DATA NOTE

The genome sequence of the Speckled Yellow, *Pseudopanthera macularia* (Linnaeus, 1758) (Lepidoptera: Geometridae)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a female specimen of *Pseudopanthera macularia* (Speckled Yellow; Arthropoda; Insecta; Lepidoptera; Geometridae). The assembly contains two haplotypes with total lengths of 717.87 megabases and 650.90 megabases. Most of haplotype 1 (99.61%) 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 15.61 kilobases.

Keywords

Pseudopanthera macularia, Speckled Yellow, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 15 Aug 2025	✓ view	✓ view

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Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Geometroidea; Geometridae; Ennominae; *Pseudopanthera*; *Pseudopanthera macularia* (Linnaeus, 1758) (NCBI:txid1007708)

Background

The Speckled Yellow – *Pseudopanthera macularia* (Linnaeus, 1758) – is a small geometrid moth with a wingspan of 23 to 28 mm. The species is very distinctive with its orange-yellow wings mottled with large brown spots. However, the extent of these spots and the intensity of the yellow-orange colouration can greatly vary between individuals and even between regions. In the past, this led to the description of numerous subspecies, forms and aberrations, which are now hardly considered. Recently, however, Beljaev (1997) attributed the populations of southern Siberia and adjacent regions to a new subspecies (*cryptica*) based on differences in female genital morphology. The species shows no sexual dimorphism.

This Eurosiberian species is widespread from the Iberian Peninsula across most of temperate Europe, the Caucasus, Central Asia and southern Russia (GBIF Secretariat, 2023) as far as northern Mongolia (Enkhtur *et al.*, 2020).

It is univoltine and flies from April to July, depending on altitude. As a mesophilic species, it can be found in shrubby meadows, open woods and forest margins, from the plains up to altitudes of over 2 000 metres. The caterpillars feed mainly on various Lamiaceae of the genera *Lamium*, *Teucrium*, *Mentha*, *Salvia*, *Stachys*, etc. (Scoble, 1999). The species hibernates as a chrysalis. The imagos are active during the day and are easy to observe, especially when foraging for nectar from many flowers or taking mineral salts from damp places or excrement.

This common species is very widespread and colonises widespread environments, so it is probably not threatened. The reference genome presented here will provide the opportunity to study the population structure of this species as well as shedding light on W chromosome evolution as this species shows intraspecific sex chromosome polymorphism (Hejníčková *et al.*, 2021). The sequence data were derived from a female specimen (Figure 1) collected from Ferreyres, Switzerland.

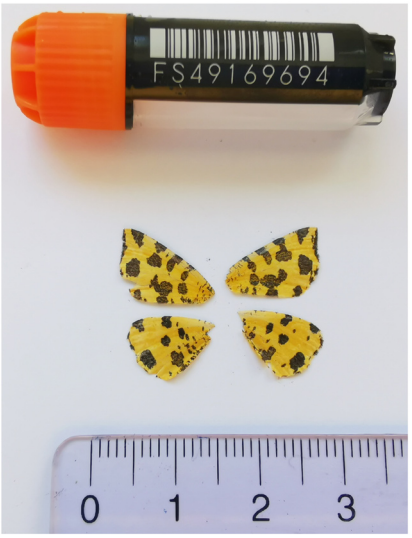

Figure 1. Voucher photograph of the *Pseudopanthera macularia* (ilPseMacu1) specimen used for genome sequencing.

Methods

Sample acquisition

The specimen used for genome sequencing was an adult female *Pseudopanthera macularia* (specimen ID SAN28000061, ToLID ilPseMacu1; Figure 1), collected from Ferreyres, Switzerland (latitude 46.6657, longitude 6.4726; elevation 629 m) on 31/05/2023. The specimen was collected and identified by Andreas Sanchez (Infofauna).

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 ilPseMacu1 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 27.51 ng/μL and a yield of 1 292.97 ng, with a fragment size of 16.1 kb. The 260/280 spectrophotometric ratio was 2, and the 260/230 ratio was 3.73.

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 ilPseMacu1 sample using the Arima-HiC v2 kit (Arima Genomics). Following the manufacturer's instructions, tissue was fixed and DNA crosslinked using TC buffer to a final formaldehyde concentration of 2%. The tissue was homogenised using the Diagnocine Power Masher-II. Crosslinked DNA was digested with a restriction enzyme master mix, biotinylated, and ligated. Clean-up was performed with SPRISelect beads before library preparation. DNA concentration was measured with the Qubit Fluorometer (Thermo Fisher Scientific) and Qubit HS Assay Kit. The biotinylation percentage was estimated using the Arima-HiC v2 QC beads.

Hi-C library preparation and sequencing

Biotinylated DNA constructs were fragmented using a Covaris E220 sonicator and size selected to 400–600 bp using SPRISelect beads. DNA was enriched with Arima-HiC v2 kit Enrichment beads. End repair, A-tailing, and adapter ligation were carried out with the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs), following a modified protocol where library preparation occurs while DNA remains bound to the Enrichment beads. Library amplification was performed using KAPA HiFi HotStart mix and a custom Unique Dual Index (UDI) barcode set (Integrated DNA Technologies). Depending on sample concentration and biotinylation percentage determined at the crosslinking stage, libraries were amplified with 10–16 PCR cycles. Post-PCR clean-up was performed with SPRISelect beads. Libraries were quantified using the AccuClear Ultra High Sensitivity dsDNA Standards Assay Kit (Biotium) and a FLUOstar Omega plate reader (BMG Labtech).

Prior to sequencing, libraries were normalised to 10 ng/µL. Normalised libraries were quantified again and equimolar and/or weighted 2.8 nM pools. Pool concentrations were checked using the Agilent 4200 TapeStation (Agilent) with High Sensitivity D500 reagents before sequencing. Sequencing was performed using paired-end 150 bp reads on the Illumina NovaSeq X.

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

Genome assembly

Prior to assembly of the PacBio HiFi reads, a database of *k*-mer counts ($k = 31$) was generated from the filtered reads using FastK. GenomeScope2 (Ranallo-Benavidez *et al.*, 2020) was used to analyse the *k*-mer frequency distributions, providing estimates of genome size, heterozygosity, and repeat content.

The HiFi reads were assembled using Hifiasm in Hi-C phasing mode (Cheng *et al.*, 2021; Cheng *et al.*, 2022), producing two haplotypes. Hi-C reads (Rao *et al.*, 2014) were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). Contigs were further scaffolded with Hi-C data in YaHS (Zhou *et al.*, 2023), using the --break option for handling potential misassemblies. The scaffolded assemblies were evaluated using Gfastats (Formenti *et al.*, 2022), BUSCO (Manni *et al.*, 2021) and MERQURY.FK (Rhie *et al.*, 2020).

Table 1. Specimen and sequencing data for BioProject.

Platform	PacBio HiFi	Hi-C
ToLID	ilPseMacu1	ilPseMacu1
Specimen ID	SAN28000061	SAN28000061
BioSample (source individual)	SAMEA115109743	SAMEA115109743
BioSample (tissue)	SAMEA115109785	SAMEA115109779
Tissue	thorax	head
Sequencing platform and model	Revio	Illumina NovaSeq X
Run accessions	ERR13605499	ERR13602162
Read count total	1.48 million	752.52 million
Base count total	17.86 Gb	113.63 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 10 breaks and 156 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 *Pseudopanthera macularia* was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 17.86 Gb (gigabases) from 1.48 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 679.30 Mb, with a heterozygosity of 0.83% and repeat content of 36.10%. These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 25× coverage. Hi-C sequencing produced 113.63 Gb from 752.52 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 717.87 Mb in 103 scaffolds, with 318 gaps, and a scaffold N50 of 23.47 Mb (Table 2).

Most of the assembly sequence (99.61%) 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). Chromosomes Z and W were assigned based on read coverage and HiC signal. The order and orientation of contigs within the W chromosome is uncertain.

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 64.7, and for haplotype 2, 66.2. When the two haplotypes are combined, the assembly achieves an estimated QV of 65.4. The *k*-mer completeness is 82.76% for haplotype 1, 78.67% for haplotype 2, and 99.40% for the combined haplotypes (Figure 4). BUSCO analysis using the lepidoptera_odb10 reference set (n = 5 286) (Kriventseva *et al.*, 2019) identified 98.6% of the expected gene set (single = 98.1%, duplicated = 0.5%) 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.Q64**, meeting the recommended reference standard.

Table 2. Genome assembly statistics.

Assembly name	ilPseMacu1.hap1.1	ilPseMacu1.hap2.1
Assembly accession	GCA_964260665.1	GCA_964260615.1
Assembly level	chromosome	scaffold
Span (Mb)	717.87	650.90
Number of chromosomes	32	N/A
Number of contigs	421	263
Contig N50	4.87 Mb	4.98 Mb
Number of scaffolds	103	84
Scaffold N50	23.47 Mb	22.79 Mb
Longest scaffold length (Mb)	35.71	N/A
Sex chromosomes	W and Z	N/A
Organelles	Mitochondrial genome: 15.61 kb	N/A

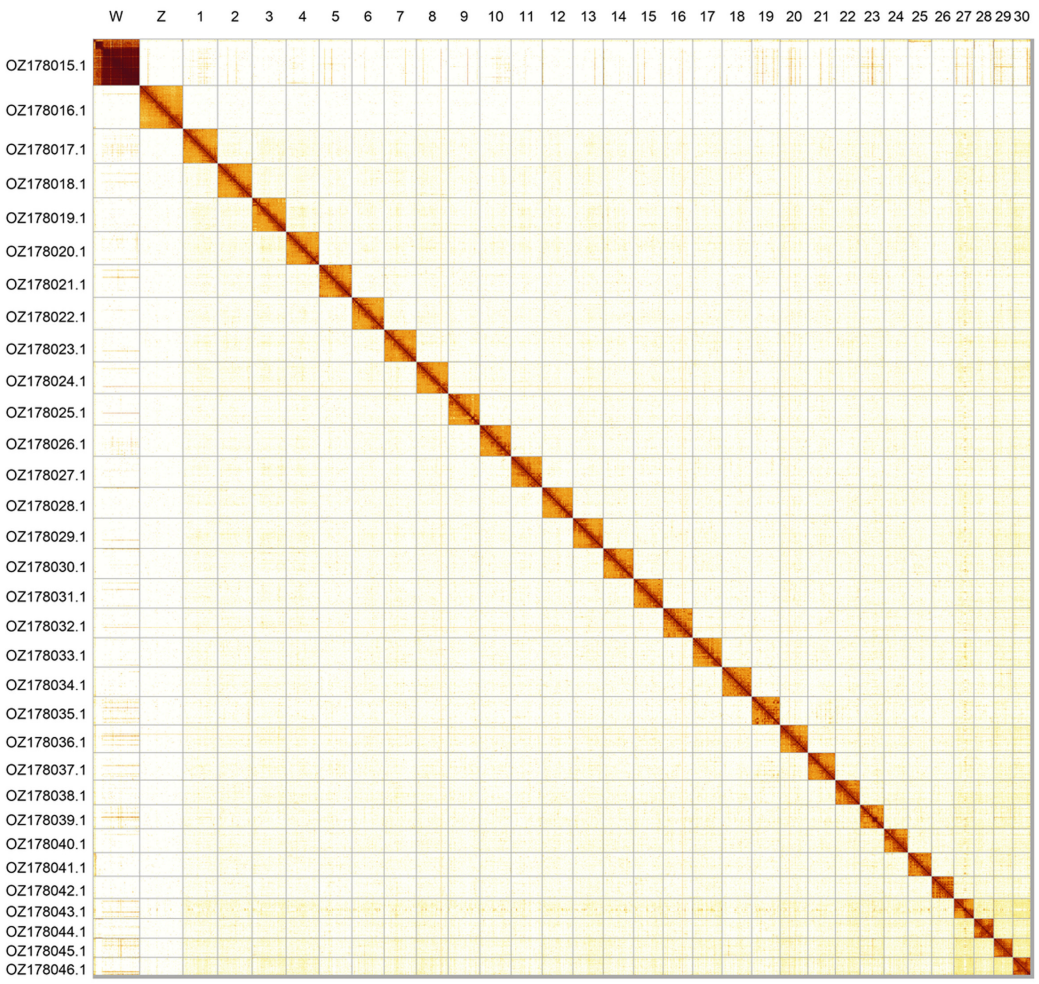

Figure 2. Hi-C contact map of the *Pseudopanthera macularia* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Pseudopanthera macularia* ilPseMacu1.

INSDC accession	Molecule	Length (Mb)	GC%	Assigned Merian elements
OZ178017.1	1	26.49	36	M1
OZ178018.1	2	26.23	36	M2
OZ178019.1	3	25.98	36	M17;M20
OZ178020.1	4	25.16	36	M3
OZ178021.1	5	24.95	35.50	M9
OZ178022.1	6	24.70	35.50	M8
OZ178023.1	7	24.57	35.50	M5
OZ178024.1	8	24.25	36	M7
OZ178025.1	9	24.02	35.50	M18
OZ178026.1	10	23.86	35.50	M12
OZ178027.1	11	23.65	36	M6
OZ178028.1	12	23.47	35.50	M16
OZ178029.1	13	23.23	36	M4
OZ178030.1	14	23.04	35.50	M21
OZ178031.1	15	22.59	36	M10
OZ178032.1	16	22.48	36	M11
OZ178033.1	17	22.47	36	M15
OZ178034.1	18	22.47	35.50	M22
OZ178035.1	19	21.71	36	M23
OZ178036.1	20	21.25	36	M14
OZ178037.1	21	20.88	35.50	M13
OZ178038.1	22	18.84	36.50	M19
OZ178039.1	23	18.28	36	M27
OZ178040.1	24	18.23	36	M24
OZ178041.1	25	18.18	35.50	M26
OZ178042.1	26	16.82	36	M28
OZ178043.1	27	15.39	38	M30
OZ178044.1	28	15.07	36	M25
OZ178045.1	29	14.48	36	M29
OZ178046.1	30	13.59	37	M31
OZ178015.1	W	35.71	36	N/A
OZ178016.1	Z	33.04	35.50	MZ

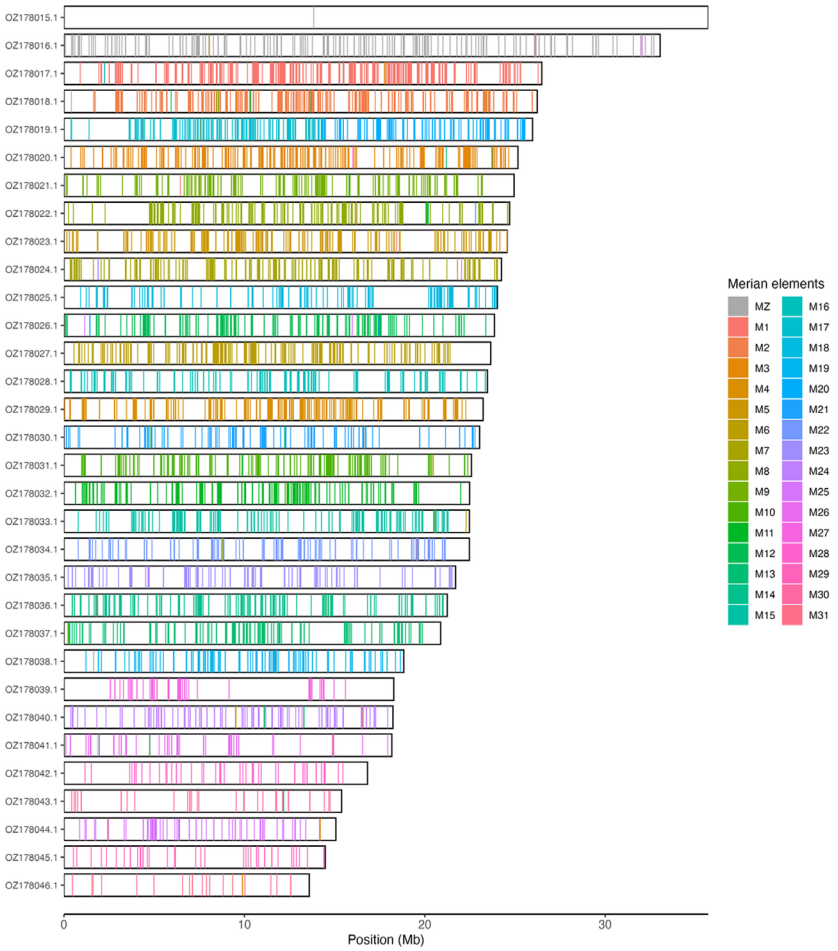

Figure 3. Merian elements painted across chromosomes in the ilPseMacu1.hap1.1 assembly of *Pseudopanthera macularia*. Chromosomes are drawn to scale, with the positions of orthologues shown as coloured bars. Each orthologue is coloured by the Merian element that it belongs to. All orthologues which could be assigned to Merian elements are shown.

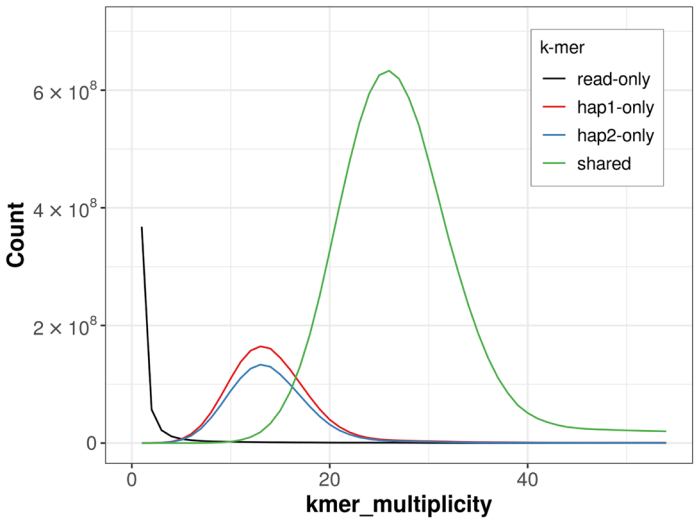

Figure 4. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers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

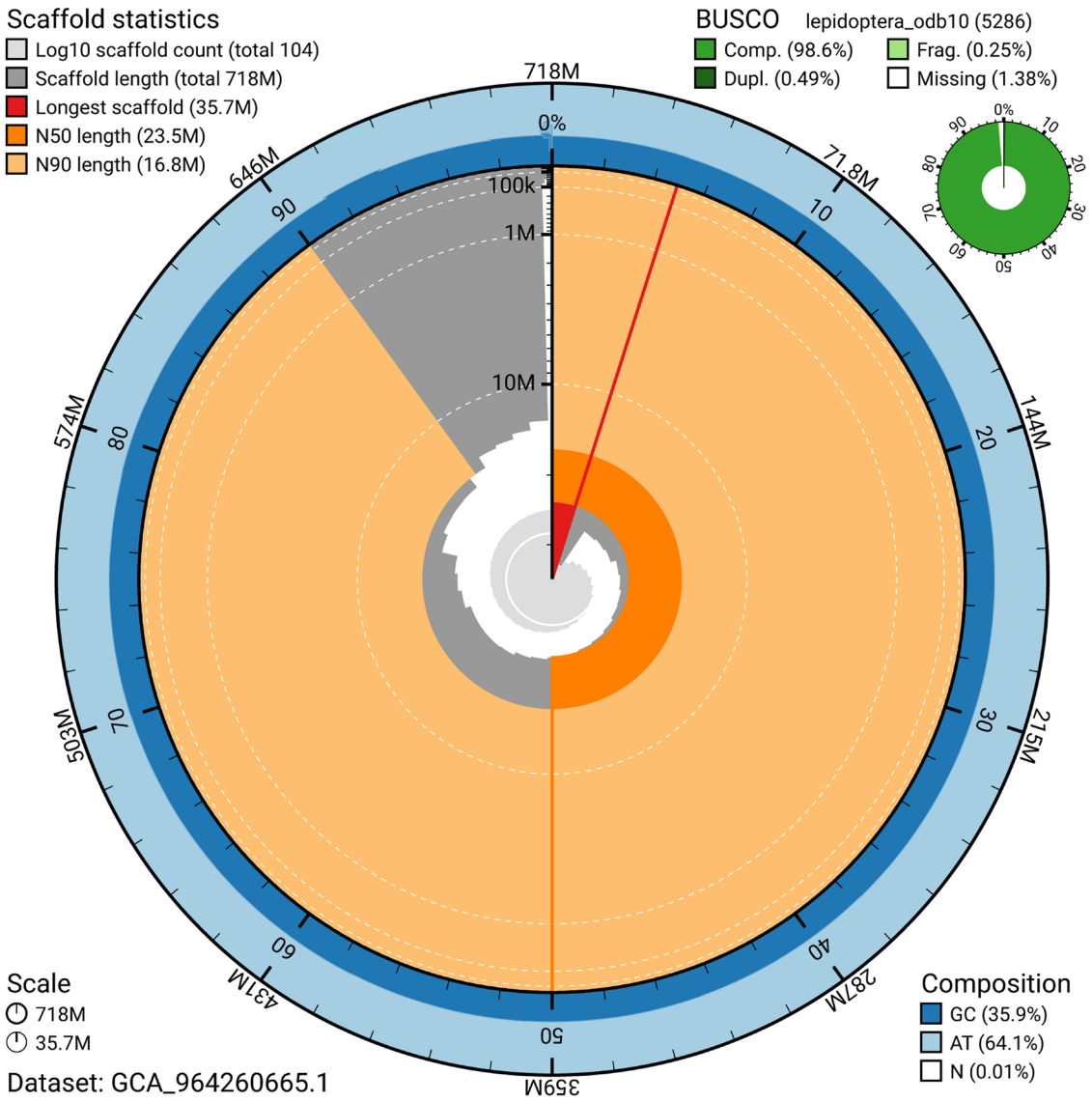

Figure 5. Assembly metrics for ilPseMacu1.hap1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. The outermost 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.

Wellcome Sanger Institute – Legal and Governance

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

- Ethical review of provenance and sourcing of the material
- Legality of collection, transfer and use (national and international).

Each transfer of samples is undertaken according to a Research Collaboration Agreement or Material Transfer Agreement

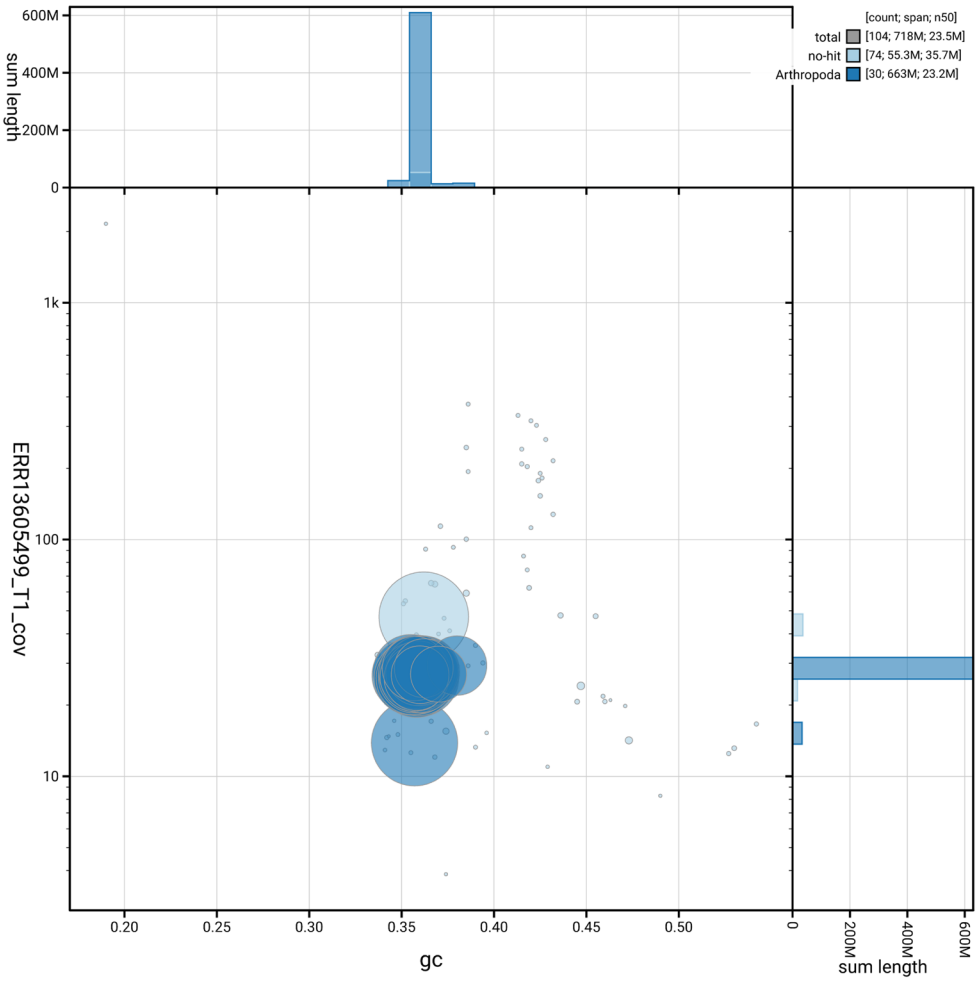

Figure 6. BlobToolKit GC-coverage plot for ilPseMacu1.hap1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes 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 *Pseudopanthera macularia* assembly.

Measure	Value	Benchmark
EBP summary (haplotype 1)	6.C.Q64	6.C.Q40
Contig N50 length	4.87 Mb	≥ 1 Mb
Scaffold N50 length	23.47 Mb	= chromosome N50
Consensus quality (QV)	Haplotype 1: 64.7; haplotype 2: 66.2; combined: 65.4	≥ 40
k-mer completeness	Haplotype 1: 82.76%; Haplotype 2: 78.67%; combined: 99.40%	≥ 95%
BUSCO	C:98.6%[S:98.1%,D:0.5%], F:0.2%,M:1.1%,n:5 286	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.61%	≥ 90%

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: Pseudopanthera macularia (speckled yellow). Accession number PRJEB79172. The genome sequence is released openly for reuse. The *Pseudopanthera macularia* 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
samtools	1.19.2	https://github.com/samtools/samtools

Software	Version	Source
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 03 November 2025

https://doi.org/10.21956/wellcomeopenres.27188.r129957

✔ **Jose Martinez**
University of Florida, Florida, USA

The dataset has been constructed in accordance with established standards, with clear and accessible explanations that ensure transparency. The protocols and procedures employed are methodologically sound, and the accompanying documentation of methods and materials is presented in a manner that facilitates reproducibility. Owing to its high degree of standardization, comprehensive illustrations, and open accessibility, the dataset constitutes a valuable resource with broad applicability.

Sanchez *et al.* present a comprehensive and meticulously assembled genome of the Speckled Yellow, *Pseudopanthera macularia* (Linnaeus, 1758), generated from sequencing a single female specimen. The assembly demonstrates exceptional completeness, as reflected by its high BUSCO score, and is organized into 32 chromosomal pseudomolecules, including both the W and Z sex chromosomes. Produced through rigorous methodological approaches, this work delivers a detailed and robust genomic resource.

The authors emphasize the significance of this genomic information for future studies, particularly regarding the population structure of the species and intraspecific sex chromosome polymorphism. In addition, they provide valuable context by outlining the biological history of *P. macularia*.

Congratulations!

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

Are the protocols appropriate and is the work technically sound?
Yes

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

Yes

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

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Evolutionary Biology, Ecology, IPM, Integrative Biology, Entomology, 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.

Reviewer Report 11 October 2025

https://doi.org/10.21956/wellcomeopenres.27188.r129954

Sivasankaran Kuppusamy
Loyola College, Chennai, Tamil Nadu, India

Authors have done the chromosome-level genome assembly of *Pseudopanthera macularia* (Linnaeus, 1758). They sequence and assemble two haplotypes with the lengths of 717.87 Mb and 650.90 Mb respectively. Through the assembly they scaffolded into 32 chromosomes. Authors have used appropriate methodology and software for high molecular weight DNA isolation and sequencing, sequence assembly and annotations.

Comments on the manuscript
Authors have given the species Linnaean hierarchy in the abstract. The species hierarchy may be removed in the abstract. The total number of protein-coding genes, non-coding genes and gene transcripts can be included in the abstract.

The author used the full name of the genus, *Pseudopanthera macularia*, in the article. Start with the full genus name once, if possible, then shorten it to *P. macularia*.

In the previous genome assemblies authors have detected protein-coding genes, non-coding genes and gene transcripts through the sequence assembly and annotations. In the present research authors haven't given above mentioned details. Any reason?

The article was well prepared and the manuscript meets the necessary scientific standard and is suitable for indexing.

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: Phylogenetic analysis of Noctuoidea moths using mitogenome sequence

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.