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Genetic Diversity and Phylogeographic Structure of Icefishes (Salangidae) from Chinese Freshwater Lakes

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ABSTRACT

Background: This study aimed to investigate genetic diversity and phylogeographic structure of seven icefish populations from Yunnan freshwater lakes, Southwestern China.

Methods: All 116 COI and 130 Cytb sequences from seven icefish populations were mainly used for genetic diversity, phylogeographic structure and demographic history analyses.

Result: Genetic diversity analysis showed low mtDNA diversity in seven icefish populations. In the COI tree, *Neosalanx* sp., *N. tangkahkeii* and *N. taihuensis* samples formed a monophyletic clade with a 100% bootstrap support rate and the same occurred in the Cytb tree where our sequences, together with those of *N. taihuensis* and *N. tangkahkeii*, formed a monophyletic clade with the same high support rate. Haplotype network analysis showed that all haplotypes did not cluster separately. Genetic variation mainly occurred within populations, as revealed by the AMOVA analysis. A Mantel test proved that there was no correlation existing between genetic and geographical distances. Moreover, the entire dataset of icefish populations showed signs of population expansion. Overall, these findings suggest relatively low genetic diversity and no significant phylogeographic structure of icefish in these lakes and also imply that besides *N. taihuensis*, *N. tangkahkeii* may inhabit the seven freshwater lakes.

Key words: COI gene, Cytb gene, Genetic diversity, Icefish, Phylogeographic structure.

INTRODUCTION

Icefishes belong to the Salangidae family (Zhang *et al.*, 2007). They exhibit ecological traits of r-selection, including high fecundity, short generation cycles, early maturity, minimal parental care and strong colonization ability in unstable environments (Zhang *et al.*, 2013). Globally, icefishes comprise approximately 20 species in six genera, primarily distributed in the coastal and inland waters of East Asian countries and regions (Saruwatari *et al.*, 2002). In China, 15 icefish species in six genera have been classified based on morphological characteristics, with main distributions in Yangtze River system (Xie and Chen, 1999). For instance, due to its great commercial significance, *Neosalanx taihuensis* has been introduced into a large number of lacustrine and reservoir systems across over 20 Chinese provinces since the 1980s (Liu, 2001). This species can rapidly adapt to new conditions by evolving phenotypic or genotypic changes within a short number of generations. Such adaptation often occurs through natural selection acting on pre-existing genetic variation or new mutations, ultimately leading to population differentiation or the emergence of new species (Fang *et al.*, 2022).

Icefishes disperse via artificial introduction and natural swimming-based diffusion. The introduction and reproduction of Chinese icefishes in freshwater lakes primarily involve *N. taihuensis*, *N. pseudotaihuensis* and *Protosalanx hyalocranius* (Wang *et al.*, 2002). Yunnan has records of successive introductions of *N. taihuensis* into Dianchi, Xingyun and Erhai lakes in the early 1980s (Chen *et al.*, 1989; Gao *et al.*, 1989; Wu *et al.*, 1998). Zhou and Xie (1991) confirmed that icefishes introduced into Dianchi Lake were *N. taihuensis* and *N. pseudotaihuensis*. Genetic differentiation may exist among these geographical populations, including potential cryptic or disputed species. However, due to overfishing, environmental pollution and habitat destruction, icefish populations have continuously declined and their distribution ranges significantly reduced. Some species are gradually becoming endangered (Zhang, 2008) and exhibit low genetic diversity, which generally indicates poor environmental adaptability (Rossi *et al.*, 2021). When environmental pressures (such as habitat destruction) reduce population size, genetic drift intensifies random fluctuations in allele frequencies, thereby rapidly decreasing genetic diversity (Frankham, 1995). Therefore, a comprehensive understanding of the genetic diversity and phylogeographic structure of these

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populations is crucial for enhancing the protection and rational utilization of icefish resources.

Mitochondrial DNA (mtDNA) is distinguished by its simple structure, maternal inheritance and rapid mutation rate (Lai *et al.*, 2015) and has been widely used in research on vertebrates genetic diversity, population structure and phylogeny (Naseer *et al.*, 2018; Utami *et al.*, 2024). Among cytochrome c oxidase subunit I (COI) and cytochrome b (Cytb) are ideal molecular markers for species and genus level phylogenetic studies (Abbas *et al.*, 2022; Sari *et al.*, 2015). Currently, researchers have used mitochondrial and microsatellite DNA markers to investigate the genetic diversity (Fang *et al.*, 2022; Xing *et al.*, 2022; Zheng *et al.*, 2024) and systematic evolution (Liu *et al.*, 2018) of icefishes. However, existing studies mainly focus on the genetic variation patterns in *Protosalanx chinensis*, *N. taihuensis*, *Salanx ariakensis* and *N. jordani* using Cytb sequences. To date, knowledge regarding the genetic diversity and phylogeographic structure of icefishes in Chinese freshwater lakes based on COI and Cytb genes remains scarce.

In this study, we utilized COI and Cytb sequences to analyze the genetic diversity and phylogeographic structure of seven icefish populations. The primary objectives were (1) to investigate genetic diversity of seven icefishes populations and formulate rational conservation strategies; (2) to determine whether significant phylogeographic structure exist within these populations; (3) to identify whether there are other icefish species besides *N. taihuensis* in these populations. These findings of this research can serve as a reference for the protection and utilization of icefish resources.

MATERIALS AND METHODS

Sample collection and DNA extraction

In total, 144 and 140 icefish samples were collected from Yunnan seven freshwater lakes. After collection, these samples were preserved in Zoology Laboratory of the College of Life Sciences of Yunnan Normal University for COI and Cytb gene analysis, respectively (Table 1). The Biomedical Research Ethics Committee of Yunnan Normal University approved all the experimental protocols applied in this study. We used the Hi-Pure Animal Genomic DNA Kit (TSINGKE TSP202-200 Trelief) following the kit's protocol precisely to extract genomic DNA from the dorsal muscle. After the DNA concentration was detected, these samples were stored at -20°C for subsequent analysis. The entire research period was from January 2023 to April 2024.

PCR amplification and sequencing

The COI fragment was amplified using primers Fish-F1 (5′- TCGACTAATCATAAAGATATCGGCAC-3′) and Fish-R1 (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′) (Ward *et al.*, 2005). The Cytb gene primers were L14321 (5′-CC AGTGACTTGAAAAACCACCG-3') and H15634 (5′-CT TAGCTTTGGGAGTTAAGGGT-3') (Zhang *et al.*, 2007). PCR amplifications of two genes took place within a 50 μL reaction system. This reaction mixture included 2 μL of each 10 μmol/L primer, 2 μL of genomic DNA, 25 μL of 2 × San Taq PCR Mix and19 μL of ddH$_2$O. PCR cycling conditions of COI and Cytb genes were initial denaturation at 94°C for 5 min, followed by 35 cycles (COI) or 30 cycles (Cytb) of denaturation at 94°C for 30 s, annealing at 50°C for 35 s (COI) or 55°C for 50 s (Cytb) and extension at 72°C for 45 s (COI) or 90 s (Cytb), with a final extension at 72°C for 8 min (COI) or 10 min (Cytb) (Ward *et al.*, 2005; Zhang *et al.*, 2007). The successfully amplified PCR products were sent to Beijing Qingke Biotech (Kunming) Co., Ltd for bidirectional sequencing. Due to double peaks in some samples causing incomplete sequencing, the final usable sequences were 116 (COI) and 130 (Cytb).

Data analyses

The SeqMan program in the DNAstar v7.1 was employed to meticulously examine the newly obtained sequences. The Clustal W program integrated in MEGA7.0 to align for two genes sequences. The obtained sequences were used to calculate sequence similarity by the BLAST sequence analysis tool. We used the MEGA7.0 to calculate base composition and genetic distance of icefish populations based on Kimura 2-parameter method. The variation sites (S), parsimony informative sites (Np), number of haplotypes (Nh), haplotype diversity (Hd), nucleotide diversity (Pi) and gene flow (*Nm*) were calculated with DnaSP v5.10.

To determine phylogenetic relationship of seven icefish populations. A Neighbor-Joining (NJ) tree was constructed in MEGA7.0 using two obtained genes sequences, along with some *Neosalanx* species and outgroup (*P. chinensis*) sequences. Node supports in NJ tree analysis were evaluated using a bootstrap analysis with 1000 replications. The POPART software was used to construct the haplotype network to detect genetic relationships among haplotypes. Additionally, analysis of molecular variance (AMOVA) and a Mantel test were executed in Arlequin v3.5.2. To detect demographic history of seven icefish populations, the Neutrality tests (Tajima's *D*, Fu's *Fs*), the sum of squared deviation (SSD) and Raggedness index (r) were computed in Arlequin software.

RESULTS AND DISCUSSION

MtDNA sequence characteristics and haplotypes

The 653 bp and 1205 bp fragment were successfully obtained for COI and Cytb gene, respectively. The proportion of A+T (45.2%, 48.6%) was lower than G+C (54.8%, 51.4%) for 116 COI and 130 Cytb sequences, which is consistent with previous research (Xiong *et al.*, 2021; Zhong *et al.*, 2016). Sequence similarity analysis revealed that COI sequence exhibited 100% identity with *N. taihuensis* and *N. tangkahkeii* and 99% identity with *Neosalanx* sp. Cytb sequences showed 100% similarity with *N. taihuensis* and *N. tangkahkeii*, confirming these icefish populations as *N. taihuensis* and *N. tangkahkeii* (Kang *et al.*, 2015; Zhang *et al.*, 2013). Amongst the 116 COI sequences, there were 8 variable sites, 4 parsimony informative sites and 8 haplotypes (Hap1C-Hap8C), with Hap1C as the core haplotype, accounting for 81.03% of all individuals. In the case of the 130 Cytb sequences, there were 17 variable sites, 12 parsimony informative sites and 13 haplotypes (Hap1B-Hap13B) (Table 1), with Hap2B as the core haplotype (67.69% frequency).

Genetic diversity and conservation of icefish resources

Genetic diversity underlies a species' adaptability to environmental changes and supports evolutionary persistence (Roberts, 2002). Haplotype and nucleotide diversities were employed to evaluate genetic richness (Xing *et al.*, 2022), with values >0.5 (Hd) and >0.005 (Pi) indicating high diversity (Grant and Bowen, 1998). This study found that the genetic diversity values (Hd=0.331±0.053, Pi=0.00059±0.00011) of the COI gene were lower than the average values (Hd=0.576±0.036, Pi=0.00112±0.00204) calculated by Zhang *et al.* (2012) and were consistent with the genetic diversity findings reported by Fang *et al.* (2022). However, the genetic diversity (Hd=0.525±0.050, Pi=0.00111±0.00018) of seven icefish populations based on the Cytb gene were greater than 0.5 and but less than 0.005. These results suggest a relatively low mtDNA diversity level, which may be attributed to long term overfishing, water quality degradation, competition with other fish species and artificial control (Avise, 2004). This phenomenon could also be due to the genetic bottleneck often occurring during the introduction process and the potential founder effects resulting from the introduction of a small number of individuals (Fang *et al.*, 2022; Zhu *et al.*, 2023). For example, Fang *et al.* (2022) analyzed the genetic diversity of *N. taihuensis* from eleven Chinese river basins and found high haplotype diversity but low nucleotide diversity, displaying that *N. taihuensis* may have undergone rapid population expansion following bottleneck effects. However, this study only used mitochondrial markers, which may not fully represent genetic variation. Nuclear markers (such as microsatellites and SNPs) are necessary for future validation, as these molecular markers have been widely used in genetic diversity research and phylogenetic studies of various animals (Veeramani *et al.*, 2023; Pan *et al.*, 2023). Hence, it is necessary to strengthen resource conservation measures to prevent the loss of genetic variation. At the population level, the YZH population's higher diversity (Table 1) may reflect larger sample size rather than true genetic variation, highlighting the need for standardized sampling across all populations.

A "unified management unit" refers to a coordinated and integrated entity that oversees various aspects of fishing activities, like resource monitoring and catch limits, to ensure sustainable yields in fisheries (Badr *et al.*, 2014). Studies on genetic diversity and genetic structure of fish

Table 1: Genetic diversity, Neutrality test indexes of seven icefish populations in Yunnan based on COI and Cytb genes.

Gene	Sample locality	Abbreviation	N	Sequences	S	Np	Nh	Hd	Pi	Tajima's *D* (P-value)	Fu's *Fs* (P-value)	SSD (P-value)	Raggedness index "r" (P-value)
COI	Fuxian Lake	FXH	20	11	1	1	2	0.182±0.144	0.00028±0.00022	-1.12850 (0.18030)	-0.40988 (0.14220)	0.00041 (0.17120)	0.43802 (0.96180)
	Yangzonghai Lake	YZH	20	19	2	2	3	0.608±0.070	0.00106±0.00018	0.49216 (0.75180)	0.37280 (0.51200)	0.03303 (0.00320)	0.22232 (0.11440)
	Dianchi Lake	DC	20	20	1	0	2	0.100±0.088	0.00015±0.00013	-1.16439 (0.13650)	-0.87930 (0.07870)	0.00006 (0.12020)	0.65000 (0.95210)
	Erhai Lake	EH	22	15	2	0	2	0.133±0.112	0.00041±0.00034	-1.49051 (0.05680)	0.23493 (0.30650)	0.00975 (0.17160)	0.78667 (0.78340)
	Qilu Lake	QLH	22	19	2	0	3	0.205±0.119	0.00032±0.00019	-1.51077 (0.04740)	-1.80438 (0.01140)	0.00045 (0.16360)	0.39308 (0.86540)
	Chenghai Lake	CHH	20	12	1	1	2	0.409±0.133	0.00063±0.00020	0.54055 (0.81110)	0.73482 (0.49610)	0.00833 (0.02550)	0.20041 (0.66950)
	Xingyun Lake	XYH	20	20	3	0	3	0.416±0.116	0.00082±0.00030	-0.97524 (0.19830)	-0.07875 (0.38050)	0.00009 (0.83670)	0.13194 (0.68760)
		Total	144	116	8	4	8	0.331±0.053	0.00059±0.00011	-1.76369 (0.00940)	-6.28176 (0.00090)	0.00029 (0.06320)	0.20859 (0.61860)
Cytb	Fuxian Lake	FXH	20	18	3	3	4	0.314±0.138	0.00028±0.00013	-1.71304 (0.02230)	-2.60267 (0.00260)	0.00204 (0.09060)	0.22953 (0.69250)
	Yangzonghai Lake	YZH	20	20	9	9	5	0.679±0.080	0.00247±0.00032	0.59934 (0.76520)	1.89845 (0.83350)	0.10774 (0.07560)	0.30922 (0.41040)
	Dianchi Lake	DC	20	20	1	1	2	0.442±0.087	0.00037±0.00007	1.02588 (0.88650)	1.16902 (0.61980)	0.01252 (0.00020)	0.20886 (0.41870)
	Erhai Lake	EH	20	19	2	0	3	0.205±0.119	0.00017±0.00010	-1.51077 (0.04730)	-1.80438 (0.01270)	0.00045 (0.16260)	0.39308 (0.87050)
	Qilu Lake	QLH	20	19	1	0	2	0.105±0.092	0.00009±0.00008	-1.16480 (0.13620)	-0.83782 (0.09020)	0.00002 (0.27140)	0.63435 (0.94290)
	Chenghai Lake	CHH	20	15	8	0	5	0.476±0.155	0.00089±0.00047	-2.08600 (0.00280)	-1.36075 (0.11250)	0.01806 (0.08320)	0.14395 (0.69560)
	Xingyun Lake	XYH	20	19	9	5	6	0.643±0.108	0.00181±0.00045	-0.54138 (0.33840)	-0.12827 (0.47800)	0.05859 (0.21140)	0.16227 (0.67100)
		Total	140	130	17	12	13	0.525±0.050	0.00111±0.00018	-1.56217 (0.03250)	-4.85524 (0.03530)	0.02779 (0.37040)	0.13055 (0.78010)

N: Sample size; S: variable site; Np: Parsimony informative sites; Nh: Number of haplotypes; Hd: Haplotype diversity; Pi: Nucleotide diversity.

populations can provide crucial references for establishing fishery resource management measures and species conservation strategies (Avise, 2004). This study found relatively low genetic diversity and limited variation among seven icefish populations, indicating poor genetic resources in these lakes. Therefore, these populations should be conserved as a unified management and protection unit. Currently, effective control measures should be implemented for these icefish populations. Such as, avoiding overfishing, protecting habitats, reducing environmental pollution and improving the ecological environment of icefish in the process of management and conservation. As icefishes are one year lived species with a short life cycle and a high reproduction rate, the breeding and release of icefish populations should be carried out scientifically and regularly. Moreover, monitoring and genetic assessment of migratory and wild populations should be strengthened to enhance genetic diversity of icefishes in Yunnan lakes.

Phylogeographic structure of populations

Calculating genetic distances in fish research distinguishes species precisely, unravels evolutionary histories and identifies cryptic species, aiding taxonomy and conservation (Hebert *et al.*, 2003). The COI sequences of *N. tangkahkeii*, *Neosalanx* sp., *N. taihuensis*, *P. chinensis* (Table S1) and our samples sequences were combined to calculate genetic distances (Table 2), indicating that the genetic distance between our measured samples and *Neosalanx* sp. was the smallest (0.1%). While the genetic distance between our measured samples and *N. tangkahkeii* (0.2%) and *N. taihuensis* (0.2%) were significantly lower than 2% (Hebert *et al.*, 2003), indicating an intraspecific level. The COI tree indicated that seven populations samples, *N. tangkahkeii*, *Neosalanx* sp. and *N. taihuensis* were gathered into a monophyletic clade with 100% bootstrap support rate (Fig 1a). Notably, icefish

Table 2: The genetic distance calculated based on the COI gene and Cytb gene.

Gene	Populations	Interspecific genetic distance										Intraspecific
		1	2	3	4	5	6	7	8	9	10	genetic distance
COI	1.FXH											0.000
	2.YZH	0.001										0.001
	3.DC	0.000	0.001									0.001
	4.EH	0.000	0.001	0.000								0.000
	5.QLH	0.000	0.001	0.000	0.000							0.000
	6.CHH	0.001	0.001	0.000	0.001	0.001						0.001
	7.XYH	0.001	0.001	0.001	0.001	0.001	0.001					0.001
	8. *N. tangkahkeii*	0.002	0.002	0.002	0.002	0.002	0.002	0.002				0.000
	9. *N. taihuensis*	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003			0.000
	10. *N.* sp.	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.002		0.001
	11. *P. chinensis*	0.125	0.126	0.125	0.125	0.125	0.125	0.126	0.125	0.127	0.125	0.000
Cytb	1.FXH											0.000
	2.YZH	0.002										0.003
	3.DC	0.001	0.002									0.000
	4.EH	0.000	0.002	0.001								0.000
	5.QLH	0.000	0.002	0.001	0.000							0.000
	6.CHH	0.001	0.002	0.001	0.001	0.001						0.001
	7.XYH	0.001	0.002	0.002	0.001	0.001	0.001					0.002
	8. *N. tangkahkeii*	0.001	0.003	0.000	0.001	0.001	0.001	0.002				0.000
	9. *N. taihuensis*	0.002	0.003	0.003	0.002	0.002	0.002	0.003	0.003			0.004
	10. *N. argentea*	0.034	0.033	0.034	0.033	0.033	0.034	0.033	0.034	0.034		0.002
	11. *P. chinensis*	0.126	0.126	0.127	0.126	0.126	0.126	0.126	0.127	0.127	0.124	0.002

Table S1: GenBank sequences and their accession numbers used in this study.

Family	Genus	Species	Accession numbers	
			COI	Cytb
Salangidae	*Neosalanx*	*Neosalanx* sp.	MK843743-47, MK843749-52	/
		Neosalanx tangkahkeii	NC_026455, KP170510	NC_026455, KP170510, DQ191112
		Neosalanx taihuensis	NC_019629, JX524196	DQ191098, DQ191105, DQ191107-08, DQ191110-11
		Neosalanx argentea	/	DQ191080-81
	Protosalanx	*Protosalanx chinensis*	JN242662-64	DQ191122-24

samples from each lake did not cluster independently, but were interspersed with samples from other lakes. The Cytb tree results was similar to that of COI tree (Fig 1b). As of 1993, *N. taihuensis* was transplanted into Yunnan eleven lakes (Zhuang *et al.*, 1996). Therefore, it is speculated that *N. taihuensis* and *N. tangkahkeii* are present in the seven lakes of Yunnan. However, Zhong *et al.* (2016) reported that sequence similarity between *N. tangkahkeii* and *N. taihuensis* was 99.9% and suggested that they belong to the same species. Thus, precise species distinction not only prevents over exploitation of specific populations and maintains ecosystem balance in fisheries, but also aids in understanding speciation mechanisms and adaptive evolution. This provides a scientific basis for addressing challenges such as habitat fragmentation and climate change (Murat, 2025).

The NJ tree and haplotype network (Fig 2) demonstrated that samples or haplotypes of each icefish population from seven lakes did not cluster separately and haplotypes were shared among individuals from different populations. The AMOVA analysis indicated that the genetic variation occurred predominantly within population (89.97% for COI, 76.61% for Cytb), despite the populations being geographically separated. A Mantel tests displayed no significant associations (R=-0.1717, P=0.7796 for COI; R=-0.2374, P=0.8017 for Cytb) between genetic and geographic distances (Table S2), which is similar to genetic structure studies on *P. chinensis* (Zheng *et al.*, 2024). This phenomenon may be explained by extensive gene flow (2.35 for COI, 0.90 for Cytb). Collectively, these results demonstrated no significant phylogeographic structure among these icefish populations. As economically valuable small fish, icefishes spread via cruise ships and human activities, promoting gene exchange. However, the absence of phylogeographic structure has significant impacts on fisheries management. In this study, treating all populations

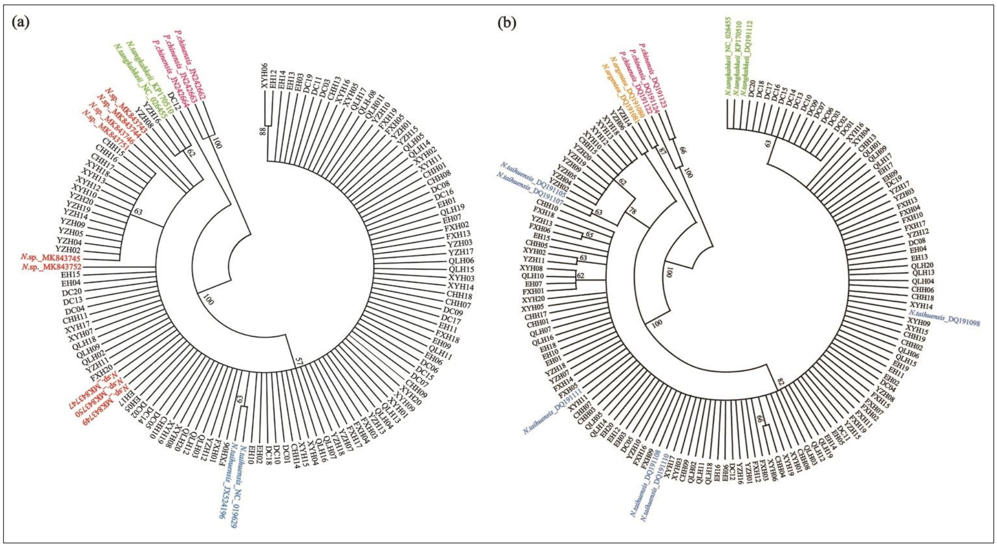

Fig 1: The NJ phylogenetic tree of *Neosalanx* based on COI (a) and Cytb (b) gene sequences. The numbers at the branches represent the bootstrap values (only shown those ≥50%) based on 1,000 replicates.

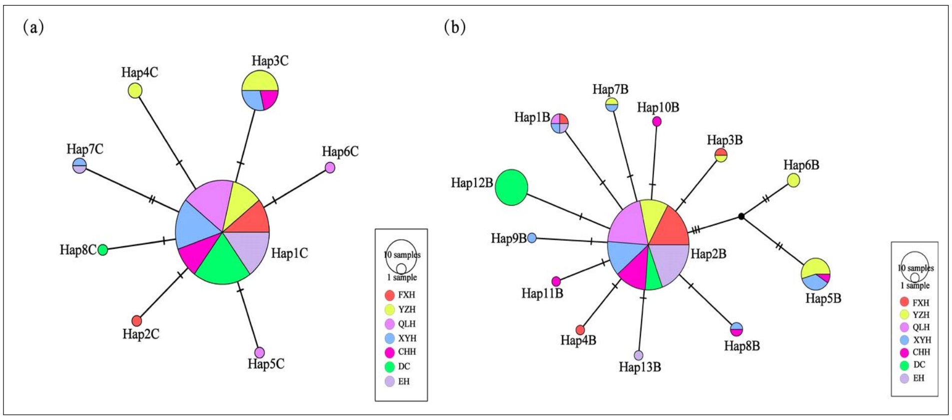

Fig 2: The haplotype network of seven icefish populations based on COI (a) and Cytb (b) gene sequences.

Table S2: The geographic distance (lower triangle, km) and genetic distance (upper triangle) among the seven icefish populations in Yunnan.

Gene	Populations	1	2	3	4	5	6	7
COI	1.FXH		0.0010	0.0000	0.0000	0.0000	0.0010	0.0010
	2.YZH	40.46		0.0010	0.0010	0.0010	0.0010	0.0010
	3.DC	35.03	30.06		0.0000	0.0000	0.0000	0.0010
	4.EH	305.87	301.20	271.79		0.0000	0.0010	0.0010
	5.QLH	41.96	82.99	73.02	328.69		0.0010	0.0010
	6.CHH	316.38	298.45	274.68	102.51	343.34		0.0010
	7.XYH	27.13	67.65	56.65	308.10	19.04	330.80	
Cytb	1.FXH		0.0020	0.0010	0.0000	0.0000	0.0010	0.0010
	2.YZH	40.46		0.0020	0.0020	0.0020	0.0020	0.0020
	3.DC	35.03	30.06		0.0010	0.0010	0.0010	0.0020
	4.EH	305.87	301.20	271.79		0.0000	0.0010	0.0010
	5.QLH	41.96	82.99	73.02	328.69		0.0010	0.0010
	6.CHH	316.38	298.45	274.68	102.51	343.34		0.0010
	7.XYH	27.13	67.65	56.65	308.10	19.04	330.80	

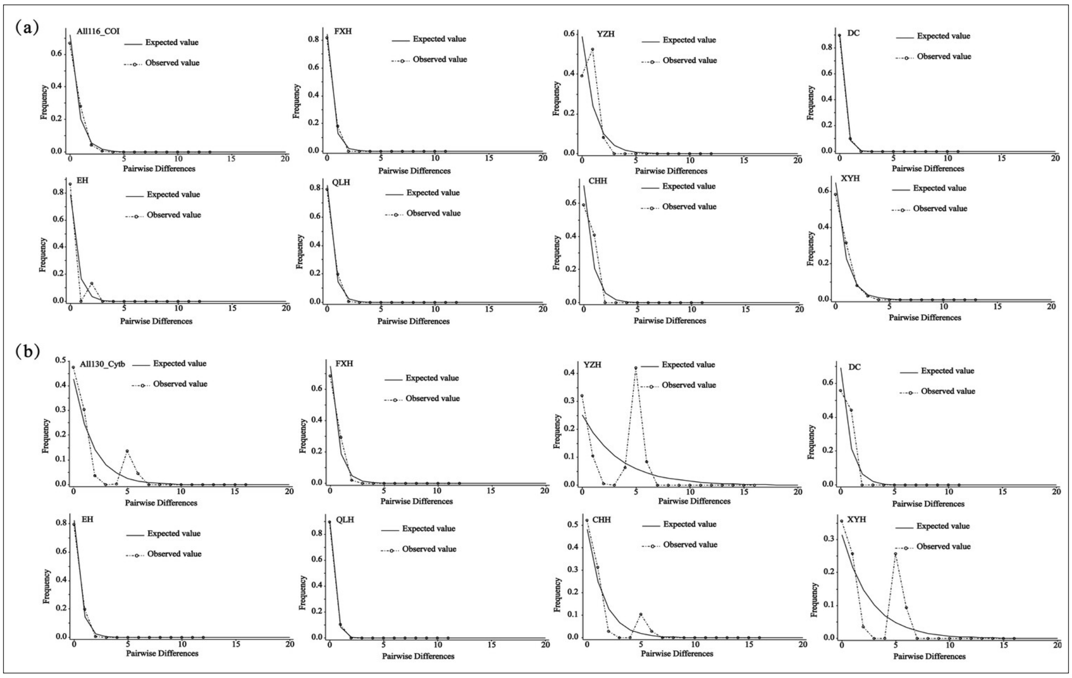

Fig 3: The mismatch distribution of seven icefish populations based on COI (a) and Cytb (b) genes.

as unified management unit has protected icefish, but may have overlooked local adaptive traits. This is because unmonitored intensive fishing could damage genetically distinct subpopulations and climate change may also disrupt gene flow, thus urgently requiring the formulation of dynamic management plans (Murat, 2025).

Demographic history

Neutrality tests (Fu, 1997; Ramos-Onsins and Rozas, 2002) and mismatch distribution analyses (Barbosa *et al.*, 2013) can be used to predict historical demographic expansions. As expected, all seven populations dataset indicated population expansion (Table 1; Fig 3). The haplotype network presented a star-like phylogeny. This is a characteristic trait typically associated with a population that has experienced a recent expansion (Avise, 2004). Additionally, the statistical tests of SSD and Raggedness were not significant, suggesting no significant deviation from the population expansion model. These results are in accordance with earlier investigations (Fang *et al.*, 2022).

CONCLUSION

Our results revealed relatively low mtDNA diversity across seven Yunnan icefish populations. As expected, no significant phylogeographic structure was detected among these populations and all seven populations datasets presented indications of population expansion. Besides *N. taihuensis*, *N. tangkahkeii* may also inhabit these seven freshwater lakes. These findings of this study indicated that different populations ought to be regarded as an integrated management unit to achieve effective conservation and management goals. Future research will incorporate nuclear molecular markers and genome-wide analysis to further characterize genetic diversity in Yunnan icefish populations.

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Disclaimers

The opinions and conclusions in the article only represent those of the authors and do not necessarily represent their affiliated institutions.

Informed consent

The Biomedical Research Ethics Committee of Yunnan Normal University had approved experimental procedure that used animals in this research.

Conflict of interest

All authors state that there are no conflicts of interest.

REFERENCES

Abbas, K., Zhou, X. and Wang, W. (2022). Mitochondrial diversity and phylogenetic structure of Yellowcheek (*Elopichthys bambusa*) in the Yangtze river. *Journal of Applied Ichthyology.* **38:** 596-603.

Avise, J.C. (2004). Molecular Markers, Natural History and Evolution. Sinauer Associates, Sunderland, Massachusetts.

Barbosa, A., Real, R., Muñoz, A.R. and Brown, J.A. (2013). New measures for assessing model equilibrium and prediction mismatch in species distribution models. *Diversity and Distributions*. **19:** 1333-1338.

Badr, L.M., Salwa, O. and Ahmed, Y. (2014). Implementing a unified methodology for fisheries management (UMFM) in fresh water in morocco. *International Journal of Fisheries and Aquaculture.* **6:** 94-103.

Chen, P., Yu, W. and He, J. (1989). Experiment of transplanting *neosalanx taihuensis* from taihu lake to Xingyun Lake, Yunnan Province. *Freshwater Fisheries.* **1:** 31-32.

Fang, D.A., He, M., Ren, Y.F., Luo, H., Zhou, Y.F., Jiang, S.L. and You, Y. (2022). Assessment of genetic diversity of the Salangid, *Neosalanx taihuensis*, based on the mitochondrial COI gene in different Chinese River basins. *Biology.* **11:** 968.

Frankham, R. (1995). Effective population size/adult population size ratios in wildlife: A review. *Genetics Research*. **66:** 95-107.

Fu, Y.X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics.* **147:** 915-925.

Gao, L.C., Zhuan, D.D., Che, J.Z., Chen, L.L. and Zhang, J.L. (1989). Test study of introducing *Neosalanx taihuensis* Chen into Dianche Lake. *Scientia Limnologica Sinica.* **1:** 79-87.

Grant, W.A.S. and Bowen, B.W. (1998). Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. *Journal of Heredity.* **89:** 415-426.

Hebert, P.D.N., Ratnasingham, S. and De Waard, J.R. (2003). Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*. **270:** S96-S99.

Kang, B., Deng, J., Wang, Z. and Zhang, J. (2015). Transplantation of Icefish (Salangidae) in China: Glory or Disaster? *Reviews in Aquaculture.* **7:** 13-27.

Lai, J., Zhao, L.J., Fan, Y.C., Qu, X.C., Liu, D.Y., Guo, Z.L., Wang, Y.H., Liu, Q.G. and Chen, Y.S. (2015). Using whole body elemental fingerprint analysis to distinguish different populations of coilia nasus in a large river basin. *Biochemical Systematics and Ecology*. **60**: 249-257.

Liu, Y., Song, C. and Chen, J. (2018). The mitochondrial genome of clearhead icefish *Protosalanx chinensis* (Salmoniformes: Salangidae). *Mitochondrial DNA Part B.* **3:** 774-775.

Liu, Z.W. (2001). The introduction of icefish, *Neosalanx taihuensis* Chen, in China with special reference to the subtropical lakes of the Yunnan Plateau (southwest China). *International Association of Theoretical and Applied Limnology*. **27:** 3877-3880.

Murat, Ö. (2025). Phylogenetic and taxonomic studies of *Paral ichthys* (Paralichthyid): Evolutionary insights from molecular and morphological data. *Fish Taxa.* **35:** 34-43.

Naseer, J., Anjum, K.M., Khan, W.A., Imran, M., Ishaque, M., Hafeez, S., Munir, M.A. and Nazir, M.A. (2018). Phylogenetic analysis based studies on genetic variation of Cytochrome b gene of Indian peafowl (*Pavo cristatus*) in Pakistan. *Indian Journal of Animal Research.* **52:** 343-346. doi: 10.188 05/ijar.v0iOF.9119.

Pan, X., Zhou, L.P., He, S.S. and Tang, L.H. (2023). Genetic diversity estimation of six goat populations from Chongqing, China using microsatellite markers. *Indian Journal of Animal Research*. **57:** 951-955. doi: 10.18805/IJAR.BF-1437.

Ramos-Onsins, S.E. and Rozas, J. (2002). Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution.* **19:** 2092-2100.

Roberts, C.M. (2002). Deep impact: the rising toll of fishing in the deep sea. *Trends in Ecology and Evolution.* **17:** 242-245.

Rossi, A.R., Petrosino, G., Crescenzo, S., Milana, V., Talarico, L., Martinoli, M., Rakaj, A., Lorenzoni, M., Carosi, A., Ciuffardi, L. and Tancioni, L. (2021). Phylogeography and population structure of *Squalius lucumonis*: A baseline for conservation of an Italian endangered freshwater fish. *Journal for Nature Conservation.* **64:** 126085.

Sari, A., Duran, M., Sen, A. and Bardakci, F. (2015). Investigation of chironomidae (Diptera) relationships using mitochondrial COI gene. *Biochemical Systematics and Ecology.* **59:** 229-238.

Saruwatari, T., Oohara, I. and Kobayashi, T. (2002). Salangid fishes: Their past, present and future. *Fisheries Science.* **68:** 71-74.

Utami, S., Jamil, A., Nidom, R.V., Nasir, M., Prayitno, H., Prakoso, D., Ansori, A.N., Indrasari, S. and Nidom, C.A. (2024). Genetic diversity of crossbred cattle using cytochrome oxidase subunit I (COI) gene in South Sulawesi, Indonesia. *Indian Journal of Animal Research.* **58:** 28-34. doi: 10.18805/IJAR.BF-1600.

Veeramani, P., Prabakaran, R., Sivaselvam, S.N., Sivakumar, T., Selvan, S.T. and Karthickeyan, S.M.K.(2023). Genetic diversity of six duck populations in India. *Indian Journal of Animal Research.* **57:** 1113-1119. doi: 10.18805/IJAR.B-4379.

Wang, Z., Fu, C. and Lei, G. (2002). Biodiversity of Chinese icefishes (Salangidae) and their conserving strategies. *Biodiversity Science.* **10:** 416-424.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. and Hebert, P.D.N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London.* Series *B, Biological Sciences.* **360:** 1847-1857.

Wu, Q., Li, W., Wang, S., Dong, Y., Zhu, J. and Zhang, H. (1998). The growth features of *Neosalanx taihuensis* in Erhai Lake and its fisheries utilization. *Journal of Fishery Sciences of China.* **5:** 110-113.

Xie, P. and Chen, Y. (1999). Threats to biodiversity in Chinese inland waters. *Ambio.* **28:** 674-681.

Xing, T.F., Li, Y.L. and Liu, J.X. (2022). Anthropogenic influences on genetic diversity and differentiation of the clearhead icefish (*Protosalanx hyalocranius*), as revealed by mitochondrial and microsatellite DNA markers. *Regional Studies in Marine Science.* **55:** 102547.

Xiong, F., Zhai, D.D., Yu, J.X., Chen, Y.Y., Wang, Y. and Liu, H.Y. (2021). The complete mitochondrial genome of the Chinese noodlefish *Leucosoma chinensis* and phylogenetic analysis of Salangidae (Osteichthyes: Osmeriformes). *Mitochondrial DNA Part B.* **6:** 176-177.

Zhang, D., Lei, C.G., Gong, C.S. and Wang, Z.S. (2012). Genetic diversity of *Neosalanx taihuensis* based on mitochondrial COI sequences. *Journal of Lake Sciences.* **24:** 299-306.

Zhang, J. (2008). Biodiversity of Chinese icefishes (Salangidae) and their diversity conservation. *Biological Bulletin.* **43:** 4-6.

Zhang, J., Deng, F.Y. and Zhou, Q.H. (2013). Weight-length relationships of 14 species of icefishes (Salangidae) endemic to East Asia. *Journal of Applied Ichthyology.* **29:** 476-479.

Zhang, J., Li, M., Xu, M., Takita, T. and Wei, F. (2007). Molecular phylogeny of icefish Salangidae based on complete mtDNA cytochrome b sequences, with comments on estuarine fish evolution. *Biological Journal of the Linnean Society.* **91:** 325-340.

Zheng, Y., Tang, F., Zheng, X., Na, R., Li, Z., Lu, W. and Lu, C. (2024). Analysis of the genetic structure of the introduced clearhead icefish (*Protosalanx chinensis*) populations in northern China. *Journal of Fish Biology.* **104:** 1743-1753.

Zhong, L., Wang, M., Li, D., Tang, S., Zhang, T., Bian, W. and Chen, X. (2016). Complete mitochondrial genome of Chinese icefish *Neosalanx tangkahkeiis* (Salmoniformes, Salangidae): comparison reveals *Neosalanx taihuensis* not a valid name. *Mitochondrial DNA Part A.* **27:** 3303-3305.

Zhou, W. and Xie, Q. (1991). Study on the ultrastructure egg surface of two species in *Neosalanx* (Pisces: Salangidae). *Oceanologia et limnologia Sinica.* **22:** 93-95.

Zhu, S.L., Chen, W.T., Wu, Z., Li, Y.F., Li, J., Li, X.H. and Yang, J.P. (2023). Mitochondrial diversity and genetic structure of common carp (*Cyprinus carpio*) in pearl River and Nandujiang river. *Journal of Fish Biology.* **102:** 1109-1120.

Zhuang, Y., Feng. Z. and Li, J. (1996). Study on the proliferation ecology of *Neosalanx taihuensis* in Yunnan plateau lakes. *Reservoir Fisheries.* **16:** 16-20.