



DATA NOTE

The genome sequence of the muscid fly *Eudasyphora cyanicolor* (Zetterstedt, 1845)

[version 1; peer review: 2 approved]

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Abstract
We present a genome assembly from an individual female specimen of *Eudasyphora cyanicolor* (Arthropoda; Insecta; Diptera; Muscidae). The genome sequence has a total length of 1,450.40 megabases. Most of the assembly (99.68%) is scaffolded into 5 chromosomal pseudomolecules. The mitochondrial genome has also been assembled and is 19.34 kilobases in length.

Keywords
Eudasyphora cyanicolor, muscid fly, genome sequence, chromosomal, Diptera



This article is included in the [Tree of Life](#) gateway.

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2. **Rakesh K Mishra** , Tata Institute for Genetics and Society, Bengaluru, India

Any reports and responses or comments on the article can be found at the end of the article.

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Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Diptera; Brachycera; Muscomorpha; Eremoneura; Cyclorrhapha; Schizophora; Calyptratae; Muscoidea; Muscidae; Muscinae; Muscini; *Eudasyphora*; *Eudasyphora cyanicolor* (Zetterstedt, 1845) (NCBI:txid1643974)

Background

Eudasyphora cyanicolor (Zetterstedt, 1845) belongs to the Muscidae family of houseflies. Muscid flies are diverse, with different members exhibiting varied feeding mechanisms such as phytophagy, saprophagy, hematophagy, and carnivory (Kutty *et al.*, 2014; Moon, 2019). These flies are synanthropes, thriving on various substrates and habitats influenced by human activities and the environment (Moon, 2019).

Some Muscidae members are important vectors of pathogens and parasites with negative impacts on the public hygiene of humans and livestock (Cook, 2020; Issa, 2019; Onwugamba *et al.*, 2018). However, most muscid flies are ecologically relevant in the breakdown and recycling of organic waste (Moon, 2019). The members of the *Eudasyphora* genus are frequent visitors to our homes and sometimes overwinter indoors (Logunov & Dennis, 2016). *E. cyanicolor* is typically associated with woodlands, scrublands, swamps, and animal dung (Taylor, 1999; Tridrih & Sorokina, 2021).

E. cyanicolor can be easily identified by its deep metallic blue body and a stripe of whitish dusting at the front of the mesonotum (Falk, 2016; Grzywacz *et al.*, 2017). These unique features distinguish it from *E. cyanella*, its closest relative, which possesses a green colour (Falk, 2016; Nihei & De Carvalho, 2007). *E. cyanicolor* is widespread in Europe, with the highest records in the United Kingdom and Finland (GBIF Secretariat, 2024). The UK records show adult emergence from March to November, with peak flight activity in summer (NBN Atlas Partnership, 2024).

This article presents a chromosomal-level genome assembly for *E. cyanicolor*, sequenced as part of the Darwin Tree of Life Project. It will contribute to future studies on the biodiversity, genomics and evolution of this species.

Genome sequence report

The genome of *Eudasyphora cyanicolor* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating a total of 45.53 Gb (gigabases) from 4.39 million reads, providing an estimated 36-fold coverage. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data, which produced 138.68 Gb from 918.42 million reads. Specimen and sequencing details are summarised in Table 1.

This reduced the assembly length by 3.24%, the scaffold number by 31.82%, and the scaffold N50 by 0.82%. The final assembly has a total length of 1,450.40 Mb in 74 sequence



Figure 1. Photograph of the *Eudasyphora cyanicolor* (idEudCyai1) specimen used for genome sequencing.

scaffolds, with 370 gaps, and a scaffold N50 of 293.4 Mb (Table 2).

The snail plot in Figure 2 provides a summary of the assembly statistics, indicating the distribution of scaffold lengths and other assembly metrics. Figure 3 shows the distribution of scaffolds by GC proportion and coverage. Figure 4 presents a cumulative assembly plot, with separate curves representing different scaffold subsets assigned to various phyla, illustrating the completeness of the assembly.

Most of the assembly sequence (99.68%) was assigned to 5 chromosomal-level scaffolds. These chromosome-level scaffolds, confirmed by the Hi-C data, are named in order of size (Figure 5; Table 3). During manual curation it was noted that there was insufficient information to identify the sex chromosomes. Sequence data from the heterogametic sex was not available and homology is unreliable for sex chromosome identification in Diptera due to frequent sex chromosome turnover (Vicoso & Bachtrog, 2015).

While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited. The mitochondrial genome was also assembled and can be found as a contig within the multifasta file of the genome submission, and as a standalone sequence on GenBank.

The primary haplotype has a QV of 61.4, and the combined primary and alternate assemblies achieve an estimated QV of 61.4. The *k*-mer completeness for the primary haplotype is 66.92%, and for the alternate haplotype it is 66.27%. The combined primary and alternate assemblies achieve a *k*-mer completeness of 99.09%. BUSCO analysis using the diptera_odb10 reference set ($n = 3,285$) indicated a completeness score of 98.7% (single = 97.8%, duplicated = 0.9%). The assembly achieves the EBP reference standard of 6.C.61. Other quality metrics are given in Table 2.

Table 1. Specimen and sequencing data for *Eudasyphora cyanicolor*.

Project information			
Study title	Eudasyphora cyanicolor		
Umbrella BioProject	PRJEB67607		
Species	<i>Eudasyphora cyanicolor</i>		
BioSample	SAMEA113425446		
NCBI taxonomy ID	1643974		
Specimen information			
Technology	ToLID	BioSample accession	Organism part
PacBio long read sequencing	idEudCyai1	SAMEA113425543	Whole organism
Hi-C sequencing	idEudCyai1	SAMEA113425543	Whole organism
Sequencing information			
Platform	Run accession	Read count	Base count (Gb)
Hi-C Illumina NovaSeq 6000	ERR12143998	9.18e+08	138.68
PacBio Revio	ERR12205263	4.39e+06	45.53

Table 2. Genome assembly data for *Eudasyphora cyanicolor*, idEudCyai1.1.

Genome assembly		
Assembly name	idEudCyai1.1	
Assembly accession	GCA_963930755.1	
Accession of alternate haplotype	GCA_963930745.1	
Span (Mb)	1,450.40	
Number of contigs	445	
Number of scaffolds	74	
Longest scaffold (Mb)	365.32	
Assembly metrics*		Benchmark
Contig N50 length (Mb)	6.9	$\geq 1 \text{ Mb}$
Scaffold N50 length (Mb)	293.4	= chromosome N50
Consensus quality (QV)	Primary: 61.4; alternate: 61.5; combined 61.4	≥ 40
k-mer completeness	Primary: 66.92%; alternate: 66.27%; combined: 99.09%	$\geq 95\%$
BUSCO**	C:98.7%[S:97.8%,D:0.9%], F:0.6%,M:0.7%,n:3,285	$S > 90\%$, $D < 5\%$
Percentage of assembly mapped to chromosomes	Primary: 66.92%; alternate: 66.27%; combined: 99.09%	$\geq 90\%$
Sex chromosomes	Not identified	localised homologous pairs
Organelles	Mitochondrial genome: 19.34 kb	complete single alleles

* Assembly metric benchmarks are adapted from Rhie *et al.* (2021) and the Earth BioGenome Project Report on Assembly Standards September 2024.

** BUSCO scores based on the diptera_odb10 BUSCO set using version 5.4.3. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison. A full set of BUSCO scores is available at https://blobtoolkit.genomehubs.org/view/Eudasyphora_cyanicolor/dataset/GCA_963930755.1/busco.

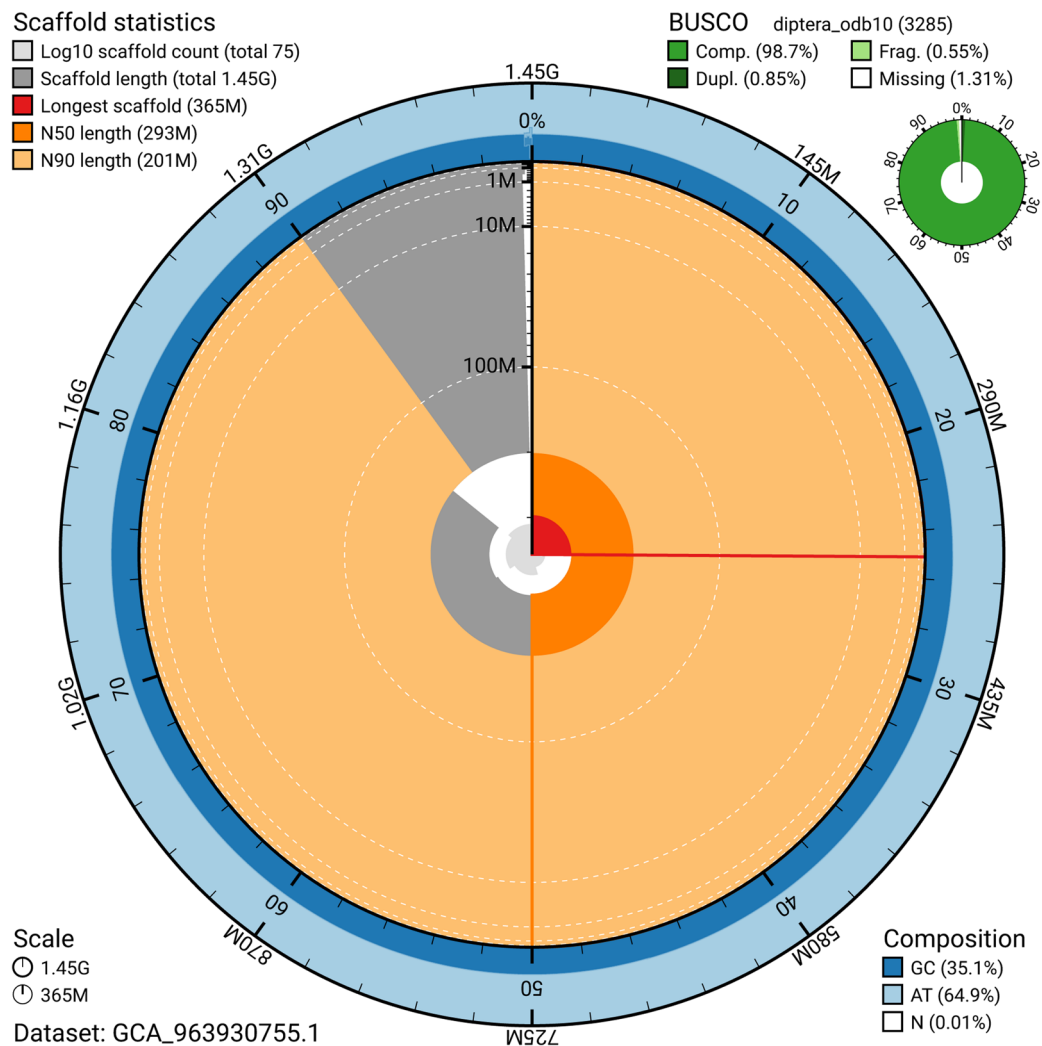


Figure 2. Genome assembly of *Eudasyphora cyanicolor*, idEudCyai1.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. The outermost blue tracks display the distribution of GC, AT, and N percentages across the bins. Scaffolds are arranged clockwise from longest to shortest and are depicted in dark grey. The longest scaffold is indicated by the red arc, and the deeper orange and pale orange arcs represent the N50 and N90 lengths. A light grey spiral at the centre shows the cumulative scaffold count on a logarithmic scale. A summary of complete, fragmented, duplicated, and missing BUSCO genes in the diptera_odb10 set is presented at the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963930755.1/dataset/GCA_963930755.1/snail.

Methods

Sample acquisition and DNA barcoding

An adult female *Eudasyphora cyanicolor* (specimen ID Ox002809, ToLID idEudCyai1) was collected from Wytham Woods, Oxfordshire, United Kingdom (latitude 51.77, longitude -1.33) on 2022-07-14 by netting. The specimen was collected by Steven Falk (independent researcher) and Liam Crowley (University of Oxford), identified by Steven Falk and preserved on dry ice.

The initial identification was verified by an additional DNA barcoding process according to the framework developed

by Twyford *et al.* (2024). A small sample was dissected from the specimens and stored in ethanol, while the remaining parts were shipped on dry ice to the Wellcome Sanger Institute (WSI). The tissue was lysed, the COI marker region was amplified by PCR, and amplicons were sequenced and compared to the BOLD database, confirming the species identification (Crowley *et al.*, 2023). Following whole genome sequence generation, the relevant DNA barcode region was also used alongside the initial barcoding data for sample tracking at the WSI (Twyford *et al.*, 2024). The standard operating procedures for Darwin Tree of Life barcoding have been deposited on protocols.io (Beasley *et al.*, 2023).

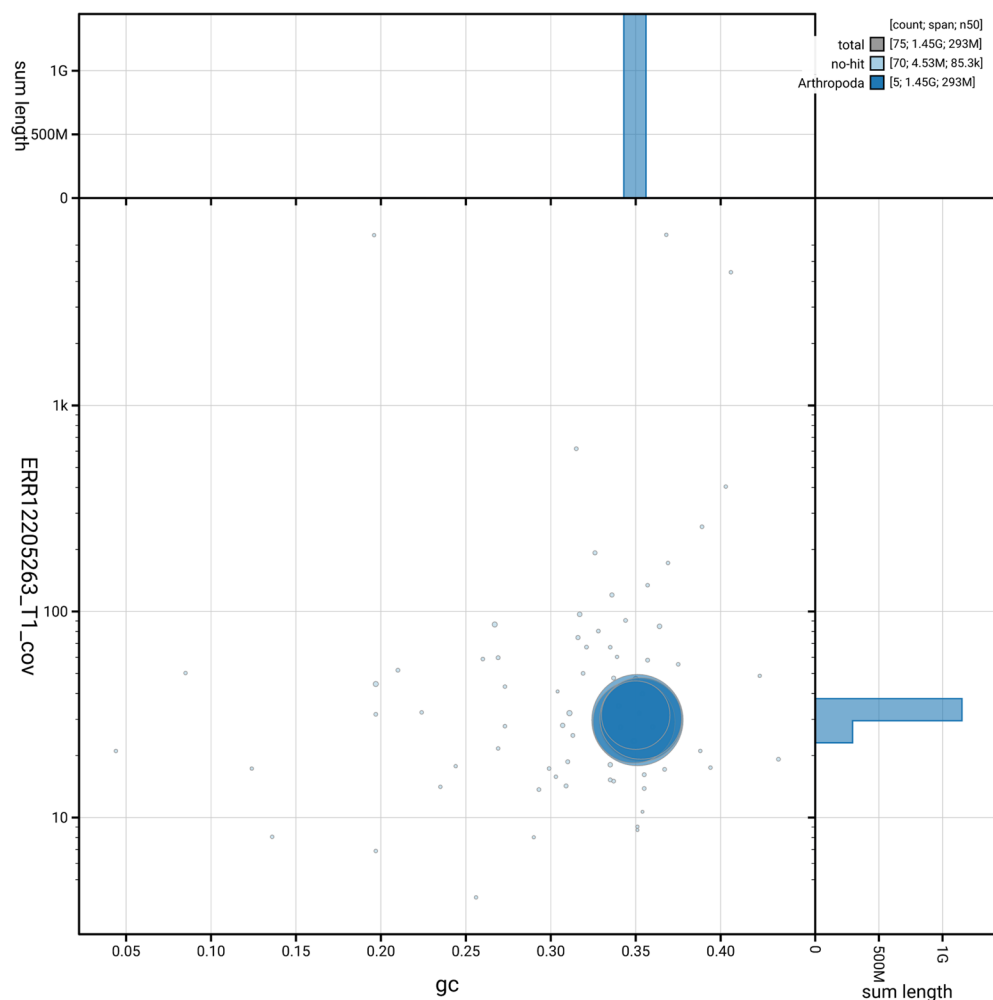


Figure 3. Genome assembly of *Eudasyphora cyanicolor*, idEudCyai1.1: BlobToolKit GC-coverage plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes display the total length of sequences distributed across different levels of coverage and GC content. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963930755.1/dataset/GCA_963930755.1/blob.

Nucleic acid extraction

The workflow for high molecular weight (HMW) DNA extraction at the Wellcome Sanger Institute (WSI) Tree of Life Core Laboratory includes a sequence of procedures: sample preparation and homogenisation, DNA extraction, fragmentation and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). The idEudCyai1 sample was prepared for DNA extraction by weighing and dissecting it on dry ice (Jay *et al.*, 2023), and was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a). HMW DNA was extracted in the WSI Scientific Operations core using the Automated MagAttract v2 protocol (Oatley *et al.*, 2023). The DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system (Bates *et al.*, 2023). Sheared DNA was purified by solid-phase reversible immobilisation, using AMPure PB beads to eliminate shorter fragments and concentrate the DNA (Strickland *et al.*, 2023). The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer

and Qubit Fluorometer using the Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

Hi-C sample preparation

Tissue from the idEudCyai1 sample was processed at the WSI Scientific Operations core, using the Arima-HiC v2 kit. Tissue (stored at -80°C) was fixed, and the DNA crosslinked using a TC buffer with 22% formaldehyde. After crosslinking, the tissue was homogenised using the Diagnocine Power Masher-II and BioMasher-II tubes and pestles. Following the kit manufacturer's instructions, crosslinked DNA was digested using a restriction enzyme master mix. The 5'-overhangs were then filled in and labelled with biotinylated nucleotides and proximally ligated. An overnight incubation was carried out for enzymes to digest remaining proteins and for crosslinks to reverse. A clean up was performed with SPRIselect beads prior to library preparation.

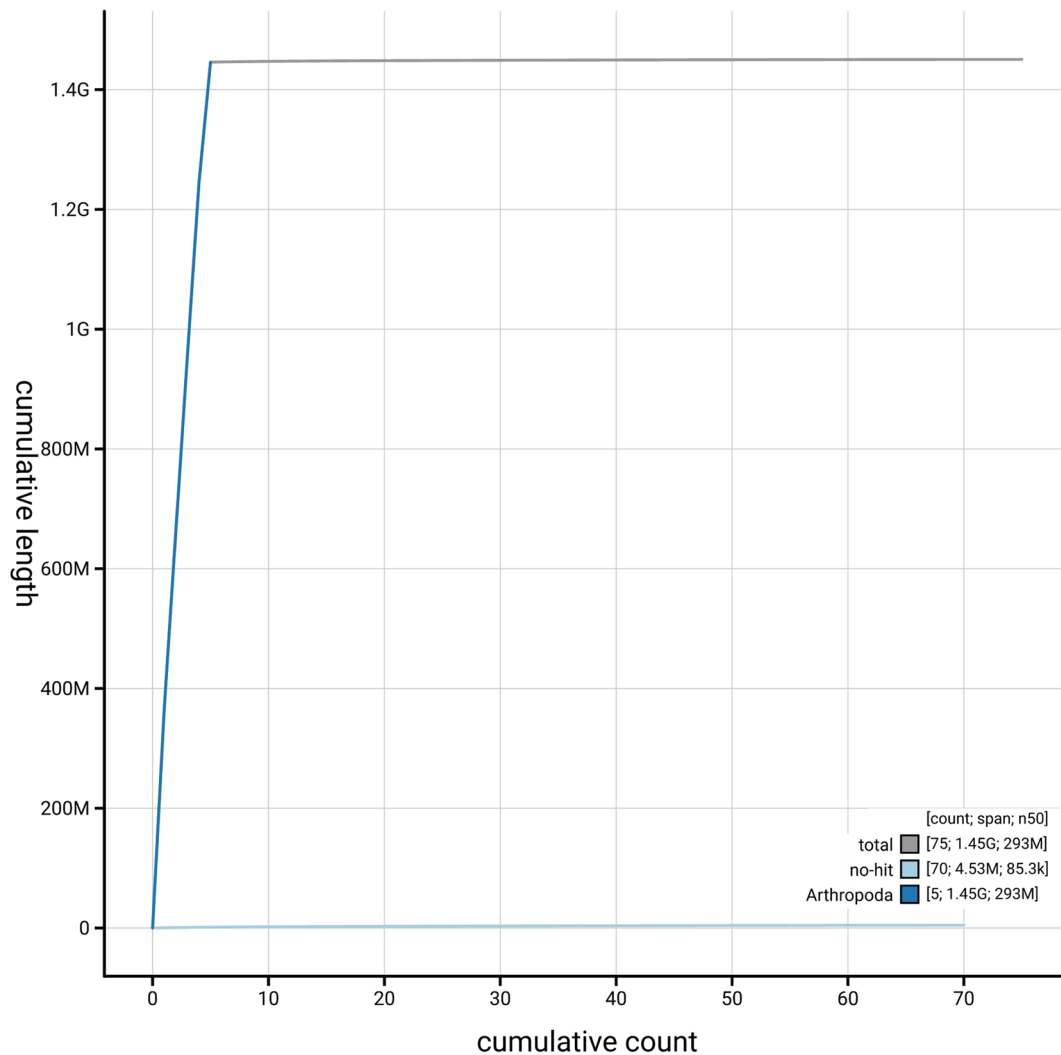


Figure 4. Genome assembly of *Eudasyphora cyanicolor* idEudCyai1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963930755.1/dataset/GCA_963930755.1/cumulative.

Library preparation and sequencing

PacBio HiFi

At a minimum, samples were required to have an average fragment size exceeding 8 kb and a total mass over 400 ng to proceed to the low input SMRTbell Prep Kit 3.0 protocol (Pacific Biosciences, California, USA), depending on genome size and sequencing depth required. Libraries were prepared using the SMRTbell Prep Kit 3.0 (Pacific Biosciences, California, USA) as per the manufacturer's instructions. The kit includes the reagents required for end repair/A-tailing, adapter ligation, post-ligation SMRTbell bead cleanup, and nuclease treatment. Following the manufacturer's instructions, size selection and clean up was carried out using diluted AMPure PB beads (Pacific Biosciences, California, USA). DNA concentration was quantified using the Qubit Fluorometer v4.0 (Thermo Fisher

Scientific) with Qubit 1X dsDNA HS assay kit and the final library fragment size analysis was carried out using the Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies) and gDNA 55kb BAC analysis kit.

Samples were sequenced on a Revio instrument (Pacific Biosciences, California, USA). Prepared libraries were normalised to 2 nM, and 15 µL was used for making complexes. Primers were annealed and polymerases were hybridised to create circularised complexes according to manufacturer's instructions. The complexes were purified with the 1.2X clean up with SMRTbell beads. The purified complexes were then diluted to the Revio loading concentration (in the range 200–300 pM), and spiked with a Revio sequencing internal control. Samples were sequenced on Revio 25M SMRT cells (Pacific Biosciences,

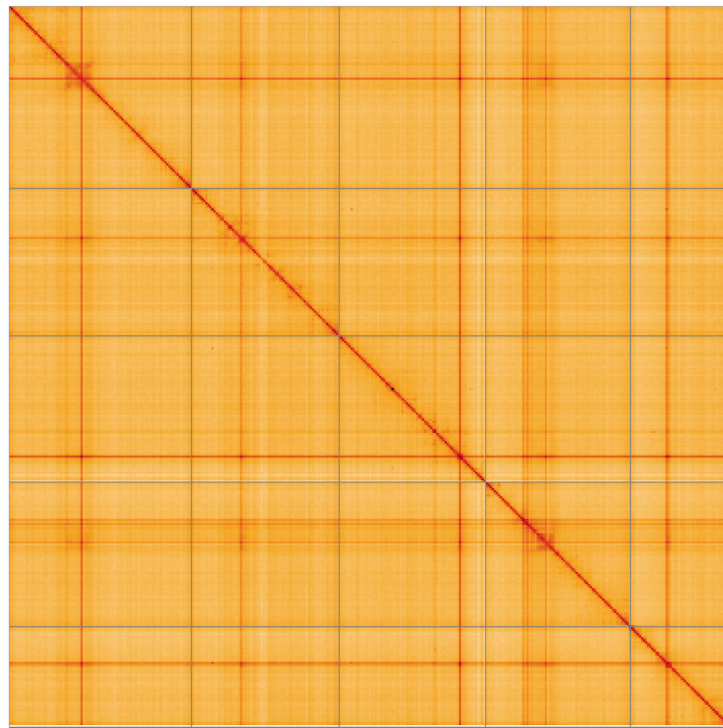


Figure 5. Genome assembly of *Eudasyphora cyanicolor* idEudCyai1.1: Hi-C contact map of the idEudCyai1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at <https://genome-note-higlass.tol.sanger.ac.uk/l/?d=VRyzap9aQCqFDjjPjdSuQA>.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Eudasyphora cyanicolor*, idEudCyai1.

INSDC accession	Name	Length (Mb)	GC%
OZ005734.1	1	365.32	35.0
OZ005735.1	2	295.72	35.5
OZ005736.1	3	293.42	35.0
OZ005737.1	4	290.28	35.5
OZ005738.1	5	201.16	35.0
OZ005739.1	MT	0.02	19.5

California, USA). The SMRT link software, a PacBio web-based end-to-end workflow manager, was used to set-up and monitor the run, as well as perform primary and secondary analysis of the data upon completion.

Hi-C

For Hi-C library preparation, DNA was fragmented using the Covaris E220 sonicator (Covaris) and size selected using SPRISelect beads to 400 to 600 bp. The DNA was then enriched using the Arima-HiC v2 kit Enrichment beads. Using the NEB-Next Ultra II DNA Library Prep Kit (New England Biolabs)

for end repair, a-tailing, and adapter ligation. This uses a custom protocol which resembles the standard NEBNext Ultra II DNA Library Prep protocol but where library preparation occurs while DNA is bound to the Enrichment beads. For library amplification, 10 to 16 PCR cycles were required, determined by the sample biotinylation percentage. The Hi-C sequencing was performed using paired-end sequencing with a read length of 150 bp on an Illumina NovaSeq 6000 instrument.

Genome assembly, curation and evaluation

Assembly

The HiFi reads were assembled using Hifiasm (Cheng *et al.*, 2021) with the --primary option. Haplotypic duplications were identified and removed using purge_dups (Guan *et al.*, 2020). The Hi-C reads were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). The contigs were further scaffolded using the provided Hi-C data (Rao *et al.*, 2014) in YaHS (Zhou *et al.*, 2023) using the --break option for handling potential misassemblies. The scaffolded assemblies were evaluated using Gfastats (Formenti *et al.*, 2022), BUSCO (Manni *et al.*, 2021) and MERQURY.FK (Rhie *et al.*, 2020).

The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2023), which runs MitoFinder (Allio *et al.*, 2020) and uses these annotations to select the final mitochondrial contig and to ensure the general quality of the sequence.

Assembly curation

The assembly was decontaminated using the Assembly Screen for Cointons and Contaminants (ASCC) pipeline (article in preparation). Flat files and maps used in curation were generated in TreeVal (Pointon *et al.*, 2023). Manual curation was primarily conducted using PretextView (Harry, 2022), with additional insights provided by JBrowse2 (Diesh *et al.*, 2023) and HiGlass (Kerpedjiev *et al.*, 2018). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Any identified contamination, missed joins, and mis-joins were corrected, and duplicate sequences were tagged and removed. The curation process is documented at <https://gitlab.com/wtsi-grit/rapid-curation> (article in preparation).

Evaluation of the final assembly

The Merqury.FK tool (Rhie *et al.*, 2020) was used to evaluate *k*-mer completeness and assembly quality for the primary and alternate haplotypes using the *k*-mer databases (*k* = 31) that were pre-computed prior to genome assembly. The analysis outputs included assembly QV scores and completeness statistics.

A Hi-C contact map was produced for the final, public version of the assembly. The Hi-C reads were aligned using bwa-mem2 (Vasimuddin *et al.*, 2019) and the alignment files were combined using SAMtools (Danecek *et al.*, 2021). The Hi-C alignments were converted into a contact map using BEDTools (Quinlan & Hall, 2010) and the Cooler tool suite (Abdennur & Mirny, 2020). The contact map is visualised in HiGlass (Kerpedjiev *et al.*, 2018).

The blobtoolkit pipeline is a Nextflow port of the previous Snakemake Blobtoolkit pipeline (Challis *et al.*, 2020). It aligns

the PacBio reads in SAMtools and minimap2 (Li, 2018) and generates coverage tracks for regions of fixed size. In parallel, it queries the GoaT database (Challis *et al.*, 2023) to identify all matching BUSCO lineages to run BUSCO (Manni *et al.*, 2021). For the three domain-level BUSCO lineages, the pipeline aligns the BUSCO genes to the UniProt Reference Proteomes database (Bateman *et al.*, 2023) with DIAMOND blastp (Buchfink *et al.*, 2021). The genome is also divided into chunks according to the density of the BUSCO genes from the closest taxonomic lineage, and each chunk is aligned to the UniProt Reference Proteomes database using DIAMOND blastx. Genome sequences without a hit are chunked using seqtk and aligned to the NT database with blastn (Altschul *et al.*, 1990). The blobtools suite combines all these outputs into a blobdir for visualisation.

The genome assembly and evaluation pipelines were developed using nf-core tooling (Ewels *et al.*, 2020) and MultiQC (Ewels *et al.*, 2016), relying on the Conda package manager, the Bioconda initiative (Grüning *et al.*, 2018), the Biocontainers infrastructure (da Veiga Leprevost *et al.*, 2017), as well as the Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017) containerisation solutions.

Table 4 contains a list of relevant software tool versions and sources.

Wellcome Sanger Institute – Legal and Governance

The materials that have contributed to this genome note have been supplied by a Darwin Tree of Life Partner. The submission of materials by a Darwin Tree of Life Partner is subject to the ‘Darwin Tree of Life Project Sampling Code of Practice’,

Table 4. Software tools: versions and sources.

Software tool	Version	Source
BEDTools	2.30.0	https://github.com/arq5x/bedtools2
BLAST	2.14.0	ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/
BlobToolKit	4.3.7	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.4.3 and 5.5.0	https://gitlab.com/e2lab/busco
bwa-mem2	2.2.1	https://github.com/bwa-mem2/bwa-mem2
Cooler	0.8.11	https://github.com/open2c/cooler
DIAMOND	2.1.8	https://github.com/bbuchfink/diamond
fasta_windows	0.2.4	https://github.com/tolkkit/fasta_windows
FastK	427104ea91c78c3b8b8b49f1a7d6bbeaa869ba1c	https://github.com/thegenemyers/FASTK
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
GoaT CLI	0.2.5	https://github.com/genomehubs/goat-cli
Hifiasm	0.19.8-r587	https://github.com/chhyllp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84aa44357826c0b6753eb28de	https://github.com/higlass/higlass

Software tool	Version	Source
Mercury.FK	d00d98157618f4e8d1a9190026b19b471055b22e	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	3	https://github.com/marcelauliano/MitoHiFi
MultiQC	1.14, 1.17, and 1.18	https://github.com/MultiQC/MultiQC
NCBI Datasets	15.12.0	https://github.com/ncbi/datasets
Nextflow	23.04.0-5857	https://github.com/nextflow-io/nextflow
PretextView	0.2.5	https://github.com/sanger-tol/PretextView
purge_dups	1.2.5	https://github.com/dfguan/purge_dups
samtools	1.16.1, 1.17, and 1.18	https://github.com/samtools/samtools
sanger-tol/ascc	-	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	0.6.0	https://github.com/sanger-tol/blobtoolkit
Seqtk	1.3	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.0.0	https://github.com/sanger-tol/treeval
YaHS	1.2a.2	https://github.com/c-zhou/yahs

which can be found in full on the Darwin Tree of Life website [here](#). By agreeing with and signing up to the Sampling Code of Practice, the Darwin Tree of Life Partner agrees they will meet the legal and ethical requirements and standards set out within this document in respect of all samples acquired for, and supplied to, the Darwin Tree of Life Project.

Further, the Wellcome Sanger Institute employs a process whereby due diligence is carried out proportionate to the nature of the materials themselves, and the circumstances under which they have been/are to be collected and provided for use. The purpose of this is to address and mitigate any potential legal and/or ethical implications of receipt and use of the materials as part of the research project, and to ensure that in doing so we align with best practice wherever possible. The overarching areas of consideration are:

- Ethical review of provenance and sourcing of the material
- Legality of collection, transfer and use (national and international)

Each transfer of samples is further undertaken according to a Research Collaboration Agreement or Material Transfer Agreement entered into by the Darwin Tree of Life Partner, Genome Research Limited (operating as the Wellcome Sanger Institute), and in some circumstances other Darwin Tree of Life collaborators.

Data availability

European Nucleotide Archive: *Eudasyphora cyanicolor*. Accession number PRJEB67607; <https://identifiers.org/ena.embl/PRJEB67607>. The genome sequence is released openly for

reuse. The *Eudasyphora cyanicolor* genome sequencing initiative is part of the Darwin Tree of Life (DTOL) project. All raw sequence data and the assembly have been deposited in INSDC databases. The genome will be annotated using available RNA-Seq data and presented through the [Ensembl](#) pipeline at the European Bioinformatics Institute. Raw data and assembly accession identifiers are reported in [Table 1](#) and [Table 2](#).

Author information

Members of the University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: <https://doi.org/10.5281/zenodo.12157525>.

Members of the Darwin Tree of Life Barcoding collective are listed here: <https://doi.org/10.5281/zenodo.12158331>.

Members of the Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team are listed here: <https://doi.org/10.5281/zenodo.12162482>.

Members of Wellcome Sanger Institute Scientific Operations: Sequencing Operations are listed here: <https://doi.org/10.5281/zenodo.12165051>.

Members of the Wellcome Sanger Institute Tree of Life Core Informatics team are listed here: <https://doi.org/10.5281/zenodo.12160324>.

Members of the Tree of Life Core Informatics collective are listed here: <https://doi.org/10.5281/zenodo.12205391>.

Members of the Darwin Tree of Life Consortium are listed here: <https://doi.org/10.5281/zenodo.4783558>.

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Rakesh K Mishra 

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Authors in this report present genome sequencing and assembly of muscid fly. They also use NGS based approach to assemble the sequence data in five molecules (chromosomes). The description of the process and approach is described in sufficient details. This will be a very useful information in the fast growing field of genome biology of insects for understanding basic biology and human health perspective as the information is of potential use in genome based technologies of vector control.

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genome organisation, comparative genomics, genetics and epigenetic regulation of genes

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 26 February 2025

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Jason Charamis 

Foundation for Research and Technology - Hellas, Irákleion, Greece

This article describes the genome sequencing of the housefly *Eudasyphora cyanicolor*. The study produced a highly contiguous and highly accurate genome assembly of all main chromosomes, as demonstrated by the BUSCO analysis and the k-mer based QV metric, except for the sex chromosomes. This is a common challenge in dipteran genome assemblies, due to the frequent evolutionary turnover of sex chromosomes in this order.

The manuscript is mostly ready for indexing. My only request is to better describe the relevance of sequencing of this species for future ecological studies. For example, because of its synanthropic behavior, this species has been probably exposed to pesticides for several decades. In this context, genome sequencing would be useful for studying insecticide resistance in non-target insects. I would like to see the Introduction section expanded to better establish the relevance of this genome assembly.

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Arthropod Comparative Genomics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.