Limnology and Freshwater Biology 2020 (6): 1084-1089 DOI:10.31951/2658-3518-2020-A-6-1084

Original Article

ISSN 2658-3518
LIMNOLOGY FRESHWATER BIOLOGY
www.limnolfwbiol.com

Study of microsatellite cross-species specificity in freshwater sponge families Lubomirskiidae and Spongillidae

Yakhnenko A.S.[1,2]*, Itskovich V.B.[1]

[1] Limnological Institute, Siberian Branch of the Russian Academy of Sciences, 3, Ulan-Batorskaya, Irkutsk, Russia, 664033
[2] International Intergovernmental Organization Joint Institute for Nuclear Research, 6 Joliot-Curie St, Dubna, Moscow Region, Russia, 141980

ABSTRACT. The endemic Baikal sponges of the Lubomirskiidae family are a unique bouquet of closely related species formed from a common ancestor with the present-day cosmopolitans, *Ephydatia muelleri*, facing today are big ecological problems that require careful study. It is necessary to analyze the genetic structure of endemic freshwater sponge populations for a better understanding of the influence of such adaptive features on permanent habitat conditions as the loss of the ability to form gemmules. Microsatellite markers are best suited for analyzing population structure. The closest species to them, for which microsatellite markers have been developed to date, is *Ephydatia fluviatilis*. In this article, we check the suitability of these markers for population genetic analysis of *Lubomirskia baikalensis* and *E.muelleri* species using bioinformatic and molecular genetic methods of analysis, since the cross-species specificity of microsatellite markers has been shown for many closely related species. Despite the revealed 45.5% cross-species specificity for both *L.baikalensis* and *E.muelleri* at the level of genomic data, qualitative population genetic analysis requires the development of specific microsatellite markers *de novo* based on the genomic data of *L.baikalensis* and *E.muelleri*.

Keywords: Genetic markers, microsatellites, interspecies specificity, sponges, Porifera

1. Introduction

Lake Baikal is a unique ancient rift lake, the deepest on the planet (Kozhov, 1962; Jaguś et al., 2015). These features contributed to the formation of endemic species, which currently make up approximately 70% of the species inhabiting Baikal. The Baikal sponges are no exception. The ancestral species of endemic Baikal sponges colonized the lake millions of years ago and formed a bouquet of closely related endemic species (Efremova, 2004; Itskovich et al., 2006; 2008; Meixner et al., 2007; Maikova et al., 2015). Overall, 19 species of sponges live in the lake today, 15 of which are endemic (Itskovich et al., 2015; Manconi and Pronzato, 2019; Bukshuk and Maikova, 2020).

During the formation of endemic species of Baikal sponges from the cosmopolitan genera *Ephydatia* (Itskovich et al., 2008), Baikal sponges have lost the ability to form gemmules as an adaptation to permanent habitat conditions. Due to the loss of this method of asexual reproduction, a significant decrease in the representation of clones in the populations of Baikal sponges and a change in the population structure are expected. Research on the population genetic structure of freshwater sponges is limited to a few studies of *Ephydatia fluviatilis* (Lucentini et al., 2013; Li et al., 2018). In this regard, the study of the population structure of *Lubomirskia baikalensis* and *E.muelleri* is highly relevant.

The study of Lubomirskiidae and Spongillidae at the molecular genetic level has been actively pursued in recent years. At the moment, the draft genome of *L.baikalensis* and four transcriptomes from the species *L.baikalensis*, *L.abietina*, *B.bacillifera*,(Kenny et al., 2019), and *Sw.papyracea* (Kenny and Itskovich, 2021) have been discovered. For cosmopolitan freshwater sponges, the transcriptome of *E.muelleri* at the chromosomal level genome was discovered (Kenny et al., 2020). Despite the great success in the study of endemic Baikal sponges at the level of genomes and transcriptomes, the issue of molecular marker development for studying the population structure of Lubomirskiidae remains uncovered.

Microsatellite markers are widely used for study the population structure of marine (Blanquer and Uriz, 2010; Dailianis et al., 2011; Pérez-Portela et al., 2015; Riesgo et al., 2019) and freshwater (Lucentini et al., 2013; Li et al., 2018) sponges. The study of the genetic diversity of endemic Baikal sponges at the population level is fundamentally important for the conservation of species, especially in the conditions of mass mortality observed in Lake Baikal during the past decade (Kaluzhnaya and Itskovich, 2015; Denikina et al., 2016; Itskovich et al., 2018; Khanaev et al., 2018; Kulakova et al., 2018; Belikov et al., 2019).

Several approaches can be used in choosing microsatellite markers for the analysis of population structure. These can be the development of markers

*Corresponding author.
E-mail address: yakhnenkoas@gmail.com (Yakhnenko A.S.)

de novo for a target species or testing of previously developed markers for closely related species. The second approach is the cheapest and shows good efficiency. The analysis of published data on the development of microsatellite markers (Barbará et al., 2007) revealed that for invertebrates, on average, 72% of markers were successfully amplified from the DNA of closely related species, 77% of which were polymorphic. Moreover, the use of cross-species microsatellite markers facilitates the comparison of closely related taxa in the study of the mechanisms involved in population divergence and speciation (Noor and Feder, 2006), which makes the approach for identifying universal microsatellite markers more attractive. Among freshwater sponges, microsatellite markers are currently developed only for the species *Ephydatia fluviatilis* (Anderson et al., 2010), which is closely related to the Baikal endemic sponges.

In this work, we investigated the cross-species specificity of microsatellite markers developed for the cosmopolitan freshwater sponge, *E. fluviatilis*, within the population genetic studies of the closely related sponge *E. muelleri* and the endemic Baikal sponge *L. baikalensis* using bioinformatic and molecular genetic methods.

2. Methods

2.1 Sampling

Specimens of *L. baikalensis* sponges were collected during the 2018 expeditions by SCUBA divers in the northern basin of Lake Baikal (55°17.067' N; 109°45.401' E) from a depth of 10 - 17 m; immediately after collection, they were fixed and stored in 70% ethanol at a temperature of +4 C°. The species were identified by morphological characteristics such as body shape and size.

2.2. DNA isolation, PCR analysis and fragment analysis

Total genomic DNA was extracted using the CTAB solution (Gustincich et al., 1991).

Microsatellite markers were published previously (Anderson et al., 2010); forward primers were marked with a fluorescent label (Table 1).

PCR amplification of gene fragments was performed in a thermal cycler Techne TC 5000 (UK) using the Encyclo Plus PCR kit (Eurogen, Russia). The PCR protocol published for these primer pairs (Li et al., 2018) did not yield PCR products for Baikal sponges; therefore, the PCR protocol was optimized:

Initial denaturation for 2 min at 94 C°, followed by 11 cycles of denaturation for 30 sec at 94 C°, annealing for 30 sec at 65-55 C° (1-degree reduction every cycle), the extension for 30 sec at 72 C°, followed by 24 cycles of denaturation for 30 sec at 94 C°, annealing for 30 sec at 55 C°, the extension for 30 sec at 72 C°, then the final extension for 8 min at 72 C°.

PCR products were visualized by electrophoresis in 2% agarose gel for 40 minutes. Fragment analysis was performed for two loci that gave clear single bands on the agarose gel. The exact length of the loci was determined using fragment analysis on an ABI 3130xl Genetic Analyzer (Syntol, Moskow Russia). The six obtained fragments were analyzed using GeneMarker 3.01 (Hulce et al., 2011).

2.3. Genome data analysis

To study the suitability of the *E.fluviatilis* microsatellite markers for population genetic analysis of the Baikal endemic sponges of the *L.baikalensis* species, we searched for the flanking regions of microsatellite markers in the draft genome of *L.baikalensis* (Kenny et al., 2019) (Table 2). To assess the level of cross-species specificity of microsatellite markers among freshwater sponges, we additionally searched for flanking regions of the *E.fluviatilis* microsatellite markers in the genome assembly of the *E.muelleri* chromosomal level containing assemblies in the form of a scaffold for each of 22 chromosomes and 2 scaffolds for 23 chromosomes likely to connect by a centromere (Kenny et al., 2020) (Table 3). In each genome, flanking sequences of microsatellite markers (left and right separately) were searched using the BLAST + software package (Camacho et al., 2009); for matches greater than 25 base pairs long, the aligned sequences plus 500 base pairs on each side were extracted using the SeqinR package in the R programming language. The resulting sequences for both species were aligned to the original sequence of the microsatellite with flanking regions of

Table 1. Fluorescent labels for primers and repeat type for the *E.fluviatilis* microsatellite markers

GenBank Accession no.	Locus	fluorescent label for forward primer	
FJ752588	Efi-3	FAM	(CA)9
GQ476799	Efi-4	R6G	(CA)22
FJ752589	Efi-5	TAMRA	(ATT)8
FJ752590	Efi-7	FAM	(TGT)5
FJ752591	Efi-9	R6G	(TATG)4 (TG)15 C(GT)11
FJ752592	Efi-10	TAMRA	[(GAAT)4 (GAA)2TT]2(GATT)5
FJ752593	Efi-12	FAM	(CA)8T(CA)3
FJ752594	Efi-14	R6G	(TG)13
FJ752595	Efi-17	TAMRA	(CA)5TGCG(CA)8TGTG(CA)6TGCG (CA)6
GQ476801	Efi-20	FAM	[(CA)2/4/6T]4CTA(CA)4A4(CA)2TCAATA(CA)3TAT(CA)3)
GQ476800	Efi-22	R6G	(TG)23(AG)4 (TG)8

Table 2. Hits found in the *L.baikalensis* draft genome for microsatellite markers Efl3 – Efl22

				Coordinates in the genome assembly (Kenny et al., 2019)			
Locus	Alignment on flanking region	Presence of microsatellite	Number of copies	Sequence name	Sequence start	Sequence end	Amplification
Efi-3	+	+	1	NODE_133600_length_504_cov_14.1171	263	1	No amplification product
Efi-4	+	+	1	NODE_15577_length_2618_cov_33.3609	269	478	Multiple non-specific amplification
Efi-5	+	-	2+	NODE_3929_length_6454_cov_36.6491	453	883	Multiple non-specific amplification
				NODE_50989_length_1056_cov_44.9704	548	977	
Efi-7	-	-	-	-	-	-	**One clear band**
Efi-9	+	+	1	NODE_5049_length_5597_cov_19.842	5186	4893	Multiple non-specific amplification
Efi-10	-	-	-	-	-	-	Two bands
Efi-12	-	-	-	-	-	-	No amplification product
Efi-14	+	-	1	NODE_100388_length_621_cov_27.2923	434	288	Multiple non-specific amplification
Efi-17	+	+	3+	NODE_4777_length_5775_cov_22.5239	5469	5732	Multiple non-specific amplification / no amplification product
				NODE_4777_length_5775_cov_22.5239	5469	5775	
				NODE_59274_length_939_cov_10.8979	645	936	
Efi-20	+	+	1	NODE_68985_length_832_cov_30.9788	219	643	**One clear band**
Efi-22	-	-	-	-	-	-	Two bands

E.fluviatilis (Anderson et al., 2010) and on the primer sequences using the BioEdit 7.0 software package (Hall, 1999) and the MAFFT v 7 online service (Katoh et al., 2018). We also carried out an analysis of the matching of the *E.fluviatilis* primer sequences with similar regions in the genomes of *L.baikalensis* and *E.muelleri* (Table 4).

3. Results and discussion

Based on the results of bioinformatic analysis of genomic data of *L.baikalensis* and *E.muelleri*, we identified and analyzed hits with flanking regions of microsatellite markers Efl3 - Efl22. For *E.muelleri*, the published genome of 1490 times total coverage (Kenny et al., 2020) allows us to assess the real picture of the representation of microsatellite markers Efl3 - Efl22 based only on bioinformatic analysis, without testing in the laboratory. When analyzing the genome, hits were found for seven markers (Table 2), while microsatellite sequences were present only in five of them. For the two markers, more than one coincidence was found in different regions of the genome.

For *L.baikalensis*, the published draft genome is incomplete. Therefore, in addition to bioinformatic analysis, we also assessed the cross-species specificity of Efl3 - Efl22 microsatellite markers using standard laboratory methods (see the Methods section). During genome analysis, we detected hits for seven markers, two of which did not match with the markers identified in the *E.muelleri* genome (Table 2). Microsatellite sequences were present only in five of seven markers identified, one of which did not coincide with those identified in the *E.muelleri* genome. More than one match was found for two markers in different regions of the genome. Each marker Efl3 - Efl22 was amplified with three samples of *L.baikalensis* and only for two markers out of 11 (Efl7 and Efl20); clear single bands were obtained on gel electrophoresis (Table 2). Based on the results of the fragment analysis, the length of the Efl7 fragment was 337 nucleotides. The lack of matches in the *L.baikalensis* draft genome may be caused by incomplete genome sequence. The Efl20 locus length was158 base pairs, although the expected fragment length was approximately 213 base pairs. All three samples at both loci were homozygous and had the same length. The rest of the markers did not produce a PCR product, or a multiple PCR product was amplified.

The analysis of the matching of the *E.fluviatilis* primer sequences with similar regions in the genomes of *L.baikalensis* and *E.muelleri* revealed that the pairs of primers published for microsatellite markers of *E.fluviatilis* (Efl3 - Efl22) are not suitable for specific

Table 3. Hits found in the *E.muelleri* genome assembly for microsatellite markers Efl3 – Efl22

				Coordinates in the genome assembly (Kenny et al., 2020)		
Locus	Alignment on flanking region	Presence of microsatellite	Number of copies	Sequence name	Sequence start	Sequence end
Efi-3	+	+	1	Scaffold 0005	13008353	13008032
Efi-4	+	+	1	Scaffold 0006	8489737	8489969
Efi-5	+	-	4+	Scaffold 0590	24234	24672
				Scaffold 0022	739868	740297
				Scaffold 0431	21422	21075
				Scaffold 0019	333069	332719
Efi-7	Low quality alignment	-	1	Scaffold 0015	4326639	4327264
Efi-9	+	+	1	Scaffold 0014	6568680	6568945
Efi-10	+	+	1	Scaffold 110	11587	11767
Efi-12	-	-	-	-	-	-
Efi-14	-	-	-	-	-	-
Efi-17	+	+	2+	Scaffold 0366	10252	10568
				Scaffold 0006	12667029	12667346
Efi-20	-	-	-	-	-	-
Efi-22	-	-	-	-	-	-

Table 4. Cross-species specificity of primer pairs

Fw 3' - 5'		Rev 3' - 5'
Efi3	CCAC - - - - - AGGACACAACT - - - - - - - - ACCACA	ACCGAGCAGACCGTTGTATT
E.muelleri	CCACAGTGGTAGCAAACACTTTCTTTTAGTGCCA	ACGGAGCAGACTGTTGTGTT
Efi3	CCAC - - AGGACAC - - - - - - AACTACCACA	ACCGAGCAGACCGTTGTATT
L.baikalensis	CCATAGTGGTCACTGTGGTGACTAACAGG	TCGGAGCAGACTGTGGTGTT
Efi4	GAAGCAGCTACGGCACTACC	TTCACACCTCACGATAAGACAAA
E.muelleri	GAAGCAGTTACGACACTACC	TGTACATATGTGTATGTGTGTGT
L.baikalensis	AAAGCAGCTAAGGCACTACC	TTCACCAGACATG-TAC - AT AAT
Efi5	AGTAA – GCCACGAAGCA - GCAT	GTGGCGA - - CATCATGCAAGTA
E.muelleri	AGTAACGATGCAAAATGTGAAG	GTGGCTAATCTTCCTGCAAGTC
L.baikalensis	AGTAATGATGCAAAATGTGGAG	GTGGCTAGTCTTCCAGCAAGTC
Efi9	GGAATGGTAAGGTTCCTGCAT	GCCATACTA CTT TCTCT CTTGTGC
E.muelleri	GGAATGGTAG G - - - -- TGTGT	CACTCAAAGCTATACTAGCTGTAC
Efi9	GGAATGGTAAGGTTCCTGCAT	GCCATACTACT - TTCTCTC - - - - - - TTGTGC
L.baikalensis	GGAATGGTAAGGACCCTGTGT	GCCACTCAACTCTTCTATCACAACATGTGT
Efi10	GGAGAAAACATATGCAAGCAA	CGTGCTATTACTTGCCTTCTAGC
E.muelleri	GGAAT CACCTGAAGATGGCAC	CGTGCCACTACTTGCCTTCTTGC
Efi14	CTGCACGTATAGGGA - ATGGA	TGATGAGATGCTTGACACACA
L.baikalensis	CTGCACGTGTAGGGATATGGA	TGCCAAGTCCTCAGCAACACA
Efi17	CCATGTGTGTGC - TCA -TGAAA	TCACACACTTGACGT - TGGAGA
E.muelleri	CCAAGTGTGCGCATCAGTGAAA	CCACACACTAGACGCGGATGTGCGTGTCTCTGCGATGGAGA
L.baikalensis	CCATGTGTGTATATAAGTGAAT	CCACACACTAGACGCGGATGTGTGTGTCTCTGCGATGGAGA
Efi20	GGTTGATGGGCAATTTAGGA	CTCCCAAACTCCAGAAGCAG
L.baikalensis	TAATAATTGGAAGTGTTGGA	CTGCCAAACTCCAGAAGCAG

amplification of markers for *L.baikalensis* and *E.muelleri* species, since the genome regions containing primer sequences contain a large number of substitutions (Table 4). This explains the lack of specific amplification for *L.baikalensis* samples.

Thus, markers Efl3, Efl4, Efl9, Efl17, and Efl20 are cross-specific for species *L.baikalensis*: 45.5% of the total number of tested markers, and for species *E.muelleri*, Efl3, Efl4, Efl9, Efl10, and Efl17 are also 45.5% of those tested. This is 10% lower than the average value of cross-specific polymorphic microsatellite markers for invertebrates (Barbará et al., 2007)

Despite the presence of microsatellites and matches in the flanking regions of these loci, all loci require the development of new specific primer pairs for population genetic analysis of *E.muelleri* and *L.baikalensis* (Table 4). Markers Efl9, Efl10, Efl17, and Efl20 contain imperfect microsatellite repeats, and their use for population genetic studies can lead to erroneous identification of alleles because microsatellite elongation can occur in different parts of the imperfect repeat; thus, PCR products of the same length will have different sequences and, hence, will be different alleles. The flanking regions of markers Efl3 and Efl4 differ significantly in *E.muelleri* and *L.baikalensis*, which indicates a high variability of this genome region.

4. Conclusions

Microsatellite markers developed and successfully used for population genetic studies of *E.fluviatilis* (Anderson et al., 2010; Lucentini et al., 2013; Li et al., 2018) are not suitable for population genetic studies of the *E.muelleri* and *L.baikalensis* species.

The *de novo* development of microsatellite markers based on the genomic data of *E.muelleri* and *L.baikalensis* is more promising. Universal microsatellite sequences with conserved flanking regions have already been identified in *E.muelleri* and *L.baikalensis* genomes (Yakhnenko and Itskovich, 2020), and work on the development and testing of specific primers is underway.

Acknowledgement

We thank Julia Vitushenko for editing a draft of this manuscript.

The reported study was funded by RFBR and the Government of the Irkutsk Region, project number 20-44-383010 and basic funding, project number 0279-2021-0011.

Conflicts of Interest

The authors declare no conflicts of interest.

References

Anderson C., Gallego M., Aparicio J. et al. 2010. Permanent genetic resources added to molecular ecology resources database 1 December 2009 – 31 January 2010. Molecular Ecology Resources 10: 576-579. DOI: 10.1111/j.1755-0998.2010.02851.x

Barbará T., Palma-Silva C., Paggi G.M. et al. 2007. Cross-species transfer of nuclear microsatellite markers: potential and limitations. Molecular Ecology 16(18): 3759-3767. DOI: 10.1111/j.1365-294X.2007.03439.x

Belikov S., Belkova N., Butina T. et al. 2019. Diversity and shifts of the bacterial community associated with Baikal sponge mass mortalities. PLoS ONE 14(3). DOI: 10.1371/journal.pone.0213926

Blanquer A., Uriz M.J. 2010. Population genetics at three spatial scales of a rare sponge living in fragmented habitats. BMC Evolutionary Biology 10. DOI: 10.1186/1471-2148-10-13

Bukshuk N.A, Maikova O.O. 2020. A new species of Baikal endemic sponges (Porifera, Demospongiae, Spongillida, Lubomirskiidae). ZooKeys 906: 113-130. DOI: 10.3897/zookeys.906.39534

Camacho C., Coulouris G., Avagyan V. et al. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10. DOI: 10.1186/1471-2105-10-421

Dailianis T., Tsigenopoulos C.S., Dounas C. et al. 2011. 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. Molecular Ecology 20(18): 3757-3772. DOI: 10.1111/j.1365-294X.2011.05222.x

Denikina N.N., Dzyuba E.V., Belkova N.L. et al. 2016. The first case of disease of the sponge *Lubomirskia baicalensis*: investigation of its microbiome. Biology Bulletin 43(3): 263-270. DOI: 10.1134/S106235901603002X

Efremova S.M. 2004. New genus and new species of sponges from family Lubomirskiidae Rezvoj, 1936. In: Timoshkin O.A. (Ed.), Index of animal species inhabiting Lake Baikal and its catchment area. Novosibirsk: Nauka, pp. 1261-1278. (in Russian)

Gustincich S., Manfioletti G., Del Sal G. et al. 1991. A fast method for high-quality genomic DNA extraction from whole human blood. BioTechniques 11(3): 298-300, 302. PMID: 1931026

Hall T.A. 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symposium Series 41: 95-98.

Hulce D., Li X., Snyder-Leiby T. et al. 2011. GeneMarker® genotyping software: tools to increase the statistical power of DNA fragment analysis. Journal of Biomolecular Techniques : JBT 22: S35-S36. PMCID: PMC3186482

Itskovich V.B., Belikov S.I., Efremova S.M. et al. 2006. Monophyletic origin of freshwater sponges in ancient lakes based on partial structures of COXI gene. Hydrobiologia 568: 155-159. DOI: 10.1007/s10750-006-0320-z

Itskovich V.B., Shigarova A.M, Glyzina O.Y. et al. 2018. Heat shock protein 70 (Hsp70) response to elevated temperatures in the endemic Baikal sponge *Lubomirskia baicalensis*. Ecological Indicators 88: 1-7. DOI: 10.1016/j.ecolind.2017.12.055

Itskovich V.B., Kaluzhnaya O.V, Veynberg E. et al. 2015. Endemic Lake Baikal sponges from deep water. 1: Potential cryptic speciation and discovery of living species known only from fossils. Zootaxa 3990(1): 123-137. DOI: 10.11646/zootaxa.3990.1.7

Itskovich V.B., Gontcharov A., Masuda Y. et al. 2008. Ribosomal ITS sequences allow resolution of freshwater sponge phylogeny with alignments guided by secondary structure prediction. Journal of Molecular Evolution 67. DOI: 10.1007/s00239-008-9158-5

Jaguś A., Rzętała M.A., Rzętała M. 2015. Water storage possibilities in Lake Baikal and in reservoirs impounded by the dams of the Angara River cascade. Environmental Earth Sciences 73(2): 621-628. DOI: 10.1007/s12665-014-3166-0

Kaluzhnaya O.V., Itskovich V.B. 2015. Bleaching of Baikalian sponge affects the taxonomic composition of symbiotic microorganisms. Genetika 51: 1153-1157. DOI: 10.7868/s0016675815110077

Katoh K., Rozewicki J., Yamada K.D. 2018. MAFFT online service: multiple sequence alignment, interactive sequence

choice and visualization. Briefings in Bioinformatics 20(4): 1160-1166. DOI: 10.1093/bib/bbx108

Kenny N., Plese J.B., Riesgo A. et al. 2019. Symbiosis, selection, and novelty: freshwater adaptation in the unique sponges of Lake Baikal. Molecular Biology and Evolution 36(11): 2462-2480. DOI: 10.1093/molbev/msz151

Kenny N., Francis J., Rivera-Vicéns R.E. et al. 2020. Tracing animal genomic evolution with the chromosomal-level assembly of the freshwater sponge *Ephydatia muelleri*. Nature Communications 11(1). DOI: 10.1038/s41467-020-17397-w

Kenny N., Itskovich V.B. 2021. Phylogenomic inference of the interrelationships of Lake Baikal sponges. Systematics and Biodiversity 19(2): 209-217. DOI: 10.1080/14772000.2020.1827077

Khanaev I.V., Kravtsova L.S., Maikova O.O. et al. 2018. Current state of the sponge fauna (Porifera: Lubomirskiidae) of Lake Baikal: sponge disease and the problem of conservation of diversity. Journal of Great Lakes Research 44. DOI: 10.1016/j.jglr.2017.10.004

Kozhov M.M. 1962. Biologiya ozera Baikal [Biology of Lake Baikal]. Moscow: Akad. Nauk SSSR press.

Kulakova N.V., Sakirko M.V., Adelshin R.V. et al. 2018. Brown rot syndrome and changes in the bacterial community of the Baikal sponge *Lubomirskia baicalensis*. Microbial Ecology 75(4): 1024-1034. DOI: 10.1007/s00248-017-1097-5

Li R., Nitsche F., Arndt H. 2018. Mesoscale investigations based on microsatellite analysis of the freshwater sponge *Ephydatia fluviatilis* in the River-Sieg system (Germany) reveal a genetic divergence. Conservation Genetics 19: 959-968. DOI: 10.1007/s10592-018-1069-4

Lucentini L., Gigliarelli L., Puletti M.E. et al. 2013. Spatially explicit genetic structure in the freshwater sponge *Ephydatia fluviatilis* (Linnaeus, 1759) within the framework of the monopolisation hypothesis. Journal of Limnology 72(1)-. DOI: 10.4081/jlimnol.2013.e14

Maikova O.O., Khanaev I.V., Belikov S.I. et al. 2015. Two hypotheses of the evolution of endemic sponges in Lake Baikal (Lubomirskiidae). Journal of Zoological Systematics and Evolutionary Research 53(2): 175-179. DOI: 10.1111/jzs.12086

Manconi R., Pronzato R. 2019. Phylum Porifera. In: Damborenea C. (Ed.), Thorp and Covich's freshwater invertebrates. Academic Press, pp. 43-92. DOI: 10.1016/b978-0-12-385024-9.00003-4