



DATA NOTE

# The genome sequence of the Red-barred Tortrix moth, *Ditula angustiorana* (Haworth, 1811)

[version 1; peer review: 2 approved]

Douglas Boyes<sup>1+</sup>, Finley Hutchinson<sup>2</sup>, Liam M. Crowley<sup>id</sup><sup>3</sup>,  
 University of Oxford and Wytham Woods Genome Acquisition Lab,  
 Darwin Tree of Life Barcoding collective,  
 Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory  
 team,  
 Wellcome Sanger Institute Scientific Operations: Sequencing Operations,  
 Wellcome Sanger Institute Tree of Life Core Informatics team,  
 Tree of Life Core Informatics collective, Darwin Tree of Life Consortium

<sup>1</sup>UK Centre for Ecology & Hydrology, Wallingford, England, UK<sup>2</sup>University of Exeter, Falmouth, England, UK<sup>3</sup>University of Oxford, Oxford, England, UK

+ Deceased author

**V1** First published: 22 Apr 2025, 10:202  
<https://doi.org/10.12688/wellcomeopenres.23994.1>

Latest published: 22 Apr 2025, 10:202  
<https://doi.org/10.12688/wellcomeopenres.23994.1>

## Abstract

We present a genome assembly from a female specimen of *Ditula angustiorana* (Red-barred Tortrix; Arthropoda; Insecta; Lepidoptera; Tortricidae). The genome sequence has a total length of 468.36 megabases. Most of the assembly (99.83%) is scaffolded into 31 chromosomal pseudomolecules, including the W and Z sex chromosomes. The mitochondrial genome has also been assembled, with a length of 16.19 kilobases.

## Keywords

*Ditula angustiorana*, Red-barred Tortrix, genome sequence, chromosomal, Lepidoptera



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

## Open Peer Review

Approval Status

	1	2
version 1 22 Apr 2025	 <a href="#">view</a>	 <a href="#">view</a>

1. **Hasin Ullah**, Northwest University, Xi'an, China
2. **Qing-Song Zhou** , Chinese Academy of Sciences, Beijing, China  
**Jing-Yi Zhang**, Institute of Zoology Chinese Academy of Sciences (Ringgold ID: 53024), Chaoyang, China

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

**Corresponding author:** Darwin Tree of Life Consortium ([mark.blaxter@sanger.ac.uk](mailto:mark.blaxter@sanger.ac.uk))

**Author roles:** **Boyes D:** Investigation, Resources; **Hutchinson F:** Investigation, Resources; **Crowley LM:** Investigation, Resources;

**Competing interests:** No competing interests were disclosed.

**Grant information:** This work was supported by Wellcome through core funding to the Wellcome Sanger Institute [206194, <https://doi.org/10.35802/206194>] and the Darwin Tree of Life Discretionary Award [218328, <https://doi.org/10.35802/218328>]. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

**Copyright:** © 2025 Boyes D *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**How to cite this article:** Boyes D, Hutchinson F, Crowley LM *et al.* **The genome sequence of the Red-barred Tortrix moth, *Ditula angustiorana* (Haworth, 1811) [version 1; peer review: 2 approved]** Wellcome Open Research 2025, 10:202 <https://doi.org/10.12688/wellcomeopenres.23994.1>

**First published:** 22 Apr 2025, 10:202 <https://doi.org/10.12688/wellcomeopenres.23994.1>

## 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; Ramapesiini; *Ditula*; *Ditula angustiorana* (Haworth, 1811) (NCBI:txid572840)

## Background

The genome of the Red-barred Tortrix or Leaf roller, *Ditula angustiorana*, 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 *Ditula angustiorana*, based on a specimen from Wytham Woods, Oxfordshire, United Kingdom (Figure 1).

## Genome sequence report

### Sequencing data

The genome of a specimen of *Ditula angustiorana* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 17.10 Gb (gigabases) from 1.22 million reads. GenomeScope analysis of the PacBio HiFi data estimated the haploid genome size at 424.80 Mb, with a heterozygosity of 0.86% and repeat content of 23.59%. These values provide an initial assessment of genome complexity and the challenges anticipated during assembly. Based on this estimated genome size, the sequencing data provided approximately 38.0x coverage of the genome. Chromosome conformation Hi-C sequencing produced 127.20 Gb from 842.36 million reads. Table 1 summarises the specimen and sequencing information.

### Assembly statistics

The primary haplotype was assembled, and contigs corresponding to an alternate haplotype were also deposited in INSDC databases. The assembly was improved by manual curation, which corrected 67 misjoins or missing joins and removed five haplotypic duplications. These interventions reduced the total assembly length by 0.64%, decreased the



**Figure 1.** Photograph of the *Ditula angustiorana* (ilDitAngu1) specimen used for genome sequencing.

scaffold count by 19.67%, and increased the scaffold N50 by 5.06%. The final assembly has a total length of 468.36 Mb in 48 scaffolds, with 83 gaps, and a scaffold N50 of 16.19 Mb (Table 2).

The snail plot in Figure 2 provides a summary of the assembly statistics, indicating the distribution of scaffold lengths and other assembly metrics. Figure 3 shows the distribution of scaffolds by GC proportion and coverage. Figure 4 presents a cumulative assembly plot, with separate curves representing different scaffold subsets assigned to various phyla, illustrating the completeness of the assembly.

Most of the assembly sequence (99.83%) 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 5; Table 3). During curation, chromosomes W and Z were assigned based on the read coverage statistics.

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.

### Assembly quality metrics

The estimated Quality Value (QV) and  $k$ -mer completeness metrics, along with BUSCO completeness scores, were calculated for each haplotype and the combined assembly. The QV reflects the base-level accuracy of the assembly, while  $k$ -mer completeness indicates the proportion of expected  $k$ -mers identified in the assembly. BUSCO scores provide a measure of completeness based on benchmarking universal single-copy orthologues.

The combined primary and alternate assemblies achieve an estimated QV of 64.5. The  $k$ -mer recovery for the primary haplotype is 85.47%, and for the alternate haplotype 73.32%; the combined primary and alternate assemblies have a  $k$ -mer recovery of 98.67%. BUSCO v.5.5.0 analysis using the lepidoptera\_odb10 reference set ( $n = 5,286$ ) identified 98.4% of the expected gene set (single = 98.0%, duplicated = 0.4%).

Table 2 provides assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project (EBP) Report on Assembly Standards September 2024. The assembly achieves the EBP reference standard of **6.C.Q64**.

## Methods

### Sample acquisition and DNA barcoding

An adult female *Ditula angustiorana* (specimen ID Ox000553, ToLID ilDitAngu1) was collected from Wytham Woods, Oxfordshire, United Kingdom (latitude 51.77, longitude -1.32) on 2020-06-25, using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford). This specimen was used for PacBio DNA sequencing.

The specimen used for Hi-C sequencing (specimen ID Ox003037, ToLID ilDitAngu2) was collected from the same location on

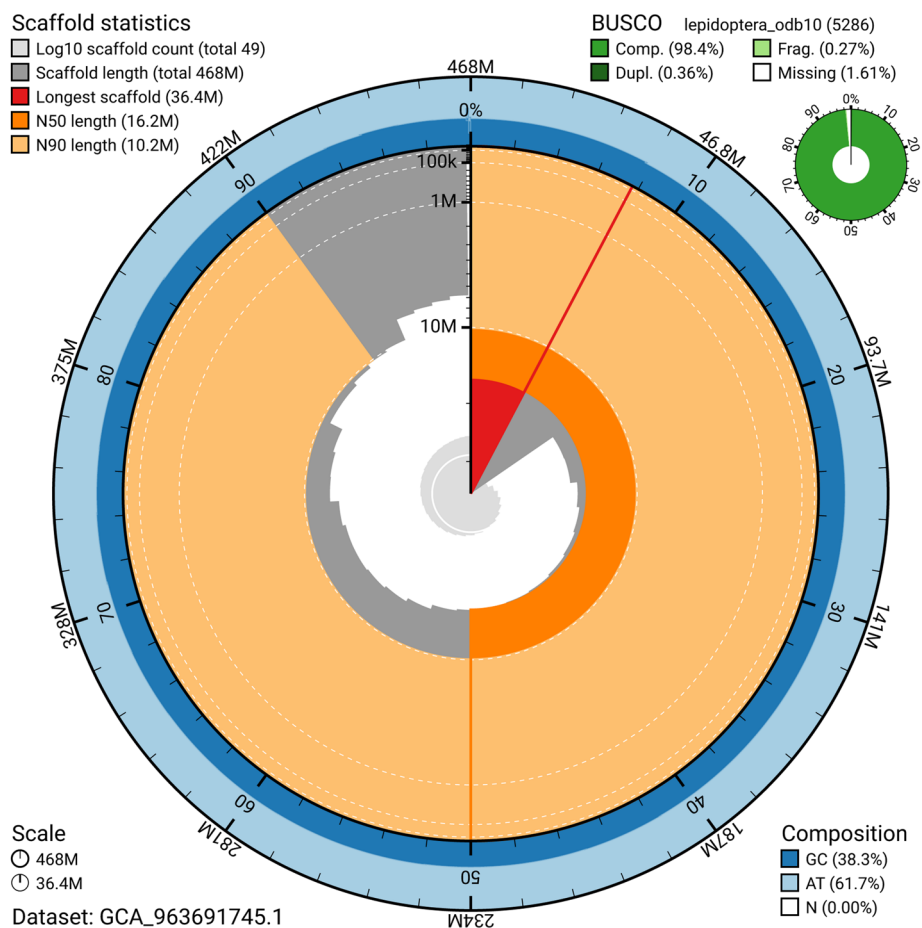
**Table 1. Specimen and sequencing data for *Ditula angustiorana*.**

Project information			
Study title	Ditula angustiorana (red-barred tortrix)		
Umbrella BioProject	PRJEB66768		
Species	<i>Ditula angustiorana</i>		
BioSpecimen	SAMEA7701319		
NCBI taxonomy ID	572840		
Specimen information			
Technology	ToLID	BioSample accession	Organism part
PacBio long read sequencing	ilDitAngu1	SAMEA7701415	whole organism
Hi-C sequencing	ilDitAngu2	SAMEA113426985	whole organism
Sequencing information			
Platform	Run accession	Read count	Base count (Gb)
Hi-C Illumina NovaSeq 6000	ERR12102442	8.42e+08	127.2
PacBio Sequel IIe	ERR12102461	1.22e+06	17.1

**Table 2. Genome assembly data for *Ditula angustiorana*.**

Genome assembly		
Assembly name	ilDitAngu1.1	
Assembly accession	GCA_963691745.1	
Alternate haplotype accession	GCA_963691855.1	
Assembly level for primary assembly	chromosome	
Span (Mb)	468.36	
Number of contigs	131	
Number of scaffolds	48	
Longest scaffold (Mb)	36.38	
Assembly metric	Measure	Benchmark
Contig N50 length	6.61 Mb	$\geq 1$ Mb
Scaffold N50 length	16.19 Mb	= chromosome N50
Consensus quality (QV)	Primary: 64.7; alternate: 64.3; combined: 64.5	$\geq 40$
k-mer completeness	Primary: 85.47%; alternate: 73.32%; combined: 98.67%	$\geq 95\%$
BUSCO*	C:98.4%[S:98.0%,D:0.4%], F:0.3%,M:1.3%,n:5,286	$S > 90\%$ ; $D < 5\%$
Percentage of assembly mapped to chromosomes	99.83%	$\geq 90\%$
Sex chromosomes	W and Z	localised homologous pairs
Organelles	Mitochondrial genome: 16.19 kb	complete single alleles

\* BUSCO scores based on the lepidoptera\_odb10 BUSCO set using version 5.5.0. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison.



**Figure 2. Genome assembly of *Ditula angustiorana*, iDitAngu1.1: metrics.** 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 is available at [https://blobtoolkit.genomehubs.org/view/GCA\\_963691745.1/dataset/GCA\\_963691745.1/snail](https://blobtoolkit.genomehubs.org/view/GCA_963691745.1/dataset/GCA_963691745.1/snail).

2022-07-22, using a light trap. The specimen was collected by Finley Hutchinson and Liam Crowley, and identified by Finley Hutchinson.

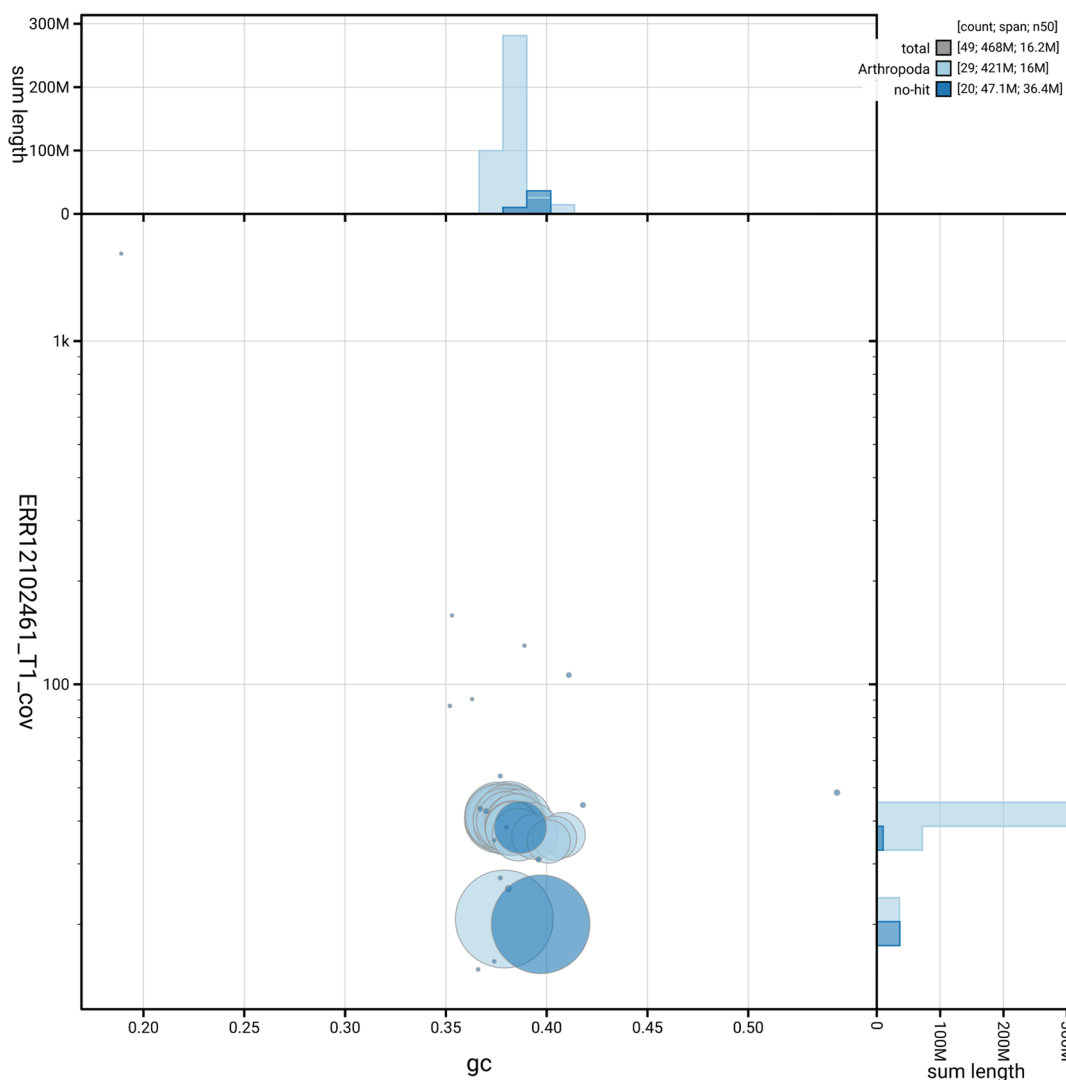
The initial identification was verified by an additional DNA barcoding process according to the framework developed by Twyford *et al.* (2024). A small sample was dissected from each specimen and stored in ethanol, while the remaining parts were shipped on dry ice to the Wellcome Sanger Institute (WSI) (Pereira *et al.*, 2022). 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 have been deposited on protocols.io (Beasley *et al.*, 2023).

Metadata collection for samples adhered to the Darwin Tree of Life project standards described by Lawniczak *et al.* (2022).

#### 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 procedures: sample preparation and homogenisation, DNA extraction, fragmentation and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). The iDitAngu1 sample was prepared for DNA extraction by weighing and dissecting it on dry ice (Jay *et al.*, 2023). Tissue from the whole organism

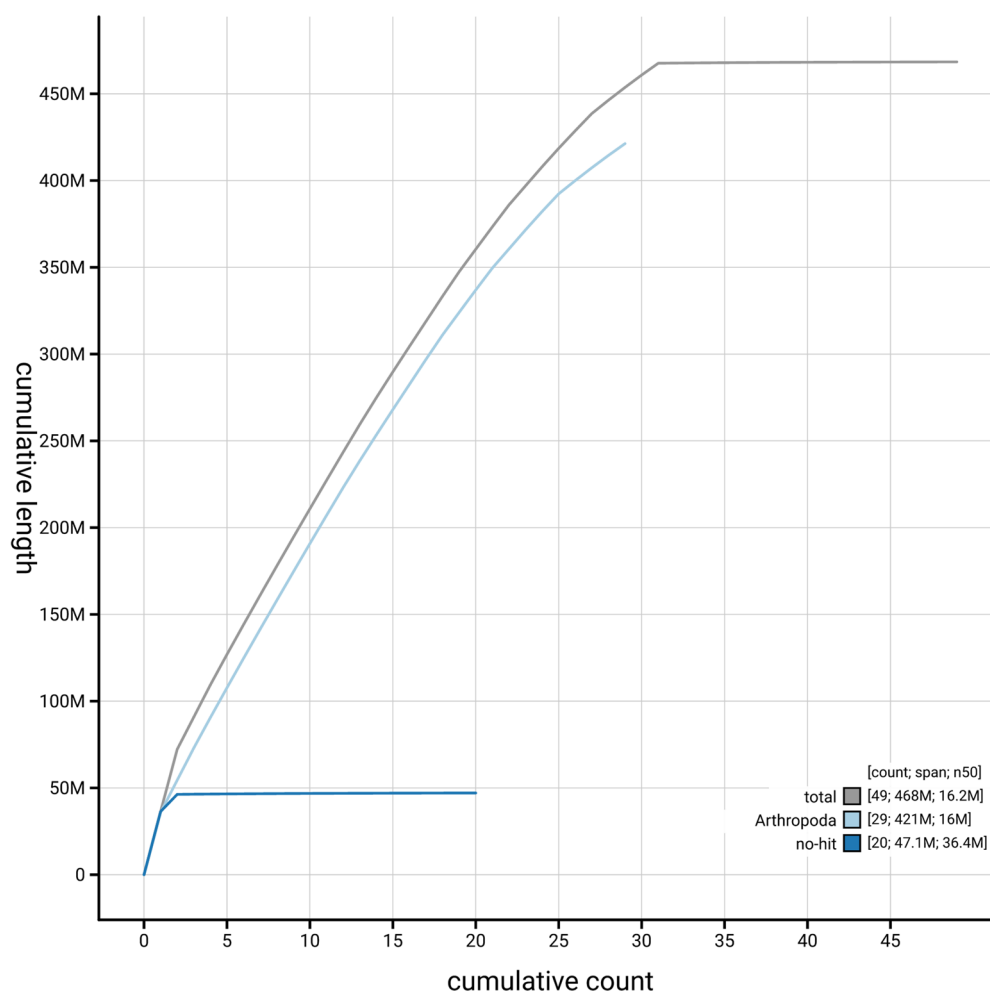


**Figure 3. Genome assembly of *Ditula angustiorana*, iDitAngu1.1: BlobToolKit GC-coverage plot.** Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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 at [https://blobtoolkit.genomehubs.org/view/GCA\\_963691745.1/dataset/GCA\\_963691745.1/blob](https://blobtoolkit.genomehubs.org/view/GCA_963691745.1/dataset/GCA_963691745.1/blob).

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 (Todorovic *et al.*, 2023). Sheared DNA was purified by solid-phase reversible 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 a Qubit Fluorometer using the Qubit dsDNA High Sensitivity Assay kit. The fragment size distribution was evaluated by running the sample on the FemtoPulse system.

### Hi-C sample preparation

Tissue from the whole organism of the iDitAngu2 sample was processed for Hi-C sequencing at the WSI Scientific Operations core, using the Arima-HiC v2 kit. In brief, 20–50 mg of frozen tissue (stored at  $-80^{\circ}\text{C}$ ) was fixed, and the DNA crosslinked using a TC buffer with 22% formaldehyde concentration (final concentration 2%). After crosslinking, the tissue was homogenised using the Diagenode Power Masher-II and BioMasher-II tubes and pestles. Following the Arima-HiC v2 kit manufacturer's instructions, crosslinked DNA was digested using a restriction enzyme master mix. The 5'-overhangs were filled in and labelled with biotinylated nucleotides and



**Figure 4. Genome assembly of *Ditula angustiorana*, iDitAngu1.1: 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/GCA\\_963691745.1/dataset/GCA\\_963691745.1/cumulative](https://blobtoolkit.genomehubs.org/view/GCA_963691745.1/dataset/GCA_963691745.1/cumulative).

proximally ligated. An overnight incubation was carried out for enzymes to digest remaining proteins and for crosslinks to reverse. A clean up was performed with SPRIselect beads prior to library preparation. Additionally, the biotinylation percentage was estimated using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and Qubit HS Assay Kit and Arima-HiC v2 QC beads.

#### Library preparation and sequencing

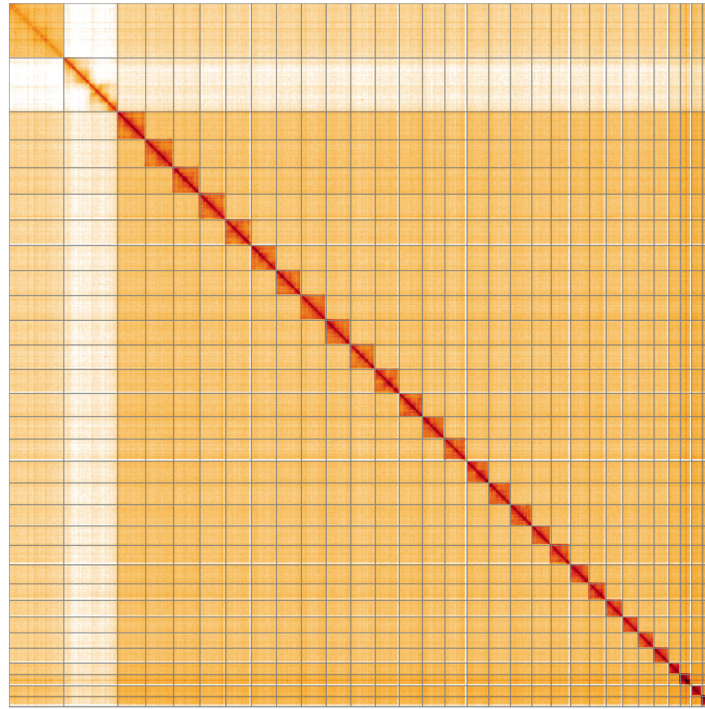
Library preparation and sequencing were performed at the WSI Scientific Operations core.

#### *PacBio HiFi*

At a minimum, samples were required to have an average fragment size exceeding 8 kb and a total mass over 400 ng to proceed to the low input SMRTbell Prep Kit 3.0 protocol (Pacific Biosciences, California, USA), depending on genome

size and sequencing depth required. Libraries were prepared using the SMRTbell Prep Kit 3.0 (Pacific Biosciences, California, USA) as per the manufacturer's instructions. The kit includes the reagents required for end repair/A-tailing, adapter ligation, post-ligation SMRTbell bead cleanup, and nuclease treatment. Following the manufacturer's instructions, size selection and clean up was carried out using diluted AMPure PB beads (Pacific Biosciences, California, USA). DNA concentration was quantified using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) with Qubit 1X dsDNA HS assay kit and the final library fragment size analysis was carried out using the Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies) and gDNA 55kb BAC analysis kit.

Samples were 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



**Figure 5. Genome assembly of *Ditula angustiorana*: Hi-C contact map of the iDitAngu1.1 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/I/?d=TrgGYcppRguJUTHkj7VOmg>.

**Table 3. Chromosomal pseudomolecules in the genome assembly of *Ditula angustiorana*, iDitAngu1.**

INSDC accession	Name	Length (Mb)	GC%
OY829473.1	1	18.61	38
OY829474.1	2	18.55	38
OY829475.1	3	17.54	38
OY829476.1	4	17.17	37.5
OY829477.1	5	16.96	37.5
OY829478.1	6	16.76	37.5
OY829479.1	7	16.58	37.5
OY829480.1	8	16.45	38
OY829481.1	9	16.34	37.5
OY829482.1	10	16.19	37.5
OY829483.1	11	16.01	38
OY829484.1	12	15.41	38
OY829485.1	13	14.94	38.5
OY829486.1	14	14.66	38
OY829487.1	15	14.5	38.5

INSDC accession	Name	Length (Mb)	GC%
OY829488.1	16	14.49	38
OY829489.1	17	14.1	38.5
OY829490.1	18	12.97	38
OY829491.1	19	12.9	38.5
OY829492.1	20	12.62	38.5
OY829493.1	21	11.01	39
OY829494.1	22	11.0	38.5
OY829495.1	23	10.61	38.5
OY829496.1	24	10.17	38.5
OY829497.1	25	9.91	38.5
OY829498.1	26	7.66	39.5
OY829499.1	27	7.39	41
OY829500.1	28	7.11	40.5
OY829501.1	29	6.79	40
OY829471.1	W	36.38	39.5
OY829472.1	Z	35.83	38
OY829502.1	MT	0.02	19

SMRT link software, a PacBio web-based end-to-end workflow manager, was used to set-up and monitor the run, as well as perform primary and secondary analysis of the data upon completion.

### Hi-C

For Hi-C library preparation, DNA was fragmented using the Covaris E220 sonicator (Covaris) and size selected using SPRISelect beads to 400 to 600 bp. The DNA was then enriched using the Arima-HiC v2 kit Enrichment beads. Using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) for end repair, A-tailing, and adapter ligation. This uses a custom protocol which resembles the standard NEBNext Ultra II DNA Library Prep protocol but where library preparation occurs while DNA is bound to the Enrichment beads. For library amplification, 10 to 16 PCR cycles were required, determined by the sample biotinylation percentage. The Hi-C sequencing was performed using paired-end sequencing with a read length of 150 bp on an Illumina NovaSeq 6000 instrument.

## Genome assembly, curation and evaluation

### 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 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 (Rao *et al.*, 2014) were mapped to the primary contigs using `bwa-mem2` (Vasimuddin *et al.*, 2019), and the contigs were scaffolded using 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. Flat files and maps used in curation were generated via the TreeVal pipeline (Pointon *et al.*, 2023). Manual curation was conducted primarily in PretextView (Harry, 2022) and HiGlass (Kerpedjiev *et al.*, 2018), with additional insights provided by JBrowse2 (Diesh *et al.*, 2023). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Any identified contamination, missed joins, and mis-joins were amended, and

duplicate sequences were tagged and removed. Sex chromosomes were identified by read coverage analysis. The curation process is documented at <https://gitlab.com/wtsi-grit/rapid-curation>.

### Assembly quality assessment

The Merqury.FK tool (Rhie *et al.*, 2020), run in a Singularity container (Kurtzer *et al.*, 2017), was used 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.

A Hi-C contact map was produced for the final version of the assembly. The Hi-C reads were aligned using `bwa-mem2` (Vasimuddin *et al.*, 2019) and the alignment files were combined using SAMtools (Danecek *et al.*, 2021). The Hi-C alignments were converted into a contact map using BEDTools (Quinlan & Hall, 2010) and the Cooler tool suite (Abdennur & Mirny, 2020). The contact map was visualised in HiGlass (Kerpedjiev *et al.*, 2018).

The blobtoolkit pipeline is a Nextflow (Di Tommaso *et al.*, 2017) port of the previous Snakemake Blobtoolkit pipeline (Challis *et al.*, 2020). It aligns the PacBio reads in SAMtools and minimap2 (Li, 2018) and generates coverage tracks for regions of fixed size. In parallel, it queries the GoAT database (Challis *et al.*, 2023) to identify all matching BUSCO lineages to run BUSCO (Manni *et al.*, 2021). For the three domain-level BUSCO lineages, the pipeline aligns the BUSCO genes to the UniProt Reference Proteomes database (Bateman *et al.*, 2023) with DIAMOND blastp (Buchfink *et al.*, 2021). The genome is also divided into chunks according to the density of the BUSCO genes from the closest taxonomic lineage, and each chunk is aligned to the UniProt Reference Proteomes database using DIAMOND blastx. Genome sequences without a hit are chunked using `seqtk` and aligned to the NT database with `blastn` (Altschul *et al.*, 1990). The blobtools suite combines all these 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), relying on the Conda package manager, the Bioconda initiative (Grüning *et al.*, 2018), the Biocontainers infrastructure (da Veiga Leprevost *et al.*, 2017), as well as the Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017) containerisation solutions.

Table 4 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

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

Software tool	Version	Source
BEDTools	2.30.0	<a href="https://github.com/ark5x/bedtools2">https://github.com/ark5x/bedtools2</a>
BLAST	2.14.0	<a href="ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/">ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/ </a>
BlobToolKit	4.3.9	<a href="https://github.com/blobtoolkit/blobtoolkit">https://github.com/blobtoolkit/blobtoolkit</a>
BUSCO	5.5.0	<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>
DIAMOND	2.1.8	<a href="https://github.com/bbuchfink/diamond">https://github.com/bbuchfink/diamond</a>
fasta_windows	0.2.4	<a href="https://github.com/tolkit/fasta_windows">https://github.com/tolkit/fasta_windows</a>
FastK	666652151335353eef2fcd58880bcef5bc2928e1	<a href="https://github.com/thegenemyers/FASTK">https://github.com/thegenemyers/FASTK</a>
Gfastats	1.3.6	<a href="https://github.com/vgl-hub/gfastats">https://github.com/vgl-hub/gfastats</a>
GoaT CLI	0.2.5	<a href="https://github.com/genomehubs/goat-cli">https://github.com/genomehubs/goat-cli</a>
Hifiasm	0.19.5-r587	<a href="https://github.com/chhylp123/hifiasm">https://github.com/chhylp123/hifiasm</a>
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84aa44357826c0b6753eb28de	<a href="https://github.com/higlass/higlass">https://github.com/higlass/higlass</a>
MercuryFK	d00d98157618f4e8d1a9190026b19b471055b22e	<a href="https://github.com/thegenemyers/MERQURY.FK">https://github.com/thegenemyers/MERQURY.FK</a>
Minimap2	2.24-r1122	<a href="https://github.com/lh3/minimap2">https://github.com/lh3/minimap2</a>
MitoHiFi	3	<a href="https://github.com/marcelauliano/MitoHiFi">https://github.com/marcelauliano/MitoHiFi</a>
MultiQC	1.14, 1.17, and 1.18	<a href="https://github.com/MultiQC/MultiQC">https://github.com/MultiQC/MultiQC</a>
Nextflow	23.04.1	<a href="https://github.com/nextflow-io/nextflow">https://github.com/nextflow-io/nextflow</a>
PretextView	0.2.5	<a href="https://github.com/sanger-tol/PretextView">https://github.com/sanger-tol/PretextView</a>
purge_dups	1.2.5	<a href="https://github.com/dfguan/purge_dups">https://github.com/dfguan/purge_dups</a>
samtools	1.19.2	<a href="https://github.com/samtools/samtools">https://github.com/samtools/samtools</a>
sanger-tol/ascc	-	<a href="https://github.com/sanger-tol/ascc">https://github.com/sanger-tol/ascc</a>
sanger-tol/blobtoolkit	0.4.0	<a href="https://github.com/sanger-tol/blobtoolkit">https://github.com/sanger-tol/blobtoolkit</a>
Seqtk	1.3	<a href="https://github.com/lh3/seqtk">https://github.com/lh3/seqtk</a>
Singularity	3.9.0	<a href="https://github.com/sylabs/singularity">https://github.com/sylabs/singularity</a>
TreeVal	1.2.0	<a href="https://github.com/sanger-tol/treeval">https://github.com/sanger-tol/treeval</a>
YaHS	1.2a.2	<a href="https://github.com/c-zhou/yahs">https://github.com/c-zhou/yahs</a>

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)

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: *Ditula angustiorana* (red-barred tortrix). Accession number PRJEB66768; <https://identifiers.org/ena.embl/PRJEB66768>. The genome sequence is released openly for reuse. The *Ditula angustiorana* genome sequencing initiative is part of the Darwin Tree of Life (DTOL) project (PRJEB40665) 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](#).

## Author information

Members of the University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: <https://doi.org/10.5281/zenodo.12157525>.

Members of the Darwin Tree of Life Barcoding collective are listed here: <https://doi.org/10.5281/zenodo.12158331>.

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>.

## References

- Abdennur N, Mirny LA: **Cooler: scalable storage for Hi-C data and other genomically labeled arrays.** *Bioinformatics.* 2020; **36**(1): 311–316. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Allio R, Schomaker-Bastos A, Romiguier J, et al.: **MitoFinder: efficient automated large-scale extraction of mitogenomic data in target enrichment phylogenomics.** *Mol Ecol Resour.* 2020; **20**(4): 892–905. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Altschul SF, Gish W, Miller W, et al.: **Basic Local Alignment Search Tool.** *J Mol Biol.* 1990; **215**(3): 403–410. [PubMed Abstract](#) | [Publisher Full Text](#)
- Bateman A, Martin MJ, Orchard S, et al.: **UniProt: the universal protein knowledgebase in 2023.** *Nucleic Acids Res.* 2023; **51**(D1): D523–D531. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Beasley J, Uhl R, Forrest LL, et al.: **DNA barcoding SOPs for the Darwin Tree of Life project.** *protocols.io.* 2023; [Accessed 25 June 2024]. [Publisher Full Text](#)
- Buchfink B, Reuter K, Drost HG: **Sensitive protein alignments at Tree-of-Life scale using DIAMOND.** *Nat Methods.* 2021; **18**(4): 366–368. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Challis R, Kumar S, Sotero-Caio C, et al.: **Genomes on a Tree (GoAT): a versatile, scalable search engine for genomic and sequencing project metadata across the eukaryotic Tree of Life [version 1; peer review: 2 approved].** *Wellcome Open Res.* 2023; **8**: 24. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Challis R, Richards E, Rajan J, et al.: **BlobToolKit – interactive quality assessment of genome assemblies.** *G3 (Bethesda).* 2020; **10**(4): 1361–1374. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Cheng H, Concepcion GT, Feng X, et al.: **Haplotype-resolved *de novo* assembly using phased assembly graphs with hifiasm.** *Nat Methods.* 2021; **18**(2): 170–175. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Crowley L, Allen H, Barnes I, et al.: **A sampling strategy for genome sequencing the British terrestrial arthropod fauna [version 1; peer review: 2 approved].** *Wellcome Open Res.* 2023; **8**: 123. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- da Veiga Leprevost F, Grüning BA, Alves Aflitos S, et al.: **BioContainers: an open-source and community-driven framework for software standardization.** *Bioinformatics.* 2017; **33**(16): 2580–2582. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Danecek P, Bonfield JK, Liddle J, et al.: **Twelve years of SAMtools and BCFtools.** *GigaScience.* 2021; **10**(2): giab008. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Denton A, Oatley G, Cornwell C, et al.: **Sanger Tree of Life sample homogenisation: PowerMash.** *protocols.io.* 2023a. [Publisher Full Text](#)
- Denton A, Yatsenko H, Jay J, et al.: **Sanger Tree of Life wet laboratory protocol collection V.1.** *protocols.io.* 2023b. [Publisher Full Text](#)
- Di Tommaso P, Chatzou M, Floden EW, et al.: **Nextflow enables reproducible computational workflows.** *Nat Biotechnol.* 2017; **35**(4): 316–319. [PubMed Abstract](#) | [Publisher Full Text](#)
- Diesh C, Stevens GJ, Xie P, et al.: **JBrowse 2: a modular genome browser with views of synteny and structural variation.** *Genome Biol.* 2023; **24**(1): 74. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

- Ewels P, Magnusson M, Lundin S, *et al.*: **MultiQC: summarize analysis results for multiple tools and samples in a single report.** *Bioinformatics.* 2016; **32**(19): 3047–3048.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ewels PA, Peltzer A, Fillinger S, *et al.*: **The nf-core framework for community-curated bioinformatics pipelines.** *Nat Biotechnol.* 2020; **38**(3): 276–278.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Formenti G, Abueg L, Brajuka A, *et al.*: **Gfastats: conversion, evaluation and manipulation of genome sequences using assembly graphs.** *Bioinformatics.* 2022; **38**(17): 4214–4216.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Grüning B, Dale R, Sjödin A, *et al.*: **Bioconda: sustainable and comprehensive software distribution for the life sciences.** *Nat Methods.* 2018; **15**(7): 475–476.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Guan D, McCarthy SA, Wood J, *et al.*: **Identifying and removing haplotypic duplication in primary genome assemblies.** *Bioinformatics.* 2020; **36**(9): 2896–2898.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Harry E: **PretextView (Paired Read Texture Viewer): a desktop application for viewing pretext contact maps.** 2022.  
[Reference Source](#)
- Howe K, Chow W, Collins J, *et al.*: **Significantly improving the quality of genome assemblies through curation.** *GigaScience.* 2021; **10**(1): g1aa153.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Jay J, Yatsenko H, Narváez-Gómez JP, *et al.*: **Sanger Tree of Life sample preparation: triage and dissection.** *protocols.io.* 2023.  
[Publisher Full Text](#)
- Kerpedjiev P, Abdennur N, Lekschas F, *et al.*: **HiGlass: web-based visual exploration and analysis of genome interaction maps.** *Genome Biol.* 2018; **19**(1): 125.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kurtzer GM, Sochat V, Bauer MW: **Singularity: scientific containers for mobility of compute.** *PLoS One.* 2017; **12**(5): e0177459.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Lawniczak MKN, Davey RP, Rajan J, *et al.*: **Specimen and sample metadata standards for biodiversity genomics: a proposal from the Darwin Tree of Life project [version 1; peer review: 2 approved with reservations].** *Wellcome Open Res.* 2022; **7**: 187.  
[Publisher Full Text](#)
- Li H: **Minimap2: pairwise alignment for nucleotide sequences.** *Bioinformatics.* 2018; **34**(18): 3094–3100.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Manni M, Berkeley MR, Seppely M, *et al.*: **BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes.** *Mol Biol Evol.* 2021; **38**(10): 4647–4654.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Merkel D: **Docker: lightweight Linux containers for consistent development and deployment.** *Linux J.* 2014; **2014**(239): 2, [Accessed 2 April 2024].  
[Reference Source](#)
- Pereira L, Sivell O, Sivess L, *et al.*: **DTOL taxon-specific standard operating procedure for the terrestrial and freshwater arthropods working group.** 2022.  
[Publisher Full Text](#)
- Pointon DL, Eagles W, Sims Y, *et al.*: **sanger-tol/treeval v1.0.0 - Ancient Atlantis.** 2023.  
[Publisher Full Text](#)
- Quinlan AR, Hall IM: **BEDTools: a flexible suite of utilities for comparing genomic features.** *Bioinformatics.* 2010; **26**(6): 841–842.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ranallo-Benavidez TR, Jaron KS, Schatz MC: **GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes.** *Nat Commun.* 2020; **11**(1): 1432.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Rao SSP, Huntley MH, Durand NC, *et al.*: **A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping.** *Cell.* 2014; **159**(7): 1665–1680.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Rhie A, McCarthy SA, Fedrigo O, *et al.*: **Towards complete and error-free genome assemblies of all vertebrate species.** *Nature.* 2021; **592**(7856): 737–746.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Rhie A, Walenz BP, Koren S, *et al.*: **Merquy: reference-free quality, completeness, and phasing assessment for genome assemblies.** *Genome Biol.* 2020; **21**(1): 245.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sheerin E, Sampaio F, Oatley G, *et al.*: **Sanger Tree of Life HMW DNA extraction: automated MagAttract v.1.** *protocols.io.* 2023.  
[Publisher Full Text](#)
- Strickland M, Cornwell C, Howard C: **Sanger Tree of Life fragmented DNA clean up: manual SPRI.** *protocols.io.* 2023.  
[Publisher Full Text](#)
- Todorovic M, Sampaio F, Howard C, *et al.*: **Sanger Tree of Life HMW DNA fragmentation: diagenode Megaruptor<sup>®</sup>3 for PacBio HiFi.** *protocols.io.* 2023.  
[Publisher Full Text](#)
- Twyford AD, Beasley J, Barnes I, *et al.*: **A DNA barcoding framework for taxonomic verification in the Darwin Tree of Life project [version 1; peer review: 2 approved].** *Wellcome Open Res.* 2024; **9**: 339.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Uliano-Silva M, Ferreira JGRN, Krashennikova K, *et al.*: **MitoHiFi: a python pipeline for mitochondrial genome assembly from PacBio high fidelity reads.** *BMC Bioinformatics.* 2023; **24**(1): 288.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Vasimuddin M, Misra S, Li H, *et al.*: **Efficient architecture-aware acceleration of BWA-MEM for multicore systems.** In: *2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS).* IEEE, 2019; 314–324.  
[Publisher Full Text](#)
- Zhou C, McCarthy SA, Durbin R: **YaHS: yet another Hi-C scaffolding tool.** *Bioinformatics.* 2023; **39**(1): btac808.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

# Open Peer Review

Current Peer Review Status:  

---

## Version 1

Reviewer Report 05 January 2026

<https://doi.org/10.21956/wellcomeopenres.26471.r139440>

© 2026 Zhou Q et al. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



**Qing-Song Zhou** 

Chinese Academy of Sciences, Beijing, China

**Jing-Yi Zhang**

Institute of Zoology Chinese Academy of Sciences (Ringgold ID: 53024), Chaoyang, Beijing, China

This article presents a high-quality chromosome-level genome assembly of *Ditula angustiorana* using PacBio HiFi and Hi-C data. with high contiguity (as reflected by a high N50) and completeness (as indicated by high BUSCO scores). The assembly demonstrates excellent contiguity and completeness, representing a valuable resource for lepidopteran genomics. The technical approach is robust and well-executed.

However, it is better to provide additional background information on *D. angustiorana*, including its ecological and economic relevance, biodiversity context, and taxonomic history. Such context would help readers to understanding the significance of this genomic resource.

Additionally, while the current assembly is already of high quality, the inclusion of Illumina short-read data for polishing and RNA-seq data for genome annotation would further enhance the accuracy and utility of this reference genome. The authors are encouraged to consider incorporating these data in future updates or supplementary analyses.

**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:** biodiversity, genomics, entomology

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

Reviewer Report 01 December 2025

<https://doi.org/10.21956/wellcomeopenres.26471.r139445>

© 2025 Ullah H. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



**Hasin Ullah**

Northwest University, Xi'an, China

The article presents the genome assembly of *Ditula angustiorana*, a species in the family Tortricidae, sequenced as part of the Darwin Tree of Life Project. The genome sequencing provides valuable insight into the genetic makeup of this insect species, with a genome size of 468.36 megabases and chromosomally complete scaffolds for 29 autosomes and sex chromosomes. The assembly also includes the mitochondrial genome, showcasing a thorough effort to document the species' genomic landscape.

The methodology section is well-detailed, explaining the use of PacBio HiFi long reads for sequencing and Hi-C sequencing for scaffolding the chromosomal structure. The Hi-Fi reads and Hi-C data were successfully combined to generate an accurate assembly, with quality metrics showing a QV of 64.5 and a BUSCO gene completeness of 98.4%. These quality benchmarks demonstrate a high-quality assembly.

Additionally, the curation process, which involved visual inspection and removal of contaminants and misjoins, ensured the assembly's accuracy. Specific issues like haplotypic duplications were addressed, and corrections improved the scaffold N50, a critical metric for assembly quality. This shows that the authors are meticulous in their approach and committed to ensuring a high-quality product.

**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:** Now I am working on insect morphology, but my previous work was on 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.**

---