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TECHNICAL NOTE

Polymorphic microsatellite DNA markers for the ark shell *Scapharca subcrenata* (bivalve: Arcidae)

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Abstract We have isolated and characterized twelve novel polymorphic microsatellite loci from *Scapharca subcrenata* to analyse the population structure. The number of observed alleles per locus ranged from 3 to 17. Observed heterozygosity (H_O) ranged from 0.321 to 0.929. Cross-species amplification was tested successfully in three other bivalve species. These microsatellite markers will be useful for genetic diversity studies of *S. subcrenata* and other Lamellibranchia species.

Keywords Ark shell · *Scapharca subcrenata* · Microsatellite locus · Polymorphism · Bivalve

The ark shell *Scapharca subcrenata* is a benthic bivalve distributed along the shallow coasts of China, Japan, and Korea. In China this natural resources has been reduced dramatically due to the unsustainable exploitation and environment impact over the last two decades. To ensure the containable development, studies have been carried out about its reproductive biology and artificial breeding (e.g., Jiang et al. 2006; Chen et al. 2006), and the genetic variation of isozyme was analyzed among three populations of *S. subcrenata* (Li et al. 2007). However, our understanding about this species' population level, its genetic structure and gene flow is still limited. Efforts to conserve genetic diversity of this species require a better understanding of the genetic architecture of its populations, and more appropriate genetic markers could play an important role in this field. Microsatellite markers are useful for informing animals genetic diversity levels in populations because of their high polymorphism and codominance. Here we characterize 12 polymorphic microsatellite loci in *S. subcrenata*.

Microsatellite loci were isolated using an enrichment protocol modified from Gardner et al. (1999). Samples were collected from Nantong, China. Genomic DNA was extracted from the adductor muscle of one individual using the DNeasy Tissue Kit (QIAGEN). DNA was then digested with the restriction enzymes *MseI* (*NEB*) and ligated simultaneously to adaptors: oligo1: 5′-TACTCAGGACTC AT-3′ and oligo2: 5′-GACGATGAGTCCTGAG-3′ at 37°C for 4 h. The ligated fragments were polymerase chain reaction (PCR) amplified using the *Mse-N* primer (5′-GA TGAGTCCTGAGTAAN-3′). Fragments of 300–800 bp were selected and hybridized to two biotinylated oligonucleotide probes $(CA)_{15}$ and $(GA)_{15}$ at room temperature for 30 min, and then captured by streptavidin-coated magnetic beads (Promega). Nonspecific binding and unbound DNA was removed by several nonstringent and stringent washes. These microsatellite-enriched DNA fragments were PCR-amplified again and then ligated overnight at 4°C into pGEM-T Easy vectors (Promega), and followed by transformation into JM109 competent cells. Transformed cells grew at 37°C for 16 h on LB agar plate containing ampicillin (100 mg/ml), X-gal and IPTG for blue/white

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Table 1 Characteristics of the 12 polymorphic microsatellite loci isolated from ark shell

Locus	Accession no.	Repeat motif	Primer sequence (5′–3′)	Size (bp)	T_a (°C)	N_A	H_O	H_E	*PIC*
N206	EU483140	$(CA)_7$	F: CAGTCGTGCTCTTCCCTCCTTC	124–154	59	3	0.929*	0.523	0.399
			R: GCATGGTGTCTGGTGCTGTCTC						
N213	EU483151	$(GT)_{19}$	F: TCTTTTATGTTCTACCGACCTT	254–344	59	12	0.414*	0.897	0.870
			R: ACAGTCAAATGTATCCACACCT						
N247	EU483141	$(CA)_{13}A(CA)_8$ $A(CA)_9A(CA)_{15}$	F: GGGTTTCAGAGTGAATGGGTGTT	162–252	59	17	0.714	0.899	0.874
			R: TTACCAGTTAGGCAAATCAGGGG						
N263	EU483142	$(CA)_{12}$	F: GTGAGAGACCCTGGATTTGAAG	127–145	59	3	0.567	0.422	0.342
			R: ACAGAATCACTGTCAGCGTGTG						
N265	EU483143	$(GA)_{12}(GT)_{12}$	F: AATCAGCCGTATCTTCAAT	163–211	59	11	0.667	0.828	0.789
			R: CACATGTATATCTACAAGGTAAG						
N268	EU483150	$(GT)_{18}$	F: CCATAACACGCCAACGCTAC	241–321	59	17	0.714	0.892	0.863
			R: CATGCATACATAAACACCCCTTC						
N506	EU483144	$(GT)_{27}AT(GT)_5$	F: TCTGTGGTTTTATTGGTGGTGCT	264–340	62	15	0.700	0.879	0.850
			R: TCTACCTTCACGCTTCGTCAGAC						
N545	EU483145	$(GA)_{16}$	F: TCATTGACATCTAAATTCGTG	232–270	58	7	0.321*	0.869	0.835
			R: AGGTCTACCACATTTCTACTAAC						
N554	EU483149	$(GA)_{14}$	F: TACAAATAATCTCTACTGGCAT	302–398	59	14	0.517*	0.925	0.902
			R: GATTTCCCCACAACTATATT						
N560	EU483146	$(CT)_{12}C_2(CT)_8$	F: CGAATCTAAGCGGTAAAAGGTGG	171–219	62	10	0.767	0.810	0.772
			R:CAAAATCCTGAATGAGACAACGG						
N566	EU483147	$(CT)_{21}$	F: TTATTACCGATTCAGTGCCC	337–403	59	8	0.833	0.799	0.758
			R: GACTATTTGGTTGTTCTTTGGAT						
N590	EU483148	$(CA)_{10}TA(CA)_6TA(CA)_7$	F: AGTTACTCCATCTGTCTATCAAT	166–236	51	12	0.552*	0.916	0.891
			R: GCCGTGCTTAGTAGTGTTT						

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

* Indicates significant departure from expected Hardy–Weinberg equilibrium ($P < 0.001$)

Table 2 Results obtained from cross-species amplification tests on three bivalve species

Species	Locus						
	N247	N263	N265	N268	N554	N560	N590
Mactra veneriformis	+	+	+	+	−	+	+
Meretix meretrix	−	+	−	+	+	−	+
Coelomactra antiquate	+	+	+	−	+	+	+

+, successful amplification; –, unsuccessful amplification

selection. Twenty-nine positive clones were screened and sequenced, and 21 contained repetitions.

Twenty primer sets were designed in the flanking regions using the PRIMER 3 software (Rozen and Skaletsky 2000) and synthesized, and one primer in each pair was modified on the 5′ end with a LI-COR's M13 f-29 sequence (5′-CACG ACGTTGTAAAACGAC-3′) to allow use of a third primer in the PCR for producing labelled DNA fragments. These primers were tested for polymorphism on 30 wild individuals collected from Nantong. Genomic DNA was extracted with Genomic DNA MiniPrep kit (AxyPrep). PCR amplification conditions were optimized using a PTC-200 thermocycler (MJ Research). PCR amplifications were conducted in 20 μl volumes containing about 50 ng template DNA, 10 μl Ex Taq premix buffer (TaKaRa), 0.8 μM of each primer, and 0.5 pmol of fluorescently labelled M13 primer (either IRD700 or IRD800 (LI-COR)). The conditions for amplification were 8 min at 94°C followed by 34 cycles of 40 s at 94°C, 50 s at the annealing temperature (Table 1) and 30 s at 72°C with a final extension time of 8 min at 72°C. PCR products were electrophoresed and visualized on 6.5% polyacrylamide gels using a LI-COR 4300 DNA analyzer. Results were analyzed using LI-COR SAGA^{GT} software.

CERVUS 2.0 software (Marshall et al. 1998) was used to calculate the number of alleles, polymorphic information content (PIC), and observed and expected heterozygosities at each polymorphic locus. The characteristics of each locus were shown in Table 1. Tests for linkage disequilibrium (LD) and deviations from Hardy–Weinberg equilibrium (HWE) were performed using GENEPOP 3.4 (Raymond and Rousset 2004). Five loci showed significant deviations from HWE, and no LD was detected between any two loci ($P > 0.05$ for all comparisons). We used MICRO-CHECKER (van Oosterhout et al. 2004) to check microsatellite data for null alleles and scoring errors. Results reveal that the general excess of homozygotes present at the five loci was most likely due to null alleles. Homozygotes excess of *S. subcrenata* was also shown in the allozyme loci analysis (Li et al. 2007). Phenomena of higher null alleles frequencies have been reported in many bivalve microsatellite DNA studies (McGoldrick et al. 2000; Hedgecock et al. 2004; Reece et al. 2004; Kelly and Rhymer 2005; Zhan et al. 2007).

Using the PCR conditions described in the preceding text, these 12 loci were also tested preliminarily in 3 other bivalve commercial species: *Meretix meretrix, Mactra quadrangularis* and *Coelomactra antiquate*. PCR products were separated on agarose gels and inspected by naked eye, and most microsatellites isolated from ark shell gave an amplification product in these species tested (Table 2).

The result obtained in this study indicates that the microsatellites developed here are highly polymorphic and differentially represented in three bivalve species, and they should be useful for further studies of conservation genetics and other applications in ark shell as well as some other bivalves.

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References

Chen JH, Yan BL, Gao H (2006) Biological characteristics of ark shell *Scapharca subcrenata* and the research progress. Hebei Fish 153:24–26

Gardner MG, Cooper SJB, Bull CM, Grant WN (1999) Isolation of microsatellite loci from a social lizard, *Egernia stokesii*, using a modified enrichment procedure. J Hered 90:301–304

Hedgecock D, Li G, Hubert S, Bucklin K, Ribes V (2004) Widespread null alleles and poor cross-species amplification of microsatellite DNA loci cloned from the Pacific oyster, *Crassostrea gigas*. J Shellfish Res 23:379–385

Jiang HL, Shi XY, Liu WJ (2006) Preliminary study for artificial breeding technique of *Scapharaca subcrenata Lischke*. J Mod Fish Inf 8: 23–25 + 31

Kelly MW, Rhymer JM (2005) Population genetic structure of a rare unionid (*Lampsilis cariosa*) in a recently glaciated landscape. Conserv Genet 6:789–802

Li XG, Yan BL, Xu GP (2007) Analysis on biochemical genetic variation in three geographical populations of *Scapharca subcrenata*. Marian Fish 29(3):207–212

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639–655

McGoldrick DJ, Hedgecock D, English LJ, Baoprasertkul P, Ward RD (2000) The transmission of microsatellite alleles in Australian and North American stocks of the Pacific oyster (*Crassostrea gigas*): selection and null alleles. J Shellfish Res 19:779–788

Raymond M, Rousset F (2004) Genepop (version 3.4): population genetics software for exact tests and ecumenicism. Available at: http://wbiomed.curtin.edu.au/genepop/.html

Reece KS, Ribeiro WL, Gaffney PM, Carnegie RB, Allen SK Jr (2004) Microsatellite marker development and analysis in the eastern oyster, *Crossaster virginica*: confirmation of null alleles and non-Mendelian segregating ratios. J Hered 95:255–361

Rozen S, Skaletsky HJ (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana, Totowa, pp 365–386

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley PF (2004) The university of hull micro-checker user guide. Available at: http://www.hull.ac.uk/evolution/Software/microchecker.html

Zhan AB, Bao ZM, Hui M et al (2007) Inheritance pattern of EST-SSRs in self-fertilized larvae of the bay scallop *Argopecten irradians*. Ann Zool Fennici 44:259–268