DATA NOTE

The genome sequence of the Pale November moth, *Epirrita christyi* (Allen, 1906)

[version 1; peer review: 3 approved]

Douglas Boyes[1+], Liam M. Crowley[2], Clare Boyes[3],
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

[1]UK Centre for Ecology & Hydrology, Wallingford, England, UK
[2]University of Oxford, Oxford, England, UK
[3]Independent researcher, Welshpool, Wales, UK

[+] Deceased author

v1 **First published:** 20 Sep 2024, **9**:540
https://doi.org/10.12688/wellcomeopenres.23060.1
Latest published: 20 Sep 2024, **9**:540
https://doi.org/10.12688/wellcomeopenres.23060.1

Abstract

We present a genome assembly from an individual female Pale November moth, *Epirrita christyi* (Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence has a total length of 474.20 megabases. Most of the assembly is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 15.99 kilobases in length. Gene annotation of this assembly on Ensembl identified 16,983 protein-coding genes.

Keywords

Epirrita christyi, Pale November moth, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓ ✓

	1	2	3
version 1 20 Sep 2024	✓ view	✓ view	✓ view

1. **Zoltan Erdos**, University of Exeter Cornwall Campus (Ringgold ID: 151778), Penryn, UK
Ben Raymond, University of Exeter, Penryn, UK

2. **Arun Arumugaperumal**, Rajalakshmi Engineering College, Tamilnadu, India

3. **Shruti Iyer**, Cold Spring Harbor Laboratory, Cold Spring Harbor, USA

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)

Author roles: Boyes D: Investigation, Resources; **Crowley LM**: Investigation, Resources; **Boyes C**: Writing – Original Draft Preparation;

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, Crowley LM, Boyes C *et al.* **The genome sequence of the Pale November moth, *Epirrita christyi* (Allen, 1906) [version 1; peer review: 3 approved]** Wellcome Open Research 2024, **9**:540 https://doi.org/10.12688/wellcomeopenres.23060.1

First published: 20 Sep 2024, **9**:540 https://doi.org/10.12688/wellcomeopenres.23060.1

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; Obtectomera; Geometroidea; Geometridae; Larentiinae; *Epirrita*; *Epirrita christyi* (Allen, 1906) (NCBI:txid247947).

Background

The Pale November Moth (*Epirrita christyi*) is a moth in the family Geometridae. It is common and widespread in Britain, although not often found in Ireland, and has declined significantly since 1970 (Randle *et al.*, 2019). It is present throughout Central and Northern Europe (GBIF Secretariat, 2024).

The adult moth is difficult to identify as there are four similar *Epirrita* species in the UK and it is therefore likely to be under-recorded. The forewing background can range from dark grey to light grey and can be plain or banded. Colouration and patterning do not indicate species as there is significant variation within and between species. Morphological features are required for correct identification although this only applies to males: females cannot reliably be determined. The moth occurs in mature woodland and can be found by tapping the lower branches of trees. It also flies to light and is on the wing between late September and November. The egg is laid on a twig where it overwinters, hatching in late April, before pupating underground. Larval foodplants are deciduous trees and includes elms, birches, hawthorns and sallows, although there is some debate about the exact requirements because of the identification difficulties (Waring *et al.*, 2017).

The genome of *Epirrita christyi* 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 *Epirrita christyi* based on one adult specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome of an adult female *Epirrita christyi* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating a total of 23.55 Gb (gigabases) from 2.22 million reads, providing approximately 48-fold coverage. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data, which produced 97.18 Gb from 643.55 million reads, yielding an approximate coverage of 205-fold. Specimen and sequencing information is summarised in Table 1.

Manual assembly curation corrected 48 missing joins or mis-joins and 19 haplotypic duplications, reducing the assembly length by 1.41% and the scaffold number by 30.65%, and increasing the scaffold N50 by 0.46%. The final assembly has a total length of 474.20 Mb in 42 sequence scaffolds with a scaffold N50 of 16.3 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

Figure 1. Photograph of the *Epirrita christyi* (ilEpiChri1) specimen used for genome sequencing.

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 3). The Z chromosome was identified by coverage, and by alignment to *Gymnoscelis rufifasciata* (GCA_929108375.1) (Boyes *et al.*, 2022), *Electrophaes corylata* (GCA_947095575.1) (Boyes *et al.*, 2023b), *Thera britannica* (GCA_939531255.2) (Boyes *et al.*, 2023c) and *Anticlea derivata* (GCA_947579855.1) (Boyes *et al.*, 2023a). While the Z chromosome has approximately half read coverage, no W chromosome could be identified, and the specimen is likely to be a ZO female. 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 64.9 with *k*-mer completeness of 100.0%, and the assembly has a BUSCO v5.3.2 completeness of 98.4% (single = 97.9%, duplicated = 0.5%), using the lepidoptera_odb10 reference set (n = 5286).

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/247947.

Genome annotation report

The *Epirrita christyi* genome assembly (GCA_951392215.1) was annotated at the European Bioinformatics Institute (EBI) on Ensembl Rapid Release. The resulting annotation includes 17,160 transcribed mRNAs from 16,983 protein-coding genes

Table 1. **Specimen and sequencing data for *Epirrita christyi*.**

Project information			
Study title	*Epirrita christyi* (pale November moth)		
Umbrella BioProject	PRJEB60639		
Species	*Epirrita christyi*		
BioSample	SAMEA8603217		
NCBI taxonomy ID	247947		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	ilEpiChri1	SAMEA8603789	Abdomen
Hi-C sequencing	ilEpiChri1	SAMEA8603785	Head
RNA sequencing	ilEpiChri3	SAMEA113425986	Whole organism
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq 6000	ERR11040168	6.44e+08	97.18
PacBio Sequel IIe	ERR11029660	2.22e+06	23.55
RNA Illumina NovaSeq X	ERR12765138	7.07e+07	10.68

(Table 2; https://rapid.ensembl.org/Epirrita_christyi_GCA_951392215.1/Info/Index). The average transcript length is 7,844.37. There are 1.01 coding transcripts per gene and 5.84 exons per transcript.

Methods

Sample acquisition and barcoding

An adult female *Epirrita christyi* (specimen ID Ox000994, ToLID ilEpiChri1) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2020-11-21 using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice. This specimen was used for PacBio HiFi and Illumina Hi-C sequencing.

The specimen used for RNA sequencing (specimen ID Ox003249, ToLID ilEpiChri3) was an adult specimen collected from the same location on 2022-10-20, using a light trap. The specimen was collected and identified by Liam Crowley (University of Oxford) and preserved on dry ice.

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 the specimens and stored in ethanol, while the remaining parts of the specimen were shipped on dry ice to the Wellcome Sanger Institute (WSI). 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).

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 core procedures: sample preparation and homogenisation, DNA extraction, fragmentation and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). In sample preparation, the ilEpiChri1 sample was weighed and dissected on dry ice (Jay *et al.*, 2023). Tissue from the abdomen 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 Qubit Fluorometer using the Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

Table 2. Genome assembly data for *Epirrita christyi*, ilEpiChri1.1.

Genome assembly		
Assembly name	ilEpiChri1.1	
Assembly accession	GCA_951392215.1	
Accession of alternate haplotype	*GCA_949802565.1*	
Span (Mb)	474.20	
Number of contigs	142	
Contig N50 length (Mb)	6.1	
Number of scaffolds	42	
Scaffold N50 length (Mb)	16.3	
Longest scaffold (Mb)	19.35	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	64.9	*≥ 50*
k-mer completeness	100.0%	*≥ 95%*
BUSCO**	C:98.4%[S:97.9%,D:0.5%], F:0.5%,M:1.1%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.96%	*≥ 95%*
Sex chromosomes	Z	*localised homologous pairs*
Organelles	Mitochondrial genome: 15.99 kb	*complete single alleles*
Genome annotation of assembly GCA_951392215.1 at Ensembl		
Number of protein-coding genes	16,983	
Number of gene transcripts	17,160	

* 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/ilEpiChri1_1/dataset/ilEpiChri1_1/busco.

RNA was extracted from whole organism tissue of ilEpiChri3 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ *mir*Vana 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 integrity of the RNA was done using the Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

Library preparation and 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 the ilEpiChri1 sample, 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 sequencing was performed using paired-end sequencing with

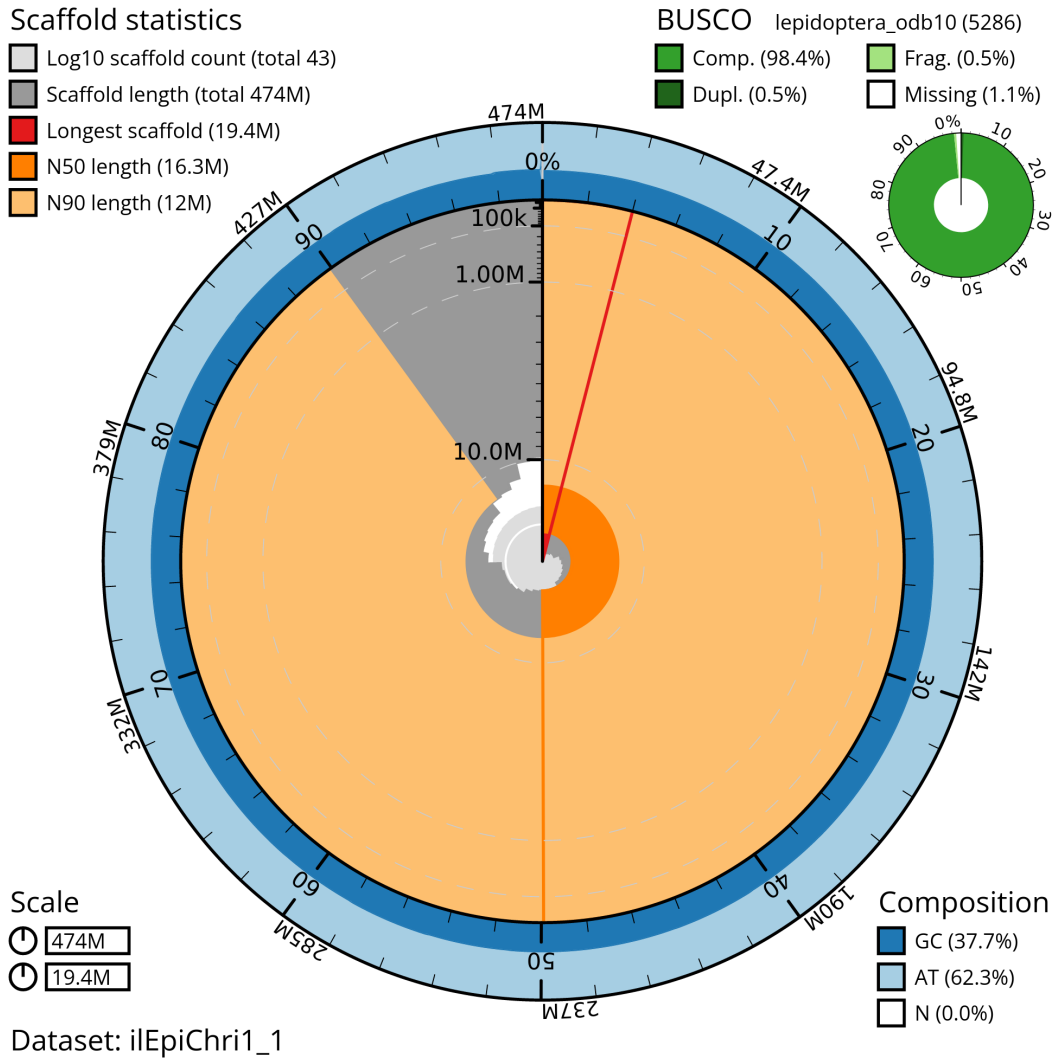

Figure 2. Genome assembly of *Epirrita christyi*, ilEpiChri1.1: metrics. The BlobToolKit snail plot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 474,169,335 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (19,350,054 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (16,313,595 and 11,999,344 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/ilEpiChri1_1/dataset/ilEpiChri1_1/snail.

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.

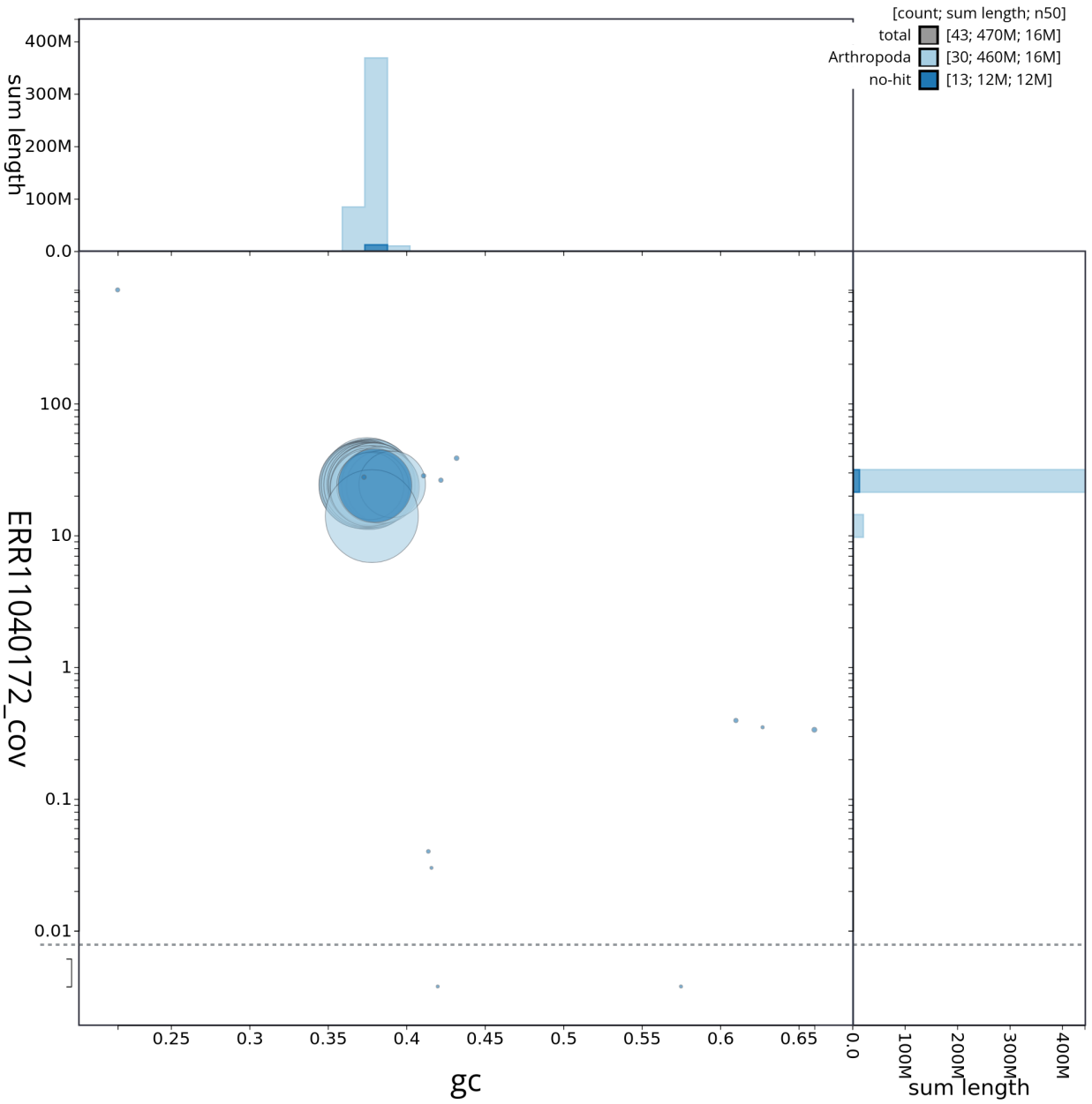

Figure 3. Genome assembly of *Epirrita christyi*, ilEpiChri1.1: Blob plot of base coverage against GC proportion for sequences in the assembly. Sequences are coloured by phylum. Circles are sized in proportion to sequence length. Histograms show the distribution of sequence length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilEpiChri1_1/dataset/ilEpiChri1_1/blob.

Assembly curation

The assembly was decontaminated using the Assembly Screen for Cobionts and Contaminants (ASCC) pipeline (article in preparation). 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. Sex chromosomes were identified by synteny analysis. The curation process is documented at https://gitlab.com/wtsi-grit/rapid-curation (article in preparation).

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,

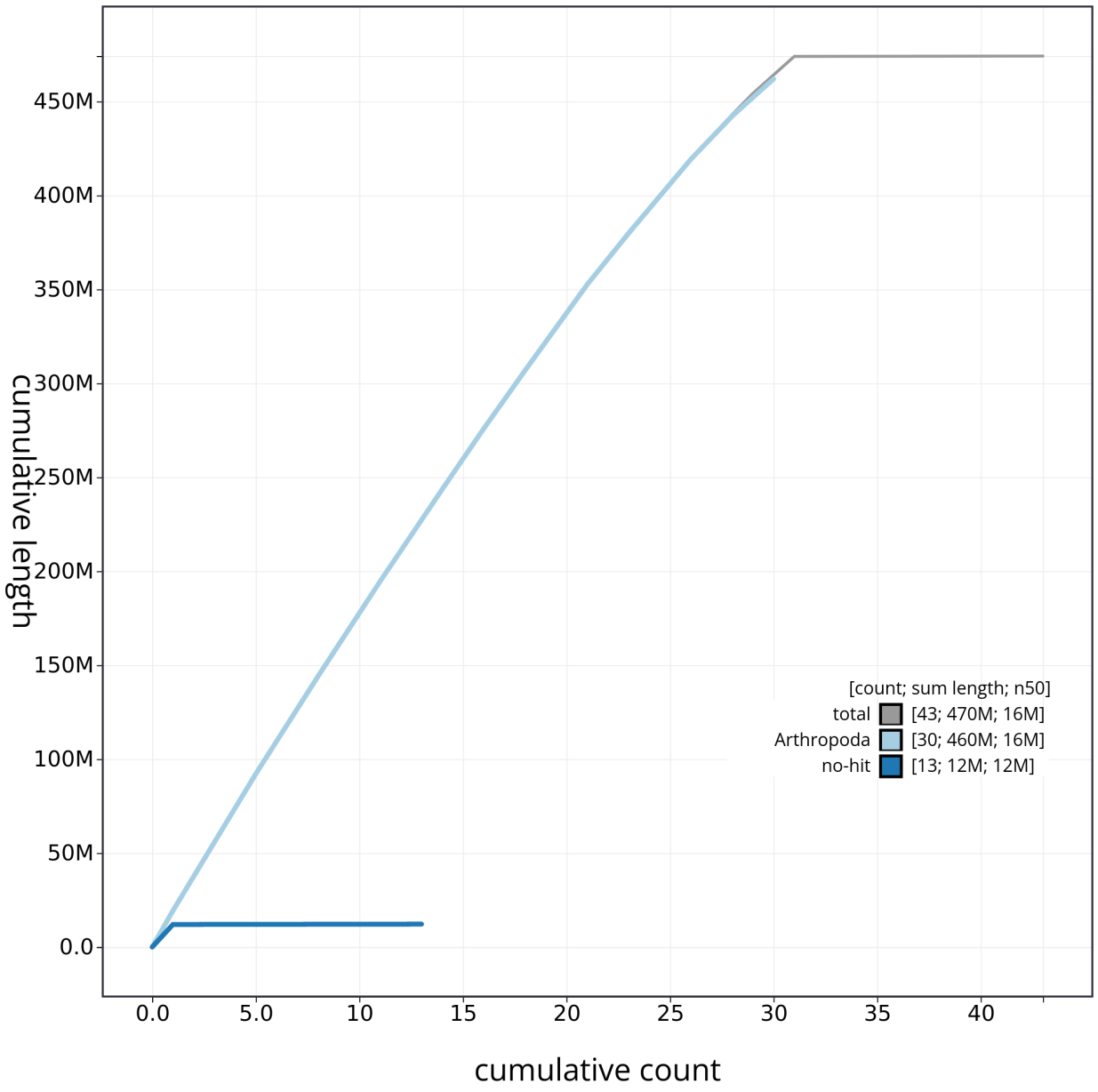

Figure 4. Genome assembly of *Epirrita christyi* ilEpiChri1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all sequences. Coloured lines show cumulative lengths of sequences assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilEpiChri1_1/dataset/ilEpiChri1_1/cumulative.

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.

Genome annotation

The BRAKER2 pipeline (Brůna *et al.*, 2021) was used in the default protein mode to generate annotation for the *Epirrita christyi* assembly (GCA_951392215.1) in Ensembl Rapid Release at the EBI.

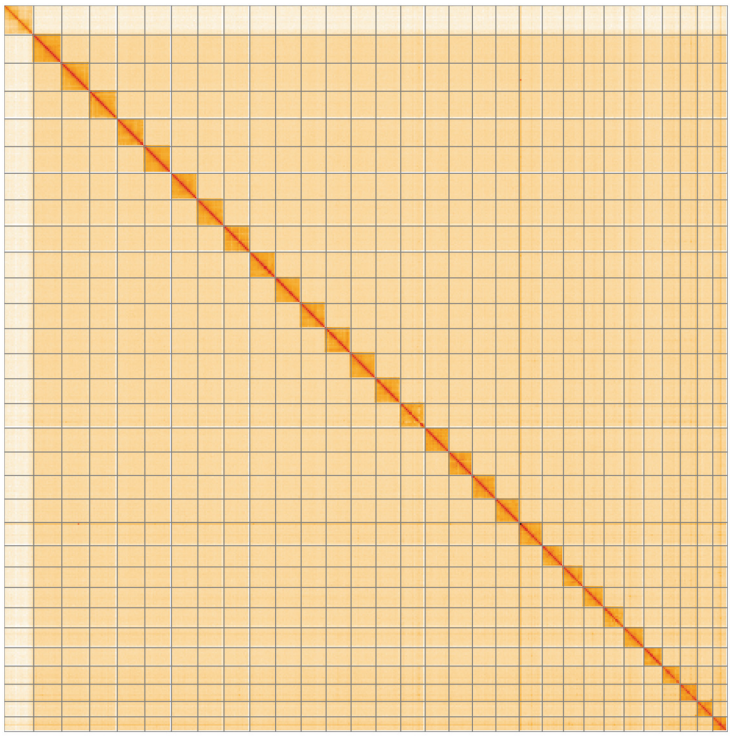

Figure 5. Genome assembly of *Epirrita christyi* ilEpiChri1.1: Hi-C contact map of the ilEpiChri1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=cXBs9OpPQsu7CmKGlsRvOA.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Epirrita christyi*, ilEpiChri1.

INSDC accession	Name	Length (Mb)	GC%
OX595834.1	1	18.39	37.5
OX595835.1	2	18.27	37.5
OX595836.1	3	18.06	37.5
OX595837.1	4	18.05	37.5
OX595838.1	5	17.46	37.5
OX595839.1	6	17.29	37.5
OX595840.1	7	17.12	37.5
OX595841.1	8	16.97	37.0
OX595842.1	9	16.84	37.5
OX595843.1	10	16.74	37.0
OX595844.1	11	16.37	37.5
OX595845.1	12	16.36	37.5
OX595846.1	13	16.31	37.5
OX595847.1	14	16.22	37.5
OX595848.1	15	16.0	38.0
OX595849.1	16	15.59	37.5
OX595850.1	17	15.41	37.5
OX595851.1	18	15.3	37.5
OX595852.1	19	15.3	38.0
OX595853.1	20	15.14	38.0
OX595854.1	21	13.9	38.0
OX595855.1	22	13.4	37.5
OX595856.1	23	13.21	37.5
OX595857.1	24	13.1	37.5
OX595858.1	25	13.0	38.5
OX595859.1	26	12.0	38.0
OX595860.1	27	11.82	38.5
OX595861.1	28	11.24	38.0
OX595862.1	29	10.01	37.5
OX595863.1	30	9.76	39.0
OX595833.1	Z	19.35	38.0
OX595864.1	MT	0.02	21.5

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
Merqury.FK	d00d98157618f4e8d1a91 90026b19b471055b22e	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	1.2a	https://github.com/c-zhou/yahs

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: *Epirrita christyi* (pale November moth). Accession number PRJEB60639; https://identifiers.org/ena.embl/PRJEB60639 (Wellcome Sanger Institute, 2023). The genome sequence is released openly for reuse. The *Epirrita christyi* 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 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

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

Boyes D, Holland PWH, University of Oxford and Wytham Woods Genome Acquisition Lab, *et al.*: **The genome sequence of the Streamer, *Anticlea derivata* (Denis & Schiffermüller, 1775) [version 1; peer review: awaiting peer review].** *Wellcome Open Res.* 2023a; **8**: 254.
Publisher Full Text

Boyes D, Lewis OT, University of Oxford and Wytham Woods Genome Acquisition Lab, *et al.*: **The genome sequence of the broken-barred carpet, *Electrophaes corylata* (Thunberg, 1792) [version 1; peer review: 2 approved].** *Wellcome Open Res.* 2023b; **8**: 283.
PubMed Abstract | Publisher Full Text | Free Full Text

Boyes D, University of Oxford and wytham woods Genome Acquisition Lab, Darwin Tree of Life barcoding collective, *et al.*: **The genome sequence of the Spruce Carpet Moth, *Thera britannica* (Turner, 1925) [version 1; peer review: 2 approved].** *Wellcome Open Res.* 2023c; **8**: 114.
PubMed Abstract | Publisher Full Text | Free Full Text

Boyes D, University of Oxford and Wytham Woods Genome Acquisition Lab, Darwin Tree of Life Barcoding collective, *et al.*: **The genome sequence of the double-striped pug, *Gymnoscelis rufifasciata* (Haworth, 1809) [version 1; peer review: 4 approved].** *Wellcome Open Res.* 2022; **7**: 135.
PubMed Abstract | Publisher Full Text | Free Full Text

Brůna T, Hoff KJ, Lomsadze A, *et al.*: **BRAKER2: automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS supported by a protein database.** *NAR Genom Bioinform.* 2021; **3**(1): lqaa108.
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

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

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

do Amaral RJV, Bates A, Denton A, *et al.*: **Sanger Tree of Life RNA extraction: automated MagMax™ mirVana.** *protocols.io.* 2023.
Publisher 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

GBIF Secretariat: ***Epirrita christyi* (Allen, 1906).** *GBIF Backbone Taxonomy.* 2024; [Accessed 15 August 2024].
Reference Source

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): giaa153.
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

Manni M, Berkeley MR, Seppey 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

Randle Z, Evans-Hill LJ, Parsons MS, *et al.*: **Atlas of Britain & Ireland's larger moths**. Newbury: NatureBureau, 2019.
Reference Source

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.*: **Merqury: 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

Surana P, Muffato M, Qi G: **Sanger-tol/readmapping: sanger-tol/readmapping v1.1.0 - Hebridean Black (1.1.0).** *Zenodo.* 2023.
Publisher Full Text

Surana P, Muffato M, Sadasivan Baby C: **Sanger-tol/genomenote (v1.0.dev).** *Zenodo.* 2023.
Publisher Full Text

Todorovic M, Sampaio F, Howard C: **Sanger Tree of Life HMW DNA fragmentation: diagenode Megaruptor®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: awaiting peer review].** *Wellcome Open Res.* 2024; **9**: 339.
Publisher Full Text

Uliano-Silva M, Ferreira JGRN, Krasheninnikova 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

Waring P, Townsend M, Lewington R: **Field guide to the moths of great Britain and Ireland: Third Edition**. Bloomsbury Wildlife Guides, 2017.
Reference Source

Wellcome Sanger Institute: **The genome sequence of the Pale November moth, *Epirrita christyi* (Allen, 1906)**. European Nucleotide Archive. [dataset], accession number PRJEB60639, 2023.

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 18 November 2024

https://doi.org/10.21956/wellcomeopenres.25396.r109742

✔ **Shruti Iyer**
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA

Review of "The genome sequence of the Pale November moth, *Epirrita christyi* (Allen, 1906)"

The authors have done a great job generating a good quality genome, while also including the mitochondrial genome and annotating the assembled genome.

In terms of the text, I think the manuscript would benefit with an expansion of the background. *Epirrita christyi* seems difficult to accurately identify. While the authors touch upon this difficulty, especially when identifying adult females, they do not elaborate on how they were confident of the sample they collected. Confirmation of species genomically is touched upon later, but addressing this earlier in the text is recommended.

With respect to the figures, I think the color scheme needs to be modified in some of them to improve visibility.

- Figure 2: using shades of green for the BUSCO plot make it difficult to discern between the categories, especially since this part of the figure isn't interactive.

- Figure 3: the colors for total (gray) and arthropoda (light blue) are too similar to distinguish between them. Changing the color scheme will help with better visualization.

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: genetics, genomics, sequencing technologies, technology development, targeted sequencing.

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.

Reviewer Report 08 November 2024

https://doi.org/10.21956/wellcomeopenres.25396.r109737

Arun Arumugaperumal
Department of Biotechnology, Rajalakshmi Engineering College, Tamilnadu, Thandalam, Chennai, 602105, India

This is the first published article on the genome sequence of the Pale November moth *Epirrita christyi*. The assembly was of length 474.2 Mb. The mitogenome was also assembled and of size 15.99 kb. The assembly had allowed identification of 16,983 genes. Long read sequencing technology has been used to arrive at a near-complete genome sequence. The methods followed have been clearly explained.

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: Bioinformatics, 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.

Reviewer Report 18 October 2024

https://doi.org/10.21956/wellcomeopenres.25396.r103842

Zoltan Erdos
University of Exeter Cornwall Campus (Ringgold ID: 151778), Penryn, England, UK
Ben Raymond
University of Exeter, Penryn, England, UK

This is a very high quality genome of a nice little geometrid that is hard to identify from morphological traits.

I have few comments - I thought the background section could also mention that the larvae in this genus are also hard to separate based on morphology- something that is complicated by the occurence of melanic forms. The implication that a genome could aid future identifiication could be stated explicitly.

I wasn't convinced of the value of all the blob plots - eg Fig 4. This could have been summarized in the text.

The chromosomal level assembly was particularly impressive..

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: Entomology, evolutionary biology, ecology

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.