DATA NOTE

The genome sequence of Erhard's wall lizard, *Podarcis erhardii* (Bedriaga, 1882)

[version 1; peer review: 3 approved]

Nathalie Feiner[1,2], Tobias Uller[2], Kinsey M. Brock[3,4], Panayiotis Pafilis[5,6], Joana Meier[7],
Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team,
Wellcome Sanger Institute Scientific Operations: Sequencing Operations,
Wellcome Sanger Institute Tree of Life Core Informatics team,
Tree of Life Core Informatics collective

[1]Max Planck Institute for Evolutionary Biology, Plön, Germany
[2]Department of Biology, Lund University, Lund, Skåne County, Sweden
[3]Department of Biology, San Diego State University, San Diego, California, USA
[4]Biodiversity Museum, San Diego State University, San Diego, California, USA
[5]Department of Biology, National and Kapodistrian University of Athens, Athens, Greece
[6]Museum of Zoology, National and Kapodistrian University of Athens, Athens, Greece
[7]Wellcome Sanger Institute, Hinxton, England, UK

v1 **First published:** 23 Apr 2025, **10**:212
https://doi.org/10.12688/wellcomeopenres.24041.1
Latest published: 23 Apr 2025, **10**:212
https://doi.org/10.12688/wellcomeopenres.24041.1

Abstract

We present a genome assembly from a female specimen of *Podarcis erhardii* (Erhard's wall lizard; Chordata; Lepidosauria; Squamata; Lacertidae). The assembly contains two haplotypes with total lengths of 1,495.98 megabases and 1,477.75 megabases. Most of haplotype 1 (99.28%) is scaffolded into 20 chromosomal pseudomolecules, including the W and Z sex chromosomes. Haplotype 2 was assembled to scaffold level. The mitochondrial genome has also been assembled, with a length of 17.14 kilobases.

Keywords

Podarcis erhardii, Erhard's wall lizard, genome sequence, chromosomal, Squamata

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓ ✓

	1	2	3
version 1 23 Apr 2025	✓ view	✓ view	✓ view

1. **Erin Westeen**, University of California Berkeley, Berkeley, USA
2. **Roberto Biello**, John Innes Centre Department of Crop Genetics (Ringgold ID: 534071), Norwich, UK
University of Ferrara, Ferrara, Italy
3. **Merly Escalona**, University of California, Santa Cruz, USA

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

Corresponding author: Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team (Mark.Blaxter@sanger.ac.uk)

Author roles: Feiner N: Investigation, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; **Uller T**: Investigation, Resources, Writing – Original Draft Preparation; **Brock KM**: Resources, Writing – Review & Editing; **Pafilis P**: Resources, Writing – Review & Editing; **Meier J**: Investigation, Resources, Writing – Review & Editing;

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Wellcome through core funding to the Wellcome Sanger Institute (220540). N.F. was funded by Starting Grants from the European Research Council (948126) and the Swedish Research Council (2020-03650). K.M.B. was supported by a United States National Science Foundation Postdoctoral Research Fellowship in Biology (Award number 2109710).
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: Feiner N, Uller T, Brock KM *et al.* **The genome sequence of Erhard's wall lizard, *Podarcis erhardii* (Bedriaga, 1882) [version 1; peer review: 3 approved]** Wellcome Open Research 2025, **10**:212 https://doi.org/10.12688/wellcomeopenres.24041.1

First published: 23 Apr 2025, **10**:212 https://doi.org/10.12688/wellcomeopenres.24041.1

Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Deuterostomia; Chordata; Craniata; Vertebrata; Gnathostomata; Teleostomi; Euteleostomi; Sarcopterygii; Dipnotetrapodomorpha; Tetrapoda; Amniota; Sauropsida; Sauria; Lepidosauria; Squamata; Bifurcata; Unidentata; Episquamata; Laterata; Lacertibaenia; Lacertidae; Lacertinae; *Podarcis*; *Podarcis erhardii* (Bedriaga, 1882) (NCBI:txid140042)

Background

The Aegean wall lizard *Podarcis erhardii* (Bedriaga 1882; Figure 1), also commonly known as Erhard's wall lizard, is named after the German naturalist Theodor Erhard. It is a diurnal lacertid lizard with a broad distribution in south-eastern Europe, occurring throughout Greece and northwards into the Balkan region. *P. erhardii* is classified as Least Concern (LC) by the IUCN (Bowles & Crnobrnja-Isailović, 2024).

The Aegean wall lizard is one of 28 currently described species of the genus *Podarcis*. The species consists of several deeply divergent lineages that could justify a taxonomic revision (Poulakakis *et al.*, 2003). *P. erhardii* is most closely related to the group of *P. cretensis*, *P. levendis*, *P. peloponnesiacus* and *P. thais*, which are also native to Greece (Kiourtsoglou *et al.*, 2021; Yang *et al.*, 2021).

Typical of generalist species, Aegean wall lizards occur in a variety of habitats, including Mediterranean shrubland, grassland, sandy or rocky habitats as well as urban environments. The species is found from sea-level up to 2000 m elevation. Individuals can be active all year, but reproduction is generally restricted to spring and early summer. *P. erhardii* is oviparous and females lay between one and five eggs per clutch and multiple clutches per year (Böhme, 1986). Meiri *et al.* (2020) reports an average clutch size of 2.53.

Populations of Aegean wall lizards are highly variable in colouration (Escoriza, 2024) and body size (Stadler *et al.*, 2023).

Figure 1. A) Female (left) and male (right) *Podarcis erhardii* photographed on the island of Andros, Greece. **B**) Female *P. erhardii* (rPodErh1) that was selected for genome sequencing upon capture on the island of Andros. Photographs by Kinsey Brock (**A**) and John David Curlis (**B**).

The snout-to-vent length varies between 45 mm and 81 mm for adult males (Valakos *et al.*, 2008) and 45 mm to 77 mm for adult females (Stadler *et al.*, 2023). Colouration varies both within and between populations, with predominantly brown, grey and green hue and a variety of dorsal patterns. This species is one of several *Podarcis* that exhibit ventral colour polymorphism with yellow, orange and white morphs (Brock *et al.*, 2020).

Podarcis erhardii belongs to the Balkan group of the genus *Podarcis*, which consists of 10 currently described species. Within this group, there is a reference genome available for *P. cretensis* (Poulakakis *et al.*, 2024), and reference genomes for two other species (*P. gaigeae* and *P. melisellensis*) have already been generated and will be published soon. Thus, the reference genome of *P. erhardii* will be a valuable resource, both for comparative studies above the species level, as well as studies addressing the evolutionary history, population structure, and phylogeography of *P. erhardii.*

The genome of the Erhard's wall lizard, *Podarcis erhardii*, was sequenced as part of the Darwin Tree of Life Project, a collaborative effort to sequence all named eukaryotic species in the Atlantic Archipelago of Britain and Ireland. Here we present a chromosomally complete genome sequence for *Podarcis erhardii*, based on a specimen from Andros (Figure 1).

Genome sequence report

Sequencing data

The genome of a specimen of *Podarcis erhardii* (Figure 1) was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 51.11 Gb (gigabases) from 4.93 million reads. GenomeScope analysis of the PacBio HiFi data estimated the haploid genome size at 1,440.47 Mb, with a heterozygosity of 0.59% and repeat content of 20.86%. These values provide an initial assessment of genome complexity and the challenges anticipated during assembly. Based on this estimated genome size, the sequencing data provided approximately 34.0x coverage of the genome. Chromosome conformation Hi-C sequencing produced 226.70 Gb from 1,501.35 million reads. Table 1 summarises the specimen and sequencing information.

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 assembly was improved by manual curation, which corrected 73 misjoins or missing joins and removed 649 haplotypic duplications. These interventions decreased the scaffold count by 65.58%. The final assembly has a total length of 1,495.98 Mb in 325 scaffolds, with 836 gaps, and a scaffold N50 of 93.52 Mb (Table 2).

The snail plot in Figure 2 provides a summary of the assembly statistics, indicating the distribution of scaffold lengths and other assembly metrics. Figure 3 shows the distribution of scaffolds by GC proportion and coverage. Figure 4

Table 1. **Specimen and sequencing data for *Podarcis erhardii*.**

Project information			
Study title	Podarcis erhardii (Erhard's wall lizard)		
Umbrella BioProject	PRJEB78688		
Species	*Podarcis erhardii*		
BioSpecimen	SAMEA115336771		
NCBI taxonomy ID	140042		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	rPodErh1	SAMEA115336780	terminal body
Hi-C sequencing	rPodErh1	SAMEA115336780	terminal body
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq X	ERR13474180	1.50e+09	226.7
PacBio Revio	ERR13485715	1.95e+06	19.48
PacBio Revio	ERR13485716	2.98e+06	31.63

Table 2. **Genome assembly data for *Podarcis erhardii*.**

Genome assembly	**Haplotype 1**	**Haplotype 2**
Assembly name	rPodErh1.hap1.1	rPodErh1.hap2.1
Assembly accession	GCA_964252035.1	GCA_964251985.1
Assembly level	chromosome	scaffold
Span (Mb)	1,495.98	1,477.75
Number of contigs	1,161	1,458
Number of scaffolds	325	647
Assembly metrics (benchmark)	**Haplotype 1**	**Haplotype 2**
Contig N50 length (≥ 1 Mb)	3.23 Mb	3.03 Mb
Scaffold N50 length *(= chromosome N50)*	93.52 Mb	93.57 Mb
Consensus quality (QV) (≥ 40)	63.9	61.5
k-mer completeness	86.58%	85.24%
Combined *k*-mer completeness (≥ 95%)	99.25%	
BUSCO* (S > 90%; D < 5%)	C:92.8%[S:91.0%,D:1.8%], F:0.8%,M:6.4%,n:7,480	C:92.3%[S:90.7%,D:1.6%], F:1.0%,M:6.7%,n:7,480
Percentage of assembly mapped to chromosomes (≥ 90%)	99.28%	-
Sex chromosomes (localised homologous pairs)	W and Z	-
Organelles (one complete allele)	Mitochondrial genome: 17.14 kb	-

* BUSCO scores based on the sauropsida_odb10 BUSCO set using version 5.5.0. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison.

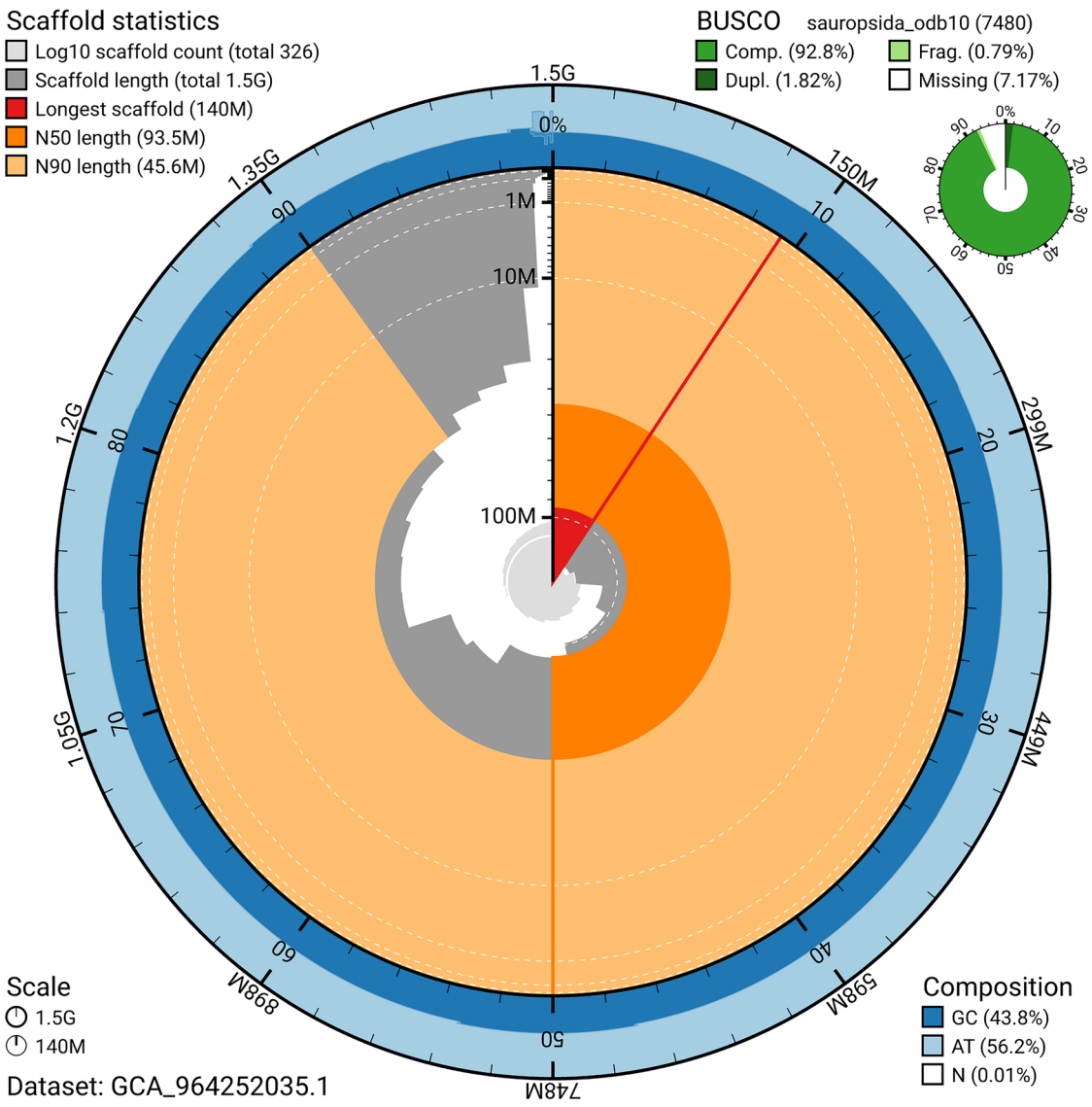

Figure 2. Genome assembly of *Podarcis erhardii*, rPodErh1.hap1.1: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the circumference. 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 sauropsida_odb10 set is presented at the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_964252035.1/dataset/GCA_964252035.1/snail.

presents a cumulative assembly plot, with separate curves representing different scaffold subsets assigned to various phyla, illustrating the completeness of the assembly.

Most of the assembly sequence (99.28%) was assigned to 20 chromosomal-level scaffolds, representing 18 autosomes and the W and Z sex chromosomes. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 5; Table 3). During curation, the Z and W sex chromosomes were identified by ploidy and read coverage.

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.

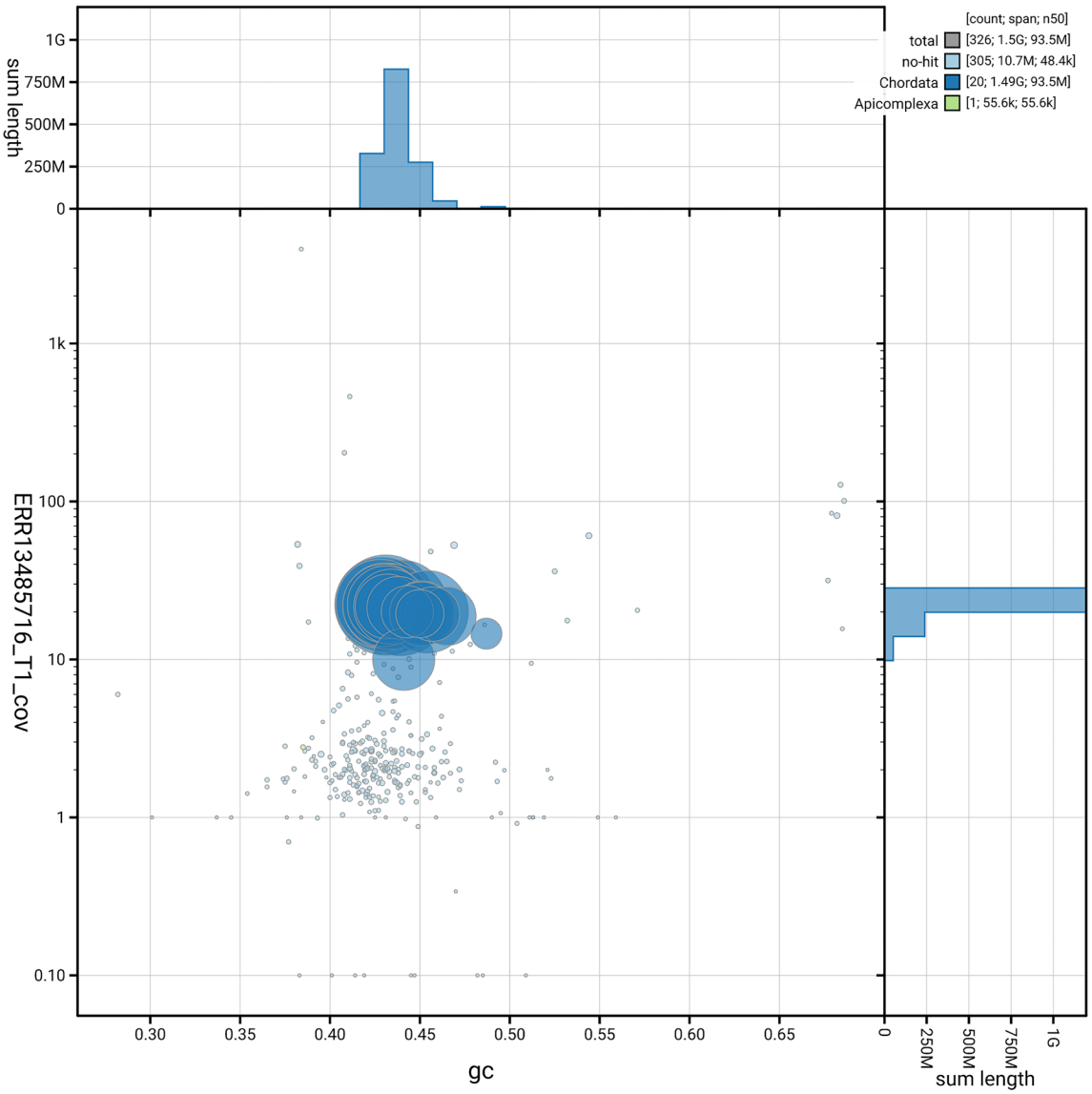

Figure 3. Genome assembly of *Podarcis erhardii*, rPodErh1.hap1.1: BlobToolKit GC-coverage plot. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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 at https://blobtoolkit.genomehubs.org/view/GCA_964252035.1/dataset/GCA_964252035.1/blob.

Assembly quality metrics

The estimated Quality Value (QV) and *k*-mer completeness metrics, along with BUSCO completeness scores, were calculated for each haplotype and the combined assembly. The QV reflects the base-level accuracy of the assembly, while *k*-mer completeness indicates the proportion of expected *k*-mers identified in the assembly. BUSCO scores provide a measure of completeness based on benchmarking universal single-copy orthologues.

For haplotype 1, the estimated QV is 63.9, and for haplotype 2, 61.5. When the two haplotypes are combined, the assembly achieves an estimated QV of 62.6. The *k*-mer recovery for haplotype 1 is 86.58%, and for haplotype 2 85.24%, while the combined haplotypes have a *k*-mer recovery of 99.25%. BUSCO 5.5.0 analysis using the sauropsida_odb10 reference set (n = 7,480) identified 92.8% of the expected gene set (single = 91.0%, duplicated = 1.8%) for haplotype 1.

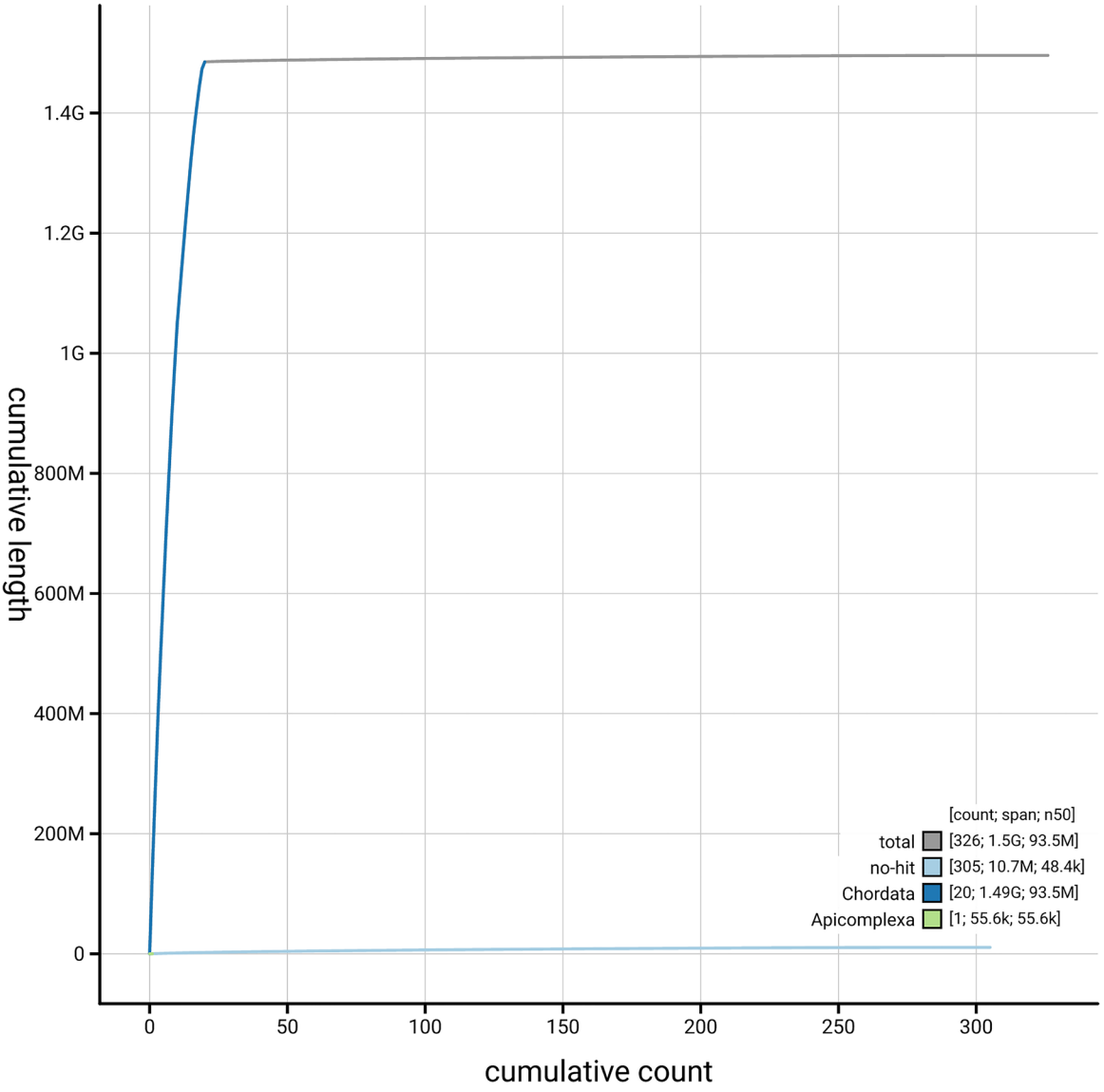

Figure 4. Genome assembly of *Podarcis erhardii*, rPodErh1.hap1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_964252035.1/dataset/GCA_964252035.1/cumulative.

Table 2 provides assembly metric benchmarks adapted from Rhie *et al.* (2021) and the Earth BioGenome Project (EBP) Report on Assembly Standards September 2024. The haplotype 1 assembly achieves the EBP reference standard of **6.C.Q63**.

Methods

Sample acquisition

The specimen, an adult female *P. erhardii* lizard (specimen ID SAN25002444, ToLID rPodErh1) was collected on 2023-05-29 from a site on the island of Andros in the northern Cyclades (latitude: 37.85005159; longitude: 24.91615335). The specimen belongs to the subspecies *P. e. mykonensis*. The specimen was caught by noosing, standard morphometric measurements were taken, and the tip of the tail (ca. 2 cm) was collected and preserved in ethanol. The specimen was released again at the site of capture. Field work was conducted under the permit ID ΑΔΑ: Ψ9Ω04653Π8-ΓΩΓ.

Nucleic acid extraction

The workflow for high molecular weight (HMW) DNA extraction at the Wellcome Sanger Institute (WSI) Tree of Life Core Laboratory includes a sequence of procedures: sample

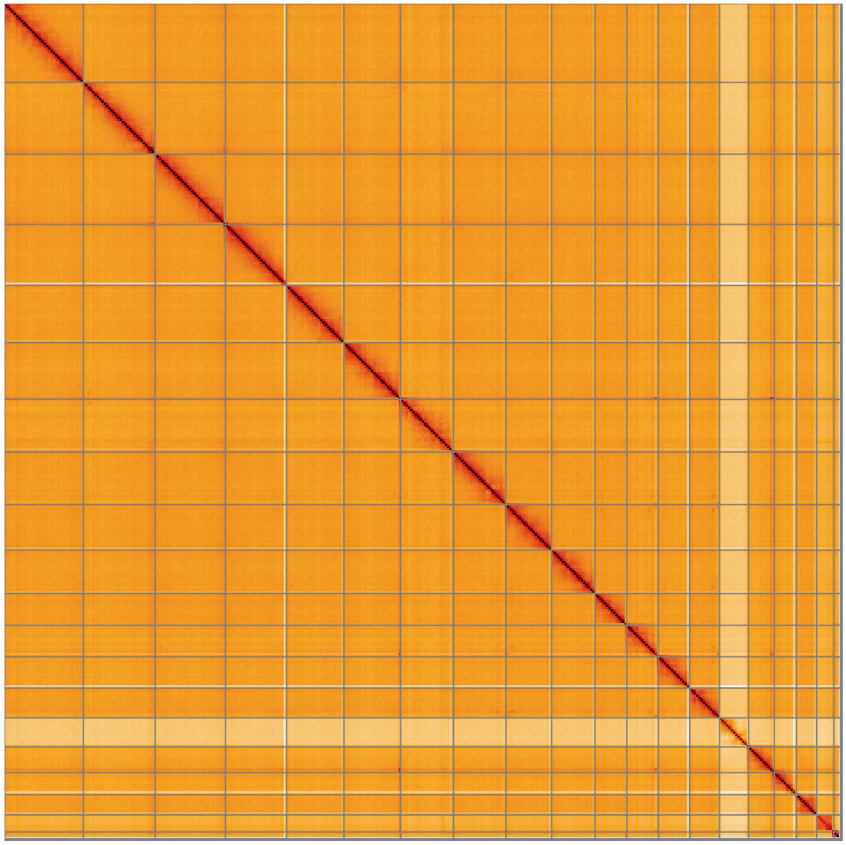

Figure 5. Genome assembly of *Podarcis erhardii*: Hi-C contact map of the rPodErh1.hap1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=Nl3EYMFBTZudopXQwDabVA.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Podarcis erhardii*, rPodErh1.

INSDC accession	Name	Length (Mb)	GC%
OZ176200.1	1	140.11	43
OZ176201.1	2	127.75	44
OZ176202.1	3	124.95	43
OZ176203.1	4	108.49	43
OZ176204.1	5	101.84	43
OZ176205.1	6	101.02	43.5
OZ176206.1	7	93.52	45.5
OZ176207.1	8	93.46	43
OZ176208.1	9	81.14	43
OZ176209.1	10	77.07	43.5
OZ176210.1	11	56.5	43
OZ176211.1	12	52.97	44
OZ176212.1	13	56.18	45
OZ176213.1	14	55.41	45
OZ176215.1	15	45.6	46.5
OZ176216.1	16	39.23	45.5
OZ176217.1	17	35.95	44.5
OZ176219.1	18	11.67	48.5
OZ176218.1	W	30.37	45
OZ176214.1	Z	51.98	44
OZ176220.1	MT	0.02	39.5

preparation and homogenisation, DNA extraction, fragmentation and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). The rPodErh1 sample was prepared for DNA extraction by weighing and dissecting it on dry ice (Jay *et al.*, 2023). Tissue from the terminal body was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a). HMW DNA was extracted using the Manual MagAttract v1 protocol (Strickland *et al.*, 2023b). DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system (Todorovic *et al.*, 2023). Sheared DNA was purified by solid-phase reversible immobilisation, using AMPure PB beads to eliminate shorter fragments and concentrate the DNA (Strickland *et al.*, 2023a). 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.

Hi-C sample preparation and crosslinking

Tissue from the terminal body of the rPodErh1 sample was processed for Hi-C sequencing at the WSI Scientific Operations core, using the Arima-HiC v2 kit. In brief, 20–50 mg of frozen tissue (stored at –80 °C) was fixed, and the DNA crosslinked using a TC buffer with 22% formaldehyde concentration. After crosslinking, the tissue was homogenised using the Diagnocine Power Masher-II and BioMasher-II tubes and pestles. Following the Arima-HiC v2 kit manufacturer's instructions, crosslinked DNA was digested using a restriction enzyme master mix. The 5'-overhangs were filled in and labelled with biotinylated nucleotides and proximally ligated. An overnight incubation was carried out for enzymes to digest remaining proteins and for crosslinks to reverse. A clean up was performed with SPRIselect beads prior to library preparation. Additionally, the biotinylation percentage was estimated using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and Qubit HS Assay Kit and Arima-HiC v2 QC beads.

Library preparation and sequencing

Library preparation and sequencing were performed at the WSI Scientific Operations core.

PacBio HiFi

At a minimum, samples were required to have an average fragment size exceeding 8 kb and a total mass over 400 ng to proceed to the low input SMRTbell Prep Kit 3.0 protocol (Pacific Biosciences, California, USA), depending on genome size and sequencing depth required. Libraries were prepared using the SMRTbell Prep Kit 3.0 (Pacific Biosciences, California, USA) as per the manufacturer's instructions. The kit includes the reagents required for end repair/A-tailing, adapter ligation, post-ligation SMRTbell bead cleanup, and nuclease treatment. Following the manufacturer's instructions, size selection and clean up was carried out using diluted AMPure PB beads (Pacific Biosciences, California, USA). DNA concentration was quantified using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) with Qubit 1X dsDNA HS assay kit and the final library fragment size analysis was carried out using the Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies) and gDNA 55kb BAC analysis kit.

Samples were sequenced on a Revio instrument (Pacific Biosciences, California, USA). Prepared libraries were normalised to 2 nM, and 15 µL was used for making complexes. Primers were annealed and polymerases were hybridised to create circularised complexes according to manufacturer's instructions. The complexes were purified with the 1.2X clean up with SMRTbell beads. The purified complexes were then diluted to the Revio loading concentration (in the range 200–300 pM), and spiked with a Revio sequencing internal control. Samples were sequenced on Revio 25M SMRT cells (Pacific Biosciences, California, USA). The SMRT link software, a PacBio web-based end-to-end workflow manager, was used to set-up and monitor the run, as well as perform primary and secondary analysis of the data upon completion.

Hi-C

For Hi-C library preparation, DNA was fragmented using the Covaris E220 sonicator (Covaris) and size selected using SPRISelect beads to 400 to 600 bp. The DNA was then enriched using the Arima-HiC v2 kit Enrichment beads. Using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) for end repair, A-tailing, and adapter ligation. This uses a custom protocol which resembles the standard NEBNext Ultra II DNA Library Prep protocol but where library preparation occurs while DNA is bound to the Enrichment beads. For library amplification, 10 to 16 PCR cycles were required, determined by the sample biotinylation percentage. The Hi-C sequencing was performed using paired-end sequencing with a read length of 150 bp on an Illumina NovaSeq X instrument.

Genome assembly, curation and evaluation

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), resulting in a pair of haplotype-resolved assemblies. The Hi-C reads were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). The contigs were further scaffolded using the provided Hi-C data (Rao *et al.*, 2014) 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. Flat files and maps used in curation were generated via the TreeVal pipeline (Pointon *et al.*, 2023). Manual curation was conducted primarily in PretextView (Harry, 2022) and HiGlass (Kerpedjiev *et al.*, 2018), with additional insights provided by JBrowse2 (Diesh *et al.*, 2023). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Any identified contamination, missed joins, and mis-joins were amended, and duplicate sequences were tagged and removed. The curation process is documented at https://gitlab.com/wtsi-grit/rapid-curation.

Assembly quality assessment

The Merqury.FK tool (Rhie *et al.*, 2020), run in a Singularity container (Kurtzer *et al.*, 2017), was used 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.

A Hi-C contact map was produced for the final version of the assembly. The Hi-C reads were aligned using bwa-mem2 (Vasimuddin *et al.*, 2019) and the alignment files were combined using SAMtools (Danecek *et al.*, 2021). The Hi-C alignments were converted into a contact map using BEDTools (Quinlan & Hall, 2010) and the Cooler tool suite (Abdennur & Mirny, 2020). The contact map was visualised in HiGlass (Kerpedjiev *et al.*, 2018).

The blobtoolkit pipeline is a Nextflow (Di Tommaso *et al.*, 2017) port of the previous Snakemake Blobtoolkit pipeline (Challis *et al.*, 2020). It aligns the PacBio reads in SAMtools and minimap2 (Li, 2018) and generates coverage tracks for regions of fixed size. In parallel, it queries the GoaT database (Challis *et al.*, 2023) to identify all matching BUSCO lineages to run BUSCO (Manni *et al.*, 2021). For the three domain-level BUSCO lineages, the pipeline aligns the BUSCO genes to the UniProt Reference Proteomes database (Bateman *et al.*, 2023) with DIAMOND blastp (Buchfink *et al.*, 2021). The genome is also divided into chunks according to the density of the BUSCO genes from the closest taxonomic lineage, and each chunk is aligned to the UniProt Reference Proteomes database using DIAMOND blastx. Genome sequences without a hit are chunked using seqtk and aligned to the NT database with blastn (Altschul *et al.*, 1990). The blobtools suite combines all these 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), relying on the Conda package manager, the Bioconda initiative (Grüning *et al.*, 2018), the Biocontainers infrastructure (da Veiga Leprevost *et al.*, 2017), as well as the Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017) containerisation solutions.

Table 4 contains a list of relevant software tool versions and sources.

Wellcome Sanger Institute – Legal and Governance

The materials that have contributed to this genome note have been supplied by a Tree of Life collaborator. 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

Table 4. Software tools: versions and sources.

Software tool	Version	Source
BEDTools	2.30.0	https://github.com/arq5x/bedtools2
BLAST	2.14.0	ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/
BlobToolKit	4.3.9	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.5.0	https://gitlab.com/ezlab/busco
bwa-mem2	2.2.1	https://github.com/bwa-mem2/bwa-mem2
Cooler	0.8.11	https://github.com/open2c/cooler
DIAMOND	2.1.8	https://github.com/bbuchfink/diamond
fasta_windows	0.2.4	https://github.com/tolkit/fasta_windows
FastK	666652151335353eef2fcd58880bcef5bc2928e1	https://github.com/thegenemyers/FASTK
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats

Software tool	Version	Source
GoaT CLI	0.2.5	https://github.com/genomehubs/goat-cli
Hifiasm	0.19.8-r603	https://github.com/chhylp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84a a44357826c0b6753eb28de	https://github.com/higlass/higlass
MerquryFK	d00d98157618f4e8d1a9190026b19b471055b22e	https://github.com/thegenemyers/MERQURY.FK
Minimap2	2.24-r1122	https://github.com/lh3/minimap2
MitoHiFi	3	https://github.com/marcelauliano/MitoHiFi
MultiQC	1.14, 1.17, and 1.18	https://github.com/MultiQC/MultiQC
Nextflow	23.10.0	https://github.com/nextflow-io/nextflow
PretextView	0.2.5	https://github.com/sanger-tol/PretextView
samtools	1.19.2	https://github.com/samtools/samtools
sanger-tol/ascc	-	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	0.5.1	https://github.com/sanger-tol/blobtoolkit
Seqtk	1.3	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.2.0	https://github.com/sanger-tol/treeval
YaHS	1.2a.2	https://github.com/c-zhou/yahs

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 undertaken according to a Research Collaboration Agreement or Material Transfer Agreement entered into by the Tree of Life collaborator, Genome Research Limited (operating as the Wellcome Sanger Institute) and in some circumstances other Tree of Life collaborators.

Data availability

European Nucleotide Archive: Podarcis erhardii (Erhard's wall lizard). Accession number PRJEB78688; https://identifiers.org/ena.embl/PRJEB78688. The genome sequence is released openly for reuse. The *Podarcis erhardii* genome assembly is provided by the Wellcome Sanger Institute Tree of Life Programme (https://www.sanger.ac.uk/programme/tree-of-life/) and is part of 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.

Author information

Members of the Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team are listed here: https://doi.org/10.5281/zenodo.12162482.

Members of Wellcome Sanger Institute Scientific Operations: Sequencing Operations are listed here: https://doi.org/10.5281/zenodo.12165051.

Members of the Wellcome Sanger Institute Tree of Life Core Informatics team are listed here: https://doi.org/10.5281/zenodo.12160324.

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.12205391.

References

Abdennur N, Mirny LA: **Cooler: scalable storage for Hi-C data and other genomically labeled arrays.** *Bioinformatics.* 2020; **36**(1): 311–316.
PubMed Abstract | Publisher Full Text | Free Full Text

Allio R, Schomaker-Bastos A, Romiguier J, *et al.*: **MitoFinder: efficient automated large-scale extraction of mitogenomic data in target enrichment phylogenomics.** *Mol Ecol Resour.* 2020; **20**(4): 892–905.
PubMed Abstract | Publisher Full Text | Free Full Text

Altschul SF, Gish W, Miller W, *et al.*: **Basic Local Alignment Search Tool.** *J Mol Biol.* 1990; **215**(3): 403–410.
PubMed Abstract | Publisher Full Text

Bateman A, Martin MJ, Orchard S, *et al.*: **UniProt: the universal protein knowledgebase in 2023.** *Nucleic Acids Res.* 2023; **51**(D1): D523–D531.
PubMed Abstract | Publisher Full Text | Free Full Text

Böhme W: **Handbuch der Reptilien und Amphibien Europas**. Aula-Verlag, 1986; **2/II**.

Bowles P, Crnobrnja-Isailović J: **Podarcis erhardii.** *The IUCN Red List of Threatened Species*. 2024; e.T207598021A137855176.
Publisher Full Text

Brock KM, Baeckens S, Donihue CM, *et al.*: **Trait differences among discrete morphs of a color polymorphic lizard, *Podarcis erhardii*.** *PeerJ.* 2020; **8**: e10284.
PubMed Abstract | Publisher Full Text | Free Full Text

Buchfink B, Reuter K, Drost HG: **Sensitive protein alignments at Tree-of-Life scale using DIAMOND.** *Nat Methods.* 2021; **18**(4): 366–368.
PubMed Abstract | Publisher Full Text | Free Full Text

Challis R, Kumar S, Sotero-Caio C, *et al.*: **Genomes on a Tree (GoaT): a versatile, scalable search engine for genomic and sequencing project metadata across the eukaryotic Tree of Life [version 1; peer review: 2 approved].** *Wellcome Open Res.* 2023; **8**: 24.
PubMed Abstract | Publisher Full Text | Free Full Text

Challis R, Richards E, Rajan J, *et al.*: **BlobToolKit – interactive quality assessment of genome assemblies.** *G3 (Bethesda).* 2020; **10**(4): 1361–1374.
PubMed Abstract | Publisher Full Text | Free Full Text

Cheng H, Concepcion GT, Feng X, *et al.*: **Haplotype-resolved *de novo* assembly using phased assembly graphs with hifiasm.** *Nat Methods.* 2021; **18**(2): 170–175.
PubMed Abstract | Publisher Full Text | Free Full Text

Cheng H, Jarvis ED, Fedrigo O, *et al.*: **Haplotype-resolved assembly of diploid genomes without parental data.** *Nat Biotechnol.* 2022; **40**(9): 1332–1335.
PubMed Abstract | Publisher Full Text | Free Full Text

da Veiga Leprevost F, Grüning BA, Alves Aflitos S, *et al.*: **BioContainers: an open-source and community-driven framework for software standardization.** *Bioinformatics.* 2017; **33**(16): 2580–2582.
PubMed Abstract | Publisher Full Text | Free Full Text

Danecek P, Bonfield JK, Liddle J, *et al.*: **Twelve years of SAMtools and BCFtools.** *GigaScience.* 2021; **10**(2): giab008.
PubMed Abstract | Publisher Full Text | Free Full Text

Denton A, Oatley G, Cornwell C, *et al.*: **Sanger Tree of Life sample homogenisation: PowerMash.** *protocols.io.* 2023a.
Publisher Full Text

Denton A, Yatsenko H, Jay J, *et al.*: **Sanger Tree of Life wet laboratory protocol collection V.1.** *protocols.io.* 2023b.
Publisher Full Text

Di Tommaso P, Chatzou M, Floden EW, *et al.*: **Nextflow enables reproducible computational workflows.** *Nat Biotechnol.* 2017; **35**(4): 316–319.
PubMed Abstract | Publisher Full Text

Diesh C, Stevens GJ, Xie P, *et al.*: **JBrowse 2: a modular genome browser with views of synteny and structural variation.** *Genome Biol.* 2023; **24**(1): 74.
PubMed Abstract | Publisher Full Text | Free Full Text

Escoriza D: **Environmental colour pattern variation in Mediterranean *Podarcis*.** *BMC Ecol Evol.* 2024; **24**(1): 53.
PubMed Abstract | Publisher Full Text | Free Full Text

Ewels P, Magnusson M, Lundin S, *et al.*: **MultiQC: summarize analysis results for multiple tools and samples in a single report.** *Bioinformatics.* 2016; **32**(19): 3047–3048.
PubMed Abstract | Publisher Full Text | Free Full Text

Ewels PA, Peltzer A, Fillinger S, *et al.*: **The nf-core framework for community-curated bioinformatics pipelines.** *Nat Biotechnol.* 2020; **38**(3): 276–278.
PubMed Abstract | Publisher Full Text

Formenti G, Abueg L, Brajuka A, *et al.*: **Gfastats: conversion, evaluation and manipulation of genome sequences using assembly graphs.** *Bioinformatics.* 2022; **38**(17): 4214–4216.
PubMed Abstract | Publisher Full Text | Free Full Text

Grüning B, Dale R, Sjödin A, *et al.*: **Bioconda: sustainable and comprehensive software distribution for the life sciences.** *Nat Methods.* 2018; **15**(7): 475–476.
PubMed Abstract | Publisher Full Text | Free Full Text

Harry E: **PretextView (Paired REad TEXTure Viewer): a desktop application for viewing pretext contact maps**. 2022.
Reference Source

Howe K, Chow W, Collins J, *et al.*: **Significantly improving the quality of genome assemblies through curation.** *GigaScience.* 2021; **10**(1): giaa153.
PubMed Abstract | Publisher Full Text | Free Full Text

Jay J, Yatsenko H, Narváez-Gómez JP, *et al.*: **Sanger Tree of Life sample preparation: triage and dissection.** *protocols.io.* 2023.
Publisher Full Text

Kerpedjiev P, Abdennur N, Lekschas F, *et al.*: **HiGlass: web-based visual exploration and analysis of genome interaction maps.** *Genome Biol.* 2018; **19**(1): 125.
PubMed Abstract | Publisher Full Text | Free Full Text

Kiourtsoglou A, Kaliontzopoulou A, Poursanidis D, *et al.*: **Evidence of cryptic diversity in *Podarcis peloponnesiacus* and re-evaluation of its current taxonomy; insights from genetic, morphological, and ecological data.** *J Zool Syst Evol Res.* 2021; **59**(8): 2350–2370.
Publisher Full Text

Kurtzer GM, Sochat V, Bauer MW: **Singularity: scientific containers for mobility of compute.** *PLoS One.* 2017; **12**(5): e0177459.
PubMed Abstract | Publisher Full Text | Free Full Text

Li H: **Minimap2: pairwise alignment for nucleotide sequences.** *Bioinformatics.* 2018; **34**(18): 3094–3100.
PubMed Abstract | Publisher Full Text | Free Full Text

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**(10): 4647–4654.
PubMed Abstract | Publisher Full Text | Free Full Text

Meiri S, Avila L, Bauer AM, *et al.*: **The global diversity and distribution of lizard clutch sizes.** *Glob Ecol Biogeogr.* 2020; **29**(9): 1515–1530.
Publisher Full Text

Merkel D: **Docker: lightweight Linux containers for consistent development and deployment.** *Linux J.* 2014; **2014**(239): 2, [Accessed 2 April 2024].
Reference Source

Pointon DL, Eagles W, Sims Y, *et al.*: **sanger-tol/treeval v1.0.0 – Ancient Atlantis.** 2023.
Publisher Full Text

Poulakakis N, Lymberakis P, Antoniou A, *et al.*: **Molecular phylogeny and biogeography of the wall-lizard *Podarcis erhardii* (Squamata: Lacertidae).** *Mol Phylogenet Evol.* 2003; **28**(1): 38–46.
PubMed Abstract | Publisher Full Text

Poulakakis N, Lymberakis P, Stratakis M, *et al.*: **The genome sequence of the Cretan wall lizard, *Podarcis cretensis* (Wettstein, 1952) [version 1; peer review: 2 approved with reservations].** *Wellcome Open Res.* 2024; **9**: 161.
Publisher Full Text

Quinlan AR, Hall IM: **BEDTools: a flexible suite of utilities for comparing genomic features.** *Bioinformatics.* 2010; **26**(6): 841–842.
PubMed Abstract | Publisher Full Text | Free Full Text

Ranallo-Benavidez TR, Jaron KS, Schatz MC: **GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes.** *Nat Commun.* 2020; **11**(1): 1432.
PubMed Abstract | Publisher Full Text | Free Full Text

Rao SSP, Huntley MH, Durand NC, *et al.*: **A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping.** *Cell.* 2014; **159**(7): 1665–1680.
PubMed Abstract | Publisher Full Text | Free Full Text

Rhie A, McCarthy SA, Fedrigo O, *et al.*: **Towards complete and error-free genome assemblies of all vertebrate species.** *Nature.* 2021; **592**(7856): 737–746.
PubMed Abstract | Publisher Full Text | Free Full Text

Rhie A, Walenz BP, Koren S, *et al.*: **Merqury: reference-free quality, completeness, and phasing assessment for genome assemblies.** *Genome Biol.* 2020; **21**(1): 245.
PubMed Abstract | Publisher Full Text | Free Full Text

Stadler SR, Brock KM, Bednekoff PA, *et al.*: **More and bigger lizards reside on islands with more resources.** *J Zool.* 2023; **319**(3): 163–174.
Publisher Full Text

Strickland M, Cornwell C, Howard C: **Sanger Tree of Life fragmented DNA clean up: manual SPRI.** *protocols.io.* 2023a.
Publisher Full Text

Strickland M, Moll R, Cornwell C, *et al.*: **Sanger Tree of Life HMW DNA extraction: manual MagAttract.** *protocols.io.* 2023b.
Publisher Full Text

Todorovic M, Sampaio F, Howard C: **Sanger Tree of Life HMW DNA fragmentation: diagenode Megaruptor®3 for PacBio HiFi.** *protocols.io.* 2023.
Publisher Full Text

Uliano-Silva M, Ferreira JGRN, Krasheninnikova K, *et al.*: **MitoHiFi: a python pipeline for mitochondrial genome assembly from PacBio high fidelity reads.** *BMC Bioinformatics.* 2023; **24**(1): 288.
PubMed Abstract | Publisher Full Text | Free Full Text

Valakos ED, Pafilis P, Lymberakis P, *et al.*: **The Amphibians and Reptiles of**

Greece. Ed. Chimaira, 2008.
Reference Source

Vasimuddin M, Misra S, Li H, *et al.*: **Efficient architecture-aware acceleration of BWA-MEM for multicore systems.** In: *2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS).* IEEE, 2019; 314–324.
Publisher Full Text

Yang W, Feiner N, Pinho C, *et al.*: **Extensive introgression and mosaic genomes of Mediterranean endemic lizards.** *Nat Commun.* 2021; **12**(1): 2762.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Zhou C, McCarthy SA, Durbin R: **YaHS: yet another Hi-C scaffolding tool.** *Bioinformatics.* 2023; **39**(1): btac808.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Open Peer Review

Current Peer Review Status: ✔ ✔ ✔

Version 1

Reviewer Report 02 July 2025

https://doi.org/10.21956/wellcomeopenres.26525.r125224

✔ **Merly Escalona**
University of California, Santa Cruz, California, USA

The authors describe the efforts for genome sequencing and assembly of a female *Podarcis erhardii* lizard. These efforts include a phased nuclear genome assembly, mitochondrial genome assembly, and annotation of both nuclear and mitochondrial assemblies. All the resources generated are of high quality and satisfy the minimum metrics of the Earth BioGenome Project and Vertebrate Genome Project as established by the Rhie et al. 2020 paper and the documentation of the EBP.

However, I have 2 minor comments and some formatting notes.

Minor comments:

- While both the nuclear and mitochondrial genomes have been assembled, annotated, and made publicly available, there are no metrics for quality description of the mitochondrial genome, and it would be good to share those as well.
- Are the contaminants from Apicomplexa expected on this species? Genus? Is that single contig the full genome of the parasite?

Formatting notes:

- There are some inconsistencies with how locations are described:
  - Section Background - Paragraph 6 - Last line: Andros -> Andros, Greece.
  - Section Methods - Sample acquisition - Paragraph 1 - Line3-4: Andros in the northern Cyclades > Andros in the northern Cyclades, Greece.
  - Section Methods - Nucleic acid extraction - Paragraph 1: WSI DToL > WSI DToL, UK.
  - Section Methods - Hi-C sample preparation and crosslinking
    - Thermo Fisher Scientific > Thermo Fisher Scientific, Massachusetts, USA or Thermo Fisher Scientific, USA.
  - Section Methods - Library preparation - HiC:
    - Covaris > Covaris, Massachusetts, USA or Covaris, USA.
- Is PacBio HiFi header level 3? If so, wouldn't Hi-C also be formatted the same way?
- Section Genome assembly, curation and evaluation - Assembly quality assessment -

Paragraph 3: Is it blobtoolkit or Blobtoolkit or BlobToolkit? Please be consistent.

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: genome assembly, bioinformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 25 June 2025

https://doi.org/10.21956/wellcomeopenres.26525.r125225

Roberto Biello
[1] John Innes Centre Department of Crop Genetics (Ringgold ID: 534071), Norwich, England, UK
[2] University of Ferrara, Ferrara, Italy

The manuscript presents a chromosome-level genome assembly of the Erhard's wall lizard (*Podarcis erhardii*). It represents an important contribution to genomic resources for the *Podarcis* genus and related taxa. Sequencing was performed using PacBio HiFi and Hi-C data, and assemblies were generated for both haplotypes. However, chromosome-level resolution was achieved only for the first haplotype. The assemblies meet high-quality standards, supported by BUSCO scores, k-mer completeness, and a high percentage of sequence mapping to chromosomes. The methods are clearly described, and the associated data have been made publicly available.

Minor comments:
- At the end of the introduction, the authors stated that this species was sequenced as part of the DToL project, which focuses on sequencing all eukaryotic species in Britain and Ireland. Since *P. erhardii* is a Balkan species, the authors should better explain why it was included in the DToL

project.
- In the sample acquisition section, the authors mentioned that they collected the tip of the tail and preserved it in ethanol. However, for the Hi-C sample preparation, they reported using 20–50 mg of frozen tissue stored at -80°C. These descriptions are inconsistent, and the authors should clarify it.
- The rationale for choosing the subspecies *mykonensis* for genome sequencing was not provided.
- The authors described that PacBio reads are aligned using SAMtools within the BlobToolKit pipeline. In fact, read alignment is performed using minimap2, while SAMtools is used for downstream processing of the SAM/BAM files. This should be corrected in the methods section.
- The authors stated that Figure 4 illustrates the “completeness of the assembly,” but this is not correct given that analysis shows the cumulative length for all scaffolds.

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

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: Comparative genomics, Genome assembly and annotation, Evolutionary biology, 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.

Reviewer Report 10 May 2025

https://doi.org/10.21956/wellcomeopenres.26525.r122613

Erin Westeen
University of California Berkeley, Berkeley, California, USA

The authors present a genome sequence of a female individual of Erhard's wall lizard, Podarcis erhardii. The genome was sequenced using PacBio long reads and Hi-C to capture chromosome conformation. The authors assembled two haplotypes and the mitochondrial genome.

The article is concise and well-written. I have only small suggestions.
when beginning a sentence with the scientific name, Podarcis should be spelled out (rather than P.).
In the methods, perhaps it should be mentioned that Andros (and the Cyclades) are in Greece?

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: phenotypic evolution, comparative biology, herpetology, 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.