

Expansion and contraction of lake basin shape the genetic structure of *Sinocyclocheilus* (Osteichthyes: Cypriniformes: Cyprinidae) populations in central Yunnan, China

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Abstract

Geological events and historical environmental change can strongly affect the genetic structures and differentiation of fish populations. Although the central region of the Yunnan-Guizhou Plateau contains the highest concentration of rift-subsidence lakes in China, the effects of key geological events on the distributions and genetic structures of the regional fauna remain poorly understood. Fishes of the genus *Sinocyclocheilus* are endemic to the Yunnan-Guizhou Plateau, where they are found in karst landforms. As a result of environmental pollution and other human activities, *Sinocyclocheilus* populations have decreased sharply, and the genus is currently listed under Class II of the nationally protected animals classification in China. Examining the phylogeographic patterns of *Sinocyclocheilus* fishes can be useful for elucidating the spatio-temporal dynamics of their population size, dispersal history and extent of geographical isolation, thereby providing a theoretical basis for their protection. Here, we used Restriction Site Associated DNA Sequencing (RAD-seq) to investigate the evolution of *Sinocyclocheilus* fishes. Our analysis supports the endemism of *Sinocyclocheilus*, and identifies the formation of Dianchi Lake and Fuxian Lake as key geological events shaping *Sinocyclocheilus* population structure. We estimate that the most recent common ancestor (MRCA) of *Sinocyclocheilus* fish occurred in the Central Yunnan Basin 3.75–3.11 Ma. It is the first time to prove that the altitude change has a great influence on the genetic variation among the populations of *Sinocyclocheilus*. We discuss the implications of our results for the protection and management of *Sinocyclocheilus* *grahami* and other cave fishes.

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ABSTRACT

Geological events and historical environmental change can strongly affect the genetic structures and differentiation of fish populations. Although the central region of the Yunnan-Guizhou Plateau contains the highest concentration of rift-subsidence lakes in China, the effects of key geological events on the distributions and genetic structures of the regional fauna remain poorly understood. Fishes of the genus *Sinocyclocheilus* are endemic to the Yunnan-Guizhou Plateau, where they are found in karst landforms. As a result of environmental pollution and other human activities, *Sinocyclocheilus* populations have decreased sharply, and the genus is currently listed under Class II of the nationally protected animals classification in China. Examining the phylogeographic patterns of *Sinocyclocheilus* fishes can be useful for elucidating the spatio-temporal dynamics of their population size, dispersal history and extent of geographical isolation, thereby providing a theoretical basis for their protection. Here, we used Restriction Site Associated DNA Sequencing (RAD-seq) to investigate the evolution of *Sinocyclocheilus* fishes. Our analysis supports the endemism of *Sinocyclocheilus*, and identifies the formation of Dianchi Lake and Fuxian Lake as key geological events shaping *Sinocyclocheilus* population structure. We estimate that the most recent common ancestor (MRCA) of *Sinocyclocheilus* fish occurred in the Central Yunnan Basin 3.75~3.11 Ma. It is the first time to prove that the altitude change has a great influence on the genetic variation among the populations of *Sinocyclocheilus*. We discuss the implications of our results for the protection and management of *Sinocyclocheilus grahami* and other cave fishes.

KEYWORDS

central Yunnan, divergence time, genetic structure, geological events, RAD-seq, *Sinocyclocheilus*

1 | INTRODUCTION

In comparison with freshwater fish in lakes in plain regions, fish communities in plateau lakes are characterized by small populations as well as high endemism, species richness, and interspecific variation (Chu & Chen, 1989). Such fishes are also more vulnerable to threats such as environmental pollution, alien species invasions, overfishing and other impacts stemming from human activities around lakes.

The Yunnan-Guizhou Plateau, located in the center of Yunnan Province, contains six of the province's nine plateau lakes. Situated along the Xiao-Jiang fault zone, these lakes were formed between the late Pliocene and early Pleistocene, with most being formed during the middle to late stages of the Pleistocene (Li et al., 1963; Yang, 1984). During the Qingzang (3.6~1.7 Ma) and Kunhuang (1.1~0.6 Ma) periods, the Yunnan-Guizhou Plateau was uplifted from an average elevation of < 1,000 m to 4,000 m. Subsequently, the region was subjected to an intensification of the Asian monsoonal climate and increased precipitation (Li & Fang, 1999) and saw the formation of numerous glaciers (Shi et al., 1999). The freshwater fish genus *Sinocyclocheilus* originated around 10.16 Ma, with most speciation events occurring in the last two million years. Such speciation was likely favored by the uplifting of the Qinghai-Tibetan Plateau and the aridification of the regional climate, which led to the isolation of *Sinocyclocheilus* populations in cave systems (Mao et al., 2021). Similar geological processes have been proposed to shape the divergence and evolution of other faunal species of the central Yunnan Plateau (Che et al., 2010; Zhao & Li, 2017; Guo et al., 2019; Deng et al., 2020; Xiang et al., 2021), including fish species in plateau lakes (Yang et al., 2016; Wen et al., 2022). Nonetheless, the relative importance of key geological events in shaping the distributions and genetic structures of faunal communities of the central Yunnan lake basin remains poorly understood.

Fishes in the genus *Sinocyclocheilus* are morphologically distinct. Their populations are distributed across karst landforms of the Yunnan-Guizhou Plateau, where they reside in vauclusian springs, karst caves or underground river outlets. As such, *Sinocyclocheilus* fishes live in extremely challenging environmental conditions where little photosynthesis occurs and food resources are low (Camacho, 1992; Bitchuette & Trajano,

2010). Due to environmental pollution and human impacts, the habitats of *Sinocyclocheilus* include some of the most threatened ecosystems in the world, and *Sinocyclocheilus* populations have decreased sharply (Shu et al., 2013). In China, the genus is listed under Class II of the nationally protected animals classification. Intraspecific phylogeographic patterns are useful for elucidating the spatio-temporal dynamics of a species' population size, dispersal history and extent of geographical isolation (Baker & Marshall, 1997; Merila et al., 1997; Zink, 1997; Kvist et al., 1999; Leonard et al., 2000; Zhang et al., 2005). Such patterns can thus provide a theoretical basis for species conservation. Despite the steep decline of *Sinocyclocheilus* populations, there has been limited understanding of the genetic population structures of these fishes as well as the phylogenetic relationships between *Sinocyclocheilus* species (Zhao & Zhang, 2009; Jiang et al., 2019; Mao et al., 2021). These gaps need to be addressed because they can result in the application of inappropriate conservation and management actions to *Sinocyclocheilus* populations (Gutierrez & Helgen, 2013; Zachos, 2013). Hence, it is necessary to clarify the phylogenetic relationships between and within different *Sinocyclocheilus* species in Yunnan.

In the present study, we measured the SNP (Single Nucleotide Polymorphisms) of 108 *Sinocyclocheilus* individuals from 24 different populations and used RAD-seq as a genetic method to analyze their population structure, with the aim of addressing the following questions: (i) What is the population structure of *Sinocyclocheilus* in central Yunnan? (ii) Have historic geological events in central Yunnan affected the genetic structures and distributional patterns of *Sinocyclocheilus* populations? (iii) What factors influence the genetic structure and differentiation patterns of the *Sinocyclocheilus* population in the central Yunnan Basin? (iv) What is the role of river capture events in shaping the distribution of genetic lineages of fishes in the central Yunnan Basin? Finally, we discussed the implications of our findings for the protection and management of *Sinocyclocheilus* and other cave fish.

2 | MATERIALS AND METHODS

2.1 | Sample collection

We collected 108 *Sinocyclocheilus* individuals from 24 populations in the central Yunnan Basin (**Fig. 1**). The caudal fin or muscle tissue from each individual was preserved in 95% ethanol for DNA extraction. The individuals were stored in 10% methanol to facilitate taxonomic identification and deposited in the Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ). Data from additional samples was sourced from the National Center of Biotechnology Information (NCBI). Information associated with each sample is provided in **Table 1**.

Sinocyclocheilus fishes are listed under Class II of the nationally protected animals classification in China. All specimens used in this study were collected with the permission of the relevant animal protection government departments in China. The study also complied with the current laws on animal research in China. and no more than five individuals were collected from each population.

2.2 | RAD library construction and sequencing

The total DNA of each individual was extracted from the muscle tissue using the standard phenolchloroform method (Sambrook et al., 1989). DNA quality was first assessed by visually inspecting for signs of fragmentation on 1% agarose gels. To avoid the potential disproportionate representation of individuals in sequencing pools owing to the varying DNA quality across samples, we reextracted and reexamined any DNA samples showing degradation on agarose gels. Only nondegraded samples were processed further and quantified (twice) with a NanoDrop ND-1000 spectrophotometer. Each sample was then diluted to 10 ng/ μ L, requantified and pooled for each population. Next, each pooled sample was quantified (twice) with the spectrophotometer and equalized to 10 ng/ μ L to construct the RAD library.

To construct RAD libraries, DNA was fragmented by the restriction enzyme *Eco* RI. A P1 adapter including a forward amplification primer, sequencing primer, and an 8-base pair (bp)-specific barcode was added to each of the fragmented DNA pools. The barcoded samples were then pooled and sheared randomly, and a P2 adapter was added to the sheared DNA fragments. DNA with a P1 adapter was selectively enriched

by PCR amplification. Finally, DNA fragments of 300–500 bp were gel-purified and sequenced on the Illumina HiSeq2000 platform to generate 100-bp paired-end reads. All pools were sequenced on a paired-ends sequencing lane. The construction of RAD libraries and sequencing were carried out by BGI Tech Solutions Co., Ltd. (Shenzhen, China). Filtered reads for the RAD data pertaining to this study can be accessed through the NCBI GenBank Short Read Archive (PRJNA990519).

2.3 | RAD read mapping

We first de-multiplexed sequences according to their barcodes using an in-house Perl script, ensuring that no mismatches occurred between the sequenced barcode and its sequence. We then used the FASTX toolkit to end-trim raw reads to a length of 150-bp, and to exclude reads containing one or more bases with a < 10 Phred quality score or which had $> 5\%$ of bases receiving a < 20 Phred quality score. We then analyzed the remaining reads in the software pipeline STACKS v.2.64. Next, we aligned clean reads to the *Sinocyclocheilus grahami* reference genome (Yang et al., 2016) using the BWA-MEM algorithm from the bwa v.0.7.15 software (Li & Durbin, 2009). The resulting SAM file was converted to a coordinate-sorted and indexed BAM file using samtools 0.1.16 (Li et al., 2009).

2.4 | Population genetic analyses

We selected high-quality SNPs which contained no more than 20% missing data, and thinned the sites such that no two sites were within the same 2000-bp region. Thereby, we eliminated the potential effects of physical linkage among variants. We then conducted a phylogenetic analysis with the final SNP set using the IQ-TREE software (version 1.6.9). We constructed a maximum likelihood (ML)-based phylogenetic tree using the GTR + F + R5 model, and ran 1000 rapid bootstrap replicates to determine the confidence values of phylogenetic branches.

To visualize patterns of genetic variation, we conducted a principal component analysis (PCA) of the final SNP set using PLINK (version 1.90), and plotted the resultant principal components against one another using the software R (version 3.4). We also used the final SNP set for a population structure analysis using ADMIXTURE (version 1.3), which was ran with K values (the number of assumed ancestral components) ranging from 1 to 10.

2.5 | Divergence time estimation

We inferred divergence times for the *Sinocyclocheilus* using the MSC model in SNAPP. The absence of a specific fossil record provides a calibration for *Sinocyclocheilus*. To investigate the divergence times of geographic populations of *Sinocyclocheilus* in the central Yunnan Basin in accordance with molecular clock theory, we set three calibration points from previous studies on this group (Chen et al., 2009; Mao et al., 2021; Yang et al., 2021) and set the differentiation rate of *S. grahami* at 3.51×10^{-9} years (Yang et al., 2016). We ran an independent SNAPP analysis with 20 million MCMC generations, sampling at every 2,000 steps. Stationarity of the BEAST 1.8.2 (Drummond et al., 2012). analysis was assessed using TRACER 1.7.1 (Rambaut et al., 2018) and was considered as evidence of convergence when ESS values greater than 200. Maximum clade credibility (MCC) trees were obtained using TREEANNOTATOR 2.4.3 to burn-in the initial 10% of the all samples.

2.6 | Fixation index analysis

Fixation index (Fst) was calculated using VCFTOOLS (Danecek et al., 2011) with a sliding window of 100 kb and step size of 10 kb (-window-pi 100000 -window-pi-step 10000). By using the Fst for correlation analysis to analyze the altitude distance and geographic distance between materials. Pearson Correlation Coefficient (PCC) (Eggers et al., 2003) is used to represent the phase between altitude distance and geographic distance. By using R 3.6.3 software for calculating the correlation coefficient (r) and significance level (p). The magnitude of the PCC is determined by the value of r. It reflects the degree of linear correlation between two variables, with a range of r values ranging from -1 to +1. Close to -1 indicates negative correlation, and close to +1 indicates positive correlation.

3 | RESULTS

3.1 | RAD-seq dataset

The RAD-seq genome library of 108 individuals of *Sinocyclocheilus* individuals yielded approximately 4.1 million reads per individual on average after splitting and filtering the original data. The proportion of bases with a mass value greater than 20 in all reads to the total reads length was more than 96.15%. Among them, 92% of reads were on average mapped to the reference genome of *Sinocyclocheilus graham* (Yang et al., 2016). After variant calling and filtering, a total of 26,318,419 SNPs were identified from 108 individuals of *Sinocyclocheilus*.

3.2 | Population structure of *Sinocyclocheilus* in the central Yunnan region

The maximum likelihood (ML) tree revealed three main branches within the genus *Sinocyclocheilus*, the first of which included the *Sinocyclocheilus* population of Hunan Province (HN). The second branch included the *Sinocyclocheilus* population of Guangxi Province (GX), and split into two subbranches, population I (GX i) and population II (GX ii). The third branch included the *Sinocyclocheilus* populations of Yunnan Province (YN), which included the Mile City population (YN-ML), Qujing City population (YN-QJ) and the Dianchi and Fuxian lake population (YN-DF) (see **Appendix S1, Fig S1**). These findings were supported by the PCA. The ADMIXTURE-based analysis of population structure also generated similar results. The error values were lowest (0.33) when clusters were set to K=4, and all populations converged to four clusters with the highest average likelihood value (**Fig. 2**). The YN population displayed more admixed and diversified genetic components than the three other populations. Collectively, the results reveal the geographical distribution of *Sinocyclocheilus* within the Yunnan-Guizhou Plateau. Since the *Sinocyclocheilus* population of HN and GX has been well discussed (Mao et al., 2021), this paper will focus on the central Yunnan region, and only a supplement to the population structure of *Sinocyclocheilus* in Hunan and Guangxi Province.

3.3 | Species delimitation

At present, *Sinocyclocheilus grahami* is the only *Sinocyclocheilus* species described from Dianchi Lake (Regan, 1904). This species is distributed in the upper reaches of the Pudu River and in a tributary on the right of the Jinsha River. In addition to the *S. grahami*, three other *Sinocyclocheilus* species, *S. guanduensis*, *S. huanglongdongensis* and *S. hei* are known from (Xiao et al., 2004). Based on our examination of type specimens of these three species, it is believed that the DNA sequence results show that they are very different from each other, which is due to the high genetic diversity of *S. grahami*. As the four *Sinocyclocheilus* species are not clearly differentiated in morphology, *S. guanduensis*, *S. huanglongdongensis* and *S. hei* are considered to be synonyms of *S. grahami* (Zhao & Zhang, 2009). The maximum likelihood (ML) tree revealed that the *Sinocyclocheilus* populations from the Chenglong vaclusian spring (C) and the Fuming vaclusian spring (F) in Dianchi Lake are clustered within *Sinocyclocheilus tingi*. Furthermore, the morphological characteristics of individuals from these populations were clearly distinct from those of *S. grahami* and the synonymized taxa *S. guanduensis*, *S. huanglongdongensis* and *S. hei*. We therefore propose that individuals from the Chenglong vaclusian spring (C) and Fuming vaclusian spring (F) populations constitute an undescribed *Sinocyclocheilus* species, which we intend to formally describe at a later stage.

3.4 | Divergence times estimates

Based on the RAD-seq data, we estimated the age of the most recent common ancestor (MRCA) of *Sinocyclocheilus* in the central Yunnan Basin at approximately 3.75~3.11 Ma. The earliest differentiation event occurred at 2.54~2.15 Ma, between the Mile (YN-ML) and Qujing (YN-QJ) populations. This was followed by the differentiation of the Dianchi and Fuxian lake population (YN-DF) and the Qujing population (YN-QJ) 1.43~1.18 Ma (**Fig. 3**). The relatively late tectonic uplift of the Tibetan Plateau 3.6 Ma (Qingzang movement: 3.6~1.7 Ma, Kunhuang movement: 1.1~0.6 Ma) may have affected the population dynamics of *Sinocyclocheilus* in the central Yunnan Basin.

4 | DISCUSSION

4.1 | Effects of geological changes on the evolution of *Sinocyclocheilus* in the central Yunnan Basin

The Yunnan-Guizhou Plateau in China has a high concentration of plateau lakes, with most forming as a result of stratigraphic fault subsidence in the late Cenozoic. Located in the hinterland of the Yunnan-Guizhou Plateau, Dianchi Lake is the largest freshwater lake in Yunnan Province. The basin of Dianchi Lake has experienced extensive geological and climatic changes over the past 3.4 million years. Specifically, with the uplift and subsidence of the lake basin and the associated expansion and contraction of the lake area, Dianchi Lake which was previously a part of the Nanpanjiang River system, has transformed into a tributary source for the Jinsha River in a “river capture event” (Zhu et al., 1991). Such changes in the landscape have made it possible for aquatic organisms that were once restricted to the Nanpanjiang River to colonize the Yangtze River Basin, thereby expanding their geographic ranges. The structural evolution of Dianchi Lake is a notable feature of the geomorphology and neotectonics of the Yunnan Plateau. The population structure of *Sinocyclocheilus* fishes inhabiting the region also reflects the complex geological development of Dianchi Lake.

Dianchi Lake is a fault subsidence lake of plateau limestone formed by the Tertiary Himalayan Orogeny. During the Yanshan Movement, the Yunnan Plateau experienced stratigraphic uplift, folding, and faulting. These processes led to the formation of high mountains and basins and shaped the present landform. As a result of strong neotectonic movement that occurred up to the Himalayan movement in the late Cenozoic, a large north-south trending fault spanning was formed in the Xishan Mountains. When the fault collapsed, Dianchi Lake was formed by the accumulation of water during the initial formation of the resulting graben. The formation of Dianchi Lake comprised three main stages, which also influenced the population structure of *Sinocyclocheilus* fishes in the region.

The first stage of lake formation occurred approximately 3.4 Ma. According to the divergence times of *Sinocyclocheilus* in the Central Yunnan Basin, we speculate that the common ancestor of *Sinocyclocheilus* in the Central Yunnan Basin, the “Central Yunnan Group,” colonized the lake during this period.

The second stage of lake formation saw the expansion of the lake basin during the early and middle Pleistocene (2.59~0.13 Ma). The early Pleistocene (2.59~0.78 Ma) saw a gradual intensification of the uplifting and depression of the Dianchi Lake Basin relative to the surrounding fault block mountains. As the northern section of the basin continued to subside, the lake deepened and its area expanded. During this time, the “Central Yunnan Group” differentiated into the “Mile Group” and the other groups. During the middle Pleistocene (0.78~0.13) Ma, the lake basin accumulated water, forming the main lake. This period corresponded with the differentiation of *Sinocyclocheilus* fish populations in central Yunnan, a process that may have been driven by the specialized ecology of *Sinocyclocheilus* and the distinct geological conditions of Dianchi Lake. Notably, populations of *Sinocyclocheilus* (C and F) in Dianchi Lake and in Fuxian Lake differentiated 0.84~0.49 Ma, which is related to the original Nanpan River system in Dianchi Lake. This is further supported by the fact that the capture of Tanglang River occurred after this period.

The third stage of lake formation occurred during the period in which the Tanglang River was captured (0.126~0.01 Ma), leading to a transformation of the water system of the ancient Dianchi Lake. The Dianchi Lake, originally belonging to the Nanpan River water system, was transformed into a tributary source lake of the Jinsha River water system. Bailong vauchusian spring (FM) and Long vauchusian spring (JW) happened to be at the intersection of the Nanpan River water system and the Jinsha River water system. Due to the contraction of lake with decline of water level, differentiation (0.12~0.05 Ma) occurred (**Fig. 3**).

4.2 | Factors influencing the genetic structures and differentiation of *Sinocyclocheilus* populations in the central Yunnan Basin

We found that altitudinal distances showed a stronger correlation ($r=0.492$, $p<0.01$) (**Fig. 4**). In our analysis of *Sinocyclocheilus* population structure, individuals that were collected from more similar altitudes tended to be more closely related genetically. This suggested that altitudinal variation had a strong effect on the genetic variation among *Sinocyclocheilus* populations. The results of our correlation analysis between

the differentiation times of *Sinocyclocheilus* and Fst indicated that the river capture event appeared to have no discernable effects on the distribution of *Sinocyclocheilus* populations in the Central Yunnan Basin. This finding contrasts studies of other freshwater fish taxa that have found river capture events to play a key role in shaping the distributions of genetic lineages (e.g., Poissant et al., 2005; Burrridge et al., 2007). We contend that geographical isolation and the unique ecological preferences of *Sinocyclocheilus* fishes were key factors driving population differentiation.

4.3 | Implications for the conservation and management

Additional studies on the morphology and genetic structures of *Sinocyclocheilus* fishes will be required to confirm our findings that populations from the Chenglong vauclyusian spring (C) and the Fuming vauclyusian spring (F) have diverged. Although much efforts have been made to protect populations of *S. grahami* from human exploitation in accordance with the national protection scheme (Yue & Chen, 1998), little remains known regarding the conservation of this species through management practices (Li, 2001). Our findings reveal that geographic populations of *S. grahami* have formed (especially within the vauclyusian spring) thereby meeting a key criterion for this species being considered an evolutionary significant unit (ESU) (Ryder, 1986). Although it is difficult, We therefore propose that the eight clades or even more of the Dianchi Lake Basin should be treated as ESUs of *S. grahami* for future conservation and management efforts. Vauclyusian spring provides a lebensraum for cave fish, but it also hinders gene exchange between species, so it should be specially protect.

Table 1 Details of sampling sites, the number of *Sinocyclocheilus* individuals collected, and additional samples sourced from NCBI.

species	River system	Abbr.	
<i>Sinocyclocheilus grahami</i>	Yangtze River	C	
	Yangtze River	F	
	Yangtze River	D	
	Yangtze River	X	
	Yangtze River	JW	
	Yangtze River	FM	
	Yangtze River	K	
	Yangtze River	M	
	Yangtze River	J	
	<i>Sinocyclocheilus multipunctatus</i>	Yangtze River	DB
<i>Sinocyclocheilus tingi</i>	Pearl River	FX	
	Pearl River	LC	
	Pearl River	XLT	
<i>Sinocyclocheilus qujingensis</i>	Pearl River	Q	
<i>Sinocyclocheilus oxycephalus</i>	Pearl River	DS	
	Pearl River	XT	
	Pearl River	QS	
	Pearl River	CL	
	Pearl River	DL	
	Pearl River	H	
	<i>Sinocyclocheilus yangzongensis</i>	Pearl River	YZH
		Pearl River	YZM
	<i>Sinocyclocheilus maitianheensis</i>	Pearl River	MT
<i>Sinocyclocheilus xichouensis</i>	Pearl River	XC	
NCBI (Accession: PRJNA764266)	NCBI (Accession: PRJNA764266)	NCBI (Accession: PRJNA764266)	
<i>Sinocyclocheilus altishoulderus</i>	Pearl River		
<i>Sinocyclocheilus angustiporus</i>	Pearl River		
<i>Sinocyclocheilus brevibarbatulus</i>	Pearl River		

species	River system	Abbr.
<i>Sinocyclocheilus brevis</i>	Pearl River	
<i>Sinocyclocheilus cf. guanyangensis</i>	Pearl River	
<i>Sinocyclocheilus cf. longibarbatus</i>	Pearl River	
<i>Sinocyclocheilus donglanensis</i>	Pearl River	
<i>Sinocyclocheilus furcodorsalis</i>	Pearl River	
<i>Sinocyclocheilus guanyangensis</i>	Pearl River	
<i>Sinocyclocheilus guilinensis</i>	Pearl River	
<i>Sinocyclocheilus huangtianensis</i>	Pearl River	
<i>Sinocyclocheilus huanjiangensis</i>	Pearl River	
<i>Sinocyclocheilus lingyunensis</i>	Pearl River	
<i>Sinocyclocheilus longibarbatus</i>	Pearl River	
<i>Sinocyclocheilus macrophthalmus</i>	Pearl River	
<i>Sinocyclocheilus macroscalus</i>	Pearl River	
<i>Sinocyclocheilus mashanensis</i>	Pearl River	
<i>Sinocyclocheilus tianeensis</i>	Pearl River	
<i>Sinocyclocheilus tianlinensis</i>	Pearl River	
<i>Sinocyclocheilus xunlensis</i>	Pearl River	
<i>Sinocyclocheilus yishanensis</i>	Pearl River	

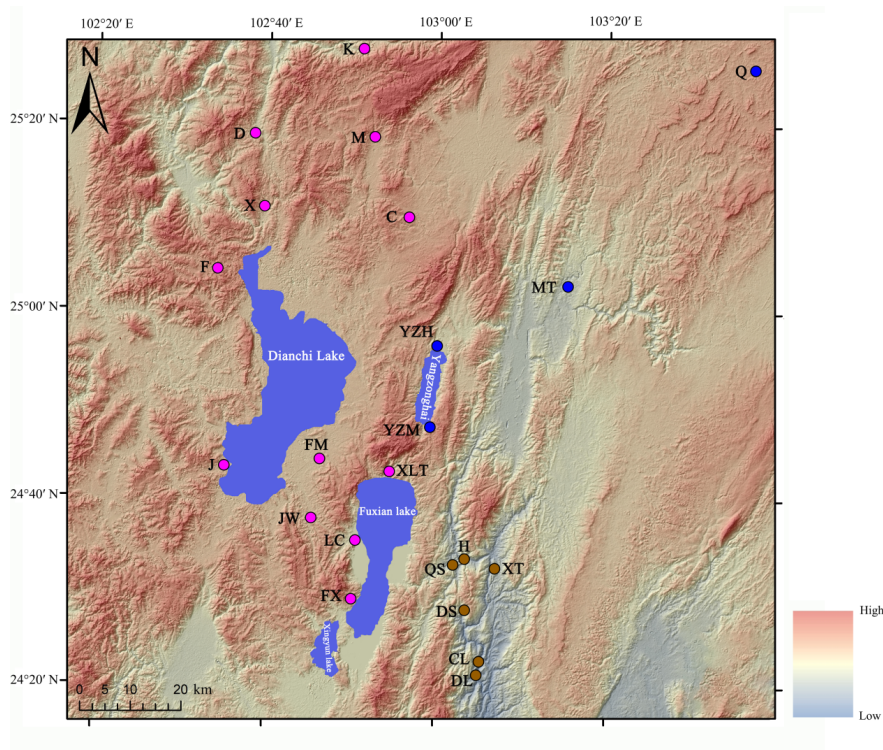


Figure 1 Distribution of the sites in the central Yunnan region where *Sinocyclocheilus* populations were sampled.

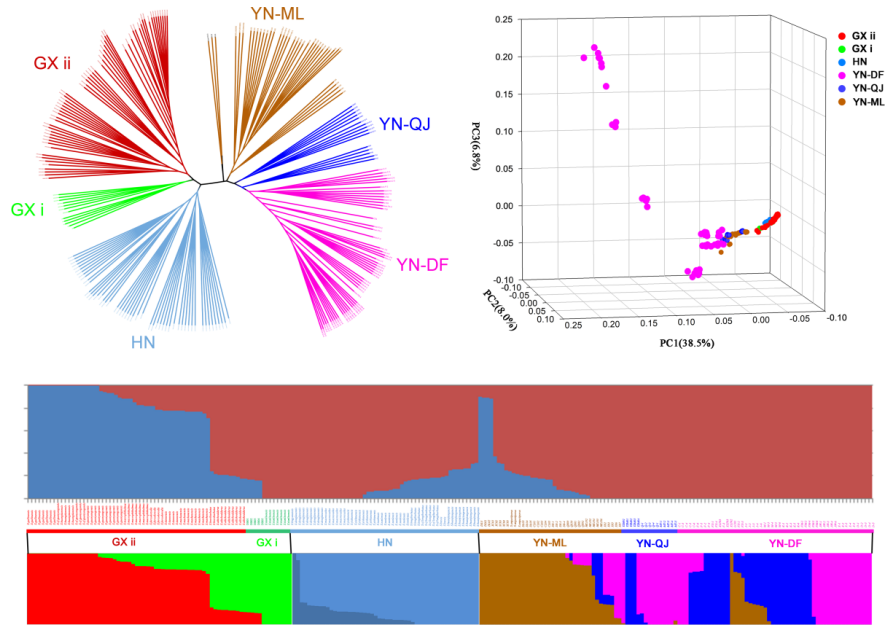


Figure 2 Genetic structure of geographic populations of *Sinocyclocheilus* based on RAD-seq.

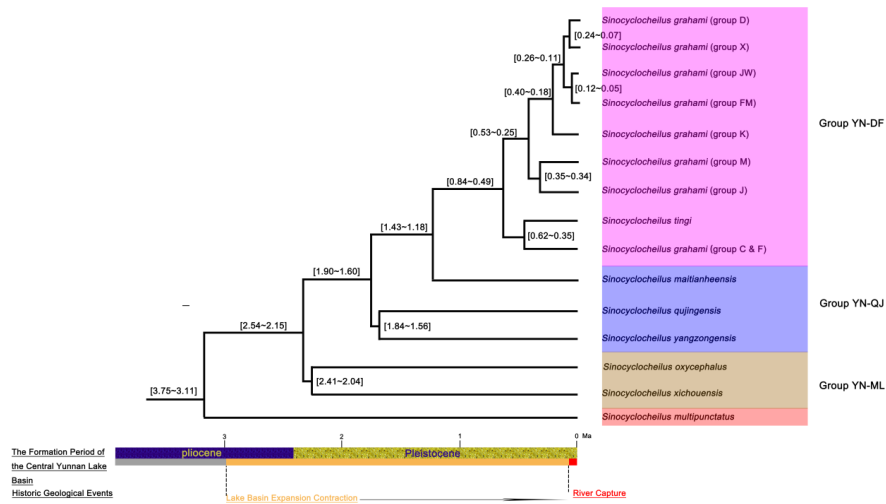


Figure 3 Time-calibrated maximum clade credibility tree of *Sinocyclocheilus* species is inferred by SNAPP in the central Yunnan region

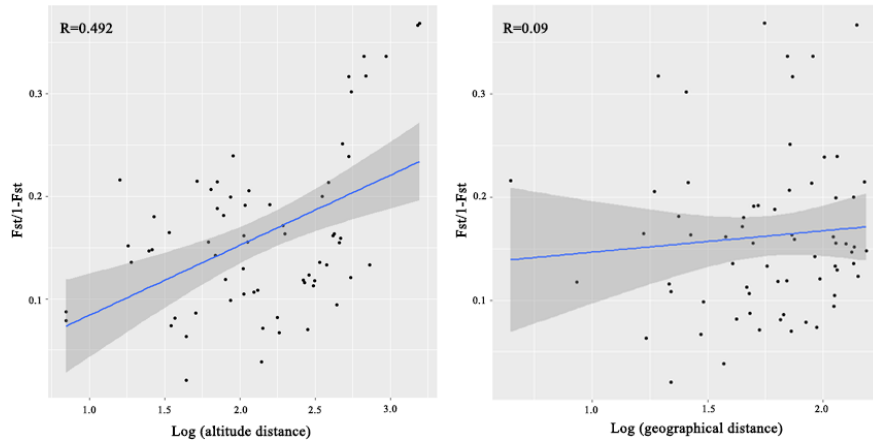


Figure 4 Pairwise genetic distance ($F_{st}/1-F_{st}$) is associated with log (geographical distance) and log (environmental distance).

AUTHOR CONTRIBUTIONS

Xing-Jin Che : writing—original draft (equal); writing—review and editing (equal); investigation (equal); methodology (equal). **Yuan-Wei Zhang** : writing—review and editing (equal); methodology (equal). **An-Li Wu** : Formal analysis (equal); data curation (equal). **Xiao-Fu Pan**: Formal analysis (equal); data curation (equal). **Mo Wang** : Formal analysis (equal). **Jun-Xing Yang** : Conceptualization (equal); data curation (equal); funding acquisition (equal). **Xiao-Ai Wang** : Conceptualization (equal); data curation (equal); funding acquisition (equal); methodology (equal); validation (equal).

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CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

This study of genetic data (Genbank) can be accessed upon acceptance of the paper. Filtered reads for the RAD data pertaining to this study can be accessed through the NCBI GenBank Short Read Archive (accession numbers: PRJNA990519).

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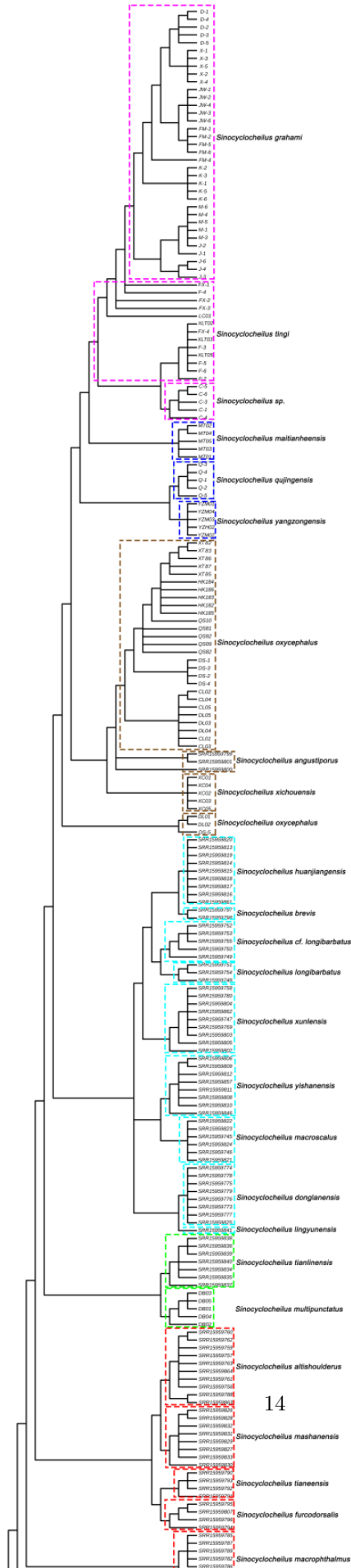
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SUPPORTING INFORMATION

Appendix S1 Phylogenetic Relationship.



the population of
Dianchi and Fuxian lake
Basin, Yunnan Province
(YN-DF)

the population of
Qujing Basin, Yunnan
Province (YN-QJ)

the population of
Mile Basin, Yunnan
Province (YN-ML)

the population of
Hunan Province
(HN)

the population II
of Guangxi
Province (GX ii)

the population I
of Guangxi
Province (GX i)

Figure S1 Phylogenomic relationships of the *Sinocyclocheilus* species based on the unpartitioned concatenated maximum likelihood (ML) analysis