Identification of microsatellite loci in sea anemones *Aulactinia stella* and *Cribrinopsis albopunctata* (family Actiniidae) [version 1; referees: awaiting peer review]

Ekaterina S. Bocharova, Alexey A. Sergeev, Aleksandr A. Volkov

Russian Federal Research Institute of Fisheries and Oceanography, Moscow, 107140, Russian Federation

**Abstract**

From the DNA libraries enriched by the repeat motifs (AAAC)$_6$, (AATC)$_6$, (ACAG)$_6$, (ACCT)$_6$, (ACTG)$_6$, (AAAT)$_6$, (AAGT)$_6$, (AGAT)$_6$, for two viviparous sea anemones *Aulactinia stella* and *Cribrinopsis albopunctata*. 41 primer pairs were developed. These primer pairs resulted in the identification of 41 candidate microsatellite loci in either *A. stella* or *C. albopunctata*. Polymorphic loci were identified in both sea anemone species for 13 of the primer pairs and can be applicable for population genetics researches.

**Corresponding author:** Ekaterina S. Bocharova (bocharova.ekaterina@gmail.com)

**Author roles:** Bocharova ES: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Project Administration, Resources, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; Sergeev AA: Data Curation, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Volkov AA: Data Curation, Methodology, Resources, Software, Validation, Visualization

**Competing interests:** No competing interests were disclosed.

**How to cite this article:** Bocharova ES, Sergeev AA and Volkov AA. Identification of microsatellite loci in sea anemones *Aulactinia stella* and *Cribrinopsis albopunctata* (family Actiniidae) [version 1; referees: awaiting peer review] F1000Research 2018, 7:232 (doi: 10.12688/f1000research.13724.1)

**Copyright:** © 2018 Bocharova ES et al. This is an open access article distributed under the terms of the Creative Commons Attribution Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Grant information:** This work is supported by Russian Fund for Basic Research 16-04-01685. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**First published:** 27 Feb 2018, 7:232 (doi: 10.12688/f1000research.13724.1)
Introduction

Sea anemones are known to live in clonal or partially clonal populations (Bocharova, 2015; Bocharova 2016; Bocharova & Mugue, 2012). Data based on sequences of mitochondrial (12S rRNA, 16S rRNA and cytochrome oxidase III) and nuclear (18S rRNA and 28S rRNA) genes, which are successfully used in phylogenetic research, are not applicable to population genetics studies because of the high amount of monomorphic samples. Sometimes it is not evident that a population is clonal, for instance, in the parthenogenetic populations of Aulactinia stella (Verrill, 1864) in the White and the Barents Seas (Bocharova & Mugue, 2012; Bocharova, 2015). Representatives of other species can combine sexual and asexual (clonal) reproduction in response to environmental changes (Bocharova & Kozevich, 2011). For Cribrinopsis albopunctata Sanamyan et Sanamyan, 2006 there is no data about its asexual or parthenogenetic reproduction and populations of this species usually consist of males and females. Thus, these two species are characterized by different reproductive modes. The development of polymorphic microsatellite markers resulted in the design of 41 primer pairs, which were subsequently screened using DNA from both A. stella and C. albopunctata to assess primer utility in different species and populations of the same species.

Methods

For this research, sea anemone specimens were collected in Avachinsky Bay of Kamchatka Peninsula at the depths of 11–18 meters and identified in vivo. The total DNA was extracted from the samples, which were preserved in 96% ethanol, using the Wizard SV Genomic DNA Purification System (Promega, USA) following the manufacturer’s protocol. Extracted DNA was amplified using the newly created primers. An amount of 50 ng of the extracted DNA was amplified in 20 µL reactions with 1x SmarNGTaq Buffer (Dialat Ltd., Russia), 25 µM of each of four deoxyribonucleotide triphosphates, 2 mM MgCl₂, 0.1 µM of each fluorescent labeled forward and unlabeled reverse primers, and 1 unit SmarNGTaq polymerase (Dialat Ltd., Russia). Amplification of all the microsatellite loci was performed by Touchdown PCR with the following conditions: 96°C for 3 minutes for initial denaturation, followed by 30 cycles at 96°C for 10s, 62°C for 30s (with a 0.2°C decrease in the second step of each cycle), 72°C for 10s; 10 cycles at 96°C for 10s, 56°C (with a 0.2°C increase in the second step of each cycle) for 30s, 72°C for 10s; 20 cycles at 96°C for 10s, 56°C for 30s, 72°C for 10s; 72°C for 10 minutes; ending with a 4°C soak.

One µL of PCR product was added to 24 µL of deionized formamide Hi-Di (Applied Biosystems, USA) and 1 µL of LIZ-labeled ladder SD-450 (Syntol, Russia) and denatured at 95°C for 3 minutes. Products were visualized in 3500 Genetic Analyzer (Applied Biosystems, USA) using POP7 gel polymer.

Validation

Analysis of the obtained chromatograms was performed by GenMapper Software (ThermoFisher Scientific, USA). Of the 41 primer pairs developed, 5 (12.2%) resulted in poor or no amplification in both A. stella and C. albopunctata. Almost half (56.1%) of the remaining loci successfully amplified was monomorphic in the two species. Finally, 13 primer pairs appeared to amplify polymorphic microsatellite loci at combined panels for the two species (Table 1).
### Table 1. Characterization of 13 polymorphic microsatellite loci in the pooled DNA of Aulactinia stella (5 individuals) and Cribrinopsis albopunctata (3 individuals).

<table>
<thead>
<tr>
<th>Primer sequence (5’–3’)</th>
<th>PCR product length (bp)</th>
<th>Repeat motif</th>
<th>No. of alleles</th>
<th>Allele size range (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Act007</td>
<td>199</td>
<td>(ACAG)₅</td>
<td>2</td>
<td>192–196</td>
</tr>
<tr>
<td>Act011</td>
<td>135</td>
<td>(AATC)₃</td>
<td>3</td>
<td>137–165</td>
</tr>
<tr>
<td>Act020</td>
<td>199</td>
<td>(AACT)₁₂</td>
<td>4</td>
<td>203–215</td>
</tr>
<tr>
<td>Act021</td>
<td>282</td>
<td>(AATC)₉</td>
<td>5</td>
<td>268–284</td>
</tr>
<tr>
<td>Act028</td>
<td>299</td>
<td>(AATC)₂₄</td>
<td>5</td>
<td>252–268</td>
</tr>
<tr>
<td>Act061t</td>
<td>289</td>
<td>(ATC)₁₀</td>
<td>3</td>
<td>273–294</td>
</tr>
<tr>
<td>Act173</td>
<td>196</td>
<td>(ACAG)₄</td>
<td>2</td>
<td>197–201</td>
</tr>
<tr>
<td>Act177</td>
<td>238</td>
<td>(AAAT)₉(AACC)₂</td>
<td>5</td>
<td>176–244</td>
</tr>
<tr>
<td>Act235</td>
<td>210</td>
<td>(AAAC)₆(AAA)₃</td>
<td>3</td>
<td>199–211</td>
</tr>
<tr>
<td>Act238</td>
<td>121</td>
<td>(ACAG)₄(ACAA)(ACAG)₃(ACAA)₃</td>
<td>3</td>
<td>122–154</td>
</tr>
<tr>
<td>Act249</td>
<td>123</td>
<td>(AAAC)₅</td>
<td>2</td>
<td>130–134</td>
</tr>
<tr>
<td>Act252</td>
<td>133</td>
<td>(AAAC)₄</td>
<td>2</td>
<td>131–135</td>
</tr>
<tr>
<td>Act304</td>
<td>200</td>
<td>(AAAC)₁₁</td>
<td>4</td>
<td>192–204</td>
</tr>
</tbody>
</table>

### Data availability
The raw data is available:
- [https://doi.org/10.5281/zenodo.1171106](https://doi.org/10.5281/zenodo.1171106) (Bocharova et al., 2018a). The dataset contains three files in different formats (*.scv, *.geneious, *.fasta) with primer sequences of all 41 STR loci for Aulactinia stella and Cribrinopsis albopunctata.

### Grant information
This work is supported by Russian Fund for Basic Research 16-04-01685.

*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

### Acknowledgments
Additional support came from the Syntol Company ([www.syn-tol.ru](http://www.syn-tol.ru)) due to help of Vera Ustinova and Julia Monakhova with DNA libraries preparation and MiSeq sequencing. Special thanks are given to Nadya and Karen Sanamyan (Kamchatka Branch of Pacific Geographical Institute, Far-Eastern Branch of the Russian Academy of Sciences) for collecting and identifying of these anemone samples.

### Competing interests
No competing interests were disclosed.
References


The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com