




## METHOD ARTICLE

# Identification of microsatellite loci in sea anemones *Aulactinia stella* and *Cribrinopsis albopunctata* (family Actiniidae) [version 1; referees: 3 approved]

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## Abstract




From the DNA libraries enriched by the repeat motifs (AAAC)<sub>6</sub>, (AATC)<sub>6</sub>, (ACAG)<sub>6</sub>, (ACCT)<sub>6</sub>, (ACTC)<sub>6</sub>, ACTG)<sub>6</sub>, (AAAT)<sub>8</sub>, (AACT)<sub>8</sub>, (AAGT)<sub>8</sub>, (AGAT)<sub>8</sub>, 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.


## Keywords

cnidaria, sea anemones, microsatellites, primers, *Aulactinia*, *Cribrinopsis*

## Open Peer Review

Referee Status: 

	Invited Referees		
	1	2	3
<b>version 1</b>			
published 27 Feb 2018	report	report	report

- Boris Levin**, Russian Academy of Sciences, Russian Federation
- Fabián H. Acuña**, National University of Mar del Plata (UNMDP), Argentina
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**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.

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## Introduction

Sea anemones are known to live in clonal or partially clonal populations (Bocharova, 2015; Bocharova 2016; Bocharova & Mogue, 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 & Mogue, 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) with previous desiccation and grinding. Extracted genomic DNA was exposed to fragmentation by Covaris S-Series (Covaris, USA) resulting in average distribution of fragment lengths of about 150–200 bp, which were additionally estimated by capillary electrophoresis (Nanofor-05, Syntol, Russia) with non-denaturing polymer and intercalating dye. DNA libraries were prepared by using TruSeq DNA LT Sample Prep Kit (Illumina, USA). Then the libraries were enriched for the repeat motifs (AAAC)<sub>6</sub>, (AATC)<sub>6</sub>, (ACAG)<sub>6</sub>, (ACCT)<sub>6</sub>, (ACTC)<sub>6</sub>, ACTG)<sub>6</sub>, (AAAT)<sub>8</sub>, (AACT)<sub>8</sub>, (AAGT)<sub>8</sub>, (AGAT)<sub>8</sub> and screened according to the protocol described in Glenn & Schable (2005). The fragments containing hybridization of biotinylated oligonucleotides with tandem microsatellite repeats was separated by magnetic Streptavidin M-280 Dynabeads (Dyna, Oslo, Norway). The enriched genomic DNA libraries were treated using Miseq Kit v2 for 300 cycles

in the paired-end read mode (250 + 250 bp) for MiSeq next-generation sequencing (Illumina, USA).

Sequences obtained were analyzed for the repeat regions by NGS analysis tool Geneious 10.2.3, which also compared sequences to determine the existence of duplicates. This software was also used to create 41 primer pairs flanking the repeat regions of interest. Primers were named Act ## and numbered sequentially.

The total DNA from pedal disc tissues of five *A. stella* specimens and three *C. albopunctata* specimens was extracted by 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 deoxyribonucleoside triphosphates, 2 mM MgCl<sub>2</sub>, 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).**

	Primer sequence (5'–3')	PCR product length (bp)	Repeat motif	No. of alleles	Allele size range (bp)
Act007	F: TGCAACTAACCCAAGCACCT R: TCGTTGGCTGCCTCTTGTC	199	(ACAG) <sub>5</sub>	2	192–196
Act011	F: AACAAACACATATAGGGTTACGTGTA R: AATAGCCATAGAAGCTGGATGAATG	135	(AATC) <sub>3</sub>	3	137–165
Act020	F: CGATGCGACTAGGACCGTC R: GCTTGGTGTGGCATTGAGG	199	(AACT) <sub>12</sub>	4	203–215
Act021	F: TTACGATCTTCTGAGATTAAGCCTT R: TAAAGGTCTACTGTTGTCTTATCCC	282	(AACT) <sub>9</sub>	5	268–284
Act028	F: TAAGCCTTTGTTCTACGATTGTTC R: GGTCTACTATTGTCTTATCCCTGAC	299	(AACT) <sub>24</sub>	5	252–268
Act061t	F: TGCAGTCATTCTACCCGCAA R: ACCACAGGGCTAAACAAGACA	289	(ATC) <sub>20</sub>	3	273–294
Act173	F: TGCAACTAACCCAAGCACCT R: CTCGTTGGCTGCCTCTTGT	196	(ACAG) <sub>4</sub>	2	197–201
Act177	F: TTGAAATACTGTAGAAATGGCACC R: AACACATATAGGGTTACGTGTAGAC	238	(AATC) <sub>18</sub> (AACC) <sub>2</sub>	5	176–244
Act235	F: TGGACTTGCATCTTATAACCCTAGA R: GGTGTTTCGACATTAACCTGCT	210	(AAAC) <sub>6</sub> (AAA)(AAAC) <sub>3</sub>	3	199–211
Act238	F: TGTCCGCTCTGATTGTCTGCC R: GGAGTTCCTGAGTTTGCTGC	121	(ACAG) <sub>2</sub> (ACAA)(ACAG) <sub>2</sub> (ACAA)	3	122–154
Act249	F: ACGGTCATCAATTCGGCTCA R: TCCAATACAACGGTCACTCACT	123	(AAAC) <sub>5</sub>	2	130–134
Act252	F: GTTGTGAGTTCCCGTCCAGT R: TTTGCCTTCCAACGAACAGC	133	(AAAC) <sub>4</sub>	2	131–135
Act304	F: GGTGTTCCGACATTAACCTGCT R: CGGTCCCTTATAACCCTAGAATCA	200	(AAAC) <sub>11</sub>	4	192–204

### Data availability

The raw data is available:

- <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*.
- <http://doi.org/10.5281/zenodo.1144120> (Bocharova *et al.*, 2018b). The dataset includes \*.fsa files (the Prism Genetic Analyzer 3500, Applied Biosystems) of pooled DNA PCR product of 13 STR polymorphic loci for *Aulactinia stella* and *Cribrinopsis albopunctata*.

### Competing interests

No competing interests were disclosed.

### Grant information

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

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[Data Source](#)

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# Open Peer Review

Current Referee Status:



Version 1

Referee Report 24 April 2018

doi:10.5256/f1000research.14908.r33175



**Svetlana V. Malysheva** 

Russian Academy of Sciences , Moscow, Russian Federation

As the title implies the article describes the method of microsatellite loci isolation from the DNA of two sea anemone species, which are not the model objects. The goal of the study is to bring some information about 41 microsatellite loci and corresponding primers for PCR than can be used for different species of sea anemones, which genomes have not been sequenced yet. It is reported that 13 of 41 microsatellite loci were polymorphic for the two species but possibly will be suitable for population genetic studies of other sea anemones. Much attention is given to the optimization of PCR conditions and this procedure is described in detail. In my opinion, this article is interesting and can be useful for scientists who deal with sea anemones or other representatives of Anthozoa because it includes elaborate technique for microsatellite loci isolation as well as prepared loci for microsatellite analysis. The manuscript is certainly worthy for publication.

**Is the rationale for developing the new method (or application) clearly explained?**

Yes

**Is the description of the method technically sound?**

Yes

**Are sufficient details provided to allow replication of the method development and its use by others?**

Yes

**If any results are presented, are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions about the method and its performance adequately supported by the findings presented in the article?**

Yes

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

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Referee Report 18 April 2018

doi:10.5256/f1000research.14908.r33174



**Fabián H. Acuña**

Laboratory of Cnidarian Biology (LABIC), Faculty of Natural and Exact Sciences, Institute of Marine and Coastal Research (IIMyC), National Scientific and Technical Research Council (CONICET), National University of Mar del Plata (UNMDP), Mar del Plata, Argentina

This article is a concise study concerning with the identification of microsatellite loci in sea anemones *Aulactinia stella* and *Cribrinopsis albopunctata*, both belonging to family Actiniidae, from Avachinsky Bay of Kamchatka Peninsula. In the introduction the authors said "Sea anemones are known to live in clonal or partially clonal populations" but this is valid only for some (not all) sea anemone species and it is not possible to generalize. The methodology is detailed and all the source data are available to ensure full reproducibility. The authors mention that total DNA from pedal disc tissues of studied species was extracted, but they must justify why they choose this part and not other (column, oral disc or tentacles) of examined sea anemones for DNA extraction. The text is correctly written and obtained results are well exposed in the text and table 1. This article is a valuable contribution for sea anemone genetic population investigations and merit approval for indexing.

**Is the rationale for developing the new method (or application) clearly explained?**

Yes

**Is the description of the method technically sound?**

Yes

**Are sufficient details provided to allow replication of the method development and its use by others?**

Yes

**If any results are presented, are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions about the method and its performance adequately supported by the findings presented in the article?**

Yes

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

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Referee Report 23 March 2018

doi:10.5256/f1000research.14908.r31280



**Boris Levin**

Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Yaroslavl, Russian Federation

MS 'Identification of microsatellite loci in sea anemones *Aulactinia stella* and *Cribrinopsis albopunctata* (family Actiniidae)' by E.S. Bocharova *et al.* is a methodical study related to the search and testing of polymorphic microsatellite loci in the sea anemones of above mentioned species of Actiniidae. Microsatellites are informative genetic markers for population genetic studies. Such approach is useful for obtaining information on many fundamental issues related to the reproductive strategy and spread of clonal organisms. Unlike well-studied commercial species, the biology of sea anemones is still remained unexplored in many aspects. Theory explaining the evolution of a larval stage and their long-distance dispersal ability requires genetic examination. The clonal nature of these organisms impedes application of mtDNA polymorphism and requires the search for other informative genetic markers such as microsatellites. The methods used in this study are modern and reproducible. Manuscript is well written and concise. Apparently, developed STR markers will be suitable in studies of other sea anemone species. I recommend the MS to be indexed.

**Is the rationale for developing the new method (or application) clearly explained?**

Yes

**Is the description of the method technically sound?**

Yes

**Are sufficient details provided to allow replication of the method development and its use by others?**

Yes

**If any results are presented, are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions about the method and its performance adequately supported by the findings presented in the article?**

Yes

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

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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