



Short Communication

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

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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.

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Authors' Contributions

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

Key words

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

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 25 µL, which contained approximately 100ng of DNA as template, 2.5 µL dNTP (2.5 mM each), 2.5 µL 10×buffer, 2 µL MgCl₂ (20 mM), 1 µL primers (10 mM each), and 0.25 µL 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 R: AAGAAGAATTAGGCAGGCACTT	54	6	198-210	0.25	0.643	0.593	0.723	MH492333
SSR39	(TC) ₇	F: GGTCATCTCTGGTAAGATTACAC R: TCTGGTTCTCCGCCTGTT	49	7	180-200	0.417	0.866	0.829	0.851	MH492334
SSR49	(AT) ₆	F: ACTGCTACGGCGACACTT R: GTTCATCTTCTTGTAACGTGGA	52	6	178-186	0.125	0.56	0.519	0.253	MH492335
SSR52	(AT) ₆	F: TACTGCCTCCTGGTTACTATGT R: CTGAATTGAACTGCACCTGAA	58	6	220-232	0.125	0.657	0.604	0.267	MH492336
SSR54	(TG) ₆	F: ACTGAAACTTGAAAGGAAGGGA R: CTGTCTGAAAGTCGTCACCTTGT	55	6	196-220	0.833	0.781	0.736	0.013	MH492337
SSR53	(AT) ₆	F: TTCCCTGATGTAAACACCAAGT R: CCGGTCAGTACACCTTCAAT	56		200-212	0.292	0.827	0.788	0.111	MH492338
SSR56	(TG) ₆	F: CCTTCCTTAACTGCTCTTCGTA R: CACACTCTCATTCACTTACACA	53	8	120-148	0.792	0.558	0.479	0.018	MH492339
SSR57	(AT) ₆	F: AACGAGGGACGCTGGAAAT R: GCAGTGCAAACAGACTCAGT	55	3	156-164	0.083	0.298	0.272	0*	MH492340
SSR58	(AG) ₆	F: AACCATAATGGTAGGCAGAGA R: CTCTTTCACTCACTCTCACTCT	54	6	140-152	0.429	0.789	0.74	0.338	MH492341
SSR62	(TAA) ₅	F: AAACGCTAACAAGACGAATGG R: GCTTCCAACACAAACCTCTATC	55	2	120-125	0.217	0.264	0.225	0.322	MH492342
SSR70	(CA) ₆	F: TCTTCCCTTCGGAACAGACATA R: CCACCTGACTCGCAATAGC	61	2	130-142	0.083	0.082	0.077	0*	MH492343
SSR73	(TGC) ₅	F: GCTTGCAGAGGAAGATGAAGG R: AGCACCATTGACAATACTACCA	58	2	120-130	0.042	0.042	0.04	0*	MH492344
SSR80	(AT) ₆	F: CCCAATAATATGTTTCTCGTCG R: GCCATCCACTGGTGTAGAT	48	3	210-222	0.25	0.228	0.206	0.083	MH492345
SSR86	(CA) ₆	F: TCCGCAAACACATTTAGAGAAC R: CCGTGATGACCTGGCAGAA	50	2	220-232	0.125	0.12	0.11	0.129	MH492346

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: CCTCACAAGCATTAAAGCTACCA	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: TCCTTCTGGCATATTCCTG	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: AACCTGTTTCGCACTTTGTCA 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 flanking sequence priority. 98 of the 120 primers were successfully amplified, but only 25 loci showed polymorphic (Table 1). 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.

References

- Chang K.M. and Wu, C.W., 2009. *J. Zhejiang Ocean Univ. (Nat. Sci.)*, **28**: 257-263.
- Csencsics, D., Brodbeck, S. and Holderegger, R., 2010. *J. Hered.*, **101**: 789-793. <https://doi.org/10.1093/jhered/esq069>
- Guo, B.Y., Qi, P.Z., Zhu, A.Y., Lv, Z.M., Wang, W.C. and Wu, C.W., 2013. *Genet. mol. Res.*, **12**: 2376-2379. <https://doi.org/10.4238/2013.December.19.5>
- Kalinowski, S.T., Taper, M.L. and Marshall, T.C., 2007. *Mol. Ecol.*, **16**: 1099-1106. <https://doi.org/10.1111/j.1365-294X.2007.03089.x>
- Li, J.J., Guo, B.Y. and Wu, C.W., 2011. *J. Zhejiang Ocean Univ. (Nat. Sci.)*, **30**: 381-386.
- Rajwant, K.K., Manoj, K.R., Sanjay, K., Rohtas, S. and Dhawan, A.K., 2011. *Euphytica*, **177**: 309-334.

- <https://doi.org/10.1007/s10681-010-0286-9>
Rice, W.R., 1989. *Evolution*, **43**: 223-225. <https://doi.org/10.1111/j.1558-5646.1989.tb04220.x>
- Rousset, F., 2008. *Mol. Ecol. Resour.*, **8**: 103-106. <https://doi.org/10.1111/j.1471-8286.2007.01931.x>
- Shan, B., Liu, Y., Yang, C., Liu, S. and Sun, D., 2018. *Pakistan J. Zool.*, **50**: 2273-2278
- Song, W.W. and Wang, C.L., 2009. *Oceanol. Limnol. Sin.*, **40**: 590-595.
- Wu, C.W., Chi, C.F., He, G.Y., Lü, Z. and Xu, M., 2010. *Acta Oceanol. Sin.*, **29**: 121-124. <https://doi.org/10.1007/s13131-010-0083-2>
- Wu, C.W., Zhao, S.J. and Xu, D.N., 2006. *Acta Oceanol. Sin.*, **37**: 231-237.
- Yu, J.N., Won, C., Jun, J., Lim, Y.W. and Kwak, M., 2011. *PLoS One*, **6**: e26933. <https://doi.org/10.1371/journal.pone.0026933>
- Zhu, H., Senalik, D., McCown, B.H., Zeldin, E.L., Speers, J., Hyman, J., Bassil, N., Hummer, K., Simon, P.W. and Zalapa, J.E., 2012. *Theoret. appl. Genet.*, **124**: 87-96. <https://doi.org/10.1007/s00122-012-1787-9>

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