



DATA NOTE

# The genome sequence of the Common Moorhen, *Gallinula chloropus* (Linnaeus, 1758) (Gruiformes: Rallidae)

[version 1; peer review: 3 approved]

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### Abstract

We present a genome assembly from an individual male *Gallinula chloropus* (Common Moorhen; Chordata; Aves; Gruiformes; Rallidae). The assembly contains two haplotypes with total lengths of 1 282.39 megabases and 1 208.56 megabases. Most of haplotype 1 (92.66%) is scaffolded into 38 chromosomal pseudomolecules, including the Z sex chromosome. Haplotype 2 was assembled to scaffold level. The mitochondrial genome has also been assembled, with a length of 17.04 kilobases. 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

*Gallinula chloropus*; Common Moorhen; genome sequence; chromosomal; Gruiformes



This article is included in the [Tree of Life gateway](#).

### Open Peer Review

#### Approval Status



|             | 1                    | 2                    | 3                    |
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## Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Deuterostomia; Chordata; Craniata; Vertebrata; Gnathostomata; Teleostomi; Euteleostomi; Sarcopterygii; Dipnotetrapodomorpha; Tetrapoda; Amniota; Sauropsida; Sauria; Archelosauria; Archosauria; Dinosauria; Saurischia; Theropoda; Coelurosauria; Aves; Neognathae; Neoaves; Gruiformes; Rallidae; *Gallinula*; *Gallinula chloropus* (Linnaeus, 1758) (NCBI:txid9123)

## Background

The common moorhen (*Gallinula chloropus*) is a small water-bird in the rail family. Adult birds are black all over with stark white rumps and a white horizontal line marking on their flanks. The white rump is easily spotted, as the bird habitually flicks its tail upwards to reveal it while moving, and also displays it during territorial disputes. Their yellow legs with long, unwebbed toes and red facial shield provide striking contrast to their monochrome plumage. The facial shield extends from above the red eyes onto the short, pointed bill, which ends in a yellow tip. Juveniles do not show a red shield until maturity and take on a duller grey plumage and leg colour until adulthood (del Hoyo J *et al.*, 1996). When flushed, individuals prefer to run or swim rather than fly, though they are capable of low, short flights between dense cover. Despite this tendency to short, hesitant flight, some northern populations can migrate distances to escape freezing winter conditions that would otherwise limit their aquatic habits (del Hoyo J *et al.*, 1996).

Moorhens are found across the Old World, with resident and breeding populations found throughout mainland Europe and northern Africa across to southern Africa and south-east Asia (eBird, 2025). In 2011, the species was split from the now recognised common gallinule (*Gallinula galeata*), a similar-looking New World species. The common moorhen itself now has five recognised geographically separated subspecies (Gill *et al.*, 2025).

Moorhens are wetland specialists of marshes and reedbeds which are used for cover and nesting material. They make small bowls of reeds with 3 to 15 eggs and both parents of seasonally monogamous pairs taking an active role in rearing, although intraspecific brood parasitism is not uncommon (Amininasab *et al.*, 2021; Foreman, 2001; Huxley & Wood, 1976; Petrie, 1987). Moorhens have a wide diet mostly consisting of vegetation such as grasses and aquatic invertebrates such as *Daphnia* spp. (Lardjane-Hamiti *et al.*, 2015), but also feed opportunistically on fruits and nuts (Thomas, 1982) and even carrion (Ciach, 2004).

The species is shot recreationally during hunting seasons (BASC, 2025). The IUCN ranks the common moorhen as least concern, due to the species' wide distribution and stable population (IUCN, 2019). As moorhens are abundant and widespread, present in high densities in many wetlands, some studies have used moorhens as subjects to examine heavy metal and metalloid build-up in bird populations in polluted environments (López-Perea *et al.*, 2019; Zamani-Ahmadmahmoodi *et al.*, 2009).

Being a wetland specialist and ground feeder, the moorhen is vulnerable to avian botulism, contracted via invertebrate prey and also through sediment disturbance. It is also infected with avian influenza through contact with high-densities of flocking birds (Anza *et al.*, 2016; Martelli *et al.*, 2025). Analysis of the genetic sequence of this species could be applied to epidemiological studies looking at immune response and disease spread.

Genetic analysis of common moorhens in the literature has focused on population dynamics such as dispersal patterns (Ruan *et al.*, 2018), genetic diversity (Ruan *et al.*, 2018; Van Duyse *et al.*, 1999), and population viability of endangered subspecies (Miller *et al.*, 2015). We present a chromosomally complete genome sequence for *Gallinula chloropus*, produced using the Tree of Life pipeline from a specimen collected in London Wetlands Centre, London, England, United Kingdom (Figure 1), as part of the Darwin Tree of Life project.

## Methods

### Sample acquisition

The *Gallinula chloropus* specimen used for genome sequencing (specimen ID NHMUK014561234, ToLID bGalCh11; Figure 1) was a wild male specimen found deceased and collected at WWT London Wetland Centre, London in 2021 as part of a disease surveillance programme carried out by WWT in contribution to the Great Britain Wildlife Health Partnership. It was collected from London Wetlands Centre, London, England, United Kingdom (latitude 51.47, longitude -0.23) on 2021-08-14 and stored at -20 °C. Several small samples of pectoral muscle were taken and stored at -80 °C. The specimen was collected and identified by Michelle O'Brien (Wildfowl & Wetlands Trust). A sample from the same specimen was used for RNA sequencing. Sample metadata were collected in line with the Darwin Tree of Life project standards described by Lawniczak *et al.* (2022).

### Nucleic acid extraction

Protocols for high molecular weight (HMW) DNA extraction developed at the Wellcome Sanger Institute (WSI) Tree



**Figure 1.** Photograph of the *Gallinula chloropus* (bGalCh11) specimen used for genome sequencing.

of Life Core Laboratory are available on [protocols.io](https://protocols.io) (Howard *et al.*, 2025). The bGalChl1 sample was weighed and triaged to determine the appropriate extraction protocol. Tissue from the muscle was homogenised by [cryogenic disruption](#) using the Covaris cryoPREP® Automated Dry Pulverizer.

HMW DNA was extracted in the WSI Scientific Operations core 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 [manual 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 12.3 ng/μL and a yield of 3 982.00 ng, with a fragment size of 12.8 kb. The 260/280 spectrophotometric ratio was 1.88, and the 260/230 ratio was 1.84.

RNA was extracted from muscle tissue of bGalChl1 in the Tree of Life Laboratory at the WSI using the [RNA Extraction: Automated MagMax™ mirVana](#) 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 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 25M 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 muscle tissue of the bGalChl1 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 Diagenode 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 and 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 to generate 150-bp 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; [Cheng et al.](#), 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 MERQURY.FK (Rhie *et al.*, 2020).

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

### Assembly curation

The assembly was decontaminated using the Assembly Screen for Cobionts and Contaminants (ASCC) pipeline. TreeVal was used to generate the flat files and maps for use in curation. MicroFinder (Mathers *et al.*, 2025) was used to order scaffolds prior to 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 7 breaks, 63 joins, and removal of 395 haplotypic duplications. The curation process is documented at <https://gitlab.com/wtsi-grit/rapid-curation>. PretextViewSnapshot was used to generate a Hi-C contact map of the final assembly.

### Assembly quality assessment

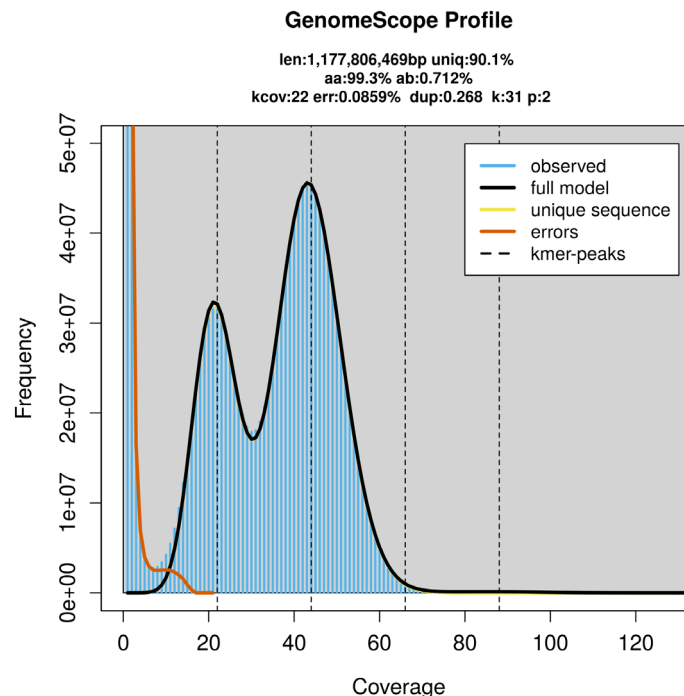
The Merqury.FK tool (Rhie *et al.*, 2020) was run in a Singularity container (Kurtzer *et al.*, 2017) to evaluate *k*-mer completeness and assembly quality for both haplotypes using the *k*-mer databases ( $k = 31$ ) computed prior to genome assembly. The analysis outputs included assembly QV scores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

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).

### Genome sequence report

#### Sequence data

PacBio sequencing of the *Gallinula chloropus* specimen generated 53.43 Gb (gigabases) from 6.61 million reads, which were used to assemble the genome. GenomeScope2.0 analysis estimated the haploid genome size at 1 177.81 Mb, with a heterozygosity of 0.71% and repeat content of 9.94% (Figure 2). These estimates guided expectations for the assembly. Based on the estimated genome size, the sequencing data provided approximately 44x coverage. Hi-C sequencing produced



**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.

56.12 Gb from 371.67 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 1 282.39 Mb in 817 scaffolds, with 603 gaps, and a scaffold N50 of 90.34 Mb ([Table 2](#)).

Most of the assembly sequence (92.66%) was assigned to 38 chromosomal-level scaffolds, representing 37 autosomes and the Z sex chromosome. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size ([Figure 3](#); [Table 3](#)).

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.

For haplotype 1, the estimated QV is 62.5, and for haplotype 2, 63.4. When the two haplotypes are combined, the assembly achieves an estimated QV of 62.9. The *k*-mer completeness is 84.64% for haplotype 1, 83.77% for haplotype 2, and 99.81% for the combined haplotypes ([Figure 4](#)).

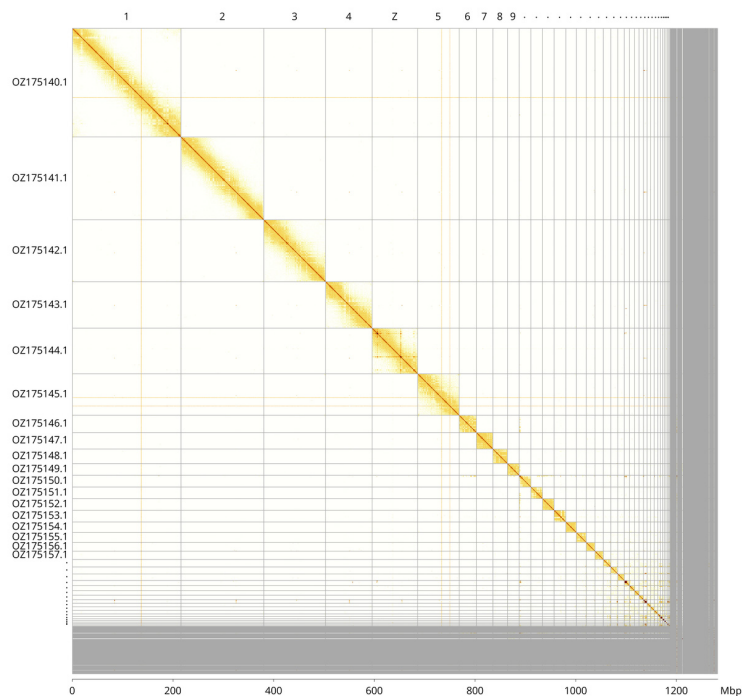
BUSCO analysis using the aves\_odb10 reference set (*n* = 8 338) identified 96.9% of the expected gene set (single = 96.5%, duplicated = 0.4%) for haplotype 1. The snail plot in [Figure 5](#) summarises the scaffold length distribution and other assembly statistics for haplotype 1. The blob plot in [Figure 6](#) shows the distribution of scaffolds by GC proportion and coverage for haplotype 1.

**Table 1.** Specimen and sequencing data for BioProject PRJEB74708.

| Platform                      | PacBio HiFi    | Hi-C               | RNA-seq            |
|-------------------------------|----------------|--------------------|--------------------|
| ToLID                         | bGalChl1       | bGalChl1           | bGalChl1           |
| Specimen ID                   | NHMUK014561234 | NHMUK014561234     | NHMUK014561234     |
| BioSample (source individual) | SAMEA113398958 | SAMEA113398958     | SAMEA113398958     |
| BioSample (tissue)            | SAMEA114299703 | SAMEA114299703     | SAMEA114299703     |
| Tissue                        | muscle         | muscle             | muscle             |
| Instrument                    | Revio          | Illumina NovaSeq X | Illumina NovaSeq X |
| Run accessions                | ERR12875176    | ERR12893025        | ERR13493939        |
| Read count total              | 6.61 million   | 371.67 million     | 71.64 million      |
| Base count total              | 53.43 Gb       | 56.12 Gb           | 10.82 Gb           |

**Table 2.** Genome assembly statistics.

| Metric                       | Haplotype 1             | Haplotype 2     |
|------------------------------|-------------------------|-----------------|
| Assembly name                | bGalChl1.hap1.1         | bGalChl1.hap2.1 |
| Assembly accession           | GCA_964237585.1         | GCA_964237395.1 |
| Assembly level               | chromosome              | scaffold        |
| Span (Mb)                    | 1 282.39                | 1 208.56        |
| Number of chromosomes        | 38                      | N/A             |
| Number of contigs            | 1 420                   | 931             |
| Contig N50                   | 3.67 Mb                 | 4.02 Mb         |
| Number of scaffolds          | 817                     | 395             |
| Scaffold N50                 | 90.34 Mb                | 87.26 Mb        |
| Longest scaffold length (Mb) | 215.89                  | N/A             |
| Sex chromosomes              | Z                       | N/A             |
| Organelles                   | Mitochondrion: 17.04 kb | N/A             |

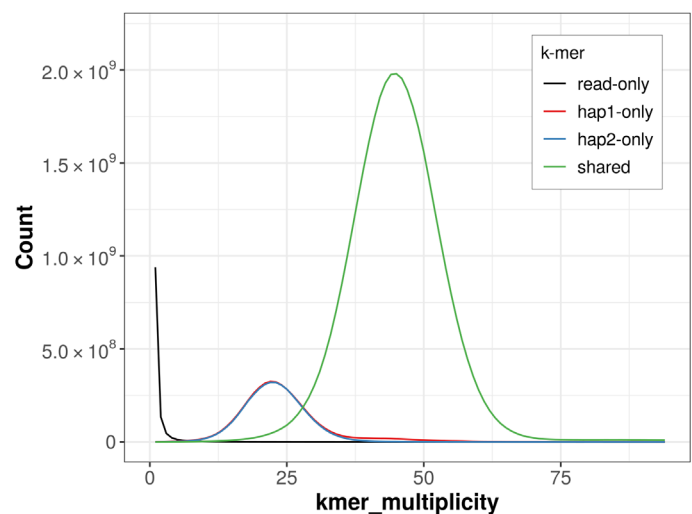


**Figure 3. Hi-C contact map of the *Gallinula chloropus* genome assembly.** Assembled chromosomes are shown in order of size and labelled along the axes, with a megabase scale shown below. The plot was generated using PretextSnapshot.

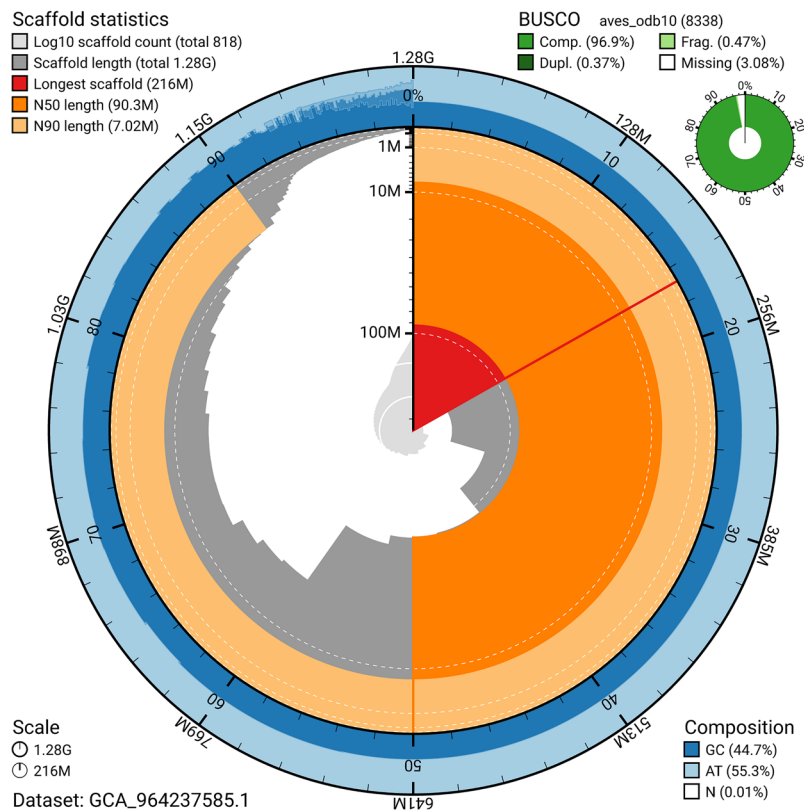
**Table 3. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *Gallinula chloropus* bGalCh1.**

| INSDC accession | Molecule | Length (Mb) | GC%   |
|-----------------|----------|-------------|-------|
| OZ175140.1      | 1        | 215.89      | 42    |
| OZ175141.1      | 2        | 164.19      | 41.50 |
| OZ175142.1      | 3        | 122.94      | 42    |
| OZ175143.1      | 4        | 92.36       | 42    |
| OZ175145.1      | 5        | 82.23       | 43    |
| OZ175146.1      | 6        | 34.21       | 43.50 |
| OZ175147.1      | 7        | 32.66       | 41.50 |
| OZ175148.1      | 8        | 28.86       | 44.50 |
| OZ175149.1      | 9        | 23.46       | 45    |
| OZ175150.1      | 10       | 23.22       | 46    |
| OZ175151.1      | 11       | 23.07       | 45    |
| OZ175152.1      | 12       | 23.04       | 45    |
| OZ175153.1      | 13       | 23.01       | 44    |
| OZ175154.1      | 14       | 21.23       | 44.50 |
| OZ175155.1      | 15       | 19.38       | 47.50 |
| OZ175156.1      | 16       | 17.44       | 48    |
| OZ175157.1      | 17       | 16.81       | 47.50 |
| OZ175158.1      | 18       | 14.42       | 49.50 |

| INSDC accession | Molecule | Length (Mb) | GC%   |
|-----------------|----------|-------------|-------|
| OZ175159.1      | 19       | 14.13       | 49    |
| OZ175160.1      | 20       | 12.82       | 48.50 |
| OZ175161.1      | 21       | 10.40       | 49    |
| OZ175162.1      | 22       | 10.09       | 52.50 |
| OZ175163.1      | 23       | 8.83        | 49.50 |
| OZ175164.1      | 24       | 8.65        | 51.50 |
| OZ175165.1      | 25       | 7.33        | 56    |
| OZ175166.1      | 26       | 7.23        | 52.50 |
| OZ175167.1      | 27       | 7.02        | 53.50 |
| OZ175168.1      | 28       | 6.91        | 54    |
| OZ175169.1      | 29       | 5.29        | 55    |
| OZ175170.1      | 30       | 5.07        | 52.50 |
| OZ175171.1      | 31       | 3.59        | 58    |
| OZ175172.1      | 32       | 3.57        | 54    |
| OZ175173.1      | 33       | 3           | 60.50 |
| OZ175174.1      | 34       | 2.55        | 53.50 |
| OZ175175.1      | 35       | 2.05        | 51.50 |
| OZ175176.1      | 36       | 0.75        | 61.50 |
| OZ175177.1      | 37       | 0.21        | 64.50 |
| OZ175144.1      | Z        | 90.34       | 42    |



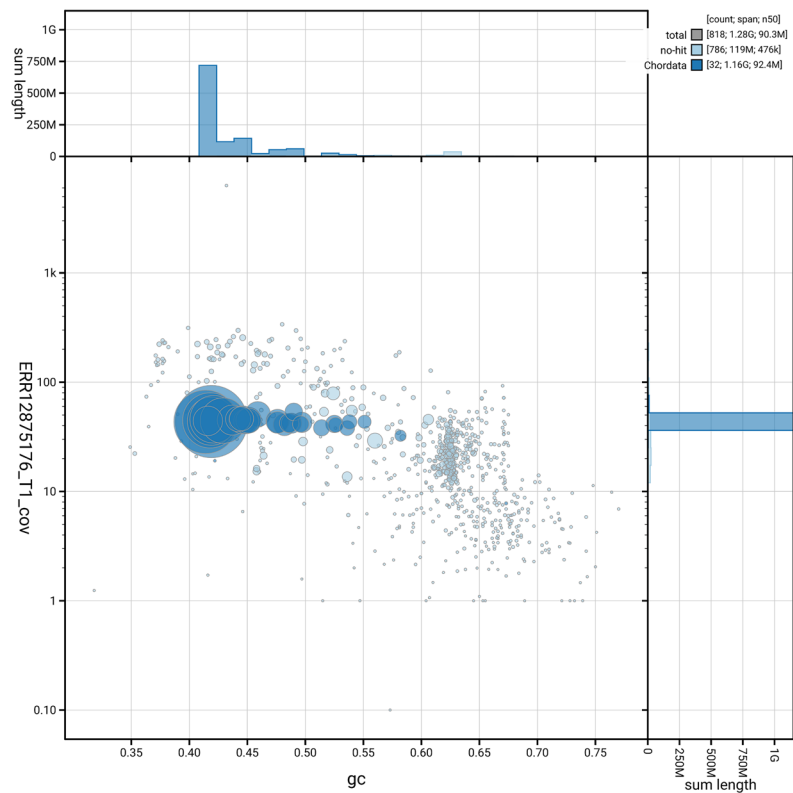
**Figure 4. Evaluation of *k*-mer completeness using MerquyFK.** This plot illustrates the recovery of *k*-mers from the original read data in the final assemblies. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers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



**Figure 5. Assembly metrics for bGalCh1.hap1.1.** 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](#).

Table 4 lists the assembly metric benchmarks adapted from Rhie *et al.* (2021) the Earth BioGenome Project Report on Assembly Standards September 2024. The EBP metric, calculated for the haplotype 1, is **6.C.Q62**, meeting the recommended reference standard.

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**’,



**Figure 6. BlobToolKit GC-coverage plot for bGalChl1.hap1.1.** 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 on the [BlobToolKit viewer](#).

**Table 4. Earth Biogenome Project summary metrics for the *Gallinula chloropus* assembly.**

| Measure                                        | Value                                                      | Benchmark        |
|------------------------------------------------|------------------------------------------------------------|------------------|
| EBP summary (haplotype 1)                      | 6.C.Q62                                                    | 6.C.Q40          |
| Contig N50 length                              | 3.67 Mb                                                    | ≥ 1 Mb           |
| Scaffold N50 length                            | 90.34 Mb                                                   | = chromosome N50 |
| Consensus quality (QV)                         | Haplotype 1: 62.5; haplotype 2: 63.4; combined: 62.9       | ≥ 40             |
| k-mer completeness                             | Haplotype 1: 84.64%; Haplotype 2: 83.77%; combined: 99.81% | ≥ 95%            |
| BUSCO                                          | C:96.9% [S:96.5%; D:0.4%]; F:0.5%; M:2.6%; n:8 338         | S > 90%; D < 5%  |
| Percentage of assembly assigned to chromosomes | 92.66%                                                     | ≥ 90%            |

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: *Gallinula chloropus* (common moorhen). Accession number [PRJEB74708](#). The genome

sequence is released openly for reuse. The *Gallinula chloropus* genome sequencing initiative is part of the Darwin Tree of Life Project (PRJEB40665), the Sanger Institute Tree of Life Programme (PRJEB43745) and the Vertebrate Genomes Project (PRJNA489243). 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](#).

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.

Author information

Contributors are listed at the following links:

- Members of the [Natural History Museum Genome Acquisition Lab](#)
- 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](#)

Table 5. Software versions and sources.

| Software       | Version     | Source                                                                                                                  |
|----------------|-------------|-------------------------------------------------------------------------------------------------------------------------|
| BEDTools       | 2.30.0      | <a href="https://github.com/arq5x/bedtools2">https://github.com/arq5x/bedtools2</a>                                     |
| BLAST          | 2.14.0      | <a href="ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/">ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/</a> |
| BlobToolKit    | 4.3.9       | <a href="https://github.com/blobtoolkit/blobtoolkit">https://github.com/blobtoolkit/blobtoolkit</a>                     |
| BUSCO          | 5.5.0       | <a href="https://gitlab.com/ezlab/busco">https://gitlab.com/ezlab/busco</a>                                             |
| bwa-mem2       | 2.2.1       | <a href="https://github.com/bwa-mem2/bwa-mem2">https://github.com/bwa-mem2/bwa-mem2</a>                                 |
| Cooler         | 0.8.11      | <a href="https://github.com/open2c/cooler">https://github.com/open2c/cooler</a>                                         |
| DIAMOND        | 2.1.8       | <a href="https://github.com/bbuchfink/diamond">https://github.com/bbuchfink/diamond</a>                                 |
| fasta_windows  | 0.2.4       | <a href="https://github.com/tolkit/fasta_windows">https://github.com/tolkit/fasta_windows</a>                           |
| FastK          | 1.1         | <a href="https://github.com/thegenemyers/FASTK">https://github.com/thegenemyers/FASTK</a>                               |
| GenomeScope2.0 | 2.0.1       | <a href="https://github.com/tbenavi1/genomescope2.0">https://github.com/tbenavi1/genomescope2.0</a>                     |
| Gfastats       | 1.3.6       | <a href="https://github.com/vgl-hub/gfastats">https://github.com/vgl-hub/gfastats</a>                                   |
| GoaT CLI       | 0.2.5       | <a href="https://github.com/genomehubs/goat-cli">https://github.com/genomehubs/goat-cli</a>                             |
| Hifiasm        | 0.19.8-r603 | <a href="https://github.com/chhyllp123/hifiasm">https://github.com/chhyllp123/hifiasm</a>                               |
| HiGlass        | 1.13.4      | <a href="https://github.com/higlass/higlass">https://github.com/higlass/higlass</a>                                     |

| Software               | Version             | Source                                                                                                            |
|------------------------|---------------------|-------------------------------------------------------------------------------------------------------------------|
| MercuryFK              | 1.1.2               | <a href="https://github.com/thegenemyers/MERQUERY.FK">https://github.com/thegenemyers/MERQUERY.FK</a>             |
| Minimap2               | 2.24-r1122          | <a href="https://github.com/lh3/minimap2">https://github.com/lh3/minimap2</a>                                     |
| MitoHiFi               | 3                   | <a href="https://github.com/marcelauliano/MitoHiFi">https://github.com/marcelauliano/MitoHiFi</a>                 |
| MultiQC                | 1.14; 1.17 and 1.18 | <a href="https://github.com/MultiQC/MultiQC">https://github.com/MultiQC/MultiQC</a>                               |
| Nextflow               | 23.10.0             | <a href="https://github.com/nextflow-io/nextflow">https://github.com/nextflow-io/nextflow</a>                     |
| PretextViewSnapshot    | N/A                 | <a href="https://github.com/sanger-tol/PretextViewSnapshot">https://github.com/sanger-tol/PretextViewSnapshot</a> |
| PretextView            | 0.2.5               | <a href="https://github.com/sanger-tol/PretextView">https://github.com/sanger-tol/PretextView</a>                 |
| samtools               | 1.19.2              | <a href="https://github.com/samtools/samtools">https://github.com/samtools/samtools</a>                           |
| sanger-tol/ascc        | 0.1.0               | <a href="https://github.com/sanger-tol/ascc">https://github.com/sanger-tol/ascc</a>                               |
| sanger-tol/blobtoolkit | 0.6.0               | <a href="https://github.com/sanger-tol/blobtoolkit">https://github.com/sanger-tol/blobtoolkit</a>                 |
| Seqtk                  | 1.3                 | <a href="https://github.com/lh3/seqtk">https://github.com/lh3/seqtk</a>                                           |
| Singularity            | 3.9.0               | <a href="https://github.com/sylabs/singularity">https://github.com/sylabs/singularity</a>                         |
| TreeVal                | 1.2.0               | <a href="https://github.com/sanger-tol/treeval">https://github.com/sanger-tol/treeval</a>                         |
| YaHS                   | 1.2a.2              | <a href="https://github.com/c-zhou/yahs">https://github.com/c-zhou/yahs</a>                                       |

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# Open Peer Review

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## Version 1

Reviewer Report 12 December 2025

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**Stephan Koblmüller** 

University of Graz, Graz, Graz, Austria

In this data note, Humby *et al.* present the genome assembly of the Common Moorhen, a rallid species widely distributed across many parts of Europe, Africa and Asia. I particularly like the background section, which gives an informative introduction to the study species. The data are clear, and the methods are well described. I have no objections to any of the data presented or the results. As far as I can tell, the datasets are deposited in publicly accessible repositories.

**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:** phylogenetics/-genomics, population genetics, phylogeography

**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 11 December 2025

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**Tsuneyuki Tatsuke** 

National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan

The authors successfully sequenced the genomic DNA of the Common Moorhen, *Gallinula chloropus*, with high accuracy, primarily using the PacBio Revio system, which resulted in the construction of a novel reference genome. This study provides detailed documentation of the collection time, location, and preservation status of the sequenced individual, and the experimental methodology is thoroughly described. This reference genomic sequence and associated data are publicly available online and are expected to serve as a valuable foundation for future research.

**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:** Molecular Biology

**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 14 October 2025

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**Xuejing Wang** 

Copenhagen University, Copenha, Denmark

This paper made a clear and thorough description of the newly assembled reference genome for

the common moorhen. Overall it is a very good work. The methods of sampling, sequencing and assembling are sound. The quality of the assembly is very high. The data of the reference genome is available online.

I only have a small question about RNA sequencing, or rather the specimen. Usually it is better to take RNA samples shortly after the death of the animal. Nevertheless, looking by the photo of the bird, it seemed very fresh, and the sequencing quality looks high. Maybe it is good to have a short description, like one sentence or a phrase, about the condition of the specimen?

**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:** Population genetics and conservation genomics

**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.**

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