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DATA NOTE

The genome sequence of the Triangle-marked Roller, *Ancylis achatana* (Denis & Schiffermüller), 1775

[version 1; peer review: 2 approved, 1 approved with reservations]

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v1 **First published:** 14 Mar 2025, **10**:128
https://doi.org/10.12688/wellcomeopenres.23897.1
Latest published: 14 Mar 2025, **10**:128
https://doi.org/10.12688/wellcomeopenres.23897.1

Abstract

We present a genome assembly from a male specimen of *Ancylis achatana* (Triangle-marked Roller; Arthropoda; Insecta; Lepidoptera; Tortricidae). The genome sequence has a total length of 547.27 megabases. Most of the assembly (99.25%) is scaffolded into 28 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled, with a length of 16.0 kilobases.

Keywords

Ancylis achatana, Triangle-marked Roller, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ? ✓

	1	2	3
version 1 14 Mar 2025	✓ view	? view	✓ view

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Author roles: Boyes D: Investigation, Resources; **Lees DC**: Investigation, Resources; **Boyes C**: Writing – Original Draft Preparation;

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Wellcome through core funding to the Wellcome Sanger Institute (220540) and the Darwin Tree of Life Discretionary Award [218328, https://doi.org/10.35802/218328].
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Boyes D, Lees DC, Boyes C *et al.* **The genome sequence of the Triangle-marked Roller, *Ancylis achatana* (Denis & Schiffermüller), 1775 [version 1; peer review: 2 approved, 1 approved with reservations]** Wellcome Open Research 2025, **10**:128 https://doi.org/10.12688/wellcomeopenres.23897.1

First published: 14 Mar 2025, **10**:128 https://doi.org/10.12688/wellcomeopenres.23897.1

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; Apoditrysia; Tortricoidea; Tortricidae; Olethreutinae; Enarmoniini; *Ancylis*; *Ancylis achatana* (Denis & Schiffermüller), 1775 (NCBI: txid1869464)

Background

Ancylis achatana (common name Triangle-marked Roller or Hedge Hook-wing) is a micro-moth in the family Tortricidae. It is common in England and Wales and local in Ireland. It is found eastwards across Europe to Russia (GBIF Secretariat, 2025).

The small moth (forewing length 6–9 mm) is mottled brown and grey with a distinctive pre-tornal triangular rich brown patch in the middle of the trailing edge of the forewings. Although the colouration is variable, the markings are consistent. The moth is on the wing between May and August and comes to light (Sterling *et al.*, 2023).

Ancylis achatana lays its eggs on hawthorn, blackthorn or occasionally *Cotoneaster* spp., and the larva spins a tubular shelter from leaves attached to a twig, and then feeds close by (Hancock *et al.*, 2015). It pupates in the leaf spinnings during May (Langmaid *et al.*, 2018).

We present a chromosome-level genome sequence for *Ancylis achatana* based on one male specimen from Wytham Woods, Oxfordshire, UK. The genome 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.

Genome sequence report

Sequencing data

The genome of a specimen of *Ancylis achatana* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 59.18 Gb (gigabases) from 5.55 million reads. GenomeScope analysis of the PacBio HiFi data estimated the haploid genome size at 575.95 Mb, with a heterozygosity of 0.29% and repeat content of 38.29%. These values provide an initial assessment of genome complexity and the challenges anticipated during assembly. Based on this estimated genome size, the sequencing data provided approximately 100.0x coverage of the genome. Chromosome conformation Hi-C sequencing produced 106.37 Gb from 704.46 million reads. Table 1 summarises the specimen and sequencing information.

Assembly statistics

The primary haplotype was assembled, and contigs corresponding to an alternate haplotype were also deposited in INSDC databases. The assembly was improved by manual curation, which corrected 55 misjoins or missing joins. These interventions decreased the scaffold count by 5.88%. The final assembly has a total length of 547.27 Mb in 111 scaffolds, with 67 gaps, and a scaffold N50 of 20.35 Mb (Table 2).

Figure 1. Photograph of the *Ancylis achatana* (ilAncAcha1) specimen used for genome sequencing.

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

Most of the assembly sequence (99.26%) was assigned to 28 chromosomal-level scaffolds, representing 27 autosomes and the Z sex chromosome. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 5; Table 3). During curation, chromosome Z was assigned by synteny to *Archips crataeganus* (GCA_947859365.1) (Boyes *et al.*, 2024).

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.

Assembly quality metrics

The estimated Quality Value (QV) and *k*-mer completeness metrics, along with BUSCO completeness scores, were calculated for each haplotype and the combined assembly. The QV reflects the base-level accuracy of the assembly, while *k*-mer completeness indicates the proportion of expected *k*-mers identified in the assembly. BUSCO scores provide a measure of completeness based on benchmarking universal single-copy orthologues.

The combined primary and alternate assemblies achieve an estimated QV of 61.9. The *k*-mer recovery for the primary haplotype is 91.92%, and for the alternate haplotype

Table 1. Specimen and sequencing data for *Ancylis achatana*.

Project information			
Study title	Ancylis achatana (triangle-marked roller)		
Umbrella BioProject	PRJEB78334		
Species	*Ancylis achatana*		
BioSpecimen	SAMEA7520689		
NCBI taxonomy ID	1869464		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	ilAncAcha1	SAMEA7520779	whole organism
Hi-C sequencing	ilAncAcha2	SAMEA114806094	whole organism
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq X	ERR13389762	7.04e+08	106.37
PacBio Revio	ERR13420778	5.55e+06	59.18

90.69%; the combined primary and alternate assemblies have a *k*-mer recovery of 99.17%. BUSCO analysis using the lepidoptera_odb10 reference set (n = 5,286) identified 98.4% of the expected gene set (single = 98.0%, duplicated = 0.5%).

Table 2 provides assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project (EBP) Report on Assembly Standards September 2024. The assembly achieves the EBP reference standard of **6.C.Q61.**

Methods

Sample acquisition and DNA barcoding

An adult male *Ancylis achatana* (specimen ID Ox000473, ToLID ilAncAcha1) was collected from Wytham Woods, Oxfordshire, United Kingdom (latitude 51.77, longitude –1.34) on 2020-06-13 by 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 NHMUK014584877, ToLID ilAncAcha2) was collected from Bure Marshes Stables, England, United Kingdom (latitude 52.69, longitude 1.45) on 2022-06-29. The specimen was collected by David Lees (Natural History Museum) and identified by Mark Sterling (Natural History Museum) and preserved by dry freezing (–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 specimen and stored in ethanol, while the remaining parts were shipped on dry ice to the Wellcome Sanger Institute (WSI) (Pereira *et al.*, 2022). The tissue was lysed, the COI marker region was amplified by PCR, and amplicons were sequenced and compared to the BOLD database, confirming the species identification (Crowley *et al.*, 2023). Following whole genome sequence generation, the relevant DNA barcode region was also used alongside the initial barcoding data for sample tracking at the WSI (Twyford *et al.*, 2024). The standard operating procedures for Darwin Tree of Life barcoding have been deposited on protocols.io (Beasley *et al.*, 2023).

Metadata collection for samples adhered to the Darwin Tree of Life project standards described by Lawniczak *et al.* (2022).

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 ilAncAcha1 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 v2 protocol (Oatley *et al.*, 2023a). DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system (Bates *et al.*, 2023). Sheared DNA was purified by solid-phase

Table 2. Genome assembly data for *Ancylis achatana*.

Genome assembly		
Assembly name	ilAncAcha1.1	
Assembly accession	GCA_964214005.1	
Alternate haplotype accession	*GCA_964214015.1*	
Assembly level for primary assembly	chromosome	
Span (Mb)	547.27	
Number of contigs	178	
Number of scaffolds	111	
Longest scaffold (Mb)	42.27	
Assembly metric	**Measure**	***Benchmark***
Contig N50 length	9.22 Mb	*≥ 1 Mb*
Scaffold N50 length	20.35 Mb	*= chromosome N50*
Consensus quality (QV)	Primary: 63.0; alternate: 61.1; combined 61.9	*≥ 40*
k-mer completeness	Primary: 91.92%; alternate: 90.69%; combined: 99.17%	*≥ 95%*
BUSCO*	C:98.4%[S:98.0%,D:0.5%], F:0.2%,M:1.3%,n:5,286	*S > 90%; D < 5%*
Percentage of assembly mapped to chromosomes	99.26%	*≥ 90%*
Sex chromosomes	Z	*localised homologous pairs*
Organelles	Mitochondrial genome: 16.0 kb	*complete single alleles*

* BUSCO scores based on the lepidoptera_odb10 BUSCO set using version 5.5.0. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison.

reversible immobilisation, using AMPure PB beads to eliminate shorter fragments and concentrate the DNA (Oatley *et al.*, 2023b). 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.

Hi-C sample preparation

Tissue from the whole organism of the ilAncAcha2 sample was processed for Hi-C sequencing at the WSI Scientific Operations core, using the Arima-HiC v2 kit. In brief, 20–50 mg of frozen tissue (stored at –80 °C) was fixed, and the DNA crosslinked using a TC buffer with 22% formaldehyde concentration. After crosslinking, the tissue was homogenised using the Diagnocine Power Masher-II and BioMasher-II tubes and pestles. Following the Arima-HiC v2 kit manufacturer's instructions, crosslinked DNA was digested using a restriction enzyme master mix. The 5'-overhangs were 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. Additionally, the biotinylation percentage was estimated using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and Qubit HS Assay Kit and Arima-HiC v2 QC beads.

Library preparation and sequencing

Library preparation and sequencing were performed at the WSI Scientific Operations core.

PacBio HiFi

At a minimum, 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

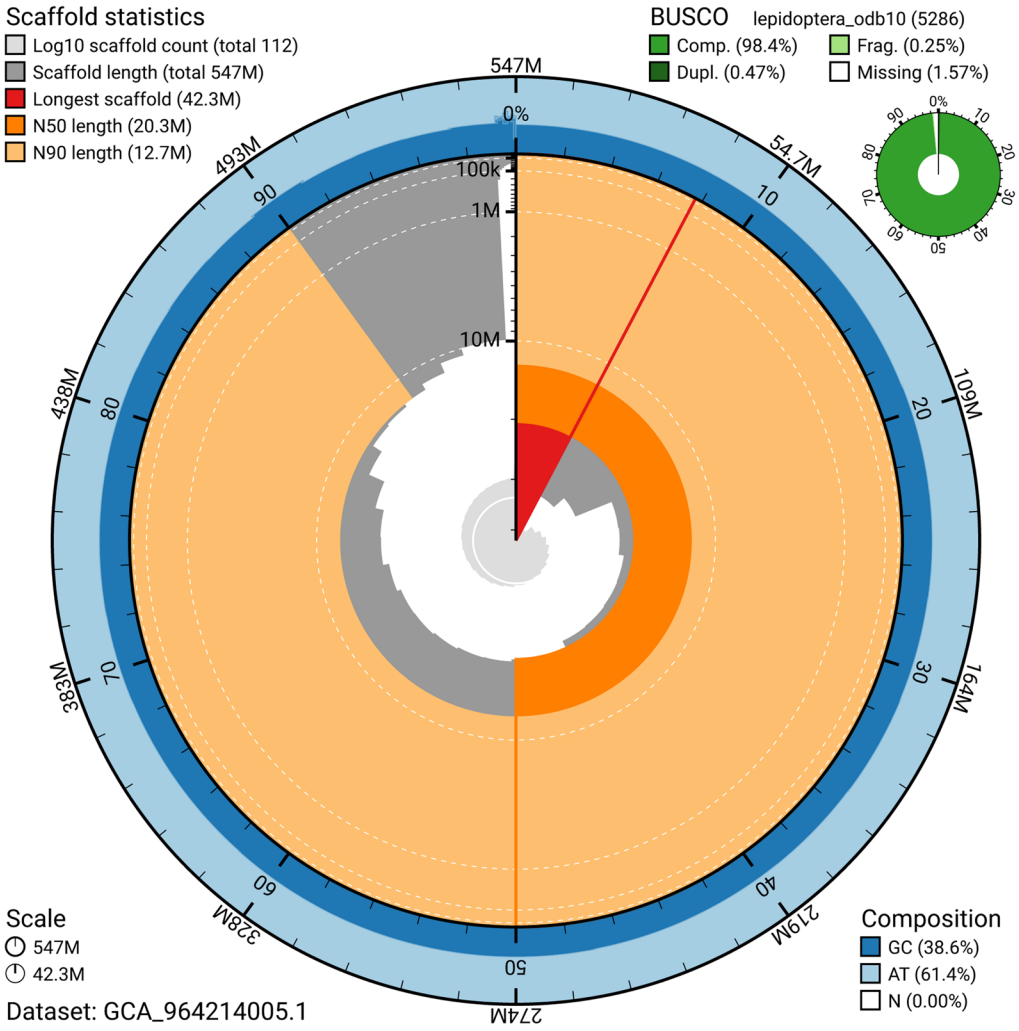

Figure 2. Genome assembly of *Ancylis achatana*, ilAncAcha1.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. 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_964214005.1/dataset/GCA_964214005.1/snail.

protocol (Pacific Biosciences, California, USA), depending on genome size and sequencing depth required. Libraries were prepared using the SMRTbell Prep Kit 3.0 (Pacific Biosciences, California, USA) as per the manufacturer's instructions. 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 Biosciences, California, USA). 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 on a Revio instrument (Pacific Biosciences, California, USA). Prepared libraries were normalised to 2 nM, and 15 µL was used for making complexes. Primers were annealed and polymerases were hybridised to create circularised complexes according to manufacturer's instructions. The complexes were purified with the 1.2X clean up with SMRTbell beads. The purified complexes were then diluted to the Revio loading concentration (in the range 200–300 pM), and spiked with a Revio sequencing internal control. Samples were sequenced on Revio 25M SMRT cells (Pacific

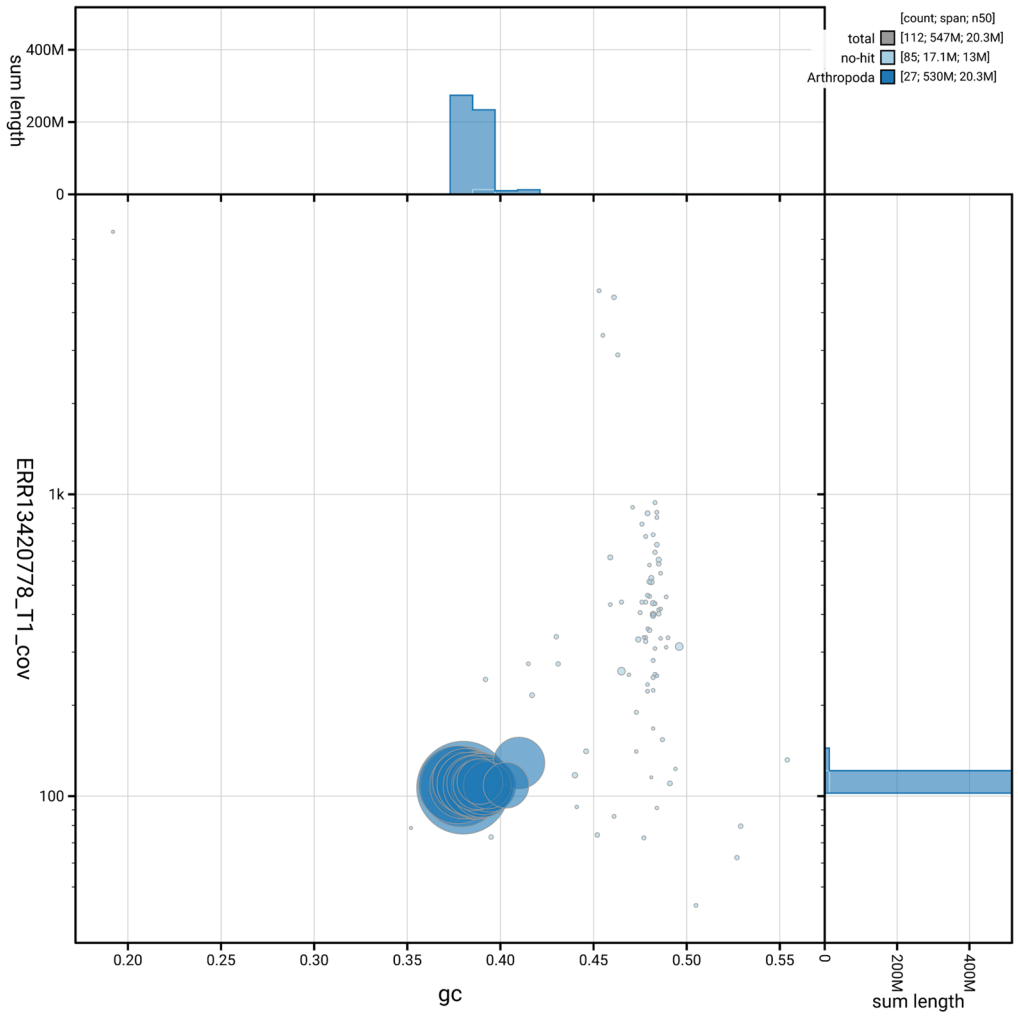

Figure 3. Genome assembly of *Ancylis achatana*, ilAncAcha1.1: BlobToolKit GC-coverage plot. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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 at https://blobtoolkit.genomehubs.org/view/GCA_964214005.1/blob.

Biosciences, California, USA). 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.

Hi-C

For Hi-C library preparation, DNA was fragmented using the Covaris E220 sonicator (Covaris) and size selected using SPRISelect beads to 400 to 600 bp. The DNA was then enriched using the Arima-HiC v2 kit Enrichment beads. Using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) for end repair, a-tailing, and adapter ligation. This uses a custom protocol which resembles the standard NEBNext Ultra II DNA Library Prep protocol but where library preparation occurs while DNA is bound to the Enrichment beads. For library amplification, 10 to 16 PCR cycles were required, determined by the sample biotinylation percentage. The Hi-C sequencing was performed using paired-end sequencing with a read length of 150 bp on an Illumina NovaSeq X instrument.

Genome assembly, curation and evaluation

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 first assembled using Hifiasm (Cheng *et al.*, 2021) with the --primary option. Haplotypic duplications were identified and removed using purge_dups (Guan *et al.*, 2020). The Hi-C reads were mapped to the primary

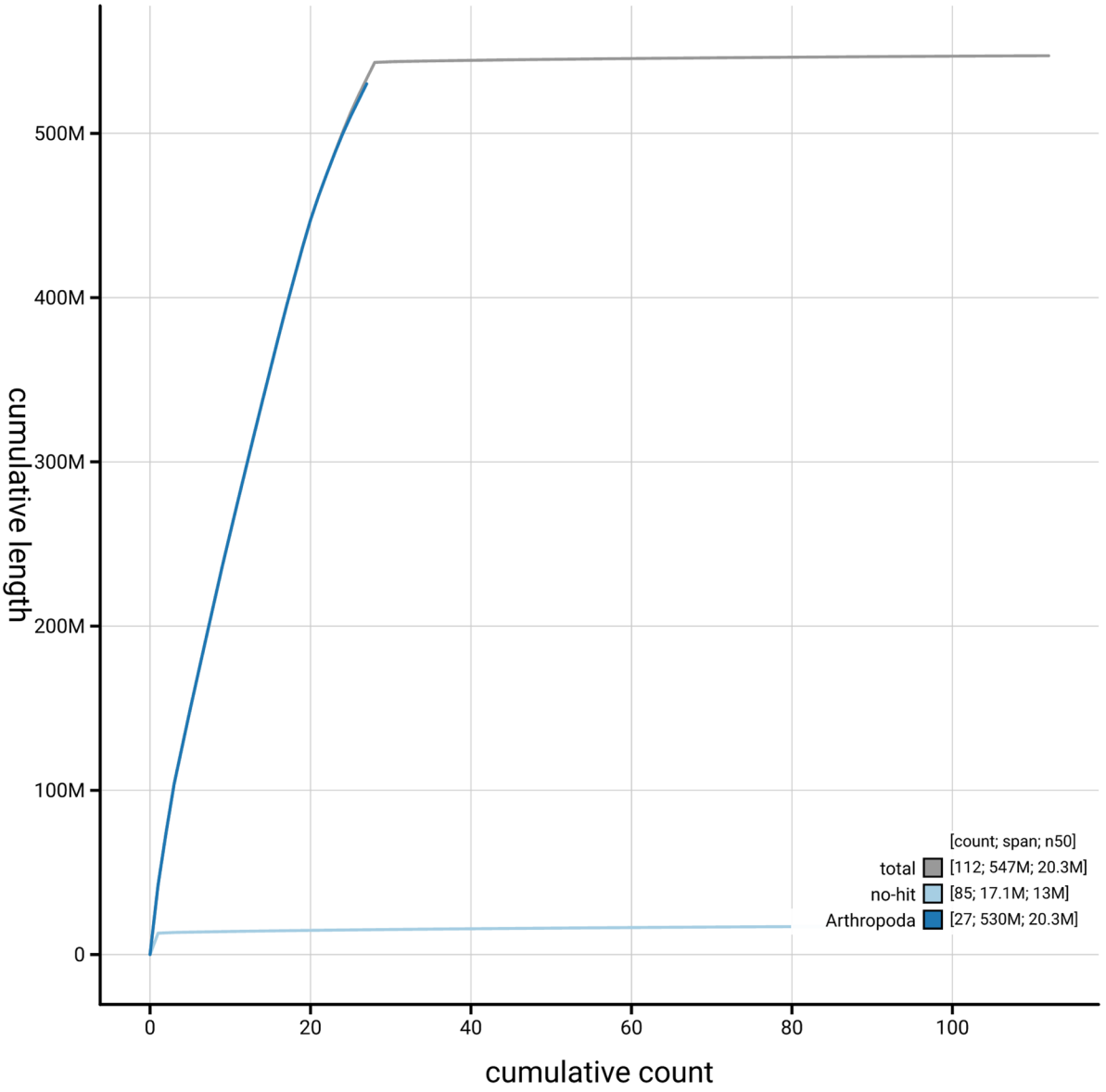

Figure 4. Genome assembly of *Ancylis achatana*, ilAncAcha1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_964214005.1/dataset/GCA_964214005.1/cumulative.

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. Flat files and maps used in curation were generated via the TreeVal pipeline (Pointon *et al.*, 2023). Manual curation was conducted primarily in PretextView (Harry, 2022) and HiGlass (Kerpedjiev *et al.*, 2018), with additional insights provided by JBrowse2 (Diesh *et al.*, 2023). Scaffolds were visually inspected and

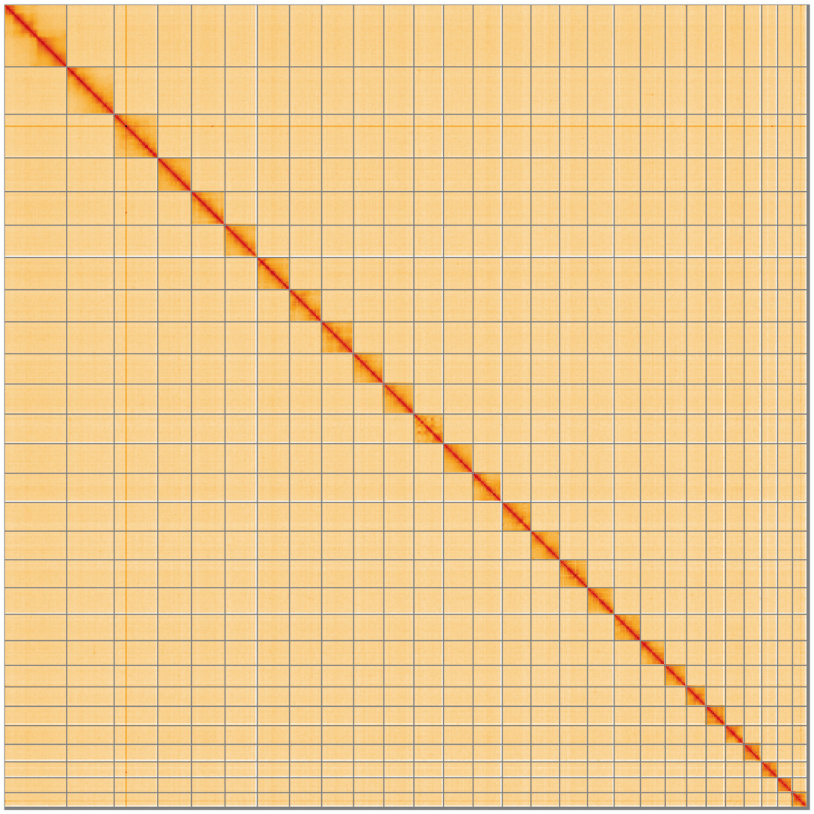

Figure 5. Genome assembly of *Ancylis achatana*: Hi-C contact map of the ilAncAcha1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=fNieo9pLR92pE1Di_iUfgA.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Ancylis achatana*, ilAncAcha1.

INSDC accession	Name	Length (Mb)	GC%
OZ172862.1	1	32.02	38
OZ172863.1	2	29.49	37.5
OZ172864.1	3	22.82	38.5
OZ172865.1	4	22.72	38.5
OZ172866.1	5	21.89	38
OZ172867.1	6	21.81	38.5
OZ172868.1	7	21.7	38
OZ172869.1	8	21.61	38
OZ172870.1	9	20.52	38
OZ172871.1	10	20.35	38.5
OZ172872.1	11	20.04	38.5
OZ172873.1	12	19.98	39
OZ172874.1	13	19.72	38.5
OZ172875.1	14	19.48	38.5
OZ172876.1	15	19.42	38.5
OZ172877.1	16	18.85	38.5
OZ172878.1	17	17.99	39
OZ172879.1	18	17.9	39
OZ172880.1	19	16.71	39
OZ172881.1	20	14.5	38.5
OZ172882.1	21	13.21	39.5
OZ172883.1	22	13.01	39
OZ172884.1	23	12.66	41
OZ172885.1	24	11.92	39
OZ172886.1	25	10.76	39.5
OZ172887.1	26	9.98	39
OZ172888.1	27	9.87	40.5
OZ172861.1	Z	42.27	38
OZ172889.1	MT	0.02	19

corrected as described by Howe *et al.* (2021). Any identified contamination, missed joins, and mis-joins were amended, and duplicate sequences were tagged and removed. Sex chromosomes were identified by synteny analysis. The curation process is documented at https://gitlab.com/wtsi-grit/rapid-curation.

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 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 was 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.

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

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**

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	4.3.9	https://github.com/blobtoolkit/blobtoolkit
BUSCO	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	666652151335353eef2fcd58880bcef5bc2928e1	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-r603	https://github.com/chhylp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84a a44357826c0b6753eb28de	https://github.com/higlass/higlass
MerquryFK	d00d98157618f4e8d1a9190026b19b471055b22e	https://github.com/thegenemyers/MERQURY.FK
Minimap2	2.24-r1122	https://github.com/lh3/minimap2
MitoHiFi	3	https://github.com/marcelauliano/MitoHiFi

Software tool	Version	Source
MultiQC	1.14, 1.17, and 1.18	https://github.com/MultiQC/MultiQC
NCBI Datasets	15.12.0	https://github.com/ncbi/datasets
Nextflow	23.10.0	https://github.com/nextflow-io/nextflow
PretextView	0.2.5	https://github.com/sanger-tol/PretextView
samtools	1.19.2	https://github.com/samtools/samtools
sanger-tol/ascc	-	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	0.5.1	https://github.com/sanger-tol/blobtoolkit
Seqtk	1.3	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.2.0	https://github.com/sanger-tol/treeval
YaHS	1.2a.2	https://github.com/c-zhou/yahs

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: Ancylis achatana (triangle-marked roller). Accession number PRJEB78334; https://identifiers.org/ena.embl/PRJEB78334. The genome sequence is released openly for reuse. The *Ancylis achatana* 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. 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.

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Members of the Natural History Museum Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.12159242.

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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

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

Bates A, Clayton-Lucey I, Howard C: **Sanger Tree of Life HMW DNA fragmentation: diagenode Megaruptor®3 for LI PacBio.** *protocols.io.* 2023.
Publisher 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

Boyes D, Gibbs M, University of Oxford and Wytham Woods Genome Acquisition Lab, *et al.*: **The genome sequence of the Brown Oak Tortrix, *Archips crataeganus* (Hübner, 1796) [version 1; peer review: 3 approved, 1 approved with reservations].** *Wellcome Open Res.* 2024; **9**: 9.
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): 170–175.
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.
Publisher Full Text

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

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

GBIF Secretariat: ***Ancylis achatana* (Denis & Schiffermüller), 1775 checklist dataset.** *GBIF Backbone Taxonomy.* 2025; [Accessed 8 February 2025].
Reference Source

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

Hancock EF, Bland KP, Razowski J: **The Moths and Butterflies of Great Britain and Ireland Tortricidae Olethreutinae.** Leiden: Brill, 2015.

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

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; **19**(1): 125.
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

Langmaid JR, Palmer S, Young MR: **A field guide to the smaller moths of Great Britain and Ireland.** 3rd ed., British Entomological and Natural History Society, 2018.
Reference Source

Lawniczak MKN, Davey RP, Rajan J, *et al.*: **Specimen and sample metadata standards for biodiversity genomics: a proposal from the Darwin Tree of Life project [version 1; peer review: 2 approved with reservations].** *Wellcome Open Res.* 2022; **7**: 187.
Publisher 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].
Reference Source

Oatley G, Denton A, Howard C: **Sanger Tree of Life HMW DNA extraction: automated MagAttract v.2.** *protocols.io.* 2023a.
Publisher Full Text

Oatley G, Sampaio F, Howard C: **Sanger Tree of Life fragmented DNA clean up: automated SPRI.** *protocols.io.* 2023b.
Publisher Full Text

Pereira L, Sivell O, Sivess L, *et al.*: **DToL taxon-specific standard operating procedure for the terrestrial and freshwater arthropods working group**. 2022.
Publisher Full Text

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

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

Ranallo-Benavidez TR, Jaron KS, Schatz MC: **GenomeScope 2.0 and smudgeplot for reference-free profiling of polyploid genomes.** *Nat Commun.* 2020; **11**(1): 1432.
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): 737–746.
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

Sterling P, Parsons M, Lewington R: **Field guide to the micro-moths of Great Britain and Ireland.** Second Edition. London: Bloomsbury Publishing, 2023.
Reference Source

Twyford AD, Beasley J, Barnes I, *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**

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**

Open Peer Review

Current Peer Review Status: ✔ ? ✔

Version 1

Reviewer Report 07 May 2025

https://doi.org/10.21956/wellcomeopenres.26364.r121274

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✔ **Arun Arumugaperumal**
Department of Biotechnology, Rajalakshmi Engineering College, Thandalam, Chennai, 602105, India

The data note describes the whole genome sequencing of *Ancylis achatana*, known as Triangle-marked Roller. This is the first report of the high quality genome sequence of this species. The authors have followed high-end methodology to sequence the DNA. The assembly was of size 547.27 Mb. The 16 kb mitogenome was also presented.

The photograph used for figure 1 is not clear. Kindly replace with a more-clear photograph, if available. All the NCBI accessions provided are working fine.

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

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 29 April 2025

https://doi.org/10.21956/wellcomeopenres.26364.r121276

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Bryan Brunet
Ottawa Research and Development Centre, Ontario, Canada

The manuscript entitled "The genome sequence of the Triangle-marked Roller, *Ancylis achatana* (Denis & Schiffermüller), 1775" by authors Boyes et al presents a chromosome length genome assembly for the tortricid leaf roller moth, *Ancylis achatana*. A combination of PacBio HiFi long reads and Arima v2 HiC chromosome conformation capture technology was used, resulting in 28 chromosome length pseudomolecules and an additional 83 unplaced scaffolds, in addition to a complete mitochondrial genome. The number of chromosome scaffolds and the size of the genome approximates the expected number of chromosomes from related tortricids with genome assemblies that are publicly available. Assembly metrics are good and the approaches used are standard approaches employed by and publicly available by the Wellcome Sanger Institute's Tree of Life projects. The authors are encourage to consider including an annotated mitogenome assembly as a separate figure, since annotations for the mitogenome are already known from the MitoHIFI output.

Other suggested edits to improve clarity around the methods are requested below:
-The relevance/importance of a genome assembly for this species should be elaborated.
-Reporting the size of the mitogenome as 0.02 Mb in Table 3 is uninformative
-Under the PacBio Hifi section, "samples" and "libraries" are referred to, but the sample collection information indicates that a single specimen was used.
-Under the Assembly section, the authors map Arima Hi-C reads to the assembly but do not mention the use of the recommended Arima mapping pipeline; this pipeline is meant to remove chimerism, low quality reads, and merge technical replicates before removing pcr duplicates – please explain why it wasn't used and if/how these steps were performed.
-Provide a reference for FastK
-How was the synteny analysis conducted?
-The two paragraphs on the blobtoolkit pipeline seem disconnected from the rest of the section (ie. there's no introduction to what the toolkit is being used for - even if most of the readership probably already knows, this should be stated). Also, the second paragraph gives the impression that the blobtoolkit pipeline (as used via nextflow: sanger-tol/blobtoolkit v0.7.1) was newly developed, but that's not the case. I believe the appropriate citation is Zaynab et al. 2025
-Which versions of uniprot and nt dbs?
-ASCC pipeline reference under Assembly curation, needs a reference.
-Table 4 is missing genomescope, purge_dups, mitofinder, ASCC pipeline, JBrowse2, singularity

Is the rationale for creating the dataset(s) clearly described?
Partly

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: hemiptera systematics and taxonomy, genomics

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, however I have significant reservations, as outlined above.

Reviewer Report 28 March 2025

https://doi.org/10.21956/wellcomeopenres.26364.r121278

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Kay Lucek
University of Neuchâtel, Neuchâtel, Switzerland

The authors present the chromosome level genome assembly of a male specimen of the Triangle-marked Roller moth (*Ancylis achatana*). The assembly consists of 28 chromosomes including the Z chromosome. The assembly is highly complete as revealed by the high BUSCO score but not fully phased. Sequencing and genome assembly follow the current state of the art and use established methods.

Overall, the presented assembly will be of great value to study the evolution of Lepidoptera through comparative studies and shed light on the diversity of this less well studied branch of the Tree of Life. Interestingly the Z chromosome was identified through synteny analyses with the distantly related *Archips crataeganus*. It would have been nice to give more details why this species was used in particular and how the synteny analysis was performed.

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: Speciation genomics

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.