DATA NOTE

The genome sequence of the Ibiza wall lizard, *Podarcis pityusensis* (Boscá, 1883)

[version 1; peer review: 2 approved, 1 approved with reservations]

Nathalie Feiner[1,2], Tobias Uller[2], Ferran de la Cruz[3-5], Roberto Garcia-Roa[2], Joana Meier[6],
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, Sweden
[3]CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO Associate Laboratory, Universidade do Porto, Porto, Portugal
[4]BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, Vairão, Portugal
[5]Departamento de Biologia, Universidade do Porto Faculdade de Ciencias, Porto, Portugal
[6]Tree of Life, Wellcome Sanger Institute, Hinxton, England, UK

v1 **First published:** 07 May 2025, **10**:235
https://doi.org/10.12688/wellcomeopenres.24143.1
Latest published: 07 May 2025, **10**:235
https://doi.org/10.12688/wellcomeopenres.24143.1

Abstract

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 pityusensis* (Boscá, 1883) (NCBI:txid74359).

Keywords

Podarcis pityusensis, Ibiza 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 07 May 2025	? view	✓ view	✓ view

1. **Hardip R. Patel**, Australian National University, Canberra, Australia
Paul Waters, University of New South Wales Science, Sydney, Australia
Arthur Georges, University of Canberra, Canberra, Australia

2. **Ranjana Bhaskar**, Zoological Survey of India, Southern Regional Centre, Santhome High Road, chennai, India

3. **Jessica Gomez-Garrido**, CNAG, Centro Nacional de Análisis Genómico, 08028,

Barcelona, Barcelona, Spain

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

Corresponding author: Joana Meier (jm66@sanger.ac.uk)

Author roles: Feiner N: Investigation, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; **Uller T**: Investigation, Resources, Writing – Original Draft Preparation; **de la Cruz F**: Resources, Writing – Review & Editing; **Garcia-Roa R**: 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). F.d.l.C. was supported by the Fundação para a Ciência e Tecnologia (FCT, Portugal; PhD contract with reference 2022.14105.BD). R.G.R. was funded by a MSCA Postdoctoral Fellowship (EvoColour, 101061275). Field work was supported by a research grant from the Swedish Research Council to T.U. (VR 2021-04656).
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, de la Cruz F *et al.* **The genome sequence of the Ibiza wall lizard, *Podarcis pityusensis* (Boscá, 1883) [version 1; peer review: 2 approved, 1 approved with reservations]** Wellcome Open Research 2025, **10**:235 https://doi.org/10.12688/wellcomeopenres.24143.1

First published: 07 May 2025, **10**:235 https://doi.org/10.12688/wellcomeopenres.24143.1

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 pityusensis* (Boscá, 1883) (NCBI:txid74359)

Background

The Ibiza wall lizard *Podarcis pityusensis* (Boscá, 1883; Figure 1) is a diurnal lacertid lizard endemic to Ibiza, Formentera and surrounding islets. More than 20 subspecies have been described, reflecting the large phenotypic diversity found in this species (Böhme, 1986). Introduced populations of *P. pityusensis* are present in Northern and Eastern Spain and on Mallorca (Colodro *et al.*, 2020; Zawadzki, 2020). As a consequence of its restricted distribution and recent decline on Ibiza, the species is classified as Endangered (EN) by the IUCN (Bowles, 2024). Populations on the main island of Ibiza are particularly threatened by the invasive horseshoe whip snakes (*Hemorrhois hippocrepis*) that were introduced with ornamental plants (Montes *et al.*, 2022). Reports suggest that these snakes may even be capable of reaching nearby islets, potentially eradicating islet populations of *P. pityusensis* (Montes *et al.*, 2022). The species has also suffered from the pet trade and is listed in Annex II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Zawadzki, 2020).

Figure 1. A) Female and **B**) male *Podarcis pityusensis* photographed in Cala Vedella (April 2022). Note that the specimen selected for genome sequencing is from the same locality, but not shown on the photographs. Photographs by Ferran de la Cruz.

Podarcis pityusensis is one of 28 currently described species of the genus *Podarcis*. It is the sister species to *P. lilfordi* (Yang *et al.*, 2021), which is another endemic to the Balearic islands inhabiting the Cabrera archipelago and islets surrounding Mallorca and Menorca. The two species likely separated following the post-Messinian rise in sea levels (Brown *et al.*, 2008).

The Ibiza wall lizard *P. pityusensis* occurs in a variety of habitats, including Mediterranean shrubland, shrubby vegetation, grassland, sandy or rocky shores as well as urbanized environments. The population densities can be very high and lizards can vary substantially across islands in aspects of their biology, such as behaviour, colouration and body size (Cooper & Pérez-Mellado, 2012). Colour variation includes reticulated, striped or plain patterns in blue, grey, black, brown, green and yellow hues. The main activity period is in spring and early summer when reproduction occurs, but lizards can be active throughout the year. The species is oviparous and Meiri *et al.* (2020) report an average clutch size of 2.65.

P. pityusensis is one of three species of the Western Islands group of *Podarcis*. This group is a descendant of a lineage formed through extensive introgressive hybridisation between lizards belonging to the eastern and western clade of *Podarcis* (Yang *et al.*, 2021). Reference genomes of its sister species *P. tiliguerta* and *P. lilfordi* are available (Gomez-Garrido *et al.*, 2023). The availability of reference genomes for all three species in this group will be a valuable resource for comparative studies above the species level. We anticipate that the reference genome of *P. pityusensis* will support ongoing conservation efforts and facilitate further studies of population history, gene flow, and causes of phenotypic diversification within this species.

Genome sequence report

Sequencing data

The genome of a specimen of *Podarcis pityusensis* was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 35.44 Gb (gigabases) from 5.36 million reads. GenomeScope analysis of the PacBio HiFi data estimated the haploid genome size at 1,461.54 Mb, with a heterozygosity of 0.57% and repeat content of 20.66%. 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 23.0x coverage of the genome. Chromosome conformation Hi-C sequencing produced 555.30 Gb from 3,677.49 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 216 misjoins or missing joins and removed 9 haplotypic duplications. These interventions increased the total assembly length by

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

Project information			
Study title	Podarcis pityusensis (Ibiza wall lizard)		
Umbrella BioProject	PRJEB73697		
Species	*Podarcis pityusensis*		
BioSpecimen	SAMEA114217797		
NCBI taxonomy ID	74359		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	rPodPit1	SAMEA114217801	terminal body
Hi-C sequencing	rPodPit1	SAMEA114217801	terminal body
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq X	ERR12743668	3.68e+09	555.3
PacBio Revio	ERR12736921	5.36e+06	35.44

1.31%, decreased the scaffold count by 11.58%, and increased the scaffold N50 by 0.63%. The final assembly has a total length of 1,515.36 Mb in 335 scaffolds, with 1,506 gaps, and a scaffold N50 of 91.58 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 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 (99.0%) 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 sex chromosomes were assigned by synteny to the genome of *Podarcis raffonei* (GCA_027172205.1) and by PacBio reads coverage observed in the Hi-C map.

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.

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 60.6, and for haplotype 2, 60.8. When the two haplotypes are combined, the assembly achieves an estimated QV of 60.7. The *k*-mer recovery for haplotype 1 is 86.42%, and for haplotype 2 82.68%, while the combined haplotypes have a *k*-mer recovery of 97.94%. BUSCO 5.5.0 analysis using the sauropsida_odb10 reference set (n = 7,480) identified 94.9% of the expected gene set (single = 92.7%, duplicated = 2.2%) for haplotype 1.

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 assembly achieves the EBP reference standard of **6.C.Q60.**

Methods

Sample acquisition

The specimen, an adult female *P. pityusensis* lizard (specimen ID SAN25001763, ToLID rPodPit1) was collected on 2022-04-19 from a site near Cala Vedella on the main island of Ibiza (latitude: 38.9105995; longitude: 1.2240528). 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 CEP 03/2022. The specimen was collected and identified by Roberto García-Roa (University of Lund, Sweden).

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

Genome assembly	**Haplotype 1**	**Haplotype 2**
Assembly name	rPodPit1.hap1.2	rPodPit1.hap2.2
Assembly accession	GCA_964106645.2	GCA_964106635.2
Assembly level	chromosome	chromosome
Span (Mb)	1,515.36	1,417.08
Number of contigs	1,841	1,548
Number of scaffolds	335	183
Longest scaffold (Mb)	139.68	139.78
Assembly metrics (benchmark)	**Haplotype 1**	**Haplotype 2**
Contig N50 length (≥ 1 Mb)	1.61 Mb	1.72 Mb
Scaffold N50 length (= chromosome N50)	91.58 Mb	92.42 Mb
Consensus quality (QV) (≥ 40)	60.6	60.8
k-mer completeness	86.42%	82.68%
Combined *k*-mer completeness (≥ 95%)	97.94%	
BUSCO* (S > 90%; D < 5%)	C:94.9%[S:92.7%,D:2.2%], F:0.9%,M:4.2%,n:7480	-
Percentage of assembly mapped to chromosomes (≥ 90%)	99.0%	99.43%
Sex chromosomes (localised homologous pairs)	W and Z	-
Organelles (one complete allele)	Mitochondrial genome: 17.19 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.

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 preparation and homogenisation, DNA extraction, fragmentation and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). The rPodPit1 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

Hi-C data were generated from the terminal body of the rPodPit1 sample using the Arima-HiC v2 kit (Arima Genomics) with 20–50 mg of frozen tissue (stored at –80 °C). As per manufacturer's instructions, tissue was fixed, and the DNA crosslinked using a TC buffer with a final formaldehyde concentration of 2%. The tissue was then homogenised using the Diagnocine Power Masher-II. The crosslinked DNA was digested using a restriction enzyme master mix, then biotinylated and ligated. A clean up was performed with SPRIselect beads prior to library preparation. DNA concentration was quantified using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and Qubit HS Assay Kit, and sample biotinylation percentage was estimated using the 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

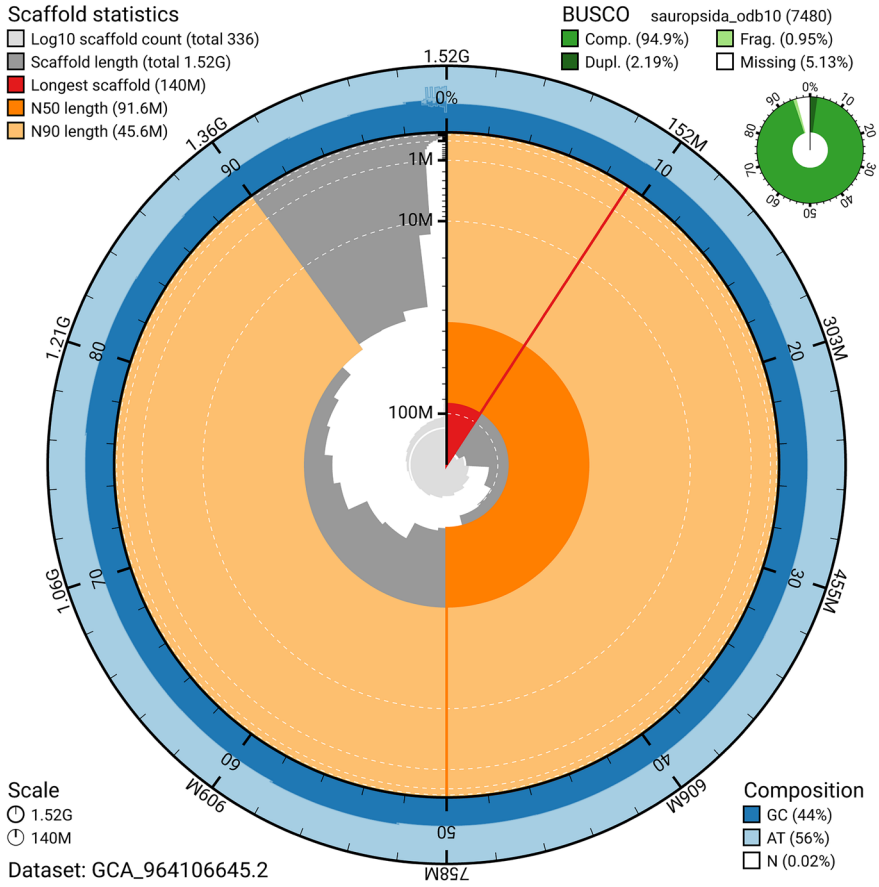

Figure 2. Genome assembly of *Podarcis pityusensis*, rPodPit1.hap1.2: metrics. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the whole genome sequence, and the main plot is divided into 1,000 bins around the 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_964106645.2/dataset/GCA_964106645.2/snail.

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, the biotinylated DNA constructs were fragmented using a Covaris E220 sonicator and size-selected to 400–600 bp using SPRISelect beads. DNA was then enriched using Arima-HiC v2 Enrichment beads. The

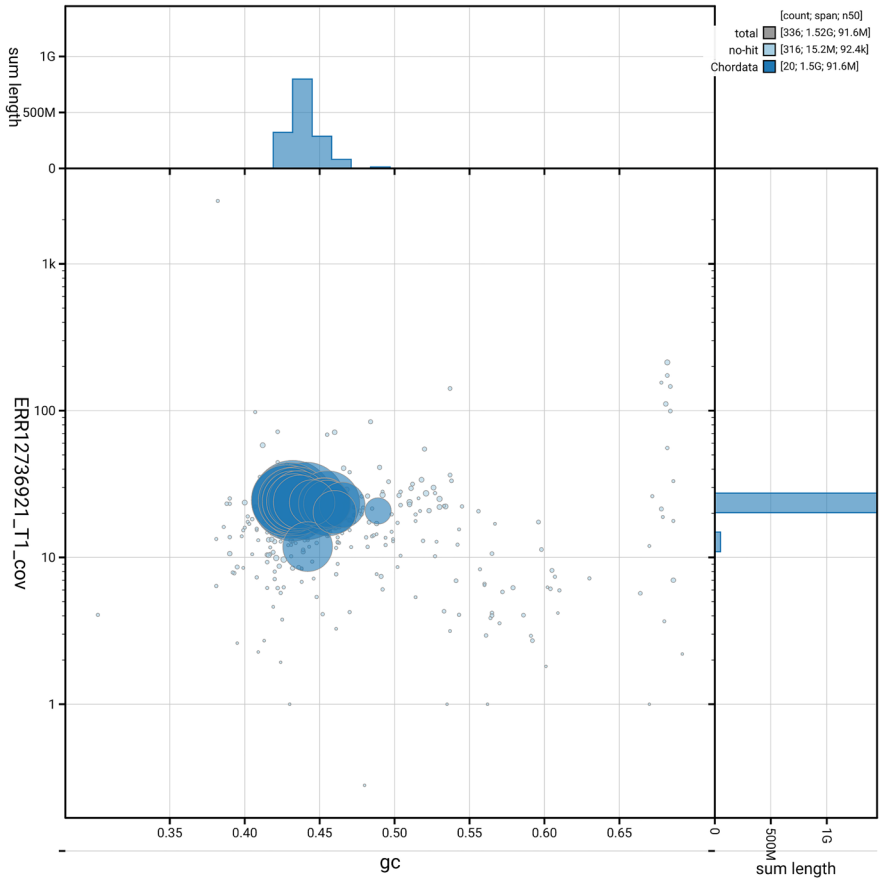

Figure 3. Genome assembly of *Podarcis pityusensis*, rPodPit1.hap1.2: BlobToolKit GC-coverage plot. Blob plot showing sequence coverage (vertical axis) and GC content (horizontal axis). The circles represent scaffolds, with the size proportional to scaffold length and the colour representing phylum membership. 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_964106645.2/dataset/GCA_964106645.2/blob.

NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) was used for end repair, A-tailing, and adapter ligation, following a modified protocol in which library preparation is carried out while the DNA remains bound to the enrichment beads. PCR amplification was performed using KAPA HiFi HotStart mix and custom dual-indexed adapters (Integrated DNA Technologies) in a 96-well plate format. Depending on sample concentration and biotinylation percentage determined at the crosslinking stage, samples were amplified for 10–16 PCR cycles. Post-PCR clean-up was carried out using SPRISelect beads. The libraries were quantified using the Accuclear Ultra High Sensitivity dsDNA Standards Assay kit (Biotium) and normalised to 10 ng/μL before sequencing. Hi-C sequencing was performed on the Illumina NovaSeq X instrument using 150 bp paired-end reads.

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.

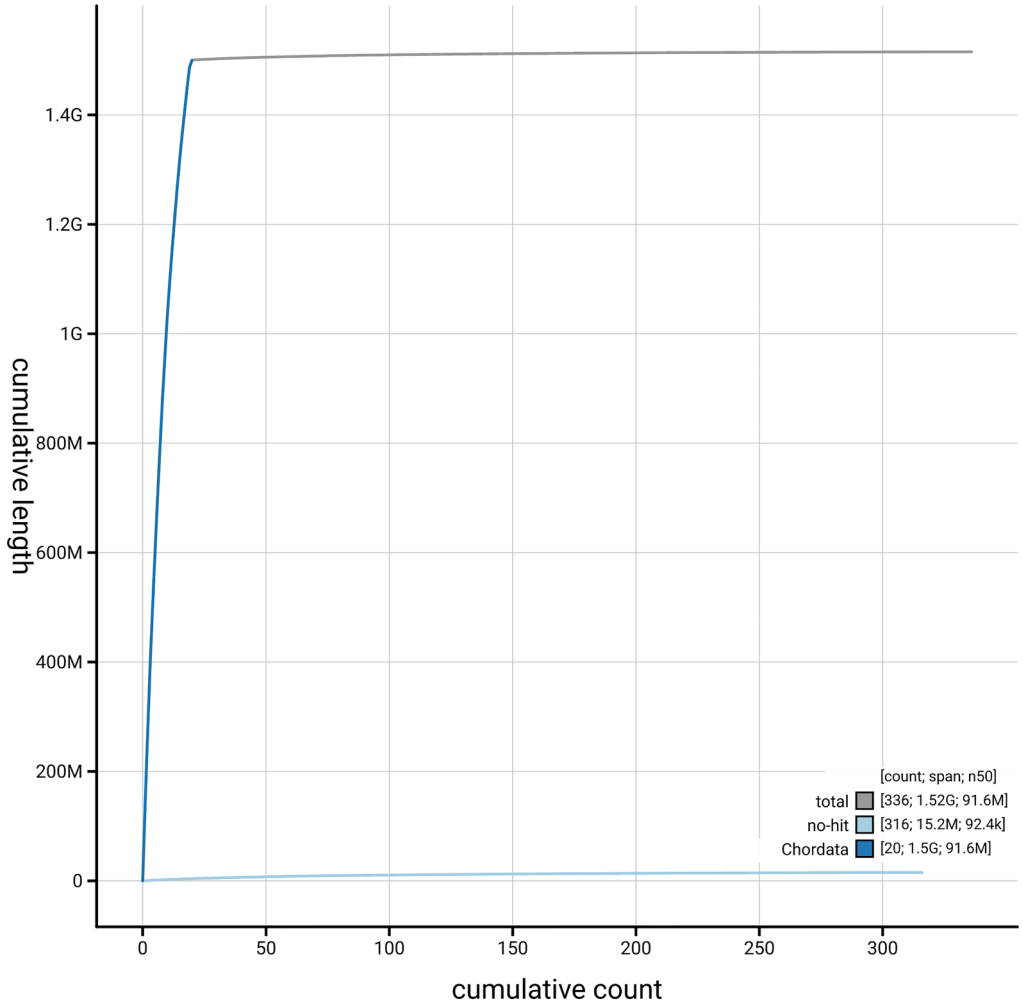

Figure 4. Genome assembly of *Podarcis pityusensis*, rPodPit1.hap1.2: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/GCA_964106645.2/dataset/GCA_964106645.2/cumulative.

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

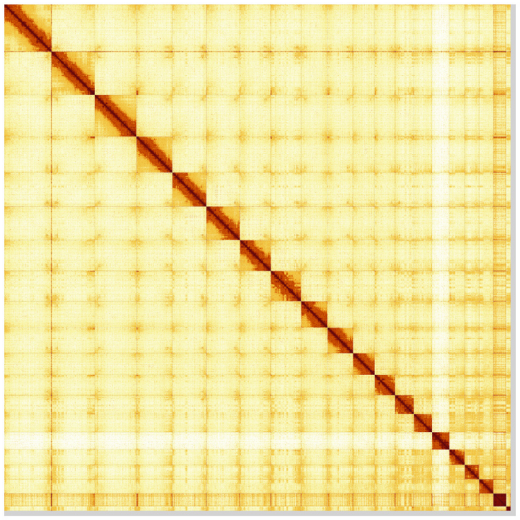

Figure 5. Genome assembly of *Podarcis pityusensis*: Hi-C contact map of the rPodPit1.hap1.2 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=X5Qm-ZHpRBSosl1GZmTKWA.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Podarcis pityusensis*, rPodPit1.

Haplotype 1				Haplotype 2			
INSDC accession	**Name**	**Length (Mb)**	**GC%**	**INSDC accession**	**Name**	**Length (Mb)**	**GC%**
OZ067038.1	1	139.68	43	OZ067003.1	1	139.78	43
OZ067039.1	2	128.38	44	OZ067004.1	2	126.52	44
OZ067040.1	3	123.67	43	OZ067005.1	3	122.66	43
OZ067041.1	4	106.34	43	OZ067006.1	4	107.44	43
OZ067042.1	5	100.81	43.5	OZ067007.1	5	100.53	43
OZ067043.1	6	99.26	43.5	OZ067008.1	6	99.09	43.5
OZ067044.1	7	91.58	43	OZ067009.1	7	92.42	43
OZ067045.1	8	89.68	45.5	OZ067010.1	8	89.26	45.5
OZ067046.1	9	78.23	43	OZ067011.1	9	78.36	43
OZ067047.1	10	75.91	43.5	OZ067012.1	10	76.05	43.5
OZ067048.1	11	63.86	43.5	OZ067013.1	11	64.48	43.5
OZ067049.1	12	60.19	43.5	OZ067014.1	12	60.35	43.5
OZ067050.1	13	55.79	45.5	OZ067015.1	13	55.12	45.5
OZ067051.1	14	54.0	45.5	OZ067016.1	14	53.75	45.5
OZ067052.1	15	45.56	45.5	OZ067017.1	15	45.55	45.5
OZ067053.1	16	42.64	46.5	OZ067018.1	16	42.68	46.5
OZ067054.1	17	42.41	44.5	OZ067019.1	17	42.29	44.5
OZ067055.1	18	13.05	49	OZ067020.1	18	12.74	49
OZ078260.1	W	38.07	46				
OZ078259.1	Z	51.1	44				
OZ067056.1	MT	0.02	39				

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 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

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
GoaT CLI	0.2.5	https://github.com/genomehubs/goat-cli
Hifiasm	0.19.8-r603	https://github.com/chhylp123/hifiasm
HiGlass	44086069ee7d4d3f6f3f0012569789ec138f42b84aa443578 26c0b6753eb28de	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
NCBI Datasets	15.12.0	https://github.com/ncbi/datasets
Nextflow	23.10.0	https://github.com/nextflow-io/nextflow

Software tool	Version	Source
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

- 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 pityusensis* (Ibiza wall lizard). Accession number PRJEB73697; https://identifiers.org/ena.embl/PRJEB73697. The genome sequence is released openly for reuse. The *Podarcis pityusensis* genome assembly is provided by the Wellcome Sanger Institute Tree of Life Programme (https://www.sanger.ac.uk/programme/tree-of-life/). 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.** Vol. 2/II. Aula-Verlag, 1986.

Bowles P: ***Podarcis pityusensis*.** *The IUCN Red List of Threatened Species.* 2024; **2024**: e.T17800A137843475.
Publisher Full Text

Brown RP, Terrasa B, Pérez-Mellado V, *et al.*: **Bayesian estimation of post-Messinian divergence times in Balearic Island lizards.** *Mol Phylogenet Evol.* 2008; **48**(1): 350–358.
PubMed Abstract | Publisher 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

Colodro I, Atiénzar F, Sancho V, *et al.*: **Nueva población peninsular de lagartija de las Pitiusas, *Podarcis pityusensis* (Boscá, 1883).** *Bol Asoc Herpetol Esp.* 2020; **31**(1): 88–93.
Reference Source

Cooper WE Jr, Pérez-Mellado V: **Historical influence of predation pressure on escape by *Podarcis* lizards in the Balearic Islands.** *Biol J Linn Soc Lond.* 2012; **107**(2): 254–268.
Publisher 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

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

Gomez-Garrido J, Cruz F, Alioto TS, *et al.*: **Chromosome-level genome assembly of Lilford's wall lizard, *Podarcis lilfordi* (Günther, 1874) from the Balearic Islands (Spain).** *DNA Res.* 2023; **30**(3): dsad008.
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

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

Montes E, Kraus F, Chergui B, *et al.*: **Collapse of the endemic lizard *Podarcis pityusensis* on the island of Ibiza mediated by an invasive snake.** *Curr Zool.* 2022; **68**(3): 295–303.
PubMed Abstract | Publisher Full Text | Free Full Text

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

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; [Accessed 24 November 2023].
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

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

Zawadzki M: **Success model "Island lizard" – or rather a bleak future for the Ibiza wall lizard *Podarcis pityusensis* (BOSCÁ, 1883)?** *L@CERTIDAE (Eidechsen Online).* 2020; **3**: 19–41.

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 12 June 2025

https://doi.org/10.21956/wellcomeopenres.26634.r123795

✓ **Jessica Gomez-Garrido**
CNAG, Centro Nacional de Análisis Genómico, 08028, Barcelona, Barcelona, Spain

This genome note presents the genome assembly of Podarcis pityusensis, a species of the genus Podarcis that inhabits in Ibiza, Formentera and close islets. It is classified as endangered by the IUCN and the availability of its genome will be a key resource to support conservation efforts.

The sequencing and assembly strategies are appropriate, and the results are good. 2 haplotypes are provided, the first one containing both sex chromosomes and the mitogenome. Both haplotypes contain one copy of each autosome. This is standard practice.

Data has been properly deposited into INSDC databases and can be accessed.

In my opinion, this genome report is good, and no clarifications are needed. I would just suggest a revision of the abstract section which just seems to be the species taxonomy. An abstract with more details should be added.

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 and annotation.

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 29 May 2025

https://doi.org/10.21956/wellcomeopenres.26634.r123353

Ranjana Bhaskar
Zoological Survey of India, Southern Regional Centre, Santhome High Road, chennai, Tamilandu, India

The author performed the genome sequencing of the Ibiza wall lizard, *Podarcis pityusensis.* Sequencing was conducted using the PacBio platform, generating 35.44 Gb (gigabases) from 5.36 million reads. The genome assembly of *Podarcis pityusensis* was also carried out by the author. Assembly quality was assessed using BlobToolKit, and the snail plot provides an overview of assembly metrics and BUSCO gene completeness. The author also conducted the quality assessment.
This study represents a novel addition to genomic databases and will allow for comparative analysis across the three species of the Western Islands group of *Podarcis*. The generated sequences will support ongoing conservation efforts and facilitate further research on population history, gene flow, and the drivers of phenotypic diversification within this species.
I recommend this study for indexing.

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: My area is conservation genetics, population genetics, Forensic science

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 29 May 2025

https://doi.org/10.21956/wellcomeopenres.26634.r123793

?

Hardip R. Patel
Australian National University, Canberra, Canberra, Australia
Paul Waters
University of New South Wales Science, Sydney, New South Wales, Australia
Arthur Georges
University of Canberra, Canberra, Australia

Review Prepared by Arthur Georges, Paul Waters and Hardip Patel

We found this genome note to be technically sound but found the communication to be wanting. Part of this deficiency arises from lack of clarity on what the genome note is designed to achieve. A genome note is an announcement of the availability of a new genome assembly made available concurrently with the assembly file becoming available in a public repository. In this the genome note succeeds. However, it should also describe the sequences and how they were generated and analysed in sufficient detail for one to be able to repeat the workflow or, if necessary, turn to the genome note for insight as to why subsequent use of the genome assembly has delivered unexpected results. It should highlight any novel approaches to the assembly for the benefit of others. In this the genome note does not succeed. More detail is required as in for example the description of the progressive gains from the hifiasm base assembly, the value add of the YAHS scaffolding, and then the manual curation required and the value add of that manual curation. Finally, the genome note should point to knowledge gaps where this assembly can be used to advance our knowledge and understanding. Pointers to the future.

We would like to see the genome note revised to bring out these sentient points. Otherwise, the value of the publication is not a lot greater than simply the submission of the assembly on NCBI.

Abstract

The abstract needs to be included. Among other details expected in abstract, it should also include (a) a brief sentence or two introducing the species, its karyotype and why it is important; (b) “a female ZW specimen” so that one can see immediately that the heterogametic sex has been sequenced; (c) a statement of the key benchmark statistics for the assembly against the standard set published by the Earth BioGenome Project; (d) a statement to the effect that the assembly is published without annotation; and (e) a final concluding sentence or two on the value of having this assembly out. At the moment it contains species taxonomy only.

Species Taxonomy

The NCBI abbreviated version would be more appropriate here. Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Lepidosauria; Squamata; Bifurcata; Unidentata; Episquamata;

Laterata; Lacertibaenia; Lacertidae; *Podarcis pityusensis* (Boscá 1883) [NCBI:txid74359]

Background

Notwithstanding the information being included in the abstract, the background needs to include a description of the karyotype and state clearly that the species has a ZZ/ZW system of sex determination, and that a female (ZW) animal has been sequenced.

The authors could consider being more specific in how the generation of this assembly will provide the basis for an acceleration of new knowledge in conservation and comparative studies, and provide opportunity to address challenges that would otherwise be intractable.

Genome Sequence Report

Consider using read depth in place of coverage, as the latter is unambiguous [throughout].

Approximately 23.0x? Do you mean approximately 23x? And in any case 35.44Gb/1.46154 gives 24.25x. 35.44Gb output from a Revio SMRT Cell seems relatively low. Details on the number of SMRT cells and how they were used can inform other readers about expectation from a sequencing platform.

As outlined earlier, it would be nice to see how the assembly progressed in terms of summary statistics from the base hifiasm assembly, to the YAHS assembly, to the final manual curated assembly. There are some details provided but not clear how different contigs/scaffolds progressed in the workflow. More detail is required on what the manual curation involved.

What do you mean exactly by curated to chromosome level. Need to specify what you mean by chromosome level.

It is not clear what the final assembly is. Is it Haplotype 1? Why not the hifiasm pseudohaplotype? Have the Z and the W been added to this final assembly? How? There is a discrepancy between the length of the final assembly and the genomescope estimate. This warrants a comment.

The authors report the percentage of the assembly mapped to chromosomes, but the assembly has not been physically mapped. Should these be referred to as putative chromosomes?

Most of the assembly sequence (99.0%) was assigned to 20 chromosomal-level scaffolds, representing 18 autosomes and the W and Z sex chromosomes. Is this consistent with the karyotype? Be sure to add citations for the karyotype.

Not really enamored with Figure 2. This information might be better presented in a table. “A light grey spiral at the centre shows the cumulative scaffold count on a logarithmic scale” – not sure what this means. Where is the log scale? Very hard to interpret.

Figure 3. I really struggled to see the different colours for the smaller dots in this graph. Suggest using more definitive colours.

The paragraph immediately following Figure 3 needs a substantial rewrite.

“The *k*-mer recovery for haplotype 1 is 86.42%, and for haplotype 2 82.68%, while the combined haplotypes have a *k*-mer recovery of 97.94%.” This likely arises from the high heterozygosity. Inclusion of a GenomeScope figure would assist the reader here. 97.94 recovery of k-mers seems lower as well.

Methods

We were disappointed to see that the specimen used for the assembly was released. It should have been vouchered and lodged with a reputable museum. Standard morphometric measurements were taken but not reported in the manuscript.

An assembly often includes mitochondria sequences as multiple contigs. Were they removed? More clarity on mitohifi would help here. Was mitohifi run on the reads or the assembly?

“Detailed protocols are available on protocols.io (Denton *et al.*, 2023b)” – a quick perusal of these two papers shows this claim is not true. Need to add the quantity and type of the lysis buffer, quantity of the tissued used after dissection, etc. The specific protocol within these papers needs to be identified. Also confused as to how tissue 20-50 mg was used for the HiC when the lizard was

released. Was the HiC from a different individual from the focal assembly individual?
Inclusion of FemtoPulse results could inform other users working with similar samples to compare their DNA quality and assembly results.
20-50 mg of frozen tissue? But only one individual. Which was it, 20 mg or 50 mg?
“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”. This is a generic statement presumably taken from the manufacturers guidelines. What did you actually do? Needs to be specific to the current study.
“Samples were sequenced on Revio 25M SMRT cells”. How many?
Hi-C wet lab experiments suggest multiple samples were processed at the same time (“*in a 96-well plate format*”). Indicate this clearly. Sample contaminations are often an issue, and readers can benefit by referring to this detail if needed.
Bioinformatics methods require parameters used to conduct analysis. If defaults were used, state that explicitly.
“The assembly was decontaminated using the Assembly Screen for Cobionts and Contaminants (ASCC) pipeline.” Results of this would be useful to readers.
“Any identified contamination, missed joins, and mis-joins were amended, and duplicate sequences were tagged and removed”. Can you provide details of how these decisions were made. For example, how is a mis-join identified?
“The Hi-C reads were aligned using bwa-mem2”. The parameters and any additional details would be useful here.
“The Hi-C alignments were converted into a contact map using BEDTools” Further details required here for reproducibility.
The blobkit pipeline – what did you use it for. There is a statement on what it can be used for, but what did you specifically use it for in this assembly?
“The genome will be annotated using available RNA-Seq data and presented through the Ensembl pipeline at the European Bioinformatics Institute”. Hmmm. Why no annotation here? What RNAseq data are available for this Endangered species? Greatly limits the utility of the assembly and thus the resource note.

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

Yes

Are the protocols appropriate and is the work technically sound?

Partly

Are sufficient details of methods and materials provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Comparative Genomics, Bioinformatics

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.