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Prospects for population genetic studies of cosmopolitan freshwater sponges of the Spongillidae family in Lake Baikal

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ABSTRACT. Cosmopolitan freshwater sponges inhabit Lake Baikal. They are of great interest for carrying out population genetic studies. Microsatellite markers are best suited for population genetic studies of sponges. To date, no markers have been developed for the species *Ephydatia muelleri* that is widespread in Lake Baikal and found across the northern hemisphere. In the course of this study, a search was carried out for microsatellite markers in the previously published complete genome of *E.muelleri*. The most promising microsatellite loci were selected among those found in the genome data. Selected loci were tested on *E.muelleri* DNA samples. A set of 11 specific variable microsatellite markers was developed and tested for further population genetic studies of *E.muelleri*. Also, the Maloye More Strait area of Lake Baikal was surveyed to determine the sites of mass accumulation of the Spongilliadae family representatives. An analysis of the species composition of cosmopolitan sponges was carried out for Site 1 (Olkhon Island). Two species were identified: *E.muelleri* (72%) and *Spongilla lacusris* (18%).

Keywords: Genetic markers, microsatellites, population genetics, sponges, Porifera, Lake Baikal

1. Introduction

Sponges are one of the oldest multicellular organisms that have survived and thrive today (Philippe et al., 2009). More than 8500 sponge species were described and accepted worldwide (van Soest et al., 2012). Sponges lead an attached lifestyle and have a filtration type of nutrition (van Soest et al., 2012). Most freshwater sponge species are capable of asexual reproduction (Maldonado and Riesgo, 2008) and can survive under unfavorable conditions, for example, desiccation (Manconi and Pronzato, 2008), in the form of resting stages, gemmules. Due to the limited swimming ability of the larvae, most sponge species are found only in a limited habitat or are endemic. However, there are cosmopolitan sponge genera that spread through gemmules (Bilton et al., 2001; Manconi and Pronzato, 2008). Sponges make a significant contribution to the ecology of both marine and freshwater ecosystems (Dröscher and Waringer, 2007; Bell, 2008; Vohmann et al., 2009). Due to the filtration type of nutrition, sponges serve as sensitive bioindicators of the pollution of aquatic ecosystems (Roveta et al., 2021).

Several studies of the population structure were conducted for marine sponges (Duran et al., 2004; Calderón et al., 2007; Hoshino et al., 2008; Blanquer et al., 2009; Blanquer and Uriz, 2010; 2011; Dailianis et al., 2011; Guardiola et al., 2012; Noyer and Becerro, 2012; Riesgo et al., 2016); however, data for freshwater sponges are very scarce.

For the freshwater sponge, *Ephydatia fluviatilis*, an analysis for compliance with the hypothesis of monopolization, which is confirmed for crustaceans (De Gelas and De Meester, 2005; Muñoz and Pacios, 2010), bryozoans (Hoare et al., 2001; Massard and Geimer, 2008) and rotifers (Mills et al., 2007; Fontaneto et al., 2008) was carried out. For samples from Central Italy located within 100 km, no genetic differentiation was found. Differentiation was found between populations from Italy and Hungary, located more than 600 km apart. Sponges in terms of their genetic relationships between populations are similar to rotifers and crustaceans and can be used in combination with these organisms for further studies of the monopolization hypothesis (Lucentini et al., 2013). Additionally, the population structure of sponges inhabiting the River-Sieg system was studied for the *E.fluviatilis* freshwater sponge. That study revealed a clear genetic differentiation between populations living at a distance of up to 50 kilometers (Li et al., 2018). The population structure of freshwater sponges has not previously been studied for large

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ancient lakes. However, the relevance of such studies is undoubtable because, for example, Lake Baikal is a unique ecosystem that has existed for millions of years (Kozhov, 1962; Jaguś et al., 2015), and is mainly represented by endemic flora and fauna.

Cosmopolitan sponges live only in shallow waters; therefore, their settlement and distribution pattern in Lake Baikal is not obvious. According to paleontological data, sponges have lived in Baikal for at least 10 Ma (Veynberg, 2009). Since the formation of Lake Baikal, catastrophic events have occurred several times, causing a dramatic change in the level of the lake (Arzhannikov et al., 2017; 2021). There is no evidence that the episodes of the water levels changes led to the disappearance and subsequent recolonization by cosmopolitan sponge species of Lake Baikal or they developed continuously in the water area of the lake. The level of migration of cosmopolitan sponges between the bays separated from the main water area of Lake Baikal is also not obvious. The search for the answers to these questions is of interest and requires the study of the population genetic structure of Baikal cosmopolitan sponges. Also, endemic sponges inhabit Lake Baikal. Baikal endemic sponges have a common ancestor with the cosmopolitan genus *Ephydatia* (Itskovich et al., 2008). During the formation of an endemic family, Baikal endemic sponges lost their ability to form gemmules and acquired a long-term life cycle. The question remains open of how such changes affected the ability of endemic sponges to settle and migrate within Lake Baikal. It is necessary to carry out a comparative analysis between the population structures of the cosmopolitan sponges of the genus *Ephydatia* inhabiting Lake Baikal and the endemic ones.

According to the data from the recent revision of Baikal sponges (Efremova, 2004), there are members of four genera of the cosmopolitan family Spongillidae: *Ephydatia* Lamouroux, 1816, including the species *Ephydatia muelleri*, *Spongilla* Lamarck, 1816, *Eunapius* Gray, 1867, and *Trochospongilla* Vejdovsky, 1888. We found massive concentrations of individuals of this species in the Maloye More Strait. Therefore, this species was chosen as a promising one for carrying out population genetic studies of cosmopolitan freshwater sponges in Lake Baikal.

Molecular genetic markers were used for carrying out population genetic studies of sponges. The low resolution of mitochondrial markers such as COI was indicated for sponges (Yakhnenko and Itskovich, 2020a); however, microsatellite markers were used quite successfully. Several sets of microsatellite markers were published for marine sponge species (Duran et al., 2002; Knowlton et al., 2003; Blanquer et al., 2005; Hoshino and Fujita, 2006; Noyer et al., 2009; Anderson et al., 2010; Dailianis and Tsigenopoulos, 2010; Guardiola et al., 2012; Giles et al., 2013; Taboada et al., 2018). For freshwater sponges, microsatellite markers were developed only for *E.fluviatilis* (Anderson et al., 2010). As we have shown previously (Yakhnenko and Itskovich, 2020b), these markers are not suitable for population genetic studies of other species of the Spongillidae family, specifically for *E.muelleri*, although these species are closely related. Thus, it is necessary to develop and test a set of microsatellite markers specific for *E.muelleri*. To solve this problem, an analysis of the chromosomal level genomic data of *E.muelleri* published previously (Kenny et al., 2020) will be carried out.

To assess the prospects for analyzing the genetic distances between the populations of cosmopolitan freshwater sponges in Lake Baikal, it is also necessary to conduct fieldwork to survey the coves and bays of the Maloye More Strait at Lake Baikal to select the site of mass accumulation of the Spongillidae members.

2. Materials and methods

2.1. Sampling

Sponges were collected in November 2019, July and November 2020 in four bays and lagoons in the area of the Maloye More Strait at Lake Baikal (Fig. 1; Fig. 2; Table 1) at depths of 0 to 1.5 meters. Most of the sponge samples were collected from the back surface of the stones.

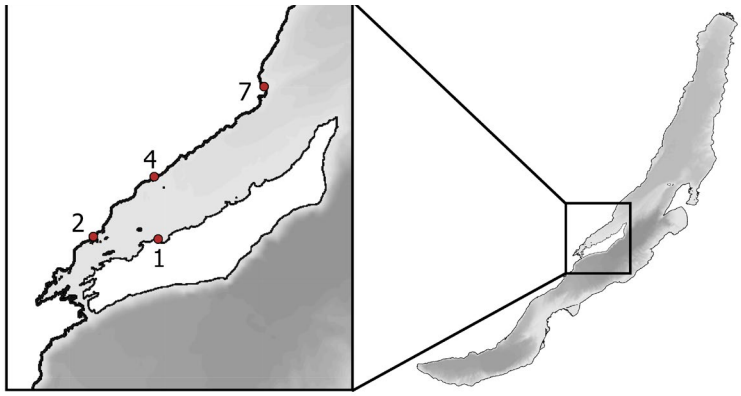

Fig.1. Spongillidae mass accumulation sites near the Maloye More Strait.

Fig.2. Sponge sample of the genus *Ephydatia* from Site 1.

The samples were fixed in 70% ethanol immediately after collection. After 24 hours ethanol was replaced with a new one for long-term storage at +4C°. Some samples in the form of gemmules were washed from the remains of the skeleton and were placed in a refrigerator at +4C° in a humid state without access to sunlight.

Species were identified based on morphological characteristics such as appearance, length and shape of spicules and gemmosclera using a light microscope. Spicules were isolated from a small fragment of the sample. The organic part was dissolved by decolorant; then, the decolorant was washed off with distilled water.

2.2. Microsatellite markers development

Tandem repeats with 7 to 30 blocks in the complete genome data on *E.muelleri* (Kenny et al., 2020) were searched for using the STR detection tool in Galaxy software (Fungtammasan et al., 2015). The most suitable microsatellites with flanking regions were selected among the identified ones. For the set of 28 microsatellites, primer pairs were designed and tested. Primers were searched for using Primer-BLAST NCBI (Ye et al., 2012). Primers were purchased from Evrogen (Moscow, Russia) and Syntol (Moscow, Russia).

2.3. Microsatellite amplification and genotyping

DNA from sponge samples was isolated using the CTAB method (Gustincich et al., 1991). PCR was performed in a Peltier Thermal Cycler (MJ Research, USA) using a ScreenMix-HS kit (Evrogen), Russia. The PCR program was optimized. Initial denaturation carried out for 2 min at 94C° followed by 11 cycles of denaturation for 30 sec at 94C°, annealing for 30 sec at 65-55C° (one-degree reduction every cycle), the extension for 30 sec at 72C° followed by 24 cycles of denaturation for 30 sec at 94C°, annealing for 30 sec at 55C°, the extension for 30 sec at 72C°, and then the final extension for 8 min at 72C°.

PCR products were visualized in a 2% agarose gel with Syber Green dye. The exact size of PCR products was estimated using fragment analysis on an ABI 3130xl Genetic Analyzer (Syntol, Moscow) and analyzed with the GenMarker 3.01 software.

Table 1. Coordinates of sampling sites

Sampling site No	Coordinates
1	53°09’10.4»N 107°10’11.4»E
2	53°09’32.5»N 106°56’56.9»E
4	53°16’59.1»N 107°09’21.4»E
7	53°28’11.1»N 107°32’02.5»E

3. Results and discussion

During the fieldwork, seven bays and lagoons were surveyed in the area of the Maloye More Strait at Lake Baikal. Mass accumulations of freshwater cosmopolitan sponges were found at four out of seven locations (Fig. 1).

We analyzed species composition for Site 1. The collections contained samples of two species: *Spongilla lacustris* (18%) and *Ephydatia muelleri* (72%).

Based on the analysis of the *E.muelleri* genomic data, we selected 28 promising microsatellite loci with flanking regions suitable for the development of primers. A pair of primers for each locus was developed. Each locus was tested on four DNA samples of *E.muelleri.* Fragment analysis was carried out for 17 loci, giving clear bands in the gel electrophoresis. Among the analyzed loci, 11 were variable, which were included in the set of microsatellite markers (Table 2). For fragment analysis, fluorescent labels were attached to the forward primers. (Table 3).

Table 2. Coordinates of microsatellite markers in genomic data on *E.muelleri* (Kenny et al., 2020)

Locus Name	Scaffold No*	Query start*	Query end*	Sequence length	Repeat type	n of alleles
Emu_241	scaffold_0019	1357021	1357338	318	(CT)14	4
Emu_249	scaffold_0019	1810514	1810717	204	(CA)13	2
Emu _257	scaffold_0019	1386901	1387195	295	(CG)6(CA)21	2
Emu_187	scaffold_0016	868299	867998	302	(CA)18	2
Emu_291	scaffold_0020	6431917	6432205	289	(TG)9	3
Emu_124	scaffold_0012	8003328	8003132	197	(GTG)5	2
Emu_369	scaffold_0023	541061	540749	313	(GT)27	3
Emu_266	scaffold_0019	6087901	6087619	283	(TGG)7	3
Emu_260	scaffold_0019	1043658	1043935	278	(CA)28	2
Emu_217	scaffold_0018	7273471	7273864	394	(AC)9	3
Emu_367	scaffold_0023	2188522	2188841	320	(AC)23	2

*In genome assembly from (Kenny et al., 2020)

Table 3. Primer pairs for the set of microsatellite markers

Locus Name	Fw. primer	Rev. Primer	Tm	Fluorescent label
Emu_241	GCTCACTACTCCAACCCGAC	CAAAGCATGGCGTGTTGTGT	59-61	Tamra
Emu_249	CATCTCTGGTGAAGTACACAGGTG	CAGAGTGCTCCAGCTGCT	59-61	Fam
Emu _257	GAGTCCGTCCTCCTGTTCAC	TGCCAGCAGAGGATATAGCATTC	59-61	R6G
Emu_187	AGCACAGCATAGCAACGATTG	ATGTGGACTTCAGGCACCTG	59-61	Fam
Emu_291	ACGTACCTCAAACACCGTAGTAC	CCCGGCTGTCTGTAAGTCAT	59-61	Tamra
Emu_124	ACTGCCACTCAGGCTCAAG	TTATCCTCAGCGAGAACGTAGTC	59-61	Rox
Emu_369	ACAAGGGTTAGTTAGGAGGCAG	GAGTACTAGGAGGATGAACCAGTG	59-61	Rox
Emu_266	GCCTGTGGTGTAAACAGTGG	CCAAGCGTCCCAGCTAAGAG	59-61	Fam
Emu_260	CCTTCGTCCACAATGGCTTG	ACCTTGGACCAGATTACTCCAAC	59-61	Fam
Emu_217	GTGTCATGGAAGACCAATGAGC	CCTTCAGGCAGGACATCAATACT	59-61	Fam
Emu_367	CCACTGTTCCTTGCCAGACA	CCAGAGGGTGTCAGGATTGAG	59-61	Rox

As shown by González-Ramos et al. (2015), a set of nine microsatellite markers is sufficient to conduct a population genetic study of sponges. It was also revealed that with 12 loci or more, the analysis resolution does not change. Blanquer and Uriz (2010) showed the presence of genetic structure within and between populations of marine sponges as well as between different geographic areas using seven microsatellite markers. Duran et al. (2004) identified the genetic structure between geographically distant locations for the *Crambe Crambe* marine sponge using six microsatellite loci. Both population genetic studies of freshwater sponges used the same set of 11 microsatellite markers (Lucentini et al., 2013; Li et al., 2018).

Thus, the most appropriate number of microsatellite loci for sponges can be from 9 to 12, taking into account that with a smaller number of microsatellite markers, it is also possible to reveal the population genetic structure, albeit with a lower resolution. Our set of microsatellite markers includes 11 loci and meets all the requirements for high-quality population genetic analysis.

4. Conclusions

Based on the results of the fieldwork, the sites of mass accumulations of Spongillidae representatives were identified. We analyzed species diversity for Site 1. The species *E.muelleri* was the predominant species. A set of 11 specific and variable microsatellite markers was successfully developed and tested for the *E.muelleri*. Thus, we prepared a basis for population genetic studies of cosmopolitan freshwater sponges in Lake Baikal and further possible studies of the population structure of *E.muelleri* around the world.

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Conflict of interests

The authors declare no conflict of interests.

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