JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

doi: 10.1111/jwas.12334

Development of Gene-derived SNP Markers and Their Application for the Assessment of Genetic Diversity in Wild and Cultured Populations in Sea Cucumber, *Apostichopus japonicus*

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Abstract

The *Apostichopus japonicus* is a valuable aquaculture species in China. In this study, 51 single nucleotide polymorphisms (SNPs) were identified from expressed sequence tags of sea cucumber using high-resolution melting. The average observed heterozygosity (*H*o) and expected heterozygosity (*H*e) were 0.2462 and 0.2897, respectively. Thirty-two of these loci were used for estimating the genetic similarity and variation between the five hatchery stocks from China and two wild stocks from Japan. No significant differences in *H*o or *H*e were observed between the wild and hatchery populations. The pairwise *F*st (which ranged from 0.0119 to 0.0236) and the genetic identity (which varied from 0.9802 to 0.9915) showed no significant differentiation between the wild and cultured stocks. The analysis of molecular variance indicated the source of variation was at the level of "within the populations." The information on the genetic variation and differentiation in cultured and wild populations of *A. japonicus* obtained in this study is useful for setting up suitable guidelines for founding and maintaining of cultured stocks and for future genetic improvement by selective breeding.

The sea cucumber, *Apostichopus* (*Stichopus*) *japonicus* (Selenka), is a common benthic detritus feeder of coastal sea habitats in China, Japan, Korea, and far eastern Russia. *A. japonicus* is an important economic species in China due to its high market value. The increasing market demand and overexploitation of wild sea cucumbers stimulated the aquaculture of *A. japonicus* along the coasts of China in the past decades (Du et al. 2012). In 2012, the production of sea cucumber was 170,830 metric tons in China (BOF 2013). However, the rapid development of sea cucumber aquaculture was accompanied by a serious problem that the diversity of *A. japonicus* may decline to some degree after nearly 30 yr of domestication. In hatchery stocks, sea cucumber breeding could persist for many generations by using the limited number of parents because of high fecundity of mature female sea cucumbers and the technique of artificial fertilization. However, their offerings may demonstrate a reduction in genetic variability compared with their wild progenitors. Moreover, the massive releases of hatchery sea cucumbers in the stock enhancement industry have raised concerns about the genetic effects on the wild populations. This may lead to reduction in the genetic diversity of released population and genetic variabilities of wild populations may be eroded by the transplantation of non-native sea cucumbers (Taniguchi 2004). The reduction in genetic diversity may result in reduction of a population's ability to adapt to environments (An et al. 2011a). Therefore, it is important to investigate the genetic diversity and genetic population structure in wild and cultured populations of sea cucumbers.

Molecular markers are widely used as an exceptional indicator of genetic variation within and between populations. As a prominent molecular marker, single nucleotide polymorphism (SNP) has been used in some marine species (Karlsson et al. 2011; Kong et al. 2014) owing to its abundance, low genotyping cost, typically biallelic, codominant and high-throughput genotyping (Bester et al. 2008; Hubert et al. 2009;

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McGlauflin et al. 2010; Clemento et al. 2011). They also have advantages of lower mutation rate and genotyping error rates which are important in many applications (Abadía-Cardoso et al. 2011). SNPs are regarded as an ideal tool for construction of high-dense maps, quantitative trait loci, assessment of genetic diversity, parentage studies, and marker-assisted breeding (Zhu et al. 2003; Hubert et al. 2009). In aquaculture animals, SNP identification and application were documented in some species including the Pacific oyster (Bai et al. 2009; Zhong et al. 2013; Jin et al. 2014), Atlantic cod (Hubert et al. 2009), black tiger shrimp (Sellars et al. 2012; Henshall et al. 2014), Pacific abalone (Qi et al. 2008), and common carp (Kongchum et al. 2010). In *A. japonicas*, the number of SNPs from the expressed sequence tag (EST) database (Sun et al. 2010; Yang et al. 2012) are limited compared with the SNPs from the transcriptome (Du et al. 2012; Gao et al. 2013; Zhou et al. 2014). Gene-derived SNPs have more advantages because they can result in a change in the amino acid sequence of encoded protein, which may affect protein function, namely nonsynonymous SNPs (Kim et al. 2003).

In this study, we developed SNP markers from the EST database of *A. japonicas* by high-resolution melting (HRM) method, and compared the genetic diversity between five cultured populations of *A. japonicas* from China and two wild populations from Japan using the SNP markers.

Materials and Methods

Experimental Populations and DNA Extraction

To screen for polymorphic SNPs, 30 hatchery individuals of *A. japonicas* were collected from Yantai, Shandong Province, China. Two wild and five cultured *A. japonicas* populations were collected for genetic diversity analysis. Two wild samples were collected from Saiki Bay (Oita) (abbreviation: WSB, 46 individuals) and Mutsu Bay (Aomori) (WMB, 50 individuals), Japan. Five cultured samples were collected from Shandong Province, the northern coast of China: Changdao (HCD, 37 individuals), Jiaonan (HJN, 59 individuals), Yantai (HYT, 59 individuals), Wendeng (HWD, 60 individuals), and Penglai (HPL, 60 individuals) (Fig. 1). No details of the founding and maintenance of the cultured populations are available; however, the parents of the sea cucumber were sampled from cultured populations. Genomic DNA was extracted from skin tissue using a cetyltrimethyl ammonium bromide procedure (Li et al. 2009). After extraction, all the DNA samples were preserved in TE buffer, then quantified and diluted to 10 ng/μL for polymerase chain reaction (PCR).

SNP Discovery in the EST Database

A total of 7739 EST sequences of *A. japonicas* were downloaded from GenBank EST database (The National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/, March 14, 2013) and assembled into contigs using the SeqMan Pro sequence assembly software (DNASTAR Inc., Madison, WI, USA). A single-base mutation that occurred in four or more ESTs and surrounded by good flanking sequences was identified as a potential SNP for validation analysis. Sequences containing SNPs were annotated using BLASTx software (U.S. Army Engineer Research and Development Center, Vicksburg, MS, USA), and sequence homology was accepted based on a cutoff E value of 1.0×10^{-6}. The informative strand and reading frame were identified by using the sequence with highest homology. The open reading frame finder (ORF Finder) was used to determine whether SNPs were synonymous, nonsynonymous, or from untranslated regions (UTRs).

SNP Genotyping and Polymorphism Evaluation

Primers were designed to have a product size of 50–250 bp and an annealing temperature at 60 ± 2 C with few exceptions, using the Primer Premier 5.0 program (PREMIER Biosoft International, USA) and Oligo 7.0 software (http://www.oligo.net/downloads.html). The polymorphic SNPs were genotyped using HRM technology. PCR was performed in a total volume of 10 μL on a LightCycler® 480 real-time PCR instrument according to Zhong et al. (2013). Data were analyzed using the LightCycler 480 Gene Scanning Software 1.5

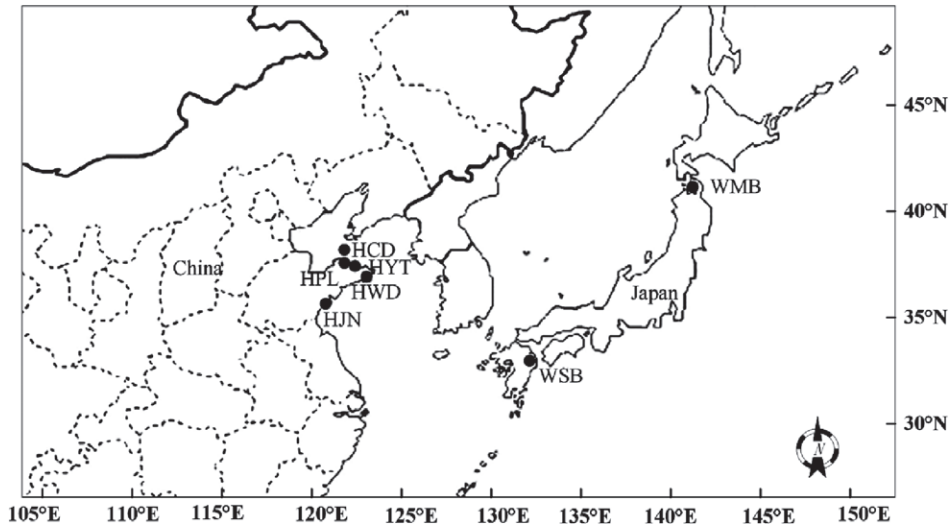

FIGURE 1. *The geographic distributions of Apostichopus japonicus samples used in this study. Two wild populations (WMB, Mutsu Bay [Aomori]; WSB, Saiki Bay [Oita], both in Japan) and five cultured populations (HCD, Changdao; HYT, Yantai; HWD, Wendeng; HPL, Penglai; HJN, Jiaonan, all in Shandong province, China) were used.*

(Roche Diagnostics). For each SNP locus, the minor allele frequency, expected heterozygosity (*H*e), observed heterozygosity (*H*o), deviations from Hardy–Weinberg equilibrium (HWE), and linkage disequilibrium (LD) were calculated using PopGen 32 software (http://cc.oulu.fi/~jaspi/popgen/popdown.htm).

Genetic Diversity of Wild and Hatchery Populations

Genetic diversity of wild and hatchery populations was assessed using the developed SNP loci. For each population and locus, *H*e, and *H*o, deviations from HWE were determined by PopGen 32 software. The polymorphic information content (PIC) was calculated by the method according to Botstein (1980):

$$\mathrm{PIC} = \sum_{i=0}^{n} P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

In the formula, the P_i and P_j represent allele frequency of *i*th and *j*th in the population, respectively, and the *n* represents the number of alleles. Fixation index (*F*is) was also calculated using PopGen 32 software to evaluate the extent of differences within and among populations.

To estimate the genetic differentiation between the wild and hatchery populations, two parameters were measured: genetic identity (*I*), which estimates the proportion of genes that are identical in two populations, and genetic distance (*D*), which estimates the proportion of gene changes that have occurred in the separate evolution of two populations (Rafferty 2010). Pairwise genetic distances and genetic identity between populations were estimated according to Nei (1978) using PopGen 32 software. The *F*st reflects the genetic relationships of different populations. The population kinship relationship has a negative correlation with the value of *F*st, so pairwise *F*st were calculated with the Weir and Cockerham (1984) method using the GenAlex 6.501 software (Peakall and Smouse 2012). The hierarchical genetic variation existing between wild and hatchery populations and within populations was analyzed by analysis of molecular variance (AMOVA) using GenAlex 6.501 software (Meirmans 2012).

Results

Marker Information

The alignment of the 7739 ESTs downloaded from GenBank produced 1878 contigs.

According to the frequency of mutation and conservation of flanking sequences, 229 putative SNPs were selected for validation. Of the 229 primer pairs, 140 primers could produce reliable amplification, and the other 89 failed to amplify. Among the 140 candidate SNPs, 51 (36.43%) were polymorphic and considered as validated in the 30 cultured sea cucumbers. The characteristics of 51 SNPs are summarized in Table 1. Of these SNPs, four EST-SNPs (SNP59, SNP109, SNP138, and SNP148) were the same as the SNPs developed before (Sun et al. 2010; Yang et al. 2012), the other 47 EST-SNPs were newly developed. The minor allele frequency ranged from 0.0333 to 0.4000, with an average of 0.1563. The *H*o and *H*e ranged from 0.0345 to 0.5000 and from 0.0345 to 0.5643, with averages of 0.2405 and 0.3055, respectively. The number of significant LD is 12 ($P < 0.05$). The deviation from HWE was observed at eight loci. Of the 51 SNPs, 38 (74.5%) could not be annotated, 28 (54.9%) were located in the coding region, and 23 (45.1%) in the UTR (Table 1). Twenty-two of the 28 SNPs located within the coding region were synonymous, and 6 nonsynonymous.

Population Genetic Variability

A total of 32 EST-SNP loci were selected for the population analysis. All 32 SNP loci were found to be polymorphic in both wild and hatchery populations. The *H*o, *H*e, probability of significant deviations from HWE, index *F*is, and the PIC are presented in Table 2. The mean *H*o of the 32 loci was the highest in the wild WMB stocks (0.2760), followed by the hatchery HCD (0.2759) and WSB (0.2664) populations. The hatchery HCD (0.3354) was the highest in the average *H*e, followed by the WSB (0.3280), HYT (0.3256), WMB (0.3188), HWD (0.3147), HPL (0.3011), and HJN (0.2689). Significant departures from HWE were observed in 90 of the 224 singlelocus (Table 2). Inbreeding coefficients (*F*is) varied from −0.2632 to 0.6580 in the hatchery stocks and from −0.2500 to 0.6617 in the wild stocks. Moderate polymorphisms were found from the PIC values of all the loci. The average PIC value (0.2687) of the hatchery HCD was higher than that of other stocks, followed by the wild WSB (0.2642) and the hatchery HYT (0.2620).

Population Genetic Differentiation

Genetic identity (*I*) and genetic distance (*D*) between the wild and hatchery populations are presented in Table 3. The high genetic identity (*I*) (from 0.9802 to 0.9915) demonstrates that both the wild and hatchery populations have a high proportion of identical genes structure. The pairwise *F*st values between the hatchery stocks and wild populations showed no significant differences in all but three comparisons (HJN and WMB, HJN and WSB, HPL and WSB), suggesting no genetic differentiation between the hatchery and wild stocks (Table 4). Significant genetic differentiation was detected in 8 of 10 comparisons in the five hatchery stocks, but not between the two wild populations. In the AMOVA, 99% of variations were found within populations, and only 1% among populations (Table 5).

Discussion

Marker Information

Gene-derived SNPs are more valuable than the genome SNPs because they are directly located in coding sequences and related to gene functions. The functions may be correlated with sea cucumber aquaculture and could be used to establish families which have particular economic traits by marker-assisted selection (Aguilar-Espinoza et al. 2014). The 51 EST-SNP loci showed polymorphic in the population and were beneficial for genetic diversity assessment, genome mapping, reproductive ecology analysis, and other SNP-based analysis in aquaculture practices for *A. japonicus*. Less than half of SNP loci can be annotated probably because several putative ORFs were found for some amplicons and only the most feasible one was chosen. The deviations from HWE were observed at eight loci with heterozygote deficiencies, which may be due to the null alleles or Wahlund effect.

A variety of methods such as tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) and melting temperature

TABLE 1. *Characteristics of the 51 single-nucleotide polymorphisms (SNPs) for* Apostichopus japonicus *derived from expressed sequences tags.*

SNP name	Accession no.	Primer sequences (5′–3′)	*T*a (C)	Amplicon length (bp)	SNP type and location	Annotation	Type	*H*e	*H*o	MAF	*P* value
SNP4	GH986253	F: GAACCTGAAGGAGGATTAAGACC R: GAAGTAGTCCGACCGTTCCAC	60	63	A/G 776	Ferritin	UTR	0.3539	0.3103	0.2241	0.5072
SNP8	GH253714	F: GCTAATTTGACAGGTATCACACACC R: CAACACCTTTAGTGGTAGCGGC	60	86	A/G 851	Unknown	UTR	0.1887	0.1379	0.1034	0.1979
SNP20	GH253420	F: ACTCACTGTGCTGGAACTGTCG R: CGCCGTAAAGGCTGACTGATT	60	67	A/G 586	Proprotein convertase subtilisin/kexin type 9	N (Asn/Ser)	0.3727	0.2759	0.2414	0.1686
SNP26	GO253762	F: GTCTTAACCAGACCTCTTTCCTATT R: ATGGGACTGATTCTATAACTATCGG	60	114	A/G 723	Cytochrome c oxidase subunit II	S (Gly)	0.3254	0.1333	0.2000	0.0024*
SNP27	GH986068	F: GGTAACCAAAGGGTGTAGCAGC R: ACCTGACTTACGTCGGTCTGAAC	60	91	A/G 412	Unknown	UTR	0.0345	0.0345	0.0172	1.0000
SNP33	GH986384	F: GACTGCTCGTAAATCAACTGGTGG R: CACTCTTTCTGGCTGCCTTGG	62	71	T/G 287	Unknown	S (Ala)	0.0345	0.3103	0.1897	0.9655
SNP48	GO254052	F: ACAACTTTTTTTTGACCCAGCAG R: GTGAGAGATCATACCAAATCCAGG	60	121	C/T 438	Cytochrome oxidase subunit 1	UTR	0.1948	0.1429	0.1071	0.2066
SNP59	GO253323	F: GCAACCGTGGAGAAAAAGAAT R: AACTTTTTCCGTTTACCAGAGTAAC	60	97	A/G 389	Unknown	UTR	0.1266	0.0667	0.0667	0.0501
SNP62	GH985579	F: GGGGAGTCACAAGTATGTATCAGAG R: TGTAGTGCCATGTACGGATTCTC	60	89	A/G 258	Unknown	S (Arg)	0.4217	0.3793	0.0667	0.5842
SNP68	GR706505	F: TATCTGCCATTTTGTTTACCTCTCT R: TTGACCTGTGTAGTAAGGCTGATAC	60	126	A/G 642	NADH dehydrogenase subunit 5	S (Gly)	0.3045	0.3667	0.0667	0.1323
SNP84	GO270701	F: GGAAGCGTGCTCTTATTAGGAAC R: GATAGAGCAGACTTTTGAAGGGAG	60	132	A/G 106	Unknown	S (Leu)	0.1307	0.069	0.069	0.0523
SNP88	GO495976	F: CCCATATTGACGAGAAGGATTGC R: GACCTGACTTACGTCGGTCTGAAC	62	134	C/T 441	Unknown	UTR	0.0655	0.0667	0.0333	0.8527
SNP92	GR706116	F: GAAGAAGTGACCAAGAAGAGGACC R: CTGACCTCTGGCTTCTGATTCTT	60	110	A/G 422	Unknown	S (Leu)	0.4401	0.3667	0.3167	0.3568
SNP102	GH985669	F: AGTGGACACCAGATTGAACAAACAT R: GAACACTCCTGATGCCTTTAGCC	60	53	C/T 369	Unknown	S (Ile)	0.1554	0.1000	0.0833	0.1072

TABLE 1. *continued*

SNP name	Accession no.	Primer sequences (5′–3′)	*T*a (C)	Amplicon length (bp)	SNP type and location	Annotation	Type	*He*	*Ho*	MAF	*P* value
SNP105	GR706656	F: GGTGCTGTATTTGCTATATTTGCC R: AGTGTTGTGGGAAGAAGGTTAGG	60	139	A/G 434	Cytochrome c oxidase subunit I	UTR	0.1831	0.1333	0.1000	0.1900
SNP106	GR706656	F: GATTACCCAGACGCTTATACAACAT R: ATTGTCATTCCAGAGATGCGG	60	163	A/G 625	Cytochrome c oxidase subunit I	UTR	0.2096	0.1000	0.1167	0.0154*
SNP109	GO253832	F: GGAGCGAAAGGTTCTTGGGT R: GCGTCAGCAAATGGTTGTAGTAAT	60	87	C/T 303	NADH dehydrogenase subunit 1	UTR	0.3254	0.2000	0.2000	0.0450*
SNP115	GO496096	F: AACATCTTGGAAACAGGAAAGTCAC R: CTGGTTTACTGTGGTTTGCTTCAT	60	64	A/G 219	Fibrinogen-like protein A	UTR	0.4271	0.3333	0.3000	0.2279
SNP121	GH551772	F: TGGAGCAAAGGAAGTTGAGGTC R: GTTTGCCACTGAACTTCTTCTCG	60	131	C/G 250	Unknown	UTR	0.2160	0.1034	0.1207	0.0167*
SNP123	GH550818	F: CTTTCAGTGTGTAATGTGTGGTTGG R: GCACAAATGAAGTGTTGACGATAAG	62	167	C/T 124	Profilin	UTR	0.4808	0.3000	0.3833	0.0359*
SNP126	GH985773	F: TGTGTTGGTGAGAGCGGTGAC R: CTTTTGAGAAGACTGGTGTCTGTCC	62	85	C/G 255	Unknown	S (Leu)	0.1266	0.1333	0.0667	0.6433
SNP131	GO269824	F: AGGGTGACAACAAGGTCCAGG R: AGCCTCTGTTGGGTTGAATCC	60	74	C/T 262	Calmodulin 2	S (Ile)	0.3339	0.4138	0.2069	0.0883
SNP136	GR706115	F: GGACGGCAAGTTCAACCAGAT R: CCAATAATGACGAAGACCACGAT	60	109	C/T 479	Unknown	S (Gly)	0.4130	0.5000	0.2833	0.2103
SNP138	GR706048	F: GAGGATGTTAGAGGCAAGAACTGTC R: GACCATAGAGCAATACTTGTCCCTG	62	78	C/T 464	Unknown	S (His)	0.2668	0.3103	0.1552	0.2240
SNP141	GH986450	F: TGAGGGAGTTGAAGGAGCAGTAGT R: ACCTCCATTCCCAGAAAGATACTC	60	120	C/T 488	Unknown	UTR	0.3977	0.3333	0.2667	0.3747
SNP143	GH985516	F: AATCTGAACTTAGCAAACTCAAACG R: CCTGGAGTTCTCGGCTGGTAT	60	111	C/T 388	Unknown	S (Thr)	0.4130	0.4333	0.2833	0.7801
SNP146	GR706693	F: GGTAACCAAAGGGTGTAGCAGC R: GTAGGGAATTAACGGACGAACAG	60	59	A/G 244	Unknown	UTR	0.4401	0.4333	0.3167	0.9314
SNP147	GO253384	F: CTTGCCAGGGTGACAGCG	60	87	A/G 736	Complement factor B-2	N (Gly/Ser)	0.0655	0.0667	0.0333	0.8527

TABLE 1. *continued*

SNP name	Accession no.	Primer sequences (5′–3′)	*T*a (C)	Amplicon length (bp)	SNP type and location	Annotation	Type	*He*	*Ho*	MAF	*P* value
		R: CATATCCCCAACTGGTGACTCC									
SNP148	DY625372	F: AGGTTCTTGTTTTGCTATCATTGTT	60	103	A/G 151	NADH dehydrogenase subunit 5	S (Gly)	0.1831	0.1333	0.1000	0.1900
		R: AGTTTACCCGTGTTCAGCCAT									
SNP149	GO253404	F: AATGTCATCAGAGTCAACTGGTCC	60	63	C/G 415	Unknown	S (Gly)	0.2825	0.2667	0.1667	0.7555
		R: GTTAGATGTGGAGACCCCATAGAG									
SNP153	GO270690	F: AACACTGGCTACAACAGAGGACC	60	67	A/G 442	Unknown	S (Gln)	0.4271	0.3333	0.3000	0.2280
		R: CTCAAAGCGACCGATTGTGC									
SNP154	GH551555	F: GTTGTTTCTGTTTGGTGCTTTTTTC	62	118	C/G 721	Unknown	UTR	0.2904	0.2069	0.1724	0.1431
		R: CCACCAAGCACACCAAAAATG									
SNP155	GR706565	F: CGCAGATGAAACTGTTGAGCAT	62	126	C/T 742	Unknown	S (Ala)	0.2987	0.2143	0.1786	0.1547
		R: TGACAAGAAGAGGCTTTTCCAGAT									
SNP163	GH550645	F: GACCCTACCCTGGAGGTTGC	60	155	T/G 765	Unknown	S (Pro)	0.3045	0.3000	0.1833	0.9330
		R: ATCTCCGTGGTGGTGAATGACT									
SNP164	GH551587	F: CCATTTGCTCTTTGAAGACTGTTT	60	124	A/C 68	Unknown	N (Gln/Pro)	0.4217	0.3103	0.2931	0.1553
		R: ATTGCTACAGTGCAAAAGACGAG									
SNP166	GO270325	F: GCCAAGGGAACCCAGGACT	62	119	A/C 289	Unknown	S (Thr)	0.4483	0.3793	0.3276	0.4024
		R: TTCAGTGCCTTGATGATGAGAGAG									
SNP175	GH985470	F: GGAGGAGAAGTTGAACAAGGCAC	62	111	A/C 301	Unknown	S (Ile)	0.4401	0.2333	0.3167	0.0097*
		R: AAACTGTCGCCATCTTGCTTG									
SNP177	DY625159	F: TGAAGTTATTTGGCAAGTGGAGC	60	113	A/G 188	Unknown	S (Ala)	0.3638	0.2000	0.2333	0.0171*
		R: CGGAAACGCTTGACCTGGT									
SNP178	DY625289	F: CAGTGCCCAGCCGTAGAAC	60	72	C/T 153	Unknown	S (His)	0.3638	0.2000	0.2333	0.0171*
		R: CATCAGTTTCTTGCCATTGTTTC									
SNP183	GH550884	F: CGGAGAAAATGTCCTGATGTAAAC	62	111	C/T 334	Unknown	UTR	0.4870	0.3478	0.3913	0.1615
		R: CCAGTGAATGTGTCTGATCAAACG									
SNP185	GO253769	F: GAAGCAGGTTTACCCCGACTACT	62	141	A/G 447	Cytochrome c oxidase subunit II	UTR	0.2668	0.1724	0.1552	0.0797
		R: CACGGCATCCATCTTTACTCCT									
SNP186	GR706604	F: CTTACTTGCTGATTTTGTGTGGTG	60	56	A/G 607	Unknown	UTR	0.1831	0.2000	0.1000	0.4555
		R: TACGGTCTACAAGGAACATACACTG									

TABLE 1. *continued*

SNP name	Accession no.	Primer sequences (5′–3′)	*T*a (C)	Amplicon length (bp)	SNP type and location	Annotation	Type	*H*e	*H*o	MAF	*P* value
SNP187	GR706604	F: ATGCTCTAGTTTCCTTCCATTACAC R: ACAAACTGTTTTCCGATTTATGGT	60	157	A/G 854	Unknown	UTR	0.0727	0.2143	0.0370	0.8445
SNP189	GH986408	F: GCTCTCAGGGTCAGTGTACTCAAGT R: ATGATGGAACGGTTTGTGTCG	62	64	A/T 263	Unknown	S (Ile)	0.1948	0.2759	0.1071	0.4380
SNP213	GH549884	F: ACTGGTCAACTTCCAAAGCGTAT R: TCATCTCACAGTAGCCCTTGGTT	60	68	C/T 241	Unknown	UTR	0.3339	0.2759	0.2069	0.3560
SNP216	GO269843	F: AATGTGCTACTCATGGGGTGATT R: AGTCGGGGATCTCTCTCCTTATT	60	104	A/G 427	Unknown	UTR	0.3339	0.2759	0.2069	0.3560
SNP217	GH985485	F: AACTGGATGTGGTTACACGAGG R: CTTTGGGGAGGTCTGATGGTC	60	156	C/G 551	Unknown	UTR	0.2987	0.2857	0.1786	0.8138
SNP222	GH551382	F: CTGCTCTATTCTGTGCTTTATGTCC R: ATTGGGAGTGCTTCAAGTCATAAC	60	60	C/T 351	Unknown	UTR	0.3539	0.3103	0.2241	0.5072
SNP223	GH550323	F: CAGGGATGGTGCTCTTTACGAT R: ACTGCCACCAGCAATTCCAG	60	92	T/G 175	Unknown	N (Ser/Ala)	0.3638	0.3333	0.2333	0.6430
SNP225	GH550784	F: GTCTTATGGTTGCTTTCCTTATCCT R: AACACCGTTCTCTCTGGTCAAAT	60	140	A/G 565	Unknown	N (Gly/Arg)	0.4881	0.4667	0.4000	0.8064
SNP228	GH551832	F: GCTATCAGACGCCCCCTACTT R: GTAACGTCAGAGAAGGACAGTGGT	60	58	A/C 447	Unknown	N (Ala/Asp)	0.1266	0.0667	0.0667	0.0520

*H*e = expected heterozygosity; *H*o = observed heterozygosity; MAF = minor allele frequency; *T*a = annealing temperature; UTR = untranslated region.

*Significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$).

TABLE 2. *Observed heterozygosity (Ho), expected heterozygosity (He), (fixation index and polymorphic information content) values at 32 SNP loci of seven populations.*

		Hatchery population					Wild population	
Locus		HJN	HYT	HWD	HPL	HCD	WMB	WSB
SNP4	*H*o	0.1034	0.2807	0.2333	0.3220	0.1622	0.3800	0.1522
	*H*e	0.1295	0.4360	0.4235	0.4231	0.3732	0.3578	0.2759
	P	0.1988	0.0075*	0.0006*	0.4952	0.0009*	0.6477	0.0060*
	*F*is	0.1944	0.3504	0.4444	0.0812	0.5595	−0.0728	0.4424
	PIC	0.1202	0.3388	0.3318	0.3123	0.3004	0.2915	0.2356
SNP8	*H*o	0.0877	0.1864	0.1167	0.2667	0.1351	0.1224	0.1739
	*H*e	0.2306	0.2238	0.1948	0.3742	0.2566	0.1851	0.1959
	P	0.0000*	0.2337	0.0094*	0.3467	0.0105*	0.0451*	0.4698
	*F*is	0.6162	0.1599	0.3961	0.1162	0.4661	0.3318	0.1024
	PIC	0.2024	0.1973	0.1745	0.2819	0.2210	0.1664	0.1750
SNP20	*H*o	0.3051	0.2931	0.4167	0.3333	0.2703	0.1800	0.3478
	*H*e	0.3060	0.4250	0.4727	0.5036	0.3999	0.2285	0.4443
	P	0.9823	0.0193*	0.3559	0.8072	0.0521	0.1686	0.1405
	*F*is	−0.0057	0.3044	0.1111	0.0231	0.3148	0.2042	0.2086
	PIC	0.2573	0.3326	0.3589	0.3356	0.3167	0.2006	0.3429
SNP26	*H*o	0.0508	0.0517	0.1897	0.0847	0.3784	0.1800	0.3043
	*H*e	0.1126	0.1144	0.2763	0.1705	0.3110	0.1655	0.3440
	P	0.0034*	0.0035*	0.0281*	0.0022*	0.0801	0.3733	0.4390
	*F*is	0.5444	0.5439	0.3077	0.4987	−0.2333	−0.0989	0.1056
	PIC	0.1053	0.1069	0.2364	0.1547	0.2597	0.1504	0.2824
SNP33	*H*o	0.4737	0.2881	0.4500	0.4667	0.1892	0.4600	0.4222
	*H*e	0.3995	0.3739	0.4021	0.3782	0.3277	0.3788	0.3848
	P	0.1347	0.0854	0.3369	0.0431*	0.0153*	0.0938	0.4944
	*F*is	−0.1963	0.2228	−0.1285	−0.2444	0.4147	−0.2267	−0.1097
	PIC	0.3176	0.3020	0.3192	0.3047	0.2710	0.3047	0.3081
SNP48	*H*o	0.0508	0.0517	0.3966	0.0847	0.0811	0.1000	0.2667
	*H*e	0.0500	0.0508	0.3207	0.1126	0.1277	0.1315	0.2337
	P	0.8193	0.8177	0.0190*	0.1335	0.0834	0.1624	0.1923
	*F*is	−0.0261	−0.0265	−0.2473	0.2407	0.3565	0.2320	−0.1538
	PIC	0.0483	0.0492	0.2674	0.1053	0.1181	0.1217	0.2044
SNP59	*H*o	0.0169	0.1017	0.0500	0.0333	0.0270	0.0408	0.0217
	*H*e	0.0500	0.0973	0.0492	0.0650	0.0270	0.0791	0.0217
	P	0.0076*	0.6047	0.8208	0.0223*	1.0000	0.0282*	1.0000
	*F*is	0.6580	−0.0536	−0.0256	0.4828	−0.0137	0.4787	−0.0110
	PIC	0.0483	0.0918	0.0476	0.0623	0.0263	0.0752	0.0213
SNP62	*H*o	0.4211	0.4746	0.3667	0.2167	0.3514	0.3400	0.2826
	*H*e	0.3552	0.3987	0.4683	0.3864	0.3869	0.4709	0.4193
	P	0.1212	0.1182	0.0914	0.0010*	0.5746	0.0479*	0.0287*
	*F*is	−0.1958	−0.2006	0.2105	0.4346	0.0794	0.2707	0.3185
	PIC	0.2901	0.3172	0.3566	0.3098	0.3089	0.3575	0.3287
SNP88	*H*o	0.0339	0.0508	0.3500	0.0667	0.0811	0.3400	0.3913
	*H*e	0.0336	0.0818	0.2912	0.0650	0.0789	0.3788	0.3679
	P	0.8955	0.0481*	0.0386*	0.7477	0.7712	0.4706	0.6536
	*F*is	−0.0172	0.3735	−0.2121	−0.0345	−0.0423	0.0933	−0.0753
	PIC	0.0327	0.0779	0.2471	0.0623	0.0747	0.3047	0.2977
SNP102	*H*o	0.1356	0.1695	0.0667	0.0333	0.1081	0.1224	0.1087
	*H*e	0.1275	0.1565	0.1815	0.0650	0.1511	0.2474	0.1785
	P	0.4753	0.3609	0.0001*	0.0223*	0.1475	0.0020*	0.0284*
	*F*is	−0.0727	−0.0926	0.6296	0.4828	0.2745	0.5000	0.3842
	PIC	0.1184	0.1430	0.1638	0.0623	0.1379	0.2150	0.1609

TABLE 2. *continued*

Locus		Hatchery population					Wild population	
		HJN	HYT	HWD	HPL	HCD	WMB	WSB
SNP109	*Ho*	0.0339	0.2542	0.1695	0.1167	0.3611	0.1042	0.3261
	He	0.0661	0.2238	0.3060	0.2688	0.3940	0.1366	0.3313
	P	0.0228*	0.1526	0.0016*	0.0001*	0.6142	0.1704	0.9129
	*F*is	0.4825	−0.1456	0.4413	0.5623	0.0705	0.2295	0.0050
	PIC	0.0634	0.1973	0.2573	0.2310	0.3130	0.1260	0.2740
SNP115	*Ho*	0.3390	0.3051	0.2545	0.3833	0.2703	0.3673	0.1333
	He	0.2839	0.3465	0.3229	0.3696	0.3110	0.3282	0.1998
	P	0.0481*	0.3656	0.1311	0.7680	0.4333	0.3643	0.0544
	*F*is	−0.2041	0.1120	0.2045	−0.0459	0.1190	−0.1308	0.3250
	PIC	0.2419	0.2845	0.2688	0.2994	0.2597	0.2721	0.1780
SNP123	*Ho*	0.3220	0.3621	0.2833	0.3559	0.4054	0.3400	0.4348
	He	0.4759	0.4790	0.4637	0.6080	0.4935	0.4709	0.4902
	P	0.0123*	0.0612	0.0024*	0.9069	0.2710	0.0479*	0.4385
	*F*is	0.3175	0.2376	0.3839	0.0067	0.1673	0.2707	0.1033
	PIC	0.3605	0.3621	0.3541	0.3724	0.3683	0.3575	0.3673
SNP131	*Ho*	0.4138	0.3390	0.2833	0.2833	0.3056	0.3000	0.2174
	He	0.3310	0.5007	0.2452	0.3696	0.2625	0.4467	0.2293
	P	0.0137*	0.0118*	0.1033	0.0782	0.1780	0.0203*	0.7245
	*F*is	−0.2609	0.3171	−0.1650	0.2270	−0.1803	0.3216	0.0417
	PIC	0.2743	0.3732	0.2137	0.2994	0.2254	0.3444	0.2011
SNP143	*Ho*	0.4576	0.4407	0.3729	0.4500	0.3514	0.4000	0.4565
	He	0.3907	0.4137	0.3824	0.3864	0.4121	0.4848	0.4365
	P	0.1608	0.6084	0.8464	0.1744	0.3697	0.2120	0.7503
	*F*is	−0.1813	−0.0742	0.0167	−0.1743	0.1357	0.1667	−0.0575
	PIC	0.3123	0.3261	0.3073	0.3098	0.3239	0.3648	0.3385
SNP146	*Ho*	0.1186	0.2069	0.1333	0.2500	0.1944	0.3600	0.0652
	He	0.1977	0.3310	0.2801	0.4873	0.3658	0.4073	0.1421
	P	0.0099*	0.0071*	0.0003*	0.0001*	0.0067*	0.4124	0.0059*
	*F*is	0.3949	0.3696	0.5200	0.4826	0.4610	0.1071	0.5361
	PIC	0.1769	0.2743	0.2392	0.3664	0.2957	0.3219	0.1307
SNP147	*Ho*	0.0172	0.4182	0.1500	0.0667	0.3235	0.1000	0.1957
	He	0.0172	0.4085	0.1399	0.1541	0.4965	0.2851	0.2129
	P	1.0000	0.8581	0.4203	0.0009*	0.0381*	0.0000*	0.5929
	*F*is	−0.0087	−0.0331	−0.0811	0.5636	0.3386	0.6456	0.0707
	PIC	0.0169	0.3229	0.1291	0.1411	0.3695	0.2424	0.1884
SNP148	*Ho*	0.0847	0.1864	0.0667	0.0339	0.1111	0.1429	0.0652
	He	0.1977	0.3165	0.1255	0.0661	0.1064	0.2013	0.1421
	P	0.0004*	0.0033*	0.0078*	0.0228*	0.6743	0.0756	0.0060*
	*F*is	0.5678	0.4059	0.4643	0.4825	−0.0588	0.2832	0.5361
	PIC	0.1769	0.2646	0.1168	0.0634	0.0995	0.1794	0.1307
SNP149	*Ho*	0.3220	0.3559	0.4167	0.3103	0.3243	0.1600	0.4348
	He	0.2951	0.2951	0.3326	0.3868	0.4235	0.2982	0.4281
	P	0.4476	0.0367*	0.0115*	0.1386	0.1553	0.0027*	0.9141
	*F*is	−0.1006	−0.2165	−0.2632	0.1907	0.2238	0.4580	−0.0268
	PIC	0.2498	0.2498	0.2754	0.3099	0.3305	0.2516	0.3338
SNP153	*Ho*	0.4237	0.1754	0.4167	0.4333	0.3243	0.4000	0.2826
	He	0.3907	0.3739	0.3326	0.3608	0.3110	0.3887	0.4517
	P	0.5016	0.0001*	0.0115*	0.0832	0.7832	0.8332	0.0109*
	*F*is	−0.0938	0.5266	−0.2632	−0.2112	−0.0571	−0.0395	0.3675
	PIC	0.3123	0.3019	0.2754	0.2938	0.2597	0.3108	0.347

TABLE 2. *continued*

Locus		Hatchery population					Wild population	
		HJN	HYT	HWD	HPL	HCD	WMB	WSB
SNP154	*H*o	0.3091	0.5085	0.2069	0.2667	0.4722	0.3800	0.3696
	*H*e	0.4239	0.4867	0.4444	0.5193	0.4754	0.4160	0.4517
	P	0.0466*	0.7289	0.0000*	0.0005*	0.9680	0.5398	0.2158
	*F*is	0.2641	−0.0536	0.5304	0.4360	−0.0074	0.0772	0.1729
	PIC	0.3318	0.3662	0.3435	0.3610	0.3589	0.3270	0.3470
SNP155	*H*o	0.4576	0.4068	0.4667	0.4667	0.4054	0.4800	0.4348
	*H*e	0.4063	0.3824	0.4364	0.3944	0.3869	0.4073	0.3899
	P	0.3128	0.6143	0.5839	0.1295	0.7632	0.1803	0.4120
	*F*is	−0.1358	−0.0727	−0.0783	−0.1932	−0.0622	−0.1905	−0.1275
	PIC	0.3218	0.3073	0.3391	0.3146	0.3089	0.3219	0.3113
SNP163	*H*o	0.2712	0.2069	0.4237	0.2203	0.3243	0.3600	0.2391
	*H*e	0.2839	0.3508	0.4463	0.2487	0.3999	0.3467	0.4001
	P	0.7303	0.0030	0.6954	0.3993	0.2533	0.7786	0.0075*
	*F*is	0.0367	0.4051	0.0425	0.1066	0.1778	−0.0490	0.3958
	PIC	0.2419	0.2873	0.3446	0.2162	0.3167	0.2843	0.3174
SNP164	*H*o	0.1017	0.1356	0.1333	0.2000	0.2162	0.1800	0.1739
	*H*e	0.2608	0.1275	0.2078	0.2331	0.2369	0.1655	0.2293
	P	0.0000*	0.4753	0.0172*	0.3002	0.6000	0.3733	0.1356
	*F*is	0.6067	−0.0727	0.3531	0.1346	0.075	−0.0989	0.2333
	PIC	0.2250	0.1184	0.1849	0.2044	0.2064	0.1504	0.2011
SNP177	*H*o	0.3036	0.40000	0.2586	0.3051	0.3784	0.3469	0.3953
	*H*e	0.4336	0.3643	0.2991	0.5088	0.4443	0.4917	0.4172
	P	0.0258*	0.4452	0.3175	0.4321	0.3640	0.0368*	0.7284
	*F*is	0.2935	−0.1081	0.1278	0.0944	0.1367	0.2871	0.0413
	PIC	0.3374	0.2959	0.2525	0.3378	0.3422	0.3683	0.3274
SNP178	*H*o	0.3559	0.2881	0.3500	0.2203	0.3514	0.2653	0.0889
	*H*e	0.4463	0.4342	0.4021	0.4521	0.3588	0.3158	0.2657
	P	0.1202	0.0103*	0.3184	0.0051*	0.8981	0.2770	0.0001*
	*F*is	0.1957	0.3307	0.1223	0.3757	0.0072	0.1512	0.6617
	PIC	0.3446	0.3378	0.3192	0.2906	0.2913	0.2637	0.2283
SNP183	*H*o	0.2982	0.2143	0.1500	0.2167	0.4054	0.2600	0.3261
	*H*e	0.4150	0.4524	0.3517	0.5335	0.4832	0.4160	0.5033
	P	0.0360*	0.0001*	0.0000*	0.0000*	0.3220	0.0089*	0.0149*
	*F*is	0.2750	0.5220	0.5699	0.5378	0.1494	0.3686	0.3450
	PIC	0.3268	0.3478	0.2879	0.3589	0.3630	0.3270	0.3739
SNP185	*H*o	0.0169	0.0847	0.0167	0.0339	0.3243	0.4000	0.2174
	*H*e	0.0169	0.1421	0.1108	0.0336	0.3436	0.3232	0.4443
	P	1.0000	0.0164*	0.0000*	0.8955	0.7308	0.0284*	0.0005*
	*F*is	−0.0085	0.3986	0.8483	−0.0172	0.0431	−0.2500	0.5054
	PIC	0.0167	0.1310	0.1038	0.0327	0.2815	0.2688	0.3429
SNP189	*H*o	0.2373	0.1017	0.1500	0.1500	0.1622	0.0200	0.1739
	*H*e	0.2109	0.2839	0.1679	0.1399	0.2755	0.0588	0.1605
	P	0.1852	0.0000*	0.4413	0.4203	0.0226*	0.0091*	0.4138
	*F*is	−0.1346	0.6388	0.0992	−0.0811	0.4032	0.6564	−0.0952
	PIC	0.1872	0.2419	0.1527	0.1291	0.2348	0.0565	0.1462
SNP213	*H*o	0.3571	0.3621	0.4500	0.4500	0.4054	0.4600	0.2609
	*H*e	0.4633	0.4610	0.4021	0.4486	0.4535	0.4160	0.4587
	P	0.0854	0.1013	0.3369	0.7155	0.5146	0.4383	0.0032*
	*F*is	0.2222	0.2078	−0.1285	−0.055	0.0939	−0.1170	0.4250
	PIC	0.3537	0.3526	0.3192	0.3356	0.3473	0.3270	0.3508

TABLE 2. *continued*

Locus		Hatchery population					Wild population	
		HJN	HYT	HWD	HPL	HCD	WMB	WSB
SNP217	*H*o	0.2586	0.4915	0.2321	0.2069	0.2500	0.3600	0.3478
	*H*e	0.4382	0.5037	0.4336	0.4510	0.4597	0.4396	0.4099
	P	0.0019*	0.8515	0.0005*	0.0003*	0.0056*	0.2005	0.3057
	*F*is	0.4047	0.0158	0.4597	0.4821	0.4485	0.1728	0.1422
	PIC	0.3400	0.3747	0.3374	0.3197	0.3506	0.3405	0.3233
SNP223	*H*o	0.4407	0.2034	0.4237	0.2667	0.3784	0.3800	0.4130
	*H*e	0.3651	0.3824	0.3559	0.2571	0.3999	0.3352	0.4365
	P	0.0768	0.0005*	0.1044	0.7623*	0.74100	0.3017	0.7136
	*F*is	−0.2175	0.4636	−0.2007	−0.0458	0.0407	−0.1453	0.0432
	PIC	0.2964	0.3073	0.2906	0.2225	0.3167	0.2768	0.3385
Mean	*H*o	0.2381	0.2624	0.2639	0.2489	0.2759	0.2760	0.2664
	*H*e	0.2689	0.3256	0.3147	0.3011	0.3354	0.3188	0.3280
	*F*is	0.1068	0.1871	0.1542	0.1661	0.1661	0.1255	0.1790
	PIC	0.2203	0.2620	0.2566	0.2434	0.2687	0.2585	0.2642

SNP = single-nucleotide polymorphism; PIC = polymorphic information content; HCD = Changdao; HJN = Jiaonan; HYT = Yantai, HWD = Wendeng; HPL = Penglai; WSB = Saiki Bay (Oita); WMB = Mutsu Bay (Aomori).

*Significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$).

TABLE 3. *Nei's genetic identity (above diagonal) and genetic distance (below diagonal) at 32 SNP loci between seven populations of* Apostichopus japonicus.

Population	Hatchery population					Wild population	
	HJN	HYT	HWD	HPL	HCD	WMB	WSB
HJN	–	0.9889	0.9898	0.9915	0.9848	0.9909	0.9859
HYT	0.0112	–	0.9870	0.9896	0.9904	0.9868	0.9802
HWD	0.0102	0.0131	–	0.9904	0.9883	0.9866	0.9901
HPL	0.0085	0.0105	0.0097	–	0.9901	0.9888	0.9837
WCD	0.0153	0.0096	0.0118	0.0100	–	0.9857	0.9913
WMB	0.0091	0.0133	0.0135	0.0113	0.0144	–	0.9883
WSB	0.0142	0.0200	0.0100	0.0164	0.0087	0.0118	–

SNP = single-nucleotide polymorphism; HCD = Changdao, HJN = Jiaonan, HYT = Yantai; HWD = Wendeng; HPL = Penglai; WSB = Saiki Bay (Oita); WMB = Mutsu Bay (Aomori).

(Tm)-shift have been used to genotype SNP markers. The method of ARMS-PCR requires four primers to amplify larger fragments containing the SNP and two smaller fragments representing each of the two allele-specific products (Sun et al. 2010). The method of the Tm-shift uses 3′-terminal base of each allele-specific primer corresponding to one of the two SNP allelic variants, and GC-rich tails of different length are attached to the allele-specific primers. Then the PCR product with distinct Tm is generated, and genotypes can be determined (Yang et al. 2012). In comparison with these two methods, the HRM analysis demonstrates great advantages because it is simple, rapid, inexpensive, and efficient. The HRM method can detect SNPs in small PCR amplicons and the processes of PCR amplification take place in the same tube in <2 h (Wu et al. 2008; Jin et al. 2014). Besides, the genotypes of SNPs can be identified by the different shapes of melting curves which make it easy to discriminate different genotypes (Garritano et al. 2009).

Population Genetic Variability

In this study, the mean *H*o and *H*e were lower than those reported by Chen et al. (2008), which analyzed the genetic variation in wild and hatchery stocks from northern China using microsatellite markers. This suggests a greater number of SNP (at least 4×) is required due to there being only three possible genotypes for

TABLE 4. *Analysis of genetic differentiation between pairs of samples based on estimates of Fst (below diagonal).*

	Hatchery population					Wild population	
Population	HJN	HYT	HWD	HPL	HCD	WMB	WSB
HJN	–						
HYT	0.0165*	–					
HWD	0.0172*	0.0163*	–				
HPL	0.0130*	0.0136*	0.0129	–			
HCD	0.0227*	0.0126	0.0148*	0.0140*	–		
WMB	0.0185*	0.0141	0.0145	0.0156	0.0163	–	
WSB	0.0228*	0.0236	0.0119	0.0215*	0.0121	0.0147	–

HCD = Changdao; HJN = Jiaonan; HYT = Yantai; HWD = Wendeng; HPL = Penglai; WSB = Saiki Bay (Oita); WMB = Mutsu Bay (Aomori).

*Significant at $P < 0.05$.

TABLE 5. *Analysis of molecular variance of 32 SNP loci in the wild and hatchery populations of* Apostichopus japonicus.

Division	Source of variation	Degrees of freedom (df)	Percentage of variation (%)	*F*st
None	Among all the populations	6	2	0.0021
	Within populations	735	98	
Wild versus hatchery	Among all the populations	1	1	0.0095
	Within populations	740	99	
Wild	Among all the populations	1	2	0.0181
	Within populations	190	98	
Hatchery	Among all the populations	4	2	0.0202
	Within populations	545	98	

SNP = single-nucleotide polymorphism.

a SNP as opposed to microsatellites. However, in some instances of microsatellites, new alleles are described, which are artifacts in fact (Vignal et al. 2002). For SNP markers, the small number of alleles allows rapid genotyping with low error rates, and the character of the most abundant variation in the entire genome increases the polymorphisms (Narum et al. 2008). With the advent of next-generation sequencing, the SNPs will likely advance population genetics rapidly because SNPs have a low cost to score and are easy to identify from nonmodel organisms (Ekblom and Galindo 2011).

DNA markers can be good monitors of genetic shifts over generations of domestication. In the study of black tiger shrimp, *Penaeus monodon*, using microsatellites, Dixon et al. (2008) noted large decline in diversity from the wild-caught stocks to the domesticated stocks and the losses of genetic diversity were observed within three generations in comparison with wild populations. The loss of genetic diversity in farmed populations is generally caused by small effective population sizes. A high level of genetic diversity is essential for long-term survival of populations, as their ability to adapt to changing environments lies in the extent of variation (Chen et al. 2008; Xing et al. 2014). The reduced genetic diversity may cause pernicious effects on some commercial traits such as survival, growth rate, and disease resistance (An et al. 2011b). Therefore, it is vital to evaluate and monitor the genetic diversity and structure of hatchery stocks using molecular markers. In this study, compared with the wild stocks of *A. japonicas*, there are no obvious differences in the average *H*o, *H*e, and the PIC of the hatchery stocks. This indicates that hatchery sea cucumbers have considerable genetic variation through the process of domestication. In hatchery stocks, the genetic variability is likely related to the number of parents used for reproductive stock (An et al. 2013a). In China, the seeds of sea cucumbers are produced in hatcheries, where hundreds

of cultured mature males and females as parents collected from different farms are used in an artificial mass spawning. In addition, the parents used for spawning at different times often have been mixed with each other. The stock enhancement programs of sea cucumbers may maintain the high genetic variability of cultivated populations instead of genetic diversity reduction. In Korea, the red sea cucumbers also maintain high genetic diversity because the parents used for spawning were caught from the near coast (An et al. 2013b). There are also other marine organisms reported to maintain high genetic diversity in the hatchery populations (Pan and Yang 2010; An et al. 2013a; Kong et al. 2014).

A total of 30 loci (except SNP143 and SNP155) exhibited deviations of HWE in both the wild and hatchery populations of sea cucumber, showing heterozygote deficiency. These deviations may account for null alleles, nonrandom-mating, more than two independent populations as parents and natural or artificial selection. Besides, the high *F*is values of most SNP loci suggests that nonrandom mating may have occurred (An et al. 2013c). Similar results were also reported in the Chen et al. (2008) and An et al. (2013b).

Population Genetic Differentiation

In this study, pairwise comparison *F*st analysis showed there is no significant differentiation between the wild and hatchery stocks. The high genetic identity (from 0.9802 to 0.9915) demonstrates that these stocks regardless of whether wild populations or the hatchery populations have high proportions of identical alleles. The AMOVA analysis revealed differentiation generated at the level of "within the populations," which explains why there is no differentiation between the wild and hatchery populations. Their current population structures might result from different founder populations and different selection procedures.

In conclusion, we developed 51 polymorphic EST-SNPs of *A. japonicas* and compared the genetic variation between Chinese hatchery and Japanese wild sea cucumbers using the SNP markers. In this study, the Chinese hatchery stocks showed no reduction in genetic diversity compared with the wild stocks from Japan. This information may be applied for future genetic improvement of *A. japonicas* by selective breeding and design suitable management guidelines for sea cucumber.

Acknowledgments

The study was supported by the grants from Scientific and Technical Supporting Program (2011BAD13B03), National Marine Public Welfare Research Program (201305005), and Doctoral Program of Ministry of Education of China (20130132110009).

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