




DATA NOTE

The genome sequence of the Barred Red, *Hylaea fasciaria* (Linnaeus, 1758) [version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual male *Hylaea fasciaria* (the Barred Red; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence is 327.9 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 17.18 kilobases in length. Gene annotation of this assembly on Ensembl identified 17,216 protein coding genes.

Keywords

Hylaea fasciaria, Barred Red, 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; Amphimesenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Geometroidae; Geometridae; Ennominae; *Hylaea*; *Hylaea fasciaria* (Linnaeus, 1758) (NCBI:txid722673).

Background

Hylaea fasciaria, commonly known as the Barred Red, is a moth species of the family Geometridae, subfamily Ennominae. It is a Palearctic species, distributed across central and northern Europe, the Urals, Caucasus, Altai, and eastern Siberia (GBIF Secretariat, 2022). It is widespread and common in Britain and Ireland in regions of coniferous woodland (Kimber, 2023).

The Barred Red is a typical size for a Geometrid moth, with a wingspan of 27–40 mm. The Barred Red has two main forms: a rust red form (*f. fasciaria*) and a green form (*f. prasinaria*) (Sihvonen *et al.*, 2014). The red form is found more commonly in Britain, while *H. fasciaria prasinaria* is found more frequently in Europe, with rare records from the south-east of England. In both forms, the front wings are slightly curved with thin transverse bands and a slightly dark closed midfield. These bands are pale whitish coloured in the rust-red form, while in the green form the colouring of the bands varies from pale whitish to beige to light brown. In addition to the two main forms, there are other colour variants with different shades of green or plain brown (Sihvonen *et al.*, 2014).

The *H. fasciaria* caterpillar is greyish or greyish brown with some brownish smears and a light brown head, with series of small warts on the back. The caterpillar feeds on coniferous trees, including *Pinus sylvestris* and *Picea abies*. The larva grows slowly and enters hibernation in winter, usually roosting on green spruce twigs, and often stretching along a single needle. This behaviour has been observed to provide the larva with sufficient food while reducing the risk of predation and protecting the body of the caterpillar from sudden fluctuations in body temperature (Dvořáčková & Kulfan, 2009).

Pupation occurs in spring, lasting 4 to 6 weeks. On the continent, the adults fly in two generations from April to October, but the species is univoltine in Britain and northern Europe, where it flies from June to August (Waring *et al.*, 2017). Adults are nocturnal, and come to light, sometimes in great numbers (Kimber, 2023).

The genome sequencing of *Hylaea fasciaria*, part of the Darwin Tree of Life project, is expected to broaden our ecological understanding of this moth. It will provide comprehensive DNA reference data for molecular surveillance of the species and offer valuable insights into its phylogenetic relationship with other similar species.

Genome sequence report

The genome was sequenced from one male *Hylaea fasciaria* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.76, -1.34). A total of 72-fold coverage in Pacific Biosciences single-molecule HiFi long reads and 97-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 8 missing joins or misjoins and removed one haplotypic duplication, reducing the scaffold number by 17.5%, and increasing the scaffold N50 by 3.08%.

The final assembly has a total length of 327.9 Mb in 33 sequence scaffolds with a scaffold N50 of 11.7 Mb (Table 1). Most (99.98%) 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 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 55.5 with *k*-mer completeness of 99.99%, 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* = 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/722673>.



Figure 1. Photograph of the *Hylaea fasciaria* (ilHylFasc1) specimen used for genome sequencing.

Table 1. Genome data for *Hylaea fasciaria*, ilHylFasc1.1.

Project accession data		
Assembly identifier	ilHylFasc1.1	
Species	<i>Hylaea fasciaria</i>	
Specimen	ilHylFasc1	
NCBI taxonomy ID	722673	
BioProject	PRJEB42128	
BioSample ID	SAMEA7520684	
Isolate information	ilHylFasc1, male: head and thorax (DNA sequencing and Hi-C scaffolding)	
Assembly metrics*	Benchmark	
Consensus quality (QV)	55.5	≥ 50
<i>k</i> -mer completeness	99.99%	≥ 95%
BUSCO**	C:98.4%[S:97.9%,D:0.5%], F:0.4%,M:1.2%,n:5,286	C ≥ 95%
Percentage of assembly mapped to chromosomes	99.98%	≥ 95%
Sex chromosomes	Z chromosome	<i>localised homologous pairs</i>
Organelles	Mitochondrial genome assembled	<i>complete single alleles</i>
Raw data accessions		
PacificBiosciences SEQUEL II	ERR6412026	
10X Genomics Illumina	ERR6002646, ERR6002648, ERR6002647, ERR6002649	
Hi-C Illumina	ERR6002645	
Genome assembly		
Assembly accession	GCA_905147375.1	
<i>Accession of alternate haplotype</i>	GCA_905147295.1	
Span (Mb)	327.9	
Number of contigs	44	
Contig N50 length (Mb)	11.1	
Number of scaffolds	33	
Scaffold N50 length (Mb)	11.7	
Longest scaffold (Mb)	17.1	
Genome annotation		
Number of protein-coding genes	17,216	
Number of gene transcripts	17,406	

* 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/ilHylFasc1.1/dataset/CAJHVH01.1/busco>.

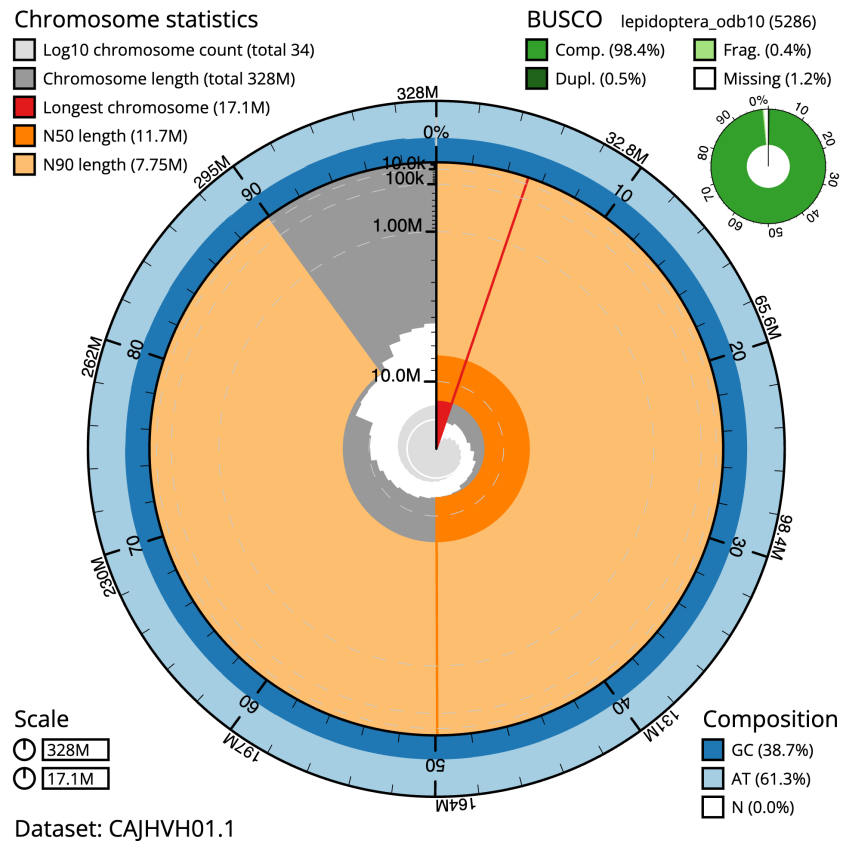


Figure 2. Genome assembly of *Hylaea fasciaria*, iHylFasc1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 327,936,668 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (17,069,495 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (11,686,647 and 7,753,621 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/iHylFasc1.1/dataset/CAJHVH01.1/snail>.

Genome annotation report

The *Hylaea fasciaria* genome assembly (GCA_905147375.1) was annotated using the Ensembl rapid annotation pipeline (Table 1; https://rapid.ensembl.org/Hylaea_fasciaria_GCA_905147375.1/Info/Index). The resulting annotation includes 17,406 transcribed mRNAs from 17,216 protein-coding genes.

Methods

Sample acquisition and nucleic acid extraction

A male *Hylaea fasciaria* (specimen ID Ox000468, individual iHylFasc1) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.76, longitude -1.34) on 2020-06-13 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 iHylFasc1 sample was weighed

and dissected on dry ice with tissue set aside for Hi-C sequencing. Head and thorax 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 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.

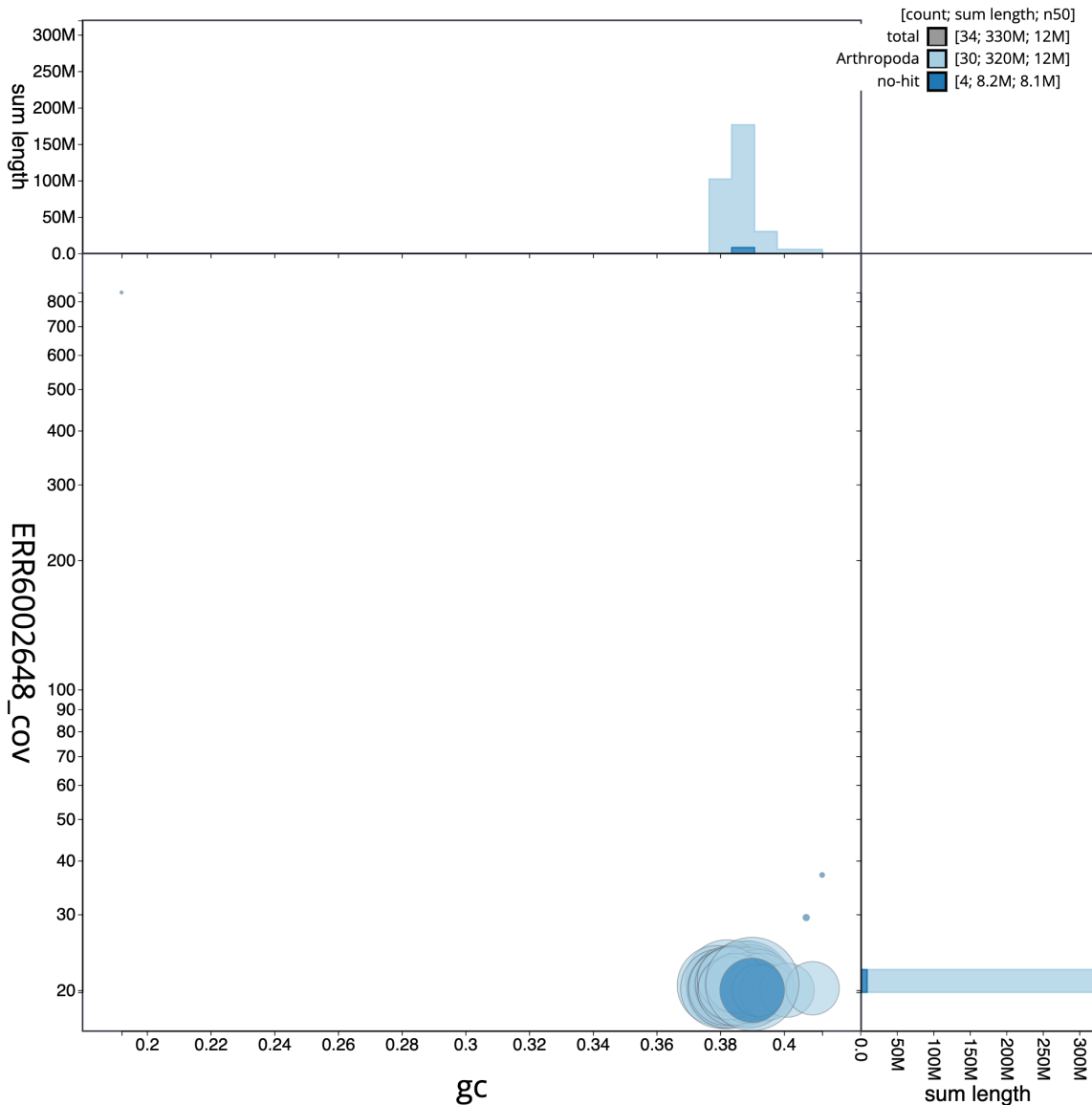


Figure 3. Genome assembly of *Hylaea fasciaria*, ilHylFasc1.1: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ilHylFasc1.1/dataset/CAJHVH01.1/blob>.

Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud DNA sequencing libraries were constructed according to the manufacturers' instructions. DNA sequencing was performed by the Scientific Operations core at the WSI on Pacific Biosciences SEQUEL II (HiFi) and HiSeq X Ten (10X) instruments. Hi-C data were also generated from head and thorax tissue of ilHylFasc1 that had been set aside, using the Arima2 kit and sequenced on the HiSeq X Ten 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

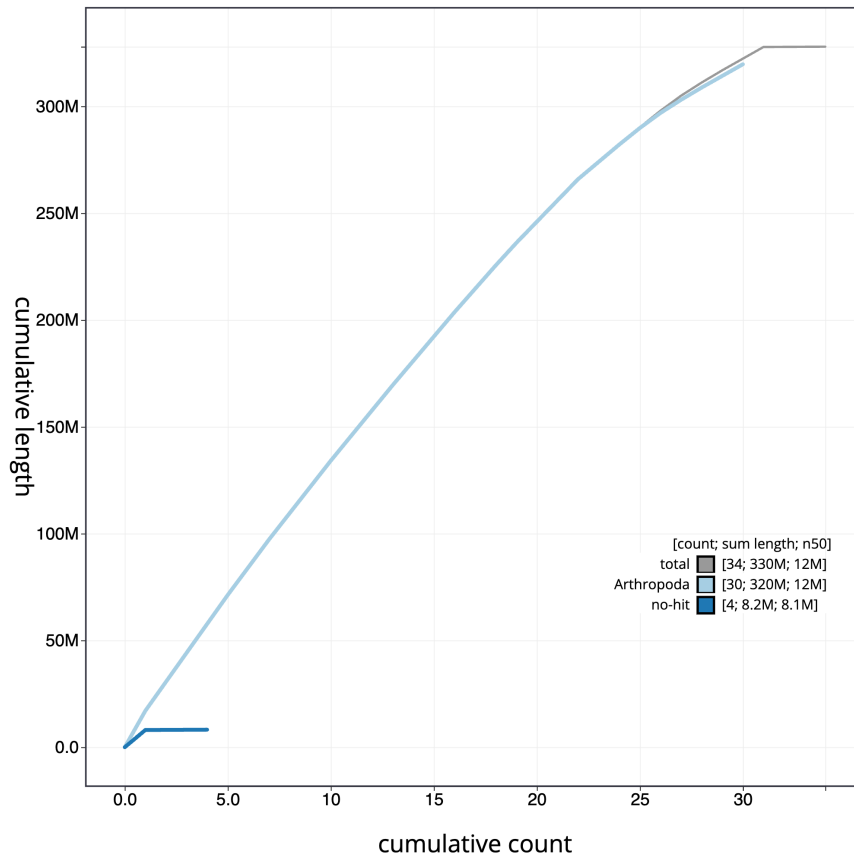


Figure 4. Genome assembly of *Hylaea fasciaria*, ilHylFasc1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ilHylFasc1.1/dataset/CAJHvh01.1/cumulative>.

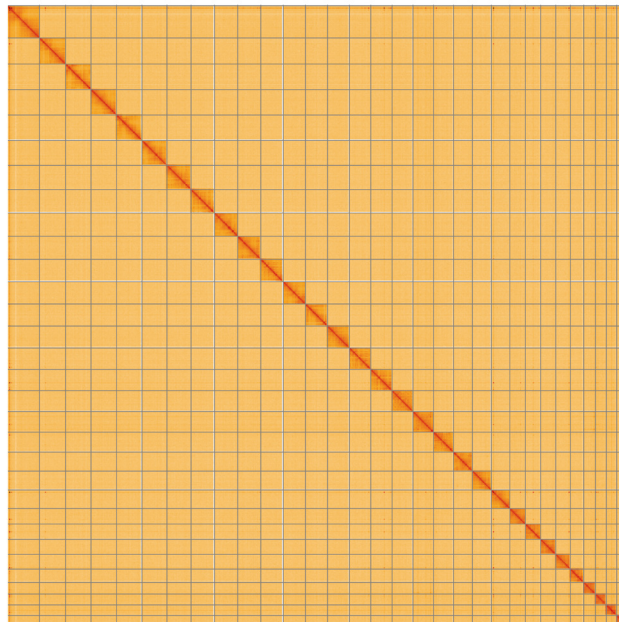


Figure 5. Genome assembly of *Hylaea fasciaria*, ilHylFasc1.1: Hi-C contact map of the ilHylFasc1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at <https://genome-note-higlass.tol.sanger.ac.uk/?d=Irlrd5-MSvOiiPqAGjoSQ>.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Hylaea fasciaria*, ilHyIFasc1.

INSDC accession	Chromosome	Length (Mb)	GC%
LR990193.1	1	13.79	38.5
LR990194.1	2	13.49	39.0
LR990195.1	3	13.49	39.0
LR990196.1	4	13.46	39.0
LR990197.1	5	13.16	38.0
LR990198.1	6	12.76	38.0
LR990199.1	7	12.41	38.0
LR990200.1	8	12.3	38.0
LR990201.1	9	12.21	38.5
LR990202.1	10	11.86	38.0
LR990203.1	11	11.77	38.5
LR990204.1	12	11.69	38.5
LR990205.1	13	11.57	38.5
LR990206.1	14	11.34	38.0
LR990207.1	15	11.25	38.5
LR990208.1	16	11.05	38.5
LR990209.1	17	10.84	39.0
LR990210.1	18	10.54	39.0
LR990211.1	19	10.04	39.0
LR990212.1	20	10.01	38.5
LR990213.1	21	9.82	39.0
LR990214.1	22	8.2	38.5
LR990215.1	23	8.08	39.0
LR990216.1	24	8.0	39.5
LR990217.1	25	7.75	38.5
LR990218.1	26	7.05	38.5
LR990219.1	27	6.16	39.5
LR990220.1	28	5.73	40.0
LR990221.1	29	5.57	41.0
LR990222.1	30	5.34	39.0
LR990192.1	Z	17.07	39.0
LR990223.1	MT	0.02	19.0

(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 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 BRAKER2 pipeline (Brůna *et al.*, 2021) was used in the default protein mode to generate annotation for the *Hylaea fasciaria* assembly (GCA_905147375.1) 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.

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	4.1.5	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	https://gitlab.com/ezlab/busco
FreeBayes	1.3.1-17-gaa2ace8	https://github.com/freebayes/freebayes
gEVAL	N/A	https://geval.org.uk/
Hifiasm	0.12	https://github.com/chhyllp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Long Ranger ALIGN	2.2.2	https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines
Mercury	MercuryFK	https://github.com/thegenemyers/MERURY.FK
MitoHiFi	1	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
SALSA	2.2	https://github.com/salsa-rs/salsa
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

Data availability

European Nucleotide Archive: *Hylaea fasciaria* (barred red). Accession number PRJEB42128; <https://identifiers.org/ena.embl/PRJEB42128>. (Wellcome Sanger Institute, 2021)

The genome sequence is released openly for reuse. The *Hylaea fasciaria* 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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