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The phylogeny and evolutionary ecology of hoverflies (Diptera: Syrphidae) inferred from mitochondrial genomes

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ABSTRACT

Hoverflies (Diptera: Syrphidae) are a diverse group of pollinators and a major research focus in ecology, but their phylogenetic relationships remain incompletely known. Using a genome skimming approach we generated mitochondrial genomes for 91 species, capturing a wide taxonomic diversity of the family. To reduce the required amount of input DNA and overall cost of the library construction, sequencing and assembly was conducted on mixtures of specimens, which raises the problem of chimera formation of mitogenomes. We present a novel chimera detection test based on gene tree incongruence, but identified only a single mitogenome of chimeric origin. Together with existing data for a final set of 127 taxa, phylogenetic analysis on nucleotide and amino acid sequences using Maximum Likelihood and Bayesian Inference revealed a basal split of Microdontinae from all other syrphids. The remainder consists of several deep clades assigned to the subfamily Eristalinae in the current classification, including a clade comprising the subfamily Syrphinae (plus Pipizinae). These findings call for a re-definition of subfamilies, but basal nodes had insufficient support to fully justify such action. Molecular-clock dating placed the origin of the Syrphidae crown group in the mid-Cretaceous while the Eristalinae-Syrphinae clade likely originated near the K/Pg boundary. Transformation of larval life history characters on the tree suggests that Syrphidae initially had sap feeding larvae, which diversified greatly in diet and habitat association during the Eocene and Oligocene, coinciding with the diversification of angiosperms and the evolution of various insect groups used as larval host, prey, or mimicry models. Mitogenomes proved to be a powerful phylogenetic marker for studies of Syrphidae at subfamily and tribe levels, allowing dense taxon sampling that provided insight into the great ecological diversity and rapid evolution of larval life history traits of the hoverflies.

1. Introduction

Hoverflies (Diptera: Syrphidae) are among the most species-rich families of Diptera, comprising over 6,300 species in more than 200 genera (Brown, 2009; Skevington et al., 2019). Well recognized for their crucial role in pollination (Larson et al., 2001; Forup et al., 2008; Inouye et al., 2015; Wotton et al., 2019; Doyle et al., 2020), they are also known as flower flies and, especially in recent years, have been a major research interest in many fields, for example, in taxonomy (Gilasian et al., 2020; Radenković et al., 2020; Jordaens et al., 2021; Moran & Skevington, 2019, 2021; Vujić et al., 2021), phylogenetics (Young et al., 2016; Pauli et al., 2018; Moran et al., 2022; Mullens et al., 2022, Mengual et al.,

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2023; Guo et al., 2023; Li et al., 2023), behaviour (Goulard et al., 2015; Moore & Hassall, 2016; Emtia & Ohno, 2017; Gao et al., 2020), aerodynamics (Geurten et al., 2010; Tian et al., 2019; Verbe et al., 2020), landscape ecology (Meyer et al., 2009; Power et al., 2016; Medeiros et al., 2019; Walcher et al., 2020), forensic science and medicine (Magni et al., 2013; Heo et al., 2020; Pérez-Bañón et al., 2020) and conservation (Rotheray et al., 2012, 2014; Vujić et al., 2016; Radenković et al., 2017; Miličić et al., 2018). Hoverflies are ecologically very diverse; adults mainly act as specialized or generalized pollinators of different flowering plants, forming intricate pollination networks of various complexity and specialism linked to distinct mouthpart structures (Klečka et al., 2018; Lucas et al., 2018a, b). Some species are involved in deceptive pollination mechanisms of orchids (Stökl et al., 2011; Jin et al., 2014; Jiang et al., 2020; Zheng et al., 2021), and many species visually and acoustically mimic well-protected hymenopteran species to avoid predators (Penney et al., 2012, 2014; Edmunds & Reader, 2014; Moore & Hassall, 2016; Taylor et al., 2017). While the adults generally are flower visiting and thus are important pollinators, the larvae have very different and varied lifestyles. Some species are closely associated with social insects like ants (Schönrogge et al., 2008; Bonelli et al., 2011; Reemer, 2013; Schmid et al., 2014; Scarparo et al., 2019), whilst others have aquatic filter-feeding larvae (Rotheray & Gilbert, 2011; Campoy et al., 2017). Larvae adopt a variety of feeding styles, such as saprophagy, mycophagy, phytophagy and zoophagy (Rotheray, 1993; Thompson & Rotheray, 1998; Rotheray & Gilbert, 2011), and the latter are viewed as effective biological control of pests like aphids (Rojo et al., 2003; Nelson et al., 2012; Pekas et al., 2016; Arcaya et al., 2017; Ramsden et al., 2017; Bellefeuille et al., 2021).

Substantial progress has been made in various areas with the help of DNA sequence data, including taxonomic classification and phylogenetic relationships (Mengual et al., 2015, 2018, 2021, 2023; Young et al., 2016; Pauli et al., 2018; Mengual, 2020; Moran et al., 2022; Mullens et al., 2022; Guo et al., 2023; Li et al., 2023; among others), population structure and phylogeography (Ståhls et al., 2016; Gojkovic et al., 2019; Liu et al., 2019), and floral resource partitioning (Klečka et al., 2018; Lucas et al., 2018a, b). Mitochondrial genomes continue to hold a special role in phylogenetics of insects (Cameron, 2014) and allow for the inference of well supported taxon-rich phylogenetic trees (Chesters, 2017). Mitogenomes can be generated by genome skimming, i.e. the low-depth shotgun sequencing and subsequent genome sequence assembly, which retrieves contigs from the high-copy portion of the sequenced DNA, including mitochondrial genomes. To reduce costs and to overcome the problem of low available DNA amounts, skimming can be conducted on specimen mixtures of up to ~ 100 individuals, following the methodology of Gillett et al. (2014). However, this procedure bears the risk of producing chimeric assemblies from multiple specimens in the mixture, which contort the phylogenetic analysis but may be detectable based on the difference in phylogenetic positions of a taxon in individual gene trees (see below). Once available in high taxon density, mitogenomes are easily linked to 'barcodes' from short sequence fragments obtained by PCR usually based on the cytochrome *c* oxidase subunit 1 (COX1) gene that offers a cost-effective, rapid and fairly accurate means for taxon identification when reference sequences are provided (Piper et al., 2019; Kirse et al., 2021), including those from mixed samples via metabarcoding (Arribas et al., 2016; Marquina et al., 2019; Piper et al., 2019). Yet, for the Syrphidae only 102 complete or partial mitogenomes of limited taxonomic scope are available on GenBank (NCBI, February 2022).

The lack of molecular information continues to hamper the inference of phylogenetic relationships within Syrphidae. The family is currently split into four subfamilies, namely Microdontinae, Eristalinae, Pipizinae and Syrphinae (Mengual et al., 2015). Recent studies on both molecular and morphological data indicate that Microdontinae is monophyletic and sister to the rest of Syrphidae, while Eristalinae has been consistently recovered as non-monophyletic, and Pipizinae is placed as sister to Syrphinae, which together are embedded within Eristalinae (Mengual et al., 2015, 2021, 2023; Young et al., 2016; Pauli et al., 2018; Moran et al., 2022; Mullens et al., 2022; Guo et al., 2023). There are currently 13 tribes of Syrphidae defined by adult morphological traits and larval life histories, being Microdontini and Spheginobacchini in Microdontinae; Brachyopini, Callicerini, Cerioidini, Eristalini, Merodontini, Milesiini, Rhingiini, and Volucellini in Eristalinae; and Bacchini, Melanostomini and Syrphini in Syrphinae. Yet many tribes may not be monophyletic or have undetermined relationships with each other, leaving the hoverfly tribal classification in a state of flux (Mengual et al., 2015, 2023; Young et al., 2016; Mengual, 2020; Moran et al., 2022).

This study builds a reference set of mitochondrial genomes for the Syrphidae and attempts to infer phylogenetic relationships covering most major taxonomic groups. The resulting phylogenetic tree is used for molecular-clock dating. Moreover, inferred phylogenetic relationships along with supplementary COX1 barcodes for species of known ecology were used to map larval life history characters to examine trends of diversification and specialization in the evolution of Syrphidae.

2. Methods

2.1. Taxon choice and DNA sequencing

Specimens were selected from the collections of the Canadian National Collection (CNC), chosen to represent the spread of tribes and subfamilies recognised in previous studies. Several species from closely related families were chosen as outgroups. Specimens were identified by coauthors JHS, KMM and KJ and associated metadata were compiled (Table S1). DNA was extracted at the CNC, followed by DNA sequencing of mitochondrial genomes by genome skimming on seven specimen pools composed of ~ 50 DNA extractions of equimolar concentrations, for a total of 200 ng of DNA per pool, prior to library construction using a TruSeq Nano kit and shotgun sequencing on an Illumina HiSeq platform (2x250 PE). Species selection for pooling aimed at maximizing phylogenetic diversity in each library, to reduce the chance of chimera formation in the assembly steps. Post-sequencing quality analysis for each library was carried out using FastQC (Babraham Bioinformatics, 2015), and remaining Illumina adapters were removed using Trimmomatic (Bolger et al., 2014). Prior to assembly, the dataset was filtered for putative mitochondrial reads against a database of dipteran mitochondrial genomes, using dc-megablast under low stringency conditions that minimized the loss of target reads. Reads from this step were extracted using FastqExtract3 and subjected to genome assembly using three different assemblers: Ray (Boisvert et al., 2010), SPAdes (Bankevich et al., 2012) and IDBA (Peng et al., 2010). Assemblies from each procedure were imported into Geneious Basic (Kearse et al., 2012) and *de novo* assembled to produce super-contigs from the primary assemblies, which generally produced more and longer contigs than any one assembler alone.

Gene predictions were obtained using the MITOS server (Bernt et al., 2013), based on existing annotations for a range of invertebrate mitochondrial genomes. The annotations were manually edited to obtain the correct start and (full or partial) stop codons, selecting among possible alternatives by minimizing the intergenic spaces and overlap of genes. For simplicity, once several full length contigs had been annotated in this way, these were used as a reference genome for the remaining unannotated genomes. Independently, the COX1 barcoding region was amplified and Sanger sequenced to obtain a reference barcode for each specimen, which was then used as bait to assign contigs from the mixture to particular input species. All contigs from the mixed libraries over 2 kb were subjected to Blast searches against the COX1 baits, and sequence similarity of greater than 98% was considered to be accurate for assignments of the mitogenomes. Some contigs could not be identified in this way because they lacked the COX1 region, but four additional identifications were obtained by unequivocal placement on a phylogenetic tree of identified and unidentified contigs. In total, out of 207 species attempted, 91 produced valid mitogenome sequences. The

success rate was relatively low compared to similar studies (Gillett et al., 2014), presumably due to the low available DNA amounts for some taxa and limited sequencing depth.

A test for chimera detection was conducted on the protein coding genes based on the assumption that in a non-chimeric contig a particular focal taxon is placed in the same position in each gene tree, while in a chimeric set it would be placed in different positions of the respective gene trees for the various portions of a contig. The discrepancy in the placement should be greatest between adjacent genes where the chimeric portions abut. The first step in this analysis was to calculate the distance in tree position for a reference set of well-established non-chimeras. Existing Genbank sequences that had not been assembled from mixtures are appropriate for this analysis, which in our dataset were represented by 27 complete sequences. We first generated phylogenetic trees for each of the 13 protein coding genes and calculated the distance in tree position between each pair of taxa. We were interested in the difference in position in the tree between adjacent genes, as indicators of chimeric sequences, and thus generated violin plots from the reference set to give the distance in tree position of a taxon between two genes adjacent in the linear mitogenome sequence, e.g. COX1 and COX2, then COX2 and ATP8, and so on, based on the node distance in the gene trees. In the next step each mitogenome sequence newly generated from genome skimming on DNA mixtures was scored for its distance in placement between two trees generated from adjacent genes. If these distances were low (i.e. within the ranges commonly observed for the non-chimeric reference set) for all gene tree comparisons, the contig was considered to be non-chimeric. In several cases the distances in tree positions jumped greatly (see Supplementary Material S1). Further investigation established that some of these cases were due to previously unrecognised frameshifts that produced erroneous placements, while only the sequence of *Hypselosyrphus* Hull appeared as a chimera detectable in this way (with the contig of *Stipomorpha* Hull) and thus the chimeric part was removed. (The sequence was also affected by an editing error in NAD3 which makes it different from COX3 and from NAD5, although the real jump due to chimera formation is probably between NAD3 and NAD5).

In addition to newly generated mitogenomes, three mitogenomes from aquatic larvae collected in Bangladesh (Rahman et al., 2022) and all complete or partial mitochondrial genomes of at least 2000 bp were downloaded from GenBank and annotated with MitoZ (Meng et al., 2019). Alignment was conducted with MAFFT v7.490 (Katoh & Standley, 2013) under default parameters. For protein coding genes, alignments were conducted at the amino acid level and back translated into nucleotides using an in-house script (Creedy, 2022). All genes were concatenated into a supermatrix. The nucleotide matrices were built based on all 37 mitochondrial genes, including 13 protein coding, two rRNA and 22 tRNA genes.

2.2. Phylogenetic analysis

Maximum likelihood (ML) trees were generated in IQ-TREE v1.6.12 (Nguyen et al., 2015), choosing the best models for the nucleotide and amino acid data according to BIC scores by ModelFinder (Kalyaanamoorthy et al., 2017). Tree searches were conducted under 1,000 ultrafast bootstrap approximations (Hoang et al., 2018) and different partitioning schemes (Chernomor et al., 2016). Partitioning was by genes, either each as a separate partition or encoded on the forward and reverse strands, and further by codon positions (for nucleotide analyses), accommodating the differences of the evolutionary process among the three codon positions (Bofkin & Goldman, 2007; Błażej et al., 2018). Trees constructed based on untranslated sequences involved all mitochondrial genes under the GTR + F + R5 model, while the amino acid based analyses only used the 13 translated protein coding genes under the WAG + F + R10 model. Relationships of the hoverfly subfamilies were tested against trees constrained according to published topologies (Mengual et al., 2015; Young et al., 2016; Mengual, 2020; Mengual et al., 2023; Moran et al., 2022; Guo et al., 2023) using approximately unbiased (AU) tests (Shimodaira, 2002). Outgroup sequences were chosen according to the Diptera tree of Wiegmann et al. (2011).

Bayesian phylogenetic analyses were conducted in PhyloBayes v4.1 (Lartillot et al., 2015) under the CAT model, running two Monte Carlo Markov chains (MCMC) with a cut-off of 0.1 for the maximum difference between the two runs. Bayesian analysis of nucleotide data was also used for dating the tree using BEAST v1.10.4 (Suchard et al., 2018) under an uncorrelated relaxed clock model. The data were partitioned by the 37 mitochondrial genes and further partitioning of the protein coding genes into three codon positions. Each gene alignment was assigned a best evolutionary model and respective prior values in jModelTest v2.1.10 (Darriba et al., 2012) based on AIC values. Four different models were chosen for sets of markers (Table S2). BEAST used a birth–death serially sampled speciation model under a lognormal uncorrelated relaxed clock, where each lineage can evolve and go extinct at a fixed rate. We set a root height for Syrphidae at no more than 180 mya based on the estimated age for Eremoneura by Wiegmann et al. (2011) and used internal nodes of outgroup taxa to calibrate the Syrphidae ingroup based on various sources (see Table S3), whereby the outgroup relationships were constrained following the topology of Wiegmann et al. (2011). BEAST was run for 50 million generations on the CIPRES Science Gateway v3.3 (Miller et al., 2010), where the chains were sampled every 5000 generations. The output log file was visualized in Tracer v1.7.1 (Rambaut et al., 2018). TreeAnnotator was used to create a maximum clade credibility tree with target node heights. Dating was also performed using least-squares dating (To et al., 2016). Starting with the best ML tree, evolutionary rates and relative divergence dates at nodes were calculated using LSD v2.1. The most recent common ancestor was assigned with a divergence time of –1 and the tips a time of 0 for the computation of the relative node divergence dates, whose confidence intervals were generated from 1,000 simulation trees, and the variances were calculated based on branch lengths produced in the first run of the analysis.

To investigate the evolution of larval life histories and larval habitat types, relevant trait data were added to the tree via the placement of COX1 barcodes of 116 syrphid species with known larval diets and habitats (Speight, 2020). Sequences were downloaded from GenBank and Barcode of Life Data System (BOLD) (Ratnasingham & Hebert, 2007) and mostly included European species, but also several non-European species exhibiting a range of larval life histories (Marín, 1969; Rotheray et al., 2000a; Nishida et al., 2002; Weng & Rotheray, 2008; Downes et al., 2017). The COX1 barcodes were aligned and concatenated with the mitogenome sequences to form a supermatrix used to generate a ML tree in IQ-TREE v1.6.12 with 1,000 ultrafast bootstrap replicates under the best partitioning scheme and backbone constrained for the mitochondrial genome-only tree, ensuring that the topology would not be affected by a large amount of missing data.

Life history traits of the hoverfly species and outgroup taxa were collated from scattered literature records (see details in Table S4). For those taxa in the mitogenome tree lacking relevant information, trait data of members of the same or closely related genera were used, or their life history was simply left as unknown (Table S4). The larval diets and habitats were examined on the preferred phylogenetic tree using models of discrete-character evolution (Lewis, 2001) implemented in the *phytool* package in R (Revell, 2012). Transition rates between character states were estimated under the equal rates, symmetric rates and all-rates-different models (Paradis et al., 2004), and the best evolutionary model was selected based on AIC values.

3. Results

3.1. Tree topologies under various partitioning schemes

A total of 127 complete or partial mitochondrial genomes were used to construct the phylogenetic tree of Syrphidae, including 111 ingroup

and 16 outgroup taxa. The dataset included 91 non-chimeric mitochondrial contigs generated *de novo*, three syrphid mitogenomes collected from Bangladesh (Rahman et al., 2022), and 28 hoverfly genomes and three others from related outgroup taxa (*Lonchoptera multiseta* Dong & Yang, *Pipunculus* sp. and *Platypeza* sp.; from Li et al., 2017) available from GenBank (see Table S1 for details). The newly generated mitogenomes comprised on average 12,284 bp, ranging from 2,649 to 17,574 bp (Table S1). Fifty-eight of the sequences were circular and considered complete, consisting of all 37 mitochondrial genes, and 83 species contained the complete set of 13 protein coding genes, 76 with both rRNA genes and 60 having all 22 tRNA genes (see Table S1). We did not observe any deviation from the presumed ancestral gene order in Diptera (Clary & Wolstenholme, 1985).

Tree topologies of the nucleotide and amino acid maximum likelihood trees built under different partitioning schemes were compared with existing studies (Table 1). All trees strongly supported Syrphidae to be monophyletic. The subfamily Syrphinae was monophyletic within a paraphyletic Eristalinae, either recovered as sister to *Milesia pendleburyi* Curran in the nucleotide trees (Figures S1, S2) or *Criorhina coquilletti* Williston in the amino acid trees (Figure S3). The monophyly of Microdontinae was supported in all trees, but its position as sister to all other Syrphidae was only obtained in the nucleotide trees (Table 1, Figures S1, S2), whereas the amino acid data placed Microdontinae as sister to *Alipumilio avispas* Vockeroth, i.e. within Eristalinae (Figure S3). The position of Pipizinae, represented only by *Pipizella viduata* (Linnaeus), based on a truncated mitogenome of fewer than 3,000 bp, fluctuated widely in different partitioning schemes but was never found to be sister to Syrphinae, contrary to expectations (see Table 1).

Out of the 13 tribes of Syrphidae, only Spheginobacchini was missing from the analysis, while Callicerini was represented by a single mitogenome only. For the remaining 11 tribes, only Cerioidini, Eristalini, Melanostomini, Microdontini, Rhingiini and Volucellini were found to be monophyletic in all trees (Table 1, Figures S1–S3). Bacchini was polyphyletic in the amino acid tree, with *Baccha elongata* (Fabricius) embedded within Syrphini or sister to *Pipizella viduata* (Figure S3), while Brachyopini, Merodontini and Milesiini were polyphyletic in all trees. The genus *Toxomerus* Macquart representing the former tribe Toxomerini was embedded within Syrphini in both nucleotide and amino acid trees, in accordance with Mengual et al. (2023) (Table 1, Figures S1–S3). The 17 genera represented by multiple species were recovered as monophyletic, apart from *Parhelophilus* Girschner in the nucleotide trees not partitioned by codon (Fig. S2a, c, e), *Criorhina* Meigen in the amino acid trees (Figure S3), and *Orthoprosopa* Macquart and *Tropidia* Fallén in all trees (Table 1, Figures S1–S3).

Table 1
Monophyly of various hoverfly clades on the unconstrained trees constructed under different partitioning methods in IQ-TREE, compared with results of recent phylogenetic studies on Syrphidae. Bootstrap support values are given for monophyletic groups. The overall bootstrap values of the trees and percentages of branches with bootstrap values being 100 and at least 90 are also shown. Only references for tribal monophyly are listed.

Partitioning method	**Nucleotide**								**Amino acid**			**References**
	Unpartitioned		**Forward + reverse**		**Gene**				**Unpartitioned**	**Forward + reverse**	**Gene**	
Partitioned by codon	No	Yes	No	Yes	No	Yes^	Yes#	Yes^ *	No	No	No	
Syrphidae monophyletic	100	100	100	100	100	100	100	100	99	99	100	
Syrphinae monophyletic within paraphyletic Eristalinae	99	99	96	97	96	97	96	100	97	98	98	1–5
Microdontinae sister to other Syrphidae	100	100	100	100	100	100	100	100	No	No	No	1, 2, 4, 5
Pipizinae sister to Syrphinae	No	No	No	No	No	No	No	No	No	No	No	1–7
Bacchini monophyletic	98	95	94	93	95	88	76	92	No	No	No	3–5, 7
Brachyopini polyphyletic	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	1, 3
Cerioidini monophyletic	100	100	100	100	100	100	100	100	100	100	100	3
Eristalini monophyletic	100	100	100	100	100	100	100	100	100	100	100	1, 3, 5
Merodontini monophyletic	No	No	No	No	No	No	No	No	No	No	No	1
Milesiini monophyletic	No	No	No	No	No	No	No	No	No	No	No	1, 2
Melanostomini monophyletic	100	100	100	100	100	100	100	100	100	100	100	3–5, 7
Rhingiini monophyletic	100	100	100	100	100	100	100	99	94	98	98	1–3, 5
Syrphini monophyletic	100	100	100	100	100	100	100	100	No	No	38	4
Volucellini monophyletic	100	100	100	100	100	100	100	100	100	100	100	1–3, 5
Brachypalpus monophyletic	100	100	100	100	100	100	100	100	100	100	100	
Criorhina monophyletic	97	94	98	98	98	98	95	97	No	No	No	
Eristalinus monophyletic	100	100	100	100	100	100	100	100	100	100	100	
Eristalis monophyletic	100	100	100	100	100	100	100	100	99	100	97	
Helophilus monophyletic	100	100	100	100	100	100	100	100	100	100	100	
Melanostoma monophyletic	100	100	100	100	100	100	100	100	100	100	100	
Orthonevra monophyletic	100	100	100	100	100	100	100	100	100	100	100	
Orthoprosopa monophyletic	No	No	No	No	No	No	No	No	No	No	No	
Parhelophilus monophyletic	No	99	No	65	No	100	99	100	100	100	99	
Platycheirus monophyletic	100	100	100	100	100	100	100	100	100	100	100	
Psilota monophyletic	100	100	100	100	100	100	100	100	100	100	100	
Serichlamys monophyletic	100	100	100	100	100	100	100	100	100	100	100	
Syrphus monophyletic	100	100	100	100	100	100	100	100	100	100	100	
Tropidia monophyletic	No	No	No	No	No	No	No	No	No	No	No	
Victoriana monophyletic	100	100	100	100	100	100	100	100	100	100	100	
Xylota monophyletic	100	100	100	100	100	100	100	100	100	100	100	
Average bootstrap	94.6	91.5	94.2	91.9	93.1	93.8	95.3	94.3	88.5	90.5	88.9	
Bootstrap = 100 (%)	53.6	51.2	54.4	54.4	60.0	53.6	53.6	54.4	39.2	41.6	40.8	
Bootstrap ≥ 90 (%)	84.8	78.4	79.2	79.2	77.6	80.0	82.4	80.0	72.8	71.2	74.4	

References and numbering used: 1: Mengual et al., 2015; 2: Young et al., 2016; 3: Moran et al., 2022; 4: Mengual et al., 2023; 5: Guo et al., 2023; 6: Pauli et al., 2018; 7: Mengual, 2020.

^ The three codon positions are treated as separate partitions.

#The first two codon positions are grouped together and partitioned against the third codon position.

*Only for protein coding genes; all tRNA genes treated as one single partition.

Given minor differences in subfamilial and tribal relationships, for further discussion we constrained tree searches by enforcing Microdontinae as sister to all others and the monophyly of Pipizinae plus Syrphinae. AU tests against the unconstrained trees did not reject the constrained topology under any partitioning schemes and for both data types ($p > 0.05$) (Table 2). The nucleotide tree with constrained subfamily relationships, partitioned by 13 protein coding genes with separate codon positions, two rRNA genes and the tRNA genes combined, was selected as the best ML phylogeny (Figure S1, inset in Fig. 1), since it met most of the taxonomic assumptions (Table 1) and had the lowest BIC score amongst all constrained topologies (Table 2).

Considering the topology below the subfamily level, *Alipumilio avispas* (Merodontini) was recovered as sister to the rest of the non-Microdontinae hoverflies, with Cerioidini and Volucellini branching off next (nodes A, B, C in Fig. 1). The remainder of species (node G in Fig. 1) poorly matched the existing classification in Eristalinae, recovering the recognized tribes as broadly polyphyletic. Major lineages could be detected (nodes D, E, F in Fig. 1), corresponding to the (monophyletic) Eristalini and the widely polyphyletic Rhingiini, Brachyopodini and Milesini. In addition, the Syrphinae-Pipzinae clade formed one of the lineages within Eristalinae, but its sister relationships were unclear. *Milesia pendleburyi* was placed sister to the Syrphinae-Pipizinae clade (Figures 1, S1) or to Syrphinae only in the unconstrained trees (Figure S2). Within Syrphinae, Melanostomini was sister to the rest of the subfamily, and Bacchini was sister to Syrphini (nodes H, I, J in Fig. 1).

Bayesian analyses using PhyloBayes were generally poorly supported (posterior probability less than 0.5 for many branches; Figure S4) and showed poor convergence of the MCMC chains. Yet, the topology was generally similar to that obtained under ML, with the main difference that Microdontinae was largely unresolved (low PP) near the base of Eristalinae in slightly different positions under nucleotide and amino acid coding (Figures S1–S4), whilst the position of Pipizinae differed widely in either tree, with low support (Figs. S3, S4b).

3.2. Dating the phylogenetic tree of Syrphidae

The BEAST search under a lognormal relaxed clock model, conducted with fixed outgroup node ages (Table S3) and constrained outgroup and subfamily relationships (Fig. 1), produced a tree that showed only small differences from the ML analysis in the placements within tribes and the relationships near the base of Eristalinae (Figure S1). The BEAST analysis dated the stem and crown group of Syrphidae to 164–160 mya and 116–103 mya, respectively (Fig. 1). The Microdontinae split from the other lineages was dated to 99–86 mya, followed by the diversification of Eristalinae at around 72–58 mya, and the origin of Syrphidae and Pipizinae at 55–45 mya (Fig. 1). Results from BEAST were compared to least-squares dating on the maximum likelihood topology using LSD (Figure S5). If the LSD tree was rescaled based on the branch of *Blera eoa* (Stackelberg) using the extinct *B. miocenica* Hadrava et al. dated to 18–20 mya in the Early Miocene (Hadrava et al., 2019), this produced a younger stem age of the Syrphidae at 135 mya, a slightly greater crown group age (126–106 mya), earlier separations of Syrphidae and Pipizinae (67–53 mya) as well as Eristalinae lineages (103–79 mya). The diversification date of Eristalinae was slightly later (81–64 mya) when omitting *A. avispas* from Eristalinae, which has a different placement in the Bayesian trees, but still earlier than suggested by the BEAST analysis (Fig. 1).

3.3. Evolution of ecological traits

A new ML tree including the COX1 barcode taxa was generated under the full partitioning scheme and using the best mitogenome ML topology (Figure S1) as a backbone constraint. All barcodes were placed in the expected subfamilies (Fig. 2). The barcodes increased taxon coverage by the addition of several genera including *Paragus* Latreille (the former tribe Paragini recently recognized to be embedded within Syrphini; Mengual et al., 2023), and others such as *Dasysyrphus* Enderlein, *Rhingia* Scopoli, *Temnostoma* Le Peletier & Serville and *Xanthogramma* Schiner. More species were added in some genera, for instance, *Cheilosia* Meigen, *Eristalis* Latreille, *Eupeodes* Osten-Sacken and *Merodon* Meigen (Fig. 2).

Table 2
Likelihood (ln(*L*)) and BIC values of the nucleotide and amino acid phylogenetic trees constructed using the GTR + F + R5 and WAG + F + R10 models respectively, under different partitioning schemes and with and without subfamily relationships in Syrphidae constrained. The trees are ordered according to BIC values of the partitioning schemes for each data type. Note that the best BIC is achieved if all tRNA genes are treated as a single partition, despite the slightly higher ML compared to the full partitioning; likewise, the partitioning by genes beyond the partitioning by forward and reverse strand improves the ML but not the BIC.

Data type	Partitioning	Subfamilies constrained	No. of partitions	No. of free parameters	ln(*L*)	BIC	ΔBIC
Nucleotide	Gene[#] + codon*[^]	No	42	1384	–391800.16	797024.83	0
		Yes			–391853.24	797130.97	106.14
	Forward + reverse + codon*	No	6	352	–397431.71	798277.66	1252.8367
		Yes			–397456.32	798326.89	1302.0578
	Gene + codon[&]	Yes	74	1508	–393060.86	800749.00	3724.16
		No			–393079.79	800786.86	3762.02
	Gene + codon*	No	111	2137	–391159.29	803047.02	6022.19
		Yes			–391253.65	803235.74	6210.90
	Codon*	Yes	3	301	–404041.90	811003.44	13978.61
		No			–404137.20	811194.04	14169.21
	Forward + reverse	Yes	2	284	–407068.52	816891.78	19866.95
		No			–407072.14	816899.02	19874.18
	Gene	Yes	37	879	–404613.48	817753.07	20728.23
		No			–404650.97	817828.05	20803.22
	Unpartitioned	No	1	267	–412800.35	828190.54	31165.70
		Yes			–412814.85	828219.55	31194.71
Amino acid	Forward + reverse	No	2	326	–125401.53	253498.67	0
		Yes			–125423.19	253541.99	43.32
	Unpartitioned	Yes	1	288	–125743.08	253867.57	368.90
		No			–125758.32	253898.05	399.38
	Gene	No	13	744	–124931.29	256014.51	2515.84
		Yes			–124944.56	256041.05	2542.38

[^] Only for protein coding genes.
[#] All tRNA genes treated as one single partition.
[*] The three codon positions are treated as separate partitions.
[&] The first and second codon positions are grouped together and partitioned against the third codon position.

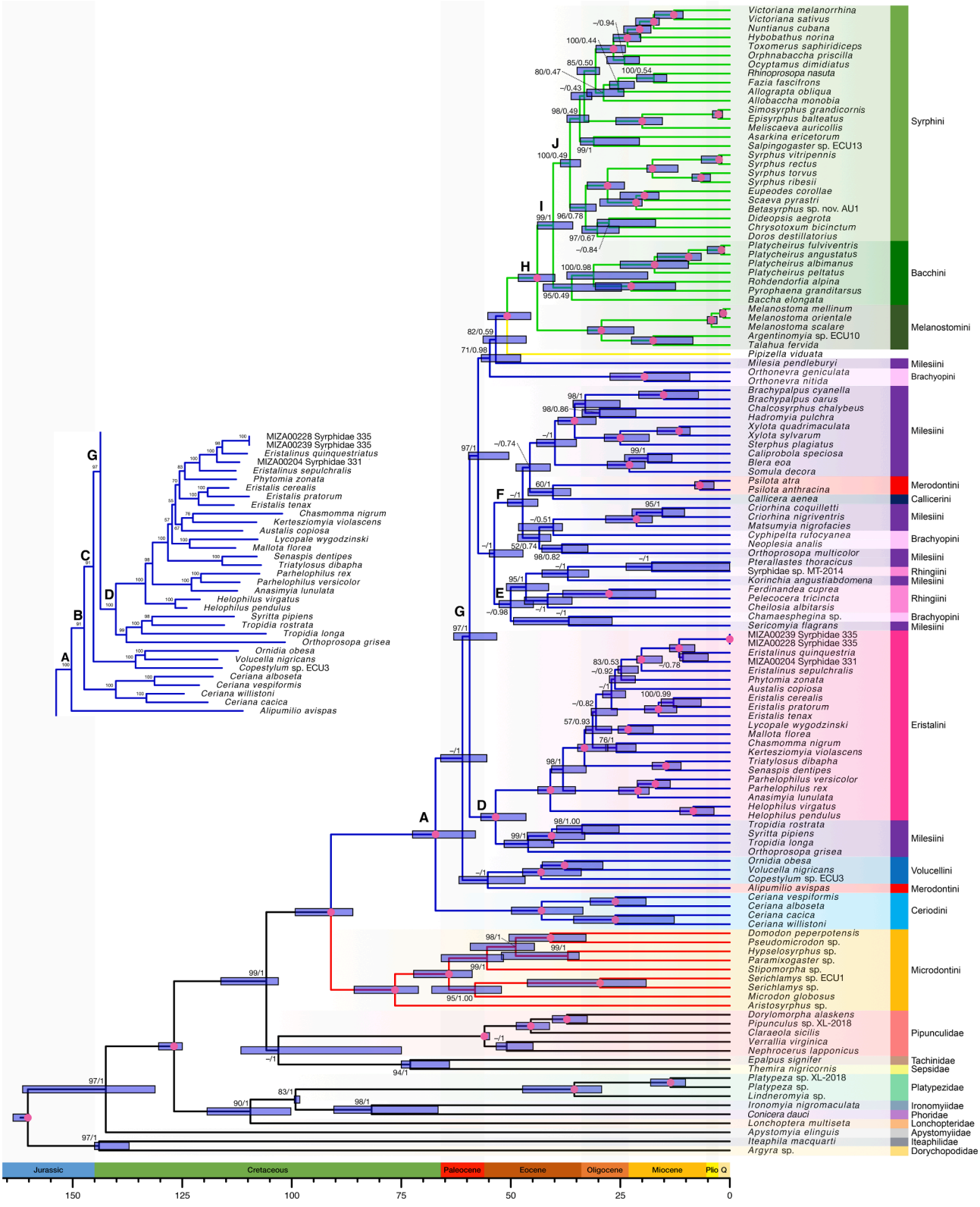

Fig. 1. The phylogeny of Syrphidae from Bayesian and ML analyses. The main topology shown is a clock constrained tree constructed with BEAST using nucleotide data, partitioned by 37 genes and the three codon positions for the protein coding genes. Each node is labelled with its respective bootstrap support value in the ML tree, followed by posterior probability values from the Bayesian inference, with pink dots marking the nodes fully supported by both methods and a dash representing the nodes missing due to a difference in tree topology. The inset shows a portion near the base of Eristalinae to illustrate key differences in the ML tree, showing recovery of nodes B and C not recovered in the BEAST tree. The scale axis shows the estimated evolutionary time (in million years) computed under a lognormal relaxed molecular clock. The four subfamilies are labelled by colours (red, Microdontinae; blue, Eristalinae; yellow, Pipizinae; green, Syrphinae), and the outgroup of closely related Diptera families is shown in black. The coloured bar at the right shows the extent of tribes of Syrphidae, as defined in the current taxonomy. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

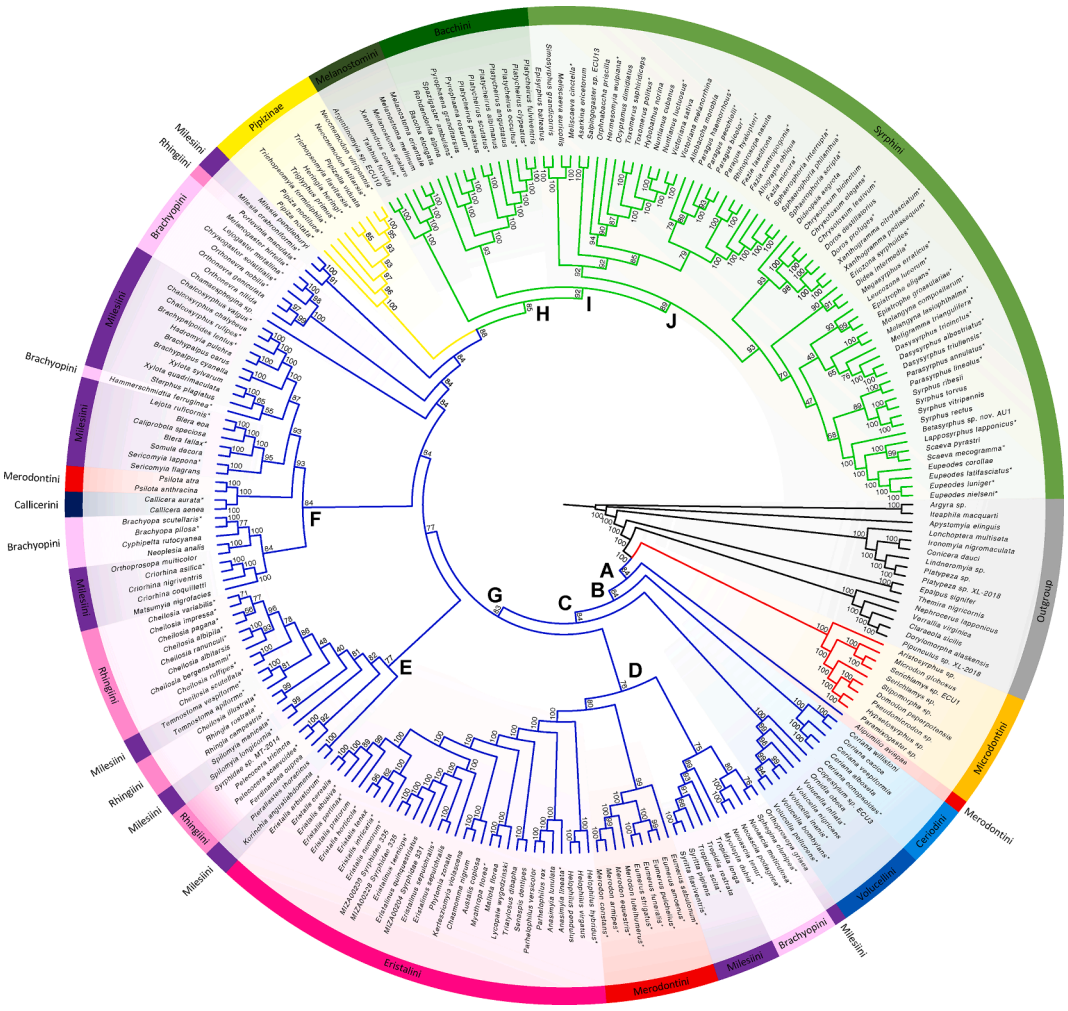

Fig. 2. Combined mitogenome and COX1 barcode tree. COX1 barcode sequences labelled with an asterisk (*) were aligned to the corresponding gene region and ML tree searches were conducted on the expanded matrix using the tree in Figure S3 as backbone constraint. Bootstrap values are shown at each node. Colour coding of subfamilies, tribes and outgroups as in Fig. 1.

Most of the newly added genera were monophyletic, except *Fazia* Shannon, *Cheilosia* and *Eumerus* Meigen which were paraphyletic for *Sphaerophoria* Le Peletier & Serville, *Temnostoma* and *Merodon*, respectively, and *Anasimyia* Schiner, *Blera* Billberg, *Pipiza* Fallén and *Trichopsomyia* Williston recovered as polyphyletic (Fig. 2). Moreover, with *Spilomyia* Meigen and *Temnostoma* of Milesiini located within Rhingiini and *Portevinia maculata* (Fallén) removed from it, Rhingiini was polyphyletic in the COX1 tree (Fig. 2).

Ecological data were collated for as many species as possible and used to define 14 different states for larval life history (one of them only in the outgroup) and 8 states for habitat type (6 states if all "aquatic/subaquatic" are combined), and character states were mapped on the COX1 barcode tree under equal rates and symmetric rates models, respectively. This produced the first ecological character reconstruction across all Syrphidae, and demonstrated the principal features of character variation that broadly divided the mainly detritivorous lifestyle of eristaline lineages and their dependence on aquatic habitats versus the mostly predaceous syrphine lineages confined to terrestrial habitats, but with great variation in the specific character state, while also showing convergence towards phytophagy and nest parasitism throughout the major subdivision.

The unresolved outgroup relationships, partly unknown lifestyle of the potential sister groups, and the divergent lifestyle (myrmecophily) in Microdontinae at the base of the tree hampered the inference of ancestral states in Syrphidae. However, the origin of the various eristaline lineages coincided with an upsurge of larval feeding styles mainly related to diets on decaying vegetation and other organic materials including dead and dying wood but also including various phytophagous and fungus feeding lineages (Fig. 3). The common ancestor of Pipizinae-Syrphinae was inferred as an aphid predator, which diversified in its prey type and also produced a few non-predatory lineages (Fig. 3). The evolution of syrphid larval life histories presented multiple incidents of convergent and parallel evolution. For example, phytophagy appeared in the common ancestor of *Eumerus* and *Merodon*, that of most Rhingiini species, *Portevinia maculata* (Speight, 2020), *Fazia centropogonis* (Nishida) (Nishida et al., 2002), *Fazia micrura* (Osten-Sacken) (Weng & Rotheray, 2008) and *Toxomerus politus* Say (Marín, 1969). Myrmecophilous lifestyles emerged in Microdontinae (Reemer, 2013) and *Trichopsomyia formiciphila* Downes, Skevington & Thompson (Downes et al., 2017), while *Brachyopa* Meigen and *Psilota* also have been reported to feed on sap, similar to the more early-branching Eristalinae species (Speight, 2020). The common ancestor of *Volucella* Geoffroy, excluding *V. inflata* Fabricius, developed insectivory, as also seen in Syrphinae and Pipizinae (Fig. 3). A few terminal lineages have acquired unique larval lifestyles, such as fungivory in *Cheilosia scutellata* (Fallén) and *C. ruffipes* (Preyssler) and parasitism in *Volucella inanis* (Linnaeus) (Speight, 2020) (Fig. 3).

Larval habitats likely were ancestrally aquatic, except for the ancestor of Microdontinae associated with ant nests (Reemer, 2013). The maggots of many Eristalinae lineages, for instance *Brachypalpus*

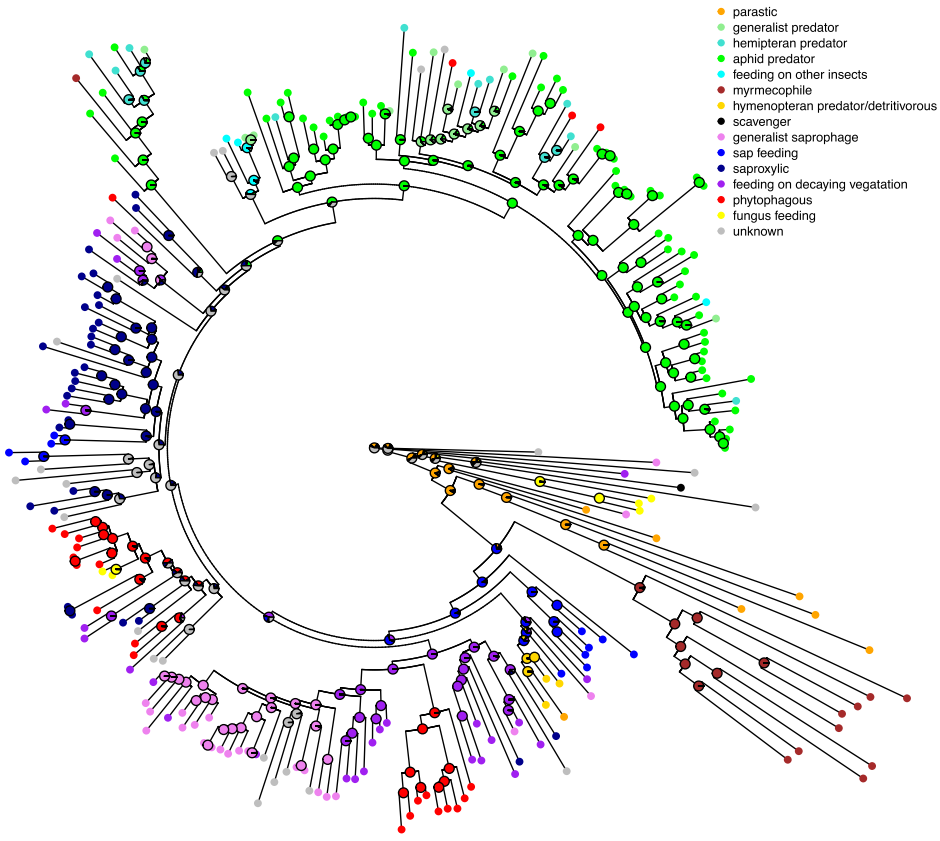

Fig. 3. Phylogenetic trees showing the evolution of larval life histories under an equal rates model, mapped on the tree topology of Fig. 2. The posterior probability for each trait over 1,000 simulations is presented at each node in the form of a pie chart.

Macquart, *Criorhina*, *Milesia* Latreille, *Xylota* Meigen and *Ferdinandea cuprea* (Scopoli), gained partial independence from aquatic habitats, while larvae of the common ancestor of Pipizinae and Syrphinae became fully terrestrial, as did the genus *Volucella* from within Eristalinae that live in nests of Hymenoptera (Fig. 4; Table S4). Likewise, the common ancestor of the non-*Pipiza* Pipizinae taxa likely was a predator living in aphid galls (Speight, 2020). Subterranean lifestyles appeared in several lineages, where *Cheilosia impressa* Loew feeds on underground plant parts, and *Xanthogramma*, *Chrysotoxum elegans* Loew, *Eupeodes latifasciatus* (Macquart) and *Pipizella viduata* live below ground to prey on root aphids (Speight, 2020). On the other hand, independent secondary returns to aquatic habitat were inferred for *Nuntianus luctuosus* (Bigot) and *Hermesomyia wulpiana* (Lynch Arribálzaga) (Rotheray et al., 2000a) (Fig. 4). Similar to the outgroup taxa with parasitoid larvae endogenous to insect hosts, some hoverfly lineages have larvae feeding inside plant or fungal tissues, including the lineage of *Eumerus* and *Merodon*, *Cheilosia*, *Portevinia maculata*, *Syritta flaviventris* Macquart (Speight, 2020) and *Fazia centropogonis* (Nishida et al., 2002) (Fig. 4).

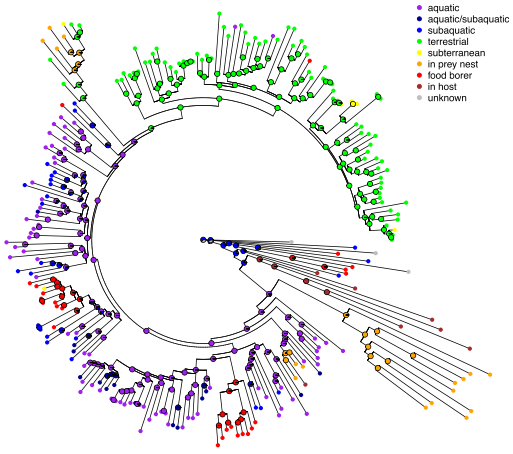

Fig. 4. Phylogenetic trees showing the evolution of larval habitats under a symmetric rates model, mapped on the tree topology of Fig. 2. The posterior probability for each trait over 1,000 simulations is presented at each node in the form of a pie chart.

4. Discussion

4.1. Tree topologies

Mitochondrial genomes produced well-supported phylogenetic trees of Syrphidae that extend the taxonomic range and gene representation of existing work (Skevington & Yeates, 2000; Ståhls et al., 2003; Mengual et al., 2015, 2023; Young et al., 2016; Pauli et al., 2018; Mengual, 2020; Leavey et al., 2021; Moran et al., 2022; Guo et al., 2023; Li et al., 2023). Comprising a total of 127 complete or partial mitochondrial genomes (Table S1), most of them newly sequenced here, and complemented by 116 publicly available COX1 barcodes, we analysed both the nucleotide data themselves and the translated amino acid sequences. The former also included the rRNA and tRNA genes that make up ~ 30% of the total data. Various partitioning schemes produced overall similar topologies (Table 1, Figures S1–S3), and tree likelihoods are not directly correlated with the number of partitions (Table 2). Over-parameterization was evident in some partitioning schemes, including those implementing separate partitions for each gene and, for the nucleotide sequences, further partitioning by codon positions, whose

higher likelihoods are not preferred based on the BIC (Table 2). Balancing various criteria, including the BIC, consistency with the Linnaean classification and recent phylogenetic studies, and average bootstrap values, we prefer a tree obtained under partitioning by gene and codon positions for protein coding genes, with the tRNA genes treated as a single partition (Figures 1 and S1). For amino acid data, partitioning had a less severe effect and for simplicity the unpartitioned model was chosen. For analyses of life histories and evolutionary time frames we constrained a monophyletic Microdontinae as sister to all other syrphids and the sister relationship of Syrphinae and Pipizinae, which were not obtained in all of the tree searches but never rejected in AU tests and in some cases even produced higher likelihoods than their unconstrained counterparts, presumably due to the more efficient search guided by the constraint (Table 2).

Maximum likelihood trees always recover Syrphidae as monophyletic and Pipunculidae as the immediate sister lineage of Syrphidae, consistent with the traditional superfamily Syrphoidea (Rotheray & Gilbert, 1999; Skevington & Yeates, 2000; Ståhls et al., 2003). However, this arrangement has been rejected by recent studies involving additional outgroups and using morphological traits together with mitochondrial and nuclear genes, which placed Pipunculidae as sister to Schizophora (a large clade that includes houseflies, tephritid fruit flies and drosophilids) (Wiegmann et al., 2011; Young et al., 2016; Pauli et al., 2018; Bayless et al., 2021; Mengual et al., 2023). Within Syrphidae, our analyses reveal similar subfamily relationships as in recent studies (Mengual et al., 2015, 2021, 2023; Young et al., 2016; Mengual, 2020; Moran et al., 2022; Mullens et al., 2022; Guo et al., 2023), where Microdontinae, here represented only by Microdontini, is sister to all other syrphids, while Syrphinae is monophyletic and embedded within the paraphyletic Eristalinae. The Pipizinae, represented only by a partial mitogenome of *Pipizella viduata*, has varied positions in different tree searches, but AU tests showed that the widely established sister relationship with Syrphinae is consistent with the mitogenome data also. Intertribal relationships within Eristalinae are similar for both ML and BEAST trees. The deepest splits in Eristalinae are defined by three early branches occupied by *Alipumilio avispas*, Cerioidini, and Volucellini (nodes A, B, C in Figs. 1, 2), with the latter being the sister to all remining Eristalinae in the ML trees (node G), and if *A. avispas* can indeed be considered a representative of Merodontini, as implied by the strong resemblance in larval morphology (Rotheray et al., 2000b), the basal topology is exactly as in Moran et al. (2022) using eight nuclear and mitochondrial makers. The mitogenomes also recover the monophyly of the tribes of Syrphinae and their relationships, with Melanostomini branching first, followed by Bacchini and Syrphini (nodes H, I, J in Figs. 1, 2). The formerly recognized tribes Toxomerini and Paragini represented only by DNA barcodes were embedded within the paraphyletic Syrphini. This arrangement matches the recent study using hybrid capture, which argued for the removal of their tribal status and formal recognition as members of Syrphini (Mengual et al., 2023).

This leaves an area of greater uncertainty in the middle portion of the tree (node G in Fig. 1) and the difficulty of resolving relationships among several clades that in the current classification are ascribed to Eristalinae. The problem is exacerbated by the polyphyly of tribes Brachyopini, Merodontini and Milesiini (Table 1, Fig. 1). The subtribes of Milesiini have been found to be widely separated and their arrangement here partly matches their relationships in previous studies (Mengual et al., 2015), e.g. the close relationship of the milesine subtribe Tropidiina with the large, monophyletic tribe Eristalini (node D in Fig. 1). However, the backbone of the tree remains weak, and thus the relationships among the major lineages relative to each other, including to the Syrphinae-Pipizinae clade, are insufficiently supported. As a working hypothesis we also define nodes E and F corresponding to two clades consistently found in our analyses that group different subtribes of Milesini predominantly with multiple lineages assigned to Rhingiini and Brachyopini, respectively (Figures 1, 2, S1, S2).

The COX1 barcodes further extended our taxon sampling, adding *Paragus* (the former Paragini) and several other genera (Fig. 2), which mostly are recovered as monophyletic, albeit in some cases only under a specific partitioning scheme (e.g. *Parhelophilus*) or evolutionary model (e.g. *Criorhina*). *Orthoprosopa* is consistently revealed as non-monophyletic, indicating potential misidentifications or mislabelling of input sequences and the need for taxonomic revisions (Moran et al., 2022). Some genera have already been considered non-monophyletic, such as *Fazia* (Mengual et al., 2021) and *Criorhina* (Moran et al., 2022), which was confirmed here.

4.2. Dating the tree and evolution of life histories

The BEAST analysis (Fig. 1) shows the early separation of Microdontinae at the beginning of the Upper Cretaceous (99–86 mya), greatly preceding the diversification of other lineages of the Syrphidae crown group. Given the notable distance in sequence divergence, morphology, larval life histories, and the lack of pollen feeding (Cheng & Thompson, 2008), the Microdontinae has sometimes been treated as a separate family (Thompson, 1969; Speight, 1987, 2020), consistent with the early separation and long independent evolution. The earliest diversification of the remaining crown group only began at the end of the Cretaceous (72–58 mya), and Pipizinae-Syrphinae emerged during the early Eocene (55–45 mya), consistent with findings of fossils of all four subfamilies only from the Eocene onwards. The node ages obtained here do not contradict the ages of syrphid fossils described in recent decades (Mengual et al., 2023; see Table 3). The pattern is generally supported by the least-squares dating, although the early-branching ingroup nodes are more closely packed (Figure S4), and when calibrated, the syrphid taxa would all have earlier emergence times ranging from the Lower Cretaceous to the late Paleocene. This may be explained by the use of rate smoothing in least-squares dating versus the model-based inferences in BEAST that are better able to estimate rates under variable clocks, especially the apparent rate increase in Microdontinae relative to the sister clade comprising the other three subfamilies. In addition, the outgroup taxa were only loosely sampled, to include species distantly related to the hoverflies, such as *Argyra* sp. (Dolichopodidae), *Iteaphila macquarti* Zetterstedt (Iteaphilidae) and *Apystomyia elinguis* Melander (Apystomyiidae) (Spasojevic et al., 2021), increasing the error from rate smoothing. Nonetheless, the dates of the BEAST tree are generally consistent with dating of the Syrphidae crown group to the Upper Cretaceous (Grimaldi, 2018) and the diversification of most modern groups in the Eocene-Oligocene (Ngô-Muller & Nel, 2020; Mengual et al., 2023).

Based on literature sources we defined 14 different character states for larval feeding styles and eight habitat types. Ancestral state reconstructions at the base of Syrphidae are not overly informative because of the disparate lifestyles of outgroups and the divergent, presumably apomorphic myrmecophily of the Microdontinae defining the earliest splits. Within the non-Microdontinae clade, aquatic and

Table 3
Fossilized hoverfly species from the same genus or tribe of the taxa depicted in the BEAST phylogenetic tree.

Species	Tribe/Subfamily	Age (mya)	References
Blera miocenica	Milesiini	~20–18	Hadrava et al., 2019
Cheilosia spheginascioides	Rhingiini	38.0–33.9	Röder, 1980
Eoxanthandrus garroustei	Bacchini–Melanostomini	Mid Eocene	Ngô-Muller & Nel, 2020
Helophilus nothobombus	Eristalini	18–16	Kotthoff & Schmid, 2005
Oligopipiza quadriguttata	Pipizinae	33.9–28.4	Nidergas et al., 2018
Prosyrphus thompsoni	Syrphidae (stem group)	99.41–98.17	Grimaldi, 2018
Tropidia tumulata	Milesiini	38.0–33.9	Lewis, 1973

subaquatic habitats represent the ancestral conditions and mainly coincide with various detritivorous and saprophagous feeding styles, from which herbivorous lineages have arisen that feed endogenously in plant tissues, as well as predatory lineages that are confined to terrestrial habitats (Fig. 3). The definition of character states may be subjective to some extent, in particular if taken from published work and if based on closely related species in a few cases, which limits the precision of the character state information. For example, aquatic maggots of many species actually filter and consume microorganisms living in decaying organic matter (Rotheray, 1993), but are recorded to have different larval diets just to separate their food sources. Similarly, some species with aphid specialist larvae may also feed on other insects as minor prey items or develop a myrmecophilous lifestyle (Rojo et al., 2003; Speight, 2020), which is being ignored here, but may be biologically relevant to indicate the opportunity for ecological transitions. We conclude that most larval ecological traits have evolved multiple times convergently or in parallel at various taxonomic levels, but despite the great variability, the feeding styles are broadly consistent with the major clades determined by mitochondrial genomes, including the sap feeding Volucellini, the Eristalini-Tropidiina clade (Fig. 1, node D) of mostly saprophages and plant detritivores, the saproxylic feeding style of the main group of Milesini (Fig. 1, node F), and the predatory lifestyle of the Pipizinae-Syrphinae clade (Figs. 3, 4).

The time estimates of lineages exhibiting larval life history traits would suggest that the earliest myrmecophilous node (85–71 mya) followed after the appearance of the earliest ants which are thought to have arisen in the Lower Cretaceous (Peters et al., 2017; Borowiec et al., 2019; Barden et al., 2020). The later diversification of all other lineages might be connected to the flower visiting behaviour of the adults; nectar bearing flowers have only become dominant in the Upper Cretaceous (Li et al., 2019; van der Kooi & Ollerton, 2020), and the origin of the Syrphidae-plant interaction as pollinators is estimated during the Palaeocene (60 mya) (Ollerton, 1999). Thus, the diversification of the main adult food source could be one of the factors explaining the diversification of non-microdontine hoverflies, albeit later than the origin of pollen collecting in bees (Anthophila) estimated to ~ 120 Mya (Peters et al., 2017), which also serve as mimicry models. The larval traits related to zoophagy also were reconstructed to arise late and clearly after the radiation of aphids, which itself has been linked to angiosperm diversification (von Dohlen & Moran, 2000). A rapid diversification of Aphidini aphids is estimated during the Eocene (Kim et al., 2011; Monnin et al., 2020), overlapping in time with the estimated diversification of the predatory Syrphinae. Thus, the emergence of Syrphidae is linked directly and indirectly to the diversification of angiosperm plants since the Cretaceous (Li et al., 2019; van der Kooi & Ollerton, 2020) and the insects on which syrphid larvae depend on either as parasites/commensals or predators. The diversification of Syrphidae apparently only accelerated after the end of the Cretaceous, akin to other angiosperm dependent insect lineages (Grimaldi & Engel, 2005; Donovan et al., 2016), possibly following the Cretaceous-Paleogene extinction event (Labandeira et al., 2002; Wilf et al., 2006) when bees received a catastrophic blow (Rehan et al., 2013). The evolutionary timeline of diversification therefore is dependent both on larval and adult features; although the ancestral larval state of detritivory/saprophagy in the Eristalinae-Pipizinae-Syrphinae clade is compatible with the biotic resources available in earlier geological times, their adult lifestyle is dependent on the presence of advanced angiosperm flowers, while the origin of the derived Syrphinae required the prior evolution of angiosperm-feeding insect prey also in the larval stages.

5. Conclusion

Our broad use of whole mitochondrial genomes for studying basal relationships in Syrphidae mostly supports recent studies of parts of the family, but here extends the taxon coverage to most major lineages, especially in the paraphyletic Eristalinae that constitute the greatest taxonomic challenge in the family. We show that delimitation of major clades and establishment of their relationships remains to be refined and will require additional taxon and gene sampling prior to the erection of new subfamilies for a more realistic evolutionary classification. Likewise, the dating of the tree requires further fossils for refining the evolutionary timeline, to confirm the proposed scenario of trait evolution in the context of the origin of aphids as the primary prey, the origin of social hymenopterans as larval hosts and Batesian mimicry models, and the coevolution with flower traits. Most recent studies of character traits focus on species in Europe (Penney et al., 2012; Ball & Morris, 2015; Klečka et al., 2018; Speight, 2020) and the Americas (Reemer, 2016; Skevington et al., 2019) but not in other parts of the world (Haffaressas et al., 2017). Given that many of these important pollinators are threatened with extinction due to habitat loss (Rotheray et al., 2012; Jauker et al., 2019) and climate change (Rotheray et al., 2014; Miličić et al., 2018; Vujić et al., 2022), a more complete, worldwide sampling will aid in studying patterns of evolutionary diversity globally and in setting conservation priorities. Mitochondrial genomes will continue to play a major role, as a marker for dense taxon sampling in phylogenetics, as an estimate of the phylogeny that is independent of the nuclear genome, and as a link to widely used (meta)barcode markers that need to be placed in a phylogenetic framework.

CRediT authorship contribution statement

Daniel Wong: Formal analysis, Writing – original draft, Conceptualization, Visualization, Data curation. **Hannah Norman:** Formal analysis, Writing – original draft, Conceptualization. **Thomas J. Creedy:** Formal analysis, Software, Data curation. **Kurt Jordaens:** Writing – review & editing. **Kevin M. Moran:** Writing – review & editing. **Andrew Young:** Resources. **Ximo Mengual:** Writing – review & editing, Resources. **Jeffrey H. Skevington:** Conceptualization, Writing – review & editing, Data curation, Resources, Supervision. **Alfried P. Vogler:** Conceptualization, Writing – original draft, Resources, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2023.107759.

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