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References


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Development and characterization of 30 polymorphic microsatellite markers for the Atlantic surfclam, Spisula solidissima (Dillwyn, 1817)

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Abstract

Thirty polymorphic microsatellite markers were developed for the Atlantic surfclam, Spisula solidissima, from an enriched library and characterized in 24 clams from a wild population. The number of alleles ranged from 3 to 16 per locus. The expected and observed heterozygosities ranged from 0.1942 to 0.9238 and 0.0833 to 0.875 respectively. Six loci showed significant \( P < 0.05 \) after Bonferroni correction deviation from Hardy–Weinberg equilibrium, probably because of the presence of null alleles. Three primer pairs amplified duplicated loci with two involving tandem mini-satellite repeats. Most of the microsatellite markers developed here should be useful for genetic studies in this species.

Keywords: Atlantic surfclam, fishery, microsatellites, Spisula solidissima

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The Atlantic surfclam, Spisula solidissima (Dillwyn, 1817), is a marine bivalve widely distributed along the Atlantic coast of North America. Its geographic distribution stretches from the southern Gulf of St. Lawrence to Cape Hatteras, North Carolina. It is mostly found on mud and sandy bottoms from the intertidal zone to 70m in depth. It occurs at high densities in certain areas and plays an important role in benthic ecology. The Atlantic surfclam is a popular model bivalve used in studies on fertilization and early development. It is also an important fishery resource and supports an annual landing of about 150000 metric tons. Studies on the population structure of the Atlantic surfclam have been limited partly because of a lack of genetic markers. Here we report the development and characterization of the first set of microsatellite makers for this species.

Microsatellite markers were developed from an enriched genomic library constructed following the method of Tong et al. (2006). Genomic DNA was extracted from ethanol-preserved adductor muscles from 12 individuals with the
Cell/Tissue Genomic DNA extraction kit (TianGen) and digested with MboI (Takara). Fragments of 400–1000bp were collected by TianGen Gel DNA Extraction Kit. Double-stranded MboI adapters, Linker A (5ʹ-GATCGTGACGG-TACCGAATTCT-3ʹ) and Linker B (3ʹ-CAGGCATGGC-TTAAAGACTG-5ʹ) (250 pmol, excess) were ligated to DNA fragments with T4 DNA ligase (Takara) at 16 °C overnight. Excess adapters were removed by washing on an Ultrafree column (FALL). DNA fragments were amplified 10 cycles with linker B and purified. The products were denatured and hybridized to biotin-labelled (CA)_{12}, (GA)_{12}, (ACA)_{10}, (AGA)_{10}, (GACA)_{8}, and (GATA)_{8} oligo-nucleotides (mixed in advance at the ratio of 3:1:1:1:2:2) in 0.5X SSC at 68 °C for 1 h. DNA fragments bound to these probes were captured with Promega Streptavidin MagneSphere® Paramagnetic Particles and eluted by DNAse-free water after being washed four times in 0.1X SSC at room temperature, and then amplified for 10 cycles with Linker B.

The enriched fragments were ligated to pGEM-T-easy vector (Promega) at 4 °C overnight, and then transformed into Promega high-efficiency competent Escherichia coli cells (DH5α), which were cultured on Luria–Bertani solid medium containing 100mg/mL ampicillin. One-hundred and ninety-nine white positive clones were picked and sequenced with the T7 primer on ABI 3730xl DNA analysers at the Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. Sequences were screened for microsatellites containing at least six di-, five tri-, five tetra-, four penta-, three hexa- and three octa-nucleotide repeats using the software MISA (http://pgrc.ipk-gatersleben.de/misa/). One-hundred and twenty-six (excluding five duplicates) microsatellite-containing sequences were identified and consisted of 28.1% di-, 7.8% tri-, 22.7% tetra-, 6.3% penta-, 1.6% hexa-, 4.7% octa-nucleotide and 35.2% compound microsatellite repeats. Fifty-seven good sequences with sufficient flanking regions were selected for primer design with Primer 3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). An M13-(21) universal leading sequence (18bp) 5ʹ-TGTAACGAGCCGAGT-3ʹ was added to the 5ʹ end of each forward primer listed in Table 1 (Schuelke 2000). PCR solution (10 μL) contained 10–50 ng of template DNA and the following reagents: 1x Promega Colorless GoTaq® Flexi Reaction Buffer (pH 8.5, content proprietary), 1.5–2.5 mm MgCl₂, 0.6 mg/mL bovine serum albumin, 0.2 mm each dNTP, 0.2 pmol M13 (−21) tailed forward primer, 0.8 pmol reverse primer, 0.8 pmol M13(−21) primer labelled with fluorescent dyes (FAM, VIC, NED or PET; Applied Biosystems) and 0.3 units of Taq polymerase. PCR was conducted on either a GeneAmp 9700 thermocycler (Perkin Elmer) or a PTC-200 DNA engine (MJ Research Inc.). Cycling parameters were 94 °C for 5 min followed by 34 cycles of 94 °C for 30 s, annealing (temperatures indicated in Table 1) for 45 s, 72 °C for 45 s, followed by 10 cycles of 94 °C for 30 s, 53 °C for 45 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min. Amplified products were detected and sized on an ABI 3130xl Prism Genetic Sequencer (Applied Biosystems) using GS-600LIZ as size standard. Allele scoring was performed with GeneMapper v4.0. Thirty primer pairs were successfully amplified and genotyped in 24 individuals from a wild population collected offshore at Rockaway Beach, Long Island, New York.

Observed (Hₒ) and expected (Hₑ) heterozygosities were calculated with ARLEQUIN 3.0 (Excoffier et al. 2005). Hardy–Weinberg equilibrium (HWE) for each locus and linkage disequilibrium (LD) for all pairs were tested with GENEPOP (Fisher’s exact test, 1000 iterations, http://genepop.curtin.edu.au/). All tests were corrected for multiple comparisons by sequential Bonferroni correction (Rice 1989). The 30 microsatellite markers showed variable levels of polymorphism, with the number of alleles ranging from 3 to 16 (mean 6.80 ± 3.78), Hₒ ranging from 0.0833 to 0.8750 (mean 0.4389 ± 0.2449) and Hₑ ranging from 0.1942 to 0.9238 (mean 0.6440 ± 0.2304) (Table 1). No LD was detected among all loci at P < 0.05 (with Bonferroni correction). Six loci (RUSS05, RUSS18, RUSS23, RUSS26, RUSS48 and RUSS59) showed significant (P < 0.05 after Bonferroni correction) deviation from HWE. These six and four other loci (RUSS10, RUSS47, RUSS51 and RUSS53) showed signs of null alleles as indicated by MICRO-CHECKER (1000 randomizations) (van Oosterhout et al. 2004), and these 10 loci had the lowest P-values when tested for HWE, suggesting that the presence of null alleles is the main cause for the deviation from HWE at these loci.

Three primer pairs RUSS33, RUSS47 and RUSS57 amplified more than two fragments per individual, suggesting that they belong to duplicated loci. RUSS33 amplified three to six fragments per individual with well-defined patterns. It can be explained by the amplification of a tandem mini-satellite repeat in a model of Fprimer-25bp-Fprimer-75bp-Fprimer-(18bp)n-Rprimer (Fisher’s exact test, 1000 iterations, http://genepop.curtin.edu.au/). RUSS57, and RUSS45, not presented here) had clusters of fragments that differed by 8bp, with a cluster-to-cluster distance of 145bp: Fprimer-[8bp]n]N-Rprimer-145bp-Rprimer-145bp-Rprimer. RUSS57 amplified one monomorphic locus at 152 bp and another polymorphic locus.

This is the first time that microsatellite markers have been developed for S. solidissima. Most of the markers developed here are highly polymorphic and in HWE. They should be useful in genetic studies on this important model and fishery species.

**Acknowledgement**

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<table>
<thead>
<tr>
<th>Locus</th>
<th>GenBank Accession no.</th>
<th>Repeat motif§</th>
<th>Primer sequences (5’–3’)</th>
<th>( T_a ) (°C)</th>
<th>( n )</th>
<th>( a )</th>
<th>( a_s )</th>
<th>( H_E )</th>
<th>( H_O )</th>
<th>( P_{\text{HWE}} )</th>
</tr>
</thead>
</table>
| RUSS02  | EU446421             | (CCCG)$_n$    | F: caacagaaacaagaatctggaac  
R: tcagattctgaagaacagtgg  | 50            | 21 | 3  | 142–150 | 0.3298 | 0.1250 | 0.0239 |
| RUSS03  | EU446422             | (ACA)$_n$     | F: tcgtaaccctgaatcaatag  
R: agttcagccttcgtcttggg  | 55            | 24 | 3  | 127–136 | 0.3697 | 0.4583 | 0.6350 |
| RUSS05† | EU446424             | (GT)$_n$      | F: aaatatggtcatcgggggtlt  
R: tgcgcgggacacacatcct  | 50            | 19 | 9  | 131–185 | 0.8635 | 0.2917 | 0.0000* |
| RUSS07  | EU446425             | (TCCG)$_n$    | F: tcaattgaaacctgggtggaacg  
R: aaccattgctttacctgctcgg  | 50            | 24 | 5  | 213–245 | 0.5576 | 0.5833 | 0.6573 |
| RUSS08  | EU446426             | (CCGT)$_n$    | F: gcaccaaatgatctgtgacag  
R: caataaagaaccaacagatggt  | 55            | 24 | 3  | 183–187 | 0.1968 | 0.3333 | 0.1017 |
| RUSS10† | EU446428             | (CAGA)$_n$    | F: caactaaactcatacataagtg  
R: aagttggggagacatattgttgt  | 55            | 23 | 7  | 108–138 | 0.6676 | 0.2917 | 0.0000* |
| RUSS11  | EU446429             | (GTCT)$_n$    | F: ttggtcgcgttctgtatct  
R: gacagttgggaacacagataac  | 55            | 26 | 4  | 230–280 | 0.7243 | 0.8333 | 0.0424 |
| RUSS13  | EU446430             | (GACA)$_n$    | F: cagttggtcataagcgacag  
R: caaggtgctgtcttaaaacagtag  | 57            | 24 | 5  | 121–137 | 0.6871 | 0.6667 | 0.8678 |
| RUSS14  | EU446431             | (CA)$_n$      | F: aagttgggaacacagataac  
R: aatgcgcegtttataaccttc  | 50            | 24 | 7  | 220–250 | 0.6809 | 0.8333 | 0.3629 |
| RUSS15  | EU446432             | (CT)$_n$      | F: aacaccgagccattcctgccg  
R: caaatagtaaatctgtgatgt  | 58            | 22 | 5  | 187–207 | 0.6286 | 0.6667 | 0.3800 |
| RUSS18† | EU446435             | (GTCC)$_n$    | F: caagttgtagtttctggttagt  
R: caagttgtagtttctggttagt  | 58            | 21 | 10 | 264–323 | 0.9034 | 0.2083 | 0.0000* |
| RUSS20  | EU446436             | (GACA)$_n$    | F: cagttggtcataagcgacag  
R: caaggtgctgtcttaaaacagtag  | 50            | 20 | 3  | 302–314 | 0.4832 | 0.8333 | 1.0000 |
| RUSS21  | EU446437             | (AAACAC)$_n$  | F: atctctttctttctttctcctc  
R: taaaggaatgagaatcggctcag  | 55            | 24 | 3  | 179–191 | 0.6489 | 0.4583 | 0.2027 |
| RUSS23† | EU446439             | (CAGA)$_n$    | F: aacagtgtatgggtgcttggt  
R: tcagctgggaacattgtgatag  | 50            | 22 | 7  | 254–314 | 0.8289 | 0.2500 | 0.0000* |
| RUSS24  | EU446440             | (TG)$_n$      | F: gttttgcccgttttctc  
R: agcaagcaatttggagaagc  | 50            | 22 | 5  | 236–246 | 0.6614 | 0.5833 | 0.8519 |
| RUSS26† | EU446441             | (AA)$_n$      | F: gacagttggagaatcggctcag  
R: caaatagtaaatctgtgatgt  | 53            | 19 | 8  | 130–289 | 0.8750 | 0.3750 | 0.0000* |
| RUSS29  | EU446443             | (GTCT)$_n$    | F: aagttgggaacacagataac  
R: gacagttggagaatcggctcag  | 55            | 24 | 3  | 249–297 | 0.2514 | 0.1250 | 0.9165 |
| RUSS33‡ | EU446445             | (GT)$_n$      | F: tgggtcttcttctctctctc  
R: aaatcaggaacccggttctcc  | 55            | 23 | 4  | 226–265 | 0.3076 | 0.1250 | 0.0520 |
| RUSS34  | EU446446             | (CA)$_n$      | F: tcaattctattaacccgcatc  
R: cagcaatgcgctgtatgatgac  | 55            | 20 | 8  | 185–205 | 0.8289 | 0.8750 | 0.0120 |
| RUSS35  | EU446447             | (TG)$_n$      | F: aagttgtgttctggtgtgcc  
R: gacacccatggattcctcatacc  | 55            | 24 | 6  | 222–252 | 0.4583 | 0.3333 | 0.1225 |
| RUSS40  | EU446451             | (AAACAG)$_n$  | F: caacagaaacaagaatctggaac  
R: ccccaaatgctgcttttgttg  | 55            | 23 | 10 | 112–229 | 0.7340 | 0.5000 | 0.1518 |
| RUSS44  | EU446452             | (GT)$_n$      | F: cgataacatggttaagacttggtg  
R: tcaagcaaatgggatgttggtgg  | 53            | 23 | 6  | 189–219 | 0.4903 | 0.5000 | 1.0000 |
| RUSS46  | EU446453             | (GT)$_n$      | F: cgacatactgttgggagtgttg  
R: taagcaaatgggatgttggtgg  | 60            | 24 | 10 | 190–220 | 0.7952 | 0.6667 | 0.0729 |
| RUSS47†‡| EU446454             | (GACTGCTT)$_n$| F: caatacaggaagcggggcagac  
R: cgcacccagcggcttggtgtgg  | 55            | 21 | 4  | 275–299 | 0.7899 | 0.2500 | 0.0152 |
| RUSS48† | EU446455             | (GATA)$_n$    | F: tcgaccccttctgataatcacc  
R: tgcgcggatagatagacag  | 55            | 24 | 16 | 104–186 | 0.9238 | 0.5417 | 0.0006* |
| RUSS51† | EU446456             | (GCA)$_n$     | F: cagcaacagtgaatcacc  
R: eggtatgcgcatatattctgg  | 53            | 21 | 11 | 334–394 | 0.8954 | 0.4583 | 0.0104 |
| RUSS53† | EU446457             | (GAGAC)$_n$   | F: tcaattgaaacctgggtggaacg  
R: tgacaccaaatgccgagtgggg  | 55            | 23 | 15 | 143–203 | 0.9202 | 0.6250 | 0.0063 |
Table 1  Continued

| Locus | GenBank no. | Repeat motif§ | Primer sequences (5'–3')                                                                 | T_a (°C) | n | a | a_s | H_E | H_O | P_HWE \\
|-------|-------------|---------------|-----------------------------------------------------------------------------------------|----------|---|---|-----|-----|-----|---------|
| RUSS54 | EU446458    | (ACA)_h      | F: cctaatcgtagtgctgcccccc  
R: gttatccttggaagagctgcctgcctgctgcccccc | 55       | 24 | 3 | 316–322 | 0.1942 | 0.2083 | 1.0000 |
| RUSS57‡ | EU446460    | (GAA)_h      | F: gttctatcatttgagacactgtgc  
R: aggaggccaaactgtgagacctgcctgctgcccccc | 57       | 24 | 15 | 190–247 | 0.9220 | 0.8750 | 0.1138 |
| RUSS59† | EU446461    | (ACA)_h      | F: gttggctgcttaaaacaggtg  
R: tgaactacagtcagtgagtccatcctgcctgctgcccccc | 55       | 20 | 4 | 134–155 | 0.6959 | 0.1667 | 0.0003* |

§N, pure; n, interrupted.  
*Significant deviation from HWE (P < 0.05) after Bonferroni correction.  
†Loci showing evidence for null alleles.  
‡Loci amplified more than two bands per individual.

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Eleven new microsatellite loci for the tiger rattlesnake  
(Crotalus tigris)

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Abstract

Eleven microsatellite loci were isolated from an enriched genomic library from the tiger rattlesnake Crotalus tigris. Average observed heterozygosities in two populations were 0.456 and 0.427, respectively, and mean number of alleles were 7.54 (range 2–14) and 4.72 (range 2–13) respectively. No evidence of linkage disequilibrium was found across pairs of loci. The markers will be used in a long-term study examining the potential effects of urbanization on population dynamics and connectivity of this species in the mountain ranges surrounding Tucson, Arizona.

Keywords: microsatellites, rattlesnake, urban development, Viperidae

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The tiger rattlesnake Crotalus tigris (Viperidae) is a mediumsized, ground-dwelling snake found primarily on rocky slopes or in washes within rocky mountains and foothills.  

The species ranges from central Arizona south to southern Sonora, Mexico, including Isla Tiburon in the Gulf of California (Grismer 2002). We developed 11 novel microsatellite loci for C. tigris, which along with six markers previously described for the species (Goldberg et al. 2003), will be used to infer patterns of fine-scale genetic structure,