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Molecular phylogenetics and piercer evolution in the bug-killing flies (Diptera: Tachinidae: Phasiinae)

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Abstract. Phasiinae (Diptera: Tachinidae) are endoparasitoid flies that attack Heteroptera, including a multitude of agricultural pests. A phylogenetically informed classification of Phasiinae has eluded systematists for over a century, primarily because of the conflicting character states and confusing morphology of certain taxa that indicate potential placement within other 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 Phasiinae is presented, including 128 worldwide taxa (80 genera) and approximately 7.6 kb of nuclear data representing four genes. Special emphasis is placed on the resolution of taxonomically ambiguous groups. The resulting robustly supported phylogenetic trees [maximum-likelihood (ML)/Bayesian] were used to trace the evolution of significant adaptive traits within Tachinidae and test hypotheses about the classification of Phasiinae. Subfamily placements of certain taxa are confidently resolved including Eutherini, Epigrimyiini, *Litophasia* Girschner within Dexiinae, and Strongygastrini and Parerigonini within Phasiinae. The members of tribe Phasiini are redistributed: *Cistogaster* Latreille, *Clytiomya* Rondani, *Ectophasia* Townsend, *Eliozeta* Rondani and *Euclytia* Townsend transferred to Gymnosomatini; *Opesia* Robineau-Desvoidy to Strongygastrini; and *Xysta* Meigen to Xystini. Similarly, members of Parerigonini are treated as belonging to Parerigonini (*Parerigone* Brauer, *Zambesomima* Walker), Cylindromyiini (*Australotachina* Curran, *Pygidimyia* Crosskey, *Neobrachelia* Townsend) or new tribe Zitini (*Zita* Curran, *Leverella* Baranov). *Penthosia* van der Wulp is transferred from Cylindromyiini to Hermyini. Ancestral state reconstruction suggests that piercing structures used to insert eggs directly into host tissues have evolved separately in a number of groups, but have also been lost or reduced in several lineages. A single potentially unequivocal morphological synapomorphy of Phasiinae, an elongated medial plate of the hypandrium in males, is identified.

This published work has been registered in ZooBank, http://zoobank.org/urn:lsid:zoobank.org:pub:8BE75122-FC7C-4809-AAF7-19575596EF78.

Introduction

Tachinidae comprise a well-supported clade of 'higher' Diptera, defined in part by an enlarged postscutellum and obligate parasitism of arthropod hosts (Wood, 1987; Pape, 1992). With ~8500 described species worldwide and perhaps an equal number still undescribed (O'Hara, 2013a), Tachinidae are considered to be among the most species-rich and morphologically diverse families of Diptera (Crosskey, 1980; Pape *et al*., 2011), and represent the largest clade of insect parasitoids outside Hymenoptera (Eggleton & Belshaw, 1992). Tachinids have likely experienced such impressive evolutionary success in part due to their varied use of hosts. The family includes both

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generalists and specialists that collectively attack a multitude of hosts from 14 orders of arthropods. They primarily attack caterpillars, beetles and bugs, but also parasitize such diverse taxa as scorpions, centipedes, ants, bees and earwigs (Arnaud, 1978; Stireman *et al*., 2006). By parasitizing insect plant pests like caterpillars and true bugs, tachinids play an important role in regulating pest populations in both natural ecosystems and controlled agricultural environments (Nishida, 1966; Stireman & Singer, 2003; Coombs, 2004). Despite their great diversity and importance, our knowledge of evolutionary relationships among tachinids is limited, and only recently have quantitative phylogenetic methods been applied to understanding the phylogeny of the family (Tachi & Shima, 2010; Cerretti *et al*., 2014; Winkler *et al*., 2015).

Phasiinae constitute the smallest of the four subfamilies of Tachinidae yet still contain over 600 described species belonging to about 100 genera (Crosskey, 1976; Herting, 1984; Wood, 1987; Herting & Dely-Draskovits, 1993; Ziegler, 1998; O'Hara & Wood, 2004; Richter, 2004; O'Hara *et al*., 2009; Evenhuis, 2013; O'Hara & Cerretti, 2016). Even so, some of the most extreme morphological variation within Tachinidae can be found in this subfamily (Fig. 1). Phasiines differ dramatically in size and appearance, from among the smallest of tachinids (*Catharosia* Rondani spp.: <2 mm) to among the largest (*Lophosia* Meigen spp.: 18 mm). Some phasiines are recognizably tachinids, being garbed in traditional black and grey with large bristles on their abdomen (e.g. Fig. 1M, N). Others are uniquely beautiful mimics of wasps and bees, with reduced setation and contrasting banding patterns or bright (i.e. aposematic) colouration (Fig. 1G, I, O, S). Among the more notable wasp mimics are *Penthosia satanica* (Bigot) (Fig. 1O), *Formicophania elegans* Townsend and *Cylindromyia mirabilis* (Townsend) (see fig. 3 in O'Hara, 2012b).

The hosts of Phasiinae are almost exclusively adults of true bugs (Hemiptera, suborder Heteroptera) and are unusual among tachinid hosts in being highly mobile and protected by a hardened cuticle (in contrast to caterpillars and other larval Holometabola). Thus, they are colloquially referred to as the bug-killing flies. Some of these hosts are prominent agricultural pests, such as *Nezara viridula* (L.) (southern green stink bug), *Euschistus servus* Say (brown stink bug), and *Lygus lineolaris* (Palisot de Beauvois) (tarnished plant bug) (Arnaud, 1978). In the United States, phasiines also attack the recently invasive pests *Halyomorpha halys* Stål (brown marmorated stink bug; Aldrich *et al*., 2007) and *Megacopta cribraria* (F.) (kudzu bug; Golec *et al*., 2013). At least some phasiines locate potential hosts through the use of specialized antennal receptors that are extremely sensitive to host pheromones (Aldrich *et al*., 2006). Remarkably, phasiines that have been tested for their ability to detect such pheromones were more sensitive to them than were the hosts themselves (Aldrich *et al*., 1989). Because of their distinctive ability to use pheromone cues, phasiines hold great promise as biological control agents but have been underutilized as such. A predictive and well-supported phylogeny of Phasiinae is needed to provide an evolutionary framework for future predictive studies in biological control, pheromone attractants and tritrophic ecological interactions.

Another notable feature of many phasiines, aside from their parasitism of heteropteran hosts, is their possession of piercing structures used to insert eggs into the bodies of hosts. Flies in general lack the ancestral, lepismatid-type ovipositor found in the parasitic Hymenoptera, and most tachinid flies lay eggs externally on or near the host. However, a number of tachinid lineages have secondarily evolved piercing structures from various abdominal sternites that allow them to inject eggs directly into the host. We know little about the evolution of these piercers (e.g. how many times they have evolved) due to the lack of rigorous phylogenetic analyses of Tachinidae and, in the present context, Phasiinae.

Objectives

Here we use sequence data from four nuclear protein coding genes to reconstruct a comprehensive molecular phylogeny of the subfamily Phasiinae. We explore relationships among major phasiine lineages that have been difficult to resolve using morphology and provide a foundation for significant classification revisions within Phasiinae and between Phasiinae and the other tachinid subfamilies. We use this phylogenetic framework to explore the evolution of piercers and other taxonomically significant structures, asking how many times they have evolved in phasiines, and if piercer structure can be used to taxonomically define clades within the subfamily. Specifically, our objectives were as follows:

- Determine whether Phasiinae are monophyletic, and if so, which (if any) morphological synapomorphies define the lineage.
- Assess the monophyly/validity of, and relationships among, tribes in Phasiinae.
- Infer the evolutionary relationships of the following taxonomically ambiguous taxa: Epigrimyiini, Eutherini, Imitomyiini, *Litophasia* Girschner, Strongygastrini and Parerigonini.
- Trace the evolution of key traits (e.g. piercers) across the phylogeny to test hypotheses about subfamily synapomorphies and the evolution of Tachinidae.

Taxonomic background

Modern experts subdivide Tachinidae into four subfamilies: Phasiinae, Dexiinae, Exoristinae and Tachininae (Herting, 1984; Tschorsnig & Richter, 1998; O'Hara & Wood, 2004; Cerretti *et al*., 2014). However, relationships within, and among, these subfamilies are poorly understood and have undergone considerable rearrangement throughout their history (O'Hara, 2013b). Phasiinae were proposed by Wood (1987) to be a basally branching tachinid lineage due to their 'primitive' oviparous condition. Richter (1992) supported this view and also considered (Tachininae + Dexiinae) to be the most derived group. This arrangement was similar to the proposed (Phasiinae + Exoristinae) and (Tachininae + Dexiinae) of Herting (1966, 1983) based on

Fig. 1. Habitus images of representative Phasiinae and selected other tachinids of disputed placement. (A) *Beskia aelops* (Walker); (B) *Euthera woodi* O'Hara; (C) *Imitomyia sugens* (Loew); (D) Nr. *Neobrachelia;* (E) *Australotachina* sp.; (F) *Pygidimyia* sp.; (G) *Hemyda aurata* Robineau-Desvoidy; (H) *Besseria brevipennis* (Loew); (I) *Cylindromyia euchenor* (Walker); (J) *Xysta holosericea* (Fabricius); (K) *Zita* sp.; (L) *Opesia americana* (Bigot); (M) *Rondaniooestrus apivorus* Villeneuve; (N) *Strongygaster didyma* (Loew); (O) *Penthosia satanica* (Bigot); (P) *Catharosia* sp.; (Q) *Clairvillia timberlakei* (Walton); (R) *Phasia diversa* (Coquillett); (S) *Trichopoda pennipes* (Fabricius); (T) *Gymnoclytia unicolor* (Brooks). Sex and body length (rounded to nearest 0.5 mm) are given for each specimen.

oviposition type (oviparous vs. ovolarviparous, respectively). However, Dexiinae were proposed as the sister group to Phasiinae by Shima (1989) who suggested that this pair is sister to (Exoristinae + Tachininae). Recently, this arrangement has found support from morphological (Cerretti *et al*., 2014) and molecular phylogenetic analyses (Winkler *et al*., 2015). Due to the small number of subfamilies, a phylogeny that defines relationships among even a single subfamily like Phasiinae can contribute much to a broader picture of tachinid evolution and history.

The subfamily Phasiinae is taxonomically distinguished from other tachinid subfamilies by their generally reduced chaetotaxy, parasitism of heteropteran hosts, oviparity (i.e. laying of unembryonated eggs) (Stireman *et al*., 2006), possession of a modified ovipositor derived from either the 8th or 10th sternite (Fig. 2H–P) (Herting, 1957) in certain lineages, and presence of an elongated medial plate of the hypandrium in males (Tschorsnig, 1985; Shima, 2015a). With respect to this last character, the medial plate of the hypandrium is elongated to such an extent that the pregonites are attached at the posterior end of the hypandrium and the hypandrial arms are apparently reduced (Tschorsnig, 1985; Fig. 2F, G). Additionally, Shima (2015a) pointed out that unlike other tachinid subfamilies (e.g., Fig. 2A), the basiphallus and distiphallus are not distinctly differentiated in Phasiinae (Fig. 2F), and this may represent a synapomorphy.

Dexiinae are the most likely sister-group candidate and are defined by potential synapomorphies in the male terminalia including an entirely membranous and strongly angled (*c*. 90°) connection between the basiphallus and distiphallus and the presence of platiform pregonites (Fig. 2E; Verbeke, 1963; Tschorsnig, 1985; Tschorsnig & Richter, 1998; Cerretti *et al*., 2014). In contrast to Phasiinae, dexiines primarily attack Coleoptera and Lepidoptera and are ovolarviparous (i.e. they lay fully embryonated and ready-to-hatch eggs) (Wood, 1987; Stireman *et al*., 2006). Given these definitions of Phasiinae and Dexiinae, several taxa cannot be confidently placed in either subfamily because they possess characters of both. These enigmatic taxa and their conflicting characters are briefly reviewed below and summarized in Table 1.

Epigrimyiini

Consistent with a placement in Phasiinae, the two genera of tribe Epigrimyiini (*Epigrimyia* Townsend and *Beskia* Brauer & Bergenstamm; Fig. 1A) parasitize Heteroptera (Guimarães, 1977; Biehler & McPherson, 1982; Sutherland & Baharally, 2002), possess 8th sternite piercers and have reduced chaetotaxy (Wood, 1987; Table 1). However, male Epigrimyiini lack the elongated hypandrium of Phasiinae. Rather, they share the male terminalic characters of Dexiinae (membranous, hinged connection between the basiphallus and distiphallus and platiform pregonites) (Tschorsnig, 1985; O'Hara & Wood, 2004) as well as that subfamily's ovolarviparity (Townsend, 1938).

Eutherini

Eutherini (*Euthera* Loew and *Redtenbacheria* Schiner; Fig. 1B) are heteropteran parasitoids (Arnaud, 1978; Nishayama *et al*., 1995), suggesting affiliation with Phasiinae. They also deposit 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 Dexiinae (Stireman *et al*., 2006; O'Hara, 2012a). However, Eutherini are ovolarviparous rather than oviparous and lack piercers (Cantrell, 1983; Townsend, 1938). Additionally, they possess the dexiine-like male terminalia trait of platiform pregonites, but the connection between the basiphallus and distiphallus is not hinged and angled as in dexiines (Fig. 2B; Tschorsnig, 1985; Cerretti *et al*., 2014). They also do not possess a phasiine-like elongated hypandrium (Table 1). Due to their oviposition strategy and egg type, the subfamily Exoristinae also has been proposed as a possible location for this enigmatic tribe (Cerretti *et al*., 2014).

Imitomyiini

Similar to the groups above, Imitomyiini (*Imitomyia* Townsend, *Proriedelia* Mesnil, and *Riedelia* Mesnil; Fig. 1C) present a confusing morphological intermediacy between Dexiinae and Phasiinae. Hosts are unknown, but *Imitomyia* females possess an unusual 8th sternite piercer and the males have an elongated hypandrium (females of other genera are unknown) – both typical phasiine traits (Townsend, 1936, 1938; Tschorsnig, 1985; O'Hara & Wood, 2004; Table 1, Fig. 2G, H). However, Imitomyiini are ovolarviparous like Dexiinae and possess the characteristic dexiine male terminalia except for the 90° hinged connection of the basiphallus to the distiphallus (Fig. 2C; Table 1; Tschorsnig, 1985; O'Hara & Wood, 2004; Cerretti *et al*., 2014). For a time, Imitomyiini were included in the subfamily Dufouriinae by Verbeke (1963) and Crosskey (1976). The tribe Dufouriini now resides within Dexiinae, which speaks to the similarity shared between imitomyiines and more traditional dexiines.

Litophasia

The genus *Litophasia* Girschner possesses a piercing 8th sternite and has highly 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 terminalia except for the 90° hinged connection of the basiphallus to the distiphallus (Tschorsnig, 1985).

Strongygastrini

In contrast to the aforementioned taxa, Strongygastrini (*Strongygaster* Macquart, *Arcona* Richter, *Melastrongygaster* Shima and *Rondaniooestrus* Villeneuve; Fig. 1M, N) have a debated phylogenetic position between the subfamilies Phasiinae and Tachininae. This tribe corresponds closely with Phasiinae with respect to male terminalia (including elongated medial plate of hypandrium) (Tschorsnig, 1985; Cerretti *et al*., 2014; Shima, 2015a). The primary argument for placing Strongygastrini within Tachininae is their unusual host associations: *Rondaniooestrus* is a parasitoid of honeybees (Villeneuve, 1924) and *Strongygaster* has been recorded parastizing heteropterans similar to other phasiines (Sabrosky & Braun, 1970; Santos & Panizzi, 1997; Panizzi & Oliveira, 1999; Golec *et al*., 2013), but also attacks a wide array of other insects including Orthoptera (Kevan & Koshnaw, 1988), Coleoptera (Arnaud, 1978), Hymenoptera (Eggleton & Belshaw, 1992; Feener, 2000),

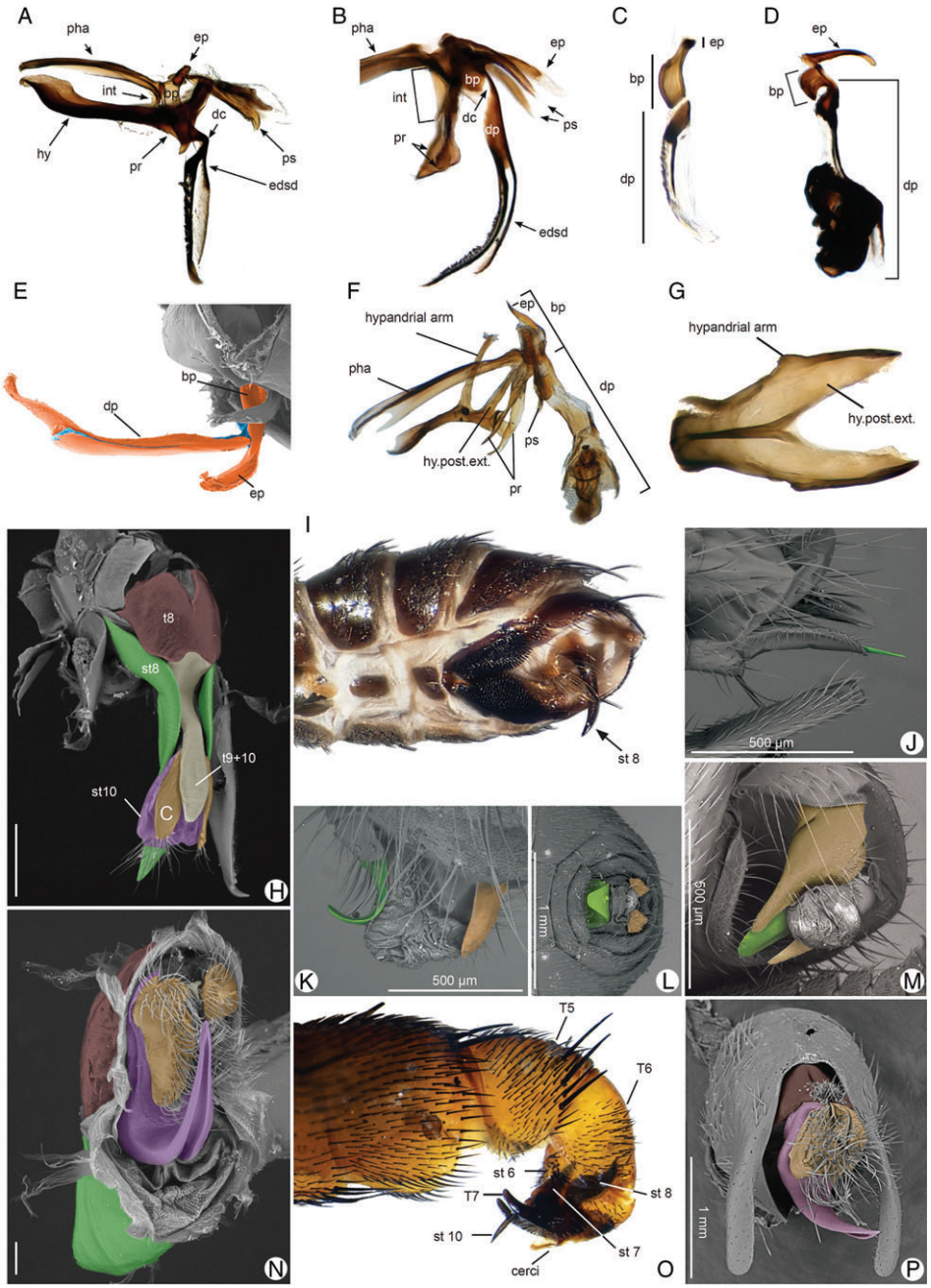

Fig. 2. Details of male terminalia in left lateral view (A–G) and details of female terminalia (H–P). (A) *Gnadochaeta* sp. (U.S.A.), hypandrial complex; (B) *Euthera* sp. (male, U.S.A.), phallus, pre- and postgonite; (C) *Imitomyia* sp. (South Africa), phallus; (D) *Opesia grandis* (Egger) (Italy), phallus; (E) *Estheria birtelei* Cerretti & Tschorsnig (Italy), phallus (colour coding: orange, sclerotized parts of basi-, epi- and distiphallus; blue, membrane); (F) *Ectophasia crassipennis* (Fabricius) (Italy), hypandrial complex; (G) *Imitomyia* sp. (South Africa), hypandrium in ventral view; (H) *Imitomyia* sp. (Kenya), oviscapt in left posterolateral view; (I) *Catharosia pygmaea* (Fallén) (Italy), abdomen with oviscapt pulled out in left lateroventral view; (J) *Phasia barbifrons* (Girschner) (Italy), posterior portion of abdomen in left lateral view; (K) *Ectophasia crassipennis* (Italy), posterior portion of abdomen in left lateral view; (L) *Gymnosoma desertorum* (Rohdendorf) (Italy), posterior portion of abdomen in ventral view; (M) *Cistogaster globosa* (Fabricius) (Italy), oviscapt in left posteroventral view; (N) *Australotachina* nr. *calliphoroides* Curran (Australia, Queensland), oviscapt in left posterolateral view; (O) *Cylindromyia bicolor* (Olivier) (Italy), abdomen with oviscapt pulled out in left lateral view; (P) *Cylindromyia bicolor* (Italy), oviscapt in left posterolateral view. Abbreviations: bp, basiphallus; dc, sclerotized dorsal connection between basiphallus and distiphallus; dp, distiphallus; edsd, extension of the dorsal sclerite of distiphallus; ep, epiphallus; hy, hypandrium; hy.post.ext., posterior extension of hypandrium; int, intermedium; pha, phallapodeme; pr, pregonite; ps, postgonite; st, sternite.

Table 1. A summary of the typical characteristics of tachinid subfamilies Phasiinae and Dexiinae and traits of several enigmatic related and/or constituent tribes.

	Phasiinae[a]	Dexiinae[a]	Epigrimyiini	Eutherini	Imitomyiini	*Litophasia*	Strongygastrini	Parerigonini
Host: Heteroptera	x		x	x	?	?	x[b]	x
Elongated hypandrium	x				x		x	x
Reduced chaetotaxy or bright colours	x		x			x		
Piercer	x		x		x	x		x
Membranous basi/distiphallus		x	x			x	x	
Hinged basi/distiphallus		x	x					
Platiform pregonites		x	x	x	x	x		
Ovolarviparity		x	x	x	x	?	x	

[a]Excluding the tribes and groups examined in the table.
[b]Not exclusively Heteroptera.

Lepidoptera (Sabrosky & Braun, 1970), Dermaptera (Sabrosky & Braun, 1970) and Diptera (Ferrar, 1977). No other phasiine regularly exploits hosts outside Heteroptera, and therefore the host use of *Strongygaster* would be unprecedented in the subfamily. Their unusual hosts, coupled with ovolarviparity, suggest affiliation with Tachininae rather than Phasiinae (Wood, 1987).

Parerigonini

The parerigonines are currently considered to be a tribe of Phasiinae (Tschorsnig, 1985; O'Hara *et al*., 2009; Shima, 2011), but have historically been placed within Tachininae (Townsend, 1936, 1938; Guimarães, 1971; Crosskey, 1973, 1976; Cantrell & Crosskey, 1989). Their external morphology is quite divergent from typical Phasiinae with superficial similarity to the Ernestiini (Tachininae; Fig. 1D–F, K). However, traits of male and female terminalia (elongated hypandrium – Tschorsnig, 1985; Shima, 2011; 8th or 10th sternite piercers – Cantrell, 1988; Shima, 2011; Fig. 2N) and hosts (Heteroptera; Mesnil, 1970; Wood & Zumbado, 2010; Shima, 2015b), where known, suggest they are more likely to belong to Phasiinae.

Materials and methods

Collection

Specimens were obtained through Malaise trapping and/or hand collection from sites around the world by the authors and a network of international collaborators (see Acknowledgements). Malaise trap specimens were preserved in 95% ethanol. Manually collected tachinids were pinned and one to three right legs from each specimen were removed and placed in 95% ethanol for DNA extraction. For some very small specimens of known species, the entire fly was preserved and used for analysis. Voucher specimens are currently housed at the following institutions (curators in brackets): Union University, Jackson, Tennessee, U.S.A. (Blaschke), University of Tennessee, Knoxville, U.S.A. (Moulton); Wright State University, Dayton, Ohio, U.S.A. (Stireman); Canadian National Collection of Insects, Ottawa, Canada (O'Hara); 'Sapienza' University of Rome, Italy (Cerretti); and Kyushu University, Fukuoka, Japan (Tachi).

Our initial goal was to collect species of representative genera of every tribe within Phasiinae as well as species from genera or tribes that have a debated affiliation with Phasiinae. This goal was nearly achieved – only the two rare South American tribes Tarassini and Euscopoliopterygini, both assigned to Phasiinae by Guimarães (1971) and known from only a few specimens, are absent from the analyses. In total, 126 specimens (80 genera; Table S1) were included in this study representing all seven widely accepted tribes of Phasiinae, an additional seven tribes or genera 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 (three species), Rhinophoridae (three species), Sarcophagidae (one species), Oestridae (one species) and Muscidae (one species).

Genes

The genes used in this study, *carbamoyl-phosphate synthetase 2/rudimentary* (*CAD*), *lethal giant larvae* (*LGL*), *methyl-accepting chemoreceptor* (*MAC*), and *molybdenum cofactor sulfurase* (*MCS*), were chosen specifically for their ability to resolve nodes within a phylogeny of rapidly evolving lineages. *CAD* was first introduced by Moulton & Wiegmann (2004), whereas the significant phylogenetic utility of *LGL*, *MAC* and *MCS* was debuted in Winkler *et al.* (2015). Each marker is a single-copy nuclear coding gene that offers significantly more phylogenetic signal in tachinids than traditional markers (i.e. *COI/II*, *28S* and *EF1-α*) which, though easier to obtain, typically exhibit too much substitutional saturation at variable sites to fully resolve the phylogeny of a young and diverse group like Tachinidae (Winkler *et al*., 2015). Sequences were processed in the Moulton Lab (University of Tennessee) with the exception of *CAD* sequences for a few taxa that were processed in the Stireman Lab (Wright State University) or the Tachi Lab (Kyushu University) (see Table S1).

Extraction and amplification

Genomic DNA was extracted using a ThermoScientific™ DNA extraction kit according to the manufacturer's protocol with few minor modifications. Post-extraction DNA samples were stored at −20°C. Custom primers for *CAD*, *LGL*, *MAC* and *MCS* were designed by Moulton (Table S2). The target genes

were amplified using 53-μL PCR reactions in MasterCycler thermal cyclers (Eppendorf North America, Westbury, NY, U.S.A.). PCR reactions combined 36 μL of ultra-pure DI H_2O, 2 μL of $MgCl_2$ (25 mM), 5 μL of 10× buffer solution, 3.5 μL dNTPs solution and 0.2 μL of *Taq* polymerase [10×, dNTPs and *Taq* from Hotstart Ex Taq kits (Takara Bio Inc., Shiga, Japan)] with 1–1.5 μL of purified template DNA and 3μL (10 μM) 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 were as follows: 30 s denaturation at 94°C; 5 cycles of 94°C for 30s, 56°C for 15 s and 72°C for 1.5 min; 5 cycles of 94°C for 30 s, 51°C for 15 s and 72°C for 1.5 min; 30 cycles of 94°C for 30 s, 46°C for 15 s 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 cleanly, annealing temperatures were increased from the default touchdown sequence of 56, 51, and 46°C to 59, 54 and 49°C with all other parameters the same.

After PCR, the amplified gene products were electrophoresed through a 1% agarose gel at 115 V for 25 min, excised from the gel, purified with various silica column-based gel extraction kits and eluted in 35μL of elution buffer (10 mM 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, CA, U.S.A.). Subsequent products were cleaned using Centri-sep purification columns (Princeton Separations, Adelphia, NJ, U.S.A.) 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, MI, U.S.A.). 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 & Wall, 1980). *CAD* and *MAC* had a single intron each, whereas *LGL* and *MCS* each contained two introns.

Phylogenetic analysis

All phylogenetic analyses were performed using the online CIPRES Science Gateway (Miller *et al*., 2010). Alignment of nucleotides was completed using the parallel version of MAFFT v6 (Katoh *et al*., 2002; Katoh & Toh, 2010). These alignments were visualized and final adjustments were made in Mesquite 3.01 (Maddison & 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 estimated from both partitioned and unpartitioned data for each individual codon position of every gene, each entire gene individually, and all data combined into a single matrix. In the final concatenated data matrix there were 12 distinct data partitions whose parameters were estimated separately but were analysed 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 robustness of phylogenetic relationships was assessed by conducting 1000 bootstrap replicates and by comparing 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 analysed. 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 2, Table S3). 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 (v2.74; Maddison & Maddison, 2015), ML methods and maximum parsimony (not shown due to similarity with ML) 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.

Results

Sequence data

In total, the nucleotide data matrix consisted of 7651 aligned sites for 126 taxa with >91% gene-by-taxa coverage. Sequences were submitted to Genbank and assigned unique accession numbers (Table S1). Sequence summary statistics are given in Table 3 including information on total/average length and base frequencies.

Table 2. Characters examined for Ancestral State Reconstruction (ASR).

Character/States	0	1	2	3	4	5
Female sternite 8	Unmodified	Shovel-like	Piercer-like	Corkscrew		
Female sternite 10	Unmodified	Piercer-like				
Connection of basi/distiphallus	Sclerotized	Membranous and hinged	Membranous and not hinged			
Posterior extension of hypandrium - elongation	Absent	Present				
Platiform pregonites	Absent	Present				
Oviposition strategy	Oviparity	Ovolarviparity				
Hosts	Heteroptera	Lepidoptera	Coleoptera	Hymenoptera	Orthoptera	Isopoda

Table 3. Summary statistics of genes used for phylogeny.

		Sequence length			Base frequencies			
Genes	# Taxa	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

CAD, carbamoyl-phosphate synthetase 2/rudimentary; *LGL, lethal giant larvae*; *MAC, methyl-accepting chemoreceptor*; *MCS*, *molybdenum cofactor sulfurase*.

Inferred trees

A total of 28 inferences resulting from phylogenetic analyses outlined below can be accessed through TreeBase (ID# 20747). Bayesian analyses were conducted on each gene individually (e.g. CADBayes, LGLBayes, MACBayes, and MCSBayes) as well as the concatenated, partitioned dataset (i.e. CLMMBayes). Maximum likelihood analyses were more exhaustive, with five analyses conducted per gene: one on each individual codon position (e.g. CAD1, CAD2, CAD3), one with third positions removed (i.e. CAD12), and one with all positions included and partitioned separately (i.e. CAD123). A total evidence ML analysis was conducted upon all three codon partitions for each gene (i.e. CLMM123). Two additional ML analyses were conducted, one with *Imitomyia* excluded (not shown) and another with *LGL* excluded (i.e. CMM123), to investigate the effects of putative long-branch attraction or conflicting phylogenetic signal,

This large number of analyses employing independent lines of evidence provides insight into which clades and relationships were strongly supported. Given the substantial variation in substitution rates among codon positions, clades supported by analyses of each codon position provide confidence in the evolutionary validity of that clade – even more so if multiple genes are also providing similar signal. The relative frequency of each clade's appearance across all phylogenies is summarized in Fig. 3.

A substantial number of clades were present in over 70% of the trees. This is interpreted as strong evidence that these clades accurately represent evolutionary relationships. These clades include Catharosiini, Strongygastrini, 'Trichopodini', Gymnosomatini s.l., Epigrimyiini + Eutherini, Hermyini, Leucostomatini, *Zita* + *Leverella*, Gymnosomatini s.s., Phasiini, Exoristinae and Rhinophoridae – the first five of which are absent only 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 (e.g. using only codon position 2 or 3 across all genes, *LGL* all individual codon positions) as expected. With the inclusion of *Imitomyia*, both Phasiinae and Dexiinae are rarely recovered as monophyletic. However, with *Imitomyia* removed, these clades are usually recovered as monophyletic sister groups.

MCS proved to be the single most important gene for this phylogenetic analysis, recovering ~85% of the final clades present in the total evidence tree. The utility of *MCS* was closely followed by *MAC*, then *CAD*, with *LGL* lagging behind. *LGL* was unable to recover several clades on its own, especially ones outside of Phasiinae. A comparison of the phylogenetic utility of each gene and their natural partitions is shown in Fig. 4.

Bayesian and ML analyses of the partitioned, concatenated dataset reconstructed identical phylogenies that had very similar support for every clade recovered (Fig. 5: Phasiinae collapsed, Fig. 6: Phasiinae tribal detail, and Figure S1: transformed, all nodes shown with supporting statistics). This tree is hypothesized to be the best estimate of the evolutionary history of these taxa using these genes. Therefore, this reconstruction's clade structure and node support is evaluated and used as a reference phylogeny for further investigation of the phylogenetic signal of individual genes and codon positions. Node support statistics for ML and Bayesian inferences, are represented by 'bs' (bootstrap) and 'pp' (posterior probability), respectively.

Outgroups

Nine non-tachinid outgroups were included in this phylogenetic analysis, with the muscid *Musca domestica* (L.) rooting the tree. The single sarcophagid (*Macronychia* sp.) and oestrid species (*Cuterebra austeni* Sabrosky) included formed a moderately supported sister group (bs = 53; pp = 96). The three rhinophorid species included formed a highly supported

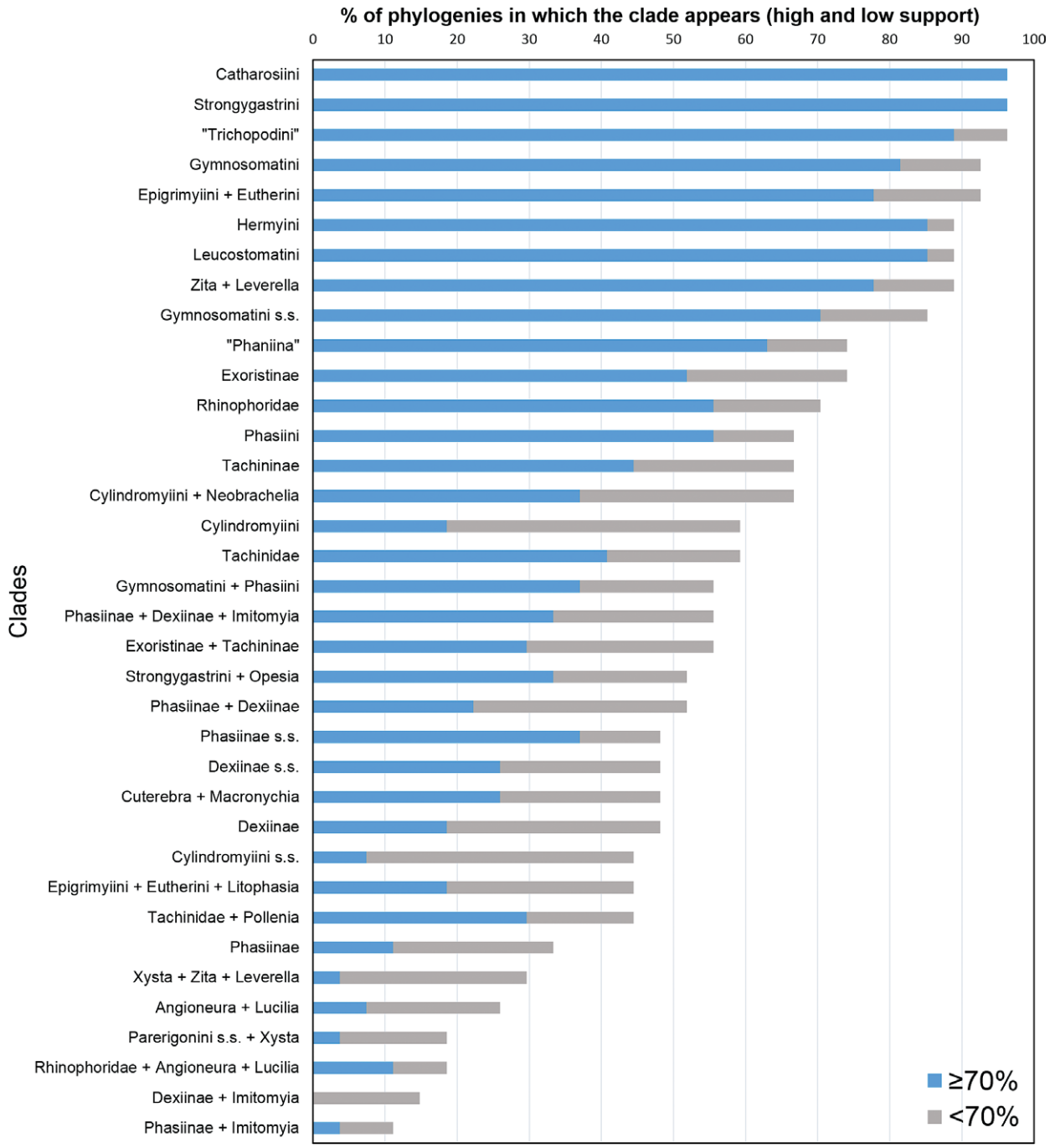

Fig. 3. Frequency of clade appearance across all 28 phylogenies as a percentage of totals. [Colour figure can be viewed at wileyonlinelibrary.com].

clade ((*Axinia* sp. + *Melanophora roralis* (L.)) + *Rhinomorinia* sp.; bs = 100; pp = 100) closely allied with the calliphorid sister taxa *Angioneura abdominalis* (Reinhard) and *Lucilia sericata* Meigen (bs = 83; pp = 55). A third calliphorid species, *Pollenia pediculata* Macquart, was reconstructed as sister to Tachinidae with very high support (bs = 99; pp = 100). The family Tachinidae itself forms a clearly monophyletic group (bs = 100; pp = 100).

Tachininae + Exoristinae

Tachinidae are represented in this phylogeny by two lineages. The first clade comprises the subfamilies Tachininae + Exoristinae, and the second comprises 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;

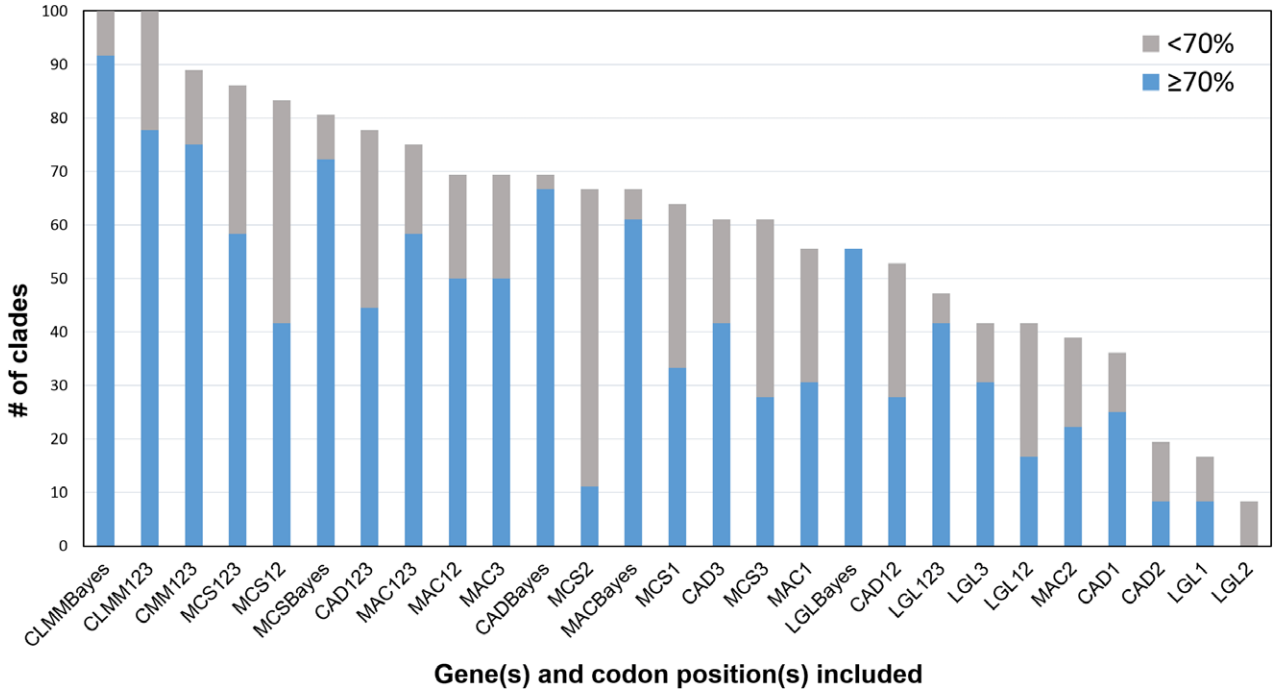

Fig. 4. Comparison of genetic data partitions by percentage of reconstructed clades. CLMM, combined data from *CAD*/*LGL*/*MCS*/*MAC*. Number suffixes indicate codon positions used. *CAD, carbamoyl-phosphate synthetase 2*/*rudimentary*; *LGL, lethal giant larvae*; *MAC, methyl-accepting chemoreceptor*; *MCS*, *molybdenum cofactor sulfurase* [Colour figure can be viewed at wileyonlinelibrary.com].

pp = 100). The included tribes of Tachininae and Exoristinae were represented by a single genus each except for the Tachinini for which two common species were included (see Table S1). 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 Exoristinae, the tribes were laddered as follows: (((((Erycini + Goniini) + Blondeliini) + Exoristini) + Winthemiini) + Acemyini). The somewhat enigmatic taxon *Ceracia dentata* (Coquillett) included in Acemyini, which has historically been placed in both of these subfamilies, was robustly reconstructed as a member of Exoristinae (as Herting, 1960 argued) – sister to all other included exoristine taxa (bs = 100; pp = 100).

Dexiinae

Dexiinae have previously been shown to have a close association with Phasiinae (Cerretti *et al*., 2014; Winkler *et al*., 2015) and most taxonomically ambiguous genera considered here are morphologically intermediate between these two subfamilies. To better assess the placement of these enigmatic groups, Dexiinae were sampled more thoroughly than Tachininae and Exoristinae. Eight tribes represented by ten genera formed a highly supported clade, here termed Dexiinae s.s. (bs = 100; pp = 100). Within this clade, the three genera of tribe Dexiini (represented by *Billaea* sp., *Euchaetogyne roederi* (Williston) and *Ptilodexia harpasa* Walker) coalesced into an expected clade (bs = 100; pp = 100). *Uramya pristis* (Walker) + *Voria ruralis* (Fallén) formed a sister group (bs = 64; pp = 95), as did *Thelaira americana* Brooks + *Campylocheta semiothisae* (Brooks) (bs = 79; pp = 95), but with lower support for each grouping. Outside these clades, most intertribal relationships within Dexiinae s.s. were less supported (e.g. Dufouriini sister to clade *Thelaira americana* + *Campylocheta semiothisae*, together forming a sister group to the rest of Dexiinae s.s.).

Most interestingly, the sister clade to Dexiinae s.s. was composed of a well-supported clade (bs = 91; pp = 100) consisting of the tribes Eutherini and Epigrimyiini, along with the genus *Litophasia*. Both genera of Epigrimyiini (*Epigrimyia* and *Beskia*) were represented (bs = 100; pp = 100) and three species of *Euthera* were included (bs = 100; pp = 100). Epigrimyiini and Eutherini were reconstructed as sister taxa with *Litophasia* sister to them. This clade forms the sister clade to Dexiinae s.s. (bs = 99; pp = 100).

Phasiinae

Phasiinae form a weakly supported clade (bs = 60; pp = 84). Statistical support for Phasiinae dramatically increases (bs = 95; pp = 100) when a single enigmatic taxon is excluded: *Imitomyia sugens* (Loew). *Imitomyia* is a strange genus with unknown hosts and morphological characteristics that defy easy placement in either Phasiinae or Dexiinae. The only analyses that place *Imitomyia* within 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 to both (ML trees) or as an unresolved polytomy of Dexiinae, Phasiinae and *Imitomyia* (Bayesian trees).

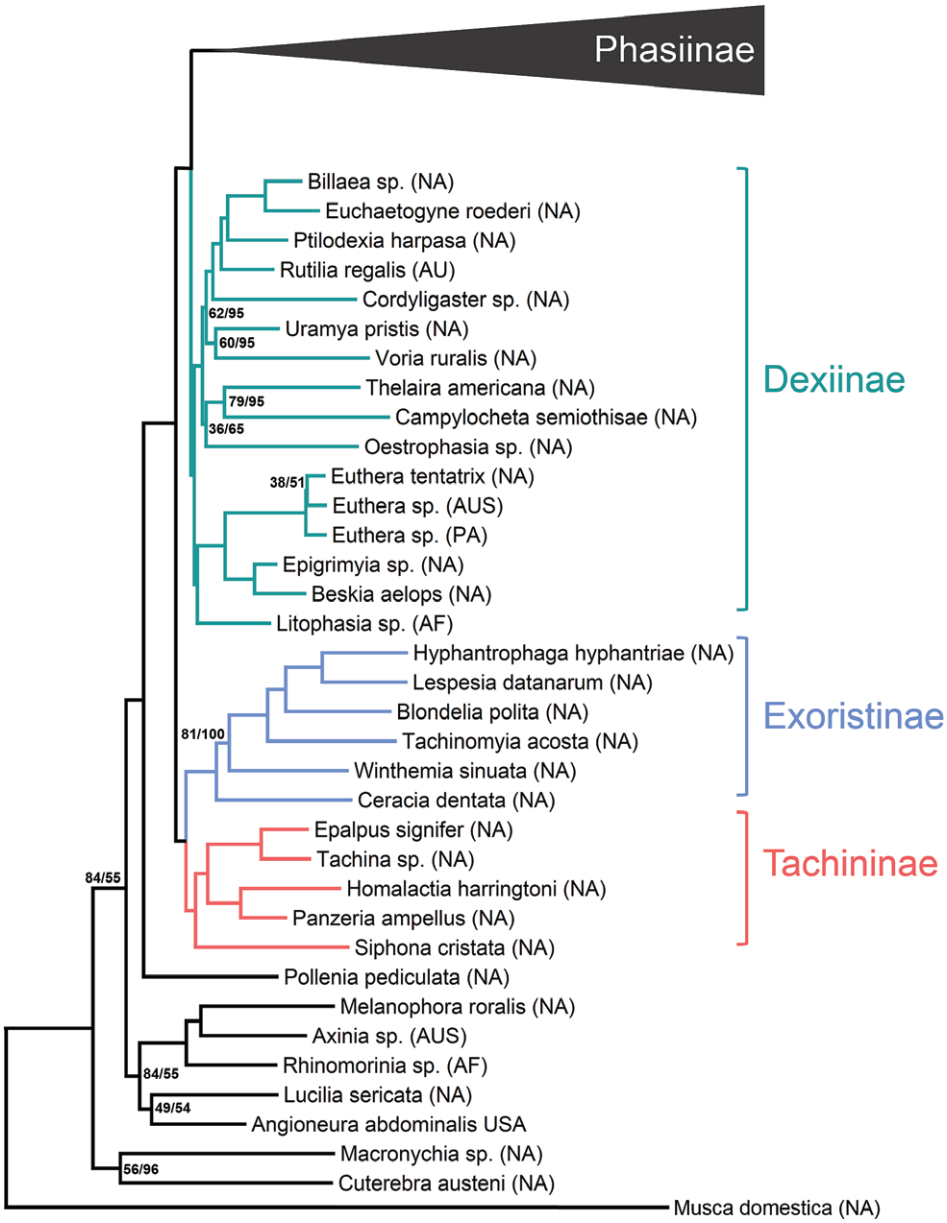

Fig. 5. Phylogram of Tachinidae with the phasiine node collapsed estimated from the concatenated and partitioned dataset. Node support is provided for nodes with <90% ML support (ML/PP).

Within Phasiinae, ten clades are clearly defined, namely the tribes Catharosiini, Cylindromyiini (+ several Parerigonini), Gymnosomatini, Hermyini, Imitomyiini, Leucostomatini, Parerigonini (most current genera + *Xysta holosericea* (Fabricius)), Phasiini, Strongygastrini and *Opesia grandis* (Egger). Each of these clades except Parerigonini (+ *Xysta holosericea*) was recovered with maximum possible support (bs = 100; pp = 100). Additionally, within the Gymnosomatini, two strongly supported clades were recovered: a Gymnosomatini s.s. clade (bs = 100; pp = 100) and a 'Trichopodini' clade (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 = 57; pp = 93, + (Strongygastrini + *Opesia grandis*) + Hermyini): bs = 100; pp = 100, Parerigonini s.s. (including *Xysta holosericea*): bs = 38; pp = 81, and (Cylindromyiini + Imitomyiini + *Pygidimyia* sp., *Australotachina* (Curran) and G. nr. *Neobrachelia* Townsend): bs = 30; pp = 55.

Conclusive subfamily placement for almost all morphologically intermediate taxa was achieved with robust support. The Epigrimyiini, *Euthera* and *Litophasia* were all recovered as a clade of Dexiinae rather than Phasiinae. *Strongygaster* Macquart and *Rondaniooestrus* Villeneuve, as well as all sampled genera

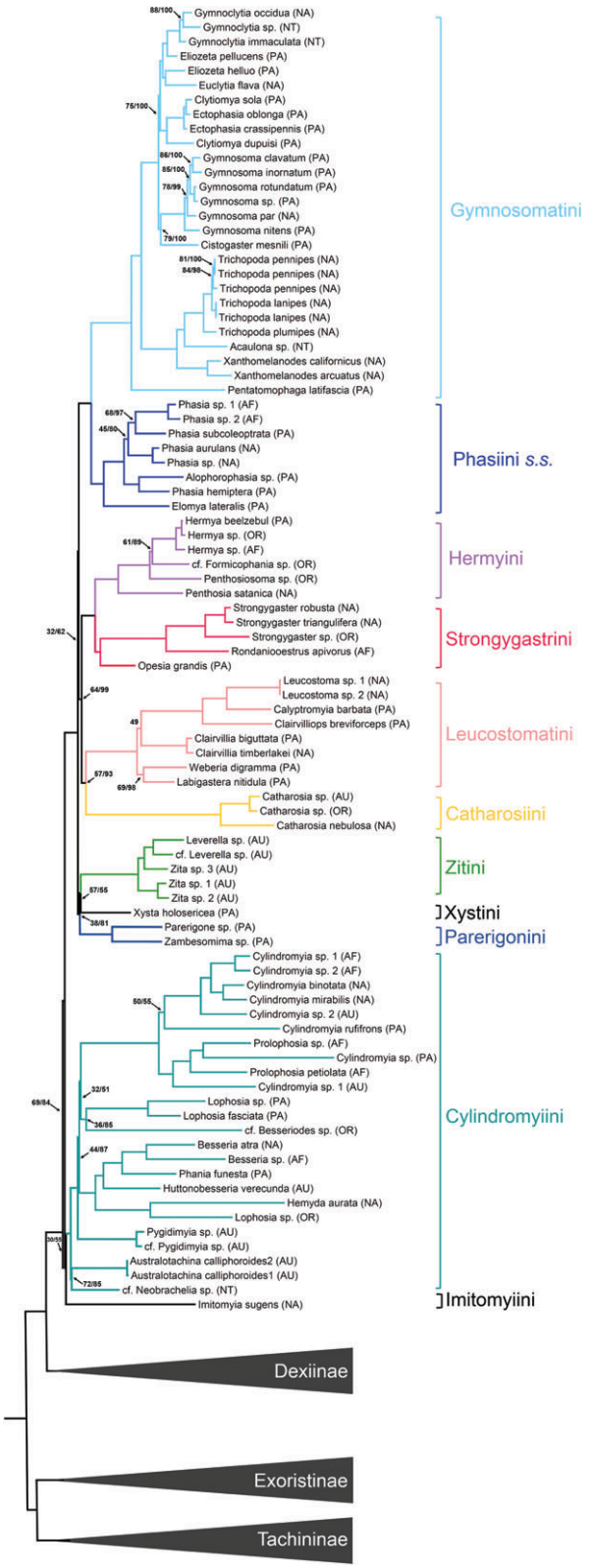

Fig. 6. Phylogram of Phasiinae with outgroup taxa collapsed estimated from the concatenated and partitioned dataset. Node support is provided for nodes with <90% ML support (ML/PP).

of Parerigonini s.l., were firmly nested within Phasiinae rather than Tachininae. Only the placement of *Imitomyia* is poorly resolved.

Clades of Phasiinae

Gymnosomatini + Phasiini (Fig. 1J, L, R–T)

The Gymnosomatini s.l. clade is composed of two subclades: the Gymnosomatini s.s. and the former 'Trichopodini' (recently included within Gymnosomatini by O'Hara & Cerretti, 2016 based on findings of Cerretti *et al*., 2014). The latter are represented by ((*Trichopoda* Berthold + *Acaulona* van der Wulp) + *Xanthomelanodes* Townsend). Most sampled genera currently assigned to Phasiini (e.g. *Clytiomya* Rondani, *Ectophasia* Townsend, *Eliozeta* Rondani and *Euclytia* Townsend) were reconstructed as members of Gymnosomatini s.s., where they formed a clade with *Gymnoclytia* Brauer and Bergenstamm. *Cistogaster* Latreille was found to be sister to the six sampled *Gymnosoma* Meigen species. *Pentatomophaga latifascia* (Villeneuve) was reconstructed as sister taxon to the rest of Gymnosomatini s.l. and as such may represent an early branching lineage. Two other genera currently in Phasiini, *Opesia* Robineau-Desvoidy and *Xysta* Meigen, were placed rather distant from Phasiini as early branching members of other lineages (see below). Only *Phasia* Latreille and *Elomya* Robineau-Desvoidy of the sampled Phasiini clustered together and this clade was sister to Gymnosomatini s.l.

Leucostomatini + Catharosiini (Fig. 1P–Q)

The Catharosiini clade consists of three species of *Catharosia* Rondani and was reconstructed as sister to the Leucostomatini – here represented by six genera in two clades: (((*Leucostoma* Meigen + *Calyptromyia* Villeneuve) + *Clairvilliops* Mesnil) + *Clairvillia* Robineau-Desvoidy) and (*Weberia* Robineau-Desvoidy + *Labigastera* Macquart). *Litophasia*, presently classified in the Catharosiini, was reconstructed as a dexiine (see above).

(Strongygastrini + Opesia) + Hermyini (Fig. 1L–O)

Three *Strongygaster* species and the honeybee parasitoid *Rondaniooestrus apivorous* form the Strongygastrini clade. Sister to Strongygastrini is *Opesia*, a genus currently assigned to Phasiini but here forming a strongly supported clade with the Strongygastrini that is in turn sister to the Hermyini (bs = 100; pp = 100). The relatively small tribe Hermyini was well represented by three species of *Hermya* Robineau-Desvoidy and three additional genera (cf. *Formicophania* Townsend, *Penthosiosoma* Townsend, and *Penthosia* van der Wulp). *Penthosia*, currently classified in the Cylindromyiini, is here recovered as the lone New World hermyiine and sister to the remaining Old World Hermyini.

Parerigonini clade 1 + Xysta (Fig. 1J, K)

Our analyses revealed two separate lineages within the currently recognized Parerigonini. The 'core' lineage is represented by

the Asian genera *Parerigone* Brauer and *Zambesomima* Mesnil, the Australasian genera *Zita* Curran and *Leverella* Baranov, and the West Palaearctic *Xysta* (currently classified in Phasiini; Herting, 1984; Cerretti, 2010). A separate lineage comprising an undescribed South American genus near *Neobrachelia* and the aberrant Australian genera *Australotachina* Curran and *Pygidimyia* Crosskey are reconstructed at the base of the tribe Cylindromyiini (see below).

The Parerigonini s.s. + *Xysta* clade is weakly supported (bs = 39; pp = 80). The position and support of the *Zita* + *Leverella* clade is consistent across genes and codon positions, but placement of *Xysta* and the remaining Parerigonini is much more unstable. For example, *MAC* weakly supports *Xysta* as sister to the Hermyini + Strongygastrini rather than with *Zita* + *Leverella*. *CAD* groups *Xysta* with *Zita* + *Leverella*, but places *Parerigone* and *Zambesomima* as sister to the large clade of (Hermyini + Strongygastrini) + (Leucostomatini + Catharosiini). In no analysis are *Parerigone* + *Zambesomima* more closely related to *Zita* + *Leverella* than is *Xysta*, implying a tribal division is needed in the classification of Parerigonini s.s.

Cylindromyiini + Parerigonini clade 2 (Fig. 1D–I)

The other lineage of Parerigonini ('clade 2'), consisting of 'cf. *Neobrachelia* sp.', *Australotachina* and *Pygidimyia*, was found to be closely allied with the Cylindromyiini (bs = 100; pp = 100). The first two 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 two genera, but was ultimately reconstructed sister to the Cylindromyiini s.s. (bs = 100; pp = 100).

Cylindromyiini s.s. is composed of two clades which correspond to Herting's (1983) 'Cylindromyiina' and 'Phaniina.' The Phaniina group has maximum statistical support (bs = 100; pp = 100) and is composed of the genera *Besseria* Robineau-Desvoidy, *Phania* Meigen, *Huttonobesseria* Curran, *Hemyda* Robineau-Desvoidy, and a single difficult to identify specimen near *Lophosia* Meigen (most likely not a *Lophosia sensu* Crosskey, 1976). Species of *Cylindromyia* Meigen are mixed with those of *Lophosia* and *Prolophosia* Townsend in a clade that enjoys far less statistical support (bs = 29; pp = 51). The grade of *Lophosia* species sometimes groups with *Pygidimyia* rather than *Cylindromyia*. The endemic African genus *Prolophosia* was recovered within *Cylindromyia*. The Cylindromyiini s.l. were weakly reconstructed as sister to the unusual and phylogenetically unstable genus *Imitomyia* as discussed above.

Evolution of Phasiinae: female piercers, male terminalia and hosts

Ancestral state reconstructions of character evolution suggest that abdominal piercing structures in females have evolved at least three times in Phasiinae, with piercers evolving from the 8th sternite at least twice and from the 10th sternite once (Fig. 7). However, reconstructions suggest that 8th sternite piercers have been reduced (i.e. evolved into shovel-like structures) and lost multiple times in Phasiinae. Piercing structures have also evolved from the 8th sternite at least three times in Dexiinae (*Oestrophasia* Brauer and Bergenstamm, Epigrimyiini and *Litophasia*).

In the male terminalia, an elongated medial plate of the hypandrium appears to be a clear synapomorphy of Phasiinae (including *Imitomyia*; Figure S2, Fig. 2G) and platiform and elongated pregonites (as defined by Tschorsnig, 1985) are a synapomorphy of Dexiinae (but present also in *Imitomyia*; Figure S2). The L-shaped, hinged connection between the basiphallus and distiphallus defines almost all of Dexiinae but is lacking in *Euthera* and is present although not strongly bent and hinged in *Litophasia* (and in *Imitomyia* as well; Figure S3, Fig. 2C). Reconstruction of this latter character state with equal probability of gains and losses suggests that the L-shaped, hinged phallus evolved independently in Dexiinae s.s. and Epigrimyiini or was lost in Eutherini and *Litophasia*.

Host-use reconstructions indicate that the ancestor of Dexiinae + Phasiinae most likely parasitized Heteroptera and this trait was subsequently lost in Dexiinae s.s. and in the Strongygastrini (Phasiinae) (Fig. 8). The ancestral reproductive strategy of Tachinidae is reconstructed as ovolarviparity, with the oviparous Phasiinae having reverted only once to ovolarviparity (Strongygastrini; Figure S4).

Discussion

This study is the largest molecular analysis of Tachinidae to date and the first to consider in depth the classification and evolution of the subfamily Phasiinae. The additional effort required to amplify our target genes resulted in relatively high phylogenetic resolution at the subfamily level, and for controversial taxa, better resolution than obtained in previous attempts at tachinid molecular systematics (Stireman, 2002; Tachi & Shima, 2010). Several previous hypotheses about tribal relationships and affinities gained substantial evidence in their favour and a number of novel hypotheses about phasiine taxonomy and evolution were generated. In general, this molecular phylogeny agrees remarkably well with current concepts of phasiine classification with some important exceptions.

Here, phasiine relationships are briefly summarized and molecular evidence for the relationships 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, we evaluate the evolutionary history of ecologically and systematically important character traits that may serve as clade-defining synapomorphies.

Phylogenetic positions of key taxa

Our results indicate that Strongygastrini and Parerigonini belong to the Phasiinae clade, but Epigrimyiini, Eutherini and

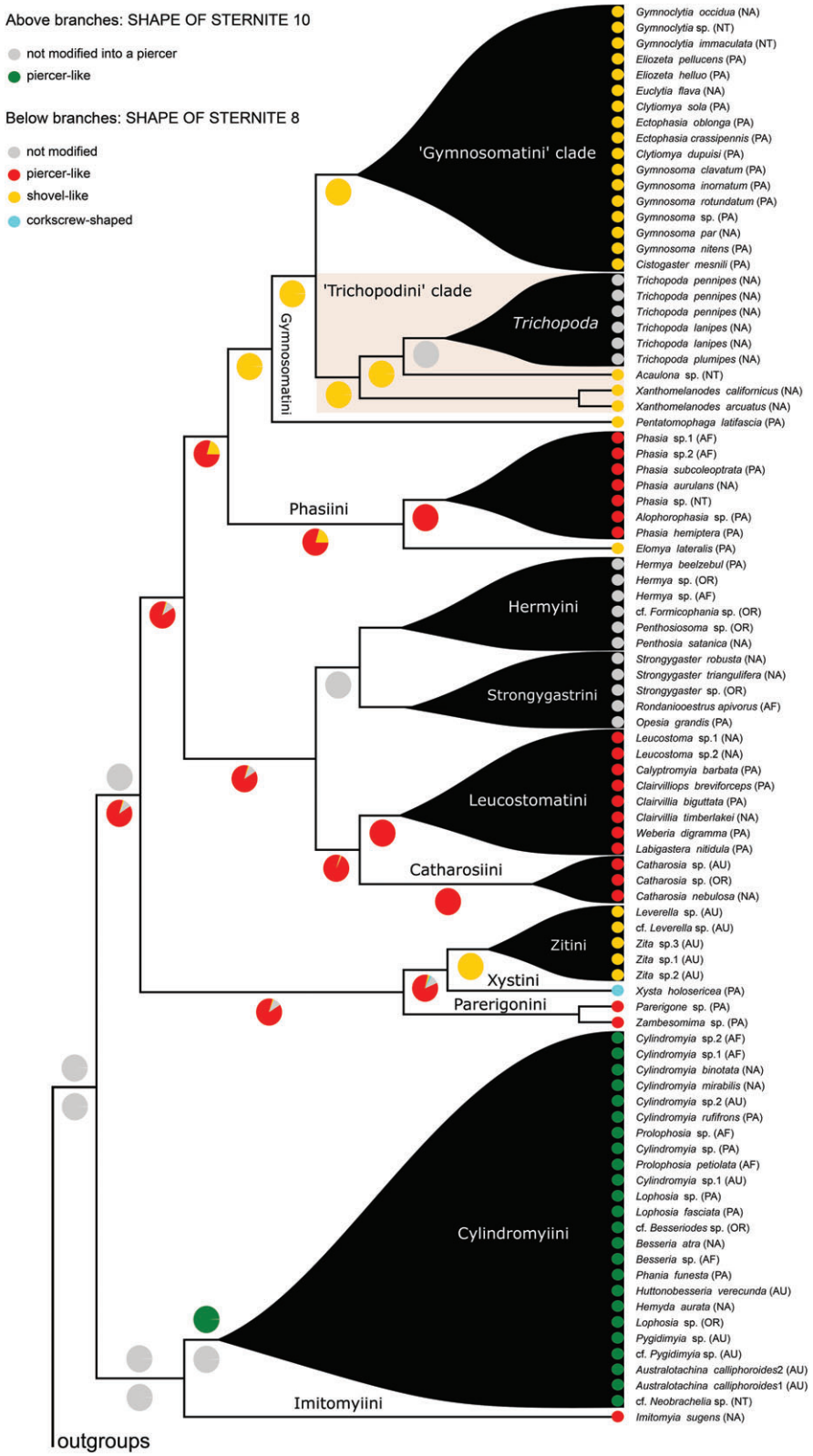

Fig. 7. Maximum-likelihood (ML) ancestral state reconstruction of piercer evolution across Phasiinae, mapped on the partitioned, concatenated ML analysis. Pie charts indicate proportional likelihood. Region abbreviations: AF, Afrotropical; AU, Australasian; NA, Nearctic; NT, Neotropical; OR, Oriental; PA, Palaearctic.

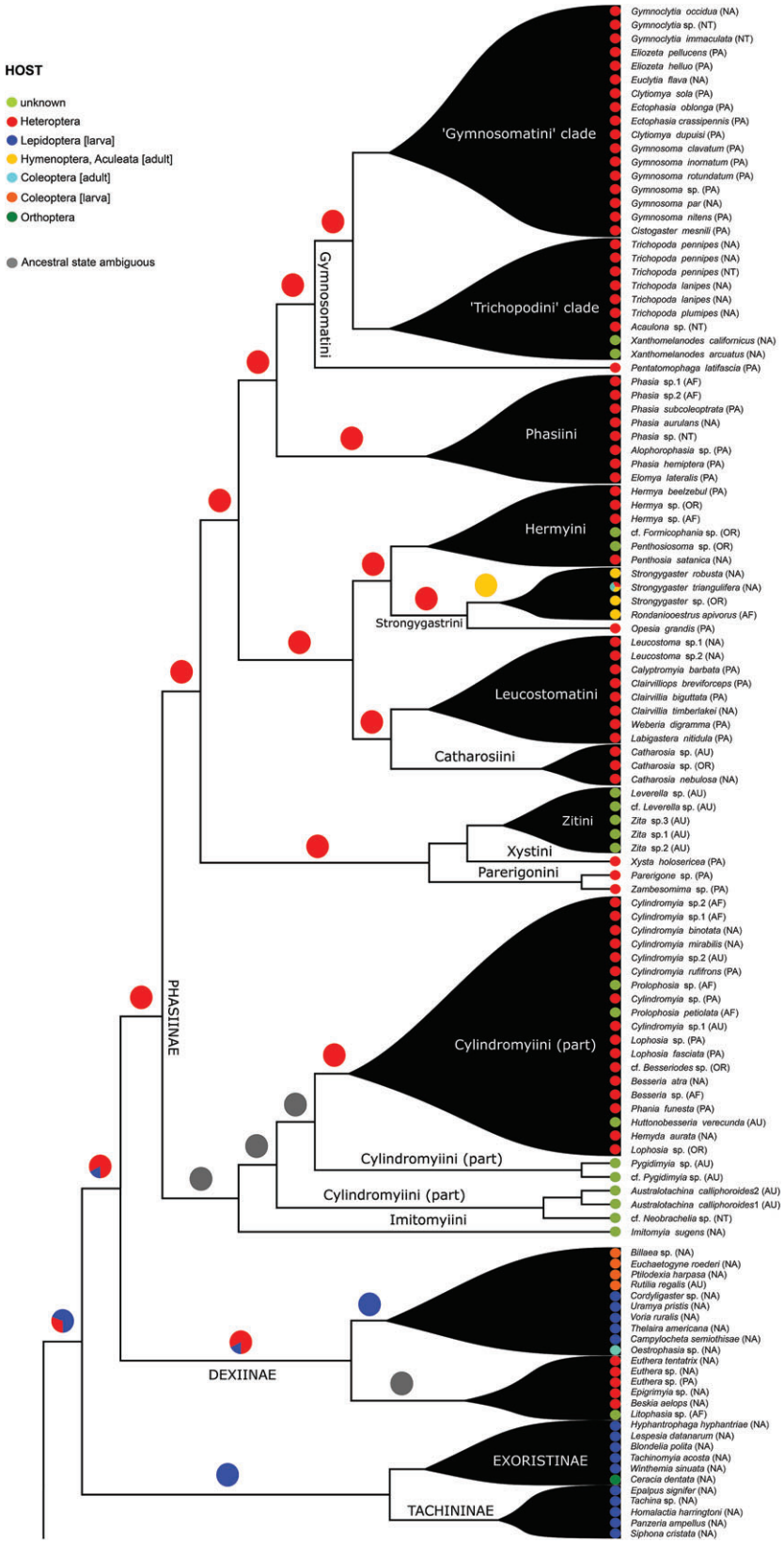

Fig. 8. Maximum likelihood ancestral state reconstruction of host use across Phasiinae, mapped on the partitioned, concatenated ML analysis. Pie charts indicate proportional likelihood. Abbreviations: AF = Afrotropical Region; AU = Australasian Region; NA = Nearctic Region; NT = Neotropical Region; OR = Oriental Region; PA = Palaearctic Region.

the genus *Litophasia* do not, whereas the position of Imitomyiini is inconclusive. Morphologically, Phasiinae can be defined by the synapomorphic trait of an elongated medial plate of the hypandrium of the male terminalia. In addition, all tachinids with a piercer derived from the 10th sternite (postgenital plate; Fig. 2N–P) are found in Phasiinae and the vast majority of phasiine species (*c*. 99%) use Heteroptera exclusively as hosts. However, not all phasiines have reduced chaetotaxy, are oviparous, possess piercers or even parasitize Heteroptera. Furthermore, a few tachinid genera that attack Heteroptera and/or have piercers derived from the 8th sternite are placed in Dexiinae rather than Phasiinae, making these traits homoplastic. Female and male terminalic characters appear to be better indicators of evolutionary history than other traits, such as reduced setation and hymenopteran mimicry, or oviposition strategy. Despite generally strong congruence with taxonomic classification, several phasiine tribes were identified as unnatural groupings of genera and will need to be reorganized into a more evolutionarily meaningful classification (see below).

Recent molecular and morphological analyses have supported Herting (1960) and Mesnil (1966) in placing Strongygastrini within Phasiinae (Cerretti *et al*., 2014; Winkler *et al*., 2015), and questions about its subfamily affinities should now be resolved. Multiple genera of Parerigonini have never before been analysed 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 in the present study – supporting the morphology-based conclusions of Mesnil (1966), Herting (1983) and others. The position of *Imitomyia* is more unstable in our analyses and its sister clade has yet to be determined, but molecular and morphological evidence suggests a placement within Phasiinae.

In contrast to the taxa above, Epigrimyiini, Eutherini and *Litophasia* were found to be allied with Dexiinae rather than Phasiinae. Although this affinity has been previously hypothesized (Tschorsnig, 1985), this is the first quantitative phylogenetic evidence that these taxa belong to Dexiinae. The morphological analysis of Tachinidae of Cerretti *et al*. (2014) found *Litophasia* to be a 'basal' phasiine (Dexiinae were paraphyletic), whereas the Eutherini were most often recovered within Exoristinae (Epigrimyiini were not represented). Here, Epigrimyiini and Eutherini were recovered as sister taxa within Dexiinae in almost every partition and their clade is one of the strongest in this analysis. The close relationship between these two tribes has not been suggested previously and is an unexpected result from the genetic evidence. The taxonomic history and phylogenetic affinities of each of these tribes is discussed below.

Epigrimyiini

Our reconstruction of Epigrimyiini (*Beskia* and *Epigrimyia*) as allied with Dexiinae supports recent arguments and classification based on distinctive secondary structures of the male terminalia (Tschorsnig, 1985; O'Hara & Wood, 2004). However, this group shares some traits with Phasiinae and may represent a lineage that has maintained several traits that were present in the common ancestor of Dexiinae and Phasiinae but lost in most extant dexiines. In addition to their external habitus, which is more reminiscent of *Cylindromyia* and relatives than any other dexiine, *Epigrimyia* and *Beskia* also share the heteropteran hosts of the phasiines. *Epigrimyia* is known to attack *Galgupha ovalis* Hussey (Hemiptera: Corimelaenidae) (Biehler & McPherson, 1982) and *Beskia* has been reared from multiple species of Pentatomidae, most notably the rice stink bug (*Oebalus pugnax* (F.)) (Guimarães, 1977; Sutherland & Baharally, 2002). Additionally, epigrimyiines possess 8th sternite piercers which, until the present study, have been associated primarily with Phasiinae (Townsend, 1938).

Eutherini

Similar to Epigrimyiini, Eutherini share morphological and behavioural traits with both Phasiinae and Dexiinae. Two genera are included in the tribe: *Euthera*, composed of 12 species and distributed worldwide (O'Hara, 2013a), whose colouration and shape resembles that of deer flies (Tabanidae: *Chrysops* Meigen), and *Redtenbacheria*, consisting of the sole species *R. insignis* Egger and found only in the Palaearctic Region (Herting, 1984; O'Hara *et al*., 2009). The phylogenetic position of Eutherini is controversial, with experts arguing for their inclusion in Phasiinae (Guimarães, 1971; Crosskey, 1977; Herting, 1984) or Dexiinae (Shima, 1989; O'Hara & Wood, 2004). Phylogenetic affiliation with Phasiinae is suggested by their parasitism of heteropterans (Arnaud, 1978; Nishayama *et al*., 1995) and laying of planoconvex eggs (Herting, 1966; Mesnil, 1966). However, the development and oviposition of these eggs is unlike any phasiine outside Strongygastrini.

Phasiinae lack a modified uterus and are therefore oviparous (i.e. lay unincubated eggs). In contrast, Eutherini are ovolarviparous (i.e. lay incubated eggs; Cantrell, 1983). Furthermore, traits of the male terminalia including the shape and location of the pregonites, postgonites, sternite 5 and the distiphallus suggest an association with Dexiinae (Tschorsnig, 1985; O'Hara & Wood, 2004; Cerretti *et al*., 2014). Yet, unlike the Epigrimyiini discussed above, Eutherini lack an L-shaped membranous connection between the basiphallus and distiphallus – the most convincing synapomorphy of Dexiinae, and neither do they possess the elongated medial plate of the hypandrium characteristic of Phasiinae. The unusual nature of *Euthera* led Shima (1989) to place *Euthera* as the most primitive member of Dexiinae on a cladogram of tachinid relationships. As with Epigrimyiini, the hypothesis that Eutherini belongs in Dexiinae and may have retained some ancestral traits as a transitional taxon between the two subfamilies is strongly supported by this molecular phylogeny. This result stands in contrast to an earlier morphological phylogeny that nested Eutherini within Exoristinae (Cerretti *et al*., 2014). Although the monophyly of Eutherini was strongly supported in that study, its placement in Exoristinae was largely a result of their egg type and oviposition strategy.

Litophasia

The atypical genus *Litophasia* is known from three species in the Palaearctic and Afrotropical regions (Herting & Dely-Draskovits, 1993; O'Hara & Cerretti, 2016). It was initially thought to belong to Rhinophoridae (van Emden, 1945; Crosskey, 1977), but is now accepted as a tachinid, despite lacking the almost universal trait of an enlarged subscutellum (Wood, 1987; Tschorsnig & Richter, 1998). Females possess a piercer (8th sternite) similar to that found in the phasiine tribes Catharosiini and Leucostomatini (Dear, 1980), leading to their current placement in the Catharosiini (Crosskey, 1980; Belshaw, 1993; Herting & Dely-Draskovits, 1993; O'Hara & Cerretti, 2016). However, *Litophasia* possesses a dexiine-type membranous connection between the basiphallus and distiphallus (although these are not attached at a right angle; Tschorsnig, 1985) and lacks the phasiine-type elongated hypandrial plate. It is thus not surprising that in the morphological analysis of Cerretti *et al*. (2014), *Litophasia* was inconsistently placed as either sister to Phasiinae or Dexiinae depending on the weighting scheme. Strong supporting evidence for a position within Dexiinae is indicated by the current analysis where *Litophasia* was reconstructed with high support as sister to Epigrimyiini + Eutherini, with this clade in turn sister to the remaining Dexiinae.

Evolution of Tachinidae

Analyses of trait evolution are strongly dependent on the accuracy of phylogenetic reconstructions, the sampling of taxa and the method of reconstruction employed. The phylogenetic reconstruction presented here largely corroborates current taxonomic hypotheses and is generally well supported. The robustness of this phylogeny provides some confidence in reconstructions of character evolution, but central to the ancestral state reconstruction is the phylogenetic position of the 'phasiine-like dexiines': Epigrimyiini, Eutherini, *Litophasia* and *Imitomyia*. With their curious amalgamation of traits, the phylogenetic positions of these taxa heavily influence any potential subfamily synapomorphies as well as interpretations of tachinid evolution in general.

To summarize our analyses of character evolution, 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 medial plate of the hypandrium in the male terminalia. In contrast, Dexiinae cannot be defined by a single unique synapomorphy, although there are several useful characters that are nearly universally present across dexiine taxa (see above). None of the potentially synapomorphic traits of the male terminalia were recovered exclusively among Dexiinae, mainly due to the tentative placement of *Imitomyia* in Phasiinae.

Piercers

Piercers that are used to inject eggs directly into the body cavity of hosts have evolved several times throughout tachinid evolutionary history and appear in many tribes. However, not all piercers are homologous. Those found in Exoristinae (e.g. Blondeliini) are derived from the 7th sternite, whereas those in Phasiinae have evolved from either the 8th or 10th sternite (Fig. 7).

Only cylindromyiines possess 10th sternite piercers (Townsend, 1938; Herting, 1957, 1983; Cantrell, 1988; Fig. 2N–P), which most likely evolved only a single time in the ancestor of Cylindromyiini s.l. (i.e. including the former parerigonine genera *Australotachina*, *Pygidimyia* and an undescribed genus near *Neobrachelia*). The evolution of piercers from the 8th sternite piercer appears more labile, appearing in both Dexiinae and Phasiinae, with an estimated three separate origins in the former, two in the latter, and multiple losses or reductions among Phasiinae (Fig. 7). Four clades of phasiines can be wholly or partially defined by the secondary absence or reduction of 8th sternite piercers: Strongygastrini + Hermyini (variable), Gymnosomatini s.l. (shovel-like or none; Fig. 2K–M), *Zita* + *Leverella* (shovel-like) and *Elomya* (shovel-like). The recently described genus *Melastrongygaster* Shima (Shima, 2015a) in the Strongygastrini possesses an 8th sternite piercer, suggesting either the maintenance of a piercer in this genus where it has been lost in other members of the clade or its evolutionary reappearance. We cannot discern between these possibilities as *Melastrongygaster* was not included in our analysis.

The wide distribution and multiple gains of piercers in Phasiinae suggest that such piercers are adaptively beneficial for members of this clade. 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 chitin-degrading proteins and/or specialized mandibles to break through the host's exoskeleton (a strategy employed by some blondeliines, for example). There is also substantial risk to the exposed egg of predation, desiccation and physical removal by the host. A piercer avoids these problems by bypassing the host's cuticle. Loss or reduction of piercers into shovel-like structures may be due to selection for more rapid oviposition and deposition of eggs in protected areas under hemelytra or between sternites/tergites.

The unique morphology of *Xysta* deserves special mention. Although derived from the 8th sternite, the piercer of *Xysta* is morphologically distinct 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. Our reconstruction suggests that the piercer in *Xysta* is a modification of the more typical 8th sternite piercer found in the related Parerigonini.

Hosts

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 ancestor and was retained in Phasiinae, Epigrimyiini, Eutherini and probably *Litophasia* as well (host unknown), with parasitism of Coleoptera and Lepidoptera arising subsequently in Dexiinae. This stands in contrast to the scenario proposed 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 evolved independently in Phasiinae and Eutherini. These conflicting interpretations are due largely to the phylogenetic placement of the ((Epigrimyiini + Eutherini) + *Litophasia*) clade. More thorough phylogenetic sampling of Dexiinae and other groups is needed to understand basal patterns of ancestral host use and host transitions. Reconstruction of the Strongygastrini in Phasiinae corroborates earlier suggestions that phasiines are not exclusively parasitoids of Heteroptera. *Strongygaster* parasitizes a plethora of hosts, including bugs, beetles and caterpillars, whereas *Rondaniooestrus* exclusively parasitizes honeybees. Heteroptera is retained as the primary host in all other phasiine lineages with known hosts.

Oviposition strategy

Another way in which the Strongygastrini are exceptional with respect to Phasiinae is their method of oviposition. Phasiinae as a whole are characterized by oviparity, but the Strongygastrini are ovolarviparous. Phasiinae represent the largest oviparous lineage of Tachinidae and likely had an oviparous ancestor (88%, Figure S4). Consequently, ovolarviparity most likely evolved secondarily in the Strongygastrini.

Ovolarviparity requires significant modifications to the female reproductive system in order to incubate large numbers of eggs (Herting, 1957; Wood, 1987). As a result, oviparity has historically been considered the more primitive condition of Tachinidae (Herting, 1960; Tschorsnig & Richter, 1998; Stireman *et al*., 2006; Tachi & Shima, 2010). However, Cerretti *et al*. (2014) found all oviparous lineages to be nested within ovolarviparous lineages, implying that ovolarviparity may have characterized the tachinid ancestor. Our analyses 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 strong statistical support for an ovolarviparous tachinid ancestor. Furthermore, this result seems unlikely to change with additional taxon sampling as most tachinid lineages not represented are ovolarviparous.

Synapomorphies of Phasiinae/Dexiinae

The placement of several taxonomically ambiguous genera within a robustly supported molecular phylogeny allows potential synapomorphic character traits of Phasiinae and Dexiinae to be evaluated. As previously discussed, neither oviparity nor parasitism of heteropteran hosts represent absolute synapomorphies of Phasiinae clade, although they remain useful in defining the subfamily with but a few exceptions (~16 of 600+ spp.). The lack of differentiation between the basiphallus and distiphallus may represent a synapomorphy of Phasiinae (as suggested by Shima, 2015a) as it appears universal in the subfamily with the exception of *Imitomyia*. Further refinement and study of this character trait is needed. In many respects (ovolarviparity, platiform pregonites and membranous basi/distiphallus connection), *Imitomyia* aligns more closely with Dexiinae than Phasiinae. However, the elongated hypandrial plate and some molecular analyses suggest that *Imitomyia* may belong to Phasiinae. If so, this would make the elongated medial plate of the hypandrium the only known absolute synapomorphy of Phasiinae (Tschorsnig, 1985; Shima, 2015a; see Figure S2).

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 our phylogeny indicates that these traits are homoplasious and not necessarily coincident with one another. This confusion is clearly seen in a summary of these states across taxa: the connection between the basiphallus and distiphallus in Dexiinae s.s. and Epigrimyiini is membranous and hinged, in *Litophasia* and *Imitomyia* it is membranous but not hinged, and in Phasiinae and Eutherini it is nonmembranous and not hinged (Figure S3). Platiform (strap-like) pregonites is a universal trait for Dexiinae with *Litophasia*, Epigrimyiini, Eutherini and all other Dexiinae possessing this state. Unfortunately, the aberrant genus *Imitomyia*, like several Acemyiini (Exoristinae) and Palpostomatini (Tachininae), also possesses platiform pregonites, making this trait a questionable synapomorphy of Dexiinae.

Classification changes

Substantial changes to, or reaffirmations of, current tachinid classification are suggested by this phylogeny and are summarized as follows. Epigrimyiini, Eutherini and *Litophasia* are considered members of Dexiinae. Strongygastrini is considered a member of Phasiinae. *Penthosia* is transferred from Cylindromyiini to Hermyini. The following genera formerly placed in Phasiini are transferred to other tribes within Phasiinae: *Cistogaster*, *Clytiomya*, *Ectophasia*, *Eliozeta* and *Euclytia* are moved to Gymnosomatini (which also includes all members of the former tribe Trichopodini); *Opesia* is moved to Strongygastrini; and *Xysta* is moved to the reinstated and monotypic tribe Xystini Lioy, 1864. Former members of Parerigonini are distributed as follows: *Parerigone* and *Zambesomima* remain in Parerigonini; *Australotachina*, *Neobrachelia* and *Pygidimyia* are transferred to Cylindromyiini; and *Zita* and *Leverella* are placed in the new tribe Zitini. These changes affect the genera we analysed genetically and we leave to future researchers a more thorough revision of phasiine classification based on both molecular and morphological data. The new tribe Zitini is erected and characterized below.

Zitini, trib.n.

Type genus. Zita Curran, 1927 (with one described species, *Z. aureopyga* Curran, 1927).

Other included genus. Leverella Baranov, 1934 (with two described species, *L. institutiimperialis* Baranov, 1934 and *L. novaeguineae* Baranov, 1934).

Remarks. Zita and *Leverella* are transferred here from their former placement in the Parerigonini. They form a strongly supported molecular clade within Phasiinae. As explained above, the apparent grouping of this lineage with *Xysta*, *Parerigone* and *Zambesomima* has low bootstrap support and was not consistently recovered in our analyses. To better reflect these uncertain relationships, Zitini *trib.n*. is erected for *Zita* and *Leverella*, tribe Xystini is resurrected for *Xysta*, and the tribe Parerigonini is restricted to *Parerigone* and *Zambesomima*. Morphologically, female zitines can be distinguished from true parerigonines (i.e. *Parerigone* and *Zambesomima*) by their 8th sternite shovel-like ovipositor, and from cylindromyiines by lack of fusion between sternite 7 and tergite 7 (Cantrell, 1988).

Distribution. Australasian and Oceanian Regions.

Conclusions

Our molecular phylogenetic analysis of Phasiinae and related Tachinidae contributes new evidence to several long-standing taxonomic debates. The subfamily placements of Eutherini, Epigrimyiini, *Litophasia*, Strongygastrini and Parerigonini are well resolved. Ancestral state reconstruction indicates multiple origins of piercing structures used to insert eggs directly into host tissues from different abdominal sternites and a trend in Phasiinae towards reduction or loss of piercers in various lineages. A single potential universal synapomorphy of Phasiinae is identified (elongated medial plate of hypandrium), but the single trait that comes closest to defining Dexiinae (platiform pregonites) is shared with a possible phasiine, *Imitomyia*. Finally, the robust framework of phasiine phylogeny presented here can serve as a basis for future taxonomic and systematic work in the subfamily.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12272

Figure S1. Transformed phylogeny estimated from the concatenated and partitioned dataset. Node support: Maximum-likelihood above branches, posterior probability below.

Figure S2. Ancestral state reconstruction of extension of medial plate of hypandrium and shape of pregonites in the Tachinidae.

Figure S3. Ancestral state reconstruction of connection between basi- and distiphallus in the Tachinidae.

Figure S4. Ancestral state reconstruction of oviposition strategy in the Tachinidae.

Table S1. List of taxa included in the phylogeny, corresponding gene coverage, and Genbank accession numbers. Organization into tribes is based on current taxonomic placement in recent catalogs (see text). *indicates that *CAD* sequences are from the Tachi Lab.

Table S2. Custom primers for *CAD*, *LGL*, *MAC* and *MCS* used in molecular analysis of Tachinidae.

Table S3. Coded character states for Tachinidae. ? unknown state.

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References

Aldrich, J.R., Lusby, W.R., Marron, B.E., Nicolaou, K.C., Hoffmann, M.P. & Wilson, L.T. (1989) Pheromone blends of green stink bugs and possible parasitoid selection. *Naturwissenschaften*, **76**, 173–175.

Aldrich, J.R., Khrimian, A., Zhang, A. & Shearer, P.W. (2006) Bug pheromones (Hemiptera, Heteroptera) and tachinid fly host-finding. *Denisia*, **19**, 1015–1031.

Aldrich, J.R., Khrimian, A. & Camp, M.J. (2007) Methyl 2, 4, 6-decatrienoates attract stink bugs and tachinid parasitoids. *Journal of Chemical Ecology*, **33**, 801–815.

Arnaud, P.H. Jr. (1978) *A Host-parasite Catalog of North American Tachinidae (Diptera)*. USDA Miscellaneous Publications 1319. Science and Education Administration, Washington D.C.

Belshaw, R. (1993) Tachinid flies. Diptera: Tachinidae. *Handbooks for the Identification of British Insects*, Vol. 10, Part 4a(i), (ed. by W.R. Dolling and R.R. Askew), pp. 115–119. Royal Entomological Society, London.

Biehler, J.A. & McPherson, J.E. (1982) Life history and laboratory rearing of *Galgupha ovalis* (Hemiptera: Corimelaenidae), with

descriptions of immature stages. *Annals of the Entomological Society of America*, **75**, 465–470.

Cantrell, B.K. (1983) Revision of the Australian species of *Euthera* Loew (Diptera: Tachinidae). *Australian Journal of Entomology*, **22**, 53–59.

Cantrell, B.K. (1988) The comparative morphology of the male and female postabdomen of the Australian Tachinidae (Diptera), with descriptions of some first-instar larvae and pupae. *Invertebrate Systematics*, **2**, 81–221.

Cantrell, B.K. & Crosskey, R.W. (1989) Family Tachinidae. *Catalog of the Diptera of the Australasian and Oceanian Regions*, Bishop Museum Special Publication 86 (ed. by N.L. Evenhuis), pp. 733–784. Bishop Museum Press, Honolulu/Leiden.

Cerretti, P. (2010) *I tachinidi della fauna italiana (Diptera Tachinidae). Con chiave interattiva dei generi ovest-paleartici*, Vol. **I**. Centro Nazionale Biodiversità Forestale, Verona.

Cerretti, P., O'Hara, J.E., Wood, D.M., Shima, H., Inclan, D.J. & Stireman, J.O. III (2014) Signal through the noise? Phylogeny of the Tachinidae (Diptera) as inferred from morphological evidence. *Systematic Entomology*, **39**, 335–353.

Coombs, M. (2004) Overwintering survival, starvation resistance, and post-diapause reproductive performance of *Nezara viridula* (L.) (Hemiptera: Pentatomidae) and its parasitoid *Trichopoda giacomellii* Blanchard (Diptera: Tachinidae). *Biological Control*, **30**, 141–148.

Crosskey, R.W. (1973) A conspectus of the Tachinidae (Diptera) of Australia, including keys to the supraspecific taxa and taxonomic and host catalogues. *Bulletin of the British Museum (Natural History), Entomology, Supplement*, **21**, 1–221.

Crosskey, R.W. (1976) A taxonomic conspectus of the Tachinidae (Diptera) of the Oriental Region. *Bulletin of the British Museum (Natural History), Entomology, Supplement*, **26**, 1–357.

Crosskey, R.W. (1977) A review of Rhinophoridae (Diptera) and a revision of the Afrotropical species. *Bulletin of the British Museum (Natural History), Entomology, Supplement*, **36**, 1–66.

Crosskey, R.W. (1980) Family Tachinidae. *Catalogue of the Diptera of the Afrotropical Region* (ed. by R.W. Crosskey), pp. 822–882. British Museum (Natural History), London.

Dear, J.P. (1980) A new species of *Litophasia* Girschner from South Africa (Diptera: Tachinidae). *Annals of the National Museum (Pietermaritzburg)*, **24**, 217–220.

Eggleton, P. & Belshaw, R. (1992) Insect parasitoids: an evolutionary overview. *Philosophical Transaction of the Royal Society of London B*, **337**, 1–20.

van Emden, F.I. (1945) 23. Keys to the Ethiopian Tachinidae. I. Phasiinae. *Proceedings of the Zoological Society of London*, **114**, 89–436 + 3 pls.

Evenhuis, N.L. (2013) Family Tachinidae. *Catalog of the Diptera of the Australasian and Oceanian Regions* (ed. by N.L. Evenhuis). (online version) [WWW document]. URL http://hbs.bishopmuseum.org/aocat/tachinidae.html [accessedon 17 July 2017].

Feener, D.H. Jr (2000) Is the assembly of ant communities mediated by parasitoids? *Oikos*, **90**, 79–88.

Ferrar, P. (1977) Parasitism of other adult Diptera by Tachinidae in Australia. *Journal of Australian Entomological Society*, **16**, 397–401.

Golec, J.R., Hu, X.P., Ray, C. & Woodley, N.E. (2013) *Strongygaster triangulifera* (Diptera: Tachinidae) as a parasitoid of adults of the invasive *Megacoptacribraria* (Heteroptera: Plataspidae) in Alabama. *Journal of Entomological Science*, **48**, 352–354.

Guimarães, J.H. (1971) 104. Family Tachinidae (Larvaevoridae). *A Catalogue of the Diptera of the Americas South of the United States*, 1–86.

Guimarães, J.H. (1977) Host-parasite and parasite-host catalogue of South American Tachinidae (Diptera). *Arquivos de Zoologia, São Paulo*, **28**, 1–131. Museu de Zoologia, Universidade de Sau Paulo, Sao Paulo.

Herting, B. (1957) Das weibliche postabdomen der calyptraten fliegen (Diptera) und sein merkmalswert für die systematik der gruppe. *Zeitschrift für Morphologie und Ökologie der Tiere*, **45**, 429–461.

Herting, B. (1960) Biologie der westpaläarktischen Raupenfliegen (Diptera Tachinidae). *Monographien zur Angewandten Entomologie*, **16**, 1–188.

Herting, B. (1966) Beiträge zur Kenntnis der europäischen Raupenfliegen (Diptera Tachinidae). IX. *Stuttgarter Beiträge zur Naturkunde*, **146**, 1–12.

Herting, B. (1983) 64c. Phasiinae. *Die Fliegen der palaearktischen Region*, Vol. **9** (Lieferung 329), (ed. by E. Lindner), pp. 1–88. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

Herting, B. (1984) Catalogue of Palearctic Tachinidae (Diptera). *Stuttgarter Beiträge zur Naturkunde. Serie A (Biologie)*, **369**, 1–228.

Herting, B. & Dely-Draskovits, A. (1993) Family Tachinidae. *Catalogue of Palaearctic Diptera*, Vol. 13: Anthomyiidae–Tachinidae (ed. by Á. Soós and L. Papp), pp. 1–624. Hungarian Natural History Museum, Budapest.

Katoh, K. & Toh, H. (2010) Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics*, **26**, 1899–1900.

Katoh, K., Misawa, K., Kuma, K.I. & Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**, 3059–3066.

Kevan, D.K. & Koshnaw, R.T.B. (1988) *Hyalomyodes* (Diptera; Tachinidae), an endoparasitoid of Tetrigoidea (Orthoptera). *Entomologist's Record and Journal of Variation*, **100**, 55–57.

Maddison, W.P. & Maddison, D.R. (2015) *Mesquite: A Modular System for Evolutionary Analysis. Version 3.03* [WWW document]. URL http://mesquiteproject.org [accessed on 5 May 2017].

Mesnil, L.P. (1966) 64g. Larvaevorinae (Tachininae). *Die Fliegen der palaearktischen Region*, Vol. 10 (Lieferung 263), (ed. by E. Linder), pp. 881–928. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

Mesnil, L.P. (1970) Descriptions de nouveaux Tachinaires de l'ancien monde, et notes synonymiques (Diptera Tachinidae). *Mushi*, **44**, 89–123.

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Gateway Computing Environments Workshop (GCE)*. New Orleans, Louisiana, 14 November 2010, pp. 1–8.

Moulton, J.K. & Wiegmann, B.M. (2004) Evolution and phylogenetic utility of CAD (*rudimentary*) among Mesozoic-aged Eremoneuran Diptera (Insecta). *Molecular Phylogenetics and Evolution*, **31**, 363–378.

Nishayama, M., Iwasa, M. & Hori, K. (1995) Parasitism by tachinid flies (Diptera, Tachinidae) of heteropterous insects in Tokachi, Hokkaido. *Japanese Journal of Entomology*, **63**, 159–165.

Nishida, T. (1966) Behavior and mortality of the southern stink bug *Nezaraviridula* in Hawaii. *Researches on Population Ecology*, **8**, 78–88.

O'Hara, J.E. (2012a) Review of *Euthera* (Diptera: Tachinidae) in North America with the description of a new species. *Canadian Entomologist*, **144**, 206–215.

O'Hara, J.E. (2012b) Review of the tachinid fly diversity in the Gila National Forest, New Mexico. *New Mexico Botanist*, Special Issue No. 3, pp. 32–41[= *Proceedings of the Third Natural History of the Gila Symposium* (ed. by W. Norris and R. Felger), 14–16 October 2010. Western New Mexico University, Silver City, NM].

O'Hara, J.E. (2013a) Where in the world are all the tachinid genera? *Tachinid Times*, Vol. 26, pp. 10–16.

O'Hara, J.E. (2013b) History of tachinid classification (Diptera, Tachinidae). *ZooKeys*, **316**, 1–34.

O'Hara, J.E. & Cerretti, P. (2016) Annotated catalogue of the Tachinidae (Insecta, Diptera) of the Afrotropical region, with the description of seven new genera. *ZooKeys*, **575**, 1–344.

O'Hara, J.E. & Wood, D.M. (2004) Catalogue of the Tachinidae (Diptera) of America north of Mexico. *Memoirs of the International Entomological Society*, **18**, 1–410.

O'Hara, J.E., Shima, H. & Zhang, C. (2009) Annotated catalogue of the Tachinidae (Insecta: Diptera) of China. *Zootaxa*, **2190**, 1–236.

Panizzi, A.R. & Oliveira, É.D. (1999) Seasonal occurrence of tachinid parasitism on stink bugs with different overwintering strategies. *Anais da Sociedade Entomológica do Brasil*, **28**, 169–173.

Pape, T. (1992) Phylogeny of the Tachinidae family-group. *Tijdschrift voor Entomologie*, **135**, 43–86.

Pape, T., Blagoderov, V. & Mostovski, M.B. (2011) Order Diptera Linnaeus, 1758, in: Z.-Q. Zhang (Ed.), Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness. *Zootaxa*, **3148**, 222–229.

Richter, V.A. (1992) Biological evolution of tachinids (Diptera, Tachinidae). *Advances in Entomology in USSR, Diptera: Systematics, Ecology, Medical and Veterinary Importance. Proceedings of the Tenth Congress of the All-Union Entomological Society*, St. Petersburg, 12–15 September 1989 (ed. by V.A. Richter and V.V. Zlobin), pp. 59–62 (in Russian).

Richter, V.A. (2004) Fam. Tachinidae – tachinids. *Key to the insects of Russian Far East*, Vol. VI, Diptera and Siphonaptera, Part 3 (ed. by V.S. Sidorenko), pp. 148–398. Dal'nauka, Vladivostok. (in Russian).

Rogers, J. & Wall, J. (1980) A mechanism for RNA splicing. *Proceedings of the National Academy of Sciences of the United States of America*, **77**, 1877–1879.

Ronquist, F., Teslenko, M., van der Mark, P. *et al.* (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**, 539–542.

Sabrosky, C.W. & Braun, B.H. (1970) A tachinid parasite of fireflies. *Entomological News*, **81**, 185–187.

Santos, C.H. & Panizzi, A.R. (1997) Tachinid parasites of adult *Megalotomus parvus* West (Hemiptera: Alydidae). *Anais da Sociedade Entomológica do Brasil*, **26**, 577–578.

Shima, H. (1989) Parasitic way of life in tachinid flies. *Insectarium*, **26**, 4–9, 46–51, 88–94, 120–126 (in Japanese; cladogram on p. 125 reproduced with English caption in *Tachinid Times*, 3, 9 (1990).)

Shima, H. (2011) Notes on *Parerigone* with a description of a new species from Nepal (Diptera: Tachinidae). *Canadian Entomologist*, **143**, 674–687.

Shima, H. (2015a) *Melastrongygaster*, a new genus of the tribe Strongygastrini (Diptera: Tachinidae), with five new species from Asia. *Zootaxa*, **3904**, 427–445.

Shima, H. (2015b) New host records of Japanese Tachinidae (Diptera). *Acta Dipterologica*, **26**, 9–19.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, **57**, 758–771.

Stireman, J.O. III (2002) Phylogenetic relationships of tachinid flies in subfamily Exoristinae (Tachinidae: Diptera) based on 28S rDNA and elongation factor-1α. *Systematic Entomology*, **27**, 409–435.

Stireman, J.O. III & Singer, M.S. (2003) Determinants of parasitoid-host associations: insights from a natural tachinid-lepidopteran community. *Ecology*, **84**, 296–310.

Stireman, J.O. III, O'Hara, J.E. & Wood, D.M. (2006) Tachinidae: evolution, behavior, and ecology. *Annual Review of Entomology*, **51**, 525–555.

Sutherland, J.P. & Baharally, V. (2002) Spatio-temporal distribution of *Beskia aelops* (Walker) (Diptera: Tachinidae) and its potential for the biocontrol of *Oebalus poecilus* (Dallas) (Hemiptera: Pentatomidae). *Biocontrol Science and Technology*, **12**, 513–517.

Tachi, T. & Shima, H. (2010) Molecular phylogeny of the subfamily Exoristinae (Diptera, Tachinidae), with discussions on the evolutionary history of female oviposition strategy. *Systematic Entomology*, **35**, 148–163.

Townsend, C.H.T. (1936) *Manual of Myiology in Twelve Parts. Part III. Oestroid Classification and Habits. Gymnosomatidae to Tachinidae*. Privately Published, Itaquaquecetuba.

Townsend, C.H.T. (1938) *Manual of Myiology in Twelve Parts. Part VII. Oestroid Generic Diagnoses and Data. Gymnosomatini to Senostomatini*. Privately Published, Itaquaquecetuba.

Tschorsnig, H.P. (1985) Taxonomie forstlich wichtiger Parasiten: Untersuchungen zur Struktur des mannlichen Postabdomens der Raupenfliegen (Diptera, Tachinidae). *Stuttgarter Beiträge zur Naturkunde, Serie A (Biologie)*, **383**, 1–137.

Tschorsnig, H.P. & Richter, V.A. (1998) 3.54. Family Tachinidae. *Contribution to a Manual of Palaearctic Diptera (with Special Reference to Flies of Economic Importance)*, *Vol. 3: Higher Brachycera* (ed. by L. Papp and B. Darvas), pp. 691–827. Science Herald, Budapest.

Verbeke, J. (1963) The structure of the male genitalia in Tachinidae (Diptera) and their taxonomic value. *Stuttgarter Beiträge zur Naturkunde*, **114**, 1–5.

Villeneuve, J. (1924) Contribution à la classification des "Tachinidae" Paléarctiques. *Annales des Sciences Naturelles, Séries Botanique et Zoologie*, **10**, 5–39.

Winkler, I.S., Blaschke, J.D., Davis, D.J., Stireman, J.O. III, O'Hara, J.E., Cerretti, P. & Moulton, J.K. (2015) Explosive radiation or uninformative genes? Origin and early diversification of tachinid flies (Diptera: Tachinidae). *Molecular Phylogenetics and Evolution*, **88**, 38–54.

Wood, D.M. (1987) Tachinidae. *Manual of Nearctic Diptera*, Agriculture Canada Monograph 28, Vol. **2** (ed. by J.F. McAlpine, B.V. Peterson, G.E. Shewell, H.J. Teskey, J.R. Vockeroth and D.M. Wood), pp. 675–1332. Biosystematics Research Institute, Ottawa.

Wood, D.M. & Zumbado, M.A. (2010) Tachinidae (tachinid flies, parasitic flies). *Manual of Central American Diptera*, Vol. **2** (ed. by B.V. Brown, A. Borkent, J.M. Cumming, D.M. Wood, N.E. Woodley and M.A. Zumbado), pp. 715–1442. NRC Research Press, Ottawa.

Ziegler, J. (1998) Die Morphologie der Puparien und der larvalen Cephalopharyngealskelette der Raupenfliegen (Diptera, Tachinidae) und ihre phylogenetische Bewertung. *Studia Dipterologica Supplement*, **3**, 1–244.

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