DATA NOTE

The genome sequence of the White-faced Tortrix, *Pandemis cinnamomeana* (Treitschke, 1830) [version 1; peer review: awaiting peer review]

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v1 **First published:** 14 Jun 2023, **8**:244
https://doi.org/10.12688/wellcomeopenres.19544.1
Latest published: 14 Jun 2023, **8**:244
https://doi.org/10.12688/wellcomeopenres.19544.1

Abstract

We present a genome assembly from an individual male *Pandemis cinnamomeana* (the White-faced Tortrix; Arthropoda; Insecta; Lepidoptera; Tortricidae). The genome sequence is 426.1 megabases in span. Most of the assembly is scaffolded into 30 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 15.82 kilobases in length. Gene annotation of this assembly on Ensembl identified 19,832 protein coding genes.

Keywords

Pandemis cinnamomeana, White-faced Tortrix, 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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Author roles: Boyes D: Investigation, Resources; **Hammond J**: Writing – Original Draft Preparation, Writing – Review & Editing;

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.

How to cite this article: Boyes D, Hammond J, University of Oxford and Wytham Woods Genome Acquisition Lab *et al.* **The genome sequence of the White-faced Tortrix, *Pandemis cinnamomeana* (Treitschke, 1830) [version 1; peer review: awaiting peer review]** Wellcome Open Research 2023, **8**:244 https://doi.org/10.12688/wellcomeopenres.19544.1

First published: 14 Jun 2023, **8**:244 https://doi.org/10.12688/wellcomeopenres.19544.1

Species taxonomy

Eukaryota; 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; Archipini; *Pandemis*; *Pandemis cinnamomeana* (Treitschke, 1830) (NCBI:txid753214).

Background

The White-faced Tortrix, *Pandemis cinnamomeana* (Treitschke, 1830) is a moth in the Tortricidae family. The species is found widely across northern Eurasia to the Korean peninsula and Japan (Bradley *et al.*, 1973; GBIF Secretariat, 2022). In Britain, the species is local and occurs north to Strathspey, however, there are no records of this species in Ireland (Bradley *et al.*, 1973; Elliott *et al.*, 2018).

The species overwinters as an egg, and the larva feeds from May to June between spun leaves. The species is remarkably polyphagous, and is one of very few Lepidoptera known to feed on the highly toxic yew (*Taxus*). Adults occur between June and July, hiding by day amongst foliage and coming to light in the evening (Bradley *et al.*, 1973; Elliott *et al.*, 2018).

A genome of *Pandemis cinnamomeana* will contribute to the understanding of lepidopteran polyphagy and its evolution. Here we present a chromosomally complete genome sequence for *Pandemis cinnamomeana*, based on one male specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome was sequenced from one male *Pandemis cinnamomeana* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, –1.34). A total of 34-fold coverage in Pacific Biosciences single-molecule HiFi long reads was generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 14 missing joins or mis-joins and removed 7 haplotypic duplications, reducing the assembly length by 0.52% and the scaffold number by 17.65%, and increasing the scaffold N50 by 3.07%.

Figure 1. Photograph of the *Pandemis cinnamomeana* (ilPanCinn1) specimen used for genome sequencing.

The final assembly has a total length of 426.1 Mb in 42 sequence scaffolds with a scaffold N50 of 15.2 Mb (Table 1). Most (99.98%) 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 2–Figure 5; Table 2). 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 64.4 with *k*-mer completeness of 100%, and the assembly has a BUSCO v5.3.2 completeness of 98.5% (single = 98.1%, duplicated = 0.3%), using the lepidoptera_odb10 reference set (n = 5,286).

Metadata for specimens, spectral estimates, sequencing runs, contaminants and pre-curation assembly statistics can be found at https://links.tol.sanger.ac.uk/species/753214.

Genome annotation report

The *Pandemis cinnamomeana* genome assembly (GCA_932294345.1) was annotated using the Ensembl rapid annotation pipeline (Table 1; https://rapid.ensembl.org/Pandemis_cinnamomeana_GCA_932294345.1/Info/Index). The resulting annotation includes 20,007 transcribed mRNAs from 19,832 protein-coding genes.

Methods

Sample acquisition and nucleic acid extraction

The specimen selected for genome sequencing was a male *Pandemis cinnamomeana* (specimen ID Ox000946, individual ilPanCinn1), collected in Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2020-09-08. The specimen was collected by Douglas Boyes (University of Oxford) using a light trap. The specimen was identified by the collector and snap frozen on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute (WSI). The ilPanCinn1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing. Tissue from the whole organism was disrupted using a Nippi Powermasher fitted with a BioMasher pestle. High molecular weight (HMW) DNA was extracted using the Qiagen MagAttract HMW DNA extraction kit. HMW DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system with speed setting 30. Sheared DNA was purified by solid-phase reversible immobilisation using AMPure PB beads with a 1.8X ratio of beads to sample to remove the shorter fragments and concentrate the DNA sample. The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and Qubit

Table 1. Genome data for *Pandemis cinnamomeana*, ilPanCinn1.1.

Project accession data		
Assembly identifier	ilPanCinn1.1	
Species	*Pandemis cinnamomeana*	
Specimen	ilPanCinn1	
NCBI taxonomy ID	753214	
BioProject	PRJEB50746	
BioSample ID	SAMEA8603177	
Isolate information	ilPanCinn1, male: whole organism (DNA sequencing and Hi-C scaffolding)	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	64.4	*≥ 50*
k-mer completeness	100%	*≥ 95%*
BUSCO**	C:98.5%[S:98.1%,D:0.3%], F:0.4%,M:1.2%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.98%	*≥ 95%*
Sex chromosomes	Z chromosome	*localised homologous pairs*
Organelles	Mitochondrial genome assembled	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR8575385	
Hi-C Illumina	ERR8571672	
Genome assembly		
Assembly accession	GCA_932294345.1	
Accession of alternate haplotype	GCA_932294335.1	
Span (Mb)	426.1	
Number of contigs	76	
Contig N50 length (Mb)	11.7	
Number of scaffolds	42	
Scaffold N50 length (Mb)	15.2	
Longest scaffold (Mb)	35.2	
Genome annotation		
Number of protein-coding genes	19,832	
Number of gene transcripts	20,007	

* 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 v5.3.2. 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/ilPanCinn1.1/dataset/CAKOAK01/busco.

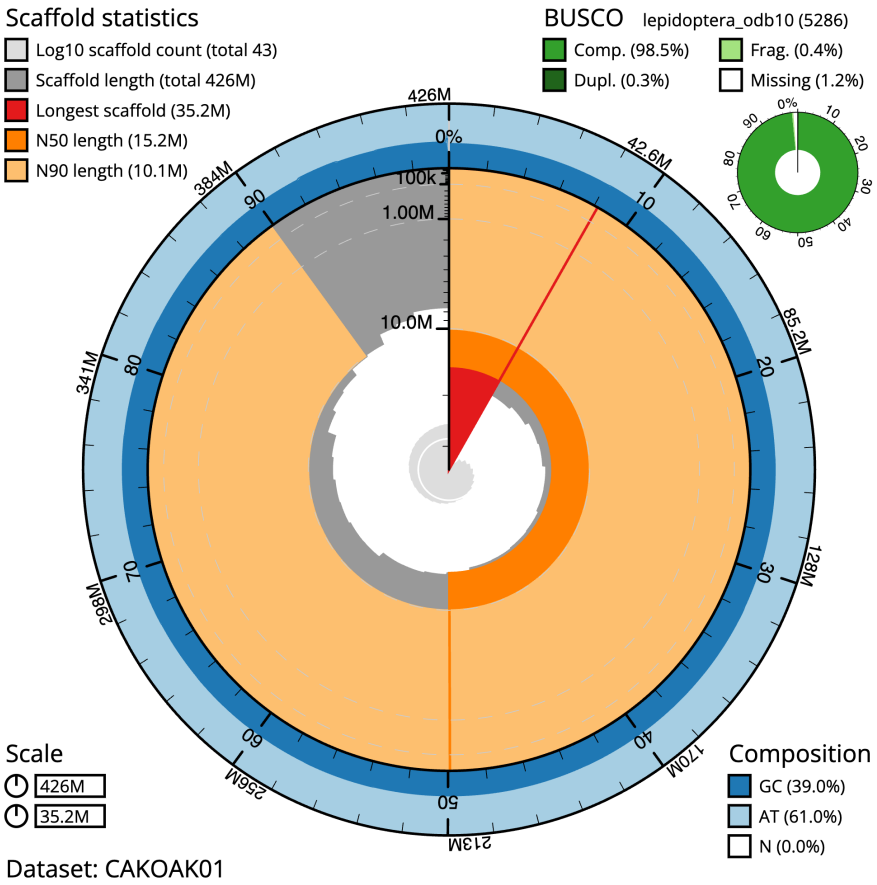

Figure 2. Genome assembly of *Pandemis cinnamomeana*, ilPanCinn1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 426,135,498 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (35,212,762 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (15,160,100 and 10,121,450 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/ilPanCinn1.1/dataset/CAKOAK01/snail.

Fluorometer and Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

Sequencing

Pacific Biosciences HiFi circular consensus DNA sequencing libraries were constructed according to the manufacturers' instructions. DNA sequencing was performed by the Scientific Operations core at the WSI on the Pacific Biosciences SEQUEL II (HiFi) instrument. Hi-C data were also generated from tissue of ilPanCinn1 using the Arima2 kit and sequenced on the Illumina NovaSeq 6000 instrument.

Genome assembly, curation and evaluation

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

A Hi-C map for the final assembly was produced using bwa-mem2 (Vasimuddin *et al.*, 2019) in the Cooler file format (Abdennur & Mirny, 2020). To assess the assembly metrics, the *k*-mer completeness and QV consensus quality values were

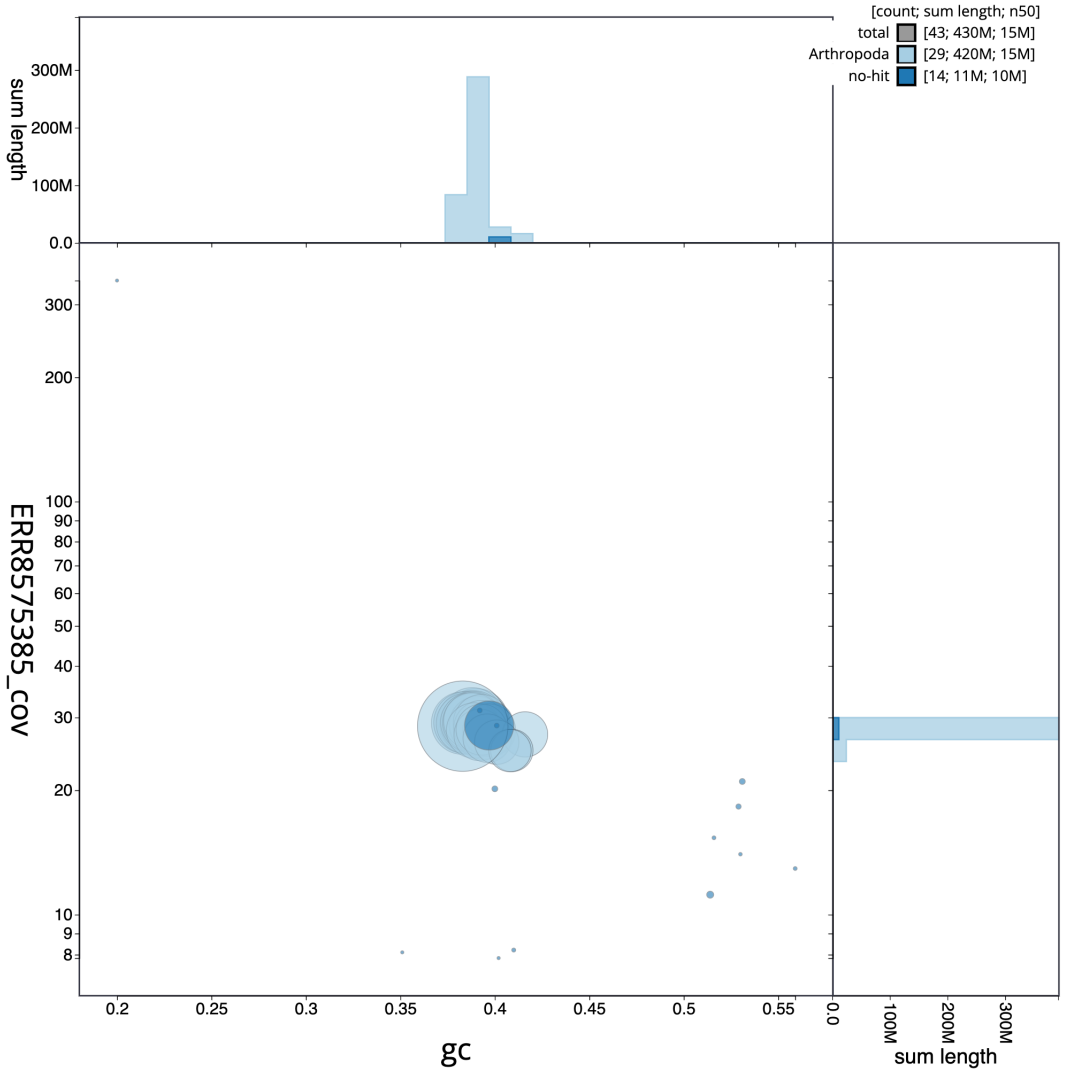

Figure 3. Genome assembly of *Pandemis cinnamomeana*, ilPanCinn1.1: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilPanCinn1.1/dataset/CAKOAK01/blob.

calculated in Merqury (Rhie *et al.*, 2020). This work was done using Nextflow (Di Tommaso *et al.*, 2017) DSL2 pipelines "sanger-tol/readmapping" (Surana *et al.*, 2023a) and "sanger-tol/genomenote" (Surana *et al.*, 2023b). The genome was analysed within the BlobToolKit environment (Challis *et al.*, 2020) and BUSCO scores (Manni *et al.*, 2021; Simão *et al.*, 2015) were calculated.

Table 3 contains a list of relevant software tool versions and sources.

Genome annotation

The BRAKER2 pipeline (Brůna *et al.*, 2021) was used in the default protein mode to generate annotation for the *Pandemis cinnamomeana* assembly (GCA_932294345.1) in Ensembl Rapid Release.

Wellcome Sanger Institute – Legal and Governance

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

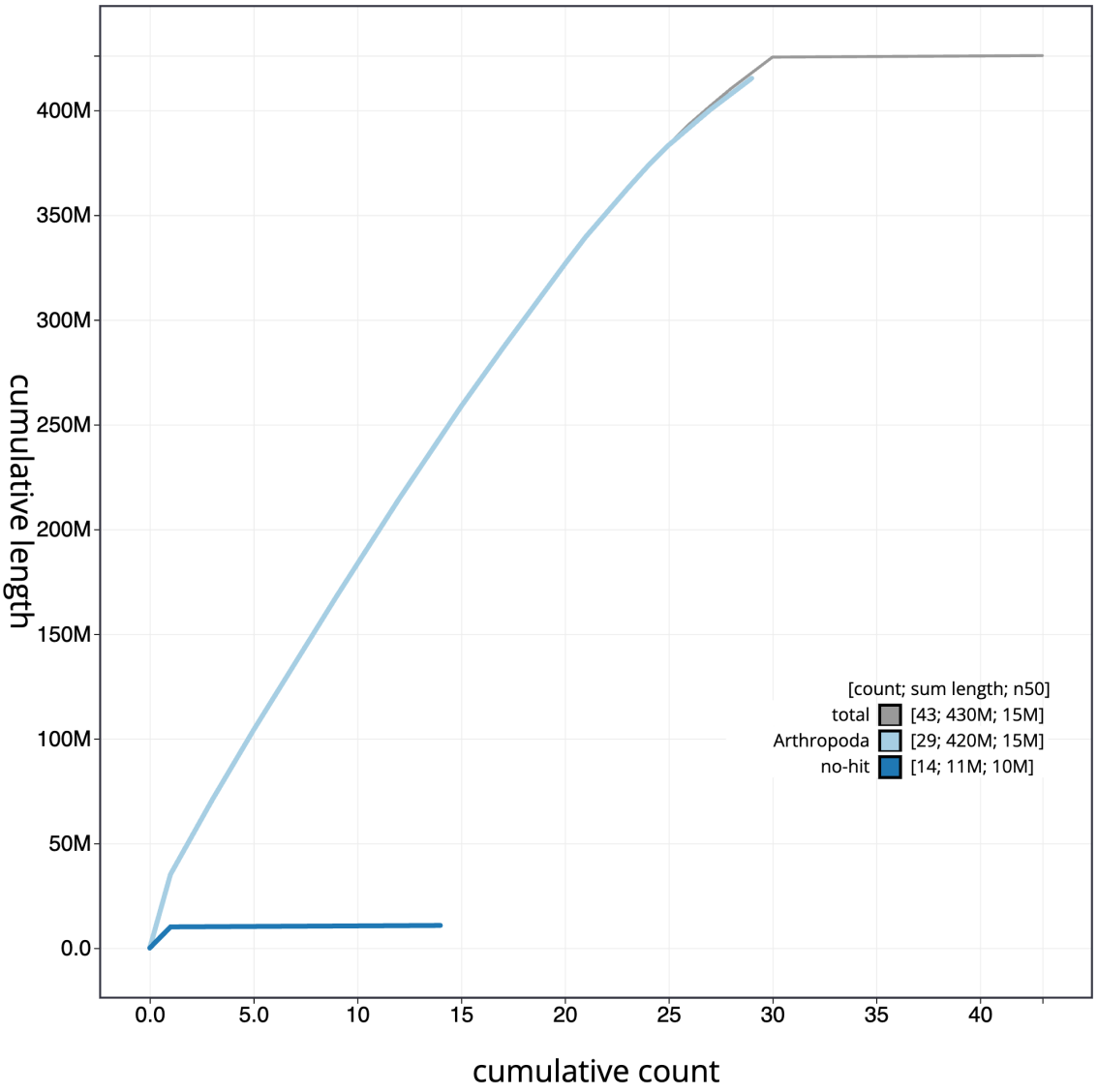

Figure 4. Genome assembly of *Pandemis cinnamomeana*, ilPanCinn1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilPanCinn1.1/dataset/CAKOAK01/cumulative.

Further, the Wellcome Sanger Institute employs a process whereby due diligence is carried out proportionate to the nature of the materials themselves, and the circumstances under which they have been/are to be collected and provided for use. The purpose of this is to address and mitigate any potential legal and/or ethical implications of receipt and use of the materials as part of the research project, and to ensure that in doing so we align with best practice wherever possible. The overarching areas of consideration are:

- Ethical review of provenance and sourcing of the material
- Legality of collection, transfer and use (national and international)

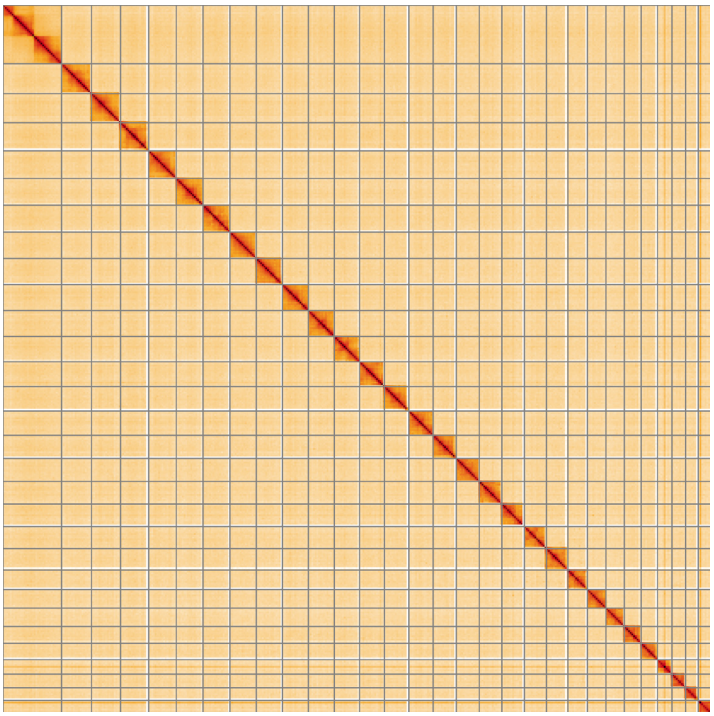

Figure 5. Genome assembly of *Pandemis cinnamomeana*, ilPanCinn1.1: Hi-C contact map of the ilPanCinn1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=Nx_NyHA9Tey8qx-4A0h1-Q.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Pandemis cinnamomeana*, ilPanCinn1.

INSDC accession	Chromosome	Length (Mb)	GC%
OW028703.1	1	17.94	39.0
OW028704.1	2	17.46	38.5
OW028705.1	3	16.83	39.0
OW028706.1	4	16.65	38.5
OW028707.1	5	16.16	38.5
OW028708.1	6	16.13	38.5
OW028709.1	7	15.83	39.0
OW028710.1	8	15.74	39.0
OW028711.1	9	15.62	38.5
OW028712.1	10	15.6	38.5
OW028713.1	11	15.16	39.0
OW028714.1	12	14.89	38.5
OW028715.1	13	14.71	39.0
OW028716.1	14	14.68	39.0
OW028717.1	15	13.87	39.0
OW028718.1	16	13.84	39.0
OW028719.1	17	13.6	39.5
OW028720.1	18	13.49	39.5
OW028721.1	19	13.2	39.5
OW028722.1	20	12.95	39.0
OW028723.1	21	11.71	39.5
OW028724.1	22	11.22	39.0
OW028725.1	23	11.0	39.5
OW028726.1	24	10.12	39.5
OW028727.1	25	9.89	39.5
OW028728.1	26	8.61	41.5
OW028729.1	27	8.27	40.0
OW028730.1	28	7.53	41.0
OW028731.1	29	7.51	41.0
OW028702.1	Z	35.21	38.5
OW028732.1	MT	0.02	20.0

Table 3. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.1.5	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	https://gitlab.com/ezlab/busco
Hifiasm	0.16.1-r375	https://github.com/chhylp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Merqury	MerquryFK	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	2	https://github.com/marcelauliano/MitoHiFi
PretextView	0.2	https://github.com/wtsi-hpag/PretextView
purge_dups	1.2.3	https://github.com/dfguan/purge_dups
sanger-tol/genomenote	v1.0	https://github.com/sanger-tol/genomenote
sanger-tol/readmapping	1.1.0	https://github.com/sanger-tol/readmapping/tree/1.1.0
YaHS	yahs-1.1.91eebc2	https://github.com/c-zhou/yahs

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: *Pandemis cinnamomeana* (white-faced tortrix). Accession number PRJEB50746; https://identifiers.org/ena.embl/PRJEB50746. (Wellcome Sanger Institute, 2022)

The genome sequence is released openly for reuse. The *Pandemis cinnamomeana* 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.

Author information

Members of the University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.4789928.

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Members of the Wellcome Sanger Institute Tree of Life programme are listed here: https://doi.org/10.5281/zenodo.4783585.

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Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.5013541.

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

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