DATA NOTE

The genome sequence of the planthopper, *Conomelus anceps* (Germar, 1821) [version 1; peer review: 2 approved]

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v1 **First published:** 17 Oct 2024, **9**:605
https://doi.org/10.12688/wellcomeopenres.23104.1
Latest published: 17 Oct 2024, **9**:605
https://doi.org/10.12688/wellcomeopenres.23104.1

Abstract

We present a genome assembly from an individual male *Conomelus anceps* (planthopper; Arthropoda; Insecta; Hemiptera; Delphacidae). The genome sequence has a total length of 957.80 megabases. Most of the assembly is scaffolded into 12 chromosomal pseudomolecules, including the X sex chromosome. The mitochondrial genome has also been assembled and is 21.98 kilobases in length.

Keywords

Conomelus anceps, planthopper, genome sequence, chromosomal, Hemiptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 17 Oct 2024	✓ 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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Author roles: Moran S: Investigation, Resources; **Griffiths A**: Investigation, Resources; **Crowley LM**: Investigation, Resources;

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Wellcome through core funding to the Wellcome Sanger Institute [206194, https://doi.org/10.35802/206194] 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: Moran S, Griffiths A, Crowley LM *et al.* **The genome sequence of the planthopper, *Conomelus anceps* (Germar, 1821) [version 1; peer review: 2 approved]** Wellcome Open Research 2024, **9**:605
https://doi.org/10.12688/wellcomeopenres.23104.1

First published: 17 Oct 2024, **9**:605 https://doi.org/10.12688/wellcomeopenres.23104.1

Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Paraneoptera; Hemiptera; Auchenorrhyncha; Fulgoromorpha; Fulgoroidea; Delphacidae; Delphacinae; *Conomelus*; *Conomelus anceps* (Germar, 1821) (NCBI: txid491269).

Background

Conomelus anceps (Germar, 1821) (Figure 1) is a species of planthopper belonging to the family Delphacidae. Its global distribution extends across Europe and parts of Asia, with records from various countries in the Palaearctic region. There are also a few records from North America (GBIF Secretariat, 2024). Within the United Kingdom, *C. anceps* is commonly found throughout England, Wales, and southern Scotland, primarily in grassland habitats. They are herbivorous, feeding on the sap of *Juncus* spp. (rushes) in meadows and woodlands in damp habitats.

Adults are active from late spring to early autumn, and although small in size (up to 4 mm in length), they can be distinguished by the reddish-brown ground colour and the wings have dark spots on the veins. *C. anceps* has two wing forms: macropterous (long-winged) and brachypterous (short-winged). The macropterous form can fly and disperse, while the brachypterous form has reduced wings and limited mobility. Both forms are commonly observed, with the brachypterous form often more prevalent in some populations (British Bugs, no date).

In this data note we present a chromosomally complete genome sequence for *Conomelus anceps*, based on a specimen from Beinn Eighe National Nature Reserve, Scotland, UK.

Figure 1. Photograph of the *Conomelus anceps* (ihConAnce5) specimen used for RNA sequencing.

Genome sequence report

The genome of an adult male *Conomelus anceps* was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating a total of 27.17 Gb (gigabases) from 2.22 million reads, providing approximately 28-fold coverage. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data, which produced 119.77 Gb from 793.15 million reads, yielding an approximate coverage of 125-fold. Specimen and sequencing information is summarised in Table 1.

Manual assembly curation corrected 75 missing joins or mis-joins and one haplotypic duplications, reducing the scaffold number by 6.83%. The final assembly has a total length of 957.80 Mb in 504 sequence scaffolds with a scaffold N50 of 73.1 Mb (Table 2). The total count of gaps in the scaffolds is. The snail plot in Figure 2 provides a summary of the assembly statistics, while the distribution of assembly scaffolds on GC proportion and coverage is shown in Figure 3. The cumulative assembly plot in Figure 4 shows curves for subsets of scaffolds assigned to different phyla. Most (94.29%) of the assembly sequence was assigned to 12 chromosomal-level scaffolds, representing 11 autosomes and the X sex chromosome. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 5; Table 3). The order and orientation of contigs along Chromosome 6 is uncertain between 31.7 Mb and 42.5 Mb. While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited. The mitochondrial genome was also assembled and can be found as a contig within the multifasta file of the genome submission.

The estimated Quality Value (QV) of the final assembly is 59.2 with *k*-mer completeness of 100.0%, and the assembly has a BUSCO v5.3.2 completeness of 98.2% (single = 96.4%, duplicated = 1.8%), using the hemiptera_odb10 reference set (n = 2,510).

Metadata for specimens, BOLD barcode results, spectra estimates, sequencing runs, contaminants and pre-curation assembly statistics are given at https://links.tol.sanger.ac.uk/species/491269.

Methods

Sample acquisition

Adult specimens of *Conomelus anceps* were hand-collected from Beinn Eighe National Nature Reserve, Scotland, UK (latitude 57.63, longitude –5.35) on 2021-08-04. The specimens were collected by Stephen Moran (Highland Biological Recording Group) and Andy Griffiths (Wellcome Sanger Institute), identified by Stephen Moran, and preserved by flash freezing. The genome was assembled from PacBio HiFi data generated from one specimen (specimen ID SAN0001820, ToLID ihConAnce4). Another specimen (specimen ID SAN0001817, ToLID ihConAnce1) was used for Hi-C sequencing.

The specimen used for RNA sequencing (specimen ID Ox001994, ToLID ihConAnce5) was an adult specimen collected

Table 1. **Specimen and sequencing data for *Conomelus anceps*.**

Project information			
Study title	*Conomelus anceps*		
Umbrella BioProject	PRJEB58256		
Species	*Conomelus anceps*		
BioSample	SAMEA12997853		
NCBI taxonomy ID	491269		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	ihConAnce4	SAMEA12997912	Whole organism
Hi-C sequencing	ihConAnce1	SAMEA12997909	Whole organism
RNA sequencing	ihConAnce5	SAMEA110451719	Whole organism
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq 6000	ERR10684084	7.93e+08	119.77
PacBio Sequel IIe	ERR10688630	2.22e+06	27.17
RNA Illumina NovaSeq 6000	ERR12642421	7.05e+07	10.64
RNA Illumina NovaSeq X	ERR12861027	5.93e+07	8.96

from Wytham Woods, Oxfordshire, UK (latitude 51.77, longitude –1.34) on 2021-12-16, using a sweep net. The specimen was collected and identified by Liam Crowley (University of Oxford) and preserved on dry ice. The image in Figure 1 shows this specimen.

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 specimens and stored in ethanol, while the remaining parts of the specimen were shipped on dry ice to the Wellcome Sanger Institute (WSI). 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).

Nucleic acid extraction

The workflow for high molecular weight (HMW) DNA extraction at the WSI Tree of Life Core Laboratory includes a sequence of core procedures: sample preparation and homogenisation, DNA extraction, fragmentation and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). The ihConAnce4 sample was weighed and dissected on dry ice (Jay *et al.*, 2023) and tissue from the whole organism was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a).

HMW DNA was extracted in the WSI Scientific Operations core using the Automated MagAttract v2 protocol (Oatley *et al.*, 2023). The 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 (Strickland *et al.*, 2023). 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.

RNA was extracted from whole organism tissue of ihConAnce5 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ *mir*Vana protocol (do Amaral *et al.*, 2023). The RNA concentration was assessed using a Nanodrop spectrophotometer and a Qubit Fluorometer

Table 2. Genome assembly data for *Conomelus anceps*, ihConAnce4.1.

Genome assembly		
Assembly name	ihConAnce4.1	
Assembly accession	GCA_948455865.1	
Accession of alternate haplotype	*GCA_948455875.1*	
Span (Mb)	957.80	
Number of contigs	2,253	
Contig N50 length (Mb)	1.1	
Number of scaffolds	504	
Scaffold N50 length (Mb)	73.1	
Longest scaffold (Mb)	169.51	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	59.2	*≥ 50*
k-mer completeness	100.0%	*≥ 95%*
BUSCO**	C:98.2%[S:96.4%,D:1.8%], F:0.9%,M:0.9%,n:2,510	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	94.29%	*≥ 95%*
Sex chromosomes	X	*localised homologous pairs*
Organelles	Mitochondrial genome: 21.98 kb	*complete single alleles*

* Assembly metric benchmarks are adapted from column VGP-2020 of "Table 1: Proposed standards and metrics for defining genome assembly quality" from Rhie *et al.* (2021).

** BUSCO scores based on the hemiptera_odb10 BUSCO set using version 5.3.2.C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison. A full set of BUSCO scores is available at https://blobtoolkit.genomehubs.org/view/CAOJWK01/dataset/CAOJWK01/busco.

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.

Sequencing

Pacific Biosciences HiFi circular consensus DNA sequencing libraries were constructed according to the manufacturers' instructions. Poly(A) RNA-Seq libraries were constructed using the NEB Ultra II RNA Library Prep kit. DNA and RNA sequencing was performed by the Scientific Operations core at the WSI on Pacific Biosciences Sequel IIe (HiFi) and Illumina NovaSeq X (RNA-Seq) instruments.

Hi-C data were generated from the whole organism tissue of ihConAnce1, using the Arima-HiC v2 kit. In brief, frozen tissue (–80°C) was fixed, and the DNA crosslinked using a TC buffer containing formaldehyde. The crosslinked DNA was then digested using a restriction enzyme master mix. The 5'-overhangs were then filled in and labelled with a biotinylated nucleotide and proximally ligated. The biotinylated DNA construct was fragmented to a fragment size of 400 to 600 bp using a Covaris E220 sonicator. The DNA was then enriched, barcoded, and amplified using the NEBNext Ultra II DNA Library Prep Kit, following manufacturers' instructions. The Hi-C sequencing was performed using paired-end sequencing with a read length of 150 bp on an Illumina NovaSeq 6000 instrument.

Genome assembly, curation and evaluation

Assembly

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 were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). The contigs were further scaffolded using the provided Hi-C data (Rao *et al.*, 2014) in YaHS (Zhou *et al.*, 2023) using the --break option. 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

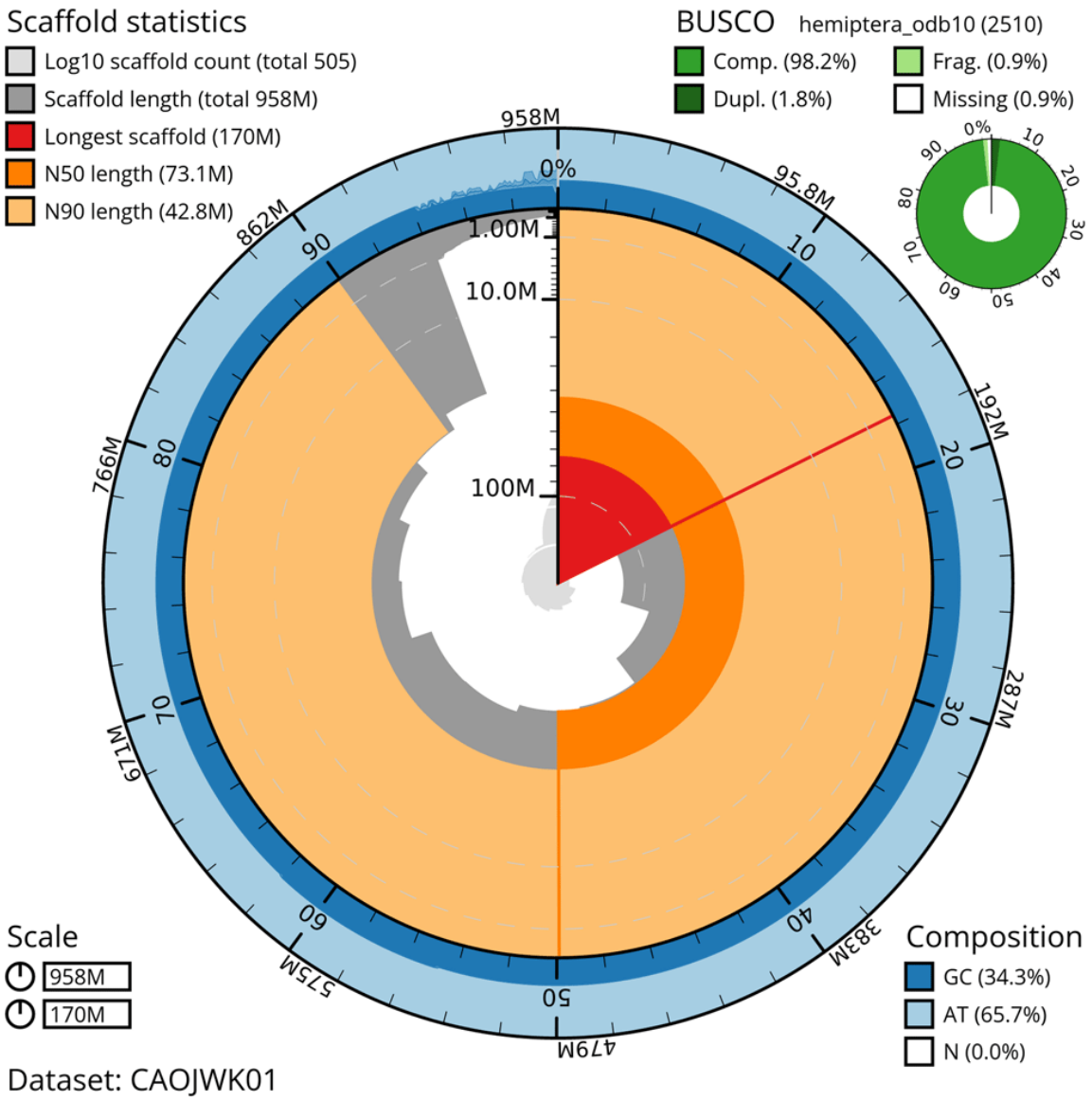

Figure 2. Genome assembly of *Conomelus anceps*, ihConAnce4.1: metrics. The BlobToolKit snail plot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 957,858,181 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (169,506,832 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (73,089,041 and 42,841,405 bp), respectively. The pale grey spiral shows the cumulative scaffold count on a log scale with white scale lines showing successive orders of magnitude. The blue and pale-blue area around the outside of the plot shows the distribution of GC, AT and N percentages in the same bins as the inner plot. A summary of complete, fragmented, duplicated and missing BUSCO genes in the hemiptera_odb10 set is shown in the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/CAOJWK01/dataset/CAOJWK01/snail.

(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 (article in preparation). Manual curation was primarily conducted using PretextView (Harry, 2022), with additional insights provided by JBrowse2 (Diesh *et al.*, 2023) and HiGlass (Kerpedjiev *et al.*, 2018). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Any identified contamination, missed joins, and mis-joins were corrected, and duplicate sequences were tagged and removed.

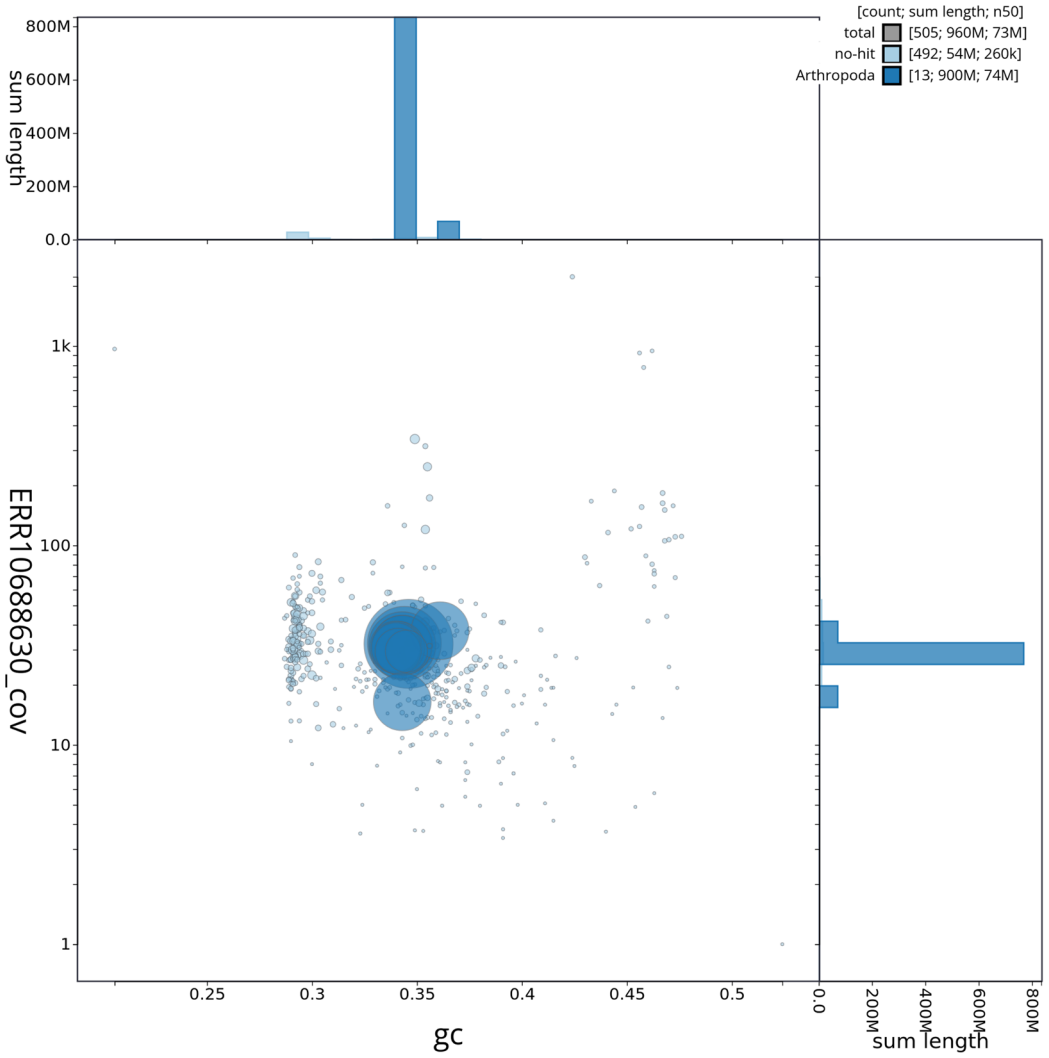

Figure 3. Genome assembly of *Conomelus anceps*, ihConAnce4.1: Blob plot of base coverage against GC proportion for sequences in the assembly. Sequences are coloured by phylum. Circles are sized in proportion to sequence length. Histograms show the distribution of sequence length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/CAOJWK01/dataset/CAOJWK01/blob.

The process is documented at https://gitlab.com/wtsi-grit/rapid-curation (article in preparation).

Evaluation of the final assembly

A Hi-C map for the final assembly was produced using bwa-mem2 (Vasimuddin *et al.*, 2019) in the Cooler file format (Abdennur & Mirny, 2020). To assess the assembly metrics, the *k*-mer completeness and QV consensus quality values were calculated in Merqury (Rhie *et al.*, 2020). This work was done using the "sanger-tol/readmapping" (Surana *et al.*, 2023a) and "sanger-tol/genomenote" (Surana *et al.*, 2023b) pipelines. The genome readmapping pipelines were developed using the nf-core tooling (Ewels *et al.*, 2020), use MultiQC (Ewels *et al.*, 2016), and make extensive use of the Conda package manager, the Bioconda initiative (Grüning *et al.*, 2018), the Biocontainers infrastructure (da Veiga Leprevost *et al.*, 2017), and the Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017) containerisation solutions. The genome was also analysed within the BlobToolKit environment (Challis *et al.*, 2020) and BUSCO scores (Manni *et al.*, 2021) were calculated.

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

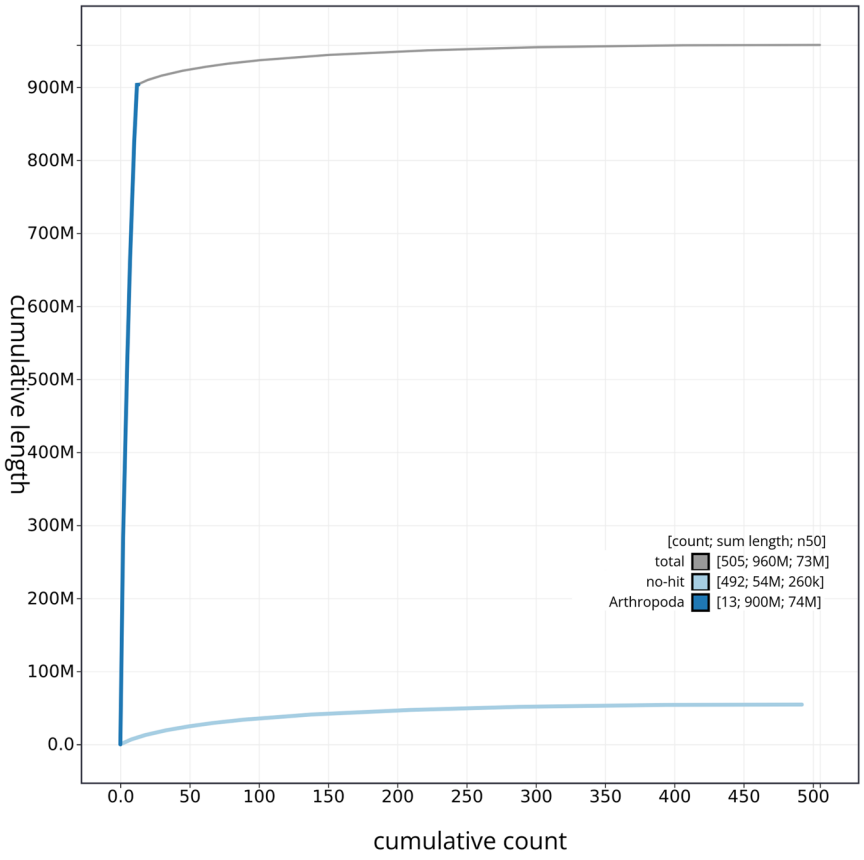

Figure 4. Genome assembly of *Conomelus anceps* ihConAnce4.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all sequences. Coloured lines show cumulative lengths of sequences assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/CAOJWK01/dataset/CAOJWK01/cumulative.

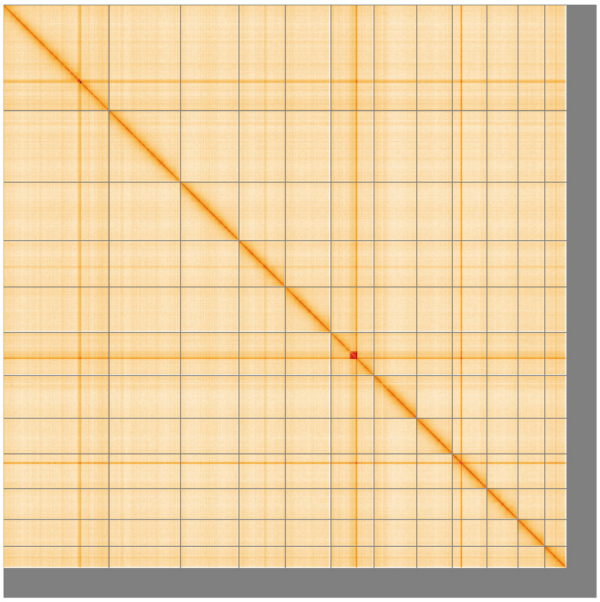

Figure 5. Genome assembly of *Conomelus anceps* ihConAnce4.1: Hi-C contact map of the ihConAnce4.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=FTonhwL0SLi-C0cN5GqwoQ.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Conomelus anceps*, ihConAnce4.

INSDC accession	Name	Length (Mb)	GC%
OX418216.1	1	169.51	34.5
OX418217.1	2	114.62	34.5
OX418218.1	3	93.62	34.5
OX418219.1	4	74.37	34.5
OX418220.1	5	73.09	34.5
OX418221.1	6	68.83	36.0
OX418223.1	7	57.1	34.0
OX418224.1	8	55.53	34.0
OX418225.1	9	49.77	34.0
OX418226.1	10	42.84	34.0
OX418227.1	11	35.43	34.5
OX418222.1	X	68.52	34.5
OX418228.1	MT	0.02	20.5

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,

Table 4. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.2.1	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	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
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
Hifiasm	0.16.1-r375	https://github.com/chhylp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Merqury.FK	d00d98157618f4e8d1a9190026b19b471055b22e	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	2	https://github.com/marcelauliano/MitoHiFi
PretextView	0.2	https://github.com/wtsi-hpag/PretextView
purge_dups	1.2.3	https://github.com/dfguan/purge_dups
sanger-tol/genomenote	v1.0	https://github.com/sanger-tol/genomenote
sanger-tol/readmapping	1.1.0	https://github.com/sanger-tol/readmapping/tree/1.1.0
Singularity	3.9.0	https://github.com/sylabs/singularity
YaHS	1.2a	https://github.com/c-zhou/yahs

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

Data availability

European Nucleotide Archive: Conomelus anceps. Accession number PRJEB58256; https://identifiers.org/ena.embl/PRJEB58256 (Wellcome Sanger Institute, 2023). The genome sequence is released openly for reuse. The *Conomelus anceps* genome sequencing initiative is part of the Darwin Tree of Life (DToL) project. 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

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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.

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 11 November 2024

https://doi.org/10.21956/wellcomeopenres.25441.r109489

✔ **Lim Li**
Universiti Sains Malaysia, George Town, Malaysia

General comment:
This manuscript provides a thorough and well-structured account of the genome sequencing of a planthopper species. The authors have successfully generated a high-quality genome assembly with extensive coverage and resolution. The methods are detailed, allowing for reproducibility.

Specific comments:
Figure 1: The insect photograph should be taken under a dissecting microscope for clarity and include an appropriate scale bar.
Genome Sequence Report Section: There is an incomplete sentence that should be revised for clarity: "The total count of gaps in the scaffolds is."

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: Molecular biology, entomology, plant pathology

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 02 November 2024

https://doi.org/10.21956/wellcomeopenres.25441.r106332

Qianquan Chen
Guizhou Normal University, Guiyang, Guizhou, China

Conomelus anceps, a species of planthopper, is a globally distributed herbivorous pest. It has two wing forms: long-winged and short-winged. Authors got a chromosomally complete genome sequence for *C. anceps*. The genome information can contribute to the study of the *C. anceps*. I advise authors to use a new photo which can clearly show the morphological characteristics of *C. anceps*.

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: Eco-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.