

First mitochondrial genome of the Amazonian marsh rat *Holochilus sciureus* Wagner 1842 (Rodentia, Cricetidae, Sigmodontinae)

Susette Castañeda-Rico^{a,b,c} , Jesús E. Maldonado^{a,b,c} , Robert C. Dowler^d  and Cody W. Edwards^{a,c} 

^aSmithsonian-Mason School of Conservation, Front Royal, VA, United States; ^bCenter for Conservation Genomics, Smithsonian National Zoo and Conservation Biology Institute, Washington, DC, United States; ^cDepartment of Biology, George Mason University, Fairfax, VA, United States; ^dDepartment of Biology, Angelo State University, San Angelo, TX, United States

ABSTRACT

The Amazonian marsh rat, *Holochilus sciureus*, is a member of the subfamily Sigmodontinae, the second-largest subfamily of murid rodents, with 410 species and ca. 84 genera in 12 tribes. This semiaquatic rodent is distributed in South America and is of great economic and epidemiological importance. In this study, we obtained the first mitochondrial genome of the genus *Holochilus* obtained from a tissue sample associated with a museum voucher specimen. The generated mitogenome sequence of *H. sciureus* is 16,358 bp length. It comprises a control region and a conserved set of 37 genes encoding for 2 rRNA genes, 22 tRNA genes and 13 protein-coding genes. We conducted a phylogenetic analysis that included *H. sciureus* and the only five other published mitochondrial genomes of this poorly studied subfamily of rodents.

ARTICLE HISTORY

Received 7 April 2022
Accepted 22 July 2022

KEYWORDS

Holochilus; mitogenome;
museum specimens;
Sigmodontinae;
South America

Introduction

The genus *Holochilus*, a sigmodontine genus of large, herbivorous, and semiaquatic rodents, is broadly distributed and inhabits several environments throughout South America (Musser and Carleton 2005). *Holochilus sciureus* is distributed in the lowlands of the Guianas, Peru, Bolivia, and Brazil (D'Elía et al. 2015; Prado et al. 2021b). It is considered a member of a species complex, whose taxonomy and phylogenetic relationships have undergone several changes including the composition and names of species (Prado et al. 2021a; 2021b). *H. sciureus* has an economic and epidemiological importance since it is considered a pest in rice and sugar cane fields (Eiris and Barreto 2009) and as a sentinel species as it is a host for parasites causing severe human diseases (da Silva-Souza et al. 2019).

In this study, we sequenced the first publicly available mitogenome of the genus *Holochilus* from a tissue sample, on loan, of *H. sciureus* associated with a museum voucher specimen, both deposited at the Museum of Vertebrate Zoology, University of California, Berkeley with catalog number MVZ:Mamm:190356 (<https://mvz.berkeley.edu/mvzmamm/>; Chris Conroy, ondatra@berkeley.edu). The specimen was collected in 1991 in Brazil, Amazonas, Eirunepé river Juruá (−6° 37' 59.88" S, −69° 52' 0.012" W).

We extracted DNA from a tissue sample using the DNeasy[®] Blood and Tissue Kit (QIAGEN, Inc., Valencia, CA, US). We quantified the DNA extraction using a Qubit[®] (Thermo Fisher) fluorometer with a 1x dsDNA HS assay kit. We used the single tube library preparation method

described by Carøe et al. (2018) and dual indexing PCR with TruSeq-style indices (Meyer and Kircher 2010) using Kapa HiFi HotStart Ready Mix (Roche Sequencing). We used the myBaits[®] UCE Tetrapods 5Kv1 kit (Arbor Biosciences) to perform target enrichment. We amplified the post-enrichment UCE library with 14 cycles of PCR using universal Illumina primers and Kapa HiFi Hotstart Ready Mix, and sequenced on a NovaSeq 6000 (Illumina, Inc., San Diego, CA, US) at the Oklahoma Medical Research Foundation, Oklahoma City (combined with samples from other projects).

We removed low-quality reads and adapter contamination using Trim Galore 0.6.5 (<https://github.com/FelixKrueger/TrimGalore>) and Prinseq-lite v0.20.4 (Schmieder and Edwards, 2011) to remove exact duplicates (−derep1,4). We mapped the cleaned reads to a reference (*Melanomys caliginosus* GenBank MH939287) using Geneious algorithm in Geneious Prime[®] 2021.2.2 with default parameters, and aligned using MAFFT 7.45 plug-in (Katoh and Standley 2013). To rule out the presence of nuclear copies of mitochondrial genes (NUMTs), we transferred annotations from the reference genome and translated all protein-coding genes (PCGs) to check for frame shifts or stop codons.

The generated complete mitogenome sequence of *H. sciureus* is 16,358 bp in length (GenBank accession number OL685394), which covers 100% of the reference genome sequence. The average sequencing depth was 1,700x with 238,757 reads mapped to the reference genome. The sequence contains the standard features present in a vertebrate mitochondrial genome including one non-coding

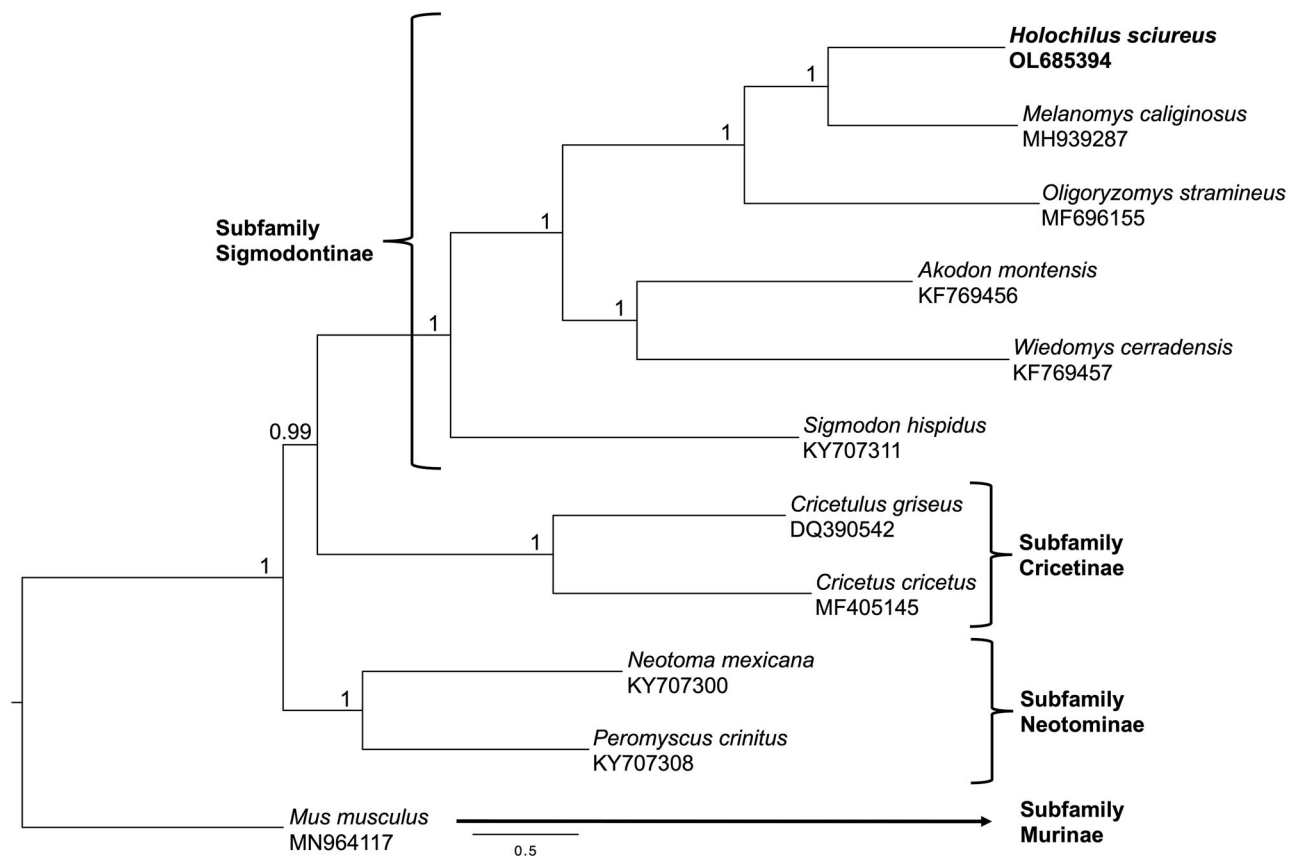


Figure 1. Bayesian Inference phylogeny of Sigmodontinae rodents using complete mitochondrial genomes. Nodal support is provided with posterior probabilities values.

control region, 2 rRNA genes (12S rRNA and 16S rRNA), 22 tRNA genes, and 13 PCGs including ones for NADH dehydrogenase (*ND1*, *ND2*, *ND3*, *ND4*, *ND4L*, *ND5* and *ND6*), ones from cytochrome c oxidase (*COX1*, *COX2*, and *COX3*), ATP synthase (*ATP6* and *ATP8*) and *cytochrome b* gene. The base composition was 34.2% A, 25.2% C, 12.3% G, 28.4% T and the GC content was 37.4%.

A Bayesian Inference (BI) analysis, including all the Sigmodontinae mitogenomes available in GenBank (accessed on 15 November 2021) and five outgroups, was performed using MrBayes 3.2.6 (Ronquist et al. 2012) under the best model and partition scheme (by gene and codon position) selected by PartitionFinder 2.1.1 (Lanfear et al. 2016). The analysis ran for 50 million generations sampling every 1,000 generations with a burn-in of 25%. Output parameters were visualized using Tracer v1.7.1 (Rambaut et al. 2018) to check for convergence between runs. The resulting phylogenetic tree was visualized using FigTree v1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). Our phylogenetic hypothesis based on a BI analysis supports the placement *H. sciureus* as sister to *M. caliginosus* and within a clade that includes the few other members of the Sigmodontinae sequenced to date (Figure 1).

Species delimitation has been challenging for the *H. sciureus* complex. Two recent studies conducted by Prado et al. (2021a; 2021b) using morphological and morphometric characters, and nuclear genomic data (Single Nucleotide Polymorphisms -SNPs) redefined the species boundaries within *H. sciureus* sensu Gonçalves et al. (2015) into three different species (*H. sciureus*, *H. nanus* and *H. oxe* spp. nov.).

However, phylogenetic analysis using complete mitochondrial genomes have shown to be invaluable for understanding the evolutionary history and taxonomy of several taxa (Duchêne et al. 2011; Abramson et al. 2021). Hence, we expect that the mitochondrial genome generated in this study will contribute to future genomic studies that aim to resolve the taxonomy and phylogenetic relationships within the genus *Holochilus* as well as adding to the few mitochondrial genomes available for the subfamily Sigmodontinae.

Acknowledgments

We would like thank the curators and collection support staff, especially Chris Conroy, at the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ), for the loaned tissue sample used in this study.

Ethics statement

Ethical review and approval was not required for this study because we used a museum specimen deposited at the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ). We obtained DNA from that museum sample formally loaned to RCD and we used published data available on GenBank for reference mitogenomes and to generate the phylogeny.

Author contributions

SC-R, JEM, RCD, and CWE designed and conceived the study. SC-R, JEM, and CWE secure funding. RCD performed the specimen sampling. SC-R

conducted the lab experiments, analyzed and archived the data, produced the figure, and wrote the manuscript with contributions from all coauthors. All authors read and approved the submitted version.

Disclosure statement

The authors report no conflicts of interest.

Funding

This project was funded by the Smithsonian-Mason School of Conservation, George Mason University and the Center for Conservation Genomics, Smithsonian National Zoo and Conservation Biology Institute.

ORCID

Susette Castañeda-Rico  <http://orcid.org/0000-0002-4301-3579>

Jesús E. Maldonado  <http://orcid.org/0000-0002-4282-1072>

Robert C. Dowler  <http://orcid.org/0000-0001-6268-7598>

Cody W. Edwards  <http://orcid.org/0000-0001-5912-6693>

Data availability statement

The mitochondrial genome sequence is available in GenBank at <https://www.ncbi.nlm.nih.gov/genbank/> under the accession number OL685394. The associated BioProject, BioSample and SRA numbers are PRJNA818347, SAMN26856163, and SRR18404174, respectively.

References

- Abramson NI, Bodrov SY, Bondareva OV, Genelt-Yanovskiy EA, Petrova TV. 2021. A mitochondrial genome phylogeny of voles and lemmings (Rodentia:Arvolidae): Evolutionary and taxonomic implications. *PLoS One*. 16(11):e0248198.
- Carøe C, Gopalakrishnan S, Vinner L, Mak SST, Sinding MHS, Samaniego JA, Wales N, Sicheritz-Pontén T, Gilbert MTP. 2018. Single-tube library preparation for degraded DNA. *Methods Ecol Evol*. 9(2):410–419.
- D'Elía G, Hanson JD, Mauldin MR, Teta P, Pardiñas UFJ. 2015. Molecular systematics of South American marsh rats of the genus *Holochilus* (Muroidea, Cricetidae, Sigmodontinae). *JMAMMAL*. 96(5):1081–1094.
- Duchêne S, Archer FI, Vilstrup J, Caballero S, Morin PA. 2011. Mitogenome phylogenetics: The impact of using single regions and partitioning schemes on topology, substitution rate and divergence time estimation. *PLoS One*. 6(11):e27138.
- Eiris GC, Barreto GR. 2009. Home range of marsh rats, *Holochilus sciureus*, a rodent pest in rice fields of Venezuela. *Interciencia*. 34(6):400–405.
- Gonçalves PR, Teta P, Bonvicino CR. 2015. Genus *Holochilus*. In Patton JL, Pardiñas UFJ, D'Elía G. (Eds.), *Mammals of South America*. University of Chicago Press, Chicago, Illinois p. 325–335.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. 30(4):772–780.
- Lanfear R, Frandsen P B, Wright AM, Senfeld T, Calcott, B. 2016. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol Biol Evol*. 34:772–773. <https://doi.org/10.1093/molbev/msw260>
- Meyer M, Kircher M. 2010. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb Protoc*. 2010(6):pdb.prot5448.
- Musser GG, Carleton MD. 2005. Superfamily Muroidea. In: Wilson DE, Reeder DA. (Eds.), *Mammal Species of the World. A Taxonomic and Geographic Reference*. Johns Hopkins University Press, Baltimore, p. 894–1531.
- Prado JR, Knowles LL, Percequillo AR. 2021a. New species boundaries and the diversification history of marsh rat taxa clarify historical connections among ecologically and geographically distinct wetlands of South America. *Mol Phylogenet Evol*. 155:106992.
- Prado JR, Knowles LL, Percequillo AR. 2021b. A new species of South America marsh rat (*Holochilus*, Cricetidae) from northeastern Brazil. *J Mammal*. gyab104:1–9.
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst Biol*. 67(5):901–904.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Syst Biol*. 61(3):539–542.
- Schmieder R, Edwards R. 2011. Quality control and preprocessing of metagenomic datasets. *Bioinformatics*. 27(6):863–864.
- Silva-Souza N d, Silva APdCd, Oliveira RMd, Lira MGS, Nogueira RA, Penha-Silva TAd, Melo SdA, Andrade FHed, Santos-Ribeiro LSD, Carvalho Neta AVd, et al. 2019. Parasitological and histological aspects of *Holochilus sciureus* naturally infected by *Schistosoma mansoni*. *Rev Bras Parasitol Vet*. 28(4):769–772.