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Isolation and characterization of microsatellite markers in *Atrina vexillum* Born

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ABSTRACT. *Atrina vexillum* Born is an economically valuable species, widely distributed in the coastal waters of temperate and tropical areas of the Asia Pacific region. Twenty one novel microsatellite loci were identified in the genome of *A. vexillum* Born using the protocol for fast isolation by amplified fragment length polymorphism of sequence containing repeats. Thirty-two wild type individuals were used to evaluate the degree of polymorphism of these markers. We identified 13 polymorphic and 8 monomorphic loci with the number of alleles per locus and the polymorphism information content ranging from 2 to 5 and 0.141 to 0.664, respectively. The observed and expected heterozygosity varied from 0.1250 to 0.7000 and 0.1223 to 0.6216, respectively. Two loci deviated significantly from Hardy-Weinberg equilibrium (HWE) after Bonferroni correction, whereas the other loci were in HWE. These loci are expected to provide useful information for population genetic studies of *A. vexillum* Born.

Key words: *Atrina vexillum* Born; Microsatellites; Genetic markers

INTRODUCTION

Atrina vexillum Born, belonging to the family Pinnidae, is an economically valuable species, widely distributed in the coastal waters of the temperate and tropical areas of the Asia-Pacific region. In China, it is found mostly in the South China Sea (Wang, 1979). A large wedge-shaped bivalve, it commonly lives 20-50 m beneath the ocean floor. The natural population of *A. vexillum* Born has decreased considerably due to overexploitation because of its high commercial value and deteriorating habitat (Yu et al., 1999). In order to conserve genetic resources and assess genetic diversity in *A. vexillum* Born, it is necessary and important to isolate some polymorphic microsatellite markers for this shellfish.

Microsatellite DNA, also called simple sequence repeat or short tandem repeat consists of repeating nucleotide units 2-6 bp (Chen et al.,2012). Microsatellite markers are non-coding, highly polymorphic, co-dominant DNA markers, which are effective tools for genetic analysis. It has been widely used in population genetics, population differentiation, linkage analysis, and evolutionary studies (Rice and Pechenik, 1992; Xu et al., 2009). Previous studies on *A. vexillum* Born focused on its morphology and isozyme patterns (Wang and Yu, 2000). Eleven polymorphic microsatellite loci have been reported in *A. vexillum* Born. However, the population structure of this economically valuable species has been insufficiently studied, and studies on the development of genetic markers are urgently needed. In the present study, a novel marker suite of 21 loci in *A. vexillum* Born was developed, which may be useful in further studies on population genetics and measures for the protection of the species.

MATERIAL AND METHODS

Twenty one microsatellite markers were identified in *A. vexillum* Born using the fast isolation by amplified fragment length polymorphism of sequences containing repeats protocol (Zane et al., 2002). Total genomic DNA was extracted from the muscle tissue of a wild *A. vexillum* Born individual collected from Hainan, China, using the genomic DNA extraction kit (Tiangen, Beijing, China) according to the manufacturer instructions. DNA concentration was estimated using an ultraviolet spectrophotometer and by electrophoresis on a 1% agarose gel. The obtained DNA was diluted to 100 ng/μL followed by digestion with 10 U restriction enzyme *FastDigestTru1I* in a 25-μL volume and incubation for 10 min at 65°C. The digested fragments, ranging from 500 to 1200 bp, were purified and ligated to *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 digestion-ligation mixture was subsequently denatured and hybridized to the biotin-labeled oligo-nucleotide probes $(CT)_{15}$ and $(GT)_{15.}$ Fragments containing microsatellite repeats were captured with Streptavidin-coated Magnetic Sphere Particles (Promega, Madison, WI, USA), and the unannealed DNA was washed away. The recovered products were amplified using *Mse*I adapter1. The PCR products were purified to remove the extra dNTPs and adaptors. The purified products were ligated to pMD19-T vector (Takara, Shiga, Japan) and then transformed into *Escherichia coli* (Invitrogen, Carlsbad, CA, USA) for further selection on ampicillin plates.

A total of 178 positive clones were amplified via colony-PCR with universal M13 primers. DNA fragments above 500 bp were sequenced by Invitrogen (Shanghai, China); 146 sequences with microsatellites were successfully obtained. Forty eight primer pairs were designed using the Primer Premier version 5.0 (Singh et al., 1998). The amplification conditions for all the primers

were optimized in an Eppendorf Mastercycler Gradient System (Eppendorf, Hamburg, Germany). Successful amplification primers were validated using the genomic DNA of 32 wild individuals of *A. vexillum* Born collected from Hainan Island, China. The 10-μL amplification reaction consisted of 50 ng genomic DNA, 10X Taq buffer, 2 mM $MgCl_2$, 0.4 μM each primer, 0.2 mM each dNTP, and 0.25 U Taq DNA Polymerase (Fermentas). PCR was carried out under the following conditions: Initial denaturation at 94°C for 5 min, followed by 37 cycles of denaturation at 94°C for 40 s, annealing at an optimal temperature (Table 1) for 40 s, and extension at 72°C for 1 min, with a final elongation step at 72°C for 10 min. The PCR products were electrophoresed on a 6% denaturing polyacrylamide gel in a Sequi-Gen Sequencing Cell (Bio-Rad, Hercules, CA, USA) and visualized using silver staining. Data matrices was analyzed to estimate the observed heterozygosity (H_O), expected heterozygosity (H_E), number of alleles (N_A) and the polymorphism information content (PIC) by the software POPGEN 32 (version 1.32) and CERVUS 3.0 (version 3.0) (Yeh et al., 2000).

Table 1. Statistical information of 21 microsatellite loci in *Atrina vexillum* Born (32 individuals).

GenBank accession No.	Locus ID	Primer sequences (5'-3')	Repeat motif	Ta (°C)	N_A	PIC	H_O	H_E	Allele size (bp)
KR704581	Q-1	F: AACGCATCGTAACATTTTC R: AGCCTACAGTTCACATCCC	$(GT)_{12}$	51	4	0.460	0.2727	0.2495	183-195
KR704582	Q-2	F: GACAAACGGACATACAGA R: TCACGAAATTACATCCC	$(CT)_{17}$	55	4	0.382	0.1923	0.2451	130-154
KR704583	Q-3	F: GAATCTCGCTGAAATAAC R: TGTCCCAGAACTGTCATA	$(TC)_{34}(TG)_{22}$	45	2	0.255	0.3667	0.3045	140-150
KR704584	Q-4	F: CCTAAATGACAATGAGCAG R: GCAAAAATCGCTGTAGTAC	$(TG)_{42}$	48	4	0.445	0.6217	0.5033	173-191
KR704585	Q-5	F: GAGATACCAAGAGTCAATG R: GACTGCCAAGTAACAAA	$(AG)_{19}$	43	5	0.351	0.1250	0.1223	132-164
KR704586	Q-6	F: GTTATGATCCCAAGAGC R: AACGGACATACATACTGAC	$(CT)_{16}CC(CT)_{46}$	54	3	0.420	0.7000	0.5011	210-232
KR704587	Q-7	F: ATTTCAAACGTAAAGGTTGTC R: CAGTTCTTGTTGGTGGTGTA	$(AC)_{23}$	48	4	0.436	0.3200	0.2865	92-115
KR704588	Q-8*	F: ATCTACCGATCCATAAGC R: GTCATCCATTTCCCAAC	$(AC)_{26}$	46	4	0.664	0.2727	0.6216	134-167
KR704589	Q-9*	F: GTCACATTCATAAAACAA R: CTCTGTCTACCCCATT	$(CT)_{12}N(CA)_{28}$	50	4	0.506	0.3750	0.3768	130-154
KR704590	Q-10	F: TAAAACAAGGCAAATAAG R: CAAGACTGGTAGTAAAAGG	$(GT)_{18}$	53	3	0.193	0.2333	0.2130	160-175
KR704591	Q-11	F: AATTCGTATTCGTTCAC R: CATAGGCTTCCTGTTT	$(CT)_{12}N(CA)_{28}$	51	3	0.227	0.2667	0.2441	143-158
KR704592	Q-12	F: CGTACAGACTGGAAGATGG R: CGTTTTCACGGCTATTT	$(CT)_5$	49	2	0.141	0.1667	0.1554	126-137
KR704593	Q-13	F: GCTTTTAGTACTCCAAG R: GAGATTTTGTTGTTGAA	$(AG)_{23}N(CAA)_3$	48	4	0.494	0.2105	0.2845	110-136
KR704594	Q-14	F: CGGACATACAGACTGACACG R: CTGGGACACTCCTAATGAAAT	$(CT)_{18}AT(CA)_{11}$	54	NA	NA	NA	NA	168
KR704595	Q-15	F: TAACAGTTGTCCCTCTG R: GACCAGATTTTAGTTATTG	$(TG)_{16}(GA)_{15}$	42	NA	NA	NA	NA	227
KR704596	Q-16	F: TTTTCCACAATCCTGT R: ACTTTCCTATAAATCACTG	$(GA)_{12}$	52	NA	NA	NA	NA	125
KR704597	Q-17	F: AATTGACATGTATTTTGTA R: TATATCTAACTTTGGTTTT	$(TC)_{17}$	60	NA	NA	NA	NA	142
KR704598	Q-18	F: CAAGTAGTGGAGGGTG R: TCTTTATCTGTTACTGAAA	$(AC)_{39}$	50	NA	NA	NA	NA	210
KR704599	Q-19	F: TTGTATTGTCCAAATGTC R: ACTGAAAGATAAGGAGAAA	$(CT)_{10}$	54	NA	NA	NA	NA	125
KR704600	Q-20	F: GCTTTCTCGCTCCTGAAC R: TGGTGGATGACACTCTTTACT	$(TC)_{17}$	53	NA	NA	NA	NA	223
KR704601	Q-21	F: GAGATACCAAGAGTCAATG R: GACTGCCAAGTAACAAA	$(AG)_{19}$	43	NA	NA	NA	NA	155

Ta = annealing temperature; N_A = number of polymorphic alleles per locus; PIC = polymorphism information content; H_O = observed heterozygosity; H_E = expected heterozygosity, *highly significant deviations ($P < 0.0038$) of locus from Hardy-Weinberg equilibrium after Bonferroni correction (k = 13).

RESULTS

Twenty one novel microsatellite markers were successfully amplified (Table 1); these included 13 polymorphic microsatellite loci and 8 monomorphic loci. The N_A and the PIC ranged from 2 to 5 and 0.141 to 0.664, respectively. The H_O and H_E varied from 0.1250 to 0.6217 and 0.1223 to 0.6216, respectively. Deviations from the Hardy-Weinberg equilibrium (HWE) and genotypic

linkage disequilibrium were tested using POPGENE 32 (version 1.32). Most loci were in HWE (P > 0.05), except locus Q-8 and Q-9, even after sequential Bonferroni correction (P < 0.0038).

DISCUSSION

Many reasons could contribute to this result, such as natural selection, inbreeding, the Wahlund effect, null alleles, and size homoplasy (Dai et al., 2013). Also, the small sample size could account for the significant deviations from HWE. Therefore, to reveal the accurate genetic background of wild type *A. vexillum* Born, more sample areas and larger sample sizes should be used for further studies.

The loci characterized in this study may be useful for further analysis of the genetic diversity and structure of *A. vexillum* populations, and for designing conservation strategies for *A. vexillum* Born.

Conflicts of interest

The authors declare no conflicts of interest.

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