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Editor's Choice Article

Molecular phylogeny and evolution of world Tachinidae (Diptera)

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ABSTRACT

We reconstructed phylogenetic relationships within the diverse parasitoid fly family Tachinidae using four nuclear loci (7800 bp) and including an exceptionally large sample of more than 500 taxa from around the world. The position of the earthworm-parasitizing Polleniinae (Calliphoridae *s.l.*) as sister to Tachinidae is strongly supported. Our analyses recovered each of the four tachinid subfamilies and most recognized tribes, with some important exceptions in the Dexiinae and Tachininae. Most notably, the tachinine tribes Macquartiini and Myiophasiini form a clade sister to all other Tachinidae, and a clade of Palpostomatini is reconstructed as sister to Dexiinae + Phasiinae. Although most nodes are well-supported, relationships within several lineages that appear to have undergone rapid episodes of diversification (basal Dexiinae and Tachininae, Blondeliini) were poorly resolved. Reconstructions of host use evolution are equivocal, but generally support the hypothesis that the ancestral host of tachinids was a beetle and that subsequent host shifts to caterpillars may coincide with accelerated diversification. Evolutionary reconstructions of reproductive strategy using alternative methods were incongruent, however it is most likely that ancestral tachinids possessed unincubated, thick shelled eggs from which incubated eggs evolved repeatedly, potentially expanding available host niches. These results provide a broad foundation for understanding the phylogeny and evolution of this important family of parasitoid insects. We hope it will serve as a framework to be used in concert with morphology and other sources of evidence to revise the higher taxonomic classification of Tachinidae and further explore their evolutionary history and diversification.

1. Introduction

The Tachinidae are one of the largest families of flies, with more than 8500 described species worldwide (O'Hara, 2013), and thousands more undescribed (Stireman et al., 2006). Within the order Diptera, they currently rank second only to the crane flies (Tipulidae) in number of described species (Pape et al., 2011). This great diversity is all the more impressive in light of the relatively recent origin for the family, which is estimated to be on the order of 30–40 mya based on recent molecular dating analyses of flies (Wiegmann et al., 2011; Cerretti et al., 2017). This young age suggests that tachinids are one of the most rapidly radiating families of Diptera and perhaps among the most rapidly diversifying lineages of metazoans (Scholl and Wiens, 2016).

The extraordinary diversification of Tachinidae may be related to their parasitoid habit and their rapid exploitation of this niche (though see Wiegmann et al., 1993). All known species are internal parasitoids of insects or other arthropods and as a group they attack hosts across at least 15 orders of Arthropoda (Arnaud, 1978; Stireman et al., 2006; von Ellenreider et al., 2015). However, the vast majority of tachinid species parasitize holometabolous insect larvae (caterpillars, beetle grubs, and sawfly larvae) or adult beetles, as well as true bugs (Stireman et al., 2006; Cerretti, 2010; Cerretti et al., 2014). As enemies of these primarily phytophagous groups, tachinids play important ecological regulatory roles in both natural and managed ecosystems.

Tachinidae are a significant component of insect communities in nearly all terrestrial ecosystems. As parasitoids, they are second only to the parasitic Hymenoptera in diversity and importance. For example, extensive rearing programs of caterpillars have estimated parasitism frequencies by tachinids averaging 10% or more, sometimes exceeding parasitism rates by Hymenoptera (Gentry and Dyer, 2002; Stireman and

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Singer, 2003; Stireman et al., 2009, 2017). Tachinids have also been shown to be effective at regulating certain host populations and suppressing outbreaks of insect pests (Brodmann et al., 1997; Maron and Harrison, 1997; Lamb et al., 1999; Hernandez et al., 2009). Since the early 1900s, dozens of tachinid species have been imported into North America and other regions to control crop and forest pests, and though the record is mixed (Grenier, 1988), an appreciable number have proven to be successful in controlling their injurious hosts (e.g., Bartlett et al., 1978; Roland and Embree, 1995; Parkman et al., 1996). More recently, the potential use of tachinids for biological control continues to be evaluated in diverse systems, including bromeliad weevils (Wood and Cave, 2006), palm weevils (Nihei and Pavarini, 2011), the prickly pear cactus moth (Pemberton and Cordo, 2001), and sugarcane borers (Vargas et al., 2015), among others.

Although tachinids are generally beneficial, they may also cause economic or ecological harm. This is especially true of certain silkworm parasitoids that attack hosts used in the commercial production of silk (Kumar et al., 1993). Introduced tachinids have been known to parasitize non-target hosts in biological control programs (e.g., *Compsilura concinnata*; Boettner et al., 2000; though see Elkinton and Boettner, 2012), although improved testing prior to the release of biocontrol agents has significantly reduced this threat in recent decades. There is also the potential for tachinids to take advantage of the growing commercialization of insects as a food resource for humans and domestic animals.

Comparative study of tachinids, particularly the evolution of their host use, will help us to better understand their ecological roles and aid in predicting host associations for taxa in which hosts are unknown. This may provide insight into the potential positive and negative ecological consequences of introduced species.

Despite their impressive diversity and ecological importance, Tachinidae have garnered relatively limited attention from basic and applied researchers. They remain, as a group, relatively poorly known, largely due to the taxonomic difficulties such an immense and relatively young group presents. Even the most recent classification schemes are thought to delimit few monophyletic groups (Tschornig, 1985; Wood, 1987). For example, in his North American revision of the tribe Polideini, O'Hara (2002) included taxa formerly assigned to nine tribes and two subfamilies. Only recently have quantitative morphological and molecular analyses been applied to understanding relationships within and among major tachinid clades (Stireman, 2002; Tachi and Shima, 2010; Cerretti et al., 2014; Winkler et al., 2015; Blaschke et al., 2018), and no study has yet examined comprehensive phylogenetic relationships of taxa across the family using molecular methods.

In addition to the practical taxonomic value of deciphering phylogenetic relationships among tachinids, they are an ideal clade in which to explore the evolution of host use and life history strategies. As a result of the myriad lifestyles of their hosts, tachinids have evolved a fascinating diversity of attack strategies. These include deposition of “typical” unincubated eggs on hosts that must develop for some time before hatching, production of “planidial” larvae that seek out hosts, minute eggs that are ingested by hosts and hatch in the gut (microtype), and membranous eggs that hatch shortly after being laid in, on, or near the host, or on the host’s food plant (O'Hara, 1985; Wood, 1987). The manner in which these oviposition strategies have evolved and how this is related to host use are controversial and unresolved questions (e.g., Cerretti et al., 2014). The evolution of host associations in tachinids is only beginning to be explored (Cerretti et al., 2014; Blaschke et al., 2018), and many questions remain. What was the ancestral host of Tachinidae? How labile are evolutionary associations within host clades? Are some host shifts more permissible than others? What is the sister group to Tachinidae and from what life-history did the parasitoid habit of tachinids evolve? Each of these questions, as well as others concerning morphological and behavioral evolution of tachinid flies, require a robust phylogenetic understanding of Tachinidae. It is towards this goal, a phylogenetic framework of Tachinidae, that we take an important step.

The primary goals of this study were to understand the evolutionary relationships among Tachinidae, to use this knowledge to improve tachinid classification, and to examine the evolution of major life history and ecological traits within the family. Previously, we have evaluated many of these phylogenetic and evolutionary questions for the bug-killing flies of the subfamily Phasiinae (Blaschke et al., 2018). Here we expand upon that work to consider the family Tachinidae as a whole. We sampled many disparate taxa from a wide variety of biogeographic regions to produce a world phylogeny of Tachinidae. Our specific objectives included:

(1) Reconstruction of a dense, informative molecular phylogeny of Tachinidae that can serve as a foundation for future evolutionary and systematic work and further test phylogenetic inferences based on morphology.
(2) Evaluation of the position of Tachinidae among the Oestroidea and its implications for the origin of the family and trait evolution.
(3) Determination of the monophyly and interrelationships of the four tachinid subfamilies (Dexiinae, Exoristinae, Phasiinae, and Tachininae) and their constituent tribes.
(4) Elucidation of the evolution of tachinid morphological, ecological, and behavioral traits important to basic and applied research, including oviposition strategy and host use.

1.1. The current state of tachinid systematics

Monophyly of Tachinidae is supported by their endoparasitism of insects, modifications of the mouthparts in the first instar (strongly developed labrum in first instars), and swollen adult subscutellum (Wood, 1987; Pape, 1992). However, relationships within the family have been somewhat unsettled for much of the historical study of the family (O’Hara, 2013). Only in the past few decades has some stability been achieved, beginning with Herting (1984), but recent morphological and molecular studies suggest even the most current classification schemes include para- and polyphyletic groups (Cerretti et al., 2015; Blaschke et al., 2018).

The classification followed here recognizes four subfamilies, the Exoristinae, Tachininae, Dexiinae, and Phasiinae. This four-subfamily classification scheme was first made popular by Herting (1984) for the Palaearctic Region and has since been adopted, with some modifications, as the standard throughout the world. The most recent iteration it appeared in is the Afrotropical catalog of O’Hara and Cerretti (2016). Most significant of the post-1984 changes were the transfer of Epigrimyiini and Eutherini from the Phasiinae to the Dexiinae and the emergence of Imitomyiini as a tribe of uncertain affinity (see Blaschke et al., 2018). The hinged phallus of nearly all Dexiinae (excluding only Eutherini) and the elongated medial plate of the hypandrium in Phasiinae are the only conserved characters serving as a synapomorphies for the four tachinid subfamilies. The remaining subfamilies are defined by the possession of a suite of characters, often with many exceptions, making their monophyly questionable.

The classification of Tachinidae at the tribal level has varied considerably over time and has yet to reach a universal consensus (O’Hara, 2013). Although some tribes are fairly homogenous and highly distinctive (e.g., Siphonini, Exoristini, Cylindromyiini), others, of questionable monophyly, contain a wide diversity of forms (e.g., Blondeliini, Eryciini, Voriini) that may overlap in appearance with other groups. Perhaps more puzzling are the relationships among tribes; for the most part, such intertribal relationships and the major morphological and ecological transitions between them remain poorly understood.

Despite improvements in recent decades in our understanding of the composition and relationships of certain tachinid taxa (e.g., Wood, 1985; Shima, 1996; O'Hara, 2002), rigorous analyses of deeper relationships have seldom been conducted. Landmark studies on female (Herting, 1957) and male (Tschorsnig, 1985; Cantrell, 1988) genitalia, larvae and puparia (Ziegler, 1998), and eggs (Gaponov, 1993, 1996a–c,

1998) were comparative in nature and informative, but did not incorporate modern phylogenetic analyses. Molecular sequence data has shown promise for addressing difficult phylogenetic questions within Tachinidae. Stireman's (2002) molecular systematic investigation of relationships within the Nearctic members of the subfamily Exoristinae using 28S rDNA and EF-1α recovered several tribes, including Winthemiini, Exoristini and Blondeliini. This analysis found support for the monophyly of Tachinidae and Exoristinae, however neither Tachininae nor Phasiinae was reconstructed as monophyletic. Subsequently, Tachi and Shima (2010) employed four genes (16S, 18S, 28S and *white*) in an analysis of Palaearctic Exoristinae and were able to resolve each of the major constituent tribes, including the microtype egg-laying Goniini. However, in both of these studies, relationships among and within subfamilies other than Exoristinae were represented by few exemplars and poorly supported.

Cerretti et al. (2014) conducted the first comprehensive quantitative morphological analyses of the family including nearly 500 species. These analyses reconstructed relationships among many major clades, questioning previously assumed monophyletic groups and proposing a number of novel relationships including: (1) A polyphyletic Tachininae with one lineage (Myiophasiini + Palpostomatini) being sister to the rest of Tachinidae, (2) Dexiinae + Phasiinae being sister to Exoristinae + (most) Tachininae, (3) Phasiinae arising from within Dexiinae, and (4) Exoristinae arising from within Tachininae. Relationships within the Exoristinae and Tachininae were poorly resolved and highly sensitive to model assumptions.

Employing a broad set of diverse loci, Winkler et al. (2015) robustly reconstructed relationships among tachinid subfamilies, largely confirming the results of Cerretti et al. (2014) and hypothesizing the Polleniinae (a subfamily of the Calliphoridae that may soon be elevated to family) to be the sister clade to Tachinidae (also see Cerretti et al., 2017). However, this study employed only a small number of tachinid taxa (22 genera/tribes), making it difficult to fully evaluate the monophyly and limits of subfamilies, let alone infer inter-tribal relationships within them. Recently, Blaschke et al. (2018) conducted a detailed molecular phylogenetic analysis of the subfamily Phasiinae based on four nuclear genes and including 128 taxa belonging to 80 genera. This is the most robust molecular phylogeny of any tachinid group to date, solidly establishing the composition of the Phasiinae and its constituent tribes and recognizing Dexiinae as sister to Phasiinae. Here, we build upon these initial efforts to understand tachinid phylogeny by expanding sampling and analyses to the family Tachinidae as a whole. Much of the data from Blaschke et al.'s study are included here, but we focus our attention on taxa and relationships not covered in that study.

1.2. Evolutionary transitions in the Tachinidae

Given their importance as parasitoids, the evolution of host use and host-attack strategies in tachinids are of particular interest. Although the parasitoid habit characterizes several families and has evolved many times independently within the order Diptera (Wiegmann et al., 2011), tachinids are the most diverse clade of dipteran parasitoids and, notably, have exploited the host-space represented by Lepidoptera to a far greater degree than any other group of extant flies. More than 60% of tachinid species attack caterpillars (Cerretti, 2010; Cerretti, O'Hara, Stireman, unpublished), but the propensity to attack this group varies widely among subfamilies. Dexiinae are mostly parasitoids of lepidopterans but diverse subgroups like the Dexiini attack beetle larvae; moreover, several small dexiine tribes such as Epigrimyiini, Eutheriini, Freraeini and Dufouriini *s.l.* attack true bugs or adult beetles. The Phasiinae develop almost exclusively on true bugs, with only a couple of genera of the tribe Strongygastrini attacking other insect orders (Lepidoptera, Coleoptera, Hymenoptera), in addition to Hemiptera. The subfamily Tachininae exhibits the greatest morphological disparity, and this is mirrored by their great diversity in ecology and host preferences. Several taxa in this subfamily parasitize Coleoptera, Orthoptera, Embioptera, Dermaptera, and even Chilopoda and Scorpiones, but the majority of species attack caterpillars. A similar condition characterizes the Exoristinae, where several taxa, especially among Ethillini, Acemyini, and Blondeliini, develop on orthopteroids and adult beetles, leaving the bulk of taxa attacking lepidopterans. Yet, we understand little of the evolutionary development of these host associations; whether caterpillars were the ancestral host of tachinids from which other groups were colonized or if this order was colonized independently by multiple lineages that then radiated extensively is an unresolved question.

A preliminary, parsimony-based character reconstruction by Cerretti et al. (2014), based on a morphology-based phylogeny, suggested that the ancestral tachinid was most likely a parasitoid of beetles. Cerretti et al. (2014) were unable to resolve the ancestral state of Tachininae + Exoristinae, but lepidopteran hosts appear to have been acquired early in this lineage. The general pattern from this analysis was that tachinids shifted onto caterpillars as a resource relatively late (and more than once) in their evolution, and this may have spurred diversification of those lineages. What key innovation(s) allowed tachinids to exploit lepidopteran host-space to such a degree remains to be discovered.

The success of tachinid flies may be tied to innovations in and the diversity of their reproductive strategies. Tachinidae have evolved a plethora of egg morphologies and a diverse array of reproductive strategies (Herting, 1960; Mellini, 1990; Stireman et al., 2006; see above). Understanding where and when evolutionary transitions in reproductive strategy occurred will aid in understanding how they relate to patterns of host use and diversification. Prior to Cerretti et al.'s (2014) analysis it was assumed that oviparity (unincubated, macrotype eggs) was the ancestral condition in Tachinidae (e.g., Wood, 1987, Tachi & Shima, 2010); however Cerretti and colleagues found ovolarvipary to be a more likely ancestral state with repeated reversals to ovipary, based on their morphological-based phylogeny. Furthermore, studies have been mixed as to whether there has been a single origin of microtype eggs or if multiple transitions have occurred from or to this state (Stireman, 2002; Tachi and Shima, 2010; Cerretti et al., 2014). A dense and robust phylogeny of Tachinidae is necessary to resolve these uncertainties in the evolution of reproductive strategies in the family and to understand their consequences.

2. Materials and methods

2.1. Specimen acquisition

Tachinid taxa were obtained by hand collecting and trapping in the author's home countries (Canada, Italy, U.S.A.), as well through collecting trips to Australia, Burundi, Chile, Costa Rica, Ecuador, Germany, Israel, Kenya and South Africa. Many additional taxa were sent to us by collaborators who obtained material from such countries as Finland, Japan, South Korea, Malaysia, New Zealand, Slovenia, Thailand and Vietnam. A small number of taxa were obtained through rearing hosts, most notably from the Caterpillars and Parasitoids of the Ecuadorian Andes project (Stireman et al., 2017). In selecting taxa for DNA extraction and sequencing, we included representatives from as many currently recognized tribes as possible, as well as from a wide diversity of genera within tribes. Genera within large clades such as Blondeliini, Goniini and Tachinini were sampled more densely. In some cases, we sequenced multiple species within a genus, particularly for large genera or genera whose placement was less certain. We also obtained and sequenced a diversity of other Oestroidea as outgroups (i.e., Calliphoridae *s.l.*, Oestridae, Rhinophoridae and Sarcophagidae) to more fully resolve the placement of Tachinidae within the superfamily.

Specimens of sampled taxa were either preserved whole in 95% ethanol (e.g., Malaise trap samples) or pinned and 1–3 legs removed from the right side of the thorax and placed in 95% ethanol shortly after

specimens were killed. For most of the former, 1–3 legs were removed for DNA extraction and the rest of the fly was chemically dried with ethyl acetate and mounted as a voucher. Voucher specimens are currently housed in the following institutions: Wright State University, Dayton, Ohio, USA (JOSC, Stireman); Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Canada (CNC, O'Hara); 'Sapienza' University of Rome, Rome, Italy (MZUR, Cerretti). See Blaschke et al. (2018) for preservation of taxa used in that study (mostly Phasiinae).

Most taxa were identified to the level of genus or below by the authors using available taxonomic literature and keys and by comparing specimens to identified material in the National Museum of Natural History (Washington), CNC, JOSC, and MZUR. A few taxa were identified by collaborators including J. Pohjoismäki (Finland), T. Tachi (Japan), and D.M. Wood (Ottawa). We were not able to identify many taxa to species, especially outside the Nearctic and Western Palaearctic regions, due to the lack of adequate revisions and keys and the large number of undescribed species. For a few taxa we were uncertain of genus and even tribe, but we considered that it would be valuable to include them to establish their phylogenetic position.

2.2. DNA extraction, amplification, and sequencing

DNA was extracted from ethanol-preserved individual adult legs using a Puregene Tissue Kit (Qiagen Inc.), with slight modifications to the manufacturer's protocol (see Winkler et al., 2015).

After extraction DNA samples were stored at −20 °C. We used primers from Winkler et al. (2015) and Blaschke et al. (2018) to amplify 28S, CAD, MAC, and MCS as well as additional primers (Table 1) we designed based on sequences generated by genomic sequencing of eight tachinid taxa (RNAseq; unpub. data). As each of the target loci, except 28S, span > 1500 bp, they were amplified as two overlapping fragments (e.g., CAD1, CAD2; see Moulton and Wiegmann, 2004). PCR reagent concentrations and cycling conditions are described in Winkler et al. (2015, Section 2.2). If we failed to achieve acceptable amplification of loci in single reactions after trying various alternate primer pairs, we attempted to re-amplify fragments using nested or hemi-nested primer pairs. In short, an initial 10 μL reaction was diluted 20-fold with DI H_2O and used as template in a second reaction employing primers located within the initially amplified fragment. After visualization on a 1.5% agarose gel, PCR products were sent to the University of Arizona Genetics Core (uagc.arl.arizona.edu) for cleanup, quantification and DNA sequencing. All amplified products were sequenced in both directions (although occasionally only one direction produced usable sequence data). Phasiinae and other taxa sequenced by JKM's lab (indicated by "JKM" in Supplementary Table 1) followed DNA extraction, amplification and sequencing protocols described in Blaschke et al (2018). Neighbor-joining trees were generated in MEGA for each separately amplified gene partition to check for anomalous results attributed to contamination or mislabeling.

2.3. Alignment and analysis

Chromatograms of forward and reverse sequences for each partition of each locus (e.g., CAD1 and CAD2) were assembled and edited manually using CodonCode Aligner (CodonCode Corp.) and/or Sequencher 4.7 (Gene Codes Corp.). IUPAC ambiguity codes were used to indicate nucleotide calls that were unclear (e.g., heterozygotes) or if forward and reverse strands appeared to differ. Final edited data for each locus was uploaded to the MAFFT v.7 alignment server (http://mafft.cbrc.jp/alignment/server/). For protein coding genes CAD and MAC we employed the FFT-NS-i iterative alignment algorithm and for MCS we employed the G-INS-i algorithm. This was followed by minor manual editing, mostly to limit gap introduction in partial sequences.

Table 1
Custom primers for CAD, MAC, and MCS used in initial PCR amplifications, re-amplifications, or both of Tachinidae. These primers are in addition to those previously described in Winkler et al. (2015). A number of these primers came from or were modified from those listed in Blaschke et al. (2018).

Gene	Primer name	F/R	Sequence (5′ → 3′)
CAD	CAD_P1FS1	F	TGYMAAACNTAYAARATGAARTAYGG
	CAD_P1FS1blond	F	ATTGGYAACTAYGGCATACCYGA
	CAD_P2F2	F	GTTTYATGACWTCYCAAAATCA
	CAD_ P2F3	F	GAAGAAAAAATYCAAACTGT
	CAD_P2R2n1	R	GCRTAYTGNATRTTRCAYTC
MCS	MCS_446F	F	TTYGTNGGNTTYGCYGARGT
	MCS_P1F3_Stire	F	GCNGARACNTTTRATTTYGG
	MCS_P1F3_gon	F	GCNGAATGCTTTGATTTYGG
	MCS_P2F1_Stire	F	GGYACNGTHAAYATWGCHATG
	MCS_P2F2_Stire	F	CGBACNGGHTGYTTTTGYAAYCC
	MCS_838R	R	TGRTCDATRCADATCANNGRCA
	Tach_MCS_P1R	R	GCRTADATRCADATYTCYTT
	MCS_P2R3.3	R	GRHRTYTCYAARCARTCRCWTAACC
	MCS_P2R3.4	R	CYAARCARTYRSWYARCC
	MCS_P2R4	R	ABTGYYTNAYYAAYCKYAADCCNG
MAC	HappyMACF	F	GAYCCHTCNCAAMGRHTNTGGTAYCG
	MAC_P1F5n	F	CCNGTRGAYATACARTATTTGG
	MAC_1.2F_Stire	F	CCHTTYCCNGTRGAYATACA
	MAC_MISS1_Stire	F	CATATCARTGGCAYARYATATTRGG
	MAC_MISS0_DEX	F	GGYAAYRTYTCHACMAYCATYTAT
	MAC_P2F3	F	AGARGTNAAYGCNCTNTAYAATGCHATGC
	MAC_P2F4	F	CAYACNATATTTGARGGNAARTCNAATGC
	MAC_NP2F_MISSING	F	TATCANTGGCAYARYATATTGG
	MAC_NP2F_MISS#2N	F	GARAAATATCTNAGAACATAYCA
	MAC_957F	F	GCNGGNGGNGCNGGNTAYAT
	MAC_957F_Stire	F	GAYGCNGGHGGHKCNGGYTAYAT
	MAC_P2RN_Stire	R	CATCCTGYTGRCACCATTCRCARCCC
	TACH_MAC_nP1R_Stire	R	GCYAARTADGAYTCYTTRTGNG
	MAC_P1R5	R	CGRTTY TGRTARTTKGCRCTDGC
	MAC_nP1R	R	GTRTCYTTYAARTARGCCAT
	MAC_P2Rev3	R	GGYTCRTARGGHGGYTCRCARTARTC

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.

For 28S sequences we explored a variety of alignment methods, including secondary structure based alignment (RNAfold webserver; RNAsalsa, Stocsits et al., 2009) and algorithms to identify and exclude questionably aligned sites (Gblocks (Castresana, 2000) and Guidance2 (Sela et al., 2015)). However, we found that the method that produced the most intuitive alignment and resulted in the most consistent phylogenetic reconstruction was the G-INS-i iterative refinement method using default parameters, including all sites but partitioned into regions of high and lower (gappy loop regions) alignment confidence.

At this scale of phylogenetic divergence, alignment of introns was not possible. We identified introns by the presence of variable-sized gaps in the alignments. Intron boundaries were determined using the GT-AG rule (Rogers and Wall, 1980) and by translating the codons and adjusting alignments in order to achieve a continuous open reading frame. A single intron in the sequenced portions of CAD and MAC and two introns in MCS were identified and excised from alignments.

A concatenated data set comprised of all examined loci was created to enable total evidence-based phylogenetic analyses. In some cases sequences derived from different individuals of the same species or closely related congeners were concatenated to achieve maximum gene coverage. In all such cases, individual gene trees were examined to confirm that the taxon's phylogenetic position was similar across loci prior to concatenation. (See Supplementary Table 1; concatenated sequences are from multiple species are indicated as "spp." in figures).

2.4. Phylogenetic analysis

Each data set (locus) was subjected to independent phylogenetic analyses in addition to analysis of the concatenated data set. Given that individual analyses suggested that 28S was poor at resolving deeper relationships, we also performed analyses with and without this locus to assess whether its inclusion improved or hindered phylogenetic resolution (see Winkler et al., 2015). Maximum Likelihood (ML) analyses were conducted using both RAxML 8.2.10 (Stamatakis, 2014) and IQ-tree (Nguyen et al., 2015) and Bayesian analysis was conducted with MrBayes (Ronquist et al., 2012).

RAxML analyses were conducted via the CIPRES Science Gateway v.3.3 (Miller et al., 2010), employing GTR + Γ models estimated for each partition (gene × codon position/high-low reliability region for 28S; 11 models for the full data set) and 500 rapid bootstrap replicates. ModelFinder (Kalyaanamoorthy et al., 2017) was used in IQtree to select the best model with free-rate heterogeneity (based on BIC scores) for each data partition. Selected models were GTR for most partitions, but models for CAD (1st pos.) and MCS (3rd) were TVM, MAC (3rd) was TIM, and 28S models were TVM and K3Pu for high and low confidence regions respectively. Branch support was evaluated with 1000 ultrafast bootstraps (ubf2; Hoang et al., 2017), as well as SH-like approximate likelihood ratio tests (SH-alrt, 1000 reps.; Guindon et al., 2010) and approximate Bayes (aBayes) tests (Anisimova et al., 2011). Ultrafast bootstraps are highly efficient, produce very low bias, are relatively robust to model violations, and the resulting clade support values are more readily interpretable as confidence in nodes, unlike conservative traditional bootstrap analysis (Minh et al., 2013). A chronogram was estimated using the Maximum likelihood function *chronos* in the R package APE (Paradis et al., 2004) employing a relaxed molecular clock model and default parameters (e.g., λ = 1). We used estimates of node ages for Oestroidea (46.7–66.5 mya) and Tachinidae (24.8–37.1 mya) from Cerretti et al. (2017) as calibrations to provide a rough approximation of the relative timing of diversification. Analysis of amino acid alignments were performed similarly but with protein evolution models WAG (Whelan and Goldman, 2001) and JTT (Jones et al., 1992) for RaxML and IQtree searches respectively. Bayesian analyses were conducted using MrBayes 3.2.6 through the CIPRES web portal with the following parameters: nst = 6, rates = invgamma, runs = 2 (combined), generations = 15,000,000, and burn in fraction = 0.25. Examination of likelihoods in Tracer v1.6. indicated that convergence may not have been reached even after searches of 15 million generations (consuming > 1500 CPU hours) due to the vast numbers of possible trees with > 500 taxa (i.e., $\gg 10^{100}$). However, incremental improvements in likelihood after ca. 5 million generations were small and likely involved minor rearrangements of poorly resolved branches.

2.5. Ancestral character state reconstruction

Reconstructions of hypothetical ancestral states of two key behavioral and morpho-functional traits, host use and reproductive strategy, were conducted using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BY) methods. Species were scored based on information available in the literature or, in a few cases, from unpublished label data from museum specimens. Host use was scored at the level of host order used by the genus for each taxon in the tree. For some genera represented by multiple species, hosts used by each species was scored (Supplementary Table 2). For a number of taxa, host use is unknown. To simplify analyses, reproductive strategies were categorized into one of five primary types: ovipary, ovolarvipary (non-microtype), ovolarvipary (microtype), larvipary, and macrolarvipary (e.g., *Mesembrinella*). Traits were mapped onto the consensus phylogram generated from the IQtree ML analysis. MP and ML analyses were conducted using the ASR package implemented in Mesquite v.3.5.1 (Maddison and Maddison, 2018); BY analyses were conducted using the "Multistate module" (Pagel et al., 2004) implemented in BayesTraits V3.0.1 (Pagel and Meade, 2017), with priors set to an exponential distribution with an average value of 10 and running each MCMC reversible-jump chain analysis for 1,000,000 iterations, sampling every 1000 iterations and setting a burn-in of 10,000 iterations. Tags to identify all nodes were created using BayesTrees V1.3. Results were visualized using TreeGraph 2 (Stöver and Müller, 2010). Trait reconstructions were evaluated based on parsimony, likelihood, and posterior probability scores respectively, and inferred evolutionary pathways of trait evolution were compared among inference methods.

3. Results and discussion

3.1. Taxa and sequence data

Our final data set consisted of 504 terminal taxa, representing 359 tachinid genera (Supp. Table 1, Fig. 1). Included are 54 of the roughly 60 currently recognized tribes of Tachinidae; only the small tribes Anacamptomyiini, Iceliini, Protohystriciini, Tarassini, Thrixionini, and Trichodurini were unsampled. Collectively these unsampled tribes amount to less than 0.5% of described tachinid species. Total taxa number and sequence lengths for each locus after removal of introns and end-trimming were: CAD: 456 taxa, 1719 bp, MAC: 430 taxa, 2226 bp, MCS: 393 taxa, 1878 bp, and 28S: 347 taxa, 1983 bp; for a total of 7806 bp. At the nucleotide level total coverage was 56.6% (excluding gaps and undetermined bases). At the locus level taxon coverage was 80.5% (84.4% excluding 28S; Supp. Table 1).

3.2. Phylogenetic inference

Phylogenetic trees inferred by different methods (ML versus Bayesian) and different approaches with varying search strategies (RAxML, IQtree) were consistent in overall structure, as were trees based on the concatenated data set with and without inclusion of 28S rDNA (Figs. 2–8, Supplemental Figs. 1–9). Inclusion of 28S sequence data added little to our ability to resolve relationships among clades, and support for some interior clades was greater without 28S rDNA data than with them. Bootstrap support was relatively strong for most nodes, however, there were several areas of the tree where support for branches was weak, and it is in these areas where we observe topological variation among individual analysis runs, inference methods and data sets (e.g., relationships among the clades of the Voriini grade of

Fig. 1. A small sample of representative genera of Tachinidae included in the current phylogenetic study. (A) *Macquartia plumbea* Richter & Wood. (B) *Gnadochaeta metallica* (Townsend). (C) *Eutrixa exilis* (Coquillett). (D). *Oestrophasia clausa* Brauer & Bergenstamm. (E) *Argyromima mirabilis* Brauer & Bergenstamm. (F) *Wagneria pacata* Reinhard. (G) *Pelycops darwini* Aldrich. (H) *Cordyligaster septentrionalis* Townsend. (I) *Rutilia* sp. (J) *Ginglymia johnsoni* (Coquillett). (K) *Eulasiona comstocki* Townsend. (l) *Leskia occidentalis* (Coquillett). (M) *Adejeania vexatrix* (Osten Sacken). (N) *Megaprosopus regalis* (Reinhard). (O) *Linnaemya comta* (Fallén). (P) *Neomintho macilenta* (Wiedemann). (Q) *Tachinomyia montana* (Smith). (R) *Medina quinteri* (Townsend). (S) *Phebellia helvina* (Coquillett). (T) *Gonia smithi* Brooks. Sex and body length (rounded to nearest 0.5 mm) are given for each specimen.

Dexiinae). Analyses of amino acid alignments resulted in some potential improvements in outgroup topology, but at a cost of generally lower bootstrap support throughout the tree and a few unlikely relationships. It appeared that inferred relationships based on protein alignments were strongly influenced by missing data, wherein the most dubious reconstructed relationships were associated with taxa with low sequence coverage (e.g., IQtree ML analysis with *Gastrolepta* (Blondeliini) outside Tachinidae; Supplemental Fig. 5). Below, we highlight some of the major features of our phylogenetic reconstructions and explore their implications for tachinid evolution and systematics.

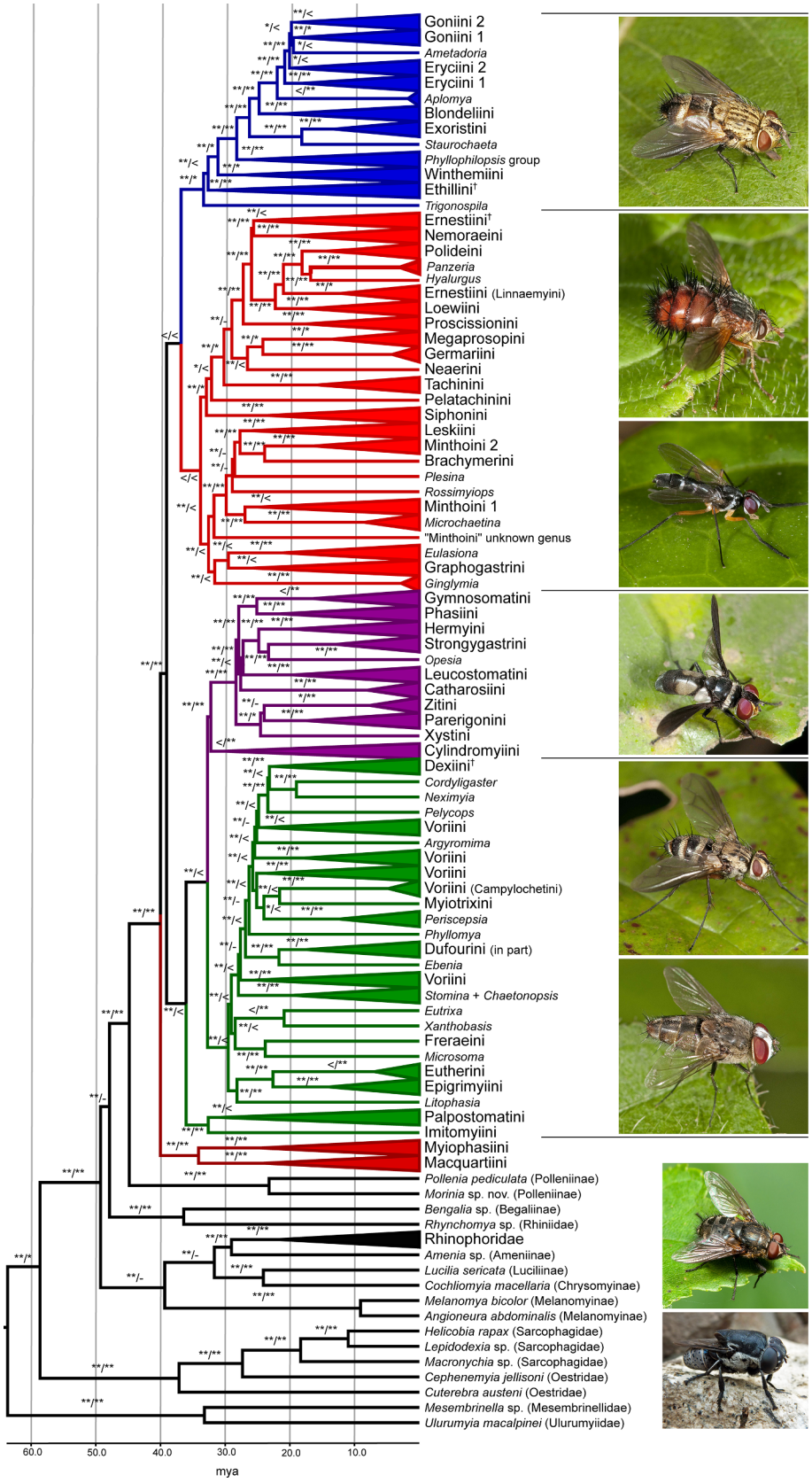


Fig. 2. A summary phylogeny of Tachinidae with major clades (tribes) collapsed. Reconstructed subfamilies are color coded (Exoristinae: top, blue, Tachininae: red, Phasiinae: purple, Dexiinae: green, outgroups: bottom, black), with tribes and other lineages indicated. Clades marked with "†" contain multiple recognized tribes (see Figs. 3–8). Branch support is indicated by ML ultrafast bootstraps (ubf)/ Bayesian posterior probabilities with: ** = > 90%, * = 80–89%, < = less than 80% and – = clade not present in Bayesian consensus tree. A rough time scale is provided at the bottom (see text). Representative tachinid species are illustrated to the right (from top): Goniini: *Frontina* sp., Tachinini: *Dejeania* sp., Minthoini: cf. *Mintho* sp., Gymnosomatini: *Bogosia* sp., Dexiini: cf. *Dexia* sp., Telothyriini: *Telothyria* sp.; outgroups: *Pollenia* sp. (Polleniinae) and *Cuterebra* sp. (Oestridae). All photos by Steve A. Marshall except *Pollenia* (Matt Bertone) and *Cuterebra* (JEOH). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. General structure and outgroups

We reconstructed Tachinidae as a monophyletic group with high confidence (Fig. 2). This is unsurprising as there has been no serious question regarding tachinid monophyly for some time, although phylogenetic analyses with limited taxon sampling have occasionally failed to reconstruct the family as a clade (Kutty et al., 2010). In contrast, the sister group to Tachinidae has been highly contentious, with virtually

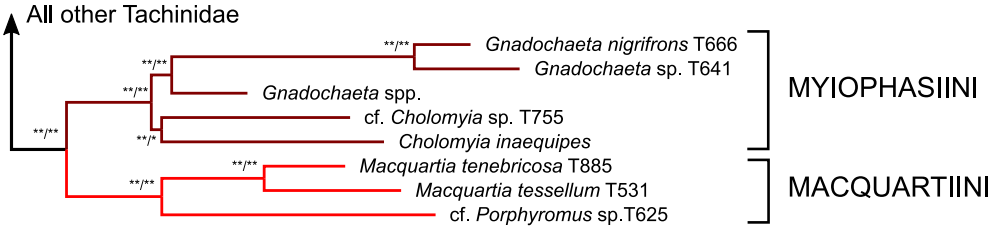


Fig. 3. The basal-most branching clade of Tachinidae comprising the tribes Macquartini + Myiophasiini. See Fig. 2 for explanation.

every other clade of Oestroidea being proposed as its sister by at least one study (e.g., McAlpine, 1989; Pape, 1992; Kutty et al., 2010; Wiegmann et al., 2011; Zhao et al., 2013; Ding et al., 2015). As in previous, taxon-limited but locus-rich analyses (Winkler et al., 2015; Zhang et al., 2016), we find strong support for a sister group relationship with Polleniinae (a subfamily of Calliphoridae), the earthworm parasitizing "cluster flies."

In terms of oestroid relationships, our results are consistent with Cerretti et al. (2017) in reconstructing Mesembrinellidae and Ulurumyiidae as sister taxa at the base of the Oestroidea with relatively strong support, although amino acid analyses suggest *Ulurumyia* is sister to the rest of Oestroidea (Supplemental Fig. 5). Oestridae and Sarcophagidae form a monophyletic clade, with the latter being paraphyletic with respect to the former or, for amino acids, both monophyletic (Winkler et al. 2014; Cerretti et al., 2017). Relationships among the various clades of the Calliphoridae *s.l.* and the Rhinophoridae are less well resolved and vary across analyses. In the IQtree ML analysis, Melanomyinae, Chrysomyinae, Luciliinae and Ameniinae form a grade of lineages from which a monophyletic Rhinophoridae is derived. Rhiniidae and Bengaliinae are consistently reconstructed as sister taxa, but their relationship to other oestroid clades varies among analyses. In ML trees this pair is sister to Tachinidae + Polleniinae, however in the Bayesian tree Melanomyinae takes up this position and the rhiniid-bengaliine clade joins with the main calliphorid-rhinophorid assemblage. Never do we see rhinophorids as sister to Tachinidae. However, the weak to moderate support for these relationships is insufficient to resolve debate concerning the phylogeny of Oestroidea. Denser taxon sampling and/or genomic scale data may be necessary to understand relationships among these rapidly branching clades, or it may be that internode intervals were so short when these lineages diversified in the early Paleogene (Cerretti et al., 2017) that we may never have high confidence in their relationships.

3.4. Subfamily phylogenetic structure

In general, our analyses support the subfamily relationships indicated in previous morphological (Cerretti et al., 2014) and molecular (Winkler et al., 2015) reconstructions, with the groups Dexiinae + Phasiinae (also see Blaschke et al., 2018) and Tachininae + Exoristinae (Fig. 2). However, two early branching clades render the Tachininae polyphyletic and the Dexiinae paraphyletic, respectively: one comprising Macquartiini and Myiophasiini that is robustly reconstructed as sister to all other Tachinidae (Figs. 2, 3) and the other comprising the morphologically unusual tribes Palpostomatini (in part) and Imitomyiini that is reconstructed with strong support as the sister to Dexiinae + Phasiinae. The early branching position of these groups was suggested by previous phylogenetic analysis of morphological data, although in a somewhat different configuration (Cerretti et al., 2014). Aside from these two small clades, each of the four major subfamilies is strongly supported as monophyletic, in contrast to analysis of morphological data (Cerretti et al., 2014).

3.4.1. Phasiinae

The subfamily Phasiinae received a recent molecular phylogenetic treatment by Blaschke et al. (2018), and much of the data from that work was included in the current analyses (CAD, MAC, and MCS sequences). Phylogenetic relationships among genera and tribes of Phasiinae recovered here are largely congruent with the findings of this previous work (Supplemental Figs. 1–5), and thus we refer readers to Blaschke et al. (2018) for an in-depth evaluation and discussion of these relationships. As in this previous work, we find relatively strong support for a monophyletic Phasiinae (bs = 77, ubf = 100, pp = 0.96), although the branch joining Cylindromyiini to the rest of Phasiinae is short and this clade sometimes joins the Dexiinae in individual gene-trees (CAD; Supplemental Fig. 6). The only notable differences in phasiine relationships between the current analysis and that of Blaschke et al. are: (1) Neither of the two genera of Zitini, *Zita* and cf. *Leverella*, is monophyletic (the latter was monophyletic in Blaschke et al.), (2) Leucostomatini and Catharosiini form a grade rather than being sister, (3) *Xysta* is sister to Zitini + Parerigonini, and (4) *Imitomyia* joins the Palpostomatini in a clade distinct from both Dexiinae and Phasiinae (see above). In light of this last phylogenetic rearrangement, fusion of the basiphallus and distiphallus (i.e., lack of differentiation) can be considered a valid synapomorphy of the Phasiinae (Shima, 2015; Blaschke et al., 2018). A few additional phasiine taxa absent from Blaschke et al.'s analysis are placed here including an unknown genus of Parerigonini from China, an additional species of *Australotachina* from Australia, and *Phaeodema* from Chile, which joins *Neobrachelia* to form a clade sister to the rest of Phasiinae (see Supplemental Table 1).

3.4.2. Dexiinae

Aside from the cluster of Palpostomatini (*Palpostoma*, *Eutrixopsis*) and Imitomyiini (*Imitomyia*) that occupies a sister group position to Dexiinae + Phasiinae, the Dexiinae comprise a well-supported monophyletic subfamily (Fig. 4). However, relationships among tribes within the subfamily exhibit more uncertainty, and some regions of the consensus tree conflict markedly with current tribal arrangements. Sister to the rest of Dexiinae is a clade composed of *Euthera* + Epigrimyiini + *Litophasia* as was found in Blaschke et al. (2018). Oddly, ML trees based on protein alignment tend to unite *Freraea* and *Litophasia* as sisters within this larger group. Above this node, relationships become complicated as a series of lineages grade into well-defined sister clades of core Voriini *s.s.* (i.e., those with a long strap-like distiphallus and bristly parafacials) and Dexiini. At the base of this grade are eclectic clades comprising a mixture of (remaining) Palpostomatini, Dufouriini (including *Ebenia*), Freraeini, and Telothyriini. The grade continues as a ladder-like series of voriine lineages (including former tribes Campylochetini, Uramyini, and Thelairini) and Myiotrixini, eventually giving rise to Dexiini + Voriini *s.s.* These phylogenetic results support, in part, the broad view of Voriini espoused by workers such as Herting (1957, 1960), Mesnil (1966), and O'Hara and Cerretti (2016), but even in this taxonomic arrangement the group remains paraphyletic with respect to Dexiini. Some of the internal branches connecting these groups are among the weakest (shortest and lowest support) of the entire tree and relationships vary considerably among single gene trees and analyses (e.g., compare parallel IQtree ML tree Fig. 4 to Supplemental Fig. 1), yet it is clear that Voriini and related lineages form a grade, suggesting the possibility that many tribes may have to be erected and defined if they are to reflect monophyletic groups.

Some well supported relationships within the "voriine grade" are worth highlighting. First, the Voriini *s.s.* are strongly supported as a clade in close association with several genera of the former Thelairini.

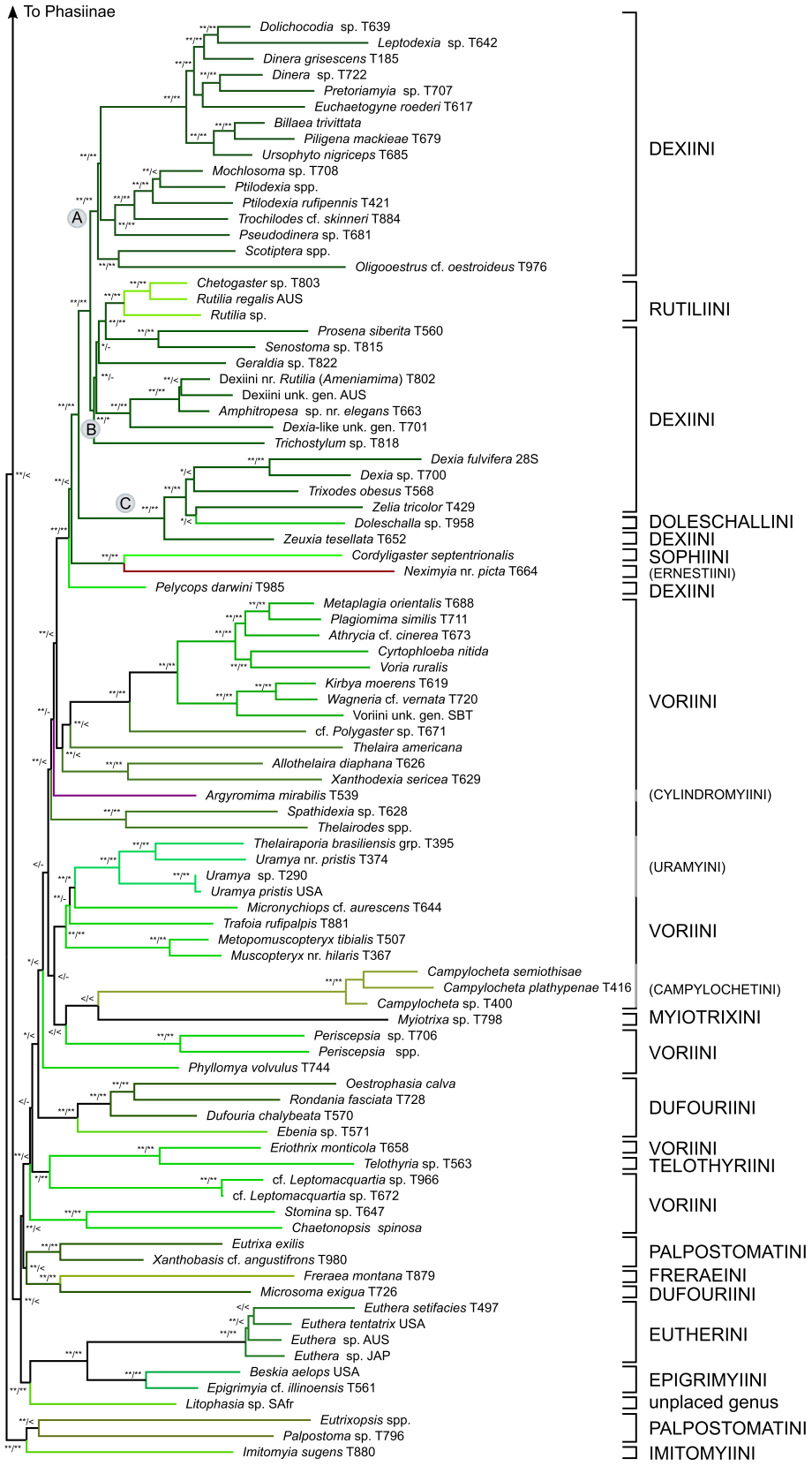


Fig. 4. Reconstruction of the subfamily Dexiinae (see Fig. 2). Smaller font names in parentheses indicate former tribes or placements that were considered doubtful. Clades referred to in the text are indicated by letters. See Blaschke et al., 2018 and Supplemental Figs. 1–5 for phylogenetic details of the subfamily Phasiinae.

The former Uramyini are also well-supported as a voriine clade with close relationships to the genera *Micronychiops*, *Trafoia*, and *Muscopteryx*. The majority of Dufouriini represented in the tree form a well-supported clade, nested among more traditional voriines. This restricted Dufouriini clade corresponds to those taxa with modified pregonites as recognized by O'Hara and Wood (2004). Finally,

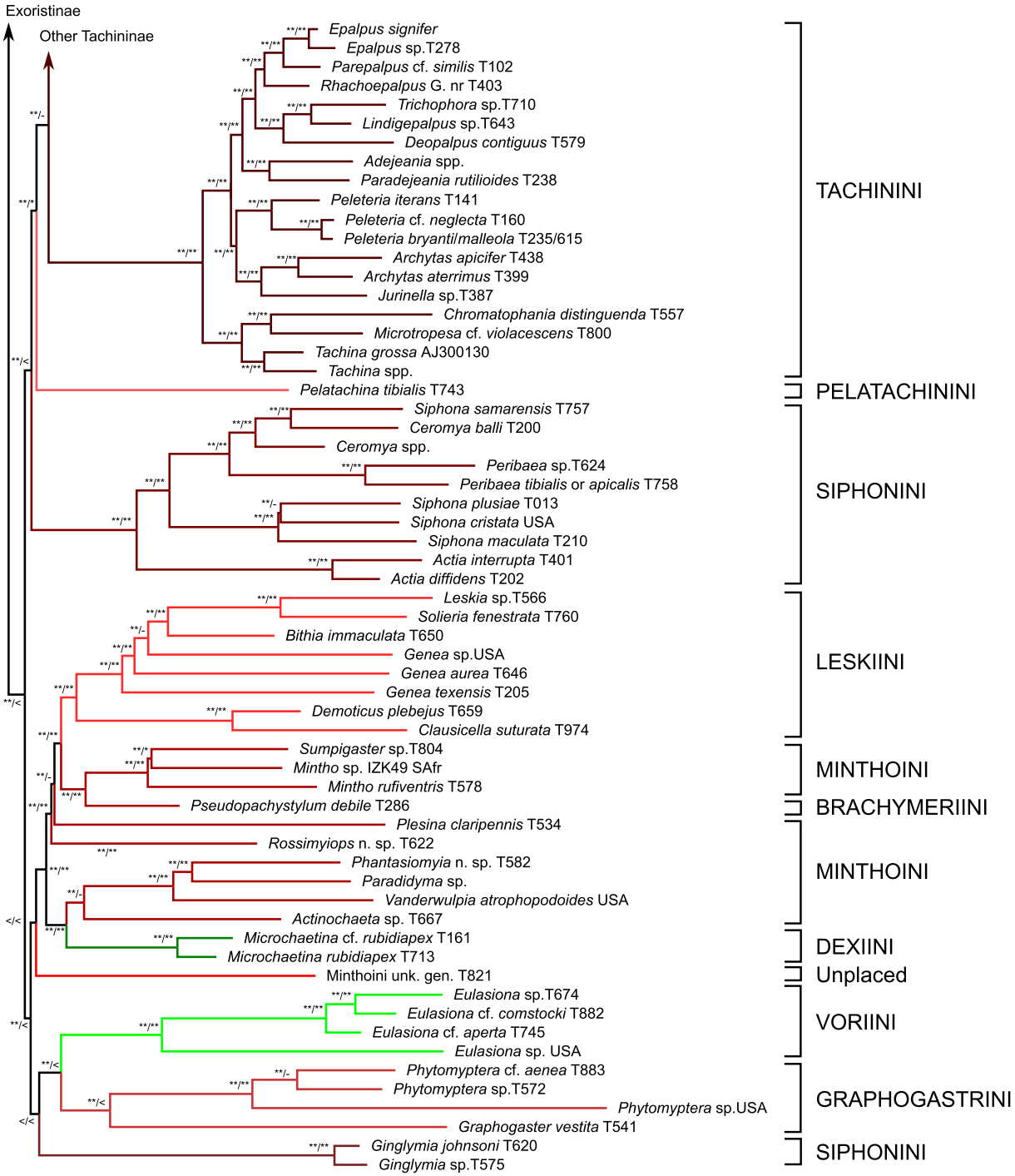


Fig. 5. Tachininae in part, including minthoine-leskiine assemblages, Siphonini and Tachinini; see Fig. 2).

Phyllomya, placed in the subtribe Phyllomyina (of a broadly circumscribed Voriini) by Mesnil (1966), is reconstructed as a lone lineage, without apparent close association with any other included taxon. Taxa that appear to be particularly mobile and troublesome in the voriine grade include *Thelaira*, *Argyromima* (considered as Cylindromyiini (Phasiinae) by Guimarães (1971)), *Phyllomya*, *Periscepsia* and *Myiotrixa* (Myiotrixini). Myiotrixini, including the genera *Myiotrixa* and *Obscuromyia*, is a highly apomorphic Australian tribe with uncertain taxonomic affinity, tentatively placed in the subfamily Tachininae by Crosskey (1973) and Barraclough and O'Hara (1998). All of our analyses, across data sets and inference methods, consistently reconstruct *Myiotrixa* within Dexiinae, although its exact position within the voriine grade varies. *Myiotrixa* possesses a hinged phallus characteristic of Dexiinae (although apparently lacking an epiphallus; Cantrell, 1988), further supporting its placement among the dexiines.

Dexiini, if considered in a broad sense as including the Australasian Rutiliini, are well-supported as a clade. As reconstructed here, the tribe consists of three major lineages: a *Dinera* group (Fig. 4, clade A), an Australasian *Rutilia* group (clade B), and sister to these, a basally branching *Dexia* group (Clade C). The *Dinera* group consists of several lineages, two early branching New World groups (including the previously unplaced genus *Oligooestrus* and the voriine genus *Trochilodes*) and a more recently diversifying and broadly distributed clade consisting of taxa from Europe, Africa, and the Americas. The *Rutilia* group

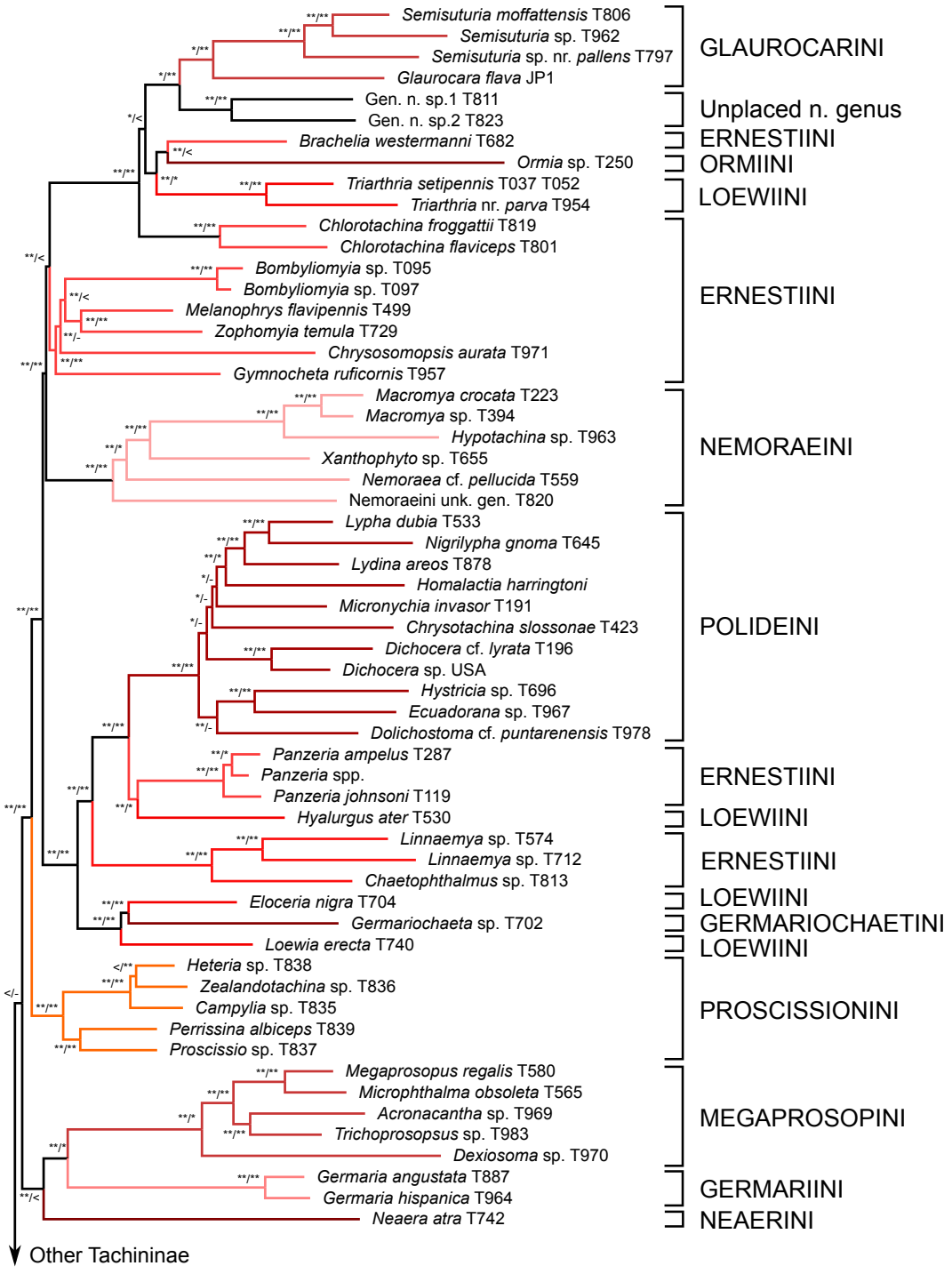


Fig. 6. Tachininae in part, including Ernestiini, Polideini and related lineages of the "Tachinini clade"; see Fig. 2).

consists of Rutiliini nested within a larger clade of primarily Australian Dexiini, with the exception of the widespread Old World genus *Prosena*. Finally, the *Dexia* group is composed of a mixture of Old and New World genera, including Doleschallini. Sister to Dexiini *s.l.* is a surprising clade of *Cordyligaster* (Sophiini) and the Australian *Neximyia* (an enigmatic genus formerly of uncertain subfamilial placement but provisionally assigned to the tachinine tribe Ernestiini by Crosskey (1973) and Cantrell (1988)). Possession of a dexiine-like phallus (Cantrell, 1988) supports our reconstruction of *Neximyia* within the Dexiinae. Sister to this group + Dexiini is a single representative of the *Dasyuromyia* group from Chile, *Pelycops darwini*.

3.4.3. Tachininae

The Tachininae are the most morphologically heterogeneous subfamily of tachinids, containing a multitude of disparate tribes. Yet, aside from the departure of the beetle-attacking Macquartiini and Myiophasiini, Tachininae are strongly supported as a clade and many relationships within the subfamily are well resolved in our analyses (Figs. 2, 4, 5). Two novel inclusions in the Tachininae recovered here are the genera *Microchaetina* and *Eulasiona* (formerly Dexiini and Voriini, respectively; Fig. 5). Due to their unexpected phylogenetic placement, multiple collections of these genera were sequenced and their position as tachinines is well-established. Cursory examination of the phalli of these taxa has revealed that although they appear to have the

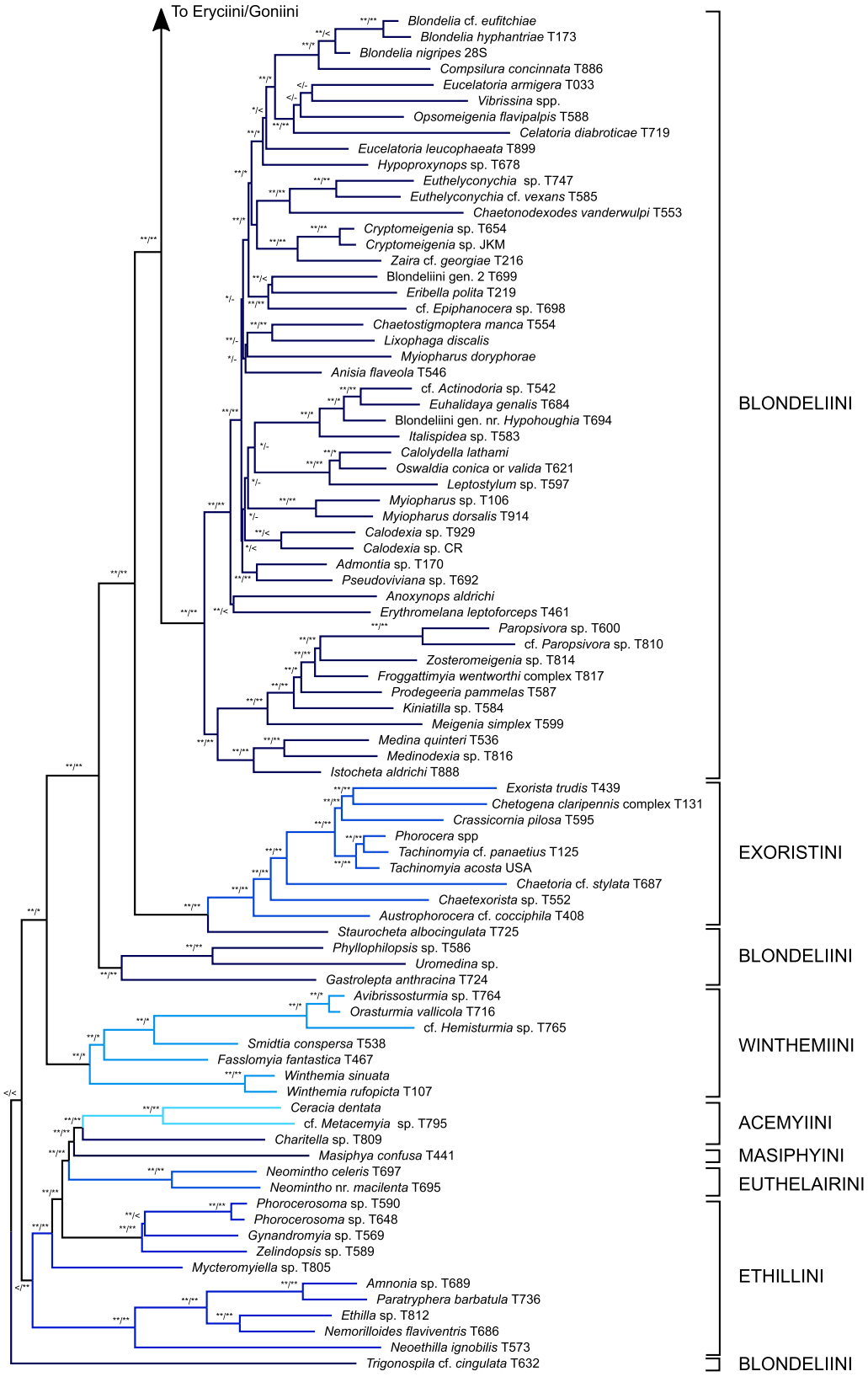


Fig. 7. Basally branching lineages of the Exoristinae (see Fig. 2).

L-shaped structure characteristic of Dexiinae (; only slightly bent in *Microchaetina*), they are not truly hinged via a thin membrane and represent somewhat convergent but unrelated forms. Neither genus was included in the morphological study of Cerretti et al. (2014).

The subfamily is divided into two major clades, each itself containing a diversity of tribes and forms.Tschorsnig, 1985 The first, here referred to as the *Mintho*-leskiine assemblage (Fig. 5 in part), comprises the tribes Graphogastrini, Minthoini, Leskiini and Brachymerini, in addition to the genera *Eulasiona* and *Microchaetina*, mentioned above. Graphogastrini form a monophyletic group, sister to

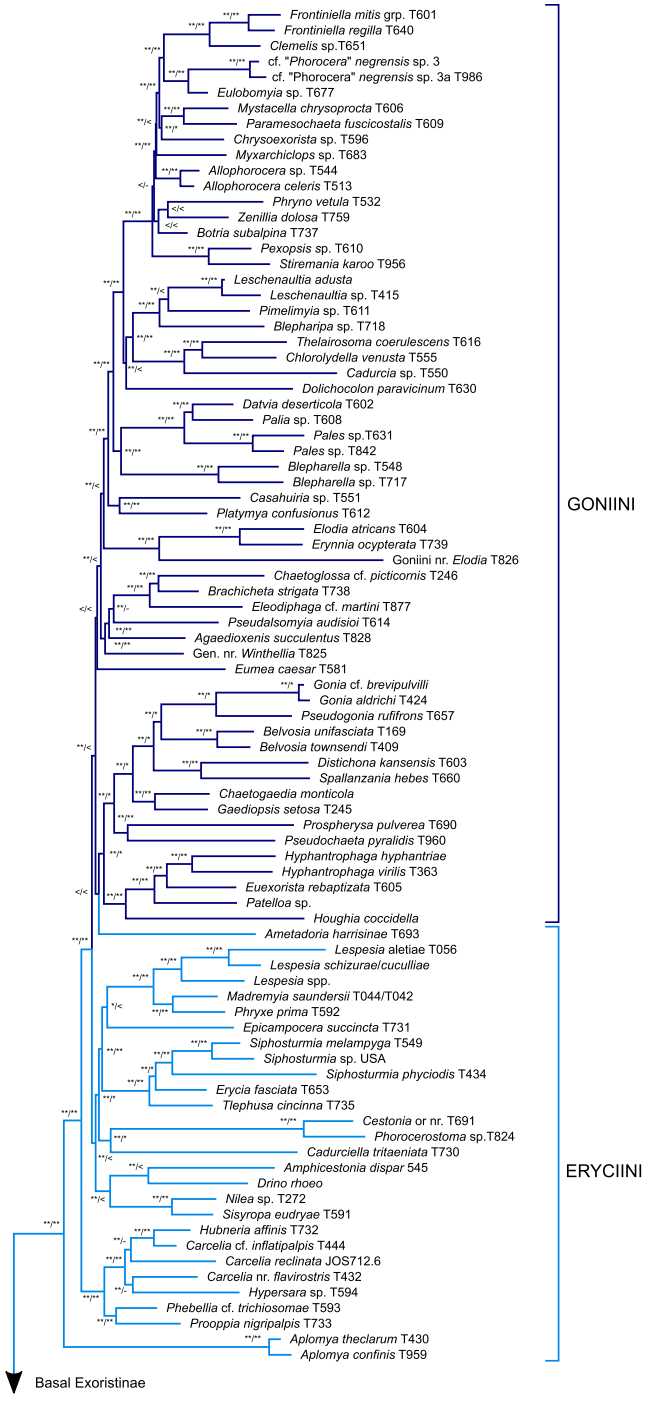


Fig. 8. Distally branching lineages of Exoristinae (Eryciini and Goniini; see Fig. 2).

Eulasiona. Leskiini are also monophyletic, with the exception of *Ginglymyia* which weakly joins Graphogastrini + *Eulasiona*. Interestingly, the Leskiini are a sizable tribe but lack a recognizable morphological synapomorphy and were reconstructed as paraphyletic in the morphological analysis of Cerretti et al. (2014). The Minthoini, however, are divided into several lineages intermixed by Brachymerini (*Pseudopachystylum*) and *Microchaetina* (see above), rendering the tribe paraphyletic with respect to Leskiini, even if broadly defined. The small embiopteran-parasitizing genus *Rossimyiops* is reconstructed among the minthoine lineages, supporting the taxonomic arguments of Cerretti et al. (2009). Paraphyly of minthoines was also found in the phylogenetic analysis of morphology (Cerretti et al., 2014). Relationships among these *Mintho*-leskiine lineages are only moderately well-supported and vary somewhat among analyses, suggesting a rapid diversification of lineages as proposed for Voriini *s.l.* above.

The second major lineage of Tachininae, the Tachinini group, contains a diverse assemblage of tribes including Siphonini, Pelatachinini, Tachinini, Neaerini, Germariini, Megaprosopini, Proscissionini, Loewiini, Ernestiini, Polideini, Nemoraeini, Ormiini, Germariochaetini and Glaurocarini (Fig. 5 [in part] and Fig. 6). The vast majority of these tribes are reconstructed as monophyletic with strong support. The major exceptions are the Ernestiini and Loewiini (and related taxa), which are each split into two or three lineages. A well-supported Siphonini is sister to the other tribes in this clade. The tribe Tachinini is one of the most easily recognized groups of Tachinidae (often large bodied and spiny) and its phylogenetic structure suggests a recent and explosive radiation. Despite this, all inferred relationships among genera within this tribe are robustly supported. The sister to this well-defined clade varies within and among analyses, either consisting of a large ernestiine-polideine complex (Figs. 5, 6) or a Megaprosopini-Germariini clade (Supplemental Figs. 2, 3). *Pelatachina*, *Germaria* and *Neaera* occupy isolated branches supporting the classification of each as a small unique tribe, however the last two tribes consistently form a clade with the dexiine-like Megaprosopini across analyses.

The tribe Loewiini is divided among three clades (Fig. 6): (1) The centipede parasitoids *Loewia* and *Eloceria* (along with *Germariochaeta*), (2) The sawfly-attacking *Hyalurgus*, sister to *Panzeria* (ernestiine parasitoids of caterpillars), and (3) The earwig parasitizing *Triarthria* in a distant clade near *Ormia*. These taxa were all tentatively included in Loewiini by O'Hara and Wood (2004), but sequence data and host associations support their dispersal among at least two tribes as advocated by Herting (1984). The polyphyletic Ernestiini *s.l.* are divided into at least four clades including a *Linnaemya* group, a *Panzeria* lineage, a core Ernestiini group (e.g., *Gymnocheta*, *Bombyliomyia*), and a series of lineages that grade into the Glaurocarini. This polyphyly has also been suspected on morphological grounds (O'Hara, 2002). The motley collection of taxa grading into Glaurocarini, comprising some Ernestiini (e.g., *Chlorotachina*), *Triarthria*, *Ormia*, and the Glaurocarini (including an undescribed genus), is very strongly supported. Interestingly, as in previous analyses (Inclán et al., 2018), we do not find evidence for a close association between the nocturnal/crepuscular Orthoptera-attacking tribes Glaurocarini and Ormiini, although they both belong to the same larger clade. The endemic New Zealand tribe Proscissionini is strongly resolved as a clade near, but not among, the Ernestiini-Loewiini lineages.

Although support for branches in this region of the tree is generally high, there are a number of relationships that remain somewhat uncertain. These include the exact position of Germariini + Neaerini, relationships among ernestiine genera, and the arrangement of the Glaurocarini-Ernestiini clade mentioned above. In addition, some intergeneric relationships within well-supported tribes, such as Polideini and Leskiini, are not robustly resolved.

3.4.4. *Exoristinae*

Our reconstruction of the (monophyletic) subfamily Exoristinae reveals a clear gradation of lineages from the basally branching ethillines, to the winthemiines and exoristines, to the "crown groups" of blondeliines and the eryciine/goniine assemblage (Figs. 7, 8). Our reconstruction largely supports current tribal divisions of Exoristinae, however, the placement of a few genera conflicts with current classification schemes. Notable among these is the placement of the blondeliine *Trigonospila* near the base of the subfamily, either as sister to all other Exoristinae (IQtree, Bayesian; Fig. 7; Supplemental Figs. 1, 2, 4) or among the ethilline lineages (RaxML, AAalign, Supplemental Figs. 3, 5). This placement is similar to the findings of Tachi and Shima (2010) and Cerretti et al. (2014) and is consistent with the oviparous reproductive strategy of this genus that is shared with Winthemiini, Exoristini, most Ethillini and a few Blondeliini (Wood, 1987). Other

"rogue" blondeliines include a *Phyllophilopsis* group (sister to Exoristini + Blondeliini + Eryciini + Goniini) and *Staurocheta* (sister to Exoristini). Aside from these taxa, most tribes of Exoristinae are recovered as monophyletic, or very nearly so. One exception is Ethillini; for this tribe to represent a monophyletic group, it must include Acemyini, Masiphyini and Euthelairini (all oviparous, orthopteroid-attacking taxa). Relationships within Ethillini *s.s.* correspond well to predictions by Cerretti et al. (2012) based on morphology, egg type, and host associations with a primary division into three major clades (*Ethilla*, *Phorocerosoma*, and *Mycteromyiella* groups).

The remaining Blondeliini are strongly supported as a clade that is split into two main groups: a predominantly Old World *Meigenia* clade, and a predominantly New World *Blondelia* clade (Fig. 7). Although inter-generic relationships within the former are well-resolved, those of the latter tend to vary among analyses and are largely inconclusive aside from closely related taxa (e.g., *Cryptomeigenia* and *Zaira*). This pattern suggests an early explosive radiation of New World Blondeliini relative to a more constant rate of diversification in the ancestrally Old World group.

Several authors have suggested that the Eryciini are likely paraphyletic (e.g., Stireman, 2002; Tachi and Shima, 2010) and our results support this view (Fig. 8). Here, we reconstruct the oviparous *Aplomya* as sister to all other Eryciini (+ Goniiini), with a grade of two additional eryciine groups arising between it and the Goniini: a relatively small and homogenous *Carcelia* group and a large and diverse *Erycia* group containing most eryciines. A single eryciine genus, *Ametadoria*, was reconstructed within the Goniini, where it joins the base of one or the other of two major clades of the tribe (the *Gonia* clade or the terminal *Pales-Frontiniella* clade; Fig. 8 versus Supplemental Fig. 1). As a result, absolute monophyly of Goniini was not indicated despite convincing morphological evidence that the tribe is monophyletic (most notably the production of specialized microtype eggs; Herting, 1957; Cerretti et al., 2014). Similar relationships are found across gene analyses except MAC where *Ametadoria* is absent. However, in no analysis was the position of this taxon strongly supported. *Ametadoria* possesses a typical membranous eryciine egg morphology and deposits eggs directly on its host caterpillars (Smith et al., 1955) and would thus appear to be misplaced in our reconstructions. We suggest that the Goniini are likely monophyletic despite our failure to recover the tribe as an unambiguous clade.

3.5. Evolution of morphological and ecological traits

3.5.1. Host use

Maximum likelihood (and MP) and Bayesian analyses yielded contrasting reconstructions of ancestral host use for the Tachinidae, as well as for all major internal lineages (Fig. 9). Both ML and MP reconstruct a coleopteran host as the most recent common ancestor of Tachinidae with high probability. This reconstruction is likely driven by the sister-group relationship between the beetle-attacking clade Macquartiini + Myiophasiini (traditionally in the subfamily Tachininae) and all remaining tachinids. Moreover, ML analyses infer that coleopterans were the most likely hosts of the ancestors of the following clades: (1) (*Imitomyia* + Palpostomatini) + (Dexiinae + Phasiinae), (2) Dexiinae + Phasiinae, and (3) Dexiinae; whereas, MP is ambiguous in reconstructing the host preference for the ancestor of both Dexiinae + Phasiinae and Dexiinae as either Coleoptera or Heteroptera. Within the Dexiinae, the ancestor of the Voriini *s.l.* + Dexiini clade likely developed on Lepidoptera, but the lineage experienced independent shifts to beetles or sawflies in more distal clades (e.g., *Phyllomya*, Dufouriini, Dexiini). Interestingly, ML and MP analyses suggest that adult Heteroptera were acquired independently as hosts, once in the ancestor of the Eutherini + Epigrimyiini clade of Dexiinae and once in the ancestor of the Phasiinae; in both instances arising from beetle-attacking ancestors. The ancestor of clade Tachininae + Exoristinae, as well as ancestors of both Tachininae and Exoristinae are robustly reconstructed as caterpillar parasitoids. This leads to the conclusion that all nested clades characterized by differing host preferences (e.g., Ormiini, Glaurocarini, Megaprosopini, *Loewia* and relatives, Acemyini, as well as several Graphogastrini, Minthoini, Ethillini and Blondeliini) diversified from ancestral lepidopteran parasitoids. Interestingly, we found no clear evidence of reversals back to Lepidoptera after these had been evolutionarily abandoned for insects in other orders. In general, broad host associations for most tachinid clades are relatively stable evolutionarily, e.g., the novel clade of orthopteroid parasitoids consisting of Acemyini, Masiphyini, Euthelairini (hosts unknown but likely orthopteroid), and part of the Ethillini (Fig. 7). However, a few groups appear to have been more evolutionarily labile, particularly the Blondeliini in which many transitions occurred between Lepidoptera and Coleoptera (and other orders).

Bayesian (BY) inference of host use evolution indicates more uncertainty in the states of basal nodes. Lepidoptera, Coleoptera, and Heteroptera are nearly equally probable hosts of the common ancestor of extant Tachinidae. Host use states are similarly ambiguous for the ancestor of all tachinids minus the Maquartiini + Myiophasiini clade (most likely Heteroptera or Lepidoptera) and the Dexiinae + Phasiinae + Palpostomatini clade (with Heteroptera being somewhat more likely (at 55%) than beetles or caterpillars). The ancestors of both Dexiinae + Phasiinae and Phasiinae were reconstructed as heteropteran parasitoids with high likelihood. The lack of host records for *Litophasia* may be hindering resolution of ancestral host use of Dexiinae, although a non-lepidopteran host is likely, whether beetle or bug. At more distal nodes within Dexiinae, BY analysis of host use tells the same story as ML and MP reconstructions; i.e., an early shift to Lepidoptera, which characterizes host preferences of the diverse grade of Voriini *s.l.* lineages, followed by multiple shifts to Coleoptera. Despite limited resolution, BY inference hints at Heteroptera being colonized as hosts only once in early-radiating tachinids, with only a handful of subsequent shifts to beetles and thence to Lepidoptera. Consistent with ML and MP reconstructions, BY results strongly support Lepidoptera as the ancestral host of clade Tachininae + Exoristinae and each of these subfamilies, with use of other hosts within these subfamilies representing secondary shifts.

3.5.2. Reproductive strategy

The reconstruction by ML and MP of ovolarvipary as the ancestral egg type (Fig. 10) is more or less consistent with the pattern of evolution proposed by Cerretti et al. (2014), with the notable difference that shifts to oviparity occurred in two primary lineages: once in the ancestor of Phasiinae and once in the ancestor of Exoristinae. Within Phasiinae a reversal to ovolarvipary occurred in the ancestor of Strongygastrini, which represents the only exception to oviparity (and to heteropteran hosts) within the subfamily. Exoristinae, on the other hand, display a more complex pattern of loss and re-acquisition of ovolarvipary from ovipary and vice versa in virtually all its major subclades.

The exoristine tribe Goniini evolved a special type of micro-ovolarvipary which is unique among Diptera and evolved independently in only one other family of insect parasitoids, the Trigonalidae (Hymenoptera). Goniini lay small ("microtype"), hard-shelled, planoconvex and fully incubated eggs on leaves that are ingested by their phytophagous hosts. Proteolytic enzymes in the host's midgut trigger the eggs to hatch and the first instars quickly migrate into the body cavity to complete development. Our ML reconstruction suggests this strategy evolved once from an ancestor that likely deposited relatively large (macrotype) membranous eggs directly onto a host. However, several other lineages of tachinids have evolved similar strategies (Wood, 1985; PC unpublished data). Interestingly, our analyses reconstruct the macrotype egg-laying *Ametadoria* as nested within the Goniini, which suggests a reversal from a microtype to macrotype egg-laying strategy if the genus is correctly placed. However, the phylogenetic position of *Ametadoria* is poorly supported (see above) and further

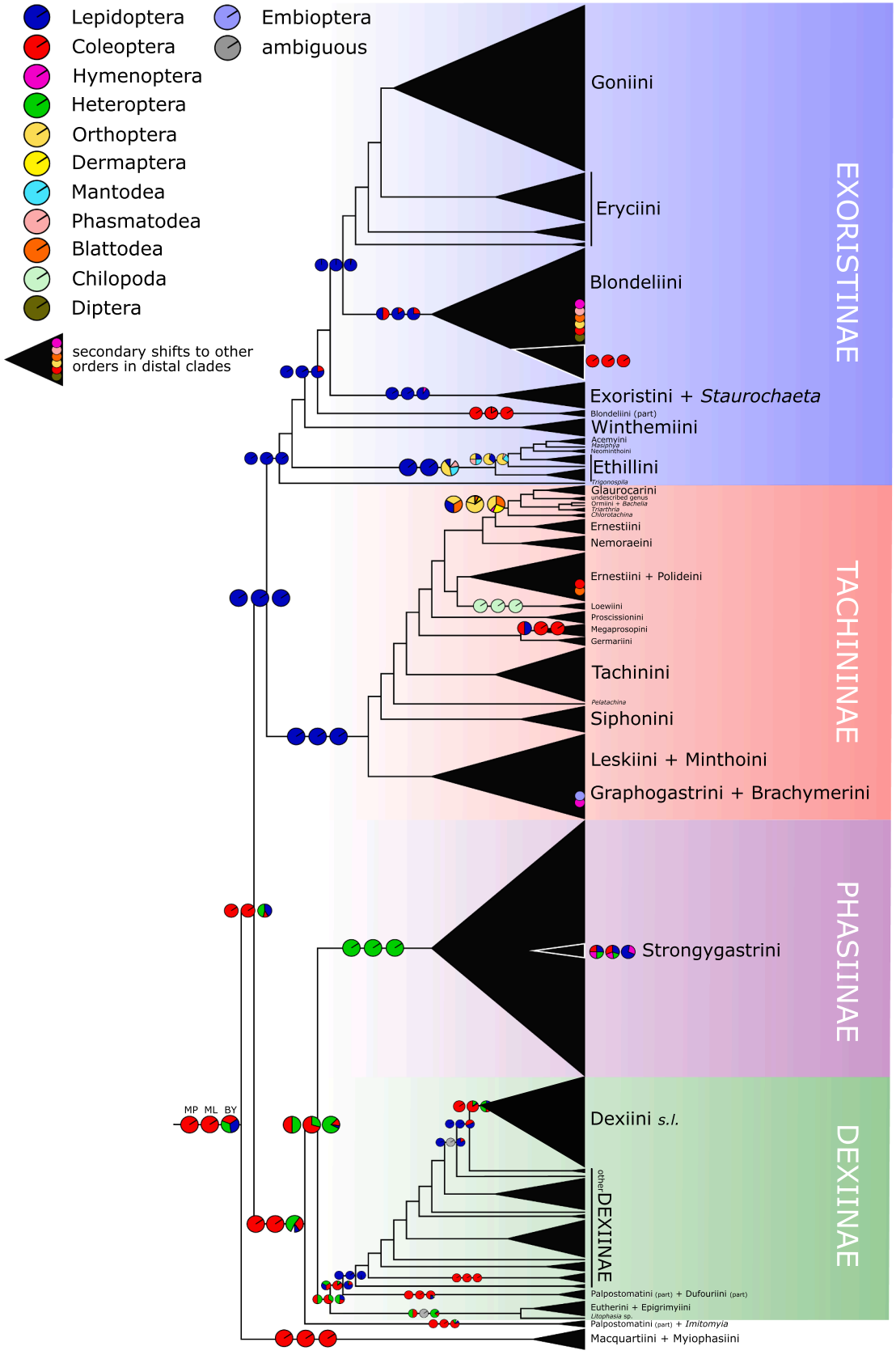
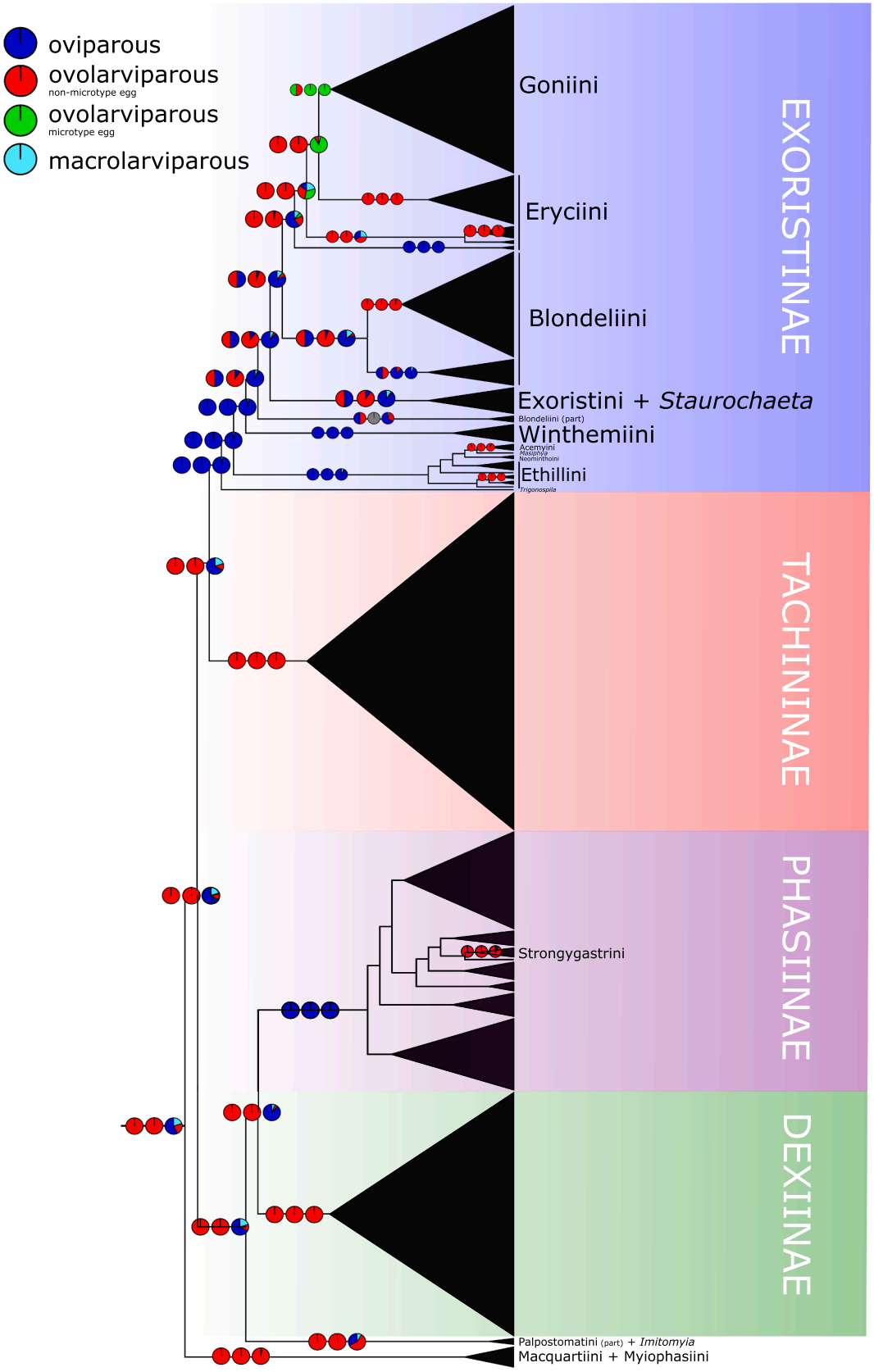


Fig. 9. Reconstruction of the evolution of host associations of the Tachinidae at the level of host order. Colored pie charts at major nodes indicate ancestral host probabilities as reconstructed by maximum parsimony (MP), maximum Likelihood (ML) and Bayesian (BY) inference methods, respectively. Secondary host shifts within major clades is indicated by colored circles inside clade triangles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

discussion of its reproductive strategy is premature.

Reconstruction of reproductive strategies inferred by BY methods yields a strikingly divergent scenario from our ML and MP analyses (Fig. 10). Here, nearly all early branches of Tachinidae (including the ancestor of the family) are most likely characterized by ovipary from which ovolarviparous lineages arose many times independently. Under



(caption on next page)

Fig. 10. Reconstruction of the evolution of major reproductive strategies (egg types) of tachinid flies. Colored pie charts at major nodes indicate ancestral state probabilities of the four major reproductive strategies as reconstructed by maximum parsimony (MP), maximum Likelihood (ML) and Bayesian (BY) inference methods, respectively. Secondary host shifts within major clades is indicated by colored circles inside clade triangles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

this reconstruction, shifts to ovolarvipary would have occurred independently in the following clades: Macquartiini + Myiophasiini, Palpostomatini + *Imitomyia*, Dexiinae, Strongygastrini, Tachininae and at least six times within the Exoristinae (once each in Ethillini, Acemyini and Eryciini + Goniini, three times in the Blondeliini). This scenario of repeated evolution of uterine incubation of eggs is more intuitively pleasing than its repeated loss as recovered by ML and MP methods. Observations of uterine incubation in oviparous taxa suggest that transitions to ovolarvipary may not represent major physiological hurdles (Herting, 1965; Terkanian, 1993) and a close look at the female reproductive system reveals that the morphology of both eggs and the common oviduct, as well as the arrangement of eggs within the common oviduct during incubation, are remarkably varied among ovolarviparous groups (Tschorsnig & Richter, 1998). This hints at multiple evolutionary origins of ovolarvipary, supporting the scenario recovered by BY inference. Moreover, if this reconstruction is an accurate reflection of evolutionary history, shifts to ovolarvipary appears to correspond to shifts in both host use and diversification rate. Ovolarvipary may thus represent an exaptation allowing host use expansion as evidenced by the diverse host spectrum of all ovolarviparous groups.

3.6. Implications for tachinid classification

We refrain here from proposing higher level classificatory changes based on our results pending a more integrated assessment of morphological and/or life history synapomorphies. Such studies will assist with the defining of identified clades and placement of unsampled taxa. Although many current higher taxonomic groups were recovered, our analyses imply that changes in tribal and subfamily classification will be necessary if these groups are to represent monophyletic lineages. We briefly examine some of these implications here, but leave formal revision for a future, more focused and thorough review of tachinid classification.

Based upon our results, the four subfamilies Dexiinae, Phasiinae, Tachininae and Exoristinae can be maintained in nearly their present forms, however our results strongly suggest that at least two additional subfamilies should be considered for the clades Macquartiini + Myophasiini and Palpostomatini + Imitomyiini.

Tribal classification of Dexiinae is likely to need major revision. The two tribes Eutherini and Epigrimyiini are well resolved, as is an expanded Dexiini (see above), but all trees inferred from our various analyses suggest that Voriini *s.l.* represent a grade of lineages that cannot be easily divided into a small number of tribes. Dufouriini also are split into at least two groups. Unfortunately, many relationships among the voriine grade are only moderately supported and additional lines of evidence will be necessary to resolve this assemblage into a functional, evolutionary tribal classification.

As mentioned previously, our results for the Phasiinae support the recent conclusions of Blaschke et al. (2018), as expected based on our shared use of much of the same taxa and loci for the subfamily.

Most of the current tribes of Tachininae were recovered with moderate to strong support and our analyses would suggest little or no taxonomic alteration. As mentioned above, the problematic taxa are primarily the ernestiine-loewiine assemblage and the Minthoini. The former may need more careful study to sort out the true relationships before assigning the genera to tribes while maintaining the well-defined tribes Ormiini, Glaurocarini and Polideini. Our results imply that the Minthoini either need to be expanded to include Leskiini and Brachymerini, or divided into at least five tribes, including an undescribed one from Australia (as “Minthoini unknown genus” in Fig. 5). Furthermore, the morphologically distinct genera *Eulasiona* and *Ginglymia* would each appear to constitute an independent lineage without a clear affiliation with any established tribe.

Our results are perhaps the least troublesome for tribal classification of Exoristinae. The unexpected placement of *Ametadoria* within the Goniini is in conflict with morphological evidence and further study is needed to better understand the reason for this incongruity. Eryciini could be easily divided into three tribes (Eryciini, *Carcelia* group, and *Aplomya* group) to preserve monophyly. As indicated previously, the Blondeliini are well-supported, although the genus *Trigonospila* and the *Phyllophilopsis* clade are distinct and may warrant tribal status and *Staurocheta* is strongly allied with Exoristini. Finally, our results suggest that the Ethillini could be expanded to include Acemyini, Euthelairini, and Masiphyini (and take the name of the oldest family-group name), or perhaps more reasonably, be divided into three morphologically distinct tribes representing the *Phorocerosoma* group, *Paratryphera* group, and *Mycteromyiella* group (Cerretti et al., 2012).

4. Conclusions

In this study, we assessed relationships within the diverse parasitoid fly family Tachinidae using an exceptionally large taxon sample (> 500 OTUs) including most recognized tribes. We found strong support for the contention that earthworm-parasitizing Polleniinae are sister to Tachinidae, suggesting that the parasitic habit predates the origin of tachinids (Winkler et al., 2015). Our analysis recovered each of the subfamilies and many currently recognized tribes, with some notable exceptions particularly in the subfamilies Dexiinae and Tachininae. With our dense sampling we provide a framework for understanding much of the evolutionary relationships among lineages within each subfamily, which will aid in the search for morphological synapomorphies of traditionally recognized and novel clades. Several lineages appear to have undergone rapid episodes of diversification including lineages of Dexiinae, basal Tachininae, and Blondeliini – perhaps in association with major host transitions.

Our evolutionary reconstructions of host use generally support the hypothesis that, although most extant tachinid species attack Lepidoptera, the ancestral host of tachinids was probably a beetle, and transitions to caterpillars as hosts may have spurred tachinid diversification. Aside from a few clades (e.g., Blondeliini, Ernestiini-Polideini complex), host use at the level of order appears relatively conserved within major clades.

Bayesian and Likelihood (and Parsimony) inference methods arrive at quite contrasting scenarios with respect to the evolution of reproductive strategies. The former method infers that ancestral tachinids possessed unincubated, thick shelled eggs from which ovolarvipary (incubated “ready to hatch” eggs) evolved repeatedly, perhaps in association with host shifts and adaptive radiations; a hypothesis that deserves further exploration. This more traditional hypothesis appears to be supported by available morphological evidence. Likelihood and MP methods support the opposite view proposed by Cerretti et al. (2014), that ovipary evolved repeatedly from ovolarviparous stem lineages of Tachinidae.

These results provide an extensive foundation for future study of the phylogeny and evolution of this important and diverse family of insects. In addition, our study can serve as a framework that can be used in concert with morphology and other sources of evidence to revise the higher taxonomic classification of Tachinidae, by identifying regions where changes are clearly needed and areas that will require further study. Although the current study is a major contribution to

understanding the composition and relationships of super-generic clades of Tachinidae, phylogenetic resolution in several regions in the present trees is unsatisfying. Phylogenomic approaches employing hundreds or thousands of loci (e.g., UCEs, AHE, RNAseq, HiMAP; Dupuis et al., 2018) may be necessary to clarify relationships among taxa in these regions of apparent rapid diversification. Unfortunately, however, genomic approaches often fail to resolve the same contentious nodes where smaller data sets flounder (Pyron, 2015). In at least some cases, these nodes may be impossible to resolve with confidence, or, as appears to be the case here with Goniini, morphology may provide the clearest source of evidence of phylogenetic structure.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2018.12.002.

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