

Metagenomic analysis of viral communities in diseased Baikal sponge *Lubomirskia baikalensis*

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ABSTRACT. Sponges are an ecologically important component of marine and freshwater bodies. Sponge community includes a variety of microorganisms: fungi, algae, archaea, bacteria and viruses. Despite active research in the field of aquatic virology, biodiversity and the role of viruses in sponges are poorly studied. The relevance of research in this area is also related to the worldwide problem of sponge diseases. The aim of this study was to elucidate the genetic diversity of viruses in the associated community of diseased endemic Baikal sponge *Lubomirskia baikalensis* using metagenomic analysis. As a result, we have shown for the first time a high genetic and taxonomic diversity of DNA viruses in the Baikal sponge community. Identified sequences belonged to 16 viral families that infect a wide range of organisms. Moreover, our analysis indicated the differences in viral communities of visually healthy and diseased branches of the sponge. The approach used in this study is promising for further studies of viral communities in sponges, obtaining more complete information about the taxonomic and functional diversity of viruses in holobionts and entire Lake Baikal, and identifying the role of viruses in sponge diseases.

Keywords: metagenomic analysis, virome, viral diversity, viral communities, sponges, Lake Baikal

1. Introduction

Viruses are the most ubiquitous, abundant and diverse biological objects on Earth. They are of particular importance in the water environments, where their concentration reaches 10^{11} particles/ml (Wilhelm and Matteson, 2008). They regulate the abundance, composition and biodiversity of numerous aquatic microorganisms and other hydrobionts, participate in biochemical processes and, in general, influence significantly on the functioning and ecological state of water bodies (Wommack and Colwell, 2000; Suttle, 2007; Jacquet et al., 2015). Despite the considerable advances in the field of aquatic virology in recent decades, we lack knowledge about viruses of marine and, especially, freshwater invertebrates including symbiotic organisms, or it is incomplete.

Sponges are the oldest multicellular invertebrates (phylum Porifera) that represent complex symbioses in marine and freshwater ecosystems and have unusual properties: high diversity, abundance and biomass; contribution to primary production and nitrification through symbioses; high chemical and physical adaptation; competitiveness; biomineralization;

production of the secondary metabolites, etc. (Diaz and Ruetzler, 2001; Bell, 2008). The sponge community includes various microorganisms: fungi, dinoflagellates, small algae, archaea, bacteria, and viruses. The abundance and diversity of viruses can be very significant, considering a great number of their potential hosts in the sponge community. However, the diversity and importance of viruses have been little studied compared to other sponge-associated microorganisms, and the role of viruses in the sponges remains largely unknown. Previously, virus-like particles (VLPs) morphologically similar to adenoviruses, picornaviruses and mimiviruses were rarely found in some sponges (Vacelet and Gallissian, 1978; Johnson, 1984; Claverie et al., 2009). Recently, the first comprehensive morphological assessment of sponge-associated viruses by transmission electron microscopy has revealed diverse communities of viral-like particles in the different marine sponge species (Pascelli et al, 2018). Moreover, the first metaviromic studies provided new molecular data on the composition, function of viruses inhabiting reef sponges, and showed a high diversity of sponge viral communities (Laffy et al., 2016; 2018).

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As natural filter feeders, sponges play a great role in the existence of any water body, which is especially relevant for Lake Baikal as a unique natural object characterized by a huge freshwater supply, ancient origin, a rich diversity of endemic flora and fauna, etc. (Kozhova and Izmesteva, 1998). According to the current taxonomy, two families represent the Baikal sponges: endemic *Lubomirskiidae* (includes 4 genera and 14 species) and cosmopolitan *Spongillidae* (3 genera and 5 species) (Efremova, 2001; 2004; Itskovich et al., 2017). Endemic species inhabit almost all depths of the lake (from 1 m to maximum depths), but the bulk of their abundance and species diversity is concentrated at depths of 5-30 m (Khanaev et al., 2018), where their biomass mostly exceeds the biomass of all zoobenthic groups (Kozhov, 1970). The most mass species *Lubomirskia baikalensis* is the only branching sponge in Lake Baikal, which colonies reach up to 1.5 m in height.

The first investigations of viruses associated with the Baikal sponges *Lubomirskia baikalensis* were based on the analysis of marker genes. We identified a high diversity of *g20* gene of cyanophages (Butina et al., 2015) and *g23* genes of T4-like bacteriophages (Butina T.V., unpublished data) in the community of *L. baikalensis*. The analysis revealed specific groups of cyanophages different from those inhabiting the lake water, which is natural and corresponds to the hologenome concept. Holobiont is a united complex of the host organism (sponge, coral, etc.) and associated microorganisms (Rohwer et al., 2002). The genetic material of holobiont (hologenome) changes faster than the genome of the host organism, which increases the potential and adaptability of the complex organism in unfavourable and changing conditions (Rosenberg et al., 2007). Sponges are one of the most diverse and complex aquatic holobionts (Webster and Thomas, 2016; Pita et al., 2018).

Over the past decades, Lake Baikal has experienced an increasing anthropogenic load. Global climate change (Shimaraev and Domysheva, 2013) and anthropogenic impact on the Baikal ecosystem in recent years have contributed to the obvious changes in the structure of benthic communities from the coastal zone: abundant growth of filamentous algae, especially in places with high anthropogenic load; the mass development of cosmopolitan small-cell algae that are untypical for the lake and inhabit eutrophic water bodies; and, at the same time, a decrease in the number of endemic phytoplankton species (Kravtsova et al., 2012; 2014; Timoshkin et al., 2016; Bondarenko and Logacheva, 2017). In addition, since 2011 there has been a mass disease and mortality of sponges (Bormotov, 2012). Several types of disease in the Baikal sponges were described, all of which are characteristic of *Lubomirskia baikalensis*: bleaching, necrosis, violet cyanobacterial biofilm, and brown spots (Timoshkin et al., 2016; Khanaev et al., 2018). Notably, sponge disease is a well-known worldwide problem, and since 1983 the sponge disease outbreaks have been reported in a wide range of geographic locations, however, no diseases have been reported in freshwater sponge

populations before (Webster, 2007). The understanding of the origin and causative agents of sponge disease is still insufficient. Some works indicated potential bacterial pathogens in sponges, however, most studies showed only shift in the microbial community of diseased sponges (dysbiosis) in comparison with healthy individuals (Webster, 2007; Belikov et al., 2018). To our knowledge, no viral putative pathogens have been found in diseased sponges.

The aim of this work was the study of the genetic diversity of DNA viruses in the associated community of the endemic Baikal sponge *Lubomirskia baikalensis* with signs of disease using metagenomic analysis. Viruses do not have universal genes, like the ribosomal genes of pro- and eukaryotes; therefore, the metagenomic analysis is still the most informative way to study the diversity of viral communities in natural samples. As far as we know, metagenomic studies of viruses in freshwater sponges have not been carried out previously.

2. Materials and methods

Sampling, obtaining the viral fraction and DNA extraction

The *Lubomirskia baikalensis* sponges were sampled using lightweight diving equipment in March 2015 in the Maloye More Strait of Lake Baikal, near the Malye Olkhonskiye Vorota Strait. Two branches of 2-3 cm³ in volume were collected from the same sponge. One branch looked healthy (sample Sv3h), and another had lesions in the form of brown spots (sample Sv3d) (Fig. 1). The sponge samples were washed twice in sterile water, frozen and transported to the laboratory. To obtain the fraction of virus-like particles, the samples were homogenized and centrifuged (3000 rpm, 30 min); the aqueous fraction was passed through a syringe filter with a pore size of 0.2 µm (Sartorius). Then, the samples were treated with DNase I and RNase A enzymes (Thermo Fisher Scientific) to remove contaminating nucleic acids. Viral DNA was extracted by the method with proteinase K, SDS and phenol-chloroform extraction (Sambrook et al., 1989).



Fig. 1. Diseased sponge *L. baikalensis* with brown spots lesions

Library preparation and sequencing

The total viral DNA was sheared in a microTUBE AFA Fiber Snap-cap using a Covaris S2 instrument (Covaris) with a medium size distribution of fragments of about 500 bp. The paired-end libraries were prepared using a NEBNext Ultra DNA library prep kit for Illumina (NEB). Sequencing of the virome was conducted on a MiSeq genome sequencer (2x300cycles, Illumina) in SB RAS Genomics Core Facility (ICBFM SB RAS, Novosibirsk, Russia).

Analysis of virome datasets

The primary processing of the received data (paired reads of 2x300 bp) was performed using the R package “ShortReads” (Morgan et al., 2009). The sequences of less than 100 nucleotides were excluded before the next analysis.

Taxonomic identification of viral sequences was performed using the BLASTn algorithm (Altschul et al., 1990) against NCBI RefSeq complete viral genomes database (September 2018 release) (Pruitt et al., 2005). The BLASTn parameters used were as follows: cost to open a gap, two; cost to extend a gap, one; word size for word finder algorithm, twelve; penalty for a nucleotide mismatch, one; reward for a nucleotide match, one. The sequence reads were considered ‘identified’ if it had a relative in the reference database with an e-value of $< 10^{-5}$ and bit score ≥ 50 . The results of BLASTn analysis were saved as a hit table. BLAST hits corresponding to the same viral genome subject ID were considered to belong to one virotype. Each subject ID from the BLASTn hit table was converted to a taxonomic annotation of the virus for a tabulated representation of the various virotypes in the sample. For further analysis, data on the representativeness of virotypes (number of reads per virotype in a sample) were normalized to the average number of reads per sample.

Rarefaction analysis was performed to assess the species richness in the samples (Heck et al., 1975). The representativeness of virotypes was used to calculate Shannon and Simpson indices of community biodiversity (Hill, 1973). Comparison of samples was carried out by cluster UPGMA method using the Bray-Curtis (Faith et al., 1987) and the Gower distances (Gower and Legendre, 1986). The clustering accuracy was estimated using the bootstrap method (1000 replicas). Before clustering, the representativeness of virotypes in samples was transformed into relative values (number of reads per virotype in a sample divided by total viral reads). The reliability of the difference between the distributions of virotypes representativeness in the compared communities was estimated using the chi square test. Statistical calculations were performed using the R packages “vegan” (Dixon, 2003) and “pvclust” (Suzuki and Shimodaira, 2006).

For a comparative analysis, other metaviromic datasets were used in this study: from the marine sponge *Rhopaloeides odorabile* (Great Barrier Reef (GBR), Davis Reef, sampled in January 2014; Laffy et al., 2016), from corals *Acropora millepora* (GBR, Orpheus Island Reef, sampled in March 2013) and *Pocillopora damicornis*

(GBR, Trunk Reef, November 2012; Weynberg et al., 2014). The reference data sets were also processed and analyzed according to the procedure described above.

3. Results

Identification of viral sequences in sponge viromes

After processing and filtering of raw paired reads, 310 080 high-quality sequences remained for the sample Sv3h and 327 901 – for the sample Sv3d. The total number of sequences similar to the genomes of viruses in the RefSeq database for the samples of the sponge *Lubomirskia baikalensis* Sv3h and Sv3d was 6903 and 13432, respectively. This averaged to 2.23% and 4.1% of the total data sets, which is comparable with the analysis of reference metagenomic data sets (2.79%, 3.35% and 4.18% for samples of *P. damicornis*, *R. odorabile* and *A. millepora*, respectively).

The majority of the sequences in virome datasets from *L. baikalensis* were similar to double-stranded DNA (dsDNA) viruses, which is attributed to the method for preparing libraries for the MiSeq platform (Illumina), in which dsDNA has a significant advantage at the amplification stage (Kim and Bae, 2011). Thus, the proportion of single-stranded DNA (ssDNA) viruses did not exceed 0.8%. We also detected a small number of RNA viruses (0.16% and 0.54% in the Sv3h and Sv3d samples, respectively), among which there were not only reverse-transcribing viruses of the *Retroviridae* but also those from other families. We cannot explain the presence of reads similar to viral RNA, since we constructed and sequenced the libraries of total viral DNA. Further, we did not consider the sequences similar to RNA viruses.

Taxonomic diversity of viral communities

In this study, we have identified 259 and 293 virotypes in samples from the Baikal sponge *Lubomirskia baikalensis* (for the samples Sv3h and Sv3d, respectively), which belong to sixteen viral families (Fig. 2). The families *Siphoviridae*, *Myoviridae*, *Podoviridae*, *Phycodnaviridae*, *Poxviridae*, and *Mimiviridae* were the most numerous (represented by more than 1% of the sequences and accounted for approximately 97% of virome reads). The significant parts of viromes (19.1% and 15.1% in the samples Sv3h and Sv3d, respectively) were unclassified to the family rank viruses (Fig. 2).

Bacteriophages (the families *Siphoviridae*, *Myoviridae* and *Podoviridae*) had the largest proportion in viromes of *L. baikalensis*, which was expectable considering the large abundance of bacteria in the sponge holobionts (up to 35% of the total sponge biomass at densities exceeding 10^9 microbial cells per cubic centimetre of sponge tissue) (Hentschel et al., 2012). In the list of potential hosts for detected bacteriophages, the representatives of the phyla *Proteobacteria* prevailed (data not shown). As known, the members of the phyla *Proteobacteria* (especially, of the classes *Alpha*-, *Gamma*- and *Deltaproteobacteria*) are well-represented in the highly diverse sponge microbial

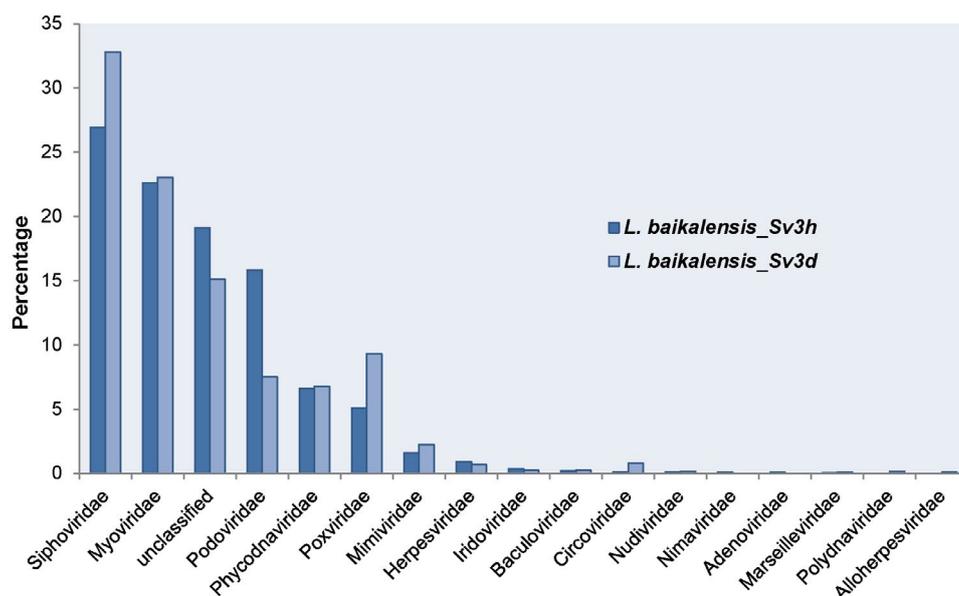


Fig. 2. Percentage of different viral families in viromes of *Lubomirskia baikalensis*

communities (Hentschel et al., 2012). In our study, the *Alphaproteobacteria virus phiJ1001*, which infects a novel marine bacterium isolated from the marine sponge *Ircinia strobilina* (Lohr et al., 2005), has been detected among the identified virotypes. In addition, a great number of sequences were unexpectedly related to unclassified *Idiomarinaceae phage 1N2-2*. The *Idiomarinaceae* is a family within the order *Alteromonadales* of the class *Gammaproteobacteria*. Members of this family have been isolated from saline habitats, such as coastal and oceanic waters, solar salterns, submarine hydrothermal fluids and inland hypersaline wetlands (Albuquerque and da Costa, 2014). The *Synechococcus phage S-CBP4* and *Prochlorococcus phage P-SSM2* (*Podoviridae* and *Myoviridae* families, respectively) were also ones of the dominant (more than 1% of reads) putative bacteriophages in the Baikal sponge viromes.

Among the most abundant virotypes, there were also some eukaryotic viruses: viruses of microalgae *Chrysochromulina ericina virus* and *Emiliania huxleyi virus 86* (the family *Phycodnaviridae*), the viruses of protozoa *Yellowstone lake mimivirus*, *Megavirus chiliensis* (*Mimiviridae*/*Megaviridae*) and *Cedratvirus A11* (unclassified, putative *Pithoviridae* family), as well as *BeAn 58058 virus* (*Poxviridae*), and *Yellowstone Lake virophage 5* (unclassified).

A comparative analysis of the diseased and the visually healthy sponge branches of *L. baikalensis* revealed that the composition of the most numerous families in two samples of the sponge was the same, but their percentage differed (Fig. 2). For example, the families *Siphoviridae*, *Myoviridae*, *Phycodnaviridae*, *Poxviridae*, and *Mimiviridae* were more abundant in the sample from the diseased branch of the sponge, and the families *Podoviridae*, *Iridoviridae* and *Herpesviridae* prevailed in the healthy one. Representatives of the families *Nimaviridae* and *Adenoviridae* were found only in the sample Sv3h, while *Polydnaviridae* and *Allohpesviridae* were found only in Sv3d, but the number of reads for these families was low (less than 22). In general, the viromes of the visually healthy and

diseased branches of *L. baikalensis* were significantly different (P value < 0.01).

It should be noted that the number of reads was insufficient to estimate the total viral diversity in the samples of *L. baikalensis*, since the rarefaction curves in our analysis did not reach the plateau (data not shown). Thus, we estimated mainly only dominant sponge-associated viruses. In the future, it is necessary to carry out a deeper sequencing of virome libraries from the existing and/or new samples of the Baikal sponges.

Comparative analysis of sponge and coral viromes

Figure 3 shows the proportions of viral families and unclassified at the rank of family viruses in the metagenomes of the Baikal sponge, marine sponge and corals. Tailed bacteriophages of the order *Caudovirales* (*Myoviridae*, *Siphoviridae* and *Podoviridae* families) dominated all datasets, but the distribution of these taxa differed between holobionts. Viral metagenome of *Rhopaloeides odorabile* was obvious for a greater number of the *Myoviridae* and vice versa – for a less number of the *Siphoviridae* in comparison with other metagenomes. The *Podoviridae* were the most numerous in the health branch of *Lubomirskia baikalensis* (Sv3h), as well as in corals *Acropora millepora* and *Pocillopora damicornis*. The families *Phycodnaviridae*, *Poxviridae*, *Mimiviridae*, and *Herpesviridae* also dominated all datasets, comprising 95-98% of reads together with tailed bacteriophages and unclassified viruses. The viromes of sponges differed from corals by a greater number of *Phycodnaviridae* and *Mimiviridae*. Unclassified viruses prevailed in *L. baikalensis* (Sv3h) and in *A. millepora*. The *Herpesviridae* were the most abundant in the sponge *R. odorabile*. The ssDNA viruses of the family *Microviridae* lacked in *L. baikalensis* while in the coral *P. damicornis* they accounted for 2.8%. Variation between holobionts was also observed in composition and percentage of less numerous families (Fig. 3). The highest number of families (thirty) was detected in the marine sponge *R. odorabile*, which is most likely due to

the largest number of reads.

UPGMA cluster analysis (considering the dominant virotypes, accounted for 95% of data sets) revealed two reliable clusters both, by using the Bray-Curtis and the Gower distances. One cluster consisted of viral metagenomes from Baikal sponge, and other – from the marine sponge and corals (Fig. 4).

4. Discussion and conclusions

In this study, we investigated viral communities of diseased sponge *L. baikalensis* through metagenomics. Despite the small number of reads, diversity indices of viruses in the *L. baikalensis* sponge were comparable to those in marine holobionts (Table 1), where the number of reads was much higher. Thus, we were able to identify a high diversity of viruses in samples of the Baikal sponge, and our analysis indicates the appropriateness of the chosen protocols for the isolation of VLPs and preparation of sponge viral DNA libraries for further studies of viral communities in the Baikal sponges.

Three bacteriophage families of the order *Caudovirales*: *Siphoviridae*, *Myoviridae* and *Podoviridae* mostly represented the *L. baikalensis* virome. This was expectable considering the high abundance and diversity of their bacterial hosts in associated communities. These viruses normally predominate in viromes of the aquatic biomes. Metagenomic studies of marine sponges also indicated the dominance of tailed bacteriophages (Laffy et al., 2016; 2018). The families *Phycodnaviridae*, *Mimiviridae* and *Poxviridae* were also numerous. Members of *Phycodnaviridae* infect microalgae; *Mimiviridae* are the largest viruses that infect amoeba and other protozoa. The dominance of viruses of these families in the sponge hologenome is also expectable considering the presence of their hosts in the communities of the Baikal sponges. At the same time, Claverie et al. (2009) show circumstantial

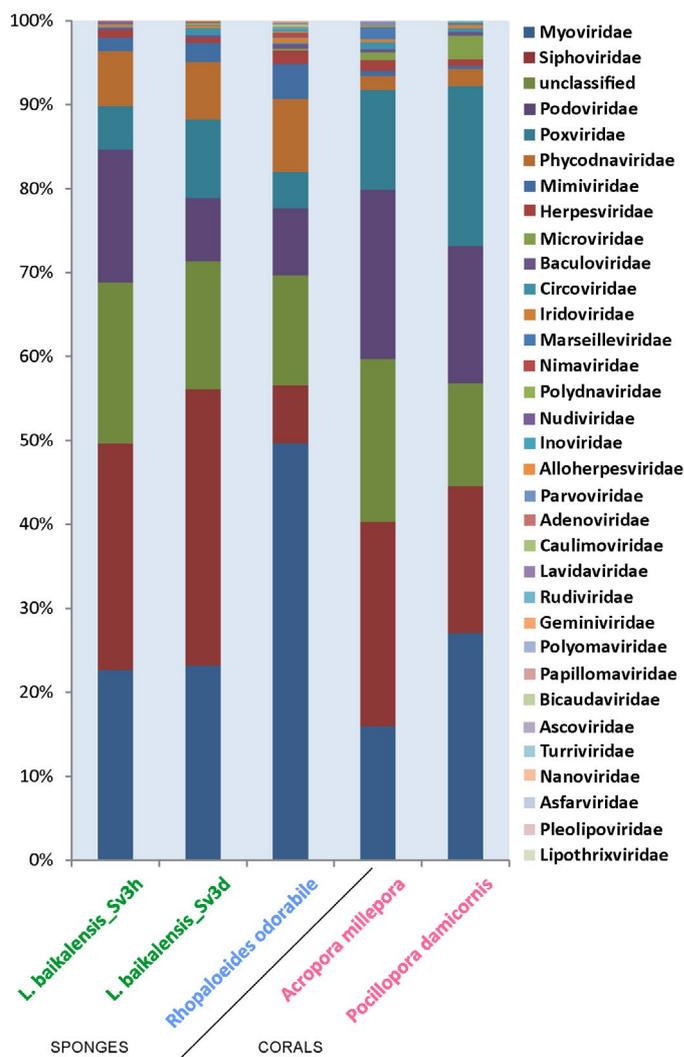


Fig. 3. The proportions of viral families and unclassified viruses in metaviromes of sponges and corals

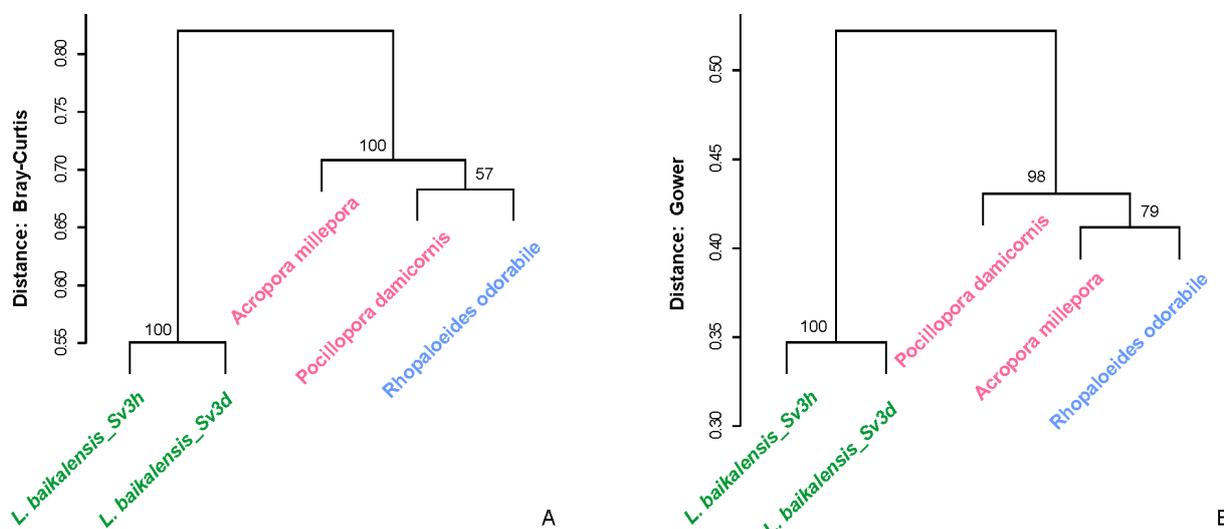


Fig. 4. UPGMA cluster analysis estimating the differences in diversity and composition of viromes performed using the Bray-Curtis (A) and the Gower (B) distances. The samples from the Baikal sponge are marked in green, and those from marine sponge and corals – in blue and pink, respectively

Table 1. Diversity indexes for the samples from sponges *L. baikalensis* and reference viromes

Samples	Alpha diversity	Shannon index	Simpson index
<i>L. baikalensis_Sv3h</i>	259	4,699	0,981
<i>L. baikalensis_Sv3d</i>	293	4,348	0,965
<i>R. odorabile</i>	697	4,378	0,955
<i>A. millepora</i>	520	4,588	0,973
<i>P. damicornis</i>	813	5,028	0,959

evidence of the possible mimivirus infection of some marine sponges (and corals).

It is worth noting that many viral families have a wide range of taxonomically distant hosts, and the list of species for the known viral families is constantly increasing. For example, known representatives of the heavily investigated family *Poxviridae* infect the widest host range among vertebrate and invertebrate taxa (Haller et al., 2014; Oliveira et al., 2017). Recently, new Salmon gill poxvirus (SGPV) has been found in the marine inhabitants (Gjessing et al., 2015). Like in our study, the sequences affiliated to *Poxviridae* were also detected in marine sponges *Amphimedon queenslandica* and *Ianthella basta* (Laffy et al., 2018). Therefore, it is most likely that the sequences of the sponge viromes similar to the known poxviruses of the terrestrial animals as well as other viruses of eukaryotes actually belong to unknown viruses that infect the aquatic invertebrates.

Despite the diversity of aquatic invertebrates, our knowledge of viral pathogens of this animal group including sponges is limited. Virus-like particles have been often identified without isolation of viruses and study of their pathogenesis in different species of invertebrates (Johnson, 1984; Munn, 2006). To date, several tens of aquatic invertebrates (mainly mollusks and crustaceans) viruses are known, and they are tentatively or more accurately assigned to the different families, including those found in the *L. baikalensis* sponge and other viromes: *Herpesviridae*, *Baculoviridae*, *Iridoviridae*, *Adenoviridae*, *Nimaviridae*, and *Coronaviridae*. In fact, previous studies have shown that herpes-like viruses are commonly observed in cnidarian viromes (Vega Thurber et al., 2008). It is suggested that herpes-like viruses infect all corals. Moreover, the sequences similar to *Herpesviridae* dominated samples of healthy corals but were less abundant in stressed or diseased coral tissues (Vega Thurber et al., 2008; Soffer et al., 2014). Thus, herpes-like viruses, as well as the abovementioned viruses of other families, may be a part of stable health sponge holobionts. The sponges provide food and habitat for many small invertebrates (mollusks, polychaetes, crustaceans and others) (Wulff, 2006), and those viruses could also be present in sponge viromes.

The diversity of a number of viral families belonging to the Nucleo-cytoplasmic large DNA viruses (NCLDVs) group was found in the sponge *L. baikalensis* and reference viromes, as well as in other studies of marine sponges (Laffy et al., 2016; 2018). NCLDVs is a

monophyletic group of eukaryotic viruses with a large double-stranded DNA genome ranging from 100 kb to 1.26 Mb (Yutin and Koonin, 2012). This group consists of seven families, five of which were found in the viromes *L. baikalensis*: *Iridoviridae*, *Marseilleviridae*, *Mimiviridae*, *Phycodnaviridae*, and *Poxviridae*. NCLDVs are known to have important roles in marine ecosystems (Hingamp et al., 2013) and most likely no less significant in sponges and other holobionts (Vega Thurber et al., 2008). Sequences similar to NCLDVs, including members of the *Phycodnaviridae*, *Poxviridae* and *Mimiviridae* were relatively more abundant in bleached coral tissues than in non-bleached ones (Soffer et al., 2014).

Mass disease of the Baikal sponges, throughout the entire water area of the lake, covering not only in the recreational but also in relatively favourable areas (Khanaev et al., 2018), indicates the infectious nature of the disease. However, the bacterial pathogens in the Baikal sponges have not been detected yet. As noted above, the viral pathogens of worldwide marine sponge diseases are also unknown and, unfortunately, the number of investigations in this field is the fewest. On the contrary, some viruses are known to be potential causative agents of coral diseases. It was suggested that eukaryotic circular Rep-encoding single-stranded DNA (CRESS-DNA) viruses (particularly *Circoviridae* and *Nanoviridae*), and their associated satellites may be responsible for white plague-like diseases of tropical corals (Soffer et al., 2014). Some sequences similar to circoviruses were also detected in the Baikal sponge viromes, but as mentioned above these ssDNA viruses were, probably, underestimated in our study. Therefore, the role of CRESS-DNA, as well as NCLDVs viruses in sponge holobionts, remains to be revealed in further examination of DNA-viruses (including ssDNA) in a number of diseased and healthy (without signs of disease) sponges.

Diseases of sponge and other holobionts are a result of stress due to the environmental perturbations and are accompanied by dysbiosis that is the substantial shifts in taxonomic and functional diversity of microbial communities (Pita et al., 2018). In this study, we analyzed the sponge with the signs of disease and, most likely, this individual also had dysbiosis and corresponding changes in abundances and composition of viral communities especially in the affected branch. This may explain a significant difference of viromes from the affected and visually healthy tissues of the *L. baikalensis* sponge. As a rule, the diversity of microbiota including viruses in diseased sponges is higher in

comparison with the stable healthy holobiont (Pita et al., 2018), and in our study the number of virotypes in the diseased branch was a little more than in the visually healthy one.

All sponge- and coral-associated viral communities were significantly different in our analysis (P value <0.01); in addition, the freshwater viromes were distant from the marine ones (Fig. 4). This is consistent with the data reported previously, where the viruses associated with sponges were species specific (Laffy et al., 2018), and the environmental conditions affected the formation of viral communities (Batista et al., 2018). It should be noted that the methods of sample preparation influence on the results of metagenomic analysis; hence, some proportion of differences between the viromes from the Baikal and marine holobionts may be a consequence of this fact.

Therefore, the metagenomic analysis of the viral community from the Baikal sponges have revealed for the first time a high genetic and taxonomic diversity of viruses in the associated communities of the *L. baikalensis* sponges, and demonstrated the differences in viral communities of visually healthy and diseased branches of the sponge. In general, our study broadens the understanding of the nature of symbiotic communities in freshwater organisms. Future investigations are necessary to assess the diversity and functions of viral communities in different species of the Baikal sponges; understand the role of viruses in mass disease and mortality of sponges; and trace the dynamics of viral communities in the course of the disease until adaptation of sponge holobionts and the onset of a new stable equilibrium state.

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