



## DATA NOTE

# The genome sequence of the Shaded Broad-bar moth,

## *Scotopteryx chenopodiata* (Linnaeus, 1758)

[version 1; peer review: awaiting peer review]

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### Open Peer Review

**Approval Status** AWAITING PEER REVIEW

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### Abstract

We present a genome assembly from a male specimen of *Scotopteryx chenopodiata* (Shaded Broad-bar; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence has a total length of 337.86 megabases. Most of the assembly (98.85%) is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled, with a length of 16.65 kilobases.

### Keywords

*Scotopteryx chenopodiata*, Shaded Broad-bar, genome sequence, chromosomal, Lepidoptera



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

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## Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Geometroidea; Geometridae; Larentiinae; *Scotopteryx*; *Scotopteryx chenopodiata* (Linnaeus, 1758) (NCBI:txid326962)

## Background

The Shaded Broad-bar (*Scotopteryx chenopodiata*) is a moth in the family Geometridae. It is very widely distributed in Britain, but nevertheless has undergone a steep decline since 1970 (Randle *et al.*, 2019). It has been recorded throughout Central Europe and Russia (GBIF Secretariat, 2023).

The moth is brown, but fairly variable in colour. However, it has an easily recognisable patterning with a darker central cross-bar. There is one generation a year which flies in July and August. It often flies by day, and rarely comes to light. The moth occurs in grassy places where its foodplants are clovers and vetches (Waring *et al.*, 2017). It overwinters as a larva and pupates in a spinning (Henwood *et al.*, 2020).

The genome of the Shaded Broad-bar, *Scotopteryx chenopodiata*, 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 *Scotopteryx chenopodiata*, based on a specimen from Wytham Woods, Oxfordshire, United Kingdom (Figure 1).

## Genome sequence report

### Sequencing data

The genome of a specimen of *Scotopteryx chenopodiata* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 23.10 Gb (gigabases) from 2.02 million reads. GenomeScope analysis of the



**Figure 1.** Photograph of the *Scotopteryx chenopodiata* (ilScoChen1) specimen used for genome sequencing.

PacBio HiFi data estimated the haploid genome size at 327.18 Mb, with a heterozygosity of 0.64% and repeat content of 22.51%. These values provide an initial assessment of genome complexity and the challenges anticipated during assembly. Based on this estimated genome size, the sequencing data provided approximately 67.0x coverage of the genome. Chromosome conformation Hi-C sequencing produced 101.82 Gb from 674.30 million reads. Table 1 summarises the specimen and sequencing information.

### Assembly statistics

The primary haplotype was assembled, and contigs corresponding to an alternate haplotype were also deposited in INSDC databases. The assembly was improved by manual curation, which corrected 49 misjoins or missing joins and removed five haplotypic duplications. These interventions increased the scaffold count by 2.6%. The final assembly has a total length of 337.86 Mb in 78 scaffolds, with 81 gaps, and a scaffold N50 of 12.07 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 (98.85%) was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 5; Table 3). During curation, chromosome Z was assigned based on synteny to the genome of *Scotopteryx bipunctaria* (GCA\_949320045.1).

The mitochondrial genome was also assembled. This sequence is included as a contig in the multifasta file of the genome submission and as a standalone record.

### Assembly quality metrics

The estimated Quality Value (QV) and *k*-mer completeness metrics, along with BUSCO completeness scores, were calculated for each haplotype and the combined assembly. The QV reflects the base-level accuracy of the assembly, while *k*-mer completeness indicates the proportion of expected *k*-mers identified in the assembly. BUSCO scores provide a measure of completeness based on benchmarking universal single-copy orthologues.

The combined primary and alternate assemblies achieve an estimated QV of 63.2. The *k*-mer recovery for the primary haplotype is 85.07%, and for the alternate haplotype 81.76%; the combined primary and alternate assemblies have a *k*-mer recovery of 99.10%. BUSCO analysis using the lepidoptera\_odb10 reference set (*n* = 5,286) identified 98.5% of the expected gene set (single = 98.1%, duplicated = 0.4%).

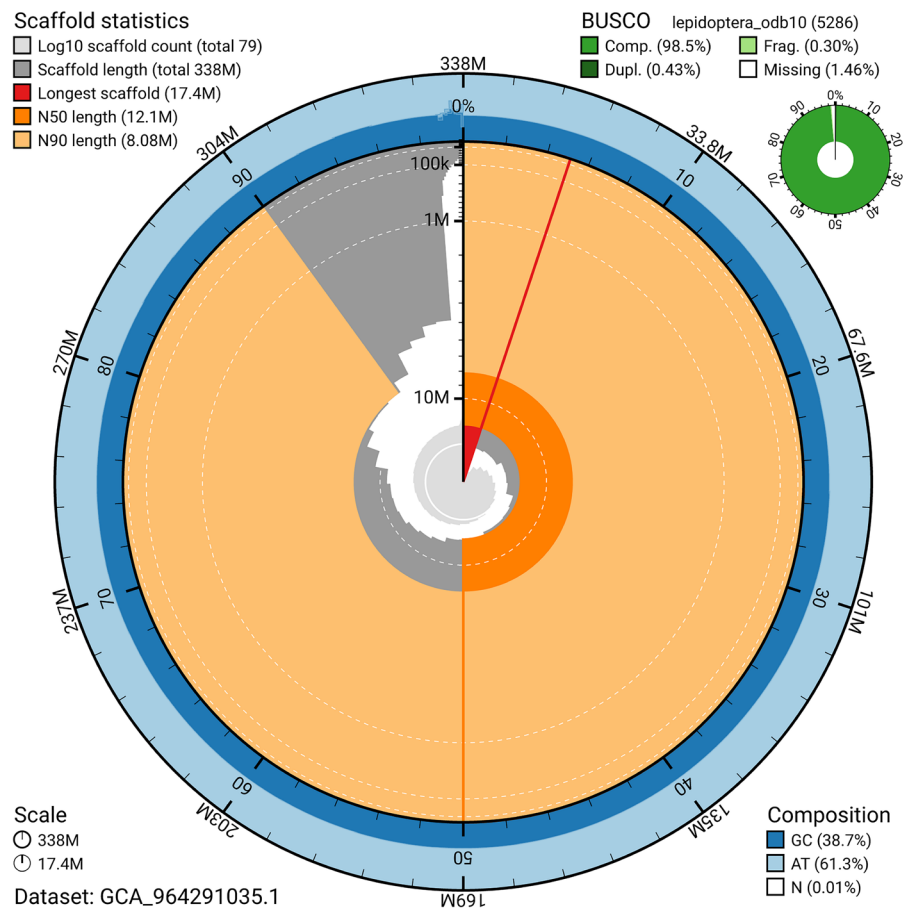
**Table 1. Specimen and sequencing data for *Scotopteryx chenopodiata*.**

Project information			
Study title	Scotopteryx chenopodiata (shaded broad-bar)		
Umbrella BioProject	PRJEB71304		
Species	Scotopteryx chenopodiata		
BioSpecimen	SAMEA7701561		
NCBI taxonomy ID	326962		
Specimen information			
Technology	ToLID	BioSample accession	Organism part
PacBio long read sequencing	ilScoChen1	SAMEA7701757	whole organism
Hi-C sequencing	ilScoChen2	SAMEA115163619	head
RNA sequencing	ilScoChen2	SAMEA115163596	abdomen
Sequencing information			
Platform	Run accession	Read count	Base count (Gb)
Hi-C Illumina NovaSeq X	ERR13702756	6.74e+08	101.82
PacBio Sequel IIe	ERR12370429	2.02e+06	23.1
RNA Illumina NovaSeq X	ERR13999094	7.49e+07	11.3

**Table 2. Genome assembly data for *Scotopteryx chenopodiata*.**

Genome assembly		
Assembly name	ilScoChen1.1	
Assembly accession	GCA_964291035.1	
Alternate haplotype accession	GCA_964290915.1	
Assembly level for primary assembly	chromosome	
Span (Mb)	337.86	
Number of contigs	159	
Number of scaffolds	78	
Longest scaffold (Mb)	17.45	
Assembly metrics	Measure	Benchmark
Contig N50 length	4.18 Mb	$\geq 1$ Mb
Scaffold N50 length	12.07 Mb	= chromosome N50
Consensus quality (QV)	Primary: 63.2; alternate: 63.1; combined: 63.2	$\geq 40$
k-mer completeness	Primary: 85.07%; alternate: 81.76%; combined: 99.10%	$\geq 95\%$
BUSCO*	C:98.5%[S:98.1%,D:0.4%], F:0.3%,M:1.2%,n:5,286	$S > 90\%$ ; $D < 5\%$
Percentage of assembly mapped to chromosomes	98.85%	$\geq 90\%$
Sex chromosomes	Z	localised homologous pairs
Organelles	Mitochondrial genome: 16.65 kb	complete single alleles

\* BUSCO scores based on the lepidoptera\_odb10 BUSCO set using version 5.5.0. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison.



**Figure 2. Genome assembly of *Scotopteryx chenopodiata*, ilScoChen1.1: 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\\_964291035.1/dataset/GCA\\_964291035.1/snail](https://blobtoolkit.genomehubs.org/view/GCA_964291035.1/dataset/GCA_964291035.1/snail).

Table 2 provides assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project (EBP) Report on Assembly Standards September 2024. The assembly achieves the EBP reference standard of **6.C.Q63**.

## Methods

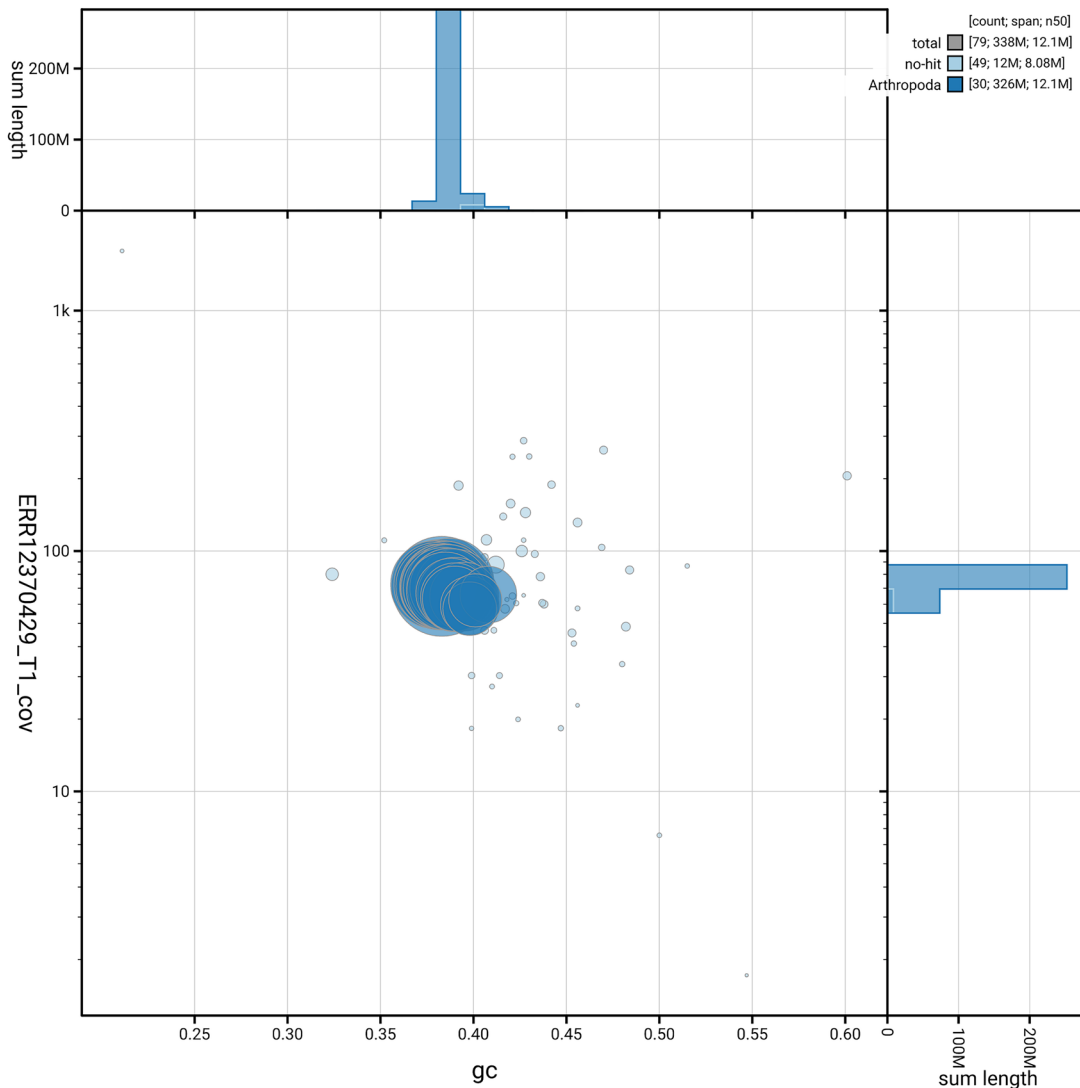
### Sample acquisition and DNA barcoding

An adult male *Scotopteryx chenopodiata* (specimen ID OX000700, ToLID ilScoChen1) was collected from Wytham Woods, Oxfordshire, United Kingdom (latitude 51.77, longitude -1.34) on 2020-07-20, using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice. This specimen was used for PacBio DNA sequencing.

The specimen used for Hi-C and RNA sequencing (specimen ID SAN00003355, ToLID ilScoChen2) was a[n] adult

specimen collected from Hinxton, Cambridgeshire, England (latitude 52.0749, longitude 0.1843) on 2023-08-08. The specimen was collected by Charlotte Wright and Witold Morek, identified by Charlotte Wright (Wellcome Sanger Institute).

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 ilScoChen1 specimen and stored in ethanol, while the remaining parts were shipped on dry ice to the Wellcome Sanger Institute (WSI) (Pereira *et al.*, 2022). 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



**Figure 3. Genome assembly of *Scotopteryx chenopodiata*, ilScoChen1.1: BlobToolKit GC-coverage plot.** Blob 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\\_964291035.1/dataset/GCA\\_964291035.1/blob](https://blobtoolkit.genomehubs.org/view/GCA_964291035.1/dataset/GCA_964291035.1/blob).

operating procedures for Darwin Tree of Life barcoding have been deposited on protocols.io (Beasley *et al.*, 2023).

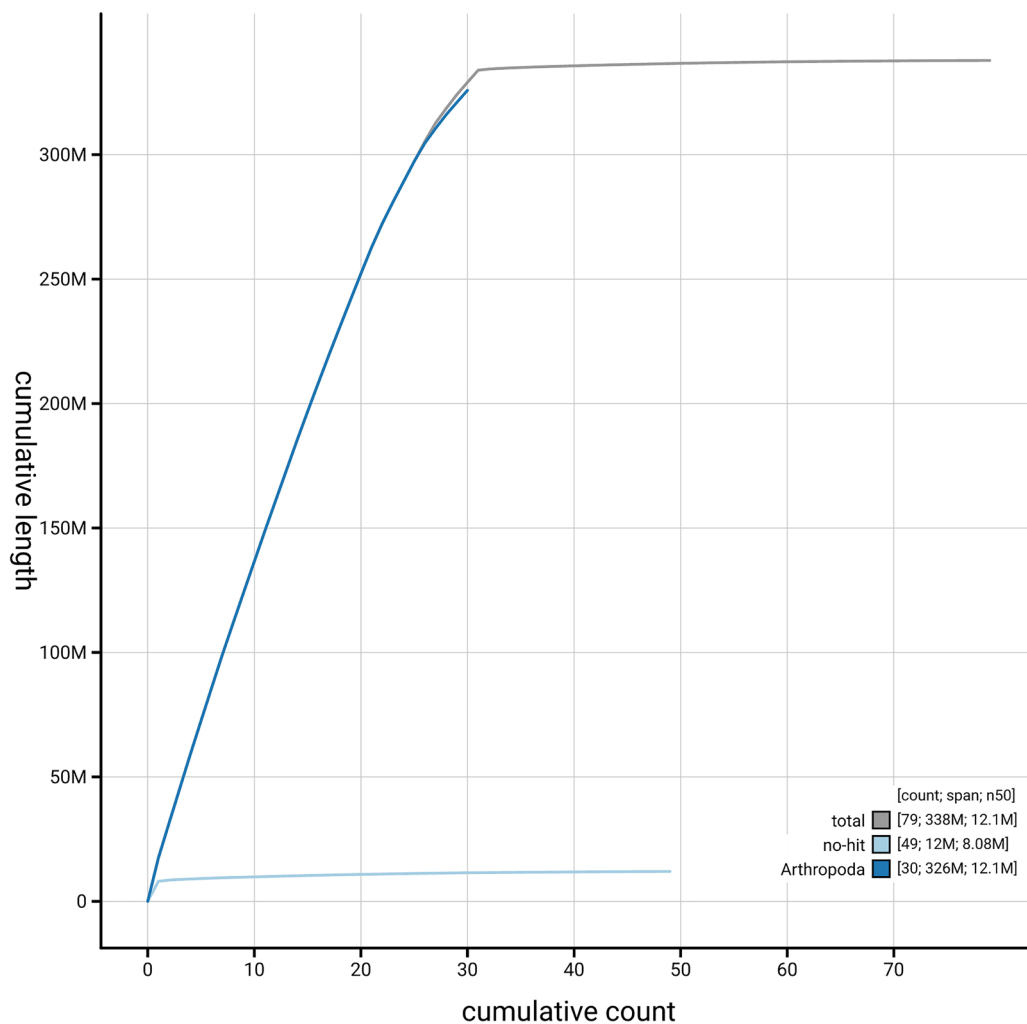
Metadata collection for samples adhered to the Darwin Tree of Life project standards described by Lawniczak *et al.* (2022).

#### 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 ilScoChen1 sample

was prepared for DNA extraction by weighing and dissecting it on dry ice (Jay *et al.*, 2023), and tissue was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a).

HMW DNA was extracted using the Automated MagAttract v2 protocol (Oatley *et al.*, 2023a). DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system (Bates *et al.*, 2023). Sheared DNA was purified by solid-phase reversible immobilisation, using AMPure PB beads to eliminate shorter fragments and concentrate the DNA (Oatley *et al.*, 2023b). The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and Qubit Fluorometer using the Qubit dsDNA High Sensitivity Assay



**Figure 4. Genome assembly of *Scotopteryx chenopodiata*, ilScoChen1.1: 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\\_964291035.1/dataset/GCA\\_964291035.1/cumulative](https://blobtoolkit.genomehubs.org/view/GCA_964291035.1/dataset/GCA_964291035.1/cumulative).

kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

RNA was extracted from abdomen tissue of ilScoChen2 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ mirVana protocol (do Amaral *et al.*, 2023). The RNA concentration was assessed using a Nano-drop 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

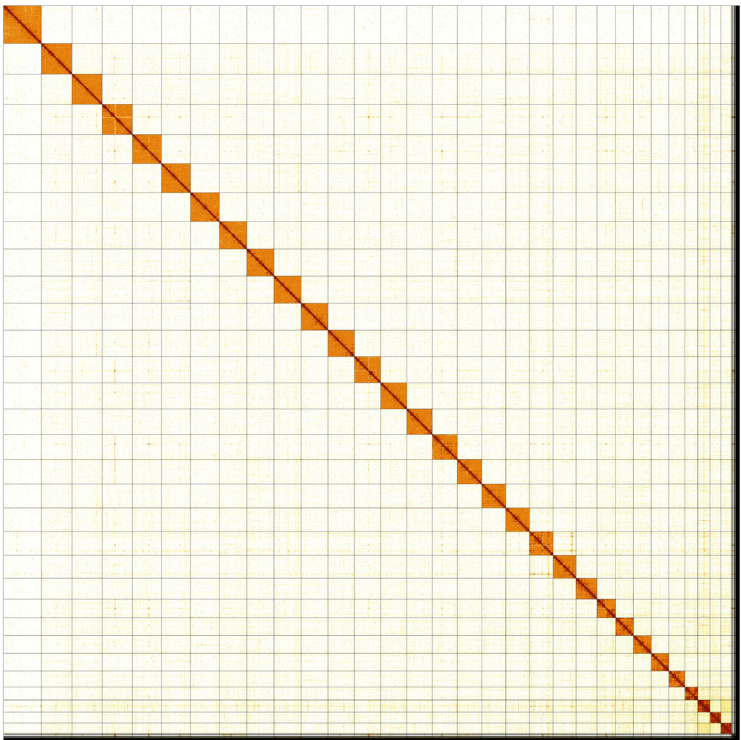
Tissue from the head of the ilScoChen2 sample was processed for Hi-C sequencing at the WSI Scientific Operations core, using the Arima-HiC v2 kit. In brief, 20–50 mg of frozen tissue (stored at –80 °C) was fixed, and the DNA crosslinked

using a TC buffer with 22% formaldehyde concentration. After crosslinking, the tissue was homogenised using the Diagenode Power Masher-II and BioMasher-II tubes and pestles. Following the Arima-HiC v2 kit manufacturer's instructions, crosslinked DNA was digested using a restriction enzyme master mix. The 5'-overhangs were 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. Additionally, the biotinylation percentage was estimated using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and Qubit HS Assay Kit and Arima-HiC v2 QC beads.

#### Library preparation and sequencing

Library preparation and sequencing were performed at the WSI Scientific Operations core.





**Figure 5.** Genome assembly of *Scotopteryx chenopodiata*: Hi-C contact map of the iScoChen1.1 assembly, produced in PretextView. Chromosomes are shown in order of size from left to right and top to bottom.

**Table 3.** Chromosomal pseudomolecules in the genome assembly of *Scotopteryx chenopodiata*, iScoChen1.

INSDC accession	Name	Length (Mb)	GC%
OZ197133.1	1	13.99	38.5
OZ197134.1	2	13.88	38
OZ197135.1	3	13.84	38.5
OZ197136.1	4	13.39	38.5
OZ197137.1	5	13.34	38
OZ197138.1	6	13.22	38.5
OZ197139.1	7	12.59	38
OZ197140.1	8	12.47	38
OZ197141.1	9	12.41	38
OZ197142.1	10	12.39	38
OZ197143.1	11	12.07	38.5
OZ197144.1	12	12.07	38.5
OZ197145.1	13	12.04	38.5
OZ197146.1	14	11.66	38.5

INSDC accession	Name	Length (Mb)	GC%
OZ197147.1	15	11.46	38.5
OZ197148.1	16	11.27	38.5
OZ197149.1	17	11.0	38.5
OZ197150.1	18	10.89	39
OZ197151.1	19	10.82	38.5
OZ197152.1	20	10.55	38.5
OZ197153.1	21	9.56	39
OZ197154.1	22	8.59	39
OZ197155.1	23	8.2	39.5
OZ197156.1	24	8.17	39
OZ197157.1	25	8.08	39.5
OZ197158.1	26	7.32	39
OZ197159.1	27	5.9	40
OZ197160.1	28	5.53	41
OZ197161.1	29	4.95	40
OZ197162.1	30	4.85	40
OZ197132.1	Z	17.45	38.5
OZ197163.1	MT	0.02	21



### **PacBio HiFi**

At a 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), depending on genome size and sequencing depth required. Libraries were prepared using the SMRTbell Prep Kit 3.0 (Pacific Biosciences, 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 using the Sequel IIe system (Pacific Biosciences, California, USA). The concentration of the library loaded onto the Sequel IIe was in the range 40–135 pM. 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. Using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) 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 to 16 PCR cycles were required, determined by the sample biotinylation percentage. The Hi-C sequencing was performed using paired-end sequencing with a read length of 150 bp on an Illumina NovaSeq X 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**

Prior to assembly of the PacBio HiFi reads, a database of  $k$ -mer counts ( $k = 31$ ) was generated from the filtered reads using FastK. GenomeScope2 (Ranallo-Benavidez *et al.*, 2020) was used to analyse the  $k$ -mer frequency distributions, providing estimates of genome size, heterozygosity, and repeat content.

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 (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. Flat files and maps used in curation were generated via the TreeVal pipeline (Pointon *et al.*, 2023). Manual curation was conducted primarily in PretextView (Harry, 2022) and HiGlass (Kerpedjiev *et al.*, 2018), with additional insights provided by JBrowse2 (Diesh *et al.*, 2023). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Any identified contamination, missed joins, and mis-joins were amended, and duplicate sequences were tagged and removed. The sex chromosome was assigned based on synteny analysis. The curation process is documented at <https://gitlab.com/wtsi-grit/rapid-curation>.

### **Assembly quality assessment**

The Merqury.FK tool (Rhie *et al.*, 2020), run in a Singularity container (Kurtzer *et al.*, 2017), 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 computed prior to genome assembly. The analysis outputs included assembly QV scores and completeness statistics.

The blobtoolkit pipeline is a Nextflow port of the previous Snakemake Blobtoolkit pipeline (Challis *et al.*, 2020). It aligns the PacBio reads in SAMtools (Danecek *et al.*, 2021) 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 blastp (Buchfink *et al.*, 2021). The genome is also divided 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 using DIAMOND blastx. Genome sequences without a hit are chunked using 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 blobtoolkit pipeline was 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.

#### 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.

**Table 4. Software tools: versions and sources.**

Software tool	Version	Source
BLAST	2.14.0	<a href="ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/">ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/</a>
BlobToolKit	4.3.9	<a href="https://github.com/blobtoolkit/blobtoolkit">https://github.com/blobtoolkit/blobtoolkit</a>
BUSCO	5.5.0	<a href="https://gitlab.com/ezlab/busco">https://gitlab.com/ezlab/busco</a>
bwa-mem2	2.2.1	<a href="https://github.com/bwa-mem2/bwa-mem2">https://github.com/bwa-mem2/bwa-mem2</a>
DIAMOND	2.1.8	<a href="https://github.com/bbuchfink/diamond">https://github.com/bbuchfink/diamond</a>
fasta_windows	0.2.4	<a href="https://github.com/tolkit/fasta_windows">https://github.com/tolkit/fasta_windows</a>
FastK	666652151335353eef2fcd58880bcef5bc2928e1	<a href="https://github.com/thegenemyers/FASTK">https://github.com/thegenemyers/FASTK</a>
Gfastats	1.3.6	<a href="https://github.com/vgl-hub/gfastats">https://github.com/vgl-hub/gfastats</a>
Goat CLI	0.2.5	<a href="https://github.com/genomehubs/goat-cli">https://github.com/genomehubs/goat-cli</a>
Hifiasm	0.19.8-r603	<a href="https://github.com/chhyllp123/hifiasm">https://github.com/chhyllp123/hifiasm</a>
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84aa44357826c0b6753eb28de	<a href="https://github.com/higlass/higlass">https://github.com/higlass/higlass</a>
MercuryFK	d00d98157618f4e8d1a9190026b19b471055b22e	<a href="https://github.com/thegenemyers/MERQURY.FK">https://github.com/thegenemyers/MERQURY.FK</a>
Minimap2	2.24-r1122	<a href="https://github.com/lh3/minimap2">https://github.com/lh3/minimap2</a>
MitoHiFi	3	<a href="https://github.com/marcelauliano/MitoHiFi">https://github.com/marcelauliano/MitoHiFi</a>
MultiQC	1.14, 1.17, and 1.18	<a href="https://github.com/MultiQC/MultiQC">https://github.com/MultiQC/MultiQC</a>
Nextflow	23.10.0	<a href="https://github.com/nextflow-io/nextflow">https://github.com/nextflow-io/nextflow</a>
PretextView	0.2.5	<a href="https://github.com/sanger-tol/PretextView">https://github.com/sanger-tol/PretextView</a>
samtools	1.19.2	<a href="https://github.com/samtools/samtools">https://github.com/samtools/samtools</a>
sanger-tol/ascc	-	<a href="https://github.com/sanger-tol/ascc">https://github.com/sanger-tol/ascc</a>
sanger-tol/blobtoolkit	0.5.1	<a href="https://github.com/sanger-tol/blobtoolkit">https://github.com/sanger-tol/blobtoolkit</a>
Seqtk	1.3	<a href="https://github.com/lh3/seqtk">https://github.com/lh3/seqtk</a>
Singularity	3.9.0	<a href="https://github.com/sylabs/singularity">https://github.com/sylabs/singularity</a>
TreeVal	1.2.0	<a href="https://github.com/sanger-tol/treeval">https://github.com/sanger-tol/treeval</a>
YaHS	1.2a.2	<a href="https://github.com/c-zhou/yahs">https://github.com/c-zhou/yahs</a>

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: *Scotopteryx chenopodiata* (shaded broad-bar). Accession number PRJEB71304; <https://identifiers.org/ena.embl/PRJEB71304>. The genome sequence is released openly for reuse. The *Scotopteryx chenopodiata* genome sequencing initiative is part of the Darwin Tree of Life (DTOL) project (PRJEB40665) and Project Psyche (PRJEB71705). 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](#) and [Table 2](#).

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## References

- Allio R, Schomaker-Bastos A, Romiguier J, *et al.*: **MitoFinder: efficient automated large-scale extraction of mitogenomic data in target enrichment phylogenomics**. *Mol Ecol Resour.* 2020; **20**(4): 892–905. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Altschul SF, Gish W, Miller W, *et al.*: **Basic Local Alignment Search Tool**. *J Mol Biol.* 1990; **215**(3): 403–410. [PubMed Abstract](#) | [Publisher Full Text](#)
- Bateman A, Martin MJ, Orchard S, *et al.*: **UniProt: the universal protein knowledgebase in 2023**. *Nucleic Acids Res.* 2023; **51**(D1): D523–D531. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bates A, Clayton-Lucey I, Howard C: **Sanger Tree of Life HMW DNA fragmentation: diagenode Megaruptor<sup>®</sup>3 for LI PacBio**. *protocols.io.* 2023. [Publisher Full Text](#)
- Beasley J, Uhl R, Forrest LL, *et al.*: **DNA barcoding SOPs for the Darwin Tree of Life project**. *protocols.io.* 2023; [Accessed 25 June 2024]. [Publisher Full Text](#)
- Buchfink B, Reuter K, Drost HG: **Sensitive protein alignments at Tree-of-Life scale using DIAMOND**. *Nat Methods.* 2021; **18**(4): 366–368. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Challis R, Kumar S, Sotero-Caio C, *et al.*: **Genomes on a Tree (GoAT): a versatile, scalable search engine for genomic and sequencing project metadata across the eukaryotic Tree of Life** [version 1; peer review: 2 approved]. *Wellcome Open Res.* 2023; **8**: 24. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Challis R, Richards E, Rajan J, *et al.*: **BlobToolKit – interactive quality assessment of genome assemblies**. *G3 (Bethesda).* 2020; **10**(4): 1361–1374. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Cheng H, Concepcion GT, Feng X, *et al.*: **Haplotype-resolved *de novo* assembly using phased assembly graphs with hifiasm**. *Nat Methods.* 2021; **18**(2): 170–175. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Crowley L, Allen H, Barnes I, *et al.*: **A sampling strategy for genome sequencing the British terrestrial arthropod fauna** [version 1; peer review: 2 approved]. *Wellcome Open Res.* 2023; **8**: 123. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- da Veiga Leprevost F, Grünig BA, Alves Aflitos S, *et al.*: **BioContainers: an open-source and community-driven framework for software standardization**. *Bioinformatics.* 2017; **33**(16): 2580–2582. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Danecek P, Bonfield JK, Liddle J, *et al.*: **Twelve years of SAMtools and BCFtools**. *GigaScience.* 2021; **10**(2): giab008. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Denton A, Oatley G, Cornwell C, *et al.*: **Sanger Tree of Life sample homogenisation: PowerMash**. *protocols.io.* 2023a. [Publisher Full Text](#)
- Denton A, Yatsenko H, Jay J, *et al.*: **Sanger Tree of Life wet laboratory protocol collection V.1**. *protocols.io.* 2023b. [Publisher Full Text](#)
- Diesh C, Stevens GJ, Xie P, *et al.*: **JBrowse 2: a modular genome browser with views of synteny and structural variation**. *Genome Biol.* 2023; **24**(1): 74. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- do Amaral RJV, Denton A, Yatsenko H, *et al.*: **Sanger Tree of Life RNA extraction: automated MagMax<sup>™</sup> mirVana**. *protocols.io.* 2023. [Publisher Full Text](#)
- Ewels P, Magnusson M, Lundin S, *et al.*: **MultiQC: summarize analysis results for multiple tools and samples in a single report**. *Bioinformatics.* 2016; **32**(19): 3047–3048. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Ewels PA, Peltzer A, Fillinger S, *et al.*: **The nf-core framework for community-curated bioinformatics pipelines.** *Nat Biotechnol.* 2020; **38**(3): 276–278.  
[PubMed Abstract](#) | [Publisher Full Text](#)

Formenti G, Abueg L, Brajuka A, *et al.*: **Gfastats: conversion, evaluation and manipulation of genome sequences using assembly graphs.** *Bioinformatics.* 2022; **38**(17): 4214–4216.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

GBIF Secretariat: ***Scotopteryx chenopodiata* (Linnaeus, 1758).** *GBIF Backbone Taxonomy.* 2023; [Accessed 17 February 2025].  
[Reference Source](#)

Grüning B, Dale R, Sjödin A, *et al.*: **Bioconda: sustainable and comprehensive software distribution for the life sciences.** *Nat Methods.* 2018; **15**(7): 475–476.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Guan D, McCarthy SA, Wood J, *et al.*: **Identifying and removing haplotypic duplication in primary genome assemblies.** *Bioinformatics.* 2020; **36**(9): 2896–2898.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Harry E: **PretextView (Paired REad TEXTure Viewer): a desktop application for viewing pretext contact maps.** 2022.  
[Reference Source](#)

Henwood B, Sterling P, Lewington R: **Field guide to the caterpillars of Great Britain and Ireland.** London: Bloomsbury, 2020.  
[Reference Source](#)

Howe K, Chow W, Collins J, *et al.*: **Significantly improving the quality of genome assemblies through curation.** *GigaScience.* 2021; **10**(1): gaa153.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Jay J, Yatsenko H, Narváez-Gómez JP, *et al.*: **Sanger Tree of Life sample preparation: triage and dissection.** *protocols.io.* 2023.  
[Publisher Full Text](#)

Kerpedjiev P, Abdennur N, Lekschas F, *et al.*: **HiGlass: web-based visual exploration and analysis of genome interaction maps.** *Genome Biol.* 2018; **19**(1): 125.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Kurtzer GM, Sochat V, Bauer MW: **Singularity: scientific containers for mobility of compute.** *PLoS One.* 2017; **12**(5): e0177459.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Lawniczak MKN, Davey RP, Rajan J, *et al.*: **Specimen and sample metadata standards for biodiversity genomics: a proposal from the Darwin Tree of Life project [version 1; peer review: 2 approved with reservations].** *Wellcome Open Res.* 2022; **7**: 187.  
[Publisher Full Text](#)

Li H: **Minimap2: pairwise alignment for nucleotide sequences.** *Bioinformatics.* 2018; **34**(18): 3094–3100.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Manni M, Berkeley MR, Seppely M, *et al.*: **BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes.** *Mol Biol Evol.* 2021; **38**(10): 4647–4654.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Merkel D: **Docker: lightweight Linux containers for consistent development and deployment.** *Linux J.* 2014; **2014**(239): 2, [Accessed 2 April 2024].  
[Reference Source](#)

Oatley G, Denton A, Howard C: **Sanger Tree of Life HMW DNA extraction: automated MagAttract v.2.** *protocols.io.* 2023a.  
[Publisher Full Text](#)

Oatley G, Sampaio F, Howard C: **Sanger Tree of Life fragmented DNA clean up: automated SPRI.** *protocols.io.* 2023b.  
[Publisher Full Text](#)

Pereira L, Sivell O, Sivess L, *et al.*: **DTOL: taxon-specific standard operating procedure for the terrestrial and freshwater arthropods working group.** 2022.  
[Publisher Full Text](#)

Pointon DL, Eagles W, Sims Y, *et al.*: **sanger-tol/treeval v1.0.0 - Ancient Atlantis.** 2023.  
[Publisher Full Text](#)

Ranallo-Benavidez TR, Jaron KS, Schatz MC: **GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes.** *Nat Commun.* 2020; **11**(1): 1432.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Randle Z, Evans-Hill LJ, Parsons MS, *et al.*: **Atlas of Britain and Ireland's larger moths.** Newbury: Pisces Publications, 2019.  
[Reference Source](#)

Rao SSP, Huntley MH, Durand NC, *et al.*: **A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping.** *Cell.* 2014; **159**(7): 1665–1680.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Rhie A, McCarthy SA, Fedrigo O, *et al.*: **Towards complete and error-free genome assemblies of all vertebrate species.** *Nature.* 2021; **592**(7856): 737–746.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Rhie A, Walenz BP, Koren S, *et al.*: **Merquy: reference-free quality, completeness, and phasing assessment for genome assemblies.** *Genome Biol.* 2020; **21**(1): 245.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Rhie A, Walenz BP, Koren S, *et al.*: **Merquy: reference-free quality, completeness, and phasing assessment for genome assemblies.** *Genome Biol.* 2020; **21**(1): 245.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Twyford AD, Beasley J, Barnes I, *et al.*: **A DNA barcoding framework for taxonomic verification in the Darwin Tree of Life project [version 1; peer review: 2 approved].** *Wellcome Open Res.* 2024; **9**: 339.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Uliano-Silva M, Ferreira JGRN, Krashenninnikova K, *et al.*: **MitoHiFi: a python pipeline for mitochondrial genome assembly from PacBio high fidelity reads.** *BMC Bioinformatics.* 2023; **24**(1): 288.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Vasimuddin M, Misra S, Li H, *et al.*: **Efficient architecture-aware acceleration of BWA-MEM for multicore systems.** In: *2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS).* IEEE, 2019; 314–324.  
[Publisher Full Text](#)

Waring P, Townsend M, Lewington R: **Field guide to the moths of Great Britain and Ireland: third Edition.** Bloomsbury Wildlife Guides, 2017.  
[Reference Source](#)

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](#)