DATA NOTE

The genome sequence of the July Highflyer, *Hydriomena furcata* (Thunberg, 1784) [version 1; peer review: awaiting peer review]

Douglas Boyes[1+], Peter W. H. Holland[2],
University of Oxford and Wytham Woods Genome Acquisition Lab,
Darwin Tree of Life Barcoding collective,
Wellcome Sanger Institute Tree of Life programme,
Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective,
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

[+] Deceased author

v1 **First published:** 26 Oct 2023, **8**:496
https://doi.org/10.12688/wellcomeopenres.20182.1
Latest published: 26 Oct 2023, **8**:496
https://doi.org/10.12688/wellcomeopenres.20182.1

Abstract

We present a genome assembly from an individual male *Hydriomena furcata* (the July Highflyer; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence is 423.3 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 15.89 kilobases in length. Gene annotation of this assembly on Ensembl identified 17,324 protein coding genes.

Keywords

Hydriomena furcata, July Highflyer, 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.

Corresponding author: Darwin Tree of Life Consortium (mark.blaxter@sanger.ac.uk)

Author roles: Boyes D: Investigation, Resources; **Holland PWH**: Writing – Original Draft Preparation, Writing – Review & Editing;

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Wellcome through core funding to the Wellcome Sanger Institute (206194) and the Darwin Tree of Life Discretionary Award (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, Holland PWH, University of Oxford and Wytham Woods Genome Acquisition Lab *et al.* **The genome sequence of the July Highflyer, *Hydriomena furcata* (Thunberg, 1784) [version 1; peer review: awaiting peer review]** Wellcome Open Research 2023, **8**:496 https://doi.org/10.12688/wellcomeopenres.20182.1

First published: 26 Oct 2023, **8**:496 https://doi.org/10.12688/wellcomeopenres.20182.1

Species taxonomy

Eukaryota; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Geometroidea; Geometridae; Larentiinae; *Hydriomena*; *Hydriomena furcata* (Thunberg, 1784) (NCBI:txid104460).

Background

The July highflyer *Hydriomena furcata* is a widely distributed moth in the superfamily Geometridae found across Europe and Asia, ranging from Portugal and Ireland to the far east of Russia and Japan. The species is also widespread in Canada and the United States (GBIF Secretariat, 2023). In the UK, *H. furcata* is univoltine with adults on the wing in July and August in woodlands, moorlands, hedgerows and suburban areas (NBN Atlas Partnership, 2023). The forewings of the adult moth are delicately patterned with a series of wavy bands of alternating dark and light shading, most likely providing protection through crypsis on patchy and irregular surfaces including tree bark and lichen. Wing shape is unusual with a distinct 'shoulder' at the base of the leading wing edge, but colouration is highly variable. One common form has cream and black bands crossing a bright olive green ground colour; other individuals are dark reddish-brown with little green visible.

Larvae of *H. furcata* have been recorded feeding on the leaves of a wide range of deciduous plants including willow (*Salix*), poplar (*Populus*), alder (*Alnus*), birch (*Betula*), bilberry (*Vaccinium myrtillus*) and heather (*Calluna vulgaris*) (South, 1961). High densities can sometimes be recorded; one study estimated that the abundant larvae of *H. furcata* consumed 50% of the annual growth of mature heather in a moorland region of northern England (Fielding, 1992). In stands of heather *Calluna vulgaris,* late instar larvae spin a small silken feeding web on mature plants (Fielding, 1992).

The genome of the July highflyer, *Hydriomena furcata*, will facilitate studies into polyphagy and insect colour polymorphism, and contribute to the growing set of resources for studying lepidopteran evolution. Here we present a chromosomally complete genome sequence for *Hydriomena furcata*, based on one male specimen of the green form, collected from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome was sequenced from one male *Hydriomena furcata* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, –1.33). A total of 42-fold coverage in Pacific Biosciences single-molecule HiFi long reads and 98-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 18 missing joins or mis-joins and removed one haplotypic duplication, reducing the scaffold number by 24.44%, and decreasing the scaffold N50 by 2.58%.

Figure 1. Photograph of the *Hydriomena furcata* (ilHydFurc1) specimen used for genome sequencing.

The final assembly has a total length of 423.3 Mb in 34 sequence scaffolds with a scaffold N50 of 16.1 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 (99.97%) of the assembly sequence was assigned to 28 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 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 61.1 with *k*-mer completeness of 100%, and the assembly has a BUSCO v5.3.2 completeness of 98.3% (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/104460.

Genome annotation report

The *Hydriomena furcata* genome assembly (GCA_912999785.1) was annotated using the Ensembl rapid annotation pipeline (Table 1; https://rapid.ensembl.org/Hydriomena_furcata_GCA_912999785.1/Info/Index). The resulting annotation includes 17,492 transcribed mRNAs from 17,324 protein-coding genes.

Table 1. Genome data for *Hydriomena furcata*, ilHydFurc1.1.

Project accession data		
Assembly identifier	ilHydFurc1.1	
Assembly release date	2021-08-18	
Species	*Hydriomena furcata*	
Specimen	ilHydFurc1	
NCBI taxonomy ID	104460	
BioProject	PRJEB45668	
BioSample ID	SAMEA7701301	
Isolate information	ilHydFurc1, male: abdomen (DNA sequencing), head and thorax (Hi-C scaffolding and RNA sequencing)	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	61.1	*≥ 50*
k-mer completeness	100%	*≥ 95%*
BUSCO**	C:98.3%[S:97.9%,D:0.5%], F:0.4%,M:1.3%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.97%	*≥ 95%*
Sex chromosomes	Z chromosome	*localised homologous pairs*
Organelles	Mitochondrial genome assembled	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR6939218	
10X Genomics Illumina	ERR6363290, ERR6363291, ERR6363292, ERR6363293	
Hi-C Illumina	ERR6363294	
PolyA RNA-Seq Illumina	ERR9434989	
Genome assembly		
Assembly accession	GCA_912999785.1	
Accession of alternate haplotype	GCA_912999755.1	
Span (Mb)	423.3	
Number of contigs	55	
Contig N50 length (Mb)	13.9	
Number of scaffolds	34	
Scaffold N50 length (Mb)	16.1	
Longest scaffold (Mb)	25.7	
Genome annotation		
Number of protein-coding genes	17,324	
Number of gene transcripts	17,492	

* 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/ilHydFurc1.1/dataset/CAJVWF01.1/busco.

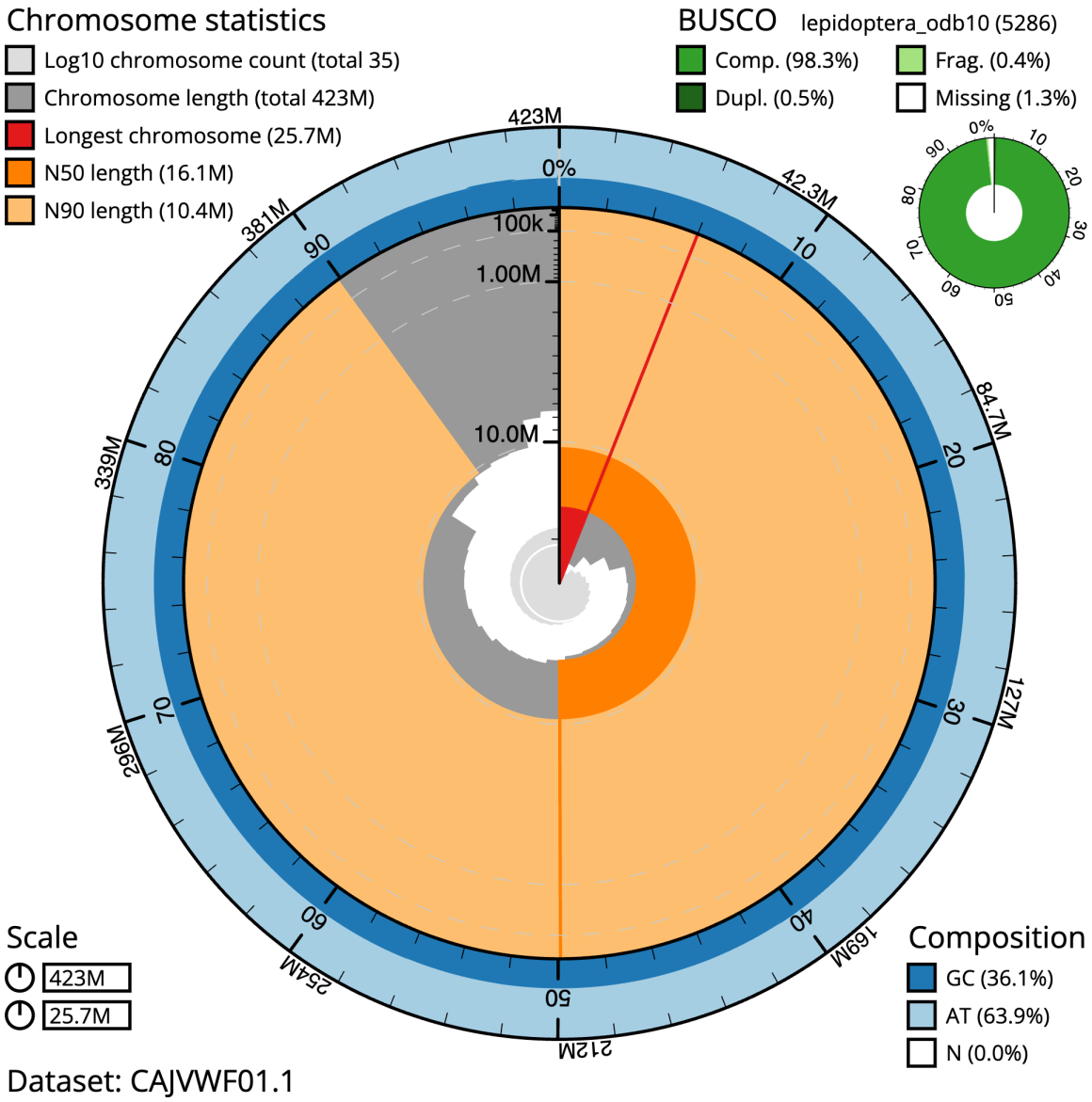

Figure 2. Genome assembly of *Hydriomena furcata*, ilHydFurc1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 423,317,948 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (25,664,163 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (16,092,901 and 10,438,587 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/ilHydFurc1.1/dataset/CAJVWF01.1/snail.

Methods

Sample acquisition and nucleic acid extraction

A male *Hydriomena furcata* (specimen ID Ox000534, ToLID ilHydFurc1) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.33) on 2020-06-25 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 ilHydFurc1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C

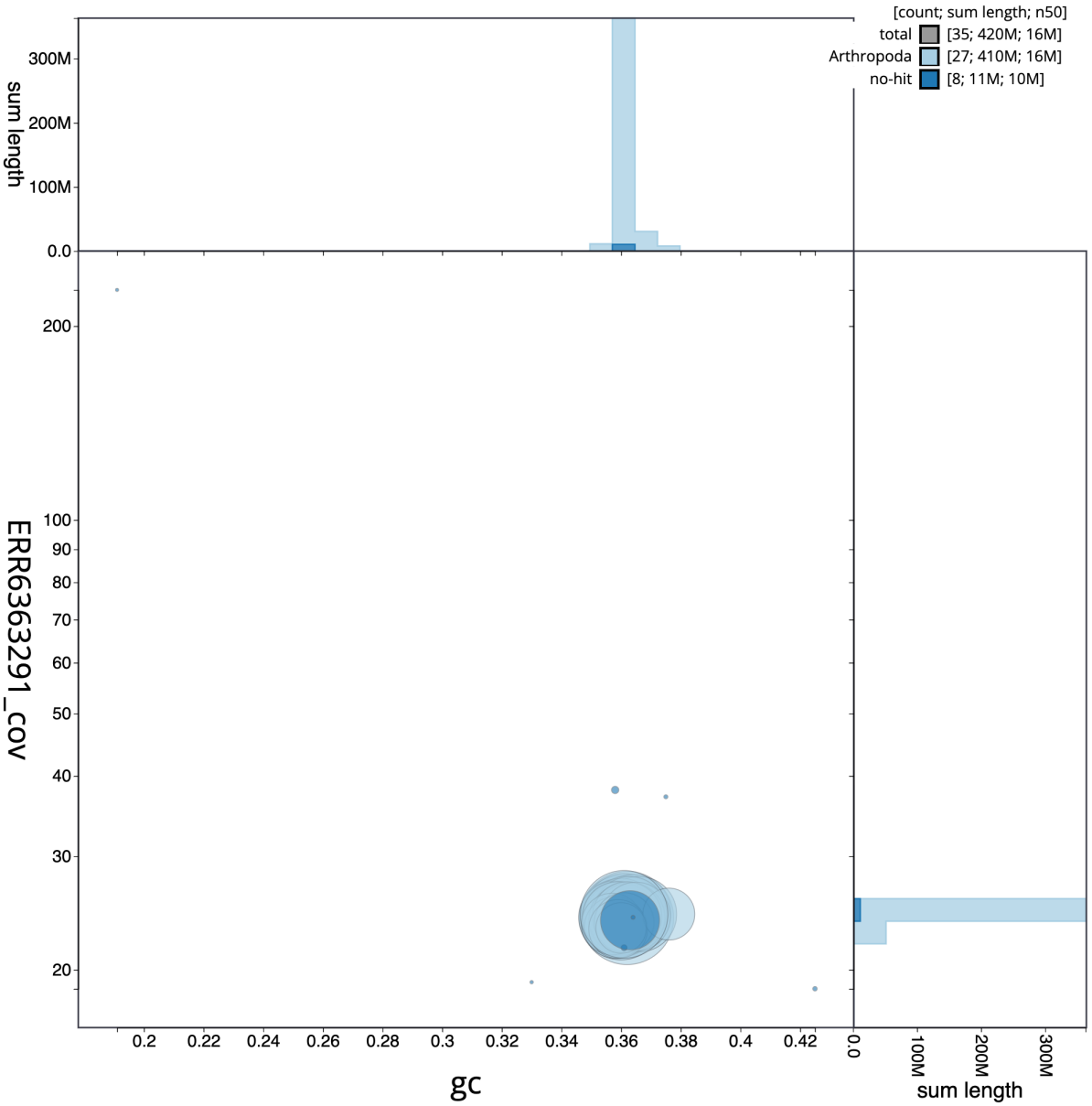

Figure 3. Genome assembly of *Hydriomena furcata*, ilHydFurc1.1: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilHydFurc1.1/dataset/CAJVWF01.1/blob.

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 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.

RNA was extracted from head and thorax tissue of ilHydFurc1 in the Tree of Life Laboratory at the WSI using TRIzol, according to the manufacturer's instructions. RNA was then eluted in 50 μl RNAse-free water and its concentration assessed using a Nanodrop spectrophotometer and Qubit Fluorometer

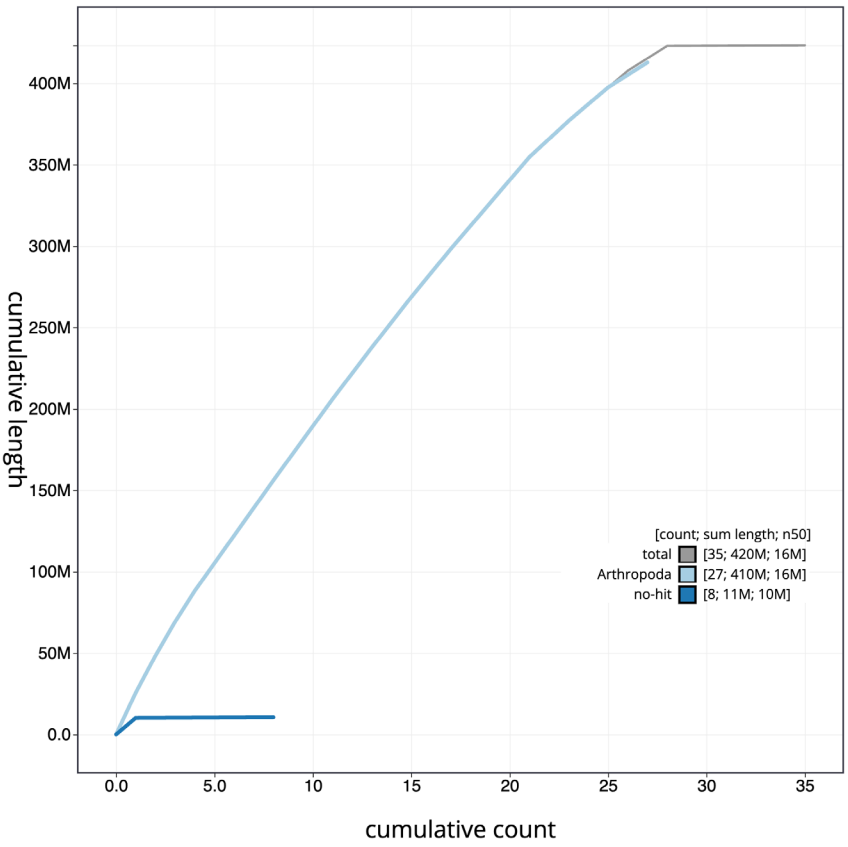

Figure 4. Genome assembly of *Hydriomena furcata*, ilHydFurc1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilHydFurc1.1/dataset/CAJVWF01.1/cumulative.

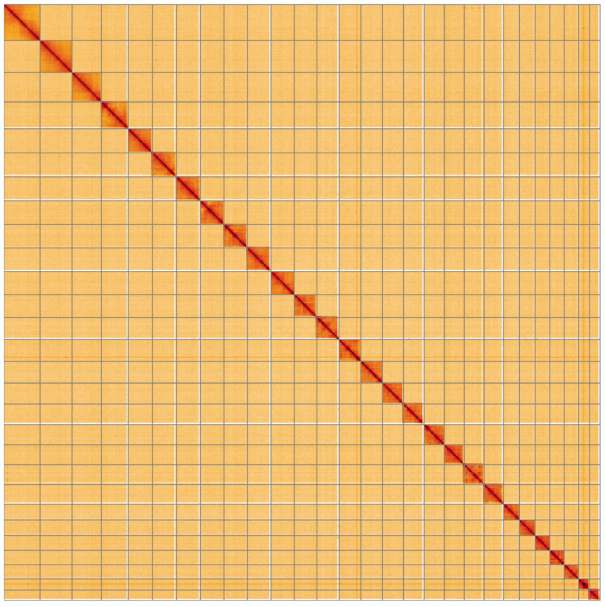

Figure 5. Genome assembly of *Hydriomena furcata*, ilHydFurc1.1: Hi-C contact map of the ilHydFurc1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=emIrPe0SQe6WaCmtohrc7A.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Hydriomena furcata*, ilHydFurc1.

INSDC accession	Chromosome	Length (Mb)	GC%
OU538820.1	1	25.66	36.2
OU538822.1	2	20.88	36
OU538823.1	3	19	36.1
OU538824.1	4	17.18	36.2
OU538825.1	5	17.02	35.9
OU538847.1	6	16.9	36.4
OU538826.1	7	16.85	36.2
OU538827.1	8	16.68	36.6
OU538828.1	9	16.65	36
OU538829.1	10	16.52	36
OU538830.1	11	16.09	36
OU538831.1	12	15.68	35.8
OU538832.1	13	15.54	36.3
OU538833.1	14	15.37	35.9
OU538834.1	15	14.89	35.9
OU538835.1	16	14.56	36.1
OU538836.1	17	14.3	36.3
OU538837.1	18	14.2	36.2
OU538838.1	19	14.07	36.2
OU538839.1	20	13.93	36.5
OU538840.1	21	11.18	35.8
OU538841.1	22	11.13	35.7
OU538842.1	23	10.44	36.1
OU538843.1	24	10.2	36.3
OU538848.1	25	10.1	35.9
OU538844.1	26	7.86	37.6
OU538845.1	27	7.43	36
OU538821.1	Z	22.68	36.1
OU538846.1	MT	0.02	19.1

using the Qubit RNA Broad-Range (BR) Assay kit. Analysis of the integrity of the RNA was done using Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud 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 II (HiFi), Illumina HiSeq 4000 (RNA-Seq) and Illumina NovaSeq 6000 (10X) instruments. Hi-C data were also generated from head and thorax tissue of ilHydFurc1 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 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.*, 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 *Hydriomena furcata* assembly (GCA_912999785.1) 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

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
FreeBayes	1.3.1-17-gaa2ace8	https://github.com/freebayes/freebayes
Hifiasm	0.15	https://github.com/chhylp123/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
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
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

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: *Hydriomena furcata* (June highflyer). Accession number PRJEB45668; https://identifiers.org/ena.embl/PRJEB45668. (Wellcome Sanger Institute, 2021)

The genome sequence is released openly for reuse. The *Hydriomena furcata* 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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