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The complete mitochondrial genome sequence of *Astyanax paranae* (Teleostei: characiformes)

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ABSTRACT

Complete mitochondrial genome of the characiform fish *Astyanax paranae* was characterized in the present study. The whole mitogenome was 16,707 bp long and consisted of 13 protein-coding genes, 22 tRNAs, 2 rRNAs genes, a control region and origin of light-strand replication. The gene order is similar to those of the congeneric blind cavefish *A. mexicanus*. The nucleotide content of *A. paranae* mitogenome was 29.5% for A, 27.6% for T, 15.8% for G, 27.1% for C. Nucleotide identity between *A. paranae* and *A. mexicanus* across all the 37 genic regions ranged from 74.9% (ND2) to 90.3% (COX3).

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Characidae; mitochondrial DNA; mtDNA; next generation sequencing

Astyanax is a specious characiform fish genus with a wide distribution throughout the Neotropical drainages, occurring from southern United States to central Argentina (Ornelas-García et al. 2008). This group is currently composed of 154 valid species (Eschmeyer 2015), but it does not represent a monophyletic entity, as evidenced by morphological and molecular characters (Javonillo et al. 2010; Mirande 2010; Oliveira et al. 2011). Additionally, the phylogenetic relationships in this group still remain confused due to the existence of 'species complexes', e.g. *A. scabripinnis*, *A. altiparanae* and *A. fasciatus* (Moreira-Filho & Bertollo 1991; Artoni et al. 2006; Castro et al. 2015) and putative cryptic species (Pansonato-Alves et al. 2013). Here we provided the complete mitochondrial genome of *A. paranae*, a species belonging to the *A. scabripinnis* complex. This is the second mitogenome available for the *Astyanax* genus after the *A. mexicanus* mitogenome.

The *A. paranae* specimen was collected at the Capivara river (22°53′57″S 48°23′11″W), Botucatu, São Paulo, Brazil. The specimen was identified and deposited in the fish collection of the Laboratório de Biologia e Genética de Peixes de Botucatu, São Paulo, Brazil, under the code LBP19572. Total genomic DNA from liver was extracted using a tissue kit (Macherey-Nagel, Düren, Germany), including a step of RNA removal with RNAse A (Invitrogen, Waltham, MA). Genomic DNA sequencing was performed at Life Sciences Core Facility (LaCTAD) of Universidade Estadual de Campinas (UNICAMP) using Illumina HiSeq2000 platform (2×101bp paired-end) (Illumina, San Diego, CA). The whole-genome sequencing generated 140,184,276 reads, from which 369,366 were used to assemble the mitogenome of *A. paranae*. The NGS reads obtained from Ilumina Sequencing were assembled using two methods. First, the MITObim mitogenome assembler (Hahn et al. 2013) was used taking the sequence of the almost complete *Astyanax mexicanus* mitogenome as a reference (accession number AP011982.1). We run MITObim with the two-step protocol performing an initial mapping with MIRA software (Chevreux et al. 2004) using the default parameters. An assembly with the '*de novo* assembly' option and with the 'reference-based genome assembly' option was performed as well. We generated a consensus sequence of the resulting contigs. The assembly was completed by selecting homologous reads pairs to 200bp sequences adjacent to the low-quality regions of the consensus sequence with custom script (https://github.com/fjruizruano/ngs-protocols/blob/master/mapping_blat_gs.py) and assembling them using the CAP3 software (Huang & Madan 1999) with default parameters. For annotation procedures, we compared the assembled mitogenome of *A. paranae* to the annotated mitogenome of *A. mexicanus* using Geneious Pro v8.1.5 software created by Biomatters (http://www.geneious.com/). To validate, the results were compared to those obtained using the MitoAnnotator software (Iwasaki et al. 2013). The final mitogenome of *A. paranae* was deposited in GenBank under the accession number KX609386.

The complete mitochondrial genome of *A. paranae* has 16,707bp and is composed by 13 PCGs, 22 transfer tRNAs, 2 ribosomal RNAs, a control region (D-loop) and origin of light-strand replication (O_L). The gene order follows the typical arrangement of vertebrates and the organization of the protein-coding genes (PCGs) of *A. paranae* mitogenome is very similar to the *A. mexicanus*. The A + T content in the *A. paranae* mitogenome is 57.1%, and its overall base composition is 29.5% for A, 27,6% for T, 15.8% for G, 27.1% for C. Most of the

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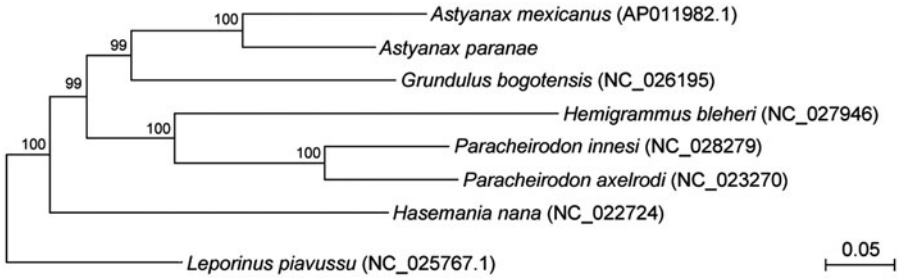

Figure 1. Maximum likelihood tree showing the phylogenetic relationships between the *A. paranae* mitogenome and all mitogenomes available for Characidae species, using as external group Anostomidae species.

genes were encoded on the H-strand, except for tRNA-Gly, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser, ND6, tRNA-Glu and tRNA-Pro. The putative O_L is composed by 33 bp (from 5334 to 5366 nucleotide) between tRNA-Asn and tRNA-CysA. The putative control region (D-loop) has 1032 bp (from 15,676 to 16,707) between tRNA-Pro and tRNA-Phe.

In addition, we studied the phylogenetic relationships between the *A. paranae* mitogenome and all mitogenomes available for Characidae species, using as external group Anostomidae species. The 13 protein-coding genes were extracted and aligned with the Muscle algorithm (Edgar 2004) implemented in the Geneious software. We used the resulting alignment to build a maximum likelihood tree using PhyML (Guindon et al. 2010) with a GTR + I + G model and 1000 bootstrap replicates. In the phylogenetic analysis all nodes were strongly supported and the closer species with *A. paranae* was *A. mexicanus* as expected (Figure 1).

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Disclosure statement

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