The Complete Mitogenome and Phylogenetic Analysis of *Acrossocheilus wuyiensis* (Osteichthyes Cyprinidae)

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**Receiving date:** Aug 21, 2017; **Acceptance date:** Sep 13, 2017; **Publication date:** Sep 18, 2017

**Abstract**

*Acrossocheilus wuyiensis* is an endemic south China stream-dwelling cyprinid species. In this study, we decoded the complete mitogenome of *Acrossocheilus wuyiensis* for the first time by using whole genome sequencing approach. The complete mitogenome is 16,594 bp in length, consisting of 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes and one D-loop control region. Only ND6 and tRNA eight tRNA genes are encoded on the L-strand while most of these genes are located in the H-strand. Its overall base composition is A: 31.1%, C: 28.0%, G: 16.2% and T: 24.7%. The complete mitogenome of the Chinese barred species of *Cyprinidae* could provide a basic data for further phylogenetic and conversational analysis.

**Keywords:** Barred species; Mitogenome; *Acrossocheilus wuyiensis*; Next generation sequencing

**Introduction**

*Acrossocheilus wuyiensis* is a fresh water fish that live in benthopelagic area in subtropical climate. This species was found in Wuyishan Nature Reserve, Fujian, China [1]. *Acrossocheilus* is also known to be found in Southern part of China and northern part of Laos. The *Acrossocheilus genera* has a distinctive characteristic from the family of *Barbinae*, a rostral fold between the lip and the snout, fleshy and continuous lips around the corners of the mouth, horned sheath and two pairs of barbels around the jaw, serrated or smooth posterior margin [2]. This genus is divided into two species groups: a striped or barred species, and non-barred species. The barred species group are characterized by possessing several dark vertical bars on each side of the body [3]. *A. wuyiensis* is considered to be in the barred species group. *A. wuyiensis* can be distinguished from other *Acrossocheilus* species in having black blatches along the back, longer maxillary barbel, and no weak serrat on the last dorsal spine [1]. This species has forked caudal fins, 8 soft-rays in anal fins, 12 soft rays in dorsal fins, dark yellow body color and also can grow up to 15.9 cm.

The complete mitogenome of *A. wuyiensis* will benefit our knowledge on phylogeny and conservation of Chinese barred species of *Cyprinidae*.

**Materials and Methods**

Sample of *A. wuyiensis* was collected in April 2015 from the Wuyishan county (Min Jiang, flowing to East Sea), Fujian Province of China and deposited to the Zhejiang Museum of Natural History (ZMNH 2015040001). The complete mitogenome of *A. wuyiensis* has been obtained from high-throughput sequencing on whole genomic DNA with HiSeq 2000 platform (Illumina, San Diego, CA). We used next generation sequencing to perform low-coverage whole genome sequencing according to previous protocol [4]. About 0.11% raw reads (29,959 out of 26,785,944) were de novo assembly by using commercial software (Geneious V9, Auckland, New Zealand) to produce a single, circular form of complete mitogenome with about an average 268 X coverage. The protein coding, rRNA and tRNA genes of *A. wuyiensis* mitogenome were predicted by using DOGMA [5], ARWEN [6], MITOS [7] tools and manually inspected. To validate the phylogenetic position of *A. wuyiensis*, we used MEGA6 software [8] to construct a Maximum likelihood tree (with 500 bootstrap replicates and Kimura 2-parameter model [9]) containing complete mitogenomes of 12 species derived from *Acrossocheilus* genus. Two species of the genus *Onychostoma* (*O. gerlachi* and *O. barbatulum*) were included as the ingroup in this study as they are thought to be closely related to the barred species of *Acrossocheilus* [10]. *Barbonymus gonionotus* was utilized as out-group for tree rooting [11].

**Results and Discussion**

The complete mitochondrial genome of *A. wuyiensis* has been submitted to GenBank under accession no. KY131977. The length of complete mitochondrial genome of *A. wuyiensis* is 16,594 bp, includes 13 protein-coding genes, 22 tRNA genes (ranging from 67 bp in tRNACys to 76 bp in tRNALeu and tRNAlys), 2 rRNA genes (957 bp in 12S rRNA and 1682 bp in 16S rRNA), and 1 D-loop control region (939 bp) (Figure 1). The complete mitogenome of *A. wuyiensis* showing 99% identities to *A. stenotaeniatus* (GenBank KJ909660) after BLAST search against NCBI nr/nt database. Only ND6 and other eight tRNA (tRNAcys, tRNAla, tRNAGlu, tRNAPro, tRNAIln, tRNAAsn, tRNATyr and tRNAAsn) genes are encoded on the L-strand while...
most of these genes are located in the H-strand. All protein-coding genes initiated with the typical start codon ATG except for COX1 and ND3 beginning with GTG. Seven of the PCGs (ATP6, COX1, COX3, CYTB, ND1, ND4L and ND5) shared TAA stop codon, four PCGs shared TAG stop codon (ATP8, ND2, ND3 and ND6), one with AGA stop codon (COX2) and one with AGG stop codon (ND4).

The D-Loop control region, with 939 bp in length, was located between tRNAPro and tRNAPhe, and the base composition reflected with a lower GC content (34.2%) than the overall average mitogenome (44.2%).

Conclusion

Phylogenetic tree analysis was employed to find the phylogenetic positions of A. wuyiensis and other Acrossocheilus species based on the complete mitogenome sequences, which were retrieved from the GeneBank databases. The resultant phylogeny (Figure 2) shows that the phylogenetic positions of the relevant species were mostly consistent with that obtained in the previous research based on the mtDNA control region [10]. However, there were also some differences between them. Firstly, 12 different Acrossocheilus species were classified into four main clusters in this study while that was three in the previous study [10]; secondly, A. wuyiensis was significantly clustered as one clade with A. beijiangensis and A. stenotaeniatus (=A. longipinnis [12]) with high bootstrap value supported in this study while it was sister to A. paradoxus in the previous study [10]; thirdly, A. paradoxus joined with A. barbodon (=A. iridescens [13]) in this study but made a sister pair to A. wuyiensis in the previous study [10]; fourthly, A. longipinnis was united with the species pair A. monticola and A. yunnanensis and these three species were recognized as recognized as belonging to their own genus in the previous study [10] but joined with A. beijiangensis in this study. The main cause for these differences in topologies of phylogenetic tree may lie in differences in phylogenetic reconstruction methods, differences in nuclear DNA markers, accuracy of species identification, or accuracy differences among the sequencing platforms [14] [15]. In the future, more complete mitogenome data are needed for further phylogenetic analysis of barred Acrossocheilus species.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. This work was jointly supported by grants from the National Natural Sciences Foundation of China [grant number 31401974 and grant number 31501581] and Zhejiang Provincial Program for the Cultivation of High-level Innovative Health Talents.

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Acknowledgements

Our sincere thanks are given to Xue Wang and Liang Cao for their assistance in data analysis and Zhuoqiang Zhou, Qingyun Huang and Xiaolu Yu for helping with field work. We also thank Dr. Chung-Der Hsiao from Chung Yuan Christian University in Taiwan for his help on Bioinformatic analysis of NGS data.


