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Genetic Diversity of the Common Black Carp Strain (*Cyprinus carpio* var. *baisenensis*)

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Abstract: The Common Black Carp Strain (*Cyprinus carpio* var. *baisenensis*), known for its black skin, is commonly cultured in the integrated rice-agriculture (IRA) system in Guangxi province, China. This study aimed to compare the genetic diversity of three common carp strains/populations (Common Black Carp Strain, Huanghe, and Songpu) using resequencing data. The genome-based method reveals a significant difference ($p < 0.05$) in identified loci and SNP frequency ($p < 1 \times 10^{-6}$) between the Songpu (Sp) or mirror carp and Huanghe (Hh) new strain. Additionally, the Common Black Carp Strain (Bk) exhibits a higher number of Tajima's D values, possibly due to its population size and mutations within its entire genome. The average value of population nucleotide diversity (π) for the Bk is 1.706×10^{-4} while the mean number for the Hh and Sp strains is 1.691×10^{-4} Heterozygosity analysis results indicate that the Bk has the highest F coefficient compared to the Sp and Hh hybrids. This suggests that the isolated population of the Bk may have experienced a decrease in population size as a result of environmental disturbances in the IRA system. PCA results further reveal that all individuals of the Bk, except for one, are clustered together, while individuals of the Hh form a separate group. On the other hand, Sp displays a distinct distribution pattern. The comparative study of the genetic diversity of the Bk provides baseline data on its genome makeup. Assessing genetic diversity and genetic structure is critical for fisheries management and the conservation of critically endangered fish species.

Keywords: Bk; genetic diversity; genome sequencing; Guangxi province

Citation: Sumana, S.L.; Liao, Y.; Zhang, C.; Jing, X.; Zhu, J.; Tang, Y.; Liu, W.; Su, S. Genetic Diversity of the Common Black Carp Strain (*Cyprinus carpio* var. *baisenensis*). *Diversity* **2024**, *16*, 413. https://doi.org/10.3390/d16070413

Academic Editor: Francesco Nardi

Received: 24 May 2024
Revised: 14 July 2024
Accepted: 15 July 2024
Published: 17 July 2024

1. Introduction

The common carp (*Cyprinus carpio* L.) is a globally significant freshwater species that belongs to the *Cyprinidae* family. It is revered for its historical and widespread cultivation, both internationally and particularly in China [1,2]. Originally from western Asia, *C. carpio* has been extensively introduced to various regions worldwide and has diversified into numerous strains and varieties [3–5].

Among these, the black strain of common carp plays a central role in Chinese freshwater aquaculture and culinary traditions. This strain, belonging to *C. carpio*, exhibits a sleek, elongated body with a slightly arched dorsal profile, small eyes, and a wide mouth. It displays significant polymorphism, including varied colorations [6]. Originally originating from traditional Asian carp farming practices, particularly in China and Japan, the Bk strain is prominently cultivated in regions such as Guangxi for commercial production [7,8]

Assessing genetic diversity and population structure is crucial for effective fishery management and conservation. Traditional markers may not adequately capture genetic patterns in freshwater and marine species, prompting the use of advanced, high-throughput

sequencing technologies. For instance, Xu et al. [9] conducted a population genomics study of *C. carpio* using SNP genotyping across 14 global populations, revealing distinct genetic structures and adaptive genetic factors like growth differentiation factor 6 (gdf6a) and bone morphogenetic protein receptor 1 (bmpr1b). Similarly, SNP arrays have proven invaluable in enhancing precision and efficiency in genetic studies of fish species, including common carp, offering insights into population structure and evolutionary processes [10,11].

These studies have used SNPs to investigate various aspects, including population structure and the effects of natural and artificial selection on a genome-wide scale. This has contributed to our understanding of fish genetics and evolutionary processes.

For instance, researchers utilized the Atlantic salmon SNP array, which contains 6176 informative SNPs, to genotype 38 anadromous and freshwater wild fish [12]. The data obtained provided insights into the genetic structure of salmon and demonstrated the adaptability of SNP allele frequencies across different populations and regions. Another study conducted by Bradbury et al. demonstrated the association between SNP allele frequency and species temperature in Atlantic cod, using the SNP array [13]. Furthermore, SNP arrays have been employed to study genetic variation in freshwater fish, leading to the identification of genomic regions that contribute to the evolution of different species pairs [14]

In carp research, researchers have identified numerous SNP markers [7,15] and developed a high-throughput 250 K carp SNP array. The average spacing between two loci of the 250,000 SNPs is 6.6 kilobase (kb), with most SNPs having a spacing of 3–8 kb, which provides a dense "molecular ruler". Validation with samples from common carp populations and closely related carp has confirmed the array's reliability and universal applicability to *Cyprinus* species.

Genome-wide association studies (GWAS) are valuable tools for assessing genetic diversity. Advancements in sequencing technologies have made it easier to obtain more genomic information, including examining single nucleotide polymorphisms (SNPs) and constructing high-density gene maps. The Carp 250 K SNP array has been instrumental in various carp genetics research projects. It has aided in the construction of ultra-high genetic maps, conducting GWAS for important traits, and studying population genetics. However, the high-density SNP genotyping array has limitations in terms of cost and flexibility [6]. To address this, researchers have developed a flexible low-density SNP genotyping platform based on Fluidigm SNP-type technology. This platform allows for the multiplexing of 48 or 96 SNPs in a single assay, enabling cost-effective genotyping of a larger number of samples.

In China, selective breeding programs have resulted in the creation of distinct strains, such as the Hh new carp strain. This particular strain was developed near the Yellow River with the intention of enhancing traits like rapid growth and disease resistance [2,16]. The Hh new carp strain is easily recognizable due to its vibrant golden-yellow coloration, streamlined body, and protective scales. These characteristics showcase the genetic adaptations that have taken place in response to local environmental conditions. On the other hand, the Sp strain, which originated from German mirror carp introduced to China, represents the intensive efforts put into selective breeding to improve growth and other specific traits [7]. This strain is characterized by a deep, thick fish with a large head and a protruding upper jaw. It often displays a bronze or golden body color and scales that shine like metal [6].

While breeding programs have successfully developed distinct genetic profiles within the Bk, Hh, and Sp strains, they have also brought attention to challenges related to genetic diversity and conservation. The varying levels of genetic diversity observed among these populations highlight the impacts of artificial selection and geographic isolation [17]. Addressing these issues is crucial for maintaining robust and sustainable aquaculture practices and informing conservation strategies that consider the genetic context of each strain.

In conclusion, comprehending the genetic foundations and adaptive potentials of common carp strains is vital for optimizing breeding programs, enhancing conservation efforts, and ensuring the long-term viability of aquaculture systems. Future research should

continue to explore genomic advancements and their applications in fisheries management and biodiversity conservation.

2. Methodology

2.1. Sampling Information

Thirty (30) mature individuals of both sexes were collected from three different sites, which included the Bk strain sourced from Integrated Rice Agriculture (IRA) farms located in Nanning, Guangxi province in the southern region. The Hh strain was developed at the Freshwater Fisheries Research Centre (FFRC) in Wuxi, Jiangsu province in the south. The Sp strain was obtained from the Heilongjiang Fisheries Research Institute in Harbin, Heilongjiang province in the northeast. Table 1 presents the information on the sample collection of the three carp populations and the DNA extraction process.

Table 1. Sample information of the three common carp strains.

Population	Source	Latitude (°N)	Longitude (°E)	Number of Samples	Age	Collection Date
Bk	Nanning, Guangxi province	22.8167	108.3669	16	2	April
Hh	FFRC, Jiangsu province	31.5653	120.3275	8	2	April
Sp	Heilongjiang River Fisheries Research Institute	47.8333	127.6666	6	2	April

The two wild carp populations were obtained from selective breeding programs that have been conducted for over a decade. The Sp population consists of artificially cultivated varieties, known as Sp, which were collected from the Heilongjiang Fisheries Research Institute. The lower lobe tissue of the carp individuals' caudal fin was cut and fixed in 90% ethanol for later use. Genomic DNA was extracted using the traditional phenol-chloroform method. The integrity of the extracted DNA was tested using 1% agarose gel electrophoresis, and its concentration and purity were determined using a NANO-DROP 2000 spectrophotometer. The DNA was then diluted with TE Buffer (pH = 8.0) to a final mass concentration of approximately 60 ng·μL^{-1} and stored at −20 °C for later use. The high-quality DNA was quantified using a Qubit 4.0 Fluorometer (Invitrogen, Waltham, MA, USA). All experimental protocols were approved by the Laboratory Animal Guidelines for Ethical Review of Animal Welfare of the China Nationalization Administration at Nanjing Agriculture University.

2.2. Read Mapping and SNP Genotyping

The raw sequencing data were filtered using fastp v0.20.1 [18] with default parameters to remove residual adapter sequences and low-quality regions. The second quality control was then performed using fastQC v0.11.9 [19]. The clean data were mapped using BWA mem v0.7.17 [20]. The mapping results were sorted and converted into BAM format using SAMtools v1.11 [21]. All variants were detected using the standard Genome Analysis Toolkit v4.1.9.0 (GATK4) [22]. The Variant Filtration and SelectVariants modules of GATK4 were subsequently used for joint genotyping and the selection of high-quality SNPs with the following filtering criteria: QD < 2.0 | | MQ < 40.0 | | FS > 60.0 | | SOR > 3.0 | | MQRankSum < 12.5 | | ReadPosRankSum < 8.0. To ensure variant concordance, all SNPs were filtered in VCFtools v0.1.16 [23] with the following thresholds: minor allele frequency (MAF) < 0.01, proportion of missing genotypes (max-missing) > 0.95, and minimum sequencing depth (minDP) < 4. The high-confidence SNPs were annotated and classified in SnpEff [24] with default parameters.

2.3. Library Information

The library types (Table S1) for these samples were prepared using standard Illumina library preparation protocols. The samples were fragmented, and the DNA fragments

then underwent end-repair, A-tailing, and ligation of Illumina-compatible adapters. Size selection or normalization steps were performed to ensure that the final libraries fell within the desired size range. The prepared libraries were amplified through PCR to increase the DNA quantity for sequencing. The high Q20 and Q30 values observed for these samples indicate the successful library preparation and subsequent sequencing, resulting in high-quality sequencing data. The sequencing was performed on an Illumina NovaSeq 6000 instrument using a paired-end 150 bp (PE150) sequencing chemistry. The effective mapping rate, approximately 99.07%, confirms the high integrity and fidelity of the sequencing data. This is further supported by the comprehensive quality assurance or quality control measures employed.

2.4. Genetic Diversity and Population Structure Analysis

Principle component analysis (PCA) was performed to explore genetic differences among populations using PLINK v1.90 [25,26]. Genetic diversity parameters such as the fixation index (Fst), nucleotide diversity (π), observed heterozygosity (Ho), expected heterozygosity (He), inbreeding coefficient (Fis), minor allele frequencies (MAFs), and the Hardy–Weinberg equilibrium (HWE) were evaluated in VCFtools v0.1.16.

3. Results

3.1. Summary of Data from Common Carp Genome Resequencing

The average 142,579,732 clean reads for all samples (Table S1) were isolated from the average raw reads of 143,920,978. Similarly, there were an average of 42.77 G clean bases from which 43.18 G raw bases were generated at an effective rate of approximately 99.07%. The Q20 and Q30 were 97.52% and 93.18%, respectively, where the *GC* content was 38.52%. After mapping to the common carp genome, 216,881,016.9 average reads were mapped to the common carp genome (Table S2) with a 97.23% mapping rate, where the average depth was 20.12 and the percentage of coverage of at least 4× was 68.06%.

3.2. Summary of SNPs in the Common Carp Genome Resequencing

Based on the results provided in Table S3, the total number of SNPs examined was 26,439,902. On average, there were 62,855 SNPs located in the 1 kb upstream region and 63,553 SNPs located in the downstream 1 kb region. The average number of SNPs located in both the downstream 1 kb region of one gene and the upstream 1 kb region of another gene was 4957. In the intronic region, the average number of SNPs was 941,814, while in the intergenic region, it was 1,033,333. Some SNPs can cause changes in the function of the target gene. On average, 242 SNPs result in the gene having a new stop codon, while 92 SNPs can cause the loss of the target gene's stop codon. The average number of exonic frameshift deletion SNPs is 6230, and the number of exonic frameshift insertion SNPs is 5364. The heter–SNP ratio is 40.95%.

The average number of each SNP type was calculated. The number of C:G > A:T SNPs is 1,090,697, and the number of C:G > G:C SNPs is 669,747. Additionally, the number of C:G > T:A SNPs is 2,004,266, and the number of T:A > A:T SNPs is 1,421,396. Furthermore, the number of T:A > C:G SNPs is 2,362,432, and the number of T:A > G:C SNPs is 1,009,322 (Table S4).

3.3. Genetic Diversity of the Common Carp Strain Compared with the Other Two Common Carp Strains

The mean Tajima D values for the Bk strain were 3.304, while for other common carp strains, they were 2.266. The average value of π for the Bk strain was 1.706×10^{-4}, whereas for the other two common carp strains, it was 1.691×10^{-4}. Therefore, there is no significant difference ($p > 0.05$) in genetic diversity between Bk and the other two common carp strains. When comparing Bk with other populations of common carps (Hh, Bk, and Sp carp), a significant difference ($p < 0.05$) in the genome makeup was observed for the identified loci ($p < 1 \times 10^{-6}$), with a total of 4591 SNPs found. The heterozygosity of these

three common carp populations was examined (Table 2). The observed heterozygosity was found to be significantly different ($p < 0.05$) among them, with the Bk strain ranking first. Additionally, a significant difference in estimated heterozygosity was observed between Sp and Hh, as well as between Sp and Bk. However, no significant difference ($p > 0.05$) in estimated heterozygosity was found between the Hh and Bk strains.

Table 2. Genetic homozygosity and inbreeding coefficient of three common carp strains.

Species	O (HOM)	E (HOM)	F
Bk	4,569,120 ± 78,758.29 [a]	4,959,505 ± 59,674.71 [a]	−0.109535 ± 0.04059818 [a]
Hh	3,002,751 ± 68,158.42 [b]	4,998,472 ± 32,348.82 [a]	−0.620822 ± 0.02159997 [c]
Sp	1,820,137 ± 77,443.48 [c]	2,923,116 ± 188,970.12 [b]	−0.480120 ± 0.04433147 [b]

Statistical significance denoted by lowercase letters ([a], [b], [c]), observed homozygotes (O (HOM)), expected homozygotes (E (HOM)), and the inbreeding coefficient (F).

3.3.1. Genetic Diversity and Population Structure of the Three Populations

To assess the genetic diversity of the three common carp populations, we examined several genome-wide genetic diversity indexes: nucleotide diversity (π), observed heterozygosity (Ho), and expected heterozygosity (He) (Table 3). Among the populations, the Sp strain displayed the highest nucleotide diversity (0.6695 ± 0.4162), while Bk had the lowest (0.4755 ± 0.4332). He was highest in Hh (0.3618 ± 0.1964) and lowest in Bk (0.2686 ± 0.2246). As for the π values, Sp had the highest (0.5998 ± 0.3893), whereas Bk had the lowest (0.3804 ± 0.3536). This indicates that Bk exhibited slightly lower genetic diversity compared to the other populations.

Table 3. Summary of genetic diversity parameter for three populations.

Statistics	Bk	Hh	Sp
Ho	0.4755 ± 0.4332	0.6506 ± 0.3928	0.6695 ± 0.4162
He	0.2686 ± 0.2246	0.3618 ± 0.1964	0.3588 ± 0.2015
π	0.3804 ± 0.3536	0.4257 ± 0.2622	0.5998 ± 0.3893
Fis	−0.1095 ± 0.0994	−0.1028 ± 0.0966	−0.4801 ± 0.0887
MAF	0.2377 ± 0.2166	0.3253 ± 0.1964	0.3348 ± 0.2081
HWE *p*-value	0.9552 ± 0.1679	0.7249 ± 0.4100	0.9866 ± 0.0905

Additionally, we analyzed the pairwise Fst values of the three populations. The Sp population showed the largest inbreeding coefficient (Fis) value of −0.4801 ± 0.0887, indicating the greatest genetic distance from the other two populations, Bk (−0.1095 ± 0.0994) and Hh (−0.1028 ± 0.0966). This suggests that there are smaller genetic distances within the Bk and Hh populations.

Furthermore, we examined the MAF among the populations. The Sp population had the highest MAF (0.3348 ± 0.2081), while the Bk population had the lowest (0.2377 ± 0.2166). Lastly, we analyzed the HWE *p*-values. The Sp population had the highest HWE *p*-value (0.9866 ± 0.0905), indicating a good fit with the equilibrium. On the other hand, the Hh population had the lowest HWE *p*-value (0.7249 ± 0.4100), suggesting a departure from the equilibrium.

3.3.2. Pairwise Population Fixation Index (Fst)

The results in Table 4 show population divergence, measured by the Fixation index (Fst), among the three populations (Bk, Hh, and Sp). The Fst values range from 0 to 1, where 0 indicates no genetic differentiation between populations and 1 indicates complete genetic differentiation (i.e., the populations are completely diverged).

Table 4. Population divergence (Fixation index, Fst) among three populations.

	Bk	Hh	Sp
Bk			
Hh	0.1612		
Sp	0.2603	0.1615	

Comparing the Fst values between the Bk and Hh populations, we find a value of 0.1612, indicating a moderate level of genetic differentiation between these two populations. The Fst value between the Bk and Sp populations is 0.2603, suggesting a greater degree of genetic differentiation between the two populations. Lastly, the Fst value between the Hh and Sp populations is 0.1615, again indicating a moderate level of genetic differentiation.

3.4. Genetic Status of the Bk in Comparison with the Other Two Common Carp

PCA was used to measure the genetic differences among different common carp populations (Figure 1). All the Bk were clustered together except one, which was found along with the Sp, whereas a similar instance was observed for the Hh individuals, which were clustered in one group. The results also revealed that one individual Sp was completely isolated from the other 2 common carp populations, and one was clustered with the Hh in one group.

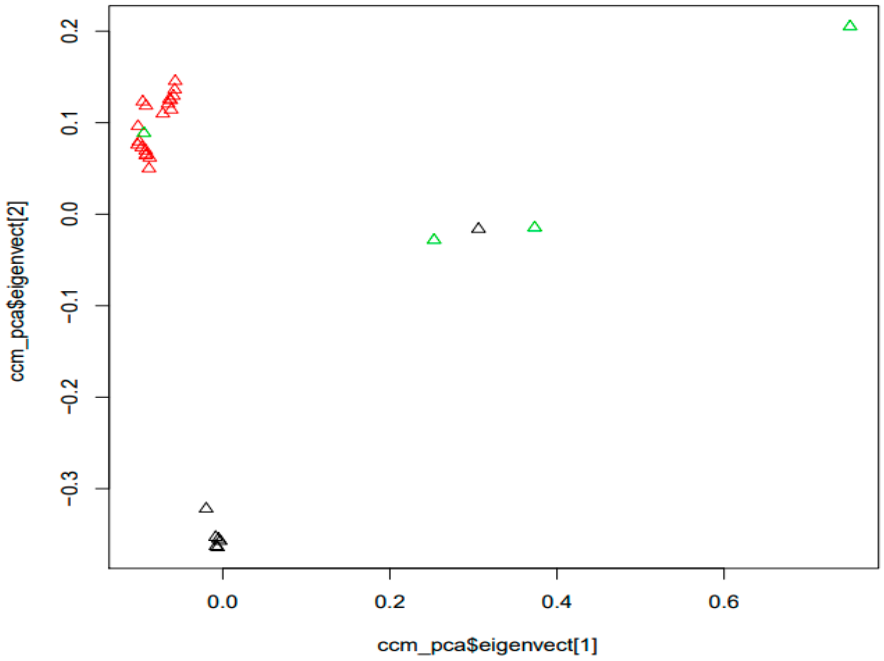

Figure 1. PCA based on the three common carp populations. Red represents the new strain of Hh, black represents the Bk, and green represents the Sp.

4. Discussions

Based on our findings, the Bk strain showed significant differences in the results of SNP genotyping. A total of 26,439,902 SNPs were obtained, with 4591 SNPs specifically found in the genetic makeup of the Bk strain compared to the other sequenced common carp. The *p*-values were $p < 1 \times 10^{-6}$.

Recently, there has been increasing interest in analyzing the genetic basis of variance in quantitative traits. Differences in the variance of a quantitative trait between genotypes of an SNP can be attributed to environmental sensitivity, gene–gene or gene–environment interactions, or linkage disequilibrium with causal variants. The long-term isolation of the Bk strain and the impact of a disturbed environment may have influenced its genetic

makeup. However, further research is needed to determine if such migration blockage affects the genetic diversity in this study.

One possible speculation is that the low genetic diversity in the Bk strain may be attributed to the effective population size of each population collected in this study, which is significantly lower compared to other carp strains [27].

As Tajima's D values for loci genotyping statistics are positive, they indicate an excess of high-frequency mutations, which can occur after a population contraction or under-balancing selection. Conversely, Tajima's D values become negative when there is an excess of low-frequency mutations, such as after population expansions, recent selective sweeps, or weak negative selection [27]. By interpreting Tajima values, negative Tajima D values indicate an excess of low-frequency mutations, population expansions, and recent selective sweeps. On the other hand, higher positive Tajima D values found in the Bk population were attributed to a decline in its population and a higher frequency of polymorphism. The interaction of the Bk with the Burau people, who isolated it, has led to migration and reproduction. Additionally, their integration into the rice system has enabled them to adapt to living in special habitats with intense human activities.

When analyzing the heterozygosity of different carp strains, it was found that the Bk strain had the highest F coefficient compared to the Sp and Hh carp hybrid. This suggests that the Bk strain may have become isolated and experienced a decline in population size due to environmental disturbances. Additionally, this could be attributed to a reduction in the effective population size [28]. The high level of isolation in the Bk strain has led to increased reproduction among closely related parents, resulting in a higher coefficient of inbreeding. Studies have shown that species with low genome-wide genetic diversity tend to have a higher abundance of long runs of homozygosity, elevated levels of inbreeding, and an overall accumulation of harmful variants. This seems to be the case for the Bk population [29].

The presence of this selection pressure has been identified in both functional genes [30] and molecular marker loci [3] within carp populations. This indicates that as a result of artificial directional selection, specific individuals experience a loss of genetic material, leading to the loss of certain original alleles from the gene pool. Consequently, this causes a reduction in heterozygotes and a deviation from HWE. Carp, which holds the distinction of being the earliest fish species to undergo domestication and cultivation in China, is extensively utilized in the realms of breeding and enhancement practices.

However, the breeding populations of Bk and Hh displayed lower values in allele locus and heterozygosity compared to the Sp population. This observation aligns with the findings of Lehoczky et al. [31] and Xu et al. [3] in the carp population. Conversely, the Sp populations analyzed exhibited a notable genetic diversity level, possibly attributed to their limited exploitation and utilization. Additionally, the genetic diversity of the two wild populations, Bk and Sp, has been influenced by their utilization as breeding materials for breed selection, as highlighted by Lu et al. [32] and Shi et al. [33]. Notably, Lu et al. [32] highlighted the resource shrinkage and germplasm degradation in the Yellow River carp.

Moreover, the two varieties of Bk and Hh showed a lower level of genetic diversity, which can be attributed to the higher germplasm purity resulting from multi-generation artificial selection. This finding is consistent with the analysis results of site selection pressure and genetic structure in this paper. Moreover, the two varieties exhibited differences in the level of genetic diversity, which is likely related to the basic breeding group and breeding technology.

Research findings indicate that the common carp population Sp, known as Songpu Mirror Carp, underwent a breeding process involving multi-generation group selection of German Mirror Carp [34]. Conversely, the Furui Carp was developed through multi-generation BLUP family selection following the crossbreeding of the Jianli and Yellow River Carp [35]. The inclusion of hybrid sources and family selection in the latter case played a significant role in preserving its genetic diversity. Comparative studies using various

molecular markers by Lu et al. [32] revealed that, in contrast to other breeding populations and original parent populations, Furui carp exhibited a higher genetic diversity.

Furthermore, the results of the PCA showed that the Bk strain formed one cluster, with only a few individuals found in the Sp population. This can be attributed to the fact that these populations have a shared ancestor. However, the effect is not significant enough to confirm because there were only a few individuals. On the other hand, the new strain, Hh, formed its own cluster, indicating that it has very little in common with the rest of the populations investigated. This is likely because it is a new variety and is likely located away from other populations.

The fact that Hh formed a single cluster also implies gene flow through dispersal mechanisms, either through human intervention or self-dispersal. This new strain has not yet been cultured throughout China, and there is no genetic introgression between Hh and the rest of the studied populations. However, there was some small similarity with the Sp population, indicating that they may share some alleles from a distant ancestor [36].

The results of this study suggest that several management practices should be adopted to conserve the three common carp populations. For the Bk strain, it is important to maintain genetic diversity through selective breeding programs and controlled breeding practices, while avoiding excessive inbreeding that could compromise desirable traits. On the other hand, the Hh strain requires genetic characterization and improvement to enhance traits such as growth performance and disease resistance [37]. It is also crucial to evaluate the suitability and performance of the Hh strain in different environments. For the Sp strain, a genetic diversity assessment is essential to understand its characteristics and potential for selective breeding. Additionally, its growth performance, disease resistance, and other economically important traits should be evaluated for commercial aquaculture production, with comparisons made to other common carp strains.

5. Conclusions

The study aimed to investigate the genetic diversity of the Common black strain in comparison to other carp strains, based on SNPs. We observed a higher level of significance among the compared loci of the local carp. The higher Tajima D values for the Common black strain suggest a decreased population size due to isolation and anthropogenic activities, such as rice-fish integration fish farming. These factors may have an impact on the survival of the population or allow for adaptation within the genome composition of the Common black strain.

This study serves as a valuable resource for the protection and conservation of Chinese Common black strain germplasm resources. It also provides guidance for breeding foundational populations with enhanced genetic diversity. Furthermore, it establishes the groundwork for future variety improvement strategies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16070413/s1, Table S1: Summary of quality and efficiency for different library sequencing metrics; Table S2: Comparative analysis of mapping efficiency and coverage for different samples; Table S3: Distribution of genetic variants and heterozygozygosity rates in various categories; Table S4: provides an analysis of the base content ratio within the genomes of selected local carps (Sp mirror, Hhs, and Bk); there were no significant differences among the studied.

Author Contributions: S.S., Y.T. and W.L. conceived, supervised and designed the experiment. J.Z. and X.J. also assisted in analyzing the data and editing. The experiments were conducted by S.L.S., who also wrote the research paper. The data were gathered, processed, and refined by Y.L. and C.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the earmarked fund for CARS (CARS-45) and Guangxi Science and Technology Project: Development and Ultilization of Indigenous Black Carp Germplasm in Rice Fields (Z202484).

Institutional Review Board Statement: The authors confirm that all experimental protocols were approved by the Laboratory Animal Guidelines for Ethical Review of Animal Welfare of the China National Standardization Administration (GB/T 35892-2018) of Nanjing Agriculture University.

Data Availability Statement: The genome-wide genetic diversity data that support the findings of the Common black strain (*Cyprinus carpio* var. *baisenensis*) are available in the GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA936928 accessed on 14 July 2024.) under the accession number SRP430518.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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