

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

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Genetics and Molecular Research 11 (4): 4501-4507 (2012)

J.-T. Tian et al.

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 *MseI* enzyme (New England Biolabs, USA). The DNA fragments were ligated to *MseI* 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- μ L 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

Genetics and Molecular Research 11 (4): 4501-4507 (2012)

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), SO₄, pH 8.4), 0.2 mM dNTPs, 2 mM MgCl, 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/BsuRI 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*.

Genetics and Molecular Research 11 (4): 4501-4507 (2012)

 H.I. F. GGTAGAGTTGTTCATGAATTAA. H.2. GGACGGTTTCATAGTACGAATCA H.3. F. CCTGGCGTTTCATAGTACGAAACC H.3. F. CCAAGGATTACTCATAAACGAATCAAA H.4. F. AGTTCAGTGCTCTCAAAACGAAACCACTAAA H.4. F. AGTGGGTTCACATTGGCTAAATTCAAAACGGGTCACAAATTCAAAACGGGTCACGAAATTCAAAACGGGTCACAAATTCACACAAATAAAGTCACGTTAGGGTCACGAAATAAAAAGGGGAAAACGCAAAATAAAAGGGGAAAACGGAAAACGGAAAACGGAAAACGGAAAACGGAAAACGGAAAACGCAAAATAAAAGGTCAGGTCACTAAAATAAAAGGTCAGGTCAAAAACGAAAAACGGAAAACGGAAAACGGAAAACGGAAAACGGAAAACGGGAAAACGGAAAACGGAAAACGGAAAACGGAAAACGGAAAACGGGAAAACGGAAAACGGGAAAACGGAAAACGGAAAACGGGAAAACGGAAAACGGAAAACGGAAAACGGAAAACGGAAAACGGAAAACGGGAAAACGGAAAACGGAAAACGGAAAACGGGAAAACGGAAAACGGGAAAACGGGAAAACGGAAAACGGGAAAACGGAAAACGGGAAAACGGGAAAACGGAAAACGGGAAAACGGAAAACGGGAAAACGGGAAAACGGAAAACGGGAAAACGGGAAAACCAAGGGGAAAACGGGAAAACCAAGGAGG	TGGT 214-253 AAG 287-336 ATTT 287-336	Repeat motif	Ta (°C)	N N	H_0	$H_{\rm E}$	PIC	P_{HW}	Accession No.
 ch-2 K: GGCTGGGTTTGACCTTTAM ch-3 K: GGCTGGGTTTGACCTTTAM ch-3 K: GGTGGGGTTTGACCCTGGCAACC ch-4 K: GGCTGGGGTTACCGTCAACCGGAAT ch-7 K: GGTGGGGTTACCGTTAGTAA ch-10 K: GGTGGGGTCAAGGGCTTAGTAA ch-10 K: AGTGGGGTCAAGGGAAACGGAAAGG ch-11 K: AGTGGGGTCAAGGGAAACGGAAAGG ch-11 K: AGTGGGGTCAAGGGAAAGG ch-12 K: AGTGGGGTCAAGGGAAAGGG ch-13 K: AGTGGGGTCAAGGGAAAGGG ch-14 K: AGTGGGGTCAAGGGAAAGG ch-15 K: AGTGGGGTCAAGGGAAAGG ch-16 K: AGTGGGGTCAAGGGAAAGG ch-16 K: ACTGGGCAAAGGGAAAGGG ch-17 K: AGTGGGGTGCGGAAAAGGGGAAAGG ch-20 K: TGGGGGGTGCGGGAAAGGG ch-21 K: GGTGGGGGTGGGGGGCCGAA ch-22 K: GGTGGGGGGGGGAAAGGG ch-23 K: GTTGGGAAACAAGGAAAGGGGAAAAGGGGAAAAGGGGAAAAGGGGAAAAGGGG	ATTT 287-336	$(GT)_{17}$	57	9	0.6552	0.8203	0.7789	0.0071	JN682059
 GH-3 K. GGTUTGGGUI GGUI LCAIAULAGA GH-5 F. GATGGGTUGGTI LCAIAULAGA GH-7 F. AGTTACGTTCGTCAAAACC GH-7 F. AGTTACGTTCCTCCGTAAAACC GH-8 K. AGTCCGGGTCAATGCTTGGTACAAAAC GH-15 F. AGTCGGGTCAAAGGGGTAAGGGAAACGG GH-15 F. AGTGGGGTCAAAAGGGGAAAACGG GH-15 F. AGTGGGTCAAAAGGGGAAAACGG GH-16 F. AGTGGGTCAAAAAGGGGAAAAAAA GH-16 F. AGTGGGTCAAAAAGGGGTCAAAAT GH-16 F. GGTTCGGGTCAAAATGGG GH-20 F. GGTTCGGGTGAAAATGGG GH-20 F. GGTTCGGTGATTGGGTCAAAATGG GH-20 F. GGTTCGGTGATTGGGTGAAAATGGG GH-20 F. GGTTCGGTGATTGGGTGAAAATGGGT GH-20 F. GGTTCGGTGGTGGTGGGTCAGAAAATGGGTGGGAAAAAAGGGGACCAAAAAGGGGACCTAAAATGGTGGTGGGGATGTAGGGTGGGGAAAAAGGGGTCGGGAAAAAGGGGTCAGGAAAAGGGGACGAAAAGGGGACGAAAAAGGGGACGAAAAAGGGGACGAAAAAGGGGACGAAAAGGGGACGAAAAAGGGGACGAAAAAGGGGACGAAAAAGGGGACGGAAAAAGGGGACGGAAAAAGGGGACGAAAAGGGGACGGAAAAAGGGGACGGAAAAAGGGGACGGGAAAAAGGGGACGGGAAAAAGGGGACGGGAAAAAGGGGACGGAAAAAGGGGACGGAAAAAGGGGACGGGAAAAAGGGGACGGAAAAAGGGGACGGCAAAAGGGGACGGAAAAAGGGGACGGAAAAAGGGGACGGAAAAAGGGGACGGAAAAAGGGGACGGAAAAAGGGGACGGAAAAAGGGGACGGAAAAAGGGGACGGAAAAAGGGGACGGAAAAGGGGACGGAAAAGGGGACGGAAAAGGGGACGCGAAAAGGGGACGCGAAAAGGGGACGGAAAAGGGGACGGAAAAAGGGGACGAAAAGGGGACGGAAAAGGGGACGCGCGGGAAAAGGGGAAAACCGGGGACGCACGGGGACGCCCGGGGACGGCGCGGGGAAAACCGGGGACGCCCGGGGACGCGCGGGGACGGCGCGGGAAAACCGGGGACGCGCGGGACGGCGCGGGGACGGCGCGGGGACGGCGCGGGAAAACCGGGGAAAACCGGGGACGCCCGGGGACGGCGCGGGGAAAACCGGGGACGCCCGGGGACGGCGCGGGGACGGCGCGGGAAAACCGGGGAAAACCGGGGAAAACCGGGGAAAACCGGGGAAAACCGGGGAAAACCGGGGACGCCCGGGGACGCCCGGGGACGCCCGGGGACGCCCGGGGACGCCCGGGGACGGCCCGGGGACGCCCGGGCGCCCGGGCGCCCGGGGACGGCCCGGGGACGCCCGGGGACGGCCCGGGGCGCCCGGGGCGCCCGGGGCCCCGGGCGCCGGGG	EC	$(GTGA)_4$	51	9	0.5333	0.7379	0.6927	0.0107	JN682060
 GH-5 CATAGGTACCTTCAANAL GH-7 F-40TTACATTCCTCCCACATAG GH-7 F-40TTACATTCCTCCACATAG GH-10 R-40TGGGTCACGTTGGTTAG GH-10 R-40TGGGTCAGGGTCAAGGGCTTAGGG GH-16 R-40TGGGGTCAGGGGAAAGGG GH-16 R-40TGGGTCAGGGTCAAATTCGGGAAAGGG GH-16 R-40AGGTCAGGGTCAAATTCGGCAAATTCGGGGGGAAAGGG GH-16 R-40AGGTCAGGGTCAAATTCGGGAAAGGGCAAAAG GH-16 R-40AGGTCAGGGGTCAAATTTGATGGG GH-16 R-40AGGTCAGGGGTCAAATTCGGGAAAGGG GH-16 R-40AGGTCAGGGGTCAAATTGATGGGG GGTTAGGTTGGGAATTGAAGGGGGCTAAA GGTTAGGTAGGGGGGGAAAAGGG GGTTGTTGGGAAGGTCAGG GGTTGTGGGAAGGTCAGGAAAAAGGG GH-26 R-47TGTCGGAAACAAAGGGTCAGG GGTTGTTGGGAAGGTCAGGAAAAAGGTCAGG GGTTGTTGGGAAGGTCAGGAAAAAGGTCAGG GGTTGTTGGGAAGGTCAGGAAAAAGGTCAGG GATCCTGTGGAAGGTCAGGAAAAAGGTCAGG GATCCTGTGGAAGGTCAGGAAAAAGGTCAGG CAACACGGTGAAACCAAGGAAAAAGGTCAGG CAACACGGTGAAACCAAGGAAAAAGGTCAGG CAACACGGTGAAACCAAGGAAAAAGGTCAGG CAACACGGGAAAACCAAGGGTCAGG CAACACGGGAAAAACCAAGGGTCAGG CAACACGGGAAAAACCAGGAAAAACCAGAAAAGGTCAGG CAACACGGGGAAAAACCAGGACAAAACCGGAAAAACCGGAAAAACCGGAAAAACCAGAAAACCAGGAAAAACCAGAAAACCAGAAAACCAGGAAAAACCAGGAAAAACCAGAAAACCGGAAAAACCGGAAAAACCAGAAAACCAGAAAACCAGAAAAACCAGAAAAACCAGAAAAACCAGGAAAAAA	GI CCT 293-341	$(TC)_{11}(TG)_{18}$	55	8	0.7667	0.8339	0.7952	0.0227	JN682061
 GH-7 K. CAGTGACCULULAULUAULAULAULAULAULAULAULAULAULAULAU	AC 190-285	$(CT)_{11}(TC)_{10}.(TC)_{\gamma}.(TC)_{11}.(TC)_{10}$	09	12	0.7333	0.9169	0.8933	0.0034	JN682063
 GH-8 GH-10 F. AGUGGGTCATTGGCCTTGTA GH-10 F. AGUGGGTCATTGGCCACATTC R. ACTGACAAAGGCTTTGGCAATC C. CAAGGGTCAAAGGGTCAAATGCGGGGGGGGGGGGGGGGG	ALCG 241-267	(CT) ₈ (TC) ₁₀	53	7	0.2000	0.1831	0.1638	1.0000	JN682067
 GH-10 F. AGCUGOUTCAROUCH IT LUNCIA B. AGGCUGOUTCAROUCATIN LUNCIA F. GTTATCGTCAACTGGGG R-16 F. GTTAGGCTGGTCAACTGGGG R-20 F. GGTGGCTGGTAATTGGC R. TGGTGGCTGGTAATTGGC R. TGGTGCCTGTAAATGGG R-20 F. GGTGGCTGGTAATGGC R. TGGTGGCTGGAATGGCTAA R-20 F. GGTGGCTGGTGGGACGAA R-20 F. GGTGGGGGGGGGGGGCCAA R-20 F. GGTGGGAGGACCAATGGCTAA R-20 F. GGTGGGAGGACCAATGGTAGGC R-20 F. GGTGGGAGGACCAATGGTAGGC R-20 F. GGTGGGAGGACAAGCAAGGGGAAAGC R-21 F. GTTGGGAGGTAGGGGAAAGCAGGAAAGC R-21 F. CTTCTGTGAACAAGGGGAAAGC R-21 F. CTTCTGTGAAGGGAAAACCAGGAAAGC R-21 F. CAACAACTGAATGGTCAGG R-21 F. GGAACCGTTGAAGGGAAAACCAGCAAACCAGCAAAACCAGCAAACCAGCAAACCAGCAAAACCAGCAAAACCAGCAAAACCAGAAACCAGCAAAACCAAAACCAGCAAAACCAGCAAAACCAGCAAAACCAGCAAAACCAGCAAAACCAGCAAAACCAGCAAAACCAGCAAAACCAGCAAAACCAGCAAAACCAGCAAAACCAGCAAAACCAGCAAAACCAAAAACCAAAAAA	1 5 414-453 ۸۳	$(GT)_{10} T (TG)_7$	56	6	0.7000	0.8475	0.8135	0.0183	JN682068
 GH-15 F. GATAGGTTCAACUGAAAAUGG GH-16 F. TGTAGGTTGCAACTACTGGGG C.AAAGGGTCAATTGGG C.AAAGGGTCAATTGGG C.AAAGGGTCAATTGGGGGGCCAAA C.B-20 F. GGTCAAAAAGGGGACCTAA C.B-21 F. GGTCGGGGTGCTGGGAAAGG C.B-25 F. GGTGGGGTGGTGAAAGGGACCAAA C.B-25 F. GGTGGGGTGTGAAAAGGGAAAGT GH-26 F. ATTGTCGCTTGAAAAAGGGCAGAAA C.B-24 C.AAAACAACAACAAGGTCAGG C.B-26 F. ATTGTCGCTGGAAAGGTCAGG C.B-26 F. ATTGTCGCTTGAACAAGGTCAGG C.B-26 F. ATTGTCGGTAAACAAGGTCAGG C.B-26 F. ATTGTCGCTGAACAAGGTCAGG C.B-26 F. ATTGTCCGTAAGCAAGGAAAAGGTCAGG C.B-26 F. ATTGTCCGTAACAACAAGGTCAGG C.B-26 F. ATTGTCCGTAACAACAAGGTCAGGAAAA C.B-26 C.CATATGTCCGTAAGCAAAGGTCAGG C.B-26 C.CATATGTCCGTAACAACAAGGTCAGGAAAA C.B-26 C.CATATGTCCGTAACAACAAGGTCAGGAAAA C.B-26 C.CATATGTCCGTAACAAAGGTCAGGAAAA C.B-26 C.CATATGTCCGTCAACTCAATTGG C.B-27 C.CATATGTCCGTCAACTCAATTGG C.B-28 C.CATATGTCCGTCAACTCAATTGG C.B-28 C.CATATGTGCCAAAAGGTCAGGAAAAACCAGGA C.B-29 C.CATATGTCGCTCCAAAGGT C.B-20 C.CATATGGGCCCCCAAAGGT C.B-20 C.CATATGGGCCCCCAAAGGT C.B-20 C.GGATCACCACTCAGTCAGTCAGGT 	AI C 268-324	$(TC)_{6}(CA)_{20}$	59	9	0.6667	0.8062	0.7605	0.0212	JN682070
 GH-16 CH-20001CARACUTGRADIATTGOC CH-20 CHCTAAAAAGGGGACTAAA CH-20 CHCTCTCTCAGAAATTGOCTAAA CH-21 CHCTCTCTCTCAGAAATTGOCTAAA CH-22 CHCTCTCTCTCAGAAATGCAAC CH-23 CHTCTTCTCTCTGTTAGTTGCTCAGT CH-25 CHTCTTCTCTCAGTTGGCTAAAAAGG CH-25 CHTCTTCTCTCAGTTGGCTAAAAAGG CH-26 CHTCTTCTCTCAGTTGGCTAAAAAAAAAAAAAAAAAAAAA	CAAI 3C 530-558 TCTAT	$(AC)_{18}(AG)_{11}$	58	5	0.6333	0.7814	0.7300	0.0068	JN682073
 CH-20 F. GUITCUCUIALI LUAUROUG CH-20 F. GGTCAAAAAGGGGACCTAA CH-22 F. GGCTCAAAAAGGGAACCTAA CH-23 F. GGTCGGAATGCACCAACTA CH-24 F. GTTGGGATGTTGGTCGTCACA CH-25 F. CTTCTTCCTCAGTTTGCTCA CH-26 F. ATTGTCGCTTGAACAAGGTCAGAAA CH-26 F. ATTGTCGCTTGAACAAGGTCAGAAA CH-26 F. ATTGTCGCTTGAACAAGGTCAGAAAA CH-27 F. CATATGTCAACAACAAGGTCAGAAAA CH-28 F. ATTGTCTCGTAACAACGGAAAAA CH-28 F. ATTGTCTCGTAACAACGGAAAAA CH-28 F. ATTGTCTCGTAACAAGGTCAGGAAAA CH-28 F. ATTGTCTCGTAAGGGAAAACGTCAAGGTCAGGAAAA CH-28 F. CCAACAACAACAACGGAAAACGTCAAGGTCAGGAAAA CH-28 F. CCAACACGAAGGGTCAAAAGGTCAGGAAAA CH-28 F. CCAACACGAAAGGGTCAAAAGGTCAGGAAAA CH-28 F. CCAACAGGAAAAACGTCAAAGGTCAGGAAAACGTGAAAAACGAGAAAGGTCCAGGAAAACCTCGAAAAGGTCAGGAAAA CH-31 F. TGTTCTGGTGGAACGTCCAAGGTCAAGGTCAAAGGTCAAAGGTCAAAGGTCAAAGGTCAAAGGTCAAAGGTCAAAGGTCAAAGGTCAAAACTCAAAGGTCAAAGGTCAAAAACGAGAAAACTCTGGTTCAGGAAAACTCTGGTCAAAAGTCAGGAAAAACGAGAAAACTCTGGTCAAAAGGTCAAAAACGAGAAAACGAGAAAACGAGAAAACGAGAAAACGAGAAAACGAGAAAACGAGAAAACGAGAAAACGAGAAAACGAGAAAACTCGGTTCAAAGGTCAAAAACGAGAAAACGAGAAAACTCGGTCAAAAACCAGGAAAACCAGGAAAACGAGAAAACTCGGTCAAAAACGAGAAAACGAGAAAACTCGGTCAAAAACGAGAAAACTCGGTCAAAAACGAGAAAACTCGGTCAAAAACTCGGTCAAAAACGAGAAAACTCGGTCAAAAACGAGAAAACTCGGTCAAAAACTCGGTCAAAACTCAGTCAAAACTCAGTCAAAACTCAGTCAAAACTCAGTCAAAACTCAGTCAAAACTCAGTCAAAAACTCAGTCAAAAACTCAGTCAAAACTCAGTCAAAACTCAGTCAAAAACTCAGTCAAAAACTCAGTCAAAAACTCAGTCAAAAACTCAGTCAAAAACTCAGTCAAAAACTCAGTCAAAACTCAGTCAAAAACTCAGTCAAAAACTCAGTCAAAAACTCAAAAACTCAAAAACTCAAAAACTCAAAAAA	CG 286-314	$(CA)_{17}$	61	8	0.6667	0.8599	0.8266	0.0024	JN682074
 KH-22 KH-11ULULUARAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	AATCG 155-211	$(AG)_{12}$	61	6	0.5667	0.8492	0.8144	0.0052	JN682079
 Kadol Control Control Control Control Kadol GGTI GTI GGG GGT GAT GGG TA GGT CA GGT CA GGT GGT GGG GGT CA GGT C	CICIIGU 260-320	$(CA)_{19}$	61	7	0.7333	0.7944	0.7535	0.0585	JN682081
 KH-25 KU LUOUNDUATIONICAUT KU L26 KUTTCTCCTCAGTTTGCTCAG KU CTTCTCCTCAGTTGGTCAG KU CTCTCACTTGAAGGTCAAAA KU CTCTCGGTAAGGAAAAAA KU CTGTGAAGGTAAAAAC KU CTGTGAAGGTCAAAATGG KU CTGTGAAGGTCAAAAAC KU CTGTGAAGGTCAAAAAC KU CTGTGAAGGTCAAAATGG KU CTGTGAAGGTCAAAAACCAGA KU CTGTGAAAACTCGATTGGTCAGGA KU CTGTGAAAACTCGAAAAGTGTAGGAAAAACTCGGAAAACTCGGTGAAAAACTCGGAAAAACTCGGAAAAACTCGGAAAAACTCGGAAAACTCGGAAAAACTCGGAAAAACTCGGAAAACTCGGAAAACTCGGTAAAACTCGGTAAAACTCGGAAAAACTCGGTAAAACTCGGTAAAAACTCGGAAAAACTCGGAAAAACTCGGAAAAACTCGGTAAAAACTCGGAAAAACTCGAAAAAAAA	CAGG 162-187	(AC) ₁₁	56	9	0.9310	0.8034	0.7615	0.7172	JN682082
 KH-26 K- GALCAAAAAAUGAAAAA K- GALCAAAAAGAAAAA K- ATTGTCGGTAAGGAAAAA K- ATTGTCGTTGAACAGAAAAT K- ATTGTCGTTGAACAGAAAAT K- ATTGTCGTTGAAGGAAAAT K- ATTGTCGAAGGTCAAAAT K- ATTGTCGAAGGTCAAAAT K- ATTGTCGAAGGTCAAAAT K- ATTGTCGAAAAACCAGA K- AGCAGACGGAAAAACCAGA K- AGCAGACGGAAAAACTCGGT K- AGCAGACGAAAACTCGGTCATGGT K- AGCAGACGAAAACTCGGTCATGGT 	314-400 314-400	(AC) ₁₃	61	4	0.4000	0.6186	0.5517	0.0281	JN682085
 KH-27 F. CATATGTCAACTCTATTTGGC KH-28 F. CATATGTCAACTCTATTTGGC R. ATCTGTGAAGGTCAAATGG R. ATCTGTGAAGGTCAAAATGGT K. AACAACTGAAAACCAGA K. AACAACTGAAAAACCAGA K. H-31 F. TGTTCTGTGTCACTCAATGT K. AGCAGACGGTGCCTCAATGT K. AGCAGACGGAAAACTCGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTG	ATCT 241-273	$(A)_{8}(AC)_{7}$	48	9	0.5667	0.7638	0.7118	0.0073	JN682086
KH-28 F: GCAACCGITTATTAGTGIT KH-28 F: GCAACCGITTATTAGTGIT R: CAACAACTGAAAACCAGA R: CAACAACTGAAAACCAGA R: TATACTGGTCCTGTTCAG KH-31 E: TGTTCTGTGACCGTCAATGT R: AGCAGACGAAAATTCTGGTG R: AGCAGACGAAAATTCTGGGC	GTG 138-156	$(GT)_8$	56	4	0.6000	0.7158	0.6556	0.0555	JN682087
KH-30 F. CATAATGTTTCCTGTTCAGGA R: TATACTGGTCCCTCATGT R: TATACTGGTCCCCTCATGT KH-31 F. TGTTCTGTGACGTC R: AGCAGACGACAACCTCGGG R: AGCAGACGACAAACTCTGG KH-32 F. GGATCACATCAGTCAGTA	ыс TG 240-294 АтС	$(AC)_{18}$	56	9	0.5667	0.7644	0.7139	0.0481	JN682088
KH-31 F. TGTTCTGTGACGTCATGGTC KH-31 F. TGTTCTGTGACGTCATGCTC R: AGCAGACGAAAACTCTGG KH-32 F. GGGATCACATCAGTCAGTA	GC 324-375	(TTA) ₆ (TAG) ₅	49	~	0.8214	0.8669	0.8337	0.2630	JN682090
KH-32 F: GGGATCACATCAGTCAGTA	IGTT 400-440	$(TC)_{22}$	63	×	0.7241	0.8766	0.8456	0.0146	JN682091
	AGC 196-246 CTT	$(CT)_{12}$	59	6	0.6333	0.8508	0.8165	0.0071	JN682093
KH-34 F: TGATCCTTCCCTCAACTTAI	NTTC 89-126	(CT) ₁₅	51	14	0.9333	0.9119	0.8877	0.7657	JN682095
KH-35 F: GCATGCAATTTGTTATGAG R: CGGAGTTGGTAGTATAGGGT	3G 225-258 TAAG	(CT) ₁₃	56	12	0.7667	0.8831	0.8558	0.0060	JN682096

J.-T. Tian et al.

Genetics and Molecular Research 11 (4): 4501-4507 (2012)

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Table	1. Continued.									
Locus	Primer sequence (5'-3')	Size range (bp)	Repeat motif	Ta (°C)	$^{N}_{v}$	H_0	$H_{\rm E}$	PIC	$P_{\rm HW}$	Accession No.
KH-41	F: CGCATTGCAGACGTTCTTTTCAT R: TCGTCAGCTTTTTGAACAACTTTCG	358-392	(CA) ₆ T(AC) ₅ (CCCA) ₂	56	×	0.6000	0.8576	0.8236	0.0021	JN682102
KH-42	F: TCCTAATGTTACCTGACACTACTTC P: ATGGTTCTTCACTACAA A ATCTGCC	235-279	$(GA)_{14}$	56	11	0.8333	0.8763	0.8469	0.1896	JN682103
KH-43	E: TGTAACAGAGTTGTCTGCTCTT R: TATAAGAGTTAGGAACCAGGG	236-271	$(GT)_{14}$	56	~	0.7667	0.8339	0.7971	0.0783	JN682104
KH-44	F: GCAGCTTGATTCALTGTTCAC B: GCACCCAGTGATTATTTCCTAT	234-278	$(TTA)_3(T)_5$	56	6	0.6333	0.8011	0.7596	0.0124	JN682105
KH-45	F: CTGAAACGCTGTTTATCATCTGTCG R: ACCATTACGCACCACCACTTTCTGT	192-245	(AG) ₁₅	56	10	0.9000	0.8768	0.8477	0.6318	JN682106
KH-50	F: TTATTGTATGTAGGGGGGGGGTG R: TGGCCATCAGTTAGAATTGTGTG	268-301	(AC) ₅ T(AC) ₅ (CG) ₆	56	9	0.5517	0.8100	0.7653	0.0126	JN682112
KH-56	F: AGCGTTTTGTCCATTTGGGGGAT R: GCATCGGGGCAGGTAATCT	282-324	$(GA)A_{5}(AG)_{13}AA(AG)_{9}$	56	9	0.7000	0.8260	0.7850	0.0114	JN682116
KH-61	F: GGACGTAAACCTCCATCT F: CCAGAACCTCCATCT	118-183	$(TC)_{19}(CA)_{20}(AC)_{12}$	51	11	0.8276	0.9002	0.8735	0.0752	JN682121
KH-65	R. CCAGCATTGTTAGTGGTGT P: CCCAGCATTGTTAGTGGTGT R: TAATCGCTCCTTTTTAGGACAGCT	180-213	$(TC)_{31}(TCA)_6$	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 P: CGTGCCATGGGTATCCGTTTTT	126-212	$(CT)_{23}$	63	9	0.7586	0.8209	0.7796	0.0601	JN682133
KH-68	F: TGTCTACTACACTGGGCATCAT R: AGTTGCTGAAATACAAACCTACACTC	466-509	$(TC)_{11}(TC)_7ACT(CT)_8(CT)_{14}(TG)_8$	63	9	0.7037	0.8155	0.7710	0.0098	JN682134
KH-71	F: GGCTAATTGCACTGAATGTCTG R: GTTCAGTGTGCGTAGCCAAT	77-124	$(GA)_8A(AG)_{17}$	09	6	0.6897	0.8947	0.8670	0.0021	JN682142
KH-74	F: ATGGAACAGGCTCTGGTGTC R: GCTCTTTCAGACTCGCGGGATAT	269-341	$(GA)_6(AG)_6$	61	9	0.8667	0.7610	0.7137	0.5528	JN682146
KH-75	F: CATTGGCCGTTTGTTTCTTT R: CCGTCTGTATACTACAATTCTCC	279-295	$(T)_{19}$	60	8	0.9655	0.8445	0.8076	0.9775	JN682148
								C	ontinued	on next page

Microsatellite markers for Scapharca broughtonii

Genetics and Molecular Research 11 (4): 4501-4507 (2012)

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4505

FUCUS	Primer sequence (5'-3')	Size range (bp)	Repeat motif	Ta (°C)	\sim^{N}	$H_{ m o}$	$H_{\rm E}$	PIC	P_{HW}	Accession No.
KH-76	F: GGTTTATGAATGTTTACGGCACTG R: ATCA A ACTGGATGGGACA GG	234-267	(C),G(TC),	63	4	0.4333	0.4000	0.3710	0.8613	JN682149
KH-78	F. GCAGCTGATGTGTGTAAAACAGT	288-322	(TC),TT(TC)5	56	8	0.3448	0.8324	0.7929	0.0000*	JN682155
KH-79	R: ACTCATTTGTCCCCGCTTT F: GCTGCGAATCCTAACACTAC	238-273	$(TG)_{\epsilon}$	47	14	0.5333	0.9006	0.8755	0.0000*	JN682157
KH-80	R: ACCCAGGGCATCTATAAAGT F: ATAGAACCGTAGCGTTTAGA	298-369	(AG)	56	6	0 7667	0 8842	0 8552	0 0069	IN682158
	R: CCAATAATAGCAGCGAAAATC		(1)12	2	,					
KH-88	F: ATCTGCACTTTGACACGACA R: TGC ATTTC ACTCGC A ATCTCT	213-261	$(GA)_{12}$	61	8	0.7667	0.8684	0.8366	0.0490	JN682169
KH-89	F: TCACTCATTCTCCCCATTTAT	234-257	(CTAGA) ₃	51	9	0.1034	0.7393	0.6788	0.0000*	JN682170
	R: AGCTTCCGATTTGGTACTTAT		5							
KH-90	F: CCATTAAGACGATTATGGACGA	124-176	$(GT)_{18}$	61	13	0.6429	0.9208	0.8964	0.0000*	JN682171
VH 02	K: TAAGCUGAAGCACAAUIA E: Caaaacuudaagcacaacia	107 731		09	9	0,000	1000	19990	0.0002	171C02INI
66-HN	R: CCAGGATTAGAATGCCGAAGT	107-161		00	D	0.4000	0.1294	0.0004	C000.0	4/1700MT
KH-95	F: ACTTTGACACGACACACACG	211-245	(GA) ₁ ,	61	10	0.9000	0.8599	0.8281	0.7117	JN682177
	R: GTGCATTTCACTCGCAATCTCTT		4							
KH-98	F: ATTGGCCGTTTGTTTCTTT	275-297	$(T)_{9}$ $(GTTT)_{3}(T)_{17}$	60	9	0.7000	0.8322	0.7927	0.0552	JN682181
	R: ACACCGTCTGTATAACTACAATT									
KH-99	F: CCCAAAGGATTATTTGGCTGTCAT	272-400	(TGAGT),	61	9	0.6897	0.7943	0.7494	0.0801	JN682182
	R: TGTCTGCACCTTCGCCTAT		1							
KH-100	F: GTTTGTACTGTGACTGAGGC R: TGTCGGTTGGTCTAAAGCAT	294-376	$(T)_4 G(T)_{10}$	60	2	0.6071	0.8117	0.7687	0.0010*	JN682183

Genetics and Molecular Research 11 (4): 4501-4507 (2012)

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4506

J.-T. Tian et al.

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Genetics and Molecular Research 11 (4): 4501-4507 (2012)