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Isolation and characterization of novel polymorphic microsatellite loci in large yellow croaker (*Larimichthys crocea*)

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ABSTRACT. The large yellow croaker (*Larimichthys crocea*) is one of the largest marine net-cage cultured species in the oceans around China. In the present study, we isolated and characterized 13 polymorphic microsatellite markers from genomic libraries of *L. crocea*. Loci were screened for 10 wild specimens from 2 sites in southeast of China. All loci were polymorphic. The number of alleles per locus ranged from 2 to 21. The expected heterozygosity ranged from 0.233 to 0.838 and observed heterozygosity ranged from 0.527 to 0.935. Eleven loci were highly informative (polymorphic information content >0.5). Significant deviation from Hardy-Weinberg equilibrium was observed at 3 loci after Bonferroni's correction. The

microsatellite loci may be valuable tools for studying the genetic diversity and genetic structure for conservation planning of the fish.

Key words: Large yellow croaker; Microsatellites; Polymorphism; *Larimichthys crocea*

INTRODUCTION

The large yellow croaker, *Larimichthys crocea*, represents the largest marine net-cage yield species in the oceans around China (Wei et al., 2009). However, because of overfishing and deterioration of the coastal environment, the natural resource of the croaker has become rare and endangered in recent decades (Li et al., 2008; Chang et al., 2009). The fish is currently successfully cultured by artificial propagation. Ye et al. (2012) isolated and characterized 21 microsatellite markers in this species. However, lower growth, earlier sex maturation and lower viability is observed in fish grown under culture conditions, and it is necessary to develop genetic markers for appropriate conservation management.

Genetic analysis has been conducted using amplified fragment length polymorphism (Wang et al., 2002). Furthermore, Guo et al. (2005) isolated and characterized 6 polymorphic microsatellite markers using an enriched library technique in croaker. Chang et al. (2009) isolated and characterized 11 microsatellite markers in croaker. However, such markers are insufficient for the species, and a more polymorphic microsatellite marker is essential for further studies. In the present study, we report a set of novel microsatellites developed from an enriched genomic library, which may benefit further studies of conservation genetics in the species.

MATERIAL AND METHODS

Genomic DNA was isolated from the tail fin following standard phenol/chloroform extraction procedures. The microsatellite library was constructed using the protocol described by Zane et al. (2002) with some modifications. Briefly, 10 µg total genomic DNA from a single fish was digested by the restriction enzyme *Sau*3AI, and fractions measuring 400 to 800 bp were recovered by 5% polyacrylamide gel electrophoresis. The digested fragments were ligated into pUC118 and transformed into DH5a competent cells. Colonies were grown on LB+AMP plates containing X-gal and ampicillin at 37°C for 16 h. Recombinant plasmids were examined using blue-white screening to test for successful ligations into the vector. The genomic libraries enriched in $(CA)_n$ were constructed by Southern hybridization. Next, positive colonies were confirmed by a second hybridization. Positive clones were detected by screening using a 5'-[g-^{32}P] ATP labeled probe $(CA)_{10}$ for screening and sequencing. Sequences were trimmed using the Sequencing Analysis 3.7 system (Hitachi, Tokyo, Japan). Eighteen primers were designed using PRIMER version 3.0 (Rozen and Skaletsky, 1999) and synthesized by Sangon Biotech (Shanghai, China).

The final polymerase chain reactions were performed in a 10-mL mixture containing 20 ng genomic DNA, 0.15 mM of each primer, 1 mL 10X polymerase chain reaction buffer, and 0.5 U *Taq* DNA polymerase (Takara, Shiga, Japan). The amplification conditions were as follows: initial denaturation at 94°C for 2 min, 30s at 65°C, 1 min at 72°C; followed by 28

cycles of denaturing at 94°C for 30s, annealing at a primer-specific optimal temperature for 40 s (Table 1), extension at 72°C for 1 min, and final extension at 72°C for 5 min. The polymerase chain reaction products were evaluated using a Bio-Rad GDY-3000 system (Hercules, CA, USA). The number of alleles, effective number for alleles, observed heterozygosity, Nei's expected heterozygosity, and heterozygosity deficiency were calculated using the POPGEN version 1.32 software (Yeh and Boyle, 1997). Hardy-Weinberg equilibrium and linkage disequilibrium were analyzed using GENEPOP 3.4 (Raymond and Rousset, 1995). Conditions and the characterization of the 13 loci are shown in Table 1.

RESULTS AND DISCUSSION

Approximately 186 positive colonies were screened and 143 recombinants were sequenced. Eighty-two positive colonies identified from 143 recombinant colonies were sequenced for primer design.

Thirteen of 18 primer pairs were polymorphic after a screening. A total of 30 unrelated samples were obtained from Guanjingyang and Taiwan Strait, East Sea, China, for genetic diversity analysis. Thirteen polymorphic loci were identified (Table 1) (GenBank accession No. EU022002 to EU022019). The number of alleles per locus ranged from 2 to 21 with an average of 10.231. The observed and expected heterozygosities ranged from

Table 1. Characterization of 13 polymorphic microsatellite loci isolated from *Larimichthys crocea*.

Locus	GenBank accession No.	Primer sequence (5'-3')	Motif	Ta (°C)	Size (bp)	N_A	H_O	H_E	PIC	P
LYC-101	EU022002	F: GTTCAGAGCGTCCTAATTGC R: GTGTGACAGAGTAGTCGTCG	$(CA)_{25}$	55.0	130-170	3	0.660	0.670	0.640	0.215
LYC-102	EU022003	F: CACACACACACACACCTACA R: CCGACATCCTAATTCTCCAG	$(CA)_8 \ldots (CA)_{13}$	55.0	180-250	14	0.581	0.613	0.797	0.043
LYC-103	EU022004	F: GCCAGTTGGTCTGTTAAGTG R: CACACACACACACACACTCC	$(GT)_{29}(GA)_6 \ldots (GT)_7$	62.0	180-250	4	0.233	0.539	0.452	0.016
LYC-106	EU022007	F: TCCTGTGTGTGCGTGTCTAT R: CACGGCGATCCTACTCATAA	$(GT)_{38}$	51.0	270-280	9	0.438	0.748	0.697	0.013
LYC-107	EU022008	F: AGGCACACACACACACACAC R: CTAATGTCACACTGTACGCG	$(CA)_{30}$	51.0	160-200	11	0.765	0.826	0.738	0.030
LYC-108	EU022009	F: GTGTAATCAGCTCAGGTGGT R: CCGTATTGTGTGTGTGTG	$(AGAT)_{25} \ldots (CA)_7$	58.5	140-170	2	0.431	0.527	0.181	0.714
LYC-109	EU022010	F: GTGAGGATGAGTGATGAACC R: GGTGACTCTCTTAGACAGTG	$(GT)_{25}$	61.5	120-140	14	0.714	0.801	0.771	0.197
LYC-110	EU022011	F: GACACAGAATGACGGAGAAG R: GTGAGAGACAGACAGACGAC	$(CA)_{26}$	51.0	150-230	14	0.645	0.790	0.865	0.001*
LYC-112	EU022013	F: CCGTTCAGACATATGCAAGC R: GCGTCCGTACATTACACATG	$(GT)_{28}(GCGT)_4$	61.5	100-120	12	0.536	0.685	0.856	0.011
LYC-114	EU022014	F: GACTCTAGAGGATCCACAGT R: CATCTACACAGACAGTCTCG	$(CA)_{28}$	55.0	180-200	12	0.542	0.588	0.861	0.002*
LYC-115	EU022016	F: CTCTCAGTAACATGACGCAG R: GGAGACACGTCTAGGAGATC	$(CA)_{13}$	60.0	190-230	21	0.838	0.935	0.915	0.561
LYC-117	EU022018	F: ACACTCTCAGGCATTCTGCT R: CAACTTCGTCACACTCAGGT	$(GT)_{30}$	55.0	210-240	6	0.674	0.798	0.754	0.373
LYC-118	EU022019	F: GACCAGGTACTCAATCACTC R: GGATGGATGTGGACCTTATC	$(CA)_{11}$	51.0	160-230	11	0.538	0.674	0.842	0.001*
Average						10.231	0.584	0.705	0.597	

Motif, repeat sequence of the isolated clone, Ta = annealing temperature; N_A = number of alleles; H_O = observed heterozygosity, H_E = expected heterozygosity, PIC = polymorphism information content, P = associated probability value of conformation with Hardy-Weinberg equilibrium (HWE). *Significant deviation from HWE at $P < 0.01$ for loci after Bonferroni's correction.

0.233 to 0.838 and 0.527 to 0.935, respectively. Significant deviations from Hardy-Weinberg equilibrium ($P < 0.05$) were observed at 3 loci after Bonferroni's correction. The sample size may have contributed to the deviations from Hardy-Weinberg equilibrium at these loci. Microsatellite markers described in the present study may be useful for examining genetic diversity and structure, as well as for providing information necessary for effective conservation management of the species.

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