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Development of novel microsatellite markers for *Holothurian scabra* (Holothuriidae), *Apostichopus japonicas* (Stichopodidae) and cross-species testing in other sea cucumbers*

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Abstract Thirty-five new microsatellite loci from the sea cucumbers *Holothurian scabra* (Jaeger, 1833) and *Apostichopus japonicas* (Selenka, 1867) were screened and characterized using the method of magnetic bead enrichment. Of the twenty-four polymorphic loci tested, eighteen were consistent with Hardy-Weinberg equilibrium after a modified false discovery rate (B-Y FDR) correction, whereas six showed statistically significant deviations (CHS2 and CHS11: $P<0.014\ 790$; FCS1, FCS6, FCS8 and FCS14: $P<0.015\ 377$). Furthermore, four species of plesiomorphous and related sea cucumbers (*Holothurian scabra*, *Holothuria leucospilota*, *Stichopus horrens* and *Apostichopus japonicas*) were tested for mutual cross-amplification using a total of ninety microsatellite loci. Although transferability and universality of all loci were generally low, the results of the cross-species study showed that the markers can be applied to identify individuals to species according to the presence or absence of specific microsatellite alleles. The microsatellite markers reported here will contribute to the study of genetic diversity, assisted breeding, and population conservation in sea cucumbers, as well as allow for the identification of individuals to closely related species.

Keyword: sea cucumber; microsatellite loci (SSR); genetic diversity; cross-amplification; species identification

1 INTRODUCTION

Sea cucumbers (Echinodermata; also known as bêche-de-mer, trepan, or gamat) are soft-bodied, flexible, elongated and worm-like marine organisms that have been harvested for food and medicinal purposes over many centuries in Asian countries including China and Malaysia (Taiyeb-Ali et al., 2003; Bordbar et al., 2011). Sea cucumbers tend to live on the sea floor in deep waters and usually feed on plankton and seabed algae (Conand, 1990). Increasing demand for trepang and a steady increase in price have led to a worldwide intensification of artificial breeding and wild harvesting of sea cucumbers (Conand, 2006).

Apostichopus japonicas is the only temperate sea cucumber found in Chinese waters, yet the species has a wider distribution along the coasts of Japan, Korea, and Far Eastern Russia. Over the past decade, large-scale aquaculture of the species throughout China has developed (Li, 2009). Conversely, *Holothuria scabra*is an abundant species, is widely distributed in shallow soft-bottom habitats throughout the Indo-Pacific, and is the only tropical holothurian species currently mass-produced in hatcheries (Battaglene and Seymour, 1998; Xia et al., 2016). The body of research regarding population genetics and aquaculture of these two species is increasing in size in response to a commercial over-exploitation of most

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wild stocks and an increasing market demand (Conand and Bryne, 1993; Tian et al., 2008). Selection of brood stocks with optimal traits, such as rapid growth and disease resistance, is urgently needed to alleviate multiple conservation concerns and achieve sustainable sea cucumber aquaculture.

Microsatellite DNA markers, also called simple sequence repeats (SSR), consist of multiple copies of 1–6 base pairs (bp) that occur as highly repetitive elements in all eukaryotic genomes, as well as in some prokaryotes and eubacteria (Tautz, 1989; Liu and Cordes, 2004). These molecular markers are suitable for use in species with relatively limited existing genomic information and for which collection might be limited due to protected status (for example, endangered species). Although Single Nucleotide Polymorphism (SNP) markers are considered the most powerful for population studies involving genome mapping and identification of candidate genes for Quantitative Trait Locus analysis, they are expensive to develop. It is currently not clear that SNP markers will become popular in aquaculture genetics due to the great financial investment (Liu and Cordes, 2004). We believe that microsatellite markers are appropriate for genetics research, especially for primary genome mapping, and thus use them here.

2 MATERIAL AND METHOD

2.1 Sample materials and DNA extraction

Thirty adults each of *H. scabra* and *A. japonicas* were collected from Chinese waters in October 2014 (*H. scabra* from Sanya in Hainan Province and *A. japonicas* from Qingdao in Shandong Province). A total of thirty genomic DNA samples of high quality were extracted from the body wall of freshly collected individuals using the TIANamp Marine Animals DNA Kit (Tiangen Biotech, Beijing, China). The resulting DNA was purified using the EZNA^{TM} Cycle-Pure Kit (Omega Bio-Tek, Norcross, GA, USA).

2.2 SSR-enriched library construction and primer design

Twenty μL of high-quality genomic DNA were sampled from a 100-ng/μL gene pool (a mixture of five randomly selected samples from the 30 collected) and used for the construction of a microsatellite library. The library was constructed following a modified version of the fast isolation method using Amplified Fragment Length Polymorphisms of sequences containing repeats (Zane et al., 2002). Two sets of SSR primers for *H. scabra* and *A. japonicas* were developed. The microsatellite fragments were then captured by streptavidin-coated magnetic beads (Promega Corporation, Madison, WI, USA) and purified by eluent. SSR-enriched libraries were constructed after amplification by polymerase chain reaction (PCR) and then ligated into a PMD19-T vector (4:1 library volume:vector volume) (TaKaRa, Shiga, Japan). Next, the products were transformed into *Escherichia coli* DH5a strains (Tiangen Biotech) for positive monoclone selection on LuriaBertani agar medium with ampicillin. Satisfactory fragments with sizes 400–1 000 bp were selected and sent for sequencing to Life Technologies (Carlsbad, CA, USA). The reads containing microsatellite motifs of at least five repetitions of 1-6 bp were screened by the software SSR hunter 1.3 (Li and Wan, 2005). Finally, the SSR primers were designed from applicable flanking sequences by the software Primer Premier 5.0 (Lalitha, 2000).

2.3 Specific and polymorphic primers screening and verification

The PCR annealing temperature (T_a) for each primer was optimized by a gradient temperature PCR. PCR amplification was conducted in a total volume of 10 μL and using a mixed genomic DNA pool. After preliminary screening of all primers, the amplification-specific primers with matched target strips were tested for polymorphisms using a panel of genomic DNA from 30 individuals at the specific T_a (Tables 1 and 2). The amplicons were checked using 6% polyacrylamide gels in a vertical Sequi-Gen Sequencing Cell (Bio-Rad, Hercules, CA, USA) at a constant temperature of 50–55°C. Additionally, a 10-bp DNA ladder (Invitrogen, Carlsbad, CA, USA) was used as a size standard. The gels were visualized by silver staining and analyzed by visually counting the alleles.

2.4 Cross-species amplification

A total of 90 microsatellite loci were selected for cross-species amplification (Table 3). Four individuals from each of the four species were used in the amplification test. The PCR conditions were performed as previously described and the results were visualized using 2% agarose gel with a 50-bp DNA ladder (Invitrogen, Carlsbad, CA, USA). The loci were considered successfully amplified when one band of the expected target size was present.

Table 1 Basic genetic information for 16 microsatellite primers in *H. scabra* (sample size=30 individuals)

Locus ID	Primer sequences (5′→3′)	T_a (°C)	Repeat motif	Allele size (bp)	Na	PIC	H_o	H_e	GenBank
CHS1	F: TTCCACTTTATCACACTAGCACTA R: AAACCTTTTATTTTATGAAACAAC	50.8	$(TAT)_4$	290–310	2	0.062	0.066 7	0.064 4	KR653160
CHS2*	F: CTTTTTCACCTTTGACCAGT R: AATGACCTCGACAGACAGAC	57.1	$(CTGT)_5$	275–290	4	0.615	0.148 1	0.609 1	KR653161
CHS3	F: CAGCAGCCACAGAGAGTGATTTC R: ATGTTGCCTTGAGTGTCCGAGAG	57.1	A_{11}	240–250	2	0.032	0.033 3	0.032 8	KR653162
CHS4	F: CATCCCCAACCCCGTCAAC R: CCCCCCCCAACAAACACAC	60.0	T_{19}	120–140	6	0.584	0.347 8	0.459 4	KR653163
CHS5	F: TACTTACTTCTTCCTCTTCCC R: TTTTGGTTTTATTTTTTTACA	50.8	$(TTTTC)_3$	175–185	4	0.324	0.083 3	0.080 7	KR653164
CHS6	F: TCCTCGATTTACTGGTACAAGACA R: AAGTTTGAAGCATTTTGGAGTTTT	50.0	$(AC)_6$	119–128	2	0.062	0.066 7	0.064 4	KR653165
CHS7	F: ACACTTGACTAGGGGAA R: AGTTTGAAGCATTTTGG	51.8	$(AC)_6$	148–156	3	0.535	0.500 0	0.375 0	KR653166
CHS8	F: GACTTTTTTTTGAGTGTTCTAC R: AATTATTTAACTGATTCCTTTA	50.8	$(GAAG)_3$	145–150	3	0.175	0.137 9	0.128 4	KR653167
CHS9	F: TAAGGGGAATGATGGAGTGTAA R: AGTAAGAGGATGGTCCCTATTG	50.8	$(ATAA)_3$	147–152	3	0.357	0.517 2	0.383 5	KR653168
CHS10	F: CCTTCTCACCTTAATGTGTATCC R: CTCTCTGTCCCTCTGTCTGTCTC	55.3	$(TG)_5$	164–170	2	0.185	0.166 7	0.206 1	KR653169
CHS11*	F: TTACTTCCTTCTCCTCC R: GTTGCTGTCTCTCTTCC	55.3	$C_{13}N(TTTG)_3NA_{14}$	138–150	6	0. 712	0.500 0	0.690 1	KR653170
CHS12	F: TCACAGAGAACAAGAAAC R: ACACACACAAACACACAT	52.0	$(GA)_{17}$	141–152	3	0.383	0.240 0	0.211 2	KR653171
CHS13	F: ATAATACTACAAGCGAAGGGC R: ACAAGCAAAGTGTCACAAACA	55.3	$(GT)_{46}A(TG)_8$	250–290	5	0.699	0.434 8	0.654 1	KR653172
CHS14	F: TAAAAATAGGAAGTCAACCAAAC R: AGACCTCAACGTAACAAAAATAA	45.0	$(GT)_{12}$	159–170	3	0.331	0.192 3	0.173 8	KR653173
CHS15	F: TAACAAGCAAAGTGTCACAA R: CAGTAAATCGAGGACATGAG	55.0	$(AC)_6$	150–160	3	0.402	0.280 0	0.240 8	KR653174
CHS16	F: TGACCACAAACTCAGATA R: GTTGCTCAGCTTTACTAG	40.2	$(AC)_{33}N(CT)_5N(CT)_{26}$	225–230	3	0.367	0.090 9	0.086 8	KR653175
CHS17	F: ACACACATACACACACGC R: GCTATCACAAGATTCCAC	40.0	$(CA)_{29}T(TC)_9N(CT)_{17}$	167	-	-	-	-	KR653176
CHS18	F: GAGCCCTCACAGTTACCC R: GAACCTATTTGCTTCCCA	60.0	$(TG)_{14}$	119	-	-	-	-	KR653177
CHS19	F: AAGAGGCAATCGGAGTT R: CTTTCAGGGGGAAGCTC	42.2	$(GA)_{39}$	237	-	-	-	-	KR653178
CHS20	F: CTATGTAGTCAGGTTGG R: GTAGTCATTGTTGTTCC	46.6	$(TG)_{16}$	357	-	-	-	-	KR653179

T_a: annealing temperature; N_a: number of polymorphic alleles per locus; PIC: polymorphic information content; H_o: observed heterozygosity; H_e: expected heterozygosity. * Significant deviations of locus from Hardy-Weinberg equilibrium after B-Y FDR correction (P<0.014 790).

2.5 Relevant data analysis

The basic population genetic information about each microsatellite locus in *H. scabra* and *A. japonicas* was quantified using a combination of software. Specifically, MICRO-CHECKER (v.2.2.3, van Oosterhout et al., 2004) was used for null alleles and scoring error assessments; CERVUS (v.3.0, Kalinowski et al., 2007) was used for to calculate the number of alleles (Na) per locus and the polymorphism information content (PIC); and POPGENE 32 (v.1.32, Yeh et al., 2000) was used to estimate observed heterozygosity (H_o), expected heterozygosity (H_e), genotypic linkage disequilibrium (LD), and deviation from Hardy-Weinberg equilibrium (HWE) for each locus. Critical significance values were adjusted for multiple comparisons by a modified false discovery rate (B-Y FDR) correction when necessary (Narum, 2006).

Table 2 Basic genetic information for 15 microsatellite primers in *A. japonicus*

Locus ID	Primer sequences (5′→3′)	T_a (°C)	Repeat motif	Allele size (bp)	Na	PIC	H_o	H_e	GenBank
FCS1*	F: GTGCTTCAGTCCCTATG R: AAGTTGTTGTGCCAGTT	53.0	$(TA)_6$	195–215	4	0.557	0.142 9	0.553 6	KR653180
FCS2	F: TTTGTAGAAAGGAAGAGAC R: AGTAATACCATTAAGGCAT	59.1	$A_{10}N\ (AAG)_4$	190–210	6	0.313	0.300 0	0.324 4	KR653181
FCS3	F: AATTACCGACGGTAGCAA R: AGACTCACGCCCAAAGAA	59.1	$(TG)_4$	115–120	2	0.315	0.466 7	0.391 1	KR653182
FCS4	F: GGCTCTTGTTCCCTTCGT R: CTGTTCGGCCATTCTGTG	42.0	$(CT)_{11}$	140–150	3	0.268	0.040 0	0.039 2	KR653183
FCS5	F: AGCTTGTGGTTTTGATA R: AGGGACAGTTGGTTTAC	48.0	$(GT)_{32}$	100–110	3	0.410	0.166 7	0.218 8	KR653184
FCS6*	F: TTTCCGTTGTTGTTTTGTTT R: TTCTGGTTGACTTTCCTGTG	59.1	C_9T_5	165–190	5	0.468	0.133 3	0.546 1	KR653185
FCS7	F: TAAGTGATTTGTGTGTGTG R: TGAAAATAATACTGACGAC	52.0	$(TG)_7\ (GT)_9$	260–270	3	0.175	0.071 4	0.068 9	KR653186
FCS8*	F: TGACGATGAATAAAAAG R: AAATGTAGAATGCTGCT	52.0	$C_{10}A_8$	320–330	3	0.479	0.233 3	0.572 8	KR653187
FCS9	F: GAGAAAGTGTGTGCATGCG R: AGGCGAGTTCCGAAATCAG	55.0	$(AG)_6$	150–160	3	0.309	0.400 0	0.337 8	KR653188
FCS10	F: GCAAATAATCAACGAAACCG R: GATGGAAAATACAATGGGGC	45.0	$(AC)_{22}$	170–180	3	0.221	0.074 1	0.071 3	KR653189
FCS11	F: CAAATTGTAGCAAACCAA R: AGATCATCCTGAAAGCAG	50.0	A_9	142–165	3	0.309	0.400 0	0.365 0	KR653190
FCS12	F: GAGCCAAATGACCCTGAA R: AATGGATGTGTGCCGAGA	45.0	$(AG)_{18}A_8N(AG)_{15}\ (GT)_6$	210–225	3	0.410	0.166 7	0.218 8	KR653191
FCS13	F: TCATCCCATAAAAAGGC R: CACGGTCAAAGGTCATT	46.6	$(TC)_{26}$	130–140	3	0.094	0.034 5	0.033 9	KR653192
FCS14*	F: TGTGTTTGTATGTGTAATGTGTC R: TTAAATACCCTGATTTTCTACTG	46.6	$(TATG)_3N(GT)_{58}$	230–250	4	0.601	0.307 7	0.663 5	KR653193
FCS15	F: CTTTCTATTTTGCAGCAGGTA R: AGAATGGCATCATAGGTAATG	50.0	$(GT)_5$	183	-	-	-	-	KR653194

Sample size=30 individuals. T_a: annealing temperature; N_a: number of polymorphic alleles per locus; PIC: polymorphic information content; H_o: observed heterozygosity; H_e: expected heterozygosity. *Significant deviations of locus from Hardy-Weinberg equilibrium after B-Y FDR correction (P<0.015 377).

Table 3 The microsatellite loci used in the cross-species amplification

Species name	Number of SSR loci	GenBank	References/Source
Holothurian scabra	9	KJ875899–KJ875907	Li et al., 2015a
	20	KR653160–KR653179	Table 1
Holothuria leucospilota	8	KF741213–KF741220	Dai et al., 2015
	16	KM880029–KM880044	Shangguan et al., 2014a
Stichopus horrens	9	KJ875908–KJ875916	Li et al., 2015b
	13	KR653147–KR653159	Shangguan et al., 2014b
Apostichopus japonicas	15	KR653180–KR653194	Table 2
Total	90		

3 RESULT AND DISCUSSION

3.1 Development and sceening of SSR markers in *H. scabra* and *A. japonicas*

H*. *scabra: Three-hundred positive clones were randomly select to sequence from the microsatellite enrichment library of *H. scabra* and 240 successful sequences were achieved. The positive rate of cloning was therefore 80%. Twenty microsatellite loci, including 16 polymorphic loci and 4 monomorphic loci, were isolated from the designed 70 pairs of specific primers. Among the 16 polymorphic loci, the Na ranged from three to eight. H_o was 0.033 3–0.517 2 and H_e was 0.032 8–0.690 1. Fitch et al. (2013) also

developed 18 microsatellite markers for *H. scabra*, and found Na ranging from two to 28. The reason for the difference in the number of alleles may be the geographic origins of the samples or the numbers of individuals used to estimate Na. Across all the loci, PIC ranged from 0.032 to 0.712. Six polymorphic loci were in the middle of the range (0.25<PIC<0.50) and five loci were highly polymorphic (PIC>0.50), according to the judgment standard (Botstein et al., 1980). Finally, 14 of the polymorphic loci were shown to not deviate from HWE after B-Y FDR correction, whereas two of the loci did deviate from HWE (CHS2* and CHS11*, $P<0.014\ 790$).

A. japonicas: One-hundred and forty-two positive clones (400–1 000 bp) were randomly selected from the microsatellite enrichment library of *A. japonicus* and generated 80 successful sequences, for a rate of positive cloning of 56.3%. Fifteen microsatellite loci (14 polymorphic, one monomorphic) were isolated from 36 designed pairs of specific primers. Among the 14 polymorphic loci, the Na range was 2–6 and the mean was 3.43, which is lower than in previous studies, such as Chen et al. (2013) (5.53), Peng et al. (2012) (7.00), Zhan et al. (2007) (5.27) and Kanno et al. (2005) (6.65). The number of repetitions or the length of the SSR repeat unit may have led to this phenomenon. Furthermore, the polymorphisms of SSR loci in noncoding regions tend to be higher because reduced evolutionary constraints (Serapion et al., 2004), and thus genome location may have played a role. H_o was 0.034 5–0.466 7 and H_e was 0.033 9–0.663 5. PIC ranged from 0.094 to 0.679, and 11 of the polymorphic loci had either medium or high rates of polymorphisms. Finally, 10 of the polymorphic loci did not deviate from HWE after B-Y FDR correction, but four of the loci did deviate from HWE (FCS1*, FCS6*, FCS8*, FCS14*; $P<0.015\ 377$).

Summing across both species, 30 polymorphic microsatellite loci were tested and 24 were found to be in accordance with HWE (Tables 1 and 2). An additional five monomorphic microsatellite loci were screened (Tables 1 and 2). The results of these analyses indicate the potential utility of these markers for sea cucumber population genetics and parentage research.

3.2 Cross-species Transferability of SSR markers

Microsatellite markers are co-dominant and highly polymorphic, and the methods used with microsatellites are reproducible and transferable to related species, making them a powerful tool for analyzing population structure and genetic diversity (Varshney et al., 2005). The transferability ratio for of the 90 sea cucumber SSR markers is presented in Table 4. The success rate of cross-species amplification of SSR loci differed among the four species, but was generally low (less than 50%). Only four loci (KR653157, KR653159, KJ875909 and KJ875911) developed for *S. horrens* were successfully applicable in all three of the other species.

Overall, the universalities of the primers were poor due to the low transferability ratios (3.45%–46.67%, mean=24.88%). However, similarly low rates of transferability were found in other taxonomic groups, for example among species of the *Indirana* genus (mean=21.2%) (Nair et al., 2012). Some taxonomic groups show high cross-species amplification success rates, e.g. *Begonia* (50%–100%) (Chan et al., 2015), *Allium* (about 50%) (Lee et al., 2011), *Cedrela* (62.8%), (Soldati et al., 2014), and Mullidae (67%–94%) (Vogiatzi et al., 2012). Different levels of transferability may results from variable species diversity among taxonomic groups. In addition, the genetic distance between the source and target species has a negative effect on the transferability of SSRs (Luo et al., 2015). A low rate of success in cross-species SSR amplification can also be due to the conservation of the flanking regions surrounding the SSR (Balloux et al., 1998), a large genome size (Barbará et al., 2007), or evolutionary divergence between the target and source species (Primmer et al., 2005; Nair et al., 2012). Conversely, the length of the microsatellite repeat in the source species may have a positive effect on the cross-species transferability (Primmer et al., 2005; Shikano et al., 2010).

In general, the SSR markers presented here could be used to identify individuals among these four species, resolve taxonomic uncertainties, and assess genetic diversity of threatened and endemic sea cucumbers.

4 CONCLUSION

Many taxonomic groups, including sea cucumbers, lack easy methods for identifying individuals to visually similar species. The microsatellite loci developed here have the potential to play an important role in aiding species identification of sea cucumbers, at least among the four species tested. These SSR loci provide a PCR-based, non-destructive, accurate, and simple test to identify individuals to genetically different but morphologically similar species of sea cucumbers. In addition, the microsatellite markers

Table 4 Cross-species amplifications of the microsatellite loci

	Locus ID	GenBank	Species				No. of successfully amplified species
			Holothuria		*Stichopus*	*Apostichopus*	
			H. scabra (*n*=4)	*H. leucospilota* (*n*=4)	*S. horrens* (*n*=4)	*A. japonicas* (*n*=4)	
H. scabra	CHS1	KR653160	+		+		2
	CHS2	KR653161	+				1
	CHS3	KR653162	+		+	+	3
	CHS4	KR653163	+				1
	CHS5	KR653164	+		+		2
	CHS6	KR653165	+	+	+		3
	CHS7	KR653166	+	+			2
	CHS8	KR653167	+				1
	CHS9	KR653168	+				1
	CHS10	KR653169	+				1
	CHS11	KR653170	+				1
	CHS12	KR653171	+				1
	CHS13	KR653172	+				1
	CHS14	KR653173	+	+			2
	CHS15	KR653174	+	+	+		3
	CHS16	KR653175	+				1
	CHS17	KR653176	+				1
	CHS18	KR653177	+				1
	CHS19	KR653178	+	+	+		3
	CHS20	KR653179	+				1
	CHS21	KJ875899	+		+		2
	CHS22	KJ875900	+				1
	CHS23	KJ875901	+		+		2
	CHS24	KJ875902	+				1
	CHS25	KJ875903	+				1
	CHS26	KJ875904	+	+	+		3
	CHS27	KJ875905	+				1
	CHS28	KJ875906	+				1
	CHS29	KJ875907	+				1
	Amplification success rate		**100%**	**20.69%**	**31.03%**	**3.45%**	
H. leucospilota	Y1-11	KM880033		+	+		2
	Y1-15*	KM880035		+			1
	Y2-7	KM880030		+			1
	Y2-8	KM880037		+			1
	Y11-1*	KM880038		+			1
	Y11-2	KM880044		+			1
	Y67-2	KM880031		+			1
	Y67-3	KM880032	+	+			2
	Y3	KM880041		+			1
	Y5	KM880043		+			1

To be continued

Table 4 Continued

	Locus ID	GenBank	Species				No. of successfully amplified species
			Holothuria		*Stichopus*	*Apostichopus*	
			H. scabra (*n*=4)	*H. leucospilota* (*n*=4)	*S. horrens* (*n*=4)	*A. japonicas* (*n*=4)	
H. leucospilota	Y16	KM880042	+	+		+	3
	Y21	KM880039		+			1
	Y28*	KM880034	+	+		+	3
	Y31	KM880029		+			1
	Y48	KM880040		+	+		2
	Y78	KM880036		+		+	2
	YZHS17	KF741213		+	+		2
	YZHS18	KF741214		+	+		2
	YZHS19	KF741215		+			1
	YZHS20	KF741216		+			1
	YZHS21	KF741217		+	+	+	3
	YZHS22	KF741218		+	+	+	3
	YZHS23	KF741219		+		+	2
	YZHS24	KF741220	+	+	+		3
	Amplification success rate		**16.67%**	**100%**	**29%**	**25%**	
S. horrens	CCS1	KR653147			+		1
	CCS2	KR653148			+		1
	CCS3	KR653149			+		1
	CCS4	KR653150			+		1
	CCS5	KR653151			+		1
	CCS6	KR653152			+		1
	CCS7	KR653153			+		1
	CCS8	KR653154			+		1
	CCS9	KR653155			+		1
	CCS10	KR653156			+		1
	CCS11	KR653157	+	+	+	+	4
	CCS12	KR653158			+		1
	CCS13	KR653159	+	+	+	+	4
	CCS14	KJ875908	+		+		2
	CCS15	KJ875909	+	+	+	+	4
	CCS16	KJ875910			+		1
	CCS17	KJ875911	+	+	+	+	4
	CCS18	KJ875912			+		1
	CCS19	KJ875913	+		+	+	3
	CCS20	KJ875914			+		1
	CCS21	KJ875915			+		1
	CCS22	KJ875916		+	+		2
	Amplification success rate		**27.27%**	**22.73%**	**100%**	**22.73%**	

To be continued

Table 4 Continued

	Locus ID	GenBank	Species				No. of successfully amplified species
			Holothuria		*Stichopus*	*Apostichopus*	
			H. scabra (*n*=4)	*H. leucospilota* (*n*=4)	*S. horrens* (*n*=4)	*A. japonicas* (*n*=4)	
A. japonicus	FCS1	KR653180		+		+	2
	FCS2	KR653181				+	1
	FCS3	KR653182				+	1
	FCS4	KR653183	+			+	2
	FCS5	KR653184		+		+	2
	FCS6	KR653185				+	1
	FCS7	KR653186				+	1
	FCS8	KR653187				+	1
	FCS9	KR653188		+	+	+	3
	FCS10	KR653189		+	+	+	3
	FCS11	KR653190	+	+	+	+	4
	FCS12	KR653191		+		+	2
	FCS13	KR653192	+	+	+	+	4
	FCS14	KR653193				+	1
	FCS15	KR653194			+	+	2
	Amplification success rate		**20%**	**46.67%**	**33.33%**	**100%**	

identified here, including the polymorphic and monomorphic loci, will increase the limited population genetic information available for *H. scabra* and *A. japonicus*. These results will aid sea cucumber resource investigations and genetic resource conservation.

5 DATA ACCESSIBILITY

Genbank accessions of DNA sequences: *H. scabra* for KR653160–KR653179, *A. japonicus* for KR653180–KR653194.

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