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## Short Communication

# Isolation and Characterization of 19 Polymorphic Microsatellite Markers of *Sepia esculenta*

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**Authors' Contributions**
**XBM conceived and designed the study, executed the experimental work, analyzed the data, and wrote the article. TXG and NS helped in conceiving and designing the study. CHL, LL and LQL helped in sampling of specimens. XMZ helped in preparation of manuscript.**

**Key words**
***Sepia esculenta*, Population structure, Genetic diversity, Polymorphic loci, Microsatellite makers.**

## ABSTRACT

*Sepia esculenta* is an economically important marine cephalopoda. The availability of highly polymorphic markers will be important to promote the conservation of this species. We performed next-generation sequencing and *de novo* assembly to obtain potential useful microsatellite markers for *S. esculenta.* Among 80 tested microsatellite markers, 19 showed polymorphism among *S. esculenta* individuals. The allele number of all polymorphic microsatellite markers ranged from 6 to 10. Expected and observed heterozygosity varied from 0.260 to 0.904 and from 0.292 to 0.861, with an average of 0.741 and 0.607, respectively. The polymorphism information content (PIC) ranged from 0.566 to 0.823. These markers will be useful in further studies and will give new insight into conservation and efficient management of this species.

*Sepia esculenta* is widely distributed in the sea areas of Russian Far East, China, Japan, Korea and Philippines (Hao *et al*., 2007). It is not only one of the most important species of coastal fisheries resource in China, South Korea and Japan, but also a good wild species with high commercial value. Unfortunately, since the 1980s, *S. esculenta* resources have been gradually declining possibly due to over-exploitation and ocean environmental change, especially deterioration of the spawning grounds (Hao *et al*., 2007). In order to prevent the reduction of *S. esculenta*, many studies which focused on mating system had been conducted. In this study, we aimed to develop a series of suitable microsatellite markers to conduct the population structure and assess genetic diversity of *S. esculenta*.

### *Material and methods*

Twenty-four individuals of *S. esculenta* were collected in October from the coastal waters near Jiaonan, China. The samples were preserved at -20°C until DNA extraction. One of them was send to Novogene Biotech Inc., for high-throughput DNA sequencing using 454 Genome Sequencer FLX platform (Roche). Thousands of DNA sequences which contained the short tandem repeat fragments were subsequently obtained and PRIMER 5 software was used to analyze these fragments. Eventually, 80 perfect microsatellite repeats were picked up to test polymorphism among *S. esculenta* individuals.

The designed primers were evaluated using 24 individuals of *S. esculenta*. PCR was performed on a Veriti Thermal Cycler (Applied Biosystems, USA) in a total volume of 25 µl containing 0.4 µM of eachprimer, 0.2 mM of each dNTP, 1×PCR buffer, 2 mM of $MgCl_2$, 1 unit Taq polymerase (TaKaRa, Japan) and 10-100 ng DNA. The amplification profile included an initial denaturing at 94°C for 5 min, 35 cycles of 45 s at 94°C, at the locus-specific annealing temperature for 1 min, and 72°C for 45 s, and final period at 72°C for 10 min. The PCR products were separated on 6% denaturing polyacrylamide gel, and visualized by silver staining.

The observed and expected heteozygosities together with tests for Hardy-Weinberg disequilibrium were calculated by GENEPOP 4.0 (Rousset, 2007).

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**Table I.- Characteristics of microsatellite loci in *Sepia esculenta.***

| Locus | Primer sequence (5'-3') | Repeat motif | Ta (°C) | Allele size range (bp) | $N_A$ | $H_O$ | $H_E$ | PIC | GenBank accession No. |
|---|---|---|---|---|---|---|---|---|---|
| S6 | F: TCAAACTGTTCTTCCCAGAC | (CAG)11 | 56 | 160-190 | 6 | 0.292 | 0.260 | 0.566 | KX687172 |
| | R: GATCAGGAATGAGGAGAGTTC | | | | | | | | |
| S23*† | F: CCTCATTTGATTGAACTTGAC | (TAC)8 | 56 | 130-160 | 9 | 0.520 | 0.723 | 0.796 | KX687173 |
| | R: TGGAACCTTACACAGAAGAAG | | | | | | | | |
| S26 | F: TCCGAGAGTGAACAAGACTC | (TG)17 | 56 | 140-180 | 7 | 0.625 | 0.738 | 0.613 | KX687174 |
| | R: AGCCAAACTGCTTTAATTCTT | | | | | | | | |
| S27 | F: TGATAATGTTGTTAGCACAACTG | (ACA)6 | 56 | 120-180 | 9 | 0.375 | 0.550 | 0.790 | KX687175 |
| | R: ATGACAAGAATGAAGAAGACG | | | | | | | | |
| S29 | F: GCACTTAGTCAAAGGGTGTC | (CAG)8 | 56 | 140-170 | 6 | 0.667 | 0.835 | 0.652 | KX687176 |
| | R: TTGTTGTCGTTGTTGAGATG | | | | | | | | |
| S33 | F: CAGCCTCAAATGTCAGTGTAT | (CAG)7 | 56 | 160-200 | 7 | 0.723 | 0.806 | 0.681 | KX687177 |
| | R: CAAGCTCAGTTGTCTGTGAAT | | | | | | | | |
| S36 | F: TTCAGATGAATACAAATGGAGA | (TGC)7 | 55 | 140-180 | 7 | 0.557 | 0.772 | 0.667 | KX687178 |
| | R: TGCAATTAGATTCAGCTTCTT | | | | | | | | |
| S38 | F: CAGCCTTTAATGACTCTGTTG | (TAT)9 | 55 | 160-200 | 6 | 0.712 | 0.765 | 0.637 | KX687179 |
| | R: CACCACCACCATTACAACTA | | | | | | | | |
| S40 | F: ATCTGTTTCCTCCCTACTCAC | (ATT)8 | 55 | 160-180 | 10 | 0.861 | 0.823 | 0.823 | KX687180 |
| | R: GTCATGATGAGAGGAATGATG | | | | | | | | |
| S41 | F: ACATCTGGGTCAGGGAGTAT | (TAA)10 | 56 | 170-200 | 6 | 0.477 | 0.745 | 0.625 | KX687181 |
| | R: CGAGTTACAACACGGTACTTC | | | | | | | | |
| S42 | F: GGAGGTCCATTTATTTTCTGT | (ATA)8 | 56 | 160-220 | 8 | 0.542 | 0.724 | 0.781 | KX687182 |
| | R: AATTATTCTTGGCAACTATTCC | | | | | | | | |
| S43 | F: TTTGGATATTGTTTCTGTCGT | (TCC)9 | 54 | 140-180 | 10 | 0.834 | 0.895 | 0.810 | KX687183 |
| | R: TAATCCACTCTCAGACAAGGA | | | | | | | | |
| S52*† | F: GGCACCCTAAAGTATGGTTAG | (TCA)6 | 56 | 130-160 | 6 | 0.500 | 0.714 | 0.650 | KX687184 |
| | R: CCTTCATGAAAGGCATAATAA | | | | | | | | |
| S53 | F: TGGTAACCAGCAGAGTTAGAG | (GAA)6 | 54 | 170-190 | 6 | 0.473 | 0.744 | 0.633 | KX687185 |
| | R: CAGAAAGAAACGGTAGTCAGA | | | | | | | | |
| S56 | F: TAAACAAGAGTGAGGGGAAAC | (GA)25 | 58 | 220-240 | 8 | 0.695 | 0.798 | 0.745 | KX687186 |
| | R: TCGGACCAGTTGTTTATGTAT | | | | | | | | |
| S58*† | F: GCAGATTATGAGGTGAGTCAA | (AGT)7 | 56 | 150-170 | 6 | 0.528 | 0.736 | 0.611 | KX687187 |
| | R: CAGAGAAGGTGGCTACAACTA | | | | | | | | |
| S60† | F: TGTAAGTTTGATCCTCATTGG | (CA)14 | 56 | 130-160 | 9 | 0.621 | 0.734 | 0.802 | KX687188 |
| | R: GGCATAGTAACAAGATGGTGA | | | | | | | | |
| S74 | F: GTTGTGTTTATTGCAGCTCTT | (TG)19 | 59 | 180-210 | 6 | 0.792 | 0.904 | 0.661 | KX687189 |
| | R: ATACTTCATGCTCCTTTCTGC | | | | | | | | |
| S85 | F: GATGCTGTTGAAGGTTGACTA | (TGG)8 | 53 | 210-240 | 7 | 0.731 | 0.814 | 0.679 | KX687190 |
| | R: TAAAACTGTTAAGCCAACCAA | | | | | | | | |

Ta, optimized annealing temperature; $N_A$, number of alleles; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity. *locus may harbor null alleles (null allele frequency > 5%). †locus deviated from Hardy-Weinberg proportions (adjusted P-value < 0.0021).

Null allele frequencies (Brookfield, 1996) were calculated by MICRO-CHECKER 2.2.3 (van Oosterhout *et al*., 2004). All results for multiple tests were corrected using Bonferroni's correction (Rice, 1989).

*Results and discussion*

Among 80 primer pairs tested, 68 amplified single PCR products of expected size, and the others had either no products or smear only. Only 19 microsatellite markers showed polymorphism. The number of alleles, observed and expected heterozygosity per locus ranged from 6 to 10, from 0.292 to 0.861 and from 0.260 to 0.904, with an average of 7.300, 0.670 and 0.741, respectively. The polymorphism information content (PIC) ranged from 0.566 to 0.823. Four loci significantly deviated from Hardy-Weinberg equilibrium after Bonferroni correction ($P < 0.0021$), which may be due to the small sample size or the presence of null alleles confirmed by MICRO-CHECKER (Table I), but no evidence for stuttering and allelic dropout were found in all loci. Three loci showed evidences of null alleles (null allele frequency > 0.05). No significant genotypic linkage disequilibrium (LD) was found among all pairs of the 19 loci after Bonferroni's correction ($P > 0.0021$).

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*Statement of conflict of interest*

Authors have declared no conflict of interest.

*References*

Brookfield, J.F.Y., 1996. *Mol. Ecol*., **5**: 453-455. https://doi.org/10.1111/j.1365-294X.1996.tb00336.x

Hao, Z.L., Zhang, X.M. and Zhang, P.D., 2007. *Chin. J. Ecol*., **26**: 601-606.

Rice, W.R., 1989. *Evolution*, **43**: 223-225. https://doi.org/10.1111/j.1558-5646.1989.tb04220.x

Rousset, F., 2007. *Mol. Ecol. Resour*., **8**: 103-106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

van Oosterhout, C., Hutchinson, W.F., Wills, D.P. and Shipley, P., 2004. *Mol. Ecol. Notes*, **4**: 535-538. https://doi.org/10.1111/j.1471-8286.2004.00684.x