DATA NOTE

The genome sequence of the Grey Shoulder-knot, *Lithophane ornitopus* (Hufnagel, 1766) [version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual male *Lithophane ornitopus* (the Grey Shoulder-knot; Arthropoda; Insecta; Lepidoptera; Noctuidae). The genome sequence is 508.6 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 15.33 kilobases in length. Gene annotation of this assembly on Ensembl identified 18,397 protein coding genes.

Keywords

Lithophane ornitopus, Grey Shoulder-knot, 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; Opisthokonta; 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 ornitopus Hufnagel, 1766 (NCBI:txid987973).

Background

Lithophane (*Lithophane*) *ornitopus* Hufnagel, 1766 (Grey Shoulder-knot) is a member of a widespread Holarctic genus of noctuid moths typically active from autumn (occasionally late summer) to early spring, with a variably prolonged period of rest in sheltered sites during the coldest winter months, depending on the latitude and elevation. Like most of the species within this genus, it is characterised by comparatively long and narrow forewings which have an obviously cryptic pattern, enabling the adult to camouflage against bark. However, the etymology of this species' name refers to another feature of its forewings, namely the strong black trifurcated mark at their base that is reminiscent of bird's foot toes (from the Greek *órnis* = bird, and *poús* = foot) (Spuler, 1908).

The moths fly by night and come readily to artificial light and alcohol–sugar lures. The species is univoltine, inhabiting a variety of broad-leaved forested environments, but it is most abundant in oak woodlands, *Quercus* spp. being its preferred host plants. Other recorded hostplants are *Prunus* spp., *Populus* spp., *Salix* spp. and *Ulmus* spp. The larvae develop during spring and early summer, usually aestivating before pupation takes place in late summer, in a tough cocoon spun in the soil (Ronkay *et al.*, 2001).

Lithophane (*L.*) *ornitopus* is widely distributed through the Palaearctic Region from western Europe and north-west Africa (Morocco) across the Urals and Mediterranean to western Siberia and western Kazakhstan. Records from central Asia should be verified with respect to the closely similar *Lithophane* (*L.*) *pruinosa* (Butler, 1878) substituting *L.* (*L.*) *ornitopus* in east Asia (Kononenko, 2016; Ronkay *et al.*, 2001).

Here we present a chromosomal-level genome assembly for *Lithophane ornitopus*, based on one male specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome was sequenced from a male *Lithophane ornitopus* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, –1.34). A total of 58-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 14 missing joins or mis-joins and removed 3 haplotypic duplications, reducing the scaffold number by 5.13%.

The final assembly has a total length of 508.6 Mb in 36 sequence scaffolds with a scaffold N50 of 17.2 Mb (Table 1).

Figure 1. Photograph of the *Lithophane ornitopus* (ilLitOrni1) specimen used for genome sequencing.

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 (99.98%) of the assembly sequence was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. 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 69.5 with *k*-mer completeness of 100.0%, and the assembly has a BUSCO v5.3.2 completeness of 98.9% (single = 98.5%, duplicated = 0.4%), 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/987973.

Genome annotation report

The *Lithophane ornitopus* genome assembly (GCA_948473465.1) was annotated using the Ensembl rapid annotation pipeline at the European Bioinformatics Institute. The resulting annotation includes 18,607 transcribed mRNAs from 18,397 protein-coding genes (Table 1 https://rapid.ensembl.org/Lithophane_ornitopus_GCA_948473465.1/Info/Index).

Methods

Sample acquisition and nucleic acid extraction

A *Lithophane ornitopus* (specimen ID Ox001087, ToLID ilLitOrni1) was collected Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2021-03-31 using a light trap. The specimen was

Table 1. Genome data for *Lithophane ornitopus*, ilLitOrni1.1.

Project accession data		
Assembly identifier	ilLitOrni1.1	
Species	*Lithophane ornitopus*	
Specimen	ilLitOrni1	
NCBI taxonomy ID	987973	
BioProject	PRJEB58424	
BioSample ID	SAMEA10107010	
Isolate information	ilLitOrni1, male: thorax (DNA sequencing), head (Hi-C sequencing), abdomen (RNA sequencing)	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	69.5	*≥ 50*
k-mer completeness	100.0%	*≥ 95%*
BUSCO**	C:98.9%[S:98.5%,D:0.4%], F:0.2%,M:0.9%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.98%	*≥ 95%*
Sex chromosomes	ZZ	*localised homologous pairs*
Organelles	Mitochondrial genome: 15.33 kb	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR10704794	
Hi-C Illumina	ERR10684091	
PolyA RNA-Seq Illumina	ERR11242517	
Genome assembly		
Assembly accession	GCA_948473465.1	
Accession of alternate haplotype	GCA_948473405.1	
Span (Mb)	508.6	
Number of contigs	95	
Contig N50 length (Mb)	10.9	
Number of scaffolds	36	
Scaffold N50 length (Mb)	17.2	
Longest scaffold (Mb)	23.1	
Genome annotation		
Number of protein-coding genes	18,397	
Number of gene transcripts	18,607	

* 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/CAOKYS01/dataset/CAOKYS01/busco.

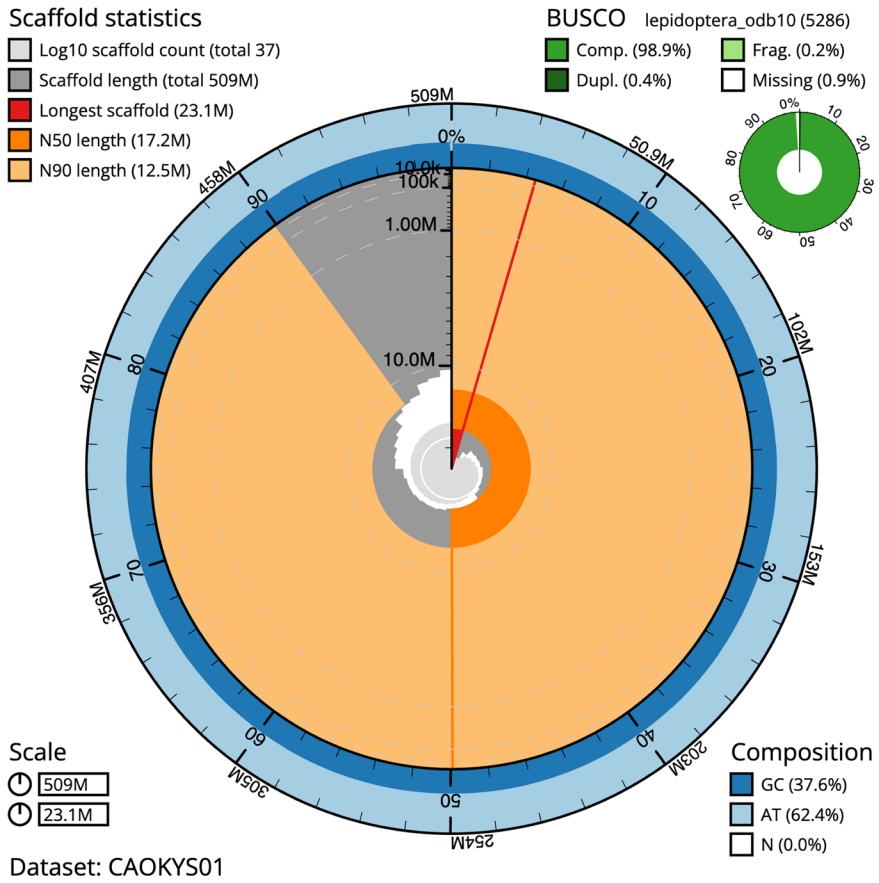

Figure 2. Genome assembly of *Lithophane ornitopus*, ilLitOrni1.1: metrics. The BlobToolKit snail plot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 508,611,498 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (23,095,587 bp, shown in red). . Orange and pale-orange arcs show the N50 and N90 scaffold lengths (17,228,122 and 12,539,399 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/CAOKYS01/dataset/CAOKYS01/snail.

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, fragmentation, and clean-up. The sample was prepared for DNA extraction at the WSI Tree of Life Core Laboratory: the ilLitOrni1 sample was weighed and dissected on dry ice (Jay *et al.*, 2023). Thorax tissue was cryogenically disrupted using the Covaris cryoPREP® Automated Dry Pulverizer (Narváez-Gómez *et al.*, 2023). 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 with speed setting 31 (Bates *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 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 abdomen tissue of ilLitOrni1 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ mirVana 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.

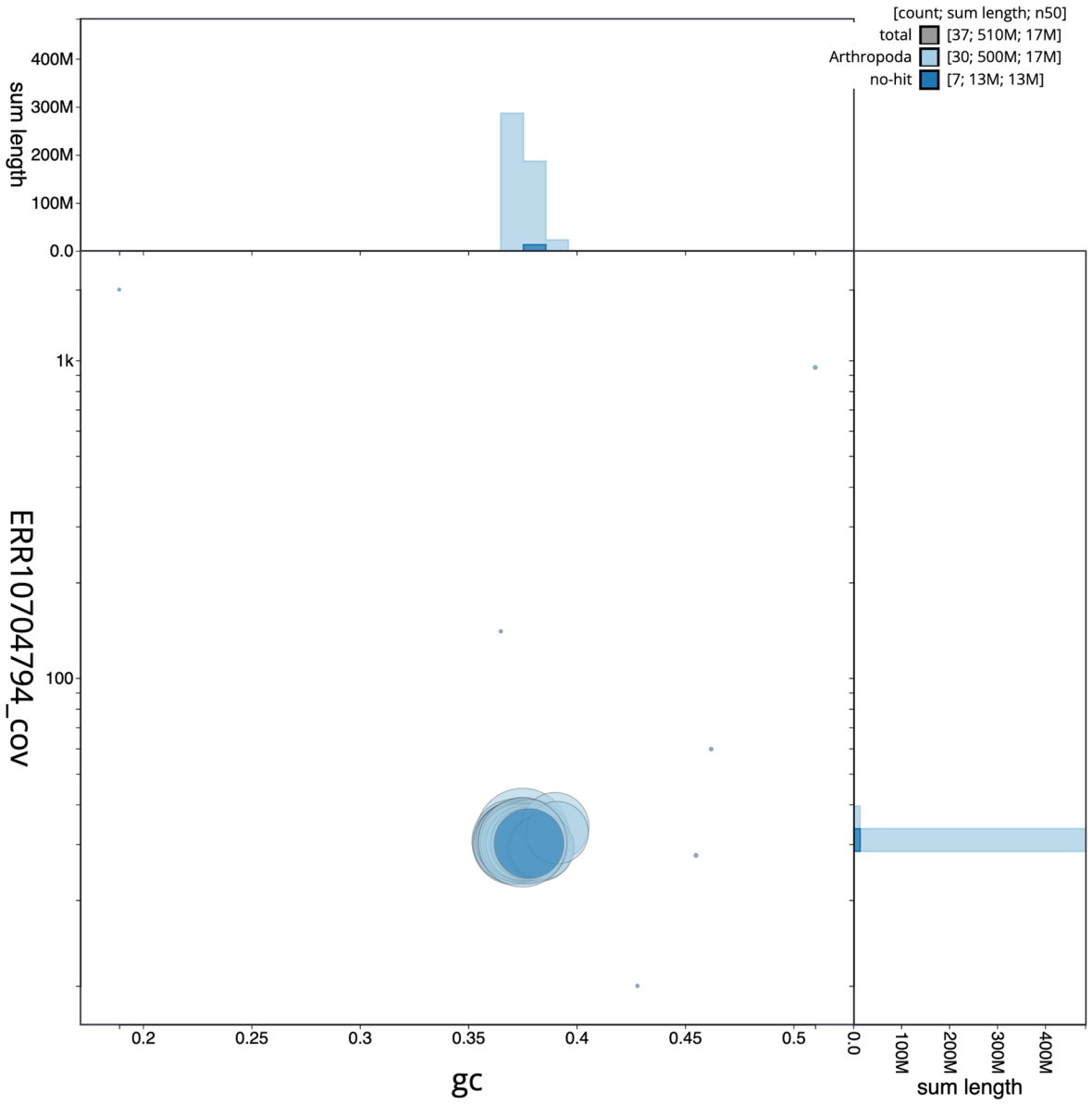

Figure 3. Genome assembly of *Lithophane ornitopus*, ilLitOrni1.1: BlobToolKit GC-coverage plot. Sequences are coloured by phylum. Circles are sized in proportion to sequence length. Histograms show the distribution of sequence length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/CAOKYS01/dataset/CAOKYS01/blob.

Protocols developed by the WSI Tree of Life laboratory are publicly available on protocols.io (Denton *et al.*, 2023).

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 Illumina NovaSeq 6000 (RNA-Seq) instruments. Hi-C data were also generated from head tissue of ilLitOrni1 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).

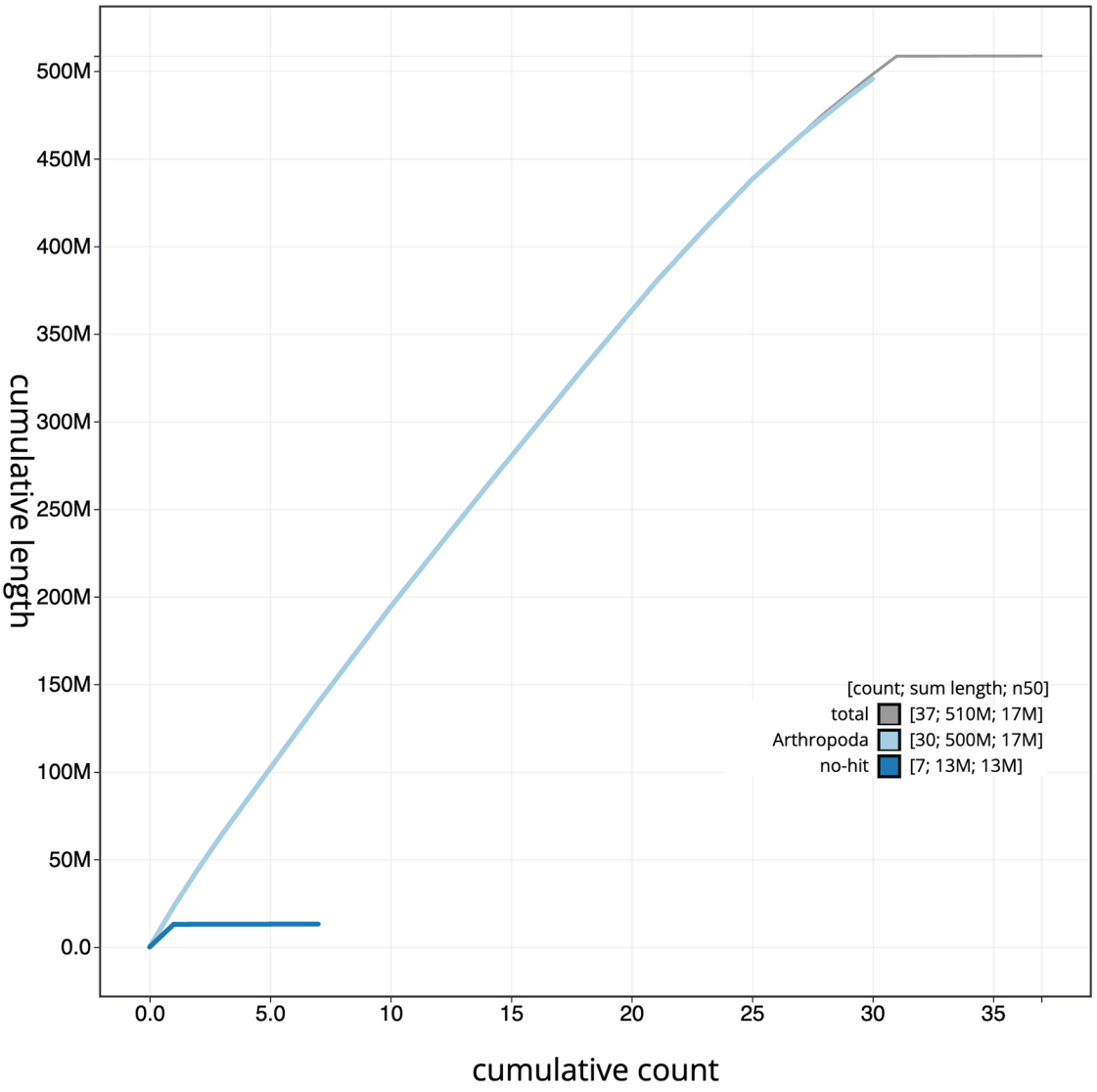

Figure 4. Genome assembly of *Lithophane ornitopus*, ilLitOrni1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all sequences. Coloured lines show cumulative lengths of sequences assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/CAOKYS01/dataset/CAOKYS01/cumulative.

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

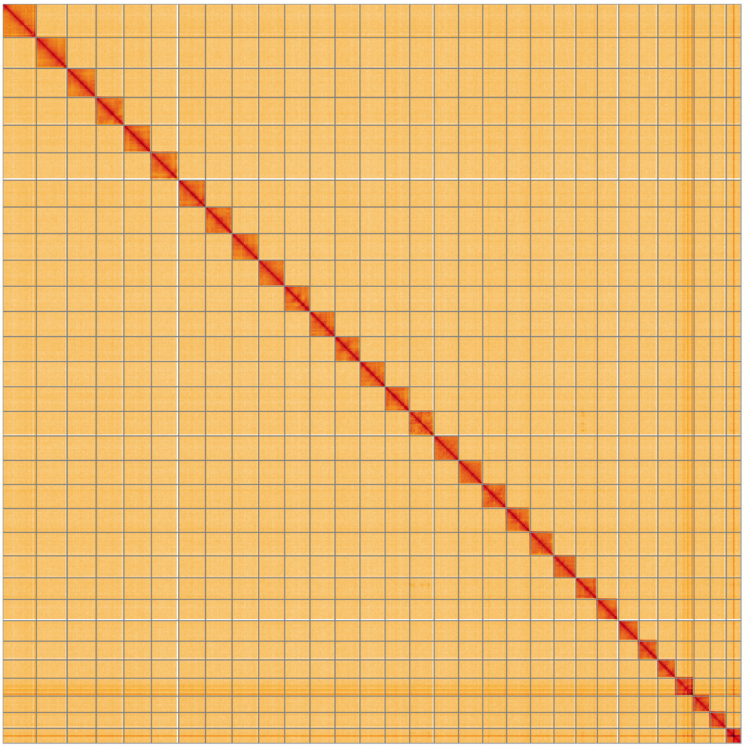

Figure 5. Genome assembly of *Lithophane ornitopus*, ilLitOrni1.1: Hi-C contact map of the ilLitOrni1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=JrYfpw0mQ46ruZibv99pQw.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Lithophane ornitopus*, ilLitOrni1.

INSDC accession	Chromosome	Length (Mb)	GC%
OX419613.1	1	23.1	37.5
OX419615.1	2	20.0	37.5
OX419616.1	3	19.14	37.5
OX419617.1	4	18.96	37.5
OX419618.1	5	18.79	37.0
OX419619.1	6	18.42	37.5
OX419620.1	7	18.33	37.0
OX419621.1	8	18.23	37.0
OX419622.1	9	17.98	37.5
OX419623.1	10	17.3	37.0
OX419624.1	11	17.28	37.5
OX419625.1	12	17.25	37.5
OX419626.1	13	17.23	37.0
OX419627.1	14	16.99	37.5
OX419628.1	15	16.85	37.5
OX419629.1	16	16.79	37.5
OX419630.1	17	16.62	37.5
OX419631.1	18	16.46	38.0
OX419632.1	19	16.44	37.5
OX419633.1	20	16.15	38.0
OX419634.1	21	15.12	37.5
OX419635.1	22	14.93	38.0
OX419636.1	23	14.5	37.5
OX419637.1	24	14.1	38.0
OX419638.1	25	12.96	38.0
OX419639.1	26	12.54	37.5
OX419640.1	27	12.29	39.0
OX419641.1	28	11.23	38.5
OX419642.1	29	10.98	38.5
OX419643.1	30	10.32	39.0
OX419614.1	Z	21.19	37.5
OX419644.1	MT	0.02	19.0

Table 3. 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
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	1.2a	https://github.com/c-zhou/yahs

Lithophane ornitopus assembly (GCA_948473465.1) in Ensembl Rapid Release at the EBI.

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: *Lithophane ornitopus* (grey shoulder-knot). Accession number PRJEB58424; https://identifiers.org/ena.embl/PRJEB58424 (Wellcome Sanger Institute, 2023). The genome sequence is released openly for reuse. The *Lithophane ornitopus* 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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