

Research Article

Open Access

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

Leyang Yuan^{1,2,3}, Xuelin Song^{2,5}, Bihao Ji⁴, Changgao Zhao⁴ and Xiaoxiang Liu^{2,6}*

¹Zhejiang Museum of Natural History, Hangzhou, Zhejiang, China

²Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei, China

³Biodiversity Research Center of Zhejiang Province, Hangzhou, Zhejiang, China

⁴Mangagement Bureau of Wangdongyang Alpine Wetland Nature Reserve, Jingning, Lishui, China

⁵University of Chinese Academy of Sciences, Beijing, China

⁶Faculty of Basic Medicine, Hangzhou Medical College, Hangzhou, Zhejiang, China

*Corresponding author: Xiao-Xiang Liu, Faculty of Basic Medicine, Hangzhou Medical College, Hangzhou, Zhejiang, China, Tel: +8615355710671, E-mail: liuxiaoxiang413@126.com

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

Copyright: © 2017 Yuang L, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Acorssocheilus wuyiensis is an endemic south China stream-dwelling cyprinid species. In this study, we decoded the complete mitogenome of Acorssocheilus 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 other 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; *Acorssocheilus 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, horny 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 blotches along the back, longer maxilarry barbel, and no weak serrate 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

J Phylogenetics Evol Biol, an open access journal ISSN: 2329-9002

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, tRNAAla, tRNAGlu, tRNAPro, tRNAGln, tRNASer, 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%).

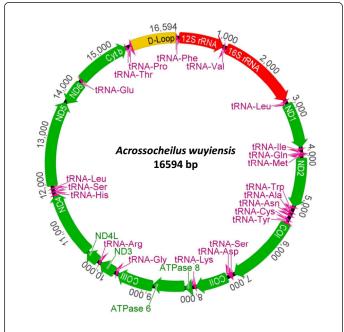


Figure 1:The complete mitogenome map of *Acrossocheilus wuyiensis.* The protein coding regions are labeled in green, tRNA genes are labeled in pink, rRNA genes are labeled in red and the putative D-loop control region is labeled in orange colors.

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.

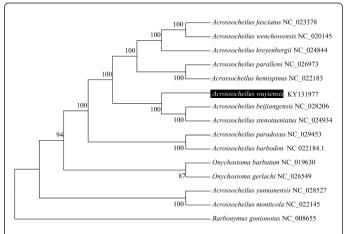


Figure 2: Molecular phylogeny of *Acrossocheilus wuyiensis* and related species based on complete mitogenome. The complete mitogenomes is downloaded from GenBank and the phylogenic tree is constructed by Maximum likelihood method with 500 bootstrap replicates. The gene's accession number for tree construction is listed behind the species name. The bootstrap values are shown at the inner nodes of the tree.

Acknowledgements

Our sincere thanks are given to Xue Wang and Liang Cao for their assistance in data analysis and Zhuocheng 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.

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.

References

- 1. Wu SH, Chen HX, Cao XY, Li SQ (1981) A new species of fish of the genus Acrossocheilus from Fujian. Wuyi Sci J 1:126-127.
- Lan ZJ, Chan BP, Zhao J (2014) Acrossocheilus multistriatus, a new species of barbine fish (Teleostei: Cyprinidae) from the Zhujiang River basin, South China. Zoological Systematics 39:433-441.
- Kottelat M (2001) Fishes of Laos. WHT Publications, Colombo, Sri Lanka 1-198.
- Shen KN, Yen TC, Chen CH, Li HY, Chen PL, et al. (2016) Next generation sequencing yields the complete mitochondrial genome of the flathead mullet, *Mugil cephalus* cryptic species NWP2 (Teleostei: Mugilidae). Mitochondrial DNA A DNA Mapp Seq Anal 27: 1758-1759.

Page 3 of 3

- Wyman SK, Jansen RK, Boore JL (2004) Automatic annotation of organellar genomes with DOGMA. Bioinformatics 20: 3252-3255.
- Laslett D, Canback B (2008) ARWEN: A program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics 24: 172-175.
- Bernt M, Donath A, Juhling F, Externbrink F, Florentz C, et al. (2013) MITOS: improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution 69: 313-319.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30: 2725-2729.
- 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.
- Yuan LY, Liu XX, Zhang E (2015) Mitochondrial phylogeny of Chinese barred species of the cyprinid genus *Acrossocheilus oshima*, 1919 (Teleostei: Cypriniformes) and its taxonomic implications. Zootaxa 4059: 151-168.
- 11. Saitoh K, Sado T, Mayden RL, Hanzawa N, Nakamura K, et al. (2006) Mitogenomic evolution and interrelationships of the Cypriniformes

(Actinopterygii: Ostariophysi): The first evidence toward resolution of higher-level relationships of the world's largest freshwater fish clade based on 59 whole mitogenome sequences. J Mol Evol 63: 826-841.

- Yuan LY, Chan BP, Zhang E (2012) Acrossocheilus longipinnis (Wu 1939), a senior synonym of Acrossocheilus stenotaeniatus Chu & Cui 1989 from the Pearl River basin (Teleostei: Cyprinidae). Zootaxa 3586: 160-172.
- Chen YS, Han M, Wang CL, Shen CN (2013) The complete mitochondrial genome of rainbow barbel *Acrossocheilus barbodon* (Nichols and Pope) (Teleostei, Cyprinidae, Barbinae). Mitochondrial DNA 26: 145-146.
- 14. Schardl CL, Young CA, Faulkner JR, Florea S, Panet J (2012) Chemotypic diversity of epichloae, fungal symbionts of grasses. Fungal Ecol 5: 331-344.
- 15. Salipante SJ, Kawashima T, Rosenthal C, Hoogestraat DR, Cummings LA, et al. (2014) Performance comparison of Illumina and ion torrent nextgeneration sequencing platforms for 16S rRNA-based bacterial community profiling. Appl Environ Microbiol 80: 7583-7591.