



DATA NOTE

The genome sequence of the Tawny Oak Tortrix, *Acleris ferrugana* (Denis & Schiffermüller), 1775 (Lepidoptera: Tortricidae)

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

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Abstract
We present a genome assembly from an individual female *Acleris ferrugana* (Tawny Oak Tortrix; Arthropoda; Insecta; Lepidoptera; Tortricidae). The genome sequence has a total length of 480.03 megabases. Most of the assembly (99.03%) is scaffolded into 31 chromosomal pseudomolecules, including the W and Z sex chromosomes. The mitochondrial genome has also been assembled, with a length of 16.58 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
Acleris ferrugana; Tawny Oak Tortrix; Rusty Oak Button; genome sequence; chromosomal; Lepidoptera

Open Peer Review

Approval Status ? ? ✓ ✓

	1	2	3	4
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; Amphimesenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Apoditrysia; Tortricodea; Tortricidae; Tortricinae; Tortricini; *Acleris*; *Acleris ferrugana* (Denis & Schiffermüller), 1775 (NCBI:txid758707)

Background

Acleris ferrugana (Tortricidae), the Tawny Oak Tortrix, is also called the Rusty Oak Button. It is a variable woodland moth, very similar to *A. notana* (also a variable species). Reliable separation of the two species is by dissection of the genitalia, though *A. ferrugana* often shows a small black scale-tuft about a quarter from the head (NatureSpot, 2025). It is fairly common across the British and Irish Isles, more scarce to the north.

In the UK, adults fly in two generations: the first is from July to September, and the second from October to May, overwintering as an adult and reappearing in spring (Sterling *et al.*, 2023). Larvae feed mainly on oak (*Quercus*) in a spinning between leaves, whereas *A. notana* uses birch (*Betula*) as its food plant (NatureSpot, 2025).

We present the first genome sequence for *Acleris ferrugana*, the Rusty Oak Button. The assembly was produced using the Tree of Life pipeline from a specimen collected in Wytham Woods, Oxfordshire, United Kingdom (Figure 1). This assembly was generated as part of the Darwin Tree of Life Project, which aims to generate high-quality reference genomes for all named eukaryotic species in Britain and Ireland to support research, conservation, and the sustainable use of biodiversity (Blaxter *et al.*, 2022).

Methods

Sample acquisition and DNA barcoding

The specimen used for genome sequencing was an adult female *Acleris ferrugana* (specimen ID Ox003294, ToLID iAcIFerr2; Figure 1), on 2022-11-01. The specimen was collected and identified by Liam Crowley. A second specimen was



Figure 1. Photograph of the *Acleris ferrugana* (iAcIFerr2) specimen used for genome sequencing.

used for Hi-C sequencing (specimen ID Ox000992, ToLID iAcIFerr1) was collected and identified by Douglas Boyes. A third specimen, used for RNA sequencing (specimen ID Ox003303, ToLID iAcIFerr8), was also collected and identified by Liam Crowley. All specimens were collected from Wytham Woods, Oxfordshire, United Kingdom (latitude 51.772, longitude -1.338). For the Darwin Tree of Life sampling and metadata approach, refer to Lawniczak *et al.* (2022).

The initial identification was verified by an additional DNA barcoding process according to the framework developed by Twyford *et al.* (2024). A small sample was dissected from each 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 iAcIFerr2 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 9.75 ng/μL and a yield of 448.50 ng, with a fragment size of 13.3 kb. The 260/280 spectrophotometric ratio was 2.36, and the 260/230 ratio was 4.02.

RNA was extracted from whole organism tissue of iAcIFerr8 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ mirVana protocol. The RNA concentration was assessed using a Nanodrop spectrophotometer and a Qubit Fluorometer using the Qubit RNA Broad-Range Assay kit. Analysis of the integrity of the RNA was done using the Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

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 of the iLacIFerr1 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 6000.

RNA library preparation and sequencing

Libraries were prepared using the NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina (New England Biolabs), following the manufacturer's instructions. Poly(A) mRNA in the total RNA solution was isolated using oligo(dT) beads, converted to cDNA, and uniquely indexed; 14 PCR cycles were performed. Libraries were size-selected to produce fragments between 100–300 bp. Libraries were quantified, normalised, pooled to a final concentration of 2.8 nM, and diluted to 150 pM for loading. Sequencing was carried out on the Illumina NovaSeq X to generate 150-bp paired-end reads.

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 Gfstats (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 104 breaks and 184 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 *Acleris ferrugana* specimen generated 24.06 Gb (gigabases) from 2.59 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 408.64 Mb, with a heterozygosity of 0.89% and repeat content of 28.48% ([Figure 2](#)).

These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 56× coverage. Hi-C sequencing produced 143.57 Gb from 950.80 million reads, which were used to scaffold the assembly. RNA sequencing data were also generated and are available in public sequence repositories. [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 480.03 Mb in 205 scaffolds, with 135 gaps, and a scaffold N50 of 13.41 Mb ([Table 2](#)).

Most of the assembly sequence (99.03%) was assigned to 31 chromosomal-level scaffolds, representing 29 autosomes and the W and Z sex chromosomes. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size ([Figure 3](#); [Table 3](#)). The Hi-C data was derived from a male, resulting in the W chromosome remaining unscaffolded. Z chromosome identified based BUSCO gene painting with ancestral Merian elements ([Wright 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.

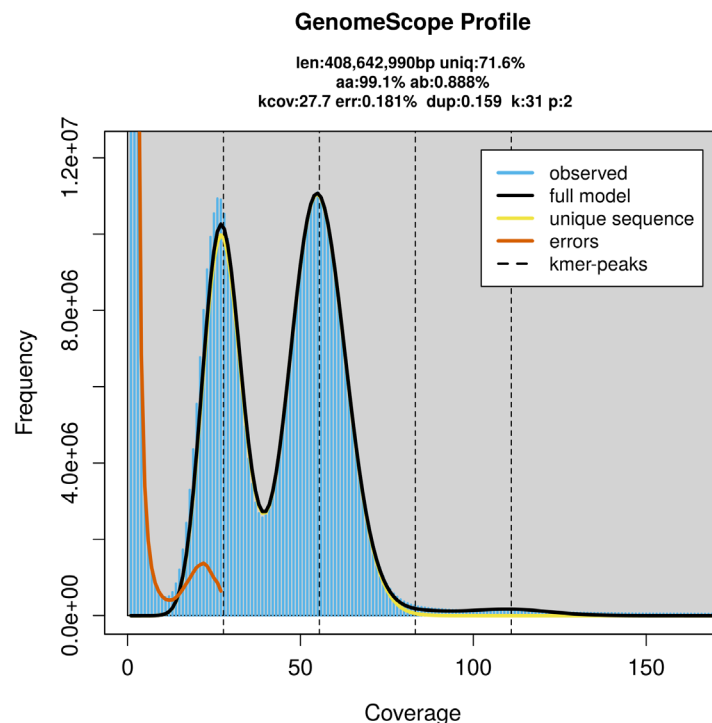


Figure 2. Frequency distribution of *k*-mers generated using GenomeScope2. The plot shows observed and modelled *k*-mer spectra, providing estimates of genome size, heterozygosity, and repeat content based on unassembled sequencing reads.

Table 1. Specimen and sequencing data for BioProject PRJEB76341.

Platform	PacBio HiFi	Hi-C	RNA-seq
ToLID	ilAcFerr2	ilAcFerr1	ilAcFerr8
Specimen ID	Ox003294	Ox000992	Ox003303
BioSample (source individual)	SAMEA113425835	SAMEA8603215	SAMEA113425841
BioSample (tissue)	SAMEA113426021	SAMEA8603781	SAMEA113426027
Tissue	whole organism	whole organism	whole organism
Instrument	Sequel IIe	Illumina NovaSeq 6000	Illumina NovaSeq X
Run accessions	ERR13245266	ERR13248929	ERR13248930
Read count total	2.59 million	950.80 million	68.68 million
Base count total	24.06 Gb	143.57 Gb	10.37 Gb

Table 2. Genome assembly statistics.

Assembly name	ilAcFerr2.1
Assembly accession	GCA_964656575.1
Alternate haplotype accession	GCA_965112395.1
Assembly level	chromosome
Span (Mb)	480.03
Number of chromosomes	31
Number of contigs	340
Contig N50	4.03 Mb
Number of scaffolds	205
Scaffold N50	13.41 Mb
Sex chromosomes	W and Z
Organelles	Mitochondrion: 16.58 kb

Table 3. Chromosomal pseudomolecules in the primary genome assembly of *Acleris ferrugana* ilAcFerr2.

INSDC accession	Molecule	Length (Mb)	GC%
OZ210445.1	1	17.97	37.50
OZ210446.1	2	16.89	37.50
OZ210447.1	3	16.18	36.50
OZ210448.1	4	15.95	37.50
OZ210449.1	5	15.16	37
OZ210450.1	6	15.09	37
OZ210451.1	7	14.51	37.50

INSDC accession	Molecule	Length (Mb)	GC%
OZ210452.1	8	14.27	37
OZ210453.1	9	14.07	37.50
OZ210454.1	10	13.98	37
OZ210455.1	11	13.78	37
OZ210456.1	12	13.77	37
OZ210457.1	13	13.41	37
OZ210458.1	14	13.02	38
OZ210459.1	15	13.02	37
OZ210460.1	16	12.99	37.50
OZ210461.1	17	12.39	37
OZ210462.1	18	12.07	37
OZ210463.1	19	11.59	37
OZ210464.1	20	11.32	37.50
OZ210465.1	21	10.45	38
OZ210466.1	22	10.21	37
OZ210467.1	23	9.97	39
OZ210468.1	24	9.61	37
OZ210469.1	25	9.15	37.50
OZ210470.1	26	9.13	36.50
OZ210471.1	27	8.83	39
OZ210472.1	28	7.27	37.50
OZ210473.1	29	6.77	37.50
OZ210474.1	W	61.44	37.50
OZ210475.1	Z	51.11	36.50

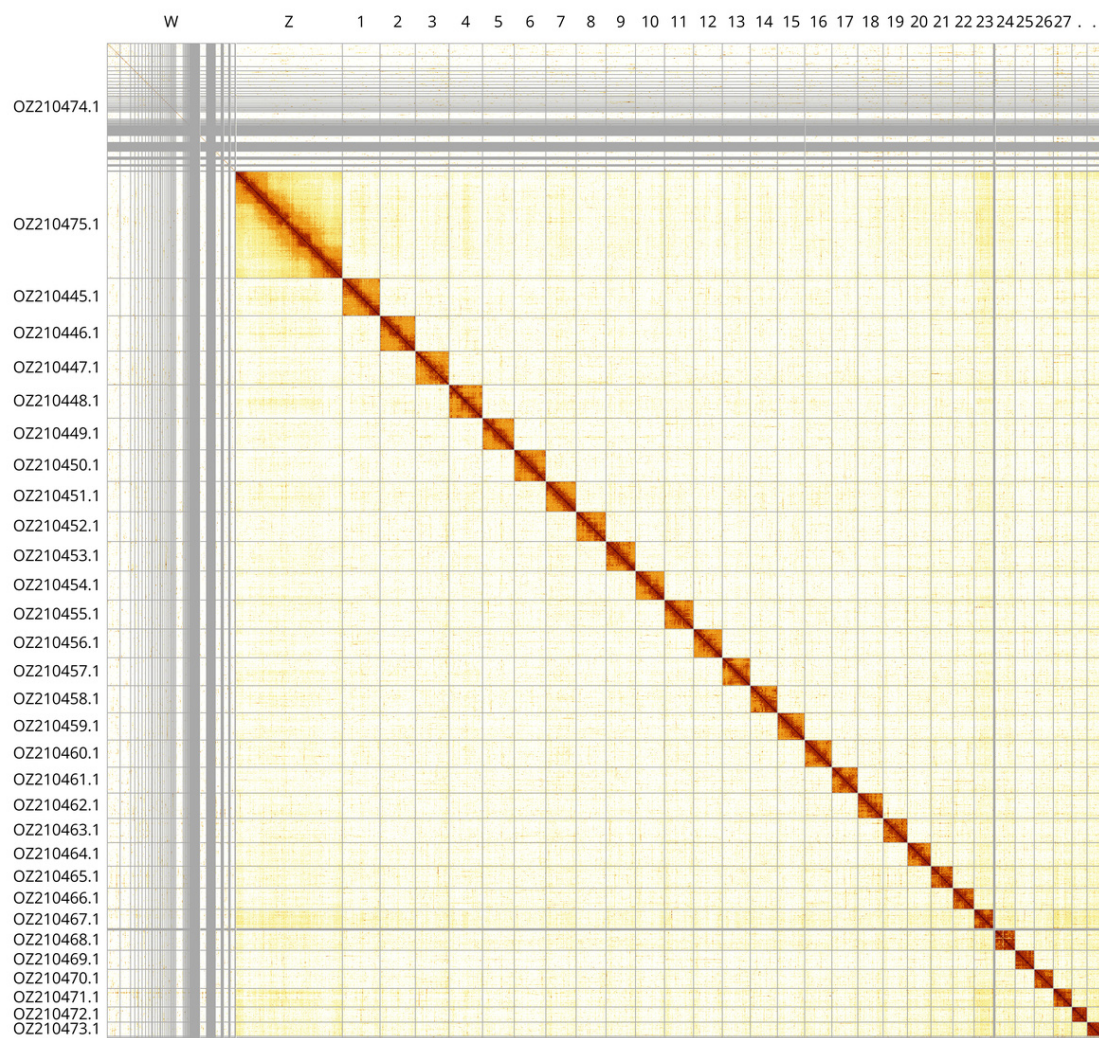


Figure 3. Hi-C contact map of the *Acleris ferrugana* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes. The plot was generated using PretextSnapshot.

The combined primary and alternate assemblies achieve an estimated QV of 63.6. The k -mer completeness is 86.30% for the primary assembly, 72.25% for the alternate haplotype, and 99.33% for the combined assemblies (Figure 4).

BUSCO v.5.5.0 analysis using the lepidoptera_odb10 reference set ($n = 5\,286$) identified 98.2% of the expected gene set (single = 97.4%, duplicated = 0.8%). 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 **6.C.Q63**, meeting the recommended reference standard.

Wellcome Sanger Institute – Legal and Governance

The materials that have contributed to this genome note have been supplied by a Darwin Tree of Life Partner. The submission of materials by a Darwin Tree of Life Partner is subject to the ‘**Darwin Tree of Life Project Sampling Code of Practice**’, which can be found in full on the [Darwin Tree of Life website](#). By agreeing with and signing up to the Sampling Code of Practice, the Darwin Tree of Life Partner agrees they will meet the legal and ethical requirements and standards set out within this document in respect of all samples acquired for, and supplied to, the Darwin Tree of Life Project. Further, the Wellcome Sanger Institute employs a process whereby due diligence is carried out proportionate to the nature of the materials themselves, and the circumstances under which they have been/are to be collected and provided for use. The purpose of this is to address and mitigate any potential legal and/or ethical implications of receipt and use of the materials as part of the research project, and to ensure that in doing so

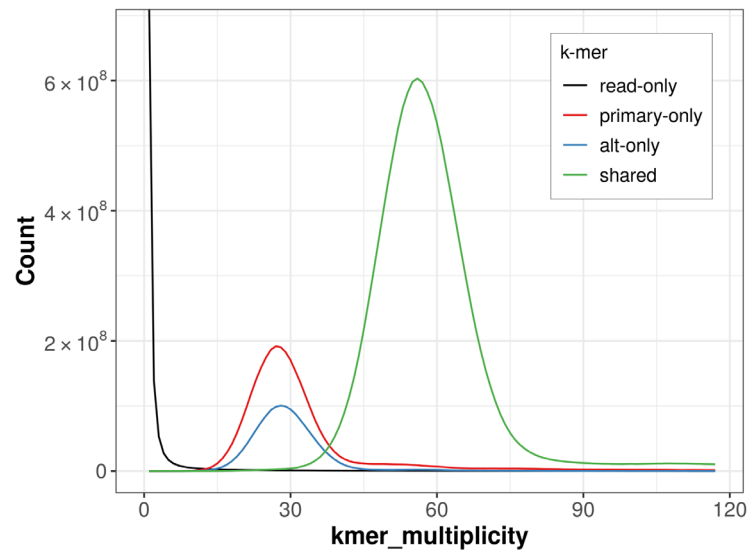


Figure 4. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers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

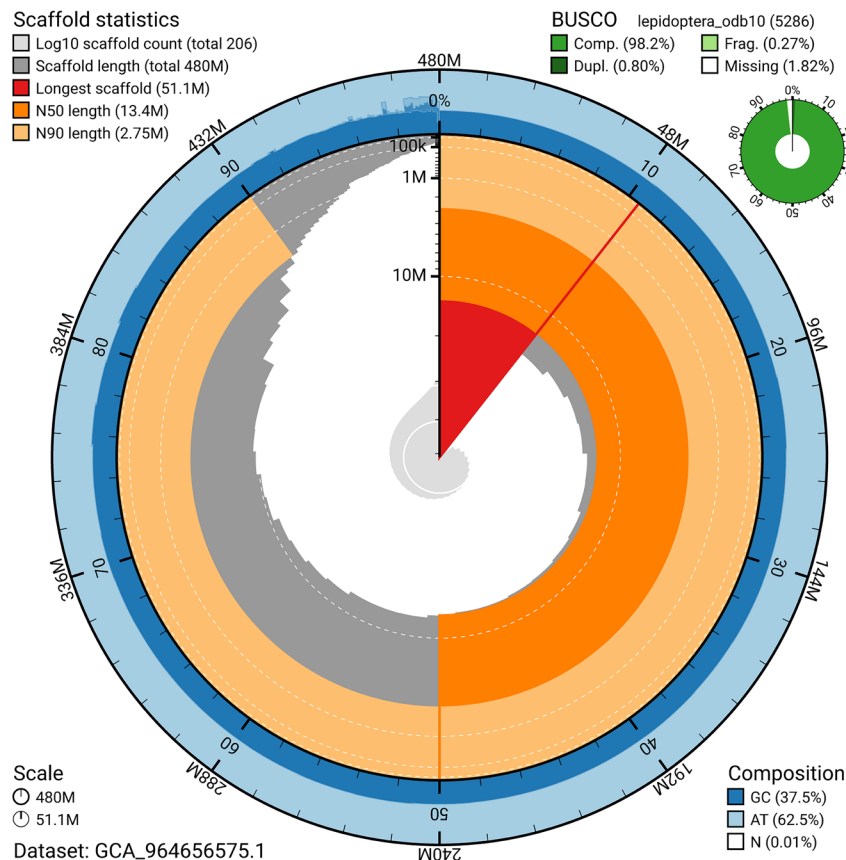


Figure 5. Assembly metrics for iAclFerr2.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1 000 bins around the circumference. The outermost blue 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](#).

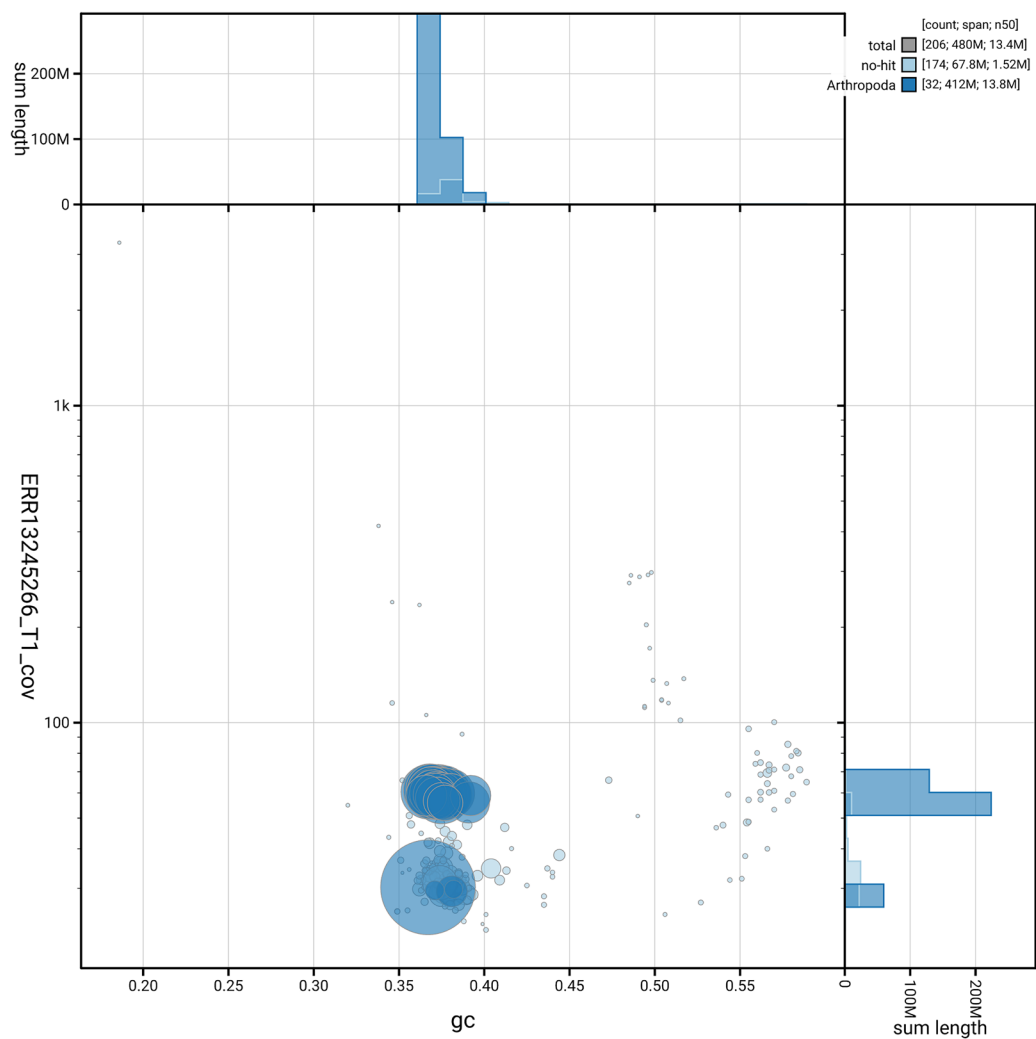


Figure 6. BlobToolKit GC-coverage plot for ilAclFerr2.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes display 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 *Acleris ferrugana* assembly.

Measure	Value	Benchmark
EBP summary (primary)	6.C.Q63	6.C.Q40
Contig N50 length	4.03 Mb	≥ 1 Mb
Scaffold N50 length	13.41 Mb	= chromosome N50
Consensus quality (QV)	Primary: 63.6; alternate: 63.6; combined: 63.6	≥ 40
<i>k</i> -mer completeness	Primary: 86.30%; alternate: 72.25%; combined: 99.33%	≥ 95%
BUSCO	C:98.2% [S:97.4%; D:0.8%]; F:0.3%; M:1.6%; n:5 286	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.03%	≥ 90%

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: *Acleris ferrugana* (rusty oak but-ton). Accession number [PRJEB76341](#). The genome sequence

is released openly for reuse. The *Acleris ferrugana* genome sequencing initiative is part of the Darwin Tree of Life Project (PRJEB40665), the Sanger Institute Tree of Life Programme (PRJEB43745) and Project Psyche (PRJEB71705). All raw sequence data and the assembly have been deposited in INSDC databases. The genome will be annotated using available RNA-Seq data and presented through the [Ensembl](#) pipeline at the European Bioinformatics Institute. Raw data and assembly accession identifiers are reported in [Table 1](#) and [Table 2](#).

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

Author information

Contributors are listed at the following links:

- Members of the [University of Oxford and Wytham Woods Genome Acquisition Lab](#)

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	4.3.9	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	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/chhy1p123/hifiasm
HiGlass	1.13.4	https://github.com/higlass/higlass
MerquryFK	1.1.2	https://github.com/thegenemyers/MERQURY.FK
Minimap2	2.24-r1122	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	23.10.0	https://github.com/nextflow-io/nextflow
PretextSnapshot	N/A	https://github.com/sanger-tol/PretextSnapshot
PretextView	0.2.5	https://github.com/sanger-tol/PretextView

Software	Version	Source
samtools	1.19.2	https://github.com/samtools/samtools
sanger-tol/ascc	0.1.0	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	0.6.0	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	1.2a.2	https://github.com/c-zhou/yahs

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- Members of the [Tree of Life Core Informatics](#) collective
- Members of the [Darwin Tree of Life Consortium](#)

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Noah H Rose 

University of California San Diego, San Diego, USA

This manuscript describes a newly assembled *Acleris ferrugana* (rusty oak button) genome. The combination of long read sequencing and Hi-C scaffolding is appropriate, the assembly methods are well suited to the data, and the resulting assembly appears to be of high quality. The species in question is common across the British and Irish Isles, so this assembly will be a valuable resource for the Darwin Tree of Life Project.

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: Evolutionary genetics, ecological 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 11 November 2025

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Piotr Zieliński

Jagiellonian University, Kraków, Poland

This genome note presents the first high-quality reference genome assembly for *Acleris ferrugana*, the Tawny Oak Tortrix, generated as part of the Darwin Tree of Life (DTOL) project. The assembly was based on PacBio HiFi, Hi-C, and RNA-seq data from specimens collected in Wytham Woods, UK. The resulting genome totals 480.03 Mb, with 99.03% of the sequence scaffolded into 31 chromosomal pseudomolecules, including the W and Z sex chromosomes. The mitochondrial genome was also assembled (16.58 kb).

The authors followed standard DTOL protocols for sample collection, barcoding, sequencing, assembly, and curation. The genome achieved a high level of completeness and accuracy (BUSCO 98.2%). Overall, this article represents a technically sound and well-documented contribution to the growing catalogue of lepidopteran genomes. The data provide valuable resources for future population genomic research on Tawny Oak Tortrix, its evolution, ecology, and comparative genomics.

Assembly completeness and haplotype representation

The authors report that the primary assembly represents 86.3% k-mer completeness. The relatively low completeness for the primary assembly might merit a short discussion — for example, whether it reflects biological reality or technical filtering?

Chromosome assignment and sex determination

The manuscript states that the W chromosome remained unscaffolded due to the Hi-C data being derived from a male individual. While this limitation is understandable, the authors could briefly note how this affects completeness of the sex chromosome assembly and whether future sequencing of a female could improve this.

Table 3 lists chromosome-level scaffolds, but sex chromosomes sizes appear very large. A brief comment on the possible reasons for that, would be useful.

Overall Assessment

This manuscript represents a high-quality genome resource note, aligning with the standards of the Darwin Tree of Life initiative. The technical execution is excellent, and the data are fully open and reusable.

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?

Yes

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

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Population genomics, molecular ecology

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

Reviewer Report 10 November 2025

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Shangzhe Zhang 

University of St Andrews, St Andrews, UK

The authors present a high-quality genome assembly for *Acleris ferrugana*, providing a valuable genomic resource that contributes meaningfully to the Tree of Life initiative. The sequencing strategy, assembly pipeline, and overall analytical workflow are robust, and the results appear well supported. I recommend acceptance of this work after addressing a few minor but important points.

(1) Sample information and mapping statistics:

Multiple samples were used across different sequencing platforms. This raises potential concerns regarding mapping consistency and sequence heterogeneity. It would be helpful to include a brief summary of the mapping statistics in the Results section to confirm data concordance. Additionally, more details about the samples should be provided—specifically, the sex of each individual and whether they were collected from the same wild population or derived from an inbred laboratory line.

(2) Identification of the Z chromosome:

The method used to identify the Z chromosome is not clearly described and appears to be missing from the Methods section. Please provide additional information or specify the pipeline used for chromosome identification to enhance reproducibility.

(3) Chromosome number and karyotype consistency:

Chromosome number is often an informative feature in Lepidoptera genomics. It would be valuable to comment on whether the assembly's inferred chromosome count aligns with previously reported karyotype stability in related species.

(4) Clarification on the Z chromosome scaffolding:

In the statement, "Most of the assembly sequence (99.03%) was assigned to 31 chromosomal-level scaffolds,..." it is unclear whether the Z chromosome was successfully scaffolded or identified. Please clarify this point.

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, Evolutionary 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, however I have significant reservations, as outlined above.

Reviewer Report 14 October 2025

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Luana Bataglia

Catholic University of the Sacred Heart - Piacenza and Cremona Campus (Ringgold ID: 96985), Piacenza, Emilia-Romagna, Italy

The article reports the sequencing of the genome of the Lepidoptera species *Acleris ferrugana*.

1) Although the article type is a data note and focuses on technical results, the background requires more biological information about the species to justify the creation of the dataset.

Some points to be considered are:

-What is the biological/conservational importance of the species?

-Is this species considered vulnerable?

-Can it be considered an insect pest?

-The authors mentioned the *Acleris ferrugana* as a variable species. Therefore, should the sequencing be related to more phylogenetic or evolutionary aims?

The authors should clarify the interest in sequencing this species.

2) The sex of the sequenced specimens should be specified in the methods section. The only specification present is for the genomic DNA (female). Only in the results section is it reported that a male was used for the HI-C sequencing. There is no information about the specimen used in RNA-seq.

3) For better and faster understanding, the authors should report in the text which body parts were used for DNA barcoding and which remaining parts were used for sequencing.

4) The mitochondrial genome data were poorly reported. The only information is about the length of 16.58 kilobases, reported in the abstract. But this information is missing in the results section.

5) The same for the RNA-seq data, which needs to be further explored.

6) Although it is written that the gene annotation is still to be done, I strongly suggest that the authors complete it as soon as possible and include this information in the article.

7) The authors should include small discussions related to the resulting data to add a minimum degree of biological interpretation:

Example: - Do the length of the nuclear and mitochondrial genomes match the expected size and resemble those of close species?

-The same kind of discussion should be done with the annotation data. Do the number of predicted genes and proteins match the expected number and resemble those of close species?

- Some groups of genes presented a significant increase or decrease in the species?

- Did the authors detect potential unique regions/highlights in the genome of this species?

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

No

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?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular Biology, Genetics, Epigenetics, Entomology, Biotechnology.

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.
