



DATA NOTE

The genome sequence of the Map, *Araschnia levana* (Linnaeus, 1758) (Lepidoptera: Nymphalidae)

[version 1; peer review: 3 approved]

Mathieu Joron ¹, Kay Lucek ², Charlotte J. Wright ³, Joana I. Meier³, Mark L. Blaxter ³,
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

¹CEFE CNRS, Montpellier, France

²University of Neuchâtel, Neuchâtel, Switzerland

³Tree of Life, Wellcome Sanger Institute, Hinxton, England, UK

V1 First published: 31 Jul 2025, 10:398
<https://doi.org/10.12688/wellcomeopenres.24633.1>
Latest published: 31 Jul 2025, 10:398
<https://doi.org/10.12688/wellcomeopenres.24633.1>

Abstract

We present a genome assembly from a female specimen of *Araschnia levana* (Map; Arthropoda; Insecta; Lepidoptera; Nymphalidae). The assembly contains two haplotypes with total lengths of 362.54 megabases and 332.31 megabases. Most of haplotype 1 (99.09%) is scaffolded into 32 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 15.96 kilobases.

Keywords

Araschnia levana, Map, genome sequence, chromosomal, Lepidoptera



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

Open Peer Review

Approval Status   

	1	2	3
version 1			
31 Jul 2025	view	view	view

1. **Gulab D Khedkar** , Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India
2. **Martijn Derks**, Wageningen University & Research, Wageningen, The Netherlands
3. **Rachel A Steward** , Lund University, Lund, Sweden

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

Corresponding author: Charlotte J. Wright (cw22@sanger.ac.uk)

Author roles: **Joron M:** Writing – Original Draft Preparation; **Lucek K:** Resources, Supervision; **Wright CJ:** Funding Acquisition, Project Administration, Supervision; **Meier JI:** Funding Acquisition, Project Administration, Supervision; **Blaxter ML:** Funding Acquisition, Project Administration, Supervision;

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Wellcome through core funding to the Wellcome Sanger Institute (220540). KL was supported by the Swiss National Science Foundation (SNSF) Grant ID 202869.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Joron M, Lucek K, Wright CJ *et al.* **The genome sequence of the Map, *Araschnia levana* (Linnaeus, 1758) (Lepidoptera: Nymphalidae) [version 1; peer review: 3 approved]** Wellcome Open Research 2025, 10:398 <https://doi.org/10.12688/wellcomeopenres.24633.1>

First published: 31 Jul 2025, 10:398 <https://doi.org/10.12688/wellcomeopenres.24633.1>

Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphimesenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Papilionoidea; Nymphalidae; Nymphalinae; Nymphalini; *Araschnia*; *Araschnia levana* (Linnaeus, 1758) (NCBI:txid171575).

Background

Araschnia levana Linnaeus, 1758, known as the map butterfly due to its underside wing pattern, is a nymphalid butterfly in a small predominantly East Asian genus (Fric *et al.*, 2004). *A. levana* has a range extending across the Palaearctic from North Japan to the Atlantic (Bozano & Floriani, 2012). In Europe, it thrives in regions where summers are warm but not dry, effectively excluding the Mediterranean area in the south, and most of the British and Irish Isles and Atlantic Scandinavia in the northwest. However, it has significantly expanded northward recently (Audusseau *et al.*, 2017; Widenfalk *et al.*, 2014) and is listed as Least Concern with an increasing trend by the IUCN (Van Swaay *et al.*, 2010).

Araschnia levana thrives in lowland, wooded countryside with an abundance of stinging nettle (*Urtica dioica* L.), its main food plant. An interesting behaviour is the unique mode of egg-laying of females, who stack eggs on top of each other in a neat pile under the host leaf (García-Barros & Fartmann, 2009). Caterpillars are gregarious but do not spin a silk nest. They are black with red-brown spines (scoli) and two prominent spines on their head capsule.

Individuals overwinter as pupae. *A. levana* produces two to three generations per year, and is famous for its striking seasonal polyphenism. Spring adults, emerging from overwintering pupae, display orange wings with black spots, while summer adults, emerging from direct development of pupae, are black with a white band. This makes it an iconic case of seasonal polyphenism (Baudach & Vilcinskis, 2021; Windig & Lammar, 1999). While the hormonal response to photoperiod that triggers this phenotypic variation is well studied (Koch & Buckmann, 1987), the evolutionary forces driving the wing pattern polyphenism are not entirely clear. Hypotheses include developmental and nutritional trade-offs (Friborg & Karlsson, 2010; Morehouse *et al.*, 2013; Windig, 1999), crypsis or mimicry (Ihalainen & Lindstedt, 2012; Joiris *et al.*, 2010), thermal regulation (Windig & Lammar, 1999), or links with immunity (Baudach & Vilcinskis, 2021; Freitak *et al.*, 2019; Vilcinskis & Vogel, 2016). Recent research into the genetic mechanisms underlying polyphenism has documented the differences in gene and miRNA expression between long-day and short-day forms (Mukherjee *et al.*, 2020; Vilcinskis & Vogel, 2016), providing new insight into functional differences between the two forms.

This reference genome for *A. levana* will be invaluable for furthering our understanding of these genetic and evolutionary processes underlying seasonal polyphenism, wing patterning, and voltinism. The sequence data was derived from a female

specimen (Figure 1) collected from Forêt de Jorat, Twann-Tüscherz, Bern, Switzerland.

Methods

Sample acquisition and DNA barcoding

The specimen used for genome sequencing was an adult female *Araschnia levana* (specimen ID SAN28000060, ToLID ilAraLeval; Figure 1), collected from Forêt de Jorat, Twann-Tüscherz, Bern, Switzerland (latitude 47.1211, longitude 7.2039; elevation 516 m) on 29/05/2023.

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 ilAraLeval 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 31.41 ng/μL and a yield of 1 476.27 ng, with a fragment size of 13.4 kb. The 260/280 spectrophotometric ratio was 2.02, and the 260/230 ratio was 3.05.



Figure 1. Voucher photograph of the *Araschnia levana* (ilAraLeval) 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), 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 ilAraLeva1 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 flag to handle potential misassemblies. The scaffolded assemblies were evaluated using Gfa-stats (Formenti *et al.*, 2022), BUSCO (Manni *et al.*, 2021) and MERQUERY.FK (Rhie *et al.*, 2020).

Table 1. Specimen and sequencing data for BioProject PRJEB78668.

Platform	PacBio HiFi	Hi-C
ToLID	ilAraLeva1	ilAraLeva1
Specimen ID	SAN28000060	SAN28000060
BioSample (source individual)	SAMEA115109736	SAMEA115109736
BioSample (tissue)	SAMEA115109761	SAMEA115109771
Tissue	thorax	head
Sequencing platform and model	Revio	Illumina NovaSeq X
Run accessions	ERR13485705	ERR13474170
Read count total	1.75 million	751.79 million
Base count total	18.32 Gb	113.52 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 17 breaks, 53 joins, and removal of 57 haplotypic duplications. The curation process is documented 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

Chromosomal painting was performed using lep_buscoPainter 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 Merquy.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 *Araschnia levana* was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 18.32 Gb (gigabases) from 1.75 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 350.24 Mb, with a heterozygosity of 0.48% and repeat content of 17.74%. These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 51× coverage. Hi-C sequencing produced 113.52 Gb from 751.79 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 362.54 Mb in 111 scaffolds, with 85 gaps, and a scaffold N50 of 12.61 Mb (Table 2).

Most of the assembly sequence (99.09%) was assigned to 32 chromosomal-level scaffolds, representing 30 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.

Table 2. Genome assembly statistics.

Assembly name	ilAraLeva1.hap1.1	ilAraLeva1.hap2.1
Assembly accession	GCA_964258735.1	GCA_964258705.1
Assembly level	chromosome	scaffold
Span (Mb)	362.54	332.31
Number of chromosomes	32	N/A
Number of contigs	196	161
Contig N50	5.14 Mb	5.54 Mb
Number of scaffolds	111	95
Scaffold N50	12.61 Mb	12.33 Mb
Longest scaffold length (Mb)	17.03	N/A
Sex chromosomes	W and Z	N/A
Organelles	Mitochondrial genome: 15.96 kb	N/A

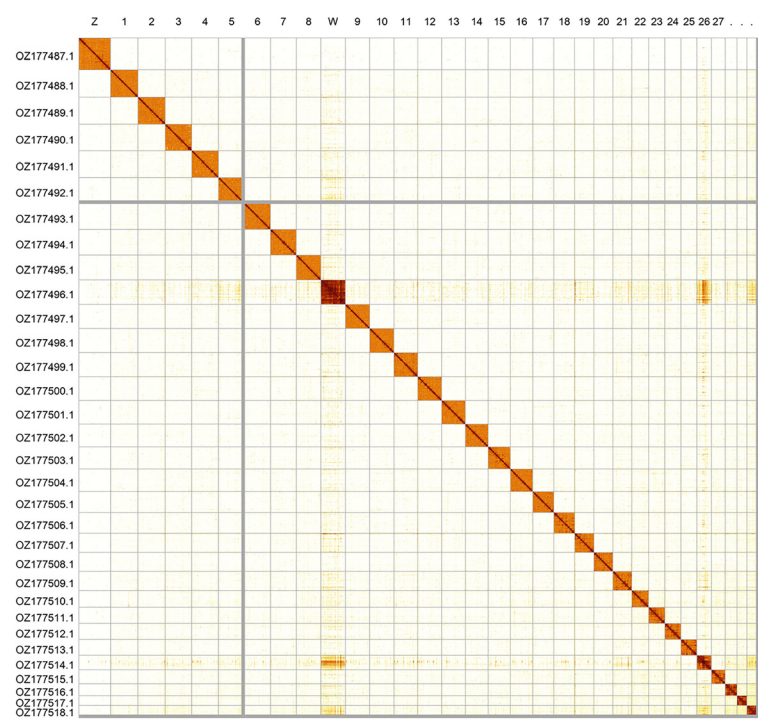


Figure 2. Hi-C contact map of the *Araschnia levana* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Araschnia levana* ilAraLeva1.

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ177488.1	1	14.57	32.50	M2
OZ177489.1	2	14.46	33	M1
OZ177490.1	3	14.20	33	M17;M20
OZ177491.1	4	14.16	33	M8
OZ177492.1	5	13.95	32.50	M21
OZ177493.1	6	13.76	32.50	M9
OZ177494.1	7	13.76	33	M3
OZ177495.1	8	13.07	33	M7
OZ177497.1	9	12.97	33	M12
OZ177498.1	10	12.86	33	M5
OZ177499.1	11	12.73	33.50	M6
OZ177500.1	12	12.73	33	M18
OZ177501.1	13	12.61	32.50	M16
OZ177502.1	14	12.16	33	M4
OZ177503.1	15	11.84	33	M15

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ177504.1	16	11.83	33	M22
OZ177505.1	17	11.24	33	M10
OZ177506.1	18	11.14	33.50	M11
OZ177507.1	19	10.24	34	M14
OZ177508.1	20	10.09	33	M13
OZ177509.1	21	10.06	33.50	M23
OZ177510.1	22	8.94	33	M26
OZ177511.1	23	8.67	33.50	M24
OZ177512.1	24	8.63	34	M19
OZ177513.1	25	8.41	33.50	M28
OZ177514.1	26	7.72	37.50	M30
OZ177515.1	27	7.34	33.50	M27
OZ177516.1	28	6.33	34.50	M25
OZ177517.1	29	5.31	34.50	M29
OZ177518.1	30	4.94	35.50	M31
OZ177496.1	W	13.05	38	N/A
OZ177487.1	Z	17.03	32.50	MZ

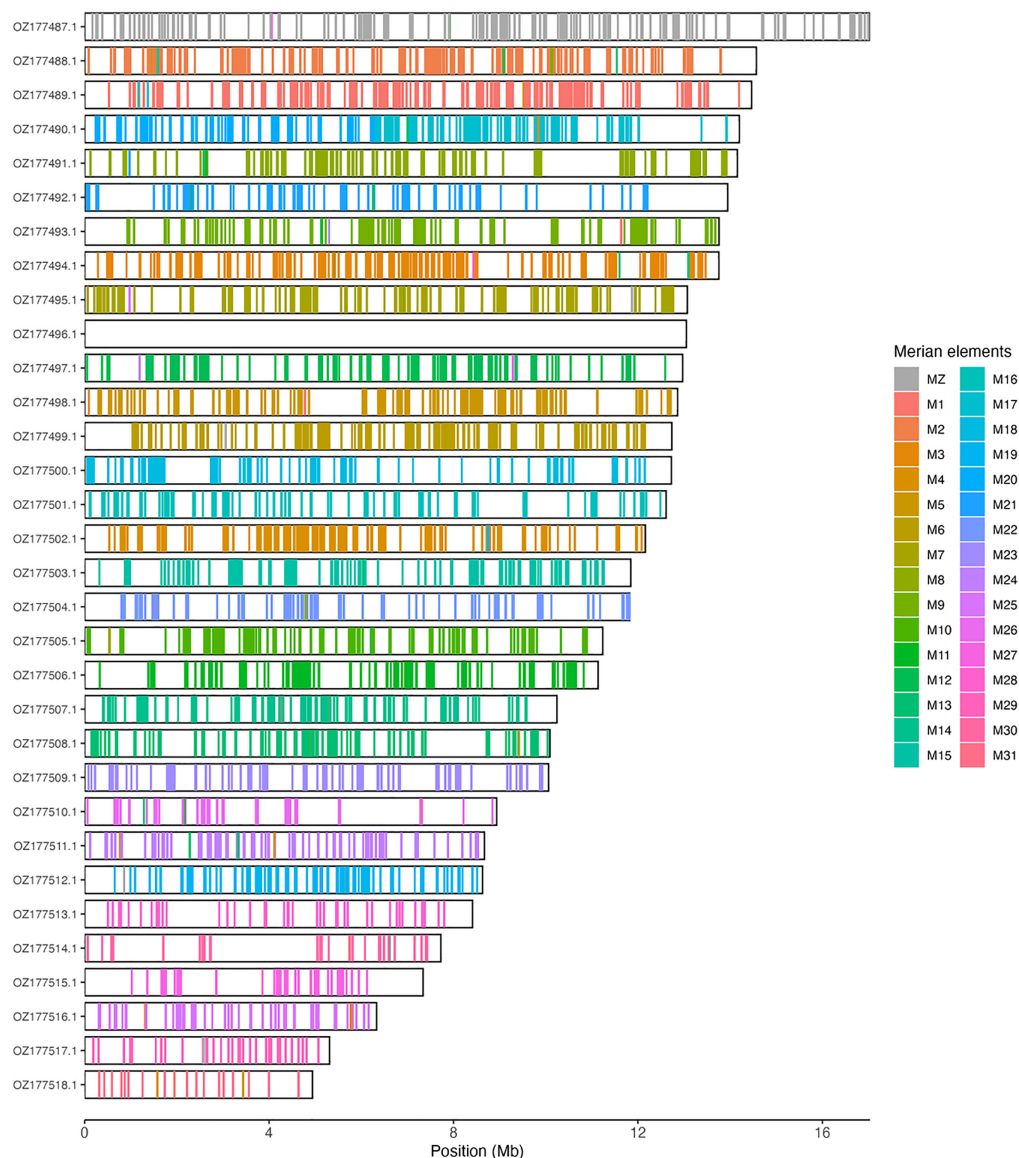


Figure 3. Merian elements painted across chromosomes in the *ilAraLeva1.hap1.1* assembly of *Araschnia levana*. Chromosomes are drawn to scale, with the positions of orthologues shown as coloured bars. Each orthologue is coloured by the Merian element that it belongs to. All orthologues which could be assigned to Merian elements are shown.

Assembly quality metrics

For haplotype 1, the estimated QV is 62.2, and for haplotype 2, 62.6. When the two haplotypes are combined, the assembly achieves an estimated QV of 62.4. The *k*-mer completeness is 89.98% for haplotype 1, 85.12% for haplotype 2, and 99.69% for the combined haplotypes (Figure 4). BUSCO analysis using the *lepidoptera_odb10* reference set (*n* = 5 286) (Kriventseva *et al.*, 2019) identified 98.8% of the expected gene set (single = 98.5%, duplicated = 0.2%) for haplotype 1. The snail plot in Figure 5 summarises the scaffold length 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.Q62**, 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

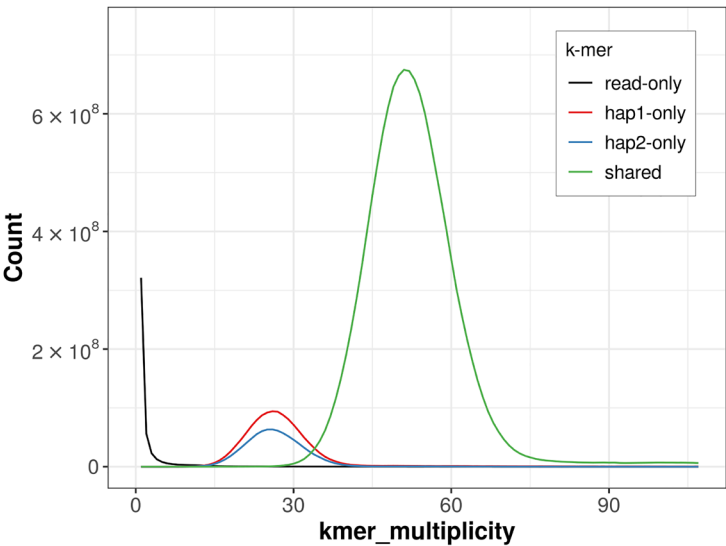


Figure 4. Evaluation of *k*-mer completeness using MerquyFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers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

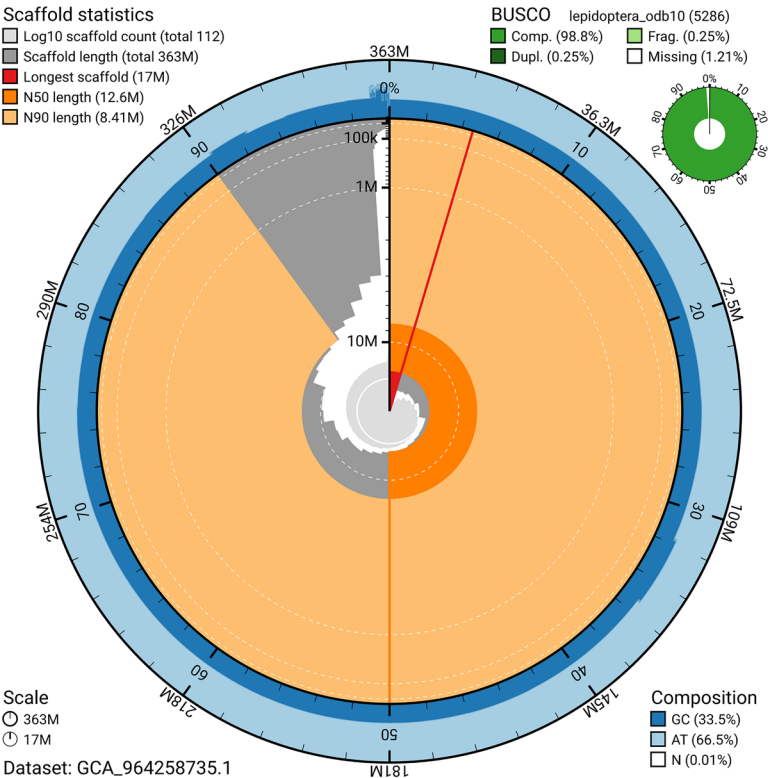


Figure 5. Assembly metrics for iAraLeva1.hap1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. The outermost 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](#).

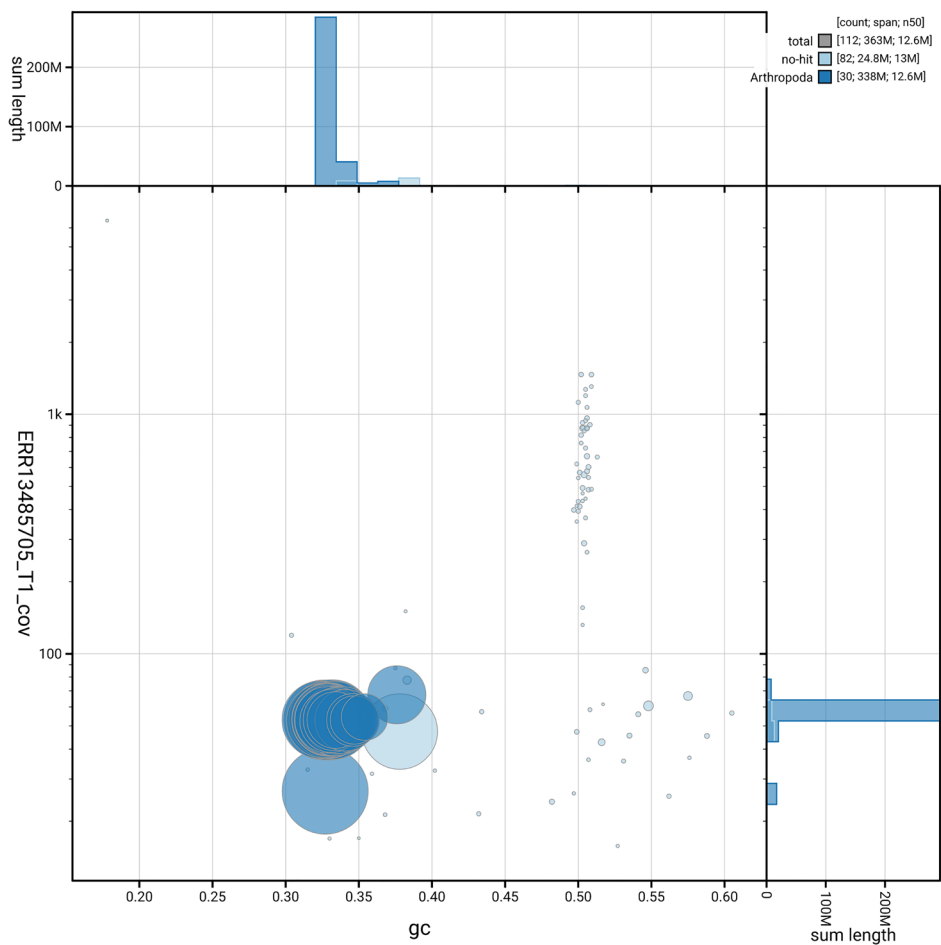


Figure 6. BlobToolKit GC-coverage plot for ilAraLeva1.hap1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes 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 *Araschnia levana* assembly.

Measure (Benchmark)	Value
EBP summary (haplotype 1)	6.C.Q62
Contig N50 length (≥ 1 Mb)	5.14 Mb
Scaffold N50 length (= chromosome N50)	12.61 Mb
Consensus quality (QV) (≥ 40)	Haplotype 1: 62.2; haplotype 2: 62.6; combined: 62.4
<i>k</i> -mer completeness (≥ 95%)	Haplotype 1: 89.98%; Haplotype 2: 85.12%; combined: 99.69%
BUSCO* (S > 90%; D < 5%)	C:98.8%[S:98.5%,D:0.2%],F:0.2%,M:1.0%,n:5286
Percentage of assembly assigned to chromosomes (≥ 90%)	99.09%

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: *Araschnia levana* (map butterfly). Accession number PRJEB78668; <https://identifiers.org/ena.embl/PRJEB78668>. The genome sequence is released openly for reuse. The *Araschnia levana* 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_buscoPainter	1.0.0	https://github.com/charlottewright/lep_buscoPainter
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

Software	Version	Source
MultiQC	1.14; 1.17 and 1.18	https://github.com/MultiQC/MultiQC
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 01 September 2025

<https://doi.org/10.21956/wellcomeopenres.27147.r129547>

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Rachel A Steward 

Lund University, Lund, Sweden

The authors present the genome of the Map butterfly, *Araschnia levana*. As the authors eloquently explain, this genome will be a valuable resource for the study of seasonal polyphenism and voltinism, especially in the context of climate-driven range expansions.

The genome, sequenced from a female sample from Bern, Switzerland, is highly complete, comprising 30 autosomes and both Z and W sex chromosomes, with nearly 99% complete Lepidopteran single copy orthologs. Similar to other Nymphalinae butterfly genomes published by Wellcome Open Research, painting with conserved Lepidopteran linkage groups suggest that the map genome has experienced very few chromosomal rearrangements (excluding ancient M17 M21 fusion).

The Genome Note presents a comprehensive overview of the sequenced genome, but does not present any annotation of gene features. Rather, the authors note that the genome will be annotated through Ensembl at EBI.

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: gene expression, alternative splicing, plant insect interactions, host use plasticity, seasonal polyphenisms

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 01 September 2025

<https://doi.org/10.21956/wellcomeopenres.27147.r129546>

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Martijn Derks

Wageningen University & Research, Wageningen, Gelderland, The Netherlands

This study presents a high-quality, chromosome-level genome assembly for the map butterfly *Araschnia levana*. The final haplotype 1 assembly spans 362.54 Mb with 99.09% placed into 32 chromosomal pseudomolecules, including the W and Z sex chromosomes. Haplotype 2 was assembled to scaffold level, and the mitochondrial genome was also reconstructed. The assembly has high completeness (98.8% BUSCO) and accuracy (QV ~62) with low levels of duplication. This reference genome will be valuable for studying seasonal polyphenism, wing patterning, and evolutionary processes in butterflies.

One thing the authors could potentially look into is also if there are large structural variants between the haplotypes?

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

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 30 August 2025

<https://doi.org/10.21956/wellcomeopenres.27147.r128418>

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Gulab D Khedkar 

Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India

Review report on "The genome sequence of the Map, *Araschnia levana* (Linnaeus, 1758) (Lepidoptera: Nymphalidae)"

This is a thoughtful and valuable genome-sequencing work that provides a high-quality, chromosome-level reference assembly for the Map butterfly, *Araschnia levana*. The authors have carefully executed research, resulting in an important resource that will be highly useful for the scientific community, particularly for researchers studying seasonal polyphenism and chromosomal evolution.

Here is a review of the preprint, highlighting its strengths and offering some suggestions for improvement.

Review Outcome Strengths

1. High-quality assembly: The assembly, particularly Haplotype 1, is of excellent quality. The use of long-read technology for initial assembly and Hi-C data for scaffolding to the chromosome level is a reliable approach. The assembly statistics, including a high scaffold N50 (12.61 Mb) and a high percentage of the assembly assigned to chromosomes (99.09%), demonstrate a near-complete representation of the genome.
2. Comprehensive methods: The methods section is thorough and transparent. They cite the protocols used, which may aid in reproducibility. The use of a female specimen allowed for the identification of both W and Z sex chromosomes, which is an important contribution.
3. Data quality assessment: The assembly meets the Earth BioGenome Project (EBP) standards, with a summary metric of 6.C.Q62, denoting the high quality of the data.
4. Relevance and context: The background section effectively establishes the biological significance of the *A. levana* genome. The seasonal polyphenism of this butterfly is a classic and fascinating case study, and the authors make an argument for how this genome will facilitate future research into the genetic basis of this trait.
5. Clear visualizations: Figures 2, 3, 4, 5, and 6 are highly effective. The Hi-C contact map (Figure 2) and the Merian element painting (Figure 3) provide clear visual evidence of the successful chromosome-level scaffolding and a valuable evolutionary context. The Snail and BlobToolKit plots (Figures 5 and 6) are standard and informative, effectively summarizing key assembly metrics and quality.
6. Open data and reproducibility: The article is open access, and the authors have made all

raw data, assemblies, and software pipelines publicly available, which is commendable and fully aligns with FAIR data principles.

Suggestive inputs for improvement

While the preprint is already of a very high standard, a few additions or clarifications could further enhance its value.

1. *Expanded abstract*: The current abstract is very concise. It would be beneficial to slightly expand it to include the key quality metrics (e.g., BUSCO score, N50, and EBP metric). This would give readers a more complete picture of the assembly's quality at a glance. For example, explicitly stating that the assembly is "chromosome-level and meets Earth BioGenome Project standards" would be a powerful summary statement.
2. *Interpretation of merian element painting*: The Merian element painting (Figure 3) is a great addition, as it shows the conservation of ancient lepidopteran linkage groups. A brief discussion of the results in the main text would be helpful. For example, are any of the identified fusions or fissions novel or consistent with other closely related Nymphalidae species? Highlighting specific observations, such as the fusion of M17 and M20 on chromosome 3, and M1 and M2 on chromosomes 1 and 2, could provide deeper insights into the chromosomal evolution of *A. levana*.
3. *Comparison with other Nymphalidae genomes*: The authors mention that *A. levana* is in the Nymphalidae family. A brief comparison of key genomic features (e.g., genome size, chromosome number) with other sequenced Nymphalidae genomes (e.g., *Heliconius*, *Danaus plexippus*) would provide valuable context. This would help position this new assembly within the broader evolutionary landscape of butterflies.
4. *Heterozygosity and haplotype assembly*: The authors assembled two haplotypes. While Haplotype 1 is well-curated, Haplotype 2 is only at the scaffold level. The reasons for this disparity could be briefly mentioned. For instance, were there technical challenges with Haplotype 2? A more detailed explanation of why one haplotype was chosen for the chromosome-level curation over the other would be a good addition. The authors could also provide more detail on how the 0.48% heterozygosity estimate from GenomeScope2.0 influenced the assembly strategy and quality.
5. *Future directions*: While the authors state that the genome will be invaluable for studying polyphenism, a concluding sentence or short paragraph outlining a few specific research questions that could be addressed with this genome would be a strong finish. For example: "This genome will be critical for identifying candidate genes involved in the photoperiodic and hormonal pathways that regulate seasonal polyphenism, as well as for comparative genomic studies across other polyphenic Nymphalidae species." This would further underscore the article's impact and value.

Overall, a good piece of work is depicted in this preprint. The suggestions are minor and primarily aimed at enriching the context and interpretation of the already robust data. This reference genome is a significant contribution to lepidopteran genomics and more specifically, the emerging area of biodiversity genomics.

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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?

Partly

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

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biodiversity genomics, evolutionary biology and 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.
