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Isolation and characterization of 16 microsatellite loci in marble goby (*Oxyeleotris marmoratus*)

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ABSTRACT. A microsatellite-enriched genomic library for marble goby (*Oxyeleotris marmoratus*), a freshwater fish of considerable economic value, was obtained. A total of 16 microsatellite markers were successfully isolated and characterized in a population from the Mekong River in Vietnam. These markers had 2-20 alleles with expected heterozygosity ranging from 0.0370 to 0.8927. Linkage equilibrium was observed in most loci, and only 1 locus revealed a significant deviation from Hardy-Weinberg equilibrium. These microsatellite markers will be useful for genetic diversity and molecular marker-assisted selection studies of wild and farmed *O. marmoratus*.

Key words: *Oxyeleotris marmoratus*; Microsatellite loci; Magnetic-bead enrichment

INTRODUCTION

The marble goby (*Oxyeleotris marmoratus*), commonly known as Soon Hock, is the largest species of goby-like fishes (Smith, 1945). It is native to the Mekong and Chao Phraya River basins, Malay Peninsula, Indonesia, and the Philippines (Kottelat, 1993). *O. marmoratus* is considered a superior-quality freshwater fish with high market demand and commercial value. Owing to its high price and cultivation potential, marble goby culture has been rapidly developed in Southeast Asian countries such as Thailand, Vietnam, Malaysia, and China. Many studies have been conducted on artificial culture, larval rearing, and nutrition physiology (Vu et al., 2005; Su et al., 2008; Nakagawa et al., 2009). However, genetic studies on the species have rarely been reported. Only 8 simple sequence repeat (SSR) primers have been developed for *O. marmoratus* (Abercrombie et al., 2009). Those loci with few alleles (2-4) are inadequate for further population genetic analysis and breeding studies.

Microsatellites or SSRs exist in both coding and noncoding regions of prokaryotic and eukaryotic genomic DNA. SSRs are usually characterized by a high degree of length polymorphism (Zane et al., 2002). Herein, we report a set of 16 polymorphic microsatellite loci and primers for *O. marmoratus* obtained using the method of fast isolation by amplified fragment length polymorphism of sequences containing repeats. These markers will be useful for genetic diversity and molecular marker-assisted selection studies of wild and farmed *O. marmoratus*.

MATERIAL AND METHODS

An enrichment library was constructed using a protocol described by Zane et al. (2002). Genomic DNA was extracted from tail fin tissues of 3 individuals using a standard proteinase K/phenol extraction protocol. Three 10 µg DNA samples were pooled and digested with *Bsp*143I restriction enzyme. Size fractions of 400-1000 bp were selected and ligated to adaptors: oligo A (5'-GCGGTACCCGGGAAGCTTGG-3') and oligo B (5'-GATCCCAAGCTTCCCGGGTACCGC-3') (Moraga et al., 1998). Pre-hybridization polymerase chain reaction (PCR) amplification was performed for the digested/ligated library using oligo A. For enrichment, the DNA was denatured and hybridized with 2 biotinylated probes [B-ATAGAATAT$(CA)_{15}$ and B-ATAGAATAT$(GA)_{15}$] at 68°C for 1 h. The fragments hybridized to the probes were then captured with streptavidin-coated magnetic beads (Streptavidin Magnesphere Paramagnetic Particles, Promega, Madison, WI, USA).

After enrichment, nonspecific binding and unbound DNAs were removed with several non-stringent and stringent washes. The microsatellite-enriched DNA fragments were collected and amplified with PCR and then ligated to T-vectors (pMD18-T, Takara, Dalian, China) and transformed into DH5α competent cells. Transformed cells grew at 37°C overnight on a Luria-Bertani agar plate containing ampicillin, X-gal, and isopropyl-beta-D-thiogalactopyranoside for blue/white selection. The positive clones were selected for post-PCR using M13/RV-p and $(CA)_{12}$/$(GA)_{12}$ primers and sequenced on an ABI 3730 Genetic Analyzer (Applied Biosystems). SSRs were detected using the SSRHunter 1.0 software (Li and Wan, 2005). All of these loci were deposited in GenBank.

Primers were designed using the Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA, USA). The primer pairs (fluorescence-labeled at the 5'-end) were tested on 53 individuals of *O. marmoratus* collected from the Mekong River in Vietnam. The allele number per locus (N_A), allele size range, observed heterozygosity (H_O), and expected heterozygosity

(H_E) were calculated using Popgene 1.31 (Yeh et al., 1999). Deviations from Hardy-Weinberg expectations were estimated using Genepop4.0 (Raymond and Rousset, 1995; Rousset, 2008).

RESULTS AND DISCUSSION

A total of 205 positive clones were selected for post-PCR, and 127 were successfully sequenced. Among the 127 clone sequences, 81 (64%) contained simple or compound microsatellites with 9 or more repeats. All of these loci were deposited in GenBank. Primers were tested on the genomic DNA of marble goby, and 29 primer pairs successfully revealed consistent amplification and polymorphic loci. The variability of 16 selected loci from 29 primer pairs (fluorescence-labeled at the 5'-end) was tested on 53 individuals of *O. marmoratus*.

N_A, allele size range, H_O, H_E, and deviations from Hardy-Weinberg expectations are listed in Table 1. N_A ranged from 2 to 20, and H_O and H_E ranged from 0.0377 to 0.8491 and 0.0370 to 0.8927, respectively. No evidence of linkage disequilibrium between locus pairs was observed. One locus showed significant departure from Hardy-Weinberg equilibrium ($P < 0.05$), which might have been due to the segregation of null alleles or the presence of too many alleles (20) in a low-detection population sample. These 16 microsatellite markers may be useful in the analysis of genetic applications for *O. marmoratus*.

Table 1. Characterization of 16 marble goby (*Oxyeleotris marmoratus*) microsatellite loci.

Locus	Repeat motif	Primer sequences (5'-3')	Size range (bp)	Ta (°C)	N_A	H_O	H_E	P (HWE)	Accession No.
H113	$(CA)_{11}$	F: HEX - GGAAGCTGCTGACCTTGACTC R: CCTATGGTCCGTCCAGAGTGT	148-188	60	10	0.6604	0.7182	0.1519	JF264394
H137	$(GT)_9$	F: FAM - GATCAGAGGGTTCAGAAAGCAG R: CATTACAGCACCGACAGAGGA	210-236	58	4	0.2075	0.2209	0.5780	JF264402
H27	$(TG)_{21}N_{105}(AC)_{5v}$	F: TAMRA - GATCAACAGTGTTTGCGTTAGG R: TCTCACCTGATGGAAAGATGG	242-302	56	13	0.7358	0.7686	0.2894	JF264376
H60	$(GT)_{13}N_{15}(TG)_6$	F: HEX - GTTTGGCTGAAATGGTAGTGTG R: TGGAATGATGCTAGTGGCTGT	160-200	56	3	0.0943	0.1255	0.0854	JF264381
H167	$(AC)_9N_{73}(AC)_6$	F: FAM - TCCATTACAGCACCGACAGAG R: GATCAGAGGGTTCAGAAAGCAG	220-232	58	3	0.2830	0.3245	0.5791	JF264409
H63	$(AC)_5AG(AC)_7$	F: TAMRA - AGAGCAGGCGTTAGCAAGAC R: AGATTAGAGGACGGAGGGAGA	302-303	58	2	0.0377	0.0370	1.0000	JF264382
H94	$(TG)_{12}$	F: HEX - GAGGATTTCCCGCTTCTATG R: GCCGTCTTTCTGTTTGTCTTG	146-166	56	5	0.2452	0.3012	0.1256	JF264391
Y12	$(CA)_7N_4(CA)_6$	F: FAM - ATTATGATCCCCCACCAGCT R: TGTGATTGTCCCCTCTCACAG	207-229	57	3	0.3208	0.3859	0.2631	JF419699
H117	$(CA)_8N_{111}(CA)_5$	F: TAMRA - ATAGCTCTGCGACGTGATTGG R: GGACTTAGCTTTACCCTGTGGA	276-286	58	4	0.3962	0.4381	0.4815	JF264411
H138	$(TC)_5(AC)_{13}$	F: HEX - TAAGCCAGTGCCAGCAGAGT R: GCCCTGATTGTGACTGTGGAG	138-172	58	6	0.4717	0.4963	0.3953	JF264403
H191	$(AC)_{11}N_{51}(GA)_{10}N_8(TG)_4T_2(TG)_6$	F: FAM - TGACATCTGTCCTGGCTTCG R: GCCTGCGTCTTTGACAACTC	254-288	58	15	0.7170	0.8033	0.2897	JF264413
H56	$(AC)_{12}$	F: TAMRA - GCGAATTGCTGCAAGTGAGA R: GGTTGGGAGGAAGTGTTAGGA	258-272	56	6	0.2453	0.2424	0.5998	JF264379
H142	$(CA)_6G(AC)_8N_5(CA)_7$	F: HEX - GAAATTGGAACGGGAGGCA R: ATGGGAGCCACGACTCACA	93-123	58	11	0.7547	0.7175	0.3493	JF264405
H97	$(CA)_{24v}$	F: FAM - AATCTGGCTTGACGCACTCT R: TTCCGCACGGTATCCTCTT	191-263	56	20	0.8491	0.8927	0.0445*	JF264392
M351	$(TTCAA)_5$	F: TAMRA - GATCCTTTGCTCTGTTTCAG R: TCCTGGGTCTGTTTAGTGTAG	247-293	54	5	0.6981	0.6759	0.1523	JF419693
H240	$(TG)_{12}$	R: HEX - GAGGATTTCCCGCTTCTATG F: CCGTCTTTCTGTTTGTCTTGAG	167-177	56	3	0.4151	0.5568	0.1927	JF264420

N_A = allele number; H_O = observed heterozygosity; H_E = expected heterozygosity; HWE = Hardy-Weinberg equilibrium. Statistically significance at *$P < 0.05$.

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