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Analysis of Cross-Species Usability of Microsatellite Markers for Baikal Endemic Sponges †

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Abstract: In the last decade, events of mass disease and mortality of sponges have been observed in Lake Baikal, which indicates an ecological crisis of the lake. Based on the crucial role of sponges as filter feeders and bioindicators, their comprehensive study in this situation is of great interest. Despite the presence of genomic and transcriptome data for several species of endemic Baikal sponges, their population structure has never been studied before. The analysis of the population structure of both marine and freshwater sponges is successfully carried out using microsatellite markers. For freshwater sponges, the only species for which microsatellite markers have been published is *Ephydatia fluviatilis*, a close relative of the Baikal endemic sponges. Microsatellite markers show a high percentage of interspecies cross-specificity among invertebrates. According to this, here we attempted to access the suitability of these microsatellite markers for population genetic studies of endemic Baikal sponge *Lubomirskia baikalensis* based on genomic data. The presence of microsatellite sequence markers homologous to the flanking regions in the *L. baikalensis* genome was shown for 63.6% of markers, 71.4% of which contained microsatellite sequences. However, all of these markers require the development of species-specific primer pairs.

Keywords: genetic markers development; microsatellites; sponges

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1. Introduction

Sponges are one of the oldest multicellular organisms capable of biomineralization. There are cosmopolitan [1,2] and endemic [3–5] families among the freshwater sponges. One of these endemic families—the Lubomirskiidae—inhabits the unique ancient Lake Baikal. A bouquet of species of endemic sponges has been forming in Lake Baikal for the last 2–4 Ma [4,6–9]. Baikal sponges have a high morphological plasticity [10–12] and a reduced rate of evolution of the mitochondrial COI [7,9,13] gene, which is commonly used as a barcode for species identification. These factors not only complicate the species identification, but can also indicate unfinished speciation processes. Despite the availability of published genomic data for *Lubomirskia baikalensis* [14], the population structure of this species has not been studied before. Therefore, such a study would be of great interest, since it is not known whether the sponges living in different basins of the lake make up different populations, how the loss of the ability to form gemmules affects the population structure compared to cosmopolitan freshwater sponges, [15,16] and whether the mass events of disease and mortality of sponges observed in Lake Baikal over the last decade [17–22] affected the population structure in heavily diseased areas.

To answer these questions, it is necessary to select molecular genetic markers suitable for a population analysis. Microsatellite markers have been successfully used for population genetic studies of marine and cosmopolitan freshwater sponges [15,16,23–26].

Microsatellites are short tandem repeats with higher evolutionary rates than other regions of the genome. Several approaches can be used to develop microsatellite markers. First is to test previously developed markers for closely related species (*E. fluviatilis* [15]), and the second is to develop markers de novo. Since the cross-species specificity testing of microsatellite markers has shown good results for invertebrates [27], here, we tried to test the suitability of microsatellite markers developed for *E. fluviatilis* on the Baikal endemic sponge *L. baikalensis,* using bioinformatics methods based on draft genome data, and using standard laboratory tests.

2. Experiments

2.1. Data Analysis

To study the suitability of *E. fluviatilis* microsatellite markers for population genetic analysis of the Baikal endemic sponges of the *L. baikalensis* species, we searched for flanking regions of microsatellite markers in the *L. baikalensis* draft genome [14] (NCBI SRA, accession number PRJNA431612). The search for flanking sequences of microsatellite markers (left and right separately) was carried out using the BLAST + software package [28]. Aligned sequences for matches of more than 25 base pairs, plus 500 base pairs on each side were extracted using the SeqinR package in the programming language R. Sequences obtained from the draft genome data for *L. baikalensis* were aligned to the original microsatellite sequence with flanking regions of *E. fluviatilis* [29], and to the primer sequences for them using the BioEdit 7.0 software package [30] and the MAFFT v 7 online service [31].

2.2. DNA Isolation, PCR, Electrophoresis

Total DNA was isolated using the CTAB method [32]. PCR was carried out using a Techne TC 5000 thermal cycler (UK) using the Encyclo Plus PCR kit (Eurogen, Moskow, Russia). PCR products were visualized by electrophoresis in 2% agarose gel for 40 min. For loci which gave clear single bands on the agarose gel, fragment analysis was performed.

3. Results

Based on the results of the bioinformatic analysis of *L. baikalensis* genomic data, hits with the flanking regions of the Efl3–Efl22 microsatellite markers were identified, extracted from the draft genome, and analyzed [29].

Since the published draft genome of the *L. baikalensis* was incomplete, in addition to the bioinformatics analysis, the cross-species specificity of the Efl3–Efl22 microsatellite markers was also assessed using standard laboratory methods (PCR, gel electrophoresis, and a fragment analysis). When analyzing the genome, hits were found for seven markers; microsatellite sequences were present only in five of them. For the remaining two markers, more than one hit was found in different regions of the genome. For Efi-3, a hit was found in NODE_133600_length_504_cov_14.1171, for Efi-4 in NODE_15577_length_2618_cov_33.3609, for Efi-5 in NODE_3929_length_6454_cov_36.6491 and in NODE_50989_length_1056_cov_44.9704, for Efi-9 in NODE_5049_length_5597_cov_19.842, for Efi-14 in NODE_100388_length_621_cov_27.2923, for Efi-17 in NODE_4777_length_5775_cov_22.5239, and for Efi-20 in NODE_68985_length_832_cov_30.9788. All sequence names was given for a draft genome assembly with NCBI SRA, accession number PRJNA431612 [14].

Each marker Efl3–Efl22 was amplified with three samples of *L. baikalensis*. Only for two markers out of eleven (Efl7 and Efl20), clear single bands were obtained on gel electrophoresis. The rest of the markers either did not produce a PCR product, or a multiple PCR product was amplified.

According to the results of the fragment analysis, the length of the Efl7 locus was 337 base pairs for all three samples. It was not possible to assess the expected length based on the genomic data of *L. baikalensis*, since no hits were found for this marker. The reason may be the incompleteness of the genome data. The Efl20 locus was 158 bp in all samples, although the expected length based on genomic data was 213 bp. The absence

of heterozygous individuals and individuals with different alleles among the analyzed samples indicated the unsuitability of these two markers for population genetic studies of *L. baikalensis*.

We also analyzed the coincidences of the *E. fluviatilis* primer sequences with similar regions in the *L. baikalensis* genome. The analysis revealed that the primer pairs, published for microsatellite markers for *E. fluviatilis* (Efl3–Efl22), were not suitable for the specific amplification of markers of the Baikal endemic sponges *L. baikalensis*, since the genome regions containing primer sequences also contained a large number of substitutions.

4. Discussion

According to the data obtained, the presence of homologous flanking regions to the microsatellite markers sequence in the *L. baikalensis* genome was shown for 63.6% of the markers, 71.4% of which contained microsatellite sequences, namely, markers Efl3, Efl4, Efl9, Efl17, and Efl20. The obtained values were 10% lower than the average value of cross-species-specific microsatellite markers for invertebrates [27]. Despite the presence of microsatellites and hits in the flanking regions of these loci, they all require the development of new specific primer pairs.

5. Conclusions

Thus, the microsatellite markers developed and successfully used for the population genetic studies of *E. fluviatilis* [15,16] were not suitable for the population genetic studies of endemic Baikal sponges *L. baikalensis*.

The development of microsatellite markers based on *L. baikalensis* genomic data was more promising. It is possible that the search for universal microsatellite sequences in the published genomes of freshwater sponges could create a basis for the development of universal microsatellite markers for many closely related species. This work is underway at the moment.

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Abbreviations

The following abbreviations are used in this manuscript:

COI Cytochrome c oxidase subunit I

References

1. Schletterer, M.; Eggers, T. Evidence of Freshwater Sponges (Porifera: Spongillidae) in the Upper Volga River (Russia). *Ber.-Nat. Med. Ver. Innsbr.* **2006**, *93*, 73–84.
2. Pronzato, R.; Pisera, A.; Manconi, R. Fossil freshwater sponges: Taxonomy, geographic distribution, and critical review. *Acta Palaeontol. Pol.* **2017**, *62*, 467–495. [CrossRef]
3. Manconi, R.; Pronzato, R. Suborder Spongillina subord. nov.: Freshwater sponges, 921–1020. In *Systema Porifera. A Guide to the Classification of Sponges*; Hooper, J.N.A., Van Soest, R.W.M., Eds.; Kluwer Academic/Plenum Publishers: New York, NY, USA, 2002; Volume 2.

4. Meixner, M.J.; Lüter, C.; Eckert, C.; Itskovich, V.; Janussen, D.; von Rintelen, T.; Bohne, A.V.; Meixner, J.M.; Hess, W.R. Phylogenetic analysis of freshwater sponges provide evidence for endemism and radiation in ancient lakes. *Mol. Phylogenetics Evol.* **2007**, *45*, 875–886. [CrossRef] [PubMed]
5. Itskovich, V.; Kaluzhnaya, O.; Ostrovsky, I.; McCormack, G. The number of endemic species of freshwater sponges (Malawispongiidae; Spongillina; Porifera) from Lake Kinneret is overestimated. *J. Zool. Syst. Evol. Res.* **2013**, *51*, 252–257. [CrossRef]
6. Efremova, S.M. New Genus and New Species of Sponges from Family Lubomirskiidae Rezvoj, 1936. In *Index of Animal Species Inhabiting Lake Baikal and Its Catchment Area*; Timoshkin, O.A., Ed.; Nauka: Novosibirsk, Russia, 2004; Volume 1.
7. Itskovich, V.B.; Belikov, S.I.; Efremova, S.M.; Masuda, Y.; Krasko, A.; Schroeder, H.C.; Mueller, W.E.G. Monophyletic origin of freshwater sponges in ancient lakes based on partial structures of COXI gene. *Hydrobiology* **2006**, *568*, 155–159. [CrossRef]
8. Itskovich, V.; Gontcharov, A.; Masuda, Y.; Nohno, T.; Belikov, S.; Efremova, S.; Meixner, M.; Janussen, D. Ribosomal ITS Sequences Allow Resolution of Freshwater Sponge Phylogeny with Alignments Guided by Secondary Structure Prediction. *J. Mol. Evol.* **2008**, *67*, 608–620. [CrossRef]
9. Maikova, O.; Khanaev, I.; Belikov, I.S.; Sherbakov, D. Two hypotheses of the evolution of endemic sponges in Lake Baikal (Lubomirskiidae). *J. Zool. Syst. Evol. Res.* **2014**, *53*, 175–179. [CrossRef]
10. Palumbi, S.R. How Body Plans Limit Acclimation: Responses of a Demosponge to Wave Force. *Ecology* **1986**, *67*, 208–214. [CrossRef]
11. Bell, J.J.; Barnes, D.K.A.; Turner, J.R. The importance of micro and macro morphological variation in the adaptation of a sublittoral demosponge to current extremes. *Mar. Biol.* **2002**, *140*, 75–81. [CrossRef]
12. Masuda, Y. Studies on the Taxonomy and Distribution of Freshwater Sponges in Lake Baikal. *Mol. Biominer.* **2009**, *47*, 81–110. [CrossRef]
13. Yakhnenko, A.S.; Itskovich, V.B. Analysis of mtDNA variability in closely related Baikal sponge species for new barcoding marker development. *Limnology* **2020**, *21*, 49–57. [CrossRef]
14. Kenny, N.J.; Plese, B.; Riesgo, A.; Itskovich, V. Symbiosis, Selection, and Novelty: Freshwater Adaptation in the Unique Sponges of Lake Baikal. *Mol. Biol. Evol.* **2019**, *36*, 2462–2480. [CrossRef] [PubMed]
15. Li, R.; Nitsche, F.; Arndt, H. Mesoscale investigations based on microsatellite analysis of the freshwater sponge Ephydatia fluviatilis in the River-Sieg system (Germany) reveal a genetic divergence. *Conserv. Genet.* **2018**, *19*, 959–968. [CrossRef]
16. Lucentini, L.; Gigliarelli, L.; Puletti, M.E.; Palomba, A.; Caldelli, A.; Fontaneto, D.; Panara, F. Spatially explicit genetic structure in the freshwater sponge Ephydatia fluviatilis (Linnaeus, 1759) within the framework of the monopolisation hypothesis. *J. Limnol.* **2013**, *72*, e14. [CrossRef]
17. Kaluzhnaya, O.V.; Itskovich, V.B. Bleaching of Baikalian Sponge Affects The Taxonomic Composition of Symbiotic Microorganisms. *Genetika* **2015**, *51*, 1153–1157. [CrossRef]
18. Itskovich, V.; Shigarova, A.; Glyzina, O.; Kaluzhnaya, O.; Borovskii, G. Heat shock protein 70 (Hsp70) response to elevated temperatures in the endemic Baikal sponge Lubomirskia baicalensis. *Ecol. Indic.* **2018**, *88*, 1–7. [CrossRef]
19. Belikov, S.; Belkova, N.; Butina, T.; Chernogor, L.; Kley, A.M.-V.; Nalian, A.; Rorex, C.; Khanaev, I.; Maikova, O.; Feranchuk, S. Diversity and shifts of the bacterial community associated with Baikal sponge mass mortalities. *PLoS ONE* **2019**, *14*, e0213926. [CrossRef] [PubMed]
20. Khanaev, I.V.; Kravtsova, L.S.; Maikova, O.O.; Bukshuk, N.A.; Sakirko, M.V.; Kulakova, N.V.; Butina, T.V.; Nebesnykh, I.A.; Belikov, S.I. Current state of the sponge fauna (Porifera: Lubomirskiidae) of Lake Baikal: Sponge disease and the problem of conservation of diversity. *J. Great Lakes Res.* **2018**, *44*, 77–85. [CrossRef]
21. Denikina, N.N.; Dzyuba, E.V.; Bel'Kova, N.L.; Khanaev, I.V.; Feranchuk, S.I.; Makarov, M.; Granin, N.G.; Belikov, S.I. The first case of disease of the sponge Lubomirskia baicalensis: Investigation of its microbiome. *Biol. Bull.* **2016**, *43*, 263–270. [CrossRef]
22. Kulakova, N.V.; Sakirko, M.V.; Adelshin, R.; Khanaev, I.V.; Nebesnykh, I.A.; Pérez, T. Brown Rot Syndrome and Changes in the Bacterial Community of the Baikal Sponge Lubomirskia baicalensis. *Microb. Ecol.* **2017**, *75*, 1024–1034. [CrossRef]
23. Blanquer, A.; Uriz, M.J. Population genetics at three spatial scales of a rare sponge living in fragmented habitats. *BMC Evol. Biol.* **2010**, *10*, 13. [CrossRef] [PubMed]
24. Dailianis, T.; Tsigenopoulos, C.S.; Dounas, C.; Voultsiadou, E. Genetic diversity of the imperilled bath sponge Spongia officinalis Linnaeus, 1759 across the Mediterranean Sea: Patterns of population differentiation and implications for taxonomy and conservation. *Mol. Ecol.* **2011**, *20*, 3757–3772. [CrossRef]
25. Pérez-Portela, R.; Noyer, C.; Becerro, M.A. Genetic structure and diversity of the endangered bath spongeSpongia lamella. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2015**, *25*, 365–379. [CrossRef]
26. Riesgo, A.; Taboada, S.; Pérez-Portela, R.; Melis, P.; Xavier, J.R.; Blasco, G.; López-Legentil, S. Genetic diversity, connectivity and gene flow along the distribution of the emblematic Atlanto-Mediterranean sponge Petrosia ficiformis (Haplosclerida, Demospongiae). *BMC Evol. Biol.* **2019**, *19*, 24. [CrossRef]
27. Barbará, T.; Palma-Silva, C.; Paggi, G.; Bered, F.; Fay, M.F.; Lexer, C. Cross-species transfer of nuclear microsatellite markers: Potential and limitations. *Mol. Ecol.* **2007**, *16*, 3759–3767. [CrossRef]
28. Camacho, C.; Coulouris, G.; Avagyan, V.; Ma, N.; Papadopoulos, J.; Bealer, K.; Madden, T.L. BLAST+: Architecture and applications. *BMC Bioinform.* **2009**, *10*, 421. [CrossRef]

29. Mol Ecology Resources Primer Molecular Ecology Resources Primer Development Consortium; Anderson, C.M.; Aparicio, G.J.; Atangana, A.R.; Beaulieu, J.; Bruford, M.; Cain, F.; Campos, T.; Cariani, A.; Carvalho, M.A.; et al. Permanent Genetic Resources added to Molecular Ecology Resources Database 1 December 2009–31 January 2010. *Mol. Ecol. Resour.* **2010**, *10*, 576–579. [CrossRef] [PubMed]
30. Hall, T.A. BIOEDIT: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. *Nucleic Acids Symp. Ser. J.* **1999**, *41*, 95–98.
31. Katoh, K.; Rozewicki, J.; Yamada, K.D. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* **2019**, *20*, 1160–1166. [CrossRef]
32. Gustincich, S.; Manfioletti, G.; Del Sal, G.; Schneider, C.; Carninci, P. A fast method for high-quality genomic DNA extraction from whole human blood. *Biotechniques* **1991**, *11*, 95–98.