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Isolation and characterization of novel polymorphic microsatellite loci in *Atrina vexillum* Born (Pinnidae)

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ABSTRACT. The pen shell, *Atrina vexillum* Born, is an edible shellfish that is widely consumed in the Asia-Pacific region. In this study, 11 polymorphic microsatellite loci were isolated from *A. vexillum*, and 30 wild individuals were used to evaluate the degree of polymorphism of these markers. The number of alleles per locus ranged from 3 to 8. The polymorphism information content varied from 0.199 to 0.831. The observed and expected heterozygosities were 0.1000-0.8667 and 0.1244-0.8356, respectively. Two loci deviated significantly from the Hardy-Weinberg equilibrium (HWE) after a Bonferroni correction, while the other nine loci were at HWE. These microsatellite loci will be useful in further studies on population genetic analyses, and will provide important genetic data for the conservation and recovery of *A. vexillum*.

Key words: Genetic markers; *Atrina vexillum* Born; Microsatellite; Magnetic bead enrichment

INTRODUCTION

The pen shell, *Atrina vexillum* Born, is a large, wedge-shaped benthic bivalve and a commercially important species. It belongs to the family Pinnidae, and is widely distributed in the Indo-West Pacific, from eastern Africa to India and the Philippines, and north to Japan and south to Indonesia. In China, the pen shell lives in 20-50 m deep mud at the bottom of the ocean, and is mainly found in the South China Sea (Wang, 1979). In recent years, wild stocks of *A*. *vexillum* have declined sharply, due to commercial catches and habitat destruction (Yu et al., 1999). In order to protect *A*. *vexillum*, it is necessary to fully understand its genetic structure. Microsatellite loci are powerful genetic markers that have the advantages of co-dominance, high DNA polymorphism, and reliability, and have been widely used in population genetics, population differentiation, linkage analyses, and evolutionary studies (Li, 2006). Most previous studies on *A*. *vexillum* have focused on its morphology (Yu et al., 1999) and isozyme patterns (Wang and Yu, 2000). However, no microsatellite loci have been reported for the species to date. To the best of our knowledge, this study is the first to identify microsatellite loci for *A*. *vexillum*, which could provide useful tools in population genetic studies and the conservation of *A*. *vexillum*.

MATERIAL AND METHODS

The microsatellite loci were developed according to the FIASCO protocol (Zane et al., 2002). High-quality DNA was extracted from the musculature of a single wild *A*. *vexillum* individual captured in Hainan, China, using the gene DNA extraction kit DP304-03 (Tiangen, Beijing, China). Genomic DNA was digested with the restriction enzyme FastDigest Tru1I at 65°C for 10 min, and the digested fragments were then ligated to a *Mse*I adapter A (5'-ACGATGAGTCCTGAG-3')/*Mse*I adapter B (5'-TACTCAGGACTCAT-3') by T4 DNA ligase at 37°C for 3.5 h. The digestion-ligation mixture was then hybridized to the biotinylated probes $(CT)_{15}$ and $(GT)_{15}$. Fragments containing microsatellite repeats were captured with Streptavidin MagneSphere® Paramagnetic Particles (Promega, Madison, USA). The recovered DNA fragments were amplified using the *Mse*I A primer. Polymerase chain reaction (PCR) products were purified using GenCleanPCR (Generay, Shanghai, China) and ligated to the PMD19-T (Takara, Shiga, Japan) at 16°C for 3 h, and subsequently transformed into *Escherichia coli*. Transformants were selected on lysogeny broth agar plates containing ampicillin. Positive clones were detected by PCR amplification using universal M13 primer. After visualizing the PCR product clones using 1% agarose gels, 236 clones, with sizes ranging from 500 to 1200 bp, were sequenced by Invitrogen (Guangzhou, China), and 11 pairs of primers were designed by Primer Premier 5.0.

The polymorphism at each locus was determined in 30 wild individuals collected from Hainan, China. PCR amplification was carried out in 10-µL volumes, with 50 ng genomic DNA, 0.25 U Taq DNA polymerase (Fermentas, Vilnius, Lithuania), 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 94°C for 5 min, followed by 32 cycles at 94°C for 45 s, annealing temperature for 45 s (Table 1), and elongation at 72°C for 45 s, with a final extension at 72°C for 10 min. PCR amplifications were separated on 6% denaturing polyacrylamide gels, and visualized using silver staining. POPGEN32 (version 1.32; Yeh et al., 2000) was used to calculate the number of alleles, the observed (H_O), and the expected (H_E) heterozygosity, the

Hardy-Weinberg equilibrium (HWE), and the genotypic linkage disequilibrium. CERVUS 3.0 was used to indicate polymorphism information.

Table 1. Characterization of 11 microsatellite loci isolated from *Atrina vexillum*.

Locus ID	GenBank accession No.	Primer sequence (5'-3')	Repeat motif	Allele size (bp)	Ta (°C)	PIC	Na	H_O	H_E
Av 1-1	KF048854	F: GAAAAAGTAAGTGAAACGC R: GGTAATAATGGCTGCTGTA	$(AAATC)_3N\text{-}(CAC)_6N(CAC)_3$	142-150	52.0	0.475	4	0.3667	0.3394
Av 1-10	KF048855	F: AGAGGCAAGGTGGCAGT R: CGGGGGAGAGAACGGAC	$(GA)_{19}N(AG)_5$	258-272	56.0	0.642	4	0.7667	0.7239
Av 1-27	KF048856	F: ACATTATTGAAAGAGGA R: ATAGAAAGTAAGGCTAG	$(AG)_{16}N(TG)_4$	188-196	52.0	0.483	4	0.4333	0.4078
Av 1-33	KF048857	F: CAGAGGTCCTTGTTTGTCCAT R: ACACCAGTCTACCGATTTGCT	$(TC)_{19}$	257-278	50.0	0.502	3	0.2759	0.3216*
Av 1-46	KF048858	F: GTGTGTGCGTGCGTGTAG R: CCGTTGCCGTGATTTTAG	$(TG)_5$	204-224	56.0	0.378	3	0.4333	0.5761
Av 1-51	KF048859	F: TGATCCATGCCATTCTATTTTA R: ATACTTTGTGACGCAGTTTGAC	$(TTTGT)_3N\text{-}(TTG)_3T_{28}$	128-150	44.0	0.656	3	0.6000	0.7350
Av 1-115	KF048860	F: TTACGCAAATCTTGAAAAT R: ACAAACTGAACTGGGTCCT	$(CT)_{17}$	191-242	52.0	0.831	8	0.8667	0.8356
Av 2-99	KF048861	F: ATAAAGATAAAGAAATTGGAAAT R: GTAAATACTTAGGCTGTGATGAA	$(CT)_{12}$	210-265	50.0	0.792	7	0.6429	0.8055
Av 3-25	KF048862	F: CATACCTTTCTTTTTTCCC R: CTAACGTCTGGTGTTCACA	$(GT)_4(AC)_3$	266-269	52.0	0.199	7	0.1000	0.3394*
Av 3-48	KF048863	F: ATTTACTGCACATTTCACGC R: GCCAGTCTCTCCTTTTGTCT	$(GACA)_3N(CA)_{13}$	242-270	50.0	0.571	7	0.1333	0.1244
Av 3-54	KF048864	F: TGGGATTTTACAATACAAC R: ACTTCAAGAGTGCTCATAC	$(AG)_{21}$	210-272	52.0	0.688	7	0.6333	0.8094

Na = number of polymorphic alleles; Ta = annealing temperature; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphism information content. *Indicates significant departure ($P < 0.0045$) from expected Hardy-Weinberg equilibrium conditions, after correction for multiple tests (k = 11).

RESULTS AND DISCUSSION

We successfully isolated 11 polymorphic microsatellite loci (Table 1). The number of polymorphic alleles per locus ranged from 3 to 8, and the polymorphism information content ranged from 0.199 to 0.831. The observed and expected heterozygosities were 0.1000-0.8667 and 0.1244-0.8356, respectively. No significant linkage disequilibrium was detected for any pairwise combination of loci. Nine loci were at HWE ($P > 0.05$); after a sequential Bonferroni correction ($P < 0.0045$), Av1-33 and Av3-25 were found not to be at HWE, possibly because of the presence of null alleles that were detected using Micro-Checker (Van Oosterhout et al., 2004). The 11 microsatellite loci identified would be useful for population genetic structure analyses and a genetic diversity assessment of *A. vexillum*, and will facilitate conservation genetic studies on this species.

This is the first identified set of polymorphic microsatellite loci in *A. vexillum*, and could provide a useful tool for future studies of genetic variation, population structure, and conservation genetics of *A. vexillum*.

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