

Genetic diversity and temporal changes of an endemic cyprinid fish species, *Ancherythroculter nigrocauda*, from the upper reaches of Yangtze River

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ABSTRACT

Small populations with low genetic diversity are prone to extinction. Knowledge on the genetic diversity and structure of small populations and their genetic response to anthropogenic effects are of critical importance for conservation management. In this study, samples of *Ancherythroculter nigrocauda*, an endemic cyprinid fish from the upper reaches of Yangtze River, were collected from five sites to analyze their genetic diversity and population structure using mitochondrial cytochrome *b* gene and 14 microsatellite loci. Haplotype diversity, nucleotide diversity, and expected heterozygosity indicated that the *A. nigrocauda* populations had low genetic diversity, and decreased heavily from 2001 to 2016. Significant genetic differentiation was found among different populations in the *cyt b* gene and SSR markers based on the genetic differentiation index (F_{ST}), whereas no differentiation was found in 2001. Haplotype genealogy showed that eight out of 15 haplotypes were private to one population. The SSR STRUCTURE analysis showed that there were four genetic clusters in the *A. nigrocauda* samples, with each population forming a single cluster, except for the Chishui River (CSR) and Mudong River (MDR) populations, which formed a common cluster.

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Therefore, loss of genetic diversity and increased genetic differentiation were found in the *A. nigrocauda* populations, which could be attributed to dam construction, overfishing, and water pollution in the upper Yangtze River. It is therefore recommended that the government should ban fishing, control water pollution, increase river connectivity, and establish artificial breeding and stocking.

Keywords: Genetic diversity; Population structure; Temporal change; Conservation

INTRODUCTION

Genetic diversity of a species determines its adaptive capacity and evolutionary potential (Altizer et al., 2003; Pinsky & Palumbi, 2014). Small populations of narrowly distributed species often have low genetic variation within populations but high genetic differentiation among populations due to genetic drift and restricted gene flow (Gibson et al., 2008; Hamrick & Godt, 1996; Young et al., 1996). Moreover, adverse anthropogenic influences can accelerate loss of genetic diversity within populations and differentiation among

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populations (Frankham, 2002). Genetic impoverishment can accelerate the process of local extinction of small populations (Hedrick & Kalinowski, 2000). An understanding of the genetic variability and structure of small populations and their genetic responses to anthropogenic effects is of critical importance for conservation management (Keyghobadi, 2007; Zhang et al., 2007) and for formulating the appropriate scales and subunits (Moritz, 1999) for sustainable long-term conservation.

The Yangtze River is the largest river in China and the third longest river in the world, with a total length of 6 300 km and a drainage area of 1 800 000 km². The Yangtze River supports 378 fish species, of which 162 are endemic (Yu et al., 2005), representing the highest fish diversity in the Palearctic region (Matthews, 1998). The upper Yangtze River refers to the reach above Yichang City and reportedly contains 124 endemic fish species (Cao, 2011). Therefore, the upper Yangtze River is a crucial area for the conservation of fish diversity and genetic resources. However, due to dam construction, overfishing, and water pollution, fish diversity in this region has decreased sharply and many species have become endangered (Fu et al., 2003; Park et al., 2003; Zhong & Power, 1996).

Ancherythroculter nigrocauda, belonging to Cyprinidae in Cypriniformes, is an important commercial and aquaculture species in China and an endemic fish from the upper Yangtze River, where it predominantly inhabits the main river and its tributaries (Ding, 1994). This species is sedentary and lays adhesive eggs from April to August during the rainy season (Cao et al., 2007; Liu et al., 2013). The minimum age of sexual maturity of *A. nigrocauda* is one year, and the body length at 50% sexual maturity estimated to be 106 and 125 mm for males and females, respectively (Liu et al., 2013). Its absolute fecundity varies from 11 300 to 504 630 eggs, with a mean of 162 377 eggs (Liu et al., 2013). In recent years, however, the natural populations and distribution areas of *A. nigrocauda* have declined significantly (Liu, 2013). While previous studies have reported on the age and growth (Xue & He, 2001), reproductive biology (Liu et al., 2013), and artificial propagation (Tan et al., 2004; Yin & Lv, 2010) of *A. nigrocauda*, very little is known about the genetic diversity and structure of populations in the upper Yangtze River. Liu et al. (2005) conducted a study on the genetic diversity and population structure of *A. nigrocauda* with samples collected from 2001 to 2002 in the upper Yangtze River, and found high genetic diversity and no genetic differentiations among different geographical populations. However, that study had a limited sample size (43 samples from three localities) and only recovered a 546 bp fragment of the cytochrome *b* (*cyt b*) gene. Moreover, due to a sharp decline in the natural populations of *A. nigrocauda* over the past few decades and substantial environmental changes in the upper Yangtze River after the impoundment of the Three Gorges Dam (TGD), it is likely that both the genetic diversity and population structure of *A. nigrocauda* in the upper Yangtze River have been impacted.

In the current study, samples of *A. nigrocauda* were collected from five sites in the upper Yangtze River. We

analyzed the genetic diversity and population structure of fish samples from the five different sites based on the *cyt b* gene and simple sequence repeat (SSR) markers and compared the results with those of Liu et al. (2005). The *cyt b* gene was used in the current study for better comparison with Liu et al. (2005). The *cyt b* gene is part of the mitochondrial genome, whereas SSR loci are distributed on genomes and have advantages of high polymorphism and codominance, therefore, the combination of *cyt b* gene and SSR markers is a powerful tool in studies on population genetics. This study aimed to determine the genetic diversity and population structure, as well as temporal changes, of *A. nigrocauda* in the upper Yangtze River, and provide important information for the conservation of this species.

MATERIALS AND METHODS

Samples collection and DNA extraction

From 2016 to 2017, a total of 239 *A. nigrocauda* samples were collected from five localities (Longxi River (LXR), Chishui River (CSR), Mudong River (MDR), Modao Stream (MDS), and Daning River (DNR)) in the upper Yangtze River (Figure 1, Table 1). Dorsal muscle used for DNA extraction was clipped from each of the fish, and then preserved in 95% alcohol in 5 mL cryogenic vials and stored at -20 °C. Total DNA was extracted from alcohol preserved muscle tissue using proteinase K digestion at 55 °C for 3–5 h, followed by phenol/chloroform extraction (Kocher et al., 1989).

Table 1 Sampling sites, sample site codes, GPS locations, and samples sizes of *A. nigrocauda* in the upper Yangtze River, China

Sample site	Code	GPS location (N E)	Sample size (<i>n</i>)
Longxi River	LXR	28°54'46" 105°30'51"	64
Chishui River	CSR	28°48'03" 105°50'22"	38
Mudong River	MDR	29°33'56" 106°50'16"	36
Modao Stream	MDS	30°49'04" 108°51'46"	39
Daning River	DNR	31°16'37" 109°49'00"	62

mtDNA amplification and sequencing

All 239 samples were used for mtDNA amplification. The mtDNA *cyt b* gene was amplified using polymerase chain reaction (PCR) in 30 µL reactions containing 3 µL of reaction buffer (200 mmol/L Tris-HCL pH 8.4, 500 mmol/L KCL, 50 mmol/L MgCL), 1.5 µL of dNTPs (1 mmol/L), 1 µL of each primer (10 µmol/L), 0.25 µL (2.5 U) of *Taq* DNA polymerase, 3 µL of template DNA, and 20.25 µL of H₂O. Primer sets were L14724 5'-GACTTGAAAAACCACCGTTG-3' and H15915 5'-CTCCGATCT CCGGATTACAAGAC-3' (Xiao et al., 2001). The PCR profile was initial denaturation at 94 °C for 4 min; followed by 35 cycles at 94 °C for 45 s, 54 °C for 45 s, and 72 °C for 1 min; then one cycle at 72 °C for 10 min. The PCR products were purified and sequenced by Shanghai DNA Biotechnologies Company.

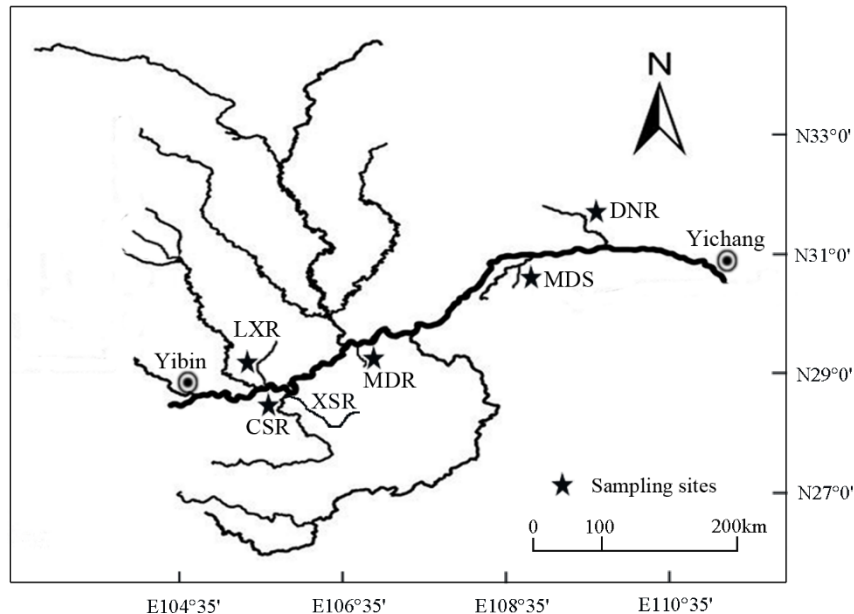


Figure 1 Sampling sites (stars) of *A. nigrocauda* in upper Yangtze River
 LXR: Longxi River; CSR: Chishui River; MDR: Mudong River; MDS: Modao Stream; DNR: Daning River.

SSR amplification and electrophoresis

A total of 161 samples from the five localities (sample size of each locality is described in Table 7) and 14 polymorphic SSR loci were used in this study. Primers for the 14 microsatellite loci were developed using fast isolation with the amplified fragment length polymorphism (AFLP) of sequences containing repeats (FIASCO) protocol. The specific sequence, optimum annealing temperature, and GenBank accession No. for each microsatellite primer are listed in Supplementary Table S1. Amplification of DNA was performed in a 10 μ L reaction mixture. The PCR profile was initial denaturation at 94 °C for 3 min; followed by 28 cycles at 94 °C for 30 s, annealing temperature for 40 s, and 72 °C for 1 min; then one cycle at 72 °C for 10 min. The PCR products were electrophoresed in 8% non-denaturing polyacrylamide gels on a Sequi-Gen GT system (Bio-Rad, USA). The gels were then stained using Ultra GelRed before being photographed. Lastly, the allele sizes were obtained manually by referring to the pBR322 DNA/Msp I marker (Tiangen Biotechnologies, China).

mtDNA sequence analysis

MEGA7 was used to align and edit the nucleotide sequences (Kumar et al., 2016). The haplotype frequency, haplotype diversity, and nucleotide diversity were calculated with DnaSP v5.10 (Librado & Rozas, 2009). Arlequin v3.0 (Excoffier et al., 2005) was used to perform analysis of molecular variance (AMOVA) and compute pairwise F_{ST} values (Excoffier et al., 1992). A neighbor-joining (NJ) phylogenetic tree was constructed using MEGA7 (Kumar et al., 2016). The median joining algorithm from Network 4.6 was used to construct a haplotype network (Bandelt et al., 1999). Tajima's D and Fu's F_s tests were implemented in Arlequin v3.0 (Excoffier et al.,

2005) to test for departure from neutrality due to population expansion or selection. Mismatch distribution analysis was used to further detect demographic expansion using DnaSP v5.10 (Librado & Rozas, 2009).

In addition, we compared our results with that of Liu et al. (2005) to determine the temporal changes in genetic diversity and population structure of *A. nigrocauda* from 2001 to 2016. Sequences from this study were downloaded from GenBank (accession Nos. AY493869–AY493886) and reanalyzed using the above methods. Because the obtained length of the *cyt b* gene was only 546 bp in Liu et al. (2005), we also aligned and edited our sequences to 546 bp to ensure accurate comparison and analysis. Our specific sample sites in the Longxi and Mudong rivers were the same as those of Liu et al. (2005), and our sample site in Chishui River was next to the sample site of Liu et al. (2005) in Xishui River, a tributary of Chishui River. Thus, we compared the present genetic diversity and population structure of *A. nigrocauda* from these three localities with the results of Liu et al. (2005).

SSR data analysis

Micro-checker v2.2.1 (Van Oosterhout et al., 2004) was used to check possible large allele dropout, scoring errors due to stuttering, and null alleles. Deviations from the Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) across all pairs of loci were assessed in GENEPOP v4.7.0 (Rousset, 2008) using the exact test with Markov chain algorithm (Guo & Thompson, 1992; P -values were estimated from 10 000 dememorizations, 100 batches, and 5 000 iterations per batch). Significance levels for multiple comparisons were adjusted using the sequential Bonferroni correction (Rice, 1989).

The number of alleles (A), observed (H_o) and expected (H_e) heterozygosity, and polymorphic information content (PIC) per locus were calculated using Cervus v3.0 (Kalinowski et al., 2007). Standardized allelic richness (A_r) was calculated using Fstat v2.9.3.2 (Goudet, 2001). Arlequin v3.0 (Excoffier et al., 2005) was used to perform analysis of molecular variance (AMOVA) and compute pairwise F_{ST} values (Excoffier et al., 1992).

The heterozygote excess test was performed to detect recent bottleneck effects using Bottleneck v1.2.02 with the Wilcoxon test under the Two-Phased Mutation Model (TPM) (Cornuet & Luikart, 1996). Piry et al. (1999) suggested that the TPM of mutation for microsatellite loci was appropriate with 95% single-step changes and a variance of 12.

Bayesian assignment analysis was applied to infer the number of genetically differentiated clusters (K) using STRUCTURE v2.3.4 (Pritchard et al., 2000). We performed 10 replications for each K starting from one to nine (400 000 iterations with 100 000 burn-in periods) under an admixture model and correlated allele frequencies within populations (Falush et al., 2003). The optimal K value was determined by comparing the mean log probability $\ln P(K)$ and calculating the ΔK value for each K (Evanno et al., 2005) using Structure Harvester (Earl & vonHoldt, 2012; available at <http://taylor0.biology.ucla.edu/structureHarvester/>). Outputs from Structure Harvester were further analyzed using CLUMPP v1.1.2 (Jakobsson & Rosenberg, 2007), which estimates membership coefficients across replicate analyses. Outputs from CLUMPP were then used directly as inputs in DISTRUCT v1.1 (Rosenberg, 2003), which assists in the visual presentation of these estimated membership coefficients. According to previous studies, individuals were able to assign to one of the inferred clusters when the corresponding membership proportion was ≥ 0.80 (Mukesh et al., 2013; Oliveira et al., 2008).

RESULTS

Cyt *b* gene marker

Genetic diversity and demographic history

Following alignment, a 1 140 bp cyt *b* gene sequence was obtained for 239 individuals. No deletions or insertions were observed. The average base composition was A=29.19%, T=27.59%, G=14.45%, and C=28.76%. Within the 1 140 bp region, 13 sites were variable, including nine parsimony informative sites and four singleton variable sites. We identified 15 haplotypes (GenBank accession Nos.: MH665369–MH665383) from the 239 individuals, and the numbers of haplotypes ranged from four to nine for each sampled population. For the five populations, haplotype diversity ranged from 0.488 to 0.794, with a mean value of 0.786. Nucleotide diversity ranged from 0.084% to 0.163%, with a mean value of 0.141% (Table 2).

Some Tajima's D and Fu's F_s test values were negative in the five populations, though none were statistically significant, which suggests no expansion for the *A. nigrocauda*

populations (Table 2). Furthermore, all mismatch distributions for each population and the whole population exhibited a multimodal distribution (Figure 2), further indicating no population expansion.

Population structure

The AMOVA based on haplotype frequencies revealed that 76.19% of the genetic variation occurred within populations, whereas 23.81% occurred among populations, thus suggesting significant genetic variation among the populations (Table 3). Pairwise F_{ST} values between the populations are listed in Table 4. Results showed significant genetic differentiation between each population pair, except for that between the MDS and DNR populations.

The NJ phylogenetic tree indicated there were four clades of the 15 haplotypes (Figure 3), which was confirmed with the haplotype network (Figure 4). Each of the four clades was shared by three to five populations; thus, no obvious genealogical geographic pattern was formed. In regard to haplotype distribution, Hap 1, Hap 2, and Hap 3 were the main haplotypes, shared by more than three populations; however, eight of the 15 haplotypes were found in one population only.

Temporal changes

A total of 43 sequences from Liu et al. (2005) were obtained for the LXR, CSR, and MDR populations. The haplotype diversities of the three analysed populations were 0.812, 1.000, and 0.833 in Liu et al. (2005), but 0.469, 0.656, and 0.707 in the present study, respectively. The nucleotide diversities of the three analysed populations were 0.436%, 0.488%, and 0.400% in Liu et al. (2005), but 0.090%, 0.204%, and 0.177% in the present study, respectively. These results indicated that the genetic diversity of *A. nigrocauda* has declined sharply in the three sampling sites from 2001 to 2016 (Table 5). The pairwise F_{ST} values among the three populations were -0.018 62, 0.013 38, and -0.044 13 (all non-significant, $P > 0.05$) in Liu et al. (2005) and 0.095 23, 0.185 18, and 0.092 84 (all significant $P < 0.05$) in the present study. These results demonstrated significant genetic differentiation in 2016 but not in 2001 (Table 6).

SSR marker

Genetic diversity and bottleneck effects

No large allele dropout or scoring errors due to stuttering were detected by Micro-Checker; however, An63 and An114 in LXR, An72 in CSR, An65 in MDS, and An63 and An65 in DNR all showed the presence of null alleles. Eleven out of 70 tests differed significantly from the HWE after Bonferroni correction, whereas no significant deviation from the HWE was detected in any locus across all populations (Supplementary Table S2). No linkage disequilibrium was found among SSR locus pairs, except for An63 and An114 in the CSR population and An63 and An76 in the DNR population.

For the five populations, the average number of alleles per population ranged from 4.929 to 10.286 and the average allelic richness per population ranged from 4.817 to 9.876.

Table 2 Haplotype frequency distribution, haplotype diversity, nucleotide diversity, and neutrality tests for five *A. nigrocauda* populations based on mitochondrial cyt b analysis

Sample site	LXR (64)	CSR (38)	MDR (36)	MDS (39)	DNR (62)	Total (239)	GenBank accession Nos.
Haplotype frequency							
Hap1	0.296 9	0.052 6	0.222 2	0.512 8	0.548 4	0.347 3	MH665369
Hap2			0.027 8	0.384 6	0.290 3	0.142 3	MH665370
Hap3	0.656 3	0.342 1	0.111 1		0.032 3	0.255 2	MH665371
Hap4					0.016 1	0.004 2	MH665372
Hap5	0.015 6	0.026 3	0.083 3		0.096 8	0.046 0	MH665373
Hap6					0.016 1	0.004 2	MH665374
Hap7		0.078 9	0.388 9	0.025 6		0.075 3	MH665375
Hap8		0.184 2				0.029 3	MH665376
Hap9		0.263 2	0.027 8			0.046 0	MH665377
Hap10		0.026 3				0.004 2	MH665378
Hap11	0.015 6	0.026 3	0.055 6			0.016 7	MH665379
Hap12	0.015 6					0.004 2	MH665380
Hap13				0.076 9		0.012 6	MH665381
Hap14			0.055 6			0.008 4	MH665382
Hap15			0.027 8			0.004 2	MH665383
Haplotype diversity	0.488±0.050	0.789±0.038	0.794±0.049	0.598±0.046	0.614±0.048	0.786±0.016	
Nucleotide diversity	0.000 84±0.000 09	0.001 63±0.000 13	0.001 55±0.000 11	0.001 09±0.000 11	0.000 92±0.000 07	0.001 41±0.000 04	
Tajima's D	0.283 64	0.326 94	-0.238 51	0.139 25	-0.039 54	0.094 36	
P-value	0.650	0.679	0.426	0.612	0.518	0.577	
Fu's F _s	-0.014 25	-1.109 99	-2.255 98	1.259 59	-0.677 17	-0.559 56	
P-value	0.496	0.323	0.132	0.759	0.386	0.419	

Data are based on 239 samples of *A. nigrocauda* from upper Yangtze River, China. Numbers in brackets indicate number of individuals from each sample site.

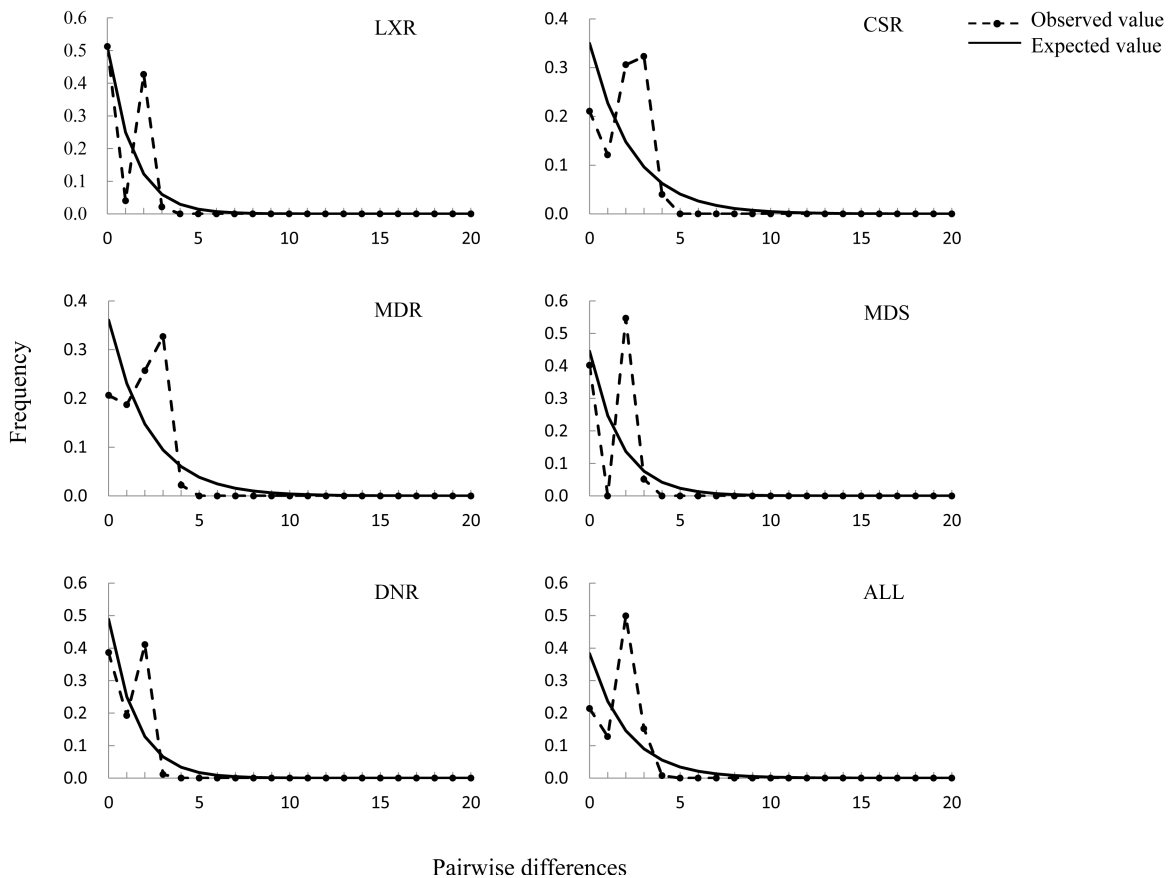


Figure 2 Mismatch distributions for each analyzed population and all 239 samples of *A. nigrocauda* sampled from upper Yangtze River

Table 3 Analysis of molecular variance (AMOVA) for five *A. nigrocauda* populations based on mtDNA and SSR analyses

Source of variation	df	Sum of squares	Variance component	Percentage variation	F_{ST}	P
mtDNA						
Among populations	4	40.543	0.201 96	23.81	0.238 14	0
Within populations	234	151.189	0.646 11	76.19		
Total	238	191.732	0.848 07			
SSR						
Among populations	4	309.614	1.127 46	18.98	0.189 83	0
Within populations	317	1 525.355	4.811 84	81.02		
Total	321	1 834.969	5.939 31			

Data are based on 239 and 161 samples of *A. nigrocauda* from upper Yangtze River, China, respectively.

The average observed heterozygosity per population ranged from 0.595 to 0.746, whereas the average expected heterozygosity per population ranged from 0.649 to 0.816. No significant heterozygote excess ($P > 0.05$) was detected for the five populations under the TPM, indicating no recent bottleneck effects (Table 7).

Population structure

Similarly, for SSR analysis, AMOVA revealed significant genetic differentiation among the five populations ($F_{ST} =$

0.18983, $P < 0.05$) (Table 3). The pairwise F_{ST} values varied from 0.088 86 to 0.246 93, which were all statistically significant ($P < 0.05$) after Bonferroni correction, suggesting significant genetic differentiation between each population pair (Table 4). When conducting STRUCTURE analysis, $\text{LnP}(K)$ showed no clear peak, but ΔK reached a maximum value when $K=4$, inferring there were four genetic clusters in the *A. nigrocauda* samples (Figure 5). The membership proportions of the four inferred genetic clusters in the five populations are listed in Table 8, which showed little gene flow among the five

Table 4 Pairwise F_{ST} values based on *cyt b* analysis of 239 samples (below diagonal) and SSR analysis of 161 samples (above diagonal) of *A. nigrocauda* from five different sites in upper Yangtze river, China

Population	LXR	CSR	MDR	MDS	DNR
LXR		0.226 56*	0.228 63*	0.246 93*	0.211 17*
CSR	0.152 51*		0.088 86*	0.187 23*	0.173 27*
MDR	0.113 45*	0.102 40*		0.163 89*	0.203 83*
MDS	0.348 66*	0.296 86*	0.260 19*		0.149 23*
DNR	0.342 81*	0.320 61*	0.282 98*	- 0.00 426	

*: $P < 0.05$ after Bonferroni correction.

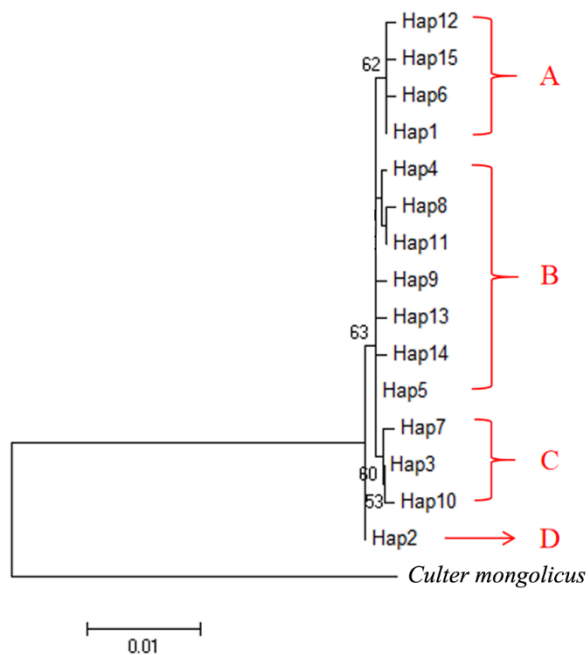


Figure 3 Neighbor-joining phylogenetic tree of 15 haplotypes of *A. nigrocauda* samples from five different sites in upper Yangtze River, China, inferred from *cyt b* sequences

A to D represent four clades.

populations, except for between CSR and MDR. The membership proportions of the four inferred clusters in each sample are listed in Supplementary Table S3. After removing the individuals that could not be assigned to one of the four clusters, the remaining samples from each population formed a single cluster, except for the CSR and MDR populations, which formed a common cluster. AMOVA revealed significant genetic differences among the four clusters ($F_{ST}=0.16596$, $P < 0.05$) (Table 9).

DISCUSSION

Genetic diversity

The present study indicated that *A. nigrocauda* had lower genetic diversity compared to other endemic fishes in the

upper Yangtze River. For instance, Li et al. (2018) reported haplotype and nucleotide diversities for the *Hemiculterella sauvaigi* from the Chishui River of 0.895 and 0.487%, respectively. Zhang & Tan (2010) reported average observed and expected heterozygosities for the largemouth bronze gudgeon (*Coreius guichenoti* Sauvage et Dabry) from the upper Yangtze River of 0.838 and 0.841, respectively. The genetic diversity of *A. nigrocauda* from Longxi River was the lowest among the five sample sites, which could be attributed to population fragmentation due to the eight constructed dams along that river (Wang, 1994). Dams can decrease genetic diversity and increase genetic drift by reducing effective population size and limiting gene flow among populations (Jager et al., 2001). Hänfling & Weetman (2006) found that the genetic diversity of isolated upstream river sculpin (*Cottus gobio*) population was lower than that of downstream population, and Zhao et al. (2016) found the same result in fragmented *Sinibrama macrops* populations in Min River, China.

Comparison between our results and those of Liu et al. (2005) indicated that the genetic diversity of *A. nigrocauda* has declined sharply in the upper Yangtze River from 2001 to 2016. This loss of genetic diversity could be attributed to adverse anthropogenic factors, such as damming, overfishing, and water pollution. *Ancherythroculter nigrocauda* is a cyprinid fish endemic to the upper Yangtze River, and has developed a high degree of adaptability to the lotic environment of this region during its long evolution. However, the Three Gorges Project and Jinsha River Project in the upper Yangtze River have fragmented the river and transformed the free-flowing water into a lacustrine environment (Neraas & Spruell, 2001; Nilsson et al., 2005), which has reduced the natural habitats of *A. nigrocauda*. Thus, the populations of *A. nigrocauda* have decreased accordingly (Park et al., 2003). Moreover, overfishing, which is common in the Yangtze River (Gao, 2008; Liu, 2013; Wang et al., 2015; Xiong et al., 2016; Zhu & Chang, 2008), has probably reduced population size and genetic diversity of *A. nigrocauda* through fishing mortality. Similarly, water pollution (He et al., 2011; Liu et al., 2009; Xu, 2010; Yi et al., 2016) in the upper Yangtze River has also probably reduced population size by imperiling the feeding and reproductive biology of the fish.

Population structure

Both *cyt b* and SSR markers showed significant genetic differentiations among the populations in the present study. Based on the pairwise F_{ST} values, no significant genetic differentiation was observed between the MDS and DNR populations in the *cyt b* analysis. However, this differed from the SSR analysis results, suggesting that the SSR marker may be more sensitive than the *cyt b* marker in studying genetic variation, especially among closely related populations or populations sampled over a reduced geographical scale (Estoup et al., 1998; Harrison & Hastings, 1996).

According to our reanalysis, however, no genetic differentiation was found among the populations by *cyt b* analysis in Liu et al. (2005), which might be due to the small

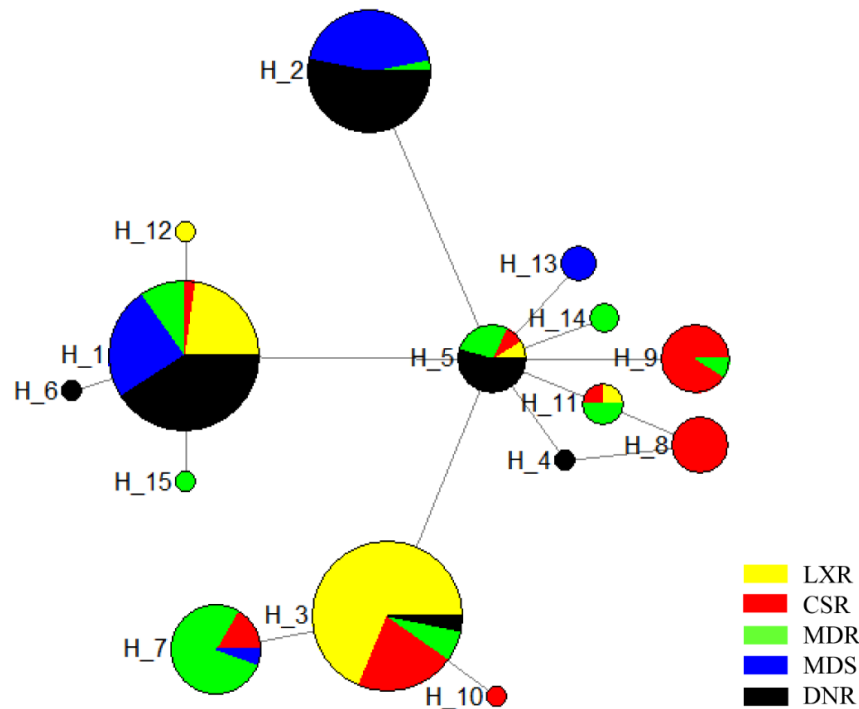


Figure 4 Median-joining network of 15 haplotypes of *A. nigrocauda* samples from five different sites in upper Yangtze River, China, inferred from *cyt b* gene sequences

Each circle represents a haplotype, and its size is proportional to its total frequency.

sample size. Studies have shown that modification of ecological environments by human activities can affect fish population genetic structure (Meldgaard et al., 2003; Zhang et al., 2011). *Ancherythroculter nigrocauda* is a sedentary fish with a relatively small scope of activity and adhesive egg reproduction (Cao et al., 2007); thus, gene flow between different populations mainly depends on the dispersal of larvae. Before the TGD impounding of water to 135 m in 2003, the upper Yangtze River was a free-flowing water environment, which allowed the *A. nigrocauda* larvae to disperse over longer distances, leading to gene flow between different populations. This likely explains the lack of genetic differentiation among the studied populations in Liu et al. (2005). After 2003, however, the TGD changed the hydrological regime in the upper Yangtze River from lotic to lentic. This reduced the dispersal range of *A. nigrocauda* larvae and decreased gene flow between the different populations, resulting in genetic differentiation due to isolation of the fish populations (Young et al., 2018). Similarly, damming and isolation of populations reduced the population sizes, leading to genetic differentiation via increased genetic drift (Jager et al., 2001). Low level gene flow among populations resulting from restricted dispersal of larvae has been well documented in many studies (Dong et al., 2012; Han et al., 2015; Yang & Li, 2018).

Demographic history

A unimodal pattern of mismatch distribution is indicative of

populations that have experienced recent expansions, whereas multimodal distributions are indicative of populations at demographic equilibrium (Rogers & Harpending, 1992; Slatkin & Hudson, 1991). Moreover, negative and statistically significant values of Tajima's D or Fu's F_s tests are suggestive of populations that have experienced expansion (Fu, 1997; Tajima, 1989). Therefore, in the present study, the neutrality test and mismatch distribution results suggested no expansion of the *A. nigrocauda* populations. Generally, bottleneck effects are always followed by population expansion. Here, thus, it was reasonable that no bottleneck effects were detected for any of the five populations.

Implications for conservation

Genetic diversity is influenced by many factors, including historical and anthropogenic factors. In the present study, no bottlenecks or population expansions were detected; thus, anthropogenic activity was likely responsible for the loss of genetic diversity of *A. nigrocauda*. Therefore, it is necessary to decrease the negative impacts of anthropogenic activity on the *A. nigrocauda* populations. As the dams in the Longxi River are producing little electric power or are deserted, it is suggested that these dams should be removed preferentially to restore river connectivity. In addition, further studies are needed to investigate and minimize the adverse impacts of the TGD on fish populations. A 10-year fishing ban in the Chishui River has been in place since January 2017 to help in the recovery of fish stocks. In the same vein, we suggest that

Table 5 Haplotype frequency distribution, haplotype diversity, and nucleotide diversity of three *A. nigrocauda* populations in 2001 and 2016 based on 546 bp cyt *b* sequence

Haplotype	LXR (2001)	LXR (2016)	MDR (2001)	MDR (2016)	CSR (2001)	CSR (2016)
Hap1			1	14		3
Hap2					1	7
Hap3	10	42	1	4	5	14
Hap4	1	21	1	16		14
Hap5		1				
Hap6				1		
Hap7				1		
Hap8	1					
Hap9	1					
Hap10	1					
Hap11	1					
Hap12	2		1		1	
Hap13	4				2	
Hap14	1					
Hap15	1					
Hap16	1					
Hap17			1			
Hap18			1			
Hap19			1		1	
Hap20					1	
Hap21					1	
Haplotype diversity	0.812	0.469	1	0.656	0.833	0.707
Nucleotide diversity	0.004 36	0.000 90	0.004 88	0.002 04	0.004 00	0.001 77

Data are based on a total of 43 and 138 samples of *A. nigrocauda* sampled from upper Yangtze River, China, in 2001 and 2016, respectively.

Table 6 Pairwise F_{ST} values between *A. nigrocauda* populations in 2016 (below diagonal) and 2001 (above diagonal) based on 546 bp cyt *b* sequences of 138 and 43 samples, respectively, from three different sites in upper Yangtze River, China

Population	LXR	CSR	MDR
LXR		- 0.018 62	0.013 38
CSR	0.095 23*		- 0.044 13
MDR	0.185 18*	0.092 84*	

*: $P < 0.05$ after Bonferroni correction.

fishing in the main stream of the Yangtze River and its tributaries should be eliminated. Moreover, as water pollution is another significant issue, the government should prevent factories from discharging wastewater into the Yangtze River and undertake centralized management of domestic wastewater.

Artificial breeding and stocking are feasible methods to increase fish abundance, although the sources and numbers of parental fish are important (Dudgeon, 2011; Fu et al., 2003). In the present study, there were significant genetic

Table 7 Parameters of genetic variation and P -values for heterozygote excess test of five *A. nigrocauda* populations, inferred from 14 pairs of SSR markers

Population	<i>A</i>	<i>Ar</i>	<i>Ho</i>	<i>He</i>	<i>P</i>
LXR(32)	4.929	4.817	0.595	0.649	0.428
CSR(31)	7.429	7.321	0.652	0.727	0.879
MDR(32)	7.000	6.817	0.594	0.665	0.998
MDS(32)	8.571	8.312	0.694	0.765	0.914
DNR(34)	10.286	9.876	0.746	0.816	0.923

Data are based on 161 samples of *A. nigrocauda* from upper Yangtze River, China. *A*: Observed allele; *Ar*: Allelic richness; *Ho*: Observed heterozygosity; *He*: Expected heterozygosity. Numbers in brackets indicate number of individuals from each sample site.

differentiations among the populations, and STRUCTURE analysis showed that there were four distinct genetic clusters. Therefore, it would be necessary to use a wide variety of parental fish in the artificial breeding of *A. nigrocauda* to increase the quality of seed stock from hatcheries, which

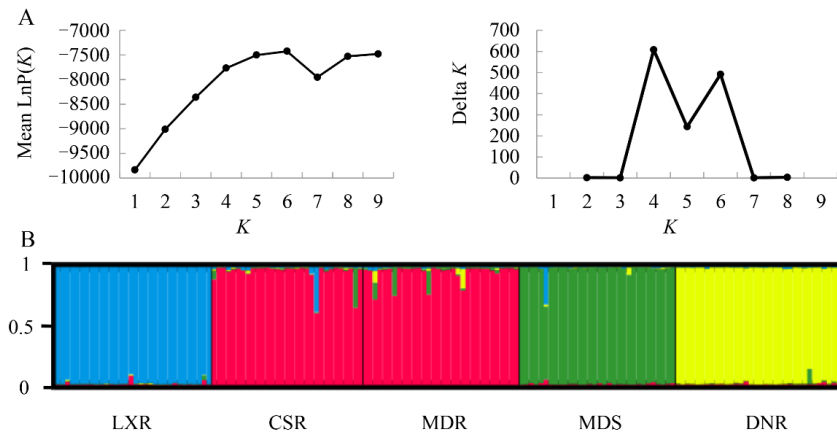


Figure 5 STRUCTURE analysis based on genotypes at 14 microsatellite loci from 161 unique *A. nigrocauda* individuals sampled from five different sites in upper Yangtze River, China

A: Estimations of mean log probability $\text{LnP}(K)$ and ΔK values for $K=1-9$ using Structure Harvester. $K=4$ is shown as the optimal value; B: Proportions of each individual assigned to each inferred cluster. Four colors indicate four inferred distinct genetic clusters. Sampling sites of individuals are shown at the bottom.

Table 8 Membership proportions of four inferred genetic clusters in five populations based on STRUCTURE analysis of SSR data

Population	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Total sample size (n)
LXR	0.008	0.003	0.003	0.986	32
CSR	0.957	0.017	0.006	0.021	31
MDR	0.954	0.026	0.014	0.006	32
MDS	0.008	0.973	0.006	0.014	32
DNR	0.006	0.008	0.981	0.004	34

Table 9 Analysis of molecular variance (AMOVA) for four inferred genetic clusters based on STRUCTURE analysis of SSR data

Source of variation	df	Sum of squares	Variance component	Percentage variation	F_{ST}	P
Among clusters	3	243.265	1.008 81	16.60	0.165 96	0
Within clusters	306	1 551.342	5.069 74	83.40		
Total	309	1 794.606	6.078 55			

would ensure better survivability during river reintroduction. Moreover, the *A. nigrocauda* populations in the upper Yangtze River need to be managed as multiple genetic units.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests

AUTHORS' CONTRIBUTIONS

H.Z.L., W.X.C., and X.G. designed the study and revised the manuscript. D. D. Z. performed the laboratory work and wrote the manuscript. W. J. L. helped in data analysis. All authors read and approved the final version of the manuscript.

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