DATA NOTE

The genome sequence of Hornet Plumehorn hoverfly, *Volucella zonaria* (Poda, 1761)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a male specimen of *Volucella zonaria* (Hornet Hoverfly; Arthropoda; Insecta; Diptera; Syrphidae). The genome sequence has a total length of 1,262.78 megabases. Most of the assembly (89.25%) is scaffolded into 7 chromosomal pseudomolecules, including the X and Y sex chromosomes. The mitochondrial genome has also been assembled and is 15.82 kilobases in length.

Keywords

Volucella zonaria, Hornet Plumehorn hoverfly, genome sequence, chromosomal, Diptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 19 Feb 2025	✓ view	✓ view

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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; Muscomorpha; Eremoneura; Cyclorrhapha; Aschiza; Syrphoidea; Syrphidae; Eristalinae; Volucellini; *Volucella*; *Volucella zonaria* (Poda, 1761) (NCBI:txid511121)

Background

Volucella zonaria (Poda, 1761), is a large and spectacular looking hoverfly, often described as a Hornet *Vespa crabro* Linnaeus, 1758 mimic (Ball & Morris, 2024; Stubbs & Falk, 2002). The species colonised the UK during the late 1930s, establishing itself in the London area. This sparked many reports in entomological literature from the 1940s to the 1970s. *V. zonaria* is now a frequent hoverfly species across England and Wales occurring north to the central belt of Scotland (Ball & Morris, 2024) The species is the largest of all British hoverflies and is easily recognisable with yellow and black abdominal banding and a yellow face (Stubbs & Falk, 2002). Confusion is only likely with *Volucella inanis* but the two can be separated through the colouring of tergite 2 which is yellow in *V. inanis* and chestnut in *V. zonaria*. A further key distinguishing characteristic is the variation in their abdominal undersides. *V. zonaria* hoverflies feature broad black bars, whereas the tergites of *V. inanis* are predominantly yellow (Ball & Morris, 2024; Stubbs & Falk, 2002; van Veen, 2014).

Larvae of the species inhabit social wasp nests, particularly those of the common wasp *Vespula vulgaris* and the German wasp *V. germanica*, where they scavenge debris at the nest's base (Ball & Morris, 2024; Rotheray, 1993). Consequently, *V. zonaria* records increase notably in the year following a summer with high wasp abundance (Stubbs & Falk, 2002).

Adults can be found from May to November with a peak during August (Stubbs & Falk, 2002). *V. zonaria* are frequently observed in urban and suburban areas and the species is considered to be a highly migratory species, with its presence strongly correlated to anthropogenic activities (Ball & Morris, 2000; van Veen, 2014). Adults are regular flower visitors and display a noted preference for *Buddleja* (Butterfly bush) plants (Ball & Morris, 2024; van Veen, 2014).

The chromosomally complete reference genome for *Volucella zonaria* as part of the Darwin Tree of Life Project provides an exciting research tool for further exploration of this captivating hoverfly species.

Genome sequence report

Sequencing data

The genome of a specimen of *Volucella zonaria* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 52.39 Gb from 4.21 million reads. Based on the genome size estimated in GenomeScope, the sequencing data provided approximately 38.0x coverage of the genome. Hi-C sequencing produced 120.31 Gb from 796.73 million reads.

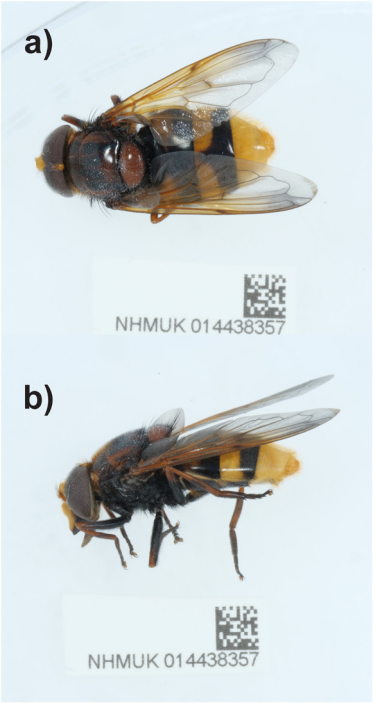

Figure 1. Photograph of the *Volucella zonaria* (idVolZona1) specimen used for genome sequencing.

Table 1 summarises the specimen and sequencing information, including the BioProject, study name, BioSample numbers, and sequencing data for each technology.

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 11 misjoins or missing joins and removed one haplotypic duplication. These interventions reduced the total assembly length by 5.08% and decreased the scaffold N50 by 3.48%. The final assembly has a total length of 1,262.78 Mb in 858 scaffolds, with 437 gaps, and a scaffold N50 of 201.04 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 (88.62%) was assigned to 7 chromosomal-level scaffolds, representing 5 autosomes and the X and Y sex chromosome. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 5; Table 3). During curation, chromosomes X and Y were assigned by analysing read coverage statistics.

Table 1. **Specimen and sequencing data for *Volucella zonaria*.**

Project information			
Study title	Volucella zonaria		
Umbrella BioProject	PRJEB71657		
Species	*Volucella zonaria*		
BioSpecimen	SAMEA11025132		
NCBI taxonomy ID	511121		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	idVolZona1	SAMEA11025419	thorax
Hi-C sequencing	idVolZona1	SAMEA11025420	head
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq 6000	ERR12411015	7.97e+08	120.31
PacBio Sequel IIe	ERR12408802	1.93e+06	22.56
PacBio Sequel IIe	ERR12408803	2.28e+06	29.83

Table 2. **Genome assembly data for *Volucella zonaria*.**

Genome assembly		
Assembly name	idVolZona1.1	
Assembly accession	GCA_963966105.1	
Alternate haplotype accession	*GCA_963966115.1*	
Assembly level for primary assembly	chromosome	
Span (Mb)	1,262.78	
Number of contigs	1,295	
Number of scaffolds	858	
Longest scaffold (Mb)	368.25	
Assembly metric	**Measure**	***Benchmark***
Contig N50 length	3.79 Mb	*≥ 1 Mb*
Scaffold N50 length	201.04 Mb	*= chromosome N50*
Consensus quality (QV)	Primary: 61.7; alternate: 61.1; combined 61.3	*≥ 40*
k-mer completeness	Primary: 69.77%; alternate: 74.82%; combined: 99.21%	*≥ 95%*
BUSCO*	C:90.6%[S:89.4%,D:1.2%], F:0.8%,M:8.6%,n:3,285	*S > 90%, D < 5%*
Percentage of assembly mapped to chromosomes	88.62%	*≥ 90%*
Sex chromosomes	X and Y	*localised homologous pairs*
Organelles	Mitochondrial genome: 15.82 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.

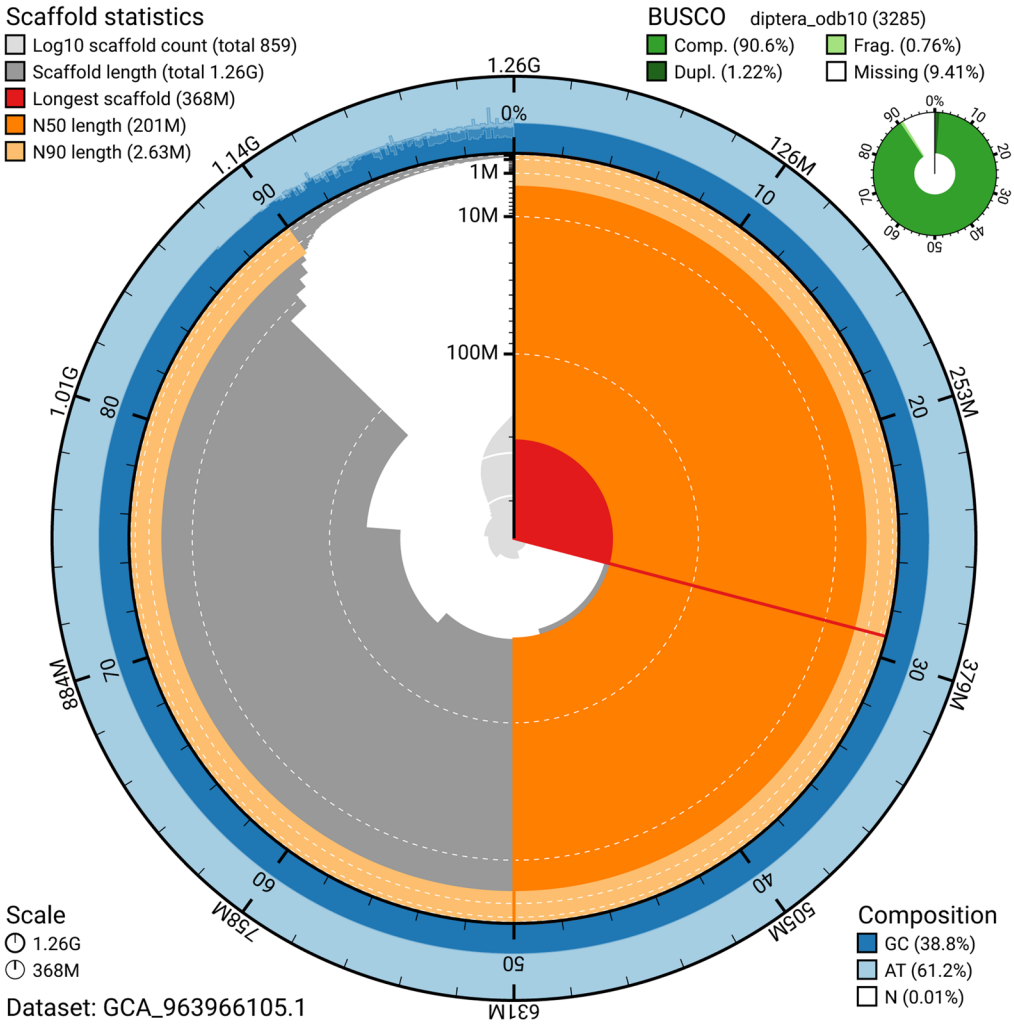

Figure 2. Genome assembly of *Volucella zonaria* idVolZona1.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. 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_963966105.1/dataset/GCA_963966105.1/snail.

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 in GenBank.

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 primary haplotype has a QV of 61.7, and the combined primary and alternate assemblies achieve an estimated QV of 61.3. The *k*-mer completeness for the primary haplotype is 69.77%, and for the alternate haplotype it is 74.82%. The combined primary and alternate assemblies achieve a *k*-mer completeness of 99.21%. BUSCO analysis using the diptera_odb10 reference set (n = 3,285) indicated a completeness score of 90.6% (single = 89.4%, duplicated = 1.2%).

Table 2 provides assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project Report on Assembly Standards September 2024. The assembly achieves the Earth Biogenome Project (EBP) reference standard of **6.8.Q61**.

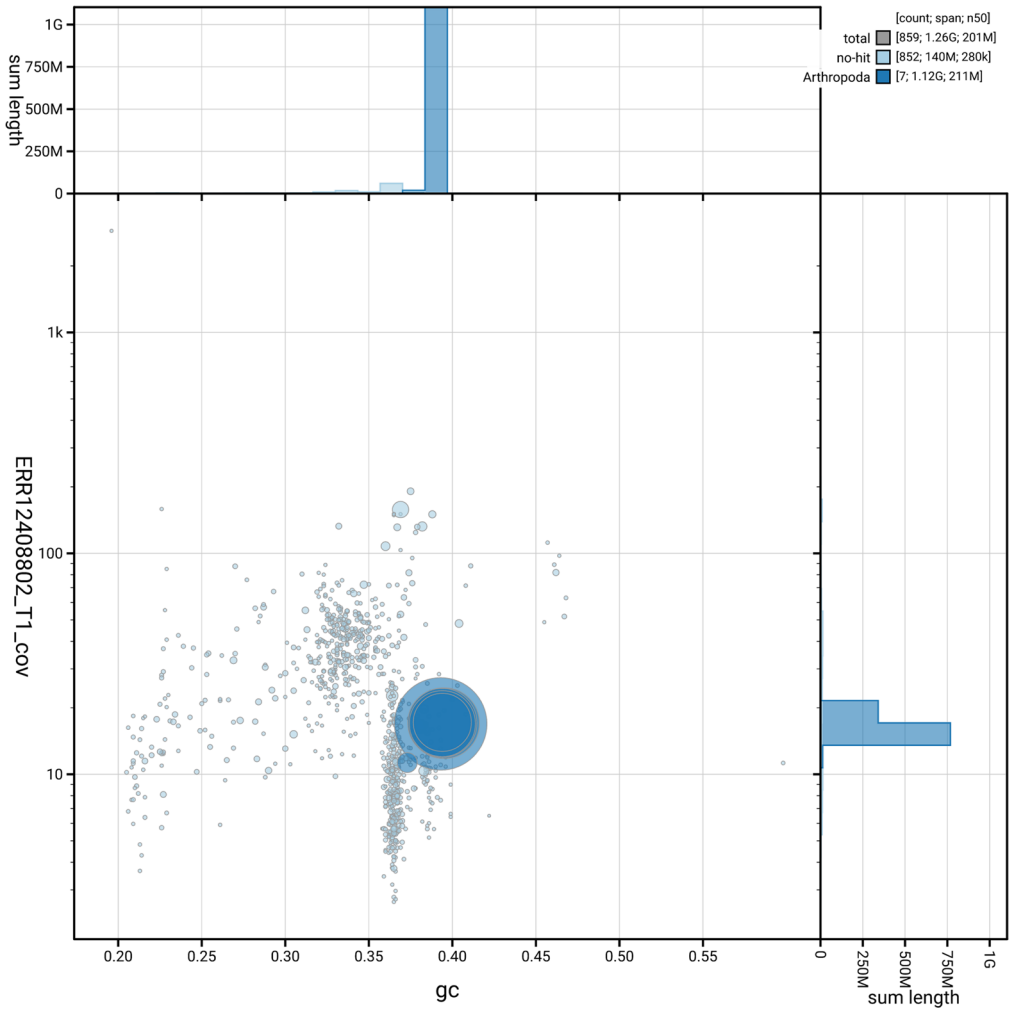

Figure 3. Genome assembly of *Volucella zonaria* idVolZona1.1: BlobToolKit GC-coverage plot. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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_963966105.1/blob.

Methods

Sample acquisition and DNA barcoding

A male *Volucella zonaria* (specimen ID NHMUK014438357, ToLID idVolZona1) was collected from Newquay SW842581 (50.384, –5.04) on 2021-07-01, using an aerial net. The specimen was collected by Robert Wolton (Dipterists Forum), identified by Olga Sivell (Natural History Museum) and preserved by dry freezing (–80 °C).

The initial identification by Morphology 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. Detailed protocols are available on

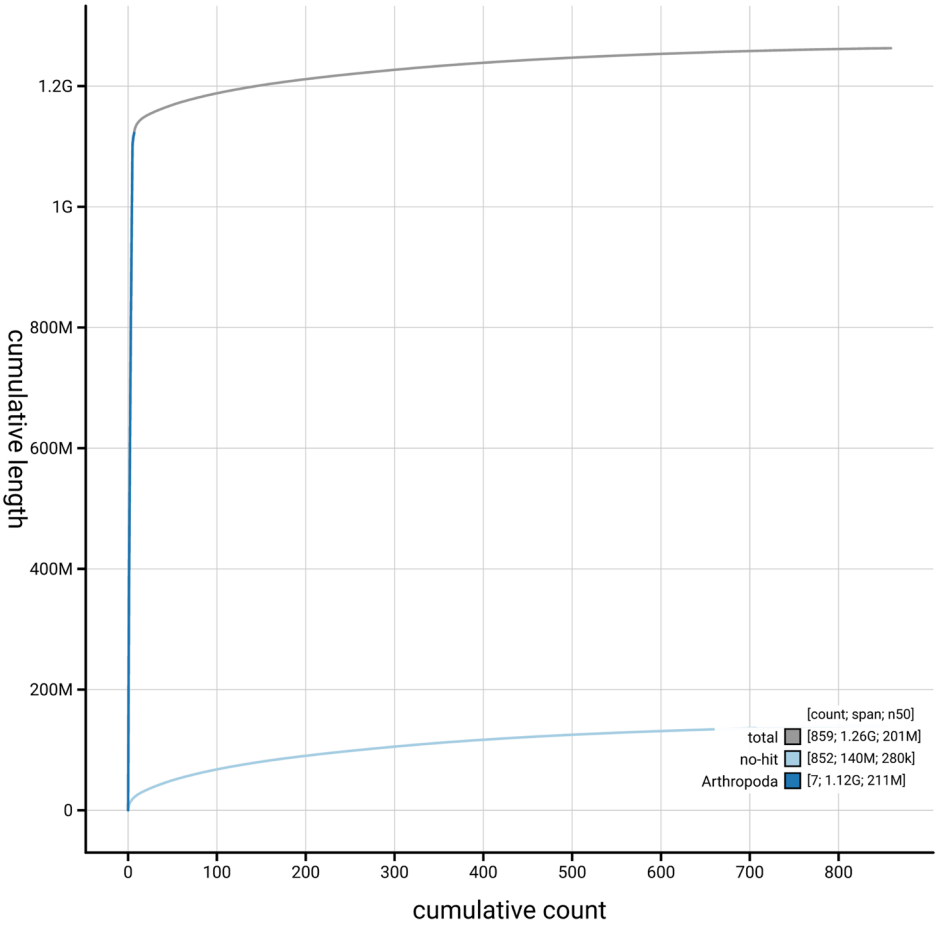

Figure 4. Genome assembly of *Volucella zonaria* idVolZona1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963966105.1/dataset/GCA_963966105.1/cumulative.

protocols.io (Denton *et al.*, 2023b). The idVolZona1 sample was prepared for DNA extraction by weighing and dissecting it on dry ice (Jay *et al.*, 2023). Tissue from the thorax was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a).

HMW DNA was extracted using the Automated MagAttract v2 protocol (Oatley *et al.*, 2023a). DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system (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 using a Nanodrop spectrophotometer and Qubit Fluorometer using the Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

Hi-C sample preparation

Tissue from the head of the idVolZona1 sample was processed for Hi-C sequencing at the WSI Scientific Operations core, using the Arima-HiC v2 kit. In brief, 20–50 mg of frozen tissue (stored at –80 °C) was fixed, and the DNA crosslinked using a TC buffer with 22% formaldehyde concentration. After crosslinking, the tissue was homogenised using the Diagnocine Power Masher-II and BioMasher-II tubes and pestles. Following the Arima-HiC v2 kit manufacturer's instructions, crosslinked DNA was digested using a restriction enzyme master mix. The 5'-overhangs were filled in and labelled with biotinylated nucleotides and proximally ligated. An overnight incubation was carried out for enzymes to digest remaining proteins and for crosslinks to reverse. A clean up was performed with SPRIselect beads prior to library preparation. Additionally, the biotinylation percentage was estimated using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and Qubit HS Assay Kit and Arima-HiC v2 QC beads.

Library preparation and sequencing

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

PacBio HiFi

At a minimum, samples were required to have an average fragment size exceeding 8 kb and a total mass over 400 ng to

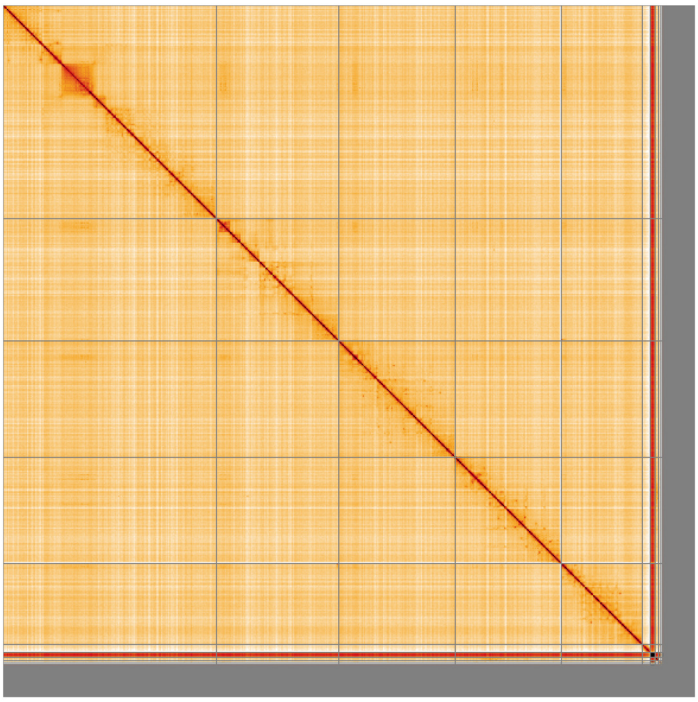

Figure 5. Genome assembly of *Volucella zonaria:* Hi-C contact map of the idVolZona1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=awO1KOTISvKLO73n_ValvA.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Volucella zonaria*, idVolZona1.

INSDC accession	Name	Length (Mb)	GC%
OZ014530.1	1	368.25	39.5
OZ014531.1	2	210.86	39.5
OZ014532.1	3	201.04	39.5
OZ014533.1	4	182.93	39.5
OZ014534.1	5	139.91	39.5
OZ014535.1	X	13.49	37.5
OZ014536.1	Y	2.63	38.5
OZ014537.1	MT	0.02	19.5

proceed to the low input SMRTbell Prep Kit 3.0 protocol (Pacific Biosciences, California, USA), depending on genome size and sequencing depth required. Libraries were prepared using the SMRTbell Prep Kit 3.0 (Pacific Biosciences, California, USA) as per the manufacturer's instructions. The kit includes the reagents required for end repair/A-tailing, adapter ligation, post-ligation SMRTbell bead cleanup, and nuclease treatment. Following the manufacturer's instructions, size selection and clean up was carried out using diluted AMPure PB beads (Pacific Biosciences, California, USA). DNA concentration was quantified using the Qubit Fluorometer v4.0 (Thermo Fisher 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 gDNA 55kb BAC analysis kit.

Samples were sequenced using the Sequel IIe system (Pacific Biosciences, California, USA). The concentration of the library loaded onto the Sequel IIe was in the range 40–135 pM. The SMRT link software, a PacBio web-based end-to-end 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, DNA was fragmented using the Covaris E220 sonicator (Covaris) and size selected using SPRISelect beads to 400 to 600 bp. The DNA was then enriched using the Arima-HiC v2 kit Enrichment beads. Using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) for end repair, a-tailing, and adapter ligation. This uses a custom protocol which resembles the standard NEBNext Ultra II DNA Library Prep protocol but where library preparation occurs while DNA is bound to the Enrichment beads. For library

amplification, 10 to 16 PCR cycles were required, determined by the sample biotinylation percentage. 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

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 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 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 (article in preparation). Flat files and maps used in curation were generated in TreeVal (Pointon *et al.*, 2023). 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. Sex chromosomes were identified by read coverage statistics. The curation process is documented at https://gitlab.com/wtsi-grit/rapid-curation (article in preparation).

Assembly quality assessment

The Merqury.FK tool (Rhie *et al.*, 2020), run in a Singularity container (Kurtzer *et al.*, 2017), was used to evaluate *k*-mer completeness and assembly quality for the primary and alternate haplotypes using the *k*-mer databases (k = 31) that were computed prior to genome assembly. The analysis outputs included assembly QV scores and completeness statistics.

A Hi-C contact map was produced for the final version of the assembly. The Hi-C reads were aligned using bwa-mem2 (Vasimuddin *et al.*, 2019) and the alignment files were combined using SAMtools (Danecek *et al.*, 2021). The Hi-C alignments were converted into a contact map using BEDTools (Quinlan & Hall, 2010) and the Cooler tool suite (Abdennur & Mirny, 2020). The contact map is visualised in HiGlass (Kerpedjiev *et al.*, 2018).

The blobtoolkit pipeline is a Nextflow port of the previous Snakemake Blobtoolkit pipeline (Challis *et al.*, 2020). It aligns the PacBio reads in SAMtools and minimap2 (Li, 2018) and generates coverage tracks for regions of fixed size. In parallel, it queries the GoaT database (Challis *et al.*, 2023) to identify all matching BUSCO lineages to run BUSCO (Manni *et al.*, 2021). For the three domain-level BUSCO lineages, the pipeline aligns the BUSCO genes to the UniProt Reference Proteomes database (Bateman *et al.*, 2023) with DIAMOND blastp (Buchfink *et al.*, 2021). The genome is also divided into chunks according to the density of the BUSCO genes from the closest taxonomic lineage, and each chunk is aligned to the UniProt Reference Proteomes database using DIAMOND blastx. Genome sequences without a hit are chunked using seqtk and aligned to the NT database with blastn (Altschul *et al.*, 1990). The blobtools suite combines all these outputs into a blobdir for visualisation.

The blobtoolkit pipeline was developed using nf-core tooling (Ewels *et al.*, 2020) and MultiQC (Ewels *et al.*, 2016), relying on the Conda package manager, the Bioconda initiative (Grüning *et al.*, 2018), the Biocontainers infrastructure (da Veiga Leprevost *et al.*, 2017), as well as the Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017) containerisation solutions.

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

Wellcome Sanger Institute – Legal and Governance

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

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)

Table 4. Software tools: versions and sources.

Software tool	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	427104ea91c78c3b8b8b49f1a7d6bbeaa869ba1c	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	0.19.5-r587	https://github.com/chhylp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84a a44357826c0b6753eb28de	https://github.com/higlass/higlass
Merqury.FK	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
NCBI Datasets	15.12.0	https://github.com/ncbi/datasets
Nextflow	23.04.1	https://github.com/nextflow-io/nextflow
PretextView	0.2	https://github.com/sanger-tol/PretextView
purge_dups	1.2.5	https://github.com/dfguan/purge_dups
samtools	1.19.2	https://github.com/samtools/samtools
sanger-tol/ascc	-	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	0.5.1	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

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: Volucella zonaria. Accession number PRJEB71657; https://identifiers.org/ena.embl/PRJEB71657. The genome sequence is released openly for reuse. The *Volucella zonaria* 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.

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Open Peer Review

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Version 1

Reviewer Report 03 June 2025

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✔ **Jonathan Wilson**
The University of Melbourne, Melbourne, Victoria, Australia
Nina Wedell
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Wolton *et al.* have reported a well assembled chromosome-level reference genome for *Volucella zonaria*. This assembly captures the majority of the genome within 5 autosomes and 2 sex chromosomes. They have summarised the results of the genome assembly nicely, and there are no obvious issues with any methods or findings that could detrimentally affect subsequent genomic analyses involving *V. zonaria*. I have some minor comments about metrics to report, and possible additional analyses that may be performed in future improvements to this genome assembly.

1. If there are any previous estimates of chromosome number available, it would be worth mentioning them (though the 7 pseudomolecules seem very likely to represent the true chromosome number). It would also help to state the genome size estimate (from GenomeScope2) explicitly in text – is it the 1262.78 Mb in Table 2?
2. The BUSCO results you have reported look adequate, however the similarity between the BUSCO completeness percentage (90.6%) and the percentage of assembly mapped to the chromosomes is interesting. Have you run the BUSCO analysis exclusively on the chromosomes to see how many BUSCOs are on the chromosomes? While not of huge importance, this might provide a better picture of how complete each chromosome is and could be important information for downstream analyses. Also, it is possible that some missing BUSCOs may lie within the alternate contigs. Including the alternate assembly in a subsequent BUSCO run could indicate whether some genes are missing only on the primary haplotype.
3. Finally, in future it would be worth further investigating the alternate assembly. It would be helpful to report metrics from the alternate haplotype, especially given the k-mer completeness appears to be higher in the alternate assembly (Table 2). Also, aligning the alternate contigs to the primary assembly would be interesting. This could help in efforts to

understand structural variation within this species and future efforts to create a pangenome for this species and/or its relatives and may also resolve some of the missing BUSCOs.

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, bioinformatics, genomics, population genetics

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 26 February 2025

https://doi.org/10.21956/wellcomeopenres.26186.r119132

Bhagya C. Thimmappa
USDA Agricultural Research Service, Chatsworth, NJ, USA

In this work, the authors report a chromosomal-level assembly of the Hornet Plumehorn hoverfly, *Volucella zonaria* (Poda, 1761). The report is concise and well-written, with a sufficient introduction and a detailed method section. The nuclear and mitochondrial genome assemblies are of good quality, and with manual curation, the authors improved the assembly by correcting 11 misjoins or missing joins and removing one haplotypic duplication.

Comments:

- The annotation of the nuclear and mitochondrial genomes would further increase the value of these datasets.
- It is mentioned that the nuclear genome will be annotated using RNA-Seq data. When is this expected to be completed? How will the annotated genome be made accessible once it is finished? How many RNA-Seq datasets will be used, and where are these datasets from? Are they publicly available?

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, Trasnscriptomics, Bioinformatics, Multi-Omics analysis

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.