

DATA NOTE

The genome sequence of the Drinker, Euthrix potatoria

(Linnaeus, 1758) (Lepidoptera: Lasiocampidae)

[version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual female *Euthrix* potatoria (Drinker; Arthropoda; Insecta; Lepidoptera; Lasiocampidae). The assembly contains two haplotypes with total lengths of 487.86 megabases and 466.06 megabases. Most of haplotype 1 (99.97%) is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. Haplotype 2 was assembled to scaffold level. The mitochondrial genome has also been assembled, with a length of 15.43 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

Euthrix potatoria; Drinker; 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; Obtectomera; Bombycoidea; Lasiocampidae; Lasiocampinae; *Euthrix; Euthrix potatoria* (Linnaeus, 1758) (NCBI:txid624169)

Background

The Drinker (*Euthrix potatoria*) is a moth in the family Lasio-campidae. It is a moth of damp habitats including gardens, and is found throughout the UK. It has increased in abundance and range since 1970 (Randle *et al.*, 2019). It occurs throughout central Europe, and as far east as Japan (GBIF Secretariat, 2025).

The common name of Drinker relates to its hairy caterpillar which has long been observed drinking dew and raindrops, but also putting its head under water to drink. The family of moths is also referred to as the Eggars because of the egg-like cocoon of their pupae (Marren, 2019).

This large moth (forewing length around 25 mm) has reddish brown males, and dark or pale yellow females. The moth can be distinguished from other eggar moths by the diagonal cross line on the forewings, above which are two white spots. There is one generation a year, flying in July and August (Waring et al., 2017). The caterpillars feed on wide range of coarse grasses, mainly at night but it can be found at rest during the day. It overwinters as a small larva (Sterling et al., 2023).

We present a chromosome-level genome sequence for *Euthrix potatoria*, the Drinker. This assembly is the first high-quality genome for the genus *Euthrix* and one of 13 genomes available for the family Lasiocampidae, as of August 2025 (data obtained via NCBI datasets, O'Leary *et al.*, 2024). The assembly was produced as part of the Darwin Tree of Life Project 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 *Euthrix potatoria* (specimen ID Ox001659, ToLID ilEutPota3; Figure 1), collected from Wytham Woods, Oxfordshire, United Kingdom (latitude 51.772, longitude –1.338) on 2021-07-17. The specimen was collected and identified by Douglas Boyes. 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 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



Figure 1. Photograph of the *Euthrix potatoria* (ilEutPota3) specimen used for genome sequencing.

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 ilEutPota3 sample was weighed and triaged to determine the appropriate extraction protocol. Tissue from the thorax was homogenised by powermashing using a Power-Masher II tissue disruptor. HMW DNA was extracted 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 automated 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 7.82 ng/µL and a yield of 3 128.00 ng.

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 on a Revio instrument (Pacific Biosciences). The prepared library was normalised to 2 nM, and $15\,\mu\text{L}$ was used for making complexes. Primers were annealed and polymerases bound to generate circularised complexes, following the manufacturer's instructions. Complexes were purified using 1.2X SMRTbell beads, then diluted to the Revio loading concentration (200–300 pM) and spiked with a Revio sequencing internal control. The sample was sequenced on a Revio 25M SMRT cell. The SMRT Link software (Pacific Biosciences), a web-based workflow manager, was used to configure and monitor the run and to carry out primary and secondary data analysis.

Hi-C

Sample preparation and crosslinking

The Hi-C sample was prepared from 20–50 mg of frozen tissue from the head of the ilEutPota3 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 10-16 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 in Hi-C phasing mode (Cheng et al., 2021; Cheng et al., 2022), producing two haplotypes. Hi-C reads (Rao et al., 2014) were mapped to the primary contigs using bwa-mem2 (Vasimuddin et al., 2019). Contigs were further scaffolded with Hi-C data in YaHS (Zhou et al., 2023), using the --break option for handling potential misassemblies. The scaffolded assemblies were evaluated using Gfastats (Formenti et al., 2022), BUSCO (Manni et al., 2021) and MERQURY.FK (Rhie et al., 2020).

The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2023), which runs MitoFinder (Allio *et al.*, 2020) and uses these annotations to select the final mitochondrial contig and to ensure the general quality of the sequence.

Assembly curation

The assembly was decontaminated using the Assembly Screen for Cobionts and Contaminants (ASCC) pipeline. 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). The curation process is documented at https://gitlab.com/wtsi-grit/rapid-curation. PretextSnapshot 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 both 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 *Euthrix potatoria* specimen generated 23.82 Gb (gigabases) from 2.37 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated

the haploid genome size at 471.62 Mb, with a heterozygosity of 0.56% and repeat content of 25.74% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 49× coverage. Hi-C sequencing produced 108.82 Gb from 720.65 million reads, which were used to scaffold the assembly. Table 1 summarises the specimen and sequencing details.

Assembly statistics

The genome was assembled into two haplotypes using Hi-C phasing. Haplotype 1 was curated to chromosome level,

GenomeScope Profile

len:471,616,374bp uniq:74.3% aa:99.4% ab:0.564% kcov:24.7 err:0.0661% dup:0.37 k:31 p:2

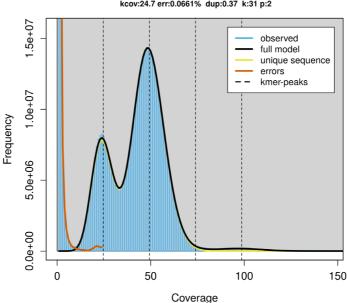


Figure 2. Frequency distribution of *k***-mers generated using GenomeScope2.** The plot shows observed and modelled *k*-mer spectra, providing estimates of genome size, heterozygosity, and repeat content based on unassembled sequencing reads.

Table 1. Specimen and sequencing data for BioProject PRJEB85378.

Platform	PacBio HiFi	Hi-C	
ToLID	ilEutPota3	ilEutPota3	
Specimen ID	Ox001659	Ox001659	
BioSample (source individual)	SAMEA10978928	SAMEA10978928	
BioSample (tissue)	SAMEA10979263	SAMEA10979262	
Tissue	thorax	head	
Instrument	Revio	Illumina NovaSeq X	
Run accessions	ERR14231585	ERR14242293	
Read count total	2.37 million	720.65 million	
Base count total	23.82 Gb	108.82 Gb	

while haplotype 2 was assembled to scaffold level. The final assembly has a total length of 487.86 Mb in 68 scaffolds, with 84 gaps, and a scaffold N50 of 18.02 Mb (Table 2).

Most of the assembly sequence (99.97%) was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 3; Table 3). Z chromosome was identified based on PacBio reads coverage on the single haplotype map.

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.

For haplotype 1, the estimated QV is 64.1, and for haplotype 2, 64.0. When the two haplotypes are combined, the assembly achieves an estimated QV of 64.0. The k-mer completeness

Table 2. Genome assembly statistics.

ilEutPota3.hap1.1	ilEutPota3.hap2.1
GCA_965178115.1	GCA_965178355.1
chromosome	scaffold
487.86	466.06
31	N/A
152	125
8.23 Mb	8.02 Mb
68	42
18.02 Mb	17.75 Mb
21.02	N/A
Z	N/A
Mitochondrion: 15.43 kb	N/A
	GCA_965178115.1 chromosome 487.86 31 152 8.23 Mb 68 18.02 Mb 21.02 Z

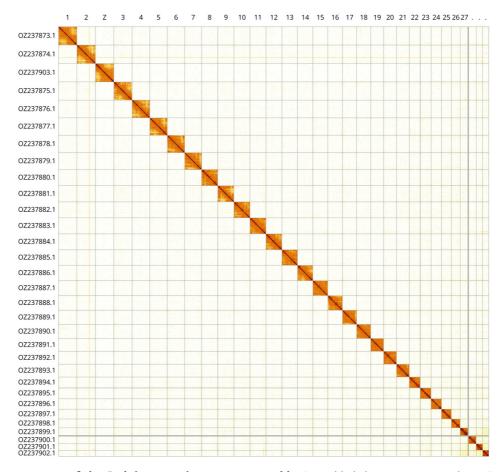


Figure 3. Hi-C contact map of the *Euthrix potatoria* **genome assembly.** Assembled chromosomes are shown in order of size and labelled along the axes. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Euthrix potatoria* ilEutPota3.

INSDC accession	Molecule	Length (Mb)	GC%
OZ237873.1	1	21.02	36.50
OZ237874.1	2	20.95	36.50
OZ237875.1	3	20.76	36.50
OZ237876.1	4	19.99	36.50
OZ237877.1	5	19.82	36
OZ237878.1	6	19.73	36
OZ237879.1	7	19.13	36
OZ237880.1	8	18.37	36
OZ237881.1	9	18.23	36.50
OZ237882.1	10	18.18	36
OZ237883.1	11	18.16	36.50
OZ237884.1	12	18.02	36.50
OZ237885.1	13	17.71	36
OZ237886.1	14	17.38	36.50
OZ237887.1	15	16.95	36.50
OZ237888.1	16	16.61	36.50
OZ237889.1	17	16.04	36.50
OZ237890.1	18	15.96	37
OZ237891.1	19	14.64	36.50
OZ237892.1	20	14.54	37
OZ237893.1	21	14.50	37
OZ237894.1	22	12.44	37
OZ237895.1	23	12.29	37.50
OZ237896.1	24	11.79	37
OZ237897.1	25	11.18	37
OZ237898.1	26	10.03	37
OZ237899.1	27	9.51	39
OZ237900.1	28	8.48	37.50
OZ237901.1	29	7.37	38
OZ237902.1	30	7.06	38.50
OZ237903.1	Z	20.86	36

is 88.38% for haplotype 1, 84.55% for haplotype 2, and 99.75% for the combined haplotypes (Figure 4).

BUSCO analysis using the lepidoptera_odb10 reference set $(n=5\,286)$ identified 98.4% of the expected gene set (single = 98.1%, duplicated = 0.3%) for haplotype 1. The snail plot in Figure 5 summarises the scaffold length distribution and other assembly statistics for haplotype 1. The blob plot in Figure 6 shows the distribution of scaffolds by GC proportion and coverage for haplotype 1.

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 haplotype 1, is **6.C.Q64**, 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 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

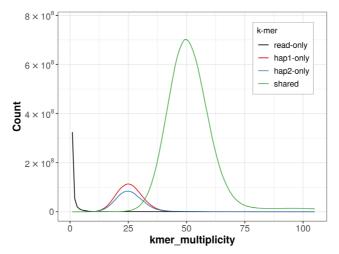


Figure 4. Evaluation of *k***-mer completeness using MerquryFK.** This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers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

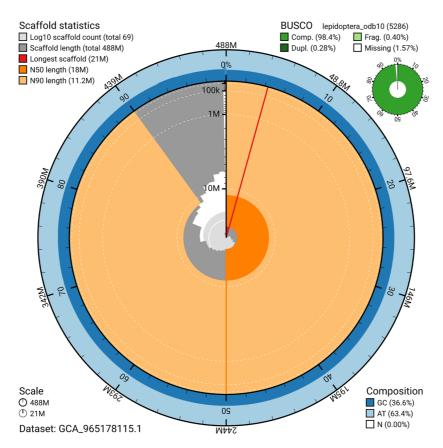


Figure 5. Assembly metrics for ilEutPota3.hap1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1 000 bins around the circumference. The outermost 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 set is presented at the top right. An interactive version of this figure can be accessed on the BlobToolKit viewer.

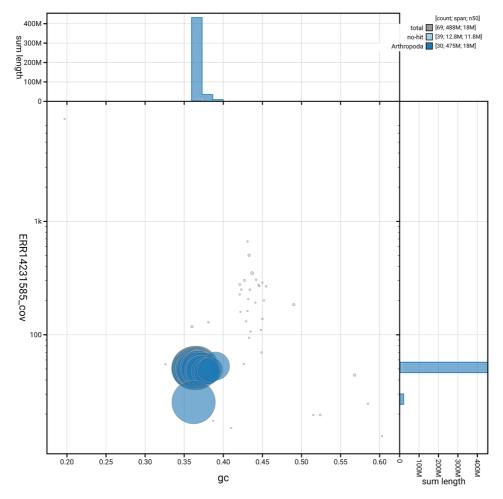


Figure 6. BlobToolKit GC-coverage plot for ilEutPota3.hap1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes 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 Euthrix potatoria assembly.

Measure	Value	Benchmark
EBP summary (haplotype 1)	6.C.Q64	6.C.Q40
Contig N50 length	8.23 Mb	≥ 1 Mb
Scaffold N50 length	18.02 Mb	= chromosome N50
Consensus quality (QV)	Haplotype 1: 64.1; haplotype 2: 64.0; combined: 64.0	≥ 40
k-mer completeness	Haplotype 1: 88.38%; Haplotype 2: 84.55%; combined: 99.75%	≥ 95%
BUSCO	C:98.4% [S:98.1%; D:0.3%]; F:0.4%; M:1.2%; n:5 286	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.97%	≥90%

Data availability

European Nucleotide Archive: Euthrix potatoria (drinker moth). Accession number PRJEB85378. The genome sequence is released openly for reuse. The *Euthrix potatoria* 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
- Members of the Darwin Tree of Life Barcoding collective
- Members of the Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team
- 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/chhylp123/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
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

Software	Version	Source
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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PubMed Abstract | Publisher Full Text

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