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Isolation and characterization of novel polymorphic microsatellite markers for *Lutjanus erythropterus*

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ABSTRACT. We isolated and characterized 22 polymorphic microsatellite loci in the crimson snapper (*Lutjanus erythropterus*) using a $(GT)_{13}$-enriched genomic library. We found three to 15 alleles per locus, with a mean of 6.68. The observed and expected heterozygosities ranged from 0.087 to 0.978 and from 0.125 to 0.904, respectively, with averages of 0.576 and 0.650, respectively. Only three loci showed significant deviation from the Hardy-Weinberg equilibrium after Bonferroni correction. Four loci showed evidence for null alleles. These markers will be useful for analyzing the population genetic structure and gene flow of *L. erythropterus*.

Key words: *Lutjanus erythropterus*; Crimson snapper; Microsatellite marker; Population structure

INTRODUCTION

Crimson snapper, *Lutjanus erythropterus*, is a pelagic fish that is widely distributed throughout the Indian Ocean and the subtropical and tropical parts of the western Pacific Ocean. This species is an important fish resource in China. Recent data collected within the past three decades indicate that the stock density and yield of *L. erythropterus* have dramatically declined (Guo et al., 2011). The conservation and sustainable utilization of *L. erythropterus* resources in Chinese coastal waters has drawn the attention of regulatory authorities. An evaluation of the population genetic structure is critical for making informed conservation and management decisions for this species. Microsatellites are important tools for examining genetic diversity and population genetic structure. Here, we report the development of 22 novel polymorphic microsatellite markers for *L. erythropterus* that will improve the ability to detect population genetic structure and gene flow of *L. erythropterus*.

MATERIAL AND METHODS

Fifty fishes were collected from the sea around Sanya, China. The samples were preserved at -20°C until DNA extraction. A dinucleotide-enriched genomic library was constructed following a previously published method (Ma and Chen, 2009). In brief, DNA was extracted from muscle tissue and digested with the restriction enzyme *Mse*I (New England Biolabs, USA). The digested DNA fragments were ligated to *Mse*I adaptor pair (5'-TACTCAGGACTCAT-3'/5'-GACGATGAGTCCTGAG-3'). The ligated products were then pre-amplified in a 25-μL reaction mixture using the adapter specific primer (5'-GATGAGTCCTGAGTAA-3') to verify successful ligation and increase DNA concentration. A biotin-labeled $(GT)_{13}$ probe was applied to hybridize with the pre-amplification products. The hybridized complexes were captured using streptavidin-coated magnetic beads (Promega, USA) and then eluted, forming a library of GT-rich DNA fragments.

Subsequently, the library DNA was amplified using the adapter-specific primer and ligated into pMD 18-T plasmid vectors (TaKaRa, Japan), and *Escherichia coli* DH5α competent cells were transformed with these plasmid vectors. The positive clones were randomly sequenced using an ABI Prism 3730 automated DNA sequencer (Applied Biosystems, USA). Microsatellite repeats were found in 62 sequenced clones. Primer pairs were designed for 53 of the microsatellite loci with suitable flanking regions for amplification by polymerase chain reaction (PCR) using the Primer Premier 5 software (PREMIER Biosoft International, USA).

The designed primer pairs were evaluated using 50 individuals of *L. erythropterus*. PCR was performed on a Veriti Thermal Cycler (Applied Biosystems, USA) in a total reaction volume of 25 μL, containing 0.4 μM primer (each), 0.2 mM dNTP (each), 1X PCR buffer, 2 mM $MgCl_2$, 1 U Taq polymerase (TaKaRa, Japan), and 10-100 ng DNA. The amplification profile consisted of an initial denaturing step of 94°C for 5 min, 35 cycles of 45 s at 94°C, 50 s at the locus-specific annealing temperature, and 45 s at 72°C, followed by a final step of 72°C for 10 min. The PCR products were separated on 6% denaturing polyacrylamide gel and visualized by silver staining. Observed and expected values of heterozygosity as well as deviation from the Hardy-Weinberg equilibrium were calculated using GENEPOP 4.0 (Rousset, 2007). Null allele frequencies (Brookfield, 1996) were estimated using MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004). All P values were corrected for multiple testing using Bonferroni correction (Rice, 1989).

RESULTS AND DISCUSSION

Twenty-two of 53 loci were cleanly amplified and shown to be polymorphic. The number of alleles per locus ranged from three to 15 with an average of 6.68 (Table 1). The observed and expected heterozygosities ranged from 0.087 to 0.978 and from 0.125 to 0.904, respectively, with averages of 0.576 and 0.650, respectively (Table 1). Only loci LE76, LE88, and LE107 showed significant deviation from Hardy-Weinberg proportions after Bonferroni correction ($P < 0.0023$). Four loci (LE76, LE88, LE95, and LE107) showed evidence of null alleles (estimated null allele frequency >5%).

Table 1. Characteristics of 22 microsatellite loci in *Lutjanus erythropterus*.

Locus	Primer sequence (5'-3')	Repeat motif	Ta (°C)	Allele size range (bp)	N_A	H_O	H_E	P_{HW}	GenBank accession No.
LE58	F: TGTGAACTTTCTTTTGGAT R: AGTAACTATAAGCCCTCGA	$(CA)_{18}$	50	330-380	9	0.756	0.871	0.004	KC006902
LE60	F: AAGCACTGTTTACAGCAG R: GAGAAAAGAAGAAAGGTTA	$(AC)_{13}N(CA)_{12}N(CAC)_5$	47	250-350	9	0.848	0.820	0.667	KC006903
LE76*†	F: CTTGGAGGAAGGTGGGTT R: TCAAGCACTCGGAGCAAA	$(TG)_8N(GT)_5$	50	200-220	7	0.476	0.625	0.000	KC006904
LE77	F: CAGGACGGTTCGCATTAC R: AGGCTGGCAGGTAGACAA	$(AC)_9$	50	250-300	8	0.773	0.802	0.675	KC006905
LE80	F: CAGCACCCATTCAAGCAA R: CGACAGACTGGCGAGAAA	$(CA)_7$	50	220-250	3	0.087	0.125	0.016	KC006906
LE88*†	F: ATTTACCAGGGTTCACAG R: CTCTTTATTTGGGTAGCG	$(CA)_{22}A(AC)_9$	47	260-320	9	0.391	0.778	0.000	KC006907
LE95*	F: AAAAGCTCCACATATCCACT R: TATCACTCCGCTAAGAACG	$(CA)_{10}$	58	180-220	8	0.643	0.766	0.003	KC006908
LE99	F: GAAATTTAGCATGGAATACAA R: TCTCAATGGAAACTTCACTG	$(CA)_5CC(CA)_{10}N(CT)_8$	47	270-290	3	0.341	0.380	0.330	KC006909
LE100	F: TACCAGAAAGGCCCATTAGA R: CAGACCAGACGAGGAGACG	$(TG)_{11}$	58	170-200	4	0.200	0.223	0.500	KC006910
LE103	F: TACTCTGGTGGCGGAGCAATG R: TCTGGGCTGATGTTCGTGGAT	$(AC)_{15}$	45	210-240	5	0.756	0.686	0.121	KC006911
LE107*†	F: GAGATGTGAGGTGCTGTG R: GTCTATTCTGACGGGAGC	$(GT)_{10}$	55	200-230	4	0.222	0.586	0.000	KC006912
LE116	F: TATGGAGACTTGCTTGTGGTC R: CTACTTTGTCCTGGGTAATGC	$(CA)_{10}$	45	270-310	6	0.651	0.711	0.014	KC006913
LE151	F: TCCAAGGATTACGTGTATG R: GAGGTGTAAGGAAAGCAGA	$(AC)_9C(CA)_5AC(CA)5$	52	200-220	3	0.556	0.646	0.493	KC006914
LE161	F: ACGAGTAAACAGCCCTTCT R: AGTCCTGAGTAATAGTGAGCC	$(AC)_{15}$	45	240-300	11	0.743	0.828	0.003	KC006915
LE170	F: TCTGCCCTCAGACCACAG R: TTCCCAAGTGCTCAAGTGT	$(AC)_{10}AA(CA)_9$	47	190-210	5	0.512	0.542	0.130	KC006916
LE172	F: CCCTACCCATGATGACGA R: GACTTGATCTGCCCCTGA	$(CA)_{19}$	47	240-300	10	0.978	0.882	0.097	KC006917
LE177	F: GGCTGTCGGCATAAGAAGTGT R: GCTGGGTGCTGATGTGACTAA	$(CA)_6$	47	190-230	9	0.696	0.810	0.007	KC006918
LE181	F: GCACGCTTGTAGTTATGGAC R: ATTAGAGGGTAAGTGGGAGG	$(AC)_{23}$	55	300-400	5	0.773	0.679	0.578	KC006919
LE183	F: CTACTTTGTCCTGGGTAATGC R: TATGGAGACTTGCTTGTGGTC	$(TG)_{10}$	53	270-310	6	0.682	0.717	0.158	KC006920
LE187	F: GCTTTTAGCCCATTTTATTC R: TACAGTTGTGCCCTGAGATT	$(CA)_6$	45	250-310	3	0.087	0.125	0.016	KC006921
LE189	F: CCCTACCCATGATGACGA R: GACTTGATCTGCCCCTGA	$(CA)_{19}$	52	240-300	15	0.913	0.904	0.107	KC006922
LE190	F: AAGGAGCGAGCGTGTTCT R: TGTGGGCAGGTATTTGAG	$(GT)_5N(TG)_5$	47	242-270	5	0.587	0.789	0.030	KC006923

Ta = optimized annealing temperature; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; P_{HW} = Hardy-Weinberg probability test, *locus may harbor null alleles (estimated null allele frequency >5%); †locus deviated from Hardy-Weinberg proportions (adjusted P value <0.0023).

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