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DATA NOTE

REVISED ERGA-BGE reference genome of the Eurasian Woodcock (*Scolopax rusticola*), a game bird species with isolated populations of conservation interest

[version 2; peer review: 2 approved]

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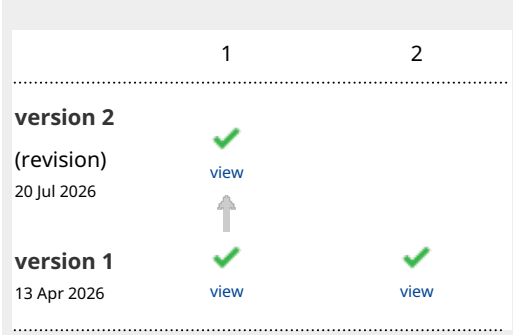
Abstract

The reference genome of the Eurasian Woodcock (*Scolopax rusticola*) is an important resource to investigate population structure across the wide breeding range of this iconic game species and the conservation status of specific management units, such as the isolated Macaronesian populations. The genome sequence was assembled into 45 contiguous chromosomal pseudomolecules and 2 sex chromosomes (W and Z). This chromosome-level assembly encompasses 1.2 Gb, composed of 1,613 contigs and 935 scaffolds, with contig and scaffold N50 values of 5.9 Mb and 34.2 Mb, respectively.

Keywords
Scolopax rusticola, genome assembly, European Reference Genome Atlas, Biodiversity Genomics Europe, Earth BioGenome Project, Aves, Scolopacidae, Eurasian Woodcock

This article is included in the Genome Reports from the Biodiversity Genomics Europe Project collection.



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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: Ferrer Obiol J: Investigation, Methodology, Project Administration, Resources, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Rubolini D**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Paris JR**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Giannelli F**: Investigation, Methodology, Project Administration, Resources, Supervision, Writing – Review & Editing; **Gonçalves D**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Andrade P**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Rodríguez B**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Tedeschi A**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Sorrenti M**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Natali C**: Investigation, Writing – Review & Editing; **Sozzoni M**: Data Curation, Formal Analysis, Writing – Review & Editing; **Böhne A**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Monteiro R**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Marcussen T**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **H. Struck T**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **A. Oomen R**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Diroma MA**: Data Curation, Formal Analysis, Writing – Review & Editing; **Ciofi C**: Investigation, Supervision, Writing – Review & Editing; **Howard C**: Investigation, Supervision, Writing – Review & Editing; **Blaxter M**: Investigation, Supervision, Writing – Review & Editing; **Bortoluzzi C**: Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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REVISED **Amendments from Version 1**

In this revised version of the genome report we addressed the two minor comments of reviewer 1, concerning the sequencing of additional samples capturing a broader genetic diversity and the clarification of terms used in the report, such as contig N50 and scaffold N50.

Any further responses from the reviewers can be found at the end of the article

Introduction

The Eurasian Woodcock is a member of the *Scolopacidae* bird family (Figure 1). Among members of its family, it stands out for its preference for terrestrial habitats, as it inhabits mature forests year-round, whereas most members of this family are strongly associated with aquatic habitats (Cramp, 1998). The species is strictly nocturnal during most of its annual cycle (Cramp, 1998; Duriez et al., 2004). The species has a broad breeding distribution range, spanning most of Eurasia (Cramp, 1998). Northern breeding populations are highly migratory - birds from the north-eastern part of the breeding range can perform migratory flights exceeding 6000 km (Arizaga et al., 2015; Tedeschi et al., 2020), spending the non-breeding period in southern Europe and the Mediterranean region (including north Africa).

The Eurasian Woodcock is currently classified as Least Concern by the IUCN, with a global population estimated at 10 to 16 million mature individuals (BirdLife International, 2025). European breeding populations are considered 'Decreasing' (BirdLife International, 2021). As an important game species, the Eurasian Woodcock is subjected to intense hunting pressure across most of its breeding and non-breeding range, including the small, isolated island populations in Macaronesia (Andrade et al., 2022). An estimated 3–4 million individuals are hunted annually in Europe, most of which in France, Italy, and Greece (Ferrand & Gossmann, 2001), and hunting pressure appears to be an important driver of survival and population dynamics (Duriez et al., 2005; Prieto et al., 2019).

The species is a peculiar component of the taxonomic diversity of boreal and temperate forests, as it is the only Western Palearctic shorebird that relies exclusively on forest habitats throughout its annual cycle (Cramp, 1998). Performing long-distance migrations, it contributes to the dispersal of many microorganisms and invertebrates (e.g., endoparasites) across Eurasia and Africa (Paoletti et al., 2016), influencing their genetic diversity, dispersal, and population structure. It feeds mainly on soil invertebrates (Cramp, 1998), thus contributing to regulating their population size and biomass.

The genome assembly presented here will serve as an important resource to investigate population structure in this widely distributed species and to deepen our understanding of the genetic and conservation status of isolated, genetically distinct,



Figure 1. An adult Eurasian Woodcock displaying its typical cryptic plumage colouration. Image by Paul Cools, some rights reserved (CC BY-NC) (https://www.inaturalist.org/photos/1935765).

island populations in Macaronesia (see Andrade et al., 2022). The generation of this reference resource was coordinated by the European Reference Genome Atlas (ERGA) initiative's Biodiversity Genomics Europe (BGE) project, supporting ERGA's aims of promoting transnational cooperation to promote advances in the application of genomics technologies to protect and restore biodiversity (Mazzoni et al., 2023).

Materials & Methods

ERGA's sequencing strategy to facilitate genome assembly and annotation includes Oxford Nanopore Technology (ONT) and/or Pacific Biosciences (PacBio) for long-read sequencing, along with Hi-C sequencing to reconstruct chromosomal conformation, Illumina Paired-End (PE) reads for polishing (i.e. recommended for ONT-only assemblies), and RNA sequencing for transcriptomic profilingn.

Sample and sampling information

Joan Ferrer Obiol opportunistically collected tissues from one female specimen from Coppo, Ancona, Italy (43.51 N, 13.59 E) on 30 November 2023. At this location, woodcocks are routinely captured at night for scientific bird ringing by authorised and licensed expert operators, following standard and safe procedures commonly adopted for capturing this species (hand-held net mounted on a long pole and a flashlight; e.g., Duriez et al., 2004; Gossmann et al., 1988; Tedeschi et al., 2020). On that night, despite every precaution taken by ringers to avoid injuries and excessive stress to animals during capture, one captured individual accidentally died shortly after capture, not showing any external sign of damage, wound or haemorrhage. Joan Ferrer Obiol inferred that death likely resulted from capture myopathy, a fatal condition that can develop following capture events and has been frequently reported in wading birds, including woodcock, for reasons that remain unclear (Breed et al., 2019; Rosenhagen, 2023). Muscle and brain tissue samples were thus opportunistically collected immediately after death, stored in dry ice shortly upon collection, and preserved at −80°C until DNA extraction. Identification was performed by Joan Ferrer Obiol based on phenotypic appearance using standard references (Cramp, 1998) and own solid ornithological expertise. The Eurasian woodcock is a very distinctive species, with no other similar taxa occurring in the sampling region (Cramp, 1998). Sex was identified *postmortem* based on gonadal inspection. Authorization for temporary capture of woodcocks for scientific purposes was issued to the Department of Environmental Science and Policy of the University of Milan by the local authorities (Regione Marche, D.D. Settore Forestazione e Politiche Faunistico-Venatorie - SDA/AP-FM n. 701).

Vouchering information

Frozen reference tissue material of muscle is available from the same individual at the Biobank of the Leibniz Institute for the Analysis of Biodiversity Change https://leibniz-lib.de/en/research/research-centres/zmb/bonn-location/biobank.html under the voucher ID ZFMK-TIS-89907.

Genetic information

The estimated genome size, based on ancestral taxa, is 1.32 Gb. This is a diploid genome with a haploid number of 44 chromosomes (2n = 88). All information for this species was retrieved from Genomes on a Tree (Challis et al., 2023).

DNA/RNA processing

DNA was extracted from muscle tissue using the MagAttract HMW DNA Kit (Qiagen) following the manufacturer's protocol. Nucleic acids yield was quantified in a Qubit fluorimeter using a Qubit 1X dsDNA HS Kit (Life Technologies), and DNA purity was assessed by comparing absorbance values at 260, 280 and 230 nm in a Tecan Infinite M200 Pro spectrophotometer using a NanoQuant plate for quantification of small volumes of nucleic acids (Tecan). Integrity of DNA was then assessed by pulsed-field gel electrophoresis. DNA was then preserved at −80°C.

RNA was extracted using the Quick-RNA MicroPrep Kit (Zymo research) according to the manufacturer's instructions. RNA was extracted from brain tissue. RNA quantification was performed using the Qubit RNA BR kit, and RNA integrity was assessed using a Bioanalyzer 2100 system RNA 6000 Nano Kit (Agilent). RNA was preserved at −80°C until library preparation.

Library preparation and sequencing

Whole DNA was sheared in a Megaruptor 2 DNA shearing system (Diagenode) using large fragment hydropores with a target mean fragment length of 20 kb. Fragment profile was assessed by capillary electrophoresis on a 5200 Fragment Analyzer system using an HS large fragment 50 kb kit (Agilent Technologies). Repair and A-tailing of DNA fragments and SMRTbell adapters ligation were performed according to the SMRTbell prep kit 3.0 protocol (Pacific Biosciences). A BluePippin automated pulsed-field gel electrophoresis system (Sage Science) was employed to remove fragments shorter than 10 kb. Primer annealing, polymerase binding and preparation of internal DNA control were performed using the Pacific Biosciences Sequel II binding kit and DNA internal control complex 3.2 according to the manufacturer's

protocol. The library was sequenced in HiFi mode in a Pacific Biosciences Sequel IIe platform using a Sequel II Sequencing plate 2.0 and two 8 M ZMW SMRT cells with a 30-hour movie time and 2 hours of pre-extension time for a target 25X genome coverage.

An RNA library was prepared using the Illumina Stranded mRNA library preparation and ligation kit, starting from 700 ng of total RNA and following the manufacturer's protocol. RNA library was quantified in a Qubit Flex Fluorometer using an RNA BR assay, and library quality was checked by capillary electrophoresis in a 5200 Fragment Analyzer System. The library was then sequenced 2 × 150 paired end on an Illumina Novaseq 6000 platform to attain a 50X genome coverage. Hi-C data were generated from muscle tissue of bScoRus1 using the Arima-HiC v2 kit. For Hi-C library preparation, DNA was fragmented to a size of 400 to 600 bp using a Covaris E220 sonicator. The DNA was then enriched, barcoded, and amplified using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) following the manufacturer's instructions. The Hi-C sequencing was performed using paired-end sequencing with a read length of

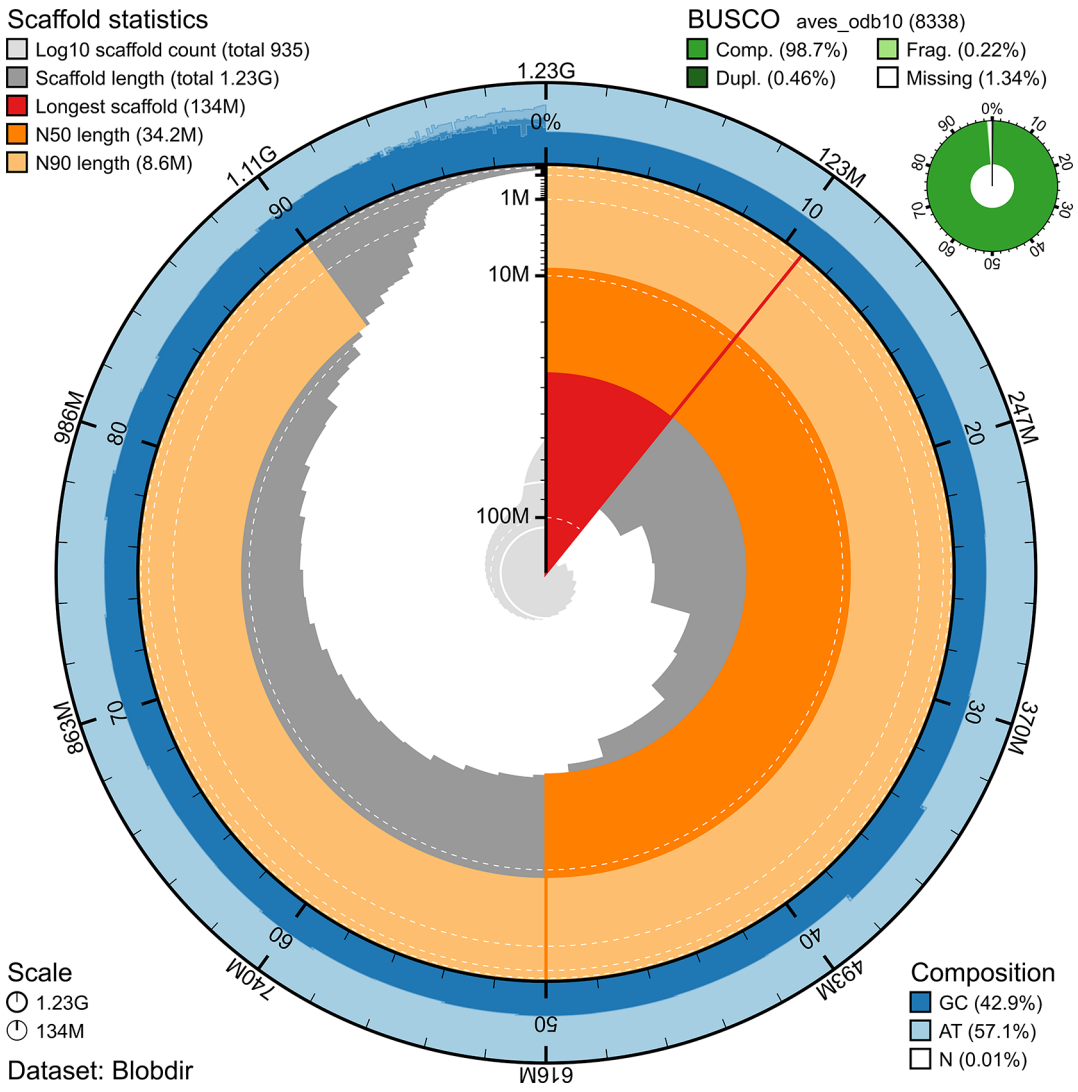

Figure 2. Snail plot summary of assembly statistics. The main plot is divided into 1,000 size-ordered bins around the circumference, with each bin representing 0.1% of the 1,232,635,287 bp assembly. The distribution of sequence lengths is shown in dark grey, with the plot radius scaled to the longest sequence present in the assembly (134 Mb, shown in red). Orange and pale-orange arcs show the scaffold N50 and N90 sequence lengths (34,162,297 and 8,602,126 bp), respectively. The pale grey spiral shows the cumulative sequence 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 found in the assembled genome from the Aves database (odb10) is shown in the top right.

150 bp on an Illumina NovaSeq X instrument. In total 33.5 Gb PacBio HiFi reads, 171 million Illumina RNA reads, and 80Gb HiC data were sequenced to generate the assembly.

Genome assembly methods

The genome was assembled using the VGP Galaxy pipeline (Larivière et al., 2024). Briefly, reads were preprocessed for quality and length using Cutadaptv Galaxy Version 5.0 + galaxy0, and initial contigs were assembled using Hifiasm Galaxy Version 0.24.0 + galaxy0, followed by scaffolding with YaHS Galaxy Version 1.2a.2 + galaxy2. Finally, assembled scaffolds were curated via manual inspection using Pretext v 1.0.0 with the Rapid Curation Toolkit (https://gitlab.com/wtsi-grit/rapid-curation) to remove any false joins and incorporate any sequences not automatically scaffolded into their respective locations in the chromosomal pseudomolecules (or super-scaffolds). We used the script MicroFinder.py (https://github.com/sanger-tol/MicroFinder) to better reconstruct microchromosomes. Summary analysis of the released assembly (GCA_976944445.1) was performed using the ERGA-BGE Genome Report ASM Galaxy workflow (https://doi.org/10.48546/workflowhub.workflow.1104.1).

Results

Genome assembly

The genome assembly has a total length of 1,232,635,287 bp in 935 scaffolds (Figures 2 & 3), with a GC content of 42.9%. The contiguity of the assembly was measured as contig N50, which is the length cutoff for the longest contigs that contain 50% of the total genome length, and scaffold N50, which is the length of the shortest contig in the set of longest contigs that collectively represent at least 50% of the total assembly size. The assembly of *Scolopax rusticola* has a contig N50 of 5,888,542 bp and L50 of 51, and a scaffold N50 of 34,162,297 bp and L50 of 10. The assembly has 678 gaps, totalling 135.6 kb in cumulative size. The single-copy gene content analysis using the Aves database (odb10) with BUSCO (Manni et al., 2021) resulted in 98.7% completeness (98.2% single and 0.5% duplicated). 84.4% of reads k-mers

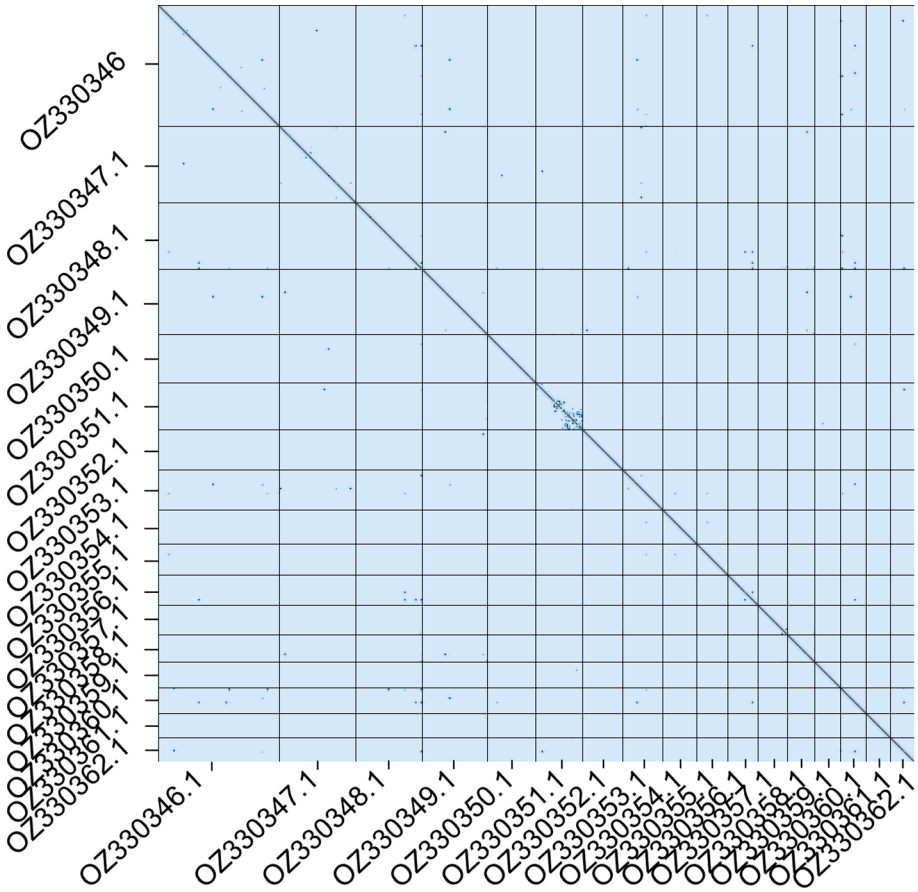

Figure 3. Hi-C contact map showing spatial interactions between regions of the genome. The diagonal corresponds to intra-chromosomal contacts, depicting chromosome boundaries. Chromosomes are shown on both x-axis and y-axis.

were present in the assembly, and the assembly has a base accuracy Quality Value (QV) of 61.84 as calculated by Merqury (Rhie et al., 2020).

Author contributions

DR, JFO, JRP, DG, PA, BR, AT and MS coordinated the project within the framework of a broader collaboration involving the study of Eurasian woodcock population genomics; JFO and FG collected the species; JFO identified the species; JFO sampled and preserved biological material and provided metadata; AsB, RM, TM, THS, and RAO provided support in sampling, shipping of biological material, metadata collection, and management; CN extracted DNA, prepared libraries, and performed sequencing under the supervision of CC; WSOTOL Management and WSISO extracted DNA, prepared libraries, and performed sequencing under the supervision of CH; MS performed genome assembly and curation; MAD performed data curation; CB generated the analysis and report. All authors contributed to the writing, review, and editing of this genome note and read and approved the final version.

Data availability

Scolopax rusticola and the related genomic study were assigned to Tree of Life ID (ToLID) 'bScoRus1' and all sample, sequence, and assembly information are available under the umbrella BioProject PRJEB77651. The sample information is available at the following BioSample accessions: SAMEA115083842, SAMEA115083846, SAMEA115083847, and SAMEA115083848. The genome assembly is accessible from ENA under accession number GCA_976944445.1 and the annotated genome will be made available through the Ensembl website (https://projects.ensembl.org/erga-bge/). Sequencing data produced as part of this project are available from ENA at the following accessions: ERX12721989, ERX12721990, ERX13270910, and ERX14241334. Documentation related to the genome assembly and curation can be found in the ERGA Assembly Report (EAR) document available at https://github.com/ERGA-consortium/EARs/tree/main/Assembly_Reports/Scolopax_rusticula/bScoRus1. Further details and data about the project are hosted on the ERGA portal at https://portal.erga-biodiversity.eu/data_portal/100826.

Acknowledgements

We thank the Parco Regionale del Conero for granting permission to capture woodcocks within its territory, and Claudio Sebastianelli and Pierfrancesco Gambelli for their invaluable assistance in the field. We acknowledge the support of the Freiburg Galaxy Team: Saim Momin and Björn Grüning, Bioinformatics, University of Freiburg (Germany), funded by the German Federal Ministry of Education and Research BMBF grant 031 A538A de. NBI-RBC and the Ministry of Science, Research and the Arts Baden-Württemberg (MWK) within the framework of LIBIS/de. NBI Freiburg. We would like to acknowledge the assembly reviewer, Michael Paulini from the Wellcome Sanger Institute.

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Version 2

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The authors have addressed all my comments; therefore, I have no further comments to make.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular biology, evolutionary biology, behavioural ecology, ornithology, conservation biology, colouration 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.

Version 1

Reviewer Report 02 July 2026

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✔ **Leilton Willians Luna**
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This Data note describes the assembly of a reference genome for *Scolopax rusticola* (Eurasian

Woodcock). Although this species is not globally threatened, the availability of this reference genome represents a valuable resource for assessing genetic diversity and population structure, particularly in potentially isolated populations such as those in Macaronesia.

The methods employed by the authors follow current best practices for reference genome generation and assembly, and the resulting genome is of high quality. I have no major concerns regarding the methodology or analyses presented, and therefore I support the indexing of this data note.

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: Biogeography, Evolution, Conservation, Molecular Ecology, Population genomics, Ornithology

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.

Author Response 09 Jul 2026

Chiara Bortoluzzi

We thank very much the reviewer for their positive feedback!

Competing Interests: No competing interests were disclosed.

Reviewer Report 16 June 2026

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Title: ERGA-BGE reference genome of the Eurasian Woodcock (Scolopax rusticola), a game bird species with isolated populations of conservation interest

The paper reports the assembly and characterization of a high-quality reference genome for the Eurasian Woodcock, an important game bird with isolated populations of conservation concern. The assembly will support research into population structure, conservation status, and the management of both widespread and isolated populations, particularly in Macaronesia.

Though I find the paper well written and the genome assembly method clear, I have a few very minor comments.

The sampling rationale is acceptable, but it would be helpful to explicitly state whether additional individuals from other populations are planned for sequencing to capture broader genetic diversity.
Since the methods section is highly technical, with detailed references to specific kits, protocols utilized, and software versions, while this is valuable for reproducibility, a brief lay summary or diagram of the sequencing assembly workflow would aid readers who are novices in genomics. I also think that terms such as contig N50 and scaffold N50 should at least be defined.

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, evolutionary biology, behavioural ecology, ornithology, conservation biology, colouration 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.

Author Response 09 Jul 2026

Chiara Bortoluzzi

We would like to thank the reviewer for their positive review. We have addressed the minor comments are follow:

1. No additional individuals are planned for sequencing in the context of this genome report. The individual sample was chosen as representative of the species for the generation of a reference genome. We note, however, that some of the coauthors of this genome report have generated whole-genome sequencing data for 35 individuals from across the species distribution, including Macaronesia. Analysis of that dataset relies on the reference genome presented here and will form the basis of a separate manuscript, currently in preparation.
2. We have now clarified in the genome report the meaning of both contig N50 and scaffold N50.

Competing Interests: No competing interests were disclosed.