DATA NOTE

The genome sequence of the ichneunomid wasp, *Ischnus inquisitorius* (Muller, 1776) (Hymenoptera: Ichneumonidae)

[version 1; peer review: 2 approved]

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v1 **First published:** 15 Aug 2025, **10**:448
https://doi.org/10.12688/wellcomeopenres.24737.1
Latest published: 15 Aug 2025, **10**:448
https://doi.org/10.12688/wellcomeopenres.24737.1

Abstract

We present a haploid genome assembly from an individual male *Ischnus inquisitorius* (ichneunomid wasp; Arthropoda; Insecta; Hymenoptera; Ichneumonidae). The genome sequence has a total length of 232.18 megabases. Most of the assembly (99.81%) is scaffolded into 13 chromosomal pseudomolecules. The mitochondrial genome has also been assembled, with a length of 29.53 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

Ischnus inquisitorius, ichneunomid wasp, genome sequence, chromosomal, Hymenoptera

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 15 Aug 2025	✓ view	✓ view

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Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Wellcome through core funding to the Wellcome Sanger Institute (220540) and the Darwin Tree of Life Discretionary Award [218328, https://doi.org/10.35802/218328].
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: McCulloch J, Crowley LM, De Ketelaere A *et al.* **The genome sequence of the ichneunomid wasp, *Ischnus inquisitorius* (Muller, 1776) (Hymenoptera: Ichneumonidae) [version 1; peer review: 2 approved]** Wellcome Open Research 2025, **10**:448 https://doi.org/10.12688/wellcomeopenres.24737.1

First published: 15 Aug 2025, **10**:448 https://doi.org/10.12688/wellcomeopenres.24737.1

Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Hymenoptera; Apocrita; Ichneumonoidea; Ichneumonidae; Cryptinae; Cryptini; Ischnina; *Ischnus*; *Ischnus inquisitorius* (Muller, 1776) (NCBI:txid904094)

Background

Ischnus inquisitorius is a widespread and common ichneumonid of the subfamily Cryptinae. Compared to many Cryptinae this is a relatively small species, about 7 mm long, but is easily identified by its distinctive markings. Females have a black mesosoma and red metasoma, with conspicuous white markings on the scutellum and orbits. Males have white stripes on the metasoma and extra white markings on the mesosoma, including in the middle of the mesoscutum. Although there are no modern keys to European *Ischnus*, *I. inquisitorius* can be identified using Morley (1903) or Townes & Townes (1962), and Martin Schwarz's manuscript keys for identification of British Cryptini have been widely circulated.

For a while, this species was known as *Ischnus migrator* (Fabricius), due to a misinterpretation of Fabricius's types, but this was clarified by Horstmann (2001) and the valid name was re-established as *I. inquisitorius*. The nature of the species involved has never been in doubt, just the valid name; for example, Schwarz & Shaw (1998) report on the ecology and distribution of *I. inquisitorius* using the name *I. migrator*.

Hosts of *I. inquisitorius* are the pupae of Tortricidae, leaf-rolling Lepidoptera. The egg is laid externally and the larva starts feeding externally but later burrows into the pupa to finish feeding (Cole, 1979). There is one generation per year (Schwarz & Shaw, 1998) and the adult females hibernate (Cole, 1979). This is a widespread woodland species in England (Schwarz & Shaw, 1998) and can readily be beaten from foliage. Ranging extensively across the Palaearctic and Nearctic (e.g., Townes & Townes, 1962), *I. inquisitorius* reaches as far South as Mexico (Kasparyan & Ruíz Cancino, 2005). It attacks some economically important forestry and agriculture pests in North America (Bennett, 2008) but is probably a minor constituent of the guild of parasitoids of tortricids.

We present a chromosomally complete genome sequence for *Ischnus inquisitorius*. The assembly was produced using the Tree of Life pipeline from a specimen collected in Wytham Woods, Oxfordshire, United Kingdom (Figure 1), as part of the Darwin Tree of Life project.

Methods

Sample acquisition and DNA barcoding

The specimen used for genome sequencing was an adult male *Ischnus inquisitorius* (specimen ID Ox002543, ToLID iyIscInqu1; Figure 1), collected from Wytham Woods, Oxfordshire, United Kingdom (latitude 51.772, longitude –1.338) on 2022-07-25. The specimen was collected by James

Figure 1. Photograph of the *Ischnus inquisitorius* (iyIscInqu1) specimen from which samples were taken for genome sequencing.

McCulloch and Liam Crowley (University of Oxford) and identified by Augustijn De Ketelaere. Another specimen was used for RNA sequencing (specimen ID NHMUK010636682, ToLID iyIscInqu2). It was collected from Imperial Road, England, United Kingdom (latitude 51.47, longitude –0.18) on 2022-03-18. This specimen was collected by Maxwell Barclay and identified by Gavin Broad (both Natural History Museum). Sample metadata were collected in line with the Darwin Tree of Life project standards described by 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 iyIscInqu1 sample was weighed and triaged to determine the appropriate extraction protocol.

Tissue from the whole organism was homogenised by powermashing using a PowerMasher II tissue disruptor. HMW DNA was extracted 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 7.72 ng/μL and a yield of 347.40 ng, with a fragment size of 11.5 kb. The 260/280 spectrophotometric ratio was 1.97, and the 260/230 ratio was 2.32.

RNA was extracted from whole organism tissue of iyIscInqu2 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ *mir*Vana protocol. 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.

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 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, and to perform primary and secondary analysis of the data upon completion.

Hi-C

Sample preparation and crosslinking

The Hi-C sample was prepared from 20–50 mg of frozen whole organism tissue of the iyIscInqu1 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 10–16 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.

RNA library preparation and sequencing

Libraries were prepared using the NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina (New England Biolabs), following the manufacturer's instructions. Poly(A) mRNA in the total RNA solution was isolated using oligo(dT) beads, converted to cDNA, and uniquely indexed; 14 PCR cycles were performed. Libraries were size-selected to produce fragments between 100–300 bp. Libraries were quantified, normalised, pooled to a final concentration of 2.8 nM, and diluted to 150 pM for loading. Sequencing was carried out on the Illumina NovaSeq 6000 to generate 150-bp paired-end reads.

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 (Cheng *et al.*, 2021) with the --primary option. The Hi-C reads (Rao *et al.*, 2014) were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019), and the contigs were scaffolded in YaHS (Zhou *et al.*, 2023) with 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. 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 and 77 joins. The curation process is documented at https://gitlab.com/wtsi-grit/rapid-curation. PretextSnapshot was used to generate a Hi-C contact map of the final assembly.

Assembly quality assessment

The Merqury.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 the haploid assembly 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 by querying the GoaT database (Challis *et al.*, 2023). 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 package management via Conda and Bioconda (Grüning *et al.*, 2018), and containerisation through Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017).

Genome sequence report

Sequence data

PacBio sequencing of the *Ischnus inquisitorius* specimen generated 22.26 Gb (gigabases) from 2.51 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 107.00 Mb (using a diploid model), with a heterozygosity of 50.00% and repeat content of 15.47% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 186× coverage. Hi-C sequencing produced 97.64 Gb from 646.65 million reads, which were used to scaffold the assembly. RNA sequencing data were also generated and are available in public sequence repositories. Table 1 summarises the specimen and sequencing details.

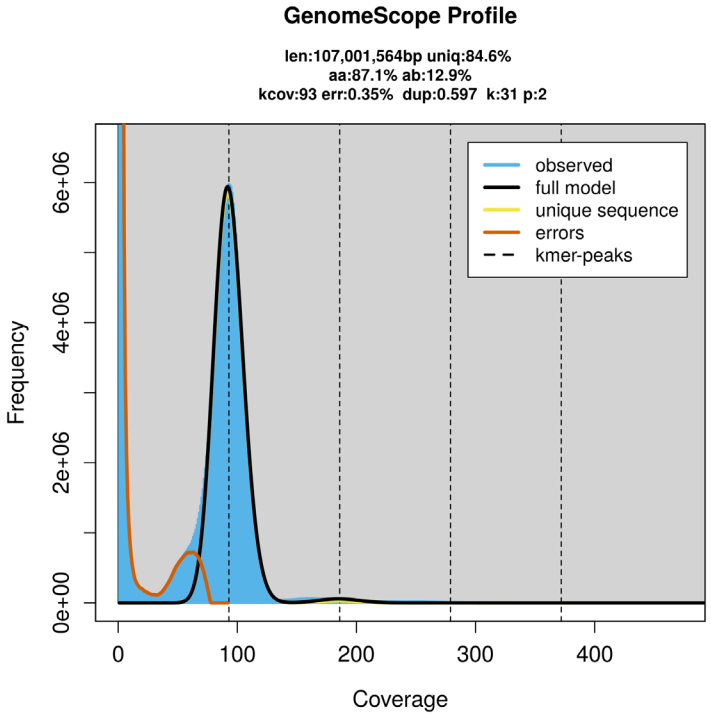

Figure 2. Frequency distribution of *k*-mers generated using GenomeScope2. The plot shows observed and modelled *k*-mer spectra, providing estimates of genome size, heterozygosity, and repeat content based on unassembled sequencing reads.

Table 1. **Specimen and sequencing data for BioProject PRJEB66033.**

Platform	PacBio HiFi	Hi-C	RNA-seq
ToLID	iyIscInqu1	iyIscInqu1	iyIscInqu2
Specimen ID	Ox002543	Ox002543	NHMUK010636682
BioSample (source individual)	SAMEA112232736	SAMEA112232736	SAMEA112964374
BioSample (tissue)	SAMEA112233228	SAMEA112233228	SAMEA112975539
Tissue	whole organism	whole organism	whole organism
Instrument	Sequel IIe	Illumina NovaSeq 6000	Illumina NovaSeq 6000
Run accessions	ERR12055572	ERR12071241	ERR12245600
Read count total	2.51 million	646.65 million	81.79 million
Base count total	22.26 Gb	97.64 Gb	12.35 Gb

Table 2. **Genome assembly statistics.**

Assembly name	iyIscInqu1.1
Assembly accession	GCA_963924025.1
Assembly level	chromosome
Span (Mb)	232.18
Number of chromosomes	13
Number of contigs	161
Contig N50	3.91 Mb
Number of scaffolds	22
Scaffold N50	18.61 Mb
Sex chromosomes	N/A
Organelles	Mitochondrion: 29.53 kb

Assembly statistics

This is a haploid assembly, with a total length of 232.18 Mb in 22 scaffolds, with 139 gaps, and a scaffold N50 of 18.61 Mb (Table 2). Most of the assembly sequence (99.81%) was assigned to 13 chromosomal-level scaffolds. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 3; Table 3). the order and orientation of the contig in the repetitive region of chromosome 13 (8.1 Mbp - telomer) is unknown.

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.

The assembly achieves a QV of 66.1 and a *k*-mer completeness of 97.42% for the primary assembly (Figure 4). BUSCO v.5.5.0 analysis using the hymenoptera_odb10 reference set (n = 5 991) identified 96.3% of the expected gene set (single = 96.0%, duplicated = 0.3%). The snail plot in Figure 5 summarises the scaffold length distribution and other assembly statistics for the primary assembly. The blob plot in Figure 6 shows the distribution of scaffolds by GC proportion and coverage.

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 primary assembly, is **6.C.Q66**, 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**

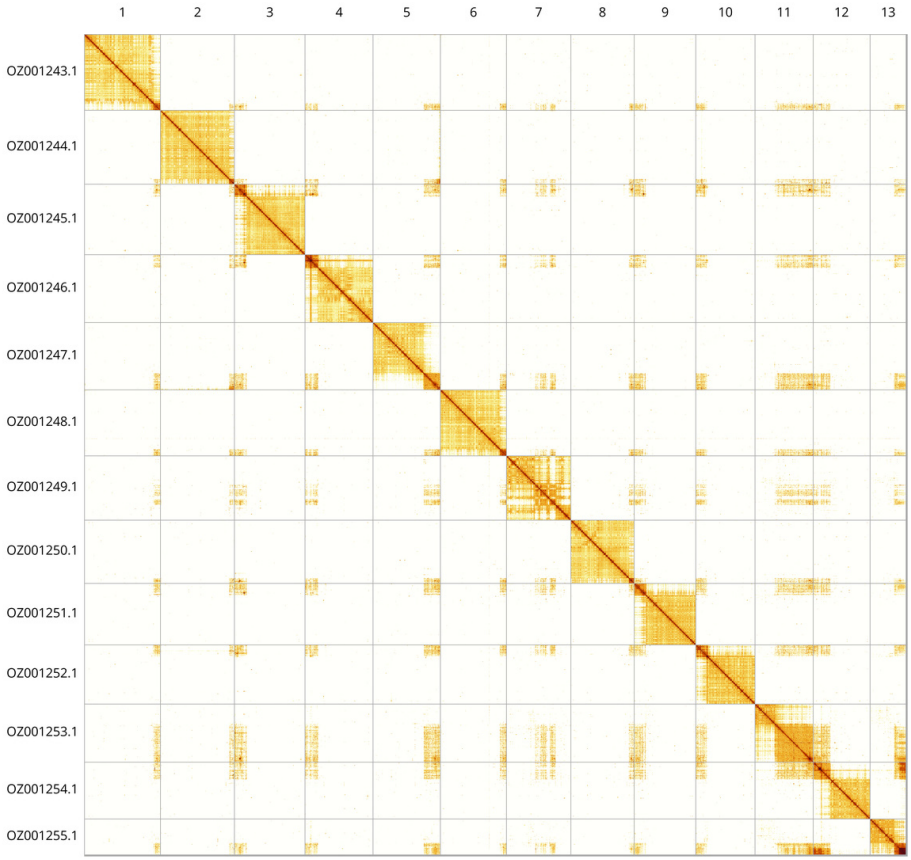

Figure 3. Hi-C contact map of the *Ischnus inquisitorius* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the primary genome assembly of *Ischnus inquisitorius* iyIscInqu1.

INSDC accession	Molecule	Length (Mb)	GC%
OZ001243.1	1	21.53	43.50
OZ001244.1	2	20.84	43.50
OZ001245.1	3	19.90	44
OZ001246.1	4	19.18	44.50
OZ001247.1	5	18.99	44.50
OZ001248.1	6	18.61	44.50
OZ001249.1	7	18.16	43
OZ001250.1	8	17.87	43.50
OZ001251.1	9	17.38	43.50
OZ001252.1	10	16.66	43.50
OZ001253.1	11	16.43	43.50
OZ001254.1	12	16.04	44.50
OZ001255.1	13	10.14	43

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,

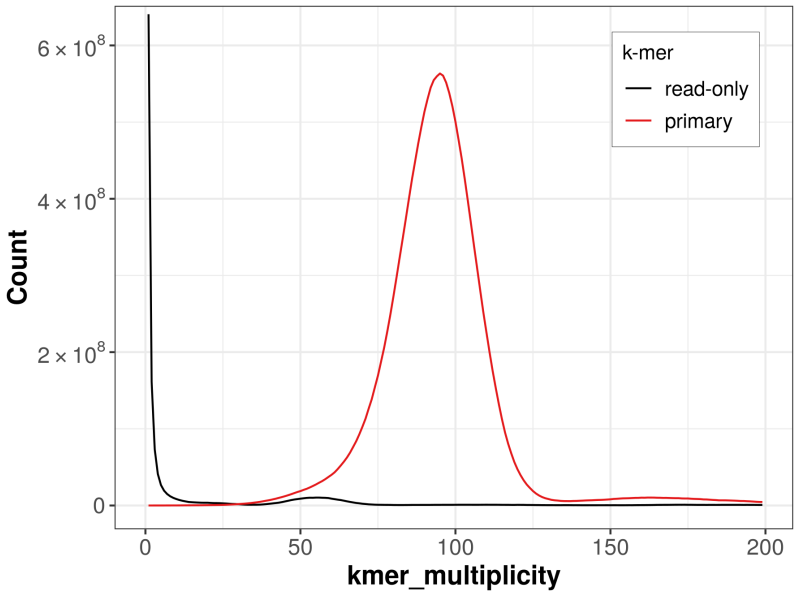

Figure 4. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers 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.

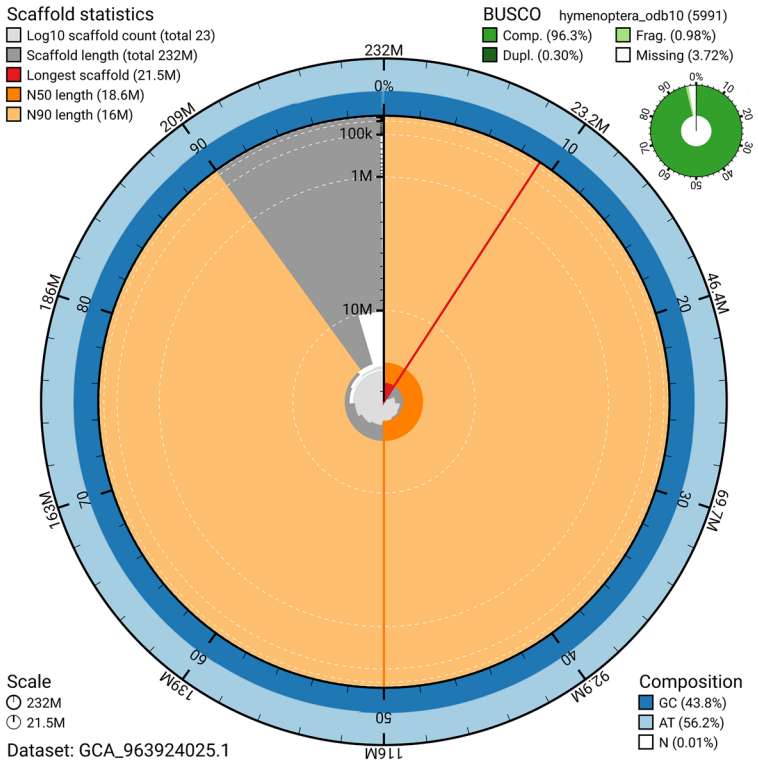

Figure 5. Assembly metrics for iyIscInqu1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. The outermost blue 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 hymenoptera_odb10 set is presented at the top right. An interactive version of this figure can be accessed on the BlobToolKit viewer.

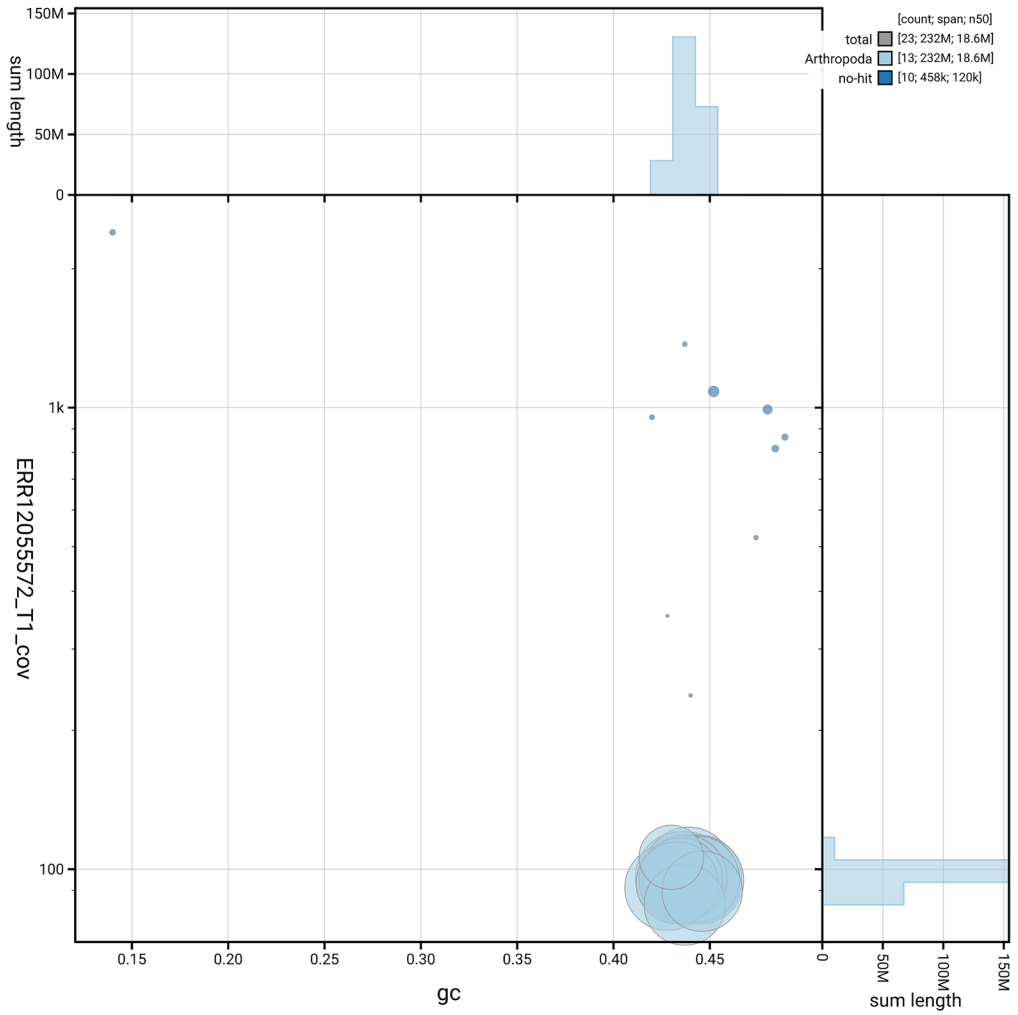

Figure 6. BlobToolKit GC-coverage plot for iyIscInqu1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes display 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 *Ischnus inquisitorius* assembly.

Measure	Value	Benchmark
EBP summary	6.C.Q66	6.C.Q40
Contig N50 length	3.91 Mb	≥ 1 Mb
Scaffold N50 length	18.61 Mb	= chromosome N50
Consensus quality (QV)	66.1	≥ 40
k-mer completeness	97.42%	≥ 95%
BUSCO	C:96.3% [S:96.0%; D:0.3%]; F:1.0%; M:2.7%; n:5 991	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.81%	≥ 90%

Genome Research Limited (operating as the Wellcome Sanger Institute), and in some circumstances, other Darwin Tree of Life collaborators.

Data availability

European Nucleotide Archive: Ischnus inquisitorius. Accession number PRJEB66033. The genome sequence is released openly for reuse. The *Ischnus inquisitorius* genome sequencing initiative is part of the Darwin Tree of Life Project (PRJEB40665) and the 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.

Production code used in genome assembly at the WSI Tree of Life are 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 Natural History Museum 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	0.16.1	https://github.com/chhylp123/hifiasm
HiGlass	1.13.4	https://github.com/higlass/higlass
MerquryFK	1.1.2	https://github.com/thegenemyers/MERQURY.FK
Minimap2	2.24-r1122	https://github.com/lh3/minimap2
MitoHiFi	3.1	https://github.com/marcelauliano/MitoHiFi
Oatk	1	https://github.com/c-zhou/oatk
MultiQC	1.14; 1.17 and 1.18	https://github.com/MultiQC/MultiQC
Nextflow	23.04.1	https://github.com/nextflow-io/nextflow
PretextSnapshot	N/A	https://github.com/sanger-tol/PretextSnapshot

Software	Version	Source
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	0.4.0	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	1.1a.2	https://github.com/c-zhou/yahs

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 02 January 2026

https://doi.org/10.21956/wellcomeopenres.27254.r139039

✔ **Julien Varaldi**
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This paper presents the full haploid genome of the ichneumonid parasitoid wasp Ischnus inquisitor. In the introduction, the authors provide an appropriate background to the biology of the wasp, including information on its host, pre-imaginal development, and geographic distribution. They took advantage of the haplo-diploid genetic system to sequence a haploid male using both long-read and Hi-C Illumina reads, enabling them to produce a highly contiguous assembly organised into chromosomes. The protocol used is described in detail, and the data are publicly available.

Minor comments:
It would have been useful to include a few words on the extra-contact that can be observed in the contact map (not only involving car 13, but all the others too). Do these regions contain repeated sequences?
In Fig. 4, there is no apparent green curve.

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: insect genomics

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 03 December 2025

https://doi.org/10.21956/wellcomeopenres.27254.r139043

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This data note describes a high-quality chromosomal assembly of the ichneunomid wasp *Ischnus inquisitorius*. The genome data are available at NCBI and data is accessible as checked in November 2025.

Comments:

- For the DNA extraction, ratios 260/280 and 260/230 were within acceptable ranges. However, for the RNA-seq sample, although the text states the use of Nanodrop, Qubit, and Agilent RNA 6000 Pico kit, neither the RNA concentration nor any RNA integrity metrics are reported.
- The other specimen collected for RNA-sequencing was also a male?
- For what purpose the RNA seq was produced? is not mentioned in the text.
- What is the sex-determination system in this species? Why could not the sex chromosomes be assembled?
- There are some software cited in Table 5 but not mentioned in the main text: Cooler, BEDTools, fasta_windows, and Oatk

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: population genetics, bioinformatics, genomics, transposable elements

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.