INTRODUCTION

In riverine ecosystems, dams have been well known as one of the most negative anthropogenic impacts on fish populations (Saunders, Hobbs, & Margules, 1991). Dams reduce fish populations by blocking migration routes, and alter fish community structure upstream and downstream through regulating flow (Agostinho, Pelicice, & Gomes, 2008; Gao, Zeng, Wang, & Liu, 2010; Neraas & Spruell, 2001; Quinn & Kwak, 2003). Moreover, dams decline genetic diversity of fish populations and cause genetic differentiation among separated populations. For instance, Meldgaard, Nielsen, and Loeschcke (2003) found the construction of dams apparently influenced the genetic structure of European grayling in the Skjern River. Faulks, Gilligan, and Beheregaray (2011) also demonstrated the appearance of genetic differentiation and loss of genetic diversity in the Macquarie Perch after damming. However, the previous studies mainly focused on the genetic impacts of dams on migratory fish, rather than resident fish.
an important economic fish and aquaculture species in China. In the past decades, the abundance and distribution area of this species were reducing. Yan (2002) found that population of this species still remained abundant in the Longxi River, a tributary of the upper Yangtze River, in Sichuan Province, China. This river is 97 km long with drainage area of 512 km². Eight dams have been constructed on the Longxi river since 1920s (Figure 1; Table S1; Wang, 1994) and fragmented the populations of *A. nigrocauda*. There were some studies suggested that the growth and reproduction of the populations of *A. nigrocauda* in Longxi River have been adversely influenced, it might be related to damming (Liu, 2013; Liu et al., 2013). However, impacts of dams on the genetic diversity and population structure of *A. nigrocauda* in Longxi River have been little investigated.

In this study, samples of *A. nigrocauda* were collected above and below the dams in the Longxi River. We analyzed genetic diversity and population structure of the fragmented populations by using cyt b gene and SSR markers. Our objective was to understand the genetic impacts of dams on resident fish populations.

2 | MATERIALS AND METHODS

2.1 | Samples collection and DNA extraction

Samples of *A. nigrocauda* were collected above and below the cascade dams in Longxi River in 2016. A total of 52 samples were collected from the Yulong Lake (YLL), which is above the cascade dams. Another 64 samples were collected from the reach between the seventh dam (Kuifeng Dam) and eighth dam (Dongwo Dam) (DK). The two sampling sites are separated by 7 hydroelectric dams (Figure 1). For DNA extraction, the tissue samples from the dorsal muscle of these samples were preserved in 95% ethanol and stored at −20°C. Total DNA was extracted using standard proteinase K digestion followed by phenol/chloroform extraction (Kocher et al., 1989).
2.2 | MtDNA amplification and sequencing

The fragments containing the mtDNA cyt b gene were obtained using polymerase chain reaction (PCR) amplification with the primer sets L14724 5′-GAC TTG AAA AAC CAC CGT TG-3′ and H15915 5′-CTC CGA TCT CGT GAT TAC AAG AC-3′ (Xiao, Zhang, & Liu, 2001). PCR was performed at an initial denaturation step at 94°C for 3 min, followed by 28 cycles at 94°C for 30 s, 54°C for 45 s, 72°C for 1 min, and a final extension at 72°C for 8 min. The amplified fragments were purified with a BioStar glass-milk DNA purification kit following the manufacturer’s instruction. The purified fragments were sequenced by Shanghai DNA Biotechnologies Company.

2.3 | MtDNA sequence analysis

Nucleotide sequences were aligned and refined manually with MEGA7 (Kumar, Stecher, & Tamura, 2016). The number of variable site, haplotype and nucleotide diversity, and haplotype frequency were calculated with DnaSP v5.10 (Librado & Rozas, 2009). Arlequin version 3.0 (Excoffier, Laval, & Schneider, 2005) was used to perform an analysis of molecular variance (AMOVA) to examine the degree of differentiation between the two populations (Excoffier, Smouse, & Quattro, 1992).

2.4 | SSR amplification and electrophoresis

PCR was performed at an initial denaturation step at 94°C for 3 min, followed by 28 cycles at 94°C for 30 s, annealing temperature for 40 s, 72°C for 1 min, and a final extension at 72°C for 8 min. The specific sequences, repeat motif, optimum annealing temperature and accession number of GenBank for each primer was listed in Table S2. The first 12 loci were developed using the fast isolation by specific sequences, repeat motif, optimum annealing temperature 40°C, 72°C for 1 min, and a final extension at 72°C for 8 min. The amplified fragments were purified with a BioStar glass-milk DNA purification kit following the manufacture’s instruction. The purified fragments were sequenced by Shanghai DNA Biotechnologies Company.

The number of alleles (A), observed (Ho) and expected (He) heterozygosity, and polymorphic information content (PIC) per locus were calculated by Cervus3.0 (Kalinowski, Taper, & Marshall, 2007). Standardized allelic richness (Ar) and allele frequency distribution of per population at each locus were calculated using Fstat software v2.9.3.2 (Goudet, 2001). The effective population size (Ne) was estimated by the Colony Version 2.0.6.4 (Jones & Wang, 2010). Arlequin version 3.0 (Excoffier et al., 2005) was used to perform an analysis of molecular variance (AMOVA) to examine the degree of differentiation between the two populations (Excoffier et al., 1992). Wilcoxon signed rank test was performed to detect the difference of allele frequency distribution between the two populations.

We calculated the M values (Garza & Williamson, 2001) to detect whether populations have suffered from recent bottleneck effect. The formula is given as $M = k/r$ where k is the number of alleles at a given loci in a population sample, and r is the difference between the maximum and the minimum number of repeats. Loci were excluded from analysis that were monomorphic or that contained different repeat unit sizes (Garza & Williamson, 2001; Hundertmark & Daele, 2010). Based on simulations and experimental data, populations that have experienced recent bottlenecks with the mean value of $M < 0.68$ (Garza & Williamson, 2001; Reid, Wilson, Mandrak, & Carl, 2007).

The detection of sibship among individuals was inferred by Colony Version 2.0.6.4 (Jones & Wang, 2010). The parameters were set according to actual situation and the number of threads for the run was set to 10. The sibships were only accepted for posterior probability values of more than 0.75 (Schunter, Pascual, Garza, Raventos, & Macpherson, 2014).

3 | RESULTS

3.1 | Cyt b marker

3.1.1 | Genetic characterization and diversity

Following alignment, 1,140 bp of cyt b gene were obtained for 116 individuals. No deletions and insertions were observed. Among the 1,140 bp, five sites were variable, of which two were parsimony informative. Only 6 haplotypes (GenBank accession number: MG756603-MG756608) were identified from the total 116 individuals. The haplotype and nucleotide diversity of the DK population were 0.488% and 0.084% respectively, and for the YLL population, they were 0.486% and 0.082% respectively (Table 1).

3.1.2 | Population differentiation

Hap1, Hap2 and Hap3 were shared by the two populations, and the frequency of each haplotype in each population was nearly equal. Hap4, Hap5 and Hap6 were only found within one population, but these three haplotypes only represented a single individual with relatively low frequency of 0.0086, respectively (Table 1). So there were only slight differences between the haplotype frequency...
### TABLE 1
Number of samples, haplotype frequencies and indices of genetic diversity with standard deviations (SE) for the two populations

<table>
<thead>
<tr>
<th>Population</th>
<th>Haplotype</th>
<th>Hap1</th>
<th>Hap2</th>
<th>Hap3</th>
<th>Hap4</th>
<th>Hap5</th>
<th>Hap6</th>
<th>Haplotype diversity</th>
<th>Nucleotide diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DK(64)</td>
<td>0.6563</td>
<td>0.2969</td>
<td>0.0156</td>
<td>0.0156</td>
<td>0.0156</td>
<td>0.488 ± 0.050</td>
<td>0.00084 ± 0.00009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YLL(52)</td>
<td>0.6538</td>
<td>0.3077</td>
<td>0.0192</td>
<td>0.0192</td>
<td></td>
<td>0.486 ± 0.053</td>
<td>0.00082 ± 0.00009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total(116)</td>
<td>0.6552</td>
<td>0.3017</td>
<td>0.0172</td>
<td>0.0086</td>
<td>0.0086</td>
<td>0.0086</td>
<td>0.483 ± 0.036</td>
<td>0.00082 ± 0.00006</td>
<td></td>
</tr>
</tbody>
</table>

Note. Numbers in brackets indicate the number of individuals from each population, Hap1-Hap6 represent the six haplotypes.

### TABLE 2
Summary statistics of SSR analysis of the two Ancherythroculter nigrocauda populations

<table>
<thead>
<tr>
<th>Population</th>
<th>Locus</th>
<th>N</th>
<th>Ho</th>
<th>He</th>
<th>A</th>
<th>Ar</th>
<th>M</th>
<th>PIC</th>
<th>Size range (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DK</td>
<td>An1</td>
<td>64</td>
<td>0.672</td>
<td>0.715</td>
<td>6</td>
<td>5.757</td>
<td>0.375</td>
<td>0.660</td>
<td>146−210</td>
</tr>
<tr>
<td></td>
<td>An10</td>
<td>64</td>
<td>0.531</td>
<td>0.585</td>
<td>5</td>
<td>4.594</td>
<td>0.509</td>
<td></td>
<td>140−156</td>
</tr>
<tr>
<td></td>
<td>An21</td>
<td>63</td>
<td>0.619</td>
<td>0.649</td>
<td>6</td>
<td>5.810</td>
<td>0.857</td>
<td>0.608</td>
<td>176−204</td>
</tr>
<tr>
<td></td>
<td>An52</td>
<td>64</td>
<td>0.547</td>
<td>0.581</td>
<td>9</td>
<td>7.944</td>
<td>0.474</td>
<td>0.508</td>
<td>189−227</td>
</tr>
<tr>
<td></td>
<td>An63</td>
<td>64</td>
<td>0.344</td>
<td>0.625</td>
<td>6</td>
<td>5.789</td>
<td>0.573</td>
<td></td>
<td>172−190</td>
</tr>
<tr>
<td></td>
<td>An68</td>
<td>64</td>
<td>0.750</td>
<td>0.777</td>
<td>8</td>
<td>7.905</td>
<td>0.727</td>
<td>0.735</td>
<td>156−178</td>
</tr>
<tr>
<td></td>
<td>An70</td>
<td>64</td>
<td>0.594</td>
<td>0.495</td>
<td>8</td>
<td>7.147</td>
<td>0.381</td>
<td>0.439</td>
<td>141−183</td>
</tr>
<tr>
<td></td>
<td>An76</td>
<td>64</td>
<td>0.641</td>
<td>0.667</td>
<td>6</td>
<td>5.797</td>
<td>0.300</td>
<td>0.624</td>
<td>262−302</td>
</tr>
<tr>
<td></td>
<td>An86</td>
<td>64</td>
<td>0.656</td>
<td>0.676</td>
<td>8</td>
<td>7.391</td>
<td>0.615</td>
<td>0.639</td>
<td>220−272</td>
</tr>
<tr>
<td></td>
<td>An95</td>
<td>64</td>
<td>0.500</td>
<td>0.589</td>
<td>5</td>
<td>4.959</td>
<td>0.556</td>
<td>0.498</td>
<td>160−178</td>
</tr>
<tr>
<td></td>
<td>An98</td>
<td>64</td>
<td>0.578</td>
<td>0.672</td>
<td>5</td>
<td>4.757</td>
<td>0.333</td>
<td>0.598</td>
<td>166−196</td>
</tr>
<tr>
<td></td>
<td>An114</td>
<td>62</td>
<td>0.387</td>
<td>0.753</td>
<td>11</td>
<td>10.108</td>
<td>0.714</td>
<td></td>
<td>144−204</td>
</tr>
<tr>
<td></td>
<td>hwb03</td>
<td>62</td>
<td>0.629</td>
<td>0.695</td>
<td>9</td>
<td>8.610</td>
<td>0.818</td>
<td>0.655</td>
<td>180−202</td>
</tr>
<tr>
<td></td>
<td>hwb08</td>
<td>62</td>
<td>0.548</td>
<td>0.637</td>
<td>4</td>
<td>3.823</td>
<td>0.267</td>
<td>0.555</td>
<td>261−291</td>
</tr>
<tr>
<td></td>
<td>hwb16</td>
<td>64</td>
<td>0.672</td>
<td>0.740</td>
<td>8</td>
<td>7.677</td>
<td>0.216</td>
<td>0.689</td>
<td>230−304</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>0.611</td>
<td>0.652</td>
<td>6.692</td>
<td>6.321</td>
<td>0.493</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YLL</td>
<td>An1</td>
<td>51</td>
<td>0.608</td>
<td>0.686</td>
<td>6</td>
<td>6.000</td>
<td>0.462</td>
<td>0.623</td>
<td>146−198</td>
</tr>
<tr>
<td></td>
<td>An10</td>
<td>52</td>
<td>0.538</td>
<td>0.656</td>
<td>4</td>
<td>3.981</td>
<td>0.580</td>
<td></td>
<td>144−156</td>
</tr>
<tr>
<td></td>
<td>An21</td>
<td>52</td>
<td>0.788</td>
<td>0.763</td>
<td>10</td>
<td>9.923</td>
<td>1.111</td>
<td>0.730</td>
<td>176−212</td>
</tr>
<tr>
<td></td>
<td>An52</td>
<td>52</td>
<td>0.654</td>
<td>0.623</td>
<td>7</td>
<td>6.961</td>
<td>0.500</td>
<td>0.570</td>
<td>189−217</td>
</tr>
<tr>
<td></td>
<td>An63</td>
<td>52</td>
<td>0.596</td>
<td>0.563</td>
<td>5</td>
<td>5.000</td>
<td>0.516</td>
<td></td>
<td>172−184</td>
</tr>
<tr>
<td></td>
<td>An68</td>
<td>51</td>
<td>0.686</td>
<td>0.787</td>
<td>7</td>
<td>7.000</td>
<td>0.778</td>
<td>0.747</td>
<td>160−178</td>
</tr>
<tr>
<td></td>
<td>An70</td>
<td>52</td>
<td>0.519</td>
<td>0.531</td>
<td>4</td>
<td>4.000</td>
<td>0.211</td>
<td>0.475</td>
<td>141−179</td>
</tr>
<tr>
<td></td>
<td>An76</td>
<td>52</td>
<td>0.673</td>
<td>0.663</td>
<td>5</td>
<td>5.000</td>
<td>0.385</td>
<td>0.613</td>
<td>262−288</td>
</tr>
<tr>
<td></td>
<td>An86</td>
<td>52</td>
<td>0.712</td>
<td>0.748</td>
<td>9</td>
<td>8.923</td>
<td>0.750</td>
<td>0.709</td>
<td>232−280</td>
</tr>
<tr>
<td></td>
<td>An95</td>
<td>52</td>
<td>0.481</td>
<td>0.584</td>
<td>5</td>
<td>4.981</td>
<td>0.556</td>
<td>0.496</td>
<td>160−178</td>
</tr>
<tr>
<td></td>
<td>An98</td>
<td>52</td>
<td>0.577</td>
<td>0.675</td>
<td>5</td>
<td>5.000</td>
<td>0.294</td>
<td>0.606</td>
<td>166−200</td>
</tr>
<tr>
<td></td>
<td>An114</td>
<td>51</td>
<td>0.392</td>
<td>0.788</td>
<td>9</td>
<td>9.000</td>
<td>0.752</td>
<td></td>
<td>144−220</td>
</tr>
<tr>
<td></td>
<td>hwb03</td>
<td>52</td>
<td>0.731</td>
<td>0.635</td>
<td>7</td>
<td>6.942</td>
<td>0.467</td>
<td>0.560</td>
<td>172−202</td>
</tr>
<tr>
<td></td>
<td>hwb08</td>
<td>52</td>
<td>0.558</td>
<td>0.658</td>
<td>5</td>
<td>4.962</td>
<td>0.250</td>
<td>0.580</td>
<td>261−301</td>
</tr>
<tr>
<td></td>
<td>hwb16</td>
<td>52</td>
<td>0.692</td>
<td>0.777</td>
<td>8</td>
<td>8.000</td>
<td>0.267</td>
<td>0.733</td>
<td>236−296</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>0.632</td>
<td>0.676</td>
<td>6.308</td>
<td>6.283</td>
<td>0.502</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. N: sample size; Ho: observed heterozygosity; He: expected heterozygosity; A: observed allele; Ar: allelic richness; PIC: polymorphism information content. The calculation of mean values excluded the loci An63 and An114.

Indicates significant deviations from HWE after standard Bonferroni correction (p < 0.05). An10 was excluded from the calculation of M value due to the presence of different repeat unit size.
distribution of the two populations. The value of $F_{st}$ between the two populations was $-0.01677 (p = 0.99707 > 0.05)$, suggesting no genetic differentiation between them.

### 3.2 SSR marker

#### 3.2.1 Characteristics of SSR loci

The same 116 samples and 15 loci were used for SSR analysis. All the loci were polymorphic in the two populations and easily scored. After Bonferroni correction, significant deviations from HWE were found at locus An63 and An114 in the DK population, An68 and An114 in the YLL population. All significant deviations from HWE resulted from a deficit of heterozygotes (Table 2). No LD was found among SSR loci pairs after Bonferroni correction. Micro-Checker detected no large allele dropout across all loci in the two populations. However, locus An63 suffered from possible scoring errors due to stutters in the DK population. And locus An63, An114 in the DK population and locus An114 in the YLL population showed an excess of homozygotes, indicating the possibility of null alleles. Therefore, due to the possible presence of null alleles and deviations from HWE, loci An63 and An114 were excluded in the subsequent data analysis.

#### 3.2.2 Genetic diversity and bottleneck effect

The average number of alleles, allelic richness and observed and expected heterozygosity of DK population were 6.692, 6.321, 0.611 and 0.652 respectively, and for YLL population, they were 6.308, 6.283, 0.632 and 0.676 respectively. The mean M values of the two populations were 0.493 and 0.502 respectively (Table 2). The $N_e$ of DK and YLL populations were estimated to be 66 and 58 respectively.

### 3.3 Population differentiation

The allele frequency distribution of the two populations at each locus was showed in Table S3, and the value of $p$ of Wilcoxon Signed Rank Test was 0.656 ($p > 0.05$), showing no significant difference between the two populations in allele frequency distribution. Similarly, for SSR analysis, the $F_{st}$ value between the two populations was 0.00259 ($p = 0.81427 > 0.05$), suggesting no genetic differentiation between them.

### 3.4 Sibship among the samples

The results of sibship test of every two individuals, one from the DK population and the other from the YLL population, showed that there was no full-sibling relationship, but included 11 pairs of half-sibling relationships. There were 10 individuals from DK population and 8 individuals from YLL population participated in the construction of 11 pairs of half-sibling relationships, and their matching relationships were listed in Table 3.

### 4 DISCUSSION

#### 4.1 Genetic diversity

In the present study, cyt $b$ gene and SSR markers were used to evaluate the genetic diversity. For cyt $b$ gene, the haplotype and nucleotide diversity of the two populations were lower compared to those of Liu, Zhu, Wang, and Tan (2005), sampled of $A. nigrocauda$ from the same locality with DK population in 2001, with the value of 0.812 and 0.44% respectively. Moreover, the haplotype frequency distribution of DK population compared with those of Liu et al. (2005) was displayed by Figure 2, it showed that nine haplotypes were lost but only increased one haplotype in the present study (The sequence of each haplotype of Liu et al. (2005) were downloaded from NCBI (GenBank accession number: AY493869-AY493886), then we recovered the data of Liu et al. (2005) by referring the haplotype frequency distribution. Because the obtained length of cyt $b$ was only 546 bp in Liu et al. (2005), we aligned and edited our sequences to 546 bp to do a better comparison). While using SSR as the genetic marker, the average expected heterozygosities were 0.652 and 0.676 for the two populations respectively, it showed a lower level of genetic diversity while compared with other freshwater fishes in the upper Yangtze River (Liu et al., 2017; Zhang, Duan, Cao, Wang, & Tan, 2011; Zhang & Tan, 2010). Three of the used loci (hwb03, hwb08, hwb16) were already published in Sun et al. (2014), and we found that the average number of alleles, observed heterozygosity and expected heterozygosity of the three loci in the present study were smaller than those in Sun et al. (2014), which the samples were even collected from cultured population in Wu Lake, Wuhan.

For cyt $b$ gene, the low haplotype diversity ($h < 0.5$) and nucleotide diversity ($\pi<0.5\%$) suggested that $A. nigrocauda$ in the two localities might have suffered from bottleneck effect recently (Grant & Bowen, 1998). For SSR analysis, both the mean values of $M$ were lower than the threshold value ($M = 0.68$), suggesting the two populations had undergone a recent bottleneck. Therefore, loss of
genetic diversity and recent bottleneck effect were obviously detected in the two populations of *A. nigrocauda* in the Longxi River, it might be related to damming.

Dams can fragment the riverine fish population and lower its genetic diversity by reducing effective population size, increasing genetic drift and limiting gene flow between populations (Jager, Chandler, Lepla, & Winkle, 2001). Lower genetic diversity had been measured in populations of steelhead (*Oncorhynchus mykiss*) (Nielsen, Hansen, & Loeschcke, 1997) and *Sinibrama macrops* (Zhao, Chenoweth, Liu, & Liu, 2016) isolated by dams. Of course, some other influences could not been excluded in this study. It had been found that the degree of fishing on *A. nigrocauda* in the Longxi River was over the appropriate limit from 2001 to 2002 analyzed by the Yield per recruit (Gao, 2007), so was the year from 2011 to 2012 (Liu, 2013). Xiao and Hu (2015) indicated that the Longxi River had suffered from quite heavy pollution because of the discharge of waste water from industry, agricultural non-point and residency in recent years.

### 4.2 Population differentiation

Generally speaking, dams can cause genetic differentiation among fragmented populations due to its blocking effect on gene flow and the reduction in effective population size and increase in genetic drift associated with small, isolated populations (Hanfling & Weetman, 2006; Jager et al., 2001; Meldgaard et al., 2003). While our results indicated that there was no genetic differentiation between the two populations, it might be attributed to dams being permeable to downstream migration in the Longxi River and the high level of fecundity of *A. nigrocauda*.

It is notable that all the dams in the Longxi River are low-head dams that look like a waterfall with water passing through above them in flooding season. Therefore, although the adhesive eggs of *A. nigrocauda* can’t drift downstream, the larvae can drift downstream passively in the flooding season. In addition, *A. nigrocauda* reproduces large number of offspring (absolute fecundity varied between 11,300 and 504,630 eggs, with the mean value of 162,377) from April to August during the flooding season (Liu et al., 2013), making its larvae drift to the downstream from the Yulong Lake largely and easily. Eleven pairs of half-sibling relationships between the two populations confirmed the presence of individual movement and gene flow between them, which could weaken the genetic differentiation caused by the blocking effect of dams. This one-way individual movement were supported by many studies, where low-head dams act as barriers to fish move upstream but allowing individuals to drift downstream (Junker et al., 2012; Meldgaard et al., 2003; Yamamoto, Morita, Koizumi, & Maekawa, 2004).

However, some studies found that small isolated populations were prone to take place genetic drift that could increase genetic differentiation (Hänfling & Weetman, 2006; Jager et al., 2001). In the present study, the two fragmented populations are smaller than before, especially for DK population, and recent bottleneck effects and small $N_e$ were found in the two populations, so the genetic drift will be more intensified in the future if the living condition doesn’t get improvement. Over time, when the genetic drift was more influential than one-way gene flow, there might be genetic differentiation between the two populations.

### ACKNOWLEDGEMENTS

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ZHAI et al.


SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.