



DATA NOTE

The genome sequence of the Little Snipefly, *Chrysopilus asiliformis* (Preyssler, 1791)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a female specimen of *Chrysopilus asiliformis* (Little Snipefly; Arthropoda; Insecta; Diptera; Rhagionidae). The genome sequence has a total length of 429.05 megabases. Most of the assembly (99.58%) is scaffolded into 5 chromosomal pseudomolecules. The mitochondrial genome has also been assembled, with a length of 16.38 kilobases. Gene annotation of this assembly on Ensembl identified 14,233 protein-coding genes.

Keywords

Chrysopilus asiliformis, Little Snipefly, genome sequence, chromosomal, Diptera



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

Open Peer Review

Approval Status

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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; Endopterygota; Diptera; Brachycera; Tabanomorpha; Tabanoidea; Rhagionidae; *Chrysopilus*; *Chrysopilus asiliformis* (Preysler, 1791) (NCBI:txid1822507)

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 Little Snipefly, *Chrysopilus asiliformis*. This genome was assembled using the Tree of Life pipeline from a specimen collected in Wytham Woods, Oxfordshire, United Kingdom (Figure 1).

Genome sequence report

Sequencing data

The genome of a specimen of *Chrysopilus asiliformis* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 26.93 Gb (gigabases) from 2.09 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 418.21 Mb, with a heterozygosity of 1.23% and repeat content of 35.15%. These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 61 coverage. Hi-C sequencing produced 101.34 Gb from 671.10 million reads, 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.

Assembly statistics

The primary haplotype was assembled, and contigs corresponding to an alternate haplotype were also deposited in INSDC

databases. The assembly was improved by manual curation, which corrected 75 misjoins or missing joins and removed 8 haplotypic duplications. These interventions decreased the scaffold count by 22.22% and increased the scaffold N50 by 1.13%. The final assembly has a total length of 429.05 Mb in 41 scaffolds, with 138 gaps, and a scaffold N50 of 116.07 Mb (Table 2).

The snail plot in Figure 2 provides a summary of the assembly statistics, indicating the distribution of scaffold lengths and other assembly metrics. Figure 3 shows the distribution of scaffolds by GC proportion and coverage. Figure 4 presents a cumulative assembly plot, with separate curves representing different scaffold subsets assigned to various phyla, illustrating the completeness of the assembly.

Most of the assembly sequence (99.58%) was assigned to 5 chromosomal-level scaffolds. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 5; Table 3). During curation, we noted that read coverage suggests this is the homogametic sex, but we did not identify the sex chromosome(s) as sequence data from the heterogametic sex was not available and homology is unreliable for sex chromosome identification in Diptera due to frequent sex chromosome turnover (Vicoso & Bachtrog, 2015).

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

Assembly quality metrics

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

The combined primary and alternate assemblies achieve an estimated QV of 63.8. The *k*-mer completeness is 76.68% for the primary haplotype and 75.42% for the alternate haplotype; and 98.85% for the combined primary and alternate assemblies. BUSCO v.5.5.0 analysis using the diptera_odb10 reference set ($n = 3,285$) identified 95.3% of the expected gene set (single = 94.9%, duplicated = 0.5%).

Table 2 provides assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project Report on Assembly Standards September 2024. The primary assembly achieves the EBP reference standard of 6.C.Q64.

Genome annotation report

The *Chrysopilus asiliformis* genome assembly (GCA_964034945.1) was annotated externally by Ensembl at the European Bioinformatics Institute (EBI). This annotation includes 21,860 transcribed mRNAs from 14,233 protein-coding and 219



Figure 1. Photograph of the *Chrysopilus asiliformis* (idChrAsil3) specimen used for genome sequencing.

Table 1. Specimen and sequencing data for *Chrysopilus asiliformis*.

Project information			
Study title	Chrysopilus asiliformis (little snipefly)		
Umbrella BioProject	PRJEB62173		
Species	<i>Chrysopilus asiliformis</i>		
BioSpecimen	SAMEA112232524		
NCBI taxonomy ID	1822507		
Specimen information			
Technology	ToLID	BioSample accession	Organism part
PacBio long read sequencing	idChrAsil3	SAMEA112232968	whole organism
Hi-C sequencing	idChrAsil3	SAMEA112232968	whole organism
RNA sequencing	idChrAsil5	SAMEA112975362	whole organism
Sequencing information			
Platform	Run accession	Read count	Base count (Gb)
Hi-C Illumina NovaSeq 6000	ERR11468745	6.71e+08	101.34
PacBio Sequel IIe	ERR11458816	2.09e+06	26.93
RNA Illumina NovaSeq X	ERR13093633	1.98e+08	29.93

non-coding genes. The average transcript length is 14,865.63 bp. There are 1.51 coding transcripts per gene and 5.79 exons per transcript. For further information about the annotation, please refer to <https://beta.ensembl.org/species/f203d201-1fcc-4c7d-af2f-be919ed4894a>.

Methods

Sample acquisition and DNA barcoding

The specimen used for genome sequencing was an adult female *Chrysopilus asiliformis* (specimen ID Ox002290, ToLID idChrAsil3), collected from Wytham Woods, Oxfordshire, United Kingdom (latitude 51.772, longitude -1.338) on 2022-07-05 by netting. The specimen was collected and identified by Liam Crowley (University of Oxford) and preserved on dry ice.

Another specimen was used for RNA sequencing (specimen ID NHMUK015059616, ToLID idChrAsil5). It was an adult specimen collected from Sheringham, England, United Kingdom (latitude 52.94, longitude 1.21) on 2022-07-07 by handpicking. The specimen was collected and identified by Sue Taylor and preserved by dry freezing (-80 °C).

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).

Nucleic acid extraction

The workflow for high molecular weight (HMW) DNA extraction at the Wellcome Sanger Institute (WSI) Tree of Life Core Laboratory includes a sequence of procedures: sample preparation and homogenisation, DNA extraction, fragmentation and purification (Howard *et al.*, 2025). Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). The idChrAsil3 sample was prepared for DNA extraction by weighing and dissecting it on dry ice (Jay *et al.*, 2023). Tissue from the whole organism was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a). HMW DNA was extracted using the Automated MagAttract v2 protocol (Oatley *et al.*, 2023a). DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system (Bates *et al.*, 2023). Sheared DNA was purified by solid-phase reversible immobilisation, using AMPure PB beads to eliminate shorter fragments and concentrate the DNA (Oatley *et al.*, 2023b). The concentration of the sheared and purified DNA was assessed

Table 2. Genome assembly data for *Chrysopilus asiliformis*.

Genome assembly		
Assembly name	idChrAsil3.1	
Assembly accession	GCA_964034945.1	
Alternate haplotype accession	GCA_964035425.1	
Assembly level for primary assembly	chromosome	
Span (Mb)	429.05	
Number of contigs	179	
Number of scaffolds	41	
Longest scaffold (Mb)	117.0	
Assembly metric	Measure	Benchmark
Contig N50 length	6.56 Mb	≥ 1 Mb
Scaffold N50 length	116.07 Mb	= chromosome N50
Consensus quality (QV)	Primary: 64.0; alternate: 63.7; combined: 63.8	≥ 40
k-mer completeness	Primary: 76.68%; alternate: 75.42%; combined: 98.85%	$\geq 95\%$
BUSCO*	C:95.3%[S:94.9%,D:0.5%], F:1.2%,M:3.5%,n:3,285	$S > 90\%$; $D < 5\%$
Percentage of assembly assigned to chromosomes	99.58%	$\geq 90\%$
Sex chromosomes	Not identified	localised homologous pairs
Organelles	Mitochondrial genome: 16.38 kb	complete single alleles

* BUSCO scores based on the diptera_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.

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 extracted DNA had a Qubit concentration of 7.72 ng/μL and a yield of 1,003.60 ng. Spectrophotometric measurements indicated 260/280 and 260/230 ratios of 1.96 and 1.52, respectively.

RNA was extracted from whole organism tissue of idChrAsil5 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ mirVana protocol (do Amaral *et al.*, 2023). The RNA concentration was assessed using a 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.

Hi-C sample preparation and crosslinking

Hi-C data were generated from the whole organism of the idChrAsil3 sample using the Arima-HiC v2 kit (Arima Genomics) with 20–50 mg of frozen tissue (stored at -80°C). As

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 Diagenode 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

Samples with an average fragment size exceeding 8 kb and a total mass over 400 ng were eligible for 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

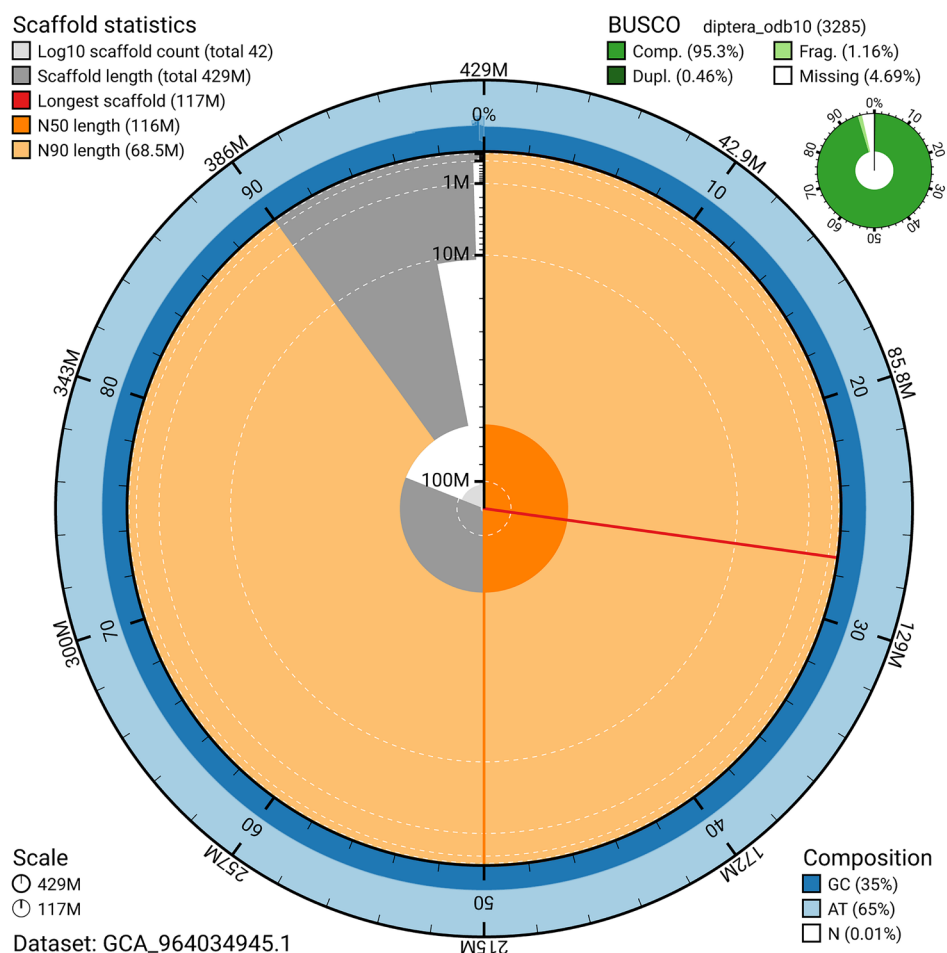


Figure 2. Genome assembly of *Chrysopilus asiliformis*, idChrAsil3.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the 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 diptera_odb10 set is presented at the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_964034945.1/dataset/GCA_964034945.1/snail.

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.

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, 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)

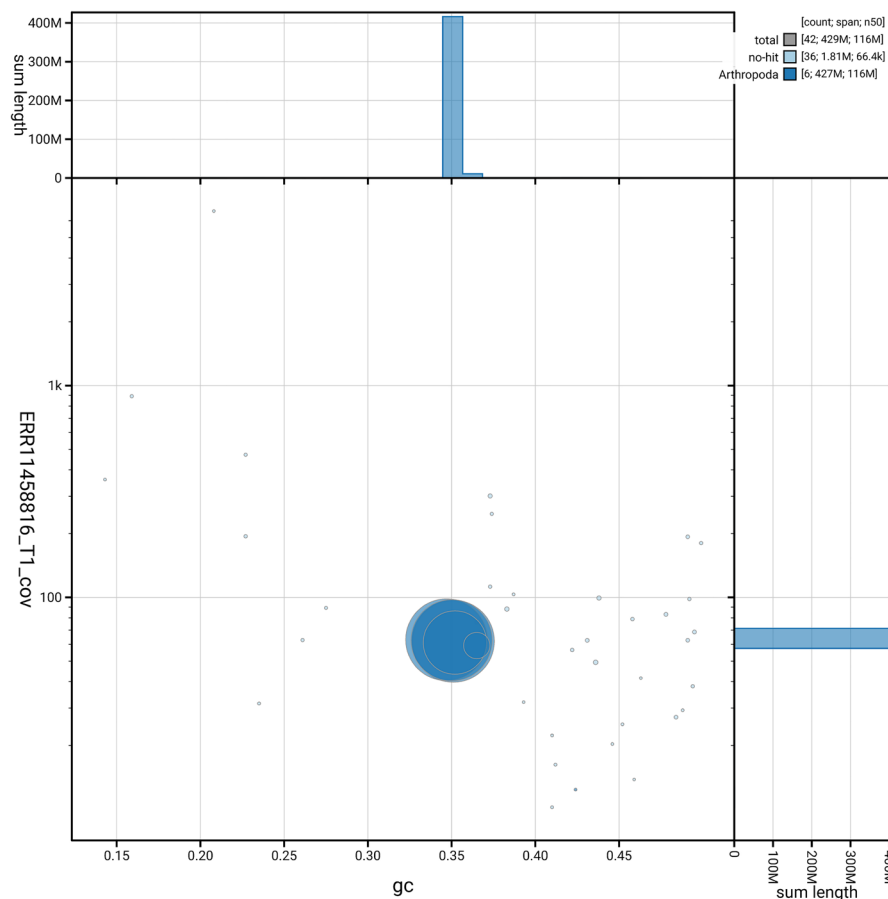


Figure 3. Genome assembly of *Chrysopilus asiliformis*, idChrAsil3.1: BlobToolkit GC-coverage plot. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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_964034945.1/dataset/GCA_964034945.1/blob.

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.

RNA

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 X instrument.

Genome assembly, curation and evaluation

Assembly

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

The HiFi reads were first assembled using Hifiasm (Cheng *et al.*, 2021) with the --primary option. Haplotypic duplications were identified and removed using purge_dups (Guan *et al.*, 2020). The Hi-C reads (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) using the --break option for handling potential misassemblies. The scaffolded assemblies were evaluated using

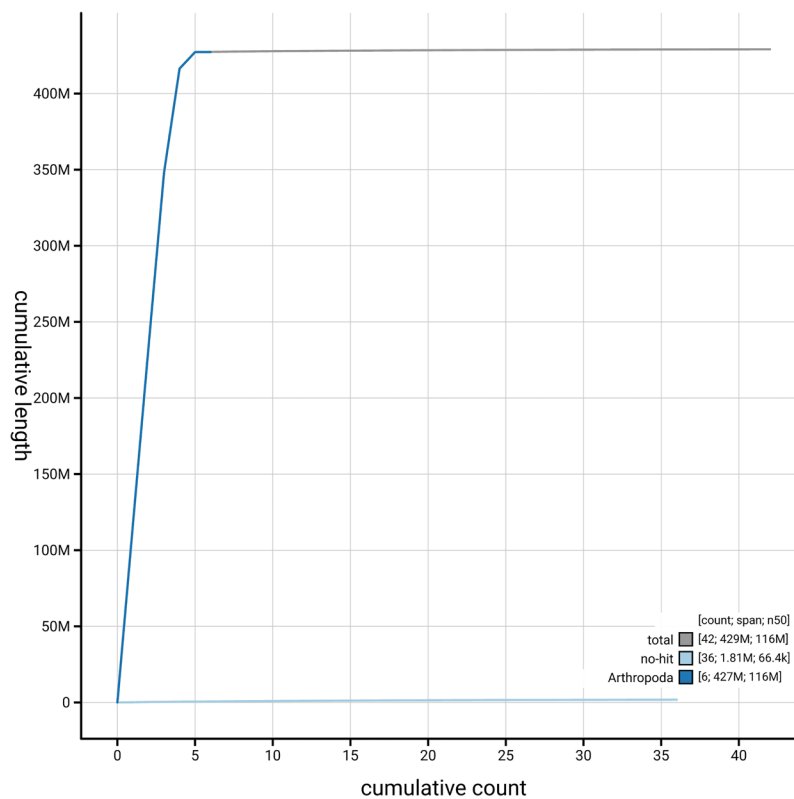


Figure 4. Genome assembly of *Chrysopilus asiliformis*, idChrAsil3.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_964034945.1/dataset/GCA_964034945.1/cumulative.

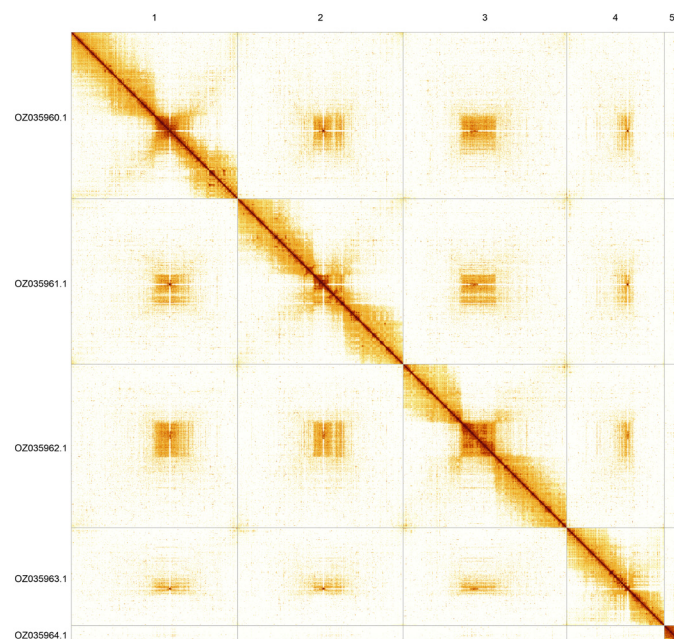


Figure 5. Genome assembly of *Chrysopilus asiliformis*. Hi-C contact map of the idChrAsil3.1 assembly, generated using PretextSnapshot. Chromosomes are shown in order of size and labelled with chromosome numbers (top) and chromosome accession numbers (left).

Table 3. Chromosomal pseudomolecules in the genome assembly of *Chrysopilus asiliformis*, idChrAsil3.

INSDC accession	Name	Length (Mb)	GC%
OZ035960.1	1	117.0	35
OZ035961.1	2	116.07	34.5
OZ035962.1	3	114.8	35
OZ035963.1	4	68.48	35
OZ035964.1	5	10.88	36.5
OZ035965.1	MT	0.02	21

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 Cointons 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 Merquery.FK tool (Rhie *et al.*, 2020), run in a Singularity container (Kurtzer *et al.*, 2017), was used to evaluate *k*-mer completeness and assembly quality for the primary and alternate haplotypes using the *k*-mer databases (*k* = 31) 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 (Di Tommaso *et al.*, 2017) implementation of the earlier Snakemake BlobToolKit pipeline (Challis *et al.*, 2020). The pipeline aligns PacBio reads using minimap2 (Li, 2018) and SAMtools (Danecek *et al.*, 2021) to generate coverage tracks. Simultaneously, it queries the GoAT database (Challis *et al.*, 2023) to identify relevant BUSCO lineages and

runs BUSCO (Manni *et al.*, 2021). For the three domain-level BUSCO 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).

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

Wellcome Sanger Institute – Legal and Governance

The materials that have contributed to this genome note have been supplied by a Darwin Tree of Life Partner. The submission of materials by a Darwin Tree of Life Partner is subject to the ‘**Darwin Tree of Life Project Sampling Code of Practice**’, which can be found in full on the Darwin Tree of Life website [here](#). By agreeing with and signing up to the Sampling Code of Practice, the Darwin Tree of Life Partner agrees they will meet the legal and ethical requirements and standards set out within this document in respect of all samples acquired for, and supplied to, the Darwin Tree of Life Project.

Further, the Wellcome Sanger Institute employs a process whereby due diligence is carried out proportionate to the nature of the materials themselves, and the circumstances under which they have been/are to be collected and provided for use. 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.

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
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-r375	https://github.com/chhylyp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84aa44357826c0b6753eb28de	https://github.com/higlass/higlass
MercuryFK	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
PretextView	0.2.5	https://github.com/sanger-tol/PretextView
PretextViewSnapshot	-	https://github.com/sanger-tol/PretextViewSnapshot
purge_dups	1.2.5	https://github.com/dfguan/purge_dups
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

Data availability

European Nucleotide Archive: *Chrysopilus asiliformis* (little snipefly). Accession number PRJEB62173; <https://identifiers.org/ena.embl/PRJEB62173>. The genome sequence is released openly for reuse. The *Chrysopilus asiliformis* 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. 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 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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Daniela H Palmer Droguett

The University of Texas at Arlington, Arlington, Texas, USA

The study presents a genome assembly and annotation for the Little Snipefly (*Chrysopilus asiliformis*). The genome assembly is 429.05 Mb in length, and 99.58% of the assembly is contained in 5 major scaffolds. The assembly contains 95.3% complete BUSCOs based on the diptera_odb10 set. The annotation includes 14,233 protein-coding genes. Based on read coverage metrics, females appear to be the homogametic sex in this species.

A few notes that would strengthen the manuscript:

1. Are there karyotype data that can corroborate the 5 major scaffolds of the assembly?
2. Verification of female homogamety and identification of the sex chromosomes incorporating data from both sexes would be ideal.
3. Was the specimen used for RNA-seq also a female? The methods don't specify.
4. Average transcript length is nearly 15kb, which seems quite high. Can the authors please clarify why this might be?

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: Evolution, 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 12 August 2025

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Cheng Sun

Capital Normal University, Beijing, Beijing, China

The current study presents a chromosome-level genome sequence for the Little Snipefly, *Chrysopilus asiliformis*. The genome sequence has a total length of 429 Mb, with 5 pseudomolecules. The mitochondrial genome has also been assembled, with a length of 16.38 kilobases. Genome annotation identified 14,233 protein-coding genes.

- 1) There was a typo in this paper. "chromosomal-level" should be "chromosomal-level".
- 2) More information on Little Snipefly, *Chrysopilus asiliformis* should be provided.

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: Genome evolution

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.
