DATA NOTE

The genome sequence of the Frosted Green moth, *Polyploca ridens* (Fabricius, 1787) [version 1; peer review: awaiting peer review]

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v1 **First published:** 24 Sep 2024, **9**:545
https://doi.org/10.12688/wellcomeopenres.23043.1
Latest published: 24 Sep 2024, **9**:545
https://doi.org/10.12688/wellcomeopenres.23043.1

Abstract

We present a genome assembly from an individual male *Polyploca ridens* (the Frosted Green; Arthropoda; Insecta; Lepidoptera; Drepanidae). The genome sequence has a total length of 493.60 megabases. Most of the assembly is scaffolded into 30 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 15.34 kilobases in length. Gene annotation of this assembly on Ensembl identified 12,321 protein-coding genes.

Keywords

Polyploca ridens, Frosted Green 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.

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Author roles: Boyes D: Investigation, Resources; **Crowley LM**: Investigation, Resources;

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, University of Oxford and Wytham Woods Genome Acquisition Lab *et al.* **The genome sequence of the Frosted Green moth, *Polyploca ridens* (Fabricius, 1787) [version 1; peer review: awaiting peer review]** Wellcome Open Research 2024, **9**:545 https://doi.org/10.12688/wellcomeopenres.23043.1

First published: 24 Sep 2024, **9**:545 https://doi.org/10.12688/wellcomeopenres.23043.1

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; Drepanoidea; Drepanidae; Thyatirinae; *Polyploca*; *Polyploca ridens* (Fabricius, 1787) (NCBI:txid988154).

Background

The Frosted Green (*Polyploca ridens*) is a moth species belonging to the family Drepanidae. This species is commonly observed from western Europe, extending eastwards into Russia and parts of Asia, including the Caucasus and Iran (GBIF Secretariat, 2024), with a local distribution in the United Kingdom, mainly in southern England and parts of Wales (NBN Atlas Partnership, 2024). The adult moths typically emerge in April and May, displaying a distinctive green colour with white and black markings on their wings, which serve as camouflage against lichen-covered tree trunks (Waring *et al.*, 2017).

Larvae of *P. ridens* feed on the leaves of oak (*Quercus*) species, developing until pupation occurs in the leaf litter or soil (Waring *et al.*, 2017). Although *P. ridens* is currently considered of least concern in terms of conservation status, its reliance on deciduous woodland habitats makes it potentially vulnerable to habitat loss and changes in woodland management practices. For example, th in Germany north of the Alps the species is in decline due to the displacement of the oak in forests in favour of fast-growing trees and increasing dark forest management (Wagner, 2024).

The Darwin Tree of Life project has sequenced the genome of *Polyploca ridens* to enhance understanding of its evolutionary history and ecological adaptations. This genetic information will contribute to broader biodiversity research and conservation efforts.

Genome sequence report

The genome of an adult male *Polyploca ridens* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating a total of 23.35 Gb (gigabases) from 2.32 million reads, providing approximately 47-fold coverage. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data, which produced 77.90 Gbp from 515.87 million reads, yielding an approximate coverage of 158-fold. Specimen and sequencing information is summarised in Table 1.

Manual assembly curation corrected 113 missing joins or misjoins and 24 haplotypic duplications, reducing the assembly length by 0.74% and the scaffold number by 9.4%, and increasing the scaffold N50 by 4.7%. The final assembly has a total length of 493.60 Mb in 481 sequence scaffolds with a scaffold N50 of 16.8 Mb (Table 2). The total count of gaps in the scaffolds is 1,505. The snail plot in Figure 2 provides a summary of the assembly statistics, and Figure 3 shows the distribution of base

Figure 1. Photograph of the *Polyploca ridens* (ilPolRide1) specimen used for genome sequencing.

coverage against position per chromosome. The cumulative assembly plot in Figure 4 shows curves for subsets of scaffolds assigned to different phyla. Most (96.6%) of the assembly sequence was assigned to 30 chromosomal-level scaffolds, representing 29 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 assigned based on synteny to *Achlya flavicornis* (GCA_947623365.1) (Crowley *et al.*, 2023a). 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 60.2 with *k*-mer completeness of 100.0%, and the assembly has a BUSCO v5.3.2 completeness of 95.1% (single = 93.8%, duplicated = 1.3%), using the lepidoptera_odb10 reference set (n = 5,286).

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/988154.

Genome annotation report

The *Polyploca ridens* genome assembly (GCA_951394255.1) was annotated at the European Bioinformatics Institute (EBI) on Ensembl Rapid Release. The resulting annotation includes 22,397 transcribed mRNAs from 12,321 protein-coding and 1,995 non-coding genes (Table 2; https://rapid.ensembl.org/Polyploca_ridens_GCA_951394255.1/Info/Index). The average transcript length is 15,390.97. There are 1.56 coding transcripts per gene and 7.12 exons per transcript.

Methods

Sample acquisition

An adult male *Polyploca ridens* (specimen ID Ox001099, ToLID ilPolRide1) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2021-03-31 using a light trap. The

Table 1. Specimen and sequencing data for *Polyploca ridens*.

Project information			
Study title	Polyploca ridens (frosted green)		
Umbrella BioProject	PRJEB61605		
Species	*Polyploca ridens*		
BioSample	SAMEA10107022		
NCBI taxonomy ID	988154		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	ilPolRide1	SAMEA10200694	thorax
Hi-C sequencing	ilPolRide1	SAMEA10200693	head
RNA sequencing	ilPolRide3	SAMEA113426048	abdomen
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq 6000	ERR11271524	5.16e+08	77.9
PacBio Sequel IIe	ERR11279083	2.32e+06	23.35
RNA Illumina NovaSeq 6000	ERR12708746	7.88e+07	11.9

specimen was collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice. The specimen used for RNA sequencing (specimen ID Ox003348, ToLID ilPolRide3) was an adult specimen collected from the same location on 2023-03-31, using a light trap. This 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.*, 2023b). 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.*, 2023).

For sample homogenisation, thorax tissue of ilPolRide1 was cryogenically disrupted using the Covaris cryoPREP® Automated Dry Pulverizer (Narváez-Gómez *et al.*, 2023). 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.

RNA was extracted from abdomen tissue of ilPolRide3 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.

Table 2. Genome assembly data for *Polyploca ridens*, ilPolRide1.1.

Genome assembly		
Assembly name	ilPolRide1.1	
Assembly accession	GCA_951394255.1	
Accession of alternate haplotype	*GCA_951394295.1*	
Span (Mb)	493.60	
Number of contigs	1,987	
Contig N50 length (Mb)	0.6	
Number of scaffolds	481	
Scaffold N50 length (Mb)	16.8	
Longest scaffold (Mb)	24.88	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	60.2	*≥ 50*
k-mer completeness	100.0%	*≥ 95%*
BUSCO**	C:95.1%[S:93.8%,D:1.3%], F:1.1%,M:3.8%,n:5286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	96.6%	*≥ 95%*
Sex chromosomes	Z	*localised homologous pairs*
Organelles	Mitochondrial genome: 15.34 kb	*complete single alleles*
Genome annotation of assembly GCA_951394255.1 at Ensembl		
Number of protein-coding genes	12,321	
Number of non-coding genes	1,995	
Number of gene transcripts	22,397	

* 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/ilPolRide1_1/dataset/ilPolRide1_1/busco.

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 6000 (RNA-Seq) instruments.

Hi-C data were generated from frozen head tissue of ilPolRide1, using the Arima-HiC v2 kit. The tissue was fixed with a TC buffer containing formaldehyde, resulting in crosslinked DNA. The crosslinked DNA was digested with a restriction enzyme master mix. The resulting 5'-overhangs were filled in and labelled with a biotinylated nucleotide. The biotinylated DNA was then fragmented, enriched, barcoded, and amplified using the NEBNext Ultra II DNA Library Prep Kit. Hi-Csequencing was performed on an Illumina NovaSeq 6000 instrument, using paired-end sequencing with a read length of 150 bp.

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

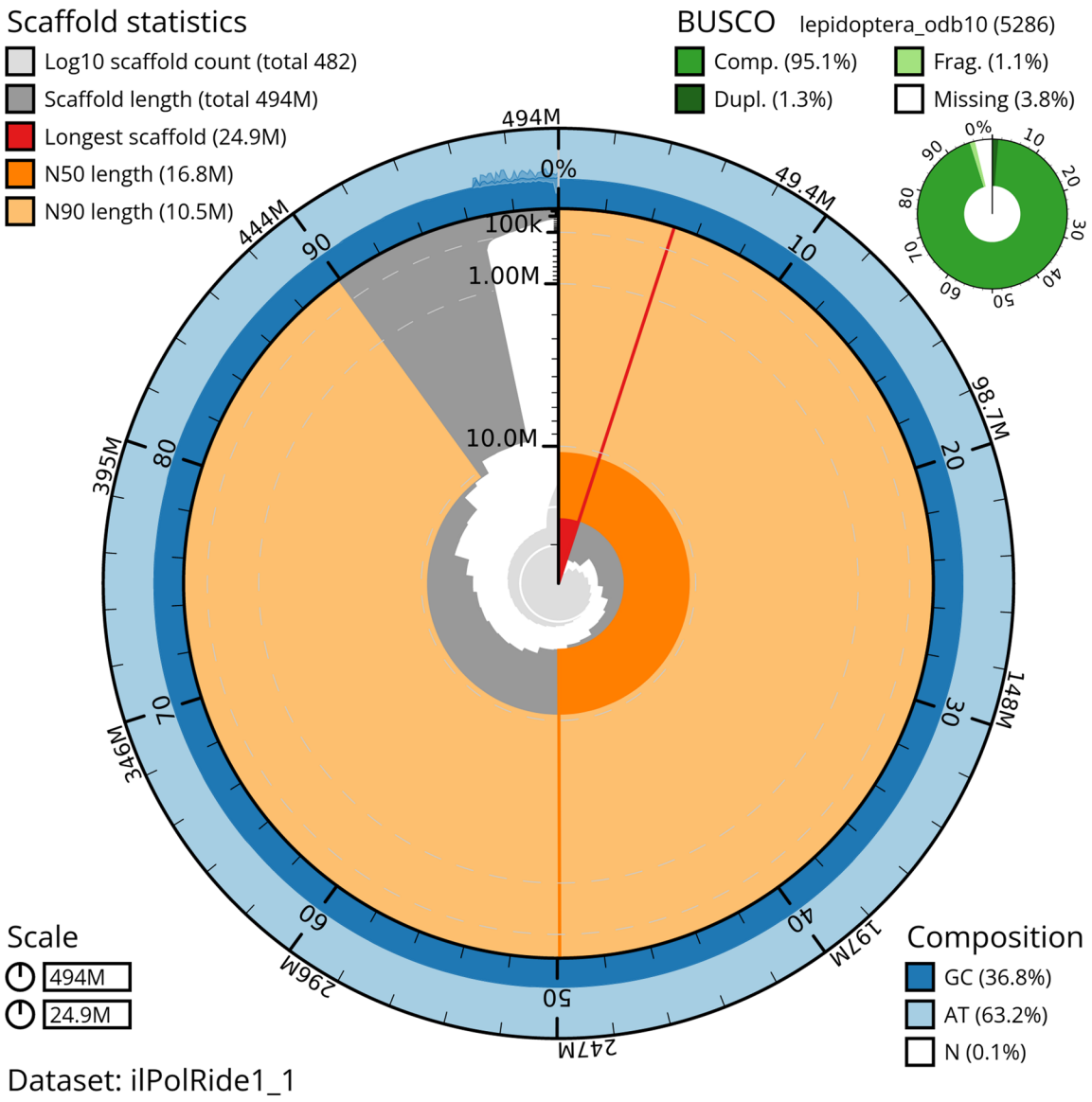

Figure 2. Genome assembly of *Polyploca ridens*, ilPolRide1.1: metrics. The BlobToolKit snail plot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 493,579,502 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (24,878,430 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (16,791,215 and 10,503,112 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/ilPolRide1_1/dataset/ilPolRide1_1/snail.

(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.

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

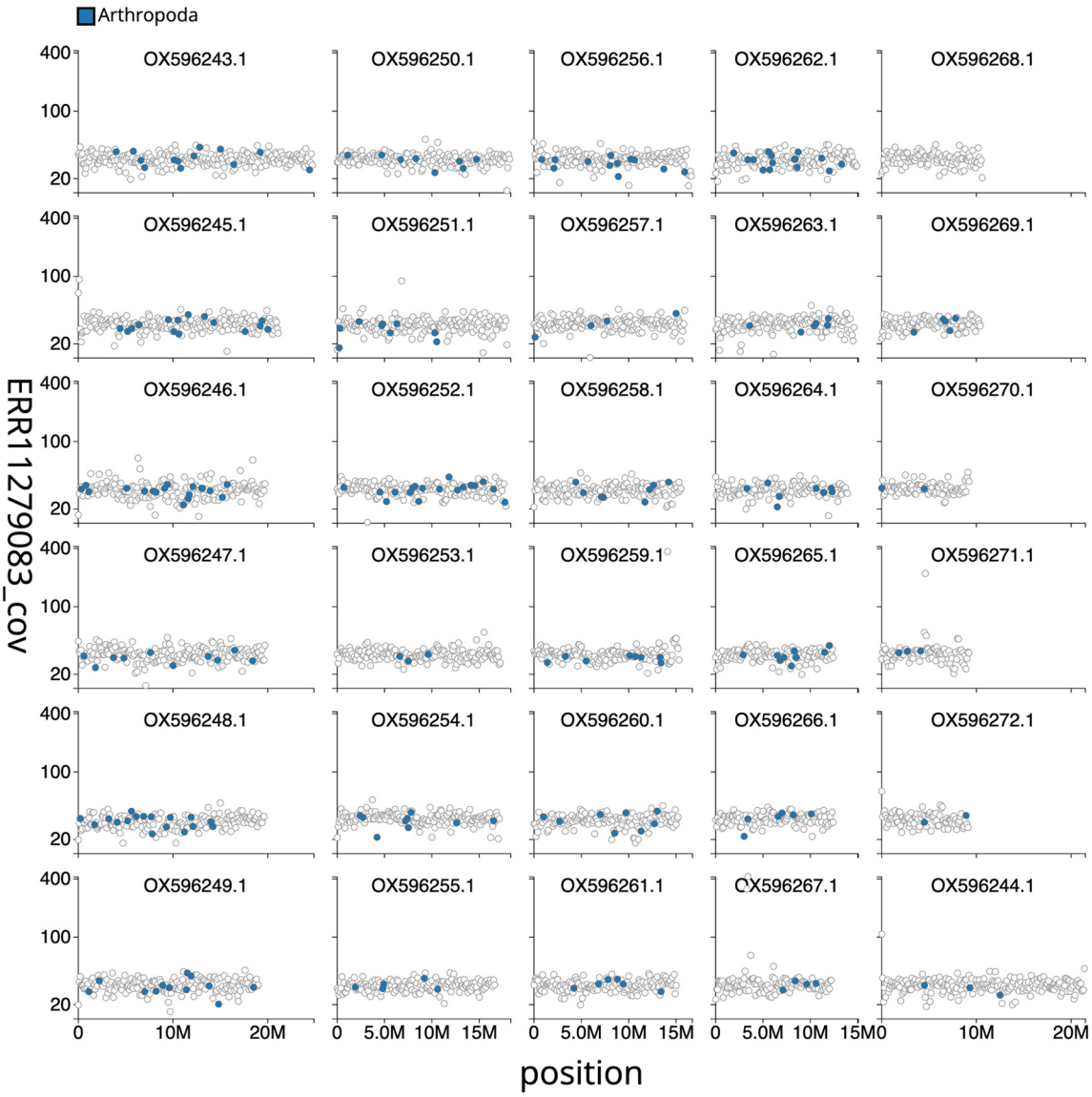

Figure 3. Genome assembly of *Polyploca ridens*, ilPolRide1.1: Distribution plot of base coverage against position for sequences in the assembly. An interactive version of this figure is available here.

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. The sex chromosome was identified by synteny. The 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, 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; Simão *et al.*, 2015) were calculated.

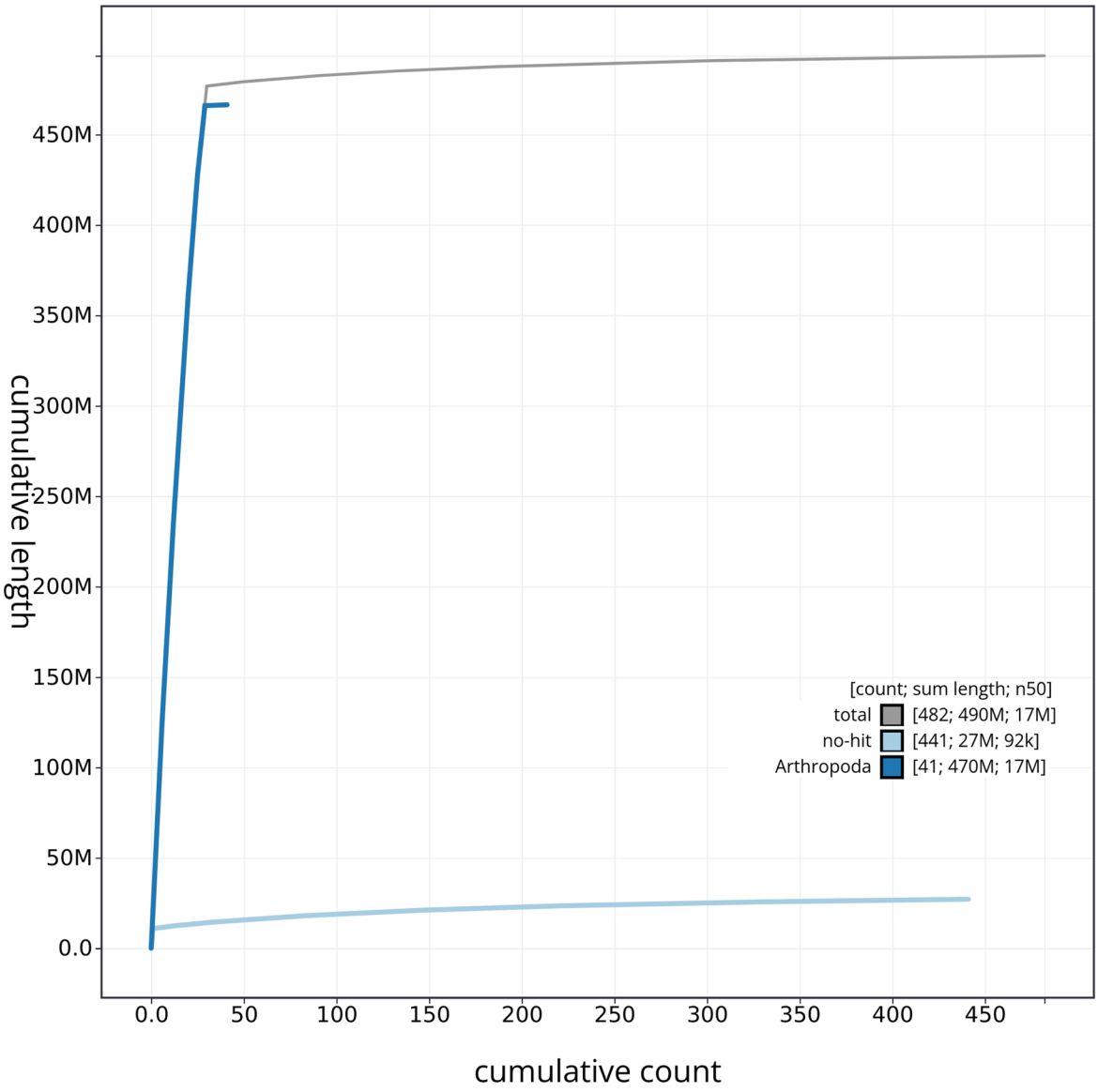

Figure 4. Genome assembly of *Polyploca ridens* ilPolRide1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all sequences. Coloured lines show cumulative lengths of sequences assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilPolRide1_1/dataset/ilPolRide1_1/cumulative.

Table 4 contains a list of relevant software tool versions and sources.

Genome annotation

The Ensembl Genebuild annotation system (Aken *et al.*, 2016) was used to generate annotation for the *Polyploca ridens* assembly (GCA_951394255.1) in Ensembl Rapid Release at the EBI. Annotation was created primarily through alignment of transcriptomic data to the genome, with gap filling via protein-to-genome alignments of a select set of proteins from UniProt (UniProt Consortium, 2019).

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

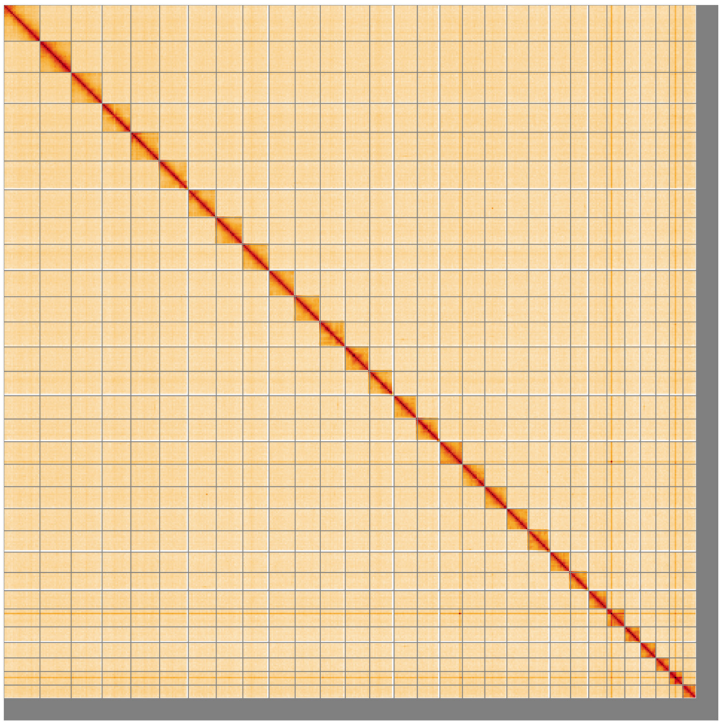

Figure 5. Genome assembly of *Polyploca ridens* ilPolRide1.1: Hi-C contact map of the ilPolRide1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=GcVGL1j_QWaHXPrrJX6azg.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Polyploca ridens*, ilPolRide1.

INSDC accession	Name	Length (Mb)	GC%
OX596243.1	1	24.88	36.5
OX596245.1	2	21.25	36.5
OX596246.1	3	19.81	36.5
OX596247.1	4	19.8	36.5
OX596248.1	5	19.79	36.5
OX596249.1	6	19.18	36.5
OX596250.1	7	18.33	36.5
OX596251.1	8	18.03	36.5
OX596252.1	9	17.96	36.5
OX596253.1	10	17.35	36.5
OX596254.1	11	17.21	36.5
OX596255.1	12	16.79	36.0
OX596256.1	13	16.72	36.5
OX596257.1	14	16.04	36.5
OX596258.1	15	15.61	36.5
OX596259.1	16	15.49	36.5
OX596260.1	17	15.4	37.0
OX596261.1	18	15.18	36.5
OX596262.1	19	15.08	37.0
OX596263.1	20	14.71	37.0
OX596264.1	21	14.0	36.5
OX596265.1	22	12.55	37.0
OX596266.1	23	12.51	37.0
OX596267.1	24	12.35	37.0
OX596268.1	25	10.8	37.0
OX596269.1	26	10.5	36.5
OX596270.1	27	9.36	37.5
OX596271.1	28	9.34	38.0
OX596272.1	29	9.27	37.5
OX596244.1	Z	21.51	36.5
OX596273.1	MT	0.02	19.0

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	MerquryFK	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	3	https://github.com/marcelauliano/MitoHiFi
MultiQC	1.14, 1.17, and 1.18	https://github.com/MultiQC/MultiQC
PretextView	0.2	https://github.com/wtsi-hpag/PretextView
purge_dups	1.2.5	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.2	https://github.com/c-zhou/yahs

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: *Polyploca ridens* (frosted green). Accession number PRJEB61605; https://identifiers.org/ena.embl/PRJEB61605 (Wellcome Sanger Institute, 2023). The genome sequence is released openly for reuse. The *Polyploca ridens* 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.

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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.

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