MITOGENOME ANNOUNCEMENT

Next generation sequencing yields the complete mitochondrial genome of the Endangered Chilean silverside *Basilichthys microlepidotus* (Jenyns, 1841) (Teleostei, Atherinopsidae), validated with RNA-seq

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Abstract

The complete sequence of the mitochondrial genome for the Chilean silverside *Basilichthys microlepidotus* is reported for the first time. The entire mitochondrial genome was 16,544 bp in length (GenBank accession no. KM245937); gene composition and arrangement was conformed to that reported for most fishes and contained the typical structure of 2 rRNAs, 13 protein-coding genes, 22 tRNAs and a non-coding region. The assembled mitogenome was validated against sequences of COI and Control Region previously sequenced in our lab, functional genes from RNA-Seq data for the same species and the mitogenome of two other atherinopsid species available in Genbank.

Basilichthys microlepidotus is an atherinopsid endemic to Chile that inhabits lakes and rivers from 28°S to 39°S in Chile (Véliz et al., 2012). It is an endangered species (Campos et al., 1998), although it has been found surviving in highly polluted rivers (Vega-Retter et al., 2014). This species is microphagous, feeding on insect larvae, small invertebrates, filamentous algae and detritus (Bahamondes et al., 1979; Duarte et al., 1971); its reproductive period ranges from August to January (Comte & Vila, 1992). Here we report the sequence of the entire mitochondrial genome of *Basilichthys microlepidotus*.

A specimen was collected in the Maipo River basin (33°46′S; 70°43′W) in October, 2012 and identified using morphological and genetic criteria (following Véliz et al., 2012). A fin clip was stored in 95% ethanol, and total genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI). The mitogenome of *B. microlepidotus* was assembled from one 454 sequencing (NGS) run on a Roche 454 GS Junior. About 23,024,550 bases were sequenced, yielding 82,789 sequences with an average read length of 278.11 bp.

A *de novo* assembly was performed, trimming low quality sequences in the 5′ and 3′ ends with the software Geneious v7.1.5 (Biomatters Ltd., Aucklans, New Zealand). Novel assembly (GenBank accession no. KM245937) was mapped against the complete mitochondrial genome of *Odontesthes* sp. and *Menidia menidia* (Setiamarga et al., 2008; GenBank Accession Numbers: AB370894 and AB370893). The proposed mtDNA assembly was validated using two regions sequenced previously by our research group. The first was the control region (912 bp), sequenced for 351 individuals (Quezada-Romegialli et al., 2010; Genbank Accession Numbers: GQ178157 to GQ178214, FJ843108 to FJ843127 and FJ380093 to FJ380105); the second

Table 1. The genomic organization of mitochondrial genes in *B. microlepidotus*.

Gene	Direction	From	To	Size (bp)	Start	Stop
tRNA-Phe	+	1	69	69		
12S rRNA	+	70	1010	941		
tRNA-Val	+	1011	1083	73		
16S rRNA	+	1099	2789	1691		
tRNA-Leu	+	2790	2863	74		
ND1	+	2864	3838	975	ATG	TAA
tRNA-Ile	+	3843	3912	70		
tRNA-Gln	−	3912	3982	71		
tRNA-Met	+	3982	4050	69		
ND2	+	4036	5097	1062	ATC	TAG
tRNA-Trp	+	5096	5167	72		
tRNA-Ala	−	5169	5237	69		
tRNA-Asn	−	5239	5311	73		
tRNA-Cys	−	5350	5415	66		
tRNA-Tyr	−	5416	5485	70		
CO1	+	5478	7037	1560	ATC	TAA
tRNA-Ser	−	7063	7133	71		
tRNA-Asp	+	7137	7208	72		
CO2	+	7209	7913	705	ATG	CTT
tRNA-Lys	+	7906	7979	74		
ATPase 8	+	7981	8148	168	ATG	TAA
ATPase 6	+	8124	8822	699	ATC	TAA
CO3	+	8822	9607	786	ATG	ATA
tRNA-Gly	+	9607	9677	771		
ND3	+	9678	10,028	351	ATG	TAG
tRNA-Arg	+	10,027	10,095	69		
ND4L	+	10,096	10,392	297	ATG	TAA
ND4	+	10,386	11,771	1374	ATG	AGA
tRNA-His	+	11,767	11,835	69		
tRNA-Ser	+	11,836	11,903	68		
tRNA-Leu	+	11,908	11,980	73		
ND5	+	11,981	13,810	1831	ATG	TAA

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(continued)

Table 1. Continued

Gene	Direction	From	To	Size (bp)	Start	Stop
ND6	−	13,817	14,359	536	ATC	T– –
tRNA-Glu	−	14,345	14,413	69		
Cytb	+	14,418	15,536	1137	ATG	AGA
tRNA-Thr	+	15,560	15,632	73		
tRNA-Pro	−	15,634	15,704	71		
Control region	+	15,705	16,543	839		

region was the COI gene (687 bp) amplified for 31 individuals (Véliz et al., 2012; Genbank Accession Numbers: FJ380197 to FJ380116). Both fragments showed 100% alignment to the expected Control region and COI gene, respectively. Additionally, 34,385 contigs (14.46 million reads, 2.13 Gb) obtained from a *de novo* assembly performed with the sequences of an RNA-Seq performed in Ion Torrent and Ion Proton platform were mapped to the novel *Basilichthys* mitogenome, checking at the same time potential indels against depth and quality of NGS 454 reads.

Finally, the validations yielded a mitogenome assembly of 16,544 bp, similar in size to the *Odontesthes* sp. (16,527 bp) and the *Menidia menidia* mtDNA genomes (16,456 bp). Annotations were confirmed by comparing with Dual Organellar GenoMe Annotator (DOGMA) (Wyman et al., 2004) and MITOchondrial genome annotation Server (MITOS)-generated annotations (Bernt et al., 2013). The complete mitochondrial genome of the silverside *B. microlepidotus* has a gene order typical of fishes and it contains 22 tRNA genes, 13 protein-coding genes, two rRNA genes and a non-coding control region (Table 1). The nucleotide composition was: A, 27.2%, T, 27.5%, G, 17.0% and C, 28.2%.

Acknowledgements

Declaration of interest

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