

Evolution and Phylogeny of the Parasitoid Subfamily Phasiinae (Diptera: Tachinidae)

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Abstract

The first molecular phylogenetic analysis of the agriculturally important parasitoid subfamily Phasiinae (Diptera: Tachinidae) is presented, estimated from 128 worldwide taxa (80 genera) and approximately 7.6 kilobases of nuclear data. Special emphasis is placed on taxa with controversial taxonomic placement. The resultant phylogenetic tree is used to reconstruct ancestral character states, trace the evolution of significant adaptive traits within the Tachinidae, and test hypotheses about the classification of Phasiinae. Subfamily placements of the taxa Eutherini, Epigrimyiini, *Litophasia*, Strongygastrini, and Parerigonini are confidently resolved, the former three within Dexiinae and the latter two within Phasiinae. Due to sparse molecular evidence, the Imitomyiini are tentatively placed among the Phasiinae. Ancestral state reconstruction suggests a dominant and persistent trend in Phasiinae to evolve piercing structures used to insert eggs directly into host tissues. A single potential synapomorphy of Phasiinae is identified (elongated hypandrium).

This phylogeny is used to update classification of worldwide phasiine genera and tribes. Many novel phylogenetic hypotheses are presented including the division of Parerigonini s. l. into three tribes: Parerigonini s. s., Zitini, and Cylindromyiini, and the division of Phasiini s. l. into four lineages: Phasiini s. s., Gymnosomatini, Opsiini, and Xystini. Two tribes are resurrected (Opsiini and Xystini) and one new tribe is proposed (Zitini *nomen novum*).

Additionally, a survey of phasiine biodiversity was conducted in Great Smoky Mountains National Park (TN, NC). Species identifications were made using morphological keys, with further evidence from 900 base pairs of the nuclear coding gene MCS. In total, 221 specimens representing 26 phasiine species were collected. Of these, 21 species are newly recorded from the park, four are new records for Tennessee, and two are new records for North Carolina. All 12 eastern Nearctic phasiine genera were represented. Updated identification keys to eastern Phasiinae are provided and DNA barcoding sequences were generated that will aid future researchers to quickly and inexpensively identify phasiine species.

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Introduction

Parasitoid flies of the subfamily Phasiinae (Diptera: Tachinidae) attack heteropteran bugs and are important members of their ecosystems as secondary consumers and pollinators. Many of their hosts are serious agricultural pests, and phasiines show great promise as biological control agents. Chapter I includes a phylogenetic reconstruction of relationships within Phasiinae based on nuclear coding data. Ancestral states of several significant traits are included, along with a discussion of synapomorphies of Phasiinae and its sister group Dexiinae. Chapter II uses this new phylogeny to review and update the classification of world Phasiinae. Discussions of the taxonomic history and new molecular data for each tribe are included. In Chapter III, the results of a biodiversity survey of phasiines found in Great Smoky Mountains National Park are presented.

Chapter I: Evolution and Phylogeny of the Parasitoid

Subfamily Phasiinae (Diptera: Tachinidae)

Abstract

Phasiinae are endoparasitoid flies that attack Heteroptera, including a multitude of agricultural pests. A phylogenetically informed classification of the Phasiinae (Diptera: Tachinidae) has eluded systematists for over a century, primarily because of the conflicting character states and confusing morphology of certain taxa that indicate placement in multiple subfamilies. The unstable nature of phasiine taxonomy discourages important research into their classification, life history, and potential use in biological control. In hopes of resolving several longstanding taxonomic debates and encouraging future research into this important group of parasitoids, the first molecular systematic analysis of the Phasiinae is presented, estimated from 128 worldwide taxa (80 genera) and approximately 7.6 kilobases of nuclear data. Special emphasis is placed on taxonomically ambiguous groups. The resultant robustly supported phylogenetic tree is used to reconstruct ancestral character states, trace the evolution of significant adaptive traits within the Tachinidae, and test hypotheses about the classification of Phasiinae. Subfamily placements of the taxa Eutherini, Epigrimyiini, *Litophasia*, Strongygastrini, and Parerigonini are confidently resolved, the former three within Dexiinae and the latter two within Phasiinae. The phylogenetic position of the Imitomyiini is more difficult to decipher, but the Imitomyiini are tentatively placed here among the Phasiinae. Phasiinae is represented by 12 tribes, with the genera of Phasiini and Parerigonini redistributed among multiple tribes. Ancestral state reconstruction suggests a dominant and persistent trend in Phasiinae to evolve piercing structures used to insert eggs directly into host tissues. A single potential synapomorphy of Phasiinae is identified (elongated hypandrium), but a suite of characters is needed to differentiate Dexiinae from the other subfamilies of Tachinidae.

Introduction and Background

The biological sub-discipline of taxonomy has been studied in various permutations for millennia by humans exploring, discovering, and organizing the diversity of life on earth. In addition to being essential for understanding biodiversity and conservation, taxonomic research creates a framework unencumbered by language barriers for investigating ecology, biomedicine, and biological control around the world. As such it is indispensable to both basic and applied research. A subtle but transformative change in taxonomy came about with the introduction of cladistics in the middle 20th century by Willi Hennig and its eventual acceptance among systematists (Hennig 1965; Wheeler *et al.* 2013). The traditional hierarchical classification of Linnaeus began to be informed by evolutionary hypotheses rather than simply nested similarity among organisms (phenetics). As a result, modern phylogenetics, both morphological and molecular, attempts to create “trees of life” that accurately reflect an organism’s evolutionary history and relationship with other organisms (Wiley and Lieberman 2011).

One hypothesis of evolutionary phylogenetics is that descendants of a common ancestor should have one or more shared derived traits that can be used to distinguish them from other clades and distant relatives/ancestors. Such traits are termed synapomorphies and form the cornerstone of cladistics (Mooi and Gill 2010). While synapomorphies can be fairly easy to identify in some clades (e.g., halteres of Diptera), evolution rarely creates such neat and tidy groups. True synapomorphies are sometimes difficult to find as exceptions occur in many clades. Contributing to this problem are the phenomena of convergent evolution (Wallace and Gibson 2002). Species in separate lineages often evolve the same or very similar morphological traits. When the same complex trait appears in two organisms, the most parsimonious explanation is that the two species shared a common ancestor that also possessed the same trait. This characteristic was then passed on to its descendants, providing an important clue as to the species’ ancestry. This explanation is beautiful in its simplicity as it requires only a single origin for a complex trait or behavior. However, it is not uncommon for evolution to produce similar complex traits in independent lineages (homoplasy), thus obscuring their evolutionary history. What are taxonomists to do then, when a species has competing homoplastic characters that imply an evolutionary relationship within different clades? A satisfying answer to this question is

often difficult to obtain through morphological systematics alone because of these conflicting characters.

For centuries, taxonomy was built solely around the physical traits and behaviors of organisms, but with recent advances in DNA sequencing technology and phylogenetic algorithms, molecular systematics has risen to the forefront of biological classification. Molecular data offers an independent line of evidence for ancestry and evolution that is often able to resolve longstanding questions in classification for which the morphological data are equivocal. While the physical traits of two species evolve independently – sometimes diverging and sometimes converging until little phenotypic difference remains – the evolution of DNA mutations (unlinked to physical traits) steadily widens the genetic gap between the species. Over many generations then, the molecular data often show a consistent diverging pattern between taxa even when the morphology is nearly indistinguishable.

In this study, we present the first molecular systematic analysis focused on the agriculturally important parasitoid subfamily Phasiinae (Diptera: Tachinidae) – a group long characterized by uncertain taxonomy due to widespread homoplasy of morphological traits – and present new evidence for the subfamily and tribal affinities of several morphologically confusing taxa. A robust phylogenetic tree is created which is then used to reconstruct ancestral character states, trace the evolution of significant adaptive traits within the Tachinidae, and test hypotheses about the classification of Phasiinae.

Tachinidae

The family Tachinidae (Superfamily Oestroidea) is a fascinating and diverse group of endoparasitoid flies (Stireman *et al.* 2006). Tachinidae form a well-supported clade defined in part by an enlarged postscutellum and obligate parasitism of arthropod hosts (Wood 1987; Pape 1992). With ~8,500 described species worldwide and perhaps an equal number still undescribed (O'Hara 2013a), Tachinidae are considered to be the most speciose (second in described species to Tipulidae) and morphologically diverse family of Diptera (Crosskey 1980) and one of the largest insect parasitoid groups (Eggleton and Belshaw 1992). Tachinids have experienced such astounding adaptive success in part due to their varied choice in hosts. The family includes both generalist species and specialists that collectively attack a multitude of hosts from 14 different orders of arthropods. They primarily attack caterpillars, beetles, and bugs (Heteroptera) (Arnaud

1978), but also parasitize scorpions (Williams and Arnaud 1990) and centipedes (Wood and Wheeler 1972). By parasitizing insect plant pests like lepidopteran caterpillars and heteropteran bugs, tachinids play an important role in regulating pest populations in both natural ecosystems and controlled agricultural environments (Nishida 1966; Karban and English-Loeb 1997; Coombs 2004; Stireman and Singer 2003).

Despite their abundance and potential for use as biological control agents, the taxonomy of Tachinidae is well known among insect systematists for being difficult and confusing (see O'Hara 2013b and Blaschke 2015 for reviews). They appear to be a recent (minimum Oligocene, O'Hara *et al.* 2013) and actively radiating group of insects and this recent evolution has created substantial problems in reconstructing phylogenetic relationships (Guimarães 1971; Stireman 2002; Cerretti *et al.* 2014; Winkler *et al.* 2015). Additionally, there is widespread homoplasy throughout the family resulting in very few clear synapomorphies that distinguish genera, tribes, or even subfamilies (Wood 1987).

Modern tachinid experts recognize four subfamilies: Phasiinae, Dexiinae, Exoristinae, and Tachininae (Herting 1984; Tschorsnig and Richter 1998; O'Hara and Wood 2004; Cerretti *et al.* 2014). However, relationships within and among the subfamilies are poorly understood and have undergone considerable rearrangement throughout their history (O'Hara 2013b). The subfamilies Tachininae and Exoristinae are defined based on the presence of a collection of multiple character traits and/or life history traits that are difficult to identify and contain many exceptions (Wood 1987). Only Dexiinae and Phasiinae have potential synapomorphic character traits, both found in the male postabdomen (Dexiinae: hinged, membranous connection of basi/distiphallus; Phasiinae: elongated hypandrium) (Tschorsnig 1985). Therefore, a phylogeny that defines relationships even among a single subfamily like Phasiinae can contribute significant information to a broader picture of tachinid evolution and history.

Phasiinae

The subfamily Phasiinae is the smallest of the Tachinidae yet still contains ~600 species in ~100 genera (Crosskey 1973, 1976; Herting 1984; Tschorsnig 1985; Wood 1987; Herting and Dely-Draskovits 1993; Tschorsnig and Richter 1998; Ziegler 1998; Richter 2004; O'Hara *et al.* 2009; Blaschke 2015). Even so, some of the most extreme morphological differences between genera within the Tachinidae can be found in Phasiinae. Phasiines differ dramatically in size and

appearance, from one of the smallest tachinids (*Catharosia* Rondani: <2mm) to one of the largest (*Lophosia* Meigen: 18mm). Some phasiines are colored in traditional blacks and greys while others mimic the banded patterns or bright colors that often indicate toxicity in other insects (aposematism). As a result, several genera closely resemble various hymenopterans, most notably *Polistiopsis* Townsend, *Formicophania* Townsend, and *Cylindromyia mirabilis* (Townsend). Most species in the tribes Cylindromyiini and Gymnosomatini (including Trichopodini) have bright red, orange, or black and yellow abdomens that mimic the warning colors of wasps and bees (Waldbauer *et al.* 1977).

The primary hosts for this group of parasitoids are heteropteran bugs – many of which are prominent agricultural pests, including *Nezara viridula* L. (southern green stink bug), *Euschistus servus* Say (brown stink bug), and *Lygus lineolaris* Beauvois (tarnished plant bug) (Coquillett 1897; Arnaud 1978). Phasiines also attack the invasive pests *Halyomorpha halys* (brown marmorated stink bug, Aldrich *et al.* 2007) and *Megacopta cribraria* (kudzu bug, Golec *et al.* 2013). Many phasiines identify potential hosts through the use of specialized antennal receptors that are extremely sensitive to host pheromones (Aldrich *et al.* 2006). Remarkably, many phasiines are more sensitive to their host's pheromones than even the hosts are themselves (Aldrich *et al.* 1989). These phasiines are most commonly attracted to aggregation pheromones of the male bugs and as a result generally parasitize more males than females. Because of their distinctive ability to use pheromone cues, phasiines hold great promise as biological control agents but have been underutilized as such due to their confusing taxonomy and difficult identification. A predictive and well-supported taxonomy of Phasiinae will provide fertile research ground for future studies in biological control, co-evolution of interspecies complexes, pheromone attractants, and tritrophic ecological interactions.

Taxonomy

The subfamily Phasiinae is taxonomically separated from other tachinid subfamilies by their reduced chaetotaxy, parasitism of heteropteran hosts, oviparity (i.e., laying of unembryonated eggs), possession of a piercer derived from either the 8th or 10th sternite (with exceptions), and an elongated hypandrium in the male postabdomen (Coquillett 1897; O'Hara 1985; Tschorsnig 1985; Stireman *et al.* 2006). Their closely related sister group, Dexiinae, is defined by potential synapomorphies in the male postabdomen including an entirely

membranous and “hinged” ($\leq 90^\circ$) connection between the basiphallus and the distiphallus and the presence of platform pregonites (Tschorsnig 1985; Tschorsnig and Richter 1998; O’Hara and Wood 2004; Cerretti *et al.* 2014). In contrast to the Phasiinae, dexiines primarily attack Coleoptera and Lepidoptera and are ovolarviparous (i.e., lay late developmental stage eggs or early instar larvae) (Wood 1987; Stireman *et al.* 2006). Given these definitions of Phasiinae and Dexiinae, several taxa cannot be confidently placed in either subfamily as they possess characters used to define both. The conflicting characters of these taxa are briefly introduced below.

Epigrimyini – In favor of a placement in Phasiinae, the two genera of tribe Epigrimyini (*Epigrimyia* Townsend and *Beskia* Brauer and Bergenstamm) parasitize Heteroptera (Guimarães 1977; Biehler and McPherson 1982; Sutherland and Baharally 2002), possess 8th sternite piercers, and have reduced chaetotaxy (Wood 1987). *Beskia* has a bright red/orange abdomen. However, Epigrimyini do not possess the elongated hypandrium of Phasiinae. Rather, they share the male terminalic characters of the Dexiinae (membranous, hinged connection between the basiphallus and the distiphallus and platform pregonites) (Tschorsnig 1985; O’Hara and Wood 2004) as well as that subfamily’s ovolarviparity (Townsend 1938).

Eutheriini – The Eutherini (*Euthera* Loew and *Redtenbacheria* Schiner) are heteropteran parasitoids (Arnaud 1978; Nishayama 1995), which usually indicates a placement within Phasiinae. They also produce planoconvex eggs (membranous on the ventral side, but thickened and convex on the dorsal surface) (Herting 1966; Mesnil 1966) rather than the entirely membranous eggs of the Dexiinae (Stireman *et al.* 2006). However, even though their eggs are morphologically similar to those found in Phasiinae, the Eutherini are ovolarviparous rather than oviparous and do not have piercers (Townsend 1938; Cantrell 1983). Additionally, they possess the dexiine-like male terminalia trait of platform pregonites, but the connection between the basiphallus and the distiphallus is neither hinged nor entirely membranous as in other dexiines (Tschorsnig 1985; Cerretti *et al.* 2014). Neither do they possess a phasiine-like elongated hypandrium. Because of the oviposition strategy and egg-type of Eutherini, the subfamily Exoristinae has also been proposed as a possible location for this enigmatic tribe (Cerretti *et al.* 2014).

Imitomyiini – Similarly, the Imitomyiini (*Imitomyia* Townsend, *Proriedelia* Mesnil, and *Riedelia* Mesnil) are found in a confusing morphological intermediacy between the Dexiinae and Phasiinae. Hosts are unknown, but *Imitomyia* females possess a 10th sternite piercer and the males an elongated hypandrium (females of other genera are unknown) – both uniquely phasiine traits (Townsend 1936, 1938; Tschorsnig 1985; O’Hara and Wood 2004). However, the Imitomyiini are ovularviparous like the Dexiinae and possess all the characteristics previously mentioned of the dexiine male postabdomen except the $\leq 90^\circ$ angle of the “hinged” connection of the basiphallus to the distiphallus (Tschorsnig 1985; O’Hara and Wood 2004; Cerretti *et al.* 2014).

Litophasia – The rare genus *Litophasia* Girschner possesses a piercing 8th sternite and has significantly reduced chaetotaxy, both of which indicate a placement in Phasiinae (Dear 1980), but its hosts and oviposition strategy are unknown. In contrast to these traits, *Litophasia*, like *Imitomyia*, has all the features of a dexiine male postabdomen except the hinged connection of the basiphallus to the distiphallus (Tschorsnig 1985). *Litophasia* is currently classified in Phasiinae: Catharosiini (Crosskey 1980; Herting 1983).

Strongygastrini/Rondaniooestrini – Two additional tribes have maintained a debated taxonomic position between Phasiinae and the subfamily Tachininae. Foremost among these are the tribes Strongygastrini (*Strongygaster* Macquart, *Arcona* Richter, and *Melastrongygaster* Shima) and the closely related tribe Rondaniooestrini (*Rondaniooestrus* Villeneuve). These genera align closely with Phasiinae in respect to the male genitalia (including elongated hypandrium) and overall habitus (Tschorsnig 1985, Cerretti 2014, pers. com.). The primary argument for placement in the Tachininae is a result of the unusual host range found in these taxa. *Rondaniooestrus* is the only known tachinid parasite of honeybees (Villeneuve 1924) and is not known to parasitize anything else. *Strongygaster*, on the other hand, parasitizes heteropterans, similar to other phasiines (Sabrosky and Braun 1970; Santos and Panizzi 1997; Panizzi and Oliveira 1999; Golec *et al.* 2013; pers. observ.), but also attacks a wide array of other insects including Orthoptera (Kevan and Koshnaw 1988), Coleoptera (Arnaud 1978), Hymenoptera (Eggleton and Belshaw 1992; Feener 2000), Lepidoptera (Sabrosky and Braun 1970), Dermaptera (Sabrosky and Braun 1970), and Diptera (Ferrar 1977). No other phasiine

regularly exploits hosts outside of Heteroptera, and therefore the host range of *Strongygaster* would be unprecedented in the subfamily. Their unusual hosts coupled with the fact that these genera are ovularviparous rather than oviparous indicates a placement in Tachininae rather than Phasiinae (Wood 1987).

Parerigonini – The Parerigonini are currently considered to be in Phasiinae (Tschorsnig 1985; O’Hara *et al.* 2009; Wood and Zumbado 2010), but have historically been members of Tachininae (Townsend 1936, 1939; Guimarães 1971; Crosskey 1973, 1976; Cantrell and Crosskey 1989). Their external morphology is quite divergent from typical Phasiinae with strong correlations to the Linnaemyini (Tachininae). However, their male and female terminalia (elongated hypandrium – Tschorsnig 1985; piercers – Cantrell 1988) and hosts, where known, indicate a placement in Phasiinae (Mesnil 1970; Shima 2015b).

Objectives

Representatives of each of the above potential phasiine tribes as well as genera from all widely accepted traditional phasiine tribes were included in this analysis in an attempt to clarify the confusion of phasiine classification. This research had the following objectives:

1. Determine whether Phasiinae are monophyletic, and if so, if there are any synapomorphies that define the clade.
2. Assess the monophyly/validity of, and relationships between, tribes in Phasiinae.
3. Infer the evolutionary relationships of the following taxonomically ambiguous taxa: Epigrimyiini, Eutheriini, Imitomyiini, *Litophasia*, Strongygastrini, Rondaniooestrini, and Parerigonini.
4. Trace the evolution of important traits across the phylogeny to test hypotheses about subfamily synapomorphies and the evolution of Tachinidae.

Materials and Methods

Collection

An international collaboration was necessary to obtain representative genera of Phasiinae and other subfamilies from around the world. We are very grateful for the specimens and sequences provided by other researchers (see Acknowledgements section) without which this phylogeny would have been much more incomplete and less supported. Specimens were obtained through Malaise trapping and/or hand collecting. Malaise specimens were preserved in 95% ethanol. Manually collected tachinids were pinned to preserve intact morphological specimens for species identification. During pinning, one to three right legs from each specimen were removed and placed in 95% ethanol for DNA extraction. For some very small specimens with known species identification, the entire fly was used. Post-extraction DNA samples were stored at -20°C. This study is a subproject of a larger ongoing phylogeny of Tachinidae and therefore voucher specimens are being temporarily housed in the Blaschke Lab – Union University (TN), the Moulton Lab – University of Tennessee, the Stireman Lab – Wright State University (OH), the Canadian National Collection (Ottawa, Canada), the Cerretti Lab – University of Rome (Italy), and the Tachi Lab – Kyushu University (Japan).

The idealistic goal was to collect representative genera of every tribe within the diverse Phasiinae as well as species from every tribe with a debated subfamily position for which Phasiinae is a possible resting place. Remarkably, this goal was almost achieved – only the two extremely rare South American tribes Tarassini and Euscopoliopterygini (possible phasiines), which are known from only a few museum specimens, are absent from the phylogeny. In total, 126 specimens (80 genera, Table 1.1) were included in this study representing all seven widely accepted tribes of Phasiinae, an additional seven tribes with uncertain subfamily affiliations, eight tribes of Dexiinae, six tribes of Exoristinae, and five tribes of Tachininae. Also included as outgroup taxa were specimens of Calliphoridae (3 species), Rhinophoridae (3 species), Sarcophagidae (1 species), Oestridae (1 species), and Muscidae (1 species).

Table 1.1: List of taxa included in the phylogeny and corresponding gene coverage. * indicates CAD sequences are from the Stireman Lab. ** indicates CAD sequences are from the Tachi Lab.

<u>Tachinidae: Phasiinae</u>						
Tribe	Genus species	Country	CAD	LGL	MCS	MAC
Catharsiini	<i>Catharosia nebulosa</i> (Coquillett)	USA	x	x	x	x
	<i>Catharosia</i> sp.	Australia	x	x	x	x
	<i>Catharosia</i> sp.	Thailand	x	x	x	x
Cylindromyiini	<i>Australotachina calliphoroides</i> Curran 1	Australia	x	x	x	x
	<i>Australotachina calliphoroides</i> Curran 2	Australia	x	x	x	x
	<i>Besseria atra</i> (Coquillett)	Canada	x	x	x	x
	<i>Besseria</i> sp.	South Africa	x	x	x	x
	cf. <i>Lophosia</i> sp. 1	Thailand	x	x	x	x
	cf. <i>Lophosia</i> sp. 2	Korea	x	x	x	x
	cf. <i>Pygidimya</i> sp.	Australia	x	x	x	x
	<i>Cylindromyia binotata</i> (Bigot)	USA	x	x	x	x
	<i>Cylindromyia mirabilis</i> (Townsend)	USA	x	x	x	x
	<i>Cylindromyia rufifrons</i> (Loew)	Sardinia	x	x	x	x
	<i>Cylindromyia</i> sp.**	Japan	x			
	<i>Cylindromyia</i> sp. 1	Australia	x	x	x	x
	<i>Cylindromyia</i> sp. 1	South Africa	x	x	x	x
	<i>Cylindromyia</i> sp. 2	Australia	x	x	x	x
	<i>Cylindromyia</i> sp. 2	South Africa	x	x	x	x
	<i>Hemyda aurata</i> Robineau-Desvoidy	USA	x	x	x	x
	<i>Huttonobesseria verecunda</i> (Hutton)	New Zealand	x	x	x	x
	<i>Lophosia fasciata</i> Meigen	Finland	x	x	x	x
	<i>Lophosia</i> sp.	Thailand	x	x	x	x
	<i>Neobrachelia</i> sp.	Ecuador	x	x	x	x
	<i>Phania funesta</i> (Meigen)	Italy	x	x	x	x
	<i>Prolophosia petiolata</i> Townsend	Burundi	x	x	x	x
	<i>Prolophosia</i> sp.	Burundi	x	x	x	x
	<i>Pygidimya</i> sp.	Australia	x	x	x	x
Gymnosomatini	<i>Acaulona</i> sp.	Costa Rica	x	x	x	x
	<i>Cistogaster mesnili</i> (Zimin)	Italy	x	x	x	x
	<i>Clytiomya dupuisi</i> Kugler	Italy	x	x	x	x
	<i>Clytiomya sola</i> (Rondani)	Italy	x	x	x	x
	<i>Ectophasia crassipennis</i> (Fabricius)	Italy	x	x	x	x
	<i>Ectophasia oblonga</i> (R.-D.)	Israel	x	x	x	x
	<i>Eliozeia helluo</i> (Fabricius)	Italy	x	x	x	x
	<i>Eliozeia pellucens</i> (Fallén)	Slovenia	x	x	x	x
	<i>Euclytia flava</i> (Townsend)	USA	x	x	x	x
	<i>Gymnoclytia immaculata</i> (Macquart)	USA	x	x	x	x
	<i>Gymnoclytia occidua</i> (Walker)	USA	x	x	x	x
	<i>Gymnoclytia</i> sp.	Costa Rica	x	x	x	x

Table 1.1 (continued)

<u>Tachinidae: Phasiinae</u>						
Tribe	Genus species	Country	CAD	LGL	MCS	MAC
Gymnosomatini	<i>Gymnosoma clavatum</i> (Rohdendorf)	Germany	x	x	x	x
	<i>Gymnosoma inornatum</i> Zimin	Italy	x	x	x	x
	<i>Gymnosoma nitens</i> Meigen	Slovenia	x	x	x	x
	<i>Gymnosoma par</i> Walker	USA	x	x	x	x
	<i>Gymnosoma rotundatum</i> (L.)	Italy	x	x	x	x
	<i>Gymnosoma</i> sp.	Korea	x	x	x	x
	<i>Pentatomophaga latifascia</i> (Villeneuve)**	Japan	x			
	<i>Trichopoda lanipes</i> (Fabricius) 1	USA	x	x	x	x
	<i>Trichopoda lanipes</i> (Fabricius) 2	USA	x	x	x	x
	<i>Trichopoda pennipes</i> (Fabricius) 1	Fr. Polynesia		x	x	x
	<i>Trichopoda pennipes</i> (Fabricius) 2	USA	x	x	x	x
	<i>Trichopoda pennipes</i> (Fabricius) 3	USA		x	x	x
	<i>Trichopoda plumipes</i> (Fabricius)	USA	x	x	x	x
	<i>Xanthomelanodes arcuatus</i> (Say)	USA	x	x	x	x
	<i>Xanthomelanodes californicus</i> Townsend	USA	x	x	x	x
Hermyini	cf. <i>Formicophania</i> sp.	Thailand	x	x	x	x
	<i>Hermya beelzebul</i> (Weidemann)**	Japan	x			
	<i>Hermya</i> sp. 1	Burundi	x	x	x	x
	<i>Hermya</i> sp. 2	Malaysia	x	x	x	x
	<i>Penthosia satanica</i> (Bigot	USA	x	x	x	x
	<i>Penthosiosoma</i> sp.	Thailand	x	x	x	x
Imitomyiini	<i>Imitomyia sugens</i> (Loew)	USA	x	x	x	x
Leucostomatini	<i>Calyptromyia barbata</i> Villeneuve**	Japan	x			
	<i>Clairvillia biguttata</i> (Meigen)	Israel	x	x	x	x
	<i>Clairvillia timberlakei</i> (Walton)	USA	x	x	x	x
	<i>Clairvilliops breviforceps</i> (van Emden)**	Japan	x			
	<i>Labigastera nitidula</i> (Meigen)	Italy	x	x	x	x
	<i>Leucostoma</i> sp. 1	USA	x	x		x
	<i>Leucostoma</i> sp. 2	USA	x	x	x	x
	<i>Weberia digramma</i> (Meigen)	Israel	x	x	x	x
Opesia clade	<i>Opesia grandis</i> (Egger)	Italy	x	x	x	x
Parerigonini	<i>Parerigone</i> sp.**	Japan	x			
	<i>Zambesomima</i> sp.**	Japan	x			
Phasiini	<i>Elomya lateralis</i> (Meigen)	Israel	x	x	x	x
	<i>Phasia aurulans</i> Meigen	USA	x	x	x	x
	<i>Phasia hemiptera</i> (Fabricius)**	Japan	x			
	<i>Phasia</i> sp. 1	South Africa	x	x	x	x
	<i>Phasia</i> sp. 2	South Africa	x	x	x	x
	<i>Phasia</i> sp. 3	Ecuador	x	x	x	x
	<i>Phasia</i> sp. 4	Japan	x			

Table 1.1 (continued)

<u>Tachinidae: Phasiinae</u>						
Tribe	Genus species	Country	CAD	LGL	MCS	MAC
Phasiini	<i>Phasia subcoleoptrata</i> (L.)	Israel	x	x	x	x
Strongygastriini	<i>Rondaniooestrus apivorous</i> Villeneuve	South Africa	x	x	x	x
	<i>Strongygaster robusta</i> (Townsend)	USA	x	x	x	x
	<i>Strongygaster</i> sp.	Thailand	x	x	x	x
	<i>Strongygaster triangulifera</i> (Loew)	USA	x	x	x	x
Xysta clade	<i>Xysta holosericea</i> (Fabricius)	Israel	x	x	x	x
Zitini	cf. <i>Leverella</i> sp.	Australia	x	x	x	x
	<i>Leverella</i> sp.	Australia	x	x	x	x
	<i>Zita</i> sp. 1	Australia	x	x	x	x
	<i>Zita</i> sp. 2	Australia	x	x	x	x
	<i>Zita</i> sp. 3	Australia	x	x	x	x
<u>Tachinidae: Dexiinae</u>						
Tribe	Genus species	Country	CAD	LGL	MCS	MAC
Campylochetaiini	<i>Campylocheta semiothisae</i> (Brooks)	USA	x	x	x	x
Dexiini	<i>Billaea</i> sp.	USA	x	x	x	x
	<i>Euchaetogyne roederi</i> (Williston)*	Canada	x	x	x	x
	<i>Ptilodexia</i> sp.	USA	x	x	x	x
	<i>Oestrophia</i> sp.	USA	x	x	x	x
Dufouriini	<i>Beskia aelops</i> (Walker)	USA	x	x	x	x
Epigrimyiini	<i>Epigrimyia illinoensis</i> Robertson	USA	x	x	x	x
	<i>Euthera tentatrix</i> Loew	Australia	x	x	x	x
Eutherini	<i>Euthera</i> sp. 1	USA	x	x	x	x
	<i>Euthera</i> sp. 2**	Japan	x			
	<i>Rutelia regalis</i> Guérin-Méneville	Australia	x	x	x	x
Sophiini	<i>Cordyligaster</i> sp.	USA	x	x	x	x
Thelairiini	<i>Thelaira americana</i> Brooks	USA	x	x	x	x
Uramyini	<i>Uramya pristis</i> (Walker)	USA	x	x	x	x
Voriini	<i>Voria ruralis</i> (Fallén)	USA	x	x	x	x
Unplaced	<i>Litophasia</i> sp.	South Africa	x	x	x	x
<u>Tachinidae: Exoristinae</u>						
Tribe	Genus species	Country	CAD	LGL	MCS	MAC
Blondeliini	<i>Blondelia eufitchiae</i> (Townsend)	USA	x	x		x
Acemyini	<i>Ceracia dentata</i> (Coquillett)	USA	x	x	x	x
Goniini	<i>Hyphantrophaga hyphantriae</i> (Townsend)	USA	x	x	x	x
Eryciini	<i>Lespesia</i> sp.	USA	x	x	x	x
Exoristini	<i>Tachinomyia nigricans</i> Webber	USA	x	x	x	x
Winthemiini	<i>Winthemia</i> sp.	USA	x	x	x	x

Table 1.1 (continued)

<u>Tachinidae: Tachininae</u>						
Tribe	Genus species	Country	CAD	LGL	MCS	MAC
Ernestiini	<i>Panzeria ampellus</i> (Walker)	USA	x	x	x	x
Siphonini	<i>Siphona plusiae</i> Coquillett	USA	x	x	x	x
Tachinini	<i>Tachina</i> sp.*	USA	x	x	x	x
Polideini	<i>Homalactia harringtoni</i> Coquillett*	USA	x	x	x	x
<u>Outgroup taxa</u>						
Subfamily	Genus species	Country	CAD	LGL	MCS	MAC
Calliphoridae	<i>Angioneura abdominalis</i> (Reinhard)*	USA	x	x	x	x
Oestridae	<i>Cuterebra austeni</i> Sabrosky*	USA	x	x	x	x
Calliphoridae	<i>Lucilia sericata</i> Meigen*	USA	x	x	x	x
Rhinophoridae	<i>Melanophora roralis</i> L.*	USA	x	x	x	
Calliphoridae	<i>Pollenia pediculata</i> Macquart*	USA	x	x	x	x
Rhinophoridae	<i>Rhinomorinia</i> sp.*	South Africa	x	x	x	x
Sarcophagidae	<i>Macronychia</i> sp.*	USA	x	x	x	x
Muscidae	<i>Musca domestica</i> L.*	USA	x	x	x	x
Rhinophoridae	<i>Axinia</i> sp.*	Australia	x	x	x	x

Genes

The genes used in this study, *carbamoyl-phosphate synthetase 2/rudimentary* (CAD), *lethal giant larvae* (LGL), *methyl-accepting chemoreceptor* (MAC), and *molybdenum cofactor sulfuryase* (MCS), were chosen specifically for their ability to resolve nodes within a phylogeny of rapidly evolving lineages. CAD was first introduced by Moulton and Weigmann (2004), while the significant phylogenetic utility of LGL, MAC, and MCS recently debuted in Winkler *et al.* (2015). Each gene is a single copy nuclear protein coding gene that offers significantly more phylogenetic signal than more traditional markers such as COI/II, 28S, EF1- α , etc. (Winkler *et al.* 2015). The standard ribosomal and mitochondrial genes are easier to obtain, but do not contain enough nonsynonymous mutations to fully resolve the phylogeny of a young and diverse group like the Tachinidae. The additional effort required to amplify our target genes resulted in substantially better phylogenetic resolution at the subfamily level and for controversial taxa than what has been produced in previous attempts at tachinid molecular systematics (Stireman 2002; Tachi and Shima 2010). All sequences for LGL, MAC, and MCS were processed in the Moulton Lab (University of Tennessee). A few taxa for CAD were processed in the Stireman Lab (Wright State University) or the Tachi Lab (Kyushu University) (see Table 1.1).

Extraction and Amplification

Genomic DNA was extracted using a ThermoScientific™ DNA extraction kit according to the manufacturer's protocol with few minor modifications. Custom primers for CAD, LGL, MAC, and MCS were designed by J.K. Moulton (Table 1.2). The target genes were amplified using 53µL PCR reactions in MasterCycler thermal cyclers (Eppendorf North America, Westbury, NY). PCR reactions combined 36µL of ultra-pure DI H₂O, 2µL of MgCl₂ (25mM), 5µL of 10x buffer solution, 3.5µL dNTPs solution, and 0.2µL of *Taq* polymerase (10x, dNTPs, and *Taq* from Hotstart Ex Taq kits (Takara Bio Inc., Shiga, Japan)) with 1-1.5µL of purified template DNA and 3µL (10µM) each of forward and reverse primers. A three-step touchdown PCR program was used to amplify the genes. The parameters of the most commonly used program "56-51-46" were as follows: 30s denaturation at 94°C; 5 cycles of 94°C for 30s, 56°C for 15s and 72°C for 1.5 min; 5 cycles of 94°C for 30s, 51°C for 15s and 72°C for 1.5 min; 30 cycles of 94°C for 30s, 46°C for 15s and 72°C for 1.5 min, and a final extension for 5 min at 72°C. For some genes/taxa that were difficult to amplify, annealing temperatures were increased to "59-54-49" with all other parameters the same.

After PCR, the amplified gene products were electrophoresed through a 1% agarose gel at 115V for 25 min, excised from the gel, purified with Qiagen© (Redwood City, California) silica column based gel extraction kits and eluted in 35µL of elution buffer (10mM Tris, pH 8.5). Purified PCR products served as templates for sequencing reactions using the same primers used in PCR reactions at 50% concentration. When required, both strands of each product were then cycle-sequenced using Big Dye Terminator Cycle Sequencing kits (Applied Biosystems, Carlsbad, California). Subsequent products were cleaned using Centri-sep purification columns (Princeton Separations, Adelphia, New Jersey) and sent to the Molecular Biology Resource Facility at the University of Tennessee for sequencing. Chromatograms of forward and reverse sequences were then reconciled and verified for accuracy using Sequencher 5.3 (Gene Codes Corp., Ann Arbor, Michigan). The boundaries of each intron were identified and the introns removed following the GT-AG rule based upon achieving a continuous open reading frame of targeted exons after the exclusion of introns (Rogers and Wall 1980). CAD and MAC had a single intron each, while LGL and MCS each contained two introns.

Table 1.2: Custom primers for CAD, LGL, MAC, and MCS used in molecular analysis of Tachinidae.

Gene	Primer Name	F/R	Sequence (5' → 3')¹
<u>CAD</u>	CAD_54F	F	GTNGTNTTYCARACNGGNATGGT
	CAD_54FM	F	GTNGTNTTYCARCCNGGYATGGT
	CAD_53F	F	GGNGARGTNGTNTTYCARAC
	CAD_57F	F	TCNATGACNGAYCGYTCRTAYAG
	CAD_405R	R	GCNGTRTGYYTCNGGRTGRAAYTG
	T'podine_CAD_P1R	R	TTCVAGRTCTTCSGGACCAGC
	Melanoph_CAD_P1R	R	CCAATGGCAAAGTCTCAGTG
	CAD_320F	F	TTYGGNATYTGYYTGGGYCAYCA
	tCAD_P2F	F	AAYTTNCCRTGYATYCAAYGG
	THAX_CAD_P2F	F	GGACGTTGCTTTATGACATCTC
	GLEX_CAD_P2F	F	TGTTTCATGACTTCWCAAAAYC
	CAD_Mesemb_P2F	F	GGCACTGGGCGTTGTTATA
	Oestroph_CAD_P2F	F	GTATACATCTTGATACAAAG
	Epigrimy_CAD_P2F	F	TTACAAATGCCAATGACGGT
	Billaea_CAD_P2F	F	CCTTGTAATACATCACGGA
	CAD_680R	R	GCRTCYCKNACMACYTCRTAYTC
	tCAD_P2R	R	GGRTCYAARTTYTCCATRTTRCA
<u>LGL</u>	LGL_38F	F	CARCAYGGNTTYCCNCAYAARCC
	LGL_Tach_P2F	F	AARGCNGGNGARGARCARGA
	LGL_36F	F	ACNGCNCARCAYGGYTTYCC
	LGL_Tach_P2F2	F	GGHCAYGGNCARAGYGT
	LGL_Intron_Fwd_CU	F	GCNTAYGGYGAYCAYAAAYTG
	LGL_472R	R	CACNCCNGTRCARTCCCARAA
	LGL_617R	R	ARYTGRTCRTGNCCYTTCCA
	LGL_Tach_P1R	R	CGRTTWATRYTYTRCA
	LGL_Tach_P2R	R	GGYAWRTCRTCYTGRTTYTCRTG

Table 1.2 (continued)

Gene	Primer Name	F/R	Sequence (5' → 3') ¹
<u>MCS</u>	Tach_MCS_P1F	F	TGYTAYYTNGAYCAYGCNCG
	MCS_55(Y)	F	TGYTAYYTNGANNNNCYGG
	MCS_TACH_P1_5SP	F	ATHCTYCARCAYTTYAAYGCGAYCC
	Tach_MCS_P1R	R	GCRTADATRCADATYTCYTT
	APWXB_MCS_P1R	R	TTAAAAGCTCCACAWGATTT
	MCS_P1R	R	ATYAAATCRTRTARTCRCTRCA
	CYL_MCS_P2F	F	CARGGNGGNGTNATTACYTTYAA
	MCS_463_P2F	F	YCTNCGNACNGGNTGYTTYTG
	TRICH_MCS_P2F	F	TTYAATATTYTACAYGAAGAYGG
	MCS_GTX_P2F	F	CATGTYTTTCAAYTRGCAAAATAYTG
	MCS_446F	F	TTYGTNGGNTTYGCGARGT
	MCS_838R	R	TGRTCDATRCADATCANNRCA
<u>MAC</u>	TMAC_P1_F3	F	GAYGARAARCGNATGTAYGARAARCA
	TMAC_P1_F3-1	F	GAYGARAARCGNATYATGTAYGA
	GEE_MAC_P1R	R	NYTTYAAATAMGCCATTATACT
	SBT/SST_MAC_P1R	R	TGCCAYTGATATGTTCTTA
	MAC_NEWTACH_P1R	R	TTNGCYAARTANGAYTCYTTRTG
	MAC_NP2F_MISSING	F	TATCANTGGCAYARYATATTGG
	MAC_NP2F_MISS#2	F	GARAAATATCTNAGAACATATCA
	TACH_MAC_P1R	R	TCYTTRTGYGTCARRTGTCRTG
	TMAC_NP1R	R	GTRTCYTTYAARTARGCCAT
	TMAC_P2FN_MOD	R	CAYGCGAYTGYGCRCTRCAAA
	TMAC_NP2_FWD	F	CCYTTYCCNGTNGAYATACARTA
	MAC_949F	F	ACNACNGARCCRTAYTTAGAYGC
	MAC_957F	F	GCNGGNGGNGCNGGRTAYAT
	NEW_TMAC_P2R	R	TCYTGYTGRCACCAAYTCRCA

¹ W=A/T; Y=C/T; R=A/G; M=A/C; K=G/T; H=A/T/C; B=C/G/T; N=A/T/C/G.

Phylogenetic Analysis

All phylogenetic analyses were performed through the online CIPRES Science Gateway (Miller *et al.* 2010). Alignment of nucleotides was completed using the parallel version of MAFFT v6 (Kato *et al.* 2002; Kato and Toh 2010). These alignments were visualized and final adjustments were made in Mesquite 3.03 (Maddison and Maddison 2015). Mesquite was also used to create partitions of codon positions for each gene.

Maximum likelihood (ML) analyses were performed using RAxML 7.0.3 (Stamatakis 2006; Stamatakis *et al.* 2008). Phylogenies were created from both partitioned and unpartitioned data for each individual codon position of every gene, each entire gene individually, and all data combined in a single matrix. In the final concatenated data matrix there were 12 distinct data partitions whose parameters were estimated separately but were analyzed together with joint branch length optimization. For each partition, the free model parameters, including base frequencies, were estimated by RAxML. The substitution matrix chosen was GTR, and GAMMA model parameters were estimated from the matrix. The statistical robustness of the phylogenies was measured by conducting 1000 bootstrap replicates and by comparing the tree topologies from each analysis.

Following the ML analysis, a Bayesian phylogeny using Markov Chain Monte Carlo methods was estimated with MrBayes 3.2.2 (Ronquist *et al.* 2012). Each gene was partitioned by codon position and evaluated separately. Additionally, a combined dataset containing all four genes with a total of 12 partitions was analyzed. Parameters were unchanged between phylogenies and included default priors. Nucleotide substitution matrix, rate variation, gamma shape parameter, and base frequencies were estimated separately for each data partition (nst = mixed; rate = invgamma; unlink statefreq = (all); revmat = (all); tratio = (all); shape = (all); pinvar = (all); prset applyto = (all) ratepr = variable). Two runs with six chains each were run for a total of 30 million generations. Markov chains were sampled at intervals of 500 generations and the first 35% of trees discarded as burn-in prior to assembling a 50% majority rule consensus tree. Verification that stationarity had been reached was measured by the standard deviation of split frequencies (value = 0.0024, should be <0.1), the Potential Scale Reduction Factor (value = 1.0, should approach 1.0 as runs converge), and the MrBayes output overlay plot which showed no directional trend for either run.

Ancestral State Reconstruction

Seven evolutionarily significant traits were chosen for Ancestral State Reconstruction (ASR) (Table 1.3, 1.4). Each specimen was scored based on information extracted from published literature or physical examination of specimens. When necessary, information from congeneric species was used. Using Mesquite's ASR package (v.2.74, Maddison and Maddison 2010), maximum likelihood (ML) methods were used to reconstruct hypothetical ancestral states. Traits were mapped onto phylogenies generated from the partitioned, concatenated ML analysis. Results were evaluated based on likelihood scores and potential evolutionary explanations for the trait evolution suggested by the ASR analysis.

Table 1.3: Character matrix used for ASR analysis.

#	Character / States	0	1	2	3	4
1	Location of piercer	No piercer	8th sternite, straight	8th sternite, corkscrew	10th sternite	7th sternite
2	Hosts	Heteroptera	Lepidoptera	Coleoptera	Hymenoptera	Orthoptera
3	Oviposition strategy	Oviparity	Ovoviviparity			
4	Elongated hypandrium	Absent	Present			
5	Membranous basi/distiphallus	Absent	Present			
6	Hinged basi/distiphallus	Absent	Present			
7	Platform pregonites	Absent	Present			

Table 1.4: Coded character states for Tachinidae. ? = unknown state.

<u>Phasiinae</u>								
Taxa	1	2	3	4	5	6	7	
<i>Acaulona</i> sp. CR	1	0	0	1	0	0	0	
<i>Australotachina calliphoroides</i> AUS1	3	0	0	1	0	0	0	
<i>Australotachina calliphoroides</i> AUS2	3	0	0	1	0	0	0	
<i>Besseria atra</i> CAN	3	0	0	1	0	0	0	
<i>Besseria</i> sp. SAfr	3	0	0	1	0	0	0	
<i>Calyptromyia barbata</i> JAP	1	0	0	1	0	0	0	

Table 1.4 (continued)

Taxa	<u>Phasiinae</u>						
	1	2	3	4	5	6	7
<i>Catharosia nebulosa</i> USA	1	0	0	1	0	0	0
<i>Catharosia</i> sp. AUS	1	0	0	1	0	0	0
<i>Catharosia</i> sp. THA	1	0	0	1	0	0	0
cf. <i>Formicophania</i> THA	0	0	0	1	0	0	0
cf. <i>Leverella</i> AUS	3	?	0	1	0	0	0
cf. <i>Lophosia</i> THA	3	0	0	1	0	0	0
cf. <i>Pygidimya</i> AUS	3	0	0	1	0	0	0
<i>Cistogaster mesnili</i> ITA	0	0	0	1	0	0	0
<i>Clairvillia biguttata</i> ISR	1	0	0	1	0	0	0
<i>Clairvillia timberlakei</i> USA	1	0	0	1	0	0	0
<i>Clairvilliops breviforceps</i> JAP	1	0	0	1	0	0	0
<i>Clytiomya dupuisi</i> ITA	0	0	0	1	0	0	0
<i>Clytiomya sola</i> ITA	0	0	0	1	0	0	0
<i>Cylindromyia binotata</i> USA	3	0	0	1	0	0	0
<i>Cylindromyia mirabilis</i> USA	3	0	0	1	0	0	0
<i>Cylindromyia rufifrons</i> ITA	3	0	0	1	0	0	0
<i>Cylindromyia</i> sp. AUS1	3	0	0	1	0	0	0
<i>Cylindromyia</i> sp. AUS2	3	0	0	1	0	0	0
<i>Cylindromyia</i> sp. JAP	3	0	0	1	0	0	0
<i>Cylindromyia</i> sp. SAfr1	3	0	0	1	0	0	0
<i>Cylindromyia</i> sp. SAfr2	3	0	0	1	0	0	0
<i>Ectophasia crassipennis</i> ITA	0	0	0	1	0	0	0
<i>Ectophasia oblonga</i> ISR	0	0	0	1	0	0	0
<i>Eliozeta helluo</i> ITA	0	0	0	1	0	0	0
<i>Eliozeta pellucens</i> SLV	0	0	0	1	0	0	0
<i>Elomya lateralis</i> ISR	0	0	0	1	0	0	0
<i>Euclytia flava</i> USA	0	0	0	1	0	0	0
<i>Gymnoclytia immaculata</i> USA	0	0	0	1	0	0	0
<i>Gymnoclytia occidua</i> USA	0	0	0	1	0	0	0
<i>Gymnoclytia</i> sp. ECU	0	0	0	1	0	0	0
<i>Gymnosoma clavatum</i> GER	0	0	0	1	0	0	0
<i>Gymnosoma inornatum</i> ITA	0	0	0	1	0	0	0
<i>Gymnosoma nitens</i> SLV	0	0	0	1	0	0	0
<i>Gymnosoma par</i> USA	0	0	0	1	0	0	0
<i>Gymnosoma rotundatum</i> ITA	0	0	0	1	0	0	0
<i>Gymnosoma</i> sp. KOR	0	0	0	1	0	0	0
<i>Hemyda aurata</i> USA	3	0	0	1	0	0	0
<i>Hermya beelzebul</i> JAP	0	0	0	1	0	0	0
<i>Hermya</i> sp. BUR	0	0	0	1	0	0	0

Table 1.4 (continued)

Taxa	<u>Phasiinae</u>						
	1	2	3	4	5	6	7
<i>Hermys</i> sp. MAL	0	0	0	1	0	0	0
<i>Huttonobesseria verecunda</i> NZ	3	0	0	1	0	0	0
<i>Imitomyia sugens</i> USA	3	?	1	1	1	0	1
<i>Labigastera nitidula</i> ITA	1	0	0	1	0	0	0
<i>Leucostoma</i> sp. USA1	1	0	0	1	0	0	0
<i>Leucostoma</i> sp. USA2	1	0	0	1	0	0	0
<i>Leverella</i> sp. AUS	3	?	0	1	0	0	0
<i>Lophosia fasciata</i> FIN	3	0	0	1	0	0	0
<i>Lophosia</i> sp. KOR	3	0	0	1	0	0	0
<i>Lophosia</i> sp. THA	3	0	0	1	0	0	0
<i>Neobrachelia</i> ECU	3	0	0	1	0	0	0
<i>Opesia grandis</i> ITA	0	0	0	1	0	0	0
<i>Parerigone</i> sp. JAP	1	0	0	1	0	0	0
<i>Pentatomophaga latifascia</i> JAP	0	0	0	1	0	0	0
<i>Penthosia satanica</i> USA	0	0	0	1	0	0	0
<i>Penthosiosoma</i> sp. THA	0	0	0	1	0	0	0
<i>Phania funesta</i> ITA	3	0	0	1	0	0	0
<i>Phasia aurulans</i> USA	1	0	0	1	0	0	0
<i>Phasia hemiptera</i> JAP	1	0	0	1	0	0	0
<i>Phasia</i> sp. ECU	1	0	0	1	0	0	0
<i>Phasia</i> sp. JAP	1	0	0	1	0	0	0
<i>Phasia</i> sp. SAfr1	1	0	0	1	0	0	0
<i>Phasia</i> sp. SAfr2	1	0	0	1	0	0	0
<i>Phasia subcoleoptrata</i> ISR	1	0	0	1	0	0	0
<i>Prolophosia petiolata</i> BUR	3	0	0	1	0	0	0
<i>Prolophosia</i> sp. BUR	3	0	0	1	0	0	0
<i>Pygidimys</i> sp. AUS	3	0	0	1	0	0	0
<i>Rondaniooestrus apivorous</i> SAfr	0	3	1	1	0	0	0
<i>Strongygaster robusta</i> USA	0	3	1	1	0	0	0
<i>Strongygaster</i> sp. THA	0	3	1	1	0	0	0
<i>Strongygaster triangulifera</i> USA	0	3	1	1	0	0	0
<i>Trichopoda lanipes</i> USA1	0	0	0	1	0	0	0
<i>Trichopoda lanipes</i> USA2	0	0	0	1	0	0	0
<i>Trichopoda pennipes</i> FP	0	0	0	1	0	0	0
<i>Trichopoda pennipes</i> USA1	0	0	0	1	0	0	0
<i>Trichopoda pennipes</i> USA2	0	0	0	1	0	0	0
<i>Trichopoda plumipes</i> USA	0	0	0	1	0	0	0
<i>Weberia digramma</i> ISR	1	0	0	1	0	0	0
<i>Xanthomelanodes arcuatus</i> USA	0	0	0	1	0	0	0
<i>Xanthomelanodes californicus</i> USA	0	0	0	1	0	0	0

Table 1.4 (continued)

<u>Phasiinae</u>							
Taxa	1	2	3	4	5	6	7
<i>Xysta holosericea</i> ISR	2	0	0	1	0	0	0
<i>Zambesomima</i> sp. JAP	1	0	0	1	0	0	0
<i>Zita</i> sp. AUS1	3	?	0	1	0	0	0
<i>Zita</i> sp. AUS2	3	?	0	1	0	0	0
<i>Zita</i> sp. AUS3	3	?	0	1	0	0	0
<u>Dexiinae</u>							
Taxa	1	2	3	4	5	6	7
<i>Beskia aelops</i> USA	1	0	1	0	1	1	1
<i>Billaea</i> sp. USA	0	2	1	0	1	1	1
<i>Campylocheta semiothisae</i> CAN	0	1	1	0	1	1	1
<i>Cordyligaster</i> sp. USA	0	1	1	0	1	1	1
<i>Epigrimyia</i> sp. USA	1	0	1	0	1	1	1
<i>Euchaetogyne roederi</i> USA	0	2	1	0	1	1	1
<i>Euthera</i> sp. AUS	0	0	0	0	0	0	1
<i>Euthera</i> sp. JAP	0	0	0	0	0	0	1
<i>Euthera tentatrix</i> USA	0	0	0	0	0	0	1
<i>Litophasia</i> sp. SAfr	1	?	1	0	1	0	1
<i>Oestrophasia</i> sp. USA	1	2	1	0	1	1	1
<i>Ptilodexia conjuncta</i> USA	0	2	1	0	1	1	1
<i>Rutilia regalis</i> AUS	0	2	1	0	1	1	1
<i>Thelaira americana</i> USA	0	1	1	0	1	1	1
<i>Uramya pristis</i> USA	0	1	1	0	1	1	1
<i>Voria ruralis</i> USA	0	1	1	0	1	1	1
<u>Exoristinae</u>							
Taxa	1	2	3	4	5	6	7
<i>Blondelia polita</i> USA	4	1	1	0	0	0	0
<i>Ceracia dentata</i> USA	0	4	1	0	0	0	0
<i>Hyphantrophaga hyphantriae</i> USA	0	1	1	0	0	0	0
<i>Lespesia</i> sp. USA	0	1	1	0	0	0	0
<i>Tachinomyia</i> sp. USA	0	1	0	0	0	0	0
<i>Winthemia</i> sp. USA	0	1	0	0	0	0	0
<u>Tachininae</u>							
Taxa	1	2	3	4	5	6	7
<i>Epalpus signifer</i> USA	0	1	1	0	0	0	0
<i>Homalactia harringtoni</i> USA	0	1	1	0	0	0	0
<i>Panzeria ampellus</i> USA	0	1	1	0	0	0	0
<i>Siphona plusiae</i> USA	0	1	1	0	0	0	0
<i>Tachina</i> sp. USA	0	1	1	0	0	0	0

Results

Sequence data

In total, the nucleotide data matrix consisted of 7651 aligned sites for 126 taxa with >91% gene-by-taxa coverage (97% coverage excluding additional taxa shared by the Tachi Lab used only for CAD). Sequence summary statistics can be found in Table 1.5 including information on total/average length and base frequencies.

Trees

Bayesian and ML analyses of the partitioned, concatenated dataset reconstructed identical phylogenies that had very similar support for every clade recovered (Figure 1.1: transformed, Figure 1.2: proportional, Figure 1.3: phasiine tribes). This tree is hypothesized to be the best estimation of the evolutionary history of these taxa using these genes. Therefore, this phylogeny's clade structure and node support will be described first, then used as a reference phylogeny for further investigation of the phylogenetic signal coming from individual genes and codon positions. Nodal support statistics are represented by “bs” (bootstrap) for ML and “pp” (posterior probability) for Bayesian analyses.

Outgroups

Nine outgroups were included in this phylogenetic analysis, with *Musca domestica* rooting the tree. The single sarcophagid (*Macronychia* sp.) and oestrid species (*Cuterebra austeni*) that were included formed a moderately supported sister group (bs = 53; pp = 96). Rhinophoridae are a potential sister group of the Tachinidae and three species were included here. These species expectedly formed a highly supported clade ((*Axinia* sp. + *Melanophora roralis*) + *Rhinomorinia* sp.) (bs = 100; pp = 100). However, this clade was not reconstructed as sister to the Tachinidae but was instead closely allied with the calliphorid sister taxa *Angioneura abdominalis* and *Lucilia sericata* (bs = 83; pp = 55). A third calliphorid species, *Pollenia pediculata*, was reconstructed as sister taxon of the Tachinidae with very high support (bs = 99; pp = 100). The family Tachinidae itself forms a distinctive monophyletic group (bs = 100; pp = 100).

Table 1.5: Summary statistics of genes used for phylogeny.

Genes	# Taxa	Sequence length			Base Frequencies			
		Longest	Shortest	Average	%A	%T	%C	%G
CAD	122	1686	771	1554	29.5	22.8	29.0	18.4
LGL	112	1546	720	1234	29.9	30.0	18.0	21.7
MAC	113	2197	828	1997	32.3	28.3	20.7	18.1
MCS	114	2003	942	1868	32.2	31.4	15.4	20.5

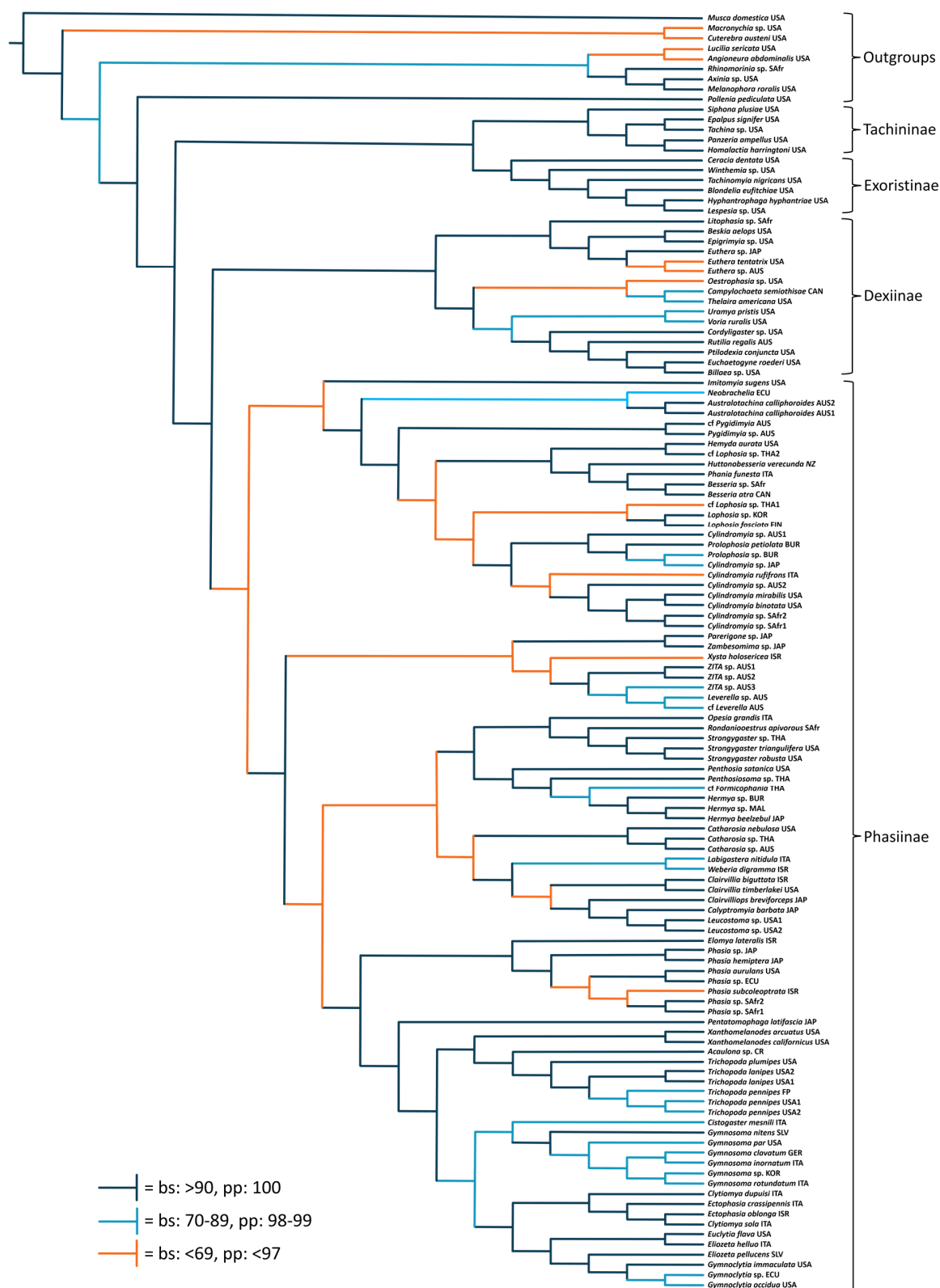


Figure 1.1: Transformed phylogeny of Tachinidae estimated from the concatenated and partitioned dataset.

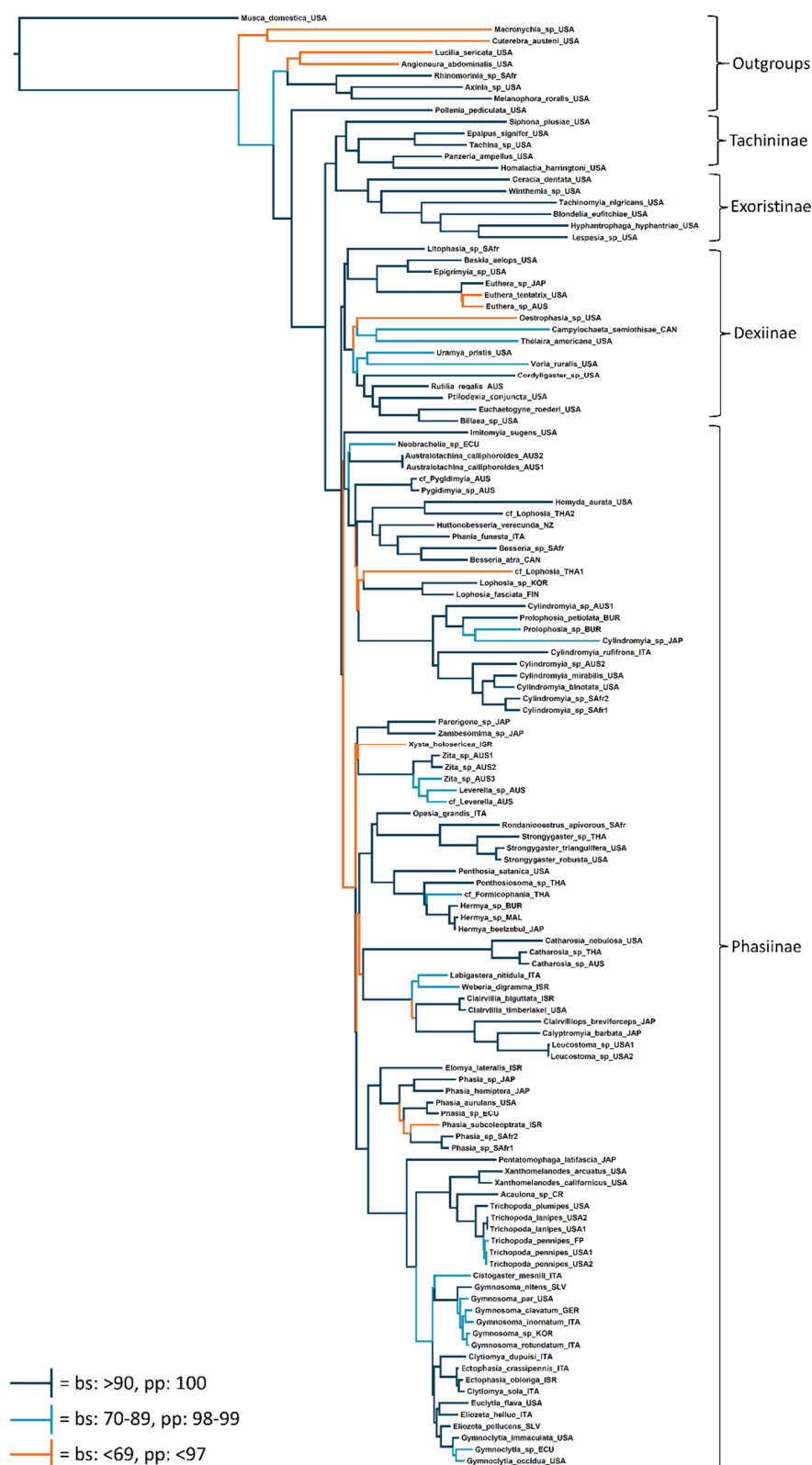


Figure 1.2: Proportional phylogeny of Tachinidae estimated from the concatenated and partitioned dataset.

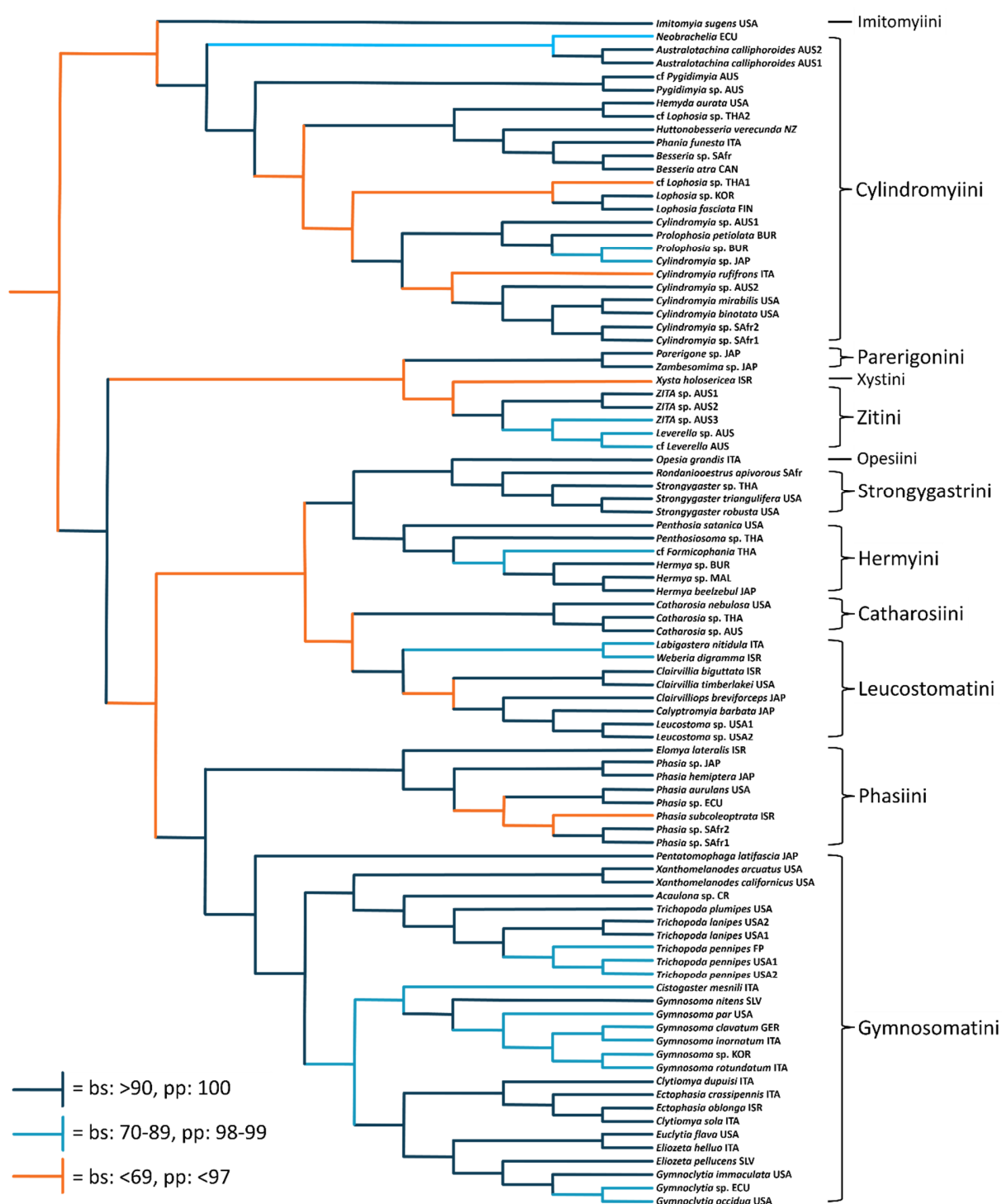


Figure 1.3: Transformed phylogeny of Tachinidae estimated from the concatenated and partitioned dataset. Only showing Phasiinae, with tribes labeled.

Tachininae + Exoristinae

The Tachinidae are represented in this phylogeny by two lineages. The first clade includes the subfamilies Tachininae + Exoristinae, and the second contains the subfamilies Dexiinae + Phasiinae. The Tachininae + Exoristinae clade is strongly supported (bs = 96; pp = 100) and is composed of two highly supported clades corresponding to the two subfamilies (Tachininae: bs = 98; pp = 100, Exoristinae: bs = 100; pp = 100). The tribes of Tachininae and Exoristinae were represented by a single genus each except for the tribe Tachinini for which two common species were included (see Table 1.1). Tribal relationships within the subfamilies were strongly supported and consistent across ML and Bayesian analyses (bs/pp >80). The tribal structure of Tachininae was reconstructed as (((Polideini + Ernestiini) + Tachinini) + Siphonini). For the Exoristinae, the tribes were ladderized as follows: (((((Eryciini + Goniini) + Blondeliini) + Exoristini) + Winthemiini) + Acemyini). The somewhat enigmatic taxon *Ceracia dentata* (Acemyini), which has historically been placed in both of these subfamilies, was reconstructed as a statistically established member of Exoristinae – sister to all other included exoristine taxa (bs = 100; pp = 100).

Dexiinae

The Dexiinae have previously been shown to have a close association with the Phasiinae (Cerretti *et al.* 2014; Winkler *et al.* 2015) and most taxonomically ambiguous genera considered here possess intermediate suites of morphological character states of these two subfamilies. In order to better assess the placement of these enigmatic groups, the Dexiinae were sampled more thoroughly than the Tachininae and Exoristinae. Eight tribes represented by ten genera formed Dexiinae s. s. and formed a highly supported clade (bs = 100; pp = 100). The three genera of tribe Dexiini (represented by *Billaea* sp., *Euchaetogyne roederi*, and *Ptilodexia conjuncta*) coalesced into their expected clade (bs = 100; pp = 100). Uramyini + Voriini formed a sister group (bs = 64; pp = 95), as did Thelairini + Campylochetini (bs = 79; pp = 95). Outside these clades, most inter-tribal relationships within the Dexiinae s. s. were ladderized or only moderately supported (e.g., Dufouriini sister to clade Thelairini + Campylochetini and together forming a sister group to the rest of the tribes).

Most interestingly, the sister clade to Dexiinae s. s. was composed of the tribes Eutherini and Epigrimyiini, as well as the genus *Litophasia*, and was reconstructed as a highly supported clade (bs = 91; pp = 100). Each of the genera included in this clade share morphological and/or

behavioral traits with both Phasiinae and Dexiinae. Both genera of Epigrimiini (*Epigrimyia* and *Beskia*) were represented (bs = 100; pp = 100) and three species of *Euthera* represented the Eutherini (the only other genus in Eutherini is the rare Palearctic genus *Redtenbacheria*) (bs = 100; pp = 100). Epigrimiini and Eutherini were reconstructed as sister taxa with *Litophasia* sister to them. This clade forms the sister clade to Dexiinae s. l. and these two clades together form a monophyletic group that is here defined as the subfamily Dexiinae (bs = 99; pp = 100).

Phasiinae

The Phasiinae form a distinct clade as well. However, support for its monophyly is low (bs = 60; pp = 84). Given the high statistical support for other clades throughout the tree, it is surprising that the target group Phasiinae was not highly supported. Interestingly, but not unexpectedly, this low support transforms to very high statistical support (bs = 95; pp = 100) when a single enigmatic taxon is excluded: *Imitomyia sugens*. *Imitomyia* is a strange genus whose characteristics are either unknown or contradict placement in either the Phasiinae or Dexiinae. The only analyses that place *Imitomyia* within the Phasiinae are the partitioned, concatenated ML and Bayesian analyses. Most phylogenies estimated from individual genes and individual codon positions place *Imitomyia* outside both Dexiinae and Phasiinae as the sister taxa to both subfamilies (ML trees) or as an unresolved polytomy of Dexiinae, Phasiinae, and *Imitomyia* (Bayesian trees). It is clear that this single genus representing the tribe Imitomyiini has an unusual evolutionary history that is difficult to decipher. Given the supportive genetic evidence, albeit low, the Phasiinae are defined here as a clade including the Imitomyiini.

Within the Phasiinae, 12 recovered groups are defined here as the tribes Catharosiini, Cylindromyiini, Gymnosomatini, Hermyini, Imitomyiini, Leucostomatini, Parerigonini, Phasiini, Strongygastrini, the clade Zitini, and the phylogenetically isolated taxa *Opesia* and *Xysta*. Every tribe was recovered with absolute support (bs = 100; pp = 100). Additionally, within the Gymnosomatini, two strongly supported clades were recovered: Gymnosomatina (bs = 100; pp = 100) and Trichopodina (bs = 100; pp = 100). The tribes of Phasiinae aggregated into five “supertribal” clades with varying support: (Gymnosomatini + Phasiini): bs = 100; pp = 100, (Leucostomatini + Catharosiini): bs = 64; pp = 93, + (Strongygastrini + *Opesia*) + Hermyini): bs = 100; pp = 100, ((Zitini + *Xysta*) + Parerigonini): bs = 39; pp = 80, and (Cylindromyiini + Imitomyiini): bs = 28; pp = 55.

(Gymnosomatini + Phasiini) – The tribe Gymnosomatini is composed of two evolutionary clades: Gymnosomatina and Trichopodina. The Trichopodina are represented in this phylogeny as ((*Trichopoda* + *Acaulona*) + *Xanthomelanodes*). Most genera currently considered to belong in Phasiini (e.g., *Cistogaster*, *Clytiomya*, *Ectophasia*, *Eliozeta*, and *Euclytia*) were reconstructed as members of Gymnosomatini where they joined *Gymnosoma* and *Gymnoclytia* to form clade Gymnosomatina. *Pentatomophaga latifascia* was reconstructed as sister taxa to the two clades and is therefore not placed in either. Two other nominal Phasiini, *Opesia* and *Xysta*, were placed rather distant from Phasiini as monotypic lineages (see below). The removal of most genera from Phasiini left this tribe with only two genera: *Phasia* and *Elomya*. These two genera form Phasiini and are closely allied with the Gymnosomatini.

(Leucostomatini + Catharosiini) – The Catharosiini were represented by three species of *Catharosia* and the apparently misclassified genus *Litophasia*. *Catharosia* species were reconstructed as sister to the Leucostomatini – here represented by six genera in two clades: (((*Leucostoma* + *Calyptromyia*) + *Clairvilliops*) + *Clairvillia*) and (*Weberia* + *Labigastera*). *Litophasia* was recovered in Dexiinae.

((Strongygastrini + Opsia) + Hermyini) – Three *Strongygaster* species and the honeybee parasitoid *Rondaniooestrus apivorous* Villeneuve form a clade defined here as the Strongygastrini. Closely allied to the Strongygastrini is *Opesia* – a genus currently belonging to Phasiini s. l. but here found apart from that clade. The Strongygastrini and *Opesia* formed a strongly supported clade as sister taxon of the Hermyini (bs = 100; pp = 100). The relatively small tribe Hermyini was thoroughly represented by three species of *Hermya* and three additional genera (cf. *Formicophania*, and *Penthosiosoma*, and *Penthosia*). The lone New World genus, *Penthosia*, was recovered sister to the remaining sampled Hermyini.

((Zitini + Xysta) + Parerigonini) – This analysis revealed three separate evolutionary lineages of Parerigonini s. l. consisting of 1) the Asian genera *Parerigone* and *Zambesomima* (here as Parerigonini *sensu* Blaschke *et al.*), 2) the Australian genera *Zita* and *Leverella* (here as the new tribe Zitini), and 3) the South American genus *Neobrachelia* coupled with the aberrant

Australian genera *Australotachina* and *Pygidimyia* (reconstructed as members of Cyldromyiini).

The (Zitini + *Xysta*) + Parerigonini clade is weakly supported (bs = 39; pp = 80). The position and support of the Zitini is consistent across genes and codon positions, but placement of *Xysta* and the Parerigonini is much more unstable. Current hypotheses place *Xysta* in Phasiini (Herting 1984), but it is here considered to occupy a phylogenetically isolated position within the Phasiinae. Even though *Xysta* has never been associated with the Parerigonini *s. l.* it was reconstructed bisecting two lineages of that nominal tribe. In no analysis is Parerigonini *sensu* Blaschke more closely related to Zitini than is *Xysta*. This suggests that Parerigonini *s. s.* consists of multiple evolutionary lineages and that *Xysta* should be considered more closely aligned to them than to the Phasiini.

(Cyldromyiini + Imitomyiini) – Another lineage of Parerigonini *s. l.*, composed of the South American genus *Neobrachelia* and the Australian genera *Australotachina* and *Pygidimyia*, was discovered to be closely allied with the Cyldromyiini (bs = 100; pp = 100). *Neobrachelia* and *Australotachina* were consistently recovered together in individual gene/codon analyses and in the complete dataset (bs= 70; pp = 85). *Pygidimyia* was more phylogenetically unstable than the other genera, but was ultimately reconstructed sister to the Cyldromyiini *s. s.* (bs= 100; pp = 100).

Cyldromyiini *s. s.* is composed of two clades for which the names of Herting (1983) can be applied: Cyldromyiina and Phaniina. The Phaniina clade has absolute statistical support (bs = 100; pp = 100) and is composed of the genera *Besseria*, *Phania*, *Huttonobesseria*, *Hemyda*, and a single difficult to identify specimen near *Lophosia*. This species is most likely not a *Lophosia sensu* Crosskey (1976). The Cyldromyiina enjoy far less statistical support (bs = 29; pp = 51) mainly due to a grade of nominal *Lophosia* species which sometimes group with *Pygidimyia* rather than other Cyldromyiina. Other than *Lophosia*, the clade Cyldromyiina also includes the speciose genus *Cyldromyia* and *Prolophosia* – an African genus that appears synonymous with *Cyldromyia*. The Cyldromyiini were found to be sister to the unusual genus *Imitomyia* whose unstable phylogenetic position is discussed above.

Taxonomically ambiguous genera

Conclusive subfamily placements for almost all morphologically intermediate taxa was achieved with significant support. Epigrimiini and *Litophasia* were placed in Dexiinae rather than Phasiinae and *Euthera* was recovered in Dexiinae rather than Phasiinae or Exoristinae. *Strongygaster* and *Rondaniooestrus*, as well as all genera of Parerigonini s. l., were firmly nested within Phasiinae rather than Tachininae. Although not overwhelmingly convincing, the best evidence from all available data places *Imitomyia* as a member of Phasiinae rather than Dexiinae.

Clade frequencies across all analyses

In total, 28 phylogenies were generated for this study. Bayesian analyses included trees consisting of each gene partitioned individually (CADBayes, LGLBayes, MACBayes, and MCSBayes) as well as the concatenated and partitioned tree (CLMMBayes). Maximum likelihood analyses were more thorough. Five trees were created for each gene: three trees consisting of each individual codon position (e.g., CAD1, CAD2, CAD3), one tree with third positions removed (e.g., CAD12), and one tree with all positions included and partitioned separately (e.g., CAD123). A complete evidence ML phylogeny was created using twelve partitions and a concatenated dataset (CLMM123). Two additional ML trees were generated to reduce potential conflicting phylogenetic signal, one with *Imitomyia* excluded (not shown) and one with data from LGL excluded (CMM123).

The numerous trees created from a variety of independent lines of evidence provided helpful insights into which clades and which arrangements of clades were strongly vs. weakly supported. Third codons are often extremely variable and thus often have little phylogenetic signal at the deeper family/subfamily levels. Second positions rarely provide significant signal because of their genetic stability. When mutations occur in the first positions, they often have important amino acid (and therefore phylogenetically important) changes. A clade supported by each codon position invites substantial confidence in the evolutionary validity of that clade – even more so if multiple genes are also providing similar signals. A summary of the relative frequency of each clade appearance across all phylogenies can be found in Figure 1.4.

A substantial amount of clades were present in over 70% of the trees. This is interpreted as considerable evidence that these clades accurately represent a reasonable evolutionary history. These clades include Catharosiini, Strongygastriini, Trichopodina, Gymnosomatini, (Epigrimiini + Eutherini), Hermyini, Leucostomatini, Zitini, Gymnosomatina, Phasiina, Exoristinae, and

Rhinophoridae – the first five of which are only absent from one or two low-signal partitions. Most other clades are consistently recovered in complete gene analyses, but are more unstable with less informative partitions (codon position 2 across all genes, LGL codon positions) as would be expected. With the inclusion of *Imitomyia*, Phasiinae and Dexiinae are rarely recovered as monophyletic. However, with *Imitomyia* removed, these clades are almost always fully resolved.

Phylogenetic informativeness of genes and codon positions

MCS proved to be the single most important gene for this phylogenetic analysis, recovering ~85% of the final clades by itself. The utility of MCS was closely followed by MAC, CAD, and lastly LGL. LGL was unable to recover several significant clades on its own, especially outside the Phasiinae. A comparison of the ability of each gene and each codon position to reconstruct clades is shown in Figure 1.5.

Discussion

This study is the largest molecular analysis of Tachinidae to date and the first to consider the classification and evolution of the subfamily Phasiinae (Stireman 2002; Tachi & Shima 2010; Winkler *et al.* 2015). Certain historical hypotheses gained substantial evidence in their favor and several novel ideas about phasiine taxonomy and evolution were generated. In general, this molecular phylogeny agrees remarkably well with current concepts of phasiine classification. However, several significant revisions to tribal classifications within Phasiinae are suggested by these evolutionary relationships. Most notably, Parerigonini and Phasiini were shown to consist of three and four different evolutionary lineages respectively. These tribes, and several others, deserve proper consideration and discussion of their historical taxonomy and the morphological and molecular evidence for their specific evolution within Phasiinae. There is not the space for such a thorough analysis here, as this present work is devoted to the phylogenetic placement of taxonomically difficult genera and tracing the evolution of traits within Tachinidae. Instead, in a separate paper, this molecular phylogeny is used as a framework for updating and revising the classification of all worldwide genera of Phasiinae (Blaschke 2015).

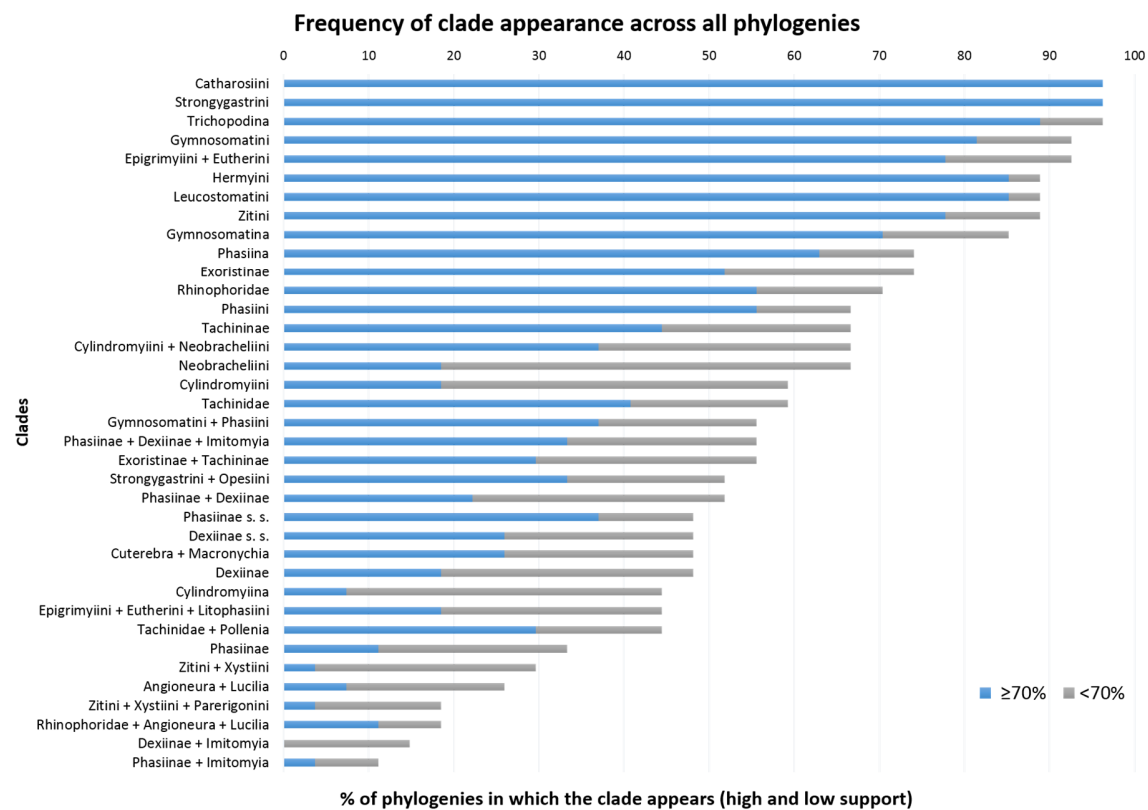


Figure 1.4: Frequency of clade appearance across all phylogenies as a percent of total.

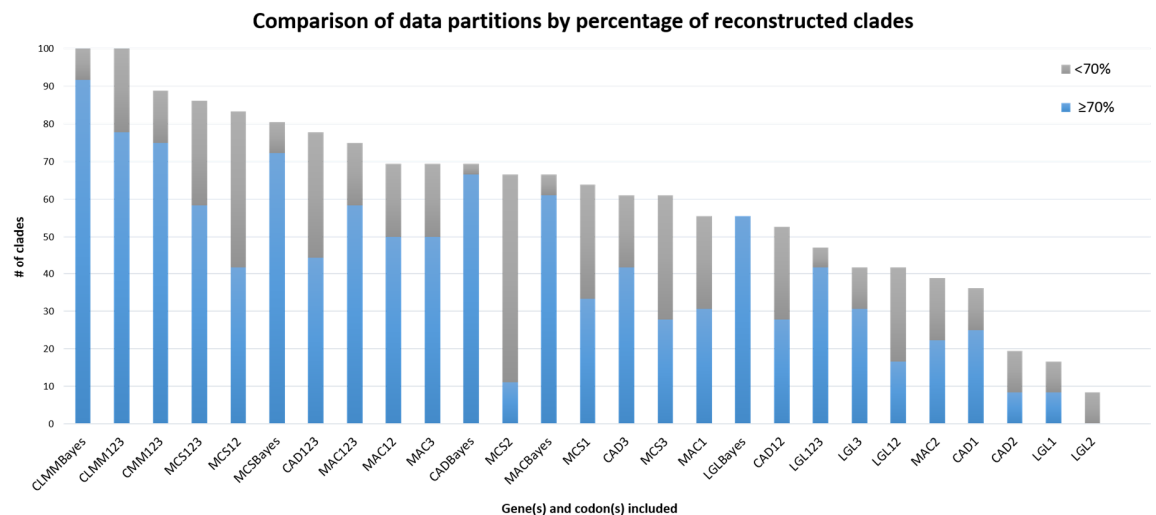


Figure 1.5: Comparison of genetic data partitions by percentage of reconstructed clades.

Here, phasiine relationships are briefly summarized and the molecular evidence for the subfamily placement of several taxonomically ambiguous genera is presented. Also, because the phylogenetic placement of the dexiine taxa *Epigrimyiini*, *Eutherini*, and *Litophasia* is critical for reconstructing the evolution of Tachinidae, their taxonomic history is explored and discussed in a phylogenetic context. Lastly, the evolution of important and/or potentially synapomorphic characters traits is applied to the phylogeny and discussed.

Classification

As a result of the molecular evidence, the Phasiinae are represented here by 12 tribes, including the *Imitomyiini*, *Strongygastrini* (including *Rondaniooestrus*), and *Parerigonini*, but excluding the *Epigrimyiini*, *Eutherini*, and *Litophasia*. Morphologically, the Phasiinae can be defined by the synapomorphic trait of an elongated hypandrium of the male postabdomen. Specifically, the median plate of the hypandrium is elongated to such an extent that the pregonites are attached at the back (first identified by Tschorsnig 1985). All tachinids with a piercer derived from the 10th sternite (postgenital plate) are found in Phasiinae. However, not all phasiines have reduced chaetotaxy, are oviparous, possess piercers, or even parasitize Heteroptera. Furthermore, some tachinid genera that do parasitize Heteroptera and/or have piercers derived from the 8th sternite are placed in the Dexiinae rather than the Phasiinae, making these traits homoplastic and of less use phylogenetically (except at the family level). Female, and especially male, terminalic characters were shown to be significantly better indicators of evolutionary history than external morphology, host range, or oviposition strategy. Several phasiine tribes were identified as unnatural groupings of genera and need to be repositioned into more evolutionarily meaningful clades (see results).

Convincing corroborative evidence that the *Strongygastrini* (including *Rondaniooestrus*), all lineages of *Parerigonini*, and the *Imitomyiini* belong in Phasiinae was recovered. Recent molecular and morphological analyses have placed *Strongygastrini* within Phasiinae (Cerretti *et al.* 2014, Winkler *et al.* 2015) and the debate about its subfamily affinities should now be completely resolved. Multiple genera of *Parerigonini* have never been analyzed phylogenetically, but Cerretti *et al.* (2014) found support for a placement in Phasiinae for the lone genus *Parerigone*. Despite their tachinine-like appearance, all parerigonine genera were found to be convincingly phasiines – thus resolving another long-standing issue in tachinid classification. The position of *Imitomyia* is more unstable and its taxonomy may still be debated. However,

molecular evidence has now been added to the morphological evidences that place *Imitomyia* within the Phasiinae. Because these tribes belong in Phasiinae, their taxonomic history and classification is more thoroughly discussed with other phasiine taxa in Blaschke (2015).

In contrast to the taxa above, Epigrimiini, Eutherini, and *Litophasia* were found to be allied with Dexiinae rather than Phasiinae. While this affinity has been previously hypothesized (Tschorsnig 1985; O'Hara and Wood 2004), this is the first molecular evidence and the first strong phylogenetic evidence that these taxa belong in Dexiinae. The morphological analysis of Tachinidae of Cerretti *et al.* (2014) found *Litophasia* to be a “basal” phasiine, while the Eutherini were most often recovered within the Exoristinae (Epigrimiini were not represented). Here, Epigrimiini and Eutherini were recovered as sister taxa in almost every partition and represent one of the strongest supported clades in this analysis. The close relationship between these two tribes has not been previously suggested and is an unexpected result from the genetic evidence. The taxonomic history and phylogenetic affinities of each of these tribes is discussed below.

Epigrimiini – Epigrimiini includes two genera (*Beskia* and *Epigrimyia*), both endemic to the Western Hemisphere (O'Hara 2014). Historically, they have been considered members of the Phasiinae (Townsend 1936, 1938; Sabrosky and Arnaud 1965; Guimarães 1971, 1977), but have been recently associated with Dexiinae due to distinctive secondary structures of the male postabdomen (Tschorsnig 1985, O'Hara and Wood 2004). The classification of Epigrimiini has never been analyzed phylogenetically until now.

Epigrimyia was the sole member of Epigrimiini from Townsend's initial designation of the tribe (1908) until O'Hara and Wood (2004) included the additional genus *Beskia*. However, their close morphological association with each other and to the modern phasiine tribe Cyldromyiini was noted by many previous authors (Brauer and Bergenstamm 1889; Wulp 1903; Townsend 1891, 1938; Sabrosky and Arnaud 1965; Herting 1984). Townsend originally placed Epigrimiini in the now outdated subfamily Pyrrhosiinae, which also included genera in the very different modern tribes of Leskiini and Leucostomatini, but he also commented on the close affinity of *Epigrimyia* with the subfamily Phaniinae (= Cyldromyiini/Hermiyini). *Beskia* was placed by Townsend with *Ocyptera* (= *Cylindromyia*) in his subfamily Ocypterinae (= Cyldromyiini). Although he initially positioned *Epigrimyia* in its own tribe, Townsend later moved the genus to the Cyldromyiini along with *Beskia* (Townsend 1919). It should be noted that Malloch (1929) insightfully did not consider *Beskia* to belong

in the same tribe as *Cylindromyia*. In Townsend's (1936) key to the Cylindromyiini, *Epigrimyia* and *Beskia* share a terminal couplet based on their elongated proboscises.

In addition to their external habitus, which is more reminiscent of *Cylindromyia* and relatives than any member of Dexiinae, *Epigrimyia* and *Beskia* also share host similarity with other phasiines. All phasiines with known hosts, except *Rondaniooestrus*, are parasitoids of heteropteran bugs and it has been proposed that this could be a synapomorphy of Phasiinae (Wood 1987; Stireman *et al.* 2006). Such an argument maintains Epigrimyini in the Phasiinae as *Epigrimyia* attacks *Galgupha ovalis* (Hemiptera: Corimelaenidae) (Biehler and McPherson 1982) and *Beskia* attacks multiple species of Pentatomidae, most notably the rice stink bug (*Oebalus pugnax*) (Guimarães 1977; Sutherland and Baharally 2002). Additional evidence for a placement of Epigrimyini in Phasiinae can be found in the female postabdomen where both genera share a modified 8th sternite used as a piercing structure for direct oviposition into a host (Townsend 1938).

Although at a glance *Epigrimyia* and *Beskia* seem strongly reminiscent of Phasiinae, recent morphological evidence indicates a position within the Dexiinae. Tschorsnig (1985) examined the various structures of the male postabdomen and concluded that *Epigrimyia* and *Beskia* belong in the Dexiinae based on the membranous connection of the distiphallus to the basiphallus (among other secondary structures) that he proposed were synapomorphic of Dexiinae. Additionally, Tschorsnig identified an elongated hypandrium as a potential synapomorphy of Phasiinae. This trait was not found in either *Epigrimyia* or *Beskia*. The placement of Epigrimyini in Dexiinae gains considerable support from this molecular analysis, which recovered a strongly supported Epigrimyini (composed of *Beskia* and *Epigrimyia*) as part of a monophyletic Dexiinae sister to the Eutherini.

Eutherini – Similar to the Epigrimyini, the Eutherini share morphological and behavioral traits with both the Phasiinae and Dexiinae. As a result, their taxonomic placement has been debated over the decades by dipterists. Two genera are included in the tribe. *Euthera* is a unique tachinid whose coloration mimics deer flies (Tabanidae: *Chrysops* spp.). It is uncommon in collections, but has a worldwide distribution (O'Hara 2013a). Reviews of the taxonomic history of *Euthera* can be found in Bezzi (1925), Cantrell (1983), and O'Hara (2012). The only other species in the Eutherini is *Redtenbacheria insignis* Schiner, which is a rare fly found only in the Palearctic (Herting 1984; O'Hara *et al.* 2009). Three species of *Euthera* were included in this molecular phylogeny.

The similarity of these two genera was first recognized by Stein (1924) who placed them in his “Gruppe *Trixa*”, and most modern tachinid experts have considered *Euthera* and *Redtenbacheria* to share a close affinity (Herting 1984; Ziegler 1998; Richter 2004; O’Hara *et al.* 2009; although see Crosskey 1976). Significant evidence for this relationship comes from the specialized shape of the phallus (Tschorsnig 1985) and the posterodorsal “window” on their eggs (Cerretti *et al.* 2014), which are both synapomorphies of the tribe. The subfamily placement of Eutherini is more controversial, with various experts preferring Phasiinae (Guimarães 1971; Crosskey 1977; Crosskey 1980; Herting 1984; Tschorsnig 1985; Cantrell and Crosskey 1989; Tschorsnig and Herting 1994; Tschorsnig and Richter 1998) or recently Dexiinae (Shima 1989, 1999; O’Hara and Wood 2004; Shima 2006; O’Hara *et al.* 2009).

The most convincing reason for placing the Eutherini in Phasiinae is their parasitism of heteropteran hosts (*Euthera* – Arnaud 1978; *Redtenbacheria* – Nishayama 1995), which has long been considered a distinctive trait of Phasiinae. They also produce planoconvex eggs (Herting 1966; Mesnil 1966) rather than the entirely membranous eggs of the Dexiinae (Stireman *et al.* 2006) and share this trait only with Phasiinae and some Exoristinae. However, the development and oviposition of these eggs is unlike any phasiine outside the Strongygastrini.

Phasiinae lack a uterus and are therefore oviparous (i.e., lay unincubated eggs). In contrast, the Eutherini are ovolarviparous (i.e., lay incubated eggs, Cantrell 1983). If included in the Phasiinae, the Eutherini would not be alone in having this trait as the Strongygastrini are also ovolarviparous, but these two groups are not closely related and such a classification would require the non-parsimonious explanation of ovolarviparity evolving in two separate lineages within the Phasiinae. In addition to their oviposition strategy, several other important morphological traits indicate a placement in Dexiinae.

When O’Hara and Wood (2004) moved the Eutherini from Phasiinae to Dexiinae, they did so based on Tschorsnig’s analysis of male postabdomen structures (1985) and their own examination of the male terminalia of *Euthera*. These authors identified several morphological traits shared between Eutherini and Dexiinae including the shape and location of the pregonites, postgonites, sternite 5, and the distiphallus (see also Cerretti *et al.* 2014). However, unlike the Epigrimyiini discussed above, the Eutherini do not possess a hinged membranous connection between the basiphallus and the distiphallus – a trait considered to be the most convincing synapomorphic character trait of the Dexiinae. To further confuse the issue, neither do they

possess the elongated hypandrium characteristic of Phasiinae. This evidence led Shima (1989) to conclude that the Eutherini represented the only extant taxa of the Dexiinae that retained the primitive trait of a sclerotized basi/distiphallus connection. As with the Epigrimyiini, the hypothesis that Eutherini belongs in Dexiinae and may have retained some ancestral traits throughout its evolution is strongly supported by this molecular phylogeny. Interestingly, these two phasiine-like dexiines were recovered as monophyletic sister groups in the phylogenetic analysis.

The molecular phylogenetic evidence stands in contrast to an earlier morphological phylogeny that nested the Eutherini within the Exoristinae (Cerretti *et al.* 2014). While the monophyly of the Eutherini was strongly supported, its placement in the Exoristinae was largely a result of their egg type and oviposition strategy and was not recovered in every analysis. The Tachinidae contains widespread homoplasy throughout the family and the morphological analysis of Cerretti *et al.*'s (op. cit.) highlights the difficulty of reconstructing the evolutionary history of such a group using solely morphological characters. Under different weighting schemes numerous trees were obtained that sometimes differed dramatically, especially for the taxonomically ambiguous groups like the Eutherini. Some of these trees agreed quite well with the molecular evidence, and in situations with extremely high morphological homoplasy, it is perhaps best to trust the genetically inferred trees rather than the structural traits. Therefore, Eutherini are considered here to be a tribe within Dexiinae that possesses several significant ancestral characters states.

Litophasia – The atypical genus *Litophasia* Girschner is composed of two diminutive and rarely observed flies, one species found in the Palearctic Region and the other from South Africa (Herting 1984; Crosskey 1984). Initially thought to belong to the Rhinophoridae (van Emden 1945, Crosskey 1977), *Litophasia* is now considered a tachinid that lacks the almost universal trait of an enlarged postscutellum (Wood 1987; Tschorsnig and Richter 1998). Females possess a piercer (8th sternite) very similar to those found in the phasiine tribes Catharsiini and Leucostomatini, but are not found in any known Rhinophoridae (Dear 1980). As a result of this unique trait and other morphological similarities, Herting considered it a member of Catharsiini (see communication in Dear 1980), an opinion with which Dear (1980) agreed and subsequent

authors have followed (Crosskey 1980, 1984; Herting 1984; Belshaw 1993; Herting and Dely-Draskovits 1993).

However, the male terminalia of *Litophasia* were not examined comparatively until Tschorsnig (1985). In the Tachinidae, the traits of the male postabdomen are generally more phylogenetically informative than those of the female postabdomen, eggs, larvae, or behavioral traits. As with the Epigrimyini and Eutherini, *Litophasia* possesses dexiine-type characters in the male postabdomen, including the distinctive membranous connection between the basiphallus and the distiphallus (Tschorsnig 1985) and does not have the phasiine-type elongated hypandrium. Given its taxonomic ambiguity, it is not surprising that in the morphological phylogeny of Cerretti *et al.* (2014) *Litophasia* was inconsistently placed as either sister to Phasiinae or Dexiinae under different weighting schemes. However, strong supporting evidence for a position within Dexiinae comes from this molecular phylogenetic analysis where *Litophasia* was reconstructed with high support as sister to Epigrimyini + Eutherini. This clade in turn was sister to the rest of Dexiinae. Therefore *Litophasia* is included here in the Dexiinae rather than the Phasiinae.

Evolution of Tachinidae

Any analysis of trait evolution using a phylogeny is only as good as the tree itself. If even a single clade is misplaced it can often have negative reverberations for ancestral state reconstruction throughout the tree, resulting in incorrect evolutionary theories and misplaced relationships. The phylogeny presented here was recovered with very high statistical support throughout the tree and largely corroborates current taxonomic hypotheses. As a result, this phylogeny was used to trace the evolution of important tachinid traits/behaviors and visually analyze any potential synapomorphies of Phasiinae and Dexiinae. The robustness of this phylogeny provides confidence in the analysis, but central to the ancestral state reconstruction is the phylogenetic position of the “phasiine-like dexiines”: Epigrimyini, Eutherini, *Litophasia*, and *Imitomyia*. With their curious amalgamation of traits, the phylogenetic position of these taxa heavily influence any potential subfamily synapomorphies as well as the evolution of Tachinidae in general. Each trait analyzed will be discussed individually below.

In summary, according to this analysis, the ancestor of Phasiinae can most likely be characterized by the homoplastic traits of parasitism of Heteroptera and oviparity, along with the plesiomorphic trait of lacking a piercer. The only recognized synapomorphy of Phasiinae is an

elongated hypandrium in the male postabdomen. In contrast, the Dexiinae cannot be defined by a universal synapomorphy. None of the potentially synapomorphic traits of the male postabdomen were recovered exclusively in Dexiinae, mainly due to the placement of *Imitomyia* in Phasiinae. The ancestor of Tachinidae was reconstructed with the following traits: lacking a piercer, parasitizing Lepidoptera, ovularviparous, and not possessing any trait or combination of traits in the male postabdomen that could be used to define either Phasiinae or Dexiinae.

Piercers – Used to inject eggs directly into the body cavity of hosts, piercers have evolved several times throughout tachinid evolution and appear in many tribes. However, not all piercers are identical in origin. Those found in the Exoristinae (e.g., Blondeliini) are derived from the 7th sternite, while those in the Phasiinae have been adapted from either the 8th or 10th sternite (Figure 1.6). Parsimoniously, it is expected that there would be three origins of the piercer – one for each type. Remarkably though, each type of piercer appears to have evolved independently in several different lineages. The focus of this analysis was on the Phasiinae, and therefore only the evolution of the 8th and 10th sternites is discussed here.

Three phasiine tribes possess 10th sternite piercers (Imitomyiini, Cylindromyiini, and Zitini) (Townsend 1938; Herting 1983, Cantrell 1988). It is partially this trait that morphologically supports the inclusion of *Imitomyia* in Phasiinae as no other tachinid subfamily is known to have this unique trait. According to this phylogeny, the 10th sternite piercer evolved once in the ancestor of (Imitomyiini + Cylindromyiini) and then separately in the ancestor of Zitini. This non-parsimonious explanation was unexpected given that the genera of Zitini (*Zita* and *Leverella*) have historically been closely allied with *Pygidimyia*, *Australotachina*, and *Neobrachelia* in Parerigonini. The latter three genera were recovered in the Cylindromyiini as a paraphyletic sister group to Cylindromyiini s. s. Morphologically, the simplest classification would be to include the Zitini in Cylindromyiini with the other former parerigonines possessing 10th sternite piercers or as a separate tribe sister to Cylindromyiini. However, no molecular analysis with any gene or codon position supports that naturally parsimonious explanation. Therefore, the Zitini are left in their own tribe apart from Cylindromyiini and the 10th sternite piercer is hypothesized to have arisen independently in the two lineages.

The evolution of the 8th sternite piercer is quite remarkable. This potentially adaptive trait appears in both the Dexiinae and Phasiinae, with at least three separate origins in the former and.

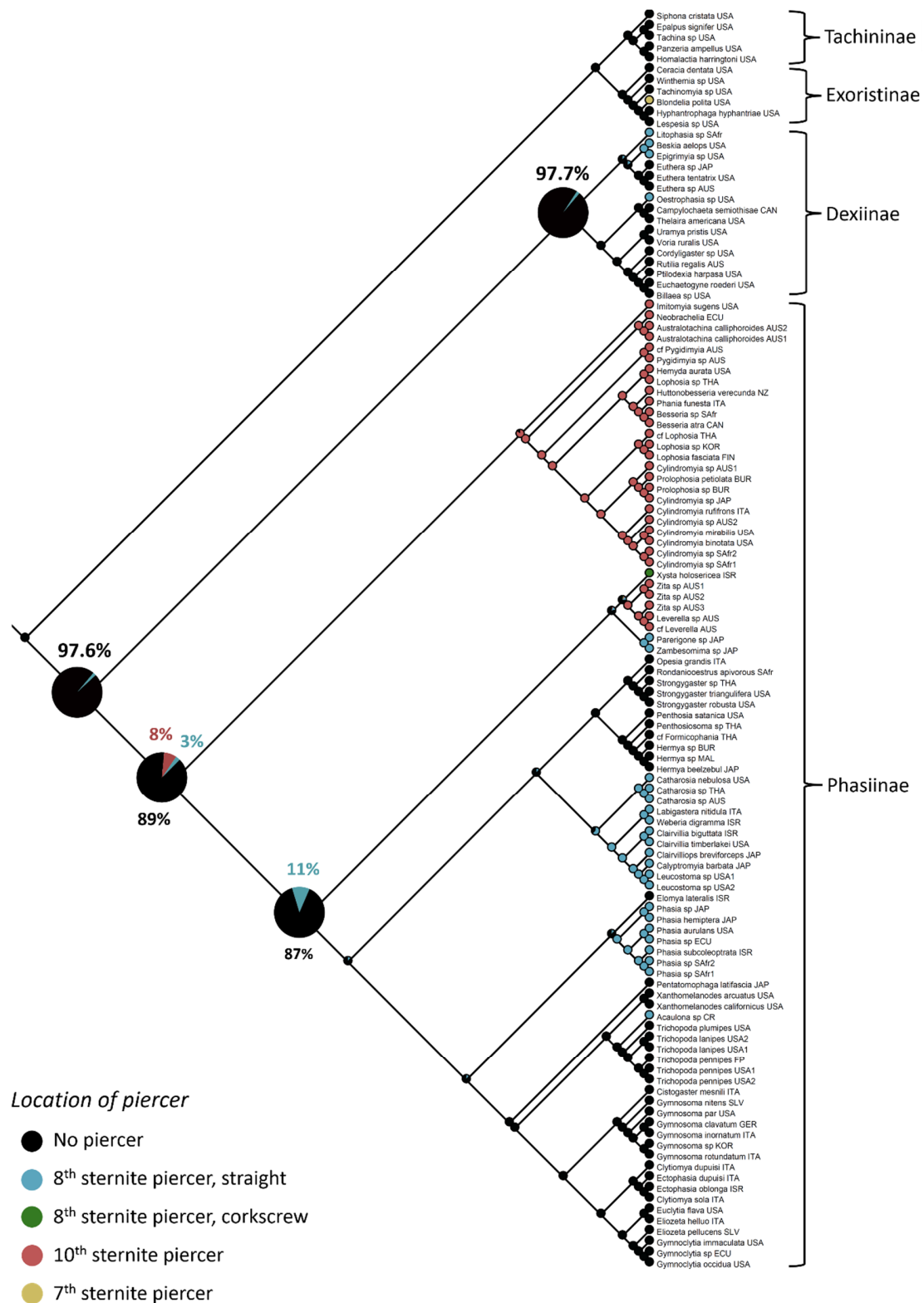


Figure 1.6: Ancestral state reconstruction of piercer location in Tachinidae.

four in the latter, as evidenced by the non-piercing ancestor of Phasiinae (89%) and Dexiinae (97.7%). Eight tribes can be wholly or partially defined by the presence of the 8th sternite piercer: Dexiinae – *Litophasia*, Epigrimyiini, and Dufouriini (here represented by *Oestrophia*); Phasiinae – *Xysta*, Catharosiini, Leucostomatini, Parerigonini *sensu* Blaschke *et al.*, and Phasiini. Additionally, at least one genera of Gymnosomatini (Trichopodina) possesses an 8th sternite piercer (*Acaulona*). Within the Phasiini, an interesting state is found. Of the two genera included, *Phasia* possesses the typical 8th sternite piercer, but the 8th sternite of *Elomya* is not developed into a fully functional piercer. Rather, *Elomya* uses its uniquely shaped “narrow point” 8th sternite to firmly insert its eggs into the intersegmental membranes of the host, but the 8th sternite does not actually pierce the exoskeleton (Herting 1960; Tschorsnig and Richter 1998). This may represent an evolutionarily intermediate state between piercing and non-piercing forms

Given the numerous independent origins of the 8th and 10th sternite piercers, the possession of such a trait seems to be highly selected for within phasiine populations. In contrast to most other tachinids that generally attack soft-bodied immature insects, phasiines exploit adult hosts with hardened exoskeletons. Attaching an egg to the outside of the host requires the phasiine larvae to utilize powerful chitinase-degrading proteins and/or specialized mandibles to break through the host’s exoskeleton. There is also substantial risk for the exposed egg from predation, desiccation, and physical removal by the host. A piercer solves these problems by bypassing the host’s cuticle. As such, natural selection would most likely encourage the evolution of postabdominal sclerites toward a piercing structure, perhaps via a structure similar to the narrow point of *Elomya* (see above). Once evolved, it seems unlikely that a piercer would be lost through natural selection. This reconstruction supports that hypothesis. Even with 10 different piercer origins theorized, there is no significant evidence of a loss of a piercer in any lineage.

The unique morphology of *Xysta* deserves special mention here. While derived from the 8th sternite, the piercer of *Xysta* is significantly divergent from other phasiine piercers. The 8th tergite of *Xysta* has special modifications that allow it to be inserted between the host’s body segments and expanded, thus exposing the inner cavity of the host to the fly’s piercer. The 8th sternite then protrudes from between the structures of the 8th tergite and injects the eggs into the body cavity of the host (Herting 1957). Most phasiine piercers are simple and needle-like, most often slightly curved but sometimes straight. However, the piercer of *Xysta* is curved around

itself like a corkscrew and rotates on its axis when inserted, thus “drilling” into the host rather than stabbing. These unusual structures strongly indicate an independent evolution of the piercer unrelated to other phasiine piercers.

Consequently, *Xysta* has been uniquely coded here for the piercer state. Due to its important phylogenetic position, coding *Xysta* independently as opposed to lumping it into an “8th sternite piercer” character state significantly changes the ASR analysis. With *Xysta* included as a typical 8th sternite piercer, the evolution of piercers is far less parsimonious. The ancestor of Phasiinae is split almost equally between the three states (non-piercer: 38%; 8th sternite piercer: 34%; 10th sternite piercer: 28%, not shown), and ancestral nodes throughout the Phasiinae are recovered as most likely possessing an 8th sternite piercer. Such a reconstruction requires multiple gains and losses of piercers throughout the Phasiinae, with Zitini specifically losing the 8th sternite piercer and gaining the 10th sternite piercer during a very short evolutionary time. This creates a quandary for both parsimony and logic, and therefore provides additional evidence that the piercer of *Xysta*, while homologous to other phasiine piercers, is independently derived and justifies its unique character coding.

Hosts – The diverse host range of Tachinidae, characterized by multiple host shifts and rapid exploitation of new hosts, has made speculating about the original tachinid host difficult. Lepidoptera is often seen as the “default” hypothesis as a majority of tachinids utilize lepidopterans as hosts (Cerretti & Tschorsnig 2010) and caterpillars are exploited by an enormous array of other insect parasitoids, which indicates they are an easy group to invade. However, the morphological phylogeny of Tachinidae conducted by Cerretti *et al.* (2014) did not find strong evidence to support a lepidopteran host in the ancestral tachinid population, instead finding some support for a coleopteran host. This was inferred primarily as a result of the coleopteran parasitoid taxa *Gnadochaeta* and the Palpostomatini being reconstructed sister to all other tachinid genera and a parsimony ASR analysis. These genera were not included here and that specific hypothesis remains unverified. In support of the historical views on host evolution, the maximum likelihood ASR analysis presented here recovered moderate support (67%) for Lepidoptera as the original host of Tachinidae (Figure 1.7). From there, tachinids radiated into Heteroptera, Coleoptera, and ever further into Lepidoptera. A more comprehensive molecular

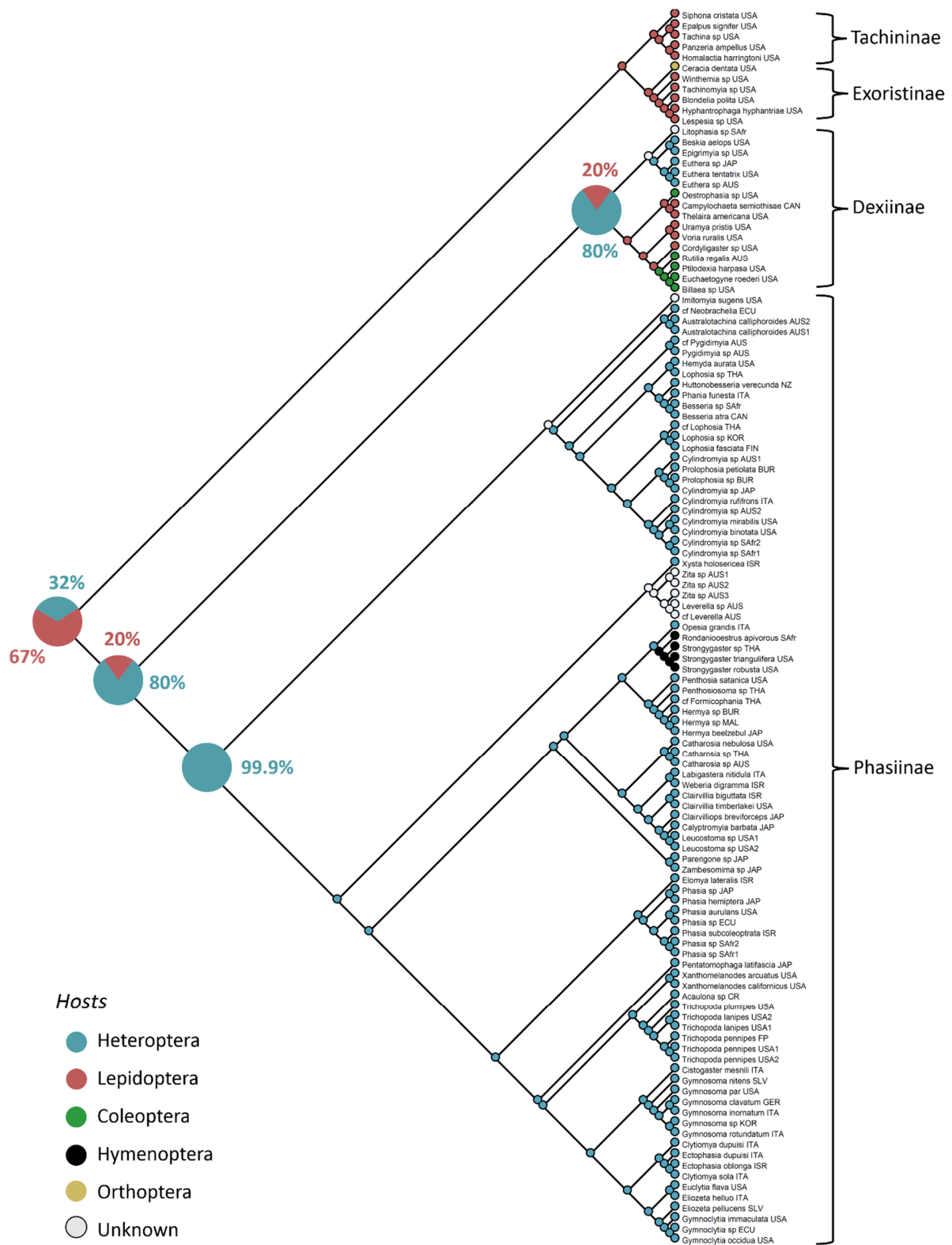


Figure 1.7: Ancestral state reconstruction of host use in Tachinidae.

phylogeny of Tachinidae, already in progress (Stireman *et al.* 2013), will allow a more confident theory of host evolution within the Tachinidae to be constructed.

The ancestor of the (Phasiinae + Dexiinae) clade was reconstructed as a parasitoid of Heteroptera (80%) as were the individual ancestral nodes for each subfamily (Phasiinae: 99.9%; Dexiinae: 80%). Thus, parasitism of Heteroptera most likely arose once in this ancestral population and was retained in Phasiinae, Epigrimyiini, Eutherini, and therefore probably *Litophasia* as well (host unknown). Inferring from this result, the ancestor of Dexiinae s. s. (i.e., excluding Epigrimyiini, Eutherini, and *Litophasia*) switched hosts from Heteroptera to Lepidoptera and later, the tribes Dexiini and Dufouriini diverged from attacking Lepidoptera to parasitizing Coleoptera. The Voriini, Uramyini, Siphonini, Thelairini, and Campylochetini retain their ancestral parasitism of Lepidoptera. These hypotheses stand in contrast to those endorsed by Cerretti *et al.* (2014) whose morphological phylogeny found Phasiinae s. s. to be derived from a paraphyletic clade of Dufouriini (coleopteran parasitoids) and thus recovered Heteroptera as a derived rather than ancestral state, having been evolved independently in Phasiinae and Eutherini. These conflicting results are due to the phylogenetic placement of the ((Epigrimyiini + Eutherini) + *Litophasia*) clade. The Epigrimyiini, Eutherini, and *Litophasia* are extremely difficult to resolve morphologically but are consistently recovered together as members of Dexiinae in molecular analyses, lending support to a heteropteran parasitoid ancestor of (Phasiinae + Dexiinae).

The Phasiinae have long been characterized by their parasitism of heteropteran hosts (Dupuis 1963; Wood 1987; Tschorsnig and Richter 1998), but here, that trait is found within Dexiinae as well, making that trait symplesiomorphic. Additionally, including the Strongygastrini in Phasiinae means that not all phasiines are parasitoids of Heteroptera. *Strongygaster* parasitizes a plethora of hosts, including bugs, beetles, and caterpillars, while *Rondaniooestrus* exclusively parasitizes honeybees. Heteroptera is retained as the primary host in all other phasiine lineages with known hosts. Hosts are unknown for *Litophasia*, but given its phylogenetic position belonging to a clade including the Epigrimyiini and Eutherini, it is hypothesized that *Litophasia* parasitizes small members of Heteroptera.

Oviposition strategy – Another way in which the Strongygastrini are exceptional with respect to Phasiinae is their method of oviposition. Phasiinae as a whole is characterized by oviparity (i.e.,

laying unembryonated eggs), but the Strongygastrini possess uteri and are therefore ovolarviparous (i.e., lay embryonated eggs). Phasiinae represent one of the largest oviparous lineages (others are found in Exoristinae) and are characterized by an oviparous ancestor (82%, Figure 1.8). Consequently, ovolarviparity most likely evolved secondarily in the Strongygastrini and in the Imitomyiini as well.

Ovolarviparity requires significant modifications to the female reproductive system in order to incubate eggs (Herting 1957; Wood 1987). As a result, oviparity has historically been considered the more primitive condition of Tachinidae (Herting 1960; Tschorsnig and Richter 1998; Stireman *et al.* 2006; Tachi and Shima 2010). However, Cerretti *et al.* (2014) found all oviparous lineages to be nested within ovolarviparous lineages, thus implying that ovolarviparity may have characterized the tachinid ancestor. This ASR analysis provides corroborating molecular evidence to support this somewhat counterintuitive hypothesis. Ovolarviparity was recovered at the ancestral node of (Dexiinae + Phasiinae) (96%) and Tachinidae as a whole (99.5%). Unlike reconstructing Lepidoptera as an ancestral host – which had only moderate support and was missing crucial taxa – there is very strong statistical support for an ovolarviparous tachinid ancestor. Furthermore, this result seems unlikely to change with more taxa included as most tachinid lineages not represented are ovolarviparous.

Synapomorphies of Phasiinae/Dexiinae – The placement of several taxonomically ambiguous genera within a robustly supported phylogeny allows potential synapomorphic character traits of Phasiinae and Dexiinae to be explored in a phylogenetic context for the first time. As already discussed, neither oviparity nor parasitism of heteropteran hosts can be used to define Phasiinae as a clade. In fact, there seems to be only a single trait that is synapomorphic for Phasiinae: an elongated hypandrium in the male postabdomen.

The elongated hypandrium was first identified as a distinguishing feature of Phasiinae by Tschorsnig (1985) and is here visualized with ASR analysis (Figure 1.9). *Imitomyia* deserves special mention as it possesses a suite of traits that characterize the dexiine male postabdomen as well as the phasiine-like elongated hypandrium. In many respects (ovolarviparous, platform pregonites, and membranous basi/distiphallus), *Imitomyia* aligns more closely with Dexiinae than Phasiinae. However, given its elongated hypandrium, 10th sternite piercer, and moderate

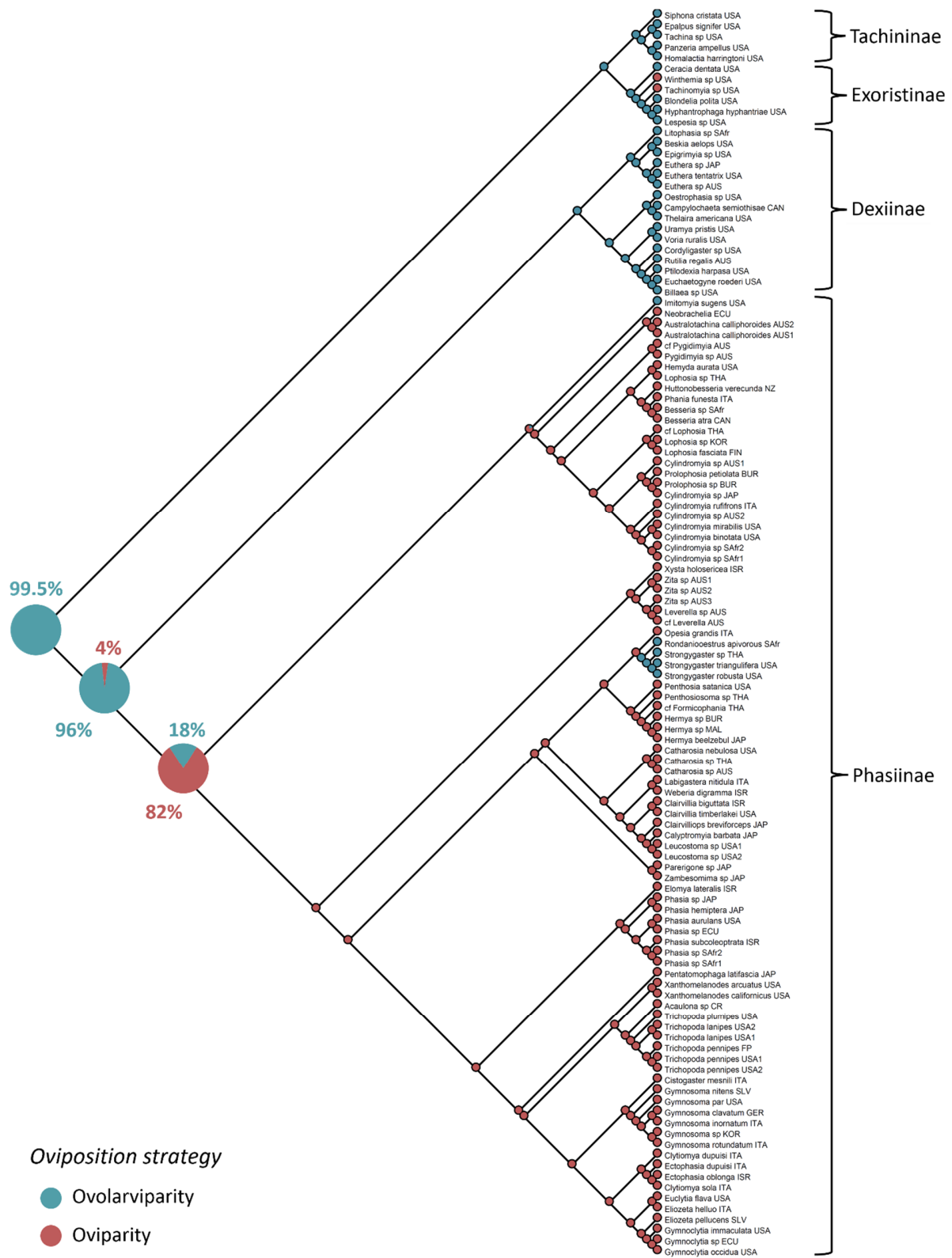


Figure 1.8: Ancestral state reconstruction of oviposition strategy in Tachinidae.

presence of platform pregonites. Taking into consideration the novel hypothesis about the phylogenetic placement of the ((Epigrimyiini + Eutherini) + *Litophasia*) clade, each of these three characters was visualized with ASR analysis to see if any of them represent true synapomorphies of Dexiinae (Figures 1.10-1.12). Each trait is a derived trait not present in the ancestor of (Phasiinae + Dexiinae) and all Dexiinae s. s. possess these traits.

The membranous and hinged connection of the basiphallus to the distiphallus is the most often cited potential synapomorphy of Dexiinae. However, mapping these states onto this phylogeny reveals that basing a classification on a single origin of one or both of these characters results in confusion. This is clearly seen in a summary of these states across taxa: the connection between the basiphallus and distiphallus is characterized in the “Dexiinae” as membranous and hinged, in the Phasiinae (excluding *Imitomyia*) as non-membranous and not hinged, in *Litophasia* and *Imitomyia* as membranous but not hinged, in the Epigrimyiini as membranous and hinged, and in the Eutherini as non-membranous and not hinged. Using only these characters, Eutherini would align with Phasiinae while Epigrimyiini has all the features of Dexiinae. *Litophasia* and *Imitomyia* are intermediate.

Platform (strap-like) pregonites present an easier character to discuss. *Litophasia*, Epigrimyiini and Eutherini all possess platform pregonites and at first glance it looks like this could be a synapomorphic trait for Dexiinae in the same way an elongated hypandrium is for Phasiinae. Unfortunately, the aberrant genus *Imitomyia* also possesses platform pregonites. In this analysis, no single trait stands out as a true synapomorphy for Dexiinae. However, when taken together as a whole, it seems that possessing most or all of a suite of characters (including additional secondary characters like position and morphology of the epiphallus and postgonites) can be used to define the “gestalt” of dexiine male terminalia. The only genus this definition would not apply to is *Imitomyia*. Nevertheless, *Imitomyia* also possesses the phasiine elongated hypandrium, and perhaps the possession of this trait taxonomically “trumps” other features of the male terminalia.

Conclusions

Molecular systematics has been shown to be a useful tool for reconstructing the evolution of Tachinidae, with an emphasis on the Phasiinae. It has contributed important information to

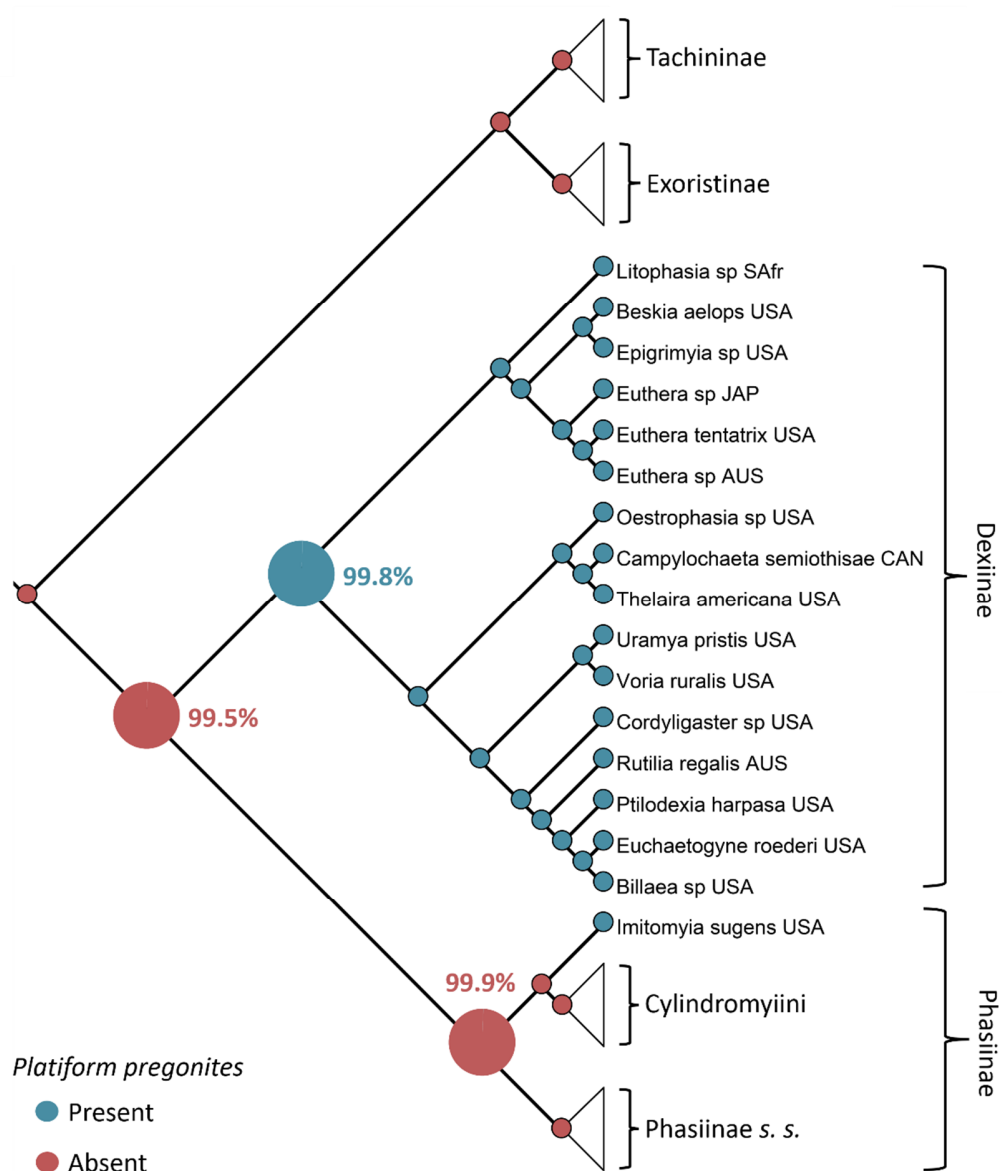


Figure 1.10: Ancestral state reconstruction of platform pregonites in Tachinidae.

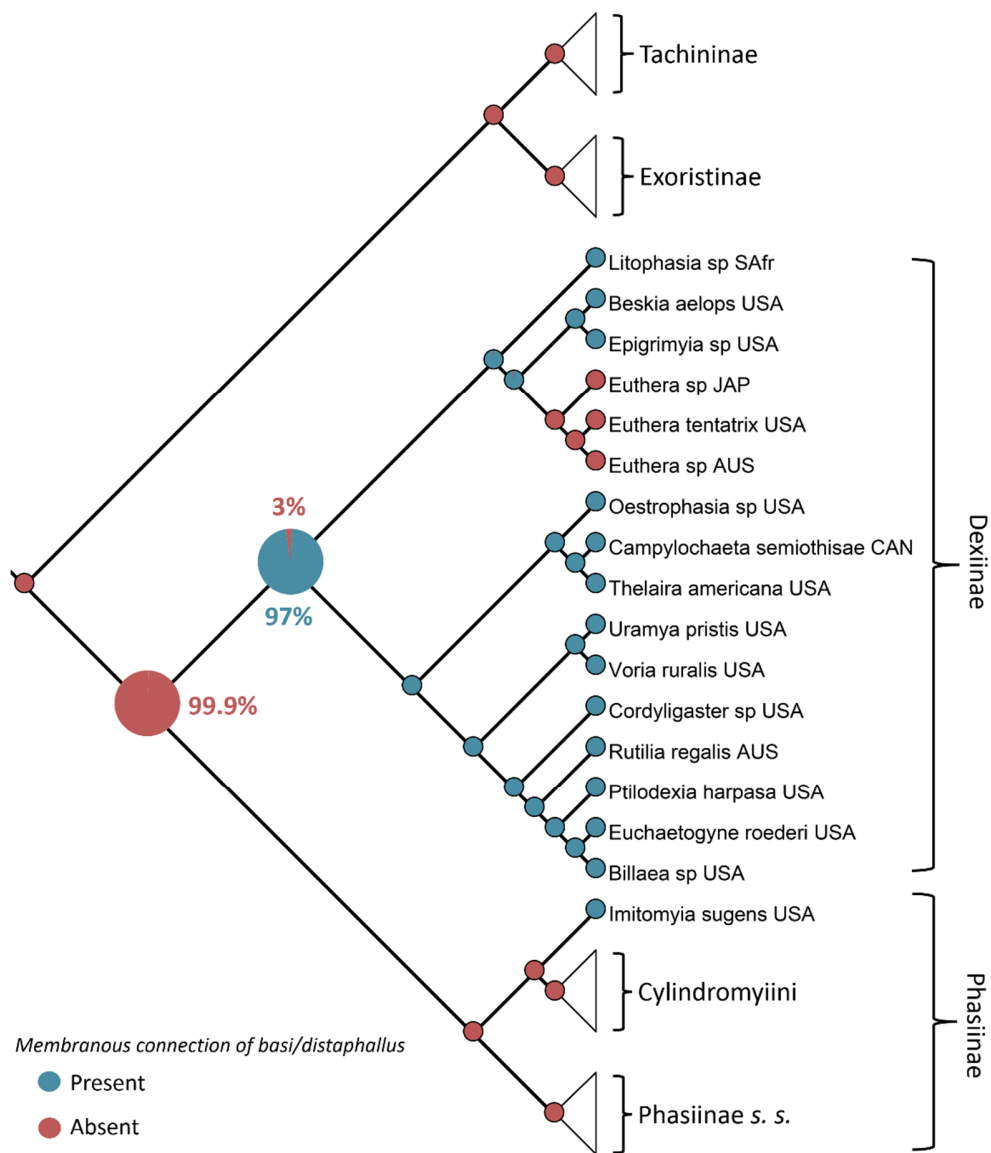


Figure 1.11: Ancestral state reconstruction of membranous connection of basiphallus to distiphallus in Tachinidae.

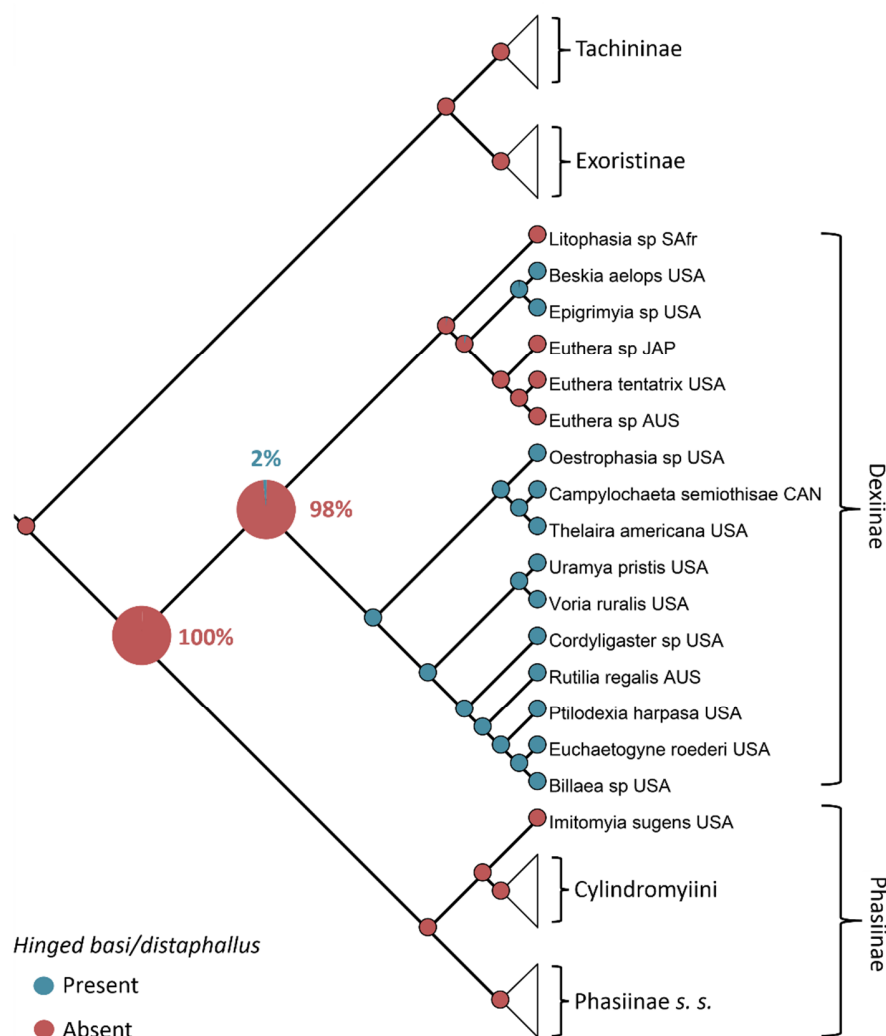


Figure 1.12: Ancestral state reconstruction of hinged connection of basiphallus to distiphallus in Tachinidae.

several longstanding taxonomic debates. The subfamily placements of Eutherini, Epigrimyiini, *Litophasia*, Strongygastrini, and Parerigonini have been all but completely resolved. The Imitomyiini were shown to be as difficult to decipher molecularly as they are morphologically, but the sum genetic and phenotypic evidence suggests a placement in Phasiinae. Ancestral state reconstruction suggests a dominant and persistent trend in Phasiinae to evolve piercing structures used to insert eggs directly into host tissues. A single potential synapomorphy of Phasiinae is identified (elongated hypandrium), but a suite of characters is needed to differentiate Dexiinae. Finally, a robust framework of phasiine classification is provided for use in future revisionary work in the subfamily.

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**Chapter II: Historical Systematics in a Modern Phylogenetic
Context: Creating a Global Classification of Phasiinae
(Diptera: Tachinidae) in Light of New Molecular and
Morphological Evidence**

Abstract

Historical hypotheses about the classification of the endoparasitoid subfamily Phasiinae (Diptera: Tachinidae) are reviewed and compared to phylogenetic trees inferred from modern phylogenetic analyses of nucleotide sequences. An updated classification of worldwide genera and tribes is created and discussions of each tribe's taxonomic and evolutionary history is included. Several historical theories about relationships within Phasiinae are affirmed, including the subfamily placements of Epigrimyiini, Eutherini, and *Litophasia* within the Dexiinae, and the placement of Imitomyiini, Parerigonini, and Strongygastrini within the Phasiinae. Trichopodini is regarded as a clade within Gymnosomatini (Trichopodina) rather than an independent tribe. Many novel phylogenetic hypotheses are also presented including the division of Parerigonini s. l. into three tribes: Parerigonini, Zitini, and Cylindromyiini, and the division of Phasiini into four lineages: Phasiini, Gymnosomatini, Opsiini, and Xystini. Two tribes are resurrected (Opsiini and Xystini) and one new tribe is proposed (Zitini *nomen novum*).

Introduction and Background

Importance of Taxonomy

Taxonomy, the discovering, naming, and organizing of life on earth, is among humanity's most ancient professions. This daunting task has been appreciated by philosophers and naturalists throughout recorded history from Aristotle to Linnaeus to Darwin. Through centuries of diverse thought and research, the purpose and importance of classification has persisted almost unchanged. Taxonomy has three primary purposes: 1) discover and document what plants and animals are valuable to humans, as food, medicine, clothing, labor, aesthetics, or companionship; 2) identify organisms that are potentially harmful to humans, including predators, parasites, and those that are venomous, poisonous, or passively or aggressively defensive; and 3) organize life into predictive categories into which new species can be easily placed and that bridges cultural and linguistic barriers to ensure all of humanity has access to the same tree of knowledge. These practical benefits of classification are well-known and incredibly important, but there is an additional, often undervalued reason to study taxonomy: scientific curiosity.

Humans are the only creatures on earth known to enjoy the diversity of nature itself as beauty and art. Among the diversity of humans, there have always been those who are deeply compelled to explore and describe, and often complete these tasks not out of obligation to produce something useful to humanity, but out of excitement and passion for the task itself. Taxonomy is a profession that provides an exciting and interesting vocation coupled with important practical applications. As such, it has been an occupation or hobby of choice for some of the brightest minds and deepest thinkers throughout history (Theophrastus, Shen Nung, John Ray, Lamarck, etc.) However, naming and organizing all life on earth is a prodigious task. Given the diversity still unknown in certain groups like prokaryotes and arthropods, it is possible that classifying all life on earth will never be a completed project. Even so, significant progress has been made throughout history and it is fascinating to examine how new ideas and new discoveries have provided clarity to puzzling taxonomic relationships.

With the ultimate goal of creating a predictive and comprehensive classification of their study taxa, modern taxonomists are challenged in two ways. First, with the recent introduction and popularization of molecular systematics, the past few decades have seen significant changes to historical hypotheses of classification based on newly acquired genetic evidence. Therefore,

phylogenetic trees generated using modern techniques and statistics should be compared to classical taxonomic hypotheses to create the best total-evidence, evolutionarily meaningful classification. Second, the level of inter-continental collaboration, cooperation, and communication in our modern world is unprecedented. This allows for synthesis of scientific research across biogeographic regions and the creation of truly worldwide taxonomic hypotheses. In this paper, these two challenges of modern taxonomy are applied to the agriculturally important parasitoid fly subfamily Phasiinae (Diptera: Tachinidae). The taxonomic history of Phasiinae is reviewed and compared to recent molecular and morphological phylogenies and a new classification of Phasiinae is presented that includes all worldwide genera placed in phylogenetic context with discussions of inter and intra-tribal evolutionary relationships.

Phasiinae

The Phasiinae are an intriguing group of insects to study. As internal parasitoids, these diverse flies primarily attack true bugs (Heteroptera) (Wood 1987) and many genera use their host's own aggregation pheromones to locate their prey (Aldrich *et al.* 2006). As a result, phasiines have largely untapped potential for use as biological control agents for both native (e. g. *Euschistus servus* Say, Coquillett 1897; *Lygus lineolaris* Beauvois, Arnaud 1978) and exotic invasive crop pests (e. g. *Halyomorpha halys* Stål, Aldrich *et al.* 2007; *Megacopta cribraria* Fabricius, Golec *et al.* 2014). One genus in particular, *Trichopoda* Berthold, has been extensively used around the world to control *Nezara viridula* (Harris & Todd 1981; Sands & Coombs 1999), but use of other genera for biological control is hampered by the difficult and confusing taxonomy of Phasiinae. Also, within Phasiinae are found numerous interesting evolutionary adaptations, including specialized piercers used to inject eggs directly into host tissue (Stireman *et al.* 2006), aposematic mimicry colors/patterns (Waldbauer *et al.* 1977), and a remarkably variable external and internal morphology (Crosskey 1976; Tschorsnig 1985). Analyzing phasiine relationships in a rigorous phylogenetic context will provide a crucial foundation for both applied research in biological control and theoretical research into the evolution of unique character traits.

Historically, the classification of Phasiinae has been unstable, with numerous genera and tribes maintaining a debated placement within Phasiinae based on contradicting morphological

and/or life history traits (see Blaschke 2015). Phasiine taxonomy is further complicated by significant dissonance between the classification systems of the six biogeographic regions of the world (O’Hara 2013a). Currently, there is no checklist of world Phasiinae and there is considerable disagreement among experts as to which genera even belong in the subfamily. Of the 17 tribes discussed here, nine have had a volatile taxonomic history with various lines of evidence indicating placement into Phasiinae or one of the other three subfamilies of Tachinidae (e.g., Dexiinae, Exoristinae, Tachininae). However, recent morphological (Cerretti *et al.* 2014) and molecular (Blaschke 2015) phylogenies have provided significant statistical support for tribal and generic relationships within Phasiinae. These studies have established the necessary robust phylogenetic framework on which to build an unprecedented, evolutionarily informed worldwide classification of the Phasiinae and help resolve several longstanding debates within dipteran systematics.

Using these phylogenies as a framework, the taxonomic history of each recognized and potential phasiine tribe is reviewed and updated, if necessary. A global list of phasiine genera is included. This is not meant to be a complete revision of the subfamily, which would require extensive examination of type specimens, but rather a gathering of historical and current taxonomic hypotheses blended with modern phylogenetic evidence. The goal is to provide an evolutionary informed classification that can be used for testing hypotheses about trait evolution and biogeography, while highlighting areas in need of future systematic research. A cladogram of proposed tribal relationships can be found in Figure 1 and a cladogram of phasiine genera is presented in Figure 2.

Methods

A global list of genera belonging to the tachinid subfamily Phasiinae was compiled from the most recent catalogs of every major biogeographic region. These regional catalogs served as indispensable primary sources for this work and are as follows:

Afrotropical Region	Crosskey 1980
Australasian Region	Cantrell & Crosskey 1989
Nearctic Region	O’Hara & Wood 2004
Neotropical Region	Guimarães 1971

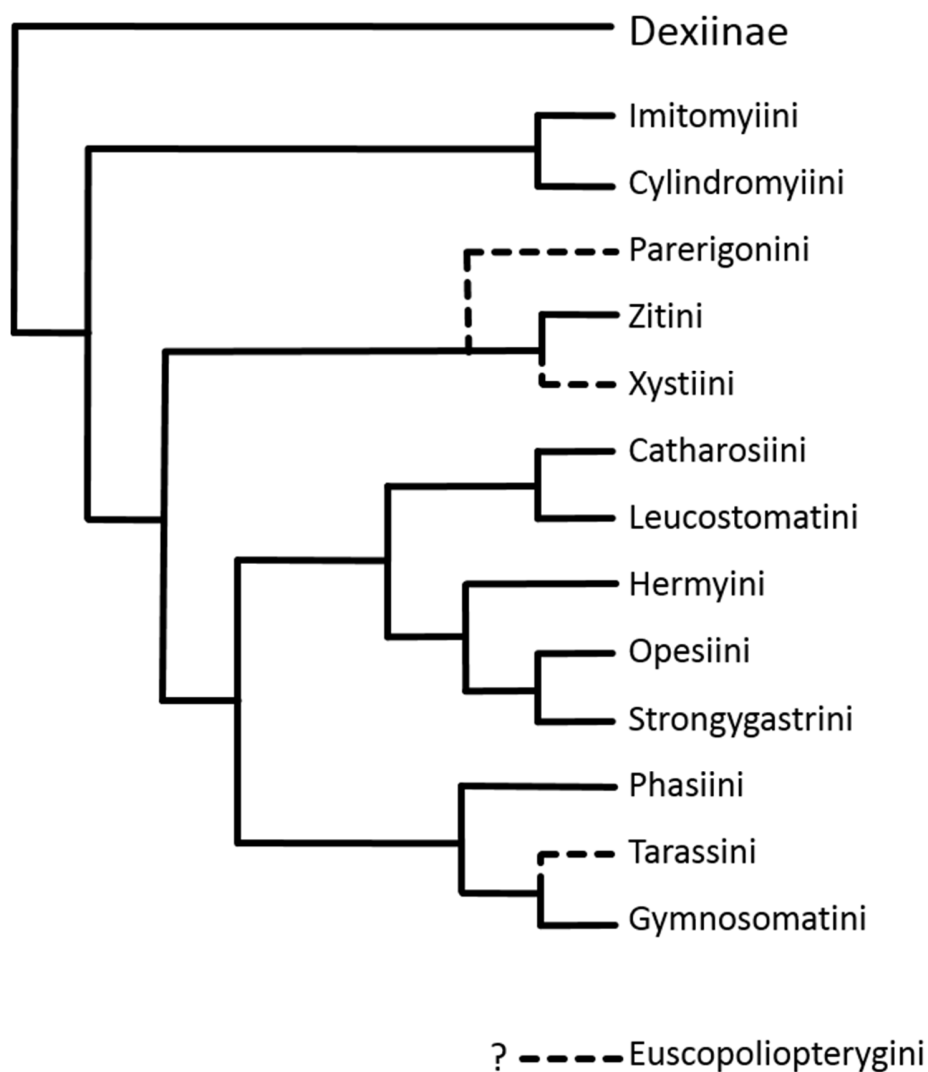


Figure 2.1: Cladogram of proposed tribal relationships within the Phasiinae. Solid lines indicate significant molecular and/or morphological support; dotted lines in the cladogram indicate weakly supported or hypothetical relationships. Euscopiopterygini are currently *incertae sedis*.

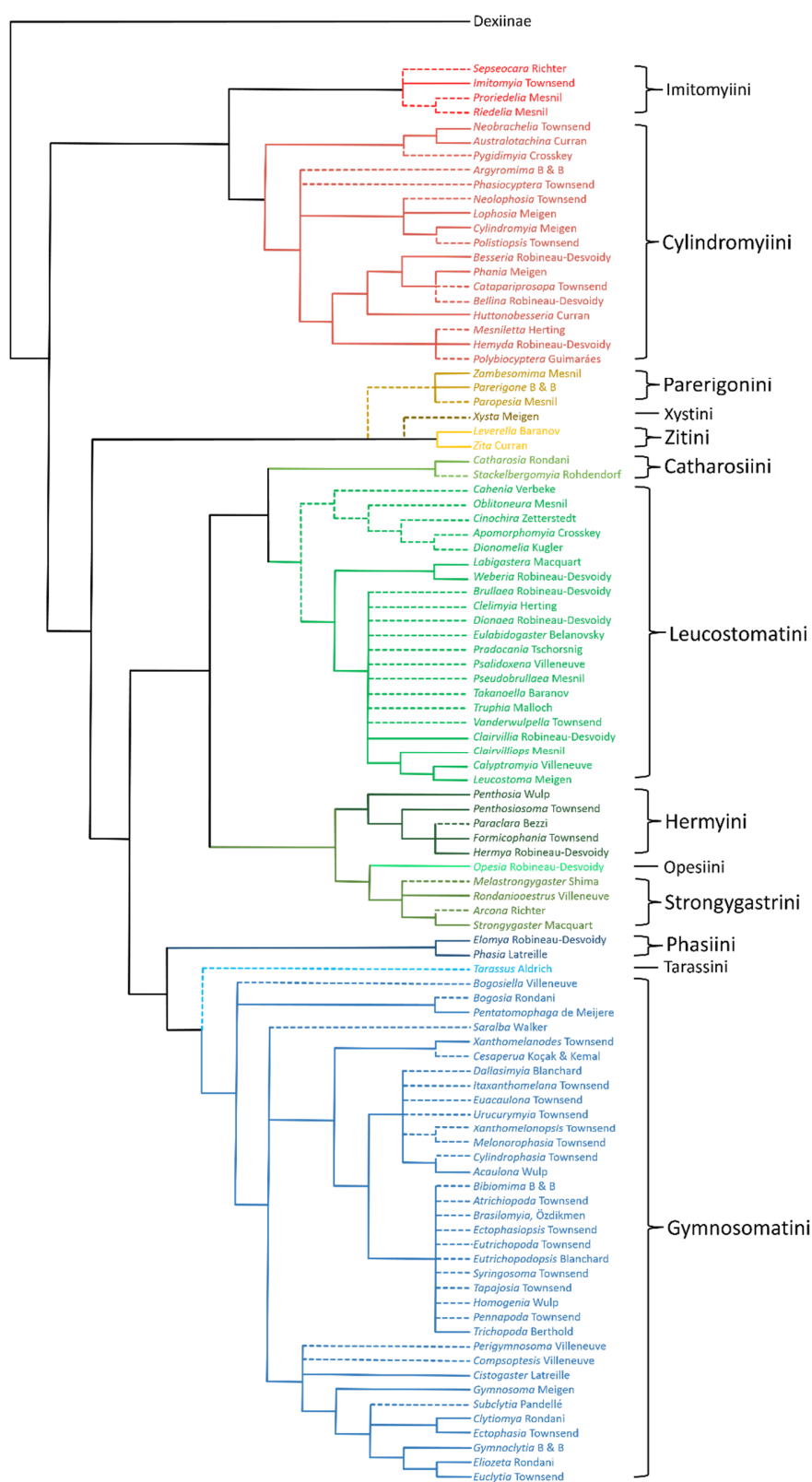


Figure 2.2: Cladogram of Phasiinae with all genera included except the Euscopoliopterygini. Solid lines indicate significant molecular and/or morphological support, dotted lines indicate weakly supported or hypothetical relationships.

Oriental Region	Crosskey 1976, 1977
Palearctic Region	Herting & Dely-Draskovits 1993
China	O'Hara <i>et al.</i> 2009

A preliminary list of genera was then cross-referenced against the world list of tachinid genera found in O'Hara 2014 and updated to reflect recent synonyms and name changes. Global species counts for each genus were documented by comparing species names and numbers across regional catalogs and creating an inclusive list of worldwide species. When relevant, generic revisions published in the years since the catalogs were first written were used to determine current estimates on species diversity. Phasiine genera that have been recently reviewed systematically include the following:

<i>Arcona</i> Richter	Richter 1988
<i>Besseria</i> Robineau-Desvoidy	Cerretti <i>et al.</i> 2010
<i>Cylindromyia</i> Meigen (Neotropical)	Guimarães 1976
<i>Cylindromyia</i> Meigen (Australia)	Cantrell 1984
<i>Cylindromyia</i> Meigen (Palearctic)	Sun & Marshall 1995
<i>Cylindromyiini</i>	Herting 1979
<i>Ectophasiopsis</i> Townsend	Dios 2014
<i>Euthera</i> Loew (Nearctic)	O'Hara 2012
<i>Euthera</i> Loew (Australia)	Cantrell 1983
<i>Lophosia</i> Meigen (China)	Sun 1995
<i>Melastrongygaster</i> Shima	Shima 2015a
<i>Parerigone</i> Brauer & Bergenstamm	Shima 2011
<i>Paropesia</i> Mesnil	Shima 2014
<i>Phania</i> Meigen	Gilasian <i>et al.</i> 2013
<i>Phasia</i> Latreille	Sun & Marshall 2003
<i>Sepseocara</i> Richter	Richter 1986
<i>Trichopoda</i> Berthold	Dios 2014
<i>Zambesomima</i> Mesnil	Wang <i>et al.</i> 2014

To understand the taxonomic history of these genera and ultimately place them in a phylogenetic context, several landmark papers on tachinid morphological systematics were

closely examined, most notably Townsend's (1936, 1938) diagnoses of adults and immatures, Herting's (1957) analysis of the female postabdomen, Tschorsnig's (1985) research on the male postabdomen, and Zeigler's (1998) work on tachinid puparia and larvae. Insightful discussions of tachinid history and systematics were found throughout Crosskey's publications (1973, 1976, 1977, 1980, and 1984) and in more current literature (Wood 1987; Tschorsnig & Richter 1998; Stireman *et al.* 2006; Wood & Zumbado 2010; O'Hara 2013b).

Modern phasiine systematics has benefited greatly from two recent phylogenetic analyses. Cerretti *et al.* (2014) provided the first cladistic analysis of Tachinidae using modern morphological techniques, while Blaschke (2015) established a statistically sound phylogeny of Phasiinae generated from multiple nuclear protein coding genes. The molecular phylogeny of Blaschke (2015) is used here as the framework for the subfamily and tribal structure of Phasiinae and is compared to the historical theories of phasiine classification to create a predictive and evolutionarily meaningful classification of world Phasiinae.

Results and Discussion

Subfamily Phasiinae (14 tribes, 100 genera, 597 species)

Tribe Catharosiini (2 genera, 13 species)

Catharosia Rondani, 1868 (12 species)

Stackelbergomyia Rohdendorf, 1948 (1 species)

Phylogenetics and Classification

The tribe Catharosiini dates back to Townsend (1936) and has been little changed since its creation. *Catharosia* Rondani species occur worldwide and are among the smallest tachinids – some measuring <2mm (Tschorsnig & Richter 1998). The only other genus included is *Stackelbergomyia* Rohdendorf, which contains a single Palearctic species (Herting 1984). Until Herting (1981) added this strange, sand-digging genus, the tribe primarily contained only *Catharosia*, albeit with numerous synonyms (Townsend 1936; Herting 1957; Crosskey 1980, 1984; Tschorsnig 1985; Herting & Dely-Draskovits 1993; Tschorsnig & Herting 1994; O'Hara 2004). Some authors have also included the rare *Litophasia* in this tribe due to the shared

terminalia and wing venation (Crosskey 1980, 1984; Belshaw 1993; Herting & Dely-Draskovits 1993; Tschorsnig & Herting 1994), but that genus is here considered a member of Dexiinae Tschorsnig (1985) and Blaschke (2015). The reduced wing venation and atypical postscutellum of *Stackelbergomyia* influenced Rohdendorf (1948) to place this unique genus in its own family Stackelbergomyiidae. Herting (1981) later examined the fly and noted similarities between it and *Catharosia*, specifically the small calypters, partly membranous postscutellum, reduced proboscis, and male genitalia. He subsequently suggested including *Stackelbergomyia* in the Catharsiini.

Catharosia females use their 8th sternite, which has been modified into a piercer, to inject unincubated eggs into their hosts (Herting 1957). This trait is shared with many other phasiine tribes (e. g. Leucostomatini, Phasiini) and is a different evolutionary adaptation than the 10th sternite (postgenital plate) piercer of the Cylindromyiini. According to Tschorsnig (1985), males of Catharsiini (*Catharosia* and *Stackelbergomyia* included) can be distinguished from other phasiines by the following post-abdominal characters: surstyli completely reduced, aedeagus bent towards back, and arms of sternite 6 fused with sternite 7 + 8. Molecular phylogenetics places the Catharsiini (only *Catharosia* spp. included) in the Phasiinae sister to the Leucostomatini (Blaschke 2015). *Stackelbergomyia* has yet to be included in a modern phylogenetic analysis and its hosts and female genitalia are unknown. Its systematic position in the Catharsiini should be verified by future molecular and morphological research.

Tribe Cylindromyiini (17 genera, 199 species)

Clade Cylindromyiina (4 genera, 155 species)

Cylindromyia Meigen, 1803 (~122 species)

Lophosia Meigen, 1824 (30 species)

Neolophosia Townsend, 1939 (1 species)

Polistiopsis Townsend, 1915 (2 species)

Clade Neobrachelina (3 genera, 7 species)

Australotachina Curran 1834 (1 species)

Neobrachelia Townsend 1931 (5 species)

Pygidimya Crosskey, 1967 (1 species)

Clade Phaniina (8 genera, 35 species)

Bellina Robineau-Desvoidy, 1863 (1 species)

Besseria Robineau-Desvoidy, 1830 (15 species)

Catapariprosopa Townsend, 1927 (4 species)

Hemyda Robineau-Desvoidy, 1830 (6 species)

Huttonobesseria Curran, 1927 (1 species)

Mesniletta Herting, 1979 (1 species)

Phania Meigen, 1824 (6 species)

Polybiocrypta Guimarães, 1979 (1 species)

Genera incertae sedis (2 genera, 2 species)

Argyromima Brauer & Bergenstamm, 1889 (1 species)

Phasiocrypta Townsend, 1927 (1 species)

Phylogenetics and Classification

The worldwide tribe *Cylindromyiini* is the most speciose of the phasiine tribes, with the extremely diverse and common *Cylindromyia* Meigen accounting for over 100 species alone. It is also one of the oldest recognized groups within Tachinidae. The *Cylindromyiini* (as various forms of *Ocypterinae*) have been associated with the *Phasiinae* for most of their taxonomic history (Villeneuve 1924; Townsend 1936; Mesnil 1939), often as a tribe but sometimes as a separate but closely related subfamily (Herting 1957). Morphologically, the *Cylindromyiini* could be considered dexiine-like *Phasiinae* in the same way that the *Epigrimyini* (historically included in the *Cylindromyiini*) are considered phasiine-like *Dexiinae*. These tribes form part of an abstract morphological “bridge” between the two subfamilies as dexiine characters merge into phasiine characters (i.e., *Dexiinae* s. s. – *Dufouriini* – *Epigrimyini*/*Eutherini*/*Litophasia*–*Cylindromyiini* – *Phasiinae* s. s.). The unique gradation of morphological features between these tribes has made creating an evolutionarily informed classification of the *Phasiinae*/*Dexiinae* nearly impossible. Recent advances in molecular phylogenetics however, have provided support for historical hypotheses and offered new insights into the systematics of these insects (Blaschke 2015).

Most *cylindromyiines* have elongated, subcylindrical abdomens with enlarged terminal segments that are often curved beneath the abdomen (Crosskey 1976). Many are fantastic mimics

of various wasp species (Townsend 1938). Although very distinct from other cylindromyiines in coloration and abdominal structure, two of the most striking mimics, *Ichneumonops mirabilis* Townsend and *Clinogaster notabilis* Wulp, have been transferred to *Cylindromyia* (Herting 1979). A third mimic, *Polistiopsis* Townsend, still remains in its own genus (Arnaud 1966; Herting 1979) even though Malloch (1929) doubted it was truly different from *Cylindromyia*. The synonymy of *Ichneumonops* with *Cylindromyia* has been confirmed molecularly (Blaschke 2015) and indicates a remarkable adaptive potential in ancestral *Cylindromyia* populations to mimic sympatric wasp species.

Unlike many phasiine tribes, the Cylindromyiini are an easy group to define morphologically. Except for the closely related Imitomyiini and Zitini, the Cylindromyiini are the only phasiines whose females possess piercers derived from the 10th sternite (postgenital plate) rather than the 8th sternite (Herting 1957). Herting (1983) further defined Cylindromyiini by the fusion of tergite 7 and sternite 7 which together contain a pair of hooks used for prying open the host's sclerites during oviposition. This fusion characterizes all cylindromyiines except those found in the clade Neobrachelina in which the fusion is incomplete and probably represents a plesiomorphic state.

Tschorsnig (1985) characterized the males of Cylindromyiini (*Cylindromyia*, *Lophosia* Meigen, *Phania* Meigen, *Hemyda* Robineau-Desvoidy, and *Besseria* Robineau-Desvoidy examined) by a horseshoe shaped basal-hypandrium and by the unique structures found in the membrane between the hypandrium and sternite 6. He also found synapomorphic characters that further divided the Cylindromyiini into two monophyletic groups corresponding to Herting's (1983) clades Cylindromyiina and Phaniina. In the Cylindromyiina (*Cylindromyia* and *Lophosia*), the pre/postgonites and part of the hypandrium form a "guide cone" to the female postabdominal cavity during copulation, while in the Phaniina (*Phania*, *Hemyda*, and *Besseria*) the surstyli perform this function (Tschorsnig 1985). The molecular phylogenies of Blaschke (2015) support the monophyly of the Cylindromyiini as well as the two clades of Herting/Tschorsnig discussed above. A third clade is here added to the Cylindromyiini based on new molecular and morphological evidence. The Neobrachelina is composed of three genera formerly included in Parerigonini. Each clade is discussed individually below.

Clade Cyldromyiina

The molecularly defined *Cylindromyiina* clade of Blaschke (2015) incorporated multiple species each of *Cylindromyia* and *Lophosia*, as well as representatives from the nominal genera *Ichneumonops* and *Prolophosia* Townsend. These analyses also confirmed the synonymy of *Ichneumonops* with *Cylindromyia* and identified another potential generic synonymy between *Prolophosia* and *Cylindromyia*.

First described from South Africa in 1932b, *Prolophosia petiolata* Townsend was placed near *Cylindromyia* in the *Cylindromyiini*. Concurrently, Curran (1934) was describing the same fly as two new species of *Cylindromyia*: *C. atypica* and *C. ugandana* – both from Uganda. After studying the variable forms of these species, Emden (1945) synonymized Curran's species to *Prolophosia petiolata* and conveyed his opinion that *Prolophosia* may be morphologically distinct enough from *Cylindromyia* to warrant generic or sub-generic rank. Crosskey retained *Prolophosia* as a separate genus in his Afrotropical catalog (1980) and keys (1984). Molecular evidence places *Prolophosia petiolata* and an undescribed *Prolophosia* species firmly within the *Cylindromyia* clade (Blaschke 2015). Townsend was a notorious splitter of tachinid taxa. Many of his genera have since been synonymized, but some still await scrutiny via a modern systematic study. It is not very surprising then to find that *Prolophosia* is not genetically distinct enough from *Cylindromyia* to merit its own generic ranking. Given the molecular evidence and a taxonomic history that has considered merging the two taxa together, *Prolophosia* is considered a synonym of *Cylindromyia*.

Although not included in the molecular analysis, *Neolophosia shannoni* Townsend is also included in this *Cylindromyiina* clade. Little is known about this fly other than what can be gleaned from Townsend's original description (1939). Townsend conveyed that the genus would key out to *Paralophosia* (= *Lophosia* Crosskey 1976) but is actually closer to *Malayia* – a genus that is now in Rhinophoridae (Cerretti *et al.* 2014). It seems probable that Townsend erred in this hypothesized connection to *Malayia* because he describes *Neolophosia* as having a large hypopygium with a piercer derived from what he interpreted could be the 9th sternite (most likely the 10th). No rhinophorids possess these characters but they are typical of *Cylindromyiini*. I therefore assume the rightful systemic position of *Neolophosia* is near *Lophosia* and thus suggest its placement here until type specimens can be examined. The wasp mimic *Polistiopsis* is also

included here in the *Cylindromyiina* based on its close morphological similarity to *Cylindromyia* (Malloch 1929).

Clade Neobrachelina

The Australian genera *Australotachina* Curran and *Pygidimyia* Crosskey are here included with the South American genus *Neobrachelia* Townsend in the *Neobrachelina*. This grouping is a result of molecular evidence that strongly indicates a close evolutionary relationship between the three genera and places them as a paraphyletic sister group to the *Cylindromyiini* (Blaschke 2015). This novel evolutionary hypothesis has since been confirmed by morphological evidence (Cerretti, O'Hara, Stireman 2015 pers. com.). Formerly members of *Parerigonini* and closely associated with the *Zitini*, these unusual genera look very different from other *cylindromyiines*. Their bristly appearance and tachinine-like head shape seem to contradict a placement in *Cylindromyiini* and it was these features that led early authors to place them in the *Tachininae* (Townsend 1938; Guimarães 1971; Crosskey 1973).

In contrast to their external morphology, a placement within *Phasiinae* is supported by male and female terminalia. In females, the *Neobrachelina* possess the distinctive 10th sternite (postgenital plate) piercers found only in *Phasiinae* (Townsend 1940, as *Xenopyxis*; Cantrell 1988). In his examination of terminal segments of Australian tachinids, Cantrell (1988) was the first to identify that the piercers of *Pygidimyia*, *Australotachina*, and *Zita* (*Zitini*), were derived from the 10th sternite rather than the 8th and were thus strongly reminiscent of the *Cylindromyiini* – indicating a placement for these taxa in *Phasiinae* rather than *Tachininae*. This hypothesis was confirmed by molecular analyses that placed *Pygidimyia*, *Neobrachelia*, and *Australotachina* within a monophyletic clade including *Cylindromyiini* s. s. (Blaschke 2015).

The most distinctive characteristic of the *Cylindromyiini* is the complete fusion of tergite 7 and sternite 7 into a complete “ring” segment (Herting 1957, 1983). Interestingly, these segments are only partially fused in the *Neobrachelina* (Cantrell 1988, Cerretti, O'Hara, Stireman 2015 pers. com.), and thus the *Neobrachelina* can be separated from other *cylindromyiines* by this unique pleisiomorphic character. Molecular data indicates a fairly close relationship between *Pygidimyia* and various *Lophosia* species, sometimes reconstructing them together in the same clade (see Blaschke 2015). It is this association with *Lophosia* that prevents a monophyletic *Neobrachelina*. These taxa are understudied and there are undescribed species

waiting to be discovered and in collections waiting to be described. Including some of these additional taxa in future phylogenies may help delineate the two groups through more accurate reconstruction of ancestral states and thus possibly result in a monophyletic Neobrachelina. However, as a consequence of their current molecular paraphyly, the Neobrachelina are here included in an expanded Cylindromyini and form a group therein rather than being considered as their own tribe.

A few notes about the genera of Neobrachelina are warranted. Although most modern tachinid experts consider *Neobrachelia* a parerigonine (Wood & Zumbado 2010; O'Hara 2014 pers. com.), the genus was initially placed in its own monogeneric tribe: Neobrachelini. In the most recent catalog of South American Diptera, Guimarães (1971) included the Neobrachelini in the subfamily Tachininae. At about the same time, DM Wood was relaying his thoughts to Mesnil (see Mesnil 1970) on the similarities of *Neobrachelia* to the Parerigonini and suggesting these taxa should share a systematic relationship. Mesnil, and later Herting (1974), agreed with Wood's assessment and realized that including *Neobrachelia* in Parerigonini finally added crucial host information for the tribe. No hosts were known for any Parerigonini except *Neobrachelia*, and because *Neobrachelia* parasitizes Heteroptera, the debate about subfamily placement of the Parerigonini swung in favor of the Phasiinae (for a more detailed history of Parerigonini see their section below).

Townsend (1940) described the confusing systematic position of *Neobrachelia* (as *Xenopyxis* Townsend) as follows:

“This genus has the weak abdominal macrochaetae of the Ernestiini, the head of the Linnaemyiini, the thoracic chaetotaxy of the Germariini and Schineriini, the male hypopygium of the Melanophryctini, the general characters of the Aphriini, the wide front of all the above tribes and the female hypopygium of none of them.”

Ultimately, he decided to place the Neobrachelini near the Aphriini and Linnaemyiini in his Pyrrhosiinae (Tachininae) (Townsend 1931). However, morphological evidence for a placement in Phasiinae comes from Tschorsnig (1985) who identified the elongated hypandrium characteristic of Phasiinae in *Neobrachelia* and *Parerigone*. Interestingly, Tschorsnig did not find much in common between these two genera other than the structures of the pregonites and postgonites which he found to be similar to the Leucostomatini as well.

Neobrachelia was once thought distinct enough to warrant its own tribe. *Australotachina* as well has long been seen as an aberrant parerigonine – one that most authors retained in Parerigonini solely for convenience until a more thorough analysis of the genus could be made (Crosskey 1973, 1976; Cantrell 1988). The last genus here included in the Neobracheliina is *Pygidimyia*. Crosskey (1976) considered it “undoubtedly” parerigonine and placed it in that tribe in the Tachininae (Crosskey 1973, 1976). However, the enlarged hypopygium that gave *Pygidimyia* its name, has strong similarity to the postabdomens found in Cylindromyiini and has hinted at a placement in Cylindromyiini for almost a century even before the internal anatomy of the genitalia was thoroughly examined.

Clade Phaniina

The molecular phylogenetic clade corresponding to Herting’s Phaniina provides corroborative evidence of a close relationship between *Phania*, *Besseria*, and *Hemyda* (Blaschke 2010). Additionally, the genus *Huttonobesseria* (originally *Phania* Hutton) was placed molecularly in the Phasiina. This rare New Zealand fly differs from *Besseria* by only a few characters (e.g., strong lateral bristles on abdomen, R4+5 open in wing margin near wing tip, and the presence of palpi (Curran 1927)), so its presence in this clade was expected. The genera *Catapariprosopa* Townsend, *Bellina* Robineau-Desvoidy, *Mesniletta* Herting, and *Polybiocyptera* Guimarães were not included in the molecular phylogeny but are included here in the Phaniina due to significant morphological similarity with other members of the clade.

The taxonomic history of the genus *Catapariprosopa* is complicated. *Catapariprosopa* closely resembles *Phania* and may be doubtfully distinct from that genus. Townsend (1927a) first described *Catapariprosopa curvicauda* from Taiwan (as “Formosa”) and treated it as a cylindromyiine distinct from *Lophosia*. Meanwhile, a two new species of the same genus were described by Villeneuve (1932) as *Weberia rubiginans* (subgenus *Chaetoweberia*) and by Emden (1945) as *Phania edwardsi*. Villeneuve (1937) again described what he thought to be a new genus (actually *Catapariprosopa*) as *Hemiphania* (with single species *H. trispana*), which he placed near *Phania*. Herting (1979) later combined Emden’s *Phania edwardsi* and Villeneuve’s *Hemiphania trispana* with *C. edwardsi* and *C. trispana* respectively. Most of these authors (Townsend, Villeneuve, and Emden) did not have our modern definition of *Phania* with which to compare their specimens. These genera, if truly separate, are not sympatric and thus no tachinid

cataloger has, to my knowledge, examined both genera comparatively (Crosskey 1976, 1977, 1980, 1984; Herting 1984; Herting & Dely-Draskovits 1993; O'Hara *et al* 2009). However, when Herting (1979) treated *P. edwardsi* and *H. trispana* as *C. edwardsi* and *C. trispana*, he surely would have had *Phania* s. l. in mind and would have merged the genera together at that time if warranted. He did not, however, so neither do I – especially without having examined the type specimens. Regardless, *Catapariprosopa* is undoubtedly very similar to *Phania* and so belongs in the Phaniina.

Another puzzling genus is the monotypic *Bellina* which was described from India in 1863. The type specimens of the type species *B. melanura* have since been lost and the species has never been identified again. Based on the original description, Townsend (1936, 1938) placed *Bellina* in Cylindromyiini. Crosskey (1976) agreed that it was a cylindromyiine and hypothesized that *Bellina* should be placed near *Catapariprosopa*. On that basis alone, *Bellina* is included here in the clade Phaniina near *Catapariprosopa* and *Phania*.

Mesniletta is a relatively recently described genus created for the previously named *Gymnosoma ventricosum* de Meijere from Java. Herting (1979) examined the species and concluded based on the genitalia that it did not belong to *Gymnosoma*, but was rather a cylindromyiine. The species was different enough from other Cylindromyiini to justify a new genus, but the postabdomen was reminiscent of *Besseria*, *Phania*, and *Hemyda*. With this association, *Mesniletta* is here placed in the clade Phaniina. Similarly, the wasp mimic *Polybiocyptera* is included in the Phaniina due to its affinities with *Hemyda*. Guimarães (1979) initially considered the genus near *Polistiopsis* and *Cylindromyia*. However, Herting (1979) classified the genus near *Hemyda* based in part on the indentation of the eye and similarity of postabdominal characters.

Genera incertae sedis

Several genera are only known from their original descriptions and thus their systematic placement within the Cylindromyiini is uncertain. *Argyromima mirabilis* Brauer & Bergenstamm was described in 1889 from a single South American specimen and has not been rediscovered (Townsend 1928, 1938; Guimarães 1971). This genus is an apparent dolichopodid-counterfeit (of *Argyra* Macquart), does not have palpi (Aldrich 1925: 122), and was placed in Cylindromyiini by Townsend (1936, 1938) and Guimarães (1971). Similarly, the only published discussion of

the Brazilian species *Phasiocyptera punctata* Townsend was by Townsend, who placed it in Cyldromyiini (1927b, 1936, and 1938) where it was retained by Guimarães (1971). These two genera remain *incertae sedis* within Cyldromyiini.

Finally, the aberrant *Tachinophasia transita* Townsend is worth mentioning. This unusual species was placed in the Cyldromyiini (Townsend 1936; Guimarães 1971) but then moved to the Linnaemyini (Tachininae) by Herting (1979). Most recently, *Tachinophasia* was not placed in any subfamily (O'Hara 2013a) and it is uncertain what ultimate systematic position this strange tachinid will occupy. Because of the uncertainty surrounding its classification, I have also excluded it from the Cyldromyiini and therefore the Phasiinae.

Tribe Euscopiopterygini (2 genera, 2 species)

***Euscopiopteryx* Townsend, 1917 (1 species)**

***Shannonomyiella* Townsend, 1939 (1 species)**

Phylogenetics and Classification

Very little information is available on these enigmatic tachinids and no molecular work has been done due to their rarity. The two genera found in Euscopiopterygini are monotypic and found only in South and Central America. *Euscopiopteryx* Townsend was described from a single damaged male specimen taken from a spider's web (another male specimen, as *Dictya externa* Fabricius was later examined by Townsend, 1931) and *Shannonomyiella* Townsend described from one male and one female (Townsend 1917, 1939). Townsend (1931, 1936) relates that Wiedemann considered *Euscopiopteryx* an acalyptate fly in the "Cypseloidea". Townsend included the Euscopiopterygini in his family Gymnosomatidae (mostly modern Phasiinae, but including some Dexiinae and Tachininae) and Guimarães (1971) maintained their position in the Phasiinae in his South American catalog. It is unknown if Guimarães examined types of these species when considering their classification. Wood & Zumbado (2010) considered *Euscopiopteryx* to be in Phasiinae but gave no indication about relationships to other genera within the subfamily. In their key to Central American Tachinidae, *Euscopiopteryx* can be found in the midst of other phasiines (due to its mostly bare parafacial) and is coupled with *Phasia*, primarily due to the petiolate wing venation. However,

Euscopoliopteryx also has slightly plumose arista which is only rarely found in Phasiinae and is considered a plesiomorphic trait (Wood & Zumbado 2010 op. cit.).

In his original description, and indicated by its name, Townsend (1917) related *Euscopoliopteryx* to *Euscopolia* Townsend – a genus that for a time was placed in the Cylindromyiini (Sabrosky & Arnaud 1965). *Euscopolia* was moved to the Linnaemyini (Tachininae) by Herting (1979) and now rests in the Polideini (Tachininae) (O'Hara 2002). It may be that the Euscopoliopterygini also belong in the Tachininae, but without examining specimens or molecular markers, the tribe is maintained in the Phasiinae due to taxonomic precedence and until such an examination can be conducted. Unfortunately, no one has ever indicated where Euscopoliopterygini belongs within the Phasiinae, therefore, the tribe is *incertae sedis*.

Tribe Gymnosomatini (35 genera, 174 species)

Clade Gymnosomatina (10 genera, 73 species)

Cistogaster Latreille, 1829 (7 species)

Clytiomya Rondani, 1861 (4 species)

Comptosia Villeneuve, 1915 (3 species)

Ectophasia Townsend, 1912 (9 species)

Eliazeta Rondani, 1856 (2 species)

Euclytia Townsend, 1908 (1 species)

Gymnoclytia Brauer & Bergenstamm, 1893 (14 species)

Gymnosoma Meigen, 1803 (30 species)

Perigymnosoma Villeneuve, 1929 (2 species)

Subclytia Pandellé, 1894 (1 species)

Clade Trichopodina (21 genera, 94 species)

Acaulona Wulp, 1884 (5 species)

Atrichiopoda Townsend, 1931 (1 species)

Bibiomima Brauer & Bergenstamm, 1889 (1 species)

Brasilomyia Özdikmen, 2010 (1 species)

Cesaperua Koçak & Kemal, 2010 (2 species)

Cylindrophasia Townsend, 1916 (4 species)

- Dallasimyia* Blanchard, 1944 (1 species)
Ectophasiopsis Townsend, 1915 (3 species)
Euacaulona Townsend, 1908 (1 species)
Eutrichopoda Townsend, 1908 (6 species)
Eutrichopodopsis Blanchard, 1966 (4 species)
Homogenia Wulp, 1892 (8 species)
Itaxanthomelana Townsend, 1927 (1 species)
Melonorophasia Townsend, 1934 (1 species)
Pennapoda Townsend, 1897 (1 species)
Syringosoma Townsend, 1917 (1 species)
Tapajosia Townsend, 1934 (1 species)
Trichopoda Berthold, 1827 (36 species)
Urucurymyia Townsend, 1934 (1 species)
Xanthomelanodes Townsend, 1892 (12 species)
Xanthomelonopsis Townsend, 1917 (3 species)
Genera incertae sedis (4 genera, 9 species)
Bogosia Rondani, 1873 (5 species)
Bogosiella Villeneuve, 1923 (1 species)
Pentatomophaga de Meijere, 1917 (2 species)
Saralba Walker, 1865 (1 species)

Phylogenetics and Classification

As presented here, the Gymnosomatini follow the proposed classification of Gymnosomatini *sensu* Tschorsnig (1985) and thus include species from Phasiini *sensu* Townsend (1936), Gymnosomatini *sensu* Townsend (1936), and Trichopodini *sensu* Townsend (1908). As a result, the Gymnosomatini form by far the largest phasiine tribe in number of genera – more than doubling the diverse Cyldromyiini. The Gymnosomatini are divided into two clades that are each individually corroborated by morphological and molecular evidence. The clade Gymnosomatina includes genera of Gymnosomatini *sensu* Townsend as well as most genera currently considered in Phasiini *sensu* Herting (1984) (excluding only *Phasia* Latreille and *Elomya* Robineau-Desvoidy). The Trichopodina are include the Neotropical genera formerly

of Trichopodini *sensu* Townsend. Four additional gymnosomatine genera cannot be confidently placed within either clade at the present.

The three nominal tribes Phasiini, Gymnosomatini, and Trichopodini have shared an interwoven and convoluted taxonomic history. These tribes are distributed worldwide and regional experts have typically followed their own regional taxonomic precedent when considering tribal placement for their local genera and only rarely considered global patterns of diversity. Over the past century or more, this has led to a confusing picture of phasiine classification. Nearctic experts have recognized Gymnosomatini and Trichopodini as separate tribes distinct from the Phasiini since Townsend (1908, 1936) first established them (Guimarães 1971; O'Hara & Wood 2004). However, Palearctic experts and the authors of the Afrotropical, Australasian, and Oriental tachinid catalogs did not recognize them as valid taxonomic units (Crosskey 1976, 1977, 1980, 1984; Herting 1984; Cantrell & Crosskey 1989; Richter 2004; O'Hara et. al 2009).

In his Australian catalog, Crosskey (1973) initially followed Townsend's concept of Trichopodini and included *Saralba* Walker and *Pentatomophaga* de Meijere in the Trichopodini. However, after examining more species from around the world, Crosskey found *Perigymnosoma* Villeneuve to be morphologically intermediate between *Gymnosoma* Meigen and typical Phasiini, and *Pentatomophaga* as intermediate between *Trichopoda* Berthold and *Ectophasia* Townsend (in his view a typical Phasiini). In Crosskey's opinion, three distinct tribes were not justifiable and in his Oriental catalog he therefore merged *Pentatomophaga* (and thus Trichopodini) into Phasiini based primarily on morphological homology (Crosskey 1976). This "lumping" was based solely on adult characters and it is interesting that Crosskey also recognizes that with an expanded Phasiini it becomes difficult to even discern that tribe from the Cyndromyiini. This hints at the futility of relying solely on tachinid external morphology for an evolutionarily meaningful taxonomy. As has been crucial for other tachinid tribes, terminalia characters and molecular evidence have recently provided a framework of taxonomic stability for the Phasiini, Gymnosomatini, and Trichopodini.

Tschorsnig (1985) was the first to consider the phylogenetic utility of male postabdomen characters for delineating phasiine groups and was able to examine species from around the globe including representative members of Gymnosomatini *sensu* Townsend (*Gymnosoma*), Phasiini *sensu* Townsend (*Ectophasia*, *Eliazeta* Rondani, *Subclytia* Pandellé, *Phasia*, and

Elomya), and Trichopodini *sensu* Townsend (*Trichopoda* Berthold, *Bogosia* Rondani, and *Bogosiella* Villeneuve). He discovered that *Phasia* and *Elomya* clearly belonged to a separate lineage, but all other genera examined were united by a very strong synapomorphy: three rigid, tubular spermatic ducts. Consequently, this group was defined as the Gymnosomatini *sensu* Tschorsnig. In his pupal and larval diagnostic guide, Zeigler (1998) followed Tschorsnig in recognizing a Gymnosomatini distinct from the Phasiini based on non-adult characters, but also considered Trichopodini a separate tribe. However, other experts have been more conservative about splitting the Phasiini into separate tribes even though the distinction between Gymnosomatini and Phasiini *s. s.* appears to be evolutionarily significant and strongly supported (Tschorsnig & Herting 1994; Richter 2004; O'Hara *et al.* 2009; Shima 2014). Whether or not Gymnosomatini itself should be divided into two tribes (Gymnosomatini and Trichopodini) is less certain.

In his revision of the “*Acaulona* complex” within the Trichopodini, Toma (2003) related the morphological work of Tschorsnig to an expanded group of trichopodines including *Homogenia* Wulp, *Brasilomyia* Özdikmen (as *Platyphasia* Townsend), *Bibiomima* Brauer & Bergenstamm, *Xanthomelanodes* Townsend, and *Cesaperua* Koçak & Kemal (as *Xenophasia* Townsend). His analysis corroborated the spermatic duct synapomorphy suggested by Tschorsnig in the male terminalia of Gymnosomatini. Toma also found a small yet consistent difference between the structure of the elongated hypandrium between his study taxa and Tschorsnig's (see Toma 2003) suggesting a natural division within the Gymnosomatini.

In recent phylogenetic analyses, Gymnosomatini *sensu* Tschorsnig has been consistently recovered as a monophyletic group separate from Phasiini *s. s.* In the tachinid morphological analysis of Cerretti *et al.* (2014), the clade Gymnosomatini (*Gymnosoma*, *Ectophasia*, *Clytiomya*, and *Trichopoda* included) was separated from Phasiini by the synapomorphic sclerotized sperm ducts. However, because only a single representative of Trichopodini was included (*Trichopoda*), nothing could be inferred about the phylogenetic validity of separating the Trichopodini from the Gymnosomatini. The molecular phylogenetic studies of Blaschke (2015) included multiple gymnosomatine and trichopodine genera and established a statistically strong phylogenetic structure of the Gymnosomatini. The Gymnosomatini were recovered as the sister tribe of Phasiini (composed of *Phasia* and *Elomya*), and within the Gymnosomatini, two additional clades were reconstructed with absolute statistical support. The first corresponds to

Gymnosomatini *s. l.* and included *Cistogaster*, *Clytiomya*, *Ectophasia*, *Eliazeta*, *Euclytia*, *Gymnoclytia*, and *Gymnosoma*. The second clade corresponds to the Trichopodini *s. l.* and included *Trichopoda*, *Xanthomelanodes*, and *Acaulona*.

Phylogenetically, these two clades reflect well the statistical support and diversity of other tribes in Phasiinae and at first glance seem to be clearly deserving of tribal status. However, inclusion of *Pentatomophaga* – the same genus whose morphological ambiguity prompted Crosskey (1976) to merge the Trichopodini into the Phasiini – in the molecular analysis resulted in an enlightening phylogenetic placement. *Pentatomophaga* was reconstructed as the sister group to the gymnosomatines plus the trichopodines. This phylogenetic placement provides the final piece of evidence for an inclusive Gymnosomatini concept. If retained in either Trichopodini or Gymnosomatini, its host tribe becomes paraphyletic with respect to the other tribe – a taxonomic situation that should be avoided if possible. Consequently, given the morphological evidence that provides a strongly synapomorphic trait to the Gymnosomatini and the molecular evidence that clearly places all the genera into a single highly supported – albeit large – clade, I endorse an inclusive concept of the tribe Gymnosomatini in which the Gymnosomatini are composed of the two clades Gymnosomatina and Trichopodina as well as a few unplaced genera.

Clade Gymnosomatina

The recent phylogenetic analyses of Cerretti *et al.* (2014) and Blaschke (2015) have provided strong support for dividing the Phasiini *sensu* Townsend/Herting into two separate groups. *Phasia* and *Elomya* are left in the Phasiini and are treated in the Phasiini section below. All other genera are here transferred to the clade Gymnosomatina. Molecularly, the Gymnosomatina are supported by genetic evidence sequenced from *Cistogaster*, *Clytiomya*, *Ectophasia*, *Eliazeta*, *Euclytia*, *Gymnoclytia*, and *Gymnosoma*. These genera create a strongly supported monophyletic clade that provides the framework for the Gymnosomatina (Blaschke 2015). Morphological support for the Gymnosomatina comes from Tschorsnig (1985) who grouped *Ectophasia*, *Gymnosoma*, *Eliazeta*, and *Subclytia* together, Tschorsnig & Herting (1994), who found a shared larval trait between *Gymnosoma* and *Ectophasia* (ventral margin of their labrum serrated), and Cerretti *et al.* (2015) who recovered a morphologically supported clade including *Ectophasia*, *Clytiomya*, and *Gymnosoma*.

The molecularly supported Gymnosomatini and the formal removal of several genera from the Phasiini solves a taxonomic problem that has been discussed since Townsend first established the Gymnosomatini consisting of *Gymnosoma*, *Perigymnosoma*, and *Stylogymnomyia* (= *Gymnosoma*) and the Phasiini including *Gymnoclytia*, *Euclytia*, *Eliozeta*, *Ectophasia*, *Clytiomya*, *Cistogaster*, *Phasia*, and *Elomya*. Even in his diagnoses, Townsend recognized that there were two groups within the Phasiini – those with piercers and those without. This difference in reproductive morphology was widely known to divide the Phasiini into the “*Phasia* complex” and the “*Gymnosoma* complex” (Brooks 1946), but most experts continued to use the established taxonomy in the hopes that future revisionary work would be possible on a global scale (Sabrosky 1950). This division within Phasiini was quantitatively defined by Herting (1957, female postabdomen) and Tschorsnig (1985, male postabdomen, discussed above). Herting separated *Ectophasia* (as *Phasia*), *Clytiomya*, and *Gymnosoma* from *Phasia* based on the functionality of the 8th sternite as a piercer (functional in *Phasia*, non-functional in others). Interestingly, Herting suggests that the blunt 8th sternite of the *Gymnosoma* group may have been derived from the functional piercer of *Phasia* – a hypothesis that is compatible with the molecular phylogeny.

Two more genera historically considered within the Phasiini, *Perigymnosoma* and *Compsoptesis*, are here included in the Gymnosomatini. Both of these genera are endemic to Southeast Asia where little is known about their habits or characters, and their original diagnoses did not include pertinent genitalia characters that seem to be essential for delineating between Phasiini and Gymnosomatini (Villeneuve 1915, 1929). The abdominal segments of *Perigymnosoma* are fused together as in *Gymnosoma* and the abdomen is yellow/orange as in most Gymnosomatini (Crosskey 1976). *Compsoptesis* has very unusual wing venation (closed R4 +5 cell) when compared to *Phasia* and also has golden coloration on the abdomen (Malloch 1930; Dear & Crosskey 1982). Genetic evidence or direct investigation of the male and female genitalia will allow us to place these genera with confidence, but until such diagnoses can be made, they seem to align more closely with the Gymnosomatina than with the Phasiini.

Clade Trichopodina

In contrast to the Cylindromyiini, whose diversity in species and morphology positively correlate with their apparent antiquity, the Trichopodina appear to be an actively radiating and

relatively new evolutionary clade within Phasiinae. This creates a unique problem in the taxonomy of Trichopodina. Many genera (e. g., *Gymnosoma*, *Trichopoda*) contain species that are not quite diverse enough to be justifiably maintained as distinct species, but they are not similar enough to be conspecific either (see Zimin 1966, Blaschke 2015). A further complication is that this speciose group is characterized by a significant reduction of morphological characters typically used to differentiate genera of tachinids (i.e., chaetotaxy, terminalia) while at the same time they possess an abundance of visually striking colors and patterns that at first glance seem ideal for species delimitation. Unfortunately, patterns vary, stripes fade, and colors tarnish given even a modest amount of time – both as the fly is living and after a lengthy museum repose – all but erasing potential taxonomic characters (Townsend 1897, Blanchard 1966, pers. obs.). As a result, the taxonomic history of this clade is characterized by various experts wrestling with where to draw species and genus lines around an extremely limited repository of specimens whose morphology weaves irascibly around and between other taxa.

Townsend chose to split the trichopodines into finer and finer taxonomic units and left future researchers with primarily monotypic genera and no reliable keys for identification (Townsend 1908, 1917, 1936, 1938). Most of his taxa still remain valid even though many are undoubtedly synonymous. Sabrosky (1950) attempted to organize the group into three “complexes” (*Trichopoda* Berthold, *Acaulona* Wulp, and *Xanthomelanodes* Townsend), but admitted there was little evolutionary significance to the distinction between the two largest groups (*Trichopoda* and *Acaulona*) and it was primarily a classification of convenience. Dupuis (1963) provided a classification that accounted for egg, larval, and female terminalia characters. Again, three groups were formed (clades Trichopodina, Acaulonina, and Cylindrophasiina), with only minor differences between Dupuis’ and Sabrosky’s subgroupings. Blanchard (1966) and Toma (2003) also contributed new species, records, and synonyms to the Trichopodina, with the latter providing the most recent modern revision of the “*Acaulona* complex”. The characters used by these authors to organize the trichopodines into various subgroups might have potential generic value if a more inclusive genus-concept within the Trichopodina was endorsed. For this checklist, it is unnecessary to subdivide the Trichopodina into smaller and smaller clades of ambiguous distinction, so I will only mention a few relationships within the clade that warrant comment and leave all other genera in a phylogenetic puddle.

There are 21 genera included in the Trichopodina. From these, two genera, *Xanthomelanodes* and *Cesaperua*, can be easily characterized as sister taxa by their long, widely diverging scutellar bristles and distinctively asymmetrical male terminalia (Sabrosky 1950, Toma 2003). Next, in contrast to all other Trichopodina, *Acaulona* and *Cylindrophasia* Townsend both have piercers derived from the 8th sternite with which they inject eggs into their hosts (Townsend 1936, Sabrosky 1950, Dupuis 1963). These two genera are split by Sabrosky and Dupuis between the *Acaulona* complex/*Acualonina* and the *Trichopoda* complex/*Cylindrophasiina*, but because the female piercer is a character that has significant phylogenetic signal throughout the Phasiinae, I consider these two genera closely related until a more thorough examination can be made. The other 17 trichopodine genera can be broadly classified as either *Trichopoda*-like or *Acaulona*-like. In the former are the taxa *Atrichiopoda* Townsend, *Bibiomima*, *Brasilomyia*, *Eutrichopoda* Townsend, *Ectophasiopsis* Townsend, *Eutrichopodopsis* Blanchard, *Homogenia*, *Syringosoma* Townsend, *Pennapoda* Townsend, *Tapajosia* Townsend, and *Trichopoda*. *Ectophasiopsis* was placed in Phasiini by Guimarães (1971, 1977) but Townsend (1915) initially considered it near *Ectophasia* and *Trichopoda* (as *Trichopodopsis* Townsend). Dios (2014) revised the genus and placed it back in Trichopodini as a close relative of *Trichopoda*.

The *Acaulona*-like trichopodines include *Dallasimyia* Blanchard, *Euacaulona* Townsend, *Itaxanthomelana* Townsend, *Melonorophasia* Townsend, *Urucurymyia* Townsend, and *Xanthomelonopsis* Townsend. *Melonorophasia* and *Xanthomelonopsis* share similarities in egg morphology (Dupuis 1963). Future research should focus on minimizing the nomenclatural challenges of this group and creating effective and consistent identification keys. This is a clade ripe for molecular systematics as genetics may be the only avenue through which the evolutionary relationships within the Trichopodina can be confidently elucidated.

Genera incertae sedis

The last four genera of Gymnosomatini remain *incertae sedis* for various reasons. Molecular evidence necessitates *Pentatomophaga* is placed in an isolated position and because *Bogosia* is very closely related, if not synonymous, with *Pentatomophaga* (Crosskey 1973, 1976; Barraclough 1985), it too is unplaced. *Bogosiella* is an African genus that was synonymized with *Phasia* by Sun & Marshall (2003), but the synonymy has not been accepted by other tachinid

experts (O’Hara 2015 pers. com.) and so I am uncertain where to place the seemingly intermediate group. Finally, *Saralba* most likely fits within the Trichopodina given its general morphology, but because it would be the only non-Neotropical genus in the clade, I think it best to leave it unplaced without more specific morphological or molecular clues as to ancestry.

Tribe Hermyini (5 genera, 23 species)

***Formicophania* Townsend, 1916 (1 species)**

***Hermya* Robineau-Desvoidy, 1830 (18 species)**

***Paraclara* Bezzi, 1908 (2 species)**

***Penthosia* Wulp, 1892 (1 species)**

***Penthosiosoma* Townsend, 1926 (1 species)**

Phylogenetics and Classification

Historically affiliated with the *Cylindromyiini*, the Hermyini share many external traits with the familiar *Cylindromyia* and *Lophosia*. Both tribes contain species with elongated subcylindrical abdomens and quite a few remarkable hymenopteran mimics. In the Hermyini, *Formicophania elegans* Townsend has an appearance reminiscent of the social wasp *Ropalidia binghami* (Crosskey 1976) and *Penthosia satanica* Wulp is a rare jet black fly known only from a few New World localities (Guimarães 1971; O’Hara & Wood 2004). Except for *Penthosia*, the Hermyini are found throughout Africa (Emden 1945; Crosskey 1980, 1984) and eastern Asia/Oceania (Crosskey 1976, 1977; O’Hara *et al.* 2009). However, only the relatively speciose genus *Hermya* Robineau-Desvoidy is distributed throughout this range. Most other genera are monotypic and are known from only a few localities.

Because of their close morphological and behavioral affinity to *Cylindromyia* and allies, the members of Hermyini were originally placed in the *Cylindromyiini* (Townsend 1936, 1938). This designation was followed by Crosskey in his Afrotropical (1980, 1984) and Oriental (1967, 1977) catalogs (also Dear & Crosskey 1982) and by other non-European authors (Guimarães 1971; O’Hara & Wood 2004). However, according to Sabrosky (1999), the family group name Hermyini dates back to Dupuis (1958, *Hermyina*) and Mesnil (1980, *Hermyini*). The impetus for these authors to separate Hermyini from *Cylindromyiini* probably stemmed from Herting’s (1957) analysis of female postabdomen characters. Herting placed his “Gruppe *Hermyia*

[sic]/*Clara* (= *Paraclara* Bezzi)” in the Phasiinae, excluding them from his subfamily Ocypterinae (= tribe Cylindromyiini) because they lacked the characteristic 10th sternite piercers that defined the Ocypterinae. Since then, Palearctic authors have maintained two distinct phasiine tribes (Herting 1983, 1984; Tschorsnig 1985; Herting & Dely-Draskovits 1993; O’Hara *et al.* 2009, Tachi & Shima 2010), and this two-tribe system is currently accepted by worldwide tachinid experts (O’Hara 2014 pers. com.).

Even though many earlier authors combined the Hermyini and Cylindromyiini, most also commented on the similarities of the modern hermyine genera to each other and their differences from cylindromyiines. In his diagnoses and descriptions of tachinid tribes/genera, Townsend (1938) documented the telescopic female terminalia in *Hermya* and *Paraclara*, which differ substantially from the greatly enlarged terminalia of the Cylindromyiini. He also noted that *Formicophania* was very similar to *Liancosmia* Speiser (= *Hermya*). Elsewhere, Townsend (1926, 1928) also suggested that *Hermya* and *Penthosiosoma* Townsend were near *Penthosia*. Crosskey (1976, 1984) mentioned that both *Formicophania* Townsend and *Paraclara* should probably be synonymized with *Hermya* because of the obvious similarities between the genera. He chose to maintain their generic status until more specimens could be examined. Crosskey (1976) also associated *Penthosiosoma* with *Hermya* based on superficial similarities in the chaetotaxy and shape of their abdomens, but noted that they were still distinct genera. Additionally, he identified “*Hermya* and allies” as a distinct group of the Cylindromyiini based on the presence of palpi and the open posteroventral declivity in the thorax. In summary, the genera belonging in Hermyini have long been associated with each other apart from Cylindromyiini s. s. by tachinid experts – whether placed in an official tribe or otherwise.

Further support for a tribal status for these genera comes from molecular phylogenetics. Blaschke (2015) recovered a monophyletic Hermyini (*Hermya*, *Penthosia*, *Penthosiosoma*, and cf. *Formicophania* included) which was placed rather far from the Cylindromyiini as sister group to the Strongygastrini + Opeini. This relationship supports several interesting hypotheses from the morphological analyses of Herting (female terminalia, 1957) and Tschorsnig (male terminalia, 1985). According to Herting, this clade contains those genera that have the primitive condition of retaining a terminal tergite, either heavily developed (*Hermya*, *Paraclara*, and *Opeia* examined) or reduced but not absent (*Strongygaster*). Additionally, Herting hypothesized that the broad and blunt 8th sternite of *Strongygaster* could have been derived through reduction

from the spoon-shaped 8th sternite of *Hermya/Paraclara* (Herting characterized the Hermyni by this distinctive feature). This hypothesis is not rejected by the molecular evidence. For his part, Tschorsnig (op. cit.) identified the extreme flattening of the basiphallus as a distinguishing character of Hermyni. Notably, Tschorsnig characterized the structure of the hypandrium and pre/postgonites as being more similar to the Phasiini than the Cylindromyiini. More work is needed to answer questions on generic synonymy, but for now, the Hermyni represent a robustly supported phasiine tribe, closely allied with the Strongygastrini and *Opesia*.

Tribe Imitomyiini (4 genera, 9 species)

***Imitomyia* Townsend, 1912 (6 species)**

***Proriedelia* Mesnil, 1953 (1 species)**

***Riedelia* Mesnil, 1942 (1 species)**

***Sepseocara* Richter, 1986 (1 species)**

Phylogenetics and Classification

The tribe Imitomyiini presents one of the most indecipherable taxonomic difficulties within all of Tachinidae. Morphologically, imitomyiines maintain traits of both the Dexiinae and Phasiinae, and unlike other tribes whose classification limits have greatly benefited from molecular research, the Imitomyiini remain somewhat ambiguous. Three genera are currently included in the Imitomyiini: *Imitomyia* Townsend (extremely rare but with an almost worldwide distribution), *Riedelia* Mesnil (Palearctic), and *Proriedelia* Mesnil (East Asia). Nothing is known about the female morphology, biology, or genetics of *Proriedelia* and *Riedelia* and no hosts are known for any imitomyiine. *Imitomyia* must therefore stand as representative of the whole tribe for purposes of classification hypothesizing. Fortunately, the male and female terminalia are known from *Imitomyia* and there is a substantial amount of molecular data for the genus as well. Unfortunately, the available evidence is largely equivocal. After considering both morphological and molecular evidence, the most appropriate placement for Imitomyiini seems to be within the Phasiinae sister to the Cylindromyiini.

Townsend (1908) was the first to assign *Imitomyia* (as *Himantostoma* Loew) a taxonomic position, placing it near *Clistomorpha* (= *Strongygaster*) in the Clistomorphi (Phasiidae) probably due to similarities in overall habitus and size. When Townsend replaced the

preoccupied *Himantostoma* with *Imitomyia* (1912), he placed *Imitomyia* without comment in Eutherini (Exoristidae: Pseudodexiinae) possibly due to the prominent facial carinas present in both genera (Aldrich 1919). Later in his career, Townsend (1936) once again repositioned *Imitomyia* to the Gymnosomatidae (= mostly Phasiinae) but this time in its own tribe Imitomyiini. Crosskey, the only author to eventually examine all three Imitomyiini genera, placed the tribe in the subfamily Dufouriinae in his Afrotropical (1980, 1984) and Oriental catalogs (1976, 1977). This classification followed that of Emden (1945) who included *Diplopota* Bezzi (= *Imitomyia*) in the tribe Dufouriini (Phasiinae). Crosskey (1976: 41-43) provided the most complete hypothesis of the evolution and classification of Imitomyiini up to that time. He considered them as potentially derived from the Prosenini (Dexiinae) with perhaps coleopterous hosts. Accordingly, *Riedelia* and *Proriedelia* with their plumose arista and short proboscises, represent a more plesiomorphic habitus than does *Imitomyia* that has gently pubescent (not plumose) antennae and a much elongated proboscis.

The best morphological evidence that suggests a position among the Phasiinae for *Imitomyia* is found in the female terminalia. Townsend (1936: 75-77; 1938: 178-179) describes the unique postabdomen of *Imitomyia*, most notably the piercer derived from what he calls the 9th sternite. An illustration of this structure can be found in Townsend (1942, fig. 477) and indicates that the structure identified as the 9th sternite is actually the 10th sternite (postgenital plate) of modern authors (see comparison of Cyldromyiini abdomens on same plate, e. g. fig. 473). If so, this provides very strong evidence for phasiine affinity, as the 10th sternite piercer is only found in the closely related phasiine tribes Cyldromyiini and Zitini (Blaschke 2015). The Imitomyiini were subsequently placed in Phasiinae by Herting (1984) and Herting & Dely-Draskovits (1993).

In contrast to the evidence above, *Imitomyia* is ovolarviparous (O'Hara & Wood 2004) which suggests a closer relationship with Dexiinae than with Phasiinae. Additionally, the male terminalia of tachinids has provided excellent phylogenetic information for many confusing tribes (Eutherini, Epigrimyiini, and *Litophasia*) and for these characters, *Imitomyia* seems to have more in common with Dexiinae than with Phasiinae. Tschorsnig (1985) examined *Imitomyia* (as *Diplopota*) and identified many similarities shared with other dexiine taxa, including the distinctive membranous connection between the basiphallus and the distiphallus. However, he also noted that this connection on *Imitomyia* is not as "hinged" as in other dexiines

(a trait shared with *Litophasia*). *Imitomyia* was also found to possess the secondary hypandrial characters of the Phasiinae, which may be a more phylogenetically important character, Tschorsnig ultimately retained *Imitomyia* in Phasiinae. Wood & O'Hara (2004) examined *Imitomyia* and decided the tribe fit better in Dexiinae, saying that *Imitomyia* has “somewhat dexiine-like genitalia”.

In recent phylogenetic analyses, there has been limited but consistent support for a placement within Phasiinae for *Imitomyia*. In their morphological phylogeny, Cerretti *et al.* (2014) recovered a weakly supported clade of Phasiinae including *Imitomyia* that was supported by two non-homoplasious apomorphies (medial plate of the hypandrium elongated and pregonite posteriorly connected to the hypandrium). Under different weighting schemes however, placement of *Imitomyia* was unstable. Molecular evidence places *Imitomyia* with moderate support in a monophyletic Phasiinae (Blaschke 2015). This analysis used four different nuclear genes under both maximum likelihood and Bayesian models. When examined individually, each gene tree pushed *Imitomyia* out of both the Dexiinae and Phasiinae and reconstructed it as sister to both subfamilies (maximum likelihood analyses) or created an unresolved polytomy composed of *Imitomyia* and the dexiine and phasiine lineages (Bayesian analyses). Only when all genes were analyzed together did *Imitomyia* take up a position in a monophyletic clade sister to *Cylindromyiini* in the Phasiinae.

Although statistical support for a monophyletic Phasiinae including *Imitomyia* is relatively low in both morphological and molecular analyses, this classification is supported by the synapomorphic phasiine-like elongated hypandrium in the male and the uniquely formed 10th sternite piercer of the female, which is only found in Phasiinae. Consequently, *Imitomyia* (and therefore the tribe *Imitomyiini*) is here considered a phasiine taxon that has retained several pleisiomorphic characters (plumose arista, ovularviparity, and dexiine-like features of the basi/distiphallus). As an indirect result of this phylogenetic placement, the unknown hosts of *Imitomyia* and relatives most likely belong to Heteroptera rather than Coleoptera.

The rare Russian genus *Sepseocara* Richter is also included here in the *Imitomyiini*, although this association is only loosely supported. When first describing the genus, Richter (1986) conveyed that *Sepseocara* possesses some features of the *Catharosiini* (wing venation, spiracle shape) but is excluded from that tribe by the piercing 10th sternite (postgenital plate). As a result, Richter placed *Sepseocara* in the *Cylindromyiini* with other genera that share this

feature. However, other than the piercer, *Sepseocara* shares little in common morphologically with Cylindromyiini and noticeably lacks the fusion of tergite/sternite 7 that characterizes that tribe (Richter 1986). Additionally, the abdomen is not recurved as in most Cylindromyiini, R_{4+5} is long petiolate, and the head is distinctly non-cylindromyiine. These characteristics are more reminiscent of Catharosiini as Richter already noted, or maybe better – *Imitomyia* and allies. Perhaps this genus is a pleisiomorphic (or highly derived) cylindromyiine, but given its general habitus and 10th sternite piercer, *Sepseocara* appears more closely allied with the Imitomyiini than any other phasiine tribe and is therefore tentatively placed here for now.

Tribe Leucostomatini (21 genera, 59 species)

- Apomorphomyia* Crosskey, 1984 (1 species)
- Brullaea* Robineau-Desvoidy, 1863 (1 species)
- Cahenia* Verbeke, 1960 (2 species)
- Calyptromyia* Villeneuve, 1915 (1 species)
- Cinochira* Zetterstedt, 1845 (2 species)
- Clairvillia* Robineau-Desvoidy, 1830 (8 species)
- Clairvilliops* Mesnil, 1959 (1 species)
- Clelimyia* Herting, 1981 (1 species)
- Dionaea* Robineau-Desvoidy, 1830 (4 species)
- Dionomelia* Kugler, 1978 (1 species)
- Eulabidogaster* Belanovsky, 1951 (1 species)
- Labigastera* Macquart, 1834 (3 species)
- Leucostoma* Meigen, 1806 (25 species)
- Oblitoneura* Mesnil, 1975 (1 species)
- Pradocania* Tschorsnig, 1997 (1 species)
- Psolidoxena* Villeneuve, 1941 (1 species)
- Pseudobrullaea* Mesnil, 1957 (1 species)
- Takanoella* Baranov, 1935 (1 species)
- Truphia* Malloch, 1930 (1 species)
- Vanderwulpella* Townsend, 1919 (1 species)
- Weberia* Robineau-Desvoidy, 1830 (1 species)

Phylogenetics and Classification

The Leucostomatini are distributed worldwide but are by far the most diverse in the Palearctic region (Herting 1984). They represent one of the few phasiine lineages that possesses an easily discernable synapomorphy and as a result their classification has been historically stable since Townsend (1908) first designated the tribe. Female leucostomatines possess unique horizontal pincers as extensions of the 6th tergite (Herting 1957) that are used to ensure cohesion of copulatory organs during mating (Tschorsnig 1985). These pincers are often discernable without dissection and have allowed researchers to confidently place genera within the Leucostomatini with little debate for over a century (Townsend 1936, Crosskey 1976, 1980; Herting 1984; O'Hara & Wood 2004; O'Hara *et al.* 2009). Female leucostomatines also possess piercers derived from the 8th sternite (Herting 1957, *Leucostoma* Meigen, *Dionaea* Robineau-Desvoidy, and *Cinochira* Zetterstedt examined) and share this trait with the closely related tribe Catharosiini among others. Phylogenetically, the Leucostomatini are consistently recovered as a monophyletic group both morphologically (Cerretti *et al.* 2014, *Weberia* Robineau-Desvoidy, *Brullaea* Robineau-Desvoidy, and *Clairvillia* Robineau-Desvoidy included) and molecularly (Blaschke 2015, *Leucostoma*, *Clairvillia*, *Calyptromyia* Villeneuve, *Clairvilliops* Mesnil, *Labigastera* Macquart, and *Weberia* included) where they are reconstructed as sister to the Catharosiini.

Intratribal relationships within the Leucostomatini remain largely unexplored. Mesnil (1966) divided the group into three tribes – Leucostomina, Takanoellina, and Calyptromyina – but this classification was not followed by other authors. In his analysis of the tachinid male postabdomen, Tschorsnig (1985) found that in most Leucostomatini the pincers are used to grasp a constricted part of the male terminalia between the epandrium and cerci (*Leucostoma*, *Clairvillia*, *Takanoella* Baranov, *Eulabidogaster* Belanovsky, *Brullaea*, and *Truphia* Malloch examined). Exceptions occur in *Labigastera* which uses its pincers to grasp the hypandrium and in *Weberia* whose pincers extend between the hypandrium and the genital membrane (Tschorsnig op. cit.). Two genera require further verification as to their placement within the Leucostomatini. *Pseudobrullaea* Mesnil was considered by Crosskey (1976) as potentially intermediate between the Leucostomatini and the Cylindromyiini, particularly in regard to head shape and chaetotaxy. However, this genus possesses the leucostomatine pincers and therefore most likely resides within the tribe. The females of *Vanderwulpella* Townsend, on the other

hand, are unknown and so a clear tribal placement cannot be ascertained. Townsend placed *Vanderwulpella* in the Leucostomatini, but Crosskey (1973) considered the placement most likely an error. These genera are left within the Leucostomatini until types can be examined.

In describing the aberrant tachinid genus *Cahenia* Verbeke, Verbeke (1960) erected the tribe Cinochirini to house *Cahenia* and *Cinochira* based on certain similarities of the male genitalia between the two genera. Although noting that Cinochirini was doubtfully distinct from the Leucostomatini, Crosskey (1984) included his new genus *Apomorphomyia* Crosskey in the Cinochirini and provided an insightful synopsis of the taxonomic relationship between the two nominal tribes. As discussed here, the Cinochirini group encompasses the genera *Cinochira*, *Cahenia*, *Apomorphomyia*, *Oblitoneura* Mesnil, and *Dionomelia* Kugler (Crosskey 1984; Kugler 1978). These genera are loosely united by various reductions in external morphology that remarkably seem to form a bridge between the Leucostomatini s. s. and the Catharosiini. All display some degree of reduced wing venation compared to Leucostomatini s. s., from the straight *M* vein and missing apical crossvein of *Cinochira* and *Dionomelia*, to the drastically reduced wing venation of *Apomorphomyia*. *Cinochira*, *Cahenia*, and *Oblitoneura* share the absence of a lunula, while *Apomorphomyia* and *Dionomelia* both possess extraordinarily reduced lower calypters. *Apomorphomyia* also lacks an enlarged subscutellum and only shares this reduced feature with the rare *Litophasia* (Dexiinae) which has been historically allied with the Catharosiini (Crosskey 1980; Herting 1983).

Given the shared morphological reductions, it is tempting to place these genera in a separate tribe or clade. However, the females of *Cinochira*, *Apomorphomyia*, and *Dionomelia* possess the distinctive pincers found only in Leucostomatini as well as 8th sternite piercers (Crosskey 1984) and thus probably represent an evolutionary clade nested within Leucostomatini s. s. The Middle Eastern genus *Oblitoneura* occupies a phylogenetically intriguing position between these genera and the Catharosiini. While possessing a piercer derived from the 8th sternite, the female genitalia of *Oblitoneura* are reminiscent of both *Catharosia* and *Clairvillia* (Mesnil 1975; see Crosskey 1984) and it is unclear if it possesses a leucostomatine pincer (Mesnil relates that there are two small triangles through which the piercer extrudes). Mesnil (1975) placed this rare fly in the Leucostomatini near *Cinochira*, but Crosskey (1984) considered the genus without a doubt to be closer to *Catharosia* than *Cinochira* or *Apomorphomyia*. When considered collectively, the Leucostomatini s. s. merge seamlessly into the Cinochirini which are

in turn linked with the Catharosiini through the morphological intermediacy of *Oblitoneura*. In light of this, future phylogenetic analyses may uncover evidence that justifies a complete merger of the tribes Leucostomatini and Catharosiini.

Lastly, from the Cinochirini group, and indeed all of Tachinidae, *Cahenia* stands in isolation. Originally associated with *Cinochira* based on similarities in the male terminalia (Verbeke 1960), *Cahenia* shares little in common with the external morphology of either Leucostomatini or Catharosiini. Unfortunately, the phylogenetically essential characters of the females as well as potential hosts of *Cahenia* remain unknown. Further obscuring its systematic position, *Cahenia* possesses the plesiomorphic characters of plumose arista, 3 postsutural *dc* bristles, and strongly developed leg chaetotaxy (Crosskey 1984). It is possible that *Cahenia* belongs in another subfamily altogether (similar to *Litophasia*) and I suspect that if *Cahenia* belongs within the Leucostomatini it would be as sister taxa to an inclusive Leucostomatini + Catharosiini clade.

Tribe Opesiini (1 genus, 4 species)

***Opesia* Robineau-Desvoidy, 1863 (4 species)**

Resurrected tribe: Opesiini Mesnil 1966: 887

Type genus: *Opesia* Robineau-Desvoidy, 1863

Includes: *Opesia americana* Bigot 1889; *Opesia cana* Meigen, 1824; *Opesia descendens* Herting, 1973; and *Opesia grandis* Egger, 1860

Phylogenetics and Classification

The rarely collected genus *Opesia* Robineau-Desvoidy contains three Palearctic/Asian species (Herting & Dely-Draschkovits 1993; O'Hara *et al.* 2009) and one western Nearctic species (O'Hara & Wood 2004). It has spent its entire modern taxonomic history closely allied with *Phasia* in the tribe Phasiini (Herting 1984; Tschorsnig 1985; Belshaw 1993; Herting & Dely-Draschkovits 1993; Tschorsnig and Richter 1998; O'Hara & Wood 2004; O'Hara *et al.* 2009). However, several authors have noted peculiarities within the genus that separate it from other phasiines.

Herting (1957) noted a strong terminal tergite in *Opesia cana*. He commented that this trait is shared with *Hermyna* and *Clara* (= *Paraclara*) and potentially represented a primitive trait of Phasiinae. More significantly, Herting discovered that the 8th sternite of *Opesia cana* was completely reduced (this is the sternite from which the modified piercer of *Phasia* is derived). This unique reduction of the 8th sternite in *Opesia cana* was confirmed by Tschorsnig & Richter (1998) and establishes a distinctive trait for the Opeini. In males, *Opesia* differs from *Phasia*, *Elomya*, and *Xysta* (Phasiini *sensu* Tschorsnig 1985) by the location of spiracle 6, the lack of lateral epandrial lobes, the shortened surstyli, and the particularly small processus longi (Tschorsnig 1985). It should also be noted that in Tschorsnig's analysis he was unable to find significant synapomorphies to define Phasiini (*Elomya*, *Phasia*, *Opesia*, and *Xysta* included).

In their morphological analysis of Tachinidae, Cerretti *et al.* (2014) did not recover the Phasiini (*Phasia*, *Opesia*, and *Xysta* included) as a monophyletic group under any weighting schemes. This lends support to the herein proposed division of Phasiini into separate tribes. Substantial evidence for *Opesia* forming its own tribe distinct from Phasiini was discovered through molecular systematics. Blaschke (2015) recovered a monophyletic Phasiini that only included *Phasia* and *Elomya*. *Opesia* was reconstructed as a well-supported sister group of the Strongygastrini. This clade together formed the sister clade to the Hermyni and supports Herting's (1957) comments on the morphological similarities of *Opesia* to *Hermyna/Paraclara*. As a result, *Opesia* is here removed from the Phasiini and is considered to belong to the newly revived tribe Opeini, closely allied with Strongygastrini and Hermyni.

Tribe Parerigonini (3 genera, 17 species)

***Parerigone* Brauer 1898 (11 species)**

***Paropesia* Mesnil, 1970 (4 species)**

***Zambesomima* Mesnil, 1967 (2 species)**

Phylogenetics and Classification

The Parerigonini *sensu* Mesnil (1966), Crosskey (1973, 1976), and Wood & Zumbado (2010) include eight genera: *Parerigone* Brauer & Bergenstamm, *Paropesia* Mesnil, *Zambesomima* Mesnil, *Zita* Curran, *Leverella* Baranov, *Pygydimyia* Crosskey, *Australotachina*, and *Neobrachelia*. This diverse group of geographically isolated genera from around the world

has never been comparatively examined in a global context and the tribe is taxonomically unstable. Recent molecular evidence indicates that this assortment of unusual taxa includes three separate evolutionary lineages (Blaschke 2015) and this suggested classification is followed here. *Australotachina*, *Neobrachelia*, and *Pygidimyia* are considered members of Cylindromyiini and are discussed above. The final five genera are separated here into two tribes corresponding to biogeography and the location of piercers in the female flies. The tribe Parerigonini s. s. includes genera found only in eastern Asia and have piercers derived from the 8th sternite (*Parerigone*, *Paropesia*, and *Zambesomima*), while the newly established tribe Zitini contains those genera with piercers derived from the 10th sternite (postgenital plate) and are found only in Australia (*Zita* and *Leverella*, see section below).

When early authors first described these genera, they routinely judged the flies based on external characters and did not place much importance on the morphology of the postabdomen (or could not discern the characters easily) (Brauer 1898; Malloch 1930; Mesnil 1957). Consequently, these unusual phasiines were initially thought to belong in Tachininae due to the similarity in head shape, chaetotaxy, and other characters that seem unlike traditional phasiine morphology but link them to genera of the Linnaemyini. A placement within Tachininae in or near the Linnaemyini has been a consistent theme in all regional catalogs since that time (Townsend 1936, 1939; Guimarães 1971; Crosskey 1973, 1976; Crosskey & Cantrell 1989). It should be noted, however, that Townsend (1936, 1939) placed *Zita* and *Pygidia* Malloch (= *Pygidimyia*) in the Leucostomatini (Gymnosomatidae), though for largely unjustified reasons (Crosskey 1976).

Even though superficially they appear more similar to tachinines than phasiines, tachinid experts have considered Parerigonini as potentially aberrant phasiines since Mesnil first described the new genus *Paropesia* (1970) which is considered very closely related to *Parerigone* (Shima 2014). Mesnil noted that *Paropesia* and *Parerigone* both possess piercers derived from the 8th sternite – a common trait among Phasiinae. Hosts were unknown for Parerigonini at that time (hosts are now known for *Parerigone* and *Zambesomima*: Heteroptera: Urostylididae, Shima 2015b), but due to the parasitism of Heteroptera by the closely related *Neobrachelia*, Mesnil (1970, and DM Wood, pers. com. in Mesnil 1970) suggested that Parerigonini may belong in Phasiinae. Herting (1981) also commented on the similarity of the piercing structure of *Parerigone* to the piercers found in Leucostomatini. The third genus of

Parerigonini, *Zambesomima*, was described by Mesnil (1967; male only) who found it difficult to classify and placed it near *Pelatachina* (Tachininae). But this genus also possesses a piercer similar to those found in *Parerigone* and *Paropesia* and seems to be a firmly established member of Parerigonini (Wang *et al.* 2014). The most recent regional tachinid catalog follows this pattern and considers Parerigonini to belong in Phasiinae (O'Hara *et al.* 2009).

Phylogenetic evidence that Parerigonini should rest in Phasiinae can be found in both morphological and molecular analyses. Morphologically, Cerretti *et al.* (2014) recovered *Parerigone* near *Hermia* and the Leucostomatini within the Phasiinae. Molecularly, phylogenetic reconstructions placed *Parerigone* firmly in Phasiinae but its placement in relation to other tribes was equivocal (Blaschke 2015). Evidence from a single gene (CAD) placed *Parerigone* and *Zambesomima* as either close to *Xysta* and *Zitini* (maximum likelihood analyses) or near Leucostomatini and/or Catharosiini (Bayesian analyses). Statistically, there was slightly more support for the former arrangement, but considering the weight of the morphological evidence and especially the similarity in piercers between the Parerigonini and Leucostomatini/Catharosiini, I here suggest that the Parerigonini be placed in the Phasiinae as sister to the diverse clade of ((Leucostomatini + Catharosiini) + (Hermiini + (Opesiini + Strongygastrini))).

Tribe Phasiini (2 genera, 76 species)

***Elomya* Robineau-Desvoidy 1830 (1 species)**

***Phasia* Latreille, 1804 (75 species)**

Phylogenetics and Classification

This proposed classification of Phasiinae differs substantially from previous hypotheses regarding the structure of the Phasiini. Molecular analyses of the subfamily have revealed the historically defined Phasiini to be composed of multiple evolutionary lineages that do not form a single monophyletic group (Blaschke 2015). Consequently, this once large tribe is here reduced to two genera: *Phasia* and *Elomya*. The genera *Eliazeta*, *Euclytia*, *Ectophasia*, *Clytiomya*, *Cistogaster*, *Subclytia*, *Perigymnosoma*, and *Compsoptesis* are transferred to the Gymnosomatini while *Opesia* and *Xysta* each form their own tribe with systematic positions relatively far from the Phasiini (see sections on Gymnosomatini, Opesiini, and Xystini for discussion).

The taxonomic history of *Phasia* is long and extremely varied (see Sun & Marshall 2003) with numerous generic synonymies, but its position in Phasiini alongside *Elomya* (when present) has been a constant (Herting 1957, 1960; Guimarães 1971; Crosskey 1977; Crosskey 1980; Herting 1984; Tschorsnig 1985; Cantrell & Crosskey 1989; Belshaw 1993; Herting & Dely-Draschkovits 1993; Tschorsnig and Herting 1994; O'Hara & Wood 2004; O'Hara *et al.* 2009). In *Phasia*, the 8th sternite forms the characteristic piercing structure found in many phasiine tribes (Herting 1957). However, the 8th sternite in *Elomya* does not form a strongly sclerotized and curving piercer. Herting (1957, 1960) describes it as a "narrow point" that is used to deposit eggs into host body niches (intersegmental membranes, Tschorsnig & Richter 1998). This trait is distinct from the piercer of *Phasia* and may be an intermediate form between non-piercing sternites and fully functional host-stabbing piercers.

Along with the molecular evidence for their close evolutionary history, these two genera also share the following morphological traits of the male postabdomen which can be used to separate them from other Phasiini *sensu* Tschorsnig (1985): tergite 6 is platform and easy to discern, the confluence of tergite 6 and sternite 7 +8 forms a nearly acute angle, the lobes of sternite 5 are developed into cone-shaped structures (as in most *Phasia* species), and the surstyli are longer than the cerci (Tschorsnig 1985). The Phasiini also share the absence of teeth on the apex of the first instar larval mouth hook (Dupuis 1963; Sun & Marshall 2003).

Tribe Strongygastrini (4 genera, 16 species)

Arcona Richter, 1988 (2 species)

Melastrongygaster Shima, 2015 (5 species)

Rondaniooestrus Villeneuve, 1924 (1 species)

Strongygaster Macquart, 1834 (8 species)

Phylogenetics and Classification

Possessing traits and behaviors of both the Phasiinae and Tachininae, the Strongygastrini is yet another puzzling tachinid tribe with uncertain taxonomic affinities. Distributions vary considerably throughout the group. The type genus *Strongygaster* Macquart is found worldwide, *Rondaniooestrus* Villeneuve is an African genus (O'Hara 2012), and *Arcona* Richter and *Melastrongygaster* Shima are known only from Russia and eastern Asia (Shima 2015a). A

position in Phasiinae is easy to support superficially as most of the genera are very similar to the phasiine genera *Opesia* and *Phasia* in overall appearance (head shape, chaetotaxy, and wing venation). Herting (1957) considered *Tamiclea* and *Hyalomyiodes* (both = *Strongygaster*) to be primitive phasiines based on host use and egg, larval, and female postabdomen characters. However, Dupuis (1963) chose to exclude the Strongygastrini from his monograph on Phasiinae.

In morphological studies of male terminalia, Verbeke (1962) identified the same general structure of the distiphallus in *Strongygaster*, *Cylindromyia*, and *Phasia*, and Tschorsnig (1985) recognized the characteristic elongated hypandrium of the Phasiinae in *Strongygaster*. Consequently, the historical classification of *Strongygaster* as a member of Phasiinae (Townsend 1936, as Gymnosomatidae) has been followed by many taxonomists (Mesnil 1966; Guimarães 1971; Herting 1984; Tschorsnig 1985; Shima 1989; Richter 1993; Tschorsnig & Herting 1994; Shima 2006, 2015). Recently, the newly discovered genus *Melastrongygaster* was described and associated with *Strongygaster* and *Arcona* based on considerable homology of the male terminalia even though the genus is otherwise morphologically uncharacteristic of Strongygastrini (Shima 2015a). The female *Melastrongygaster* differs from other Strongygastrini in that the 8th sternite is modified into a piercing structure. This character strongly implies a phasiine ancestry, as similar piercers are found throughout the Phasiinae and occur almost nowhere else in Tachinidae (e. g. Epigrimyiini, Dufouriini).

In contrast to the characters above, *Strongygaster* develops eggs in a uterus (Wood 1987) and is ovolarviparous (lays incubated eggs or early instar larvae), thus setting it apart from the oviparous Phasiinae – although Herting (1960) considered this a derived feature. *Strongygaster* also differs from other Phasiinae in its choice of hosts. It commonly attacks the phasiine staple of heteropterans (Sabrosky & Braun 1970; Santos & Panizzi 1997; Panizzi & Oliveira 1999; Golec *et al.* 2013; Blaschke 2014 pers. ops.) but also attacks a wide array of other insects including Orthoptera (Kevan & Koshnaw 1988), Coleoptera (Arnaud 1978), Hymenoptera (Eggleton & Belshaw 1992; Feener 2000), Lepidoptera (Sabrosky & Braun 1970), Dermaptera (Sabrosky & Braun 1970), and Diptera (Ferrar 1977). As a result of this evidence, many recent authors have chosen to associate *Strongygaster* with the Tachininae (O'Hara & Wood 2004; Stireman *et al.* 2006; Cerretti *et al.* 2009; Inclan & Stireman 2011).

The genera of Strongygastrini have also been historically aligned with the Dufouriini (Dexiinae). The genera *Rondania* and *Campogaster* (= *Microsoma*), now in Dufouriini (Herting

1984), were considered members of the Strongygastrini by Townsend (1936), *Rondaniooestrus* was initially placed in the Dufouriina (in Phasiinae) (Mesnil 1939), and Dear & Crosskey (1982) positioned *Strongygaster* in the subfamily Dufouriinae. However, this relationship does not have any specific morphological synapomorphies in its favor.

Hosts are unknown for *Melastrongygaster* and *Arcona* (although see Richter 1998 [in Russian] for possible mention of hosts), and *Rondaniooestrus* is the only known tachinid parasitoid of honey bees (Villeneuve 1924). If included in the Phasiinae then, *Rondaniooestrus* would be the only phasiine with known hosts that do not include heteropterans. This distinction in part led Mesnil (1980) and Crosskey (1984) to maintain *Rondaniooestrus* in its own tribe Rondaniooestrina/i in the Tachininae, although Herting (1983) considered it belonging to Strongygastrini. Evidence for the inclusion of *Rondaniooestrus* in Strongygastrini comes from molecular phylogenetics which places the genus with high support as sister to *Strongygaster* and the entire clade firmly within Phasiinae (Blaschke 2015). The male terminalia of *Rondaniooestrus*, *Arcona*, and *Melastrongygaster* contain the same reduced phallus and other derived characters identified in *Strongygaster* (Tschorsnig 1985), which help define the Strongygastrini (Richter 1988; Cerretti 2014 pers. com.; Shima 2015a). The molecular phylogeny also revealed a close relationship with the genus *Opesia* which was recovered as sister to the Strongygastrini (Blaschke op. cit.). Morphological phylogenetics provides additional confirmatory evidence that the Strongygastrini belong in Phasiinae (Cerretti *et al.* 2014).

The evolution of this clade within the Phasiinae is unique in a number of ways. First, it represents the only one of two origins of ovarviparity within the subfamily – a complex reproductive strategy that dominates the Dexiinae and Tachininae (found also in Imitomyiini). Second, the Strongygastrini includes a species not known to parasitize Heteroptera (*Rondaniooestrus*) and a species with one of the widest host ranges of all Tachinidae (*Strongygaster*). These traits are unknown in other phasiines. Third, if *Melastrongygaster* does belong to the Strongygastrini, its piercer probably represents an ancestral character for the tribe. The piercer was most likely lost in the ancestor of *Strongygaster* + *Arcona* + *Rondaniooestrus* after the ancestor of *Melastrongygaster* diverged. The loss of the piercer is a potential factor in the explosion of host diversity in these genera as the flies were no longer specialized to attack hosts with hardened cuticles (Blaschke 2015). Discovering additional hosts of Strongygastrini,

especially *Arcona* and *Melastrongygaster*, could shed light on this interesting evolutionary question.

Tribe Tarassini (1 genus, 1 species)

Tarassus Aldrich, 1933 (1 species)

Phylogenetics and Classification

The genus *Tarassus* Aldrich was named from the Greek work *tarassein* meaning to “stir up trouble, throw into confusion” (Aldrich 1933) and is an apt moniker for this unusual fly. Described from a single female specimen, *Tarassus* lacks the usual tachinid characters of enlarged calypters, meral bristles, and enlarged subscutellum. As a result, Townsend (1935) did not even consider it belonging to Calypttratae (Guimarães 1971). Other experts, however, including Malloch and Aldrich (see Aldrich 1933) and Hennig and Guimarães (see Guimarães 1971) considered it an anomalous tachinid.

Aldrich (1933) originally suggested a new subfamily for *Tarassus* near “Trichiopodinae”. Guimarães (1971) only bestowed tribal status on the fly and placed it near the Trichopodini in Phasiinae. The gestalt of *Tarassus* is certainly Tachinidae and in my opinion it looks like a much-reduced trichopodine (Figure 3). Given the dark coloration of its body and wings, the virtual absence of stout macrochaetae, and the almost petiolate abdomen, *Tarassus* may be mimicking a sympatric wasp species (e.g., *Cylindromyia mirabilis* or *Polistiopsis* spp.).

The only specimens known to me include the holotype, which Aldrich originally described from Brazil and is housed in the National Museum of Natural History (Smithsonian Institution, Washington DC, USA), and one from Colombia housed in the National History Museum (London, UK). There may be others scattered throughout regional museums but this is undoubtedly an extremely rare and fascinating fly. Genetic evidence would undoubtedly place this genus with more certainty. Unfortunately, such rare species are difficult to obtain for molecular analysis. Therefore, *Tarassus* is left here in its own tribe near Trichopodini until further research is completed.

Tribe Xystini (1 genus, 1 species)

***Xysta* Meigen, 1924 (1 species)**

Resurrected tribe: Xystini Lioy, 1864: 882

Type genus: *Xysta* Meigen, 1824

Includes: *Xysta holosericea* Fabricius, 1805

Phylogenetics and Classification

The general habitus of *Xysta* Meigen is strongly reminiscent of fellow phasiine genera *Phasia* and *Opesia*. As a result, *Xysta* has been included in the tribe Phasiini for most of its taxonomic history (Herting 1957, 1984; Tschorsnig 1985; Herting & Dely-Draskovits 1993). However, when internal structures are examined, *Xysta* contains notable differences from these taxa that set it apart as unique.

In his examination of female terminalia, Herting (1957) commented that *Xysta* held a very isolated position within the Phasiinae. Unlike any other phasiine, *Xysta* possesses a normally developed postgenital plate which Herting suggested could be an ancestral trait of Phasiinae. Additionally, Herting hypothesized that the uniquely shaped 8th tergite of *Xysta* was used by the fly to spread open its host's elytra during oviposition. The characteristic corkscrew ovipositor (8th sternite) of *Xysta* would then penetrate the host and deposit eggs. Similarly, Tschorsnig (1985) examined the male postabdomen of *Xysta* and found it to be quite distinctive when compared with other Phasiini. Probably as a direct consequence of the corkscrew ovipositor and the mating requirements for such a modification to the abdomen, *Xysta* males have a uniquely asymmetrical postabdomen. These traits are not found anywhere else in the Tachinidae.

Molecular phylogenetics supports the hypothesis that *Xysta* occupies an isolated position within the Phasiinae and suggests a closer evolutionary history with the Zitini rather than the Phasiini (Blaschke 2015). As a result, this unique genus is here removed from the Phasiini and placed in its own tribe Xystini.

Tribe Zitini (2 genera, 3 species)

Leverella Baranov, 1934 (2 species)

Zita Curran, 1927 (1 species)

New tribe: Zitini

Type genus: *Zita* Curran, 1927

Includes: *Zita aureopyga* Curran, 1927; *Leverella institutiimperialis* Baranov, 1934; and *Leverella novaeguineae* Baranov, 1934

Phylogenetics and Classification

The new tribe Zitini is erected for the under-studied Australian genera *Zita* Curran and *Leverella* Baranov. Formerly of the Parerigonini (see their section above), these genera are distinct from the Parerigonini s. s. by their geography (Australia vs. Asia) and more importantly by their possession of female piercers derived from the 10th sternite rather than the 8th (Cantrell 1988). No hosts are known for *Zita* or *Leverella*, few specimens have been caught, and there are undoubtedly new species waiting to be described. Externally, *Zita* and *Leverella* do not appear phasiine-like but rather share tachinine-like affinities similar to the Parerigonini, with *Zita* initially related to *Arctophyto* Townsend (= *Ateloglossa* Coquillett, Dexiini; Curran 1927). The Zitini were therefore placed in Tachinae by Townsend (1936, see section on Parerigonini for full history), but now reside firmly in Phasiinae (Blaschke 2015).

Molecular phylogenetic analyses have positioned *Zita* and *Leverella*, along with some unknown species near these genera, in the Phasiinae as sister taxa to all other phasiine tribes outside of clade Cylindromyiini + Imitomyiini (see Blaschke 2015). The tribes Imitomyiini, Cylindromyiini, and Zitini share the unique positioning of the female piercer (10th sternite) that is not found anywhere else in Tachinidae (Cantrell 1988) and may have characterized the early phasiine ancestor. The Zitini are also closely related to *Xysta*, another unusual tachinid with an aberrant piercer (derived from 8th sternite, but is corkscrew unlike other phasiine piercers). Surprisingly, *Pygidimyia*, another Australian genus formerly of Parerigonini and closely associated with *Zita* and *Leverella*, was not reconstructed within the Zitini but was rather placed within Cylindromyiini (see Cylindromyiini section for discussion), leaving only *Zita* and *Leverella* as strongly supported members of Zitini. These genera are distinct from the

Cylindromyiini morphologically due to the lack of fusion of sternite 7 and tergite 7, which is a characteristic feature of Cylindromyiini (Cantrell 1988).

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**Chapter III: Biodiversity Survey and DNA Barcoding of the
Phasiinae (Diptera: Tachinidae) of Great Smoky Mountains
National Park, USA**

Abstract

In contribution to the All Taxa Biodiversity Inventory (ATBI), a survey of the subfamily Phasiinae (Diptera: Tachinidae) was conducted in Great Smoky Mountains National Park. Phasiines are brightly colored endoparasitoid flies that attack true bugs (Heteroptera) and can be important pollinators in meadow habitats. Collections were made from August to October over a two year period at two sites: Purchase Knob (2013: NC: Haywood Co.) and Cades Cove, Hyatt Lane (2014: TN: Blount Co.). Phasiines were collected by hand and with the aid of Malaise traps baited with host pheromone lures. Collected specimens were brought back to the Moulton Lab at the University of Tennessee for sorting and identification. The three right legs of each specimen were removed and placed in 95% ethanol for potential molecular analysis. Species identifications were made using morphological keys, with further evidence from 900 base pairs of the nuclear coding gene MCS. In total, 221 specimens representing 26 phasiine species were collected. Of these, 21 species are newly recorded from the park, four are new records for Tennessee, and two are new records for North Carolina. All 12 eastern Nearctic phasiine genera were represented. Phasiines of the genus *Gymnoclytia* were shown to be exceptionally attracted to pheromones of the brown stink bug (*Euschistus* spp.). Updated identification keys and DNA barcoding sequences are provided for future researchers to quickly and inexpensively identify phasiine species.

Introduction and Background

Great Smoky Mountains National Park

Containing over 1700 species of native trees and vascular plants, Great Smoky Mountains National Park (GSMNP) is one of the most biologically diverse temperate areas in the world and a protected International Biosphere Reserve (Nichols and Langdon 2007). However, the persistence of the park's diversity and beauty is threatened by habitat fragmentation and pollution (Cai *et al.* 2012). In 1998, GSMNP and Discover Life in America began the All Taxa Biodiversity Inventory, or ATBI (Sharkey 2001). This project has the ambitious goal of identifying and cataloging every species in the park in order to better protect and steward this valuable natural resource. Since its inception, over 8000 species have been identified as new to the park, with a remarkable 951 of these being new to science as of March 2015 (Nichols pers. com.). The inventory relies extensively on professional taxonomists who primarily identify organisms in their personal group of expertise. As a result, many taxa still await their own specialist for identification. Among these under-studied groups are the Phasiinae (Diptera: Tachinidae), a diverse subfamily of insects composed entirely of endoparasitoid flies.

Phasiinae

Phasiines vary significantly in size (2mm – 17mm) and color (drab to vivid) but the average phasiine is a medium-sized brightly colored fly with a red/orange abdomen often accented by various black markings. They are commonly observed feeding on flowering plants, especially Queen Anne's lace (*Daucus carota* L.) and goldenrod (*Solidago* L. spp.). Phasiines attack a variety of insects in the suborder Heteroptera (Hemiptera), from minute tarnished plant bugs (*Lygus* Hahn spp.) to leaf-footed bugs (*Leptoglossus* Guérin-Méneville spp.), including several important agricultural and invasive pests (*Nezara viridula* L., *Halyomorpha halys* Stål, *Megacopta cribraria* Fabricius etc.) (Arnaud 1978; Aldrich *et al.* 2007). Host location is often accomplished through specialized antennal receptors that are extremely sensitive to their host's pheromones (Aldrich *et al.* 2006). Many phasiines deposit eggs on the external cuticle of their prey, but others have a modified piercing structure that enables them to inject eggs directly into the host's tissue (Wood 1987).

Phasiines serve two primary roles in the ecosystem as secondary consumers and pollinators. As secondary consumers, phasiines help regulate populations of plant damaging insects and thereby affect the health and diversity of the plants themselves. This can be especially useful in agricultural systems where they can achieve almost 100% parasitization rates of target pests (Davis 1964). As pollinators, phasiines contribute to the continued reproductive health of flowering plants and are thus important members of meadow habitats (Kearns 2001; Nihei and Schwarz 2011).

Although phasiines are ecologically important and can be quite abundant and diverse in the right habitat, this is the first attempt at an inventory of Phasiinae in GSMNP. There are currently five unofficial records of Phasiinae from the park (Nichols pers. com.) but actual phasiine diversity is shown herein to be much higher. In the Eastern US, there are 47 species of Phasiinae organized into 12 genera and 7 tribes (O'Hara & Wood 2004, plus the inclusion of *Strongygaster* Macquart). Some of these species (e.g., *Catharosia frontalis* Smith), are known from only from their type localities, but it is probable that a majority of eastern Nearctic phasiine species inhabit GSMNP.

Materials and Methods

Site Selection

Collections were made over a two year period (2013-2014) at two separate sites, one site per year: Purchase Knob (2013: NC: Haywood Co. ~4500 ft. /1370 m elevation) and Cades Cove, Hyatt Lane (2014: TN: Blount Co. ~1900 ft. /580 m elevation). These sites were chosen to maximize potential climatic variability in phasiine species, as an increase in elevation provides a similar change in species abundance and diversity as would an increase in latitude (Rahbek 1995). Both sites contained meadow habitats with abundant flowering plants that are attractive to phasiines (Apiaceae and Asteraceae) surrounded by forest and nearby streams. Because species diversity is greatest at the borders of habitats the collections at Cades Cove were made along a mown corridor through a meadow that borders Abrams Creek. This provided a natural flight way and helped concentrate the phasiines along the path. There were no such natural corridors at Purchase Knob so collections were made throughout the meadow and along the forest and road edge.

Collection

A Malaise trap was placed at each site and monitored 8-10 times over the fall collecting season (mid-August to mid-October). Because many phasiines exploit pentatomid bugs as hosts, the Malaise trap at Purchase Knob was baited with pheromones from *Euschistus* spp. (brown stink bug) and *Halyomorpha halys* (brown marmorated stink bug) purchased from AgBio (AgBio, Inc., Westminster, CO). The Malaise trap was operated for six weeks, the first three without pheromone lures and the last three with pheromone lures.

Malaise traps are incredibly useful for catching diverse species of flying insects, but unfortunately, phasiines are rarely caught in them- even if locally abundant. In addition to Malaise trapping, manual collecting of phasiines using an aerial sweep net was conducted during each visit to the selected sites to maximize species capture. Specimens were frozen, pinned, and taken to the Moulton Lab at the University of Tennessee for sorting and identification. During pinning, the three right legs of each specimen were removed and placed in 95% ethanol for potential molecular barcoding.

Identification

Phasiines belong to the diverse family Tachinidae, one of the largest and most morphologically variable families of flies. External morphology in the Tachinidae has only a limited ability to distinguish subfamilies, tribes, and even genera due to the widespread homoplasy of character traits throughout the family (Wood 1987). Identification keys to genera are by necessity long, cumbersome, and difficult to use for all but the most experienced dipterist. Although dichotomous keys may be unwieldy, the Nearctic genera of Phasiinae can be relatively easily recognized without keys by examining their general habitus, size, and coloration. Identifying specimens to species, however, remains a challenge.

Identifying species of Phasiinae required a thorough search of the literature to find the most recent keys and generic revisions, some of which are close to 100 years old. These papers were then examined for generic and species synonymies and updated, using O'Hara & Wood (2004) as a guide, to reflect the decades of taxonomic work since their publication. The following keys were used in this study for non-monotypic eastern Nearctic genera: *Cylindromyia* – Aldrich (1926); *Gymnosoma* and *Gymnoclytia* – Brooks (1946); *Leucostoma* – Reinhard (1956); *Phasia* – Sun & Marshall (2003); *Trichopoda* – Coquillett (1897); *Xanthomelanodes* –

Sabrosky (1950); and *Strongygaster* – Brooks (1942). The modified species keys to eastern Nearctic genera of Phasiinae used in this study are included in Appendix I. Specimens were first sorted by morphospecies, then keyed out using the literature listed above and assigned a tentative identification when possible. When dissection of the postabdomen was necessary (*Cylindromyia* and *Gymnosoma*), terminalia were cleared using lactic acid according to the protocols found in O’Hara (2002). Several specimens did not fit descriptions provided in the keys and were set aside as “unknowns”. Specimens were then selected from each morpho-group that would best encompass the variation found within the group (i.e. smallest/largest specimens, various localities). The previously removed ethanol-preserved legs from each corresponding specimen was then used for a DNA barcoding analysis. When possible, the gene sequences obtained were compared to sequences from known specimens that were named to species by tachinid experts (P. Cerretti, J.E. O’Hara, and/or J.O. Stireman). However, the inherent difficulty in identifying phasiines to species means that there was still some uncertainty even for these “known” species. The final species identification then, was only finalized when all three lines of evidence, when available, agreed on the same identification. In most cases, the groups of morphospecies aligned remarkably well with the molecular clades and, when present, the known specimens were recovered in their expected clades.

There are three main benefits to this thorough method of identification. First, confidence can be placed in the final species identification as it will have both morphological and molecular evidence supporting it. Second, this initial barcoding analysis will provide reference sequences for future researchers who can then quickly and cheaply identify phasiine species. Third, it has the potential to reveal cryptic species diversity, provide phylogenetic insight into each phasiine genus, and highlight areas that are in need of future research.

Barcoding

DNA barcoding was carried out on 59 morphologically diverse specimens from GSMNP and 13 specimens with known species identifications. Fifty-five other phasiine specimens from around the world were included to provide phylogenetic context and test species ranges. Approximately 900bp of the nuclear gene MCS were sequenced. This gene was chosen over the more traditional “barcoding gene” COI/II for this specific analysis due to the inconsistency of COI/II when used for distinguishing species of Diptera (Meier *et al.* 2006) and the much greater

phylogenetic informativeness of MCS (Winkler *et al.* 2015). Ideally, both genes would have been sequenced and our phylogeny created from multiple independent genetic lineages, but time prohibited us from such an analysis.

Phylogenetics

Genomic DNA was extracted with a ThermoScientific™ DNA extraction kit and amplified using the forward primers 446F (TTYGTNGGNTTYGCGYGARGT) and CYL2F (CARGGNGGNGTNATTACYTTYAA), and the reverse primer 838R (TGRTCDATRCADATCANNRCA) in a Scientis Inc. thermal cycler using the following reaction: 30s denaturation at 94°C; 5 cycles of 94°C for 30s, 56°C for 15s and 72°C for 1.5 min; 5 cycles of 94°C for 30s, 51°C for 15s and 72°C for 1.5 min; 30 cycles of 94°C for 30s, 46°C for 15s and 72°C for 1.5 min, and a final extension for 5 min at 72°C. Final sequences were obtained from the Molecular Biology Research Facility at the University of Tennessee. Chromatograms were inspected in Sequencher 5.3 and aligned using the MAFFT algorithm (Kato *et al.* 2005) through the online CIPRES portal (Miller *et al.* 2010). Phylogenetic relationships were reconstructed using the maximum likelihood program RAxML (Stamatakis 2014) and visualized in FigTree v1.4.2.

Results

Inventory (Tribes, Genera, Species)

Over a two year period (2013-2014), 518 tachinids were collected from GSMNP at Purchase Knob, NC and Cades Cove, TN. Two hundred and twenty-one of these belonged to the subfamily Phasiinae, representing 8 tribes, 12 genera, and 26 species (Table 3.1). Of these, 23 species are new to the park, five are new state records for Tennessee, and two are newly recorded from North Carolina. All 12 eastern Nearctic phasiine genera (O'Hara & Wood 2004, including *Strongygaster*) were collected in a relatively short time from only a few sites, indicating high biodiversity in the park. Two additional species, *Beskia aelops* Walker and *Epigrimyia*

Townsend sp., that have historically been classified in Phasiinae but are currently placed in the closely related subfamily Dexiinae were collected and are here included.

Table 6: Identification and specimen counts of Tachinidae (primarily Phasiinae) collected in GSMNP from 2013-2014.
PK= Purchase Knob, CC= Cades Cove.

<u>Subfamily Phasiinae</u>			<u>Net</u>		<u>Malaise</u>		Total	<u>New to:</u>			
Tribe	Genus	Species	PK	CC	PK	CC		GSMNP	NC	TN	
Catharosiini	<i>Catharosia</i>	<i>nebulosa</i> (Coquillett)			1		1	1			
Cylindromyiini	<i>Besseria</i>	<i>atra</i> Coquillett	1				1	1			
	<i>Cylindromyia</i>	<i>binotata</i> (Bigot)	6	11			17	1			
	<i>Cylindromyia</i>	<i>euchenor</i> (Walker)	2				2	1			
	<i>Cylindromyia</i>	<i>fumipennis</i> (Bigot)	1	1	2		4	1			
	<i>Cylindromyia</i>	<i>interrupta</i> (Meigen)		6			6	1			
	<i>Cylindromyia</i>	<i>nana</i> (Townsend)		1			1	1		1	
	<i>Hemyda</i>	<i>aurata</i> Robineau-Desvoidy	1				1				
Gymnosomatini	<i>Gymnoclytia</i>	<i>immaculata</i> (Macquart)		1			1	1			
	<i>Gymnoclytia</i>	<i>minuta</i> Brooks		10	2		12	1			
	<i>Gymnoclytia</i>	<i>occidua</i> (Walker)	14	19	44	1	78	1			
	<i>Gymnoclytia</i>	<i>unicolor</i> (Brooks)		2			2	1		1	
	<i>Gymnosoma</i> cf. <i>filiola</i>	Loew		1			1	1			
	<i>Gymnosoma</i>	<i>occidentale</i> Curran				1	1	1		1	
	<i>Gymnosoma</i> cf. <i>par</i>	Walker	21	9	1		31	1	1	1	
	<i>Euclytia</i>	<i>flava</i> (Townsend)	1				1	1	1		
Leucostomatini	<i>Leucostoma</i> cf. <i>acirostre</i>	Reinhard	1		1		2	1			
	<i>Leucostoma</i> cf. <i>simplex</i>	(Fallén)	1		1		2	1			
Phasiini	<i>Phasia</i> cf. <i>aurulans</i>	Meigen	1		1		2				
	<i>Phasia</i> cf. <i>purpurascens</i>	(Townsend)	1		1		2	1			
Strongygastrini	<i>Strongygaster</i>	<i>triangulifera</i> Loew	2	6	3	2	13				
Trichopodini	<i>Trichopoda</i>	<i>lanipes</i> (Fabricius)	1	2			3	1			
	<i>Trichopoda</i>	<i>pennipes</i> (Fabricius)	6	19		1	26				
	<i>Trichopoda</i>	<i>plumipes</i> (Fabricius)	1				1	1			
	<i>Xanthomelanodes</i>	<i>arcuatus</i> (Say)			1		1	1			
	<i>Xanthomelanodes</i>	<i>atripennis</i> (Say)		7			7				
<u>Subfamily Dexiinae</u>			<u>Net</u>		<u>Malaise</u>		Total	<u>New to:</u>			
Tribe	Genus	Species	PK	CC	PK	CC		GSMNP	NC	TN	
Epigrimyini	<i>Beskia</i>	<i>aelops</i> (Walker)		1			1	1		1	
	<i>Epigrimyia</i>	sp. Townsend	1				1	1			
Totals:	8	14	28	63	95	59	4	221	23	2	5

Purchase Knob displayed the most species diversity (20 species, 7 unique) compared to Cades Cove (14 species, 3 unique) but excluding the pheromone traps had less total specimens than Cades Cove (76 vs. 99). Many of the species collected at Purchase Knob were represented by only a single specimen. The most commonly encountered phasiines were *Gymnoclytia occidua* Walker (78 specimens), *Gymnosoma* Meigen cf. *par* Walker (31), *Trichopoda pennipes* Fabricius (26 specimens), and *Cylindromyia binotata* Bigot (17 specimens). These four species represent almost 70% of the phasiines collected. Sixteen species were represented by two or fewer specimens.

Barcoding

Phylogenetic trees provided corroborative evidence for preliminary morphospecies identifications and in some cases revealed additional species. In the tribe Cylindromyiini, 16 specimens from GSMNP representing four morpho-species yielded five distinct phylogenetic clades (Figure 3.1). Specimens from the extra clade were dissected and identified as *Cylindromyia euchenor*, a species that is externally indistinguishable from *C. binotata*. In the tribe Trichopodini, 11 specimens representing four morphospecies yielded only three phylogenetic species (Figure 3.2). *Trichopoda lanipes* Fabricius and *T. pennipes* form a single monophyletic clade rather than two independent evolutionary groups. In the Gymnosomatini there is significant morphological variation within species, therefore 32 specimens representing the morphological extremes of 12 potential morpho-species were included. These 12 different groups formed seven separate phylogenetic species (Figure 3.3). One *Gymnosoma* sp. remains unknown.

Pheromones

Most phasiines (158) were hand collected as they visited flowers, primarily *Solidago* spp. and *Daucus* spp., but the Malaise trap provided a unique opportunity to study pheromone lures. The aggregation pheromones from *Euschistus* Dallas spp. that were placed in the Malaise trap at Purchase Knob attracted 44 specimens of *Gymnoclytia occidua* over a three week period. No *Gymnoclytia* specimens were found in the Malaise trap during the first three weeks when the lures were not in use.

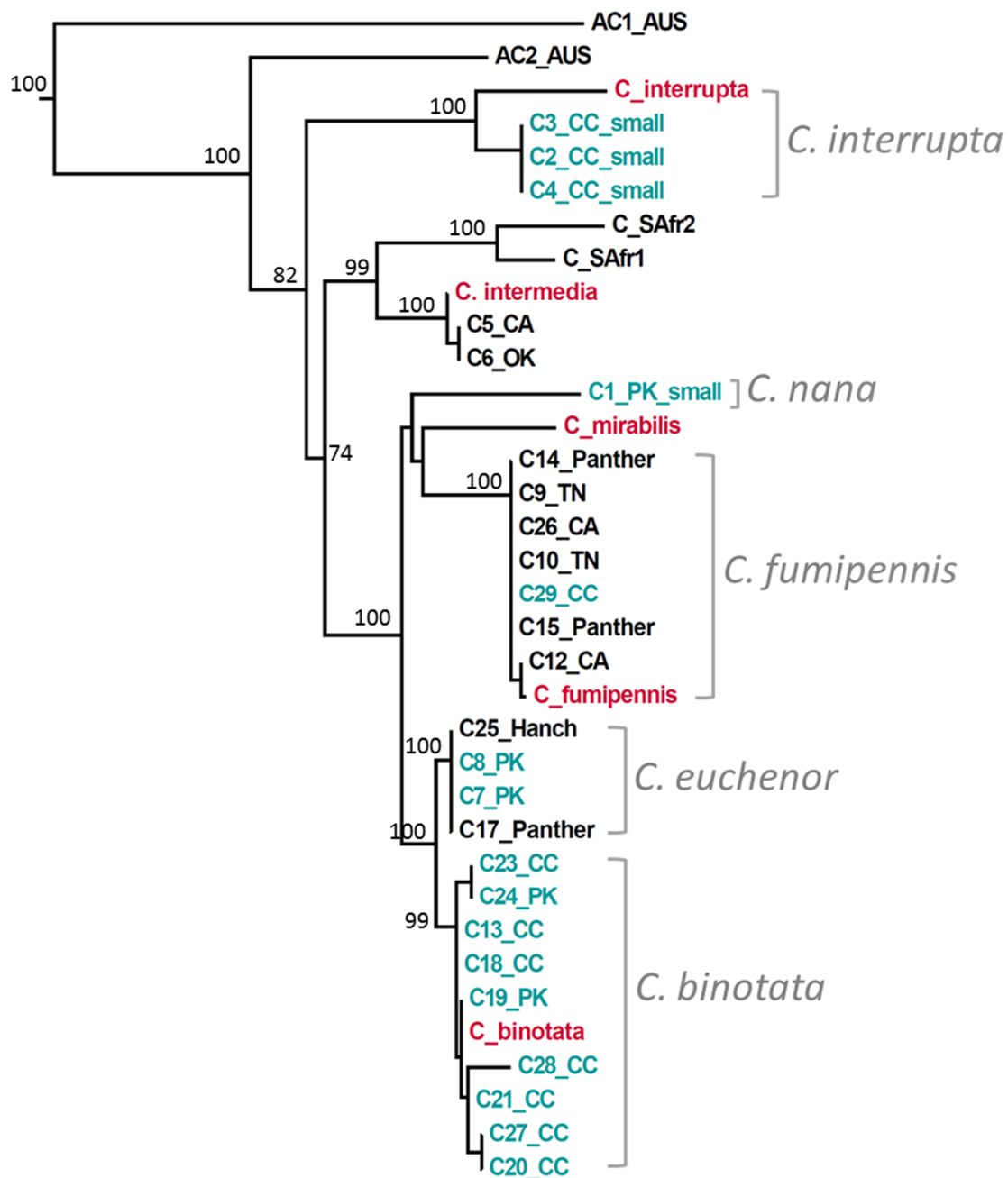


Figure 3.1: Maximum Likelihood tree of *Cylindromyia* species. Aqua = specimen from GSMNP; Red = specimens identified by other researchers. Bootstrap support is shown on significant nodes.

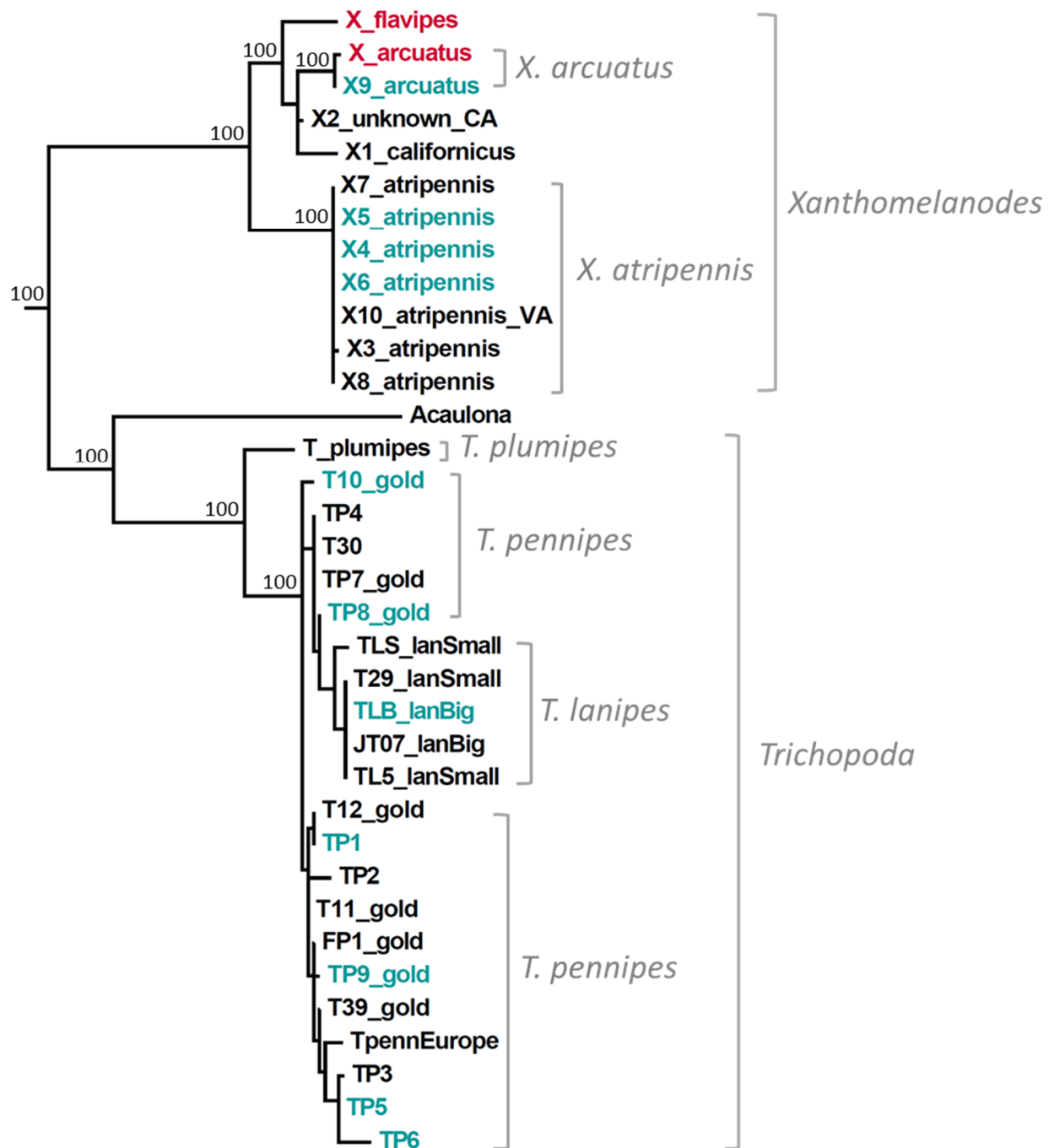


Figure 3.2: Maximum Likelihood tree of tribe Trichopodini. Aqua = specimen from GSMNP; Red = specimens identified by other researchers. Bootstrap support is shown on significant nodes.

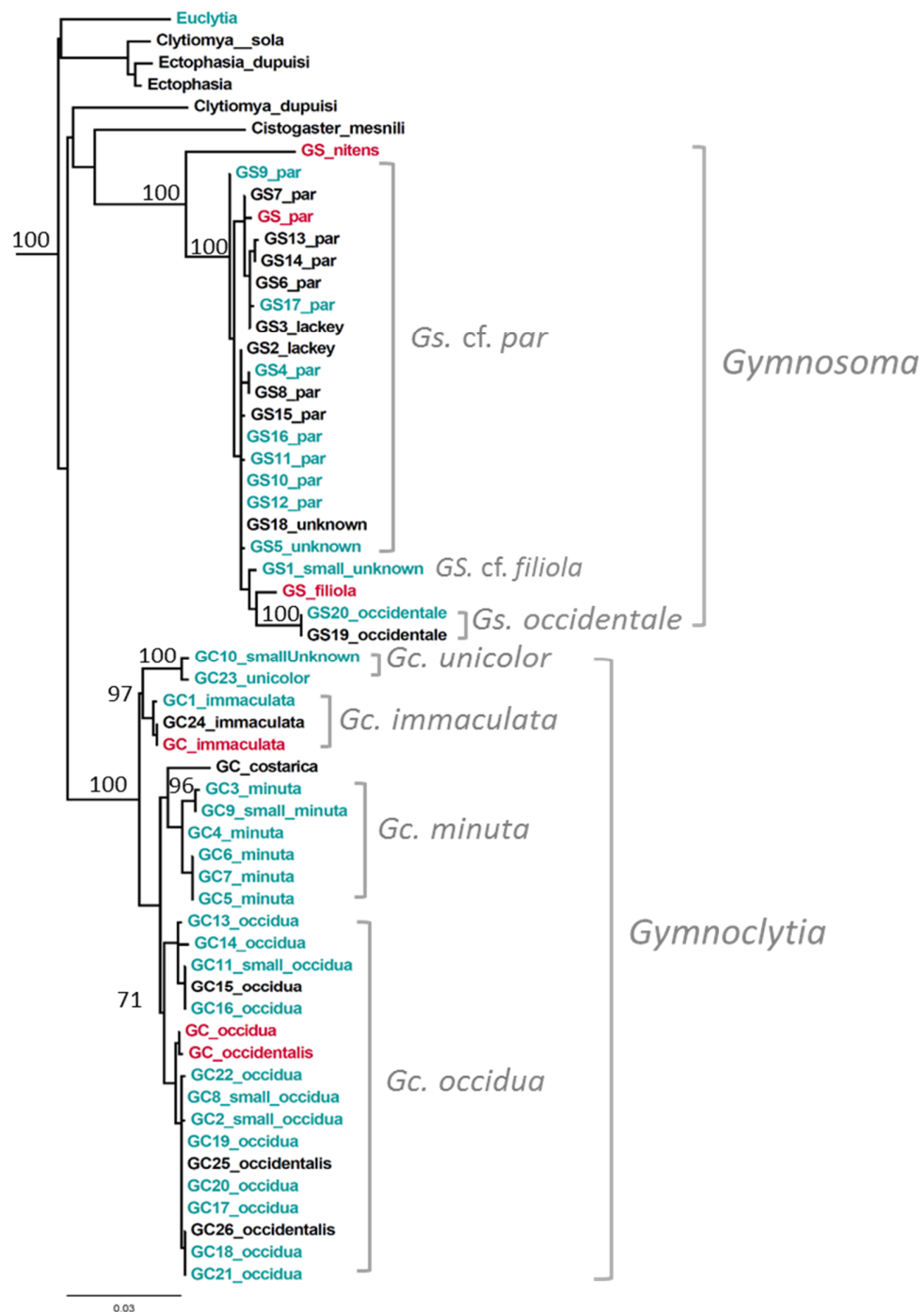


Figure 3.3: Maximum Likelihood tree of tribe Gymnosomatini. Aqua = specimen from GSMNP; Red = specimens identified by other researchers. Bootstrap support is shown on important nodes.

Discussion

Usefulness of barcoding

Even though the ATBI is over fifteen years in progress and nearly 19,000 species are currently recorded from the park, the Phasiinae are a group that has received little attention. From personal observation in this study, phasiines were found to be among the most abundant floral visitors outside of common hymenopterans in GSMNP. All tribes and over half of all known eastern Nearctic species of Phasiinae were found to inhabit the park. Such diversity accents their importance as pollinators in meadow ecosystems and highlights the diversity that is still undocumented in GSMNP. Because little research has been conducted on phasiine diversity in the park, almost all species discovered are new records for the park.

One of the primary reasons phasiines are often overlooked as research subjects is the difficulty of species identification. As with many insects, species keys are difficult to find, outdated, and are often unreliable. Additionally, many phasiines require dissecting genitalia for confident species placement—a difficult task for amateur entomologists. Accurate species identification is essential for future research in biodiversity, pollinator populations, or biological control of heteropteran crop pests. To that end, the available keys to phasiine species have been collected, their terminology updated, and will soon be available as an online photographic key. Another excellent resource for species identification is DNA barcoding which has the potential to accurately identify phasiine species while at the same time providing insight into evolutionary relationships and cryptic speciation.

This is the first attempt at creating a reference database specifically for phasiine DNA sequences. In total, 131 sequences for MCS will be uploaded to GenBank to serve as reference sequences for future researchers. By using a combined morphological and molecular species identification method, fewer specimens required dissection, and therefore remained undamaged, and several areas of future research were illuminated. For example, the *Trichopoda pennipes/lanipes* complex deserves further scrutiny into their apparent interbreeding populations. This is the first molecular evidence to show that these two species may not be as distinct as currently thought. Also, it is well known that *Gymnosoma* species are highly variable and difficult to differentiate (Zimin 1966). With further more comprehensive molecular analysis, many of the nominal species within *Gymnosoma* may be revealed to be genetically similar

enough to warrant synonymy. The phasiine barcodes provided here will aid future research into each species' evolutionary history and their relationship to other species.

The phylogenies generated during this study combined the phasiine species diversity of GSMNP with specimens collected personally from across the US or borrowed from other tachinid researchers from around the world (P. Cerretti, J.E. O'Hara, and J.O. Stireman). This resulted in trees that contained almost all known Nearctic species of *Gymnoclytia*, *Gymnosoma*, *Trichopoda*, and *Cylindromyia* and that revealed numerous interesting relationships among and between these species. These relationships deserve a more comprehensive analysis and discussion, however, the focus of this specific study is on identifying the species, not reworking their taxonomy. Consequently, details of specimen collections/vouchering of those species not from GSMNP and a thorough discussion of the phylogenetic relationships among Phasiinae is reserved for future papers and is not included here.

Collecting techniques

Hand collecting phasiines with an aerial net yielded more specimens and species diversity than the Malaise traps. However, one disadvantage of using a net is the overreliance on sight and dexterity to catch phasiines, many of which are small, dark, and easily missed. As a result, larger phasiines in the tribes Cylindromyiini, Gymnosomatini, and Trichopodini were disproportionately represented in this study (90%). Thirteen species were only caught by hand compared with three species that were found only in Malaise traps: *Catharosia nebulosa* Coquillett, *Gymnosoma occidentale* Curran, and *Xanthomelanodes arcuatus* Say. The Malaise traps were more successful at capturing the inconspicuous phasiines, like the species above, and those found in the tribes Phasiini and Leucostomatini.

The Phasiini and Leucostomatini are among the most common and speciose groups of phasiines, but only eight specimens representing four morpho-species were found during this study. There are undoubtedly more species of these tribes in GSMNP than were collected here. It may be possible to target these flies with yellow pan traps or random sweep netting through grass. Because of the lack of diversity and specimens collected, species identifications were difficult and remain "cf. species" until they can be compared with a larger museum collection of known species.

Pheromones

Pheromones for the stink bugs *Euschistus* spp. and *Halyomorpha halys* were placed in the Malaise trap at Purchase Knob to test phasiine attraction to these pheromones. *Gymnoclytia occidua* exhibited a strong attraction to the pheromones and numerous individuals were caught in the baited Malaise traps. As *Euschistus* is a known host for *Gymnoclytia* (Arnaud 1978), this pheromone was probably the predominant attractant. Unexpectedly, the vast majority of these specimens were male rather than female flies (42 males vs. 2 females). This unusual result is difficult to explain as these pheromones are host pheromones that are expected to draw in large numbers of gravid females (Krupke & Brunner 2003; Aldrich *et al.* 2006). Males seem to be as sensitive to host pheromones as females, if not more so (see Higaki and Adachi 2011), perhaps as a way to increase mating opportunities. Pheromones of other host species would increase Malaise trap yield and allow for research into pheromone strains of phasiine species.

An interesting observation was made during the collecting trip of September 17, 2014 at Cades Cove. A cluster of the invasive kudzu bug (*Megacopta cribraria*) was found on a goldenrod plant. Thirteen individuals were collected alive, fed with green beans, and monitored daily until they died. After four days, parasitic larvae had emerged from two specimens and pupated. Ten and eleven days later, adult flies emerged and were identified as the generalist phasiine parasitoid *Strongygaster triangulifera* Loew. Three other kudzu bugs that were dissected after eventual death had parasitic larvae in the abdominal cavity. This new host relationship was recently discovered in Alabama (Golec *et al.* 2013), and is here reported in Tennessee. This is a remarkable example of a native species quickly adapting to a new invasive host and provides encouragement that biological control of the damaging kudzu bug may be possible using native species.

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Appendix A: Identification Keys and Known Distributions of Eastern Nearctic Species of Phasiinae (Diptera: Tachinidae)

The following species keys were used to identify phasiines of eastern North America: *Cylindromyia*- Aldrich (1926); *Gymnosoma* and *Gymnoclytia*- Brooks (1946); *Leucostoma*- Reinhard (1956); *Phasia*- Sun & Marshall (2003); *Trichopoda*- Coquillett (1897); and *Xanthomelanodes*- Sabrosky (1950). The species names in these keys were cross-referenced against the most recent catalog of North American tachinids (O'Hara & Wood 2004, online revision 2013) and updated to reflect taxonomic revisions and species synonyms since they were first published. All species with entirely western Nearctic distributions were removed to create a more manageable sized geographic-specific key to eastern North American Phasiinae. Nomenclatural changes are summarized at the beginning of each genus in the key below.

Morphological terminology was updated to reflect the most recent terms in dipterology (Wood & Zumbado 2010; Cerretti *et al.* 2014; O'Hara 2015 pers. com.). A summary of significant vocabulary changes is as follows: sternopleuron/al = katepisternum/al; macrochaetae = bristles; anterior forceps (male) = surstyli; posterior forceps (male) = cerci; last genital segment (*Cylindromyia*, female) = T8; genital hooks (*Cylindromyia*, female) = hooks of T8; third antennal segment = postpedicel; apical cell = cell r4+5; pollen (pollenosity) = pruinescence; forceps (*Leucostoma*, female) = posterior clasping lobes; cheek groove = genal dilation; front = frons.

Tribes are presented alphabetically. Monotypic eastern Nearctic genera do not have individual keys. Distributions of each species can be found following the species keys. These were created by following the official distributions of these species through the literature listed above to the catalog of Sabrosky & Arnaud (1965) and double-checked in O'Hara & Wood (2004). The distribution maps are essentially a visual representation of the states and ranges listed in the above literature with syntax maintained. New state records for Tennessee and North Carolina are included and verified with a literature search for the species name and the state.

Each of these genera is in need of modern revision both morphologically and molecularly. This key is not meant to be such a revision but merely provides a workable identification key for general entomologists and a foundation for future taxonomic research.

Tribe Catharosiini
Genus *Catharosia* Rondani, 1868

Known distribution of *Catharosia* spp. in America north of Mexico found in Figures 3.4-5.

Tribe Cyldromyiini
Genus *Besseria* Robineau-Desvoidy, 1830

Known distribution of *Besseria* spp. in America north of Mexico found in Figure 3.6.

Genus *Hemyda* Robineau-Desvoidy, 1830

Known distribution of *Hemyda* spp. in America north of Mexico found in Figure 3.7.

Genus *Cylindromyia* Meigen, 1803

Adapted from Aldrich (1926) including *C. dosiades* (= *C. interrupta*), *C. nigra* (= *C. propusilla*), *C. argentea* (= *C. binotata*), and *C. vulgaris* (= *C. fumipennis*). Some species can be identified with external morphology, but most require dissecting the genitalia. Known distribution of *Cylindromyia* spp. in America north of Mexico found in Figures 3.8-16.

- 1) Scutellum with only *one* pair of bristles. Abdomen with discal bristles.....
.....Subgenus *Neocyptera interrupta*
 - Scutellum with *two* pairs of bristles, a small crossed apical pair and another much larger pair not far from them.....2
 - Scutellum with *three* pairs of bristles, the additional pair being near the base at the sides..3
- 2) Abdomen *without red* on sides, wholly black. Front basitarsus shorter than the four following joints.....*propusilla*
 - Abdomen *broadly red* on sides. Female with distinct, symmetrical hooks on T8 which are placed quite far apart. Male with the front basitarsus as long as the following four joints and concave on outer side. Hind tibia villous on inner side.....Subgenus *Calocyptera intermedia*

- 3) Males.....4
- Females.....8
- 4) Cerci *long* and soft, basal half swollen, suddenly reduced at middle, the apical portion reaching almost to tips of surstyli.....*binotata*
- Cerci *short* and greatly swollen, soft and cushion-like but suddenly contracted into a small apical beak. Sternite 6 with a wide, shallow emargination in the middle.....*euchenor*
- Cerci not as described.....5
- 5) Cerci and surstyli yellow, shining, *alike in consistency*, cerci chitinized. Both small and somewhat concealed by the thin yellow expanded margin of T8.....Subgenus *Apinocyptera nana*
- Cerci *much less* chitinized than surstyli which are hard and shining.....6
- 6) Surstyli *broad* apically, broader than at the base, subtruncate.....*armata*
- Surstyli *tapering* apically.....7
- 7) Cerci *short* and in profile rather thick, but not strikingly swollen, about half as long as surstyli which are *grooved* behind at the bend. Back of head with some *black* hair on each side of the occipital region above.....*decora*
- Cerci more than *two-thirds as long* as surstyli which are *not grooved* behind at the bend. Back of head with only *pale* hair inside the orbital row.....*fumipennis*
- 8) Third abdominal segment ventrally with *large crescent shaped area of short stubby spines* on a prominence behind which is a concavity. Terminal hooks of T8 *unsymmetrical* on only one present.....*nana*

- Third abdominal segment ventrally *lacking* such spiny protuberances. Terminal hooks of T8 *symmetrical*.....9
- 9) The hooks of T8 attached below a *square upper apical shoulder* of the segment.....10
 - T8 *without* such shoulder, sloping down to the hook.....12
- 10) Only *two* katapisternal bristles.....*decora*
 - *Three* katapisternal bristles.....11
- 11) Hooks of T8 pointing decidedly forward *gradually turned upward*.....*armata*
 - Hooks of T8 *turned upward from base* standing close to shoulder the tips divergent.....*euchenor*
- 12) Usually with *less than six* bristles on fifth abdominal tergite segment. Hooks of T8 small.....*binotata*
 - With *more than six* bristles on fifth abdominal tergite. Hooks of T8 large.....*fumipennis*

Tribe Gymnosomatini

- 1) *With* marginal bristles on abdomen.....*Euclytia*
 - *Without* marginal bristles on abdomen.....2
- 2) Postpedicel *elongated*, nearly as long as face. *Without* crossed apical scutellar bristles.....*Gymnosoma*

- Postpedicel *not elongated*, almost oval in shape. With crossed apical scutellar bristles.....*Gymnoclytia*

Genus *Gymnoclytia* Brauer & Bergenstamm, 1893

Adapted from Brooks (1946) as *Gymnoclytia* (*G. minuta* and *G. occidua*), *Procistogaster* (*P. immaculata* and *P. unicolor*), and *Siphopallasia* (*S. dubia*). Known distribution of *Gymnoclytia* spp. in America north of Mexico found in Figures 3.17-21.

- 1) Cell r4+5 *narrowly open, closed, or with a short petiole* which is not longer than the length of the small crossvein. When present, the petiole is bent forward in line with M1. One katepisternal bristle.....2
 - Cell r4+5 *closed with a long petiole* distinctly longer than small crossvein, bent forward but not in line with M1.....3
- 2) Species about 4mm. Cell r4+5 *closed* in wing margin. Parafrontal of female with one row of hairs outside the frontal row.....*minuta*
 - Species 6-7mm. Cell r4+5 *open, closed, or with a short petiole* in line with M1. Parafrontal of female with two or more rows of hairs outside the frontal row.....*occidua*
- 3) Antennae and palpi *wholly black*. Lower facial margin strongly and abruptly warped forward beyond antennal prominence. One katepisternal bristle.....*dubia*
 - Antennae and palpi largely *reddish*. Lower facial margin not warped beyond antennal prominence. Two katepisternal bristles.....4
- 4) Male.....5
 - Female.....6

- 5) Postpedicel *deep black on apical two thirds*. Abdomen wholly reddish with golden or greyish pruinescence.....*unicolor*
- Postpedicel reddish (dorsal anterior edge sometimes black). Abdomen reddish or with black markings, pruinescence grey/yellow-grey.....*immaculata*
- 6) Postpedicel *deep black* except at base. Abdomen wholly reddish. Genital segments black. Pruinescence golden or greyish.....*unicolor*
- Postpedicel *reddish*, dark apically and dorsally. Abdomen partly or wholly black. Pruinescence greyish.....*immaculata*

Genus *Gymnosoma* Meigen, 1803

Adapted from Brooks (1946) as *Rhodogyne* (*R. occidentalis*, *R. canadensis*, *R. filiola*, *R. fuliginosa*, and *R. par*). Different keys are provided for males and females. Identification relies almost exclusively on genitalia characters. Known distribution of *Gymnosoma* spp. in America north of Mexico found in Figures 3.22-26.

Males

- 1) Species <5mm. Abdomen red with isolated black spots, rarely connected. Surstyli long and narrow, strongly curved dorsally.....*occidentale*
- Species >5mm. Surstyli not as above.....2
- 2) Surstyli large and bulky, broadly triangular with a blunt tip, convex to flat dorsally. Abdomen red with small connected spots. 7mm.....*canadense*
- Surstyli concave to flat dorsally, short triangular or elongate and parallel-sided.....3
- 3) Surstyli long and nearly parallel-sided with a rounded-truncate tip that is slightly turned up. Abdominal spots small and rounded, never connected. 5.5-6.5 mm.....*filiola*

- Surstyli short triangular.....4

4) Surstyli very broad, short triangular with rounded tip. Abdominal spots rarely connected. 7mm...
.....*fuliginosum*

- Surstyli narrower, acute. Abdominal spots usually connected. 5.5-6mm.....*par*

Females

1) Species <5mm. Sternite 8 not triangular, sparsely haired.....*occidentale*

- Species >5mm.....2

2) Cerci very large, curved up at the tips. Sternite 8 large and triangular, evenly haired on the margin. Sternite 9 abruptly constricted before the tip. Abdomen black with at most the narrow lateral edges reddish.....*canadense*

- Cerci not curved up at the tips. Sternite 9 not constricted. Abdomen generally red with black spots or triangles.....3

3) Sternite 8 not triangular, evenly haired on the margin. Abdomen red with small, isolated black spots.....*filiola*

- Sternite 8 triangular and with a bunch of heavier bristles at the apex of the triangle.....4

4) Tergites 6 and 7 mostly black, sternite 9 short and broad, rounded acute. Abdominal spots broadly triangular and widely connected. 5.5-6mm.....*par*

- Tergites 6 and 7 mostly red. Sternite 9 longer, equibroad or widened towards the tip. Abdominal spot small, separated or only narrowly connected. 7mm.....*fuliginosum*

Genus *Euclytia* Townsend, 1908

Known distribution of *Euclytia* spp. in America north of Mexico found in Figure 3.27.

Tribe Leucostomatini **Genus *Leucostoma* Meigen, 1803**

Adapted from Reinhard (1956). Different keys are provided for males and females. Females are easily distinguished from males by the easy to spot posterior clasping lobes at the end of the abdomen. Known distribution of *Leucostoma* spp. in America north of Mexico found in Figures 3.28-31.

Males

- 1) Abdomen *without* pruinescence, wholly shining. Palpi yellow, postpedicel tinged with red.....*simplex*
 - Abdomen *with* one or more segments pollinose.....2
- 2) *Abdominal pruinescence confined to basal half or less of last three segments*. Hairs on intermediate abdominal segments appressed. Head pruinescence silvery. Surstyli tapering sharply to middle leaving a slender curved beak which is as long as broader basal part...*acirostre*
 - Abdominal pruinescence not as described above.....3
- 3) *Fore tibia with two posterolateral bristles*. Frontal vitta wider than parafrontal which is lightly dusted with gray pruinescence on a blackish subshiny background. Last three abdominal segments thinly gray pollinose. Calypters not much enlarged.....*gravipes*
 - *Fore tibia with one median posterolateral bristle*. Two katepisternal bristles. Abdomen narrowed to apex, last two segments silvery pollinose. Surstyli rather narrow, gradually tapering from base to apex and transversely rounded or convex on hind side.....*aterrimum*

Females

- 1) *Abdomen longer and narrower than usual*, tapering gradually from base to apex. Three katepisternal bristles. Arms of posterior clasping lobes strongly bowed inward and bearing a series of *minute teeth* on ventral edge on inner margin.....*aterrimum*
 - *Abdomen ordinary in form*, segments two and three much broader than long.....2
- 2) Abdomen with silvery pruinescence on narrow basal margin of intermediate segments. Calypters moderately enlarged, opaque white. Posterior clasping lobes slender, strongly bowed inward and with *only fine hairs* on inner margin.....*acirostre*
 - Abdomen without pruinescence, wholly shining black.....3
- 3) Fore tibia with *two* posterolateral bristles. Calypters not enlarged. Posterior clasping lobes strongly bowed inward with a series of *stubby dentations* on inner ventral edge.....*gravipes*
 - Fore tibia with *only one* median posterolateral bristle. Calypters enlarged. Posterior clasping lobes *denticulate* on inner margin.....*simplex*

Tribe Phasiini Genus *Phasia* Latreille, 1804

Adapted from Sun & Marshall (2003). Species are first keyed to species-group than keys to eastern Nearctic members of each species-groups are provided. Known distribution of *Phasia* spp. in America north of Mexico found in Figures 3.32-41.

- 1) Fronto-orbital plate *bare* laterally. Lower margin of face weakly to moderately prominent. Sublunular bulla usually not developed. Subscutellum evenly rounded, not extended beyond the scutellar apex. Male without scale-like setae.....*Phasia pusilla* species-group
 - Fronto-orbital plate *haired* laterally.....2

2) Fronto-orbital plate *densely haired*, haired area sometimes reaching eye or nearly so..
***Phasia hemiptera* species-group**

– Fronto-orbital plate with only a *few rows of hairs*, usually less than 3-4; never reaching eye..
***Phasia subcoleoprata* species-group**

***Phasia pusilla* species-group**

1) Hind tibia *strongly arched*, *shorter* than hind femur. Presutural supraalar seta absent. Sternite 6 of female *extremely long*, longer than the total length of the previous sternites.....*punctigera*

– Hind tibia *straight or slightly arched*, as *long* as hind femur. Presutural supraalar seta usually present; sternite 6 of female *shorter* than the total length of other sternites.....2

2) Male- dorsum of abdomen *with* a purple shining area. Female- abdomen greyish *yellow* pruinose, hair spots *very distinct*. Sternite 7 *long*. Frontal vitta less than 1.5 times as wide as Fronto-orbital plate anteriorly.....*purpurascens*

– Male- dorsum of abdomen *without* purple shining area. Surstylus ball-like. Female- abdomen greyish *white* pruinose, hair spots *indistinct*. Sternite 7 *short*.....*aldrichii*

***Phasia hemiptera* species-group** (different keys are provided for males and females)

Males

1) Mesoscutum with a distinct *golden* pruinose spot, at least on the median postsutural part; tergite 5 *with* V-shaped golden pruinosity posterolaterally. Lower margin of face *strongly projecting*, pruinose spot usually limited to postsutural scutum; surstylus straight; distiphallus swollen, not branched.....*aurulans*

– Mesoscutum *evenly* pruinose, or with vitta-like pattern; tergite 5 *without* such pruinosity...2

- 2) *Two or three* katapisternal bristles.....3
- *One or fewer* katapisternal bristles.....4
- 3) M1 meeting R4+5 at *acute* angle. Syncercus deeply notched posteriorly.....*grandis*
- M1 meeting R4+5 *almost at right angle*. Syncercus not or shallowly notched posteriorly...
.....*diversa* (in part)
- 4) Phallus *not haired*; abdomen always uniformly thinly grey pruinose except syntergite 1+2.....*diversa* (in part)
- Phallus *haired*; tergites and terminalia not as above.....5
- 5) *Abdominal tergites clearly transversely grey pruinose*; Fronto-orbital plate yellow pruinose; wing not enlarged.....*subopaca*
- *Abdominal tergites shining*, or not pruinose as above; Fronto-orbital plate grey or yellow pruinose; wing enlarged or not. Ventrolateral process of distiphallus bent, and hook-like.....*robertsonii*

Females

- 1) Sternite 7 bent, *apex directed ventrally*. Ovipositor bent, apex slightly or strongly directed ventrally.....*aurulans*
- Sternite 7 *straight, or bent but apex directed dorsally*.....2
- 2) Posterior margin of sternite 7 *rounded or linear* in ventral view. Sternite 7 short and wide, boat-like.....*diversa*
- Posterior margin of sternite 7 *pointed* in ventral view.....3

- 3) Two katepisternal bristles. Lower margin of face perpendicular. Gena greyish yellow pruinose. Sternite 7 bent upward gradually.....*grandis*
- One katepisternal bristle. Lower margin of face strongly projecting, visible in profile.....4
- 4) Abdomen *silvery* pruinose, always *with* black longitudinal vitta; abdomen with black transverse vittae posteriorly (at least tergite 3); sternite 7 thin, triangular, apex pointed.....*subopaca*
- Abdomen *black, shining purple, or grey* (or yellowish grey) pruinose; if pruinosity present, black longitudinal vitta and transverse vitta *absent* or indistinct. Sternite 7 tapered.....*robertsonii*

***Phasia subcoleoprata* species-group**

- 1) One katepisternal bristle. Tibia and usually femora *yellow*, at least tibia. Thorax and abdomen greyish yellow pruinose. Female- Sternite 7 abruptly bent ventrally.....*fenestrata*
- Two or more katepisternal bristles. Legs *black*. Female- lower margin of face perpendicular, nor projecting.....*chilensis*

Tribe Strongygastrini
Genus *Strongygaster* Macquart, 1834

Adapted from Brooks (1942) as *Clistomorpha* (*C. didyma*) and *Hyalomyodes* (*H. robusta* and *H. triangulifera*). Known distribution of *Strongygaster* spp. in America north of Mexico found in Figures 3.42-44.

- 1) Cell r4+5 ending in wing margin or extremely short petiolate.....*didyma*
- Cell r4+5 long petiolate.....2

- 2) *Parafacials broad* (more than 0.5 clypeal length); lower facial margin broadly triangular and warped forward. First abdominal segment *with* pruinescence. Third arisal segment abruptly enlarged at the base.....*robusta*
- *Parafacials narrow* (0.2 to 0.25 as wide as distance between vibrissae); lower facial margin not distinct; First abdominal segment *without* pruinescence.....*triangulifera*

Tribe Trichopodini **Genus *Trichopoda* Berthold, 1827**

Modified from Coquillett (1897) including *T. cilipes* (= *T. pennipes*) and *T. formosa* (= *T. lanipes*). Known distribution of *Trichopoda* spp. in America north of Mexico found in Figures 3.45-47.

- 1) *Abdomen black with golden pruinose spots*.....*plumipes*
- Abdomen variable, orange to dark brown. *Without* spotted golden pruinosity.....2
- 2) Post sutural markings *golden*, usually a “w” in shape (three longitudinal lines, one transverse), often with similar markings on mesoscutum.....*pennipes*
- Post sutural markings *silver*, almost always in “u” shape (two longitudinal lines, one transverse).....*lanipes*

Genus *Xanthomelanodes* Townsend, 1893

Modified from Sabrosky (1950). Known distribution of *Xanthomelanodes* spp. in America north of Mexico found in Figures 3.48-50.

- 1) Legs and antennae predominantly *orange*.....*flavipes*

- Legs and antennae predominantly *black*.....2
- 2) Abdominal segments with distinct black markings on dorsal surface, last two-three segments completely black, usually with three katepisternal bristles. Frons relatively narrow, avg. 0.21 times the width of the head.....*arcuatus*
- Abdominal segments sometimes with light black markings on dorsal surface, last two-three segments rarely completely black, usually with two katepisternal bristles. Frons broader, avg. 0.25 times the width of the head.....*atripennis*

Appendix B: Figures



Figure 3.4: Known distribution of *Catharosia frontalis* Smith, 1917.— Massachusetts.

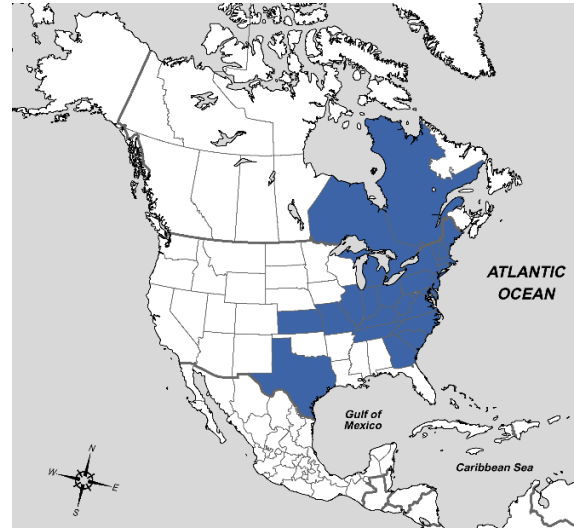


Figure 3.6: Known distribution of *Besseria atra* Coquillett, 1897.— Québec and Maine to Kansas and Georgia, also Texas, Ontario.

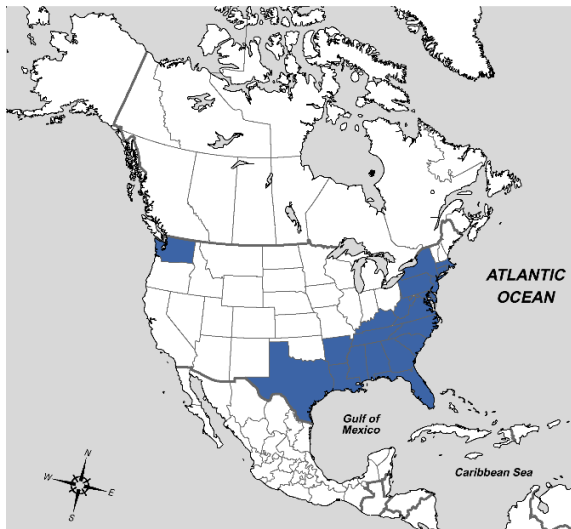


Figure 3.5: Known distribution of *Catharosia nebulosa* Coquillett, 1897.— Massachusetts to Texas and Florida, Washington.

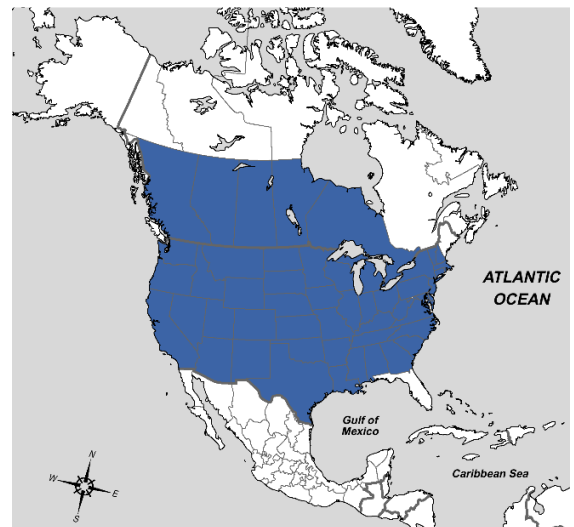


Figure 3.7: Known distribution of *Hemyda aurata* Robineau-Desvoidy, 1830.— British Columbia to New Hampshire, south to California, Mexico, and Georgia.

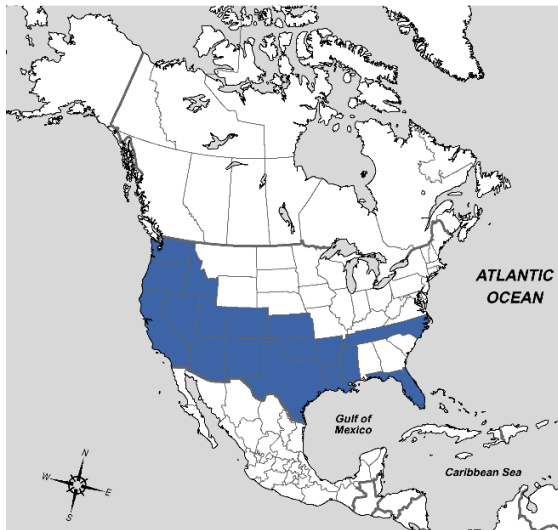


Figure 3.8: Known distribution of *Cyindromyia nana* Townsend, 1915.— Washington to Kansas, south to California, Mexico, and Mississippi, also North Carolina, Florida, and Tennessee (new record).

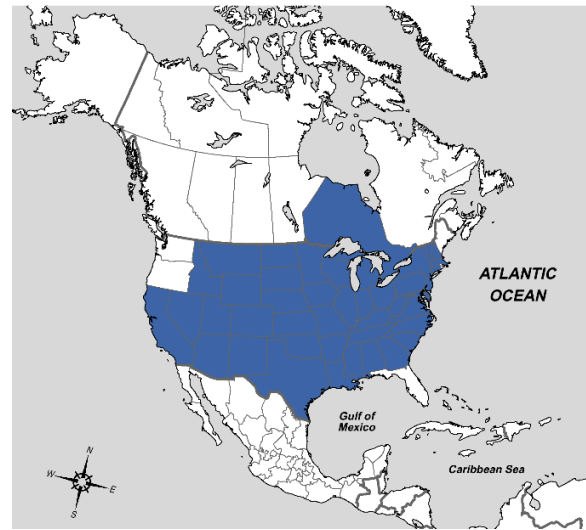


Figure 3.10: Known distribution of *Cyindromyia armata* Aldrich, 1926.— Montana, California to Kansas and New Mexico, Michigan, Massachusetts to Georgia, Texas, Jamaica, Arizona, Idaho, South Dakota, Ontario, New Hampshire.

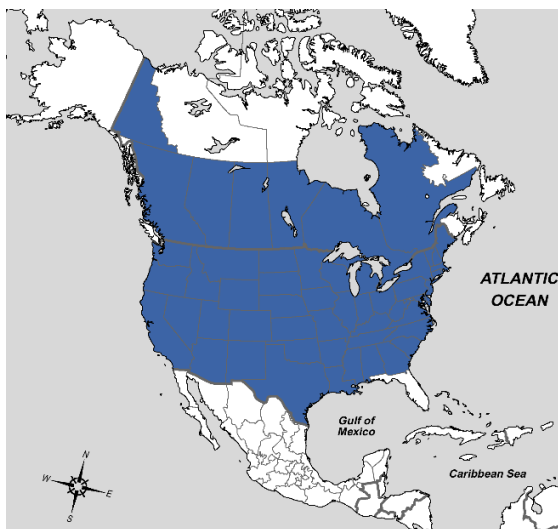


Figure 3.9: Known distribution of *Cyindromyia intermedia* Meigen, 1824.— Palearctic, British Columbia to Québec and Maine, south to California, Texas, South Carolina, Yukon.

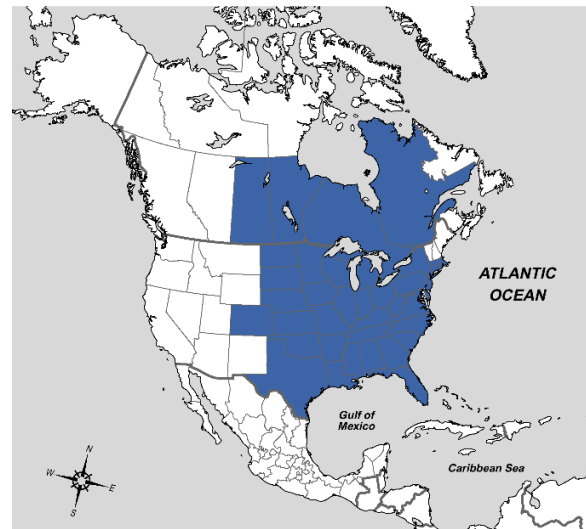


Figure 3.11: Known distribution of *Cyindromyia binotata* Bigot, 1878.— Manitoba, Colorado and South Dakota to New York, south to Texas and Florida, Saskatchewan, Ontario, Québec, Massachusetts.

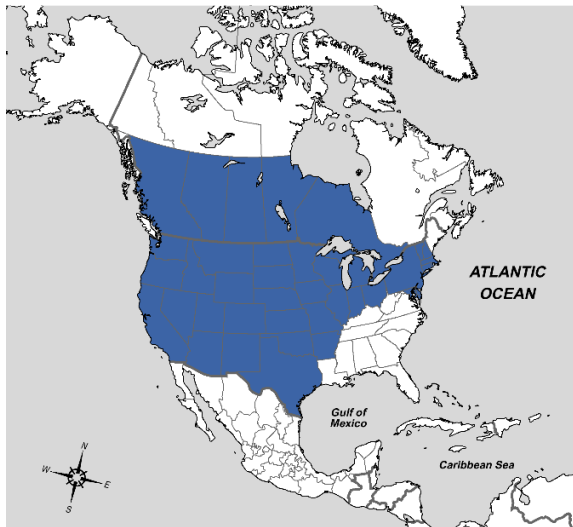


Figure 3.12: Known distribution of *Cyindromyia decora* Aldrich, 1926.– British Columbia to New Hampshire, Maryland, south to California, Texas, Mexico.

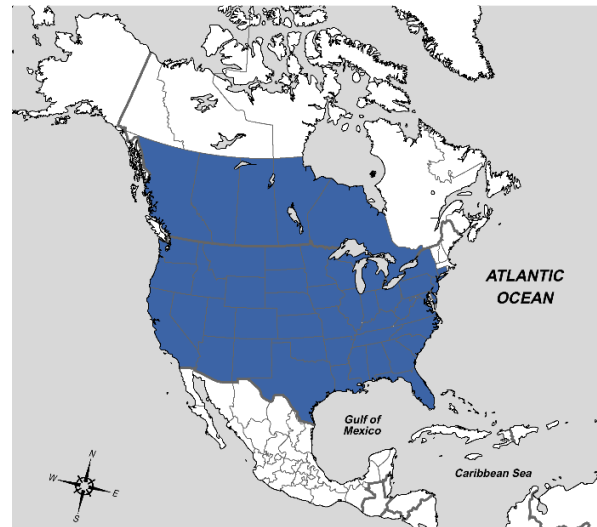


Figure 3.14: Known distribution of *Cyindromyia fumipennis* Bigot, 1878.– British Columbia to Connecticut, south to California and Florida, Mexico.

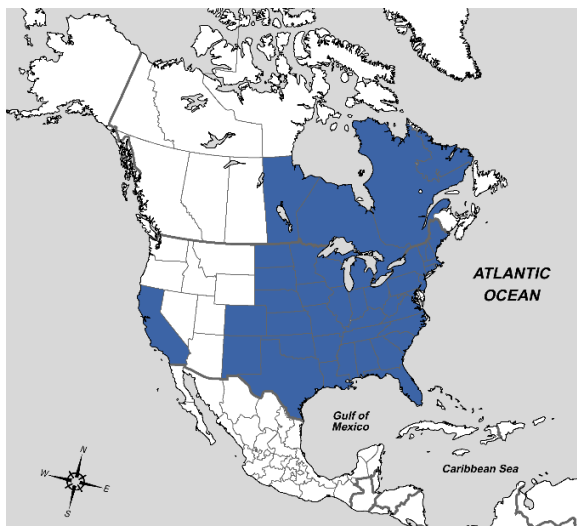


Figure 3.13: Known distribution of *Cyindromyia euchenor* Walker, 1849.– Minnesota to Newfoundland, south to New Mexico and Florida, also California, Mexico, Manitoba, North Dakota.



Figure 3.15: Known distribution of *Cyindromyia propusilla* Sabrosky & Arnaud, 1965.– Wisconsin to Massachusetts, south to Virginia, also Mexico, Texas, Florida, Ontario, Québec, New Hampshire, Kentucky.

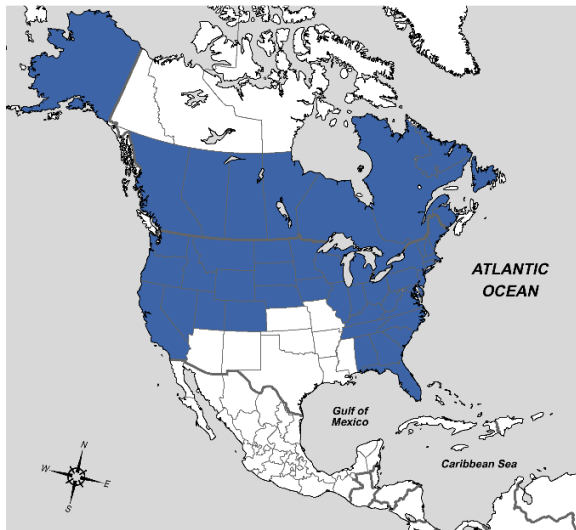


Figure 3.1613: Known distribution of *Cylindromyia interrupta* Meigen, 1824.— Palearctic, Yukon, British Columbia, Wyoming east to New Brunswick, south to Iowa and Florida, California, Nevada, Utah, Colorado, Nova Scotia.

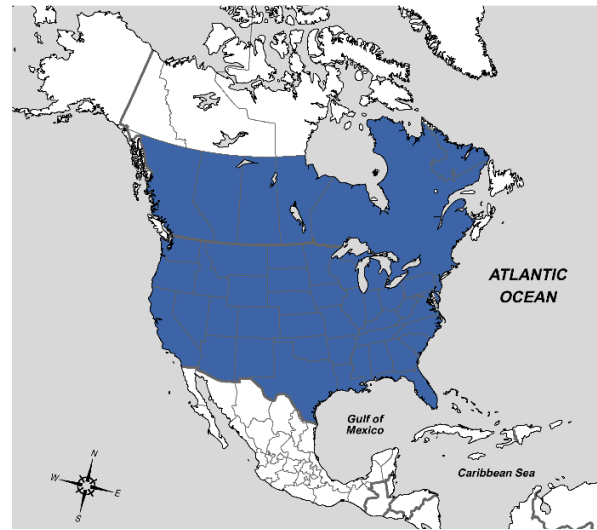


Figure 3.18: Known distribution of *Gymnoclytia immaculata* Macquart, 1844.— British Columbia to Québec, entire United States, Mexico.

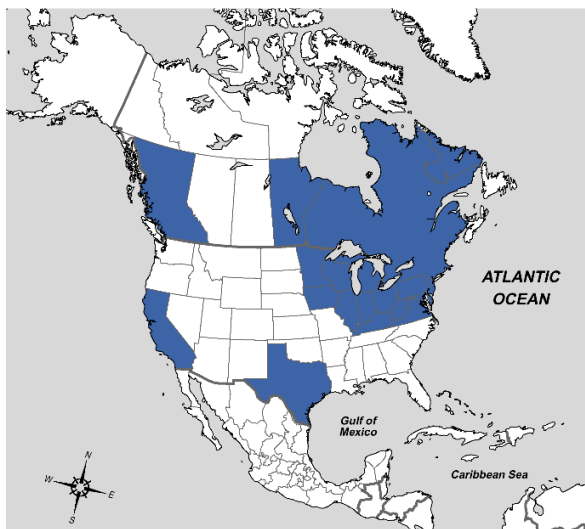


Figure 3.17: Known distribution of *Gymnoclytia dubia* West, 1925.— Manitoba to Nova Scotia, south to Virginia, also ?California, British Columbia, Texas.

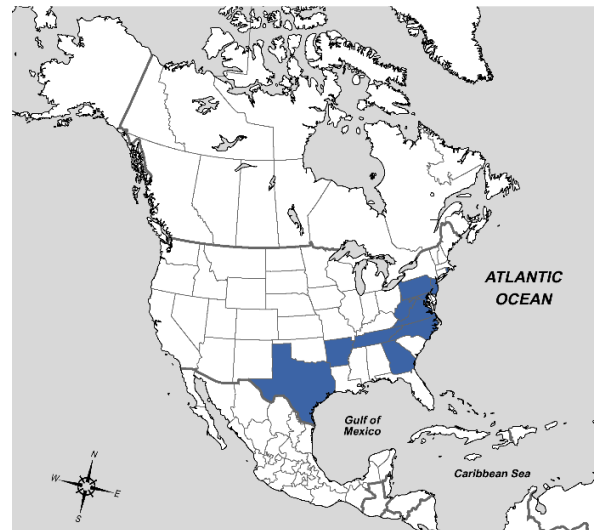


Figure 3.19: Known distribution of *Gymnoclytia minuta* Brooks, 1946.— New Jersey, District of Columbia, Virginia, Texas, Arkansas, Tennessee, Georgia, North Carolina, Rhode Island.

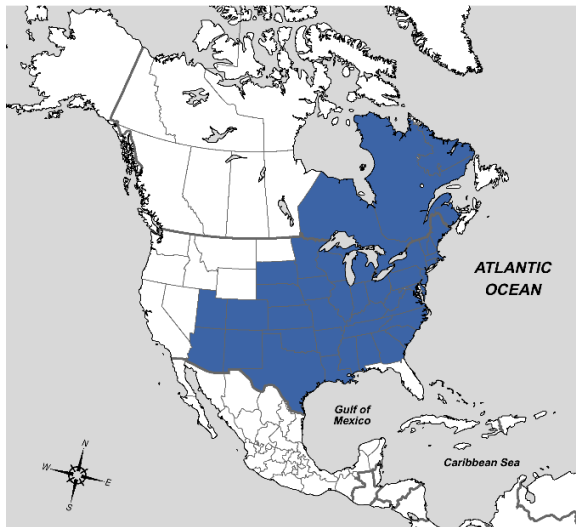


Figure 3.20: Known distribution of *Gymnoclytia occidua* Walker, 1849.— Michigan to Nova Scotia, southwest to Arizona, Mexico, and Georgia, Illinois to Virginia, Texas.

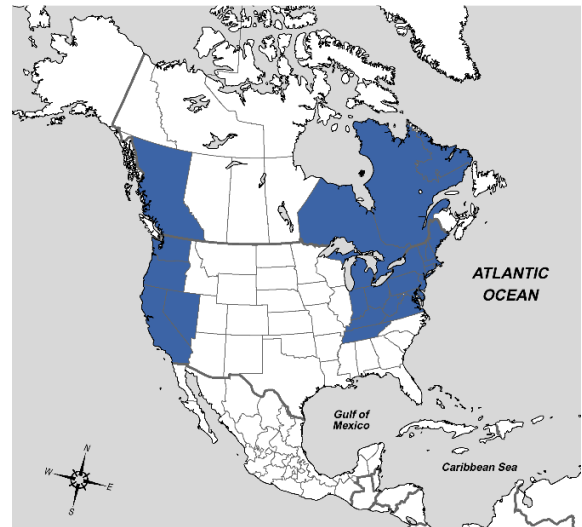


Figure 3.22: Known distribution of *Gymnosoma canadense* Brooks, 1946.— Michigan to Québec and Nova Scotia, south to North Carolina, also British Columbia to Nevada, California, Tennessee.

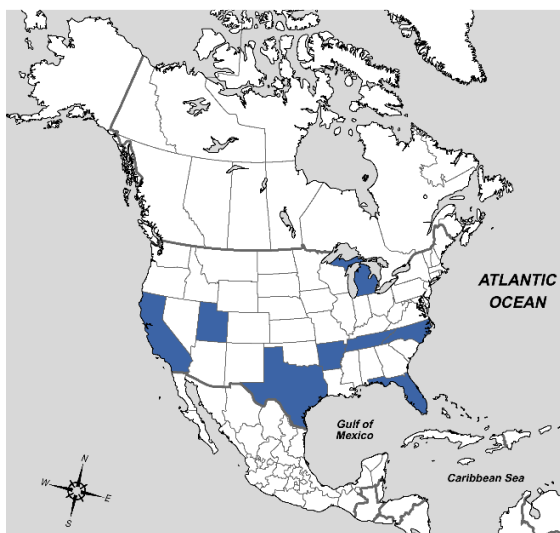


Figure 3.21: Known distribution of *Gymnoclytia unicolor* Brooks, 1946.— California, Utah, Texas, Arkansas, North Carolina, Florida, Michigan, and Tennessee (new record).

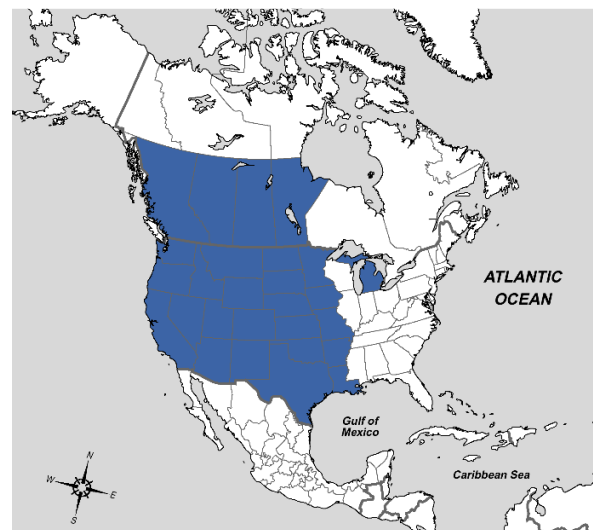


Figure 3.23: Known distribution of *Gymnosoma filiola* Loew, 1872.— British Columbia to Manitoba, south to California and Louisiana, also Michigan.



Figure 3.24: Known distribution of *Gymnosoma fuliginosum* Robineau-Desvoidy, 1830.– British Columbia to New Hampshire, south to California, Mexico, and South Carolina.

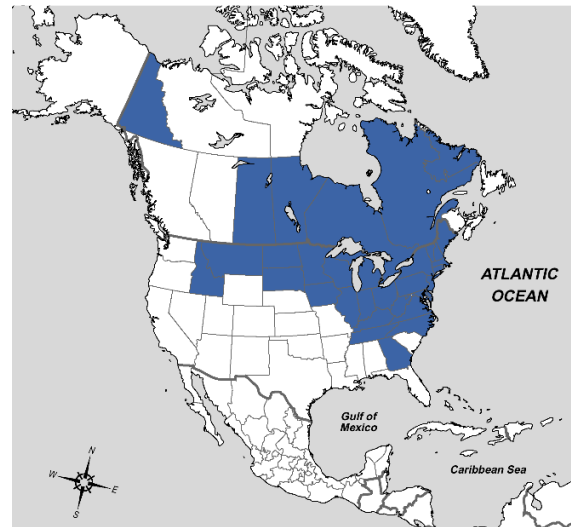


Figure 3.26: Known distribution of *Gymnosoma par* Walker, 1849.– Idaho, Saskatchewan to Nova Scotia, south to Virginia, also Georgia, Yukon, Tennessee (new record, and North Carolina (new record).

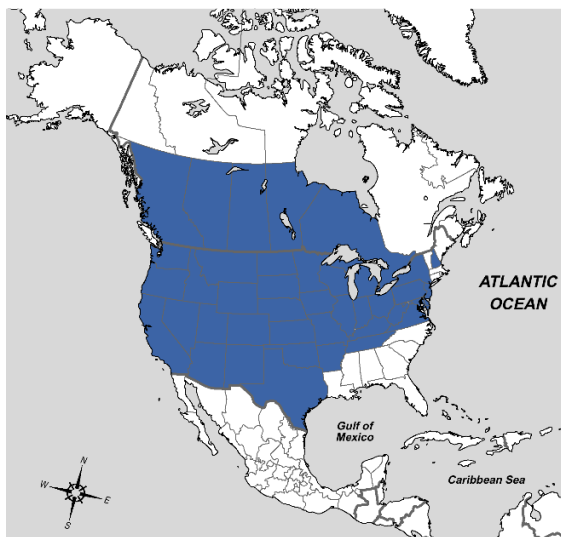


Figure 3.25: Known distribution of *Gymnosoma occidentale* Curran, 1927.– British Columbia to Ontario, south to California, Texas, and Virginia, New Hampshire, and Tennessee (new record).

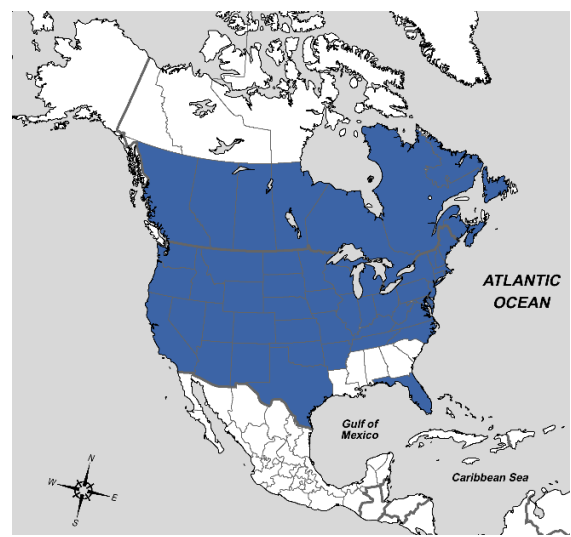


Figure 3.27: Known distribution of *Euclytia flava* Townsend, 1891.– British Columbia, California to Maine, south to Texas and Virginia, Alberta to Nova Scotia, Labrador, also Utah, Arizona, New Mexico, Florida, and North Carolina (new record).

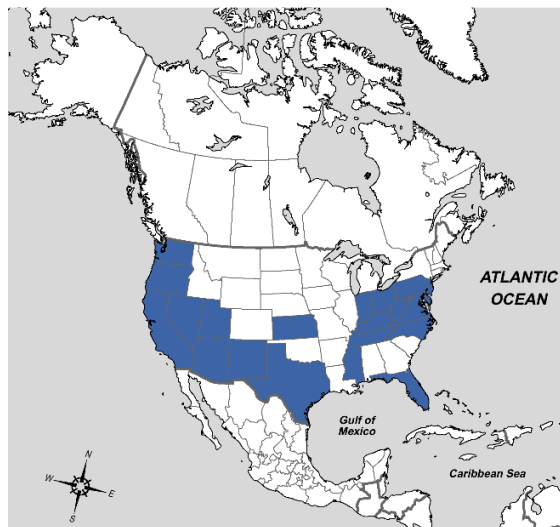


Figure 3.28: Known distribution of *Leucostoma acirostre* Reinhard, 1956.– Washington to California and Texas, also Indiana to New Jersey and Virginia, Mississippi, Florida, ?Kansas, ?Tennessee.

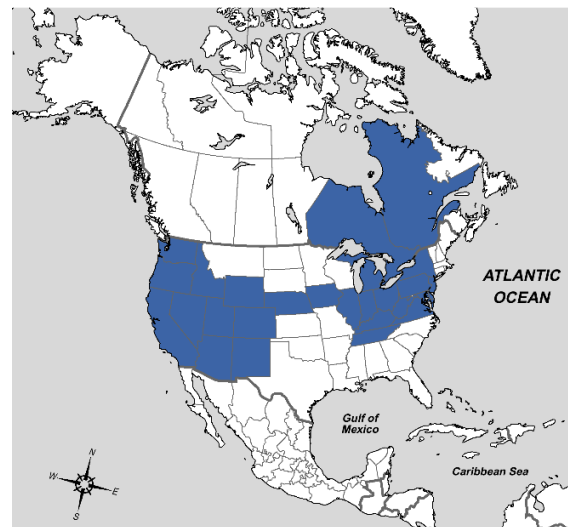


Figure 3.30: Known distribution of *Leucostoma gravipes* van der Wulp, 1890.– Washington to Nebraska, south to California, Mexico, and Texas, also Michigan to New York, south to Virginia, Iowa, Illinois, Tennessee, Ontario, Québec.

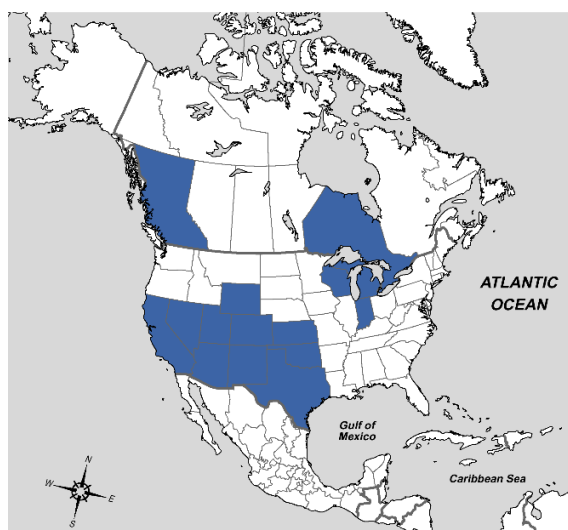


Figure 3.29: Known distribution of *Leucostoma aterrimum* Villers, 1789.– Europe, California to Wyoming and Kansas, south to Mexico and Texas, also Wisconsin, Michigan, Indiana, British Columbia, Ontario.

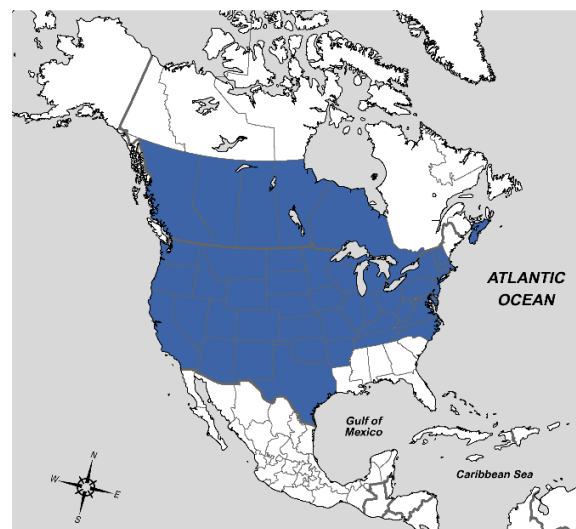


Figure 3.31: Known distribution of *Leucostoma simplex* Fallén, 1815.– Palearctic, British Columbia to New Hampshire, south to California, Texas, and Virginia, Prince Edward Island.

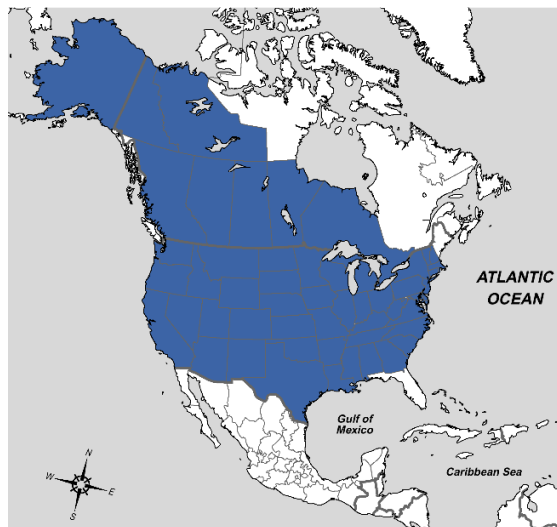


Figure 3.32: Known distribution of *Phasia aldrichii* Townsend, 1891.– Alaska, Northwest Territories, British Columbia to New Hampshire, south to California, Mexico and Georgia, also Hungary, Russia, Kazakhstan and Mongolia.

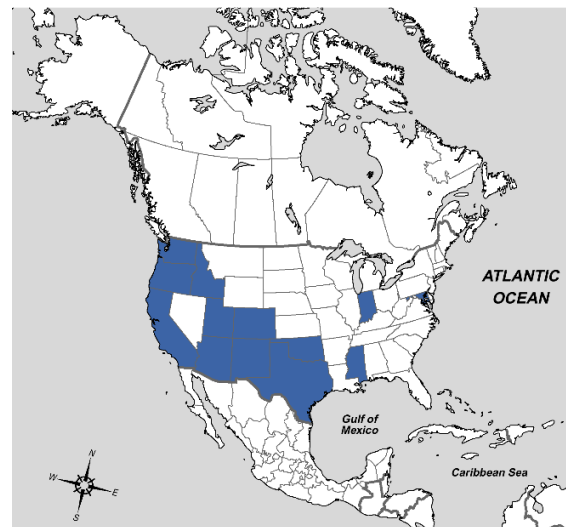


Figure 3.34: Known distribution of *Phasia chilensis* Macquart, 1851.– Washington, Idaho, Oregon, California, Utah, Arizona, Colorado, New Mexico, Oklahoma, Texas, Mississippi, Indiana, District of Columbia, Maryland, Mexico, South America.

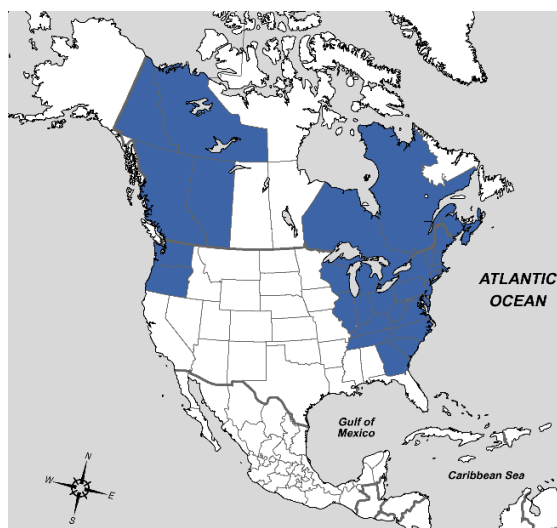


Figure 3.33: Known distribution of *Phasia aurulans* Meigen, 1824.– Ontario to Nova Scotia, south to Tennessee and Georgia, also Northwest Territories, British Columbia, Alberta, Washington, Oregon

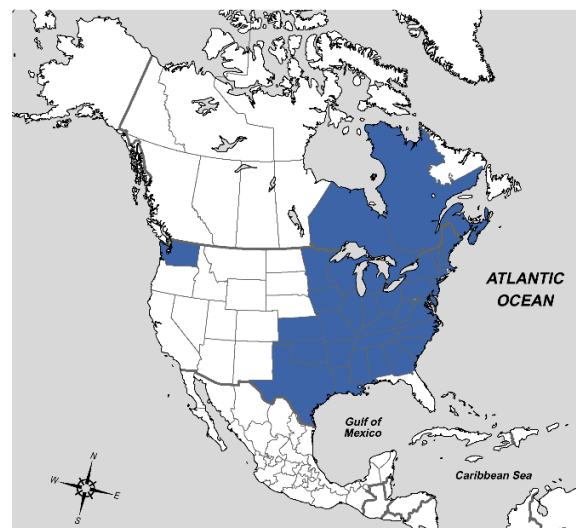


Figure 3.35: Known distribution of *Phasia diversa* Coquillett, 1897.– Ontario to Nova Scotia, south to Texas and Georgia, also Washington.

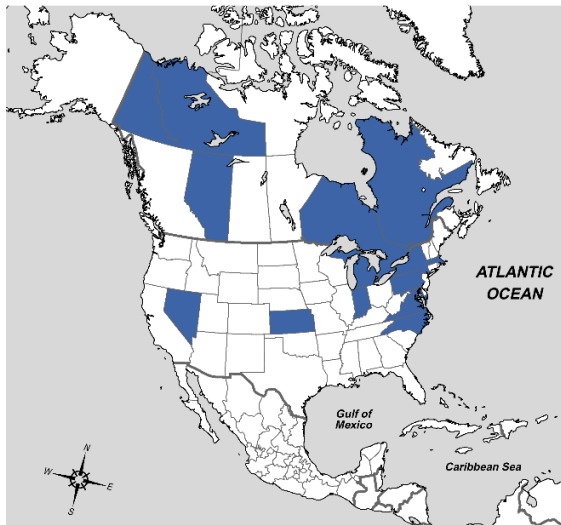


Figure 3.36: Known distribution of *Phasia fenestrata* Bigot, 1889.— Northwest Territories, Alberta, Nevada, Kansas, Indiana, Michigan, Ontario, Québec, New York, Massachusetts, Pennsylvania, District of Columbia, Virginia, North Carolina.

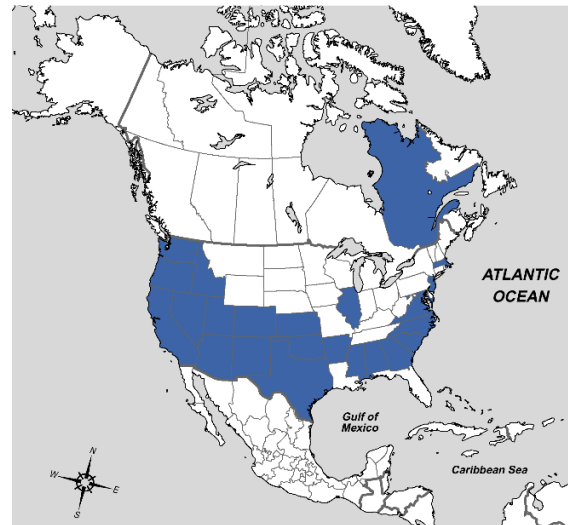


Figure 3.38: Known distribution of *Phasia punctigera* Townsend, 1891.— Washington, Idaho, Oregon, California, Nevada, Utah, Arizona, Colorado, New Mexico, Kansas, Oklahoma, Texas, Arkansas, Mississippi, Indiana, Québec, Massachusetts, New Jersey, District of Columbia, Maryland, Virginia, North Carolina, South Carolina, Alabama, Georgia, Mexico.

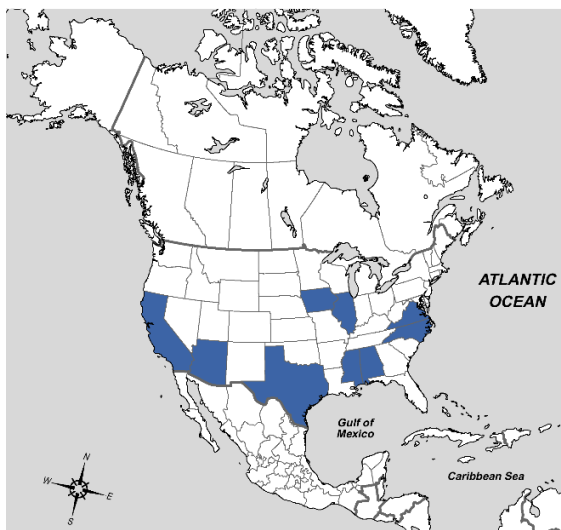


Figure 3.37: Known distribution of *Phasia grandis* Coquillett, 1897.— California, Arizona, Texas, Mississippi, Alabama, Iowa, Illinois, Virginia, North Carolina.

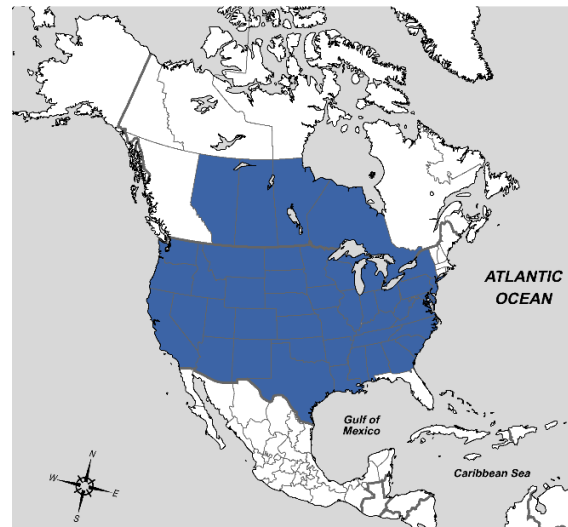


Figure 3.39: Known distribution of *Phasia purpurascens* Townsend, 1891.— Washington and Alberta to Ontario and New York, south to California and Georgia.



Figure 3.40: Known distribution of *Phasia robertsonii* Townsend, 1891.— Nebraska, Minnesota and Ontario east to Nova Scotia, south to Oklahoma, Mississippi and Florida.

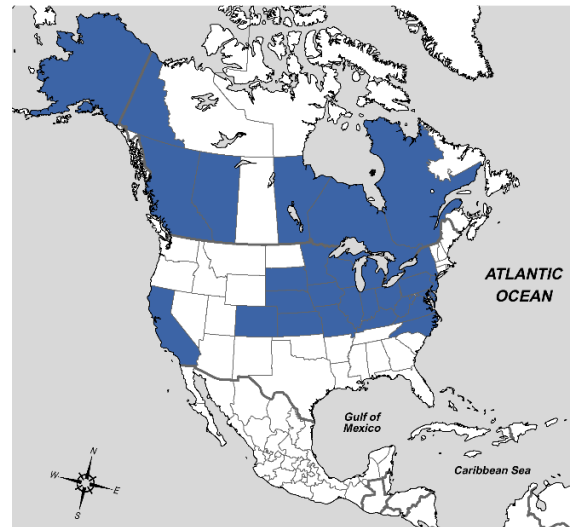


Figure 3.42: Known distribution of *Strongygaster didyma* Loew, 1863.— California, Alberta, South Dakota to New York, south to Colorado and Virginia, Alaska, Yukon, British Columbia, Manitoba, Ontario, Québec.

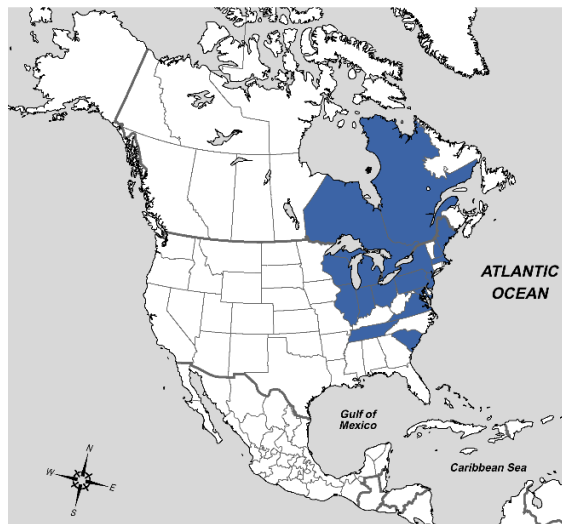


Figure 3.41: Known distribution of *Phasia subopaca* Coquillett, 1897.— Ontario, Wisconsin, Illinois, Indiana, Michigan, Ohio, Québec, Maine, New Hampshire, New York, Massachusetts, Pennsylvania, New Jersey, Maryland, Virginia, Tennessee, South Carolina.

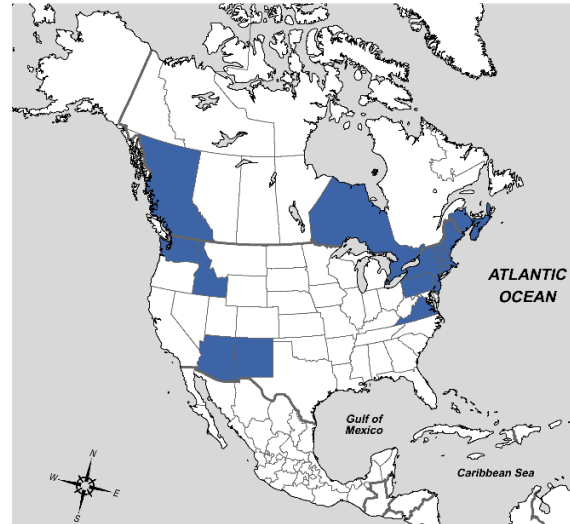


Figure 3.43: Known distribution of *Strongygaster robusta* Townsend, 1908.— British Columbia, Washington, Idaho, Arizona, New Mexico, Nova Scotia to Pennsylvania, Ontario, New Brunswick, North Carolina.

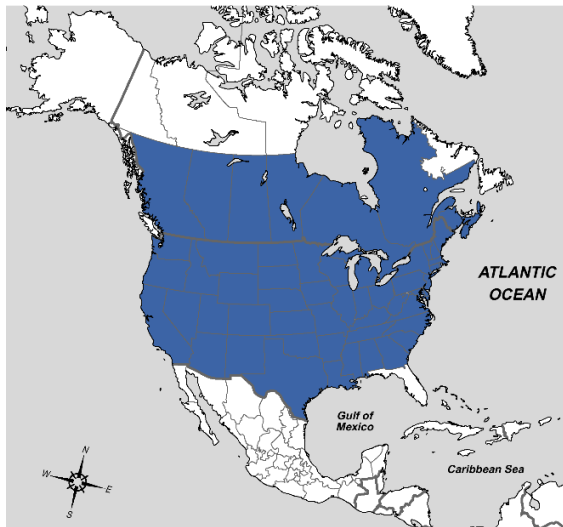


Figure 3.44: Known distribution of *Strongygaster triangulifera* Loew, 1863.— British Columbia to Nova Scotia, south to California, Mexico, and Georgia.

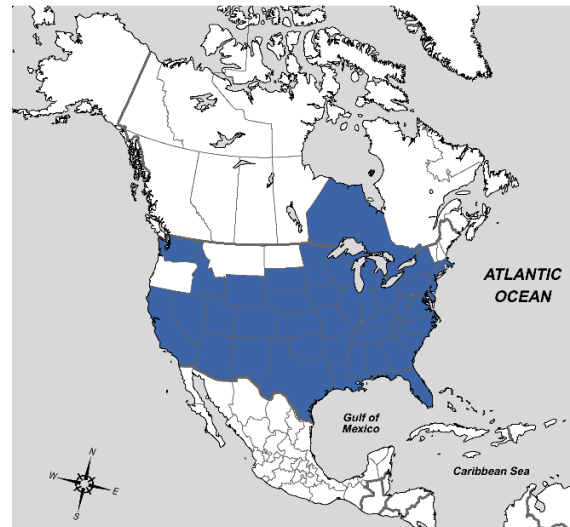


Figure 3.46: Known distribution of *Trichopoda pennipes* Fabricius, 1781.— Washington (introduced), California to Ontario and Massachusetts, south to Mexico and Florida, Idaho.



Figure 3.45: Known distribution of *Trichopoda lanipes* Fabricius, 1805.— Kansas to Connecticut, south to Mexico and Florida, Iowa, Ontario.



Figure 3.47: Known distribution of *Trichopoda plumipes* Fabricius, 1805.— Kansas to Connecticut, south to Texas and Florida, Ontario.

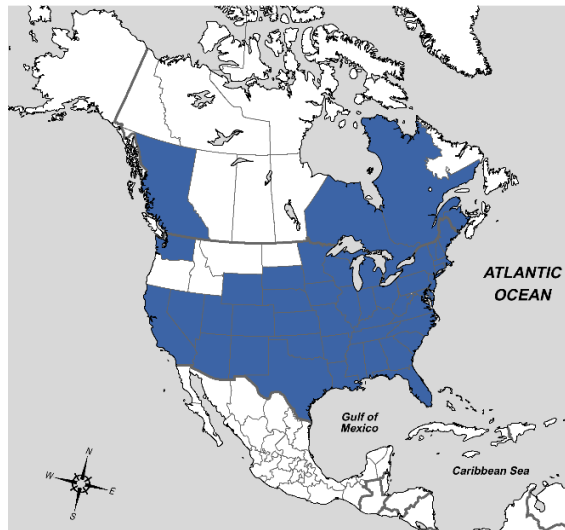


Figure 3.48: Known distribution of *Xanthomelanodes arcuatus* Say, 1829.— Washington, California to Wisconsin to New Hampshire, south to Mexico and Florida, Ontario to New Brunswick and Maine, also British Columbia.

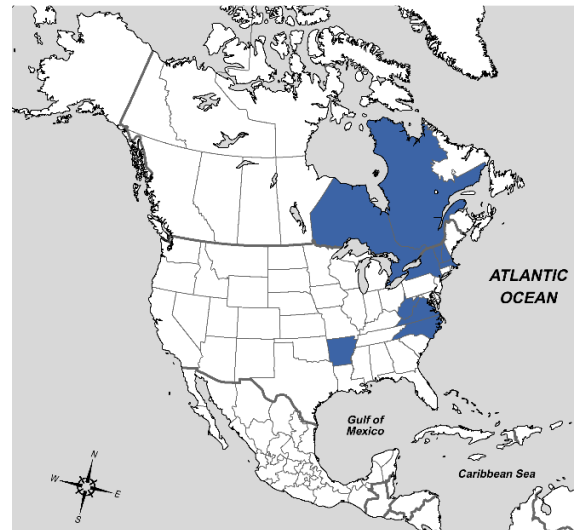


Figure 3.50: Known distribution of *Xanthomelanodes flavipes* Coquillett, 1897.— Québec, New York, Vermont, Massachusetts, Virginia, Arkansas, Ontario, New Hampshire, West Virginia, Virginia.



Figure 3.49: Known distribution of *Xanthomelanodes atripennis* Say, 1829.— Michigan to Vermont, south to Arkansas and Florida, also ?Mexico.

Conclusions

In Chapter I, the nuclear coding genes CAD, LGL, MCS, and MAC were used to reconstruct phylogenetic relationships within the subfamily Phasiinae. In total, 128 worldwide taxa (80 genera) and approximately 7.6 kilobases of nuclear data were used to estimate a phylogeny of Phasiinae. The resultant phylogeny was robustly supported throughout, with Maximum Likelihood and Bayesian analyses returning identical trees. Tachinidae was recovered as monophyletic, as were the four subfamilies therein (Phasiinae, Dexiinae, Exoristinae, Tachininae). Dexiinae was recovered sister to Phasiinae. Phasiinae is represented by 12 tribes, all robustly supported, including Strongygastrini, Parerigonini, and Imitomyiini but excluding Eutherini, Epigrimyiini, and Litophasia, which were recovered as members of Dexiinae.

Ancestral state reconstruction revealed a single synapomorphy of Phasiinae: an elongated hypandrium in the male postabdomen. No universal trait was found to define Dexiinae, but the following characters used in combination can identify the “dexiine-type” male terminalia: platform pregonites and a membranous and hinged connection of the distiphallus to the basiphallus. Oviparity was reconstructed as the ancestral oviposition strategy within the Tachinidae, supporting earlier morphological phylogenetics. The ancestral host of the Phasiinae + Dexiinae clade was strongly recovered as Heteroptera, while the ancestral host of Tachinidae was weakly recovered as Lepidoptera. A piercer, derived from the 8th or 10th sternite, is hypothesized to have evolved independently in at least ten different lineages within the Dexiinae and Phasiinae.

Chapter II compared historical hypotheses about phasiine taxonomy to the newly generated molecular phylogeny. As a result, the Phasiini were divided between four tribes: Phasiini, Gymnosomatini, Opsiini (resurrected tribe), and Xystini (resurrected tribe). Similarly, the Parerigonini were split into three lineages: Parerigonini, Cylindromyiini, and Zitini (new tribe). The tribe Trichopodini is considered a subclade of the Gymnosomatini (Trichopodina). The subfamily Phasiinae is composed of 14 tribes, 100 genera, and 597 species.

The focus of Chapter III was on the diversity of Phasiinae in Great Smoky Mountains National Park. In total, 221 specimens representing 26 phasiine species were collected. Of these, 21 species were newly recorded from the park, four were new records for Tennessee, and two were new records for North Carolina. All 12 eastern Nearctic phasiine genera were represented.

Updated identification keys to eastern Phasiinae and 131 DNA barcoding sequences of phasiine species were created to aid future research into phasiine identification and systematics.

Vita

Jeremy Daniel Blaschke was born in Santa Fe, New Mexico and spent many hours of his childhood collecting insects in his southwestern backyard. Jeremy was home-educated throughout high school and after graduating in 2006 left the plains and deserts of New Mexico for the hills and forests of Tennessee where he attended Bryan College in Dayton. Jeremy earned a Bachelor of Science degree in Biology with a minor in Origins Research and graduated *cum laude* in 2010. After college, Jeremy worked at an environmental testing company in Knoxville, TN as a chemical analyst for eighteen months. In 2011 he accepted a position in the Entomology and Plant Pathology department at the University of Tennessee to study molecular systematics under the mentoring of J.K. Moulton. Jeremy earned his Master's degree in Entomology in August 2013. Jeremy remained in the Moulton lab for his PhD, and focused on a molecular analysis of the phylogeny and evolution of Phasiinae (Diptera: Tachinidae) as well as a survey of phasiines of Great Smoky Mountain National Park. Jeremy has accepted a position as Assistant Professor of Biology at Union University (Jackson, TN) and expects to receive his PhD in August 2015.