Genetic structure and variation of Liobagrus nigricauda of the upper Yangtze River basin based on cytochrome b

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Research Article

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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 bp 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 from within populations was 93.03%. And the mainstream populations of the upper Yangtze River had preliminary genetic differentiation with tributary.

Introduction

The Upper Yangtze River basin is one of the most abundant biodiversity regions in China (Cao, 2000). 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 (Fish Base 2012), and listed as an endangered species. For conserving and recovering the species through appropriate measures, genetic studies on the population are necessary.

In this study, 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 (Zane et al. 2002). Primer pairs were designed using Premier Prime 5.0 (F:5'-CTCACCAAGACTTTAACTAGGACCAATG-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 (Kumar et al. 2001). The calculation include nucleotide variations, number of haplotypes, nucleotide and haplotype diversities, values of gene flow (Nm), haplotype distribution (Nei 1987), mismatch distribution pattern and the neutral test including indexes of Fu's Fs and Tajima's D (Bandelt et al. 2001). Molecular variance analysis (AMOVA) and the level of genetic differentiation between populations by estimating the fixation index (Fst) were summerized by ARLEQUIN 3.1 (Schneider et al. 2000).

Results

21 nucleotides sites detected within 1086 bp fragment of cytochrome *b were* belonging to 16 haplotypes. Nucleotide diversity and haplotype diversity were obtained (Tab 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.

Tab 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	0.40000
Juexi	0	1	0.00000	0.00000
Nancong	4	5	0.00029	0.29892
Shuifu	12	6	0.00067	0.28409
Zhuyangxi	10	6	0.00200	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 in 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 in five populations (Table 2).

Table 2. Geographical distribution of *L. nigricauda* haplotypes based on the seqence of cyt b

Haplotype	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 in 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. In totally, the haplotype network demonstrated some corresponding relationship between geographical information and haplotype distribution.

2. The molecular variance analysis

Analysis of molecule variance (AMOVA) showed that the F_{ST} was 0.05519 $0.05<F_{ST}<0.15$, $P<0.02$). 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 these were 5.52% of the variation within 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 (Tab.3). This result was in accordance with the value of F_{ST} mentioned above.

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

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	

In addition, by analysing the pairwise value of *FST* and genetic distance from *6* populations, FST showed a direct connection with genetic distance. It infeded that the mainstream populations (Jiang Jin and Zhu Yangxi) of the upper Yangtze River had preliminary genetic differentiation with tributary (Nan Cong, Gao Chang, Jue Xi and Shui Fu).

Conclusion

In recent years, some hydropower projects were builted in the Upper Yangtze River basin, and damaged the nature habitat of fishes in this area (Chen et al. 2002). 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 among differenct populations. Zhu Yangxi had the most derived haplotypes, and others had unique haplotype. According to geographical positions, we infered 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 tributary. Mismatch distribution and neutrality tests showed *L. nigricauda* had experienced a population expansion in history, but the effect was not sgnificant, 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 coule be protected as one big management unit, or it could treated as two units that divided into mainstream population and tributary population.

Declarations

Acknowledgments

The authors deny any conflicts of interest related to this study.

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Figures

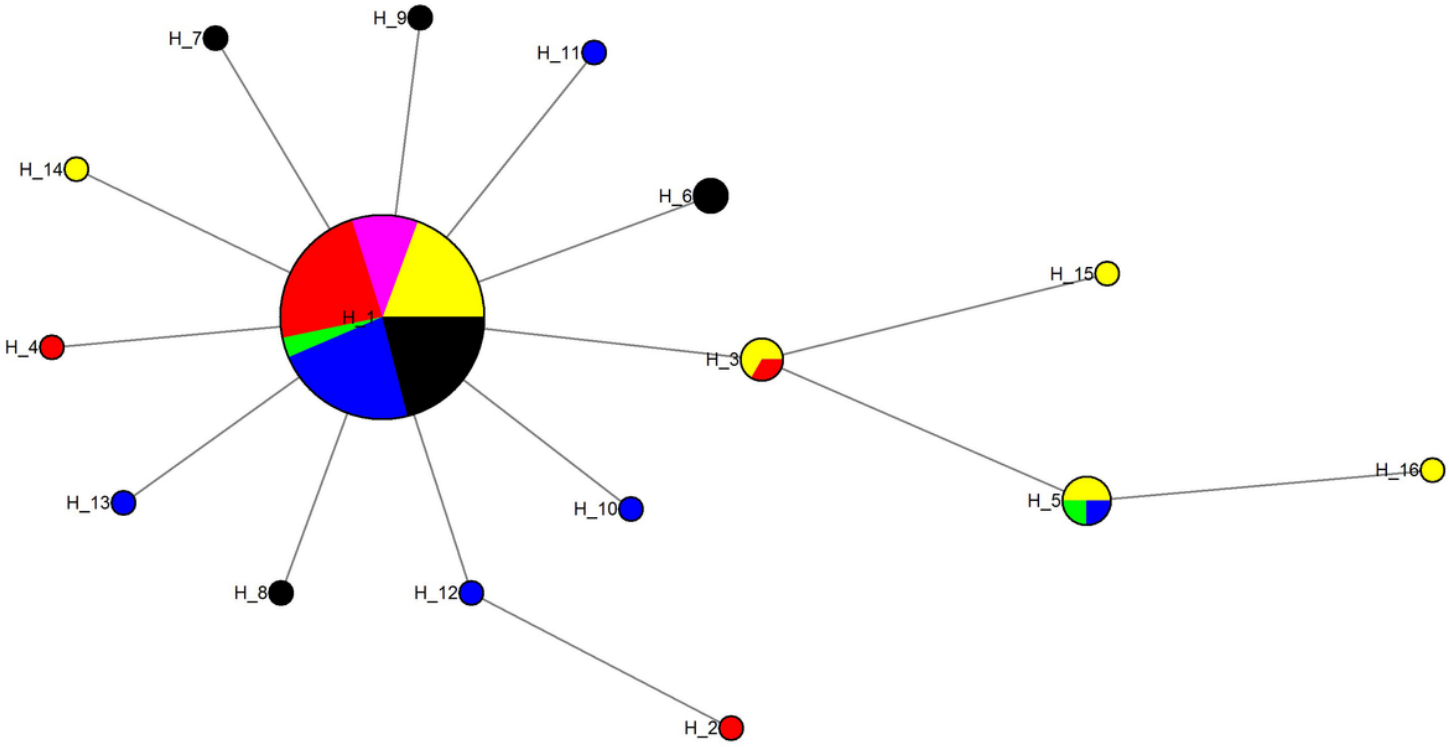

Figure 1

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.