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Universal microsatellite markers for Baikal endemic sponge family Lubomirskiidae

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Motivation and Aim: Lake Baikal is a unique natural ecosystem with a high level of endemism. Baikal endemic sponges are representatives of a bunch of closely related species of the family Lubomirskiidae. Due to the close relationship between species, some molecular genetic markers are universal for most species of this family, for example classic barcoding marker COI. Despite great progress in studying the evolution and phylogeny of the Baikal endemic sponges, the population structures of the species have not been studied at all. The study of the population structure is also of interest in connection with the events of mass mortality and disease of sponges that have been identified in the last decade on Lake Baikal. The most suitable molecular genetic markers for studying the population structure of sponges are microsatellites. There are no developed sets of microsatellite markers for Baikal endemic sponges. For other freshwater sponges, only a few studies of the population structure for members of the Spongillidae family have been published.

Methods and Algorithms: In this work, we tested the universality of a set of 28 microsatellites developed based on the complete genome of *Ephydatia muelleri* and the draft genome of *Lubomirskia baikalensis* among representatives of *Ephydatia muelleri, Lubomirskia abietina, Baikalospongia bacillifera, Swartschewskia papyracea* and *Rezinkovia echinata* species.

For all species at least three samples were tested. DNA from sponge samples was isolated using the CTAB method. PCR was performed in a Peltier Thermal Cycler using a ScreenMix-HS kit. For *E.muelleri* microsatellite markers specificity and the exact size of PCR products were estimated using fragment analysis on an ABI 3130xl Genetic Analyzer and analyzed with the GenMarker 3.01 software. For other species marker specificity was tested by PCR products visualization in a 2 % agarose gel with Syber Green dye.

Results: For *E.muelleri* 17 loci specifically amplified and gave clear bands in agarose gel electrophoresis. Of 17 loci 11 were selected based on the results of fragment analysis. There were eight dinucleotide microsatellites, two trinucleotide microsatellites and one interrupted repeat. All microsatellite sequences can be found in *Ephydatia muelleri* genome data [1] in the following positions: scaffold_0019 – 1357021-1357338, 1810514-1810717, 1386901-1387195, 6087901-6087619, 1043658-1043935; scaffold_0016 – 868299-867998; scaffold_0020 – 6431917-6432205; scaffold_0012 – 8003328-8003132; scaffold_0023 – 541061-540749, 2188522-2188841; scaffold_0018 – 7273471-7273864. For *Lubomirskia abietina, Baikalospongia bacillifera, Swartschewskia papyracea* and *Rezinkovia echinata* species same 17 loci from 28 tested were successfully amplified and gave clear bands on gel electrophoresis. All samples of

Lubomirskiidae species will be further tested for these 17 loci by fragment analysis for exact microsatellite marker sets determination.

Conclusion: A set of 11 microsatellite markers for *E.muelleri* was successfully developed. Specific amplification of 17 out of 28 microsatellite loci for representatives of every genus from Lubomirskiidae family gives strong evidence for high cross-species specificity of microsatellites in freshwater sponges. For microsatellite marker sets development for all studied species more analyses will be carried out.

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References

1. Kenny N.J. et al. Tracing animal genomic evolution with the chromosomal-level assembly of the freshwater sponge Ephydatia muelleri. *Nat Commun.* 2020:11(1):3676.