frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 29 January 2026
DOI 10.3389/fevo.2025.1742741

OPEN ACCESS

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RECEIVED 09 November 2025
REVISED 29 November 2025
ACCEPTED 01 December 2025
PUBLISHED 29 January 2026

CITATION
Su Q, He K, Yang P and Zhang C (2026) Genetic diversity and population structure of three *Schizothorax curvilabiatus* populations in the Yarlung Zangbo River based on mitochondrial genes.
Front. Ecol. Evol. 13:1742741.
doi: 10.3389/fevo.2025.1742741

Genetic diversity and population structure of three *Schizothorax curvilabiatus* populations in the Yarlung Zangbo River based on mitochondrial genes

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Background: *Schizothorax curvilabiatus* is a commercially important freshwater fish endemic to the lower reaches of the Yarlung Zangbo River. However, its fishery resources have been severely depleted due to human activities, leading to a scarcity of data regarding its genetic characteristics. This study aims to assess the genetic diversity and population structure of this species to provide a scientific basis for its conservation.
Methods: Three mitochondrial DNA markers—Cytochrome b (Cyt *b*), Control Region (D-loop), and Cytochrome Oxidase Subunit I (COI)—were sequenced and analyzed. A total of 100 samples were collected from three natural populations in the lower Yarlung Zangbo River: Motuo (MT), Palong (PL), and Chayu (CY).
Results: High overall haplotype diversity (H_d: 0.889–0.951) but relatively low nucleotide diversity (P_i: 0.00450–0.0111) were observed. Notably, the PL population exhibited the lowest genetic diversity across all markers. Significant genetic differentiation was detected between the PL population and the other two populations (MT and CY), supported by F_{ST} indices, gene flow (N_m) estimates, and Analysis of Molecular Variance (AMOVA), which revealed that most variation resided within populations (>83%). Both Neighbor-Joining (NJ) phylogenetic trees and haplotype network analyses indicated a distinct geographical lineage pattern, with the PL and CY populations showing a distant genetic relationship.
Discussion: Although *S. curvilabiatus* maintains substantial overall genetic diversity, the low diversity within specific populations (particularly PL) and the significant inter-population differentiation highlight the vulnerability of its germplasm resources. These findings provide crucial insights into the genetic background of the species and offer valuable guidance for the conservation and sustainable management of its genetic resources.

KEYWORDS

genetic diversity, genetic structure, geographical population, mitochondrial gene, *Schizothorax curvilabiatus*

1 Introduction

Schizothorax curvilabiatus is a freshwater fish species belonging to the order Cypriniformes, family Cyprinidae, subfamily Schizothorinae, and genus *Schizothorax*. This species is widely distributed in the lower reaches of the Yarlung Zangbo River, including the mainstream of Motuo and its tributaries, Palong Zangbo and Chagyu River, at elevations ranging from 500 to 2300 meters. Historically, it has served as an important contributor to the local economy and remains a dominant fish species in the region (Wu and Wu, 1992; Wu, 1985). Due to the unique conditions of its high-altitude habitat, indigenous fish in Tibet exhibit slow growth rates, prolonged resource replenishment cycles, and late sexual maturity (Liu et al., 2021), with females reaching maturity at approximately 12 years and males at around 6 years (Wang, 2018). In recent years, the wild population of *S. curvilabiatus* has experienced a significant decline due to various factors, including overfishing for economic purposes, habitat destruction caused by the construction of water and hydropower facilities, and the introduction of alien species (Wang et al., 2022; Zhang et al., 2023). This decline has been accompanied by a noticeable trend of resource depletion, as well as an increase in individual miniaturization and a shift toward younger age groups. There is an urgent need for in-depth basic research to establish a scientific basis for the conservation of its resources. Existing studies have primarily focused on the biological characteristics (Zhang et al., 2021; Liu and Wei, 2022) and muscle nutrient composition (Jin et al., 2022) of *S. curvilabiatus*, with limited research on the differentiation between geographical populations. Therefore, it is crucial to investigate the germplasm resources of *S. curvilabiatus* in the lower reaches of the Yarlung Zangbo River. This research should include an analysis of its genetic diversity and structure, as well as an evaluation of its historical dynamics. Such studies are essential for the conservation and sustainable utilization of *S. curvilabiatus* germplasm resources.

Genetic diversity is a core aspect of species conservation research and a prerequisite for survival, adaptation, and evolution (David et al., 2018; Cao et al., 2024). Mitochondrial DNA (mtDNA), characterized by maternal inheritance, a simple structure, and a rapid evolution rate, has been widely applied in phylogenetic analysis and population genetic studies of fish (He et al., 2022; Xu et al., 2022). Among mtDNA segments, Cytochrome *b* (Cyt *b*) evolves at a moderate rate due to selection pressure, making it a frequently used marker for analyzing phylogenetic origins, geographic lineage, and population historical dynamics (Sun et al., 2023; Purohit et al., 2023). The control region (D-loop), a non-coding sequence with the fastest evolution rate in mtDNA, contains abundant genetic variation, thereby providing more comprehensive insights in phylogenetic and population studies (Fang et al., 2022; Roy et al., 2024). The cytochrome c oxidase subunit I (COI) gene, known for its slow evolutionary rate and strong conservation in its 5' end (500–700 bp), is frequently used in vertebrate species identification and population genetics studies due to its abundant base variation (Liu et al., 2020). Although the mtDNA sequence and structure of *S. curvilabiatus* have been described (Wang et al., 2017), its population genetic structure and diversity have not been systematically studied. Ma (2019) analyzed genetic differences among populations in the Motuo Section of Yarlung Zangbo River and Yigong Lake (a first-level tributary of the Palong Zangbo River) using the SLAF-seq method and developed SNP markers. However, due to insufficient sample size, a lack of analysis of inter-population gene flow, as well as the omission of the CY population, the genetic information available for *S. curvilabiatus* remains limited.

In this study, we analyzed the genetic diversity and population genetic structure of 100 samples collected from three sections of the lower reaches of the Yarlung Zangbo River (Motuo(MT), Palong (PL), and Chayu(CY)) using mitochondrial Cyt *b*, D-loop, and COI sequences. Our aim was to provide a scientific basis for the effective conservation strategies and germplasm resource managing of *S. curvilabiatus*.

2 Materials and methods

2.1 Sample collection and DNA extraction

In this study, samples of *S. curvilabiatus* were collected from the MT (35), PL (30) and CY (35) Rivers in the lower reaches of the Yarlung Zangbo River (Table 1; Figure 1). Fin clips were taken from the samples, preserved in anhydrous ethanol (95%), and stored at -20°C. DNA extraction was performed using a commercial DNA extraction kit (Sangon Biotech (Shanghai) Co., Ltd). The purity of the extracted DNA was verified via 1% agarose gel electrophoresis, and its concentration and $A_{260/280}$ ratio were measured using a Nanodrop 2000 spectrophotometer (Thermo Scientific, USA).

2.2 Cyt *b*, D-loop and COI gene sequence primer design and PCR amplification

Primers were designed using Primer 6 software based on the Cyt *b*, D-loop, and COI sequences (Genebank Accession no. NC_035994.1) from NCBI. Their specificity was verified prior to synthesis by Biomek,

TABLE 1 Sample collection information of *S.curvilabiatus*.

Population	River section	Sample time	Abbreviation	Sample size
MT Population	Yarlung Zangbo River (Motuo river section)	2024.02	MT	35
PL Population	Palong river	2024.02	PL	30
CY Population	Chayu river	2024.02	CY	35

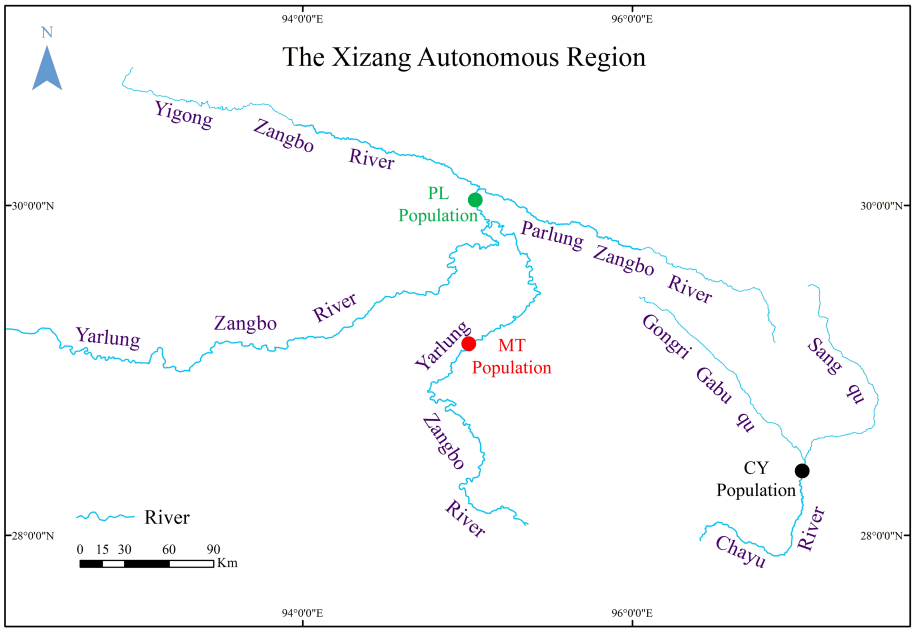

FIGURE 1
Sample sites of *S. curvilabiatus*. The red points represent MT population; the green points represent PL population; the black points represent CY population.

as detailed in Table 2. The PCR reaction consisted of a total volume of 20 µL, including 10 µL of 2× Taq PCR Master Mix (with dye), 6.5 µL of ddH_2O, 1 µL each of upstream and downstream primers (at a concentration of 10 µmol/L), and 1.5 µL of template DNA. The PCR cycling conditions were as follows: an initial denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 40 seconds. A final extension was performed at 72°C for 10 minutes, followed by a hold at 15°C for 5 minutes. The PCR products were subsequently sent to Biomek for sequencing.

2.3 Data analysis

The sequencing results were edited using BioEdit 7.0.5 (Hall, 1999) and manually proofread. Homology comparisons with the Cyt *b*, D-loop, and COI sequences with mtDNA (Gene bank Accession no. NC_035994.1) from NCBI were conducted using MEGA 7.0 (Kumar et al., 2016) to confirm sequence accuracy. Base composition, sequence variation, and inter-population genetic distances were analyzed. A phylogenetic tree was constructed using the Kimura 2-parameter (K2-P) model via the Neighbor-Joining (NJ) method, with branch confidence evaluated through Bootstrap analysis (1000 replicates). Genetic metrics, including the number of polymorphic sites (S), nucleotide diversity (P_i), number of haplotypes (H), and haplotype diversity index (H_d), were calculated using DnaSP 6.0 (Rozas et al., 2017). The haplotype network was constructed using PopART 1.7 (Leigh and Bryant, 2016). Additionally, Arlequin 3.5 (Excoffier et al., 2007) was used to perform an Analysis of Molecular Variance (AMOVA) to assess genetic variation within and between populations. To investigate potential population expansion, neutrality tests including Tajima's D (Zhang et al., 2022) and Fu's Fs (Zhao et al., 2021) were conducted, alongside with nucleotide mismatch distribution analysis.

TABLE 2 Mitochondrial gene primer sequence information.

Genes	Forward / Reverse	Primer sequences (from 5′ to 3′)	GenBank accession no.	Size (bp)
Cyt *b*	Forward	CTACGAAAAACACATCCCCT	NC_035994.1	1100
	Reverse	CCTGCTAGTGGGATAAAGAC		
D-Loop	Forward	CCACCCCTTATGGTCTAGTA	NC_035994.1	904
	Reverse	ATTTAGTGCCGGGTTACTTT		
COI	Forward	GCGCTGATTCTTCTCTACT	NC_035994.1	1503
	Reverse	TATGTGTGATAAGGAGGGGG		

3 Results

3.1 Sequence characteristics of mitochondrial genes

Following calibration and comparison of the sequencing results, Cyt *b* (1007 bp), D-loop (886 bp) and COI (1371 bp) sequences were selected for subsequent analysis. Analysis using MEGA revealed a significant AT bias in the mitochondrial gene sequences of the three populations of *S. curvilabiatus*, with the A+T content considerably exceeding the G+C content (Table 3). This observation is consistent with the typical characteristics of fish mtDNA. Additionally, the Cyt *b* sequence showed 107 polymorphic loci, consisting of 97 parsimony-informative sites and 10 singleton variable sites. The D-loop sequence contained 59 polymorphic sites, including 42 parsimony informative sites and 17 singleton variable sites. The COI sequence displayed 97 polymorphic sites, with 82 parsimony informative sites and 15 singleton variable sites (Table 4). The transition/transversion ratios for the three sequence regions were 11.4, 7.0 and 7.4, respectively.

3.2 Analysis of population genetic diversity

The genetic diversity parameters of three populations of *S. curvilabiatus* were analyzed using DnaSP 6.0, and the results are presented in Table 4. Among the 100 samples, a total of 41 Cyt *b* haplotypes were identified. The H_d for the populations ranged from 0.813 to 0.929, with an overall value of 0.945. The P_i ranged from 0.00551 to 0.01684, with an overall value of 0.0111. The *K* ranged from 5.55347 to 16.95429, with an overall average of 11.16662. Notably, the CY population exhibited the highest haplotype diversity, while the MT population showed the highest nucleotide diversity and average number of nucleotide differences. For the D-loop sequence, 48 haplotypes were identified. The H_d, P_i and *K* were 0.951, 0.01191 and 9.353, respectively. The CY population exhibited the highest haplotype diversity, while the MT population had the highest nucleotide diversity and average number of nucleotide differences. Additionally, 31 haplotypes were detected in the COI sequence, with an overall H_d of 0.889 and P_i index of 0.00450. Notably, the CY population also showed the highest haplotype diversity, nucleotide diversity, and average number of nucleotide differences among the three populations.

Comparatively, the MT population had the highest nucleotide diversity and average number of nucleotide differences, while the CY population consistently demonstrated the highest haplotype diversity across all analyses. Furthermore, the genetic parameters derived from D-loop analysis were superior to those obtained from the COI sequence, which reflected the lowest values.

3.3 Analysis of population genetic structure

Statistical analyses of genetic distance and genetic differentiation indices revealed the following: based on the Cyt *b* gene, genetic distances ranged from 0.00864 to 0.0134, while genetic differentiation indices varied from 0.06293 to 0.20213. For the D-loop gene, genetic distances ranged from 0.0116 to 0.0134, with differentiation indices between 0.08634 and 0.24561. Regarding the COI gene, genetic distances ranged from 0.00238 to 0.00578, and differentiation

TABLE 3 Nucleotide composition of mtDNA Cyt *b*, D-loop and COI gene sequences of *S. curvilabiatus*.

Genes	Base	MT (Percentage)	PL (Percentage)	CY (Percentage)	Total (Percentage)
Cyt *b*	A	25.7	25.7	25.7	25.7
	T	28.8	28.8	28.8	28.8
	G	17.0	17.0	17.0	17.0
	C	28.5	28.6	28.5	28.5
	A+T	54.5	54.5	54.5	54.5
D-loop	A	33.5	33.5	33.5	33.5
	T	33.1	33.1	33.0	33.0
	G	13.6	13.5	13.6	13.6
	C	19.8	19.9	19.9	19.9
	A+T	66.6	66.6	66.5	66.5
COI	A	25.5	25.6	25.6	25.6
	T	29.5	29.5	29.5	29.5
	G	19.0	18.9	18.9	18.9
	C	26.0	26.0	26.0	26.0
	A+T	55.0	55.1	55.1	55.1

TABLE 4 Genetic diversity parameters in mtDNA Cyt *b* gene, D-loop region and COI gene sequence of three populations of *S. curvilabiatus*.

Genes	Population	Genetic diversity parameters						
		H	H_d	P_i	*K*	*S*	*P-i-s*	*S-v-s*
Cyt *b*	MT	8	0.813	0.01684	16.95429	107	97	10
	PL	24	0.886	0.00828	8.32571	45	37	8
	CY	20	0.929	0.00551	5.55347	29	20	9
	Total	41	0.945	0.0111	11.16662	117	102	15
D-loop	MT	16	0.927	0.01133	8.904	30	24	6
	PL	12	0.801	0.0098	7.696	32	28	4
	CY	24	0.951	0.01046	8.22	39	24	15
	Total	48	0.951	0.01191	9.353	59	42	17
COI	MT	13	0.861	0.00875	11.972	77	69	8
	PL	10	0.713	0.00218	2.974	20	17	3
	CY	11	0.863	0.00176	2.411	20	12	8
	Total	31	0.889	0.00450	6.143	97	82	15

H, Haplotypes; H_d, Haplotype diversity; P_i, Nucleotide diversity; *K*, Average number of nucleotide differences; *S*, Variable(polymorphic) sites; *P-i-s*, Parsimony informative sites; *S-v-s*, Singleton variable sites.

indices ranged from 0.05183 to 0.16328 (Table 5). The N_m values for gene flow between the MT and PL populations ranged from 2.65 to 4.57, between the MT and CY populations from 1.48 to 2.74 and between the PL and CY populations from 0.77 to 1.28 (Table 6).

Results from the AMOVA analysis (Table 7) revealed that the contribution rates within population genetic variation were 88.59% (Cyt *b*), 83.72% (D-loop) and 91.58% (COI). Conversely, the contribution rates of among population genetic variation were 11.41%, 16.28% and 8.42%, respectively. These findings indicate that the primary source of genetic variation is within the populations. Furthermore, analysis of the genetic differentiation indices showed a significant degree of genetic differentiation among populations, with F_{ST} values of 0.11414 (Cyt *b*), 0.16276 (D-loop) and 0.08423 (COI) ($P < 0.05$).

TABLE 5 Population genetic differentiation and genetic distances.

Gene	Population	MT	PL	CY
Cyt *b*	MT		0.06293	0.10825
	PL	0.00864		0.20213
	CY	0.01253	0.0134	
D-loop	MT		0.08634	0.14482
	PL	0.0116		0.24561
	CY	0.0127	0.0134	
COI	MT		0.05183	0.08427
	PL	0.00238		0.16328
	CY	0.00573	0.00578	

Genetic distance among populations was the below diagonal value, the fixation index was the above diagonal value.

3.4 Haplotype distribution and phylogenetic analysis

Based on multilocus sequence data, this study constructed a neighbor-joining (NJ) phylogenetic tree with *S. oconnori* and *S. waltoni* as out groups to investigate the population evolutionary relationships of *S. curvilabiatus*. The results revealed significant phylogeographic structure among populations (Figure 2), demonstrating that the MT population formed a monophyletic clade with the PL and CY populations, while the PL and CY populations each constituted independent subclades. To validate the phylogenetic topology, a haplotype network analysis was performed (Figure 3), showing that the distribution patterns of haplotypes in Cyt *b* (47 haplotypes), D-loop (48 haplotypes), and COI (31 haplotypes) highly corresponded with the NJ tree topology. Haplotype sharing analysis indicated that the MT-PL population shared 3 (Cyt *b*), 3 (D-loop) and 2 (COI) haplotypes, while the MT-CY population shared 2 (Cyt *b*), 1 (D-loop) and 1 (COI) haplotype. Notably, no shared haplotypes were detected between the PL and CY populations.

TABLE 6 The gene flow among *S. curvilabiatus* populations.

Gene	Population		
	MT/PL	MT/CY	PL/CY
Cyt *b*	3.72	2.10	0.99
D-loop	2.65	1.48	0.77
COI	4.57	2.74	1.28

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TABLE 7 Analysis of molecular variances (AMOVA).

Gene	Source of variation	Degree of freedom	Sum of species	Variance components	Percentage of variation	Genetic differentiation index
Cyt *b*	Among populations	2	76.493	0.66216	11.41	F_{ST}=0.11414
	Within population	97	755.42	5.13891	88.59	
	Total	99	831.913	5.80107	100	
D-loop	Among populations	2	88.693	0.8042	16.28	F_{ST}=0.16276
	Within population	97	608.1	4.13673	83.72	
	Total	99	696.793	4.94093	100	
COI	Among populations	2	32.393	0.26608	8.42	F_{ST}=0.08423
	Within population	97	425.24	2.89279	91.58	
	Total	99	457.633	3.15887	100	

3.5 Population historical dynamic information

The results of the neutral tests, Tajima's *D* and Fu's *Fs*, are presented in Table 8. For the Cyt *b* sequence, negative Tajima's *D* values were observed across all populations, while a positive Fu's *Fs* value was found only in the MT population. However, Tajima's *D* values for all populations were not statistically significant ($P > 0.05$). In contrast, the Fu's *Fs* values for the PL and CY populations were significantly different ($P < 0.05$). The overall values for Tajima's *D* and Fu's *Fs* were -1.48953 ($P < 0.05$) and -21.13796 ($P < 0.01$), respectively.

Analysis using the D-loop sequence indicated that only the CY population had negative values for both Tajima's *D* and Fu's *Fs*, with the difference in Fu's *Fs* being significant ($P < 0.05$). The *D* and *Fs* values for the PL population were positive but not significant, while the MT population exhibited positive *D* values and negative *Fs* values, with both results also being non-significant. The overall *D* and *Fs* values across the D-loop sequence were -0.35407 ($P > 0.05$) and -19.13379 ($P < 0.05$), respectively.

In the analysis of the COI sequence, all three populations had negative Tajima's *D* values. The Fu's *Fs* values were negative for the PL and CY populations, while the overall *Fs* values for all three populations were positive. Among these, only the CY population showed a significant difference ($P < 0.05$). The total values of *D* and *Fs* for the COI gene were -2.04701 ($P < 0.01$) and -13.29356 ($P < 0.01$), respectively.

Additionally, the nucleotide mismatch distribution curves based on Cyt *b* and D-loop sequences exhibited multi-peaks, indicating

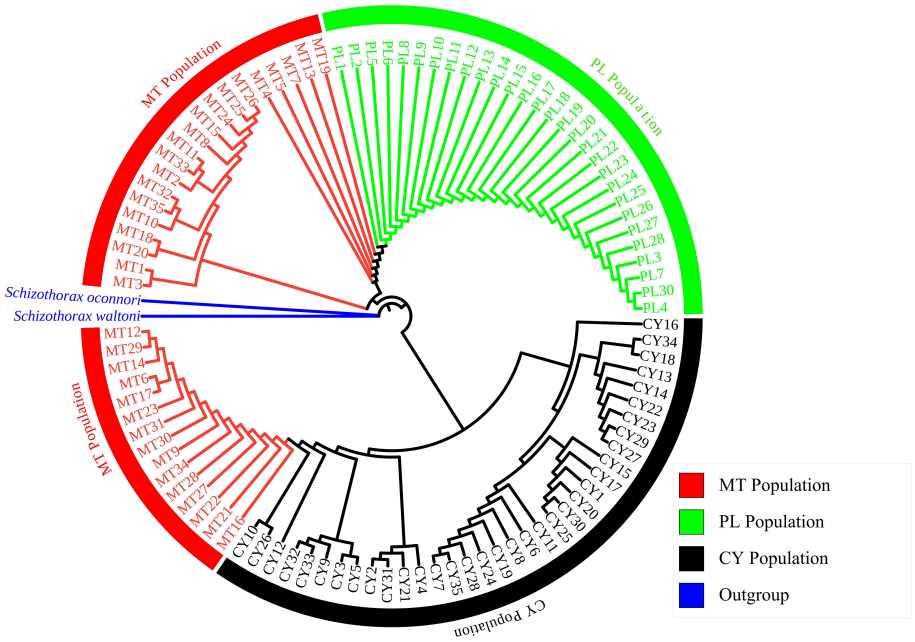

FIGURE 2
NJ phylogenetic trees based on Cyt *b*, D-loop and COI sequences using Kimura 2-parameter (K2-P).

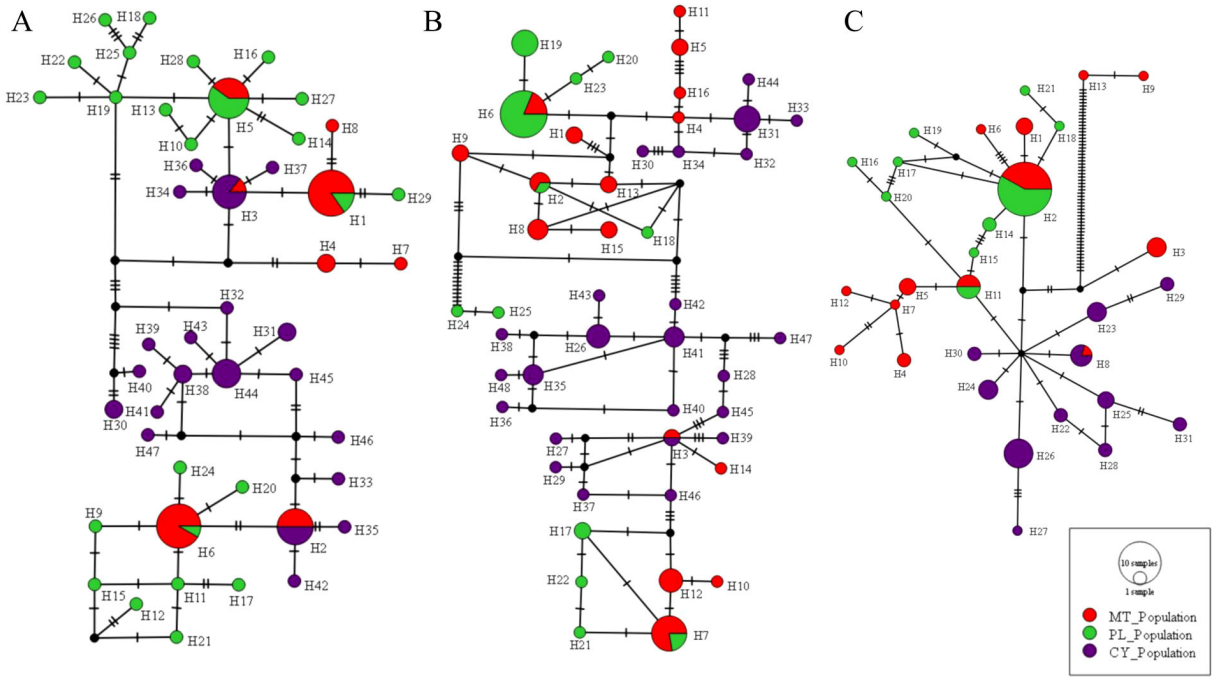

FIGURE 3
Median-Joining network of Cyt *b* **(A)**, D-loop **(B)** and COI **(C)** haplotypes from three populations of *S. curvilabiatus*.

that the populations were in a stable state. The historical dynamic analysis based on these two genes showed that the MT population was in a relatively stable state without evidence of population expansion events (Figures 4, 5). Although there were significant differences in the Fu's *Fs* values of the PL population, Tajima's *D* values were not significantly different, and the nucleotide mismatch map showed multiple peaks, indicating that no population expansion event had occurred historically (Figures 4, 5).

The mismatch analysis for COI gene indicated population expansion (Table 7), with a single-peak overall nucleotide mismatch map (Figure 6). Among the three populations, only the CY population exhibited significantly negative Tajima's *D* and Fu's *Fs* values, alongside a double-peak nucleotide mismatch map (Figure 6), suggesting historical expansion followed by stabilization.

Overall, the historical dynamics of *S. curvilabiatus* are complex. Based on neutral tests and nucleotide mismatch analysis, it is inferred that the recent expansion events are rare, and the species is currently in a relatively stable state.

TABLE 8 Neutrality test for three *S. curvilabiatus* populations.

Gene	Neutral test	MT	PL	CY	Total
Cyt *b*	*D*	-0.37681	-0.58747	-0.47182	-1.48953*
	Fs	8.63173	-10.7623*	-6.04096*	-21.13796**
D-loop	*D*	1.09633	0.25861	-0.19014	-0.35407
	Fs	-0.1548	0.76461	-7.30463*	-19.13379*
COI	*D*	-1.06943	-1.06507	-1.46737*	-2.04701**
	Fs	2.5865	-2.45241	-6.81541**	-13.29356**

*represent $P < 0.05$; ** represent $P < 0.01$.

4 Discussion

4.1 Genetic diversity of different geographical populations of *S. curvilabiatus*

Genetic diversity is a crucial prerequisite for assessing the status of population resources and informing conservation efforts. It serves as a key analytical method for studying the origin and evolution of species while providing insights into their evolutionary potential (Ward, 2000; Chen et al., 2020). Environmental selection pressures significantly influence population dynamics. Under low selection pressure, populations are more likely to survive, indicating higher adaptability to their environment. This adaptability is often associated with greater genetic diversity, which helps populations remain resilient to environmental changes. Conversely, low genetic diversity renders populations vulnerable to environmental pressures, potentially leading to bottlenecks or extinction (Manel et al., 2020; Williams and Johnson, 2022; Yao et al., 2022).

In this study, mitochondrial DNA sequences, including the Cyt *b* gene, D-loop region, and COI gene, were used as genetic markers to analyze the genetic diversity of three populations of *S. curvilabiatus*. Sequence analysis of the homologous fragments of the Cyt *b*, D-loop, and COI genes revealed a higher content of adenine (A) and thymine (T) compared to guanine (G) and cytosine (C), exhibiting a significant AT bias and a bias against G/C. This observation aligns with the known nucleotide distribution patterns

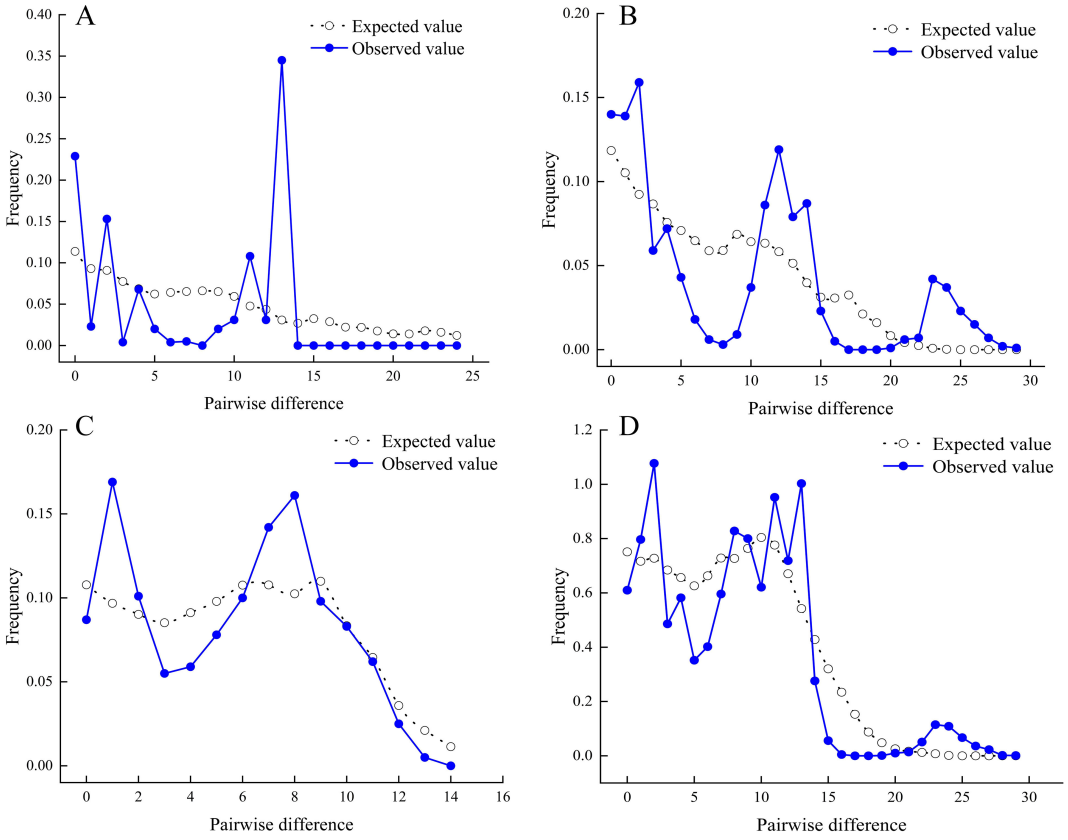

FIGURE 4
Mismatch distribution of based on mtDNA Cyt *b* gene sequences. **(A)** MT; **(B)** PL; **(C)** CY; **(D)** Total.

in the mitochondrial genomes of fish (Tsuji et al., 2020; Choi et al., 2021; Tesfaye et al., 2021; Modeel et al., 2023).

The genetic diversity of fish populations is primarily assessed through H_d and P_i. Based on established classification criteria (H_d = 0.5, P_i = 0.005), genetic diversity can be categorized into four levels (Grant and Bowen, 1998; Bowen et al., 2001), the combination of low H_d - low P_i is indicative of the founder effect, whereas a high H_d- low P_i combination reflects post-bottleneck expansion. A low H_d- high P_i combination suggests geographic isolation and subsequent differentiation, while a high H_d- high P_i combination indicates a stable large population. In this study, the overall H_d and P_i values indicate a pattern of high haplotype diversity coupled with low nucleotide diversity. This pattern likely arises because haplotype diversity accumulates more rapidly, whereas nucleotide diversity increases over a longer time frame. It is speculated that a bottleneck effect or post-colonization expansion occurred within the *S. curvilabiatus* populations, resulting in limited nucleotide variation accumulation in a short period. Among the three genetic markers, the D-loop exhibited the highest H_d and P_i values, suggesting that it is more sensitive to changes than the Cyt *b* and COI genes. This increased sensitivity may be attributed to the location of the D-loop in the non-coding region, which tends to evolve faster than coding regions due to the absence of natural selection pressures. Therefore, the D-loop region is particularly well-suited for detecting fine-scale population structure. Nevertheless, owing to the inherent limitations in the amount of genetic information provided by any single locus, the protein-coding genes COI and Cyt *b* continue to hold significant value for phylogenetic reconstruction and species-level taxonomic assessments. Consequently, a comprehensive understanding of population genetic structure is best achieved through the integrated analysis of genetic variation across multiple, independently evolving loci. This finding is consistent with the mitochondrial gene patterns observed in *Cyprinus carpio* var. *baisenensis* and *Labeo gonius* (Roy et al., 2024; Sumana et al., 2024). When analyzing the genetic diversity among the three populations, both the MT and CY populations exhibited high genetic diversity, while the PL population displayed significantly lower genetic diversity. This pattern may align with the theory that environmental gradients, including altitude, climate, and habitat complexity, influence the dispersal and population genetic structure of plateau species. At the altitudinal limits of a species' range, diversity and adaptation may be accelerated, potentially leading to a reduction in genetic diversity at higher altitudes (Schiffers et al., 2013; Halbritter et al., 2015).

4.2 Genetic structure and genetic differentiation of different geographical populations of *S. curvilabiatus*

The genetic structure and differentiation among fish populations were assessed using genetic distance, gene flow (N_m),

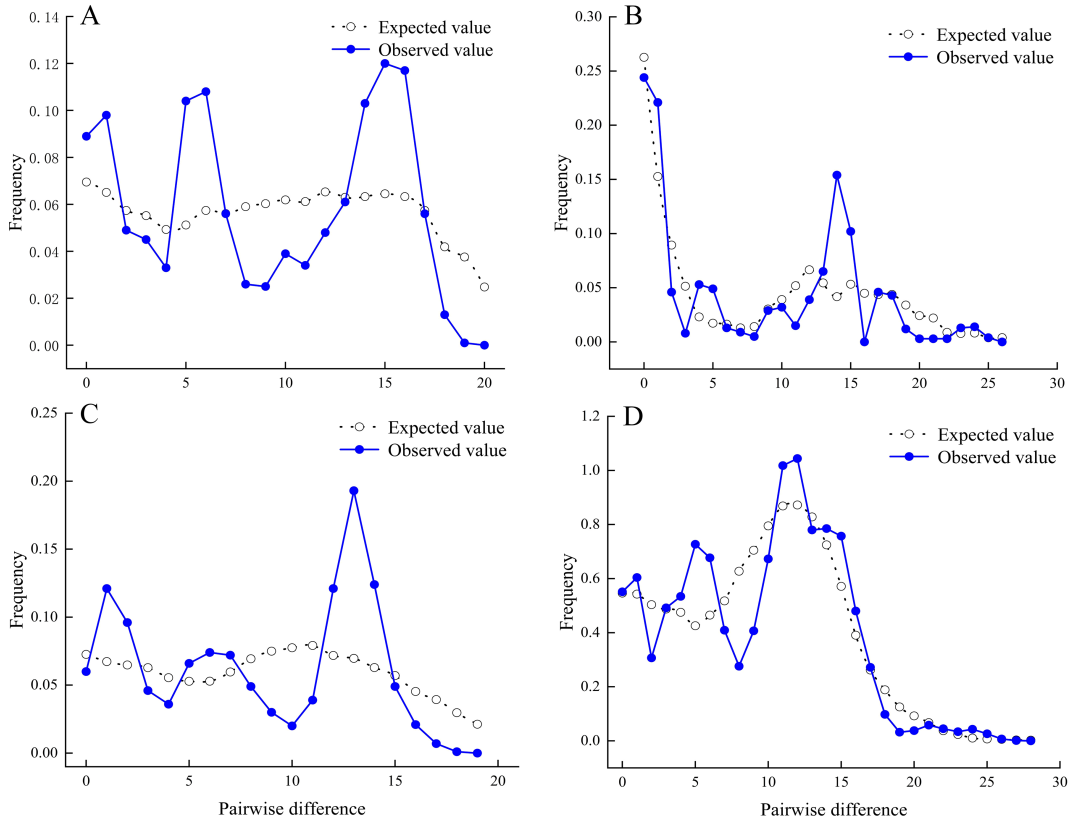

FIGURE 5
Mismatch distribution of based on mtDNA D-loop region sequences. **(A)** MT; **(B)** PL; **(C)** CY; **(D)** Total.

and genetic differentiation index (F_{ST}). In this study, the three genes exhibited a consistent distribution trend, arranged from closest to farthest: MT and PL populations, MT and CY populations, and CY and PL populations. This pattern corresponds with the geographical distribution of *S. curvilabiatus*. Based on genetic distance classifications, distances greater than 0.9 were categorized as genus level, distances over 0.3 as species level, and those exceeding 0.05 as population level (Shaklee et al., 1982). None of the three populations reached the population classification standard, suggesting that they may have originated from a common ancestor. Geographical isolation and differing habitats within the same water restricted gene exchange among the populations, contributing significantly to their differentiation and leading to a genetic structure that mirrors the species distribution (Vilcot et al., 2022; Jia et al., 2024).

The genetic differentiation index (F_{ST}) is a critical measure for evaluating population differentiation. Classification criteria for F_{ST} are as follows: $0 < F_{ST} < 0.05$: low differentiation; $0.05 < F_{ST} < 0.15$: moderate differentiation; $0.15 < F_{ST} < 0.25$: high differentiation; $F_{ST} > 0.25$: very high differentiation (Wright, 1931, 1978). Analysis of the three genes indicated that the F_{ST} values of the populations ranged from 0.05 to 0.25. Specifically, the F_{ST} values between the MT and PL populations were 0.05183 to 0.08634, indicating low differentiation. In contrast, the F_{ST} values for MT and CY populations ranged from 0.08427 to 0.14482, reflecting moderate differentiation. The values for the PL and CY populations were between 0.16328 to 0.24561, indicating a high level of genetic differentiation.

Gene flow (N_m) is another key metric for evaluating population genetic structure and differentiation. In this study, the N_m value of gene flow between the MT and PL populations ranged from 2.65 to 4.57, significantly exceeding 1, which suggests close geographical proximity and potential channels for gene exchange. This indicates that random mating is likely among individuals of the MT and PL populations (Petit and Excoffier, 2009). The N_m value between the MT and CY populations was 1.48 to 2.74, signifying relatively close geographical distance and the possibility of gene exchange. Conversely, the N_m value for the PL and CY populations was 0.77 to 1.28, indicating substantial genetic differentiation between these groups.

In summary, analyses of genetic distance, genetic differentiation index (F_{ST}), and gene flow (N_m) among three populations revealed a similar differentiation structure. The MT and PL populations were the closest, exhibiting the lowest F_{ST} values and the highest N_m values, suggesting low differentiation and a high probability of gene exchange. The MT and CY populations demonstrated moderate differentiation, accompanied by a lower likelihood of gene flow (N_m). In contrast, the PL and CY populations were highly differentiated, with minimal gene exchange. The results of the AMOVA analysis among the three populations indicated that over 83% of the genetic variation was within populations, suggesting that the within-population variation is the primary source of overall genetic diversity.

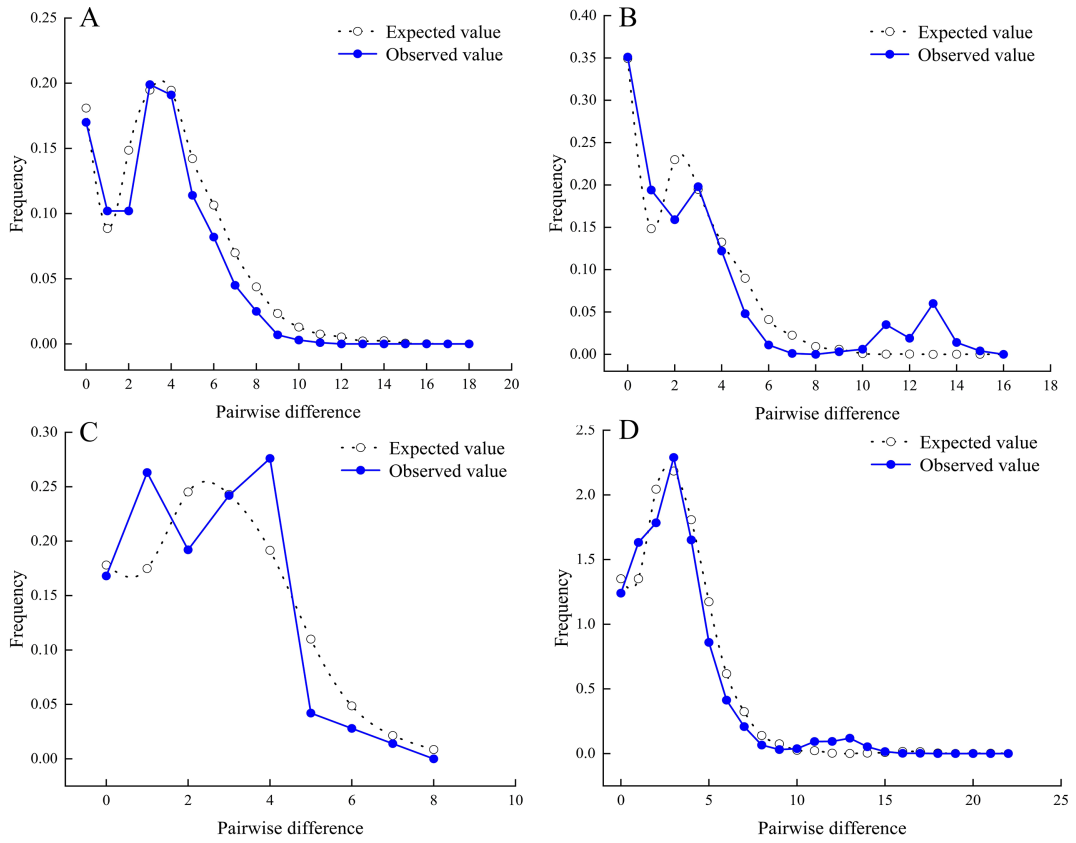

FIGURE 6
Mismatch distribution of based on mtDNA COI gene sequences. **(A)** MT; **(B)** PL; **(C)** CY; **(D)** Total.

The phylogenetic tree constructed from three genes and the haplotype network indicated that the three populations formed two distinct geographic clusters: one composed of the MT and PL populations, with mixed haplotypes but no obvious phylogenetic relationship, and the other composed of the MT and CY populations, with no obvious phylogenetic relationship between their haplotypes. This suggests that the PL and CY populations formed distinct geographical populations and lineage structures, consistent with the above genetic differentiation pattern.

Based on the above analysis results, the genetic differentiation pattern of the three populations was closely related to their geographic location. The MT population was distributed in the main stream of the Yarlung Zangbo River, while the PL population was distributed in the Palong River basin, a first-level tributary of the Yarlung Zangbo River. The Palong River flows into the Yarlung Zangbo River in the Yarlung Zangbo Grand Canyon, providing opportunities for gene exchange. The CY population is distributed in the Chayu River, which flows into the Yarlung Zangbo River overseas, geographically distant. There is a theoretical possibility of gene exchange between the MT population in the main stream of the Yarlung Zangbo River and the PL population in the Palong River. However, the analysis results show that the genetic diversity of the CY population is high, and there is essentially no possibility of gene exchange. Thus, it became distinctly differentiated from the other populations.

4.3 Population historical dynamics of different geographical populations of *S. curvilabiatus*

This study employed neutrality tests for three genes and nucleotide mismatch analysis to infer whether the populations of *S. curvilabiatus* had experienced expansion events. According to the neutrality analysis, negative and statistically significant values of Tajima's *D* and Fu's *Fs* indicate that a population deviates significantly from the neutral model, suggesting a historical expansion event (Tajima, 1989). In conjunction with nucleotide mismatch analysis, a unimodal distribution, represented by a single peak in the Poisson distribution, further supports the occurrence of population expansion (Mária et al., 2013). The overall Tajima's *D* and Fu's *Fs* values for the three genes varied, suggesting that the entire *S. curvilabiatus* population may have experienced expansion events. However, notable differences in these values indicated that only the CY population might have undergone distinct population expansion events.

The nucleotide mismatch analysis showed that only the overall distribution of the COI gene exhibited a unimodal pattern. In contrast, the remaining populations displayed multimodal distributions. Specifically, the MT and PL populations indicated a stable state without evidence of recent expansion, whereas the CY population may have experienced a recent expansion event or a bottleneck effect, followed by a period of relative stability. These

findings align with results observed in *Scophthalmus maximus* and *Pangasius* spp (Firidin et al., 2020; Ha et al., 2020).

While this study has revealed the genetic differences among various geographical populations of *S. curvilabiatus* from a mitochondrial perspective, the limited genetic information available does not fully explain the reasons behind the current genetic structure of this species. Future research could expand the sample size or delve deeper into genetic information to analyze the timing and influencing factors of genetic differentiation among geographical populations at the genomic level.

In recent years, the fishery resources in the lower reaches of the Yarlung Zangbo River have drastically declined due to frequent geological disasters and human activities. As a representative fish species in this region, *S. curvilabiatus* is inevitably experiencing population decline and bottleneck effects, leading to a significant reduction in genetic diversity.

5 Conclusion

This study found that the genetic diversity of *S. curvilabiatus* in the MT and CY populations was relatively high overall, but significant differences existed in the genetic diversity within each population. The three populations exhibited two main phylogenetic relationships, revealing the genetic background of the germplasm resources of *S. curvilabiatus* in the lower reaches of the Yarlung Zangbo River. Moreover, the results provide valuable insights into the conservation, sustainable development, and utilization of the germplasm resources of *S. curvilabiatus*.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: NCBI, accession PX806090–PX806188.

Ethics statement

The animal study was approved by Animal Ethics Committee of the Academy of Agriculture and Animal Husbandry of Xizang Autonomous Region. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

QS: Conceptualization, Validation, Investigation, Data curation, Funding acquisition, Methodology, Writing – original draft, Formal analysis, Visualization. KH: Writing – original draft, Investigation, Supervision, Software, Resources, Methodology. PY: Writing – original draft, Investigation, Methodology. CZ: Formal analysis, Resources, Visualization, Validation, Writing – review & editing, Investigation, Supervision, Funding acquisition, Conceptualization.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This work supported by the National Key R&D Program of China (Grant no. 2024YFD12007000); the Key Science and Technology Plan project of Tibet Autonomous Region (Grant no. XZ202301ZY0012N) and the financial special project of the Ministry of Agriculture and Rural Affairs (Grant no. CJW2023034).

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

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