



DATA NOTE

The genome sequence of the Treble Lines, *Charanyca*

trigrammica (Hufnagel, 1766) (Lepidoptera: Noctuidae)

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

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Abstract

We present a genome assembly from an individual male *Charanyca trigrammica* (Treble Lines; Arthropoda; Insecta; Lepidoptera; Noctuidae). The assembly contains two haplotypes with total lengths of 546.43 megabases and 546.58 megabases. Most of haplotype 1 (99.97%) is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. Haplotype 2 was assembled to scaffold level. The mitochondrial genome has also been assembled, with a length of 15.44 kilobases. This assembly was generated as part of the Darwin Tree of Life project, which produces reference genomes for eukaryotic species found in Britain and Ireland.

Keywords

Charanyca trigrammica; Treble Lines; genome sequence; chromosomal; Lepidoptera



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

Open Peer Review

Approval Status ✓✓?

	1	2	3
version 1	✓	✓	?
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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; Noctuoidea; Noctuidae; Xyleninae; *Charanyca*; *Charanyca trigrammica* (Hufnagel, 1766) (NCBI:txid987901)

Background

Charanyca trigrammica (Treble Lines) is a moth in the family Noctuidae. It is a common species in the southern half of Britain where it occurs in a variety of open habitats (Waring *et al.*, 2017). It has significantly increased its range and abundance in the UK since the 1970s (Randle *et al.*, 2019) and it occurs throughout Europe (GBIF Secretariat, 2025).

As its common name suggests, this medium sized moth (forewing length 15–17 mm) is distinguished by three straight dark-brown crosslines across the forewing. The ground colour is pale brown although there is some variation. There is one generation a year and it flies in May and June (Waring *et al.*, 2017). The larvae feed at night on a variety of herbaceous plants including Knapweeds and Greater Plantain. The larvae eat the plants at ground level and it pupates underground (Heath & Emmet, 1983).

We present a chromosome-level genome sequence for *Charanyca trigrammica*, the Treble Lines. The assembly was produced using the Tree of Life pipeline from a specimen collected in Wytham Woods, Oxfordshire, United Kingdom (Figure 1). This assembly was generated as part of the Darwin Tree of Life Project, which aims to generate high-quality reference genomes for all named eukaryotic species in Britain and Ireland to support research, conservation, and the sustainable use of biodiversity (Blaxter *et al.*, 2022).

Methods

Sample acquisition and DNA barcoding

The specimen used for genome sequencing was an adult male *Charanyca trigrammica* (specimen ID Ox001875, ToLID ilChaTrig1; Figure 1), collected from Wytham Woods,



Figure 1. Photograph of the *Charanyca trigrammica* (ilChaTrig1) specimen used for genome sequencing.

Oxfordshire, United Kingdom (latitude 51.774, longitude –1.324) on 2021-05-28. The specimen was collected and identified by Douglas Boyes. For the Darwin Tree of Life sampling and metadata approach, refer to Lawniczak *et al.* (2022).

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 specimen and stored in ethanol, while the remaining parts were shipped on dry ice to the Wellcome Sanger Institute (WSI) (see the protocol). 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 are available on protocols.io.

Nucleic acid extraction

Protocols for high molecular weight (HMW) DNA extraction developed at the Wellcome Sanger Institute (WSI) Tree of Life Core Laboratory are available on protocols.io (Howard *et al.*, 2025). The ilChaTrig1 sample was weighed and triaged to determine the appropriate extraction protocol. Tissue from the thorax was homogenised by powermashing using a Power-Masher II tissue disruptor. HMW DNA was extracted in the WSI Scientific Operations core using the Automated MagAttract v2 protocol. DNA was sheared into an average fragment size of 12–20 kb following the Megaruptor®3 for LI PacBio protocol. Sheared DNA was purified by manual SPRI (solid-phase reversible immobilisation). 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. For this sample, the final post-shearing DNA had a Qubit concentration of 28.6 ng/μL and a yield of 1344.20 ng, with a fragment size of 14.1 kb. The 260/280 spectrophotometric ratio was 1.91, and the 260/230 ratio was 3.38.

PacBio HiFi library preparation and sequencing

Library preparation and sequencing were performed at the WSI Scientific Operations core. Libraries were prepared using the SMRTbell Prep Kit 3.0 (Pacific Biosciences, California, USA), following the manufacturer's instructions. The kit includes reagents for end repair/A-tailing, adapter ligation, post-ligation SMRTbell bead clean-up, and nuclease treatment. Size selection and clean-up were performed using diluted AMPure PB beads (Pacific Biosciences). DNA concentration was quantified using a Qubit Fluorometer v4.0 (ThermoFisher Scientific) and the Qubit 1X dsDNA HS assay kit. Final library fragment size was assessed with the Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies) using the gDNA 55 kb BAC analysis kit.

The sample was sequenced on a Revio instrument (Pacific Biosciences). The prepared library was normalised to 2 nM,

and 15 µL was used for making complexes. Primers were annealed and polymerases bound to generate circularised complexes, following the manufacturer's instructions. Complexes were purified using 1.2X SMRTbell beads, then diluted to the Revio loading concentration (200–300 pM) and spiked with a Revio sequencing internal control. The sample was sequenced on a Revio 25M SMRT cell. The SMRT Link software (Pacific Biosciences), a web-based workflow manager, was used to configure and monitor the run and to carry out primary and secondary data analysis.

Hi-C

Sample preparation and crosslinking

The Hi-C sample was prepared from 20–50 mg of frozen tissue from the head of the *ilChaTrig1* sample using the Arima-HiC v2 kit (Arima Genomics). Following the manufacturer's instructions, tissue was fixed and DNA crosslinked using TC buffer to a final formaldehyde concentration of 2%. The tissue was homogenised using the Diagnocine Power Masher-II. Crosslinked DNA was digested with a restriction enzyme master mix, biotinylated, and ligated. Clean-up was performed with SPRISelect beads before library preparation. DNA concentration was measured with the Qubit Fluorometer (Thermo Fisher Scientific) and Qubit HS Assay Kit. The biotinylation percentage was estimated using the Arima-HiC v2 QC beads.

Hi-C library preparation and sequencing

Biotinylated DNA constructs were fragmented using a Covaris E220 sonicator and size selected to 400–600 bp using SPRISelect beads. DNA was enriched with Arima-HiC v2 kit Enrichment beads. End repair, A-tailing, and adapter ligation were carried out with the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs), following a modified protocol where library preparation occurs while DNA remains bound to the Enrichment beads. Library amplification was performed using KAPA HiFi HotStart mix and a custom Unique Dual Index (UDI) barcode set (Integrated DNA Technologies). Depending on sample concentration and biotinylation percentage determined at the crosslinking stage, libraries were amplified with 10 to 16 PCR cycles. Post-PCR clean-up was performed with SPRISelect beads. Libraries were quantified using the AccuClear Ultra High Sensitivity dsDNA Standards Assay Kit (Biotium) and a FLUOstar Omega plate reader (BMG Labtech).

Prior to sequencing, libraries were normalised to 10 ng/µL. Normalised libraries were quantified again and equimolar and/or weighted 2.8 nM pools. Pool concentrations were checked using the Agilent 4200 TapeStation (Agilent) with High Sensitivity D500 reagents before sequencing. Sequencing was performed using paired-end 150 bp reads on the Illumina NovaSeq 6000.

Genome 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 assembled using Hifiasm in Hi-C phasing mode (Cheng *et al.*, 2021; Cheng *et al.*, 2022), producing two haplotypes. Hi-C reads (Rao *et al.*, 2014) were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). Contigs were further scaffolded with Hi-C data 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 MERQUERY.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. TreeVal was used to generate the flat files and maps for use in curation. Manual curation was conducted primarily in PretextView and HiGlass (Kerpedjiev *et al.*, 2018). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Manual corrections included 5 breaks, 20 joins, and removal of 13 haplotypic duplications. The curation process is documented at <https://gitlab.com/wtsi-grit/rapid-curation>. PretextView-Snapshot was used to generate a Hi-C contact map of the final assembly.

Assembly quality assessment

The Merquery.FK tool (Rhie *et al.*, 2020) was run in a Singularity container (Kurtzer *et al.*, 2017) to evaluate *k*-mer completeness and assembly quality for both haplotypes using the *k*-mer databases (*k* = 31) computed prior to genome assembly. The analysis outputs included assembly QV scores and completeness statistics.

The genome was analysed using the BlobToolKit pipeline, a Nextflow implementation of the earlier Snakemake version (Challis *et al.*, 2020). The pipeline aligns PacBio reads using minimap2 (Li, 2018) and SAMtools (Danecek *et al.*, 2021) to generate coverage tracks. It runs BUSCO (Manni *et al.*, 2021) using lineages identified from the NCBI Taxonomy (Schoch *et al.*, 2020). For the three domain-level lineages, BUSCO genes are aligned to the UniProt Reference Proteomes database (Bateman *et al.*, 2023) using DIAMOND blastp (Buchfink *et al.*, 2021). The genome is divided into chunks based on the density of BUSCO genes from the closest taxonomic lineage, and each chunk is aligned to the UniProt Reference Proteomes database with DIAMOND blastx. Sequences without hits are chunked using seqtk and aligned to the NT database with blastn (Altschul *et al.*, 1990). The BlobToolKit suite consolidates all 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), with

containerisation through Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017).

Genome sequence report

Sequence data

PacBio sequencing of the *Charanyca trigrammica* specimen generated 56.74 Gb (gigabases) from 5.41 million reads, which were used to assemble the genome. GenomeScope2.0 analysis

estimated the haploid genome size at 597.33 Mb, with a heterozygosity of 0.64% and repeat content of 28.73% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 78× coverage. Hi-C sequencing produced 138.94 Gb from 920.12 million reads, which were used to scaffold the assembly. Table 1 summarises the specimen and sequencing details.

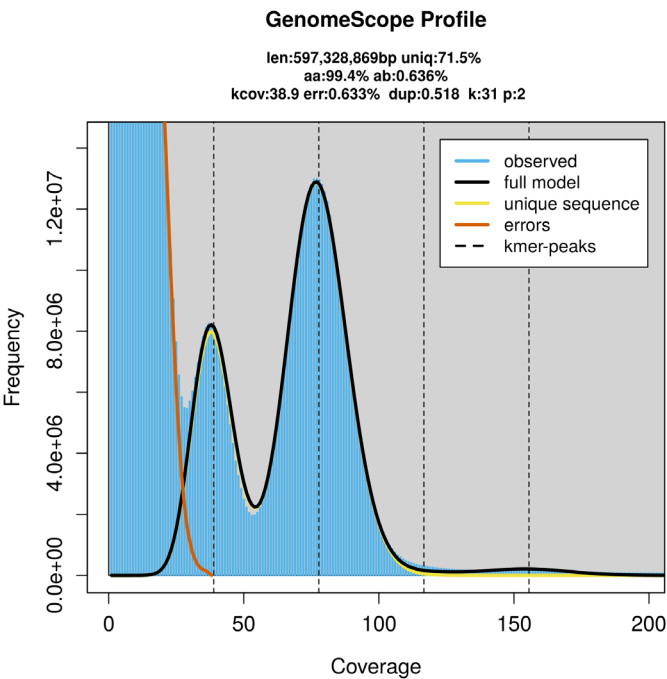


Figure 2. Frequency distribution of *k*-mers generated using GenomeScope2. The plot shows observed and modelled *k*-mer spectra, providing estimates of genome size, heterozygosity, and repeat content based on unassembled sequencing reads.

Table 1. Specimen and sequencing data for BioProject PRJEB85024.

Platform	PacBio HiFi	Hi-C
ToLID	ilChaTrig1	ilChaTrig1
Specimen ID	Ox001875	Ox001875
BioSample (source individual)	SAMEA10979135	SAMEA10979135
BioSample (tissue)	SAMEA10979548	SAMEA10979547
Tissue	thorax	head
Instrument	Revio	Illumina NovaSeq 6000
Run accessions	ERR14209139; ERR14209138	ERR14224619
Read count total	5.41 million	920.12 million
Base count total	56.74 Gb	138.94 Gb

Assembly statistics

The genome was assembled into two haplotypes using Hi-C phasing. Haplotype 1 was curated to chromosome level, while haplotype 2 was assembled to scaffold level. The final assembly has a total length of 546.43 Mb in 38 scaffolds, with 133 gaps, and a scaffold N50 of 18.96 Mb (Table 2).

Most of the assembly sequence (99.97%) 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 3; Table 3). The Z chromosome was identified based on BUSCO gene painting with ancestral Merian elements (Wright *et al.*, 2024).

Table 2. Genome assembly statistics.

Assembly name	ilChaTrig1.hap1.1	ilChaTrig1.hap2.1
Assembly accession	GCA_965151675.1	GCA_965151685.1
Assembly level	chromosome	scaffold
Span (Mb)	546.43	546.58
Number of chromosomes	31	N/A
Number of contigs	171	176
Contig N50	6.0 Mb	6.25 Mb
Number of scaffolds	38	58
Scaffold N50	18.96 Mb	18.85 Mb
Longest scaffold length (Mb)	28.41	N/A
Sex chromosomes	Z	N/A
Organelles	Mitochondrion: 15.44 kb	N/A

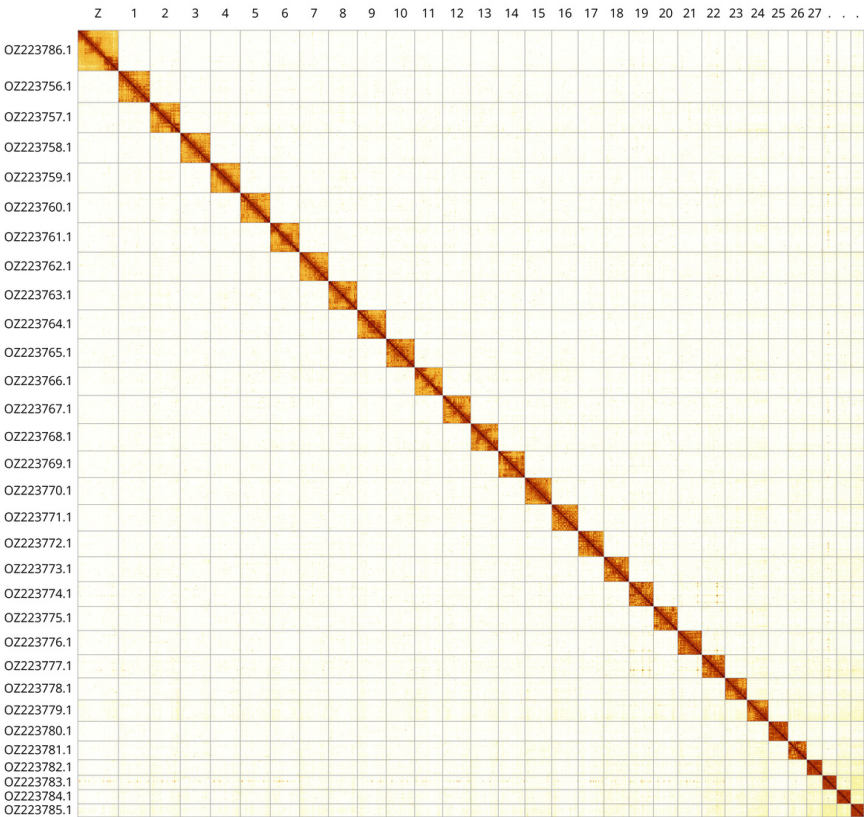


Figure 3. Hi-C contact map of the *Charanyca trigrammica* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Charanyca trigrammica* ilChaTrig1.

INSDC accession	Molecule	Length (Mb)	GC%
OZ223756.1	1	21.93	37
OZ223757.1	2	20.98	37.50
OZ223758.1	3	20.90	37
OZ223759.1	4	20.82	37.50
OZ223760.1	5	20.73	37
OZ223761.1	6	20.42	37
OZ223762.1	7	20.09	37
OZ223763.1	8	20.06	37
OZ223764.1	9	20.03	37
OZ223765.1	10	19.75	37
OZ223766.1	11	19.56	37
OZ223767.1	12	19.50	37
OZ223768.1	13	18.96	37
OZ223769.1	14	18.61	37
OZ223770.1	15	18.57	37.50
OZ223771.1	16	18.40	37
OZ223772.1	17	17.89	37
OZ223773.1	18	17.44	37.50
OZ223774.1	19	16.99	37.50
OZ223775.1	20	16.86	37.50
OZ223776.1	21	16.80	37
OZ223777.1	22	16.10	37.50
OZ223778.1	23	15.18	37.50
OZ223779.1	24	14.94	38.50
OZ223780.1	25	13.71	37.50
OZ223781.1	26	12.90	37.50
OZ223782.1	27	10.87	38
OZ223783.1	28	9.89	39.50
OZ223784.1	29	9.85	38.50
OZ223785.1	30	9.15	38
OZ223786.1	Z	28.41	37

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.

For haplotype 1, the estimated QV is 67.0, and for haplotype 2, 67.6. When the two haplotypes are combined, the assembly achieves an estimated QV of 67.3. The *k*-mer completeness is 35.64% for haplotype 1, 35.63% for haplotype 2, and 41.22% for the combined haplotypes (Figure 4). The low completeness values are due to removal of contaminant sequences prior to assembly.

BUSCO analysis using the lepidoptera_odb10 reference set ($n = 5\,286$) identified 99.0% of the expected gene set (single = 98.7%, duplicated = 0.3%) for haplotype 1. The snail plot in Figure 5 summarises the scaffold length distribution and other assembly statistics for haplotype 1. The blob plot in Figure 6 shows the distribution of scaffolds by GC proportion and coverage for haplotype 1.

Table 4 lists the assembly metric benchmarks adapted from Rhie *et al.* (2021) the Earth BioGenome Project Report on Assembly Standards September 2024. The EBP metric, calculated for the haplotype 1, is **6.C.Q67**, meeting the recommended reference standard.

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](#). 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.

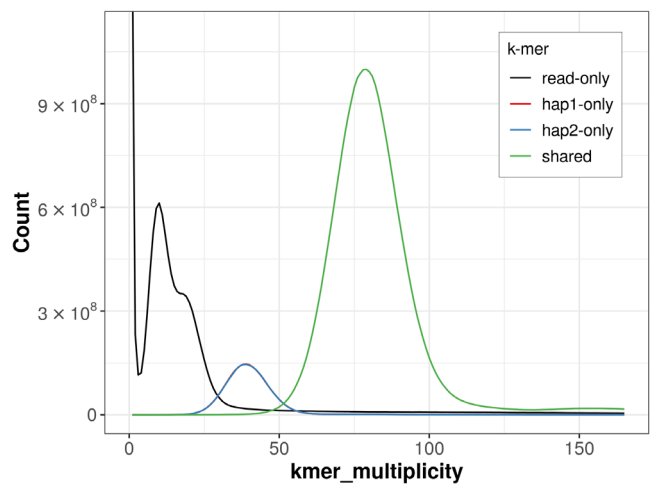


Figure 4. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers that appear in the reads but are not assembled. The green curve corresponds to *k*-mers shared by both haplotypes, and the red and blue curves show *k*-mers found only in one of the haplotypes.

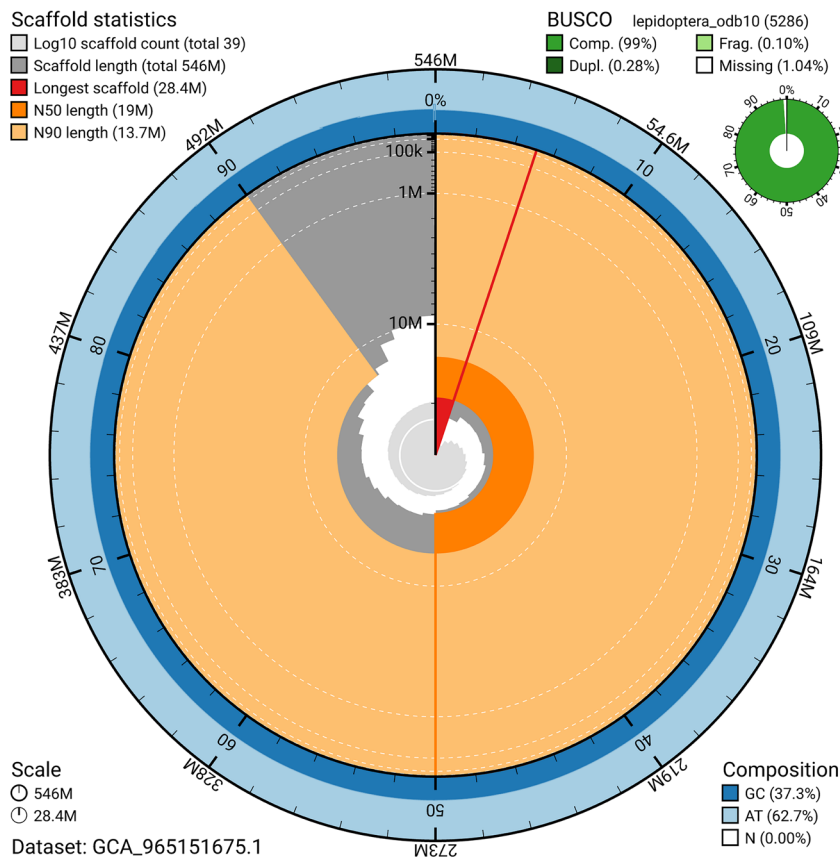


Figure 5. Assembly metrics for *ilChaTrig1.hap1.1*. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1 000 bins around the circumference. The outermost 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 set is presented at the top right. An interactive version of this figure can be accessed on the [BlobToolKit viewer](#).

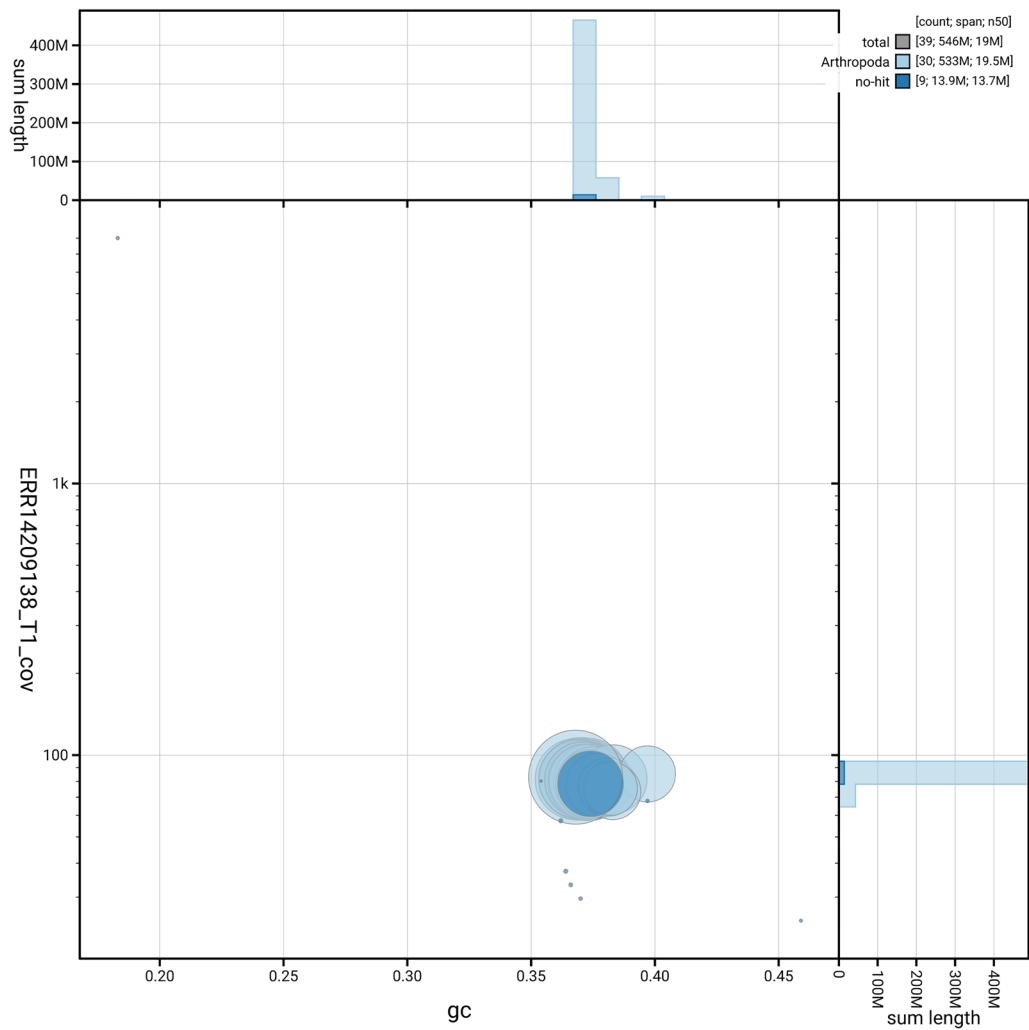


Figure 6. BlobToolKit GC-coverage plot for ilChaTrig1.hap1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes display the total length of sequences distributed across different levels of coverage and GC content. An interactive version of this figure is available on the [BlobToolKit viewer](#).

Table 4. Earth Biogenome Project summary metrics for the *Charanyca trigrammica* assembly.

Measure	Value	Benchmark
EBP summary (haplotype 1)	6.C.Q67	6.C.Q40
Contig N50 length	6 Mb	≥ 1 Mb
Scaffold N50 length	18.96 Mb	= chromosome N50
Consensus quality (QV)	Haplotype 1: 67.0; haplotype 2: 67.6; combined: 67.3	≥ 40
<i>k</i> -mer completeness	Haplotype 1: 35.64%; Haplotype 2: 35.63%; combined: 41.22%	≥ 95%
BUSCO	C:99.0% [S:98.7%; D:0.3%]; F:0.1%; M:0.9%; n:5 286	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.97%	≥ 90%

Data availability

European Nucleotide Archive: *Charanyca trigrammica* (treble lines). Accession number [PRJEB85024](#). The genome sequence is released openly for reuse. The *Charanyca trigrammica* genome sequencing initiative is part of the Darwin Tree of Life Project (PRJEB40665), the Sanger Institute Tree of Life Programme (PRJEB43745) 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](#).

Production code used in genome assembly at the WSI Tree of Life is available at <https://github.com/sanger-tol>. [Table 5](#) lists software versions used in this study.

Author information

Contributors are listed at the following links:

- Members of the [University of Oxford and Wytham Woods Genome Acquisition Lab](#)
- Members of the [Darwin Tree of Life Barcoding collective](#)
- Members of the [Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team](#)
- Members of [Wellcome Sanger Institute Scientific Operations – Sequencing Operations](#)
- Members of the [Wellcome Sanger Institute Tree of Life Core Informatics team](#)
- Members of the [Tree of Life Core Informatics collective](#)
- Members of the [Darwin Tree of Life Consortium](#)

Table 5. Software versions and sources.

Software	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	4.3.9	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.5.0	https://gitlab.com/ezlab/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	1.1	https://github.com/thegenemyers/FASTK
GenomeScope2.0	2.0.1	https://github.com/tbenavi1/genomescope2.0
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-r603	https://github.com/chhy123/hifiasm
HiGlass	1.13.4	https://github.com/higlass/higlass
MercuryFK	1.1.2	https://github.com/thegenemyers/MERQURY.FK
Minimap2	2.24-r1122	https://github.com/lh3/minimap2
MitoHiFi	3	https://github.com/marcelauliano/MitoHiFi
MultiQC	1.14; 1.17 and 1.18	https://github.com/MultiQC/MultiQC
Nextflow	23.10.0	https://github.com/nextflow-io/nextflow
PretextSnapshot	N/A	https://github.com/sanger-tol/PretextSnapshot
PretextView	0.2.5	https://github.com/sanger-tol/PretextView
samtools	1.19.2	https://github.com/samtools/samtools

Software	Version	Source
sanger-tol/ascc	0.1.0	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	0.6.0	https://github.com/sanger-tol/blobtoolkit
sanger-tol/curationpretext	1.4.2	https://github.com/sanger-tol/curationpretext
Seqtk	1.3	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.4.0	https://github.com/sanger-tol/treeval
YaHS	1.2.2	https://github.com/c-zhou/yaHS

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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](#)

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Arun Arumugaperumal 

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The genome note describes the whole genome sequencing of *Charanyca trigrammica*, also known as the Treble Lines. The article has been written well, and it is very interesting to read. The assembly reported here is of size 546.43 Mb, and the mitogenome size is 15.44 kb. The assembly has been scaffolded into 31 chromosomal molecules. Since the authors have sequenced a male specimen, they could capture only the Z sex chromosome. If they had sequenced a female specimen, they could have captured the W chromosome also. Already published, *Charanyca* member has the ZW sex system [1]. BUSCO completeness of 99% indicates that the genome sequence reported is a near-complete one. The annotation from Ensembl has not been released yet.

In Table 1, the run accession ERR14209139 was given. But the text does not describe any sequencing using Sequel Ii instrument or the inclusion of such data in the assembly process. I can only assume that data from both runs are pooled together, from the number of bases mentioned in Table 1. Since this article is very nicely written and very appealing to the reader, anybody will naturally have the same doubt. Can the author include a line or two to clear this doubt?

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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, however I have significant reservations, as outlined above.

Reviewer Report 06 October 2025

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Jhon Alex Dziechciarz Vidal 

Universidade Federal de São Carlos (UFSCar), São Carlos, Brazil

The manuscript presents the genome assembly of *Charanyca trigrammica*, describing the process of sample collection, species identification, tissue selection for DNA extraction, sequencing, and assembly. The background information about the species is valuable, as it helps readers better understand the evolutionary and ecological context of this moth. The methods are clearly and consistently described, and the level of detail is appropriate.

I particularly appreciate the detailed description of how the species was identified, as morphological identification can be challenging for some taxa. The genome assembly methods rely on widely used tools, and since the pipeline follows the standards established by the consortium, it represents a consistent and reliable approach for genome assembly.

The results are well presented, providing a thorough assessment of assembly quality, including Hi-C contact maps, figures, and tables that effectively illustrate the assembly metrics. The decision to assemble only haplotype 1 to the chromosome level was appropriate, considering that the specimen used was a male and therefore lacks heterogametic sex chromosomes to resolve. In conclusion, I have no concerns regarding the data report. The methodology and assembly quality meet current standards for genome assembly projects, and the manuscript represents a solid contribution to the field.

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: My background is in cytogenetics, genetics, genomics, and bioinformatics, using different animal models, both vertebrates and invertebrates. Yes, I feel capable of assessing several aspects of this paper.

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 03 October 2025

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Stephan Koblmüller 

University of Graz, Graz, Austria

In this manuscript, Boyes et al. present the whole genome assembly of the Treble Lines, *Charanyca trigrammica*, a noctuid moth that is widely distributed across Europe. Some background info about the target species is provided, which I appreciate. Also, the rationale for sequencing this particular species (part of a large whole genome sequencing initiative) is given, something that's often forgotten in this kind of publications.

The data are clear; the methods are well described. I have no objections to any of the data presented or the results. As far as I can tell, the datasets are deposited in publicly accessible repositories.

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: phylogenetics/-genomics, population genetics

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.
