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DATA NOTE

The genome sequence of the Bronze Shieldbug, *Troilus luridus* (Fabricius, 1775)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a male specimen of *Troilus luridus* (Bronze Shieldbug; Arthropoda; Insecta; Hemiptera; Pentatomidae). The assembly contains two haplotypes with total lengths of 1,044.50 megabases and 842.58 megabases. Most of haplotype 1 (93.48%) is scaffolded into 8 chromosomal pseudomolecules, including the X and Y sex chromosomes. Most of haplotype 2 (93.27%) is scaffolded into 6 chromosomal pseudomolecules. The mitochondrial genome has also been assembled, with a length of 17.9 kilobases.

Keywords

Troilus luridus, Bronze Shieldbug, genome sequence, chromosomal, Hemiptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 20 May 2025	✓ view	✓ view

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

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Species taxonomy
Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Paraneoptera; Hemiptera; Prosorrhyncha; Heteroptera; Euheteroptera; Neoheteroptera; Panheteroptera; Pentatomomorpha; Pentatomoidea; Pentatomidae; Asopinae; *Troilus*; *Troilus luridus* (Fabricius, 1775) (NCBI:txid1545368)

Background
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 – we present a chromosomally complete genome sequence for the Bronze Shieldbug, *Troilus luridus*. This genome was assembled using the Tree of Life pipeline from a specimen collected in Harcourt Arboretum, Oxfordshire, United Kingdom (Figure 1).

Genome sequence report
Sequencing data
The genome of a specimen of *Troilus luridus* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 108.06 Gb (gigabases) from 8.03 million reads, which were used to assemble the genome. GenomeScope analysis estimated the haploid genome size at 887.94 Mb, with a heterozygosity of 1.51% and repeat content of 28.62%. These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 116 coverage. Hi-C sequencing produced 119.18 Gb from 789.25 million reads, used to scaffold the assembly. Table 1 summarises the specimen and sequencing details.

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 assembly was improved by manual curation, which corrected 155 misjoins or missing joins and removed 14 haplotypic duplications. These interventions increased the total assembly length by 5.63%, decreased the scaffold count by 8.26%, and increased the scaffold N50 by 9.05%. The final assembly has a total length of 1,044.50 Mb in 521 scaffolds, with 286 gaps, and a scaffold N50 of 126.18 Mb (Table 2).

Figure 1. Photograph of the *Troilus luridus* (ihTroLuri1) specimen used for genome sequencing.

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 (93.48%) was assigned to 8 chromosomal-level scaffolds, representing 6 autosomes and the X and Y sex chromosomes. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 5; Table 3). During curation, we noted that order and orientation of contigs along the Y chromosome are uncertain from ~ 28.47 Mb to the end.

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.

For haplotype 1, the estimated QV is 60.5, and for haplotype 2, 60.9. When the two haplotypes are combined, the assembly achieves an estimated QV of 60.7. The *k*-mer completeness is 77.78% for haplotype 1 and 66.82% for haplotype 2; and 98.88% for the combined haplotypes. BUSCO v.5.5.0 analysis using the hemiptera_odb10 reference set (n = 2,510) identified 98.8% of the expected gene set (single = 96.4%, duplicated = 2.4%) for haplotype 1.

Table 2 provides assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project Report on Assembly Standards September 2024. The haplotype 1 assembly achieves the EBP reference standard of **6.C.Q60**.

Methods
Sample acquisition and DNA barcoding
The specimen used for genome sequencing was an adult male *Troilus luridus* (specimen ID Ox001206, ToLID ihTroLuri1), collected from Harcourt Arboretum, Oxfordshire, United Kingdom (latitude 51.683, longitude -1.203) on 2021-04-24

Table 1. Specimen and sequencing data for *Troilus luridus*.

Project information			
Study title	Troilus luridus (bronze shieldbug)		
Umbrella BioProject	PRJEB73932		
Species	*Troilus luridus*		
BioSpecimen	SAMEA10157799		
NCBI taxonomy ID	1545368		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	ihTroLuri1	SAMEA10200853	head and thorax
Hi-C sequencing	ihTroLuri1	SAMEA10200853	head and thorax
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq 6000	ERR12765191	7.89e+08	119.18
PacBio Revio	ERR12760817	6.30e+06	87.85
PacBio Sequel IIe	ERR12760818	1.74e+06	20.21

Table 2. Genome assembly data for *Troilus luridus*.

Genome assembly	**Haplotype 1**	**Haplotype 2**
Assembly name	ihTroLuri1.hap1.1	ihTroLuri1.hap2.1
Assembly accession	GCA_964056715.1	GCA_964056705.1
Assembly level	chromosome	chromosome
Span (Mb)	1,044.50	842.58
Number of contigs	807	459
Number of scaffolds	521	263
Longest scaffold (Mb)	207.7	209.09
Assembly metrics (benchmark)	**Haplotype 1**	**Haplotype 2**
Contig N50 length (≥ 1 Mb)	5.47 Mb	6.44 Mb
Scaffold N50 length (= chromosome N50)	126.18 Mb	130.5 Mb
Consensus quality (QV) (≥ 40)	60.5	60.9
k-mer completeness	77.78%	66.82%
Combined *k*-mer completeness (≥ 95%)	98.88%	
BUSCO* (S > 90%; D < 5%)	C:98.8%[S:96.4%,D:2.4%], F:0.2%,M:1.1%,n:2510	-
Percentage of assembly assigned to chromosomes (≥ 90%)	93.48%	93.27%
Sex chromosomes (localised homologous pairs)	X and Y	-
Organelles (one complete allele)	Mitochondrial genome: 17.9 kb	-

* BUSCO scores based on the hemiptera_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.

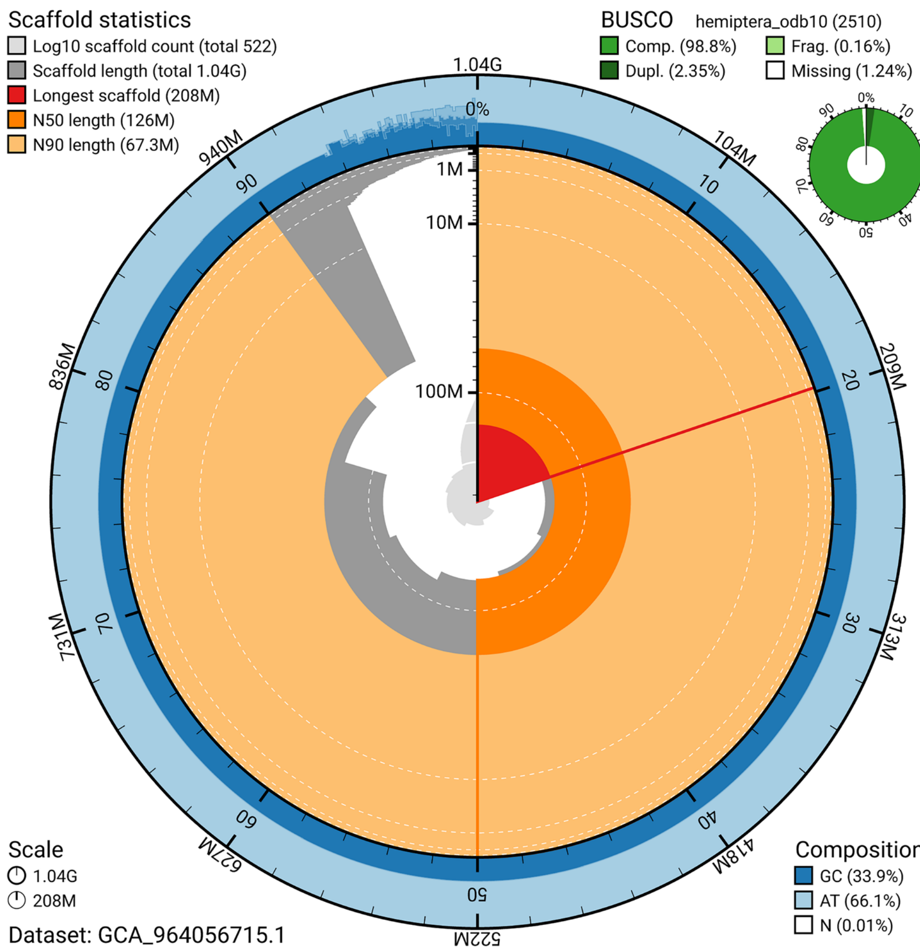

Figure 2. Genome assembly of *Troilus luridus*, ihTroLuri1.hap1.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. 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 hemiptera_odb10 set is presented at the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_964056715.1/dataset/GCA_964056715.1/snail.

by potting. The specimen was collected and identified by Liam Crowley (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 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 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).

DNA 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 (Howard *et al.*, 2025). Detailed protocols are available on protocols.io (Denton *et al.*, 2023b).

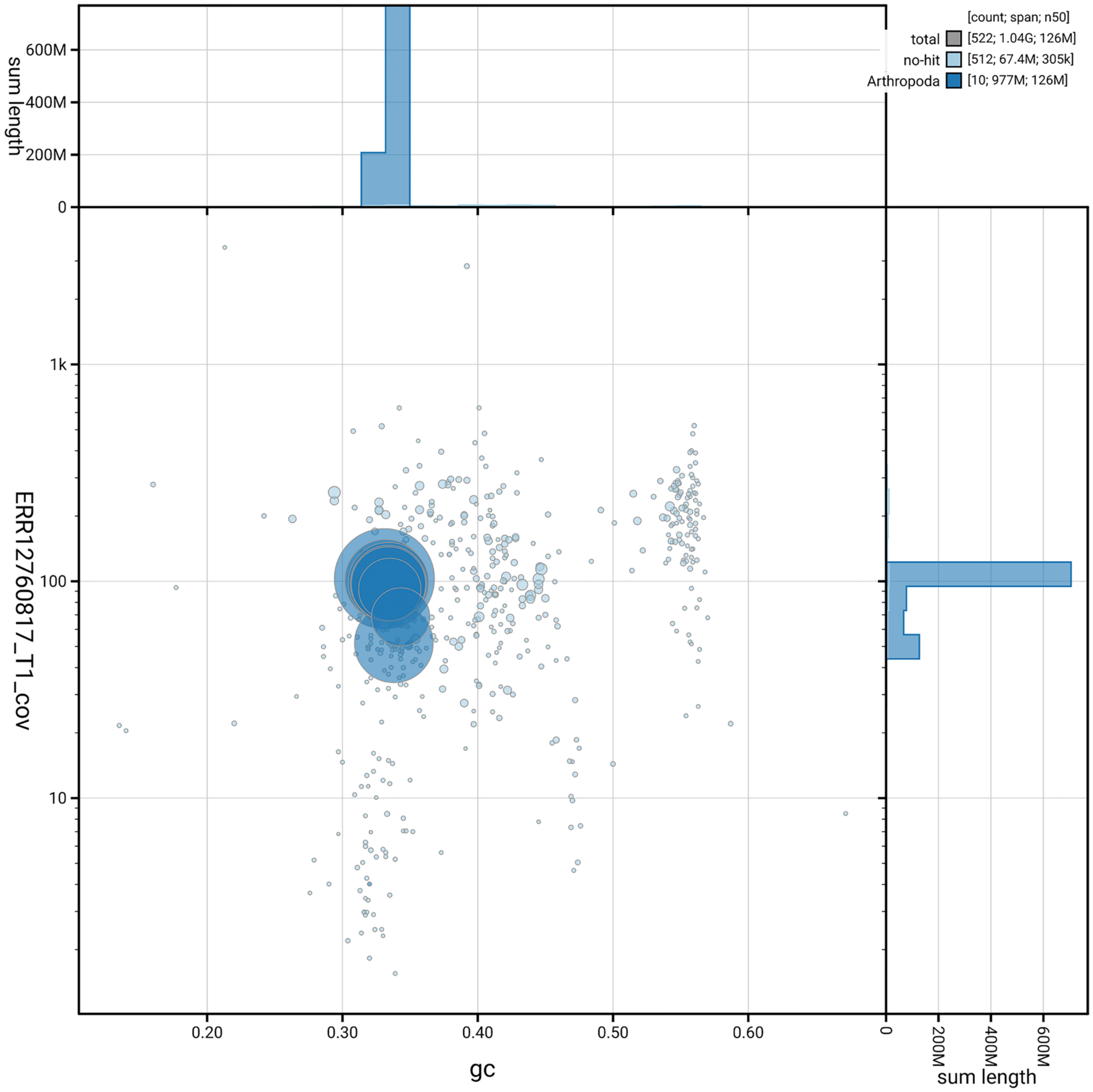

Figure 3. Genome assembly of *Troilus luridus*, ihTroLuri1.hap1.1: BlobToolKit GC-coverage plot. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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_964056715.1/dataset/GCA_964056715.1/blob.

The ihTroLuri1 sample was prepared for DNA extraction by weighing and dissecting it on dry ice (Jay *et al.*, 2023). Tissue from the head and thorax 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 (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 (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 kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

Hi-C sample preparation and crosslinking

Hi-C data were generated from head and thorax tissue of the ihTroLuri1 sample using the Arima-HiC v2 kit (Arima Genomics) with 20–50 mg of frozen tissue (stored at –80 °C). As

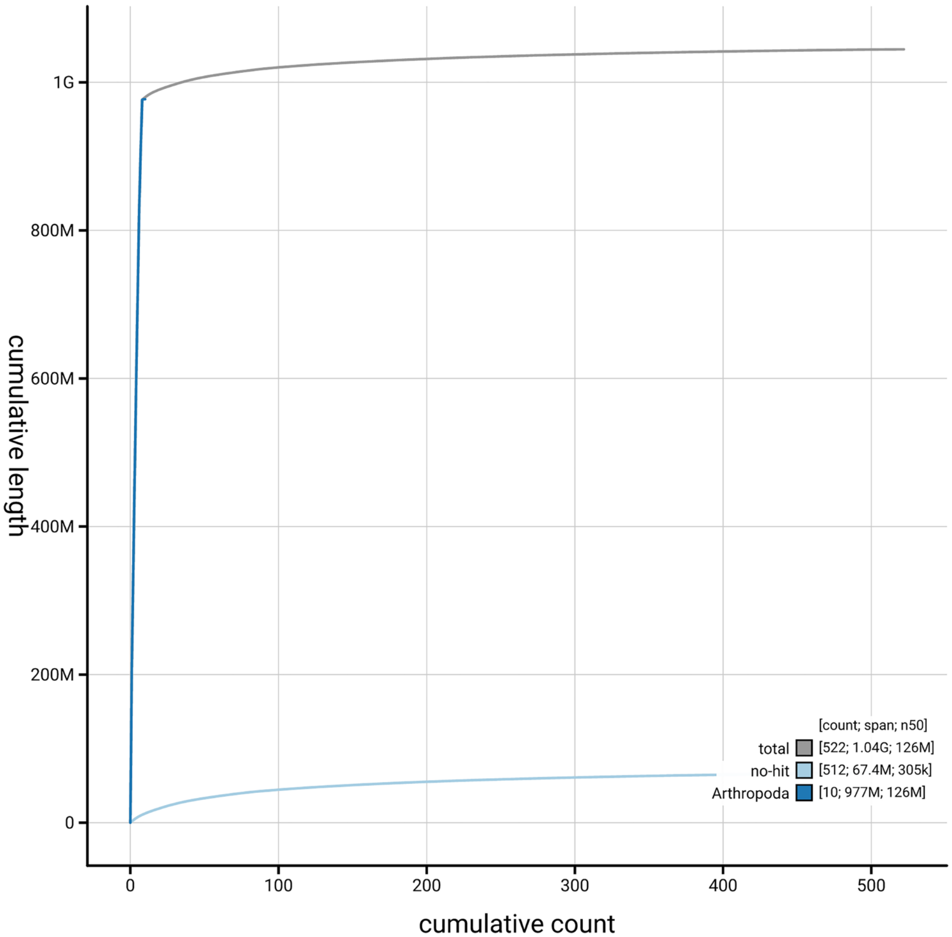

Figure 4. Genome assembly of *Troilus luridus*, ihTroLuri1.hap1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_964056715.1/dataset/GCA_964056715.1/cumulative.

per manufacturer's instructions, tissue was fixed, and the DNA crosslinked using a TC buffer, with a final formaldehyde concentration of 2%. The tissue was then homogenised using the Diagnocine Power Masher-II. The crosslinked DNA was digested using a restriction enzyme master mix, then biotinylated and ligated. A clean up was performed with SPRIselect beads prior to library preparation. DNA concentration was quantified using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and Qubit HS Assay Kit, and sample biotinylation percentage was estimated using the Arima-HiC v2 QC beads.

Library preparation and sequencing

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

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), depending on genome size and sequencing depth required. Libraries were prepared using the SMRTbell Prep Kit 3.0 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. Size-selection and clean-up were carried out using diluted AMPure PB beads (Pacific Biosciences). DNA concentration was quantified using the Qubit Fluorometer v4.0 (ThermoFisher 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 the 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

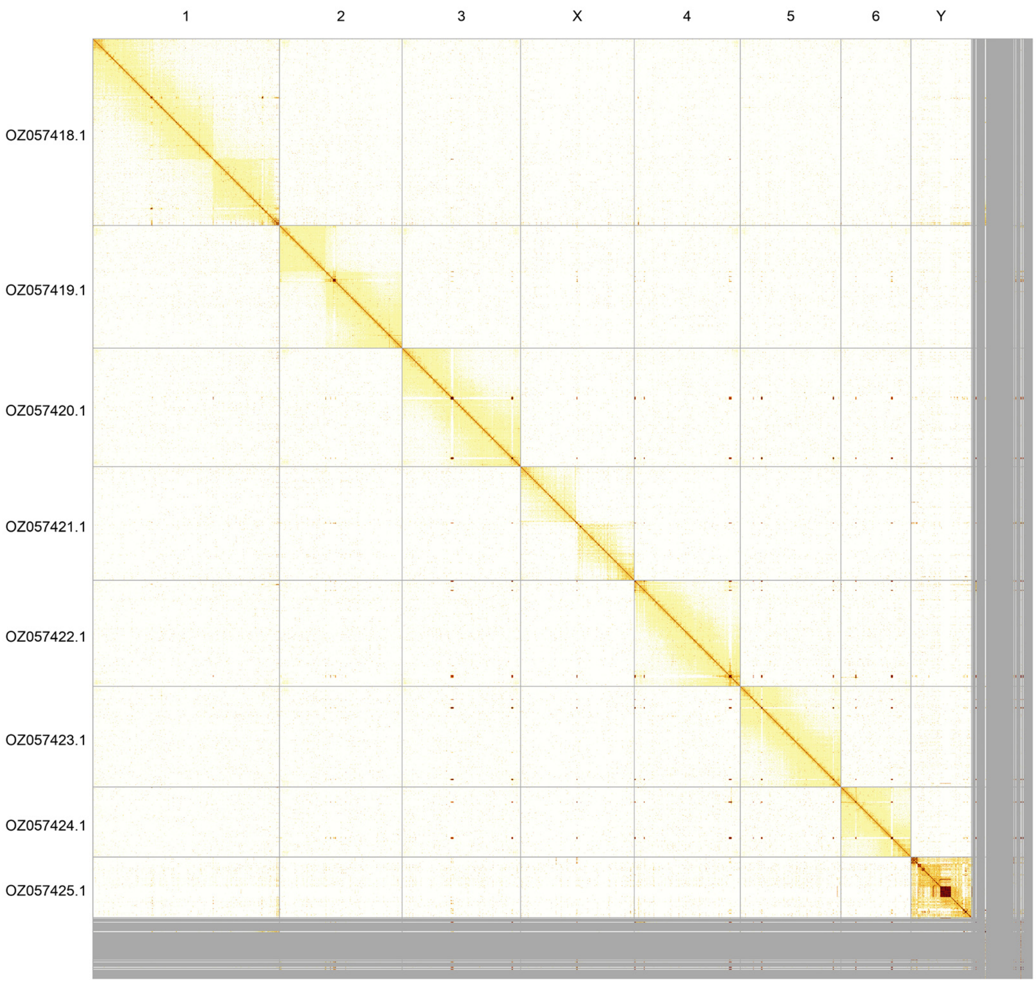

Figure 5. Genome assembly of *Troilus luridus*: Hi-C contact map of the ihTroLuri1.hap1.1 assembly, generated using PretextSnapshot. Chromosomes are shown in order of size and labelled along the axes.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Troilus luridus*, ihTroLuri1.

Haplotype 1				Haplotype 2			
INSDC accession	Name	Length (Mb)	GC%	INSDC accession	Name	Length (Mb)	GC%
OZ057418.1	1	207.7	33	OZ057412.1	1	209.09	33
OZ057419.1	2	136.25	33	OZ057413.1	2	138.2	33.5
OZ057420.1	3	131.61	33.5	OZ057414.1	3	130.5	33.5
OZ057422.1	4	117.76	33.5	OZ057415.1	4	116.36	33.5
OZ057423.1	5	112.02	33.5	OZ057416.1	5	112.83	33.5
OZ057424.1	6	77.6	33.5	OZ057417.1	6	78.86	33.5
OZ057421.1	X	126.18	34				
OZ057425.1	Y	67.32	34.5				
OZ057426.1	MT	0.02	21.5				

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, the biotinylated DNA constructs were fragmented using a Covaris E220 sonicator and size-selected to 400–600 bp using SPRISelect beads. DNA was then enriched using Arima-HiC v2 Enrichment beads. The NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) was used for end repair, A-tailing, and adapter ligation, following a modified protocol in which library preparation is carried out while the DNA remains bound to the enrichment beads. PCR amplification was performed using KAPA HiFi HotStart mix and custom dual-indexed adapters (Integrated DNA Technologies) in a 96-well plate format. Depending on sample concentration and biotinylation percentage determined at the crosslinking stage, samples were amplified for 10–16 PCR cycles. Post-PCR clean-up was carried out using SPRISelect beads. The libraries were quantified using the Accuclear Ultra High Sensitivity dsDNA Standards Assay kit (Biotium) and normalised to 10 ng/μL before sequencing. Hi-C sequencing was performed on the Illumina NovaSeq 6000 instrument using 150 bp paired-end reads.

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 assembled using Hifiasm in Hi-C phasing mode (Cheng *et al.*, 2021; Cheng *et al.*, 2022), resulting in a pair of haplotype-resolved assemblies. The Hi-C reads (Rao *et al.*, 2014) were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). The 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 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 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 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 in the blobtoolkit pipeline, a Nextflow (Di Tommaso *et al.*, 2017) 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.

Table 4. Software tools: versions and sources.

Software tool	Version	Source
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
DIAMOND	2.1.8	https://github.com/bbuchfink/diamond
fasta_windows	0.2.4	https://github.com/tolkit/fasta_windows
FastK	666652151335353eef2fcd58880bcef5bc2928e1	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/chhylp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84aa 44357826c0b6753eb28de	https://github.com/higlass/higlass
MerquryFK	d00d98157618f4e8d1a9190026b19b471055b22e	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	-	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
sanger-tol/ascc	0.1.0	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	1.2.0	https://github.com/sanger-tol/treeval
YaHS	1.2a.2	https://github.com/c-zhou/yahs

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: Troilus luridus (bronze shieldbug). Accession number PRJEB73932; https://identifiers.org/ena.embl/PRJEB73932. The genome sequence is released openly for reuse. The *Troilus luridus* genome sequencing initiative is part of the Darwin Tree of Life Project (PRJEB40665) and Sanger Institute Tree of Life Programme (PRJEB43745). 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 Natural History Museum Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.12159242.

Members of the Marine Biological Association Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.8382513.

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.14870789.

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

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.12205391.

Members of the Darwin Tree of Life Consortium are listed here: https://doi.org/10.5281/zenodo.4783558.

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Open Peer Review

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Version 1

Reviewer Report 13 May 2026

https://doi.org/10.21956/wellcomeopenres.26757.r153625

✔ **Annabel Whibley**
The University of Auckland, Auckland, New Zealand

In this Data Note, Crowley and colleagues report the genome assembly of the Hemipteran Bronze Shieldbug, *Troilus luridus*. Sample identity was verified by barcoding and PacBio HiFi (Revio and Sequel IIe) and HiC data contributed to the assembly. The methods and reporting follow DToL templating and are well-structured and comprehensive, with public accession links and extensive analysis and metadata to support the excellent quality of the resulting assembly.

I note that there is considerable sub-structure to the HiC heatmap, but the diagonal signal remains strong and so I interpret this as interactions within chromosome arms rather than misassembly. The BlobPlot shows a few clouds of small scaffolds, including a cluster with high GC content, reflecting the challenges of assembling a relatively large and heterozygous genome. Nevertheless, >93% of the assembly was scaffolded to haplotypes and completeness and contiguity stats are excellent for the taxon.

The assembly is presented as two haplotypes- one curated to chromosome level and containing both sex chromosomes, the other curated just to scaffold level. Whilst this is a practical solution- a useful reference for population studies would indeed include X and Y- I did also find it triggered a bit of a semantic debate about what constitutes a haplotype. I wonder whether there might be different term or qualifier that should be applied for a haplotype which is not, strictly, a “real” biological unit but nevertheless represents a single copy of each chromosome within a genome.

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: Genomics, Bioinformatics

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 19 March 2026

https://doi.org/10.21956/wellcomeopenres.26757.r149489

Sivasankaran Kuppusamy
Loyola College, Chennai, Tamil Nadu, India

The chromosome-level genome sequence of *Troilus luridus* (Fabricius, 1775) has been generated by the authors. Using whole-genome sequencing data, the authors assembled two haplotypes with total lengths of 1,044.50 Mb and 842.58 Mb, respectively. The haplotypes were further scaffolded into chromosomal pseudomolecules. Appropriate methodologies appear to have been used for sequencing, genome assembly, and annotation.

Comments on the manuscript:

In the Abstract, the authors state: "Most of haplotype 1 (93.48%) is scaffolded into 8 chromosomal pseudomolecules, including the X and Y sex chromosomes. Most of haplotype 2 (93.27%) is scaffolded into 6 chromosomal pseudomolecules." However, in the Assembly Statistics section, it is mentioned that "haplotype 2 was assembled to scaffold level." This appears inconsistent with the statement in the Abstract. The authors are requested to verify and correct this discrepancy.
The full genus name *Troilus luridus* has been used throughout the manuscript. According to standard scientific writing conventions, the full name may be used at the first mention, after which it should be abbreviated as *T. luridus* in subsequent occurrences.

Overall, the research article is well prepared, and the manuscript meets the necessary scientific standards. It is suitable 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: Phylogenetic analysis of superfamily Noctuoidea moths using mitogenome sequences

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.