



DATA NOTE

The genome sequence of the Pine-tree Lappet moth, *Dendrolimus pini* (Linnaeus, 1767) [version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual male *Dendrolimus pini* (the Pine-tree Lappet moth; Arthropoda; Insecta; Lepidoptera; Lasiocampidae). The genome sequence spans 611.10 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 15.41 kilobases in length. Gene annotation of this assembly on Ensembl identified 11,847 protein-coding genes.

Keywords

Dendrolimus pini, Pine-tree Lappet 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; Bombycoidea; Lasiocampidae; Lasiocampinae; *Dendrolimus*; *Dendrolimus pini* (Linnaeus, 1767) (NCBI:txid151304).

Background

Dendrolimus pini, commonly known as the Pine-tree Lappet moth (Figure 1), is a distinctive but quite variable moth in the Lasiocampidae family (Eggar moths). It is a large moth, with females having a wingspan of up to 90 mm and males up to 70 mm. The species undergoes a lifecycle that typically spans two years under natural conditions, although it can vary depending on environmental factors. In mainland Europe it overwinters as a part-grown larvae among fallen tree needles or moss on the ground, reascending the trees in spring. Adult moths emerge in midsummer, and while male moths can fly several kilometres, females are less mobile due to their egg load (Forest Research, 2024).

Found throughout Europe and Western Asia the larvae feed on leaves of coniferous trees, in particular Scots pine (*Pinus sylvestris*), often leading to extensive defoliation. In severe infestations, the caterpillars can strip trees of their needles, weakening them and making them more susceptible to other pests and diseases, or even causing tree death and is regarded as a significant pest species (GBIF Secretariat, 2024).

In the UK, *D. pini* is currently only known to be established in a small area around Beaulieu, Scotland. Ongoing containment efforts, including restrictions on timber movement, have been implemented to prevent the spread of this potentially devastating pest (Forest Research, 2024). Outbreaks in Europe



Figure 1. Photograph of *Dendrolimus pini* by Hannes Lemme (not the specimen used for genome sequencing).

appear to be occurring more frequently with an expansion in range, and this is assumed to be caused by climate warming (Skrzecz *et al.*, 2020).

A research project undertaken by Forest Research aims to establish whether the Pine-lappet it is a recent introduction to the UK, or a previously undiscovered native species (Moore, 2024). Analysis of mitochondrial DNA sequence data has delineated three genetic groups of *D. pini*: a central and northern group including species from Norway, through central Europe and down to Hungary; a group from Spain and western France, and a third group from Italy, Turkey and Mongolia as well as moths from the Scottish population.

The genome of the pine lappet, *Dendrolimus pini*, 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 *Dendrolimus pini*, based on a male specimen from Glen Strathfarrar, Scotland, UK. This genome sequence will be useful in pest-control research.

Genome sequence report

The genome of an adult male *Dendrolimus pini* was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating a total of 26.00 Gb (gigabases) from 2.56 million reads, providing approximately 40-fold coverage. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data, which produced 83.82 Gbp from 555.11 million reads, yielding an approximate coverage of 137-fold. Specimen and sequencing information is summarised in Table 1.

Manual assembly curation corrected 9 missing joins or mis-joins and one haplotypic duplications, reducing the scaffold number by 6.25%. The final assembly has a total length of 611.10 Mb in 44 sequence scaffolds with a scaffold N50 of 22.3 Mb (Table 2). 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. Most (99.85%) 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). 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 62.3 with *k*-mer completeness of 100.0%, and the assembly has a BUSCO v5.3.2 completeness of 98.5% (single = 98.1%, duplicated = 0.5%), using the lepidoptera_odb10 reference set (*n* = 5,286).

Table 1. Specimen and sequencing data for *Dendrolimus pini*.

Project information			
Study title	Dendrolimus pini (pine lappet)		
Umbrella BioProject	PRJEB60712		
Species	<i>Dendrolimus pini</i>		
BioSample	SAMEA112198464		
NCBI taxonomy ID	151304		
Specimen information			
Technology	ToLID	BioSample accession	Organism part
PacBio long read sequencing	ilDenPini1	SAMEA112198489	thorax
Hi-C sequencing	ilDenPini1	SAMEA112198487	head
RNA sequencing	ilDenPini1	SAMEA112198491	abdomen
Sequencing information			
Platform	Run accession	Read count	Base count (Gb)
Hi-C Illumina NovaSeq 6000	ERR11040191	5.55e+08	83.82
PacBio Sequel IIe	ERR11029700	2.56e+06	26.0
RNA Illumina NovaSeq X	ERR12765139	6.61e+07	9.98

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/151304>.

Genome annotation report

The *Dendrolimus pini* genome assembly (GCA_949752895.1) was annotated at the European Bioinformatics Institute (EBI) on Ensembl Rapid Release. The resulting annotation includes 21,804 transcribed mRNAs from 11,847 protein-coding and 1,890 non-coding genes (Table 2; https://rapid.ensembl.org/Dendrolimus_pini_GCA_949752895.1/Info/Index). The average transcript length is 16,359.77. There are 1.59 coding transcripts per gene and 6.94 exons per transcript.

Methods

Sample acquisition and nucleic acid extraction

An adult male *Dendrolimus pini* (specimen ID SAN00002584, ToLID ilDenPini1) was collected from Glen Strathfarrar, Scotland, UK (latitude 57.41, longitude -4.73) on 2022-06-27, using a moth trap and lure. The specimen was collected by Marc Botham (UK Centre for Ecology & Hydrology) and Katrina Dainton (Forest Research), identified by Marc Botham and preserved by flash freezing.

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.*, 2023a). The sample was prepared for DNA extraction at the WSI Tree of Life Core Laboratory. The ilDenPini1 sample was weighed and dissected on dry ice (Jay *et al.*, 2023) and tissue from the thorax was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023b).

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 immobilisation, using AMPure PB beads to eliminate shorter fragments and concentrate the DNA (Strickland *et al.*, 2023): in brief, the method employs a 1.8X ratio of. 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 ilDenPini1 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ mirVana 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

Table 2. Genome assembly data for *Dendrolimus pini*, ilDenPini1.1.

Genome assembly		
Assembly name	ilDenPini1.1	
Assembly accession	GCA_949752895.1	
Accession of alternate haplotype	GCA_949752875.1	
Span (Mb)	611.10	
Number of contigs	151	
Contig N50 length (Mb)	6.9	
Number of scaffolds	44	
Scaffold N50 length (Mb)	22.3	
Longest scaffold (Mb)	28.59	
Assembly metrics*		Benchmark
Consensus quality (QV)	62.3	≥ 50
<i>k</i> -mer completeness	100.0%	≥ 95%
BUSCO**	C:98.5%[S:98.1%,D:0.5%], F:0.3%,M:1.1%,n:5,286	C ≥ 95%
Percentage of assembly mapped to chromosomes	99.85%	≥ 95%
Sex chromosomes	Z	localised homologous pairs
Organelles	Mitochondrial genome: 15.41 kb	complete single alleles
Genome annotation of assembly GCA_949752895.1 at Ensembl		
Number of protein-coding genes	11,847	
Number of non-coding genes	1,890	
Number of gene transcripts	21,804	

* 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/ilDenPini1_1/dataset/ilDenPini1_1/busco.

integrity of the RNA was done using the Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

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 X (RNA-Seq) instruments.

Hi-C data were generated from the head tissue of ilDenPini1 using the Arima-HiC v2 kit. In brief, frozen tissue (−80°C) was fixed, and the DNA crosslinked using a TC buffer containing formaldehyde. The crosslinked DNA was then digested using a restriction enzyme master mix. The 5’-overhangs were then filled in and labelled with a biotinylated nucleotide and proximally ligated. The biotinylated DNA construct was fragmented to a fragment size of 400 to 600 bp using a Covaris E220 sonicator. The DNA was then enriched, barcoded, and amplified using the NEBNext Ultra II DNA Library Prep Kit, following manufacturers’ instructions. The Hi-C

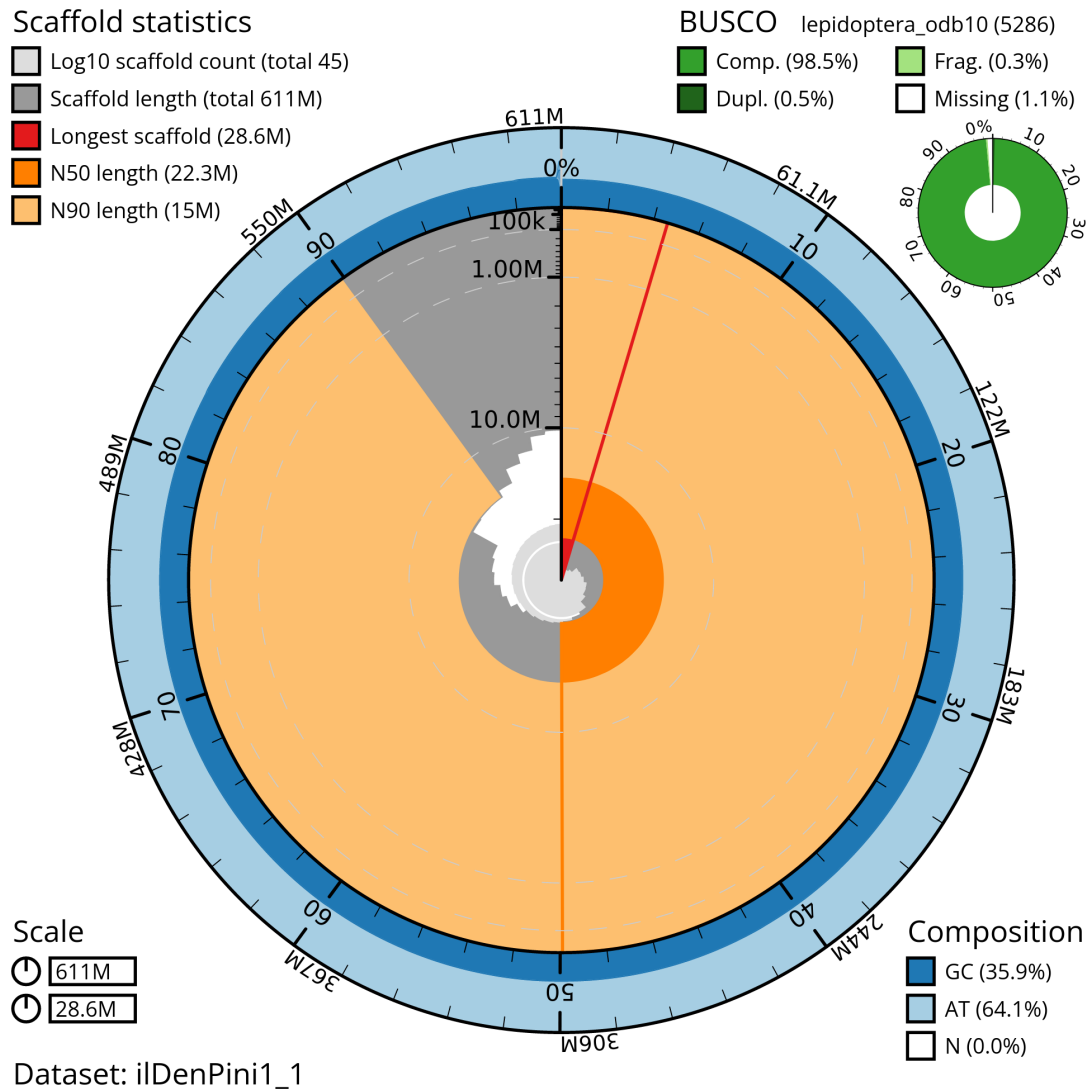


Figure 2. Genome assembly of *Dendrolimus pini*, iIDenPini1.1: metrics. The BlobToolKit snail plot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 611,147,457 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (28,593,271 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (22,307,560 and 15,032,431 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/iIDenPini1_1/dataset/iIDenPini1_1/snail.

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

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.

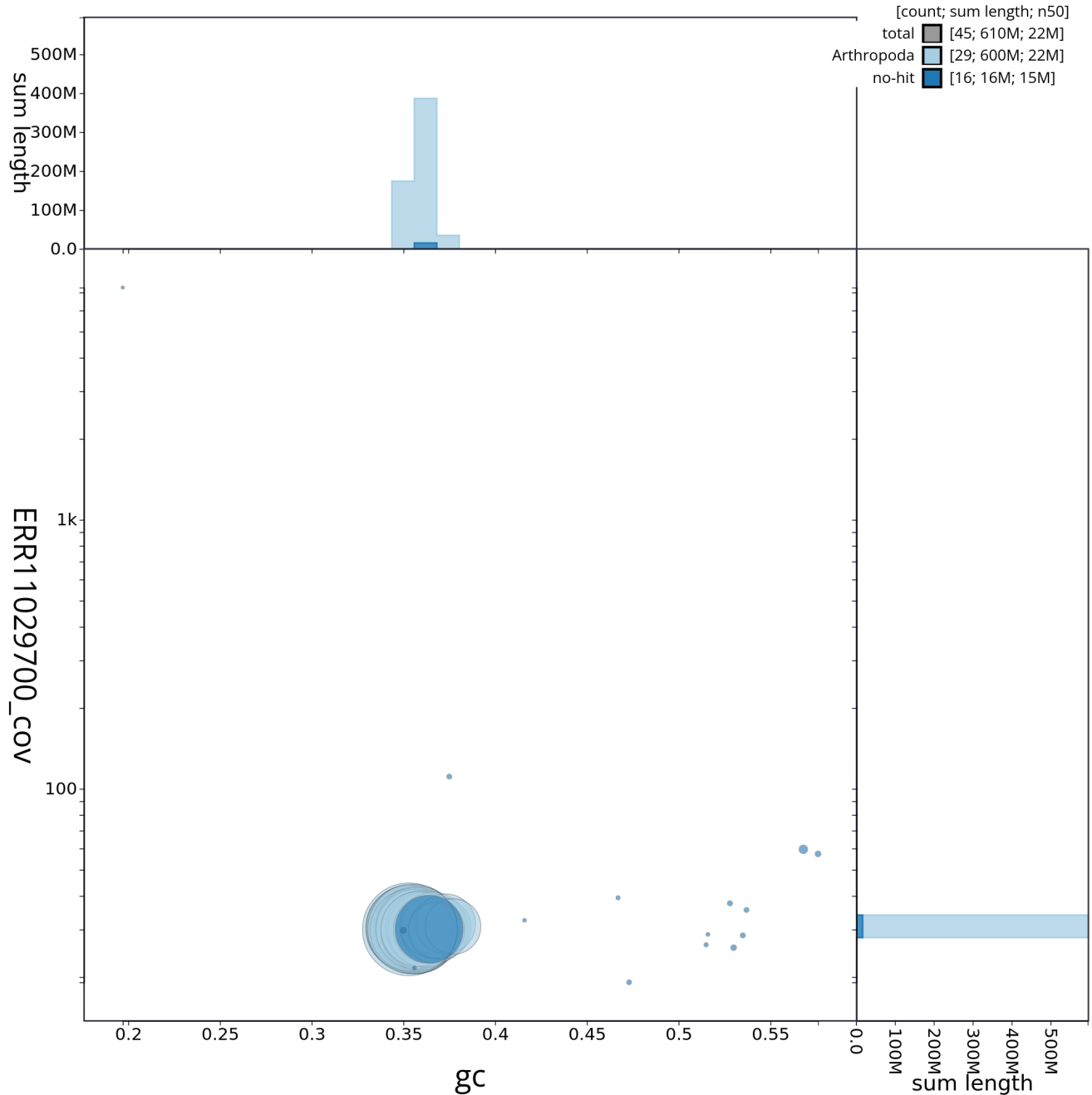


Figure 3. Genome assembly of *Dendrolimus pini*, iIDenPini1.1: BlobToolKit GC-coverage plot. Sequences are coloured by phylum. Circles are sized in proportion to sequence length. Histograms show the distribution of sequence length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/iIDenPini1_1/dataset/iIDenPini1_1/blob.

Assembly curation

The assembly was decontaminated using the Assembly Screen for Cobionts and Contaminants (ASCC) pipeline (article in preparation). Flat files and maps used in curation were generated in TreeVal (Pointon *et al.*, 2023). 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. The sex chromosome was identified based on read coverage statistics. The entire process is documented at <https://gitlab.com/wtsi-grit/rapid-curation> (article in preparation).

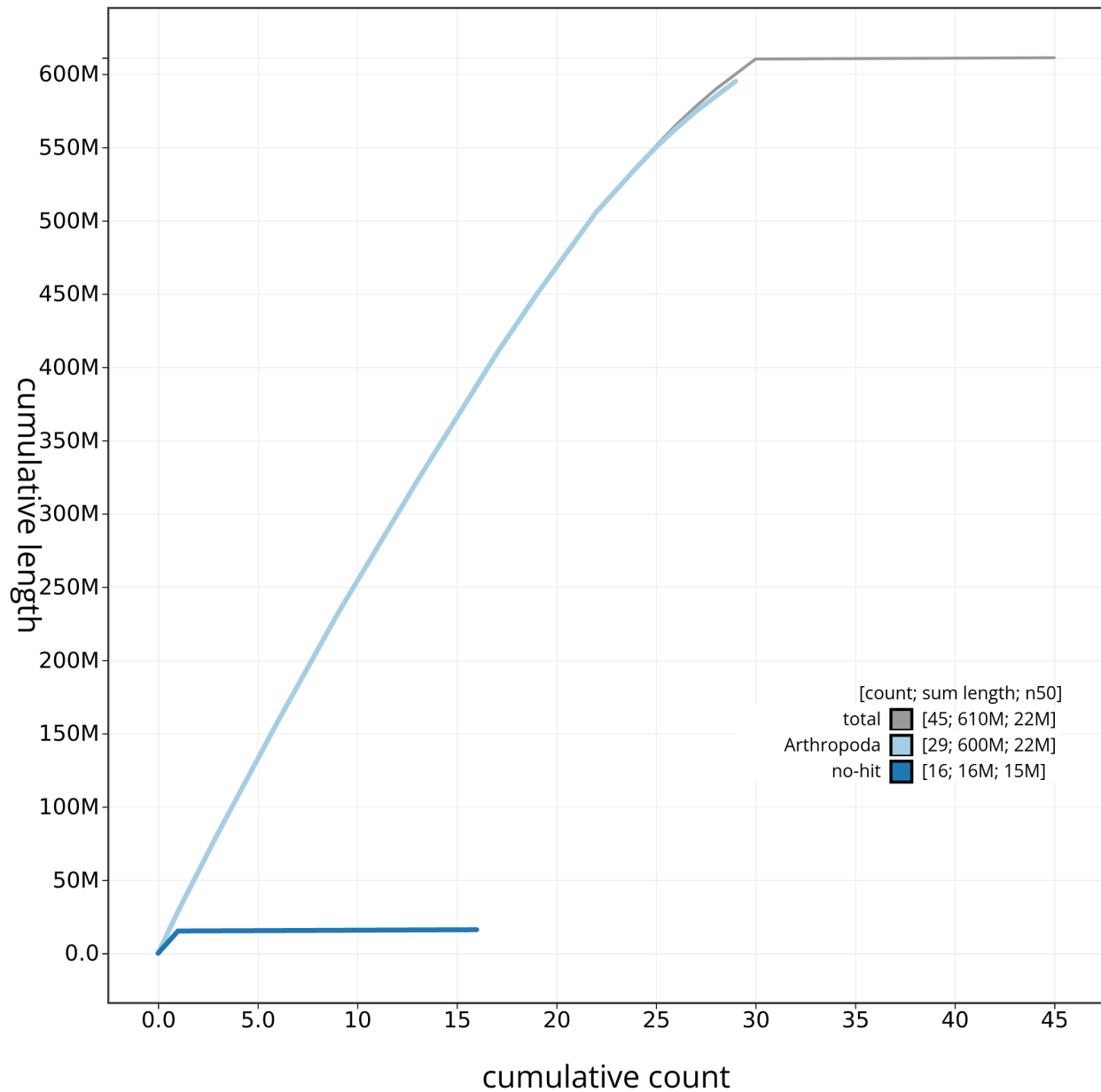


Figure 4. Genome assembly of *Dendrolimus pini* iDenPini1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all sequences. Coloured lines show cumulative lengths of sequences assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/iDenPini1_1/dataset/iDenPini1_1/cumulative.

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.

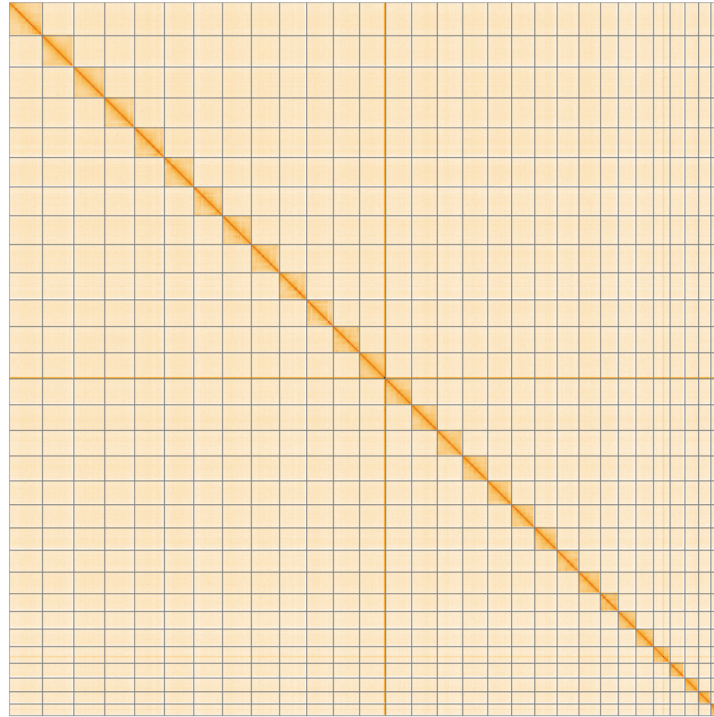


Figure 5. Genome assembly of *Dendrolimus pini* iDenPini1.1: Hi-C contact map of the iDenPini1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at <https://genome-note-higlass.tol.sanger.ac.uk/?d=HhN6EPTUSjih4IF2duiGsw>.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Dendrolimus pini*, iDenPini1.

INSDC accession	Name	Length (Mb)	GC%
OX457124.1	1	28.59	35.5
OX457126.1	2	26.43	35.5
OX457127.1	3	25.55	35.5
OX457128.1	4	25.42	35.5
OX457129.1	5	25.1	35.5
OX457130.1	6	24.67	35.5
OX457131.1	7	24.6	35.5
OX457132.1	8	24.17	35.5
OX457133.1	9	23.12	35.5
OX457134.1	10	22.82	35.5
OX457135.1	11	22.49	35.5
OX457136.1	12	22.31	35.5
OX457137.1	13	22.17	35.5
OX457138.1	14	21.99	35.5

INSDC accession	Name	Length (Mb)	GC%
OX457139.1	15	21.82	35.5
OX457140.1	16	21.28	35.5
OX457141.1	17	20.38	36.0
OX457142.1	18	19.84	36.0
OX457143.1	19	19.08	36.0
OX457144.1	20	18.75	36.0
OX457145.1	21	18.43	36.0
OX457146.1	22	15.17	36.5
OX457147.1	23	15.06	36.5
OX457148.1	24	15.03	36.5
OX457149.1	25	14.23	36.5
OX457150.1	26	12.66	37.0
OX457151.1	27	11.73	37.5
OX457152.1	28	10.46	37.0
OX457153.1	29	10.16	37.5
OX457125.1	Z	26.74	35.5
OX457154.1	MT	0.02	20.0

Table 4. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.2.1	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	https://gitlab.com/ezlab/busco
bwa-mem2	2.2.1	https://github.com/bwa-mem2/bwa-mem2
Cooler	0.8.11	https://github.com/open2c/cooler
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
Hifiasm	0.16.1-r375	https://github.com/chhylp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Mercury.FK	d00d98157618f4e8d1a9190026b19b471055b22e	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	2	https://github.com/marcelauliano/MitoHiFi
PretextView	0.2	https://github.com/wtsi-hpag/PretextView
purge_dups	1.2.3	https://github.com/dfguan/purge_dups
sanger-tol/genomenote	v1.0	https://github.com/sanger-tol/genomenote
sanger-tol/readmapping	1.1.0	https://github.com/sanger-tol/readmapping/tree/1.1.0
Singularity	3.9.0	https://github.com/sylabs/singularity
YaHS	yahs-1.1.91eabc2	https://github.com/c-zhou/yahs

Genome annotation

The [Ensembl Genebuild](#) annotation system (Aken *et al.*, 2016) was used to generate annotation for the *Dendrolimus pini* assembly (GCA_949752895.1) in Ensembl Rapid Release at the EBI. Annotation was created primarily through alignment of transcriptomic data to the genome, with gap filling via protein-to-genome alignments of a select set of proteins from UniProt (UniProt Consortium, 2019).

Wellcome Sanger Institute – Legal and Governance

The materials that have contributed to this genome note have been supplied by a Darwin Tree of Life Partner. The submission of materials by a Darwin Tree of Life Partner is subject to the **‘Darwin Tree of Life Project Sampling Code of Practice’**, which can be found in full on the Darwin Tree of Life website [here](#). By agreeing with and signing up to the Sampling Code of Practice, the Darwin Tree of Life Partner agrees they will meet the legal and ethical requirements and standards set out within this document in respect of all samples acquired for, and supplied to, the Darwin Tree of Life Project.

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: *Dendrolimus pini* (pine lappet). Accession number PRJEB60712; <https://identifiers.org/ena.embl/PRJEB60712> (Wellcome Sanger Institute, 2023).

The genome sequence is released openly for reuse. The *Dendrolimus pini* 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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