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Isolation and characterization of novel microsatellite markers for molecular genetic diversity in *Siganus fuscescens*

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ABSTRACT. The rabbitfish *Siganus fuscescens* is an economically valuable species that is widely distributed throughout the estuaries, intertidal, and offshore coasts of the Indo-Pacific and eastern Mediterranean. Ten novel microsatellite loci from the genome of *S. fuscescens* were developed using the fast isolation protocol with amplified fragment length polymorphism of sequences containing repeats. Polymorphisms in these 10 microsatellite markers were determined from 32 wild individuals. The number of alleles per locus and the polymorphism information content ranged from 2 to 5 and from 0.059 to 0.668, respectively. The observed and expected heterozygosities varied from 0.063 to 0.781 and from 0.062 to 0.731, respectively. Although 1 locus (LZY-X7, $P < 0.005$) showed significant deviation from the Hardy-Weinberg equilibrium, no deviations were detected in the other 9 loci. These microsatellite loci may be useful for further population genetic studies, conservation studies, population structure assessment, and linkage map construction of *S. fuscescens*.

Key words: Genetic markers; Microsatellite; *Siganus fuscescens*; Fragment length polymorphism of sequences containing repeats

INTRODUCTION

The rabbitfish S*iganus fuscescens* belongs to the order Perciformes, family Siganidae, which is widely distributed throughout the estuaries, intertidal, and offshore coasts of the Indo-Pacific and eastern Mediterranean. In China, this species is mainly distributed in the southeastern coastal area. As an omnivorous habit fish, its fingerlings feed mainly on benthic algae and organic detritus (Yang et al., 2000). It is becoming increasingly sought after not only because of its high protein content and good taste but also because it plays an important role in the ecological purification of the aquaculture water quality (Ma and Liu, 2006). However, increasing evidence suggests that overfishing, high exploitation, and habit destruction have sharply decreased the population of this species (Jumawan-Nanual and Metilla, 2008). Currently, the study of population dynamics, genetic diversity, and conservation of this economically valuable species is insufficient and limited, making the development of genetic markers in *S. fuscescens* urgent and necessary. Microsatellite simple sequence repeats, because of their extensive applications and advantages in the study of population genetics, population differentiation, linkage analysis, and evolutionary studies, are considered to be powerful tools for researching species genetic conservation (Wang et al., 1994). In this study, we identified 10 novel microsatellite markers in *S. fuscescens* with the goal of protecting this natural resource.

MATERIAL AND METHODS

Genetic DNA was sampled from the muscle tissues from a wild *S. fuscescens* individual captured in Xiamen, China, by using the Genomic DNA Extraction kit (Tiangen, Beijing, China). The microsatellite-enriched library was derived from a single *S. fuscescens* using fast isolation by amplified fragment length polymorphism (AFLP) of sequences containing repeats (FIASCO) protocol (Zane et al., 2002) using 2 biotinylated probes [$(GT)_{15}$ and $(CT)_{15}$] (Cao et al., 2012). A total volume of 2000 μg (100 μg/μL) DNA was digested by the restriction enzyme *FastDigestTru1*I at 37°C for 5 min. The enzyme was inactivated by heating for 5 min at 65°C. Digested fragments approximately 400-1200 bp in size were then ligated to the *Mse*I adapter 1 (5'-ACGATGAGTCCTGAG-3')/*Mse*I adapter 2 (5'-TACTCAGGACTCAT-3') by T4 DNA ligase (Fermentas, Vilnius, Lithuania) overnight at 22°C. The linker-ligated mixture was denatured at 95°C for 10 min and then hybridized to the biotin-labeled oligonucleotide probes $(CT)_{15}$ and $(GT)_{15}$ at 61°C for 1 h. Microsatellite-containing fragments were captured via streptavidin-coated magnetic sphere particles (Promega, Madison, WI, USA). Target DNA fragments were released from the beads after washing and were re-amplified using *Mse*I adapter 1. After purification by GenCleanPCR (Generay, Shanghai, China), the products were ligated into the PMD19-T vector (Takara, Shiga, Japan) at 16°C for 3.5 h and transformed into OneShot chemically competent *Escherichia coli* (Invitrogen, Carlsbad, CA, USA) grown overnight at 37°C. Transformants were cultured on Luria-Bertani agar plates supplemented with 60 μg/mL ampicillin. After polymerase chain reaction (PCR) amplification of positive colonies via using M13 universal primers, the sizes of inserted products were determined using 1% agarose gel electrophoresis. A total of 128 positive clones with DNA fragments bands of 500-1200 bp were sequenced by the Majorbio Company (Shanghai, China), 117 of which contained microsatellites according to the SSRhunter1.3 software, while 40 pairs of microsatellite amplification primers were designed by the Primer Premier version 5.0 software.

After optimizing the amplification conditions for each primer pair in an Eppendorf Mastercycler Gradient System (Eppendorf, Hamburg, Germany), 34 primer pairs were successfully selected for testing by amplifying genomic DNA of 32 wild *S. fuscescens* individuals collected from Xiamen, China. PCR was carried out in a 10-μL volume consisting of 50 ng genomic DNA, 10X Taq buffer, 2 mM $MgCl_2$, 0.4 μM of each primer, 0.2 mM of each dNTP, and 0.25 U *Taq* DNA polymerase (Fermentas). The reaction protocol was as follows: predenature at 94°C for 5 min, followed by 32 cycles of denaturation at 94°C for 30 s, annealing at the optimal temperature (Table 1) for 30 s, and 72°C for 1 min, with a final 10-min elongation step at 72°C. The PCR products were resolved on 6% denaturing polyacrylamide gels in a Sequi-Gen Sequencing Cell (Bio-Rad, Hercules, CA, USA) and visualized by silver staining. A 10-bp DNA ladder (Fermentas) was used to identify the alleles. The number of alleles per locus, the observed and expected heterozygosities, tests for linkage disequilibrium (LD), and Hardy-Weinberg equilibrium (HWE) were obtained using the POPGEN 32 (version 1.32) software (Yeh et al., 2000). Polymorphic information content was calculated using CERVUS 3.0 (version 3.0).

RESULTS AND DISCUSSION

Error analysis was carried out using MICRO-CHECKER (Van Oosterhaut et al., 2004). Nine loci were in HWE ($P > 0.005$) except locus (LZY-X7), which significantly deviated from the HWE after Bonferroni's correction ($P < 0.005$; Table 1). Characterization of the loci is presented in Table 1. Ten loci were shown to be polymorphic with 2-5 alleles and polymorphism information content ranged from 0.059 to 0.668. The observed and expected heterozygosities ranged from 0.063 to 0.781 and from 0.062 to 0.731, respectively.

Table 1. Primer sequence and characterization of 10 novel microsatellite loci in *Siganus fuscescens* (sample size = 32 individuals).

GenBank accession No.	Locus ID	Primer sequences (5'-3')	Repeat motif	Ta (°C)	N_A	Allele size (bp)	PIC	H_O	H_E
KF773749	LZY-14	F: ATCACTGGTGGAATGTTGT R: TCCTCTGCTCCTTTGTCT	$(GT)_{14}(GTGC)_3(GT)_7$	55	5	149-187	0.601	0.781	0.659
KF773750	LZY-39	F: TGTTGGTAATCGCTCTG R: GATGGAAGACACGGATG	$(GT)_{33}$	42	5	126-144	0.539	0.500	0.594
KF773751	LZY-36	F: GAGCGAGGACCACAAACA R: TCCCACCCTACCACCAC	$(AG)_{27}$	58	3	109-117	0.444	0.469	0.510
KF773752	LZY-7	F: TTACAATCTTCCTGGCTCTG R: GGGCTCACCCACTTCTG	$(TCC)_{10}$	55	5	195-223	0.598	0.688	0.669
KF773753	LZY-X7	F: CTGGTTGTGCGATCCCTG R: CCCTTCCTGACATACCCTCC	$(GT)_8$	53	5	260-290	0.659*	0.219	0.720
KF773754	LZY-50	F: GCCTTTGGTATCCGTCTG R: TGCGTCGCCTCACTTTT	$(AG)_7AC(AG)_{23}(TG)_{13}$	50	5	170-195	0.668	0.719	0.731
KF773755	LZY-71	F: TAAGGGTTTGTTAGAGTGT R: GGGGCTGTATTATTGTC	$(GT)_{19}(GA)_6$	48	2	251-260	0.195	0.250	0.222
KF773756	LZY-43	F: GTCAGTGTTAGTAGAGCAAGT R: CAGATAAATCACCAGGAA	$(AAGTC)_4$	45	4	190-220	0.491	0.688	0.559
KF773757	LZY-61	F: CGACCAGCACAGGATAG R: CCATGTTGTCATAGGGAG	$(TG)_{16}$	42	2	115-120	0.305	0.250	0.381
KF773758	LZY-19	F: AGGGCTGAAGACGTAGAT R: AAAGGACAGTGGAAATGG	$(AG)_7T(GA)_{33}$	42	2	230-240	0.059	0.063	0.062

Ta = annealing temperature; N_A = number of polymorphic alleles per locus; PIC = polymorphism information content; H_O = observed heterozygosity; H_E = expected heterozygosity. *Indicates significant departure ($P < 0.005$) from expected Hardy-Weinberg equilibrium conditions after correction for multiple tests (k = 10).

The 10 novel polymorphic microsatellite loci presented may be useful for further population genetic studies, conservation studies, population structure assessment, and linkage map construction of *S. fuscescens*.

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