



DATA NOTE

The genome sequence of the Scorched Wing moth, *Plagodis dolabraria* (Linnaeus, 1767)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a male specimen of *Plagodis dolabraria* (Scorched Wing; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence has a total length of 939.07 megabases. Most of the assembly (99.59%) is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 16.97 kilobases in length.

Keywords

Plagodis dolabraria, Scorched Wing, genome sequence, chromosomal, Lepidoptera



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

Open Peer Review

Approval Status  

	1	2
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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; *Plagodis*; *Plagodis dolabraria* (Linnaeus, 1767) (NCBI:txid934889)

Background

Plagodis dolabraria, the Scorched Wing, is a striking geometrid moth that cannot be mistaken for any other species in Britain. The fore wings are ‘crumpled’ and traversed by many fine brown vermiculations (Figure 1). The trailing corners of the fore and hind wings have darkened areas which give rise to the ‘scorched’ in the English name. At rest, the abdomen points upwards. Emmet (1991) explains that Linnaeus’s enigmatic Latin name (Linnaeus, 1767), from *dolabra*, ‘pickaxe’, refers to the natural shape of the wings at rest, which Linnaeus appreciated as he did not set his Lepidoptera specimens.

Found across the Palaearctic as far East as Japan, *P. dolabraria* is one of only two European *Plagodis* species, the other being the morphologically dissimilar *P. pulveraria* (Linnaeus), but in North America there are several species apparently closely related to *P. dolabraria* (Rupert, 1949). In Britain, *P. dolabraria* is widespread, at least in England and Wales, but seldom numerous (Waring *et al.*, 2017). There has been a very substantial increase in range since the 1970s, into northern England and Scotland, but no change in abundance over time (Randle *et al.*, 2019).

Like many other relatives, the larvae feed on deciduous trees and are superbly camouflaged as twigs. The main foodplants are oaks (*Quercus*) and birches (*Betula*) but a variety of other trees can be eaten (Henwood *et al.*, 2020). The winter is spent as a cocooned pupa at ground level, adults are on the wing from mid-May to early July with larvae feeding from July to September. Although males are readily attracted to light, females are rarely seen (Waring *et al.*, 2017).



Figure 1. Photograph of the *Plagodis dolabraria* (iIPlaDola) 1 specimen used for Hi-C sequencing.

We present a chromosome-level genome sequence for *Plagodis dolabraria*, based on a male specimen from Gilbert White’s House, Selborne, England, UK.

Genome sequence report

Sequencing data

The genome of a specimen of *Plagodis dolabraria* was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 89.08 Gb from 9.53 million reads. GenomeScope analysis of the PacBio HiFi data estimated the haploid genome size at 931.66 Mb, with a heterozygosity of 0.83% and repeat content of 36.69%. These values provide an initial assessment of genome complexity and the challenges anticipated during assembly. Based on this estimated genome size, the sequencing data provided approximately 90.0x coverage of the genome. Chromosome conformation Hi-C sequencing produced 133.17 Gb from 881.95 million reads.

Table 1 summarises the specimen and sequencing information, including the BioProject, study name, BioSample numbers, and sequencing data for each technology.

Assembly statistics

The primary haplotype was assembled, and contigs corresponding to an alternate haplotype were also deposited in INSDC databases. The assembly was improved by manual curation, which corrected 14 misjoins or missing joins and removed 8 haplotypic duplications. These interventions reduced the total assembly length by 0.67% and decreased the scaffold count by 4.76%. The final assembly has a total length of 939.07 Mb in 59 scaffolds, with 82 gaps, and a scaffold N50 of 32.42 Mb (Table 2).

The snail plot in Figure 2 provides a summary of the assembly statistics, indicating the distribution of scaffold lengths and other assembly metrics. Figure 3 shows the distribution of scaffolds by GC proportion and coverage. Figure 4 presents a cumulative assembly plot, with separate curves representing different scaffold subsets assigned to various phyla, illustrating the completeness of the assembly.

Most of the assembly sequence (99.6%) was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 5; Table 3). During curation, the Z chromosome was assigned by synteny to *Agriphila geniculea* (GCA_950108535.1) (Boyes *et al.*, 2023).

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 in GenBank.

Assembly quality metrics

The estimated Quality Value (QV) and *k*-mer completeness metrics, along with BUSCO completeness scores, were calculated for each haplotype and the combined assembly. The QV reflects the base-level accuracy of the assembly, while *k*-mer completeness indicates the proportion of expected *k*-mers

Table 1. Specimen and sequencing data for *Plagodis dolabraria*.

Project information			
Study title	Plagodis dolabraria (scorched wing)		
Umbrella BioProject	PRJEB66013		
Species	<i>Plagodis dolabraria</i>		
BioSpecimen	SAMEA111457906		
NCBI taxonomy ID	934889		
Specimen information			
Technology	ToLID	BioSample accession	Organism part
PacBio long read sequencing	ilPlaDola2	SAMEA111458674	thorax
Hi-C sequencing	ilPlaDola1	SAMEA7520626	Head and thorax
Sequencing information			
Platform	Run accession	Read count	Base count (Gb)
Hi-C HiSeq X Ten	ERR12071220	8.82e+08	133.17
PacBio Revio	ERR12055557	9.53e+06	89.08

Table 2. Genome assembly data for *Plagodis dolabraria*.

Genome assembly		
Assembly name	ilPlaDola2.1	
Assembly accession	GCA_963854805.1	
<i>Alternate haplotype accession</i>	GCA_963854775.1	
Assembly level for primary assembly	chromosome	
Span (Mb)	939.07	
Number of contigs	141	
Number of scaffolds	59	
Longest scaffold (Mb)	44.36	
Assembly metrics	Measure	Benchmark
Contig N50 length	13.27 Mb	≥ 1 Mb
Scaffold N50 length	32.42 Mb	= chromosome N50
Consensus quality (QV)	Primary: 64.2; alternate: 63.7; combined 63.9	≥ 40
<i>k</i> -mer completeness	Primary: 81.30%; alternate: 80.98%; combined: 99.01%	≥ 95%
BUSCO*	C:98.6%[S:97.7%,D:0.8%], F:0.3%,M:1.1%,n:5,286	S > 90%; D < 5%
Percentage of assembly mapped to chromosomes	99.6%	≥ 90%
Sex chromosomes	Z	localised homologous pairs
Organelles	Mitochondrial genome: 16.97 kb	complete single alleles

* BUSCO scores based on the lepidoptera_odb10 BUSCO set using version 5.5.0. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison.

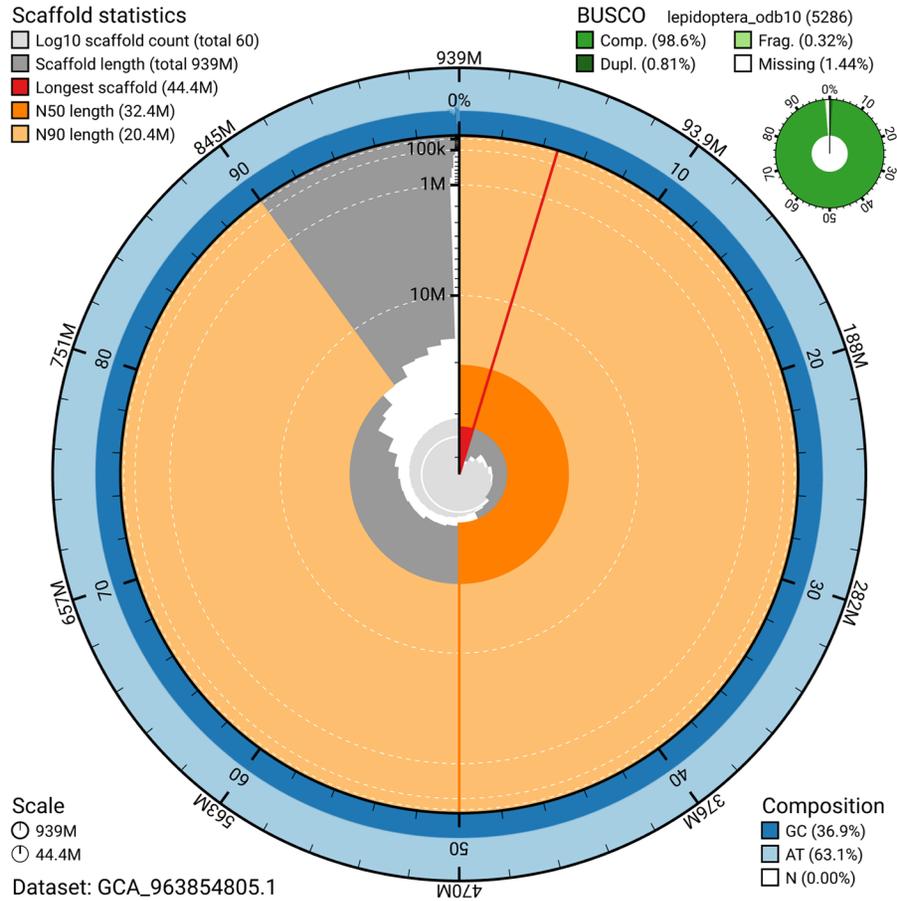


Figure 2. Genome assembly of *Plagodis dolabraria*, ilPlaDola2.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. 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, and missing BUSCO genes in the lepidoptera_odb10 set is presented at the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963854805.1/dataset/GCA_963854805.1/snail.

identified in the assembly. BUSCO scores provide a measure of completeness based on benchmarking universal single-copy orthologues.

The primary haplotype has a QV of 64.2, and the combined primary and alternate assemblies achieve an estimated QV of 63.9. The k -mer completeness for the primary haplotype is 81.30%, and for the alternate haplotype it is 80.98%. The combined primary and alternate assemblies achieve a k -mer completeness of 99.01%. BUSCO analysis using the lepidoptera_odb10 reference set ($n = 5,286$) indicated a completeness score of 98.6% (single = 97.7%, duplicated = 0.8%).

Table 2 provides assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project Report on Assembly Standards September 2024. The assembly achieves the EBP reference standard of 7.C.Q64.

Methods

Sample acquisition and DNA barcoding

An adult male *Plagodis dolabraria* (specimen ID NHMUK014536885, ToLID ilPlaDola2) was collected from Selborne, Gilbert White's House, England, UK (latitude 51.09, longitude -0.94) on 2021-06-10, using a light trap. The specimen was collected by Steph Holt, Gavin Broad and Laura Sivess (Natural History Museum), identified by Gavin Broad and preserved by dry freezing (-80°C). The specimen used for Hi-C sequencing (specimen ID Ox0410a, ToLID ilPlaDola1), was collected and identified by Douglas Boyes (University of Oxford) from Wytham Woods, Oxfordshire, UK on 2020-05-22.

The initial identification by morphology was verified by an additional DNA barcoding process according to the framework developed by Twyford *et al.* (2024). A small sample was dissected from each specimen and stored in ethanol, while the

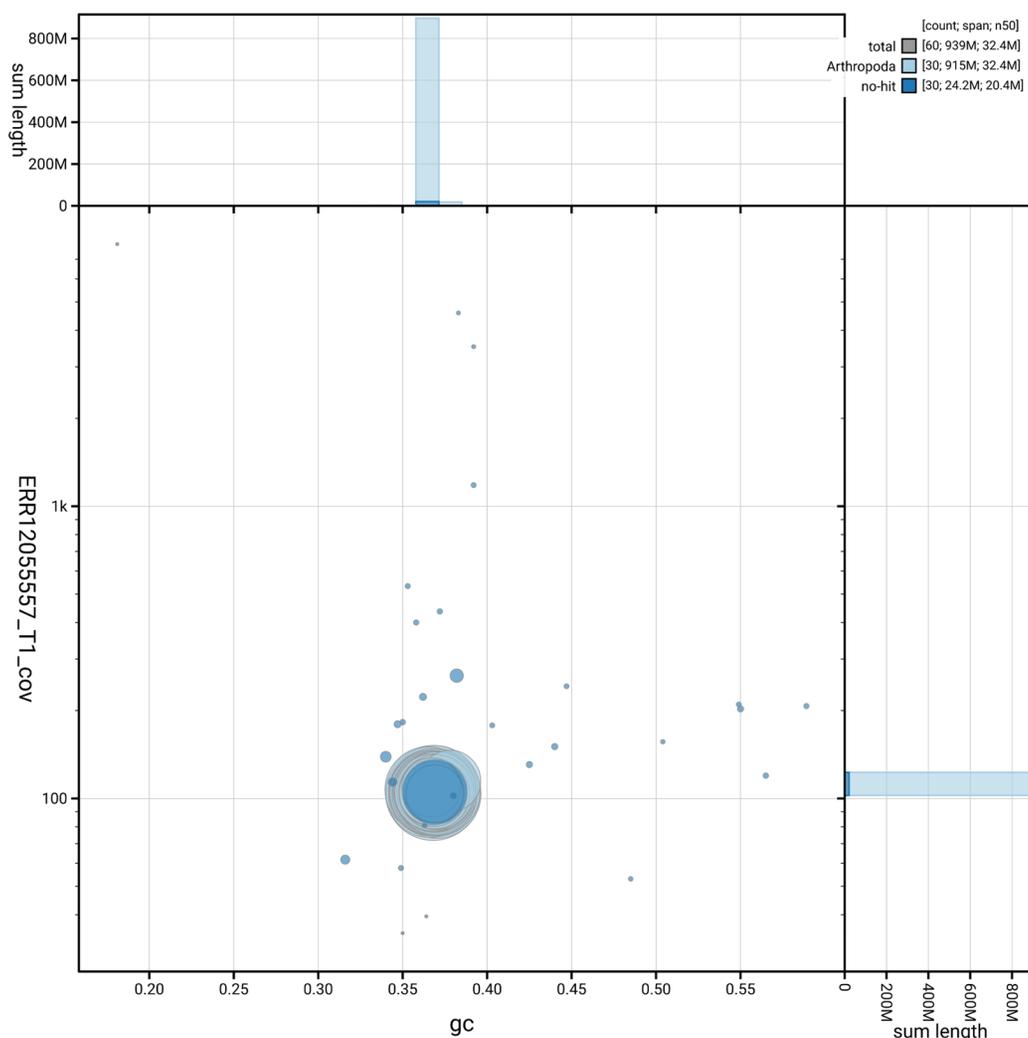


Figure 3. Genome assembly of *Plagodis dolabraria*, ilPlaDola2.1: BlobToolKit GC-coverage plot. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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 at https://blobtoolkit.genomehubs.org/view/GCA_963854805.1/blob.

remaining parts were shipped on dry ice to the Wellcome Sanger Institute (WSI) (Pereira *et al.*, 2022). The tissue was lysed, the COI marker region was amplified by PCR, and amplicons were sequenced and compared to the BOLD database, confirming the species identification (Crowley *et al.*, 2023). Following whole genome sequence generation, the relevant DNA barcode region was also used alongside the initial barcoding data for sample tracking at the WSI (Twyford *et al.*, 2024). The standard operating procedures for Darwin Tree of Life barcoding have been deposited on protocols.io (Beasley *et al.*, 2023).

Metadata collection for samples adhered to the Darwin Tree of Life project standards described by Lawniczak *et al.* (2022).

Nucleic acid extraction

The workflow for high molecular weight (HMW) DNA extraction at the Wellcome Sanger Institute (WSI) Tree of Life Core Laboratory includes a sequence of procedures: sample preparation and homogenisation, DNA extraction, fragmentation and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). The ilPlaDola2 sample was prepared for DNA extraction by weighing and dissecting it on dry ice (Jay *et al.*, 2023). Tissue from the thorax was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a).

HMW DNA was extracted in the WSI Scientific Operations core using the Automated MagAttract v2 protocol (Oatley *et al.*, 2023). The DNA was sheared into an average fragment size of

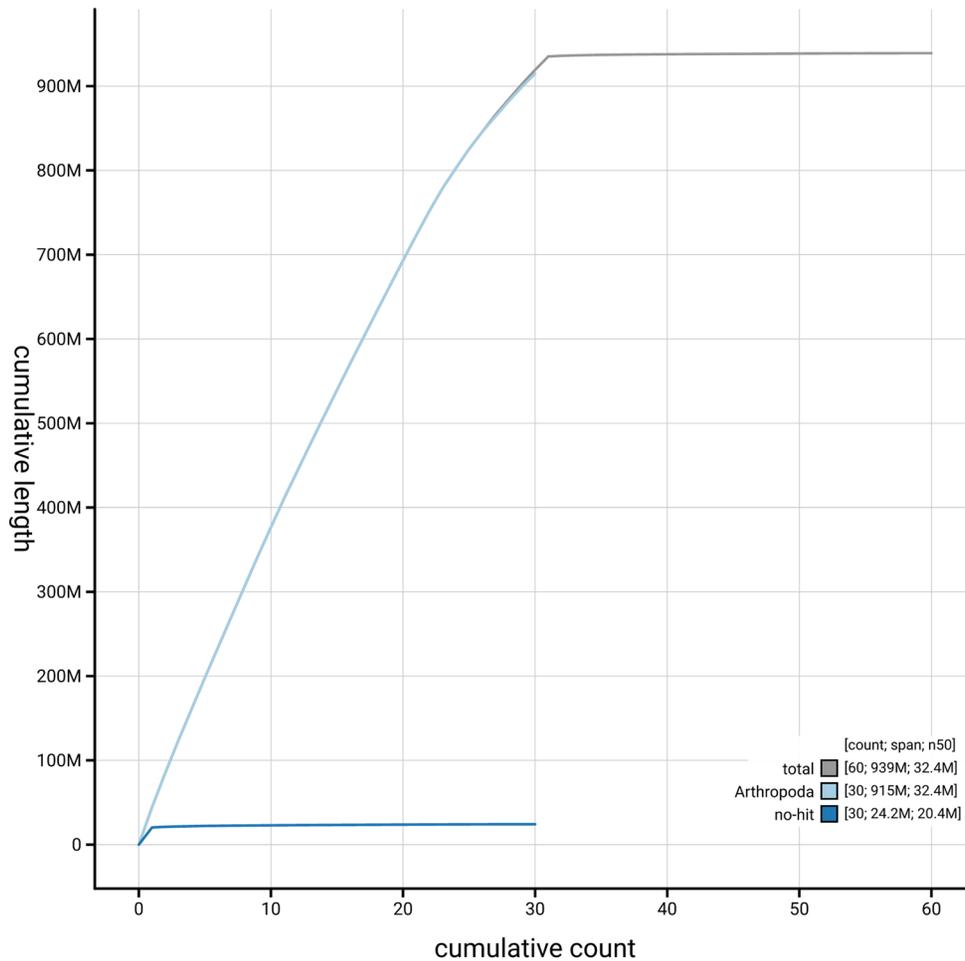


Figure 4. Genome assembly of *Plagodis dolabraria*, iPlaDola2.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963854805.1/dataset/GCA_963854805.1/cumulative.

12–20 kb in a Megaruptor 3 system (Bates *et al.*, 2023). Sheared DNA was purified by solid-phase reversible immobilisation, using AMPure PB beads to eliminate shorter fragments and concentrate the DNA (Strickland *et al.*, 2023). 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.

Hi-C sample preparation

Tissue from the head and thorax of the sample was processed for Hi-C sequencing at the WSI Scientific Operations core, using the Arima-HiC v2 kit. In brief, 20–50 mg of frozen tissue (stored at -80°C) was fixed, and the DNA crosslinked using a TC buffer with 22% formaldehyde concentration. After crosslinking, the tissue was homogenised using the Diagenode Power Masher-II and BioMasher-II tubes and pestles. Following the Arima-HiC v2 kit manufacturer's

instructions, crosslinked DNA was digested using a restriction enzyme master mix. The 5'-overhangs were filled in and labelled with biotinylated nucleotides and proximally ligated. An overnight incubation was carried out for enzymes to digest remaining proteins and for crosslinks to reverse. A clean up was performed with SPRIselect beads prior to library preparation. Additionally, the biotinylation percentage was estimated using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and Qubit HS Assay Kit and Arima-HiC v2 QC beads.

Library preparation and sequencing

Library preparation and sequencing were performed at the WSI Scientific Operations core.

PacBio HiFi

At a minimum, samples were required to have an average fragment size exceeding 8 kb and a total mass over 400 ng to proceed to the low input SMRTbell Prep Kit 3.0 protocol (Pacific Biosciences, California, USA), depending on genome size

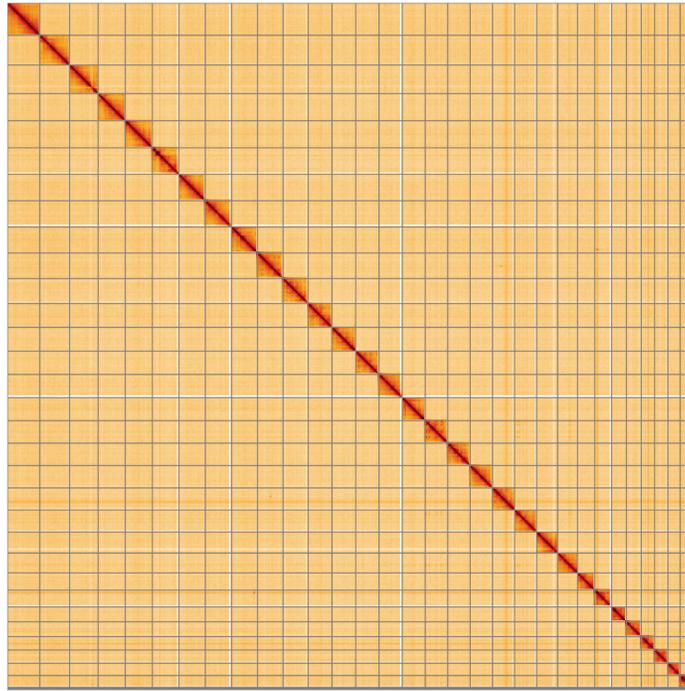


Figure 5. Genome assembly of *Plagodis dolabraria*: Hi-C contact map of the iPlaDola2.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/?d=B_zdvPqKT-aqIvovyTb0g.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Plagodis dolabraria*, iPlaDola2.

INSDC accession	Name	Length (Mb)	GC%
OY978441.1	1	40.63	37
OY978442.1	2	38.9	37
OY978443.1	3	37.05	36.5
OY978444.1	4	37.04	37
OY978445.1	5	36.2	36.5
OY978446.1	6	36.06	36.5
OY978447.1	7	35.86	37
OY978448.1	8	35.8	37
OY978449.1	9	34.63	37
OY978450.1	10	34.36	36.5
OY978451.1	11	32.47	37
OY978452.1	12	32.42	37
OY978453.1	13	31.94	37
OY978454.1	14	31.61	37
OY978455.1	15	31.53	37

INSDC accession	Name	Length (Mb)	GC%
OY978456.1	16	30.8	37
OY978457.1	17	30.73	37
OY978458.1	18	30.44	37
OY978459.1	19	30.24	37
OY978460.1	20	29.89	37
OY978461.1	21	29.02	37
OY978462.1	22	27.05	37
OY978463.1	23	23.61	37
OY978464.1	24	22.78	37
OY978465.1	25	20.39	37
OY978466.1	26	20.26	36.5
OY978467.1	27	18.29	38
OY978468.1	28	18.04	37
OY978469.1	29	16.87	36.5
OY978470.1	30	16.03	37
OY978440.1	Z	44.36	37
OY978471.1	MT	0.02	18

and sequencing depth required. Libraries were prepared using the SMRTbell Prep Kit 3.0 (Pacific Biosciences, California, USA) as per the manufacturer's instructions. The kit includes the reagents required for end repair/A-tailing, adapter ligation, post-ligation SMRTbell bead cleanup, and nuclease treatment. Following the manufacturer's instructions, size selection and clean up was carried out using diluted AMPure PB beads (Pacific Biosciences, California, USA). DNA concentration was quantified using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) with Qubit 1X dsDNA HS assay kit and the final library fragment size analysis was carried out using the Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies) and gDNA 55kb BAC analysis kit.

Samples were sequenced on a Revio instrument (Pacific Biosciences, California, USA). Prepared libraries were normalised to 2 nM, and 15 µL was used for making complexes. Primers were annealed and polymerases were hybridised to create circularised complexes according to manufacturer's instructions. The complexes were purified with the 1.2X clean up with SMRTbell beads. The purified complexes were then diluted to the Revio loading concentration (in the range 200–300 pM), and spiked with a Revio sequencing internal control. Samples were sequenced on Revio 25M SMRT cells (Pacific Biosciences, California, USA). The SMRT link software, a PacBio web-based end-to-end workflow manager, was used to set-up and monitor the run, as well as perform primary and secondary analysis of the data upon completion.

Hi-C

For Hi-C library preparation, DNA was fragmented using the Covaris E220 sonicator (Covaris) and size selected using SPRISelect beads to 400 to 600 bp. The DNA was then enriched using the Arima-HiC v2 kit Enrichment beads. Using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) for end repair, a-tailing, and adapter ligation. This uses a custom protocol which resembles the standard NEBNext Ultra II DNA Library Prep protocol but where library preparation occurs while DNA is bound to the Enrichment beads. For library amplification, 10 to 16 PCR cycles were required, determined by the sample biotinylation percentage. The Hi-C sequencing was performed using paired-end sequencing with a read length of 150 bp on an HiSeq X Ten instrument.

Genome assembly, curation and evaluation

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 first assembled using Hifiasm (Cheng *et al.*, 2021) with the --primary option. Haplotypic duplications were identified and removed using purge_dups (Guan *et al.*, 2020). The Hi-C reads were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). The contigs

were further scaffolded using the provided Hi-C data (Rao *et al.*, 2014) 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 (article in preparation). Flat files and maps used in curation were generated in TreeVal (Pointon *et al.*, 2023). Manual curation was primarily conducted using PretextView (Harry, 2022), with additional insights provided by JBrowse2 (Diesh *et al.*, 2023) and HiGlass (Kerpedjiev *et al.*, 2018). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Any identified contamination, missed joins, and mis-joins were corrected, and duplicate sequences were tagged and removed. Sex chromosomes were identified by synteny. The curation process is documented at <https://gitlab.com/wtsi-grit/rapid-curation> (article in preparation).

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 the primary and alternate haplotypes using the k -mer databases ($k = 31$) that were computed prior to genome assembly. The analysis outputs included assembly QV scores and completeness statistics.

A Hi-C contact map was produced for the final version of the assembly. The Hi-C reads were aligned using bwa-mem2 (Vasimuddin *et al.*, 2019) and the alignment files were combined using SAMtools (Danecek *et al.*, 2021). The Hi-C alignments were converted into a contact map using BEDTools (Quinlan & Hall, 2010) and the Cooler tool suite (Abdennur & Mirny, 2020). The contact map was visualised in HiGlass (Kerpedjiev *et al.*, 2018).

The blobtoolkit pipeline is a Nextflow port of the previous Snakemake Blobtoolkit pipeline (Challis *et al.*, 2020). It aligns the PacBio reads in SAMtools and minimap2 (Li, 2018) and generates coverage tracks for regions of fixed size. In parallel, it queries the GoT database (Challis *et al.*, 2023) to identify all matching BUSCO lineages to run BUSCO (Manni *et al.*, 2021). For the three domain-level BUSCO lineages, the pipeline aligns the BUSCO genes to the UniProt Reference Proteomes database (Bateman *et al.*, 2023) with DIAMOND blastp (Buchfink *et al.*, 2021). The genome is also divided into chunks according to the density of the BUSCO genes from the closest taxonomic lineage, and each chunk is aligned to the UniProt Reference Proteomes database using DIAMOND blastx. Genome sequences without a hit are chunked using seqtk

and aligned to the NT database with blastn (Altschul *et al.*, 1990). The blobtools suite combines all these 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), relying on the Conda package manager, the Bioconda initiative (Grüning *et al.*, 2018), the Biocontainers infrastructure (da Veiga Leprevost *et al.*, 2017), as well as the Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017) containerisation solutions.

Table 4 contains a list of relevant software tool versions and sources.

Wellcome Sanger Institute – Legal and Governance

The materials that have contributed to this genome note have been supplied by a Darwin Tree of Life Partner. The submission of materials by a Darwin Tree of Life Partner is subject to the ‘**Darwin Tree of Life Project Sampling Code of Practice**’, which can be found in full on the Darwin Tree of Life website [here](#). By agreeing with and signing up to the

Table 4. Software tools: versions and sources.

Software tool	Version	Source
BEDTools	2.30.0	https://github.com/arq5x/bedtools2
BLAST	2.14.0	http://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/tolkkit/fasta_windows
FastK	427104ea91c78c3b8b8b49f1a7d6bbeaa869ba1c	https://github.com/thegenemyers/FASTK
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
Goat CLI	0.2.5	https://github.com/genomehubs/goat-cli
Hifiasm	0.19.5-r587	https://github.com/chhylp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84aa44357826c0b6753eb28de	https://github.com/higlass/higlass
MercuryFK	d00d98157618f4e8d1a9190026b19b471055b22e	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
NCBI Datasets	15.12.0	https://github.com/ncbi/datasets
Nextflow	23.04.1	https://github.com/nextflow-io/nextflow
PretextView	0.2.5	https://github.com/sanger-tol/PretextView
purge_dups	1.2.5	https://github.com/dfguan/purge_dups
samtools	1.19.2	https://github.com/samtools/samtools
sanger-tol/ascc	-	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	0.5.1	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

Sampling Code of Practice, the Darwin Tree of Life Partner agrees they will meet the legal and ethical requirements and standards set out within this document in respect of all samples acquired for, and supplied to, the Darwin Tree of Life Project.

Further, 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 further undertaken according to a Research Collaboration Agreement or Material Transfer Agreement entered into by the Darwin Tree of Life Partner, Genome Research Limited (operating as the Wellcome Sanger Institute), and in some circumstances other Darwin Tree of Life collaborators.

Data availability

European Nucleotide Archive: *Plagodis dolabraria* (scorched wing). Accession number PRJEB66013; <https://identifiers.org/ena.embl/PRJEB66013>. The genome sequence is released openly for reuse. The *Plagodis dolabraria* genome sequencing initiative is part of the Darwin Tree of Life (DTOL) project.

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 the [Ensembl](#) pipeline at the European Bioinformatics Institute. Raw data and assembly accession identifiers are reported in [Table 1](#) and [Table 2](#).

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Maríndia Deprá

Federal University of Rio Grande do Sul, Porto Alegre, Brazil

The data note titled “The genome sequence of the Scorched Wing moth, *Plagodis dolabraria* (Linnaeus, 1767)” presents a well-structured genome assembly from a male specimen of *Plagodis dolabraria*. The study generated nuclear and mitochondrial genome sequences using advanced technologies, ensuring robust chromosomal assembly and accurate gene annotation.

The haploid genome size is 931.66 Mb, assigned to 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome is 16.97 kb long.

The genomic data are publicly accessible through the NCBI database (GCA_963854805.1), ensuring transparency and availability for further research. Additionally, the curation pipeline is available on GitHub, providing an open-source resource for validation and improvement. The study's protocols are appropriate, and the methodologies employed are technically sound. The detailed description of methods and materials allows for reproducibility, facilitating further research in this area. Moreover, the datasets are clearly presented in a usable and accessible format, enhancing their utility for the scientific community.

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: genetics and genomics

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

Reviewer Report 10 March 2025

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Laurence Despres 

Universite Savoie Mont Blanc, Auvergne-Rhône-Alpes, France

The authors report on the chromosome-level genome assembly of an individual male specimen of *Plagodis dolabraria* (the Scorched Wing; Geometridae). The initial identification by morphology of the two collected specimens was verified by an additional DNA barcoding process. Given the distinctiveness of the Scorched Wing, this additional sequencing step could have been avoided. I have some reserves regarding the systematic inclusion of a barcode process in the standardized DTOL procedures when it is not necessary; my main concern is keeping science as sober as possible.

The genome sequence is 939 megabases in span. Most of the assembly is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The Z chromosome was assigned by synteny to *Agriphila geniculea* (GCA_950108535.1). The assembly was performed using appropriate methods : Pac Bio HiFi long reads (90-fold coverage) and Hi-C data to refine scaffolding, manual curation of the final assembly, and contamination check, following the analysis pipeline and high standard of the Darwin Tree of Life Project for results presentation.

The mitogenome was also assembled. The quality of the assembly was further assessed by the proportion of complete BUSCO genes recovered, which is very high (>98% of Lepidoptera database BUSCO genes lepidoptera_odb10 set). Annotation was apparently not performed, although protein-coding predictions would be a useful addition to this high quality genome.

This high quality genome assembly will provide a reference genome for further genomic studies on this geometrid moth.

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

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.
