Complete mitochondrial genome of Korean endemic species, *Iksookimia yongdokensis* (Actinopterygii, Cypriniformes, Cobitidae)

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**ABSTRACT**

*Iksookimia yongdokensis* (Actinopterygii, Cypriniformes, Cobitidae) is known as endemic freshwater fish species in Korea. The total length of *I. yongdokensis* mitogenome is 16,640 bp, consisting of 13 protein-coding genes (PCGs), 22 tRNAs, 2 rRNAs, and 1 control region (D-loop). The gene arrangement and content were identical with previously recorded mitogenomes of *Iksookimia* species. Phylogenetic analysis using PCGs and rRNA was examined by maximum likelihood method indicated that genus *Iksookimia* is taxonomically not stable status according to *Iksookimia* species and *Cobitis* species were grouped together.

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Genus *Iksookimia* was newly classified as endemic species which are well-known as high geographic variation (Nalbant 1993). Genus *Iksookimia* included a total of seven species, *Iksookimia koreensis*, *I. pacifica*, *I. longicorpa*, *I. yongdokensis*, *I. hugowolfeld*, *I. pumila*, and *I. choii* (Kim 1997, 2009) and the complete mitogenome revealed only three species (Kim et al. 2008; Yu et al. 2016). Here, we first report the complete mitogenome of *I. yongdokensis* (MNS581868) for contributing a phylogenetic study of genus *Iksookimia*.

A specimen was collected in Ulsan, South Korea (35°45'38.03"N, 128°59'05.05"E) on 1 July 2019. After collection, the specimen was immediately identified based on the morphological characteristics (Kim 2009). In laboratory steps, we isolated mitochondrial DNA (mt-DNA) using Qproteome Mitochondria Isolation Kit (Qiagen, Hilden, Germany) and DNeasy Blood and Tissue DNA isolation kit (Qiagen) from caudal fin. PCR product was amplified using mt-DNA by REPLI-g Mitochondrial DNA kit (Qiagen) and sequencing library was prepared by QIseq FX single cell DNA library kit (Qiagen). NGS analysis was conducted in GNC Bio Co. (Daejeon, South Korea) followed by sequencing on the Illumina Hi-Seq 2500 platform (San Diego, CA, USA). The specimen (NSMK-FI00002) and isolated mt-DNA (NSMK-DN00002) were stored in a freezer (−80°C) of storage in Natural History Laboratory of National Science Museum (Daejeon, Korea).

The complete mitogenome of *I. yongdokensis* (MNS581868) was 16,640 bp in length and contained 13 PCGs, 22 tRNA genes, 2 rRNA genes. The mitogenome of *I. yongdokensis* has the same gene order with other Cobitidae species, such as genus *Cobitis* and *Iksookimia*. The overall nucleotide compositions were 33.3% A, 26.1% T, 24.6% C, 15.9% G. All PCGs have initiation codons ‘ATG’, except for COI started with GTG. The seven PCGs (ND1, COX1, COX2, ATP8, ATP6, ND4, ND6) and ND5 have complete stop codon, TAA and TAG, respectively. The four PCGs (ND2, COX3, ND3, CytB) completed with incomplete stop codon ‘T–’, and ND5 has incomplete stop codon ‘TA–’.

To reveal the taxonomical relationship based on molecular phylogenetic analysis of *Iksookimia* species, which has included several species revised genus from *Cobitis*, 21 Cypriniformes species nucleotide dataset (13 PCGs and 2 rRNA) was included four *Cobitis* species and four *Iksookimia* species. Also, two species of order Siluriformes were used as outgroups (Slechtová et al. 2007). For ML analysis, the dataset was analyzed using PhyML 3.1 with GTR+I+G model estimating jModelTest 2.1.1 (Guindon and Gascuel 2003; Guindon et al. 2010). Bootstrap resampling was accomplished with 1000 iterations.

In ML tree, *I. yongdokensis* (MNS581868) was clearly distinguished from other *Iksookimia* and *Cobitis* species in Cobitidae clade (Figure 1). The family Cobitidae was grouped as a monophyletic clade and formed a larger clade with the families of Cypriniformes. The phylogenetic analysis showed genus *Iksookimia* and *Cobitis* were unseparated in Cobitidae. This result revealed they were unstable in taxonomic status. We expect the mitogenome sequence of *I. yongdokensis* in this study to contribute to establishing a taxonomical relationship between genus *Iksookimia* and *Cobitis*. 

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Figure 1. Phylogenetic tree of maximum likelihood (ML) method based on the nucleotide sequences of 13 PCGs and 2 rRNAs of 21 Cypriniformes species, included I. yongdokensis (MN581868), and two outgroup species belongs to order Siluriformes. Bootstrap support values are indicated on each node as >70.