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Development and characterization of novel microsatellite markers in the rock bream fish *Oplegnathus fasciatus*

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ABSTRACT. The rock bream fish *Oplegnathus fasciatus* is one of the most popular aquaculture species in China. In the present study, 15 novel polymorphic microsatellite loci were isolated and characterized from a wild population of *O. fasciatus* from the Zhoushan coast of China. The number of alleles per polymorphic locus ranged from 4 to 9 in a sample of 30 individuals. Observed and expected heterozygosities per locus varied from 0.267 to 0.767 and from 0.395 to 0.859, respectively. Eleven of the 15 microsatellite loci conformed to Hardy-Weinberg equilibrium. No significant linkage disequilibrium between pairs of loci was detected. The present microsatellite markers could provide a useful tool for the genetic analyses of *O. fasciatus*.

Key words: *Oplegnathus fasciatus*; Microsatellite; Polymorphism

INTRODUCTION

The rock bream *Oplegnathus fasciatus* is a subtropical and carnivorous fish species that belongs to the Oplegnathidae family (Meng et al., 1995). This species is an economically important marine fish in East Asia. It has recently been considered as a potential species for commercial aquaculture and stock enhancement. For the purpose of fishery management and aquaculture development programs in *O. fasciatus*, studies on the genetic structure of *O. fasciatus* are urgently required. Microsatellite DNA loci are the most popular and powerful molecular markers for genetic analysis (Liu and Cordes, 2004). However, only 66 microsatellite sequences have been published for *O. fasciatus* in GenBank to date (An et al., 2006; Xu et al., 2009). More microsatellite markers are required for genetic analyses in *O. fasciatus*. In the present study, we report an additional 15 microsatellite markers for *O. fasciatus*.

MATERIAL AND METHODS

Thirty *O. fasciatus* individuals were collected from the coast of Zhoushan Island (122°30'N, 30°08'E). Microsatellite loci were isolated and cloned according to the protocol described by Yue et al. (2000). Genomic DNA was extracted from fin clips of two individuals (one male and one female) using a method described previously (Yue and Orban, 2005). The genomic DNA extracted was then digested with *Rsa*I and DNA fragments of 250-1000 bps were selected on agarose gel and recovered using a DNA purification kit (Tiangen, Beijing). Fragments were then ligated with 20 pmol 21- and 25-mer oligo adaptors (Fischer and Bachmann, 1998). Using the linker sequences as specific primers, the ligation products were amplified and the amplified products were hybridized to biotinylated probes $(CA)_{10}$ and streptavidin-coated magnetic beads (Dynal). Magnetic beads captured DNA containing microsatellites were eluted. The eluted fragments were amplified using 21-mer as primers, ligated to a pGEM-T-vector (Promega, Shanghai), and then transformed into XL-blue 1 competent cells (Stratagene). Colony polymerase chain reaction (PCR) products were tested in an ABI3100 sequencer (Applied Biosystems). Primers were designed for 15 microsatellites in the regions flanking repeats using the Primer 3.0 software (Rozen and Skaletsky, 2000).

All of the 15 pairs of primers were examined in a wild population of 30 individuals collected from the coastal water of Zhoushan. PCR amplification was performed in a 25-µL reaction mixture including 10 pmol of each primer set, 100 µM dNTPs, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 1U Taq polymerases (TaKaRa), and 40 ng template DNA. The reactions were carried out using the following conditions separately: 5 min at 94°C; 35 cycles of 30 s at 94°C, 45 s at annealing temperature, and 1 min at 72°C, and a final extension step of 7 min at 72°C. Following amplification, PCR products were mixed with 5 µL of sequencing dye and heated for 5 min at 95°C. Amplified products were separated on 6% denaturing polyacrylamide gels and visualized by silver staining. A 10-bp DNA ladder (Fermentas) was used to identify the alleles. The number of alleles, observed heterozygosity (H_O), and expected heterozygosity (H_E) were calculated using the POPGENE version 3.2 software (Raymond and Rousset, 1995). Conformance of genotypic proportions to Hardy-Weinberg equilibrium was tested using exact tests in the ARLEQUIN 3.01 software (Excoffier et al., 2005). Probability values were determined based on 10,000 dememorizations of 1000 batches with 10,000 iterations per batch.

RESULTS AND DISCUSSION

Fifteen polymorphic microsatellite markers were successfully screened in *O. fasciatus* (Table 1). The microsatellite data were analyzed using POPGENE version 3.2 (Raymond and Rousset, 1995), which indicated that the number of alleles ranged from four to nine, with an average of 5.93 alleles per locus. The observed and expected heterozygosity at the population level varied from 0.267 to 0.767 and from 0.395 to 0.859, respectively. The average H_O and H_E was 0.516 and 0.724, respectively. Four microsatellite loci, namely Opfa01, Opfa03, Opfa09, and Opfa11, deviated significantly from the Hardy-Weinberg equilibrium ($P < 0.01$). No evidence of linkage disequilibrium was observed in any pair of loci. In summary, these new informative microsatellite markers will be useful in genetic studies of *O. fasciatus*, and will thereby help to improve natural resource protection and genetic breeding studies.

Table 1. Characteristics of 15 microsatellite markers developed for *Oplegnathus fasciatus*.

Locus	Repeat motif	Primer sequence (5'-3')	Ta (°C)	N_A	Allele-size range	H_O	H_E	P_{HW}
Opfa01	$(GT)_{11}(GT)_5$	F: TTGTCTGACTGTCTGTGCTTTTGTT R: GTTGACTAATGCGATGCTGGTTC	54	7	236-260	0.333	0.841	0.000
Opfa02	$(CA)_{13}$	F: TGTTTGAGTGATGTTGTGCGTC R: GCTCCTCCAAGGGCTTTAGAT	54	4	101-251	0.267	0.395	0.215
Opfa03	$(TG)_8(GTGC)_{10}$	F: TGATGTTGTCTGGTCCTCTTCGT R: GGGTCCTGACTCACTCTACACTGG	55	5	157-215	0.567	0.781	0.000
Opfa04	$(AC)_{14}$	F: GCAGACACTTCTTCCCACAC R: GCGCACACTCACACGCCTT	55	5	228-306	0.467	0.680	0.384
Opfa05	$(AC)_{16}$	F: AGCGGAGTCCACACAGGT R: TTGGGGGGACACATAGTTT	51	5	201-219	0.767	0.782	0.017
Opfa06	$(CA)_7(AC)_{16}$	F: GTCCACCAAAAAATCCCATCT R: TGTTCTGTGTGCGACTCCTAAA	51	6	180-217	0.433	0.664	0.117
Opfa07	$(CA)_{17}$	F: TGAAGCCCAGGAGGCAACAC R: GCTAACAAAACCGCCGCAGT	55	6	471-621	0.433	0.723	0.059
Opfa08	$(CA)_9(AC)_5$	F: GAGAGGAGAAGTGAAATCTGAAT R: GCGTCTGCCTACCTAAAGC	52	4	184-203	0.633	0.623	0.068
Opfa09	$(GT)_{10}(GA)_5$ $(AG)_6(GA)_{17}$	F: AACACAGGGAACGGGCAG R: TTAGTCAGAGAAGAGGCAGAGAAAT	54	7	275-457	0.300	0.844	0.000
Opfa10	$(GA)_{17}$	F: ACAACGCTGGTAAATGGCTC R: TAAAACGATGGTGAAAGACGC	51	5	457-707	0.667	0.662	0.083
Opfa11	$(AC)_{24}(AC)_{13}$	F: TTGTTTACTGATTTTGTCTGTGGTTGT R: TCACGCACACACGCAGACTAAT	53	6	625-779	0.467	0.759	0.006
Opfa12	$(CT)_{19(}TC)_{11}$	F: AGTAAGGACAAACTCTGAATAGCA R: AGCAACAGCACAGTGGTAGC	52	6	445-514	0.633	0.786	0.089
Opfa13	$(TG)_{13}(GA)_{15}$ $(AG)_5$	F: CCACCTCACTGATTCCTACAT R: TCTCTCTTTTTCCCTCCCTA	51	9	455-549	0.700	0.859	0.075
Opfa14	$(GA)_{15}(AG)_5$	F: CAGCCCACCTCACTGATTCCTA R: CCTATTTGTCTGTGCTTCTTCGG	55	9	480-549	0.700	0.843	0.095
Opfa15	$(TG)_{13}(GT)_5$	F: CGATGGCAAGATACAGCACG R: AACACAGCGGAGACCTGATGA	55	5	523-655	0.367	0.615	0.011

Ta = annealing temperature, N_A = number of alleles, H_O = observed heterozygosity, H_E = expected heterozygosity, and P_{HW} = probability of departure from Hardy-Weinberg equilibrium.

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