Development and Characterization of 37 SNP Markers For The Largemouth Bass (*Micropterus Salmoides*) By Using PCR-RFLP Method

Zhou Jiang
Henan Normal University
Jiao Cui
Henan Normal University
Jiaqi Shao
Henan Normal University
Chuanju Dong
Henan Normal University
Jinxing Du
Chinese Academy of Fishery Sciences Pearl River Fisheries Research Institute
Yubang Shen
Shanghai Ocean University
Shengjie Li
Chinese Academy of Fishery Sciences Pearl River Fisheries Research Institute
Meng Zhang (zhangmeng@htu.edu.cn)
Henan Normal University https://orcid.org/0000-0002-7918-0811
Xuejun Li
Henan Normal University

Research Article

Keywords: Micropterus salmoides, SNP, PCR-RFLP, Conservation genetics

Posted Date: May 24th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-472661/v1

Abstract

Largemouth bass (*Micropterus salmoides*) is an economically important species in China. Contrary to its rapidly increasing yield during the last decades, the domestic genetic diversity of largemouth bass has gradually declined. For further rationally excavation and utilization of largemouth bass germplasm resources, 37 single nucleotide polymorphism (SNP) markers were developed based on genotyping-by-sequencing (GBS) data and characterized by genotyping 32 individuals using the PCR-RFLP method. The effective number of alleles (*Ne*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and polymorphic information content (*PIC*) of these SNPs ranged from 1.168 to 1.998, 0.156 to 0.844, 0.146 to 0.507, and 0.134 to 0.375, respectively. Totally, five loci deviated significantly from Hardy-Weinberg equilibrium ($p < 0.05$), while there existed no linkage disequilibrium at all loci. These novel polymorphic markers will lay the foundation for future population and conservation genetics of *M. salmoides*.

Main Text

Largemouth bass (*Micropterus salmoides*) is a wildly distributed and indigenous species in North American freshwaters, and can be considered a typical representative carnivorous fish (Chen et al. 2015; Gaeta et al. 2015). As famous for its superior growth rate and broad adaptability, largemouth bass has been introduced into Chinese mainland in the 1980s. Nowadays, the annual production of largemouth bass exceeds 500,000 tons, and its cultivation areas are almost all over China. Correspondingly, the conservation of its germplasm resources has gradually attracted the attention of researchers. It is worth mentioning that molecular markers about largemouth bass were mainly about simple sequence repeat (SSR) markers (Kubota et al. 2014), while few single nucleotide polymorphism (SNP) markers have been developed and reported, which may be caused by the high SNP-genotyping costs.

SNPs represent the most profuse form of genetic variations that can be wildly used in molecular marker-assisted selection (MAS) breeding (Qu et al. 2019). At present, the common SNP genotyping methods include direct sequencing, high resolution melting-curve (HRM) (Guo et al. 2018), denaturing high-performance liquid chromatography (DHPLC) (Wolford et al. 2000), kompetitive allele specific PCR (KASP) (Semagn et al. 2014), etc. However, due to the high requirements for instruments or high costs, most of the above genotyping methods are difficult to be widely used in aquatic animals. PCR-RFLP is a cheap and efficient SNP genotyping method with PCR technology as the core to improve the resolution of traditional RFLP, and this genotyping method has low requirements for instruments, which can be carried out in most laboratories. In addition, with the publication of largemouth bass genome (Sun et al. 2020), a large number of restriction enzyme sites could provide the basis for SNP mining and application, especially using PCR-RFLP genotyping method (Lozano-Duque et al. 2018).

Pectoral fins from 32 cultured largemouth bass (Zhoukou, Henan) were randomly sampled. Genomic DNA was extracted by “Rapid Animal Genomic DNA Isolation Kit” (Sangon Biotech (Shanghai) Co., Ltd.) and diluted to 50 ng/μL. According to our previous GBS data (unpublished), 37 SNP loci, which could be used for PCR-RFLP genotyping method, were randomly selected and primers were designed according to the flanking sequences (Electronic supplementary 1). The amplification reaction volume was 20 μL, which contained 10.0 μL of 2×Taq PCR Mastermix (Tiangen Biotech (Beijing) Co., Ltd.), 1 μL of primer pairs, 2 μL of genomic DNA and 7 μL of ddH_2O. The amplification parameters were as follows: an initial denaturation at 95 °C for 5 min; total of 35 cycles of denaturation at 95 °C for 30 s, annealing at 46.6-63 °C (Table 1) for 30 s, extension at 72 °C for 30 s; and finally extension at 72 °C for 5 min. Subsequently, PCR products were digested by specific enzyme at 37 °C for 45 min. The digestion system included 5 μL of PCR product, 1 μL of 10×SpeedyOne Buffer, 0.5 μL of SpeedyCut enzyme and 3.5 μL of ddH_2O. Finally, the digestion products were genotyped by 2% agarose gel electrophoresis.

The effective number of alleles (*Ne*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and polymorphic information content (*PIC*) of these SNP loci were analyzed by software Cervus 3.0. Results showed that the *Ne*, *Ho*, *He* and *PIC* of 37 SNP loci ranged from 1.168 to 1.998 (mean 1.794), 0.156 to 0.844 (mean 0.470), 0.146 to 0.507 (mean 0.443) and 0.134 to 0.375 (mean 0.338), respectively (Table 1). Results from software Popgen 32 showed that five SNP loci significantly deviated from Hardy–Weinberg equilibrium (Table 1, superscript with ‘*’), while there was no linkage disequilibrium at all loci. In summary, 37 SNP loci, of which 35 were moderate polymorphic, of largemouth bass were successfully developed and characterized by PCR-RFLP genotyping method. These polymorphic SNP loci could provide reference for the excavation and rational utilization of largemouth bass germplasm resources.

Declarations

Funding

This study was supported by the Ph.D. Foundation of Henan Normal University (qd19067); the Open Funding Project of the Key Laboratory of Freshwater Aquatic Genetic Resources, Ministry of Agriculture and Rural Affairs (KF-2021-03); and the Central Public-interest Scientific Institution Basal Research Fund (CAFS-2021SJ-CG1).

Conflicts of interest

The authors have no conflicts of interest to declare.

Availability of data and material

Provided as supporting information.

Authors' contributions

Meng Zhang and Xuejun Li conceived the project and designed the scientific objectives. Zhou Jiang, Jiao Cui and Jiaqi Shao collected and prepared the fish samples. Jinxing Du and Zhou Jiang conducted bioinformatics analysis. Meng Zhang, Zhou Jiang and Chuanju Dong prepared the manuscript. Shengjie Li, Yubang Shen and Xuejun Li revised the manuscript. All authors have read and approved the final manuscript.

Ethics statement

This study was conducted under the permission of the Animal Conservation and Utilization Committee of Fisheries College of Henan Normal University. Largemouth bass were treated appropriately to minimize suffering.

Informed consent

The authors provide consent to participate. The authors provide consent for publication.

References

1. Chen YJ, Yuan RM, Liu YJ, Yang HJ, Liang GY, Tian LX (2015) Dietary vitamin C requirement and its effects on tissue antioxidant capacity of juvenile largemouth bass, *Micropterus salmoides*. Aquaculture 435:431-436. https://doi.org/10.1016/j.aquaculture.2014.10.013
2. Gaeta JW, Guarascio MJ, Sass GG, Carpenter SR (2011) Lakeshore residential development and growth of largemouth bass (*Micropterus salmoides*): A cross-lakes comparison. Ecology of Freshwater Fish 20:92-101. https://doi.org/10.1111/j.1600-0633.2010.00464.x
3. Guo YM, Zeng WW, Wang Q, Wang YY, Li YY, Yin JY, Ren Y, Shi CB (2018) Use of high-resolution melting curve analysis to differentiate vaccine and wild type strains of grass carp reovirus genotype II. Journal of Virological Methods 256:111-115. https://doi.org/10.1016/j.jviromet.2018.03.006
4. Kubota S, Qi L, Kessuwan K, Okamoto N, Sakamoto T, Nakamura Y, Shigenobu Y, Sugaya T, Sano M, Uji S, Nomura K, Ozaki A (2014) High-throughput simple sequence repeat (SSR) markers development for the kelp grouper (*Epinephelus bruneus*) and cross-species amplifications for Epinephelinae species. Advances in Bioscience and Biotechnology 5:117-130. https://doi.org/10.4236/abb.2014.52016
5. Lozano-Duque Y, Richlen ML, Smith TB, Anderson DM, Erdner DL (2018) Development and validation of PCR-RFLP assay for identification of *Gambierdiscus* species in the Greater Caribbean Region. Journal of Applied Phycology 30:3529-3540. https://doi.org/10.1007/s10811-018-1491-5
6. Qu JY, Wang SS, Cui YT, Guo XY, Wang YH (2019) Development and characterization of 26 SNP markers in *Nipponacmea schrenckii*. Conservation Genetics Resources 12:195-198. https://link.springer.com/article/10.1007%2Fs12686-019-01095-x
7. Sun CF, Li j, Dong JJ, Niu YC, Hu J, Lian JM, Li WH, Li J, Tian YY, Shi Q, Ye X (2020) Chromosome-level genome assembly for the largemouth bass *Micropterus salmoides* provides insights into adaptation to fresh and brackish water. Molecular Ecology Resources 21:301-315. https://doi.org/10.1111/1755-0998.13256
8. Semagn K, Babu R, Hearne S, Olsen M (2014) Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. Molecular Breeding 33:1-14. https://doi.org/10.1007/s11032-013-9917-x
9. Wolford J, Blunt D, Ballecer C, Prochazka M (2000) High-throughput SNP detection by using DNA pooling and denaturing high performance liquid chromatography (DHPLC). Human Genetics 107(5):483-487. https://doi.org/10.1007/s004390000396

Tables

Table 1
Characterization of 37 SNP markers in largemouth bass, Micropterus salmoides

Locus	SNP type	Primer sequences(5′-3′)		Ta (°C)	Size (bp)	Restriction enzyme	*Ne*	*Ho*	*He*	*PIC*
		Forward	Reverse							
Msa01	C/A	CAGCCACCGTGAAGAACTA	TTTCTAGGTGATTGCGTTATG	54.0	720	EcoRI	1.853	0.594	0.468	0.354
Msa02	T/A	GCAGATTGGGTCTAATGTGG	GGTGTATGCTTGCCCTTG	54.0	681	EcoRI	1.952	0.844	0.496	0.369
Msa03	A/G	CAGGCCAGGGCTCTACACTA	TGGACCACTTCACCTTCACTCTA	63.0	900	EcoRI	1.853	0.594	0.468	0.354
Msa04	A/T	CAGATCCAGTCTCCAGGACTAATA	TGGGTAGCAGCCTTGTCG	51.5	305	HinfI	1.998	0.531	0.507	0.375
Msa05	T/G	CAAAAGGCAACCAAAGTGA	CACTGTGGGATGTTCTGTTCTA	52.0	703	EcoRI	1.822	0.500	0.458	0.349
Msa06	G/T	TACCCTGTCATACCACCCA	CAGCCAGCCTCTTTAGCA	55.0	551	EcoRI	1.600	0.375	0.381	0.305
Msa07	C/T	TCTGACAGTCTGGGTTTCG	CCAATCTCCTCCTGGTTTT	51.0	700	EcoRI	1.679	0.563	0.411	0.323
Msa08	C/T	GCTGTCATCTCCCTTGAA	AGTAGTAGGGCACAGCAAT	53.0	704	EcoRI	1.789	0.656	0.448	0.344
Msa09	A/T	TTCAGCGAAAGTGAGCAA	AAGTGATACGGAGATGCTGT	50.2	265	EcoRI	1.398	0.281	0.289	0.244
Msa10	A/C	TGTTCGCAGTCTTCTTTCC	GAGCCCAACTCCTTATCC	53.5	885	EcoRI	1.753	0.438	0.437	0.337
Msa11	C/T	TGCTCCCGATTGGTAGATAG	AACCAGCGGCATCATAGTC	51.8	527	BamHI	1.600	0.188	0.381	0.305
Msa12	C/T	GGTGATAAACCCACTGTATGC	CTTGGATGTGCTGCTCAAT	52.0	831	EcoRI	1.998	0.531	0.507	0.375
Msa13	A/C	ACTCACAGCCTTCATGTCGA	GCCACCCTACCAGGTCTGT	57.0	591	EcoRI	1.952	0.469	0.496	0.369
Msa14	C/T	TCAGCATTTCCCTGTTTGTAG	CTGCCACTCGGAGGATGA	54.0	685	EcoRI	1.853	0.281	0.468	0.354
Msa15	A/G	GACCTGTGGGACACTTGAT	AATAAACACTGGCTAAACTTCT	56.0	572	EcoRI	1.600	0.500	0.381	0.305
Msa16	T/C	GTTTAATGCGTATCTTAGACAGC	CCTGAGGACCTAACTGGAAG	52.0	525	EcoRI	1.789	0.594	0.448	0.344
Msa17	T/A	CAACTAAAGGAAAGGCTGATT	GGAAGACGAGAAACACGAAA	49.7	230	PvuII	1.882	0.563	0.476	0.359
Msa18	C/A	GGGGAAGGAACAGAAGCA	GACCACAACTAACAATTACTTTCG	48.9	417	HindIII	1.909	0.344	0.483	0.363
Msa19	C/T	ATCTTCTATCAAGTCGCCTCC	GACACGAGCTGGGCTTATC	55.0	539	EcoRI	1.519	0.375	0.347	0.283
Msa20	A/G	TAAAGTGTAGTTTGTACCCTTACTG	GCAGTAGGTGGCGGTAGA	54.0	635	EcoRI	1.998	0.406	0.507	0.375
Msa21	A/G	TGCTAATGATGAACCCACG	CAACGAATAGCCAATACCG	62.0	376	EcoRI	1.822	0.500	0.458	0.349
Msa22	C/A	GGGAACATACAAAGGTAAAGATT	CCTGGCGTGACATTAGCA	52.0	718	EcoRI	1.789	0.469	0.448	0.344
Msa23	G/A	TCGGGAAATCCGTGTTGA	CAGGTTAAACTTTGTTCCTGGTC	46.6	358	XhoI	1.969	0.625	0.500	0.371
Msa24	G/A	GCAGACTATGCTGGATGTGG	AGTGGTCATCTCACGCACC	58.0	506	EcoRI	1.789	0.406	0.448	0.344
Msa25	C/A	GGTGTCAGAGTGAATGGGTAAG	TCACTGGCAACTGAAAGGAG	58.0	511	EcoRI	1.717	0.469	0.424	0.330
Msa26	A/C	CACTTACTTGGCAAAGAATGA	CATCTTGAATGCTGCCCTA	50.0	374	EcoRI	1.932	0.375	0.490	0.366
Msa27	A/T	TAATAACCTGCCTCTGTGCT	ATCGTCAACCGCTTATCC	52.5	453	EcoRI	1.998	0.469	0.507	0.375
Msa28	A/T	CCTTTCCCTCCAGGCTTTC	CAGATGAAGTGTTTGACGAGGA	54.0	791	EcoRI	1.992	0.625	0.506	0.374
Msa29	A/T	GGGTTCAGGACCACAGAC	ATTGAAGCAACTGCCATG	49.0	556	EcoRI	1.909	0.594	0.484	0.363
Msa30	G/C	CTTCATGCAGTTGGGTATT	AATACTTATGTTTGCCCTTG	50.0	648	PvuII	1.909	0.531	0.484	0.363
Msa31	C/T	GATAGGACTAGGGTTTAAGAGG	TTTGCTGCTCTTTCTTGGT	50.0	352	EcoRV	1.168	0.156	0.146	0.134
Msa32	A/G	TAAGAAGCGTGGTGATGC	AAGGGAATAATACAGCAAGTC	54.0	505	EcoRI	1.952	0.594	0.496	0.369
Msa33	T/C	AATGTGAAATGGGTTGCT	TGAACAGGGATGTAGGAAT	47.7	476	PstI	1.717	0.281	0.424	0.330
Msa34	G/A	ATGTGAAGTTAGCGAAGCC	CAGAAAGTCTACTGTTGTGGGT	53.0	564	EcoRI	1.789	0.344	0.448	0.344
Msa35	C/A	GTCAGTACCCGCTCATTATATGT	GCAACTTCCTTCCCACTTTCT	49.5	661	PstI	1.932	0.500	0.490	0.366
Msa36	C/A	ATCTCCCACGCCAAGTCA	AAAATCCAAGTGCGGTCTG	51.0	376	XbaI	1.717	0.531	0.424	0.330
Msa37	G/T	TGACTGCTGGACTACTCGC	GGCTAGATTCTGCTCCGATA	51.0	569	XbaI	1.600	0.500	0.381	0.305

Notes: Effective number of alleles (*Ne*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), polymorphic information content (*PIC*) and probability for Hardy–Weinberg equilibrium tests (P_{HW}).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Electronicsupplementary1.docx