# DATA NOTE



# The genome sequence of the Lunar Underwing, Omphaloscelis

# *lunosa* (Haworth, 1809) [version 1; peer review: awaiting peer

# review]

Douglas Boyes<sup>1+</sup>, Harriet F. Johnson<sup>2</sup>, University of Oxford and Wytham Woods Genome Acquisition Lab, Darwin Tree of Life Barcoding collective, Wellcome Sanger Institute Tree of Life programme, Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective, Tree of Life Core Informatics collective, Darwin Tree of Life Consortium

<sup>1</sup>UK Centre for Ecology & Hydrology, Wallingford, England, UK <sup>2</sup>Wellcome Sanger Institute, Hinxton, England, UK

+ Deceased author

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# Abstract

We present a genome assembly from an individual female *Omphaloscelis lunosa* (the Lunar Underwing; Arthropoda; Insecta; Lepidoptera; Noctuidae). The genome sequence is 661.9 megabases in span. Most of the assembly is scaffolded into 32 chromosomal pseudomolecules, including the W and Z sex chromosomes. The mitochondrial genome has also been assembled and is 15.47 kilobases in length. Gene annotation of this assembly on Ensembl identified 18,931 protein coding genes.

# **Keywords**

Omphaloscelis lunosa, Lunar Underwing, 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.

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

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# Species taxonomy

Eukaryota; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Noctuoidea; Noctuidae; Xyleninae; *Omphaloscelis; Omphaloscelis lunosa* (Haworth, 1809) (NCBI:txid2492375).

# Background

*Omphaloscelis lunosa*, the lunar underwing, is a moth of the family *Noctuidae* (Haworth, 1809). It has a wingspan of 32–38 mm, and its colouration varies across individuals from yellowish orange to dark brown. Darker individuals often show a 'netted' appearance due to contrasting pale veins. It gains its common name, the lunar underwing, due to the dark crescent moon shape visible on its lighter coloured hind wings. It is not considered endangered. It is common across southern UK, with a scattered distribution across western Europe. It has recently been recorded in the US (Roble, 2018). The single generation flies from August to October and the larvae overwinter feeding on various grasses (Kimber, 2023; Waring *et al.*, 2017).

*Noctuidae* is the largest superfamily within Lepidoptera and has a controversial phylogeny (Zahiri *et al.*, 2011). As a nocturnal pollinator and prey item, the lunar underwing is ecologically important and has been included in biodiversity monitoring of farmland management (Taylor & Morecroft, 2009) and molecular analyses of diet in cryptic bat species (Razgour *et al.*, 2011). The growing availability of a complete reference genomes such as this one, have already contributed to large scale evolutionary comparisons (Mulhair & Holland, 2024), and will improve future ecological and phylogenetic studies.

# Genome sequence report

The genome was sequenced from one female *Omphaloscelis lunosa* (Figure 1) collected from Wytham Woods, Oxfordshire,



Figure 1. Photograph of the *Omphaloscelis lunosa* (ilOmpLuno1) specimen used for genome sequencing.

UK (51.77, -1.34). A total of 29-fold coverage in Pacific Biosciences single-molecule HiFi long reads and 82-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 10 missing joins or mis-joins, reducing the scaffold number by 10.91%.

The final assembly has a total length of 661.9 Mb in 49 sequence scaffolds with a scaffold N50 of 22.1 Mb (Table 1). A summary of the assembly statistics is shown in Figure 2, while the distribution of assembly scaffolds on GC proportion and coverage is shown in Figure 3. The cumulative assembly plot in Figure 4 shows curves for subsets of scaffolds assigned to different phyla. Most (99.85%) of the assembly sequence was assigned to 32 chromosomal-level scaffolds, representing 30 autosomes and the W and Z sex chromosomes. Chromosomescale scaffolds confirmed by the Hi-C data are named in order of size (Figure 5; Table 2). While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited. The mitochondrial genome was also assembled and can be found as a contig within the multifasta file of the genome submission.

The estimated Quality Value (QV) of the final assembly is 59.3 with *k*-mer completeness of 100%, and the assembly has a BUSCO v5.3.2 completeness of 99.0% (single = 98.5%, duplicated = 0.5%), using the lepidoptera\_odb10 reference set (n = 5,286).

Metadata for specimens, spectral estimates, sequencing runs, contaminants and pre-curation assembly statistics can be found at https://links.tol.sanger.ac.uk/species/2492375.

# Genome annotation report

The *Omphaloscelis lunosa* genome assembly (GCA\_916610215.1) was annotated using the Ensembl rapid annotation pipeline (Table 1; https://rapid.ensembl.org/Omphaloscelis\_lunosa\_GCA\_916610215.1/Info/Index). The resulting annotation includes 19,123 transcribed mRNAs from 18,931 protein-coding genes.

# Methods

#### Sample acquisition and nucleic acid extraction

A female *Omphaloscelis lunosa* (specimen ID Ox000964, ToLID ilOmpLuno1) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, lon-gitude –1.34) on 2020-09-08 using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice.

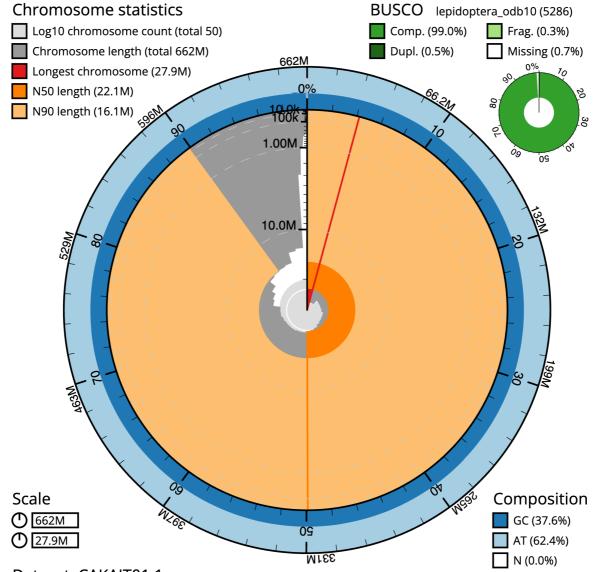
DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute (WSI). The ilOmpLuno1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing. Thorax tissue was cryogenically disrupted to a fine powder using a Covaris cryoPREP Automated Dry Pulveriser, receiving multiple impacts. High molecular weight (HMW) DNA was extracted using the Qiagen MagAttract HMW DNA extraction kit. Low molecular weight DNA was removed from a

| Project accession data                       |   |                            |  |
|--|---|----------------------------|--|
| Assembly identifier                          | ilOmpLuno1.1  |                            |  |
| Assembly release date                        | 2021-10-01  |                            |  |
| Species                                      | Omphaloscelis lunosa  |                            |  |
| Specimen                                     | ilOmpLuno1  |                            |  |
| NCBI taxonomy ID                             | 2492375   |                            |  |
| BioProject                                   | PRJEB46322  |                            |  |
| BioSample ID                                 | SAMEA8603195  |                            |  |
| Isolate information                          | ilOmpLuno1, female: thorax (DNA sequencing), head (Hi-C<br>scaffolding), abdomen (RNA sequencing) |                            |  |
| Assembly metrics*                            |   | Benchmark                  |  |
| Consensus quality (QV)                       | 59.3  | ≥ 50                       |  |
| k-mer completeness                           | 100%  | ≥ 95%                      |  |
| BUSCO**                                      | C:99.0%[S:98.5%,D:0.5%], F:0.3%,<br>M:0.7%,n:5,286  | <i>C</i> ≥ 95%             |  |
| Percentage of assembly mapped to chromosomes | 99.85%  | ≥ 95%                      |  |
| Sex chromosomes                              | W and Z chromosomes   | localised homologous pairs |  |
| Organelles                                   | Mitochondrial genome assembled  | complete single alleles    |  |
| Raw data accessions                          |   |                            |  |
| PacificBiosciences SEQUEL II                 | ERR6808005  |                            |  |
| 10X Genomics Illumina                        | ERR6688543, ERR6688540, ERR6688541, ERR6688542  |                            |  |
| Hi-C Illumina                                | ERR6688539  |                            |  |
| PolyA RNA-Seq Illumina                       | ERR9435009  |                            |  |
| Genome assembly                              |   |                            |  |
| Assembly accession                           | GCA_916610215.1   |                            |  |
| Accession of alternate haplotype             | GCA_916610225.1   |                            |  |
| Span (Mb)                                    | 661.9   |                            |  |
| Number of contigs                            | 61  |                            |  |
| Contig N50 length (Mb)                       | 21.6  |                            |  |
| Number of scaffolds                          | 49  |                            |  |
| Scaffold N50 length (Mb)                     | 22.1  |                            |  |
| Longest scaffold (Mb)                        | 28.0  |                            |  |
| Genome annotation                            |   |                            |  |
| Number of protein-coding genes               | 18,931  |                            |  |
| Number of gene transcripts                   | 19,123  |                            |  |
|  |   |                            |  |

#### Table 1. Genome data for *Omphaloscelis lunosa*, ilOmpLuno1.1.

\* Assembly metric benchmarks are adapted from column VGP-2020 of "Table 1: Proposed standards and metrics for defining genome assembly quality" from (Rhie *et al.*, 2021).

\*\* BUSCO scores based on the lepidoptera\_odb10 BUSCO set using v5.3.2. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison. A full set of BUSCO scores is available at https://blobtoolkit.genomehubs.org/view/Omphaloscelis%20lunosa/dataset/CAKAIT01.1/busco.

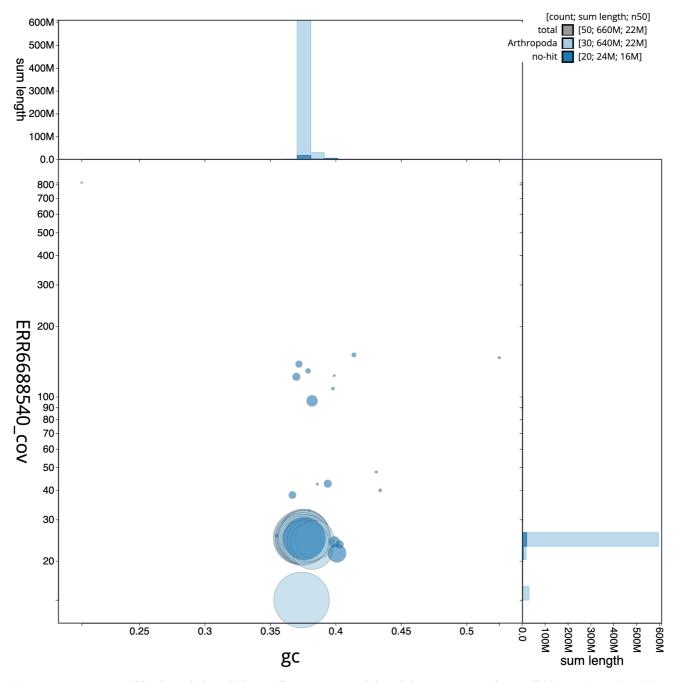


Dataset: CAKAIT01.1

**Figure 2. Genome assembly of Omphaloscelis lunosa, ilOmpLuno1.1: metrics.** The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 661,874,710 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (27,948,822 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (22,138,921 and 16,118,763 bp), respectively. The pale grey spiral shows the cumulative scaffold count on a log scale with white scale lines showing successive orders of magnitude. The blue and pale-blue area around the outside of the plot shows the distribution of GC, AT and N percentages in the same bins as the inner plot. A summary of complete, fragmented, duplicated and missing BUSCO genes in the lepidoptera\_odb10 set is shown in the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/Omphaloscelis%20lunosa/dataset/CAKAIT01.1/snail.

20 ng aliquot of extracted DNA using the 0.8X AMpure XP purification kit prior to 10X Chromium sequencing; a minimum of 50 ng DNA was submitted for 10X sequencing. HMW DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system with speed setting 30. Sheared DNA was purified by solid-phase reversible immobilisation using AMPure PB beads with a 1.8X ratio of beads to sample to remove the shorter fragments and concentrate the DNA sample. The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and Qubit Fluorometer and Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

RNA was extracted from abdomen tissue of ilOmpLuno1 in the Tree of Life Laboratory at the WSI using TRIzol, according

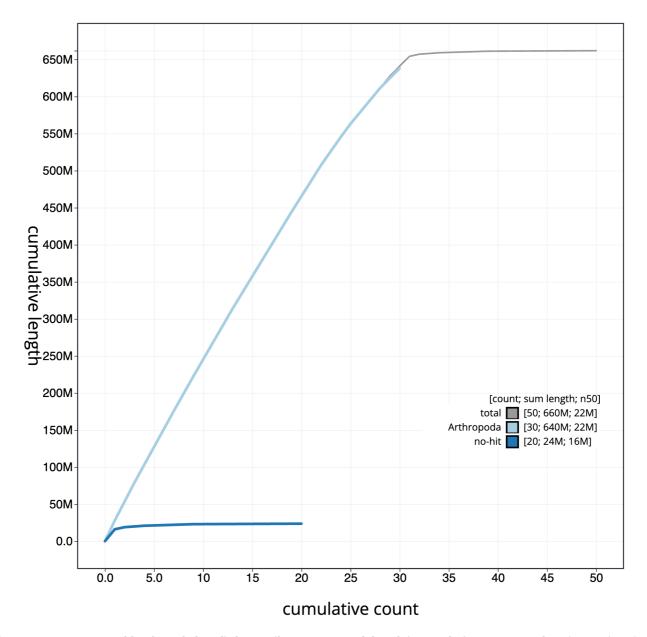


**Figure 3. Genome assembly of** *Omphaloscelis lunosa*, **ilOmpLuno1.1: BlobToolKit GC-coverage plot.** Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/Omphaloscelis%20lunosa/dataset/CAKAIT01.1/blob.

to the manufacturer's instructions. RNA was then eluted in 50 µl RNAse-free water and its concentration assessed using a Nanodrop spectrophotometer and Qubit Fluorometer using the Qubit RNA Broad-Range (BR) Assay kit. Analysis of the integrity of the RNA was done using Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

#### Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud DNA sequencing libraries were constructed according to the manufacturers' instructions. Poly(A) RNA-Seq libraries were constructed using the NEB Ultra II RNA Library Prep kit. DNA and RNA sequencing was performed



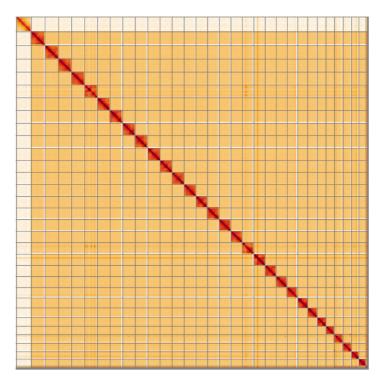
**Figure 4. Genome assembly of** *Omphaloscelis lunosa*, **ilOmpLuno1.1: BlobToolKit cumulative sequence plot.** The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/Omphaloscelis%20lunosa/dataset/ CAKAIT01.1/cumulative.

by the Scientific Operations core at the WSI on Pacific Biosciences SEQUEL II (HiFi), Illumina HiSeq 4000 (RNA-Seq) and Illumina NovaSeq 6000 (10X) instruments. Hi-C data were also generated from head tissue of ilOmpLuno1 using the Arima2 kit and sequenced on the Illumina NovaSeq 6000 instrument.

#### Genome assembly, curation and evaluation

Assembly was carried out with Hifiasm (Cheng et al., 2021) and haplotypic duplication was identified and removed with

purge\_dups (Guan *et al.*, 2020). One round of polishing was performed by aligning 10X Genomics read data to the assembly with Long Ranger ALIGN, calling variants with FreeBayes (Garrison & Marth, 2012). The assembly was then scaffolded with Hi-C data (Rao *et al.*, 2014) using SALSA2 (Ghurye *et al.*, 2019). The assembly was checked for contamination and corrected as described previously (Howe *et al.*, 2021). Manual curation was performed using HiGlass (Kerpedjiev *et al.*, 2018) and Pretext (Harry, 2022). The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2023),



**Figure 5. Genome assembly of** *Omphaloscelis lunosa*, **ilOmpLuno1.1: Hi-C contact map of the ilOmpLuno1.1 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=YRuJM2bKQyigC7BtsU1A8Q.

| INSDC<br>accession | Chromosome | Length<br>(Mb) | GC%  |
|--------------------|------------|----------------|------|
| OU744244.1         | 1          | 25.6           | 37.5 |
| OU744245.1         | 2          | 25.48          | 37.6 |
| OU744246.1         | 3          | 24.23          | 37.6 |
| OU744247.1         | 4          | 24.2           | 37.4 |
| OU744248.1         | 5          | 24.14          | 37.5 |
| OU744249.1         | 6          | 23.95          | 37.6 |
| OU744250.1         | 7          | 23.51          | 37.4 |
| OU744251.1         | 8          | 23.46          | 37.2 |
| OU744252.1         | 9          | 23.35          | 37.2 |
| OU744253.1         | 10         | 23.1           | 37.6 |
| OU744254.1         | 11         | 22.62          | 37.4 |
| OU744255.1         | 12         | 22.39          | 37.3 |
| OU744256.1         | 13         | 22.14          | 37.6 |
| OU744257.1         | 14         | 21.86          | 37.5 |
| OU744258.1         | 15         | 21.83          | 37.3 |

| INSDC<br>accession | Chromosome | Length<br>(Mb) | GC%  |
|--------------------|------------|----------------|------|
| OU744259.1         | 16         | 21.25          | 37.6 |
| OU744260.1         | 17         | 21.64          | 37.4 |
| OU744261.1         | 18         | 21.57          | 37.5 |
| OU744262.1         | 19         | 21.48          | 37.7 |
| OU744263.1         | 20         | 20.91          | 37.7 |
| OU744264.1         | 21         | 20.85          | 37.4 |
| OU744265.1         | 22         | 19.14          | 37.4 |
| OU744266.1         | 23         | 18.76          | 37.5 |
| OU744267.1         | 24         | 17.89          | 37.6 |
| OU744268.1         | 25         | 16.38          | 37.6 |
| OU744269.1         | 26         | 16.12          | 37.6 |
| OU744270.1         | 27         | 15.97          | 38.2 |
| OU744271.1         | 28         | 15.75          | 37.9 |
| OU744272.1         | 29         | 13.6           | 38.4 |
| OU744273.1         | 30         | 13.19          | 38   |
| OU744274.1         | W          | 2.87           | 40.1 |
| OU744243.1         | Z          | 27.95          | 37.4 |
| OU744275.1         | MT         | 0.02           | 20.8 |

Table 2. Chromosomal pseudomoleculesin the genome assembly of Omphaloscelislunosa, ilOmpLuno1.

which runs MitoFinder (Allio *et al.*, 2020) or MITOS (Bernt *et al.*, 2013) and uses these annotations to select the final mitochondrial contig and to ensure the general quality of the sequence.

A Hi-C map for the final assembly was produced using bwamem2 (Vasimuddin *et al.*, 2019) in the Cooler file format (Abdennur & Mirny, 2020). To assess the assembly metrics, the *k*-mer completeness and QV consensus quality values were calculated in Merqury (Rhie *et al.*, 2020). This work was done using Nextflow (Di Tommaso *et al.*, 2017) DSL2 pipelines "sanger-tol/readmapping" (Surana *et al.*, 2023a) and "sangertol/genomenote" (Surana *et al.*, 2023b). The genome was analysed within the BlobToolKit environment (Challis *et al.*, 2020) and BUSCO scores (Manni *et al.*, 2021; Simão *et al.*, 2015) were calculated.

Table 3 contains a list of relevant software tool versions and sources.

#### Genome annotation

The BRAKER2 pipeline (Brůna *et al.*, 2021) was used in the default protein mode to generate annotation for the *Omphalos-celis lunosa* assembly (GCA\_916610215.1) in Ensembl Rapid Release.

#### 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 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.

| Software tool          | Version           | Source  |  |  |
|------------------------|-------------------|---|--|--|
| BlobToolKit            | 4.1.7             | https://github.com/blobtoolkit/blobtoolkit  |  |  |
| BUSCO                  | 5.3.2             | https://gitlab.com/ezlab/busco  |  |  |
| FreeBayes              | 1.3.1-17-gaa2ace8 | https://github.com/freebayes/freebayes  |  |  |
| Hifiasm                | 0.15.3            | https://github.com/chhylp123/hifiasm  |  |  |
| HiGlass                | 1.11.6            | https://github.com/higlass/higlass  |  |  |
| Long Ranger ALIGN      | 2.2.2             | https://support.10xgenomics.com/genome-exome/<br>software/pipelines/latest/advanced/other-pipelines |  |  |
| Merqury                | MerquryFK         | https://github.com/thegenemyers/MERQURY.FK  |  |  |
| MitoHiFi               | 2                 | https://github.com/marcelauliano/MitoHiFi   |  |  |
| PretextView            | 0.2               | https://github.com/wtsi-hpag/PretextView  |  |  |
| purge_dups             | 1.2.3             | https://github.com/dfguan/purge_dups  |  |  |
| SALSA                  | 2.2               | https://github.com/salsa-rs/salsa   |  |  |
| sanger-tol/genomenote  | v1.0              | https://github.com/sanger-tol/genomenote  |  |  |
| sanger-tol/readmapping | 1.1.0             | https://github.com/sanger-tol/readmapping/tree/1.1.0  |  |  |

#### Table 3. Software tools: versions and sources.

#### Data availability

European Nucleotide Archive: Omphaloscelis lunosa (lunar underwing). Accession number PRJEB46322; https://identifiers.org/ena.embl/PRJEB46322. (Wellcome Sanger Institute, 2021) The genome sequence is released openly for reuse. The Omphaloscelis lunosa 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.

#### Author information

Members of the University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: https://doi.org/10.5281/ zenodo.4789928.

Members of the Darwin Tree of Life Barcoding collective are listed here: https://doi.org/10.5281/zenodo.4893703.

Members of the Wellcome Sanger Institute Tree of Life programme are listed here: https://doi.org/10.5281/ zenodo.4783585.

Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: https://doi.org/10.5281/ zenodo.4790455.

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.5013541.

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