0 0	0 1 0	1 0 0	0 1 0	0 0 0	0 0 0	0 0 0	14 4 5	122,717,495	83,654	94	4%
	2	0	0	1	1	1	1	1	1	1	1	1	0	0	26				
2	0 1 1	0 0 0	0 0 0	1 0 0	0 0 1	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	0 1 0	0 0 1	0 0 0	0 0 0	16 3 7	123,887,601	87,214	87	3%
	2	1	1	1	1	1	1	1	1	1	1	1	1	0	37				
1	0 0 2	1 0 0	1 0 0	0 0 1	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	0 0 0	28 1 8	125,029,492	71,794	148	12%
	1	2	2	1	0	0	0	0	0	1	0	2	2	0	15				
0	1 0 0	2 0 0	0 2 0	1 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 1 0	0 0 0	1 0 1	0 0 2	0 0 0	5 5 5	112,460,424	290,153	105	4%
	1	1	0	1	0	0	0	0	1	0	0	0	0	0	7				
0	0 0 1	0 1 0	0 0 0	1 0 0	0 0 0	0 0 0	0 0 0	0 0 0	1 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	2 4 1	109,979,891	416,607	102	6%
	0	6	5	3	3	0	2	1	8	2	5	6	3	2	71				
0	0 0 0	0 5 1	0 3 2	0 2 1	0 3 0	0 0 0	0 1 1	0 1 0	1 6 1	0 2 0	1 3 1	1 5 0	0 2 1	0 0 2	6 48 17	122,084,715	157,929	94	4%
	0	1	0	1	0	2	1	2	0	2	2	3	0	1	26				
0	0 0 0	0 1 0	0 0 0	1 0 0	0 0 0	1 1 0	1 0 0	1 1 0	0 0 0	0 0 2	0 1 1	0 1 2	0 0 0	0 1 0	9 8 9	119,227,408	131,566	106	5%
	1	0	2	1	2	0	1	2	1	1	2	1	2	3	34				
1	1 0 0	0 0 0	1 0 1	1 0 0	1 1 0	0 0 0	1 0 0	1 1 0	1 0 0	1 0 0	1 1 0	1 0 0	1 0 1	1 1 1	20 8 6	128,083,191	68,560	90	5%
	1	0	2	1	2	2	2	1	0	0	2	1	2	2	30				
0	1 0 0	0 0 0	2 0 0	1 0 0	1 1 0	0 2 0	1 1 0	0 1 0	0 0 0	0 0 0	1 1 0	1 0 0	2 0 0	1 1 0	17 10 3	129,001,494	112,390	79	3%
	18	14	17	16	11	15	16	13	19	14	19	19	13	8	435				
13	6 4 8	5 7 2	4 9 4	9 2 5	4 5 2	9 6 0	9 5 2	8 5 0	9 9 1	9 3 2	4 9 6	6 6 7	6 2 5	2 3 3	192 124 119				
														Average	27				
															12 8 7				

Table 5.4: Number of complete (C), potential pseudogene (P), and fragmented (F) copies of each of 11 distinct viral ankyrin (ANK) genes by *Parapanteles* species.

Species	ANK 1			ANK 2			ANK 3			ANK 4			ANK 5			ANK 6			ANK 7			ANK 8			ANK 9			ANK 10			ANK 11			Tallies			Assembly size	N50	Average coverage	Missing BUSCOs
	n			n			n			n			n			n			n			n			n			n			n			n						
	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F	C	P	F				
Parapanteles continua	2			1			0			1			1			1			0			1			3			2			1			13			119,429,209	156,077	149	15%
	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	1	0	2	1	0	2	0	0	0	1	0	6	7				
Parapanteles tessares	2			0			0			1			1			1			1			0			2			2			1			11			140,495,598	119,018	71	4%
	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	1	0	1	0	0	5	3	3				
Parapanteles sicpolus	1			0			0			1			1			1			1			1			2			2			0			10			121,184,736	170,933	100	4%
	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0	0	1	0	2	0	0	2	0	0	0	0	1	5	4				
Parapanteles sp. G	1			0			1			0			1			0			0			0			1			0			0			4			124,624,894	137,255	92	3%
	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3	0	1				
Parapanteles sp. D	2			1			1			1			1			1			1			1			3			2			1			15			116,790,194	117,898	110	7%
	2	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0	2	1	0	2	0	1	0	0	9	4	2				
Parapanteles sp. valerio05	2			1			0			0			0			0			1			1			2			1			2			10			133,812,320	114,323	100	5%
	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	2	0	0	1	0	0	2	1	0	9				
Parapanteles em	1			1			1			0			0			1			0			1			2			1			1			9			130,144,387	107,957	104	6%
	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	2	0	0	0	1	1	0	0	1	4	4				
Parapanteles paradoxus	1			1			0			1			1			1			0			0			0			0			1			6			122,717,495	83,654	94	4%
	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	4				
Parapanteles sp. I	1			1			0			1			1			1			0			1			0			0			1			7			123,887,601	87,214	87	3%
	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	5	0	2				
Parapanteles tlinea	2			1			0			1			1			1			1			1			1			1			1			11			125,029,492	71,794	148	12%
	2	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	1	0	0	1	1	0	0	1	0	0	1	0	0	8	0	3				
Parapanteles sp. F	2			1			1			0			0			0			0			0			4			4			1			13			112,460,424	290,153	105	4%
	0	0	2	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	4	0	0	0	1	0	8	5				
Parapanteles sp. whitfield08	2			0			0			0			1			0			0			0			1			2			0			6			109,979,891	416,607	102	6%
	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	2	1	3				
Parapanteles sp. B	3			6			0			0			5			1			0			0			2			5			7			29			122,084,715	157,929	94	4%
	0	0	3	2	2	2	0	0	0	0	0	0	2	2	1	0	0	1	0	0	0	0	0	0	1	1	0	0	4	1	4	1	2	9	10	10				
Parapanteles sp. C	2			2			0			0			2			1			0			0			0			0			3			10			119,227,408	131,566	106	5%
	2	0	0	0	0	2	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	7	1	2				
Parapanteles sp. J	0			2			2			1			1			0			0			0			1			1			1			9			128,083,191	68,560	90	5%
	0	0	0	1	1	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	4	2	3				
Parapanteles sp. K	0			2			2			0			0			2			0			0			0			0			1			7			129,001,494	112,390	79	3%
	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3	3	1				
Counts	24			20			8			8			17			12			5			7			24			23			22			170						
	8	2	14	7	4	9	3	3	2	2	0	6	12	4	1	4	2	6	2	1	2	0	0	7	5	14	5	3	16	4	14	1	7	60	47	63				
																															Average			11						
																																		4	3	4				

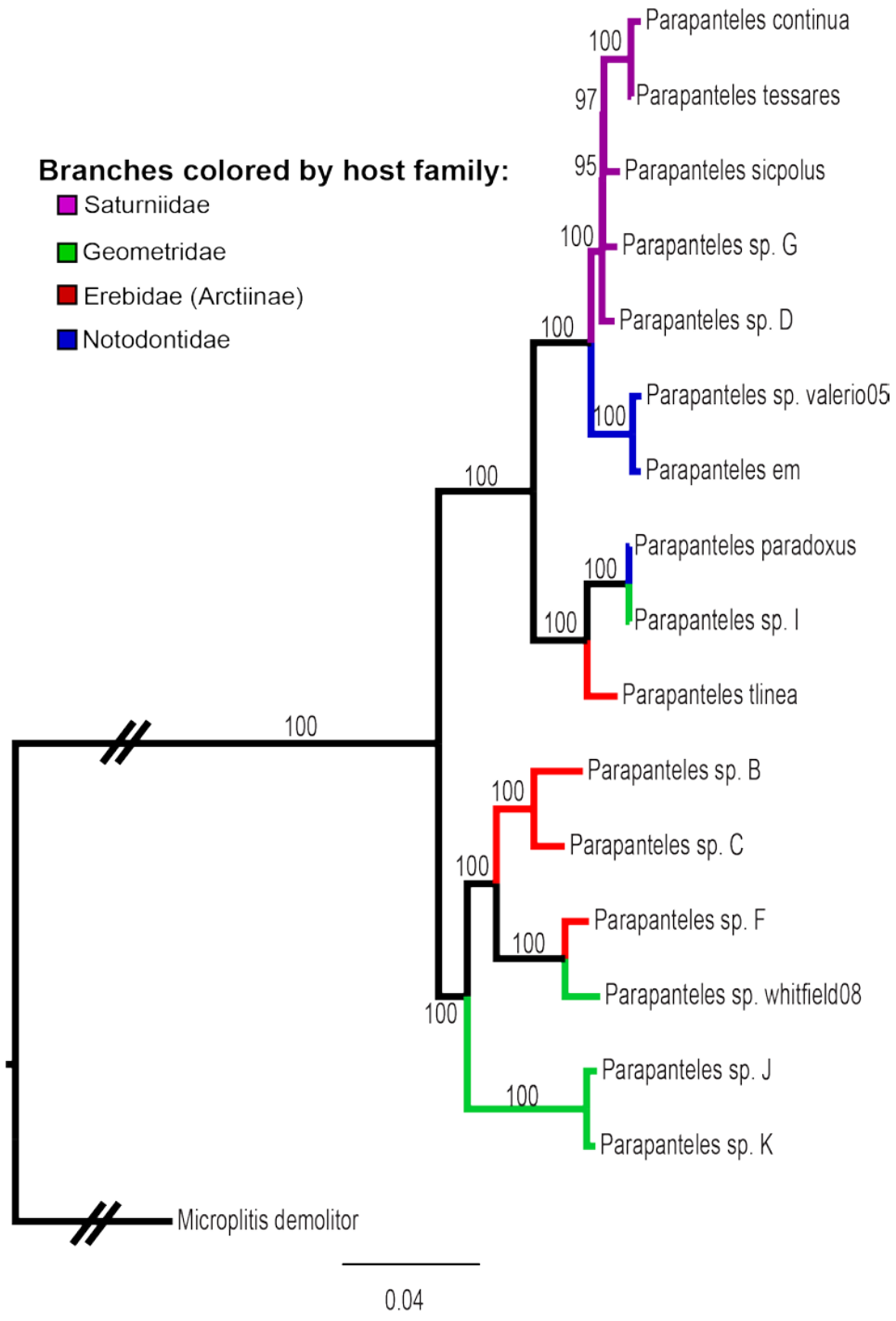


Figure 5.1: Maximum-likelihood RAxML species tree of fragments from 101 distinct gene models produced by BUSCO for 16 *Parapanteles* species and *Microplitis demolitor.* Brach colors correspond to parasitoid host family.

Figure 5.2: Maximum-likelihood RAxML gene tree of *Parapanteles* protein tyrosine phosphatase (PTP) gene annotation nucleotide sequences. Branches are colored by PTP (1.1-26) and tips are colored by species and host use: Species that parasitize caterpillars in Saturniidae are in shades of purple, species that parasitize caterpillars in Notodontidae are in shades of blue, species that parasitize caterpillars in Erebidae are in shades of red, and species that parasitize caterpillars in Geometridae are in shades of green. Stars with yellow fill on branches indicate duplication events within *Parapanteles*, stars with white fill on branches indicate potential duplications that predate *Parapanteles* but are not seen in *Cotesia congregata* or *Microplitis demolitor*. Red circles at the ends of tips indicate potential pseudogenes. Branch labels indicate bootstrap support based on 1000 bootstrap replicates.

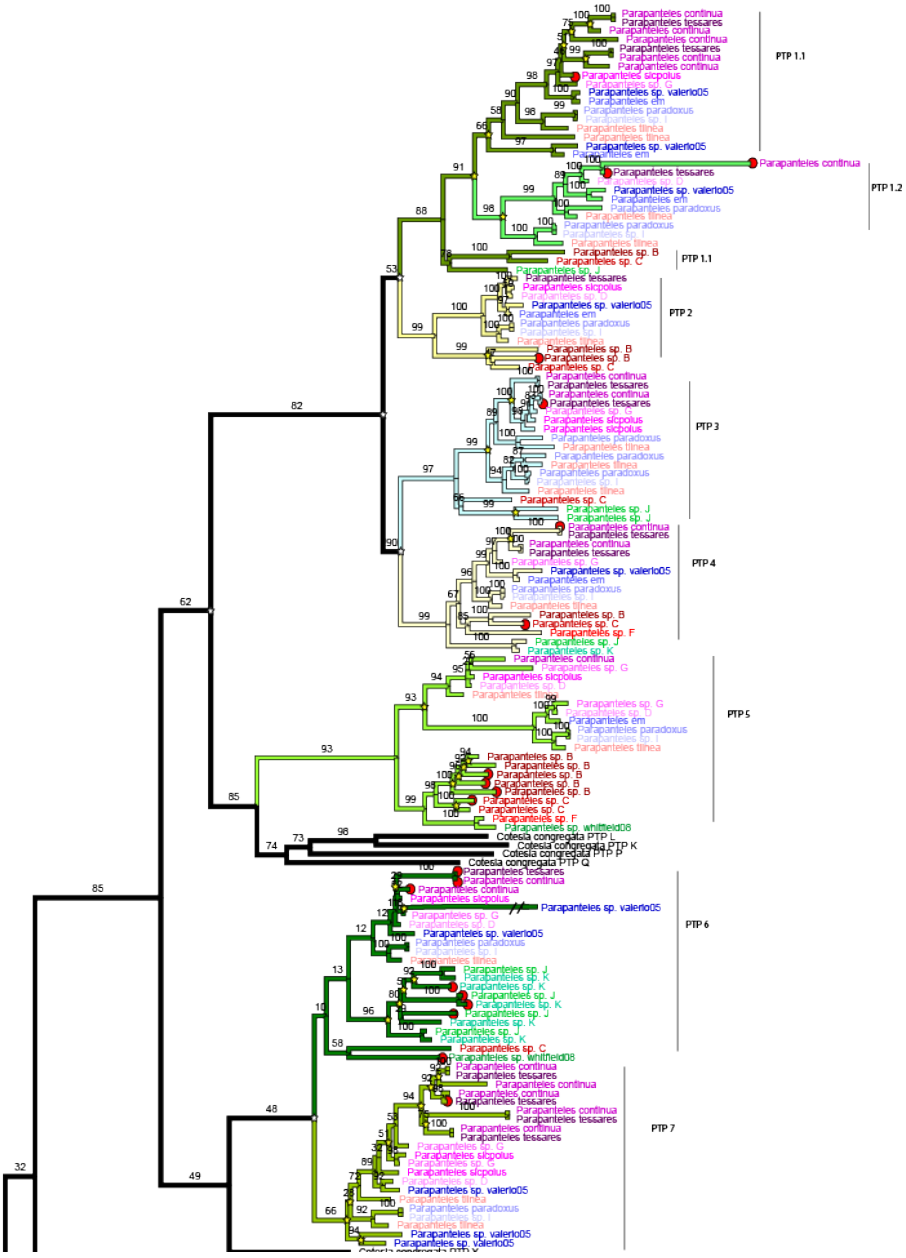


Figure 5.2 continued

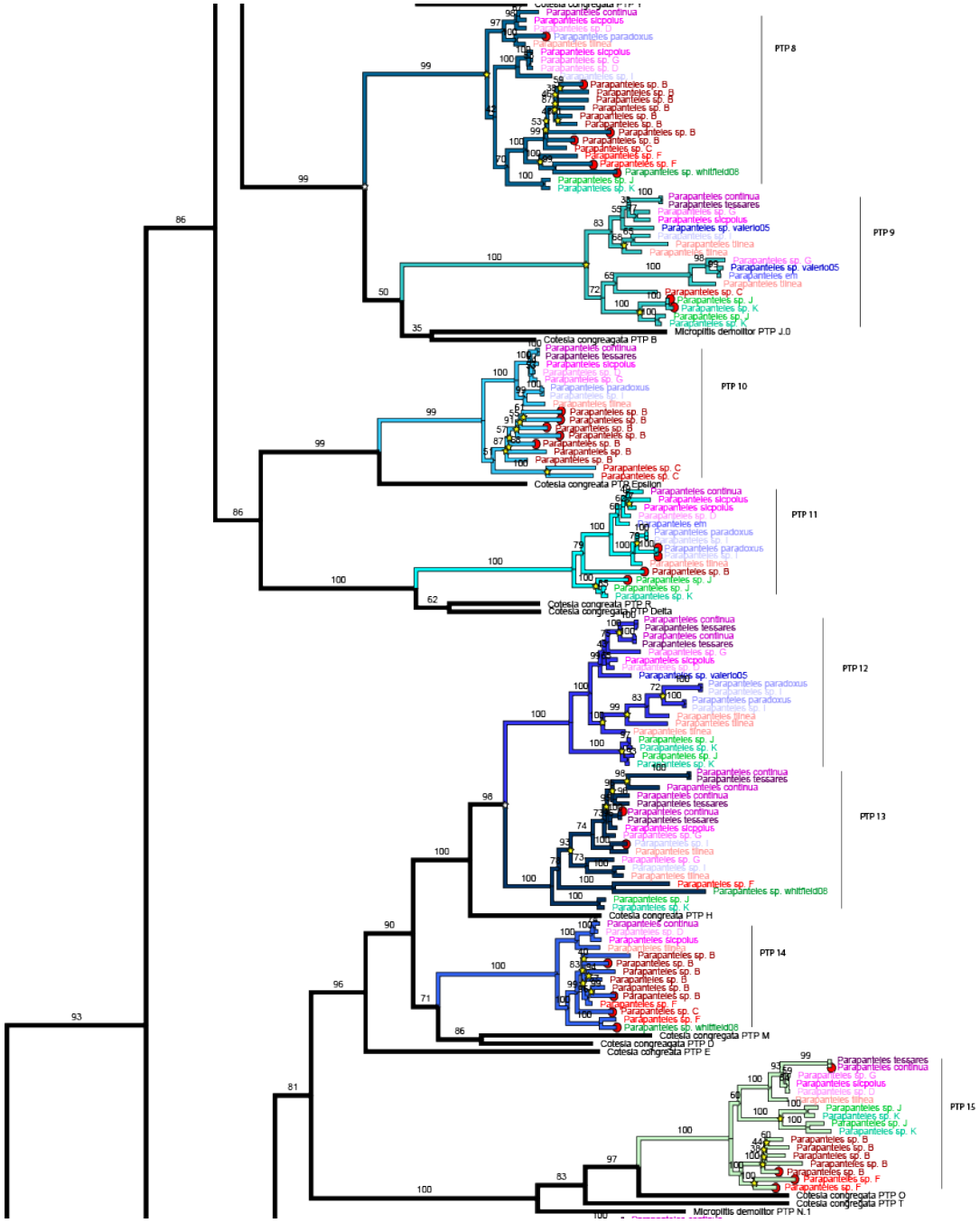


Figure 5.2 continued

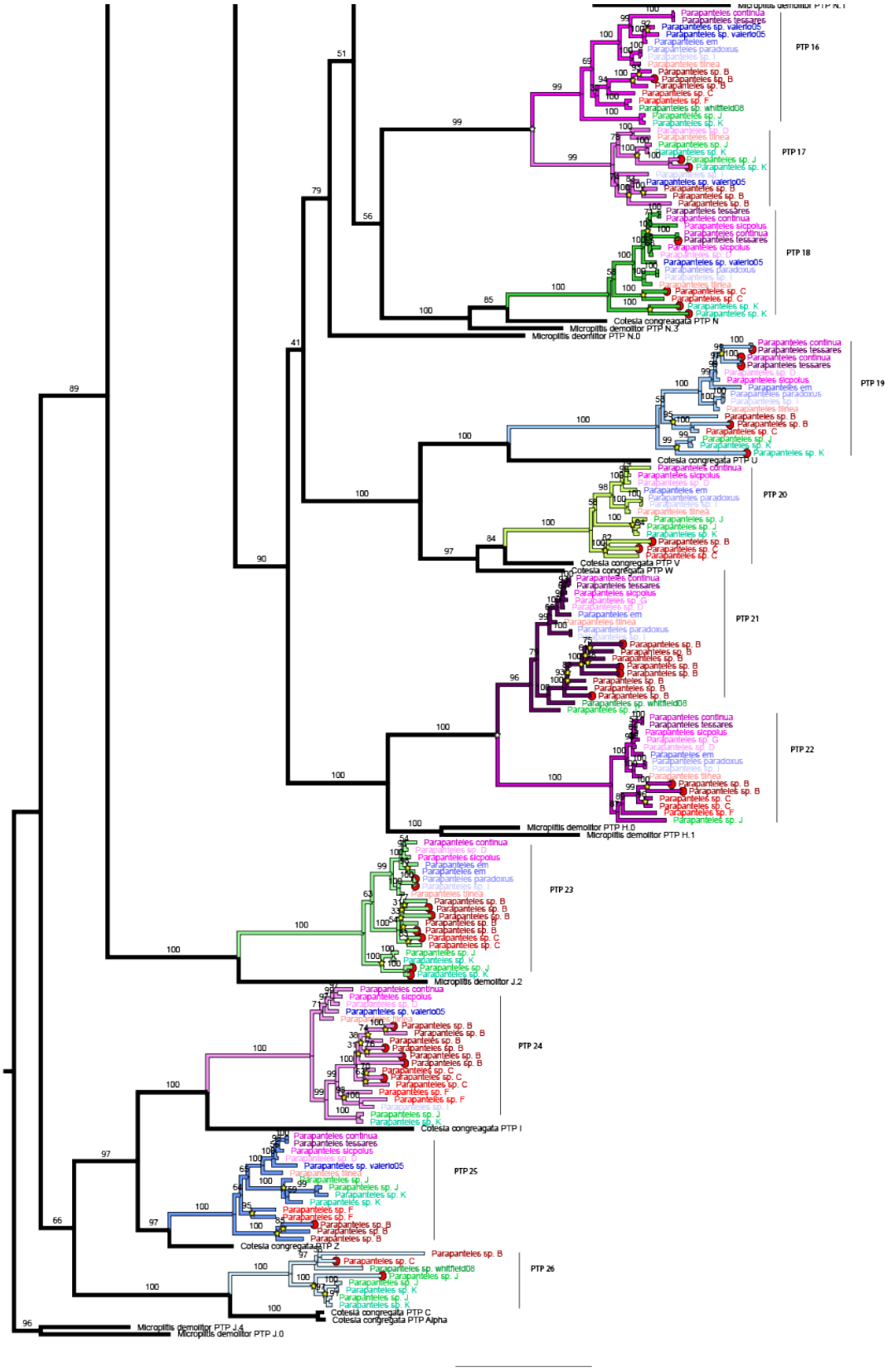


Figure 5.3: Maximum-likelihood RAxML gene tree of *Parapanteles* viral ankyrin (ANK) gene annotation nucleotide sequences. Branches are colored by ANK (1-11) and tips are colored by species and host use: Species that parasitize caterpillars in Saturniidae are in shades of purple, species that parasitize caterpillars in Notodontidae are in shades of blue, species that parasitize caterpillars in Erebidae are in shades of red, and species that parasitize caterpillars in Geometridae are in shades of green. Stars with yellow fill on branches indicate duplication events within *Parapanteles*, stars with white fill on branches indicate potential duplications that predate *Parapanteles* but are not seen in *Cotesia congregata* or *Microplitis demolitor*. Red circles at the ends of tips indicate potential pseudogenes. Branch labels indicate bootstrap support based on 1000 bootstrap replicates.

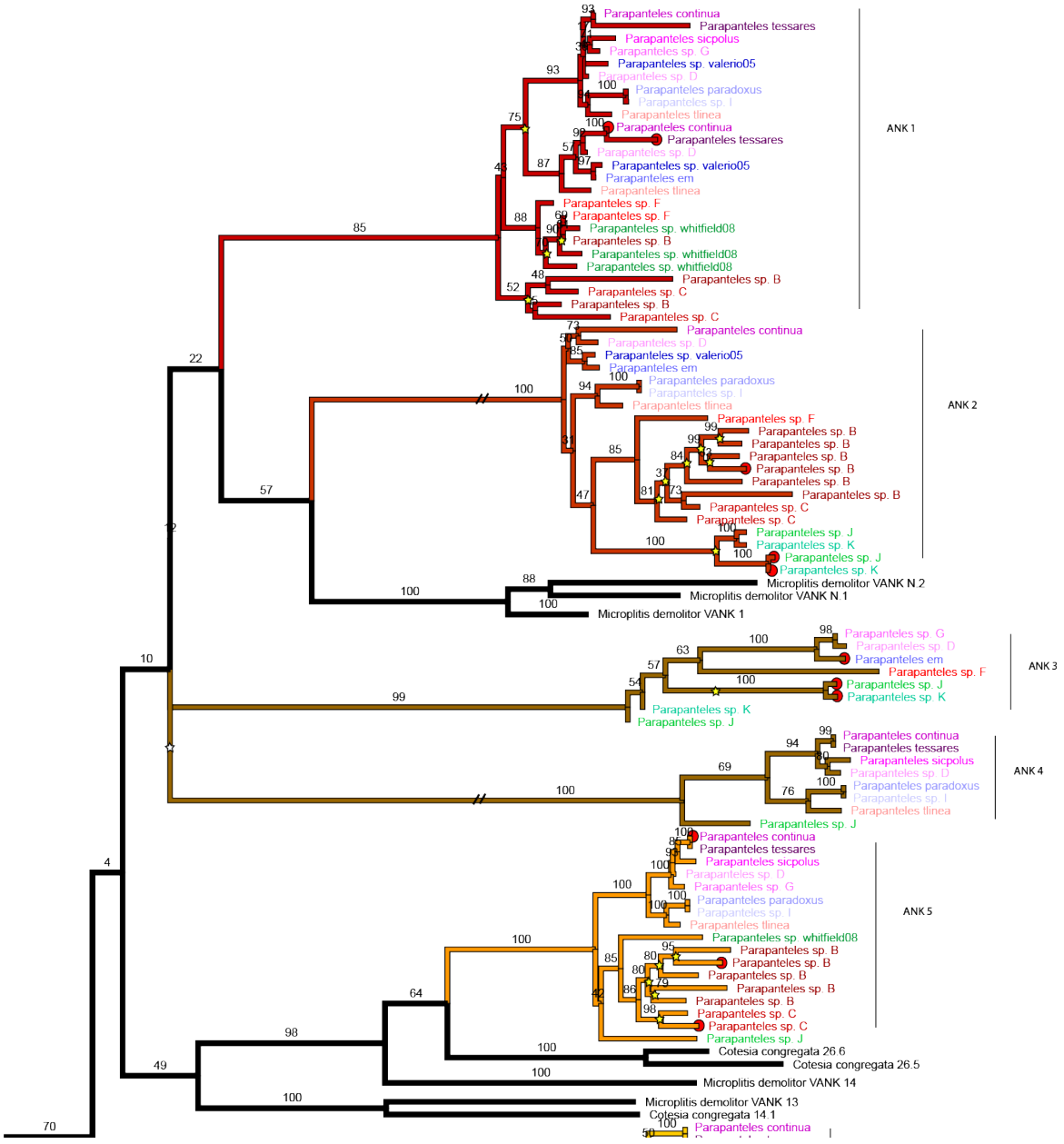
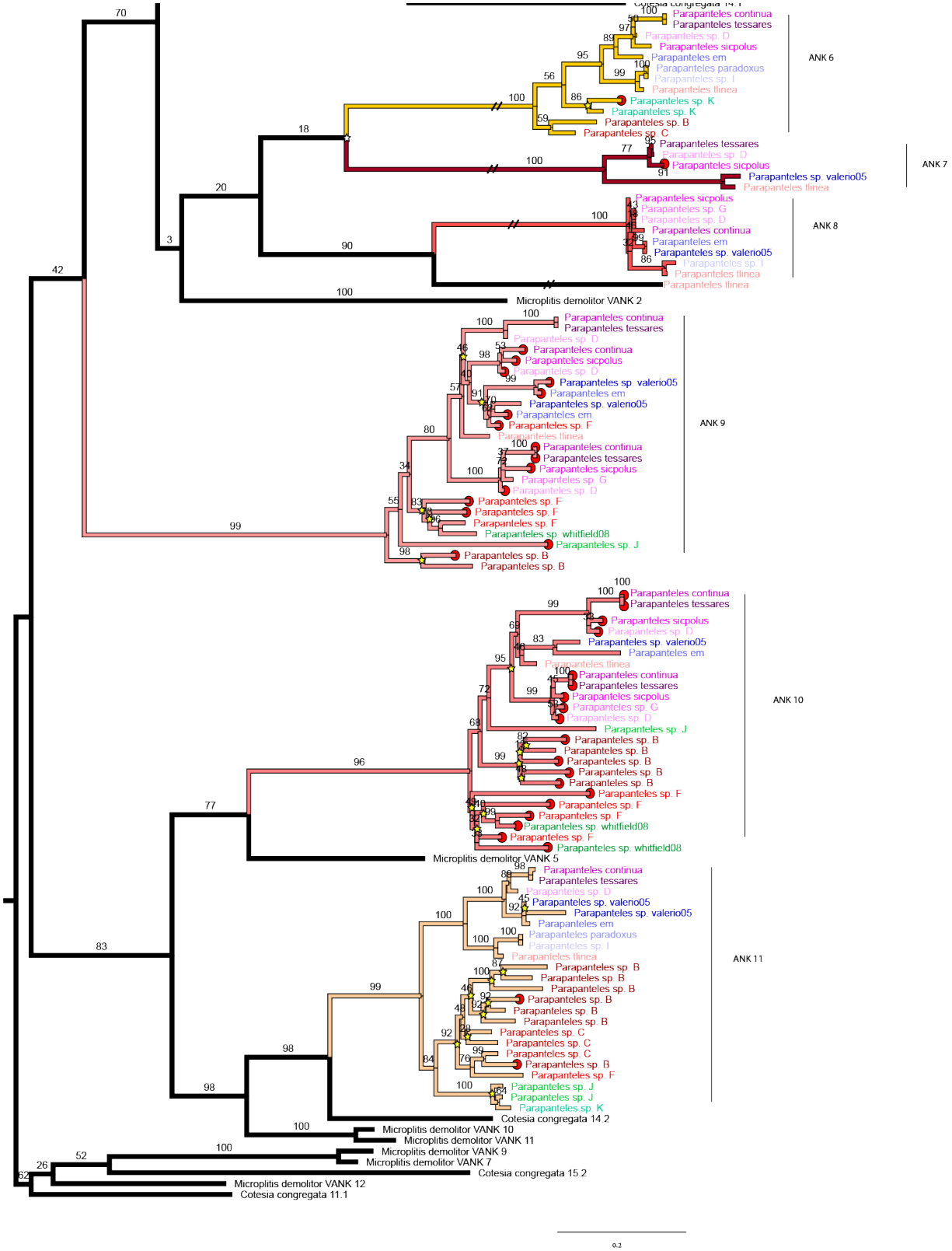


Figure 5.3 continued



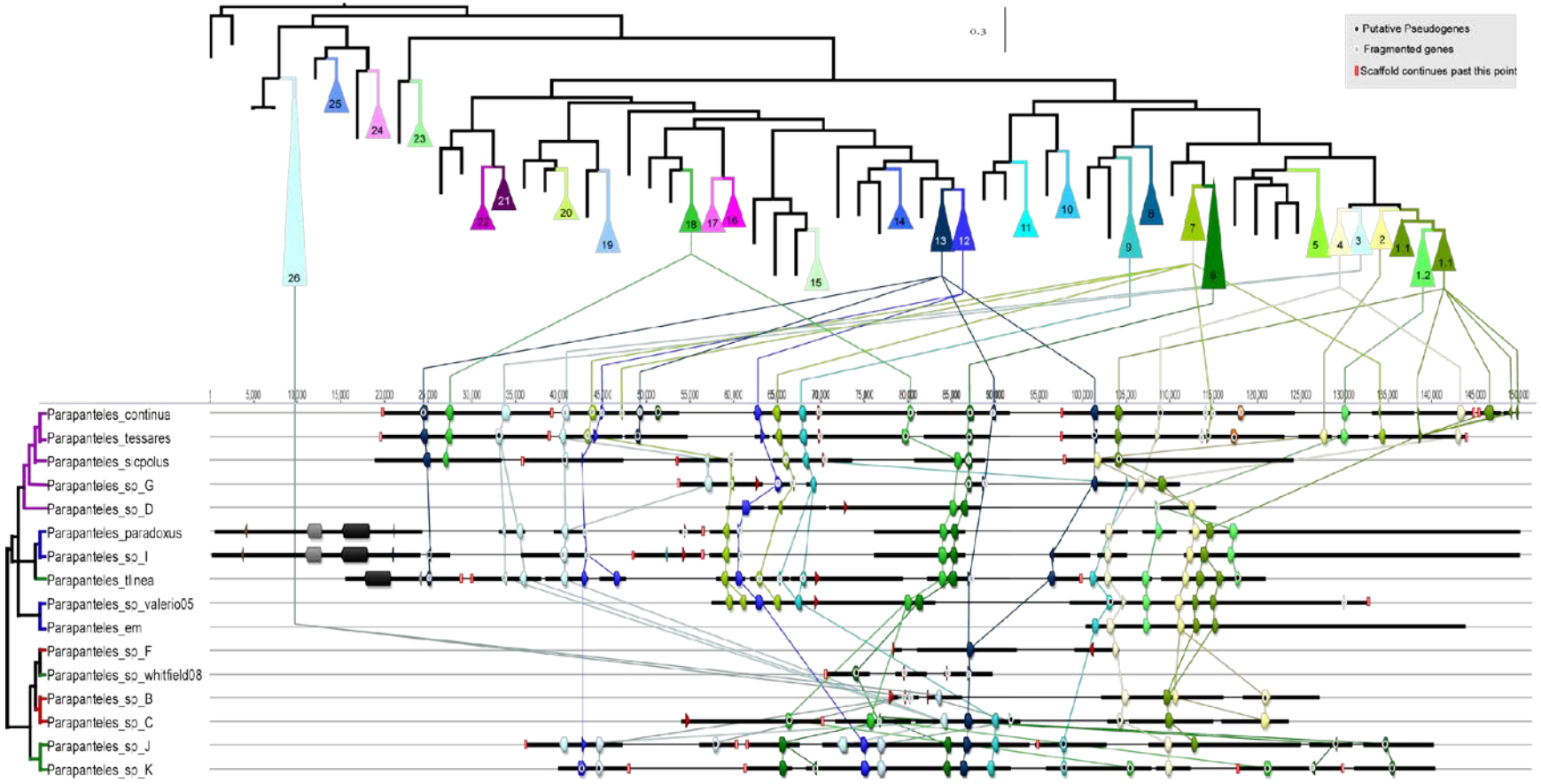


Figure 5.4: Rough-alignment of scaffold set 11 (SM 5.22). Protein tyrosine phosphatase (PTP) genes are colored by homology in shades of light yellow, green, blue, and purple. Viral ankyrin (ANK) genes are colored by homology in shades of red and orange. All other genes are colored in shades of white, grey, and black. Segments between gaps are separate scaffolds or contigs. Red rectangles with white fill indicate where scaffolds were truncated to visually simplify the figure. A simplified PTP gene tree is shown at the top of the figure with each PTP collapsed, labelled, and color-coded. Lines from the PTP gene tree and between genes on scaffolds indicate homology. White circles with black fill indicate potential pseudogenes and white circles with grey fill indicate fragmented genes (incomplete due to partial sequencing). A cladogram of species relationships based on Figure 5.1 is to the left of the species labels with branches colored by host family (purple for Saturniidae, blue for Notodontidae, red for Erebidae, and green for Geometridae).

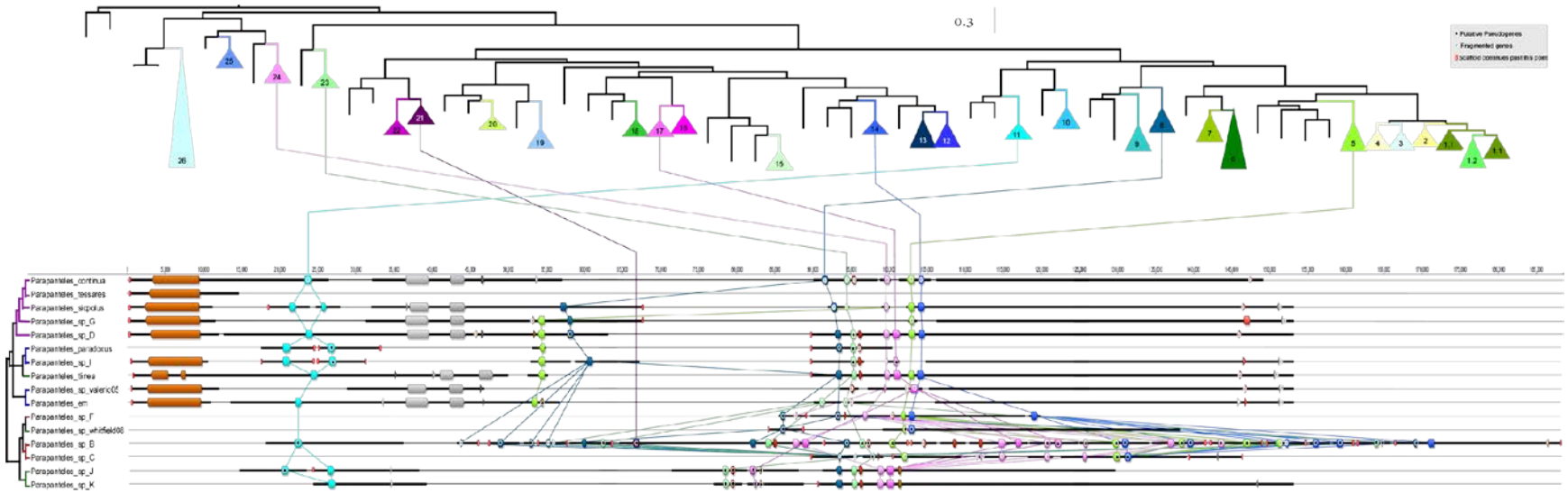


Figure 5.5: Rough-alignment of scaffold sets 15 & 18 (SM 5.22). Protein tyrosine phosphatase (PTP) genes are colored by homology in shades of light yellow, green, blue, and purple. Viral ankyrin (ANK) genes are colored by homology in shades of red and orange. All other genes are colored in shades of white, grey, and black. Segments between gaps are separate scaffolds or contigs. Red rectangles with white fill indicate where scaffolds were truncated to visually simplify the figure. A simplified PTP gene tree is shown at the top of the figure with each PTP collapsed, labelled, and color-coded. Lines from the PTP gene tree and between genes on scaffolds indicate homology. White circles with black fill indicate potential pseudogenes and white circles with grey fill indicate fragmented genes (incomplete due to partial sequencing). A cladogram of species relationships based on Figure 5.1 is to the left of the species labels with branches colored by host family (purple for Saturniidae, blue for Notodontidae, red for Erebidae, and green for Geometridae).

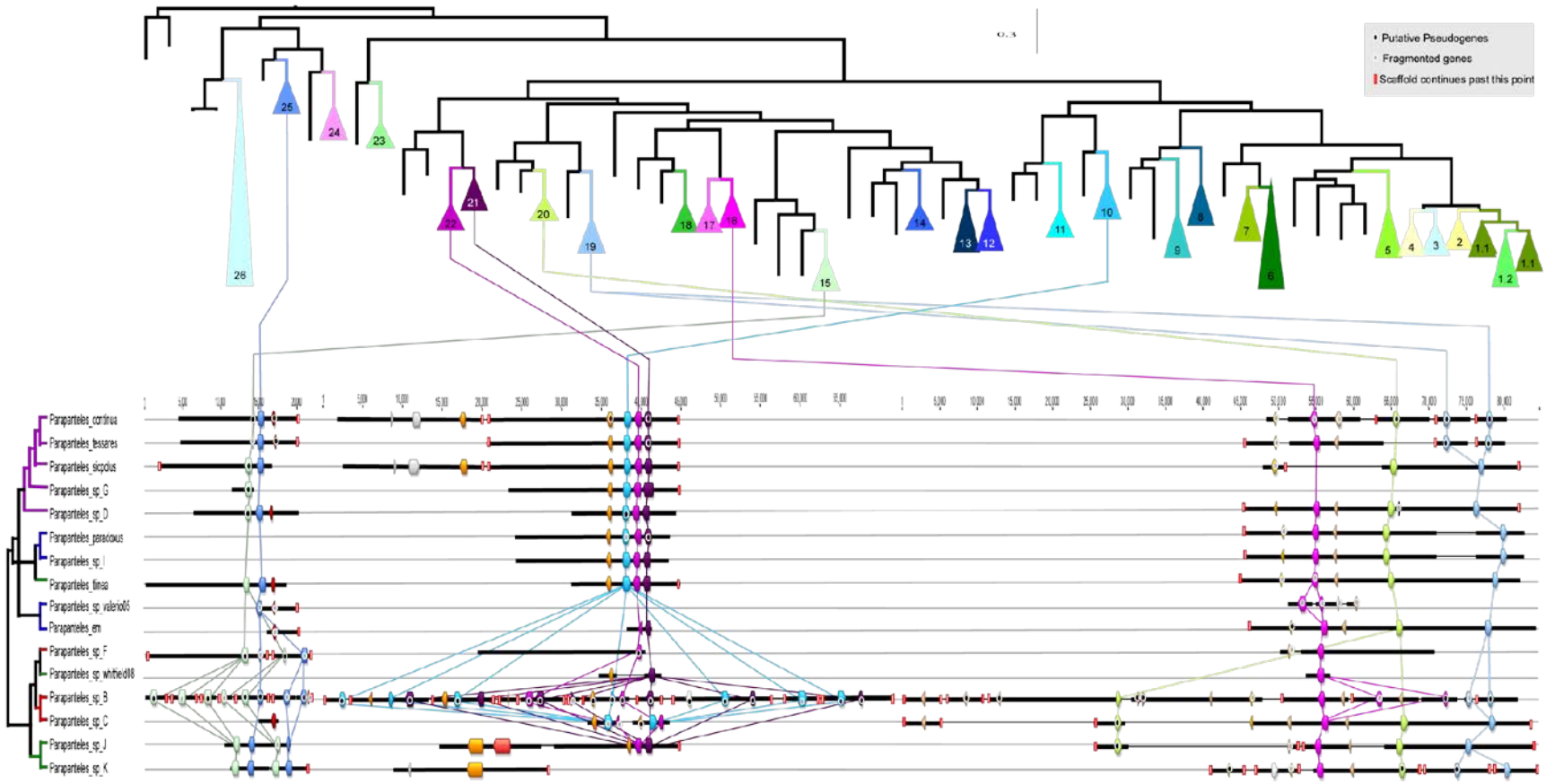


Figure 5.6: Rough-alignment of scaffold sets 10, 11 (partial), and 16. (SM 5.22). Protein tyrosine phosphatase (PTP) genes are colored by homology in shades of light yellow, green, blue, and purple. Viral ankyrin (ANK) genes are colored by homology in shades of red and orange. All other genes are colored in shades of white, grey, and black. Segments between gaps are separate scaffolds or contigs. Red rectangles with white fill indicate where scaffolds were truncated to visually simplify the figure. A simplified PTP gene tree is shown at the top of the figure with each PTP collapsed, labelled, and color-coded. Lines from the PTP gene tree and between genes on scaffolds indicate homology. White circles with black fill indicate potential pseudogenes and white circles with grey fill indicate fragmented genes (incomplete due to partial sequencing). A cladogram of species relationships based on Figure 5.1 is to the left of the species labels with branches colored by host family (purple for Saturniidae, blue for Notodontidae, red for Erebidae, and green for Geometridae).

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APPENDIX A: CHAPTER 2 SUPPLEMENTAL MATERIALS

The supplementary file “Chapter_2_SM.zip” includes:

Chapter 2 Supplemental Materials (SM):

SM 2.1: Genbank accession numbers of sequences used in 14247 sample COI tree of microgastrine genera.

SM 2.2: Pasta Alignment of sequences used in 14247 sample COI tree of microgastrine genera

SM 2.3: Fasttree approximated maximum-likelihood phylogeny of 14247 microgastrine COI samples. 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.

SM 2.4: List of primers and annealing temperatures used in this study.

SM 2.5: Genbank accession numbers of sequences used in 5-gene concatenated analysis and individual gene trees.

SM 2.6: Partitionfinder model scheme used in Bayesian analysis.

SM 2.7: Alignment of sequences used in COI trees.

SM 2.8: Alignment of sequences used in WG trees.

SM 2.9: Alignment of sequences used in ND1 trees.

SM 2.10: Alignment of sequences used in 28s trees.

SM 2.11: Alignment of sequences used in EF1a trees.

SM 2.12: Alignment of sequences used in 5-gene concatenated trees.

SM 2.13: Unedited RAxML COI tree.

SM 2.14: Unedited Bayesian COI tree.

SM 2.15: Unedited RAxML WG tree.

SM 2.16: Unedited Bayesian WG tree.

SM 2.17: Unedited RAxML ND1 tree.

SM 2.18: Unedited Bayesian ND1 tree.

SM 2.19: Unedited RAxML 28s tree.

SM 2.20: Unedited Bayesian 28s tree.

SM 2.21: Unedited RAxML EF1a tree.

SM 2.22: Unedited Bayesian EF1a tree.

SM 2.23: Unedited RAxML concatenated tree.

SM 2.24: Unedited Bayesian concatenated tree.

APPENDIX B: CHAPTER 4 SUPPLEMENTAL MATERIALS

The supplementary file “Chapter_4_SM.zip” includes:

Chapter 4 Supplemental Materials (SM):

SM 4.1: Phylip-format alignment of 370 concatenated anchored hybrid enrichment loci.

SM 4.2: Partitions of each anchored hybrid enrichment locus used in RAxML maximum likelihood analysis.

APPENDIX C: CHAPTER 5 SUPPLEMENTAL MATERIALS (GENOME ASSEMBLIES 1 OF 2)

The supplementary file “Chapter_5_SM_5.1-5.8.zip” includes:

Chapter 5 Supplemental Materials (SM) Genome Assemblies 1 of 2:

SM 5.1: *De novo* genome assembly of single male *Parapanteles tessares* genomic DNA.

SM 5.2: *De novo* genome assembly of single male *Parapanteles continua* genomic DNA.

SM 5.3: *De novo* genome assembly of single male *Parapanteles sicpolus* genomic DNA.

SM 5.4: *De novo* genome assembly of single male *Parapanteles* sp. G genomic DNA.

SM 5.5: *De novo* genome assembly of single male *Parapanteles* sp. D genomic DNA.

SM 5.6: *De novo* genome assembly of single male *Parapanteles* sp. Valerio05 genomic DNA.

SM 5.7: *De novo* genome assembly of single male *Parapanteles em* genomic DNA.

SM 5.8: *De novo* genome assembly of single male *Parapanteles paradoxus* genomic DNA.

APPENDIX D: CHAPTER 5 SUPPLEMENTAL MATERIALS (GENOME ASSEMBLIES 2 OF 2)

The supplementary file “Chapter_5_SM_5.9-5.16.zip” includes:

Chapter 5 Supplemental Materials (SM) Genome Assemblies 2 of 2:

SM 5.9: *De novo* genome assembly of single male *Parapanteles* sp. I genomic DNA.

SM 5.10: *De novo* genome assembly of single male *Parapanteles tlinea* genomic DNA.

SM 5.11: *De novo* genome assembly of single male *Parapanteles* sp.F genomic DNA.

SM 5.12: *De novo* genome assembly of single male *Parapanteles* sp. Whitfield08 genomic DNA.

SM 5.13: *De novo* genome assembly of single male *Parapanteles* sp. C genomic DNA.

SM 5.14: *De novo* genome assembly of single male *Parapanteles* sp. B genomic DNA.

SM 5.15: *De novo* genome assembly of single male *Parapanteles* sp. J genomic DNA.

SM 5.16: *De novo* genome assembly of single male *Parapanteles* sp. K genomic DNA.

APPENDIX E: CHAPTER 5 SUPPLEMENTAL MATERIALS (PHYLOGENETIC FILES)

The supplementary file "Chapter_5_SM_5.17-5.30.zip" includes:

Chapter 5 Supplemental Materials (SM) Phylogenetic Files:

SM 5.17: MUSCLE alignment of 101 BUSCO gene models for 16 *Parapanteles* species.

SM 5.18: Unedited RAxML maximum likelihood tree of 101 BUSCO gene models for 16 *Parapanteles* species.

SM 5.19: List of BUSCOs by OrthoDB ID number missing from all *Parapanteles* species and all *Parapanteles* species plus *Microplitis demolitor.*

SM 5.20: Tallies of gene and gene fragment annotations by gene family and *Parapanteles* species of the initial 890 polydnavirus virulence genes annotated.

SM 5.21: List of top BLAST hit-containing scaffold or contig in each *Parapanteles* species for each of the initial annotated 890 polydnavirus virulence genes, excluding 238 genes which only returned conspecific hits.

SM 5.22: Sets of scaffolds and contigs with similar virulence genes. Similarity is based on BLAST search results of virulence genes listed in SM 5.21. The initially annotated 890 virulence genes are located on highlighted scaffolds and contigs. Sets in bold typeface were annotated completely for this study.

SM 5.23: Nucleotide sequences of PTP gene annotations.

SM 5.24: Translated protein sequences of PTP gene annotations.

SM 5.25: Nucleotide sequences of ANK gene annotations.

SM 5.26: Translated protein sequences of ANK gene annotations.

SM 5.27: MAFFT alignment of PTP nucleotide sequences.

SM 5.28: Unedited RAxML maximum likelihood PTP gene tree.

SM 5.29: MAFFT alignment of ANK nucleotide sequences.

SM 5.30: Unedited RAxML maximum likelihood ANK gene tree.