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Isolation and characterization of 48 polymorphic microsatellite markers for the blood clam *Scapharca broughtonii* (Arcidae)

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ABSTRACT. Blood clams (*Scapharca broughtonii*) are widely cultivated and consumed in noutheast Asia. Forty-eight polymorphic microsatellite loci were developed for this clam using magnetic-bead hybridization enrichment. The number of alleles per locus ranged from 2 to 14. Polymorphism of these loci was assessed in 30 individuals from a population collected from coastal areas of Qingdao, China. The values of observed heterozygosity, expected heterozygosity and polymorphism information content per locus ranged from 0.1034 to 0.9655, from 0.1831 to 0.9208, and from 0.1638 to 0.8964, respectively. Forty-three of 48 loci conformed to Hardy-Weinberg equilibrium. These microsatellite loci would be useful for molecular

genetic breeding, population genetics, genome mapping, and other relevant research on *S. broughtonii*.

Key words: Blood clam; *Scapharca broughtonii*; Microsatellite loci; Genetic structure

INTRODUCTION

The blood clam *Scapharca broughtonii* belongs to the family Arcidae, distributed in coastal waters of China, Japan, Korean Peninsula, and southeast Russia. In China, *S. broughtonii* is one of the most commercially important shellfish resources. It is highly valued because of taste and nutrients. However, the wild resources of *S. broughtonii* have sharply declined due to overfishing, annual cultivation, environmental pollution, and deterioration of marine ecosystems. Successful management of recovery for the blood clam depends on fishing restriction, artificial breeding programs, and protection of Fine-Breed (Luan et al., 2003; Zhang et al., 2007).

Little information exists so far on the genetic structure and genetic diversity of the wild populations. Molecular markers, especially microsatellites, are effective markers that have been widely applied to the development of marker-assisted breeding technology and conservation of this species, because of the high degree of polymorphism, abundance, neutrality, and codominant inheritance (Zhan et al., 2005; Schwartz et al., 2007). Therefore, the development of new polymorphic microsatellite markers opens new perspectives for population genetics (Chen et al., 2005). Ten and twelve microsatellite markers from *S. broughtonii* have been isolated by An and Park (2005) and Li and Li (2008). However, additional loci will be beneficial to both population genetics and construction of a genome map. Here, we developed 48 polymorphic microsatellite markers for *S. broughtonii* by the method of magnetic-bead hybridization enrichment.

MATERIAL AND METHODS

Thirty individuals were collected from the coastal waters of Qingdao, China. Samples were preserved in alcohol until DNA extraction. An enriched genomic library was constructed essentially following the FIASCO (fast isolation by AFLP of sequences containing repeats) protocol (Zane et al., 2002), which has been described in detail by Shao et al. (2008). In brief, genomic DNA was extracted from the adductor muscle using a modified phenol-chloroform procedure (Li et al., 2006) and digested with *Mse*I enzyme (New England Biolabs, USA). The DNA fragments were ligated to *Mse*I adaptors (5'-GATCATGAGTCCTGCT-3' / 5'-CGAGCAGGACTCAGAA-3'). DNA fragments ranging from 400 to 1200 bp were isolated by separation on 1% agarose gels. The products were then pre-amplified in a 20-µL reaction system using an adapter-specific primer (5'-GATCATGAGTCCTGCT-3') to verify successful ligation. The PCR products were purified using a DNA Purification kit (TaKaRa, Japan) and hybridized to biotin-labeled $(AC)_{15}$ and $(AG)_{15}$ probes. The mixture was denatured at 95°C for 5 min and then at 68°C for 1 h. Subsequently, the hybrids were captured by streptavidin-coated magnetic beads (Promega, USA). After washing, DNA fragments obtained were eluted from

the magnetic beads and amplified using the corresponding primer. The amplification products were ligated to pMD18-T vectors (TaKaRa) and transformed into *Escherichia coli* TOP10 competent cells. From the library, 295 positive clones were randomly selected for sequencing using an ABI Prism 3730 automated DNA sequencer (ABI), of which 208 clones contained microsatellite repeats. Finally, DNA primer pairs were designed using PRIMER PREMIER 5.0 (Premier Biosoft International, USA), and we obtained 124 primer pairs based on the regions flanking the microsatellite motifs. These relevant sequences have been deposited in GenBank (JN682059, JN682183).

The capability of the PCR primer pairs was tested using 8 individuals from the population. The PCR mixtures (25 µL) contained 80-100 ng genomic DNA, 0.8 µM of each primer, 1X PCR buffer (20 mM Tris-HCl, 20 mM KCl, 10 mM $(NH4)_2SO_4$, pH 8.4), 0.2 mM dNTPs, 2 mM $MgCl_2$ and 1 U Taq DNA polymerase (TaKaRa). Amplification was carried out with the following thermal cycle profile: 94°C for 5 min, 30 cycles of 94°C for 45 s, annealing temperature for 40 s and 72°C for 45 s, and a final extension at 72°C for 8 min. Denatured PCR products were separated on 8% denaturing polyacrylamide gels, and visualized by silver staining. Denatured pBR322DNA/*Bsu*RI marker (Fermentas, Canada) was used as the size standard to identify alleles. Once the polymorphism was confirmed for a given locus, 30 individuals were genotyped to determine heterozygosity. The observed and expected heterozygosities with tests for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were calculated using GENEPOP 4.0 (Rousset, 2008). The MICRO-CHECKER software (Van Oosterhout et al., 2004) was employed to infer the most probable technical cause of departures from HWE, including null alleles, scoring error due to stuttering, and large allelic dropout due to short allele dominance. All results were corrected for multiple simultaneous comparisons using a Bonferroni's correction (Rice, 1989).

RESULTS AND DISCUSSION

In total, 48 of the 124 microsatellite loci were polymorphic (Table 1), while the remaining 76 loci were monomorphic or resulted in poor or no amplification in *S. broughtonii*. Among these 48 polymorphic microsatellite loci, the number of alleles at each locus ranged from 2 to 14 (mean 7.8333). The observed heterozygosity, expected heterozygosity and polymorphism information content ranged from 0.1034 to 0.9655 (mean 0.6661), from 0.1831 to 0.9208 (mean 0.8068), and from 0.1638 to 0.8964 (mean 0.7685), respectively. Five loci deviated significantly from HWE ($P < 0.05$) after sequential Bonferroni's correction (adjusted $P < 0.00104$), and as a result, there were 43 loci without deviation from HWE. Such deviations might have been caused by the limited sample size used in our test or the presence of null alleles confirmed by the MICRO-CHECKER version 2.2.3 software (Van Oosterhout et al., 2004), but no evidence of stuttering and allelic dropout was found in all markers. No significant genotypic linkage disequilibrium was detected between any pairs of loci.

Consequently, these microsatellite loci will enrich the microsatellite marker resources and be useful for the investigation of population structure, assessment of genetic diversity and construction of a genetic linkage map in future studies of *S. broughtonii.*

Table 1. Characterization and genetic parameters of 48 polymorphic microsatellite markers in *Scapharca broughtonii*.

Locus	Primer sequence (5'-3')	Size range (bp)	Repeat motif	Ta (°C)	N_A	H_O	H_E	PIC	P_{HW}	Accession No.
KH-1	F: CGTAGAGTTGTTCATGAATGGT R: GGACGTTGCTTGCTATTTAAG	214-253	$(GT)_{17}$	57	6	0.6552	0.8203	0.7789	0.0071	JN682059
KH-2	F: CCTGCCGTTTGACCTTTAATTT R: GGCTGGGTTTCATAGTACGT	287-336	$(GTGA)_4$	51	6	0.5333	0.7379	0.6927	0.0107	JN682060
KH-3	F: TCAACGATTTTCGCAAACCCT R: GTCTTGACACCCCGCATT	293-341	$(TC)_{11}(TG)_{18}$	55	8	0.7667	0.8339	0.7952	0.0227	JN682061
KH-5	F: ATCAGTGCCTCTCATCAAAC R: CATAGGTTACCGTTAGTCATCG	190-285	$(CT)_{11}...(TC)_{10}.(TC)_7.(TC)_{11}.(TC)_{10}$	60	12	0.7333	0.9169	0.8933	0.0034	JN682063
KH-7	F: AGTTACATTCCTCCACATAGTG R: AGTCCTGCTACCATTGTTT	241-267	$(CT)_8...(TC)_{10}$	53	2	0.2000	0.1831	0.1638	1.0000	JN682067
KH-8	F: ACCGTGTCATTTGGCTTAG R: AGTGGGTCAAGGCTTTCTAT	414-453	$(GT)_{10}$ T $(TG)_7$	56	9	0.7000	0.8475	0.8135	0.0183	JN682068
KH-10	F: AGCCTGATCTGCCACATTC R: ACTGACAAACGGAAACGCAAT	268-324	$(TC)_6(CA)_{20}$	59	6	0.6667	0.8062	0.7605	0.0212	JN682070
KH-15	F: GTTATCGTCAACTACTCGGC R: CAAAGGTCAGGGTCAAATCTAT	530-558	$(AC)_{18}(AG)_{11}$	58	5	0.6333	0.7814	0.7300	0.0068	JN682073
KH-16	F: TGTAGCTTGTCTTAATTGCCG R: TGGTTCCGTATTTGATGGGT	286-314	$(CA)_{17}$	61	8	0.6667	0.8599	0.8266	0.0024	JN682074
KH-20	F: GGTCAAAAAGGGACCTAAATCG R: TCTCTCTCAGAAATACAACTCTTGG	155-211	$(AG)_{12}$	61	9	0.5667	0.8492	0.8144	0.0052	JN682079
KH-22	F: GGCGTGCCTGTGATAGCT R: AGCTCACCGTGTCCTACATCA	260-320	$(CA)_{19}$	61	7	0.7333	0.7944	0.7535	0.0585	JN682081
KH-23	F: GGTTGTTGGTAAACAAGTCAGG R: GTTGGGATGTATGGTCAGTG	162-187	$(AC)_{11}$	56	6	0.9310	0.8034	0.7615	0.7172	JN682082
KH-25	F: CTTCTTCCTCAGTTTGCTCC R: GATCCAAACAAAGGTCAGGG	314-400	$(AC)_{13}$	61	4	0.4000	0.6186	0.5517	0.0281	JN682085
KH-26	F: ATTGTCGCTTGAACAGAAATCT R: ATGTCTCGTAAGCGAAAAGG	241-273	$(A)_8(AC)_7$	48	6	0.5667	0.7638	0.7118	0.0073	JN682086
KH-27	F: CATATGTCAACTCTATTGGGTG R: ATCTGTGAAGGTCAAAATGC	138-156	$(GT)_8$	56	4	0.6000	0.7158	0.6556	0.0555	JN682087
KH-28	F: GCAACCGTTTATTAAGTGTG R: CAACAACTGAAAACCAGATC	240-294	$(AC)_{18}$	56	6	0.5667	0.7644	0.7139	0.0481	JN682088
KH-30	F: GATAATGTTTCCTGTTCAGC R: TATACTGGTCCCTCAATGT	324-375	$(TTA)_6(TAG)_5$	49	8	0.8214	0.8669	0.8337	0.2630	JN682090
KH-31	F: TGTTCTGTGACGTCATGCTGTT R: AGCAGACGAAAACTCTGGTTGA	400-440	$(TC)_{22}$	63	8	0.7241	0.8766	0.8456	0.0146	JN682091
KH-32	F: GGGATCACATCAGTCAGTAGC R: ATGTAGATAGTGCGGTCTCGTT	196-246	$(CT)_{12}$	59	9	0.6333	0.8508	0.8165	0.0071	JN682093
KH-34	F: TGATCCTTCCCTCAACTTATTC R: CAGCTACTTGCAGTATCCTTT	89-126	$(CT)_{15}$	51	14	0.9333	0.9119	0.8877	0.7657	JN682095
KH-35	F: GCATGCAATTTGTTATGAGG R: CGGAGTTGGTATAATAGGTAAG	225-258	$(CT)_{13}$	56	12	0.7667	0.8831	0.8558	0.0060	JN682096

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Table 1. Continued.

Locus	Primer sequence (5'-3')	Size range (bp)	Repeat motif	Ta (°C)	N_A	H_O	H_E	PIC	P_{HW}	Accession No.
KH-41	F: CGCATTGCAGACGTTCTTTTCAT R: TCGTCAGCTTTTGAACAACTTTCG	358-392	$(CA)_6T(AC)_3(CCCA)_2$	56	8	0.6000	0.8576	0.8236	0.0021	JN682102
KH-42	F: TCCTAATGTTACCTGACACTACTTC R: ATGGTTCTCACTAAAATCTGCC	235-279	$(GA)_{14}$	56	11	0.8333	0.8763	0.8469	0.1896	JN682103
KH-43	F: TGTAACAGAGTTGTCTGCTCTT R: TATAAGCGTTAGGAACCAGCG	236-271	$(GT)_{14}$	56	8	0.7667	0.8339	0.7971	0.0783	JN682104
KH-44	F: GCAGCTTGATTCATTGTTCTAC R: GCACCCAGTGATTATTTCCTAT	234-278	$(TTA)_3(T)_5$	56	9	0.6333	0.8011	0.7596	0.0124	JN682105
KH-45	F: CTGAAACGCTGTTTATCATCTGTCG R: ACCATTACGCACCACTTTCTGT	192-245	$(AG)_{15}$	56	10	0.9000	0.8768	0.8477	0.6318	JN682106
KH-50	F: TTATTGTATGTAGGCGGGGGTTG R: TGGCCATCAGTTAGAATTGTGTG	268-301	$(AC)_3T(AC)_3(CG)_6$	56	6	0.5517	0.8100	0.7653	0.0126	JN682112
KH-56	F: AGCGTTTTGTCCATTTGGGGAT R: GCATCGGGGCAGGTAATCT	282-324	$(GA)A_3(AG)_{13}AA(AG)_9$	56	6	0.7000	0.8260	0.7850	0.0114	JN682116
KH-61	F: GGACGTAAACCTCCATCT R: CCAGAACCTCATAGAACGATT	118-183	$(TC)_{19}(CA)_{20}(AC)_{12}$	51	11	0.8276	0.9002	0.8735	0.0752	JN682121
KH-65	F: CCCAGCATTGTTAGTGGTGT R: TAATCGCTCCTTTTAGGACAGCT	180-213	$(TC)_{31}\ldots(TCA)_6$	61	13	0.8333	0.9062	0.8812	0.2087	JN682129
KH-66	F: CAGAACTACTGATCGACGCTGAA R: CTCCTCCCACTCAAACATCAAAG	420-471	$(GT)_{19}$	61	7	0.6552	0.8409	0.8029	0.0159	JN682132
KH-67	F: TTTTCGCCAAGCACTCAGGGTC R: CGTGCCATTGGGTATCCGTTTT	126-212	$(CT)_{23}$	63	6	0.7586	0.8209	0.7796	0.0601	JN682133
KH-68	F: TGTCTACTACACTGGGCATCAT R: AGTTGCTGAAATACAAACCTACACTC	466-509	$(TC)_{11}\ldots(TC)_7ACT(CT)_8\ldots(CT)_{14}(TG)_8$	63	6	0.7037	0.8155	0.7710	0.0098	JN682134
KH-71	F: GGCTAATTGCACTGAATGTCTG R: GTTCAGTGTGCGTAGCCAAT	77-124	$(GA)_8A(AG)_{17}$	60	9	0.6897	0.8947	0.8670	0.0021	JN682142
KH-74	F: ATGGAACAGGCTCTGGTGTC R: GCTCTTTCAGACCTGGCGATAT	269-341	$(GA)_6(AG)_6$	61	6	0.8667	0.7610	0.7137	0.5528	JN682146
KH-75	F: CATTGGCCGTTTGTTTCTTT R: CCGTCTGTATAACTACAATTCTCC	279-295	$(T)_{19}$	60	8	0.9655	0.8445	0.8076	0.9775	JN682148

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Table 1. Continued.

Locus	Primer sequence (5'-3')	Size range (bp)	Repeat motif	Ta (°C)	N_A	H_O	H_E	PIC	P_{HW}	Accession No.
KH-76	F: GGTTTATGAATGTTTACGGCACTG R: ATCAAACTGGATGGGACAGG	234-267	$(C)_9G(TC)_8$	63	4	0.4333	0.4000	0.3710	0.8613	JN682149
KH-78	F: GCAGCTGATGTGTAAAAACAGT R: ACTCATTTGTCCCCGCTTT	288-322	$(TC)_9TT(TC)_5$	56	8	0.3448	0.8324	0.7929	0.0000*	JN682155
KH-79	F: GCTGCGAATCCTAACACTAC R: ACCCAGGGCATCTATAAAAGT	238-273	$(TG)_6$	47	14	0.5333	0.9006	0.8755	0.0000*	JN682157
KH-80	F: ATAGAACCGTAGCGTTTAGA R: CCAATAATAGCAGCGAAAATC	298-369	$(AG)_{12}$	56	9	0.7667	0.8842	0.8552	0.0069	JN682158
KH-88	F: ATCTGCACTTTGACACGACA R: TGCATTTCACTCGCAATCTCT	213-261	$(GA)_{12}$	61	8	0.7667	0.8684	0.8366	0.0490	JN682169
KH-89	F: TCACTCATTCTCCCCATTTAT R: AGCTTCCGATTTGGTACTTAT	234-257	$(CTAGA)_3$	51	6	0.1034	0.7393	0.6788	0.0000*	JN682170
KH-90	F: CCATTAAGACGATTATGGACGA R: TAAAGCCGAAAGCACAACTA	124-176	$(GT)_{18}$	61	13	0.6429	0.9208	0.8964	0.0000*	JN682171
KH-93	F: CAAACATACACGGTGAGACGA R: CCAGGATTAGAATGCCGAAGT	197-231	$(AAAT)_5A_{11}$	60	6	0.4000	0.7294	0.6664	0.0083	JN682174
KH-95	F: ACTTTGACACGACACACACG R: GTGCATTTCACTCGCAATCTCTT	211-245	$(GA)_{12}$	61	10	0.9000	0.8599	0.8281	0.7117	JN682177
KH-98	F: ATTGGCCGTTTGTTTCTTT R: ACACCGTCTGTATAACTACAATT	275-297	$(T)_9...(GTTT)_3...(T)_{17}$	60	6	0.7000	0.8322	0.7927	0.0552	JN682181
KH-99	F: CCCAAAGGATTATTTGGCTGTCAT R: TGTCTGCACCTTCGCCTAT	272-400	$(TGAGT)_3$	61	6	0.6897	0.7943	0.7494	0.0801	JN682182
KH-100	F: GTTTGTACTGTGACTGAGGC R: TGTCGGTTGGTCTAAAGCAT	294-376	$(T)_4G(T)_{10}$	60	7	0.6071	0.8117	0.7687	0.0010*	JN682183

Ta = optimized annealing temperature; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphism information content; P_{HW} = Hardy-Weinberg probability. *Significant deviation from HWE after Bonferroni's correction (adjusted P = 0.00104).

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