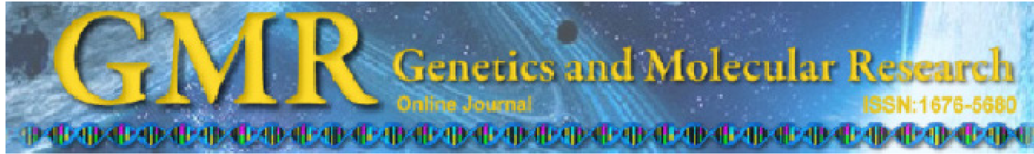

Polymorphic microsatellite loci for the crimson snapper (*Lutjanus erythropterus*)

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ABSTRACT. We isolated and characterized 22 polymorphic microsatellite loci in *Lutjanus erythropterus* using a $(GT)_{13}$-enriched genomic library. We found between 2 and 8 alleles per locus, with a mean of 4.85. The observed and expected heterozygosities ranged from 0.065 to 0.867 and from 0.085 to 0.832, respectively, with means of 0.461 and 0.529, respectively. Allele frequencies in three loci were found to deviate from Hardy-Weinberg equilibrium. Evidence for null alleles was found for three loci. These markers will be useful for distinguishing released captive-bred *L. erythropterus* individuals from wild individuals.

Key words: *Lutjanus erythropterus*; Crimson snapper; Microsatellite loci; Hatchery releasing

INTRODUCTION

Crimson snapper (*Lutjanus erythropterus*) is a pelagic fish widely distributed in the tropical and subtropical waters of the west Pacific and the Indian Ocean. This species is an economically important fish in the Chinese fishing industry. Over the last 30 years, the *L. erythropterus* stock of Chinese coastal waters has seriously declined, mainly due to overexploitation and environmental change (Guo et al., 2011). In order to help this fishery resource to recover, many large-scale hatchery release programs have been conducted in China since the 1990s (Zhu et al., 2009). Since no physical or chemical markers were applied to the captive-bred individuals that had been released, the inability to distinguish the released individuals from wild fish has become a major obstruction to assessing the impact of these release programs.

Molecular marker-based parentage analysis has been widely confirmed as an effective method for identifying captive-bred individuals, and microsatellites have been approved as one of the most appropriate markers for parentage analysis (Jones and Ardren, 2003). Twenty-five microsatellite markers have previously been isolated in *L. erythropterus* (Lo et al., 2006; Guo et al., 2011). Here we report the development of an additional 22 loci that will increase the power available for distinguishing released individuals from wild fish.

MATERIAL AND METHODS

Forty-six individuals were collected from the sea around Sanya, China. These fish were preserved at -20°C until DNA extraction. A microsatellite-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 adapters (5'-TAC TCA GGA CTC AT-3'/5'-GAC GAT GAG TCC TGA G-3'). The ligated products were then pre-amplified in a 25-μL reaction using the adapter specific primer (5'-GAT GAG TCC TGA GTA A-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. Library DNA was amplified using the adapter-specific primer and ligated into pMD 18-T plasmid vectors (TaKaRa, Japan), which were then transformed into *Escherichia coli* DH5α competent cells. The positive clones were randomly sequenced using an ABI Prism 3730 automated DNA sequencer (Applied Biosystems, USA). Microsatellite repeats were found in 106 sequenced clones. Primer pairs were designed for 50 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 DNA samples from all 46 *L. erythropterus* individuals. 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) 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, 1 min at the locus-specific annealing temperature (Table 1), 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, together with tests for deviation from Hardy-Weinberg equilibrium, were calculated using GENEPOP 4.0 (Rousset, 2008). 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 a Bonferroni's correction (Rice, 1989).

RESULTS AND DISCUSSION

Twenty-two of 50 loci amplified cleanly and were found to be polymorphic. The number of alleles per locus ranged from 2 to 8 with an average of 4.32. The observed and expected heterozygosities ranged from 0.065 to 0.867 and from 0.085 to 0.832, respectively, with an average of 0.461 and 0.529, respectively (Table 1).

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

Locus	Premier sequence (5'-3')	Repeat motif	Ta (°C)	Allele size range (bp)	N_A	H_O	H_E	GenBank accession No.
Le1*†	F: GCTCCTCACCACTGTATCTG R: CCTTGGCATCCATTAGAAA	$(AC)_9$	52	250-270	5	0.478	0.670	KC006885
Le5	F: ACAGCCAATCATTTAGGG R: CTGAGTAAGGGTGGAGGG	$(TGGA)_5$	52	245-280	5	0.304	0.353	KC006886
Le6	F: AGTGACAGGCTTCATCTT R: ATGTGGCCTTCAACTTAT	$(CA)_{28}$	45	270-350	8	0.667	0.832	KC006887
Le10.1	F: TGAACATACCCTCAAAAT R: AAAGTGAAGGACAAGGAG	$(AC)_6$	52	190-210	3	0.348	0.441	KC006888
Le10.2	F: CTCTGGCATCCCTGCTAAT R: GTCCACGGTGGTTCTTCTC	$(CCT)_7$	52	250-300	4	0.391	0.450	KC006888
Le16.1	F: CACATGAACATACCCTCAA R: TTCACCAAGGCTACAAGA	$(AC)_6ATT(CCT)_7$	55	250-280	3	0.378	0.489	KC006933
Le16.2*	F: TGTGCATCTCCACAGTTC R: CAAAAGTGAAGGACAAGG	$(AC)_8CAA(AC)_6CA(AC)_6$	55	280-320	3	0.326	0.421	KC006933
Le11	F: CTCCAACCACCACTGAAA R: AGAACAACGTCCGAACTG	$(CA)_8$	55	190-210	3	0.239	0.220	KC006889
Le14†	F: AAACTGAAGTCTTCGAGGGA R: TGAACGTCTGCTGGGAGT	$(CT)_7$	55	260-300	2	0.739	0.471	KC006890
Le17	F: TTTACCTCCGCAGGCACTA R: TCGCTGCTGTGACTCCATT	$(AC)_{27}CA(AC)_5$	52	280-320	2	0.543	0.465	KC006891
Le30	F: CCACCATACCGCCCTTCAT R: GGCCCATATTCCTCCTCCC	$(CA)_7CC(AC)_7$	52	170-200	3	0.239	0.339	KC006892
Le32	F: GCCCAGGTTACTCTGTTGTTG R: AGTGAGCGTACAGGTGGTTTG	$(AC)_5AATC(AC)_{17}$	55	210-240	5	0.591	0.646	KC006893
Le38	F: CCCCTGCTTCTCCTGTAA R: TTGCTCCTGGGATTTCAC	$(GT)_9$	55	280-320	4	0.705	0.701	KC006894
Le41	F: GCTGTCTCAACGAAACGCTCCA R: GAAGGTGGGTTCTTGGCTGGAT	$(CA)_5AAC(CA)_8$	50	170-200	4	0.545	0.489	KC006895
Le42	F: CTGTAGTGATGAGGGTTGACG R: CATTGGCACTGACATGAGC	$(GT)_{10}$	52	210-240	6	0.522	0.756	KC006896
Le43	F: TGTTCTTAGAGGGGATTATG R: GGTGTAGACTAAGTAACAAAGC	$(CA)_6TAA(AC)_9$	47	180-200	3	0.217	0.234	KC006897
Le49	F: AGAGGAGCGTATGGTGTA R: TAGGGTAAGTGGGATGTT	$(AC)_8$	55	260-300	4	0.435	0.541	KC006898
Le51.1	F: GGAGTAACCAATCTGTCTATG R: TTATGGAGTCGCTAATGAAAC	$(TG)_6$	45	190-300	8	0.867	0.813	KC006899
Le57.1	F: GGAGTAACCAATCTGTCTATG R: TTATGGAGTCGCTAATGAAAC	$(TG)_5(TA)_5$	55	190-220	3	0.444	0.628	KC006934
Le57.2*†	F: TCTGCCTCTGTTGAGTTTT R: ATGGAGTCGCTAATGAAAC	$(TG)_5(TA)_5(TG)_6$	58	210-260	8	0.422	0.832	KC006934
Le52	F: CTGGAGCGAGGACAAACAT R: TTGGGATTGGTCAGTGAAG	$(CA)_{13}$	55	230-280	6	0.674	0.770	KC006900
Le56	F: CGATTCATTTCGGATACAG R: TTTCGCCTTCCTACTTCA	$(AGA)_5$	58	240-260	3	0.065	0.085	KC006901

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

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No loci showed significant deviation from Hardy-Weinberg equilibrium after Bonferroni's correction ($P < 0.0023$), except for loci Le1, Le14 and Le57.2. Three loci (Le1, Le16.2 and Le57.2) showed evidence of null alleles (estimated null allele frequency >5%). Significant gametic disequilibrium was detected between one pair of loci (Le6 and Le52).

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REFERENCES

Brookfield JF (1996). A simple new method for estimating null allele frequency from heterozygote deficiency. *Mol. Ecol.* 5: 453-455.

Guo YS, Wang ZD, Xie ZQ and Liu CW (2011). Isolation and genetic diversity analysis of microsatellite DNA in *Lutjanus erythropterus*. *J. Guangdong Ocean Univ.* 31: 13-16.

Jones AG and Ardren WR (2003). Methods of parentage analysis in natural populations. *Mol. Ecol.* 12: 2511-2523.

Lo LC, Zhu ZY and Yue GH (2006). Multiplex genotyping of novel tetranucleotide microsatellites from a marine foodfish species crimson red snapper (*Lutjanus erythropterus*). *Mol. Ecol. Notes* 6: 524-526.

Ma HY and Chen SL (2009). Isolation and characterizaion of 31 polymorphic microsatellite markers in barfin flounder (*Verasper moseri*) and the cross-species amplification in spotted halibut (*Verasper variegatus*). *Conserv. Genet.* 10: 1591-1595.

Rice WR (1989). Analyzing tables of statistical tests. *Evolution* 43: 223-225.

Rousset F (2008). GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol. Ecol. Notes* 8: 103-106.

Van Oosterhout C, Hutchinson WF, Wills DPM and Shipley P (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4: 535-538.

Zhu LX, Hou G, Lu HS and Liu JD (2009). Preliminary study of age and growth of chimson snapper *Lutjanus erythropterus* in Beibu Gulf. *Trans. Oceanol. Limnol.* 2: 19-25.