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DATA NOTE

The genome sequence of the Flame Shoulder, *Ochropleura plecta* (Linnaeus, 1761) [version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual female *Ochropleura plecta* (the Flame Shoulder; Arthropoda; Insecta; Lepidoptera; Noctuidae). The genome sequence is 643.9 megabases in span. Most of the assembly is scaffolded into 32 chromosomal pseudomolecules, including the W and Z sex chromosomes. The mitochondrial genome has also been assembled and is 15.34 kilobases in length. Gene annotation of this assembly on Ensembl identified 19,016 protein coding genes.

Keywords

Ochropleura plecta, Flame Shoulder, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status *AWAITING PEER REVIEW*

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

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Author roles: Boyes D: Investigation, Resources; **Eagles M**: Writing – Original Draft Preparation;

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Species taxonomy

Eukaryota; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Noctuoidea; Noctuidae; Noctuinae; Noctuini; *Ochropleura*; *Ochropleura plecta* (Linnaeus, 1761) (NCBI:txid320037).

Background

Widespread and abundant throughout Great Britain, the Isle of Man, Ireland and the Channel Islands (Butterfly Conservation, 2023), *Ochropleura plecta*, of the Noctuidae family, is a resident and common species of moth found in gardens, farmland, hedgerows, moorland, woodland and wetlands. Adults may be seen between late April through to September, having two generations in southern Britain, while mainly single brooded in the north, flying in June and July. The bright straw-coloured stripe, or flame shoulder, along the leading edge of the forewing, together with the black streak from the base through the oval and kidney markings make it easily recognisable (Waring *et al.*, 2017). The nearest confusion species is *Ochropleura leucogaster*, Radford's Flame Shoulder, a rare migrant to the south coast, first recorded in 1983 and seen regularly since then (Lewis, 2022).

A completed genome sequence should add evidence to the discussion on evolutionary relationships that are still not completely understood in the noctuid family (Waring *et al.*, 2017). Research by Sisson (2022) on the phylogenetic relationships of noctuid moths from museum specimens highlighted this complexity and found a contradiction of previously documented phylogenetic relationships of *Ochropleura plecta*.

We present a chromosomally complete genome sequence for *Ochropleura plecta* based on one female specimen from Wytham Woods as part of the Darwin Tree of Life Project. This project is a collaborative effort to sequence all named eukaryotic species in the Atlantic Archipelago of Britain and Ireland

Genome sequence report

The genome was sequenced from one female *Ochropleura plecta* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, –1.34). A total of 46-fold coverage in Pacific Biosciences single-molecule HiFi long reads and 46-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 138 missing joins or mis-joins and removed 34 haplotypic duplications, reducing the assembly length by 0.98% and the scaffold number by 56.41%, and increasing the scaffold N50 by 12.81%.

The final assembly has a total length of 643.9 Mb in 34 sequence scaffolds with a scaffold N50 of 21.9 Mb (Table 1). A summary of the assembly statistics is shown in Figure 2,

Figure 1. Photograph of the *Ochropleura plecta* (ilOchPlec1) specimen used for genome sequencing.

while the distribution of assembly scaffolds on GC proportion and coverage is shown in Figure 3. The cumulative assembly plot in Figure 4 shows curves for subsets of scaffolds assigned to different phyla. Most (99.98%) of the assembly sequence was assigned to 32 chromosomal-level scaffolds, representing 30 autosomes and the W and Z sex chromosomes. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 5; Table 2). 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.

The estimated Quality Value (QV) of the final assembly is 58.1 with *k*-mer completeness of 100%, and the assembly has a BUSCO v5.3.2 completeness of 98.9% (single = 98.5%, duplicated = 0.5%), using the lepidoptera_odb10 reference set (n = 5,286).

Metadata for specimens, spectral estimates, sequencing runs, contaminants and pre-curation assembly statistics can be found at https://links.tol.sanger.ac.uk/species/320037.

Genome annotation report

The *Ochropleura plecta* genome assembly (GCA_905475445.1) was annotated using the Ensembl rapid annotation pipeline (Table 1; https://rapid.ensembl.org/Ochropleura_plecta_GCA_905475445.1/Info/Index). The resulting annotation includes 19,223 transcribed mRNAs from 19,016 protein-coding genes.

Methods

Sample acquisition and nucleic acid extraction

A female *Ochropleura plecta* (specimen ID Ox000401, ToLID ilOchPlec1) was collected from Wytham Woods,

Table 1. Genome data for *Ochropleura plecta*, ilOchPlec1.1.

Project accession data		
Assembly identifier	ilOchPlec1.1	
Assembly release date	2021-04-15	
Species	*Ochropleura plecta*	
Specimen	ilOchPlec1	
NCBI taxonomy ID	320037	
BioProject	PRJEB43802	
BioSample ID	SAMEA7520524	
Isolate information	ilOchPlec1, female: abdomen (DNA sequencing); head and thorax (Hi-C scaffolding and RNA sequencing)	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	58.1	*≥ 50*
k-mer completeness	100%	*≥ 95%*
BUSCO**	C:98.9%[S:98.5%,D:0.5%],F:0.3%,M:0.8%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.98%	*≥ 95%*
Sex chromosomes	W and Z chromosome	*localised homologous pairs*
Organelles	Mitochondrial genome assembled	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR6406207	
10X Genomics Illumina	ERR6054637, ERR6054639, ERR6054638, ERR6054640	
Hi-C Illumina	ERR6054641	
PolyA RNA-Seq Illumina	ERR9434971	
Genome assembly		
Assembly accession	GCA_905475445.1	
Accession of alternate haplotype	GCA_905475425.1	
Span (Mb)	643.9	
Number of contigs	122	
Contig N50 length (Mb)	9.6	
Number of scaffolds	34	
Scaffold N50 length (Mb)	21.9	
Longest scaffold (Mb)	29.0	
Genome annotation		
Number of protein-coding genes	19,016	
Number of gene transcripts	19,223	

* Assembly metric benchmarks are adapted from column VGP-2020 of "Table 1: Proposed standards and metrics for defining genome assembly quality" from (Rhie *et al.*, 2021).

** BUSCO scores based on the lepidoptera_odb10 BUSCO set using v5.3.2. 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/Ochropleura%20plecta/dataset/CAJQFZ01.1/busco.

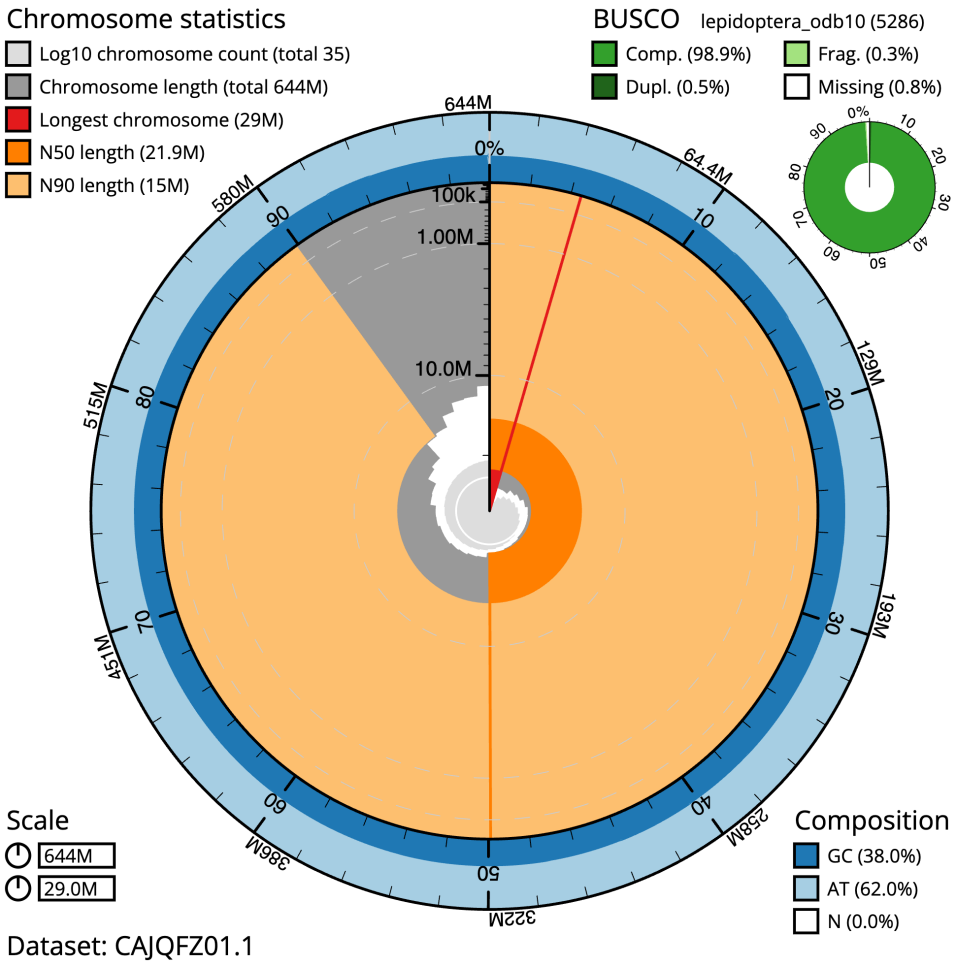

Figure 2. Genome assembly of *Ochropleura plecta*, ilOchPlec1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 643,962,443 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (28,980,428 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (21,894,754 and 14,971,023 bp), respectively. The pale grey spiral shows the cumulative scaffold count on a log scale with white scale lines showing successive orders of magnitude. The blue and pale-blue area around the outside of the plot shows the distribution of GC, AT and N percentages in the same bins as the inner plot. A summary of complete, fragmented, duplicated and missing BUSCO genes in the lepidoptera_odb10 set is shown in the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/Ochropleura%20plecta/dataset/CAJQFZ01.1/snail.

Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2020-05-22 using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute (WSI). The ilOchPlec1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing. Abdomen tissue was disrupted using a Nippi Powermasher fitted with a BioMasher pestle. High molecular weight (HMW) DNA was extracted using the Qiagen MagAttract HMW DNA extraction kit. Low molecular weight DNA was removed from a 20 ng aliquot of extracted DNA using the 0.8X AMpure XP purification kit prior to 10X Chromium sequencing; a minimum of 50 ng DNA was submitted for 10X sequencing. HMW DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system with speed setting 30. Sheared DNA was purified by solid-phase reversible immobilisation using AMPure PB beads with a 1.8X ratio of beads to sample to remove the shorter fragments and concentrate the DNA sample. The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and Qubit Fluorometer and Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

RNA was extracted from head and thorax tissue of ilOchPlec1 in the Tree of Life Laboratory at the WSI using TRIzol, according to the manufacturer's instructions. RNA was then

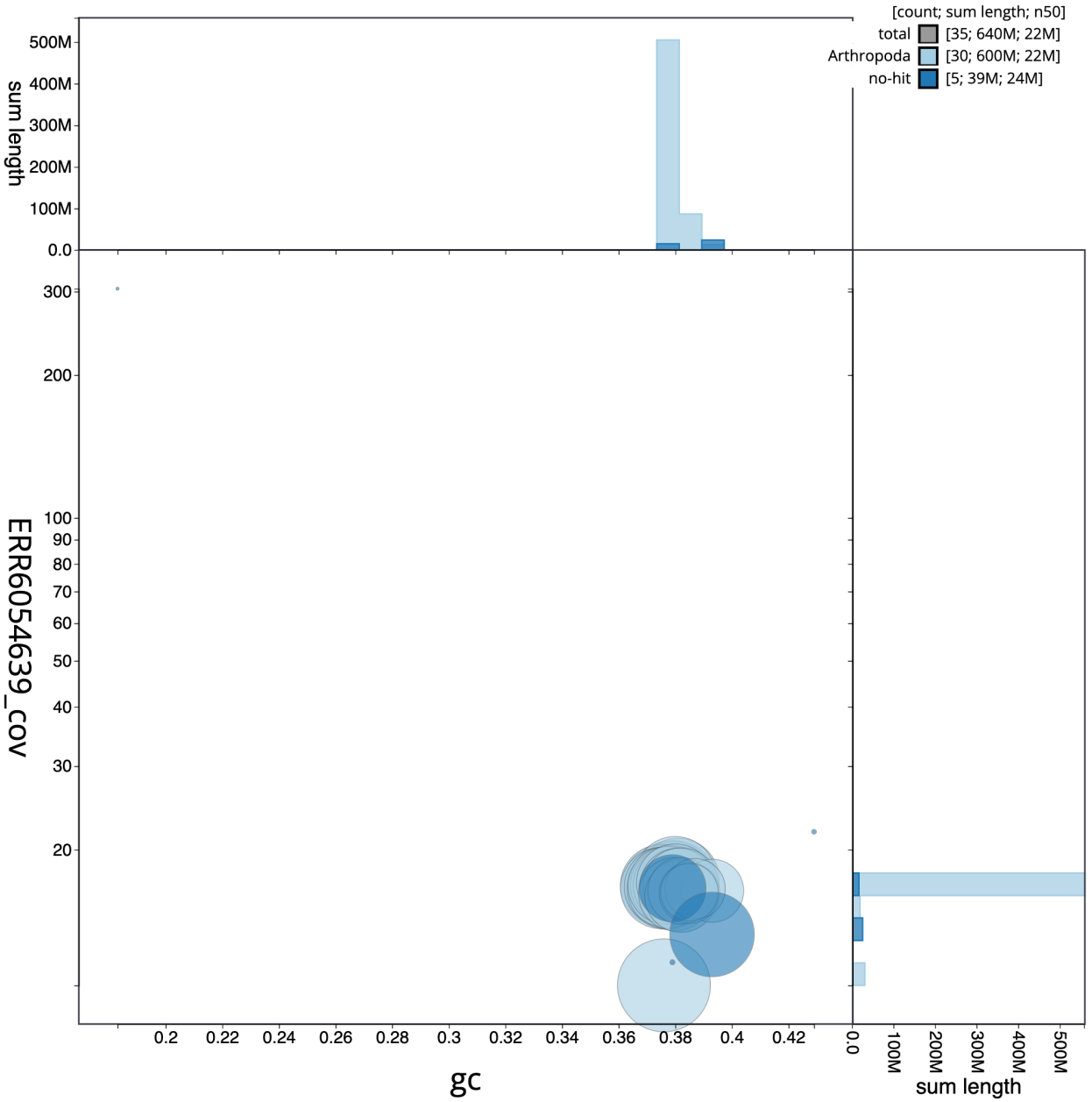

Figure 3. Genome assembly of *Ochropleura plecta*, ilOchPlec1.1: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/Ochropleura%20plecta/dataset/CAJQFZ01.1/blob.

eluted in 50 μl RNAse-free water and its concentration assessed using a Nanodrop spectrophotometer and Qubit Fluorometer using the Qubit RNA Broad-Range (BR) Assay kit. Analysis of the integrity of the RNA was done using Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud DNA sequencing libraries were constructed according to the manufacturers' instructions. Poly(A) RNA-Seq libraries were constructed using the NEB Ultra II RNA Library Prep kit. DNA and RNA sequencing was performed by the Scientific Operations core at the WSI on Pacific Biosciences SEQUEL II (HiFi), Illumina HiSeq 4000 (RNA-Seq) and HiSeq X Ten (10X) instruments. Hi-C data were also generated from remaining head and thorax tissue of ilOchPlec1 using the Arima2 kit and sequenced on the HiSeq X Ten instrument.

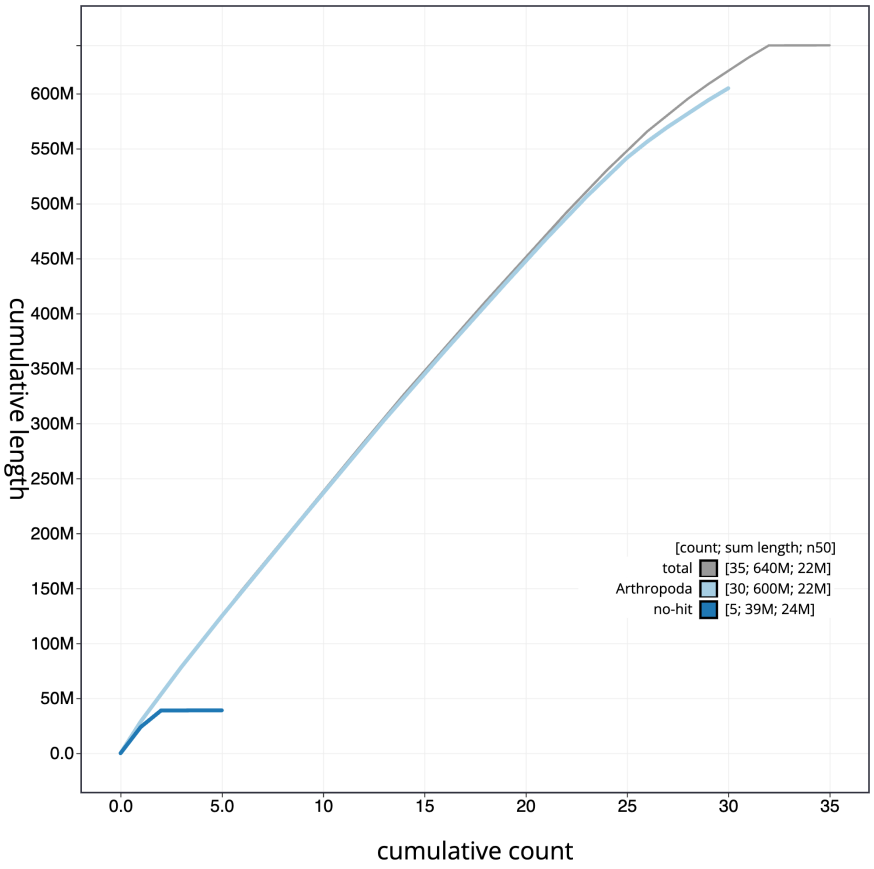

Figure 4. Genome assembly of *Ochropleura plecta*, ilOchPlec1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/Ochropleura%20plecta/dataset/CAJQFZ01.1/cumulative.

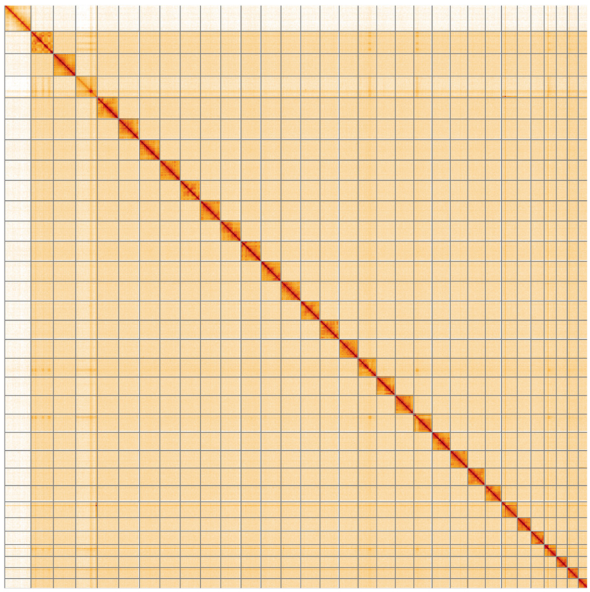

Figure 5. Genome assembly of *Ochropleura plecta*, ilOchPlec1.1: Hi-C contact map of the ilOchPlec1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=eAJ5-r_fT3WUaYFQcGactw.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Ochropleura plecta*, ilOchPlec1.

INSDC accession	Chromosome	Length (Mb)	GC%
FR997722.1	1	24.73	37.9
FR997723.1	2	24.69	37.9
FR997725.1	3	23.48	38
FR997726.1	4	22.89	37.6
FR997727.1	5	22.48	38.1
FR997728.1	6	22.43	37.7
FR997729.1	7	22.42	37.7
FR997730.1	8	22.41	37.7
FR997731.1	9	22.22	37.5
FR997732.1	10	22.13	38
FR997733.1	11	22	37.5
FR997734.1	12	21.89	37.9
FR997735.1	13	21.21	37.8
FR997736.1	14	21.1	37.6
FR997737.1	15	20.84	37.7
FR997738.1	16	20.69	37.9
FR997739.1	17	20.46	37.8
FR997740.1	18	20.46	37.9
FR997741.1	19	20.15	37.7
FR997742.1	20	20.11	38
FR997743.1	21	19.31	38.1
FR997744.1	22	19.21	38.2
FR997745.1	23	17.94	38
FR997746.1	24	17.48	38.2
FR997747.1	25	14.97	37.9
FR997748.1	26	14.66	38.2
FR997749.1	27	13.25	39.3
FR997750.1	28	12.22	38.5
FR997751.1	29	12.1	38.7
FR997752.1	30	11	38.5
FR997724.1	W	23.94	39.3
FR997721.1	Z	28.98	37.6
FR997753.1	MT	0.02	18.6

Genome assembly, curation and evaluation

Assembly was carried out with HiCanu (Nurk *et al.*, 2020), and haplotypic duplication was identified and removed with purge_dups (Guan *et al.*, 2020). One round of polishing was performed by aligning 10X Genomics read data to the assembly with Long Ranger ALIGN, calling variants with FreeBayes (Garrison & Marth, 2012). The assembly was then scaffolded with Hi-C data (Rao *et al.*, 2014) using SALSA2 (Ghurye *et al.*, 2019). The assembly was checked for contamination and corrected using the gEVAL system (Chow *et al.*, 2016) as described previously (Howe *et al.*, 2021). Manual curation was performed using gEVAL, HiGlass (Kerpedjiev *et al.*, 2018) and Pretext (Harry, 2022). The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2023), which runs MitoFinder (Allio *et al.*, 2020) or MITOS (Bernt *et al.*, 2013) and uses these annotations to select the final mitochondrial contig and to ensure the general quality of the sequence.

A Hi-C map for the final assembly was produced using bwa-mem2 (Vasimuddin *et al.*, 2019) in the Cooler file format (Abdennur & Mirny, 2020). To assess the assembly metrics, the *k*-mer completeness and QV consensus quality values were calculated in Merqury (Rhie *et al.*, 2020). This work was done using Nextflow (Di Tommaso *et al.*, 2017) DSL2 pipelines "sanger-tol/readmapping" (Surana *et al.*, 2023a) and "sanger-tol/genomenote" (Surana *et al.*, 2023b). The genome was analysed within the BlobToolKit environment (Challis *et al.*, 2020) and BUSCO scores (Manni *et al.*, 2021; Simão *et al.*, 2015) were calculated.

Table 3 contains a list of relevant software tool versions and sources.

Genome annotation

The BRAKER2 pipeline (Brůna *et al.*, 2021) was used in the default protein mode to generate annotation for the *Ochropleura plecta* assembly (GCA_905475445.1) in Ensembl Rapid Release.

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'**, 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

Table 3. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.1.7	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	https://gitlab.com/ezlab/busco
FreeBayes	1.3.1-17-gaa2ace8	https://github.com/freebayes/freebayes
gEVAL	N/A	https://geval.org.uk/
HiCanu	2.1	https://github.com/marbl/canu
HiGlass	1.11.6	https://github.com/higlass/higlass
Long Ranger ALIGN	2.2.2	https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines
Merqury	MerquryFK	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	1	https://github.com/marcelauliano/MitoHiFi
PretextView	0.2	https://github.com/wtsi-hpag/PretextView
purge_dups	1.2.3	https://github.com/dfguan/purge_dups
SALSA	2.2	https://github.com/salsa-rs/salsa
sanger-tol/genomenote	v1.0	https://github.com/sanger-tol/genomenote
sanger-tol/readmapping	1.1.0	https://github.com/sanger-tol/readmapping/tree/1.1.0

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: *Ochropleura plecta* (flame shoulder). Accession number PRJEB43802; https://identifiers.org/ena.embl/PRJEB43802. (Wellcome Sanger Institute, 2021) The genome sequence is released openly for reuse. The *Ochropleura plecta* 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. Raw data and assembly accession identifiers are reported in Table 1.

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Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: https://doi.org/10.5281/zenodo.4790455.

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.5013541.

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

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