



DATA NOTE

The genome sequence of the Light Arches, *Apamea lithoxylaea* (Denis & Schiffermüller), 1775 (Lepidoptera: Noctuidae)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from an individual female *Apamea lithoxylaea* (Light Arches; Arthropoda; Insecta; Lepidoptera; Noctuidae). The assembly contains two haplotypes with total lengths of 577.73 megabases and 521.26 megabases. Most of haplotype 1 (98.15%) is scaffolded into 32 chromosomal pseudomolecules, including the W and Z sex chromosomes. Haplotype 2 was assembled to scaffold level. The mitochondrial genome has also been assembled, with a length of 16.43 kilobases. This assembly was generated as part of the Darwin Tree of Life project, which produces reference genomes for eukaryotic species found in Britain and Ireland.

Keywords

Apamea lithoxylaea; Light Arches; genome sequence; chromosomal; Lepidoptera



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

Open Peer Review

Approval Status

	1	2
version 1		
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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; Noctuinae; Apameini; *Apamea*; *Apamea lithoxyla* (Denis & Schiffermüller, 1775 (NCBI:txid987881))

Background

Light Arches, *Apamea lithoxyla*, is a moth in the family Noctuidae. The moth is common and widespread throughout most of Britain and has a mainly European distribution although there are a handful of records from the eastern United States (GBIF Secretariat, 2025). It can be found in dry grassy areas. The moth is variable in size and colouration with a forewing length between 19 and 26 mm. Most commonly, the forewing is greyish-brown, but ranges from light brown through to blackish. There is usually one generation a year, flying between June and August, although a partial second generation can occur. The moth readily comes to light (Waring *et al.*, 2017). The egg is laid on grasses, on which the larvae feed until they pupate underground to emerge the following year (Heath & Emmet, 1983).

We present a chromosome-level genome sequence for *Apamea lithoxyla*, the Light Arches. The assembly was produced using the Tree of Life pipeline from a specimen collected in Wytham Woods, Oxfordshire, United Kingdom (Figure 1), as part of the Darwin Tree of Life Project.

Methods

Sample acquisition and DNA barcoding

The specimen used for genome sequencing was an adult female *Apamea lithoxyla* (specimen ID Ox001683, ToLID ilApaLith1; Figure 1), collected from Wytham Woods, Oxfordshire, United Kingdom (latitude 51.772, longitude -1.338) on 2021-07-17. The specimen was collected and identified by Douglas Boyes (University of Oxford). For the Darwin

Tree of Life sampling and metadata approach, refer to Lawniczak *et al.* (2022).

The initial identification 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) (see the protocol). 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 are available on protocols.io.

Nucleic acid extraction

Protocols for high molecular weight (HMW) DNA extraction developed at the Wellcome Sanger Institute (WSI) Tree of Life Core Laboratory are available on protocols.io (Howard *et al.*, 2025). The ilApaLith1 sample was weighed and triaged to determine the appropriate extraction protocol. Tissue from the abdomen was homogenised by cryogenic disruption using the Covaris cryoPREP® Automated Dry Pulverizer. HMW DNA was extracted using the Automated MagAttract v2 protocol. We used centrifuge-mediated fragmentation to produce DNA fragments in the 8–10 kb range, following the Covaris g-TUBE protocol for ultra-low input (ULI). Sheared DNA was purified by automated SPRI (solid-phase reversible immobilisation). 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. For this sample, the final post-shearing DNA had a Qubit concentration of 11.8 ng/μL and a yield of 1534.00 ng. The 260/280 spectrophotometric ratio was -0.17, and the 260/230 ratio was -0.06.

PacBio HiFi library preparation and sequencing

Library preparation and sequencing were performed at the WSI Scientific Operations core. Prior to library preparation, the DNA was fragmented to ~10 kb. Ultra-low-input (ULI) libraries were prepared using the PacBio SMRTbell® Express Template Prep Kit 2.0 and gDNA Sample Amplification Kit. Samples were normalised to 20 ng DNA. Single-strand overhang removal, DNA damage repair, and end-repair/A-tailing were performed according to the manufacturer's instructions, followed by adapter ligation. A 0.85× pre-PCR clean-up was carried out with Promega ProNex beads.

The DNA was evenly divided into two aliquots for dual PCR (reactions A and B), both following the manufacturer's protocol. A 0.85× post-PCR clean-up was performed with ProNex beads. DNA concentration was measured using a Qubit Fluorometer v4.0 (Thermo Fisher Scientific) with the Qubit



Figure 1. Photograph of the *Apamea lithoxyla* (ilApaLith1) specimen used for genome sequencing.

HS Assay Kit, and fragment size was assessed on an Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies) using the gDNA 55 kb BAC analysis kit. PCR reactions A and B were then pooled, ensuring a total mass of ≥ 500 ng in 47.4 μ L.

The pooled sample underwent another round of DNA damage repair, end-repair/A-tailing, and hairpin adapter ligation. A 1 $\times$ clean-up was performed with ProNex beads, followed by DNA quantification using the Qubit and fragment size analysis using the Agilent Femto Pulse. Size selection was performed on the Sage Sciences PippinHT system, with target fragment size determined by Femto Pulse analysis (typically 4–9 kb). Size-selected libraries were cleaned with 1.0 $\times$ ProNex beads and normalised to 2 nM before sequencing.

The sample was sequenced on a Revio instrument (Pacific Biosciences). The prepared library was normalised to 2 nM, and 15 μ L was used for making complexes. Primers were annealed and polymerases bound to generate circularised complexes, following the manufacturer's instructions. Complexes were purified using 1.2X SMRTbell beads, then diluted to the Revio loading concentration (200–300 pM) and spiked with a Revio sequencing internal control. The sample was sequenced on a Revio 25M SMRT cell. The SMRT Link software (Pacific Biosciences), a web-based workflow manager, was used to configure and monitor the run and to carry out primary and secondary data analysis.

Hi-C

Sample preparation and crosslinking

The Hi-C sample was prepared from 20–50 mg of frozen tissue from the head of the iLApalith1 sample using the Arima-HiC v2 kit (Arima Genomics). Following the manufacturer's instructions, tissue was fixed and DNA crosslinked using TC buffer to a final formaldehyde concentration of 2%. The tissue was homogenised using the Diagenode Power Masher-II. Crosslinked DNA was digested with a restriction enzyme master mix, biotinylated, and ligated. Clean-up was performed with SPRISelect beads before library preparation. DNA concentration was measured with the Qubit Fluorometer (Thermo Fisher Scientific) and Qubit HS Assay Kit. The biotinylation percentage was estimated using the Arima-HiC v2 QC beads.

Hi-C library preparation and sequencing

Biotinylated DNA constructs were fragmented using a Covaris E220 sonicator and size selected to 400–600 bp using SPRISelect beads. DNA was enriched with Arima-HiC v2 kit Enrichment beads. End repair, A-tailing, and adapter ligation were carried out with the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs), following a modified protocol where library preparation occurs while DNA remains bound to the Enrichment beads. Library amplification was performed using KAPA HiFi HotStart mix and a custom Unique Dual Index (UDI) barcode set (Integrated DNA Technologies). Depending on sample concentration and biotinylation percentage determined at the crosslinking stage, libraries were

amplified with 10 to 16 PCR cycles. Post-PCR clean-up was performed with SPRISelect beads. Libraries were quantified using the AccuClear Ultra High Sensitivity dsDNA Standards Assay Kit (Biotium) and a FLUOstar Omega plate reader (BMG Labtech).

Prior to sequencing, libraries were normalised to 10 ng/ μ L. Normalised libraries were quantified again and equimolar and/or weighted 2.8 nM pools. Pool concentrations were checked using the Agilent 4200 TapeStation (Agilent) with High Sensitivity D500 reagents before sequencing. Sequencing was performed using paired-end 150 bp reads on the Illumina NovaSeq 6000.

Genome 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 assembled using Hifiasm in Hi-C phasing mode (Cheng *et al.*, 2021; Cheng *et al.*, 2022), producing two haplotypes. Hi-C reads (Rao *et al.*, 2014) were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). Contigs were further scaffolded with Hi-C data 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 MERQUERY.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. TreeVal was used to generate the flat files and maps for use in curation. Manual curation was conducted primarily in PretextView and HiGlass (Kerpedjiev *et al.*, 2018). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Manual corrections included 15 breaks and 28 joins. The curation process is documented at <https://gitlab.com/wtsi-grit/rapid-curation>. PretextViewSnapshot was used to generate a Hi-C contact map of the final assembly.

Assembly quality assessment

The Merquery.FK tool (Rhie *et al.*, 2020) was run in a Singularity container (Kurtzer *et al.*, 2017) to evaluate k -mer completeness and assembly quality for both haplotypes using the k -mer databases ($k = 31$) computed prior to genome assembly. The analysis outputs included assembly QV scores and completeness statistics.

The genome was analysed using the BlobToolKit pipeline, a Nextflow implementation of the earlier Snakemake version

(Challis *et al.*, 2020). The pipeline aligns PacBio reads using minimap2 (Li, 2018) and SAMtools (Danecek *et al.*, 2021) to generate coverage tracks. It runs BUSCO (Manni *et al.*, 2021) using lineages identified from the NCBI Taxonomy (Schoch *et al.*, 2020). For the three domain-level lineages, BUSCO genes are aligned to the UniProt Reference Proteomes database (Bateman *et al.*, 2023) using DIAMOND blastp (Buchfink *et al.*, 2021). The genome is divided into chunks based on the density of BUSCO genes from the closest taxonomic lineage, and each chunk is aligned to the UniProt Reference Proteomes database with DIAMOND blastx. Sequences without hits are chunked using seqtk and aligned to the NT database with blastn (Altschul *et al.*, 1990). The BlobToolKit suite consolidates all 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), with

containerisation through Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017).

Genome sequence report

Sequence data

PacBio sequencing of the *Apamea lithoxyloea* specimen generated 99.19 Gb (gigabases) from 12.45 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 503.73 Mb, with a heterozygosity of 1.10% and repeat content of 21.96% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 70× coverage. Hi-C sequencing produced 118.29 Gb from 783.35 million reads, which were used to scaffold the assembly. Table 1 summarises the specimen and sequencing details.

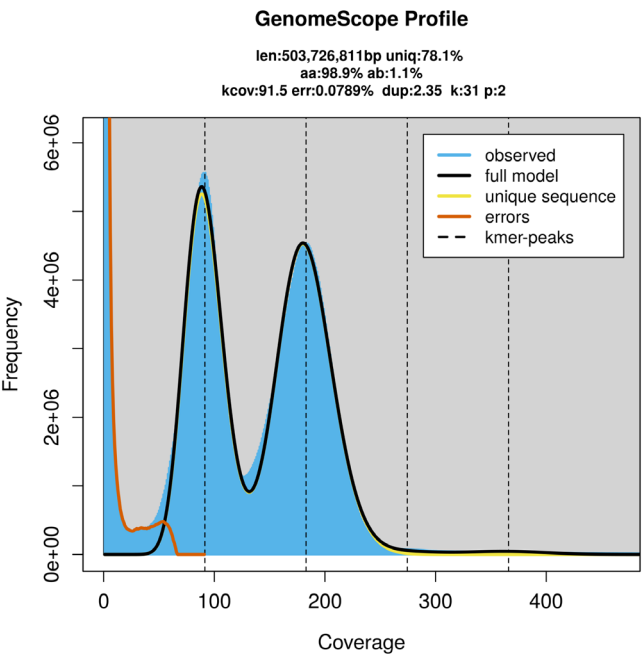


Figure 2. Frequency distribution of *k*-mers generated using GenomeScope2. The plot shows observed and modelled *k*-mer spectra, providing estimates of genome size, heterozygosity, and repeat content based on unassembled sequencing reads.

Table 1. Specimen and sequencing data for BioProject PRJEB84087.

Platform	PacBio HiFi	Hi-C
ToLID	ilApaLith1	ilApaLith1
Specimen ID	Ox001683	Ox001683
BioSample (source individual)	SAMEA10978950	SAMEA10978950
BioSample (tissue)	SAMEA10979303	SAMEA10979301
Tissue	abdomen	head
Instrument	Revio	Illumina NovaSeq 6000
Run accessions	ERR14121422; ERR15170315	ERR14125352
Read count total	12.45 million	783.35 million
Base count total	99.19 Gb	118.29 Gb

Assembly statistics

The genome was assembled into two haplotypes using Hi-C phasing. Haplotype 1 was curated to chromosome level, while haplotype 2 was assembled to scaffold level. The final assembly has a total length of 577.73 Mb in 623 scaffolds, with 581 gaps, and a scaffold N50 of 18.85 Mb (Table 2).

Most of the assembly sequence (98.15%) was assigned to 32 chromosomal-level scaffolds, representing 30 autosomes and the W and Z sex chromosomes. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 3; Table 3). Chromosomes Z and W were assigned based on copy number in the diploid genome and the Hi-C signal.

Table 2. Genome assembly statistics.

Metric	Haplotype 1	Haplotype 2
Assembly name	ilApaLith1.hap1.1	ilApaLith1.hap2.1
Assembly accession	GCA_965234015.1	GCA_965234145.1
Assembly level	chromosome	scaffold
Span (Mb)	577.73	521.26
Number of chromosomes	32	N/A
Number of contigs	1 204	891
Contig N50	1.54 Mb	1.59 Mb
Number of scaffolds	623	372
Scaffold N50	18.85 Mb	18.7 Mb
Longest scaffold length (Mb)	33.09	N/A
Sex chromosomes	W and Z	N/A
Organelles	Mitochondrion: 16.43 kb	N/A

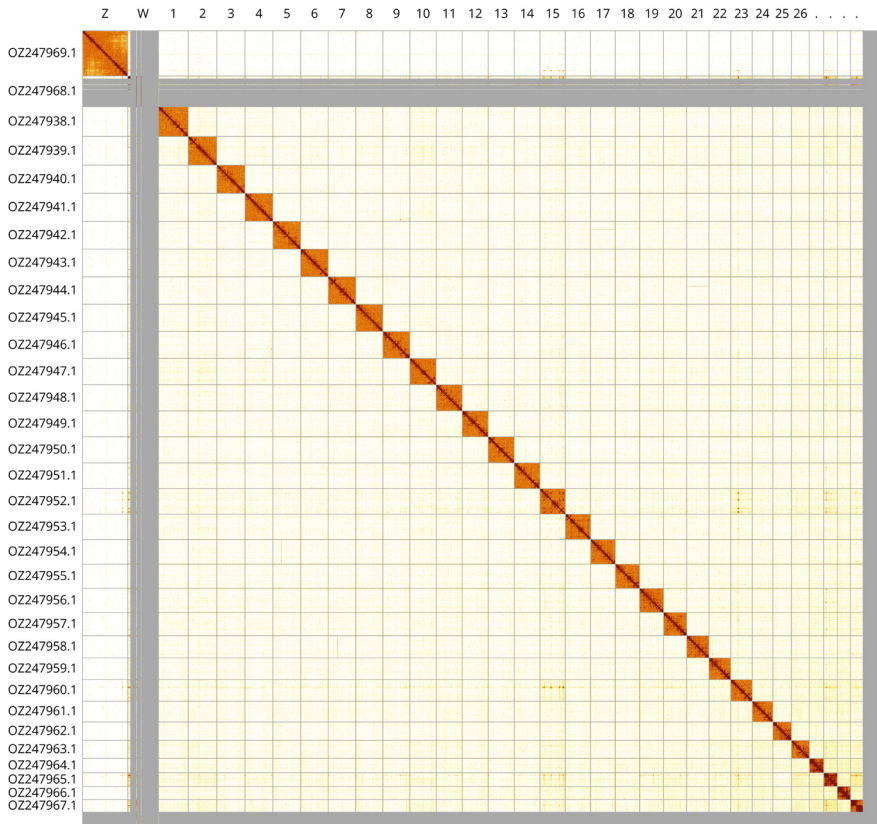


Figure 3. Hi-C contact map of the *Apamea lithoxyloae* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Apamea lithoxylaea* iApoLith1.

INSDC accession	Molecule	Length (Mb)	GC%
OZ247938.1	1	21.60	38.50
OZ247939.1	2	20.84	39
OZ247940.1	3	20.46	38
OZ247941.1	4	20.25	38.50
OZ247942.1	5	20.24	38
OZ247943.1	6	19.95	38.50
OZ247944.1	7	19.94	38.50
OZ247945.1	8	19.82	38.50
OZ247946.1	9	19.50	38.50
OZ247947.1	10	19.19	38.50
OZ247948.1	11	18.87	38.50
OZ247949.1	12	18.85	38.50
OZ247950.1	13	18.85	38.50
OZ247951.1	14	18.78	38.50
OZ247952.1	15	18.53	39
OZ247953.1	16	18.27	38.50
OZ247954.1	17	17.97	39
OZ247955.1	18	17.58	38.50
OZ247956.1	19	17.50	39
OZ247957.1	20	16.78	39
OZ247958.1	21	16.13	38.50
OZ247959.1	22	15.71	39
OZ247960.1	23	15.64	39.50
OZ247961.1	24	15.09	39.50
OZ247962.1	25	13.35	39
OZ247963.1	26	13.06	39
OZ247964.1	27	10.49	40
OZ247965.1	28	9.97	40
OZ247966.1	29	9.50	40
OZ247967.1	30	9.03	41
OZ247968.1	W	22.24	44
OZ247969.1	Z	33.09	38.50

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.

For haplotype 1, the estimated QV is 64.7, and for haplotype 2, 64.9. When the two haplotypes are combined, the assembly achieves an estimated QV of 64.8. The *k*-mer completeness is 81.85% for haplotype 1, 77.01% for haplotype 2, and 99.31% for the combined haplotypes (Figure 4).

BUSCO analysis using the lepidoptera_odb10 reference set (*n* = 5 286) identified 98.8% of the expected gene set (single = 97.0%, duplicated = 1.8%) for haplotype 1. The snail plot in Figure 5 summarises the scaffold length distribution and other assembly statistics for haplotype 1. The blob plot in Figure 6 shows the distribution of scaffolds by GC proportion and coverage for haplotype 1.

Table 4 lists the assembly metric benchmarks adapted from Rhie *et al.* (2021) the Earth BioGenome Project Report on Assembly Standards September 2024. The EBP metric, calculated for the haplotype 1, is **6.C.Q64**, meeting the recommended reference standard.

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](#). 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.

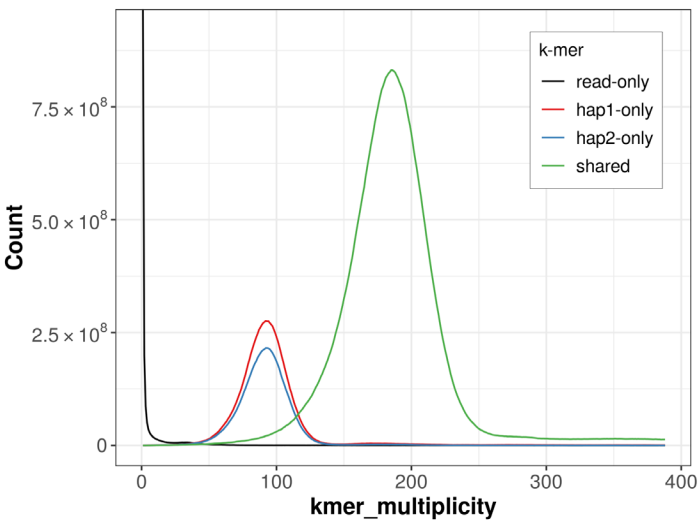


Figure 4. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers that appear in the reads but are not assembled. The green curve corresponds to *k*-mers shared by both haplotypes, and the red and blue curves show *k*-mers found only in one of the haplotypes.

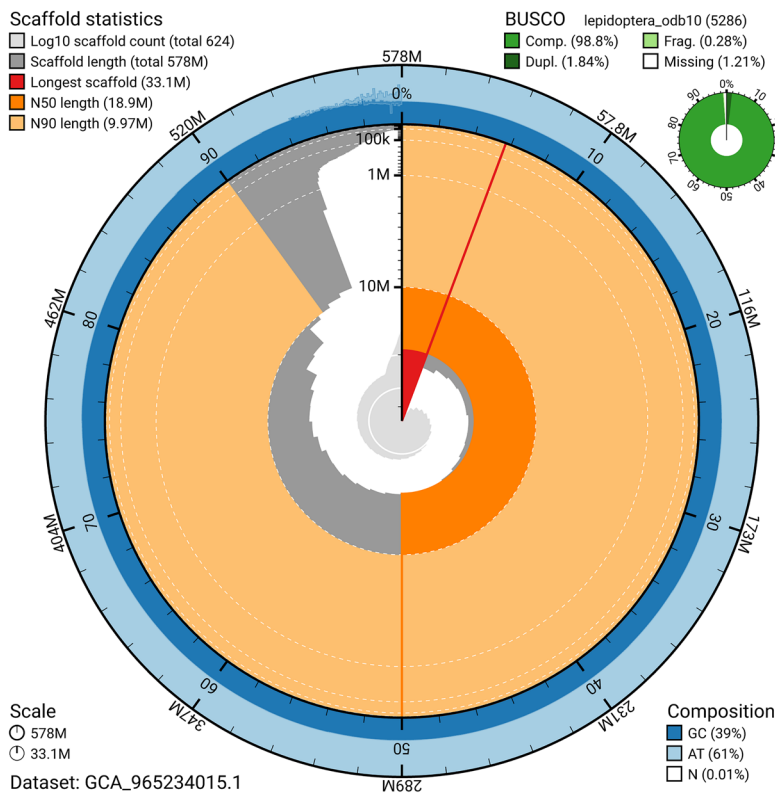


Figure 5. Assembly metrics for ilApaLith1.hap1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1 000 bins around the circumference. The outermost 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 set is presented at the top right. An interactive version of this figure can be accessed on the [BlobToolKit viewer](#).

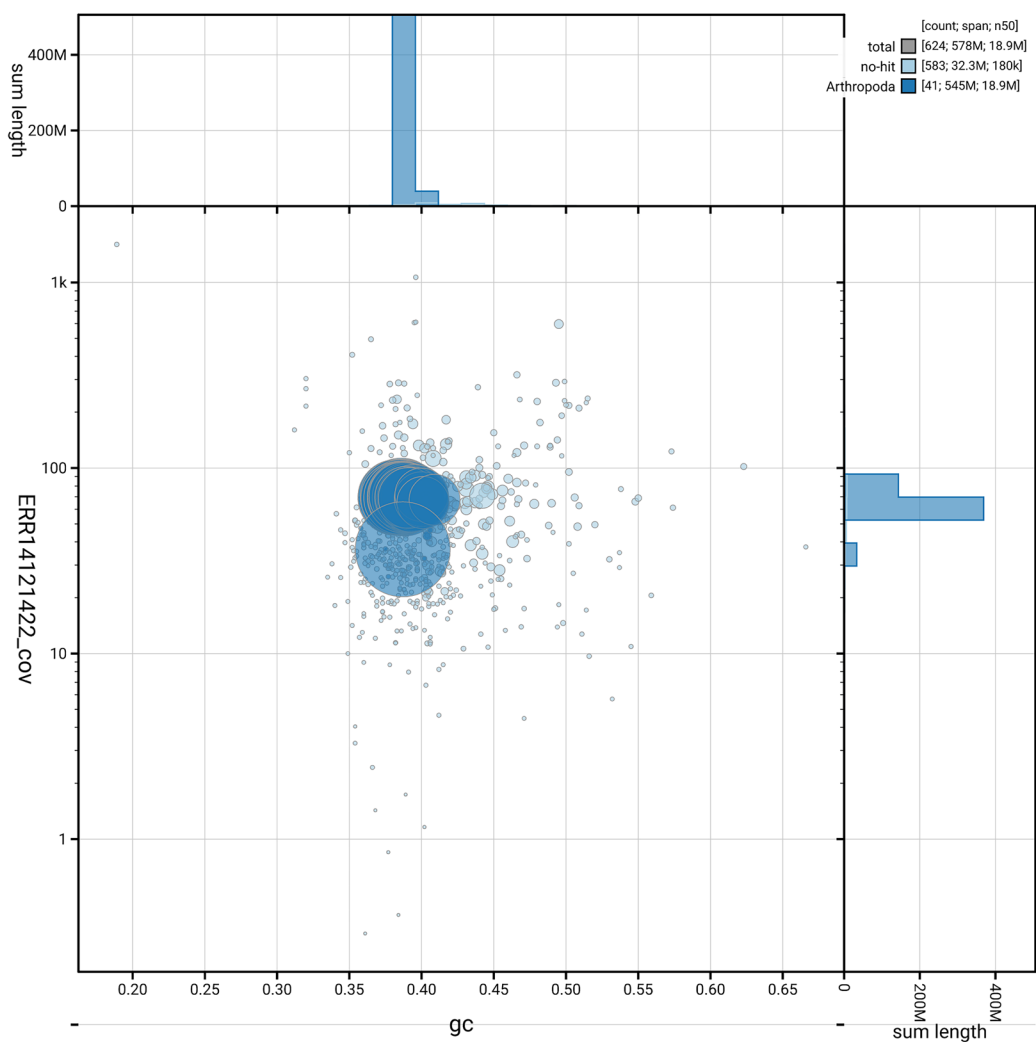


Figure 6. BlobToolKit GC-coverage plot for iLApaLith1.hap1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes display the total length of sequences distributed across different levels of coverage and GC content. An interactive version of this figure is available on the [BlobToolKit viewer](#).

Table 4. Earth Biogenome Project summary metrics for the *Apamea lithoxylaea* assembly.

Measure	Value	Benchmark
EBP summary (haplotype 1)	6.C.Q64	6.C.Q40
Contig N50 length	1.54 Mb	≥ 1 Mb
Scaffold N50 length	18.85 Mb	= chromosome N50
Consensus quality (QV)	Haplotype 1: 64.7; haplotype 2: 64.9; combined: 64.8	≥ 40
<i>k</i> -mer completeness	Haplotype 1: 81.85%; Haplotype 2: 77.01%; combined: 99.31%	≥ 95%
BUSCO	C:98.8% [S:97.0%; D:1.8%]; F:0.3%; M:0.9%; n:5 286	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	98.15%	≥ 90%

Data availability

European Nucleotide Archive: *Apamea lithoxylaea* (light arches). Accession number [PRJEB84087](#). The genome sequence is released openly for reuse. The *Apamea lithoxylaea* genome sequencing initiative is part of the Darwin Tree of Life Project (PRJEB40665), the Sanger Institute Tree of Life Programme (PRJEB43745) 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](#).

Production code used in genome assembly at the WSI Tree of Life is available at <https://github.com/sanger-tol>. [Table 5](#) lists software versions used in this study.

Author information

Contributors are listed at the following links:

- Members of the [University of Oxford and Wytham Woods Genome Acquisition Lab](#)
- Members of the [Darwin Tree of Life Barcoding collective](#)
- Members of the [Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team](#)
- Members of [Wellcome Sanger Institute Scientific Operations – Sequencing Operations](#)
- Members of the [Wellcome Sanger Institute Tree of Life Core Informatics team](#)
- Members of the [Tree of Life Core Informatics collective](#)
- Members of the [Darwin Tree of Life Consortium](#)

Table 5. Software versions and sources.

Software	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	4.4.5	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.7.1	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/tolkifasta_windows
FastK	1.1	https://github.com/thegenemyers/FASTK
GenomeScope2.0	2.0.1	https://github.com/tbenavi1/genomescope2.0
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
GoaT CLI	0.2.5	https://github.com/genomehubs/goat-cli
Hifiasm	0.19.8-r603	https://github.com/chhylp123/hifiasm
HiGlass	1.13.4	https://github.com/higlass/higlass
MerquryFK	1.1.2	https://github.com/thegenemyers/MERQURY.FK
Minimap2	2.28-r1209	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	24.10.4	https://github.com/nextflow-io/nextflow
PretextSnapshot	N/A	https://github.com/sanger-tol/PretextSnapshot
PretextView	0.2.5	https://github.com/sanger-tol/PretextView
samtools	1.21	https://github.com/samtools/samtools

Software	Version	Source
sanger-tol/ascc	0.1.0	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	v0.7.1	https://github.com/sanger-tol/blobtoolkit
sanger-tol/curationpretext	1.4.2	https://github.com/sanger-tol/curationpretext
Seqtk	1.3	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.4.0	https://github.com/sanger-tol/treeval
YaHS	1.2.2	https://github.com/c-zhou/yahs

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Annabel Whibley 

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Boyes and colleagues report the genome assembly of *Apamea lithoxyaea* (the noctuid moth commonly known as Light Arches). Two haplotypes are reported, with haplotype 1 scaffolded to pseudochromosome level and including both W and Z sex chromosomes since the individual sample was female. Sample identification was verified by barcoding and followed the standard DToL pipeline for sample processing, sequencing with the ultra-low-input PacBio Hifi protocol and HiC, and assembly.

As seems often the case, the spectrophotometric ratios deviate from typical expectations of a high quality, high purity nucleic acid extraction with no apparent consequences for downstream processing- is it valuable to continue to report these?

The reporting follows DToL templating and provides clear methodological information, sample and analysis metadata, and public accession links are active. The assembly is of high quality, as assessed by a suite of metrics, and will be a valuable addition to the genome catalogue.

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: Bioinformatics, Genomics, Evolution

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 16 October 2025

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Erin Toffelmier 

Virginia Polytechnic Institute and State University Department of Fish and Wildlife Conservation, Blacksburg, Virginia, USA

This article describes a genome assembly for *Apamea lithoxylaea* as part of the Darwin Tree of Life Project. The methods and results are technically sound, and the assembly metrics and completeness reflect a high-quality reference genome.

Notes:

I suggest re-wording or clarify the sentence about the COI species identification method to reflect more directly that the method used followed Crowley et al., 2023. As it's written and cited, it suggests that this particular identification was carried out in Crowley et al., 2023.

The Hi-C methods sections are broken up with two different bolded subheadings while the HiFi section is not. It might be cleaner to have just one section for each method.

I think it would be good to state the SPRI ratios used throughout the HiC methods section.

A synteny analysis to support the naming of the Z and W chromosomes, along with any karyotype or other *a priori* expectation of genome size would make this more robust.

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?

Partly

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: population and conservation genomics, ecology

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.
