



DATA NOTE

The genome sequence of Red Underwing, *Catocala nupta*

Linnaeus, 1767

[version 1; peer review: 2 approved, 1 approved with reservations]

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V1 First published: 30 Jan 2025, 10:36
<https://doi.org/10.12688/wellcomeopenres.23621.1>
Latest published: 30 Jan 2025, 10:36
<https://doi.org/10.12688/wellcomeopenres.23621.1>

Abstract

We present a genome assembly from an individual female specimen of *Catocala nupta* (Red Underwing; Arthropoda; Insecta; Lepidoptera; Erebidae). The genome sequence has a total length of 930.40 megabases. Most of the assembly (99.82%) is scaffolded into 32 chromosomal pseudomolecules, including the W and Z sex chromosomes. The mitochondrial genome has also been assembled and is 15.57 kilobases in length. Gene annotation of this assembly on Ensembl identified 13,889 protein-coding genes.

Keywords

Catocala nupta, Red Underwing, genome sequence, chromosomal, Lepidoptera



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

Open Peer Review

Approval Status ? ✓ ✓

	1	2	3
version 1 30 Jan 2025	? view	✓ view	✓ view

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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.*

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How to cite this article: Boyes D, Lewis OT, University of Oxford and Wytham Woods Genome Acquisition Lab *et al.* **The genome sequence of Red Underwing, *Catocala nupta* Linnaeus, 1767 [version 1; peer review: 2 approved, 1 approved with reservations]** Wellcome Open Research 2025, 10:36 <https://doi.org/10.12688/wellcomeopenres.23621.1>

First published: 30 Jan 2025, 10:36 <https://doi.org/10.12688/wellcomeopenres.23621.1>

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; Noctuoidea; Erebidae; Erebininae; *Catocala*; *Catocala nupta* Linnaeus, 1767 (NCBI:txid423320)

Background

The red underwing *Catocala nupta* is a large and distinctive moth in the family Erebidae, with a broad distribution from western Europe extending eastwards across Asia (GBIF Secretariat, 2025). Geographically, it is the most widespread of the Palaearctic *Catocala* species, of which approximately seven species form a species group with very similar phenotypes to *C. nupta* (Borth et al., 2017).

Catocala nupta has boldly patterned red and black hindwings and the forewings are cryptically patterned in shades of brown and grey. The hindwings are usually concealed when the moth is at rest, and exposed as a startle display if it is disturbed (Ford, 1955). This species is common in much of England and parts of Wales, and in recent years has been expanding its range northwards into Scotland and westwards to Ireland (Randle et al., 2019). It is typically associated with lowland habitats, including woodlands, parklands, and gardens, where its larval food plants, poplar (*Populus* spp.) and willow (*Salix* spp.), are abundant (Waring et al., 2017). The adults flight period in the British Isles extends from late July to early October. Adults are nocturnal; they are readily attracted to sugary baits and less frequently occur at light (Waring et al., 2017). Adults are also often encountered during the day at rest on walls and fences. Eggs are laid singly on bark or branches of the host trees, overwinter and hatch in the spring. Larvae are nocturnal and extremely well-camouflaged, resembling twigs (Henwood et al., 2020).

We present a chromosomally complete genome sequence for *Catocala nupta*, based on a male specimen from Behind Chalet, Wytham, United Kingdom (Figure 1). The genome 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.

Genome sequence report

The genome of *Catocala nupta* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating a total of 79.04 Gb (gigabases) from 7.12 million reads, providing an estimated 85-fold coverage. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data, which produced 80.25 Gb from 531.42 million reads. Specimen and sequencing details are summarised in Table 1.

Assembly errors, including 16 missing joins or mis-joins and 7 haplotypic duplications, were corrected by manual curation. This reduced the assembly length by 2.05% and the scaffold



Figure 1. Photograph of the *Catocala nupta* (ilCatNupt1) specimen used for genome sequencing.

number by 11.59%, and increased the scaffold N50 by 2.78%. The final assembly has a total length of 930.40 Mb in 60 sequence scaffolds, with 100 gaps, and a scaffold N50 of 31.3 Mb (Table 2).

The snail plot in Figure 2 provides a summary of the assembly statistics, indicating the distribution of scaffold lengths and other assembly metrics. Figure 3 shows the distribution of scaffolds by GC proportion and coverage. Figure 4 presents a cumulative assembly plot, with separate curves representing different scaffold subsets assigned to various phyla, illustrating the completeness of the assembly.

Most of the assembly sequence (99.82%) was assigned to 32 chromosomal-level scaffolds, representing 30 autosomes and the W and Z sex chromosomes. These chromosome-level scaffolds, confirmed by the Hi-C data, are named in order of size (Figure 5; Table 3). During manual curation, chromosomes Z and W were assigned based on read coverage statistics. However, the order and orientation of the W is not fully resolved.

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, and as a separate fasta file with accession OY776110.1.

The final assembly has a Quality Value (QV) of 67.1. The *k*-mer completeness value was estimated as 97.75% for the combined assemblies (76.20% for the primary assembly and 71.73% for the alternate haplotype). BUSCO (v5.4.3) analysis using the lepidoptera_odb10 reference set ($n = 5,286$) indicated a completeness score of 98.9% (single = 98.1%, duplicated = 0.8%). The assembly achieves the EBP reference standard of 6.C.67. Other quality metrics are given in Table 2.

Table 1. Specimen and sequencing data for *Catocala nupta*.

Project information			
Study title	Catocala nupta (red underwing)		
Umbrella BioProject	PRJEB65729		
Species	<i>Catocala nupta</i>		
BioSample	SAMEA7520180		
NCBI taxonomy ID	423320		
Specimen information			
Technology	ToLID	BioSample accession	Organism part
PacBio long read sequencing	ilCatNupt1	SAMEA7520263	abdomen
Hi-C sequencing	ilCatNupt1	SAMEA7520262	head
RNA sequencing	ilCatNupt1	SAMEA7520263	abdomen
Sequencing information			
Platform	Run accession	Read count	Base count (Gb)
Hi-C Illumina NovaSeq 6000	ERR12035302	5.31e+08	80.25
PacBio Revio	ERR12015772	7.12e+06	79.04
RNA Illumina NovaSeq X	ERR12765149	6.49e+07	9.8

Genome annotation report

The *Catocala nupta* genome assembly (GCA_963675205.1) was annotated at the European Bioinformatics Institute (EBI) on Ensembl Rapid Release. The resulting annotation includes 27,031 transcribed mRNAs from 13,889 protein-coding and 3,026 non-coding genes (Table 2; https://rapid.ensembl.org/Catocala_nupta_GCA_963675205.1/Info/Index). The average transcript length is 21,717.61, with 1.60 coding transcripts per gene and 7.07 exons per transcript.

Methods

Sample acquisition and DNA barcoding

An adult female *Catocala nupta* (specimen ID Ox000197, ToLID ilCatNupt1) was collected from Behind Chalet, Wytham, United Kingdom (latitude 51.77, longitude -1.34) on 2019-08-24 by light trap. The specimen was collected and identified by Douglas Boyes (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 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.*, 2023). 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 procedures: sample preparation and homogenisation, DNA extraction, fragmentation and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). The ilCatNupt1 sample was prepared for DNA extraction by weighing and dissecting it 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 (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 a Qubit Fluorometer using the Qubit dsDNA High Sensitivity Assay kit. The fragment size distribution was evaluated by running the sample on the FemtoPulse system.

Table 2. Genome assembly data for *Catocala nupta*, ilCatNupt1.1.

Genome assembly		
Assembly name	ilCatNupt1.1	
Assembly accession	GCA_963675205.1	
Accession of alternate haplotype	GCA_963675195.1	
Span (Mb)	930.40	
Number of contigs	161	
Number of scaffolds	60	
Longest scaffold (Mb)	44.58	
Assembly metrics*		Benchmark
Contig N50 length (Mb)	11.3	≥ 1 Mb
Scaffold N50 length (Mb)	31.3	= chromosome N50
Consensus quality (QV)	67.1	≥ 40
k-mer completeness	primary: 76.20%; alternate: 71.73%; combined: 97.75%	≥ 95%
BUSCO v5.4.3 lineage: lepidoptera_odb10	C:98.9%[S:98.1%,D:0.8%], F:0.2%,M:0.9%,n:5,286	S > 90%, D < 5%
Percentage of assembly mapped to chromosomes	99.82%	≥ 90%
Sex chromosomes	WZ	localised homologous pairs
Organelles	Mitochondrial genome: 15.57 kb	complete single alleles
Genome annotation of assembly GCA_963675205.1 at Ensembl		
Number of protein-coding genes	13,889	
Number of non-coding genes	3,026	
Number of gene transcripts	27,031	

* Assembly metric benchmarks are adapted from [Rhie et al. \(2021\)](#) and the Earth BioGenome Project Report on Assembly Standards [September 2024](#).

** BUSCO scores based on the lepidoptera_odb10 BUSCO set using version 5.4.3. 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/Catocala_nupta/dataset/GCA_963675205.1/busco.

RNA was extracted from the abdomen of ilCatNupt1 using the RNA Extraction: Automated MagMax™ mirVana 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.

Hi-C sample preparation

Tissue from the head of the ilCatNupt1 sample was processed at the WSI Scientific Operations core, using the Arima-HiC v2 kit. Tissue (stored at -80 °C) was fixed, and the DNA crosslinked using a TC buffer with 22% formaldehyde. After crosslinking, the tissue was homogenised using the Diagnocine Power Masher-II and BioMasher-II tubes and pestles.

Following the kit manufacturer's instructions, crosslinked DNA was digested using a restriction enzyme master mix. The 5'-overhangs were then filled in and labelled with biotinylated nucleotides and proximally ligated. An overnight incubation was carried out for enzymes to digest remaining proteins and for crosslinks to reverse. A clean up was performed with SPRIselect beads prior to library preparation.

Library preparation and sequencing

PacBio HiFi

At the minimum, samples were required to have an average fragment size exceeding 8 kb and a total mass over 400 ng to proceed to the low input SMRTbell Prep Kit 3.0 protocol (Pacific Biosciences, California, USA). Libraries were prepared using the SMRTbell Prep Kit 3.0 (Pacific Biosciences,

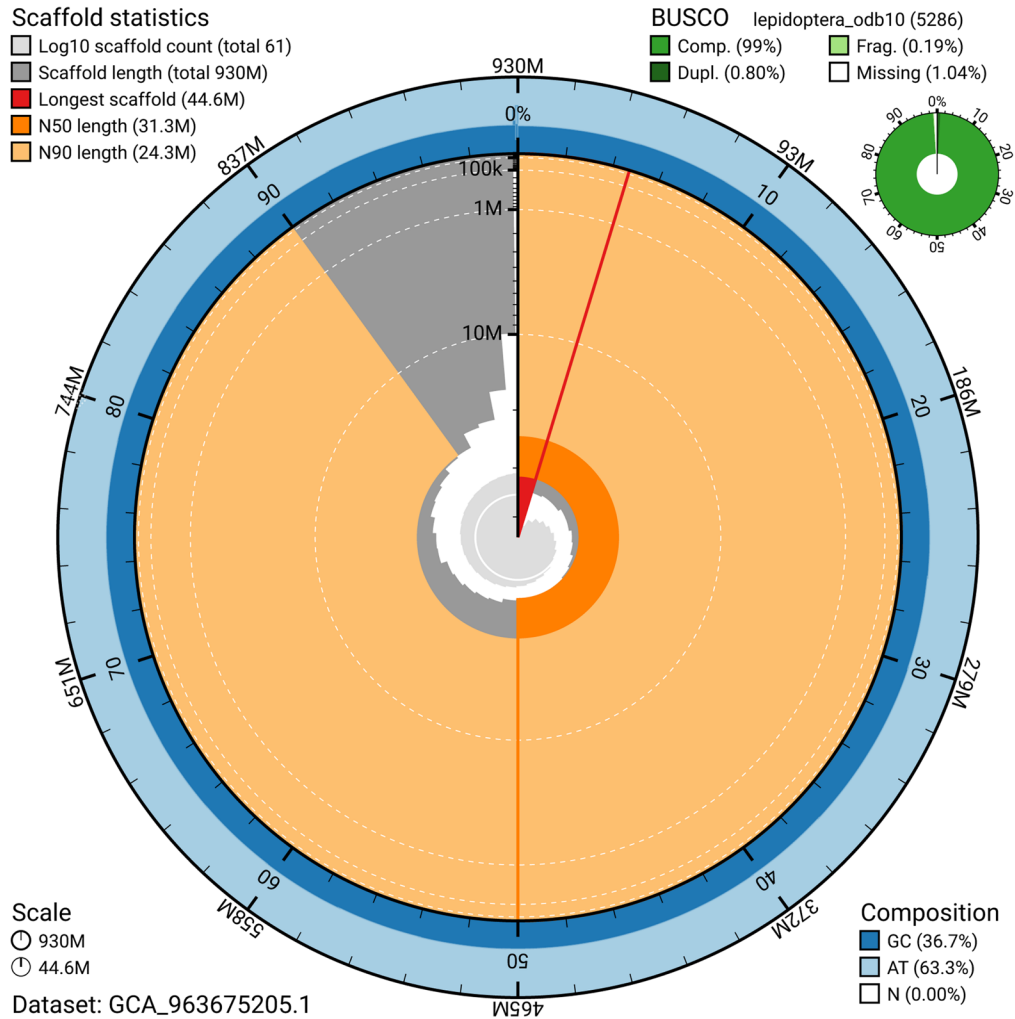


Figure 2. Genome assembly of *Catocala nupta*, ilCatNupt1.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. The outermost blue tracks display the distribution of GC, AT, and N percentages across the bins. Scaffolds are arranged clockwise from longest to shortest and are depicted in dark grey. The longest scaffold is indicated by the red arc, and the deeper orange and pale orange arcs represent the N50 and N90 lengths. A light grey spiral at the centre shows the cumulative scaffold count on a logarithmic scale. A summary of complete, fragmented, duplicated, and missing BUSCO genes in the lepidoptera_odb10 set is presented at the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963675205.1/dataset/GCA_963675205.1/snail.

California, USA) as per the manufacturer's instructions. The kit includes the reagents required for end repair/A-tailing, adapter ligation, post-ligation SMRTbell bead cleanup, and nuclease treatment. Following the manufacturer's instructions, size selection and clean up was carried out using diluted AMPure PB beads (Pacific Biosciences, California, USA). DNA concentration was quantified using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) with Qubit 1X dsDNA HS assay kit and the final library fragment size analysis was carried out using the Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies) and gDNA 55kb BAC analysis kit.

Samples were sequenced on a Revio instrument (Pacific Biosciences, California, USA). Prepared libraries were normalised to 2 nM and 15 μ L used for making complexes. For libraries below 2 nM, a volume of 10 μ L was used for making complexes. Primers were annealed and polymerases were hybridised to create circularised complexes, according to manufacturer's instructions. The complexes were purified with the 1.2X clean up with SMRTbell beads. The purified complexes were then diluted to the Revio loading concentration, in the range 200–300 pM, and spiked with a Revio sequencing internal control. Samples were sequenced using the Revio system on Revio 25M SMRT cells (Pacific Biosciences, California, USA).

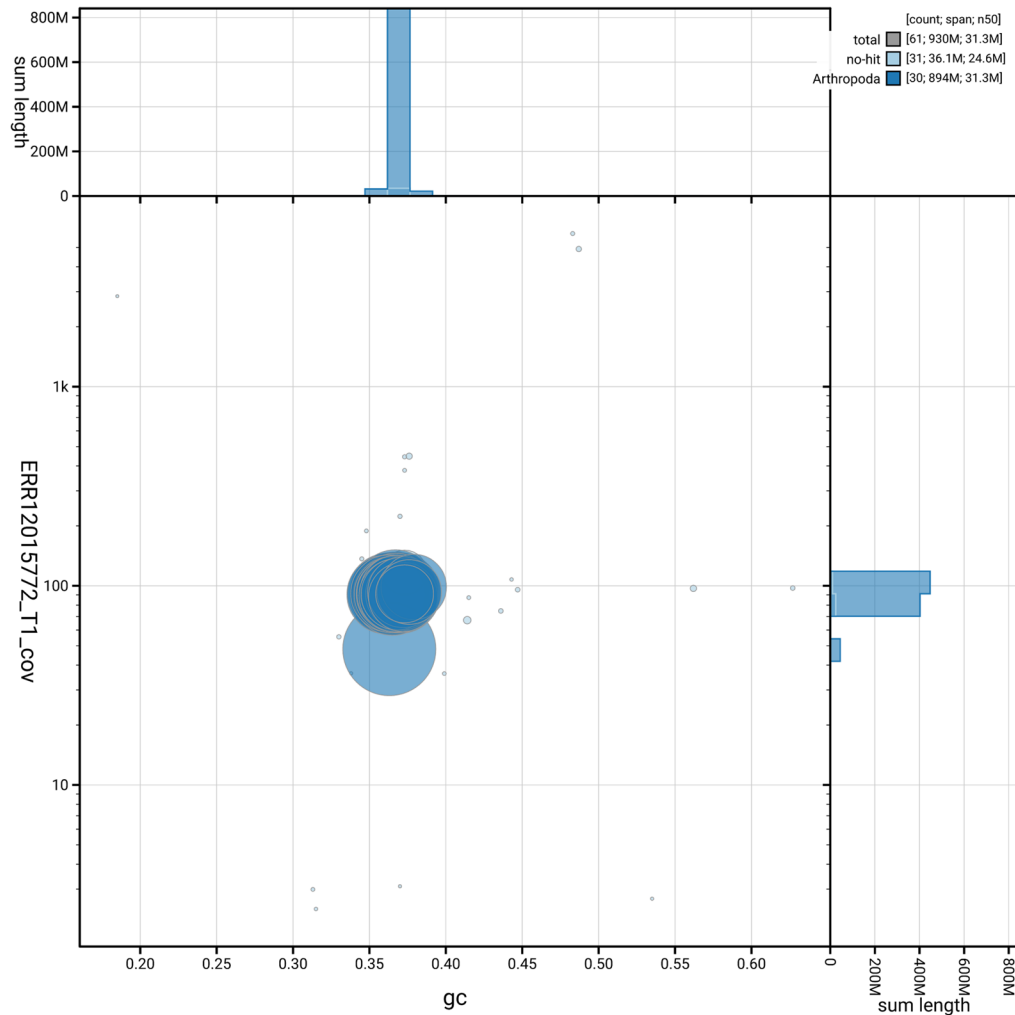


Figure 3. Genome assembly of *Catocala nupta*, ilCatNupt1.1: BlobToolKit GC-coverage plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes display the total length of sequences distributed across different levels of coverage and GC content. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963675205.1/dataset/GCA_963675205.1/blob.

The SMRT link software, a PacBio web-based end-to-end workflow manager, was used to set-up and monitor the run, as well as perform primary and secondary analysis of the data upon completion.

Hi-C

For Hi-C library preparation, DNA was fragmented using the Covaris E220 sonicator (Covaris) and size selected using SPRISelect beads to 400 to 600 bp. The DNA was then enriched using the Arima-HiC v2 kit Enrichment beads. The NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) was used for end repair, a-tailing, and adapter ligation. This uses a custom protocol which resembles the standard NEBNext Ultra II DNA Library Prep protocol but where library preparation occurs while DNA is bound to the Enrichment beads.

For library amplification, 10–16 PCR cycles were required, determined by the sample biotinylation percentage. Hi-C sequencing was performed using paired-end sequencing with a read length of 150 bp on an Illumina NovaSeq 6000 instrument.

RNA

Poly(A) RNA-Seq libraries were constructed using the NEB Ultra II RNA Library Prep kit, following the manufacturer's instructions. RNA sequencing was performed on the Illumina NovaSeq X instrument.

Genome assembly, curation and evaluation

Assembly

The HiFi reads were assembled using Hifiasm (Cheng *et al.*, 2021) with the --primary option. Haplotypic duplications were

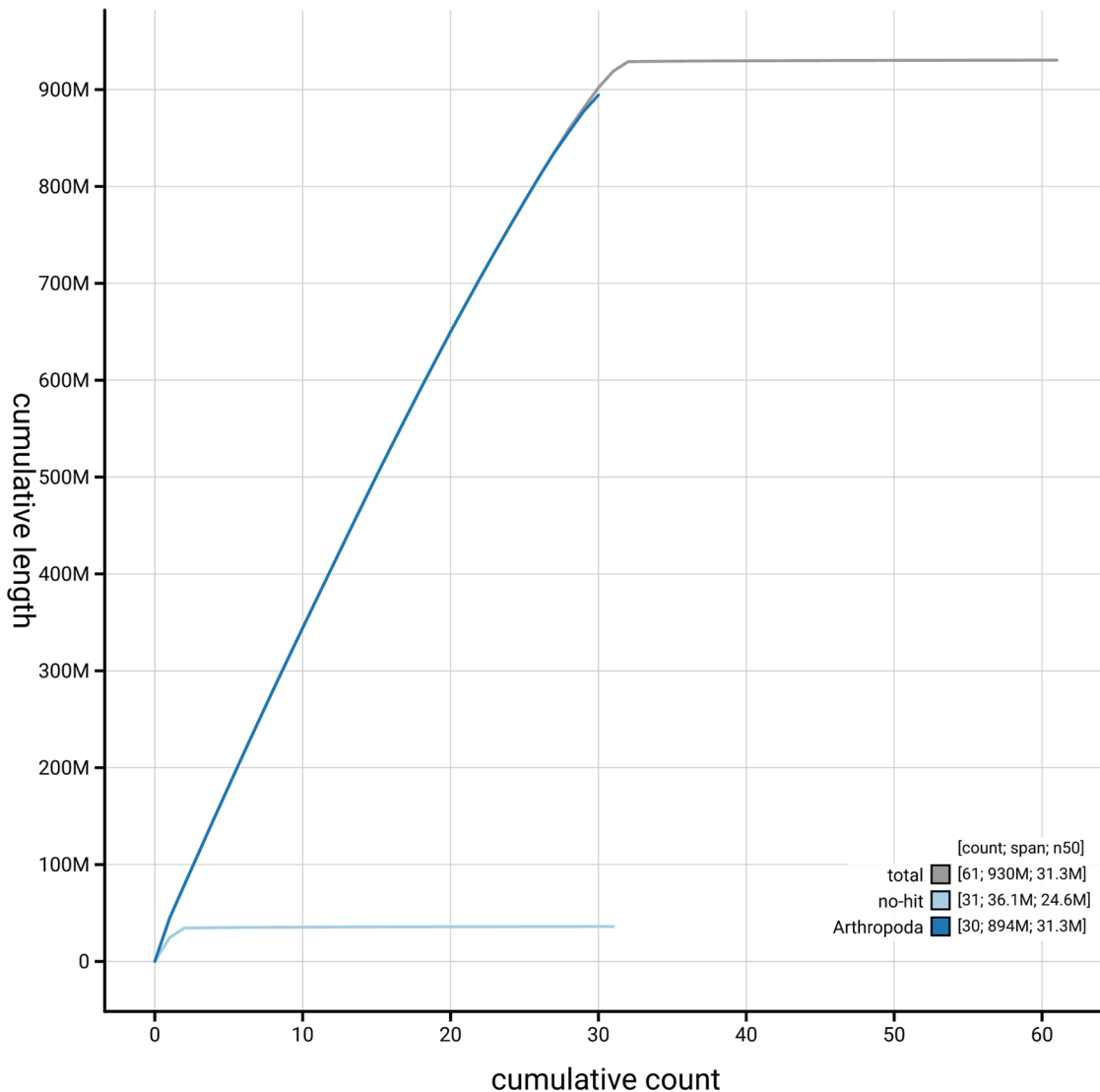


Figure 4. Genome assembly of *Catocala nupta* ilCatNupt1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963675205.1/dataset/GCA_963675205.1/cumulative.

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 (Rao *et al.*, 2014) in YaHS (Zhou *et al.*, 2023) using the `--break` option for handling potential misassemblies. 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). Flat files and maps used in curation were generated in `TreeVal` (Pointon *et al.*, 2023). Manual curation was primarily conducted using `PretextView` (Harry, 2022), with additional insights provided 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).

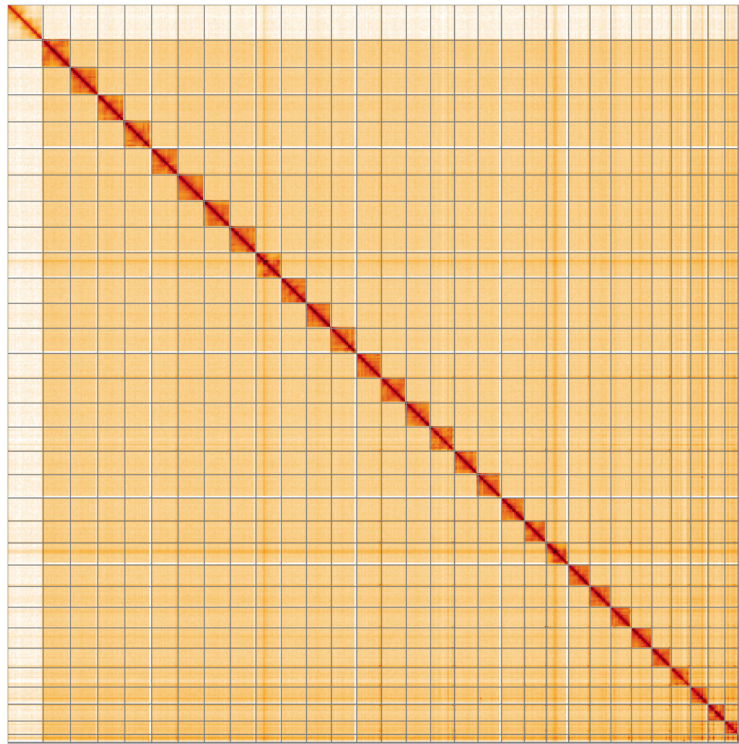


Figure 5. Genome assembly of *Catocala nupta* ilCatNupt1.1: Hi-C contact map of the ilCatNupt1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at <https://genome-note-higlass.tol.sanger.ac.uk/I/?d=U8taQOB8RpuWjrv3WnpNA>.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Catocala nupta*, ilCatNupt1.

INSDC accession	Name	Length (Mb)	GC%
OY776079.1	1	34.46	36.5
OY776080.1	2	34.16	36.5
OY776081.1	3	34.15	36.5
OY776082.1	4	33.54	36.5
OY776083.1	5	33.51	36.5
OY776084.1	6	32.97	37.0
OY776085.1	7	32.76	36.0
OY776086.1	8	32.18	37.0
OY776087.1	9	31.88	36.5
OY776088.1	10	31.64	36.0
OY776089.1	11	31.58	36.5
OY776090.1	12	31.54	36.5
OY776091.1	13	31.35	36.5
OY776092.1	14	31.17	36.5
OY776093.1	15	30.43	36.5
OY776094.1	16	30.13	37.0

INSDC accession	Name	Length (Mb)	GC%
OY776095.1	17	29.73	36.5
OY776096.1	18	29.24	37.0
OY776097.1	19	28.94	36.5
OY776098.1	20	27.8	36.5
OY776099.1	21	27.67	37.0
OY776100.1	22	27.03	36.5
OY776101.1	23	26.25	37.0
OY776102.1	24	26.01	37.0
OY776103.1	25	25.56	37.0
OY776104.1	26	24.57	37.0
OY776105.1	27	24.25	37.0
OY776106.1	28	21.72	38.0
OY776107.1	29	21.18	37.5
OY776108.1	30	16.92	37.5
OY776109.1	W	9.9	37.5
OY776078.1	Z	44.58	36.5
OY776110.1	MT	0.02	18.5

Any identified contamination, missed joins, and mis-joins were corrected, and duplicate sequences were tagged and removed. The curation process is documented at <https://gitlab.com/wtsi-grit/rapid-curation> (article in preparation).

Evaluation of final assembly

The Merqury.FK tool (Rhie *et al.*, 2020) was used to evaluate k -mer completeness and assembly quality for the primary and alternate haplotypes using the k -mer databases ($k = 31$) that were pre-computed prior to genome assembly. The analysis outputs included assembly QV scores and completeness statistics.

A Hi-C contact map was produced for the final, public version of the assembly. The Hi-C reads were aligned using bwa-mem2 (Vasimuddin *et al.*, 2019) and the alignment files were combined using SAMtools (Danecek *et al.*, 2021). The Hi-C alignments were converted into a contact map using BEDTools (Quinlan & Hall, 2010) and the Cooler tool suite (Abdennur & Mirny, 2020). The contact map is visualised in HiGlass (Kerpedjiev *et al.*, 2018).

The blobtoolkit pipeline is a Nextflow port of the previous Snakemake Blobtoolkit pipeline (Challis *et al.*, 2020). It aligns the PacBio reads in SAMtools and minimap2 (Li, 2018) and

generates coverage tracks for regions of fixed size. In parallel, it queries the GoAT database (Challis *et al.*, 2023) to identify all matching BUSCO lineages to run BUSCO (Manni *et al.*, 2021). For the three domain-level BUSCO lineages, the pipeline aligns the BUSCO genes to the UniProt Reference Proteomes database (Bateman *et al.*, 2023) with DIAMOND (Buchfink *et al.*, 2021) blastp. The genome is also split into chunks according to the density of the BUSCO genes from the closest taxonomic lineage, and each chunk is aligned to the UniProt Reference Proteomes database with DIAMOND blastx. Genome sequences with no hits are chunked with seqtk and aligned to the NT database with blastn (Altschul *et al.*, 1990). The blobtools suite combines all these outputs into a blobdir for visualisation.

The genome evaluation pipelines were developed using nf-core tooling (Ewels *et al.*, 2020) and MultiQC (Ewels *et al.*, 2016), relying on the Conda package manager, the Bioconda initiative (Grüning *et al.*, 2018), the Biocontainers infrastructure (da Veiga Leprevost *et al.*, 2017), as well as the Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017) containerisation solutions.

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

Table 4. Software tools: versions and sources.

Software tool	Version	Source
BEDTools	2.30.0	https://github.com/arq5x/bedtools2
BLAST	2.14.0	ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast/
BlobToolKit	4.3.7	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.4.3 and 5.5.0	https://gitlab.com/e2lab/busco
bwa-mem2	2.2.1	https://github.com/bwa-mem2/bwa-mem2
Cooler	0.8.11	https://github.com/open2c/cooler
DIAMOND	2.1.8	https://github.com/bbuchfink/diamond
fasta_windows	0.2.4	https://github.com/tolkit/fasta_windows
FastK	427104ea91c78c3b8b8b49f1a7d6bbeaa869ba1c	https://github.com/thegenemyers/FASTK
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
GoAT CLI	0.2.5	https://github.com/genomehubs/goat-cli
Hifiasm	0.19.8-r587	https://github.com/chhylyp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84aa44357826c0b6753eb28de	https://github.com/higlass/higlass
Merqury.FK	d00d98157618f4e8d1a9190026b19b471055b22e	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
NCBI Datasets	15.12.0	https://github.com/ncbi/datasets
Nextflow	23.04.0-5857	https://github.com/nextflow-io/nextflow

Software tool	Version	Source
PretextView	0.2.5	https://github.com/sanger-tol/PretextView
purge_dups	1.2.5	https://github.com/dfguan/purge_dups
samtools	1.16.1, 1.17, and 1.18	https://github.com/samtools/samtools
sanger-tol/ascc	-	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	0.6.0	https://github.com/sanger-tol/blobtoolkit
Seqtk	1.3	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.0.0	https://github.com/sanger-tol/treeval
YaHS	1.2a.2	https://github.com/c-zhou/yahs

Genome annotation

The [Ensembl Genebuild](#) annotation system (Aken *et al.*, 2016) was used to generate annotation for the *Catocala nupta* assembly (GCA_963675205.1) 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 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: *Catocala nupta* (red underwing). Accession number PRJEB65729; <https://identifiers.org/ena.embl/PRJEB65729>. The genome sequence is released openly for reuse. The *Catocala nupta* 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](#).

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/423320>.

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Open Peer Review

Current Peer Review Status: ? ✓ ✓

Version 1

Reviewer Report 24 February 2025

<https://doi.org/10.21956/wellcomeopenres.26061.r118072>

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Arun Arumugaperumal 

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This is the first report of the whole genome sequence of the Red Underwing *Catocala nupta*. The genome size was 930.4 Mbp. The assembly reported here has 32 chromosomes. The mitochondrial genome of size 15.57 kb was also reported. The BUSCO analysis shows 99% completeness of genome sequence. The data note can be accepted for indexing.

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: Bioinformatics, 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 18 February 2025

<https://doi.org/10.21956/wellcomeopenres.26061.r118070>

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Maríndia Deprá

Federal University of Rio Grande do Sul, Porto Alegre, Brazil

The article presents a robust and well-documented genomic assembly of a female specimen of *Catocala nupta*, following well-established protocols. The description of the methods is largely sufficient to allow replication of the study, with detailed information on the collection processes, DNA/RNA extraction, sequencing, and genome assembly. However, it would be beneficial to provide more details on the criteria for excluding haplotypic duplications and manual curation, and better clarify the parameters used in the assembly and annotation tools. Please, replace “male” by “female” in the end of the background section.

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: genetics, DNA barcoding, molecular evolution

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 04 February 2025

<https://doi.org/10.21956/wellcomeopenres.26061.r118073>

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Christophe Klopp 

INRAE, Castanet-Tolosan, France

The authors are presenting a first genome assembly, in chromosomes, of *Catocala nupta*. The

article is well written and globally the methods are standards.

Comparing principal and alternative assembly sizes (930Mb vs 866Mb) shows that the authors are very close to haplotyped assemblies of a quite heterozygous individual. Therefore it would have been interesting to change strategy and provide the community with two haplotyped assemblies in chromosomes enabling to study heterozygosity patterns between haplotypes as well as to create the foundation of a species pangenome.

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

Yes

Are the protocols appropriate and is the work technically sound?

Partly

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?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genome assembly and annotation

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, however I have significant reservations, as outlined above.
