



DATA NOTE

# The genome sequence of the common dung beetle, *Aphodius fimetarius* (Linnaeus, 1758) (Coleoptera: Scarabaeidae)

[version 1; peer review: 2 approved]

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## Abstract

We present a genome assembly from an individual male *Aphodius fimetarius* (Common dung beetle; Arthropoda; Insecta; Coleoptera; Scarabaeidae). The genome sequence has a total length of 1 343.38 megabases. Most of the assembly (72.44%) is scaffolded into 11 chromosomal pseudomolecules, including the X and Y sex chromosomes. The mitochondrial genome has also been assembled, with a length of 22.01 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

*Aphodius fimetarius*; Common dung beetle; genome sequence; chromosomal; Coleoptera

## Open Peer Review

Approval Status

	1	2
<b>version 1</b>		
18 Sep 2025	<a href="#">view</a>	<a href="#">view</a>

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Any reports and responses or comments on the article can be found at the end of the article.



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

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## Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Coleoptera; Polyphaga; Scarabaeiformia; Scarabaeoidea; Scarabaeidae; Aphodiinae; *Aphodius*; *Aphodius*; *Aphodius fimetarius* (Linnaeus, 1758) (NCBI:txid207148)

## Background

*Aphodius fimetarius* (Linnaeus, 1758), commonly known as the European Dung Beetle or the Red Dung Beetle, is a widespread and abundant member of the family Scarabaeidae, subfamily Aphodiinae. It is distributed throughout the Holarctic region (Dellacasa & Dellacasa, 2003), with introduced populations in parts of East Asia and Australia (Tyndale-Biscoe, 1990). Within the British and Irish Isles, *A. fimetarius* is widespread, but unevenly distributed, with most records concentrated in England and Wales and comparatively sparse occurrence in Ireland (NBN Atlas Partnership, 2025).

*Aphodius fimetarius* is primarily coprophagous, with both adults and larvae specialising in the decomposition of mammalian dung, particularly that of large herbivores (Holter, 2016). This species is classified as a dweller rather than a tunneler or roller, typically feeding and ovipositing within the dung pat itself rather than relocating dung resources (Hanski, 1991). *A. fimetarius* exhibits a bivoltine life cycle in most of its range, with population peaks in spring and autumn, though this pattern varies geographically with latitude (Finn & Giller, 2002). Adults are capable fliers, dispersing effectively between dung resources, and show a preference for fresh dung in open pasture habitats rather than woodland areas (Roslin, 2000). The species demonstrates some habitat plasticity but is most abundant in traditional pastoral landscapes with consistent dung availability. As an ecosystem engineer, *A. fimetarius* contributes significantly to nutrient cycling, soil aeration, and the suppression of pest flies and parasites through rapid dung removal, with a single beetle capable of processing several times its body weight in dung daily (Nichols *et al.*, 2008). Despite its ecological importance, populations have shown declines in regions with intensive livestock management practices, particularly where antiparasitic drugs such as ivermectin are routinely administered to livestock (Floate *et al.*, 2005).

The ontogeny of *A. fimetarius* includes three distinct larval instars (Christensen & Dobson, 1976). The larvae are typical scarabaeiform, cream-colored with a distinctly darker abdominal region. The pre-pupal stage is characterised by minimal activity and a uniform white coloration, which progressively darkens to reddish-brown during pupation. Teneral adults emerge with pale, creamy-white elytra contrasting with the dark head and pronotum; the elytra darken to their characteristic reddish-brown within hours of eclosion (Jessop, 1986).

Adult *A. fimetarius* are characterised by a black head with semi-oval genae that extend laterally but remain relatively flat compared to congeners. The antennae are short with yellowish-brown clubs positioned above the genae. The black tibiae bear three prominent tibial teeth, with the apical tooth approximately

equal in length to the tibial width. The pronotum is predominantly black with distinctive yellow to amber patches laterally and exhibits increasing punctation posteriorly. A small black triangular scutellum separates the pronotum from the diagnostic reddish-brown elytra. These elytra display fine reticulation with shallow striae and moderately convex intervals (Stebnicka, 2001). Sexual dimorphism is subtle but consistent, with males distinguished by a characteristic anterior indentation on the pronotum and differences in the metasternal plate (Dellacasa *et al.*, 2001).

Long considered among the most abundant dung beetles in Europe and globally (Roslin & Heliövaara, 2009), *A. fimetarius* has recently been recognised as part of a cryptic species complex. The morphologically similar *Aphodius pedellus* (De Geer, 1774) was first distinguished through karyotypic analysis (Wilson, 2001) and confirmed as a separate species through comprehensive genetic and morphological studies (Miraldo *et al.*, 2014). Despite living in sympatry across much of their range, no hybrid karyotypes have been documented. Reliable morphological differentiation between these species is possible based on several subtle characteristics: *A. fimetarius* exhibits less protruding and flatter lateral pronotal lobes, duller elytral apices lacking the strong wrinkles, shine, or raised spots present in *A. pedellus*, and more strongly convex elytral intervals (Röbner, 2012; Whitehead, 2006). Additionally, the two species show differences in seasonal phenology, with *A. fimetarius* typically active earlier in the season (Hanski, 1991). Biogeographically, *A. pedellus* demonstrates a more northerly distribution than *A. fimetarius*, being more prevalent in Canada, Scandinavia, and northern Russia (Floate *et al.*, 2022; Miraldo *et al.*, 2014).

We present a chromosome-level genome sequence for *Aphodius fimetarius*, produced using the Tree of Life pipeline from a specimen collected in Wytham Woods, Oxfordshire, United Kingdom (Figure 1). Although numerous genomes exist for the family Scarabaeidae, this assembly provides the first whole



**Figure 1.** Photograph of the *Aphodius fimetarius* (icAphFime1) specimen used for genome sequencing.

genome sequence for *Aphodius fimetarius*, enabling comparative analyses (data obtained via NCBI datasets, O'Leary *et al.*, 2024).

## Methods

### Sample acquisition and DNA barcoding

The specimen used for genome sequencing was an adult male *Aphodius fimetarius* (specimen ID Ox002480, ToLID icAphFime1; Figure 1), collected from Wytham Woods Oxfordshire, United Kingdom (latitude 51.783, longitude -1.318) on 2022-06-13. The specimen was collected by Darren Mann and Liam Crowley (University of Oxford) and identified by Darren Mann. Another specimen was used for RNA sequencing (specimen ID NHMUK014552939, ToLID icAphFime2). It was collected from Knepp Castle Estate, England, United Kingdom (latitude 50.98, longitude -0.39) on 2022-04-21. The specimen was collected by Chris Fletcher and identified by Robert Angus. 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 icAphFime1 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 manual 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 26.2 ng/μL and a yield of 1 179.00 ng, with a fragment size of 14.0 kb. The 260/280 spectrophotometric ratio was 1.95, and the 260/230 ratio was 1.79.

RNA was extracted from whole organism tissue of icAphFime2 in the Tree of Life Laboratory at the WSI using the

### RNA Extraction: Automated MagMax™ mirVana protocol.

The RNA concentration was assessed using a Nanodrop spectrophotometer and a Qubit Fluorometer using the Qubit RNA Broad-Range Assay kit. Analysis of the integrity of the RNA was done using the Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

### 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 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, and to perform primary and secondary analysis of the data upon completion.

### Hi-C

#### Sample preparation and crosslinking

The Hi-C sample was prepared from 20–50 mg of frozen whole organism tissue of the icAphFime1 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 Diagenode 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 6000.

### RNA library preparation and sequencing

Libraries were prepared using the NEBNext<sup>®</sup> Ultra<sup>™</sup> II Directional RNA Library Prep Kit for Illumina (New England Biolabs), following the manufacturer's instructions. Poly(A) mRNA in the total RNA solution was isolated using oligo(dT) beads, converted to cDNA, and uniquely indexed; 14 PCR cycles were performed. Libraries were size-selected to produce fragments between 100–300 bp. Libraries were quantified, normalised, pooled to a final concentration of 2.8 nM, and diluted to 150 pM for loading. Sequencing was carried out on the Illumina NovaSeq X to generate 150-bp paired-end reads.

### 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 (Cheng *et al.*, 2021) with the --primary option. Haplotypic duplications were identified and removed using purge\_dups (Guan *et al.*, 2020). The Hi-C reads (Rao *et al.*, 2014) were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019), and the contigs were scaffolded in YaHS (Zhou *et al.*, 2023) with 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 37 breaks, 53 joins, and removal of 15 haplotypic duplications. The curation process is documented at <https://gitlab.com/wtsi-grit/rapid-curation>. PretextView 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 the primary and alternate haplotypes using the *k*-mer databases (*k* = 31) computed prior to genome assembly. The analysis outputs included assembly QV scores and completeness statistics.

The genome was analysed using the BlobToolKit pipeline, a Nextflow 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

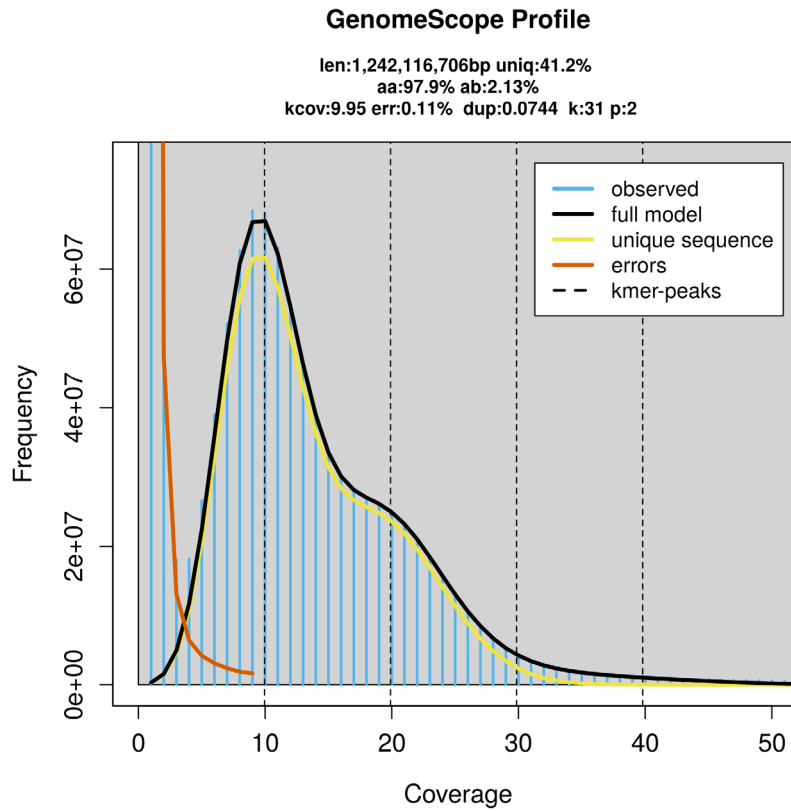
PacBio sequencing of the *Aphodius fimetarius* specimen generated 25.65 Gb (gigabases) from 2.39 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 1 242.12 Mb, with a heterozygosity of 2.13% and repeat content of 59.31% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 20× coverage. Hi-C sequencing produced 117.18 Gb from 776.03 million reads, which were used to scaffold the assembly. RNA sequencing data were also generated and are available in public sequence repositories. Table 1 summarises the specimen and sequencing details.

#### Assembly statistics

The primary haplotype was assembled, and contigs corresponding to an alternate haplotype were also deposited in INSDC databases. The final assembly has a total length of 1 343.38 Mb in 3 057 scaffolds, with 495 gaps, and a scaffold N50 of 78.76 Mb (Table 2).

Most of the assembly sequence (72.44%) was assigned to 11 chromosomal-level scaffolds, representing 9 autosomes and the X and Y sex chromosomes. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 3; 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.



**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 PRJEB64100.**

Platform	PacBio HiFi	Hi-C	RNA-seq
ToLID	icAphFime1	icAphFime1	icAphFime2
Specimen ID	Ox002480	Ox002480	NHMUK014552939
BioSample (source individual)	SAMEA112232683	SAMEA112232683	SAMEA115574760
BioSample (tissue)	SAMEA112233161	SAMEA112233161	SAMEA115599859
Tissue	whole organism	whole organism	whole organism
Instrument	Sequel Iie	Illumina NovaSeq 6000	Illumina NovaSeq X
Run accessions	ERR11673250	ERR11679414	ERR15140873
Read count total	2.39 million	776.03 million	111.56 million
Base count total	25.65 Gb	117.18 Gb	16.85 Gb

**Table 2. Genome assembly statistics.**

<b>Assembly name</b>	icAphFime1.1
<b>Assembly accession</b>	GCA_964211865.1
<b>Alternate haplotype accession</b>	GCA_964211875.1
<b>Assembly level</b>	chromosome
<b>Span (Mb)</b>	1 343.38
<b>Number of chromosomes</b>	11
<b>Number of contigs</b>	3 552
<b>Contig N50</b>	2.8 Mb
<b>Number of scaffolds</b>	3 057
<b>Scaffold N50</b>	78.76 Mb
<b>Sex chromosomes</b>	X and Y
<b>Organelles</b>	Mitochondrion: 22.01 kb

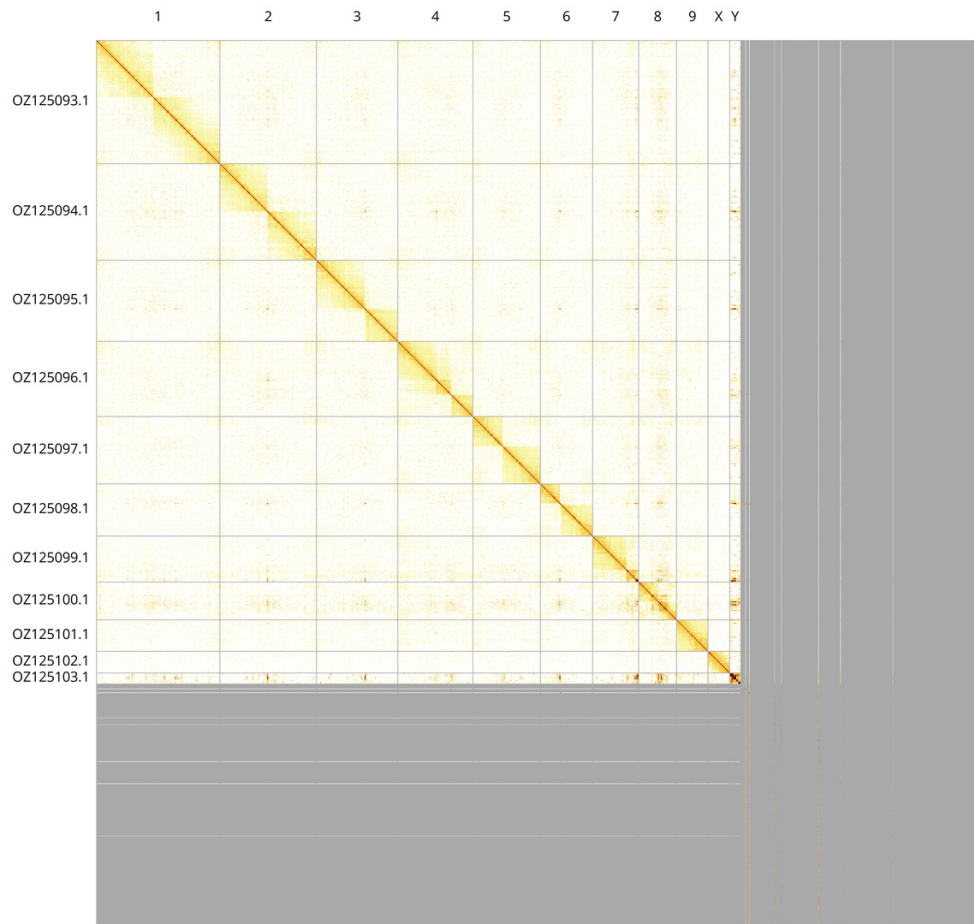
The combined primary and alternate assemblies achieve an estimated QV of 55.5. The  $k$ -mer completeness is 68.45% for the primary assembly, 63.68% for the alternate haplotype, and 97.65% for the combined assemblies (Figure 4).

BUSCO v.5.5.0 analysis using the endopterygota\_odb10 reference set ( $n = 2\,124$ ) identified 99.1% of the expected gene set (single = 97.6%, duplicated = 1.5%). The snail plot in Figure 5 summarises the scaffold length distribution and other assembly statistics for the primary assembly. The blob plot in Figure 6 shows the distribution of scaffolds by GC proportion and coverage.

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 primary assembly, is **6.7.Q54**.

#### Wellcome Sanger Institute – Legal and Governance

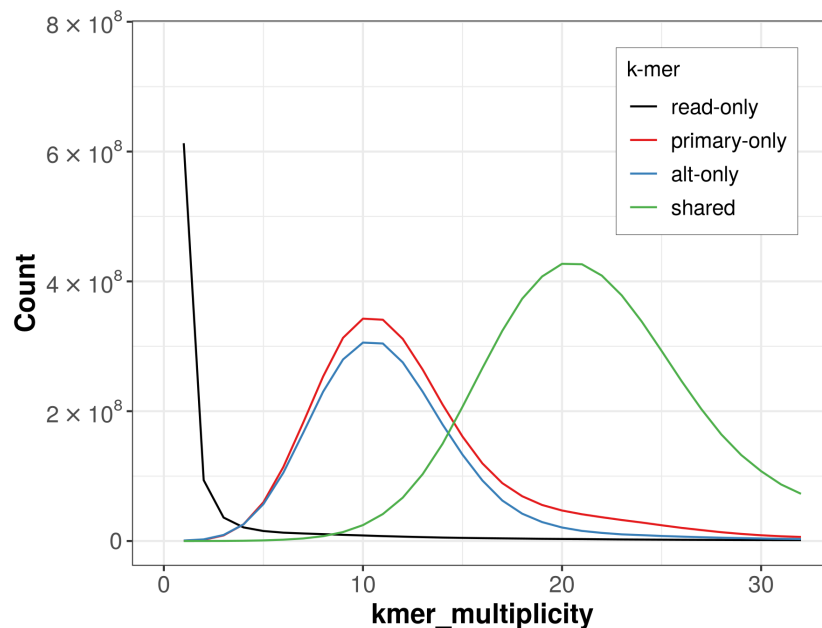
The materials that have contributed to this genome note have been supplied by a Darwin Tree of Life Partner. The submission



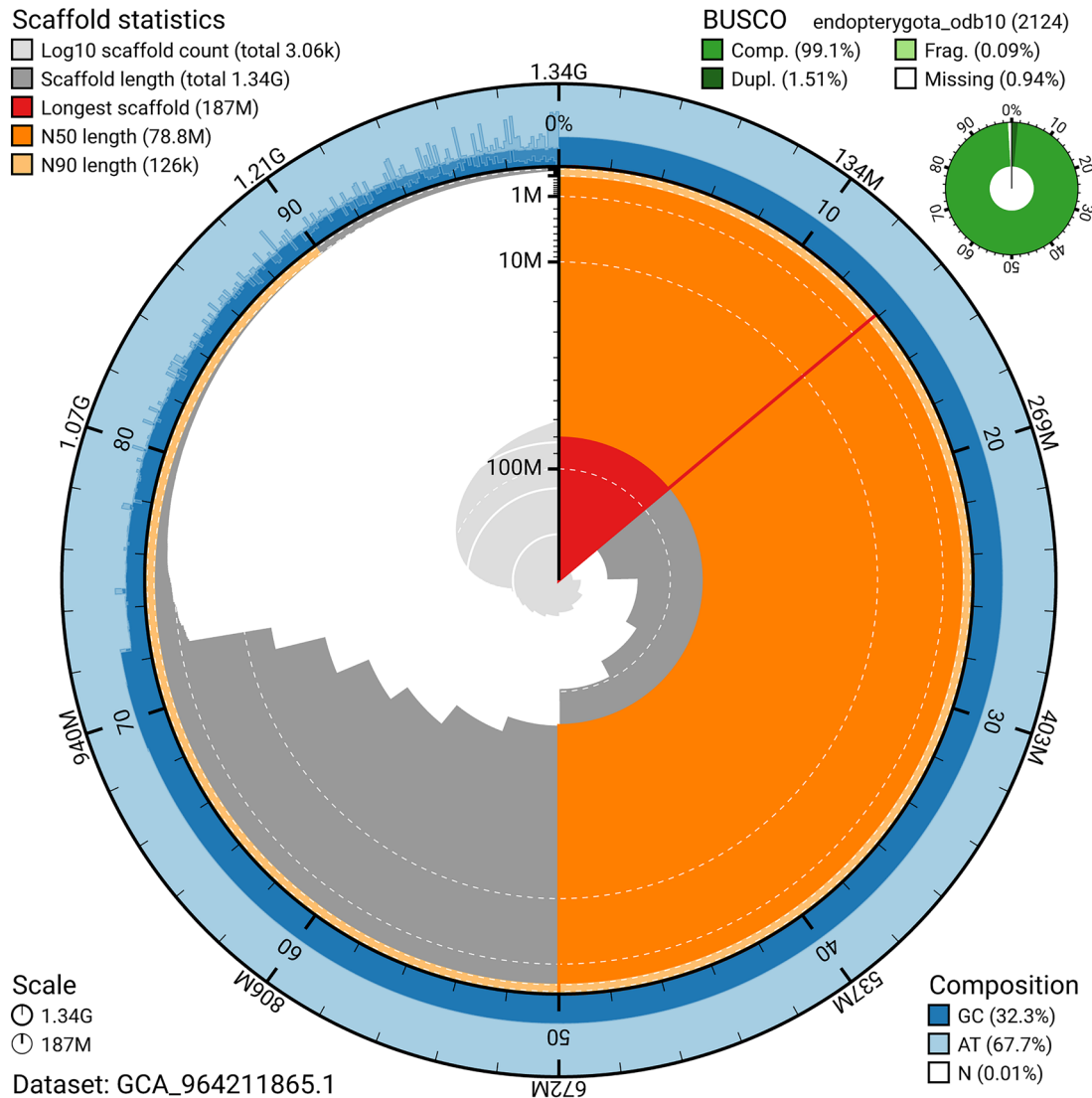
**Figure 3. Hi-C contact map of the *Aphodius fimetarius* genome assembly.** Assembled chromosomes are shown in order of size and labelled along the axes. The plot was generated using PretextSnapshot.

**Table 3. Chromosomal pseudomolecules in the primary genome assembly of *Aphodius fimetarius* icAphFime1.**

INSDC accession	Molecule	Length (Mb)	GC%
OZ125093.1	1	187.01	36
OZ125094.1	2	145.68	36
OZ125095.1	3	122.71	36
OZ125096.1	4	113.71	35.50
OZ125097.1	5	101.66	36
OZ125098.1	6	78.76	35.50
OZ125099.1	7	69.76	35.50
OZ125100.1	8	57.13	36
OZ125101.1	9	47.33	36
OZ125102.1	X	32.96	37
OZ125103.1	Y	16.49	36



**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.

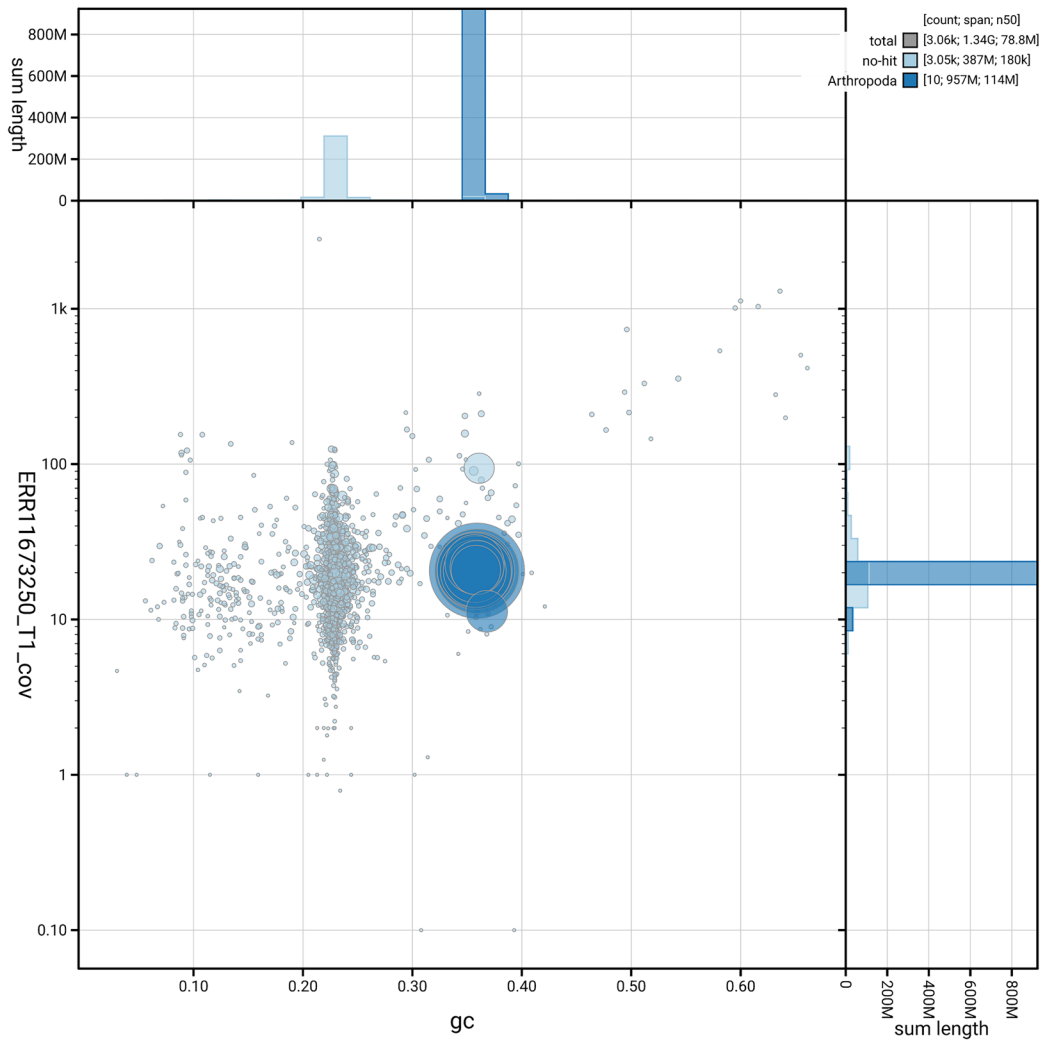


**Figure 5. Assembly metrics for icAphFime1.1.** The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1 000 bins around the circumference. The outermost 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 endopterygota\_odb10 set is presented at the top right. An interactive version of this figure can be accessed on the [BlobToolKit viewer](#).

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



**Figure 6. BlobToolKit GC-coverage plot for icAphFime1.1.** 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 *Aphodius fimetarius* assembly.**

Measure	Value	Benchmark
EBP summary (primary)	6.7.Q54	6.C.Q40
Contig N50 length	2.80 Mb	≥ 1 Mb
Scaffold N50 length	78.76 Mb	= chromosome N50
Consensus quality (QV)	Primary: 54.9; alternate: 55.9; combined: 55.5	≥ 40
<i>k</i> -mer completeness	Primary: 68.45%; alternate: 63.68%; combined: 97.65%	≥ 95%
BUSCO	C:99.1% [S:97.6%; D:1.5%]; F:0.1%; M:0.8%; n:2 124	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	72.44%	≥ 90%

- 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: *Aphodius fimetarius*. Accession number [PRJEB64100](#). The genome sequence is released openly for reuse. The *Aphodius fimetarius* 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.

### Author information

Contributors are listed at the following links:

- Members of the [University of Oxford and Wytham Woods Genome Acquisition Lab](#)
- Members of the [Natural History Museum Genome Acquisition Lab](#)
- Members of the [Darwin Tree of Life Barcoding collective](#)
- Members of the [Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team](#)
- 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](#)

**Table 5. Software versions and sources.**

Software	Version	Source
BEDTools	2.30.0	<a href="https://github.com/arq5x/bedtools2">https://github.com/arq5x/bedtools2</a>
BLAST	2.14.0	<a href="ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/">ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/</a>
BlobToolKit	4.3.9	<a href="https://github.com/blobtoolkit/blobtoolkit">https://github.com/blobtoolkit/blobtoolkit</a>
BUSCO	5.5.0	<a href="https://gitlab.com/ezlab/busco">https://gitlab.com/ezlab/busco</a>
bwa-mem2	2.2.1	<a href="https://github.com/bwa-mem2/bwa-mem2">https://github.com/bwa-mem2/bwa-mem2</a>
Cooler	0.8.11	<a href="https://github.com/open2c/cooler">https://github.com/open2c/cooler</a>
DIAMOND	2.1.8	<a href="https://github.com/bbuchfink/diamond">https://github.com/bbuchfink/diamond</a>
fasta_windows	0.2.4	<a href="https://github.com/tolkit/fasta_windows">https://github.com/tolkit/fasta_windows</a>
FastK	1.1	<a href="https://github.com/thegenemyers/FASTK">https://github.com/thegenemyers/FASTK</a>
GenomeScope2.0	2.0.1	<a href="https://github.com/tbenavi1/genomescope2.0">https://github.com/tbenavi1/genomescope2.0</a>
Gfastats	1.3.6	<a href="https://github.com/vgl-hub/gfastats">https://github.com/vgl-hub/gfastats</a>
GoaT CLI	0.2.5	<a href="https://github.com/genomehubs/goat-cli">https://github.com/genomehubs/goat-cli</a>
Hifiasm	0.19.5-r587	<a href="https://github.com/chhylp123/hifiasm">https://github.com/chhylp123/hifiasm</a>
HiGlass	1.13.4	<a href="https://github.com/higlass/higlass">https://github.com/higlass/higlass</a>
MerquryFK	1.1.2	<a href="https://github.com/thegenemyers/MERQURY.FK">https://github.com/thegenemyers/MERQURY.FK</a>
Minimap2	2.24-r1122	<a href="https://github.com/lh3/minimap2">https://github.com/lh3/minimap2</a>
MitoHiFi	3	<a href="https://github.com/marcelauliano/MitoHiFi">https://github.com/marcelauliano/MitoHiFi</a>
MultiQC	1.14; 1.17 and 1.18	<a href="https://github.com/MultiQC/MultiQC">https://github.com/MultiQC/MultiQC</a>

Software	Version	Source
Nextflow	23.10.0	<a href="https://github.com/nextflow-io/nextflow">https://github.com/nextflow-io/nextflow</a>
PretextSnapshot	N/A	<a href="https://github.com/sanger-tol/PretextSnapshot">https://github.com/sanger-tol/PretextSnapshot</a>
PretextView	0.2.5	<a href="https://github.com/sanger-tol/PretextView">https://github.com/sanger-tol/PretextView</a>
purge_dups	1.2.5	<a href="https://github.com/dfguan/purge_dups">https://github.com/dfguan/purge_dups</a>
samtools	1.19.2	<a href="https://github.com/samtools/samtools">https://github.com/samtools/samtools</a>
sanger-tol/ascc	0.1.0	<a href="https://github.com/sanger-tol/ascc">https://github.com/sanger-tol/ascc</a>
sanger-tol/blobtoolkit	0.6.0	<a href="https://github.com/sanger-tol/blobtoolkit">https://github.com/sanger-tol/blobtoolkit</a>
sanger-tol/curationpretext	1.4.2	<a href="https://github.com/sanger-tol/curationpretext">https://github.com/sanger-tol/curationpretext</a>
Seqtk	1.3	<a href="https://github.com/lh3/seqtk">https://github.com/lh3/seqtk</a>
Singularity	3.9.0	<a href="https://github.com/sylabs/singularity">https://github.com/sylabs/singularity</a>
TreeVal	1.4.0	<a href="https://github.com/sanger-tol/treeval">https://github.com/sanger-tol/treeval</a>
YaHS	1.2a.2	<a href="https://github.com/c-zhou/yahs">https://github.com/c-zhou/yahs</a>

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# Open Peer Review

Current Peer Review Status:  

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## Version 1

Reviewer Report 22 October 2025

<https://doi.org/10.21956/wellcomeopenres.27387.r133834>

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**Arun Arumugaperumal** 

Department of Biotechnology, Rajalakshmi Engineering College, Thandalam, Chennai, Tamil Nadu, 602105, India

The data note describes the whole genome sequencing of the dung beetle *Aphodius fimetarius*. The genome has been assembled using a combination of PacBio long-read sequencing and Hi-C sequencing. The assembly size reported was 1,343.38 Mb, spread among 11 chromosomes. Since a male specimen was used, the X and Y chromosomes have been captured. The mitogenome was also assembled, which is of size 22.01 kb. The other member of the same genus, *Aphodius granarius*, has a genome size of 397.20 Mb [1]. It would be interesting to study the difference in the genome sizes as the genome sequence is made available now. BUSCO completeness of 99.1% indicates that the genome is a near-complete one as per the sequences available in the database.

Figure 3 can be cropped to eliminate the unwanted regions.

In Figure 6, the link provided is not showing blobs. It is showing squares instead. If the authors want to modify the link, they can do it at this stage.

If possible, a link to the annotation of the genome can be updated in the manuscript.

The article can be indexed.

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**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:** Bioinformatics; 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.**

Reviewer Report 27 September 2025

<https://doi.org/10.21956/wellcomeopenres.27387.r133842>

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**Feng Zhang** 

Nanjing Agricultural University, Nanjing, China

This manuscript presents a high-quality, chromosome-level genome assembly for *Aphodius fimetarius*, representing the first whole-genome sequence for this ecologically important dung beetle species. The technical approach is sound and follows established best practices. However, several aspects require improvement to enhance the scientific rigor and interpretative value of this work.

### 1. Inadequate Discussion of Genome Quality Limitations

1. The k-mer completeness of only 68.45% for the primary assembly falls significantly below the 95% benchmark standard, yet this critical limitation receives insufficient attention in the manuscript. The authors should provide a thorough analysis of the causes underlying the low k-mer completeness in the Results section and discuss the potential relationship between this metric and the high repeat content (59.31%)

### 1. Methodological Details Require Clarification

1. The moderate heterozygosity level (2.13%) identified by GenomeScope2 analysis warrants

- discussion of its impact on assembly strategy
- 2. The manual curation process involving 37 breaks and 53 joins needs clearer justification and criteria
- 3. More detailed explanation of scaffold validation procedures using Hi-C data

### 1. Writing and Presentation Issues

1. The Abstract should explicitly state that this is the first chromosome-level genome for the species
2. The extensive discussion of *A. pedellus* in the Background section is overly detailed; focus on genomically relevant aspects
3. Add a concluding section discussing future applications in ecological and evolutionary research

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**Are the protocols appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and materials provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Yes

***Competing Interests:*** No competing interests were disclosed.

***Reviewer Expertise:*** genome

**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.**

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