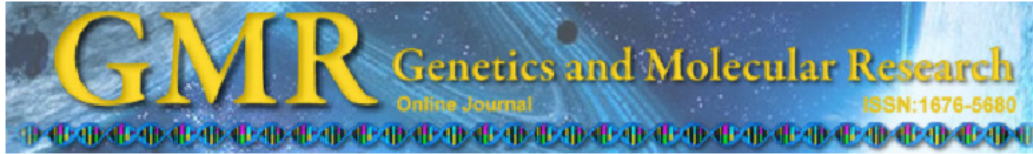

Isolation and characterization of new microsatellite markers in the pen shell *Atrina pectinata* (Pinnidae)

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ABSTRACT. The pen shell, *Atrina pectinata*, is a commercially important bivalve species, widely consumed in the Asian Pacific region. We identified 16 new microsatellite makers for *A. pectinata* using a modified fast isolation by AFLP of sequences containing repeat protocols; 27 individuals were collected from Xiamen to evaluate the degree of polymorphism. The number of polymorphic alleles per locus ranged from 2 to 11. The observed and expected heterozygosities were 0.050-0.913 and 0.049-0.869, respectively. The loci identified in this study could provide a useful tool for research on genetic diversity and genetic differentiation of *A. pectinata* populations.

Key words: Genetic markers; Microsatellite; FIASCO; *Atrina pectinata*

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INTRODUCTION

The pen shell *Atrina pectinata* (Linnaeus, 1767) is a large, fan-shaped bivalve (Liang, 1996). It belongs to the family Pinnidae and is widely distributed in temperate and tropical marine waters, especially in the Indian and Pacific Oceans. *A. pectinata* is a kind of popular seafood in China, Korea and Japan. In recent decades, due to overfishing and habitat destruction, the wild stocks of *A. pectinata* have declined sharply in China (Ren and Guo, 2005). For the purpose of protecting resources and rational exploitation, studies on the genetic structure of *A. pectinata* at both the species and population levels are urgently required (Waples, 1998). Due to their high degree of polymorphism and co-dominance, microsatellites are considered efficient molecular markers and have been widely used in population genetics, population differentiation, kinship analysis, linkage analysis and evolutional studies (Li, 2006). In the present research, a new marker suite of 16 loci in *A. pectinata* was identified, which provides a useful tool in population genetic studies and the protection of species resources.

MATERIAL AND METHODS

The microsatellite loci were developed according to the FIASCO protocol (Zane et al., 2002). Genomic DNA was extracted from musculature using the DNA extraction kit (Sangon) and digested by restriction enzymes FastDigest *Tru*1I (Fermentas) for 10 min at 65°C. The restriction fragments were then ligated with *Mse*I adaptor A (5'-ACGATGAGTCCTGAG-3')/*Mse*I adaptor B (5'-TACTCAGGACTCAT-3') by T4 DNA ligase (Fermentas) for 3.5 h at 37°C. Afterwards, the digestion-ligation fragments were hybridized to the biotin-labeled oligonucleotide probes $(GT)_{15}$ and $(CT)_{15}$, and the fragments containing microsatellites were captured with Streptavidin-coated Magnetic Sphere Particles (Promega). The recovered DNA fragments were amplified by *Mse*I primer (5'-TACTCAGGACTCAT-3') and purified with the GenClean Cycle Pure kit (Generay) to remove the extra adaptors and dNTPs. The purified products were ligated to pMD19-T vector (Takara) at 16°C for 2h and then transformed into Escherichia coli competent cells. Transformants were selected on LB agar plates containing ampicillin. The positive clone fragments were amplified using universal M13 primer, and the PCR products were visualized on 1% agarose gels. Two hundred and five clones with size ranging from 500 to 1000 bp were selected for sequencing by the BGI Company (Beijing), and 58 pairs of primers were designed by Primer Premier 5.0.

All 58 pairs of primers were tested on 27 wild individuals collected from Xiamen, China. PCR amplification was performed on a gradient thermal cycler (Bio-RAD) in a 10 µL reaction mixture containing 50 ng genomic DNA, 10X *Taq* buffer, 2 mM $MgCl_2$, 0.25 U *Taq* DNA polymerase (Fermentas), 0.2 mM of each dNTP, 0.4 µM forward primer, 0.4 µM reverse primer for all loci. The reactions were carried out using the following conditions separately: 5 min at 94°C, 32 cycles of 45 s at 94°C, 45 s at anneling temperature (Table 1) and 1 min at 72°C, and a final extension step of 10 min at 72°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.

RESULTS AND DISCUSSION

Error analysis was carried out by MICRO-CHECKER (Van Oosterhaut et al., 2004).

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The observed and expected heterozygosities, deviation from Hardy-Weinberg equilibrium (HWE) and genotypic linkage disequilibrium (LD) were calculated by POPGENE 32 (version1.32) (Yeh et al., 2000). All results were adjusted for multiple simultaneous comparisons using a sequential Bonferroni's correction (Rice, 1989). Polymorphic information content (PIC) was estimated using CERVUS (version 3.0).

The characterization of the loci is presented in Table 1. The number of polymorphic alleles per locus ranged from 2 to 11. The observed and expected heterozygosities were 0.050~0.913 and 0.049~0.869, respectively. The PIC varied from 0.224 to 0.871. Three loci (Ap2-4, Ap2-24 and Ap2-113) deviated significantly from HWE after Bonferroni's correction ($P < 0.05$, Table 1).

The new polymorphic microsatellite markers developed in this study should provide a useful tool to the study of population structure of *A. pectinata*.

Table 1. Characterization of 16 microsatellite loci isolated from *Atrina pectinata*.

Locus ID	GenBank accession No.	Primer sequence (3'-5')	Repeat motif	N_A	Ta (°C)	Allele size (bp)	H_O	H_E	PIC
Ap1-35	JN636856	F: CTGTTTTCAGGGTTCAGGTGTT R: CTCCTGGTCTGTCAAGATTATGC	$(CA)_6$	5	52	150~186	0.600	0.718	0.722
Ap1-39	JN636857	F: GCGTTCTGTCGCTAATAAA R: GAGATACCCAATGTGCTGA	$(ATC)_{10}$	5	55	224~244	0.417	0.492	0.556
Ap1-46	JN636858	F: TCCTTTTCCCTTCAGTTCATA R: TACTCTTGTTCTAAACGGTGT	$(GA)_{32}$	9	50	252~308	0.708	0.825	0.833
Ap1-50	JN636859	F: GTGCCACTTATTCTTGTATTCAG R:AACGAATAGACATCACGTAATGA	$(CT)_{20}$	6	52	250~316	0.615	0.782	0.768
Ap2-4	JN636860	F: GAATAAAACTTTGGGACG R: TTCTGACATAATTGACGC	$(ATG)_8$	5	54	200~218	0.318	0.731	0.754*
Ap2-6	JN636861	F: GAGAAATCGTTCCAACTTGAC R: CTTTATTCCTGCCTACTGCTT	$(AGAC)_4$	3	60	206~220	0.500	0.384	0.374
Ap2-11	JN636862	F: CGTGACCCGAACCTATTA R: TGCTCTGATTACGCAAGA	$(TAGA)_{10}$	11	63	208~262	0.913	0.869	0.871
Ap2-12	JN636863	F: AAGGTTATTAGCCGTGTTTAC R: AACCCAGTTTGTCATTTATTC	$(TC)_{31}$	3	58	242~252	0.192	0.242	0.282
Ap2-22	JN636864	F: TGTAACAGAGCAAATGGA R: GTTTGGATGTGCGAGTAT	$(GA)_{30}$	2	58	218~222	0.050	0.049	0.340
Ap2-24	JN636865	F: TTATGCGGATGATTCTACT R: TCCCCATTCCTATGACGAG	$(TC)_9TG(TC)_6N$ $(TC)_5N(TC)_{17}$	5	63	258~312	0.320	0.690	0.683*
Ap2-77	JN636866	F: ACGCACATGCTGAGCTTAGTTT R: GAGTCCTGAGACCCATTTACCC	$(TTTGAT)_5$	3	58	198~210	0.167	0.155	0.299
Ap2-113	JN636867	F: TAGCCCATTATGTACTTTC R: TCAACATCAGTCAGGGA	$(GA)_6$	2	60	292~302	0.118	0.457	0.572*
Ap3-8	JN636868	F: ACTGGACCTGATTTTATT R: TTTTATCTATTCGCCTCA	$(TC)_{26}$	3	48	164~168	0.208	0.518	0.526
Ap3-19	JN636869	F: AGGTCGTCCTTCAAAT R: CTGTTGGGTCATTAGG	$(CT)_{23}$	3	43	158~162	0.115	0.266	0.224
Ap3-50	JN636870	F: AGCACCTGTGACAATG R: GTTTCCCGCTAATCTA	$(GT)_{17}$	5	43	270~298	0.417	0.355	0.451
Ap3-58	JN636871	F: ACCTGTGATGGTCATAAAT R: GAAACCTAAATGTGCTTGT	$(AG)_5N(AG)_9N(GA)_5$	5	55	258~292	0.583	0.768	0.777

N_A = number of polymorphic alleles; Ta = annealing temperature; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphism information content *Indicates significant departure ($P < 0.05$) from expected Hardy-Weinberg equilibrium conditions after correction for multiple tests (k = 16).

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