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DATA NOTE

The genome sequence of the Vestal Cuckoo Bee, *Bombus vestalis* (Geoffroy, 1785)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a haploid male specimen of *Bombus vestalis* (Vestal Cuckoo Bee; Arthropoda; Insecta; Hymenoptera; Apidae). The genome sequence has a total length of 280.01 megabases. Most of the assembly (91.96%) is scaffolded into 25 chromosomal pseudomolecules. The mitochondrial genome has also been assembled and is 24.37 kilobases in length. Gene annotation of this assembly on Ensembl identified 11,600 protein-coding genes.

Keywords

Bombus vestalis, Vestal Cuckoo Bee, genome sequence, chromosomal, Hymenoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 25 Apr 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; Hymenoptera; Apocrita; Aculeata; Apoidea; Anthophila; Apidae; Apinae; Bombini; *Bombus*; *Psithyrus*; *Bombus vestalis* (Geoffroy, 1785) (NCBI:txid30202)

Background

Bombus vestalis (Geoffroy, 1785), commonly known as the vestal cuckoo bee, is a social parasite of *Bombus terrestris* (Edwards, 2020). It belongs to the subgenus *Psithyrus* within *Bombus*, which was previously classified as a separate genus. This species is widely distributed across England, Wales, and southeastern Scotland, with a range extending into much of Europe and parts of Asia, though it is scarce in Scandinavia.

Females emerge from hibernation in April or May and seek out small *B. terrestris* nests. After infiltrating a host colony, the female adopts its scent, may kill or dominate the host queen, and takes over egg-laying. The colony then produces only *B. vestalis* males and females, while all worker activity is carried out by the host species (Edwards, 2020).

Identification can be challenging due to its similarity to *Bombus bohemicus*. Both species have a black body and a white tail with yellow patches at its base, but *B. vestalis* typically has more intense and extensive yellow markings (Edwards, 2020). Reliable separation requires microscopic examination of tergite puncturation. Males are sleek, with elongated antennae, and often display a weak yellow band on tergite 1 (Falk, no date).

Bombus vestalis occurs in a wide range of habitats, mirroring its host's distribution. It visits a diverse range of flowering plants, including sallow (*Salix*), white dead-nettle (*Lamium album*), dandelions (*Taraxacum*), ground-ivy (*Glechoma hederacea*), thistles (*Cirsium*), brambles (*Rubus*), knapweeds (*Centaurea*), umbellifers, and garden plants such as lavenders (*Lavandula*) (Falk, no date).

Although most common in southern Britain, it may be expanding its range northward, possibly in response to climate change (Falk, no date). We present a chromosome-level genome sequence for *Bombus vestalis*, based on a male specimen from Wytham Woods, Oxfordshire, United Kingdom (Figure 1).

Genome sequence report
Sequencing data

The genome of a specimen of *Bombus vestalis* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 31.11 Gb (gigabases) from 2.38 million reads. GenomeScope analysis of the PacBio HiFi data estimated the haploid genome size at 263.27 Mb, with a heterozygosity of 0.02% and repeat content of 13.31%. These values provide an initial assessment of genome complexity and the challenges anticipated during assembly. Based on this estimated genome size, the sequencing data provided

Figure 1. Photograph of the *Bombus vestalis* (iyBomVest1) specimen used for genome sequencing.

approximately 111.0x coverage of the genome. Chromosome conformation Hi-C sequencing produced 135.03 Gb from 894.26 million reads. Table 1 summarises the specimen and sequencing information, including the BioProject, study name, BioSample numbers, and sequencing data for each technology.

Assembly statistics

The specimen is a haploid male, and a single haplotype was assembled. The assembly was improved by manual curation, which corrected 50 misjoins or missing joins. These interventions decreased the scaffold count by 4.51% and increased the scaffold N50 by 5.43%. The final assembly has a total length of 280.01 Mb in 232 scaffolds, with 99 gaps, and a scaffold N50 of 11.16 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 (91.96%) was assigned to 25 chromosomal-level scaffolds. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 5; Table 3).

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

Table 1. Specimen and sequencing data for *Bombus vestalis*.

Project information			
Study title	Bombus vestalis (vestal cuckoo bee)		
Umbrella BioProject	PRJEB61036		
Species	*Bombus vestalis*		
BioSpecimen	SAMEA7520656		
NCBI taxonomy ID	30202		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	iyBomVest1	SAMEA7520733	abdomen
Hi-C sequencing	iyBomVest1	SAMEA7520732	head and thorax
RNA sequencing	iyBomVest1	SAMEA7520733	abdomen
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C HiSeq X Ten	ERR11182526	8.94e+08	135.03
PacBio Sequel IIe	ERR11180451	2.38e+06	31.11
RNA Illumina NovaSeq 6000	ERR11242536	7.86e+07	11.87

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 65.3. BUSCO analysis using the hymenoptera_odb10 reference set (n = 5,991) indicated a completeness score of 97.6% (single = 97.3%, duplicated = 0.3%).

Table 2 provides assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project (EBP) Report on Assembly Standards September 2024. The assembly achieves the EBP reference standard of **6.C.Q65**.

Genome annotation report
The *Bombus vestalis* genome assembly (GCA_963556215.1) was annotated at the European Bioinformatics Institute (EBI) on Ensembl Rapid Release. The resulting annotation includes 31,400 transcribed mRNAs from 11,600 protein-coding and 4,683 non-coding genes (Table 2; https://rapid.ensembl.org/Bombus_vestalis_GCA_963556215.1/Info/Index). The average transcript length is 11,516.57. There are 1.93 coding transcripts per gene and 6.15 exons per transcript.

Methods
Sample acquisition and DNA barcoding
An adult male *Bombus vestalis* (specimen ID Ox000435, ToLID iyBomVest1) was collected from Wytham Woods, Oxfordshire, United Kingdom (latitude 51.78, longitude –1.34) on 2020-06-01 by netting. The specimen was collected and identified by Liam Crowley (University of Oxford) and preserved on dry ice.

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. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). The iyBomVest1 sample was

Table 2. Genome assembly data for *Bombus vestalis*.

Genome assembly		
Assembly name	iyBomVest1.1	
Assembly accession	GCA_963556215.1	
Assembly level for primary assembly	chromosome	
Span (Mb)	280.01	
Number of contigs	331	
Number of scaffolds	232	
Longest scaffold (Mb)	15.65	
Assembly metric	**Measure**	**Benchmark**
Contig N50 length	3.43 Mb	≥ 1 Mb
Scaffold N50 length	11.16 Mb	= chromosome N50
Consensus quality (QV)	65.3	≥ 40
BUSCO*	C:97.6%[S:97.3%,D:0.3%], F:0.5%,M:1.9%,n:5,991	S > 90%; D < 5%
Percentage of assembly mapped to chromosomes	91.96%	≥ 90%
Sex chromosomes	None	localised homologous pairs
Organelles	Mitochondrial genome: 24.37 kb	complete single alleles
Genome annotation of assembly GCA_963556215.1 at Ensembl		
Number of protein-coding genes	11,600	
Number of non-coding genes	4,683	
Number of gene transcripts	31,400	

* BUSCO scores based on the hymenoptera_odb10 BUSCO set using version 5.4.3. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison.

prepared for DNA extraction by weighing and dissecting it on dry ice (Jay *et al.*, 2023). Tissue from the abdomen was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a). HMW DNA was extracted using the Automated MagAttract v1 protocol (Sheerin *et al.*, 2023). DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system (Todorovic *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 a Qubit Fluorometer using the Qubit dsDNA High Sensitivity Assay kit. The fragment size distribution was evaluated by running the sample on the FemtoPulse system.

Hi-C sample preparation

Tissue from the head and thorax of the 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 proceed to the low input SMRTbell Prep Kit 3.0 protocol
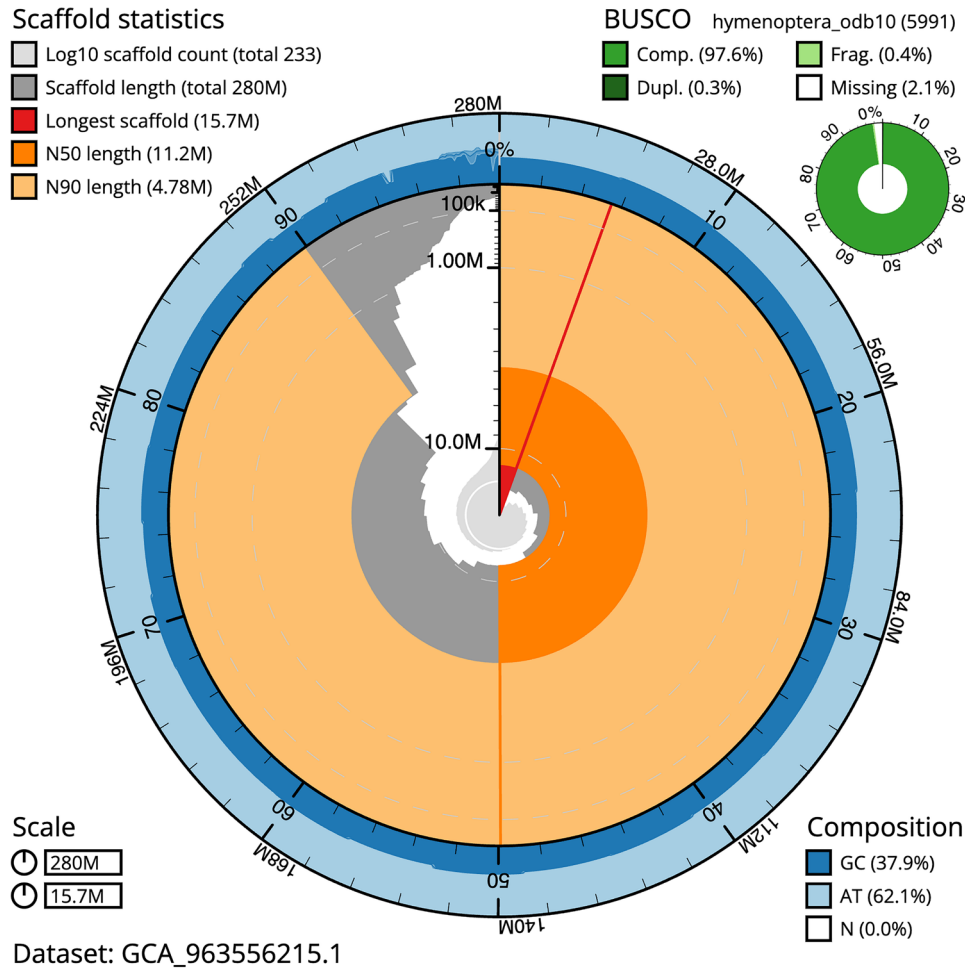
Figure 2. Genome assembly of *Bombus vestalis*, iyBomVest1.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. 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 hympenoptera_odb10 set is presented at the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963556215.1/dataset/GCA_963556215.1/snail.

(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.
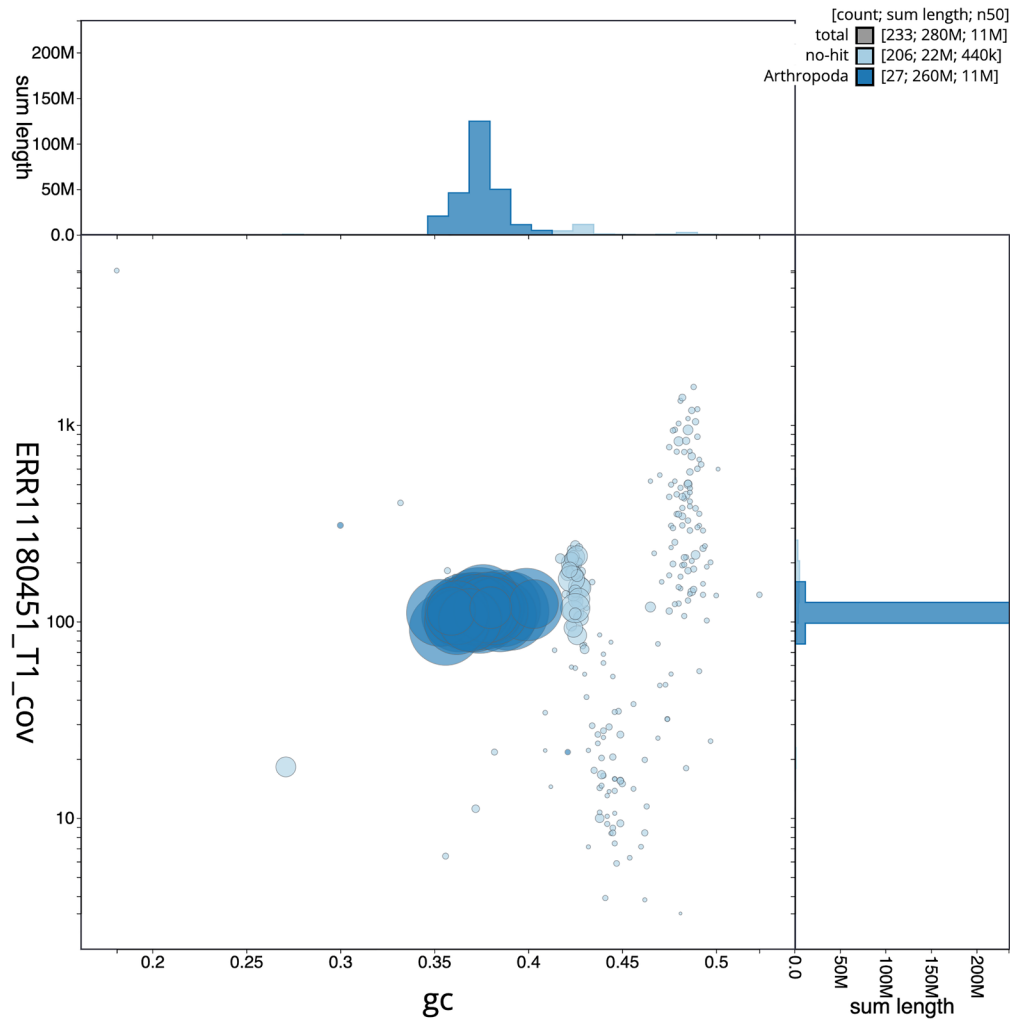
Figure 3. Genome assembly of *Bombus vestalis*, iyBomVest1.1: BlobToolKit GC-coverage plot. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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_963556215.1/blob.

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 HiSeq X Ten 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
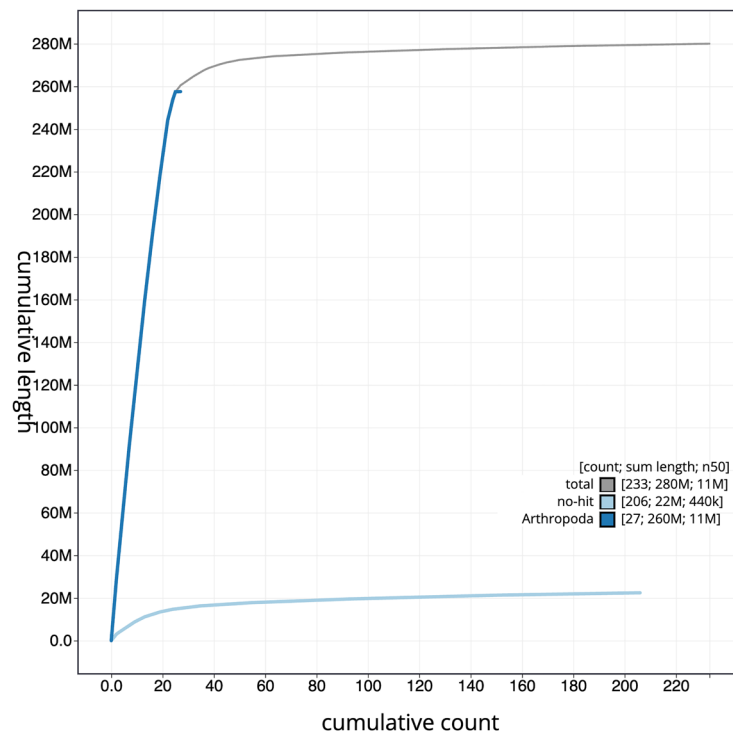
Figure 4. Genome assembly of *Bombus vestalis*, iyBomVest1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963556215.1/dataset/GCA_963556215.1/cumulative.
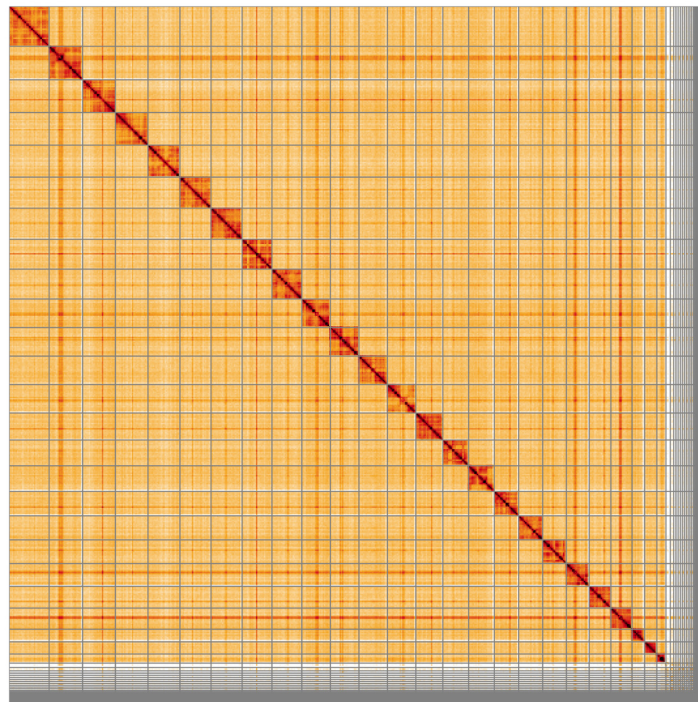

Figure 5. Genome assembly of *Bombus vestalis*: Hi-C contact map of the iyBomVest1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=Bzh3gCT-Q1WUzcNNt62flg.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Bombus vestalis*, iyBomVest1.

INSDC accession	Name	Length (Mb)	GC%
OY744576.1	1	15.65	37.5
OY744577.1	2	13.14	39
OY744578.1	3	12.83	37
OY744579.1	4	12.79	37
OY744580.1	5	12.55	38.5
OY744581.1	6	12.18	36.5
OY744582.1	7	12.16	37
OY744583.1	8	11.72	37.5
OY744584.1	9	11.69	36.5
OY744585.1	10	11.22	38.5
OY744586.1	11	11.21	37.5
OY744587.1	12	11.16	40
OY744588.1	13	11.12	35.5
OY744589.1	14	10.59	38
OY744590.1	15	10.09	37.5
OY744591.1	16	10.09	37
OY744592.1	17	9.52	38
OY744593.1	18	9.45	35.5
OY744594.1	19	9.27	38
OY744595.1	20	8.92	36
OY744596.1	21	8.54	36
OY744597.1	22	8.3	37
OY744598.1	23	4.94	40.5
OY744599.1	24	4.78	36
OY744600.1	25	3.56	38
OY744601.1	MT	0.02	18.5

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. 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 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 was 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.

Genome annotation

The Ensembl Genebuild annotation system (Aken *et al.*, 2016) was used to generate annotation for the *Bombus vestalis* assembly (GCA_963556215.1) in Ensembl Rapid Release at the EBI. Annotation was created primarily through alignment of transcriptomic data to the genome, with gap filling via protein-to-genome alignments of a select set of proteins from UniProt (UniProt Consortium, 2019).

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

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	4.3.3	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.4.3	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	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	https://github.com/chhylp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84aa44357826c0b6753eb28de	https://github.com/higlass/higlass
MerquryFK	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	23.04.1	https://github.com/nextflow-io/nextflow
PretextView	0.2	https://github.com/sanger-tol/PretextView
samtools	1.18	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	1.1a.2	https://github.com/c-zhou/yahs

Partner agrees they will meet the legal and ethical requirements nd 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.

Data availability
European Nucleotide Archive: Bombus vestalis (vestal cuckoo bee). Accession number PRJEB61036; https://identifiers.org/ena.embl/PRJEB61036. The genome sequence is released openly for reuse. The *Bombus vestalis* 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. 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 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.12165051.

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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Version 1

Reviewer Report 23 May 2025

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✔ **Wiebke Sickel** (iD)

Thünen-Institute of Biodiversity, Braunschweig, Braunschweig, Germany

The data note presents the first genome assembly of the vestal cuckoo bee. The assembly is of high-quality, completeness and the methods are described in detail.
I have two very small comments:

(1) Under "Assembly curation", the author writes "The assembly was decontaminated using the Assembly Screenfor Cobionts and Contaminants (ASCC) pipeline (article in preparation)." - I wonder whether a repository or similar could be referenced at this stage?

(2) Under "Wellcome Sanger Institute – Legal and Governance", the author writes "[...] the Darwin Tree of Life Partner agrees they will meet the legal and ethical requirements nd [sic] standards set out within this document in respect of all samples acquired for [...] - there is a typo, should read "and"

Otherwise I recommend to index this data note.

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: biodiversity, monitoring, molecular ecology

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 13 May 2025

https://doi.org/10.21956/wellcomeopenres.26482.r122716

✔️ **Fatih Dikmen** 🆔
Istanbul University, Istanbul, Turkey

This data note presents a high-quality chromosome-level genome assembly for the Vestal Cuckoo Bee (*Bombus vestalis*), which is part of the broader Darwin Tree of Life project. The authors describe the collection, sequencing, assembly, and annotation processes in comprehensive detail. The resulting genome spans 280.01 megabases with 91.96% of the assembly scaffolded into 25 chromosomal pseudomolecules, and includes a mitochondrial genome of 24.37 kilobases. Gene annotation identified 11,600 protein-coding genes. The paper successfully meets the standards of a data note, providing all necessary technical information without unnecessary speculation or interpretation.I recommend this data note for publication without revisions. The authors have produced a high-quality genome resource that will be valuable for future comparative genomic studies of bees, particularly social parasites. The assembly meets all technical quality standards, and the accompanying documentation is excellent. This resource will contribute significantly to understanding the evolution and biology of social parasitism in bees.

https://biorisk.pensoft.net/article/4749

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: Entomology and Bee Taxonomy

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.