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Development and characterization of microsatellite loci in *Megalonibea fusca*

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ABSTRACT. *Megalonibea fusca* is a commercially important large edible fish. In this study, the first set of 10 polymorphic microsatellite loci for *M. fusca* was developed and characterized. The number of alleles per locus ranged from two to five, with the observed and expected heterozygosities ranging from 0.0667 to 0.7667, and from 0.0644 to 0.5828, respectively. Most of the loci were in Hardy-Weinberg equilibrium ($P > 0.05$), except for two loci (Mf25 and Mf30) after a Bonferroni's correction ($P < 0.005$). These informative microsatellite markers will be useful in further studies of the population and conservation genetics of this species.

Key words: *Megalonibea fusca*; Magnetic bead enrichment; Microsatellite; Genetic markers

INTRODUCTION

Megalonibea fusca, which belongs to the family Sciaenidae, is a large offshore warm water fish, and is widely distributed in the East China Sea, the Southern Yellow Sea, and the Taiwan Strait (Zhu et al., 1963). It is a commercially valuable species in China because of its bladder, which is considered to be particularly valuable for human health and fitness. The resource distribution space of *M. fusca* is limited, and it has a small population size. Furthermore, overfishing and the deterioration of environmental conditions have resulted in wild stocks of *M. fusca* dramatically decreasing since 1970, and it has become an endangered species (Fu et al., 2009). To protect and sustainably exploit the species, a profound knowledge of species-level genetic structure and gene flow between populations is required. However, most studies have investigated its morphology (Sun et al., 2005), as well as artificial propagation and cultivation techniques (Chen, 2012). Wu et al. (2012) used the amplified fragment length polymorphism (AFLP) method to analyze the genetic diversity of cultured populations of *M. fusca*. However, no microsatellite loci have been reported in *M. fusca*. Microsatellite markers have been widely employed in population genetics and the conservation of biological resources, as they are highly polymorphic and co-dominant. In this study, we isolated the first set of 10 microsatellite markers of *M. fusca*, which will be useful in population genetic studies and the conservation of this species.

MATERIAL AND METHODS

A microsatellite genomic library was constructed using the fast isolation by AFLP of sequences containing repeats (FIASCO) protocol (Zane et al., 2002). Genomic DNA was extracted from the muscle of an individual *M. fusca* captured in Xiamen, China, using a gene DNA extraction kit DP304-03 (Tiangen), according to the manufacturer protocol, for automated DNA extraction and digestion by the restriction enzyme *Mse*I at 65°C for 3.5 h. Small fragments (300-1200 bp) were ligated to *Mse*I adapter 1 (5'-ACGATGAGTCCTGAG-3')/ *Mse*I adapter 2 (5'-TACTCAGGACTCAT-3') by T4 DNA ligase at 37°C for 3.5 h, denatured at 95°C for 10 min, then immediately hybridized to the biotinylated probes $(CT)_{15}$ and $(GT)_{15}$. The hybridization complex was lifted out with streptavidin-coated magnetic spheres (Promega). Enriched, bound single DNA fragments containing microsatellite repeats were captured after washing from the beads. The microsatellite-enriched DNA fragments were amplified by polymerase chain reaction (PCR) and purified using GenCleanPCR (Generay), ligated into a pMD19-T vector (Takara) at 16°C for 3 h, and transformed into competent *Escherichia coli* cells for further selection on ampicillin plates. Positive clones were detected by PCR amplification using universal M13 primers, and the PCR products were visualized on 1% agarose gels. A total of 242 recombinant clones in the size range of 500-1000 bp were shake-cultured for 8 h (37°C, 300 rpm) and then sequenced by Invitrogen (Guangzhou). After analyzing the sequences, 58 clones containing microsatellites were selected to design primers. Forty-one pairs of primers were successfully designed using Primer Premier 5.0.

Polymorphisms in all the primers were detected using 30 individuals collected from Xiamen, China. The PCR was performed in a 10-μL volume that contained 50 ng genomic DNA, 0.25 U Taq DNA polymerase (Fermentas), 10X Taq buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM forward primer, and 0.4 μM reverse primer. The cycling conditions were

as follows: 94°C for 5 min, followed by 30 cycles at 94°C for 45 s, annealing temperature for 45 s (Table 1), and elongation at 72°C for 45 s, followed by one cycle at 72°C for 10 min. The amplified products were electrophoresed on Sequi-Gen™ Sequencing Cell and visualized by silver staining. The number of alleles, the observed and expected heterozygosities, the Hardy-Weinberg equilibrium (HWE), and the genotypic linkage disequilibrium were estimated using the POPGEN32 software. The polymorphic information content was calculated using CERVUS 3.0.

RESULTS AND DISCUSSION

Five monomorphic loci and 10 polymorphic loci were screened. The genetic information for these 10 polymorphic loci is presented in Table 1.

Table 1. Basic genetic information of 10 microsatellite primers in *Megalonibea fusca*.

Locus ID	GenBank accession No.	Primer sequence (5'-3')	Allele size	Ta	PIC	N_A	H_O	H_E
Mf8	KF292220	F: AGTGGTCAGTCATTCGCT R: ATTGCTCTCTTTCTCCGT	189-198	56	0.563	3	0.7667	0.5617
Mf 9	KF292221	F: AACTCTGTTGTGCTGCTCA R: TTCTTTGTCTCCTTTTCCA	160-167	50	0.375	2	0.4	0.32
Mf 11	KF292222	F: ATCAAGACACCAGGACA R: ATAGGCACATAAAAGCA	188-194	48	0.032	2	0.0667	0.0644
Mf 22	KF292223	F: CACAAAAGCAACAATGAC R: ACTGAACCACAGAGAACG	213-218	52	0.624	4	0.3	0.255
Mf 25	KF292224	F: GTGAAAAACGAAAACAATGA R: TAAAAAATCTGAAGAAACGC	300-311	52	0.495	5	0.2333	0.5450*
Mf 27A	KF292225	F: AGCTGATGACTTTTTGT R: CAGAGATGTGAAGACTG	168-194	44	0.393	4	0.5	0.5828
Mf 30	KF292226	F: AATGGAGGGAGTCAGTGTA R: TGTGTATGCCTTTCTGTTC	200-211	48	0.421	4	0.3103	0.3442*
Mf 56	KF292227	F: ATTTATCATCCAACCCAG R: CGTCTCTCATACTTTCCC	141-158	47	0.283	3	0.0667	0.0644
Mf 69	KF292228	F: TAACAGAGGAGCACGGGAG R: CCAAAACGTCAAAGGCAAT	120-123	56	0.406	3	0.3667	0.2994
Mf 77	KF292229	F: AGACGCAACACGCTACAT R: ACAGCCCGTTACATCAAA	149-161	56	0.399	3	0.3667	0.3761

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

The number of alleles per locus ranged from two to five, and the PIC was 0.032-0.624. The observed and expected heterozygosities were 0.0667-0.7667 and 0.0644-0.5828, respectively. Most of the 10 loci were in HWE ($P > 0.05$), except for two loci (Mf25 and Mf30) after a Bonferroni's correction ($P < 0.005$). The 10 polymorphic microsatellite loci screened in this study will be useful in future studies of genetic diversity and population genetic structure analysis, and will subsequently provide genetic data for breeding and conservation genetic studies.

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