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Genetic Structure of *Spinibarbus caldwelli* Based on mtDNA D-Loop

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Abstract

Spinibarbus caldwelli was an endemic species to China, and its germplasm protection and resources utilization had become more and more concerned. To know its genetic diversity and differentiation, the mitochondrial DNA D-loop was amplified and sequenced for 148 individuals from four regions of Pearl River and Yangtze River Basin. Altogether 9 variable nucleotide sites existed among the aligned sequences of 748 bp, and 8 haplotypes were found within 148 individuals. The average nucleotide diversity (Pi) was high 0.00297, while haplotype diversity (Hd) was 0.706. The average genetic distance was 0.00298, most value occurred between LJ and CL populations, and small value occurred between HJ and QZ populations.

Keywords

Spinibarbus caldwelli, Genetic Structure, mtDNA D-Loop

1. Introduction

Spinibarbus caldwelli is a teleost fish species belonging to the order Cypriniformes. It is an important economic freshwater fish mainly distributing in the Yuanjiang, Yangtze, Jiulong, and Minjiang River, Pearl River basin and Hainan Island [1]. The wild stocks of seabream have declined substantially in recent years, owing to overexploitation, pollution, illegal fishing practices and the development of hydroelectricity. The gene diversity of *S. caldwelli* has been seriously depleted and the population has been seriously degraded. It has been listed in the Red Book of Endangered Species by the World Conservation Union [2]. There were many studies on the description, classification, age and growth, reproductive biology and diet of *S. caldwelli* [3] [4] [5] [6]. Tang *et al.* [7] and

Huang *et al*. [8] have studied the genetic relationship of different geographic groups by mtDNA.

Mitochondrial DNA (mtDNA) is a nucleic acid component in mitochondria, which has such characteristics as simple molecular structure, high coding efficiency, rapid evolution rate, strict maternal inheritance, and almost no recombination [9]. The control region of mitochondrial DNA (D-Loop) is the region with the largest variation in the whole mitochondrial DNA genome sequence and length. Its evolution rate is 5 - 10 times higher than that of other sequences, resulting in more mutations, such as base replacement, insertion or deletion, and numerous tandem repeats [10] [11]. By detecting the DNA variation in this region, we can effectively explore the genetic variation relationship between related species, species or individuals. It had become the primary tasks for the protection and development of its resources, formulate scientific and targeted protection measures to study the genetic structure. The main purpose of this study is to use the mtDNA D-loop as a molecular marker to analyze the population genetic structure of the species and provide a scientific basis for the development resource conservation and development and utilization measures.

2. Materials and Methods

2.1. Materials

The samples were collected from the Pearl River basin (Longjiang Tugong = 31) and the Yangtze River basin (the Xiangjiang River tributary Ocean River Quanzhou = 41, the tributary Minshui Chaling = 36 and the Yuan river Hongjiang = 40) from January 2013 to January 2015, a total of 148 (**Figure 1**). The fins or

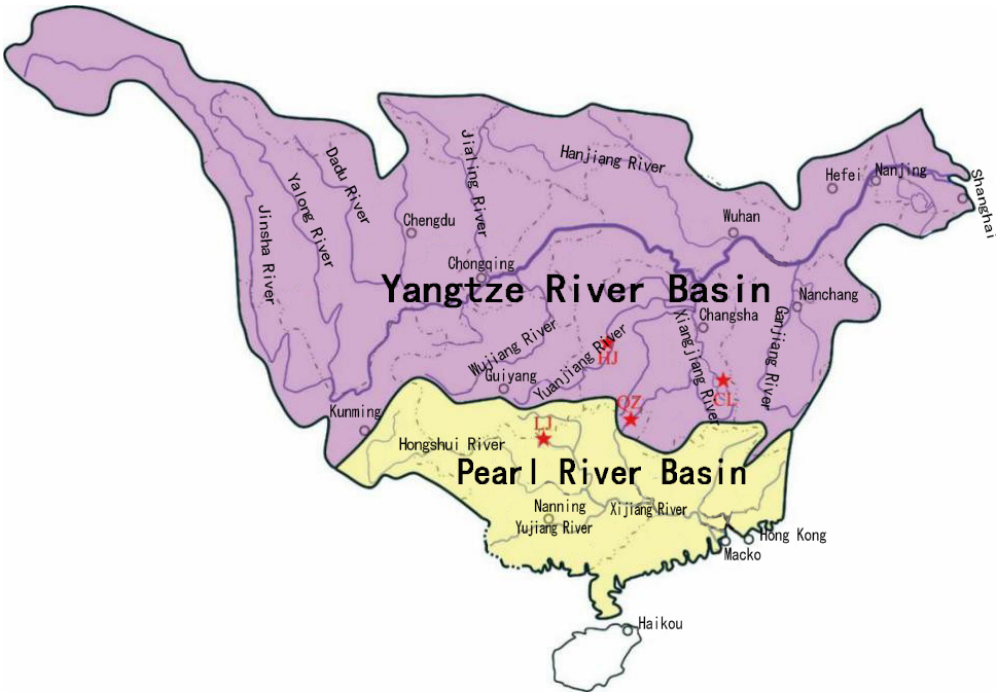


Figure 1. Distributions of *S. caldwelli* in Pearl River and Yangtze River basin.

whole individuals were collected from fresh specimens and immediately stored at 100% ethanol until DNA extraction.

2.2. Genomic DNA Extraction

Approximately 0.2 g of tissue from each specimen was placed in double distilled water for 5 h to remove all traces of ethanol. The tissue was subsequently digested with sodium dodecyl sulfate (SDS) and proteinase k. The genomic DNA was extracted using high NaCl method [12].

2.3. Amplification and Sequencing

The mtDNA D-Loop was amplified in a 50 μL reaction volume with a final concentration of 1× *Taq* polymerase buffer and 0.6 U of *Taq* polymerase, 1.5 mM $MgCl_2$, 50 mM of each dNTP and 2.5 pM of each primer. Primers used in the experiment were as follows: DL1 (5'-ACCCCTGGCTCCCAAAGC-3'); DH2 (5'-ATCTTAGCA TCTTCAGTG-3'). The thermal profile for hot-start polymerase chain reaction (PCR) included an initial denaturation 94˚ for 5 min, followed by 35 cycles of 30 s at 94˚C, 30 s at 54˚C, 60 s at 72˚C and a final cycle of 10 min at 72˚C. Amplicons were purified with QIAquick kit (QIAGEN) following the manufacturer's instructions. Both strands were sequenced using an ABI 3730XL Genetic analyser (Applied Biosystems, Inc.).

2.4. Data Analysis

All sequences were checked for quality using DNAStar Lasergene Version 7.0 software and aligned using the program ClustalX. Summary statistics (haplotype frequencies, number of polymorphic sites, number of transitions and transversions, and nucleotide composition) were estimated in Arlequin 3.0. Genetic diversity within localities was measured as the number of haplotypes, haplotype diversity (*Hd*), and nucleotide diversity (*Pi*), which were estimated in DnaSP4.0. Population subdivision and structure were estimated using an analysis of molecular variance (AMOVA) and pairwise population F_{ST} significance test as implemented in Arlequin. Significance of F_{ST} was determined via nonparametric permutation with 1000 data permutations.

3. Results

3.1. Base Composition

The average base composition in the sequences from the 148 individuals was 31.9% T, 20.1% C, 21.2% A, 26.8% G. Approximately 1.21% of sites (9 out of 745 base pairs) were found to be variable. There were 9 were transitions and no transversion, insertion or Missing sites of these variable sites (Table 1).

3.2. Genetic Diversity

Based on the polimorfic sites, it was defined 8 haplotypes in 148 individuals (Table 2). Only Hap3 was shared by Hongjiang HJ and QZ populations. Hap1

Table 1. Nucleotide compositions of mtDNA control region in *S. caldwelli* (%).

Populations	Base					
	A	T (U)	C	G	(A + T)	(C + G)
LJ	21.3	31.8	20.2	26.7	53.1	46.9
HJ	21.2	31.8	20.2	26.8	53.0	47.0
CL	21.1	31.9	20.0	27.0	53.0	47.0
QZ	21.2	31.8	20.2	26.8	53.0	47.0
Total	21.2	31.9	20.1	26.8	53.1	46.9

Table 2. Polymorphic sites in different haplotypes and haplotypes distribution.

Haplotypes	mtDNA D-loop base									Haplotypes distribution			
	2	3	4	4	4	6	6	6	7				
	6	8	0	8	8	5	9	9	2	LJ	CL	HJ	QZ
	7	9	0	1	7	7	5	6	2				
Hap 1	G	C	A	C	A	G	C	A	A	21			
Hap 2			G							1			
Hap 3					G	A			G			23	19
Hap 4	A				G	A	T	G	G		4		
Hap 5				T	G	A	T	G	G		5		
Hap 6					G	A	T	G	G		14		
Hap 7		T							G				2
Hap 8									G				1

and Hap2 were unique to LJ population. Hap4, Hap5 and Hap6 were unique to CL population. Hap7 and Hap8 were unique to QZ populations. The highest haplotype frequency was Hap3, with 42 individuals, accounting for 28.4%, shared by Hongjiang HJ and Quanzhou QZ populations, followed by Hap1, with 21 individuals, accounting for 14.2% (Table 2).

Overall haplotype diversity and nucleotide diversity was 0.706 and 0.00297, respectively. While the highest nucleotide diversity index were CL population, the highest nucleotide diversity were QZ population. Genetic diversity was poor in HJ population (Table 3).

3.3. Genetic Distance

Pairwise F_{ST} values ranged from 0.00049 to 0.00735. The mean F_{ST} values were 0.00298 (Table 4). The F_{ST} values between LJ population and CL population was the largest, being 0.00735; The F_{ST} values between HJ population and QZ population was 0.00049, which was relatively minimal.

The mtDNA homologous sequence of the carp (*Cyprinus carpio*) (Genbank accession number: AB158812.1) was used as the outer group sequence, and the haplotype NJ tree and all individual haplotypes were constructed as shown in Table 5. Haplotype 4, haplotype 5 and haplotype 6 were far away from other

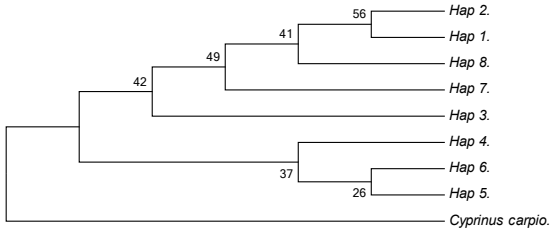
Table 3. Nucleotide diversity (π), Haplotype diversity (Hd) and Number of haplotypes (H) in 4 *S. caldwelli* populations.

Populations	π	Hd	H
LJ	0.00655	0.091	2
HJ	0.00000	0.000	1
CL	0.00767	0.577	3
QZ	0.01347	0.255	3
Total	0.00297	0.706	8

Table 4. Genetic distance of 4 *S. caldwelli* populations.

Populations	LJ	HJ	CL	QZ
LJ				
HJ	0.00410			
CL	0.00735	0.00322		
QZ	0.00386	0.00049	0.00371	

Table 5. Distribution and N-J phylogenetic of haplotype.

Populations	LJ	HJ	CL	QZ
	1			
	21			
				1 2 19
		23		
			4	
			14	
			5	
Total (Number of Haplotype)	22 (2)	23 (1)	23 (3)	22 (3)

haplotypes. However, it could be seen that the haplotype clustering of the four populations could be followed regularly. The haplotypes of various groups could be clustered closer, and the whole genetic structure was relatively clear. The LJ population was first clustered with the QZ population. And then clustered with HJ population, and finally clustered with CL population (Table 5).

4. Discussion

MtDNA is maternally inherited, has almost no recombination, has a higher variation than the nuclear gene [13], and the D-loop region is a non-coding region, which is highly likely to retain mutations and is the region with the fastest evolution rate in mtDNA [14], suitable for the study of genetic diversity of the following taxonomic factors. The mtDNA D-loop region has been widely used as a

molecular marker for genetic management of species, conservation of endangered species, genetic structure of populations, and evolutionary history [15] [16] [17]. In this study, 745 bp of mtDNA D-loop gene in 148 wild *Spinibarbus caldwelli* from four populations were analyzed providing information about the genetic structure of the species from a new biogeographical region. The overall genetic diversity ($\pi = 0.00297$, with 8 haplotypes) of the four populations showed a low level. The genetic diversity of QZ population in the whole state was relatively rich ($\pi = 0.01347$, with 3 haplotype), the genetic diversity of HJ population is extremely poor ($\pi = 0$, only one haplotype). However, Zheng *et al*. [18] based on the mtDNA D-loop region to assess the genetic diversity of *Spinibarbus caldwelli* population in Yunnan, showed that their genetic diversity was higher. In addition, studies on other carp species also showed a higher genetic diversity [19] [20].

The results of the haplotype clustering showed that the LJ population was first clustered with QZ population, and then clustered with the HJ population, and finally clustered with the CL population. The genetic distance between QZ population and LJ population was the closest. However, from the perspective of geographical location and river flow, the QZ, CL and HJ groups should be clustered together and then clustered with the LJ group. Longjiang LJ and Quanzhou QZ are located in Guangxi, but the Quanzhou QZ belongs to the Xiangjiang River system, and Longjiang belongs to the Pearl River system. Qin Shihuang built Lingqu in Xing'an, the upper reaches of the Yanghe River, a tributary of the Xiangjiang River, connecting the Xiangjiang River system with the Pearl River system. From then on, there has been the saying that "Lixiang is the same origin" since ancient times. Quanzhou is adjacent to Xing'an. It is possible that there is gene exchange between Xiangjiang River and Pearl River by Lingqu channel. Therefore, the LJ group and the QZ group can be first gathered together. The HJ population shared the haplotype with the QZ population, while the HJ population was poorly genetically diverse and the degree of differentiation among the populations was weak. In addition, floods, water conservancy projects, geological changes and human activities would also cause different levels of genetic communication between different groups. According to Shaklee *et al*., the genetic distance D values at the three levels of genus, species and population are 0.90, 0.30 and 0.05, respectively (Shaklee 1982). In this study, the populations of four populations could not reach the level of population differentiation. The P value of the eight haplotypes of the four populations was 0.00298 ($P < 0.01$), indicating that the degree of genetic differentiation among the four populations was low.

5. Conclusion

Spinibarbus caldwelli was an endemic species to China, and its germplasm protection and resources utilization had become more and more concerned. The mitochondrial DNA D-loop was amplified and sequenced for 148 individuals

from four regions of Pearl River and Yangtze River Basin. The overall genetic diversity of the four populations showed a low level. The degree of genetic differentiation among the four populations was low.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Fish Research Laboratory, Hubei Institute of Aquatic Biology (1995) Yangtze River Fish. Science Press, Beijing.

[2] Huckstorf, V. (2013) *Spinibarbus caldwelli*. The IUCN Red List of Threatened Species 2013: e.T187914A1834637.

[3] Lin, B.M. and Huang, M.M. (1999) Morphological and Distribution Differences of Other Species of *Robinia pseudoacacia* and Acridoids and Investigation of Ecological Habits of *Robinia pseudoacacia*. *Scientific Fish Farming*, **10**, 39.

[4] Jiang, L.Y., Yu, X.L., Chen, F.Y., Yang, X.M. and Huang, G.H. (2003) Age and Growth of Barbed Cockroaches under Culture Conditions. *Journal of Ocean University*, **23**, 6-13.

[5] Wen, C.Y., Zou, P.Y., Chen, J.R., Zhong, L.M. and Luo, Q.H. (2005) A Preliminary Study on the Ecology of Light Barbed Hedgehog in the Upper Reaches of the Beijiang River. *Journal of Shaoguan University* (*Natural Science*), **26**, 79-80, 95.

[6] Wang, F.Q., Li, Q., Yi, Z.S., Fan, Z.S., Ning, D.R., Yang, X.L., Peng, J.M., Huang, W.Y., Han, W., Zhong, L.M. and Lan, Z.J. (2013) Analysis of Morphological Differences of Different Populations of *G. serrata*. *Anhui Agricultural Sciences*, **41**, 2464-2465, 2479.

[7] Tang, Q.Y., Yang, X.P. and Liu, H.Z. (2003) Biogeographical Processes Based on Mitochondrial Cytochrome b Gene in *Robinia pseudoacacia*. *Journal of Hydrobiology*, **27**, 352-356.

[8] Huang, Z.Z., Huang, L.T., Lin, X.W. and Ling, W.Z. (2008) Analysis of Mitochondrial DNA Diversity of Black Ridge Thorn Mites. *Journal of Ocean University of China* (*Natural Science Edition*), **38**, 259-262, 220.

[9] Xiao, W.H. and Zhang, Y.P. (2000) Genetics and Evolution of Mitochondrial DNA in Fish. *Acta Hydrobiologica Sinica*, **24**, 384-391.

[10] Fischer, C., Koblmüller, S., Gülly, C., *et al*. (2013) Complete Mitochondrial DNA Sequences of the Threadfin Cichlid (*Petrochromis trewavasae*) and the Blunthead Cichlid (*Tropheus moorii*) and Patterns of Mitochondrial Fenome Evolution in Cichlid Fishes. *PLoS One*, **8**, e67048. https://doi.org/10.1371/journal.pone.0067048

[11] Bremer, J.R.A., Mejuto, J., Greig, T.W., *et al*. (1996) Global Population Structure of the Swordfish (*Xiphias gladius* L.) as Revealed by Analysis of the Mitochondrial DNA Control Region. *Journal of Experimental Marine Biology and Ecology*, **197**, 295-310. https://doi.org/10.1016/0022-0981(95)00164-6

[12] Yuan, X.P., Yan, L., Xu, S.Y, *et al*. (2008) Genetic Diversity of Copperfish and Round-Mouthed Copper Fish in the Yangtze River. *Chinese Journal of Fisheries Science*, **3**, 311-315.

[13] Cann, R.L., Brown, W.M. and Wilson, A.C. (1984) Polymorphic Sites and the Mechanism of Evolution in Human Mitochondrial DNA. *Genetics*, **106**, 479-490.

[14] Sbisà, E., Tanzariello, F., Reyes, A., Pesole, G. and Saccone, C. (1997) Mammalian Mitochondrial D-Loop Region Structural Analysis: Identification of New Conserved Sequences and Their Functional and Evolutionary Implications. *Gene*, **205**, 125-140. https://doi.org/10.1016/S0378-1119(97)00404-6

[15] Avise, J.C. (1991) Ten Unorthodox Perspectives on Evolution Prompted by Comparative Population Genetic Findings on Mitochondria DNA. *Annual Review of Genetics*, **25**, 45-69. https://doi.org/10.1146/annurev.ge.25.120191.000401

[16] Arise, J.C. (1994) Molecular Markers, Natural History and Evolution. Chapman and Hall, New York.

[17] Bennetts, R.Q., Grady, J.M., Rohde, F.C., *et al.* (1999) Discordant Patterns of Morphological and Molecular Change in Broadtail Madtoms (Genus *Noturus*). *Molecular Ecology*, **8**, 1563-1569.

[18] Zheng, B.R. and Zhang, Y.P. (2002) Genetic Diversity of Mt DNA D-Loop Region Sequence of Yunnan Barb, Waterfowl Fishery, 15-16.

[19] Liang, H.W., Zou, G.W., Luo, X.Z., Wang, C.Z., Hu, G.F. and Li, Z. (2009) Polymorphism and Phylogenetic Study of mtDNA D-Loop Sequences in Three Chinese Ticks. *Journal of Northwest A&F University*, **3**, 55-59.

[20] Dai, Y.G., Han, X. and Zhang, X.J. (2010) Sequence Variation and Genetic Diversity of mtDNA D Loop in Liujiang Population of Xiaokoubai Turtle. *Journal of Zoology*, **45**, 115-120.