

Complete mitochondrial genome of endangered *Rhodeus pseudosericeus* and its implications for the reconstruction of phylogenetic relationship among Acheilognathinae species

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ABSTRACT

Rhodeus pseudosericeus is a native bitterling to the Korean Peninsula and found in very limited areas with small census size. Here, its complete mitochondrial genome was analyzed to provide novel data for the reconstruction of phylogenetic relationship among Acheilognathinae species. The genome was a 16,574 bp long consisting of 1 putative control region, 2 rRNA genes, 22 tRNA and 13 protein-coding genes. The gene arrangement was completely identical to those observed in other Acheilognathinae species as well as in other cyprinid species. In our phylogenetic analyses, three major genera of Acheilognathinae independently formed monophyletic groups in the tree reconstructed based on the whole genome sequences, whereas *Rhodeus* was not recovered as a single monophyly when solely considering protein-coding genes, indicating that the taxonomic reevaluation is still required in this subfamily.

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Rhodeus pseudosericeus (Arai et al. 2001) is an endangered endemic species in South Korea belonging in Acheilognathinae (Cypriniformes, Cyprinidae). In a previous study, *R. pseudosericeus* had a sister relationship to the *R. sericeus-amarus* complex, indicating that Amur and European bitterlings originated from the dispersal of *R. pseudosericeus* or its ancestral lineage (Jeon & Suk 2014). Here, the complete mitogenome of *R. pseudosericeus* was analyzed to provide novel data for the reconstruction of phylogenetic relationship among Acheilognathinae. Genomic DNA was extracted from the caudal fin-clip of a male collected in Daecheon stream (N 36° 23' 25"/E 126° 39' 49") under the permission of the Ministry of Environment, South Korea. The fish and genomic DNA samples were stored in collection storage at the Department of Life Sciences, Yeungnam University as the voucher numbers, YUSS07 and YUSS-RP07, respectively. The mitogenome was amplified using long-range PCR and sequenced using the primer-walking strategy.

The mitogenome sequence was deposited in NCBI GenBank under KF425517. The whole sequence was 16,574 bp long consisting of 13 protein-coding genes (ATP6, ATP8 COX1 – 3, CYTB, ND1 – 6 and ND4L), 2 ribosomal RNA genes (12S and 16S) and 22 tRNA genes. One putative control region was found between tRNA^{Pro} and tRNA^{Phe}. The gene arrangement was completely identical to those found in Acheilognathinae species (Hwang et al. 2014; Luo

et al. 2014; Xu et al. 2015) as well as in cyprinid species (Yin et al. 2015; Sun et al. 2016).

The sequence size of 13 protein-coding genes was 11,256 bp. As shown in the mitogenome of Amur bitterling (Xu et al. 2015), all of the protein-coding genes were initiated by ATG, with the exceptions in COX1, and ND2, ND3, COX2, COX3 and CYTB had incomplete stop codons. Partial overlaps were found between four pairs of adjacent protein-coding genes, ATP8-ATP6, ND4L-ND4 and ND5-ND6 with 7, 1, 7 and 4 bp, respectively, as typically observed in cyprinid mitogenomes (Chen et al. 2013; Yin et al. 2015). Two rRNA genes were separated by a tRNA^{Val} and the total length of rRNA region was 2642 bp. All 22 tRNA genes, ranging from 68 to 76 bp, were interspersed throughout mitochondrial genome and were predicted to form cloverleaf-like secondary structures in the analyses using DAMBE (Xia & Xie 2001).

Bayesian inference analyses (MrBayes 3.2; Ronquist et al. 2012) were performed based on the complete mitogenome sequences (Figure 1(A)) or 13 protein-coding genes (Figure 1(B)) obtained from 17 Acheilognathinae species. Three major genera of bitterlings, *Rhodeus*, *Tanakia* and *Acheilognathus* independently formed monophyletic groups in the tree reconstructed based on the whole genome sequences (Figure 1(A)), providing little evidence to support the previous claim (Xu et al. 2015). When protein-coding sequences were only considered, however, the genus

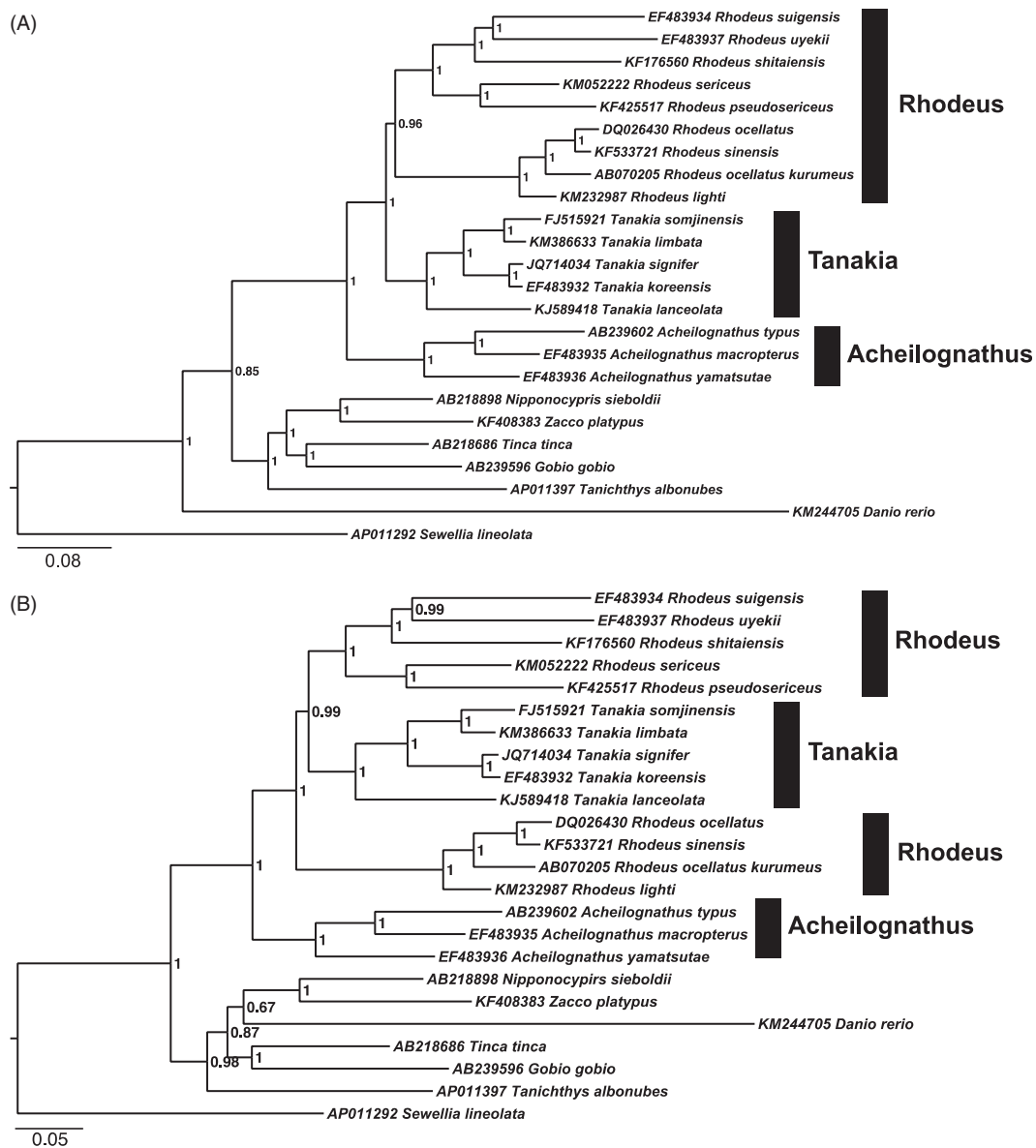


Figure 1. Bayesian inference tree of the subfamily Acheilognathinae reconstructed by MrBayes 3.2 using complete mitogenome sequences (A) or solely 13 protein-coding genes (B) obtained from 17 Acheilognathinae species. Six cyprinids (*Nipponocypris sieboldii*, *Zacco platypus*, *Danio rerio*, *Tinca tinca*, *Gobio gobio* and *Tanichthys albonubes*) and a Gastromyzontidae species (*Sewellia lineolata*) were included in the analyses as outgroups. $GTR + I + G$ was selected as the best-fit substitution model by jModeltest (Posada 2008). High posterior probabilities (>0.6) were symbolized on the node.

Rhodeus was not recovered as a single monophyly, with several *Rhodeus* species including *R. pseudosericeus* clustering primarily with *Tanakia* species (Figure 1(B)). Our study showed a more complicated pattern of Acheilognathinae phylogenetic differentiation, indicating that the taxonomic reevaluation is still required in this subfamily.

Disclosure statement

The authors report no conflict of interest.

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