Twenty-one microsatellite loci from sea cucumber *Apostichopus japonicus* (Selenka, 1867), an endangered species on the IUCN Red List

Zhiguo Dong^{1,2,3} · Yihua Chen^{1,2,3} · Guangen Xu^{1,2,3} · Chuanlei Kang^{1,3,5} · Guanghui Fu⁴ · Baiyao Chen⁴ · Jiale Li⁵

Received: 18 July 2017 / Accepted: 10 July 2018 / Published online: 17 July 2018
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Abstract

The sea cucumber *Apostichopus japonicus*—an endangered species on the IUCN Red List—is economically and ecologically important echinoderm species. Here we describe a set of 21 amplified microsatellites for *A. japonicus* isolated through biotinylated probes for hybridization and a library screening method, which were used to genotype an artificially propagated stock of sea cucumber offspring from Lianyungang City of China. The observed and expected heterozygosity at the population level ranged from 0.2083 to 0.1000 and 0.6260 to 0.9304, respectively. The number of alleles varied between 6 and 19, and none of the loci deviated significantly from the Hardy–Weinberg equilibrium ($P < 0.01$). These data might be useful in future analyses of sea cucumber genetic diversity, determination of genetic relationships, and the conservation of genetic resources.

Keywords *Apostichopus japonicus* · Microsatellites · Molecular markers · Genetic conservation

The sea cucumber *Apostichopus japonicus*, an endangered echinoderm species of the superfamily Stichopodidae, naturally distributes in the Northwest Pacific, including Japan, China (from the Liaoning coasts to Qianshan Island of Jiangsu), the Korean Peninsula, and Far Eastern Russia. As a rare seafood with high nutrition and health care function, sea cucumber farming is gaining popularity. However, except Japan, all of the Asian region countries lack conservation measures for the sea cucumber fisheries (Choo 2008). Therefore, here we developed microsatellites for the future identification and conservation of sea cucumbers germplasm resources.

Microsatellite DNA loci are popular and powerful molecular markers in population genetics and systematic geography (Liu and Cordes 2004). At present, the methods of developing microsatellites include the simple sequence repeat (SSR) method, enrichment library method, and transcriptome sequencing method. The use of magnetic beads is relatively well-established. However, the majority of reports of microsatellites involved the use of single biotinylated probes. In this study, we adopted the method by Dong et al. (2014), using two biotinylated probes simultaneously to determine the efficacy of this method to screen microsatellites.

Using the phenol–chloroform method (Sambrook et al. 1989), total DNA was extracted from the muscle of 50 sea cucumber samples obtained from an aquatic farm in Ganyu County, Lianyungang City. Total DNA was used to construct a partially enriched genomic library for GA- and CA-motif microsatellite sequences. Genomic DNA was digested with Tru1 (Fermentas, China). The fragments were ligated to an MseI adaptor pair (Oligo-MseI A: 5'-TACTCAGGACTC AT-3', Oligo-MseI B: 5'-GACGATGAGTCCTGAG-3'). The adaptor-ligated products were used as templates for PCR amplification, and the PCR products were hybridized to biotinylated oligonucleotide probes (including microsatellite GA10 or CA10 motifs) attached to streptavidin-coated magnetic beads (Dynabeads M-280 Streptavidin, Invitrogen Dynal AS, Oslo, Norway). Following the second round

✉ Zhiguo Dong
dzg7712@163.com

¹ Jiangsu Key Laboratory of Marine Bioresources and Environment, Huaihai Institute of Technology, Lianyungang 222005, China

² Jiangsu Key Laboratory of Marine Biotechnology, Huaihai Institute of Technology, Lianyungang 222005, China

³ Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Huaihai Institute of Technology, Lianyungang 222005, China

⁴ Lianyungang City Marine and Fishery Development Promotion Center, Lianyungang 222000, China

⁵ Shanghai Ocean University, Shanghai 201306, China

Table 1 The characterization of 21 *Apostichopus japonicus* microsatellite loci

Locus	Primer sequence (5'–3')	Repeat motif	Ta (°C)	Size range (bp)	No. of alleles	Sample size (N)	Ho	H _E	GenBank accession number
AJA1-103	F: TGATTACAT TACTGCTAT TC R: CACCAGAAAG TAGATCACTC	AATTTTC(3)	50	94–94	8	54	0.0370	0.8182	KP342290
AJA3-123	F: TCGTTTACG GTAGCGAGA AT R: AACCGTTTA GGTGGACTG AC	TTTCG(3)	56	94–128	11	60	0.0333	0.8494	KP342291
AJA4-229	F: GGCAGGATT ATGGTTCTT R: ATGGTTGTG ATTATTTCA GTG	GGAC(3),ATC(11),ACA AAA(6)	52	201–250	13	42	0.0432	0.8980	KP342292
AJA5-186	F: TCGAAGATA ACCGTGCTGT R: CATTGATGC GCTTGAAAAT	AAC(4)	52	162–216	14	60	0.1000	0.6260	KP342293
AJA7-346	F: TCCATCAGT AGACATTTAT R: CTTTTCAGG TAGACACTT	TTGACC(5),TGACCT(3)	49	244–342	17	54	0.6248	0.9300	KP342294
AJA8-157	F: AGCTCCATA AACCATTTC R: TACTTTCCC CGTTAAGAT	GAGG(20)	51	76–157	19	44	0.7727	0.9174	KP342295
AJA9-148	F: CAGAAAGGG AACGCAACAC R: CAGAAAGGG AACGCAACAC	CA(6),A(21)	58	258–374	19	58	0.0690	0.9304	KP342296
AJA10-189	F: GGCAACATA GTGAGACCC R: AGCAGTGGC ACAATCTTAG	AC(24),AGTC(3)	56	103–162	9	48	0.0577	0.8542	KP342297
AJA12-119	F: TGAGAGAGC GACAGAAATG R: GAGTGGGTT GTATGTTGTG	AC(18)	55	55–71	9	60	0.3667	0.8594	KP342298
AJA13-129	F: CGATCTTAT AATGTTGTCT R: ACTTTGGAA GCATACTAC	TTAT(9)	49	77–102	10	60	0.0333	0.8550	KP342299
AJA14-111	F: ACTCCTCCG TCTCTGTTA CT R: ATCCTATCT TGTCATCCC TCT	AG(3)	50	90–163	7	46	0.6522	0.7798	KT884910
AJA15-178	F: GAACAGAAT AGGCACTGA AAAAG R: GAGGAGGTG TTGTTTAAA GAAAAT	GT(3) GT(5)	56	76–107	12	46	0.9130	0.8837	KT884911

Table 1 (continued)

Locus	Primer sequence (5'–3')	Repeat motif	Ta (°C)	Size range (bp)	No. of alleles	Sample size (N)	Ho	H _E	GenBank accession number
AJA16-143	F: GCTTTCCGA GGTCAACAT R: GGATAACTT ACGGTTCCTG TCG	ACA(3)	52	125–143	6	44	0.0455	0.7882	KT884912
AJA17-106	F: AACCCGATA AGTTTCATG TCTG R: ATGTTTCCA GCCAGGGGT	GC(3) TG(3)	56	84–123	9	46	0.4783	0.7448	KT884913
AJA18-128	F: CAACAGCTA CGTTTGCA R: GAGAATATT AGATAGTTA AATGGGA	A(6) A(15) A(3) A(10) A(3) A(3) A(3)	52	85–123	10	44	0.7083	0.8186	KT884914
AJA19-143	F: CCCTGGCAT TCATATCTTT R: CAACCCTAT TCATGTACC TCTT	GA(20)	52	111–148	7	44	0.0455	0.6808	KT884915
AJA20-144	F: CCAGGAAAA CCGTTTAGG TG R: GGGCTCTCA GGCAATCGT	AAAAC(3)	57	111–146	6	48	0.0562	0.7465	KT884916
AJA21-144	F: TCGATAAAA ATAAATTAG CGG R: ATTGAAAAT GAAGACAGT ATAGAAG	TTC(4)	52	138–163	7	44	0.0455	0.6808	KT884917
AJA22-145	F: CAATAGTGA ACTTAGAAC TGTGG R: AGCAATGTC TGCAGGAAA	TAA(3)	51	122–162	10	48	0.2083	0.8459	KT884918
AJA23-185	F: CTCACGATG TCTAATTTG CAG R: CAGTACAGT CCTTTCCTC CC	AT(3)	53	178–198	10	48	0.6250	0.8715	KT884919
AJA24-185	F: GGCAACATA GTGAGACCC R: AGCAGTGGC ACAATCTTA	AC(9)AC(12)	48	104–151	10	44	0.7273	0.7934	KT884920

Ta annealing temperature, Na observed number of alleles, Ho observed heterozygosity, H_E expected heterozygosity

of PCR, the re-amplified fragments were ligated into the pUCm-T vector (Shanghai Sangon, China) for cloning and sequencing. In total, 24 microsatellite primers were successfully designed using the Primer Premier Software (version 5.0). Twenty-one individual DNA samples were amplified by PCR for screening these primers. For amplification, each PCR mix contained 12.5 µl of 2×EasyTaq PCR SuperMix

(TransGen Biotech, Beijing, China), 2 µl of DNA template, 1 µM of forward and reverse primers, and molecular biology grade water (TransGen Biotech) was added to make a total volume of 25 µl. The PCR reactions were conducted with the following cycling parameters: initial denaturation at 95 °C for 4 min; followed by 35 cycles of 95 °C for 30 s, 56–60 °C for 30 s, and 72 °C for 60 s; and then 72 °C for 7 min for

elongation and 4 °C for storage. The PCR products were electrophoresed on a 8% polyacrylamide gel for genotyping (Sambrook et al. 1989). The PAGE products were visualized by silver nitrate staining following the procedure of Gustavo and Gresshoff (1994).

Among the 24 primer pairs, 21 polymorphic microsatellite markers were screened successfully in the sea cucumber samples (Table 1). The microsatellite data were analyzed by POPGENE (version 3.2; Raymond and Rousset 1995), indicating that the number of alleles ranged from 6 to 19, and the observed and expected heterozygosity at the population level varied from 0.0333 to 0.9130 and 0.6260 to 0.9304, respectively. None of the loci deviated significantly from Hardy–Weinberg equilibrium ($P < 0.01$). These 21 novel and informative microsatellite markers will be useful in future for studies focused on the population genetics and conservation of sea cucumbers.

Acknowledgements This study was supported in part by grants from the Key Technology Research and Development Program of Jiangsu Province (no. BE2012421), the Project Jiangsu Key Laboratory of Marine Biotechnology (Grant no.2014HS001), the Innovation and Training Programs for College Students in Huaihai Institute of Technology (Grant no. HYDC16-31) and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest in the publication.

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