DATA NOTE

The genome sequence of the Straw-barred Pearl moth, *Pyrausta despicata* Scopoli, 1763

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a male specimen of *Pyrausta despicata* (Straw-barred Pearl; Arthropoda; Insecta; Lepidoptera; Crambidae). The genome sequence has a total length of 481.83 megabases. Most of the assembly (99.61%) is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled, with a length of 15.29 kilobases.

Keywords

Pyrausta despicata, Straw-barred Pearl, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 19 Mar 2025	✓ view	✓ view

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

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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; Pyraloidea; Crambidae; Pyraustinae; *Pyrausta*; *Pyrausta despicata* Scopoli, 1763 (NCBI:txid1281420)

Background

Found throughout Britain, *Pyrausta despicata* is a rather unassuming crambid moth, basically greyish-brown with obscure cross-lines and stigmata. The vernacular, Straw-barred Pearl, references the sharply defined cream-coloured lines across the hind wing. Coastal specimens, especially from the Isles of Scilly and the Shetlands, are more contrastingly patterned on the fore wings, apparently more closely resembling southern European specimens (Beirne, 1954). In the older literature, this species was usually called *Pyrausta cespitalis*.

As with other *Pyrausta* species, *P. despicata* is partly diurnal, flying in the sunshine, and comes to light at night. While the more strikingly patterned *Pyrausta* species feed on Lamiaceae, particularly mints and thymes, *P. despicata* larvae feed gregariously on the leaves of plantains, *Plantago* (Plantaginaceae). They stay within silken galleries near the base of the plant by day, feeding on the undersides of leaves by night (Beirne, 1954; Goater *et al.*, 1986). *Pyrausta despicata* requires open areas but accepts a wide variety of such habitats, including coastal shingle, grasslands and heath. Davis (2012) classified this as a common species in Britain, upgrading it from its previous categorisation as 'Local'. Adults are bivoltine, the first generation on the wing in the spring until early June and the second generation from July to September (Sterling *et al.*, 2023). The geographic range of *P. despicata* is extensive, from North Africa to Scandinavia and from Portugal to the Russian Far East, according to records on the GBIF.

The mitogenome of *P. despicata* has been sequenced (Wu *et al.*, 2023), but this is only the second full genome for a *Pyrausta* species, following that of *P. nigrata* (Lees *et al.*, 2024), with others in production. Comparative genomics of these closely related species will help in understanding why some *Pyrausta* species are thriving and others declining (Parsons & Clancy, 2023) and help resolve the increasingly well-sampled Crambidae phylogenetic tree (Léger *et al.*, 2021).

Genome sequence report

Sequencing data

The genome of a specimen of *Pyrausta despicata* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 78.73 Gb (gigabases) from 7.48 million reads. GenomeScope analysis of the PacBio HiFi data estimated the haploid genome size at 479.08 Mb, with a heterozygosity of 1.97% and repeat content of 31.24%. 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 159.0x coverage of the genome. Chromosome conformation Hi-C sequencing produced 121.30 Gb from 803.29 million reads. Table 1 summarises the specimen and sequencing information.

Figure 1. Photograph of the *Pyrausta despicata* (ilPyrDesp2) specimen used for genome sequencing.

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 four haplotypic duplications. These interventions increased the scaffold count by 1.49%. The final assembly has a total length of 481.83 Mb in 67 scaffolds, with 29 gaps, and a scaffold N50 of 16.62 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 (99.62%) 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, chromosome Z was assigned based on synteny to the genome of *Pyrausta aurata* (GCA_963584085.1).

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

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 *Pyrausta despicata*.**

Project information			
Study title	Pyrausta despicata (straw-barred pearl)		
Umbrella BioProject	PRJEB65670		
Species	*Pyrausta despicata*		
BioSpecimen	SAMEA111458735		
NCBI taxonomy ID	1281420		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	ilPyrDesp2	SAMEA111458806	whole organism
Hi-C sequencing	ilPyrDesp1	SAMEA8603673	whole organism
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq 6000	ERR12035243	8.03e+08	121.3
PacBio Revio	ERR12015734	7.48e+06	78.73

Table 2. **Genome assembly data for *Pyrausta despicata*.**

Genome assembly		
Assembly name	ilPyrDesp2.1	
Assembly accession	GCA_963921415.1	
Alternate haplotype accession	*GCA_963921445.1*	
Assembly level for primary assembly	chromosome	
Span (Mb)	481.83	
Number of contigs	96	
Number of scaffolds	67	
Longest scaffold (Mb)	19.84	
Assembly metric	**Measure**	***Benchmark***
Contig N50 length	11.59 Mb	*≥ 1 Mb*
Scaffold N50 length	16.62 Mb	*= chromosome N50*
Consensus quality (QV)	Primary: 67.3; alternate: 68.0; combined 67.6	*≥ 40*
k-mer completeness	Primary: 68.87%; alternate: 67.39%; combined: 99.79%	*≥ 95%*
BUSCO*	C:98.7%[S:98.5%,D:0.2%], F:0.2%,M:1.0%,n:5,286	*S > 90%; D < 5%*
Percentage of assembly mapped to chromosomes	99.62%	*≥ 90%*
Sex chromosomes	Z	*localised homologous pairs*
Organelles	Mitochondrial genome: 15.29 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.

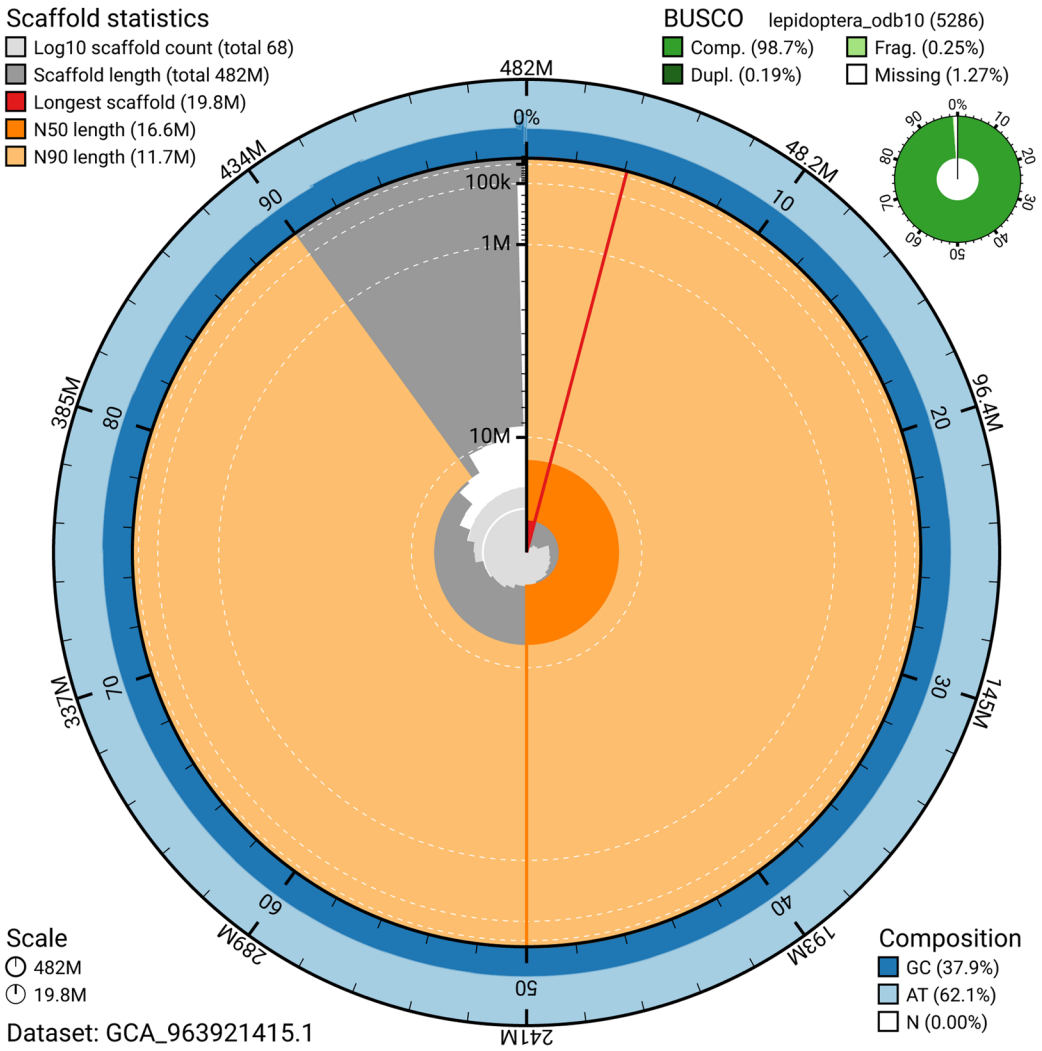

Figure 2. Genome assembly of *Pyrausta despicata*, ilPyrDesp2.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. 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, duplicated, 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_963921415.1/dataset/GCA_963921415.1/snail.

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

The combined primary and alternate assemblies achieve an estimated QV of 67.6. The *k*-mer recovery for the primary haplotype is 68.87%, and for the alternate haplotype 67.39%; the combined primary and alternate assemblies have a *k*-mer recovery of 99.79%. BUSCO analysis using the lepidoptera_odb10 reference set (n = 5,286) identified 98.7% of the expected gene set (single = 98.5%, duplicated = 0.2%).

Table 2 provides assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project (EBP) Report on Assembly Standards September 2024. The assembly achieves the EBP reference standard of **7.C.Q67**.

Methods

Sample acquisition and DNA barcoding

An adult male *Pyrausta despicata* (specimen ID NHMUK 014425859, ToLID ilPyrDesp2) was collected from Tonbridge, England, United Kingdom (latitude 51.18, longitude 0.29) on 2021-09-01 by actinic light. The specimen was collected by

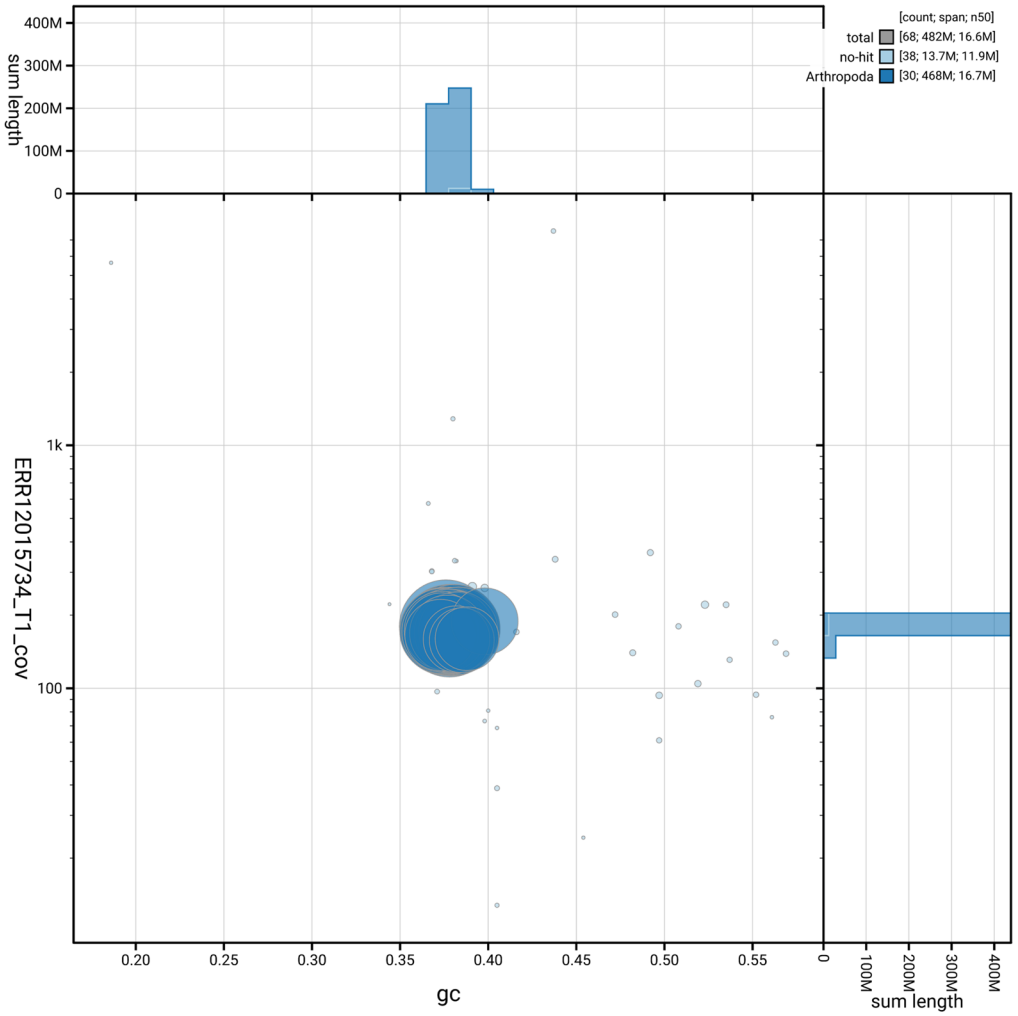

Figure 3. Genome assembly of *Pyrausta despicata*, ilPyrDesp2.1: BlobToolKit GC-coverage plot. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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_963921415.1/dataset/GCA_963921415.1/blob.

Gavin Broad (Natural History Museum), identified by David Lees (Natural History Museum) and preserved by dry freezing (–80 °C).

The specimen used for Hi-C sequencing (specimen ID Ox000962, ToLID ilPyrDesp1) was a collected from Wytham Woods, Oxfordshire, United Kingdom (latitude 51.77, longitude –1.34) on 2020-09-08 using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice.

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 the specimen and stored in ethanol, while the 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

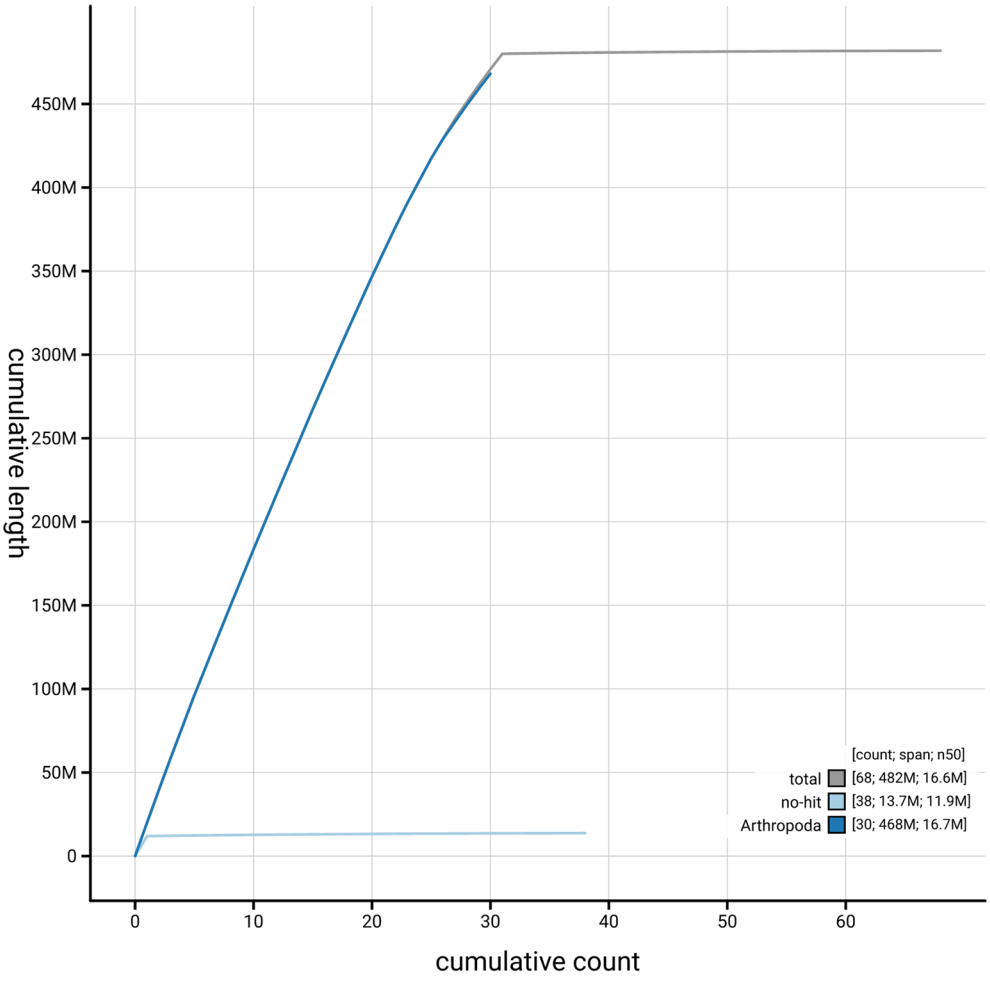

Figure 4. Genome assembly of *Pyrausta despicata,* ilPyrDesp2.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963921415.1/dataset/GCA_963921415.1/cumulative.

purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). The ilPyrDesp2 sample was prepared for DNA extraction by weighing and dissecting it on dry ice (Jay *et al.*, 2023). Tissue from the whole organism 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 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 whole organism of the ilPyrDesp1 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 Diagnocine 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.

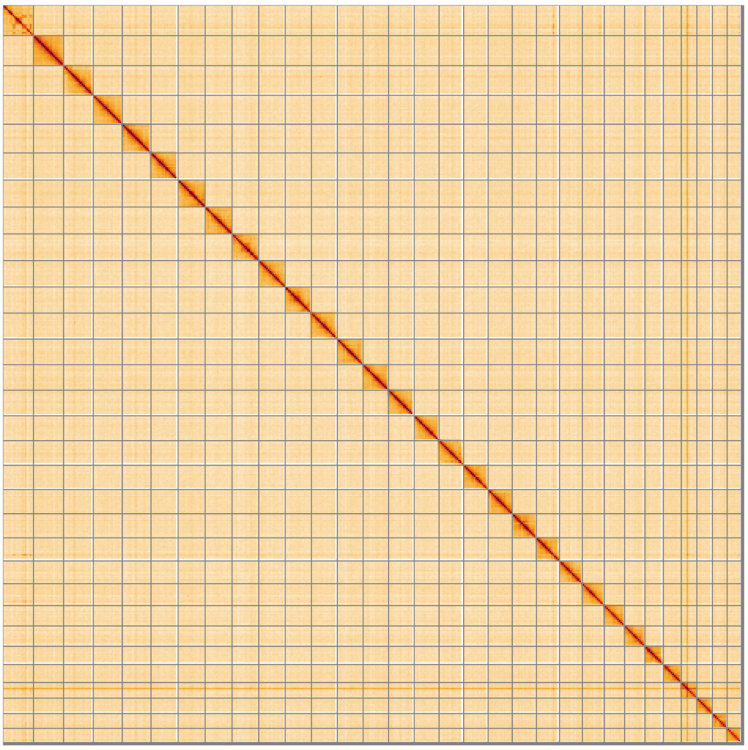

Figure 5. Genome assembly of *Pyrausta despicata:* Hi-C contact map of the ilPyrDesp2.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=Rgrq5JlSRYWvdUb5eRNsLg.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Pyrausta despicata*, ilPyrDesp2.

INSDC accession	Name	Length (Mb)	GC%
OY992844.1	1	19.84	37.5
OY992846.1	2	19.21	38
OY992847.1	3	18.84	37.5
OY992848.1	4	18.71	38
OY992849.1	5	17.62	37.5
OY992850.1	6	17.62	38
OY992851.1	7	17.5	37.5
OY992852.1	8	17.34	37.5
OY992853.1	9	17.22	38
OY992854.1	10	16.97	37.5
OY992855.1	11	16.93	37.5
OY992856.1	12	16.7	38
OY992857.1	13	16.62	38
OY992858.1	14	16.61	37.5
OY992859.1	15	16.23	38
OY992860.1	16	15.94	38.5
OY992861.1	17	15.92	38
OY992862.1	18	15.71	38
OY992863.1	19	15.67	37.5
OY992864.1	20	14.98	37.5
OY992865.1	21	14.89	38
OY992866.1	22	14.24	38.5
OY992867.1	23	13.28	37.5
OY992868.1	24	13.24	37.5
OY992869.1	25	11.88	38
OY992870.1	26	11.67	37.5
OY992871.1	27	10.16	40
OY992872.1	28	10.09	38
OY992873.1	29	9.55	38.5
OY992874.1	30	9.23	39
OY992845.1	Z	19.59	38
OY992875.1	MT	0.02	19

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 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 Illumina NovaSeq 6000 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. Flat files and maps used in curation were generated via the TreeVal pipeline (Pointon *et al.*, 2023). Manual curation was conducted primarily in PretextView (Harry, 2022) and HiGlass (Kerpedjiev *et al.*, 2018), with additional insights provided by JBrowse2 (Diesh *et al.*, 2023). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Any identified contamination, missed joins, and mis-joins were amended, and duplicate sequences were tagged and removed. The sex chromosome was identified by synteny analysis. The curation process is documented at https://gitlab.com/wtsi-grit/rapid-curation.

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 GoaT 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

Table 4. Software tools: versions and sources.

Software tool	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	666652151335353eef2fcd58880bcef5bc2928e1	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	44086069ee7d4d3f6f3f0012569789ec138f42b84a a44357826c0b6753eb28de	https://github.com/higlass/higlass
MerquryFK	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
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

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 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: Pyrausta despicata (straw-barred pearl). Accession number PRJEB65670; https://identifiers.org/ena.embl/PRJEB65670. The genome sequence is released openly for reuse. The *Pyrausta despicata* genome sequencing initiative is part of the Darwin Tree of Life (DToL) project (PRJEB40665) 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 the Ensembl pipeline at the European Bioinformatics Institute. Raw data and assembly accession identifiers are reported in Table 1 and Table 2.

Author information

Members of the University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.12157525.

Members of the Natural History Museum Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.12159242.

Members of the Darwin Tree of Life Barcoding collective are listed here: https://doi.org/10.5281/zenodo.12158331.

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Members of Wellcome Sanger Institute Scientific Operations: Sequencing Operations are listed here: https://doi.org/10.5281/zenodo.14870789.

Members of the Wellcome Sanger Institute Tree of Life Core Informatics team are listed here: https://doi.org/10.5281/zenodo.12160324.

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Members of the Darwin Tree of Life Consortium are listed here: https://doi.org/10.5281/zenodo.4783558.

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Open Peer Review

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Version 1

Reviewer Report 29 May 2025

https://doi.org/10.21956/wellcomeopenres.26369.r121029

✔ **Alan G Buddie**
CABI, Wallingford, England, UK

'The genome sequence of the Straw-barred Pearl moth, *Pyrausta despicata* Scopoli, 1763' is a very clearly written description of the genome assembly of the genome sequence of this moth which, the website 'https://www.ukmoths.org.uk/species/pyrausta-despicata/' confirms, is found throughout the British Isles. The 'Background' section notes the broader distribution of *P. despicata*, its common name, general appearance, feeding habits and reproduction mode. There is a strong justification for the publication of this genome: only one other species of the genus has had its full genome sequenced (although the mitogenome of a specimen of *P. despicata* has been described previously). Such gap-filling will help with the family-level phylogeny.

I am certainly no expert in this field but the 'Genome sequence report' section appears lucid, with relevant data and statistics being provided and the respective tables and figures being introduced with appropriate justification.

The 'Methods' section has sufficient clarity to enable anyone, who wishes to do so, to replicate the study. The section explains that the two specimens used for HiC and HiFi sequencing, respectively, were each examined morphologically and DNA-barcoded. Whilst many of the software packages used (and the parameters mentioned) were unfamiliar to me, they seemed to have been selected with a clear and sensible rationale.

Information on 'Data availability' and the 'Author information' are presented clearly, with appropriate (functional) web links. The reference list appears full and accurate.
Overall, then, this manuscript appears to me to be a worthy addition to the growing list of DToL 'gold standard' genomes and, for me, warrants its publication in *Wellcome Open Research*. Whilst this genome note sits well on its own, its overall significance will only be fully realised when all the other species in the genus (and in the family) have been sequenced, too.

I have couple of very minor typographical edits and two comments, but these do not detract from the very good quality of the manuscript.

Typographical edits:
In the paragraph regarding the specimen for HiC sequencing (p6) there is a rogue 'a' immediately before "collected from Wytham Woods..." Please delete the 'a'.
There is inconsistency in the term 'cleanup'. It is referred to, as "cleanup" or "clean up". I would prefer to see it given as the former (or as 'clean-up') when referring to DNA clean-up, or similar.

Comments:
For interested non-experts, it would be great to have terms such as 'BUSCO' defined – but I appreciate that this may make the manuscript considerably longer.
For additional clarity, it would be good to have program parameters (e.g. '- -primary option') given in a different font. This could also be applied usefully to software package names.

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: Only the most tangential of competing interests: I have worked, in a general way, with the senior author and other DToL consortium members involved in this paper, in other parts of the DToL project/programme. Despite this loose connection, I consider my review to have been entirely objective and not, in any way, influenced by my involvement in the DToL project/programme.

Reviewer Expertise: Mycology, molecular biology and phylogenetics.

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 May 2025

https://doi.org/10.21956/wellcomeopenres.26369.r122258

Nicola J Nadeau
The University of Sheffield, Sheffield, UK

There is sufficient background on the species. The methods are appropriate and the resulting genome assembly appears to be of good quality. The data and assemblies are available and accessible. Transciptome data are also available in the same project accession. The annotation using this data will increase the value to untility of this genome.

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 genetics and genomics. Butterfly evolution and genetics.

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.