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Genetic diversity analysis and DNA fingerprinting of different populations of largemouth bass (*Micropterus salmoides*) in China with fluorescence-labeled microsatellite markers

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Abstract

Background Largemouth bass (*Micropterus salmoides*, LMB) is an economic fish in China, which has developed into many different cultured populations in the past decades. These populations exhibit different growth rates, morphological traits, stress resistance, and genetic diversity. Analyzing genetic diversity and molecular identification of these populations is crucial for conserving and utilizing germplasm resources, as well as for breeding new varieties.

Result In this study, ten distinct LMB populations from China were collected and examined using fluorescence-labeled microsatellite markers. A total of 53 alleles were identified using seven microsatellite primer pairs, with allele counts ranging from 5 to 11 and an average of 7.571. The observed heterozygosity among the ten LMB populations varied from 0.210 to 0.967, while expected heterozygosity ranged from 0.204 to 0.651, and the polymorphism information content was between 0.175 and 0.597. Genetic distance varied from 0.019 to 0.457, the genetic differentiation index ranged from 0.013 to 0.258, and the number of effective migrants (*N*m) was between 0.719 and 18.981. The genetic structure analysis indicated that the ten LMB populations could be classified into two or four groups. The analysis of molecular variance (AMOVA) revealed that 83.77% of genetic variation was found within individuals, with only 16.23% attributed to differences among populations. Through construction of DNA fingerprinting, we discovered unique fragments at several loci were detected in the populations such as the reintroduced Northern LMB population, "Youlu No.3" population, and the hybrid populations. Additionally, we also created digital DNA fingerprint maps of these LMB populations. Through analysis the digital DNA fingerprints from four candidate LMB populations, three known populations corresponded with the populations collected in this study. These results indicated high identification efficiencies of the digital DNA fingerprinting created in this study.

Conclusion We established a method to distinguish 10 different LMB populations in China, which will assist in identification, traceability management, protection, and intellectual property rights of LMB in the future.

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Keywords Largemouth bass, Microsatellite marker, Genetic diversity, DNA fingerprinting, Identification

Background

Largemouth bass (*Micropterus salmoides*, LMB) is native to freshwater lakes and rivers in North America and was introduced from Taiwan (China) to mainland China in 1983 [1]. Due to its good meat, rapid growth, absence of intermuscular bone, strong adaptability, and each to capture, LMB has gained popularity among both farmers and consumers [1]. Over the past two decades, two new varieties of LMB have been selectively bred: "YouLu No.1" (Registration number: GS01-004-2010), which was selected for growth traits in 2010, and "YouLu No.3" (Registration number: GS-01-001-2018), which was selected for growth traits and food preference in 2018 [2]. With the cultivation of "YouLu No.3", as well as the development of feed formulation, culture technology, and disease prevention and treatment methods, the production of LMB increased from 477,808 tons in 2019 to 888,030 tons in 2023. However, as an imported species, the limitations in replenishing wild genetic resources have led to a significant decline in genetic diversity over time [3, 4]. Addressing the challenge of effectively enhancing genetic diversity in LMB remains a pressing issue.

LMB is classified into two subspecies, the Northern LMB (*M. salmoides*, NLMB) and the Florida LMB (*M. floridanus*, FLMB) [5, 6]. Between 2010 and 2011, our team reintroduced the wild populations of NLMB and FLMB from the United States [1]. Using microsatellite, SNP, and InDel markers, we determined that the "YouLu No.1" and "YouLu No.3" LMB are both NLMB subspecies [2, 7]. Additionally, several local populations, such as those in Taiwan and Foshan, have developed over the years and also belong to the NLMB subspecies [8]. Through mitochondrial D-loop gene analysis [9] and microsatellite markers [3, 10, 11], researchers have indicated that the genetic diversity of cultured LMB populations in China has significantly decreased as compared to that of the reintroduced NLMB population. For instance, an analysis involving six microsatellite markers across 19 populations, including four cultured LMB populations in China, five FLMB andeight NLMB wild populations, revealed that the genetic diversity of cultured LMB populations had decreased by nearly 40% compared to the wild populations [1]. Hybridization is a significant method for fish breeding, effectively transferring desirable parental traits and increasing the genetic variation of offspring [2, 12]. Currently, several studies have reported on hybridization and the comparison of genetic diversity among hybrid offspring and their parents in LMB. For example, the offspring of YL1♀*NLMB♂ exhibited higher genetic diversity and growth rates than those of YL1 and NLMB [13]. Additionally, hybrid offspring from the Foshan and Taiwan populations demonstrated superior growth performance and variation coefficients for body mass compared to the inbred populations from Foshan and Taiwan [8]. Furthermore, FLMB♀*NLMB♂ displayed the highest number of effective alleles (*Ne*), expected heterozygosity (*He*), and polymorphic information content when compared to NLMB♀*FLMB♂, NLMB, and FLMB [14]. This hybrid also exhibited the highest critical and chronic thermal maximum [15]. To effectively utilize these genetic resources, it is essential to analyze their hereditary traits, identify their differences, and estabolish methods for their identification.

DNA fingerprinting is a powerful method for identifying relationships within species, offering benefits such as high individual specificity and environmental stability. This technique can effectively differentiate between closely related species or individuals by examining variations in genomic sequences. Markers used in DNA fingerprinting include restriction fragment length polymorphisms (RFLP), random amplification of polymorphic DNAs (RAPD), microsatellite, and single nucleotide polymorphism (SNP) [16, 17]. Among these, microsatellite markers are preferred due to their high reproducibility, co-dominance, and abundance in genomes [16]. They have been extensively utilized for DNA fingerprinting in various fish species. For example, Quan et al. amplified 50 microsatellite markers to differentiate between different strains of the Animal Swordtail (*Xiphophorus helleri*) and constructed a fingerprint identification database for three strains. This database provides essential information related to identification, purity, and genetic monitoring of Swordtail populations [18]. Song et al. utilized 82 microsatellite primer pairs to investigate the genetic variation and germplasm identification of *Oreochromis aureus*, *O. niloticus* and *O. mossambicus*. The results for *He*, *Ho*, and PIC indicated that the genetic diversity of *O. niloticus* was the highest, while that of *O. aureus* was the lowest. Sixteen microsatellite primer pairs were employed to construct a digital microsatellite DNA fingerprinting for the three tilapia species, providing a technique for addressing the germplasm classification for tilapia [19]. Zhang et al. used 18 microsatellite primer pairs to analyze genetic diversity of *Parabramis* and *Megalobrama* populations. From the microsatellite DNA fingerprinting, 9 pairs of specific microsatellite markers were identified, which can be used to distinguish most genera within the *Parabramis* and *Megalobrama* populations [20]. In the case of LMB, Fan et al. developed a DNA fingerprinting method using 43 microsatellite markers, based on one cultured population from China, two reintroduced FLMB populations, and one reintroduced NLMB population

[17]. However, it remains unclear whether this DNA fingerprinting can effectively differentiate between various cultured NLMB populations.

Based on data from the China Fisheries Statistical Yearbook, a total of 10 LMB populations were collected from Guangdong, Zhejiang, Anhui, and Fujian Province. The production in these areas accounts for 69.34–76.11% of the total production from 2020 to 2023. The populations collected from Guangdong Province included "YouLu No.1", "YouLu No.3", the F7 generation of the reintroduced wild NLMB population, the Taiwan population, and the Shaoguan population. Additionally, one population was collected from Anhui, one from Zhejiang, and one from Fujian Province, respectively. Furthermore, two hybrid offspring resulting from crosses between "YouLu No.3" and the FLMB population were collected. We employed a total of 7 microsatellite markers to assess genetic diversity and create DNA fingerprint chromatograms for these 10 LMB populations. Our findings offer valuable insights for the conservation, identification, utilization, and breeding of LMB germplasm resources in the future.

Materials and methods

Collection of different LMB populations

From 2022 to 2024, a total of 300 LMB individuals were collected from ten different populations in China, with 30 individuals sourced from each population. The "Youlu No.1" (YL1) population was gathered from Jiyurunda Fishery Technology Co., Ltd, Foshan, Guangdong Province in 2022, while the Zhangzhou population (ZZ) was collected from Tongda aquatic products Co., Ltd, Zhangzhou, Fujian Province in the same year. The F4 generation of "Youlu No.3" (YL3), the F7 generation of reintroduced NLMB wild population (NB), and the Taiwan population (TW) were obtained from Guangdong Liangshi Aquatic Seed Industry Co., Ltd in Foshan, Guangdong Province in 2022. The Shaoguan population (SG) was collected from a reservoir in Shaoguan City, Guangdong Province in 2023. The Chizhou population (CZ) was sourced from Chizhou city Naiming aquatic products Co., Ltd, Chizhou, Anhui Province, and Huzhou population (HZ) was collected from Huzhou Huwang aquaculture seed industry Co., LTD, Huzhou, Zhejiang Province in 2024. In March 2024, hybridization between F6 generation of YL3 and F3 generation of reintroduced FLMB wild population (from the Pearl River Fisheries Research Institute) was conducted at Guangdong Liangshi Aquatic Seed Industry Co. The offsprings from the cross FLMB♂*YL3♀ was labeled as NF, while those from FLMB♀*YL3♂ was labeled as FN. The samples of candidate LMB populations used to test the accuracy of DNA fingerprinting were collected from 2023 to 2024. Five offsprings of YL1 (C-YL1) were collected from Jiyurunda Fishery Technology Co., Ltd, Foshan in 2024. In Guangdong Liangshi Aquatic Seed Industry Co., Ltd, five hybrid offspring of FLMB*YL3 (C-HY) were collected in 2023, five offspring of YL3 (C-YL3) and NB (C-NB) were collected in 2024. Besides, an unknown LMB population was randomly collected from a farm in Foshan City in 2024. After being anesthetized with 200 mg/L MS-222, the fin tissues were collected and kept at -20℃ until DNA extraction.

Genomic DNA extraction and PCR amplification

Genomic DNA was extracted from fin tissues for each sample using TIANamp Genomic DNA Kit DP304, following the manufacturer's instructions. The extracted DNA was quantified using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and its quality was evaluated using 1.0% agarose gel electrophoresis. The DNA concentration was adjusted to 50 ng/μL and stored at -20 ℃ until needed.

Fifteen microsatellite primer pairs were randomly selected from existing literature [17, 21]. After evaluation, seven primer pairs that produced clear and repeated bands were selected for further study. The forward primers were synthesized and labelled with FAM, ROX, TMRA, or HEX at the 5'end by Huayu Gene (Wuhan) Co., Ltd, Wuhan, China (Table S1). PCR was conducted in a total volume of 10 μL which included 1.0 μL of genomic DNA (20 ng/μL), 0.5 μL (10 pmol/L) each primer, 5 μL 2×Taq PCR Master Mix, and 3.0 μL ddH_2O. The PCR amplification process involved an initial step at 95 ℃ for 5 min, followed by 10 cycles of each consisting of 30 s at 95 ℃, 30 s at a decreasing temperature from 62 ℃ to 52 ℃ (decreasing by 1℃ each cycle), and 30 s at 72 ℃. This was followed by 25 additional cycles each consisting of 30 s at 95 ℃, 30 s at 52 ℃, and 30 s at 72 ℃. A final extension was performed at 72 ℃ for 10 min before storing the samples at 4℃.

Capillary electrophoresis detection

A mixture of highly deionized-formamide (HIDI) and GeneScan™500 LIZ molecular weight internal standard was prepared in a volume ratio of 85:5 and placed in a 96-well reaction plate with a continuous pipette. Each well contained a total volume of 9 μL, to which 1 μL of PCR amplification product diluted 10 times was added. Then, the mixture was centrifuged and placed in the PCR machine to undergo the denaturation procedure (95 ℃ for 3 min and 16℃ for 1 min), followed by immediate cooling after denaturation. According to the operational protocol of the ABI 3730XL DNA Analyzer (Applied Biosystems Inc., USA), the PCR amplification product was detected and analyzed.

Data analysis

The peak patterns generated by the sequence analyzer were examined using GeneMapper v5.0. The analysis involved counting data based on peak features and the fragment sizes of the corresponding peaks. Genetic statistics for the seven microsatellite primer pairs were calculated using Popgene version 1.32, which included metrics such as the number of alleles (*N*a), the effective number of alleles (*Ne*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), Shannon's information index (*I**), and Fixation index (F) [22]. The Polymorphism information content (PIC) was determined using Powermarker v3.0 [23], and UPGMA cluster analysis was conducted based on Nei's genetic distance (H*) matrix with MEGA 6.0. 0 [24]. STRUCTURE 2.3.4 was used to infer the population structure [25]. A Burn-in period of 10,000 Markov Chain Monte Carlo iterations and a run length of 100,000 were established to capture the main structure in the data, with twenty independent runs for each simulated value of K, ranging from 1 to 20. For each K, the statistical value DK was calculated using the formula described by Evanno et al. (2005) [26], and the optimal K was identified using Structure Harvester [27]. Based on the population structure results, GenAlEx 6.501 was used to calculate the inbreeding coefficient within populations (*F*is), the overall populations (*F*it), the genetic differentiation index (*F*st), the number of effective migrants (*N*m), as well as conducting an Analysis of Molecular Variance (AMOVA) and Principal Coordinates Analysis (PCoA) [28].

Establishment of microsatellite DNA fingerprint database

Using the results from capillary electrophoresis detection, a microsatellite DNA fingerprint was created in Excel by compiling the seven alleles amplified from each individual in the population. To facilitate analysis and statistical calculations of the numerous detected fragments, the electrophoresis peak at the same alleles was identified and recorded as "1" if present and "0" if absent, forming a 0/1 system in which all the samples were eventually represented by a series of 0/1 numbers. For example, if there were four alleles with lengths of 141, 147, 153, 155, and 157 bp, and only the alleles for 141 and 147 bp were found, the digital DNA fingerprint would be recorded as 11,000.

The identification of the candidate LMB populations is assessed using two indicators: (1) the number of valid alleles that are ultimately matched, with a higher number of alleles indicating a greater likelihood of identifying distinct germplasm. For instance, if one microsatellite locus in a known population was recorded as 11,001 and the candidate individual as 11,000, the number of matched effective loci would be noted as 2. (2) The presence of unique amplified fragments within a specific population. For example, a 201 bp fragment was identified as unique to the NB population. If the 201 bp fragment is detected in the candidate population, it could be classified as belonging to the NB population.

Results

Selection of microsatellite primers

Fifteen pairs of microsatellite primers were chosen for initial screening. After amplifying 64 LMB individuals (with 6 to 7 randomly selected from each population) using these primer pairs, 7 pairs that produced clear bands and demonstrated good reproducibility were selected for further analysis (Table 1, and Supplement materials). These 7 primer pairs generated a total of 53 alleles, with a range of 5 (Mdo6) to 11(MisaTPW12) alleles per pair and an average of 7.571. The total effective number of alleles (*Ne*) was 14.729, varying from 1.341 (Msal21) to 2.896 (MisaTPW12), with an average of 2.104. Shannon's information index (*I**) ranged from 0.599 (Msal21) to 1.451 (MisaTPW12), averaging 0.988. Observed heterozygosity (*Ho*) and expected heterozygosity (*He*) ranged from 0.237 to 0.605 (mean of 0.438) and 0.255 to 0.655 (mean of 0.484), respectively. The average Polymorphism information content (PIC) was 0.443, with a maximum of 0.620 for the MisaTPW12 locus and a minimum of 0.246 for the Msal21 locus. The mean values of the inbreeding coefficient within populations (*F*is), inbreeding coefficient in the overall populations (*F*it),

Table 1 Results for the seven microsatellite primers

Locus	*Na*	*Ne*	*I**	*Ho*	*He*	F	PIC	*Fis*	*Fit*	*Fst*	Allele sizes
Lma120	6	2.364	0.971	0.533	0.577	0.076	0.490	-0.121	0.075	0.175	192, 194, 197, 201, 203, 207
Mdo6	5	1.421	0.638	0.297	0.297	-0.001	0.283	-0.139	-0.002	0.120	141, 147, 153, 155, 157
Msal21	6	1.341	0.599	0.237	0.255	0.067	0.246	-0.054	0.072	0.119	195, 197, 199, 201, 203, 207
MisaTPW11	10	2.522	1.271	0.500	0.603	0.171	0.557	-0.080	0.172	0.233	159, 163, 167, 171, 175, 183, 187, 191, 196, 204
MisaTPW12	11	2.896	1.451	0.605	0.655	0.075	0.620	-0.074	0.076	0.140	266, 270, 274, 317, 319, 321, 323, 329, 331, 335, 343
MisaTPW25	7	1.657	0.738	0.397	0.397	0	0.348	-0.104	-0.002	0.093	257, 261, 264, 268, 272, 276, 280
MisaTPW76	8	2.527	1.250	0.500	0.604	0.173	0.554	-0.076	0.173	0.231	253, 256, 261, 264, 268, 272, 280, 287
Mean	7.571	2.104	0.988	0.438	0.484	0.080	0.443	-0.092	0.081	0.159	/

Note: *N*a, the number of alleles; *N*e, the effective number of alleles; *I**, Shannon's information index; *Ho*, observed heterozygosity; *He*, expected heterozygosity; *F*, Fixation index; PIC, Polymorphism information content; *F*is, inbreeding coefficient within populations; *F*it, inbreeding coefficient in the overall populations; *F*st, genetic differentiation

and genetic differentiation (*F*st) were – 0.092, 0.081, and 0.159, respectively.

Genetic diversity analysis among different LMB populations

The number of alleles (*N*a) across the 10 LMB populations varied from 1.714 (SG) to 5.143 (NF and FN), with an average of 3.443. The number of effective alleles (*N*e) ranged from 1.336 (HZ) to 2.943 (NF), averaging at 1.923. The Shannon's information index (*I**) was between 0.352 (HZ) and 1.253 (NF), with an average of 0.730. Observed heterozygosity (*H*o) ranged from 0.210 (HZ) to 0.967 (NF), averaging 0.438. Expected heterozygosity (*H*e) ranged from 0.204 (HZ) to 0.651 (NF), with an average of 0.402. The interpopulation genetic differentiation index (*F*st) ranged from – 0.492 (NF) to 0.145 (YL3), averaging – 0.047. The Polymorphism information content (PIC) varied from – 0.175 (HZ) to 0.597 (NF), with an average of 0.356 (Table 2).

Genetic structure among different LMB populations

The 300 LMB individuals were categorized into two or four main primary clusters using STRUCTURE 2.3.4 when the peaks of DK were observed at K = 2 and K = 4 (Fig. 1A). At K = 2, the individuals were divided into two clusters: cluster 1 contained 93 individuals (31.00%), while cluster 2 had 207 individuals (69.00%) (Fig. 1B). Cluster 1 comprised 2 YL1, 13 NLMB, 3 YL3, 25 ZZ, 30 NF, 14 FN, and 6 TW individuals. Cluster 2 included 28 YL1, 17 NLMB, 27 YL3, 5 ZZ, 30 HZ, 30 CZ, 16 FN, 30 SG, and 24 TW individuals (Table S2).

At K = 4, the 300 LMB individuals were sorted into four clusters (Fig. 1B). Cluster 1 consisted of 12 YL1, 6 MLMB, 14 YL3, 29 HZ, 25 CZ, 13 FN, and 21 TW individuals. Cluster 2 had 1 MLMB, 5 ZZ, 30 NF, and 4 FN individuals. Cluster 3 included 17 YL1, 23 MLMB, 13 YL3, 25 ZZ, 1 HZ, 3 CZ, 13 FN, and 9 TW individuals. Finally, cluster 4 contained 1 YL1, 3 YL3, 2 CZ, and 30 SG individuals (Table S3).

Phylogenetic cluster analysis among different LMB populations

The genetic distance among the ten populations varied from 0.019 to 0.457. The greatest genetic distance was found between the NF and SG populations, whereas the smallest distance was between the CZ and HZ populations (Table 3). The genetic differentiation index (*F*st) for the 10 LMB populations ranged from 0.013 to 0.258. The largest genetic distance was noted between the TW and SG populations, while the smallest distances were observed between the YL1 and YL3 populations, as well as between the NLMB and FN populations (Table 3).

The UPMGA cluster tree for the 10 LMB populations was created using Nei's genetic distance matrix. The findings indicated that the HZ population initially grouped with the CZ population, and subsequently with the TW population. The YL1 population first clustered with the YL3 population, and then both were grouped with the HZ, CZ, and TW populations. The FN population initially clustered with the NLMB population and then joined the HZ, CZ, TW, YL3, and YL1 populations. Eventually, these populations were grouped with the ZZ population, followed by the SG and NF populations (Fig. 2A). The UPMGA cluster tree for the 300 LMB individuals was illustrated in Fig. 2B. The results revealed that the 30 individuals from SG and the 30 individuals from NF formed distinct groups, suggesting significant differences from other varieties and low relatedness. However, the other individuals from the same population did not cluster together.

Population genetic variation analysis among different LMB populations

The number of effective migrants (*N*m) across the ten populations varied from 0.719 to 18.981. The lowest *N*m

Table 2 Genetic diversity of 10 different LMB populations

Populations	Number	*Na*	*Ne*	*I**	*Ho*	*He*	*Fst*	PIC
YL1	30	2.571	1.869	0.671	0.452	0.420	-0.074	0.350
NB	30	4.714	2.304	0.972	0.524	0.509	-0.036	0.459
YL3	30	4.000	1.796	0.744	0.357	0.410	0.145	0.359
ZZ	30	3.857	2.442	0.906	0.595	0.479	-0.194	0.441
HZ	30	2.143	1.336	0.352	0.210	0.204	-0.035	0.175
CZ	30	2.571	1.463	0.511	0.310	0.296	-0.029	0.257
NF	30	5.143	2.943	1.253	0.967	0.651	-0.492	0.597
FN	30	5.143	2.165	0.988	0.457	0.492	0.079	0.455
SG	30	1.714	1.456	0.380	0.252	0.256	0.043	0.202
TW	30	2.571	1.458	0.519	0.259	0.299	0.124	0.261
Mean	30	3.443	1.923	0.730	0.438	0.402	-0.047	0.356

Note: *Na*, the number of alleles; *Ne*, the effective number of alleles; *I**, Shannon's information index; *Ho*, observed heterozygosity; *He*, expected heterozygosity; *F*st, genetic differentiation; PIC, Polymorphism information content

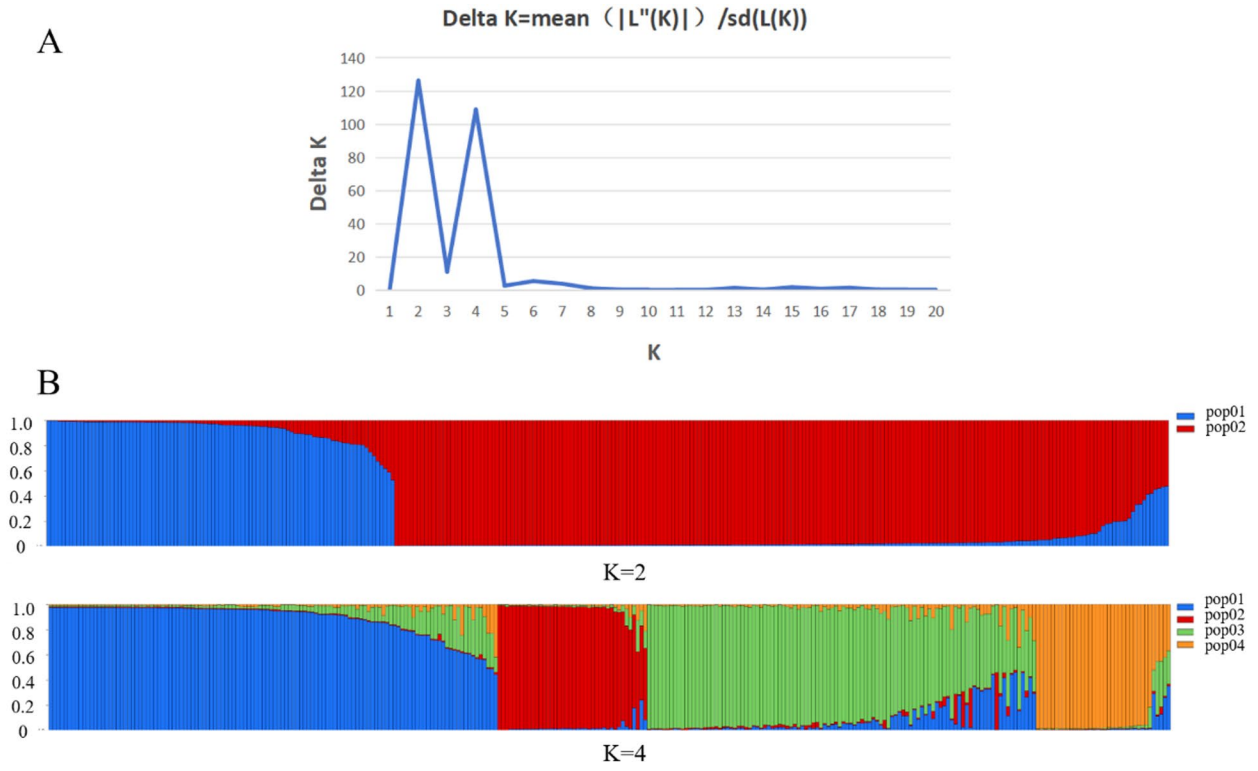

Fig. 1 Estimation of the maximum of Delta K and classification of 300 LMB individuals. Note: (**A**). Delta K values for 300 LMB individuals in the STRUCTURE analysis. (**B**) Classification of 300 LMB individuals at K = 2 and K = 4. The distribution of the accessions to different individuals is indicated by the color code. Numbers on the y-axis show the subgroup membership and the x-axis shows the different individuals

Table 3 Genetic distance (above diagonal) and genetic differentiation index (*Fst*, below diagonal) among ten LMB populations

	YL1	NB	YL3	ZZ	HZ	CZ	NF	FN	SG	TW
YL1	-	0.076	0.029	0.127	0.059	0.036	0.300	0.094	0.196	0.059
NB	0.024	-	0.061	0.095	0.121	0.095	0.246	0.052	0.262	0.090
YL3	0.013	0.026	-	0.103	0.060	0.034	0.279	0.079	0.198	0.047
ZZ	0.054	0.028	0.047	-	0.160	0.155	0.296	0.110	0.273	0.151
HZ	0.083	0.096	0.061	0.109	-	0.019	0.276	0.119	0.270	0.042
CZ	0.049	0.060	0.029	0.083	0.021	-	0.284	0.095	0.241	0.027
NF	0.111	0.083	0.099	0.102	0.153	0.118	-	0.162	0.457	0.267
FN	0.037	0.013	0.027	0.036	0.074	0.041	0.069	-	0.328	0.079
SG	0.150	0.159	0.164	0.165	0.257	0.226	0.220	0.208	-	0.300
TW	0.054	0.060	0.024	0.079	0.035	0.017	0.119	0.036	0.258	-

was found between the SG and TW populations, whereas the highest *N*m was recorded between the YL1 and YL3 populations, as well as between the NLMB and FN populations (Table 4). The AMOVA analysis indicated that the majority of genetic variation, 83.77%, was found within individuals, while only 16.23% was attributed to differences between populations (Table 5). Additionally, the PCoA results demonstrated that the 10 LMB populations did not group by region (Fig. 3), which aligned with the findings from the UPGMA analysis. These findings suggest that there is a high degree of genetic similarity among the 10 LMB populations.

Construction and application of DNA fingerprinting in LMB

To differentiate the 10 distinct LMB populations, we examined the DNA fragments produced by seven microsatellite markers. The DNA fingerprints of the 10 LMB populations, drawn in Excel based on the amplification results for the seven microsatellite sites in each population (Table 6; Fig. 4). In the DNA fragments amplified from the Lma120 locus, the NB population exhibited a unique 201 bp fragment, the YL3 population exhibited a unique 207 bp fragment, while the NF population showed a unique 197 bp fragment. Additionally, the 194 bp fragment was absent in the HZ, CZ, SG, and TW populations. For the Mdo6 locus, only a 147 bp

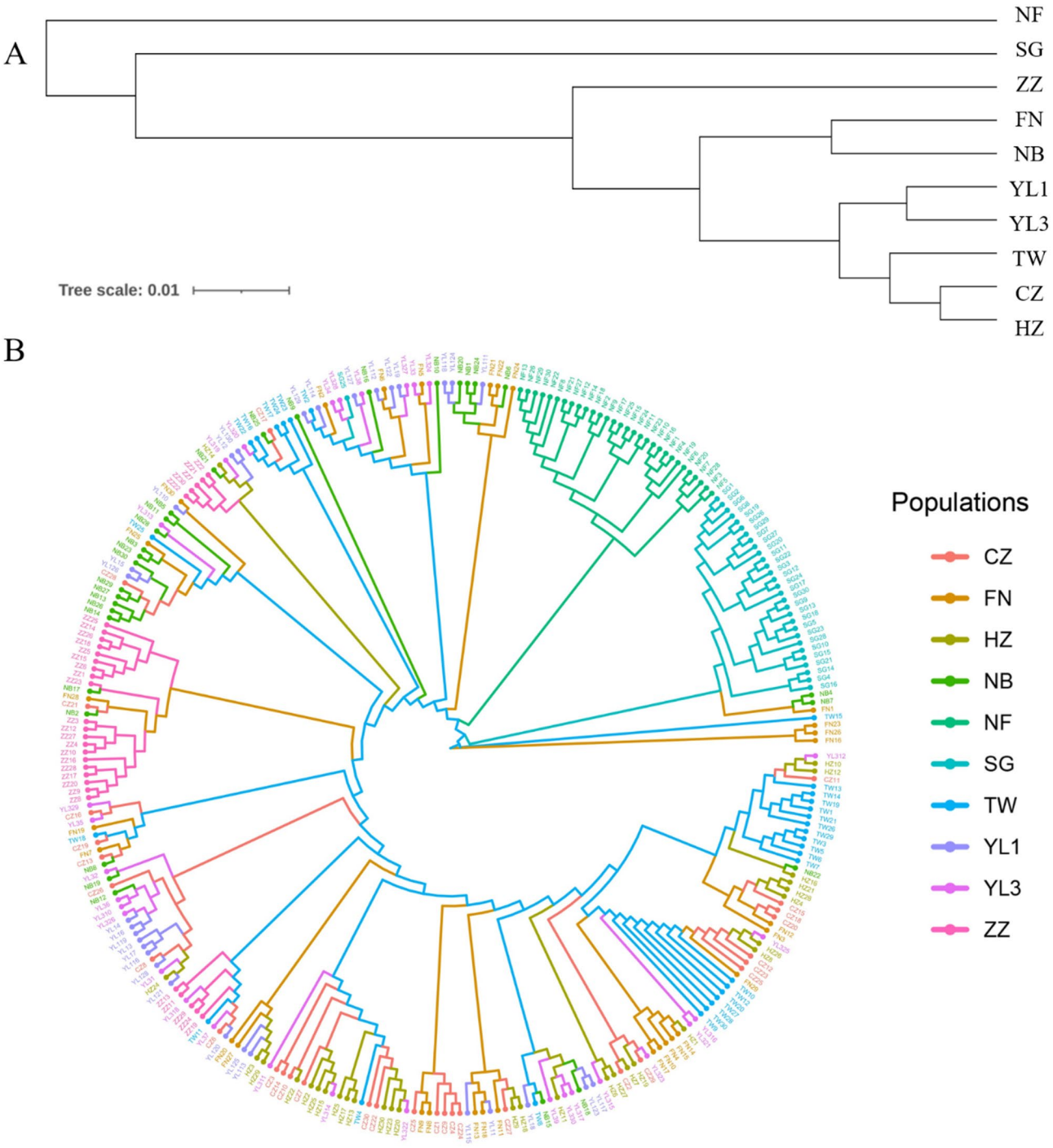

Fig. 2 Phylogenetic tree of different LMB populations and individuals based on seven microsatellite markers

fragment was found in the HZ population. In the case of the Msa121 locus, only a 197 bp fragment was identified in the SG population, and a unique 199 bp fragment was found in the FN population. The MiSaTPW11 locus revealed a unique 196 bp fragment in the NB population, and the ZZ population had unique fragments of 187 bp and 204 bp. Furthermore, fragments smaller than 183 bp were not detected in the SG population. The MiSaTPW12 locus showed a unique 317 bp fragment in the NF population, a unique 343 bp fragment in the YL3 population, and lack of 266 bp fragment in the SG population. For the MiSaTPW25 locus, unique fragments

Table 4 Number of effective migrants (*Nm*) indices among 10 LMB populations

	YL1	NB	YL3	ZZ	HZ	CZ	NF	FN	SG	TW
YL1	-									
NB	10.167	-								
YL3	18.981	9.365	-							
ZZ	4.380	8.679	5.069	-						
HZ	2.762	2.354	3.848	2.044	-					
CZ	4.852	3.917	8.371	2.762	11.655	-				
NF	2.002	2.762	2.275	2.201	1.384	1.869	-			
FN	6.507	18.981	9.009	6.694	3.128	5.848	3.373	-		
SG	1.417	1.322	1.274	1.265	0.723	0.856	0.886	0.952	-	
TW	4.380	3.917	10.167	2.915	6.893	14.456	1.851	6.694	0.719	-

Table 5 Genetic diversity parameters of 10 LMB populations

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	9	172.343	0.297	16.23%
Inter-populations	290	385.917	0.000	0%
Within individuals	300	460.000	1.533	83.77%
Total	599	1018.260	1.830	100%

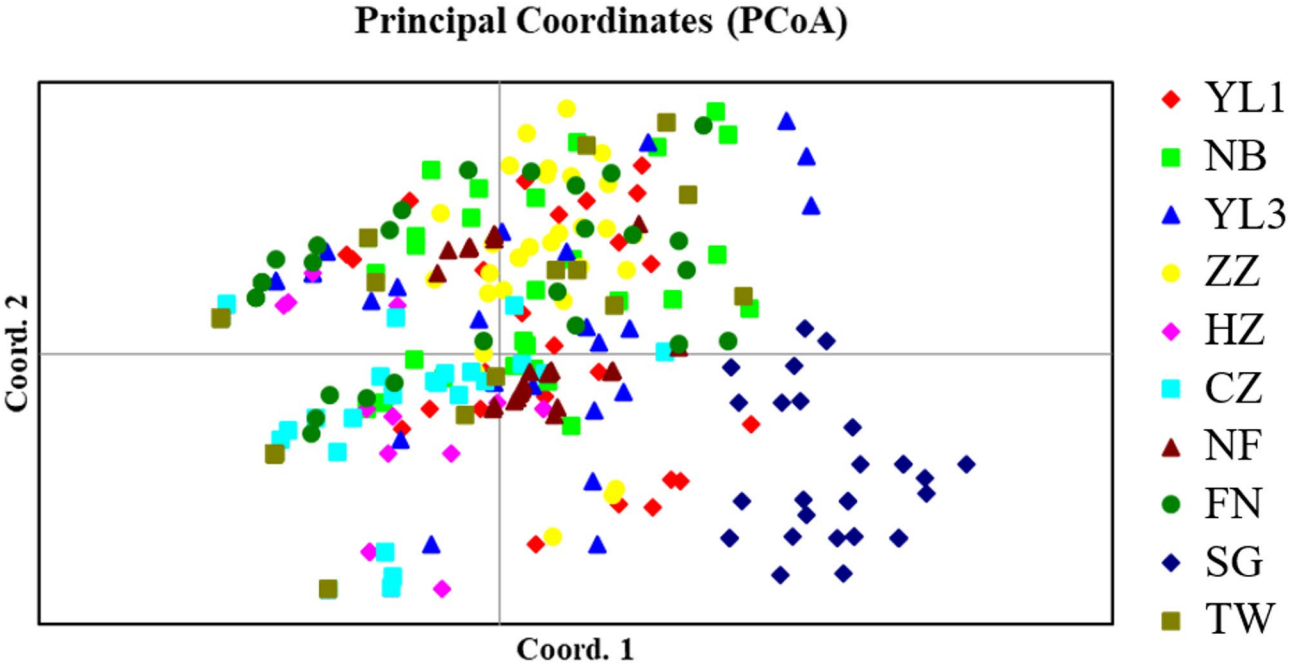

Fig. 3 PCoA of the 300 LMB individuals came from 10 different LMB populations

of 261 bp and 264 bp were found in the NF population. In the MiSaTPW76 locus, only a 253 bp fragment was detected in the SG population. Additionally, several unique fragments were identified solely in the two hybrid populations, including 201 bp in the Msa121 locus, 274 bp and 329 bp in the MiSaTPW12 locus, 268 bp in the MiSaTPW25 locus, and 272 bp and 280 bp in the MiSaTPW76 locus. However, distinguishing the YL1, HZ, CZ, and TW populations proved challenging due to their high similarity in the amplified fragments (Table 6).

Digital DNA fingerprints of the 10 LMB populations were generated based on using "1" to represent the presence and "0" to represent the absence of a band (Table S4). Furthermore, 25 individuals from five populations were used to test the accuracy of the digital DNA fingerprints. The digital DNA fingerprints of these individuals

Table 6 DNA fragments amplified by seven microsatellite markers in ten LMB populations

Locus	YL1	NB	YL3	ZZ	HZ	CZ	NF	FN	SG	TW
Lma120	192	192	192	192	192	192	192	192	192	192
	194	194	194	194			194	194		
							197			
		201								
	203	203	203	203	203	203	203	203	203	203
			207							
Mdo6	141	141	141	141		141		141	141	141
	147	147	147	147	147	147	147	147	147	147
		153		153			153	153		
		157					157	157		
Msal21	195	195	195		195	195	195	195		195
	197	197	197	197	197	197	197	197	197	197
								199		
							201	*201*		
		203	203	203			203	203		203
							207	207		207
MiSaTPW11	159	159	159	159	159	159	159	159		159
		163					163			
		167					167	167		
		171	171	171						
		175						175		
	183	183	183	183	183	183		183	183	183
				187						
		191	191	191		191			191	
		196								
				204						
MiSaTPW12	266	266	266	266	266	266	266	266		266
				270				270		
							274	*274*		
							317			
		319	319			319		319		
		321	321	321			321	321		
	323	323	323	323	323	323	323	323	323	323
							329	*329*		
	331	331	331	331	331	331	331	331	331	
		335	335							
			343							
MiSaTPW25							**261**			
							264			
							268	*268*		
	272	272	272	272	272	272	272	272	272	272
	276		276	276				276		
	280	280	280	280	280	280	280	280	280	280
MiSaTPW76	253	253	253	253	253	253	253	253	253	253
	256	256	256	256	256	256	256	256		256
	261	261	261	261	261	261		261		261
		264	264				264	264		
		268		268				268		
							272	*272*		
							280	*280*		
				287			287			

Note: Black bold fonts indicate specific fragments within a population, and Italic indicate bands that are characteristic in the hybridization offspring

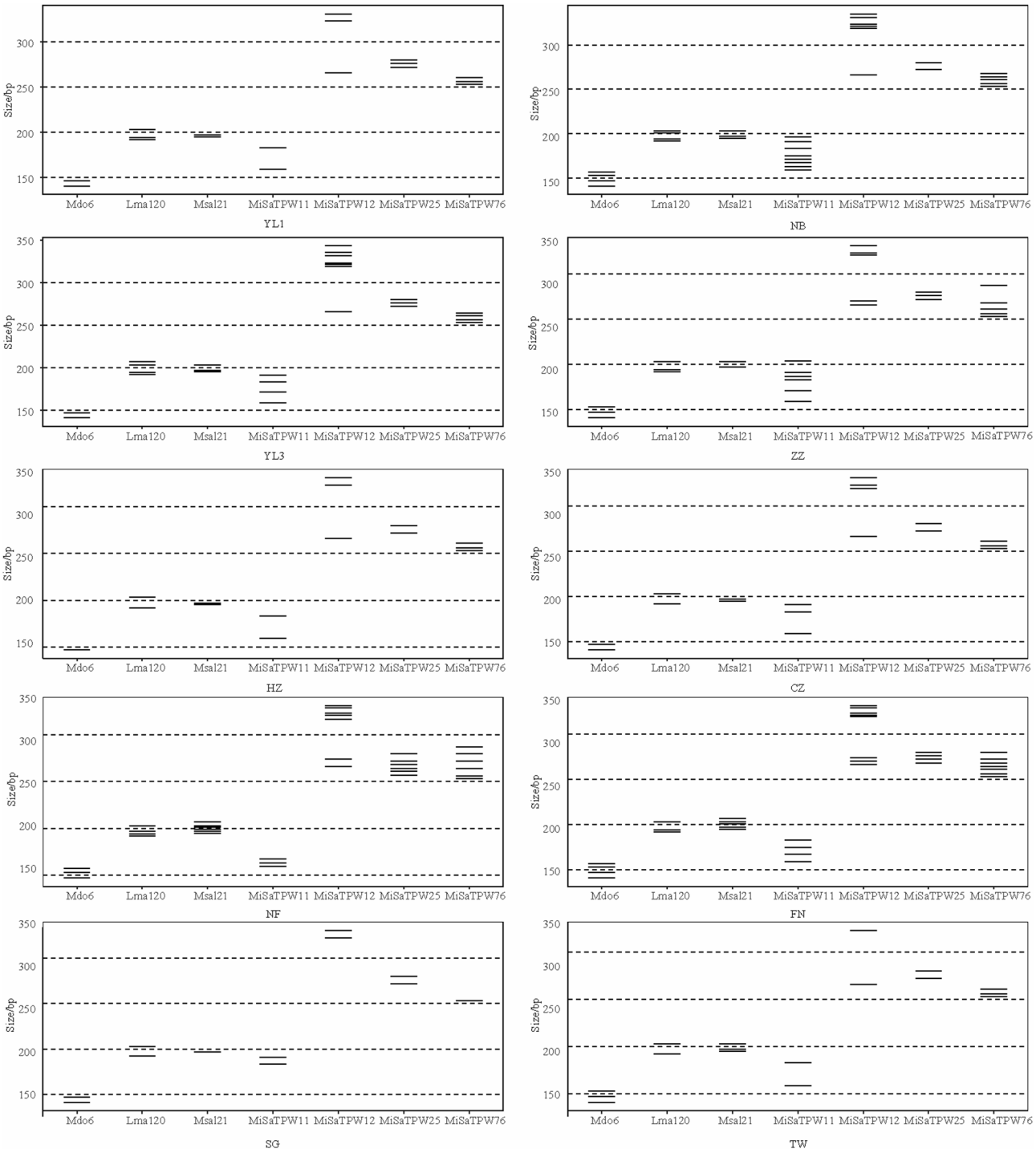

Fig. 4 DNA fingerprint patterns of 10 LMB populations with seven microsatellite markers

are shown in Table S5. The C-YL3, C-NB, and C-HY populations can be matched to the target population with the highest number of effective gene matches, respectively. Besides, C-HY-4 exhibited a unique fingerprint at the Msal21 locus with the sequence "011000," which was exclusively detected in the FN population. Similarly, C-NB-1 and C-NB-4 displayed unique fingerprints at the Mdo5 locus with the sequence "01010," which were only found in the NB population. Additionally, C-YL3-4 had a distinct fingerprint at the Lma120 locus with the sequence "000011," which was specifically identified in the YL3 population (Table S5). The C-YL1 population matches with YL3, NB, or FN populations with a high

Table 7 Matched results of five candidate LMB populations

Population	C-YL1 (1–5)	C-YL3 (1–5)	C-HY (1–5)	C-NB (1–5)	Unknown (1–5)
YL1	8.60	10.80	8.60	8.60	8.60
NB	*9.00*	11.20	9.60	*9.00*	*9.60*
YL3	*9.00*	*11.40*	8.60	8.60	9.00
ZZ	8.60	11.00	9.80	8.60	9.00
HZ	8.60	10.00	8.20	8.60	8.00
CZ	8.60	10.40	8.40	8.60	8.00
NF	8.60	9.00	8.60	7.60	8.60
FN	*9.00*	11.00	*10.00*	8.60	9.40
SG	6.80	8.00	6.40	6.40	6.20
TW	8.00	9.40	9.00	8.60	8.40
Identification	NB or YL3 or FN	YL3	FN	NB	NB

Note: The Italic indicates the population most likely to match

probability. The unknown population was eventually matched to the NB population (Table 7).

Discussion

Profile of the microsatellite alleles

Microsatellite markers have been extensively utilized to analyze genetic diversity and structure in various fish species, including black carp (*Mylopharyngodon piceus*) [29], bighead carp (*Hypophthalmichthys nobilis*) [30], dojo loach (*Misgurnus anguillicaudatus*) [31], and blunt snout bream (*Megalobrama amblycephala*) [32]. In LMB, multiple studies have reported on genetic diversity and population structure [13, 17, 33–35]. The effectiveness of microsatellites largely depends on the quality of the markers and the precision of the genotyping data. In a previous study, 42 alleles were identified using seven microsatellite markers with silver staining [17], while 53 alleles were detected using capillary electrophoresis, indicating that the latter method is more efficient and accurate.

The PIC is a crucial metric for evaluating the effectiveness of microsatellite markers. According to the formulation by Botstein et al. [36], six of the microsatellite markers (excluding Msal21) showed moderate to high polymorphism in this study, suggesting they are more informative and possess greater discriminatory power. Msal21 has been commonly used to differentiate between FB and NB populations in earlier researches [7, 17, 21]. To further distinguish between the different hybrid offsprings from the FB and YL3 populations, Msal21 was also chosen as a candidate marker in this study. Our findings revealed a broader PIC range (0.246 to 0.620) compared to previous studies, which reported values between 0.278 and 0.570 [4, 13, 14, 17, 34]. The lower PIC value observed may be linked to a decline in germplasm quality due to prolonged self-crossing [4], while the higher PIC value could be attributed to the incorporation of FB genetic information during hybridization [14].

Genetic diversity, population structure, phylogenetic tree, and population genetic variation analysis

Genetic diversity results from the long-term evolution of species or populations and serves as a crucial measure for assessing the breeding potential of germplasm [37]. This diversity is primarily indicated by metrics such as the number of alleles (*N*a) and the value of heterozygosity. Unlike *H*o, which is easily affected by population size, *H*e is solely determined by inheritance, making it a more reliable indicator of genetic diversity levels [38]. In this study, the *N*a and *H*e values for the NB population were found to be 4.714 and 0.509, respectively, the highest among the eight NLMB populations studied. These findings align with those of Su et al. [3] and Zhang et al. [34], suggesting that the NB population exhibits greater genetic diversity. The *H*e values for the YL1 and YL3 populations were 0.420 and 0.410, respectively, indicating that these populations possess moderate breeding potential.

The introduction of new germplasm resources has been extensively utilized to enhance the genetic diversity of LMB in China. For example, the YL3 was developed through selective breeding from the YL1 and NB populations, which had higher *H*o and *H*e compared to the YL1 population [13]. Additionally, the offspring of YL3 and FB populations was suggested to have higher *H*e than those from YL3 and NB populations, as well as the offspring of FB and NB populations [39]. In this study, similar to our previous research [14], we found that the NF and FN populations displayed higher *N*a, *N*e, I*, *H*o, and *H*e compared to the YL3 population. These findings suggest that hybridizing the YL3 and FB populations could be an effective strategy for enhancing the genetic diversity of LMB in China. Furthermore, our earlier findings indicate that the FB population possesses superior heat tolerance compared to YL3 [40], which could aid in developing LMB varieties with improved stress resistance.

Genetic differentiation among populations is typically assessed using genetic distance and the genetic differentiation index (*F*st). A higher genetic distance and *F*st value between populations indicates a greater level of genetic differentiation [38]. In this study, the lowest genetic distance and *F*st were found between YL1 and YL3 populations, suggesting they share a similar genetic background. This aligns with the breeding method for the YL3 variety, which was selectively bred from YL1 and re-imported NB populations. The second lowest genetic distance and *F*st were noted between CZ and HZ populations, indicating they also have a similar genetic background, possibly stemming from shared ancestry or similar parental lines. Furthermore, YL1, YL3, CZ, and HZ populations were grouped with the TW population, likely due to their common origins. It is known that LMB was first introduced from TW to Guangdong Province in

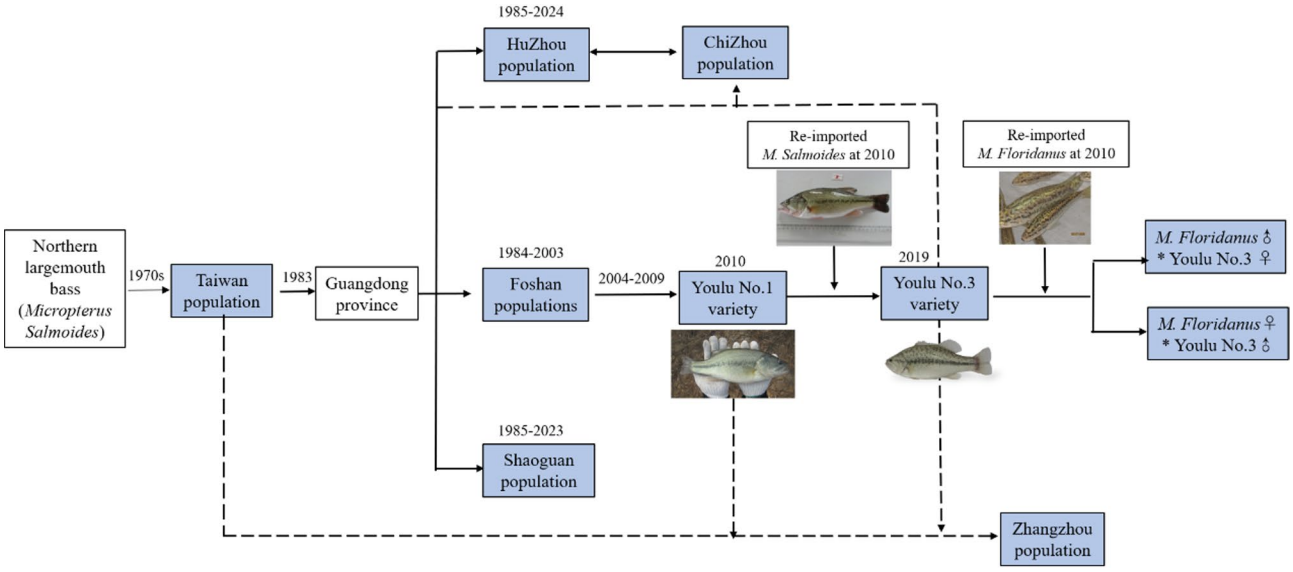

Fig. 5 History of LMB industry development and potential formation process of LMB in China. Note: The solid black line is the evolutionary process that can be accurately determined, and the dashed black line is the inferred evolutionary process

1983. Our sample collection revealed that the HZ population originated from a reservoir in Zhejiang Province, which was introduced from Guangdong around 1990. This long-term inbreeding in isolated waters has likely led to reduced genetic diversity. YL1 was selectively bred from four LMB populations in Foshan, which primarily descended from the TW population. Thus, YL1, YL3, CZ, and HZ populations are all descendants of the TW population, explaining their clustering with it. The SG population has been cultivated in a reservoir in Shaoguan since 1985, and its lower genetic diversity may be due to similar factors affecting CZ and HZ populations. However, SG exhibited a greater genetic distance and *F*st from other populations, likely due to differences in the original TW population. Reports suggest that 300 LMB from major reservoirs in northeastern Mexico likely represent four ancestral populations, indicating regional variations in the distribution of NLMB subspecies [41]. A previous study by Fan et al. also noted a significant genetic distance between LMB in China and re-imported NLMB, attributed to the wild distribution of the Northern subspecies in its native region [17]. Additionally, the lowest genetic distance and *F*st were found between FN and NB populations, with FN clustering more closely with NB than with NF, suggesting that the male parent may have a greater influence on offspring than the female. Finally, the *F*st values for ZZ compared to NB and YL3 were both below 0.05, indicating minimal genetic differentiation among the three populations. This implies that the ZZ population could potentially be a hybrid offspring of NB and YL3.

To better under the relationships between the 10 LMB populations, we conducted an analysis of the Number of effective migrants (*N*m). The findings indicated that the highest *N*m (18.981) occurred between the YL1 population and YL3 population, aligning with the clustering results. The second higher *N*m (14.456) was found between the TW and CZ populations, indicating that the CZ population may have originated from the TW population. Additionally, a high *N*m (8.371) was noted between the CZ and YL3 populations, suggesting that the CZ population might have a source of germplasm for YL3. The third higher *N*m (11.655) was observed between the CZ and HZ populations, which also matched the clustering results. The fourth higher *N*m (10.167) was between the YL1 and NB populations, implying that the TW population, introduced to mainland China in 1983, is similar to the reintroduced NB population brought back by our team in 2010. The *N*m values for ZZ compared to YL1, NB, and YL3 were 4.380, 8.679, and 5.069, respectively, suggesting that the ZZ population may have been developed from the YL3 population as well as the YL1 and NB populations. The *N*m values among FN and the NLMB populations were higher than those among NF and the NLMB populations, indicating that the male parent contributes more to the offspring than the female parent in LMB. Furthermore, AMOVA results indicated that only 16.23% of the variation was attributed to differences among populations, while 83.77% of the total variation was due to differences within individuals, highlighting that individual variation is the primary source of diversity among the LMB populations. The relationships among the ten populations are illustrated in Fig. 5.

Construction and application of DNA fingerprinting in LMB

LMB was first introduced to mainland China in 1983, but systematic research on the species began in 2004, focusing on areas such as genetic germplasm resources, quantitative genetics, and selective breeding [1]. In 2008, studies using microsatellites [10] and mitochondrial markers [9] revealed that the LMB in China is classified as the NLMB subspecies. By 2012, Fan et al. created a DNA fingerprinting for LMB based on four distinct populations, which included one cultured population from China, two re-imported FB populations, and one re-imported NB population [17]. In 2014, Li et al. identified SNP markers that could assess hybridization between NLMB and FLMB [42]. In 2022, our team developed three InDels markers and provided a straightforward PCR-based method to differentiate NLMB, FLMB, and their F1 progeny [2]. However, while these studies have concentrated on distinguishing germplasm between subspecies, there has been no research on identifying different populations within the same subspecies.

Since the breeding of YL1 at 2010, various cultured LMB populations have emerged across different regions over the past fourteen years. These populations belong to the NLMB subspecies and are difficult to differentiate based on morphological characteristics. Although a DNA fingerprint for LMB was established in 2012 [17], it only included one cultured NLMB population and requires further enhancement. This study selected seven microsatellite markers with clear bands and good reproducibility from a total of 15 markers [17]. Based on the 53 polymorphic loci, a new DNA fingerprint for LMB was created. The two hybrid populations were easily distinguishable from the eight NLMB populations. Notably, the NF population exhibited unique amplified sizes compared to others, such as 197 bp at the Lma120 locus, 199 bp at the Msal21 locus, and 270 and 274 bp at the MisaTPW12 locus. While the eight NLMB populations showed a high degree of similarity, some specific amplified DNA fragments were identified in certain populations. For instance, the YL3 population had a unique amplified size of 207 bp at the Lma120 locus and 343 bp at the MisaTPW12 locus; the NB population had a unique size of 201 bp at the Lma120 locus and 155 bp at the Mdo6 locus; and the SG population exhibited homozygous sites at the MisaTPW12 and MisaTPW76 loci, as well as lack of 264 bp at the MiSaTPW12 locus. However, it is hard to distinguish the YL1, HZ, CZ, and TW populations with the seven microsatellite markers. This result aligns with their origin, because of the absence of new germplasm additions and the stability of microsatellite markers, resulting in high similarity of these microsatellite markers in the four populations.

Furthermore, we created a new digital DNA fingerprint of the 10 LMB populations. Using the probability calculation model proposed by Wu et al. [43], we determined the resolution of this fingerprint map, revealing that the likelihood of two individuals having identical band patterns at 53 loci is $(1/2)^{53}$, or approximately $1.11\times e^{-16}$. This suggests that it is extremely unlikely for two individuals to share the same banding pattern in this fingerprint map, allowing for individual-level identification within a certain range. After analyzing the digital DNA fingerprints of 25 individuals from five candidate LMB populations, we ranked them based on the number of matched effective loci, with the highest match count serving and presence or absence of specially amplified sizes as the determining factors. The three known populations (C-YL3, C-NB, and C-HY) corresponded with the populations collected in this study, which met our expectations and demonstrated the high identification efficiency of the digital DNA fingerprint maps created. The candidate C-YL1 population matches with YL3, NB, or FN populations with a high probability, suggesting that it is hard to distinguish the YL1 population based on the microsatellite markers. Moreover, the unknown population was ultimately matched to the NB population. In conclusion, we established a method to distinguish the YL3, NB, ZZ, NF, FN and SG populations, which can serve as identifiers for individuals in the genetic breeding of LMB.

Conclusion

In this study, seven fluorescence-labeled microsatellite markers were utilized to assess genetic diversity and construct DNA fingerprints for 300 individuals from 10 different LMB populations. A total of 53 alleles were identified, surpassing the findings of a previous study [17], which indicates that the capillary electrophoresis method is more efficient and accurate. Among the populations, YL3 exhibited moderate genetic diversity, with observed heterozygosity (*Ho*), expected heterozygosity (*He*), and polymorphic information content (PIC) values of 0.357, 0.410, and 0.359, respectively. The NF and FN populations demonstrated higher values for *Ho*, *He*, and PIC, suggesting that hybridization may be a viable strategy to enhance genetic diversity in LMB. By comparing the DNA fragments produced by the seven microsatellite markers, specifically amplified fragments at several microsatellite loci were observed to distinguish the YL3, NB, ZZ, NF, FN, and SG populations, respectively. Furthermore, digital fingerprinting was conducted for the 10 different LMB populations. The verification results for five candidate populations indicated a high identification efficiency of the digital DNA fingerprint maps. Our study will contribute to the protection, identification, utilization, and breeding of LMB germplasm resources in the future.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11721-8.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Author contributions

J. D. conceptualized the project, acquired funding, designed, and conducted the experiments, analyzed the data, and wrote the manuscript. T. Z. helped in analyzed the data. T. T., H. S., C. L., and J. T. helped in collecting the samples, measuring the morphological traits. L. H. provided YL3 and NB germplasm resources, and helped to carry out the hybridization between YL3 and FLMB. S. L.* conceptualized the project, acquired funding, supervised the study, and modified the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets generated and/or analysed during the current study are available in the Supplement materials. The sequences of the 7 microsatellite markers used in this study are available at GenBank repository (https://www.ncbi.nlm.nih.gov/), under the accession number: PV607034 to PV607040.

Declarations

Ethics approval and consent to participate

The experiments involving LMB in this study were approved by the Animal Research and Ethics Committee of Pearl River Fisheries Research Institute, Chinese Academy of Sciences.

Competing interests

The authors declare no competing interests.

Conflict of interest

L. H. was employed by Guangdong Liangshi Aquatic Seed Industry Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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