



DATA NOTE

The genome sequence of the Dotted Footman moth, *Pelosia muscerda* (Hufnagel, 1767)

[version 1; peer review: 3 approved, 1 approved with reservations]

Chris Fletcher ¹, David C. Lees ¹,

Natural History Museum Genome Acquisition Lab,
Darwin Tree of Life Barcoding collective,
Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory
team,

Wellcome Sanger Institute Scientific Operations: Sequencing Operations,
Wellcome Sanger Institute Tree of Life Core Informatics team,
Tree of Life Core Informatics collective, Darwin Tree of Life Consortium

¹Natural History Museum, London, England, UK

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Abstract

We present a genome assembly from a male *Pelosia muscerda* (Dotted Footman; Arthropoda; Insecta; Lepidoptera; Erebidæ). The genome sequence has a total length of 500.49 megabases. Most of the assembly (99.98%) is scaffolded into 30 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 15.42 kilobases in length.

Keywords

Pelosia muscerda, Dotted Footman, genome sequence, chromosomal, Lepidoptera



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

Open Peer Review

Approval Status

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version 1				
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1. **Bhagya C. Thimmappa** , USDA
Agricultural Research Service (Ringgold ID: 17123), New Jersey, USA
2. **Arun Arumugaperumal** , Rajalakshmi
Engineering College, Thandalam, Chennai,
India
3. **Christophe Klopp** , INRAE, Castanet-
Tolosan, France
4. **Vlad Dinca** , University of Oulu, Oulu,
Finland

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

Corresponding author: Darwin Tree of Life Consortium (mark.blaxter@sanger.ac.uk)

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Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Noctuoidea; Erebidae; Arctiinae; Lithosiini; *Pelosia*; *Pelosia muscerda* (Hufnagel, 1767) (NCBI:txid988016)

Background

The genome of the Dotted Footman, *Pelosia muscerda*, was sequenced as part of the Darwin Tree of Life Project, a collaborative effort to sequence all named eukaryotic species in the Atlantic Archipelago of Britain and Ireland. Here we present a chromosomally complete genome sequence for *Pelosia muscerda*, based on a male specimen from Bure Marshes Stables Nature Reserve, England, UK (Figure 1).

Genome sequence report

Sequencing data

The genome of a specimen of *Pelosia muscerda* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 28.26 Gb from 2.68 million reads. GenomeScope analysis of the PacBio HiFi data estimated the haploid genome size at 492.75 Mb, with a heterozygosity of 1.98% and repeat content of 22.67%. 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 approximately 55.0x coverage of the genome. Chromosome conformation Hi-C sequencing produced 137.27 Gb from 909.05 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 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 10 misjoins or missing joins and removed one haplotypic duplication. These interventions decreased the scaffold count by 5.26%. The final assembly has a total length of 500.49 Mb in 35 scaffolds, with 42 gaps, and a scaffold N50 of 18.06 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 (99.97%) was assigned to 30 chromosomal-level scaffolds, representing 29 autosomes and the Z sex chromosome. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 5; Table 3). During curation the Z chromosome was identified based on synteny with the genome of *Eilema depressum* (GCA_914767945.1) (Boyes *et al.*, 2023).

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 66.5, and the combined primary and alternate assemblies achieve an estimated QV of 66.4. The *k*-mer completeness for the primary haplotype is 68.50%, and for the alternate haplotype it is 66.90%, while the combined primary and alternate assemblies achieve a *k*-mer completeness of 99.57%. BUSCO analysis using the lepidoptera_odb10 reference set ($n = 5,286$) indicated a completeness score of 98.6% (single = 98.0%, duplicated = 0.6%).

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 EBP reference standard of 7.C.Q66.

Methods

Sample acquisition and DNA barcoding

An adult male *Pelosia muscerda* (specimen ID NHMUK015054455, ToLID iIPelMusc1) was collected from Bure Marshes Stables Nature Reserve, England, United Kingdom (latitude 52.69, longitude 1.45) on 2022-06-28, using a light trap. The specimen was collected by Chris Fletcher



Figure 1. Photographs of the *Pelosia muscerda* (iIPelMusc1) specimen used for genome sequencing.

Table 1. Specimen and sequencing data for *Pelosia muscerda*.

Project information			
Study title	Pelosia muscerda		
Umbrella BioProject	PRJEB68294		
Species	<i>Pelosia muscerda</i>		
BioSpecimen	SAMEA112963098		
NCBI taxonomy ID	988016		
Specimen information			
Technology	ToLID	BioSample accession	Organism part
PacBio long read sequencing	ilPelMusc1	SAMEA112963194	head and thorax
Hi-C sequencing	ilPelMusc1	SAMEA112963194	head and thorax
Sequencing information			
Platform	Run accession	Read count	Base count (Gb)
Hi-C Illumina NovaSeq 6000	ERR12259846	9.09e+08	137.27
PacBio Sequel IIE	ERR12257437	2.68e+06	28.26

(Natural History Museum), identified by David Lees (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 protocols.io ([Denton et al., 2023b](#)). The ilPelMusc1 sample was prepared for DNA extraction by weighing and dissecting it on dry ice ([Jay et al., 2023](#)). Tissue from the head and

thorax was homogenised using a PowerMasher II tissue disruptor ([Denton et al., 2023a](#)).

HMW DNA was extracted in the WSI Scientific Operations core using the Automated MagAttract v2 protocol ([Oatley et al., 2023](#)). The DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system ([Bates et al., 2023](#)). Sheared DNA was purified by solid-phase reversible immobilisation, using AMPure PB beads to eliminate shorter fragments and concentrate the DNA ([Strickland et al., 2023](#)). The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and Qubit Fluorometer using the Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

Hi-C sample preparation

Tissue from the head and thorax of the ilPelMusc1 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.

Table 2. Genome assembly data for *Pelosia muscerda*.

Genome assembly		
Assembly name	ilPelMusc1.1	
Assembly accession	GCA_963691645.1	
Alternate haplotype accession	GCA_963691735.1	
Assembly level for primary assembly	chromosome	
Span (Mb)	500.49	
Number of contigs	77	
Number of scaffolds	35	
Longest scaffold (Mb)	33.69	
Assembly metrics	Measure	Benchmark
Contig N50 length	11.35 Mb	$\geq 1 \text{ Mb}$
Scaffold N50 length	18.06 Mb	= chromosome N50
Consensus quality (QV)	Primary: 66.5; alternate: 66.2; combined 66.4	≥ 40
k-mer completeness	Primary: 68.50%; alternate: 66.90%; combined: 99.57%	$\geq 95\%$
BUSCO*	C:98.6%[S:98.0%,D:0.6%], F:0.3%,M:1.1%,n:5,286	$S > 90\%$, $D < 5\%$
Percentage of assembly mapped to chromosomes	99.97%	$\geq 90\%$
Sex chromosomes	Z	localised homologous pairs
Organelles	Mitochondrial genome: 15.42 kb	complete single alleles

* BUSCO scores based on the lepidoptera_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.

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 (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.

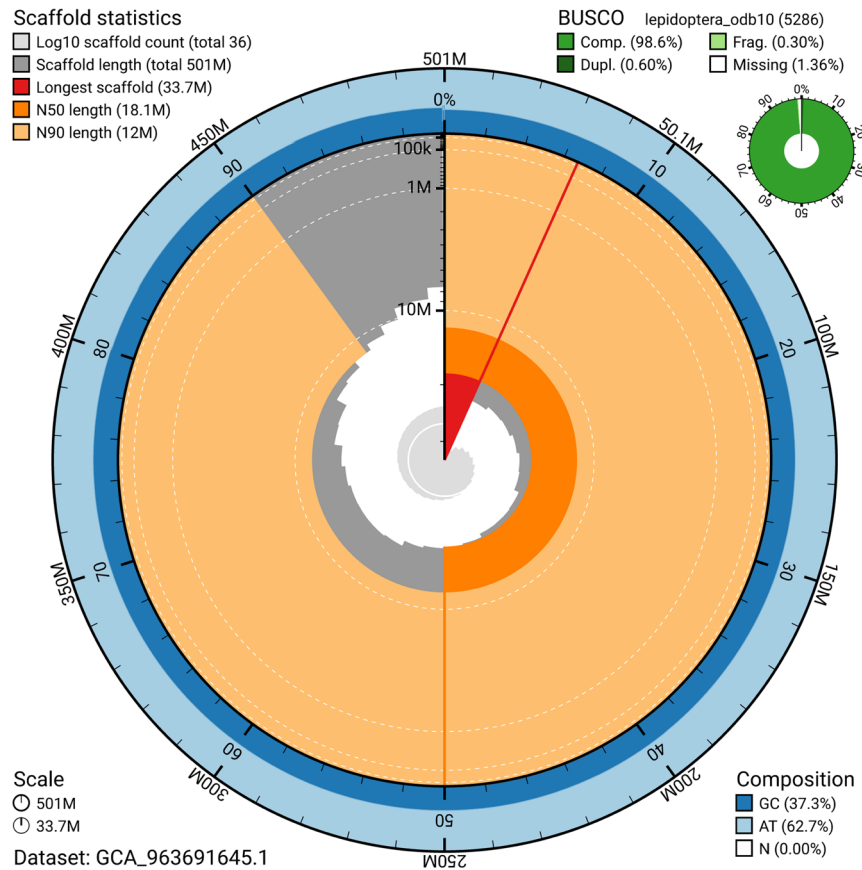


Figure 2. Genome assembly of *Pelosia muscerda*, ilPelMusc1.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. 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 lepidoptera_odb10 set is presented at the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963691645.1/dataset/GCA_963691645.1/snail.

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).

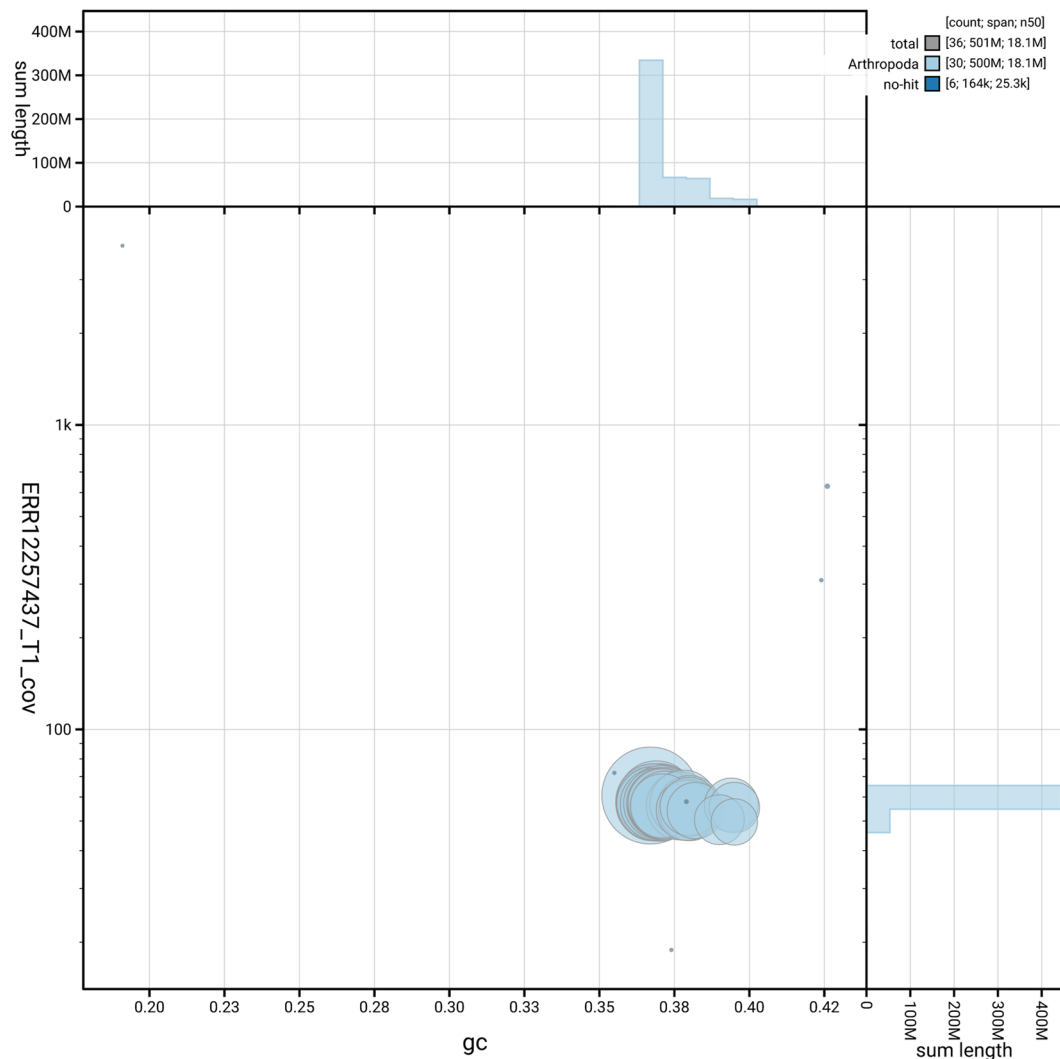


Figure 3. Genome assembly of *Pelosia muscerda*, ilPelMusc1.1: BlobToolKit GC-coverage plot. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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_963691645.1/blob.

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 Cointons 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 synteny analysis. 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

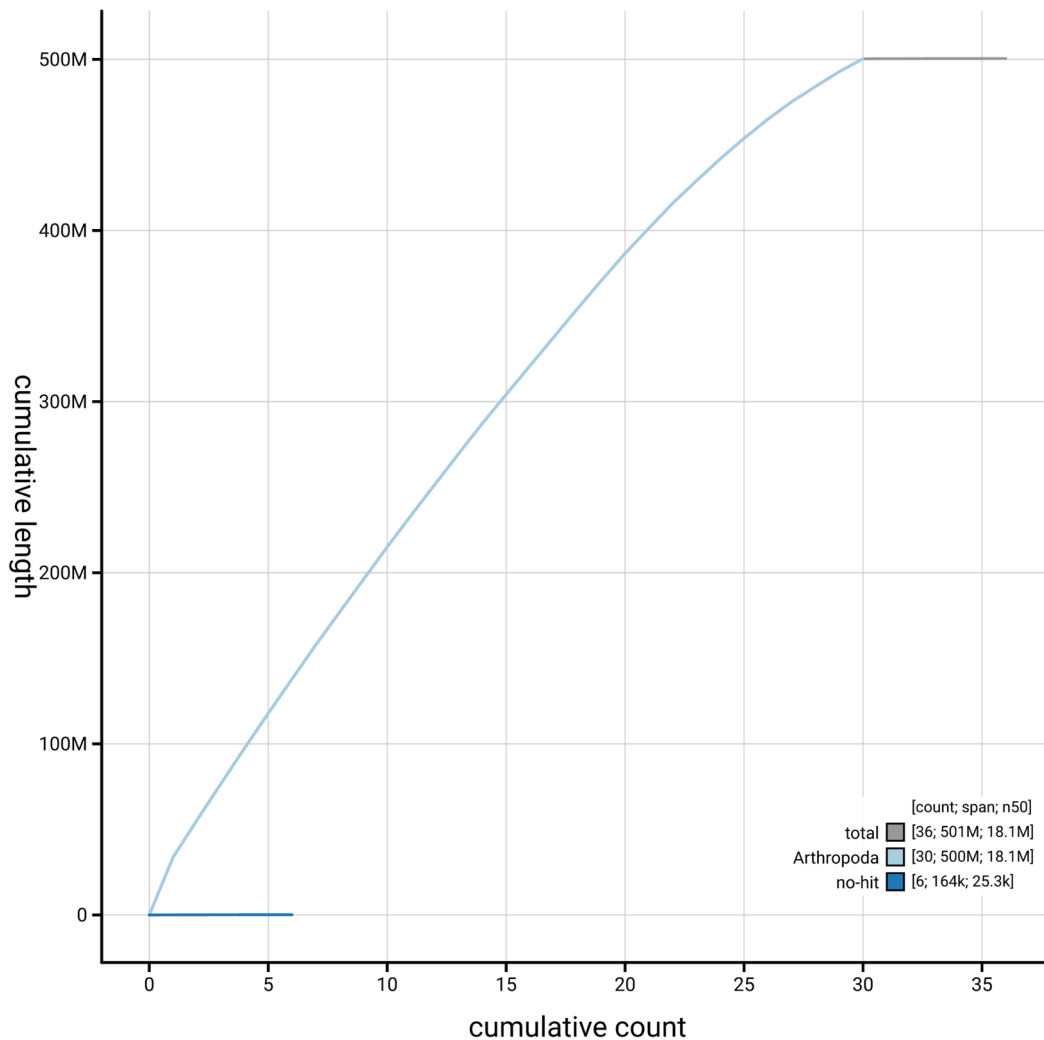


Figure 4. Genome assembly of *Pelosia muscerda*, ilPelMusc1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963691645.1/dataset/GCA_963691645.1/cumulative.

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 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.

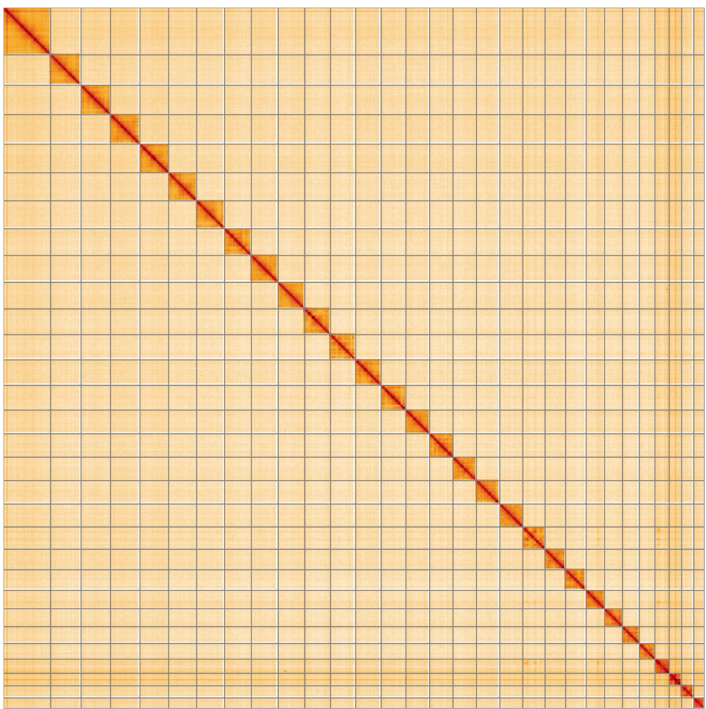


Figure 5. Genome assembly of *Pelosia muscerda*: Hi-C contact map of the ilPelMusc1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=XXnQN_OLQ3uk03WLSRDWEw.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Pelosia muscerda*, ilPelMusc1.

INSDC accession	Name	Length (Mb)	GC%
OY829099.1	1	21.66	37
OY829100.1	2	21.08	37
OY829101.1	3	20.96	37
OY829102.1	4	20.47	37
OY829103.1	5	20.07	37
OY829104.1	6	19.93	37
OY829105.1	7	19.15	37
OY829106.1	8	19.04	37
OY829107.1	9	19.0	37
OY829108.1	10	18.42	37
OY829109.1	11	18.06	37
OY829110.1	12	18.02	37
OY829111.1	13	17.72	37
OY829112.1	14	16.93	37

INSDC accession	Name	Length (Mb)	GC%
OY829113.1	15	16.74	37
OY829114.1	16	16.73	37
OY829115.1	17	16.71	37
OY829116.1	18	16.37	37.5
OY829117.1	19	15.85	38
OY829118.1	20	14.74	38
OY829119.1	21	14.6	37
OY829120.1	22	13.13	38
OY829121.1	23	12.83	38
OY829122.1	24	11.98	38
OY829123.1	25	11.19	38
OY829124.1	26	10.16	39.5
OY829125.1	27	8.91	39.5
OY829126.1	28	8.68	39
OY829127.1	29	7.53	39.5
OY829098.1	Z	33.69	36.5
OY829128.1	MT	0.02	19.5

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

Table 4. Software tools: versions and sources.

Software tool	Version	Source
BEDTools	2.30.0	https://github.com/ark5x/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/e2lab/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/chhyllp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84aa44357826c0b6753eb28de	https://github.com/higlass/higlass
MercuryFK	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	0.2.5	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

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: *Pelosia muscerda*. Accession number PRJEB68294; <https://identifiers.org/ena.embl/PRJEB68294>. The genome sequence is released openly for reuse. The *Pelosia muscerda* genome sequencing initiative is

part of the Darwin Tree of Life (DToL) project. All raw sequence data and the assembly have been deposited in INSDC databases. The genome will be annotated using available RNA-Seq data and presented through the [Ensembl](#) pipeline at the European Bioinformatics Institute. Raw data and assembly accession identifiers are reported in [Table 1](#) and [Table 2](#).

Author information

Members of the Natural History Museum Genome Acquisition Lab are listed here: <https://doi.org/10.5281/zenodo.12159242>.

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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Vlad Dinca 

University of Oulu, Oulu, Finland

In this data note, the authors report a chromosome-level genome assembly of a male *Pelosia muscerda* (Lepidoptera, Erebidae, Arctiinae). The mitochondrial genome has been assembled as well.

Although I am not an expert in several of the methodological approaches used, as far as I can tell, the manuscript uses methodologies and pipelines that are fairly well-established through use by the Sanger Institute. Data quality seems good (e.g. BUSCO score of 98.6% and other parameters summarized in Table 2).

I suggest including a bit of additional information in the Background section. For example, a few words about the general distribution of the species, its larval host plant(s), phenology, preferred habitat etc.

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: Lepidoptera speciation, taxonomy and phylogeography, with a focus on

Western Palearctic butterflies.

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 March 2025

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Christophe Klopp 

INRAE, Castanet-Tolosan, France

The authors present the first chromosomal assembly of the Dotted Footman moth, *Pelosia muscerda*.

The article is simple to read but the methods are a bit outdated. It is not clear from the material and methods if the authors have used the Hi-C data during the assembly but it seems that is was the case. This is good practice because it enables to build better haplotypes.

The authors have chosen to produce principal and alternative assemblies and not to scaffold the alternative which is as long as the principal. Producing scaffolded hap1 and hap2 would have been better for future analysis such as building a pangenome which could be of high interest in a species showing high heterozygosity.

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Partly

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

Partly

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

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, genome assembly and annotation

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, however I have

significant reservations, as outlined above.

Reviewer Report 11 March 2025

<https://doi.org/10.21956/wellcomeopenres.26214.r120277>

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Arun Arumugaperumal 

Department of Biotechnology, Rajalakshmi Engineering College, Thandalam, Chennai, Tamilnadu, 602015, India

This is the first whole genome report of the Dotted Footman moth, *Pelusia muscerda*. The assembly reported here is of size 500.49 Mb and the mitogenome is of size 15.42 kb. The authors can shed some more light on how they identified misjoins. Did they use any software or script to compare sequence contigs? In Figure 1 caption there is no explanation of what is a and b labels in the figure. BUSCO analysis has shown that the genome is 98.6% complete. The report can be accepted for indexing.

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 10 March 2025

<https://doi.org/10.21956/wellcomeopenres.26214.r120285>

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Bhagya C. Thimmappa 

US Department of agriculture, USDA Agricultural Research Service (Ringgold ID: 17123), New Jersey, Chatsworth, USA

In the article “The genome sequence of the Dotted Footman moth, *Pelosia muscerda* (Hufnagel, 1767)”, the authors report the nuclear and mitochondrial genome of the Dotted Footman moth. They performed sequencing using Pacific Biosciences single-molecule HiFi long reads and chromosome conformation Hi-C sequencing. The reported assembly is 500.49 Mb in length across 35 scaffolds, which is highly complete, with a BUSCO score of 98.6%.

Major comments:

1. Upon cross-checking NCBI taxonomy, *Pelosia muscerda* (Hufnagel, 1767) should be *Pelosia muscerda* (Hufnagel, 1766).
2. Background information is limited. What is the geographical distribution of *Pelosia muscerda*, its life cycle, and does it cause any issues for plants? In other words, more information is needed to highlight why the reported dataset is essential.

Minor comments:

1. More information on the RNA-Seq data files and when the annotation will be done would be helpful.
2. If possible, mitochondrial annotation would be helpful for further evolutionary studies.

Is the rationale for creating the dataset(s) clearly described?

Partly

Are the protocols appropriate and is the work technically sound?

Yes

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

Yes

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

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genomics, Bioinformatics, multi-omics

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.