

DATA NOTE

The genome sequence of the Small Dotted Buff moth,

Photedes minima Haworth, 1809

[version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from a male specimen of *Photedes* minima (Small Dotted Buff; Arthropoda; Insecta; Lepidoptera; Noctuidae). The genome sequence has a total length of 694.66 megabases. Most of the assembly (99.95%) is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled, with a length of 15.38 kilobases.

Keywords

Photedes minima, Small Dotted Buff, genome sequence, chromosomal, Lepidoptera



This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status AWAITING PEER REVIEW

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

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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; Noctuidae; Noctuinae; Apameini; *Photedes; Photedes minima* Haworth, 1809 (NCBI:txid988148)

Background

Photedes minima, or the Small Dotted Buff, is a moth from the Noctuidae family with a wingspan of 20–26 mm (Kimber, 2025). Males typically have a plain buff appearance with faint dark dots and a slight sheen, while females are smaller with markings that are more distinct. Their forewings range from pale ochreous to light brown, often dusted with darker shades, and females usually have a darker median area. The hindwings vary from yellow-orange to greyish near the edges (Waring et al., 2017).

Distributed across Europe, from the Iberian Peninsula to western Russia (GBIF Secretariat, 2023), *P. minima* is found in marshes, damp woodlands, and wet meadows (Kimber, 2025). In the UK, it widespread across Britain and Ireland (Waring et al., 2017). Its reliance on moist environments ties closely to the availability of its larval host plant, tufted hair-grass (Deschampsia cespitosa) (Kimber, 2025).

The moth has one generation per year, with adults active from late June to August in a single brood (Waring *et al.*, 2017). After mating, females lay eggs near host plants and the larvae feed on grass stems before entering diapause over winter. They pupate in an underground cocoon.

We present a chromosome-level genome sequence for *Photedes minima*, based on a specimen from Wytham Woods, Oxfordshire, United Kingdom (Figure 1). The genome was sequenced as part of the Darwin Tree of Life Project and will provide insights into its evolutionary biology and inform conservation strategies (Blaxter *et al.*, 2022).

Genome sequence report

Sequencing data

The genome of a specimen of *Photedes minima* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 43.48 Gb (gigabases) from 3.17 million reads, which were used to assemble the genome. GenomeScope analysis estimated the haploid genome size at 683.69 Mb, with a heterozygosity of 0.63% and repeat content of 39.57%. These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 60 coverage. Hi-C sequencing produced 125.82 Gb from 833.27 million reads, 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.



Figure 1. Photograph of the *Photedes minima* (ilPhoMini1) specimen used for genome sequencing.

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 12 misjoins or missing joins and removed 5 haplotypic duplications. These interventions decreased the scaffold count by 4.76%. The final assembly has a total length of 694.66 Mb in 39 scaffolds, with 8 gaps, and a scaffold N50 of 24.27 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 (99.95%) was assigned to 31 chromosomal-level scaffolds, representing 30 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 BUSCO gene painting with ancestral Merian elements (Wright *et al.*, 2024).

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

Table 1. Specimen and sequencing data for Photedes minima.

Project information				
Study title	Photedes minima (small dotted buff)			
Umbrella BioProject	PRJEB71302			
Species	Photedes minima			
BioSpecimen	SAMEA7701533			
NCBI taxonomy ID	988148			
Specimen information				
Technology	ToLID	BioSample accession	Organism part	
PacBio long read sequencing	ilPhoMini1	SAMEA7701719	whole organism	
Hi-C sequencing	ilPhoMini2	SAMEA114645394	whole organism	
RNA sequencing	ilPhoMini2	SAMEA114645394	whole organism	
Sequencing information				
Platform	Run accession	Read count	Base count (Gb)	
Hi-C Illumina NovaSeq X	ERR13702755	8.33e+08	125.82	
PacBio Sequel IIe	ERR12370428	1.36e+06	16.62	
PacBio Sequel IIe	ERR12370427	1.81e+06	26.86	
RNA Illumina NovaSeq X	ERR13999093	9.15e+07	13.82	

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 67.1. The k-mer completeness is 84.63% for the primary haplotype and 84.13% for the alternate haplotype; and 98.68% for the combined primary and alternate assemblies. BUSCO v.5.5.0 analysis using the lepidoptera_odb10 reference set (n = 5,286) identified 99.0% of the expected gene set (single = 98.6%, duplicated = 0.5%).

Table 2 provides assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project Report on Assembly Standards September 2024. The primary assembly achieves the EBP reference standard of **7.C.Q68**.

Methods

Sample acquisition and DNA barcoding

The specimen used for genome sequencing was an adult male *Photedes minima* (specimen ID Ox000672, ToLID ilPho-Mini1), collected from Wytham Woods, Oxfordshire, United Kingdom (latitude 51.772, longitude –1.338) on 2020-07-20, using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice. A second specimen was used for Hi-C and RNA

sequencing (specimen ID Ox003713, ToLID ilPhoMini2), was collected from the same location on 2023-06-20. The specimen was collected and identified by Liam Crowley (University of Oxford) and preserved on dry ice.

The initial identification was verified by an additional DNA barcoding process according to the framework developed by Twyford et al. (2024). A small sample was dissected from each 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

Table 2. Genome assembly data for Photedes minima.

Genome assembly				
Assembly name	ilPhoMini1.1			
Assembly accession	GCA_964662195.1			
Alternate haplotype accession	GCA_964662255.1			
Assembly level for primary assembly	chromosome			
Span (Mb)	694.66			
Number of contigs	47			
Number of scaffolds	39			
Longest scaffold (Mb)	33.31			
Assembly metric	Measure	Benchmark		
Contig N50 length	24.18 Mb	≥ 1 Mb		
Scaffold N50 length	24.27 Mb	= chromosome N50		
Consensus quality (QV)	Primary: 68.6; alternate: 66.5; combined: 67.1	≥ 40		
k-mer completeness	Primary: 84.63%; alternate: 84.13%; combined: 98.68%	≥ 95%		
BUSCO*	C:99.0%[S:98.6%,D:0.5%], F:0.1%,M:0.9%,n:5,286	S > 90%; D < 5%		
Percentage of assembly assigned to chromosomes	99.95%	≥ 90%		
Sex chromosomes	Z	localised homologous pairs		
Organelles	Mitochondrial genome: 15.38 kb	complete single alleles		

^{*} BUSCO scores based on the lepidoptera_odb10 BUSCO set using version 5.5.0. C = complete [S = single copy, D = duplicated],

Laboratory includes a sequence of procedures: sample preparation and homogenisation, DNA extraction, fragmentation and purification (Howard et al., 2025). Detailed protocols are available on protocols.io (Denton et al., 2023b). The ilPho-Mini1 sample was prepared for DNA extraction by weighing and dissecting it on dry ice (Jay et al., 2023). Tissue from the whole organism was homogenised using a PowerMasher II tissue disruptor (Denton et al., 2023a). HMW DNA was extracted using the Automated MagAttract v1 protocol (Sheerin et al., 2023). DNA was sheared into an average fragment size of 12-20 kb in a Megaruptor 3 system (Todorovic et al., 2023). Sheared DNA was purified by solid-phase reversible immobilisation, using AMPure PB beads to eliminate shorter fragments and concentrate the DNA (Strickland et al., 2023). The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and a Qubit Fluorometer using the Qubit dsDNA High Sensitivity Assay kit. The fragment size distribution was evaluated by running the sample on the FemtoPulse system.

RNA was extracted from whole organism tissue of ilPho-Mini2 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMaxTM *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 and crosslinking

Hi-C data were generated from the whole organism of the ilPhoMini2 sample using the Arima-HiC v2 kit (Arima Genomics) with 20–50 mg of frozen tissue (stored at –80 °C). As per manufacturer's instructions, tissue was fixed, and the DNA crosslinked using a TC buffer with 22% formaldehyde concentration, and a final formaldehyde concentration of 2%. The tissue was then homogenised using the Diagnocine Power Masher-II. The crosslinked DNA was digested using a restriction enzyme master mix, then biotinylated

F = fragmented, M = missing, n = number of orthologues in comparison.

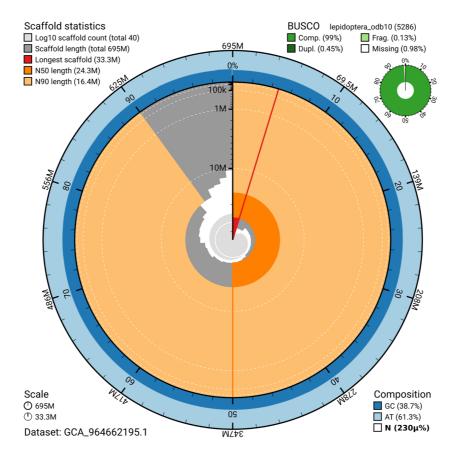


Figure 2. Genome assembly of *Photedes minima*, **ilPhoMini1.1: metrics.** The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. 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 964662195.1/dataset/GCA 964662195.1/snail.

and ligated. A clean up was performed with SPRIselect beads prior to library preparation. DNA concentration was quantified using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and Qubit HS Assay Kit, and sample biotinylation percentage was estimated using the 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), depending on genome size and sequencing depth required. Libraries were prepared using the SMRTbell Prep Kit 3.0 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. Size-selection and clean-up were carried out using diluted AMPure PB beads (Pacific Biosciences). DNA concentration was quantified using the Qubit Fluorometer v4.0 (ThermoFisher 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 the gDNA 55kb BAC analysis kit.

Samples were sequenced using the Sequel IIe system (Pacific Biosciences, California, USA). The concentration of the library loaded onto the Sequel IIe was in the range 40–135 pM. The SMRT link software, a PacBio web-based end-to-end workflow manager, was used to set-up and monitor the run, as well as perform primary and secondary analysis of the data upon completion.

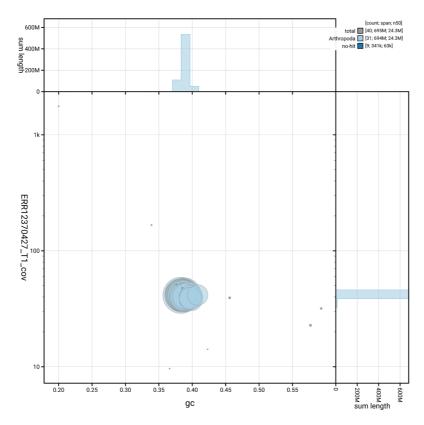


Figure 3. Genome assembly of *Photedes minima*, **ilPhoMini1.1: BlobToolKit GC-coverage plot.** Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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_964662195.1/blob.

Hi-C

For Hi-C library preparation, the biotinylated DNA constructs were fragmented using a Covaris E220 sonicator and sizeselected to 400-600 bp using SPRISelect beads. DNA was then enriched using Arima-HiC v2 Enrichment beads. The NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) was used for end repair, A-tailing, and adapter ligation, following a modified protocol in which library preparation is carried out while the DNA remains bound to the enrichment beads. PCR amplification was performed using KAPA HiFi HotStart mix and custom dual-indexed adapters (Integrated DNA Technologies) in a 96-well plate format. Depending on sample concentration and biotinylation percentage determined at the crosslinking stage, samples were amplified for 10-16 PCR cycles. Post-PCR clean-up was carried out using SPRISelect beads. The libraries were quantified using the Accuclear Ultra High Sensitivity dsDNA Standards Assay kit (Biotium) and normalised to 10 ng/µL before sequencing. Hi-C sequencing was performed on the Illumina NovaSeq X instrument using 150 bp paired-end reads.

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 (Rao et al., 2014) were mapped to the primary contigs using bwa-mem2 (Vasimuddin et al., 2019), and the contigs were scaffolded in YaHS (Zhou et al., 2023) 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 Mito-HiFi (Uliano-Silva et al., 2023), which runs MitoFinder

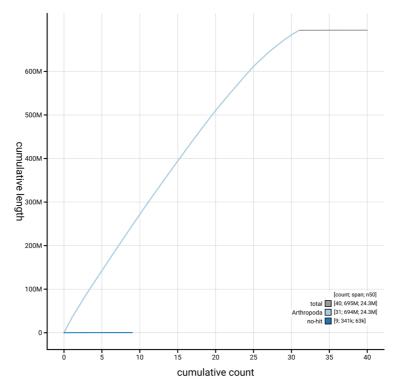


Figure 4. Genome assembly of *Photedes minima*, **ilPhoMini1.1: BlobToolKit cumulative sequence plot.** The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_964662195.1/dataset/GCA_964662195.1/cumulative.

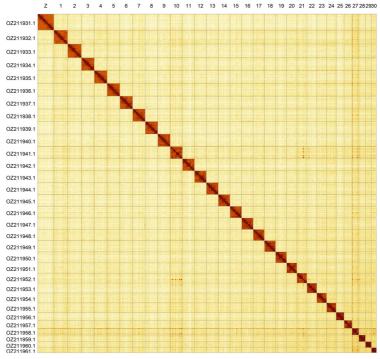


Figure 5. Genome assembly of *Photedes minima*: **Hi-C contact map of the ilPhoMini1.1 assembly, generated using PretextSnapshot.** Chromosomes are shown in order of size and labelled with chromosome numbers (top) and chromosome accession numbers (left).

Table 3. Chromosomal pseudomolecules in the genome assembly of *Photedes minima*, ilPhoMini1.

INSDC accession	Name	Length (Mb)	GC%
OZ211931.1	Z	33.31	38.5
OZ211932.1	1	28.32	39
OZ211933.1	2	28.12	38.5
OZ211934.1	3	26.43	38.5
OZ211935.1	4	26.32	38.5
OZ211936.1	5	26.16	38.5
OZ211937.1	6	26.05	38.5
OZ211938.1	7	25.87	38.5
OZ211939.1	8	25.7	38.5
OZ211940.1	9	25.6	38.5
OZ211941.1	10	25.0	39
OZ211942.1	11	24.63	38.5
OZ211943.1	12	24.3	38.5
OZ211944.1	13	24.27	38.5
OZ211945.1	14	24.18	38.5
OZ211946.1	15	23.82	38.5
OZ211947.1	16	23.76	38.5
OZ211948.1	17	22.94	39
OZ211949.1	18	22.92	39
OZ211950.1	19	22.57	38.5
OZ211951.1	20	21.02	39
OZ211952.1	21	20.52	39.5
OZ211953.1	22	20.27	38.5
OZ211954.1	23	20.17	39
OZ211955.1	24	19.33	39.5
OZ211956.1	25	16.36	39
OZ211957.1	26	16.31	39
OZ211958.1	27	13.93	40
OZ211959.1	28	13.54	40
OZ211960.1	29	12.16	39.5
OZ211961.1	30	10.45	41
OZ211962.1	MT	0.02	20.5

(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. Sex chromosomes were identified by Merian element painting. 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) computed prior to genome assembly. The analysis outputs included assembly QV scores and completeness statistics.

The genome was analysed in the blobtoolkit pipeline, a Nextflow (Di Tommaso et al., 2017) port of the previous Snakemake Blobtoolkit pipeline (Challis et al., 2020). It aligns the PacBio reads in SAMtools (Danecek et al., 2021) 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

Table 4. Software tools: versions and sources.

Software tool	Version	Source
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
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	0.19.8-r603	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	23.10.0	https://github.com/nextflow-io/nextflow
PretextView	0.2.5	https://github.com/sanger-tol/PretextView
PretextSnapshot	-	https://github.com/sanger-tol/PretextSnapshot
samtools	1.19.2	https://github.com/samtools/samtools
sanger-tol/ascc	0.1.0	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	0.6.0	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
GenomeScope2.0	2.0.1	https://github.com/tbenavi1/genomescope2.0

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 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: Photedes minima (small dotted buff). Accession number PRJEB71302; https://identifiers.org/ena.embl/PRJEB71302. The genome sequence is released openly for reuse. The *Photedes minima* genome sequencing initiative is part of the Darwin Tree of Life Project (PRJEB40665), the Sanger Institute Tree of Life Programme (PRJEB43745) and Project Psyche (PRJEB71705). 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 University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.12157525.

Members of the Darwin Tree of Life Barcoding collective are listed here: https://doi.org/10.5281/zenodo.12158331.

Members of the Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team are listed here: https://doi.org/10.5281/zenodo.12162482.

Members of Wellcome Sanger Institute Scientific Operations: Sequencing Operations are listed here: https://doi.org/10.5281/zenodo.12165051.

Members of the Wellcome Sanger Institute Tree of Life Core Informatics team are listed here: https://doi.org/10.5281/zenodo.12160324.

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.12205391.

Members of the Darwin Tree of Life Consortium are listed here: https://doi.org/10.5281/zenodo.4783558.

References

Allio R, Schomaker-Bastos A, Romiguier J, et al.: MitoFinder: efficient automated large-scale extraction of mitogenomic data in target enrichment phylogenomics. Mol Ecol Resour. 2020; 20(4): 892–905. PubMed Abstract | Publisher Full Text | Free Full Text

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

Bateman A, Martin MJ, 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

Beasley J, Uhl R, Forrest LL, et al.: **DNA barcoding SOPs for the Darwin Tree of Life project**. protocols.io. 2023; [Accessed 25 June 2024]. **Publisher Full Text**

Blaxter M, Mieszkowska N, Di Palma F, et al.: Sequence locally, think globally: the Darwin Tree of Life project. Proc Natl Acad Sci U S A. 2022; 119(4): e2115642118.

PubMed Abstract | Publisher Full Text | Free Full Text

Buchfink B, Reuter K, Drost HG: 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, Kumar S, Sotero-Caio C, et al.: Genomes on a Tree (GoaT): a versatile, scalable search engine for genomic and sequencing project metadata across the eukaryotic Tree of Life [version 1; peer review: 2

approved]. Wellcome Open Res. 2023; 8: 24.
PubMed Abstract | Publisher Full Text | Free Full Text

Challis R, Richards E, Rajan J, et al.: BlobToolKit – interactive quality assessment of genome assemblies. G3 (Bethesda). 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):

PubMed Abstract | Publisher Full Text | Free Full Text

Crowley L, Allen H, Barnes I, et al.: A sampling strategy for genome sequencing the British terrestrial arthropod fauna [version 1; peer review: 2 approved]. Wellcome Open Res. 2023; 8: 123.

PubMed Abstract | Publisher Full Text | Free Full Text

da Veiga Leprevost F, Grüning BA, Alves Aflitos S, et al.: BioContainers: an open-source and community-driven framework for software standardization. Bioinformatics. 2017; 33(16): 2580-2582.

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): giab008.

PubMed Abstract | Publisher Full Text | Free Full Text

Denton A, Oatley G, Cornwell C, et al.: Sanger Tree of Life sample

homogenisation: PowerMash. protocols.io. 2023a.

Publisher Full Text

Denton A, Yatsenko H, Jay J, et al.: Sanger Tree of Life wet laboratory protocol collection V.1. protocols.io. 2023b.

Publisher Full Text

Di Tommaso P, Chatzou M, Floden EW, et al.: Nextflow enables reproducible computational workflows. Nat Biotechnol. 2017; **35**(4): 316–319. PubMed Abstract | Publisher Full Text

Diesh C, Stevens GJ, Xie P, et al.: **JBrowse 2: a modular genome browser with views of synteny and structural variation.** Genome Biol. 2023; **24**(1): 74. **PubMed Abstract | Publisher Full Text | Free Full Text**

do Amaral RJV, Bates A, Denton A, et al.: Sanger Tree of Life RNA extraction: automated MagMax™ mirVana. protocols.io. 2023.

Publisher 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

GBIF Secretariat: *Photedes minima* Haworth, 1809. *GBIF Backbone Taxonomy*. 2023; [Accessed 20 April 2025].

Reference Source

Grüning B, Dale R, Sjödin A, *et al.*: **Bioconda: sustainable and comprehensive software distribution for the life sciences.** *Nat Methods.* 2018; **15**(7): 475–476. **PubMed Abstract | Publisher Full Text | Free Full Text**

Guan D, McCarthy SA, Wood J, et al.: Identifying and removing haplotypic duplication in primary genome assemblies. Bioinformatics. 2020; 36(9): 2896–2898.

PubMed Abstract | Publisher Full Text | Free Full Text

Harry E: PretextView (Paired REad TEXTure Viewer): a desktop application for viewing pretext contact maps. 2022.

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, $\it et~al.$: Significantly improving the quality of

genome assemblies through curation. GigaScience. 2021; 10(1): giaa153. PubMed Abstract | Publisher Full Text | Free Full Tex

Jay J, Yatsenko H, Narváez-Gómez JP, et al.: Sanger Tree of Life sample preparation: triage and dissection. protocols.io. 2023. Publisher Full Text

Kerpedjiev P, Abdennur N, Lekschas F, et al.: HiGlass: web-based visual exploration and analysis of genome interaction maps. Genome Biol. 2018;

PubMed Abstract | Publisher Full Text | Free Full Text

Kimber I: Small Dotted Buff Photedes minima (Haworth, 1809). UKMoths. 2025; [Accessed 20 April 2025].

Reference Source

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**

Lawniczak MKN, Davey RP, Raian I, et al.: Specimen and sample metadata standards for biodiversity genomics: a proposal from the Darwin Tree of Life project [version 1; peer review: 2 approved with reservations]. Wellcome Open Res. 2022; 7: 187.

Publisher 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): 2. [Accessed 2 April 2024].

Pereira L, Sivell O, Sivess L, et al.: DToL Taxon-specific Standard Operating Procedure for the terrestrial and freshwater arthropods working group. 2022.

Publisher Full Text

Pointon DL, Eagles W, Sims Y, et al.: sanger-tol/treeval v1.0.0 - Ancient Atlantis, 2023.

Publisher Full Text

Ranallo-Benavidez TR, Jaron KS, Schatz MC: GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes. Nat Commun. 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;

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): 245

PubMed Abstract | Publisher Full Text | Free Full Text

Sheerin E, Sampaio F, Oatley G, et al.: Sanger Tree of Life HMW DNA extraction: automated MagAttract v.1. protocols.io. 2023.

Publisher Full Text

Strickland M, Cornwell C, Howard C: Sanger Tree of Life fragmented DNA clean up: manual SPRI. protocols.io. 2023.

Publisher Full Text

Todorovic M, Sampaio F, Howard C: Sanger Tree of Life HMW DNA fragmentation: diagenode Megaruptor®3 for PacBio HiFi. protocols.io. 2023. **Publisher Full Text**

Twyford AD, Beasley J, Barnes I, et al.: A DNA barcoding framework for taxonomic verification in the Darwin Tree of Life project [version 1; peer review: 2 approved]. Wellcome Open Res. 2024; 9: 339.

PubMed Abstract | Publisher Full Text | Free 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. In: 2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS). IEEE, 2019; 314-324. **Publisher Full Text**

Waring P, Townsend M, Lewington R: Field guide to the Moths of Great Britain and Ireland: third edition. Bloomsbury Wildlife Guides, 2017. **Reference Source**

Wright CJ, Stevens L, Mackintosh A, et al.: Comparative genomics reveals the dynamics of chromosome evolution in Lepidoptera. Nat Ecol Evol. 2024; 8(4):

PubMed Abstract | Publisher Full Text | Free Full Text

Zhou C, McCarthy SA, Durbin R: YaHS: yet another Hi-C scaffolding tool. Bioinformatics. 2023; **39**(1): btac808.

PubMed Abstract | Publisher Full Text | Free Full Text