DATA NOTE

The genome sequence of the Grouse Louse Fly, *Ornithomya chloropus* (Bergroth, 1901)

[version 1; peer review: 2 approved, 1 approved with reservations]

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Abstract

We present a genome assembly from a male specimen of *Ornithomya chloropus* (Grouse Louse Fly; Arthropoda; Insecta; Diptera; Hippoboscidae). The genome sequence has a total length of 193.17 megabases. Most of the assembly (99.94%) is scaffolded into 5 chromosomal pseudomolecules, including the X and Y sex chromosomes. The mitochondrial genome has also been assembled, with a length of 16.78 kilobases.

Keywords

Ornithomya chloropus, Grouse Louse Fly, genome sequence, chromosomal, Diptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ? ✓ ✓

	1	2	3
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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; Hippoboscoidea; Hippoboscidae; Ornithomyinae; *Ornithomya*; *Ornithomya chloropus* (Bergroth, 1901) (NCBI:txid352856)

Background

The Grouse Louse Fly *Ornithomya chloropus* is a fly in the family Hippoboscidae. Until the 1960s, many entomologists considered it to be the same species as the Finch Louse Fly *Ornithomya fringillina* (Curtis, 1836) (Hill, 1962). In the United Kingdom (UK), those entomologists who recognised it as a separate species knew it as *Ornithomya lagopodis* (Sharp, 1907) until it was recognised as the same species as that described by Bergroth (Hill, 1964).

Ornithomya chloropus is a typical louse (or flat) fly. It is dorsoventrally flattened to help it move amongst its host's feathers and protected by a tough cuticle. It can move sideways, as well as backwards and forwards. Strong claws help it to cling on to a moving bird and the spines covering the body can stick to feathers if it loses its grip. *Ornithomya chloropus* can be distinguished from the slightly smaller *O. fringillina* by its generally darker colour, and the presence of clearly defined dark markings on the gena and mesothoracic basisternum. It also lacks the clear area towards the base of the apical wing cell (3r) seen in *O. fringillina*, as *O. chloropus* has microtrichia on the wing membrane in that area. It usually has six bristles in a row near the posterior edge of the scutellum, compared to the four seen in *O. fringillina* (Hutson, 1984).

Ornithomya chloropus's range extends across the Palaearctic from Europe to Japan (Maa, 1967). In the UK it is found at more northerly latitudes and higher altitudes than other species, but, with climate change, is undergoing range contraction at lower altitudes and in more southerly areas (Wawman, 2025). Both sexes of *O. chloropus* are obligate haematophagous ectoparasites of birds and can be found on a range of species (Hutson, 1984). In the UK and Ireland, *O. chloropus* emerge from puparia after a winter diapause in May, with the population peaking in June, before numbers gradually decrease until late autumn (Wawman, 2025).

We present a chromosome-level genome sequence for *Ornithomya chloropus*, based on one specimen, raised from a puparium. The puparium was produced by a female collected from a Meadow Pipit *Anthus pratensis* at the Carse of Ardersier, Inverness-shire, during the previous autumn, as part of the Mapping the UK's Flat Flies Project (Wawman, 2025) and the adult fly was donated to the Darwin Tree of Life Project.

Genome sequence report

Sequencing data

The genome of a specimen of *Ornithomya chloropus* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 24.93 Gb (gigabases) from 2.15 million reads. GenomeScope analysis of the PacBio HiFi data estimated the haploid genome size at 125.83 Mb, with a heterozygosity of 2.26% and repeat content of 17.88%. These values provide an initial assessment of genome complexity and the challenges anticipated during assembly. Based on this estimated genome size, the sequencing data provided approximately 180.0x coverage of the genome. Chromosome conformation Hi-C sequencing produced 114.07 Gb from 755.44 million reads. Table 1 summarises the specimen and sequencing information.

Figure 1. Photograph of the *Ornithomya chloropus* (idOrnChlo1) specimen used for genome sequencing.

Assembly statistics

The primary haplotype was assembled, and contigs corresponding to an alternate haplotype were also deposited in INSDC databases. The assembly was improved by manual curation, which corrected 45 misjoins or missing joins. These interventions decreased the scaffold count by 82.35% and increased the scaffold N50 by 96.93%. The final assembly has a total length of 193.17 Mb in 8 scaffolds, with 116 gaps, and a scaffold N50 of 56.87 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 (99.95%) was assigned to 5 chromosomal-level scaffolds, representing 3 autosomes and the X and Y sex chromosomes. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 5; Table 3). During curation, chromosomes X and Y were assigned based on read coverage statistics and BUSCO gene presence.

The mitochondrial genome was also assembled. This sequence is included as a contig in the multifasta file of the genome submission and as a standalone record.

Table 1. **Specimen and sequencing data for *Ornithomya chloropus*.**

Project information			
Study title	Ornithomya chloropus		
Umbrella BioProject	PRJEB68250		
Species	*Ornithomya chloropus*		
BioSpecimen	SAMEA112232563		
NCBI taxonomy ID	352856		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	idOrnChlo1	SAMEA112233011	whole organism
Hi-C sequencing	idOrnChlo1	SAMEA112233011	whole organism
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq 6000	ERR12259810	7.55e+08	114.07
PacBio Revio	ERR12257388	2.15e+06	24.93

Table 2. **Genome assembly data for *Ornithomya chloropus*.**

Genome assembly		
Assembly name	idOrnChlo1.1	
Assembly accession	GCA_963971445.1	
Alternate haplotype accession	*GCA_963971455.1*	
Assembly level for primary assembly	chromosome	
Span (Mb)	193.17	
Number of contigs	124	
Number of scaffolds	8	
Longest scaffold (Mb)	60.72	
Assembly metric	**Measure**	***Benchmark***
Contig N50 length	2.67 Mb	*≥ 1 Mb*
Scaffold N50 length	56.87 Mb	*= chromosome N50*
Consensus quality (QV)	Primary: 62.9; alternate: 63.6; combined: 63.1	*≥ 40*
k-mer completeness	Primary: 83.96%; alternate: 51.53%; combined: 99.00%	*≥ 95%*
BUSCO*	C:96.0%[S:93.3%,D:2.7%], F:0.6%,M:3.5%,n:3,285	*S > 90%; D < 5%*
Percentage of assembly mapped to chromosomes	99.95%	*≥ 90%*
Sex chromosomes	X and Y	*localised homologous pairs*
Organelles	Mitochondrial genome: 16.78 kb	*complete single alleles*

* BUSCO scores based on the diptera_odb10 BUSCO set using version 5.5.0. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison.

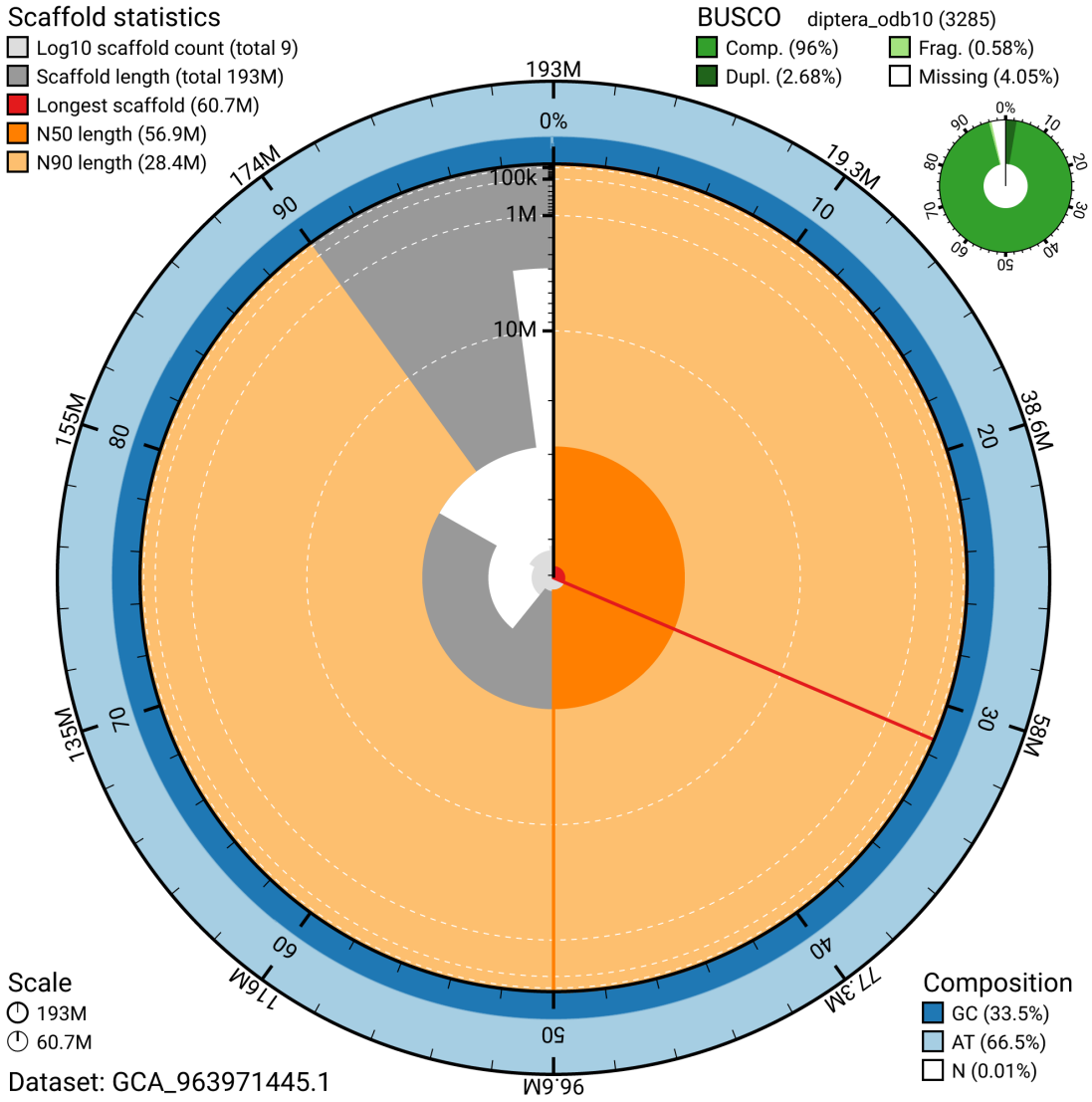

Figure 2. Genome assembly of *Ornithomya chloropus*, idOrnChlo1.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. 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_963971445.1/dataset/GCA_963971445.1/snail.

Assembly quality metrics

The estimated Quality Value (QV) and *k*-mer completeness metrics, along with BUSCO completeness scores, were calculated for each haplotype and the combined assembly. The QV reflects the base-level accuracy of the assembly, while *k*-mer completeness indicates the proportion of expected *k*-mers identified in the assembly. BUSCO scores provide a measure of completeness based on benchmarking universal single-copy orthologues.

The combined primary and alternate assemblies achieve an estimated QV of 63.1. The *k*-mer recovery for the primary haplotype is 83.96%, and for the alternate haplotype 51.53%; the combined primary and alternate assemblies have a *k*-mer

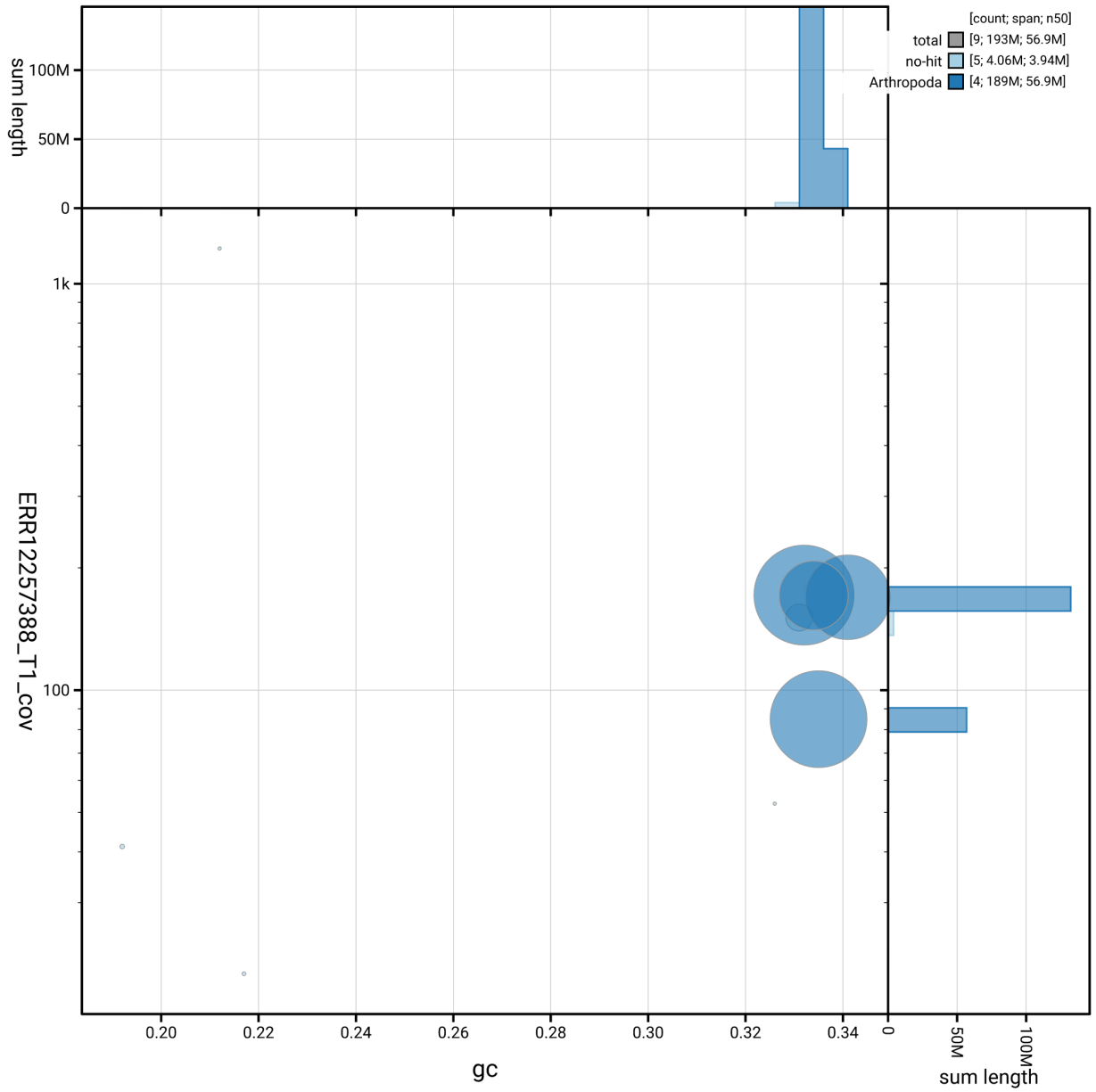

Figure 3. Genome assembly of *Ornithomya chloropus*, idOrnChlo1.1: BlobToolKit GC-coverage plot. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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_963971445.1/dataset/GCA_963971445.1/blob.

recovery of 99.00%. BUSCO v.5.5.0 analysis using the diptera_odb10 reference set (n = 3,285) identified 96.0% of the expected gene set (single = 93.3%, duplicated = 2.7%).

Table 2 provides assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project (EBP) Report on Assembly Standards September 2024. The assembly achieves the EBP reference standard of **6.C.Q63.**

Methods

Sample acquisition and DNA barcoding

An adult male *Ornithomya* chloropus (*specimen* ID Ox002336, ToLID idOrnChlo1) was raised from a puparium larviposited by a female collected from Carse of Ardersier, East Inverness-Shire, United Kingdom (latitude 57.59, longitude –4.01) on 2021-09-08 by potting. It emerged from the puparium on 2022-05-02. The female specimen was collected

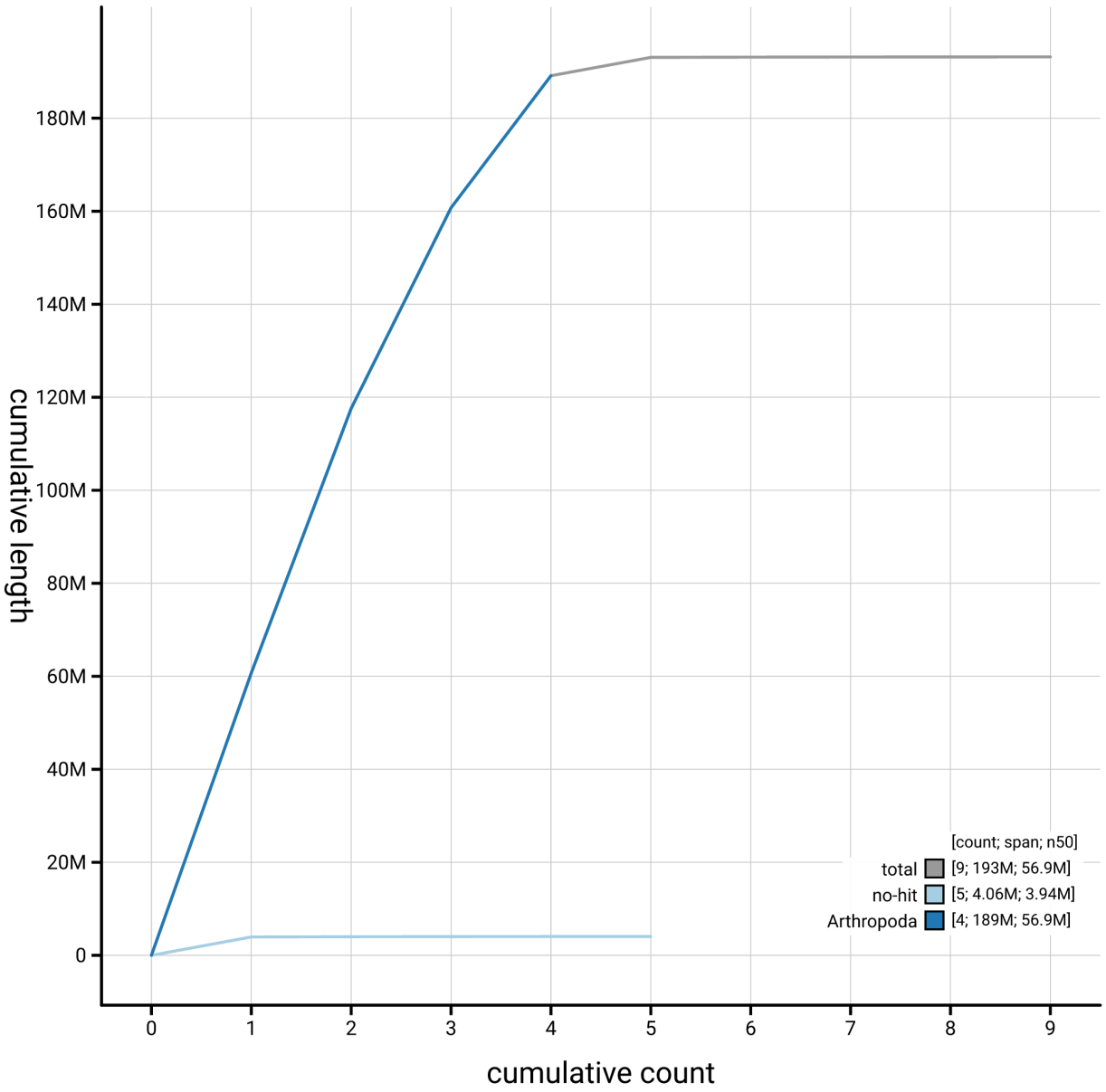

Figure 4. Genome assembly of *Ornithomya chloropus*, idOrnChlo1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963971445.1/dataset/GCA_963971445.1/cumulative.

by Hugh Insley, the puparium was raised by Denise Wawman (University of Oxford), the flies were identified by Denise Wawman and the sequenced specimen was 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 specimen and stored in ethanol, while the remaining parts were shipped on dry ice to the Wellcome Sanger Institute (WSI) (Pereira *et al.*, 2022). 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).

Metadata collection for samples adhered to the Darwin Tree of Life project standards described by Lawniczak *et al.* (2022).

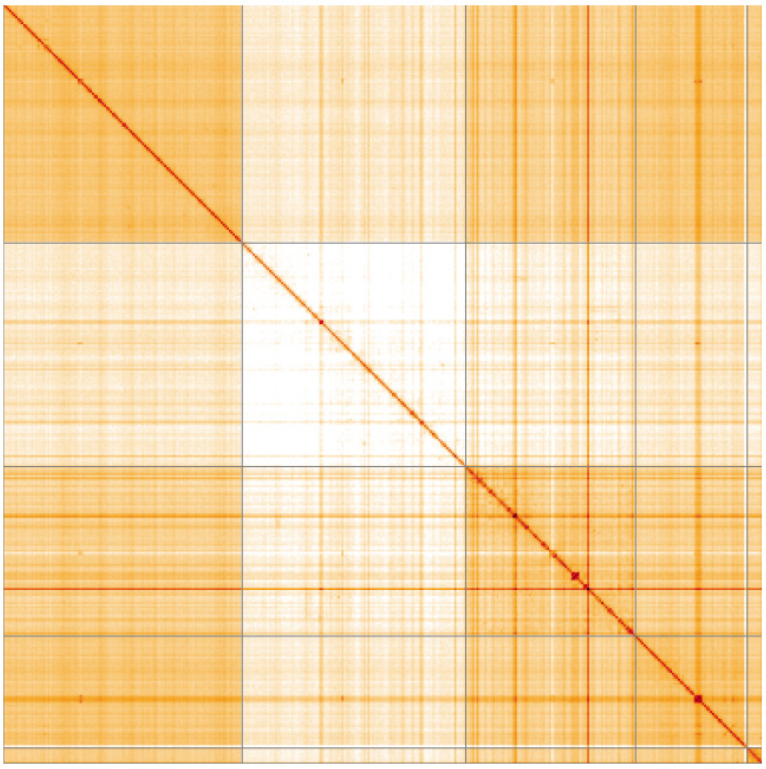

Figure 5. Genome assembly of *Ornithomya chloropus:* Hi-C contact map of the idOrnChlo1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=CsP3VJjZTfaWEcisNUfnfw.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Ornithomya chloropus*, idOrnChlo1.

INSDC accession	Name	Length (Mb)	GC%
OZ020529.1	1	60.72	33
OZ020532.1	2	28.39	33.5
OZ020533.1	3	3.94	33
OZ020530.1	X	56.87	33.5
OZ020531.1	Y	43.15	34
OZ020534.1	MT	0.02	21

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 idOrnChlo1 sample was prepared for DNA extraction by weighing and dissecting it on dry ice (Jay *et al.*, 2023). Tissue from the whole organism was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a). HMW DNA was extracted using the Automated MagAttract v2 protocol (Oatley *et al.*, 2023a). For ultra-low input (ULI) PacBio sequencing, DNA was fragmented using the Covaris g-TUBE method (Oatley *et al.*, 2023c). Sheared DNA was purified by solid-phase reversible immobilisation, using AMPure PB beads to eliminate shorter fragments and concentrate the DNA (Oatley *et al.*, 2023b). 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 whole organism of the idOrnChlo1 sample was processed for Hi-C sequencing at the WSI Scientific Operations core, using the Arima-HiC v2 kit. In brief, 20–50 mg of frozen tissue (stored at –80 °C) was fixed, and the DNA crosslinked using a TC buffer with 22% formaldehyde concentration. After crosslinking, the tissue was homogenised using the Diagnocine Power Masher-II and BioMasher-II tubes

and pestles. Following the Arima-HiC v2 kit manufacturer's instructions, crosslinked DNA was digested using a restriction enzyme master mix. The 5'-overhangs were 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. Additionally, the biotinylation percentage was estimated using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and Qubit HS Assay Kit and Arima-HiC v2 QC beads.

Library preparation and sequencing

PacBio HiFi (ULI)

The sample requires Covaris g-TUBE shearing to approximately 10 kb prior to library preparation. Ultra-low input libraries were prepared using PacBio SMRTbell® Express Template Prep Kit 2.0 and PacBio SMRTbell® gDNA Sample Amplification Kit. To begin, samples were normalised to 20 ng of DNA. Initial removal of single-strand overhangs, DNA damage repair, and end repair/A-tailing were performed per manufacturer's instructions. From the SMRTbell® gDNA Sample Amplification Kit, amplification adapters were then ligated. A 0.85X pre-PCR clean-up was performed with Promega ProNex beads and the sample was then divided into two for a dual PCR. PCR reactions A and B each followed the PCR programs as described in the manufacturer's protocol. A 0.85X post-PCR clean-up was performed with ProNex beads for PCR reactions A and B and DNA concentration was quantified using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and Qubit HS Assay Kit and fragment size analysis was carried out using the Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies) and gDNA 55kb BAC analysis kit. PCR reactions A and B were then pooled, ensuring the total mass was ≥500 ng in 47.4 μl. The pooled sample then repeated the process for DNA damage repair, end repair/A-tailing and additional hairpin adapter ligation. A 1X clean-up was performed with ProNex beads and DNA concentration was quantified using the Qubit and fragment size analysis was carried out using the Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies). Size selection was performed using Sage Sciences' PippinHT system with target fragment size determined by analysis from the Femto Pulse, usually a value between 4000 and 9000 bp. Size selected libraries were then cleaned-up using 1.0X ProNex beads and normalised to 2 nM before proceeding to sequencing.

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, 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 NEBNext 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

Prior to assembly of the PacBio HiFi reads, a database of *k*-mer counts (k = 31) was generated from the filtered reads using FastK. GenomeScope2 (Ranallo-Benavidez *et al.*, 2020) was used to analyse the *k*-mer frequency distributions, providing estimates of genome size, heterozygosity, and repeat content.

The HiFi reads were first 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 (Rao *et al.*, 2014) were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019), and the contigs were scaffolded using 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 Cobionts and Contaminants (ASCC) pipeline. Flat files and maps used in curation were generated via the TreeVal pipeline (Pointon *et al.*, 2023). Manual curation was conducted primarily in PretextView (Harry, 2022) and HiGlass (Kerpedjiev *et al.*, 2018), with additional insights provided by JBrowse2 (Diesh *et al.*, 2023). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Any identified contamination, missed joins, and mis-joins

were amended, and duplicate sequences were tagged and removed. Sex chromosomes were identified by read coverage statistics and the presence of BUSCO genes. The curation process is documented at https://gitlab.com/wtsi-grit/rapid-curation.

Assembly quality assessment

The Merqury.FK tool (Rhie *et al.*, 2020), run in a Singularity container (Kurtzer *et al.*, 2017), was used to evaluate *k*-mer completeness and assembly quality for the primary and alternate haplotypes using the *k*-mer databases (k = 31) 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 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 was visualised in HiGlass (Kerpedjiev *et al.*, 2018).

The blobtoolkit pipeline is a Nextflow (Di Tommaso *et al.*, 2017) 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 blobtoolkit pipeline was 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**

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	4.3.9	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.5.0	https://gitlab.com/ezlab/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/tolkit/fasta_windows
FastK	666652151335353eef2fcd58880bcef5bc2928e1	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	0.19.5-r587	https://github.com/chhylp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84 aa44357826c0b6753eb28de	https://github.com/higlass/higlass
MerquryFK	d00d98157618f4e8d1a9190026b19b471055b22e	https://github.com/thegenemyers/MERQURY.FK
Minimap2	2.24-r1122	https://github.com/lh3/minimap2
MitoHiFi	3	https://github.com/marcelauliano/MitoHiFi

Software tool	Version	Source
MultiQC	1.14, 1.17, and 1.18	https://github.com/MultiQC/MultiQC
Nextflow	23.04.1	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	1.19.2	https://github.com/samtools/samtools
sanger-tol/ascc	-	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	0.4.0	https://github.com/sanger-tol/blobtoolkit
Seqtk	1.3	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.2.0	https://github.com/sanger-tol/treeval
YaHS	1.2a.2	https://github.com/c-zhou/yahs

Practice', 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: Ornithomya chloropus. Accession number PRJEB68250; https://identifiers.org/ena.embl/PRJEB68250. The genome sequence is released openly for reuse. The *Ornithomya chloropus* 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.

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Open Peer Review

Current Peer Review Status: ? ✓ ✓

Version 1

Reviewer Report 22 July 2025

https://doi.org/10.21956/wellcomeopenres.26438.r126114

✓ **Hans-Peter Fuehrer**
University of Veterinary Medicine, Vienna, Austria

The authors describe the genome sequence of a grouse louse fly Ornithomya chloropus. The manuscript is of very good quality and just some minor modifications are recommended.

Background: Please change fly to dipteran or ked.
The English name for louse flies is keds.

Initial identification by barcoding. Where there sequences to compare with?

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: Medical and veterinary entomology

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 12 July 2025

https://doi.org/10.21956/wellcomeopenres.26438.r126113

Komal Kumar Bollepogu Raja
Baylor College of Medicine, Houston, Texas, USA

Wawman D.C. and Insley H. present the genome sequence of the species *Ornithimya chloropus*. The genome sequence length is 193.17 megabases. The authors have sufficiently provided the ecological and the morphological features of this species in the background. The experimental methods and the bioinformatics tools used in the manuscript are standard and can be reproducible. The genome assembly quality metrics look great with 96% BUSCO and 99% combined k-mer completeness. Further, 180x genome coverage with 99.5% mapped to chromosomes is great. Overall, the manuscript is well written and the data is a good genomic resource.

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: Genomics, single cell genomics, bioinformatics and neurobiology

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 30 June 2025

https://doi.org/10.21956/wellcomeopenres.26438.r126118

?

Henrique Antoniolli
Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

The present study reports a genome assembly for the grouse louse fly, *Ornithomya chloropus*. The Background section provides a brief overview of the species' general biology but does not clearly state the reasons for sequencing its genome. The Methods are well described, with appropriate references to protocols and software used. The assembly shows satisfactory completeness, supporting its publication.
I approve indexing the paper but recommend that the authors include a few sentences explaining the rationale behind sequencing this genome.

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

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: Genomics, evolution

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, however I have significant reservations, as outlined above.