



DATA NOTE

The genome sequence of the Yellow-barred Brindle, *Acasis viretata* (Hübner, 1799) (Lepidoptera: Geometridae)

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

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Abstract

We present a genome assembly from an individual female *Acasis viretata* (Yellow-barred Brindle; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence has a total length of 297.68 megabases. Most of the assembly (99.98%) is scaffolded into 17 chromosomal pseudomolecules, including the W and Z sex chromosomes. The mitochondrial genome has also been assembled, with a length of 16.01 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

Acasis viretata; Yellow-barred Brindle; genome sequence; chromosomal; Lepidoptera



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

Open Peer Review

Approval Status ✓ ✓ ?

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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; Geometroidea; Geometridae; Larentiinae; *Acasis*; *Acasis viretata* (Hübner, 1799) (NCBI:txid934820)

Background

Acasis viretata, the Yellow-barred Brindle, is a moth in the family Geometridae. It is widespread in southern England and is expanding northwards (Randle *et al.*, 2019); it has significantly increased in range and abundance this century (Boyes *et al.*, 2019). It is common throughout Europe and there are scattered records across Asia to Japan (GBIF Secretariat, 2025).

Yellow-barred Brindle has a forewing length of between 10 and 14 mm and, although when freshly emerged the moth is green, this soon fades to yellow with a dark grey/black central crossband (Waring *et al.*, 2017). The species is bivoltine, flying between May and June; and again from late July into September. The moth can be found in woodlands and gardens and its foodplants include Holly, Ivy, Hawthorn, Dogwood and Privet. It overwinters in the pupal stage (Waring *et al.*, 2017).

We present a chromosome-level genome sequence for *Acasis viretata*, the Yellow-barred Brindle. This assembly is the first high-quality genome for the genus *Acasis* as of August 2025 (data obtained via NCBI datasets, O'Leary *et al.*, 2024). The assembly was produced using the Tree of Life pipeline from a specimen collected in Wytham Woods, Oxfordshire, United Kingdom (Figure 1).

Methods

Sample acquisition and DNA barcoding

The specimen used for genome sequencing was an adult female *Acasis viretata* (specimen ID Ox001865, ToLID ilAcaVire1; Figure 1), collected from Wytham Woods, Oxfordshire, United



Figure 1. Photograph of the *Acasis viretata* (ilAcaVire1) specimen used for genome sequencing.

Kingdom (latitude 51.772, longitude -1.338) on 2021-08-11. The specimen was collected and identified by Douglas Boyes (University of Oxford). A second specimen was used for Hi-C sequencing (specimen ID SAN28000458, ToLID ilAcaVire2). It was collected from Saint-Étienne-De-Baïgorry, Pyrénées-Atlantiques, Pays Basques, France (latitude 43.1861, longitude -1.358) by a group of collectors from Oreina from 2024-05-20. For the Darwin Tree of Life sampling and metadata approach, refer to Lawniczak *et al.* (2022).

The initial identification of ilAcaVire1 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 ilAcaVire1 sample was weighed and triaged to determine the appropriate extraction protocol. Tissue from the whole organism was homogenised by powermashing using a PowerMasher II tissue disruptor.

HMW DNA was extracted in the WSI Scientific Operations core using the Automated MagAttract v2 protocol. DNA was sheared into an average fragment size of 12–20 kb following the Megaruptor®3 for LI PacBio protocol. Sheared DNA was purified by manual 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 28 ng/μL and a yield of 1 271.20 ng, with a fragment size of 16.9 kb. The 260/280 spectrophotometric ratio was 2.12, and the 260/230 ratio was 1.54.

PacBio HiFi library preparation and sequencing

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

Libraries were prepared using the SMRTbell Prep Kit 3.0 (Pacific Biosciences, California, USA), following the manufacturer's instructions. The kit includes reagents for end repair/A-tailing, adapter ligation, post-ligation SMRTbell bead clean-up, and nuclease treatment. Size selection and clean-up were performed

using diluted AMPure PB beads (Pacific Biosciences). DNA concentration was quantified using a Qubit Fluorometer v4.0 (ThermoFisher Scientific) and the Qubit 1X dsDNA HS assay kit. Final library fragment size was assessed with the Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies) using the gDNA 55 kb BAC analysis kit.

The sample was sequenced using the Sequel IIe system (Pacific Biosciences, California, USA). The concentration of the library loaded onto the Sequel IIe was in the range 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, and to perform primary and secondary analysis of the data upon completion.

Hi-C

Sample preparation and crosslinking

The Hi-C sample was prepared from 20–50 mg of frozen tissue from the head of the iAcaVire2 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 Diagnocine 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/μL. 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 X.

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 (Cheng *et al.*, 2021) with the --primary option. The Hi-C reads (Rao *et al.*, 2014) were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019), and the contigs were scaffolded in YaHS (Zhou *et al.*, 2023) with 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. 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 4 breaks and 9 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 Merqury.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 the primary and alternate 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 *Acasis viretata* specimen generated 19.54 Gb (gigabases) from 1.70 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 290.20 Mb, with a heterozygosity of 1.14% and repeat content of 24.47% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 64× coverage. Hi-C sequencing produced 112.00 Gb from 741.74 million reads, which were used to scaffold the assembly. Table 1 summarises the specimen and sequencing details.

Assembly statistics

The primary haplotype was assembled, and contigs corresponding to an alternate haplotype were also deposited in INSDC databases. The final assembly has a total length of 297.68 Mb in 34 scaffolds, with 7 gaps, and a scaffold N50 of 17.88 Mb (Table 2).

Most of the assembly sequence (99.98%) was assigned to 17 chromosomal-level scaffolds, representing 15 autosomes and

the W and Z sex chromosomes. Chromosomes Z and W were identified by copy number in the diploid assembly as well as the Hi-C signal. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 3; Table 3).

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.

The combined primary and alternate assemblies achieve an estimated QV of 61.1. The *k*-mer completeness is 78.42% for the primary assembly, 71.98% for the alternate haplotype, and 98.80% for the combined assemblies (Figure 4).

BUSCO v.5.7.1 analysis using the lepidoptera_odb10 reference set ($n = 5\,286$) identified 98.0% of the expected gene set (single = 97.5%, duplicated = 0.5%). The snail plot in Figure 5 summarises the scaffold length distribution and other assembly statistics for the primary assembly. The blob plot in Figure 6 shows the distribution of scaffolds by GC proportion and coverage.

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 primary assembly, is **7.C.Q61**, 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

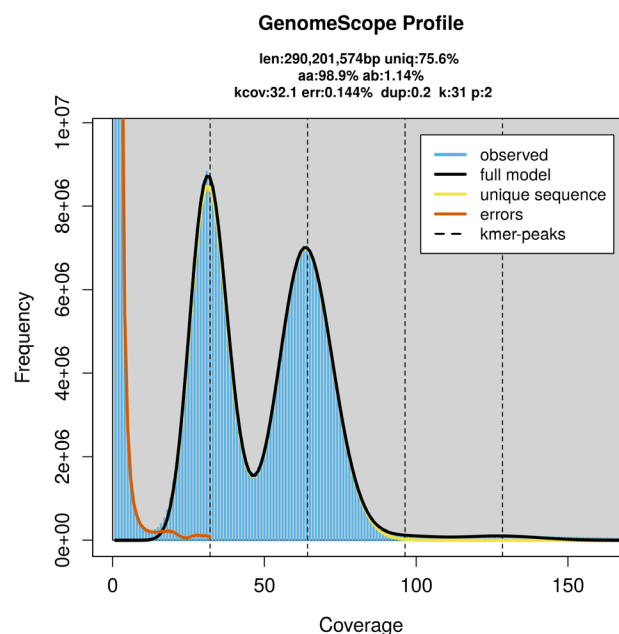


Figure 2. Frequency distribution of *k*-mers generated using GenomeScope2. The plot shows observed and modelled *k*-mer spectra, providing estimates of genome size, heterozygosity, and repeat content based on unassembled sequencing reads.

Table 1. Specimen and sequencing data for BioProject PRJEB71292.

Platform	PacBio HiFi	Hi-C
ToLID	ilAcaVire1	ilAcaVire2
Specimen ID	Ox001865	SAN28000458
BioSample (source individual)	SAMEA10979125	SAMEA115949033
BioSample (tissue)	SAMEA10979535	SAMEA115949380
Tissue	whole organism	head
Instrument	Sequel IIe	Illumina NovaSeq X
Run accessions	ERR12373386	ERR14988471
Read count total	1.70 million	741.74 million
Base count total	19.54 Gb	112.00 Gb

Table 2. Genome assembly statistics.

Assembly name	ilAcaVire1.1
Assembly accession	GCA_965285885.1
Alternate haplotype accession	GCA_965285845.1
Assembly level	chromosome
Span (Mb)	297.68
Number of chromosomes	17
Number of contigs	41
Contig N50	17.28 Mb
Number of scaffolds	34
Scaffold N50	17.88 Mb
Sex chromosomes	W and Z
Organelles	Mitochondrion: 16.01 kb

‘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)

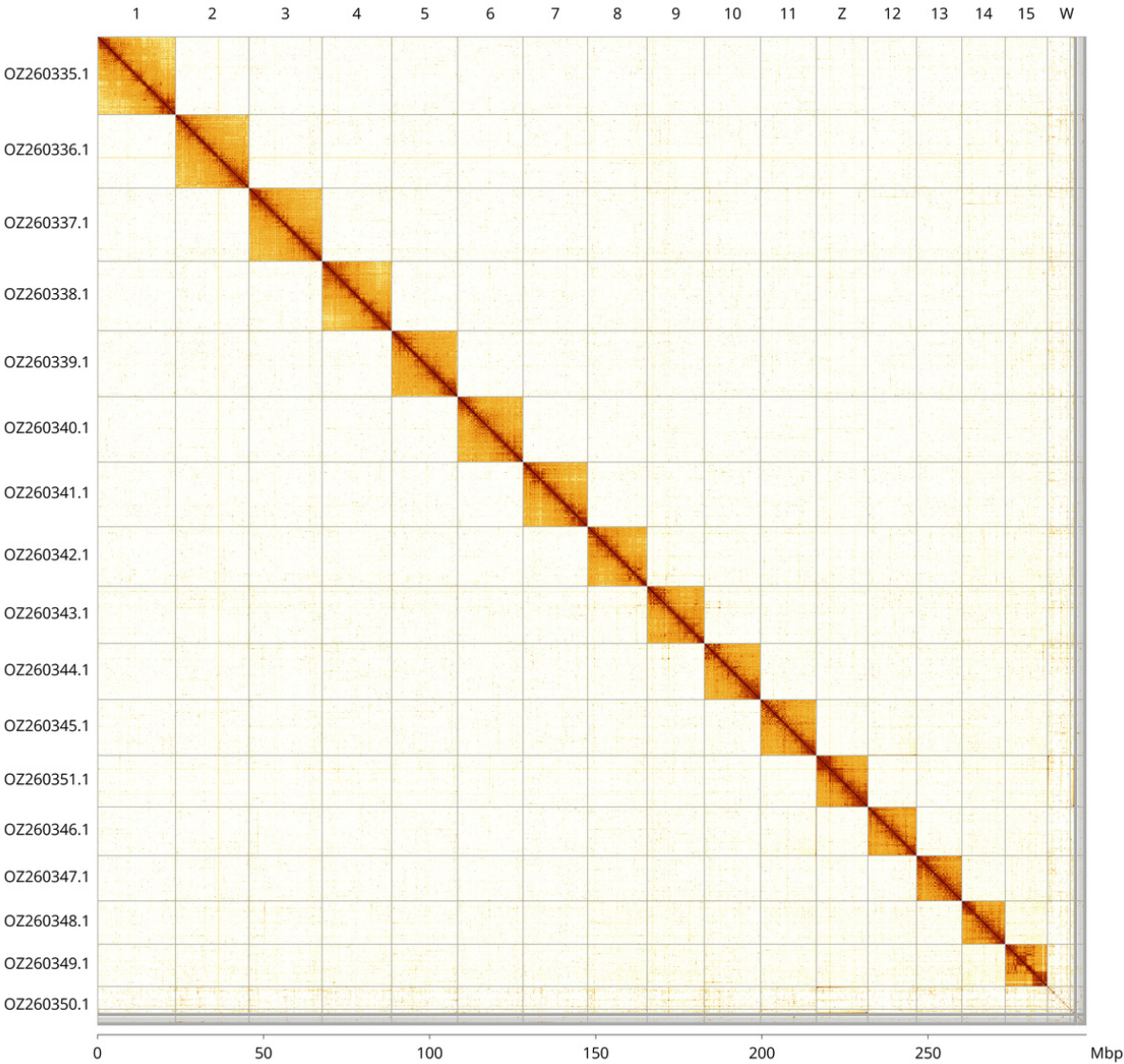


Figure 3. Hi-C contact map of the *Acasis viretata* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes, with a megabase scale along the bottom axis. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the primary genome assembly of *Acasis viretata* ilAcaVire1.

INSDC accession	Molecule	Length (Mb)	GC%
OZ260335.1	1	23.50	37.50
OZ260336.1	2	22.09	37.50
OZ260337.1	3	22.03	37.50
OZ260338.1	4	20.95	37.50
OZ260339.1	5	19.87	37.50
OZ260340.1	6	19.67	37.50
OZ260341.1	7	19.46	37.50
OZ260342.1	8	17.88	37.50

INSDC accession	Molecule	Length (Mb)	GC%
OZ260343.1	9	17.28	38
OZ260344.1	10	16.89	38
OZ260345.1	11	16.77	37.50
OZ260346.1	12	14.70	37.50
OZ260347.1	13	13.65	38
OZ260348.1	14	12.99	38
OZ260349.1	15	12.70	38
OZ260350.1	W	11.70	38.50
OZ260351.1	Z	15.50	38

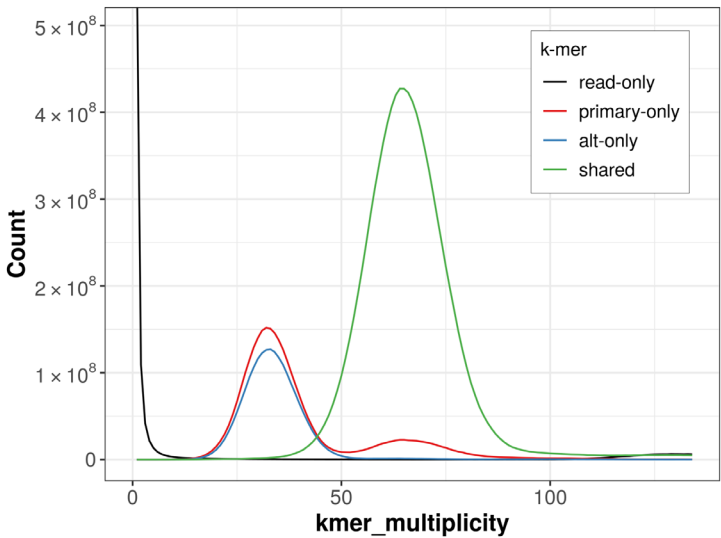


Figure 4. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers 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.

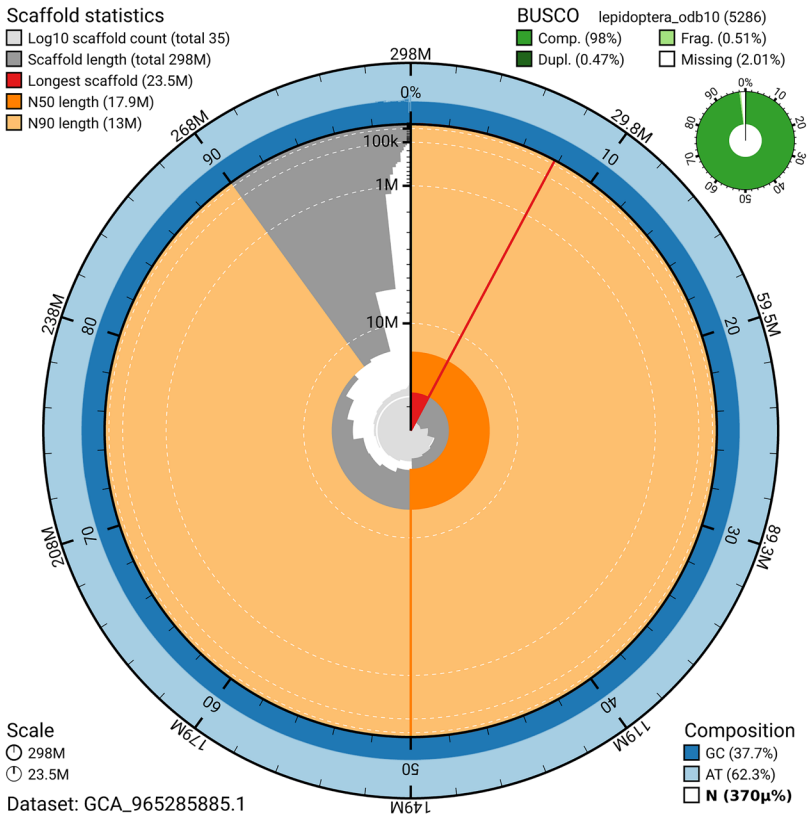


Figure 5. Assembly metrics for iAcaVire1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1 000 bins around the circumference. The outermost blue 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 lepidoptera_odb10 set is presented at the top right. An interactive version of this figure can be accessed on the [BlobToolKit viewer](#).

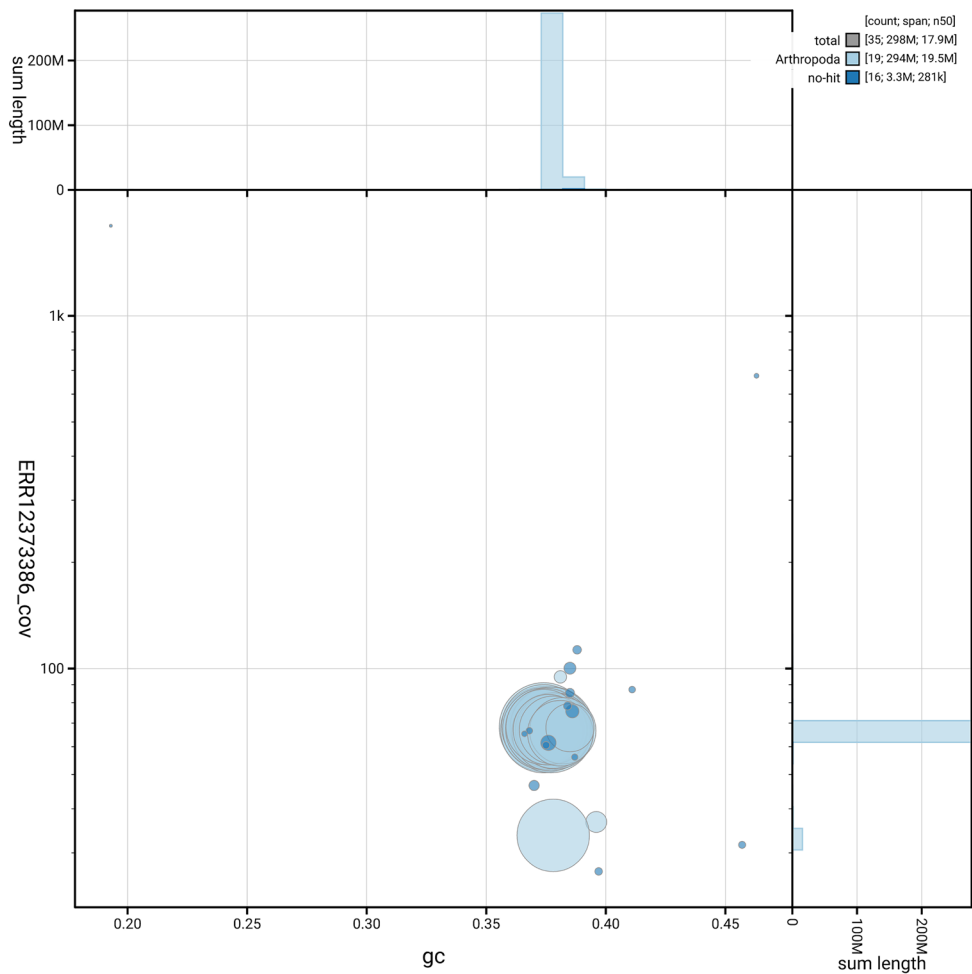


Figure 6. BlobToolKit GC-coverage plot for ilAcaVire1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes display 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 *Acasis viretata* assembly.

Measure	Value	Benchmark
EBP summary (primary)	7.C.Q61	6.C.Q40
Contig N50 length	17.28 Mb	≥ 1 Mb
Scaffold N50 length	17.88 Mb	= chromosome N50
Consensus quality (QV)	Primary: 61.8; alternate: 60.8; combined: 61.1	≥ 40
<i>k</i> -mer completeness	Primary: 78.42%; alternate: 71.98%; combined: 98.80%	≥ 95%
BUSCO	C:98.0% [S:97.5%; D:0.5%]; F:0.5%; M:1.5%; n:5 286	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.98%	≥ 90%

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: *Acasis viretata* (yellow-barred brindle). Accession number [PRJEB71292](#). The genome sequence is released openly for reuse. The *Acasis viretata* genome sequencing initiative is part of the Darwin Tree of Life Project (PRJEB40665), 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:

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- 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/tolkit/fasta_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/chhy123/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

Software	Version	Source
PretextView	0.2.5	https://github.com/sanger-tol/PretextView
samtools	1.21	https://github.com/samtools/samtools
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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This manuscript presents a high-quality, chromosome-level genome assembly of *Acasis viretata*, the Yellow-barred Brindle moth. The study forms part of the Darwin Tree of Life (DTOL) initiative and follows well-established protocols for DNA extraction, sequencing, assembly, and curation. The genome exhibits strong assembly metrics, including a scaffold N50 of 17.88 Mb, a BUSCO completeness score of 98%, and 99.98% assignment to 17 chromosomes (including the W and Z sex chromosomes).

Overall, the manuscript is technically robust, clearly written, and well aligned with the goals of the Tree of Life gateway. It can be recommended for publication after addressing the following minor but important clarifications.

1. Mitochondrial Genome Characterization

- While the mitochondrial genome (16,010 bp) has been assembled, it is only briefly described. The unit “kb” is not appropriate for this scale; it should be expressed in base pairs (bp).
- The authors should verify and explicitly state whether the mitochondrial genome is circularized and complete, as circularity is a key quality metric for mitogenome assemblies.
- It is also recommended to clarify whether the mitochondrial genome sequence was validated or polished, since linear assembly outputs can occasionally represent incomplete circular genomes.

2. Assembly Completeness and Curation

- The k-mer completeness discrepancy (primary = 78.42%, alternate = 71.98%, combined = 98.80%) warrants a short discussion. The authors should clarify whether the missing content in the primary assembly is represented in the alternate haplotype or reflects possible heterozygosity collapse.
- **Assembly curation:** Although the manuscript briefly mentions the chromosome curation process, the discussion is limited. The statement “manual corrections included 4 breaks and 9 joins” provides insufficient detail. It would be helpful to specify whether these corrections

occurred at the scaffold or chromosome level and to summarize their rationale or impact on overall assembly quality.

3. Clarification in the "Nucleic Acid Extraction" Section

In the subsection "*Nucleic acid extraction*" within the *Methods* section, the second paragraph includes the sentence:

"For this sample, the final post-shearing DNA had a Qubit concentration of 28 ng/μL and a yield of 1 271.20 ng, with a fragment size of 16.9 kb."

The reported DNA yield value ("1 271.20 ng") appears unusually formatted and may require verification. The authors should confirm whether this is a typographical error (e.g., a misplaced space) or an accurate measurement.

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, Phylogenetics, Evolutionary

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 13 October 2025

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The data note describes the whole genome of the Yellow-barred Brindle, *Acasis viretata*. The authors have used standard tree of life sequencing protocols to sequence and assemble the genome of this moth. The assembly size reported here is 297.68 Mb spread among 17 chromosome molecules. The mitochondrial genome was also reported which is of size 16.01 kb.

Since a female specimen was used the authors have obtained the W and Z sex chromosomes also. A contigN50 value of 17 Mb indicates the good quality of the genome. BUSCO completeness of 98% indicate that the genome is a near-complete one according to existing databases. The accession id provided are active. The article can be indexed. The bioproject data has an endosymbiont genome sequence also. But it was not discussed whether it was obtained during this same sequencing procedure in the data note.

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.

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Axel Künstner 

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Overall Assessment

The study presents a chromosome-level genome assembly for *Acasis viretata* (Yellow-barred Brindle), an ecologically important moth species widespread in Britain and Europe. The authors sequenced an individual female specimen using PacBio HiFi and Hi-C technologies, producing a high-quality genome sequence scaffolded into 17 chromosomal pseudomolecules, including sex chromosomes W and Z. This represents the first high-quality genome assembly for the genus *Acasis* and provides a valuable genomic resource for the Darwin Tree of Life project.

Strengths

Technical Quality: The assembly achieves excellent metrics with a scaffold N50 of 17.88 Mb, 99.98% of sequence assigned to chromosomes, and strong BUSCO completeness (98.0%). The EBP

metric of 7.C.Q61 exceeds the recommended reference standard of 6.C.Q40. The combined use of PacBio HiFi and Hi-C data with appropriate coverage (64× and extensive Hi-C data) is well-suited for chromosome-level assembly.

Methodological Rigor: The authors employed standard, well-validated bioinformatics pipelines including Hifiasm, YaHS scaffolding, and comprehensive quality assessment tools. The manual curation process using PretextView with documented corrections (4 breaks, 9 joins) demonstrates careful attention to assembly quality.

Resource Value: This genome provides an important reference for Geometridae family genomics and supports conservation genomics for this ecologically expanding species. The identification and proper scaffolding of sex chromosomes W and Z adds particular value for lepidopteran genomics.

Documentation: Comprehensive reporting of methods, software versions, and data availability meets reproducibility standards. All data are properly deposited with appropriate accession numbers.

Areas for Improvement

Mitochondrial Genome Characterization: The mitochondrial assembly (16.01 kb) is mentioned but not characterized. Even basic details about gene content, organization, or comparison to related lepidopteran species would significantly enhance the resource's utility for phylogenetic and evolutionary studies.

Assembly Completeness Discussion: The k-mer completeness of 78.42% for the primary assembly, while the combined assemblies reach 98.80%, suggests some genomic content may be relegated to the alternate haplotype. A brief discussion of this pattern and its implications would be valuable.

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: Genomics, Tumor biology

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.