DATA NOTE

The genome sequence of the Oriental Meadow Brown, *Cercyonis lupina* (Lepidoptera: Nymphalidae)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a female specimen of *Cercyonis lupina* (Oriental Meadow Brown; Arthropoda; Insecta; Lepidoptera; Nymphalidae). The assembly contains two haplotypes with total lengths of 508.65 megabases and 467.75 megabases. Most of haplotype 1 (99.9%) is scaffolded into 30 chromosomal pseudomolecules, including the W and Z sex chromosomes. Haplotype 2 was assembled to scaffold level. The mitochondrial genome has also been assembled, with a length of 15.22 kilobases. This work is part of Project Psyche, a collaborative programme generating genomes for European butterflies and moths.

Keywords

Cercyonis lupina; Oriental Meadow Brown; genome sequence; chromosomal; Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

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Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Papilionoidea; Nymphalidae; Satyrinae; Satyrini; Maniolina; *Cercyonis*; *Hyponephele*; *Cercyonis lupina* (NCBI:txid2599366)

Background

The Oriental Meadow Brown (*Cercyonis lupina*) is a species of butterfly in the family Nymphalidae, subfamily Satyrinae. The dorsal surface of the wing is dark brown, while the ventral surface is orange with grey margins on the forewing and grey with black marbling on the hindwing, which is also festooned. This species is sexually dimorphic, with dorsal forewings presenting androconia in males and subapical black and orange spots in females. It can be distinguished from the Dusky Meadow Brown (*C. lycaon*) by the male's androconia (wider in *C. lupina*) and the extent of orange colouration on the forewing dorsal side in females (more orange in *C. lycaon*).

C. lupina is found in north Africa, southern Europe, Anatolia and Arabia, up to Tian Shan and Altai mountains in Central Asia (Vila *et al.*, 2018). It is univoltine, and adults are observed between May and September with an aestivation period (García-Barros *et al.*, 2013). This mildly xerophilous species inhabits rocky shrublands with dispersed trees, between 700 m and 1 500 m asl (García-Barros *et al.*, 2013; Vila *et al.*, 2018). Larvae feed on multiple Poaceae, such as *Stipa* spp. and *Aegilops geniculata* (Vila *et al.*, 2018); oviposition takes place near the ground, on dry plants or gravel (Jutzeler & Lafranchis, 2005).

The haplotype of this species was determined to be $n = 29$ based on karyotyping studies on specimens from Turkey (de Lesse, 1960). DNA barcoding revealed the occurrence of two deeply diverged lineages, one in north Africa and the Iberian Peninsula (*C. lupina mauritanica*) and another throughout the rest of its distribution (*C. lupina lupina*), inferred to have diverged ca. 3 Mya (Dapporto *et al.*, 2022; Hinojosa *et al.*, 2018). Some authors have proposed that these subspecies should be raised to specific status, given the large mitochondrial genetic divergence correlated with morphological differences – however, no nuclear genomic sequence data is yet available (Lukhtanov & Pazhenkova, 2021). The specimen here sequenced corresponds to the subspecies *mauritanica*. In addition, *C. lupina* has historically been classified in the genus *Hyponephele*, which Zhang *et al.* (2020) consider a subgenus. Although we follow the taxonomy of NCBI, given the reciprocal monophyly of *Hyponephele* and *Cercyonis sensu stricto* and the lack of precedent for using *Cercyonis* for Old World taxa, we favour the use of *Hyponephele* as genus to maintain taxonomic stability, at least until further evidence with a more extensive taxon sampling is gathered.

The species is listed as Least Concern in the European Red List of Butterflies (van Swaay *et al.*, 2025), although some regional populations are very local and endangered – e.g. in Catalonia; where it is considered to be endangered (Vila *et al.*, 2018). Given its regionally endangered status and the taxonomic uncertainty in this species, the reference genome presented here will prove invaluable for conservation as well as for alpha taxonomy. The sequence data were derived from a female specimen (Figure 1) collected from Tàrrega, Urgell, Catalonia, Spain.

Methods

Sample acquisition

The specimen used for genome sequencing was an adult female *Cercyonis lupina* (specimen ID SAN28000318, ToLID ilHypLupi1; Figure 1), collected from Tàrrega, Urgell, Catalonia, Spain (latitude 41.6856, longitude 1.1752) on 2022-09-23. The specimen was collected and identified by Joan Carles Hinojosa (Institut de Biologia Evolutiva).

Nucleic acid extraction

Protocols for high molecular weight (HMW) DNA extraction developed at the Wellcome Sanger Institute (WSI) Tree of Life Core Laboratory are available on protocols.io (Howard *et al.*, 2025). The ilHypLupi1 sample was weighed and triaged to determine the appropriate extraction protocol. Tissue from the whole organism was homogenised by powermashing using a PowerMasher II tissue disruptor.

HMW DNA was extracted in the WSI Scientific Operations core using the Automated MagAttract v2 protocol. DNA was sheared into an average fragment size of 12–20 kb following the Megaruptor®3 for LI PacBio protocol. Sheared DNA was purified by automated SPRI (solid-phase reversible immobilisation). 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. For this sample, the final post-shearing DNA had a Qubit concentration of 44.6 ng/μL and a yield of 2 096.20 ng, with a fragment size of 13.3 kb.

PacBio HiFi library preparation and sequencing

Library preparation and sequencing were performed at the WSI Scientific Operations core. Libraries were prepared using the SMRTbell Prep Kit 3.0 (Pacific Biosciences, California, USA), according to the manufacturer's instructions. The kit includes reagents for end repair/A-tailing, adapter ligation,

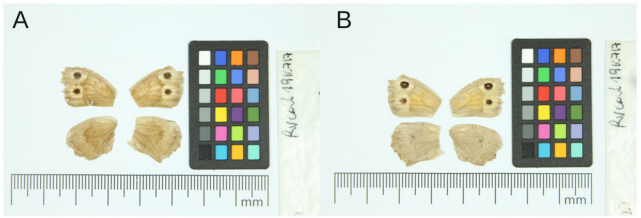

Figure 1. Voucher photographs of the *Cercyonis lupina* (ilHypLupi1) specimen used for genome sequencing.

post-ligation SMRTbell bead clean-up, and nuclease treatment. Size selection and clean-up were performed using diluted AMPure PB beads (Pacific Biosciences). DNA concentration was quantified using a Qubit Fluorometer v4.0 (ThermoFisher Scientific) and the Qubit 1X dsDNA HS assay kit. Final library fragment size was assessed with the Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies) using the gDNA 55 kb BAC analysis kit.

The sample was sequenced on a Revio instrument (Pacific Biosciences). The prepared library was normalised to 2 nM, and 15 µL was used for making complexes. Primers were annealed and polymerases bound to generate circularised complexes, following the manufacturer's instructions. Complexes were purified using 1.2X SMRTbell beads, then diluted to the Revio loading concentration (200–300 pM) and spiked with a Revio sequencing internal control. The sample was sequenced on a Revio 25M SMRT cell. The SMRT Link software (Pacific Biosciences), a web-based workflow manager, was used to configure and monitor the run and to carry out primary and secondary data analysis.

Specimen details, sequencing platforms, and data yields are summarised in Table 1.

Hi-C

Sample preparation and crosslinking

The Hi-C sample was prepared from 20–50 mg of frozen tissue from the whole organism of the ilHypLupi1 sample using the Arima-HiC v2 kit (Arima Genomics). Following the manufacturer's instructions, tissue was fixed and DNA crosslinked using TC buffer to a final formaldehyde concentration of 2%. The tissue was homogenised using the Diagnocine Power Masher-II. Crosslinked DNA was digested with a restriction enzyme master mix, biotinylated, and ligated. Clean-up was performed with SPRISelect beads before library preparation. DNA concentration was measured with the Qubit Fluorometer (Thermo Fisher Scientific) and Qubit HS Assay Kit. The biotinylation percentage was estimated using the Arima-HiC v2 QC beads.

Hi-C library preparation and sequencing

Biotinylated DNA constructs were fragmented using a Covaris E220 sonicator and size selected to 400–600 bp using SPRISelect beads. DNA was enriched with Arima-HiC v2 kit Enrichment beads. End repair, A-tailing, and adapter ligation were carried out with the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs), following a modified protocol where library preparation occurs while DNA remains bound to the Enrichment beads. Library amplification was performed using KAPA HiFi HotStart mix and a custom Unique Dual Index (UDI) barcode set (Integrated DNA Technologies). Depending on sample concentration and biotinylation percentage determined at the crosslinking stage, libraries were amplified with 10–16 PCR cycles. Post-PCR clean-up was performed with SPRISelect beads. Libraries were quantified using the AccuClear Ultra High Sensitivity dsDNA Standards Assay Kit (Biotium) and a FLUOstar Omega plate reader (BMG Labtech).

Prior to sequencing, libraries were normalised to 10 ng/µL. Normalised libraries were quantified again to create equimolar and/or weighted 2.8 nM pools. Pool concentrations were checked using the Agilent 4200 TapeStation (Agilent) with High Sensitivity D500 reagents before sequencing. Sequencing was performed using paired-end 150 bp reads on the Illumina NovaSeq X.

Specimen details, sequencing platforms, and data yields are summarised in Table 1.

Genome assembly

Prior to assembly of the PacBio HiFi reads, a database of *k*-mer counts ($k = 31$) was generated from the filtered reads

Table 1. Specimen and sequencing data for BioProject PRJEB85022.

Platform	PacBio HiFi	Hi-C
ToLID	ilHypLupi1	ilHypLupi1
Specimen ID	SAN28000318	SAN28000318
BioSample (source individual)	SAMEA115768879	SAMEA115768879
BioSample (tissue)	SAMEA115768898	SAMEA115768898
Tissue	whole organism	whole organism
Instrument	Revio	Illumina NovaSeq X
Run accessions	ERR14209136; ERR14209137	ERR14224618
Read count total	2.85 million	727.73 million
Base count total	24.30 Gb	109.89 Gb

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 assembled using Hifiasm in Hi-C phasing mode (Cheng *et al.*, 2021; Cheng *et al.*, 2022), producing two haplotypes. Hi-C reads (Rao *et al.*, 2014) were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). Contigs were further scaffolded with Hi-C data 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 Cobionts and Contaminants (ASCC) pipeline. TreeVal was used to generate the flat files and maps for use in curation. Manual curation was conducted primarily in PretextView and HiGlass (Kerpedjiev *et al.*, 2018). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Manual corrections included two breaks, 22 joins, and removal of two haplotypic duplications. This increased the scaffold count by 24.5% and increased the total assembly length by 1.1%. The curation process is described at https://gitlab.com/wtsi-grit/rapid-curation. PretextSnapshot was used to generate a Hi-C contact map of the final assembly.

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 both haplotypes using the *k*-mer database (k = 31) computed prior to genome assembly. The analysis outputs included assembly QV scores and completeness statistics.

The genome was analysed using the BlobToolKit pipeline, a Nextflow (Di Tommaso *et al.*, 2017) implementation of the earlier Snakemake version (Challis *et al.*, 2020). The pipeline aligns PacBio reads using minimap2 (Li, 2018) and SAMtools (Danecek *et al.*, 2021) to generate coverage tracks. It runs BUSCO (Manni *et al.*, 2021) using lineages identified from the NCBI Taxonomy (Schoch *et al.*, 2020). For the three domain-level lineages, BUSCO genes are aligned to the UniProt Reference Proteomes database (Bateman *et al.*, 2023) using DIAMOND blastp (Buchfink *et al.*, 2021). The genome is divided into chunks based on the density of BUSCO genes from the closest taxonomic lineage, and each chunk is aligned to the UniProt Reference Proteomes database with DIAMOND blastx. Sequences without hits are chunked using seqtk and aligned to the NT database with blastn (Altschul *et al.*, 1990). The BlobToolKit suite consolidates all 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), with containerisation through Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017).

We used lep_busco_painter to paint Merian elements along chromosomes (Wright *et al.*, 2024). Merian elements represent the 32 ancestral linkage groups in Lepidoptera. The painting process utilised BUSCO gene locations from the lepidoptera_odb10 set (Kriventseva *et al.*, 2019) and chromosome lengths from NCBI Datasets. Each complete BUSCO gene (both single-copy and duplicated) was assigned to a Merian element based on a reference database, then plotted along chromosomes drawn to scale.

Genome sequence report

Sequence data

PacBio sequencing of the *Cercyonis lupina* specimen generated 24.30 Gb (gigabases) from 2.85 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 482.22 Mb, with a heterozygosity of 2.03% and repeat content of 34.68% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 49× coverage. Hi-C sequencing produced 109.89 Gb from 727.73 million reads, which were used to scaffold the assembly. Table 1 summarises the specimen and sequencing details.

Assembly statistics

The genome was assembled into two haplotypes using Hi-C phasing. Haplotype 1 was curated to chromosome level, while haplotype 2 was assembled to scaffold level. The final assembly has a total length of 508.65 Mb in 116 scaffolds, with 56 gaps, and a scaffold N50 of 18.45 Mb (Table 2).

Most of the assembly sequence (99.9%) was assigned to 30 chromosomal-level scaffolds, representing 28 autosomes and the W and Z sex chromosomes. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 3; Table 3). Chromosome painting with Merian elements illustrates the distribution of orthologues along chromosomes and highlights patterns of chromosomal evolution relative to Lepidopteran ancestral linkage groups (Figure 4). Chromosomes Z and W were identified by copy number in the diploid assembly. The Z chromosome was assigned based on the presence of the MZ Merian element.

The mitochondrial genome was also assembled (length 15.22 kb, OZ243821.1). This sequence is included as a contig in the multifasta file of the genome submission and as a standalone record.

Assembly quality metrics

For haplotype 1, the estimated QV is 64.9, and for haplotype 2, 67.9. When the two haplotypes are combined, the assembly

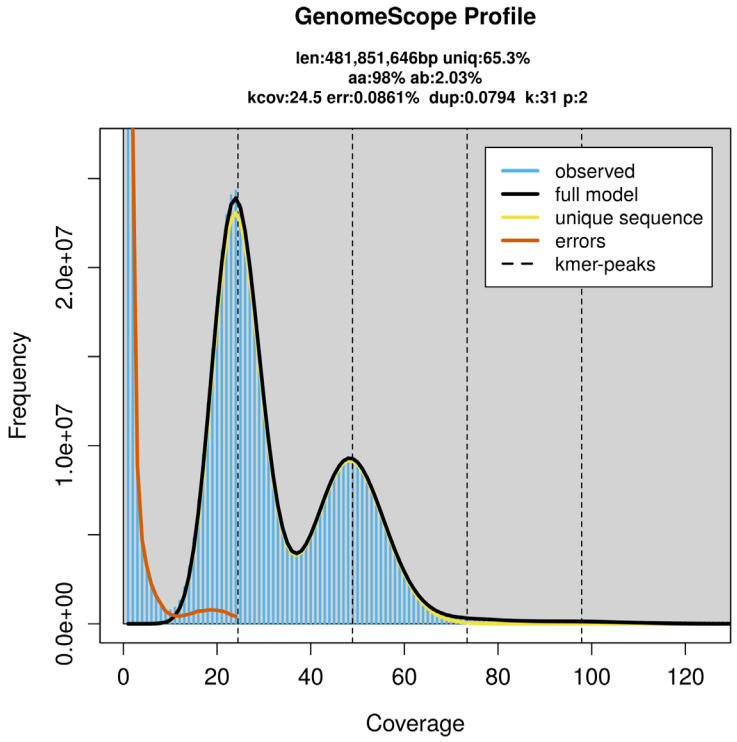

Figure 2. Frequency distribution of *k*-mers generated using GenomeScope2. The plot shows observed and modelled *k*-mer spectra, providing estimates of genome size, heterozygosity, and repeat content based on unassembled sequencing reads.

Table 2. Genome assembly statistics.

Assembly name	ilHypLupi1.hap1.1	ilHypLupi1.hap2.1
Assembly accession	GCA_965213455.1	GCA_965213415.1
Assembly level	chromosome	scaffold
Span (Mb)	508.65	467.75
Number of chromosomes	30	scaffold-level
Number of contigs	172	109
Contig N50	10.31 Mb	9.38 Mb
Number of scaffolds	116	48
Scaffold N50	18.45 Mb	18.39 Mb
Longest scaffold length (Mb)	21.63	-
Sex chromosomes	W and Z	-
Organelles	Mitochondrion: 15.22 kb	-

achieves an estimated QV of 66.1. The *k*-mer completeness is 69.61% for haplotype 1, 66.45% for haplotype 2, and 99.73% for the combined haplotypes (Figure 5). BUSCO analysis using the lepidoptera_odb10 reference set (n = 5 286) identified 98.4% of the expected gene set (single = 97.9%, duplicated = 0.5%) in haplotype 1. For haplotype 2, BUSCO analysis identified 94.2% of the expected gene set (single = 93.9%, duplicated = 0.3%). The snail plot in Figure 6 summarises the scaffold length distribution and other assembly statistics for haplotype 1. The blob plot in Figure 7 shows the distribution of scaffolds by GC proportion and coverage for haplotype 1.

Table 4 lists the assembly metric benchmarks adapted from Rhie *et al.* (2021) the Earth BioGenome Project Report

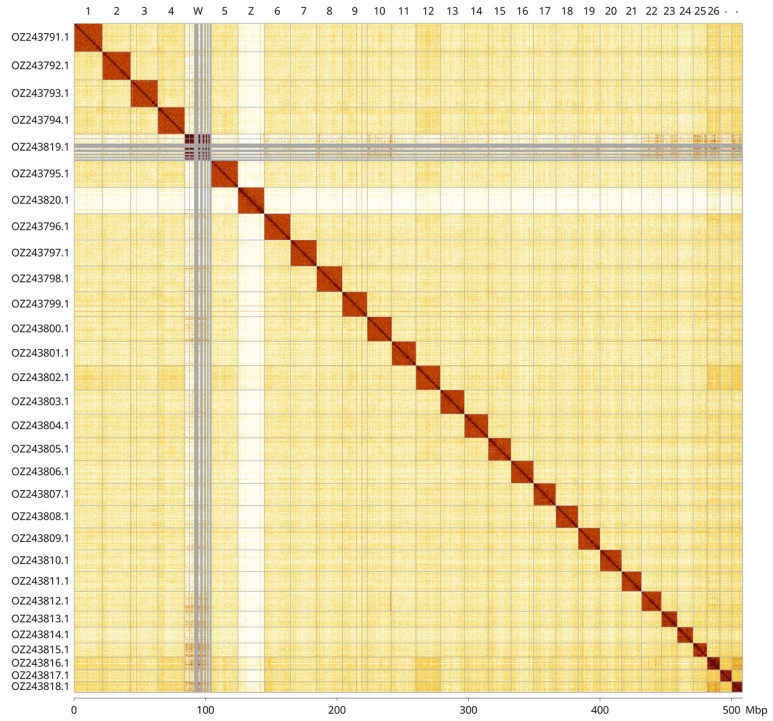

Figure 3. Hi-C contact map of the *Cercyonis lupina* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes, with a megabase scale shown below. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Cercyonis lupina* ilHypLupi1.

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ243791.1	1	21.63	36.50	M1
OZ243792.1	2	21.55	36.50	M2
OZ243793.1	3	20.56	36.50	M8
OZ243794.1	4	20.42	36.50	M17;M20
OZ243795.1	5	20.15	36.50	M3
OZ243796.1	6	19.99	36.50	M19;M26
OZ243797.1	7	19.83	36	M9
OZ243798.1	8	19.51	36.50	M12
OZ243799.1	9	18.97	36.50	M5
OZ243800.1	10	18.61	36.50	M4
OZ243801.1	11	18.49	36	M18
OZ243802.1	12	18.45	36.50	M14;M29
OZ243803.1	13	18.30	36	M16
OZ243804.1	14	18.17	36.50	M7
OZ243805.1	15	17.36	36.50	M6
OZ243806.1	16	17.07	36.50	M21
OZ243807.1	17	16.98	36.50	M15

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ243808.1	18	16.91	36.50	M22
OZ243809.1	19	16.70	36.50	M11
OZ243810.1	20	16.45	36.50	M10
OZ243811.1	21	15.10	36.50	M13
OZ243812.1	22	15.07	37	M23
OZ243813.1	23	12.08	36.50	M24
OZ243814.1	24	12.05	36.50	M28
OZ243815.1	25	10.60	36.50	M27
OZ243816.1	26	9.98	38.50	M30
OZ243817.1	27	8.77	37	M25
OZ243818.1	28	7.95	37.50	M31
OZ243819.1	W	20.35	36.50	-
OZ243820.1	Z	20.09	36.50	MZ

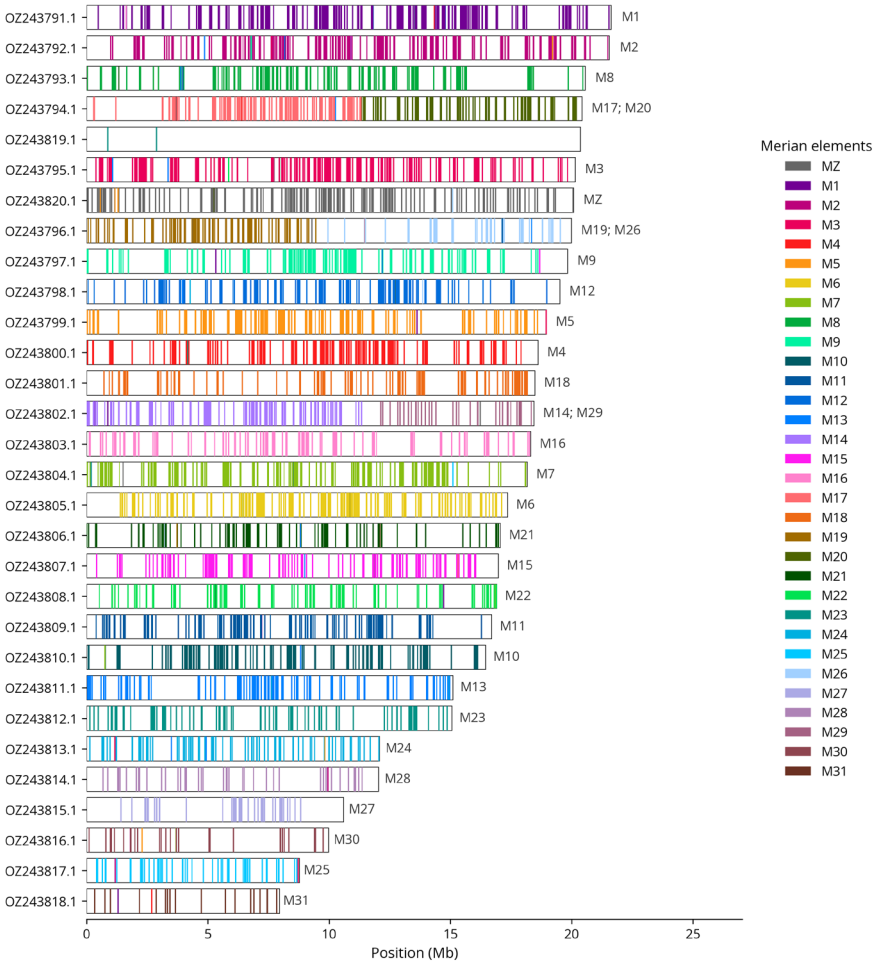

Figure 4. Merian elements painted across chromosomes in the ilHypLupi1.hap1.1 assembly of *Cercyonis lupina*. Chromosomes are drawn to scale, with the positions of orthologues shown as coloured bars. Each orthologue is coloured by the Merian element that it belongs to. All orthologues which could be assigned to Merian elements are shown.

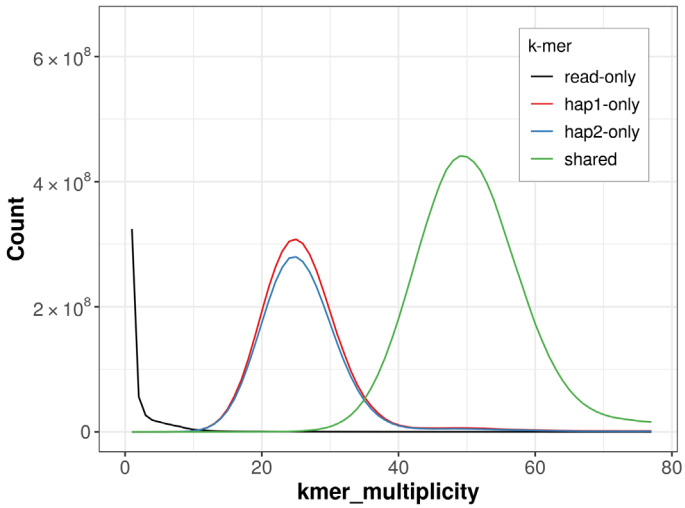

Figure 5. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers that appear in the reads but are not assembled. The green curve (the homozygous peak) corresponds to *k*-mers shared by both haplotypes and the red and blue curves (the heterozygous peaks) show *k*-mers found only in one of the haplotypes.

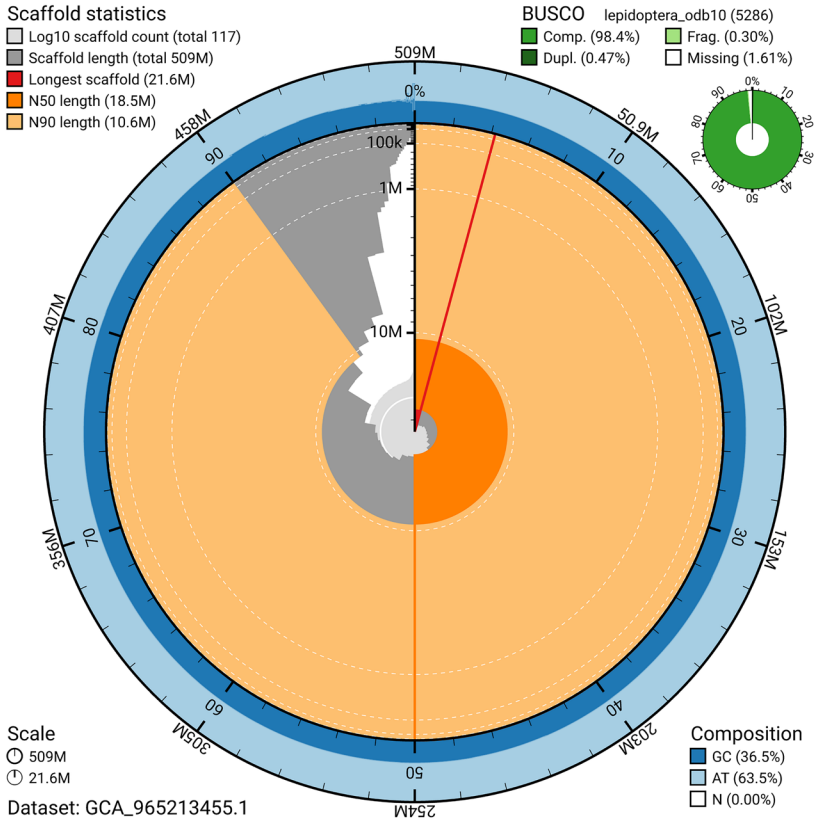

Figure 6. Assembly metrics for ilHypLupi1.hap1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. The outermost 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 set is presented at the top right. An interactive version of this figure can be accessed on the BlobToolKit viewer.

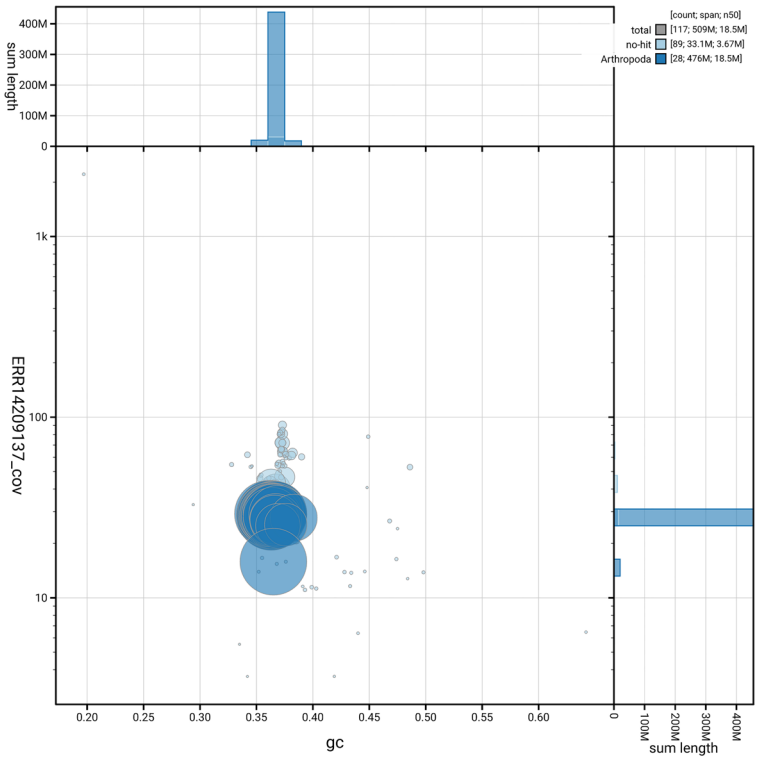

Figure 7. BlobToolKit GC-coverage plot for ilHypLupi1.hap1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes display the total length of sequences distributed across different levels of coverage and GC content. An interactive version of this figure is available on the BlobToolKit viewer.

Table 4. Earth Biogenome Project summary metrics for the *Cercyonis lupina* assembly.

Measure	Value	Benchmark
EBP summary (haplotype 1)	7.C.Q64	6.C.Q40
Contig N50 length	10.31 Mb	≥ 1 Mb
Scaffold N50 length	18.45 Mb	= chromosome N50
Consensus quality (QV)	Haplotype 1: 64.9; haplotype 2: 67.9; combined: 66.1	≥ 40
k-mer completeness	Haplotype 1: 69.61%; Haplotype 2: 66.45%; combined: 99.73%	≥ 95%
BUSCO	C:98.4% [S:97.9%; D:0.5%]; F:0.3%; M:1.3%; n:5 286	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.90%	≥ 90%

Notes: EBP summary uses log10(Contig N50); chromosome-level (C) or log10(Scaffold N50); Q (Merqury QV). BUSCO: C=complete; S=single-copy; D=duplicated; F=fragmented; M=missing; n=orthologues

on Assembly Standards September 2024. The EBP metric, calculated for the haplotype 1, is **7.C.Q64**, meeting the recommended reference standard.

Wellcome Sanger Institute – Legal and Governance

The materials that have contributed to this genome note have been supplied by a Tree of Life collaborator. 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 undertaken according to a Research Collaboration Agreement or Material Transfer Agreement entered into by the Tree of Life collaborator, Genome Research Limited (operating as the Wellcome Sanger Institute), and in some circumstances, other Tree of Life collaborators.

Data availability

European Nucleotide Archive: Cercyonis lupina. Accession number PRJEB85022. The genome sequence is released openly for reuse. The *Cercyonis lupina* genome sequencing initiative is part of the Sanger Institute Tree of Life Programme (PRJEB43745) and Project Psyche (PRJEB71705). 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 Ensembl at the European Bioinformatics Institute. Raw data and assembly accession identifiers are reported in Table 1 and Table 2.

Pipelines used for genome assembly at the WSI Tree of Life are available at https://pipelines.tol.sanger.ac.uk/pipelines. Table 5 lists software versions used in this study.

Author information

Contributors are listed at the following links:

- Members of the Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team
- Members of Wellcome Sanger Institute Scientific Operations – Sequencing Operations
- Members of the Wellcome Sanger Institute Tree of Life Core Informatics team
- Members of the Tree of Life Core Informatics collective
- Members of the Project Psyche Community

Table 5. Software versions and sources.

Software	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	4.4.5	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.7.1	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	1.1	https://github.com/thegenemyers/FASTK
GenomeScope2.0	2.0.1	https://github.com/tbenavi1/genomescope2.0
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
Hifiasm	0.19.8-r603	https://github.com/chhylp123/hifiasm
HiGlass	1.13.4	https://github.com/higlass/higlass
lep_busco_painter	1.0.0	https://github.com/charlottewright/lep_busco_painter
MerquryFK	1.1.2	https://github.com/thegenemyers/MERQURY.FK
Minimap2	2.28-r1209	https://github.com/lh3/minimap2
MitoHiFi	3	https://github.com/marcelauliano/MitoHiFi
MultiQC	1.14; 1.17 and 1.18	https://github.com/MultiQC/MultiQC
Nextflow	24.10.4	https://github.com/nextflow-io/nextflow
PretextSnapshot	0.0.5	https://github.com/sanger-tol/PretextSnapshot
PretextView	1.0.3	https://github.com/sanger-tol/PretextView

Software	Version	Source
samtools	1.21	https://github.com/samtools/samtools
sanger-tol/ascc	0.1.0	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	v0.7.1	https://github.com/sanger-tol/blobtoolkit
sanger-tol/curationpretext	1.4.2	https://github.com/sanger-tol/curationpretext
Seqtk	1.3	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.4.0	https://github.com/sanger-tol/treeval
YaHS	1.2.2	https://github.com/c-zhou/yahs

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Open Peer Review

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Version 1

Reviewer Report 30 March 2026

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✔ **Frederique Hilliou**
Universite Cote d'Azur, Provence-Alpes-Côte d'Azur, France

The manuscript presents the genome of the Oriental Meadow Brown (Cercyonis lupina), a species of butterfly in the family Nymphalidae, subfamily Satyrinae. *C. lupina* is found in north Africa, southern Europe, Anatolia and Arabia, and in some part of Central Asia where it feeds on Poaceae. Two different lineages are found, based on DNA bar-coding, grouping north Africa and Iberian Peninsula versus the rest of the population. Divergence has been inferred ca. 3 Mya. The sample used here for full genome sequencing is an adult female collected in Catalonia.
The haplotype 1 was curated at chromosome level (508.65Mb; 30 chromosomes including W and Z, 116 scaffolds, 172 contigs, and a 15.22kb mitochondrion) and the haplotype 2 at scaffold level (467.75Mb; 48 scaffolds, 109 contigs). BUSCO analysis using the lepidoptera_odb10 reference set identified 98.4% of this gene set in haplotype 1.
The assembly metric for haplotype 1 is 7.C.Q64 according to the Earth BioGenome Project Report on Assembly Standards September 2024.
Position of Merian elements is given across chromosomes of the haplotype 1.

What is the % of heterozygoty between the 2 haplotypes?
What was the method used for this genome annotation?
The mitochondrial genome is available (OZ243821.1) however I was not able to find its annotation.
The acquisition of this *C lupina* genome should help in the taxonomic classification of this species.

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.

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 27 March 2026

https://doi.org/10.21956/wellcomeopenres.28389.r150596

Liang Lü
Hebei Normal University, Shijiazhuang, Hebei, China

The authors present a valuable de novo genome assembly for the Oriental Meadow Brown butterfly (Cercyonis lupina), utilizing modern long-read sequencing and Hi-C technologies. While generating high-quality genomic resources for non-model Lepidoptera is a commendable contribution to comparative genomics, the manuscript currently reads more like a technical data report than a comprehensive scientific article. Assuming the journal's scope accommodates purely descriptive data notes, I have two specific methodological concerns that still need to be addressed:

1. Treatment of Heterozygosity: Lepidopteran genomes are known for their high levels of heterozygosity. Although the authors describe the assembly of a primary pseudo-haplotype, the manuscript lacks a sufficient discussion or characterization of the alternate haplotigs. But it can be left to other data-miners.

2. Depth of Annotation: The current functional annotation is insufficient. Simply mapping ancestral linkage groups (Merian elements) does not constitute a thorough genomic annotation. The authors state that the genome "will be annotated" using available RNA-Seq data via Ensembl. This suggests a reliance on purely automated pipelines. For a resource of this type, it is highly recommended to include manual curation of ecologically significant gene families, which are vital for understanding butterfly biology.

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: phylogenomics, 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.