



DATA NOTE

# The genome sequence of the August Thorn, *Ennomos quercinarius* (Hufnagel, 1767) [version 1; peer review: awaiting peer review]

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## Open Peer Review

**Approval Status** AWAITING PEER REVIEW

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## Abstract

We present a genome assembly from an individual male *Ennomos quercinarius* (the August Thorn; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence is 491.9 megabases in span. Most of the assembly is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 15.68 kilobases in length. Gene annotation of this assembly on Ensembl identified 11,355 protein coding genes.

## Keywords

*Ennomos quercinarius*, August Thorn, genome sequence, chromosomal, Lepidoptera



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

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## Species taxonomy

Eukaryota; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Geometroidea; Geometridae; Ennominae; *Ennomos*; *Ennomos quercinaria* (Hufnagel, 1767) (NCBI:txid875883).

## Background

*Ennomos quercinaria*, the August Thorn, is a moth of the Geometridae family. Males often are orange in colour with females being paler with a straw colouration. Both sexes have speckled forewings (plain in some cases) and occasional brown outer shading (Waring *et al.*, 2017). The key diagnostic features for this species are the cross-lines on the forewing, kinked on the leading edge and distinctly curved (Lewis, 2023; Waring *et al.*, 2017).

*E. quercinaria* is a univoltine species, flying from July to Late September. Larvae feed on pedunculate oak (*Quercus robur*) and sessile oak (*Quercus petraea*), on which they overwinter as eggs, then to pupate amongst hostplant leaves (Waring *et al.*, 2017). *E. quercinaria* has also been recorded on beech, lime, birch, hawthorn and blackthorn.

The August Thorn is distributed through much of western Europe from Belgium, to the Iberian Peninsula, and then eastwards from Greece through to Sicily. It has also been recorded as a pest species in western Asia. In the UK, *E. quercinaria* is found in wooded habitats, hedgerows and downlands; it is widespread in England and Wales with very few records through Scotland and widespread in the Channel Islands and Ireland (Waring *et al.*, 2017).

*Ennomos quercinaria* has been investigated as a pest species feeding on oak trees in Iran (Babaei, 2013; Barimani Varandi *et al.*, 2006), causing high levels of defoliation and in some cases tree mortality. However, in a study by Conrad *et al.* (2006), *E. quercinaria* was recognised as a species vulnerable to current insect declines, declining by 30 to 50% every 10 years. The full genome of this species will allow investigation into why this species is declining steadily in the UK whilst proving a successful pest species in Iran and assist in not only its potential conservation in the UK, and also to pest control in Iran.

This genome of *Ennomos quercinaria* 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 *Ennomos quercinaria*, based on three specimens collected from Wytham Woods, Oxfordshire.

## Genome sequence report

The genome was sequenced from one male *Ennomos quercinarius* (Figure 1) collected from Wytham Woods, Oxfordshire,



**Figure 1.** Photograph of the *Ennomos quercinarius* (ilEnnQuei1) specimen used for genome sequencing.

UK (51.77, -1.34). A total of 47-fold coverage in Pacific Biosciences single-molecule HiFi long reads and 105-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 32 missing joins or mis-joins and removed 10 haplotypic duplications, reducing the assembly length by 1.2% and the scaffold number by 15.91%, and increasing the scaffold N50 by 0.25%.

The final assembly has a total length of 491.9 Mb in 36 sequence scaffolds with a scaffold N50 of 17.4 Mb (Table 1). A summary of the assembly statistics is shown in Figure 2, 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 (99.96%) of the assembly sequence was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (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 57.7 with *k*-mer completeness of 99.99%, and the assembly has a BUSCO v5.3.2 completeness of 98.6% (single = 98.1%, duplicated = 0.5%), 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/875883>.

## Genome annotation report

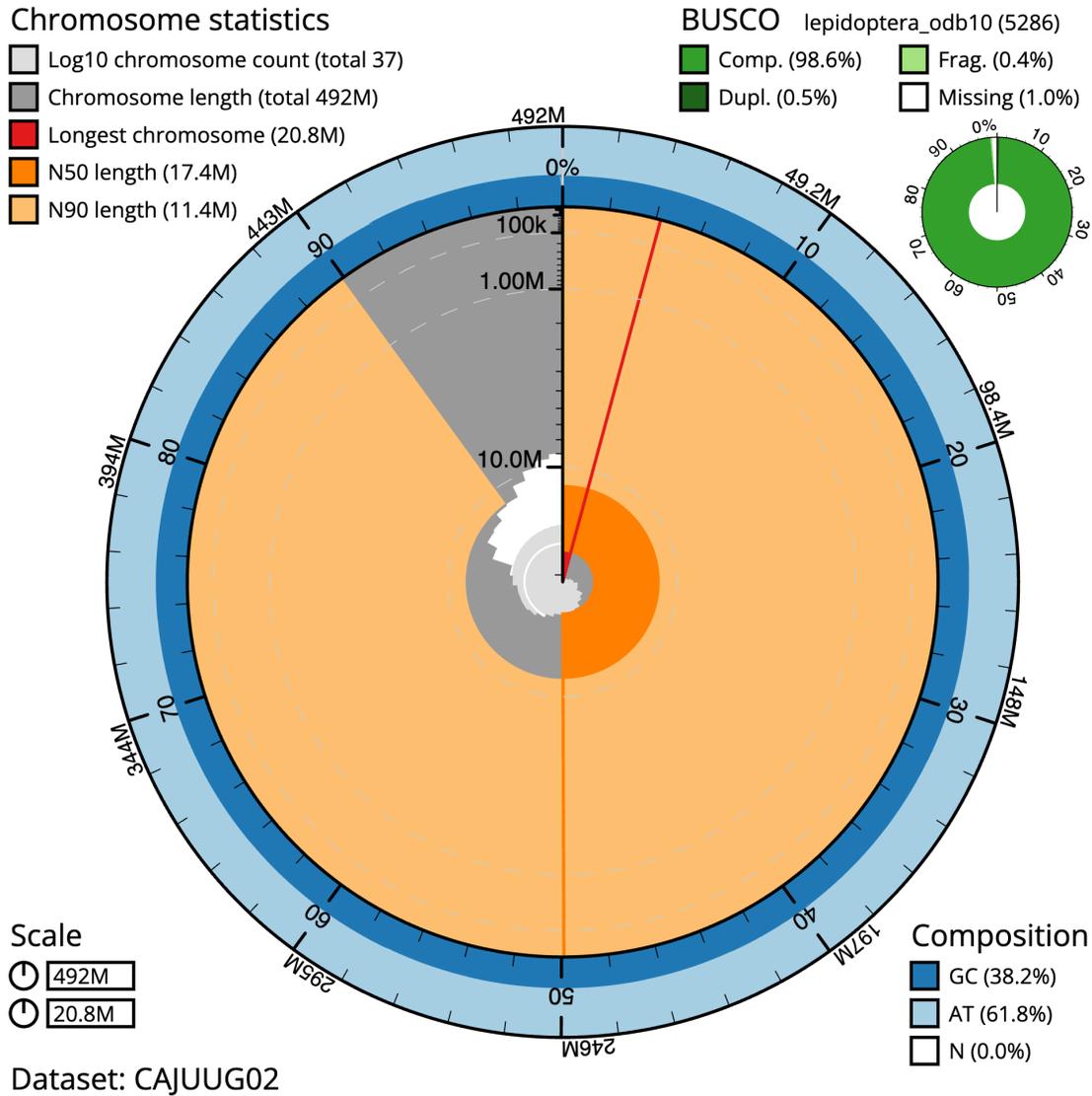
The *Ennomos quercinarius* genome assembly (GCA\_910589525.2) was annotated using the Ensembl rapid annotation pipeline

**Table 1. Genome data for *Ennomos quercinarius*, iEnnQuei1.2.**

<b>Project accession data</b>		
Assembly identifier	iEnnQuei1.2	
Species	<i>Ennomos quercinarius</i>	
Specimen	iEnnQuei1	
NCBI taxonomy ID	875883	
BioProject	PRJEB45117	
BioSample ID	SAMEA7701560	
Isolate information	iEnnQuei1, male: abdomen (DNA sequencing); head and thorax (Hi-C scaffolding)	
<b>Assembly metrics*</b>		<b>Benchmark</b>
Consensus quality (QV)	57.7	≥ 50
<i>k</i> -mer completeness	99.99%	≥ 95%
BUSCO**	C:98.6%[S:98.1%,D:0.5%],F:0.4%,M:1.0%,n:5,286	C ≥ 95%
Percentage of assembly mapped to chromosomes	99.96%	≥ 95%
Sex chromosomes	Z chromosome	<i>localised homologous pairs</i>
Organelles	Mitochondrial genome assembled	<i>complete single alleles</i>
<b>Raw data accessions</b>		
PacificBiosciences SEQUEL II	ERR6412368	
10X Genomics Illumina	ERR6054765, ERR6054762, ERR6054763, ERR6054764	
Hi-C Illumina	ERR6054761	
PolyA RNA-Seq Illumina		
<b>Genome assembly</b>		
Assembly accession	GCA_910589525.2	
<i>Accession of alternate haplotype</i>	GCA_910589225.1	
Span (Mb)	491.9	
Number of contigs	55	
Contig N50 length (Mb)	16.1	
Number of scaffolds	36	
Scaffold N50 length (Mb)	17.4	
Longest scaffold (Mb)	20.8	
<b>Genome annotation</b>		
Number of protein-coding genes	11,355	
Number of non-coding genes	1,354	
Number of gene transcripts	20,363	

\* 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/Ennomos%20quercinarius/dataset/CAJUUG02/busco>.



**Figure 2. Genome assembly of *Ennomos quercinarius*, iEnnQuei1.2: 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 491,884,259 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 (20,767,864 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (17,369,662 and 11,419,399 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/Ennomos%20quercinarius/dataset/CAJUUG02/snail>.

(Table 1; [https://rapid.ensembl.org/Ennomos\\_quercinarius\\_GCA\\_910589525.2/Info/Index](https://rapid.ensembl.org/Ennomos_quercinarius_GCA_910589525.2/Info/Index)). The resulting annotation includes 20,363 transcribed mRNAs from 11,355 protein-coding and 1,354 non-coding genes.

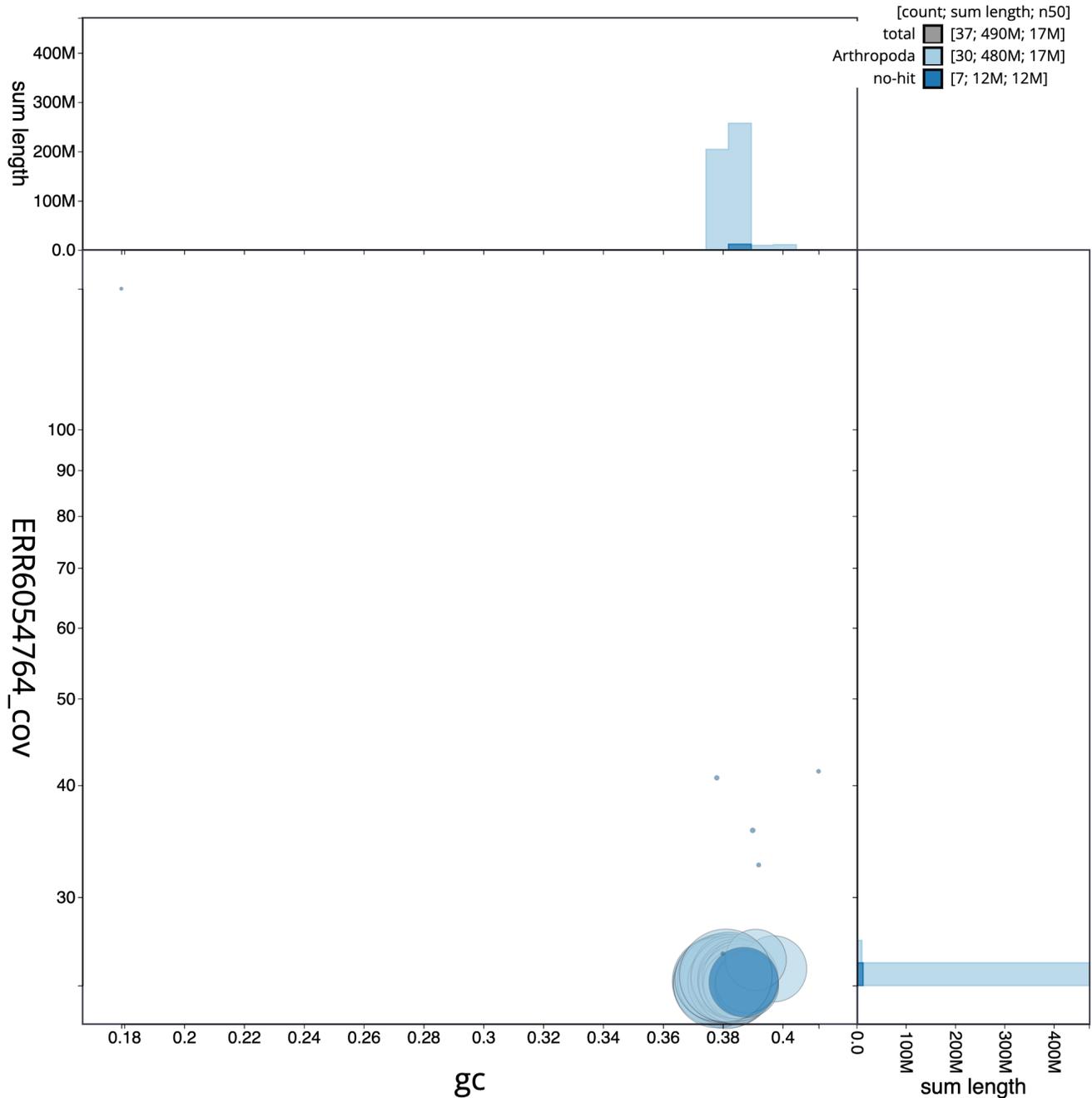
## Methods

### Sample acquisition and nucleic acid extraction

A male *Ennomos quercinarius* (specimen ID Ox000699, individual iEnnQuei1) was collected from Wytham woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude -1.34) on 2020-07-20 using a light trap.

The specimen was collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute (WSI). The iEnnQuei1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing. Abdomen tissue 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. Low molecular weight DNA was removed from a 20 ng aliquot of extracted DNA using the 0.8X AMPure XP



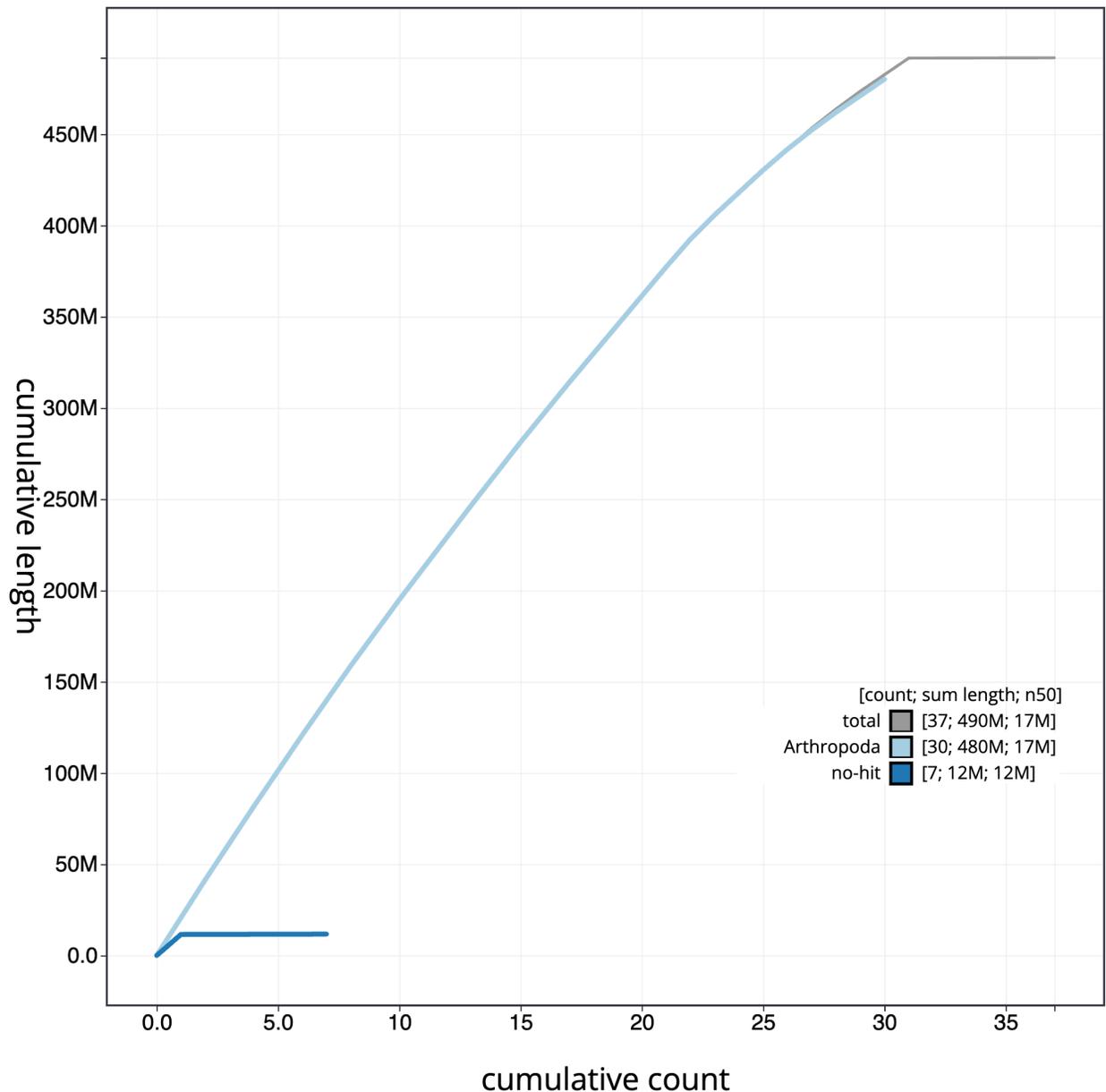
**Figure 3. Genome assembly of *Ennomos quercinarius*, iEnnQuei1.2: BlobToolKit GC-coverage plot.** Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/Ennomos%20quercinarius/dataset/CAJUUG02/blob>.

purification kit prior to 10X Chromium sequencing; a minimum of 50 ng DNA was submitted for 10X sequencing. 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 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 and 10X Genomics read cloud DNA sequencing libraries were constructed



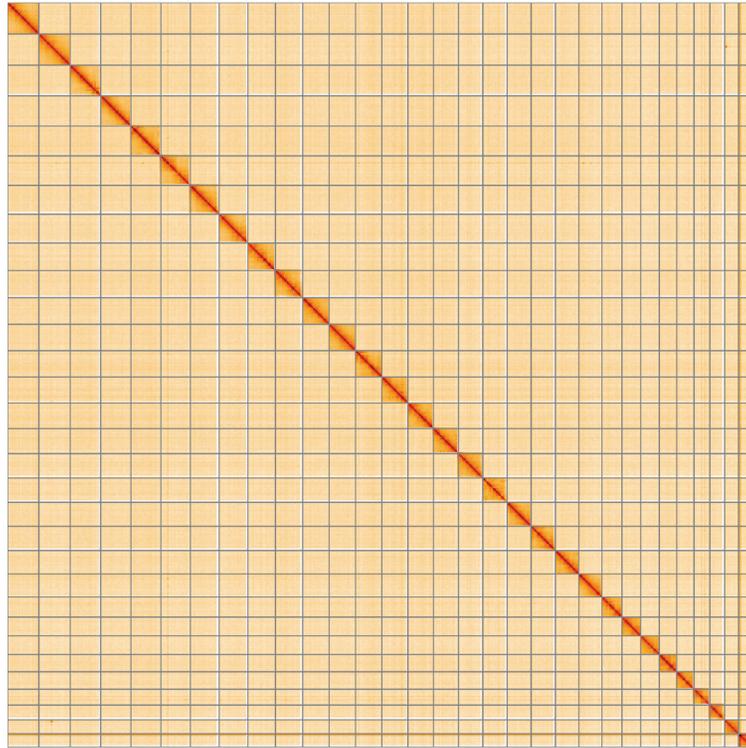
**Figure 4. Genome assembly of *Ennomos quercinarius*, iEnnQuei1.2: BlobToolKit cumulative sequence plot.** The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/Ennomos%20quercinarius/dataset/CAJUUG02/cumulative>.

according to the manufacturers' instructions. DNA sequencing was performed by the Scientific Operations core at the WSI on Pacific Biosciences SEQUEL II (HiFi) and Illumina NovaSeq 6000 (10X) instruments. Hi-C data were also generated from head and thorax tissue of iEnnQuei1 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). One round of polishing was performed by aligning 10X Genomics read data to the assembly with Long Ranger ALIGN, calling variants with FreeBayes (Garrison & Marth, 2012). The assembly was then scaffolded with Hi-C data (Rao *et al.*, 2014) using SALSA2 (Ghurye *et al.*, 2019). The assembly was checked for contamination and corrected using the gEVAL system (Chow *et al.*, 2016) as described previously (Howe *et al.*, 2021). Manual curation was performed using gEVAL, HiGlass (Kerpedjiev *et al.*, 2018) and Pretext (Harry, 2022). The mitochondrial genome was



**Figure 5. Genome assembly of *Ennomos quercinarius*, ilEnnQuei1.2: Hi-C contact map of the ilEnnQuei1.2 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=Y-cOiO6vT\\_6b8GHP17M3\\_g](https://genome-note-higlass.tol.sanger.ac.uk/?d=Y-cOiO6vT_6b8GHP17M3_g).

**Table 2. Chromosomal pseudomolecules in the genome assembly of *Ennomos quercinarius*, ilEnnQuei1.**

INSDC accession	Chromosome	Length (Mb)	GC%
OU342488.1	1	20.77	38.0
OU342490.1	2	20.12	38.0
OU342491.1	3	20.04	38.0
OU342492.1	4	19.7	38.0
OU342493.1	5	19.48	38.0
OU342494.1	6	19.02	38.0
OU342495.1	7	18.93	38.0
OU342496.1	8	18.23	38.0
OU342497.1	9	17.96	38.0
OU342498.1	10	17.52	38.0
OU342499.1	11	17.41	38.0
OU342500.1	12	17.37	38.0
OU342501.1	13	17.2	38.0
OU342502.1	14	16.86	38.0
OU342503.1	15	16.48	38.5

INSDC accession	Chromosome	Length (Mb)	GC%
OU342504.1	16	16.14	38.0
OU342505.1	17	15.97	38.5
OU342506.1	18	15.95	38.5
OU342507.1	19	15.93	38.5
OU342508.1	20	15.5	38.5
OU342509.1	21	15.19	38.5
OU342510.1	22	13.32	38.5
OU342511.1	23	12.35	38.5
OU342512.1	24	12.23	38.5
OU342513.1	25	11.55	38.5
OU342514.1	26	11.42	38.5
OU342515.1	27	10.46	39.5
OU342516.1	28	9.75	39.0
OU342517.1	29	9.25	39.0
OU342518.1	30	8.91	39.0
OU342489.1	Z	20.69	38.0
OU342519.2	MT	0.02	18.0

assembled using MitoHiFi (Uliano-Silva *et al.*, 2023), 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 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 Ensembl gene annotation system (Aken *et al.*, 2016) was used to generate annotation for the *Ennomos quercinarius* assembly (GCA\_910589525.2). 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.

**Table 3. Software tools: versions and sources.**

Software tool	Version	Source
BlobToolKit	4.0.7	<a href="https://github.com/blobtoolkit/blobtoolkit">https://github.com/blobtoolkit/blobtoolkit</a>
BUSCO	5.3.2	<a href="https://gitlab.com/ezlab/busco">https://gitlab.com/ezlab/busco</a>
FreeBayes	1.3.1-17-gaa2ace8	<a href="https://github.com/freebayes/freebayes">https://github.com/freebayes/freebayes</a>
gEVAL	N/A	<a href="https://geval.org.uk/">https://geval.org.uk/</a>
Hifiasm	0.14	<a href="https://github.com/chhylp123/hifiasm">https://github.com/chhylp123/hifiasm</a>
HiGlass	1.11.6	<a href="https://github.com/higlass/higlass">https://github.com/higlass/higlass</a>
Long Ranger ALIGN	2.2.2	<a href="https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines">https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines</a>
Merqury	MerquryFK	<a href="https://github.com/thegenemyers/MERQURY.FK">https://github.com/thegenemyers/MERQURY.FK</a>
MitoHiFi	2	<a href="https://github.com/marcelauliano/MitoHiFi">https://github.com/marcelauliano/MitoHiFi</a>
PretextView	0.2	<a href="https://github.com/wtsi-hpag/PretextView">https://github.com/wtsi-hpag/PretextView</a>
purge_dups	1.2.3	<a href="https://github.com/dfguan/purge_dups">https://github.com/dfguan/purge_dups</a>
SALSA	2.2	<a href="https://github.com/salsa-rs/salsa">https://github.com/salsa-rs/salsa</a>
sanger-tol/genomenote	v1.0	<a href="https://github.com/sanger-tol/genomenote">https://github.com/sanger-tol/genomenote</a>
sanger-tol/readmapping	1.1.0	<a href="https://github.com/sanger-tol/readmapping/tree/1.1.0">https://github.com/sanger-tol/readmapping/tree/1.1.0</a>

## Data availability

European Nucleotide Archive: *Ennomos quercinarius* (August thorn). Accession number PRJEB45117; <https://identifiers.org/ena.embl/PRJEB45117>. (Wellcome Sanger Institute, 2021) The genome sequence is released openly for reuse. The *Ennomos quercinarius* 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>.

Members of the Darwin Tree of Life Barcoding collective are listed here: <https://doi.org/10.5281/zenodo.4893703>.

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

Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: <https://doi.org/10.5281/zenodo.4790455>.

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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