DATA NOTE

The genome sequence of the Lesser Mountain Ringlet, *Erebia melampus* Füssly, 1775 (Lepidoptera: Nymphalidae)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a female specimen of *Erebia melampus* (Lesser Mountain Ringlet; Arthropoda; Insecta; Lepidoptera; Nymphalidae). The assembly contains two haplotypes with total lengths of 479.19 megabases and 424.26 megabases. Most of haplotype 1 (99.7%) is scaffolded into 21 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 15.2 kilobases.

Keywords

Erebia melampus, Lesser Mountain Ringlet, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 30 Jul 2025	✓ 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; Satyrinae; Erebiini; *Erebia*; *Erebia melampus* Füssly, 1775 (NCBI:txid1660630)

Background

The Lesser Mountain Ringlet *Erebia melampus* was first described by Johann Caspar Fuessli in 1775, based on his observations in the Swiss Alps in the cantons of Grisons and Glarus (Fuessli, 1775). The species is endemic to the European Alps and occurs throughout from France to Austria. Allozyme data further suggests strong genetic differentiation between *E. melampus* from the Western and the Eastern Alps, likely due to differentiation in distinct glacial refugia (Haubrich & Schmitt, 2007). While the sister species *Erebia sudetica* (Staudinger, 1861) also occurs in the Alps around Grindelwald, *E. melampus* does not occur with it, as the two species spatially exclude each other (Sonderegger, 2005).

The forewings are less than 20 mm long, making *E. melampus* one of the smaller Erebia species (Kleckova *et al.*, 2023). *E. melampus* are mostly brown with orange spots. The dorsal and ventral side of the wings often look alike. The anterior-most orange spots on the dorsal forewings lack a black dot, while the dorsal hindwings mostly carry a total of three black dots in the orange spots. The orange dot in the fourth cell on the dorsal side of the hindwing is following the bow-shaped pattern of the neighbouring orange spots. The orange spot on the ventral side of the hindwings is bigger than its neighbouring ones and positioned centrally within the respective wing-cell (Baudraz & Baudraz, 2021; Bühler-Cortesi, 2012).

During the caterpillar stage, *E. melampus* have been found to feed on sheep fescue (*Festuca ovina*) and sweet vernal grass (*Anthoxanthum odoratum*). In the central Alps, they occur on a broad range of habitats, ranging from dry grassland to wet meadows, occurring from 800 to 2 200 m a.s.l. (Bühler-Cortesi, 2012; Kleckova *et al.*, 2023). Adults fly from the end of June until late September in the Alps (Bühler-Cortesi, 2012; Kleckova *et al.*, 2023; Savchenko *et al.*, 2018).

While *E. melampus* is a species of Least Concern for the IUCN Red List, its genome will provide invaluable opportunities to study genome evolution in one of the most karyological groups of butterflies (Augustijnen *et al.*, 2024).

We present a chromosome-level, haplotype-resolved genome sequence of the Lesser Mountain Ringlet, *Erebia melampus*, sequenced as part of Project Psyche. The sequence data was derived from a female specimen (Figure 1) collected from Meiringen, Bern, Switzerland.

Methods

Sample acquisition

The specimen used for genome sequencing was an adult female *Erebia melampus* (specimen ID SAN28000025, ToLID ilEreMelm1; Figure 1), collected from Meiringen, Bern, Switzerland (latitude 46.6743, longitude 8.1334; elevation 1 466 m) on 09/07/2023. The specimen was collected and identified by Kay Lucek (University of Neuchâtel).

Figure 1. Voucher photograph of the *Erebia melampus* (ilEreMelm1) specimen used for genome sequencing.

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 ilEreMelm1 sample was weighed and triaged to determine the appropriate extraction protocol. Tissue from the thorax 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 34.48 ng/μL and a yield of 1 620.56 ng, with a fragment size of 15.2 kb. The 260/280 spectrophotometric ratio was 1.95, and the 260/230 ratio was 2.86.

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), following 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 head tissue of the ilEreMelm1 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 and 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).

Table 1. Specimen and sequencing data for BioProject PRJEB78676.

Platform	PacBio HiFi	Hi-C
ToLID	ilEreMelm1	ilEreMelm1
Specimen ID	SAN28000025	SAN28000025
BioSample (source individual)	SAMEA115109522	SAMEA115109522
BioSample (tissue)	SAMEA115109661	SAMEA115109663
Tissue	thorax	head
Sequencing platform and model	Revio	Illumina NovaSeq X
Run accessions	ERR13485709	ERR13474174
Read count total	1.20 million	832.73 million
Base count total	13.31 Gb	125.74 Gb

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 13 breaks and 37 joins. The curation process is documented 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

Chromosomal painting was performed using lep_busco_painter using Merian elements, which represent the 32 ancestral linkage groups in Lepidoptera (Wright *et al.*, 2024). Painting was based on gene locations from the lepidoptera_odb10 BUSCO analysis and chromosome lengths from the genome index produced using SAMtools faidx (Danecek *et al.*, 2021). Each complete BUSCO (including both single-copy and duplicated BUSCOs) was assigned to a Merian element using a reference database, and coloured positions were plotted along chromosomes drawn to scale.

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 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 implementation of the earlier Snakemake BlobToolKit pipeline (Challis *et al.*, 2020). The pipeline aligns PacBio reads using minimap2 (Li, 2018) and SAMtools (Danecek *et al.*, 2021) to generate coverage tracks. Simultaneously, it queries the GoaT database (Challis *et al.*, 2023) to identify relevant BUSCO lineages and runs BUSCO (Manni *et al.*, 2021). For the three domain-level BUSCO 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 package management via Conda and Bioconda (Grüning *et al.*, 2018), and containerisation through Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017).

Genome sequence report

Sequence data

The genome of a specimen of *Erebia melampus* was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 13.31 Gb (gigabases) from 1.20 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 450.00 Mb, with a heterozygosity of 0.91% and repeat content of 32.75%. These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 29× coverage. Hi-C sequencing produced 125.74 Gb from 832.73 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 479.19 Mb in 39 scaffolds, with 135 gaps, and a scaffold N50 of 21.41 Mb (Table 2).

Table 2. Genome assembly statistics.

Assembly name	ilEreMelm1.hap1.1	ilEreMelm1.hap2.1
Assembly accession	GCA_964261625.1	GCA_964261565.1
Assembly level	chromosome	scaffold
Span (Mb)	479.19	424.26
Number of chromosomes	21	N/A
Number of contigs	174	164
Contig N50	5.65 Mb	5.03 Mb
Number of scaffolds	39	55
Scaffold N50	21.41 Mb	20.89 Mb
Longest scaffold length (Mb)	47.86	N/A
Sex chromosomes	W and Z	N/A
Organelles	Mitochondrial genome: 15.2 kb	N/A

Most of the assembly sequence (99.7%) was assigned to 21 chromosomal-level scaffolds, representing 19 autosomes and the W and Z sex chromosomes. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 2; 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 3).

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

Assembly quality metrics

For haplotype 1, the estimated QV is 66.2, and for haplotype 2, 65.5. When the two haplotypes are combined, the assembly achieves an estimated QV of 65.8. The *k*-mer completeness

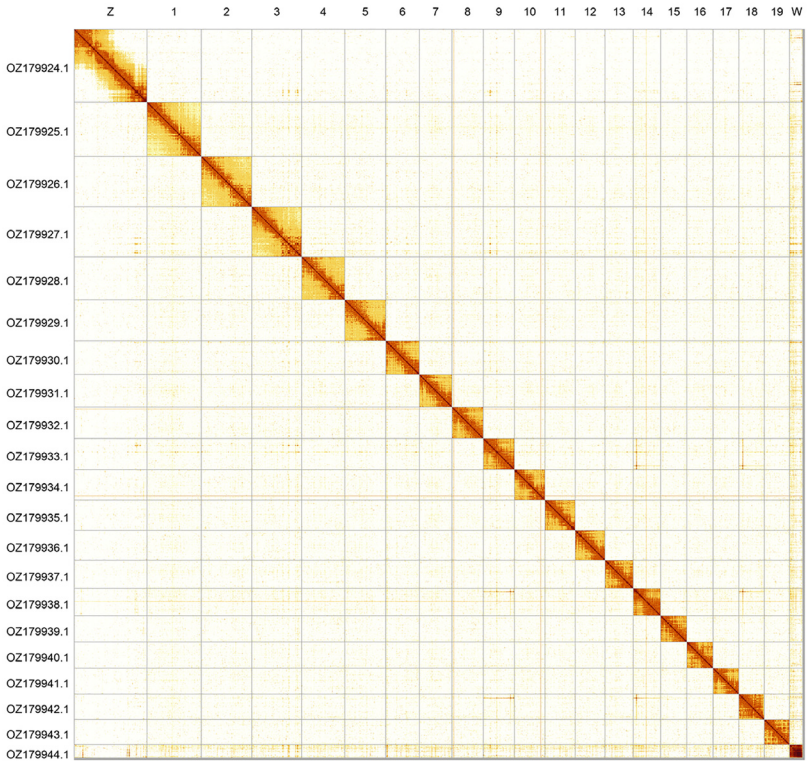

Figure 2. Hi-C contact map of the *Erebia melampus* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Erebia melampus* ilEreMelm1.

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ179925.1	1	35.55	37	M10;M14;M29
OZ179926.1	2	33.11	37.50	M11;M12
OZ179927.1	3	32.83	37	M23;M7
OZ179928.1	4	28.17	37	M27;M9
OZ179929.1	5	26.92	37	M22;M28
OZ179930.1	6	22.01	37.50	M13;M25
OZ179931.1	7	21.41	37.50	M1
OZ179932.1	8	20.51	37	M2

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ179933.1	9	20.47	37.50	M19;M26
OZ179934.1	10	20.05	37.50	M3
OZ179935.1	11	19.77	37.50	M17;M20
OZ179936.1	12	19.48	37	M8
OZ179937.1	13	18.59	37	M5
OZ179938.1	14	17.88	37.50	M24;M31
OZ179939.1	15	17.27	37	M16
OZ179940.1	16	17.06	37.50	M6
OZ179941.1	17	17.01	37	M4
OZ179942.1	18	16.62	37.50	M15
OZ179943.1	19	16.53	37	M21
OZ179944.1	W	8.63	37.50	N/A
OZ179924.1	Z	47.86	37	M18;M30;MZ
OZ179945.1	MT	0.02	19.50	N/A

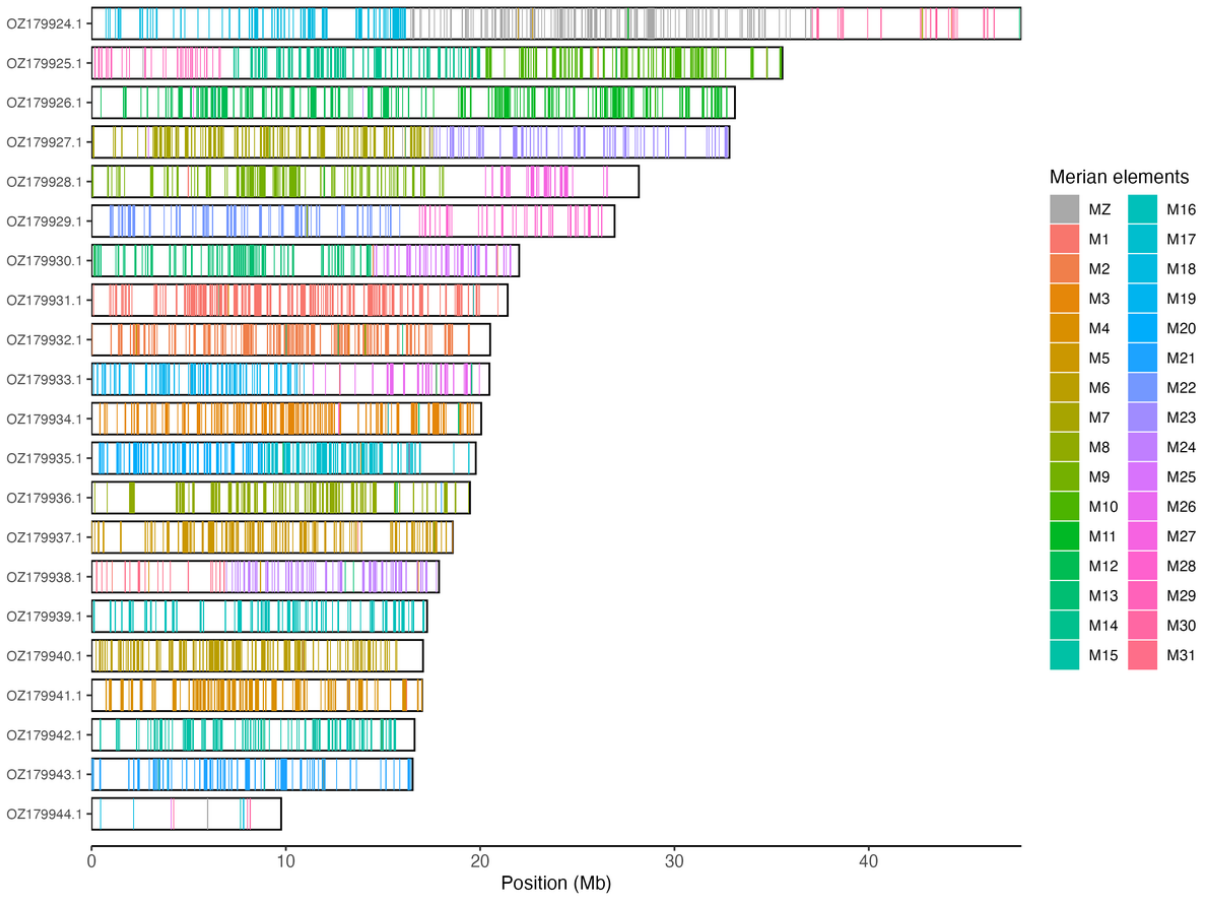

Figure 3. Merian elements painted across chromosomes in the ilEreMelm1.hap1.1 assembly of *Erebia melampus*. Chromosomes are drawn to scale, with the positions of orthologues shown as coloured bars. Each orthologue is coloured by the Merian element that it belongs to. All orthologues which could be assigned to Merian elements are shown.

is 83.58% for haplotype 1, 75.19% for haplotype 2, and 99.23% for the combined haplotypes (Figure 4). BUSCO analysis using the lepidoptera_odb10 reference set (n = 5 286) (Kriventseva *et al.*, 2019) identified 98.6% of the expected gene set (single = 98.0%, duplicated = 0.5%) for haplotype 1. The snail plot in Figure 5 summarises the scaffold length

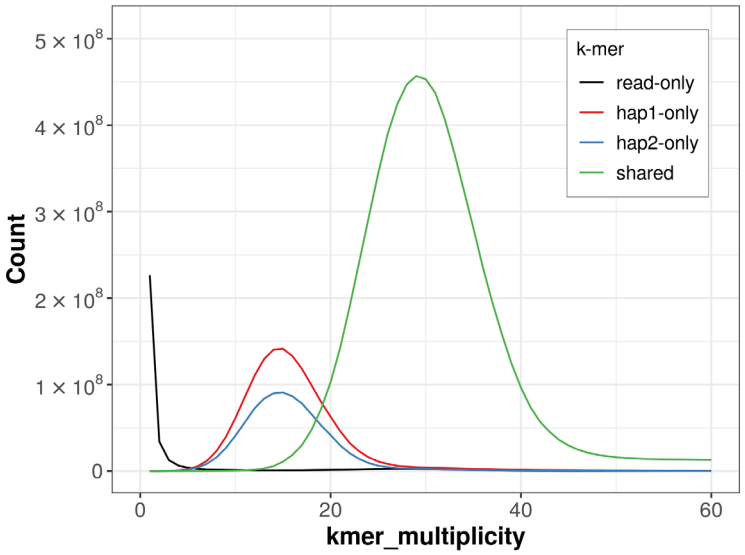

Figure 4. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers 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.

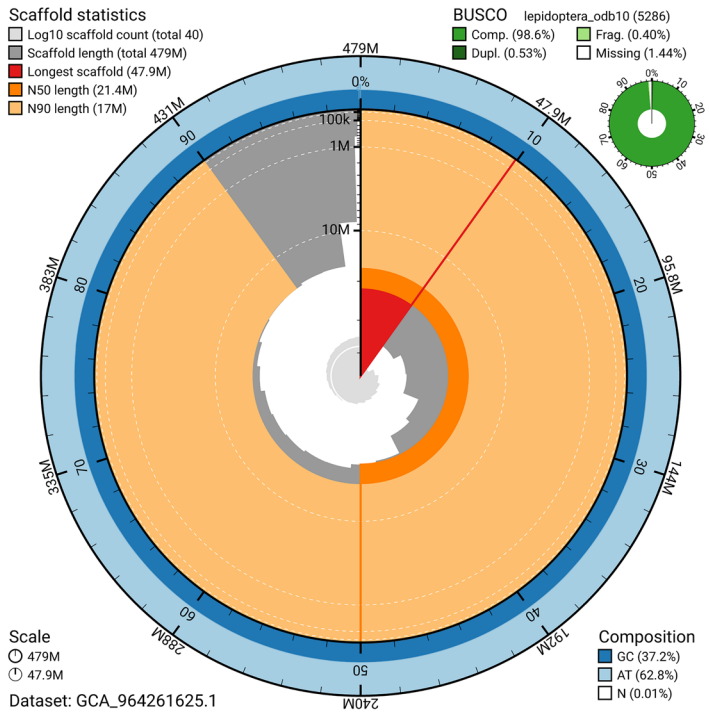

Figure 5. Assembly metrics for ilEreMelm1.hap1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. The outermost 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.

distribution and other assembly statistics for haplotype 1. The blob plot in Figure 6 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.Q66**, 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

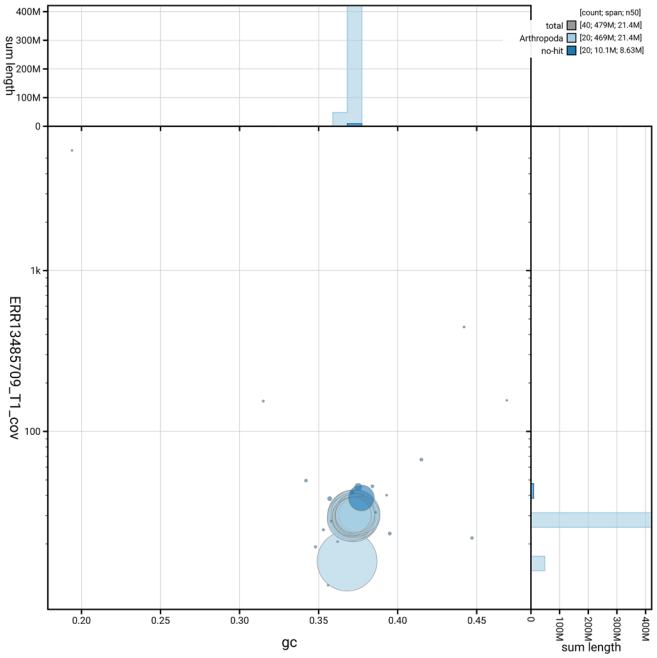

Figure 6. BlobToolKit GC-coverage plot for ilEreMelm1.hap1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes 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 *Erebia melampus* assembly.

Measure	Value	Benchmark
EBP summary (haplotype 1)	6.C.Q66	6.C.Q40
Contig N50 length	5.65 Mb	≥ 1 Mb
Scaffold N50 length	21.41 Mb	= chromosome N50
Consensus quality (QV)	Haplotype 1: 66.2; haplotype 2: 65.5; combined: 65.8	≥ 40
k-mer completeness	Haplotype 1: 83.58%; Haplotype 2: 75.19%; combined: 99.23%	≥ 95%
BUSCO	C:98.6%[S:98.0%,D:0.5%], F:0.4%,M:1.0%,n:5 286	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.70%	≥ 90%

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: Erebia melampus (lesser mountain ringlet). Accession number PRJEB78676. The genome sequence is released openly for reuse. The *Erebia melampus* 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:

- Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team
- Wellcome Sanger Institute Scientific Operations – Sequencing Operations
- Wellcome Sanger Institute Tree of Life Core Informatics team
- Tree of Life Core Informatics collective
- 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
GoaT CLI	0.2.5	https://github.com/genomehubs/goat-cli
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	1.1.1	https://github.com/thegenemyers/MERQURY.FK
Minimap2	2.24-r1122	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

Software	Version	Source
Nextflow	23.10.0	https://github.com/nextflow-io/nextflow
PretextSnapshot	N/A	https://github.com/sanger-tol/PretextSnapshot
PretextView	0.2.5	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
Seqtk	1.3	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.2.0	https://github.com/sanger-tol/treeval
YaHS	1.2a.2	https://github.com/c-zhou/yahs

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 22 September 2025

https://doi.org/10.21956/wellcomeopenres.27174.r131605

✔ **Ricardo Rodriguez-de-la-vega**
CNRS, Université Paris-Saclay, Gif-sur-Yvette, France

In this Psyche project data note, the authors present a reference-standard, haplotype-resolved genome assembly of *Erabia melampus*, a brush-footed butterfly endemic to the Alps. As with the other genomes released by the project (77 as of September 20, 2025), the assembly combines high-quality HiFi reads with long-range Hi-C data. The hifiasm assemblies are excellent in terms of contiguity (the 424 Mb alternative haplotype assembled into just 164 scaffolds) and completeness (very few 31-mers exclusive to the reads).

The presentation is well structured, the background offers a good primer to the species' ecology and the figures and tables adequately represent the underlying data. Readers are left with no explanation of what is the 8.4 Mb scaffold not assigned to arthropoda, but that's always the case in genome notes. I warmly welcome the inclusion of the Merian elements; even though there is understandably no room here for detailed discussion, it would have been interesting to place these findings in a comparative evolutionary genomics context.

As far as I can tell, there's no RNAseq available yet. So I don't think the disclaimer "The genome will be ... at the European Bioinformatics Institute" in the "Data availability" section is justified here.

I leave here the link to the GoaT link of the genome, as I think is a good entry point to access the numerous underlying data analysis.

https://goat.genomehubs.org/record?recordId=1660630&result=taxon&taxonomy=ncbi#Erebia%20melampus

I couldn't find a quality control page like the ones produced by the Darwin Tree of Life.

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

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genomics, Bioinformatics, Evolution

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 09 September 2025

https://doi.org/10.21956/wellcomeopenres.27174.r131608

Laurence Despres
[1] Universite Savoie Mont Blanc, Chambéry, France
[2] Université Grenoble Alpes, Laboratory of Alpine Ecology (Ringgold ID: 56837), Grenoble, Auvergne-Rhône-Alpes, France

The authors report on the chromosome-level, haplotype-resolved genome sequence of a female specimen of the Lesser Mountain Ringlet *Erebia melampus* (Nymphalidae). The final assembly includes 21 chromosomes : 19 autosomes and both sex chromosomes Z and W. The mitogenome was also assembled. The quality of the assembly was further assessed by the proportion of complete BUSCO genes recovered, which is very high (>98% of Lepidoptera database BUSCO genes).
The genome assembly is of good quality and was constructed using appropriate methods : Pac Bio HiFi long reads (29-fold coverage) and Hi-C data to confirm scaffolding, following the high standard of the Darwin Tree of Life Project for results presentation.
Minor comment :
A word (« diversified ») is missing in this sentence :
....its genome will provide invaluable opportunities to study genome evolution in one of the most karyological groups of butterflies (Augustijnen *et al.*, 2024).

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 genomics

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.