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Research article

Genetic diversity of the common carp black strain population based on mtDNA (*D-loop* and *cytb*)

Sahr Lamin Sumana [a], Peipei Wang [c,1], Chengfeng Zhang [a], Xiaojun Jing [a,b], Jian Zhu [a,b], Yongkai Tang [a,b], Wenting Liu [a,b], Shengyan Su [a,b,*], Yu Liao [c,**]

[a] *Wuxi Fisheries College, Nanjing Agricultural University, Wuxi, 214081, PR China*
[b] *Key Laboratory of Integrated Rice-Fish Farming Ecology, Ministry of Agriculture and Rural Affairs, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi, 214081, PR China*
[c] *Guangxi Fisheries Introduction and Cultivation Center, Nanning, PR China*

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ABSTRACT

The common strain black carp (*Cyprinus carpio* var. *baisenensis*) is a culturally important carp strain that is raised and cultured in Guangxi Province, China. Its color reflects the interactions between the Burau people and their surrounding environment. The population of the common carp black strain was isolated and cultured in a rice-fish integration system. To explore the genetic diversity and protection of germplasm resources, we analyzed mitochondrial DNA (mtDNA) sequences, specifically the displacement loop (*D-loop*) and cytochrome *b* (*Cytb*), using single-nucleotide polymorphisms (SNP). We compared these sequences with those from four other local common carp populations. The study included a total of 136 adult common carps from five strain populations: the common black carp strain (HJ = 31), Jian (F = 30), Heilongjiang (H = 10), Songpu (S = 31), and Saijiang (SJ = 34). The results of the *Cytb* and *D-loop* analyses showed that the Heilongjiang carp (H) and Saijiang (SJ) populations had the highest levels of haplotype diversity (0.867 ± 0.034785) and nucleotide diversity (π = 0.0063 ± 0.000137 and 0.0093 ± 0.000411), respectively. On the other hand, the Common carp black strain population (HJ) exhibited the lowest haplotype diversity in both *Cytb* and *D-loop*, with haplotype 2 being the most commonly observed among the populations. Private haplotypes dominated the five common carp populations, which were significantly different at $P<0.001$. Furthermore, analyzing the coefficient of genetic differentiation (Fst), the highest genetic difference was observed between Saijiang (SJ) and Heilongjiang (H) (Fst = 0.963), whereas the lowest was observed between Songpu (S) and the Common carp black strain population (HJ) (Fst = 0.019) for the *Cytb* gene sequences. For the *D-loop*, the Common carp black strain population (HJ) and Songpu (S) (Fst = 0.7) had the highest values, and Heilongjiang (H) and Common black carp strain (HJ) had an Fst of 0.125. Additionally, the AMOVA analysis revealed a higher level of variance for the *Cytb* and *D-loop* genes, indicating lower genetic diversity within the local carp community. On the other hand, the phylogenetic tree analysis showed that the five carp populations were closely related and formed a distinct cluster. The distinct cluster of populations suggests a common ancestor or recent gene flow, possibly due to geographic proximity or migration history, and unique genetic characteristics, possibly due to adaptations or selective pressures. The results of this study provide valuable

* Corresponding author. Wuxi Fisheries College, Nanjing Agricultural University, Wuxi 214081, PR China.
** Corresponding author.
E-mail addresses: ouhaicourse@hotmail.com (S. Su), Liaoyu_252@163.com (Y. Liao).
[1] Contribution to the paper equally and link to first two authors.

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insights into the genetic diversity of the common strain black carp, which can have implications for conservation, breeding programs, evolutionary studies, and fisheries management.

1. Background

The common carp, *Cyprinus carpio L.*, is a common freshwater fish of the Cyprinidae family that is native to western Asia, but has since been introduced or translocated to other parts of the world. It has since become one of the most significant edible fishes, with numerous strains and varieties in various regions of the world [1–3]. Because of its rapid growth and delicious flavor, the species has been raised in China during the past few years [4], it is a common aquarium fish that is also edible due to its domestication and cultural background, it is well known and holds great significance for Chinese history and culture, which has been in existence for over 8000 years in China [5]. However, the country has been the highest carp-exporting country since 2017, with 46,504 metric tons and the highest culture production of 64.14 % in 2018, although there was a decrease of 10.72 % from 1998 to 2018 [6].

Carps have also been introduced into numerous areas during domestication, and their ancestors have undergone genetic modifications. These factors, along with the natural and artificial selection of various elements such as mutation accumulation and long-term geographic isolation, have led to the development of numerous carp species. The scales, body shape, skin tone, and stress tolerance differ from one another. Carp transported by humans to various sites result in significant gene flow [7]. In the long history of aquaculture, distinctive artificial and regional strains have been created worldwide, including Jian fish and carp, with various traits that form the basis of their adaptability and variety.

The black-loving Burau population of Guangxi has a long history of the Common carp black strain population (*Cyprinus carpio* var. *baisenensis*). The relationship between Bourau and the environment is reflected in the color of the subspecies. There was no microbial contamination because this species was geographically separated, isolated, and reproduced. Integrated fish and rice culture technology was used to grow a Common carp black strain population. Anthropogenic activities, including agriculture (pesticides, herbicides, and excess use of inorganic fertilizers) (International Union for Conservation of Nature), have contributed to the decline of freshwater fish species such as *Cyprinus carpio* var. *baisenensis* and wild populations have decreased because of habitat degradation and the introduction of unchecked alien species [8,9].

For the successful implementation of conservation measures, a thorough understanding of gene flow, population genetic differentiation, and genetic population identification is required [10]. To deal with the dramatic reduction in economically significant species, genetic diversity studies have been carried out. Many molecular techniques have been applied to better understand the patterns of genetic diversity and stock genetic resource management. In general, the genetic diversity of aquaculture populations (including hatchery populations) of various fish species in China has been reported to be lower than in wild populations, for example, *Ctenopharyngodon idella* [11], *Siniperca chuatsi* [12], and India *Labeo gonius* [13].

There are still challenges in investigating genetic diversity based on mtDNA because of single-gene analysis, while genetic diversity is caused by the different evolutionary rates of genes and regions in the metagenome. However, the challenges in studying common carp genetic variation include biased results due to small sample sizes, sensitive detection methods, and careful data analysis [14,15]. Therefore, researchers have proposed a new idea, which is to analyze multiple genes or gene clusters in the metagenome. Not a single gene or genomic region. This method increases the possibility of revealing the actual evolutionary history of a particular species [16, 17].

The study of genetic research on the common black strain of common carp is crucial for its economic and ecological importance. Understanding genetic diversity and structure within and among populations is essential for effective conservation efforts. Previous studies by Shuli et al. [18] have assessed the genetic diversity of farmed common carp populations, suggesting that applied fish farming practices can preserve and improve genetic diversity for generations. However, the current study compared the common black strain of common carp with other local strains, as little is known about the genetic diversity and structure of the common black strain's populations based on mtDNA (*D-loop* and *Cytb*) in Guangxi Province.

2. Materials and methods

2.1. Fish samples and DNA isolation

A total of 136 mature common carps were collected from several lakes and farms, of which 5 strain populations (Table 1) were sampled, including the common black carp strain (HJ = 31), Jian (F = 30), Heilongjiang (H = 10), Songpu (S = 31), and Saijiang (SJ = 34) (see Table 2). In addition, the Common carp black strain population (8), Huanghe carp (16), and Songpu carp (6) have been sequenced using SNP-based studies. However, the procedures outlined by Quan et al. [19] and Kohlmann and Kersten [20], were used to extract DNA from fin tissue. The fins of live fish were clipped and immediately soaked in 95 % ethanol during and then stored at −20 °C in a freezer until DNA isolation. The total genomic DNA was extracted from fin tissues and preserved in ethanol using proteinase-K digestion, a traditional method. This method was employed, along with a phenol-chloroform extraction protocol [21].

To test the credentials of the samples, sequencing results were compared using the BLAST tool in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). We numbered the haplotypes, uploaded them to the NCBI database, and obtained their accession numbers. The sequences were edited and analyzed using MEGA 7.0 [22] and the results were combined with artificial correction. The number of haplotypes, polymorphic sites, haplotype diversity (h), and nucleotide diversity (π) were calculated using DnaSP 6 software

Table 1
primer used for amplification of both *Cytb* and *D-loop* genes.

Gene	Primer Name	Forward (5'→→')	Based pairs	Reverse (5'→→')	Based pairs	Size	Accession number
Cytb	*CYTB*	TTCAACTACAAGAACCACTA	A-T, T-A, C-G, A-T, T-A, A-T, C-G, A-T, A-T, C-G, C-G, A-T, C-G, T-A, A-T	GATTACAAGACCGATGCTT	G-C, A-T, T-A, T-A, A-T, C-G, A-T, A-T, G-C, A-T, C-G, G-C, A-T, G-C, T-A	930	MT780875.1
D-loop	*D-LOOP*	ATCTTAGCATCTTCAGTG	A-T, T-A, C-G, T-A, T-A, G-C, C-G, A-T, T-A, C-G, T-A, A-T, C-G, T-A, G-C, T-A	ACCCCTGGCTCCCAAAGC	A-T, T-A, G-C, G-C, G-C, A-T, C-G, G-C, C-G, T-A, T-A, G-C, G-C, A-T, C-G	1126	MT780875.1

Table 2
The Morphological differences and physical description of the 5 different common carp studied.

Common carp populations	Morphological differences	Physical appearance
Jian carp (F)	The Jian carp is a streamlined fish with a slightly compressed body shape, a concave forehead, and a pointed snout. It is often silver or grayish with a reflective sheen.	
Heilongjiang carp (H)	Heilongjiang carp has a large head, broad forehead, and blunt snout, with a robust body structure and lighter shades on the belly, typically dark brown or blackish coloration	
Common black carp strain (HJ)	The common black carp strain has a sleek, elongated body shape with a slightly arched dorsal profile, rounded head, small eyes, and wide mouth, typically dark, ranging from black or gray to olive or brownish.	
Songpu carp (S)	The Songpu carp has a deep, thick, and protruding fish with a large head and protruding upper jaw, often displaying bronze or golden body color and metallic-shining scales.	
Saijiang carp (SJ)	The Saijiang carp has a slender, elongated body shape with a pointed head and slightly upturned mouth, typically silver or grayish with a reflective appearance.	

[23]. Neutrality tests were performed using Arlequin 3.5 [24] to infer historic demographic and spatial expansion events, and deviations from the neutral model of evolution were determined using Tajima's D [25] and Fu's Fs [26] tests with 10,000 random permutations. *D-loop* and *Cytb* haplotype networks were created via the median joining method using Network 5.1 [27].

To conduct population genetic analysis, we used single-nucleotide polymorphism (SNP) array technology. In several studies, it has been effective in improving the resolution of the differentiation of genetic stocks [28,29]. SNP array technology is also considered an

Table 3
Comparative analysis of mtDNA (*Cytb & D-loop* Genes) in 5 common carp populations.

Cytb					D-loop			
Sample	Size	N	h	π	size	N	h	π
F	30	2	0.370 ± 0.014606	0.001 ± 0.000037	30	3	0.384 ± 0.016432	0.0044 ± 0.000183
H	10	7	0.867 ± 0.034785	0.0037 ± 0.000443	10	7	0.867 ± 0.034785	0.0093 ± 0.000411
S	31	3	0.374 ± 0.016164	0.0004 ± 0.000036	30	3	0.384 ± 0.016432	0.0042 ± 0.000183
SJ	34	6	0.781 ± 0.006860	0.0063 ± 0.000137	34	4	0.711 ± 0.006860	0.0073 ± 0.000120
HJ	31	2	0.065 ± 0.010776	0.0004 ± 0.000072	31	2	0.065 ± 0.010776	0.0004 ± 0.000018
Total	136	15	0.839 ± 0.001286	0.0064 ± 0.000034	135	16	0.844 ± 0.01205	0.009 ± 0.000017

Number of haplotypes (N), haplotype diversity (h), nucleotide diversity (π) and mean ± SE.

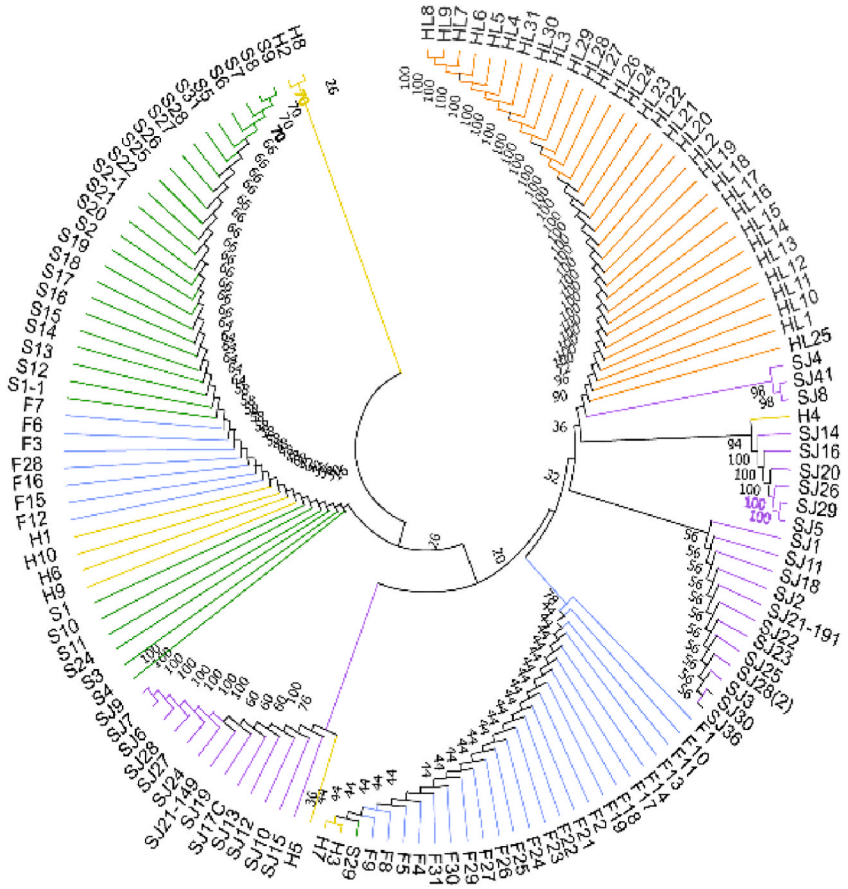

Fig. 1. *Cytb* clustered neighbor-joining tree for 5 common carp populations, with the numbers at the nodes representing the bootstrap values.

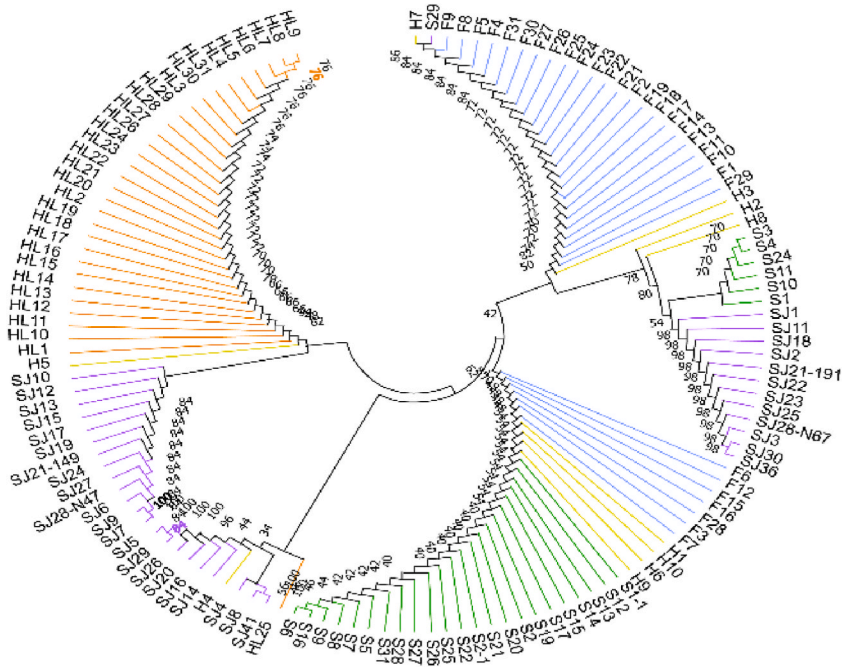

Fig. 2. *D-loop* clustered neighbor-joining tree for 5 common carp populations, with the numbers at the nodes representing the bootstrap values.

Table 4
15 Haplotype-based genetic statistics of *Cytb* in 5 common carp populations.

haplotype	Number	F	H	S	SJ	HJ
Hap_1	25	24		1		
Hap_2	35	7	4	24		
Hap_3	1		1			
Hap_4	1		1			
Hap_5	1		1			
Hap_6	1		1			
Hap_7	7		1	6		
Hap_8	30					30
Hap_9	1					1
Hap_10	12				12	
Hap_11	9				9	
Hap_12	6				6	
Hap_13	1				1	
Hap_14	3				3	
Hap_15					3	1

Table 5
16 Haplotype-based genetic statistics of *D-loop* in 5 common carp populations.

Haplotype	Number	F	H	S	SJ	HJ
Hap_1	25	24		1		
Hap_2	32	6	4	22		
Hap_3	1	1				
Hap_4	1		1			
Hap_5	1		1			
Hap_6	1		1			
Hap_7	1		1			
Hap_8	1		1			
Hap_9	30					30
Hap_10	1					1
Hap_11	6			6		
Hap_12	1				1	
Hap_13	12				12	
Hap_14	13				13	
Hap_15	6				6	
Hap_16	3				3	

Table 6
Tajima's D and Fu's Fs neutrality tests for *D-loop* and *Cytb* fragments in 5 common carp populations.

Cytb						D-loop				
F	H	S	SJ	HJ	F	H	S	SJ	HJ	
Tajima'sD	1.097	−1.434	−0.809	0.903	−2.176	1.110	−0.140	−0.079	1.768	−2.008
P	0.842	0.076	0.242	0.847	0.001	0.899	0.449	0.529	0.963	0.003
Fu'sF$_s$	3.704	−1.190	0.045	6.991	1.459	7.900	0.518	5.667	10.348	0.864
P	0.942	0.180	0.401	0.987	0.668	0.997	0.601	0.977	0.999	0.475

effective tool for studying population structure and the effects of natural and artificial selection at the genomic scale [30]. Additionally, the population structure was investigated using analysis of molecular variance (AMOVA) [31] in Arlequin 3.5 [24]. The levels of genetic differentiation and mobility in the population were estimated using F-statistics (paired FST). The P value of FST was derived using 10,000 permutations.

3. Results

3.1. Genetic diversity and phylogenetic analyses

For the *cytb* gene, 136 individuals and 16 haplotypes comprised the entire sample, and the overall genetic diversity was relatively high. The *Cytb* and *D-loop* results revealed that Heilongjiang carp (H) and Saijiang (SJ) exhibited the highest haplotype diversity ($h = 0.867 \pm 0.034785$) and nucleotide diversity ($\pi = 0.0063 \pm 0.000137$ and 0.0093 ± 0.000411), respectively. However, the lowest haplotypes of the Common carp black strain population population (HJ) of both Cytb and *Dloop* were (0.065 ± 0.010776), whereas the nucleotide diversity was ($\pi = 0.0004 \pm 0.000072$ and 0.0004 ± 0.000018), respectively. This result implies that the Common carp

Table 7
Gene flow (Nm) analysis of 5 common carp populations by *Cytb* and *D-loop* mitochondrial markers.

	Cytb					Dloop				
	F	H	S	SJ	HJ	F	H	S	SJ	HJ
F		1.567	0.296	0.944	0.034		1.434	0.444	0.702	0.125
H	0.242[a]		2.292	3.511	0.061	0.258[a]		0.152	0.180	2.603
S	0.629[a]	0.179[a]		0.964	0.019	0.530[a]	0.132[a]		0.770	3.948
SJ	0.346[a]	0.125[a]	0.341[a]		0.188	0.416[a]	0.152[a]	0.435[a]		1.322
HJ	0.936[a]	0.892[a]	0.963[a]	0.726[a]		0.800[a]	0.722[a]	0.798[a]	0.569[a]	

Note: The data above the diagonal are Nm; the data below the diagonal are Fst.
[a] indicates highly significant difference ($P < 0.01$).

black strain population population (HJ) had less genetic variation than the other common carp populations (Table 3). In addition, the findings also showed that different base pair sizes in the *D-loop* area have variable levels of genetic variation and haplotype diversity. Smaller sizes may have greater values, presumably reflecting the genetic make-up and evolutionary history of the 5 common carp strains, whereas larger sizes typically have more haplotypes and nucleotide diversity. The phylogenetic tree among the 5 different carp populations comprising 135 sequences was analyzed using Mega 7.0 for both *Cytb* and *D-loop* genes, which shows that the genes of the 5 common cap populations were clustered and closely related to one another, as shown in Figs. 1 and 2, respectively.

3.2. Haplotype analysis

3.2.1. Haplotypes of cytb

15 haplotypes were identified in all 5 common carp populations in *Cytb* (Table 4). The results showed that several haplotypes were detected in all 5 common carp populations. However, Hap_1 found 25 carps, primarily in the Jian (F) population; Hap_2 was spread across several populations, including Jian (F), Heilongjiang carp (H), Songpu (S), and Saijiang (SJ); Hap_3 to Hap_6 was only found in H; Hap_7 and Hap_8 were specifically found in the Common carp black strain population (HJ) population; Hap_9 to Hap_14 were specific to Saijiang (SJ) and Common carp black strain population (HJ) populations; and Hap_15 was found in Saijiang (SJ) and Common carp black strain population (HJ) populations.

3.2.2. Haplotypes of the D-loop

Table 5 shows the 16 *D-loop* haplotypes detected in 5 populations of common carp, of which 2 populations (one apiece) were found in Jian (F) and Heilongjiang carp (H). In addition, 32 haplotypes were found in Hap_2; of these, 6 were from the Jian (F) population, 4 were from the Heilongjiang carp (H) population, and 22 were from the Songpu (S) population. From Hap_3 to Hap_8, each haplotype appeared only once and was from the Jian (F) population. Hap_10 occurred only in the Common carp black strain population (HJ) and was not associated with a specific population in the table. Hap_11 was detected only once, specifically in Songpu (S). Furthermore, Hap_12, Hap_13, Hap_14 and Hap_15 were detected in Saijiang (SJ) 1, 12, 13, 6, and 3, respectively.

3.3. Neutrality test and mismatch analysis

Tajima's D and Fu's Fs tests were performed to determine whether the *D-loop* and *Cytb* fragments were subjected to evolutionary forces under neutral conditions. The results were negative in most populations (Table 6) and not statistically significant ($P > 0.05$). According to the findings, the average Fu's Fs value for the *Cytb* gene was 2.2018 ($P > 0.05$), while the *D-loop* gene had a value of 5.0594 ($P > 0.05$). This implies that the populations did not deviate much from the neutral model of evolution for either the *D-loop* or *Cytb* genes. Furthermore, the results indicated a possible population increase, with Songpu (S) having the highest *Dloop* of 10.348 and a *P-value* of 0.999. There was also no significant deviation in Jian's (F) *Dloop* genes with 7.900 (*P-value* = 0.997 and 0.899). Tajima's D value for the common black strain (HJ) populations was also −2.008, indicating a significant deviation from neutrality. Fu's Fs was 0.864 with a *P-value* of 0.475, indicating no significant divergence from neutrality. Tajima's D values for the Heilongjiang (H) populations show a slight deviation from neutrality, with *P-values* of 0.601 and 0.518, respectively. There was a positive selection in the Saijing (SJ) population, although there was no significant distinction.

Table 8
Analysis of molecular variance (AMOVA) for common carp populations.

	Source of variation	*df*	Sum of squares	Variance component	Percentage of variation
Cytb	Among populations	4	319.89	2.97**	70.09
	Within populations	131	166.02	1.27	29.91
	Total	135	485.91	4.24	100
D-loop	Among populations	4	276.25	2.55**	55.04
	Within populations	130	270.33	2.08	44.96
	Total	134	546.58	4.63	100

3.4. Genetic differences of 5 carp populations

The Fst values between the 5 common carp populations are shown in Table 7, based on two genetic markers, *Cytb* and *D-loop*. Higher levels of Fst indicate greater genetic diversity between populations.

A higher value of Nm (gene flow) was observed between common carp populations (HJ) and Songpu (S) for *Cytb*, and with Fst = 0.963 and Fst = 1.434 between common carp populations (HJ) and Songpu (S) for *D-loop* genes, respectively. The strong variation in Fst between the populations in terms of both genes indicates that there was gene flow between the populations, although it could be small (Table 7). The findings show that Fst values differ among populations, as observed in Jian carp (F) and Heilongjiang (H), which had Fst values of 1.567 for *Cytb* and 0.296 for the *D-loop*. This indicates that these two populations have a sizable degree of genetic differences. Similarly, various levels of genetic divergence between other population pairs are indicated by their FST values. In addition, the Fst values for Heilongjiang (H) and Songpu (S) were 0.179 and 3.511 for *Cytb* and *D-loop*, respectively, indicating a significant genetic divergence between these two groups.

Furthermore, the results offer important insights into the genetic variety and differentiation among the 5 populations analyzed, but the significant values underlined the genetic distinction between the common carp populations. The results also indicate that there are distinct genetic differences between the common carp populations, which may be caused by several different factors, including geographic isolation, genetic drift, or the process of natural selection.

3.5. Population variation

Table 8 displays the results of the Analysis of Molecular Variance (AMOVA), which enabled us to understand how genetic variation is distributed both within and among the 5 common carp populations for the genetic variation of *Cytb* and *D-loop*.

According to statistics, differences among populations accounted for 70.09 % of the genetic variation in *Cytb*, whereas variance within populations accounted for 29.91 % of the variation. Given that the variation in the variance component among populations was 2.97, the sum of squares among populations was 319.89, indicating a significant level of genetic differences among groups.

Similar results were found for the *D-loop*, where differences among populations were responsible for 55.04 % of the genetic variance and population variation accounted for 44.96 %. Likewise, the variance component was 2.55, and the sum of squares across populations was 276.25.

4. Discussion

The common carp strain is cultured across all provinces of China and worldwide and has been subjected to numerous artificial and natural genetic modifications. Several evolutionary forces, such as overfishing, development of coastal economies, increasing pollution that damages natural habitats, and breeding techniques, have influenced their genetic diversity. This has been generalized to almost all aquatic animals and is considered a driver of genomic modification in aquatic species, including the common carp population [32,33].

However, several subspecies of common carp have been identified on the basis of location or region. For instance, the common carp has been divided into two subspecies based on the location or region of origin. For instance, *C. c. haematopterus* is from Asia and *C. carpio* from Europe [20,30]. However, with the extensive use of mitochondrial DNA sequencing in research, many researchers have proposed that carp originated in East Asia and then spread to Europe because there is no polymorphism in the *D-loop* region of wild carp in Europe and Central Asia [18,34]. Also, there are many factors that have led to the genetic variation of the common carp strain, as proposed by Xu et al. (30), as this has given rise to Jian carp in China through multiple rounds of hybridization and genetic introgression [16].

On a global scale, the genetic diversity of Chinese carp was compared with that of the Hungarian common carp using mtDNA. The phylogenetic tree showed that the Chinese common carp is the basis of the Hungarian common carp, and they are placed in a single clade. This finding is consistent with those of Kohlmann and Kersten [17,35], Gross et al. [36], Kohlmann et al. [37,38], and Thai et al. [39,40]. However, some haplotypes and specimens of Chinese common carp were found in common carp in Hungary, but they were not found in the reports by Kohlmann and Kersten [17,35], Gross et al. [36], and Kohlmann et al. [37,38], although their research included the Amur carp (this may be the offspring of the Amur carp with some eggs, which were produced in the gene bank of the Czech Republic rather than the original wild population of the Amur. In addition, the haplotype network showed that some haplotypes of Chinese wild common carp appeared in Hungarian common carp, indicating that there is no isolated and possible genetic link between Chinese and Hungarian common carp.

The common carp strain is cultured across all provinces of China and worldwide, and has been subjected to numerous artificial and natural genetic modifications. Several evolutionary forces, such as overfishing, the development of coastal economies, increasing pollution that damages natural habitats, and breeding techniques, have influenced their genetic diversity. This has been generalized to almost all aquatic animals and is considered to be a driver of genomic modification in aquatic species, including the common carp population [32,33].

Based on the study, it was discovered that the Heilongjiang carp exhibited the highest levels of *Cytb* and *Dloop* haplotype diversity, with values of $h = 0.867 \pm 0.034785$ for both. Additionally, the Heilongjiang carp also had the highest nucleotide diversity found in the *Dloop*, with a value of $\pi = 0.0093 \pm 0.000411$. On the other hand, the Common carp black strain population had the lowest haplotype diversity and nucleotide diversity when compared to the other populations, as shown in Table 3. This could be attributed to the low recombination rate, demographic history, and dispersal hindrance [41]. This could also be attributed to the predominance of other common carp population haplotypes or to genetic isolation triggered by many environmental determinants [42]. The results of this study are also consistent with those of Shuli et al. [18], who reported the lowest genetic diversity of common carp in the

Nandujiang River in Hainan, China. The current study also found variable levels of genetic variation and haplotype diversity in different base pair sizes in the common carp population, which could be attributed to biological factors, including breeding patterns along the population [43], environmental factors [44], and genetic drift, which may cause random frequency fluctuations of alleles across the general population [44,45].

The results shown in Table 4 indicates that the haplotypes were not highly shared. This was probably due to the limited gene flow and demographic history of the populations; however, the most common haplotype of the 5 common carp populations was haplotype 2, which was found in the Jian Carp (J), Heilongjiang (H), and Songpu (S) populations; these populations could probably have the same ancestral and easy dispersal mechanisms. The lower frequency of haplotype 2 in the Jian (J) and Common carp black strain population (HJ) populations can be attributed to their divergence many centuries ago. Almost all haplotypes were shared by a small population, indicating little differentiation. This means that almost all haplotypes had the same origin, and their genetic distance was small.

Furthermore, AMOVA demonstrated that *Cytb* accounts for 70.09 % (Table 8) of the total variation, which corresponds with differences among common carp populations, whereas the *D-loop* accounts for 55.04 % of the variation within the population [46], which could possibly decrease haplotype diversity. These findings demonstrated substantial genetic variation in the *D-loop* genes among populations. The data from the *Cytb* and *D-loop* markers show considerable genetic variation among populations, which raises the possibility of population structuring or genetic differentiation. These results were different from those of a previous study conducted by Zhao et al. [43] in China on the genetic variation of common carp, which revealed that 72.71 % of the common carp populations were within population variation, whereas 27.79 % were among populations.

Similar results revealed that genetic variance within populations (86.2 %) was higher than that among populations (13.8 %) [18]. These results may have been due to several factors. First, a small and close population with effectiveness and a parental sex ratio imbalance can result in a disproportion in the proportion of gametes [47], which may influence gamete binding and cause loss of some haplotypes.

4.1. Genetic structure of 5 common carp populations

Generally, there are factors that can lead to the differentiation of organisms, such as the biological characteristics of the species, ecological requirements of various stages of life, physical and biological barriers, and human activities [50]. Our results revealed that the 5 common carp populations were highly different ($P < 0.001$) by analyzing their coefficients of genetic differentiation (Fst). The highest genetic difference was observed between Saijing (SJ) and Heilongjiang (H) (Fst = 0.963), whereas the lowest was observed between Songpu (S) and the Common carp black strain population (HJ) (Fst = 0.019) for *Cytb* gene sequences. For the *D-loop*, the Common carp black strain population (HJ) and Songpu (S) (Fst = 0.7) were the highest, and the Heilongjiang (H) and Common carp black strain population (HJ) had an Fst of 0.125. Generally, genetic differentiation is higher among populations, which may be attributed to several factors. However, research has found that phenotypic divergence undoubtedly increases as taxonomic relatedness decreases [48], although this pattern has rarely been documented systematically. The greater the divergence, the higher the probability of creating a new species [49].

The study reveals low gene flow in the 5 common carp populations due to intensification and long-term domestication, with genetic interventions like selective breeding, chromosome manipulation, sex reversal, and transgenesis [50,51], resulting in various breeds and strains. Although the number of studies assessing genetic variability and phenotype performance using molecular markers continues to increase, most domesticated carp strains remain genetically characterized. Several studies have documented that the Common carp black strain population population, as with other common carps, has faced different threats, including environmental degradation [52] and the invasion of "exotic" genotypes into natural populations of Japanese carp as a result of domestication and translocation [53]. It is not surprising that low haplotype and nucleotide diversity can be attributed to a reduction in the stock. Thai et al. [40] conducted research on Vietnamese common carp with molecular markers and proved its homogeneity, which can be a warning for conservation of common carp and effective management of both domestic and wild stock.

As assessed using molecular markers, it is relatively genetically homogeneous and could represent a unique genetic resource for common carp that needs to be conserved. Exploiting molecular markers should provide comprehensive DNA-based datasets.

4.2. Neutrality test

The neutrality test results showed that the Common carp black strain population (HJ) population had low values of Tajima's D, -2.176 and $-2.008 < 0$ respectively (Table 8). For both *Cytb* and *D-loop* genes, this implies population expansion toward biased rare alleles. The population of the Common carp black strain population accounted for some individuals compared with other local carp populations. Its habit has been degraded because of human activities such as integration, rice production, and farming, which could be the effect of its disappearance. The combination of anthropogenic and natural activities has caused some changes in its genome; thus, the differences we found when compared with the common carp populations [54]. The frequency distribution of segregating sites, as reflected by Tajima's D, did not significantly deviate from the expectations for Tajima's D and Fu's Fs for each of the *D-loop* and *Cytb* genes, respectively. From our results, both *Cytb* and *D-loop* genes showed positive Tajima's D coefficients for both Jian (F) and Saijing (SJ) of 1.097 and 0.903, respectively, whereas others were negative. Tajima's D was significantly negative for one gene compared with another, and Fu's Fs was significantly negative for two mtDNA genes. Generally, the sharing of haplotypes between individuals was quite low for all populations, which was considered the reason for low population expansion. The sharing of haplotypes may be due to common ancestral origins and subsequent gene flow among populations [55].

5. Conclusion

This study aimed to explore the genetic diversity of a Common carp black strain population (*Cyprinus carpio* var. *baisenensis*) in 5 populations of common carp species. The results showed that the population of Common carp black strain populations had lower haplotypes and nucleotide diversity, which probably reflects its low individual recombination rates and dispersal barriers. A higher variation was found among the populations than within, implying strong genetic differences among the 5 populations of common carp. Despite the variation, the results from the phylogenetic tree population analysis of *Cytb* and *D-loop* revealed that the 5 carp populations were clustered and closely related to one another. The more private haplotypes found were probably due to lower population differentiation, demographic factors, and dispersal mechanism issues such as barriers.

Data availability

The genome-wide genetic diversity data that support the findings of Common carp black strain population (*Cyprinus carpio* var. *baisenensis*) are available in the GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA936928) under the accession number SRP430518. BankIt2783164 Seq1Heilongjiang PP085168 BankIt2783164 Seq2Heilongjiang PP085169 BankIt2783164 Seq3Heilongjiang PP085170 BankIt2783164 Seq4Heilongjiang PP085171 BankIt2783164 Seq5Heilongjiang PP085172 BankIt2783164 Seq6Heilongjiang PP085173 BankIt2783164 Seq7Heilongjiang PP085174 BankIt2783164 Seq8Heilongjiang PP085175 BankIt2783164 Seq9Heilongjiang PP085176 BankIt2783164 Seq10Heilongjiang PP085177.

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Ethnic approval and consent to participate

A statement to confirm that all experimental protocols were approved by the Laboratory Animal Guidelines for Ethical Review of Animal Welfare in the China National Standardization Administration (GB/T 35892-2018) of Nanjing Agriculture University.

CRediT authorship contribution statement

Sahr Lamin Sumana: Writing – original draft. **Peipei Wang:** Supervision. **Chengfeng Zhang:** Validation, Data curation. **Xiaojun Jing:** Supervision. **Jian Zhu:** Supervision. **Yongkai Tang:** Software, Methodology. **Wenting Liu:** Resources. **Shengyan Su:** Supervision, Funding acquisition, Conceptualization. **Yu Liao:** Writing – review & editing, Data curation.

Declaration of competing interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing paper.

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