The Israeli Journal of Aquaculture IJA.73.2021.1496554, 8 pages
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Development and characterization of 20 microsatellite markers in spotted sea bass (*Lateolabrax maculatus*) and cross-amplification in related species

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Key words: Spotted Sea bass, *Lateolabrax maculatus*, largemouth bass, *Micropterus salmoides*, Microsatellite, Cross-species amplification

Abstract

The spotted sea bass (*Lateolabrax maculatus*) is an economically valuable cultured fish species in China. In this study, 20 novel polymorphic microsatellite loci of *L. maculatus* were isolated from genomic data and characterized using 40 wild individuals. The number of alleles and the effective number of alleles ranged from 2 to 12 (average of 5.1000) and from 1.180 to 8.000 (average of 3.3097). The observed and expected heterozygosities ranged from 0.083 to 0.875 (average of 0.4405) and from 0.153 to 0.875 (average of 0.5633), respectively. Deviation from the Hardy-Weinberg equilibrium was observed in 11 loci ($P < 0.05$). Polymorphism information content ranged from 0.141 to 0.862 (average of 0.5265) and most loci were heterozygous. Cross-amplification trials in two cryptic congeneric species, the largemouth bass, *Micropterus salmoides*, and the barramundi, *Lates calcarifer*, achieved successful amplification of 16 primers. The microsatellite markers developed in this study could be used for research on genetic breeding of *L. maculatus* and genetic relationships among tested taxa.

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Introduction

The spotted sea bass (*Lateolabrax maculatus*), which belongs to the family Moronidae (Perciformes), is widely distributed along the coast of China, reaching south to the borders of Vietnam and north to Korea (Wang et al., 2016). *L. maculatus* is an economically important cultured fish species in China (Shao et al., 2018), with an annual production of 18.02 thousand tons in 2019 (Bureau of Fisheries of Ministry of Agriculture, PRC, 2020). It is characterized by many black dots along the lateral body region and is found along inshore rocky reefs and estuaries, with limited movement to deeper marine water except during spawning and cold winters (Chen et al., 2019). The natural population has been continuously declined for two decades due to overfishing (An et al., 2013). Thus, establishing broodstock sampled from wild populations is necessary for the effective long-term management and sustainability of this species.

Microsatellites are polymorphic molecular markers (1–6 bp in length) found in simple tandem repeat sequences (Dhyani et al., 2020). These markers are widely used to analyze fish's genetic background and population structure (Kapoor et al., 2020; YU et al., 2021; Wang et al., 2020). The use of next-generation sequencing in non-model fish species has also proven to help discover in discovering molecular markers (Patel et al., 2016; Du et al., 2018; Ariede et al., 2017; MA et al., 2020; PENG et al., 2021; Jiang et al., 2014). To the best of our knowledge, however, only a few microsatellite markers have been developed for *L. maculatus* (An et al., 2013; Zhang et al., 2016; An et al., 2014; HUANG et al., 2021; Shao et al., 2009), which is insufficient for accurate population structure and parentage analyses.

Here, we developed 20 novel whole-genome-derived microsatellite markers for *L. maculatus* and tested cross-species amplification in two cryptic congeneric species (largemouth bass, *Micropterus salmoides* and barramundi, *Lates calcarifer*). Therefore, this study provides critical molecular resources for *L. maculatus*.

Materials and Methods

A total of 46 *L. maculatus* individuals were sampled in Xiamen, Fujian Province, China. Genomic DNA was extracted using a TIANamp Marine Animals DNA Kit (Tiangen, Beijing, China). Total DNA quality and concentration were measured using agarose gel (1%) electrophoresis and spectrophotometry (NanoDrop™2000, Thermo Fisher Scientific, USA), respectively .

The genome sequences of *L. maculatus* were obtained from the NCBI Sequence Read Archive and GenBank (accession number PRJNA407434). Microsatellite loci were identified with SSRHunter v.1.3 (Li and Wan, 2005). The parameters for screening microsatellite loci included: dinucleotides with repeats motifs, repeat times ≥ 6; other repeat motifs (e.g., tri-, quad-, penta- and hexa-nucleotides with repeat motifs), repeat times ≥ 5; compound microsatellites, interval between two repeats motifs < 100 nt. Simple sequence repeat (SSR)-containing sequences with sufficient flanking sequences (no less than 150 bp) were selected for characterization. The primers were designed by Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized by Sangon Biotech Co., Ltd (Shanghai, China).

The primers were verified by polymerase chain reaction (PCR) with two *L. maculatus* individuals. PCR was performed in 20-μL reactions containing 50 ng genomic DNA, 1 U ExTaq (Takara, Japan), 2 μL 10 × ExTaq Buffer, 0.2 μM each primer, 0.2 mM dNTPs, and 14 μL double-distilled water. The PCR conditions were: 5 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 50 °C for 30 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 7 min. PCR was performed using a T100 Thermal Cycler (BioRad, USA). PCR products were examined with electrophoresis on 8% non-denaturing polyacrylamide gel (180 V, 150 min) and visualized with silver staining. Successfully amplified primers were further characterized with 46 *L. maculatus* individuals. PCR amplification and profiles were the same as above .

Number of alleles (Na), effective number of alleles (Ne), expected heterozygosity (He), and observed heterozygosity (Ho) were determined using GenAlex 6.5.1 (Peakall and

Smouse, 2006). Polymorphic information content (PIC) and the Hardy-Weinberg Equilibrium (HWE) were calculated by Cervus v3.0.7 (Kalinowski et al., 2007.(
Cross-species transferability of the polymorphic markers was tested in *M. salmoides*, and *L. calcarifer.* DNA extraction and PCR amplification conditions were as described above. Loci with at least one band of the expected size were considered transferable.

Results

A total of 20 polymorphic microsatellite loci were isolated from the *L. maculatus* genome sequences and characterized in this study (**Table 1**). Results showed that Na per locus ranged from 2 to 12 (average of 5.1000); Ne ranged from 1.180 (Lm16–281) to 8.000 (Lm16–260) (average of 3.3097); and Ho and He ranged from 0.083 (Lm16–270) to 0.875 (Lm16–260) (average of 0.4405) and 0.153 (Lm16–280) to 0.875 (Lm16–260) (average of 0.5633), respectively. PIC ranged from 0.141 to 0.862 (average of 0.5265). Seven loci deviated from the HWE after Bonferroni correction ($P < 0.0025$), which may be due to the presence of null alleles or the small sample size. According to the criteria proposed by Botstein and co-workers (1980), 11 loci presented a high level of informativeness for polymorphism (PIC > 0.5). Nine microsatellite markers were amplified successfully in *M. salmoides*, with a transferability rate of 45.00% (**Table 2**). In addition, 16 microsatellite markers were amplified successfully in *L. calcarifer*, with a high transferability rate of 75% (**Table 2**).

Discussion

SSR locus screening from published whole-genome data is a low-cost, time-saving, and highly efficient method. The draft genomes of 30 marine animals were examined in previous research, resulting in the identification of more than a million genomic SSRs (Jiang et al., 2014). In this study, we developed 20 polymorphic microsatellite loci of *L. maculatus*, 11 of which showed high polymorphism (PIC > 0.5), as per Botstein et al. (1980). The transferability rate of *L. maculatus* SSR loci is reported to be 3.8%–11.5% across 10 species from the Latidae and Serranidae families (Zhang et al., 2016). Thus, cross-species transferability in this study was higher than that in the previous study.

In conclusion, 20 novel SSR markers were identified and chartered from published genomic sequences of *L. maculatus*. These microsatellite loci should facilitate studies on population genetics as well as the genetic breeding of *L. maculatus*.

Acknowledgements

Funding for this research was supported by the Central Public-interest Scientific Institution Basal Research Fund, CAFS (NO. 2021SD11, 2020TD21), the National Natural Science Foundation of China, China (NO.31802281), Rural Science and Technology Correspondent Fund of Guangzhou （20212100051）, and the Guangzhou Municipal Science and Technology Project (NO. 201906010028).

Table 1 Characteristics of 20 novel polymorphic microsatellite loci in spotted sea bass (*L. maculatus*)

Locus	Repeat motif	Primer sequence (5′–3′)	Range (bp)	*Na*	*Ne*	*Ho*	*He*	*PIC*	Accession number
Lm2-1091	$(CA)_9$	F:CTGAGTCTGGGTATCTGAAGCATT R:TTAAGTGTGTATGAAATAGGCGGC	189-221	4	2.749	0.583	0.636	0.592	MW556445
Lm2-7539	$(AC)_{17}$	F:TACTAGAGTACGTGTTTACCTGCC R:AAACATAACCTAACAAGCAGAGCG	295-307	5	2.977	0.417	0.664	0.604	MW556446
Lm2-7554*	$(TG)_{16}$	F:TGTGCAGACCCATCCTAAAATACA R:AGGCCTGATGACTTTCTGATTACA	117-125	4	2.018	0.333	0.504	0.458	MW556447
Lm2-7557	$(AC)_{15}$	F:ATGCTTTGTGTATGCTTGACCATG R:TCAAGTAGCAGCAGACATATGGAA	159-171	3	1.598	0.208	0.374	0.336	MW556448
Lm3-103*	$(AC)_{21}aa(AC)_{10}$	F:AGAGGGATAGATACACACTGGAGT R:TAGAGAAAGACAGGTTAGACGAGC	160-164	3	1.724	0.143	0.420	0.475	MW556449
Lm16-221*	$(AC)_{20}$	F:CAGAGAACCAACACTTTGCTACAG R:TCCCTTCAAACTGATGCATCCTAA	129-165	9	6.221	0.718	0.839	0.828	MW556450
Lm16-245	$(TGA)_6$	F:GGCACTGGAAATCAAACACAATCA R:AAACAGTAGAAACCGTCAGAGCTC	197-209	2	1.969	0.575	0.492	0.371	MW556451
Lm16-249	$(GT)_6ta(GT)_8$	F:TTTTGTCGCCCATCTGTGATTTAC R:GAGATGCTGCTTTTGGTTTGATCT	273-299	4	1.186	0.167	0.157	0.153	MW556452
Lm16-254	$(AC)_{12}$	F:TGCTCACCATTCACACAGTAGAAA R:TTTATCACTTTACCCTGCAGCAAC	253-273	6	3.646	0.500	0.726	0.683	MW556453

Locus	Repeat motif	Primer sequence (5′–3′)	Range (bp)	*Na*	*Ne*	*Ho*	*He*	*PIC*	Accession number
Lm16-260	$(ATG)_{13}$	F:CAGTAAAAGTAGCAAAACCACCAAG R:TCCATCAGCAGGTGTTTATCT	282-336	10	8.000	0.875	0.875	0.862	MW556454
Lm16-264	$(TTA)_7$	F:CTGGATTTCTCATCGACAGTCTGA R:GACCCTCGTGTTATTGAAACCTTC	210-222	2	1.653	0.208	0.395	0.317	MW556455
Lm16-267	$(AAT)_7$	F:GACTGTTGATTCCTCTCTGACTGT R:GTGCTTTGTAACTTGTTTGGGA	104-122	5	3.611	0.417	0.723	0.676	MW556456
Lm16-269	$(AC)_{21}$	F:TGACCTGCTTACTTACAACCTTCA R: AAGCTGTCATATCACCTCCATCTC	215-263	10	4.331	0.833	0.823	0.801	MW556457
Lm16-270*	$(AGG)_7$	F:GACAAAGGGAGGAAATGGAAACTG R:GTGCGTCAACATCTCATATCTTCC	166-178	2	5.647	0.083	0.330	0.275	MW556458
Lm16-273	$(CA)_{19}$	F:AAAATCGGGGTCAGACATTTGTTC R:CACGAGACTAACCTTAACCGTACA	215-251	12	1.492	0.792	0.867	0.853	MW556459
Lm16-277*	$(CA)_{12}$	F:GACTCTGTTAGCCTCCCTTACTCT R:AGATGGATTCAGAGAACAACGAGT	141-169	6	7.529	0.500	0.721	0.684	MW556460
Lm16-280	$(TG)_{10}$	F:TTACTGAGATGGACTGCTGTTGAA R:TATACCCTGTTCATCGTGACAGTG	272-276	2	3.589	0.167	0.153	0.141	MW556461
Lm16-281	$(GT)_{23}$	F:TTCCCAGATTTTCCATGAATTGCC R:TTTTGGGGAAATATTGAGCATCCG	268-290	6	1.180	0.833	0.731	0.691	MW556462
Lm16-283*	$(CA)_{13}$	F:CTTCCAGCCATCCATTTCTCTA R:CTGCGTTTGGTGCTGCTATTAATA	259-295	4	3.716	0.208	0.263	0.252	MW556463

Locus	Repeat motif	Primer sequence (5′–3′)	Range (bp)	*Na*	*Ne*	*Ho*	*He*	*PIC*	Accession number
Lm16-285*	$(TTGG)_5$	F:GGCTTTGAAATGTGGCTAAGACTT R:GAGGACGCTGATGAAAATGTTTGA	189-223	3	1.357	0.250	0.572	0.478	MW556464
Mean				5.1000	3.3097	0.4405	0.5633	0.5265	

N, number of individuals; ***Na***, number of alleles; ***Ne***, effective number of alleles; ***Ho***, observed heterozygosity; ***He***, expected heterozygosity; ***PIC***, polymorphism information content; * departure from Hardy-Weinberg equilibrium after Bonferroni's correction ($P < 0.0025$).

Table 2 Cross-species amplification of 20 microsatellite markers in two cryptic congeneric species

Locus	*Micropterus salmoides*	*Lates calcarifer*
Lm2-1091	-	-
Lm2-7539	-	+
Lm2-7554	-	+ +
Lm2-7557	-	+
Lm3-103	+	-
Lm16-221	-	-
Lm16-245	-	+
Lm16-249	+	+
Lm16-254	-	+
Lm16-260	+	+
Lm16-264	-	+
Lm16-267	+	+
Lm16-269	+	+
Lm16-270	+	+ +
Lm16-273	-	-
Lm16-277	+	+
Lm16-280	-	+
Lm16-281	-	-
Lm16-283	+	+ +
Lm16-285	+	+

− primer did not amplify; + primer amplified but was monomorphic; + + primer amplified and was polymorphic.

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