

The evolutionary origin of parasitoid flies in the suborder Brachycera

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Abstract

Parasitoidy is a life-history strategy demonstrated by organisms that develop on or within a host organism, using the host as an essential trophic resource which is killed as a consequence of parasitoid development. Parasitoids represent over 3% of eukaryotes and have been shown to play an important role in structuring natural communities. However, we know very little about the evolutionary origins of parasitoidy including (i) how many independent shifts to parasitoidy have occurred, (ii) from what ancestral feeding habits parasitoids are most often derived and (iii) whether evolutionary pathways to parasitoidy differ between endo- and ectoparasitoid lineages. I investigated the evolutionary origin of parasitoid lineages in the suborder Brachycera (Order Diptera) using a supermatrix approach to estimate a proportionally sampled molecular phylogeny that is robust to model violations and appropriate for hypothesis testing. The resulting alignment sampled 565 taxa and 17 genetic loci using sequence data mined from the NCBI's Nucleotide database. I then used Bayesian inference to reconstruct the ancestral larval feeding habits of all major brachyceran lineages, and estimate the number of transitions to parasitoidy within the Brachycera. In total, twenty-two transitions were recovered, seventeen gains and five losses. Parasitism was found to be the most common larval feeding habit directly preceding shifts to parasitoidy. The evolutionary history of endo- and ectoparasitoids could not be reconstructed independently due to limitations on parameter estimation given the available data. The results of this thesis contribute to our overall understanding of the origin of parasitoid flies and contribute to their continued study by producing a phylogeny which is more suitable for further hypothesis testing.

Résumé

La parasitoïdie est un mode de vie de certains organismes qui se développent sur ou dans un organisme hôte, utilisant l'hôte comme ressource trophique essentielle qui est tuée en conséquence du développement du parasitoïde. Les parasitoïdes représentent plus de 3% des eucaryotes et il a été démontré qu'ils jouent un rôle important dans la structuration des communautés naturelles. Cependant, nous savons très peu de choses sur les origines évolutives de la parasitoïdie, y compris (i) combien de changements indépendants vers la parasitoïdie se sont produits, (ii) à partir de quelles habitudes alimentaires ancestrales les parasitoïdes sont le plus souvent dérivés et (iii) si les voies évolutives vers la parasitoïdie diffèrent entre endo - et les lignées ectoparasitoïdes. J'ai étudié l'origine évolutive des lignées de parasitoïdes dans le sous-ordre des Brachycères (ordre des diptères) en utilisant une approche de supermatrice pour estimer une phylogénie moléculaire échantillonnée proportionnellement qui est robuste aux violations du modèle et appropriée pour les tests d'hypothèses. L'alignement résultant a échantillé 565 taxons et 17 locus génétiques à l'aide de données de séquence extraites de la base de données Nucleotide du NCBI. J'ai ensuite utilisé l'inférence bayésienne pour reconstruire les habitudes alimentaires ancestrales des larves de toutes les principales lignées de brachycères et estimer le nombre de transitions vers l'ecto- et l'endoparasitoïde au sein des Brachycères. Au total, vingt-deux transitions ont été récupérées, dix-sept gains et cinq pertes. Le parasitisme s'est avéré être l'habitude alimentaire larvaire la plus courante précédant directement le passage à la parasitoïdie. L'histoire évolutive des parasitoïdes n'a pas pu être reconstruite indépendamment en raison des limites de l'estimation des paramètres compte tenu des données disponibles. Les résultats de cette thèse contribuent à notre compréhension globale de l'origine des mouches

parasitoïdes et contribuent à leur étude continue en produisant une phylogénie qui est plus appropriée pour d'autres tests d'hypothèses.

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Thesis Format

This thesis is prepared in monograph format. All tables are presented at the end of the chapter subsection in which they are first mentioned, apart from appendices which are presented as supplementary material after the *Literature Cited*. Due to their size, all figures are placed at the end of the text, after the *Literature Cited* and before the appendices.

Contribution of Authors

Linley Sherin collected the data, performed all data analysis, and wrote this thesis. Christopher Buddle contributed scientific mentorship and guidance during planning and writing phases. Jessica Gillung contributed significantly to the concept and design of this project and provided critical suggestions throughout the data collection and data analysis phases. Christopher and Jessica both provided constructive comments on multiple drafts of this thesis. Morgan Jackson provided mentorship on many aspects of the project, most notably during phylogeny estimation and interpretation.

1. Introduction

1.1 Thesis rationale

A parasitoid is any organism that develops on or within a host organism, using the host as an essential trophic resource that is killed as a consequence of parasitoid development (Eggleton & Gaston, 1990). Parasitoids can be categorized into ectoparasitoids, which develop on the external surface of their hosts, and endoparasitoids, which develop within the body cavity of their host (Feener & Brown, 1997). The parasitoid life-history strategy (parasitoidy) is observed in more than 3% of eukaryote species across multiple phyla (Eggleton & Belshaw, 1992; Mora *et al.*, 2011). However, few studies have investigated patterns of parasitoid evolution beyond strict systematic treatments. Those that have center overwhelmingly on the parasitoid wasps (order Hymenoptera; e.g. Murray *et al.*, 2013; Sharanowski *et al.*, 2021; Tschopp *et al.*, 2013), and to a lesser extent other individual parasitoid lineages (e.g., Stireman III, 2005; Winkler *et al.*, 2015; Yeates and Greathead, 1997). By focussing on specific parasitoid lineages, these studies provide insights on the evolution of various parasitoid traits, including host breadth, time of attack, and ancestral host shifts. However, they are limited in their ability to draw conclusions about the origin of parasitoidy and consequently, many fundamental questions regarding the repeated evolutionary origins of parasitoidy remain outstanding (Feener & Brown, 1997). For instance, there is currently no accurate estimate of the number of independent shifts to parasitoidy across the tree of life, and we do not know from what ancestral feeding habits parasitoid most parasitoid lineages are derived, though this is thought to inform the host use and physiology of extant species (Eggleton & Belshaw, 1993; Eggleton & Gaston, 1990). In addition, many previous studies have not differentiated between endo- and ectoparasitoid lineages despite key differences in their biology. By broadening the scope of our research efforts to investigate the evolution of

multiple diverse parasitoid lineages, we can begin to address these knowledge gaps concerning the evolutionary origin of parasitoidy.

True flies (order Diptera) have been proposed as an alternative model for quantitative studies of parasitoid evolution due to the repeated convergent evolution of parasitoidy within the group, which has been conservatively estimated at 21 independent shifts (Eggleton & Belshaw, 1992; Feener & Brown, 1997). As nearly all parasitoid flies reside in the suborder Brachycera (Godfray, 1994; Wiegmann *et al.*, 2011), I propose this clade as an appropriate model for the testing of evolutionary hypotheses regarding parasitoid evolution. A limitation on the use of Brachycera as an evolutionary model is the lack of a well-supported phylogenetic framework to act as a foundation for the reconstruction of ancestral character states (Pagel *et al.*, 2004). Thus, the estimation of a new phylogeny of the Brachycera which addresses previous limitations is an essential precursor to confronting unresolved questions about the origin of parasitoidy within the group.

1.2 Research objectives

My thesis expands our knowledge of the evolutionary origins of parasitoidy by addressing the following three research questions, using the suborder Brachycera as a model study system:

- 1) How many independent shifts to parasitoidy have occurred within Brachycera?
- 2) From what ancestral larval feeding habit are parasitoid lineages most often derived?
- 3) Do evolutionary pathways to parasitoidy differ between endoparasitoid and ectoparasitoid lineages?

To address these questions, I estimated a phylogeny of the Brachycera with higher taxonomic coverage than previous phylogenetic estimates. I accomplished this by mining published DNA sequence data from multiple studies on a large scale and combining them into a single concatenated dataset (supermatrix) using a largely reproducible *R*-based workflow. Brachycera were sampled proportionally with the goal of including 0.5% of described diversity for each family within the suborder. This included the targeted sampling of parasitoid lineages to ensure that the dataset was suitable for testing the proposed hypotheses. Sequence data was harvested only for loci deemed informative in previous phylogenetic studies of dipteran evolution, including a mix of nuclear, mitochondrial, and ribosomal genes. Using the newly estimated phylogeny, I reconstructed the larval feeding habit of all ancestral nodes using Bayesian inference to evaluate the previously stated hypotheses, thus contributing to our growing knowledge of parasitoid evolution. The estimation of this phylogeny has the additional benefit of providing a foundation for the further study of parasitoid flies in the suborder Brachycera, as well as the study of parasitoids more broadly.

2. Literature review

2.1 Why parasitoids?

A parasitoid is any species that develops on or within a single host organism, using the host as a primary trophic resource that is killed due to parasitoid development. Previous research on parasitoidy has focused largely on the ecology and physiology of parasitoid insects (Feener & Brown, 1997; Godfray, 2016). These studies highlight the role that parasitoids occupy in structuring natural communities through indirect interactions (Godfray, 1994; Hassell, 2000; Morris, Lewis & Godfray, 2004; Settle & Wilson, 1990), as well as their applied use as biocontrol agents of crop and forestry pests (DeBach & Rosen, 1991; Godfray, 2016; Jervis, 2005; Pijnakker *et al.*, 2020; Pimentel *et al.*, 1997). Despite considerable scientific interest in parasitoids, many parasitoid taxa remain undescribed or biologically unknown, and parasitoids are often neglected by conservation efforts despite exhibiting many traits of extinction-prone groups (Hochberg, 2000; Shaw & Hochberg, 2001). To increase our knowledge of parasitoids, the continued study of all aspects of parasitoid biology and diversity has been highly recommended (Lasalle and Gauld 1991).

2.2 Defining parasitoidy

Previous estimates suggest that parasitoids represent ~3% of all eukaryote species. This value was calculated following Eggleton and Belshaw (1992), who state that parasitoids represent 10% of all insects, and Mora *et al.*, (2011), who estimate that insects represent nearly 30% of all eukaryotes. However, higher estimates of these values have been proposed. For instance, Godfray (1994) suggests that the proportion of parasitoids within Insecta could be as high as 20-25%, thus shifting the percentage of parasitoids within eukaryotes to above 7%.

LaSalle and Gould (1991) suggest that parasitoid wasps alone could represent 20% of all insects when accounting for undescribed species. Most recently, Sharanowski *et al.* (2021) state that parasitoid wasps alone represent 3% of all life. Thus, it appears that the proportion of parasitoids continues to grow with their continued study. However, despite discrepancies between these values, even the largest of these estimates can be considered highly conservative as they all ignore non-insect parasitoids, which are often excluded from classification as parasitoids despite meeting the requirements under a functional definition.

A functional definition of parasitoidy states that a parasitoid is any organism which develops on or within a single host organism, using the host as an essential trophic resource which is killed, either directly or indirectly, due to parasitoid development (following Eggleton & Gaston, 1990). Optionally, this definition can be further expanded to add that parasitoids all possess a free-living reproductive stage that occupies a niche that is different from their developmental stage (following Yeates & Greathead, 1997). Using the functional definition, nematodes and fungi can be classified as parasitoid taxa. This challenges alternative definitions which define parasitoidy as taxonomically constrained to insects (reviewed in Eggleton & Gaston, 1990). In fact, some definitions further limit parasitoidy to only insects which target arthropod hosts (Gauld & Bolton, 1988), having largely been informed by the biology of parasitoid wasps (order Hymenoptera; Eggleton & Belshaw, 1993). By adhering to a functional definition of parasitoidy, we ensure that parasitoidy is comparable with other life-history strategies such as phytophagy, predation and parasitism, which are defined functionally. Under the functional definition, it becomes clear that parasitoids represent a much higher proportion of eukaryote life than calculated under a taxonomically constrained definition as above. An actual estimate of this value is difficult to ascertain due to the lack of research on fungi and nematodes

within a parasitoid framework. However, there is no doubt that parasitoids represent a significant proportion of Earth's biodiversity. Thus, the study of lineages beyond parasitoid wasps can challenge traditional perspectives on parasitoid biology and provide a more holistic view of parasitoid evolutionary and ecological patterns.

2.3 Brachycera as a model

Parasitoid wasps are the most frequently studied parasitoid group primarily because they are highly species-rich, representing approximately 78% of all parasitoid insects (Eggleton & Belshaw, 1992). However, they have been criticized as an evolutionary model due to several features of the group (Eggleton & Belshaw, 1992; Feener & Brown, 1997). Namely, they represent a single evolutionary lineage and thus do not exhibit the convergence required to test certain hypotheses about repeated patterns of parasitoid evolution. Furthermore, parasitoid wasps display several unique physiological and genetic characteristics (ex. haplodiploidy) which are not generalizable to other parasitoid groups. Feener and Brown (1997) suggest true flies (order Diptera) as an alternative evolutionary model for parasitoid studies as parasitoid flies are also highly species-rich, representing 20% of parasitoid insects, and exhibit repeated convergence on parasitoidy across the order. Eggleton and Belshaw (1992) estimate that the number of transitions to parasitoidy within flies is at least 21 based on comparison at the family level. However, this is a highly conservative estimate, and they suggest the number could be as high as 100 when accounting for shifts within families. Parasitoid flies are also an ideal model for evolutionary investigations due to the extreme ecological diversity demonstrated throughout the order, including high diversity in feeding habits (Feener & Brown, 1997; Marshall, 2012). However, a limitation of the order Diptera is the large number of species that are essentially

biologically unknown, as these species cannot contribute life-history data to studies of trait evolution. Certain groups are disproportionately impacted by this issue due to a dearth of scientific experts and a large number of cryptic species (Borkent *et al.*, 2018; Savage *et al.*, 2019). In Diptera, both major suborders exhibit this limitation, but the paraphyletic Nematocera is more dramatically affected due to the small size and cryptic nature of many nematoceran flies which remain poorly known at the species level (Marshall, 2012). Furthermore, the majority of parasitoid flies reside in suborder Brachycera, a lineage comprising approximately 66% of total dipteran diversity and over 99% of known parasitoid flies (Eggleton & Belshaw, 1992; Godfray, 1994; Kirk-Spriggs & Sinclair, 2017; Pape *et al.*, 2011). Thus, by focussing on the suborder Brachycera as a parasitoid model rather than all flies, we can limit missing data and noise and achieve proper conditions for hypothesis testing. Another limitation of Brachycera as an evolutionary model is the lack of a well-supported and proportionately sampled molecular phylogeny, which is required to meet the model assumptions of phylogenetic comparative methods like ancestral character state reconstruction (Pagel *et al.*, 2004; Young & Gillung, 2020). Consequently, the estimation of a robustly sampled phylogeny of the Brachycera is a necessary first step towards addressing outstanding questions concerning parasitoid evolution.

2.4 A phylogeny of Brachycera

The monophyly of the Brachycera, which are also termed ‘short-horned flies’ due to their reduced antennal flagellomeres, has been confirmed in multiple molecular and morphological investigations of the Diptera (Lambkin *et al.*, 2013; Wiegmann *et al.* 2003, 2011; Yeates & Wiegmann, 1999). The infraorder Bibionomorpha, marsh flies and gall midges, has also been repeatedly recovered as monophyletic and sister to the Brachycera in the same studies. Multiple

phylogenetic hypotheses exist for Brachycera, however higher-level relationships within the suborder remain contentious (Bayless *et al.*, 2021; Lambkin *et al.*, 2013; Shin *et al.* 2018; Song *et al.*, 2022; Wiegmann *et al.*, 2011). Difficulty recovering these relationships is thought to stem from multiple rapid radiations which occurred in deep time and are therefore difficult to recover with limited taxon sampling and discordant molecular data (Shin *et al.*, 2018; Wiegmann *et al.*, 2011). Despite varying levels of genetic coverage, previous molecular estimates of the Brachycera have included a similar range of taxonomic coverage. Wiegmann *et al.* (2011) sampled 160 brachyceran species in their phylogeny of the entire Diptera, which combined data from 14 nuclear loci, mitochondrial genomes and morphological data into an incomplete data matrix. More recently, Song *et al.* (2022) estimated a phylogeny of Brachycera by sampling 171 ingroup taxa using complete mitochondrial genomes. It could be argued that both studies had low taxonomic sampling given the suborder Brachycera presently contains 122 families and nearly 105,000 species. Taxon sampling has been shown repeatedly to impact the results of phylogenetic inference, so it has been suggested that greater sampling can aid in the resolution of deep phylogenies even when this results in a higher proportion of missing data (though the percentage of acceptable missing data remains a topic of debate; Heath *et al.*, 2008; Hedtke *et al.*, 2006; Nabhan & Sarkar, 2011; Roure *et al.*, 2013). Accordingly, a phylogeny estimated with a higher number of taxa that are evenly distributed across families is a viable approach to resolving recalcitrant nodes, thus resulting in a strong phylogenetic framework with which to test hypotheses.

Another limitation of previous estimates is sampling methodology. Previous studies have approached phylogenetic estimation from a systematics perspective, thus focusing primarily on the resolution of relationships between major clades, and sampling taxa disproportionately based

on the relationships being tested. This method is at odds with the model assumptions of phylogenetic comparative methods, which assume proportional sampling of taxa based on real world diversity (reviewed in Young and Gillung, 2020). Thus, to address specific hypotheses about parasitoid evolution, taxon sampling should aim to be proportional and should also include sufficient sampling of parasitoid lineages to test the proposed hypotheses. In particular, there are multiple parasitoid lineages which returned low support in previous phylogenetic estimates that should be targeted in future estimations (ex. Anthomyiidae, Conopidae, Nemestrinidae, Piophilidae and Sciomyzidae; Wiegmann *et al.*, 2011).

Supermatrix analyses, which are concatenated datasets produced by combining data from multiple sources, make it possible to harness publicly available data to address untested hypotheses without additional sequencing (de Queiroz & Gatesy, 2007). Therefore, modern advances in bioinformatics and models of sequence evolution make it more attainable than ever to generate new datasets which are larger and more evenly sampled than previous iterations, and to use these datasets to produce well-supported phylogenetic hypotheses. This approach has become widely adopted for the reconstruction of a diverse range of taxonomic lineages (summarized in Shin *et al.*, 2018). Using this supermatrix approach, it is thus possible to address the sampling limitations of previous brachyceran phylogenetic studies while also estimating a phylogeny which is suitable for the testing of specific hypotheses regarding parasitoid evolution.

2.5 The origin of parasitoidy

Central to the question of how parasitoids evolve and diversify is the issue of their evolutionary origin. Under a functional definition of parasitoidy, parasitoids are distinguished by the feeding habit of their developmental stage which results in the death of their host (Eggleton

& Gaston, 1990; Godfray, 1994). Thus, by reconstructing the ancestral larval feeding habit of parasitoid lineages we can uncover common evolutionary pathways to parasitoidy. Previous qualitative work has proposed saprophagy as the most common pathway to parasitoidy within the flies, with fewer lineages derived from predatory or mycophagous ancestors (Eggleton & Belshaw, 1992). However, this work was conducted largely at the family level and entirely by outgroup comparison (Madison *et al.*, 1984) rather than with the use of statistical methods for ancestral character state reconstruction. Therefore, this hypothesis remains to be quantitatively tested. Furthermore, Eggleton and Belshaw (1992) did not differentiate between ectoparasitoids and endoparasitoids when inferring their origin, despite clear differences in the ecological, functional, and morphological constraints imposed on each group.

Gauld (1988), working with parasitoid wasps, proposed that endoparasitoidy is the derived state, and that transitions to endoparasitoidy may occur as a result of shifts to cocooned hosts by ectoparasitoid ancestors. Sharanowski *et al.* (2021) investigated this hypothesis in the wasp superfamily Ichneumonoidea and found some support for multiple transitions between endo- and ectoparasitoidy, though results were largely inconclusive due to limited taxon sampling. Posing the same question in bee flies (Diptera, Bombyliidae), Yeates and Greathead (1997) found support for the hypothesis that endoparasitoidy is derived. However, both Ichneumonoidea and Bombyliidae possess similar characteristics in that they are both single parasitoid lineages with a mix of endo- and ectoparasitoid species. In contrast, most parasitoid fly lineages consist entirely of endoparasitoids with no close ectoparasitoid relatives (Eggleton & Belshaw, 1992; Feener & Brown, 1997). This could suggest that endoparasitoidy can arise directly from life-history strategies other than ectoparasitoidy, or that endoparasitoidy is derived

from ectoparasitoidy but that ectoparasitoidy often acts as a transient life-history strategy and thus may not be recovered by ancestral character state reconstruction.

It is also worth investigating whether we observe shifts away from parasitoidy when investigating life-history transitions within a clade that contains both parasitoid and non-parasitoid lineages, such as Brachycera. Due to the highly specialized nature of the parasitoid life-history strategy, we could predict that parasitoidy is an evolutionary dead-end (Day *et al.*, 2016; Futuyuma & Moreno, 1988; Siddall *et al.*, 1993). This implies that the adaptations acquired by parasitoids could be irreversible from an evolutionary perspective. This is arguably more likely of endoparasitoidy, as many more specialized adaptations are required to survive inside the tissue of a host versus externally, which may be more comparable to a predatory life-history. Eggleton and Belshaw (1992) suggest that shifts away from parasitoidy are rare in both flies and beetles (order Coleoptera) but have been observed in wasps – most notably in the ants, bees and stinging wasps (Aculeata, non-parasitoids) which are thought to have derived from the same parasitoid ancestor as all parasitoid wasps (Peters *et al.*, 2017). These outstanding questions underline the importance of investigating the evolutionary history of multiple parasitoid lineages in order to draw conclusions on the origin of parasitoidy, as well as any other broad evolutionary patterns.

3. Methods

3.1 Data mining

Before mining any sequence data, I compiled a working list of families in the suborder Brachycera following Wiegmann *et al.*, 2011 and Pape *et al.*, 2011. This list was then corroborated with current literature to determine the status of contentious taxa. The final list of 122 brachyceran families with taxonomic notes and all sources used is available in Appendix 1.

All sequence data included in this study were mined from the publicly available National Center for Biotechnology (NCBI) Nucleotide database (NCBI Resource Coordinators, 2018) using the *R* package *rentrez* (Winter, 2017). This package acts as a wrapper for NCBI's Entrez Utilities API, providing an *R* interface with which to query all NCBI databases on a large scale. Using the *entrez_search()* command, I queried the NCBI Nucleotide database for all brachyceran species records for each gene of interest (see 3.2 *Gene selection*). For the mitochondrial gene *cytochrome c oxidase subunit I* (COI), the number of records at the suborder level exceeded the limit I could pull using *rentrez* (n=418,632, limit is 10,000 [Accessed April 03 2022]). Therefore, I queried for COI using only the list of species that had returned results from at least one of the other gene searches. I also queried the NCBI Taxonomy database to extract family and genus level classifications for each species. These queries returned 157,370 sequence records from 8,085 species. All records were summarized in a data frame by the number of sequence records available per gene per species, along with taxonomic data. A small number of records were edited manually to correct for changes in family level classifications (noted in Appendix 1). This data frame was then used as the basis for taxon selection (see 3.3 *Taxon sampling*).

After taxa were chosen, I conducted sequence selection. This was necessary as many species had multiple sequences available for commonly sequenced genes such as COI. To address

this, I automated the selection of sequences by using *R* to filter sequence records by length (bp) and select the longest sequence. A new data frame was then populated with the accession numbers of all selected sequences. Using these accession numbers and the *entrez_fetch()* command (*rentrez* package), I downloaded all sequences in fasta file format. Sequences were then concatenated into a single file for each gene using the *bash* command *cat* in preparation for DNA alignment.

3.2 Gene selection

Target genes were selected by first compiling a master list of 38 candidate genes that were considered informative in previous studies (Caravas & Friedrich, 2013; Han *et al.* 2002; Shin *et al.*, 2018; Simon *et al.*, 2010; Wahlberg & Johanson, 2018; Wiegmann *et al.*, 2011; Winkler *et al.*, 2015; Yang *et al.*, 2019). I then conducted preliminary searches within NCBI's Nucleotide database using *rentrez* to estimate the taxonomic coverage of each gene at the family level. To limit missing data, only the 18 genes which returned hits for >20% of brachyceran families were included in this study. The original list of candidate genes and the results of this search are included in Table 1. Again, COI was excluded from this search as the number of results returned exceeded the search limit. However, due to the extensive usage of COI for species barcoding, I assumed taxonomic coverage would be evenly distributed and thus COI is included as one of the final 18 genes. Despite passing this initial search, the gene ND5 was later excluded due to issues with correct identification and alignment (see 3.5 *DNA alignment*). The final 17 genes include eight nuclear protein-coding genes (AATS, CAD, EF1A, PER, PGD, SINA, SNF, TPI), five mitochondrial protein-coding genes (COI, COII, CYTB, ND1, ND3), two nuclear ribosomal RNA genes (18s, 28s), and two mitochondrial ribosomal RNA genes (12s, 16s).

Table 1: List of candidate genes and results of preliminary searches within NCBI's Nucleotide database to estimate the taxonomic coverage of each gene at the family level. Only the 18 genes which returned hits for >20% of brachyceran families were included in this study. Genes which returned hits for >40% of brachyceran families are highlighted in blue; genes which returned hits for <40% and >20% of brachyceran families are highlighted in green.

Gene	Type	# Families	% Families (/134)	Taxonomic breadth
COI	mito	NA	NA	could not compute (see note in text)
28S	nuc ribo	116	86.57%	evenly distributed throughout
CAD	nuc	105	78.36%	evenly distributed throughout
AATS	nuc	102	76.12%	evenly distributed throughout
TPI	nuc	98	73.13%	evenly distributed throughout
PGD	nuc	84	62.69%	evenly distributed throughout
16S	mito ribo	74	55.22%	evenly distributed throughout
12S	mito ribo	68	50.75%	evenly distributed throughout
COII	mito	62	46.27%	evenly distributed throughout
EF1A	nuc	57	42.54%	evenly distributed throughout
18S	nuc ribo	56	41.79%	no Opomyzoidea
cytB	mito	55	41.04%	evenly distributed throughout
ND5	mito	32	23.88%	sparse coverage throughout (missing many superfamilies)
ND3	mito	30	22.39%	sparse coverage throughout (missing many superfamilies)
ND1	mito	29	21.64%	sparse coverage throughout (missing many superfamilies)
SINA	nuc	28	20.90%	no Opomyzoidea or Tephritoidea
PER	nuc	27	20.15%	sparse coverage (lots of Tephritids)
SNF	nuc	27	20.15%	no Opomyzoidea or Tephritoidea
COIII	ribo	25	18.66%	sparse coverage throughout (missing many superfamilies)
G6PD	nuc	24	17.91%	sparse coverage throughout
ND2	mito	23	17.16%	sparse coverage throughout (missing many superfamilies)
PEPCK	nuc	24	17.91%	sparse coverage throughout
ATP6	mito	23	17.16%	sparse coverage throughout (no Opomyzoidea, lots of Drosophilidae)
ND4L	mito	22	16.42%	sparse coverage throughout (missing many superfamilies)
ND6	mito	19	14.18%	sparse coverage throughout (missing many superfamilies)
SYX	nuc	19	14.18%	sparse coverage throughout (more Calyptratae)
ATP8	mito	18	13.43%	sparse coverage throughout (no Opomyzoidea, lots of Drosophilidae)
GART	nuc	18	13.43%	very sparse coverage throughout
PUG	nuc	18	13.43%	very sparse coverage throughout
WG	nuc	13	9.70%	very sparse coverage throughout
MAC	nuc	11	8.21%	mostly Calyptratae
MCS	nuc	9	6.72%	mostly Calyptratae
LGL	nuc	8	5.97%	mostly Calyptratae
ace	nuc	6	4.48%	only sparse coverage in Orthorrhapha
RH1	nuc	5	3.73%	very sparse coverage throughout
H3	nuc	4	2.99%	mostly Drosophilidae
DDC	nuc	3	2.24%	mostly Drosophilidae
LW-opsin	nuc	0	0.00%	no coverage

3.3 Taxon sampling

To meet model assumptions, lineages were proportionally sampled based on described diversity at the family level. In total, I aimed to sample 0.5% of suborder diversity (total number of described brachyceran species = 104,977, derived from summing the number of species per family, see Appendix 1 for citations). I calculated this for each family by first gathering an estimate of described species from primary literature, and then multiplying that value by 0.005 to produce sampling targets. These targets were rounded to the nearest whole number, except when this value was zero. In those cases, I assigned targets a value of one to avoid excluding smaller families entirely. Sampling targets for each family are presented in Appendix 1.

Selection of individual species was done manually using the sequence record data mined from NCBI's Nucleotide database (see *3.1 Data mining*). This was structured as a presence/absence matrix that displayed the number of genes with sequences available per species. Within each family, species with the most genetic data available were selected preferentially to minimize missing data. When more than one species had the same level of genetic coverage, I sought to select species from a diversity of genera and subfamilies. This was particularly important in families that display a wide range of larval feeding habits, as these characters are often shared at the generic or subfamily levels. For some families, it was not possible to sample a large variety of genera due to limited available data. In some instances, I generated chimera taxa by combining sequences from two species within the same genus to minimize missing data. In those cases, taxa are clearly labelled (Appendix 1). Some families had no data available to sample and are thus not represented in the phylogeny.

3.4 Outgroup rooting

To root the phylogeny by outgroup rooting, I sampled an additional five taxa from the Bibionomorpha, a monophyletic infraorder which is sister to the Brachycera. This included one representative each from the following families: Scatopsidae, Anisopodidae, Keroplatidae, Cecidomyiidae, and Diadocidiidae. As a large clade, Bibionomorpha was not proportionally sampled as this would have minimally added an additional 75 taxa to the sampling effort (# of species = 15,000, Ševčík *et al.*, 2016). Instead, the five outgroup taxa were included for phylogeny estimation but excluded from ancestral character state reconstruction to meet the assumption of proportional sampling.

3.5 DNA alignment

Sequences were aligned using the multiple sequence alignment program MAFFT version 7 (Online version) with strategy set to ‘iterative refinement method E-INS-I’ (Katoh *et al.*, 2013; Katoh *et al.*, 2019). Quality control of alignments was conducted by eye using the alignment viewing software AliView (Larsson, 2014) and Mesquite (Maddison & Maddison, 2021). This allowed unalignable sequences to be easily flagged and investigated further. I used the Basic Local Alignment Search Tool (BLAST; Boratyn *et al.*, 2013) website at NCBI to verify the identity of flagged sequences against confirmed sequences from the same gene. This often uncovered mislabeled or contaminated sequences which were then removed from the alignment. When possible, removed sequences were replaced with others from the same gene and species. As the majority of ND5 sequences were mislabeled and could not be reliably replaced, ND5 was excluded from the final alignment.

Alignments were then concatenated into a single phylip file with all genes and species using the *concat* command from the *Python* program AMAS (Borowiec, 2016). This command also output a partition file which was then manually edited. Ambiguously aligned regions, where positional homology could not be inferred with a reasonable level of confidence were excluded from the phylogenetic analyses. Sites where data was only available for a single species were also removed.

3.6 Testing model assumptions

Before phylogeny estimation, DNA sequence data were partitioned by gene (Chernomor *et al.*, 2016). I then performed three maximum matched-pair tests of symmetry in IQ-TREE version 2 (Minh *et al.*, 2020; Nguyen *et al.*, 2015) to test for the presence of model violations in the partitioned alignment (Naser-Khdour *et al.*, 2019). These tests assess whether each partition meets the model assumptions of stationarity and homogeneity by comparing their most divergent sequences. IQ-TREE then output a ‘badremoved’ alignment by excluding partitions which rejected model assumptions.

3.7 Phylogeny estimation

Phylogenies for both the full nucleotide alignment (‘complete’) and the alignment with only those partitions which did not reject model assumptions (‘badremoved’) were estimated under maximum likelihood (ML) using IQ-TREE. Starting trees were set to ‘random’ and seed was set to ‘12345’ for reproducibility. Partition model was set with command -p so that branch lengths were linked across partitions (edge-linked), but substitution rates were allowed to vary. Substitution rate and nucleotide frequency parameters were estimated for each partition under the

GTR+G nucleotide substitution model with 4 discrete rate categories (default; Yang, 1994).

Branch support was evaluated by implementing ultrafast bootstraps (Minh *et al.*, 2013; Hoang *et al.*, 2018) and the Shimodaira–Hasegawa approximate likelihood ratio test (SH-aLRT; Guindon *et al.*, 2010; Anisimova *et al.*, 2011), which were both set to 2,000 replicates. I also attempted to implement the GHOST mixture model (Crotty *et al.*, 2020), however there was insufficient data for parameter estimation (see *5.3 Model selection and assumption violations*).

Following phylogeny estimation, tree files were imported into *R* and rooted with outgroup taxa using the package *ape 5.0* (Paradis & Schliep, 2019) with command *root()*.

3.8 Topology test

To test whether topologies differ significantly between the ‘badremoved’ tree and the ‘complete’ tree, I ran an approximately unbiased (AU) topology test (Shimodaira, 2002) with 10,000 RELL replicates. The approximately unbiased (AU) topology test calculates the log-Likelihood values for multiple input trees to find the single ‘best’ tree, and then assesses whether the other tree topologies differ significantly from the best tree ($p<0.05$). If the other tree(s) are found to be significantly different, they are rejected in favour of the best tree. The best supported tree was then used as the basis of ancestral state reconstruction.

3.9 Fossil calibration

The reconstruction of ancestral character states requires an ultrametric phylogeny, where all tips are equal distance to the root. Therefore, the ‘best tree’ as determined by the AU topology test was time-calibrated using five fossil calibration points and the *chronos()* command in *R* (*ape 5.0*; Paradis & Schliep, 2019).

Chronos() uses the penalized maximum likelihood method (Sanderson, 2002) to estimate divergences times under multiple models of substitution rate variation (Paradis, 2013). I tested both the correlated and relaxed models of molecular evolution with smoothing parameter $\lambda=1$. Note that the *chronos()* ‘relaxed model’ refers to a model which allows tree branches to have variable substitution rates and makes no assumption of correlation between neighbouring branches. This may not correspond to other ‘relaxed’ models of molecular evolution in the literature, including those implemented under a Bayesian framework. For both the relaxed and correlated models, the maximum number of function evaluations (eval.max) was set to $1e^5$, maximum number of iterations (iter.max) was set to $5e^4$, and maximum number of alternative iterations (dual.iter.max) was set to 500. All other optimization parameters were set to defaults. During model testing, the correlated model returned a larger log-Likelihood score and a smaller Φ IIC score ($\text{log-Lik} = -229.5416$, $\Phi\text{IIC} = 3843.08$) than the relaxed model ($\text{log-Lik} = -239.6968$, $\Phi\text{IIC} = 3875.44$), and was appropriately accepted as the better fitting model.

Fossil calibration points were selected following two previous estimations of a brachyceran phylogeny (Song *et al.*, 2022; Wiegmann *et al.*, 2011). In total, five fossils representing major clades across the Brachycera were used with minimum and maximum age limits set in millions of years (my): Brachycera crown, age.min = 189.6, age.max = 196.5; Stratiomyomorpha crown, age.min = 141.27, age.max = 166.73; Empidoidea crown, age.min = 112, age.max = 163; Eremoneura crown, age.min = 155.14, age.max = 167.86; and Hippoboscoidea crown, age.min = 53.96, age.max = 61.04.

After calibration, the five outgroup taxa were removed in *R* using the *drop.tip()* command in *ape* in preparation for ancestral state reconstruction analyses.

3.10 Life history data

To inform the reconstruction of ancestral states, larval feeding habit data for extant taxa were collected from the literature. Species-level data were collected preferentially, but generic-level data were often included when feeding habit was unknown at the species-level and could be reliably assumed to be shared within a genus based on the literature.

I attempted to record only primary or obligate feeding habits; however, it was not always clear whether habits were obligate or facultative, especially among polyphagous species. This was specifically an issue in the calyprate families Calliphoridae, Muscidae, and Sarcophagidae which are well-known to exhibit polyphagy, with multiple observations of saprophagy, predation and myiasis exhibited by single species. All feeding habits for these species which were observed outside of laboratory conditions were recorded and included. The species *Megaselia scalaris* (Phoridae) is also known for its flexible feeding habits that include observations of saprophagy, mycophagy, phytophagy, predation, and myiasis, in addition to facultative predation, parasitism and parasitoidy observed solely in restrictive conditions. I included all primary/obligate feeding habits for *M. scalaris* following the review by Disney (2008).

For the classification of parasitoid taxa, I did not classify all species as parasitoids, which are defined as such in the literature based on varying definitions. I instead followed the functional definition laid out in 2.2 *Defining parasitoidy*: A parasitoid is any organism which develops on or within a single host organism, using the host as an essential trophic resource which is killed, either directly or indirectly, due to parasitoid development (following Eggleton & Gaston, 1990). This resulted in multiple difficult classification decisions which raised further questions on the relationship between predation, parasitoidy and parasitism. This is revisited in the *Discussion* section 5.2 *Parasitoidy on a continuum*.

3.11 Ancestral state reconstruction

The program BayesTraits V4.0.0 (Pagel *et al.*, 2004) was used for ancestral state reconstruction (ASR) within a Bayesian framework. The time calibrated ‘best’ tree, as determined by the AU topology test, was used as an input tree. Posterior probability distributions for all internal nodes were estimated using Markov chain Monte Carlo (MCMC) and the MultiState option for reconstructing traits with more than two discrete states. The directionality of transitions was not restricted, meaning that states were assumed to be unordered, and rates were estimated for each between-state transition independently. All priors were set to an exponential distribution with mean=1. The analysis was run for 20,000,000 generations with a burn-in of 20,000 generations.

Larval feeding habit data were initially categorized into seven states coded as follows: (0) predator, (1) phytophage, (2) saprophage, (3) parasite, (4) endoparasitoid, (5) ectoparasitoid, and (6) non-feeding. Where; ‘phytophage’ is inclusive of palynivore, malivore, and mycophage species; ‘saprophage’ is inclusive of detritivore, scavenger, and coprophage species; and hyperparasitoids were classified as either endo- or ectoparasitoids depending on their feeding location. Myiasis, which is the infection and consumption of the tissue of a living animal by dipteran larvae, was classified as parasitism. However, it was sometimes unclear in the literature whether the infection of existing wounds should be classified as parasitism or saprophagy. Unknown data were coded as (-) and interpreted as having an equal probability of each trait state. Taxa with polyphagous feeding habits were coded with multiple states (i.e. ‘02’ for a species which is both a predator and a saprophage).

However, this coding scheme resulted in the estimation of too many parameters given the available data, producing a fatal error in BayesTraits. Therefore, data were recategorized to limit the number of states being reconstructed, as the number of parameters estimated is approximately equal to the number of states to the 2nd power (Pagel *et al.*, 2004). As a result, parasitoid taxa were combined into a single category and non-feeding taxa were lumped with unknowns. I justified the lumping of non-feeding taxa with unknowns as only seven non-feeding taxa were recovered during the literature search, which represented all seven taxa sampled from the monophyletic superfamily Hippoboscoidea. Thus, the exclusion of this clade from ASR was unlikely to impact the interpretation of parasitoid evolutionary transitions. These adjustments yielded five states: (0) predator, (1) phytophage, (2) saprophage, (3) parasite, and (4) parasitoid.

4. Results

4.1 Supermatrix

In total, 562 ingroup taxa and five outgroup taxa were sampled from NCBI's Nucleotide database to construct a concatenated supermatrix. However, two taxa were later excluded during the maximum tests of symmetry and were thus removed from all datasets to facilitate statistical comparison of tree topologies (see *4.2 Maximum tests of symmetry* and *4.5 Approximately unbiased topology test*). For that reason, the final supermatrix consists of 560 ingroup taxa and five outgroup taxa (Appendix 2). This failed to meet the 584-taxon sampling target representing 0.5% of described brachyceran diversity due to insufficient molecular data in NCBI's Nucleotide database. The following families were impacted by data scarcity: Chloropidae, Gobryidae, Homalocnemidae, Milichiidae, and Nothybidae. Consequently, Chloropidae and Milichiidae were underrepresented in the final alignment, and the families Homalocnemidae, Gobryidae, and Nothybidae were excluded entirely. The two taxa removed during tests of symmetry were both chloropids (*Cryptonevra nigritarsis* and *Lasiosina albipila*), further reducing their representation in the dataset.

4.2 Maximum tests of symmetry

The results of the maximum tests of symmetry removed three of the 17 gene partitions in the full nucleotide alignment after rejecting the assumption of stationarity ($p<0.05$; COII, SINA, SNF). Consequently, two taxa which were only represented by COII sequence data were removed from the alignment entirely and excluded from subsequent phylogeny estimation (*Cryptonevra nigritarsis* and *Lasiosina albipila* (both Chloropidae)). As topology tests require that all trees

include the same taxa, I also excluded *Cryptonevra nigritarsis* and *Lasiosina albipila* from the ‘complete’ 17 partition alignment before phylogeny estimation.

4.3 Phylogeny estimation

Two phylogenies were estimated using subsets of the full nucleotide alignment with 565 taxa (560 ingroup, five outgroup);

- (i) the ‘complete’ tree estimated with all 17 gene partitions (including: 12s, 16s, 18s, 28s, AATS, CAD, COI, COII, CYTB, EF1A, ND1, ND3, PER, PGD, SINA, SNF, and TPI).

This alignment contained 23,671 total sites with 52.1% missing data. The full annotated tree with bootstrap and SH-aLRT support is available in Fig.1.

- (ii) the ‘badremoved’ tree estimated with only the 14 partitions which were not rejected by the tests of symmetry (including: 12s, 16s, 18s, 28s, AATS, CAD, COI, CYTB, EF1A, ND1, ND3, PER, PGD, and TPI; excluding: COII, SINA, and SNF). This alignment contained 22,210 total sites with 50.1% missing data. The full annotated tree with bootstrap and SH-aLRT support is available in Fig.2.

After initial phylogeny estimation, I flagged several taxa as potential ‘rogue taxa’ if they were both (i) placed far outside their anticipated family or superfamily clades based on existing systematic hypotheses and (ii) variably placed between tree estimates. Rogue taxa are often attributed to systematic error caused by missing data or long branch lengths (Sanderson & Shaffer, 2002). However, the observed pattern could also be the product of contaminated or mislabeled samples. Therefore, I used BLAST to verify all sequences from taxa flagged as potential rogues and all were found to be uncontaminated. As a result, no further taxa were removed from either the ‘complete’ or ‘badremoved’ alignment. However, these taxa are noted

with red text in the full tree figures (Fig.1 and Fig.2) and are referred to during discussions of topology and parasitoid transitions due to their impact on the perceived monophyly of multiple groups. In total, six taxa in the ‘complete’ tree met the above criteria and were thus flagged, and eleven taxa in the ‘badremoved’ tree were flagged. Notably, six of the taxa flagged in the ‘badremoved’ tree were parasitoids, whereas only two parasitoids were flagged in the ‘complete’ tree, and only one of these species (*Lixophaga latigena* (Tachinidae)) was flagged in both trees.

4.4 Placement of major groups

Topologies of both trees are discussed below with key similarities and differences as summarized in Fig.3 (excluding flagged taxa for readability). Existing superfamily and higher-level classifications following Pape *et al.* 2011 and Wiegmann *et al.* 2011 are used to highlight and discuss these differences (classifications defined in Appendix 1).

The ‘complete’ tree recovered a monophyletic Tabanomorpha as sister to the remaining Brachycera. After Tabanomorpha, the following clades were recovered as monophyletic in decreasing proximity to the root: Nemestrinidae, Xylophagidae, Stratiomyoidea, Acroceridae, and an Asiloidea+Hilarimorphidae grouping. However, these clades each received low bootstrap support (<80%) compared with later diverging nodes. Empidoidea was recovered as non-monophyletic with respect to *Nanodromia narmkroi* (Hybotidae) and *Sybistroma obscurellum* (Dolichopodidae), which were both placed within the Schizophora and flagged as potential rogues following the criteria laid out in 4.3 *Phylogeny estimation*. After the empids, a monophyletic Apystomyiidae diverged, followed by a non-monophyletic Phoroidea with respect to *Megaselia abdita* (Phoridae), which was also flagged for its placement within Schizophora. A monophyletic Syrphidae diverged after Phoroidea, followed by a non-monophyletic Pipunculidae

with respect to *Neophrocerus daeckeii* (Pipunculidae) placed within the Schizophora. Excepting *N. daeckeii*, this Pipunculidae group was recovered as sister to the Schizophora.

Though Schizophora has been repeatedly recovered as monophyletic in molecular investigations of the Diptera, relationships within the Schizophora have remained largely unresolved (Bayless *et al.*, 2021; Shin *et al.* 2018; Song *et al.*, 2022; Wiegmann *et al.*, 2011). Specifically, the acalyprates, a designation referring to all schizophoran taxa excluding the monophyletic Calyptratae, have been variably placed in all previous hypotheses, including those using genomic data (Bayless *et al.*, 2021) Therefore, it is not entirely surprising that the ‘complete’ tree did not recover the majority of proposed schizophoran superfamilies, and instead recovered two large clades I designated ‘Schizophora clade #1’ and ‘Schizophora clade #2’, which consisted of a mix of all acalyprate families excluding those within the Agromyzidae, Odiniidae, Hippoboscoidea and Calyptratae. These clades also included many of the flagged taxa I expected to recover elsewhere based on existing systematic hypotheses. Diverging after ‘Schizophora clade #2’, an Agromyzidae+Odiniidae clade was recovered as non-monophyletic with respect to the flagged *Scaptomyza crassifemur* (Drosophilidae), followed by a monophyletic Hippoboscoidea. Finally, the Calyptratae were recovered as non-monophyletic with respect to the aforementioned hybotid *Nanodromia narmkroi* and the flagged *Lixophaga latigena* (Tachinidae), which was placed within Schizophora.

In contrast, the ‘badremoved’ tree recovered the parasitoid family Acroceridae as sister to the remaining Brachycera. This was followed by Nemestrinidae, Tabanomorpha (non-monophyletic with respect to the flagged *Adersia oestroides* (Rhagionidae)), Xylophagaidae, Stratiomyoidea (non-monophyletic with respect to the flagged *Microchyrsa polita* (Stratiomyidae)), and an Asiloidea+Hilarimorphidae clade which was non-monophyletic with

respect to three bombyliid species recovered within the Schizophora (*Neosardus principius*, *Comptosia quadripennis*, *Thraxan acutus*). Overall, this section of the tree, before the divergence of Eremoneura, received higher bootstrap support along the backbone of the phylogeny than the ‘complete’ tree, despite recovering fewer clades as monophyletic (3/6, vs. 6/6). Empidoidea was recovered as non-monophyletic with respect to a flagged chloropid recovered within the group (*Elachiptera bimaculata*) as well as a dolichopodid (*Dolichopus ungulates*) placed in the Schizophora. A monophyletic Apystomyiidae diverged after the empids, followed by a monophyletic Phoroidea and Syrphidae. For a second time, a non-monophyletic Pipunculidae was recovered as sister to the Schizophora, this time with respect to *Cephalops cochlearatus* (Pipunculidae) which was flagged for its placement in the Schizophora. Similar to the ‘complete’ tree, acalyprate groups were largely unrecovered, apart from Ephydriidae and Hippoboscoidea. Again, these unplaced Schizophora fell into two ambiguously defined clades – however the composition of these clades did not match those from the ‘complete’ tree and thus they received their own designations, ‘Schizophora clade #3’ and ‘Schizophora clade #4’ respectively (see Fig.2). Notably, in the ‘badremoved’ tree, Ephydriidae is recovered as monophyletic and sister to the Hippoboscoidea and Calyptratae, diverging after ‘Schizophora clade #4’. This contrasts with the ‘complete’ tree, where the family Ephydriidae was recovered as monophyletic but nested within ‘Schizophora clade #1’. Additionally, the Agromyzidae+Odiniidae group recovered as sister to the Hippoboscoidea and Calyptratae in the ‘complete’ tree (non-monophyletic with respect to a flagged drosophilid) was recovered as non-monophyletic and split within both ‘Schizophora clade #3’ and ‘Schizophora clade #4’ in the ‘badremoved’ tree. Following the monophyletic Ephydriidae, Hippoboscoidea was recovered as non-monophyletic with respect to Streblidae (*Trichobius longipes*), which was placed in ‘Schizophora clade #3’. The resulting

Hippoboscidae+Nycteriidae+Glossinidae clade was nested with a flagged tachinid *Lixophaga latigena* as sister to the Calyptratae. Finally, the Calyptratae were recovered as non-monophyletic with respect to several flagged taxa. Two chloropids (*Meromyza columbi*, *Oscinella frit*), two bombyllids (*Comptosia quadripennis*, *Thraxan acutus*), and one stratiomyid *Mircochrysa polita* were recovered within the Calyptratae, and one tachinid which was anticipated within the Calyptratae was instead recovered within ‘Schizophora clade #4’.

The results of both trees are plausible given the placement of major clades in existing phylogenetic hypotheses of the Brachycera (summarized in Fig.4), excluding the acalyptates which were poorly recovered (though this also aligns with the results of previous dipteran explorations). The placement of major groups before the divergence of Eremoneura is variable between existing hypotheses, however the placement of major clades within the Eremoneura recovered in both the ‘complete’ and ‘badremoved’ trees supports previous work by Shin *et al.*(2018), Song *et al.* (2022) and Wiegmann *et al.* (2011). Though the placement of major clades is conceivable, many brachyceran families were recovered as non-monophyletic below the superfamily level in one or both phylogenies, as seen in the full tree figures. The impact the non-monophyly of these lineages had on the reconstruction of ancestral larval feeding habits is considered later in the *Results* and *Discussion*.

4.5 Approximately unbiased topology test

The AU test with the ‘complete’ and ‘badremoved’ tree inputs recovered the ‘complete’ tree as the best tree and found that the ‘badremoved’ tree had a significantly worse likelihood score ($p=1.64e^{-4}$) and could therefore be rejected. Consequently, only the ‘complete’ tree is used for the ancestral character state reconstruction of larval feeding habits.

4.6 Reconstruction of ancestral larval feeding habits

The reconstruction of ancestral larval feeding habits in BayesTraits estimated the transition rate between each state (larval feeding habit; see Appendix 3) in both directions and used these parameter values to output the posterior probability of each state occurring at each internal node over millions of iterations. These posterior probabilities were then averaged across all iterations and plotted as pie charts at each respective node using the *ggtree* package in R (Yu *et al.*, 2017; mapped on the ‘complete’ tree in Fig.5). The pie charts communicate uncertainty in the reconstruction of ancestral character states given the results of all BayesTraits iterations. Consequently, there is inherent ambiguity in determining in which node a transition has taken place. However, it is necessary to make assumptions about where transitions occur to draw conclusions regarding the ancestral character state of parasitoid lineages. Thus, I considered a ‘gain’ between two nodes when the posterior probabilities showed an **increase** in the probability of parasitoidy from an ancestral node to a derived node, such that parasitoidy became the state with the largest probability when it had not been before. To assess what state parasitoidy derived from during each gain, I recorded the state with the highest probability at the most recent ancestral node. Conversely, I considered a ‘loss’ between two nodes when the posterior probabilities showed a **decrease** in the probability of parasitoidy from an ancestral node to a derived node, such that a non-parasitoid state became the state with the largest probability where parasitoidy had been before. To assess what state was derived from parasitoidy at each loss, I recorded the state with the highest probability at the derived node.

Using these criteria, I recorded a total of 22 transitions to and from parasitoidy across the Brachycera, which comprised 17 gains and five losses (listed in Table 2, transitions are also

labelled on Fig.5 following the same numbering scheme). Of the 17 transitions to the ‘Parasitoid’ state (gains); nine were transitions from the ‘Parasite’ state, five were from ‘Saproophage’, two were from ‘Phytophage’ and one was from ‘Predator’. Of the five transitions away from the ‘Parasitoid’ state (losses); three were to the ‘Parasite’ state, and two were to the ‘Predator’ state. All rate parameters estimated by BayesTraits are reported in Table 3.

Table 2: Transitions to (gain) and from (loss) parasitoidy recovered during ancestral state reconstruction of larval feeding habits in the Brachycera.

Transition #	Major clade (as labelled on Figure)	Family	Gain/Loss	Transition from	Transition to	Note
1	Nemestrinidae	Nemestrinidae	Gain	Saprophage	n/a	
2	Acroceridae	Acroceridae	Gain	Saprophage	n/a	
3	Asiloidea+Hilarimorphidae	Bombyliidae	Gain	Predator	n/a	
4	Asiloidea+Hilarimorphidae	Bombyliidae	Loss	n/a	Predator	
5	Asiloidea+Hilarimorphidae	Bombyliidae	Gain	Parasite	n/a	Parasite used as a transition state (Predator)
6	Phoroidea	Phoridae	Gain	Parasite	n/a	Parasite used as a transition state (Saprophage)
7	Phoroidea	Phoridae	Loss	n/a	Parasite	Parasite used as a transition state (Saprophage)
8	Phoroidea	Phoridae	Gain	Parasite	n/a	Parasite used as a transition state (Parasitoid)
9	Phoroidea	Phoridae	Loss	n/a	Parasite	
10	Phoroidea	Phoridae	Gain	Parasite	n/a	
11	Pipunculidae	Pipunculidae	Gain	Saprophage	n/a	
12	Schizophora clade #1	Pyrgotidae+Conopidae	Gain	Parasite	n/a	Parasite used as a transition state (Saprophage)
13	Schizophora clade #1	Pipunculidae	Gain	Saprophage	n/a	Species flagged as potential rogue
14	Schizophora clade #2	Sciomyzidae+Conopidae	Gain	Parasite	n/a	Parasite used as a transition state (Saprophage)
15	Schizophora clade #2	Sciomyzidae	Loss	n/a	Predator	
16	Schizophora clade #2	Pyrgotidae	Gain	Phytophage	n/a	
17	Schizophora clade #2	Tephritidae	Gain	Phytophage	n/a	
18	Schizophora clade #2	Tachinidae	Gain	Parasite	n/a	Parasite used as a transition state (Saprophage/Phytophage), Species flagged as potential rogue
19	Schizophora clade #2	Cryptochetidae	Gain	Saprophage	n/a	
20	Calypratae	Tachinidae	Gain	Parasite	n/a	Parasite used as a transition state (Saprophage)
21	Calypratae	Hybotidae	Loss	n/a	Parasite	Parasite used as a transition state (Predator), Species flagged as potential rogue
22	Calypratae	Tachinidae	Gain	Parasite	n/a	Parasite used as a transition state (Parasitoid), Sister species flagged as potential rogue

Table 3: Transition rates between larval feeding habit states, as estimated in BayesTraits. The presented rates are the average over 20,000,000 iterations. Transition rates away from parasitism are highlighted to draw attention to the much higher estimates when compared to all other rates.

From	To	Rate (averaged across iterations)
Predator	Phytophage	0.000133
Predator	Saprophase	0.000149
Predator	Parasite	0.000239
Predator	Parasitoid	0.000246
Phytophage	Predator	0.001079
Phytophage	Saprophase	0.00239
Phytophage	Parasite	0.002384
Phytophage	Parasitoid	0.001715
Saprophase	Predator	0.001329
Saprophase	Phytophage	0.002371
Saprophase	Parasite	0.00571
Saprophase	Parasitoid	0.001249
Parasite	Predator	0.80505
Parasite	Phytophage	0.629989
Parasite	Saprophase	1.299277
Parasite	Parasitoid	0.664851
Parasitoid	Predator	0.001207
Parasitoid	Phytophage	0.000427
Parasitoid	Saprophase	0.000682
Parasitoid	Parasite	0.00102

5. Discussion

5.1 Parasitoid evolutionary history

The results of this study were largely unexpected given previous investigations into parasitoid life-history transitions and my stated hypotheses. The number of total transitions to and from parasitoidy recovered within the Brachycera was only 22, with 17 gains and five losses (Table 2, Fig.5). This is less than the previous estimate made by Eggleton & Belshaw (1992) who proposed a minimum of 18 independent gains within the Brachycera (21 within the Diptera) reported solely on qualitative outgroup comparison at the family level. Based on previous knowledge of the group, my results are likely a significant underestimate of the actual number of transitions that have occurred. I propose that this underestimation is the result of several sampling limitations that hindered the reconstruction of the true evolutionary history of brachyceran parasitoids:

- (i) Several known parasitoid lineages could not be sampled due to a dearth of molecular data (Anthomyiidae, Asilidae, Calliphoridae, Chloropidae, Muscidae, Sarcophagidae, and Syrphidae). Under the conservative assumption that parasitoids within each of these families are members of a single lineage (rather than multiple lineages within a single family), I propose that an additional seven potential transitions to parasitoidy could be recovered if sampling limitations were removed.
- (ii) At least one family (Sciomyzidae), which exhibits a diversity of larval feeding habits, including parasitoidy, was not extensively sampled due to restrictions imposed by proportional sampling. It is not clear whether expanding the sampling of sciomyzid flies would return multiple transitions below the family level.

However, if multiple transitions did occur, they would not have been recovered in this study due to limitations of the sampling and thus, this hypothesis remains to be tested.

- (iii) At least one family (Bombyliidae), which is thought to exhibit a diversity of larval feeding habits, including parasitoidy, was largely excluded from the reconstruction of ancestral feeding habits due to a scarcity of species or generic level larval feeding data in the literature. This resulted in predominantly unrecovered nodes in this section of the tree where we might expect to see multiple transitions to parasitoidy.
- (iv) At least one other family (Ctenostylidae), which is thought to exhibit parasitoid larval feeding habits, was excluded from analysis due to a lack of confirmed larval feeding habit data in the literature at any level (species, genera, family).
- (v) Finally, given the general limitations of our knowledge regarding brachyceran flies and life in general, it would not be surprising to learn that there are multiple unknown parasitoid lineages (either undescribed or biologically unknown) within the Brachycera that were excluded from this reconstruction, but that would represent independent shifts to parasitoidy if we were able to successfully reconstruct their evolutionary history.

The results of rate estimation during ancestral state reconstruction in BayesTraits were also unexpected. Notably, the transition rates estimated for transitions from the ‘Parasite’ state to other states were consistently multiple orders of magnitude higher than transitions to and from other states. This resulted in an unexpected pattern of transitions being observed across internal nodes, where the ‘Parasite’ state was repeatedly reconstructed as a transitional state even in

clades where there were few or no reported parasite taxa. This was observed during ten of the 22 observed transitions to or from the ‘Parasitoid’ state (Table 2, Fig.5). For example, Transition #20 shows a transition from ‘Saprophage’ to ‘Parasite’ to ‘Parasitoid’ at the root of a large parasitoid clade in the Calyptratae consisting of mostly tachinid flies. Within the same clade, transition #21 records a second transition where the ‘Parasitoid’ state is lost and the most recent common ancestor of *Nanodromia narmkroi* (Hybotidae; predator) and *Euthera tentatrix* (Tachinidae; parasitoid) is recovered as a ‘Parasite’.

Though it is possible that this is a biologically accurate reconstruction, and that parasitism represents a transitional state between various larval feeding habits, it seems highly unrealistic given the constraints of the parasite biology. This feels particularly doubtful in the Brachycera given the number of parasites found in the group, which represented only 14 of the 560 taxa sampled, many of which were polyphagous. Instead, I propose that this is an artefact produced by a large amount of missing data unevenly distributed across the tree, which is interacting poorly with the way that BayesTraits interprets unknown states. It is likely that additional larval feeding habit data is necessary to resolve this anomaly (or verify its legitimacy).

Though it is not statistically supported, I present the transitions to and from parasitoidy again, this time while ignoring the ‘Parasite’ transition state to assess the impact of this pattern on the final results. I produced these estimates by simply ignoring the posterior probability of the ‘Parasite’ state at these transition nodes and focussing only on the proportion of the other four states. Consequently, I recorded only 14 transitions to the ‘Parasitoid’ state (gains): nine transitions from ‘Saprophage’, two transitions from ‘Predator’, two transitions from ‘Phytophage’, one transition from an ancestor ambiguously reconstructed as

‘Saprophage/Phytophage’, and one legitimate transition from the ‘Parasite’ state (transition #10). Two additional transitions are eliminated, given that the ‘Parasite’ state was acting as a transition state between two ‘Parasitoid’ nodes (transition #8 and transition #22). I also record three transitions away from the ‘Parasitoid’ state (losses): two transitions to ‘Predator’, and one transition to ‘Parasite’. Again, two additional transitions are eliminated (transition #7 and transition #21). It is impossible to say conclusively whether the transitional ‘Parasite’ states are inaccurate in any or all cases without further investigation, however the transition pattern observed when the ‘Parasite’ transition state is ignored is much closer to that suggested by Eggleton & Belshaw (1992), who proposed saprophagy as the most common ancestral feeding habit of parasitoid taxa with the Diptera, with transitions from predation, phytophagy and mycophagy (considered separately) also present.

It is also notable that transitions from parasitoidy to other states were recovered multiple times, whether the ‘Parasite’ transition state was ignored or not. Looking exclusively at the losses maintained when ignoring the ‘Parasite’ state, each occurred within a family which is known for containing species with a diversity of larval feeding habits alongside parasitoidy: Phoridae, Bombyliidae, and Sciomyzidae. Therefore, I propose that future studies that aim to reconstruct the evolutionary history of parasitoidy on a finer scale focus on these three families, as they display the highest potential for recovering multiple transitions below the family level. However, as Bombyliidae was plagued by large amount of missing larval feeding habit data, it is likely that a family level reconstruction would face the same limitations described here.

In addition to issues with the estimation of rate parameters, the reliability of several transitions is questioned given uncertainty in the input topology. Specifically, the presence of

taxa flagged as potential rogues contributed to the presence of at least four transitions which I consider unreliable;

- (i) Transition #13 counts a transition from saprophage to parasitoid due to the placement of the pipculid species *Nephrocerus daeckeii* within Schizophora clade #1 rather than with the remaining Pipunculidae.
- (ii) Transition #17 counts a transition from parasite to parasitoid due to the presence of the tachinid species *L. latigena* placed far outside its expected clade (Calyptatae), within a clade that is otherwise largely saprophagous and nested within the unresolved Schizophora clade #2;
- (iii) Transition #20 counts a transition from parasitoid to parasite due to the presence of the hybotid species *N. narmkroi* far outside its expected clade (Empidoidea), within a clade of parasitoid Calyptatae;
- (iv) Transition #21, following Transition #20, counts a transition from parasite to parasitoid in the sister species to the hybotid *N. narmkroi*.

One way to mitigate the impact of potential rogues in future reconstructions would be to identify and exclude them either before or after phylogeny estimation (methods for doing so are addressed in *5.4 Inconsistent topologies and flagged taxa*). However, another approach is to input multiple trees into BayesTraits to incorporate uncertainty in the estimated topology within the reconstruction analysis (Pagel *et al.* 2004). This is easily accommodated if phylogeny estimation is implemented within a Bayesian framework.

5.2 Parasitoidy on a continuum

A scientific consensus on the definition of parasitoidy does not currently exist. I adhered to the functional definition of parasitoidy following Eggleton & Belshaw (1990; 1992) to classify taxon larval feeding habits in this study. This definition states that a parasitoid is any organism which develops on or within a single host organism, using the host as an essential trophic resource which is killed, either directly or indirectly, due to parasitoid development. Using this definition led to multiple instances where my classification of specific taxa did not agree with the literature. In addition, there were multiple difficult classification decisions which I summarize here, and which highlight the ambiguity still present in this definition and the limitations of existing life history data.

Following Eggleton & Belshaw (1992), I did not classify egg predators, which develop within an egg sac and consume multiple eggs as parasitoids, though they are sometimes referred to as egg parasitoids in the literature. In addition, species within the family Sciomyzidae (snail-killing flies), which are specialized mollusc feeders, are often classified as predator/parasitoids by sciomyzid researchers due to the flexibility they exhibit in the number of prey consumed. Meaning that an individual larva may be classified as an ectoparasitoid if they receive all their nourishment from a single individual rather than multiple, even if other larvae in the same species feed on multiple prey. This behaviour is commonly observed within the sciomyzids due to the size of their prey, which are often many orders of magnitude larger than the larvae consuming them, and which could easily provide all the nourishment required for larval development. Eggleton & Belshaw (1990) address this confusion by adding a clause to their definition that parasitoidy is always an obligate life history strategy. Thus, excluding any polyphagous species which exhibit facultative predation, saprophagy, or other larval feeding

habits in addition to ‘parasitoid like behaviour’. Therefore, I classified these species as predators within my analysis. However, whether that exclusion is biologically justifiable remains unclear. The family Sciomyzidae also contains multiple obligate parasitoid species, which raises questions regarding the nature of parasitoidy and its relationship to the predator life-history strategy. For this reason, I reiterate my recommendation from *5.2 Proportional sampling and missing data* that the evolutionary history of parasitoids in the family Sciomyzidae should be investigated more extensively at the family-level, as further study could provide novel insight into parasitoid/predator transitions.

Another example of difficult classification concerns the species *Pollenia rufa* (Polleniidae) which is classified as both an ecto- and endoparasitoid in the literature depending on the larval instar. However, this species has been observed moving to a new host if its current host dies and begins decomposing, thus excluding it from a functional definition of parasitoidy for not meeting the requirement of having a ‘single host’. Consequently, I classified *P. rufa* as a predator during ASR. However, it remains an ambiguous case, as *P. rufa* also displays many characteristics of parasitoids including specialized host-seeking behaviour and a dependence on the host body cavity for survival during early instars.

The example of *P. rufa* also brought forward the issue with classifying larval feeding habits when they are not shared across larval instars. For instance, there are species in the family Muscidae which are known to proceed from saprophagy to predation during larval development, which we could call an example of obligate polyphagy (Marshall, 2012). However, making this classification requires a thorough knowledge of the life history of a species, and our knowledge of the feeding habits of many dipteran larvae are extremely limited at both the generic and species levels (and sometimes family level, as noted with Ctenostylidae). Thus, it is highly likely

that even when larval feeding habit data is present in the literature, that it is an incomplete picture of the actual species biology. In a parasitoid example, Robinson (1971) found that repeated rearing of *Megaselia* (Phoridae) led to a decrease in the number of species recorded as obligate parasitoids – which would remove them from classification as parasitoids at all under the current definition. It is important to acknowledge that errors in classification are possible as they could have a profound impact on the reconstruction of larval feeding habits and transition estimates. Unfortunately, these errors are nearly impossible to detect without extensive investigation of all species included in a study—which is entirely unrealistic at the suborder scale or in any study where the sampling exceeds ~20 species.

5.3 Model selection and assumption violations

Nucleotide substitution models are based on assumptions about sequence evolution that are frequently violated by actual biological data (Naser-Khdour *et al.*, 2019; Young and Gillung, 2020). These violations can have a large influence on the inferred tree topology. Therefore, it is important to understand the assumptions and parameters of any substitution model used to avoid violating model assumptions or introducing systematic error. In this section, I discuss the results of my investigations into model selection, parameterization, and assumption violations using the brachyceran supermatrix.

The selection of a nucleotide substitution model for phylogeny estimation can have profound impacts on the resulting tree. Frequently, the most parameter-rich model is selected, especially when using popular model selection tools such as ModelFinder (IQ-TREE; Kalyaanamoorthy *et al.*, 2017) and PartitionFinder2 (Lanfear *et al.*, 2016), as this is often assumed to be the best option when data is not a limiting factor. However, this is not always the

case, as the estimation of some parameters can introduce systematic error if they are confounded with others. During tree estimation, I implemented the GTR+G substitution model, which accounts for variation in substitution rates across sites following a gamma distribution with shape parameter α (Yang, 1994). This model was selected over the more parameter rich GTR+G+I model, which incorporates an additional parameter permitting a proportion of invariable sites (p_{inv} ; Gu *et al.*, 1995). Though the GTR+G+I model is widely used, it has been criticized due to fundamental flaws in parameter assumptions (Jia *et al.*, 2014; Mayrose *et al.* 2005; Sullivan *et al.*, 1999). As the +G α parameter already accounts for a proportion of sites which evolve at such a slow rate as to appear invariable, α and p_{inv} parameters under the GTR+G+I model are correlated and cannot be independently optimized. Therefore, the GTR+G model has been recommended as an alternative which accounts for both rate variation across sites and a proportion of invariable sites without introducing systematic error (Young and Gillung 2020).

In addition to the GTR+G, I tested the GHOST heterotachy mixture model (Crotty *et al.*, 2020). Heterotachy refers to the variability of substitution rates at a given site across time or across lineages, which can impact the estimation of frequency and rate parameters, and thus phylogenetic inference (Lopez *et al.*, 2002). Heterotachy is not accounted for by most widely used substitution models, including the GTR+G, which instead allows data to be partitioned *a priori* to allow for variability in rate between partitions. However, this does not accommodate rate variability within partitions, and relies entirely on data being partitioned in a biologically relevant way (Crotty *et al.*, 2020). Unfortunately, the GHOST model was unable to estimate a phylogeny with the given dataset due to numerical instability. This suggests that the supermatrix alignment has too many taxa and not enough sites to estimate parameters reliably. However, this

could also be the result of limitations of the current version of the GHOST model, which has not been reliably tested on datasets as large as the one used in this study. This is a common problem with mixture models, which are so parameter-rich that they become computationally unreliable or inaccessible, and thus have not been widely adopted for ML inference (Crotty *et al.*, 2020).

I also investigated the presence of model assumption violations in the supermatrix alignment before phylogeny estimation by conducting three maximum matched-pair tests of symmetry as implemented in IQ-TREE. These tests removed three partitions for rejecting the assumption of stationarity, where stationarity assumes that marginal frequencies of nucleotides are constant over time. This is the most common cause of model violation according to Naser-Khdour *et al.* (2019). Notably, the three genes removed (COII, SINA, SNF) were all genes with fewer than average number of sites (1,461 total, 6% of the full alignment), and higher proportions of missing data when compared to the non-rejected gene partitions. A ‘badremoved’ tree was then estimated with the reduced 14-partition alignment. However, when compared with the ‘complete’ tree estimated with all 17 partitions, this ‘badremoved’ tree presented more taxa flagged as potential ‘rogues’ due to uncertain placement. The ‘complete’ tree also better supported previous hypotheses of a brachyceran phylogeny based on mitochondrial genomes (Song *et al.*, 2022). Finally, the results of an AU topology test on the two trees rejected the ‘badremoved’ tree as having a log-Likelihood score that was significantly worse than the ‘complete’ tree, and thus the ‘complete’ tree was used for further hypothesis testing. However, these results do not conclusively resolve the question of which topology is better supported statistically given the discordant results of the symmetry tests, topology tests and comparisons to previous phylogenetic hypotheses.

In summary, many of my attempts to avoid model assumption violations with proper model selection or statistical tests resulted in inconclusive results. In the case of the GHOST mixture model, I propose that more molecular data (loci) are needed to permit accurate estimation of model parameters. The successful implementation of this model would accommodate the presence of heterotachy in the supermatrix alignment, which we may anticipate given the broad taxonomic and temporal scale that the data currently samples. Additionally, the use of non-stationarity models could present another option for addressing the results of the symmetry tests without excluding phylogenetically relevant data (Naser-Khdour *et al.* 2019). However, there is not currently an accessible way to implement these models under ML and thus a Bayesian framework must be adopted, which may introduce new computational constraints.

5.4 Inconsistent topologies and flagged taxa

The results presented here provide a plausible hypothesis for the divergence of major clades within the Brachycera, though the two presented trees differ in the placement of some groups. Furthermore, the monophyly of many families were not recovered, which in some cases conflicts with the current scientific consensus. For example, the Bombyliidae (bee flies) have been recovered as monophyletic in previous investigations of the group (e.g. Trautwein *et al.*, 2010), but were not recovered as monophyletic in either of the presented phylogenies. There are multiple potential explanations for this, the simplest being that the topologies presented here are more biologically accurate than previous estimates and that these families are non-monophyletic. However, this seems unlikely (or at least unverifiable) given the variable placement of taxa between estimates, and the topology of the largely unplaced acalyprates. In this section, I

present future steps for resolving these ambiguities, including increased sampling and tests to assess the legitimacy of potential rogue taxa.

It is tempting to suggest that more data is the answer to resolve poorly supported or unrecovered clades. In the case of the Brachycera, I suspect the inclusion of more genetic loci would increase overall support for the resultant topology. However, more loci were not included in this alignment due to a lack of available data. Some ‘available’ loci were also excluded when it was considered doubtful that their inclusion would present a net positive, given the amount of missing data they would introduce and the uneven distribution of that missing data across taxonomic groups (see Table 2). An option not pursued in this thesis is the inclusion of genomic scale data available through NCBI’s Sequence Read Archive (SRA) database. The availability of this data is highly restricted to specific groups which have been the target of previous investigation (Bayless *et al.*, 2021; Buenaventura *et al.*, 2020; Gillung *et al.*, 2018; Kutty *et al.*, 2019; Li *et al.*, 2020; Pauli *et al.*, 2018; Yan *et al.*, 2020), however it is possible that even the inclusion of limited genomic data would provide increased support for the backbone of the tree(s) by providing stronger phylogenetic signal for divergences in deep time and thus, further resolving the placement of major clades which did not receive high bootstrap support in the presented trees (Fig.3, see clades diverging before the Eremoneura in the ‘complete’ tree). However, working with genomic data introduces new challenges, including high computational and temporal requirements, which is why the inclusion of SRA data was not pursued in the presented thesis. It is also not definitively clear whether genomic data would resolve topological ambiguities given the results of a recent transcriptomic investigation into the Schizophora—which did not greatly improve the resolution of the group (Bayless *et al.*, 2021). Another approach to increasing genetic coverage is the incorporation of full mitochondrial genomes into

the current supermatrix, as the most recent phylogenetic study of brachycera presented promising results using only mitochondrial genomes (Song *et al.*, 2022). This study had limited sampling (187 species across 40 families) and excluded several major groups due to missing data (Fig.4). However, it was largely successful in recovering major groups without the inclusion of nuclear genomic data. This study also produced six new mitochondrial genomes, including two from the family Milichiidae and one from the family Chloropidae, both of which were underrepresented in my brachyceran supermatrix. Thus, the combination of data in that study and the current presented thesis may produce a better supported topology that is suited for the testing of evolutionary hypotheses.

A complement to the increased sampling of genetic loci is increased taxon sampling. However, I am not convinced that additional taxon sampling would result in a better resolved topology of the Brachycera given the constraints discussed in the next section *5.5 Proportional sampling and missing data*. Nevertheless, changes in the composition of taxa sampled may resolve the monophyly of certain groups which were recovered as non-monophyletic with respect to taxa flagged as potential rogues. Rogue taxa are often attributed to either missing data, or long branch attraction, which is a type of systematic error where distantly related taxa are inferred to be closely related due to the large amount of change exhibited by a lineage (long branch) rather than genuine shared descent (Sanderson & Shaffer, 2002). Thus, the identification and removal of true ‘rogues’ can address systematic error in an alignment and lead to an overall increase in branch support and phylogenetic confidence. There are multiple ways to approach the assessment and removal of rogues. Trautwein *et al.* (2011) proposed a method for the identification of rogue taxa in their Bayesian inferred phylogeny of the Bombyliidae using consensus networks. They subsequently pruned rogue taxa, resulting in increased posterior

probabilities at impacted nodes. Alternatively, Song *et al.* (2022) conducted a test of compositional heterogeneity on their brachyceran dataset using pairwise Euclidean distances, and then removed taxa deemed heterogeneous from their dataset prior to phylogeny estimation. Between these two methods, the identification and removal of potential rogues prior to phylogeny estimation seems to be the more principled approach. However, the test of compositional heterogeneity implemented by Song *et al.* (2022) was conducted using unpublished *Perl* scripts from a colleague rather than a test implemented in existing inference software like IQ-TREE. Consequently, there are obstacles to the widespread implementation of this method by the scientific community.

5.5 Proportional sampling and missing data

The assumption of proportional sampling made by phylogenetic comparative methods such as ancestral state reconstruction is largely ignored in phylogenetic studies (Young and Gillung, 2020). However, even when proportional sampling is attempted, there are limitations on the level of accuracy that can be achieved due to missing data.

I aimed for proportional sampling at the family-level as there exists some level of taxonomic consensus with respect to the classification of brachyceran families that is not present at the subfamily- or generic-level for most groups. This decision was also informed by the data available, which was already extremely limited for certain families and thus, proportional sampling of finer-scale taxonomic units was considered unrealistic. Even so, three families were excluded from the final alignment due to unavailable data (Homalocnemiidae, Gobryidae, and Nothybidae), and two families were severely underrepresented in the final alignment for the same reason (Chloropidae and Milichiidae). Apart from Homalocnemiidae (Empidoidea), these

families are all acalyprates, which were largely unplaced in both presented phylogenies. It is unclear how the proportional inclusion of these families would have impacted the resultant topologies. Given the inconclusive placement of acalyprate groups in previous studies, I am doubtful that supplementary sampling of these families would further resolve the placement of any major groups in either the ‘complete’ or ‘badremoved’ trees. However, the Chloropidae are a family with a few known parasitoid species – thus the inclusion of those species may have impacted the results of ancestral state reconstruction.

Furthermore, I suspect that the sampling constraints imposed by proportional sampling resulted in the exclusion of additional parasitoid lineages from certain families which may have recovered further independent shifts to parasitoidy below the family level had they been more broadly sampled. Specifically, the family Sciomyzidae, which consists of a mixture of predator, ectoparasitoid, and endoparasitoid species, was sampled with only three species (1 parasitoid, 1 predator, 1 unknown). Thus, only one transition was recovered above the family level, though the current hypothesis is that multiple transitions have occurred within the group (Knutson and Vala, 2012). It is certainly possible that this single transition is biologically accurate, however given the sampling limitations imposed in this study, it is not yet possible to reject the hypothesis that there are multiple transitions within the group that would be recovered upon more targeted sampling. However, the results presented here suggest that the limit is already being met regarding the amount of available data that can be sampled without further violating the assumption of proportional sampling.

5.6 Data mining and reliability

Data mining facilitates the repurposing of existing data to answer novel scientific questions, either exclusively or as a complement to newly generated data. In this thesis, data mining of NCBI's Nucleotide database was the sole sampling method used to construct a supermatrix alignment with more comprehensive sampling than previous phylogenetic studies of the Brachycera. However, combining data from multiple sources, made available through an inconsistently maintained online database, introduced several problems with data reliability which were difficult to counteract without the implementation of non-reproducible quality control measures. In this section, I discuss the limitations of data mining given the reality of online databases, with specific commentary on the structure of NCBI's Nucleotide and Taxonomy databases.

An example of data unreliability is the presence of bycatch (non-target results), during queries for specific loci. In NCBI'S Nucleotide database, bycatch is nearly impossible to avoid entirely due to how genetic loci are reported, as sequence record metadata does not include a mandatory field for designating which gene is included in a sequence. Instead, authors often communicate the genes included in a sequence by referencing them in the title of the sequence record. Consequently, it is easy for non-target sequences to be returned if the title is ambiguous. i.e. a title that reads “Genus_sp1, mitochondrial genome excluding COI” will be returned when the database is queried for “Genus_sp1 AND COI” even though COI is not present in this sequence. It is also relatively easy for sequences to be mislabelled at the time of input due to human error. Together, these features of the NCBI database system introduce multiple issues when trying to scale up the mining of sequence data, as misidentified or contaminated sequences are nearly impossible to detect prior to sequence alignment and verification in BLAST.

Of the 3,458 sequences initially selected for inclusion in the brachyceran supermatrix, roughly 4.5% of sequences were replaced or removed due to mislabeling or contamination flagged during sequence alignment (total: n=155; by gene: 16S, n=18; 18S, n=14; 28S, n=10; AATS, n=19; CAD, n=4; COII, n=23; CYTB, n=23; EF1A, n=2; ND1, n=3; ND3, n=4; ND5, n=30 (all); PER, n=1; PGD, n=2; SINA, n=1; SNF, n=1). I found mislabeled sequences were more common for mitochondrial genes, especially *cytochrome c oxidase subunit II* (COII), *cytochrome B* (CYTB), and *NADH dehydrogenase subunit 5* (ND5). As a result, many COII and CYTB sequences (n=23 for both) had to be removed or replaced, and ND5 was completely removed from the alignment (n=30). Using BLAST, I found many of the mislabeled sequences from these genes corresponded to other portions of the mitochondrial genome. This underlines a specific issue with how mitochondrial genes are uploaded to the Nucleotide database, as they appear to be grouped together more often than nuclear or ribosomal genes, and uploaded under single records with shared titles.

Another source of data unreliability is the assumption of correct taxonomic classification. This assumption is made at multiple levels, it is assumed that: (i) species were correctly identified before sequencing, (ii) no errors were made during sequence submission, and that (iii) NCBI's Taxonomy database (which is linked to the Nucleotide database to provide associated taxonomic data for sequence records) represents the current taxonomic consensus. The first two instances are often impossible to verify, however the correct classification of species at the family or genus level within the Taxonomy database is possible to confirm by referencing recent literature (provided a taxonomic consensus exists). However, performing this due diligence at scale is time-consuming and the manual reclassification of hundreds of species is beyond the scope of most large-scale phylogenetic studies, including this one. For taxa included in this

study, I manually updated any incorrect classifications at the family level, which became apparent during the collection of described species counts from the literature. However, it is still highly likely that the classifications of some species have been revised since their initial submission to the Nucleotide database and that a baseline level of error exists within the data which could be impacting interpretation of the resultant topology.

It is clear that the public availability of sequence data is a positive scientific advancement, as the production of new molecular data is a costly and time-consuming endeavor. However, the current structure and curation of the NCBI databases does not guarantee reliable data and thus presents an obstacle to fully reproducible data mining without reliance on manual adjustments to sequence metadata. In some ways, this is unavoidable when sourcing data from a myriad of sources. However, the implementation of a few key changes to how data is stored in this database could greatly improve user experience and facilitate the use of this data for larger scale phylogenetic studies without the introduction of systematic or human error.

6. Thesis summary and conclusion

The primary objective of this thesis was to expand our knowledge of the evolutionary origins of parasitoid species by answering the following questions using the suborder Brachycera as a model system:

- 1) How many independent shifts to parasitoidy have occurred?
- 2) From what ancestral larval feeding habit are parasitoid lineages most often derived?
- 3) Do evolutionary pathways to parasitoidy differ between endoparasitoid and ectoparasitoid lineages?

To address these questions, I estimated two proportionally sampled phylogenies of the Brachycera with subsets of a supermatrix alignment that I compiled using sequence data mined entirely from the NCBI's Nucleotide database. The final alignment samples 17 genetic loci and 560 ingroup taxa, representing 119 of the 122 brachyceran families currently recognized. This constitutes the largest sampling effort of the Brachycera to date. Both recovered topologies were considered plausible given existing phylogenetic hypotheses, however the Schizophora were largely unrecovered in both trees. Several ambiguously placed taxa were flagged as potential rogues and considered with caution. I conducted statistical tests to determine the best supported tree and reconstructed the larval feeding habit of all internal nodes (559) using Bayesian inference to unravel the evolutionary origin of parasitoidy.

In total, 17 independent gains of parasitoidy, and five independent losses were identified within the Brachycera. Parasitism was recovered as the most common ancestral state preceding shifts to parasitoidy. However, there is evidence that this is an artifact caused by the uneven distribution of missing data on the tree. It was not possible to reconstruct the evolutionary origins

of ecto- and endoparasitoidy separately given limitations on the number of parameters that could be reliably estimated from the data.

The results of this study greatly contribute to our understanding of the evolutionary history of parasitoidy within the Brachycera. Namely, these results support previous studies which found that multiple transitions to parasitoidy have occurred within the Brachycera, and that these convergent events are distributed evenly across the Brachycera tree of life, making them an ideal model for the continued study of parasitoid evolution. Additionally, this study recovered multiple shifts away from parasitoidy, suggesting that parasitoidy is not an evolutionary dead-end and that parasitoids may not be as morphologically or ecologically constrained as has been previously suggested. Finally, the results presented here underline the need for more extensive studies of parasitoid taxa, to overcome limitations imposed by missing or insufficient data.

Future studies should seek to increase sampling of genetic loci to address the constraints presented here, where certain methods were rendered inaccessible or unreliable when attempting to estimate parameters with insufficient molecular data. Increasing genetic coverage should also improve topological resolution and branch support in future estimations of a brachyceran phylogeny. I suggest the incorporation of mitochondrial genomes and genomic-scale nuclear data to address these issues.

Additionally, the following families should be the subject of targeted sampling of parasitoid species so that they may be included in future reconstructions of the parasitoid evolutionary history: Anthomyiidae, Asilidae, Calliphoridae, Chloropidae, Muscidae, Sarcophagidae, and Syrphidae. Bombyliidae should also be the subject of studies of natural history to further elucidate their larval feeding habits at the species level in order to reconstruct

the history of parasitoidy within the group more accurately, and to limit the concentration of missing data during future reconstructions.

The results of this study also suggest that there are limitations to working at the suborder level, given the restrictions of proportional sampling and available data. Thus, future studies should also investigate the evolutionary history of parasitoid lineages below the family level to acquire finer-scale resolution of parasitoid transitions. I suggest the family Sciomyzidae as an ideal starting point for such investigations as it is a mid-size group which is relatively well-known ecologically. It is also composed of a mixture of predator and obligate parasitoid species that could provide unique insights into the transitions between these two life-history strategies.

In conclusion, this study contributes novel insights into the evolutionary history of parasitoids within the suborder Brachycera and confirms that they are a suitable model system for the continued study of parasitoid evolution. The results presented here also bring attention to specific areas in need of further investigation and underline the importance of the continued study of parasitoids, which remain largely unknown despite being an ecologically important and diverse group.

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AGROMYZIDAE + ODINIIDAE

HIPPOBOSCOIDEA

SCHIZOPHORA CLADE #2

CALYPTRATAE

Hippoboscidae
Strebidae
Pteribidae
Cecidomyiidae
Odnidae
prosopiphidae

Agromyzidae

Liriomyzidae

Drosophilidae

Cryptochetidae

Drosophilidae

Cartonidae

Bentidae

Caridae

Ephydriidae

Sepsidae

Protopselidae

Neridae

Nemidae

Micropodidae

Chrysomidae

Calophyanidae

Acalyptratae

Rhopalidae

Xenocalliphoridae

Austromyopidae

Acrotyphlopidae

Oscinellidae

Pteromalidae

Tephritisidae

Platystomatidae

Ulididae

Loucheidae

Tanypidae

Streblidae

Clusiidae

Semidiptera

Tetanopteridae

Neurotidae

Pallopteridae

Polydora

Pioptilidae

Laxuaniidae

Glyaphyidae

Laxuaniidae

Natillimyidae

Chamanyidae

Sciomyzidae

Conopidae

Heteroceridae

Chloropidae

Miltichidae

Onthophagidae

Margarinotidae

Histeridae

Aulacidae

Syrphidae

Dipteridae

SCHIZOPHORA CLADE #4

SCHIZOPHORA CLADE #3

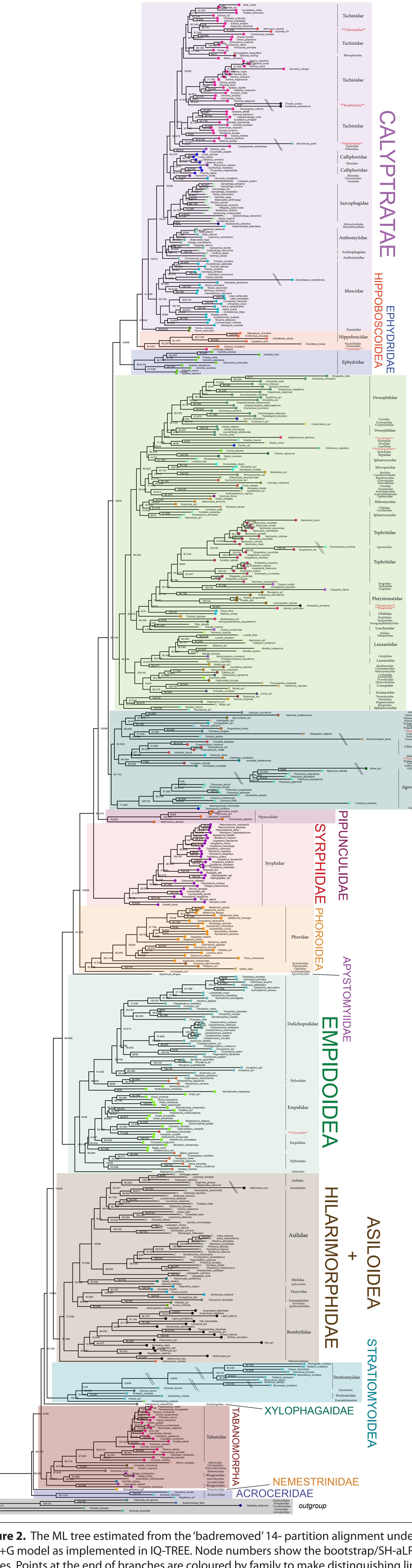
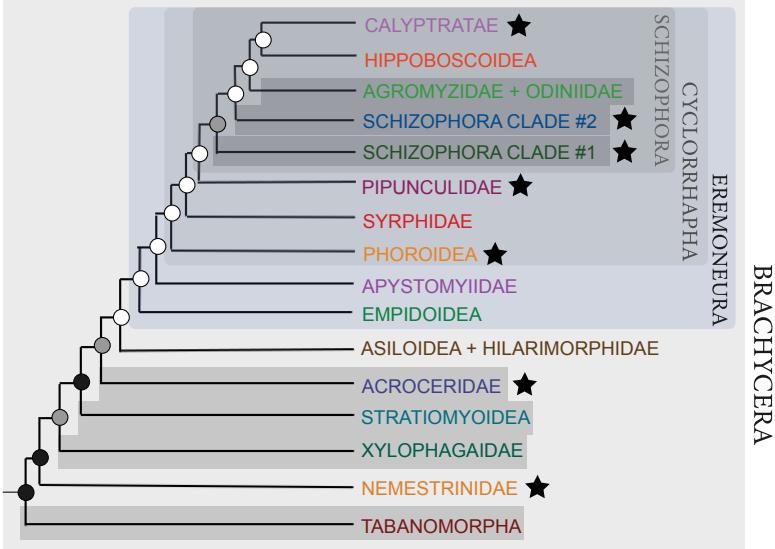


Figure 2. The ML tree estimated from the 'badremoved' 14- partition alignment under the GTR+G model as implemented in IQ-TREE. Node numbers show the bootstrap/SH-aLRT support values. Points at the end of branches are coloured by family to make distinguishing lineages more accessible.

'COMPLETE' TREE



'BADREMOVED' TREE

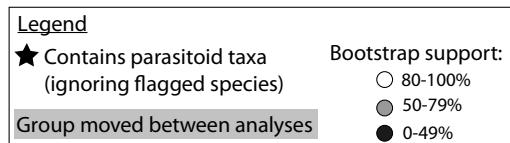
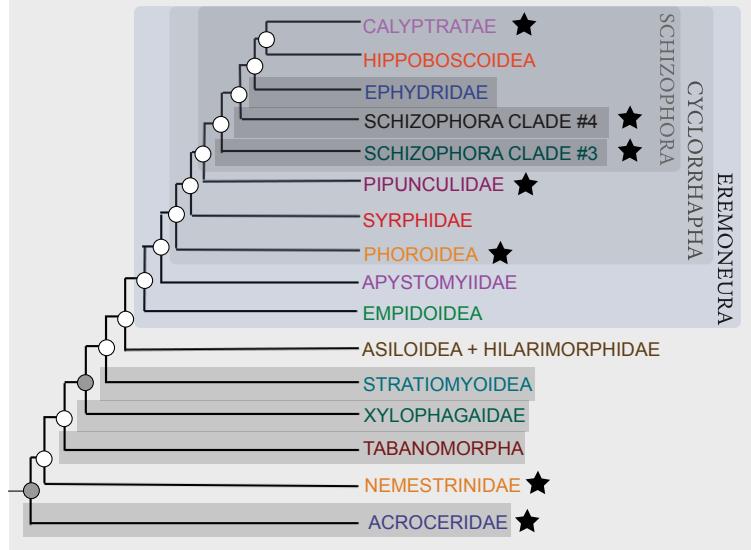
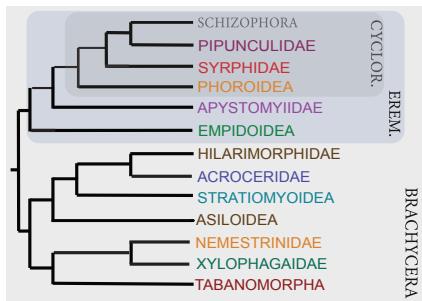
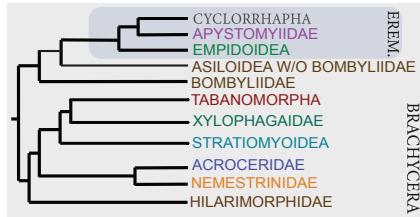


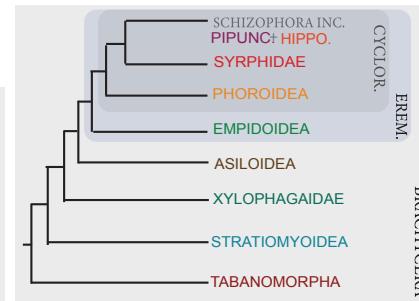
Figure 3. Topological summary of the relationships between major groups in the suborder Brachycera as recovered in this study. **A**, 'Complete' tree, estimated from the full 17-partition alignment; **B**, 'Badremoved' tree, estimated from the 14-partition subset alignment.



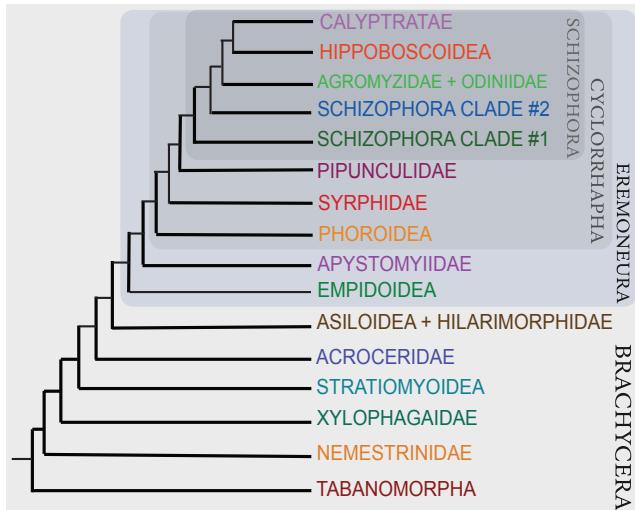
A. WIEGMANN ET AL. 2011



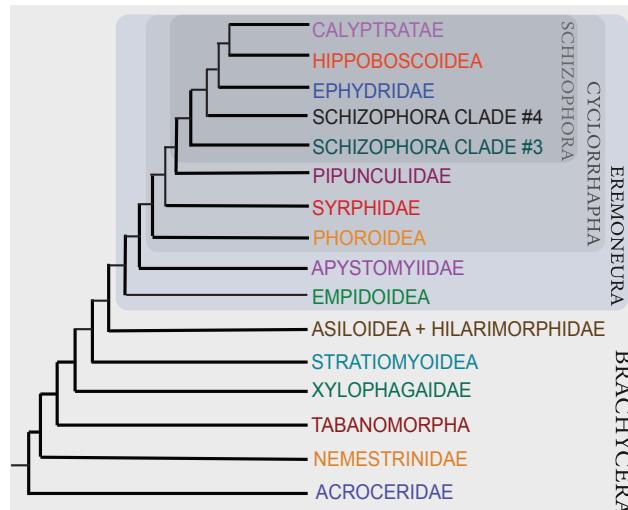
B. SHIN ET AL. 2018



C. SONG ET AL. 2022



D. 'COMPLETE' TREE



E. 'BADREMOVED' TREE

Figure 4. Topological summary of phylogenetic hypotheses for the relationships among major groups in the suborder Brachycera (Diptera) based on molecular data. **A**, Wiegmann et al., 2011; **B**, Shin et al., 2018; **C**, Song et al., 2022; **D**, 'Complete' tree, newly presented. Estimated from the full 17-partition alignment; **E**, 'Badremoved' tree, newly presented. Estimated from the 14-partition subset alignment.

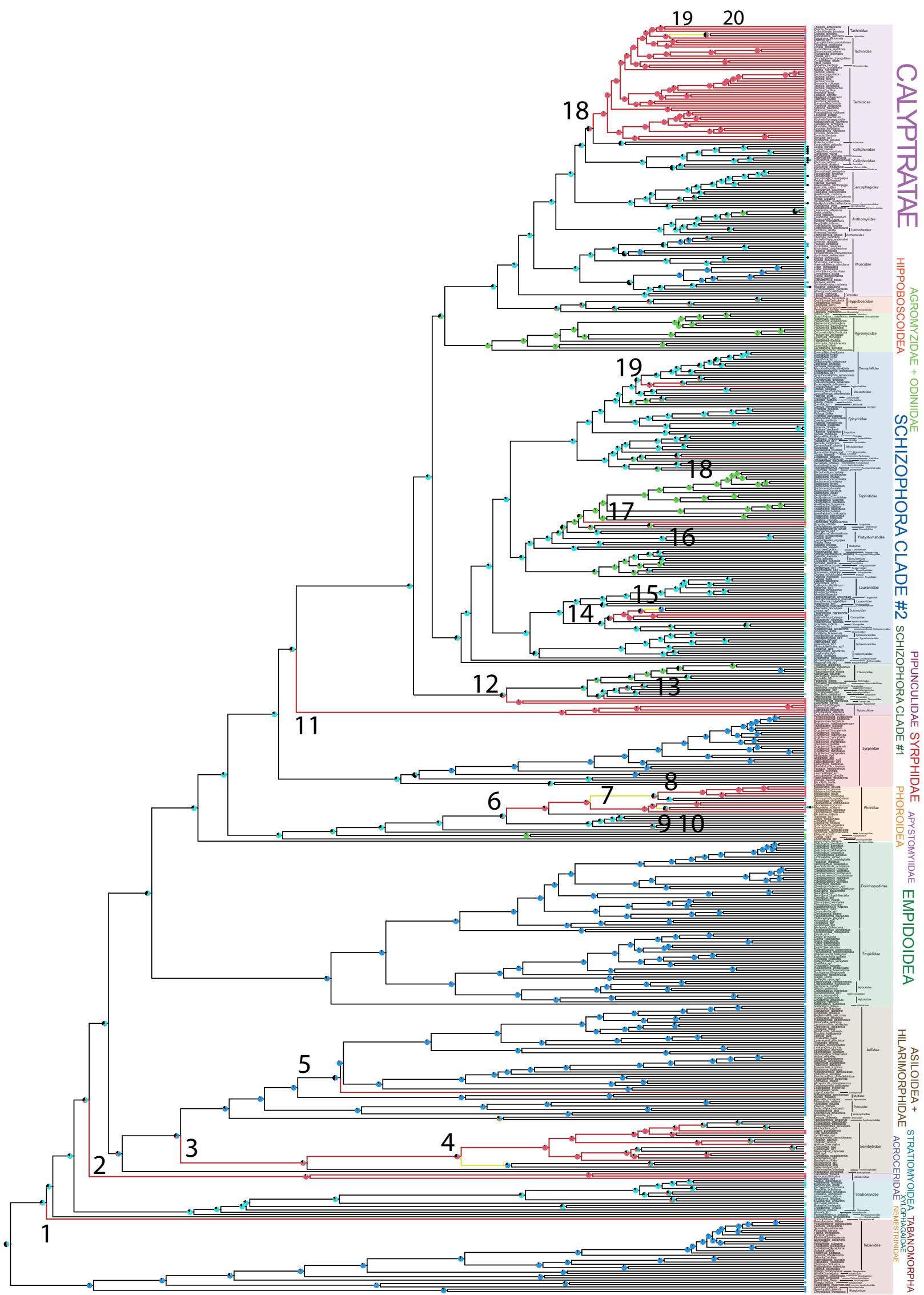


Figure 5. Bayesian ancestral state reconstruction of larval feeding habits. Pie charts on each node and terminal indicate posterior probability of state being present in the ancestor at that node: blue indicates predator, green indicates phytophage; cyan indicates saprophage, black indicates parasite, and red indicates parasitoid. Branch colours indicate transitions to and from parasitoidy: red indicates a gain (and all parasitoid lineages derived from that gain); yellow indicates a loss. Transitions are numbered following Table 6.

Appendix 1: List of families currently recognized in the suborder Brachycera with number of described species and sampling targets for the current study (0.5% of described species, plus 1 for all families where 0.5% rounds to zero).

Unranked taxon	Unranked taxon	Infraorder	Unranked taxon	Superfamily	Family	Described species (#)	Sampling targets	Source	Classification notes
			Nemestrinoidea	Nemestrinidae		275	1	Kirk-Spriggs & Sinclair, 2017	
			Nemestrinoidea	Arcoperidae		530	3	Gillung & Winterton, 2019	
			Hilarimorphidae			36	1	Pape et al., 2011	
			Vermileonidae			61	1	Kirk-Spriggs & Sinclair, 2017	
			Asiloidea	Bombyliidae		4500	23	Kirk-Spriggs & Sinclair, 2017	Includes Mythicomyiidae (not supported as a family in Trautwein et al., 2010), all Mythicomyiidae records manually changed to Bombyliidae
			Asiloidea	Asilidae		7600	38	Kirk-Spriggs & Sinclair, 2017	
			Asiloidea	Mydidae		476	2	Kirk-Spriggs & Sinclair, 2017	
			Asiloidea	Aploceridae		143	1	Kirk-Spriggs & Sinclair, 2017	
			Asiloidea	Ocoidae		1	1	Winterton & Ware, 2015	Includes records classified within Ocoidae, which is the name that predaes Evocoidae (Yeates et al., 2003), all Ocoidae records manually changed to Evocidae
			Asiloidea	Apsiocephalidae		4	1	Winterton & Ware, 2015; Zhang et al., 2018	
			Asiloidea	Scenopinidae		420	2	Kirk-Spriggs & Sinclair, 2017; Winterton & Ware, 2015	
			Asiloidea	Theridiidae		1170	6	Kirk-Spriggs & Sinclair, 2017; Winterton & Ware, 2015	
			Stratiomyoidea	Panthophthalmidae		20	1	Pape et al., 2011	Also spelled Panthophthalmidae
			Stratiomyoidea	Stratiomyidae		2,850	1	Kirk-Spriggs & Sinclair, 2017	
			Stratiomyoidea	Xylomyidae		138	4	Woodley, 2017	
Tabanomorpha			Rhagionoidea	Australozeptidae		8	1	Pape et al., 2011	
Tabanomorpha			Rhagionoidea	Bolbomyiidae		4	14	Pape et al., 2011	In NCBI's Nucleotide database as Bolbomyia (Rhagionidae) but has since been elevated from Rhagionidae (Kerr, 2010), all Bolbomyia records manually changed to Bolbomyiidae
Tabanomorpha			Rhagionoidea	Rhagionidae		700	1	Kirk-Spriggs & Sinclair, 2017	
Tabanomorpha			Tabanoidea	Athericidae		100	1	Woodley, 2017	
Tabanomorpha			Tabanoidea	Oreoleptidae		1	1	Pape et al., 2011; Zloty et al., 2005	
Tabanomorpha			Tabanoidea	Pelecorhynchidae		49	1	Pape et al., 2011	
Tabanomorpha			Tabanoidea	Tabanidae		4500	23	Kirk-Spriggs & Sinclair, 2017	
			Xylophagoidea	Xylophagidae		133	1	Brown et al., 2010	Often spelled Xylophagidae
Eremoneura			Empidoidea	Atelestidae		22	1	Pape et al., 2011; Kirk-Spriggs & Sinclair, 2017	
Eremoneura			Empidoidea	Dolichopodidae		7700	39	Kirk-Spriggs & Sinclair, 2017	
Eremoneura			Empidoidea	Empididae		5000	25	Brown et al., 2010	
Eremoneura			Empidoidea	Homalocnemidae		7	1	Pape et al., 2011	
Eremoneura			Empidoidea	Hybotidae		2005	10	Pape et al., 2011; Kirk-Spriggs & Sinclair, 2017	
Eremoneura			Apystomyoidea	Apystomyiidae		1	1	Pape et al., 2011	In NCBI's Nucleotide database as Hilarimorphidae, all Apystomyia (single extant genus) records manually changed to Apystomyiidae
Eremoneura	Cyclorrhapha		Phoridae	Lonchoppteridae		65	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Phoridae	Opeltidae		10	1	Pape et al., 2011	In NCBI's Nucleotide database as Opeltinae (Platypzidae) but referenced as Opeltidae in (Stahls, 2014) and (Bayless et al. 2020), all Opeltinae records manually changed to Opeltidae
Eremoneura	Cyclorrhapha		Phoridae	Platypzidae		277	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Phoridae	Ironomyiidae		17	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Phoridae	Phoridae		4202	21	Pape et al., 2011	Includes Sciadoceridae (rejected as a family in Disney, 2001), all Sciadoceridae records manually changed to Phoridae
Eremoneura	Cyclorrhapha		Syrphoidea	Pipunculidae		1428	7	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Syrphoidea	Syrphidae		6200	31	Young et al., 2016	
Eremoneura	Cyclorrhapha		Schizophora	Conopidae		831	4	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Carnoidea	9	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Canaceidae	323	2	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Carnidae	92	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Caroidea	2885	14	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Imbomyiidae	11	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Milichiidae	360	2	Swann, 2016	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Caroidea	5	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Ephydriidae	1994	10	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Drosophilidae	4017	20	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Ephydriidae	7	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Braulidae	34	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Cryptochetidae	42	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Ephydriidae	65	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Curtonotidae	59	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Diatatidae	115	1	Pape et al., 2011	Includes Campichoetidae (not supported as a family in Disney, 2001), all Campichoetidae records manually changed to Diastatidae
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Lauxaniidae	351	2	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Lauxaniidae	1900	10	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Neriidae	35	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Micropezidae	583	3	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Neriidae	112	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Opomyzoidea	6	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Acaroptophthalmidae	3017	15	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Anthomyzidae	95	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Asteidae	138	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Aulacigastriidae	19	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Clusiidae	363	2	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Fergusoninidae	29	1	Pape et al., 2011; Scheffer et al., 2017	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Margarinidae	3	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Megamerinidae	16	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Nemidiidae	14	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Neurochaetidae	22	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Odiniidae	65	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Opomyzidae	61	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Pallotoperidae	71	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Periscelididae	91	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Sciaromyzidae	8	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Xenastelidae	13	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Coelopidae	35	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Sciaromyzidae	30	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Heleomyzidae	12	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Helcomyzidae	23	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Heterocoeiliidae	2	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Sciaromyzidae	1	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Ropalomeridae	33	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Sciomyzidae	618	3	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Sepsidae	345	2	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Sphaeroceridae	139	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Sphaeroceridae	738	4	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Heteromyzidae	7	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Mormotomiyidae	1	1	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Sphaeroceridae	1571	8	Pape et al., 2011	
Eremoneura	Cyclorrhapha		Schizophora	Acalyptratae	Tanypezoidea	194	1	Pape et al., 2011	

Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tanypezoidea	Gobryidae	5	1	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tanypezoidea	Nothibidae	8	1	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tanypezoidea	Psilidae	322	2	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tanypezoidea	Somatidae	7	1	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tanypezoidea	Syringogastridae	10	1	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tanypezoidea	Strongylophthalmyidae	45	1	Barber, 2006
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tanypezoidea	Tanypezidae	68	1	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tephritoidea	Richardidae	178	1	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tephritoidea	Lonchaeidae	504	3	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tephritoidea	Piophiidae	83	1	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tephritoidea	Ulididae	678	3	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tephritoidea	Platystomatidae	1164	6	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tephritoidea	Otenostylidae	10	1	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tephritoidea	Pygotidae	351	2	Brown <i>et al.</i> , 2010; Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Tephritoidea	Tephritidae	4716	24	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Hippoboscidae	Glossinidae	25	1	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Hippoboscidae	Hippoboscidae	782	4	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Hippoboscidae	Nycteribiidae	274	1	Brown <i>et al.</i> , 2010
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Hippoboscidae	Strebidae	237	1	Soares <i>et al.</i> , 2013
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Muscoidea	Fannidae	359	2	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Muscoidea	Muscidae	5000	25	Kutty <i>et al.</i> , 2014
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Muscoidea	Anthomyiidae	1941	10	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Muscoidea	Scathophagidae	419	2	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Oestroidea	Calliphoridae	1525	8	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Ostroidea	Mesembrinellidae	36	1	Marinho <i>et al.</i> , 2017 In NCBI's Nucleotide database as Mesembrinellinae (Calliphoridae) but family supported in (Buenaventura <i>et al.</i> , 2020), all Mesembrinellinae records manually changed to Mesembrinellidae
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Ostroidea	Mystacinobiidae	1	1	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Ostroidea	Oestridae	176	1	Brown <i>et al.</i> , 2010; Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Ostroidea	Pollenidae	145	1	Cerretti <i>et al.</i> , 2019
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Ostroidea	Rhinidae	376	2	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Ostroidea	Rhinophoridae	174	1	Brown <i>et al.</i> , 2010; Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Ostroidea	Sarcophagidae	3094	15	Pape <i>et al.</i> , 2011
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Ostroidea	Tachinidae	10000	50	Stireman <i>et al.</i> , 2006; Stireman <i>et al.</i> , 2019
Eremoneura	Cyclorrhapha	Schizophora	Acalyptratae	Ostroidea	Ulurumiidae	1	1	Michelsen & Pape, 2017

Appendix 2: Taxa and genes sampled with accession numbers corresponding to sequence records in NCBI's Nucleotide database.

Family	Binomial name	Chimera	12S	16S	18S	28S	AATS	CAD	COI	cytB	EF1A	ND1	ND3	PER	PGD	SINA	SNF	TPI	
Scatopsidae	<i>Coboldia fuscipes</i>	NA	KJ136692	KJ136727	KC177282	KJ136764	KC178154	FJ040623	MZ723346	KC192986	NA	NA	KC176854	NA	NA	FJ040730	KC178367	KC177054	FJ040678
Anisopodidae	<i>Sylvicola fenestralis</i>	NA	KC177473	NA	KC177287	KC177637	KC178141	FJ040627	HM432769	AF547667	NA	NA	KC176814	KC178048	FJ040729	KC178329	KC177059	FJ040677	
Keroplatidae	<i>Arachnocampa flava</i>	NA	KC177467	AY576351	KC177277	KC177644	NA	FJ040615	NA	AY575705	NA	NA	KC176808	NA	FJ040725	KC178335	KC177051	KC177874	
Cecidomyiidae	<i>Mayetiola destructor</i>	NA	AY460205	LC228121	KC177284	KC177649	KC178152	MN191457	JN638239	NA	AF488423	NA	NA	NA	FJ040727	NA	NA	FJ040679	
Diadocidiidae	<i>Diadocidia ferruginosa</i>	NA	KC435526	MG554126	KP288786	KC177641	KC178145	KC178394	MZ632888	NA	KC435680	NA	NA	NA	FJ040694	NA	NA	FJ040688	
Nemestrinidae	<i>Trichophthalmalpa sp1</i>	NA	NA	NA	NA	KC177695	KC178198	KC177140	NA	NA	NA	NA	NA	KC177363	NA	NA	KC177925		
Acroceridae	<i>Acrocera orbicularis</i>	NA	NA	AY140863	NA	AY144414	NA	AF539893	DQ631983	NA									
Acroceridae	<i>Carvalhoa micella</i>	NA	NA	MH793692	NA	MH793700	NA	MK002243	MK002274	NA									
Acroceridae	<i>Megalybus sp1</i>	NA	NA	AY140874	NA	AY144429	NA	AF539885	DQ631974	NA									
Hilarimorphidae	<i>Hilarimorpha mentata</i>	NA	NA	NA	NA	KC177709	KC178211	KC177145	NA	NA	NA	NA	NA	NA	NA	NA	NA	KC177938	
Vermileonidae	<i>Vermileo opacus</i>	NA	NA	NA	NA	KC177686	KC178189	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	KC177916	
Bombyliidae	<i>Bombylius major</i>	NA	KC177474	KC177450	EF650090	KC177708	KC178210	AY280675	KC192961	KC192985	KC177585	EU144683	KC176838	KC176815	KC178051	NA	KC178366	KC177062	KC177937
Bombyliidae	<i>Neosardus principius</i>	NA	NA	NA	NA	HM183042	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Bombyliidae	<i>Villa sp1</i>	NA	NA	AF456846	NA	AF456858	NA	NA	NA	NA	NA	NA	NA	AF484027	NA	NA	NA	NA	
Bombyliidae	<i>Hemipenthes jaennickeana</i>	NA	NA	AY325005	AY325036	AY325067	NA	NA	AY325098	NA									
Bombyliidae	<i>Mythicomyia sp1</i>	NA	NA	NA	NA	KC177706	KC178208	KC177143	NA	NA	NA	NA	NA	NA	NA	NA	NA	KC177935	
Bombyliidae	<i>Paracosmus sp1</i>	NA	NA	NA	NA	KC177707	KC178209	NA	NA	NA	NA	NA	NA	NA	KC177371	NA	NA	KC177936	
Bombyliidae	<i>Megapalpus capensis</i>	NA	NA	NA	NA	NA	NA	KC562074	KC562107	NA	KC562140	NA							
Bombyliidae	<i>Corsomyza sp1</i>	NA	NA	NA	NA	NA	NA	KC562069	KC562102	NA	KC562135	NA							
Bombyliidae	<i>Corsomyza sp2</i>	NA	NA	NA	NA	NA	NA	KC562070	KC562103	NA	KC562136	NA							
Bombyliidae	<i>Pseudopenthes fenestrata</i>	NA	NA	AJ391527	NA	NA	NA	NA	NA	NA	AJ391527	NA							
Bombyliidae	<i>Ligyrus satyrus</i>	NA	NA	AJ391526	NA	NA	NA	NA	NA	NA	AJ391526	NA							
Bombyliidae	<i>Heterotropus sp1</i>	NA	NA	NA	NA	AY279198	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Bombyliidae	<i>Thraxan acutus</i>	NA	NA	AJ391516	NA	NA	NA	NA	NA	NA	AJ391516	NA							
Bombyliidae	<i>Exoprosopa latelimbata</i>	NA	NA	AJ391518	NA	NA	NA	NA	NA	NA	AJ391518	NA							
Bombyliidae	<i>Atrichochira sp1</i>	NA	NA	AJ391528	NA	NA	NA	NA	NA	NA	AJ391528	NA							
Bombyliidae	<i>Ligyrus punctipennis</i>	NA	NA	AJ391523	NA	NA	NA	NA	NA	NA	AJ391523	NA							
Bombyliidae	<i>Villa fuscicostata</i>	NA	NA	AJ391529	NA	NA	NA	NA	NA	NA	AJ391529	NA							
Bombyliidae	<i>Mythicomyia atra</i>	NA	NA	NA	U65158	U65212	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Bombyliidae	<i>Anthrax maculatus</i>	NA	NA	AJ391514	NA	NA	NA	NA	HQ945435	NA	NA	AJ391514	NA	NA	NA	NA	NA	NA	
Bombyliidae	<i>Comptosia quadripennis</i>	NA	NA	AJ391511	NA	NA	NA	NA	NA	NA	AJ391511	NA							
Bombyliidae	<i>Thraxan ebenus</i>	NA	NA	AJ391517	NA	NA	NA	NA	NA	NA	AJ391517	NA							
Bombyliidae	<i>Exoprosopa adelaidica</i>	NA	NA	AJ391519	NA	NA	NA	NA	NA	NA	AJ391519	NA							
Asilidae	<i>Lasiopogon cinctus</i>	NA	NA	NA	EF650102	EF650189	MG189305	EF650341	KT733400	NA	NA	EF650415	NA	NA	NA	NA	NA	NA	
Asilidae	<i>Machimus occidentalis</i>	NA	NA	NA	EF650157	EF650250	EF650278	EF650395	JN289688	NA	NA	EF650466	NA	NA	NA	NA	NA	NA	
Asilidae	<i>Philiocnus albiceps</i>	NA	NA	NA	EF650124	EF650216	EF650279	EF650368	KT733427	NA	NA	EF650440	NA	NA	NA	NA	NA	NA	
Asilidae	<i>Asilus crabroniformis</i>	NA	KC177475	KC177451	KC177289	KC177704	KC178206	EF650383	KC192962	KC192983	KC177586	EF650454	KC176839	KC176816	KC178050	KC177507	KC178340	KC177061	KC177933
Asilidae	<i>Diogmites grossus</i>	NA	NA	AY324988	AY325019	EF650246	EF650282	EF650391	MG967752	AY325081	NA	EF650475	NA						
Asilidae	<i>Stichopogon trifasciatus</i>	NA	NA	AY324989	EF650175	EF650256	KY906302	EF650399	KY914518	AY325082	NA	EF650471	NA						
Asilidae	<i>Astrotericus chiastoneurus</i>	NA	NA	NA	EF650120	EF650210	EF650301	EF650360	KT733200	NA	NA	EF650434	NA						
Asilidae	<i>Asilus sericeus</i>	NA	NA	NA	EF650138	EF650236	EF650276	EF650384	JN289750	NA	NA	EF650455	NA						
Asilidae	<i>Choreothes bella</i>	NA	NA	NA	EF650110	EF650198	EF650290	EF650348	KT733173	NA	NA	EF650424	NA						
Asilidae	<i>Connomyia varipennis</i>	NA	NA	NA	EF650128	EF650220	EF650304	EF650369	KT733202	NA	NA	EF650444	NA						
Asilidae	<i>Damalis monochaetes</i>	NA	NA	NA	EF650149	EF650260	EF650318	EF650403	KT733097	NA	NA	EF650473	NA						
Asilidae	<i>Dasygaster diadema</i>	NA	NA	NA	EF650103	EF650190	EF650281	EF650342	NC_045239	NA	NA	EF650416	NA						
Asilidae	<i>Dioclea hyalinipennis</i>	NA	NA	NA	EF650143	EF650244	KY906300	EF650390	KY914516	NA	NA	EF650461	NA						
Asilidae	<i>Dysmachus trigonus</i>	NA	NA	NA	EF650156	EF650214	EF650277	EF650366	KT733425	NA	NA	EF650438	NA						
Asilidae	<i>Emphysomera pallidapex</i>	NA	NA	NA	EF650139	EF650239	EF650300	EF650387	KT733227	NA	NA	EF650457	NA						
Asilidae	<i>Holcocephala abdominalis</i>	NA	NA	AY324999	EF650131	EF650224	NA	KM879072	MN411228	AY325092	NA								
Asilidae	<i>Lamyra gulo</i>	NA	NA	NA	EF650161	EF650258	EF650291	EF650401	KT733095	NA	NA	EF650476	NA						
Asilidae	<i>Lasiopogon aldrichi</i>	NA	NA	NA	EF650174	EF650248	MG189364	EF650393	MG189011	NA	NA	EF650464	NA						
Asilidae	<i>Laxenecera albincincta</i>	NA	NA	NA	EF650118	EF650208	EF650292	EF650358	KT733429	NA	NA	EF650432	NA						
Asilidae	<i>Leptogaster arida</i>	NA	NA	NA	EF650135	EF650229	EF650298	EF650362	KT733213	NA	NA	EF650451	NA						
Asilidae	<i>Leptogaster cylindrica</i>	NA	NA	NA	EF650101	EF650188	EF650299	EF650339	MN411149	NA	NA	EF650414	NA						
Asilidae	<i>Lestomyia fraudiger</i>	NA	NA	NA	EF650132	EF650226	EF650283	EF650376	KT733207	NA	NA	EF650448	NA						
Asilidae	<i>Lycostommyia albifacies</i>	NA	NA	NA	EF650116	EF650205	EF650306	EF650356	KT733185	NA	NA	EF650430	NA						

Tabanidae	Copidapha aureohirta	NA	NA	NA	NA	KC592507	KC592804	KC592730	KC592641	KC593039	NA									
Tabanidae	Dasybasis neobasalis	NA	NA	NA	NA	KM243409	KC592828	KC593157	KC592670	KC593064	NA									
Tabanidae	Goniops chrysocoma	NA	NA	NA	NA	KM243424	KC592838	KC593164	KC592648	KC593066	NA									
Tabanidae	Myioscapta calliphora	NA	NA	NA	NA	KC592458	KC592770	KC593096	KC592589	KC592987	NA									
Tabanidae	Anzomyia pegasus	NA	NA	NA	NA	KC592419	KC592749	KC593112	KC592606	KC593005	NA									
Tabanidae	Pityocera cervus	NA	NA	NA	NA	KM243451	KC592827	KC593156	KC592649	KC593050	NA									
Tabanidae	Scione equatoriensis	NA	NA	NA	NA	KC592468	KC592817	KC593105	KC592657	KC592997	NA									
Tabanidae	Pseudoscioni vittata	NA	NA	NA	NA	KC592506	KC592803	KC592728	KC592640	KC593038	NA									
Tabanidae	Triclista guttata	NA	NA	NA	NA	KM243481	KC592748	KC593156	KC592673	KC592958	NA									
Tabanidae	Oscia lata	NA	NA	NA	NA	KM243444	KC592761	KC593136	KC592629	KC593027	NA									
Tabanidae	Parosca viridiventris	NA	NA	NA	NA	KC592492	KC592802	KC593128	KC592638	KC593037	NA									
Tabanidae	Plinthina arnhemensis	NA	NA	NA	NA	KC592448	KC592763	KC593090	KC592867	KC592978	NA									
Tabanidae	Adersia oestroides	NA	NA	NA	NA	KM243387	KM243597	NA	KM243486	NA										
Tabanidae	Copidapha guttipennis	NA	NA	NA	NA	KC592517	KC592756	KC593074	KC592651	KC593055	NA									
Tabanidae	Pseudoscioni dorsoguttata	NA	NA	NA	NA	KM243454	KC592796	KC593127	KC592624	KC593021	NA									
Xylophagidae	Exeretonevra angustifrons	NA	KC177477	KC177453	KC177293	FJ040532	KC178188	FJ040628	KC192964	KC192992	KC177588	NA	KC176841	KC176818	KC178053	FJ040733	KC178337	KC177065	FJ040682	
Atelestidae	Meghyperus sudeticus	NA	NA	NA	NA	AF503079	NA	AY280688	NA	NA	AF503140	NA								
Dolichopodidae	Syntormon flexibile	NA	FJ080125	KM282992	FJ080279	FJ080345	NA	KM28180	KM282746	KM282826	FJ080484	KM362989	NA							
Dolichopodidae	Campsicnemus curvipes	NA	KM283013	EU864028	HQ449058	DO496188	NA	DQ369278	DO456892	KM282765	NA	KM362925	NA							
Dolichopodidae	Neurigonoides quadrifasciata	NA	DQ464870	EU864024	HQ449060	KC177115	KC178217	AY280690	DQ456911	NA	KC177943									
Dolichopodidae	Acropilus sp1	NA	FJ080706	FJ080151	FJ080223	FJ080301	NA	NA	FJ792973	NA	FJ080436	NA								
Dolichopodidae	Acropilus sp2	NA	FJ080707	FJ080152	FJ080224	FJ080302	NA	NA	FJ792974	NA	FJ080437	NA								
Dolichopodidae	Campsicnemus amblytylus	NA	KM282996	KM282907	NA	NA	NA	KM283087	KM282663	KM282749	NA	KM362910	NA							
Dolichopodidae	Campsicnemus distinctus	NA	KM283016	KM282927	NA	NA	NA	KM283109	KM282681	KM282768	NA	KM362927	NA							
Dolichopodidae	Campsicnemus loriopes	NA	KM283035	HQ449014	HQ449061	NA	NA	KM283126	DQ456897	NA	NA	KM362945	NA							
Dolichopodidae	Campsicnemus modicus	NA	KM283038	KM282948	NA	NA	NA	KM283131	KM282700	KM282784	NA	KM362948	NA							
Dolichopodidae	Campsicnemus mucronatus	NA	KM283051	KM282960	NA	NA	NA	KM283145	KM282703	KM282796	NA	KM362950	NA							
Dolichopodidae	Campsicnemus scambus	NA	KM283067	KM282976	NA	NA	NA	KM283161	DQ456904	KM282809	NA	KM362975	NA							
Dolichopodidae	Chaetogonopteron chaeturum	NA	FJ080801	FJ080155	FJ080229	FJ080307	NA	NA	FJ792978	NA	FJ080442	NA								
Dolichopodidae	Chaetogonopteron sp1	NA	FJ080803	FJ080157	FJ080231	FJ080309	NA	NA	FJ792979	NA	FJ080444	NA								
Dolichopodidae	Chrysosoma bearni	NA	FJ080806	FJ080160	FJ080234	FJ080311	NA	NA	FJ792980	NA	FJ080446	NA								
Dolichopodidae	Chrysosoma sp1	NA	FJ080807	FJ080161	FJ080235	FJ080312	NA	NA	FJ792981	NA	FJ080447	NA								
Dolichopodidae	Chrysotus sp1	NA	FJ080808	FJ080162	FJ080236	FJ080313	NA	NA	FJ792982	NA	FJ080448	NA								
Dolichopodidae	Dolichopus latilimbatus	NA	DQ464845	EU863916	HQ449066	FJ0706111	NA	NA	AY744200	NA	AY744241	NA								
Dolichopodidae	Dolichopus pennatus	NA	DQ464862	EU863927	HQ449069	FJ0706121	NA	NA	AY744209	NA	AY744250	NA								
Dolichopodidae	Dolichopus plumipes	NA	DQ464841	EU863940	HQ449048	FJ0706134	NA	NA	EU847549	NA	AY744227	NA								
Dolichopodidae	Dolichopus ungulatus	NA	DQ464826	EU863962	HQ449054	FJ0706157	NA	NA	EU847559	NA	AY744235	NA								
Dolichopodidae	Dolichopus tanytrix	NA	DQ464844	EU863951	HQ449065	NA	NA	NA	AY744199	NA	AY744240	NA								
Dolichopodidae	Gymnopternus aerosus	NA	DQ464827	EU863970	HQ449056	FJ0706165	NA	NA	AY744194	NA	AY744236	NA								
Dolichopodidae	Hercostomus brevidigitalis	NA	FJ080100	FJ080172	FJ080249	FJ080320	NA	NA	FJ792995	NA	FJ080458	NA								
Dolichopodidae	Lichtwardtia ziczac	NA	FJ080102	FJ080174	FJ080251	FJ080322	NA	NA	FJ792996	NA	FJ080460	NA								
Dolichopodidae	Medetera griseescens	NA	FJ080104	FJ080175	FJ080253	FJ080324	NA	NA	FJ792997	NA	FJ080461	NA								
Dolichopodidae	Nanothinophilus hoplites	NA	FJ080110	FJ080181	FJ080258	FJ080330	NA	NA	FJ793003	NA	FJ080467	NA								
Dolichopodidae	Neurigonoides sp1	NA	FJ080114	FJ080186	FJ080264	FJ080335	NA	NA	FJ793007	NA	FJ080472	NA								
Dolichopodidae	Neurigonoides squamifera	NA	FJ080116	FJ080188	FJ080266	FJ080336	NA	NA	FJ793009	NA	FJ080474	NA								
Dolichopodidae	Paracilius digitatus	NA	FJ080118	FJ080190	FJ080268	FJ080338	NA	NA	FJ793010	NA	FJ080476	NA								
Dolichopodidae	Phacaspis mitis	NA	MF928549	MF928577	FJ080270	FJ080339	NA	NA	MF944166	NA	FJ080477	NA								
Dolichopodidae	Plagiopozepma flavipodex	NA	FJ080121	FJ080192	FJ080271	FJ080340	NA	NA	FJ793012	NA	FJ080478	NA								
Dolichopodidae	Poecilobothrus nobilitatus	NA	DQ464784	EU864009	HQ449085	DQ496206	NA	DQ369296	MT410808	NA										
Dolichopodidae	Scotiomyia sp1	NA	FJ080122	FJ080193	FJ080272	FJ080341	NA	NA	FJ793013	NA	FJ080479	NA								
Dolichopodidae	Sybistroma obscurellum	NA	DQ464874	EU864016	HQ449073	FJ0706170	NA	NA	DQ456918	NA	AY744265	NA								
Dolichopodidae	Tachytrechus tessellatus	NA	FJ080127	FJ080199	FJ080281	FJ080348	NA	NA	FJ793021	NA	FJ080486	NA								
Dolichopodidae	Thambemyia pagdeni	NA	FJ080133	FJ080205	FJ080287	FJ080349	NA	NA	FJ793029	NA	FJ080492	NA								
Dolichopodidae	Thinophilus asiosbates	NA	FJ080135	FJ080207	FJ080289	FJ080350	NA	NA	FJ793031	NA	FJ080494	NA								
Dolichopodidae	Thinophilus murphyi	NA	FJ080137	FJ080209	FJ080290	FJ080352	NA	NA	FJ793033	NA	FJ080495	NA								
Dolichopodidae	Thinophilus nitens	NA	FJ080139	FJ080210	FJ080291	FJ080353	NA	NA	FJ793035	NA	FJ080497	NA								
Empididae	Oreogenet scopifer	NA	NA	NA	NA	KC177291	KC178213	DQ369287	KP264784	NA	NA	NA	NA	NA	NA	KC178052	KC177508	KC178365	KC177063	KC177940
Empididae	Empis barbatoides	NA	KC177476	KC177443	NA	NA	NA	KC192963	KC192990	KC177587	NA	KC176840	KC176817	NA						
Empididae	Heterophlebus versabilis	NA	HM062581	NA	NA	HM062609	HM062635	HM062728	NA	NA	NA	HM062558	NA	NA	NA	HM062752	NA	NA	NA	HM062682

Empididae	Trichopeza longicornis	NA	NA	HQ449035	NA	DQ496207	NA	DQ369289	HQ449172	NA	MG823617	MG823685	NA								
Empididae	Empis producta	NA	FJ808095	FJ808167	FJ808243	FJ808316	NA	NA	FJ792989	NA	FJ808453	NA									
Empididae	Empis sp1	NA	FJ808096	FJ808168	FJ808244	FJ808317	NA	NA	FJ792990	NA	FJ808454	NA									
Empididae	Gloea fuscipennis	NA	NA	NA	NA	AF503008	NA	DQ369290	MG823418	NA	MG823589	MG823669	NA								
Empididae	Hemerodromia sp1	NA	NA	FJ808170	FJ808247	FJ808319	NA	NA	FJ792993	NA	FJ808456	NA									
Empididae	Microphor holosericeus	NA	HQ449039	HQ448954	HQ449104	NA	NA	NA	MT410831	NA	MG823657	NA									
Empididae	Ragas unica	NA	NA	NA	NA	NA	NA	MG823499	MG823412	NA	MG823583	MG823665	NA								
Empididae	Dolichocephala guttata	NA	NA	NA	NA	NA	NA	MG823567	MG823482	NA	MG823652	MG823703	NA								
Empididae	Heleodromia immaculata	NA	NA	NA	NA	NA	NA	MG823557	KT22595	NA	MG823642	MG823699	NA								
Empididae	Heleodromia borealpina	NA	NA	NA	NA	NA	NA	MG823522	MG823435	NA	MG823606	MG823674	NA								
Empididae	Wiedemannia bistigma	NA	NA	NA	NA	NA	NA	MG823570	MG823486	NA	MH755994	MH764291	NA								
Empididae	Clinocera maculata	NA	NA	NA	NA	NA	NA	MG823523	MG823436	NA	MG823607	MG823675	NA								
Empididae	Empis bicuspidata	NA	HQ449046	HQ449033	HQ449107	NA	NA	NA	HQ449170	NA											
Empididae	Hilara interstincta	NA	NA	NA	NA	NA	NA	MG823527	MG823440	NA	MG823611	MG823678	NA								
Empididae	Hilara abdominalis	NA	NA	NA	NA	NA	NA	MG823533	MG823446	NA	MG823616	MG823684	NA								
Empididae	Rhamphomyia crassirostris	NA	NA	NA	NA	NA	NA	MG823532	MG823445	NA	MG823615	MG823683	NA								
Empididae	Chelifera sp1	NA	NA	NA	NA	NA	NA	MG823554	MG823469	NA	MG823639	MG823698	NA								
Empididae	Phyllodromia melanocephala	NA	NA	NA	NA	NA	NA	MG823558	MG823473	NA	MG823643	MG823700	NA								
Empididae	Microphorella malaysiana	NA	FJ808108	NA	NA	FJ808328	NA	NA	FJ793001	NA	FJ808466	NA									
Empididae	Parathalassius candidatus	NA	NA	NA	NA	KC177714	KC178216	NA	KY883174	NA	KC177942										
Empididae	Anthepiscopus sp1	NA	NA	NA	NA	NA	NA	MG823563	MG823478	NA	MG823648	MG823702	NA								
Hybotidae	Stilpon graminum	NA	NA	NA	NA	NA	NA	MG823514	MG823427	NA	MG823598	MG823672	NA								
Hybotidae	Hybos culiciformis	NA	KC699286	KC699337	NA	KC177712	KC178214	MG823562	MT410806	NA	MG823647	NA									
Hybotidae	Ocydromia glabricula	NA	NA	NA	NA	KC177713	KC178215	KC177148	MG823423	NA	MG823594	NA	KC177941								
Hybotidae	Oedalea hybotina	NA	HQ449047	HQ449037	HQ449109	NA	NA	MG823572	HQ449173	NA	MG823656	NA									
Hybotidae	Hybos femoratus	NA	NA	HQ449036	HQ449105	NA	NA	MG823506	HQ449126	NA	MG823590	NA									
Hybotidae	Elaphropeza neesoensis	NA	FJ808094	FJ808166	FJ808242	NA	NA	FJ792988	NA	FJ808452	NA										
Hybotidae	Chersodromia nigripennis	NA	FJ808085	FJ808159	FJ808233	FJ808310	NA	NA	FJ808445	NA											
Hybotidae	Nanodromia narmkroi	NA	FJ808109	FJ808180	FJ808257	FJ808329	NA	NA	FJ793002	NA											
Hybotidae	Tachypeza nubila	NA	NA	HQ449038	HQ449108	NA	NA	MG823559	HQ449174	NA	MG823644	NA									
Hybotidae	Crossopalpus nigritellus	NA	NA	NA	NA	NA	NA	MG823500	MG823413	NA	MG823584	MG823666	NA								
Apystomyiidae	Apystomyia elinguis	NA	NA	NA	NA	KC177483	KC177456	KC177298	FJ040531	KC178220	FJ040630	KC192977	KC192997	KC177600	NA	KC176853	KC176828	KC178058	FJ040732	KC178343	KC177069
Lonchopteridae	Lonchoptera sp1	y	KC177483	KC177456	KC177298	FJ040531	KC178220	FJ040630	KC192977	KC192997	KC177600	NA	KC176853	KC176828	KC178058	FJ040732	KC178343	KC177069	FJ040681		
Opetiidae	Opetia nigra	NA	KC699294	AF126347	NA	KC177711	KC178211	KC178221	FJ280692	MN868809	NA	KC699403	AF503146	NA	NA	KC177373	NA	NA	KC177946		
Platypozidae	Platypozea sp1	NA	HM062590	NA	NA	HM062616	HM062644	HM062736	NA	NA	HM062563	HM062666	NA	NA	NA	NA	HM062759	NA	NA	HM062691	
Ironomyiidae	Ironomyia nigromaculata	NA	NA	NA	NA	KC177717	KC178219	KC177150	KR260222	NA	NA	NA	NA	NA	NA	KC177372	NA	NA	NA	NA	
Phoridae	Megaselia abdita	NA	NA	NA	NA	NA	NA	KY836639	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Phoridae	Megaselia scalaris	NA	KC177486	KC177442	KC177299	KC177221	KC178223	NA	NC_023794	KC192998	KC177604	GU362007	KC176858	KC176833	KC178059	KC177510	KC178349	KC177070	KC177948		
Phoridae	Sciadocera rufomaculata	NA	GU559903	JN196447	NA	KC177723	KC178225	KC177154	GU559946	NA	NA	GU559968	NA	NA	KC177353	NA	NA	KC177950			
Phoridae	Conicera dauci	NA	JN664648	NA	NA	HM062616	JN664786	HM062735	JF73345	NA	HM062562	HM062664	NA	NA	NA	NA	HM062757	NA	NA	HM062688	
Phoridae	Beckerina luteola	NA	EU068666	EU068632	NA	MH318679	NA	EU068571	KT862036	NA	NA	EU068506	NA	MH318964							
Phoridae	Melanconcha horologia	NA	EU068645	EU068613	NA	GU559930	NA	EU068551	GU559951	NA	NA	EU068485	NA	MH318967							
Phoridae	Apoccephalus spinosus	NA	GU559911	MH318621	NA	MH318681	NA	MH318809	GU559954	NA	NA	MH318905	NA	MH318978							
Phoridae	Gymnophora spiracularis	NA	GU559905	MH318618	NA	GU559927	NA	MH318790	KT862035	NA	NA	GU559970	NA	MH318962							
Phoridae	Diplonevra nitidula	NA	AF126298	AF126326	NA	KX529297	NA	JN196449	HM352601	NA											
Phoridae	Phora holosericea	NA	GU361976	AY078062	NA	KX529470	NA	NA	MN597122	NA	GU362001	KX775119	NA								
Phoridae	Dohrniphora gigantea	NA	EU068665	EU068631	NA	EU068599	NA	EU068570	HM352597	NA											
Phoridae	Triphleba sp1	NA	GU559910	JN196446	NA	GU559932	NA	JN196452	GU559953	NA	NA	GU559975	NA								
Phoridae	Borophaga verticalis	NA	GU559899	JN196444	NA	GU559921	NA	JN196450	GU559942	NA	NA	GU559964	NA								
Phoridae	Melaloncha acoma	NA	EU068639	GU550187	NA	GU550273	NA	EU068546	GU550340	NA	NA	EU068479	NA								
Phoridae	Melaloncha feleoae	NA	EU068649	GU550206	NA	EU068585	NA	EU068555	EU068523	NA	NA	EU068489	NA								
Phoridae	Melaloncha pilula	NA	EU068654	EU068621	NA	EU068590	NA	EU068560	EU068528	NA	NA	EU068494	NA								
Phoridae	Melaloncha adusta	NA	GU550175	GU550231	NA	GU550279	NA	GU550332	GU550386	NA	NA	GU550439	NA								
Phoridae	Dohrniphora cornuta	NA	GU361987	HM366981	NA	NA	NA	JN196448	MN832849	NA	NA	GU362009	HM357185	NA							
Phoridae	Apoccephalus curtus	NA	NA	MH318668	NA	MH318723	NA	MH318831	MH318887	NA	NA	NA	MH318950	NA	NA	NA	NA	NA	MH319002		
Phoridae	Apoccephalus flexiseta	NA	NA	MH318646	NA	MH318699	NA	MH318815	MH318861	NA	NA	NA	MH318930	NA	NA	NA	NA	NA	MH318983		
Phoridae	Apocephalus orthocladius	NA	NA	MH318635	NA	MH318690	NA	MH318804	MH318850	NA	NA	NA	MH318919	NA	NA	NA	NA	NA	MH318973		
Pipunculidae	Pipunculus sp1	NA	HM062589	NA	NA	HM062617	HM062643	NA	NA	NA	NA	NA	HM062665	NA	NA	NA	NA	HM062758	NA	HM062686	
Pipunculidae	Cephalops longistylis	NA	NA	NA	NA	KC177724	KC178226	KC177155	NA	NA	NA	NA	NA	NA	NA	NA	KC177374	NA	NA	KC177951	

Pipunculidae	Nephrocerus daeckeii	NA	AF154720	AF154796	NA	NA	NA	MG295263	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Pipunculidae	Elmohardya atlantica	NA	NA	NA	NA	AF503020	NA	NA	MG295551	NA	NA	AF503148	NA							
Pipunculidae	Pipunculus houghi	NA	NA	NA	NA	DQ496182	NA	AY280691	DQ337706	NA										
Pipunculidae	Cephalops cocheatus	NA	AF154682	AF154752	NA	NA	NA	JF872529	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Syrphidae	Episyphus balteatus	NA	AY573076	AY573115	KC177297	KC177227	KC178228	NA	NC_036481	AY573151	KC177603	NA	KC176857	KC176832	KC178057	NA	KC178344	KC177068	KC177954	
Syrphidae	Rhingia nasica	NA	NA	NA	NA	KC177725	KC178227	AY280697	KM571828	NA	NA	NA	NA	KC177375	NA	NA	KC177952			
Syrphidae	Micronotus tristis	NA	NA	NA	NA	KC177726	NA	KC177157	KR672698	NA	NA	NA	NA	KC177376	NA	NA	KC177953			
Syrphidae	Toxomerus marginatus	NA	AF154745	AF154820	EU409277	HM062624	HM062649	HM062742	EU409160	NA	HM062568	HM062672	NA	NA	HM062762	NA	NA	HM062690		
Syrphidae	Allograptia alta	NA	KP402891	NA	NA	KP698557	KP403097	KP402951	KP402773	NA	KP402834	NA								
Syrphidae	Allograptia sp1	NA	KP402892	NA	NA	KP698532	KP403098	KP402952	KP402774	NA	KP402835	NA								
Syrphidae	Atylobaccha flukiella	NA	KP402893	NA	NA	KP698525	KP403099	KP402953	KP402775	NA	KP402836	NA								
Syrphidae	Baccha elongata	NA	KP402894	NA	EU431540	KP698563	KP403100	KP402954	KP402776	NA	KP402837	NA								
Syrphidae	Eristalis tenax	NA	AY573077	AY123344	AY431517	AY123356	NA	NA	NC_041143	AY573152	NA									
Syrphidae	Leucopodella gracilis	NA	KP402901	NA	EU431547	KP698551	KP403107	KP402961	KP402783	NA	KP402844	NA								
Syrphidae	Leucopodella sp1	NA	KP402900	NA	NA	KP698566	KP403106	KP402960	KP402782	NA	KP402843	NA								
Syrphidae	Ocyptamus antiphates	NA	KP402904	NA	EU241847	KP698569	KP403109	KP402963	KP402786	NA	KP402847	NA								
Syrphidae	Ocyptamus dimidiatus	NA	KP402916	NA	EU409240	KP698527	KP403121	KP402973	KP402798	NA	KP402857	NA								
Syrphidae	Ocyptamus fascipennis	NA	KP402917	NA	EU409241	KP698531	KP403122	KP402974	KP402799	NA	KP402858	NA								
Syrphidae	Ocyptamus funebris	NA	KP402919	NA	EU409242	KP698570	KP403124	KP402976	KP402801	NA	KP402860	NA								
Syrphidae	Ocyptamus fuscipennis	NA	KP402920	NA	EU409243	KP698535	KP403125	KP402977	KP402802	NA	KP402861	NA								
Syrphidae	Ocyptamus macropygia	NA	KP402896	NA	NA	KP698571	KP403102	KP402956	KP402778	NA	KP402839	NA								
Syrphidae	Ocyptamus norina	NA	KP402898	NA	EU409247	KP698549	KP403104	KP402958	KP402779	NA	KP402841	NA								
Syrphidae	Ocyptamus rubricosus	NA	KP402899	NA	NA	KP698538	KP403105	KP402959	KP402781	NA	KP402842	NA								
Syrphidae	Paragus haemorrhous	NA	KP402930	NA	EU409259	KP698522	KP403134	KP402987	KP402812	NA	KP402870	NA								
Syrphidae	Pelecinobaccha adpersa	NA	KP402932	NA	NA	KP698565	KP403136	KP402988	KP402814	NA	KP402872	NA								
Syrphidae	Pelecinobaccha alicia	NA	KP402934	NA	NA	KP698521	KP403138	KP402990	KP402816	NA	KP402874	NA								
Syrphidae	Pelecinobaccha ovipositoria	NA	KP402940	NA	NA	KP698543	KP403143	KP402996	KP402822	NA	KP402879	NA								
Syrphidae	Pseudodoros clavatus	NA	KP402944	NA	KM270807	KP698568	KP403146	KP402999	KP402826	NA	KP402882	NA								
Syrphidae	Relictanum crassum	NA	KP402945	NA	NA	KP698537	KP403147	KP403000	KP402827	NA	KP402883	NA								
Syrphidae	Relictanum magisadspersum	NA	KP402946	NA	NA	KP698517	KP403148	KP403001	KP402828	NA	KP402884	NA								
Syrphidae	Salpingogaster sp1	NA	KP402947	NA	NA	KP698523	KP403149	KP403002	KP402829	NA	KP402885	NA								
Syrphidae	Salpingogaster sp2	NA	KP402948	NA	NA	KP698530	KP403150	KP403003	KP402830	NA	KP402886	NA								
Syrphidae	Tenomostoma vespiforme	NA	NA	NA	NA	AY261746	NA	KF936845	AY261699	NA	NA	NA	NA	NA	NA	KF936135	NA	NA	KF937203	
Syrphidae	Toxomerus politus	NA	KP402949	NA	EU241863	KP698554	KP403151	KP403004	EU241755	NA	KP402888	NA								
Syrphidae	Toxomerus virgulatus	NA	KP402950	NA	EU409291	KP698536	KP403152	KP403005	KP402833	NA	KP402889	NA								
Conopidae	Myopa vesciculosa	NA	JN664611	NA	NA	JN664680	JN664749	HM062721	HM417169	NA	HM062549	HM062652	NA	NA	NA	HM062746	NA	NA	HIM062677	
Conopidae	Physocephala marginata	NA	KC177481	KC177452	KC177304	KC177729	JN664730	HM062719	KC192973	KC193000	KC177596	HM062651	KC176849	NA	KC178063	KC177511	KC178362	KC177074	KC177956	
Conopidae	Stylopaster neglecta	NA	KR262582	KR262609	NA	HM062260	HM062626	HM062623	KR262677	KR262708	HM062553	NA	NA	NA	NA	HM062749	NA	NA	HM062675	
Conopidae	Dalmannia nigriceps	NA	JN664601	NA	NA	HM062260	HM062630	HM062725	JN664802	NA	HM062551	HM062656	NA	NA	NA	NA	NA	NA	HM062676	
Australimyzidae	Australimyza sp1	NA	NA	NA	NA	KC177808	KC178304	KC177226	NA	NA	NA	NA	NA	NA	NA	KC177433	NA	NA	KC178022	
Canacidae	Tethinosoma fulvifrons	NA	NA	NA	NA	KC177816	KC178312	KC177234	NA	NA	NA	NA	NA	NA	NA	KC177436	NA	NA	KC178029	
Carnidae	Carnus hemapterus	NA	NA	NA	NA	NA	NA	KC871417	KC871417	NA										
Chloropidae	Thaumatomyia notata	NA	NA	KC177449	KC177306	KC177813	KC178309	KC177231	KC192976	KC193005	KC177599	NA	KC176852	NA	KC178065	KC177519	KC178357	KC177076	NA	
Chloropidae	Incertella albipalpis	NA	NA	NA	NA	KC177812	KC178308	KC177230	NA	NA	NA	NA	NA	NA	NA	KC177435	NA	NA	KC178026	
Chloropidae	Oscinella frit	NA	AF304346	NA	NA	NA	NA	NA	HQ081992	MT318997	NA									
Chloropidae	Chaetochlorops inquilinus	NA	NA	NA	NA	GU299234	NA	GU299261	HQ984632	NA										
Chloropidae	Thaumatomyia sp1	NA	NA	NA	NA	GU299235	NA	GU299280	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Chloropidae	Incertella incerta	NA	NA	NA	NA	NA	NA	KM571510	NA	KP037172	NA									
Chloropidae	Meromyza columbi	NA	NA	NA	NA	NA	NA	KP037172	NA	KP037359	NA									
Chloropidae	Elachiptera bimaculata	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Chloropidae	Cryptonevra nigritarsis	NA	NA	NA	NA	NA	NA	NA	NA	NA	MT318987	NA								
Chloropidae	Lasiosina albipila	NA	NA	NA	NA	NA	NA	NA	NA	NA	MT318991	NA								
Inbionyiidae	Inbionyia mcapineorum	NA	NA	NA	NA	KC177817	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Milichiidae	Paramyia nitens	NA	NA	NA	NA	KC177815	KC178311	KC177233	KR517963	NA	KC178028									
Nannodastidae	Azorastia mediterranea	NA	NA	NA	NA	KC177782	NA	KC177203	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Ephydriidae	Scatella hawaiiensis	NA	NA	EU494299	NA	NA	NA	NA	EU493565	EU493697	NA	NA	EU494299	NA	NA	NA	NA	NA	NA	
Ephydriidae	Hydrellia tritici	NA	EU494420	EU494420	NA	NA	NA	NA	EU493564	EU493696	EU494082	NA	EU494197	EU493825	NA	NA	NA	NA	NA	
Ephydriidae	Hydrellia pakistanae	NA	NA	EU494298	NA	NA	NA	NA	EU493563	EU493695	EU494081	NA	EU494298	EU493824	NA	NA	NA	NA	NA	
Ephydriidae	Ephydria packardi	NA	EU494419	EU494297	NA	NA	NA	NA	EU493562	EU493694	NA	NA	EU494297	NA	NA	NA	NA	NA	NA	

Ephydriidae	Hydrellia griseola	NA	NA	NA	NA	KC177806	KC178302	KC177224	MF059320	NA	NA	NA	NA	NA	NA	KC177431	NA	NA	KC178020
Ephydriidae	Psilopa polita	NA	NA	NA	NA	KC177805	KC178301	KC177223	NA	KC177430	NA	NA	KC178019						
Ephydriidae	Coenia palustris	NA	NA	NA	NA	KC177807	KC178303	KC177225	NA	KC177432	NA	NA	KC178021						
Ephydriidae	Limnellia quadrata	NA	AF126305	AF126333	NA	NA													
Ephydriidae	Ephydra riparia	NA	NA	NA	NA	GU597374	NA	NA	JF867574	NA	NA								
Ephydriidae	Discocerina obscurella	NA	NA	NA	NA	GU597408	NA	NA	JF875838	NA	NA								
Drosophilidae	Drosophila sp1	y	EU494480	M37275	M21017	NR_13562	KC178299	X04813	KT174474	MH925016	M37275	X06870	M37275	M37275	X03636	KC177518	KC178331	NM_078490	X57576
Drosophilidae	Phortica picta	NA	EU494422	EU494301	NA	AY081424	NA	NA	EU493567	EU493699	NA	NA	EU494301	EU493828	NA	NA	NA	NA	NA
Drosophilidae	Drosophila immigrans	NA	EU494439	EU494320	AF348906	AB932824	NA	NA	JQ679115	EU493716	EU494102	NA	EU494320	EU493846	L06337	MK016876	MK017576	NA	MK017333
Drosophilidae	Drosophila hydei	NA	EU494459	MK659821	NA	GU597386	NA	NA	EU493606	EU493736	NA	NA	EU494230	EU493866	FJ267301	NA	JF736328	JF736388	NA
Drosophilidae	Drosophila virilis	NA	AF185058	AF508180	HG798349	GU597420	NA	NA	HQ849831	EU493751	EU494135	NA	EU494353	EU493881	AY854874	NA	M77282	JF736417	NA
Drosophilidae	Chymomyza amoena	NA	EU494425	M93986	NA	GQ244450	NA	NA	EU493570	EU493702	EU494086	NA	EU494304	EU493831	NA	NA	NA	NA	NA
Drosophilidae	Chymomyza procnemis	NA	EU494426	EU494426	NA	GU597413	NA	NA	EU493571	EU493703	EU494087	NA	EU494305	EU493832	NA	NA	NA	NA	NA
Drosophilidae	Mycodrosophila dimidiata	NA	EU494530	EU494408	NA	GU597440	NA	NA	EU493682	EU493811	EU494184	NA	EU494408	EU493939	NA	NA	NA	NA	NA
Drosophilidae	Samoia leonensis	NA	EU494531	EU494409	NA	KC192913	NA	NA	EU493683	EU493812	EU494185	NA	EU494409	EU493940	NA	NA	NA	NA	NA
Drosophilidae	Scaptodrosophila latifasciaeformis	NA	EU494532	EU494410	NA	GU597377	NA	NA	EU493684	EU493813	EU494186	NA	EU494410	EU493941	NA	NA	NA	NA	NA
Drosophilidae	Scaptodrosophila lebanonensis	NA	EU494534	MK659851	NA	HQ110555	NA	NA	EU493686	EU493815	EU494188	NA	EU494411	EU493943	NA	NA	NA	NA	NA
Drosophilidae	Scaptomyza crassifemur	NA	EU494581	KC609614	NA	AF059860	NA	NA	EU493677	EU493806	EU494180	NA	EU494282	EU493934	NA	NA	NA	NA	NA
Drosophilidae	Amiota leucostoma	NA	EU494421	EU494300	NA	NA	NA	NA	EU493566	NA	EU494083	NA	EU494300	EU493827	NA	NA	NA	NA	NA
Drosophilidae	Amiota setigera	NA	EU494423	EU494302	NA	NA	NA	NA	EU493568	EU493700	EU494084	NA	EU494302	EU493829	NA	NA	NA	NA	NA
Drosophilidae	Leucophenga albofasciata	NA	EU494424	EU494303	NA	NA	NA	NA	EU493569	EU493701	EU494085	NA	EU494303	EU493830	NA	NA	NA	NA	NA
Drosophilidae	Scaptomyza caliginosa	NA	EU494525	EU494402	NA	NA	NA	NA	EU493676	EU493805	EU494179	NA	EU494402	EU493933	NA	NA	NA	NA	NA
Drosophilidae	Zygothrica sp1	NA	EU494539	EU494539	NA	NA	NA	NA	EU493692	EU493821	EU494194	NA	EU494417	EU493948	NA	NA	NA	NA	NA
Drosophilidae	Zaprionus lineatus	NA	EU494536	EU494414	NA	NA	NA	NA	EU493689	EU493818	EU494191	NA	EU494414	NA	NA	NA	NA	NA	NA
Drosophilidae	Parastegana brevivena	NA	NA	NA	NA	KJ813905	NA	MH372863	KJ813938	MH373057	NA	NA							
Drosophilidae	Pseudostegana bifasciata	NA	NA	NA	NA	JF313867	NA	MH372871	JF273079	MH373065	NA	NA							
Braulidae	Braula coeca	NA	NA	NA	NA	KC177809	KC178305	KC177227	NA	KC177434	NA	NA	KC178023						
Cryptochetidae	Cryptochetum sp1	NA	NA	NA	NA	KC177814	KC178310	KC177232	NA	KC178027									
Camillidae	Camilla sp1	NA	NA	NA	NA	KC177800	KC178297	KC177220	NA	KC177429	NA	NA	KC178015						
Curtonotidae	Curtonotus saheliense	NA	NA	NA	NA	NA	NA	JQ765780	JQ765818	NA	JQ765810								
Diastatidae	Diastata fuscula	NA	NA	NA	NA	KC177802	KC178298	KC177221	NA	KC178016									
Celyphidae	Spaniocelyphus umsinduzi	NA	NA	NA	NA	KC177761	KC178261	KC177183	NA	KC177400	NA	NA	KC177985						
Chamaemyiidae	Cremifania nearctica	NA	NA	NA	NA	KC177758	KC178258	KC177181	NA	KC177398	NA	NA	KC177982						
Lauxaniidae	Sapromyza sexpunctata	NA	NA	NA	KT956368	NA	KT956419	NA	NA	AJ439153	NA	NA	KU991646	NA	NA	NA	NA	NA	NA
Lauxaniidae	Minettia flaveola	NA	NA	NA	KC177448	KC177305	KC177760	KC178260	NA	KC192974	KC192999	KC177597	NA	KC176850	KC176826	KC178064	KC177515	KC178345	KC177075
Lauxaniidae	Minettia lupulina	NA	JN664646	KT956349	NA	HM062610	HM062636	HM062729	KM571706	NA	HM062556	KU991627	NA	NA	NA	HM062753	NA	NA	NA
Lauxaniidae	Melanina sp1	NA	HM062583	NA	NA	HM062611	HM062637	HM062730	NA	NA	HM062557	HM062660	NA	NA	NA	NA	NA	NA	HM062694
Lauxaniidae	Lyciella decipiens	NA	NA	NA	KT956334	NA	KC177759	KC178259	KC177182	NA	NA	KU991613	NA	NA	NA	KC177399	NA	NA	KC177983
Lauxaniidae	Calliopum simillimum	NA	NA	NA	KT956322	NA	KT956376	NA	NA	MN868801	NA	NA	KU991600	NA	NA	NA	NA	NA	NA
Lauxaniidae	Homoneura mayrhoferi	NA	KR262566	KR262593	NA	NA	NA	NA	KR262638	KR262693	NA	NA							
Lauxaniidae	Minettia longipennis	NA	NA	NA	KT956348	NA	KT956402	NA	NA	MN868895	NA	NA	KU991626	NA	NA	NA	NA	NA	NA
Lauxaniidae	Lyciella illota	NA	NA	NA	KT956336	NA	KT956391	NA	NA	MG673800	NA	NA	KU991614	NA	NA	NA	NA	NA	NA
Lauxaniidae	Protrigonometopus maculifrons	NA	KR262579	KR262606	NA	NA	NA	NA	KR262665	KR262705	NA	NA							
Cypselosomatidae	Rhinopomyzella sp1	NA	NA	NA	NA	KC177738	KC178238	KC177165	NA	KC177965									
Micropezidae	Micropeza sp1	NA	KM287346	NA	NA	NA	NA	KM287265	KM287301	NA	NA								
Micropezidae	Taeniptera trivittata	NA	KM287371	NA	NA	HM062613	HM062639	HM062732	KM287330	NA	HM062559	HM062662	NA	NA	NA	HM062755	NA	NA	HM062693
Micropezidae	Compsobata cibaria	NA	NA	NA	NA	KC177733	KC178233	KC177161	MG823463	NA	MG823633	MG823695	NA	NA	NA	KC177381	NA	NA	KC177960
Neriidae	Telostylinus sp1	NA	NA	NA	NA	KC177734	KC178234	KC177162	NA	KC177961									
Acartophthalmidae	Acartophthalmus nigrinus	NA	NA	NA	NA	KC177818	KC178313	KC177235	KM80533	NA	NA	NA	NA	NA	NA	KC177437	NA	NA	KC178030
Agromyzidae	Melanagromyza minimoides	y	AY573083	AY573120	NA	EF104831	NA	EF104743	KR262643	AY573158	NA	NA							
Agromyzidae	Phytomyza ilicicola	NA	NA	NA	KC177307	KC177784	KC178281	EF104814	AF276860	AF276860	NA	NA	NA	NA	KC178066	KC177517	KC178347	KC177077	NA
Agromyzidae	Liriomyza trifolii	NA	NA	NA	MF141914	KY563323	EF104879	NA	EF104791	GU327644	AY375176	NA	KY558636	NA	MF141934	NA	NA	NA	NA
Agromyzidae	Liriomyza huidobrensis	NA	NA	NA	AY842478	FJ890888	NA	EF104788	JQ862474	AY375174	NA	AY035339	NA	NA	NA	NA	NA	NA	NA
Agromyzidae	Phytomyza glabricola	NA	NA	NA	NA	EF104901	NA	EF104813	AF276838	AF276838	NA	JX658435	NA	NA	NA	EU367830	NA	NA	NA
Agromyzidae	Phytomyza syngenesiae	NA	NA	NA	NA	EF104867	NA	EF104779	KR476575	NA	NA	NA	NA	NA	MF141930	EU367821	NA	NA	NA
Agromyzidae	Cerodontha dorsalis	NA	NA	NA	NA	KC177783	KC178280	EF104771	EF104686	NA	NA	NA	NA	NA	NA	KC177415	NA	NA	KC178002
Agromyzidae	Phytomyza aconiti	NA	NA	NA	NA	EU367930	NA	EU367687	AF276828	AF276828	NA	NA	NA	NA	NA	EU367885	NA	NA	NA
Agromyzidae	Phytomyza aquilegivora	NA	NA	NA	NA	EF104899	NA	EF104811	AF276830	AF276830	NA	NA	NA	NA	NA	EU367903	NA	NA	NA
Agromyzidae	Phytomyza erigerophila	NA	NA	NA	NA	EF104900	NA	EF104812	AF276833	AF276833	NA	NA	NA	NA	NA	EU367869	NA	NA	NA

Platystomatidae	Platystoma seminationis	NA	AY573093	AY573128	NA	NA	NA	NA	KR262657	AY573168	NA	NA	NA	NA	NA	NA	NA	NA	
Platystomatidae	Pterogenia sp1	NA	KR262578	KR262605	NA	NA	NA	NA	KR262664	KR262704	NA	NA	NA	NA	NA	NA	NA	NA	
Ctenostylidae	Sinolochmostyia sinica	NA	KR262581	KR262608	NA	NA	NA	NA	KR262673	KR262707	NA	NA	NA	NA	NA	NA	NA	NA	
Pyrgotidae	Pyrgota undata	NA	AY573097	AY123352	U01264	JN664714	JN664781	HMO62739	KR262666	AY573172	HMO62565	HMO62669	NA	NA	NA	HMO62761	NA	NA	
Pyrgotidae	Eupyrgota tigrina	NA	AY573079	AY573117	NA	NA	NA	NA	KR262631	AY573154	NA	NA	NA	NA	NA	NA	NA	NA	
Tephritisidae	Ceratitis capitata	NA	NA	AF177123	AH006961	KC177754	KC178254	EU717163	NC_000857	DQ011889	JX500798	MG683583	AH014646	NA	AH015344	KC177514	KC178346	KC177071	
Tephritisidae	Bactrocera dorsalis	NA	EU926853	EU926922	U01251	GU323792	NA	MG683277	KT343905	AB972850	NA	GU339154	NA	MG916968	KC446693	NA	NA	NA	
Tephritisidae	Zeugodacus cucurbitae	NA	EU926852	EU926921	U01250	NA	NA	MG683199	JN635562	AB192452	DQ006904	MG683520	KC594985	NA	AB517621	NA	NA	NA	
Tephritisidae	Bactrocera correcta	NA	AY037332	AY037375	KF246551	JQ906101	NA	MG683252	NC_018787	AB192423	KP055591	MG6833572	FJ665832	NA	MG683722	NA	NA	NA	
Tephritisidae	Bactrocera tryoni	NA	AY037345	JQ671089	XM_040115129	KC446993	NA	MG683298	HQ130030	AB192442	JQ420932	MG683620	AY037474	NA	AF480840	NA	NA	NA	
Tephritisidae	Bactrocera carambolae	NA	AY037324	AY037367	AF033940	KC447278	NA	MG683317	MN104217	AB192421	KC439576	MG683640	AY037453	NA	KC446709	NA	NA	NA	
Tephritisidae	Anastrepha obliqua	NA	AY037507	AF152078	NA	AJ634703	NA	KY428152	HQ677064	MG020784	NA	KY428459	HM592861	NA	NA	KY428620	NA	KT594599	
Tephritisidae	Zeugodacus tau	NA	AY037348	FJ168028	AY209010	GU323789	NA	MG683201	MF966383	AB192461	KC439660	MG683522	AY037477	NA	MG683678	NA	NA	NA	
Tephritisidae	Bactrocera cacuminata	NA	AY037327	JQ671096	NA	KC447052	NA	MG683310	MG683459	JQ671162	NA	MG683362	AY037456	NA	KC446489	NA	NA	NA	
Tephritisidae	Anastrepha ludens	NA	NA	AF152074	EU179519	MH990661	NA	KU527434	HQ677057	AB192462	NA	KY428448	NA	NA	KY428613	NA	KT594565		
Tephritisidae	Anastrepha suspensa	NA	NA	U39380	U01249	DQ279855	NA	KY428163	KY428318	NA	NA	KY428470	NA	NA	MG683835	KY428626	NA	NA	
Tephritisidae	Rhagoletis pomonella	NA	EU926900	AF177127	U01265	MH99122	MH998810	KU527451	DQ006862	U53229	DQ006893	NA	NA	NA	MG825196	NA	NA	NA	
Tephritisidae	Anastrepha fraterculus	NA	NA	AF152067	AF187101	AY686688	NA	KY428135	NC_034912	MG020783	NA	MF028812	NA	NA	KY428608	NA	KT594623		
Tephritisidae	Bactrocera musae	NA	AY037328	JQ671086	NA	KC447012	NA	MG683312	AB192432	AB192432	NA	MG683364	AY037457	NA	KC446450	NA	NA	NA	
Tephritisidae	Bactrocera oleae	NA	AY037337	AF164590	NA	NA	NA	MG683222	GU108467	GQ175825	NA	MG683538	AF164590	NA	KM023545	AM158090	NA	NA	
Tephritisidae	Bactrocera occipitalis	NA	AY037343	KM023483	NA	KC447134	NA	MG683308	AB192435	AB192435	NA	MG683630	AY037472	NA	KC446579	NA	NA	NA	
Tephritisidae	Rhagoletis cingulata	NA	EU926896	U39427	NA	KU511180	MH998796	MG825286	DQ006863	KT221479	DQ006894	NA	NA	NA	MG825197	NA	NA	NA	
Tephritisidae	Zeugodacus cucumis	NA	AY037306	JQ671092	AF114473	NA	NA	MG683268	AB192448	AB192448	NA	MG683590	AY037435	NA	MG863741	NA	NA	NA	
Tephritisidae	Bactrocera frauenfeldi	NA	AY037315	JQ671085	NA	NA	NA	MG683285	AB192428	AB192428	AF033906	MG683606	AY037444	NA	MG83757	NA	NA	NA	
Tephritisidae	Bactrocera umbrosa	NA	AY037322	KM023489	NA	NA	NA	MG683208	KT881558	AB192443	DQ006899	MG683521	AY037451	NA	KM023549	NA	NA	NA	
Tephritisidae	Campiglossa pygmaea	NA	HM062597	NA	NA	HM062625	HM062650	HM062743	NA	NA	HM062569	HM062673	NA	NA	NA	HM062763	NA	NA	
Tephritisidae	Zeugodacus caudatus	NA	AY037320	KM023474	NA	KP694326	NA	MG683273	KT625492	MG020791	NA	MG683594	AY037449	NA	KM023535	NA	NA	KC177977	
Tephritisidae	Tachinisa cyaneiventris	NA	EU926907	EU926949	NA	KC177752	KC178252	KC177177	KR262678	EU926838	NA	NA	NA	NA	KC177394	NA	NA	KC177977	
Tephritisidae	Anastrepha curvicauda	NA	EU926910	U39381	U01273	NA	NA	KY428117	HQ677147	EU926841	NA	KY428419	NA	NA	KY428594	NA	NA	NA	
Glossinidae	Glossina morsitans	NA	NA	EF531110	KC177312	KC177834	KC178323	EF531178	KC192971	KR820741	KC177594	JF439518	KC176847	KC176824	KC178070	KC177524	KC178334	KC177081	
Hippoboscidae	Orthofiersia minuta	NA	NA	EF531123	NA	EF531156	NA	EF531182	EF531221	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Hippoboscidae	Ornithomya avicularia	NA	NA	MF495919	AF322421	EF531146	KC178325	EF531168	MF495992	NA	NA	MF495969	NA	NA	NA	NA	NA	KC178041	
Hippoboscidae	Stenopteryx hirundinis	NA	NA	EF531121	NA	KC177835	KC177046	KC177246	EF531215	NA	NA	NA	NA	NA	KC177439	NA	NA	KC178040	
Hippoboscidae	Lipoptena cervi	NA	NA	AF322437	AF322426	EF531139	NA	MN370852	MF496025	DQ133114	NA	MF495964	NA	NA	NA	NA	NA	NA	
Nycteribiidae	Penicillidia fulvida	NA	NA	EF531124	KF156697	EF531157	NA	EF531174	KF021520	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Strebidae	Trichobius longipes	NA	NA	DQ133052	DQ133088	NA	NA	NA	MH282298	DQ133124	DQ133158	NA	NA	NA	NA	NA	NA	NA	
Fanniidae	Fannia canicularis	NA	DQ656884	DQ648647	FJ025489	KC177820	NA	KC177237	MF511734	AJ879606	DQ657051	AJ871202	NA	NA	NA	NA	NA	KC178031	
Fanniidae	Fannia manicata	NA	DQ656885	FJ025435	FJ025490	DO656962	NA	DQ657038	NA	DQ657052	NA	NA	NA	NA	NA	NA	NA	NA	
Muscidae	Musca domestica	NA	DQ377075	AY123346	QG465780	KC177822	KC178316	AY280698	KM200723	KT272857	AF503149	KT272857	KT272857	KC178071	GQ265612	KC178355	KC177082	KC178033	
Muscidae	Stomoxys calcitrans	NA	DQ377080	HM245737	FJ025499	KC177824	KC178317	KC177247	DQ533708	EU029770	KU932178	AJ605062	HM245737	EU029770	NA	KC177438	NA	NA	KC178035
Muscidae	Helina evecta	NA	FJ025377	FJ025439	NA	FJ025522	KP161777	AJ867936	FJ025619	AJ879607	FJ025719	AJ871203	NA	NA	NA	NA	NA	NA	
Muscidae	Atherigona orientalis	NA	DQ377077	KC347602	NA	KC538817	KP161761	KP161814	EU627707	KC347607	NA	KP161729	KC524782	NA	NA	NA	NA	NA	
Muscidae	Muscinia stabulans	NA	FJ025396	EF531117	NA	EF531145	NA	EF531167	NC_026292	EU627738	FJ025736	AJ871205	NA	NA	NA	NA	NA	NA	
Muscidae	Musca autumnalis	NA	FJ025394	FJ025457	NA	KJ476355	NA	FJ025590	MT410827	JQ821709	KU932173	KU932198	NA	NA	NA	NA	NA	NA	
Muscidae	Hydrotea aeneescens	NA	DQ377079	NA	NA	KJ439011	KP161794	KP161837	NC_042952	AY184819	KU932156	KU932183	NA	NA	NA	NA	NA	NA	
Muscidae	Drymeia alpicola	NA	FJ025370	FJ025430	NA	KC177823	NA	KC177239	FJ025608	NA	FJ025710	FJ025669	NA	NA	NA	NA	NA	KC178034	
Muscidae	Huckettomyia watanabei	NA	KJ476276	KJ476311	NA	KJ476346	KP161779	KP161824	KJ510624	NA	KJ510557	KP161738	NA	NA	NA	NA	NA	NA	
Muscidae	Dichaetomyia bibax	NA	KJ476267	KJ476303	NA	KJ476337	KP161771	KP161821	KJ510615	NA	KJ510549	KJ510588	NA	NA	NA	NA	NA	NA	
Muscidae	Poliectes lardarius	NA	KJ476289	FJ025471	NA	FJ025557	KP161798	FJ025597	KF919035	NA	FJ025695	NA	NA	NA	NA	NA	NA	NA	
Muscidae	Thriocops cunctans	NA	FJ025418	FJ025478	NA	FJ025564	NA	FJ025600	FJ025661	NA	FJ025753	FJ025700	NA	NA	NA	NA	NA	NA	
Muscidae	Synthesiomyia nudiseta	NA	NA	JN26649	NA	NA	KP161811	KP161849	NC_042953	DQ345099	NA	MK29743	NA	NA	NA	NA	NA	NA	
Muscidae	Limnophora exuta	NA	FJ025384	FJ025446	NA	FJ025530	NA	FJ025581	FJ025626	NA	FJ025725	FJ025684	NA	NA	NA	NA	NA	NA	
Muscidae	Lispe tentaculata	NA	KX856038	KX856044	NA	KX856039	NA	KX856018	KX856027	NA	FJ025729	KX856032	NA	NA	NA	NA	NA	NA	
Muscidae	Haematobosca stimulans	NA	FJ025375	FJ025437	NA	FJ025518	NA	FJ025576	MT410787	NA	FJ025716	FJ025673	NA	NA	NA	NA	NA	NA	
Muscidae	Mydaea ancilla	NA	FJ025398	FJ025460	NA	FJ025547	NA	FJ025592	FJ025639	NA	FJ025737	FJ025690	NA	NA	NA	NA	NA	NA	
Muscidae	Potamia littoralis	NA	FJ025412	FJ025472	NA	FJ025558	NA	FJ025598	KU932145	NA	KU932176	KU932201	NA	NA	NA	NA	NA	NA	
Muscidae	Cyrtoneuriopsis veniseta	NA	KJ476266	KJ476302	NA	NA	KP161769	KP161819	KJ510614	NA	KJ510548	KP161735	NA	NA	NA	NA	NA	NA	
Muscidae	Achanthiptera rohrelliformis	NA	KJ476258	KJ476294	NA	KJ476331	KP161760	NA	KJ510606	NA	KJ510542	KJ510580	NA	NA	NA	NA	NA	NA	
Muscidae	Helina lasiophthalma	NA	FJ025379	NA	NA	FJ025524	NA	AJ605055	FJ025621	AJ627899	FJ025720	AJ605067	NA	NA	NA	NA	NA	NA	

Appendix 3: Larval feeding habit data for all ingroup taxa.

Family	Binomial name	Larval feeding habit	Source
Nemestrinidae	Trichophthalma sp1	Endoparasitoid	Kirk-Spriggs & Sinclair, 2017
Acroceridae	Acrocera orbiculus	Endoparasitoid	Gillung & Winterton, 2019
Acroceridae	Carvalhoa micella	Endoparasitoid	Gillung & Winterton, 2019
Acroceridae	Megalybus sp1	Endoparasitoid	Gillung & Winterton, 2019
Hilarimorphidae	Hilarimorpha mentata	Unknown	Marshall, 2012
Vermileonidae	Vermileo opacus	Predator	Kirk-Spriggs & Sinclair, 2017
Bombyliidae	Bombylius major	Ectoparasitoid	Yeates & Greathead, 1997
Bombyliidae	Neosardus principius	Unknown	
Bombyliidae	Villa sp1	Endoparasitoid	Yeates & Greathead, 1997
Bombyliidae	Comptosia sp1	Unknown	
Bombyliidae	Hemipenthes jaennickeana	Unknown	Yeates & Greathead, 1997; Kirk-Spriggs & Sinclair, 2017
Bombyliidae	Mythicomyia sp1	Unknown	
Bombyliidae	Paracosmus sp1	Unknown	
Bombyliidae	Megapalpus capensis	Unknown	
Bombyliidae	Corsomyza sp1	Unknown	Kirk-Spriggs & Sinclair, 2017
Bombyliidae	Corsomyza sp2	Unknown	Kirk-Spriggs & Sinclair, 2017
Bombyliidae	Pseudopenthes fenestrata	Unknown	
Bombyliidae	Ligyrus satyrus	Ectoparasitoid	Yeates & Greathead, 1997
Bombyliidae	Heterotropus sp1	Predator	Kirk-Spriggs & Sinclair, 2017
Bombyliidae	Thraxan acutus	Unknown	
Bombyliidae	Exoprosopa latelimbata	Unknown	Yeates & Greathead, 1997; Kirk-Spriggs & Sinclair, 2017
Bombyliidae	Atrichochira sp1	Unknown	Kirk-Spriggs & Sinclair, 2017
Bombyliidae	Ligyrus punctipennis	Ectoparasitoid	Yeates & Greathead, 1997
Bombyliidae	Villa fuscicostata	Endoparasitoid	Yeates & Greathead, 1997
Bombyliidae	Mythicomyia atra	Unknown	
Bombyliidae	Anthrax maculatus	Ectoparasitoid	Yeates & Greathead, 1997; Kirk-Spriggs & Sinclair, 2017
Bombyliidae	Comptosia quadripennis	Unknown	
Bombyliidae	Thraxan ebenus	Unknown	
Bombyliidae	Exoprosopa adelaidica	Unknown	Yeates & Greathead, 1997; Kirk-Spriggs & Sinclair, 2017
Asilidae	Lasiopogon cinctus	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Machimus occidentalis	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Philonicus albiceps	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Asilus crabroniformis	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Diogmites grossus	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Stichopogon trifasciatus	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Astroestricus chiastoneurus	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Asilus sericeus	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Choerades bella	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Connomyia varipennis	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Damalis monochaetes	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Dasygaster diadema	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Dioclea hyalinipennis	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Dysmachus trigonus	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Emphysomera pallidapex	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Holcocephala abdominalis	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Lamyra gulo	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Lasiopogon aldrichi	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Laxenecera albicincta	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Leptogaster arida	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Leptogaster cylindrica	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Lestornya fraudiger	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Lycostommyia albifacies	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Molobratia teutonus	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Neoitamus cyanurus	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Neolophonotus bimaculatus	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Ommatius tibialis	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Pegesimallus laticornis	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Philodicus tenuipes	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Pogonioeffera pogonias	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Proctacanthus philadelphicus	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Prolepsis tristis	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Saropogon luteus	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Stichopogon punctum	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Tolmerus atricapillus	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Trichardis effrena	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Willistonia bilineata	Predator	Kirk-Spriggs & Sinclair, 2017
Asilidae	Ceraturgus fasciatus	Predator	Kirk-Spriggs & Sinclair, 2017
Mydidae	Mydas clavatus	Predator	Kirk-Spriggs & Sinclair, 2017
Mydidae	Nemomydas pantherinus	Predator	Kirk-Spriggs & Sinclair, 2017
Apoceridae	Apocera haruspex	Predator	Kirk-Spriggs & Sinclair, 2017
Evocoidae	Evocoa chilensis	Unknown	Marshall, 2012
Apsioceridae	Apsiocerida longistyla	Unknown	
Scenopinidae	Riekiella sp1	Predator	Kirk-Spriggs & Sinclair, 2017, Marshall, 2012
Scenopinidae	Scenopinus fenestralis	Predator	Kirk-Spriggs & Sinclair, 2017, Marshall, 2012
Therevidae	Acrosathet novella	Predator	Kirk-Spriggs & Sinclair, 2017, Marshall, 2012
Therevidae	Henicomia hubbardi	Predator	Kirk-Spriggs & Sinclair, 2017, Marshall, 2012
Therevidae	Patanothrix wilsoni	Predator	Kirk-Spriggs & Sinclair, 2017, Marshall, 2012
Therevidae	Hemigephrya atra	Predator	Kirk-Spriggs & Sinclair, 2017, Marshall, 2012
Therevidae	Phycus kroberi	Predator	Kirk-Spriggs & Sinclair, 2017, Marshall, 2012
Austroleptidae	Austroleptis multimaculata	Unknown	Marshall, 2012
Bolbomyiidae	Bolbomyia nana	Unknown	Marshall, 2012
Rhagionidae	Spaniopsis clelandi	Predator	Marshall, 2012
Rhagionidae	Chrysopilus thoracicus	Predator	Marshall, 2012

Appendix 3: Larval feeding habit data for all ingroup taxa.

Family	Binomial name	Larval feeding habit	Source
Rhagionidae	<i>Rhagio hirtus</i>	Predator	Marshall, 2012
Rhagionidae	<i>Rhagio scolopaceus</i>	Predator	Marshall, 2012
Pantophthalmidae	<i>Pantophthalmus bellardii</i>	Saprophase	Marshall, 2012
Stratiomyidae	<i>Myxosargus knowltoni</i>	Unknown	
Stratiomyidae	<i>Hermetia illucens</i>	Saprophase	Kirk-Spriggs & Sinclair, 2021; McAlpine, 1981
Stratiomyidae	<i>Chloromyia formosa</i>	Saprophase	Kirk-Spriggs & Sinclair, 2021
Stratiomyidae	<i>Clitellaria ephippium</i>	Unknown	
Stratiomyidae	<i>Sargus bipunctatus</i>	Saprophase	Lessard <i>et al.</i> , 2020
Stratiomyidae	<i>Microchrysa polita</i>	Saprophase	Kirk-Spriggs & Sinclair, 2021
Stratiomyidae	<i>Antissa sp1</i>	Saprophase	Kirk-Spriggs & Sinclair, 2021
Stratiomyidae	<i>Dieuryneura stigma</i>	Saprophase	Kirk-Spriggs & Sinclair, 2021
Stratiomyidae	<i>Pachygaster montana</i>	Saprophase	Marshall, 2012
Stratiomyidae	<i>Neoberis brasiliiana</i>	Unknown	
Stratiomyidae	<i>Stratiomys laticeps</i>	Unknown	
Stratiomyidae	<i>Hedriodus binotatus</i>	Unknown	
Stratiomyidae	<i>Rosapha variegata</i>	Saprophase	Kirk-Spriggs & Sinclair, 2021
Stratiomyidae	<i>Oplodontha viridula</i>	Unknown	Kirk-Spriggs & Sinclair, 2021
Xylomyidae	<i>Xylomya parens</i>	Saprophase	Kirk-Spriggs & Sinclair, 2017; Marshall, 2012
Athericidae	<i>Atherix variegata</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Oreoleptidae	<i>Oreoleptis torrenticola</i>	Predator	Zloty <i>et al.</i> , 2005
Pelecorhynchidae	<i>Glutops singularis</i>	Predator	McAlpine, 1981
Tabanidae	<i>Scaptia patula</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Fidenia rhinophora</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Haematopota pluvialis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Tabanus atratus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Rhigioglossa edentula</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Chrysops viduatus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Apocampta subcana</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Copidapha aureohirta</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Dasybasis neobasalis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Gonioips chrysocoma</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Myioscaptia calliphora</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Anzomyia pegasus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Pityocera cervus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Scione equatoriaensis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Pseudoscione vittata</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Triclista guttata</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Oscia lata</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Parosca viridiventris</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Plinthina arnhemensis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Adersia oestroides</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Copidapha guttipennis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Tabanidae	<i>Pseudoscione dorsoguttata</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Xylophagidae	<i>Exeretonevra angustifrons</i>	Predator	Palmer & Yeates, 2000
Atelestidae	<i>Meghyperus sudeticus</i>	Unknown	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Syntormon flexible</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Campsicnemus curvipes</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Neurigona quadrifasciata</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Acropilus sp1</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Acropilus sp2</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Campsicnemus amblytylus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Campsicnemus distinctus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Campsicnemus loripes</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Campsicnemus modicus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Campsicnemus mucronatus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Campsicnemus scambus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Chaetogonopteron chaeturum</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Chaetogonopteron sp1</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Chrysosoma bearni</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Chrysosoma sp1</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Chrysotus sp1</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Dolichopus latilimbatus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Dolichopus pennatus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Dolichopus plumipes</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Dolichopus unguatus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Dolichopus tanythrix</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Gymnopternus aerosus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Hercostomus brevidigitalis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Lichtwardtia ziczac</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Medetera griseescens</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Nanothonophilus hoplites</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Neurigona sp1</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Neurigona squamifera</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Paracilius digitatus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Phacaspis mitis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Plagiopelma flavipodex</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Poecilobothrus nobilitatus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Scotiomyia sp1</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Sybistroma obscurellum</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Tachytrechus tessellatus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Thambemyia pagdeni</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Thinophilus asiobates</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Dolichopodidae	<i>Thinophilus murphyi</i>	Predator	Kirk-Spriggs & Sinclair, 2017

Appendix 3: Larval feeding habit data for all ingroup taxa.

Family	Binomial name	Larval feeding habit	Source
Dolichopodidae	<i>Thinophilus nitens</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Oreogeton scopifer</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Empis barbatoides</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Heterophlebus versabilis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Trichopeza longicornis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Empis producta</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Empis sp1</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Gloma fuscipennis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Hemerodromia sp1</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Microphor holosericeus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Ragas unica</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Dolichocephala guttata</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Heleodromia immaculata</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Heleodromia borealpina</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Wiedemannia bistigma</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Clinocera maculata</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Empis bicuspidata</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Hilara interstincta</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Hilara abdominalis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Rhamphomyia crassirostris</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Chelifera sp1</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Phylldromia melanocephala</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Microphorella malaysiana</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Parathalassius candidatus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Empididae	<i>Anthepiscopus sp1</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Hybotidae	<i>Stilpon graminum</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Hybotidae	<i>Hybos culiciformis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Hybotidae	<i>Ocydromia glabricula</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Hybotidae	<i>Oedalea hybotina</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Hybotidae	<i>Hybos femoratus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Hybotidae	<i>Elaphropeza neesoensis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Hybotidae	<i>Chersodromia nigripennis</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Hybotidae	<i>Nanodromia narmkroi</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Hybotidae	<i>Tachypeza nubila</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Hybotidae	<i>Crossopalpus nigritellus</i>	Predator	Kirk-Spriggs & Sinclair, 2017
Apystomyiidae	<i>Apystomyia elinguis</i>	Unknown	Marshall, 2012
Lonchopteridae	<i>Lonchoptera sp1</i>	Saprophage	Kirk-Spriggs & Sinclair, 2017
Opetidae	<i>Opetia nigra</i>	Mycophagous	Tkoc & Rohacek, 2014
Platypezidae	<i>Platypeza sp1</i>	Mycophagous	Kirk-Spriggs & Sinclair, 2017
Ironomyiidae	<i>Ironomyia nigromaculata</i>	Unknown	Li & Yeates, 2019
Phoridae	<i>Megaselia abdita</i>	Saprophage	Manlove & Disney, 2008
Phoridae	<i>Megaselia scalaris</i>	Saprophage, Predator, Parasite, Phytophage	Disney, 2008
Phoridae	<i>Sciadocera rufomaculata</i>	Saprophage	Kavazos <i>et al.</i> , 2011
Phoridae	<i>Conicera dauci</i>	Saprophage	Brown <i>et al.</i> , 2010
Phoridae	<i>Beckerina luteola</i>	Unknown	
Phoridae	<i>Melaloncha horologia</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Phoridae	<i>Apocephalus spinosus</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Phoridae	<i>Gymnophora spiracularis</i>	Saprophage	Brown <i>et al.</i> , 2010
Phoridae	<i>Diplonevra nitidula</i>	Unknown	Brown <i>et al.</i> , 2010
Phoridae	<i>Phora holosericea</i>	Unknown	Brown <i>et al.</i> , 2010
Phoridae	<i>Dohrniphora gigantea</i>	Unknown	
Phoridae	<i>Triphleba sp1</i>	Saprophage	Brown <i>et al.</i> , 2010
Phoridae	<i>Borophaga verticalis</i>	Unknown	
Phoridae	<i>Melaloncha acoma</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Phoridae	<i>Melaloncha feleoae</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Phoridae	<i>Melaloncha pilula</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Phoridae	<i>Melaloncha adusta</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Phoridae	<i>Dohrniphora cornuta</i>	Unknown	
Phoridae	<i>Apocephalus curtus</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Phoridae	<i>Apocephalus flexiseta</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Phoridae	<i>Apocephalus orthocladus</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Pipunculidae	<i>Pipunculus sp1</i>	Endoparasitoid	Marshall, 2012
Pipunculidae	<i>Cephalops longistylis</i>	Endoparasitoid	Marshall, 2012
Pipunculidae	<i>Nephrocerus daeckei</i>	Endoparasitoid	Marshall, 2012
Pipunculidae	<i>Elmohardyia atlantica</i>	Endoparasitoid	Marshall, 2012
Pipunculidae	<i>Pipunculus houghi</i>	Endoparasitoid	Marshall, 2012
Pipunculidae	<i>Cephalops cochleatus</i>	Endoparasitoid	Marshall, 2012
Syrphidae	<i>Episyrrhus balteatus</i>	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Rhingia nasica</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Microdon tristis</i>	Predator	Reemer, 2013
Syrphidae	<i>Toxomerus marginatus</i>	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Allograptia alta</i>	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Allograptia sp1</i>	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Atylabaccha flukiella</i>	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Baccha elongata</i>	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Eristalis tenax</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Leucopodella gracilis</i>	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Leucopodella sp1</i>	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Ocyptamus antiphates</i>	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Ocyptamus dimidiatus</i>	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Ocyptamus fascipennis</i>	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Ocyptamus funebris</i>	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	<i>Ocyptamus fuscipennis</i>	Predator	Kirk-Spriggs & Sinclair, 2021

Appendix 3: Larval feeding habit data for all ingroup taxa.

Family	Binomial name	Larval feeding habit	Source
Syrphidae	Ocyptamus macropyga	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Ocyptamus norina	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Ocyptamus rubricosus	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Paragus haemorrhoous	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Pelecinobaccha adpersa	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Pelecinobaccha alicia	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Pelecinobaccha ovipositoria	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Pseudodoros clavatus	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Relictanum crassum	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Relictanum magisadpersum	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Salpingogaster sp1	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Salpingogaster sp2	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Temnostoma vespiforme	Saprophage	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Toxomerus politus	Predator	Kirk-Spriggs & Sinclair, 2021
Syrphidae	Toxomerus virgulatus	Predator	Kirk-Spriggs & Sinclair, 2021
Conopidae	Myopa sp1	Endoparasitoid	Marshall, 2012
Conopidae	Physocephala marginata	Endoparasitoid	Marshall, 2012
Conopidae	Stylogaster neglecta	Endoparasitoid	Marshall, 2012
Conopidae	Dalmannia nigriceps	Endoparasitoid	Marshall, 2012
Australimyzidae	Australimyza sp1	Saprophage	Brake & Mathis, 2007
Canacidae	Tethinosoma fulvifrons	Unknown	Kirk-Spriggs & Sinclair, 2021
Carnidae	Carnus hemapterus	Saprophage	Kirk-Spriggs & Sinclair, 2021
Chloropidae	Thaumatomya notata	Predator	Kirk-Spriggs & Sinclair, 2021
Chloropidae	Incertella albipalpis	Phytophage	Deeming & Al-Dhafer, 2012
Chloropidae	Oscinella frit	Phytophage	Kirk-Spriggs & Sinclair, 2021
Chloropidae	Chaetochlorops inquilinus	Unknown	
Chloropidae	Thaumatomya sp1	Predator	Kirk-Spriggs & Sinclair, 2021
Chloropidae	Incertella incerta	Phytophage	Deeming & Al-Dhafer, 2012
Chloropidae	Meromyza columbi	Phytophage	Kirk-Spriggs & Sinclair, 2021
Chloropidae	Elachiptera bimaculata	Saprophage	Kirk-Spriggs & Sinclair, 2021
Chloropidae	Cryptonevra nigritarsis	Phytophage	Kirk-Spriggs & Sinclair, 2021
Chloropidae	Lasiosina albipila	Unknown	
Inbiomyiidae	Inbiomyia mcalpineorum	Unknown	Brown <i>et al.</i> , 2010
Milichiidae	Paramyia nitens	Saprophage	Kirk-Spriggs & Sinclair, 2021
Nannodastiidae	Azorastia mediterranea	Unknown	Brown <i>et al.</i> , 2010
Ephydriidae	Scatella hawaiiensis	Saprophage	Kirk-Spriggs & Sinclair, 2021
Ephydriidae	Hydrellia tritici	Saprophage	Kirk-Spriggs & Sinclair, 2021
Ephydriidae	Hydrellia pakistanae	Saprophage	Kirk-Spriggs & Sinclair, 2021
Ephydriidae	Ephydra packardi	Saprophage	Kirk-Spriggs & Sinclair, 2021
Ephydriidae	Hydrellia griseola	Saprophage	Kirk-Spriggs & Sinclair, 2021
Ephydriidae	Psilopa polita	Saprophage	Kirk-Spriggs & Sinclair, 2021
Ephydriidae	Coenia palustris	Saprophage	Kirk-Spriggs & Sinclair, 2021
Ephydriidae	Limnella quadrata	Saprophage	Kirk-Spriggs & Sinclair, 2021
Ephydriidae	Ephydra riparia	Saprophage	Kirk-Spriggs & Sinclair, 2021
Ephydriidae	Discocerina obscurella	Saprophage	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Drosophila sp1	Saprophage	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Phortica picta	Unknown	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Drosophila immigrans	Saprophage	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Drosophila hydei	Saprophage	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Drosophila virilis	Saprophage	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Chymomyza amoena	Unknown	Brown <i>et al.</i> , 2010
Drosophilidae	Chymomyza procnemis	Unknown	Brown <i>et al.</i> , 2010
Drosophilidae	Mycodrosophila dimidiata	Mycophage	Brown <i>et al.</i> , 2010
Drosophilidae	Samoaea leonensis	Unknown	
Drosophilidae	Scaptodrosophila latifasciaeformis	Unknown	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Scaptodrosophila lebanonensis	Unknown	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Scaptomyza crassifemur	Phytophage	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Amiota leucostoma	Saprophage	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Amiota setigera	Saprophage	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Leucophenga albofasciata	Unknown	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Scaptomyza caliginosa	Phytophage	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Zygothrica sp1	Unknown	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Zaprionus lineosus	Saprophage	Kirk-Spriggs & Sinclair, 2021
Drosophilidae	Parastegana brevivena	Unknown	
Drosophilidae	Pseudostegana bifasciata	Unknown	
Braulidae	Braula coeca	Palynivore, Malivore	Kirk-Spriggs & Sinclair, 2021
Cryptochetidae	Cryptochetum sp1	Endoparasitoid	Kirk-Spriggs & Sinclair, 2021
Camillidae	Camilla sp1	Saprophage	Kirk-Spriggs & Sinclair, 2021
Curtonotidae	Curtonotum sp1	Unknown	
Diastatidae	Diastata fuscula	Unknown	Brown <i>et al.</i> , 2010
Celyphidae	Spaniocelyphus umsinduzi	Saprophage	Kirk-Spriggs & Sinclair, 2021
Chamaemyiidae	Cremifanía nearctica	Predator	Kirk-Spriggs & Sinclair, 2021
Lauxaniidae	Sapromyza sp1	Saprophage	Kirk-Spriggs & Sinclair, 2021
Lauxaniidae	Minettia flaveola	Saprophage	Kirk-Spriggs & Sinclair, 2021
Lauxaniidae	Minettia lupulina	Saprophage	Kirk-Spriggs & Sinclair, 2021
Lauxaniidae	Melanina sp1	Saprophage	Kirk-Spriggs & Sinclair, 2021
Lauxaniidae	Lyciella decipiens	Saprophage	Kirk-Spriggs & Sinclair, 2021
Lauxaniidae	Calliopum simillimum	Saprophage	Kirk-Spriggs & Sinclair, 2021
Lauxaniidae	Homoneura mayrhoferi	Saprophage	Kirk-Spriggs & Sinclair, 2021
Lauxaniidae	Minettia longipennis	Saprophage	Kirk-Spriggs & Sinclair, 2021
Lauxaniidae	Lyciella illoca	Saprophage	Kirk-Spriggs & Sinclair, 2021
Lauxaniidae	Protrigonometopus maculifrons	Saprophage	Kirk-Spriggs & Sinclair, 2021
Cypselosomatidae	Rhinopomyzella sp1	Saprophage	Marshall, 2012

Appendix 3: Larval feeding habit data for all ingroup taxa.

Family	Binomial name	Larval feeding habit	Source
Micropezidae	<i>Micropeza</i> sp1	Saprophage	Marshall, 2012
Micropezidae	<i>Taeniptera trivittata</i>	Saprophage	Marshall, 2012
Micropezidae	<i>Compsobata cibaria</i>	Saprophage	Marshall, 2012
Neridiidae	<i>Telostylinus</i> sp1	Saprophage	Kirk-Spriggs & Sinclair, 2021
Acartophthalmidae	<i>Acartophthalmus nigrinus</i>	Saprophage	Andrade et al., 2015
Agromyzidae	<i>Melanagromyza minimoides</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Phytomyza ilicicola</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Liriomyza trifolii</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Liriomyza huidobrensis</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Phytomyza glabricola</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Phytomyza syngenesiae</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Cerodontha dorsalis</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Phytomyza aconiti</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Phytomyza aquilegivora</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Phytomyza erigerophila</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Phytomyza plantaginis</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Liriomyza chinensis</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Chromatomyia horticola</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Phytomyza spinaciae</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Agromyzidae	<i>Napomyza lateralis</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Anthomyzidae	<i>Mumetopia occipitalis</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Asteiidae	<i>Leiomysza curvinervis</i>	Mycophagous	Brown et al., 2010
Aulacastridae	<i>Aulacaster</i> sp1	Unknown	Kirk-Spriggs & Sinclair, 2021
Clusiidae	<i>Clusia lateralis</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Clusiidae	<i>Clusiodes ruficollis</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Fergusoninidae	<i>Fergusonina turneri</i>	Phytophage	Scheffer et al., 2017
Marginidae	<i>Margo</i> sp1	Unknown	Kirk-Spriggs & Sinclair, 2021
Megamerinidae	<i>Megamerina</i> sp1	Predator	Rohacek, 2016
Nemidiidae	<i>Nemula longarista</i>	Unknown	Kirk-Spriggs & Sinclair, 2021
Neurochaetidae	<i>Neurochaeta</i> sp1	Unknown	Kirk-Spriggs & Sinclair, 2021
Odiniidae	<i>Odinia</i> sp1	Unknown	Kirk-Spriggs & Sinclair, 2021
Opomyzidae	<i>Opomyza florum</i>	Phytophage	Vickerman, 1982
Pallopteridae	<i>Toxonevra superba</i>	Unknown	
Periscelididae	<i>Cyamops nebulosus</i>	Unknown	Kirk-Spriggs & Sinclair, 2021
Teratomyzidae	<i>Teratomyza</i> sp1	Unknown	
Xenasteiidae	<i>Xenasteia shalam</i>	Unknown	Kirk-Spriggs & Sinclair, 2021
Coelopidae	<i>Coelopa</i> sp1	Saprophage	Brown et al., 2010
Dryomyzidae	<i>Dryomyza anilis</i>	Saprophage	Mathis & Sueyoshi, 2011
Helosciomyzidae	<i>Neosciomyza luteipennis</i>	Unknown	
Heterocheilidae	<i>Heterocheila buccata</i>	Saprophage	Mathis, 2011
Natalimyzidae	<i>Natalimyza</i> sp1	Saprophage	Barraclough & McAlpine, 2006
Ropalomeridae	<i>Willistoniella pleuropunctata</i>	Unknown	Brown et al., 2010
Sciomyzidae	<i>Limnia</i> sp1	Unknown	Knutson & Vala, 2011
Sciomyzidae	<i>Pelidnoptera nigripennis</i>	Endoparasitoid	Kirk-Spriggs & Sinclair, 2021
Sciomyzidae	<i>Pherbellia annulipes</i>	Predator	Knutson & Vala, 2011
Sepsidae	<i>Themira nigricornis</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Sepsidae	<i>Sepsis cynipsea</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Chyromyidae	<i>Gymnochiromyia</i> sp1	Phytophage	Kirk-Spriggs & Sinclair, 2021
Heleomyzidae	<i>Epistomyia</i> sp1	Unknown	
Heleomyzidae	<i>Suillia variegata</i>	Saprophage	Rotheray, 2012
Heleomyzidae	<i>Heteromyza atricornis</i>	Saprophage	Rotheray, 2012
Sphaeroceridae	<i>Spelobia bifrons</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021; Marshall, 2012
Sphaeroceridae	<i>Lotophila atra</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021; Marshall, 2012
Sphaeroceridae	<i>Rachispoda</i> sp1	Saprophage	Kirk-Spriggs & Sinclair, 2021; Marshall, 2012
Sphaeroceridae	<i>Frutillaria edenensis</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021; Marshall, 2012
Sphaeroceridae	<i>Apteromyia</i> sp1	Saprophage	Kirk-Spriggs & Sinclair, 2021; Marshall, 2012
Sphaeroceridae	<i>Paraspheocera</i> sp1	Saprophage	Kirk-Spriggs & Sinclair, 2021; Marshall, 2012
Sphaeroceridae	<i>Archiborborus annulatus</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021; Marshall, 2012
Diopsidae	<i>Teleopsis dalmanni</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Psilidae	<i>Chyliza scrobiculata</i>	Phytophage	Brown et al., 2010
Somatiidae	<i>Somatia aestivalis</i>	Unknown	Brown et al., 2010
Syringogastridae	<i>Syringogaster</i> sp1	Unknown	Brown et al., 2010
Strongylophthalmyiidae	<i>Strongylophthalmyia angustipe</i>	Saprophage	Lonsdale, 2013
Tanypezidae	<i>Neotanypeza</i> sp1	Unknown	
Richardiidae	<i>Richardia teevani</i>	Saprophage	Brown et al., 2010
Lonchaeidae	<i>Lonchaea polita</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Lonchaeidae	<i>Silba adipata</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Lonchaeidae	<i>Dasiops inedulis</i>	Phytophage	Kirk-Spriggs & Sinclair, 2021
Piophilidae	<i>Piophila nigriceps</i>	Saprophage	Rochefer et al., 2015
Uliidae	<i>Melieria omissa</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Uliidae	<i>Tritoxa flexa</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Platystomatidae	<i>Lamprogaster nigripes</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Platystomatidae	<i>Rivellia syngenesiae</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Platystomatidae	<i>Rivellia alini</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Platystomatidae	<i>Platystoma seminationis</i>	Saprophage	Kirk-Spriggs & Sinclair, 2021
Platystomatidae	<i>Pterogenia</i> sp1	Saprophage	Kirk-Spriggs & Sinclair, 2021
Ctenostylidae	<i>Sinolochmostylia sinica</i>	Unknown	
Pyrgotidae	<i>Pyrgota undata</i>	Endoparasitoid	Kirk-Spriggs & Sinclair, 2021
Pyrgotidae	<i>Eupyrgota tigrina</i>	Endoparasitoid	Kirk-Spriggs & Sinclair, 2021
Tephritisidae	<i>Ceratitis capitata</i>	Phytophage	Brown et al., 2010
Tephritisidae	<i>Bactrocera dorsalis</i>	Phytophage	Brown et al., 2010
Tephritisidae	<i>Zeugodacus cucurbitae</i>	Phytophage	Brown et al., 2010
Tephritisidae	<i>Bactrocera correcta</i>	Phytophage	Brown et al., 2010

Appendix 3: Larval feeding habit data for all ingroup taxa.

Family	Binomial name	Larval feeding habit	Source
Tephritidae	Bactrocera tryoni	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Bactrocera carambolae	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Anastrepha obliqua	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Zeugodacus tau	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Bactrocera cacuminata	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Anastrepha ludens	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Anastrepha suspensa	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Rhagoletis pomonella	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Anastrepha fraterculus	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Bactrocera musae	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Bactrocera oleae	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Bactrocera occipitalis	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Rhagoletis cingulata	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Zeugodacus cucumis	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Bactrocera frauenfeldi	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Bactrocera umbrosa	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Campiglossa pygmaea	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Zeugodacus caudatus	Phytophage	Brown <i>et al.</i> , 2010
Tephritidae	Tachinisa cyaneiventris	Endoparasitoid	Brown <i>et al.</i> , 2010
Tephritidae	Anastrepha curvicauda	Phytophage	Brown <i>et al.</i> , 2010
Glossinidae	Glossina morsitans	Non-feeding	Marshall, 2012
Hippoboscidae	Orthoffersia minuta	Non-feeding	Marshall, 2012
Hippoboscidae	Ornithomya avicularia	Non-feeding	Marshall, 2012
Hippoboscidae	Steneapteryx hirundinis	Non-feeding	Marshall, 2012
Hippoboscidae	Lipoptena cervi	Non-feeding	Marshall, 2012
Nycteribiidae	Penicillidia fulvida	Non-feeding	Marshall, 2012
Strebidae	Trichobius longipes	Non-feeding	Marshall, 2012
Fanniidae	Fannia canicularis	Saprophage	Marshall, 2012
Fanniidae	Fannia manicata	Saprophage	Marshall, 2012
Muscidae	Musca domestica	Saprophage, Parasite	Brown <i>et al.</i> , 2010
Muscidae	Stormoxys calcitrans	Saprophage	Brown <i>et al.</i> , 2010
Muscidae	Helina evecta	Predator	Brown <i>et al.</i> , 2010
Muscidae	Atherigona orientalis	Saprophage	Brown <i>et al.</i> , 2010
Muscidae	Muscina stabulans	Saprophage, Parasite, Predator	Brown <i>et al.</i> , 2010
Muscidae	Musca autumnalis	Saprophage	Brown <i>et al.</i> , 2010
Muscidae	Hydrotaea aenescens	Saprophage	Brown <i>et al.</i> , 2010
Muscidae	Drymeia alpicola	Predator	Brown <i>et al.</i> , 2010
Muscidae	Huckettomyia watanabei	Unknown	
Muscidae	Dichaetomyia bibax	Unknown	
Muscidae	Polietes lardarius	Saprophage, Predator	Skidmore, 1985
Muscidae	Thricops cunctans	Unknown	
Muscidae	Synthesiomyia nudiseta	Saprophage	Ivorra <i>et al.</i> , 2021
Muscidae	Limnophora exuta	Predator	Brown <i>et al.</i> , 2010
Muscidae	Lispe tentaculata	Predator	Brown <i>et al.</i> , 2010
Muscidae	Haematobosca stimulans	Saprophage	Skidmore, 1985
Muscidae	Mydaea ancilla	Saprophage, Predator	Brown <i>et al.</i> , 2010
Muscidae	Potamia littoralis	Predator	Skidmore, 1985
Muscidae	Cyrtoneuropis veniseta	Unknown	
Muscidae	Achanthiptera rohrelliformis	Predator	Skidmore, 1985
Muscidae	Helina lasiopthalma	Predator	Brown <i>et al.</i> , 2010
Muscidae	Hydrotaea cyrtoneurina	Saprophage	Brown <i>et al.</i> , 2010
Muscidae	Hydrotaea dentipes	Saprophage, Predator	Brown <i>et al.</i> , 2010
Muscidae	Limnophora maculosa	Predator	Brown <i>et al.</i> , 2010
Muscidae	Lispe sericipalpis	Predator	Brown <i>et al.</i> , 2010
Anthomyiidae	Delia radicum	Phytophage	Kirk-Spriggs & Sinclair, 2021; Brown <i>et al.</i> , 2010
Anthomyiidae	Lasiomma seminitidum	Saprophage	Kirk-Spriggs & Sinclair, 2021; Brown <i>et al.</i> , 2010
Anthomyiidae	Lasiomma latipenne	Saprophage	Kirk-Spriggs & Sinclair, 2021; Brown <i>et al.</i> , 2010
Anthomyiidae	Emmesomyia grisea	Saprophage	Kirk-Spriggs & Sinclair, 2021; Brown <i>et al.</i> , 2010
Anthomyiidae	Delia platura	Phytophage	Kirk-Spriggs & Sinclair, 2021; Brown <i>et al.</i> , 2010
Anthomyiidae	Botanophila fugax	Saprophage	Kirk-Spriggs & Sinclair, 2021
Anthomyiidae	Hydrophoria lancifer	Saprophage	Kirk-Spriggs & Sinclair, 2021
Anthomyiidae	Hylemya variata	Unknown	Kirk-Spriggs & Sinclair, 2021
Anthomyiidae	Paregle coerulescens	Unknown	Kirk-Spriggs & Sinclair, 2021
Anthomyiidae	Pegoplata infirma	Saprophage	Kirk-Spriggs & Sinclair, 2021; Brown <i>et al.</i> , 2010
Scathophagidae	Scathophaga stercoraria	Saprophage	Blanckenhorn <i>et al.</i> , 2010
Scathophagidae	Cordilura atrata	Phytophage	James, 1955
Calliphoridae	Eurychaeta palpalis	Saprophage	Brown <i>et al.</i> , 2010; Nasser <i>et al.</i> , 2021
Calliphoridae	Chrysomya megacephala	Saprophage, Predator, Parasite	Brown <i>et al.</i> , 2010; Nasser <i>et al.</i> , 2021
Calliphoridae	Lucilia sericata	Saprophage, Parasite	Brown <i>et al.</i> , 2010; Nasser <i>et al.</i> , 2021
Calliphoridae	Calliphora vomitoria	Saprophage, Parasite	Brown <i>et al.</i> , 2010; Nasser <i>et al.</i> , 2021
Calliphoridae	Cochliomyia macellaria	Saprophage, Parasite	Brown <i>et al.</i> , 2010; Nasser <i>et al.</i> , 2021
Calliphoridae	Calliphora vicina	Saprophage, Parasite	Brown <i>et al.</i> , 2010; Nasser <i>et al.</i> , 2021
Calliphoridae	Lucilia caesar	Saprophage, Parasite	Brown <i>et al.</i> , 2010; Nasser <i>et al.</i> , 2021
Calliphoridae	Phormia regina	Saprophage, Parasite	Brown <i>et al.</i> , 2010; Nasser <i>et al.</i> , 2021
Mesembrinellidae	Mesembrinella bellardiana	Saprophage, Parasite	Nasser <i>et al.</i> , 2021
Mystacimobiidae	Mystacimobia zelandica	Saprophage	Gleeson <i>et al.</i> , 2000
Oestridae	Cuterebra austeni	Parasite	Marshall, 2012
Polleniidae	Pollenia rufis	Predator	Nasser <i>et al.</i> , 2021
Rhiniiidae	Stomorrhina lunata	Predator	Marshall, 2012
Rhiniiidae	Rhyncomya nigripes	Unknown	
Rhinochoridae	Stevenia hertingi	Endoparasitoid	Marshall, 2012
Sarcophagidae	Wohlfahrtia trina	Unknown	
Sarcophagidae	Sarcophaga peregrina	Saprophage	Brown <i>et al.</i> , 2010

Appendix 3: Larval feeding habit data for all ingroup taxa.

Family	Binomial name	Larval feeding habit	Source
Sarcophagidae	<i>Sarcophaga crassipalpis</i>	Saprophage	Brown <i>et al.</i> , 2010
Sarcophagidae	<i>Sarcophaga dux</i>	Saprophage, Parasite	Sukontason <i>et al.</i> , 2014
Sarcophagidae	<i>Helicobia rapax</i>	Unknown	
Sarcophagidae	<i>Blaesoxiphia plinthopyga</i>	Saprophage, Parasite	Wells & Smith, 2013
Sarcophagidae	<i>Sphenometopa claripennis</i>	Unknown	
Sarcophagidae	<i>Taxigramma multipunctata</i>	Unknown	
Sarcophagidae	<i>Nyctia lugubris</i>	Unknown	
Sarcophagidae	<i>Titanogrypa luculenta</i>	Unknown	
Sarcophagidae	<i>Sarcophaga omikron</i>	Saprophage	Brown <i>et al.</i> , 2010
Sarcophagidae	<i>Ravinia querula</i>	Saprophage	Pape, 1996
Sarcophagidae	<i>Peckia intermutans</i>	Saprophage	Brown <i>et al.</i> , 2010
Sarcophagidae	<i>Boettcheria cimbicis</i>	Unknown	
Sarcophagidae	<i>Villegrasia postuncinata</i>	Saprophage	Brown <i>et al.</i> , 2010
Tachinidae	<i>Triarthria setipennis</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Epalpus signifer</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Gymnosoma nitens</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Pseudogondonia rufifrons</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Mintho rufiventris</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Exorista larvarum</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Phania funesta</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Tachina grossa</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Gymnocheta viridis</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Ceracia dentata</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Hyphantrophaga virilis</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Lespesia aletiae</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Voria ruralis</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Tachina magnicornis</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Peleteria rubescens</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Tachinomyia nigricans</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Cylindromya binotata</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Panzeria ampelus</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Ptilodexia conjuncta</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Siphona plusiae</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Trichopoda pennipes</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Winthemia sinuata</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Campyocheata semiothisae</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Epigrimyia illinoensis</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Thelaira americana</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Archytas nivalis</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Nowickia ferox</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Gymnosoma nudifrons</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Dinera griseascens</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Blondelia hyphantriae</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Dufouria chalybeata</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Strongygaster triangulifera</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Aplomya theclarum</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Eucelatoria armigera</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Exorista sorbillans</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Belvosia sp1</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Lixophaga latigena</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Germaria ruficeps</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Cyrtophleba nitida</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Siphona flavifrons</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Euthera tentatrix</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Phasia sp1</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Uramya sp1</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Metadrinomyia flavifrons</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Tachina nupta</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Tachina corsicana</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Tachina fera</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Tachina lurida</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Tachina nigrohirta</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Tachinidae	<i>Tachina ursina</i>	Endoparasitoid	Brown <i>et al.</i> , 2010
Ulurumiidae	<i>Ulurumya macalpinei</i>	Saprophage	Michelson & Pape, 2017