Mitochondrial DNA

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MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of the southern purple-spotted gudgeon *Mogurnda adspersa* (Perciformes: Eleotridae) through pyrosequencing

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Abstract

The complete mitochondrial genome of the Critically Endangered southern purple-spotted gudgeon, *Mogurnda adspersa*, was determined for the first time using 1/8 of a 454 pyrosequencing plate. The mitogenome was assembled using the bioinformatic software MIRA. The *M. adspersa* genome organization was very similar to most vertebrates, being 16,523 bp in length. It contained 13 protein-coding genes, 22 transfer RNA, 2 ribosomal RNA genes, and 1 non-coding control region. The current methodology was effective to assemble the whole mitogenome using 2008 mitochondrial reads representing 0.23% of total genomic reads produced and providing average mitogenome coverage of 17.4 reads per site. The whole mitogenome sequence provided here is deposited in NCBI with the accession number KJ130031 and may benefit systematics studies and conservation programs of *M. adspersa*.

Keywords

Fish, mitogenome, next-generation-sequencing

History

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Introduction

The advent of next-generation sequencing associated with the advance of bioinformatics tools is allowing the quick production of partial animal genomes and population genetic markers, such as microsatellites and whole mitogenomes (Mardis, 2008). Here we present the complete mitogenome of the southern purple-spotted gudgeon, *Mogurnda adspersa*, a small freshwater fish native to southeastern Australia. This taxon was once widespread in the Murray–Darling Basin (MDB), but its distribution declined dramatically in the last 30 years and the species is currently classified as Critically Endangered in the lower MDB (Hammer et al., 2013). The lower MDB population is a relatively divergent lineage that appears isolated from other populations of the species and represents a separate Evolutionarily Significant Unit (Faulks et al., 2008).

The complete mitogenome of *M. adspersa* may benefit further phylogenetic and evolutionary studies, which are essential for the ongoing conservation efforts to manage this endangered species.

Materials and methods

A specimen of *M. adspersa* was collected in the lower MDB (35.05°S, 139.32°E). Genomic DNA was extracted from muscle samples using a modified salting-out method (Sunnucks & Hales, 1996). Extracted DNA was nebulized and entered the 454 protocol for sequencing in 1/8 of a 70-9-75 PicoTiterPlate using the Roche GS FLX (454) system (Margulies et al., 2005).

Sequencing reads in FASTA format were automated, trimmed and screened for a number of contaminants, low quality and low-complexity sequences using Seqclean script (Pertea et al., 2003).

The MIRA software (Chevreux et al., 1999) was run using default parameters for ''de novo assembly''. The preliminary mitogenome assembly was used as input for the algorithm MITObim (Hahn et al., 2013) which performs MIRA iterations and improves the assembly. The MITObim assembled mitogenome was visualized using Tablet (Milne et al., 2013) to check for the genome coverage.

Mitogenome annotation was performed using both the Mitos WebServer (Bernt et al., 2013) and MitoFish (Iwasaki et al., 2013) software. Careful manual annotation was conducted using the Artemis software (Carver et al., 2012) aided by BLAST searches (Altschul et al., 1997) to confirm gene boundaries. Transfer RNA predictions were confirmed using tRNAscan-SE software (Lowe & Eddy, 1997). Ribosomal RNA annotation was estimated through nucleotide sequence alignments with related species.

Results and discussion

The complete mitochondrial genome of *M. adspersa* is 16,523 bp in length and contains 13 protein-coding genes, 22 tRNAs, 2 rRNAs, and 1 non-coding control region (Table 1). This organization is identical to other teleosts mitogenomes (Prosdocimi et al., 2012; Song et al., 2012; Zhang et al., 2013). From a total of 883,035 pyrosequencing reads, 2008 (0.23%) were used to assemble the organelar genome, providing a coverage of 17.4×. Evidence of mitogenome circularization was found since

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Table 1. Mitochondrial genome characteristics of *M. adspersa*.

	Position		Size		Codon			
Gene	From	To	Nucleotide (bp)	Aminoacid	Start	Stop	Intergenic nucleotide	Strand
tRNA(Phe)	1	68	68				0	H
12S rRNA	69	1019	951				0	H
tRNA(Val)	1020	1099	80				−7	H
16S rRNA	1093	2775	1683				0	H
tRNA(Leu)	2776	2850	75				0	H
ND1	2851	3822	972	324	ATG	TAG	6	H
tRNA(Ile)	3829	3898	70				−2	H
tRNA(Gln)	3897	3967	71				−1	L
tRNA(Met)	3967	4035	69				0	H
ND2	4036	5079	1044	348	ATG	TAA	3	H
tRNA(Trp)	5083	5153	71				2	H
tRNA(Ala)	5156	5224	69				1	L
tRNA(Asn)	5226	5298	73				36	L
tRNA(Cys)	5335	5400	66				0	L
tRNA(Tyr)	5401	5471	71				1	L
COX1	5473	7023	1551	517	GTG	TAA	3	H
tRNA(Ser)	7027	7097	71				3	L
tRNA(Asp)	7101	7172	72				5	H
COX2	7178	7867	690	230	ATG	T−−	1	H
tRNA(Lys)	7869	7943	75				1	H
ATP8	7945	8109	165	55	ATG	TAA	−7	H
ATP6	8103	8783	681	227	ATG	TAA	2	H
COX3	8786	9568	783	261	ATG	TA−	1	H
tRNA(Gly)	9570	9641	72				0	H
ND3	9642	9989	348	116	ATG	TAG	1	H
tRNA(Arg)	9991	10,059	69				0	H
ND4L	10,060	10,353	294	98	ATG	TAA	−4	H
ND4	10,350	11,729	1380	460	ATG	T−−	1	H
tRNA(His)	11,731	11,799	69				0	H
tRNA(Ser)	11,800	11,867	68				12	H
tRNA(Leu)	11,880	11,952	73				0	H
ND5	11,953	13,791	1839	613	ATG	TAG	2	H
ND6	13,794	14,312	519	173	ATG	TAA	0	L
tRNA(Glu)	14,313	14,381	69				6	L
CYTB	14,388	15,527	1140	380	ATG	T−−	1	H
tRNA(Thr)	15,529	15,600	72				0	H
tRNA(Pro)	15,601	15,670	70				0	L
Control region	15,671	16,523	853					

the start and end regions of the MITObim initial assembly overlapped.

The described methodology proved to be effective to mitogenome assembly using sequences derived from Next Generation Sequencers such as 454, Illumina (San Diego, CA) and Ion Torrent (Life Technologies, Carlsbad, CA). Our results should contribute to improve the resolution of phylogenetic and phylogeographic studies and aid species identification via molecular barcoding.

Declaration of interest

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