# Genetic Structure and Variation of *Liobagrus nigricauda* of the Upper Yangtze River Basin Based on Cytochrome B

Wei Li\* and Bin Tu

Department of Medicine, Xianning First People's Hospital, Xianning, P. R. China

## **Corresponding Author\***

Wei Li

Department of Medicine

Xianning First People's Hospital

Xianning 437100, P. R. China

E-mail: kaixin9xiao@163.com

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# Abstract

*Liobagrus nigricauda* is endemic to the upper Yangtze River basin. By using the fast isolation by amplified fragment polymorphism of sequences containing cytochrome b, we identified the genetic variation in 6 populations of *L. nigricauda*. A total 21 nucleotide sites were detected in 1086 bps from cytochrome b, and 16 haplotypes were defined from 145 sequences. Nucleotide diversity index was 0.00087, and haplotype diversity index was 0.26858. Hierarchical Analysis of Molecular Variance (AMOVA) showed that the variation on within populations was 93.03%. And the mainstream populations of the upper Yangtze River had preliminary genetic differentiation with the tributary.

**Keywords:** *Liobagrus nigricauda* • mtDNA *Cyt b* • Hereditary diversity • Genetic structure

## Description

The Upper Yangtze River basin is one of the most abundant biodiversity regions in China [1]. However, for the over-fishing and water pollution, fish resource in the Upper Yangtze River basin was severely damaged. *Liobagrus nigricauda* was firstly described by Wu, and listed as an endangered species. For conserving and recovering the species through appropriate measures, genetic studies on the population are necessary.

In this article, we collected 145 samples of *L. nigricauda* from 6 geographic loci in the upper Yangtze River basin. Mitogenomic DNA was extracted from fin tissues of *L. nigricauda* preserved in ethanol [2]. Primer pairs were designed using Premier Prime 5.0 (F:5'CTCACCAAGACTTTA ACTAGGACCAATG-3', R:5'-GCGCTATTTATG TCTA AG CTACTAGAGC-3'). Subsequently, a 1086 bp fragment was amplified, and PCR products were purified and sequenced. Totally, 145 sequences containing *Cyt b* from L. nigricauda were obtained. Then the average content of nucleotides and genetic distance were calculated using MEGA 3.1 [3]. The calculation include nucleotide variations, number of haplotypes, nucleotide and haplotype diversities, values of gene flow (Nm), haplotype distribution [4], mismatch distribution pattern and the neutral test including indexes of Fu's Fs and Tajima's D [5]. Molecular Variance Analysis (AMOVA) and the level of genetic differentiation between populations by estimating the Fixation index (Fst) were summerized by ARLEQUIN 3.1 [6].

21 nucleotides sites detected within 1086 bp fragments of cytochrome b were belonging to 16 haplotypes. Nucleotide diversity and haplotype diversity were obtained (Table 1). Among these 6 groups, Jiagnjin nucleotide diversity was the highest and the lowest was from Juexi. Zhuyangxi population had the highest haplotype diversity and the lowest was from Juexi.

Table 1: Polymorphic site, number of haplotypes, haplotype diversity and nucleotide diversity of *L. nigricauda* mtDNA *cyt b*.

Populations	Polymorphic sites	Number of haplotypes	Nucleotide diversity	Haplotype diversity
Gaochang	8	4	0.00046	0.18145
Jiagnjin	7	2	0.00258	0.4
Juexi	0	1	0	0
Nancong	4	5	0.00029	0.29892
Shuifu	12	6	0.00067	0.28409
Zhuyangxi	10	6	0.002	0.40215
Total	21	16	0.00087	0.26858

Geographical distribution of haplotypes showed that haplotypes H\_1 had the largest amount in *L. nigricauda*, and distributed among 6 geographical populations. Shuifu haplotypes were six, and Zhuyangxi population with the same. Juexi had only one haplotype. Besides, there were 13 unique haplotypes distributed among five populations (Table 2).

 
 Table 2: Geographical distribution of L. nigricauda haplotypes based on the sequnce of cyt b.

Haplo type	Juexi	Gaochang	Jiangjing	Shuifu	Zhuyangxi	Nancong	Total
H_1	13	29	4	28	24	26	124
H_2		1					1
H_3		1			2		3
H_4		1					1
H_5			1	1	2		4
H_6						2	1
H_7						1	1
H_8						1	1
H_9						1	1
H_10				1			1
H_11				1			1
H_12				1			1
H_13				1			1
H_14					1		1
H_15					1		1
H_16					1		1

Haplotype network analysis showed that H\_1 might be the ancestral haplotype for its central position in the haplotype network and the highest haplotype frequency of each population. H\_15, H\_5, H\_16 and H\_2 belonged to secondary haplotypes. And there were some haplotype communications among 6 populations. For instance, a certain degree of haplotype communication was generated among Shuifu/Jiangjin populations and Gaochang/Zhu Yangxi populations (Figure 1). In totally, the haplotype network demonstrated some corresponding relationship between geographical information and haplotype distribution [7].



Figure 1. Haplotype network of L. nigricauda based on cyt b of mtDNA, the sizes of the circle represents haplotype frequencies. Yellow, green, blue, Black, red and purple represent Zhu Yangxi, Jiang Jin, Shui Fu, Nan Cong, Gao Chang and Jue Xi population, respectively.

#### The molecular variance analysis

Analysis of Molecule Variance (AMOVA) showed that the FST was 0.05519 0.05<FST<0.15, P<0.02). It was indicated that an initial genetic differentiation existed between the populations; the effect of genetic differentiation among them was not significant. Analysis of the percentage of variance displayed there was 5.52% of the variation on populations, and 94.48% were from among populations. It demonstrated that genetic variance mainly came from among populations, and only 5.52% of variance came from within populations (Table 3). This result was in accordance with the value of FST mentioned above [8,9].

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation			
Within populations	5	5.518	0.02739 Va	5.52			
Among populations	139	65.186	0.46896 Vb	94.48			
Total	144	70.703	0.49635				
Note: Va: Variance for group; Vb: Variance for population							

 Table 3: The AMOVA analysis of L. nigricauda between and in populations based on cyt b.

In addition, by analysing the pairwise value of FST and genetic distance from 6 populations, FST showed a direct connection with genetic distance. It inferred that the mainstream populations [10] of the upper Yangtze River had preliminary genetic differentiation with tributary [11].

### Conclusion

In recent years, some hydropower projects were built in the Upper Yangtze River basin, and damaged the nature habitat of fishes in this area. The wild resource of L. nigricauda was severely damaged many geographical sites of the Upper Yangtze River basin could not collect the samples. Only 145 samples of L.nigricauda were got from 6 sampling sites. The level of L. nigricauda' genetic diversity was low. From the result of molecular phylogenetic and haplotype network, there was some haplotype communications between differenct populations. Zhu Yangxi had the most derived haplotypes, and others had unique haplotype. According to geographical positions, we inferred that Zhu Yangxi population might be the diffusion Center of haplotypes. Based on analysing haplotype evolution and genetic structure, there was an initial genetic differentiation among the population of L. nigricauda. Specifically, the mainstream population of the upper Yangtze River had an initial genetic differentiation compared with populations tributaries. Mismatch distribution and neutrality tests showed L. nigricauda had experienced a population expansion in history, but the effect was not significant, it indicated human factors might be main factor destroied the genetic resources. According to the wild resource and genetic diversity of L. nigricauda, some actions should be necessary for conservation issue. So we suggested L. nigricauda population could be protected as one big management unit, or it could treat as two units that divided into mainstream population and tributary population.

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