




DATA NOTE

# The genome sequence of the White-barred Knot-horn, *Elegia similella* (Zincken, 1818) [version 1; peer review: 2 approved, 1 approved with reservations]

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**V1** First published: 12 Apr 2024, 9:186  
<https://doi.org/10.12688/wellcomeopenres.21213.1>  
Latest published: 12 Apr 2024, 9:186  
<https://doi.org/10.12688/wellcomeopenres.21213.1>

## Abstract

We present a genome assembly from an individual male *Elegia similella* (the White-barred Knot-horn; Arthropoda; Insecta; Lepidoptera; Pyralidae). The genome sequence is 780.4 megabases in span. Most of the assembly is scaffolded into 30 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 15.3 kilobases in length. Gene annotation of this assembly on Ensembl identified 18,805 protein coding genes.

## Keywords




*Elegia similella*, White-barred Knot-horn, genome sequence, chromosomal, Lepidoptera



This article is included in the [Tree of Life gateway](#).

## Open Peer Review

Approval Status 

	1	2	3
<b>version 1</b> 12 Apr 2024	 <a href="#">view</a>	 <a href="#">view</a>	 <a href="#">view</a>

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**Author roles: Hammond J:** 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) 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.*

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**How to cite this article:** Hammond J, University of Oxford and Wytham Woods Genome Acquisition Lab, Darwin Tree of Life Barcoding collective *et al.* **The genome sequence of the White-barred Knot-horn, *Elegia similella* (Zincken, 1818) [version 1; peer review: 2 approved, 1 approved with reservations]** Wellcome Open Research 2024, 9:186 <https://doi.org/10.12688/wellcomeopenres.21213.1>

**First published:** 12 Apr 2024, 9:186 <https://doi.org/10.12688/wellcomeopenres.21213.1>

## 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; Pyraloidea; Pyralidae; Phycitinae; *Elegia*; *Elegia similella* (Zincken, 1818) (NCBI:txid1101167).

## Background

The genome of the white-barred knot-horn, *Elegia similella*, 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 *Elegia similella*, based on one male specimen from Wytham Woods, Oxfordshire, UK.

## Genome sequence report

The genome was sequenced from one male *Elegia similella* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, -1.34). A total of 32-fold coverage in Pacific Biosciences single-molecule HiFi long reads was generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 4 missing joins or mis-joins and removed 4 haplotypic duplications, reducing the assembly length by 0.63% and the scaffold number by 2.86%.

The final assembly has a total length of 780.4 Mb in 33 sequence scaffolds with a scaffold N50 of 28.7 Mb (Table 1). The snail plot 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 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 2). While not



**Figure 1.** Photograph of the *Elegia similella* (iEleSimi1) specimen used for genome sequencing.

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 66.4 with  $k$ -mer completeness of 100.0%, and the assembly has a BUSCO v5.3.2 completeness of 98.8% (single = 98.3%, duplicated = 0.5%), 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/1101167>.

## Genome annotation report

The *Elegia similella* genome assembly (GCA\_947532085.1) was annotated using the Ensembl rapid annotation pipeline at the European Bioinformatics Institute (EBI). The resulting annotation includes 18,942 transcribed mRNAs from 18,805 protein-coding genes (Table 1; [https://rapid.ensembl.org/Elegia\\_similella\\_GCA\\_947532085.1/Info/Index](https://rapid.ensembl.org/Elegia_similella_GCA_947532085.1/Info/Index)).

## Methods

### Sample acquisition and nucleic acid extraction

A male *Elegia similella* (specimen ID Ox001596, ToLID iEleSimi1) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude -1.34) on 2021-06-30 using a light trap. The specimen was collected and identified by James Hammond (University of Oxford) and snap-frozen on dry ice.

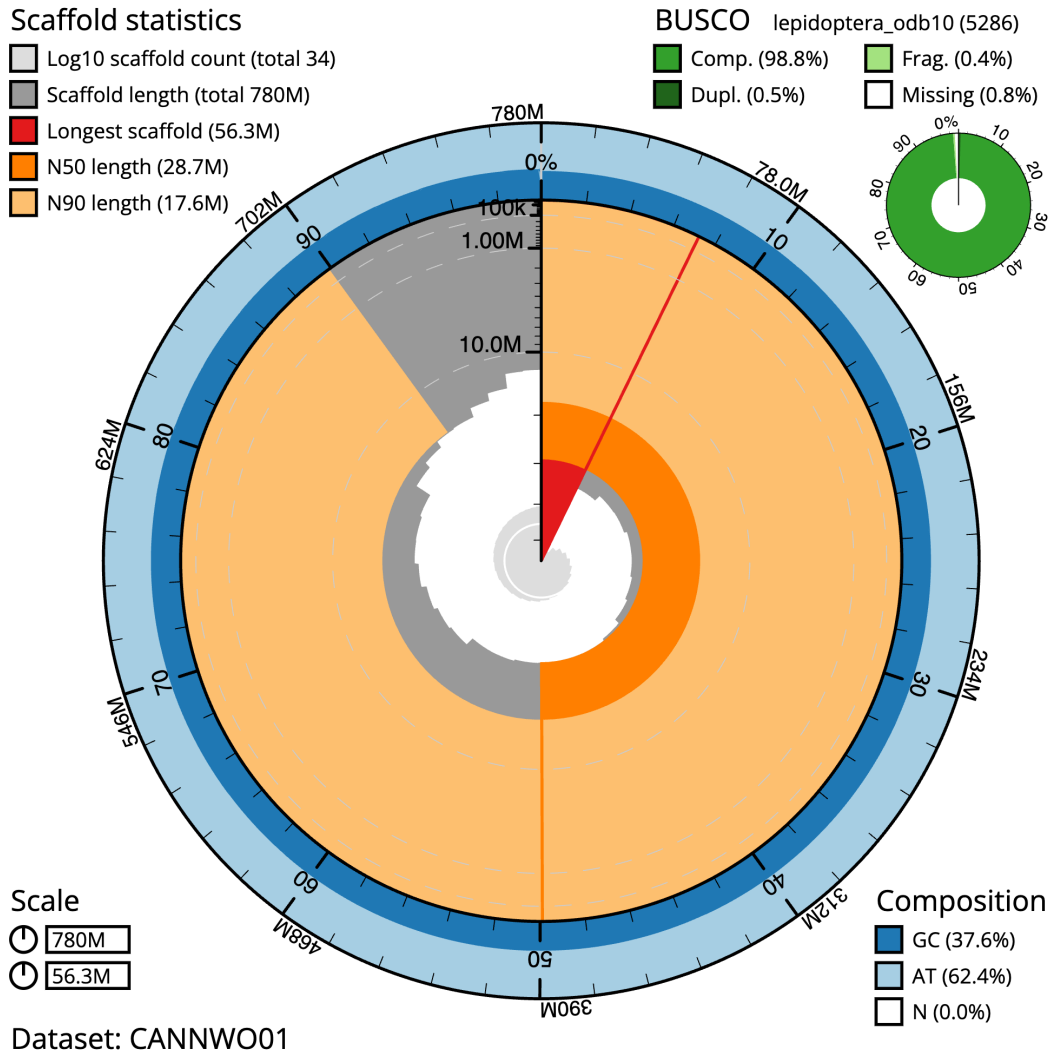
Protocols developed by the Wellcome Sanger Institute (WSI) Tree of Life core laboratory have been deposited on protocols.io (Denton *et al.*, 2023b). The workflow for high molecular weight (HMW) DNA extraction at the WSI includes a sequence of core procedures: sample preparation; sample homogenisation, DNA extraction, fragmentation, and clean-up. In sample preparation, the iEleSimi1 sample was weighed and dissected on dry ice, with tissue set aside for Hi-C sequencing (Jay *et al.*, 2023). Tissue from the whole organism was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a). HMW DNA was extracted in the WSI Scientific Operations core using the Automated MagAttract v2 protocol (Oatley *et al.*, 2023). HMW DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system with speed setting 31 (Bates *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 and Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

**Table 1. Genome data for *Elegia similella*, iEleSimi1.1.**

<b>Project accession data</b>		
Assembly identifier	iEleSimi1.1	
Species	<i>Elegia similella</i>	
Specimen	iEleSimi1	
NCBI taxonomy ID	1101167	
BioProject	PRJEB56060	
BioSample ID	SAMEA10978763	
Isolate information	iEleSimi1, male: whole organism (DNA and Hi-C sequencing)	
<b>Assembly metrics*</b>		<b>Benchmark</b>
Consensus quality (QV)	66.4	≥ 50
<i>k</i> -mer completeness	100.0%	≥ 95%
BUSCO**	C:98.8%[S:98.3%,D:0.5%], F:0.4%,M:0.8%,n:5,286	C ≥ 95%
Percentage of assembly mapped to chromosomes	99.99%	≥ 95%
Sex chromosomes	ZZ	<i>localised homologous pairs</i>
Organelles	Mitochondrial genome: 15.3 kb	<i>complete single alleles</i>
<b>Raw data accessions</b>		
PacificBiosciences SEQUEL II	ERR10224929	
Hi-C Illumina	ERR10297823	
<b>Genome assembly</b>		
Assembly accession	GCA_947532085.1	
<i>Accession of alternate haplotype</i>	GCA_947532095.1	
Span (Mb)	780.4	
Number of contigs	50	
Contig N50 length (Mb)	23.1	
Number of scaffolds	33	
Scaffold N50 length (Mb)	28.7	
Longest scaffold (Mb)	56.26	
<b>Genome annotation</b>		
Number of protein-coding genes	18,805	
Number of gene transcripts	18,942	

\* 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/CANNWO01/dataset/CANNWO01/busco>.



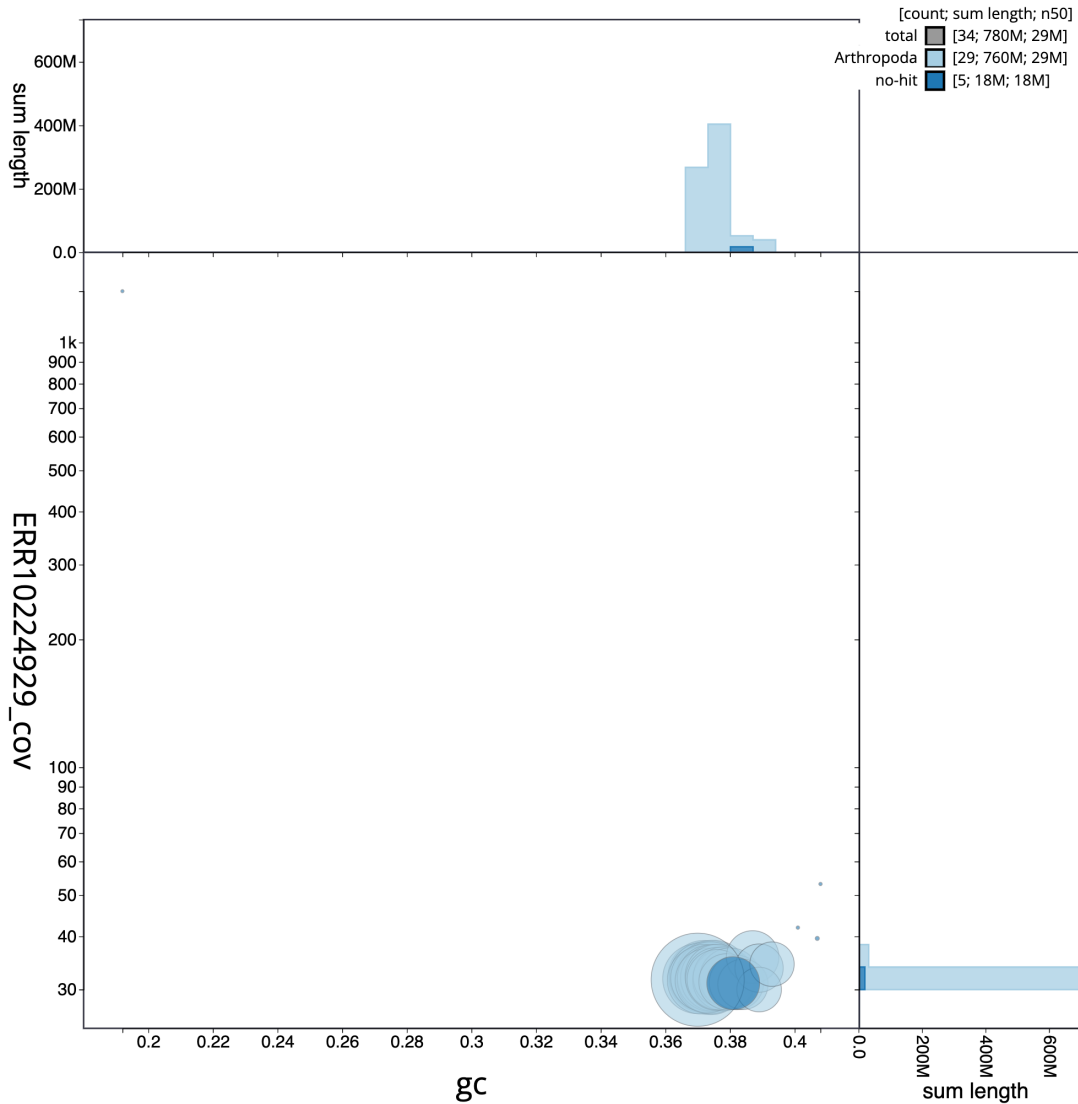
**Figure 2. Genome assembly of *Elegia similella*, iEleSimi1.1: metrics.** The BlobToolKit snail plot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 780,464,592 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (56,259,732 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (28,718,319 and 17,594,767 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/CANNWO01/dataset/CANNWO01/snail>.

### Sequencing

Pacific Biosciences HiFi circular consensus DNA sequencing libraries were constructed according to the manufacturers' instructions. DNA sequencing was performed by the Scientific Operations core at the WSI on a Pacific Biosciences SEQUEL II instruments. Hi-C data were also generated from remaining tissue of iEleSimi1 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 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)

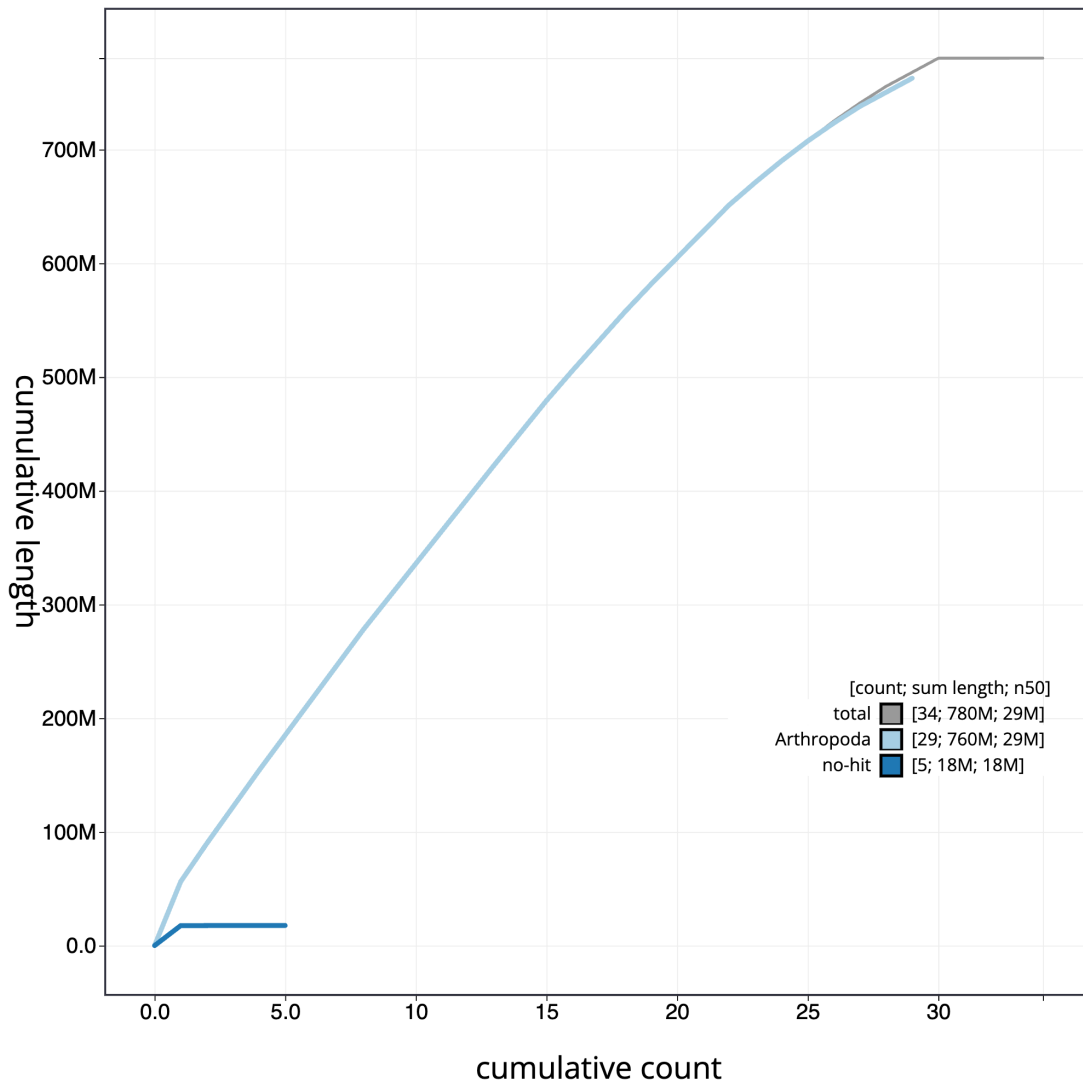


**Figure 3. Genome assembly of *Elegia similella*, iEleSimi1.1: BlobToolKit GC-coverage plot.** Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/CANNWO01/dataset/CANNWO01/blob>.

and PretextView (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.



**Figure 4. Genome assembly of *Elegia similella*, iEleSimi1.1: 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/CANNWO01/dataset/CANNWO01/cumulative>.

Table 3 contains a list of relevant software tool versions and sources.

#### Genome annotation

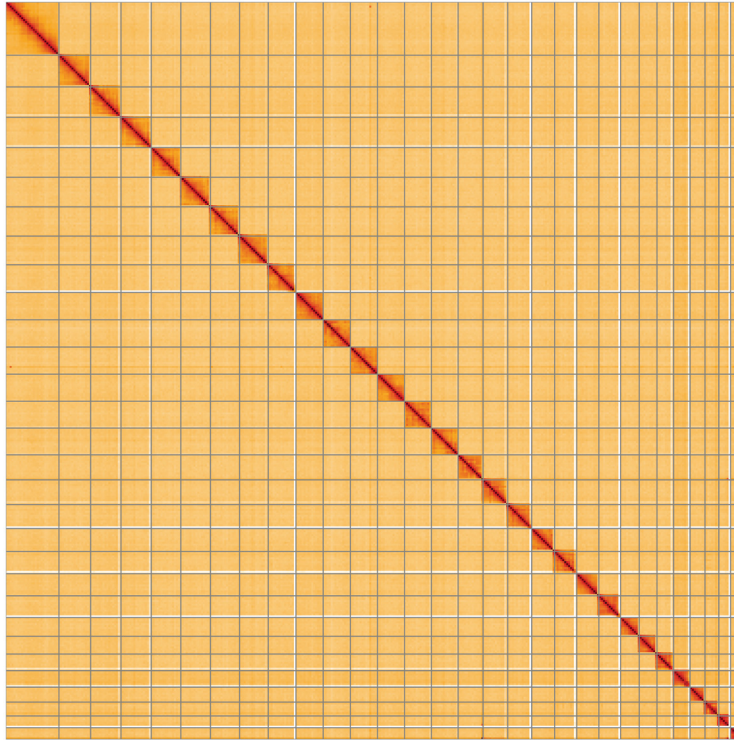
The BRAKER2 pipeline (Brůna *et al.*, 2021) was used in the default protein mode to generate annotation for the *Elegia similella* assembly (GCA\_947532085.1) in Ensembl Rapid Release at the EBI.

#### 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



**Figure 5. Genome assembly of *Elegia similella*, iEleSimi1.1: Hi-C contact map of the iEleSimi1.1 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=APAUOQonQm-CcdQ6gbWXEQ>.

**Table 2. Chromosomal pseudomolecules in the genome assembly of *Elegia similella*, iEleSimi1.**

INSDC accession	Chromosome	Length (Mb)	GC%
OX383926.1	1	33.62	37.0
OX383927.1	2	32.15	37.5
OX383928.1	3	32.05	37.5
OX383929.1	4	31.37	37.0
OX383930.1	5	31.33	37.0
OX383931.1	6	30.94	37.0
OX383932.1	7	30.41	37.5
OX383933.1	8	29.3	37.5
OX383934.1	9	28.87	37.5
OX383935.1	10	28.86	37.0
OX383936.1	11	28.72	37.0
OX383937.1	12	28.67	37.5
OX383938.1	13	28.36	37.5
OX383939.1	14	28.35	37.5
OX383940.1	15	26.52	37.5

INSDC accession	Chromosome	Length (Mb)	GC%
OX383941.1	16	26.14	37.5
OX383942.1	17	25.36	37.5
OX383943.1	18	24.3	38.0
OX383944.1	19	23.48	38.0
OX383945.1	20	23.39	38.0
OX383946.1	21	23.0	37.5
OX383947.1	22	19.86	38.0
OX383948.1	23	18.77	38.0
OX383949.1	24	17.59	38.0
OX383950.1	25	17.43	38.5
OX383951.1	26	15.88	38.5
OX383952.1	27	14.64	39.0
OX383953.1	28	12.43	39.0
OX383954.1	29	12.32	39.5
OX383925.1	Z	56.26	37.0
OX383955.1	MT	0.02	19.5



**Table 3. Software tools: versions and sources.**

Software tool	Version	Source
BlobToolKit	4.1.7	<a href="https://github.com/blobtoolkit/blobtoolkit">https://github.com/blobtoolkit/blobtoolkit</a>
BUSCO	5.3.2	<a href="https://gitlab.com/ezlab/busco">https://gitlab.com/ezlab/busco</a>
Hifiasm	0.16.1-r375	<a href="https://github.com/chhylp123/hifiasm">https://github.com/chhylp123/hifiasm</a>
HiGlass	1.11.6	<a href="https://github.com/higlass/higlass">https://github.com/higlass/higlass</a>
Merqury	MerquryFK	<a href="https://github.com/thegenemyers/MERQURY.FK">https://github.com/thegenemyers/MERQURY.FK</a>
MitoHiFi	2	<a href="https://github.com/marcelauliano/MitoHiFi">https://github.com/marcelauliano/MitoHiFi</a>
PretextView	0.2	<a href="https://github.com/wtsi-hpag/PretextView">https://github.com/wtsi-hpag/PretextView</a>
purge_dups	1.2.3	<a href="https://github.com/dfguan/purge_dups">https://github.com/dfguan/purge_dups</a>
sanger-tol/genomenote	v1.0	<a href="https://github.com/sanger-tol/genomenote">https://github.com/sanger-tol/genomenote</a>
sanger-tol/readmapping	1.1.0	<a href="https://github.com/sanger-tol/readmapping/tree/1.1.0">https://github.com/sanger-tol/readmapping/tree/1.1.0</a>
YaHS	yahs-1.1.91eebc2	<a href="https://github.com/c-zhou/yahs">https://github.com/c-zhou/yahs</a>

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: *Elegia similella* (white-barred knot-horn). Accession number PRJEB56060; <https://identifiers.org/ena.embl/PRJEB56060> (Wellcome Sanger Institute, 2022). The genome sequence is released openly for reuse. The *Elegia similella* 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.

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Members of the Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team are listed here: <https://doi.org/10.5281/zenodo.10066175>.

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Members of the Darwin Tree of Life Consortium are listed here: <https://doi.org/10.5281/zenodo.4783558>.

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Wellcome Sanger Institute: **The genome sequence of the White-barred Knot-horn, *Elegia similella* (Zincken, 1818).** European Nucleotide Archive, [dataset], accession number PRJEB56060, 2022.

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 02 November 2024

<https://doi.org/10.21956/wellcomeopenres.23460.r104972>

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**Panagiotis Ioannidis** 

Foundation for Research & Technology - Hellas, Crete, Greece

This manuscript presents the sequencing, assembly and annotation of a lepidopteran insect. The quality of the genome assembly is very good (chromosome level).

I have to say that I'm happy with the fact that the genome assemblies published in this journal, now include a predicted gene set. However, it is absolutely necessary that the authors also measure the quality of this gene using BUSCO. It is very important for whoever wants to use this genome to know how good this gene set is, both in absolute terms (e.g. a gene set having 70% complete BUSCOs can't be very good), as well as in relative terms (i.e. how good the gene set is, compared to the corresponding genome assembly). I would suggest that the authors run BUSCO (using the same lineage they used for the evaluation of the genome assembly) and add the BUSCO scores in the manuscript (Table 1 seems the most fitting place).

An additional point has to do with the number of chromosomes. The authors say that they have assembled 30 chromosomes, but it would be nice to add whether this is expected. For example, is the number of chromosomes known for other closely related Lepidoptera?

**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:** Insect 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 01 November 2024

<https://doi.org/10.21956/wellcomeopenres.23460.r104971>

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**Bryan Brunet** 

Ottawa Research and Development Centre, Ontario, Canada

The manuscript entitled “The genome sequence of the White-barred Knot-horn, *Elegia similella* (Zincken, 1818)” by Hammond *et al.* presents a chromosomal genome assembly for *Elegia similella* from a single male individual originating from the UK. The authors use a combination of Pacific Biosciences HiFi long reads and Hi-C data to assemble 30 chromosome-scale scaffolds for this species at a total genome size of 780.4 Mb. The data were also used to recover and assemble the mitogenome. The genome has very good assembly metrics, and all molecular and most bioinformatic methods employed have been sufficiently detailed either in the manuscript itself or with appropriate references to methods described in other publications. Aside from a few minor criticisms noted below, the genome should be a useful contribution to the field and is of scientific soundness and rigor.

- The authors present a photograph of the specimen in Figure 1. It is unknown whether this is sufficient to diagnose the accuracy of the identification, but at very least the authors should identify where morphological vouchers (if any) remain and/or have been deposited so that identification can be confirmed should it be necessary.
- Manual curation steps are described but the precise corrections that were made are not detailed in the manuscript. Such information should be made available in order to allow for repeatability of these steps.
- Annotation was completed using BRAKER2 in protein mode but no information is provided on what proteomic data and/or taxa were used to make these predictions. It would be useful to know the taxonomic extent for the set proteins used for annotation.

**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?**

Partly

**Are the datasets clearly presented in a useable and accessible format?**

Yes

**Competing Interests:** No competing interests were disclosed.**Reviewer Expertise:** I am an aphid systematist with expertise in genomic approaches in the context of phylogenetics and population genetics. I've been involved in several genome assembly projects.**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 October 2024

<https://doi.org/10.21956/wellcomeopenres.23460.r100551>

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**Nataliia Kopchak**

University of Manitoba (Ringgold ID: 124615), Winnipeg, Manitoba, Canada

**Jeffrey Marcus**

University of Manitoba, Winnipeg, Canada

In this manuscript, the author describes the sequencing and assembly of the *Elegia similella* genome using DNA from an adult male specimen collected in the UK. The primary genome sequence assembly includes proposed chromosomal pseudomolecule sequences for 29 autosomes, the Z sex chromosome, and a complete mitochondrial genome. On the whole, this is a useful contribution to the scientific literature, but please see our comments below, especially regarding the title, background, identification of the specimen, and details of mitogenome assembly.

Some suggestions to the author:

- Title:** The title indicates the species' genome sequence, which implies the complete genome, including that autosomes and both Z and W sex chromosomes are being analyzed. However, the paper only discusses the Z chromosome data and doesn't include an analysis of the W chromosome from females. To name the title more accurately with the content, one might write that the study focuses specifically on the male genome. We also note that in the metadata found at <https://links.tol.sanger.ac.uk/species/1101167>, the sex of the specimen is listed as "not collected", which appears to be incorrect.
- Background:** We recommend extending background information about the species

(morphology, ecology, distribution, larval host plants, etc.) to provide more context about *Elegia similella*. This will help readers unfamiliar with the species better understand this organism.

3. **Method of Specimen identification:** The specimen identification was named, but which keys/species descriptions were consulted and the morphological characters used for the identification have not been included in the manuscript.
4. **Figure #1:** It would be useful to add a scale bar on the photo, to better understand the size of the organism.
5. The author wrote: "Manual assembly curation corrected 4 missing joins or mis-joins and removed 4 haplotypic duplications, reducing the assembly length..." It would be beneficial to describe how 4 missing joins or mis-joins were identified and how they were corrected.
6. The authors describe how "The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva et al. 2022) which runs MitoFinder (Allio et al. 2020) or MITOS (Bernt et al. 2013) and uses these annotations to select the final mitochondrial contig...". The authors do not describe which of the algorithms generated the selected contig for the mitogenome.
7. **Genome Annotation:** In addition to the total number of protein-coding genes, additional description of the preliminary details of the number/proportion of novel genes identified through this analysis should be included in the text of the manuscript.
8. **Reference sequence:** Any reference genomes or sequences used in this genome assembly (for example for the mitochondrial genome assembly) should be described in the main text of the manuscript and formally cited.

**Is the rationale for creating the dataset(s) clearly described?**

Partly

**Are the protocols appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and materials provided to allow replication by others?**

Partly

**Are the datasets clearly presented in a useable and accessible format?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Evolutionary biology of insects, phylogenomics

**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, however we have significant reservations, as outlined above.**