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DATA NOTE

ERGA-BGE reference genome of the Mediterranean monk seal (*Monachus monachus*), an IUCN Vulnerable species

[version 1; peer review: 2 approved]

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Abstract

The Mediterranean monk seal, *Monachus monachus*, is the only pinniped that lives in the Mediterranean Sea and one of the rarest marine mammals in the world. The species was recently classified as "vulnerable" by the IUCN, considering an improvement in its overall status. However, the species' populations have undergone severe bottlenecks due to systematic persecution by humans over the past centuries. Today, the global population of *M. monachus* is estimated to be no more than 1,000 individuals. The Mediterranean monk seal is mainly using marine caves as resting and pupping sites. It is an opportunistic apex predator and as a result its role is considered important for maintaining the structure and function of marine ecosystems. Nowadays, the species is threatened mainly by the

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 09 Jun 2026	✓ view	✓ view

1. **Luis A Escobedo-Morales**, Universidad Nacional Autónoma de México, Mexico City, Mexico

2. **Anna Brüniche-Olsen**, University of Copenhagen, Copenhagen, Denmark

Any reports and responses or comments on the

destruction of its habitat (due to coastal development, mass tourism, and pollution) and by the depletion of its prey due to overfishing. The Mediterranean monk seal is an emblematic species; its ecological importance and its vulnerable status render its protection and effective management necessary. The entirety of the genome sequence of a female specimen was assembled into 16 contiguous chromosomal pseudomolecules, one sex chromosome (X), and one mitochondrial genome. This chromosome-level assembly encompasses 2.4 Gb, composed of 316 contigs and 275 scaffolds, with contig and scaffold N50 values of 90.9 Mb and 157 Mb, respectively.

Keywords
Monachus monachus, genome assembly, European Reference Genome Atlas, Biodiversity Genomics Europe, Earth Biogenome Project, Mediterranean monk seal, Phocidae, Μεσογειακή φώκια

article can be found at the end of the article.

This article is included in the Horizon Europe gateway.

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

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Author roles: Manousaki T: Investigation, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; **Dendrinos P**: Investigation, Resources, Writing – Review & Editing; **Vasileiadou K**: Investigation, Resources, Writing – Review & Editing; **Sarropoulou X** : Investigation, Resources, Writing – Review & Editing; **Tsigenopoulos CS**: Investigation, Resources, Writing – Review & Editing; **Dailianis T**: Investigation, Resources, Writing – Review & Editing; **Tounta E**: Investigation, Resources, Writing – Review & Editing; **Vogiatzis N**: Investigation, Resources, Writing – Review & Editing; **Komnenou A**: Investigation, Resources, Writing – Review & Editing; **Böhne A**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Monteiro R**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Fernández R**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Escudero N**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Bunikis I**: Investigation, Writing – Review & Editing; **Tellgren-Roth C**: Investigation, Writing – Review & Editing; **Mosbech MB**: Investigation, Writing – Review & Editing; **Dias GB**: Data Curation, Formal Analysis, Writing – Review & Editing; **Vinnere Pettersson O**: Investigation, Supervision, Writing – Review & Editing; **Bortoluzzi C**: Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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Introduction

The Mediterranean monk seal *Monachus monachus*, also known in Greek as Μεσογειακή φώκια, is a monotypic pinniped species that belongs to the family, *Phocidae* (subfamily *Monachinae*) (Figure 1). It is a medium sized seal with a total body length varying between 2.42 m – 2.51 m and an average weight reaching up to 300 kg (Karamanlidis et al., 2023; Karamanlidis & Dendrinos, 2023). Recently analysed data support that monk seals probably evolved from the southern hemisphere seals about 12.7 Mya and later moved northward (Karamanlidis & Dendrinos, 2023; Nebenführ et al., 2025).

The species was once widely distributed in the Mediterranean and Black Seas, and in north Atlantic waters from the Western Sahara and the Canary Islands to Morocco, the Azores and the Madeira archipelago (Karamanlidis et al., 2023). Today, the distribution of the species is highly fragmented into only three, isolated subpopulations. In the North Atlantic, two subpopulations remain: a larger one at the Cabo Blanco, W. Sahara (approx. 350 individuals) and a much smaller (approx. 30 individuals) at the archipelago of Madeira. In the Mediterranean, the main stronghold of the species, monk seals are found mainly in the eastern part of the basin (approx. 500 individuals), around the islands of the Ionian and the Aegean Seas and the mainland coast of Greece, the western and southern coast of Turkey as well as of Cyprus (Karamanlidis et al., 2023; Karamanlidis & Dendrinos, 2023; Kurt & Gücü, 2021; Nicolaou et al., 2021). Mediterranean monk seals prefer to use sea caves of a specific morphology to rest and, above all, to give birth and raise their young. This adaptation appears to be the result of the species' long-term and systematic hunting by humans. The birth of pups in the Mediterranean takes place mainly during the autumn months, peaking in October. Newborns are about 1 m long, weigh 15–26 kg, and nurse for 3–4 months.

The Mediterranean monk seal is the only seal species living in the Mediterranean Sea and one of the rarest marine mammals on the planet. For this reason, it is considered an emblematic species for the conservation of biodiversity in the Mediterranean. It is an opportunistic top predator and therefore its presence is considered of great importance for the functioning and sustainable conservation of marine ecosystems. The protection of the Mediterranean monk seal in its natural environment essentially requires the effective management and protection of marine and coastal ecosystems.

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 encouraging transnational cooperation and promoting advances in the application of high-throughput and state-of-the-art genomic technologies to protect and restore biodiversity (Mazzoni et al., 2023).

Materials & Methods

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

Sample and sampling information

On 10th of October 2024, Tereza Manousaki, Katerina Vasileiadou, Panagiotis Dendrinos, Eleni Tounta and Nikitas Vogiatzis organized the sample blood from one female specimen of *Monachus monachus* in MOm's Monk Seal Rehabilitation facilities, located at Attica Zoological Park, Spata, Athens, Greece. The species was identified by experts. Sampling was conducted under the permit 104104/3848/06-10-2025 issued by the Hellenic Ministry for Environment and Energy, General Secretariat of Forests. General Directorate of Forests and Forest Environment, Directorate of Forest Management, Department of Wildlife and Hunting Management. The blood sample was taken by an experienced veterinary assistant from the live animal undergoing treatment. The sampling had no impact on the animal's health status and behaviour. The blood sample was immediately flash-frozen in liquid nitrogen and preserved at −80 °C until DNA extraction.

Vouchering information

Photographs of the specimen have been submitted to BioImage Archive (https://www.ebi.ac.uk/bioimage-archive/) (Figure 2) and are here used as a voucher.

Data availability

Monachus monachus and the related genomic study were assigned to Tree of Life ID (ToLID) 'mMonMoa1' and all sample, sequence, and assembly information are available under the umbrella BioProject PRJEB96355. The sample information is available at the following BioSample accessions: SAMEA117387579, SAMEA117387581, and SAMEA117387583. The genome assembly is accessible from ENA under accession number GCA_976986665.1 and



Figure 1. Constantina, the herein sequenced Mediterranean monk seal individual. Photo credit: Panagiotis Dendrinos, MOm/Hellenic Society for the Study and Protection of the Monk Seal.



Figure 2. Costantina, BioSample: SAMEA117387578. This picture corresponds to the voucher deposited in Bio-Image Archive and represents the specimen used here for genome assembly.

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: ERX14538616, ERX16161577, and ERX14869051. 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/Monachus_monachus/mMonMoa1. Further details and data about the project are hosted on the ERGA portal at https://portal.erga-biodiversity.eu/data_portal/248254.

Genetic information

The estimated genome size, based on ancestral taxa, is 2.88 Gb. This corresponds to a diploid genome with an expected haploid number of 17 chromosomes (2n = 34). All information for this species was retrieved from Genomes on a Tree (Challis et al., 2023).

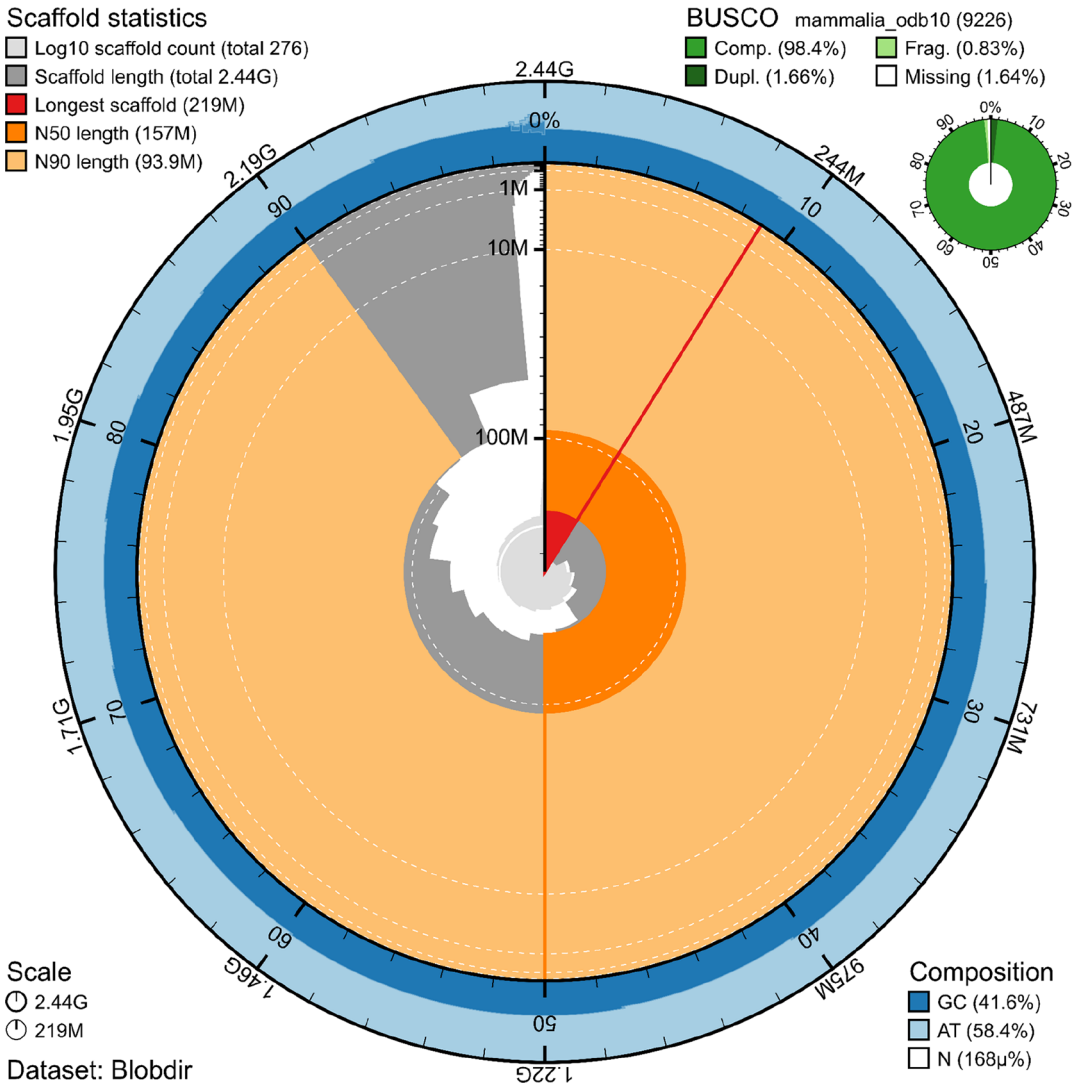

Figure 3. Snail plot summary of assembly statistics. The main plot is divided into 1,000 size-ordered bins around the circumference, with each bin representing 0.1% of the 2,437,322,148 bp assembly including the mitochondrial genome. The distribution of sequence lengths is shown in dark grey, with the plot radius scaled to the longest sequence present in the assembly (219 Mb, shown in red). Orange and pale-orange arcs show the scaffold N50 and N90 sequence lengths (157,001,452 bp and 93,858,021 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 Mammalia database (odb10) is shown in the top right.

DNA/RNA processing

DNA was extracted from 200 μl of frozen blood following PacBio's instruction described in "Procedure checklist Extracting HMW DNA from human whole blood using Nanobind® kits" (REV04 AUG2024). Quantification was performed using a Qubit dsDNA BR Assay kit (Thermo Fisher Scientific) and integrity was assessed using a FemtoPulse system (Agilent). DNA was stored at 4 °C until usage. RNA was extracted from blood using the TRIzol Reagent and Phasemaker Tubes Complete System (Invitrogen Cat #A33250) following the Invitrogen user guide (Pub. No. MAN0016163 Rev. A.0) except for steps 2.a. and 3.d., which were omitted. RNA was resuspended in 87.5 μl RNase-free water and immediately subjected to DNase treatment followed by purification according to the RNeasy Micro Handbook (pages 74 and 53). Quantity and integrity were assessed using the Agilent 2100 Bioanalyzer instrument. The eluted RNA was stored at −70 °C.

Library preparation and sequencing

One PacBio SMRTbell library was constructed using the instructions described in "Procedure & Checklist - Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0" (PN 102–182-700, REV06JAN2025). Prior to library construction, the DNA was sheared using the Megaruptor 3 (Diagenode), followed by a clean-up step using SMRTbell cleanup beads. In total, 3 µg of sheared and cleaned DNA were used for library construction. Size-selection of the ready SMRTbell was performed using the PippinHT system. Primer annealing and polymerase binding were performed using the Revio polymerase kit. Finally, two Revio SMRTcell 25 M were sequenced on the PacBio Revio System. Hi-C was generated from blood as described in the User Guide for Library Preparation for Arima High Coverage HiC Kit (A160186 v02) (Arima Genomics) and sequenced on the NovaSeq X system and XLEAP-SBS sequencing chemistry (Illumina Inc.). Sequencing library was prepared from 100 ng total RNA using the TruSeq stranded mRNA library preparation kit (cat# 20020595, Illumina Inc.) including polyA selection. Unique dual indexes (cat# 20022371, Illumina Inc.) were used. The library preparation was performed according to the manufacturers' protocol (#1000000040498). The library was sequenced on an Illumina NovaSeq X Plus system with a 25B flow cell using paired-end 150 bp read length and XLEAP-SBS sequencing chemistry. RNA-Seq data are currently being used to generate the genome annotation of *M. monachus.* In total, 91x PacBio data were sequenced to generate the assembly. The Hi-C library failed, and it was not used in scaffolding.

Genome assembly methods

The genome was assembled using the National Bioinformatics Infrastructure Sweden (NBIS) Earth-Biogenome-Pilot pipeline (https://github.com/NBISweden/Earth-Biogenome-Project-pilot), and reference-scaffolded using RagTag v2.1.0 (Alonge et al., 2022). Briefly, PacBio HiFi reads were assembled using Hifiasm with default parameters (Cheng et al., 2021). The resulting contigs were screened for contaminants using FCS-GX (Astashyn et al., 2024) and purged for haplotypic duplications using purge_dups (Guan et al., 2020). The decontaminated, purged contigs were then reference-scaffolded using RagTag and the genome of the Hawaiian Monk Seal, *Neomonachus schauinslandi*, as reference (GCF_002201575.2). RagTag was run in scaffold mode, and no breaks were introduced in the hifiasm contigs. Prior to reference scaffolding, the contigs of *M. monachus* and the scaffolds of *N. schauinslandi* were analysed for synteny and collinearity using protein-coding genes alignments (https://github.com/gbdias/quick_synteny). Finally, the mitochondrial genome was assembled as a single circular contig of 16,781 bp using the MitoHiFi pipeline (Uliano-Silva et al., 2023) and included in the released assembly (GCA_976986665.1).

Results

Genome assembly

The genome assembly has a total length of 2,437,322,148 bp in 276 scaffolds including the mitogenome (Figure 3), with a GC content of 41.5%. The assembly has a contig N50 of 90,875,510 bp and L50 of 10 and a scaffold N50 of 157,001,452 bp and L50 of 7. The assembly has a total of 41 gaps, totaling 4.1 kb in cumulative size. The single-copy gene content analysis using the Mammalia database (odb10) with BUSCO v5.8.0 (Manni et al., 2021) resulted in 98.4% completeness (96.7% single and 1.7% duplicated). 99.47% of reads *k*-mers were present in the assembly and the assembly has a base accuracy Quality Value (QV) of 68.6 as calculated by Merqury (Rhie et al., 2020).

Author contributions

TM, KV and PD coordinated the project; PD, ET, NV, AK collected the species; PD, ET, NV, AK identified the species; TM, PD, KV, XS, CST, TD, ET, NV, AK sampled and preserved biological material and provided metadata; AsB, RM, RF, and NE provided support in sampling, shipping of biological material, metadata collection, and management; M-BM extracted DNA and RNA under supervision of OVP; CTR and IB performed raw data processing and data QC; GBD performed genome assembly and 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.

Acknowledgements

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 07 August 2026

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✔ **Anna Brüniche-Olsen**
University of Copenhagen, Copenhagen, Denmark

The manuscript provides a high quality reference genome for the monk seal, a species of conservation concern, where this genomic resource will contribute to future genomic studies of the focal and other marine mammal species. Overall the manuscript is well written and concise. I only have a few comments, that I hope the authors will consider.
INTRODUCTION
A map with past and current ranges would help the reader. In the second paragraph is detailed information on past distribution and current population structure. It would be good to have a visual for this, as it would also provide context for the substantial population decline and conservation listing.
Its described that the monk seal uses sea caves to rest, give birth and raise young in. Are these caves locally protected, so the species can raise young undisturbed by humans?
What is an “opportunistic top predator”? Do you mean, that when no other large predators are around, then the monk seal moves up in the trophic cascade? I would label it as either apex or meso predator, depending on that the majority of the species are.
MATERIALS AND METHODS
DNA/RNA processing: delete “frozen”. (DNA was extracted from 200ul of **frozen** blood…”).
Library prep and seq: In the last sentence is stated that the Hi-C library failed. What implications does this have for the quality of the final genome assembly? Would it be beneficial to construct a new Hi-C library and use it for scaffolding?

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 genomics, conservation genomics, macrogenetics

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 17 July 2026

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Luis A Escobedo-Morales
Universidad Nacional Autónoma de México, Mexico City, Mexico

The manuscript provides a valuable genomic resource for future studies on a relevant marine mammal species. The manuscript is well-rounded, and the Methods are clearly described. Some numbers must be revised to ensure consistency between Figure 3 and the manuscript text. An additional review of the language would be helpful to round out scores and avoid wordy phrases. The document only needs a review of some details, but it has sufficient quality and merit for the immediate publication of the report. It would be interesting to briefly discuss the validation tools, including the failed Hi-C library.

I list a few suggestions to enhance readability for the readers:

Introduction
First paragraph
Line 2: Please remove the comma after "family". Also include Order for better reference for readers of the species taxonomy.
Line 2: Please change "medium sized" to "medium-sized".
Second paragraph
Line 3: Please change "into only three, isolated subpopulations" to "with only three isolated subpopulations".
Lines 4, 5, and 6: Please substitute "approx." for "approximately".
Line 7: Please change "as well as of" to "and".
Line 9: Please change "sea caves of" to "sea caves with".
Lines 10 and 11: Please change "The birth of pups in the Mediterranean takes place" to "In the Mediterranean, pupping occurs".
Third paragraph
Line 2: Please change "the conservation of biodiversity" to "biodiversity conservation".
Line 4: Please change "The protection of" to "Protecting".

Materials & Methods
Second paragraph (Sample and sampling information)
Line 1: Please change "On 10th of October 2024" to "On October 10, 2024".
Line 2: Please change "organized the sample blood" to "collected a blood sample".
Line 2: Please change "one female specimen of Monachus monachus" to "a female Monachus monachus specimen".
Line 3: Please remove "located".
Third paragraph (Vouchering information)
Line 2: Please change "here used" to "used here".
Fourth paragraph (Data availability)
Line 2: Please change "The sample" to "Sample".
Line 8: Please remove "document".
Fifth paragraph (Genetic information)
Line 1: Please clarify this genome size estimate (2.88 Gb) and standardize the decimal formatting throughout the manuscript (2.44 Gb).
Sixth paragraph (DNA/RNA processing)
Line 7: Please change "subjected to DNase treatment followed by purification" to "treated with DNase, then purified"
Seventh paragraph (Library preparation and sequencing)
Line 1: Please change "using the instructions described in" to "following the instructions in".
Line 3: Please change "the DNA" to "DNA".
Line 3: Please change "followed by a clean-up step using" to "and then cleaned with".
Line 9: Please change "Sequencing" to "The sequencing".
Eighth paragraph (Genome assembly methods)
Line 3: Please remove "Briefly,".
Line 5: Please change "purge_dups" to "the purge_dups tool".
Line 8: Please change "analysed" to "analyzed".

Figure 3. Please review the scaffold count (276 in the figure, 275 in the Abstract).

Results
Line 1: Please verify that the scaffold count is correct in the Abstract, Methods, and Results sections. Also, verify that the numbers in Figure 3 and the manuscript match.
Line 3: Please change "totaling 4.1 kb in cumulative size" to "for a cumulative size of 4.1 kb".

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: Phylogenomics, museomics, high-throughput sequencing.

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.