DATA NOTE

The genome sequence of the White-triangle Button, *Acleris holmiana* (Linnaeus, 1758) [version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual female *Acleris holmiana* (the White-triangle Button; Arthropoda; Insecta; Lepidoptera; Tortricidae). The genome sequence is 650.2 megabases in span. Most of the assembly is scaffolded into 31 chromosomal pseudomolecules, including the Z and W sex chromosomes. The mitochondrial genome has also been assembled and is 16.28 kilobases in length.

Keywords

Acleris holmiana, White-triangle Button, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status *AWAITING PEER REVIEW*

Any reports and responses or comments on the article can be found at the end of the article.

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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; Apoditrysia; Tortricoidea; Tortricidae; Tortricinae; Tortricini; *Acleris*; *Acleris holmiana* (Linnaeus, 1758) (NCBI:txid572802).

Background

Acleris holmiana is a micro-moth in the family Tortricidae with the common name of White-triangle Button. This is a small but distinctive species (forewing 6–7 mm). The forewing is dark orange with reddish-brown bands and a large, bright white, triangular mark on the costa (Sterling *et al.*, 2012). The moth is found throughout Europe excluding the Iberian Peninsula (GBIF Secretariat, 2023), and was accidentally introduced to British Columbia in 1977 (TortAI, 2014).

The moth can be disturbed by day, and comes to light at night (Langmaid *et al.*, 2018). In the UK, the moth is single brooded and flies between mid-June and early September (Sterling *et al.*, 2012) however, in British Columbia it is double or triple brooded (TortAI, 2014). The eggs are laid on twigs of various rosaceous shrubs including hawthorn, *Prunus* spp. and apple, and do not hatch until the following spring. The larvae feed between two leaves of the foodplant which it spins together at the edge to create a shelter in which it feeds, and later (Hancock *et al.*, 2015). It is considered a minor pest of fruit trees in Europe.

The genome of *A. holmiana* 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. Here we present a chromosomally complete genome sequence for *A. holmiana* based on one female specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome was sequenced from one female *Acleris holmiana* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, –1.34). A total of 40-fold coverage in Pacific Biosciences single-molecule HiFi long reads was generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 116 missing joins or mis-joins and removed 16 haplotypic duplications, reducing the assembly length by 0.38% and the scaffold number by 4.19%.

The final assembly has a total length of 650.2 Mb in 296 sequence scaffolds with a scaffold N50 of 21.2 Mb (Table 1). The snailplot in Figure 2 provides a summary of the assembly statistics, while the distribution of assembly scaffolds on GC proportion and coverage is shown in Figure 3. The cumulative assembly plot in Figure 4 shows curves for subsets of scaffolds assigned to different phyla. Most (97.85%) of the assembly sequence was assigned to 31 chromosomal-level

Figure 1. Photograph of the *Acleris holmiana* (ilAclHolm1) specimen used for genome sequencing.

scaffolds, representing 29 autosomes and the Z and W sex chromosomes. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 5; Table 2). The Z chromosome was assigned by alignment to *Acleris literana* (GCA_946894065.1) (Crowley *et al.*, 2023). While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited. The mitochondrial genome was also assembled and can be found as a contig within the multifasta file of the genome submission.

The estimated Quality Value (QV) of the final assembly is 61.0 with *k*-mer completeness of 100.0%, and the assembly has a BUSCO v5.3.2 completeness of 97.3% (single = 96.4%, duplicated = 0.9%), using the lepidoptera_odb10 reference set (n = 5,286).

Metadata for specimens, barcode results, spectra estimates, sequencing runs, contaminants and pre-curation assembly statistics are given at https://links.tol.sanger.ac.uk/species/572802.

Methods

Sample acquisition and nucleic acid extraction

The specimen used for genome sequencing was a female *Acleris holmiana* (specimen ID Ox000637, ToLID ilAclHolm1). The specimen was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2020-07-20 using a light trap. The specimen used for Hi-C sequencing (specimen ID Ox001677, ToLID ilAclHolm2) was collected from the same location on 2021-07-17 also using a light trap. The specimens were collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice.

The workflow for high molecular weight (HMW) DNA extraction at the Wellcome Sanger Institute (WSI) includes a sequence of core procedures: sample preparation; sample homogenisation, DNA extraction, fragmentation, and clean-up.

Table 1. Genome data for *Acleris holmiana*, ilAclHolm1.1.

Project accession data		
Assembly identifier	ilAclHolm1.1	
Species	*Acleris holmiana*	
Specimen	ilAclHolm1	
NCBI taxonomy ID	572802	
BioProject	PRJEB58938	
BioSample ID	SAMEA7701499	
Isolate information	ilAclHolm1, whole organism (DNA sequencing) ilAclHolm2, whole organism (Hi-C sequencing)	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	61.0	*≥ 50*
k-mer completeness	100.0%	*≥ 95%*
BUSCO**	C:97.3%[S:96.4%,D:0.9%], F:0.6%,M:2.1%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	97.85%	*≥ 95%*
Sex chromosomes	ZW	*localised homologous pairs*
Organelles	Mitochondrial genome: 16.28 kb	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR10798423	
Hi-C Illumina	ERR10786010	
Genome assembly		
Assembly accession	GCA_949316455.1	
Accession of alternate haplotype	GCA_949316255.1	
Span (Mb)	650.2	
Number of contigs	1240	
Contig N50 length (Mb)	1.1	
Number of scaffolds	296	
Scaffold N50 length (Mb)	21.2	
Longest scaffold (Mb)	88.82	

* Assembly metric benchmarks are adapted from column VGP-2020 of "Table 1: Proposed standards and metrics for defining genome assembly quality" from (Rhie *et al.*, 2021).

** BUSCO scores based on the lepidoptera_odb10 BUSCO set using version 5.3.2. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison. A full set of BUSCO scores is available at https://blobtoolkit.genomehubs.org/view/ilAclHolm1_1/dataset/ilAclHolm1_1/busco.

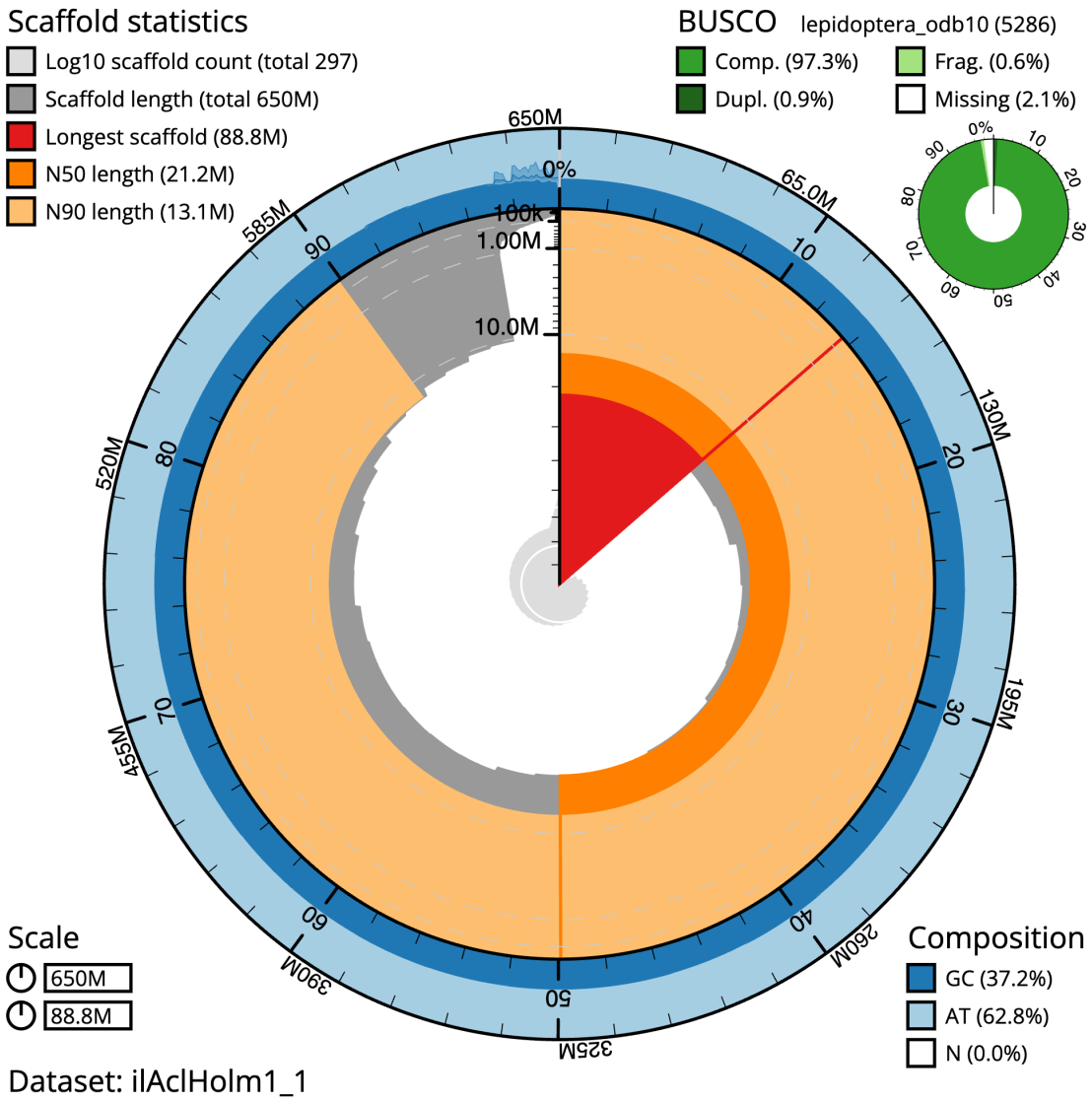

Figure 2. Genome assembly of *Acleris holmiana*, ilAclHolm1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 650,224,491 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (88,816,612 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (21,176,203 and 13,133,638 bp), respectively. The pale grey spiral shows the cumulative scaffold count on a log scale with white scale lines showing successive orders of magnitude. The blue and pale-blue area around the outside of the plot shows the distribution of GC, AT and N percentages in the same bins as the inner plot. A summary of complete, fragmented, duplicated and missing BUSCO genes in the lepidoptera_odb10 set is shown in the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilAclHolm1_1/dataset/ilAclHolm1_1/snail.

In sample preparation, the ilAclHolm1 sample was weighed and dissected on dry ice (Jay *et al.*, 2023). Tissue of the whole organism was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a). HMW DNA was extracted using the Automated MagAttract v1 protocol (Sheerin *et al.*, 2023). DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system with speed setting 30 (Todorovic *et al.*, 2023). Sheared DNA was purified by solid-phase reversible immobilisation (Strickland *et al.*, 2023): in brief, the method employs a 1.8X ratio of AMPure PB beads to sample to eliminate shorter fragments and concentrate the DNA. The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and Qubit Fluorometer and Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

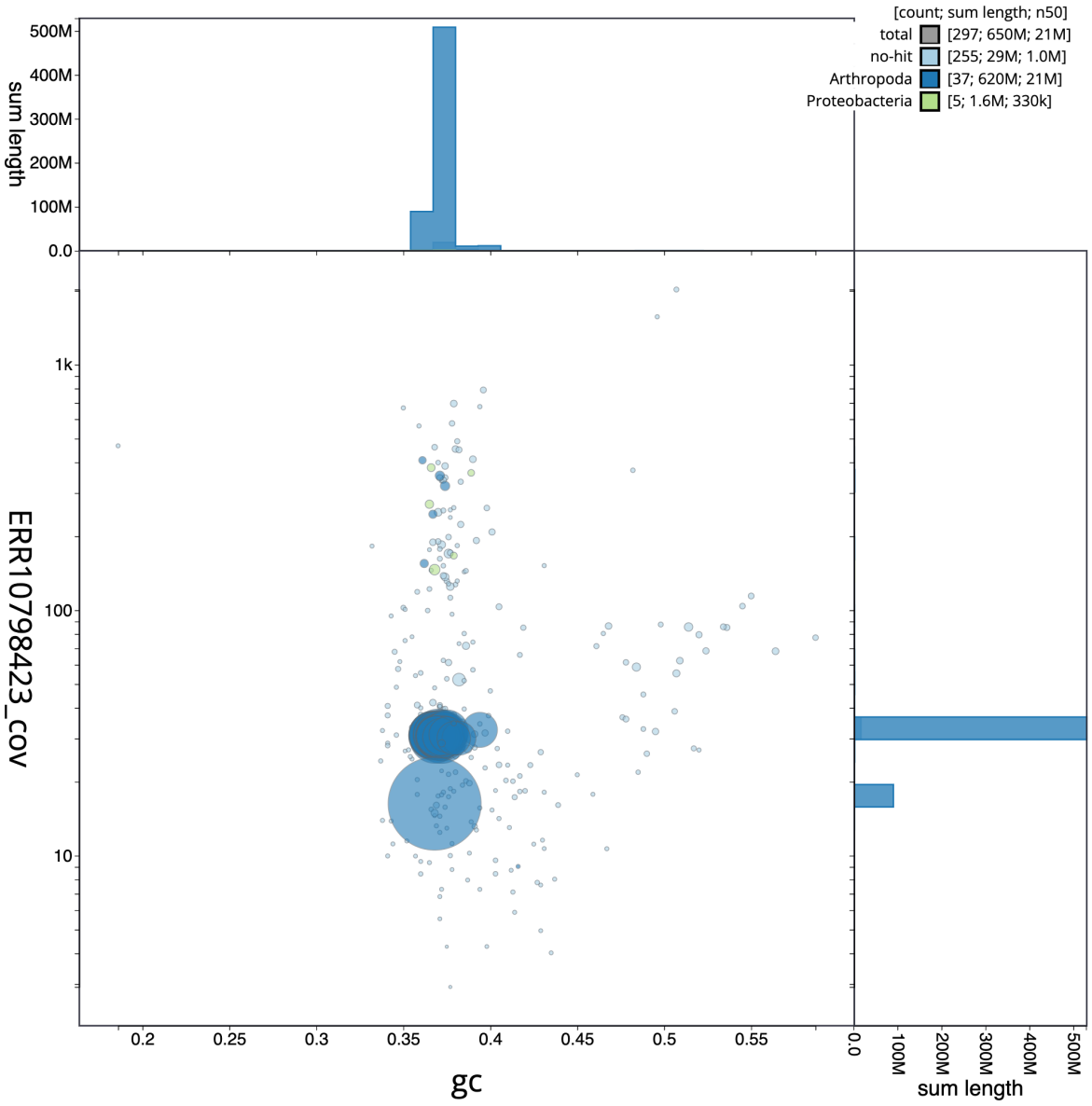

Figure 3. Genome assembly of *Acleris holmiana*, ilAclHolm1.1: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilAclHolm1_1/dataset/ilAclHolm1_1/blob.

Protocols developed by the WSI Tree of Life laboratory are publicly available on protocols.io (Denton *et al.*, 2023b).

Sequencing

Pacific Biosciences HiFi circular consensus DNA sequencing libraries were constructed according to the manufacturers' instructions. DNA sequencing was performed by the Scientific Operations core at the WSI on a Pacific Biosciences SEQUEL II instrument. Hi-C data were also generated from whole organism tissue of ilAclHolm2 using the Arima2 kit and sequenced on the Illumina NovaSeq 6000 instrument.

Genome assembly, curation and evaluation

Assembly was carried out with Hifiasm (Cheng *et al.*, 2021) and haplotypic duplication was identified and removed with purge_dups (Guan *et al.*, 2020). The assembly was then scaffolded with Hi-C data (Rao *et al.*, 2014) using YaHS (Zhou *et al.*, 2023). The assembly was checked for contamination and corrected as described previously (Howe *et al.*, 2021). Manual curation was performed using HiGlass (Kerpedjiev *et al.*, 2018) and Pretext (Harry, 2022). The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2023), which runs MitoFinder (Allio *et al.*, 2020) or MITOS (Bernt *et al.*, 2013)

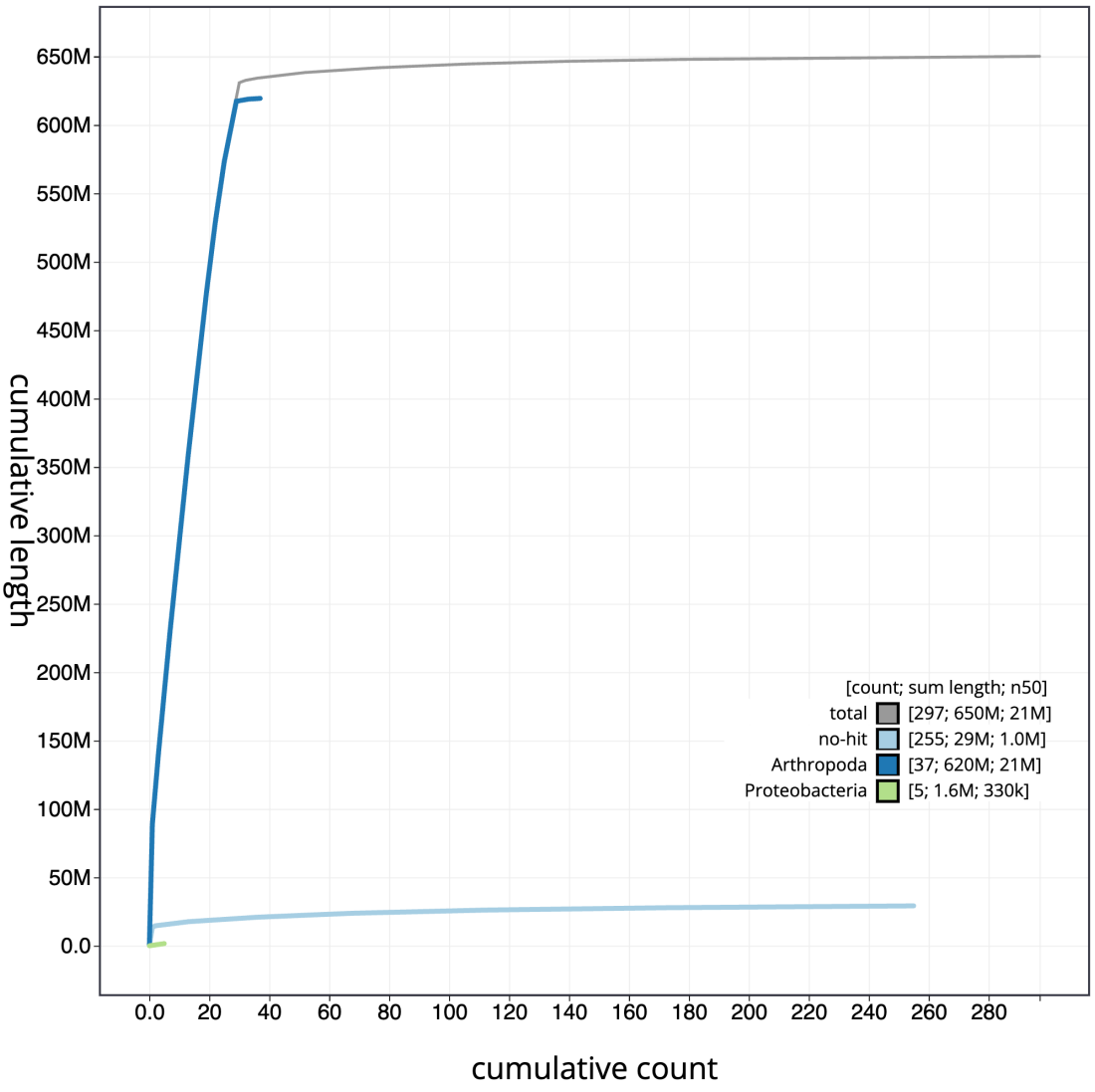

Figure 4. Genome assembly of *Acleris holmiana*, ilAclHolm1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilAclHolm1_1/dataset/ilAclHolm1_1/cumulative.

and uses these annotations to select the final mitochondrial contig and to ensure the general quality of the sequence.

A Hi-C map for the final assembly was produced using bwa-mem2 (Vasimuddin *et al.*, 2019) in the Cooler file format (Abdennur & Mirny, 2020). To assess the assembly metrics, the *k*-mer completeness and QV consensus quality values were calculated in Merqury (Rhie *et al.*, 2020). This work was done using Nextflow (Di Tommaso *et al.*, 2017) DSL2 pipelines "sanger-tol/readmapping" (Surana *et al.*, 2023a) and "sanger-tol/genomenote" (Surana *et al.*, 2023b). The genome was analysed within the BlobToolKit environment (Challis *et al.*, 2020) and BUSCO scores (Manni *et al.*, 2021; Simão *et al.*, 2015) were calculated.

Table 3 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 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.

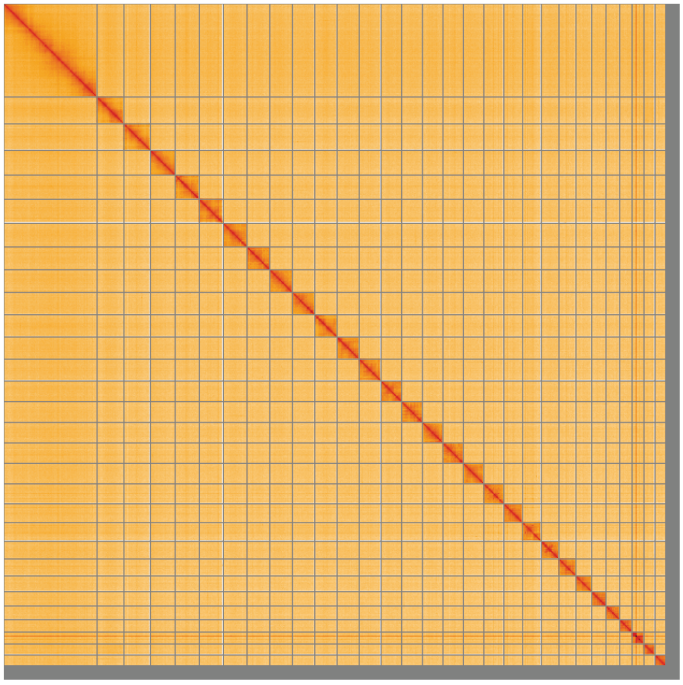

Figure 5. Genome assembly of *Acleris holmiana*, ilAclHolm1.1: Hi-C contact map of the ilAclHolm1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=B67WVoD-RpCarufRFZ8dAQ.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Acleris holmiana*, ilAclHolm1.

INSDC accession	Chromosome	Length (Mb)	GC%
OX438763.1	1	25.64	37.0
OX438764.1	2	25.22	37.0
OX438765.1	3	23.53	37.0
OX438766.1	4	23.11	37.0
OX438767.1	5	22.88	36.5
OX438768.1	6	22.58	36.5
OX438769.1	7	21.67	36.5
OX438770.1	8	21.66	37.0
OX438771.1	9	21.22	36.5
OX438772.1	10	21.19	37.0
OX438773.1	11	21.18	37.0
OX438774.1	12	20.78	37.0
OX438775.1	13	19.77	37.0
OX438776.1	14	19.72	37.0
OX438777.1	15	19.58	37.0
OX438778.1	16	19.47	37.5
OX438779.1	17	19.46	37.0
OX438780.1	18	19.09	37.5
OX438781.1	19	17.92	37.0
OX438782.1	20	17.81	37.5
OX438783.1	21	16.84	37.5
OX438784.1	22	16.15	37.0
OX438785.1	23	15.04	37.0
OX438786.1	24	13.55	37.5
OX438787.1	25	13.13	37.0
OX438788.1	26	11.76	37.5
OX438789.1	27	11.36	39.5
OX438790.1	28	10.52	38.0
OX438791.1	29	10.25	38.0
OX438792.1	W	0.66	37.0
OX438762.1	Z	88.82	37.0
OX438793.1	MT	0.02	19.0

Table 3. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.2.1	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	https://gitlab.com/ezlab/busco
Hifiasm	0.16.1-r375	https://github.com/chhylp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Merqury	MerquryFK	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	2	https://github.com/marcelauliano/MitoHiFi
PretextView	0.2	https://github.com/wtsi-hpag/PretextView
purge_dups	1.2.3	https://github.com/dfguan/purge_dups
sanger-tol/genomenote	v1.0	https://github.com/sanger-tol/genomenote
sanger-tol/readmapping	1.1.0	https://github.com/sanger-tol/readmapping/tree/1.1.0
YaHS	1.2a	https://github.com/c-zhou/yahs

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: Acl*eris holmiana* (white-triangle button). Accession number PRJEB58938; https://identifiers.org/ena.embl/PRJEB58938 (Wellcome Sanger Institute, 2023). The genome sequence is released openly for reuse. The *Acleris holmiana* 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.

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