



Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Regulatory and coding changes underlying development and host detection in vertebrate-parasitizing blowflies

Pedro Mariano-Martins, Diniz Lima Ferreira, Vanessa Araujo Soares da Cunha, Carolina Kurotuschi Canettieri, Letícia Chiara Baldassio de Paula, Tatiana Teixeira Torres *

Department of Genetics and Evolutionary Biology. Institute of Biosciences. University of São Paulo, Rua do Matão, 277, São Paulo, SP 05508-090, Brazil

ARTICLE INFO

Article history:

Received 17 February 2025

Received in revised form 14 October 2025

Accepted 16 October 2025

Available online xxx

Keywords:

Calliphoridae

Genome

Molecular evolution

Myiasis

Parasitism

ABSTRACT

The evolutionary transition to parasitism in the family Calliphoridae serves as a compelling model to study the genetic and molecular basis of ecological adaptations. In this study, we sequenced and annotated the genomes of *Chrysomya megacephala*, *Chrysomya putoria*, and *Lucilia eximia* to provide a foundation to investigate the evolution of coding and non-coding sequences within the family Calliphoridae. Combining these new resources with publicly available genomes from 11 representatives of the family, we focused on two species with distinct parasitic strategies, *Cochliomyia hominivorax* –a flesh-eating parasite–, and *Protophormia azurea* –a blood-feeding parasite– to identify unique and shared genomic features associated with the evolution of the parasitic lifestyle. Comparative analyses of conserved non-exonic elements revealed 44 genes inferred to convergently evolve at an accelerated evolutionary rate, 41 % of which associate with development, highlighting their potential role in larval feeding specialization. Analyses of coding regions identified genes, including those linked to olfaction, taste, chitin and cuticle formation, inferred to evolve at accelerated rates in both *Co. hominivorax* and *P. azurea*. This study advances our understanding of aspects of the evolution of genomic regions of members of the family under natural selection and their potential contribution to parasitism.

© 2025 Australian Society for Parasitology. Published by Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

1. Introduction

Symbiosis, the intimate association between organisms, is a major driver of evolutionary and ecological processes (Matthews et al., 2019). These interactions range from mutualism, where both partners benefit, to parasitism, where one organism gains at the expense of its host's fitness (Price, 1980; Ewald, 1987). Parasitism, in particular, has long been considered as a major source of lineage diversification (Price, 1980; Karvonen and Seehausen, 2012). Unlike free-living organisms, parasites face distinct selective pressures that allow them to live either in or on another organism.

Large-scale comparative analysis of animal lineages suggests that the diversification rates are often similar between parasitic

and free-living sister groups. However, arthropods, especially mites and flies, stand out as groups in which parasitism has been repeatedly associated with elevated diversification rates (Weinstein and Kuris, 2016). This pattern suggests that the transition to a parasitic lifestyle may have played a key role in the evolutionary success of several arthropod clades, including flies.

The remarkable diversity of fly species is tied to their adaptive radiation to a wide range of functional and trophic niches (Wiegmann et al., 2011). Within Diptera, the calyptrate superfamily Oestroidea is particularly successful in exploring diverse food sources (Wiegmann et al., 1993). This radiation has been associated with the variety of feeding habits these flies have acquired throughout their evolution (Stevens et al., 2006; Stevens and Wallman, 2006). One of the models explaining this phenomenon is the evolutionary transition from a saprophagous to a parasitic lifestyle (Zumpt, 1965; Stevens et al., 2006). A remarkable case is the family Calliphoridae, also known as blowflies. These flies are able to feed on a wide range of vertebrate or invertebrate hosts, consuming either flesh or blood (Stevens and Wallman, 2006; McDonagh and Stevens, 2011; Marinho et al., 2012; Nasser et al., 2021).

* Corresponding author at: Depto. Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, 05508-090, São Paulo, SP, Brazil.

E-mail addresses: pmariano83@gmail.com (P. Mariano-Martins), dinizferreira@usp.br (D.L. Ferreira), vanscunha@gmail.com (V. A. S. d. Cunha), carolina.kc@usp.br (C.K. Canettieri), leticia.baldassio@gmail.com (L.C.B. de Paula), ttorres@ib.usp.br (T.T. Torres).

<https://doi.org/10.1016/j.ijpara.2025.10.004>

0020-7519/© 2025 Australian Society for Parasitology. Published by Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

Parasitism has evolved independently multiple times within the family Calliphoridae (Stevens et al., 2006). Blowflies exhibit a specialized form of parasitism known as myiasis, in which their larvae develop on or within a vertebrate host (Zumpt, 1965). One of the most studied species in this group is *Cochliomyia hominivorax*, an obligate parasite of humans and other mammals in the Americas (Wyss, 2000). Females oviposit in open wounds, and larvae feed on living tissue, worsening the wounds and causing both cutaneous (Pezzi et al., 2021) and traumatic injuries (Hall et al., 2016). Another obligate parasitic blowfly of vertebrates is *Protocalliphora azurea*, an ectoparasite of wild birds in Europe and Asia. Females lay their eggs in nests and larvae feed on the blood of nestlings (Matyukhin and Krivosheina, 2008), causing several detrimental effects to their host brood (Puchala, 2004).

Despite their ecological, veterinary and sanitary importance (Stevens and Wallman, 2006), the genetic basis of parasitism in the family Calliphoridae remains poorly studied. However, this family, characterized by recent shifts in feeding habits, serves as a key model to investigate the genetic bases and evolution of trophic specialization among arthropods.

Phenotypic changes, whether convergent or not, can arise from mutations in coding sequences or from changes in the expression levels of orthologous genes. Variation in coding sequences can impact gene function, and the ratio of synonymous to non-synonymous mutations can provide evidence of selective pressures acting on these regions (Nielsen, 2005; Yang, 2007). Additionally, mutations in non-coding regions of the genome, whether proximal or distal to a target of selection, can influence gene expression differences and, consequently, affect phenotypic traits.

The regulation of gene expression is a complex process, involving both *cis* and *trans* factors. *Cis* elements include physically connected non-coding regions, such as promoters, enhancers, and repressors, which serve as binding sites for transcription factors that modulate gene expression levels. *Trans* elements, act more diffusely to regulate gene transcription, either in a direct form (as a transcription factor) or in an indirect form (e.g., proteins that interact with transcription factors, hindering their binding) (Signor and Nuzhdin, 2018; Hill et al., 2021). Studying the evolution of these non-coding regions is challenging, as synonymous and non-synonymous distinctions do not apply. Furthermore, high-quality, contiguous genome assemblies are necessary to identify and compare potentially homologous regions among species.

Previous studies of evolutionary patterns in the transcriptomes of members of family Calliphoridae have revealed genes associated with foraging and feeding habits, with marked variation both in expression profiles and nucleotide sequence divergence among distinct species (Cardoso et al., 2016; Cardoso et al., 2025b). Recent advances in methods and an increasing number of genomes available for members of the family Calliphoridae now allow new analyses within this family.

Here, we aimed to advance the understanding of the evolution of vertebrate parasitism within the Calliphoridae by focusing on two distinct parasitic strategies: those of *Co. hominivorax* and *P. azurea*. We sought to identify evolutionary patterns that could reflect the genetic basis of these adaptations, exploring how genetic variation at both functional and regulatory levels contributes to ecological transitions within this family.

To allow the study within a broader phylogenetic framework, we sequenced the genomes of three species –*Chrysomya megacephala*, *Chrysomya putoria*, and *Lucilia eximia*– and combined them with 11 other publicly available blowfly genomes. The newly sequenced genomes belong to facultative parasites, commonly associated with decaying organic matter, with occasional cases of myiasis in vertebrates. Larvae of *Ch. megacephala* and *Ch. putoria* feed on necrotic tissues in carcasses or wounds (Zumpt, 1965; Amendt et al., 2010; Gaedke and Mougá, 2017), while *Lucilia eximia*

exhibits greater dietary flexibility, feeding on both fresh and necrotic tissues in wounds (Moretti and Thyssen, 2006; Muñoz-García et al., 2016) or exclusively on necrotic tissues in carcasses (Gaedke and Mougá, 2017). These three species were thus selected to explore the transition between saprophagy and parasitism and to enhance the resolution of evolutionary analyses by including representative genomes from across a spectrum of feeding habits and parasitic tendencies.

Given the complexity linked to phenotypic changes driven by variations in both coding and non-coding sequences, we used high-quality genomic data sets to explore the selective pressures shaping these regions, offering a solid framework to investigate the genetic basis of parasitism in the family Calliphoridae. Specifically, we hypothesize that parasitism in blowflies involves convergent genomic adaptations in both coding and regulatory regions, particularly in pathways linked to host-seeking and interaction. By contrasting parasitic and non-parasitic blowflies, we aim to disentangle lineage-specific changes from convergent patterns, testing whether molecular convergence underlies the repeated evolution of parasitism.

2. Materials and methods

2.1. Fly rearing

We collected adults of *L. eximia*, *Ch. putoria* and *Ch. megacephala* using Van Someren-Rydon traps baited with chicken liver in São José do Rio Preto (São Paulo State), Taubaté (São Paulo State), and Capão do Leão (Rio Grande do Sul State), Brazil, under the permit #67867-3 from the *Sistema de Autorização e Informação em Biodiversidade* (SISBio). We identified specimens following blowfly taxonomic keys (Kosmann et al., 2013; Whitworth, 2014).

In the laboratory, we maintained adults of all three species at 25 ± 2 °C, 60 % relative humidity, and a 12:12 h light:dark cycle. We provided fresh bovine ground meat (~7% fat) to 12-to-15-day-old females for egg-laying. We reared larvae on fresh bovine ground meat at 25 ± 2 °C until the third instar, then we transferred them to sawdust at the same temperature until adult emergence.

We kept laboratory populations of *Ch. putoria* and *Ch. megacephala* under random mating conditions. We isolated third-generation females and reared their offspring separately for DNA extraction and whole-genome sequencing. Due to challenges in maintaining *L. eximia* under laboratory conditions, we reared the offspring from field-collected females for genomic sampling. We starved adults for two days, flash-froze them in liquid nitrogen and stored the samples at -80 °C.

For RNA sequencing, we froze living individuals under the same conditions. Our samples represent all life stages: eggs (n = 50), first (n = 10), second (n = 5) and third instar larvae (n = 2), pupae (n = 1), adult virgin females (n = 1), adult gravid females (n = 1), and adult males (n = 1).

2.2. Isolation of nucleic acids and sequencing

We shipped samples of *Ch. megacephala*, *Ch. putoria*, and *L. eximia* on dry ice to Dovetail Genomics® (Scotts Valley, CA, USA) for genome sequencing. Dovetail personnel performed all following procedures. DNA was individually extracted from three males of *Ch. megacephala*, two males of *Ch. putoria*, and two males of *L. eximia* with the QIAGEN Blood & Cell Culture DNA Kit (QIAGEN, Hilden, Germany), and quantified with the Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). Samples were pooled to reach the required DNA amount before sequencing. PacBio SMRTbell libraries (~20 kb) were prepared with the SMRTbell Express Template Prep Kit 2.0 (PacBio, Menlo Park, CA, USA), fol-

lowing the manufacturer's recommended protocol. Libraries were then bound to the Sequel II polymerase using the Sequel II Binding Kit 2.0 (PacBio), and sequenced on PacBio Sequel II system using 8M SMRT cells.

We used RNA-seq as coding sequence evidence for genome annotation. We extracted total RNA from each developmental stage of *Ch. putoria* and *Ch. megacephala* using TRIzol™ (Life Technologies, Carlsbad, CA, USA) and from *L. eximia* using the RNeasy® Mini Kit (QIAGEN, Hilden, Germany). We isolated RNA from each life stage sample separately, pooled samples in equimolar amounts and shipped them on dry ice to GenOne® (Rio de Janeiro, RJ, Brazil) for sequencing. Additionally, we sequenced a second RNA pool for *Ch. putoria* at NGS Soluções Genômicas® (SP, Brazil). RNA libraries were prepared using NEBNext® Ultra™ II RNA Library Prep Kit for Illumina (New England Biolabs Inc., Ipswich, MA, USA). All samples were sequenced on Illumina® (San Diego, CA, USA) NovaSeq6000 platform with paired-end reads (PE reads 2 x 150 bp).

2.3. Genome assembly and annotation

PacBio CCS (Circular Consensus Sequencing) sequences were assembled using hifiasm v0.15.4-r347 (Cheng et al., 2021). Contigs were assigned to their respective taxonomic units with blastn from BLAST v.2.11 (Altschul et al., 1990), using the nucleotide and taxonomic databases from the National Center for Biotechnology Information (NCBI). Non-arthropod scaffolds were filtered using BlobTools v1.1 (Laetsch and Blaxter, 2017) and redundant haplotigs and overlapping contigs were removed with purge_dups v1.2.5. We evaluated the final assemblies using QUAST v5.0.2 (Gurevich et al., 2013) and BUSCO v5.3.2 (Simão et al., 2015) with the Diptera OrthoDB v10 database.

We evaluated RNA-seq read quality using FastQC v0.11.9 (Andrews, 2010) and summarized the results with MultiQC v1.14 (Ewels et al., 2016). We trimmed low-quality bases and adapters with Trimmomatic v0.39 (Bolger et al., 2014), and rechecked read quality after processing. We generated *de novo* transcriptome assemblies with Trinity v2.15.1 (Haas et al., 2013), using these reads in combination with RNA-seq data from previous projects (BioProject PRJNA1159025). We excluded *Ch. putoria* from this step due to the lack of prior RNA-seq data for this species. We then evaluated transcriptome completeness with BUSCO v5.4.4 (Simão et al., 2015) using Diptera OrthoDB v10 database.

We identified mitochondrial genomes using blastn from BLAST, v.2.11 (Altschul et al., 1990) and removed them from the final assemblies. For *Ch. megacephala* and *Ch. putoria*, we used publicly available mitochondrial sequences (NC_019633.1 and AF352790.1, respectively) as references. For *L. eximia*, we used the mitochondrial genome of *L. cuprina* (NC_019573.1) as a proxy due to the lack of a species-specific reference. We annotated mitochondrial genomes using MITOS2 (Bernt et al., 2013) web server with default parameters and the genetic code for invertebrates.

To identify and analyze repetitive genomic regions, we built custom repeat libraries with RepeatModeler v2.0.4 (Flynn et al., 2020). For long terminal repeat (LTR) elements, we employed LTRharvest (Ellinghaus et al., 2008), implemented within GenomeTools v1.6.0 (Gremme et al., 2013) and LTRretriever v2.9.0 (Ou and Jiang, 2018). We merged outputs from RepeatModeler and LTRretriever, reduced redundancy using vsearch v2.22.1 (Rognes et al., 2016), and used the consolidated repeat libraries to soft-mask the genomes with RepeatMasker v4.1.2 (Smit et al., 2013).

We aligned RNA-seq reads to the genomes of each species using STAR v2.7.10a (Dobin et al., 2013), and used the resulting sorted BAM files for structural annotation and gene prediction with BRAKER3, version 3.0.3 (Gabriel et al., 2023). We performed a second round of annotation using the hints file from the first round. We evaluated the final gene predictions completeness using

BUSCO v5.4.4 (Simão et al., 2015) with the Diptera OrthoDB v10 database, and functionally annotated the predicted genes with EnTAP v0.10.8 (Hart et al., 2020) using SwissProt and RefSeq Invertebrate databases. We supplemented the Invertebrate database with annotated proteins from *L. cuprina* (GCF_022045245.1) and *Co. hominivorax* (Tandonnet et al., 2022) genomes.

2.4. Phylogenetic analysis

We inferred a phylogeny of the 14 species of Calliphoridae as no existing tree included our target species. We used BUSCO v5.4.4 (Simão et al., 2015) to analyze genome- or transcriptome-predicted proteomes, depending on data availability (Supplementary Material 1 and 2).

We obtained high-quality blowfly genomes from public repositories or requested from authors (Supplementary Material 1). We selected genomes based on quality metrics from QUAST v5.0.2 (Gurevich et al., 2013) and BUSCO v5.4.4 (Simão et al., 2015). Specifically, we prioritized genomes with more than 90 % of their total length assembled into scaffolds exceeding 50 kb (Supplementary Material 1).

For the transcriptomes (Supplementary Material 2), we predicted proteins using TransDecoder v5.7.0 (Haas, 2024), and reduced redundancy using CDHIT v4.8.1 (Li and Godzik, 2006) by applying a 0.97 sequence identity threshold.

We filtered BUSCO results to retain only complete, single-copy orthologs with an e-value threshold of $< 1e^{-100}$. We aligned orthogroups present in at least 11 species (>75 % occupancy) using MAFFT v7.505 (Katoh and Standley, 2013). We then inferred phylogenetic trees with IQ-TREE v2.2.2.6 (Minh et al., 2020), applying 1000 ultrafast bootstraps (Hoang et al., 2018), and using the model finder algorithm to estimate the best substitution model (Kalyaanamoorthy et al., 2017). We rooted the resulting tree at the split between the subfamily Chrysomyinae and (Calliphorinae + Luciliinae) using FigTree v1.4.4 (<https://tree.bio.ed.ac.uk/software/figtree/>), producing a topology (Supplementary Material 3A) that was congruent with recent phylogenetic studies of the Calliphoridae (Yan et al., 2021). Finally, we pruned the tree to retain only species with available genomic sequences (including *Ch. megacephala*, *Ch. putoria* and *L. eximia*) for further analyses using the R packages *phytools* (Revell, 2011) and *ape* (Paradis and Schliep, 2019).

2.5. Analysis of the evolution of coding sequences

We extracted coding sequences from the genomes of 10 blowfly species (Supplementary Material 3B). Using AGAT v1.2.1 (Dainat, n. d.), we removed isoforms and retained only the longest transcript for each gene. We inferred orthogroups with OrthoFinder v2.5.4 (Emms and Kelly, 2019) and aligned orthogroups with at least four species using MAFFT v7.520 (Katoh and Standley, 2013).

To remove paralogous sequences, we processed the alignments with PhyloPyPruner v1.2.4 (<https://pypi.org/project/phylopy-pruner/>) in MI mode, retaining monophyletic subtrees from the phylogenetic tree inferred for each alignment. We applied the *--jackknife* option with a minimum occupancy threshold of five taxa, yielding 11,347 final orthogroups.

We selected orthogroups containing sequences from *Co. hominivorax* (n = 9462) or *P. azurea* (n = 8926) for molecular evolution analyses. We performed codon-wise multiple alignments for each orthogroup using TranslatorX v1.1 (Abascal et al., 2010), and conducted molecular evolution tests with CodeML (Yang, 1997, 2007) under ETE3 v3.1.3 (Huerta-Cepas et al., 2016), using the phylogeny described in the 2.4 Phylogenetic analysis section.

We analyzed each orthogroup with *Co. hominivorax* or *P. azurea* as the foreground branch and tested three models: *M0* (single syn-

onymous to non-synonymous substitution ratio – d_N/d_S or ω – across the tree), b_neut ($\omega_{\text{foreground}}$ fixed at 1 and $\omega_{\text{background}}$ allowed to vary), and b_free (both ω values may vary). We first tested b_free against $M0$ to evaluate whether a two- ω model provided a better fit. When this null hypothesis was rejected, we compared b_free against b_neut to detect deviations from neutrality. We used likelihood ratio tests (LTR) for model comparisons, and rejected the null hypothesis at p -value < 0.05 after false discovery rate (FDR) correction.

We assigned Gene Ontology (GO) terms to *Co. hominivorax*'s and *P. azurea*'s genes using EnTAP v0.10.8 (Hart et al., 2020). For each species, we identified GO terms associated with genes evolving at higher or lower rates relative to the genome-wide ω distribution. We compared GO terms associated with at least 30 genes to the genome-wide ω using Mann-Whitney U tests (p -value < 0.05 after FDR correction). Additionally, we identified enriched GO terms among genes whose evolutionary patterns fit the b_free model, indicating that they are evolving at a different rate in the parasitic lineages compared with the other species in the phylogeny. We conducted GO enrichment analyses using GOATOOLS v1.4.12 (Klopfenstein et al., 2018), with a GO.obo file downloaded on July 13, 2024.

2.6. Analysis of the evolution of non-coding sequence

We used soft-masked versions of 10 blowfly genomes (Supplementary Material 3B), prepared as described in the 2.3 Genome assembly and annotation section. We generated a whole-genome alignment (WGA) using ProgressiveCactus (Armstrong et al., 2020; Paten et al., 2011) with the soft-masked genomes. We selected assemblies with an L50 value of 3 (*Belardia pandia*, *Ca. vomitoria*, *Co. hominivorax*, *L. cuprina* and *P. azurea*) as reference genomes to guide the WGA, as this value corresponds to a six-chromosome haploid assembly, consistent with the characteristic $2n = 12$ karyotype of blowflies (Ullerich and Schöttke, 2006). We converted the final HAL output into MAF files using *Co. hominivorax* as reference.

We predicted conserved regions within the WGA using PHAST v1.6 (Hubisz et al., 2011). We divided the WGA into 1 Mb segments and estimated neutral (non-conserved) and conserved evolution models for each segment using phyloFit and phastCons. We averaged the resulting models using phyloBoot and then generated genome-wide models with phastCons. Simultaneously, we extracted conserved regions into a BED file using the `--most-conserved` parameter. We refined the dataset with bedtools v2.30.0 (Quinlan and Hall, 2010) by collapsing regions separated by < 5 bp, excluding sequences shorter than 250 bp, and retaining only regions that did not overlap with annotated exons, hereon referred as conserved non-exonic elements (CNEEs) (Sackton and Clark, 2019).

We extracted the sequences for each CNEE in each species from the WGA, compiling them into multi-species FASTA files. We removed sequences shorter than half or longer than twice the length of the *Co. hominivorax* reference sequence, and retained only alignments containing all species and lacked duplicate taxa. We aligned each CNEE using MAFFT v7.505 (Katoh and Standley, 2013) and assessed accelerated evolutionary rates with PhyloAcc v2.2.0 (Hu et al., 2019), testing the branches of obligate parasites (*Co. hominivorax* and *P. azurea*) against the rest of the phylogeny.

We analyzed the subset of accelerated CNEEs for enrichment of transcription factor binding motifs using AME. We used a *Drosophila* motif database and compared CNEEs to random DNA sequences with nucleotide frequencies estimated from the input set using Fisher's exact test. We further analyzed the enriched motifs using GOMo to associate them with genes and GO terms, using *Drosophila* annotations as a reference. Both AME and GOMo

software tools are available online in The MEME Suite v5.5.4 (Bailey et al., 2009).

We determined the physical association between CNEEs and genes using the *closest* function from bedtools v2.30.0 (Quinlan and Hall, 2010), which identifies genes whose transcription start site lies nearest to each CNEE. Finally, we investigated the function of these genes based on orthology to *D. melanogaster*, as available on FlyBase v. FB2023_05 (accessed September 26, 2023).

3. Results

3.1. Genomes for *Ch. megacephala*, *Ch. putoria* and *L. eximia*

Here, we describe the sequencing, assembly and annotation of the genomes of three species of blowflies: *Ch. megacephala*, *Ch. putoria*, and *L. eximia*. The nuclear genome assembly of *Ch. megacephala* was the largest, with a total length of 671,207,201 bp across 759 contigs, with an N50 greater than 2 Mb (Table 1). The *Ch. putoria* genome measured 587,066,816 bp across 652 contigs, while *L. eximia* had 579,241,898 bp across 369 contigs, both with N50 values exceeding 4.2 Mb (Table 1). Sequencing achieved average coverage depths of 59x for *Ch. megacephala* and *Ch. putoria*, and 64x for *L. eximia*. The GC content was consistent across species, with *Ch. megacephala* at 29.14 %, *Ch. putoria* at 28.98 %, and *L. eximia* at 29.30 %. Mapping the RNA-seq data to the respective genomes resulted in 63.97 % uniquely mapped reads for *Ch. megacephala*, 45.49 % for *Ch. putoria*, and 65.11 % for *L. eximia*.

We characterized repetitive elements in the novel and in publicly available Calliphoridae genomes using RepeatModeler and RepeatMasker. Across species, repeat content (e.g., retroelements, DNA transposons, low complexity regions) represented 44–68 % of the genome (Supplementary Material 4). Most repeats consisted of unclassified elements and Class I Transposons – LTRs (Long Terminal Repeats), LINEs (Long Interspersed Nuclear Elements) and SINEs (Short Interspersed Nuclear Elements). The proportions of repeat classes were consistent among species, except for the LTR content in *Ch. putoria*'s genome (21.31 %, compared to an average of 5.63 % in other genomes) and for the DNA transposon content, which varied greatly among all genomes (Supplementary Material 4).

Using a structural annotation pipeline, we predicted a varied number of genes and proteins for the three new genomes: 28,718 in *Ch. megacephala*; 27,312 in *L. eximia*; and 20,414 in *Ch. putoria*. Functional annotation yielded fewer annotated proteins than predicted genes in all three species. The mitochondrial genome annotation revealed the same content as in any other animal lineage (Boore, 1999), comprising 37 genes: 13 protein-coding genes; 22 transfer RNAs; and two ribosomal RNAs.

We assessed genome completeness with BUSCO (Diptera odb10 database), which showed high completeness for all three species (Table 2; Fig. 1). Overall, the *L. eximia* genome contained 98.9 % of all curated dipteran orthologs, slightly exceeding *Ch. putoria* (98.6 %) and *Ch. megacephala* (97.7 %; Table 2; Fig. 1). The *L. eximia* genome, derived from F₁ individuals, exhibited 33.7 % of duplicated orthologs, likely reflecting a higher level of heterozygosity.

3.2. Chemoreceptor, cuticle and endopeptidase genes evolve faster than the genome average

We excluded three *Co. hominivorax* and two *P. azurea* sequences from the analysis due to poor alignments. Among the remaining genes, 734 *Co. hominivorax* genes best fit the b_free model, rejecting both $M0$ and b_neut null hypotheses. Of these, 136 genes showed a higher ω value than the background, and 598 showed lower values. Additional 12 genes fit the b_neut model better than $M0$, but did

Table 1

Summary statistics for genome assemblies of *Chrysomya megacephala*, *Chrysomya putoria*, and *Lucilia eximia*. The table lists species, genome length (bp), number of scaffolds, N50 (bp), L50, number of predicted genes, number of predicted proteins, and number of annotated proteins.

Species	Length (bp)	Number of scaffolds	N50 (bp)	L50	Number of predicted genes	Number of predicted proteins	Number of annotated proteins
<i>Ch. megacephala</i>	647,336,116	758	2,157,473	87	28,718	29,156	18,516
<i>Ch. putoria</i>	587,066,816	652	4,322,519	45	20,414	21,303	16,843
<i>L. eximia</i>	579,241,898	369	4,214,109	42	27,312	28,884	21,835

Table 2

Benchmarking Universal Single-Copy Orthologs (BUSCO) evaluation of genome assemblies and proteome predictions for *Chrysomya megacephala*, *Chrysomya putoria*, and *Lucilia eximia*. The table reports BUSCO results for genome assemblies and proteome predictions, including counts of single-copy, duplicated, fragmented, and missing orthologs. BUSCOs were done using Siptera OrthoDB v10 database.

Species	BUSCO (genome assembly)				BUSCO (proteome prediction)			
	Single-copy	Duplicated	Fragmented	Missing	Single-copy	Duplicated	Fragmented	Missing
<i>Ch. megacephala</i>	84.4 %	11.2 %	0.2 %	4.2 %	78.4 %	18.5 %	0.9 %	2.2 %
<i>Ch. putoria</i>	93.3 %	5.3 %	0.3 %	1.1 %	83.7 %	14 %	0.9 %	1.4 %
<i>L. eximia</i>	65.2 %	33.7 %	0.3 %	0.8 %	56.5 %	41.5 %	0.9 %	1.1 %

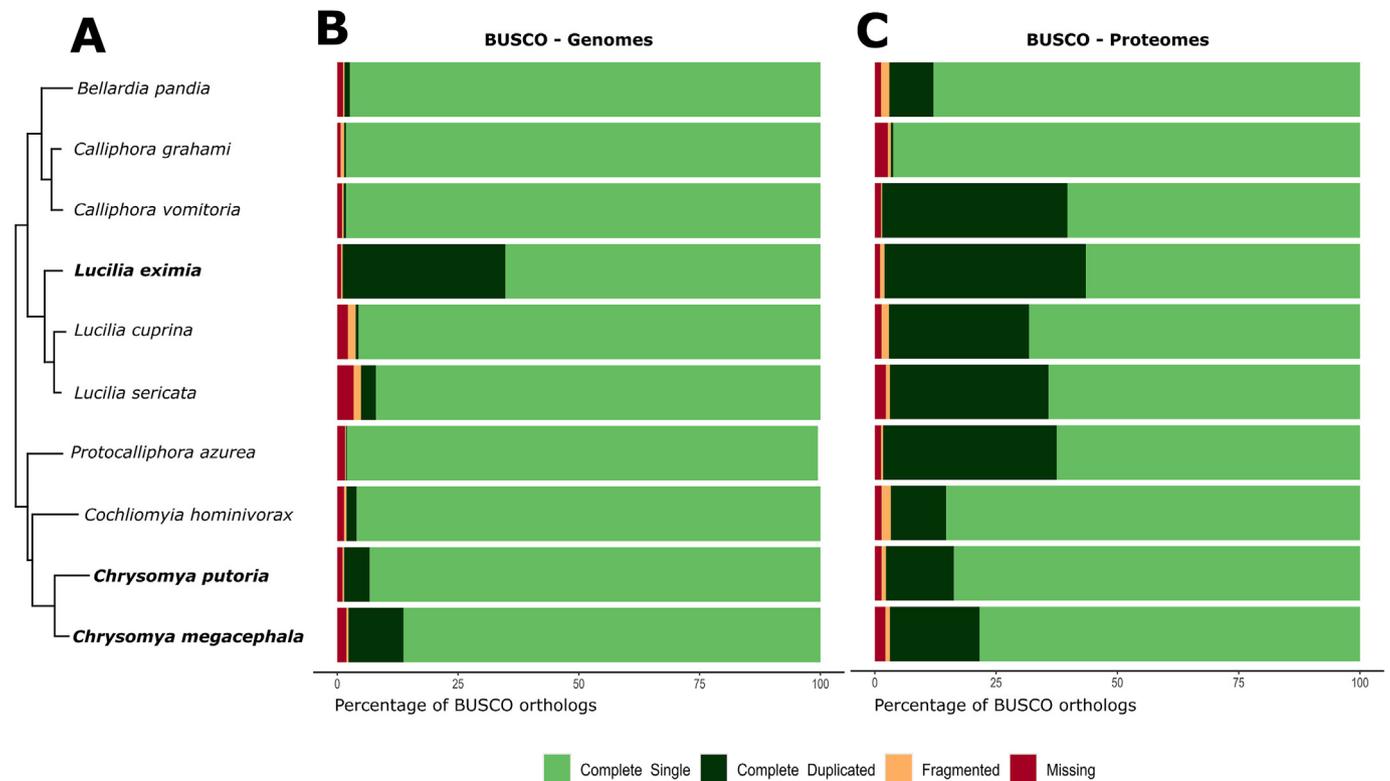


Fig. 1. Phylogenetic relationships and genome completeness in blowflies. (A) Phylogenetic tree of Calliphoridae species with high-quality genome assemblies. Species with genomes newly sequenced in this study are highlighted in bold. (B, C) Proportion of Benchmarking Universal Single-Copy Orthologs (BUSCO) identified in each category for each genome assessed using (B) the whole genome sequence or (C) the genome-predicted proteomes.

not reject *b_neut*. For 8713 genes, neither null hypothesis (*M0* nor *b_neut*) was rejected. In *P. azurea*, 848 genes best fit the *b_free* model (655 with higher ω value than the background and 193 with lower), 38 fit the *b_neut* model and 8037 fit *M0* (Supplementary Material 5 and 6).

We found mean ω values for all tested genes (genome ω) of 0.073 for *Co. hominivorax* and 0.082 for *P. azurea*. We compared genome-wide distribution of ω values of each species (i.e., all tested orthologs) with subsets of genes assigned to specific GO terms. In *Co. hominivorax*, genes associated with 1074 GO terms displayed higher or lower ω values than the genome-wide average, while in *P. azurea*, we observed this pattern in genes associated

with 762 GO terms (Supplementary Material 7 and 8 and Fig. 3). In both species, genes related to taste and olfactory perception, cuticle and chitin formation, and endopeptidase activities evolved the fastest. On the other hand, genes predominantly related to DNA and RNA-involved processes, especially transcription and splicing, evolved the slowest.

3.3. Developmental genes have different evolutionary rates in parasites

To better characterize the pathways involving genes with evidence of evolutionary shifts in the parasitic species compared to

the phylogeny (i.e., genes best fit to the *b_free* model), we performed a GO enrichment analysis. For each species, we divided these genes into two groups based on whether their ω values were above or below the genome-wide means. Enrichment analysis revealed 59 and 322 enriched GO terms for above- and below-average genes in *Co. hominivorax*, and 2 and 144 terms for *P. azurea* (Supplementary Material 9). Genes with below-average ω values showed enrichment for functions related to nervous system and eye development, reproduction and nucleic acid metabolism. In *Co. hominivorax*, above-average ω genes showed enrichment for terms related to wing and epithelium development, synaptic signaling and memory.

Finally, we intersected genes under the *b_free* model to identify those common to both parasites. This comparison revealed 145 common genes (Supplementary Material 10), which showed enrichment for 96 GO terms, mainly related to development and metabolism, particularly nucleic acid metabolism (Supplementary Material 11).

3.4. Accelerated evolution of non-coding elements neighbouring developmental genes

We identified 20,785 CNEEs, of which 273 showed evidence of accelerated evolutionary rates. Among these, 53 sequences evolved faster parallelly in both *Co. hominivorax* and *P. azurea* compared to the other species (Supplementary Material 12). Additionally, 197 sequences showed evidence of acceleration only in *Co. hominivorax* and 23 exclusively in *P. azurea* (Fig. 2).

The mean substitution rate of all these elements for accelerated branches was ~ 1.03 , compared to ~ 0.19 for conserved branches. Focusing on the 53 sequences with parallel acceleration, the mean

accelerated and conserved rates were ~ 0.74 and ~ 0.18 , respectively (Fig. 2).

Based on the positions of the CNEEs mapped onto the *Co. hominivorax* genome, we identified the closest gene for nearly all sequences, except one located on a scaffold without annotated genes (Fig. 2). The distances between CNEE and their nearest genes vary greatly, ranging from 14 bp (*Co. hominivorax*-accelerated CNEE closest to the gene CG5958; Supplementary Material 12) to 249 kb (*Co. hominivorax*-accelerated CNEE closest to the gene *turtle*; Supplementary Material 12), with a mean distance of approximately 23.7 kb.

We found that the 53 sequences with parallel acceleration were enriched for seven transcription factor binding motifs (Table 3). In *Drosophila*, these motifs are located in the promoter regions of genes enriched for 161 GO terms, which are mostly related to development-associated processes, such as organ morphogenesis, hormone regulation, the control of cell death (Supplementary Material 13).

4. Discussion

The evolutionary transition to parasitism in the family Calliphoridae represents a model to understand how natural selection operates on genomes. In this study, we characterized patterns of selection acting on both coding and non-coding sequences, some of which may be related to vertebrate parasitism in the Calliphoridae. To address this objective, we sequenced, assembled and annotated the genomes of *Ch. megacephala*, *Ch. putoria* and *L. eximia*, and integrated these new genomic resources with publicly available data. This approach allowed us to investigate selection patterns in coding and non-coding regions across calliphorids, with a partic-

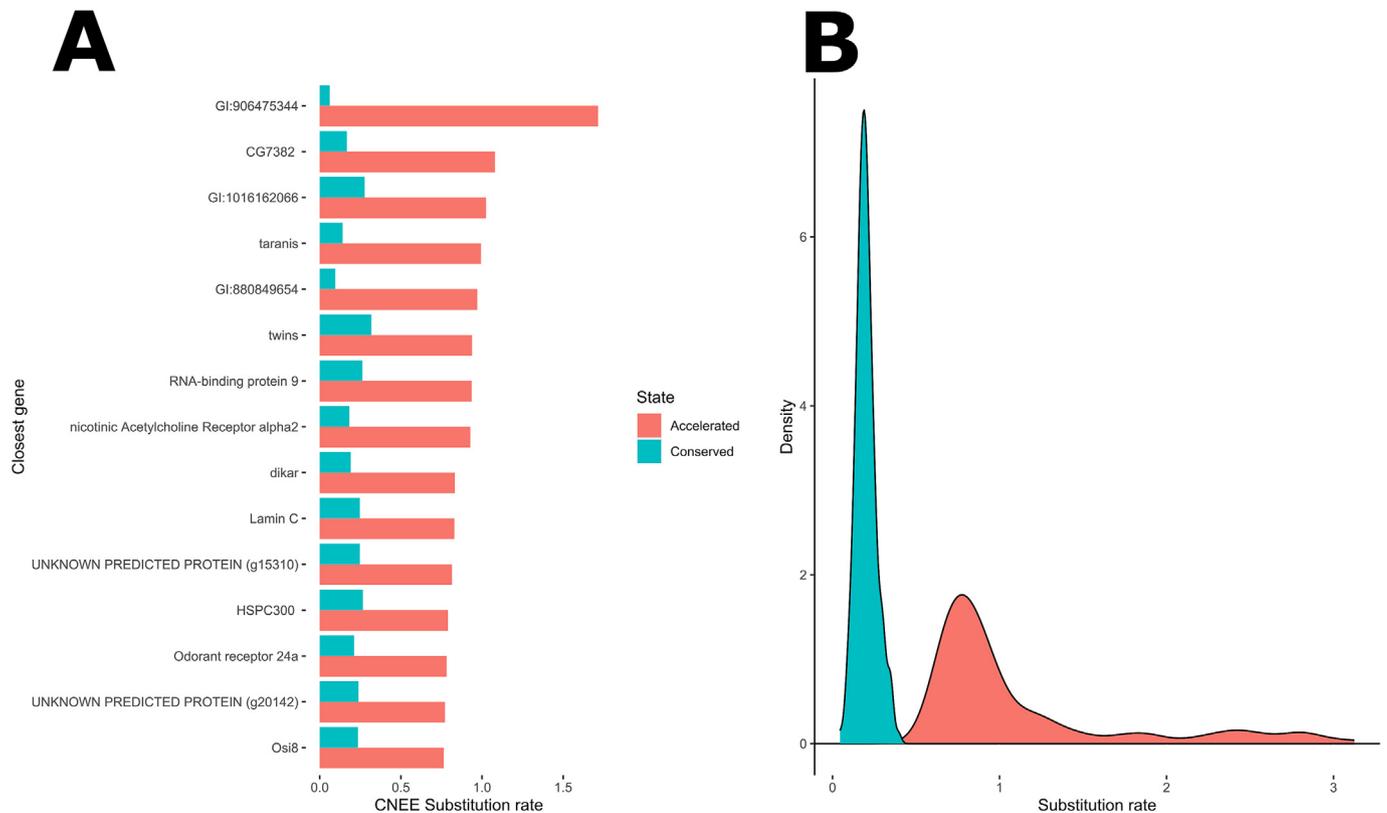


Fig. 2. Substitution rates in conserved non-exonic elements (CNEEs) in parasitic blowfly species. (A) Barplot of substitution rates for 15 most accelerated (CNEEs) in parasites. CNEE is represented by its nearest gene. (B) Density plot of substitution rates for 53 CNEEs that exhibit parallel acceleration in the target branches (*Cochliomyia hominivorax* and *Protocalliphora azurea*) compared to the conserved branches (remaining lineages in the phylogeny). Dashed lines indicate the mean substitution rates for accelerated (blue) and conserved (red) branches. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

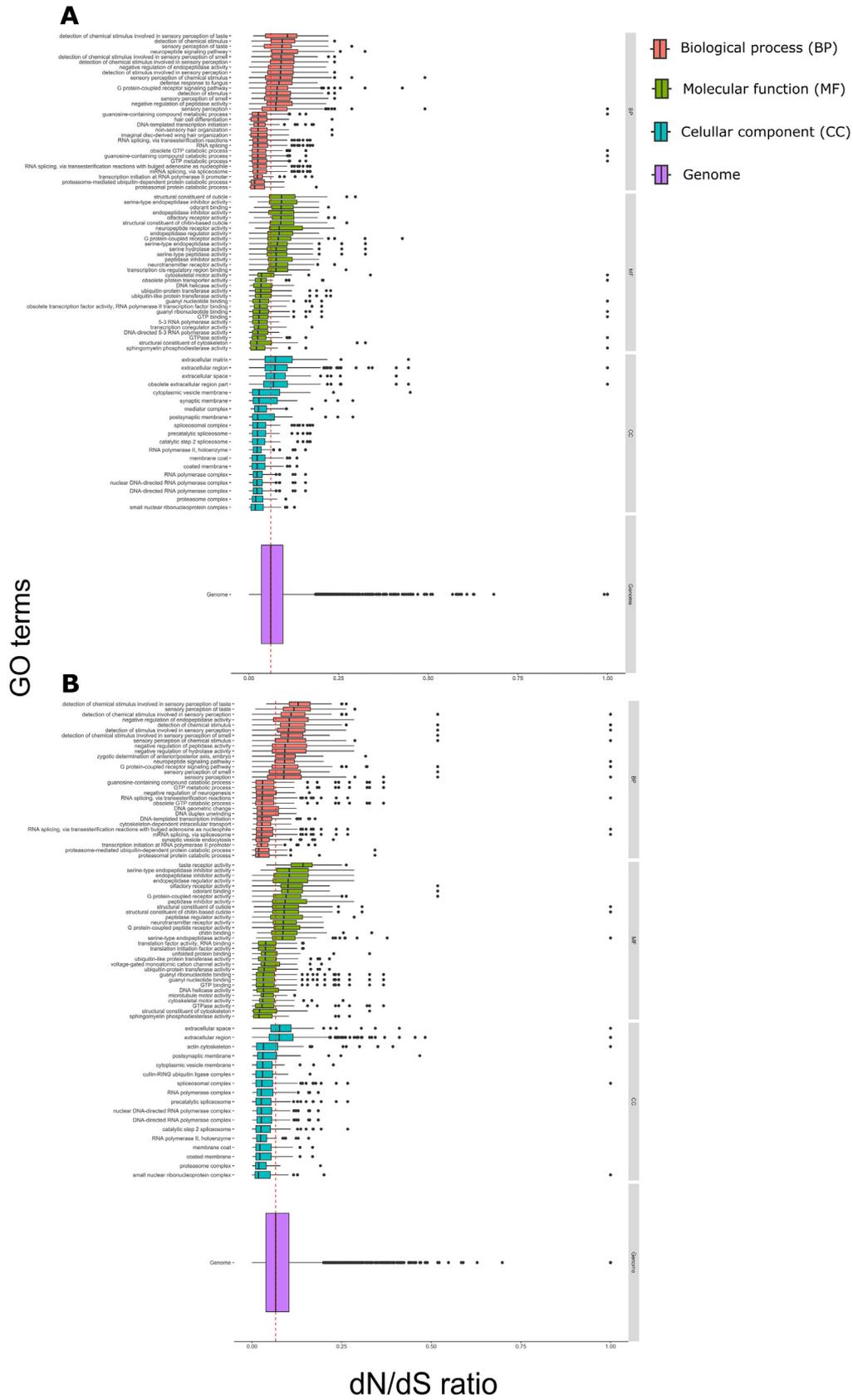


Fig. 3. Gene ontology (GO) categories associated with genes showing deviations in evolutionary rates. GO categories containing genes with significantly higher or lower nonsynonymous-to-synonymous substitution rate ratios (d_N/d_S) compared to the genome-wide mean in (A) *Cochliomyia hominivorax* e (B) *Protocalliphora azurea*. Only the GOs with the largest deviations from the genome-wide median are displayed.

Table 3

Enriched transcription factor binding motifs among the parallelly accelerated conserved non-exonic elements (CNEEs). The table lists motif code, consensus sequence, and adjusted p-values for each transcription factor binding motif.

Motif code	Consensus sequence	Adjusted p-value
FBgn0005694	CAACWACW	2.80E-08
FBgn0005694_2	RCAACAACAAC	3.78E-07
FBgn0038787_2	KTTGTTGTTGTWRTN	4.24E-07
FBgn0005694_3	KGTTGTTGTTGTWRT	1.66E-06
FBgn0004870	TATWAWTRIT	1.66E-05
FBgn0038787	MCAACAACAAMVS	2.58E-04
FBgn0035160	WSRRACAGACAV	1.40E-03

ular emphasis on *Co. hominivorax* and *P. azurea*, which have distinct parasitic strategies.

The three newly sequenced genomes improve the representation of calliphorids in genomic databases, which previously contained only 16 species, representing less than 1 % of the family's estimated diversity (Parmar et al., 2024). A previous study highlighted the value of genome sequences as important resources for elucidating the evolutionary adaptations of calyptrate flies (Wiegmann and Richards, 2018). Here, we expand that knowledge and identify genomic features with evidence of natural selection and potential links to parasitism – a paradigmatic life-history trait in the Calliphoridae.

The application of long read sequencing has greatly improved the contiguity and completeness of blowfly genomes, reducing the number of contigs from hundreds of thousands in earlier studies (Vicoso and Bachtrog, 2015; Andere et al., 2016, 2020) to just a few hundred scaffolds in more recent work (Meng et al., 2020; Davis et al., 2021; Tandonnet et al., 2022; see Supplementary Material 1 for a complete list). By providing these three high-quality genomes, the present study not only expands genomic resources for phylogenetic and population studies but also provides a robust comparative framework for investigating parasitism-associated traits. Increasing taxon sampling is critical for improving parameter estimation and reducing phylogenetic artifacts, particularly in studies of selection and evolutionary transitions (Anisimova et al., 2001, 2002; Jordan and Goldman, 2012; Jeffares et al., 2015).

With this more robust framework, we explored evolutionary trends in two important vertebrate parasites: *Co. hominivorax* and *P. azurea*. Analyses of coding regions revealed gene groups associated with specific GO terms that evolve at rates distinct from the genome-wide average in these species. Both parasites showed remarkably consistent patterns: genes associated with olfaction and taste, chitin and cuticle formation, and serine endopeptidase activity evolved the most rapidly, whereas genes involved in the regulation of nucleic acid metabolism, mainly RNA, evolved the most slowly (Fig. 3), emphasizing shared selective pressures.

Chemoreceptors are key components of the arthropod sensory systems and are essential for their ability to respond to environmental cues (Robertson, 2019). For instance, *Co. hominivorax* females locate hosts by detecting volatiles emitted by bacteria in wounds (Chaudhury et al., 2010), highlighting the importance of chemoreceptors in host-seeking behaviors. Differences in chemoreceptor repertoires have also been observed between subspecies of *L. cuprina*, one being an obligate parasite and the other saprophagous, which may contribute to the different behavior displayed by each subspecies (Kapoor et al., 2025). Although functional validation remains pending, we hypothesize that the transition to a parasitic lifestyle requires adaptation to new chemical signals, potentially explaining the higher-than-average evolutionary rates observed in genes related to olfaction and taste.

Rapid evolution of cuticle-associated genes can be interpreted as adaptation to multiple host-related challenges. Chitin, the main

component of the insect cuticle, provides protection against various environmental stressors, and acts as mediator of immune defense and desiccation resistance (Andersen, 2010; Mallick and Eleftherianos, 2024). In parasitic blowflies, larvae must withstand both mechanical constraints of penetrating host tissues and immune defenses mounted by the vertebrate host, as observed in myiasis-causing lineages (Hall and Wall, 1995), while also coping with insecticides used in population control (Balabanidou et al., 2018). Moreover, studies on organophosphate insecticide resistance in *Co. hominivorax* have implicated cuticle remodeling in tolerance to chemical exposure (Tandonnet et al., 2020), supporting the idea that cuticular genes are hotspots for rapid adaptation under strong selective pressures. These parallels reinforce the interpretation that selection on cuticular pathways is a key feature of the parasitic lifestyle.

The enrichment of serine proteases among fast-evolving genes suggests selection on digestive efficiency or on interactions with host tissues and immunity. These enzymes, members of a multi-functional protease family, participate in cell signaling, digestion and nutrition, protein turnover in proteasomes, signal peptide removal, and immunity (Page and Di Cera, 2008), all of which are relevant to a parasitic lifestyle. Serine proteases are known virulence factors in diverse parasites, including nematodes, ticks and mosquitoes, where they facilitate host tissue degradation and immune evasion (Miyoshi et al. 2007; Saboia-Vahia et al., 2014; Yang et al., 2015). In blowflies, larval feeding involves proteolytic breakdown of host tissue (Giglioti et al., 2016), a process that must balance efficient digestion with evasion of antimicrobial host responses. Accelerated evolution in these enzymes may therefore reflect fine-tuning of protease activity to optimize nutrient acquisition while minimizing host detection. This pattern parallels findings in blood-feeding arthropods, where diversification of salivary proteases contributes to host exploitation. On the other hand, slowly evolving genes, such as those involved in transcription and splicing, align with the high conservation of these essential cellular processes (Anantharaman et al., 2002). These findings are expected given the critical roles these genes play in cellular function.

After analyzing patterns within each parasite genome, we identified genes evolving at different rates in parasites compared to non-parasitic species (i.e., genes best fit to the *b_{free}* model). Although several genes exhibited contrasting evolutionary patterns, none showed evidence of positive selection (absence of $\omega > 1$) in either *Co. hominivorax* or *P. azurea*. We classified these genes as below- or above-average ω (slow- or fast-evolving genes, respectively) and performed GO enrichment analysis.

We identified some slow-evolving genes linked to nucleic acid metabolism, reinforcing the general pattern of evolutionary conservation observed across each genome. Additionally, we found developmental genes with signs of rapid evolution, while others with more conserved functions evolved more slowly. These results suggest that ecological pressures, such as parasitism, drive fast-evolving changes in developmental processes, whereas core functions remain constrained to maintain proper physiological functioning. Blowflies display contrasting feeding habits during the larval stage (Hall and Wall, 1995; Stevens, 2003; Cardoso et al., 2025a), with development and survival strongly influenced by the conditions of different substrates. The larvae's ability, or lack thereof, to thrive under these contrasting conditions is a key factor shaping species-specific behavior, revealing the reciprocal relationship among nutrition, metabolism, and development (Cardoso et al., 2025b). Therefore, it is plausible that evolution co-opted genes controlling development to support new lifestyles, such as parasitism, within Calliphoridae.

Even though the overlap of genes showing shifts in evolutionary rates across the two parasitic species was limited, GO enrichment

analysis revealed that most of these genes are involved in nucleic acid metabolism. This highlights a key finding: while both parasites share general trends, with genes performing similar functions showing accelerated evolutionary rates, the specific genes responding to selection differ between the two species. The differences are not entirely unexpected for two main reasons. First, the last common ancestor of *P. azurea* and *Co. hominivorax* lived nearly 10 million years ago, during a rapid radiation that began following the Cretaceous-Paleogene mass extinction (Wiegmann et al., 2011; Cerretti et al., 2017). Second, the two species parasitize different hosts and tissues (Wyss, 2000; Puchala, 2004; Matyukhin and Krivosheina, 2008; Hall et al., 2016; Pezzi et al., 2021). Therefore, both temporal divergence and ecological specialization likely drove the distinct genomic trajectories in these species.

We also investigated the evolution of non-coding regions, focusing on CNEEs, given their potential role as regulators of gene expression. Although direct functional validations were beyond the scope of this study, we identified transcription factor binding motifs within CNEEs, providing *in silico* evidence of their regulatory roles. Our analysis of promoter motifs indicates that, in *D. melanogaster*, genes with promoter regions containing these motifs are enriched for development-related GO terms, a pattern we also observed in the vertebrate-parasitizing calliphorids.

Accelerated CNEEs frequently occur in proximity to genes annotated for developmental functions, supporting the hypothesis that regulatory modifications have contributed to changes in developmental programs linked to trophic specialization. We identified 53 CNEEs with accelerated evolutionary rates in both *Co. hominivorax* and *P. azurea*. Among the 44 genes located closest to these 53 CNEEs (some CNEEs are near the same gene), 18 (41 %) relate to development. Although these genes are not the same as those identified in the coding region analysis, they are involved in similar developmental processes, reflecting parallel targets of evolutionary change at both coding and regulatory levels. This is consistent with evolutionary scenarios proposed for other insects, where modifications in developmental timing and growth regulation are critical for ecological specialization (Rebeiz and Tsiantis, 2017). In blowflies, larvae represent the main trophic stage, and shifts from saprophagy to parasitism likely required fine-scale tuning of developmental processes to synchronize growth with host availability (Stevens, 2003). Such regulatory reprogramming echoes findings in birds, where accelerated non-coding elements were tied to flight loss (Sackton and Clark, 2019), illustrating a general principle that regulatory evolution underlies key life-history transitions.

As observed for coding regions, chemosensory genes also appear as targets of accelerated evolution at the regulatory level. We found accelerated CNEEs near genes involved in chemoreception, including *Odorant receptor 24a*, *Odorant receptor 63a*, and *Gustatory receptor 63a* (*Gr63a*), indicating that both coding and regulatory changes may contribute to sensory adaptation. As previously discussed, chemoreceptors such as these are likely key factors in the transition to parasitic lifestyles. A remarkable example is the *Gr63a*, a CO₂ receptor essential for host identification in *Aedes aegypti* mosquitoes (McMeniman et al., 2014; Kumar et al., 2021). In *Co. hominivorax*, we identified an accelerated CNEE near this locus, which is expressed in the antennae (Hickner et al., 2020) and shows transcript levels nearly 18 times higher than in its congeneric saprophagous counterpart, *Co. macellaria* (Cardoso et al., 2025b).

Chemosensory gene families are among the most dynamic in insects, with documented expansions in *Drosophila* species adapting to new diets (McBride and Arguello, 2007) and in mosquitoes adapting to blood-feeding (Rinker et al., 2013). Host-seeking in obligate parasites such as *Co. hominivorax* depends on detecting volatiles emitted from wounds or decaying tissue (Chaudhury et al., 2010), a sensory challenge distinct from saprophagous rela-

tives. The presence of accelerated CNEEs near the CO₂ receptor gene *Gr63a* in *Co. hominivorax* suggests regulatory adaptation, consistent with studies showing that CO₂ perception is a critical cue in host location for hematophagous mosquitoes (McMeniman et al., 2014). Together, these findings indicate that chemosensory evolution in blowflies parallels adaptive processes observed in other parasitic and hematophagous insects. The differences in gene expression previously reported further suggest a role of *Gr63a* in regulating host-seeking behavior and in driving behavioral divergence between the two species, making it an excellent candidate for future functional screenings.

The full regulatory potential of these CNEEs may be underestimated. Regulatory regions often interact with multiple targets over varying distances (Jeziorska et al., 2009; Long et al., 2016), and trans-acting elements from distant genomic regions frequently modulate the expression of many genes (Signor and Nuzhdin, 2018; Hill et al., 2021). Consequently, our approach probably captured only a subset of the genes influenced by the identified CNEEs, reflecting limitations of current tools and methodologies.

Despite these constraints, our findings highlight the evolutionary dynamics shaping parasitic calliphorid genomes, revealing both species-specific adaptations and broader convergent patterns. While divergence between *Co. hominivorax* and *P. azurea* is expected due to their distinct ecological niches and evolutionary trajectories, the identification of shared functional categories, such as sensory perception, developmental processes and exoskeleton formation, suggest a potential genomic toolkit repeatedly co-opted during the evolution of parasitism in these lineages.

The observed pattern of convergence at the functional level combined with divergence at the gene level is expected under models where ecological pressures select for similar phenotypic outcomes but permit distinct molecular solutions. Such a mosaic of convergent and lineage-specific signatures has been described in bats and whales evolving echolocation (Parker et al., 2013). In Calliphoridae, *Co. hominivorax* and *P. azurea* share a parasitic life-history trait but differ markedly in host use and ontogenetic stage of parasitism. These ecological differences may channel adaptation through alternative molecular routes while maintaining convergence in functional categories such as sensory perception and development.

Recent comparative analyses of behavioral phenotypes and transcriptomic profiles in blowflies (Cardoso et al., 2025b) reinforce this view: while oviposition preferences and larval survival assays revealed behavioral differences aligned with feeding habits, gene expression and coding sequence analyses showed that distinct sets of genes underlie parasitism in each lineage. Together, these findings support a scenario where functional convergence in vertebrate parasitism emerges from heterogeneous molecular and behavioral pathways, highlighting the complexity of adaptive evolution in this group.

Our findings suggest that similar ecological pressures have driven parallel adaptations at functional level, even when the specific genetic targets differ, offering promising candidates for future experimental studies. To move from genomic correlations to causal inference, we propose targeted experiments. Comparative RNA-seq of larval tissues and sensory organs across parasitic and saprophagous species would test expression shifts predicted by accelerated regulatory elements. Chromatin accessibility assays such as ATAC-seq and targeted ChIP-seq for transcription factors would help connect CNEEs to active regulatory function, following approaches applied in model insects (Ling et al., 2023). Functional tests including enhancer-reporter assays and gene perturbation by RNAi or CRISPR would directly assess phenotypic impacts on host-seeking behavior, oviposition preference and larval survival, bringing Calliphoridae genomics closer to mechanistic causality.

CRediT authorship contribution statement

Pedro Mariano-Martins: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Diniz Lima Ferreira:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Vanessa Araujo Soares da Cunha:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Carolina Kurotusch Canetieri:** Software, Resources, Methodology, Formal analysis. **Letícia Chiara Baldassio de Paula:** Software, Resources, Methodology, Formal analysis. **Tatiana Teixeira Torres:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Funding

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo [grant numbers 20/05636-4 to T.T.T., 21/10022-8 to D.L.F., 23/14660-4 to V.A.S.C., 21/01641-6 to P.M.M., and 23/00104-2 to C.K.C.]; Conselho Nacional de Desenvolvimento Científico e Tecnológico [grant numbers 141391/2019-7 to V.A.S.C., and 310906/2022-9 to T.T.T.]; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior [grant number 88887.816569/2023-00 to L.C.B.P.]; Student Research Grant Assistance Award from Animal Behavior Society to V.A.S.C.; and Dovetail Genomics Funding Assistance Award to P.M.M., D.L.F. and V.A.S.C.. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior— Brasil (CAPES)—Finance Code 001.

Acknowledgments

We thank Laís Cardoso Valadão and Marco Antonio Tonus Marinho for their invaluable assistance with fly collection in the field and species identification.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2025.10.004>.

References

- Abascal, F., Zardoya, R., Telford, M.J., 2010. TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. *Nucleic Acids Res.* 38, W7–W13. <https://doi.org/10.1093/nar/gkq291>.
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Amendt, J., Goff, M.L., Campobasso, C.P., Grassberger, M. (Eds.), 2010. *Current Concepts in Forensic Entomology*. Springer Netherlands, Dordrecht.
- Anantharaman, V., Koonin, E.V., Aravind, L., 2002. Comparative genomics and evolution of proteins involved in RNA metabolism. *Nucleic Acids Res.* 30, 1427–1464.
- Andere, A.A., Pimsler, M.L., Tarone, A.M., Picard, C.J., 2020. The genomes of a monogenic fly: views of primitive sex chromosomes. *Sci. Rep.* 10, 15728. <https://doi.org/10.1038/s41598-020-72880-0>.
- Andere, A.A., Platt, R.N., Ray, D.A., Picard, C.J., 2016. Genome sequence of *Phormia regina* Meigen (Diptera: Calliphoridae): implications for medical, veterinary and forensic research. *BMC Genom.* 17, 842. <https://doi.org/10.1186/s12864-016-3187-z>.
- Andersen, S.O., 2010. Insect cuticular sclerotization: a review. *Insect Biochem. Mol. Biol.* 40, 166–178. <https://doi.org/10.1016/j.ibmb.2009.10.007>.
- Andrews, S., 2010. FastQC: A Quality Control Tool for High Throughput Sequence Data [Online].

- Anisimova, M., Bielawski, J.P., Yang, Z., 2002. Accuracy and power of bayes prediction of amino acid sites under positive selection. *Mol. Biol. Evol.* 19, 950–958. <https://doi.org/10.1093/oxfordjournals.molbev.a004152>.
- Anisimova, M., Bielawski, J.P., Yang, Z., 2001. Accuracy and power of the likelihood ratio test in detecting adaptive molecular evolution. *Mol. Biol. Evol.* 18, 1585–1592. <https://doi.org/10.1093/oxfordjournals.molbev.a003945>.
- Armstrong, J., Hickey, G., Diekhans, M., Fiddes, I.T., Novak, A.M., Deran, A., Fang, Q., Xie, D., Feng, S., Stiller, J., Genereux, D., Johnson, J., Marinescu, V.D., Alföldi, J., Harris, R.S., Lindblad-Toh, K., Haussler, D., Karlsson, E., Jarvis, E.D., Zhang, G., Paten, B., 2020. Progressive Cactus is a multiple-genome aligner for the thousand-genome era. *Nature* 587, 246–251. <https://doi.org/10.1038/s41586-020-2871-y>.
- Bailey, T.L., Boden, M., Buske, F.A., Frith, M., Grant, C.E., Clementi, L., Ren, J., Li, W.W., Noble, W.S., 2009. MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res.* 37, W202–W208. <https://doi.org/10.1093/nar/gkp335>.
- Balabanidou, V., Grigoraki, L., Vontas, J., 2018. Insect cuticle: a critical determinant of insecticide resistance. *Curr. Opin. Insect Sci. Pests Resist.* * Behav. Ecol. 27, 68–74. <https://doi.org/10.1016/j.cois.2018.03.001>.
- Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsche, G., Pütz, J., Middendorf, M., Stadler, P.F., 2013. MITOS: improved *de novo* metazoan mitochondrial genome annotation. *Mol. Phylogenet. Evol. Mitogenom.* 69, 313–319. <https://doi.org/10.1016/j.ympev.2012.08.023>.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Boore, J.L., 1999. Animal mitochondrial genomes. *Nucleic Acids Res.* 27, 1767–1780. <https://doi.org/10.1093/nar/27.8.1767>.
- Cardoso, G.A., Cunha, V.A.S., Genevicius, B.C., Madeira-Ott, T., Costa, B.M.D.A., Rossoni, D.M., Thyssen, P.J., Torres, T.T., 2025a. Origins and diversification of myiasis across blowflies. *Ecol. Evol.* 15, e70993. <https://doi.org/10.1002/ece3.70993>.
- Cardoso, G.A., Mariano-Martins, P., Faria, G.A., Karunaratne, I., Thyssen, P.J., Torres, T.T., 2025b. Divergent genetic pathways underlying convergent parasitic behaviours in blowflies. *Mol. Ecol. n/a*, e17785. <https://doi.org/10.1111/mec.17785>.
- Cardoso, G.A., Marinho, M.A.T., Monfardini, R.D., Espin, A.M.L.D.A., Torres, T.T., 2016. Evolution of genes involved in feeding preference and metabolic processes in Calliphoridae (Diptera: Calyptratae). *PeerJ* 4, e2598.
- Cerretti, P., Iii, J.O.S., Pape, T., O'Hara, J.E., Marinho, M.A.T., Rognes, K., Grimaldi, D.A., 2017. First fossil of an oestroid fly (Diptera: Calyptratae: Oestroidea) and the dating of oestroid divergences. *PLoS One* 12, e0182101. <https://doi.org/10.1371/journal.pone.0182101>.
- Chaudhury, M.F., Skoda, S.R., Sagel, A., Welch, J.B., 2010. Volatiles emitted from eight wound-isolated bacteria differentially attract gravid screwworms (Diptera: Calliphoridae) to oviposit. *J. Med. Entomol.* 47, 349–354. <https://doi.org/10.1093/jmedent/47.3.349>.
- Cheng, H., Concepcion, G.T., Feng, X., Zhang, H., Li, H., 2021. Haplotype-resolved *de novo* assembly using phased assembly graphs with hifiasm. *Nat. Methods* 18, 170–175. <https://doi.org/10.1038/s41592-020-01056-5>.
- Dainat, J., n.d. AGAT: Another Gff Analysis Toolkit to handle annotations in any GTF/GFF format. (Version v1.4.1). Zenodo. <https://www.doi.org/10.5281/zenodo.3552717>.
- Davis, R.J., Belikoff, E.J., Dickey, A.N., Scholl, E.H., Benoit, J.B., Scott, M.J., 2021. Genome and transcriptome sequencing of the green bottle fly, *Lucilia sericata*, reveals underlying factors of sheep flystrike and maggot debridement therapy. *Genomics* 113, 3978–3988. <https://doi.org/10.1016/j.ygeno.2021.10.003>.
- Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R., 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29, 15–21. <https://doi.org/10.1093/bioinformatics/bts635>.
- Ellinghaus, D., Kurtz, S., Willhoeft, U., 2008. LTRharvest, an efficient and flexible software for *de novo* detection of LTR retrotransposons. *BMC Bioinf.* 9, 18. <https://doi.org/10.1186/1471-2105-9-18>.
- Emms, D.M., Kelly, S., 2019. OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biol.* 20, 238. <https://doi.org/10.1186/s13059-019-1832-y>.
- Ewald, P.W., 1987. Transmission modes and evolution of the parasitism-mutualism continuum. *Ann. N. Y. Acad. Sci.* 503, 295–306. <https://doi.org/10.1111/j.1749-6632.1987.tb40616.x>.
- Ewels, P., Magnusson, M., Lundin, S., Käller, M., 2016. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* 32, 3047–3048. <https://doi.org/10.1093/bioinformatics/btw354>.
- Flynn, J.M., Hubley, R., Goubert, C., Rosen, J., Clark, A.G., Feschotte, C., Smit, A.F., 2020. RepeatModeler2 for automated genomic discovery of transposable element families. *Proc. Natl. Acad. Sci.* 117, 9451–9457. <https://doi.org/10.1073/pnas.1921046117>.
- Gabriel, L., Bruna, T., Hoff, K.J., Ebel, M., Lomsadze, A., Borodovsky, M., Stanke, M., 2023. BRAKER3: fully automated genome annotation using RNA-Seq and protein evidence with GeneMark-ETP, AUGUSTUS and TSEBRA (preprint). *Bioinformatics*. <https://doi.org/10.1101/2023.06.10.544449>.
- Gaedke, A., Mougá, D.M.D., 2017. Diptera survey in human corpses in the north of the state of Santa Catarina, Brazil. *Acta Biológica Catarin.* 4, 42–51.
- Gigliotti, R., Guimarães, S., Oliveira-Sequeira, T.C., David, E.B., Brito, L.G., Huacca, M. E., Chagas, A.C.S., Oliveira, M.C., 2016. Proteolytic activity of excretory/secretory products of *Cochliomyia hominivorax* larvae (Diptera: Calliphoridae). *Pesquisa Veterinária Brasileira* 36, 711–718. <https://doi.org/10.1590/S0100-736X2016000800006>.

- Gremme, G., Steinbiss, S., Kurtz, S., 2013. GenomeTools: a comprehensive software library for efficient processing of structured genome annotations. *IEEE/ACM Trans. Comput. Biol. Bioinform.* 10, 645–656. <https://doi.org/10.1109/TCBB.2013.68>.
- Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUILT: quality assessment tool for genome assemblies. *Bioinformatics* 29, 1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Haas, B.J., 2024. TransDecoder/TransDecoder.
- Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Couger, M.B., Eccles, D., Li, B., Lieber, M., MacManes, M.D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C.N., Henschel, R., LeDuc, R.D., Friedman, N., Regev, A., 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat. Protoc.* 8, 1494–1512. <https://doi.org/10.1038/nprot.2013.084>.
- Hall, M., Wall, R., 1995. Myiasis of humans and domestic animals. In: *Advances in Parasitology*. Elsevier, pp. 257–334. [https://doi.org/10.1016/S0065-308X\(08\)60073-1](https://doi.org/10.1016/S0065-308X(08)60073-1).
- Hall, M.J.R., Wall, R.L., Stevens, J.R., 2016. Traumatic myiasis: a neglected disease in a changing world. *Annu. Rev. Entomol.* 61, 159–176. <https://doi.org/10.1146/annurev-ento-010715-023655>.
- Hart, A.J., Ginzburg, S., Xu, M.(Sam), Fisher, C.R., Rahmatpour, N., Mitton, J.B., Paul, R., Wegryzn, J.L., 2020. EnTAP: bringing faster and smarter functional annotation to non-model eukaryotic transcriptomes. *Mol. Ecol. Resour.* 20, 591–604. <https://doi.org/10.1111/1755-0998.13106>.
- Hickner, P.V., Mittapalli, O., Subramoniam, A., Sagel, A., Watson, W., Scott, M.J., Arp, A.P., de León, A.A.P., Syed, Z., 2020. Physiological and molecular correlates of the screwworm fly attraction to wound and animal odors. *Sci. Rep.* 10, 20771. <https://doi.org/10.1038/s41598-020-77541-w>.
- Hill, M.S., Vande Zande, P., Wittkopp, P.J., 2021. Molecular and evolutionary processes generating variation in gene expression. *Nat. Rev. Genet.* 22, 203–215. <https://doi.org/10.1038/s41576-020-00304-w>.
- Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* 35, 518–522. <https://doi.org/10.1093/molbev/msx281>.
- Hu, Z., Sackton, T.B., Edwards, S.V., Liu, J.S., 2019. Bayesian detection of convergent rate changes of conserved noncoding elements on phylogenetic trees. *Mol. Biol. Evol.* 36, 1086–1100. <https://doi.org/10.1093/molbev/msz049>.
- Hubisz, M.J., Pollard, K.S., Siepel, A., 2011. PHAST and RPHAST: phylogenetic analysis with space/time models. *Brief. Bioinform.* 12, 41–51. <https://doi.org/10.1093/bib/bbq072>.
- Huerta-Cepas, J., Serra, F., Bork, P., 2016. ETE 3: reconstruction, analysis, and visualization of phylogenomic data. *Mol. Biol. Evol.* 33, 1635–1638. <https://doi.org/10.1093/molbev/msw046>.
- Jeffares, D.C., Tomiczek, B., Sojo, V., dos Reis, M., 2015. A Beginners guide to estimating the non-synonymous to synonymous rate ratio of all protein-coding genes in a genome. In: Peacock, C. (Ed.), *Parasite Genomics Protocols*. Springer, New York, NY, pp. 65–90. https://doi.org/10.1007/978-1-4939-1438-8_4.
- Jeziorska, D.M., Jordan, K.W., Vance, K.W., 2009. A systems biology approach to understanding cis-regulatory module function. *Semin. Cell Dev. Biol. Struct. Func. Golgi Appar. Syst. Approaches Cell Dev. Biol.* 20, 856–862. <https://doi.org/10.1016/j.semcdb.2009.07.007>.
- Jordan, G., Goldman, N., 2012. The effects of alignment error and alignment filtering on the sitewise detection of positive selection. *Mol. Biol. Evol.* 29, 1125–1139. <https://doi.org/10.1093/molbev/msr272>.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermini, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14, 587–589. <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., Davis, R.J., Tandonnet, S., Canettieri, C.K., Bertone, M.A., Szpila, K., Hall, R.S., Young, N.D., Korhonen, P.K., Gasser, R.B., Perry, T., Jex, A.R., Bowles, V.M., Wiegmann, B.M., Torres, T.T., Anstead, C.A., Scott, M.J., 2025. Comparative genomic analysis of necrophagous and parasitic subspecies of *Lucilia cuprina* (Diptera: Calliphoridae) provides important insights into their divergent biologies. *Int. J. Parasitol.* <https://doi.org/10.1016/j.ijpara.2025.06.001>.
- Karvonen, A., Seehausen, O., 2012. The role of parasitism in adaptive radiations—when might parasites promote and when might they constrain ecological speciation? *Int. J. Ecol.* 2012, 280169. <https://doi.org/10.1155/2012/280169>.
- Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780. <https://doi.org/10.1093/molbev/mst010>.
- Klopfenstein, D.V., Zhang, L., Pedersen, B.S., Ramirez, F., Warwick Vesztrocy, A., Naldi, A., Mungall, C.J., Yunes, J.M., Botvinnik, O., Weigel, M., Dampier, W., Dessimoz, C., Flick, P., Tang, H., 2018. GOATOOLS: a Python library for gene ontology analyses. *Sci. Rep.* 8, 10872. <https://doi.org/10.1038/s41598-018-28948-z>.
- Kosmann, C., De Mello, R.P., Souza, É.S.H., Luz, J.R.P., 2013. A list of current valid blow fly names (Diptera: Calliphoridae) in the Americas South of Mexico with key to the Brazilian species. *EntomolBrasilis* 6, 74–85.
- Kumar, K., Mhetre, A., Ratnaparkhi, G.S., Kamat, S.S., 2021. A superfamily-wide activity atlas of serine hydrolases in drosophila melanogaster. *Biochemistry* 60, 1312–1324. <https://doi.org/10.1021/acs.biochem.1c00171>.
- Laetsch, D.R., Blaxter, M.L., 2017. BlobTools: interrogation of genome assemblies. <https://doi.org/10.12688/f1000research.12232.1>.
- Li, W., Godzik, A., 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22, 1658–1659. <https://doi.org/10.1093/bioinformatics/btl158>.
- Ling, L., Mühlhling, B., Jaenichen, R., Gompel, N., 2023. Increased chromatin accessibility promotes the evolution of a transcriptional silencer in *Drosophila*. *Sci. Adv.* 9, eade6529. <https://doi.org/10.1126/sciadv.ade6529>.
- Long, H.K., Prescott, S.L., Wyszocka, J., 2016. Ever-changing landscapes: transcriptional enhancers in development and evolution. *Cell* 167, 1170–1187. <https://doi.org/10.1016/j.cell.2016.09.018>.
- Mallick, S., Eleftherianos, I., 2024. Role of cuticular genes in the insect antimicrobial immune response. *Front. Cell. Infect. Microbiol.* 14. <https://doi.org/10.3389/fcimb.2024.1456075>.
- Marinho, M.A.T., Junqueira, A.C.M., Paulo, D.F., Esposito, M.C., Villet, M.H., Azeredo-Espin, A.M.L., 2012. Molecular phylogenetics of Oestroidea (Diptera: Calyptratae) with emphasis on Calliphoridae: insights into the inter-familial relationships and additional evidence for paralogy among blowflies. *Mol. Phylogenet. Evol.* 65, 840–854. <https://doi.org/10.1016/j.ympev.2012.08.007>.
- Matthews, A.C., Mikonranta, L., Raymond, B., 2019. Shifts along the parasite-mutualist continuum are opposed by fundamental trade-offs. *Proc. R. Soc. B Biol. Sci.* 286, 20190236. <https://doi.org/10.1098/rspb.2019.0236>.
- Matyukhin, A.V., Krivosheina, M.G., 2008. Contribution to the knowledge of Diptera (Insecta) parasitizing on birds. *Entomol. Rev.* 88, 258–259. <https://doi.org/10.1134/S0013873808020115>.
- McBride, C.S., Arguello, J.R., 2007. Five *Drosophila* genomes reveal nonneutral evolution and the signature of host specialization in the chemoreceptor superfamily. *Genetics* 177, 1395–1416. <https://doi.org/10.1534/genetics.107.078683>.
- McDonagh, L.M., Stevens, J.R., 2011. The molecular systematics of blowflies and screwworm flies (Diptera: Calliphoridae) using 28S rRNA, COX1 and EF-1 α : insights into the evolution of dipteran parasitism. *Parasitology* 138, 1760–1777. <https://doi.org/10.1017/S0031182011001089>.
- McMeniman, C.J., Corfas, R.A., Matthews, B.J., Ritchie, S.A., Vosshall, L.B., 2014. Multimodal integration of carbon dioxide and other sensory cues drives mosquito attraction to humans. *Cell* 156, 1060–1071. <https://doi.org/10.1016/j.cell.2013.12.044>.
- Meng, F., Liu, Z., Han, H., Finkelbergs, D., Jiang, Y., Zhu, M., Wang, Y., Sun, Z., Chen, C., Guo, Y., Cai, J., 2020. Chromosome-level genome assembly of *Aldrichina grahami*, a forensically important blowfly. *GigaScience* 9, giaa020. <https://doi.org/10.1093/gigascience/giaa020>.
- Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., Von Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* 37, 1530–1534. <https://doi.org/10.1093/molbev/msaa015>.
- Miyoshi, T., Tsuji, N., Islam, M.K., Huang, X., Motobu, M., Alim, M.A., Fujisaki, K., 2007. Molecular and reverse genetic characterization of serine proteinase-induced hemolysis in the midgut of the ixodid tick *Haemaphysalis longicornis*. *J. Insect Physiol.* 53, 195–203. <https://doi.org/10.1016/j.jinsphys.2006.12.001>.
- Moretti, T.C., Thyssen, P.J., 2006. Miíase primária em coelho doméstico causada por *Lucilia eximia* (Diptera: Calliphoridae) no Brasil: relato de caso. *Arq. Bras. Med. Veterinária E Zootec.* 58, 28–30. <https://doi.org/10.1590/S0102-09352006000100005>.
- Muñoz-García, C.I., Lorenzo-Burgunder, D., Gumi-Castillo, G., Perelló-Undreiner, D. B., Zenteno-Nava, E., Orozco-Gregorio, H., 2016. Canine myiasis by *Lucilia eximia* in North America. *Trop. Biomed.* 33, 494–499.
- Nasser, M.G., Hosni, E.M., Kenawy, M.A., Alharbi, S.A., Almoallim, H.S., Rady, M.H., Merdan, B.A., Pont, A.C., Al-Ashaal, S.A., 2021. Evolutionary profile of the family Calliphoridae, with notes on the origin of myiasis. *Saudi J. Biol. Sci.* 28, 2056–2066. <https://doi.org/10.1016/j.sjbs.2021.01.032>.
- Nielsen, R., 2005. Molecular signatures of natural selection. *Annu. Rev. Genet.* 39, 197–218. <https://doi.org/10.1146/annurev.genet.39.073003.112420>.
- Ou, S., Jiang, N., 2018. LTR_retriever: a highly accurate and sensitive program for identification of long terminal repeat retrotransposons. *Plant Physiol.* 176, 1410–1422. <https://doi.org/10.1104/pp.17.01310>.
- Page, M.J., Di Cera, E., 2008. Serine peptidases: classification, structure and function. *Cell. Mol. Life Sci. CMLS* 65, 1220–1236. <https://doi.org/10.1007/s00018-008-7565-9>.
- Paradis, E., Schliep, K., 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35, 526–528. <https://doi.org/10.1093/bioinformatics/bty633>.
- Parker, J., Tsagkogeorga, G., Cotton, J.A., Liu, Y., Provero, P., Stupka, E., Rossiter, S.J., 2013. Genome-wide signatures of convergent evolution in ecolocating mammals. *Nature* 502, 228–231. <https://doi.org/10.1038/nature12511>.
- Parmar, D.R., Johnston, N.P., Wallman, J.F., Szpila, K., 2024. Blowfly genomics: current insights, knowledge gaps, and future perspectives. *Curr. Opin. Insect Sci.* 101305. <https://doi.org/10.1016/j.cois.2024.101305>.
- Paten, B., Earl, D., Nguyen, N., Diekhans, M., Zerbino, D., Haussler, D., 2011. Cactus: algorithms for genome multiple sequence alignment. *Genome Res.* 21, 1512–1528. <https://doi.org/10.1101/gr.123356.111>.
- Pezzi, M., Scapoli, C., Chicca, M., Leis, M., Marchetti, M.G., Del Zingaro, C.N.F., Vicentini, C.B., Mamolini, E., Giangaspero, A., Bonacci, T., 2021. Cutaneous myiasis in cats and dogs: cases, predisposing conditions and risk factors. *Vet. Med. Sci.* 7, 378–384. <https://doi.org/10.1002/vms3.370>.
- Price, P.W., 1980. *Evolutionary Biology of Parasites*. Princeton University Press.
- Puchala, P., 2004. Detrimental effects of larval blow flies (Protocalliphora azurea) on nestlings and breeding success of Tree Sparrows (*Passer montanus*). *Can. J. Zool.* 82, 1285–1290. <https://doi.org/10.1139/z04-111>.
- Quinlan, A.R., Hall, I.M., 2010. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26, 841–842. <https://doi.org/10.1093/bioinformatics/btq033>.

- Rebeiz, M., Tsiantis, M., 2017. Enhancer evolution and the origins of morphological novelty. *Curr. Opin. Genet. Dev.* 45, 115–123. <https://doi.org/10.1016/j.gde.2017.04.006>.
- Revell, L.J., 2011. *phytools: an R package for phylogenetic comparative biology (and other things)*. *Methods Ecol. Evol.* 7.
- Rinker, D.C., Pitts, R.J., Zhou, X., Suh, E., Rokas, A., Zwiebel, L.J., 2013. Blood meal-induced changes to antennal transcriptome profiles reveal shifts in odor sensitivities in *Anopheles gambiae*. *Proc. Natl. Acad. Sci.* 110, 8260–8265. <https://doi.org/10.1073/pnas.1302562110>.
- Robertson, H.M., 2019. Molecular evolution of the major arthropod chemoreceptor gene families. *Annu. Rev. Entomol.* 64, 227–242. <https://doi.org/10.1146/annurev-ento-020117-043322>.
- Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4, e2584. <https://doi.org/10.7717/peerj.2584>.
- Saboia-Vahia, L., Cuervo, P., Borges-Veloso, A., de Souza, N.P., Britto, C., Dias-Lopes, G., De Jesus, J.B., 2014. The midgut of *Aedes albopictus* females expresses active trypsin-like serine peptidases. *Parasit Vectors* 30, 253. <https://doi.org/10.1186/1756-3305-7-253>.
- Sackton, T.B., Clark, N., 2019. Convergent evolution in the genomics era: new insights and directions. *Philos. Trans. R. Soc. B Biol. Sci.* 374, 20190102. <https://doi.org/10.1098/rstb.2019.0102>.
- Signor, S.A., Nuzhdin, S.V., 2018. The Evolution of Gene Expression in cis and trans. *Trends Genet.* 34, 532–544. <https://doi.org/10.1016/j.tig.2018.03.007>.
- Simão, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V., Zdobnov, E.M., 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31, 3210–3212. <https://doi.org/10.1093/bioinformatics/btv351>.
- Smit, A., Hubley, R., Green, P., 2013. RepeatMasker Open-4.0.
- Stevens, J.R., 2003. The evolution of myiasis in blowflies (Calliphoridae). *Int. J. Parasitol.* 33, 1105–1113. [https://doi.org/10.1016/S0020-7519\(03\)00136-X](https://doi.org/10.1016/S0020-7519(03)00136-X).
- Stevens, J.R., Wallman, J.F., 2006. The evolution of myiasis in humans and other animals in the Old and New Worlds (part I): phylogenetic analyses. *Trends Parasitol.* 22, 129–136. <https://doi.org/10.1016/j.pt.2006.01.008>.
- Stevens, J.R., Wallman, J.F., Otranto, D., Wall, R., 2006. The evolution of myiasis in humans and other animals in the Old and New Worlds (part II): biological and life-history studies. *Trends Parasitol.* 22, 181–188. <https://doi.org/10.1016/j.pt.2006.02.010>.
- Tandonnet, S., Cardoso, G.A., Mariano-Martins, P., Monfardini, R.D., Cunha, V.A.S., de Carvalho, R.A., Torres, T.T., 2020. Molecular basis of resistance to organophosphate insecticides in the New World screw-worm fly. *Parasite Vectors* 13, 562. <https://doi.org/10.1186/s13071-020-04433-3>.
- Tandonnet, S., Krsticevic, F., Basika, T., Papathanos, P.A., Torres, T.T., Scott, M.J., 2022. A chromosomal-scale reference genome of the New World Screwworm, *Cochliomyia hominivorax*. *DNA Res.* dsac042. <https://doi.org/10.1093/dnares/dsac042>.
- Ullerich, F.-H., Schöttke, M., 2006. Karyotypes, constitutive heterochromatin, and genomic DNA values in the blowfly genera *Chrysomya*, *Lucilia*, and *Protophormia* (Diptera: Calliphoridae). *Genome* 49, 584–597. <https://doi.org/10.1139/g06-013>.
- Vicoso, B., Bachtrog, D., 2015. Numerous transitions of sex chromosomes in Diptera. *PLoS Biol.* 13, e1002078. <https://doi.org/10.1371/journal.pbio.1002078>.
- Weinstein, S.B., Kuris, A.M., 2016. Independent origins of parasitism in Animalia. *Biol. Lett.* 12, 20160324. <https://doi.org/10.1098/rsbl.2016.0324>.
- Whitworth, T., 2014. A revision of the Neotropical species of *Lucilia* Robineau-Desvoidy (Diptera: Calliphoridae). *Zootaxa* 3810, 1. <https://doi.org/10.11646/zootaxa.3810.1.1>.
- Wiegmann, B.M., Farrell, C., Farrell, B., 1993. Diversification of carnivorous parasitic insects: extraordinary radiation or specialized dead end? *Am. Nat.* 142, 737–754. <https://doi.org/10.1086/285570>.
- Wiegmann, B.M., Richards, S., 2018. Genomes of Diptera. *Curr. Opin. Insect Sci. Insect Genom.* * Dev. Regul. 25, 116–124. <https://doi.org/10.1016/j.cois.2018.01.007>.
- Wiegmann, B.M., Trautwein, M.D., Winkler, I.S., Barr, N.B., Kim, J.-W., Lambkin, C., Bertone, M.A., Cassel, B.K., Bayless, K.M., Heimberg, A.M., Wheeler, B.M., Peterson, K.J., Pape, T., Sinclair, B.J., Skevington, J.H., Blagoderov, V., Caravas, J., Kutty, S.N., Schmidt-Ott, U., Kampmeier, G.E., Thompson, F.C., Grimaldi, D.A., Beckenbach, A.T., Courtney, G.W., Friedrich, M., Meier, R., Yeates, D.K., 2011. Episodic radiations in the fly tree of life. *Proc. Natl. Acad. Sci.* 108, 5690–5695. <https://doi.org/10.1073/pnas.1012675108>.
- Wyss, J.H., 2000. Screwworm eradication in the Americas. *Ann. N. Y. Acad. Sci.* 916, 186–193. <https://doi.org/10.1111/j.1749-6632.2000.tb05289.x>.
- Yan, L., Pape, T., Meusemann, K., Kutty, S.N., Meier, R., Bayless, K.M., Zhang, D., 2021. Monophyletic blowflies revealed by phylogenomics. *BMC Biol.* 19, 230. <https://doi.org/10.1186/s12915-021-01156-4>.
- Yang, Y., Wen, Y.J., Cai, Y.N., Vallée, I., Boireau, P., Liu, M.Y., Cheng, S.P., 2015. Serine proteases of parasitic helminths. *Korean J. Parasitol.* 53, 1–11. <https://doi.org/10.3347/kjp.2015.53.1.1>.
- Yang, Z., 2007. PAML 4: phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* 24, 1586–1591. <https://doi.org/10.1093/molbev/msm088>.
- Yang, Z., 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. *Comput. Appl. Biosci.* 13, 555–556.
- Zumpt, F., 1965. Myiasis in man and animals in the Old World. *A Textbook for Physicians, Veterinarians and Zoologists*. Butterworths, London.