DATA NOTE

The genome sequence of the Spanish Fritillary, *Euphydryas desfontainii* (Godart, 1819) (Lepidoptera: Nymphalidae)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a male specimen of *Euphydryas desfontainii* (Spanish Fritillary; Arthropoda; Insecta; Lepidoptera; Nymphalidae). The assembly contains two haplotypes with total lengths of 755.68 megabases and 751.57 megabases. Most of haplotype 1 (99.77%) is scaffolded into 30 chromosomal pseudomolecules, including the Z sex chromosome. Haplotype 2 was assembled to scaffold level. The mitochondrial genome has also been assembled, with a length of 16.56 kilobases. Gene annotation of this assembly on Ensembl identified 14 303 protein-coding genes. This work is part of Project Psyche, a collaborative programme generating genomes for European butterflies and moths.

Keywords

Euphydryas desfontainii; Spanish Fritillary; genome sequence; chromosomal; Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 16 Feb 2026	✓ view	✓ view

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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; Nymphalinae; Melitaeini; Euphydryina; *Euphydryas*; *Euphydryas desfontainii* (Godart, 1819) (NCBI:txid104507)

Background

Euphydryas desfontainii, commonly known as the Spanish Fritillary, is a medium-sized butterfly in the family Nymphalidae. Its distribution encompasses the Iberian Peninsula and North Africa, including Spain, Portugal, the French Pyrenees, as well as parts of the Rif and the Atlas Mountains in Morocco and Algeria (Tolman & Lewington, 2009). The species inhabits dry, rocky grasslands and open scrublands, often favouring areas with sparse vegetation and a rich diversity of herbaceous plants at elevations up to 1 800 m. Host-plant abundance and quality strongly drives larval occupancy whereas oviposition by females is strongly influenced by local microclimates and nearby vegetation (Pennekamp *et al.*, 2013). Larva get attacked by a wide range of parasitoids suggesting parasitoid pressure as a key factor affecting population dynamics (Stefanescu *et al.*, 2009).

Adults are univoltine, flying primarily from April to June with separate peaks for males and females, and show limited dispersal abilities (Pennekamp *et al.*, 2014). The wingspan ranges from 40 to 45 mm (Tolman & Lewington, 2009). Morphologically, *E. desfontainii* is characterised by its richly coloured wings with deep reds and pinks, bold black discal and postdiscal transverse markings, and distinct yellow submarginal halfmoons on the dorsal side of the forewing.

Larvae feed on various plants, including *Dipsacus*, *Scabiosa*, *Cephalaria*, and *Knautia* species and in captivity on *Succisa pratensis* (Tolman & Lewington, 2009). The species overwinters in the larval stage, with pupation occurring in spring.

Conservation concerns for *E. desfontainii* are primarily due to habitat loss and fragmentation. In Portugal, it is considered one of the rarest butterflies (Maravalhas, 2003), with studies highlighting the importance of preserving remaining habitat patches, safeguarding microclimate diversity and promoting host plant abundance (Pennekamp *et al.*, 2014). Genetically, *E. desfontainii* is well separated from its sister species *E. aurinia* (the Marsh Fritillary) (Zimmermann *et al.*, 2000). The IUCN Red List currently assesses the species as of Least Concern, although its population trends are not well-documented (International Union for Conservation of Nature, 2025).

We present a chromosome-level genome sequence for *Euphydryas desfontainii*, sequenced as part of Project Psyche (Wright *et al.*, 2025). The sequence data were derived from a male specimen (Figure 1) collected from Navès, Lleida, Catalonia, Spain.

Figure 1. Voucher photographs of the *Euphydryas desfontainii* (ilEupDesf1) specimen used for genome sequencing. Top: dorsal view; bottom: ventral view.

Methods

Sample acquisition

The specimen used for genome sequencing was an adult male *Euphydryas desfontainii* (specimen ID SAN28000296, ToLID ilEupDesf1; Figure 1), collected from Navès, Lleida, Catalonia, Spain (latitude 42.0453, longitude 1.6557) on 2019-05-20. 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 ilEupDesf1 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 46.24 ng/μL and a yield of 2 173.28 ng, with a fragment size of 15.3 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, 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 ilEupDesf1 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).

Table 1. Specimen and sequencing data for BioProject PRJEB80931.

Platform	PacBio HiFi	Hi-C
ToLID	ilEupDesf1	ilEupDesf1
Specimen ID	SAN28000296	SAN28000296
BioSample (source individual)	SAMEA115768743	SAMEA115768743
BioSample (tissue)	SAMEA115768857	SAMEA115768857
Tissue	whole organism	whole organism
Instrument	Revio	Illumina NovaSeq X
Run accessions	ERR13800484	ERR13802628
Read count total	1.78 million	641.26 million
Base count total	22.03 Gb	96.83 Gb

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 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 four breaks and seven joins, which reduced the scaffold count by 2.3%. 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 *Euphydryas desfontainii* specimen generated 22.03 Gb (gigabases) from 1.78 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 744.19 Mb, with a heterozygosity of 1.12% and repeat content of 42.85% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 29× coverage. Hi-C sequencing produced 96.83 Gb from 641.26 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 755.68 Mb in 84 scaffolds, with 126 gaps, and a scaffold N50 of 26.3 Mb (Table 2).

Most of the assembly sequence (99.77%) was assigned to 30 chromosomal-level scaffolds, representing 29 autosomes and the Z sex chromosome. 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). Chromosome Z was assigned based on synteny to the genome of *Melitaea cinxia* (GCF_905220565.1) (Vila *et al.*, 2021).

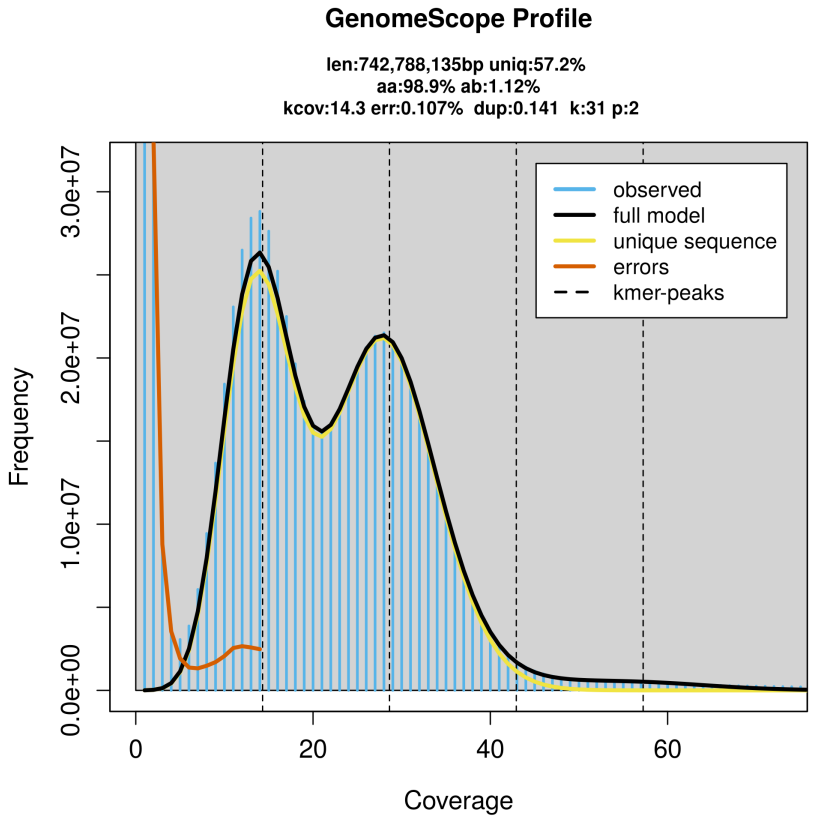

Figure 2. Frequency distribution of *k*-mers generated using GenomeScope2. The plot shows observed and modelled *k*-mer spectra, providing estimates of genome size, heterozygosity, and repeat content based on unassembled sequencing reads.

Table 2. Genome assembly statistics.

Assembly name	ilEupDesf1.hap1.1	ilEupDesf1.hap2.1
Assembly accession	GCA_964277145.1	GCA_964277265.1
Assembly level	chromosome	scaffold
Span (Mb)	755.68	751.57
Number of chromosomes	30	scaffold-level
Number of contigs	210	177
Contig N50	9.01 Mb	7.95 Mb
Number of scaffolds	84	63
Scaffold N50	26.3 Mb	26.19 Mb
Longest scaffold length (Mb)	53.52	-
Sex chromosomes	Z	-
Organelles	Mitochondrion: 16.56 kb	-

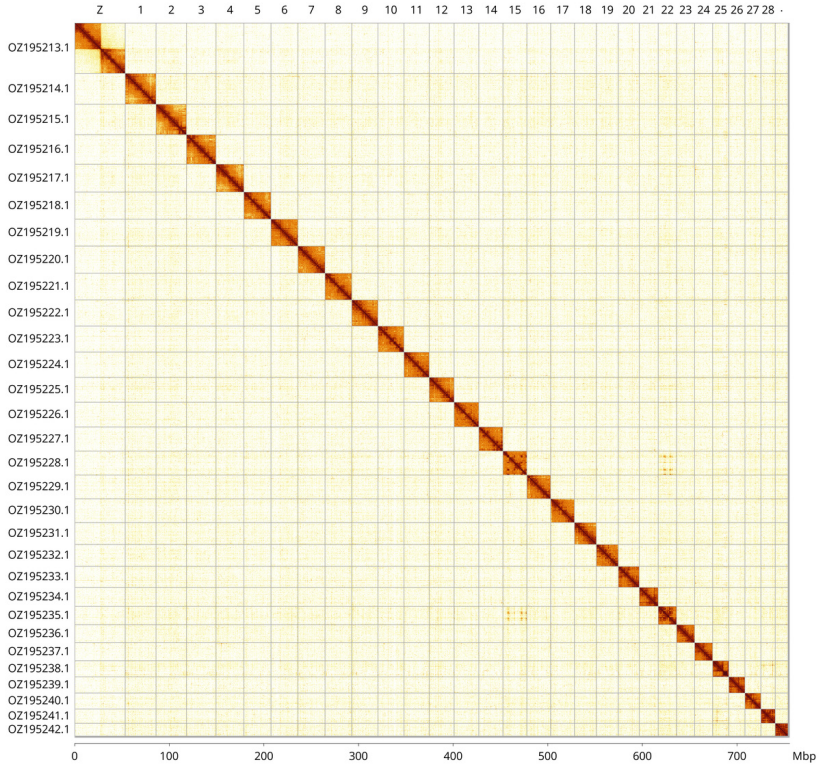

Figure 3. Hi-C contact map of the *Euphydryas desfontainii* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes, with a megabase scale shown below. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Euphydryas desfontainii* ilEupDesf1.

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ195214.1	1	32.53	34.50	M2
OZ195215.1	2	32.26	35	M17;M20
OZ195216.1	3	31.22	34.50	M8
OZ195217.1	4	29.42	35	M1
OZ195218.1	5	28.62	34.50	M9
OZ195219.1	6	28.52	35	M5
OZ195220.1	7	28.52	34.50	M7
OZ195221.1	8	28.35	35	M12
OZ195222.1	9	27.65	34.50	M6
OZ195223.1	10	27.47	34.50	M18
OZ195224.1	11	26.70	34.50	M16
OZ195225.1	12	26.30	35	M4
OZ195226.1	13	26.11	35	M22
OZ195227.1	14	25.56	34.50	M21
OZ195228.1	15	25.22	35	M23
OZ195229.1	16	25.21	35	M10
OZ195230.1	17	25.09	35	M15
OZ195231.1	18	23.19	35.50	M14
OZ195232.1	19	23.13	35	M11
OZ195233.1	20	22.22	35	M13
OZ195234.1	21	20.08	34.50	M24
OZ195235.1	22	19.42	36.50	M30
OZ195236.1	23	19.03	35.50	M19
OZ195237.1	24	18.98	35	M26
OZ195238.1	25	17.11	36	M31
OZ195239.1	26	16.95	34.50	M28
OZ195240.1	27	16.93	35	M27
OZ195241.1	28	15.15	35.50	M25
OZ195242.1	29	13.47	36	M29
OZ195213.1	Z	53.52	34	M3;MZ

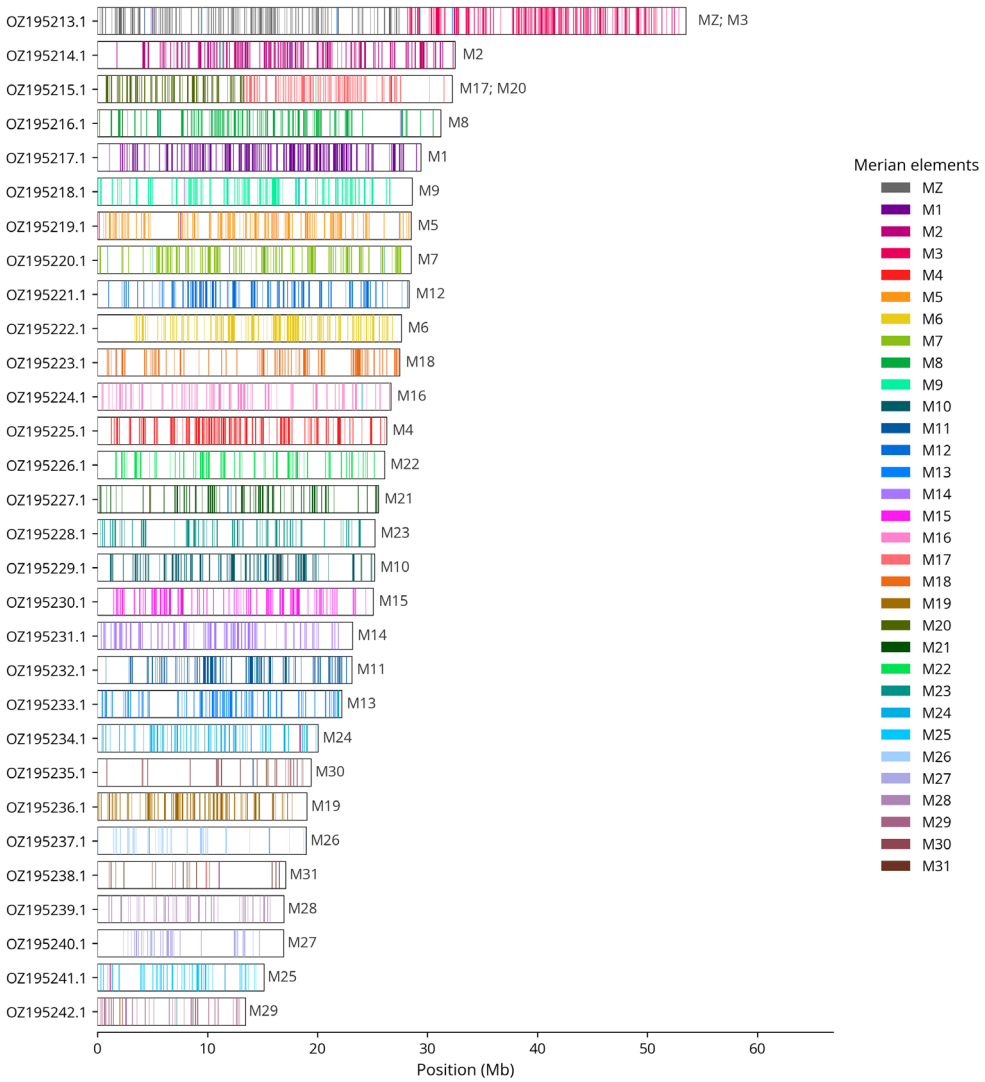

Figure 4. Merian elements painted across chromosomes in the ilEupDesf1.hap1.1 assembly of *Euphydryas desfontainii*. Chromosomes are drawn to scale, with the positions of orthologues shown as coloured bars. Each orthologue is coloured by the Merian element that it belongs to. All orthologues which could be assigned to Merian elements are shown.

The mitochondrial genome was also assembled (length 16.56 kb, OZ195243.1). 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 60.4, and for haplotype 2, 60.5. When the two haplotypes are combined, the assembly achieves an estimated QV of 60.4. The *k*-mer completeness is 76.44% for haplotype 1, 76.41% for haplotype 2, and 99.27% for the combined haplotypes (Figure 5). BUSCO analysis using the lepidoptera_odb10 reference set (n = 5 286) identified 98.5% of the expected gene set (single = 98.0%, duplicated = 0.5%) in haplotype 1. For haplotype 2, BUSCO analysis identified 98.6% of the expected gene set (single = 98.1%, duplicated = 0.5%). 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 on Assembly Standards September 2024. The EBP metric, calculated for the haplotype 1, is **6.C.Q60**, meeting the recommended reference standard.

Genome annotation report

The *Euphydryas desfontainii* genome assembly (GCA_964277145.1) was annotated by Ensembl at the European Bioinformatics Institute (EBI). This annotation includes 32 069 transcribed mRNAs from 14 303 protein-coding and 5 778 non-coding genes. The average transcript length is 20 118.45 bp,

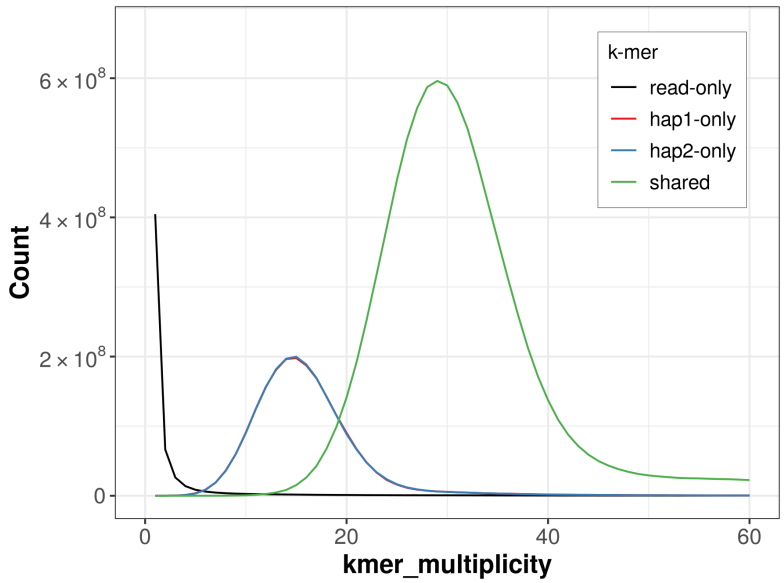

Figure 5. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers 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.

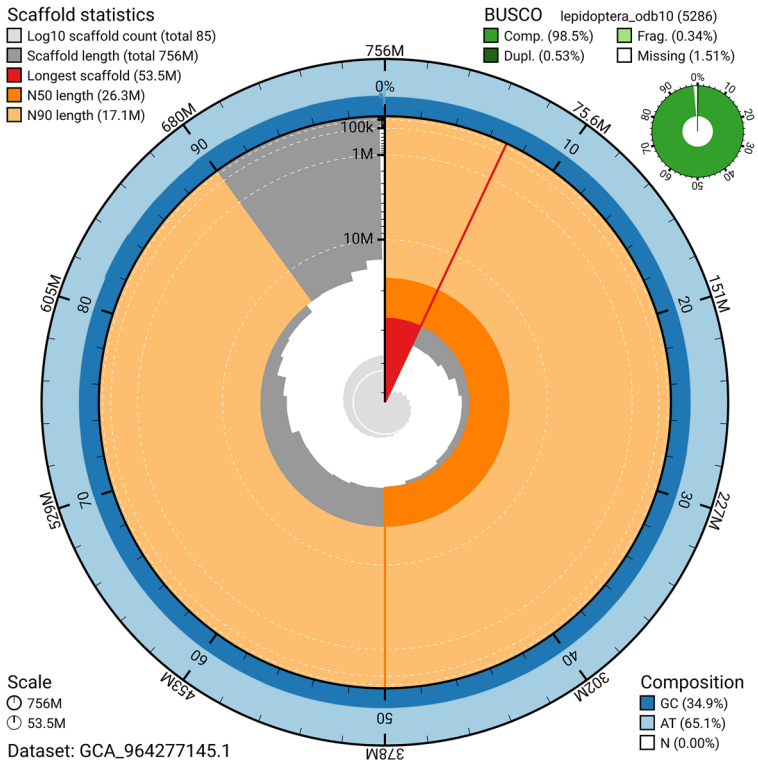

Figure 6. Assembly metrics for ilEupDesf1.hap1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. The outermost 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.

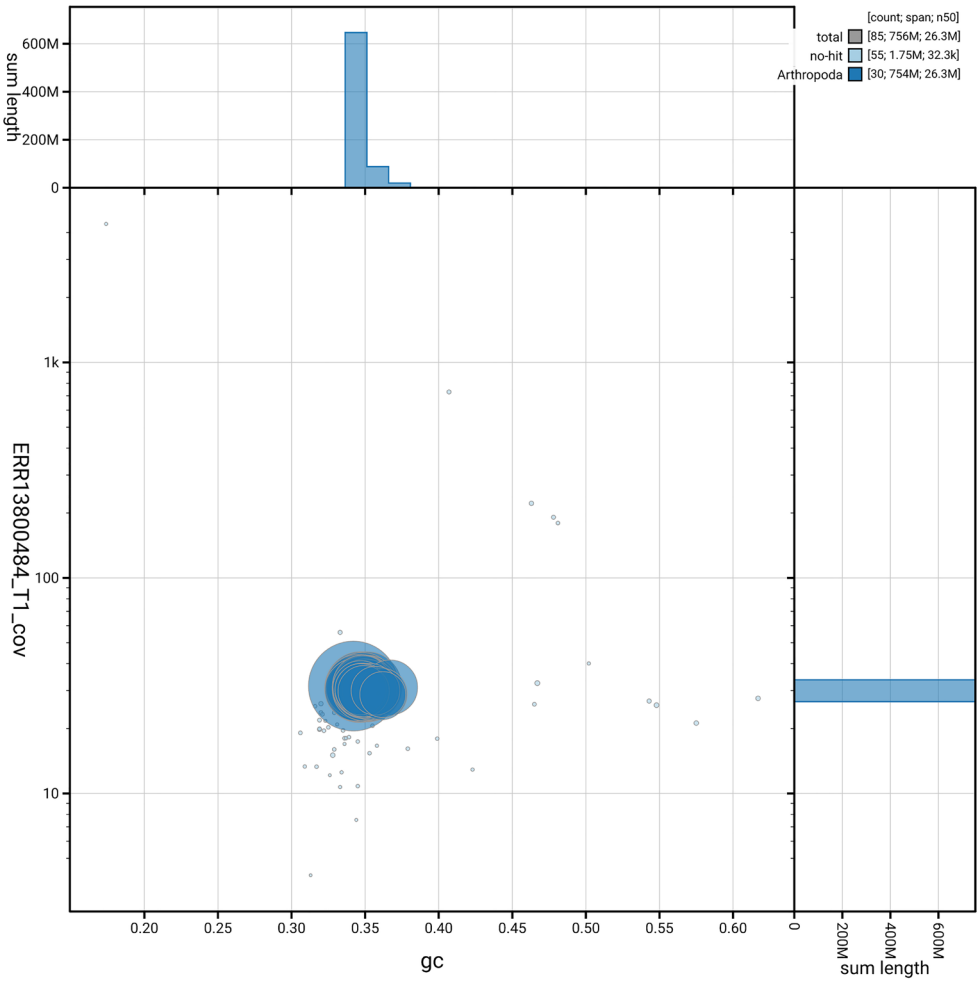

Figure 7. BlobToolKit GC-coverage plot for ilEupDesf1.hap1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes 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 *Euphydryas desfontainii* assembly.

Measure	Value	Benchmark
EBP summary (haplotype 1)	6.C.Q60	6.C.Q40
Contig N50 length	9.01 Mb	≥ 1 Mb
Scaffold N50 length	26.30 Mb	= chromosome N50
Consensus quality (QV)	Haplotype 1: 60.4; haplotype 2: 60.5; combined: 60.4	≥ 40
k-mer completeness	Haplotype 1: 76.44%; Haplotype 2: 76.41%; combined: 99.27%	≥ 95%
BUSCO	C:98.5% [S:98.0%; D:0.5%]; F:0.3%; M:1.2%; n:5 286	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.77%	≥ 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

with an average of 1.60 coding transcripts per gene and 6.65 exons per transcript. For further information, please refer to the Ensembl annotation page.

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: Euphydryas desfontainii. Accession number PRJEB80931. The genome sequence is released openly for reuse. The *Euphydryas desfontainii* 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. 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	4.3.9	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	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	2.24-r1122	https://github.com/lh3/minimap2

Software	Version	Source
MitoHiFi	3	https://github.com/marcelauliano/MitoHiFi
MultiQC	1.14; 1.17 and 1.18	https://github.com/MultiQC/MultiQC
Nextflow	23.10.0	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
samtools	1.19.2	https://github.com/samtools/samtools
sanger-tol/ascc	0.1.0	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	0.6.0	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	1.2a.2	https://github.com/c-zhou/yahs

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Version 1

Reviewer Report 13 March 2026

https://doi.org/10.21956/wellcomeopenres.28495.r148391

✔ **Taslima Sheikh**
Baba Ghulam Shah Badshah University, Rajouri, Jammu and Kashmir, India

The manuscript presents a high-quality chromosome-level genome assembly of *Euphydryas desfontainii* using PacBio HiFi and Hi-C data. The study is well structured and provides valuable genomic resources for Lepidoptera research. Minor improvements include clearer interpretation of assembly metrics, brief comparison with related genomes, and slight language refinement to improve readability and context.

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: lepidoptera, entomofauna

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 25 February 2026

https://doi.org/10.21956/wellcomeopenres.28495.r148393

Wai Lok So
[1] The Chinese University of Hong Kong, Hong Kong, Hong Kong
[2] The Hang Seng University of Hong Kong, Hong Kong, Hong Kong

The manuscript presents a high-quality genome assembly of *Euphydryas desfontainii*. The sequencing and assembly methods are technically sound. The project yielded two haplotypes with one that is with ideal completeness, including the z sex chromosome. The mitochondrial genome was also completed in standardised procedures.
The resulting resource would be very useful for conservation of such species and research on Lepidoptera evolution, and genomics.
One thing that needs to be addressed is the figure. Since whole individual was used to prepare the DNA, and in a more appropriate way, I would advise to include the whole specimen in Figure 1, not merely the wings, despite the fact that the wing is an iconic morphological identifier.
Otherwise, I have no major concerns with the manuscript.

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: Evolution, genomics, arthropods

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.