

The mitochondrial genome of the endemic Brazilian paradoxical frog *Pseudis tocantins* (Hylidae)

Kaleb Pretto Gatto^a, Jeremiah J. Smith^b and Luciana Bolsoni Lourenço^a

^aLaboratory of Chromosome Studies, Department of Structural and Functional Biology, Institute of Biology, University of Campinas, Campinas, Brazil; ^bDepartment of Biology, College of Arts and Sciences, University of Kentucky, Lexington, USA

ABSTRACT

In this work, we present for the first time the mitochondrial genome of a paradoxical frog (*Pseudis tocantins*). This genome is 15.56 kb, excluding the control region, and is similar in gene content to other hylid mitogenomes. Maximum likelihood analysis, using the mitogenomes of several anurans, indicated *P. tocantins* as closely related to other hylid species.

ARTICLE HISTORY

Received 16 July 2018
Accepted 29 July 2018

KEYWORDS

Mitochondrion; mtDNA; Anura; next-generation sequencing; paradoxical frog

The paradoxical frog *Pseudis tocantins* Caramaschi and Cruz 1998 is endemic to Brazilian savannas associated with Araguaia and Tocantins river basins (Frost 2018). Interesting features of the paradoxical frogs are related to (1) their extended larval period that results in tadpoles attaining body sizes that are substantially larger than metamorphosed adults (Shaw 1802; Garda et al. 2010; Santana et al. 2016), and (2) the study of sex chromosome differentiation, as *P. tocantins* has highly heteromorphic sex chromosomes (Busin et al. 2008; Gatto et al. 2016). Here, we describe the complete mitochondrial genome of *P. tocantins*.

Total genomic DNA was isolated from the liver of a female *P. tocantins* collected from the type locality of this species (i.e. Porto Nacional, Tocantins state in Brazil – 10°44'38.6"S 48°26'09.2"W), using a standard phenol:chloroform extraction protocol (Sambrook et al. 1989). The remaining tissue is deposited in the tissue collection of the Laboratory of Chromosome Studies of the University of Campinas, Brazil (LabEsC – UNICAMP) under the accession number SMRP 190.15 and the specimen voucher is deposited at the amphibian collection of the Museu de Zoologia Professor Adão José Cardoso at the University of Campinas, Brazil, under the accession number ZUEC 22351. Permission for collecting the specimen was granted by SISBIO (process 45183) and Committee for Ethics in Animal Use of the University of Campinas (CEUA/UNICAMP) (process 3419-1).

A paired-end genomic library was prepared using Nextera DNA Flex Library Prep Kit (Illumina, EUA) and sequenced on an Illumina HiSeq X Ten (Hudson-Alpha Institute for Biotechnology, Alabama, USA). We detected *k*-mers ($k = 31$)

occurring at high frequencies among genomic reads using RepARK script (Koch et al. 2014) and default parameters. These high-frequency *k*-mers were then assembled by Velvet (Zerbino and Birney 2008) using default parameters. Genome annotation was performed using MITOS (Bernt et al. 2013). The mitochondrial genome contig was compared with available hylid mitochondrial genomes using BLAST (Altschul et al. 1990). The complete mitochondrial genome of *P. tocantins*, excluding control region, was used in a phylogenetic analysis in comparison with other 13 anurans species. Sequences were aligned using Muscle (Edgar 2004) and used for a Maximum likelihood analysis in RAxML v.0.4.1b (Stamakis 2014), under the GTR + G evolutionary model.

The assembled mitogenome of *P. tocantins* (GenBank accession number MH571152) spanned 15.56 kb, with a GC content of 43.16%. This genome is similar to those of other hylids in size, gene content, and arrangement, with an average nucleotide similarity of 70% when compared to six species from *Hyla* + *Dryophytes*. There are 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes. These genes are coded in the heavy strand, except for eight tRNA genes (tRNA-Pro, tRNA-Gln, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser, tRNA-Glu) and one protein-coding gene (ND6).

The resulting phylogenetic tree placed *P. tocantins* as the sister taxon of all extant hylid species analyzed in this paper (Figure 1). This result agrees with the large-scale phylogenetic inferences published thus far, as they place *Pseudis* as a member of the Hylidae family (Faivovich et al. 2005; Wiens et al. 2010; Pyron and Wiens 2011; Duellman et al. 2016).

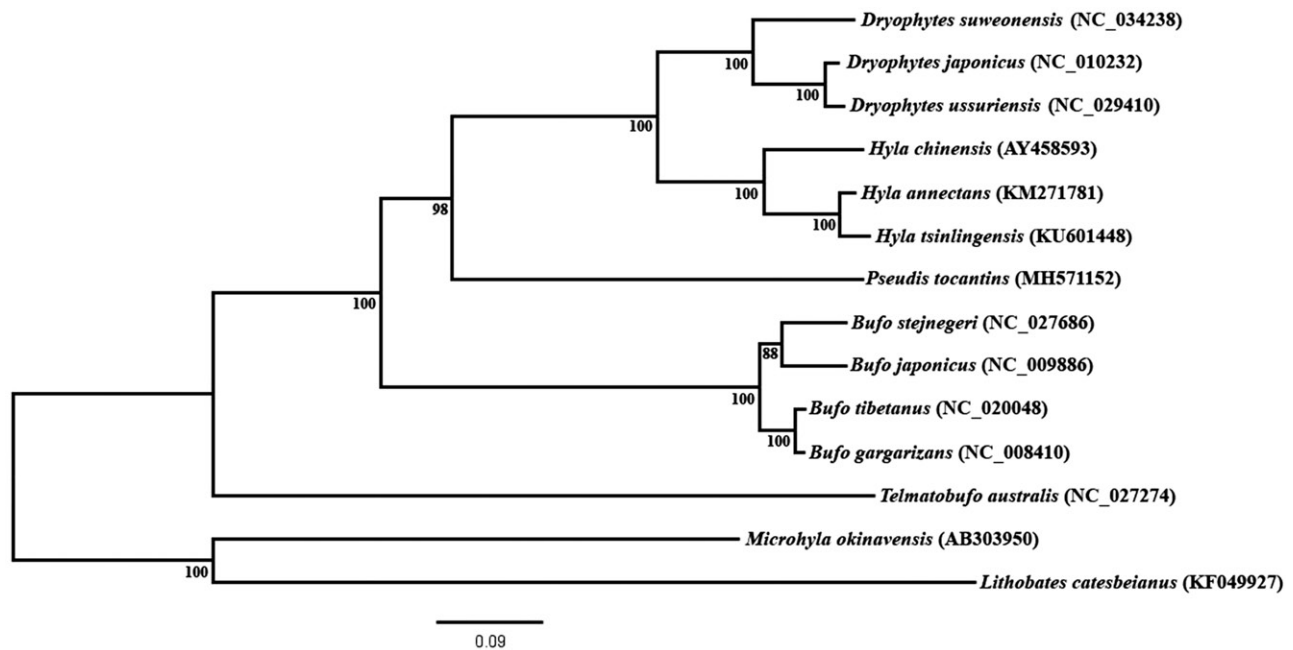


Figure 1. Phylogenetic inference obtained using maximum likelihood based on mitochondrial genomes, excluding control region, of the anuran species *Dryophytes japonicus*, *D. ussuriensis*, (as *Hyla ussuriensis* in Sun et al. 2017), *D. suweonensis* (as *H. suweonensis* in Lee et al. 2017), *Hyla chinensis*, *H. annectans*, *Hyla tsinlingensis*, *Pseudis tocantins*, *Bufo gargarizans*, *B. tibetanus* (as in Wang et al. 2013), *stejnegeri*, *B. japonicus*, *Telmatobufo australis*, *Microhyla okinavensis* and *Lithobates catesbeianus*. *M. okinavensis* and *L. catesbeianus* were used as outgroups. The phylogenetic inference was constructed under GTR + G evolutionary model. Bootstrap analysis was performed using 1000 pseudoreplicates for node support (numbers at the nodes).

Disclosure statement

No potential conflict of interest was reported by authors.

Funding

This work was supported by São Paulo Research Foundation (FAPESP) (#2014/23542-6). PhD studentships were provided by National Council for Scientific and Technological Development (CNPq) (140814/2015-9) and Coordination for the Improvement of Higher Education Personnel (CAPES) (PDSE 99999.006901/2015-08).

References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol.* 215:403–410.
- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Mol Phylogenet Evol.* 69:313–319.
- Busin CS, Andrade GV, Bertoldo J, Del Grande ML, Uetanabaro JA, Recco-Pimentel SM. 2008. Cytogenetic analysis of four species of *Pseudis* (Anura, Hylidae), with the description of ZZ/ZW sex chromosomes in *P. tocantins*. *Genetica.* 133:119–127.
- Caramaschi U, Cruz CAG. 1998. Notas taxonômicas sobre *Pseudis fusca* Garman e *P. bolbodactyla* A. Lutz, com a descrição de uma nova espécie correlata (Anura, Pseudidae). *Rev Bras Zool.* 15:929–944.
- Duellman WE, Marion AB, Hedges SB. 2016. Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arboranae). *Zootaxa.* 4104:1–109.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32: 1792–1797.
- Faivovich J, Haddad CFB, Garcia PCA, Frost DR, Campbell JA, Wheeler WC. 2005. Systematic review of the frog family Hylidae, with special reference to Hyliinae: phylogenetic analysis and taxonomic revision. *Bull Am Museum Nat Hist.* 294:1–240.
- Frost DR. 2018. Amphibian Species of the World 6.0: An Online Reference. New York: American Museum of Natural History; [accessed 2018 Jul 06]. <http://research.amnh.org/vz/herpetology/amphibia/>.
- Garda AA, Santana DJ, São-Pedro V. d A. 2010. Taxonomic characterization of paradoxical frogs (Anura, Hylidae, Pseudae): geographic distribution, external morphology, and morphometry. *Zootaxa.* 2666:1–28.
- Gatto KP, Busin CS, Lourenço LB. 2016. Unraveling the sex chromosome heteromorphism of the paradoxical frog *Pseudis tocantins*. *PLoS One.* 11:e0156176.
- Koch P, Platzer M, Downie BR. 2014. RepARK – de novo creation of repeat libraries from whole-genome NGS reads. *Nucleic Acids Res.* 42:e80.
- Pyron RA, Wiens JJ. 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Mol Phylogenet Evol.* 61:543–583.
- Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual. 2nd ed. New York (NY): Cold Spring Harbor Laboratory Press.
- Santana DJ, Magalhães F. de M, São-Pedro V. de A, Mângia S, Amado TF, Garda AA. 2016. Calls and tadpoles of the species of *Pseudis* (Anura, Hylidae, Pseudae). *Herpetol J.* 26:139–148.
- Shaw G. 1802. General zoology or systematic natural history. Volume III, Part 1. Amphibia. London (UK): Thomas Davison.
- Stamakis A. 2014. RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics.* 30: 1312–1313.
- Sun Q, Xie Y, Zhao W, Liu P. 2017. Sequencing and analysis of the complete mitochondrial genome of *Hyla ussuriensis* (Anura: Hylidae). *Mitochondr DNA part A.* 28: 328–329.
- Wang X, Wang Y, Yue B, Zhang X, Liu S. 2017. The complete mitochondrial genome of the *Bufo tibetanus* (Anura: Bufonidae). *Mitochondr DNA.* 24: 186–188.
- Wiens JJ, Kuczynski CA, Hua X, Moen DS. 2010. An expanded phylogeny of treefrogs (Hylidae) based on nuclear and mitochondrial sequence data. *Mol Phylogenet Evol.* 55:871–882.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* 18:821–829.