



DATA NOTE

# The genome sequence of the Small Argent and Sable moth, *Epirrhoe tristata* (Linnaeus, 1758) [version 1; peer review: awaiting peer review]

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## 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.

## Abstract

We present a genome assembly from an individual male Small Argent and Sable moth *Epirrhoe tristata* (Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence spans 313.80 megabases. Most of the assembly is scaffolded into 30 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 16.92 kilobases in length. Gene annotation of this assembly on Ensembl identified 16,469 protein-coding genes.

## Keywords

*Epirrhoe tristata*, Small Argent and Sable moth, genome sequence, chromosomal, Lepidoptera



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

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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; *Epirrhoe*; *Epirrhoe tristata* (Linnaeus, 1758) (NCBI:txid934838).

## Background

*Epirrhoe tristata*, the Small Argent And Sable, is a moth of the genus *Epirrhoe* in the family Geometridae. The genome of *E. tristata* was sequenced as part of the Darwin Tree of Life Project, a collaborative effort to sequence all named eukaryotic species in the Atlantic Archipelago of Britain and Ireland (Blaxter *et al.*, 2022). Here we present a chromosomally complete genome sequence for *Epirrhoe tristata*, based on a male specimen from Glen Strathfarrar, Scotland, UK.

## Genome sequence report

The genome of an adult male *Epirrhoe tristata* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating a total of 27.05 Gb (gigabases) from 2.34 million reads, providing approximately 84-fold coverage. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data, which produced 104.25 Gbp from 690.41 million reads, yielding an approximate coverage of 332-fold. Specimen and sequencing information is summarised in Table 1.

Manual assembly curation corrected four missing joins or misjoins, reducing the scaffold number by 2.94%. The final assembly has a total length of 313.80 Mb in 32 sequence scaffolds with a scaffold N50 of 11.3 Mb (Table 2). The total count of gaps in the scaffolds is 44. The snail plot 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.



**Figure 1.** Photograph of *Epirrhoe tristata* by AfroBrazilian (not the specimen used for genome sequencing).

Most (99.97%) of the assembly sequence was assigned to 30 chromosomal-level scaffolds, representing 29 autosomes and the Z sex chromosome. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 5; Table 3). Chromosome Z was assigned by alignment to *Xanthorhoe spadicearia* (GCA\_947086425.1) (Boyes *et al.*, 2024). 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 69.4 with *k*-mer completeness of 100.0%, and the assembly has a BUSCO v5.3.2 completeness of 98.4% (single = 98.0%, duplicated = 0.4%), using the lepidoptera\_odb10 reference set (*n* = 5,286).

Metadata for specimens, BOLD barcode results, spectra estimates, sequencing runs, contaminants and pre-curation assembly statistics are given at <https://links.tol.sanger.ac.uk/species/934838>.

## Genome annotation report

The *Epirrhoe tristata* genome assembly (GCA\_951394285.1) was annotated at the European Bioinformatics Institute (EBI) on Ensembl Rapid Release. The resulting annotation includes 16,639 transcribed mRNAs from 16,469 protein-coding genes (Table 2; [https://rapid.ensembl.org/Epirrhoe\\_tristata\\_GCA\\_951394285.1/Info/Index](https://rapid.ensembl.org/Epirrhoe_tristata_GCA_951394285.1/Info/Index)). The average transcript length is 6,435.52. There are 1.01 coding transcripts per gene and 6.00 exons per transcript.

## Methods

### Sample acquisition

An adult male *Epirrhoe tristata* (specimen ID SAN00002622, ToLiD iEpiTris1) was collected from Glen Strathfarrar, Scotland, UK (latitude 57.41, longitude -4.73) on 2022-06-27 using a moth trap. The specimen was collected and identified by Marc Botham (UK Centre for Ecology & Hydrology) and preserved by flash freezing.

### 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 core procedures: sample preparation and homogenisation, DNA extraction, fragmentation and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). In sample preparation, the iEpiChr1 sample was weighed and dissected on dry ice (Jay *et al.*, 2023). Tissue from the thorax was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a).

HMW DNA was extracted in the WSI Scientific Operations core using the Automated MagAttract v2 protocol (Oatley *et al.*, 2023). The 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 reversible

**Table 1. Specimen and sequencing data for *Epirrhoe tristata*.**

Project information			
Study title	<i>Epirrhoe tristata</i> (small argent and sable)		
Umbrella BioProject	PRJEB61371		
Species	<i>Epirrhoe tristata</i>		
BioSample	SAMEA112198543		
NCBI taxonomy ID	934838		
Specimen information			
Technology	ToLID	BioSample accession	Organism part
PacBio long read sequencing	ilEpiTris1	SAMEA112198599	thorax
Hi-C sequencing	ilEpiTris1	SAMEA112198599	thorax
RNA sequencing	ilEpiTris1	SAMEA112198600	abdomen
Sequencing information			
Platform	Run accession	Read count	Base count (Gb)
Hi-C Illumina NovaSeq 6000	ERR11242569	6.90e+08	104.25
PacBio Sequel Iie	ERR11242145	2.34e+06	27.05
RNA Illumina NovaSeq 6000	ERR11837486	5.60e+07	8.46

immobilisation, using AMPure PB beads to eliminate shorter fragments and concentrate the DNA (Strickland *et al.*, 2023). The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and Qubit Fluorometer using the Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

RNA was extracted from abdomen tissue of ilEpiTris1 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ *mir*Vana protocol (do Amaral *et al.*, 2023). 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.

#### Library preparation and sequencing

Pacific Biosciences HiFi circular consensus DNA sequencing libraries were constructed according to the manufacturers' instructions. Poly(A) RNA-Seq libraries were constructed using the NEB Ultra II RNA Library Prep kit. DNA and RNA sequencing was performed by the Scientific Operations core at the WSI on Pacific Biosciences Sequel Iie (HiFi) and Illumina NovaSeq 6000 (RNA-Seq) instruments.

Hi-C data were generated from frozen thorax tissue of the ilEpiTris1 sample, using the Arima-HiC v2 kit. The tissue

was fixed with a TC buffer containing formaldehyde, resulting in crosslinked DNA. The crosslinked DNA was digested with a restriction enzyme master mix. The resulting 5'-overhangs were filled in and labelled with a biotinylated nucleotide. The biotinylated DNA was then fragmented, enriched, bar-coded, and amplified using the NEBNext Ultra II DNA Library Prep Kit. Hi-C sequencing was performed on an Illumina NovaSeq 6000 instrument, using paired-end sequencing with a read length of 150 bp.

#### Genome assembly, curation and evaluation

##### Assembly

The HiFi reads were first assembled using Hifiasm (Cheng *et al.*, 2021) with the --primary option. Haplotypic duplications were identified and removed using purge\_dups (Guan *et al.*, 2020). The Hi-C reads were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). The contigs were further scaffolded using the provided Hi-C data (Rao *et al.*, 2014) in YaHS (Zhou *et al.*, 2023) using the --break option. 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.

**Table 2. Genome assembly data for *Epirrhoe tristata*, iLEpiTris1.1.**

Genome assembly		
Assembly name	iLEpiTris1.1	
Assembly accession	GCA_951394285.1	
Accession of alternate haplotype	GCA_951394275.1	
Span (Mb)	313.80	
Number of contigs	77	
Contig N50 length (Mb)	6.5	
Number of scaffolds	32	
Scaffold N50 length (Mb)	11.3	
Longest scaffold (Mb)	17.63	
Assembly metrics*		Benchmark
Consensus quality (QV)	69.4	≥ 50
k-mer completeness	100.0%	≥ 95%
BUSCO**	C:98.4%[S:98.0%,D:0.4%], F:0.4%,M:1.2%,n:5,286	C ≥ 95%
Percentage of assembly mapped to chromosomes	99.97%	≥ 95%
Sex chromosomes	Z	localised homologous pairs
Organelles	Mitochondrial genome: 16.92 kb	complete single alleles
Genome annotation of assembly GCA_951394285.1 at Ensembl		
Number of protein-coding genes	16,469	
Number of gene transcripts	16,639	

\* 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/iLEpiTris1\\_1/dataset/iLEpiTris1\\_1/busco](https://blobtoolkit.genomehubs.org/view/iLEpiTris1_1/dataset/iLEpiTris1_1/busco).

### Assembly curation

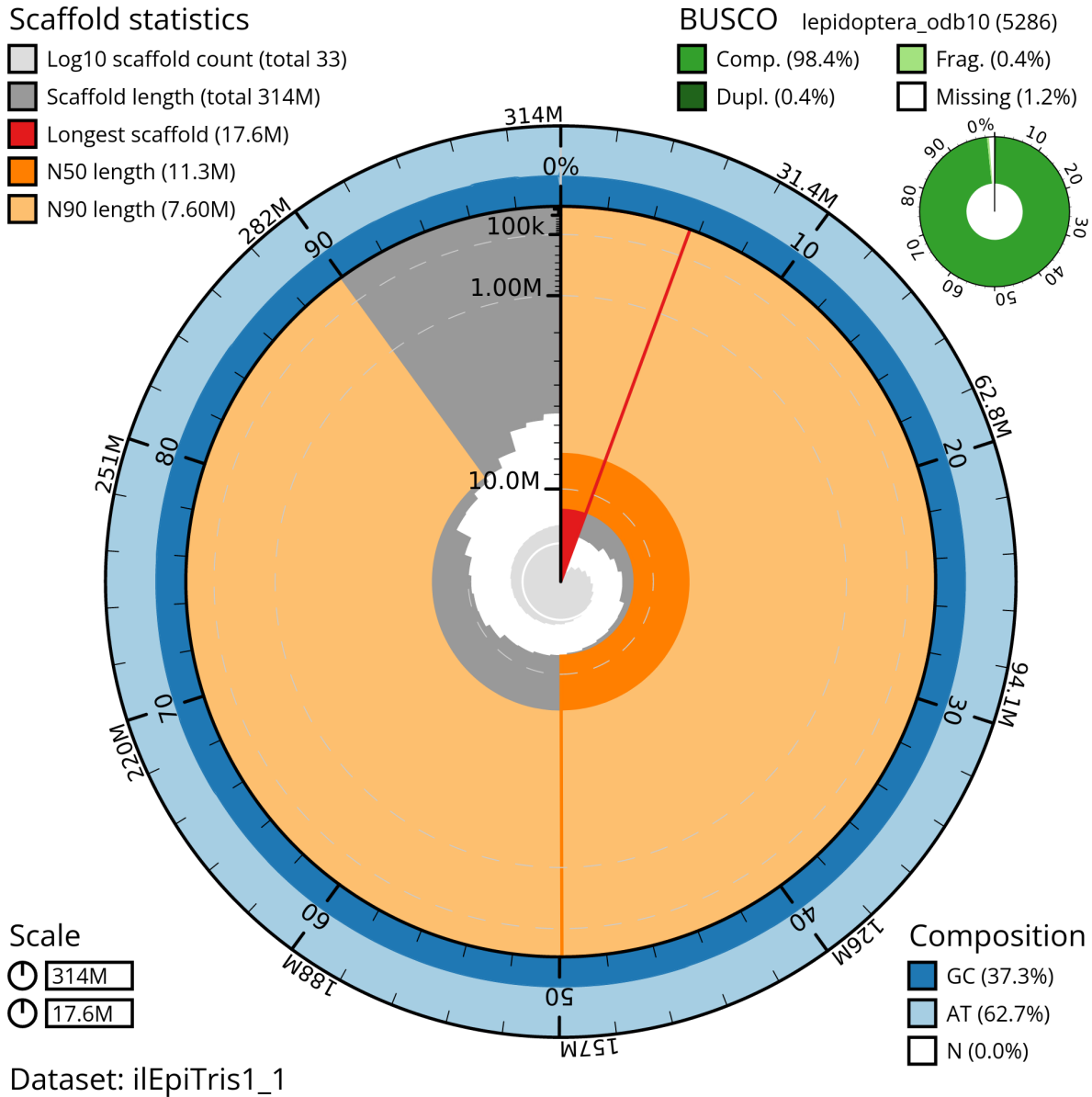
The assembly was decontaminated using the Assembly Screen for Cobionts and Contaminants (ASCC) pipeline (article in preparation). Manual curation was primarily conducted using PretextView ([Harry, 2022](#)), with additional insights provided by JBrowse2 ([Diesh et al., 2023](#)) and HiGlass ([Kerpedjiev et al., 2018](#)). Scaffolds were visually inspected and corrected as described by [Howe et al. \(2021\)](#). Any identified contamination, missed joins, and mis-joins were corrected, 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> (article in preparation).

### Evaluation of the final assembly

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 the “sanger-tol/readmapping” ([Surana et al., 2023a](#)) and “sanger-tol/genomenote” ([Surana et al., 2023b](#)) pipelines. The genome readmapping pipelines were developed using the nf-core tooling ([Ewels et al., 2020](#)), use MultiQC ([Ewels et al., 2016](#)), and make extensive use of the Conda package manager, the Bioconda initiative ([Grüning et al., 2018](#)), the Biocontainers infrastructure ([da Veiga Leprevost et al., 2017](#)), and the Docker ([Merkel, 2014](#)) and Singularity ([Kurtzer et al., 2017](#)) containerisation solutions. The genome was also analysed within the BlobToolKit environment ([Challis et al., 2020](#)) and BUSCO scores ([Manni et al., 2021](#)) were calculated.

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



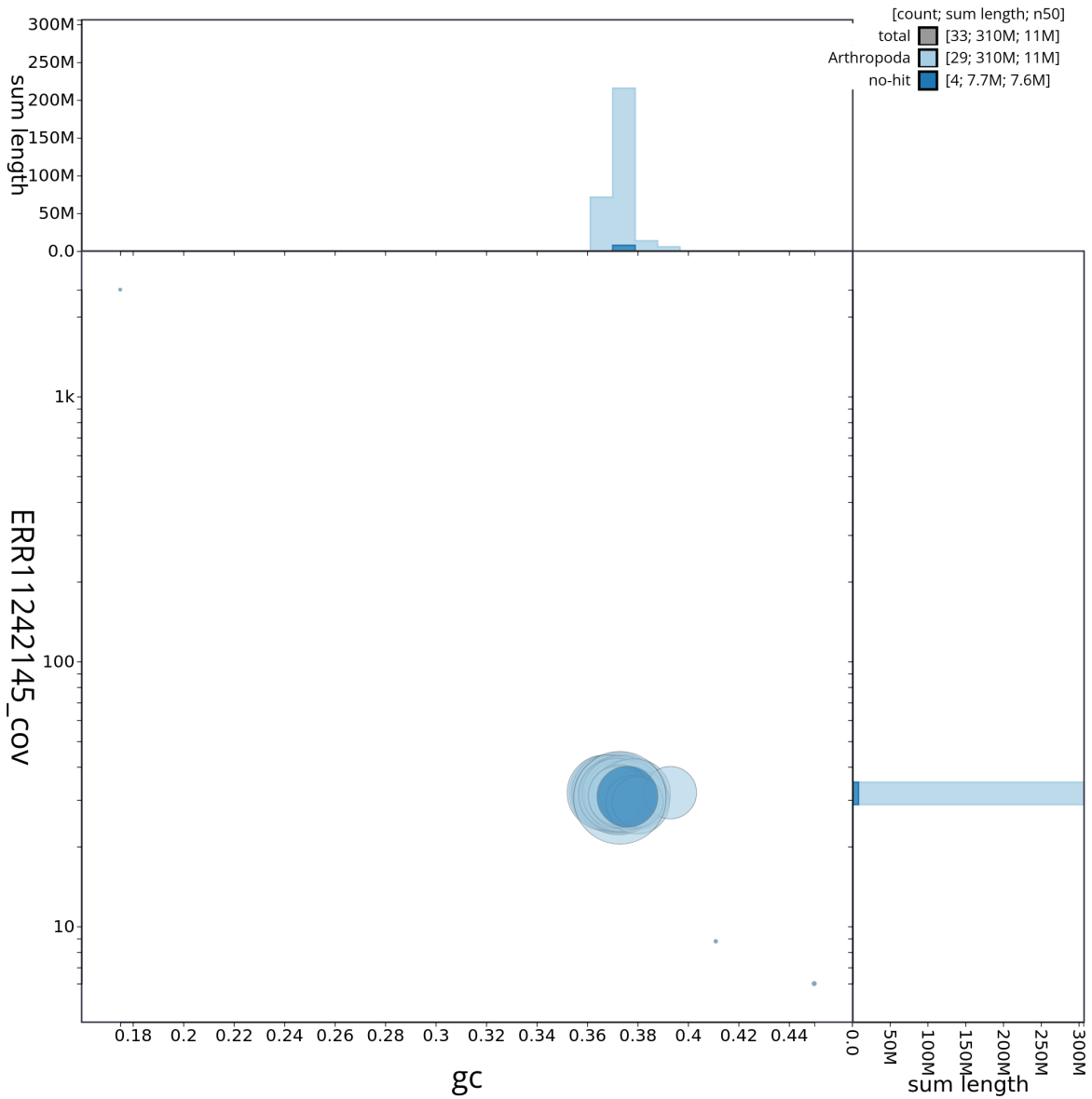
**Figure 2. Genome assembly of *Epirrhoe tristata*, ilEpiTris1.1: metrics.** The BlobToolKit snail plot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 313,821,882 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (17,631,079 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (11,321,105 and 7,599,468 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/ilEpiTris1\\_1/dataset/ilEpiTris1\\_1/snail](https://blobtoolkit.genomehubs.org/view/ilEpiTris1_1/dataset/ilEpiTris1_1/snail).

**Genome annotation**

The BRAKER2 pipeline (Brůna *et al.*, 2021) was used in the default protein mode to generate annotation for the *Epirrhoe tristata* assembly (GCA\_951394285.1) in Ensembl Rapid Release at the EBI.

**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’,



**Figure 3. Genome assembly of *Epirrhoe tristata*, ilEpiTris1.1: BlobToolKit GC-coverage plot.** Sequences are coloured by phylum. Circles are sized in proportion to sequence length. Histograms show the distribution of sequence length sum along each axis. An interactive version of this figure is available at [https://blobtoolkit.genomehubs.org/view/ilEpiTris1\\_1/dataset/ilEpiTris1\\_1/blob](https://blobtoolkit.genomehubs.org/view/ilEpiTris1_1/dataset/ilEpiTris1_1/blob).

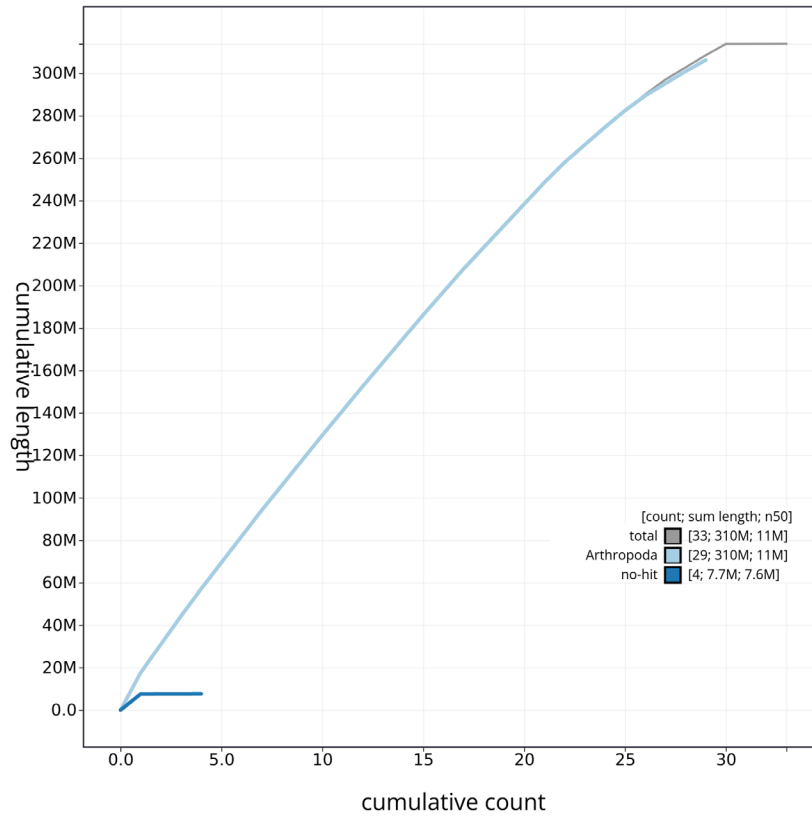
which can be found in full on the Darwin Tree of Life website [here](#). By agreeing with and signing up to the Sampling Code of Practice, the Darwin Tree of Life Partner agrees they will meet the legal and ethical requirements and standards set out within this document in respect of all samples acquired 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

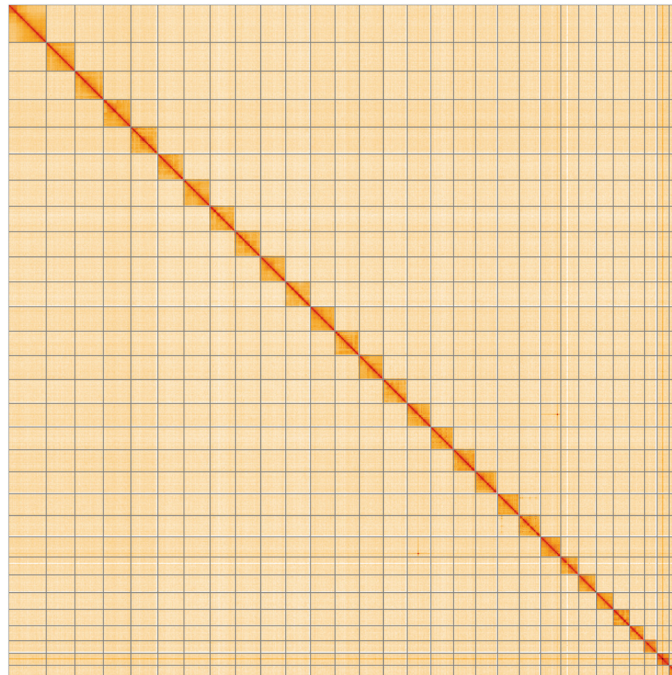
under which they have been/are to be collected and provided for use. The purpose of this is to address and mitigate any potential legal and/or ethical implications of receipt and use of the materials as part of the research project, and to ensure that in doing so we align with best practice wherever 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)





**Figure 4. Genome assembly of *Epirrhoe tristata* ilEpiTris1.1: BlobToolKit cumulative sequence plot.** The grey line shows cumulative length for all sequences. Coloured lines show cumulative lengths of sequences assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at [https://blobtoolkit.genomehubs.org/view/ilEpiTris1\\_1/dataset/ilEpiTris1\\_1/cumulative](https://blobtoolkit.genomehubs.org/view/ilEpiTris1_1/dataset/ilEpiTris1_1/cumulative).



**Figure 5. Genome assembly of *Epirrhoe tristata* ilEpiTris1.1: Hi-C contact map of the ilEpiTris1.1 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=Wq3YluJmRzynlqosrwyeaA>.



**Table 3. Chromosomal pseudomolecules in the genome assembly of *Epirrhoe tristata*, ilEpiTris1.**

INSDC accession	Name	Length (Mb)	GC%
OX596137.1	1	13.35	37.0
OX596138.1	2	13.3	37.5
OX596139.1	3	12.85	37.5
OX596140.1	4	12.52	37.5
OX596141.1	5	12.24	37.0
OX596142.1	6	12.23	37.0
OX596143.1	7	11.83	37.0
OX596144.1	8	11.79	37.0
OX596145.1	9	11.69	37.0
OX596146.1	10	11.58	36.5
OX596147.1	11	11.46	37.5
OX596148.1	12	11.32	37.0
OX596149.1	13	11.24	37.0
OX596150.1	14	11.1	37.0
OX596151.1	15	11.04	37.5

INSDC accession	Name	Length (Mb)	GC%
OX596152.1	16	10.61	37.0
OX596153.1	17	10.31	38.0
OX596154.1	18	10.26	37.5
OX596155.1	19	10.14	37.0
OX596156.1	20	9.95	37.5
OX596157.1	21	9.43	38.0
OX596158.1	22	8.32	38.0
OX596159.1	23	8.26	37.5
OX596160.1	24	7.89	37.5
OX596161.1	25	7.6	37.5
OX596162.1	26	7.0	37.0
OX596163.1	27	5.85	38.0
OX596164.1	28	5.62	39.5
OX596165.1	29	5.31	38.0
OX596136.1	Z	17.63	37.5
OX596166.1	MT	0.02	17.5

**Table 4. Software tools: versions and sources.**

Software tool	Version	Source
BlobToolKit	4.2.1	<a href="https://github.com/blobtoolkit/blobtoolkit">https://github.com/blobtoolkit/blobtoolkit</a>
BUSCO	5.3.2	<a href="https://gitlab.com/ezlab/busco">https://gitlab.com/ezlab/busco</a>
bwa-mem2	2.2.1	<a href="https://github.com/bwa-mem2/bwa-mem2">https://github.com/bwa-mem2/bwa-mem2</a>
Cooler	0.8.11	<a href="https://github.com/open2c/cooler">https://github.com/open2c/cooler</a>
Gfastats	1.3.6	<a href="https://github.com/vgl-hub/gfastats">https://github.com/vgl-hub/gfastats</a>
Hifiasm	0.16.1-r375	<a href="https://github.com/chhylp123/hifiasm">https://github.com/chhylp123/hifiasm</a>
HiGlass	1.11.6	<a href="https://github.com/higlass/higlass">https://github.com/higlass/higlass</a>
Merqury.FK	d00d98157618f4e8d1a9190026b19b471055b22e	<a href="https://github.com/thegenemyers/MERQURY.FK">https://github.com/thegenemyers/MERQURY.FK</a>
MitoHiFi	3	<a href="https://github.com/marcelauliano/MitoHiFi">https://github.com/marcelauliano/MitoHiFi</a>
PretextView	0.2	<a href="https://github.com/wtsi-hpag/PretextView">https://github.com/wtsi-hpag/PretextView</a>
purge_dups	1.2.5	<a href="https://github.com/dfguan/purge_dups">https://github.com/dfguan/purge_dups</a>
sanger-tol/genomenote	v1.0	<a href="https://github.com/sanger-tol/genomenote">https://github.com/sanger-tol/genomenote</a>
sanger-tol/readmapping	1.1.0	<a href="https://github.com/sanger-tol/readmapping/tree/1.1.0">https://github.com/sanger-tol/readmapping/tree/1.1.0</a>
Singularity	3.9.0	<a href="https://github.com/sylabs/singularity">https://github.com/sylabs/singularity</a>
YaHS	1.2a.2	<a href="https://github.com/c-zhou/yahs">https://github.com/c-zhou/yahs</a>

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: *Epirrhoe tristata* (small argent and sable). Accession number PRJEB61371; <https://identifiers.org/ena.embl/PRJEB61371> (Wellcome Sanger Institute, 2023). The genome sequence is released openly for reuse. The *Epirrhoe tristata* genome sequencing initiative is part of the Darwin Tree of Life (DTOL) project. All raw sequence data and the assembly have been deposited in INSDC databases. Raw data and assembly accession identifiers are reported in [Table 1](#) and [Table 2](#).

## Author information

Members of the Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team are listed here: <https://doi.org/10.5281/zenodo.12162482>.

Members of Wellcome Sanger Institute Scientific Operations: Sequencing Operations are listed here: <https://doi.org/10.5281/zenodo.12165051>.

Members of the Wellcome Sanger Institute Tree of Life Core Informatics team are listed here: <https://doi.org/10.5281/zenodo.12160324>.

Members of the Tree of Life Core Informatics collective are listed here: <https://doi.org/10.5281/zenodo.12205391>.

Members of the Darwin Tree of Life Consortium are listed here: <https://doi.org/10.5281/zenodo.4783558>.

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