DATA NOTE

The genome sequence of the Ear Moth, *Amphipoea oculea* (Linnaeus, 1761) [version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual male *Amphipoea oculea* (the Ear Moth; Arthropoda; Insecta; Lepidoptera; Noctuidae). The genome sequence is 669.2 megabases in span. Most of the assembly is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 15.36 kilobases in length.

Keywords

Amphipoea oculea, Ear 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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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; Noctuoidea; Noctuidae; Noctuinae; Apameini; *Amphipoea*; *Amphipoea oculea* (Linnaeus, 1761) (NCBI:txid987876).

Background

The Ear Moth *Amphipoea oculea* (Linnaeus, 1761) is a moth in the Noctuidae family. Adult moths of this species have reddish- to purplish-brown coloured forewings, highlighted with a typically white reniform stigma, although this marking can be yellow or orange in some specimens (Leverton, 2001; Skinner & Wilson, 2009). The forewing markings of this species are typically indistinguishable from the other British and Irish *Amphipoea* (*A. crinanensis*, *fucosa*, *lucens*), but specimens can sometimes be correctly identified by a relatively shorter forewing and darker colouration, amongst other factors such as habitat (Leverton, 2001). This species has little restriction in which habitats it uses, but it does have a preference for damp locales (Skinner & Wilson, 2009).

The species overwinters as an egg from October to March, and upon hatching, the larva feeds internally within the foodplant from April to June (Emmet, 1991). A variety of larval foodplants are used, feeding on grasses, such as *Deschampsia cespitosa*, and low-growing herbaceous plants and roots such as *Petasites hybridus* (Emmet, 1991). Pupation occurs in an underground cell (Waring *et al.*, 2017). Adults are on the wing between July and September, any while most often seen at light or sugar after dark, can be found by day nectaring on thistle or ragwort flowers (Skinner & Wilson, 2009).

The genome of *Amphipoea oculea* 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 *Amphipoea oculea*, based on one male specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome was sequenced from one male *Amphipoea oculea* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, –1.34). A total of 38-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 12 missing joins or mis-joins and removed two haplotypic duplications, reducing the assembly length by 0.44% and the scaffold number by 4.88%, and decreasing the scaffold N50 by 0.69%.

The final assembly has a total length of 669.2 Mb in 39 sequence scaffolds with a scaffold N50 of 23.1 Mb (Table 1). Most (99.99%) of the assembly sequence was assigned to

Figure 1. Photograph of the *Amphipoea oculea* (ilAmpOcul1) specimen used for genome sequencing.

31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 2–Figure 5; Table 2). While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited. The mitochondrial genome was also assembled and can be found as a contig within the multifasta file of the genome submission.

The estimated Quality Value (QV) of the final assembly is 69.6 with *k*-mer completeness of 100%, and the assembly has a BUSCO v5.3.2 completeness of 98.9% (single = 98.3%, duplicated = 0.6%), 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/987876.

Methods

Sample acquisition and nucleic acid extraction

A male *Amphipoea oculea* (ilAmpOcul1) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2021-07-17. The specimen was taken from woodland habitat using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford) and was snap-frozen on dry ice.

The sample was prepared for DNA extraction at the Tree of Life laboratory, Wellcome Sanger Institute (WSI). The ilAmpOcul1 sample was weighed and dissected on dry ice with head tissue set aside for Hi-C sequencing. Thorax tissue was disrupted using a Nippi Powermasher fitted with a

Table 1. Genome data for *Amphipoea oculea*, ilAmpOcul1.1.

Project accession data		
Assembly identifier	ilAmpOcul1.1	
Species	*Amphipoea oculea*	
Specimen	ilAmpOcul1	
NCBI taxonomy ID	987876	
BioProject	PRJEB53731	
BioSample ID	SAMEA10978951	
Isolate information	ilAmpOcul1, male: thorax (DNA sequencing), abdomen (RNA sequencing), head (HiC scaffolding)	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	69.6	*≥ 50*
k-mer completeness	100%	*≥ 95%*
BUSCO**	C:98.9%[S:98.3%,D:0.6%], F:0.3%,M:0.8%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.99%	*≥ 95%*
Sex chromosomes	Z chromosome	*localised homologous pairs*
Organelles	Mitochondrial genome assembled	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR9878389	
Hi-C Illumina	ERR9881693	
PolyA RNA-Seq Illumina	ERR10890686	
Genome assembly		
Assembly accession	GCA_945859645.1	
Accession of alternate haplotype	GCA_945859585.1	
Span (Mb)	669.2	
Number of contigs	53	
Contig N50 length (Mb)	22.3	
Number of scaffolds	39	
Scaffold N50 length (Mb)	23.1	
Longest scaffold (Mb)	34.1	

* 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/ilAmpOcul1.1/dataset/CAMAOK01/busco.

BioMasher pestle. DNA was extracted from thorax tissue of ilAmpOcul1 at the Wellcome Sanger Institute (WSI) Scientific Operations core using the Qiagen MagAttract HMW DNA kit, according to the manufacturer's instructions.

RNA was extracted from abdomen tissue of ilAmpOcul1 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

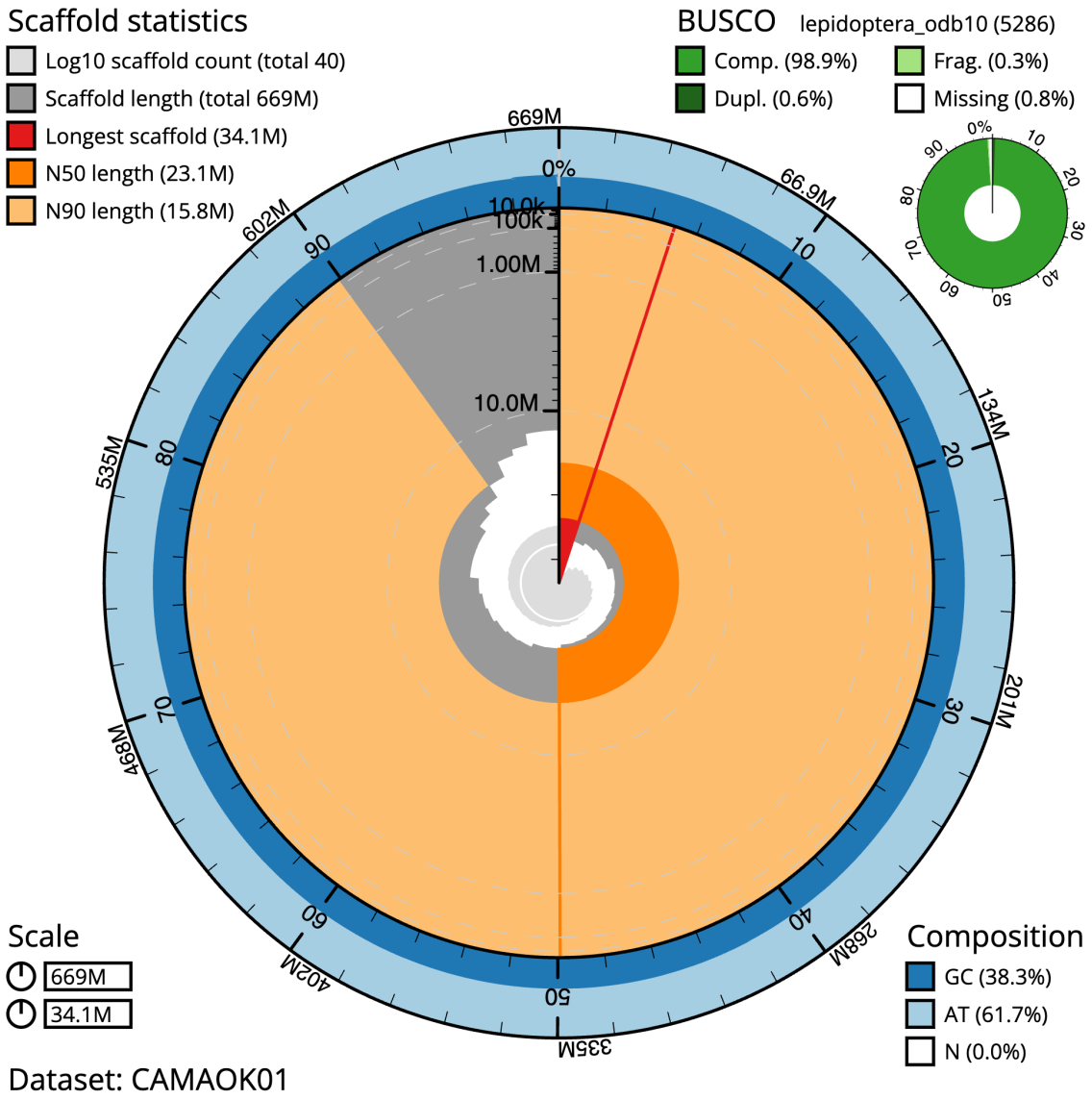

Figure 2. Genome assembly of *Amphipoea oculea*, ilAmpOcul1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 669,264,192 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (34,130,626 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (23,095,043 and 15,755,278 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/ilAmpOcul1.1/dataset/CAMAOK01/snail.

a Nanodrop spectrophotometer and Qubit Fluorometer 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 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 ilAmpOcul1 using the Arima2 kit and sequenced on the Illumina NovaSeq 6000 instrument.

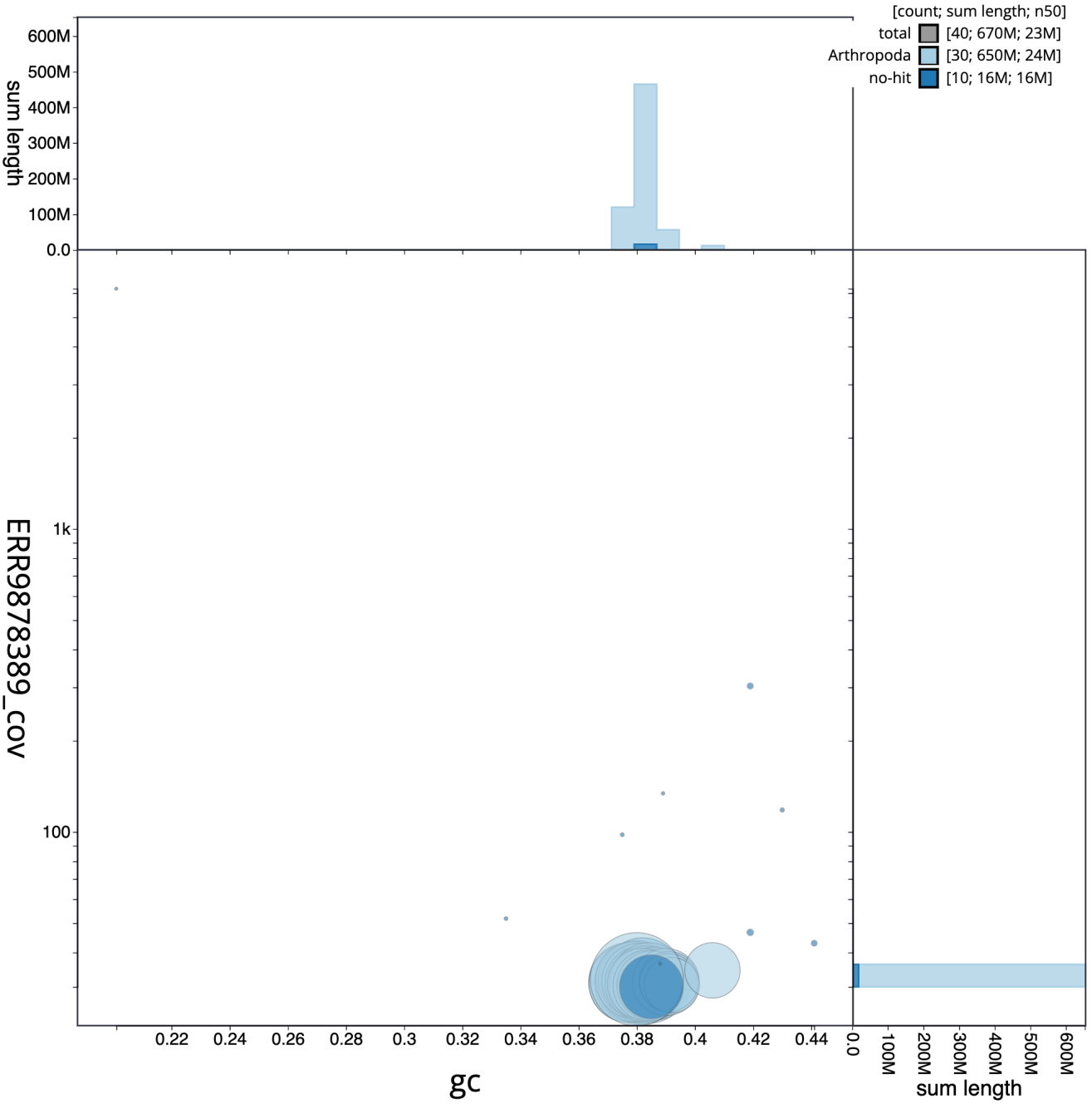

Figure 3. Genome assembly of *Amphipoea oculea*, ilAmpOcul1.1: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilAmpOcul1.1/dataset/CAMAOK01/blob.

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 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.*, 2022), 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.

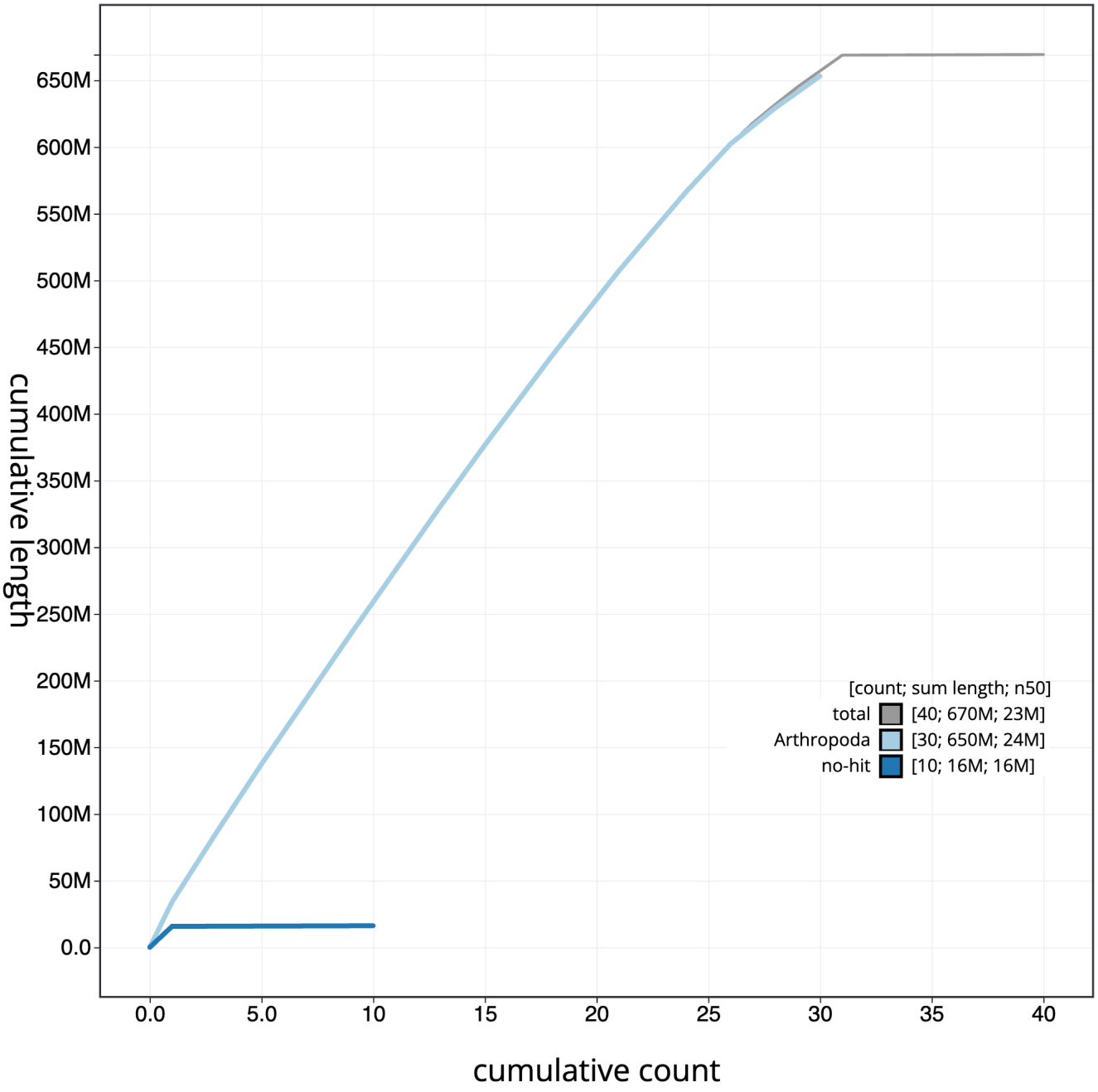

Figure 4. Genome assembly of *Amphipoea oculea*, ilAmpOcul1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilAmpOcul1.1/dataset/CAMAOK01/cumulative.

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.

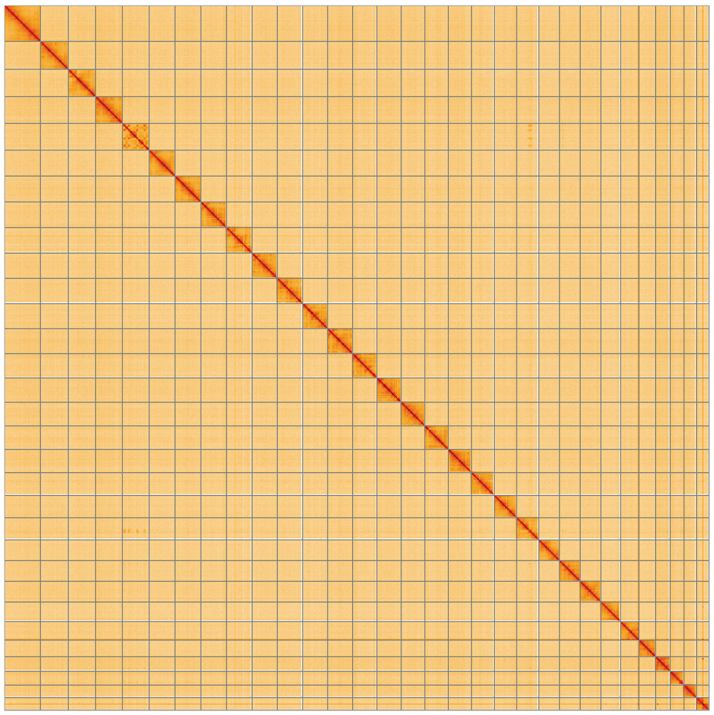

Figure 5. Genome assembly of *Amphipoea oculea*, ilAmpOcul1.1: Hi-C contact map of the ilAmpOcul1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=GD8YdSSkQxK_olmjIC2F6A.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Amphipoea oculea*, ilAmpOcul1.

INSDC accession	Chromosome	Length (Mb)	GC%
OX243862.1	1	26.43	38.0
OX243863.1	2	25.88	38.5
OX243864.1	3	25.46	38.0
OX243865.1	4	25.41	38.0
OX243866.1	5	24.61	38.0
OX243867.1	6	24.54	38.0
OX243868.1	7	24.4	37.5
OX243869.1	8	24.2	38.0
OX243870.1	9	24.01	38.0
OX243871.1	10	23.96	37.5
OX243872.1	11	23.74	38.0
OX243873.1	12	23.64	38.0
OX243874.1	13	23.1	38.0
OX243875.1	14	23.05	38.0
OX243876.1	15	22.47	38.0
OX243877.1	16	22.32	38.0
OX243878.1	17	22.03	38.5
OX243879.1	18	21.59	38.5
OX243880.1	19	21.32	38.5
OX243881.1	20	20.92	38.5
OX243882.1	21	19.68	38.5
OX243883.1	22	19.68	38.0
OX243884.1	23	19.65	38.5
OX243885.1	24	18.58	38.5
OX243886.1	25	17.52	39.0
OX243887.1	26	15.76	38.5
OX243888.1	27	13.81	39.0
OX243889.1	28	13.16	39.0
OX243890.1	29	11.91	39.0
OX243891.1	30	11.9	40.5
OX243861.1	Z	34.13	38.0
OX243892.1	MT	0.02	20.5

Table 3. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.1.3	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	yahs-1.1.91eebc2	https://github.com/c-zhou/yahs

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: *Amphipoea oculea* (ear moth). Accession number PRJEB53731; https://identifiers.org/ena.embl/PRJEB53731. (Wellcome Sanger Institute, 2022)

The genome sequence is released openly for reuse. The *Amphipoea oculea* 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. The genome will be annotated using available RNA-Seq data and presented through the Ensembl pipeline at the European Bioinformatics Institute. Raw data and assembly accession identifiers are reported in Table 1.

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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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