DATA NOTE

The genome sequence of Vine's Rustic moth, *Hoplodrina ambigua* (Denis & Schiffermüller, 1775) [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 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

[+] Deceased author

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Abstract

We present a genome assembly from an individual female *Hoplodrina ambigua* (Vine's Rustic; Arthropoda; Insecta; Lepidoptera; Noctuidae). The genome sequence is 438.1 megabases in span. Most of the assembly is scaffolded into 32 chromosomal pseudomolecules, including the Z and W sex chromosomes. The mitochondrial genome has also been assembled and is 15.39 kilobases in length. Gene annotation of this assembly on Ensembl identified 18,878 protein coding genes.

Keywords

Hoplodrina ambigua, Vine's Rustic moth, 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)

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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; Noctuoidea; Noctuidae; Xyleninae; *Hoplodrina*; *Hoplodrina* ambigua (Denis & Schiffermüller, 1775) (NCBI:txid875924).

Background

The genus *Hoplodrina* in the family Noctuidae contains several similar looking moths with grey-brown forewings and rounded, white-edged reniform and orbicular stigmata. In Britain and Ireland, three species of *Hoplodrina* can be difficult to distinguish with certainty from wing pattern alone: the Rustic *H. blanda*, the Uncertain *H. octogenaria* (synonym *H. alsines*) and Vine's Rustic *H. ambigua*. The last of these was unknown in the UK before the 1880s when a few specimens were recorded independently by Vine, Tutt and Hodges from sites along the south coast of England and on the Isle of Wight (Hodges, 1890; Hodges, 1895; South, 1907; Tutt, 1891). For several decades the species remained a rarity, with specimens likely to be occasional vagrants from mainland Europe plus some individuals from temporary colonies (Heath & Emmet, 1983).

In the 1940s *H. ambigua* became established as a breeding species in the south of the UK, and has continued to extend its range northwards ever since. The species also spread into Scandinavia around the same time (Heath & Emmet, 1983). *H. ambigua* is now well established and common across the midlands and south of England and south Wales (NBN Atlas Partnership, 2023). Adults are first seen in May and June, with the resultant eggs and larvae developing rapidly over summer leading to a more numerous second generation in August and September (NBN Atlas Partnership, 2023). The larvae of *H. ambigua* are polyphagous and feed on low-growing herbaceous plants; adults are strongly attracted to light and sugar, sometimes in abundance.

The moth is also bivoltine in much of mainland Europe, although it has a single generation at higher altitudes (Wagner, 2023). Further east and south, *H. ambigua* has been recorded sporadically from Turkey, Russia, Ukraine, Georgia, Azerbaijan, Turkmenistan, Iran and Egypt (Ahmadi *et al.*, 2022; Amer *et al.*, 2018; GBIF Secretariat, 2023).

The genome sequence of *Hoplodrina ambigua* was determined and assembled as part of the Darwin Tree of Life project. The complete genome sequence will contribute to the growing set of resources for studying molecular evolution in the Lepidoptera.

Genome sequence report

The genome was sequenced from one female *Hoplodrina ambigua* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, –1.34). A total of 57-fold coverage in Pacific Biosciences single-molecule HiFi long reads was generated. Primary assembly contigs were scaffolded with chromosome

Figure 1. Photograph of the *Hoplodrina ambigua* (ilHopAmbi1) specimen used for genome sequencing.

conformation Hi-C data. Manual assembly curation corrected 8 missing joins or mis-joins and removed 2 haplotypic duplications, reducing the scaffold number by 7.89%, and increasing the scaffold N50 by 4.27%.

The final assembly has a total length of 438.1 Mb in 34 sequence scaffolds with a scaffold N50 of 15.3 Mb (Table 1). The snailplot in Figure 2 provides a summary of the assembly statistics, 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 (99.99%) of the assembly sequence was assigned to 32 chromosomal-level scaffolds, representing 30 autosomes and the Z and W sex chromosomes. 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 72.9 with *k*-mer completeness of 100.0%, and the assembly has a BUSCO v5.3.2 completeness of 98.9% (single = 98.5%, duplicated = 0.4%), using the lepidoptera_odb10 reference set (n = 5,286).

Metadata for specimens, barcode results, spectra estimates, sequencing runs, contaminants and pre-curation assembly statistics are given at https://links.tol.sanger.ac.uk/species/875924.

Genome annotation report

The *Hoplodrina ambigua* genome assembly (GCA_949774945.1) was annotated using the Ensembl rapid annotation pipeline

Table 1. Genome data for *Hoplodrina ambigua*, ilHopAmbi1.1.

Project accession data		
Assembly identifier	ilHopAmbi1.1	
Species	*Hoplodrina ambigua*	
Specimen	ilHopAmbi1	
NCBI taxonomy ID	875924	
BioProject	PRJEB60635	
BioSample ID	SAMEA8603197	
Isolate information	ilHopAmbi1	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	72.9	*≥ 50*
k-mer completeness	100.0%	*≥ 95%*
BUSCO**	C:98.9%[S:98.5%,D:0.4%], F:0.2%,M:0.9%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.99%	*≥ 95%*
Sex chromosomes	ZW	*localised homologous pairs*
Organelles	Mitochondrial genome: 15.39 kb	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR11029658	
Hi-C Illumina	ERR11040162	
PolyA RNA-Seq Illumina	ERR12245544	
Genome assembly		
Assembly accession	GCA_949774945.1	
Accession of alternate haplotype	GCA_949774985.1	
Span (Mb)	438.1	
Number of contigs	71	
Contig N50 length (Mb)	10.5	
Number of scaffolds	34	
Scaffold N50 length (Mb)	15.3	
Longest scaffold (Mb)	23.22	
Genome annotation		
Number of protein-coding genes	18,878	
Number of gene transcripts	19,063	

* 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/ilHopAmbi1_1/dataset/ilHopAmbi1_1/busco.

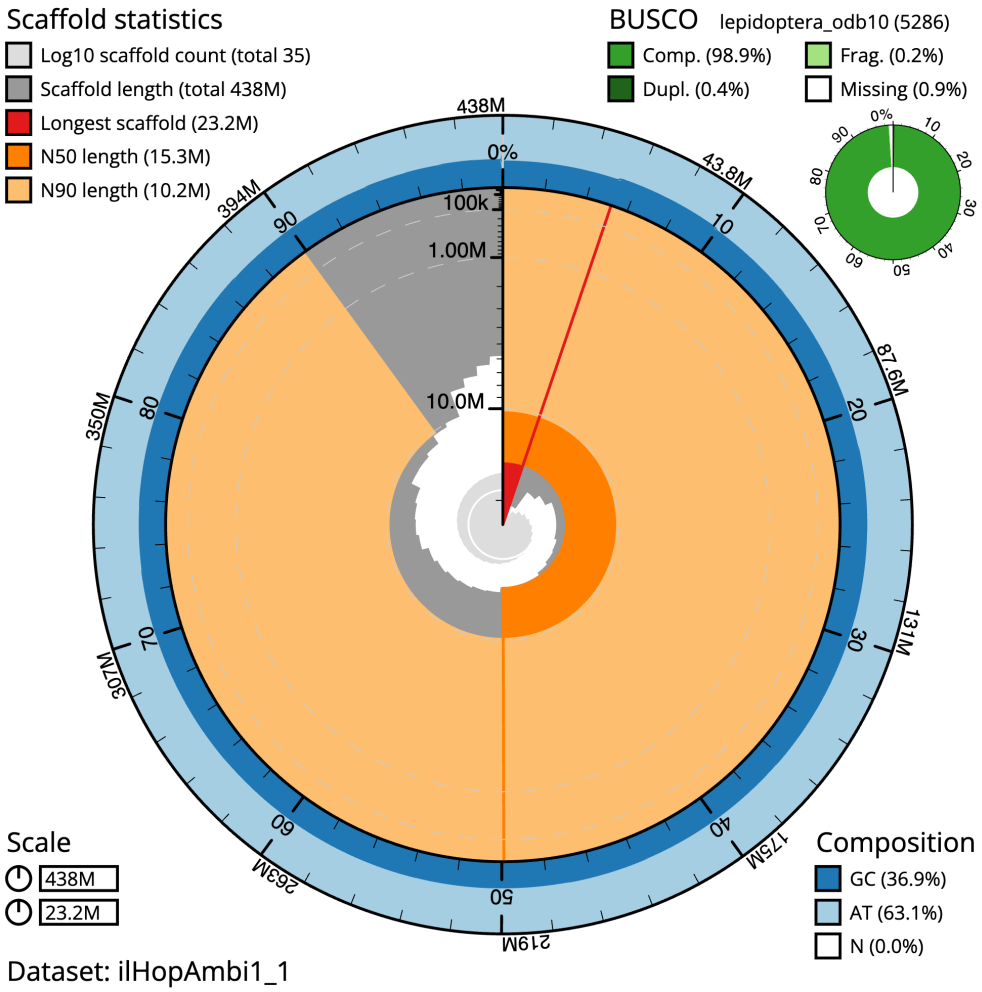

Figure 2. Genome assembly of *Hoplodrina ambigua*, ilHopAmbi1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 438,113,995 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (23,217,168 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (15,265,479 and 10,232,876 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/ilHopAmbi1_1/dataset/ilHopAmbi1_1/snail.

(Table 1; https://rapid.ensembl.org/Hoplodrina_ambigua_GCA_949774945.1/Info/Index). The resulting annotation includes 19,063 transcribed mRNAs from 18,878 protein-coding genes.

Methods

Sample acquisition and nucleic acid extraction

A female *Hoplodrina ambigua* (specimen ID Ox000966, ToLID ilHopAmbi1) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2020-09-08 using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice.

The workflow for high molecular weight (HMW) DNA extraction at the Wellcome Sanger Institute (WSI) includes a sequence of core procedures: sample preparation; sample homogenisation, DNA extraction, fragmentation, and clean-up. In sample preparation, the ilHopAmbi1 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 with speed setting 30 (Todorovic *et al.*, 2023). Sheared DNA was purified by solid-phase reversible immobilisation (Strickland *et al.*, 2023): in brief, the method employs a 1.8X ratio of AMPure PB beads to sample to eliminate shorter fragments and concentrate the DNA. The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and Qubit Fluorometer

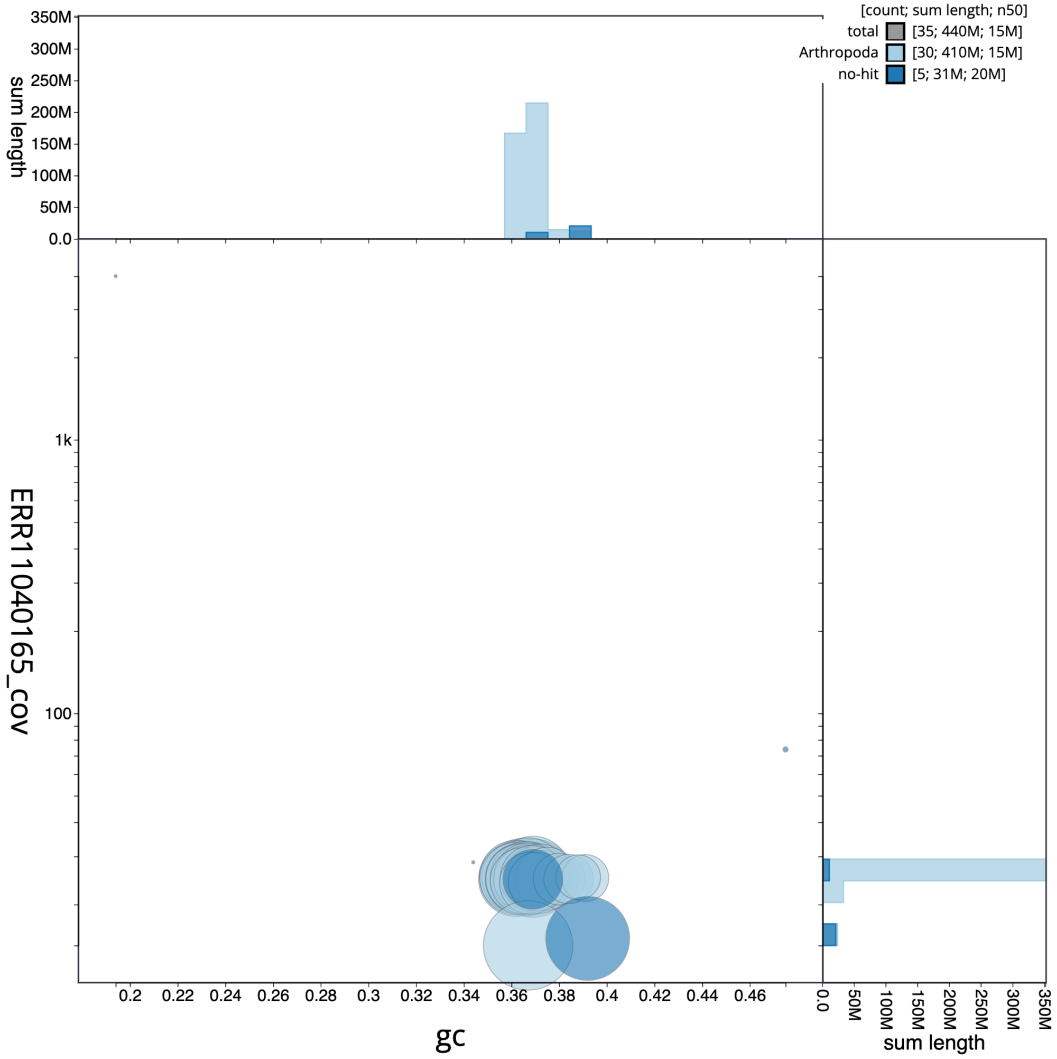

Figure 3. Genome assembly of *Hoplodrina ambigua*, ilHopAmbi1.1: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilHopAmbi1_1/dataset/ilHopAmbi1_1/blob.

and Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

RNA was extracted from remaining abdomen tissue of ilHopAmbi1 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.

Protocols developed by the WSI Tree of Life laboratory are publicly available on protocols.io (Denton *et al.*, 2023b).

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 II (HiFi) and Illumina NovaSeq 6000 (RNA-Seq) instruments. Hi-C data were also generated from head tissue of ilHopAmbi1 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

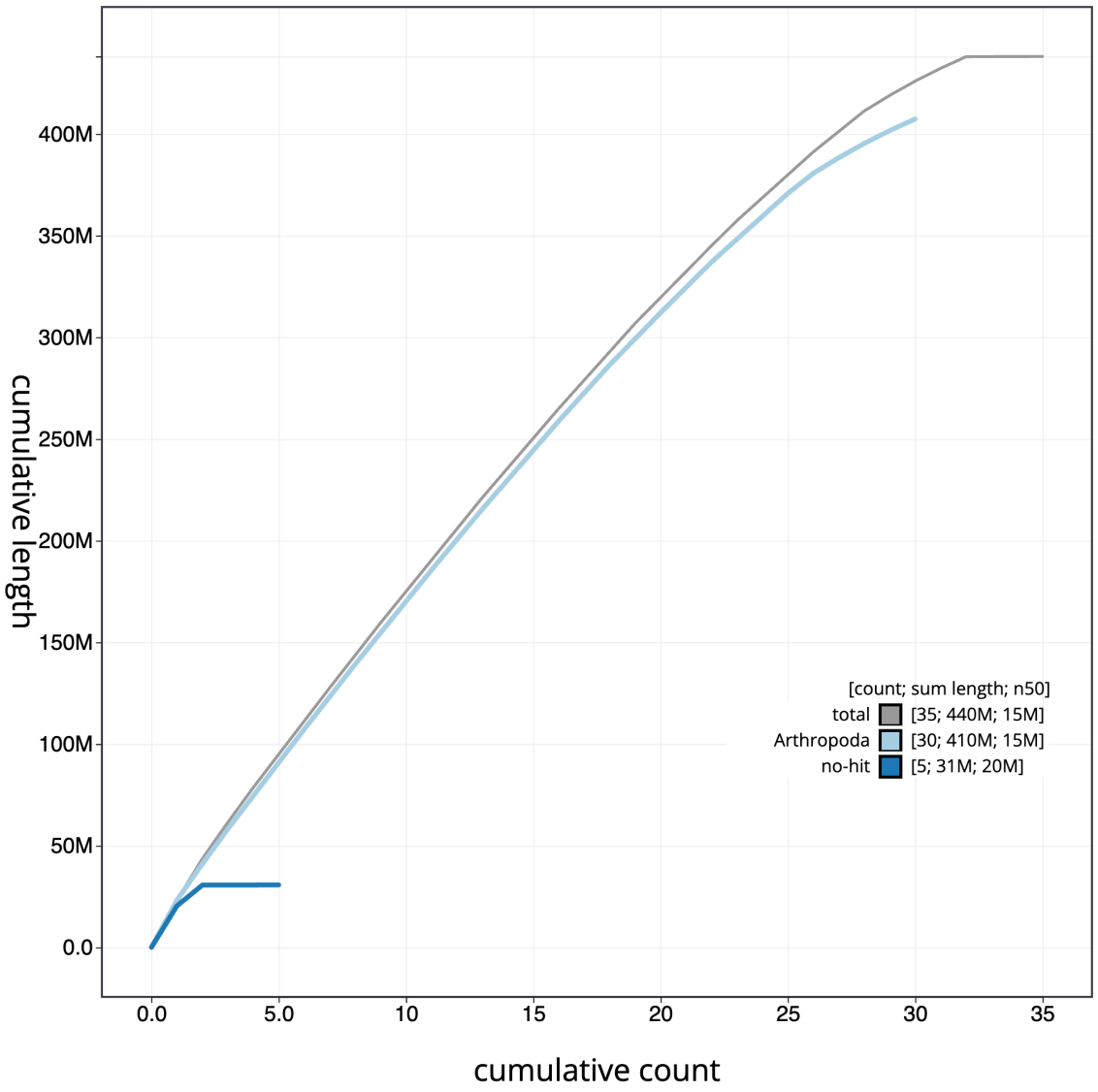

Figure 4. Genome assembly of *Hoplodrina ambigua*, ilHopAmbi1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilHopAmbi1_1/dataset/ilHopAmbi1_1/cumulative.

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.*, 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.

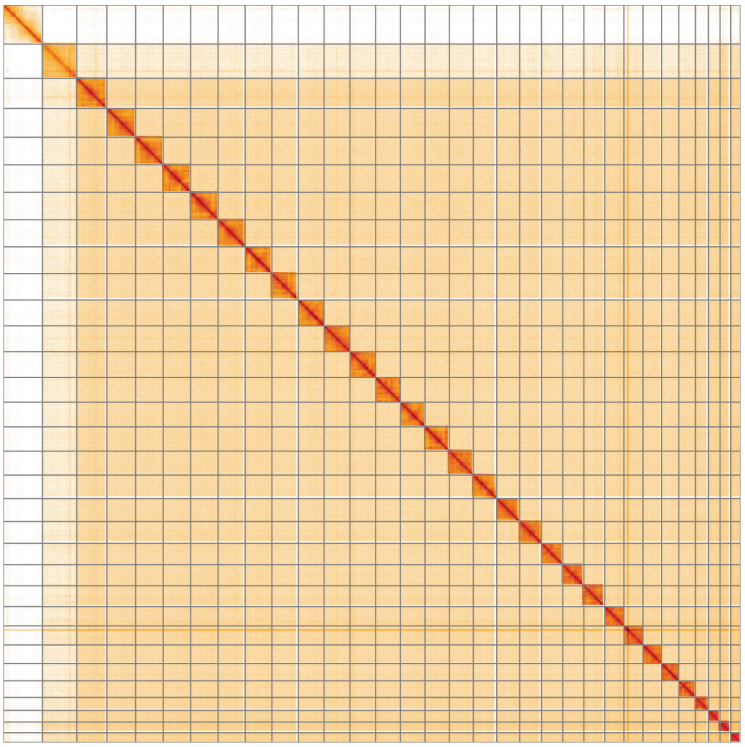

Figure 5. Genome assembly of *Hoplodrina ambigua*, ilHopAmbi1.1: Hi-C contact map of the ilHopAmbi1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=R74USs_CR364ohg8bWGpig.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Hoplodrina ambigua*, ilHopAmbi1.

INSDC accession	Chromosome	Length (Mb)	GC%
OX458965.1	1	17.84	37.0
OX458966.1	2	17.14	36.5
OX458967.1	3	16.34	36.0
OX458968.1	4	16.34	37.0
OX458969.1	5	16.34	37.0
OX458970.1	6	16.05	36.5
OX458971.1	7	15.93	36.5
OX458972.1	8	15.66	36.0
OX458973.1	9	15.37	36.5
OX458974.1	10	15.35	36.5
OX458975.1	11	15.27	36.0
OX458976.1	12	14.73	36.5
OX458977.1	13	14.64	36.5
OX458978.1	14	14.42	36.5
OX458979.1	15	14.24	36.5
OX458980.1	16	13.97	36.5
OX458981.1	17	13.65	36.5
OX458982.1	18	12.94	37.0
OX458983.1	19	12.67	36.5
OX458984.1	20	12.56	37.0
OX458985.1	21	12.35	37.0
OX458986.1	22	11.39	37.0
OX458987.1	23	11.36	37.5
OX458988.1	24	11.06	37.0
OX458989.1	25	10.23	37.0
OX458990.1	26	9.88	37.0
OX458991.1	27	7.67	38.0
OX458992.1	28	7.0	38.5
OX458993.1	29	6.31	39.0
OX458994.1	30	5.73	39.0
OX458964.1	W	20.39	39.0
OX458963.1	Z	23.22	36.5
OX458995.1	MT	0.02	19.5

Table 3. 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
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	1.2a	https://github.com/c-zhou/yahs

Genome annotation

The BRAKER2 pipeline (Brůna *et al.*, 2021) was used in the default protein mode to generate annotation for the *Hoplodrina ambigua* assembly (GCA_949774945.1) 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.

Data availability

European Nucleotide Archive: *Hoplodrina ambigua* (Vine's rustic). Accession number PRJEB60635; https://identifiers.org/ena.embl/PRJEB60635 (Wellcome Sanger Institute, 2023). The genome sequence is released openly for reuse. The *Hoplodrina ambigua* 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.7125292.

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 Management, Samples and Laboratory team are listed here: https://doi.org/10.5281/zenodo.10066175.

Members of Wellcome Sanger Institute Scientific Operations: Sequencing Operations are listed here: https://doi.org/10.5281/zenodo.10043364.

Members of the Wellcome Sanger Institute Tree of Life Core Informatics team are listed here: https://doi.org/10.5281/zenodo.10066637.

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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