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DATA NOTE

# The genome sequence of the sallow kitten, *Furcula furcula* (Clerck, 1759) [version 1; peer review: 2 approved]

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## Abstract
We present a genome assembly from an individual male *Furcula furcula* (the sallow kitten; Arthropoda; Insecta; Lepidoptera; Notodontidae). The genome sequence is 736 megabases in span. The entire assembly (100%) is scaffolded into 29 chromosomal pseudomolecules, with the Z sex chromosome assembled. The complete mitochondrial genome was also assembled and is 17.2 kilobases in length.

## Keywords
Furcula furcula, sallow kitten, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

## Open Peer Review

**Approval Status** ✓ ✓

|  | 1 | 2 |
| --- | --- | --- |
| **version 1**<br>12 Sep 2022 | ✓<br>view | ✓<br>view |

1. **Sivasankaran Kuppusamy**, Loyola College, chennai, India

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

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**Author roles: Boyes D**: Investigation, Resources; **Parker B**: Writing – Original Draft Preparation; **Plotkin D**: Writing – Original Draft Preparation; **Kawahara AY**: Writing – Original Draft Preparation;

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## Species taxonomy

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Notodontidae; Stauropinae; *Furcula*; *Furcula furcula* (Clerck, 1759) (NCBI:txid987943).

## Background

The sallow kitten, *Furcula furcula* (Clerck, 1759), is a holarctic moth belonging to the family Notodontidae (prominent moths) that is commonly found throughout Europe, Asia, and North America (Heath *et al.*, 1983; Miller *et al.*, 2018; Okagaki, 1958). Adults have a wingspan ranging from 30–36 mm, varying slightly between regions of its geographic distribution, and are white to grey in colour with a large grey band on the dorsal wing surface (Heath *et al.*, 1983). Larvae can grow up to 35 mm and are bright green with a purple or brown marking on the dorsal side and can be identified by their modified anal prolegs that form a forked tail-like appendage, for which the genus derives its name from ("furca" being Latin for fork) (Heath *et al.*, 1983).

Larvae have been observed to feed on leaves of poplar, willow, birch, and beech trees (Robinson *et al.*, 2010; Vorbrodt & Müller-Rutz, 1911). Once mature, larvae crawl down the tree trunk and make a hardened cocoon, consisting of silk and wood pulp, in which they pupate (Heath *et al.*, 1983; Okagaki, 1958). The moth emerges in the summer, between May and September, with 1–3 generations emerging per year (Heath *et al.*, 1983; Okagaki, 1958; Robinson *et al.*, 2010). The number of generations depends on the climate of the region, with more generations emerging per year in warmer regions (Okagaki, 1958). Until recently, *F. occidentalis* (Lintner, 1878) was treated as a subspecies of *F. furcula*. Given the global distribution of *F. furcula*, a fully annotated genome will help provide data needed to understand the link between its genotype and its broad larval host breadth and help distinguish this species from similar ones with overlapping distributions.

## Genome sequence report

The genome was sequenced from a single male *F. furcula* collected from Wytham Woods, Berkshire, UK (Figure 1). A total of 28-fold coverage in Pacific Biosciences single-molecule HiFi long reads and 62-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 20 missing/misjoins and removed 5 haplotypic duplications, reducing the assembly size by 0.44% and the scaffold number by 30.43%.

The final assembly has a total length of 736 Mb in 32 sequence scaffolds with a scaffold N50 of 27.06 Mb (Table 1). 100% of the assembly sequence was assigned to 29 chromosomal-level scaffolds, representing 28 autosomes (numbered by sequence length) and the Z sex chromosome (Figure 2–Figure 5; Table 2).



**Figure 1. Image of the *Furcula furcula* specimen taken prior to preservation and processing.**

**Table 1. Genome data for *Furcula furcula*, ilFurFurc1.1.**

| Project accession data | |
|---|---|
| Assembly identifier | ilFurFurc1.1 |
| Species | *Furcula furcula* |
| Specimen | ilFurFurc1 (genome assembly, Hi-C, RNA-Seq) |
| NCBI taxonomy ID | 987943 |
| BioProject | PRJEB45669 |
| BioSample ID | SAMEA7746637 |
| Isolate information | Male. Thorax tissue (genome assembly); head tissue (Hi-C); abdomen tissue (RNA-Seq) |
| **Raw data accessions** | |
| PacificBiosciences SEQUEL II | ERR6939219 |
| 10X Genomics Illumina | ERR6363296-ERR6363299 |
| Hi-C Illumina | ERR6363295 |
| PolyA RNA-Seq Illumina | ERR9434990 |
| **Genome assembly** | |
| Assembly accession | GCA_911728495.1 |
| *Accession of alternate haplotype* | GCA_911728485.2 |
| Span (Mb) | 736 |
| Number of contigs | 736 |
| Contig N50 length (Mb) | 24.9 |
| Number of scaffolds | 32 |
| Scaffold N50 length (Mb) | 27.06 |
| Longest scaffold (Mb) | 32.75 |
| BUSCO* genome score | C:98.9%[S:98.4%,D:0.5%],F:0.2%, M:0.9%,n:5,286 |

*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 com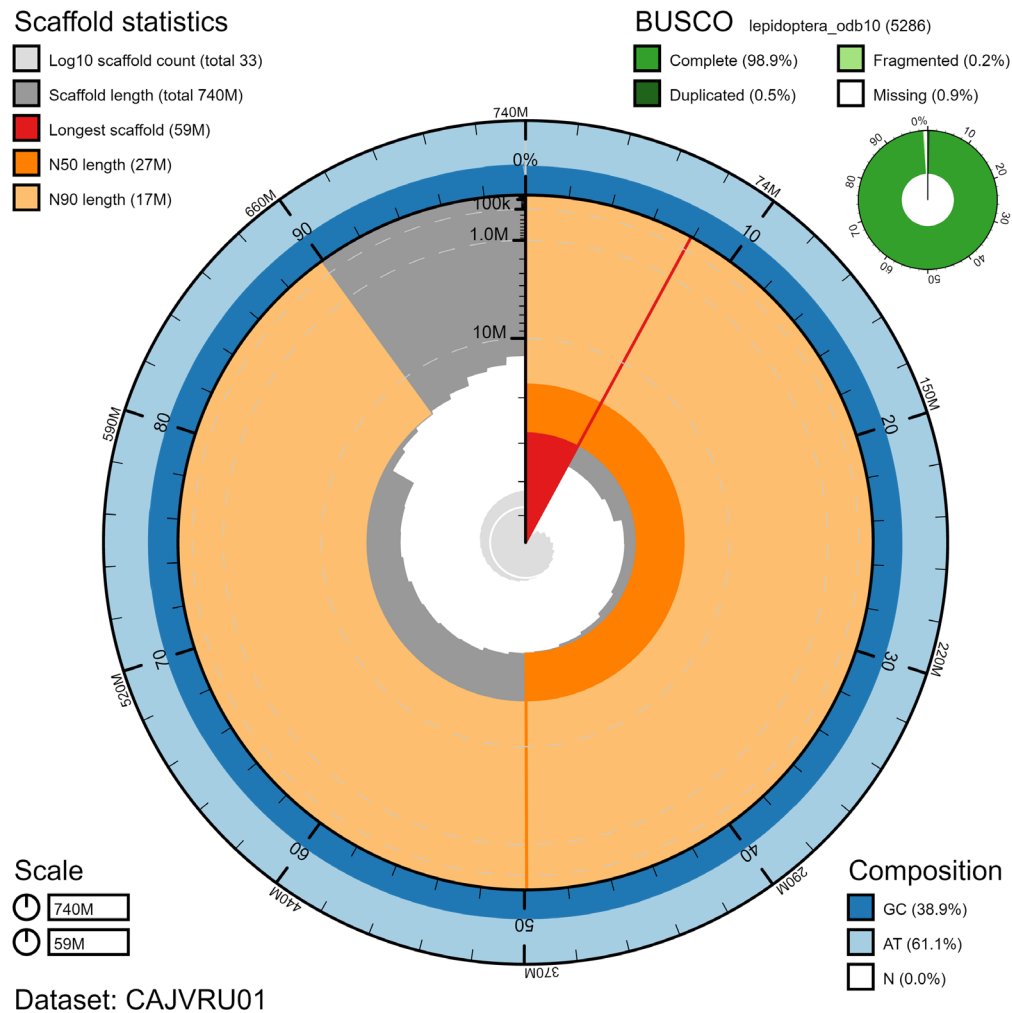parison. A full set of BUSCO scores is available at https://blobtoolkit.genomehubs.org/view/ilFurFurc1.1/dataset/CAJVRU01/busco.

**Figure 2. Genome assembly of *Furcula furcula*, ilFurFurc1.1: 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 736,123,039 bp assembly. The distribution of chromosome lengths is shown in dark grey with the plot radius scaled to the longest chromosome present in the assembly (58,744,975 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 chromosome lengths (27,058,741 and 17,271,334 bp), respectively. The pale grey spiral shows the cumulative chromosome 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/ilFurFurc1.1/dataset/CAJVRU01/snail.

The assembly has a BUSCO v5.3.2 (Manni *et al.*, 2021) completeness of 98.9% (single 98.4%, duplicated 0.5%) using the lepidoptera_odb10 reference set (n=5,286). While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited.

## Methods
### Sample acquisition and nucleic acid extraction
A single male *F. furcula* specimen (ilFurFurc1) was collected using a light trap from Wytham Woods, Berkshire, UK (latitude 51.772, longitude -1.338) by Douglas Boyes (University of Oxford). The specimen was identified by Douglas Boyes and snap-frozen on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute. The ilFurFurc1 sample was weighed and dissected on dry ice with head 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. Fragment size analysis of 0.01–0.5 ng of DNA was then performed using an Agilent FemtoPulse. High molecular weight (HMW) DNA was extracted using the Qiagen MagAttract HMW DNA extraction kit. Low molecular weight DNA was removed from a 200-ng aliquot of extracted DNA using 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 between 12–20 kb in a
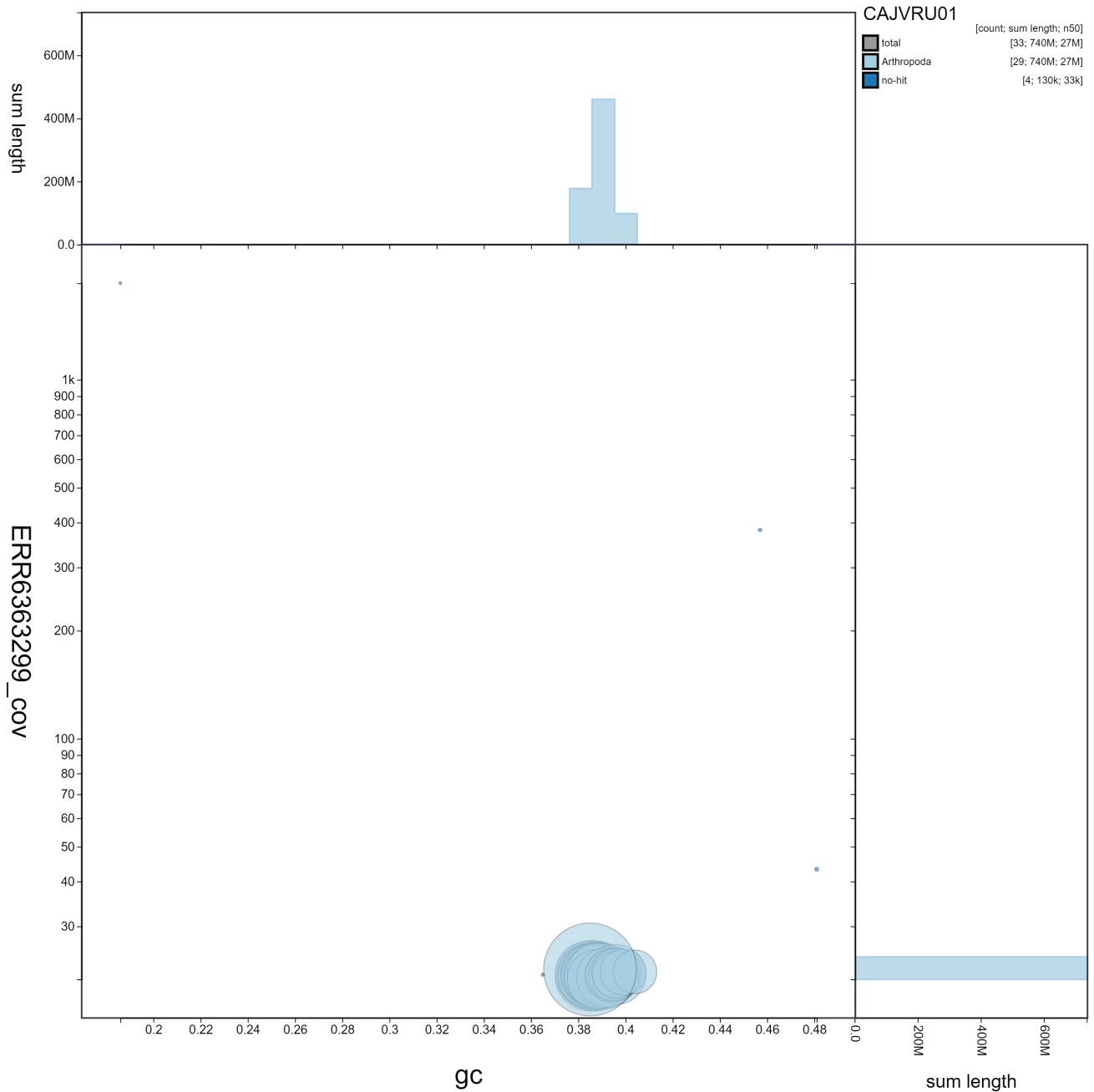
**Figure 3. Genome assembly of *Furcula furcula*, ilFurFurc1.1: GC coverage.** BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilFurFurc1.1/dataset/CAJVRU01/blob.

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 ilFurFurc1 in the Tree of Life Laboratory at the WSI using TRIzol, according to the manufacturer's instructions. RNA was then eluted in 50 μl RNAse-free water and its concentration RNA assessed
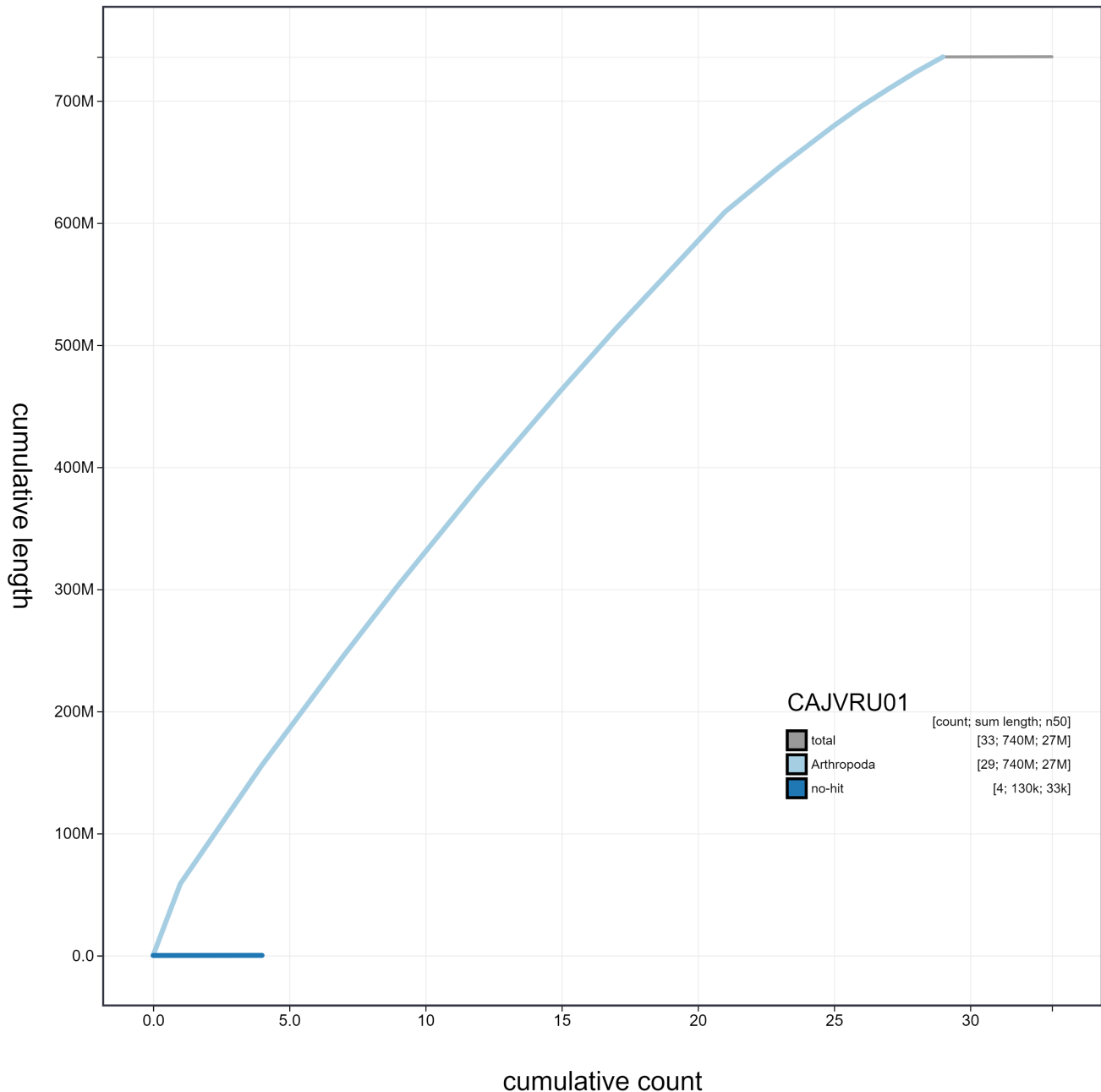
**Figure 4. Genome assembly of *Furcula furcula*, ilFurFurc1.1: cumulative sequence.** BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilFurFurc1.1/dataset/CAJVRU01/cumulative.

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 Chromium read cloud sequencing libraries were constructed according to the manufacturers' instructions. Sequencing was performed by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL II (HiFi), Illumina NovaSeq 6000 (10X) and Illumina HiSeq 4000 (RNA-Seq) instruments. Hi-C data were generated in the Tree of Life laboratory from head tissue of ilFurFurc1 using the Arima v2 kit and sequenced on a NovaSeq 6000 instrument.
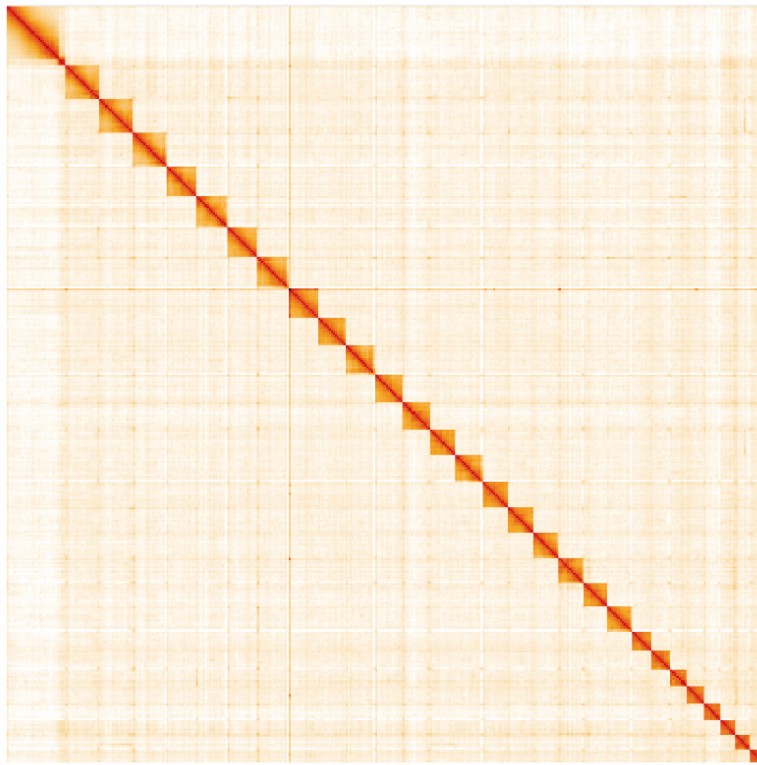
**Figure 5. Genome assembly of *Furcula furcula*, ilFurFurc1.1: Hi-C contact map.** Hi-C contact map of the ilFurFurc1.1 assembly, visualised in HiGlass. Chromosomes are arranged in size order from left to right and top to bottom. The interactive Hi-C map can be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=IqL6OXWLRbG3Rb-xprK0Zg.

**Table 2. Chromosomal pseudomolecules in the genome assembly of *Furcula furcula*, ilFurFurc1.1.**

| INSDC accession | Chromosome | Size (Mb) | GC% |
|---|---|---|---|
| OU452243.1 | 1 | 32.75 | 38.7 |
| OU452244.1 | 2 | 32.66 | 38.5 |
| OU452245.1 | 3 | 31.92 | 38.6 |
| OU452246.1 | 4 | 29.95 | 38.5 |
| OU452247.1 | 5 | 29.86 | 38.6 |
| OU452248.1 | 6 | 29.83 | 38.6 |
| OU452249.1 | 7 | 29.22 | 38.9 |
| OU452250.1 | 8 | 28.36 | 38.4 |
| OU452251.1 | 9 | 27.86 | 38.5 |
| OU452252.1 | 10 | 27.37 | 38.9 |
| OU452253.1 | 11 | 27.06 | 38.6 |
| OU452254.1 | 12 | 26.56 | 38.7 |
| OU452255.1 | 13 | 25.8 | 38.6 |
| OU452256.1 | 14 | 25.6 | 38.7 |
| OU452257.1 | 15 | 25.18 | 38.7 |
| OU452258.1 | 16 | 24.89 | 39.1 |
| OU452259.1 | 17 | 24.26 | 38.8 |
| OU452260.1 | 18 | 23.89 | 39.6 |
| OU452261.1 | 19 | 23.77 | 38.8 |
| OU452262.1 | 20 | 23.58 | 39.2 |
| OU452263.1 | 21 | 18.67 | 39.4 |
| OU452264.1 | 22 | 17.96 | 39.6 |
| OU452265.1 | 23 | 17.27 | 39.4 |
| OU452266.1 | 24 | 16.66 | 39.5 |
| OU452267.1 | 25 | 15.79 | 39.6 |
| OU452268.1 | 26 | 14.31 | 39.6 |
| OU452269.1 | 27 | 13.74 | 39.9 |
| OU452270.1 | 28 | 12.47 | 40.4 |
| OU452242.1 | Z | 58.75 | 38.5 |
| OU452271.1 | MT | 0.02 | 19 |
| - | Unplaced | 0.11 | 44.5 |

## Genome assembly

Assembly was carried out with Hifiasm (Cheng *et al.*, 2021); 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 longranger 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 as described previously (Howe *et al.*, 2021). Manual curation was performed using HiGlass (Kerpedjiev *et al.*, 2018) and Pretext. The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2021), which performs annotation using MitoFinder (Allio *et al.*, 2020). The genome was analysed and BUSCO scores generated within the BlobToolKit environment (Challis *et al.*, 2020). Table 3 contains a list of all software tool versions used, where appropriate.

## Ethics/compliance issues

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.

**Table 3. Software tools used.**

| Software tool | Version | Source |
|---|---|---|
| Hifiasm | 0.15.1 | Cheng *et al.*, 2021 |
| purge_dups | 1.2.3 | Guan *et al.*, 2020 |
| SALSA2 | 2.2 | Ghurye *et al.*, 2019 |
| longranger align | 2.2.2 | https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines |
| freebayes | 1.3.1-17-gaa2ace8 | Garrison & Marth, 2012 |
| MitoHiFi | 2.0 | Uliano-Silva *et al.*, 2021 |
| HiGlass | 1.11.6 | Kerpedjiev *et al.*, 2018 |
| PretextView | 0.2.x | https://github.com/wtsi-hpag/PretextView |
| BlobToolKit | 3.2.6 | Challis *et al.*, 2020 |

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. 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: Furcula furcula (sallow kitten). Accession number PRJEB45669; https://identifiers.org/ena.embl/PRJEB45669.

The genome sequence is released openly for reuse. The *F. furcula* genome sequencing initiative is part of the Darwin Tree of Life (DToL) project. All raw sequence data and the assembly have been deposited in INSDC databases. The genome will be annotated using the 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.

## Author information

Members of the University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.6418202.

Members of the Darwin Tree of Life Barcoding collective are listed here: https://doi.org/10.5281/zenodo.6418156.

Members of the Wellcome Sanger Institute Tree of Life programme are listed here: https://doi.org/10.5281/zenodo.6866293.

Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: https://doi.org/10.5281/zenodo.5746904.

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.6125046.

Members of the Darwin Tree of Life Consortium are listed here: https://doi.org/10.5281/zenodo.6418363.

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# Open Peer Review

## Current Peer Review Status: ✓ ✓

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**Version 1**

Reviewer Report 03 March 2023

https://doi.org/10.21956/wellcomeopenres.20083.r55051

✓   **Danilo T Amaral** [iD]

Graduate School of Biotechnology and Environmental Monitoring (UFSCar),, Sorocaba,, Brazil

I was pleased to review the date note by Boyes et al. entitled "The genome sequence of the sallow kitten, Furcula furcula (Clerck, 1759)". Genomic information, with high-quality sequencing and assembly, from different species groups, has great importance nowadays for distinct fields, such as evolution, phylogeography, biotechnology, and so forth. Even more, those that present chromosome quality assembly, such as here. The manuscript methodology and results were well-written and detailed.

As two minor considerations/comments, despite all figures and tables being quite essential to demonstrate the assembly quality, I suggest including figures 3 to 5 and table 3 as supplementary material. Based on the sequencing and assembly methodologies. I would like to know if any other study performed the karyotype of this species (or close species) to determine the number of chromosomes. If so, you may add this information to the text and briefly discuss it.

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

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

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

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

*Competing Interests:* No competing interests were disclosed.

*Reviewer Expertise:* Genomics, 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 06 February 2023

https://doi.org/10.21956/wellcomeopenres.20083.r54246

✔     **Sivasankaran Kuppusamy**
       Loyola College, chennai, India

I appreciate the author for assembling the whole genome sequence of sallow kitten, *Furcula furcula*. The standard methods for sequencing the whole genome, genome assembling technique and software were followed properly.

**The comments on the manuscripts:**

Total lengths of raw reads (paired) were not mentioned in the text.

How many nuclear genes received in the whole genome sequence assembly? The nuclear gene numbers haven't mentioned in the text.

The maximum and minimum contig length was not indicated. Total contigs length was not indicated.

Gene recovery statistics can be given in the 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:* Taxonomy and Phylogenetic analysis of superfamily  Noctuoidea moths

**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.**