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DATA NOTE

The genome sequence of the Two-tailed Pasha, *Charaxes jasius* (Linnaeus, 1767) (Lepidoptera: Nymphalidae)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a female specimen of *Charaxes jasius* (Two-tailed Pasha; Arthropoda; Insecta; Lepidoptera; Nymphalidae). The assembly contains two haplotypes with total lengths of 465.23 megabases and 401.60 megabases. Most of haplotype 1 (99.91%) is scaffolded into 26 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 15.3 kilobases. This work is part of Project Psyche, a collaborative programme generating genomes for European butterflies and moths.

Keywords

Charaxes jasius; Two-tailed Pasha; genome sequence; chromosomal; Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 23 Jan 2026	✓ view	✓ view

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Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Papilionoidea; Nymphalidae; Charaxinae; Charaxini; *Charaxes*; *Charaxes jasius* (Linnaeus, 1767) (NCBI:txid658804)

Background

Charaxes jasius (Linnaeus, 1767), commonly known as the Two-tailed Pasha, is the only representative of the mainly tropical nymphalid subfamily Charaxinae in Europe (Wiemers *et al.*, 2020). Phylogenetic analyses suggest that the genus *Charaxes* originated in Africa (where most of the diversity occurs) about 45 Mya, and only a few lineages expanded into other Old-World regions (Aduse-Poku *et al.*, 2009; Müller & Wahlberg, 2010). This large and striking butterfly is a characteristic species of Mediterranean ecosystems. Its dorsal wings are dark brown with vivid orange bands, while the ventral side features a complex mosaic of reddish-brown, white, and orange markings, providing effective camouflage (García-Barros *et al.*, 2013).

Distributed across the Mediterranean Basin, North Africa, as well as the Canary Islands and Madeira (Aduse-Poku *et al.*, 2009; GBIF Secretariat, 2023), *C. jasius* thrives in warm, dry woodlands, particularly in cork oak forests where its primary larval host plant, *Arbutus unedo* (strawberry tree), is abundant. The larvae have also been recorded feeding on *Annona cherimola* (cherimoya) and *Vaccinium* × *corymbosum* (the northern highbush blueberry), among a few other bushes (García-Barros *et al.*, 2013; Molina, 2000).

The species exhibits a bivoltine life cycle, with adults emerging in two distinct generations: the first from May to June and the second from late July to October and even November. Warmer winters can lead to earlier emergences, with records as early as March in certain regions. The second generation is significantly larger due to higher larval mortality in the first generation, which overwinters and requires 6 to 8 months for development. Although *C. jasius* has the potential to be polyvoltine, cooler autumn and winter temperatures prevent a third generation from completing development (Guan *et al.*, 2020; Stefanescu, 2004). *C. jasius* has a strong dispersal ability and territorial behaviour, particularly among males that engage in hill-topping displays to compete for mates. Unlike many butterflies, adults primarily feed on fermenting fruit and tree sap rather than nectar, and are also strongly attracted to mammalian faeces (García-Barros *et al.*, 2013). *C. jasius* has a haploid chromosome number of $n = 25$ (Trentini & Marini, 1986).

C. jasius is currently listed as Least Concern (IUCN, 2024). However, new reports from northern latitudes suggest a northward expansion of the species, likely driven by climate change (Siméoni, 2018). Understanding its life cycle, genetics, and evolutionary history is crucial for conservation efforts aimed at preserving Mediterranean biodiversity. The reference genome may be useful to clarify the relationship with closely related species in Africa, until recently considered subspecies, as well as to study adaptation to the temperate climate and potential northward expansion.

We present a chromosome-level, haplotype-resolved genome sequence for *C. jasius*, sequenced as part of Project Psyche. The sequence data were derived from a female specimen (Figure 1) collected from Barcelona, Catalonia, Spain.

Methods

Sample acquisition

The specimen used for genome sequencing was an adult female *Charaxes jasius* (specimen ID SAN28000275, ToLID ilChaJasi1; Figure 1), collected from Llac De Santa Margarida, Sant Esteve De Palautordera, Vallès Oriental, Barcelona, Catalonia, Spain (latitude 41.7169, longitude 2.4155; elevation 305 m) on 2023-08-09. The specimen was collected by Roger Vila and Gerard Talavera and identified by Roger Vila.

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 ilChaJasi1 sample was weighed and triaged to determine the appropriate extraction protocol.

Tissue from the whole organism was homogenised by powermashing using a PowerMasher II tissue disruptor. HMW DNA was extracted in the WSI Scientific Operations

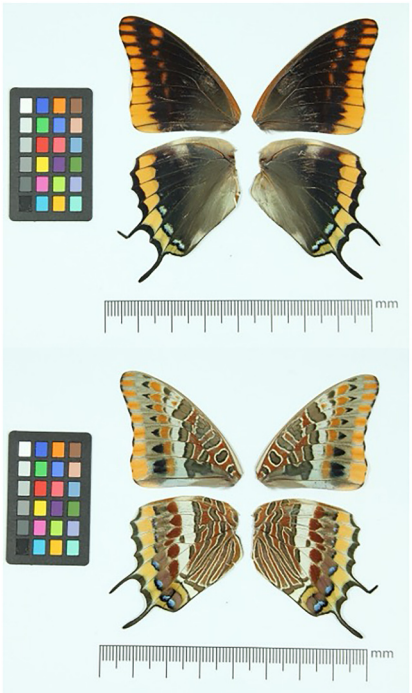

Figure 1. Voucher photographs of the *Charaxes jasius* (ilChaJasi1) specimen used for genome sequencing.

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 25.12 ng/μL and a yield of 1 180.64 ng, with a fragment size of 15.2 kb.

PacBio HiFi library preparation and sequencing

Library preparation and sequencing were performed at the WSI Scientific Operations core. Libraries were prepared using the SMRTbell Prep Kit 3.0 (Pacific Biosciences, California, USA), according to the manufacturer's instructions. The kit includes reagents for end repair/A-tailing, adapter ligation, post-ligation SMRTbell bead clean-up, and nuclease treatment. Size selection and clean-up were performed using diluted AMPure PB beads (Pacific Biosciences). DNA concentration was quantified using a Qubit Fluorometer v4.0 (ThermoFisher Scientific) and the Qubit 1X dsDNA HS assay kit. Final library fragment size was assessed with the Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies) using the gDNA 55 kb BAC analysis kit.

The sample was sequenced on a Revio instrument (Pacific Biosciences). The prepared library was normalised to 2 nM, and 15 μL was used for making complexes. Primers were annealed and polymerases bound to generate circularised complexes, following the manufacturer's instructions. Complexes were purified using 1.2X SMRTbell beads, then diluted to the Revio loading concentration (200–300 pM) and spiked with a Revio sequencing internal control. The sample was sequenced on a Revio 25M SMRT cell. The SMRT Link software (Pacific Biosciences), a web-based workflow manager, was used to configure and monitor the run and to carry out primary and secondary data analysis.

Specimen details, sequencing platforms, and data yields are summarised in Table 1.

Hi-C

Sample preparation and crosslinking

The Hi-C sample was prepared from 20–50 mg of frozen tissue from the whole organism of the ilChaJasi1 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 to create equimolar and/or weighted 2.8 nM pools. Pool concentrations were checked using the Agilent 4200 TapeStation (Agilent) with High Sensitivity D500 reagents before sequencing. Sequencing was performed using paired-end 150 bp reads on the Illumina NovaSeq X.

Table 1. Specimen and sequencing data for BioProject PRJEB81919.

Platform	PacBio HiFi	Hi-C
ToLID	ilChaJasi1	ilChaJasi1
Specimen ID	SAN28000275	SAN28000275
BioSample (source individual)	SAMEA115768776	SAMEA115768776
BioSample (tissue)	SAMEA115768848	SAMEA115768848
Tissue	whole organism	whole organism
Instrument	Revio	Illumina NovaSeq X
Run accessions	ERR13917230	ERR13925952
Read count total	1.49 million	641.71 million
Base count total	15.76 Gb	96.90 Gb

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 six breaks and 35 joins. This reduced the scaffold count by 16.5% and increased the total assembly length by 2.1%. The curation process is described at https://gitlab.com/wtsi-grit/rapid-curation. PretextSnapshot was used to generate a Hi-C contact map of the final assembly.

Assembly quality assessment

The Merqury.FK tool (Rhie *et al.*, 2020), run in a Singularity container (Kurtzer *et al.*, 2017), was used to evaluate *k*-mer completeness and assembly quality for both haplotypes using the *k*-mer database (k = 31) computed prior to genome assembly. The analysis outputs included assembly QV scores and completeness statistics.

The genome was analysed using the BlobToolKit pipeline, a Nextflow (Di Tommaso *et al.*, 2017) implementation of the earlier Snakemake version (Challis *et al.*, 2020). The pipeline aligns PacBio reads using minimap2 (Li, 2018) and SAMtools (Danecek *et al.*, 2021) to generate coverage tracks. It runs BUSCO (Manni *et al.*, 2021) using lineages identified from the NCBI Taxonomy (Schoch *et al.*, 2020). For the three domain-level lineages, BUSCO genes are aligned to the UniProt Reference Proteomes database (Bateman *et al.*, 2023) using DIAMOND blastp (Buchfink *et al.*, 2021). The genome is divided into chunks based on the density of BUSCO genes from the closest taxonomic lineage, and each chunk is aligned to the UniProt Reference Proteomes database with DIAMOND blastx. Sequences without hits are chunked using seqtk and aligned to the NT database with blastn (Altschul *et al.*, 1990). The BlobToolKit suite consolidates all outputs into a blobdir for visualisation. The BlobToolKit pipeline was developed using nf-core tooling (Ewels *et al.*, 2020) and MultiQC (Ewels *et al.*, 2016), with containerisation through Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017).

We used lep_busco_painter to paint Merian elements along chromosomes (Wright *et al.*, 2024). Merian elements represent the 32 ancestral linkage groups in Lepidoptera. The painting process utilised BUSCO gene locations from the lepidoptera_odb10 set (Kriventseva *et al.*, 2019) and chromosome lengths from NCBI Datasets. Each complete BUSCO gene (both single-copy and duplicated) was assigned to a Merian element based on a reference database, then plotted along chromosomes drawn to scale.

Genome sequence report

Sequence data

PacBio sequencing of the *Charaxes jasius* specimen generated 15.76 Gb (gigabases) from 1.49 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 432.64 Mb, with a heterozygosity of 1.30% and repeat content of 18.36% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 35× coverage. Hi-C sequencing produced 96.90 Gb from 641.71 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 465.23 Mb in 65 scaffolds, with 100 gaps, and a scaffold N50 of 16.32 Mb (Table 2).

Most of the assembly sequence (99.91%) was assigned to 26 chromosomal-level scaffolds, representing 24 autosomes and the W and Z sex chromosomes. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 3; Table 3). Chromosome painting with Merian elements illustrates the distribution of orthologues along chromosomes and highlights patterns of chromosomal evolution relative to Lepidopteran ancestral linkage groups (Figure 4). The mitochondrial genome was also assembled (length 15.3 kb, OZ243167.1). This sequence is included as a contig in the multifasta file of the genome submission and as a standalone record.

Assembly quality metrics

For haplotype 1, the estimated QV is 62.5, and for haplotype 2, 62.6. When the two haplotypes are combined, the assembly achieves an estimated QV of 62.6. The *k*-mer completeness is 78.63% for haplotype 1, 71.73% for haplotype 2, and 99.76% for the combined haplotypes (Figure 5).

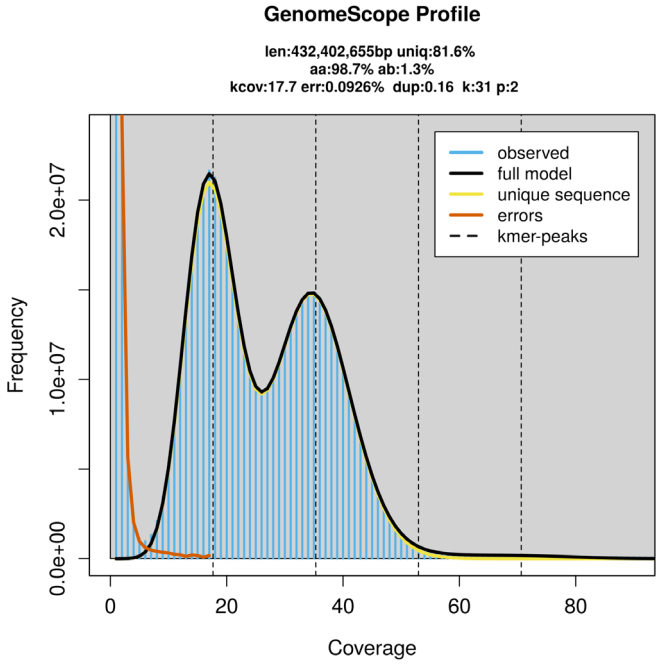

Figure 2. Frequency distribution of *k*-mers generated using GenomeScope2. The plot shows observed and modelled *k*-mer spectra, providing estimates of genome size, heterozygosity, and repeat content based on unassembled sequencing reads.

Table 2. Genome assembly statistics.

Assembly name	ilChaJasi1.hap1.1	ilChaJasi1.hap2.1
Assembly accession	GCA_965212305.1	GCA_965212895.1
Assembly level	chromosome	scaffold
Span (Mb)	465.23	401.60
Number of chromosomes	26	scaffold-level
Number of contigs	165	132
Contig N50	6.43 Mb	6.51 Mb
Number of scaffolds	65	61
Scaffold N50	16.32 Mb	16.31 Mb
Longest scaffold length (Mb)	29.46	-
Sex chromosomes	W and Z	-
Organelles	Mitochondrion: 15.3 kb	-

BUSCO analysis using the lepidoptera_odb10 reference set (n = 5 286) identified 98.7% of the expected gene set (single = 98.4%, duplicated = 0.3%) in haplotype 1. For haplotype 2, BUSCO analysis identified 92.1% of the expected gene set (single = 91.8%, duplicated = 0.2%). The snail plot in Figure 6 summarises the scaffold length distribution and other assembly statistics for haplotype 1. The blob plot in Figure 7 shows the distribution of scaffolds by GC proportion and coverage for haplotype 1.

Table 4 lists the assembly metric benchmarks adapted from Rhie *et al.* (2021) and 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

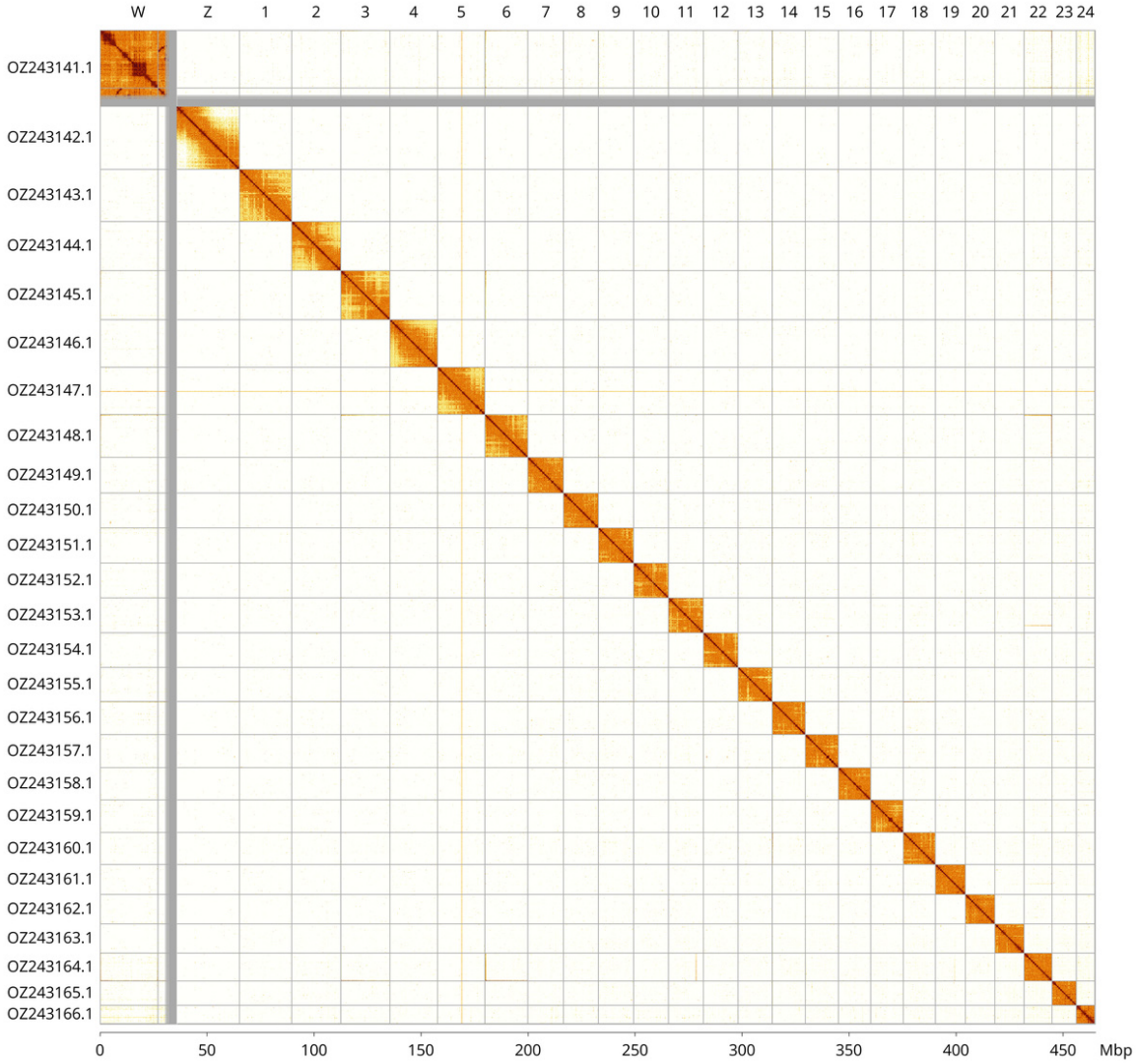

Figure 3. Hi-C contact map of the *Charaxes jasius* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes, with a megabase scale shown below. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Charaxes jasius* ilChaJasi1.

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ243143.1	1	24.39	35	M10;M16;M22;M27;M29
OZ243144.1	2	22.98	35.50	M17;M25;M3;M31;M5
OZ243145.1	3	22.95	35	M2;M9
OZ243146.1	4	22.23	35.50	M13;M15;M28;M4;M6;M8
OZ243147.1	5	22.19	35.50	M1;M26;M5;M8
OZ243148.1	6	20.06	36	M12;M17;M20
OZ243149.1	7	16.64	36	M10;M11;M6
OZ243150.1	8	16.38	36	M11;M22;M7;M9

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ243151.1	9	16.32	36	M10;M15;M18
OZ243152.1	10	16.32	36	M12;M14;M23
OZ243153.1	11	16.31	36	M16;M2;M3
OZ243154.1	12	16.23	36	M15;M27;M8
OZ243155.1	13	16	36	M16;M21;M4;M6
OZ243156.1	14	15.47	36.50	M21;M7
OZ243157.1	15	15.38	36	M1;M16;M26
OZ243158.1	16	15.18	36	M2;M25;M5
OZ243159.1	17	15.14	36	M18;M23
OZ243160.1	18	15.08	36	M21;M22
OZ243161.1	19	14.03	36	M1;M14
OZ243162.1	20	13.69	36.50	M13;M28
OZ243163.1	21	13.67	36.50	M19;M3
OZ243164.1	22	13.09	37	M2;M24
OZ243165.1	23	11.33	37	M14;M26
OZ243166.1	24	8.58	37.50	M30;M5
OZ243141.1	W	35.71	38	-
OZ243142.1	Z	29.46	35	M19;M24;M4;M6;MZ

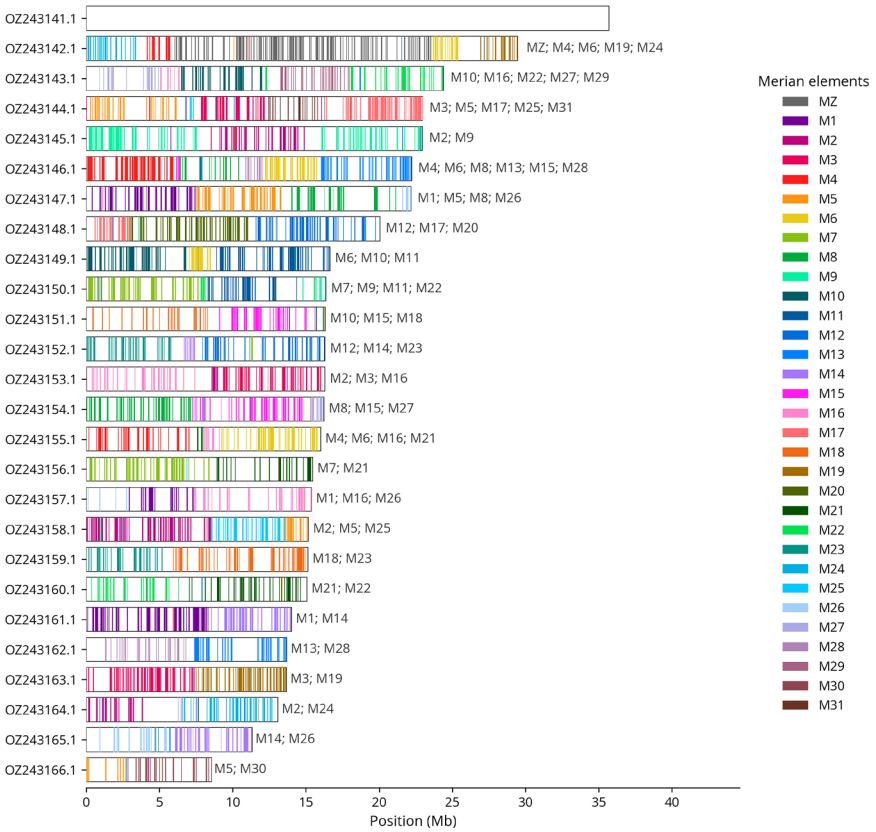

Figure 4. Merian elements painted across chromosomes in the ilChaJasi1.hap1.1 assembly of *Charaxes jasius*. Chromosomes are drawn to scale, with the positions of orthologues shown as coloured bars. Each orthologue is coloured by the Merian element that it belongs to. All orthologues which could be assigned to Merian elements are shown.

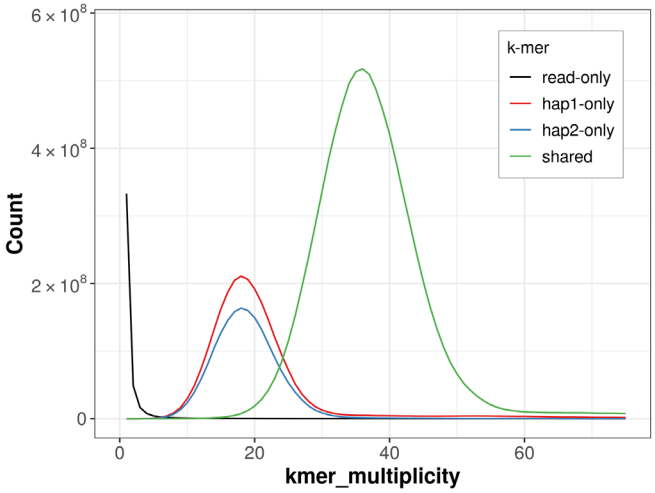

Figure 5. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers that appear in the reads but are not assembled. The green curve (the homozygous peak) corresponds to *k*-mers shared by both haplotypes and the red and blue curves (the heterozygous peaks) show *k*-mers found only in one of the haplotypes.

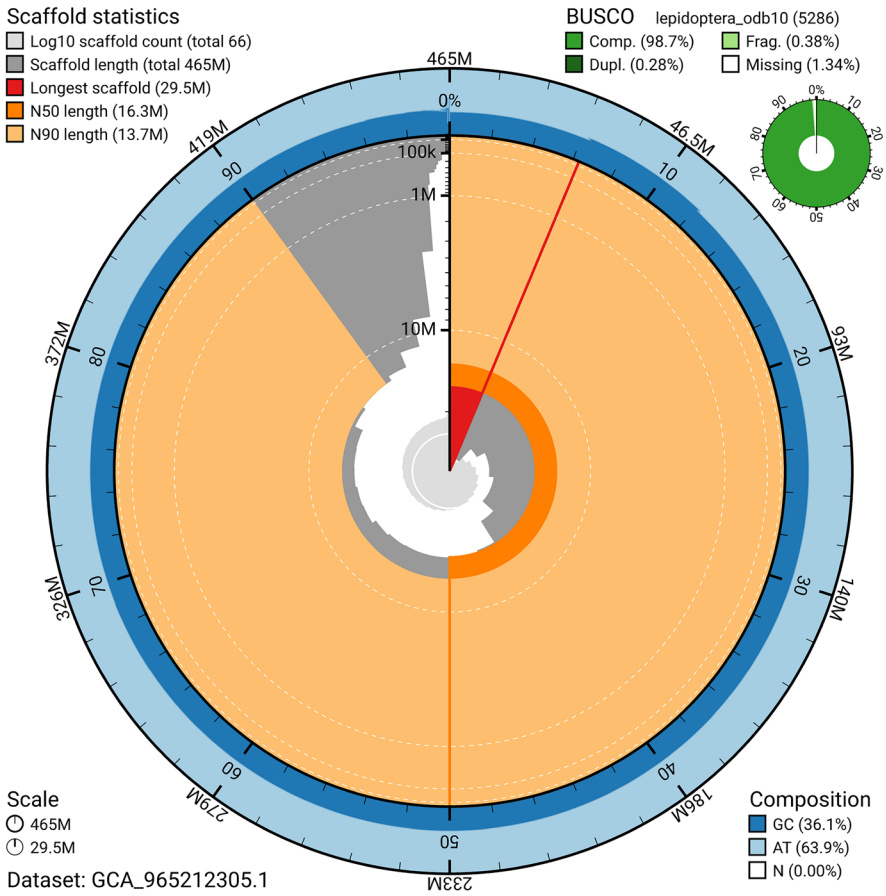

Figure 6. Assembly metrics for ilChaJasi1.hap1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. The outermost blue tracks display the distribution of GC, AT, and N percentages across the bins. Scaffolds are arranged clockwise from longest to shortest and are depicted in dark grey. The longest scaffold is indicated by the red arc, and the deeper orange and pale orange arcs represent the N50 and N90 lengths. A light grey spiral at the centre shows the cumulative scaffold count on a logarithmic scale. A summary of complete, fragmented, duplicated, and missing BUSCO genes in the set is presented at the top right. An interactive version of this figure can be accessed on the BlobToolKit viewer.

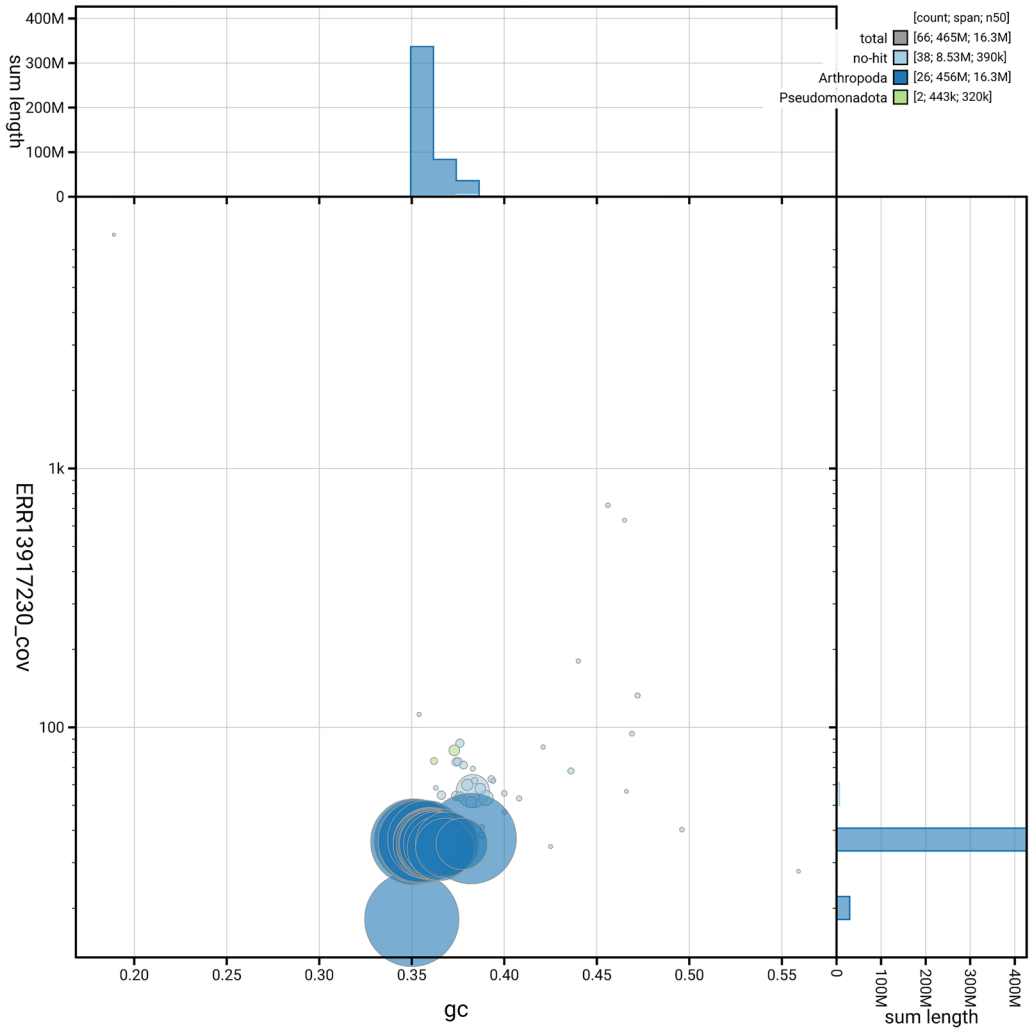

Figure 7. BlobToolKit GC-coverage plot for ilChaJasi1.hap1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes 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 *Charaxes jasius* assembly.

Measure	Value	Benchmark
EBP summary (haplotype 1)	6.C.Q62	6.C.Q40
Contig N50 length	6.43 Mb	≥ 1 Mb
Scaffold N50 length	16.32 Mb	= chromosome N50
Consensus quality (QV)	Haplotype 1: 62.5; haplotype 2: 62.6; combined: 62.6	≥ 40
k-mer completeness	Haplotype 1: 78.63%; Haplotype 2: 71.73%; combined: 99.76%	≥ 95%
BUSCO	C:98.7% [S:98.4%; D:0.3%]; F:0.4%; M:1.0%; n:5 286	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.91%	≥ 90%

Notes: EBP summary uses log10(Contig N50); chromosome-level (C) or log10(Scaffold N50); Q (Merqury QV). BUSCO: C=complete; S=single-copy; D=duplicated; F=fragmented; M=missing; n=orthologues

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- 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: Charaxes jasius. Accession number PRJEB81919. The genome sequence is released openly for reuse. The *Charaxes jasius* 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:

- Members of the Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team
- Members of Wellcome Sanger Institute Scientific Operations – Sequencing Operations
- Members of the Wellcome Sanger Institute Tree of Life Core Informatics team
- Members of the Tree of Life Core Informatics collective
- Members of the Project Psyche Community

Table 5. Software versions and sources.

Software	Version	Source
BEDTools	2.30.0	https://github.com/arq5x/bedtools2
BLAST	2.14.0	ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/
BlobToolKit	4.4.5	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.7.1	https://gitlab.com/ezlab/busco
bwa-mem2	2.2.1	https://github.com/bwa-mem2/bwa-mem2
Cooler	0.8.11	https://github.com/open2c/cooler
DIAMOND	2.1.8	https://github.com/bbuchfink/diamond
fasta_windows	0.2.4	https://github.com/tolkit/fasta_windows
FastK	1.1	https://github.com/thegenemyers/FASTK
GenomeScope2.0	2.0.1	https://github.com/tbenavi1/genomescope2.0
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
Hifiasm	0.19.8-r603	https://github.com/chhylp123/hifiasm
HiGlass	1.13.4	https://github.com/higlass/higlass
lep_busco_painter	1.0.0	https://github.com/charlottewright/lep_busco_painter
MerquryFK	1.1.2	https://github.com/thegenemyers/MERQURY.FK
Minimap2	2.28-r1209	https://github.com/lh3/minimap2

Software	Version	Source
MitoHiFi	3	https://github.com/marcelauliano/MitoHiFi
MultiQC	1.14; 1.17 and 1.18	https://github.com/MultiQC/MultiQC
Nextflow	24.10.4	https://github.com/nextflow-io/nextflow
PretextSnapshot	0.0.5	https://github.com/sanger-tol/PretextSnapshot
PretextView	1.0.3	https://github.com/sanger-tol/PretextView
samtools	1.21	https://github.com/samtools/samtools
sanger-tol/ascc	0.1.0	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	v0.7.1	https://github.com/sanger-tol/blobtoolkit
sanger-tol/curationpretext	1.4.2	https://github.com/sanger-tol/curationpretext
Seqtk	1.3	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.4.0	https://github.com/sanger-tol/treeval
YaHS	1.2a.2	https://github.com/c-zhou/yahs

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✔ **Yoshiaki Morino**
University of Tsukuba, Tsukuba, Japan

This manuscript presents a high-quality, chromosome-level, haplotype-resolved genome assembly for *Charaxes jasius*. The reported assembly metrics are strong and support the authors' conclusion that this is a reference-quality resource. In particular, haplotype 1 has a total span of 465.23 Mb, with 99.91% of the assembly assigned to 26 chromosomal pseudomolecules, including the W and Z sex chromosomes. The scaffold N50 is 16.32 Mb, indicating a highly contiguous and well-resolved assembly.

The quality assessment is also convincing. BUSCO completeness is 98.7% using the *lepidoptera_odb10* dataset, with 98.4% single-copy and only 0.3% duplicated BUSCOs, which is consistent with a highly complete assembly. The consensus quality is likewise very high, with a reported QV of 62.5 for haplotype 1 and 62.6 for the combined haplotypes, meeting the recommended reference standard.

The sequencing and assembly strategy is appropriate and clearly described. The genome was generated from 15.76 Gb of PacBio HiFi data, corresponding to approximately 35× coverage based on the estimated haploid genome size, and scaffolded with 96.9 Gb of Hi-C data. The use of HiFi reads, Hi-C phasing, manual curation, and standard quality-control tools is fully in line with current best practices.

Overall, I found the manuscript technically sound, clearly written, and suitable for Indexing in its present form. The assembly statistics, quality metrics, and data presentation are all of a high standard, and I have no major concerns regarding the methodology or the utility of the dataset. This genome should represent a valuable resource for future studies of butterfly comparative genomics, chromosome evolution, and the evolutionary biology of *Charaxes* and related nymphalid lineages.

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: marine invertebrate, Evo-Devo

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 20 February 2026

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James B Whitfield
University of Illinois at Urbana-Champaign, Champaign, USA

This paper presents a report on a reference-quality genome obtained for the only member of the mainly tropical nymphalid butterfly subfamily Charaxinae to be found in Europe. The Background section of the paper provides a nice summary of the biology and conservation status of this species. I was a bit surprised that there was no mention. of the significance various nymphalid butterflies in studying mechanisms of developmental evolution of color patterns in mimicry and aposematism (e .g. Beldade & Brakefield 2002 Nature; Abbasi & Marcus 2015 Evolution & Development; Tian and Monteiro 2026 Curr Opin Insect Science; just to name a very few examples). No doubt having a genome from a distinct nymphalid subfamily might be useful for phylogenetic perspective, especially for the relevant evo-devo genes?
Otherwise, the paper seems very well prepared and clearly written, and the methods are certainly appropriate and relatively state of the art. The presentation of genomic details seems rather standard for these reports, and straightforward to interpret.

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: Systematics, phylogeny and comparative genomics of parasitoid wasps and their associated mutualistic viruses

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.