Data Article

The first complete mitochondrial genome data of the pygmy rabbit *Brachylagus idahoensis*, the world’s smallest leporid

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\section*{Abstract}
The pygmy rabbit *Brachylagus idahoensis* (Merriam, 1891) is the smallest extant leporid, which naturally occurs in the Great Basin and adjacent areas in western parts of the United States of America. Its distribution is strongly associated with the sagebrush (*Artemisia* ssp.) vegetation. Here we present, for the first time, the complete mitochondrial genome of *Brachylagus idahoensis*, de novo assembled from Illumina short reads of fragmented probe-enriched DNA. The circular mitogenome is 17,021 bp in length and contains 13 protein-coding genes (PCGs), two ribosomal RNAs (16S rRNA and 12S rRNA), 22 transfer RNA genes, and a control region. The gene NAD6 and the tRNA(Gln), tRNA(Ala), tRNA(Asn), tRNA(Cys), tRNA(Tyr), tRNA(Ser), tRNA(Glu) and tRNA(Pro) are encoded on the light strand while the rest are encoded on the heavy strand.

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strand. The overall nucleotide composition was 30.78% for A, 28.5% for T, 13.62% for G and 27.08% for C. The mitogenome data are available in the GenBank under the accession number OL436257.

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### Specifications Table

| Subject | Genomics |
|---------|----------|
| Specific subject area | Mitogenomics |
| Type of data | Mitogenome sequence data in FASTA file format, tables, mitogenome map in figure format (.jpg), phylogenetic tree in figure format (.jpg) |
| Supplementary information | Supplementary information (.zip) contains the following documents: S1: List of taxa and corresponding accession numbers used to design baits for target enrichment (.xls) S2: Assembled mitogenome sequence data in FASTA file format (.fasta) S3: Alignment file for phylogenetic analysis in PHYLIP file format (.phy) S4: Gene partition file for phylogenetic analysis in NEXUS file format (.nex) S5: Phylogenetic tree in NEWICK file format (.newick) |
| How the data were acquired | Target enrichment; Illumina NextSeq 550 high-throughput sequencing |
| Data format | Raw and analyzed |
| Description of data collection | Genomic DNA was extracted with Qiagen DNeasy Blood & Tissue Kit (Valencia, CA, USA); double-indexed and double-stranded library preparation; mitogenome enrichment with myBaits Custom 20–40 K (Daicel Arbor Biosciences, USA); sequencing: Illumina NextSeq 550 platform; mitogenome assembled de novo in NOVOPlasty v.4.3.1 and annotated in MITOS2 web server. The circular mitogenome map was drawn using OGDRAW. Phylogenetic relationships were inferred using IQ-TREE |
| Data source location | This individual was collected from Christmas Valley, Lake County, OR, USA and is preserved under the voucher number UWBM 82570 at the Burke Museum, University of Washington, WA, USA |
| Data accessibility | The mitogenome data are available in the GenBank under the accession number OL436257 (https://www.ncbi.nlm.nih.gov/nuccore/OL436257) and Mendeley data repository (http://dx.doi.org/10.17632/g799t3s5s9.1) [1]. Raw sequence data are available in Sequence Read Archive (BioProject: PRJNA839569, BioSample: SAMN28539370; http://www.ncbi.nlm.nih.gov/bioproject/839569) |

### Value of the Data

- The mitogenome will be useful in conservation studies of North American mammalian diversity.
- The data will be useful for monitoring of potential changes in the range of the species.
- The data will contribute to our knowledge on species diversification and the micro-evolution of extant leporids, in particular the North American *Brachylagus–Sylvilagus* complex.
- The data generated will be useful in research on hybridization in Lagomorpha.

#### 1. Data Description

The pygmy rabbit, *Brachylagus idahoensis* (Merriam, 1891) is the world’s smallest leporid from North America, and belongs to a monotypic genus. This rabbit occurs in the Great Basin and adjacent intermontane areas from western Wyoming (easternmost), southwestern Oregon (westernmost), southwestern Montana (northernmost) to southwestern Utah (southernmost) [2,3].
Table 1
Mitogenome sequence data of Brachylagus idahoensis specimen (UWBM 82570).

| Metric                          | Value  |
|--------------------------------|--------|
| Total reads obtained           | 363,630|
| Total mapped reads             | 46,294 |
| Mapped paired reads            | 27,185 |
| Mean Coverage (x)              | 266.0656|
| Mean Mapping Quality           | 58.98  |

The species is adapted to semiarid sagebrush habitat and it feeds mostly on the sagebrush (Artemisia ssp.), especially during winter. B. idahoensis is considered Least Concern (LC) on the IUCN Red List of Threatened Species [3]. It is the only true fossorial rabbit in North America, digging extended burrow systems [2]. The species is important for reconstruction of the lagomorph phylogeny and evolution, especially for the history of the divergence within the Leporidae family [4], as it shows an array of derived features in the skull and dentition. Furthermore, it is important species for landscape genetics [5] and climate dynamics as an indicator species. Its strong habitat dependence can be used to monitor the subtle climate changes, correlated with changes in humidity and thus, contraction or expansion of the pygmy rabbit habitats [6].

Here we report the first complete mitogenome of Brachylagus idahoensis, which is 17,021 bp in length (GenBank No. OL436257). The sequenced mitogenome data are summarized in Table 1. The mitogenome comprises two ribosomal RNAs (16S rRNA and 12-S rRNA), 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, and a noncoding control region (D-loop). The arrangement of these 37 genes encoded on either the heavy (H) or the light (L) strand is presented in Fig. 1. The total length of PCGs is 11,389 bp, transcribing 3796 amino acids, which accounts for 66.91% of the entire mitogenome. The gene NAD6 and the tRNA(Gln), tRNA(Ala), tRNA(Asn), tRNA(Cys), tRNA(Tyr), tRNA(Ser), tRNA(Glu) and tRNA(Pro) are encoded on the light strand while rest are encoded on the heavy strand (Fig. 1; Table 2). The overall nucleotide composition is estimated as 30.78% for A, 28.5% for T, 13.62% for G and 27.08% for C.

The phylogenetic inference is limited by the incomplete taxon sampling due to the unavailability of mitogenomes of other leporid species. Although the phylogenetic position of B. idahoensis appears resolved in the tree (Fig. 2; bootstrap value=100), the precise relationship within the Leporidae cannot be ascertained due to the many species lacking. However, the description of this novel mitogenome is a crucial input to lagomorph evolutionary studies.

2. Experimental Design, Materials and Methods

2.1. Biological Sample

An individual of Brachylagus idahoensis was collected on August 23, 2011 in Christian Valley, Lake County, OR, USA. It was preserved in 100% ethanol at the collection of the Burke Museum, University of Washington, WA, USA (UWBM 82570; tissue number JEB1781; https://www.burkemuseum.org/collections-and-research/biology/mammalogy/collections-database/search.php). A sample of muscle tissue was obtained by ZM and UO from the museum collection and transferred to AS for genomic analyzes. Total genomic DNA was extracted from the tissue with Qiagen DNeasy Blood & Tissue Kit (Cat#69-504; Valencia, CA, USA) following the manufacturer’s protocol. The extracted DNA was subjected to spectrophotometric quantification, followed by fragmentation in M220 ultrasonicator (Covaris) and size-selection with magnetic beads (1.5X) to remove fragments <300 bp.

Next, an input of ~200 ng size-selected DNA was used as a template for the preparation of double indexed, double-stranded DNA library based on the protocol of Meyer and Kircher [7]. The library (~150 ng) was further enriched for the mitogenome following the manufacturer’s protocol, using a total of 28,756 unique custom designed RNA probes with 3 × tilling that were based on mitogenomes of 71 species representing all five extant orders of Euarchontoglires,
obtained from GenBank (Supplementary file S1.xls; list of species, taxonomic classification and corresponding accession numbers). The custom-designed probes were included in a myBaits Custom 20–40 K kit (Cat#300-248.V5 201119–92; Daicel Arbor Biosciences, USA). Libraries were pooled and sequenced on Illumina NextSeq 550 using 2 × 75 bp mode.

2.2. Complete Mitogenome Generation

Raw reads were demultiplexed using bcl2fastq (Illumina), adapters and low-quality bases were trimmed and overlapping reads were collapsed using AdapterRemoval v2 [8]. The mitogenome was de novo assembled in NOVOPlasty v.4.3.1 [9] with default parameters and kmer
Table 2
Mitogenome features of *Brachylagus idahoensis* (GenBank accession number OL436257; BioProject: PRJNA839569; BioSample: SAMN28539370). Protein coding genes (PCGs) are represented in bold letters and the genes encoded on light (L) strand are italicized.

| Gene      | Start | End  | Size (bp) | Amino acid length | Strand |
|-----------|-------|------|-----------|-------------------|--------|
| tRNA(Phe) | 1     | 70   | 70        |                   | H      |
| 12S rRNA  | 70    | 1029 | 960       |                   | H      |
| tRNA(Val) | 1030  | 1095 | 66        |                   | H      |
| 16S rRNA  | 1096  | 2678 | 1583      |                   | H      |
| tRNA(Leu) | 2678  | 2754 | 77        |                   | H      |
| NAD1      | 2756  | 3710 | 955       | 318               | H      |
| tRNA(Ile) | 3710  | 3780 | 71        |                   | H      |
| tRNA(Gln) | 3777  | 3848 | 72        | L                 |        |
| tRNA(Met) | 3856  | 3924 | 69        |                   | H      |
| NAD2      | 3925  | 4968 | 1044      | 348               | H      |
| tRNA(Trp) | 4975  | 5041 | 67        |                   | H      |
| tRNA(Ala) | 5043  | 5109 | 67        | L                 |        |
| tRNA(Asn) | 5110  | 5182 | 73        | L                 |        |
| rep_origin| 5182  | 5224 | 43        |                   |        |
| tRNA(Cys) | 5214  | 5281 | 68        |                   | L      |
| tRNA(Tyr) | 5282  | 5347 | 66        |                   | L      |
| COX1      | 5356  | 6897 | 1542      | 514               | H      |
| tRNA(Ser) | 6900  | 6968 | 69        | H                 |        |
| tRNA(Asp) | 6972  | 7040 | 69        |                   | H      |
| COX2      | 7041  | 7724 | 684       | 228               | H      |
| tRNA(Lys) | 7727  | 7799 | 73        |                   | H      |
| ATP8      | 7800  | 8006 | 207       | 69                | H      |
| ATP6      | 7961  | 8641 | 681       | 227               | H      |
| COX3      | 8641  | 9424 | 784       | 261               | H      |
| tRNA(Gly) | 9425  | 9495 | 71        |                   | H      |
| NAD3      | 9495  | 9841 | 347       | 116               | H      |
| tRNA(Arg) | 9842  | 9908 | 67        |                   | H      |
| NAD4-L    | 9910  | 10,206 | 297     | 99                | H      |
| NAD4      | 10,200 | 11,577 | 1378     | 459               | H      |
| tRNA(His) | 11,578 | 11,646 | 69     |                   | H      |
| tRNA(Ser) | 11,647 | 11,705 | 59     |                   | H      |
| tRNA(Leu) | 11,706 | 11,775 | 70     |                   | H      |
| NAD5      | 11,776 | 13,584 | 1809    | 603               | H      |
| NAD6      | 13,581 | 14,101 | 521    | 174               | L      |
| tRNA(Glu) | 14,102 | 14,169 | 68     |                   | L      |
| COB       | 14,173 | 15,312 | 1140   | 380               | H      |
| tRNA(Thr) | 15,312 | 15,377 | 66     |                   | H      |
| tRNA(Pro) | 15,378 | 15,443 | 66     |                   | L      |
| Control region | 15,444 | 17,021 | 1578 | |

The value of 23 to reproduce the candidate mitogenome. The sequence assembled by NOVOPlasty was used as a reference for mapping using BWA-MEM [10]. Duplicates and reads with low mapping quality (mapQ<30) were removed using samtools [11]. Number of unique mapped reads, mean coverage and mapping quality of the assembled genome was estimated by Qualimap 2 [12], followed by a manual checkinTablet software [13]. The mitogenome was annotated on the MITOS2 web server ([14]; http://mitos2.bioinf.uni-leipzig.de/index.py). The annotations for individual PCG’s start and stop codons were manually confirmed by nucleotide BLAST analysis. The circular mitogenome map was drawn using OGDRAW [15].

To reconstruct the mitogenomic phylogeny of available leporid species we used an alignment of eight closely related Leporidae taxa and two outgroup taxa (*Cavia porcellus* MT017565, a rodent, and the ochotonid lagomorph *Ochotona princeps* NC_005358), aligned with MAFFT [16] and subsequently removed ambiguously aligned sites in GBLOCKS [17,18]; http://molevol.cmima.csic.es/castresana/Gblocks_server.html). A Maximum-Likelihood (ML) based phylogeny (Fig. 2) was
inferred from the resulting alignment (15,999 bp) on IQ-TREE web server ([19]; http://iqtree.cibiv.univie.ac.at/), simultaneously using the model selection (auto) algorithm [20] and ultrafast bootstrap method [21] for 1000 iterations.

Ethics Statement

This study is based on non-living animal individuals, the only tissue sample has been collected from a museum specimen. Therefore, no ethic statement is required.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

*Brachylagus idahoensis* complete mitogenome sequence (Original data) (GenBank).
CRediT Author Statement

Anwesha Saha: Methodology, Software, Investigation, Formal analysis, Writing – original draft; Mateusz Baca: Methodology, Software, Writing – review & editing, Project administration; Danijela Popović: Methodology, Data curation; Zeinolabedin Mohammadi: Resources, Validation, Writing – review & editing; Urban Olsson: Resources, Writing – review & editing; Lucja Fostowicz-Frelik: Conceptualization, Visualization, Supervision, Funding acquisition, Writing – review & editing.

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Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.dib.2022.108314.

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