DATA NOTE

The genome sequence of the Pale Pinion, *Lithophane socia* (Hufnagel, 1766) [version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual male *Lithophane socia* (the Pale Pinion; Arthropoda; Insecta; Lepidoptera; Noctuidae). The genome sequence is 489.3 megabases in span. Most of the assembly is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 15.39 kilobases in length. Gene annotation of this assembly on Ensembl identified 18,342 protein coding genes.

Keywords

Lithophane socia, Pale Pinion, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status *AWAITING PEER REVIEW*

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; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Noctuoidea; Noctuidae; Cuculliinae; *Lithophane*; *Lithophane socia* (Hufnagel, 1766) (NCBI:txid997538).

Background

The Pale Pinion *Lithophane socia* (synonym *L. hepatica*) is a moth in the family Noctuidae with a patchy distribution across Eurasia. The species has been recorded most frequently from southern Britain, southern Scandinavia, Austria and Switzerland, with scattered records from several other European countries, together with Russia, China and Japan (GBIF Secretariat, 2023). The adult moth has pale buff wings, suffused with black in females. The species is found predominantly in woodland, although usually at low densities (Waring *et al.*, 2017).

L. socia has a single generation each year in Britain. Adults are on the wing in September and October when they are attracted to ivy blossom and other nectar sources, and then again in March and April after winter hibernation (NBN Atlas Partnership, 2023; South, 1971). Following mating and egg-laying in spring, the larvae feed on the leaves of various broad-leaved trees including oak, birch and ash (South, 1971). Anecdotal reports suggest that larvae shift to succulent, fresher leaves around the fourth instar (Allan, 1943). There are also reports of larval cannibalism although this may be infrequent and a consequence of larval overcrowding when rearing in captivity (Allan, 1943). Larvae pupate in an underground cocoon during summer, before adult emergence in autumn (Waring *et al.*, 2017).

A complete genome sequence for the Pale Pinion *L. socia* will facilitate research into biochemical adaptations permitting insect hibernation and will contribute to the growing set of resources for understanding the genomic evolution of Lepidoptera.

Genome sequence report

The genome was sequenced from one male *Lithophane socia* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, –1.32). A total of 59-fold coverage in Pacific Biosciences single-molecule HiFi long reads was generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 9 missing joins or mis-joins and removed 4 haplotypic duplications, reducing the scaffold number by 13.95%.

The final assembly has a total length of 489.3 Mb in 36 sequence scaffolds with a scaffold N50 of 16.9 Mb (Table 1). The snailplot 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

Figure 1. Photograph of the *Lithophane socia* (ilLitSoci1) specimen used for genome sequencing.

cumulative assembly plot in Figure 4 shows curves for subsets of scaffolds assigned to different phyla. Most (99.97%) of the assembly sequence was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. The Z chromosome was identified based on synteny with *Monopis laevigella* (ilMonLaev2.1; GCA_947359455.1) (Boyes *et al.*, 2023). Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 5; Table 2). 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 68.5 with *k*-mer completeness of 100%, and the assembly has a BUSCO v5.3.2 completeness of 99.0% (single = 98.6%, duplicated = 0.5%), using the lepidoptera_odb10 reference set (n = 5,286).

Metadata for specimens, barcode results, spectra estimates, sequencing runs, contaminants and pre-curation assembly statistics are given at https://links.tol.sanger.ac.uk/species/997538.

Genome annotation report

The *Lithophane socia* genome assembly (GCA_947522985.1) was annotated using the Ensembl rapid annotation pipeline (Table 1; https://rapid.ensembl.org/Lithophane_socia_GCA_947522985.1/Info/Index). The resulting annotation includes 18,551 transcribed mRNAs from 18,342 protein-coding genes.

Methods

Sample acquisition and nucleic acid extraction

A male *Lithophane socia* (specimen ID Ox001894, ToLID ilLitSoci1) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77,

Table 1. Genome data for *Lithophane socia*, ilLitSoci1.1.

Project accession data		
Assembly identifier	ilLitSoci1.1	
Species	*Lithophane socia*	
Specimen	ilLitSoci1	
NCBI taxonomy ID	997538	
BioProject	PRJEB57658	
BioSample ID	SAMEA10979155	
Isolate information	ilLitSoci1, male: thorax (DNA and RNA sequencing), head (Hi-C sequencing)	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	68.5	*≥ 50*
k-mer completeness	100%	*≥ 95%*
BUSCO**	C:99.0%[S:98.6%,D:0.5%],F:0.2%, M:0.8%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.97%	*≥ 95%*
Sex chromosomes	Z chromosome	*localised homologous pairs*
Organelles	Mitochondrial genome assembled	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR10499351	
Hi-C Illumina	ERR10500999	
PolyA RNA-Seq Illumina	ERR11641112	
Genome assembly		
Assembly accession	GCA_947522985.1	
Accession of alternate haplotype	GCA_947522845.1	
Span (Mb)	489.3	
Number of contigs	85	
Contig N50 length (Mb)	11.7	
Number of scaffolds	36	
Scaffold N50 length (Mb)	16.9	
Longest scaffold (Mb)	21.7	
Genome annotation		
Number of protein-coding genes	18,342	
Number of gene transcripts	18,551	

* 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 v5.3.2. 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/Lithophane%20socia/dataset/CANNUV01/busco.

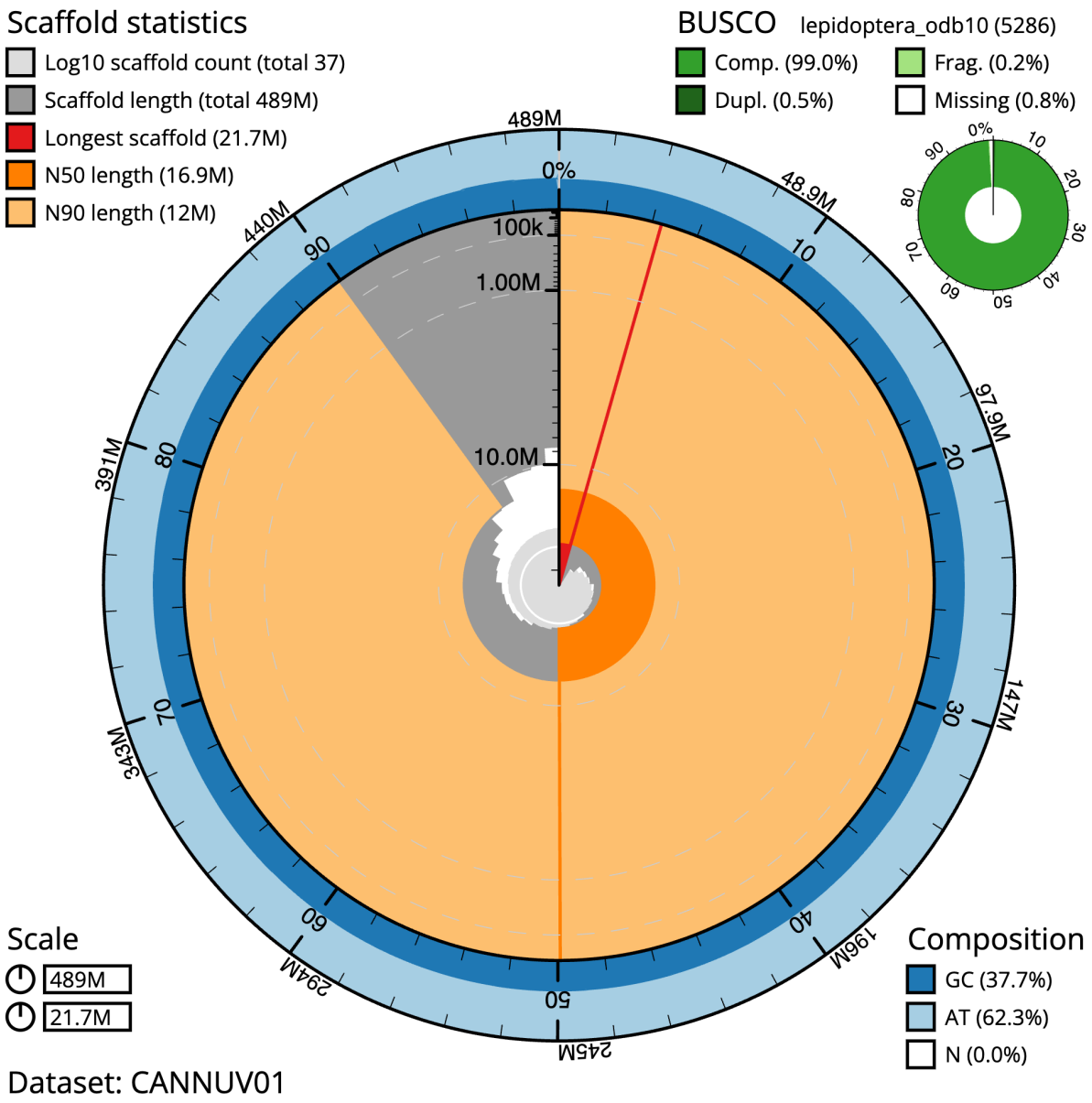

Figure 2. Genome assembly of *Lithophane socia*, ilLitSoci1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 489,345,261 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (21,701,345 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (16,922,983 and 11,981,223 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/Lithophane%20socia/dataset/CANNUV01/snail.

longitude –1.32) on 2021-05-28 using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice.

The workflow for high molecular weight (HMW) DNA extraction at the Wellcome Sanger Institute (WSI) includes a sequence of core procedures: sample preparation; sample homogenisation; DNA extraction; HMW DNA fragmentation; and fragmented DNA clean-up, for which protocols are publicly available on protocols.io (Denton *et al.*, 2023). The ilLitSoci1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing (Jay *et al.*, 2023). For sample homogenisation, thorax tissue was cryogenically disrupted using the Sample Homogenisation: Covaris cryoPREP®

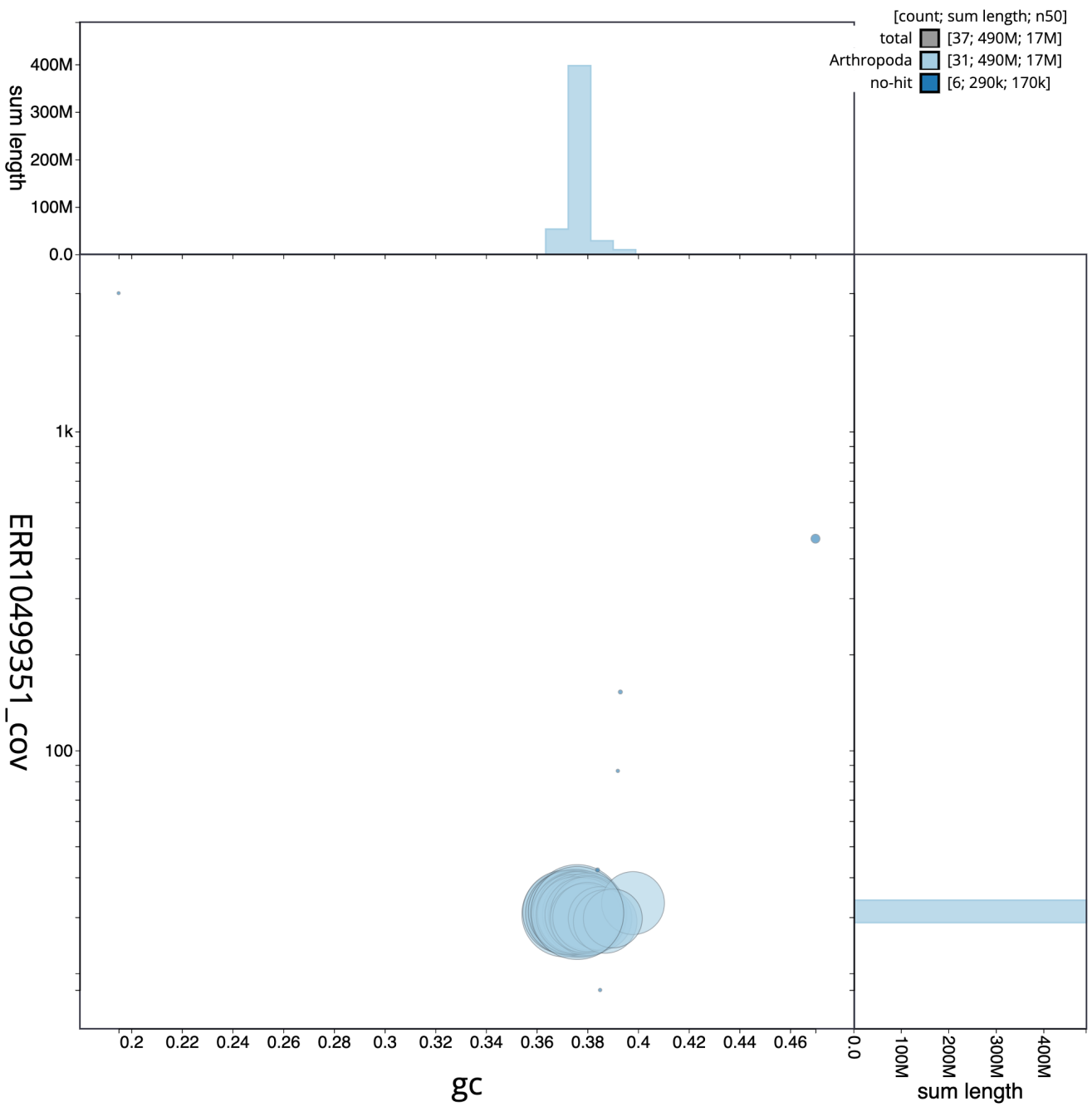

Figure 3. Genome assembly of *Lithophane socia*, ilLitSoci1.1: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at $BTK_FIG2_URL.

Automated Dry Pulverizer protocol (Narváez-Gómez *et al.*, 2023). HMW DNA was extracted using the Automated MagAttract v1 protocol (Sheerin *et al.*, 2023). The DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system with speed setting 30 (Todorovic *et al.*, 2023). Sheared DNA was purified by solid-phase reversible immobilisation (Strickland *et al.*, 2023): in brief, the method employs a 1.8X ratio of AMPure PB beads to sample to eliminate shorter fragments and concentrate the DNA. The concentration of the sheared and purified DNA was assessed using a Nanodrop

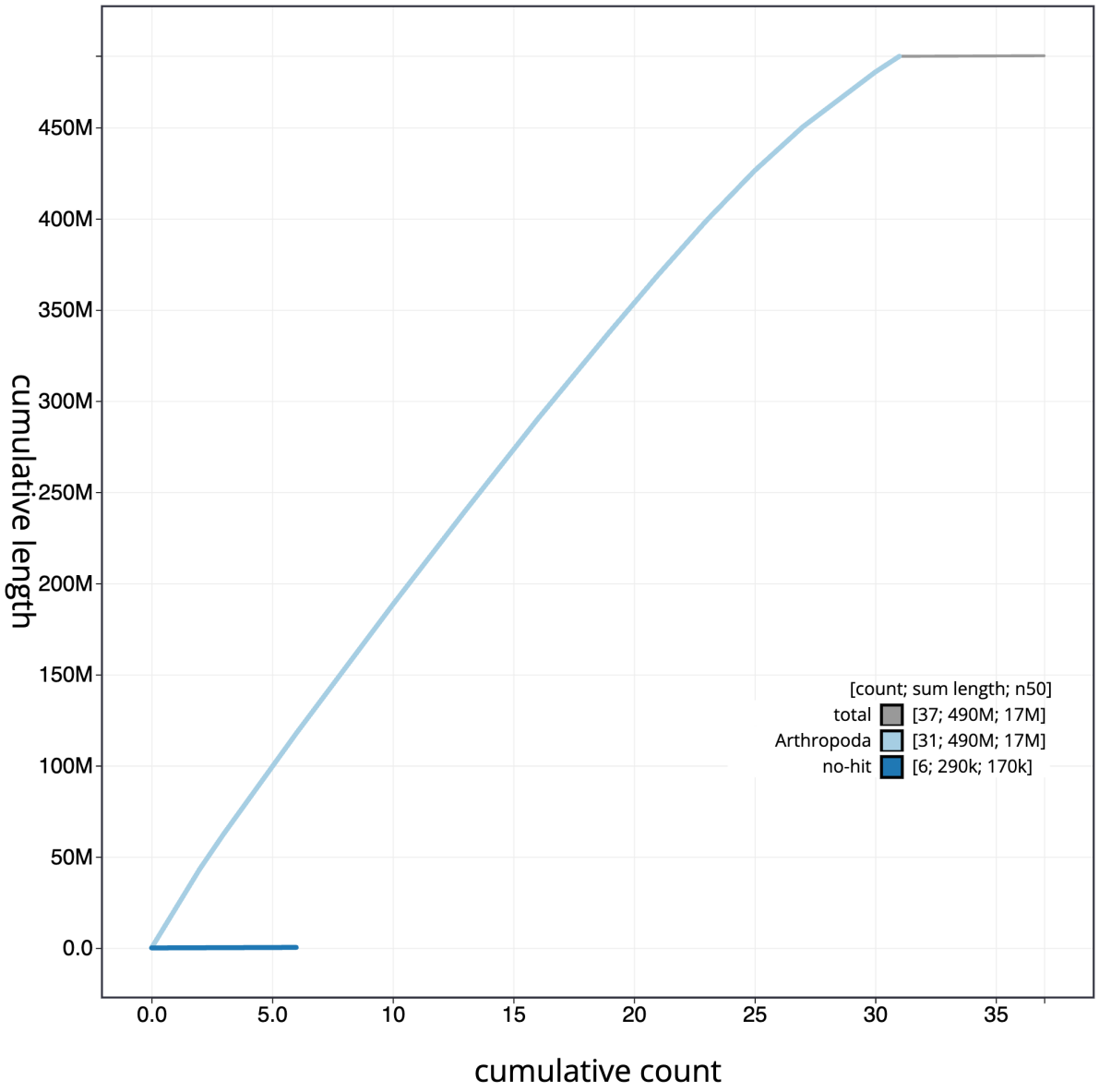

Figure 4. Genome assembly of *Lithophane socia*, ilLitSoci1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/Lithophane%20socia/dataset/CANNUV01/cumulative.

spectrophotometer and Qubit Fluorometer and Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

RNA was extracted from remaining thorax tissue of ilLitSoci1 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 core at the WSI on Pacific Biosciences SEQUEL II (HiFi) and

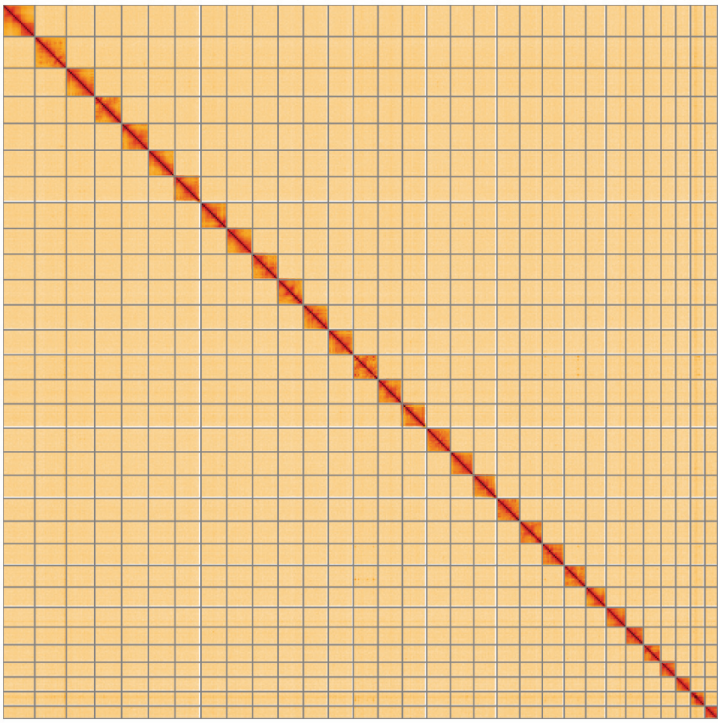

Figure 5. Genome assembly of *Lithophane socia*, ilLitSoci1.1: Hi-C contact map of the ilLitSoci1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=XzxemFD2QUGtJx8bGuOcrg.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Lithophane socia*, ilLitSoci1.

INSDC accession	Chromosome	Length (Mb)	GC%
OX383252.1	1	21.58	37.5
OX383253.1	2	19.39	37.5
OX383254.1	3	18.5	38.0
OX383255.1	4	18.34	37.0
OX383256.1	5	18.17	38.0
OX383257.1	6	17.76	37.5
OX383258.1	7	17.71	37.5
OX383259.1	8	17.57	37.5
OX383260.1	9	17.53	37.0
OX383261.1	10	17.29	37.0
OX383262.1	11	17.14	37.5
OX383263.1	12	17.01	37.5
OX383264.1	13	16.92	38.0
OX383265.1	14	16.71	37.5
OX383266.1	15	16.64	37.5
OX383267.1	16	16.3	37.5
OX383268.1	17	15.97	38.0
OX383269.1	18	15.82	37.5
OX383270.1	19	15.64	38.0
OX383271.1	20	15.48	38.0
OX383272.1	21	14.92	37.5
OX383273.1	22	14.78	38.0
OX383274.1	23	13.98	38.0
OX383275.1	24	13.45	38.0
OX383276.1	25	12.08	38.0
OX383277.1	26	11.98	38.0
OX383278.1	27	10.1	38.5
OX383279.1	28	10.08	38.5
OX383280.1	29	9.86	40.0
OX383281.1	30	8.64	39.0
OX383251.1	Z	21.7	37.5
OX383282.1	MT	0.02	19.5

Illumina NovaSeq 6000 (RNA-Seq) instruments. Hi-C data were also generated from head tissue of ilLitSoci1 using the Arima2 kit and sequenced on the Illumina NovaSeq 6000 instrument.

Genome assembly, curation and evaluation

Assembly was carried out with Hifiasm (Cheng *et al.*, 2021) and haplotypic duplication was identified and removed with purge_dups (Guan *et al.*, 2020). The assembly was then scaffolded with Hi-C data (Rao *et al.*, 2014) using YaHS (Zhou *et al.*, 2023). The assembly was checked for contamination and corrected as described previously (Howe *et al.*, 2021). Manual curation was performed using HiGlass (Kerpedjiev *et al.*, 2018) and Pretext (Harry, 2022). The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2023), which runs MitoFinder (Allio *et al.*, 2020) or MITOS (Bernt *et al.*, 2013) and uses these annotations to select the final mitochondrial contig and to ensure the general quality of the sequence.

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 Nextflow (Di Tommaso *et al.*, 2017) DSL2 pipelines "sanger-tol/readmapping" (Surana *et al.*, 2023a) and "sanger-tol/genomenote" (Surana *et al.*, 2023b). The genome was analysed within the BlobToolKit environment (Challis *et al.*, 2020) and BUSCO scores (Manni *et al.*, 2021; Simão *et al.*, 2015) were calculated.

Table 3 contains a list of relevant software tool versions and sources.

Genome annotation

The BRAKER2 pipeline (Brůna *et al.*, 2021) was used in the default protein mode to generate annotation for the *Lithophane socia* assembly (GCA_947522985.1) in Ensembl Rapid Release.

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.

Table 3. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.1.7	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	https://gitlab.com/ezlab/busco
Hifiasm	0.16.1-r375	https://github.com/chhylp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Merqury	MerquryFK	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	2	https://github.com/marcelauliano/MitoHiFi
PretextView	0.2	https://github.com/wtsi-hpag/PretextView
purge_dups	1.2.3	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	1.1a.2	https://github.com/c-zhou/yahs

Data availability

European Nucleotide Archive: *Lithophane socia* (pale pinion). Accession number PRJEB57658; https://identifiers.org/ena.embl/PRJEB57658 (Wellcome Sanger Institute, 2022). The genome sequence is released openly for reuse. The *Lithophane socia* 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.

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Members of the Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team are listed here: https://doi.org/10.5281/zenodo.10066175.

Members of Wellcome Sanger Institute Scientific Operations: Sequencing Operations are listed here: https://doi.org/10.5281/zenodo.10043364.

Members of the Wellcome Sanger Institute Tree of Life Core Informatics team are listed here: https://doi.org/10.5281/zenodo.10066637.

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.5013541.

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