DATA NOTE

The genome sequence of the White-spotted Pug moth, *Eupithecia tripunctaria* Herrich-Schäffer, 1852 [version 1; peer review: awaiting peer review]

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Abstract
We present a genome assembly from an individual female *Eupithecia tripunctaria* (the White-spotted Pug moth; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence has a total length of 494.80 megabases. Most of the assembly is scaffolded into 32 chromosomal pseudomolecules, including the Z and W sex chromosomes. The mitochondrial genome has also been assembled and is 16.51 kilobases in length. Gene annotation of this assembly on Ensembl identified 12,799 protein-coding genes.

Keywords
Eupithecia tripunctaria, White-spotted Pug moths, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

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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; Larentiinae; *Eupithecia*; *Eupithecia tripunctaria* Herrich-Schäffer, 1852 (NCBI:txid934860).

Background

The genus *Eupithecia*, commonly known as the 'Pug moths', contains over a thousand species of geometrid moth found across Eurasia, Africa and the Americas, and is one of the largest genus in the family Geometridae (Mironov & Galsworthy, 2014). Although Pug moths may easily be distinguished from other groups, it is difficult to separate different species within the genus, as most are small and brown or grey in colour (Waring *et al.*, 2017). Even in a limited geographic region such as Britain and Ireland, with fewer than 50 species, several are difficult to distinguish based on wing pattern alone.

A distinguishing feature of the White-spotted Pug, *Eupithecia tripunctaria*, is the presence of white spots on the trailing corner of the fore- and hindwings, although sometimes the spots may become worn with age or damaged (Waring *et al.*, 2017). The spots are sometimes linked by a pale waved line that follows the margin of both wings. Care should still be taken to separate it from other related species.

Eupithecia tripunctaria is distributed through the Palaearctic realm as well as North America. The species prefers lowland forests, forest edges and damp meadows. It is widely distributed throughout Britain, with the exception of northern Scotland, and it can be locally common (Kimber, 2024). It has two generations in the UK, with adults on the wing in May and June and then in July and August (Waring *et al.*, 2017). It overwinters as a pupa in plant debris or loose soil, and the larval stage is from late June to late September. The first generation of larvae feed on the flowers of elder (*Sambucus nigra*), and the second on the flowers and seeds of wild angelica (*Angelica sylvestris*), hogweed (*Heracleum*), and common ragwort (*Senecio jacobaea*) (Waring *et al.*, 2017).

The genome of the White-spotted Pug, *Eupithecia tripunctaria*, was sequenced as part of the Darwin Tree of Life Project, a collaborative effort to sequence all named eukaryotic species in the Atlantic Archipelago of Britain and Ireland. Here we present a chromosomally complete genome sequence for *Eupithecia tripunctaria*, based on one female specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome of an adult female of *Eupithecia tripunctaria* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating a total of 15.45 Gb (gigabases) from 1.06 million reads, providing approximately 29-fold coverage. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data, which produced 92.91 Gb from 615.29 million reads, yielding an approximate coverage of 188-fold. Specimen and sequencing information is summarised in Table 1.

Figure 1. Photograph of the *Eupithecia tripunctaria* (ilEupTrip1) specimen used for genome sequencing.

Manual assembly curation corrected 19 missing joins or mis-joins and 7 haplotypic duplications, reducing the assembly length by 0.69% and the scaffold number by 1.16%. The final assembly has a total length of 494.80 Mb in 84 sequence scaffolds with a scaffold N50 of 16.3 Mb (Table 2). The total count of gaps in the scaffolds is 40. The snail plot in Figure 2 provides a summary of the assembly statistics, while the distribution of assembly scaffolds on GC proportion and coverage is shown in Figure 3. The cumulative assembly plot in Figure 4 shows curves for subsets of scaffolds assigned to different phyla. Most (99.89%) of the assembly sequence was assigned to 32 chromosomal-level scaffolds, representing 30 autosomes and the Z and W sex chromosomes. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 5; Table 3). The W chromosome not scaffolded due to lack of Hi-C signal (Hi-C data was likely from a ZZ male). While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited. The mitochondrial genome was also assembled and can be found as a contig within the multifasta file of the genome submission.

The estimated Quality Value (QV) of the final assembly is 65.4 with *k*-mer completeness of 100.0%, and the assembly has a BUSCO v5.3.2 completeness of 98.0% (single = 97.2%, duplicated = 0.8%), using the lepidoptera_odb10 reference set (n = 5,286).

Metadata for specimens, BOLD barcode results, spectra estimates, sequencing runs, contaminants and pre-curation assembly statistics are given at https://links.tol.sanger.ac.uk/species/934860.

Genome annotation report

The *Eupithecia tripunctaria* genome assembly (GCA_955876795.1) was annotated at the European Bioinformatics Institute (EBI) on Ensembl Rapid Release. The resulting annotation includes 24,118 transcribed mRNAs from

Table 1. Specimen and sequencing data for *Eupithecia tripunctaria*.

Project information			
Study title	*Eupithecia tripunctaria* (white-spotted pug)		
Umbrella BioProject	PRJEB55976		
Species	*Eupithecia tripunctaria*		
BioSample	SAMEA7701546		
NCBI taxonomy ID	934860		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	ilEupTrip1	SAMEA7701735	whole_organism
Hi-C sequencing	ilEupTrip2	SAMEA112233221	whole_organism
RNA sequencing	ilEupTrip2	SAMEA112233221	whole_organism
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq 6000	ERR11468727	6.15e+08	92.91
PacBio Sequel IIe	ERR10224914	1.06e+06	15.45
RNA Illumina NovaSeq 6000	ERR11837456	7.85e+07	11.85

Table 2. Genome assembly data for *Eupithecia tripunctaria*, ilEupTrip1.1.

Genome assembly		
Assembly name	ilEupTrip1.1	
Assembly accession	GCA_955876795.1	
Accession of alternate haplotype	*GCA_955838965.1*	
Span (Mb)	494.80	
Number of contigs	125	
Contig N50 length (Mb)	8.4	
Number of scaffolds	84	
Scaffold N50 length (Mb)	16.3	
Longest scaffold (Mb)	29.0	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	65.4	*≥ 50*
k-mer completeness	100.0%	*≥ 95%*
BUSCO**	C:98.0%[S:97.2%,D:0.8%], F:0.6%,M:1.5%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.89%	*≥ 95%*
Sex chromosomes	ZW	*localised homologous pairs*
Organelles	Mitochondrial genome: 16.51 kb	*complete single alleles*
Genome annotation of assembly GCA_955876795.1 at Ensembl		
Number of protein-coding genes	12,799	
Number of non-coding genes	2,014	
Number of gene transcripts	24,118	

* Assembly metric benchmarks are adapted from column VGP-2020 of "Table 1: Proposed standards and metrics for defining genome assembly quality" from Rhie *et al.* (2021).

** BUSCO scores based on the lepidoptera_odb10 BUSCO set using version 5.3.2. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison. A full set of BUSCO scores is available at https://blobtoolkit.genomehubs.org/view/ilEupTrip1_1/dataset/ilEupTrip1_1/busco.

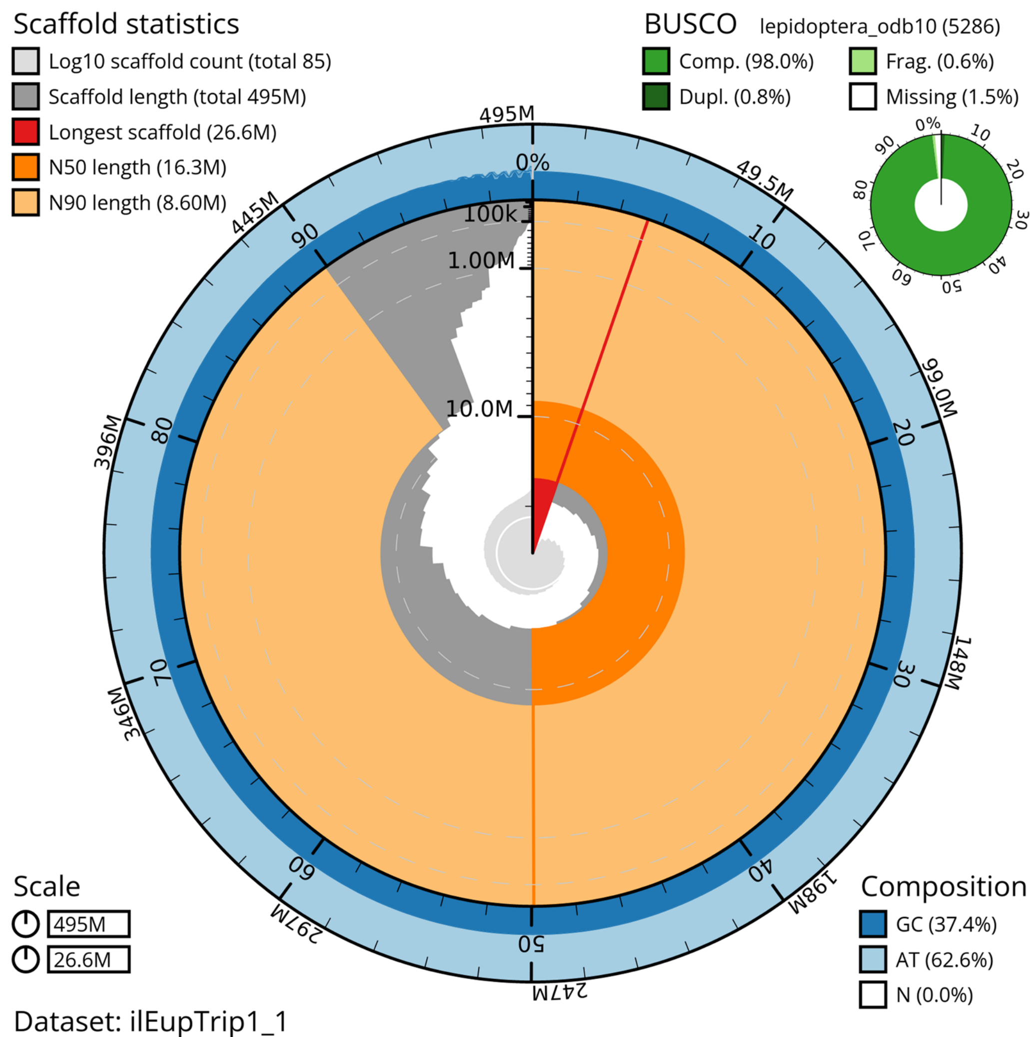

Figure 2. Genome assembly of *Eupithecia tripunctaria*, ilEupTrip1.1: metrics. The BlobToolKit snail plot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 494,829,581 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (26,572,000 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (16,304,492 and 8,597,702 bp), respectively. The pale grey spiral shows the cumulative scaffold count on a log scale with white scale lines showing successive orders of magnitude. The blue and pale-blue area around the outside of the plot shows the distribution of GC, AT and N percentages in the same bins as the inner plot. A summary of complete, fragmented, duplicated and missing BUSCO genes in the lepidoptera_odb10 set is shown in the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilEupTrip1_1/dataset/ilEupTrip1_1/snail.

12,799 protein-coding and 2,014 non-coding genes (Table 2; https://rapid.ensembl.org/Eupithecia_tripunctaria_GCA_955876795.1/Info/Index). The average transcript length is 16,849.91. There are 1.63 coding transcripts per gene and 7.66 exons per transcript.

Methods

Sample acquisition and DNA barcoding

An adult female of *Eupithecia tripunctaria* (specimen ID Ox000685, ToLID ilEupTrip1) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2020-07-20, using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice.

The specimen used for Hi-C and RNA sequencing (specimen ID Ox002534, ToLID ilEupTrip2) was an adult specimen collected from the same location on 2022-07-22, also using a light trap. The specimen was collected by James McCulloch and Liam Crowley (University of Oxford) and identified by James McCulloch, and then preserved on dry ice.

The initial species identification was verified by an additional DNA barcoding process according to the framework

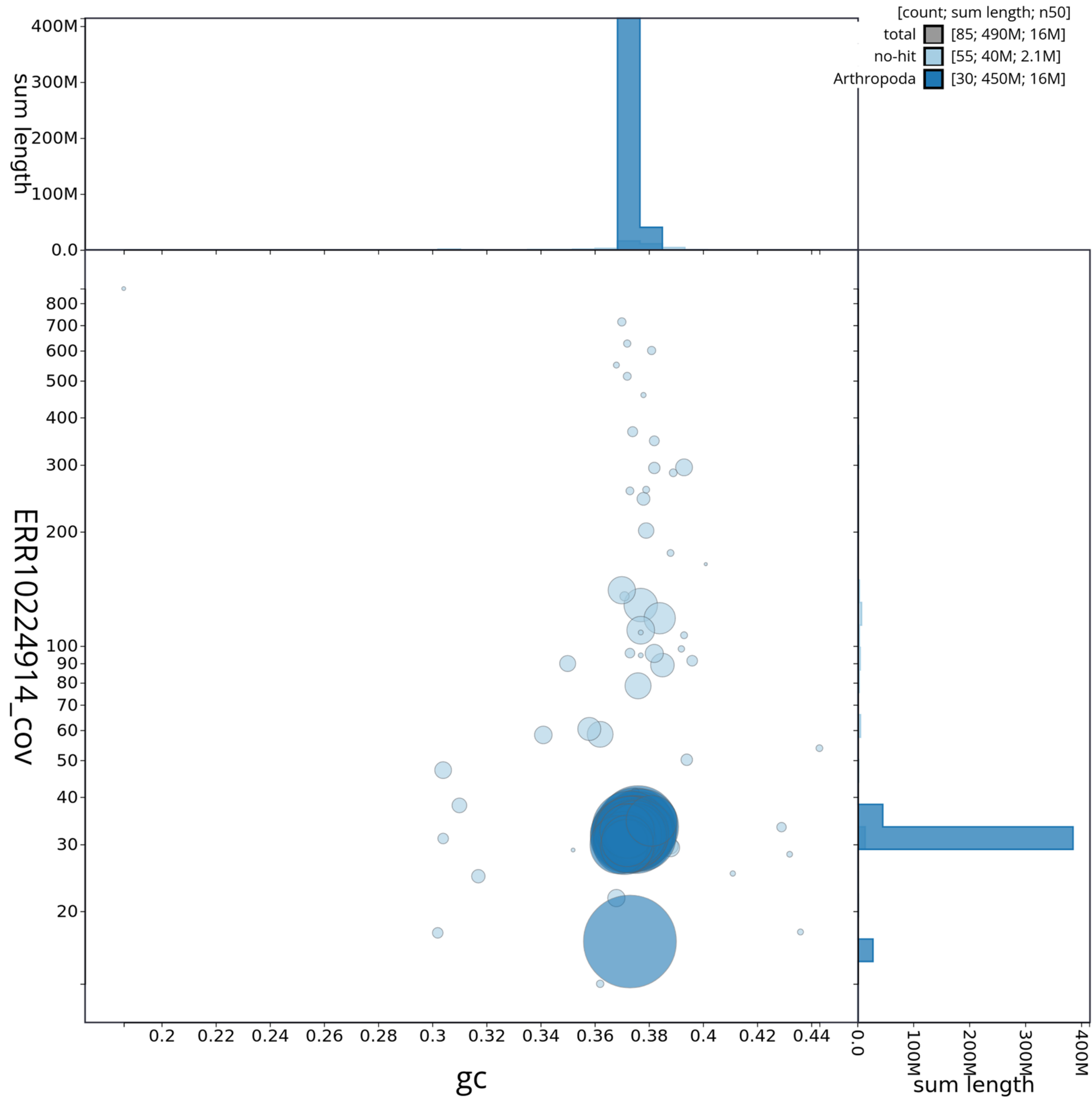

Figure 3. Genome assembly of *Eupithecia tripunctaria*, ilEupTrip1.1: Blob plot of base coverage against GC proportion for scaffolds in the assembly. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilEupTrip1_1/dataset/ilEupTrip1_1/blob.

developed by Twyford *et al.* (2024). A small sample was dissected from the specimens and stored in ethanol, while the remaining parts of the specimen were shipped on dry ice to the Wellcome Sanger Institute (WSI). 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).

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 core procedures: sample preparation and homogenisation, DNA extraction, fragmentation and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). The ilEupTrip1 sample was weighed and dissected on dry ice (Jay *et al.*, 2023).

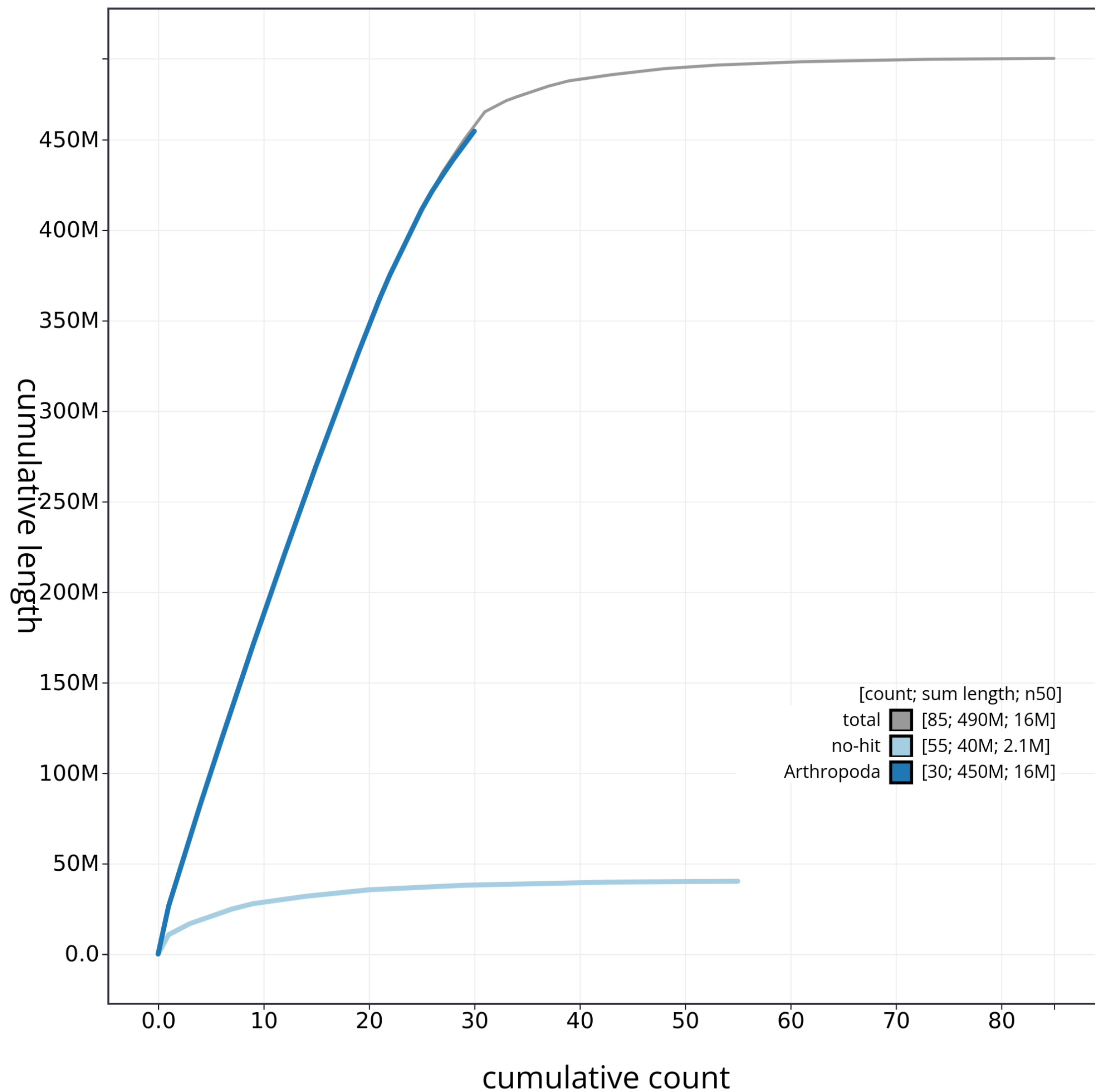

Figure 4. Genome assembly of *Eupithecia tripunctaria* ilEupTrip1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all sequences. Coloured lines show cumulative lengths of sequences assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilEupTrip1_1/dataset/ilEupTrip1_1/cumulative.

Tissue from the whole organism of the sample was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a).

HMW DNA was extracted using the Automated MagAttract v1 protocol (Sheerin *et al.*, 2023). DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system (Todorovic *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.

RNA was extracted from whole organism tissue of ilEupTrip2 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ *mir*Vana protocol (do Amaral *et al.*, 2023). The RNA concentration was assessed using a Nanodrop spectrophotometer and a Qubit Fluorometer using the Qubit RNA Broad-Range Assay kit. Analysis of the integrity of the RNA was done using the Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

Sequencing

Pacific Biosciences HiFi circular consensus DNA sequencing libraries were constructed according to the manufacturers' instructions. Poly(A) RNA-Seq libraries were constructed using the NEB Ultra II RNA Library Prep kit. DNA and RNA sequencing was performed by the Scientific Operations

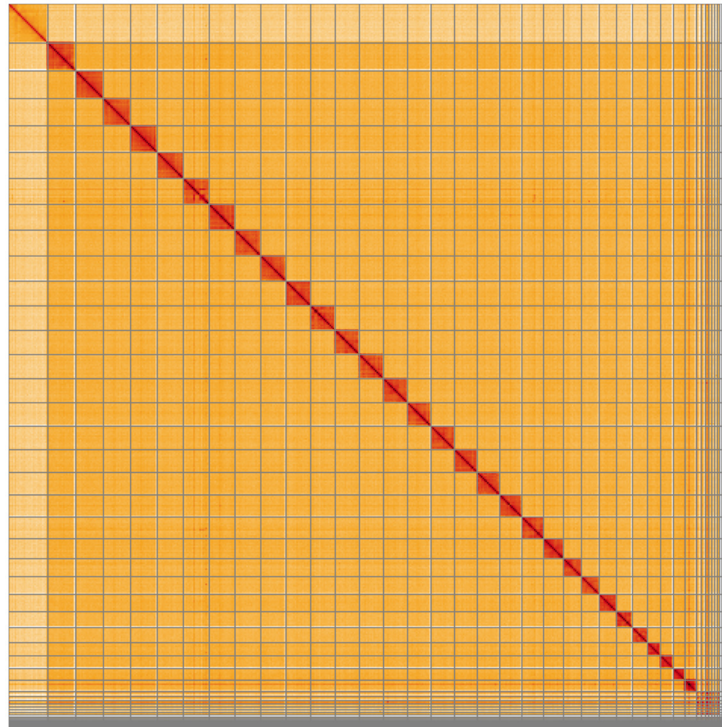

Figure 5. Genome assembly of *Eupithecia tripunctaria* ilEupTrip1.1: Hi-C contact map of the ilEupTrip1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=FpU-jysxR7mODySu8zuRcg.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Eupithecia tripunctaria*, ilEupTrip1.

INSDC accession	Name	Length (Mb)	GC%
OY041600.1	1	18.9	37.5
OY041601.1	2	18.72	37.5
OY041602.1	3	18.34	37.5
OY041603.1	4	18.06	37.5
OY041604.1	5	17.65	37.5
OY041605.1	6	17.48	37.5
OY041606.1	7	17.46	37.5
OY041607.1	8	17.43	37.5
OY041608.1	9	17.05	37.5
OY041609.1	10	16.67	37.5
OY041610.1	11	16.66	37.0
OY041611.1	12	16.32	37.5
OY041612.1	13	16.3	37.0
OY041613.1	14	16.29	37.5
OY041614.1	15	15.88	37.5
OY041615.1	16	15.86	37.5
OY041616.1	17	15.39	37.5
OY041617.1	18	15.2	37.5
OY041618.1	19	14.96	37.5
OY041619.1	20	14.63	37.5
OY041620.1	21	13.51	37.5
OY041621.1	22	12.2	37.5
OY041622.1	23	12.0	37.0
OY041623.1	24	11.74	37.5
OY041624.1	25	10.69	37.5
OY041625.1	26	10.21	37.0
OY041626.1	27	8.89	37.0
OY041627.1	28	8.6	37.0
OY041628.1	29	7.94	37.0
OY041629.1	30	7.72	38.0
OY041598.1	W	3.25	37.5
OY041599.1	Z	26.57	37.5
OY041630.1	MT	0.02	18.5

core at the WSI on Pacific Biosciences Sequel IIe (HiFi) and Illumina NovaSeq 6000 (RNA-Seq) instruments.

Hi-C data were generated from frozen tissue of ilEupTrip2, using the Arima-HiC v2 kit. The tissue was fixed with a TC buffer containing formaldehyde, resulting in crosslinked DNA. The crosslinked DNA was digested with a restriction enzyme master mix. The resulting 5'-overhangs were filled in and labelled with a biotinylated nucleotide. The biotinylated DNA was then fragmented, enriched, barcoded, and amplified using the NEBNext Ultra II DNA Library Prep Kit. Hi-C sequencing was performed on an Illumina NovaSeq 6000 instrument, using paired-end sequencing with a read length of 150 bp.

Genome assembly, curation and evaluation

Assembly

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. 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). 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 based on read coverage statistics. The curation process is documented at https://gitlab.com/wtsi-grit/rapid-curation (article in preparation).

Evaluation of the final assembly

A Hi-C map for the final assembly was produced using bwa-mem2 (Vasimuddin *et al.*, 2019) in the Cooler file format (Abdennur & Mirny, 2020). To assess the assembly metrics, the *k*-mer completeness and QV consensus quality values were calculated in Merqury (Rhie *et al.*, 2020). This work was done using the "sanger-tol/readmapping" (Surana *et al.*, 2023a) and "sanger-tol/genomenote" (Surana *et al.*, 2023b) pipelines. The genome readmapping pipelines were developed using the nf-core tooling (Ewels *et al.*, 2020), use MultiQC (Ewels *et al.*, 2016), and make extensive use of the Conda package manager, the Bioconda initiative (Grüning *et al.*, 2018), the Biocontainers infrastructure (da Veiga Leprevost *et al.*, 2017), and the Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017) containerisation solutions. The genome was also analysed within the BlobToolKit environment (Challis *et al.*, 2020) and BUSCO scores (Manni *et al.*, 2021) were calculated. Table 4 contains a list of relevant software tool versions and sources.

Table 4. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.2.1	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	https://gitlab.com/ezlab/busco
bwa-mem2	2.2.1	https://github.com/bwa-mem2/bwa-mem2
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
Hifiasm	0.16.1-r375	https://github.com/chhylp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Merqury.FK	d00d98157618f4e8d1a91 90026b19b471055b22e	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	3	https://github.com/marcelauliano/MitoHiFi
PretextView	0.2	https://github.com/wtsi-hpag/PretextView
purge_dups	1.2.5	https://github.com/dfguan/purge_dups
sanger-tol/genomenote	v1.0	https://github.com/sanger-tol/genomenote
sanger-tol/readmapping	1.1.0	https://github.com/sanger-tol/readmapping/tree/1.1.0
YaHS	1.2a.2	https://github.com/c-zhou/yahs

Genome annotation
The Ensembl Genebuild annotation system (Aken *et al.*, 2016) was used to generate annotation for the *Eupithecia tripunctaria* assembly (GCA_955876795.1) in Ensembl Rapid Release at the EBI. Annotation was created primarily through alignment of transcriptomic data to the genome, with gap filling via protein-to-genome alignments of a select set of proteins from UniProt (UniProt Consortium, 2019).

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 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: *Eupithecia tripunctaria* (white-spotted pug). Accession number PRJEB55976; https://identifiers.org/ena.embl/PRJEB55976 (Wellcome Sanger Institute, 2023). The genome sequence is released openly for reuse. The *Eupithecia tripunctaria* 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. 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 Darwin Tree of Life Barcoding collective are listed here: https://doi.org/10.5281/zenodo.12158331.

Members of the Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team are listed here: https://doi.org/10.5281/zenodo.12162482.

Members of Wellcome Sanger Institute Scientific Operations: Sequencing Operations are listed here: https://doi.org/10.5281/zenodo.12165051.

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

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.12205391.

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

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