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Short Communication

Isolation and Characterization of 25 Polymorphic Microsatellite Markers of *Sepiella japonica*

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XZ and TG designed the research. XW performed experiments. LL and XW analyzed the data. JG wrote the paper.

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***Sepiella japonica*, Common Chinese cuttlefish, Microsatellite loci, High-throughput transcriptoeme sequencing.**

ABSTRACT

Sepiella japonica was once one of the major fisheries in Zhejiang province of China. The availability of highly polymorphic markers was important to conduct the conservation of *S. japonica.* In this study, next-generation sequencing and *de novo* assembly were used for potential useful microsatellite markers obtaining of *S. japonica.* A total of 120 microsatellite markers were designed and tested, 25 primer pairs showed polymorphism among *S. japonica* individuals. Number of alleles, observed and expected heterozygosity per locus ranged from 2 to 8, 0.083 to 0.922 and 0.042 to 0.866, respectively. The PIC ranged from 0.04 to 0.829. These markers will be useful in evaluation germplasm and genetic composition in the further research.

Sepiella japonica was once one of major fisheries in Zhejiang province China (Li *et al*., 2011). The peak annual catch production reached sixty seven thousand tones in Zhejiang province (Li *et al*., 2011). But the natural population of *S. japonica* was decreasing dramatically because of over-fishing since the 1980s (Li *et al*., 2011). *S. japonica* artificial breeding were broken through in 2003 (Chang and Wu, 2009). With the implement of artificial breeding and stock enhancement, *S. japonica* resources was recovering (Wu *et al*., 2006). Reproduction cycle of *S. japonica* was half a year in artificial cultured condition, which is different from that in the nature condition (Song and Wang, 2009). The faster reproduction rate in cultured population may affect the structure of cultured *S. japonica* population. Then finally affect wild *S. japonica* populations though artificial populations releasing. Thus, it is urgent to understand germplasm situation and genetic composition of the *S. japonica* population accurately. More loci need to be developed for evaluation germplasm and genetic composition of the *S. japonica.*

Microsatellite markers were one of the most powerful tools for population structure and genetic diversity analysis due to their genetic co-dominance, multi-allelic variation, relative abundance, and high reproducibility (Rajwant *et al*., 2011). In recent years, there have been a few related reports on the development of microsatellite markers of the *S. japonica* (Wu *et al*., 2010; Guo *et al*., 2013). Former researches used clones and enrichment methods mostly. The traditional methods were time-consuming, laborious, and require a good knowledge of genomic information. Thus, more efficient and accurate methods were needed for microsatellite markers development.

In recent years, high-throughput sequencing developed rapidly (Csencsics *et al*., 2010) and be used in some aquatic species (Shan *et al*., 2018). It is widely used in the development of microsatellites for animals and plants (Yu *et al*., 2011; Zhu *et al*., 2012). The objective of our study was to develop polymorphic microsatellite markers of *S. japonica* from transcriptome. It will be benefit for the genetic research of the *S. japonica* in future researches.

Materials and methods

Samples of *S. japonica* were collected from the aquaculture farm of Zhoushan (China) and one of them (female) was send to BioMarker Biotech Inc. for High-throughput transcriptome. Tissue samples of eyestalk, peduncle, tentacle, gill, muscle and ovary were rapidly sampled, snap-frozen in liquid nitrogen and stored at -80°C prior to RNA extraction. And tissues samples were pooled in equal amounts for total RNA extraction, transcriptome Illumina sequencing and de novo assembly. Putative SSR markers were screened using the SSRHUNTER 1.3 software (http://www.biosoft.net/dna/SSRHunter.htm). The criteria used in SSRHUNTER to

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identify microsatellites were as follows: 6 repeats for di-nucleotide, 5 repeats for tri-nucleotide and tetranucleotide. Primers were designed using the PRIMER 5.0 program (http://www.premierbiosoft.com/).

Polymorphism evaluation was tested using 30 *S. japonica* individuals. Genomic DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenol-chloroform method. Amplification was carried out in a volume of 25μL, which contained approximately 100ng of DNA as template, 2.5μL dNTP (2.5 mM each), 2.5 μL 10×buffer,2μL MgCl2 (20 mM), 1μL primers (10 mM each), and 0.25μL of Taq DNA polymerase (5U/μL). Polymerase chain reaction were run under the following thermal cycle condition: at 94°C for 5 min, followed by 35 cycles of denaturing at 94°C for 1 min, 45s at the optimal annealing temperature of primers (Table I), and extending 72°C for 1.5 min, and an extra extension 72°C for 10 min. The products of PCR were electrophoresed on 8% denaturing polyacrylamide gels for 4-5 h at 12 W. The sizes of the alleles were estimated according to the 8 bp DNA ladder. Finally, the target bands were visualized by silver staining.

The expected and observed heterozygosity (H_E and H_O, respectively), allele number (N_A), polymorphism information content (PIC) were analyzed using the CERVUS 3.0 (Kalinowski *et al*., 2007). Hardy-Weinberg equilibrium (HWE) and Tested for linkage disequilibrium (LD) were calculated by GENEPOP 4.0 (Rousset, 2008). All results for multiple tests were corrected using Bonferroni's correction (Rice, 1989).

Table I.- Characterization of 25 polymorphic microsatellite loci in *Sepiella japonica.*

Locus	Repeat motif	Primer sequence (5'-3')	T_A (°C)	N_A	Expected size (bp)	H_O	H_E	PIC	P_{HWE}	Accession No.
SSR40	(AGC)5	F: CCAGACACAGTAGGTTGCTT	54	6	198-210	0.25	0.643	0.593	0.723	MH492333
		R: AAGAAGAATTAGGCAGGCACTT								
SSR39	(TC)7	F: GGTCATCTCTGGTAAGATTCAC	49	7	180-200	0.417	0.866	0.829	0.851	MH492334
		R: TCTGGTTCTCCGCCTGTT								
SSR49	(AT)6	F: ACTGCTACGGCGACACTT	52	6	178-186	0.125	0.56	0.519	0.253	MH492335
		R: GTTCATCTTCTTGTAACGTGGA								
SSR52	(AT)6	F: TACTGCCTCCTGGTTACTATGT	58	6	220-232	0.125	0.657	0.604	0.267	MH492336
		R: CTGAATTGAAACTGCACCTGAA								
SSR54	(TG)6	F: ACTGAAACTTGAAAGGAAGGGA	55	6	196-220	0.833	0.781	0.736	0.013	MH492337
		R: CTGTCTGAAAGTCGTCACTTGT								
SSR53	(AT)6	F: TTCCCTGATGTAAACACCAAGT	56		200-212	0.292	0.827	0.788	0.111	MH492338
		R: CCGGTCAGTACACCTTCAAT								
SSR56	(TG)6	F: CCTTCCTTAACTGCTCTTCGTA	53	8	120-148	0.792	0.558	0.479	0.018	MH492339
		R: CACACTCTCATTCACTTACACA								
SSR57	(AT)6	F: AACGAGGGACGCTGGAAAT	55	3	156-164	0.083	0.298	0.272	0*	MH492340
		R: GCAGTGCAAACAGACTCAGT								
SSR58	(AG)6	F: AACCATAATGGTAGGCAGAGA	54	6	140-152	0.429	0.789	0.74	0.338	MH492341
		R: CTCTTTCACTCACTCTCACTCT								
SSR62	(TAA)5	F: AAACGCTAACAAAGACGAATGG	55	2	120-125	0.217	0.264	0.225	0.322	MH492342
		R: GCTTCCAACACAAACCTCTATC								
SSR70	(CA)6	F: TCTTCCCTTCGGAACAGACATA	61	2	130-142	0.083	0.082	0.077	0*	MH492343
		R: CCACCTGACTCGCAATAGC								
SSR73	(TGC)5	F: GCTTGCGAGGAAGATGAAGG	58	2	120-130	0.042	0.042	0.04	0*	MH492344
		R: AGCACCATTGACAATACTACCA								
SSR80	(AT)6	F: CCCAATAATATGTTTCTCGTCG	48	3	210-222	0.25	0.228	0.206	0.083	MH492345
		R: GCCATCCACTGGTGTTAGAT								
SSR86	(CA)6	F: TCCGCAAACACATTTAGAGAAC	50	2	220-232	0.125	0.12	0.11	0.129	MH492346
		R: CCGTGATGACCTGGCAGAA								

Locus	Repeat motif	Primer sequence (5'-3')	T_A (°C)	N_A	Expected size (bp)	H_O	H_E	PIC	P_{HWE}	Accession No.
SSR89	(AG)6	F: TGAGCAGCACTAAACAGAATCT R: AGAGACAGCACTAACTGGAATG	55	5	152-164	0.922	0.624	0.533	0.622	MH492347
SSR91	(CAC)5	F: CTGTATCTCTTCTGCCTCTTCA R: CGTTGTTGTTGTTGTTGCTATC	58	6	100-120	0.375	0.629	0.572	0.322	MH492348
SSR95	(GT)6	F: AGCATTACAACAATGACAAGGC R: AGAATGTTCCCAGGCAATGAAA	55	5	155-180	0.864	0.807	0.754	0.122	MH492349
SSR98	(TA)6	F: CACTAATACTGCAACACACA R: ATCAGGCAGTGGTCTCTT	56	6	215-227	0.579	0.747	0.694	0.085	MH492350
SSR100	(AT)6	F: CAATACGAACATCGCCAGAAC R: TGTTGGTAGTGTTGGAATGGAA	55	4	170-185	0.892	0.611	0.519	0.255	MH492351
SSR103	(GT)6	F: ATGTGACCTCTACTGCTGACC R: CCTCACAAGCATTAAGCTACCA	54	4	200-220	0.565	0.699	0.623	0.877	MH492352
SSR104	(GA)6	F: CTTCAGAGCCAAAGAAAGTCAT R: CCCTCACAACATCTTCCAGTTA	55	4	108-120	0.333	0.698	0.624	0.144	MH492353
SSR3	(ATA)7	F: CAAGCTGATGAATTAGCGATGA R: TCCTTCTGGCATATTCCCTG	52	4	200-221	0.231	0.702	0.622	0.089	MH492329
SSR6	(AG)9	F: ATCAGGATGCGACATTAGGC R: GCTTGACAACACTTGGCTCA	55	8	180-210	0.625	0.855	0.806	0.058	MH492330
SSR16	(GCAC)5	F: TGACCAAATGACAGGGAACA R: ACTTCTCCTCATGGTGGTGG	54	7	140-170	0.846	0.794	0.732	0.061	MH492331
SSR17	(TCG)7	F: AACCTGTTCGCACTTTGTCA R: CAGTGAAGAGGCACGTTCAA	56	8	220-240	0.211	0.801	0.752	0.051	MH492332

T_A, annealing temperature; N_A, observed number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphic information content. *Significant deviation from HWE ($P < 0.05$).

Results and discussion

A total of 13,471 simple sequence repeats were identified from 58,224 unigenes, which included 1693 di-nucleotide, 1761 tri-nucleotide, and 139 quad-nucleotide simple sequence repeats. 120 primers were designed base on repetition times and flaking sequence priority. 98 of the 120 primers were successfully amplified, but only 25 loci showed polymorphic (Table I). Number of alleles, observed and expected heterozygosity per locus ranged from 2 to 8, 0.083 to 0.922 and 0.042 to 0.866, respectively. The PIC ranged from 0.04 to 0.829. Three loci significantly deviated from Hardy-Weinberg equilibrium after Bonferroni correction ($P < 0.05$), but no significant linkage disequilibrium was found between all these loci.

The results of this study indicated that high throughput sequencing technology based on transcriptome was an effective method for developing microsatellite markers of *S. japonica*. These microsatellite markers will be helpful for further *S. japonica* germplasm and genetic composition evaluation.

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Statement of conflict of interest

The authors declare no conflict of interest.

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