

# Extraordinary diversification of the “bristle flies” (Diptera: Tachinidae) and its underlying causes

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Received 20 October 2020; revised 18 January 2021; accepted for publication 19 January 2021

The family Tachinidae (“bristle flies”) is the most diverse and ecologically important group of insect parasitoids outside the parasitic wasps. It is among the most species rich families of flies (Diptera) and has experienced a recent adaptive radiation across the globe. We make use of a molecular phylogeny of the family to examine its rapid radiation and explore the traits of tachinid lineages that may have contributed to variation in their diversification. We apply a range of diversification analyses to assess the consistency and robustness of effects. We find that the Tachinidae are among the most rapidly diversifying families of animals. Six to eight clades of bristle flies, distributed across the phylogeny, exhibit strong evidence of accelerated diversification. Our results suggest that the use of holometabolous insect larvae, and specifically caterpillars (Lepidoptera), as hosts, is associated with increased diversification rates. However, these effects were inconsistent across analyses. We detected little influence of oviposition strategy (egg type) or host feeding habit, and we recovered evidence that unmeasured “hidden” traits may explain greater variance in diversification. We evaluated the strengths and weaknesses of different Maximum Likelihood and Bayesian approaches for analysing diversification and the potential for extrinsic factors, such as geography, to influence patterns of richness and diversification. In general, we conclude that although certain traits may provide opportunities for diversification, whether this is capitalized on may depend on additional traits and/or historical contingency.

**ADDITIONAL KEYWORDS:** adaptive radiation – BAMM – BiSSE – diversification rate – extinction – host use – parasitoid – speciation – state dependent diversification - tachinid.

## INTRODUCTION

It has become increasingly apparent that the diversification of life on Earth has not been a constant metronomic splitting of lineages with uniformly increasing richness over time, but a series of fits and starts, characterized by rapid and extensive radiations and episodic extinction. Indeed, much of the current diversity of plants and animals has arisen through the process of adaptive radiation, with lineages exploding in diversity in response to new ecological opportunities

and novel adaptive innovations (Schluter, 2000). Classic, well studied “island” examples of adaptive radiation such as African cichlids (Kocher, 2004; Wagner *et al.*, 2012), Galapagos finches (Sato *et al.*, 2001) and Caribbean anoles (Losos *et al.*, 1998), represent but a minute sample of recent and conspicuous cases in a long history of adaptive radiations. Much greater are the massive radiations of angiosperm lineages [e.g. Asteraceae (Panero *et al.*, 2016), Orchidaceae (Tremblay *et al.*, 2005)] and the unrivalled diversification of holometabolous insects (Kristensen, 1999; Zhang, 2013; Peters *et al.*, 2014). Understanding why groups such as these have experienced such dramatic radiations and

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why diversification rates vary so greatly within and among major lineages is a central question in evolutionary biology. Of particular interest is the role of phenotypic innovation in igniting episodes of diversification and whether common causes underlie adaptive radiations or if they tend to be idiosyncratic and contingent upon particular intrinsic (lineage traits) and extrinsic (environmental) conditions.

In the case of insects, phytophagy is a significant predictor of extant diversity and coevolutionary interactions with host plants are thought to underlie much of this diversification (Ehrlich & Raven, 1964; Mitter *et al.*, 1988; Wiens *et al.*, 2015). Such coevolutionary interactions in which “diversity begets diversity” provide a conceptually simple yet powerful model to explain patterns of evolutionary diversification and extant diversity in many groups of parasites as well as mutualists (Stireman *et al.*, 2005; Feder & Forbes, 2010; Cruaud *et al.*, 2012). Insect parasitoids encompass a number of hyperdiverse lineages that have experienced relatively recent and dramatic explosions in diversity. The Hymenoptera, for example, may be the most species rich order of insects (Forbes *et al.*, 2018), with the majority of species being parasitoids, particularly in the superfamilies Ichneumonoidea and Chalcidoidea (Aguilar *et al.*, 2013). Most of the inferred but undescribed species of Hymenoptera thought to exist are also parasitoids (Forbes *et al.*, 2018).

Among the flies (order Diptera), the bristle flies (family Tachinidae) represent the dominant lineage of parasitoids, with over 8500 described species and perhaps an equal number remaining to be discovered (O’Hara *et al.*, 2020). The Tachinidae currently represent the second largest family of flies [of > 150 families (Pape *et al.*, 2011)] and yet recent studies have suggested that it is among the youngest dipteran families (Cerretti *et al.*, 2017). This high diversity and young age suggest that the Tachinidae have undergone a recent and rapid evolutionary radiation of lineages. Our goal here is to use information on tachinid biology, diversity and phylogeny to provide insight into the extent and causes of the dramatic radiation of this family of parasitoid flies. We focus on variation in diversification within the Tachinidae to provide clues for understanding why this clade is so species rich. Specifically, we address the following questions:

1. Is the evolutionary radiation of tachinids exceptional among animals?
2. How is diversity and diversification rate distributed phylogenetically within the family? Are particular lineages responsible for the high diversity of the family, and if so, which ones?
3. Is diversification of bristle fly lineages related to the hosts they use or particular modes of attack?
4. Do different inference methods of trait-dependent diversification reveal consistent patterns?

We explore these questions using a variety of methods and approaches to evaluate how consistent and robust our inferences are about diversification in tachinids and its underlying causes. In addition, we consider the role that geography may play in diversification (e.g. ecological opportunity) and explore methodological limitations in understanding the evolutionary determinants of diversity. We find that although association with particular host groups is implicated in driving accelerated diversification, models are inconsistent in their inferences and there appear to be no simple answers as to why certain lineages have diversified extensively and others have not. The parasitoid lifestyle may have provided tachinids with the potential for rapid diversification; however, whether this opportunity has been capitalized on by various lineages may depend upon additional idiosyncratic traits and historical contingency.

## BACKGROUND

The Tachinidae are distributed worldwide in terrestrial environments and most major lineages are broadly distributed across major biogeographic realms. All known tachinids are endoparasitoids of insects or other arthropods (e.g. centipedes, scorpions). Probably less than half of described species have known hosts (Stireman *et al.*, 2006); however, of those known, most attack larval stages of holometabolous insects, especially Lepidoptera and Coleoptera. Exceptions include the relatively frequent use of juvenile and adult Heteroptera and other hemimetabolous orders, and adult Coleoptera (Wood, 1987; Cerretti, 2010). As parasitoids of mostly herbivorous insects, tachinids are important components of terrestrial food webs in both natural and managed ecosystems (Stireman *et al.*, 2006). Variation in the richness of host clades as well as host traits that affect the potential for isolation and speciation of tachinids (e.g. host defences and feeding habits) are likely factors in promoting or constraining diversification of tachinid clades. Although traditionally considered to be polyphagous, evidence is mounting that tachinids are often highly host-specific (Smith *et al.*, 2007; Stireman *et al.*, 2017). Colonization of rapidly diversifying phytophagous hosts, in particular the higher Lepidoptera, with their often highly specialized host plant and habitat associations, could underlie much of the recent radiation of tachinid lineages (Cerretti *et al.*, 2014).

Tachinidae have evolved multiple oviposition strategies and egg types to ensure that their larvae obtain ingress into hosts. These include: (1) ovipary of hard-shelled eggs that are laid on the host and must develop for some time before hatching; (2) ovolarvipary of incubated, thin-shelled, “ready-to-hatch” eggs that may be deposited on hosts, or away from them such that

the first instars seek out or ambush potential hosts; and (3) micro-ovularvipary in which tiny (“microtype”), incubated, hard-shelled eggs are ingested by the host while feeding, hatch in the gut, and the first instars subsequently migrate into the hemocoel. Although it has long been assumed that ovipary was the ancestral state in tachinids with multiple transitions to ovularvipary (Wood, 1987), recent analyses suggest that their common ancestor was ovularviparous, and that ovipary is derived (Cerretti *et al.*, 2014; Stireman *et al.*, 2019). Because ovularvipary (and micro-ovularvipary) allows attack of otherwise inaccessible hosts (e.g. nocturnal feeders, physically defended taxa, shelter builders), this strategy might be expected to promote diversification.

The family Tachinidae is widely considered a clade that is in the flowering of its diversity. Age estimates based on molecular clocks suggest a phylogenetic origin of the family from 24–34 Mya (Wiegmann *et al.*, 2011; Zhao *et al.*, 2013; Junqueira *et al.*, 2016; Cerretti *et al.*, 2017). This recent origin, combined with vast numbers of known and inferred species, suggest that the Tachinidae may represent one of the more dramatic recent radiations of animals.

## MATERIAL AND METHODS

### PHYLOGENY

To analyse diversification of the Tachinidae we used a recent molecular phylogenetic reconstruction based on four nuclear loci (7.8 kb) that included 359 tachinid genera and all major tribes (Stireman *et al.*, 2019). Our analyses focus on the Maximum Likelihood (ML) tree recovered using IQ-Tree (Nguyen *et al.*, 2015). We trimmed outgroups and transformed the tree into a chronogram with correlated rates using *makeChronosCalib* in the R package *ape* (Paradis *et al.*, 2004), setting the age of the most recent common ancestor at 33.7 Mya (Cerretti *et al.*, 2017). This age is uncertain due to the lack of fossils, but it is conservative with respect to other recent age estimates (above). For some analyses (e.g. phylogenetic generalized least squares (PGLS) regression) we employed a reduced tree, trimmed to representative taxa of 55 monophyletic tribes or other distinct subclades represented in the phylogeny, for which we were able to estimate richness (“trimmed tree”). For this, we attempted to retain the most typical or diagnostic taxon of each clade, often the “type” genus for a tribe.

### DIVERSITY DATA AND ANALYSIS

We obtained data on the species richness of tachinid genera from O’Hara *et al.*’s (2019) world checklist with

some additions from recent publications (e.g. Fleming *et al.*, 2020). For analyses, we assumed a total of 8552 described species of the Tachinidae [though the current estimate is 8592 (O’Hara *et al.*, 2020)]. Tachinid genera omitted from the original phylogenetic reconstruction were assigned to 55 clades based on previous taxonomic placement and morphological similarity with represented taxa. Some small, closely related tribes were lumped together into larger clades for estimates of clade diversification (e.g. Glaurocarini-Ormiini-Bigonichetini-Erneestiini-in part). In a few cases, placement of genera was uncertain, e.g. the basal grade of the Dexiinae (Stireman *et al.*, 2019); however, these were genera with few species and any errors in placement are unlikely to substantially affect our results. The tribes Iceliini, Protohystriciini and Thrixionini could not be placed and were omitted, but collectively these groups account for less than 0.25% of tachinid species. The Anacamptomyini are thought to belong within the Eryciini (Zeegers, 2014), closely allied with the “Carceliini” (Crosskey, 1976), and we include them in the “*Carcelia*-group” clade. We used these diversity data to estimate the sampled proportion of each tribe or major clade as well as to estimate clade-specific rates of speciation, extinction and diversification.

To examine variation in diversification rates across the phylogeny of the Tachinidae and evaluate the most likely configuration of rate shifts we used the software BMM v.2.5.0 [“Bayesian Analysis of Macroevolutionary Mixtures” (Rabosky, 2014)]. This method uses a Markov chain Monte Carlo (MCMC) approach to sample from the possible space of rate shift configurations according to their posterior probability. We accounted for missing taxa by including clade-specific sampling probabilities (fraction sampled) based on the total number of species in each tribe or major clade ( $N = 55$ , see above), and we estimated a backbone sampling fraction of 0.9, indicating that we have likely sampled 90% of all major tribes/lineages. The BMM analysis was run for 10 million generations using four chains with the following priors: expectedNumberOfShifts = 1, lambdaInitPrior = 1.7035, lambdaShiftPrior = 0.02465, muInitPrior = 1.7035, lambdaIsTimeVariablePrior = 1, minCladeSizeForShift = 5, segLength = 0.01. We analysed and visualized BMM output using the R package *BMMtools* (Rabosky *et al.*, 2014). MCMC convergence was assessed by plotting posterior probabilities and calculating effective sample size. The first million generations were discarded (10% burn-in). Effective sample sizes of log-likelihoods and N-shifts were found to be high (1031 and 924, respectively) using the R package *coda* (Plummer *et al.*, 2006). We used the BMM output to estimate the most likely number of rate shifts, the best rate shift configuration, the

maximum shift credibility configuration, cumulative shift probabilities of branches, and speciation and extinction rates for clades experiencing shifts in diversification. Multiple BAMM runs were conducted using varying estimates of time intervals and clade-specific sampling probabilities and all resulted in the same basic pattern of diversification rate variation (Supporting Information, Fig. S6).

As an alternative approach to assessing variation in diversification rates, we used the method-of-moments (MoM) approach of Magallón & Sanderson (2001) to estimate diversification rates for 55 lineages using both stem and crown-group ages based on the tachinid chronogram. MoM diversification rates were estimated using the R package *geiger* 2.0.6 (Harmon *et al.*, 2008) with extinction fractions ( $\epsilon = \mu/\lambda$ ) of 0.05, 0.1, 0.5 and 0.9. For each clade, we calculated the probability of observing a clade of that size given overall net diversification rate ( $r$ ), extinction fraction and clade age. Confidence limits (95%) for expected diversity were calculated and plotted to visualize clades with exceptionally high or low diversification rates.

We estimated constant-rate diversification parameters using ML in the R package *Diversitree* v.0.9–10 (Fitzjohn, 2012), employing a sampling fraction of  $481/8500 = 0.057$  and starting point estimates derived from BAMM analysis (above). In addition, we estimated diversification parameters via Bayesian analysis using MCMC (number of steps = 10 000) and tested if a birth-death model of diversification is significantly more likely than yule (pure-birth model) by comparing log likelihoods of each model. Diversification rate estimates for tachinids (using MoM) were compared to the distribution of rates estimated for 1710 families of animals by Scholl & Wiens (2016).

#### TRAIT DEPENDENT DIVERSIFICATION

Host associations and other biological data were compiled from the literature (e.g. Crosskey, 1973b, 1984; Guimarães, 1977; Arnaud, 1978; Cerretti, 2010; Tschorsnig, 2017) and our observations (see Cerretti *et al.*, 2014; Stireman *et al.*, 2019). Included genera were scored for host order (Lepidoptera, Coleoptera, Hemiptera or other), host stage (larva vs. adult or nymph) and host feeding habit (phytophagous or not). In cases where multiple host types were used, we scored genera for the predominant host used across known species. Many tachinid genera have no known hosts, and thus we conducted two parallel analyses: one in which data for these taxa were excluded and one in which we inferred likely hosts based on taxonomic affiliation or other biological characteristics. We also scored taxa according to their reproductive mode [oviparous, ovolarviparous or

micro-ovolarviparous (Cerretti *et al.*, 2014; Stireman *et al.*, 2019)]. For the trimmed tree, we estimated the proportion of lepidopteran, holometabolous larvae or phytophagous hosts used by members of each clade as well as the proportion of species in each clade that are ovolarviparous. Micro-ovolarvipary probably evolved only once in the Goniini (Tachi & Shima, 2010; Cerretti *et al.*, 2014; though see Stireman, 2002; Stireman *et al.*, 2019). Therefore, we were unable to effectively test if this trait has influenced tachinid diversification.

We used a range of approaches to assess effects of tachinid host traits and egg type on diversification including BiSSE/MuSSE [Binary/Multi State Speciation and Extinction (Maddison *et al.*, 2007; Fitzjohn *et al.*, 2009)], HiSSE [Hidden State Speciation and Extinction (Beaulieu & O'Meara, 2016)], FiSSE [Fast, intuitive State dependent Speciation and Extinction (Rabosky & Goldberg, 2017)], PGLS [e.g. Martins & Hansen (1997)] and STRAPP [Structured Rate Permutations on Phylogenies (Rabosky & Huang, 2016)].

We employed BiSSE/MuSSE ML models in *Diversitree* to estimate speciation rates, extinction rates and transition rates relative to trait states of taxa (i.e. state dependent diversification, SDD) using the *make.bisse* and *find.mle* functions. All parameters (speciation, extinction, state transition rates) were free to vary and ML starting points were estimated with *starting.point.bisse*. Models of highest likelihood were compared to constrained models with equal speciation or extinction rates to assess significant effects of traits on these rates. Speciation and extinction rates were also estimated using Bayesian MCMC sampling, to visualize and assess differences in diversification rates (exponential prior with rate  $1/(2r)$ , where  $r$  = overall diversification rate; 10 000 steps). Models were evaluated with both missing data and with inferred trait states. MuSSE was employed similarly for a more nuanced multi-state analysis of the effect of host order on diversification with four states (above). We also employed HiSSE models (Beaulieu & O'Meara, 2016) to assess the likelihood that associations between traits and diversification could be explained by states of an unknown trait co-occurring with the observed trait. HiSSE models were constructed using the R package *HiSSE* to compare BiSSE models to models in which 'hidden states' are responsible for variation in diversification. Specifically, we used this method to compare likelihoods and AIC scores between the following models: BiSSE null (no SDD), BiSSE (SDD based on focal trait), HiSSE null8 [Character Independent Diversification (CID-2) based only on hidden states with six transition rates (Beaulieu & O'Meara, 2016)] and HiSSE full (SDD based on focal and hidden traits).

FiSSE analyses were conducted using the R source code of Rabosky & Goldberg (2017). This method tests if mean tip speciation rates [using the inverse equal

splits measure (Jetz *et al.*, 2012)] of taxa with binary trait states differ significantly from overall mean rates across the phylogeny. We examined the effects of host (Lepidoptera or not), host stage, host habitat and egg type.

For PGLS analyses we used the trimmed tree to test for significant relationships between clade richness or clade diversification rate and the frequency of hosts in a particular category for that clade. In this way, we treated clade richness as a trait that may be correlated with other traits. These analyses were repeated for host stage, egg type and for combinations of these traits. We explored trait-richness relationships using the *gls* function in the *nlme* R package (Pinheiro *et al.*, 2019), assuming Martins's correlation structures (Martins & Hansen, 1997) with  $\alpha = 0.1$  (values less than  $c$ . 0.5 resulted in lower AIC scores). Brownian motion correlation structures resulted in lower AIC scores. We conducted parallel analyses using the *pgls* function in the *caper* R package (Orme *et al.*, 2018), where lambda, kappa and delta branch length transformations were estimated with ML.

Finally, we conducted STRAPP analyses within BAMMtools using the *traitdependentBAMM* function. STRAPP assesses correlations between traits and diversification using the posterior distribution resulting from the BAMM diversification analysis (Rabosky & Huang, 2016). Traits were scored as categorical variables and significance was assessed using two-tailed Mann-Whitney U-tests (binary traits) or Kruskal-Wallis tests (multistate).

## RESULTS

### FAMILY LEVEL DIVERSIFICATION

Estimates of overall diversification rate for the family Tachinidae were high, ranging from 0.184 to 0.248 depending on the method used (Table 1). To put this into perspective, when compared to diversification rates calculated for animal families using the MoM estimator [ $x = 0.048 \pm 0.49$  ( $\sigma$ ) using stem ages and  $\varepsilon = 0.5$  (Scholl & Wiens, 2016)], the Tachinidae fall among the top 1% of animals ( $r = 0.248$ ; Fig. 1).

**Table 1.** Estimates of speciation ( $\lambda$ ), extinction ( $\mu$ ) and diversification ( $r$ ) rates for the family Tachinidae

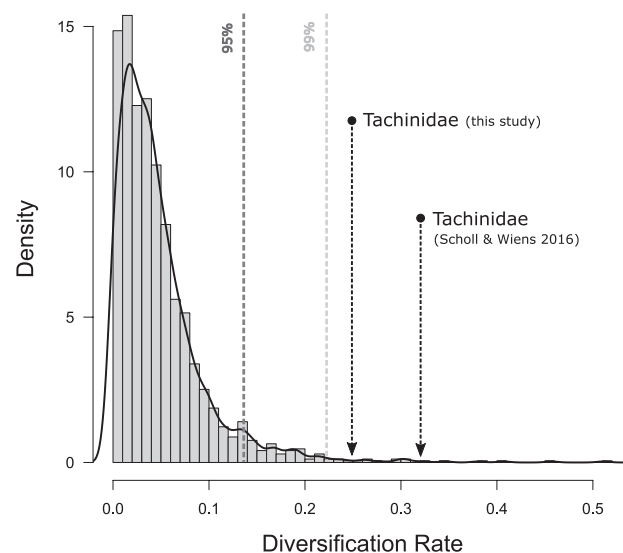
Method	Spec. ( $\lambda$ )	Ext. ( $\mu$ )	Div. ( $r$ )
ML ( <i>Diversitree</i> )	0.281	0.095	0.186
Bayes ( <i>Diversitree</i> )	0.287	0.102	0.184
Bayes BAMM	0.241	0.053	0.188
Method-of-moments	-	-	0.248

Although we acknowledge that family ranks are subjective, the Tachinidae are clearly exceptional, ranking 13<sup>th</sup> in diversification rate among animal families ( $N = 1710$ ) and 18<sup>th</sup> among all families of organisms examined ( $N = 2546$ ).

Speciation rate estimates were high (0.24–0.29); however, equally important may be that estimated rates of extinction were low, 0.1 or lower (Table 1). Still, the birth-death model including extinction provided a significantly better fit than a pure-birth Yule model ( $\chi^2 = 6.46$ ,  $P = 0.011$ ), suggesting that extinction may play a significant role in understanding tachinid diversification. Bayesian posterior distributions estimated in BAMM suggest that speciation rate has experienced a slow irregular decline through time, and that extinction rates are low but increasing (Supporting Information, Fig. S1). A plot of the number of lineages through time matches well the classic diminishing curve of adaptive radiation with diversification slowing through time as niches are filled (McPeck, 2008; Rabosky, 2013; Supporting Information, Fig. S2; but see *Methodological issues* below).

### HETEROGENEITY IN DIVERSIFICATION RATES WITHIN THE TACHINIDAE

BAMM analyses resulted in 1095 distinct rate shift configurations. Overall, the analysis indicated that 4–13 shifts in macroevolutionary rate regimes have likely occurred across the phylogeny of the



**Figure 1.** The distribution of diversification rates calculated for 1710 families of animals from Scholl & Wiens (2016) with 95% and 99% percentiles indicated. Diversification rates for the Tachinidae estimated in the current study as well as by Scholl & Wiens (2016) are indicated with arrows.

Tachinidae, with eight shifts having the highest posterior probability ( $pp = 0.3$ ) and between 6–11 accounting for 96.8% of the posterior distribution (Supporting Information, Fig. S3). Bayes factors also strongly support the existence of at least six shifts in diversification rate within the family (i.e. Bayes factors  $> 50$ ; Supporting Information, Table S1). The overall best shift configuration reconstructs seven shifts in macroevolutionary rates, all towards higher rates of diversification (Fig. 2; Table 2). The clades indicated as experiencing increases in diversification rate are dispersed across the phylogeny and include: the crown Exoristinae (Exoristiini, Blondeliini, Eryciini, Goniini), the tachinine tribes Polideini, Tachinini and Siphonini, the phasiine clade Phasiini+Gymnosomatini (P-G clade), and the dexiine tribes Dexiini and Voriini s.s. Most other credible shift configurations with non-trivial probabilities were similar, with rates increasing in the same primary clades (Supporting Information, Fig. S4). These clades were also identified as experiencing increased diversification in the maximum shift credibility configuration (an alternate method of identifying rate variation; Supporting Information, Fig. S5). As further support that shifts towards increased diversification have occurred in these clades, each of them, aside from Voriini s.s. and Siphonini, exhibit cumulative shift probabilities of greater than 95%.

Diversification rate estimates based on the MoM estimator revealed accelerated diversification rates in many of the same clades identified in the BAMM analysis. Clades identified as having significantly greater numbers of species than expected include the tribes Goniini, Eryciini (and *Carcelia* group), Blondeliini, Voriini s.s., Exoristini, Dexiini, Polideini and Tachinini (Fig. 3; Table 3). As might be expected, more clades departed from expected diversity when crown ages and low extinction fractions (e.g. 0.05, 0.1) were assumed. The phasiine tribes Phasiini and Gymnosomatini, as well as the Siphonini never fell outside of expected confidence intervals, and the “long-stemmed” tribe Tachinini was only an outlier when crown ages were used to estimate diversification rates.

#### TRAIT DEPENDENT DIVERSIFICATION

##### Hosts

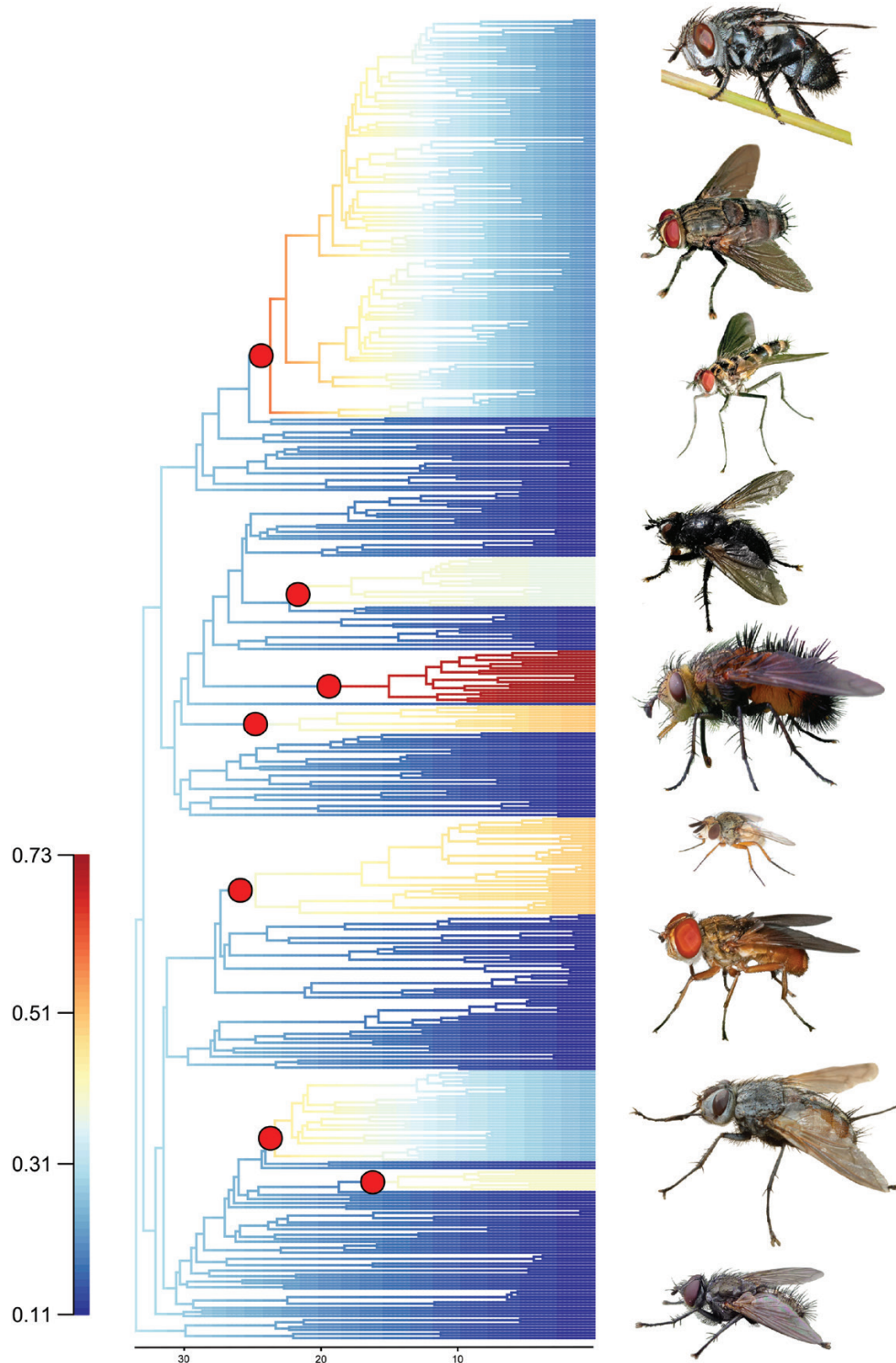
Host use at the ordinal level varies widely in the Tachinidae; however, most bristle flies attack Lepidoptera, Coleoptera or Hemiptera (Fig. 4). BiSSE analyses suggest that Lepidoptera-attacking lineages experience elevated speciation rates (Table 4); however, Bayesian analysis indicated that this does not translate into greater overall diversification rates

(see Supporting Information, Fig. S7), as extinction rates are also somewhat elevated. Multistate MuSSE analysis of host use with four states (Lepidoptera, Coleoptera, Hemiptera and other) identified significant variation in speciation, extinction and diversification rates (Table 5). In this analysis, hemipteran parasitoids displayed the highest speciation rates (but also high extinction rates); however, lepidopteran parasitoids experienced the most rapid rates of diversification (oddly, coleopteran parasitoid extinction was estimated to be zero). Posterior distributions suggest that the significant effect of host is primarily due to greater diversification rates of caterpillar parasitoids than beetle or true bug parasitoids (Supporting Information, Fig. S8). FiSSE analyses, which took into account only the taxa represented in the tree and not the full clade diversity, revealed no significant effects of hosts on diversification (including host stage and feeding habit; Supporting Information, Table S2).

Analysis of host stage, which divides tachinids into those attacking holometabolous larvae vs. adults or hemimetabolous taxa, significantly affected diversification in BiSSE models, with the former group exhibiting greater speciation and diversification rates (Table 4). In contrast, host feeding habit had no discernable effect; however, this may be due to the rarity of lineages that attack non-phytophagous hosts.

PGLS analyses, employing the trimmed tree representing major tribes and lineages, resulted in similar, but somewhat inconsistent, results regarding the effect of host use on diversification (Table 6). Total richness of lineages increased with the proportion of lepidopteran hosts and decreased with the proportion of adult/nymphal hosts, but this depended on the model framework (Table 6). When diversification rate was used as the dependent variable, significant or marginally significant effects were observed under the *nlme gls* model framework, whereas no significant variation was observed under the *caper pgl*s model framework. Models including multiple factors indicate a significant interactive effect on diversity and diversification of the proportion of caterpillar hosts and the proportion of adult hosts. Although host associations significantly influenced diversification, explained variance was low across models, with  $R^2$  values less than 0.1 for models with single factors.

Finally, STRAPP analysis revealed no significant effects of any traits on diversification (Supporting Information, Table S3). In general, estimated rates followed predictions with higher diversification rates in caterpillar-attacking taxa and those attacking larval stages; however, these rates did not differ significantly based on non-parametric tests. In multi-state analyses, tachinid taxa attacking lepidopteran and coleopteran hosts had similar elevated estimated rates of diversification over those using Hemiptera or other host orders.



**Figure 2.** A phylogenetic reconstruction of the Tachinidae indicating diversification rate variation across clades. Warmer colours indicate higher diversification rates and cooler colours lower. Red dots indicate the most likely position of shifts in rates. Taxa from the bottom are: *Macquartia tessellum* (Spain) (P. Alvarez), Dexiini ‘gen. sp.’ (South Africa) (S. Marshall), *Ectophasia leucoptera* (Greece) (S. Marshall), *Siphona* sp. (Norway) ([https://www.diptera.info/forum/viewthread.php?thread\\_id=95049](https://www.diptera.info/forum/viewthread.php?thread_id=95049)), *Protodejeania* sp. (USA) (PC), *Panzeria vivida* (Italy) (PC), *Trigonospila* sp. (Vietnam) (S. Marshall), *Sturmia* sp. (Tanzania) (S. Marshall), *Pseudogonia rufifrons* (Spain) (P. Alvarez).

**Table 2.** Mean speciation rates ( $\lambda$ ) and standard deviation (SD) of tachinid clades experiencing significant diversification rate shifts in the best shift configuration from BAMM analysis. Host orders, host stage and oviposition type (ovipary vs. ovolarvipary) are indicated

Subfamily	Clade	$\lambda$	SD	Order	Stage	Oviposition type
Exoristinae	Crown Exoristinae <sup>†</sup>	0.294	0.038	Lep./Col.*	Larvae*	Both
Tachininae	Polideini <sup>‡</sup>	0.341	0.090	Diverse	Mixed	Ovolarvipary
Tachininae	Tachinini	0.660	0.178	Lep.	Larvae	Ovolarvipary
Tachininae	Siphonini	0.438	0.165	Lep.*	Larvae	Ovolarvipary
Phasiinae	Phasiini+Gymnosomatini	0.425	0.131	Hem.	Adult	Ovipary
Dexiinae	Dexiini <sup>†</sup>	0.282	0.082	Col.	Larvae	Ovolarvipary
Dexiinae	Voriini s.s. <sup>‡</sup>	0.339	0.153	Lep.*	Larvae	Ovolarvipary
Tachinidae	(All)	0.241	0.017			

\*Indicates mixed states in the lineage but with the predominant state indicated.

<sup>†</sup>Includes the tribes Goniini, Eryciini, Blondeliini (mostly) and Exoristiini.

<sup>‡</sup>Including Ernestiini in part: *Panzeria*, *Hyalurgus* and *Linnaemya*.

<sup>§</sup>Excluding Sophiini, *Neximyia* and *Pelycops*.

<sup>¶</sup>*Sensu* Crosskey, 1984 with the addition of *Wagneria* and *Polygaster*.

### Oviposition mode

In contrast to expectation, BiSSE models suggest that oviparous taxa experience higher speciation rates than ovolarviparous tachinids (Table 4). Extinction rates were also inferred to be higher in this group, and thus more rapid speciation did not translate into greater diversification rates. FiSSE also supported higher speciation rates in oviparous taxa ( $P = 0.049$ ; Supporting Information, Table S2), but again this analysis only considered taxa present in the phylogenetic reconstruction. Oviposition mode had no significant effect on diversity or diversification rates in PGLS models (Table 6). Although not significant, STRAPP analysis suggested the opposite pattern, with ovolarviparous taxa experiencing greater speciation and net diversification rates than oviparous taxa. In multistate analyses, taxa with microtype ovolarviparous eggs had the highest estimated rate of diversification, followed by ovolarvipary (macrotype) and ovipary.

### Hidden states

Comparisons of models with and without hidden states using the HiSSE framework suggest that caution should be used in interpreting the results from BiSSE analyses. In none of the four traits examined, does the BiSSE model, which ascribes variation in diversification to the observed traits, exhibit substantially greater likelihood or a lower AIC score than the BiSSE null model (which assumes no pattern of diversification rate variation across the tree), despite clear evidence that rates are not equal (Supporting Information, Table S4). However, for host order (Lepidoptera or not), host stage and oviposition mode, the full HiSSE model including both observed and hidden states exhibited

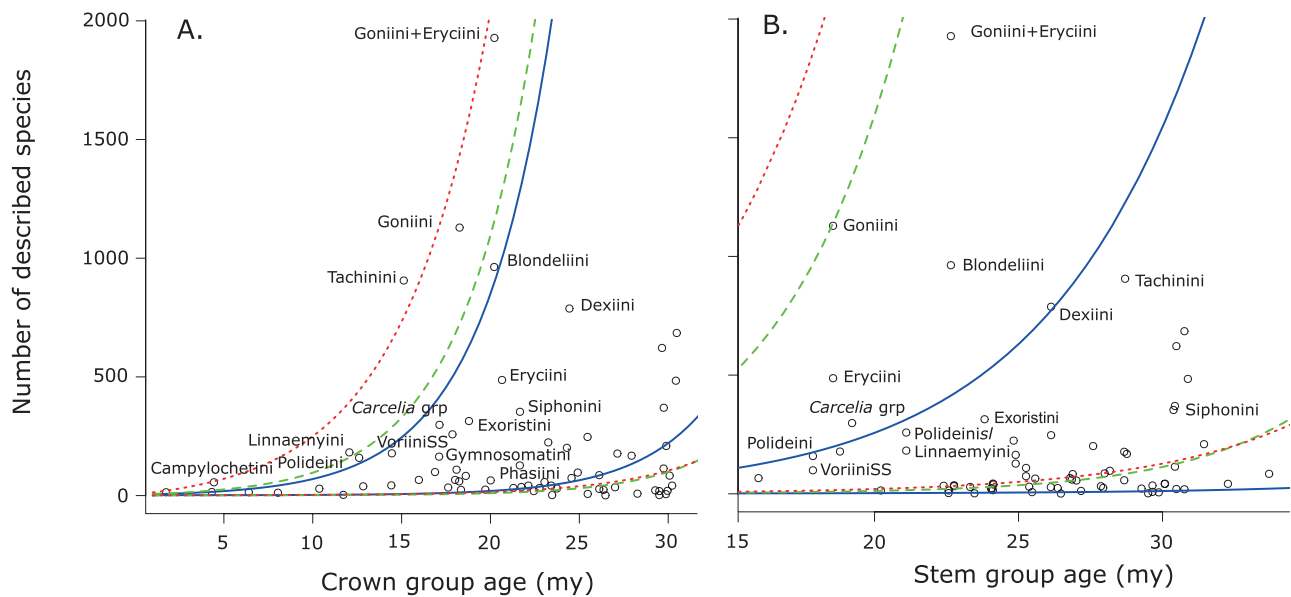
significantly greater likelihood and lower AIC scores (all  $P < 0.001$ ) than any other model. For host feeding habit, the full HiSSE model was no more likely than the HiSSE CID-2 model, indicating that the observed trait had no discernable impact on diversification alone or in combination with a hidden trait.

## DISCUSSION

### EXTRAORDINARY DIVERSIFICATION OF THE TACHINIDAE

Our estimates confirm that the fly family Tachinidae is exceptional among animals in its rate of diversification, ranking among the most rapidly diversifying families of organisms. In their examination of diversification rates among families, Scholl & Wiens (2016) estimated an even faster rate for tachinids (0.322), 6<sup>th</sup> among animal families and 9<sup>th</sup> among families across kingdoms. This rate calculation was based on an overestimate of described tachinid species (9626 vs. 8552) and an underestimate of tachinid age (26.4 vs. 33.7 Mya). However, that study also underestimated ages of other rapidly diversifying clades. For example, the top ranked animal family in their data set, the Furnariidae (ovenbirds), is likely at least twice as old as indicated by Scholl & Wiens [(2016); 25–30 Mya rather than 9.4 Mya (Claramunt *et al.*, 2012)]. The top ranked insect families of Scholl & Wiens (2016) included the moth clades Arctiidae (= Arctiinae; #2), Lymantriidae (= Lymantriinae; #3) and Noctuidae (#7), as well as the mantid family Iridopterygidae (#4). These taxa have certainly radiated explosively; however, ages of these groups were also underestimated (Kawahara *et al.*, 2019) and diversification rates overestimated, dramatically in some cases [e.g. the Iridopterygidae are polyphyletic





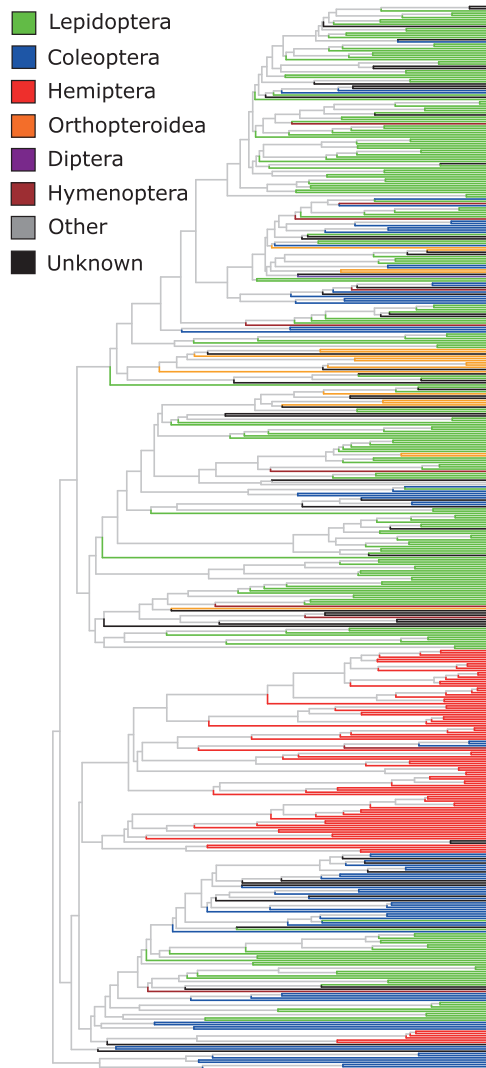
**Figure 3.** Species richness vs. crown age group (A) or stem group age (B) of tachinid clades. Coloured lines represent approximate 95% confidence intervals for extinction fractions of 0.1 (solid, blue), 0.5 (dashed, green) and 0.9 (dotted, red).

**Table 3.** Diversification rates ( $r$ ) of select tachinid clades based on MoM calculations. “ $N$  species” is the approximate number of described species.  $P$  values are the probability of observing a clade of that size or larger given the overall diversification rate of the family, with an extinction fraction of 0.1, and estimated stem or crown clade ages. Significant  $P$  values are highlighted in bold aside from those that are likely spurious (\*); strong significance based on crown ages in these lineages is likely due to limited sampling within them. Marginally non-significant trends are indicated in italics.

Subfamily	Clade	$N$ species	$r_{\text{crown}}$	$P_{\text{crown}}$	$r_{\text{stem}}$	$P_{\text{stem}}$
Dexiinae	Campylochetini*	56	0.748	$3.2 \times 10^{-8}$	0.145	0.7517
Dexiinae	Dexiini	788	0.244	0.4506	0.251	<b>0.0080</b>
Dexiinae	Voriini s.s.	178	0.309	<i>0.0530</i>	0.270	<b>0.0116</b>
Exoristinae	<i>Aplomya</i> group*	14	1.107	$6.4 \times 10^{-5}$	0.126	0.7797
Exoristinae	Blondeliini	963	0.305	<b>0.0176</b>	0.299	<b><math>9.9 \times 10^{-6}</math></b>
Exoristinae	<i>Carcelia</i> group	298	0.292	<i>0.0889</i>	0.291	<b>0.0010</b>
Exoristinae	Eryciini	487	0.266	0.2211	0.328	<b><math>2.7 \times 10^{-6}</math></b>
Exoristinae	Exoristini	314	0.269	0.2197	0.237	<b>0.0500</b>
Exoristinae	Goniini	1129	0.346	<b>0.0002</b>	0.373	<b><math>1.2 \times 10^{-13}</math></b>
Phasiinae	Gymnosomatini	164	0.257	0.3293	0.201	0.2815
Phasiinae	Phasiini	127	0.191	0.8448	0.190	0.3753
Tachininae	Linnaemyini*	182	0.373	<b>0.0018</b>	0.242	<i>0.0537</i>
Tachininae	Polideini	159	0.346	<b>0.0107</b>	0.278	<b>0.0084</b>
Tachininae	Siphonini	353	0.238	0.5055	0.190	0.3835
Tachininae	Tachinini	906	0.404	<b><math>6.4 \times 10^{-8}</math></b>	0.234	<b>0.0334</b>

and probably > 70 Mya rather than the estimated 10 Mya (Svenson & Whiting, 2009; Legendre et al., 2015)]. Our point here is that tachinid diversification rates are likely even more exceptional than indicated by our estimates, placing them among the uppermost handful of family-ranked clades of animals based on current estimates of richness, phylogeny and age.

Species richness is also probably dramatically underestimated in tachinids relative to many other rapidly radiating taxa. Diversification rate estimates of clades typically employ numbers of described species in their calculations. For vertebrate groups, such as ovenbirds and rodents, these estimates are likely not far off the true diversity; however, for tachinid flies they are likely off by a



**Figure 4.** The distribution of host use at the ordinal/superordinal level across the phylogeny of tachinids.

factor of two or more (Stireman *et al.*, 2006). Furthermore, diversification rate estimates here and in Scholl & Wiens (2016) employed stem group ages, which can underestimate diversification rates for well-sampled groups. It is unlikely that the dense phylogenetic reconstruction we used omitted many extant basally branching lineages, as nearly all tribes were included, and therefore the estimated crown group diversification rate (0.374 with  $\varepsilon = 0.5$ ) may more accurately reflect the rapidity of extant tachinid radiation.

#### BIASES IN DIVERSIFICATION RATE ESTIMATES

That tachinid flies exhibit greater diversification rates than such megadiverse insect families as weevils [Curculionidae, 51 000 spp. (Ślipiński *et al.*, 2011)], rove beetles [Staphylinidae, 56 000 spp. (Ślipiński *et al.*, 2011)] and ichneumon wasps [Ichneumonidae,

23 000 sp. (Quicke, 2015)] is surprising. However, these groups are all far older than the Tachinidae, and this reveals the somewhat misleading nature of such comparisons, as the ranking of lineages as families is somewhat subjective. Certainly diversification rates in some tribes and subfamilies of comparable age within these families would rival or surpass the Tachinidae.

One pattern that emerges from comparisons of diversification rates is that young clades tend to exhibit high rates of diversification (Scholl & Wiens, 2016; Henao Diaz *et al.*, 2019), and this may be at least partly an artifact. Recently diverging lineages tend to have higher estimated rates of diversification because extinction has had less time to operate [i.e. the “pull of the present” (Budd & Mann, 2018)]. Furthermore, only in old clades can low diversification rates be effectively detected (Magallón & Sanderson, 2001). Negative relationships between diversification rate and clade age may also be due to density dependent diversification, in which diversification rates saturate over time as ecological niches become filled (Rabosky, 2009, 2013; though see Wiens, 2011). Under this scenario, the youngest clades that are in the exponential phase of diversification, not having approached ecological limits, will be inferred to have the greatest rates of diversification. Finally, Henao Diaz *et al.* (2019) argue that the phenomenon of high diversification in young clades is not an artifact of sampling or niche-filling, but reflects the episodic nature of organismal diversification.

In addition to their outlying position in calculated diversification rates, tachinids bear other hallmarks of an explosively radiating lineage. The Tachinidae are regarded as one of the more difficult fly families in which to identify taxa (Crosskey, 1976; Wood, 1987), which may be attributed to a recent, rapid radiation of species with limited extinction. This, along with their great diversity, has discouraged research on their systematics despite their ubiquity and ecological importance. The family contains a great profusion of morphologically similar species and genera, which makes supraspecific classification difficult. The relative lack of clearly defined groups distinguished by external morphology has led to conflicting interpretations of generic limits, where at one extreme of the spectrum a new genus has been erected for almost every species (e.g. Townsend, 1927; see O’Hara, 2013a). Even terminalia, which often evolve rapidly and can be used to separate morphologically cryptic taxa in many insect groups, often vary little among tachinid species and genera (e.g. O’Hara, 1983; Fleming *et al.*, 2019). These morphological patterns are indicative of recent and rapid diversification with limited time for extinction to winnow away lineages and erode morphological space between them.

**Table 4.** BiSSE estimates of speciation ( $\lambda$ ), extinction ( $\mu$ ) and diversification ( $r$ ) rates of taxa according to traits with associated  $P$  values. Traits include: host order [Lepidoptera (Lep.) or other], host stage (adult or nymph vs. larva), host feeding habit [phytophagous (Phyt.) vs. other] and oviposition mode (oviparous or ovolarviparous). Separate analyses were conducted for only known trait states and for interpolated trait states (inferred) based on phylogenetic relationships. Marginally non-significant trends are indicated in italics

Trait:	Host order (known)			Host order (inferred)		
	Lep.	Other	$P$	Lep.	Other	$P$
$\lambda$	0.329	0.221	0.095	0.354	0.202	<b>0.009</b>
$\mu$	0.134	0.050	NS*	0.161	0.025	<i>0.094</i>
$r$	0.195	0.171	NS	0.193	0.177	NS
Trait:	Host stage (known)			Host stage (inferred)		
	Larva	Adult	$P$	Larva	Adult	$P$
$\lambda$	0.251	0.154	<b>0.049</b>	0.280	0.183	<i>0.089</i>
$\mu$	0.048	0.006	NS	0.077	0.034	NS
$r$	0.203	0.121	<b>0.008</b>	0.205	0.123	<b>0.038</b>
Trait:	Host feeding habit			Oviposition mode		
	Phyt.	Other	$P$	Ovipary	Ovolarvipary	$P$
$\lambda$	0.304	0.136	NS	0.389	0.221	<b>0.024</b>
$\mu$	0.121	0.0	<i>0.053</i>	0.252	0.013	<b>0.008</b>
$r$	0.222	0.147	NS	0.139	0.208	NS

\*NS = not significant.

**Table 5.** MuSSE estimates of speciation ( $\lambda$ ), extinction ( $\mu$ ) and diversification ( $r$ ) rates of taxa according to traits with associated  $P$  values (see Table 4). Marginally non-significant trends are indicated in italics

Metric	Host order (known)					Host order (inferred)				
	Lep.*	Col.	Het.	Oth.	$P$	Lep.	Col.	Het.	Oth.	$P$
$\lambda$	0.244	0.166	0.504	0.238	<b>0.003</b>	0.269	0.170	0.346	0.174	<b>0.001</b>
$\mu$	0.013	0.000	0.374	0.060	<b>0.003</b>	0.056	0.000	0.194	0.000	<b>0.010</b>
$r$	0.231	0.166	0.130	0.178	<b>&lt; 0.001</b>	0.218	0.162	0.151	0.178	<b>&lt; 0.001</b>

\*Lep. = Lepidoptera, Col. = Coleoptera, Het. = Heteroptera, Oth. = other.

#### WHY ARE BRISTLE FLIES SPECIAL?

The rapid diversification of bristle flies is likely related to evolutionary innovations. Indeed, tachinids are considerably more diverse than any other similarly aged clade of calyptrate flies (e.g. see Cerretti *et al.*, 2017), and their recognition as a family may be due to the novel traits they possess that underlie their radiation. An obvious explanation for their great diversity is their parasitoid lifestyle. The intimate association between parasitoids and their hosts should encourage specialization and coevolutionary interactions, which may facilitate speciation and diversification [i.e. “diversity begets diversity” (Janz *et al.*, 2006)]. For example, the vast and underestimated (Forbes *et al.*, 2018) diversity of parasitoid Hymenoptera is undoubtedly related to their parasitoid

habit and the diverse range of hosts they attack. Although tachinids are thought to be somewhat less specialized than their hymenopteran counterparts (Belshaw, 1994, though see Smith *et al.*, 2007; Stireman *et al.*, 2017), they are still likely to experience diversifying selection associated with resource specialization. An initial attempt to discern such diversifying effects of coevolutionary interactions for “carnivorous” insects, however, failed to demonstrate a clear relationship (Wiegmann *et al.*, 1993). This may be due to the relatively conservative analytical methods applied (sister-group comparisons) as well as inappropriate grouping of predators, parasitoids and parasite groups that vary in their opportunities for diversification. Notably, a recent analysis of animal phyla identified a positive relationship between parasitism

**Table 6.** Phylogenetic generalized least squares (PGLS) analyses using generalized least square models with Martins correlation structure (*gls*) and generalized linear models with optimized branch length transformations (*caper*). For *Richness* models, the number of extant described species per clade was used as the response variable and for *Diversification rate* models, estimated rates of diversification (*r*) calculated using the MoM estimator based on stem group ages is the response. Statistics of single traits are from individual models, but interaction terms are from full models. For the *gls* model  $R^2$  values are Cox and Snell pseudo  $R^2$  values, for *caper*  $R$ -values are adjusted. Marginally non-significant trends are indicated in italics

Trait	PGLS— <i>gls</i>				PGLS— <i>caper</i>			
	Est.	T	<i>P</i>	$R^2$	Est.	T	<i>P</i>	$R^2$
<i>Richness</i>								
Proportion Lepidoptera	120.5	1.69	<i>0.096</i>	0.051	130.2	2.49	<b>0.016</b>	0.088
Proportion adult	-145	-2.07	<b>0.043</b>	0.075	-84.1	-1.37	0.176	0.016
Oviposition mode	109.5	1.52	0.135	0.042	67.8	1.08	0.285	0.003
Prop. Lep. × prop. adult	2589	2.25	<b>0.029</b>	0.158	4423	3.21	<b>0.002</b>	0.246
<i>Diversification rate</i>								
Proportion Lepidoptera	0.041	1.97	<i>0.055</i>	0.068	0.016	0.57	0.568	-0.012
Proportion adult	-0.044	-2.15	<b>0.037</b>	0.080	-0.008	-0.47	0.641	-0.015
Oviposition mode	0.028	1.33	0.19	0.032	0.021	1.11	0.270	0.004
Prop. Lep. × prop. adult	0.889	2.70	<b>0.009</b>	0.199	0.742	2.20	<b>0.032</b>	0.043

and diversification (Jezkova & Wiens, 2017). Still, many parasitoid lineages exhibit more moderate diversification rates, including the sister group to tachinids, the Polleniidae, which are parasitoids of earthworms (Cerretti *et al.*, 2019). Interestingly, parasitoids and parasites are widespread among the other families of the superfamily Oestroidea to which tachinids belong (i.e. Calliphoridae, Oestridae, Polleniidae, Rhiniidae, Rhinophoridae and Sarcophagidae) and several of these groups also exhibit exceptional rates of diversification (Scholl & Wiens, 2016). Furthermore, this region of Diptera phylogeny as a whole shows evidence of an increased diversification rate (Wiegmann *et al.*, 2011), and these two observations may be related.

#### VARIATION IN DIVERSIFICATION WITHIN THE TACHINIDAE

Both BMM and MoM analyses indicate that no single clade is responsible for high diversification rate estimates for the family. We observed evidence of accelerated diversification in each of the four subfamilies, with all but the Phasiinae experiencing at least two shifts (Table 2). MoM estimators pointed to the Exoristinae as harbouring the most rapidly radiating lineages (e.g. Goniini, Blondeliini, Eryciini); however, several tachinine lineages also stood out (Tachinini and Polideini) as well as the Dexiini and Voriini *s.s.* (Dexiinae) when crown ages are considered (Table 3). Given the extensive sampling of tribes and genera in the phylogeny, estimates based on crown group ages may more accurately reflect diversification

rates in most major clades. However, there is always the uncertainty of whether the earliest branching lineages have been sampled. Relative evolutionary “losers” include taxa such as the Pelatachinini (5 spp.), Palpostomatini, Ethillini and other small tribes, as well as constituents of lineage grades including the Voriini *s.l.*, Minthoini and Ernestiini (see Stireman *et al.*, 2019). The Tachinini, which includes many large, spiny, brightly coloured tachinids (Fig. 5), have experienced perhaps the most dramatic radiation among the Tachinidae. It is possible, however, that lineages of some smaller bodied tachinids, including the Siphonini, Graphogastrini and Blondeliini, may rank far higher in inferred diversification rate once they are better studied.

#### EFFECTS OF HOST ASSOCIATIONS ON DIVERSIFICATION

Our results provide some support for the hypothesis that variation in diversification rate among tachinid clades is related to host use. Lineages attacking caterpillars, or more generally larval stages of holometabolous insects, exhibit elevated diversification rates in several analyses (BiSSE, PGLS, MuSSE). This finding is intuitively appealing given that the higher Lepidoptera have radiated recently and dramatically, and that a majority of tachinid species (~60%) use caterpillars as hosts. Several of the most rapidly diversifying clades including the Goniini, Eryciini and Tachinini are associated with the rapidly diversifying Macroheterocera (e.g. Bombycoidea, Noctuoidea, Geometroidea). The interactive effect of host type



**Figure 5.** A representative member of the rapidly diversifying tribe Tachinini [*Adejeania vexatrix* (Osten Sacken)] from the southwest U.S. (photo by S. Marshall).

(proportion Lepidoptera) and host stage (proportion adult) on diversification in PGLS analyses appears due to greater diversification as the proportion of species attacking caterpillars increases in clades that predominantly attack adult insects. In contrast, in lineages dominated by larvae-attacking species, shifts to caterpillars (e.g. as opposed to larval Coleoptera) have little effect on diversification, because rates are already high. This supports the hypothesis that shifts to caterpillars or other larval insect groups can favour diversification. Caterpillars and other phytophagous insect larvae represent an abundant, diverse, specialized and relatively vulnerable resource for parasitoids—conditions that should favour parasitoid persistence and diversification. Given the abundant caterpillar species available in most environments, it is in fact surprising that they have not been colonized more extensively by other dipteran parasitoids. The Tachinidae are the exception rather than the rule in having mastered the exploitation of caterpillars, explosively diversifying within this vulnerable niche. Finally, host feeding habit may influence diversification; however, because *c.* > 95% of tachinid hosts are phytophagous, feeding habit has little power to explain variation in diversification across the family.

It must be noted, however, that effects of host-association on diversification are inconsistent. In BiSSE models significant effects are dependent on inferred hosts, and for PGLS they depend on the model framework. Furthermore, both FiSSE

and STRAPP methods found no significant effects. The STRAPP approach to assessing SDD was highly conservative in assessing significance. Rate estimates by this method were often nearly double for taxa with one state vs. the other (e.g. larval vs. adult hosts; [Supporting Information, Table S3](#)); however, no comparisons approached statistical significance except for host feeding habit ( $P = 0.07$ ). The highly conservative nature of these tests is evident when assessing whether diversification rates are correlated with the number of extant species in each genus. This would be expected to be highly correlated, yet the estimated correlation was only 0.238 and not significant ( $P = 0.133$ ). Even in analyses where host use (order or stage) appears to have significant effects on diversification, the amount of variance explained is low.

Results from hidden state (HiSSE) models suggest that trait states identified as being important in BiSSE (or MuSSE) models may not actually be those underlying diversification. Given that full HiSSE models with both hidden and observed states were consistently of highest likelihood, host use traits appear to interact or be confounded with more influential traits. For example, parasitism of caterpillar hosts may often involve the use of plant volatiles in host location, and the use of those volatiles could be associated with diversification. This could result in a weak effect of host order on diversification, when behavioural mechanisms of host location are actually the key factor.

In summary, using caterpillars as hosts, or shifting to holometabolous insect larvae, appears to have detectable impacts; however, is neither necessary nor sufficient to drive enhanced diversification. For example, the P-G clade, which exhibits elevated diversification, develops entirely on Hemiptera. Furthermore, many tachinid clades that use caterpillar hosts (e.g. Pelatachinini, vorini and ernestiine grade lineages) are relatively depauperate. However, this does not mean that host-associations do not contribute to variation in diversification. Lineages attacking less diverse host orders such as the Orthoptera, Dermaptera, Mantodea and Phasmida, never comprise species rich or rapidly diversifying clades, whereas all significantly outlying clades in terms of diversification use species rich host clades (Lepidoptera, Coleoptera, Heteroptera). Thus, attacking diverse lineages may provide opportunities for diversification; however, other factors may determine whether that opportunity is capitalized on.

#### EFFECTS OF EGG TYPE ON DIVERSIFICATION

One interesting result from recent phylogenetic analyses of tachinids is the reconstruction of incubated eggs (ovolarvipary) as ancestral, with multiple reversions to unincubated eggs (ovipary; though see [Stireman \*et al.\*, 2019](#)). This contrasts with traditional views of ovipary being ancestral. In either case, diversification is not strongly correlated with egg type. For example, two oviparous groups show evidence of elevated diversification rates (Exoristini and Phasiini); however, other rapidly diversifying clades are predominantly ovolarviparous. Curiously, some SDD analyses suggested that oviparous taxa experience higher speciation rates, and also highly elevated extinction, resulting in a relatively moderate net rate of diversification. However, no effect was observed in other analyses, and hidden state models suggest that other, unknown, traits are likely more influential than our division of taxa into oviparous and ovolarviparous groups.

Microtype eggs may be a key evolutionary innovation for the tribe Goniini; however, because this state appears to have only evolved once (with a few other lineages sprinkled across the phylogeny having evolved similar strategies), it is difficult to assign causation. Several tachinid lineages in addition to the Goniini possess indirect attack strategies by which the larval stage contacts the host [e.g. host searching larvae ([Stireman \*et al.\*, 2006](#))], and this could influence diversification by permitting attack of otherwise inaccessible hosts. However, rapidly diversifying clades exhibit a mix of

direct (e.g. most Eryciini and Blondeliini, P-G clade) and indirect strategies (Goniini, Tachinini, Dexiini, Polideini). As with host associations, particular egg types or oviposition strategies appear neither necessary nor sufficient to drive diversification.

#### GEOGRAPHIC CONSIDERATIONS

Intrinsic traits of lineages are not the only factors that facilitate or limit diversification. Variation in diversification may involve an interplay between extrinsic factors like geographic opportunity (e.g. [Winkler \*et al.\*, 2018](#)) and intrinsic factors like dispersal ability, host location mechanisms and oviposition strategy. As examples of the former, geographic isolation provided by island archipelagos and island-like patches of mainland habitat are associated with many of our most impressive examples of adaptive radiation ([Losos & Ricklefs, 2009](#)) and such geographic contingencies may represent “hidden traits” driving shifts in diversification.

Tachinid biogeographic history is little understood; however, we can examine general patterns to evaluate the potential role of geography and geologic processes in their diversification. Each of the four subfamilies is well-represented in all biogeographic regions of the world ([O’Hara \*et al.\*, 2020](#)), and their descending rank in number of described species is constant: Exoristinae, Tachininae, Dexiinae and Phasiinae, except in the Australasian Region where the positions of dexiines and tachinines are reversed. In addition, although the Tachinidae are recent, there has been so much dispersal among regions that it is difficult to pinpoint where most major lineages have arisen and diversified. Bristle flies are diverse on all continents, and diverse clades tend to be diverse everywhere.

These distribution patterns imply that particular geographic or geologic features are not central to tachinid diversification, at least at a broad scale. Still, some tribes and genera are limited in distribution and there has been substantial diversification within particular regions. The great Australasian radiation of the almost endemic Rutiliini ([Crosskey, 1973a](#)) represents one of the few examples of a significant tribal diversification (~130 species) within a single region. Another is the radiation of the Proscissionini (Tachininae), a clade of about 80 species endemic to New Zealand. The vast majority of species in the rapidly diversifying tribe Polideini are endemic to the Americas (especially the Neotropics), whereas several tribes such as the Macquartiini, Glaurocarini and Hermyini are largely absent from this region. At the generic level, there is a high degree of endemism within certain regions, and overall about 70% of all

tachinid genera in the world are endemic to a single region (O'Hara & Henderson, 2020), varying from 14% (Oriental region) to 76% (Neotropical region).

There is no obvious consistent geographic pattern among clades in which shifts in diversification were inferred. The large “crown-clade” of the Exoristinae (i.e. Goniini, Eryciini and Blondeliini) appear to have diversified extensively in every major region of the world (O'Hara *et al.*, 2020). This also appears to be the case for Siphonini (Tachininae; JEOH, PC, JOS, pers. obs.). The bristly Tachinini are unusually rich in the Neotropics, especially in the Andes Mountains. The recent rise of the Andes (Garziona *et al.*, 2017), with their isolating effects, may have contributed to the extremely rapid radiation of this lineage as well as several other groups of Tachinidae [and other insects, e.g. Lisa De-Silva *et al.* (2017)]. In contrast to their Neotropical diversity, Tachinini are relatively poorly represented in the Afrotropical and Australasian regions. A similar pattern is apparent in the Polideini, an almost entirely American lineage (O'Hara, 2002) that possesses impressive, yet mostly undescribed, diversity in the Andes. However, the related genus *Panzeria* (Ernestiini) that comprises part of the “Polideini *s. l.*” clade exhibiting elevated diversification, is absent from the Neotropics and has diversified extensively across the Holarctic Region. Another related genus, *Linnaemya*, has the most described species of any tachinid genus (*c.* 150) and has diversified almost entirely within the Palearctic and Afrotropical regions. Like most major clades, Dexiini appear to be diverse everywhere; however, they make up a somewhat larger fraction of tachinid diversity in Australia. Voriini *s.s.* exhibit no clear geographic pattern, being reasonably diverse in all biogeographic regions. Finally, within the Phasiinae, the Phasiini+Gymnosomatini clade is somewhat over-represented in the Palearctic, Afrotropical and Oriental regions; however, the clade has diversified extensively in the Americas as well.

Like most insect groups, most of the undescribed diversity of tachinids lies in the tropics, especially the Neotropics where the more than 3000 described species is likely just a small fraction of the true richness (O'Hara, 2013b; Burington *et al.*, 2020). Tachinid diversity is also severely underestimated in the Oriental and Australasian regions (e.g. O'Hara *et al.*, 2004). Although more comprehensive surveys and descriptions of these enormous faunas could alter inferences about the relative diversity of lineages, our experience suggests that most of the unknown diversity in these regions lies in hyperdiverse lineages that already stand out [e.g. Blondeliini, Goniini, Tachinini, Siphonini, etc. (Stireman *et al.*, 2017; Brown *et al.*, 2018)].

## METHODOLOGICAL CONCERNS

There are a number of fundamental difficulties in making inferences about diversification, both its variation, and its causes, based on phylogenies. For example, taxon sampling can strongly influence inferred rates of diversification, and including sampling fractions may not always improve reliability (Chang *et al.*, 2020; Sun *et al.*, 2020). In our case, the vast numbers of undescribed species that are probably not randomly distributed phylogenetically accentuate these problems. Stochastic polytomy resolution is also difficult to apply to tachinids due to lack of understanding of composition and relationships even at the tribal level (Stireman *et al.*, 2019). Furthermore, estimating extinction rates from molecular phylogenies in the absence of fossil data can be problematic (Rabosky, 2010, 2016), and the fossil record of the Tachinidae is essentially non-existent (O'Hara, 2013b). Even more concerning, a recent analytical examination of the phenomenon of lineage diversification concluded that an infinite number of diversification histories are consistent with any particular time-calibrated phylogeny (Louca & Pennell, 2020). Thus, diversification dynamics and how they change over time (e.g. niche filling processes of adaptive radiation) cannot be inferred from time-calibrated phylogenies or lineage-through-time plots. Although we can identify lineages with varying net diversification rates, we cannot necessarily infer whether this was due to high speciation rates, low extinction rates or some combination of these variables.

Inconsistencies in our results across analyses give us pause in inferring too much from any one of them. All of the analytical methods and approaches used here have drawbacks and most have been subject to criticism for either being too conservative or too liberal in detecting significant patterns. In terms of variation in diversification, BAMM has come under criticism for improper likelihood functions (Moore *et al.*, 2016), underestimating numbers of rate shifts and providing misleading rate estimates (Meyer & Wiens, 2018; Meyer *et al.*, 2018), although these criticisms have been vigorously refuted (Rabosky *et al.*, 2017; Rabosky, 2018, 2019). On the other hand, the MoM approach is relatively crude—it fails to utilize phylogenetic information (e.g. branching patterns within clades), and it does not indicate where rate shifts happen on trees, nor does it evaluate the contribution of speciation vs. extinction to diversification rates. Furthermore, it is unclear whether stem ages (Meyer & Wiens, 2018) or crown ages (Stadler *et al.*, 2014) are more appropriate when estimating diversification rates. Still, in our analyses, both methods (BAMM and MoM) highlight primarily the same clades as outliers and these make

intuitive sense—all stand out as diverse, relatively young groups containing many genera and species.

Inference methods for SDD such as BiSSE, though an improvement upon simple sister-group comparisons, are also subject to biases that may mislead. “SSE-type” methods have been criticized due to bias towards identifying significant effects even in cases where none exist (Rabosky & Goldberg, 2015; Beaulieu & O’Meara, 2016). In addition, positive signals of diversification can be driven by a single clade under some circumstances (Maddison & FitzJohn, 2015). Alternatively, our PGLS analyses were likely less sensitive than SSE models, employing a stripped-down skeletal phylogeny and simple models.

In general, SDD analyses are hampered by high proportions of missing taxa. Despite the extensive taxon sampling of the tree used here, it still only includes 359 of 1477 genera (< 25%), and but a tiny fraction of species (O’Hara & Henderson, 2020). These missing taxa are considered to some extent in BiSSE/MuSSE analyses (as estimates of sampling fractions) as well as STRAPP and PGLS (as estimates of clade richness), but not in FiSSE analyses. As mentioned, specific inclusion of missing richness can be problematic due to uncertainty in where unsampled taxa should be placed phylogenetically.

Aside from potential analytical biases and artifacts, the trait coding used here was quite simplified, which may have obscured patterns. Host order (Lepidoptera or other), for example, is a coarse measure of host association, and finer scale or more functionally relevant states (e.g. host feeding niche, host specialization) might provide more insight. Oviposition strategy is also highly simplified here, as there is much variation within states in terms of egg morphology, where they are deposited and how the host is contacted, not to mention how hosts are located in the first place. Hidden state models hint at such a phenomenon, where additional states or interactions among multiple trait states may provide greater power in explaining variation in diversification rates.

## IDIOSYNCRATIC EVOLUTION AND CONCLUSIONS

We also must consider the possibility that there is no single trait or character state underlying shifts in diversification across the tachinid family tree. Parasitism, diverse host clades, oviposition strategies or other traits may provide potential opportunities for diversification; however, whether these opportunities are realized may depend on additional idiosyncratic traits or historical contingencies that vary from group to group. Each of the identified shifts towards rapid diversification across the tachinid phylogeny occurs in lineages that possess combinations of

traits that may facilitate diversification. In the subfamily Dexiinae, Dexiini attack diverse and relatively unexploited hosts (primarily concealed larval Scarabaeoidea) with novel planidial host-searching larvae, and Voriini are primarily parasitoids of the rapidly radiating Noctuoidea and Geometroidea, typically laying their incubated eggs directly on hosts. The tribes Phasiini and Gymnosomatini (Phasiinae) attack the Heteroptera (a diverse clade), and are known to use host pheromones to locate them (Stireman *et al.*, 2006), which could facilitate ecological speciation (Aldrich & Zhang, 2002). In the Tachininae, the diverse clades Tachinini, Polideini and Siphonini are all predominantly parasitoids of caterpillars; however, the Tachinini have ambushing larvae, the Polideini have host-seeking larvae and the Siphonini deposit eggs on or near the host. Furthermore, the Polideini have colonized a diverse array of additional, somewhat unusual, host taxa (e.g. Orthoptera, Blattodea, Chilopoda, Scorpiones), and the small-bodied Siphonini are able to exploit additional host resources such as microlepidoptera. The Tachinini, which appear to have achieved their impressive diversity (900+ spp.) over a short period (c. 15 Mya) may owe some of their evolutionary success to the rise of the Andes Mountains of South America, where a substantial portion of their diversity lies. Finally, the immense radiation of the “higher” Exoristinae (Exoristiini, Blondeliini, Eryciini and Goniini) may have been facilitated by their use of diverse hosts (predominantly Lepidoptera, but also many Coleoptera) and diverse oviposition strategies (including ovipary, ovarvipary and micro-ovolarvipary). In short, multiple factors involving host use, oviposition strategies, geography and other traits may be interacting to permit each of these lineages to take advantage of potential host resources, facilitating their diversification.

There is an emphasis on “simple stories” in the modern scientific literature. That is, straightforward causal relationships with high explanatory power. This makes sense, as our goal is often to uncover general patterns and predictive relationships that can be broadly applied across systems. However, in evolutionary biology the “stories” are not always so simple, and may depend strongly on chance and contingency as well as deterministic forces (Blount *et al.*, 2018). Here, we have recovered evidence that host associations may contribute to variation in diversification; however, the signal is inconsistent and the variation explained is relatively low. Our inability to recover consistent, robust effects of such traits on diversification may be due to inappropriate models, focus on the wrong traits, incomplete taxon sampling (and limited knowledge of true richness) or other biases and artifacts. However, it is also possible that the underlying factors facilitating elevated diversification vary among the clades themselves and are the product of complicated suites of interacting traits. Instead of one story, there may be many.



## ACKNOWLEDGEMENTS

We thank our collaborators that helped produce the phylogenetic reconstruction on which this study was based including Jeremy Blaschke, Isaac Winkler, Matt Duncan, Beth Stayrook and Alia Eckhardt. We thank Steve Marshall and Piluca Alvarez for their generous sharing of images of live tachinid flies (Figs 2, 5). We also thank two anonymous reviewers for their constructive comments. This research was supported by NSF DEB 1416269 to J.O.S., J.E.O. and P.C., 1146290 to J.K.M. and NSF DEB 1442134 to J.O.S.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Table S1.** Bayes factors comparing the likelihoods of diversification rate shifts (row vs. column). Bayes factors greater than *c.* 10 (bold) provide strong evidence of one model of rate shifts of the other (Jeffries, 1961).

**Table S2.** Speciation rates and *P*-values from FiSSE diversification analyses of binary variables.  $\lambda_0$  indicates estimated speciation rates of the first listed state and  $\lambda_1$  the second.

**Table S3.** State dependent diversification (*r*), speciation ( $\lambda$ ) and extinction rates ( $\mu$ ), estimated using the STRAPP method based on BAMM analyses of diversification (with inferred states for all taxa). *P* values are derived from Mann-Whitney U tests for binary traits, and Kruskal-Wallis tests for multistate traits.

**Table S4.** Likelihoods (lnL), AIC, and corrected AIC (AICc) values of binary trait dependent diversification models including BiSSE null (no variation in diversification), BiSSE full (observed trait dependent diversification), HiSSE CID2 (hidden trait dependence only), and HiSSE (both observed and hidden trait dependence; see text).

**Figure S1.** Speciation and extinction rates over time inferred from BAMM diversification analysis.

**Figure S2.** Lineages through time plot of the Tachinidae

**Figure S3.** Prior (blue) and posterior (red) probabilities of the number of diversification rate shifts from BAMM analysis.

**Figure S4.** Top six most credible rate shift configurations of 1095 distinct configurations. Each of these is quite similar to the best shift configuration (Figure 2). F indicates the frequency of each configuration in the posterior distribution; grey circles indicate inferred positions of shifts, with size proportional to their posterior probability.

**Figure S5.** The maximum shift credibility configuration of diversification rates shifts (see Figure 2).

**Figure S6.** Best shift configurations from BAMM runs using varied parameters including root ages (median, 33.7 Mya vs. maximum 46.7 Mya), global or species specific sampling probabilities, backbone sampling probabilities (1 or 0.9), and initial or revised estimates (based on O'Hara *et al.*, 2019) of taxon diversities (genera) and species specific sampling fractions.

**Figure S7.** Posterior probability densities of diversification rates for tachinids attacking Lepidoptera (beige, right) or other orders (blue, left) from BiSSE analyses.

**Figure S8.** Posterior probability densities of diversification rates for tachinids attacking Lepidoptera (Lep, green), Coleoptera (Col, blue), Hemiptera (Hem, red) or other orders (Other, violet) from MuSSE analyses.