Development and characterization of microsatellite markers for molecular genetic diversity in *Atrina pectinata*

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ABSTRACT. The pen shell, *Atrina pectinata*, is an economically valuable species that is widely distributed along the coastal waters of temperate and tropical areas, mainly growing in the Indian and Pacific Oceans. Eight novel microsatellite loci from the genome of *A. pectinata* were developed using fast isolation by amplified fragment length polymorphism of sequence containing repeats. The loci were screened in 30 wild individuals. The results showed that the number of alleles per locus and the polymorphism information content ranged from 2-6 and from 0.233-0.447, respectively. Observed and expected heterozygosities varied from 0.2069-0.7931 and 0.1887-0.5124, respectively. No significant deviations from Hardy-Weinberg equilibrium were detected. These microsatellite loci will be useful for further population studies of genetic diversity, population structure assessment, and conservation of *A. pectinata*.

Key words: *Atrina pectinata*; Genetic markers; Microsatellite; Fragment length polymorphism; Sequence containing repeats

INTRODUCTION

The pen shell *Atrina pectinata* belongs to the order Mytiloida, family Pinnidae, which is widely distributed along the coastal waters of temperate and tropical zones in China and mainly grows in southeastern coastal provinces such as Fujian and Guangdong (Suck An et al., 2011). *A. pectinata* is a bivalve animal with high economic value for its rich nutrition and good taste (Fu et al., 2010). However, the natural resource of *A. pectinata* has sharply decreased because of overexploitation and worsening habitat conditions in recent decades (Ren and Yang, 2005). Currently, studies of the population structure of this economically valuable species remain insufficient and are limited for use in genetic resource conservation. Thus, studies on the development of genetic markers in *A. pectinata* are urgently needed.

Because they are non-coding, highly polymorphic, co-dominant genetic markers, microsatellites are efficient tools for researching population genetics (Li, 2006). In this study, a novel marker suite of 8 loci in *A. pectinata* was developed, which may be useful for further studies of the population genetics and the protection of species resources.

MATERIAL AND METHODS

The microsatellite-enriched library was developed from a single *A. pectinata* using fast isolation by amplified fragment length polymorphism of sequences containing repeats (Zane et al., 2002). Thirty individuals of *A. pectinata* were collected from Hainan, China, and were assayed for the 8 microsatellite markers. Genomic DNA was extracted from muscle tissues using a Genomic DNA Extraction kit (Tiangen, Beijing, China), followed by 1% agarose gel electrophoresis and ultraviolet spectrophotometry. Samples were digested with restriction enzyme FastDigestTruII by incubation for 10 min at 65°C. The *MseI* adapter 1 (5'-ACGAT-GAGTCCTGAG-3') and *MseI* adapter 2 (5'-TACTCAGGACTCAT-3') were then ligated to the digestion fragments using T4 DNA ligase (Fermentas, Vilnius, Lithuania) overnight at 22°C. The linker-ligated mixture was denatured at 95°C for 10 min and hybridized to the biotin-labeled oligonucleotide probes (CT)₁₅ and (GT)₁₅, then captured using Streptavidin-coated Magnetic Sphere Particles (Promega, Madison, WI, USA), with washing away of the non-annealed DNA. The recovered DNA fragments were amplified using *MseI* adapter 1. After purification using GenCleanPCR (Generay, Shanghai, China), the products were ligated into the PMD19-T vector (Takara, Shiga, Japan) at 16°C for 8 h and transformed into *Escherichia coli* cells (Invitrogen, Carlsbad, CA, USA). The transformants were cultured on Luria-Bertani agar plates supplemented with 60 µg/mL ampicillin.

A total of 128 positive clones were amplified via colony-polymerase chain reaction with universal M13 primers. Sixty positive clones with DNA fragments above 500 bp were sequenced by Invitrogen, and 25 pairs of primers were designed using Primer Premier version 5.0 (Clarke and Gorley, 2001). The amplification conditions for the 20 pairs of primers were successfully selected by optimization in an Eppendorf Mastercycler Gradient System (Eppendorf, Hamburg, Germany). Genomic DNA samples extracted from 30 wild individuals of *A. pectinata* from Hainan, China, were assayed for polymorphisms. The polymerase chain reaction was performed in a 10-µL volume containing 50 ng genomic DNA, 10X Taq buffer, 2 mM MgCl₂, 0.4 µM of each primer, 0.2 mM of each dNTP, and 0.25 U *Taq* DNA polymerase (Fermentas). The reaction protocol was as follows: pre-denaturation at 94°C for 5 min, fol-

lowed by 37 cycles of denaturation at 94°C for 40 s, annealing at an optimal temperature (Table 1) for 40 s, and 72°C for 1 min, with a final 10-min elongation step at 72°C. Amplified products were separated by electrophoresis in a Sequi-Gen Sequencing Cell (Bio-Rad, Hercules, CA, USA) and visualized by silver staining. The data matrix was analyzed to estimate the observed heterozygosity, expected heterozygosity, number of alleles, and the polymorphic information content using the POPGEN 32 (version 1.32) (Yeh et al., 2000) and CERVUS 3.0 (version 3.0) softwares.

RESULTS AND DISCUSSION

Eight loci were successfully developed from the 30 *A. pectinata* individuals assayed. The loci were polymorphic, with the number of alleles ranging from 2-6 and polymorphism information content ranging from 0.233-0.447 (Table 1). Observed and expected heterozygosities ranged from 0.2069-0.7931 and from 0.1887-0.5124, respectively.

Table 1. Basic genetic information of 8 polymorphic microsatellite loci in *Atrina pectinata* (sample size = 30 individuals).

GenBank accession No.	Locus ID	Primer sequences (5'-3')	Repeat motif	Ta (°C)	N _A	PIC	H _O	H _E	P-HWE	Allele size (bp)
KM588371	ZJY1-2	F: ATTTGTATTAACGCCTCTGC R: CCACCTACGGATGAACCT	(CA) ₄ N(AT) ₄	55	3	0.371	0.5517	0.4301	0.2722	168-180
KM588372	ZJY1-14	F: AACTGGCAAAATAATGAG R: AATGAAGGCAAAAGTGTA	(AG) ₃₈	45	2	0.439	0.2069	0.1887	0.5736	252-264
KM588373	ZJY1-21	F: AACTTGTGCAAGAATCG R: CAGGGAATCTACAGGAA	(AT) ₅	55	2	0.269	0.4138	0.3339	0.1815	145-150
KM588374	ZJY1-23	F: TCTACCCAATCAAAACACT R: CGATAATGAATAAGAGCGT	(AAAT) ₃	58	4	0.233	0.3103	0.2825	0.9905	200-220
KM588375	ZJY1-25	F: TTTCAGCGTCTTTCCT R: ATATCGACCAATTCCT	(CAA) ₃	53	3	0.407	0.7931	0.5124	0.0077	265-290
KM588376	ZJY1-29	F: ATCCCAAAGAGTAATAAACA R: CAAAAGTTACTGGCTGAC	(AG) ₁₂	50	6	0.447	0.5517	0.4882	0.9945	260-308
KM588377	ZJY1-30	F: GTAAATGCCAAGAAACT R: CCTTAACTGCGATAA	(AGG) ₃	57	4	0.300	0.3448	0.3079	0.9808	180-198
KM588378	ZJY1-39	F: AGAAACTTTTGATGGATACTC R: AAATAGCGTCATTCATTGT	(AATT) ₃	60	2	0.371	0.5172	0.4991	0.8419	290-313

Ta = annealing temperature; N_A = number of polymorphic alleles per locus; PIC = polymorphism information content; H_O = observed heterozygosity; H_E = expected heterozygosity; P-HWE = P values for the Hardy-Weinberg equilibrium test (adjusted P = 0.00625).

No deviation from Hardy-Weinberg equilibrium in the population tested after Bonferroni's correction was observed (adjusted P = 0.00625). No loci showed significant linkage disequilibrium after Bonferroni's correction. Additional analysis using the MICRO-CHECKER software (van Oosterhout et al., 2004) showed no evidence for the presence of null alleles for any loci. The 8 novel polymorphic microsatellite loci presented may be useful for further population genetic studies of *A. pectinata*.

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