




DATA NOTE

The genome sequence of the Sprawler moth, *Asteroscopus*

sphinx Hufnagel, 1766

[version 1; peer review: 1 approved]

James Hammond ¹,

University of Oxford and Wytham Woods Genome Acquisition Lab,
Darwin Tree of Life Barcoding collective,
Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory
team,

Wellcome Sanger Institute Scientific Operations: Sequencing Operations,
Wellcome Sanger Institute Tree of Life Core Informatics team,
Tree of Life Core Informatics collective, Darwin Tree of Life Consortium

¹University of Oxford, Oxford, England, UK

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Abstract

We present a genome assembly from an individual male *Asteroscopus sphinx* (the Sprawler moth; Arthropoda; Insecta; Lepidoptera; Noctuidae). The genome sequence has a total length of 857.30 megabases. Most of the assembly is scaffolded into 32 chromosomal pseudomolecules, including the Z sex chromosome and a putative B chromosome. The mitochondrial genome has also been assembled and is 15.35 kilobases in length.

Keywords

Asteroscopus sphinx, the Sprawler moth, genome sequence, chromosomal, Lepidoptera



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

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1


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1. **Pritha Dey** , National Centre for Biological Sciences-TIFR, Bangalore, India

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Darwin Tree of Life Consortium (mark.blaxter@sanger.ac.uk)

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Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphimesenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Noctuoidea; Noctuidae; Amphipyriinae; *Asteroscopus*; *Asteroscopus sphinx* Hufnagel, 1766 (NCBI:txid988069).

Background

The genome of the Sprawler, *Asteroscopus sphinx*, 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 *Asteroscopus sphinx*, based on one male specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome of an adult *Asteroscopus sphinx* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating a total of 25.79 Gb (gigabases) from 2.16 million reads, providing approximately 29-fold coverage. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data, which produced 125.57 Gbp from 831.61 million reads, yielding an approximate coverage of 146-fold. Specimen and sequencing information is summarised in Table 1.

Manual assembly curation corrected 8 missing joins or mis-joins and one haplotypic duplications. The final assembly has a total length of 857.30 Mb in 50 sequence scaffolds with a scaffold N50 of 29.1 Mb (Table 2). 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 (99.8%) of the assembly sequence was assigned



Figure 1. Photograph of the *Asteroscopus sphinx* (ilAstSphi1) specimen used for genome sequencing.

to 32 chromosomal-level scaffolds, representing 31 autosomes and the Z sex chromosome. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 5; Table 3). Chromosome 23 is a putative B chromosome (Fraïsse *et al.*, 2017). 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 64.9 with *k*-mer completeness of 100.0%, and the assembly has a BUSCO v5.3.2 completeness of 98.8% (single = 97.6%, duplicated = 1.1%), using the lepidoptera_odb10 reference set (*n* = 5,286).

Metadata for specimens, BOLD barcode results, spectra estimates, sequencing runs, contaminants and pre-curation assembly statistics are given at <https://links.tol.sanger.ac.uk/species/988069>.

Methods

Sample acquisition

An adult female *Asteroscopus sphinx* (specimen ID Ox001963, ToLID ilAstSphi1) was collected from Wytham Woods, Oxfordshire, UK (latitude 51.78, longitude -1.33) on 2021-11-03 by potting. The specimen was collected and identified by James Hammond (University of Oxford) and preserved on dry ice.

The initial identification was verified by an additional DNA barcoding process according to the framework developed by Twyford *et al.* (2024). A small sample was dissected from the specimens and stored in ethanol, while the remaining parts of the specimen were shipped on dry ice to the Wellcome Sanger Institute (WSI). The tissue was lysed, the COI marker region was amplified by PCR, and amplicons were sequenced and compared to the BOLD database, confirming the species identification (Crowley *et al.*, 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 core procedures: sample preparation and homogenisation, DNA extraction, fragmentation, and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). In sample preparation, the ilAstSphi1 sample was weighed and dissected on dry ice (Jay *et al.*, 2023). Tissue from the thorax 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

Table 1. Specimen and sequencing data for *Asteroscopus sphinx*.

Project information			
Study title	Asteroscopus sphinx (the sprawler)		
Umbrella BioProject	PRJEB57684		
Species	<i>Asteroscopus sphinx</i>		
BioSample	SAMEA110451502		
NCBI taxonomy ID	988069		
Specimen information			
Technology	ToLID	BioSample accession	Organism part
PacBio long read sequencing	ilAstSphi1	SAMEA110451677	thorax
Hi-C sequencing	ilAstSphi1	SAMEA110451677	thorax
RNA sequencing	ilAstSphi1	SAMEA110451678	abdomen
Sequencing information			
Platform	Run accession	Read count	Base count (Gb)
Hi-C Illumina NovaSeq 6000	ERR10501030	8.32e+08	125.57
PacBio Sequel IIE	ERR10499364	2.16e+06	25.79
RNA Illumina NovaSeq 6000	ERR11641115	7.40e+07	11.17

Table 2. Genome assembly data for *Asteroscopus sphinx*, ilAstSphi1.1.

Genome assembly		
Assembly name	ilAstSphi1.1	
Assembly accession	GCA_949699075.1	
Accession of alternate haplotype	GCA_949699085.1	
Span (Mb)	857.30	
Number of contigs	183	
Contig N50 length (Mb)	9.6	
Number of scaffolds	50	
Scaffold N50 length (Mb)	29.1	
Longest scaffold (Mb)	36.2	
Assembly metrics*		Benchmark
Consensus quality (QV)	64.9	≥ 50
k-mer completeness	100.0%	≥ 95%
BUSCO**	C:98.8%[S:97.6%,D:1.1%],F:0.2%,M:1.0%,n:5,286	C ≥ 95%
Percentage of assembly mapped to chromosomes	99.8%	≥ 95%
Sex chromosomes	Z	localised homologous pairs
Organelles	Mitochondrial genome: 15.35 kb	complete single alleles

* 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/ilAstSphi1_1/dataset/ilAstSphi1_1/busco.

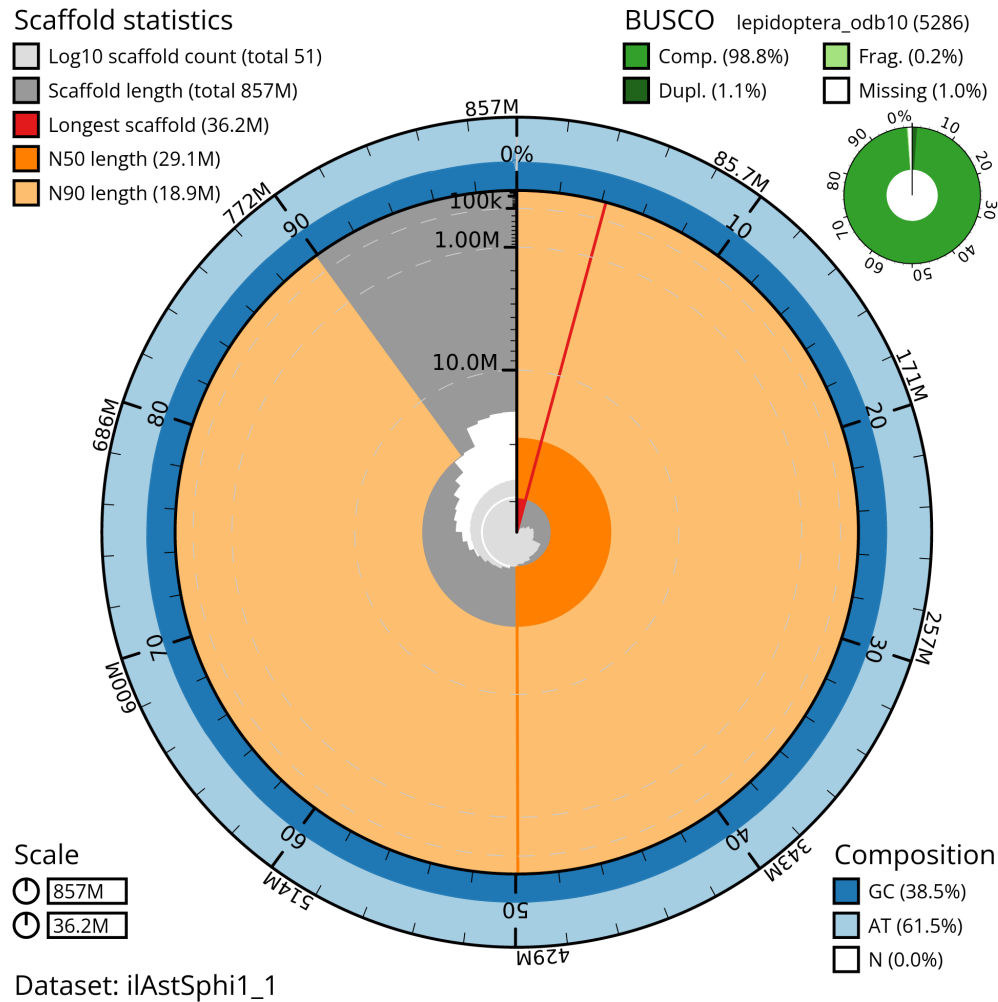


Figure 2. Genome assembly of *Asteroscopus sphinx*, ilAstSphi1.1: metrics. The BlobToolKit snail plot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 857,355,149 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (36,197,790 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (29,090,398 and 18,941,627 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/ilAstSphi1_1/dataset/ilAstSphi1_1/snail.

(Oatley *et al.*, 2023). The 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 (Strickland *et al.*, 2023). The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and Qubit Fluorometer using the Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

RNA was extracted from abdomen tissue of ilAstSphi1 in the Tree of Life Laboratory at the WSI using the RNA Extraction:

Automated MagMax™ *mir*Vana protocol (do Amaral *et al.*, 2023). The RNA concentration was assessed using a Nanodrop spectrophotometer and a Qubit Fluorometer using the Qubit RNA Broad-Range Assay kit. Analysis of the integrity of the RNA was done using the Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

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

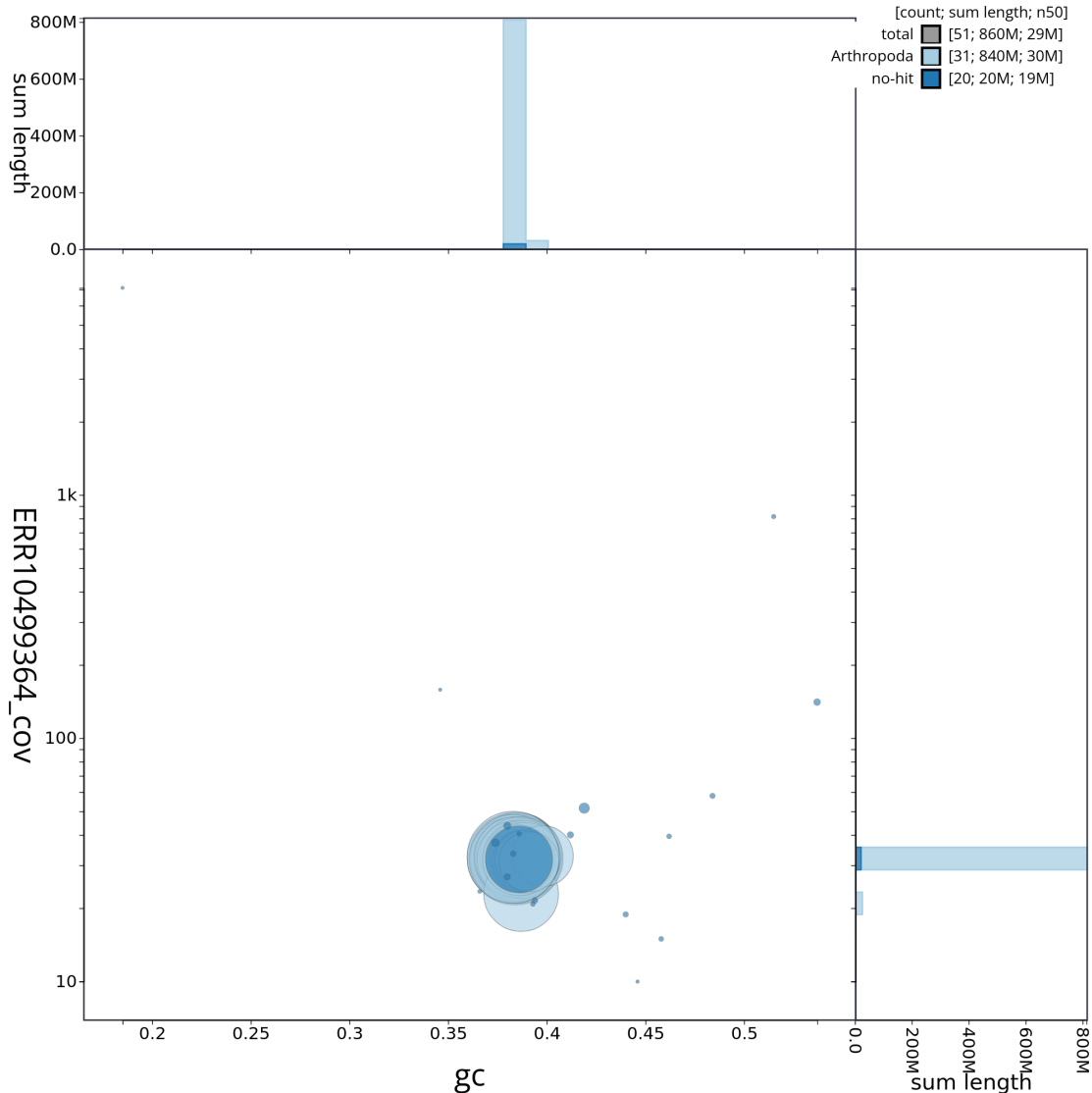


Figure 3. Genome assembly of *Asteroscopus sphinx*, ilAstSphi1.1: BlobToolKit GC-coverage plot. Sequences are coloured by phylum. Circles are sized in proportion to sequence length. Histograms show the distribution of sequence length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilAstSphi1_1/dataset/ilAstSphi1_1/blob.

WSI on Pacific Biosciences Sequel IIe (HiFi) and Illumina NovaSeq 6000 (RNA-Seq) instruments. Hi-C data were also generated from thorax tissue of ilAstSphi1 using the Arima-HiC v2 kit. The Hi-C sequencing was performed using paired-end sequencing with a read length of 150 bp on the Illumina NovaSeq 6000 instrument.

Genome assembly, curation and evaluation

Assembly

The HiFi reads were first assembled using Hifiasm (Cheng *et al.*, 2021) with the --primary option. Haplotypic duplications were identified and removed using purge_dups (Guan *et al.*, 2020). The Hi-C reads were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). The contigs were further scaffolded using the provided Hi-C data (Rao *et al.*, 2014)

in YaHS (Zhou *et al.*, 2023) using the --break option. The scaffolded assemblies were evaluated using Gfastats (Formenti *et al.*, 2022), BUSCO (Manni *et al.*, 2021) and MERQURY.FK (Rhie *et al.*, 2020).

The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2023), which runs MitoFinder (Allio *et al.*, 2020) and uses these annotations to select the final mitochondrial contig and to ensure the general quality of the sequence.

Assembly curation

The assembly was decontaminated using the Assembly Screen for Cobionts and Contaminants (ASCC) pipeline (article in preparation). Manual curation was primarily conducted

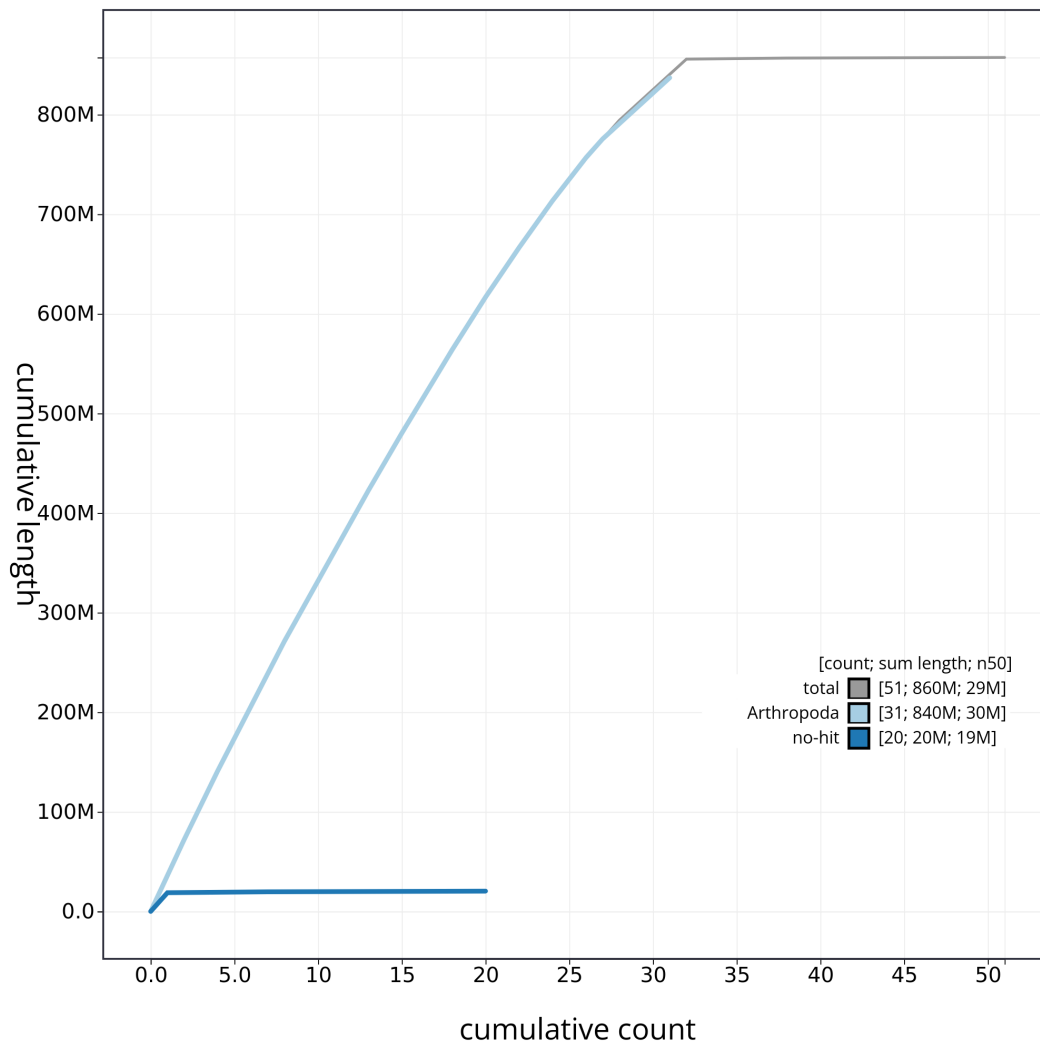


Figure 4. Genome assembly of *Asteroscopus sphinx* ilAstSphi1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all sequences. Coloured lines show cumulative lengths of sequences assigned to each phylum using the buscodegenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilAstSphi1_1/dataset/ilAstSphi1_1/cumulative.

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

Evaluation of the final assembly

A Hi-C map for the final assembly was produced using bwa-mem2 (Vasimuddin *et al.*, 2019) in the Cooler file format (Abdennur & Mirny, 2020). To assess the assembly metrics, the *k*-mer completeness and QV consensus quality values were calculated in Merqury (Rhie *et al.*, 2020). This work was done

using the “sanger-tol/readmapping” (Surana *et al.*, 2023a) and “sanger-tol/genomenote” (Surana *et al.*, 2023b) pipelines. The genome readmapping pipelines were developed using the nf-core tooling (Ewels *et al.*, 2020), use MultiQC (Ewels *et al.*, 2016), and make extensive use of the Conda package manager, the Bioconda initiative (Grüning *et al.*, 2018), the Biocontainers infrastructure (da Veiga Leprevost *et al.*, 2017), and the Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017) containerisation solutions. The genome was also analysed within the BlobToolKit environment (Challis *et al.*, 2020) and BUSCO scores (Manni *et al.*, 2021; Simão *et al.*, 2015) were calculated.

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

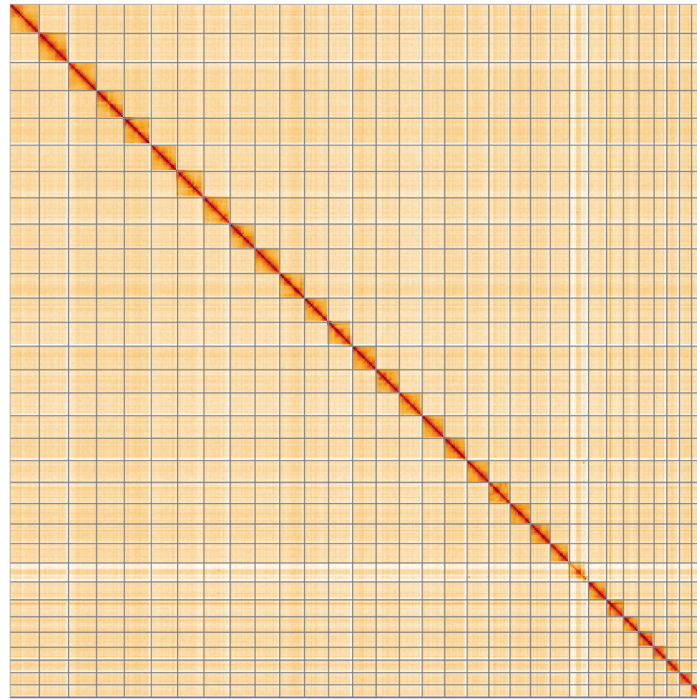


Figure 5. Genome assembly of *Asteroscopus sphinx* ilAstSphi1.1: Hi-C contact map of the ilAstSphi1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=FAkje8YkT_GPw9jqsE-o2Q.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Asteroscopus sphinx*, ilAstSphi1.

INSDC accession	Name	Length (Mb)	GC%
OX453001.1	1	36.2	38.5
OX453003.1	2	34.74	38.5
OX453004.1	3	34.22	38.5
OX453005.1	4	33.19	38.5
OX453006.1	5	32.54	38.0
OX453007.1	6	32.49	38.5
OX453008.1	7	32.4	38.5
OX453009.1	8	30.61	38.5
OX453010.1	9	30.46	38.5
OX453011.1	10	30.38	38.5
OX453012.1	11	29.82	38.5
OX453013.1	12	29.57	38.0
OX453014.1	13	29.09	38.5
OX453015.1	14	28.61	38.5
OX453016.1	15	28.11	38.5

INSDC accession	Name	Length (Mb)	GC%
OX453017.1	16	27.96	38.5
OX453018.1	17	27.41	38.5
OX453019.1	18	26.93	39.0
OX453020.1	19	26.12	38.5
OX453021.1	20	25.37	38.5
OX453022.1	21	24.26	38.5
OX453023.1	22	23.75	39.0
OX453024.1	23	23.39	38.5
OX453025.1	24	22.19	38.5
OX453026.1	25	21.04	39.0
OX453027.1	26	18.94	38.5
OX453028.1	27	18.7	38.5
OX453029.1	28	15.79	39.0
OX453030.1	29	15.41	40.0
OX453031.1	30	15.09	39.0
OX453032.1	31	15.04	39.0
OX453002.1	Z	35.86	38.5
OX453033.1	MT	0.02	19.0

Table 4. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.2.1	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	https://gitlab.com/ezlab/busco
bwa-mem2	2.2.1	https://github.com/bwa-mem2/bwa-mem2
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
Hifiasm	0.16.1-r375	https://github.com/chhylp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Merqury	MerquryFK	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	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/ascc	-	https://github.com/sanger-tol/ascc
sanger-tol/genomenote	v1.0	https://github.com/sanger-tol/genomenote
sanger-tol/readmapping	1.1.0	https://github.com/sanger-tol/readmapping/tree/1.1.0
YaHS	1.2a	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: *Asteroscopus sphinx* (the sprawler). Accession number PRJEB57684; <https://identifiers.org/ena.embl/PRJEB57684> (Wellcome Sanger Institute, 2023). The genome sequence is released openly for reuse. The *Asteroscopus sphinx* 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](#) and [Table 2](#).

Author information

Members of the University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: <https://doi.org/10.5281/zenodo.12157525>.

Members of the Darwin Tree of Life Barcoding collective are listed here: <https://doi.org/10.5281/zenodo.12158331>

Members of the Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team are listed here: <https://doi.org/10.5281/zenodo.12162482>.

Members of Wellcome Sanger Institute Scientific Operations: Sequencing Operations are listed here: <https://doi.org/10.5281/zenodo.12165051>.

Members of the Wellcome Sanger Institute Tree of Life Core Informatics team are listed here: <https://doi.org/10.5281/zenodo.12160324>.

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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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Pritha Dey 

National Centre for Biological Sciences-TIFR, Bangalore, India

I found the article to be technically very sound, and the detailed methods will allow future research to replicate them. The article is also very important and timely, in the era where new genome resources are expanding possibilities for developing insect model systems.

However, I felt in the background section, the species taxonomy, life history and ecology is missing. This is crucial information which along with the genome resources would be critical for further research.

In the sample acquisition sections under 'Methods', it is mentioned that an adult female was acquired. Whereas, in the background it is written that the genome was assembled from a male. Could the authors kindly clarify?

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: A moth-biologist, interested in diversity, distribution and evolutionary ecology of moths.

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.
