



DATA NOTE

The genome sequence of the Spanish Festoon, *Zerynthia rumina* (Linnaeus, 1758) (Lepidoptera: Papilionidae)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a female specimen of *Zerynthia rumina* (Spanish Festoon; Arthropoda; Insecta; Lepidoptera; Papilionidae). The assembly contains two haplotypes with total lengths of 852.84 megabases and 775.60 megabases. Most of haplotype 1 (99.64%) is scaffolded into 31 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 15.26 kilobases. This work is part of Project Psyche, a collaborative programme generating genomes for European butterflies and moths.

Keywords

Zerynthia rumina; Spanish Festoon; genome sequence; chromosomal; Lepidoptera



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

Open Peer Review

Approval Status

	1	2
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Any reports and responses or comments on the article can be found at the end of the article.

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Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Papilionoidea; Papilionidae; Parnassiinae; Zerynthini; *Zerynthia*; *Zerynthia rumina* (Linnaeus, 1758) (NCBI:txid132710)

Background

The Spanish Festoon, *Zerynthia rumina* (Linnaeus, 1758), is a medium-sized butterfly with a distinctive and colourful appearance. It belongs to the family Papilionidae and is found in the Iberian Peninsula, south-east France, and north-western Africa (García-Barros *et al.*, 2013). The species inhabits a range of Mediterranean habitats, including open *Quercus* woodlands, semi-arid areas, and coastal zones from sea level to around 1 200 m a.s.l. (García-Barros *et al.*, 2013).

Z. rumina is a univoltine species, overwintering as a pupa and emerging as an adult in spring. Adults are present from February to May (García-Barros *et al.*, 2013). A second summer-autumn generation can occur in coastal areas of southern Iberia (Olivares Villegas *et al.*, 1991) and north Africa (Mokhles, 1984), possibly due to an extended flowering period of the host plant. Single eggs are laid primarily on the underside of leaves or flowers of various species of birthwort (*Aristolochia* spp.). In many Iberian populations, the preferred host plant is *Aristolochia pistolochia* L., a species adapted to arid environments (García-Barros *et al.*, 2013). Larvae are parasitised by the wasp *Agrypon polyxeniae* (Szépligeti, 1899) (Ichneumonidae: Anomaloniinae) (Shaw *et al.*, 2009).

Zerynthia rumina is a highly variable species, with several described forms (e.g. *f. canteneri* and *f. honnoratii*). It shows high mitochondrial variation and spatial structure, with one lineage present on the European continent and two highly divergent lineages in north Africa (Dapporto *et al.*, 2022). Some authors consider the African populations to be a different species, *Z. africana* Stichel, 1907 (Leraut, 2016).

The species is currently listed as Least Concern in the IUCN Red List for Europe (Van Swaay *et al.*, 2025). The karyotype of *Zerynthia rumina* is unknown. The first reference genome for *Z. rumina* presented here is an important resource for clarifying the evolutionary history and systematics of this butterfly. The sequence data were derived from a female specimen (Figure 1) collected from Catalonia, Spain.

Methods

Sample acquisition

The specimen used for genome sequencing was an adult female *Zerynthia rumina* (specimen ID SAN28000382, ToLID ilZerRumi1; Figure 1), collected from Torrebesses, Garrigues, Catalonia, Spain (latitude 41.4161, longitude 0.6099) on 2021-04-13. The specimen was collected by Roger Vila, Mattia Menchetti and Joan Carles Hinojosa (Institut De Biologia Evolutiva) and identified by Joan Carles Hinojosa and Roger Vila (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 ilZerRumi1 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 45.06 ng/μL and a yield of 2 117.82 ng, with a fragment size of 15.3 kb.



Figure 1. Voucher photographs of the *Zerynthia rumina* (ilZerRumi1) specimen used for genome sequencing.

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 ilZerRumi1 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 Diagenode 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](#).

Table 1. Specimen and sequencing data for BioProject PRJEB85016.

Platform	PacBio HiFi	Hi-C
ToLID	ilZerRumi1	ilZerRumi1
Specimen ID	SAN28000382	SAN28000382
BioSample (source individual)	SAMEA115770359	SAMEA115770359
BioSample (tissue)	SAMEA115770379	SAMEA115770379
Tissue	whole organism	whole organism
Instrument	Revio	Illumina NovaSeq X
Run accessions	ERR14209133; ERR14209132	ERR14224615
Read count total	3.17 million	736.63 million
Base count total	34.45 Gb	111.23 Gb

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 MERQUERY.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 21 breaks and 172 joins. This reduced the scaffold count by 42.4% and increased the total assembly length by 2.4%. The curation process is described at <https://gitlab.com/wtsi-grit/rapid-curation>. PretextViewSnapshot was used to generate a Hi-C contact map of the final assembly.

Assembly quality assessment

The Merquery.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 databases ($k = 31$) 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_buscoPainter 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 coloured and plotted along chromosomes drawn to scale.

Genome sequence report

Sequence data

PacBio sequencing of the *Zerynthia rumina* specimen generated 34.45 Gb (gigabases) from 3.17 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 759.45 Mb, with a heterozygosity of 1.37% and repeat content of 25.19% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 43× coverage. Hi-C sequencing produced 111.23 Gb from 736.63 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 852.84 Mb in 113 scaffolds, with 306 gaps, and a scaffold N50 of 27.4 Mb (Table 2).

Most of the assembly sequence (99.64%) was assigned to 31 chromosomal-level scaffolds, representing 29 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. During curation we noted that the contigs making up the W chromosome have uncertain order and orientation.

The mitochondrial genome was also assembled (length 15.26 kb, OZ238067.1). This sequence is included as a contig in the multifasta file of the genome submission and as a standalone record.

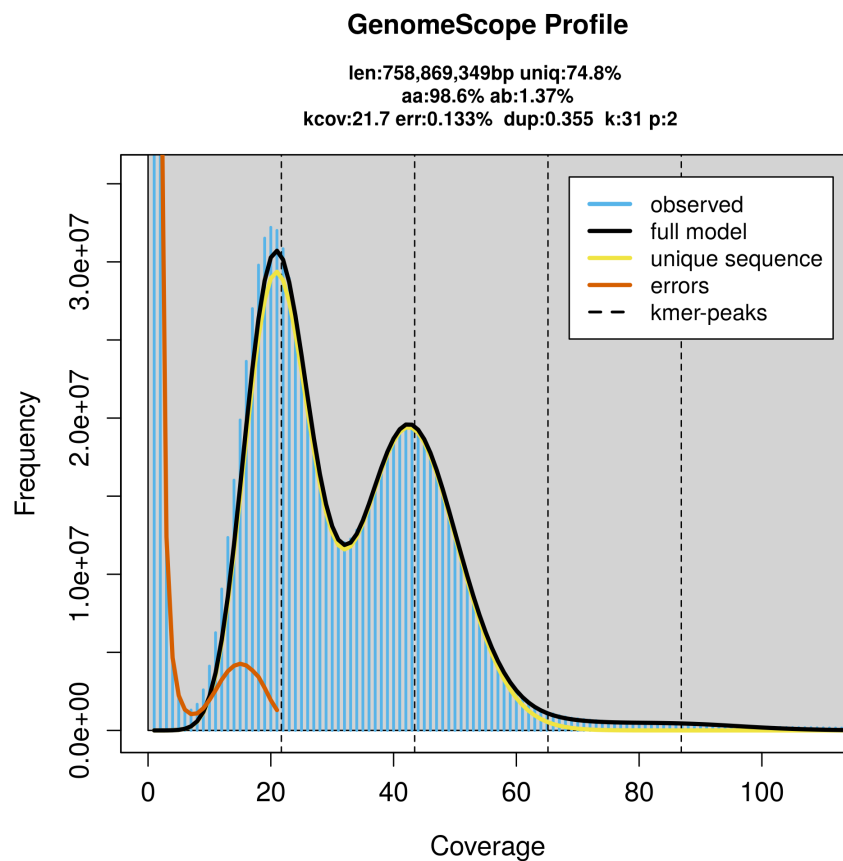


Figure 2. Frequency distribution of *k*-mers generated using GenomeScope2. The plot shows observed and modelled *k*-mer spectra, providing estimates of genome size, heterozygosity, and repeat content based on unassembled sequencing reads.

Table 2. Genome assembly statistics.

Assembly name	ilZerRumi1.hap1.1	ilZerRumi1.hap2.1
Assembly accession	GCA_965178335.1	GCA_965178255.1
Assembly level	chromosome	scaffold
Span (Mb)	852.84	775.60
Number of chromosomes	31	scaffold-level
Number of contigs	419	307
Contig N50	6.08 Mb	5.85 Mb
Number of scaffolds	113	55
Scaffold N50	27.4 Mb	27.37 Mb
Longest scaffold length (Mb)	33.56	-
Sex chromosomes	W and Z	-
Organelles	Mitochondrion: 15.26 kb	-

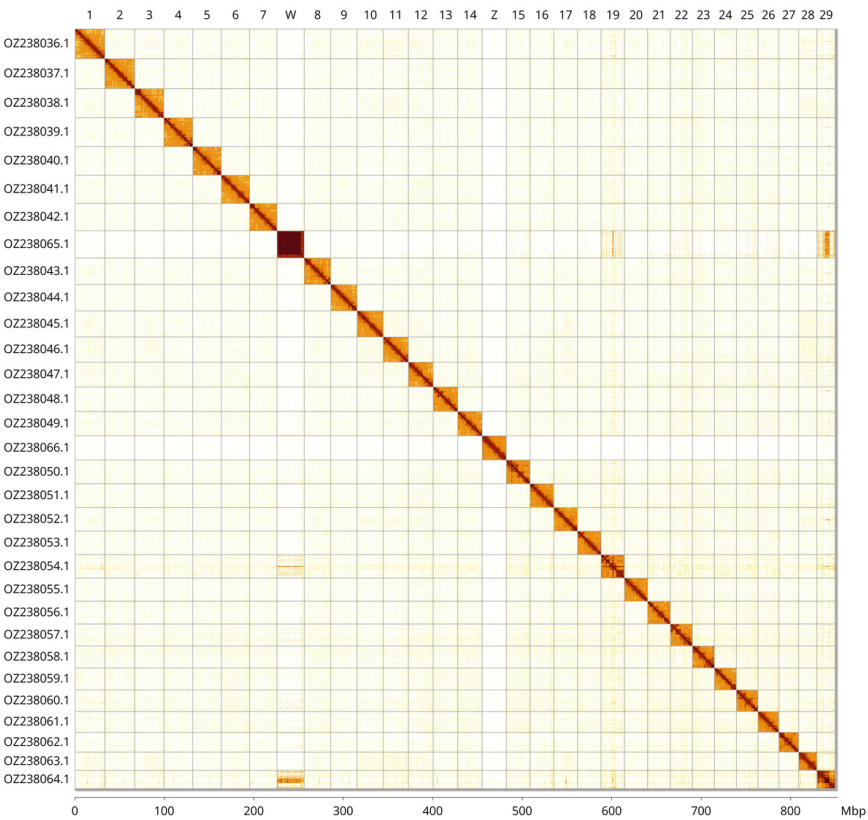


Figure 3. Hi-C contact map of the *Zerynthia rumina* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes, with a megabase scale shown below. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Zerynthia rumina* ilZerRumi1.

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ238036.1	1	33.56	35	M17;M20
OZ238037.1	2	33.43	34.50	M8
OZ238038.1	3	32.59	35	M1
OZ238039.1	4	32.27	35	M12
OZ238040.1	5	31.96	35	M22
OZ238041.1	6	31.63	35	M7
OZ238042.1	7	30.54	34.50	M9
OZ238043.1	8	29.67	35	M10
OZ238044.1	9	29.48	35	M5
OZ238045.1	10	29.14	35	M2
OZ238046.1	11	28.06	35	M3
OZ238047.1	12	27.87	35	M6
OZ238048.1	13	27.40	35	M18
OZ238049.1	14	27.32	35.50	M11
OZ238050.1	15	26.54	35.50	M24

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ238051.1	16	26.45	35.50	M15
OZ238052.1	17	26.45	35.50	M4
OZ238053.1	18	26.33	35	M16
OZ238054.1	19	26.21	36.50	M31
OZ238055.1	20	25.86	35	M21
OZ238056.1	21	25.52	35.50	M23
OZ238057.1	22	24.69	36	M27
OZ238058.1	23	24.50	36	M14
OZ238059.1	24	24.49	35.50	M26
OZ238060.1	25	24.25	36	M19
OZ238061.1	26	23.33	35.50	M13
OZ238062.1	27	22.05	35.50	M28
OZ238063.1	28	20.43	36	M25;M29
OZ238064.1	29	20.26	40	M30
OZ238065.1	W	30.52	37	-
OZ238066.1	Z	27.01	34.50	MZ

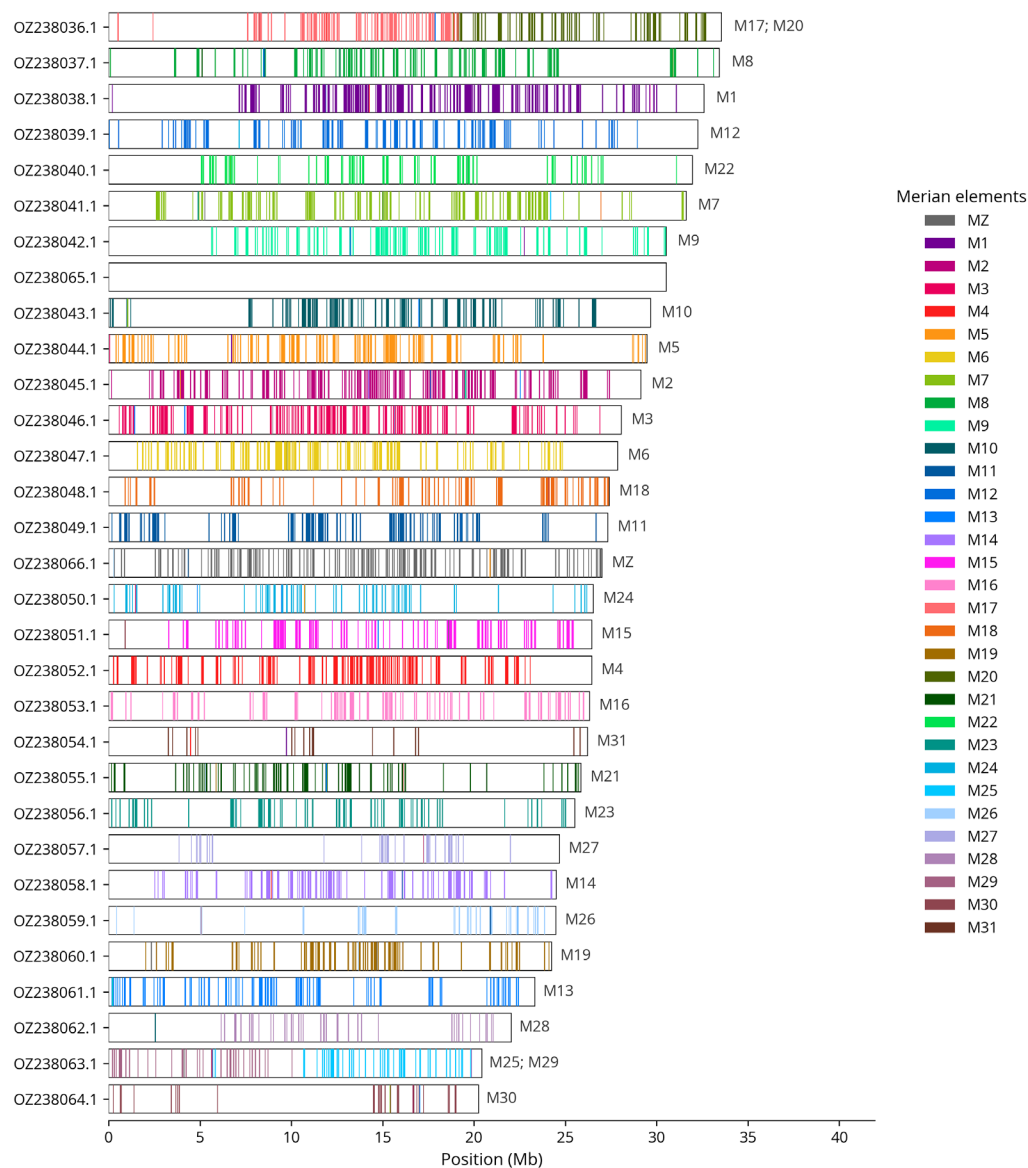


Figure 4. Merian elements painted across chromosomes in the ilZerRumi1.hap1.1 assembly of *Zerynthia rumina*. Chromosomes are drawn to scale, with the positions of orthologues shown as coloured bars. Each orthologue is coloured by the Merian element that it belongs to. All orthologues which could be assigned to Merian elements are shown.

Assembly quality metrics

For haplotype 1, the estimated QV is 64.1, and for haplotype 2, 64.5. When the two haplotypes are combined, the assembly achieves an estimated QV of 64.3. The *k*-mer completeness is 75.81% for haplotype 1, 72.00% for haplotype 2, and 99.73% for the combined haplotypes (Figure 5). BUSCO analysis using the lepidoptera_odb10 reference set (*n* = 5 286) identified 99.0% of the expected gene set (single = 98.4%, duplicated = 0.6%) in haplotype 1. For haplotype 2, BUSCO analysis identified 94.5% of the expected gene set (single = 94.2%, duplicated = 0.2%).

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.Q64**, meeting the recommended reference standard.

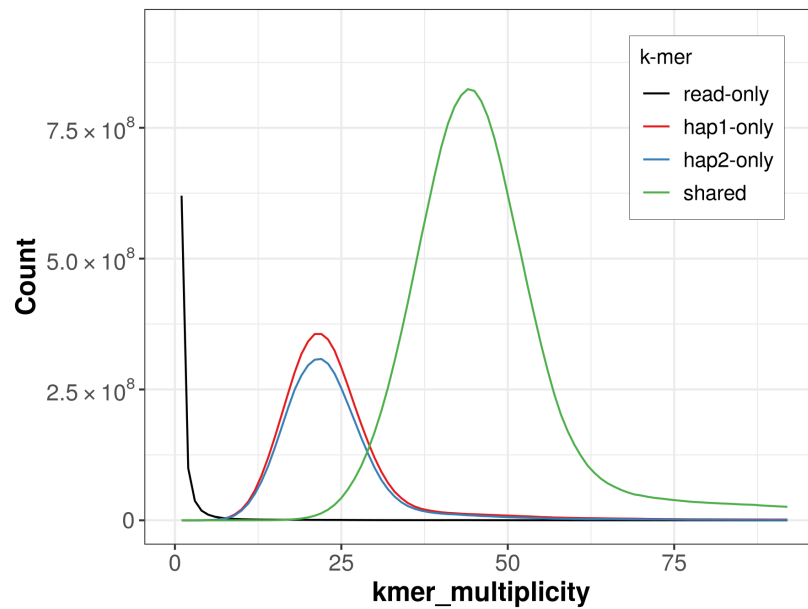


Figure 5. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers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

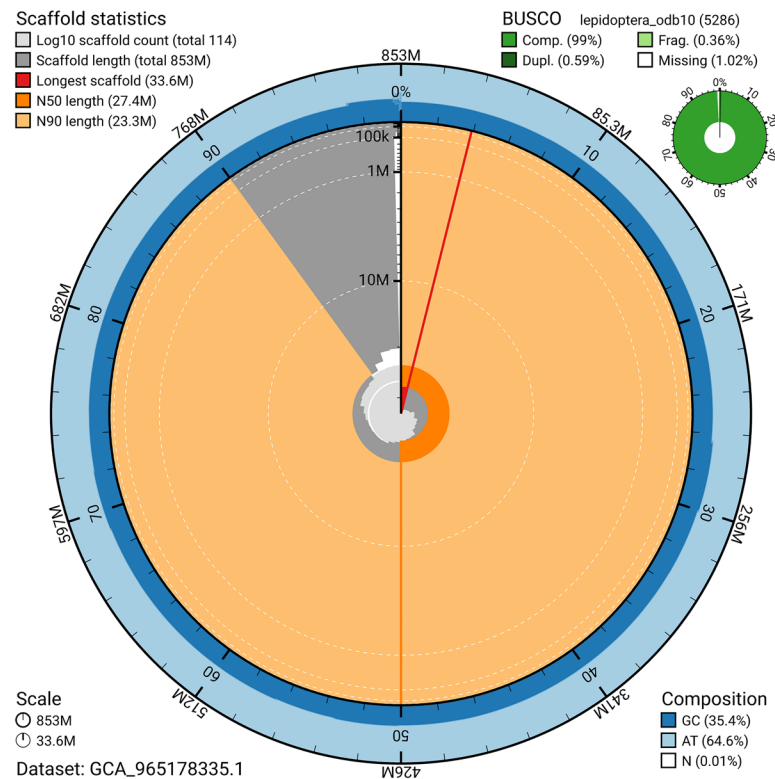


Figure 6. Assembly metrics for ilZerRumi1.hap1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. The outermost 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](#).

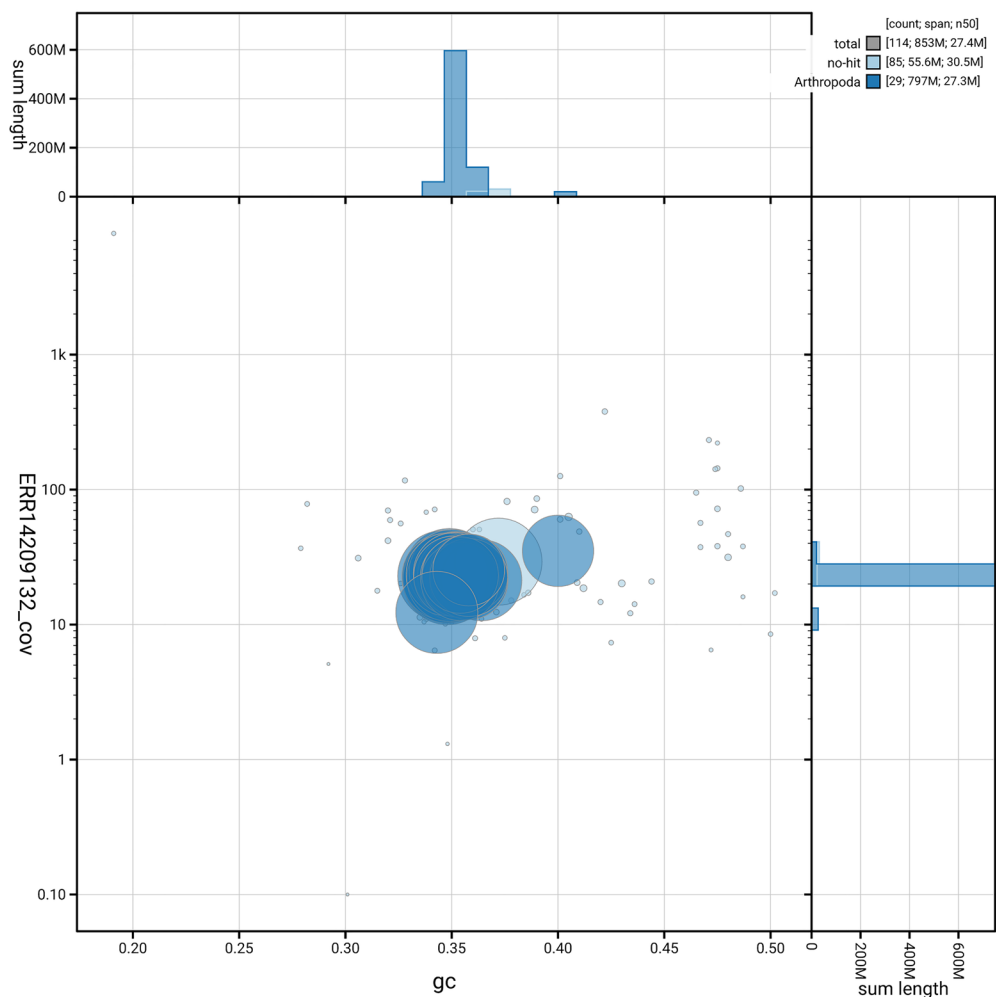


Figure 7. BlobToolKit GC-coverage plot for ilZerRumi1.hap1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes 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 *Zerynthia rumina* assembly.

Measure	Value	Benchmark
EBP summary (haplotype 1)	6.C.Q64	6.C.Q40
Contig N50 length	6.08 Mb	≥ 1 Mb
Scaffold N50 length	27.40 Mb	= chromosome N50
Consensus quality (QV)	Haplotype 1: 64.1; haplotype 2: 64.5; combined: 64.3	≥ 40
<i>k</i> -mer completeness	Haplotype 1: 75.81%; Haplotype 2: 72.00%; combined: 99.73%	≥ 95%
BUSCO	C:99.0% [S:98.4%; D:0.6%]; F:0.4%; M:0.7%; n:5 286	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.64%	≥ 90%

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: *Zerynthia rumina* (southern fescue). Accession number [PRJEB85016](#). The genome sequence is released openly for reuse. The *Zerynthia rumina* 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.

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/chhy123/hifiasm
HiGlass	1.13.4	https://github.com/higlass/higlass
lep_buscoPainter	1.0.0	https://github.com/charlottewright/lep_buscoPainter
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

Software	Version	Source
PretextView	1.0.3	https://github.com/sanger-tol/PretextView
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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Yoshiaki Morino

University of Tsukuba, Tsukuba, Japan

A high-quality reference genome assembly is presented for the Spanish festoon butterfly *Zerynthia rumina*, generated from a female collected in Catalonia, Spain, using PacBio Revio HiFi and Hi-C data. The assembly was produced as a phased diploid assembly with two haplotypes using Hi-C-based phasing: haplotype 1 comprises 852.84 Mb, with 99.64% assigned to 31 chromosome-scale pseudomolecules, while haplotype 2 is provided at the scaffold level. A complete mitochondrial genome is also included. Data deposition and methodological reporting are thorough, with clear documentation of the software, pipelines, and manual curation steps, supporting transparency and reproducibility. Assembly quality metrics are strong, including high BUSCO completeness (lepidoptera_odb10; 99.0% complete for haplotype 1), and the reported EBP summary metrics meet recommended standards. Overall, this resource should be highly valuable for downstream studies in evolutionary biology and taxonomy.

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: Evolutionary developmental biology, Invertebrate

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 03 February 2026

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Maria Nilsson 

Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany

The nuclear genome has been sequenced from a butterfly, the Spanish festoon *Zerynthia rumina*. The species occur in the Mediterranean region, and the sample originate from Spain.

The assembly is based on pacbio revio longread sequences and HiC. The final length of the manual curated assembly is 759Mb and contain 31 chromosomal pseudomolecules including both sex chromosomes. The assembly was done according to current best practice. All quality values and statistics is reported and indicate a high quality butterfly genome. The methodology and all tools and software used is reported.

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, transposable elements, mitogenomics

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.