DATA NOTE

The genome sequence of the Camberwell Beauty, *Nymphalis antiopa* (Linnaeus, 1758) (Lepidoptera: Nymphalidae)

[version 1; peer review: 3 approved]

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Abstract

We present a genome assembly from a male specimen of *Nymphalis antiopa* (Camberwell Beauty; Arthropoda; Insecta; Lepidoptera; Nymphalidae). The assembly contains two haplotypes with total lengths of 392.57 megabases and 391.53 megabases. Most of haplotype 1 (99.42%) is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. Haplotype 2 was assembled to scaffold level. The mitochondrial genome has also been assembled, with a length of 15.26 kilobases.

Keywords

Nymphalis antiopa, Camberwell Beauty, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓ ✓

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Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Papilionoidea; Nymphalidae; Nymphalinae; Nymphalini; *Nymphalis*; *Euvanessa*; *Nymphalis antiopa* (Linnaeus, 1758) (NCBI:txid171592)

Background

The Camberwell Beauty (UK) or Mourning Cloak (North America) is a large butterfly in the family Nymphalidae. It typically breeds in cool deciduous or mixed forests across the Holarctic, being especially common in North America and the continental climatic region of western Eurasia (GBIF Secretariat, 2023). Despite being closely related to the tortoiseshell butterflies such as *Nymphalis polychloros*, it has unique wing markings, which do not differ between the sexes. The upper side is mainly dark maroon to dark brown with thick pale-yellow borders and a row of small iridescent blue spots in between. The underside is less colourful, with grey striations, providing good camouflage for the adult, which overwinters and can live up to 12 months.

Nymphalis antiopa is univoltine and – as with several of its close relatives – uses various tree species as host plants, such as willow (*Salix*), birch (*Betula*) and poplar (*Populus*). It is a powerful flyer and, while it breeds poorly in the Atlantic climate of western Europe, in some years can be found there in large numbers, even in the UK where it has never established a population. It is classified as LC (least concern) in the IUCN red list (Van Swaay *et al.*, 2010). In North America, it is the state insect of Montana ("State Symbols USA"). There are 146 mitochondrial COI barcode sequences on the BOLD database (Ratnasingham *et al.*, 2024), from across the Holarctic but concentrated in Europe and north America, showing low genetic variation in Europe (Dapporto *et al.*, 2022). As well as the reference individual described here from Switzerland, there are four North American re-sequenced samples recorded on NCBI SRA (Clark *et al.*, 2016). This genome sequence can help in understanding the transition to using trees and woody plants as larval hosts, the timing and direction of crossing between the Palaearctic and Nearctic in a forest-adapted butterfly, and the evolution of wing colouration.

We present a chromosome-level, haplotype-resolved genome sequence of the Camberwell Beauty, *Nymphalis antiopa*, sequenced as part of Project Psyche. The sequence data were derived from a male specimen (Figure 1) collected from Euseigne VS, Switzerland.

Methods

Sample acquisition

The specimen used for genome sequencing was an adult male *Nymphalis antiopa* (specimen ID SAN28000136, ToLID ilNymAnti1; Figure 1), collected from Euseigne VS, Switzerland (latitude 46.1773, longitude 7.4159; elevation 600 m) on 26/04/2023. The specimen was collected and identified by Yannick Chittaro (Info Fauna, Neuchâtel, Switzerland).

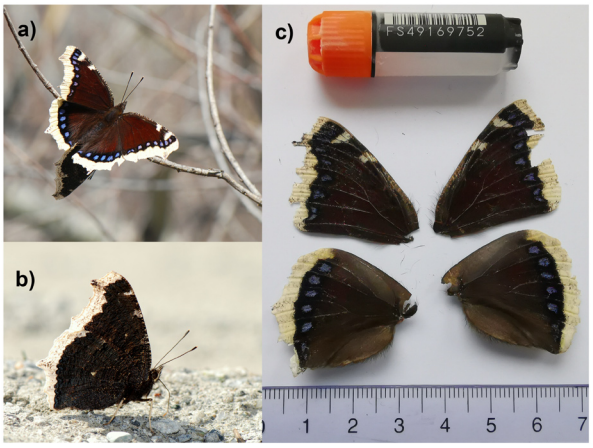

Figure 1. a) and **b**) Live specimens of *Nymphalis antiopa* photographed by Yannick Chittaro. **c**) Voucher image of the *N. antiopa* specimen used for genome sequencing (SAN28000136, ilNymAnti1).

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 ilNymAnti1 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 40.31 ng/μL and a yield of 1 894.57 ng, with a fragment size of 14.5 kb. The 260/280 spectrophotometric ratio was 1.87, and the 260/230 ratio was 2.73.

PacBio HiFi library preparation and sequencing

Library preparation and sequencing were performed at the WSI Scientific Operations core. Samples with an average fragment size greater than 8 kb and total mass exceeding 400 ng were eligible for the low-input SMRTbell Prep Kit 3.0 protocol (Pacific Biosciences, California, USA), depending on genome size and required sequencing depth. Libraries were prepared using the SMRTbell Prep Kit 3.0 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 head tissue of the ilNymAnti1 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.

Table 1. Specimen and sequencing data for BioProject.

Platform	PacBio HiFi	Hi-C
ToLID	ilNymAnti1	ilNymAnti1
Specimen ID	SAN28000136	SAN28000136
BioSample (source individual)	SAMEA115110006	SAMEA115110006
BioSample (tissue)	SAMEA115110032	SAMEA115110031
Tissue	thorax	head
Sequencing platform and model	Revio	Illumina NovaSeq X
Run accessions	ERR13605498	ERR13602161
Read count total	2.10 million	413.42 million
Base count total	20.23 Gb	62.43 Gb

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).

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 10 breaks and 22 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 *Nymphalis antiopa* was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 20.23 Gb (gigabases) from 2.10 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 389.52 Mb, with a heterozygosity of 0.53% and repeat content of 19.34%. These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 50× coverage. Hi-C sequencing produced 62.43 Gb from 413.42 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 392.57 Mb in 70 scaffolds, with 50 gaps, and a scaffold N50 of 13.9 Mb (Table 2).

Most of the assembly sequence (99.42%) was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. Chromosome Z was assigned by synteny to the genome of *Nymphalis c-album* (GCA_963151315.1). 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 63.1, and for haplotype 2, 63.7. When the two haplotypes are combined, the assembly

Table 2. Genome assembly statistics.

Assembly name	ilNymAnti1.hap1.1	ilNymAnti1.hap2.1
Assembly accession	GCA_964258955.1	GCA_964258945.1
Assembly level	chromosome	scaffold
Span (Mb)	392.57	391.53
Number of chromosomes	31	N/A
Number of contigs	120	99
Contig N50	7.03 Mb	8.62 Mb
Number of scaffolds	70	51
Scaffold N50	13.9 Mb	13.96 Mb
Longest scaffold length (Mb)	17.81	N/A
Sex chromosomes	Z	N/A
Organelles	Mitochondrial genome: 15.26 kb	N/A

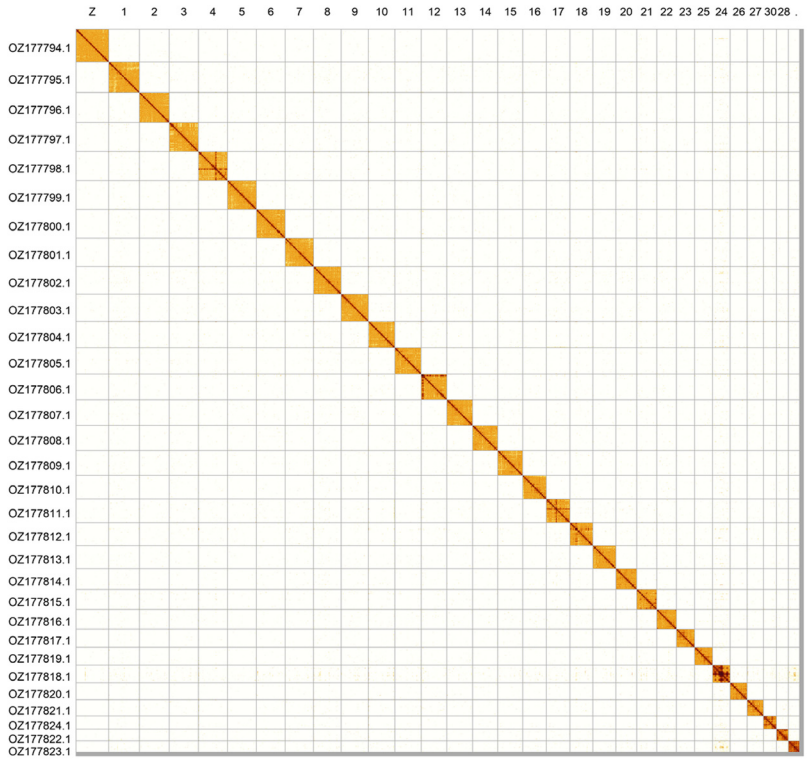

Figure 2. Hi-C contact map of the *Nymphalis antiopa* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Nymphalis antiopa* ilNymAnti1.

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ177795.1	1	16.47	34.50	M12
OZ177796.1	2	16.13	33.50	M2
OZ177797.1	3	15.76	34	M17;M20
OZ177798.1	4	15.64	34	M3
OZ177799.1	5	15.63	34	M1
OZ177800.1	6	15.49	33.50	M8
OZ177801.1	7	15.35	33.50	M9
OZ177802.1	8	14.82	34	M5
OZ177803.1	9	14.73	33.50	M7
OZ177804.1	10	14.26	34	M16
OZ177805.1	11	14.14	33.50	M18
OZ177806.1	12	13.90	33.50	M21
OZ177807.1	13	13.86	33.50	M6
OZ177808.1	14	13.54	33.50	M22
OZ177809.1	15	13.51	33.50	M4
OZ177810.1	16	12.74	33.50	M15
OZ177811.1	17	12.66	34	M11
OZ177812.1	18	12.41	34.50	M14
OZ177813.1	19	12.37	33.50	M10
OZ177814.1	20	11.30	33.50	M13
OZ177815.1	21	10.76	34	M23
OZ177816.1	22	10.57	34.50	M26
OZ177817.1	23	9.87	34	M24
OZ177818.1	24	9.61	34.50	M19
OZ177819.1	25	9.70	38.50	M30
OZ177820.1	26	9.14	34.50	M28
OZ177821.1	27	8.80	35	M27
OZ177822.1	28	7.03	35.50	M25
OZ177823.1	29	6.38	36.50	M29
OZ177824.1	30	7.40	37	M31
OZ177794.1	Z	17.81	33	MZ

achieves an estimated QV of 63.4. The *k*-mer completeness is 86.84% for haplotype 1, 86.82% for haplotype 2, and 99.77% for the combined haplotypes (Figure 4). BUSCO analysis using the lepidoptera_odb10 reference set (n = 5 286) (Kriventseva *et al.*, 2019) identified 98.7% of the expected gene set (single = 98.4%, duplicated = 0.3%) 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.

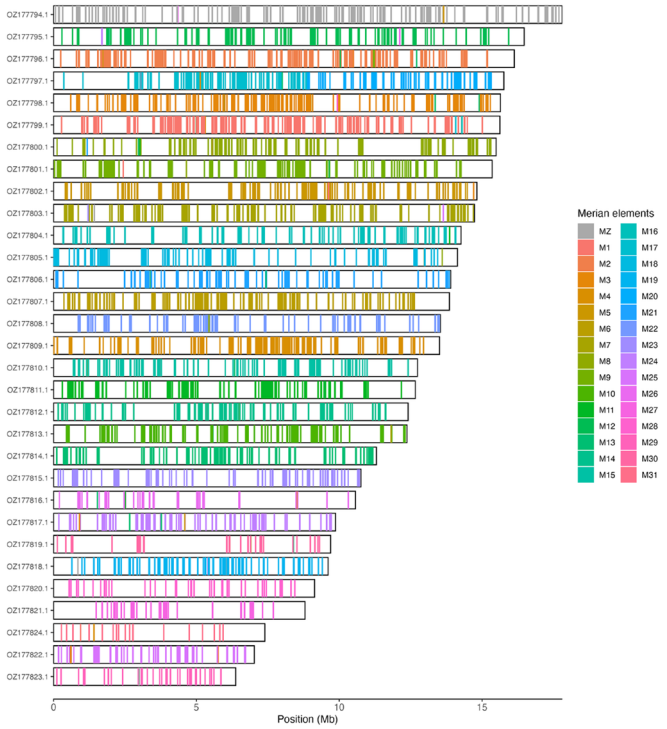

Figure 3. Merian elements painted across chromosomes in the ilNymAnti1.hap1.1 assembly of *Nymphalis antiopa*. Chromosomes are drawn to scale, with the positions of orthologues shown as coloured bars. Each orthologue is coloured by the Merian element that it belongs to. All orthologues which could be assigned to Merian elements are shown.

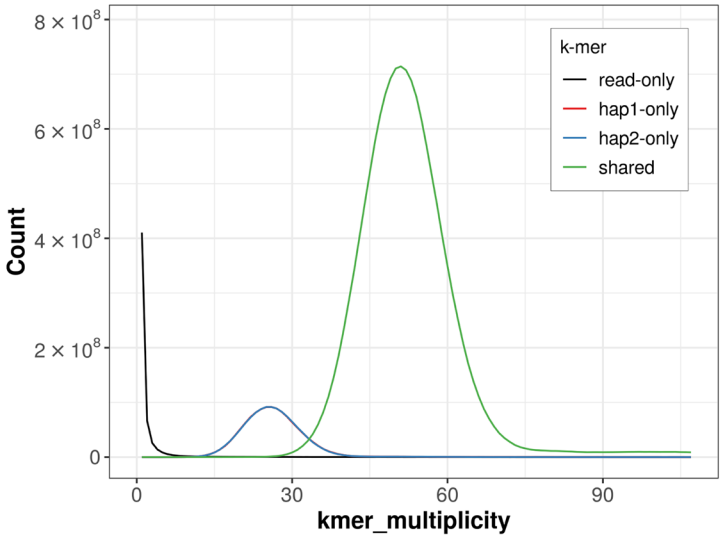

Figure 4. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers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

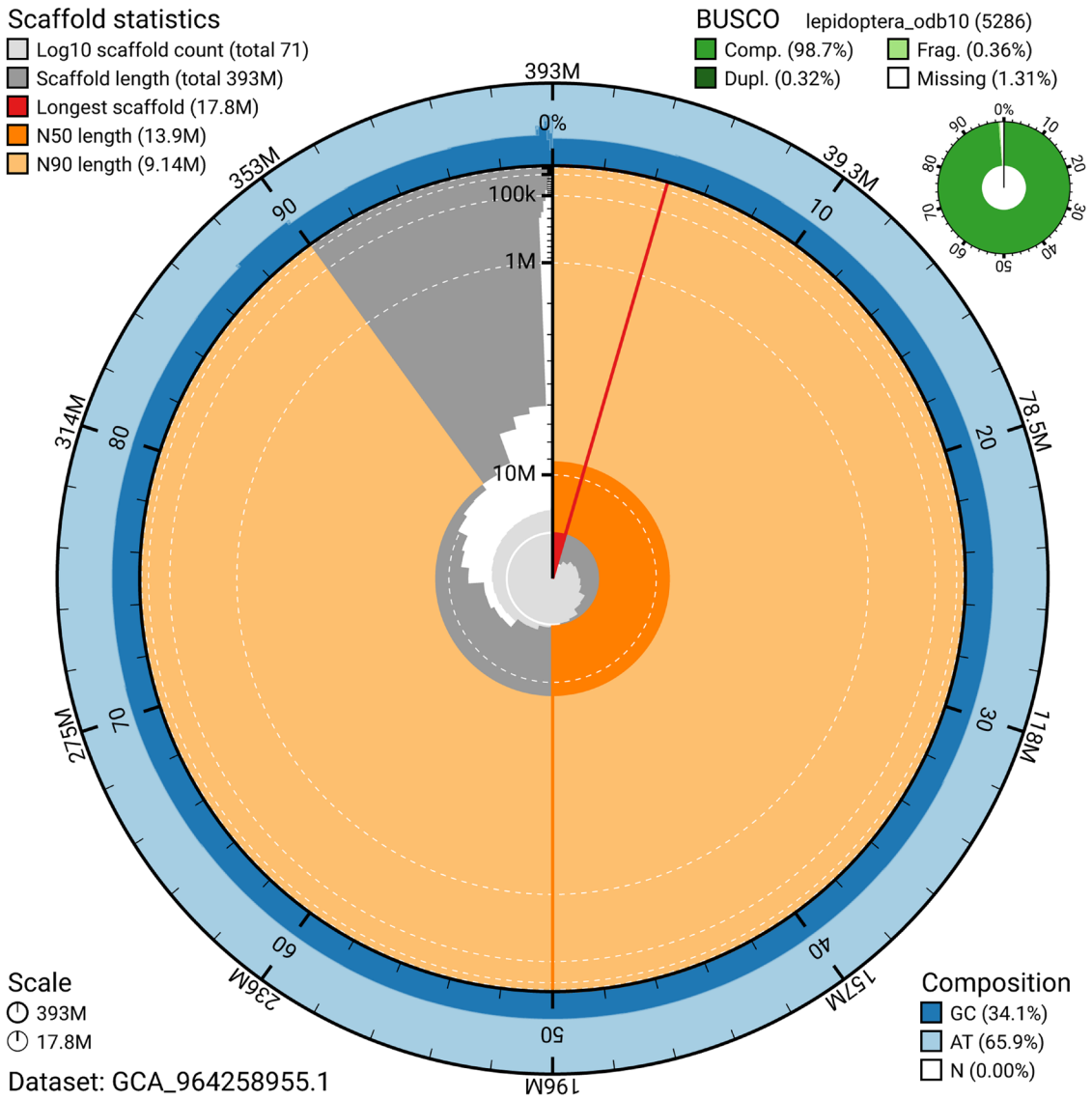

Figure 5. Assembly metrics for ilNymAnti1.hap1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. The outermost 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.

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.Q63**, meeting the recommended reference standard.

Wellcome Sanger Institute – Legal and Governance

The materials that have contributed to this genome note have been supplied by a Tree of Life collaborator. The Wellcome Sanger Institute employs a process whereby due diligence is carried out proportionate to the nature of the materials themselves, and the circumstances under which they have been/are to be collected and provided for use. The purpose of this is to address and mitigate any potential legal and/or ethical implications of receipt and use of the materials as part of the research project, and to ensure that in doing so, we align with best practice wherever possible. The overarching areas of consideration are:

- Ethical review of provenance and sourcing of the material

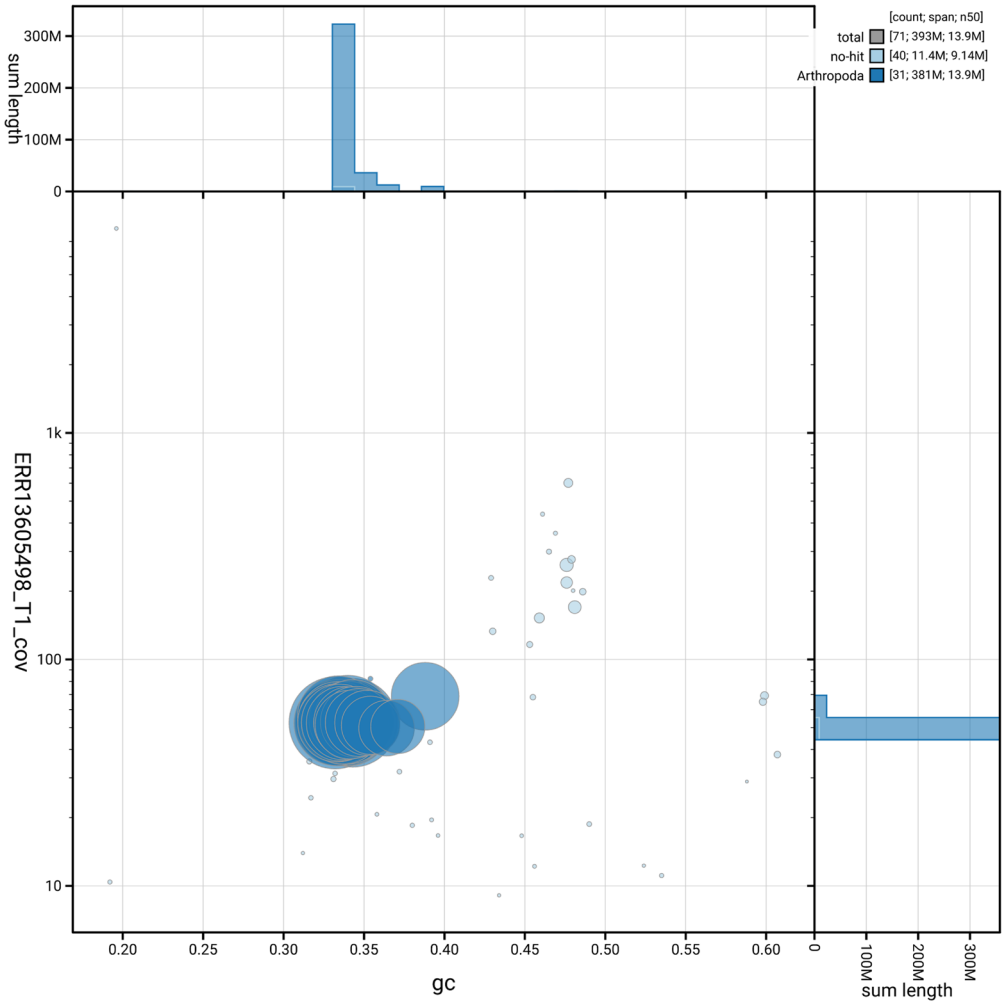

Figure 6. BlobToolKit GC-coverage plot for ilNymAnti1.hap1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes 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 *Nymphalis antiopa* assembly.

Measure	Value	Benchmark
EBP summary (haplotype 1)	6.C.Q63	6.C.Q40
Contig N50 length	7.03 Mb	≥1 Mb
Scaffold N50 length	13.90 Mb	= chromosome N50
Consensus quality (QV)	Haplotype 1: 63.1; haplotype 2: 63.7; combined: 63.4	≥ 40
k-mer completeness	Haplotype 1: 86.84%; Haplotype 2: 86.82%; combined: 99.77%	≥ 95%
BUSCO	C:98.7%[S:98.4%,D:0.3%], F:0.4%,M:0.9%,n:5 286	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.80%	≥ 90%

- 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: Nymphalis antiopa (mourning cloak). Accession number PRJEB79170. The genome sequence is released openly for reuse. The *Nymphalis antiopa* 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
MerquryFK	1.1.2	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
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

Software	Version	Source
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 08 October 2025

https://doi.org/10.21956/wellcomeopenres.27191.r132661

✔ **Panagiotis Ioannidis**
Foundation for Research & Technology - Hellas, Crete, Greece

This paper describes the genome sequencing and assembly of the lepidopteran Nymphalis antiopa.

The standard methodology the authors used resulted in a high quality genome assembly, as shown by all quality metrics.

I particularly like the Background part where the authors provided details about the genomic resources currently available for this species, as well as a brief statement about how the herein reported genome assembly could shed light in the evolution of Lepidoptera. I also liked how the authors included a figure on Merian elements (figure 3).

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: insect 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 25 September 2025

https://doi.org/10.21956/wellcomeopenres.27191.r132659

Arun Arumugaperumal
Department of Biotechnology, Rajalakshmi Engineering College, Thandalam 602105, Chennai, Tamil Nadu, India

The data note describes the genome sequence of a butterfly species named *Nymphalis antiopa*. The authors have clearly documented the photograph of the specimen in Figure 1. It will be very helpful for the readers to recognize the butterfly species. This is the first report of the high-quality genome sequence of the butterfly species. The authors have used advanced sequencing technology to obtain the genome sequence of *Nymphalis antiopa*. The assembly reported here is of size 392.57 Mb, spread among 31 chromosomes, including the Z sex chromosome. The collected specimen was a male. The genome of another Nymphalis genus member, a female *N. antiopa,* was reported to have ZW sex chromosomes. Hence, the authors could have chosen a female butterfly to get the sequence of the W chromosome. A BUSCO score of 98.7% completeness indicates that the genome sequence is a near-complete one. The genome is yet to be annotated through Ensembl. The accession IDs and links are in working condition. The article can be indexed.

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 25 September 2025

https://doi.org/10.21956/wellcomeopenres.27191.r132660

Leonardo Dapporto
Università di Firenze, Florence, Italy

In this article the authors present a genome assembly from a specimen of *Nymphalis antiopa*. To the extent of my ability to evaluate genome quality, the sequencing and assembly appear to be of high standard, consistent with other assemblies produced within the Psyche project.
However, the sequenced specimen was a male, which means that the assembly includes the Z chromosome but not the W. It would be useful if the authors clarified this point and explained why a male was chosen, since this may not be obvious to all readers. Given that the species is often common, one might expect that a female specimen could also have been available to investigate the W chromosome.
I do not see other major issues with the manuscript. It might be worth mentioning that analyses of BOLD data show that North American and Palearctic populations are separated by only a few COI mutations and are both characterised by low genetic differentiation, as reported by Dapporto et al. (2022, already cited) and D'Ercole et al. (2024) [Reference 1]

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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?
Yes

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

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Comparative hylogeography, taxonomy, ecology

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.