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Genetic Diversity of Chinese Longsnout Catfish (*Leiocassis longirostris*) in Four Farmed Populations Based on 20 New Microsatellite DNA Markers

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Abstract: Freshwater aquaculture has a long and vibrant tradition in China. The Chinese longsnout catfish (*Leiocassis longirostris*) is a popular economic freshwater fish native to China. Understanding the genetic structure of L. *longirostris* populations is important for ensuring the efficacy of management practices and the sustainability of future increases in production. In this study, we used Illumina sequencing technology to isolate 20 novel polymorphic microsatellites from the genome of L. *longirostris*. These microsatellites were used to analyze the genetic diversity of 240 L. *longrostris* individuals from four populations. Genetic diversity parameters (N_A, H_O, H_E, I, PIC, and F_{ST}) of the four farmed L. *longirostris* populations were analyzed. The level of genetic differentiation among the four farmed L. *longirostris* populations (inferred by pairwise comparisons of F_{ST} values) was low, but the genetic diversity of these populations was high, indicating that they still provide useful sources of genetic variation that could aid in breeding efforts. The STRUCTURE and ADMIXTURE analyses indicated that admixture might be occurring in the four L. *longirostris* populations, especially between the MS and YB populations. Understanding the genetic diversity of farmed L. *longirostris* populations and inbreeding prevention could greatly aid in breeding and production. These newly isolated microsatellite markers and the high genetic diversity of L. *longirostris* populations in the main breeding areas have important implications for the breeding and stock management of L. *longirostris*.

Keywords: *Leiocassis longirostris*; microsatellite; polymorphic; genetic diversity

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1. Introduction

Freshwater aquaculture has a long and vibrant tradition in China [1]. The Chinese longsnout catfish (*Leiocassis longirostris*) belongs to the family Bagridae, which is an economically important freshwater fish native to China [2]. L. *longirostris* has no scales and no intramuscular spines, and its meat is soft and flavorsome [3], which makes it highly popular among consumers. L. *longirostris* is rich in protein and fat, possessing a crude protein content of 15.85% and a crude fat content of 5.31%; additionally, the content of unsaturated fatty acids in L. *longirostris*, such as DHA, EPA, and DPA n-3, is higher than that in many freshwater fish [4,5]. Global diversity is declining, and wild fish stocks are being depleted in various places worldwide [6]. L. *longirostris* populations have declined rapidly and have nearly disappeared in many river systems due to overfishing, pollution, and other human disturbances [7]. Fishing in the Yangtze River, where L. *longirostris* is most common, has been banned for 10 years to protect the fish resources [8]. Aquaculture has promoted rapid increases in production and has become one of the world's most important food-production technologies [1]. L. *longirostris* production in China reached 21,195 tons in 2020, and Sichuan Province is responsible for 49.9% of the L. *longirostris* supply [9].

Since the successful domestication and artificial propagation of L. *longirostris* were achieved in the 1980s, artificial breeding technology has developed rapidly, and the domestic supply of L. *longirostris* is now almost completely derived from artificially bred individuals [10–12]. For most aquaculture species, a small number of parents are used to establish a base population for reproduction that is sufficient to produce a large number of offspring populations for the market, and the genetic diversity of these offspring populations can decline significantly [13]. The loss of genetic diversity has been observed over recent decades in many captive aquatic animal populations, such as *Salmo salar* [14], *Lates calcarifer* [15], *Larimichthys crocea* [16], *Colossoma macropomum* [17], and *Acanthopagrus schlegelii* [18]. The average number of eggs conceived by each sexually mature L. *longirostris* ranges from approximately 1,184 to 145,410; a female L. *longirostris* can produce tens of thousands of offspring every year, and a large number of offspring can be obtained using a small number of parents [19]. In addition, obtaining semen by artificial extrusion is extremely difficult due to the highly branched and finger-like testis of L. *longirostris* [20]; thus, it is often necessary to euthanize male L. *longirostris* and remove the testis to obtain semen for artificial insemination, which precludes the reuse of male parents [12,21]. The genetic diversity of L. *longirostris* cultures has decreased significantly after several generations of artificial breeding due to inbreeding and genetic drift [22]. Previous studies on the genetic diversity of wild L. *longirostris* populations have employed various molecular genetic methods, but few studies have examined the genetic background of L. *longirostris* parents used for reproduction [22–27]. Assessments of the genetic structure of populations are necessary for the management of farmed L. *longirostris*.

Microsatellites are neutral, codominant, and highly polymorphic markers, and these properties make them useful in population genetic studies of freshwater fish [28]. L. *longirostris* microsatellites have been developed, including 17 dinucleotide microsatellite loci isolated by the magnetic bead enrichment method in 2009 and 16 polymorphic trinucleotide and tetranucleotide microsatellite loci isolated by enrichment methods in 2010 [29,30]. However, few studies of farmed L. *longirostris* have been conducted using microsatellites. Illumina sequencing technology has been used to enrich molecular markers for L. *longirostris*; this is a less expensive and more efficient method for developing microsatellites compared with creating enriched libraries by the magnetic bead enrichment method [28]. The high-quality chromosome-level reference genome for L. *longirostris* was published in 2021 [2], and this has greatly facilitated the development and utilization of microsatellite molecular markers. Using Illumina sequencing technology, we isolated 20 new microsatellites with trinucleotide and tetranucleotide motifs and validated all of them in 240 individuals from four L. *longirostris* populations. These newly isolated microsatellite markers have important implications for the management of L. *longirostris* populations.

In this study, L. *longirostris* were collected from four seed farms in four different areas in Sichuan Province and Hubei Province. These two provinces are the main breeding and seedling production areas of L. *longirostris*, and one of the L. *longirostris* populations was sampled from the national L. *longirostris* seed farm in Hubei Province. The genetic diversity of the four L. *longirostris* populations was characterized using 20 microsatellites. Overall, the aim of this study was to clarify the genetic background of the main farmed L. *longirostris* populations.

2. Materials and Methods

A total of 240 L. *longirostris* were collected, 60 individuals each, from four different areas: Meishan City (MS) (29°44′ N, 103°47′ E) and Yibin City (YB) (28°44′ N, 104°35′E) in Sichuan Province, and Wuhan City (WH) (30°58′ N, 114°17′ E) and Shishou City (SS) (29°40′ N, 112°38′E) in Hubei Province. Tail fin tissue samples of L. *longirostris* were collected, fixed in alcohol, and stored at −20 °C. All experiments that involved handling and treating fish were conducted in strict accordance with the recommendations in the Guide for the Animal Care and Use Committee of the Fishery Institute of the Sichuan Academy of Agricultural Sciences (20200116001A). All samples in this study were collected in accordance with ethical requirements. Genomic DNA was extracted using a special DNA purification kit (B518251,

Sangon Biotech Co., Ltd., Shanghai, China) following the manufacturer's instructions. A spectrophotometer (Nanodrop ND-2000; Thermo Fisher Scientific, Waltham, MA, USA) was used to check the quality of genomic DNA.

Fin tissue samples of L. *longirostris* used for Illumina sequencing were collected from Yibin City, Sichuan Province. The L. *longirostris* DNA genome sequence library was constructed by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China). Clustering of all qualified libraries was conducted using a cBot Cluster Generation System and Illumia Cluster Kit (Illumina, Inc., San Diego, CA, USA), followed by sequencing using Illumina NovaSeq 6000 platform (Illumina, Inc., San Diego, CA, USA). Isolation of microsatellites and primer design were performed using Perl scripts; minimum repeat count parameters were defined to determine the numbers of dinucleotide–hexanucleotide motifs, which were 10, 6, 5, 5, and 5, respectively. We randomly selected 50 primer pairs to generate PCR products; 20 pairs with good stability and polymorphism were used in 240 individuals. The total volume of the PCR amplification reaction was 25 μL; each reaction contained 12.5 μL of 2 × Taq PCR Mix, 1 μL of DNA (concentration 100 ng/μL), 1 μL of fluorescent primer (Table 1), and ddH_2O for the remainder. The thermal cycling conditions for the PCR reactions were 94 °C for 5 min; t36 cycles of 94 °C for 30 s, specified temperatures (values in Table 1) for 30 s, and 72 °C for 30 s; and 72 °C for 5 min. The PCR products were genotyped by Sangon Biotech Co., Ltd. (Shanghai, China).

Cervus 3.0 software [31] and GenALEx 6.5 [32] were used to estimate the following genetic diversity parameters: number of alleles (N_A), number of effective alleles (N_E), expected heterozygosity (H_E), observed heterozygosity (H_O), Shannon's information index (I), polymorphic information content (PIC), Hardy–Weinberg Equilibrium (HWE), and the null allele frequency. The principal coordinates of four L. *longirostris* populations were estimated using GenALEx 6.5. The pairwise population genetic differentiation index (F_{ST}) and Nei's genetic distance were estimated using GENEPOP V4 software [33]. An unweighted pair group method with arithmetic mean (UPGMA) tree based on Nei's genetic distance among the four populations was constructed using MEGA 5.2 software [34]. STRUCTURE 2.3 software [35] was used to estimate the most likely value of genetic clusters (K). The number of K populations was set from 1 to 10, the number of replicates was 20,000, and the MCMC parameter was set to 100,000 times for calculation of the most likely number of genetic clusters (K). ADMIXTURE software [36] was used to estimate genetic admixture, he number of K populations was set from 1 to 10.

Table 1. Twenty new microsatellite primers for L. *longirostris*.

Locus Name	Accession Number	Primer Sequence (5′ - 3′)	Fluorescent Type	*N*	N_A	Size (bp)	Repeat Motif	*Ta* (°C)	H_o	H_E	PIC	P_{HWE}	Null Alleles
CLC01	OM868049	F:CCCATTCTGTCTTCAAATCTAAGC R:GCCAATGCTCTACTATGCTTGTC	5′6-FAM	240	10	108-144	(AGAT)14	54	0.688	0.689	0.650	0.2951	0.0016
CLC02	OM868050	F:GGAAGAGAAACCAGTGTGTAGCA R:CATGAGGTGCTGAAGTCCACTAT	5′6-FAM	240	7	106-124	(AAG)16	56	0.596	0.582	0.545	0.5313	−0.0138
CLC03	OM868051	F:GGGTGAGAAGGATAGGAAAGAAA R:TTTAACAACCCATGAATTAAAAACC	5′6-FAM	240	12	88-140	(AGAT)17	54	0.883	0.861	0.844	0.0069	−0.0164
CLC04	OM868052	F:TTTAATGGGAAAGTTTAATGGATCA R:CTGTACTGCTTCCACCTGATTG	5′6-FAM	240	9	103-135	(TAGA)14	55	0.817	0.842	0.822	0.6146	0.0157
CLC05	OM868053	F:AATAAACAAGGAAAATAATTGCTGG R:ATTGATGGCTAATTTTGCTGGTA	5′6-FAM	240	9	100-127	(TAG)18	54	0.813	0.840	0.817	0.0011 *	0.0158
CLC06	OM868054	F:ATGTTGGTATATGAAGCCTGGAT R:TGACAGTATTTCCTCCATCATCA	5′6-FAM	240	11	106-136	(AAG)15	54	0.854	0.834	0.811	0.2034	−0.0133
CLC07	OM868055	F:TCTGAAGTTGACCGTATGCTTTT R:CTTTCTTTCTCCATTGTCACCAC	5′6-FAM	240	12	153-193	(AGAT)15	56	0.879	0.859	0.840	0.1704	−0.0128
CLC08	OM868056	F:CGTAGTGCTATTTGGGGTATTGA R:TTGTCCTACAATATTCCATGTTTGTT	5′6-FAM	240	8	156-188	(ATAG)15	55	0.829	0.810	0.782	0.1714	−0.0112
CLC09	OM868057	F:GAACCACTTGCAGAATAAACACC R:TCATGATCAAAGTTCCTGACTTAAA	5′6-FAM	240	8	150-182	(TAGA)15	56	0.825	0.820	0.794	0.5680	−0.004
CLC10	OM868058	F:CGCTCTGAGAAAGAAAAACTCAT R:GAACTTTAGATTCTCGGAAGGAAA	5′6-FAM	240	8	159-191	(TAGA)15	55	0.779	0.835	0.814	0.5609	0.0312
CLC11	OM868059	F:TGAGCAAACATGATTTGAATTTG R:TGTTCAAACATTTGCATCATTTC	5′-HEX	240	9	168-207	(AAG)17	54	0.725	0.741	0.707	0.3128	0.0088
CLC12	OM868060	F:GCAATCCTCCAAAGATATTCCTC R:CATGTTTTTGAGGATGAGACTTTTT	5′-HEX	240	17	194-258	(TAGA)21	54	0.863	0.900	0.890	0.0003 **	0.022
CLC13	OM868061	F:TCCCAGGTTATGAGTTATGGTGT R:GCTTTACTTCTCTAAAACAGCTCTGA	5′-HEX	240	9	221-245	(AAG)17	55	0.813	0.803	0.779	0.0217	−0.0098
CLC14	OM868062	F:TTACTGGGGATAGATAGATGGCT R:TCTTTGTCTGTCTATGTATCTGCCT	5′-HEX	240	17	138-238	(TAGA)22	54	0.804	0.836	0.817	0.4236	0.0207
CLC15	OM868063	F:TGAGGTTGAGGTATAAAGGGAAAC R:CACTATCTTTTTCCATCCTTTCCA	5′-HEX	240	10	206-246	(TAGA)16	53	0.867	0.802	0.781	0.0053	−0.0474
CLC16	OM868064	F:GGAGAAACGTCTCAATTCACTGT R:CGTGCACATAGTTTATGCTGAGA	5′-HEX	240	13	202-259	(AGAT)15	56	0.825	0.838	0.820	0.2843	0.0067
CLC17	OM868065	F:AAATACCGTATACACATGGGGGT R:CACAAGGACAAAAATGGTGTTTT	5′-HEX	240	8	218-246	(TAGA)15	56	0.800	0.833	0.808	0.0296	0.0199
CLC18	OM868066	F:TTTACAAGCCAAGCTGAAAGAAT R:CCACTCTCATAATGTCTCTGTTTCA	5′-HEX	240	12	197-243	(ATAG)20	56	0.771	0.833	0.816	0.0044	0.044
CLC19	OM868067	F:GCCTGAAAAATGTGTTCCTTTTA R:TGCCTCTTATTCAAAGGCTTTAC	5′-HEX	240	9	223-256	(AGAT)15	54	0.846	0.800	0.773	0.0296	−0.0273
CLC20	OM868068	F:AAAATTGGTTTGAAGATGAAGCA R:TATGGCAATGTGGTGCAAAT	5′-HEX	240	12	216-256	(TAGA)18	54	0.867	0.850	0.833	0.2817	−0.0132

N, number of samples; N_A, number of different alleles; *Ta*, annealing temperature; H_O, observed heterozygosity; H_E, unbiased expected heterozygosity; PIC, polymorphic information content; P_{HWE}, *p*-value for the Hardy–Weinberg equilibrium; * $p < 0.005$ and ** $p < 0.0025$ significance after Bonferroni correction.

3. Results

A total of 399,740 microsatellite sequences were identified in this study, and the most repeats were dinucleotide motifs (297,200, 74.35%), trinucleotide motifs (46,879, 11.73%), and tetranucleotide motifs (49,767, 12.45%); pentanucleotide motifs (5,471, 1.37%) and hexanucleotide motifs (423, 0.10%) were relatively uncommon.

A total of 50 microsatellite loci primers were designed, of which 5 trinucleotide and 15 tetranucleotide microsatellite loci (GenBank accession numbers OM868049-OM868068) were selected to generate PCR products. N_A ranged from 7 to 17, average N_A for all 20 loci was 10.3, and average H_O and H_E were 0.8072 and 0.8104, respectively (Table 1). The PIC values of all 20 microsatellite loci of L. *longirostris* analyzed in this study ranged from 0.545 to 0.890. There were three loci (CLC5, CLC12, and CLC18) showing significant deviation from HWE ($p < 0.05$); after Bonferroni correction, CLC5 and CLC12 still showed significant deviation from HWE (Table 1).

The results of the genetic diversity analysis of the four L. *longirostris* populations using all 20 newly isolated microsatellite markers are shown in Table 2. The mean values of N_A and N_E indicate that fewer alleles were observed in the WH and SS populations collected from Hubei Province than in the MS and YB populations collected from Sichuan Province. H_E, I, and PIC were lower in the WH and SS populations than in the MS and YB populations; the SS population had the lowest values among all populations.

Table 2. Genetic diversity of four L. *longirostris* populations by 20 microsatellite loci (Mean ± SE).

Population	N	N_A	N_E	I	H_O	H_E	PIC
MS	60	9.750 ± 0.542	5.771 ± 0.384	1.897 ± 0.067	0.791 ± 0.019	0.813 ± 0.020	0.784 ± 0.021
WH	60	8.700 ± 0.449	4.624 ± 0.304	1.695 ± 0.066	0.828 ± 0.021	0.768 ± 0.019	0.730 ± 0.022
SS	60	7.850 ± 0.519	4.065 ± 0.259	1.588 ± 0.055	0.806 ± 0.022	0.740 ± 0.018	0.701 ± 0.018
YB	60	9.750 ± 0.542	5.683 ± 0.277	1.915 ± 0.050	0.803 ± 0.019	0.821 ± 0.012	0.792 ± 0.013

N, number of samples; N_A, number of different alleles; N_E, number of effective alleles; I, Shannon's information index; H_O, observed heterozygosity; H_E, unbiased expected heterozygosity; PIC, polymorphic information content.

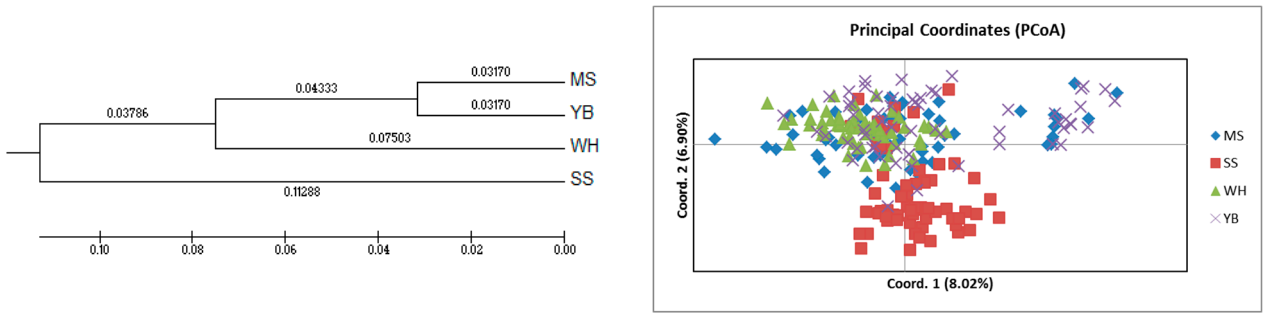
Pairwise population F_{ST} values are shown in Table 3. The F_{ST} value was lowest between MS and YB (F_{ST} = 0.009) and highest between WH and SS (F_{ST} = 0.039). Overall, low levels of genetic differentiation were observed among the four L. *longirostris* populations.

Table 3. Pairwise population genetic differentiation index (F_{ST}) of four L. *longirostris* populations.

Population	MS	SS	WH	YB
MS	0.000			
SS	0.028	0.000		
WH	0.019	0.039	0.000	
YB	0.009	0.031	0.023	0.000

A UPGMA phylogenetic tree and principal coordinates of four L. *longirostris* populations are shown in Figure 1. The MS population was situated close to the YB population within small-clustered groups; the two populations from Sichuan Province have similar genetic characteristics and are closer genetically compared with the WH and SS populations from Hubei Province. The SS population is genetically distant from the other populations.

The STRUCTURE analysis revealed that the delta-K value was highest when K = 3, indicating that the optimal number of populations of the four L. *longirostris* populations was three. When three clusters (K = 3) were assumed, the three populations were admixed with >85% of the SS population in the "blue" cluster and most individuals of the WH population in the "red" cluster (Figure 2). When three clusters (K = 3) were assumed, the results of the ADMIXTURE analysis were similar to that of the STRUCTURE analysis (Figure 3).



(**a**) (**b**)

Figure 1. (**a**) UPGMA phylogenetic tree of four L. *longirostris* populations according to 20 microsatellite loci; (**b**) principal coordinates of four L. *longirostris* populations according to 20 microsatellite loci.

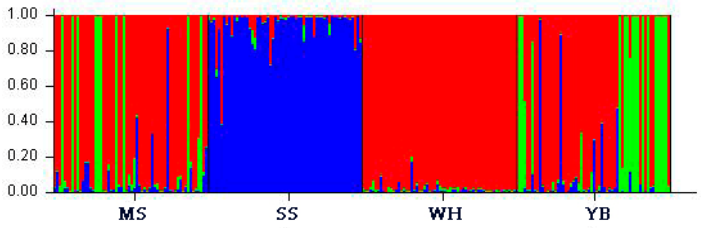


Figure 2. STRUCTURE bar plots for four populations of L. *longirostris* (K = 3).

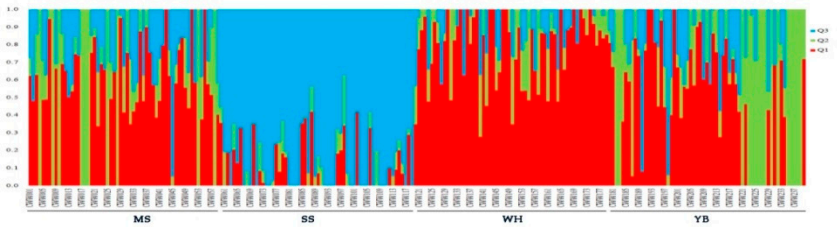


Figure 3. ADMIXTURE bar plots for four populations of L. *longirostris* (K = 3).

4. Discussion

Microsatellite markers are highly stable, polymorphic, and identifiable in genetic analyses [37]; thus, they have become a practical tool for population genetic studies of aquatic animals, such as *Xenocypris davidi* [28], *Paralichthys adspersus* [38], and *Procypris rabaudi* Tchang [39]. The trinucleotide and tetranucleotide repeats are less likely to be scored ambiguously and slip compared with dinucleotide repeats [30,40]. In our study, 5 trinucleotide and 15 tetranucleotide microsatellite loci were isolated in L. *longirostris*; all the new microsatellite loci were able to generate consistent and reliable polymorphic PCR products in 240 individuals from four L. *longirostris* populations. N_A ranged from 7 to 17; after Bonferroni correction, CLC5 and CLC12 still significantly deviated from HWE (Table 1). Null alleles might cause deviation from HWE in the aforementioned loci because null alleles occur widely in organisms and are one of the main drivers of changes in population structure [28]. PIC takes into account not only the number of detected alleles but also the relative relatedness of those alleles [41]. According to previous studies, loci with PIC values greater than 0.5 are highly informative [42]. The PIC values of all the 20 microsatellite loci of L. *longirostris* analyzed in this study ranged from 0.545 to 0.890; thus, all loci were highly informative. These 20 microsatellite markers, therefore, are sufficient for discriminating among L. *longirostris* individuals and populations according to the results of previous studies employing combinations of microsatellite markers [42].

Artificial breeding technology of many aquatic animals, including L. *longirostris*, is relatively mature; nursery technology is well-developed; modern water treatment tech-

nology and Internet of Things technology have been increasingly used; and the degree of industrialization and informatization of nursery production has been greatly improved. This large-scale breeding technology system for aquatic fry has promoted the artificial propagation of fry and their transportation to different areas for breeding [43,44]. Unlike in wild populations, the genetic distance between farmed populations is not entirely determined by geographic location, but more so by the source of fry [45]. Due to sharp decreases in wild L. *longirostris* populations, the parents are mostly individuals that have been artificially bred for multiple generations. This, coupled with the introduction of parents and the use of unplanned fry production practices by many seedling farms, has resulted in the mixing of germplasm resources and a lack of uniformity in the quality of the fry produced and has restricted the development of the aquaculture industry [2,22]. Clarifying the genetic structure of L. *longirostris* populations is important for ensuring the efficacy of management practices and the sustainability of the growth in L. *longirostris* production. In this study, we characterized the genetic diversity of four representative L. *longirostris* fry production farms in two main L. *longirostris* production areas in Sichuan Province and Hubei Province. The results of the genetic diversity analysis for the four L. *longirostris* populations revealed that N_A ranged from 7.85 to 9.75; H_O (0.791–0.828) and H_E (0.740–0.821) were greater than 0.6; and PIC (0.701–0.792) was greater than 0.5 (Table 2). The genetic diversity of the four L. *longirostris* populations was high according to the results of previous studies employing multiple microsatellite markers [42].

Polymorphism is an important parameter for selecting informative loci for primer evaluation and population analyses [41]. Microsatellite markers have been used to analyze genetic diversity in wild L. *longirostris* populations. For example, Wang et al. [26] used eight pairs of microsatellite primers to amplify sequences in 86 samples from four wild L. *longirostris* populations. N_A counts in the four populations were 7.3, 5.6, 4.8, and 3.3, and H_O values were 0.732, 0.312, 0.681, and 0.877. N_A was lower in the study by Wang et al. than in this study (N_A ranged from 7.85 to 9.75); additionally, H_O of three of the four wild populations in the study by Wang et al. was lower than that of the four populations in this study (H_O ranged from 0.791 to 0.828), which might be related to the number of microsatellite markers and the total number of samples. The sample size used for microsatellite analysis is positively correlated with the average PIC and N_A [46]. Yang et al. [23] used 20 microsatellite markers (10 dinucleotide and 10 tri- and tetranucleotide) to analyze the genetic diversity of 132 samples which were collected in the upper–lower reaches of the Yangtze River; H_O ranged from 0.8205 to 0.8697, and H_E ranged from 0.7757 to 0.8300. H_O and H_E were higher in wild L. *longirostris* populations than in farmed L. *longirostris* (H_O, 0.791–0.828; H_E, 0.740–0.821), indicating that the genetic diversity of farmed L. *longirostris* was lower. Many seedling production farms pair multiple females with the same male to save costs, but this might result in the loss of genetic diversity.

Genetic distance calculations involve using allele frequency to describe the process of population differentiation, which is an effective method for analyzing populations [47]. The UPGMA tree and principal coordinates of the four L. *longirostris* populations revealed that the MS population was situated close the YB populations within small-clustered groups, and the two populations collected from Sichuan Province were closely related compared with the WH and SS populations from Hubei Province. The genetic characteristics of the MS and YB populations were similar, and the genetic structure of the SS population differed from that of the other populations. However, the F_{ST} values (0.009–0.039) among the four populations were less than 0.05 (Table 3); low levels of genetic differentiation were observed among these populations, and $0 < F_{ST} < 0.05$ indicated that there was little genetic differentiation among the populations based on the results of a previous study [48]. Xiao et al. used mitochondrial DNA control region sequences to analyze the genetic diversity of three farmed L. *longirostris* populations in Meishan City, Shishou City, and Huainan City in 2013; the F_{ST} values among the three populations ranged from 0.0391 to 0.1024 [22]. The level of genetic differentiation among the four L. *longirostris* populations in this study was lower. The STRUCTURE analysis revealed the optimal number of populations of the four

L. *longirostris* populations was three (K = 3). The STRUCTURE analysis indicated that MS and YB farms may collect some additional individuals from a common unknown origin. Results in Figure 3 indicate that there might be much admixture in all farms, thus suggesting adequate diversification policies in all farms. We speculated that the introduction of parents and the use of unplanned fry production practices in fry breeding farms have resulted in the mixing of germplasm resources and a reduction in the degree of genetic differentiation among populations.

5. Conclusions

Overall, the level of genetic differentiation among the four farmed L. *longirostris* populations was relatively low according to pairwise comparisons of F_{ST} values among the populations; however, the genetic diversity of these populations was high, which indicates that they still provide useful sources of genetic variation that could aid in breeding efforts. Preventing inbreeding is important for ensuring that the production of L. *longirostris* derived from farmed populations is sustainable over the long term. In our study, we isolated 20 new polymorphic microsatellite loci from the genome sequences of L. *longirostris* and validated the efficacy of these markers in four L. *longirostris* populations. These newly isolated microsatellite markers and the high genetic diversity of L. *longirostris* populations in the main breeding areas have important implications for the breeding and stock management of L. *longirostris*.

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Data Availability Statement: Data available on request from the authors.

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