DATA NOTE

The genome sequence of the Birch Bell, *Epinotia demarniana* (Fischer von Röslerstamm, 1839) [version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual male *Epinotia demarniana* (the Birch Bell; Arthropoda; Insecta; Lepidoptera; Tortricidae). The genome sequence is 735.8 megabases in span. Most of the assembly is scaffolded into 28 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 15.46 kilobases in length.

Keywords

Epinotia demarniana, Birch Bell, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status *AWAITING PEER REVIEW*

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

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Species taxonomy

Eukaryota; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Apoditrysia; Tortricoidea; Tortricidae; Olethreutinae; Eucosmini; *Epinotia*; *Epinotia demarniana* (Fischer von Röslerstamm, 1839) (NCBI:txid1594294).

Background

Epinotia demarniana (Fischer von Röslerstamm, 1839) is a moth of the Tortricidae family. The adult moths have an attractive pied appearance, showing little variation in colour or pattern (Bradley *et al.*, 1979). In Great Britain, the adults are at large between June and July, flying high around treetops in the evening. By day the adult moths rest high up amongst foliage and can be caught by shaking or kicking suitable trees, then catching the adult moths as they flutter to the ground (Bradley *et al.*, 1979; Elliott *et al.*, 2018). After oviposition larvae begin feeding in September. The larval stages feed within the catkins of *Alnus*, *Salix caprea*, and *Betula* until May, subsequently pupating in a cocoon amongst leaf litter until June (Bradley *et al.*, 1979; Elliott *et al.*, 2018).

This species frequents heaths and open woodland, as well as wetter habitats such as fens, river banks, or boggy moorland (Bradley *et al.*, 1979). The species is broadly distributed across England and into Wales, but is absent from Scotland and Ireland (Elliott *et al.*, 2018). The species is regarded within the British Isles as being local or scarce1. Globally the species occurs across northern Eurasia in a possibly continuous distribution stretching from Great Britain at its western extremity to Hokkaido in the east (GBIF Secretariat, 2022; Hancock *et al.*, 2015). The species has also been recorded from eastern Russia and north-eastern China (Byun *et al.*, 2008), suggesting a broad distribution at the eastern edge of its range.

The genome of *Epinotia demarniana* 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 *E. demarniana*, based on one male specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome was sequenced from one male *Epinotia demarniana* (Figure 1) collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (51.77, –1.34). A total of 30-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 22 missing joins or mis-joins and removed 6 haplotypic duplications, reducing the assembly length by 0.2% and the scaffold number by 12.5%.

The final assembly has a total length of 735.8 Mb in 42 sequence scaffolds with a scaffold N50 of 26.9 Mb (Table 1). Most (99.85%) of the assembly sequence was assigned to 28

Figure 1. Photograph of the *Epinotia demarniana* (ilEpiDema1) specimen used for genome sequencing.

chromosomal-level scaffolds, representing 27 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 63.6 with *k*-mer completeness of 100%, and the assembly has a BUSCO v5.3.2 completeness of 98.4% (single = 97.7%, duplicated = 0.7%), 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/1594294.

Methods

Sample acquisition and nucleic acid extraction

Two *Epinotia demarniana* specimens (specimen ID Ox001904, ilEpiDema1 and specimen ID Ox001904, ilEpiDema2) were collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2021-06-16 using a light trap. The specimens were collected and identified by Douglas Boyes (University of Oxford) and were then preserved on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute (WSI). The ilEpiDema1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing. Whole organism 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. 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

Table 1. Genome data for *Epinotia demarniana*, ilEpiDema1.1.

Project accession data		
Assembly identifier	ilEpiDema1.1	
Species	*Epinotia demarniana*	
Specimen	ilEpiDema1	
NCBI taxonomy ID	1594294	
BioProject	PRJEB54049	
BioSample ID	SAMEA10979166	
Isolate information	ilEpiDema1, male, whole organism (DNA sequencing) ilEpiDema2 (Hi-C scaffolding)	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	63.6	*≥ 50*
k-mer completeness	100%	*≥ 95%*
BUSCO**	C:98.4%[S:97.7%,D:0.7%], F:0.4%,M:1.2%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.85%	*≥ 95%*
Sex chromosomes	Z chromosome	*localised homologous pairs*
Organelles	Mitochondrial genome assembled	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR9924611	
Hi-C Illumina	ERR9930686	
Genome assembly		
Assembly accession	GCA_945867215.1	
Accession of alternate haplotype	GCA_945869435.1	
Span (Mb)	735.8	
Number of contigs	109	
Contig N50 length (Mb)	13.5	
Number of scaffolds	42	
Scaffold N50 length (Mb)	26.9	
Longest scaffold (Mb)	64.2	

* 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/ilEpiDema1.1/dataset/CAMBMD01/

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 DNA sequencing libraries were constructed according to the manufacturers' instructions. DNA sequencing was performed by the Scientific Operations core at the WSI on a Pacific Biosciences SEQUEL II (HiFi) instrument. Hi-C data were also generated from

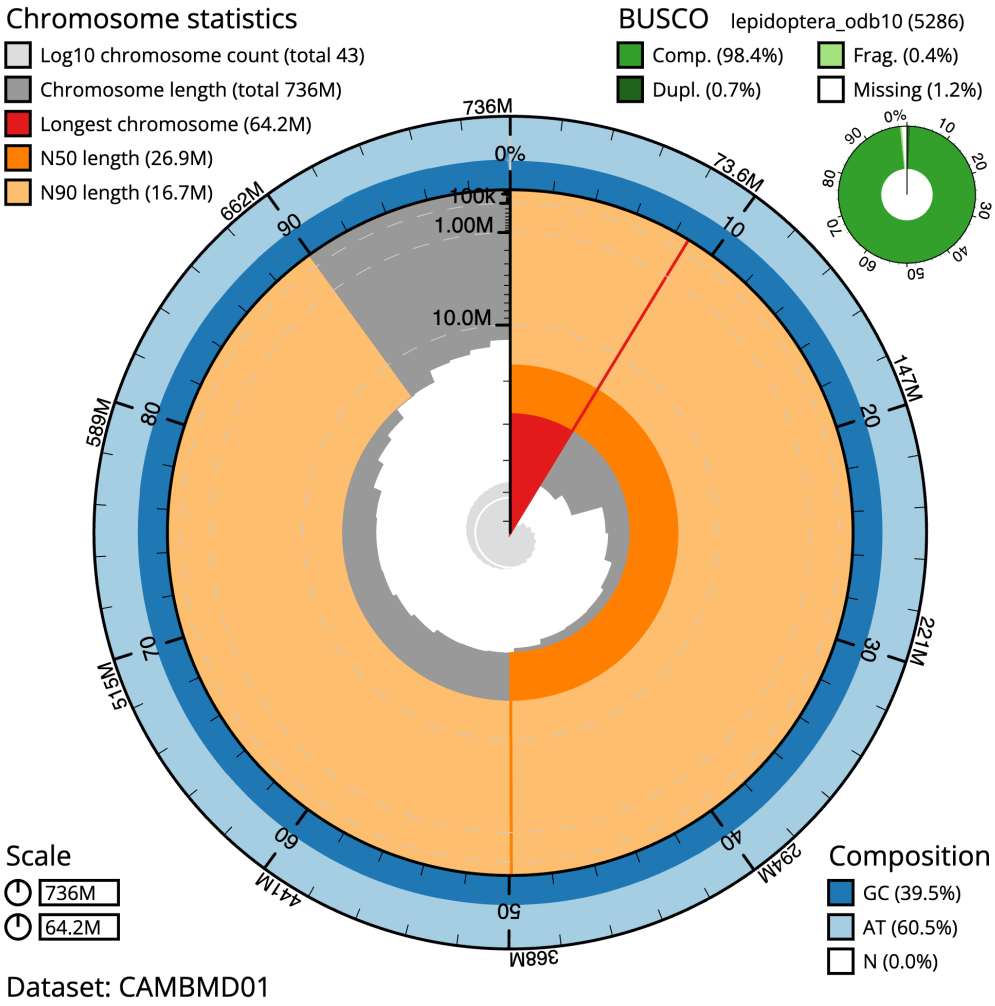

Figure 2. Genome assembly of *Epinotia demarniana*, ilEpiDema1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 735,811,797 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (64,174,388 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (26,883,300 and 16,660,370 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/ilEpiDema1.1/dataset/CAMBMD01/snail.

whole organism tissue of ilEpiDema2 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 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

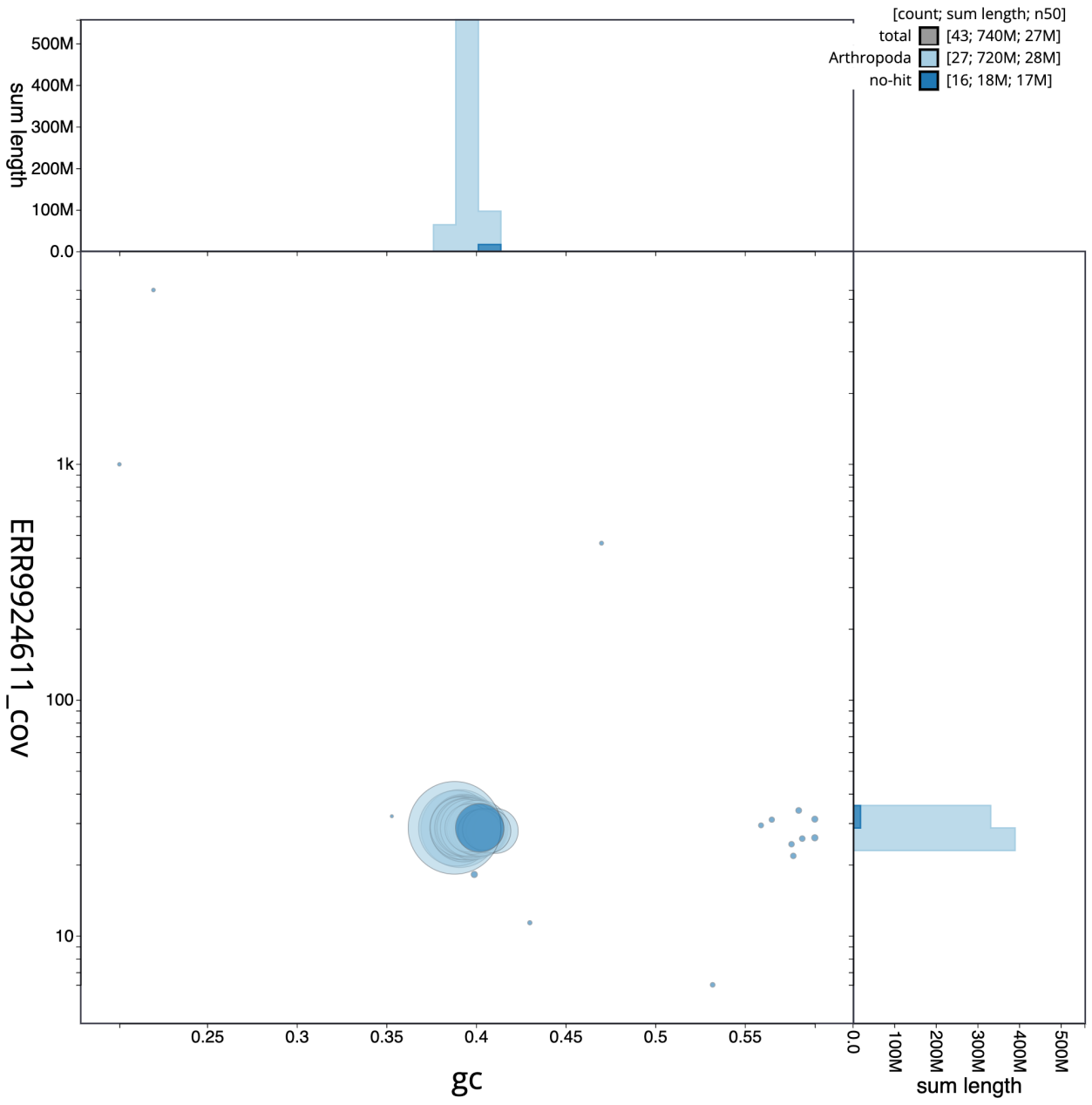

Figure 3. Genome assembly of *Epinotia demarniana*, ilEpiDema1.1: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilEpiDema1.1/dataset/CAMBMD01/blob.

"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.

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

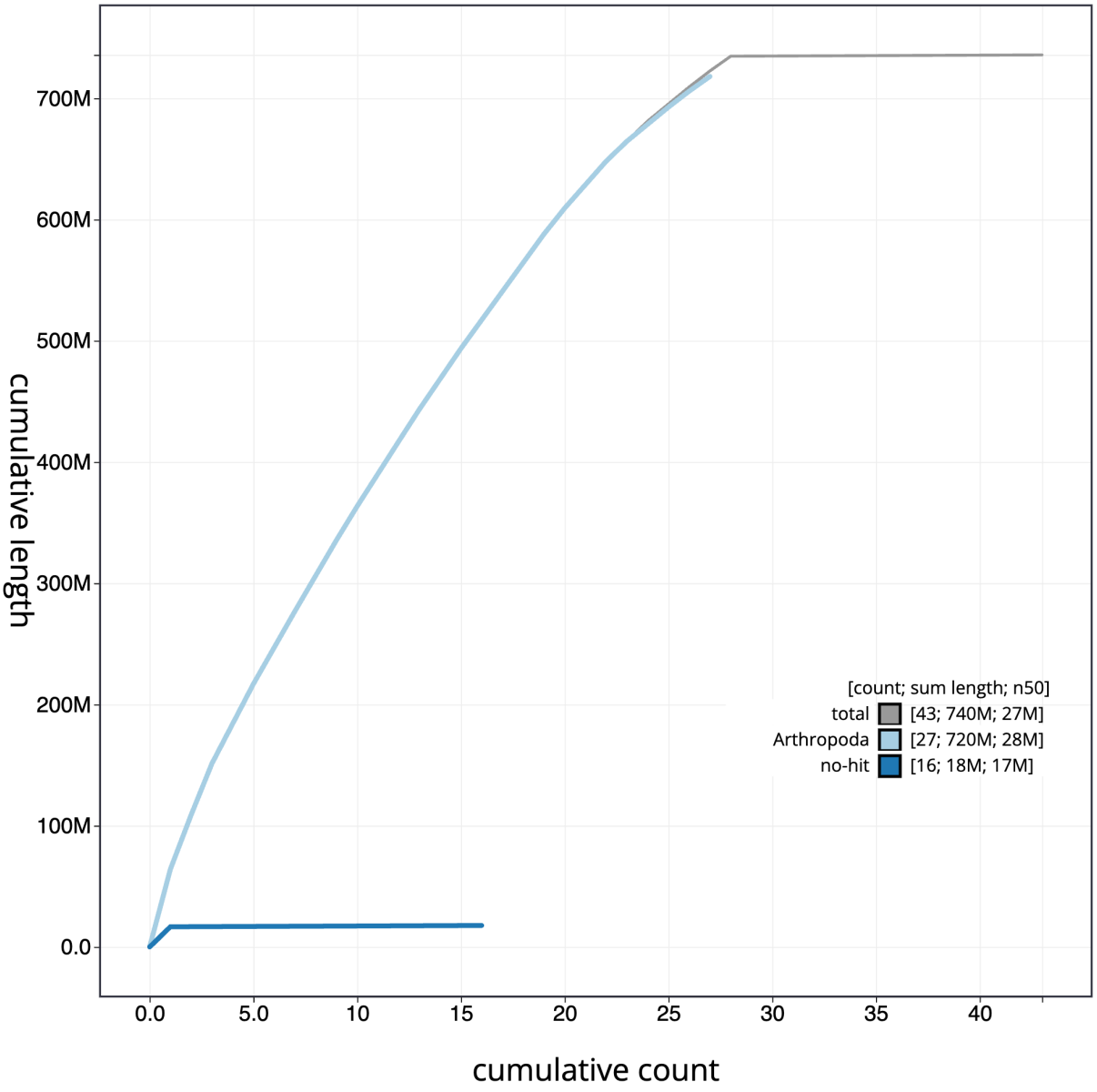

Figure 4. Genome assembly of *Epinotia demarniana*, ilEpiDema1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilEpiDema1.1/dataset/CAMBMD01/cumulative.

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

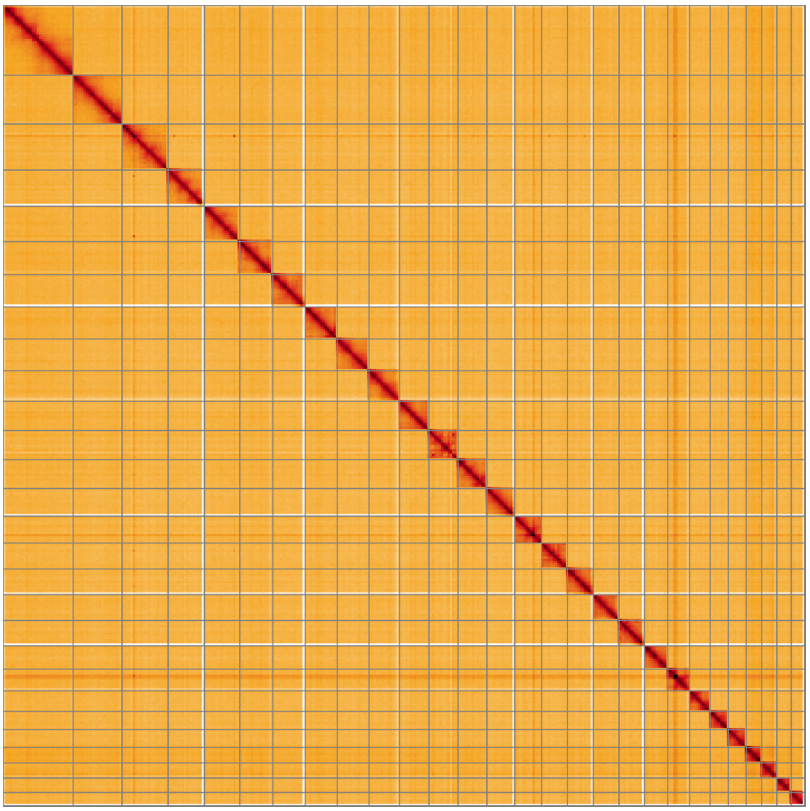

Figure 5. Genome assembly of *Epinotia demarniana*, ilEpiDema1.1: Hi-C contact map of the ilEpiDema1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=ItZbq6kISSSzVWjNKlcIiQ.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Epinotia demarniana*, ilEpiDema1.

INSDC accession	Chromosome	Length (Mb)	GC%
OX244260.1	1	44.8	39.0
OX244261.1	2	42.27	39.0
OX244262.1	3	33.22	39.5
OX244263.1	4	32.51	39.5
OX244264.1	5	30.44	39.5
OX244265.1	6	29.56	39.0
OX244266.1	7	29.39	39.5
OX244267.1	8	29.17	39.0
OX244268.1	9	27.99	39.5
OX244269.1	10	26.88	39.5
OX244270.1	11	26.63	40.0
OX244271.1	12	26.52	39.5
OX244272.1	13	25.44	39.0
OX244273.1	14	24.64	39.5
OX244274.1	15	23.82	39.5
OX244275.1	16	23.64	39.5
OX244276.1	17	23.61	39.5
OX244277.1	18	23.32	40.0
OX244278.1	19	21.41	40.0
OX244279.1	20	19.94	40.0
OX244280.1	21	18.8	40.0
OX244281.1	22	16.66	40.0
OX244282.1	23	16.44	39.5
OX244283.1	24	14.23	41.0
OX244284.1	25	13.91	40.5
OX244285.1	26	13.19	40.0
OX244286.1	27	12.14	40.5
OX244259.1	Z	64.17	39.0
OX244287.1	MT	0.02	20.0

Table 3. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.0.7	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

- 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: *Epinotia demarniana* (birch bell). Accession number PRJEB54049; https://identifiers.org/ena.embl/PRJEB54049. (Wellcome Sanger Institute, 2022)

The genome sequence is released openly for reuse. The *Epinotia demarniana* 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. 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.

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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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