DATA NOTE

The genome sequence of Schreibers's Long-fingered Bat, *Miniopterus schreibersii* (Kuhl, 1817) (Chiroptera: Miniopteridae)

[version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from an individual male *Miniopterus schreibersii* (Schreibers's Long-fingered Bat; Chordata; Mammalia; Chiroptera; Miniopteridae). The assembly contains two haplotypes with total lengths of 1 850.57 megabases and 1 699.63 megabases. Most of haplotype 1 (97.59%) is scaffolded into 24 chromosomal pseudomolecules, including the X and Y sex chromosomes. Haplotype 2 was assembled to scaffold level. The mitochondrial genome has also been assembled, with a length of 16.63 kilobases. Gene annotation of this assembly on Ensembl identified 18 289 protein-coding genes. 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

Miniopterus schreibersii; Schreibers's Long-fingered Bat; genome sequence; chromosomal; Chiroptera

Open Peer Review

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Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Deuterostomia; Chordata; Craniata; Vertebrata; Gnathostomata; Teleostomi; Euteleostomi; Sarcopterygii; Dipnotetrapodomorpha; Tetrapoda; Amniota; Mammalia; Theria; Eutheria; Boreoeutheria; Laurasiatheria; Chiroptera; Yangochiroptera; Miniopteridae; Miniopterus; *Miniopterus schreibersii* (Kuhl, 1817) (NCBI:txid9433).

Background

The Schreiber's bent-winged bat, *Miniopterus schreibersii* is a medium-sized bat with long, narrow wings with a pronounced "bend" at the third finger, a short domed head, and soft brownish-grey fur (Kuhl, 1819). Its common name, 'common bent-wing bat', derives from the curvature of the wing, and it is also referred as 'Schreiber's long-fingered bat' or 'Schreiber's bat'. Identification is primarily based on its characteristic wing shape and cranial features, although cryptic species within the complex can only be reliably distinguished using genetic and cranio-dental analyses, as subtle external differences are insufficient for field identification (Puechmaille *et al.*, 2014).

The species has a broad distribution extending across southern Europe, North Africa, the Middle East, the Caucasus, Central and East Asia, and into Australasia. GBIF.org records confirm this extensive range, with populations documented from the Iberian Peninsula through the Balkans and Anatolia to regions of the Palaearctic and Ethiopian biogeographic zones (Appleton *et al.*, 2004; Bilgin *et al.*, 2016; Puechmaille *et al.*, 2014). Phylogeographic studies indicate that *M. schreibersii* recolonized Europe from glacial refugia in the eastern Mediterranean following the last glacial maximum, while additional cryptic lineages persist in North Africa (Bilgin *et al.*, 2016; Puechmaille *et al.*, 2014). This evolutionary history reflects both the species' high dispersal capability and its ability to adapt to diverse climatic and ecological conditions.

Typical habitats include caves, tunnels, and abandoned mines, where large colonies form dense aggregations. The species is highly gregarious and migratory, with some individuals undertaking long-distance seasonal movements, particularly across eastern and southeastern Europe (Wright *et al.*, 2020). Maternity colonies form in spring, consisting of females that give birth to a single pup annually, exhibiting strong philopatry to natal roosts (Appleton *et al.*, 2004; Gürün *et al.*, 2019; Wright *et al.*, 2020).

Miniopterus schreibersii displays remarkable biological adaptations, including exceptional flight performance suited to long-distance migration, high gene flow among populations, and a complex social structure, characterised by spatial separation between mating and breeding sites (Dufresnes *et al.*, 2023; Wright *et al.*, 2020). The genus *Miniopterus* is also notable for its cryptic speciation and was recently reclassified into its own family, Miniopteridae, based on molecular and morphological evidence (Demos *et al.*, 2023; Miller-Butterworth *et al.*, 2007).

The species is listed as Near Threatened on the IUCN Red List due to population declines and habitat fragmentation, particularly in Europe (Dufresnes *et al.*, 2023; Wood *et al.*, 2011). Conservation efforts are increasingly informed by molecular and tracking studies, which reveal population connectivity and migratory behavior across the continent.

High-resolution genomic and phylogenomic analyses, including RAD-seq and ultraconserved element (UCE) studies, have advanced understanding of population structure, cryptic diversity, and evolutionary history (Demos *et al.*, 2023; Dufresnes *et al.*, 2023). The completed genome sequence provides a powerful resource for research into population genetics, disease ecology – given its role as a potential reservoir for zoonotic viruses – and conservation management. It further supports the development of tools for accurate species identification, long-term monitoring, and investigation of adaptive traits underlying flight, migration, and longevity (Demos *et al.*, 2023; Dufresnes *et al.*, 2023).

Another scaffold-level genome assembly for this species is available (GCA_004026525.1; submitted by the Broad Institute). Here we present a chromosome-level genome sequence for *Miniopterus schreibersii.* The sequence is based on tissue obtained from a male collected in an abandoned mine in the Troodos mountains, Cyprus (Figure 1). This species occupies a range of elevations depending on the season, occurring at low altitudes near the sea level (around 50 m) and at higher elevations up to about 1305 m above sea level (Benda *et al.*, 2007). This sampling is part of the Bat1K Project (Teeling *et al.*, 2018) and the Darwin Tree of Life Project (DToL) (Darwin Tree of Life Project Consortium, 2022). The Bat1K Project is a collaborative effort to sequence all extant bat species, and DToL aims to sequence all named eukaryotic species in the Atlantic Archipelago of Britain and Ireland.

Methods

Sample acquisition

The specimen used for genome sequencing was an adult male *Miniopterus schreibersii* (specimen ID SAN00002494, ToLID mMinSch1; Figure 1). Groups of *M. schreibersii* consisting of 2 to 10 individuals were observed roosting on the ceiling of an abandoned mine in the Troodos mountains, Cyprus (latitude 35.1464, longitude 33.4098) on 2022-12-05. Sites of this species in Cyprus have been previously documented (Benda *et al.*, 2007). One male individual was collected from these groups. The specimen was approximately 5.5 cm from head to bottom (excluding the tail). It was transported to a laboratory in Nicosia, where it was euthanised, dissected and multiple organs were extracted. Samples were placed in Eppendorf tubes and snap frozen in liquid nitrogen, and stored at −80 °C until it was shipped for sequencing.

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 mMinSch1 sample was weighed and triaged to determine the appropriate extraction protocol. Heart tissue was homogenised by powermashing using a PowerMasher II tissue disruptor. HMW DNA was extracted 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 automated 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 22.0 ng/μL and a yield of 2 860.00 ng.

RNA was extracted from brain tissue of mMinSch1 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ *mir*Vana 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

Figure 1. Common bent-wing bat, *Miniopterus schreibersii*. Individuals of *M. schreibersii*. (A-B) The specimen was collected from a group roosting in a cave in Cyprus. (Photos taken by Ine Alvarez van Tussenbroek).

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 on a Revio instrument (Pacific Biosciences). The prepared library was normalised to 2 nM, and 15 µL was used for making complexes. Primers were annealed and polymerases bound to generate circularised complexes, following the manufacturer's instructions. Complexes were purified using 1.2X SMRTbell beads, then diluted to the Revio loading concentration (200–300 pM) and spiked with a Revio sequencing internal control. The sample was sequenced on a Revio 25 M SMRT cell. The SMRT Link software (Pacific Biosciences), a web-based workflow manager, was used to configure and monitor the run and to carry out primary and secondary data analysis.

Hi-C

Sample preparation and crosslinking

The Hi-C sample was prepared from 20–50 mg of frozen heart tissue from the mMinSch1 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 Diagnocine 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 10–16 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 to create 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 X.

RNA library preparation and sequencing

Libraries were prepared using the NEBNext® Ultra™ 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, generating 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 in Hi-C phasing mode (Cheng *et al.*, 2021, 2022), producing two haplotypes. Hi-C reads (Rao *et al.*, 2014) were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). Contigs were further scaffolded with Hi-C data 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 MerquryFK (Rhie *et al.*, 2020).

The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2023).

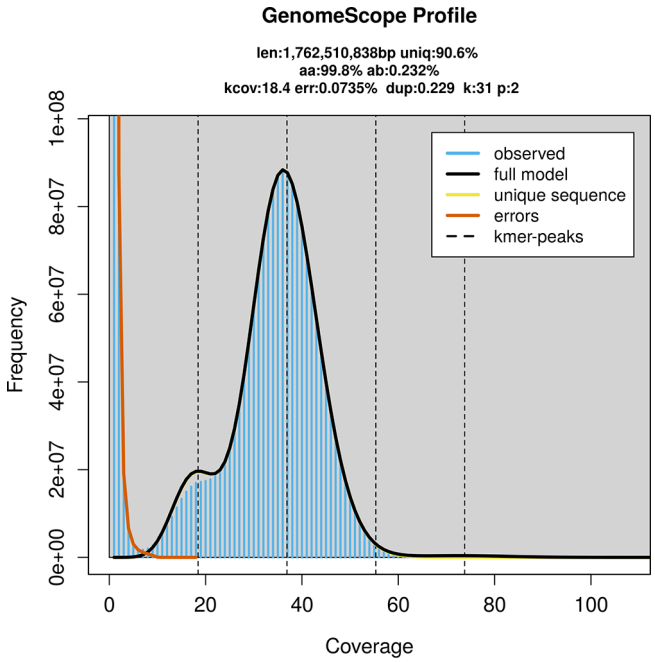

Figure 2. Frequency distribution of *k*-mers generated using GenomeScope2. The plot shows observed and modelled *k*-mer spectra, providing estimates of genome size, heterozygosity, and repeat content based on unassembled sequencing reads.

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 seven breaks and 55 joins. This reduced the scaffold count by 14.3%, increased the scaffold N50 by 3.4%, and increased the total assembly length by 0.5%. The curation process is described at https://gitlab.com/wtsi-grit/rapid-curation. PretextSnapshot was used to generate a Hi-C contact map of the final assembly.

Assembly quality assessment

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

Table 1. Specimen and sequencing data for BioProject PRJEB74978.

Platform	PacBio HiFi	Hi-C	RNA-seq
ToLID	mMinSch1	mMinSch1	mMinSch1
Specimen ID	SAN00002494	SAN00002494	SAN00002494
BioSample (source individual)	SAMEA113980738	SAMEA113980738	SAMEA113980738
BioSample (tissue)	SAMEA113980757	SAMEA113980757	SAMEA113980764
Tissue	heart	heart	brain
Instrument	Revio	Illumina NovaSeq X	Illumina NovaSeq X
Run accessions	ERR12921321	ERR12945475	ERR13493944
Read count total	6.43 million	1 355.39 million	89.43 million
Base count total	66.71 Gb	204.66 Gb	13.50 Gb

Table 2. **Genome assembly statistics.**

Assembly name	mMinSch1.hap1.2	mMinSch1.hap2.2
Assembly accession	GCA_964146895.2	GCA_964145185.2
Assembly level	chromosome	scaffold
Span (Mb)	1 850.57	1 699.63
Number of chromosomes	24	scaffold-level
Number of contigs	325	254
Contig N50	28.01 Mb	31.77 Mb
Number of scaffolds	185	168
Scaffold N50	87.8 Mb	87.05 Mb
Longest scaffold length (Mb)	202.31	-
Sex chromosomes	X and Y	-
Organelles	Mitochondrion: 16.63 kb	-

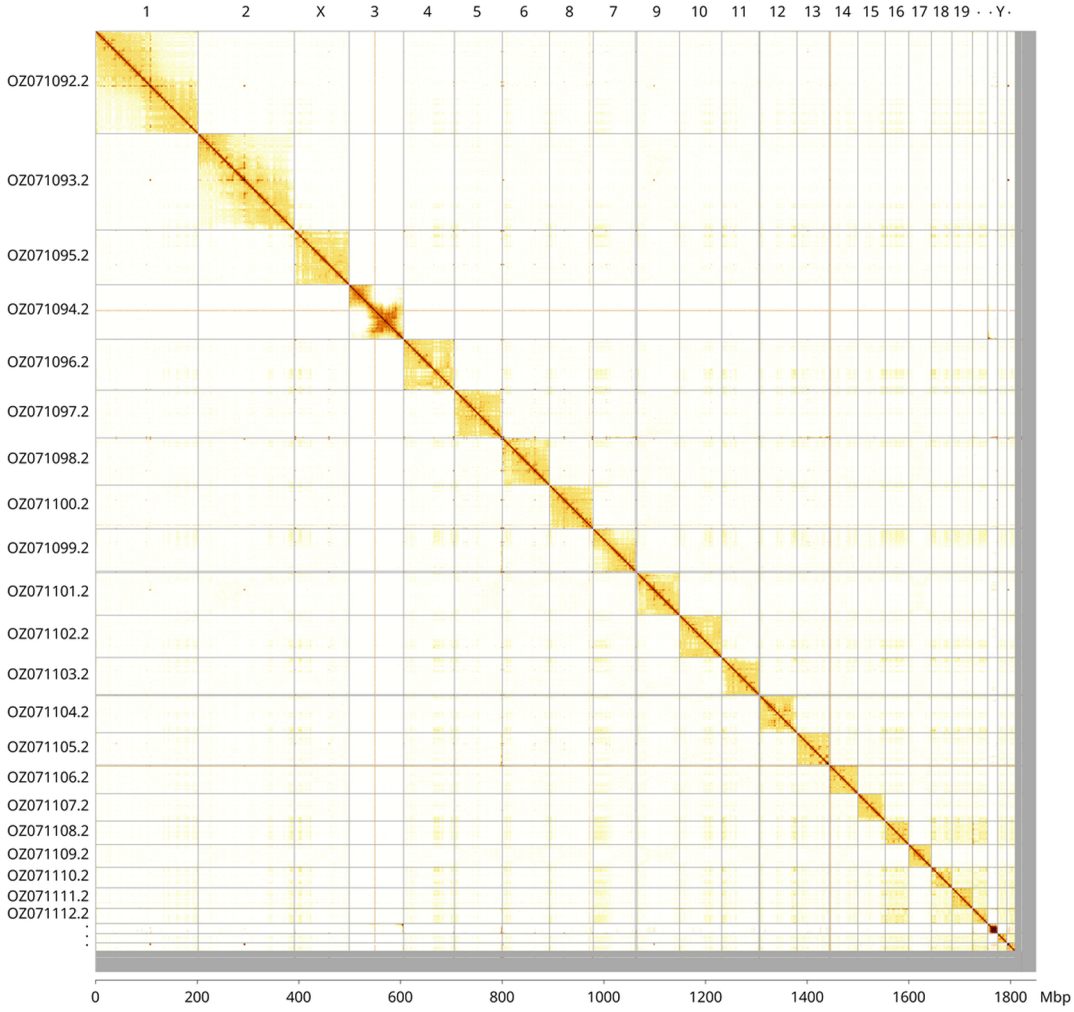

Figure 3. **Hi-C contact map of the *Miniopterus schreibersii* genome assembly.** Assembled chromosomes are shown in order of size and labelled along the axes, with a megabase scale shown below. The plot was generated using PretextSnapshot.

Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Miniopterus schreibersii* mMinSch1.

INSDC accession	Molecule	Length (Mb)	GC%
OZ071092.2	1	202.31	41
OZ071093.2	2	187.81	40
OZ071094.2	3	105.36	42
OZ071096.2	4	102.19	44
OZ071097.2	5	92.84	40.50
OZ071098.2	6	91.04	41
OZ071099.2	7	85.52	41
OZ071100.2	8	87.80	44
OZ071101.2	9	84.58	40.50
OZ071102.2	10	83.37	43.50
OZ071103.2	11	74.81	43.50
OZ071104.2	12	74.20	43.50
OZ071105.2	13	63.12	43.50
OZ071106.2	14	56.44	44
OZ071107.2	15	53.95	44
OZ071108.2	16	46.13	47
OZ071109.2	17	44.60	43
OZ071110.2	18	40.65	48
OZ071111.2	19	40.17	45.50
OZ071112.2	20	28.13	48
OZ071114.2	21	19.08	46.50
OZ071115.2	22	16.11	48
OZ071095.2	X	106.82	39
OZ071113.2	Y	18.87	45

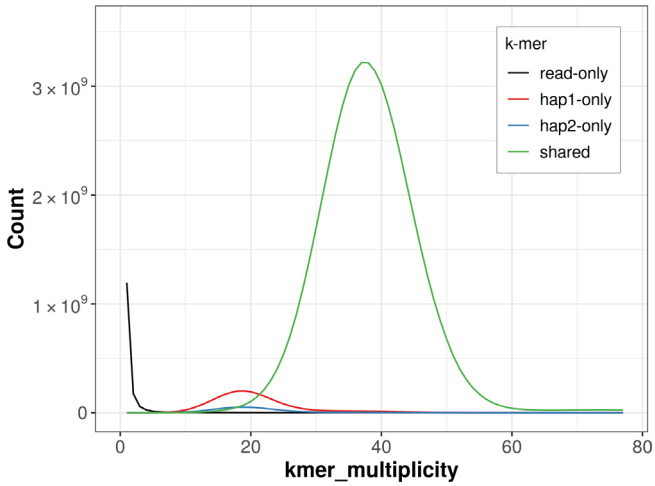

Figure 4. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers 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.

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).

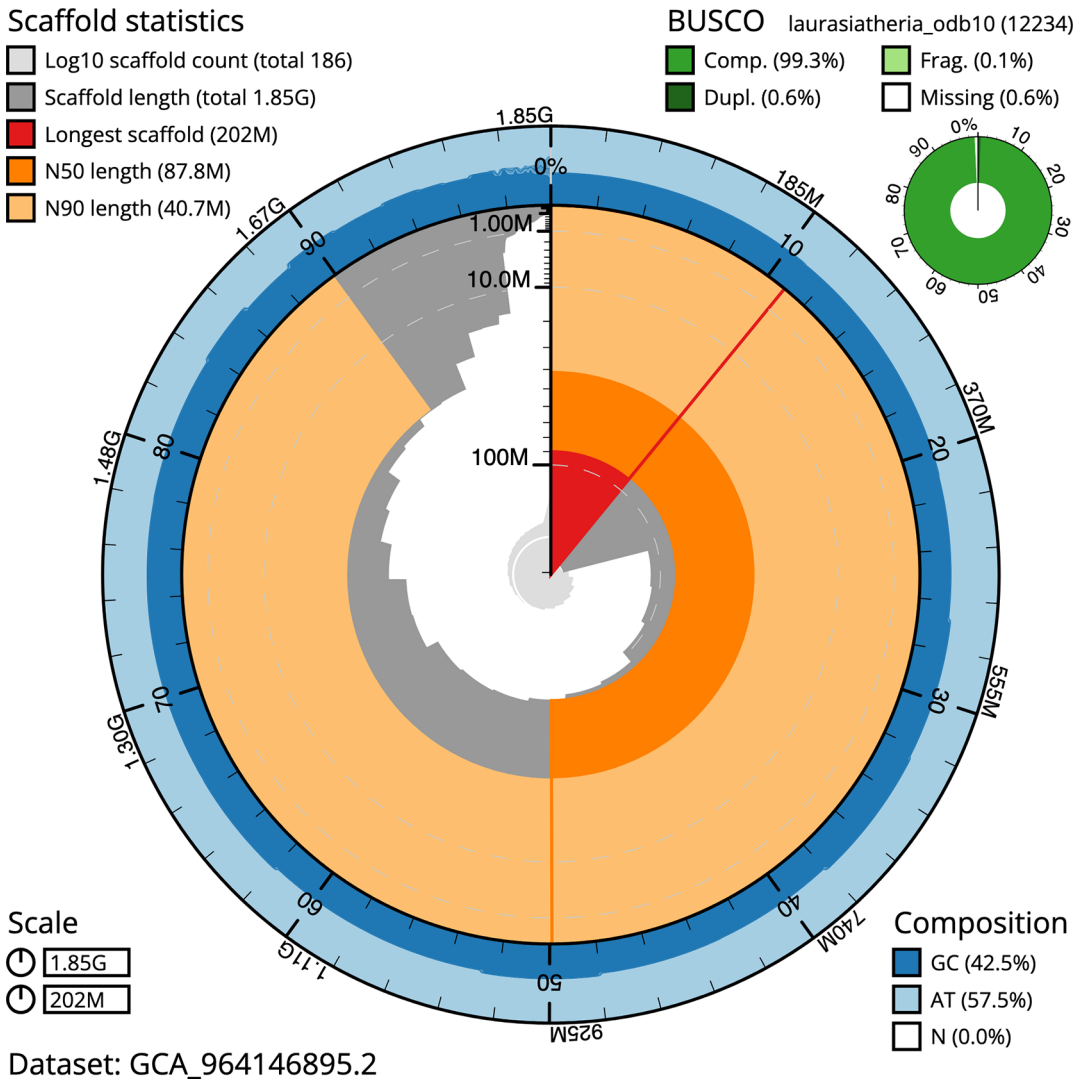

Figure 5. Assembly metrics for mMinSch1.hap1.2. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1 000 bins around the circumference. The outermost blue 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 set is presented at the top right. An interactive version of this figure can be accessed on the BlobToolKit viewer.

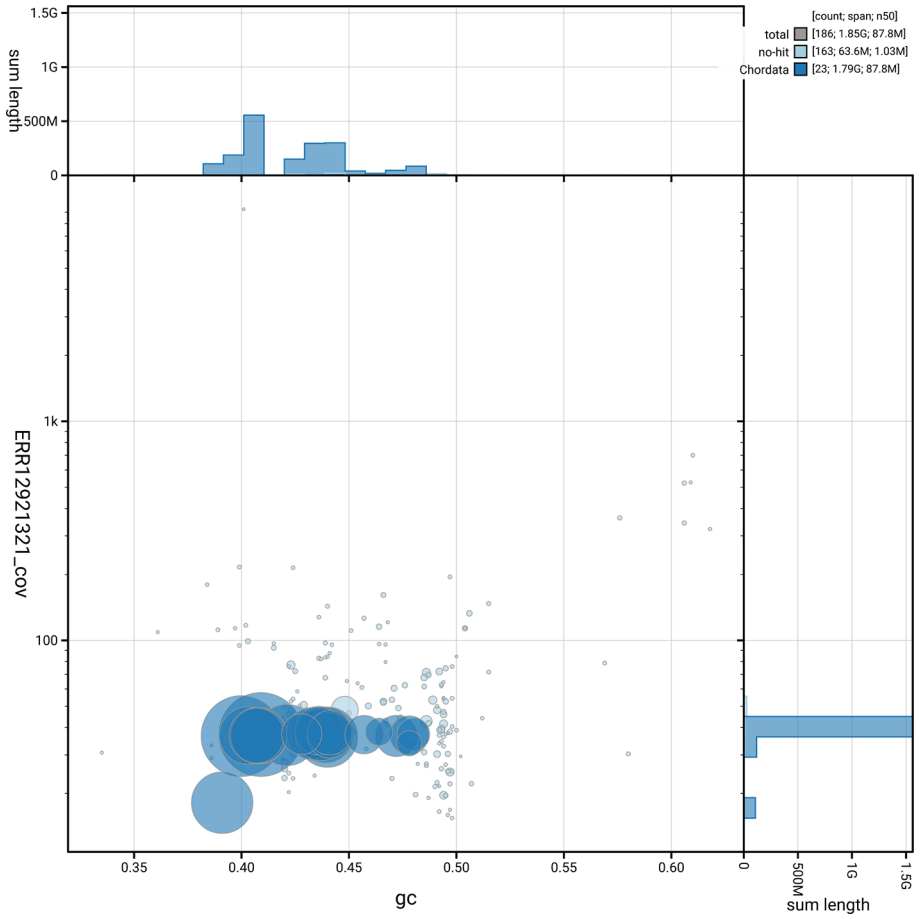

Figure 6. BlobToolKit blob plot for mMinSch1.hap1.2. The plot shows base coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. The histograms along the axes display the total 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 *Miniopterus schreibersii* assembly.

Measure	Value	Benchmark
EBP summary (haplotype 1)	7.C.Q61	6.C.Q40
Contig N50 length	28.01 Mb	≥ 1 Mb
Scaffold N50 length	87.80 Mb	= chromosome N50
Consensus quality (QV)	Haplotype 1: 61.8; haplotype 2: 61.7; combined: 61.8	≥ 40
k-mer completeness	Haplotype 1: 97.74%; Haplotype 2: 91.88%; combined: 99.79%	≥ 95%
BUSCO	C:99.9% [S:95.5%, D:4.4%], F:0.0%, M:0.1%, n:954	S > 90%; D < 5%
Percentage of assembly assigned to chromosomes	97.59%	≥ 90%

Notes: The EBP summary uses log10(Contig N50); chromosome-level (C) or log10(Scaffold N50); Q (Merqury QV). BUSCO: C = complete; S = single-copy; D = duplicated; F = fragmented; M = missing; n = orthologues

Genome sequence report

Sequence data

PacBio sequencing of the *Miniopterus schreibersii* specimen generated 66.71 Gb (gigabases) from 6.43 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 1 762.51 Mb, with a heterozygosity of 0.23% and repeat content of 9.47% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 37× coverage. Hi-C sequencing produced 204.66 Gb from 1 355.39 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 genome was assembled into two haplotypes using Hi-C phasing. Haplotype 1 was curated to chromosome level, while haplotype 2 was assembled to scaffold level. The final assembly has a total length of 1 850.57 Mb in 185 scaffolds, with 140 gaps, and a scaffold N50 of 87.8 Mb (Table 2).

Most of the haplotype 1 assembly sequence (97.59%) was assigned to 24 chromosomal-level scaffolds, representing 22 autosomes and the X and Y sex chromosomes. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to synteny (Figure 3; Table 3). The order and orientation of contigs along the Y chromosome are uncertain in the region 6.6–15.5 Mb.

Table 5. Software versions and sources.

Software	Version	Source
BLAST	2.14.0	ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/
BlobToolKit	4.4.6	https://github.com/blobtoolkit/blobtoolkit
BUSCO	6.0.0	https://gitlab.com/ezlab/busco
bwa-mem2	2.2.1	https://github.com/bwa-mem2/bwa-mem2
DIAMOND	2.1.8	https://github.com/bbuchfink/diamond
fasta_windows	0.2.4	https://github.com/tolkit/fasta_windows
FastK	1.1	https://github.com/thegenemyers/FASTK
GenomeScope2.0	2.0.1	https://github.com/tbenavi1/genomescope2.0
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
Hifiasm	0.19.8-r603	https://github.com/chhylp123/hifiasm
HiGlass	1.13.4	https://github.com/higlass/higlass
MerquryFK	1.1.2	https://github.com/thegenemyers/MERQURY.FK
Minimap2	2.28-r1209	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	24.10.4	https://github.com/nextflow-io/nextflow
PretextSnapshot	0.0.5	https://github.com/sanger-tol/PretextSnapshot
PretextView	1.0.3	https://github.com/sanger-tol/PretextView
samtools	1.21	https://github.com/samtools/samtools
sanger-tol/ascc	0.1.0	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	v0.9.0	https://github.com/sanger-tol/blobtoolkit
sanger-tol/curationpretext	1.4.2	https://github.com/sanger-tol/curationpretext
Seqtk	1.3	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.4.0	https://github.com/sanger-tol/treeval
YaHS	1.2.2	https://github.com/c-zhou/yahs

The mitochondrial genome was also assembled (length 16.63 kb, OZ071116.2). This sequence is included as a contig in the multifasta file of the genome submission and as a standalone record.

Assembly quality metrics

For haplotype 1, the estimated QV is 61.8, and for haplotype 2, 61.7. When the two haplotypes are combined, the assembly achieves an estimated QV of 61.8. The *k*-mer completeness is 97.74% for haplotype 1, 91.88% for haplotype 2, and 99.79% for the combined haplotypes (Figure 4).

BUSCO analysis using the metazoa_odb10 reference set (n = 954) identified 99.9% of the expected gene set (single = 95.5%, duplicated = 4.4%) in haplotype 1. For haplotype 2, BUSCO v.6.0.0 analysis identified 98.5% of the expected gene set (single = 95.7%, duplicated = 2.8%). The snail plot in Figure 5 summarises the scaffold length distribution and other assembly statistics for haplotype 1. The blob plot in Figure 6 shows the distribution of scaffolds by GC proportion and coverage for haplotype 1.

Table 4 lists the assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project Report on Assembly Standards September 2024. The EBP metric, calculated for the haplotype 1, is **7.C.Q61**, meeting the recommended reference standard.

Genome annotation report

The *Miniopterus schreibersii* genome assembly (GCA_964146895.1) was annotated by Ensembl at the European Bioinformatics Institute (EBI). This annotation includes 43 915 transcribed mRNAs from 18 289 protein-coding and 7 391 non-coding genes. The average transcript length is 42 035.13 bp, with an average of 1.69 coding transcripts per gene and 9.66 exons per transcript. Further details of this annotation are available from the Ensembl annotation page.

Author information

Contributors are listed at the following links:

- 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

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. By agreeing with and signing up to the Sampling Code of Practice, the Darwin Tree of Life Partner agrees they will meet the legal and ethical requirements and standards set out within this document in respect of all samples acquired for, and supplied to, the Darwin Tree of Life Project. Further, the Wellcome Sanger Institute employs a process whereby due diligence is carried out proportionate to the nature of the materials themselves, and the circumstances under which they have been/are to be collected and provided for use. The purpose of this is to address and mitigate any potential legal and/or ethical implications of receipt and use of the materials as part of the research project, and to ensure that in doing so we align with best practice wherever possible. The overarching areas of consideration are:

- Ethical review of provenance and sourcing of the material
- Legality of collection, transfer and use (national and international)

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: Miniopterus schreibersii (Schreibers' long-fingered bat). Accession number PRJEB74978. The genome sequence is released openly for reuse. The *Miniopterus schreibersii* genome sequencing initiative is part of the Darwin Tree of Life Project (PRJEB40665), the Sanger Institute Tree of Life Programme (PRJEB43745), the Vertebrate Genomes Project (PRJNA489243) and the Bat1K project (PRJNA489245). All raw sequence data and the assembly have been deposited in INSDC databases. Raw data and assembly accession identifiers are reported in Tables 1 and 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.

Acknowledgements

With thanks to Elena Erotokritou for help collecting samples and the Department of Forests and the Department of Environment of the Ministry of Agriculture, Rural Development and Environment of Cyprus.

References

Altschul SF, Gish W, Miller W, *et al.*: **Basic Local Alignment Search Tool.** *J. Mol. Biol.* 1990; **215**(3): 403–410.
PubMed Abstract | Publisher Full Text

Appleton BR, McKenzie JA, Christidis L: **Molecular systematics and biogeography of the bent-wing bat complex *Miniopterus schreibersii* (Kuhl, 1817) (Chiroptera: Vespertilionidae).** *Mol. Phylogenet. Evol.* 2004; **31**(2): 431–439.
PubMed Abstract | Publisher Full Text

Bateman A, Martin M-J, Orchard S, *et al.*: **UniProt: The Universal Protein Knowledgebase in 2023.** *Nucleic Acids Res.* 2023; **51**(D1): D523–D531.
PubMed Abstract | Publisher Full Text | Free Full Text

Benda P, Hanák V, Horáček I, *et al.*: **Bats (Mammalia: Chiroptera) of the Eastern Mediterranean. Part 5. Bat fauna of Cyprus: Review of records with confirmation of six species new for the island and description of a new subspecies.** *Acta Societatis Zoologicae Bohemicae.* 2007; **71**: 71–130.

Bilgin R, Gürün K, Rebelo H, *et al.*: **Circum-Mediterranean phylogeography of a bat coupled with past environmental niche modeling: A new paradigm for the recolonization of Europe?.** *Mol. Phylogenet. Evol.* 2016; **99**: 323–336.
PubMed Abstract | Publisher Full Text

Buchfink B, Reuter K, Drost H-G: **Sensitive protein alignments at tree-of-life scale using DIAMOND.** *Nat. Methods.* 2021; **18**(4): 366–368.
PubMed Abstract | Publisher Full Text | Free Full Text

Challis R, Richards E, Rajan J, *et al.*: **BlobToolKit – interactive quality assessment of genome assemblies.** *G3: Genes, Genomes, Genetics.* 2020; **10**(4): 1361–1374.
PubMed Abstract | Publisher Full Text | Free Full Text

Cheng H, Concepcion GT, Feng X, *et al.*: **Haplotype-resolved *de novo* assembly using phased assembly graphs with Hifiasm.** *Nat. Methods.* 2021; **18**(2): 170–175.
PubMed Abstract | Publisher Full Text | Free Full Text

Cheng H, Jarvis ED, Fedrigo O, *et al.*: **Haplotype-resolved assembly of diploid genomes without parental data.** *Nat. Biotechnol.* 2022; **40**(9): 1332–1335.
PubMed Abstract | Publisher Full Text | Free Full Text

Danecek P, Bonfield JK, Liddle J, *et al.*: **Twelve years of SAMtools and BCFtools.** *GigaScience.* 2021; **10**(2).
PubMed Abstract | Publisher Full Text | Free Full Text

Darwin Tree of Life Project Consortium: **Sequence locally, think globally: The Darwin Tree of Life Project.** *Proc. Natl. Acad. Sci. USA.* 2022; **119**(4): e2115642118.
PubMed Abstract | Publisher Full Text | Free Full Text

Demos TC, Webala PW, Goodman SM, *et al.*: **Ultraconserved elements resolve phylogenetic relationships and biogeographic history of African-Malagasy bent-winged bats (*Miniopterus*).** *Mol. Phylogenet. Evol.* 2023; **188**: 107890.
PubMed Abstract | Publisher Full Text

Dufresnes C, Dutoit L, Brelsford A, *et al.*: **Inferring genetic structure when there is little: Population genetics versus genomics of the threatened bat *Miniopterus schreibersii* across Europe.** *Sci. Rep.* 2023; **13**(1): 1523.
PubMed Abstract | Publisher Full Text | Free Full Text

Ewels P, Magnusson M, Lundin S, *et al.*: **MultiQC: Summarize analysis results for multiple tools and samples in a single report.** *Bioinformatics.* 2016; **32**(19): 3047–3048.
PubMed Abstract | Publisher Full Text | Free Full Text

Ewels PA, Peltzer A, Fillinger S, *et al.*: **The nf-core framework for community-curated bioinformatics pipelines.** *Nat. Biotechnol.* 2020; **38**(3): 276–278.
PubMed Abstract | Publisher Full Text

Formenti G, Abueg L, Brajuka A, *et al.*: **Gfastats: Conversion, evaluation and manipulation of genome sequences using assembly graphs.** *Bioinformatics.* 2022; **38**(17): 4214–4216.
PubMed Abstract | Publisher Full Text | Free Full Text

Gürün K, Furman A, Juste J, *et al.*: **A continent-scale study of the social structure and phylogeography of the bent-wing bat, *Miniopterus schreibersii* (Mammalia: Chiroptera), using new microsatellite data.** *J. Mammal.* 2019; **100**(6): 1865–1878.
Publisher Full Text

Howard C, Denton A, Jackson B, *et al.*: **On the path to reference genomes for all biodiversity: Lessons learned and laboratory protocols created in the Sanger Tree of Life core laboratory over the first 2000 species.** *bioRxiv.* 2025.
Publisher Full Text

Howe K, Chow W, Collins J, *et al.*: **Significantly improving the quality of genome assemblies through curation.** *GigaScience.* 2021; **10**(1).
PubMed Abstract | Publisher Full Text | Free Full Text

Kerpedjiev P, Abdennur N, Lekschas F, *et al.*: **HiGlass: Web-based visual exploration and analysis of genome interaction maps.** *Genome Biol.* 2018; **19**(1): 125.
PubMed Abstract | Publisher Full Text | Free Full Text

Kuhl H: *Beyträge Zur Kenntniß Der Chiroptern.* Hanau: Verlag der Hermannschen Buchhandlung; 1819.

Kurtzer GM, Sochat V, Bauer MW: **Singularity: Scientific containers for mobility of compute.** *PLOS ONE.* 2017; **12**(5): e0177459.
PubMed Abstract | Publisher Full Text | Free Full Text

Li H: **Minimap2: Pairwise alignment for nucleotide sequences.** *Bioinformatics.* 2018; **34**(18): 3094–3100.
PubMed Abstract | Publisher Full Text | Free Full Text

Manni M, Berkeley MR, Seppey M, *et al.*: **BUSCO update: Novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes.** *Mol. Biol. Evol.* 2021; **38**(10): 4647–4654.
PubMed Abstract | Publisher Full Text | Free Full Text

Merkel D: **Docker: Lightweight Linux containers for consistent development and deployment.** *Linux J.* 2014; **2014**(239).
Publisher Full Text

Miller-Butterworth CM, Murphy WJ, O'Brien SJ, *et al.*: **A family matter: Conclusive resolution of the taxonomic position of the long-fingered bats.** *Miniopterus. Molecular Biology and Evolution.* 2007; **24**(7): 1553–1561.
Publisher Full Text

Puechmaille SJ, Allegrini B, Benda P, *et al.*: **A new species of the *Miniopterus schreibersii* species complex (Chiroptera: Miniopteridae) from the Maghreb region, North Africa.** *Zootaxa.* 2014; **3794**(1):

108–124.
Publisher Full Text

Ranallo-Benavidez TR, Jaron KS, Schatz MC: **GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes.** ***Nature.*** *Communications.* 2020; **11**(1): 1432.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Rao SSP, Huntley MH, Durand NC, *et al.*: **A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping.** *Cell.* 2014; **159**(7): 1665–1680.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Rhie A, McCarthy SA, Fedrigo O, *et al.*: **Towards complete and error-free genome assemblies of all vertebrate species.** *Nature.* 2021; **592**(7856): 737–746.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Rhie A, Walenz BP, Koren S, *et al.*: **Merqury: Reference-free quality, completeness, and phasing assessment for genome assemblies.** *Genome Biol.* 2020; **21**(1).
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Schoch CL, Ciufo S, Domrachev M, *et al.*: **NCBI taxonomy: A comprehensive update on curation, resources and tools.** *Database.* 2020; **2020**: baaa062.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Teeling EC, Vernes SC, Dávalos LM, *et al.*: **Bat biology, genomes, and the Bat1K project: To generate chromosome-level genomes for all living bat species.** *Annu. Rev. Anim. Biosci.* 2018; **6**: 23–46.
Publisher Full Text

Uliano-Silva M, Ferreira JGRN, Krasheninnikova K, *et al.*: **MitoHiFi: A Python pipeline for mitochondrial genome assembly from PacBio high fidelity reads.** *BMC Bioinformatics.* 2023; **24**(1): 288.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Vasimuddin M, Misra S, Li H, *et al.*: **Efficient architecture-aware acceleration of BWA-MEM for multicore systems.** *2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS).* IEEE; 2019; 314–24.
Publisher Full Text

Wood R, Weyeneth N, Appleton B: **Development and characterisation of 20 microsatellite loci isolated from the large bent-wing bat, *Miniopterus schreibersii* (Chiroptera: Miniopteridae) and their cross-taxa utility in the family Miniopteridae.** *Mol. Ecol. Resour.* 2011; **11**(4): 675–685.
PubMed Abstract | **Publisher Full Text**

Wright PGR, Newton J, Agnelli P, *et al.*: **Hydrogen isotopes reveal evidence of migration of *Miniopterus schreibersii* in Europe.** *BMC Ecol.* 2020; **20**(1): 52.
Publisher Full Text

Zhou C, McCarthy SA, Durbin R: **YaHS: Yet another Hi-C scaffolding tool.** *Bioinformatics.* 2023; **39**(1).
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 22 May 2026

https://doi.org/10.21956/wellcomeopenres.28948.r155046

✔ **Edgar G Gutierrez**
National Polytechnic Institute, México, Santo Tomás, Mexico

The manuscript presents a valuable contribution to genomic resources, specifically providing a high-quality, chromosome-level genome assembly for the bat species *Miniopterus schreibersii*. The genomic data and assemblies presented are robust and will undoubtedly serve as an important tool for future research.
The manuscript is generally well-structured and covers the methodological steps thoroughly. However, there are areas where the narrative flow, readability, and consistency could be significantly improved. In particular, some sections within the Background and Methods would benefit from a more logical restructuring to guide the reader more naturally from general concepts to specific findings. Additionally, there are minor technical inconsistencies between the text and figures (such as the colony size description and the BUSCO datasets) and a few formatting discrepancies in the software citations and references that need to be addressed. Restructuring these sections and addressing these minor revisions will greatly enhance the clarity, precision, and reproducibility of the study. I hope the following specific comments help the authors refine their manuscript.
C: Unless the journal guidelines suggest otherwise, it is recommended to choose keywords that do not already appear in the title. This helps expand the article's discoverability and reach.
C: The background section is informative, but the organization of ideas could be improved for better flow. In particular, the discussion of cryptic species and identification challenges appears before the manuscript introduces the taxonomic complexity of the genus *Miniopterus*. I suggest restructuring the paragraph so that the text progresses from general information about the species toward the more specific discussion of cryptic diversification and taxonomic reclassification. This may improve readability and narrative coherence.
C: I suggest reorganizing the last background paragraph for a more natural narrative flow. Rather than beginning with the mention of a previously available scaffold-level assembly, the paragraph could first introduce the chromosome-level genome generated in this study and the sampled specimen, followed by contextual information about prior assemblies and related sequencing initiatives.
C: The first Methods paragraph some sentences could be refined for smoother readability and more precise scientific wording. In particular, the sentence “One male individual was collected

from these groups" appears somewhat redundant, as the paragraph already states that the sequenced specimen was an adult male *Miniopterus schreibersii*.
C: In the Nucleic acid extraction paragraph, the repeated descriptions of nucleic acid quantification methods for both DNA and RNA could potentially be consolidated. Additionally, the introductory sentence regarding the availability of protocols may be integrated more smoothly into the paragraph for better narrative flow.
C: The statement "Groups of *M. schreibersii* consisting of 2 to 10 individuals were observed roosting…" appears somewhat inconsistent with Figure 1, where a noticeably larger aggregation seems to be visible. The authors may wish to clarify whether the reported number refers to specific subgroups within the roost rather than the entire observed colony, in order to avoid potential confusion between the text and the figure.
C: Unless the journal guidelines state otherwise, once a program is cited, it is no longer necessary to cite it each time it is mentioned in the text (e.g., the MerquryFK tool (Rhie et al., 2020); Singularity (Kurtzer et al., 2017), etc.).
C: Could you please clarify which results table is being referenced here?: "The analysis outputs included assembly QV scores and completeness statistics."
C: Chromosome 3 appears to show a distinct Hi-C interaction pattern relative to the other chromosomes. Although this may represent normal chromosomal organization, a brief explanation or comment in the manuscript would help readers interpret this feature and rule out potential assembly artefacts.
C: In the sequence data paragraph, several sentences are repetitive and somewhat fragmented, particularly the repeated use of "which were used." Consider reorganizing the sequencing and assembly information into a more concise structure, while clearly separating PacBio, Hi-C, and RNA-seq results. Additionally, the sentence "These estimates guided expectations for the assembly" is vague and could either be clarified or removed.
Minor stylistic revisions would substantially improve clarity and readability. For instance, it is not entirely clear how to visualize the repetitive content mentioned in the text ("repeat content of 9.47% (Figure 2)") within Figure 2 itself.
C: Consider defining the abbreviation "Mb" (megabases) at its first appearance, as was done for "Gb" (gigabases). Additionally, please standardize the capitalization of these units throughout the text; for example, "kb" is currently used in lowercase.
C: In Table 5, consider removing the "v" from "sanger-tol/blobtoolkit v0.9.0" to maintain a standardized format in the version column across all listed software.
C: The BUSCO software version should be reported in the Methods section, ideally at the first mention of the analysis, rather than appearing only in the second reference to BUSCO within the Results section. The current wording is somewhat inconsistent and may suggest that different BUSCO versions were used for each haplotype.
C: Please check the BUSCO lineage dataset mentioned in the manuscript. The Results section indicates *metazoa_odb10*, while Figure 2 shows *laurasiatheria_odb10*; consequently, the values do not match. This inconsistency should be corrected for clarity and reproducibility.
C: The species name *Miniopterus schreibersii* should be italicized throughout the manuscript, including in the Data Availability section.
C: In the following references:

- Miller-Butterworth CM, Murphy WJ, O'Brien SJ, et al.:
- Ranallo-Benavidez TR, Jaron KS, Schatz MC:

The reference formatting appears inconsistent. Please abbreviate "Molecular Biology and Evolution" in accordance with the style used throughout the bibliography, and verify the

punctuation and formatting (e.g., bold text) within the complete article title.

C: Regarding the reference for Vasimuddin M, Misra S, Li H, et al., please check the page number formatting, as it appears inconsistent with the rest of the bibliography.

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: immunogenetics, evolution, genomics, bioinformatics

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 18 May 2026

https://doi.org/10.21956/wellcomeopenres.28948.r155043

Ricardo Durães-Carvalho
Federal University of São Paulo, São Paulo, Brazil

Mariana Dias Guilardi
Institute of Chemistry, University of Sao Paulo, São Paulo, São Paulo, Brazil

Review report n. 1

Summary
The authors present a high-quality chromosome-level genome assembly for the Schreibers's long-fingered bat, *Miniopterus schreibersii*, generated from a male individual collected in Cyprus. The primary assembly (haplotype 1) spans 1.85 Gb and is scaffolded into 24 chromosomal pseudomolecules, including the X and Y chromosomes, with 97.59% of the sequence assigned to chromosomes. A secondary haplotype assembly (1.70 Gb) is also provided at scaffold level. In addition, the authors assembled the mitochondrial genome (16.63 kb) and report Ensembl

annotation identifying 18,289 protein-coding genes.
The assembly is of excellent quality, with a consensus QV of 61.8, BUSCO completeness of 99.9%, and combined k-mer completeness of 99.79%, surpassing current Earth BioGenome Project benchmarks. This work was produced as part of the Darwin Tree of Life and Bat1K initiatives and constitutes a valuable genomic resource for studies of bat evolution, conservation, comparative genomics, and host-virus interactions.

Strengths

Technically robust approach. The study uses state-of-the-art sequencing technologies, including PacBio HiFi long reads, Hi-C scaffolding, and RNA-seq support. Assembly and curation were performed using well-established tools such as Hifiasm, YaHS, MerquryFK, and BlobToolKit.

Excellent assembly quality. The resulting assembly exceeds Earth BioGenome Project standards (7.C.Q61), with high contiguity (contig N50 of 28.01 Mb), chromosomal completeness, and outstanding consensus accuracy.

Comprehensive methodological description. The manuscript provides sufficient details regarding sample collection, nucleic acid extraction, library preparation, sequencing, assembly, and quality assessment to enable reproducibility.

Open and accessible data. All raw sequencing data, assemblies, and associated metadata are publicly available through the European Nucleotide Archive under BioProject PRJEB74978.

Clear biological relevance. The background section effectively highlights the importance of this species for conservation genetics, phylogeography, and zoonotic disease research.

Minor concerns and suggestions
The manuscript is suitable for indexing in its current form. The following minor points may improve clarity and consistency:

Species name consistency. There are a few instances where the species epithet appears as *schreiberisii* rather than the correct *schreibersii*. The authors should verify and correct these typographical errors throughout the manuscript.

Role of haplotype 2. Although the second haplotype assembly is provided, its intended use is not discussed. A brief statement clarifying whether this assembly is included primarily for phasing and heterozygosity analyses, rather than for annotation, would be helpful.

Single-individual reference. As expected for a reference genome, the assembly is based on one specimen. It would be useful to acknowledge that this resource does not capture population-level genetic variation.

Euthanasia details. The legal and governance framework is clearly described, but the specific euthanasia method is not indicated. Including this information, or stating that it followed approved institutional protocols, would improve methodological transparency.

Annotation accession version. The annotation section cites accession GCA_964146895.1,

whereas Table 2 lists GCA_964146895.2. The authors should confirm that the annotation corresponds to the latest assembly version.

Aspects not assessed
I did not independently download or reanalyze the raw sequencing data, genome assembly, or annotation files. My evaluation is based on the information presented in the manuscript and the reported quality metrics.

_____

Review report n. 2

The authors provide a high-quality genome assembly of *Miniopterus schreibersii,* comprising two haplotypes at chromosome and scaffold-levels, respectively. This work presents an accurate description of the methods, including PacBIO HiFi and Hi-C and RNA-Seq, and the results of the assemblies. Results include haplotype-resolved assemblies, genome annotation, mitochondrial genome assembly, and quality evaluation, following the standards for vertebrate reference genomes. Some suggestions to improve the text are listed as follows:

- Since external morphological features are insufficient for field identification, it would be important to include if wing, cranial or genetic data were used to identify the species prior NGS sequencing
- If the specimen is deposited in a museum collection, the Methods could include its voucher number
- The authors should include in the Background the information about cytogenetic data, considering that the assembly resulted in 24 chromosomal pseudomolecules, and the diploid number for the species is 2n=46 with polymorphisms in the autosomes (Aulagnier and Presetnik, 2020).

References
1. Aulagnier S, Presetnik P: Schreibers' Bent-Winged Bat Miniopterus schreibersii (Kuhl, 1817). 1-26
Publisher Full Text

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; Phylogeny and Molecular evolution; Genome assembly; Virology; Cytogenetics; Small mammals.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.