DATA NOTE

The genome sequence of the Bog Ant Fly, *Microdon myrmicae* Schönrogge *et al.*, 2002

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from a male specimen of *Microdon myrmicae* (Bog Ant Fly; Arthropoda; Insecta; Diptera; Syrphidae). The genome sequence has a total length of 1,366.18 megabases. Most of the assembly (99.91%) is scaffolded into 8 chromosomal pseudomolecules, including the X and Y sex chromosomes. The mitochondrial genome has also been assembled, with a length of 16.14 kilobases. Gene annotation of this assembly at Ensembl identified 13,398 protein-coding genes.

Keywords

Microdon myrmicae, Bog Ant Fly, genome sequence, chromosomal, Diptera

This article is included in the Tree of Life gateway.

Open Peer Review

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	1	2
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Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Diptera; Brachycera; Muscomorpha; Eremoneura; Cyclorrhapha; Aschiza; Syrphoidea; Syrphidae; Microdontinae; *Microdon*; *Microdon myrmicae* Schönrogge, *et al*, 2002 (NCBI:txid2767528)

Background

Microdon myrmicae is a hoverfly belonging to the outlying subfamily Microdontinae within the Syrphidae (Reemer & Ståhls, 2013). Members of this subfamily are atypical hoverflies in that they are myrmecophiles, their larvae inhabiting ant nests where they feed upon the ant brood. As a consequence, they have been the subject of intensive research (e.g., Scarparo *et al.*, 2019). Morphologically the adults are also unusual for hoverflies because they have an extra partial cross-vein that largely divides cell r_5 into two. Furthermore, they do not hover, indeed they are characteristically sedentary, spending much time sitting on low vegetation. The adults are dumpy with short wings and long forward-pointing antennae (Stubbs & Falk, 2002).

M. myrmicae is one of four *Microdon* species known to occur in Britain and Ireland and the most widespread, occurring in suitable habitat across much of western Britain and in the Thames Basin and New Forest. Elsewhere it occurs across Europe from France to Russia, excluding Mediterranean countries and the far north (GBIF Secretariat, 2023). A further two *Microdon* species occur elsewhere in Europe (Scarparo *et al.*, 2017).

M. myrmicae inhabits wet heaths, mires and bogs, where its hosts, red ants of the genus *Myrmica*, occur. The adults, which are not known to feed, live for up to three weeks (Wolton, 2011). Often the easiest way to find the species is to search for the strange slug-like white larvae and reddish-brown puparia within ant nests. Both second and third instar larvae and the puparia are hemispherical and covered by a tough cuticle. The first instar is more mobile, flatter and much more delicate, being more susceptible to predation and dehydration (Scarparo *et al.*, 2020; Wolton, 2011). It is thought that *Microdon* larvae gain protection from their ant hosts through a combination of chemical mimicry and structural defences such as the multi-layered cuticle, retractable head, dome-shaped tergum and a flat and strongly adhesive "foot" (sternum) (Scarparo *et al.*, 2019).

M. myrmicae is very closely related phylogenetically to *M. mutabilis* (Linnaeus, 1758), from which it was split in 2002 (Schönrogge *et al.*, 2002). Morphologically, neither larvae nor adults can be told apart, only the puparia differ, by the shape and length of their anterior spiracles and presence or absence of long hairs on the dorsum. In terms of both their host ant and habitat, however, the two species are very different: *M. mutabilis* larvae inhabit the nests of black ants of the genus *Formica* in well-drained habitats such as limestone pavement (Scarparo *et al.*, 2017). Thus, although the two *Microdon* species may be nearly identical morphologically, they exploit hosts of two distant ant subfamilies (Formicinae and Myrmicinae).

Here we present a chromosome-level genome sequence for *Microdon myrmicae*, based on a specimen from Locks Park Farm, Devon, United Kingdom (Figure 1).

Genome sequence report

Sequencing data

The genome of a specimen of *Microdon myrmicae* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 39.58 Gb (gigabases) from 4.04 million reads. GenomeScope analysis of the PacBio HiFi data estimated the haploid genome size at 1,241 Mb, with a heterozygosity of 1.47% and repeat content of 49.88%. 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 31.0x coverage of the genome. Chromosome conformation Hi-C sequencing produced 223.63 Gb from 1,480.96 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 9 misjoins or missing joins. These interventions decreased the scaffold count by 8.33%. The final assembly has a total length of 1,366.18 Mb in 65 scaffolds, with 579 gaps, and a scaffold N50 of 194.8 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 (98.86%) was assigned to 8 chromosomal-level scaffolds, representing 6 autosomes and the

Figure 1. Photograph of the *Microdon myrmicae* (idMicMyrm1) specimen used for genome sequencing.

Table 1. **Specimen and sequencing data for *Microdon myrmicae*.**

Project information			
Study title	Microdon myrmicae (bog ant hoverfly)		
Umbrella BioProject	PRJEB67421		
Species	*Microdon myrmicae*		
BioSpecimen	SAMEA112232574		
NCBI taxonomy ID	2767528		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	idMicMyrm1	SAMEA112233024	head and thorax
Hi-C sequencing	idMicMyrm1	SAMEA112233024	head and thorax
RNA sequencing	idMicMyrm1	SAMEA112233025	abdomen
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq 6000	ERR12121871	1.48e+09	223.63
PacBio Revio	ERR12120047	4.04e+06	39.58
RNA Illumina NovaSeq X	ERR12861040	7.09e+07	10.7

Table 2. **Genome assembly data for *Microdon myrmicae*.**

Genome assembly		
Assembly name	idMicMyrm1.1	
Assembly accession	GCA_963931805.1	
Alternate haplotype accession	*GCA_963931795.1*	
Assembly level for primary assembly	chromosome	
Span (Mb)	1,366.18	
Number of contigs	644	
Number of scaffolds	65	
Longest scaffold (Mb)	269.14	
Assembly metric	**Measure**	***Benchmark***
Contig N50 length	4.01 Mb	*≥ 1 Mb*
Scaffold N50 length	194.8 Mb	*= chromosome N50*
Consensus quality (QV)	Primary: 61.6; alternate: 61.6; combined 61.6	*≥ 40*
k-mer completeness	Primary: 80.70%; alternate: 70.64%; combined: 98.37%	*≥ 95%*
BUSCO*	C:94.8%[S:92.8%,D:2.0%], F:0.8%,M:4.4%,n:3,285	*S > 90%; D < 5%*
Percentage of assembly mapped to chromosomes	98.86%	*≥ 90%*
Sex chromosomes	X and Y	*localised homologous pairs*
Organelles	Mitochondrial genome: 16.14 kb	*complete single alleles*

* BUSCO scores based on the diptera_odb10 BUSCO set using version 5.5.0. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison.

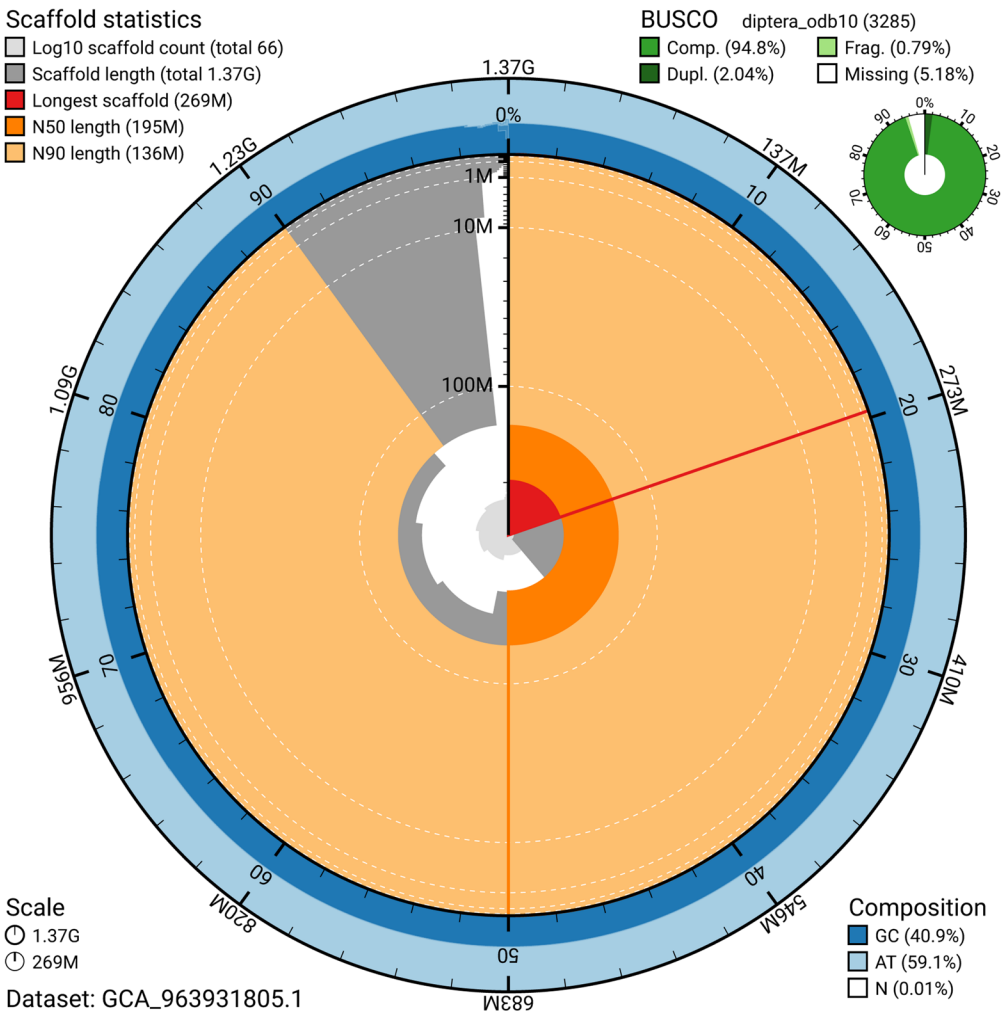

Figure 2. Genome assembly of *Microdon myrmicae*, idMicMyrm1.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. The outermost blue tracks display the distribution of GC, AT, and N percentages across the bins. Scaffolds are arranged clockwise from longest to shortest and are depicted in dark grey. The longest scaffold is indicated by the red arc, and the deeper orange and pale orange arcs represent the N50 and N90 lengths. A light grey spiral at the centre shows the cumulative scaffold count on a logarithmic scale. A summary of complete, fragmented, duplicated, and missing BUSCO genes in the diptera_odb10 set is presented at the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963931805.1/dataset/GCA_963931805.1/snail.

X and Y sex chromosome. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 5; Table 3). During curation, chromosomes X and Y were assigned based on read coverage statistics.

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.

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 combined primary and alternate assemblies achieve an estimated QV of 61.6. The *k*-mer recovery for the primary haplotype is 80.70%, and for the alternate haplotype 70.64%; the combined primary and alternate assemblies have a *k*-mer recovery of 98.37%. BUSCO analysis using the diptera_odb10 reference set (n = 3,285) identified 94.8% of the expected gene set (single = 92.8%, duplicated = 2.0%).

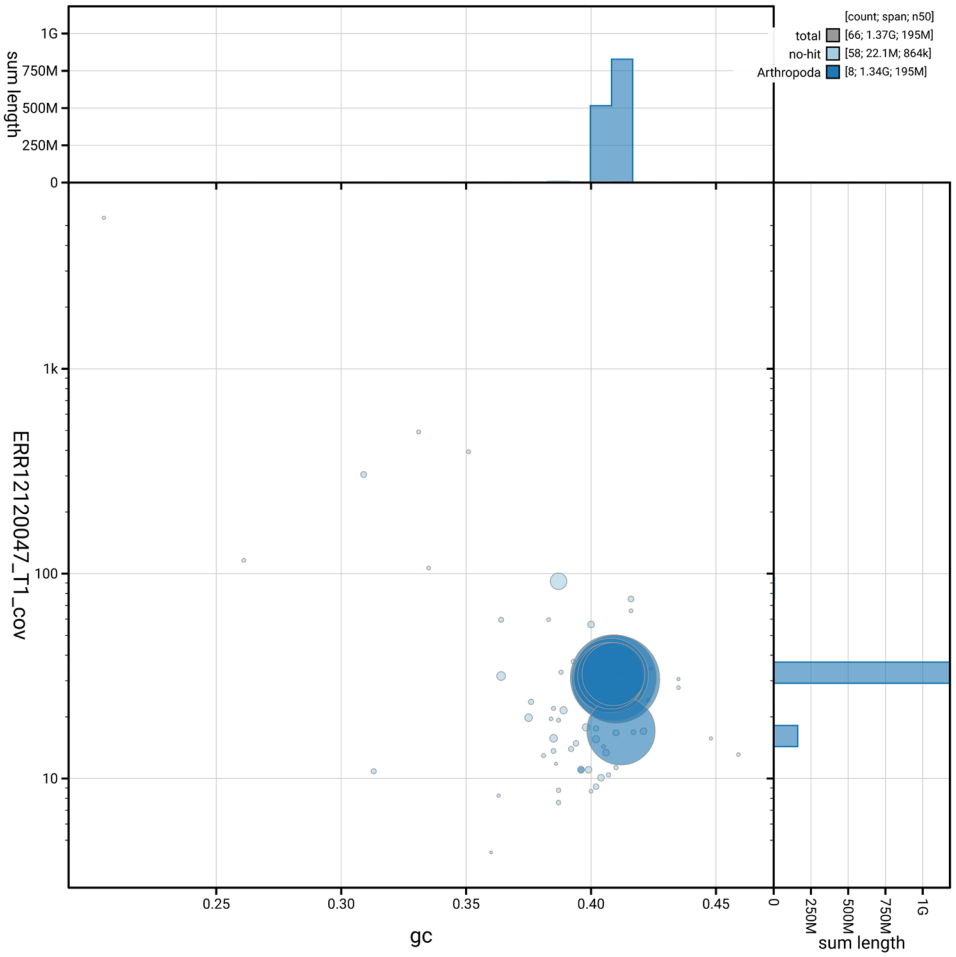

Figure 3. Genome assembly of *Microdon myrmicae*, idMicMyrm1.1: BlobToolKit GC-coverage plot. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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_963931805.1/blob.

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.Q61.**

Genome annotation report

The *Microdon myrmicae* genome assembly (GCA_963931805.1) was annotated externally by Ensembl at the European Bioinformatics Institute (EBI). This annotation includes 48,566 transcribed mRNAs from 13,398 protein-coding and 28,058 non-coding genes. The average transcript length is 12,927.67. There are 1.17 coding transcripts per gene and 2.88 exons per transcript. For further information about the annotation, please refer to https://rapid.ensembl.org/Microdon_myrmicae_GCA_963931805.1/Info/Index.

Methods

Sample acquisition and DNA barcoding

An adult male *Microdon myrmicae* (specimen ID Ox002352, ToLID idMicMyrm1) was collected from Locks Park Farm, Devon, United Kingdom (latitude 50.8, longitude –4.10) on 2022-05-19 by netting. This was the study site used for an in-depth behavioural and ecological study of the species, where numerous puparia had been found within *Myrmica scabrinodis* nests and identified as *Microdon myrmicae* (Wolton, 2011). The specimen was collected by Robert Wolton (Dipterists Forum) and preserved on dry ice. The specimen was collected and identified by Robert Wolton (Dipterists Forum) 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

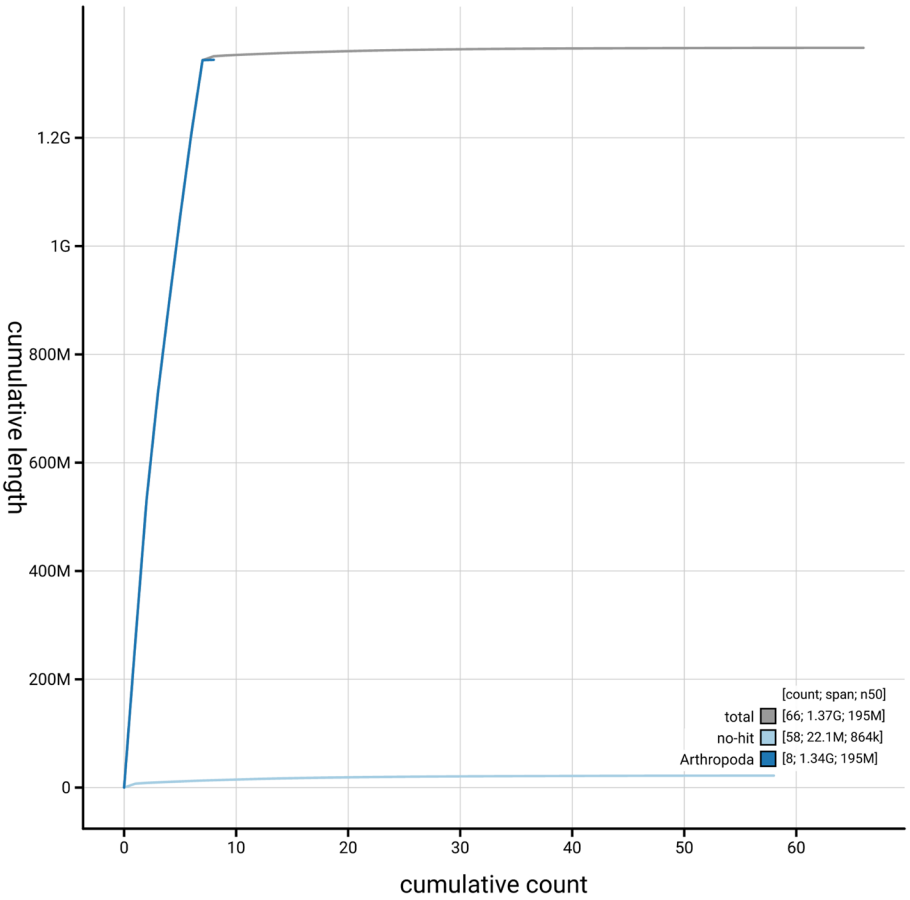

Figure 4. Genome assembly of *Microdon myrmicae*, idMicMyrm1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_963931805.1/dataset/GCA_963931805.1/cumulative.

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 idMicMyrm1 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).

RNA was extracted from abdomen tissue of idMicMyrm1 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ *mir*Vana protocol (do Amaral *et al.*, 2023). The RNA concentration was assessed using a Nanodrop spectrophotometer and a Qubit Fluorometer 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.

Hi-C sample preparation

Tissue from the head and thorax of the idMicMyrm1 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

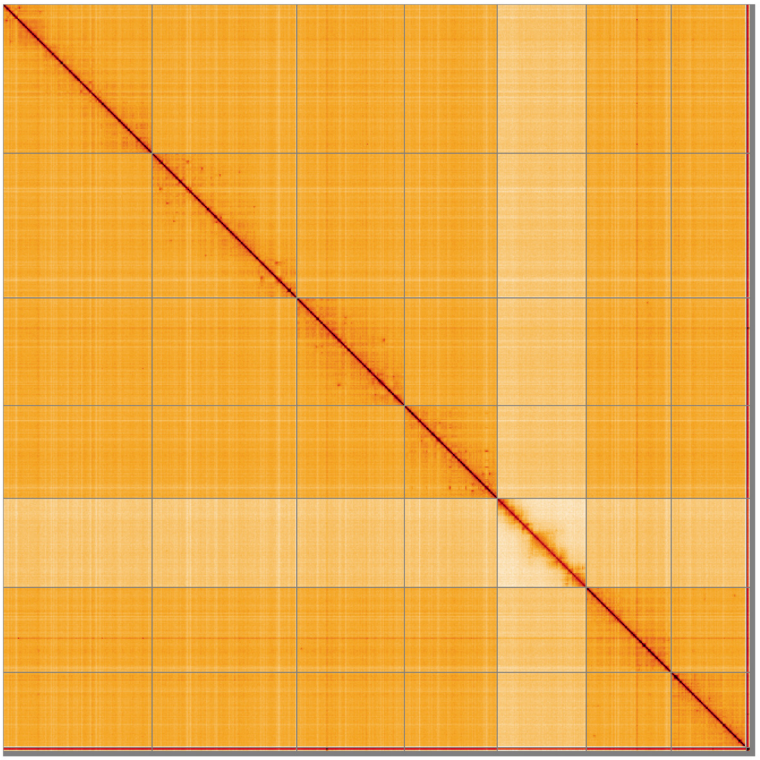

Figure 5. Genome assembly of *Microdon myrmicae:* Hi-C contact map of the idMicMyrm1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=HZwQMEbcR9-3lI5N0P917A.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Microdon myrmicae*, idMicMyrm1.

INSDC accession	Name	Length (Mb)	GC%
OZ007496.1	1	269.14	41
OZ007497.1	2	261.53	41
OZ007498.1	3	194.8	41
OZ007499.1	4	167.82	40.5
OZ007501.1	5	153.37	41
OZ007502.1	6	135.83	41
OZ007500.1	X	160.93	41
OZ007503.1	Y	7.22	38.5
OZ007504.1	MT	0.02	21

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 (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 on a Revio instrument (Pacific Biosciences, California, USA). Prepared libraries were normalised to 2 nM, and 15 µL was used for making complexes. Primers were annealed and polymerases were hybridised to create circularised complexes according to manufacturer's instructions. The complexes were purified with the 1.2X clean up with SMRTbell beads. The purified complexes were then diluted to the Revio loading concentration (in the range 200–300 pM), and spiked with a Revio sequencing internal control. Samples were sequenced on Revio 25M SMRT cells (Pacific Biosciences, California, USA). The SMRT link software, a PacBio web-based end-to-end workflow manager, was used to set-up and monitor the run, as well as perform primary and secondary analysis of the data upon completion.

Hi-C

For Hi-C library preparation, DNA was fragmented using the Covaris E220 sonicator (Covaris) and size selected using SPRISelect beads to 400 to 600 bp. The DNA was then enriched using the Arima-HiC v2 kit Enrichment beads. Using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) for end repair, a-tailing, and adapter ligation. This uses a custom protocol which resembles the standard NEBNext Ultra II DNA Library Prep protocol but where library preparation occurs while DNA is bound to the Enrichment beads. For library amplification, 10 to 16 PCR cycles were required, determined by the sample biotinylation percentage. The Hi-C sequencing was performed using paired-end sequencing with a read length of 150 bp on an Illumina NovaSeq 6000 instrument.

RNA

Poly(A) RNA-Seq libraries were constructed using the NEB Ultra II RNA Library Prep kit, following the manufacturer's instructions. RNA sequencing was performed on the Illumina NovaSeq X instrument.

Genome assembly, curation and evaluation

Assembly

Prior to assembly of the PacBio HiFi reads, a database of *k*-mer counts (k = 31) was generated from the filtered reads using FastK. GenomeScope2 (Ranallo-Benavidez *et al.*, 2020) was used to analyse the *k*-mer frequency distributions, providing estimates of genome size, heterozygosity, and repeat content.

The HiFi reads were first assembled using Hifiasm (Cheng *et al.*, 2021) with the --primary option. Haplotypic duplications were identified and removed using purge_dups (Guan *et al.*, 2020). The Hi-C reads were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). The contigs were further scaffolded using the provided Hi-C data (Rao *et al.*, 2014) in YaHS (Zhou *et al.*, 2023) using the --break option for handling potential misassemblies. The scaffolded assemblies were evaluated using Gfastats (Formenti *et al.*, 2022), BUSCO (Manni *et al.*, 2021) and MERQURY.FK (Rhie *et al.*, 2020).

The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2023), which runs MitoFinder (Allio *et al.*, 2020) and uses these annotations to select the final mitochondrial contig and to ensure the general quality of the sequence.

Assembly curation

The assembly was decontaminated using the Assembly Screen for Cobionts and Contaminants (ASCC) pipeline. Flat files and maps used in curation were generated via the TreeVal pipeline (Pointon *et al.*, 2023). Manual curation was conducted primarily in PretextView (Harry, 2022) and HiGlass (Kerpedjiev *et al.*, 2018), with additional insights provided by JBrowse2 (Diesh *et al.*, 2023). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Any identified contamination, missed joins, and mis-joins were amended, and duplicate sequences were tagged and removed. The curation process is documented at https://gitlab.com/wtsi-grit/rapid-curation.

Assembly quality assessment

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

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

Wellcome Sanger Institute – Legal and Governance

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

Further, the Wellcome Sanger Institute employs a process whereby due diligence is carried out proportionate to the nature

Table 4. Software tools: versions and sources.

Software tool	Version	Source
BEDTools	2.30.0	https://github.com/arq5x/bedtools2
BLAST	2.14.0	ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/
BlobToolKit	4.3.9	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.5.0	https://gitlab.com/ezlab/busco
bwa-mem2	2.2.1	https://github.com/bwa-mem2/bwa-mem2
Cooler	0.8.11	https://github.com/open2c/cooler
DIAMOND	2.1.8	https://github.com/bbuchfink/diamond
fasta_windows	0.2.4	https://github.com/tolkit/fasta_windows
FastK	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	0.19.5-r587	https://github.com/chhylp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84a a44357826c0b6753eb28de	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	0.2.5	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

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: Microdon myrmicae (bog ant hoverfly). Accession number PRJEB67421; https://identifiers.org/ena.embl/PRJEB67421. The genome sequence is released openly for reuse. The *Microdon myrmicae* 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.

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Open Peer Review

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Version 1

Reviewer Report 09 May 2025

https://doi.org/10.21956/wellcomeopenres.26370.r121552

✔ **Komal Kumar Bollepogu Raja**
Baylor College of Medicine, Houston, USA

The manuscript by Wolton J., Robert presents the genome assembly of *Microdon myrmicae* with a total length of 1,366.18 Mb and 8 chromosomal pseudomolecules. The author has identified 13,398 protein-coding genes. The background sufficiently introduces the taxonomical, biological and ecological aspects of the organism. The assembly quality metrics suggest that the genome sequence is of high quality. For instance, the BUSCO is 94.8% and the kmer completeness id 98.37%. Further, the percentage of assembly mapped to chromosome is also impressive at 98.86%. The methodology is robust and allows for reproducibility. Overall, all standard bioinformatics tools have been used for the assembly and the manuscript is well written. The genome sequence is a good resource for other researchers.

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: Single cell genomics, Bioinformatics, genomics, and neurobiology

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 05 May 2025

https://doi.org/10.21956/wellcomeopenres.26370.r121549

Henrique Antoniolli
Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

The present study reports a highly contiguous genome assembly for a bog ant fly, *Microdon myrmicae*. The background section brings an interesting overview on its general biology, however does not clearly state the motivations for sequencing its genome. Protocols are well described and software are clearly referenced. The mitochondrion sequence should be deposited along with its gene annotation. The data produced yielded satisfactory results on assembly statistics, therefore I recommend indexing this paper.

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

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

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

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

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genomics, evolution

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.