Complete sequence determination and analysis of mitochondrial genome in *Oreonectes daqikongensis*

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MITOGENOME ANNOUNCEMENT

**ABSTRACT**

*Oreonectes daqikongensis* revealed that the complete length of its mitochondrial genome was 16,578 bp, composed of A (30.7%), T (25.6%), G (16.2%), C (27.5%) and A + T (56.3%). Its genetic constitution and arrangement were consistent with those of other Osteichthyes, including 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes, and 1 control area (D-loop). All genes were encoded by the H-strand, except for 1 protein-coding gene (ND6) and 8 tRNA genes (tRNA-Gln, tRNA-Ala, tRNA-Cys, tRNA-Asn, tRNA-Tyr, tRNA-Ser, tRNA-Glu and tRNA-Pro) are encoded by the L-strand. The mitochondrial genomes were arranged very closely. There were four gene overlapping regions, with an overall length of 21 bp and a base covering number range of 1–14 bp, and 23 intergenic regions, with an overall length of 147 bp and an intergenic length range of 1–19 bp. There were 10 gene pairs that were neither overlapping nor intergenic.

**Introduction**

Oreonectes belongs to the Subfamily Nemacheilinae of the Family Balitoridae, which are found only in southwest China and northern Vietnam (Zhu 1989; Du et al. 2008). Balitoridae and their phylogenetic analyses are very important for studying the environmental adaptability of freshwater fishes (Doadrio & Perdices 2005; Perdices et al. 2012). In this study, complete sequences of mitochondrial DNA of *Oreonectes daqikongensis* were tested. The specimen was collected from a subterranean river of the Daqikong area (25°17′05.1″N, 107°44′54.3″E), Libo county of China in January 2011. It was stored in the animal specimen room of the School of Life Sciences, Guizhou Normal University, Guiyang, China.

**Method**

Total DNA was extracted from the fish muscle tissues and 26 pairs of primers were used to amplify genomic DNA. Then, complete mitochondrial genome was submitted into GenBank (Accession no. KU987436)

Complete mitochondrial DNA sequences of 7 genus 31 species of Nemacheilinae were obtained from GenBank, from which Cytb protein-coding genes were extracted. The phylogenetic tree was established using the neighbor-joining (NJ) method, while reliability was tested using the Kimma2-Parameter method (Kimura 1980) in MEGA 6.0.

**Results and discussion**

The overall length of the mitochondrial genome of *O. daqikongensis* was 16,578 bp, including 22 tRNA genes, 2 rRNA genes, 13 protein-coding genes and 1 control area (D-loop). All genes were encoded by the H-strand, except for 1 protein-coding gene (ND6) and 8 tRNA genes (tRNA-Gln, tRNA-Ala, tRNA-Cys, tRNA-Asn, tRNA-Tyr, tRNA-Ser, tRNA-Glu and tRNA-Pro), which were encoded by the L-strand. There were four gene overlapping regions, with an overall length of 21 bp (tRNA-Ile and tRNA-Gln, ATP8 and ATP6, ND4L and ND4, tRNA-Pho and D-loop), with a base covering number range of 1–14 bp, and there were 10 gene pairs that were neither overlapping nor intergenic, while there were 23 intergenic regions, with an overall length of 147 bp and an intergenic length range of 1–19 bp. The complete nucleotide sequence was composed of A (30.7%), T (25.6%), G (16.2%), C (27.5%) and A + T (56.3%), and had a certain degree of AT (66.2%) bias in the D-loop region.

The NJ phylogenetic tree established using Cytb gene sequence at the genus level is shown in Figure 1. *O. daqikongensis* was closer genetically to *O. shuilongensis* with a high bootstrap value (BP = 99), than clustered with *O. platycephalus* (BP = 73) supporting the inclusion of *O. daqikongensis* in *Oreonectes*.

Gene sequences in different segments of mitochondrial DNA may have different evolutionary rates (Guo et al. 2004). The ND and Cytb genes are more reliable to establish phylogenetic relationships for class group with further kinship (Zardoya & Meyer 1996). In this study, we used Cytb genes though NJ method to build phylogenetic tree. The results revealed that the fish of Nemacheilinae were divided into two branches. One branch is *Oreonectes, Schistura, Homalota, Paracobitis* and *Nemacheilus*, which is distributed in the low altitude area. Another branch is *Triplophysa* fishes distributed in the high elevation area. This may be due to the uplift of Qinghai-Tibet plateau which makes the geographical isolation and form different species.
Figure 1. NJ tree established using CytB gene sequence. Genbank accession number for the published sequences are Oreonectes shuiiongensis (KF640641), Oreonectes platycephalus (DQ105197), Oreonectes daqikongensis (KU987436), Schistura fasciolata (HM010565), Schistura shuangjiangensis (JN837651), Schistura desmotes (GQ174368), Schistura callichroma (JN837652), Schistura latifasciata (JN837653), Schistura bucculenta (JN837654), Schistura macrotaenia (JN837655), Schistura amplizona (JN837656), Schistura cryptofasciata (JF340401), Schistura sikmaensis (JF340405), Schistura poculi (JF340407), Schistura longa (JF340408), Homatula pycnolepis (KF041000), Homatula acuticephala (HM010527), Homatula longidorsalis (HM010550), Homatula potanini (JF340388), Homatula anguillioide (HM010582), Traccatichthys pulcher (JF340402), Triplophysa xiangxiensis (JN964407), Triplophysa stoliczkai (DQ105249), Triplophysa stoliczkai (EF212443), Triplophysa stoliczkai (FJ406605), Triplophysa stenura (JN837657), Triplophysa orientalis (DQ105251), Nemacheilus maysae (GQ174377), Nemacheilus ornatus (GQ174363), Nemacheilus pallidus (GQ174370).
Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding information

The data in this research comes from the database of National Digital-Museum of Animal Specimens; this project was supported by key project of Science-technology basic condition platform from The Ministry of Science and Technology of the People’s Republic of China (Grant No. 2005DKA21402) and Provincial Governor Fund of Guizhou (Qian-Sheng-Zhuan-He-Zi (2010) 14) project.

References

Doadrio I, Perdices A. 2005. Phylogenetic relationships among the Ibero-African cobitids (Cobitis, cobitidae) based on cytochrome b sequence data. Mol Phylogenet Evol. 37:484–493.

Du L, Chen X, Yang J-X. 2008. A review of the Nemacheilinae genus Oreonectes Günther with description of two new species (Teleostei: Balitoridae). Zootaxa. 1729:23–26.

Guo XH, Liu SJ, Liu Q, Liu Y. 2004. Progress of mitochondrial DNA in fish. J Genet Genom. 31:983–1000.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 16:111–120.

Perdices A, Vasil’ev V, Vasil’eva E. 2012. Molecular phylogeny and intraspecific structure of loaches (genera Cobitis and Misgurnus) from the far East region of Russia and some conclusions on their systematics. Ichthyol Res. 59:113–123.

Zardoya R, Meyer A. 1996. The complete nucleotide sequence of the mitochondrial genome of the lungfish (Protopterus dolloi) supports its phylogenetic position as a close relative of land vertebrates. Genetics. 142:1249–1263.

Zhu SQ. (1989). The loaches of the subfamily Nemacheilinae in China (Cypriniformes: Cobitidae). Nanjing (China): Jiangsu Science and Technology Publishing House.