Received: 20 March 2025 | Accepted: 28 October 2025

DOI: 10.1111/syen.70018

ORIGINAL ARTICLE

Systematic Entomology

Royal Entomological Society

Phylogenomics and the evolution of larval feeding habits in the blow flies (Diptera: Calliphoridae)

Cristian F. Beza-Beza[1,2] | John Soghigian[3] | Ezra Bailey[1] | Nikolas P. Johnston[4] | Brian K. Cassel[1] | Keith M. Bayless[5] | Jeffrey D. Wells[6] | David K. Yeates[5] | James F. Wallman[7] | Liping Yan[8] | Arianna Thomas-Cabianca[9] | Paul V. Hickner[10] | Andrzej Grzywacz[11] | Kelly A. Meiklejohn[12] | Tatiana T. Torres[13] | Maxwell J. Scott[1] | Aram Mikaelyan[1] | Dong Zhang[8] | Pierfilippo Cerretti[14] | Krzysztof Szpila[11] | Thomas Pape[15] | Brian M. Wiegmann[1]

[1]Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, North Carolina, USA

[2]Department of Entomology, University of Minnesota, St Paul, Minnesota, USA

[3]Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada

[4]Molecular Horizons, School of Science, University of Wollongong, Wollongong, New South Wales, Australia

[5]Australian National Insect Collection, CSIRO National Research Collections, Canberra, Australian Capital Territory, Australia

[6]Department of Biological Sciences, Florida International University, Miami, Florida, USA

[7]Faculty of Science, University of Technology Sydney, Sydney, New South Wales, Australia

[8]School of Ecology and Nature Conservation, Beijing Forestry University, Beijing, China

[9]Senckenberg Natural History Collections Dresden, Dresden, Germany

[10]USDA-ARS, Knipling-Bushland United States Livestock Insects Research Laboratory, Kerrville, Texas, USA

[11]Faculty of Biological and Veterinary Sciences, Department of Ecology and Biogeography, Nicolaus Copernicus University in Toruń, Toruń, Poland

[12]Department of Population Health and Pathobiology, North Carolina State University, Raleigh, North Carolina, USA

[13]Departamento de Genética e Biologia Evolutiva, Universidade de São Paulo, São Paulo, Brazil

[14]Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Rome, Italy

[15]Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

Correspondence
Cristian F. Beza-Beza and Brian M. Wiegmann, Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA.
Email: cbezabez@umn.edu, bwiegman@ncsu.edu

Funding information
National Biodiversity Future Center, Italian National Recovery and Resilience Plan (NRRP) Mission 4 Component 2 Investment 1.4, Grant/Award Numbers: CUP, B83C22002950007; National Science Centre, Poland, Grant/Award Number: 2018/31/B/

Abstract

Blow flies (Diptera: Calliphoridae) occur worldwide and exhibit a wide range of larval feeding habits, including saprophagy, coprophagy, parasitism and predation. Understanding their biology is critical for medical and veterinary science and ecology. Calliphorids thrive across a range of habitats and exhibit complex life histories, with larvae developing immersed in their food substrate, while adults are free-living and have diverse feeding strategies. Some species have evolved specialized parasitic associations with vertebrate or invertebrate hosts, which are behaviors with important implications for agriculture and for understanding evolutionary transitions between saprophagy and parasitism. This study presents a comprehensive phylogenetic analysis of the Calliphoridae, utilizing 711 of

NZ8/02113; National Science Foundation, Grant/Award Number: DEB-2030345

Editor: Emmanuel Toussaint

736 analysed nuclear genes, using anchored hybrid enrichment, from a global collection of blow flies and their relatives. Our results provide a robust and novel reconstruction of the evolutionary history of this group, pinpointing major transitions in larval feeding habits. We argue that saprophagy evolved independently multiple times from invertebrate parasitic ancestors, with vertebrate parasitism emerging from a number of different feeding strategies. These findings challenge prior hypotheses and offer new insights into the adaptive traits driving trophic specialization and diversification in this group.

KEYWORDS
anchored hybrid enrichment, blow flies, classification, parasitism

BACKGROUND

Calliphoridae, the blow flies, are a diverse assemblage of calyptrate Diptera (true flies) widely recognized for their role in decomposing carrion and other decaying materials. As larvae, they exhibit a wide range of feeding habits, including saprophagy, coprophagy, parasitism and predation (Ferrar, 2024; Stevens, 2003). Some saprophagous species are widely used in forensic science for estimating the post-mortem interval (e.g., Weidner et al., 2021; Wells, 2019). Additionally, other species, such as the Australian sheep blow fly, *Lucilia cuprina* (Wiedemann), the New World screwworm, *Cochliomyia hominivorax* (Coquerel), and the Old World screwworm, *Chrysomya bezziana* Villeneuve, are important livestock pests (Dallwitz et al., 1984; Hall et al., 2009; Wyss, 2000). The New World screwworm was the first species to be successfully controlled through the sterile insect technique (Concha et al., 2016; Wyss, 2000). A taxonomically diverse array of blow fly species has evolved highly specialized interactions with either vertebrate or invertebrate host and prey species (Altson, 1932; Banerjee et al., 2018; Clark, 2009; Colless, 1998; Prado E Castro et al., 2016; Rognes, 2011; Wood et al., 2018).

Comprising over 1800 species (Yan et al., 2021), blow flies belong to the superfamily Oestroidea, a clade of calyptrate flies that includes flesh flies, bot flies, cluster flies, tachinid flies and several other groups (Buenaventura et al., 2021; Kutty et al., 2019; Yan et al., 2021). Collectively oestroids account for approximately 15,000 extant species (Pape et al., 2011). Calliphoridae are abundant in all biogeographical regions and thrive in a wide range of terrestrial environments, from deserts to savannas, forests and montane areas (Marshall, 2012; Rognes, 1990, 1998). The life cycle of blow flies is highly compartmentalized, consisting of an egg and a number of larval instars that develop immersed in the substrate they feed on, a pupa and a free-living adult. Adult blow flies often have complex and varied diets, obtaining nutrients like sugars from honeydew, nectar, fruits or plant exudates and minerals, fats and proteins from excrement, carrion or more rarely from invertebrate prey (Rognes, 1990). Blow flies are effective pollinators of a wide range of plant species, including various crops (Rader et al., 2020), and are key components in pollination networks (Willcox et al., 2019). Other Calliphoridae, such as adults of *Bengalia* Robineau-Desvoidy, are highly specialized as predators or kleptoparasites of other insects (Zhao et al., 2024).

Larval feeding within the blow flies includes necrophagy/saprophagy (Rognes, 1990), by feeding on dead animals or any decaying matter. Some species that primarily feed on dead animals may also occasionally oviposit on a living host through wounds, natural body openings or soiled fur or wool (facultative parasitism) (Hall & Wall, 1995). Other species are obligate vertebrate parasites (Rognes, 1990) and oviposit and feed on a living vertebrate host. Likewise, some lineages have evolved obligate invertebrate parasitism (Rognes, 1990), where a parasitic association with an invertebrate host is required for part or all of their development; these species ultimately kill their host before pupating. Additionally, larvae of some blow fly species exhibit predation (Overstreet & Lotz, 2016), characterized by the ingestion of animal tissue from living individuals without depending on the prey for any period of development; some even devour several entire individuals as in larvae of *Caiusa coomani* Séguy, which prey on frog spawn (Banerjee et al., 2018). Less commonly, blow fly larvae may also feed on faeces (coprophagy). Although feeding habits are well known for certain groups (e.g., Calliphorinae, Chrysomyinae, Luciliinae and Rhinophorinae), larval feeding has been rarely documented and is poorly understood for others (e.g., Phumosiinae, Ameniinae, Bengaliinae and Rhiniinae).

The diverse, specialized feeding habits of Calliphoridae make them an excellent model for investigating and tracking the impact of adaptations associated with parasitism, for example, characterizing the different repertoires of chemosensory genes necessary for host-finding (Hickner et al., 2020; Kapoor et al., 2025) or changes in nutritional requirements necessary for the use of blood or blood-infused tissue in their diet (Cardoso et al., 2021; Graca-Souza et al., 2006). It has been proposed that Calliphoridae larval feeding habits have evolved in a linear progression from saprophagy to parasitism, with facultative parasitism serving as a “stepping stone” between these two habits (Erzinclioglu, 1989; Zumpt, 1965). One hypothesis underlying this scenario suggests that adaptations to survive extreme conditions, such as the presence of bacterial and fungal communities in decomposing tissues, may have served as “pre-adaptation” to overcome the immunological defenses of living hosts (Charabidze et al., 2021; Stireman et al., 2006). Testing this hypothesis requires the reconstruction of ancestral habits of parasitic lineages and their non-parasitic relatives. Understanding whether facultative parasitism is an intermediate step towards obligate parasitism can highlight the role of phenotypic plasticity in the evolution of complex feeding strategies. Facultative parasites could exhibit plasticity in their feeding strategy to exploit various host conditions (e.g., living and dead/necrotic tissue), thereby facilitating their transition to obligate parasitism through gradual selection for traits that enhance fitness (Luong & Mathot, 2019).

To fully assess the impact of these changes on trophic specialization and diversification, a detailed understanding of the phylogenetic history of the clades in which these changes have occurred is required. Despite substantial recent attention to the higher-level relationships of Calliphoridae and taxonomically comprehensive genomic resources (Parmar, Johnston, Wallman, & Szpila, 2025), a well-sampled and robustly supported phylogeny was still lacking. To address this gap, we present this study of phylogenetic relationships and subsequent macroevolutionary analyses, including, to our knowledge, the largest taxonomic and genomic sample of these clades ever attempted. Phylogenomic studies have revolutionized our ability to resolve relationships for groups that have confounded investigation due to sampling limitations from both the large number of species and the difficulty of large-scale genome-level sequencing (Kawahara et al., 2019; McKenna et al., 2019; Misof et al., 2014). Here, we used anchored hybrid enrichment (AHE) (Lemmon et al., 2012; Young et al., 2016), a probe-based exon capture method, to sample hundreds of orthologous nuclear genes from freshly collected and museum-preserved specimens of a global sample of blow flies and their relatives. Our dataset included representatives of all eight subfamilies of Calliphoridae, including 244 species, representing 77 genera. With these data, we present phylogenetic analyses of Calliphoridae and their relatives and use this inferred history of diversification to pinpoint major transitions in the evolution of larval feeding habits.

METHODS

Taxon sampling and specimen identification

Our dataset comprised 361 tips, representing 292 species (Table S1), including representatives from all subfamilies of Calliphoridae, all families of Oestroidea and some representatives of non-oestroid Calyptratae. Following Yan et al. (2021), *Drosophila melanogaster* Meigen (Diptera: Ephydroidea: Drosophilidae) was included as the root of the tree. Specimens were authoritatively identified by different experts in the group (Table S1). When necessary, identification was corroborated by extracting the mitochondrial Cytochrome Oxidase 1 (COXI) barcoding region from the assemblies. COXI sequences were analysed using the BOLD Identification System (Ratnasingham & Hebert, 2007) and NCBI BLAST (Camacho et al., 2009). When identification using COXI disagreed with morphological identification, molecular identification was chosen.

The representation of genera was as follows: Ameniinae (4 of 9), Bengaliinae (8 of 13), Calliphorinae (16 of 30), Chrysomyinae (9 of 15), Luciliinae (4 of 5), Phumosiinae (3 of 4), Rhiniinae (14 of 35) and Rhinophorinae (19 of 33). As outgroups, we included species from all remaining families of Oestroidea: Ulurumyiidae (1 sp.), Mesembrinellidae (1 genus, 3 spp.), Mystacinobiidae (1 sp.), Oestridae (2 genera, 2 spp.), Sarcophagidae (2 genera, 3 spp.), Tachinidae (8 genera, 8 spp.), Polleniidae (5 genera, 17 spp.). We also included representatives of the following calyptrate families: Glossinidae (1 sp.), Hippoboscidae (1 sp.), Muscidae (6 genera, 6 spp.), Anthomyiidae (3 genera, 3 spp.) and Scathophagidae (1 sp.).

Genomic data capture

Five different data sources were included in this study. For most specimens, we performed targeted genomic capture using the AHE method developed by Lemmon et al. (2012). Additionally, we used three unpublished transcriptomes (*Euphumosia papua*, Guérin, *Neorutilia* sp., Malloch, *Oestrus ovis*, *Linnaeus*). The dataset was complemented by publicly available transcriptomes, genomes and AHE data. See Supplementary Table S1 for details and NCBI accession numbers.

For AHE specimens, whole-genomic DNA was extracted from the ethanol-preserved and pinned insects. Voucher specimens were deposited at various institutions (Table S1). When possible, extractions were performed using destructive sampling of the full specimen; alternatively, two legs were used (Table S1). All extractions were performed using the DNeasy Blood & Tissue Kit (Qiagen Inc., Hilden, Germany) following the manufacturer's protocol, with slight modifications. The initial tissue incubation (ATL buffer + Proteinase K) was done overnight, and the final elution was obtained by eluting the DNA twice in 30 μL of the AE elution buffer. The concentration of DNA was quantified with a Qubit® 2.0 fluorometer (Invitrogen by Life Technologies Inc., Oregon, USA) using the dsDNA High Sensitivity Assay Kit. For a subset of samples with low concentrations (Table S1), genomic DNA was amplified using the REPLI-g Mini Kit (Qiagen Inc., Hilden, Germany) following the manufacturer's protocol.

The genomic DNA of each sample was sheared into fragments of approximately 300 base pairs via sonication on a Covaris S220/E220 Focused-ultrasonicator (Covaris Inc., Massachusetts, USA). Library preparation and indexing were performed using the NEBNext® Ultra TM II DNA Library Prep Kit (New England Biolabs Inc., Massachusetts, USA) following Meyer and Kircher (2010). Pools of 48 samples were hybridized with the Diptera AHE probe kit of Young et al. (2016) using standardized methods from previous Diptera AHE studies (Soghigian et al., 2023; Young et al., 2016). High-throughput sequencing was performed at the North Carolina State Genomic Science Laboratory (NCSU-GSL), either on an Illumina HiSeq 2500 or on an Illumina NovaSeq 6000.

Transcriptome data were obtained as detailed in Yan et al. (2021). In short, total RNA was extracted using TRIzol (Invitrogen Life Technologies), with total RNA concentration and RNA integrity number (RIN) for each extraction assessed using an Agilent 2100 Bioanalyzer with the RNA 6000 Nano kit (Agilent Technologies, Santa Clara, CA, USA). Approximately 200 ng to 1 μg of total RNA was purified to construct a cDNA library for each sample using the TruSeq RNA Sample Prep Kit v2 (Illumina, San Diego, USA) following the manufacturer's protocol. Sequencing was carried out using an Illumina NovaSeq 6000 sequencer to generate paired-end reads for each library.

Raw sequences processing and assembly

Demultiplexing of RAW reads was done by NCSU-GSL. Demultiplexed raw reads were processed using the AHE Tree Analysis pipeline described by Soghigian et al. (2023). For both transcriptome and

AHE samples sequenced using the NovaSeq platform and a subset of publicly available data without assemblies, raw reads were deduplicated using BBMap's Clumpify tool (Joint Genome Institute, https://sourceforge.net/projects/bbmap). Subsequently, we trimmed and filtered poor-quality reads, and merged paired-end reads using fastp v 0.20 (Chen et al., 2018), with the following parameters: --detect_adapter_for_pe, −-cut_front, −g and −x. Finally, the reads were assembled using SPAdes v 3.14.1 (Bankevich et al., 2012). For samples sequenced on the HiSeq platform, quality trimming was performed with Trimmomatic v 0.36 (Bolger et al., 2014), and the assembly step was performed using Trinity v2.4 (Grabherr et al., 2011).

Orthology assessment and decontamination

Orthology assessment was done for assemblies of all five data sources using Orthograph v 0.7.1 (Petersen et al., 2017). Orthograph performs a graph-based reciprocal blast approach with hidden Markov model predictions (pHMMs) for orthology assessment. For our orthologue reference set, we used a custom orthologue database (Brachybase) constructed from single-copy orthologue genes obtained from OrthoDB v10.1 (Kriventseva et al., 2019) using six fly species as references: *Ceratitis capitata* (Wiedemann) (Tephritidae), *Drosophila melanogaster* (Drosophilidae), *Musca domestica* Linnaeus (Muscidae), *Lucilia cuprina* (Wiedemann) (Calliphoridae), *Glossina austeni* Newstead (Glossinidae), and *Stomoxys calcitrans* (Linnaeus) (Muscidae). Subsequently, we verified the identity of all putative orthologues recovered from each assembly using BLAST (Camacho et al., 2009). Each orthologue was blasted against a custom reference database rich in fly genomes and complemented with genomes of other insects, other animals, fungi, bacteria and protozoa (reference database available for download in FigShare at https://doi.org/10.6084/m9.figshare.28100567.v2; Beza-Beza, 2025). When the top BLAST v 2.12.0+ hit for an orthologue was not from a fly genome, the sequence was discarded from further analyses.

Alignment, outlier pruning and detection of potential non-orthologous genes

We selected orthologues found in at least 75% of the species using the alignment and quality control pipeline described by Soghigian et al. (2023). The amino acid sequences of each orthologue were aligned with MAFFT v 7.453 (Katoh & Standley, 2013) GINSI-I (1000 iterations, add-fragments flag on), using the alignments from the Brachybase orthologue database as a reference. Nucleotide alignments were obtained in trimAL (Capella-Gutiérrez et al., 2009) using gappy-out with the back-translation option.

To prune individual orthologue alignments from outlier taxa, we obtained amino acid gene trees. Substitution model selection and fast tree search for each ortholog were conducted using IQTree v. 2.2.2.7 (Kalyaanamoorthy et al., 2017; Minh et al., 2020). Subsequently, we assessed the contribution of each taxon to the length of the gene tree using the methods described by Soghigian et al. (2023). This approach calculates the median cophenetic distance from each taxon to all other taxa in the tree and subtracts this from the total tip-to-tip distance for all taxa. This relative measure was then scaled by dividing the interquartile range of the tip distances, resulting in a branch length ratio per taxon per gene. Taxa were defined as outliers if their branch length ratio was five times the interquartile range for that tip and were removed from the alignments (see Tables S2, and S3 for summary of outliers removed per loci, and per taxon).

Finally, we screened our pruned alignments for the detection of putative non-orthologous genes using the distribution of pairwise genetic distances and visual inspection of the alignments and gene trees. For the trees, we reconstructed amino acid gene trees from the pruned alignments using IQTree2 (Minh et al., 2020) and 1000 ultrafast bootstrap (Hoang et al., 2018) replicates to measure support. Then, we were interested in identifying alignments that showed a pronounced bimodal distribution of pairwise genetic distance, as that distribution pattern could be indicative of alignments containing sequences from non-orthologous genes. To reduce the number of loci for visual inspection, we used a custom R script (Supplementary File S11), which allowed us to select alignments with pronounced multimodal distributions. First, we calculated pairwise genetic distances for the amino acid alignments of each putative orthologue. We then used Kernel Density to estimate the number of modes in the distribution of genetic distances. All putative orthologues with a single mode were considered safe alignments. For orthologues with a multimodal distribution, we obtained the value of the two highest peaks in the kernel density and divided the low peak by the high peak value. This measure allowed us to differentiate between distributions with one prominent mode and those with multiple. Distributions with only one prominent mode were considered safe alignments (peak ratio ≤0.20), as this distribution can be the result of differences caused by missing data. Finally, all multimodal distributions with 2 to 10 modes were considered potentially problematic alignments and were selected for visual inspection of the alignment and gene tree in Geneious Prime v2023.2.1 (https://www.geneious.com).

Phylogenetic analyses

The bioinformatics pipeline described in Soghigian et al. (2023) was optimized to leverage bycatch from AHE capture and allow the integration of these data with different genomic data sources (e.g., transcriptome, genomes and ultra-conserved elements). We selected two sets of loci for phylogenetic analyses: one included all recovered loci (including bycatch) with at least 75% taxa (dataset prefix 75) and the second set included the loci targeted by our AHE probe set with at least 75% taxa (dataset prefix TL). For each set, we analysed the amino acid and nucleotide sequences for a total of four matrices (75.aa, 75.12, TL.aa, TL.12). For the nucleotide matrices, only 1st and 2nd codon positions were used because of the documented effects of saturation of the 3rd codon position in the phylogenetic inference of similar-aged fly divergences (e.g., Kutty et al., 2019; Soghigian et al., 2023).

For each matrix, we performed a maximum-likelihood (ML) tree search of the concatenated data implemented in IQTree2. Amino acid analyses were partitioned by gene, and nucleotide analyses were partitioned by gene and codon positions. For the amino acid matrices, we used the substitution models selected in the amino acid gene tree reconstruction of the pruned alignments (see *Alignment, outlier pruning and detection of potential non-orthologous genes*). For the nucleotide matrices, we used the best substitution models selected by ModelFinder implemented in IQTree2. Support of the ML tree was assessed with 1000 replicates of the SH-like approximate likelihood ratio test and 1000 ultrafast bootstrap replicates with an additional step to further optimize UFBoot trees by nearest neighbour interchange (−bnni). In addition to our ML analyses, we conducted coalescence-based species tree inference using a weighted analysis in ASTRAL (Zhang et al., 2018). For amino acid matrices, we used the gene trees estimated in the previous step. For the nucleotide matrices, we estimated gene trees for each locus using the substitution models from the concatenated analysis and 1000 ultrafast bootstrap replicates to measure support. To assess the influence of taxon completeness we performed ASTRAL analyses in three sets of gene trees: loci with at least (1) 75% taxa coverage (pre-pruning), (2) 80% taxa coverage (post-pruning) and (3) 90% taxa coverage (post-pruning).

Divergence time estimation

Divergence time estimation was performed using MCMCtree implemented in PAML4 (Yang, 2007) based on the workflow described by dos Reis & Yang (Dos Reis & Yang, 2019). As input, we selected the tree resulting from our ML tree search of the 75.aa matrix, removing the root (*D. melanogaster*). Because our tree contained multiple specimens for some species, we pruned the tree to include only one tip per species using the drop.tip function in the R package phytools (Revell, 2024), we selected the representatives of each species with the most loci. For calibration, we used the same strategy employed by Cerretti et al. (2017), which included four fossils as calibration points: (1) two fossils belonging to Phaoniinae (Muscidae) from 15 to 20 million years ago (Mya) Dominican amber (Pont & Carvalho, 1997), (2) *Glossina* sp. Wiedemann (Glossinidae) from 35 Mya Colorado shale (Grimaldi & Engel, 2005), (3) *Protanthomyia minuta* Michelsen (stem group Anthomyiidae) from approximately 42 Mya Baltic amber (Michelsen, 2000) and (4) *Mesembrinella caenozoica* Cerretti, Stireman, Pape, O'Hara, Marinho, Rognes & Grimaldi from 15 to 20 Mya Dominican amber (Cerretti et al., 2017). These fossil constraints were each modelled using lower bound truncated Cauchy distributions (Dos Reis & Yang, 2019). In each case the, minimum age truncated Cauchy distribution was set with $p = 0.1$ (the distance the mode of the distribution is from the fossil minimum age), $c = 1$ (the shape of decay to zero), and $p_L = 0.025$ (the left tail constraint violation probability). Similar to other studies that have estimated divergence times for Diptera (e.g., Wiegmann et al. (2011); Xuan et al. (2023)), we set a hard root node maximum age of 65 Mya based on the complete absence of schizophoran families in the Mesozoic fossil record (Grimaldi, 2018).

To optimize the computational load, we selected the top 100 clock-like genes using the SortaDate pipeline (Smith et al., 2018). Additionally, we used the approximate likelihood method of clock dating (Thorne et al., 1998) as implemented in MCMCtree. This approach allows us to use the gradient and Hessian (gH) matrix of the branch length of the topology rather than our alignment to sample the posterior distribution. The calculation of the gH matrix was performed in BASEML implemented in PAML4; for this, we used the nucleotide sequences under two partitions (1st and 2nd codon positions) and general time-reversible + gamma (GTR+ G) substitution model.

We selected our clock model using the marginal likelihood approach implemented in the R package mcmc3r (Reis et al., 2018) following the tutorial of dos Ries (Dos Reis, 2017). We ran 64 beta values to calculate the marginal likelihood for each model. Sampling of the posterior for clock model selection was performed using the gH matrix, a burn-in of 4000 generations and a sampling frequency of two until the process reached 10,000 samples. The standard error for the likelihood was calculated with 100 bootstrap replicates using the stationary block bootstrap method of Politis and Romano (1994) described by Álvarez-Carretero et al. (2022). Model selection was performed using the Akaike information criterion (AIC).

Finally, we sampled the posterior distribution of node ages in MCMCtree using the approximate likelihood method. We used a uniform prior on node ages for uncalibrated nodes (BDparas = 1 1 0) and a diffuse prior on the mean substitution rate prior (2 40 1) and the rate variance parameter (sigma2_gamma = 1 10 1). Prior information using fossil calibrations was modelled as lower bound Cauchy distributions. We ran five independent runs with a burn-in of 50,000 generations, with a sampling frequency of 1000 until the process reached 5000 samples, for a total of 5000,000 generations. To ensure that we ran each chain sufficiently long, we looked at the effective sample size (ESS) for all parameters using Tracer v1.7.2 (Rambaut et al., 2018) and ensured that all ESS values were greater than 1000. To assess whether sufficient burn-in time was given to each run, we visually inspected the trace using Tracer and ensured that the trace oscillated around the mean. The convergence between each run was assessed by comparing the posterior means among all five runs. As all runs converged on the same posterior distribution, we combined the mcmc.txt files and summarized the results in MCMCtree (print = −1). To assess the impact of the sequence data on the analyses, we ran an analysis without sequence data (use data = 0).

Ancestral state reconstruction of feeding habits

We mapped the evolution of larval feeding habits across Oestroidea, as parasitism takes place during the larval stage. We used two coding strategies: one in which we scored six larval feeding habits: coprophagy, saprophagy, facultative parasitism, vertebrate parasitism, invertebrate parasitism and predation. Alternatively, we coded the organism according to its ability to feed on faeces, necrotic/dead (saprophagous and facultative parasites) and living (obligate parasites, facultative parasites and predators) tissue. Based on reports in the peer-reviewed literature and expert knowledge, we created a probability matrix in which we codified the tips with one known feeding habit (Table S4) as discrete characters. For species with multiple known feeding habits, we assigned an equal probability to each. Tips without species-level identification and those with unknown biology were assigned an equal probability (0.166, 0.33) to all larval feeding habits.

Saprophagy, vertebrate parasitism and invertebrate parasitism are common larval feeding strategies across all Oestroidea families. Reconstructing the ancestral larval feeding habits of Calliphoridae would benefit from the phylogenetic context provided by their relatives. However, our taxon sampling was optimized to resolve relationships within Calliphoridae. To assess the potential bias introduced by uneven taxon sampling in other Calliphoridae relatives, we evaluated both coding strategies using three phylogenetic subsets: (1) all sample members of Oestroidea, (2) Calliphoridae + (Polleniidae+Tachinidae) and (3) Calliphoridae only. Ancestral state reconstruction was performed using stochastic character mapping in the R package phytools. For each coding strategy, we ran 1000 simulations under three different character evolutionary models: Equal Rates (ER), Symmetrical Rates (SYM) and All Different Rates (ARD). Model performance was evaluated using AIC by comparing the AIC weights of each model, where we chose a single best model for each experimental condition.

RESULTS

Loci recovery and matrix construction

Including bycatch, we obtained an average of 3145 loci per sample, with a minimum of 117 loci and a maximum of 6180 loci (see Table S1 for individual specimen loci count). We did not observe any relationship between the recovered number of loci and the year of specimen collection (Supplementary Figure S1). Prior to screening for potential orthologue misassignment, we recovered 736 loci with at least 75% taxon coverage. Following all pruning methods and visual inspection, 26 of the 737 alignments were identified as containing non-orthologous sequences and were removed from further analyses. The final datasets consisted of four matrices: the 75.aa and 75.12 matrices included 711 loci with 603,231 AAs and 1,206,462 NTs respectively; the TL.aa and TL.12 matrices comprised 395 loci, 175,702 AAs and 351,404 NTs, respectively.

Phylogenetic relationships

We consistently recovered Calliphoridae sensu Yan et al. (2021) as monophyletic with high support and as sister to the clade Tachinidae +Polleniidae. Six of eight subfamilies were also recovered as monophyletic, but the subfamilies Ameniinae and Phumosiinae are not monophyletic as currently defined, with *Euphumosia papua* (Phumosiinae) recovered within Ameniinae (Figure 1a). To address this result, the genus *Euphumosia* Malloch is transferred to Ameniinae. All analyses support the following sister-group relationships among subfamilies with full branch support: Chrysomyinae+Phumosiinae, Ameniinae+Rhinophorinae, Bengaliinae+Rhiniinae and Calliphorinae +Luciliinae (Figure 1a). We found general agreement among higher-level relationships in all maximum-likelihood (ML) analyses, with minor disagreements in some relationships among the families of Oestroidea sampled as outgroups (Figure 1, Supplementary Figures S1–S4).

Regarding the relationships among the subfamilies of Calliphoridae, all ML analyses recovered the same topology (Figure 1, Supplementary Figures S1–S4), and all three ASTRAL amino acid analyses support a different topology (Supplementary Figures S5–S7); equally, all three ASTRAL nucleotide analyses converge in a different topology (Supplementary Figures S8–S10). The difference in the topologies is due to the phylogenetic position of the clades Chrysomyinae+Phumosiinae and Bengaliinae+Rhiniinae. In all ML analyses, we recovered Chrysomyinae+Phumosiinae as sister to the rest of Calliphoridae; Bengaliinae+Rhiniinae was recovered as the sister group of Calliphorinae+Luciliinae (Figure 1). In contrast, for the amino acid ASTRAL trees Chrysomyinae+Phumosiinae was recovered as sister to Bengaliinae + Rhiniinae + Calliphorinae + Luciliinae (Supplementary Figures S5–S7). On the other hand, for the nucleotide ASTRAL trees Bengaliinae+Rhiniinae was recovered sister to Chrysomyinae+Phumosiinae. However, local posterior probabilities for nodes pertaining to disagreements were low in the ASTRAL analyses (Supplementary Figures S5–S10).

All genera of Ameniinae, Bengaliinae, Chrysomyinae and Phumosiinae represented by multiple species were recovered as monophyletic (Figure 2), and there is no disagreement between the species- and genus-level relationships among the ML datasets (Figures S2–S5). Within Luciliinae, all analyses agree in topology; however, *Lucilia* Robineau-Desvoidy, the largest genus in the subfamily, was recovered as polyphyletic, and the genera *Hemipyrellia* Townsend and *Hypopygiopsis* Townsend render each other paraphyletic (Figures 2h, S6). For Rhiniinae and Rhinophorinae, there is minor disagreement between the results from the 75.12 matrix and all the other matrices, and although some genera were recovered as monophyletic, our data suggest non-monophyly among other genera within both subfamilies (Figure 2e,g, S7, S8). For Calliphorinae, there is minor disagreement between the results from the 75.12 matrix and all the other matrices; importantly, the genus *Calliphora* Robineau-Desvoidy is recovered as paraphyletic (Figure 2i, S9), with all Australasian *Calliphora* placed in a clade from which emerge other Australasian genera of Calliphorinae (including the former Aphyssurinae) as well as the widely distributed *Onesia* Robineau-Desvoidy and *Bellardia* Robineau-Desvoidy.

Divergence time estimation

All five independent MCMCtree runs yielded similar divergence estimates and converged on a similar posterior distribution (Figure S10); analysis with sequence data removed (priors only) suggests that sampling from prior distributions was significantly impacted by the use of sequence data. Our data suggest that most families of Oestroidea originated during the mid-Eocene, with Calliphoridae splitting from

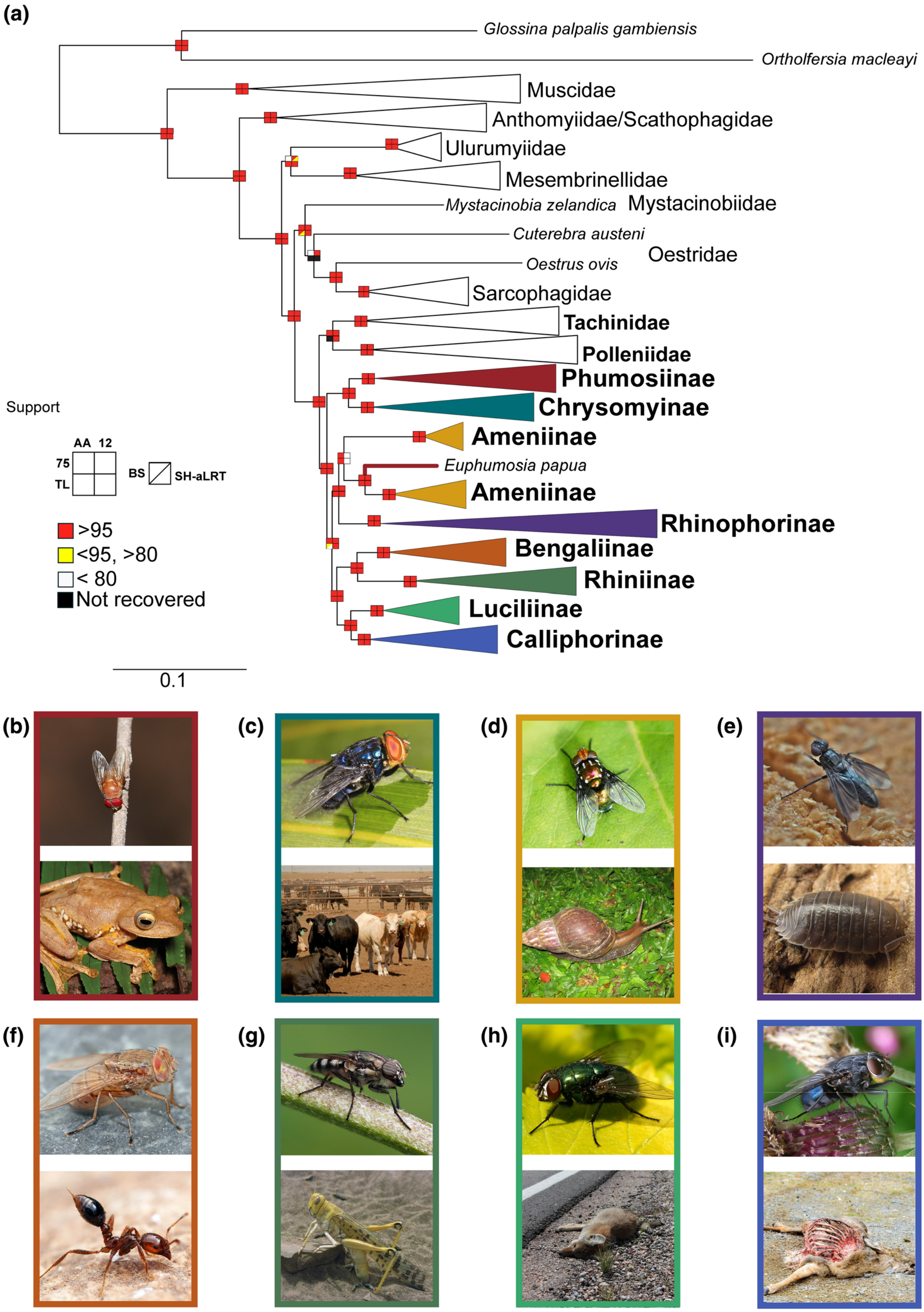

FIGURE 1 Legend on next page.

Tachinidae+Polleniidae around 40.91 Mya (95% CI = 44.93–36.53), and the crown Calliphoridae originated in the Late Eocene and diversified predominantly during the Oligocene (Figures 3, S11).

Ancestral state reconstruction of larval feeding habits

Application of the AIC weight on our ancestral state reconstruction strongly favoured (AICw >0.95) SYM as the best fitting model of evolution for larval feeding habits in all three phylogenetic sets. While all rates different (ARD) model was selected as the best feeding model for our feeding substrate strategy (Table S5). Inclusion or exclusion of outgroups did not influence the recovered ancestral feeding habits (Figures 4, S12, S13), but it differs for feeding substrate reconstruction (Figures S14–S16).

According to our reconstruction, the ancestral state for larval feeding habit in Calliphoridae is suggested to be invertebrate obligate parasitism (Figure 4). Additionally invertebrate parasitism evolved secondarily twice within Calliphorinae. Saprophagy likely evolved at least twice: once in Chrysomyinae and a second time in the common ancestor of Luciliinae and Calliphorinae. Predation evolved at least five times: at least once within Phumosiinae, *Eurychaeta* and Rhiniinae, and twice within Chrysomyinae. Additionally, vertebrate parasitism evolved independently at least five times from a number of different ancestral feeding habits.

The ancestral reconstruction of feeding substrate is largely consistent with the results inferred from the ancestral reconstruction of larval feeding habits. For instance, nodes identified as saprophagous were associated with dead tissue consumption, whereas those classified as obligate parasites were linked to live tissue consumption (Figures 4, S14–S16). The only exceptions to this pattern occur in deep nodes within the Calliphoridae-only phylogenetic dataset, where ancestral states were reconstructed as dead tissue consumers (Figures 4, S16).

DISCUSSION

Phylogenetic relationships and nomenclatural implications at the family and subfamily level

The delimitation of Calliphoridae has been challenging, and their taxonomic circumscription has undergone several changes since the first quantitative phylogenetic studies of this group. Our results consistently recover Calliphoridae sensu Yan et al. (2021) and Parmar, Johnston, and Szpila (2025) as monophyletic, supporting the recently changed concept of the family. Additionally, the phylogenetic position of Calliphoridae as the sister group to Tachinidae+Polleniidae is corroborated (Figure 1). With systematics increasingly being based in phylogenomics, classifications have become more stable, informative and thus useful to an increasing number of scientific disciplines. Nevertheless, the 'translation' of phylogenetic trees into a Linnaean classification scheme is based on a single general principle: the assignment of scientific names exclusively to monophyletic groups (clades) (De Queiroz & Gauthier, 1990; De Queiroz & Gauthier, 1992). Deciding on ranks (Class, Order, Family, etc.) is inherently subjective and as such influenced by cultural factors. Some examples include the phenotypic homogeneity within the clade (preferably supported by unambiguous autapomorphies), consistency with pre-existing classifications, agreement among contemporary taxonomists, or compromises and syntheses between these factors.

The classification of Calliphoridae is an emblematic case. Originally, the family included essentially all or nearly all current Oestroidea (Brauer & Bergenstamm, 1924; Girschner, 1894), only to lose and regain various taxa based on often contradictory data. The family circumscription has changed frequently in recent years. The suggestion by Guimarães (1977) to raise Mesembrinellidae to family rank was consistently rejected until molecular data placed them close to the base of the Oestroidea (Cerretti et al., 2017; Kutty et al., 2019). The classification of Polleniinae as a subfamily of the Calliphoridae had been almost unanimously accepted, but they are now consistently treated at family rank as a sister taxon to the Tachinidae (Kutty et al., 2019; Marinho et al., 2017). Rhiniinae had a short period at family rank but are now treated as a subfamily of the Calliphoridae (Rognes, 1990). Rhinophorinae have shifted between being classified as a subfamily of Tachinidae or of Calliphoridae but were treated at family level for years (Crosskey, 1977; Rognes, 1990, 1997) and have recently been re-included in the Calliphoridae (Yan et al., 2021). Our data corroborate the inclusion of Rhiniinae and Rhinophorinae within the blow flies (Figure 1).

Yan et al. (2021) proposed a system of eight subfamilies which, in addition to including Rhiniinae and Rhinophorinae, merged the former Aphyssurinae, Melanomyinae and Toxotarsinae into a redefined Calliphorinae, and Helicoboscinae into Ameniinae. According to this classification, the Calliphoridae as a whole, and the Calliphorinae and Ameniinae in particular, exhibit remarkable diversity in both morphology and life history strategies. Our topology agrees with that of Yan et al. (2021) (Figure 1) and further highlights that different evolutionary trajectories may have shaped the larval and adult phenotypes of blow flies to such an extent that the phylogenetic affinities of their extant representatives have remained obscure.

In contrast to Yan et al. (2021), Chrysomyinae were always recovered as sister to Phumosiinae (Figure 1). All our ML analyses converged onto the same topology regarding the relationship between

FIGURE 1 (a) Family and subfamily level relationships recovered from all the Maximum Likelihood analyses of the 75.AA matrix. Squares in the nodes denote support matrix, including all other ML analyses. Each square within the matrix is split into ultrafast bootstrap (upper) and SH-aLRT support (lower). Red Squares indicate support >95%, yellow support <95% and >80% and white support <80%. Picture of adult representative (upper) and most common larval feeding habit of (b) Phumosiinae, (c) Chrysomyinae, (d) Ameniinae, (e) Rhinophorinae, (f) Bengaliinae, (g) Rhiniinae, (h) Luciliinae, (i) Calliphorinae.

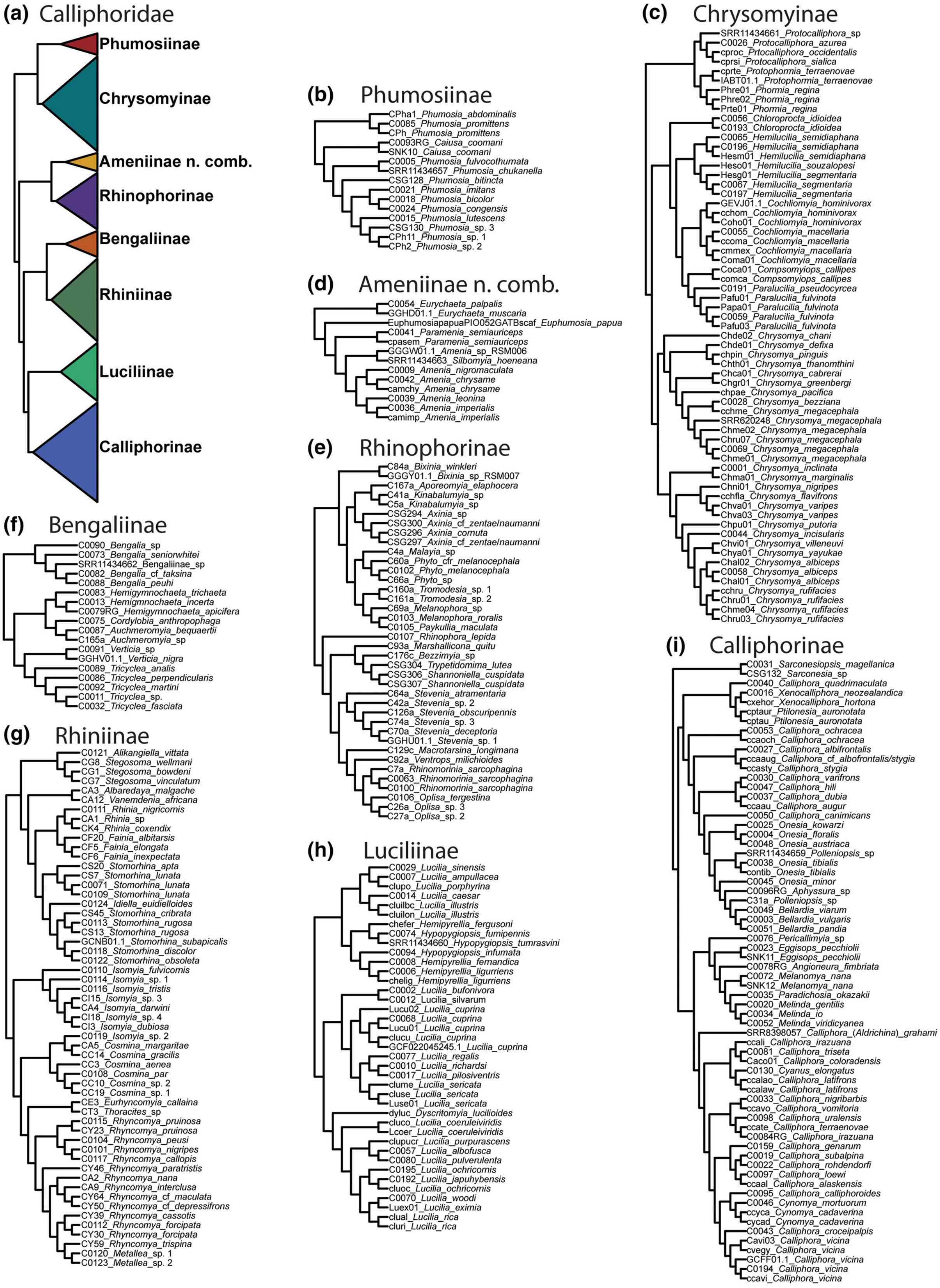

FIGURE 2 Cladograms representing phylogenetic relationships recovered in the Maximum Likelihood analysis of the 75.AA matrix: (a) Calliphoridae, (b) Phumosiinae, (c) Chrysomyinae, (d) Ameniinae n. comb., (e) Rhinophorinae, (f) Bengaliinae, (g) Rhiniinae, (h) Luciliinae, (i) Calliphorinae.

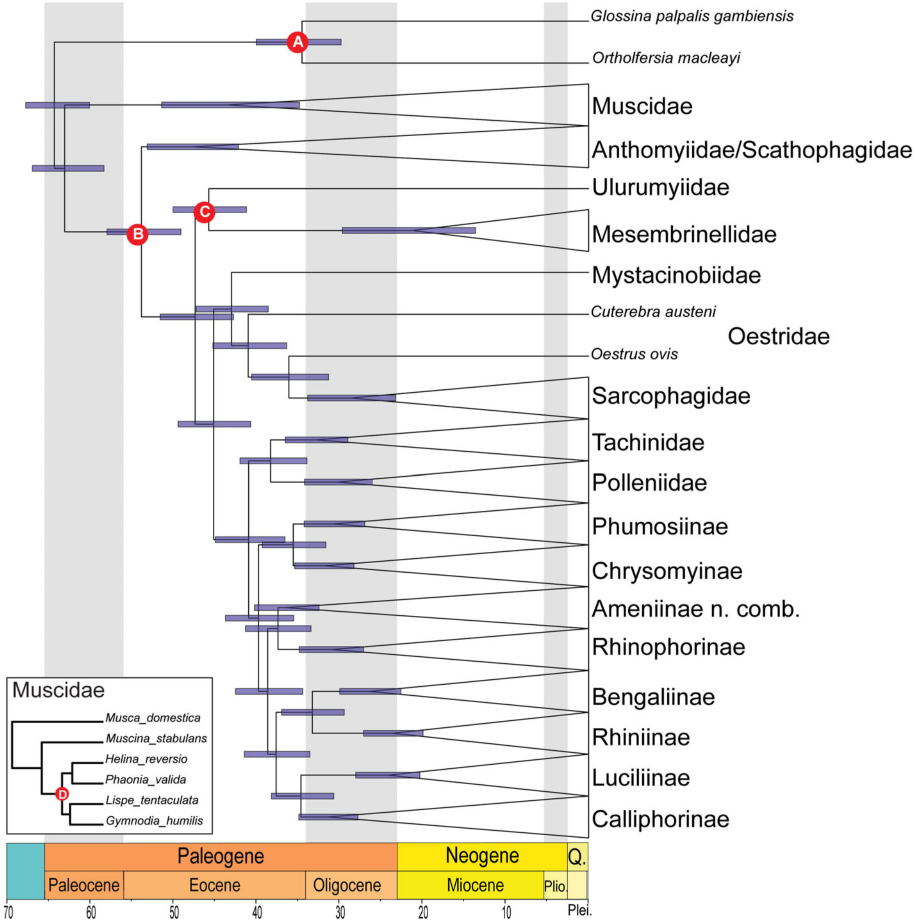

FIGURE 3 Divergence time estimation analysis. Chronogram from the summary of five independent MCMCtree runs. Blue bars at nodes represent 95% confidence intervals, numbers on scale represent million years ago. Red dots represent fossil calibrations: (a) *Glossina* sp. (Glossinidae) from 35 Mya Colorado shale (Grimaldi & Engel, 2005); (b) *Protanthomyia minuta* (stem group Anthomyiidae) from approximately 42 Mya Baltic amber (Michelsen, 2000); (c) *Mesembrinella caenozoica* from 15 to 20 Mya Dominican amber (Cerretti et al., 2017); (d) two fossils belonging to Phaoniinae (Muscidae) from 15 to 20 Mya Dominican amber (Pont & Carvalho, 1997).

these two clades, but we observed discrepancies between the ML and ASTRAL analyses. Species tree estimation for this group may be influenced by errors in gene tree estimation, which is known to affect coalescence-based tree inference (Zhang & Mirarab, 2022).

Systematic implications below the subfamily level

Calliphora and *Lucilia* are two long-established genera of blow flies with an extensive appearance in the literature due to a few synanthropic carrion-breeding species, but their monophyly has never been rigorously tested nor corroborated by molecular data (e.g., Wallman & Adams, 1997). Our analysis definitively rejects the monophyly of both of these genera (Figure 2h,i), which presents significant implications for the systematics of these two nominal groups. Additionally, our analysis recovered a novel relationship with *Euphumosia papua* emerging within the Ameniinae (Figure 1, 2b). These results underscore the need for further systematics work in the family and reassessment of the current classification.

The genus *Calliphora*, as currently defined, comprises about 100 species (Evenhuis & Pape, 2024) and has a virtually cosmopolitan distribution, largely due to a few widespread, synanthropic species [e.g., *Calliphora vicina* Robineau-Desvoidy, *Calliphora vomitoria* (Linnaeus)]; however, the bulk of species diversity within *Calliphora* is found in the Holarctic and Australasian regions. In our analyses, *Calliphora* consistently fragments into a paraphyletic grade of lineages (Supplementary File S1–S10, Figures 2i, S8). Calliphorinae are divided into two clades, one including two species of the endemic South American *Toxotarsus* Macquart and another comprising all remaining Calliphorinae sensu Yan et al. (2021). The latter clade is subdivided into two clades, one clade recovering *Pericallimyia* Villeneuve together with all genera formerly classified in the Melanomyinae (i.e., *Eggisops* Rondani, *Melanomya* Rondani, *Melinda* Robineau-Desvoidy) in a sister-group relationship to a large clade comprising a grade of Holarctic *Calliphora* species and also containing the Holarctic *Cynomya* Robineau-Desvoidy and the Nearctic *Cyanus* Hall (Figure 2i). The other clade includes all Australasian *Calliphora* species plus the genera *Xenocalliphora* Malloch, *Ptilonesia* Bezzi, *Polleniopsis* Townsend,

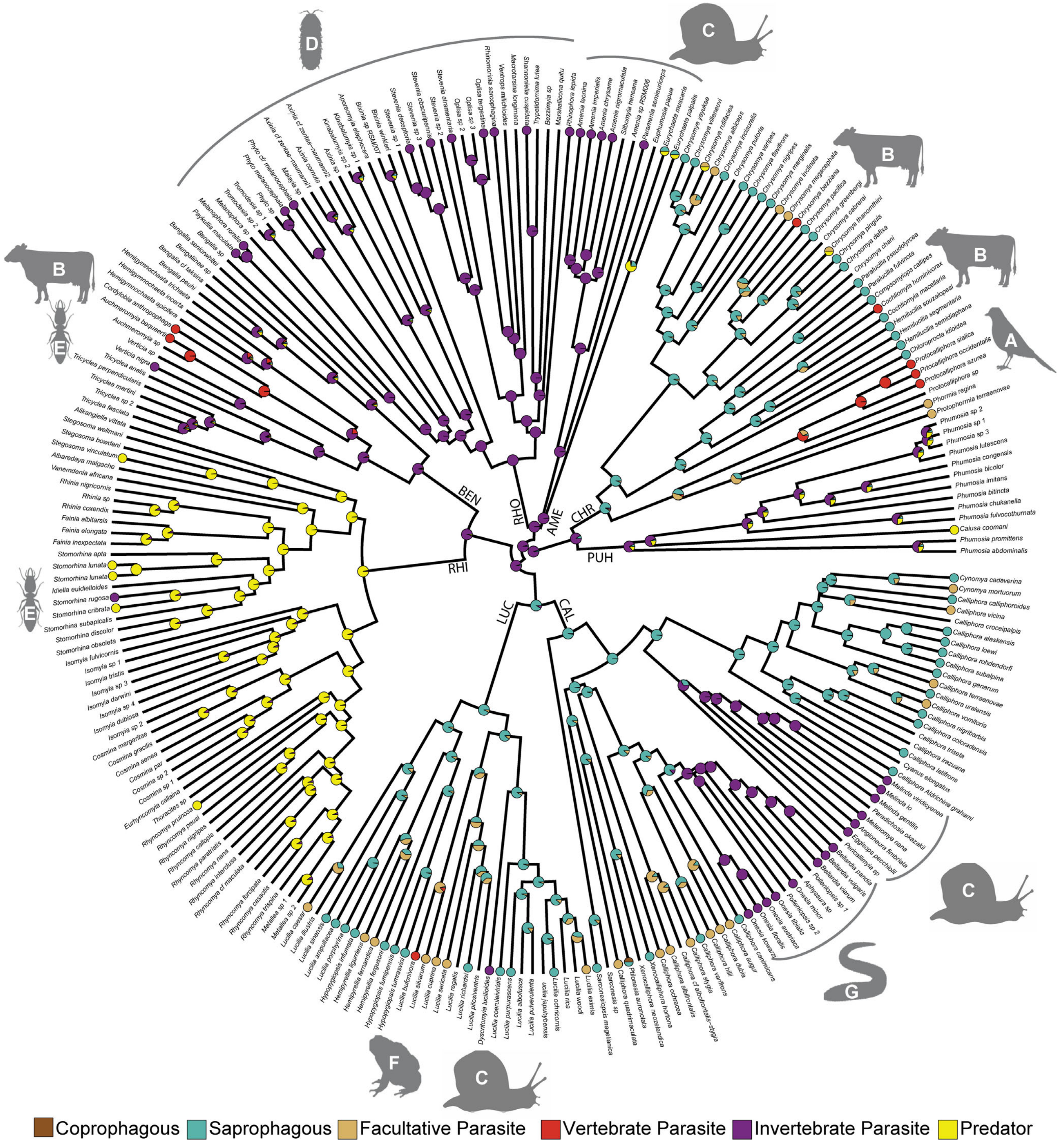

FIGURE 4 Ancestral state reconstruction of larval feeding habits of Calliphoridae. Summary of 1000 stochastic character mapping runs, circles at the tips indicate known feeding habits, samples without circles are species with unknown larval feeding habits. Abbreviations in the branches represent subfamily level names: Ameniinae (AME), Bengaliinae (BEN), Calliphorinae (CAL), Chrysomyinae (CHR), Luciliinae (LUC), Phumosiinae (PHU), Rhiniinae (RHI) and Rhinophorinae (RHO). Grey silhouettes indicate the host specialization of each clade at the class level: (a) Aves, (b) Mammalia, (c) Gastropoda, (d) Isopoda, (e) Hexapoda, (f) Amphibia and (g) Clitellata.

Aphyssura Hardy, *Onesia* and *Bellardia*. The recovery of this latter clade is an intriguing and unexpected result of this analysis. However, there are morphological clues that could support this finding, particularly the structure and sclerotization patterns of the male terminalia (Johnston et al., 2025; Kurahashi, 1971; Kurahashi, 1972a; Kurahashi, 1972b; Kurahashi, 1978; Rognes, 1990). Additionally, Kurahashi (1971, 1972b, 1978) proposed the *Onesia*-group, including the nominal genus (*Onesia*) along with *Bellardia*, *Polleniopsis* and *Tainanina* (the latter treated here as a junior synonym of *Polleniopsis*), based on two possibly derived character states, namely, a short oviscapt and a common oviduct (uterus) with a pair of lateral incubatory pouches. Furthermore, Rognes (1990, 66), referring to Kurahashi's (1971) illustrations, noted that the broad and shortened sternite 8 in the female oviscapt of *Bellardia* and *Onesia* closely resembles that of certain Australasian *Calliphora*, thus distinguishing it from the sternite 8 of Palaearctic *Calliphora* species, which is narrow, elongated and posteriorly bilobed. Interestingly, a broad and shortened sternite 8 is also characteristic of female *Aphyssura* (Norris 1999), which our analysis identifies as a sister group to *Bellardia*. Moreover, Rognes (1990, 32) proposed the presence of an "accessory oral sclerite" in the cephaloskeleton of third instars as a potential synapomorphy for the then defined Calliphorinae but noted that species of *Bellardia* and *Onesia* lack this structure. Our phylogenetic analysis suggests that the absence of an accessory oral sclerite may also be shared by both *Polleniopsis* and *Aphyssura* and may even characterize species of the Australasian *Calliphora* grade. This raises the question of whether features like a shortened oviscapt, the lateral incubatory pouches and the lack of accessory oral sclerite in third instar may be autapomorphies for this clade and whether these may be key characters in understanding the evolution of functional aspects such as oviposition behaviour, developmental strategies and larval feeding habit in the group.

Lucilia currently comprises 64 species and is distributed worldwide (Evenhuis and Pape, n.d.). Our analysis includes representatives of four of the five genera classified within Luciliinae (sensu Rognes (1990) and Evenhuis and Pape (2024)), excluding the monotypic genus *Blepharicnema* Macquart, and supports a paraphyletic *Lucilia*, with *Dyscritomyia* Grimshaw, *Hemipyrellia* and *Hypopygiopsis* nested within it (Figure 2h,S,6). Furthermore, while *Hemipyrellia* and *Hypopygiopsis* together are reconstructed as monophyletic, they exhibit mutual paraphyly. Rognes (1990) was the first to suggest the probable non-monophyly of *Lucilia* based on morphological data, noting that this genus "is differentiated from *Hemipyrellia* and *Hypopygiopsis* only by an obvious plesiomorphy", specifically the absence of long setae on the katatergite.

By scoping the generic boundaries of *Calliphora* and *Lucilia* differently, different taxonomic solutions can be explored. The most inclusive approach would be to circumscribe *Calliphora* and *Lucilia* to include all genus-group taxa nested within them. However, this would make *Calliphora* almost identical to the entire subfamily Calliphorinae, with the exception of *Toxotarsus*, and *Lucilia* would be identical to Luciliinae. Conversely, narrowing the generic scope (i.e., cutting genera at more distal branches of the tree) would result in progressively closer boundaries, leading to a greater number of genera, some of which would inevitably be monotypic or contain only a few species. The latter approach, regardless of the degree of inclusivity, has the disadvantage of requiring a global revision of the groups, and extensive generic splitting often leads to nomenclatural instability and will be difficult to manage for non-taxonomists dealing with practical applications (e.g., ecology and forensic entomology).

Additionally, there is growing evidence that adult morphology in Calliphoridae can be misleading when used alone to reconstruct phylogenetic affinities (Cerretti et al., 2019; Cerretti et al., 2024; Gisondi et al., 2023; Rognes, 1997). Our analyses confirm this not only for *Calliphora* (Figure 2i) and *Lucilia* (Figure 2h), but also for *Euphumosia papua*. This species, traditionally assigned to Phumosiinae based on external characters such as habitus and chaetotaxy (Rognes, 1990), is consistently recovered within the Ameniinae with strong statistical support (Figure 1, 2d). The Ameniinae, recently circumscribed by Yan et al. (2021) based on robust phylogenomic evidence and further confirmed by the present study, now includes the former Helicoboscinae (*Eurychaeta* Brauer & Bergenstamm and *Gulmargia* Rognes) and is characterized by a reproductive strategy involving lecithotrophic unilarviparity (Crosskey, 1965; Ferrar, 1976; Ferrar, 1978; Rognes, 1986). This strategy involves an egg (usually a single egg) containing large amounts of yolk being retained in the uterus while the larva develops to late first instar, second or early third instar before hatching from the egg during deposition. The confirmation of this reproductive strategy in *Euphumosia papua* by Ferrar (1978) significantly strengthens the evidence for inclusion of *Euphumosia* in the Ameniinae. Lecithotrophic unilarviparity is not only a strong potential synapomorphy for members of the newly proposed circumscription of Ameniinae but is also a feature that may be detectable even in pinned specimens preserved in museum collections by dissection and could therefore serve as a predictive character state of the affiliation of other species currently assigned to *Euphumosia*, at least until genomic data become available.

Timing of diversification of Calliphoridae

Our divergence time estimates provide a new historical framework for Calliphoridae divergences and the timing of oestroid family level origins (Figures 3, S11), which historically has been impaired by uncertain phylogeny and the lack of relevant fossils (Stevens et al., 2006). Despite their remarkable diversity and ecological success, fossils of Calliphoridae have yet to be discovered. More broadly, while some 22,000 species of extant calyptrates have been described (Evenhuis & Pape, 2024), palaeontological evidence is limited to a few specimens. These include a few muscids (Pont & Carvalho, 1997), a 15–17 million years old fossil of Mesembrinellidae in Dominican amber (Cerretti et al., 2017), compression fossils of tse-tse flies found in Nearctic Eocene and Palaearctic Late Oligocene deposits from 35 to 25 million years ago (Grimaldi & Engel, 2005) and anthomyiids in Dominican amber (40–25 million years ago) (Michelsen, 1996) and Baltic amber from about 42 million years ago (Michelsen, 2000). Notably, no fossils unambiguously assignable to calyptrate or other schizophoran Diptera have been found in Mesozoic deposits (Amorim & Silva, 2002). This fact underlies our decision to impose a hard constraint of a maximum age of 65 My on the root node of our analysis. Nevertheless, the estimated age of Oestroidea in our study is broadly consistent with other recent divergence time estimates (Cerretti et al., 2017; Wiegmann et al., 2011; Zhao et al., 2013). Although our new calibration is based on the same temporal calibration points of Cerretti et al. (2017)—no new oestroid fossils have been discovered in the interim—the topology and dates are supported by new genomic evidence. The most notable difference from previous works lies in the age of the oestroid clade itself. While the difference is not substantial, our analysis suggests that this clade arose slightly later, in the early Eocene, rather than the late Palaeocene. Additionally, our findings indicate that the stem Calliphoridae likely diverged from the stem Polleniidae +Tachinidae in the late Eocene, albeit with a topology for Calliphoridae that is different from those used in previous studies.

According to our results, parasitism of invertebrates originated in early oestroids long before the appearance of saprophagous or vertebrate-parasitic forms. In the Calliphoridae, saprophagous forms differentiated independently at slightly different times in the early ancestors of both the Chrysomyinae and the Luciliinae+Calliphorinae (Figure 4). This differentiation occurred before the Oligocene, a period during which many mammalian and passerine lineages underwent significant radiation (Álvarez-Carretero et al., 2022; Oliveros et al., 2019). The near-concomitant radiation of mammals and passerine birds likely led to a rise in vertebrate biomass (Benevento et al., 2023), and therefore in the availability of carcasses, which could have provided a significant boost for saprophagous species, allowing them to become prominent components of the Cenozoic terrestrial ecosystems at virtually all latitudes. The reconstruction of saprophagy in the Calliphoridae and its temporal calibration suggest a scenario in which certain lineages capitalized on an increase in available resources. In contrast, vertebrate parasitism appears to have evolved more recently in all lineages where it is found, first appearing between the middle and late Miocene (Figure 4.).

The divergence of Ameniinae and Rhinophorinae occurred in the late Eocene. Since then, these two groups have radiated, exploiting different trophic substrates, although both remain associated with hosts tied to humid soil environments. Ameniinae use land snails and Rhinophorinae oniscid isopods. The hosts of the common ancestor of Ameniinae and Rhinophorinae were probably soil-dwelling invertebrates. The stem Bengaliinae and Rhiniinae diverged during the early Oligocene; both lineages probably had trophic habits involving invertebrates, although, as noted below, there are significant exceptions within Bengaliinae. This analysis corroborates a scenario of oestroid evolution—and of Calliphoridae in particular—as a relatively short burst of episodic radiation events (Wiegmann et al., 2011) driven by adaptations that allowed efficient exploitation of emerging trophic resources during larval development.

Larval feeding and the origins of parasitism within Calliphoridae

Overall, our analyses suggest larval feeding habits of Calliphoridae have a high degree of phylogenetic conservatism (Figure 4). The clade comprising Ameniinae and Rhinophorinae is largely obligate invertebrate parasites; Ameniinae are recorded as parasites of land snails (Colless, 1998), and Rhinophorinae are parasites of woodlice (Crosskey, 1977; Rognes, 1990). The larval feeding habits of the Bengaliinae and Rhiniinae are poorly known, but adults and larvae of many species have been associated with insects, especially termites and ants (Arce et al., 2020; Rognes, 2011; Thomas-Cabianca et al., 2023). Additionally, in Bengaliinae the genera *Auchmeromyia* Brauer & Bergenstamm, *Booponus* Aldrich, *Cordylobia* Grünberg, and *Pachychoeromyia* Villeneuve are obligate vertebrate parasites of mammals (Rognes, 2011). We consistently recover *Auchmeromyia* and *Cordylobia* as sister groups with strong statistical support (Figures S1–S10, Figure S3); however, because our sampling lacked the remaining rare vertebrate parasite genera, we cannot assess if this feeding habit had single or multiple origins in the subfamily. Calliphorinae comprise a substantial proportion of carrion feeders, but certain large lineages contain parasites; for example, the former "Melanomyinae" and *Pericallimyia* are land snail parasites, whereas some Australian *Calliphora*, *Bellardia* and *Onesia* feed on earthworms. The Luciliinae and Chrysomyinae are largely carrion feeders, but in contrast to the previous lineages, the larval feeding habits are less phylogenetically conserved.

Based on larval morphology, Keilin (1915) suggested that parasitism was the ancestral state of Cyclorrhapha. Our taxon sample is insufficient to test this, but our ancestral state reconstruction suggests the ancestral Calliphoridae were larval parasites of invertebrates. Furthermore, we have recovered the independent emergence of saprophagy from obligate larval parasite ancestors in the Chrysomyinae and the Luciliinae+Calliphorinae clade (Figure 4). At least for the origins of obligate invertebrate parasitism, these results challenge earlier models arguing that the parasitic lifestyle evolved in a progression from ancestral saprophagy to facultative parasitism and finally to obligate parasitism (Zumpt, 1965).

Additionally, our analysis suggests that vertebrate parasitism evolved at least five times, with hosts recruited from amphibians, birds and mammals (Figure 4). This feeding habit has evolved in only three major groups—Chrysomyinae, Bengaliinae and Luciliinae—and this pattern is unlikely to result from biased sampling, given current knowledge of larval feeding habits in the family. In the Chrysomyinae, obligate vertebrate parasitism appears to have evolved three times: once likely from facultative parasitism as seen in *Protocalliphora* Hough spp., whose larvae feed intermittently as blood suckers on nestling birds (Prado E Castro et al., 2016), and twice independently from saprophagy, as seen in *Cochliomyia hominivorax* and *Chrysomyia bezziana*, whose larvae feed on the living tissues of mammals. In Bengaliinae, vertebrate parasitism has evolved at least once, possibly from invertebrate parasitism or predation, although the probabilities of the ancestral feeding habits of the surrounding nodes are low. Finally, in Luciliinae, vertebrate parasitism likely evolved once from facultative parasitism in two *Lucilia* species that specialize in amphibian parasitism (Figure 4). The different hosts and multiple origins of vertebrate parasitism observed across the phylogeny suggest that the transition to vertebrate parasitism has different ecological and/or physiological causes.

Our results corroborate the multiple origins of vertebrate parasitism consistently recovered in previous studies (McDonagh & Stevens, 2011; Nasser et al., 2021; Stevens, 2003; Stevens & Wall, 1997). However, the evidence for the ancestral feeding habits that likely precede vertebrate parasitism is inconclusive. Stevens and Wall (1997) found no evidence for a gradual progression towards obligatory myiasis among species of the genus *Lucilia*. In contrast, Arias-Robledo et al. (2019) found support for this progression in *L. silvarum* (Meigen). McDonagh and Stevens (2011) suggested that vertebrate parasitism evolved and possibly disappeared in several blow fly lineages. Our results do not indicate the loss or regain of vertebrate parasitism, but multiple losses and regains were observed for obligate invertebrate parasitism.

Understanding the evolution of larval feeding habits within Calliphoridae is critical due to their veterinary and forensic significance, making this economically and societally relevant. Although previous studies have attempted to elucidate this topic, key knowledge gaps remained due to three major constraints: (1) poor taxon sampling, (2) lack of phylogenetic resolution and (3) limited knowledge of larval feeding habits in understudied groups. Our dataset allowed us to address the first two; however, empirical documentation of larval feeding behaviour remains strikingly limited, with significant knowledge gaps persisting in Phumosiinae, Bengaliinae and Rhiniinae. Addressing these gaps will require integrative natural history studies focused on these understudied groups (e.g., Schär et al., 2025). Consequently, incorporating these new data may significantly influence ancestral state reconstructions, particularly in the case of Phumosiinae due to its critical phylogenetic position.

CONCLUSION

Our phylogeny provides a detailed reconstruction of the history of blow fly divergences, with the origin of the family (40.91 mya) and subfamilies ($\sim$ 35 mya) in the Eocene and major radiations within the subfamilies occurring predominantly in the Miocene (23–10 mya). Ultimately, our well-sampled and renewed phylogenetic understanding of blow flies and their relatives provides a more accurate context for comparative and functional genomic studies to characterize the adaptations that propel trophic specialization. Here, we reinterpret the evolution of saprophagy in one of the most iconic groups involved in decomposing and recycling animal carcasses. Our analysis rejects previous hypotheses and suggests that larval saprophagy is derived in the Calliphoridae and has evolved independently at least twice from ancestors characterized by larval parasitism on invertebrates. Furthermore, vertebrate parasitism has evolved independently from different feeding habits at least five times.

AUTHOR CONTRIBUTIONS

Study design: Cristian F. Beza-Beza, John Soghigian, Ezra Bailey, Kelly A. Meiklejohn, Tatiana T. Torres, Maxwell J. Scott, Paul V. Hickner, Aram Mikaelyan and Brian M. Wiegmann. Data collection: Cristian F. Beza-Beza, John Soghigian, Ezra Bailey, Nikolas P. Johnston, Brian K. Cassel, Keith M. Bayless, Jeffrey D. Wells, James F. Wallman, Arianna Thomas-Cabianca, Andrzej Grzywacz, Kelly A. Meiklejohn, Pierfilippo Cerretti, Krzysztof Szpila and Thomas Pape. Data analyses: Cristian F. Beza-Beza and John Soghigian. Results interpretation: Cristian F. Beza-Beza, Pierfilippo Cerretti, Krzysztof Szpila, Thomas Pape and Brian M. Wiegmann. Writing—primary draft preparation: Cristian F. Beza-Beza, Pierfilippo Cerretti, Thomas Pape and Brian M. Wiegmann. Writing—review and editing: Ezra Bailey, Nikolas P. Johnston, Jeffrey D. Wells, David K. Yeates, James F. Wallman, Liping Yan, Arianna Thomas-Cabianca, Paul V. Hickner, Andrzej Grzywacz, Kelly A. Meiklejohn, Tatiana T. Torres, Maxwell J. Scott, Dong Zhang and Krzysztof Szpila. Supervision: Thomas Pape and Brian M. Wiegmann.

ACKNOWLEDGEMENTS

This study was supported by the NSF Dimensions of Biodiversity Program (DEB-2030345) and National Science Centre, Poland project (2018/31/B/NZ8/02113), PC received support from The as part of the National Biodiversity Future Center, Italian National Recovery and Resilience Plan (NRRP) Mission 4 Component 2 Investment 1.4 (CUP: B83C22002950007). NPJ received support from the Australian Biological Resources Study (4-G046X5I). Many thanks to Dr. George Heimpel for providing comments on the manuscript. The US Department of Agriculture, Agricultural Research Service is an equal opportunity/affirmative action employer, and all agency services are available without discrimination. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

Specimen collection and export were conducted under the following permits: **Arianna Thomas-Cabianca:** DNA Extractions of Rhiniinae specimens deposited in BMSA, NHMD, AMSA, SAMC, CEUA, and MZUR were done with the permission of the curators during her PhD research. **Krzysztof Szpila:** Australia (New South Wales; Department of Planning, Industry and Environment, licence SL101850), Australia (Northern Territory; Parks and Wildlife Commission, permit 61438), Ethiopia (2013; Ethiopian Wildlife Conservation Authority, permit 79/2013), French Guiana (2021, 2022; Ministère de la Transition Écologique, permits 28/09/2021, 28/09/2022), Poland (collected under project NCN 2018/31/B/NZ8/02113; no permit required), South Africa (2023; Province of the Eastern Cape, permit HO/BABS/RSH/06/2023). **Nikolas Johnston** Australia (New South Wales; Office of Environment and Heritage, licence SL101850). **Pierfilippo Cerretti:** Australia (New South Wales, 2013; Office of Environment and Heritage, licence SL101236), Australia (Queensland, 2013; Department of Environment and Heritage Protection, permit WITK13349513), Chile (2015; Corporación Nacional Forestal, permit 030/2015), Ecuador (2017; INABIO, permit 097-17-EXP-ICFAU-DNB/MA), Italy (collected outside protected areas; no permit required), Namibia (2018; National Commission on Research Science and Technology, authorization AN20181007). **Brazilian specimens**: *Paralucilia pseudolyrcea* (Marco Marinho; Brazil: ND; SISGEN No A47F26D), *Lucilia japuhybensis* (Vanessa A.S. Cunha, Leticia C.B. de Paula, Diniz L. Ferreira; Brazil: RS, Pelotas; SISGEN No A01B3B3), *Chloroprocta idioidea* (Marco Marinho; Brazil: RR, Maraca; SISGEN No A01B3B3), *Lucilia ochricornis* (Vanessa A.S. Cunha, Leticia C.B. de Paula, Diniz L. Ferreira; Brazil: RS, Pelotas; SISGEN No A01B3B3).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Sequence data generated for this study are available in the NCBI repository (https://www.ncbi.nlm.nih.gov/) under BioProject PRJNA1238278. Supplementary files, fly genome reference database and nucleotide and amino acid alignments can be accessed at Figshare (https://doi.org/10.6084/m9.figshare.28100567.v2). Supplementary files include nexus files of the trees obtained from the analyses of each matrix, a custom R script for detection of misassigned orthologues, concatenated alignments and IQTree partition files.

ORCID

Ezra Bailey https://orcid.org/0009-0000-4555-5972
Pierfilippo Cerretti https://orcid.org/0000-0002-9204-3352
Thomas Pape https://orcid.org/0000-0001-6609-0609
Brian M. Wiegmann https://orcid.org/0000-0002-1869-9759

REFERENCES

Altson, A.M. (1932) On the feeding habits and breeding of *Ochromyia* (*Bengalia*) *depressa*, R.-D., and *O. Peuhi*. *Proceedings of the Royal Entomological Society of London*, 7, 36–40.

Álvarez-Carretero, S., Tamuri, A.U., Battini, M., Nascimento, F.F., Carlisle, E., Asher, R.J. et al. (2022) A species-level timeline of mammal evolution integrating phylogenomic data. *Nature*, 602, 263–267. Available from: https://doi.org/10.1038/s41586-021-04341-1

Amorim, D.D.S. & Silva, V.C. (2002) How far advanced was Diptera evolution in the Pangaea? *Annales De La Société Entomologique De France*, 38, 177–200.

Arce, B.J., Clout, S., Pat, D.L., Bharti, M., Pape, T. & Marshall, S.A. (2020) Viviparity and oviparity in termitophilous Rhiniidae (Diptera: Oestroidea) in the Western Ghats, India. *Oriental Insects*, 54, 259–264. Available from: https://doi.org/10.1080/00305316.2019.1618407

Arias-Robledo, G., Wall, R., Szpila, K., Shpeley, D., Whitworth, T., Stark, T. et al. (2019) Ecological and geographical speciation in *Lucilia bufonivora*: the evolution of amphibian obligate parasitism. *International Journal for Parasitology: Parasites and Wildlife*, 10, 218–230. Available from: https://doi.org/10.1016/j.ijppaw.2019.09.005

Banerjee, A., Rognes, K. & Whitmore, D. (2018) Two species of *Caiusa* Surcouf (Diptera: Calliphoridae) new to India, with data on larval behaviour and morphology. *Biodiversity Data Journal*, 6, e27736. Available from: https://doi.org/10.3897/BDJ.6.e27736

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S. et al. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, 19, 455–477. Available from: https://doi.org/10.1089/cmb.2012.0021

Benevento, G.L., Benson, R.B.J., Close, R.A. & Butler, R.J. (2023) Early Cenozoic increases in mammal diversity cannot be explained solely by expansion into larger body sizes. *Palaeontology*, 66, e12653. Available from: https://doi.org/10.1111/pala.12653

Beza-Beza, C. (2025) Data of Phylogenomics and the evolution of larval feeding habits in the blow flies (Diptera: Calliphoridae), Beza-Beza et al.; figshare; Version 2. https://doi.org/10.6084/m9.figshare.28100567.v2

Bolger, A.M., Lohse, M. & Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30, 2114–2120. Available from: https://doi.org/10.1093/bioinformatics/btu170

Brauer, F. & Bergenstamm, V.E.J. (1924) Vorarbeiten zu Einer Monographie der Muscaria Schizometopa. In: *Die Zweiflügler (Dipteren) des Kais. Museums zu Wien*. Dordrecht: Springer Netherlands, pp. 290–422. Available from: https://doi.org/10.1007/978-94-010-3712-9_4

Buenaventura, E., Lloyd, M.W., Perilla López, J.M., González, V.L., Thomas-Cabianca, A. & Dikow, T. (2021) Protein-encoding ultraconserved elements provide a new phylogenomic perspective of Oestroidea flies (Diptera: Calyptratae). *Systematic Entomology*, 46, 5–27. Available from: https://doi.org/10.1111/syen.12443

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K. et al. (2009) BLAST+: architecture and applications. *BMC Bioinformatics*, 10, 421. Available from: https://doi.org/10.1186/1471-2105-10-421

Capella-Gutiérrez, S., Silla-Martínez, J.M. & Gabaldón, T. (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, 25, 1972–1973. Available from: https://doi.org/10.1093/bioinformatics/btp348

Cardoso, G.A., Deszo, M.S. & Torres, T.T. (2021) Evolution of coding sequence and gene expression of blowflies and botflies with contrasting feeding habits. *Genomics*, 113, 699–706. Available from: https://doi.org/10.1016/j.ygeno.2020.09.066

Cerretti, P., Stireman, J.O., Badano, D., Gisondi, S., Rognes, K., Giudice, G.L. et al. (2019) Reclustering the cluster flies (Diptera: Oestroidea, Polleniidae). *Systematic Entomology*, 44, 957–972. Available from: https://doi.org/10.1111/syen.12369

Cerretti, P., Stireman, J.O., Pape, T., O'Hara, J.E., Marinho, M.A.T., Rognes, K. et al. (2017) First fossil of an oestroid fly (Diptera: Calyptratae: Oestroidea) and the dating of oestroid divergences. *PLoS One*, 12, e0182101. Available from: https://doi.org/10.1371/journal.pone.0182101

Cerretti, P., Yan, L., Narayanan Kutty, S., Szpila, K., Nania, D., Tintea, R. et al. (2024) Phylogenomics resolves long-standing questions about the affinities of an endangered Corsican endemic fly. *Journal of Insect Science*, 24, 9. Available from: https://doi.org/10.1093/jisesa/ieae073

Charabidze, D., Trumbo, S., Grzywacz, A., Costa, J.T., Benbow, M.E., Barton, P.S. et al. (2021) Convergence of social strategies in carrion breeding insects. *Bioscience*, 71, 1028–1037. Available from: https://doi.org/10.1093/biosci/biab068

Chen, S., Zhou, Y., Chen, Y. & Gu, J. (2018) Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, 34, i884–i890. Available from: https://doi.org/10.1093/bioinformatics/bty560

Clark, S.A. (2009) A review of the land snail genus *Meridolum* (Gastropoda: Camaenidae) from central New South Wales, Australia. *Molluscan Research*, 29, 61–120. Available from: https://doi.org/10.11646/mr.29.2.1

Colless, D.C. (1998) Morphometrics in the genus *Amenia* and revisionary notes on the Australian Ameniinae (Diptera: Calliphoridae), with the description of eight new species. *Records of the Australian Museum*, 50, 85–123. Available from: https://doi.org/10.3853/j.0067-1975.50.1998.1275

Concha, C., Palavesam, A., Guerrero, F.D., Sagel, A., Li, F., Osborne, J.A. et al. (2016) A transgenic male-only strain of the New World screwworm for an improved control program using the sterile insect technique. *BMC Biology*, 14, 72. Available from: https://doi.org/10.1186/s12915-016-0296-8

Crosskey, R.W. (1965) A systematic revision of the Ameniidae (Diptera: Calliphoridae). *Bulletin of the British Museum (Natural History) Entomology*, 16, 33–140.

Crosskey, R.W. (1977) A review of the Rhinophoridae (Diptera) and a revision of the Afrotropical species. *Bulletin of the British Museum (Natural History) Entomology*, 36, 1–67.

Dallwitz, R., Roberts, J.A. & Kitching, R.L. (1984) Factors determining the predominance of *Lucilia cuprina* larvae in blowfly strikes of sheep in southern New South Wales. *Australian Journal of Entomology*, 23, 175–177. Available from: https://doi.org/10.1111/j.1440-6055.1984.tb01940.x

De Queiroz, K. & Gauthier, J. (1990) Phylogeny as a central principle in taxonomy: phylogenetic definitions of taxon names. *Systematic Zoology*, 39, 307. Available from: https://doi.org/10.2307/2992353

De Queiroz, K. & Gauthier, J. (1992) Phylogenetic taxonomy. *Annual Review of Ecology and Systematics*, 23, 449–480.

Dos Reis, M. (2017) Estimating the marginal likelihood of a relaxed-clock model with MCMCTree.

Dos Reis, M. & Yang, Z. (2019) Bayesian molecular clock dating using genome-scale datasets. In: Anisimova, M. (Ed.) *Evolutionary genomics, methods in molecular biology*. New York, NY: Springer New York, pp. 309–330. Available from: https://doi.org/10.1007/978-1-4939-9074-0_10

Erzinclioglu, Y.Z. (1989) The evolution of parasitism in blowflies. *British Journal of Entomology and Natural History*, 2, 125–127.

Evenhuis, N.L. & Pape, T. (2024) Systema Dipterorum. https://doi.org/10.48580/DGDWL-3BZ

Ferrar. (2024) *Guide to the breeding habits and immature stages of Diptera Cyclorrhapha*. Leiden, The Netherlands: BRILL.

Ferrar, P. (1976) Macrolarviparous reproduction in Ameniinae (Diptera: Calliphoridae). *Systematic Entomology*, 1, 107–116. Available from: https://doi.org/10.1111/j.1365-3113.1976.tb00343.x

Ferrar, P. (1978) Macrolarviparous reproduction in *Euphumosia* (Diptera: Calliphoridae). *Australian Journal of Entomology*, 17, 13–17. Available from: https://doi.org/10.1111/j.1440-6055.1978.tb01739.x

Girschner, E. (1894) Beitrag zur Systematik der Musciden. *Berliner Entomologische Zeitschrift*, 38, 297–312. Available from: https://doi.org/10.1002/mmnd.18940380305

Gisondi, S., Buenaventura, E., Jensen, A.R., Stireman, J.O., Nihei, S.S., Pape, T. et al. (2023) Phylogenetic relationships of the woodlouse flies (Diptera: Rhinophorinae) and the cluster flies (Diptera: Polleniidae). *PLoS One*, 18, e0285855. Available from: https://doi.org/10.1371/journal.pone.0285855

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I. et al. (2011) Full-length transcriptome assembly from RNA-seq data without a reference genome. *Nature Biotechnology*, 29, 644–652. Available from: https://doi.org/10.1038/nbt.1883

Graca-Souza, A., Mayamonteiro, C., Paivasilva, G., Braz, G., Paes, M., Sorgine, M. et al. (2006) Adaptations against heme toxicity in blood-feeding arthropods. *Insect Biochemistry and Molecular Biology*, 36, 322–335. Available from: https://doi.org/10.1016/j.ibmb.2006.01.009

Grimaldi, D.A. (2018) Basal Cyclorrhapha in amber from the cretaceous and tertiary (Insecta: Diptera), and their relationships: Brachycera in cretaceous Amber part IX. *Bulletin of the American Museum of Natural History*, 423, 1–97. Available from: https://doi.org/10.1206/0003-0090-423.1.1

Grimaldi, D.A. & Engel, M.S. (2005) *Evolution of the insects*. Cambridge: Cambridge university press.

Guimarães, J.H. (1977) A systematic revision of the Mesembrinellidae, stat. Nov. (diptera, cyclorrhapha). *Arquivos de Zoologia*, 29, 1. Available from: https://doi.org/10.11606/issn.2176-7793.v29i1p1-109

Hall, M. & Wall, R. (1995) Myiasis of humans and domestic animals. In: *Advances in parasitology*. Elsevier, pp. 257–334. Available from: https://doi.org/10.1016/S0065-308X(08)60073-1

Hall, M.J.R., Wardhana, A.H., Shahhosseini, G., Adams, Z.J.O. & Ready, P.D. (2009) Genetic diversity of populations of Old World screwworm fly, *Chrysomya bezziana*, causing traumatic myiasis of livestock in the Gulf region and implications for control by sterile insect technique. *Medical and Veterinary Entomology*, 23, 51–58. Available from: https://doi.org/10.1111/j.1365-2915.2008.00778.x

Hickner, P.V., Mittapalli, O., Subramoniam, A., Sagel, A., Watson, W., Scott, M.J. et al. (2020) Physiological and molecular correlates of the screwworm fly attraction to wound and animal odors. *Scientific Reports*, 10, 20771. Available from: https://doi.org/10.1038/s41598-020-77541-w

Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q. & Vinh, L.S. (2018) UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35, 518–522. Available from: https://doi.org/10.1093/molbev/msx281

Johnston, N.P., Szpila, K., Pape, T., Meiklejohn, K.A., Foley, L.B. & Wallman, J.F. (2025) A species-group framework to unravel blowfly diversity: integrative revision of the *Calliphora clarki*-group (Diptera: Calliphoridae). *European Journal of Taxonomy*, 989, 94–118. Available from: https://doi.org/10.5852/ejt.2025.989.2887

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Von Haeseler, A. & Jermiin, L.S. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14, 587–589. Available from: https://doi.org/10.1038/nmeth.4285

Kapoor, S., Hickner, P.V., Dickey, A.N., Bailey, E.M., Baldassio de Paula, L.C., Belikoff, E.J. et al. (2025) Comparative genomic analysis of necrophagous and parasitic subspecies of *Lucilia cuprina* (Diptera: Calliphoridae) provides important insights into their divergent Biologies. *International Journal for Parasitology*. Available from: https://doi.org/10.1016/j.ijpara.2025.06.001

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780. Available from: https://doi.org/10.1093/molbev/mst010

Kawahara, A.Y., Plotkin, D., Espeland, M., Meusemann, K., Toussaint, E.F.A., Donath, A. et al. (2019) Phylogenomics reveals the evolutionary timing and pattern of butterflies and moths. *Proceedings of the National Academy of Sciences*, 116, 22657–22663. Available from: https://doi.org/10.1073/pnas.1907847116

Keilin, D. (1915) Cycle évolutif de *Pollenia rudis* Fabr., parasite d'*Allolobophora chlorotica* Sav.: biologie comparée des larves de Diptères. *Bulletin Scientifique de la France et de la Belgique*, 49, 15–198.

Kriventseva, E.V., Kuznetsov, D., Tegenfeldt, F., Manni, M., Dias, R., Simão, F.A. et al. (2019) OrthoDB v10: sampling the diversity of animal, plant, fungal, protist, bacterial and viral genomes for evolutionary and functional annotations of orthologs. *Nucleic Acids Research*, 47, D807–D811. Available from: https://doi.org/10.1093/nar/gky1053

Kurahashi, H. (1971) The tribe Calliphorini from Australian and oriental regions II. *Calliphora*-group (Diptera: Calliphoridae). *Pacific Insects*, 13, 141–204.

Kurahashi, H. (1972a) Tribe Calliphorini from Australian and oriental regions, I. *Melinda*-group (Diptera: Calliphoridae). *Pacific Insects*, 14, 709–724.

Kurahashi, H. (1972b) Tribe Calliphorini from Australian and oriental regions, IV. *Onesia*-group: genus *Polleniopsis*. *Pacific Insects*, 14, 709–724.

Kurahashi, H. (1978) Tribe Calliphorini from Australian and oriental regions, V. *Onesia*-group: genus *Tainanina*. *Pacific Insects*, 18, 1–8.

Kutty, S.N., Meusemann, K., Bayless, K.M., Marinho, M.A.T., Pont, A.C., Zhou, X. et al. (2019) Phylogenomic analysis of Calyptratae: resolving the phylogenetic relationships within a major radiation of Diptera. *Cladistics*, 35, 605–622. Available from: https://doi.org/10.1111/cla.12375

Lemmon, A.R., Emme, S.A. & Lemmon, E.M. (2012) Anchored hybrid enrichment for massively high-throughput phylogenomics. *Systematic Biology*, 61, 727–744. Available from: https://doi.org/10.1093/sysbio/sys049

Luong, L.T. & Mathot, K.J. (2019) Facultative parasites as evolutionary stepping-stones towards parasitic lifestyles. *Biology Letters*, 15, 20190058. Available from: https://doi.org/10.1098/rsbl.2019.0058

Marinho, M.A.T., Wolff, M., Ramos-Pastrana, Y., De Azeredo-Espin, A.M. L. & Amorim, D.D.S. (2017) The first phylogenetic study of Mesembrinellidae (Diptera: Oestroidea) based on molecular data: clades and congruence with morphological characters. *Cladistics*, 33, 134–152. Available from: https://doi.org/10.1111/cla.12157

Marshall, S.A. (2012) *Flies: the natural history and diversity of Diptera*. Richmond Hill, Ont Buffalo, NY: Firefly Books.

McDonagh, L.M. & Stevens, J.R. (2011) The molecular systematics of blowflies and screwworm flies (Diptera: Calliphoridae) using 28S rRNA, COX1 and EF-1α: insights into the evolution of dipteran parasitism. *Parasitology*, 138, 1760–1777. Available from: https://doi.org/10.1017/S0031182011001089

McKenna, D.D., Shin, S., Ahrens, D., Balke, M., Beza-Beza, C., Clarke, D.J. et al. (2019) The evolution and genomic basis of beetle diversity. *Proceedings of the National Academy of Sciences*, 116, 24729–24737. Available from: https://doi.org/10.1073/pnas.1909655116

Meyer, M. & Kircher, M. (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protocols*, 2010, pdb.prot5448. Available from: https://doi.org/10.1101/pdb.prot5448

Michelsen, V. (1996) First reliable record of a fossil species of Anthomyiidae (Diptera), with comments on the definition of recent and fossil clades in phylogenetic classification. *Biological Journal of the Linnean Society*, 58, 441–451. Available from: https://doi.org/10.1111/j.1095-8312.1996.tb01445.x

Michelsen, V. (2000) Oldest authentic record of a fossil calyptrate fly (Diptera): a species of Anthomyiidae from early Coenozoic Baltic amber. *Studia Dipterologica*, 7, 11–18.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., Von Haeseler, A. et al. (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37, 1530–1534. Available from: https://doi.org/10.1093/molbev/msaa015

Misof, B., Liu, S., Meusemann, K., Peters, R.S., Donath, A., Mayer, C. et al. (2014) Phylogenomics resolves the timing and pattern of insect evolution. *Science*, 346, 763–767. Available from: https://doi.org/10.1126/science.1257570

Nasser, M.G., Hosni, E.M., Kenawy, M.A., Alharbi, S.A., Almoallim, H.S., Rady, M.H. et al. (2021) Evolutionary profile of the family Calliphoridae, with notes on the origin of myiasis. *Saudi Journal of Biological Sciences*, 28, 2056–2066. Available from: https://doi.org/10.1016/j.sjbs.2021.01.032

Oliveros, C.H., Field, D.J., Ksepka, D.T., Barker, F.K., Aleixo, A., Andersen, M.J. et al. (2019) Earth history and the passerine superradiation. *Proceedings of the National Academy of Sciences*, 116, 7916–7925. Available from: https://doi.org/10.1073/pnas.1813206116

Overstreet, R.M. & Lotz, J.M. (2016) Host–symbiont relationships: understanding the change from guest to pest. In: Hurst, C.J. (Ed.) *The Rasputin effect: when commensals and symbionts become parasitic, advances in environmental microbiology*. Cham: Springer International Publishing, pp. 27–64. Available from: https://doi.org/10.1007/978-3-319-28170-4_2

Pape, T., Blagoderov, V. & Mostovski, M.B. (2011) Order Diptera Linnaeus, 1758. In: Zhang, Z.-Q. (Ed.) *Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness*. Zootaxa, 3148 (1), 222–229. Available from: https://doi.org/10.11646/zootaxa.3148.1.42

Parmar, D.R., Johnston, N.P. & Szpila, K. (2025) Large-scale comparative analysis of blowfly mitogenomes, with an emphasis on Calliphorinae (Diptera: Calliphoridae), provides evolutionary insights into mitochondrial DNA structure, gene rearrangements, and inter- and intra-subfamilial relationships among blowflies. *International Journal of Biological Macromolecules*, 323(2), 147063. Available from: https://doi.org/10.1016/j.ijbiomac.2025.147063

Parmar, D.R., Johnston, N.P., Wallman, J.F. & Szpila, K. (2025) Blowfly genomics: current insights, knowledge gaps, and future perspectives. *Current Opinion in Insect Science*, 68, 101305. Available from: https://doi.org/10.1016/j.cois.2024.101305

Petersen, M., Meusemann, K., Donath, A., Dowling, D., Liu, S., Peters, R.S. et al. (2017) Orthograph: a versatile tool for mapping coding nucleotide sequences to clusters of orthologous genes. *BMC Bioinformatics*, 18, 111. Available from: https://doi.org/10.1186/s12859-017-1529-8

Politis, D.N. & Romano, J.P. (1994) The stationary bootstrap. *Journal of the American Statistical Association*, 89, 1303–1313. Available from: https://doi.org/10.1080/01621459.1994.10476870

Pont, A. & Carvalho, C. (1997) Three species of Muscidae (Diptera) from Dominican amber. *Studia Dipterologica*, 4, 173–181.

Prado E Castro, C., Szpila, K., Martínez-Sánchez, A.I., Rego, C., Silva, I., Serrano, A.R.M. et al. (2016) The blowflies of the Madeira archipelago: species diversity, distribution and identification (Diptera, Calliphoridae s. l.). *ZooKeys*, 634, 101–123. Available from: https://doi.org/10.3897/zookeys.634.9262

Rader, R., Cunningham, S.A., Howlett, B.G. & Inouye, D.W. (2020) Non-bee insects as visitors and pollinators of crops: biology, ecology, and management. *Annual Review of Entomology*, 65, 391–407. Available from: https://doi.org/10.1146/annurev-ento-011019-025055

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarization in Bayesian phylogenetics using tracer 1.7. *Systematic Biology*, 67, 901–904. Available from: https://doi.org/10.1093/sysbio/syy032

Ratnasingham, S. & Hebert, P.D.N. (2007) BOLD: the barcode of life data system (http://www.barcodinglife.org). *Molecular Ecology Notes*, 7, 355–364. Available from: https://doi.org/10.1111/j.1471-8286.2007.01678.x

Reis, M.D., Gunnell, G.F., Barba-Montoya, J., Wilkins, A., Yang, Z. & Yoder, A.D. (2018) Using phylogenomic data to explore the effects of relaxed clocks and calibration strategies on divergence time estimation: primates as a test case. *Systematic Biology*, 67, 594–615. Available from: https://doi.org/10.1093/sysbio/syy001

Revell, L.J. (2024) Phytools 2.0: an updated R ecosystem for phylogenetic comparative methods (and other things). *PeerJ*, 12, e16505. Available from: https://doi.org/10.7717/peerj.16505

Rognes, K. (1986) The systematic position of the genus *Helicobosca* Bezzi with a discussion of the monophyly of the calyptrate families Calliphoridae, Rhinophoridae, Sarcophagidae and Tachinidae (Diptera). *Insect Systematics & Evolution*, 17, 75–92. Available from: https://doi.org/10.1163/187631286X00125

Rognes, K. (1990) *Blowflies (Diptera, Calliphoridae) of Fennoscandia and Denmark*, 1st edition. Leiden, The Netherlands: BRILL.

Rognes, K. (1997) The Calliphoridae (blowflies) (Diptera: Oestroidea) are not a monophyletic group [1]. *Cladistics*, 13, 27–66. Available from: https://doi.org/10.1111/j.1096-0031.1997.tb00240.x

Rognes, K. (1998) Family Calliphoridae. In: Papp, L. & Darvas, B. (Eds.) *Manual of Palaearctic Diptera, vol 3: higher Brachycera*. Budapest, Hungary: Science Herald, pp. 648–671.

Rognes, K. (2011) *A review of the monophyly and composition of the Bengaliinae with the description of a new genus and species, and new evidence for the presence of Melanomyinae in the Afrotropical region (Diptera, Calliphoridae)*. Auckland, New Zealand: Magnolia Press.

Schär, S., Talavera, G., Dapporto, L., Bruschini, C., Dincă, V., Beza-Beza, C. et al. (2025) Blow fly larvae socially integrate termite nests through morphological and chemical mimicry. *Current Biology*, 35, 1121–1127.e3. Available from: https://doi.org/10.1016/j.cub.2025.01.007

Smith, S.A., Brown, J.W. & Walker, J.F. (2018) So many genes, so little time: a practical approach to divergence-time estimation in the genomic era. *PLoS One*, 13, e0197433. Available from: https://doi.org/10.1371/journal.pone.0197433

Soghigian, J., Sither, C., Justi, S.A., Morinaga, G., Cassel, B.K., Vitek, C.J. et al. (2023) Phylogenomics reveals the history of host use in mosquitoes. *Nature Communications*, 14, 6252. Available from: https://doi.org/10.1038/s41467-023-41764-y

Stevens, J. & Wall, R. (1997) The evolution of ectoparasitism in the genus Lucilia (Diptera: Calliphoridae). *International Journal for Parasitology*, 27, 51–59. Available from: https://doi.org/10.1016/S0020-7519(96)00155-5

Stevens, J.R. (2003) The evolution of myiasis in blowflies (Calliphoridae). *International Journal for Parasitology*, 33, 1105–1113. Available from: https://doi.org/10.1016/S0020-7519(03)00136-X

Stevens, J.R., Wallman, J.F., Otranto, D., Wall, R. & Pape, T. (2006) The evolution of myiasis in humans and other animals in the old and new worlds (part II): biological and life-history studies. *Trends in Parasitology*, 22, 181–188. Available from: https://doi.org/10.1016/j.pt.2006.02.010

Stireman, J.O., O'Hara, J.E. & Wood, D.M. (2006) TACHINIDAE: evolution, behavior, and ecology. *Annual Review of Entomology*, 51, 525–555. Available from: https://doi.org/10.1146/annurev.ento.51.110104.151133

Thomas-Cabianca, A., Villet, M., Martínez-Sánchez, A. & Rojo, S. (2023) South African nose flies (Diptera, Calliphoridae, Rhiniinae): taxonomy, diversity, distribution and biology. *Biodiversity Data Journal*, 11, e72764. Available from: https://doi.org/10.3897/BDJ.11.e72764

Thorne, J.L., Kishino, H. & Painter, I.S. (1998) Estimating the rate of evolution of the rate of molecular evolution. *Molecular Biology and Evolution*, 15, 1647–1657. Available from: https://doi.org/10.1093/oxfordjournals.molbev.a025892

Wallman, J.F. & Adams, M. (1997) Molecular systematics of Australian carrion-breeding blowflies of the genus *Calliphora* (Diptera: Calliphoridae). *Australian Journal of Zoology*, 45, 337–356. Available from: https://doi.org/10.1071/ZO97006

Weidner, L.M., Byrd, J., Sutton, L. & Hans, K.R. (2021) Forensic entomology: an introduction to the literature of the United States. *WIREs Forensic Science*, 3, e1435. Available from: https://doi.org/10.1002/wfs2.1435

Wells, J.D. (2019) A forensic entomological analysis can yield an estimate of postmortem interval, and not just a minimum postmortem interval: an explanation and illustration using a case. *Journal of Forensic Sciences*, 64, 634–637. Available from: https://doi.org/10.1111/1556-4029.13912

Wiegmann, B.M., Trautwein, M.D., Winkler, I.S., Barr, N.B., Kim, J.-W., Lambkin, C. et al. (2011) Episodic radiations in the fly tree of life. *Proceedings of the National Academy of Sciences*, 108, 5690–5695. Available from: https://doi.org/10.1073/pnas.1012675108

Willcox, B.K., Howlett, B.G., Robson, A.J., Cutting, B., Evans, L., Jesson, L. et al. (2019) Evaluating the taxa that provide shared pollination services across multiple crops and regions. *Scientific Reports*, 9, 13538. Available from: https://doi.org/10.1038/s41598-019-49535-w

Wood, C.T., Nihei, S.S. & Araujo, P.B. (2018) Woodlice and their parasitoid flies: revision of isopoda (crustacea, Oniscidea) – Rhinophoridae (Insecta, Diptera) interaction and first record of a parasitized neotropical woodlouse species. *ZooKeys*, 801, 401–414. Available from: https://doi.org/10.3897/zookeys.801.26052

Wyss, J.H. (2000) Screwworm eradication in the Americas. *Annals of the new York Academy of Sciences*, 916, 186–193. Available from: https://doi.org/10.1111/j.1749-6632.2000.tb05289.x

Xuan, J.-L., Scheffer, S.J., Lewis, M., Cassel, B.K., Liu, W.-X. & Wiegmann, B.M. (2023) The phylogeny and divergence times of leaf-mining flies (Diptera: Agromyzidae) from anchored phylogenomics. *Molecular Phylogenetics and Evolution*, 184, 107778. Available from: https://doi.org/10.1016/j.ympev.2023.107778

Yan, L., Pape, T., Meusemann, K., Kutty, S.N., Meier, R., Bayless, K.M. et al. (2021) Monophyletic blowflies revealed by phylogenomics. *BMC Biology*, 19, 230. Available from: https://doi.org/10.1186/s12915-021-01156-4

Yang, Z. (2007) PAML 4: phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution*, 24, 1586–1591. Available from: https://doi.org/10.1093/molbev/msm088

Young, A.D., Lemmon, A.R., Skevington, J.H., Mengual, X., Ståhls, G., Reemer, M. et al. (2016) Anchored enrichment dataset for true flies (order Diptera) reveals insights into the phylogeny of flower flies (family Syrphidae). *BMC Evolutionary Biology*, 16, 143. Available from: https://doi.org/10.1186/s12862-016-0714-0

Zhang, C. & Mirarab, S. (2022) Weighting by gene tree uncertainty improves accuracy of quartet-based species trees. *Molecular Biology and Evolution*, 39, msac215. Available from: https://doi.org/10.1093/molbev/msac215

Zhang, C., Rabiee, M., Sayyari, E. & Mirarab, S. (2018) ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics*, 19, 153. Available from: https://doi.org/10.1186/s12859-018-2129-y

Zhao, Y., Yu, Y., Chen, Z., Li, Y., Teng, H., Chomicki, G. et al. (2024) Predator–prey encounters: deciphering the robbery relationship between highwayman flies and their ant victims. *Integrative Zoology*, 19, 253–261. Available from: https://doi.org/10.1111/1749-4877.12732

Zhao, Z., Su, T., Chesters, D., Wang, S., Ho, S.Y.W., Zhu, C. et al. (2013) The mitochondrial genome of *Elodia flavipalpis* Aldrich (Diptera: Tachinidae) and the evolutionary timescale of tachinid flies. *PLoS One*, 8, e61814. Available from: https://doi.org/10.1371/journal.pone.0061814

Zumpt, F. (1965) *Myiasis in man and animals in the Old World. A textbook for physicians, veterinarians and zoologists*. Kingsway, London W.C.2., UK: Butterworth & Co. (Publishers) Ltd.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Relationship between recovered loci and specimen age.

Figure S2. Comparison of ML topologies within Ameniinae: (A) 75.aa versus TL.aa, (B) 75.12 versus TL.12, (C) 75.aa versus 75.12 and (D) TL.aa versus TL.12.

Figure S3. Comparison of ML topologies within Bengaliinae: (A) 75.aa versus TL.aa, (B) 75.12 versus TL.12, (C) 75.aa versus 75.12 and (D) TL.aa versus TL.12.

Figure S4. Comparison of ML topologies within Chrysomyiinae: (A) 75.aa versus TL.aa, (B) 75.12 versus TL.12, (C) 75.aa versus 75.12 and (D) TL.aa versus TL.12.

Figure S5. Comparison of ML topologies within Phumosiinae: (A) 75.aa versus TL.aa, (B) 75.12 versus TL.12, (C) 75.aa versus 75.12 and (D) TL.aa versus TL.12.

Figure S6. Comparison of ML topologies within Luciliinae: (A) 75.aa versus TL.aa, (B) 75.12 versus TL.12, (C) 75.aa versus 75.12 and (D) TL.aa versus TL.12.

Figure S7. Comparison of ML topologies within Rhiniinae: (A) 75.aa versus TL.aa, (B) 75.12 versus TL.12, (C) 75.aa versus 75.12 and (D) TL.aa versus TL.12.

Figure S8. Comparison of ML topologies within Rhinophorinae: (A) 75.aa versus TL.aa, (B) 75.12 versus TL.12, (C) 75.aa versus 75.12 and (D) TL.aa versus TL.12.

Figure S9. Comparison of ML topologies within Calliphorinae: (A) 75.aa versus TL.aa, (B) 75.12 versus TL.12, (C) 75.aa versus 75.12 and (D) TL.aa versus TL.12.

Figure S10. MCMCtree runs convergent plots.

Figure S11. Chronogram resulted from the summary of five independent MCMCtree runs. Grey bars in nodes represent 95% confidence intervals and red dots fossil calibration points.

Figure S12. Summary of ancestral state reconstruction using 1000 stochastic character mapping runs for the larval feeding habits of Oestroidea.

Figure S13. Summary of ancestral state reconstruction using 1000 stochastic character mapping runs for the larval feeding habits of Calliphoridae + (Pollenidae + Tachinidae).

Figure S14. Summary of ancestral state reconstruction using 1000 stochastic character mapping runs for the feeding substrate of Oestroidea.

Figure S15. Summary of ancestral state reconstruction using 1000 stochastic character mapping runs for the feeding substrate of Calliphoridae + (Pollenidae + Tachinidae).

Figure S16. Summary of ancestral state reconstruction using 1000 stochastic character mapping runs for the feeding substrate of Calliphoridae only.

Data S1: Supporting Information.

Table S1. Voucher information. Vouchers with * were amplified using REPLI-g (see details in methods). Identifications marked with COI refers to species identification using molecular barcodes and the databases of the National Center for Biotechnology Information and the Barcode Of Life Data System. List of voucher depositories: Albany Museum, Grahamstown, South Africa (AMSA); National Museum, Bloemfontein, South Africa (BMSA); Entomological Collection University of Alicante, Alicante, Spain (CEUA); Museu de Zoologia,São Paulo, São Paulo, Brazil (MZSP); Museum of Zoology, Sapienza University of Rome, Rome, Italy (MZUR); North Carolina State University (NCSU); Nicolaus Copernicus University, Toruń, Poland (NCUT); Natural History Museum of Denmark, Copenhagen, Denmark (NHMD); Swedish Museum of Natural History, Stockholm, Sweden (NHRS); KwaZulu-Natal Museum, Pietermaritzburg, South Africa (NMSA); Diptera Collection, Iziko South African Museum, Cape Town, South Africa (SAMC); Universidade Estadual de Campinas, Campina, Brazil (DZIB); University of Technology Sydney (UTS).

Table S2. Summary of pruned taxa per individual loci alignment.

Table S3. Summary of number of times a tip was pruned from an individual loci alignment.

Table S4. Feeding habits and tissue type consumption for the species of Oestroidea in this.

Table S5. Model selection of discreet character evolution using AICw. Models tested were equal rates (ER), symmetrical rates (SYM) and all rates different (ARD). Models with AICw over 0.95 were considered strongly favoured.

How to cite this article: Beza-Beza, C.F., Soghigian, J., Bailey, E., Johnston, N.P., Cassel, B.K., Bayless, K.M. et al. (2026) Phylogenomics and the evolution of larval feeding habits in the blow flies (Diptera: Calliphoridae). *Systematic Entomology*, 51(1), e70018. Available from: https://doi.org/10.1111/syen.70018