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Population Structure of Wild *Schizothorax kozlovi* in the Upper Yangtze River Based on mtDNA and Stable Isotopes, and Their Relationship with Ambient Temperature

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Abstract: *Schizothorax kozlovi*, as an endemic and vulnerable fish of the upper Yangtze River in China, faces many threats. In order to expose the population structure of wild *S. kozlovi*, the carbon and oxygen isotopic ratios in the otoliths, and the gene sequences of two common mitochondrial markers (Cyt*b* and COI) were investigated in four sampling locations, and then their relationship with ambient temperature was further investigated. In general, it exhibits limited geographic population structuring of *S. kozlovi* in the upper Yangtze River by both mtDNA and stable isotopes. The values of otolith stable isotope ratios varied from −15.30‰ to −12.37‰ for $\delta^{18}O$ and from −10.10‰ to −6.13‰ for $\delta^{13}C$. Significant relationships were revealed between stable isotope ratios and specific mean monthly water temperature variables (from November to March), indicating low temperature effect on otolith stable isotope ratios. Haplotype diversity and nucleotide diversity were 0.928 and 0.00778, both exhibiting high levels. A median-joining haplotype network indicated a mixture of geographical distribution but exhibited two distinct haplotype lineages (Clade I and Clade II). AMOVA detected that the higher percentage of genetic variance was within sampling locations (96.94%) and between two haplotype lineages (72.82%). Most F_{ST} values between sampling locations showed small levels of genetic differentiation except the differentiation between population SJ (Sanjiangkou) and JP (Jinping). Therefore, two haplotype lineages and population JP of *S. kozlovi* in the upper Yangtze River are suggested as three management units for conservation due to their moderate-to-great genetic differentiation and isolated habitat.

Keywords: *Schizothorax kozlovi*; otolith; stable isotope; mitochondrial DNA; temperature

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1. Introduction

Schizothorax kozlovi Nikolsky (Cypriniformes: Cyprinidae) is an endemic and economic fish of the upper Yangtze River in China, preferring a cold and running-water environment [1]. They are only found in the relatively low altitude area of the Qinghai–Tibet Plateau (namely as the third pole in the earth) and the Yunnan–Guizhou Plateau, such as the Jinsha River, Yalong River, Chishui River and Wujiang River [2–4]. As a locally distributed freshwater fish, no typical migration exists in *S. kozlovi*, but after the melting of ice and snow in spring, *S. kozlovi* may swim upstream to the tributaries, where the water temperature of the tributaries tends to recover rapidly [5]. Due to the influences of overfishing, water pollution, hydropower station and global climate changes, most habitats and spawning grounds of *S. kozlovi* are destroyed [1,6]. Their population magnitude has decreased significantly through field investigation by authors and other researchers in recent years, and wild individuals are rare in recent catches of the upper Yangtze River. This indicates that their ecological stability is vulnerable. In order to explore how these environmental changes influence the stability and suitability of their population, knowledge

of their population structure is fundamental for a sustainable management of conservation. However, minimal knowledge of its population structure is known so far.

Three pairs of fish otoliths (namely lapillus, sagitta and asteriscus) are mainly composed of calcium carbonate ($CaCO_3$). Otoliths grow as a series of layers that are variously interpreted as having annual and daily periodicities, depending on their dimensions, indicating the application of age estimation of fish from otoliths. Actually, otoliths are deposited undisturbed as layers, exhibiting acellular and metabolically inertness. Otoliths record the internal and external environment of the fish, remaining chemically (and isotopically) inert [7]. Stable isotopes of otoliths, especially stable oxygen and carbon isotope ratios ($^{18}O/^{16}O$ or $\delta^{18}O$; and $^{13}C/^{12}C$ or $\delta^{13}C$), have been reported to be highly correlated with the living environment of fish, such as temperature, salinity, the primary productivity and food resources [8–11], inferring that different environments inhabited by fish populations or stocks will originate signatures in the stable isotopes of otoliths. The stable isotopes of $\delta^{18}O$ and $\delta^{13}C$ from fish can also differ with latitude and location [12,13], hence, the variations in stable isotope compositions can serve as natural tags of different locations, suggesting different population management units [14]. They have been proven to be a cost-effective tool for fish stock identification and a natural marker for exploring the spatial structure and connectivity of fish populations in many species [14–21].

On the other hand, genetic diversity is defined as an important measure of population structure, exhibiting genetic composition patterns and genetic variability in the current population, or reflecting vicarious events from its past that may have shaped their population history. Because of the ability of mitochondrial DNA (mtDNA) sequences to detect population differences, phylogeographic patterns [22], historical demographic events [23] and the existence of cryptic species or subspecies [24], mtDNA genes, including Cyt*b*, COI and ND2, have been widely used [25–28].

In the present study, the carbon and oxygen isotopic ratios in the otoliths, and the gene sequences of two common mitochondrial markers (Cyt*b* and COI) of wild *S. kozlovi* captured from four locations in the upper Yangtze River were used to explore the spatial population structure, and then the correlations between stable isotopes and ambient temperature were further investigated. It was expected to seek out the population management units of *S. kozlovi*, which could provide scientific basis for the conservation of this species.

2. Materials and Methods

2.1. Field Sampling

Fishes from four locations along the upper Yangtze River were collected: Gangtuo in the Jinsha River (one section from Yushu to Yibin of the mainstream of the upper Yangtze River is called as Jinsha River) (GT), Shigu in the Jinsha River (SG), Sanjiangkou in the Shuiluo River (SJ) and Jinping in the Yalong River (JP) (Figure 1, Table 1). GT and SG were from the mainstream of the upper Yangtze River, while SJ and JP were from the tributaries (Shuiluo River and Yalong River) of the upper Yangtze River. For all samples, standard length (mm), body weight (g) and sex were recorded. One pair of lapillus (hereafter referred to as otoliths) were removed and stored in labelled plastic tubes. Collected fin rays were stored in 95% alcohol at −20 °C for genetic analyses.

During the fish collections, environmental surveys of the sampling locations recorded longitude, latitude and altitude. Water temperature (including mean annual water temperature, minimum annual water temperature, maximum annual water temperature and mean monthly water temperature) were automatically measured by HOBO Temperature Data Loggers UA-002-64 (Onset Company, Massachusetts, USA) every day for one year during the sampling. For example, water temperature was measured in 2017 at GT population and SJ population, in 2016 at SG population and JP population. In 2016 and 2017, all the otolith samples were collected, while in 2018 and 2019 some genetic samples were added in further analysis.

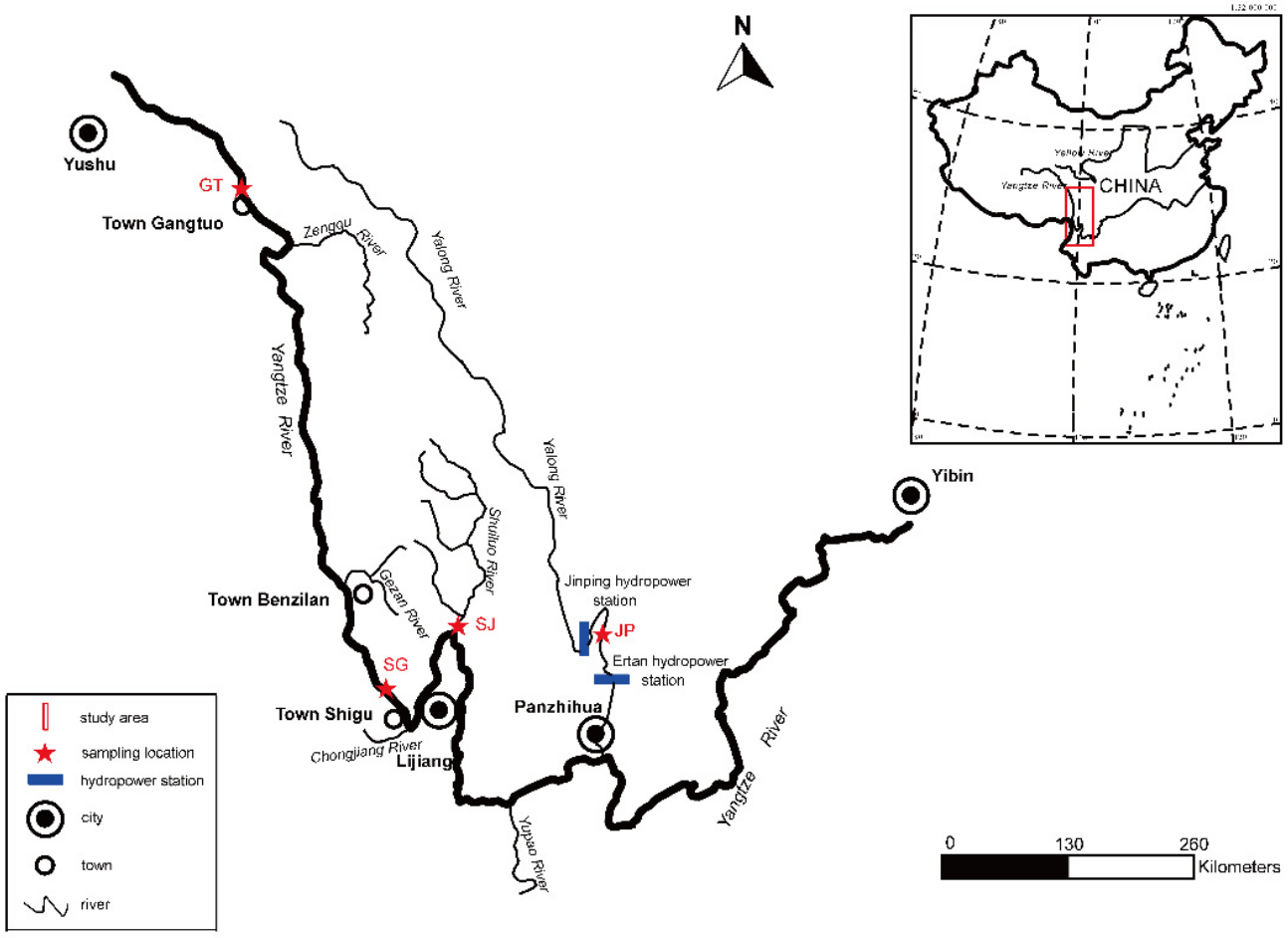

Figure 1. Sampling locations of *Schizothorax kozlovi* in the upper Yangtze River.

Table 1. Summary table of *Schizothorax kozlovi*, collected in the upper Yangtze River.

Location	Sample Year	SL (mm)	Otolith Mass (mg)	Estimated Age Range (Years)	Sampling Numbers
GT	2017, 2019	278.3 (11.3)	5.47 (0.61)	4–6	23, 7 (4:3)
SG	2016, 2017, 2018	172.0 (9.3)	2.18 (0.27)	1–3	45, 12 (5:7)
SJ	2017, 2018	244.9 (16.6)	3.98 (0.34)	3–6	18, 12 (6:6)
JP	2016	266.8 (6.2)	3.45 (0.24)	4–6	13, 12 (2:10)

Sampling numbers—Number of samples for genetic analysis, isotope analysis (Female:Male); GT—Gangtuo in the Jinsha River; SG—Shigu in the Jinsha River; SJ—Sanjiangkou in the Shuiluo River; JP—Jinping in the Yalong River. Mean and standard error (in brackets) are given for standard length (SL) and otolith mass.

2.2. Isotope Analysis

For each sample location, seven to twelve left otoliths were selected for stable isotope analysis, while right otoliths were used for age estimation. Prior to analysis, all otoliths were rinsed with deionized water, cleaned ultrasonically for 3 min, dried under vacuum at a temperature of 55 °C for 12 h, and weighted by an electronic analytical balance. Each otolith was enclosed by silver paper, ground with a pestle and the powder was carefully collected into a labelled plastic tube. The isotopic composition ($\delta^{18}O$ and $\delta^{13}C$) of the powdered otolith samples and water samples were analyzed by GasBench II-IRMS Delta V Advantage stable isotope mass spectrometry (Thermo Fisher Scientific Co., Massachusetts, USA). The $\delta^{18}O$ and $\delta^{13}C$ values were reported in conventional delta (δ) notation in per mil (‰), relative to the VPDB (otolith samples) and VSMOW (water samples): $\delta = [(Rsample - Rstandard)/Rstandard] \times 1000$ (‰), where R is the $^{18}O/^{16}O$ or $^{13}C/^{12}C$ isotopic ratio in

the sample or standard. Replicate analysis of internal standards gave an analytical precision of 0.2‰.

2.3. DNA Extraction, Amplification and Sequencing

Total genomic DNA was extracted using Tissue DNA Kit (D3396) (Omega Bio-Tek, Georgia, USA), following the manufacturer's specifications. Fragments of the mitochondrial cytochrome *b* (Cyt*b*) and cytochrome oxidase subunit I (COI) genes were amplified by means of polymerase chain reaction (PCR). The primers L14724 and H15915 for Cyt*b* [29] were used, and, for COI, the primers COI-F and COI-R [30] were used. Polymerase chain reactions (PCRs) were performed in a volume of 30 µL, containing the following: 15 µL of 2 × PowerTaqPCRMasterMix, 1 µL of each primer (10 mM), 1 µL DNA and 12 µL Milli-Q water to a final volume. The thermocycling protocol for both molecular markers involved denaturation for 5 min at 95 °C, 35 cycles for 30 s at 95 °C, 30 s for 58 °C, 1 min extension at 72 °C, and a final extension for 5 min at 72 °C. Amplifications were verified by electrophoresis on 1% agarose gels. Successful amplifications were sequenced in both directions (TIANYI HUIYUAN Inc., Wuhan, China) by using the same primers as employed for amplification.

2.4. Data Analysis

Before all analysis, the values from $\delta^{13}C$, $\delta^{18}O$, otolith mass and environmental variables were standardized by using z-score method. After testing for normality (Shapiro–Wilk Test, $p > 0.05$) and homogeneity of variances (Levene's Test, $p > 0.05$), $\delta^{13}C$ and $\delta^{18}O$ values of *S. kozlovi* were analyzed among locations by an analysis of covariance (ANCOVA). Considering that fish samples were comprising of multiple age classes, ANCOVA was analyzed with otolith mass as a covariate, including the assessment of the interaction effect (locations × otolith mass). A post hoc Dunnett's T3 test was used to examine the existence of any significant differences in the isotopic ratios of carbon and oxygen between any two locations. To determine whether there was greater separation of locations when both stable isotopes were considered simultaneously in a multivariate analysis, a Permutational ANOVA (PERMANOVA) [31,32] was used to test for effects of location on both $\delta^{18}O$ and $\delta^{13}C$. Regression analysis was used to evaluate relationships between ambient temperature variables and stable isotopes of wild *S. kozlovi*. All the analyses were conducted in SPSS software, except for PERMANOVA in R software.

The mitochondrial Cyt*b* and COI gene sequences were edited and aligned in MEGA v6.0 software [33]. The two genes were concatenated in PhyloSuite software [34] to analyze the information jointly and to increase the possibility of detecting differences among sampling sites. Haplotype diversity, nucleotide diversity, variable sites, singleton variable sites and parsimony-informative sites were estimated in DnaSP v6 [35]. To explore the phylogeographic pattern, a minimal spanning network of concatenated sequence data was constructed using the median joining method [36] in the PopART software [37]. Population genetic structure and pairwise F_{ST} was investigated to test for genetic variation within and among populations by using analysis of molecular variance (AMOVA) in Arlequin 3.5 software [38]. In addition, we assessed population structure with a genetic-mixture analysis based on Bayesian inference in BAPS 6.0 software [39]. The range of *K*-population values (clusters: 2–10) was run 10 times and with 1000 iterations to increase the probability of finding the best partition. Whether *S. kozlovi* showed signs of population expansion, bottleneck or decline was tested with Tajima's *D* and Fu's F_S statistic in Arlequin 3.5 software, as well as by drawing a diagram of the frequency distribution of pair-wise genetic differences in the program DnaSP v6.

3. Results

3.1. Stable Isotope Ratios of S. kozlovi Otolith

The values of *S. kozlovi* otolith stable isotope ratios varied from −15.30‰ to −12.37‰ for $\delta^{18}O$ and from −10.10‰ to −6.13‰ for $\delta^{13}C$ (Figure 2a). On average, population

JP (Yalong River) showed the highest value of $\delta^{18}O$ and the lowest value of $\delta^{13}C$, when comparing with other sampling locations. ANCOVA yielded significant variation of $\delta^{18}O$ and $\delta^{13}C$ among different locations with otolith mass as a covariate ($p < 0.001$). For $\delta^{13}C$, 77.7% of the sum of squares was explained by location (ANCOVA, $p = 0.000$, Table 2) and otolith mass was not significant (ANCOVA, $p = 0.09$). For $\delta^{18}O$, 70.7% of the sum of squares was explained by location (ANCOVA, $p = 0.000$, Table 2) and otolith mass was not significant (ANCOVA, $p = 0.52$). The pairwise comparison tests showed significant differences among all locations for each isotopic signature ($p < 0.05$), with the exceptions of population GT and population SG ($\delta^{18}O$: Dunnett's T3 Test, $p = 0.69$), and population GT and population JP ($\delta^{18}O$: Dunnett's T3 Test, $p = 0.15$) values.

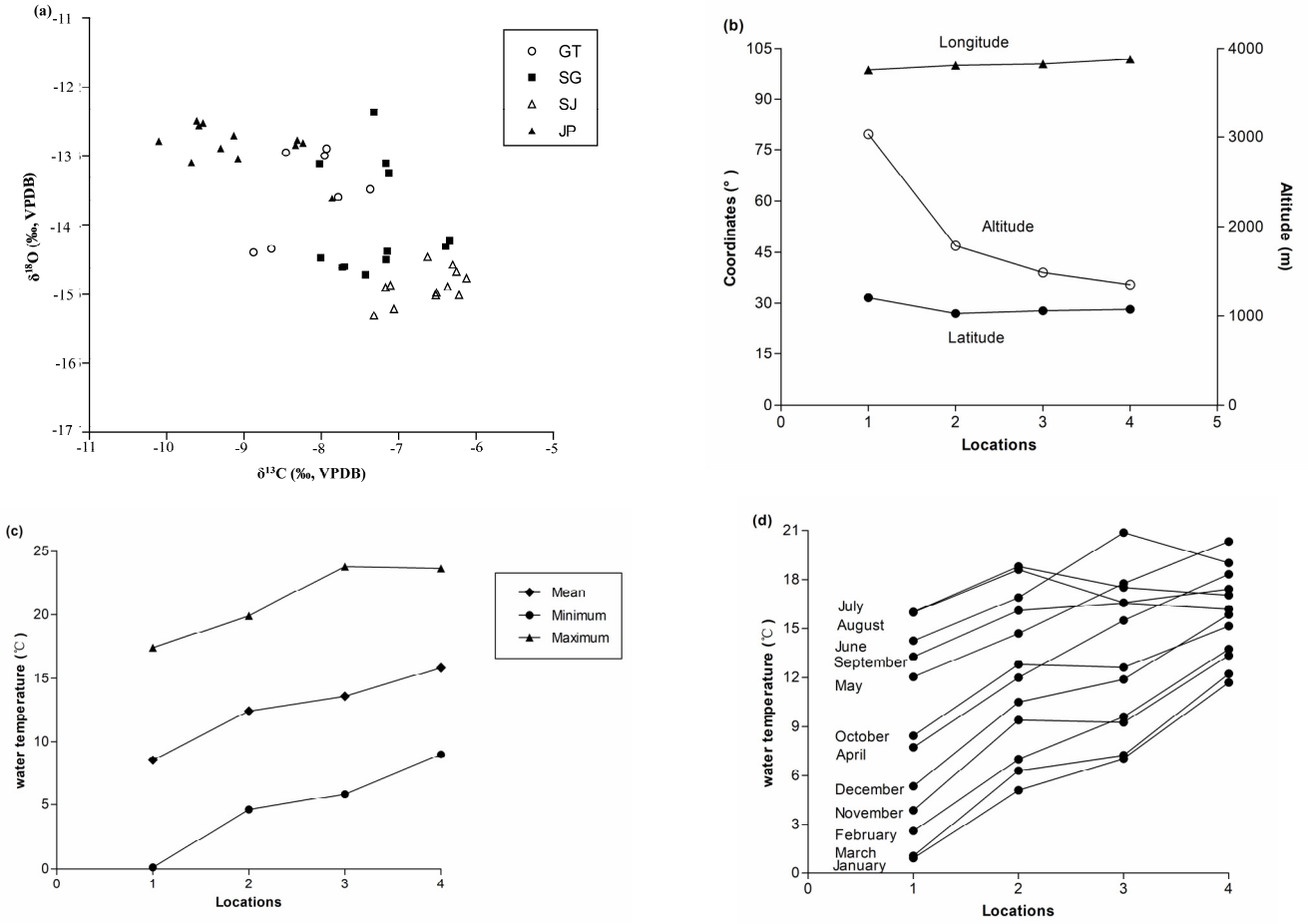

Figure 2. Stable isotope ratios, geographical location and ambient water temperature of wild *Schizothorax kozlovi* in each sampling location (1—location GT, 2—location SG, 3—location SJ, 4—location JP). (**a**) $\delta^{18}O$ and $\delta^{13}C$ variation; (**b**) coordinates and altitude; (**c**) mean, minimum and maximum water temperature; (**d**) mean monthly water temperature.

The PERMANOVA revealed that 99.5% of the sum of squares was explained by location for multi-isotopic signatures ($p = 0.001$, Table 2), which was more clearly based on single stable isotopes than ANCONA. The a posteriori pairwise tests indicated that all locations were insignificantly different from each other at $p > 0.05$ except for population GT and population SJ ($p = 0.001$), and population SJ and population JP ($p = 0.001$). The bi-plot using the two variables (oxygen and carbon) suggest that the isotopic signatures appear to be site-specific (Figure 2a).

Table 2. Summary of ANCOVA and PERMANOVA ($\delta^{18}O$ and $\delta^{13}C$) results across all locations of *Schizothorax kozlovi* based on Type III sums of squares of the standardized data.

Method	Source	d.f.	Sums of Squares	Mean Squares	F	*p*
ANCOVA ($\delta^{13}C$)	Locations	3	32.635	10.878	44.182	0.000
	Otolith mass	1	0.725	0.725	2.943	0.094
	Residual	38	9.356	0.246		
	Total	43	42.000			
ANCOVA ($\delta^{18}O$)	Locations	3	29.621	9.874	30.485	0.000
	Otolith mass	1	0.137	0.137	0.423	0.519
	Residual	38	12.308	0.324		
	Total	43	42.000			
PERMANOVA	Locations	3	65,551.787	21,850.596	2425.994	0.001
	Residual	39	351.268	9.007		
	Total	42	65903.055	1		

3.2. Correlations between Ambient Temperature and Otolith Stable Isotope Ratios

Population GT with the highest altitude located on the area with largest latitude and smallest longitude, compared with other populations (Figure 2b). Population JP with the lowest altitude was located in the area with largest longitude, while population SG was located in the area with smallest latitude (Figure 2b). Annual changes of ambient water temperature in each sampling location are quite different, such as population GT varying from 0.12 °C to 17.38 °C, population SG from 4.60 °C to 19.90 °C, population SJ from 5.86 °C to 23.77 °C, population JP from 9.00 °C to 23.63 °C (Figure 2c,d).

There were weak but significant negative relations between $\delta^{13}C$ and five mean monthly water temperature variables (January (r^2 = 0.15, p = 0.01), February (r^2 = 0.11, p = 0.03), March (r^2 = 0.10, p = 0.04), November (r^2 = 0.12, p = 0.03), December (r^2 = 0.15, p = 0.01)) while a weak but significant positive relation between $\delta^{13}C$ and mean July water temperature was obtained (r^2 = 0.17, p = 0.01). There were weak but significant positive relationships between $\delta^{18}O$ and three mean monthly water temperature variables (January (r^2 = 0.10, p = 0.04), November (r^2 = 0.09, p = 0.04) and December (r^2 = 0.10, p = 0.03)).

3.3. Genetic Diversity and Structure

Fragments 683 bp in length encoding the COI gene (GenBank Accession numbers: MW403091-MW403127) and 1141 bp in length encoding the Cyt*b* gene (GenBank Accession numbers: MW401231-MW401267) were obtained from 99 specimens, yielding a total length of 1824 bp of concatenated sequence. For the Cyt*b* gene, variable sites, singleton variable sites, and parsimony-informative sites were 84, 26 and 58, respectively. For the COI gene, variable sites, singleton variable sites, and parsimony-informative sites were 16, 4 and 12, respectively. In total, 37 haplotypes were identified based on the concatenated sequences of Cyt*b* and COI, with the number of haplotypes varying between 7 and 23 among locations. Population SJ exhibited the highest haplotype diversity (0.967 ± 0.030), while population SG exhibited the highest nucleotide diversity (0.00871 ± 0.00124) (Table 3). Population JP presented the lowest haplotype diversity (0.846 ± 0.085) and nucleotide diversity (0.00361 ± 0.00199) (Table 3).

The most frequent haplotype (Hap1, n = 23) was found at all sampling locations. Four haplotypes (Hap2, Hap5, Hap8, Hap10) were unique to population GT in the Jinsha River, fifteen haplotypes (Hap13, Hap15-Hap24, Hap26-Hap27, Hap30-Hap31) were unique to population SG in the Jinsha River, five haplotypes (Hap32-Hap36) were unique to population SJ in the Shuiluo River, while only one haplotype (Hap37) was unique to population JP in the Yalong River.

Table 3. Genetic diversity of *Schizothorax kozlovi* at different locations of the upper Yangtze River based on the concatenated Cyt*b* and COI genes sequences.

Locations	Number of Haplotypes	Haplotypes	Haplotype Diversity	Nucleotide Diversity
GT	12	Hap1-Hap12	0.913 (±0.040)	0.00708 (±0.00127)
SG	23	Hap1, Hap6-Hap7, Hap11, Hap13-Hap31	0.932 (±0.024)	0.00871 (±0.00124)
SJ	14	Hap1, Hap3-Hap4, Hap6, Hap9, Hap14, Hap25, Hap28-Hap29, Hap32-Hap36	0.967 (±0.030)	0.00795 (±0.00081)
JP	7	Hap1, Hap6-Hap7, Hap12, Hap25, Hap28, Hap37	0.846 (±0.085)	0.00361 (±0.00199)
Total	37	Hap1-Hap37	0.928 (±0.017)	0.00778 (±0.00078)

Values in the brackets represent standard deviation.

The haplotype network based on the concatenated sequences resulted in two distinct lineages (Figure 3) while the Bayesian inference also detected two different clusters, with a log (marginal likelihood) optimal partition of −1691.8028 (Figure 4). Clade I, included 73/99 (73.7%) of the samples and was documented in all sampling locations while Clade II included 26/99 (26.3%) of the samples and was also found in all sampling locations.

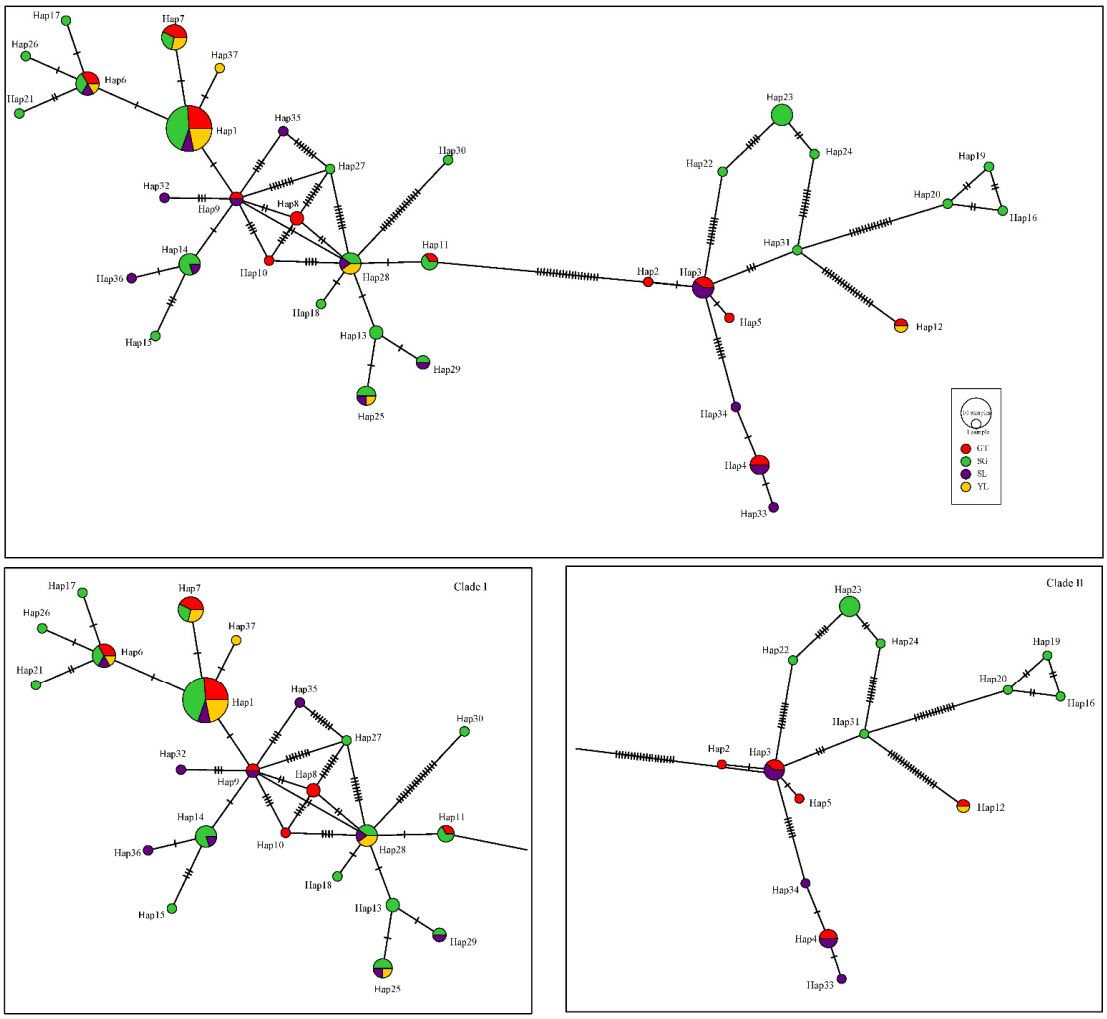

Figure 3. The haplotype network of *Schizothorax kozlovi* based on the concatenated sequences of gene Cyt*b* and COI.

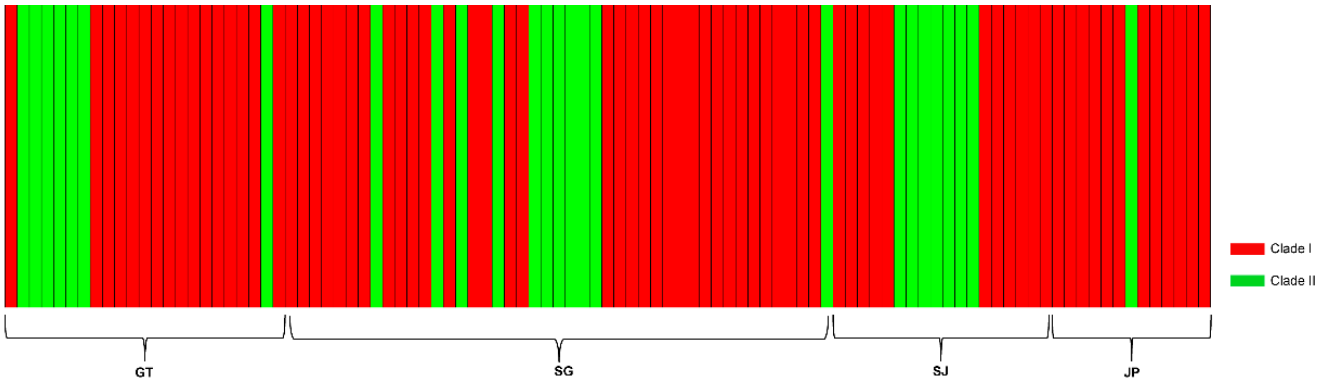

Figure 4. Bayesian analysis of population structure of *Schizothorax kozlovi* based on clustering with linked loci for both Cyt*b* and COI gene. Each color represents a separate genetic cluster, and each bar represents an individual.

The AMOVA detected that the highest percentage of genetic variance was within sampling locations (96.94%), and little genetic variance was found among sampling locations (3.06%) (Table 4). Meanwhile, the larger percentage of genetic variation was found between two clades (72.82%), and a smaller genetic variance was found within clades (27.18%). Pairwise F_{ST} comparison indicated significant differences between SJ and JP (F_{ST} = 0.159, p = 0.017), while no significant differences were found between other sampling locations (Table 5).

Table 4. Analysis of molecular variance analysis among locations of *Schizothorax kozlovi* from the upper Yangtze River based on the concatenated Cyt*b* and COI genes sequences.

Category	Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation (%)
Location	Among locations	3	38.714	0.23704	3.06
	Within locations	95	713.862	7.51433	96.94
	Total	98	752.862	7.75137	
Lineage	Among lineages	1	388.875	10.04411	72.82
	Within lineages	97	363.701	3.74949	27.18
	Total	98	752.576	13.79360	

Table 5. Pairwise F_{ST} values among different locations of *Schizothorax kozlovi* from the upper Yangtze River based on the concatenated Cyt*b* and COI genes sequences.

Locations	GT	SG	SJ	JP
GT		0.214	0.398	0.122
SG	0.010		0.126	0.112
SJ	−0.009	0.028		0.017
JP	0.055	0.040	0.159	

Values in the right upper part represent p values.

There was no direct evidence of population expansion, decline or bottleneck in the species, by the distribution of pair-wise sequence differences (Figure 5), Tajima's D (−0.91, p = 0.19) or Fu's F_S (−2.13, p = 0.34).

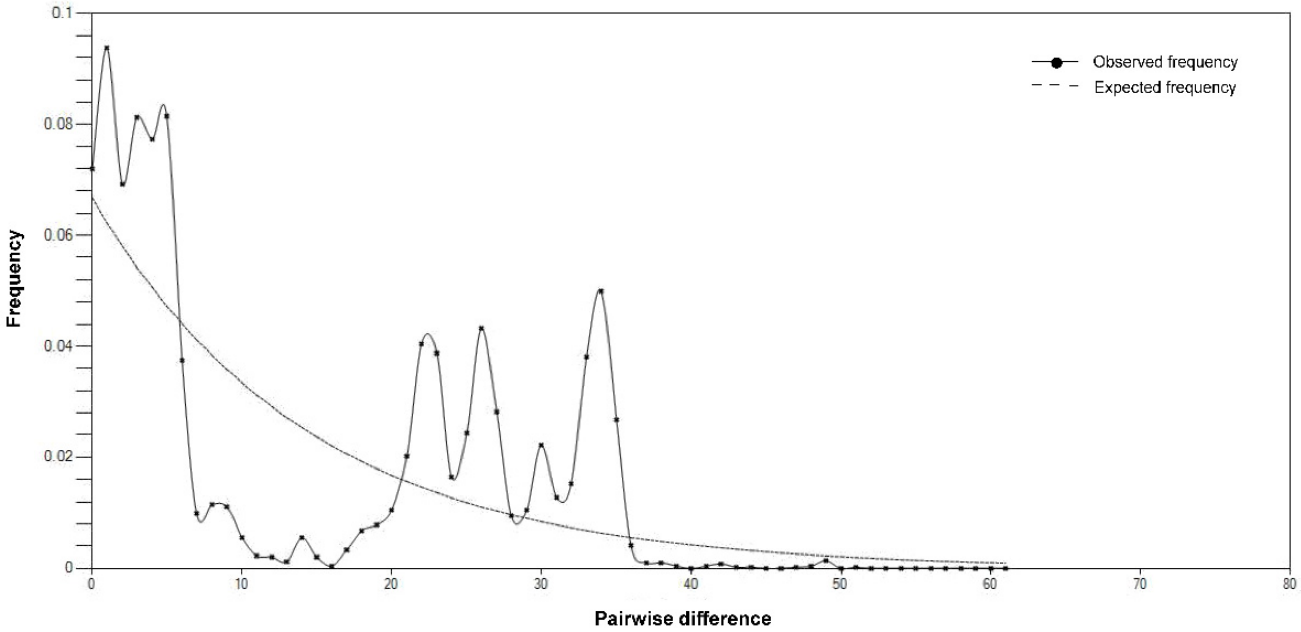

Figure 5. Observed and expected distributions of pair-wise sequence differences in mitochondrial concatenated sequences of Cyt*b* and COI of *Schizothorax kozlovi* in the upper Yangtze River.

4. Discussion

Using a combination of markers may help reach a better understanding of population structure and connectivity. The mitochondrial DNA of *S. kozlovi* in the present study shows limited geographic structuring between populations but shows high structuring between haplotype lineages. Carbon and oxygen isotopic ratios varied significantly between most geographic locations. By and large, it exhibits a limited geographic population structuring of wild *S. kozlovi* in the upper Yangtze River.

The genetic diversity of populations is usually evaluated by haplotype diversity and nucleotide diversity. In the present study, haplotype diversity and nucleotide diversity of wild *S. kozlovi* in the upper Yangtze River was 0.928 and 0.00778, which is larger than 0.5 and 0.5%, both exhibiting high levels. It indicates that the high level of divergence between haplotypes of wild *S. kozlovi* in the upper Yangtze River may be attributed to secondary contact between previously differentiated allopatric lineages [40]. Comparing with the results from Wujiang population of *S. kozlovi*, based on mtDNA control region sequences [41], higher haplotype diversity and nucleotide diversity of wild *S. kozlovi* in the upper Yangtze River was brought to light in the present study. In contrast to *S. molesworthi* in the Yarlung Zangbo River [27], both haplotype diversity and nucleotide diversity of *S. kozlovi* was higher.

Most of the F_{ST} values between the populations of *S. kozlovi* were in small levels of genetic differentiation ($F_{ST} < 0.05$), except for one in a moderate level (JP_GT, $F_{ST} = 0.055$) and one in a great level (JP_SJ, $F_{ST} = 0.159$) [42]. In addition, the population JP of *S. kozlovi* possessed the highest oxygen isotopic ratios, the lowest carbon isotopic ratios, the lowest haplotype diversity and nucleotide diversity. According to the findings, the population JP of *S. kozlovi* in the Yalong River should be paid more attention. Population JP is located between two large hydropower dams (Ertan hydropower station and Jinping hydropower station) in the Yalong River, which is the tributary of the upper Yangtze River. Ertan hydropower station and Jinping hydropower station, with the dam height of 240 m and 305 m, have been impounding water since 1998 and 2012, respectively. This means population JP of *S. kozlovi* has been isolated for eighteen years, and the water temperature of its habitat has been regulated by Jinping hydropower station but not following natural environmental changes. Gene flow between population JP and other populations has been greatly limited in recent years. Furthermore, the water temperature of population JP's habitat fluctuates

within ±1.3 °C in each 12 h by the effect of Jinping hydropower station, while population GT varied within ±2.8 °C in each 12 h by natural environmental changes through our field investigation. Mean water temperature of population JP's habitat from each month was generally much higher than other populations. That is, the habitat environment of population JP was quite different from other populations.

Nevertheless, AMOVA revealed a significant genetic differentiation between two haplotype lineages of *S. kozlovi* (Clade I and Clade II, F_{ST} = 0.728 > 0.25). No matter how large the sample size is, there are two lineages in each geographical population. Clade I possess 73.7% individuals of all populations, while Clade II has only 26.3% individuals. In population GT, SG and SJ, 69.6%, 75.6% and 61.1% individuals belong to Clade I, respectively, while only 30.4%, 24.4% and 38.9% individuals belong to Clade II. Simultaneously, 92.3% of individuals in population JP belong to Clade I, while only 7.7% individuals belong to Clade II. In general, Clade I was relatively the most primitive lineage, while Hap 1 in Clade I was the most primitive haplotype.

Studies on the genetic structure and phylogenetic geographical pattern of fish populations can provide scientific basis for the conservation management of species. Although haplotype diversity and nucleotide diversity of wild *S. kozlovi* in the upper Yangtze River were in relatively high levels, genetic differentiation between populations or between lineages began to emerge, which is the most important signal for the conservation of this fish species. Therefore, the conservation of this species should be conducted because of its isolated and declining populations. According to the principle of evolutionarily significant units (ESUs) and management units (MUs) [43], two haplotype lineages (Clade I and Clade II) of *S. kozlovi* in the upper Yangtze River should be suggested as two management units for independent conservation. Furthermore, it is suggested that population JP should also be conserved as a management unit because of its low genetic diversity, low heterogeneity of habitat environment and high effect of anthropogenic activities.

5. Conclusions

Both mitochondrial DNA markers (Cyt*b* and COI) and otolith stable isotope ratios ($\delta^{18}O$ and $\delta^{13}C$) were used to explore the spatial population structure of wild *S. kozlovi* in the upper Yangtze River, and then to correlate the structure with ambient temperature changes. Exhibiting high haplotype diversity and nucleotide diversity, wild *S. kozlovi* showed limited geographic population structuring and two distinct haplotype lineages (Clade I and Clade II). Significant relationships between otolith stable isotope ratios and specific mean monthly water temperature (from November to March) were also revealed, indicating low temperature effect on otolith stable isotope ratios. In particular, the population JP (Jinping) of *S. kozlovi* with low genetic diversity and low heterogeneity of habitat environment is suggested as a management unit for independent conservation.

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