GENOME NOTE

The genome sequence of the red-legged partridge, *Alectoris rufa* Linnaeus 1758

[version 1; peer review: 2 approved]

Abderrahmane Eleiwa[1-4], Pol Mestre[1], Ana Usie[1,5,6], Ester Vilaprinyo[1,2,4], Alberto Marin-Sanguino[1,2,4], Albert Sorribas[1,2,4], Cristobal Richart[7], Judit Salces-Ortiz[8], Rosa Fernández[8], Mar Biarnés[3], Noélia Antilles[3], Jesus Nadal[1,9], Rui Alves[1,2,4]

[1]Departament de Ciències Mèdiques Bàsiques, Universitat de Lleida, Lleida, Catalonia, Spain
[2]IRBLleida, Institut de Recerca Biomedica, Barcelona, Lleida, Spain
[3]Centre de Sanitat Avícola de Catalunya i Aragó (CESAC), Reus, Spain
[4](MathSy2Bio) group 2021 SGR 01353, Mathematical Systems & Synthetic Biology in Biomedicine and Biotechnology, Lleida, Spain
[5]Centro de Biotecnologia do Alentejo (CEBAL), Beja, Portugal
[6]MED – Mediterranean Institute for Agriculture, Environment and Development and CHANGE – Global Change and Sustainability Institute, Beja, Portugal
[7]Universitat Rovira i Virgili, Tarragona, Catalonia, Spain
[8]Metazoa Phylogenomics and Genome Evolution Lab, Institute of Evolutionary Biology (CSIC-UPF), Barcelona, Spain
[9]Departament de Ciència Animal, Universitat de Lleida, Lleida, Catalonia, Spain

v1 **First published:** 02 Sep 2025, **14**:855
https://doi.org/10.12688/f1000research.162894.1
Latest published: 02 Sep 2025, **14**:855
https://doi.org/10.12688/f1000research.162894.1

Abstract

The red-legged partridge (*Alectoris rufa*) is a socio-economically important game bird in southern Europe. Despite previous efforts, achieving a high-quality, chromosome-level genome assembly has remained challenging. Here, we present a *de novo* phased, gapless reference genome of *A. rufa*, combining Nanopore long-read sequencing and Hi-C data from both sexes. The assembly resolves 40 nuclear chromosomes (38 autosomes and the 2 sex chromosomes, Z and W) and the mitochondrial genome, achieving chromosome-scale resolution and 99.1% completeness based on the Aves BUSCO dataset. This high-quality genome provides a critical resource for studying genetic diversity, sex-linked traits, and evolutionary adaptations.

Keywords

Catalan Initiative for the Earth Biogenome Project, Birds, Phasianidae, Genome assembly, Reference Genome, Aves

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 02 Sep 2025	✓ view	✓ view

1. **Marc Palmada-Flores**, University of Girona, Girona, Spain

2. **Greta Bellinzona**, University of Pavia, Pavia, Italy

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

This article is included in the Nanopore Analysis gateway.

This article is included in the Genomics and Genetics gateway.

Corresponding authors: Jesus Nadal (jesus.nadal@udl.cat), Rui Alves (rui.alves@udl.cat)

Author roles: Eleiwa A: Data Curation, Formal Analysis, Investigation, Methodology, Software, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Mestre P**: Formal Analysis, Investigation; **Usie A**: Formal Analysis, Investigation, Validation, Writing – Review & Editing; **Vilaprinyo E**: Formal Analysis, Investigation, Writing – Review & Editing; **Marin-Sanguino A**: Formal Analysis, Investigation, Writing – Review & Editing; **Sorribas A**: Formal Analysis, Investigation, Writing – Review & Editing; **Richart C**: Funding Acquisition, Investigation; **Salces-Ortiz J**: Formal Analysis, Writing – Review & Editing; **Fernández R**: Investigation, Methodology; **Biarnés M**: Formal Analysis, Investigation, Writing – Review & Editing; **Antilles N**: Formal Analysis, Investigation, Writing – Review & Editing; **Nadal J**: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Resources, Supervision, Writing – Review & Editing; **Alves R**: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Project Administration, Resources, Supervision, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: AE is the recipients of an industrial Ph. D. fellowship from Generalitat de Catalunya and CESAC. AU acknowledges support by Fundação Nacional para a Ciência e a Tecnologia (FCT), Portugal, through contract grant CEECINST/00100/2021/CP2774/CT0001, and projects UIDB/05183, and LA/P/0121/2020. CR was supported by grant IT20041S from Fundació URV. RF acknowledges support from the following sources of funding: the European Research Council (this project has received funding from the European Research Council (ERC) under the European's Union's Horizon 2020 research and innovation programme (grant agreement no. 948281) and the Secretaria d'Universitats i Recerca del Departament d'Economia i Coneixement de la Generalitat de Catalunya (AGAUR 2021-SGR00420).
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Eleiwa A, Mestre P, Usie A *et al.* **The genome sequence of the red-legged partridge, *Alectoris rufa* Linnaeus 1758 [version 1; peer review: 2 approved]** F1000Research 2025, **14**:855 https://doi.org/10.12688/f1000research.162894.1

First published: 02 Sep 2025, **14**:855 https://doi.org/10.12688/f1000research.162894.1

Introduction

The red-legged partridge (*Alectoris rufa*) is a species of significant ecological and economic importance, particularly in southwestern Europe,[1] where it is a key game bird for hunting and rural economies. Despite its prominence, wild populations are declining due to habitat degradation and hunting pressure, leading to increased reliance on farm-reared partridges to meet hunting demands.

Limited insights into the bird's evolution were obtained from the analysis of previous efforts that created assemblies with high to moderate genome fragmentation.[1–4] That fragmentation limits our ability to fully explore the genomic basis of traits relevant to both wild and farmed populations, such as behavior, physiology, and adaptation.

Here, we present a *de novo* high-quality, chromosome-level genome assembly of *A. rufa*, incorporating both macro- and micro chromosomes, sex chromosomes, and the mitochondrial chromosome. We created this assembly through a hybrid approach combining long-read sequencing technologies (Oxford Nanopore) with Hi-C Illumina data, significantly improving contiguity, accuracy, and completeness over previous efforts. The inclusion of sex chromosomes provides a critical resource for understanding sex-linked traits and genetic diversity, which are essential for both conservation and breeding programs.

This chromosome-level assembly provides a foundation for identifying genetic markers associated with desirable traits, facilitating the development of genomic tools to ensure the genetic purity of wild partridges and enhancing the sustainability and productivity of farmed partridges.

Methods

Table 1 provides the details on all software tools used for the assembly of the *A. rufa* genome.

Sample collection

We randomly selected a wild female from Ciudad Real (central Iberian Peninsula) and a farm-raised male from Lleida (northeastern Iberian Peninsula). They were both anesthetized by inhaling isoflurane, before blood samples (0.25 ml) were drawn from the brachial vein of the wing using a sterile syringe with a 20G needle. After the procedure, the birds were allowed to recover and closely monitored for any signs of distress. The protocol was approved by the Ethics Committee on Animal Experimentation of the University of Lleida in 2016 (Ref. CEEA 09-06/16).

Sample extraction, library construction and sequencing

High molecular weight (HMW) DNA was extracted from that blood for library preparation with the genomic DNA sequencing kit of Oxford Nanopore technology (ONT) as described in[2] and then sequenced the libraries using a GridION platform.

Chromatin conformation capture sequencing (Hi-C) libraries were prepared using the Hi-C High-Coverage kit (Arima Genomics) in the Metazoa Phylogenomics Lab (Institute of Evolutionary Biology [CSIC-UPF]). Sample concentration

Table 1. Software tools used.

Tool	Version	Parameters	Source
Poreshop	0.2.4	Default	https://github.com/rrwick/Porechop
Fastp	0.23.4	Default	8
NextDenovo	2.5.21	Default	21
NextPolish	1.4.0	Default	6
purge_haplotigs	1.1.3	-wind_min 10000	7
Gfastats	1.3.6	Default	15
BUSCO	5.7.1	-l aves_odb10	16
HapHiC	1.0.6	--correct_nrounds 2 max_inflation 10 --bin_size 200	9
Juicebox	1.6	Default	22
Ragtag	v2.1.0	Default	11
BlobToolKit	2.6.2	Default	17

was assessed by Qubit DNA HS Assay kit (Thermo Fisher Scientific), and library preparation was carried out using the ACCEL-NGS 2S PLUS DNA LIBRARY KIT (Swift Bioscience) and using the 2S Set A single indexes (Swift Bioscience). We carried out library amplification with the KAPA HiFi DNA polymerase (Roche). The amplified libraries were sequenced on the NovaSeq 6000 (Illumina) with a read length of 2x151bp and a ~60Gb coverage, resulting in ~400M reads per library.

Nuclear genome assembly

Nuclear genome assembly was performed using Nanopore raw data from one female and one male, as described in our previous work.[2] We implemented and used the Dorado base caller[5] to improve per-base quality of the original noisy ONT raw reads.

Assembly was carried out with NextDenovo pipeline.[6] The yielded contig-level assembly was polished using the NextPolish v1.4.0[6] based on the ONT data. Haplotypic duplications were identified and removed using purge_haplotigs.[7] The purge_haplotigs contigs were used to filter out mitochondrial genome form the nuclear contig, as described below.

Fastp[8] was used to process the Hi-C removing low quality and duplication raw reads and HapHiC[9] was used on the Hi-C data to scaffolds the purged contigs. HapHiC is an allele-aware tool that enables scaffolding the haplotype-phased genome assembly into chromosome-scale pseudomolecules without the need for a preexistent reference genome. Chromosome assignment results were validated based on their synteny with respect to the *Gallus gallus* reference genome GCA_024206055.2,[10] using Ragtag v2.1.0.[11] Note that RagTag orders, orients, and joins sequences with gaps without altering the input query. We performed a final round of gap filling with Medaka[12] using the ONT raw reads. This was sufficient to generate the final gapless genome and capture the chromatin signal of both sex chromosomes of *A. rufa.* We independently assembled the male and female autosomes using sex-specific Hi-C data. We choose the autosome with the highest quality as the reference. However, due to the lower quality of the male Hi-C data, we used only female Hi-C data to assemble the sex chromosomes.

Table 2. Genome data for the *Alectoris rufa.*

Project accession data	
Assembly identifier	A_rufa_assembly2
Species	*Alectoris rufa*
Specimen	Arufa2401F
NCBI taxonomy ID	9079
BioProject	PRJNA1164475
Biosample ID	SAMEA114518447
Isolate information	Blood tissue (female & male)
Raw data accessions	
Nanopore raw data	ERR12165669, ERR12165668, ERR12165667, ERR12165666, ERR12165665, ERR14375934
Hi-C Illumina	ERR14648049, ERR14648048
Genome assembly	
Assembly accession	GCA_963854145
Span (Mb)	1,233
Number of contigs	46
Contig N50 length (Mb)	92
Number of scaffolds	46
Scaffold N50 length (Mb)	92
Longest scaffold (Mb)	198
BUSCO* genome score	C:99.1%[S:99.0%,D:0.1%], F:0.1%,M:0.8%,n:8338

*BUSCO scores based on the aves_odb10 BUSCO set using v5.7.1. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison.

Mitochondrial genome assembly and annotation

The mitogenome was extracted from the polished and purged nuclear contigs using a combined approach. First, the get_organelle_from_assembly.py script from GetOrganelle[13] was applied to the nuclear contigs, producing an uncircularized, gapped sequence. Next, Illumina short reads were used to circularize the mitogenome assembly. For annotation, MitoZ v3.6[14] was employed. The genome assembly metrics were estimated using the Gfastats tool.[15] The BUSCO completeness score[16] was calculated within the BlobtoolKit2.[17]

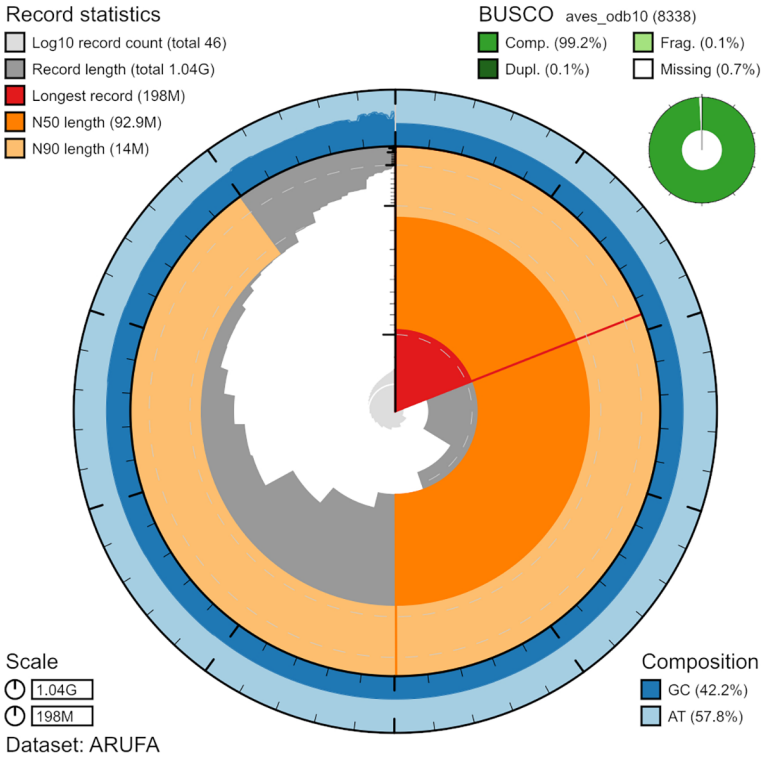

Figure 1. BlobToolKit Snailplot, representing the N50 metrics for *A. rufa* assembly and BUSCO scores for the Aves set of orthologues. The distribution of chromosome lengths is shown in dark grey with the plot radius scaled to the longest chromosome present in the assembly shown in red.

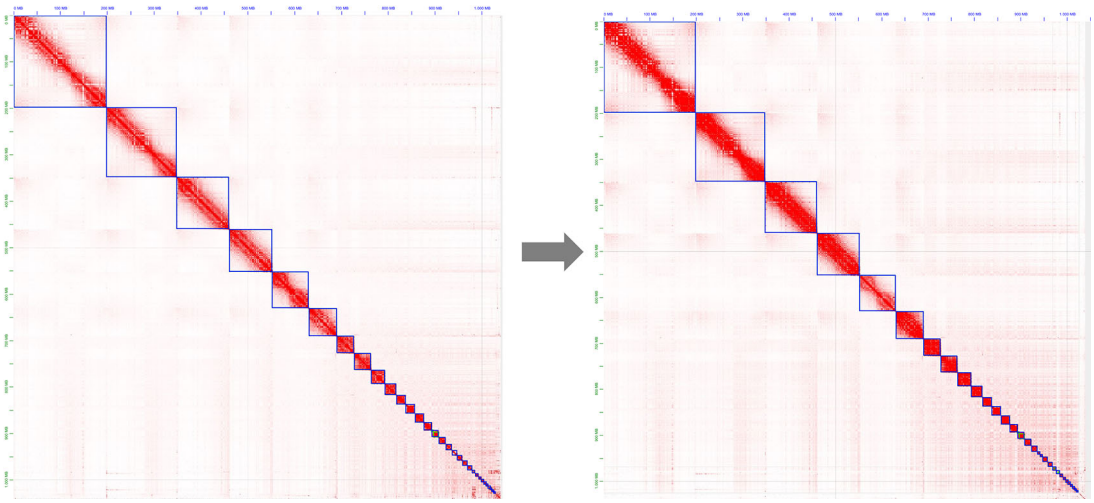

Figure 2. Juicebox visualization of the manually curated Hi-C contact map for the *A. rufa* assembly. Left-hand side: Original mapping. Right-hand side: Contact map after manual curation.

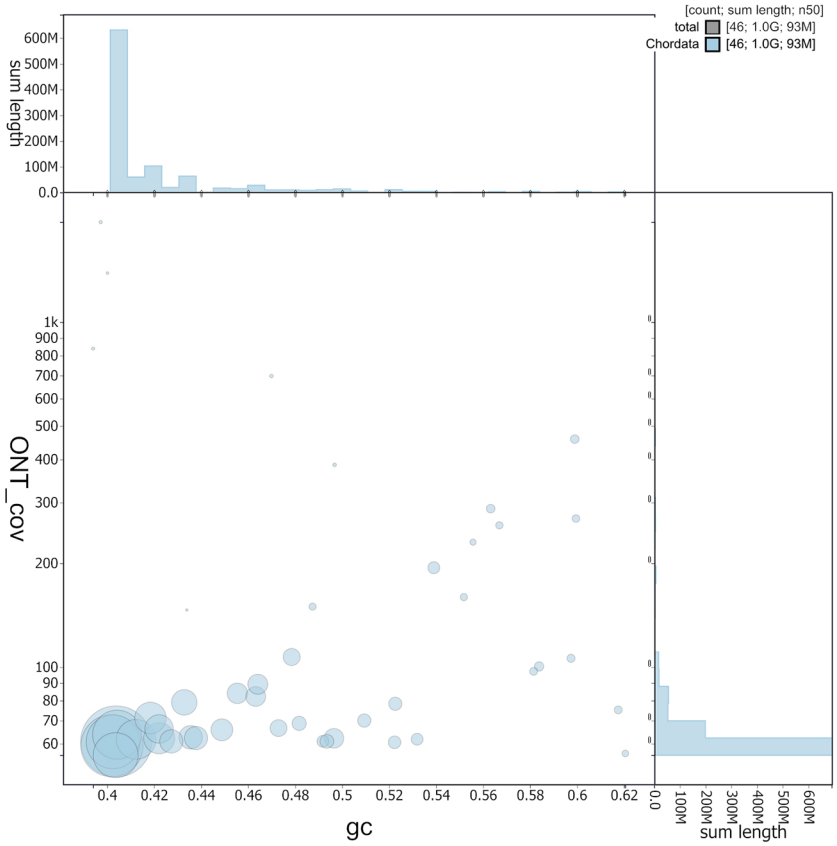

Figure 3. BlobToolKit GC-coverage plot. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. Y-axis ontogene covariance (Chordata).

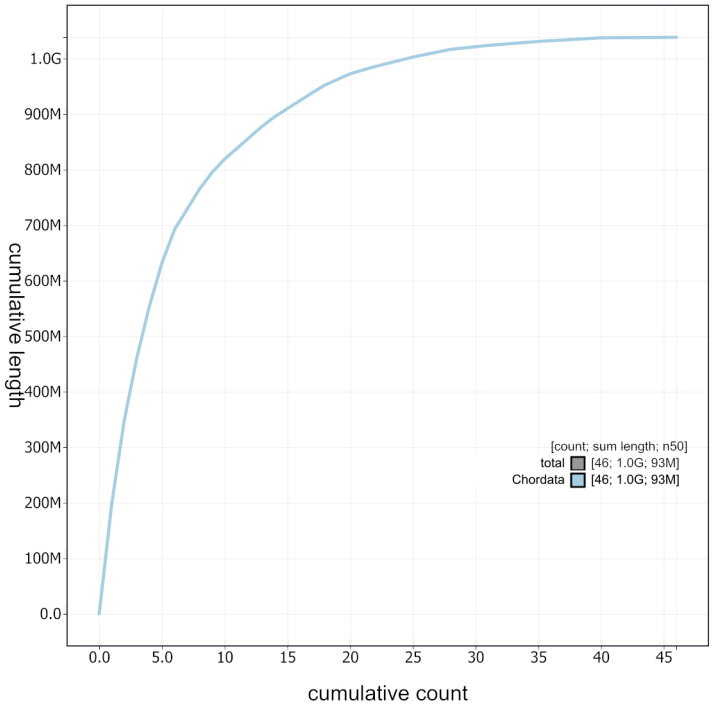

Figure 4. Cumulative sequence length plot of the *A. rufa* assembly. The grey line shows cumulative length for all sequences.

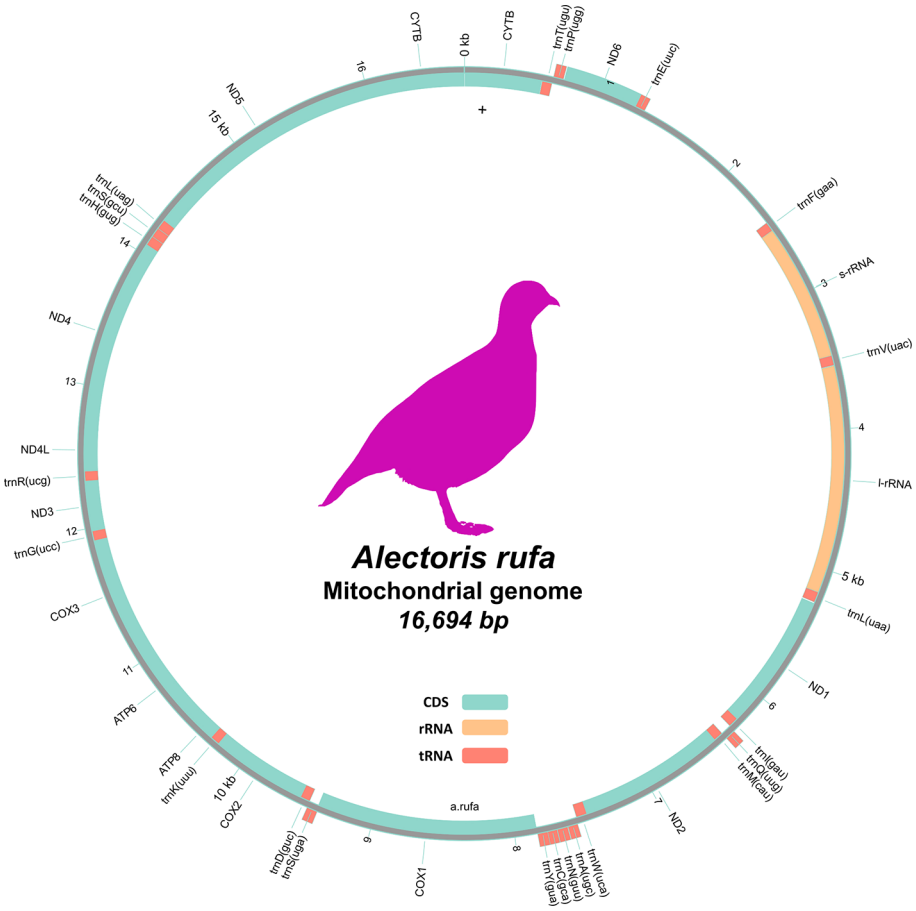

Figure 5. Mitochondrial genome gene map of *A. rufa*. The outermost track displays the annotated genes features. The middle track shows the coverage depth from ONT sequencing in 100 bp windows. The innermost track illustrates the GC content across the mitogenome, also in 100 bp windows.

Results and discussion

Genome sequence report

We sequenced the genome of two *A. rufa* individuals, one male and one female. We summarize the statistics and deposition data in Table 2. We generated a total of 60-fold coverage data in Nanopore Ultra-long reads (N50 20 kb). We also generated 60-fold coverage data in Illumina NovaSeq6000 Hi-C sequencing. We scaffolded the primary assembly contigs from nanopore sequences with chromosome conformation HiC data. We also performed a final quality control step for the HiC scaffolds by validating them through synteny comparison to the *Gallus gallus* reference genome. The final assembly has a total length of 1.38Gb in 46 sequence scaffolds with scaffold/contig N50 of 92 Mb. 99.91% of the assembly sequence was assigned to 40 chromosomal-level scaffolds representing 38 autosomes plus the W and Z sex chromosomes (Figures 1, 2, 3, and 4 and Table 2). The mitochondrial genome was 16.694 kbp long and contained 38 genes, including 22 tRNAs and 14 protein coding genes, with a GC percentage of 45.27 % (Figure 5). The genome has a BUSCO completeness of 99.1% using the aves_odb10 reference dataset of orthologue single copy genes. The statistics for each chromosome, together with their ENA accession numbers are given in Table 3.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Alectoris rufa*.

ENA accession	*Chromosome*	*Size (bp)*	*GC%*	*ENA accession*	*Chromosome*	*Size (bp)*	*GC%*
OZ238766.1	Chr01	198016086	40.38	**OZ238786.1**	Chr021	6832952	48.17
OZ238770.1	Chr02	150934939	40.20	**OZ238787.1**	Chr022	4537674	49.18
OZ238768.1	Chr03	112339401	40.25	**OZ238788.1**	Chr023	5891266	50.94

Table 3. *Continued*

ENA accession	Chromosome	Size (bp)	GC%	ENA accession	Chromosome	Size (bp)	GC%
OZ238769.1	Chr04	92881468	40.42	**OZ238789.1**	Chr024	6361550	49.35
OZ238770.1	Chr05	60168630	41.23	**OZ238790.1**	Chr025	993629	55.57
OZ238771.1	Chr06	36086843	42.21	**OZ238791.1**	Chr026	5251705	52.23
OZ238772.1	Chr07	37189634	41.84	**OZ238792.1**	Chr027	5836382	52.26
OZ238773.1	Chr08	29910164	42.21	**OZ238793.1**	Chr028	4642635	53.19
OZ238774.1	Chr09	23946095	43.28	**OZ238794.1**	Chr029	1487215	56.69
OZ238775.1	Chr010	20032715	43.55	**OZ238795.1**	Chr030	2250405	59.90
OZ238776.1	Chr011	19678379	42.73	**OZ238796.1**	Chr031	2219045	56.32
OZ238777.1	Chr012	19532764	43.78	**OZ238797.1**	Chr032	1812957	61.75
OZ238778.1	Chr013	17277913	44.88	**OZ238798.1**	Chr033	2650367	58.38
OZ238779.1	Chr014	15001852	45.54	**OZ238799.1**	Chr034	1468626	55.18
OZ238780.1	Chr015	13971621	46.32	**OZ238800.1**	Chr035	1892499	59.74
OZ238781.1	Chr016	4664116	53.90	**OZ238801.1**	Chr036	1635412	59.95
OZ238782.1	Chr017	10178771	47.85	**OZ238802.1**	Chr037	1157391	62.05
OZ238783.1	Chr018	13644036	49.65	**OZ238803.1**	Chr038	1811234	58.15
OZ238784.1	Chr019	9980371	47.29	**OZ238804.1**	Chr0W	1397749	48.74
OZ238785.1	Chr020	14276749	46.41	**OZ238805.1**	Chr0Z	77739472	40.36
				OZ238806.1	Chr0M	16694	45.27
					unplaced	914206	43.19

Reporting guidelines

Figshare: ARRIVE Checklist for the Genome Note "The genome sequence of the red-legged partridge, Alectoris rufa Linnaeus 1758", https://doi.org/10.6084/m9.figshare.29501561.v1[23]

This project contains the following data:

ARRIVE Author Checklist - E10 only.pdf

Data availability

ENA Chromosome Sequences: All sequence data is deposited at the ENA, under accession numbers GCA_963854145.2, OZ238766.1, OZ238770.1, OZ238768.1, OZ238769.1, OZ238770.1, OZ238771.1, OZ238772.1, OZ238773.1, OZ238774.1, OZ238775.1, OZ238776.1, OZ238777.1, OZ238778.1, OZ238779.1, OZ238780.1, OZ238786.1, OZ238787.1, OZ238788.1, OZ238789.1, OZ238790.1, OZ238791.1, OZ238792.1, OZ238793.1, OZ238794.1, OZ238795.1, OZ238796.1, OZ238797.1, OZ238798.1, OZ238799.1, OZ238800.1, OZ238801.1, OZ238802.1, OZ238803.1, OZ238804.1, OZ238805.1, OZ238806.1, OZ238781.1, OZ238782.1, OZ238783.1, OZ238784.1, and OZ238785.1. NCBI raw data sequences: Nanopore raw read data are available via NCBI (Bioproject accession numbers PRJNA1050768, Biosample accession numbers: ERS16499794, ERS16499793, ERS16499792, ERS16499791, ERS16499790, SRX23440923).

Links to the data:

https://www.ncbi.nlm.nih.gov/bioproject/1050768[18]

https://www.ncbi.nlm.nih.gov/nuccore/2724812281[18]

https://www.ncbi.nlm.nih.gov/sra/SRP486622[19]

https://www.ebi.ac.uk/ena/browser/view/GCA_963854145.2[20]

References

1. Chattopadhyay B, *et al.*: **Novel genome reveals susceptibility of popular gamebird, the red-legged partridge (Alectoris rufa, Phasianidae), to climate change.** *Genomics.* 2021; **113**: 3430–3438.
PubMed Abstract | Publisher Full Text

2. Eleiwa A, *et al.*: **Hybrid assembly and comparative genomics unveil insights into the evolution and biology of the red-legged partridge.** *Sci. Rep.* 2024; **14**: 19531.
PubMed Abstract | Publisher Full Text | Free Full Text

3. Ros-Freixedes R, Pena RN, Richart C, *et al.*: **Genomic diversity and signals of selection processes in wild and farm-reared red-legged partridges (Alectoris rufa).** *Genomics.* 2023; **115**: 110591.
PubMed Abstract | Publisher Full Text

4. González-Prendes R, Pena RN, Richart C, *et al.*: **Long-read de novo assembly of the red-legged partridge (Alectoris rufa) genome.** *Sci Data.* 2024; **11**: 908.
PubMed Abstract | Publisher Full Text | Free Full Text

5. nanoporetech/dorado: **Oxford Nanopore Technologies.** 2025.

6. Hu J, Fan J, Sun Z, *et al.*: **NextPolish: a fast and efficient genome polishing tool for long-read assembly.** *Bioinformatics.* 2020; **36**: 2253–2255.
PubMed Abstract | Publisher Full Text

7. Roach MJ, Schmidt SA, Borneman AR: **Purge Haplotigs: allelic contig reassignment for third-gen diploid genome assemblies.** *BMC Bioinformatics.* 2018; **19**: 460.
PubMed Abstract | Publisher Full Text | Free Full Text

8. Chen S, Zhou Y, Chen Y, *et al.*: **Fastp: An ultra-fast all-in-one FASTQ preprocessor.** *Bioinformatics.* 2018; **34**: i884–i890.
PubMed Abstract | Publisher Full Text | Free Full Text

9. Zeng X, *et al.*: **Chromosome-level scaffolding of haplotype-resolved assemblies using Hi-C data without reference genomes.** *Nat Plants.* 2024; **10**: 1184–1200.
PubMed Abstract | Publisher Full Text

10. **Gallus gallus genome assembly bGalGal1.mat.broiler. GRCg7b.** *NCBI.*
Reference Source

11. Alonge M, *et al.*: **RaGOO: fast and accurate reference-guided scaffolding of draft genomes.** *Genome Biol.* 2019; **20**: 224.
PubMed Abstract | Publisher Full Text | Free Full Text

12. **GitHub - nanoporetech/medaka: Sequence correction provided by ONT Research.** *GitHub.*
Reference Source

13. Jin J-J, *et al.*: **GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes.** *Genome Biol.* 2020; **21**: 241.
PubMed Abstract | Publisher Full Text | Free Full Text

14. Meng G, Li Y, Yang C, *et al.*: **MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization.** *Nucleic Acids Res.* 2019; **47**: e63.
PubMed Abstract | Publisher Full Text | Free Full Text

15. Formenti G, *et al.*: **Gfastats: conversion, evaluation and manipulation of genome sequences using assembly graphs.** *Bioinformatics.* 2022; **38**: 4214–4216.
PubMed Abstract | Publisher Full Text | Free Full Text

16. 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**: 4647–4654.
PubMed Abstract | Publisher Full Text | Free Full Text

17. Challis R, Richards E, Rajan J, *et al.*: **Interactive Quality Assessment of Genome Assemblies.** *G3 GenesGenomesGenetics.* 2020; **10**: 1361–1374.
PubMed Abstract | Publisher Full Text | Free Full Text

18. Eleiwa A, Nadal J, Alves R: **Alectoris rufa isolate: AQ0044 (ID 1050768) - BioProject - NCBI.**
Reference Source

19. Eleiwa A, Nadal J, Alves R: **Long-read WGS of Alectoris rufa - SRA - NCBI.**
Reference Source

20. Eleiwa A, Nadal J, Alves R: **Chromosome Assembly Alectoris Rufa.**
Reference Source

21. Hu J, *et al.*: **NextDenovo: an efficient error correction and accurate assembly tool for noisy long reads.** *Genome Biol.* 2024; **25**: 107.
PubMed Abstract | Publisher Full Text | Free Full Text

22. Durand NC, *et al.*: **Juicebox Provides a Visualization System for Hi-C Contact Maps with Unlimited Zoom.** *Cell Syst.* 2016; **3**: 99–101.
PubMed Abstract | Publisher Full Text | Free Full Text

23. Alves R: **ARRIVE Checklist for the Genome Note "The genome sequence of the red-legged partridge, Alectoris rufa Linnaeus 1758" (Version 1).** *figshare.* 2025.
Publisher Full Text

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 25 September 2025

https://doi.org/10.5256/f1000research.179167.r412834

✔ **Greta Bellinzona**
University of Pavia, Pavia, Italy

The manuscript by Eleiwa et al. reports a chromosome-level assembly of the red-legged partridge (*Alectoris rufa*), a species of ecological and economic importance. The methods integrate long-read Nanopore sequencing with Hi-C Illumina data and produce a genome with good contiguity and completeness. The study makes a valuable contribution to the genomic resources available for Galliformes.
While the importance is clear and the genome is of good quality (based on the statistics reported by the authors), the presentation of the Methods and Results would benefit from a bit of clarification:

1. *Hi-C library preparation*: The workflow is described in detail but without sufficient clarity on whether both individuals were used for Hi-C or just one (later text implies only female Hi-C was used for sex chromosomes, is that correct?)

2. Methods, Nuclear genome assembly. “haplotype-phased genome assembly” is claimed for HapHiC scaffolding, but it is unclear whether phasing was retained in the final assembly. Clarify whether the assembly is a haploid representation, phased, or partially phased

3. The coverage values are inconsistently reported: “~60 Gb coverage” in Methods vs. “60-fold coverage” in Results. Please clarify the exact sequencing depth (fold coverage vs. Gb generated).

4. The mitogenome section states “38 genes” but later specifies “22 tRNAs and 14 protein coding genes”: this adds up to 36. Were 2 rRNAs omitted in the description?

5. Figure 3. What the “ontogene covariance (Chordata)” axis represents? Add details in the caption.

6. The protocol approval is included, but it would be good, if possible, to mention adherence to relevant guidelines for wildlife sampling (e.g., minimizing harm, use of anesthesia)

Minor edits:
- filter out mitochondrial genome "form" the nuclear contig" -- "from"
- "to scaffolds the purged contigs" -- "scaffold"

Are the rationale for sequencing the genome and the species significance clearly described?
Yes

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

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genomics, parasitology

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 19 September 2025

https://doi.org/10.5256/f1000research.179167.r412828

Marc Palmada-Flores
University of Girona, Girona, Spain

In this article the authors describe and make publicly available a chromosome-level genome assembly of the reg-legged partridge (*Alectoris rufa*). The use of HiC data and ONT data allowed to reach a much better contiguity and completeness than previously fragmented references.

Firstly, I want to congratulate the authors on the clear description of the reasons why they sequenced this species and the methodology used.

I have several questions and comments regarding the paper:

1- In the first paragraph of the introduction you mention a hunting pressure and habitat degradation without citing any paper that support this claim, although habitat degradation might be common knowledge I am reluctant to think hunting is currently a big pressure to this specific species of partridge, could you cite any paper or document that can support these claims?

2- In the second paragraph of the introduction you give a great reason to generate a chromosome-level genome assembly. You could compare the resulting assembly to the previously available fragmented genomes to make clear that point in the present paper and even support the claims by comparing the results of a test analyses using one assembly or the other.

3- In the first paragraph, second line of the nuclear genome assembly section, basecaller is written jointly, not seperately as you do.

4- In the second paragraph, first line of the nuclear genome assembly section, I would not describe NextDenovo as a pipeline, but rather as a tool and the citation is wrong, it should be changed to the one you have as 21.

5- In the second paragraph, second line of the nuclear genome assembly section, it would be great to know which ONT mapping procedure you used to be able to replicate the polishing step.

6- In the third paragraph of the nuclear genome assembly section, I do not understand why you use Ragtag if you already performed an scaffolding reference-free step, it would be great to see the differences between the scaffolded and the rescaffolded assemblies to value this extra step.

7- In the third paragraph of the nuclear genome assembly section, I think it would be more clear if we could know how many gaps and contigs/scaffolds you had in every step, prior to scaffold, after first scaffolding with HapHiC, second scaffolding with RagTag and after the gap filling with Medaka.

8- In the third paragraph of the nuclear genome assembly section, using RagTag and gap filling are not as common as other steps in the genome assembly at chromosome-level of vertebrates. It would be valuable to have more insights into the outputs and value of these two steps.

9- In the third paragraph of the nuclear genome assembly section, you write Ragtag v2.1.0. and then RagTag, for consistency I would stick with RagTag. Also in Table 1 I think you should change it to keep consistency.

10- In the mitochondrial genome assembly and annotation section the version, database and annotation software used to check BUSCO completeness in BlobtoolKit2 should be mentioned.

11- In the results' genome sequence report section I miss some explanation related to having 0 gaps in the resulting scaffolds (which are actually contigs) and would change to talk to contigs instead because you no longer have any kind of gaps.

Overall, I think this work should be indexed and made a great and good effort to achieve the chromosome-level genome reference for this species.

Are the rationale for sequencing the genome and the species significance clearly described?

Yes

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

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?
Partly

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: genomics, genome assembly, reference genomes, population genomics, conservation genomics, biodiversity 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.