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SHORT COMMUNICATION

Isolation and characterization of 15 polymorphic microsatellite markers for comb pen shell (*Atrina pectinata*)

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Comb pen shell (*Atrina pectinata*), which belongs to the family Pinnidae, is a large, fan-shaped bivalve (Wang 1997) and is widely distributed in Indian and western Pacific Oceans. It is a popular seafood in coastal areas, especially in the southern China. The large posterior adductors of *A. pectinata* have been dried in a traditional way to preserve this seafood for many centuries in China. In more recent years, dried adductor of *A. pectinata*, which is known as *kongyiuchu* in Cantonese Chinese, has become popular as one of the most delicious seafood, and its market price has been increasing. Therefore, *A. pectinata* fishery has become an important industry in the coastal areas of China. However, the wild stocks of *A. pectinata* have declined drastically in the past decades as a result of overfishing, diseases and pollution in China (Ren & Guo 2005). For the purposes of suitable conservation and sustainable exploitation, studies on the molecular population genetics of *A. pectinata* are urgently required. Given their codominant inheritance, high level of polymorphism and ease in assay using polymerase chain reaction (PCR), microsatellite markers have been widely applied in population genetics and fisheries management (Chistiakov, Hellemans & Volckaert 2006). Here, we report a set of novel microsatellite markers in *A. pectinata*, which may represent a powerful tool in genetic studies and fisheries management of the species. These microsatellites may also be potentially useful in two other economically important mussel species.

Thirty-two adult individuals of *A. pectinata* were sampled as a test population from the Sanya coast of the Hainan Province of China. Their adductor muscles were soaked in 95% alcohol and then stored at − 20 °C. Genomic DNA was purified using a traditional proteinase-K digestion and phenol–chloroform extraction protocol (Sambrook & Russell 2001). A microsatellite-enriched partial genomic library for the repeat motif $(GT)_n$ was constructed essentially following the FIASCO (Fast Isolation by AFLP of Sequences COntaining repeats) protocol (Zane, Bargelloni & Patarnello 2002), with the following modifications: (i) preamplified DNA was denatured at 95 °C for 3 min and annealed with 80 pmol biotinylated $(GT)_{13}$ probes at 65 °C for 1 h; (ii) those DNA molecules hybridized to biotinylated probes were selectively captured by Streptavidin MagneSphere® Paramegnetic Particles (Promega, Beijing, China); and (iii) re-amplified target DNA molecules were ligated into pMD18-T vector (TaKaRa, Dalian, China) and transformed into *Escherichia coli* DH5α competent cells (Stratagene, Wuhan, China).

After PCR confirmation, 73 positive clones were sequenced using the BigDye termination kit (Perkin-Elmer Applied Biosystems, Shanghai, China), with the

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products resolved on an ABI 3730 Sequencer (Sangon Company, Shanghai, China). Repeat regions of the determined sequences were analysed using 'TANDEM REPEATS FINDER' software (Benson 1999) and inspected visually. The PRIMER PREMIER v. 5.00 software (Premier Biosoft International , Palo Alto, CA, USA) was then used to design 38 pairs of primers flanking the repeat regions of interest. Polymerase chain reaction amplifications were carried out in 25 μL volume on a PTC-100 thermocycler (MJ Research, Waltham, MA, USA), with the mixture containing 1 × PCR buffer (Tiangen, Beijing, China), 30–50 ng genomic DNA, 0.25 μM each primer, 150 μmol L^{-1} dNTPs, 1.5 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Tiangen). The amplifications were programmed under the following conditions: an initial denaturation at 94 °C for 3 min; 40 cycles including denaturation at 94 °C for 40 s, annealing at 50–54 °C (Table 1) for 40 s and elongation at 72 °C for 40 s; and a final elongation at 72 °C for 5 min. PCR products were size-fractionated on 6% polyacrylamide gels and visualized by silver staining. The pBR 322 DNA/*Msp* I molecular weight marker (Tiangen) was used as the size standard to identify alleles.

All primers were screened in the above-mentioned test population, and genotypes of polymorphic loci were scored. The probability of the occurrence of null alleles and scoring error were tested using MICRO-CHECKER (Van Oosterhout, Hutchinson, Wills & Shipley 2004). Calculations of the allele number (N_a), observed heterozygosity (H_O), expected heterozygosity (H_E) and polymorphic information content (PIC) were performed using CERVUS 3.0 (Kalinowski, Taper & Marshall 2007). The genotypes of polymorphic microsatellites were tested for linkage

Table 1 Characterization of 15 polymorphic microsatellites in comb pen shell (*Atrina pectinata*)

Locus	Accession no.	Primer sequences (5′–3′)	Repeat motif	Size (bp)	T_a (°C)	N_a	H_O	H_E	PIC
Ape1-2	FJ949089	F: GTGTTTGGAGTTCCGTTG R: CATATTGCTGCTTTGAC	$(AC)_9$	266–270	50	3	0.313	0.598	0.501†
Ape1-9	FJ949090	F: CCGCCTACTACACTTCTAC R: AGTCCGTCTGTTCTAATCTT	$(CT)_{25}$	213–219	54	3	0.688	0.659	0.574
Ape1-12	FJ949091	F: GGGCATTAGACAGAA R: ATCCAATACAGTAGCCAGT	$(CT)_{64}$*	175–181	50	3	0.438	0.629	0.539
Ape1-24	FJ949092	F: GACAGACGCGGACCATTA R: GTCAGGACTCGCAAGAAACA	$(GA)_{41}$	230–238	50	4	0.688	0.707	0.643
Ape1-25	FJ949093	F: GGTCCCAACAATGTATGAT R: GAACACTGGCCAGGTAAT	$(GT)_7$	132–136	50	2	0.094	0.091	0.085
Ape1-46	FJ949094	F: TTCTTCAGGGCTCACTTCTA R: GACATTTGTCTCCATTTTACTT	$(GT)_{15}$*	205–211	50	3	0.750	0.569	0.496
Ape2-4	FJ949095	F: CTTGCACAAACATTACCACA R: CTGCCTGAGTCTCTCACTATCT	$(GA)_{64}$*	191–199	50	4	0.719	0.613	0.559
Ape2-5	FJ949096	F: ATAAAGCAACAATAAACAAAGA R: CAAACCTCATCCTAAACAGA	$(TG)_{26}$	130–136	50	4	0.938	0.721	0.656
Ape2-9	FJ949097	F: AACTTCCCCACGAAACCA R: ACAACAAGGAAAAATCACAGG	$(CA)_7 \ldots (CA)_6$* $\ldots (CA)_7$*	171–181	50	4	0.844	0.605	0.537
Ape2-21	FJ949098	F: GTCGGTGGGTTGATAAGTTG R: GATTTATTGAATGGGTAGAT	$(CT)_{15}(AT)_9$	176–180	50	3	0.250	0.278	0.254
Ape2-23	FJ949099	F: GGTCATGTTGAGACCAGACTAT R: CAACCAAAGGGACAAAGAA	$(GT)_{17}$	166–174	50	5	0.906	0.743	0.686
Ape2-57	FJ949100	F: CAATGAACGGCAACTTCTATC R: ACTCGACTCGGGCAACTA	$(GA)_{32}$	200–212	54	4	0.688	0.753	0.694
Ape2-59	FJ949101	F: CCGGTAATTTATCAGTGG R: GCTGTAATGTCTTGCTATCA	$(GT)_6 \ldots (GT)_{10} \ldots (GT)_9$	177–185	50	4	0.875	0.753	0.693
Ape2-65	FJ949102	F: TGGTATTTATGGACTTTCA R: AGCATACACAATACGCCTA	$(TG)_{26}$*	160–166	50	4	0.813	0.696	0.626
Ape2-145	FJ949103	F: GTCAATTCTATGAGGTCCAA R: AGATCCTTCAGGCTGTGTA	$(CT)_{36}$	262–274	50	4	0.563	0.662	0.603

*Imperfect repeat.

†Significant deviation from HWE after Bonferroni correction ($P < 0.003$).

T_a, annealing temperature; N_a, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphic information content.

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disequilibrium and Hardy–Weinberg equilibrium (HWE) using GENEPOP v. 4.0 (Rousset 2008), and Bonferroni corrections were applied to correct for multiple simultaneous comparisons (Rice 1989).

Finally, 24 out of 38 pairs of primers amplified specific products. Among 24 detectable loci, 15 loci were polymorphic in the test population with 32 individuals. The sequences of these polymorphic microsatellite loci have been deposited in GenBank (accession numbers: FJ949089–FJ949103). The number of alleles per locus ranged from two to five, with an average of 3.6, and the mean PIC value was 0.543, ranging from 0.085 to 0.694. The observed heterozygosity (H_O) and expected (H_E) heterozygosity ranged from 0.094 to 0.938 (mean 0.638) and from 0.091 to 0.753 (mean 0.605) respectively (Table 1). All pairwise tests for linkage disequilibrium among loci were non-significant after applying sequential Bonferroni correction. Analyses of allele frequencies using MICRO-CHECKER software (Van Oosterhout *et al.* 2004) showed that there were null alleles at the loci Ape1-2 and Ape1-12. Detectable null alleles provided a possible explanation for the significant deviation from HWE within the test population at the locus Ape1-2.

We then tested all these 15 polymorphic loci for their cross-species utility in two other economically important mussel species. Sixteen unrelated individuals of Mediterranean mussel (*Mytilus galloprovincialis*) and Asian green mussel (*Perna viridis*) were genotyped. Cross-species amplifications were successful at three loci (Ape2-57, Ape2-9 and Ape1-24) in *M. galloprovincialis* and one locus (Ape2-9, with a low level of reproducibility) in *P. viridis*. However, these loci did not show polymorphism in the test DNA panels. Allele sizes were overall similar to those observed in *A. pectinata*, except for Ape2-57 in *M. galloprovincialis*. Ape2-57 exhibited a slightly larger size in *M. galloprovincialis* than the allele in *A. pectinata*, probably as a consequence of an insertion in the DNA sequence.

Although the microsatellites isolated in this study did not show any polymorphism in the test DNA panel of *M. galloprovincialis* and *P. viridis*, these microsatellite markers in *A. pectinata* would be expected to have a higher level of transferability in more closely related species (e.g. *A. fragilis*, *A. rigida*). The FIASCO protocol has been well documented as a preferable strategy for isolating microsatellites, because it requires only basic skills in molecular biology (cloning is the most difficult step) and limited laboratory equipments (Zane *et al.* 2002). These novel polymorphic microsatellites developed in the present study represent candidate codominant markers with enough polymorphism to study the population genetics and stock management of comb pen shell.

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