

DATA NOTE

The genome sequence of the Warted Knot-Horn moth,

Acrobasis repandana Fabricius, 1798

[version 1; peer review: 2 approved]

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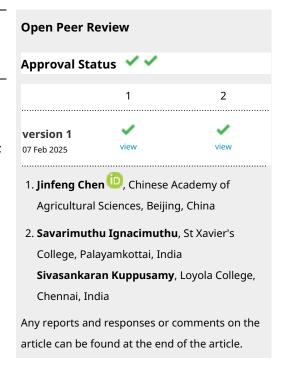
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We present a genome assembly from an individual female specimen of Acrobasis repandana (Warted Knot-Horn moth; Arthropoda; Insecta; Lepidoptera; Pyralidae). The genome sequence has a total length of 620.40 megabases. Most of the assembly (99.78%) is scaffolded into 32 chromosomal pseudomolecules, including the Z and W sex chromosomes. The mitochondrial genome has also been assembled and is 15.21 kilobases in length. Gene annotation of this assembly on Ensembl identified 11,522 protein-coding genes.

Keywords

Acrobasis repandana, Warted Knot-Horn moth, genome sequence, chromosomal, Lepidoptera



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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; Pyralidae; Phycitinae; Acrobasis; Acrobasis repandana Fabricius, 1798 (NCBI:txid1100902)

Background

We present a chromosome-level genome sequence for *Acrobasis repandana* (Figure 1), based on a female specimen from Wytham Woods, Oxfordshire, United Kingdom. It 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 (Blaxter *et al.*, 2022).

Genome sequence report

The genome of *Acrobasis repandana* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating a total of 43.03 Gb (gigabases) from 3.93 million reads, providing an estimated 36-fold coverage. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data, which produced 108.14 Gb from 716.19 million reads. Specimen and sequencing details are summarised in Table 1.

Assembly errors were corrected by manual curation, including 26 missing joins or mis-joins and three haplotypic duplications. This reduced the scaffold number by 15.38%. The final assembly has a total length of 620.40 Mb in 54 sequence scaffolds, with 23 gaps, and a scaffold N50 of 22.2 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



Figure 1. Photograph of the *Acrobasis repandana* (ilAcrRepa1) specimen used for genome sequencing.

different scaffold subsets assigned to various phyla, illustrating the completeness of the assembly.

Most of the assembly sequence (99.78%) was assigned to 32 chromosomal-level scaffolds, representing 30 autosomes and the Z and W sex chromosomes. These chromosome-level scaffolds, confirmed by the Hi-C data, are named in order of size (Figure 5; Table 3). During manual curation, chromosomes W and Z were assigned by read coverage statistics.

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, and as a separate fasta file with accession OY756246.1.

The final assembly has a Quality Value (QV) of 64.0 and k-mer completeness of 99.06% (combined primary assembly and alternate haplotype). BUSCO (v5.4.3) analysis using the lepidoptera_odb10 reference set (n=5,286) indicated a completeness score of 99.0% (single = 98.5%, duplicated = 0.5%). The assembly achieves the EBP reference standard of 6.C.64. Other quality metrics are given in Table 2.

Genome annotation report

The *Acrobasis repandana* genome assembly (GCA_963576875.1) was annotated at the European Bioinformatics Institute (EBI) on Ensembl Rapid Release. The resulting annotation includes 20,378 transcribed mRNAs from 11,522 protein-coding and 1,600 non-coding genes (Table 2; https://rapid.ensembl.org/Acrobasis_repandana_GCA_963576875.1/Info/Index). The average transcript length is 16,452.98, with 1.55 coding transcripts per gene and 6.98 exons per transcript.

Methods

Sample acquisition and DNA barcoding

An adult female *Acrobasis repandana* (specimen ID Ox000620, ToLID ilAcrRepa1) was collected from Wytham Woods, Berkshire, United Kingdom (latitude 51.77, longitude –1.34) on 2020-07-05, 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 sequencing (specimen ID NHMUK015059184, ToLID ilAcrRepa3) was collected from Marston Marsh, Norwich, England, United Kingdom (latitude 52.6, longitude 1.27) on 2022-07-04. The specimen was collected by Inez Januszczak (Natural History Museum) and identified by Ryan Mitchell (National Museums Northern Ireland) and preserved by dry freezing at -80 °C.

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 specimens and stored in ethanol, while the remaining parts were shipped on dry ice to the Wellcome Sanger Institute (WSI). The tissue was lysed, the COI marker region was

Table 1. Specimen and sequencing data for Acrobasis repandana.

Project information				
Study title	Acrobasis repandana (warted knot-horn)			
Umbrella BioProject	PRJEB65199			
Species	Acrobasis repand	Acrobasis repandana		
BioSample	SAMEA7701484			
NCBI taxonomy ID	1100902			
Specimen information				
Technology	ToLID	BioSample accession	Organism part	
PacBio long read sequencing	ilAcrRepa1	SAMEA7701659	Whole organism	
Hi-C sequencing	ilAcrRepa3	SAMEA112963129	Whole organism	
Sequencing information				
Platform	Run accession	Read count	Base count (Gb)	
Hi-C Illumina NovaSeq 6000	ERR11872558	7.16e+08	108.14	
PacBio Sequel IIe	ERR11867202	2.04e+06	19.88	
PacBio Sequel IIe	ERR11867200	6.23e+05	8.08	
PacBio Sequel IIe	ERR11867201	1.26e+06	15.06	

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 procedures: sample preparation and homogenisation, DNA extraction, fragmentation and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b).

The ilAcrRepa1 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 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 a Qubit

Fluorometer using the Qubit dsDNA High Sensitivity Assay kit. The fragment size distribution was evaluated by running the sample on the FemtoPulse system.

Hi-C preparation

Whole organism tissue of the ilAcrRepa3 sample was processed at the WSI Scientific Operations core, using the Arima-HiC v2 kit. Tissue (stored at -80 °C) was fixed, and the DNA crosslinked using a TC buffer with 22% formaldehyde. After crosslinking, the tissue was homogenised using the Diagnocine Power Masher-II and BioMasher-II tubes and pestles. Following the kit manufacturer's instructions, crosslinked DNA was digested using a restriction enzyme master mix. The 5'-overhangs were then 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.

Library preparation and sequencing

For PacBio DNA sequencing, 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. 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

Table 2. Genome assembly data for Acrobasis repandana, ilAcrRepa1.1.

Genome assembly			
ilAcrRepa1.1			
GCA_963576875.1			
GCA_963576835.1			
620.40			
78			
54			
29.01			
	Benchmark		
20.9	≥ 1 <i>Mb</i>		
22.2	= chromosome N50		
64.0	≥ 40		
99.06% (combined primary and alternate haplotypes)	≥ 95%		
C:99.0%[S:98.5%,D:0.5%], F:0.2%,M:0.8%,n:5,286	S > 90%, D < 5%		
99.78%	≥ 90%		
ZW	localised homologous pairs		
Mitochondrial genome: 15.21 kb	complete single alleles		
Genome annotation of assembly GCA_963576875.1 at Ensembl			
11,522			
1,600			
20,378			
	GCA_963576875.1 GCA_963576835.1 620.40 78 54 29.01 20.9 22.2 64.0 99.06% (combined primary and alternate haplotypes) C:99.0%[S:98.5%,D:0.5%],F:0.2%,M:0.8%,n:5,286 99.78% ZW Mitochondrial genome: 15.21 kb bly GCA_963576875.1 at Ensembl 11,522 1,600		

^{*} Assembly metric benchmarks are adapted from Rhie *et al.* (2021) and the Earth BioGenome Project Report on Assembly Standards September 2024.

Biosciences). 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 using the Sequel IIe system (Pacific Biosciences, California, USA). The concentration of the library loaded onto the Sequel IIe was in the range of 40–135 pM. 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.

For Hi-C library preparation, DNA was fragmented to a size of 400 to 600 bp using a Covaris E220 sonicator. The DNA was then enriched, barcoded, and amplified using the NEBNext Ultra II DNA Library Prep Kit following manufacturers' instructions. 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

The HiFi reads were first assembled using Hifiasm (Cheng et al., 2021) with the --primary option. Haplotypic duplications were

^{**} BUSCO scores based on the lepidoptera_odb10 BUSCO set using version 5.4.3. 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/Acrobasis_repandana/dataset/GCA_963576875.1/busco.

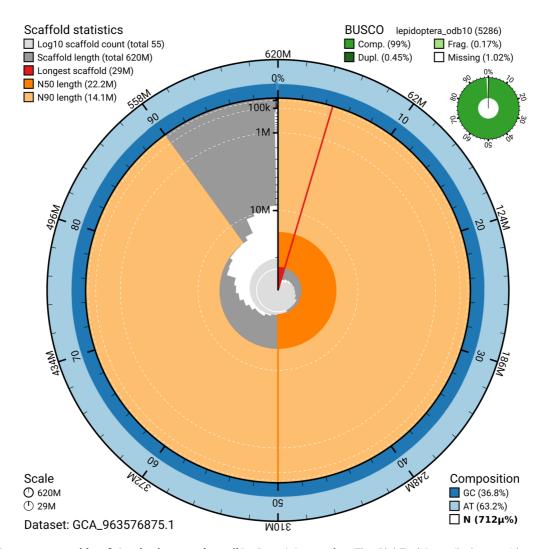


Figure 2. Genome assembly of *Acrobasis repandana*, **ilAcrRepa1.1: metrics.** The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the 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_963576875.1/dataset/GCA_963576875.1/snail.

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 (article in preparation). Flat files and maps used in curation were generated in TreeVal (Pointon *et al.*, 2023). 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).

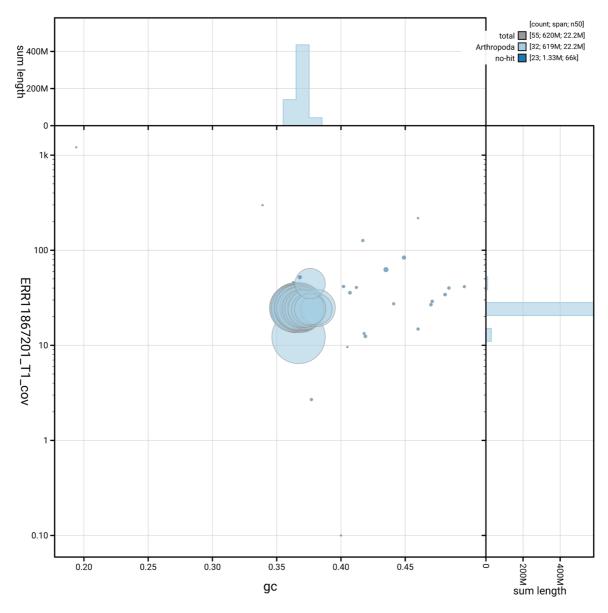


Figure 3. Genome assembly of *Acrobasis repandana*, **ilAcrRepa1.1: BlobToolKit GC-coverage plot showing sequence coverage (vertical axis) and GC content (horizontal axis).** The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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_963576875.1/blob.

Any identified contamination, missed joins, and mis-joins were corrected, and duplicate sequences were tagged and removed. The curation process is documented at https://gitlab.com/wtsi-grit/rapid-curation (article in preparation).

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

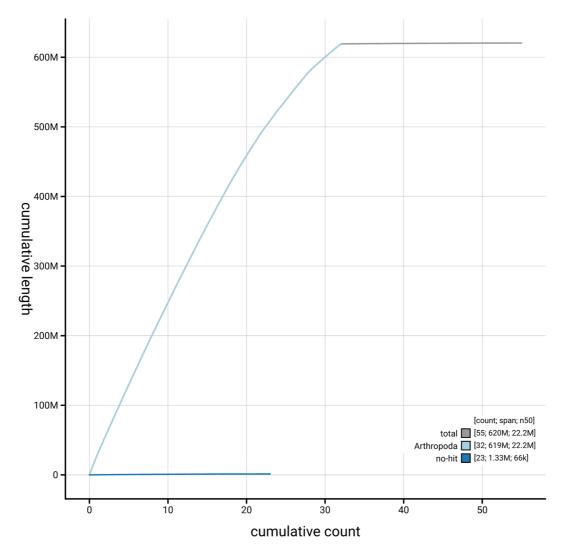


Figure 4. Genome assembly of *Acrobasis repandana* **ilAcrRepa1.1: BlobToolKit cumulative sequence plot.** The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963576875.1/dataset/GCA_963576875.1/cumulative.

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 is 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.

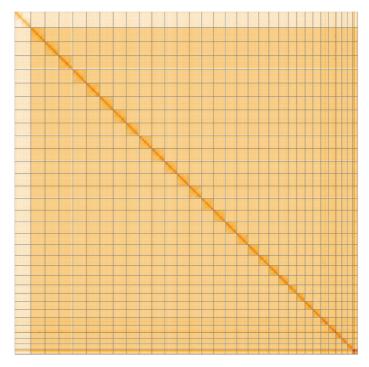


Figure 5. Genome assembly of *Acrobasis repandana* ilAcrRepa1.1: Hi-C contact map of the ilAcrRepa1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=CnJqXb37SUC-ktxRbhhHxQ.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Acrobasis repandana*, ilAcrRepa1.

INSDC accession	Name	Length (Mb)	GC%
OY756215.1	1	25.66	36.5
OY756216.1	2	25.16	36.5
OY756217.1	3	24.87	37.0
OY756218.1	4	24.61	36.5
OY756219.1	5	24.31	36.5
OY756220.1	6	24.1	36.5
OY756221.1	7	23.7	36.5
OY756222.1	8	23.11	36.5
OY756223.1	9	23.06	36.5
OY756224.1	10	23.01	37.0
OY756225.1	11	22.47	36.5
OY756226.1	12	22.18	36.5
OY756227.1	13	22.1	36.5
OY756228.1	14	21.64	36.5
OY756229.1	15	20.97	36.5

INSDC accession	Name	Length (Mb)	GC%
OY756230.1	16	20.95	36.5
OY756231.1	17	20.19	37.0
OY756232.1	18	19.35	37.0
OY756233.1	19	18.39	37.0
OY756234.1	20	17.77	36.5
OY756235.1	21	17.29	37.0
OY756236.1	22	15.23	37.0
OY756237.1	23	14.98	37.0
OY756238.1	24	14.28	37.5
OY756239.1	25	14.23	37.0
OY756240.1	26	14.13	38.0
OY756241.1	27	13.28	37.5
OY756242.1	28	10.37	37.5
OY756243.1	29	9.85	38.0
OY756244.1	30	9.7	37.5
OY756245.1	W	9.18	37.5
OY756214.1	Z	29.01	36.5
OY756246.1	MT	0.02	20.0

Table 4 contains a list of relevant software tool versions and sources.

Genome annotation

The Ensembl Genebuild annotation system (Aken *et al.*, 2016) was used to generate annotation for the *Acrobasis repandana* assembly (GCA_963576875.1) 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

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	4.3.7	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.4.3 and 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	427104ea91c78c3b8b8b49f1a7d6bbeaa869ba1c	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	0.19.8-r587	https://github.com/chhylp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84 aa44357826c0b6753eb28de	https://github.com/higlass/higlass
Merqury.FK	d00d98157618f4e8d1a9190026b19b471055b2 2e	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	3	https://github.com/marcelauliano/MitoHiFi
MultiQC	1.14, 1.17, and 1.18	https://github.com/MultiQC/MultiQC
NCBI Datasets	15.12.0	https://github.com/ncbi/datasets
Nextflow	23.04.0-5857	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	1.16.1, 1.17, and 1.18	https://github.com/samtools/samtools
sanger-tol/ ascc	-	https://github.com/sanger-tol/ascc
sanger-tol/ blobtoolkit	0.6.0	https://github.com/sanger-tol/blobtoolkit
Seqtk	1.3	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.0.0	https://github.com/sanger-tol/treeval
YaHS	1.2a.2	https://github.com/c-zhou/yahs

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: Acrobasis repandana (warted knot-horn). Accession number PRJEB65199; https://identifiers. org/ena.embl/PRJEB65199. The genome sequence is released openly for reuse. The Acrobasis repandana 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 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.

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.

References

Abdennur N, Mirny LA: Cooler: scalable storage for Hi-C data and other genomically labeled arrays. *Bioinformatics*. 2020; **36**(1): 311–316. PubMed Abstract | Publisher Full Text | Free Full Text

Aken BL, Ayling S, Barrell D, et al.: The ensembl gene annotation system. Database (Oxford). 2016; 2016: baw093.

PubMed Abstract | Publisher Full Text | Free Full Text

Allio R, Schomaker-Bastos A, Romiguier J, et al.: MitoFinder: efficient automated large-scale extraction of mitogenomic data in target enrichment phylogenomics. Mol Ecol Resour. 2020; 20(4): 892–905. PubMed Abstract | Publisher Full Text | Free Full Text

Altschul SF, Gish W, Miller W, et al.: Basic local alignment search tool. J Mol Biol. 1990; 215(3): 403-410

PubMed Abstract | Publisher Full Text

Bateman A, Martin MJ, Orchard S, et al.: UniProt: the universal protein knowledgebase in 2023. Nucleic Acids Res. 2023; 51(D1): D523-D531. PubMed Abstract | Publisher Full Text | Free Full Text

Beasley J, Uhl R, Forrest LL, et al.: DNA barcoding SOPs for the Darwin Tree of Life project. protocols.io. 2023; [Accessed 25 June 2024] **Publisher Full Text**

Blaxter M, Mieszkowska N, Di Palma F, et al.: Sequence locally, think globally: the Darwin Tree of Life project. Proc Natl Acad Sci U S A. 2022; 119(4):

PubMed Abstract | Publisher Full Text | Free Full Text

Buchfink B, Reuter K, Drost HG: Sensitive protein alignments at Tree-of-Life scale using DIAMOND. Nat Methods. 2021; 18(4): 366–368.
PubMed Abstract | Publisher Full Text | Free Full Text

Challis R, Kumar S, Sotero-Caio C, et al.: Genomes on a Tree (GoaT): a versatile, scalable search engine for genomic and sequencing project metadata across the eukaryotic Tree of Life [version 1; peer review: 2 approved]. Wellcome Open Res. 2023; 8: 24.

PubMed Abstract | Publisher Full Text | Free Full Text

Challis R, Richards E, Rajan J, et al.: BlobToolKit - interactive quality assessment of genome assemblies. G3 (Bethesda). 2020; 10(4): 1361-1374. PubMed Abstract | Publisher Full Text | Free Full Text

Cheng H, Concepcion GT, Feng X, et al.: Haplotype-resolved de novo assembly using phased assembly graphs with hifiasm. Nat Methods. 2021; 18(2):

PubMed Abstract | Publisher Full Text | Free Full Text

Crowley L. Allen H. Barnes I. et al.: A sampling strategy for genome sequencing the British terrestrial arthropod fauna (version 1; peer review: 2 approved]. Wellcome Open Res. 2023; 8: 123.

PubMed Abstract | Publisher Full Text | Free Full Text

da Veiga Leprevost F, Grüning BA, Alves Aflitos S, et al.: BioContainers: an open-source and community-driven framework for software standardization. Bioinformatics. 2017; 33(16): 2580-2582 PubMed Abstract | Publisher Full Text | Free Full Text

Danecek P, Bonfield JK, Liddle J, *et al.*: **Twelve years of SAMtools and BCFtools.** *GigaScience*. 2021; **10**(2): giab008. **PubMed Abstract** | **Publisher Full Text** | **Free Full Text**

Denton A, Oatley G, Cornwell C, et al.: Sanger Tree of Life sample homogenisation: PowerMash. protocols.io. 2023a.

Denton A, Yatsenko H, Jay J, *et al.*: **Sanger Tree of Life wet laboratory protocol collection V.1.** *protocols.io.* 2023b. **Publisher Full Text**

Diesh C, Stevens GJ, Xie P, et al.: JBrowse 2: a modular genome browser with views of synteny and structural variation. Genome Biol. 2023; 24(1): 74. PubMed Abstract | Publisher Full Text | Free Full Text

Ewels P, Magnusson M, Lundin S, et al.: MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics. 2016; 32(19): 3047-3048.

PubMed Abstract | Publisher Full Text | Free Full Text

Ewels PA, Peltzer A, Fillinger S, et al.: The nf-core framework for communitycurated bioinformatics pipelines. Nat Biotechnol. 2020; 38(3): 276-278. PubMed Abstract | Publisher Full Text

Formenti G, Abueg L, Brajuka A, et al.: Gfastats: conversion, evaluation and manipulation of genome sequences using assembly graphs. *Bioinformatics*. 2022; **38**(17): 4214–4216.

PubMed Abstract | Publisher Full Text | Free Full Text

Grüning B, Dale R, Sjödin A, et al.: Bioconda: sustainable and comprehensive software distribution for the life sciences. *Nat Methods.* 2018; **15**(7): 475–476. PubMed Abstract | Publisher Full Text | Free Full Text

Guan D, McCarthy SA, Wood J, et al.: Identifying and removing haplotypic duplication in primary genome assemblies. *Bioinformatics*. 2020; **36**(9): 2896-2898

PubMed Abstract | Publisher Full Text | Free Full Text

Harry E: PretextView (Paired REad TEXTure Viewer): a desktop application for viewing pretext contact maps. 2022. Reference

Howe K, Chow W, Collins J, et al.: Significantly improving the quality of genome assemblies through curation. GigaScience. 2021; 10(1): giaa153. PubMed Abstract | Publisher Full Text | Free Full Text

Jay J, Yatsenko H, Narváez-Gómez JP, et al.: Sanger Tree of Life sample preparation: triage and dissection. protocols.io. 2023. **Publisher Full Text**

Kerpedjiev P, Abdennur N, Lekschas F, et al.: HiGlass: web-based visual exploration and analysis of genome interaction maps. Genome Biol. 2018;

PubMed Abstract | Publisher Full Text | Free Full Text

Kurtzer GM, Sochat V, Bauer MW: Singularity: scientific containers for mobility of compute. PLoS One. 2017; 12(5): e0177459. PubMed Abstract | Publisher Full Text | Free Full Text

Li H: Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics. 2018; 34(18): 3094–3100.
PubMed Abstract | Publisher Full Text | Free Full Text

Manni M, Berkeley MR, Seppey M, et al.: BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic

coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Mol Biol Evol. 2021: 38(10): 4647-4654.

PubMed Abstract | Publisher Full Text | Free Full Text

Merkel D: **Docker: lightweight Linux containers for consistent development and deployment.** *Linux J.* 2014; **2014**(239): 2, [Accessed 2 April 2024].

Pointon DL, Eagles W, Sims Y, et al.: sanger-tol/treeval v1.0.0 - Ancient Atlantis. 2023.

Publisher Full Text

Quinlan AR, Hall IM: BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics. 2010; 26(6): 841-842.

PubMed Abstract | Publisher Full Text | Free Full Text

Rao SSP, Huntley MH, Durand NC, et al.: A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell. 2014; **159**(7): 1665-1680.

PubMed Abstract | Publisher Full Text | Free Full Text

Rhie A, McCarthy SA, Fedrigo O, et al.: Towards complete and error-free genome assemblies of all vertebrate species. Nature. 2021; 592(7856):

PubMed Abstract | Publisher Full Text | Free Full Text

Rhie A, Walenz BP, Koren S, et al.: Merqury: reference-free quality, completeness, and phasing assessment for genome assemblies, Genome Biol. 2020; 21(1): 245

PubMed Abstract | Publisher Full Text | Free Full Text

Sheerin E, Sampaio F, Oatley G: Sanger Tree of Life HMW DNA extraction: automated MagAttract v.1. protocols.io. 2023.

Publisher Full Text

Strickland M, Cornwell C, Howard C: Sanger Tree of Life fragmented DNA clean up: manual SPRI. protocols.io. 2023.

Publisher Full Text

Todorovic M, Sampaio F, Howard C: Sanger Tree of Life HMW DNA fragmentation: diagenode Megaruptor®3 for PacBio HiFi. protocols.io. 2023. **Publisher Full Text**

Twyford AD, Beasley J, Barnes I, $\it et\,al.$: A DNA barcoding framework for taxonomic verification in the darwin Tree of Life project [version 1; peer review: 2 approved]. Wellcome Open Res. 2024; 9: 339.

PubMed Abstract | Publisher Full Text | Free Full Text

Uliano-Silva M, Ferreira JGRN, Krasheninnikova K, et al.: MitoHiFi: a python pipeline for mitochondrial genome assembly from PacBio high fidelity

reads. BMC Bioinformatics. 2023; 24(1): 288.
PubMed Abstract | Publisher Full Text | Free Full Text

UniProt Consortium: UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 2019; 47(D1): D506-D515.

PubMed Abstract | Publisher Full Text | Free Full Text

Vasimuddin M, Misra S, Li H, et al.: Efficient architecture-aware acceleration of BWA-MEM for multicore systems. In: 2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS). IEEE, 2019; 314-324.

Publisher Full Text

Zhou C, McCarthy SA, Durbin R: YaHS: yet another Hi-C scaffolding tool. Bioinformatics. 2023; 39(1): btac808.

PubMed Abstract | Publisher Full Text | Free Full Text

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The authors have done chromosome-level genome assembly of Warted Knot-Horn moth, Acrobasis repandana Fabricius, 1798. They assembled the sequence using appropriate softwares and obtained the genome 620.40 megabases in size. The assembly was scaffolded into 32 chromosomes. The materials and methods were properly described.

Minor comments on the manuscript

The first sentence of the background may be modified as "We present a chromosome-level genome assembly for Acrobasis repandana Fabricius, 1798..."

The authors consistently used the complete genus name, Acrobasis repandana, throughout the manuscript. If possible, to use the full genus name at first, and later to shorten it to A. repandana. The complete length of the mitochondrial genome sequence was not given in the text. The length of the sequence can be included in the text.

The manuscript is well prepared therefore I confirm that the manuscript meets the necessary scientific standard and is suitable for indexing.

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: Molecular biology

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 24 February 2025

https://doi.org/10.21956/wellcomeopenres.26107.r118569

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Jinfeng Chen 🗓

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The manuscript describes the genome assembly of the Warted Knot-Horn moth, *Acrobasis repandana*. The authors generated 36-fold genome coverage HiFi reads and assembled a 620.40 of very high quality as shown by the 99% BUSCO score and many other parameters. The methods were well described and the related data were available in the public database. Therefore, I think this is a well-organized manuscript for describing a genome. I don't any further questions on indexing it.

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: Comparative genomics and transposable elements

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.

Comments on this article

Version 1

Reader Comment 10 Feb 2025

jim mallet, Organismic and Evolutionary Biology, Harvard University Faculty of Arts and Sciences, Cambridge, USA

The work is up to the usual high standards I have come to expect from this pipeline. However, the photograph on Fig. 1, purportedly of *Acrobasis repandana*, is clearly of another British moth, the Rosy Footman *Miltochrista miniata*. Please correct this paper! Is the genome sequence documented from a specimen of *Miltochrista* or of *Acrobasis*? In any case Erebidae (Arctiinae) - for *Miltochrista* - is very far from Pyraloidea (Pyralidae) - for *Acrobasis*. This I would say is a major problem for approval of this paper by peer-reviewers.

Competing Interests: Mutual interest in Lepidoptera and their genetics?

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