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Development of novel microsatellite markers in the Korean rockfish *Sebastes schlegeli*

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ABSTRACT. The Korean rockfish *Sebastes schlegeli* is a valuable recreational and commercial fish in China, and is cultured in land-based tanks and net cages. Fifteen microsatellite markers were developed for this species, and their polymorphisms were examined in a population. The allele number of the 15 markers ranged from 2 to 13, with an average of 5.933 per locus. The observed and expected heterozygosity values ranged from 0.063 to 0.938 (averaging 0.585), and 0.062 to 0.908 (averaging 0.642), respectively. Thirteen loci were at Hardy-Weinberg equilibrium (HWE), whereas the other two significantly deviated from the HWE after a Bonferroni's correction. No significant linkage disequilibrium was detected between the comparisons of these loci. These markers are useful for studies of population genetics, linkage mapping, and other relevant studies on *S. schlegeli.*

Key words: Microsatellites; Polymorphism; *Sebastes schlegeli*

INTRODUCTION

The Korean rockfish, *Sebastes schlegeli*, of the live-bearing genus *Sebastes*, belongs to the most species-rich genus of the family Scorpaenidae (Nelson, 1994). It inhabits coastal rocky reefs and feeds on benthic animals, and is generally known as a nonmigratory marine species. This species is a valuable recreational and commercial fish in China, and has been cultured in land-based tanks and net cages, respectively. In recent years, the rising consumption of rockfish products and the decline in natural resources have led to an increase in aquaculture practices and stock transfers of this species. Consequently, stock improvement and resource management are required for the sustainable development of this industry, for both the industrial sector and the scientific community. Microsatellites are a popular marker system for pedigree analysis, genetic linkage mapping, and trait-marker association studies (Zhan et al., 2006; Ma and Yu, 2009; Xiao et al., 2011). Although some microsatellite loci are available for this species (Yoshida et al., 2005; An et al., 2009; Bai et al., 2011; Yasuike et al., 2013), more loci are required for further research. Here, we describe the development and characterization of 15 new microsatellite loci from *S. schlegeli.*

MATERIAL AND METHODS

An adductor muscle sample was used to extract genomic DNA, using a standard phenol-chloroform protocol (Sambrook et al., 1989). Approximately 4 µg DNA was restricted with *Sau*3AI, and size fractions ranging from 400 to 1000 bp were recovered from 1.2% agarose gel using a gel extraction column kit (TaKaRa, Dalian, China). Fragments were then ligated to a blunt-end adapter (SAULA: GCGGTACCCGGGAAGCTTGG, SAULB: GATCCCAAGCTTCCCGGGTACCGC) with T4 DNA ligase (TaKaRa). The ligated fragments were subsequently amplified by polymerase chain reaction (PCR), using SAULA as a primer, before the enrichment of the microsatellite motifs.

A partial genomic library enriched with CA- and GA-microsatellite repeats was constructed using the method described by Carleton et al. (2002). Amplified fragments were denatured and hybridized to a combination of the biotinylated probes $(CA)_{16}$ GCTTGA-Biotin and $(GA)_{16}$ GCTTGA-Biotin, in 6X SSC (saline sodium citrate)/0.1% SDS (sodium dodecyl sulfate) at 58°C for 1 h. The DNA hybridized to the probes was then captured using streptavidin magnetic beads (Dynal, Shanghai, China) and washed. Following purification and elution from the beads, the enriched DNA was denatured in 0.1X TE at 95°C and amplified by post-hybridization PCR using OligoA, and the same cycling program as the pre-hybridization PCR. The DNA was then spin-cleaned, quantified, and TA-cloned into a pGEM®-T Easy Vector (Promega, Madison, WI, USA). The vector was subsequently transformed into JM109 competent cells and plated for cloning. Positive clones were identified by PCR using SP6, T7 primers, and $(CA)_{12}$ for CA-repeat or $(GA)_{12}$ for GA-repeat. The positive clones, containing fragments that ranged between 500-900 bp in length, were sent for sequencing.

Forty-five sequences contained microsatellites, and 32 possessed a sufficient flanking sequence that was appropriate for primer design. Twenty-three pairs of primers were designed by the Primer Premier 5.0 program (Premier Biosoft International, Palo Alto, CA, USA). The microsatellite loci were evaluated using a population of 30 individuals collected from Yantai, Shandong Province, China. Primer pairs were amplified in 20-µL reactions containing 0.25-0.5 U Taq polymerase (Tiangen, Beijing, China), 1X PCR buffer, 1.0-2.0 mM $MgCl_2$, 0.2 mM

dNTPs, 0.2-1 μM of each primer, and 20-100 ng of total DNA. The PCR conditions were as follows: initial denaturation at 95°C for 5 min, followed by 28 cycles at 94°C for 40 s, a primer-specific annealing temperature for 40 s, 72°C for 1 min, and a final extension at 72°C for 10 min. A total of 15 primers, which amplified clear and specific products, were selected and labeled with one of three fluorescent dyes (FAM, HEX, or TAMRA; Sangon, Shanghai, China) for polymorphism detection. The size of the PCR products was measured using an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) and the GENEMAPPER software (Applied Biosystems). The number of alleles, the heterozygosity, the Hardy-Weinberg equilibrium (HWE), and the linkage disequilibrium (LD) were analyzed using Genepop 3.4 (Raymond and Rousset, 1995). The polymorphism information content (PIC; Botstein et al., 1980) was estimated using PARFEX (v1.0; Sekino and Kakehi, 2012).

RESULTS AND DISCUSSION

Fifteen polymorphic microsatellite markers were successfully screened in *S. schlegeli* (Table 1). The allele number of the 15 polymorphic markers ranged from 2 to 13, with an average of 5.933 per locus.

Table 1. Characterization of 15 microsatellite loci in the black rockfish, Sebastes schlegelii.

Locus/ Accession No.	Primer sequence (5'-3')	Ta (°C)	Repeat motif	Size range (bp)	N_A	H_O	H_E	PIC	HWE P value
HJ1-21 KM079261	F: AGGAGGGAGGAATGGAAA R: TCAGTAAACGGCTGGTAGG	60	$(CT)_5$	352-376	2	0.063	0.062	0.059	1.0000
HJ5-5 KM079262	F: AAACATCACAACAAAGAG R: TTGCCTGTAAGTATTACA	54	$(TG)_{24}$	258-271	3	0.563	0.451	0.401	0.3365
HJ5-9 KM079263	F: GCCTTTAGATGCTTGTGG R: TCTTCTCGTACTCCTCGTC	60	$(CCT)_7$	205-233	4	0.469	0.696	0.633	0.0375
HJ5-13 KM079264	F: CCCGATTACTGGGAAGAG R: CTGAGGACAGATGCTGGA	63	$(GATA)_3(GA)_{13}$ $(GA)_{10}T(AG)_6$	250-326	13	0.906	0.908	0.885	0.7615
HJ5-50 KM079265	F: CGCGAGTGATGTTTGAGA R: CGATTGCCGTAGTTGGTC	50	$(CA)_6$	214-239	4	0.719	0.634	0.576	0.0249
HJ5-62 KM079266	F: CTCGGTCACCATAACAAT R: ACATAGAGCCCAAAACAG	63	$(CT)_{15}$	155-228	11	0.781	0.857	0.829	0.0924
HJ5-69 KM079267	F: ACAGCAGCTTCTACAACTGAA R: CAACACTGCGTCACATCAA	50	$(TCT)_4$	95-113	4	0.156	0.179	0.171	0.2375
HJ5-79 KM079268	F: CCAATGAGCTGGATGTGA R: AGACGAGGCAGAGCAAGT	60	$(CCTC)_3(CCTC)_4$ $(CCTC)_3$	328-369	3	0.469	0.506	0.436	0.0180
HJ5-97 KM079269	F: TTACGACCCGCCATTAAC R: ATCAGCAAAGGGCAACGA	65	$(TC)_{19}G(CT)_{13}$	184-267	9	0.938	0.867	0.836	0.3679
HJ5-102 KM079270	F: GCTTATTGCCAAGACGAG R: AGTCCGACCAGTATCCAA	60	$(AG)_6...(AG)_8$	160-193	5	0.531	0.796	0.748	0.0136
HJ5-111 KM079271	F: CACCTTGCCAAGCTCATC R: GGTTTCCAGGGTTTCAGA	60	$(TGA)_8$	231-272	9	0.686	0.842	0.809	0.0678
HJ5-114 KM079272	F: TGACGGGAGTCTTTGACC R: GAGGAAACACCAAGCACC	66	$(AG)_7$	91-127	6	0.937	0.753	0.700	0.0000
HJ5-116 KM079273	F: GAAAGGCAGGAACCAAAC R: GTGATGTGAAGGAGACCC	52	$(CTAA)_6$	132-164	8	0.688	0.841	0.806	0.3116
HJ5-123 KM079274	F: GGCAGCCTGGAGAATAAC R: GAAACCTGGAGCAGAACC	50	$(TC)_{18}$	150-171	4	0.250	0.494	0.447	0.0000
HJ5-139 KM079275	F: TCCGTAACGTGAACCATG R: GCAGAAGGCTCAGCAGTT	60	$(CTG)_4...(CA)_{19}$	344-375	4	0.625	0.744	0.683	0.2759

F = forward primer; R = reverse primer; Ta = annealing temperature; N_A = of alleles; H_o = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content; HWE = Hardy-Weinberg equilibrium.

Four loci were at a medium or a medium-high level of polymorphism in terms of the number of alleles (≥9). The locus HJ5-13 was the most polymorphic, with 13 distinct alleles, whereas the locus HJ1-21 was the least polymorphic, with two alleles.

The observed heterozygosity varied from 0.063 to 0.938, with an average of 0.585, while the expected values for these loci ranged from 0.062 to 0.908, with an average of 0.642. In addition, the PIC ranged from 0.059 to 0.885, with an average value of 0.601. Thirteen loci exhibited medium, or medium-high, levels of polymorphism in terms of PIC (>0.25). After a Bonferroni's correction, exact tests for the HWE revealed that 13 of the 15 markers accorded with the equilibrium, but the rest exhibited significant departures from the HWE. No significant LD was detected between the comparisons of these loci. These markers should be useful in population genetics, pedigree analysis, linkage mapping, and other relevant studies on *S. schlegeli.*

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