DATA NOTE

The genome sequence of the plant bug, *Alydus calcaratus* (Linnaeus, 1758) (Hemiptera: Alydidae)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from an individual *Alydus calcaratus* (plant bug; Arthropoda; Insecta; Hemiptera; Alydidae). The assembly contains two haplotypes with total lengths of 1 007.28 megabases and 1 001.78 megabases. Most of haplotype 1 (99.21%) is scaffolded into 7 chromosomal pseudomolecules, including the X sex chromosome. Haplotype 2 was assembled to scaffold level. The mitochondrial genome has also been assembled, with a length of 18.53 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

Alydus calcaratus; plant bug; genome sequence; chromosomal; Hemiptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓

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version 1 03 Oct 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; Coreoidea; Alydidae; *Alydus*; *Alydus calcaratus* (Linnaeus, 1758) (NCBI:txid881521)

Background

Alydus calcaratus (Linnaeus, 1758) is the only British and Irish representative of the family Alydidae. It is a large, dark bug (length 11 mm) with long legs bearing spines on the hind femora, and a distinctly curved fourth antennal segment. The front of the head, viewed from above, is evenly rounded (Unwin, 2001). The dorsal abdomen is orange-red and exposed in flight, contributing to its resemblance to spider-hunting wasps (British Bugs, 2025; Unwin, 2001). The nymphs are convincing ant mimics, and may develop in association with ant nests.

In Britain, the species is found in coastal areas and other sandy habitats (Unwin, 2001) in southern England and parts of Wales. It has only been recorded once in Northern Ireland (County Down), and is a Northern Ireland priority species (National Museums Northern Ireland, 2025).

Adults feed mainly on legumes such as broom (*Cytisus scoparius*) and gorse (*Ulex europaeus*), although carrion-feeding has been observed. The life cycle is incompletely understood; adults overwinter and a new generation appears in July. The species has a wide distribution across Europe, Asia, and northern North America, but threats in the UK and Ireland remain unknown (British Bugs, 2025; National Museums Northern Ireland, 2025).

We present the first chromosome-level genome sequence for *Alydus calcaratus*, one of two genomes available for the family Alydidae as of August 2025 (data obtained via NCBI datasets, O'Leary *et al.*, 2024). The assembly was produced using the Tree of Life pipeline from a specimen collected in Hartlebury Common, Worcestershire, 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).

Figure 1. Photograph of the *Alydus calcaratus* (ihAlyCalc2) specimen used for genome sequencing.

Methods

Sample acquisition and DNA barcoding

The specimen used for genome sequencing was a juvenile female *Alydus calcaratus* (specimen ID Ox003781, ToLID ihAlyCalc2; Figure 1), collected from Hartlebury Common, Worcestershire, United Kingdom (latitude 52.334, longitude –2.263) on 2023-07-17. The specimen was collected by Liam Crowley, Aaron Bhambra and Clare Boyes and identified by Liam Crowley. 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 ihAlyCalc2 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 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 automated 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 32.31 ng/μL and a yield of 1 518.57 ng, with a fragment size of 16.7 kb. The 260/280 spectrophotometric ratio was 1.91, and the 260/230 ratio was 1.6.

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 whole organism tissue of the ihAlyCalc2 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 X.

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 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 32 breaks and 114 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 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 *Alydus calcaratus* specimen generated 70.61 Gb (gigabases) from 6.06 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 927.38 Mb, with a heterozygosity of 0.65% and repeat content of 31.20% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 73× coverage. Hi-C sequencing produced 687.54 Gb from 4 553.26 million reads, which were 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 final assembly has a total length of 1 007.28 Mb in 248 scaffolds, with 899 gaps, and a scaffold N50 of 152.52 Mb (Table 2).

Most of the assembly sequence (99.21%) was assigned to 7 chromosomal-level scaffolds, representing 6 autosomes and the X sex chromosome. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 3; Table 3). X chromosome identified based on synteny with the genome of *Riptortus pedestris* (GCA_019009955.1). During curation, we noted that there is a heterozygous inversion on chromosome 5, with breakpoints at 90.5 Mb and 118.5 Mb.

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 58.1, and for haplotype 2, 57.5. When the two haplotypes are combined, the assembly achieves an estimated QV of 57.8. The *k*-mer completeness is 84.22% for haplotype 1, 84.69% for haplotype 2, and 98.29% for the combined haplotypes (Figure 4).

BUSCO analysis using the hemiptera_odb10 reference set (n = 2 510) identified 98.4% of the expected gene set (single = 96.8%, duplicated = 1.6%) 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.

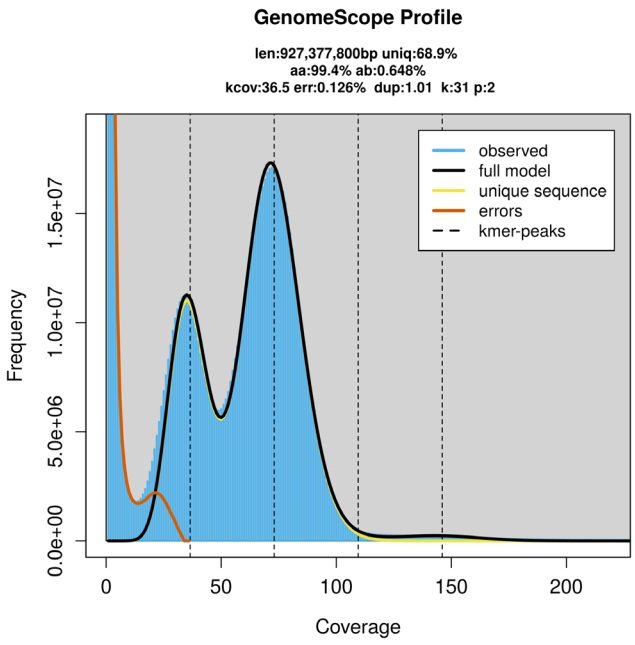

Figure 2. Frequency distribution of *k*-mers generated using GenomeScope2. The plot shows observed and modelled *k*-mer spectra, providing estimates of genome size, heterozygosity, and repeat content based on unassembled sequencing reads.

Table 1. Specimen and sequencing data for BioProject PRJEB80991.

Platform	PacBio HiFi	Hi-C
ToLID	ihAlyCalc2	ihAlyCalc2
Specimen ID	Ox003781	Ox003781
BioSample (source individual)	SAMEA114644830	SAMEA114644830
BioSample (tissue)	SAMEA114645486	SAMEA114645486
Tissue	whole organism	whole organism
Instrument	Revio	Illumina NovaSeq X
Run accessions	ERR13800524	ERR13802662
Read count total	6.06 million	4 553.26 million
Base count total	70.61 Gb	687.54 Gb

Table 2. Genome assembly statistics.

Assembly name	ihAlyCalc2.hap1.1	ihAlyCalc2.hap2.1
Assembly name	ihAlyCalc2.hap1.1	ihAlyCalc2.hap2.1
Assembly accession	GCA_964341005.1	GCA_964341015.1
Assembly level	chromosome	scaffold
Span (Mb)	1 007.28	1 001.78
Number of chromosomes	7	N/A
Number of contigs	1 147	1 330
Contig N50	2.35 Mb	2.35 Mb
Number of scaffolds	248	467
Scaffold N50	152.52 Mb	149.53 Mb
Longest scaffold length (Mb)	249.32	N/A
Sex chromosomes	X	N/A
Organelles	Mitochondrion: 18.53 kb	N/A

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.Q58**, 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)

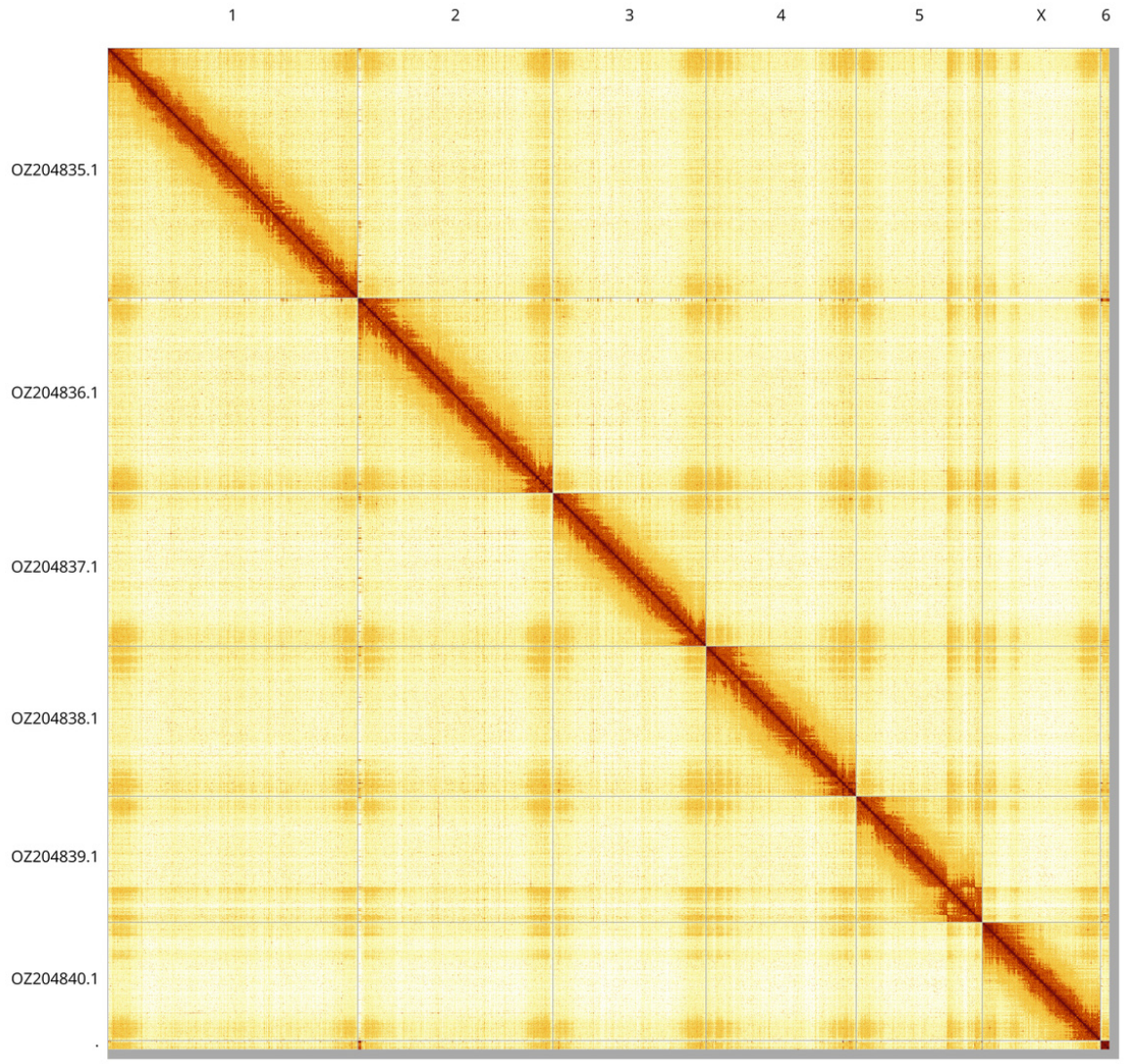

Figure 3. Hi-C contact map of the *Alydus calcaratus* genome assembly. Assembled chromosomes are shown in order of size and labelled along the axes. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Alydus calcaratus* ihAlyCalc2.

INSDC accession	Molecule	Length (Mb)	GC%
OZ204835.1	1	249.32	33
OZ204836.1	2	194.39	33.50
OZ204837.1	3	152.52	33.50
OZ204838.1	4	149.24	33.50
OZ204839.1	5	125.70	33.50
OZ204841.1	6	10.35	39
OZ204840.1	X	117.78	33

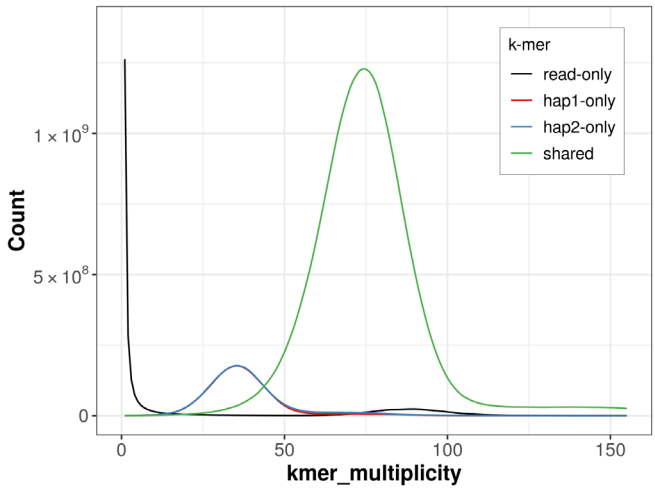

Figure 4. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers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

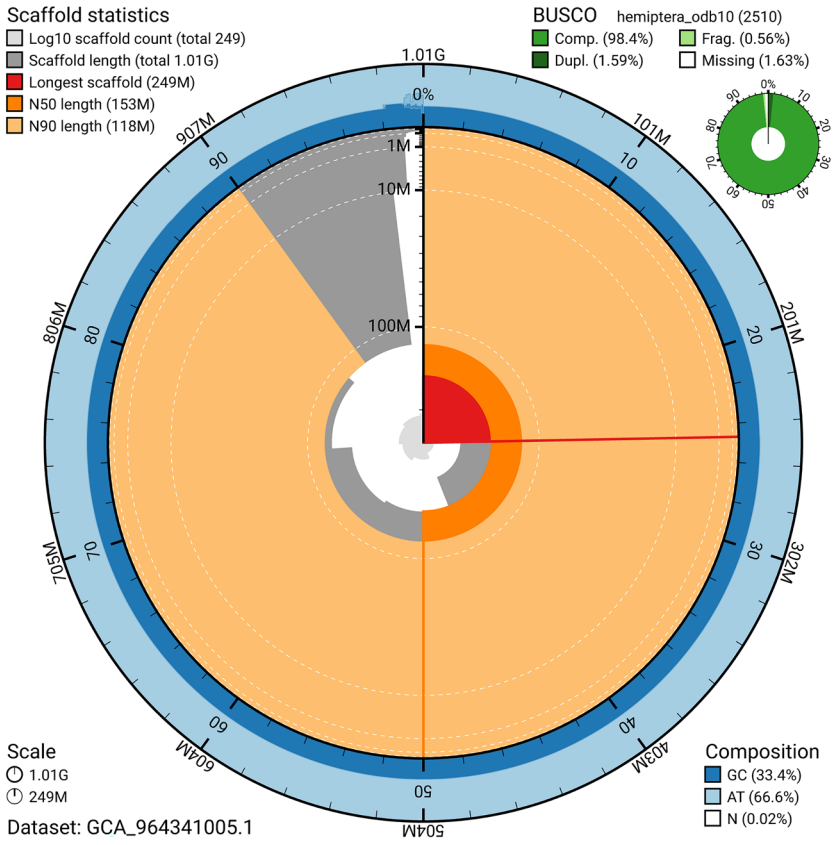

Figure 5. Assembly metrics for ihAlyCalc2.hap1.1. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1 000 bins around the circumference. The outermost 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.

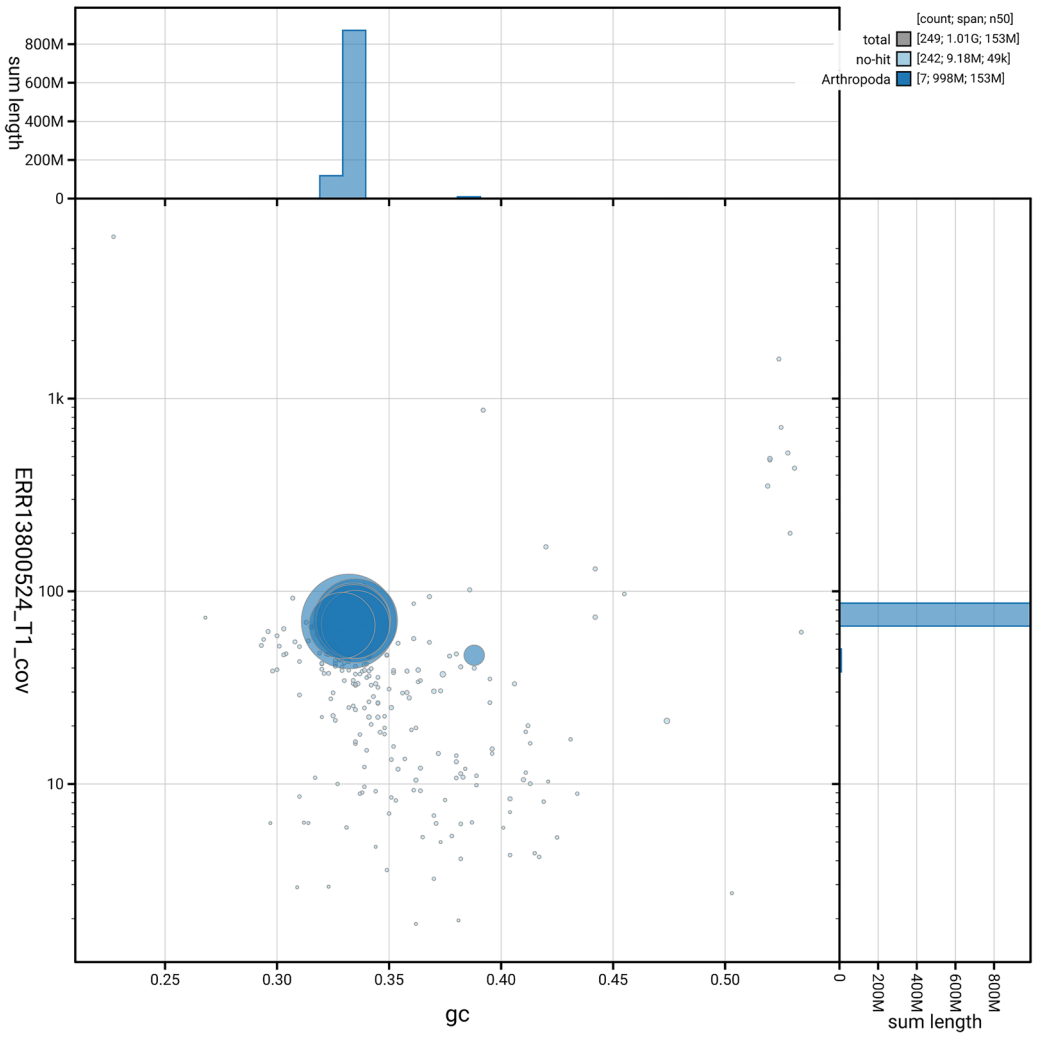

Figure 6. BlobToolKit GC-coverage plot for ihAlyCalc2.hap1.1. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes 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 *Alydus calcaratus* assembly.

Measure	Value	Benchmark
EBP summary (haplotype 1)	6.C.Q58	6.C.Q40
Contig N50 length	2.35 Mb	≥ 1 Mb
Scaffold N50 length	152.52 Mb	= chromosome N50
Consensus quality (QV)	Haplotype 1: 58.1; haplotype 2: 57.5; combined: 57.8	≥ 40
k-mer completeness	Haplotype 1: 84.22%; Haplotype 2: 84.69%; combined: 98.29%	≥ 95%
BUSCO	C:98.4% [S:96.8%; D:1.6%]; F:0.6%; M:1.1%; n:2 510	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	99.21%	≥ 90%

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: Alydus calcaratus. Accession number PRJEB80991. The genome sequence is released openly for reuse. The *Alydus calcaratus* 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 is available at https://github.com/sanger-tol. Table 5 lists software versions used in this study.

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/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	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
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	1.2a.2	https://github.com/c-zhou/yahs

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- 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

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 26 December 2025

https://doi.org/10.21956/wellcomeopenres.27451.r142905

✔ **Abdoallah Sharaf**
University of Konstanz, Konstanz, Germany

The article is scientifically sound in its current form, with no major flaws in rationale, methodology, reproducibility, or data presentation. All evaluation criteria can be confidently answered "Yes," reflecting high technical quality, methodological transparency, and strong data integrity throughout the study.

The work adheres closely to the standards and best practices of the Darwin Tree of Life (DToL) and Earth BioGenome Project (EBP) initiatives, delivering a high-quality, chromosome-level reference genome for *Alydus calcaratus*. The assembly demonstrates excellent performance metrics, including near-complete chromosomal assignment (99.21% scaffolded into 7 pseudomolecules), a scaffold N50 of 152.52 Mb, BUSCO completeness of 98.4% (Hemiptera odb10), and high consensus quality values (QV ≈ 58). These results indicate careful experimental design, robust sequencing depth, rigorous assembly curation, and thorough quality control.

The methodological description is comprehensive and reproducible, covering specimen provenance, DNA extraction, PacBio HiFi and Hi-C sequencing, assembly, curation, and validation in sufficient detail. The integration of k-mer–based analyses, BUSCO, Merqury, Hi-C contact maps, and BlobToolKit visualisations provides a transparent and convincing assessment of assembly completeness and accuracy. Identification and reporting of a heterozygous inversion further demonstrate careful manual curation rather than uncritical pipeline output.

No substantive revisions are required to support the scientific soundness or validity of the work. Overall, this genome sequence note represents a valuable and well-executed contribution to hemipteran genomics, biodiversity genomics, and the growing genomic resources generated by the Darwin Tree of Life Project.

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, and biodiversity research

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 21 November 2025

https://doi.org/10.21956/wellcomeopenres.27451.r137266

Roberto Biello
John Innes Centre Department of Crop Genetics, Norwich, England, UK

The authors present a high-quality, chromosome-level genome assembly for *Alydus calcaratus*, generated using PacBio HiFi and Hi-C sequencing technologies. This assembly, which includes both haplotypes, represents a valuable genomic resource for studying the evolution of species adapted to unique ecological niches such as coastal areas and sand dunes. The assembly meets high-quality standards, as demonstrated by high BUSCO scores and k-mer completeness. The methods are clearly described, and all associated data are publicly available.
Minor comments:
1. The manuscript reports the presence of 6 autosomes and an X sex chromosome system. Is this karyotype consistent with previously published cytogenetic data for this species or related species? Furthermore, chromosome 6 is notably small (~10 Mb) and has a higher GC content. Could the authors comment on whether this was an expected finding and its potential biological significance, if any?
2. In Figure 3, the scaffold name for the last chromosome on the y-axis appears to be missing. Please ensure all labels are present for clarity.

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: Evolutionary biology, conservation 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.