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Brown Rot Syndrome and Changes in the Bacterial Community of the Baikal Sponge *Lubomirskia baicalensis*

Nina V. Kulakova, Maria V. Sakirko, Renat V. Adelshin, Igor V. Khanaev, Ivan A. Nebesnykh, Thierry Pérez

Abstract Mass mortality events have led to a collapse of the sponge fauna of Lake Baikal. We describe a new Brown Rot Syndrome affecting the endemic species *Lubomirskia baicalensis*. The main symptoms are the appearance of brown patches at the sponge surface, necrosis, and cyanobacterial fouling. 16S rRNA gene sequencing was used to characterize the bacterial community of healthy *versus* diseased sponges, in order to identify putative pathogens. The relative abundance of 89 eubacterial OTUs out of 340 detected has significantly changed between healthy and diseased groups. This can be explained by the depletion of host-specific prokaryotes and by the appearance and proliferation of disease-specific OTUs. In diseased sponges, the most represented OTUs belong to the families Oscillatoriaceae, Cytophagaceae, Flavobacteriaceae, Chitinophagaceae, Sphingobacteriaceae, Burkholderiaceae, Rhodobacteraceae, Comamonadaceae, Oxalobacteraceae, and Xanthomonadaceae. Although these families may contain pathogenic agents, the primary causes of changes in the sponge bacterial community and their relationship with Brown Rot Syndrome remain unclear. A better understanding of this ecological crisis will thus require a more integrative approach.

Keywords Disease outbreak, Mass mortality, Porifera, Brown Rot Syndrome, Opportunistic pathogens, Freshwater

Introduction

Sponges (Porifera) are ancient Metazoa inhabiting marine and freshwater environments. They have a wide array of functional roles which make them keystone components of benthic-pelagic couplings, filtering ambient water, feeding on picoplanktonic and nanoplanktonic preys together with suspended detritus, and thus recycling organic matter into mineral material. They may also contribute to primary production and excrete secondary metabolites, thus acting on complex and poorly known networks of biotic interactions [1–4]. Sponges can host a huge diversity of symbiotic microorganisms, including viruses, archaea, eubacteria, fungi, and protista [5]. In high microbial abundance (HMA) species, microorganisms can represent up to 40% of the sponge biomass [6] and show great taxonomic diversity, with up to 47 bacterial or candidate phyla recorded so far [7–9]. Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Proteobacteria (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria), Verrucomicrobia, and Nitrospira phyla are the most common both in marine [4, 10] and freshwater sponges [11–14]. Sponge disease outbreaks or mass mortality events have been reported for more than a century, with more than 20 events studied, sometimes affecting several species and large areas [4, 15–28]. However, reports and investigations on the conditions of freshwater sponge

disease outbreaks are rare. The most commonly observed symptoms are bleaching, necrosis, and the development of filamentous cyanobacteria overgrowing the sponge surface. Such diseases are widely distributed in the Caribbean region, the Great Barrier Reef, the Indo-Pacific, and the Mediterranean [4, 29], and in some cases, they have severely affected population densities [30–38]. The causal relationships are poorly known. Global warming and extreme thermal events are considered to be the most important environmental contexts favoring the emergence of pathogens or the expression of their virulence [33, 36, 37]. Infectious agents have often been considered to be the main factors triggering mass mortalities [15, 16, 18, 38, 39], although the pathogens responsible have rarely been identified. Several studies have hypothesized on the putative role of viruses, fungi, cyanobacteria, Alphaproteobacteria, Gammaproteobacteria, Epsilonproteobacteria, Firmicutes, and Bacteroidetes [16, 19, 34, 40–47]. However, there is only one case in which the fulfillment of Koch's Postulate has enabled the identification of a new Alphaproteobacteria, *Pseudoalteromonas agarivorans*, as the pathogen behind the disease of *Rhopaloeides odorabile* [38, 48].

Freshwater sponges (order Spongillida) include both cosmopolitan species living in lakes and rivers and endemic species. Compared to other freshwater systems, Lake Baikal harbors a highly diverse and abundant sponge fauna which belongs to two families, Spongillidae with 5 species and Lubomirskiidae with 13 valid species to date [49]. In the lake, species diversity varies with depth, light, and availability of solid (stone, rock) substrates. Most Baikal sponges host eukaryotic photosymbionts which may favor their high occurrence in the photic zone [49, 50]. *Lubomirskia baicalensis* (Pallas, 1771) is the most common and emblematic sponge species of Lake Baikal [49]. This endemic species presents variable growth morphologies, and its branching forms—which measure up to 1.5 m high—shape the underwater scape, in particular between 10- and 20-m depths where their maximal biomass has been recorded [50, 51]. The first observations of a disease-like syndrome in *L. baicalensis* sponges were recorded in Central Baikal in 2010 (Khanaev, personal video records) and in 2011 [52]. Over the past 6 years, diseased sponges have been found along the near-shore zone from South to North Baikal. The most obvious signs of disease are sponge surfaces covered with reddish colored filamentous cyanobacteria, oscule deformation [53], and bleaching [54]. This disease outbreak developed into a sponge mass mortality in 2013. The most visible effects concerned the branching forms of *L. baicalensis* with, in this case, a disease occurrence affecting between 30 and 100% in the Southern Basin of Lake Baikal [55].

This study focuses on individuals of *L. baicalensis* presenting the same syndrome of dark brown necrotic patches. Sponge-associated bacterial communities were studied using deep sequencing of the 16S ribosomal RNA gene in order to identify microbial taxa associated with diseased sponges, and thus provide initial clues about the mechanisms behind the outbreak.

Materials and Methods

Sponge and Water Sampling

Healthy and diseased individuals (Fig. 1) of *L. baicalensis* were collected by scuba diving during a field trip conducted in May–June 2015.

Sponges were collected at three sites along the littoral zone of Lake Baikal: 51° 51' 46" N, 104° 50' 51" E (site L); 53° 01' 03" N, 106° 55' 47" E (site OV); 55° 17' 16" N, 109° 45' 31" E (site T) (Fig.2). The population of sponges at these sites was well represented by *L. baicalensis*. Sponge samples were

divided into healthy ($n = 11$) and diseased ($n = 11$) groups, based on absence or presence of dark brown patches (Table 1). Samples were obtained from individual sponges, with the exception of samples T1H and T5D, which consisted of healthy and diseased parts of the same individual. Pieces of sponges, measuring 4–8 cm long, were collected by divers, placed in 50-mL tubes filled with sterile water, lifted to the ship, and gently washed three times with sterile water. Each sample was photographed, divided into pieces, and frozen at $-20\text{ }^{\circ}\text{C}$ before DNA extraction. Extraction of DNA was performed within 2 months following the expedition. Additionally, pieces of samples were stored in 70% ethanol at room temperature for morphology identification. Sponge species were identified based on morphological characteristics of skeletons and spicules, according to the guidelines of Rezvoi [56] and Efremova [15].

Ambient water samples (200 mL per site) were collected and filtered through a sterile filter (Minisart NML, Sartorius) with a $0.22\text{-}\mu\text{m}$ pore size. Filters were stored at $-20\text{ }^{\circ}\text{C}$ before DNA extraction. Extracts of DNA from each filter from different locations were mixed in one sample in order to optimize our chances of obtaining good amplification. According to Mikhailov et al. [57], at this season, the bacterioplankton composition is rather homogenous across the three different basins of Lake Baikal.

Chemical Water Analysis

Water samples (1.5 L) were collected in each site at 5–15- and 15–30-m depths, to measure pH, temperature, nutrients, and dissolved oxygen using standard methods described in Semenov [58], Stroganov and Buzinova [59], and Wetzel and Likens [60]. Results were compared to reference values obtained after long-term monitoring in Lake Baikal [61–64].

454 Pyrosequencing of Bacterial 16S rRNA Genes

The genomic DNA was extracted from 30 to 50 mg of sponge tissue and water filters using TRIzol LS reagent, following the manufacturer's instructions (Invitrogen, Ambion, USA). In total, 22 sponge samples and one water sample were used for 454 sequencing.

For each sample, the hypervariable region V4–V6 of the 16S rRNA gene was amplified by PCR with bacterial primers 518F and 1064R [65], incorporated into the forward primer A (Lib-L) with sample-specific MID tags at the 5' end and reverse primer B, respectively, as described in the Guidelines for Amplicon Experimental Design (April 2014) for the GS FLX Titanium 454 Sequencing System. The three replicates of the 15- μL PCR mixture contained 1 $\times$ Tersus buffer, 1 $\times$ high-fidelity polymerase Tersus, 2.5 M Mg^{2+} , 0.2 mM of each dNTP (Tersus PCR kit, Evrogen, Russia), 10 pmol of forward and reverse primers, and 50 ng DNA. Negative (no template) controls were used in the PCR with each MID. The PCR conditions included an initial denaturation step at $96\text{ }^{\circ}\text{C}$ for 3 min; 30 cycles of $94\text{ }^{\circ}\text{C}$ for 20 s, $55\text{ }^{\circ}\text{C}$ for 20 s, and $72\text{ }^{\circ}\text{C}$ for 1 min; and a final extension at $72\text{ }^{\circ}\text{C}$ for 5 min, using a DNA Engine Dyad Thermal Cycler (MJ Research, USA). Products from triplicate PCR reactions were combined, and 25 μL of the mix was cleaned to remove products smaller than 300 bp, using a SeqCap Pure Capture Bead Kit (Roche). The DNA pool was diluted in 10 mM Tris-EDTA to a final concentration of 1×10^6 molecules/ μL , and one molecule per bead was used in emulsion PCR.

Amplicons were unidirectionally sequenced (two runs for all samples) using a Roche Genome Sequencer, the GS Junior System. The GS Run Browser 3.0 (Roche) was used for primary data

processing, and the script Extended MIDConfig.parse (Roche) was used for automated sorting of MID-containing reads. The number of reads was normalized to 4500 reads per sample. Sequences were trimmed, quality-controlled, and aligned using Mothur [66]. Pipeline and SeqClean software image processing and signal calling were performed using the Roche amplicon-processing pipeline (version 2.53) with a recursive phase correction algorithm to maximize the number of long reads. The ChimeraSlayer algorithm [67] inside a Mothur package was used to eliminate chimeras, and singletons were discarded. The Silva database [68], with the alignment, taxonomy, and operational taxonomic units (OTUs) assigned according to Greengenes repository [69], was used as a template for annotation of input sequences. Grouping of input data with $\geq 97\%$ identity threshold was implemented by a greedy complete-linkage clustering following the recommendations of He et al. [70]. Unclassified reads and chloroplast sequences were removed from downstream analysis. The percentage values of sequence reads in groups of healthy and dis-eased sponges were used to analyze differences between sponge-associated bacterial communities. Nucleotide sequences were submitted to the NCBI Sequence Read Archive SRP073411.

Statistical Analysis

The Shannon biodiversity index and species richness estimates Chao1 and ACE were calculated from canonical formulas, as documented in the Mothur manual [66]. The principal component analysis and the Mann-Whitney test were performed using XLSTAT 2016. Between-group comparisons of bacteria prevalence in healthy and diseased *L. baicalensis* sponges (at 97% of homology) were calculated using the U-criterion of the two-tailed Mann-Whitney test; values of $p < 0.05$ were considered significant. Actual p values can be found in Supplementary S1. Standard deviation of the mean was used for data presentation.

Results

Disease Occurrence

Diseased *L. baicalensis* were found at all sampling sites between 3 and 30 m. Dark brown patches were the most common external signs that we thereafter defined as Brown Rot Syndrome (BRS). Bleaching sponges were found only occasionally. Patches could cover parts of the sponge surface or entire individuals. They usually led to necrosis, collapse of the tissue, and death. Filamentous cyanobacteria covered the affected branches, giving them a brownish-pink color. The incidence disease on sponge populations was variable between sites, from a few scattered diseased individuals (site T) to extensive areas with dozens of decaying sponges (sites L and OV).

Chemical Water Analysis

The obtained data on temperature, pH, and concentrations of biogenic elements generally corresponded to average back-ground values [62, 64], the exception being a drop in pH and an increase in N and P concentrations in the water layer near the bottom at site L (Table 2).

16S Taxonomic Richness and Bacterial Composition in Healthy and Diseased Sponges

The total number of reads of 16S rRNA gene was 125,560. The average sequence length of filtered reads was 524 ± 14 nucleotides. The Shannon diversity index was 2.5 ± 0.6 for the healthy sponge group and 3.5 ± 0.4 for the diseased sponge group (mean $\pm$ SD). Diversity indices for each sample are

given in Table 3. A total of 340 OTUs was detected among our sponge samples (Supplementary file S2). Rarefaction curves for each sample indicated that the major part of the sequence diversity was identified (Supplementary file S3). However, deeper sequencing would certainly allow to recover more minor OTUs. The PCA analysis on the relative OTU abundance based on the Pearson correlation matrix clearly separates the two groups: diseased and healthy sponges with samples of healthy tissue taken from diseased individuals grouped with healthy sponges (Fig. 3). In healthy sponges, the bacterial community appeared to be composed by 19 phyla: Crenarchaeota, Acidobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chlorobi, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Elusimicrobia, Firmicutes, Gemmatimonadetes, Lentisphaerae, Nitrospira, Planctomycetes, Fibrobacteres, Proteobacteria (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Epsilonbacteria), Spirochaetes, Verrucomicrobia, and five candidate phyla BD 1–5, OD1, OP10, TM6, and WCHB1–60. Two additional candidate phyla, SM2F11 and SR1, were found in diseased sponges. The most abundant bacterial phyla were Bacteroidetes and Proteobacteria and, to a lesser extent, Actinobacteria, Cyanobacteria, Planctomycetes, and Verrucomicrobia. The microbial community of the water sample differed from that obtained from sponge samples and was dominated by Actinobacteria (Fig. 4).

Significant changes were detected in the composition of the bacterial community of diseased sponges. Some taxa showing more than 5-fold increases in abundance belonged to Cyanobacteria, Bacteroidetes, Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. At the family level, members of Oscillatoriaceae, Cytophagaceae, Flavobacteriaceae, Chitinophagaceae, Sphingobacteriaceae, Burkholderiaceae, Rhodobacteraceae, Comamonadaceae, Oxalobacteraceae, Xanthomonadaceae, and Verrucomicrobiaceae were over-represented in diseased sponges. At the genus level, 19 genera and unclassified taxa were more abundant in diseased sponges (Fig. 5). In addition, other genera such as *Cytophaga*, *Phormidium*, *Bosea*, *Pseudorhodobacter*, *Luteolibacter*, and *Prostheco bacter* were recorded in low quantity (< 0.9%), but only in diseased sponges.

At the lowest taxonomic level (97% similarity), significant quantitative changes between groups of healthy and diseased sponges were highlighted by 89 bacterial OTUs (Supplementary Table S1). Among the changes detected at this level, the relative read abundance increased for 64 OTUs, whereas it decreased for 25 OTUs. In each genus, increased abundance was found in one-to-two closely related OTUs (96–96.9%) dominating bacterial communities of diseased sponges, and was actually absent—or found only in trace amounts—in healthy sponges or water samples.

Oscillatoriales cyanobacteria (Subsection III) are the most increased OTUs in diseased sponges. In particular, the abundance of the genera *Chamaesiphon* and *Limnothrix* and of an unclassified group increased by 5% in diseased sponges, whereas they accounted for only 0–0.1% of bacterial communities in healthy samples, and were totally absent in the water sample. Several other cyanobacterial OTUs belonging to genera *Leptolyngbya* and *Phormidium* also prevailed in the diseased sponge group. (*Sediminibacterium*, *Fabibacter*), candidate division OD1, Planctomycetes (CL500-3), Alphaproteobacteria (LD12), Betaproteobacteria (LD28, OM43, *Polynucleobacter*, *Limnohabitans*), Deltaproteobacteria (*Bacteriovorax*), and Verrucomicrobia (vadinH A64, *Candidatus* Methylacidiphilum).

Discussion

The results of chemical analysis showed that water parameters were in the range of the reference values; however, in site L some changes were detected in terms of pH and nutrient concentrations. In particular, a drop in pH from 7.9 to 7.2, along with increased concentrations of nitrate (1.4-fold), phosphate (1.5-fold), ammonium (from 0.005 to 0.56 mg/L), and nitrite ions (from 0.002 to 0.05 mg/L), was detected in the water sampled near the bottom. At this site, the sponge disease appeared concomitantly with a green algae bloom described by Kravtsova et al. [71, 72] and attributed to a local eutrophication. The relationship between the sponge BRS and this eutrophication event is unclear; however, the green algae proliferation significantly affected the sponge environment, the substrate and light availability, and the water quality, and thus likely disturbed sponge filter-feeding, growth, and reproduction ability.

The analysis of 16S rRNA gene of the bacterial community associated with sponges highlighted a more diverse microbial community in healthy *L. baicalensis* than in previously studied specimens [13, 14]. This might be explained by differences in sample preparation and amplification strategy. Under reference condition, the *L. baicalensis* bacterial community includes Crenarchaeota and 23 Eubacteria phyla and candidate phyla. The major bacterial phyla are Bacteroidetes, Proteobacteria, Actinobacteria, Verrucomicrobia, and Planctomycetes, which also dominate bacterial communities of other freshwater sponges such as *Ephydatia fluviatilis* [12], *Eunapius carteri*, and *Corvospongilla lapidosa* [73]. Moreover, Cyanobacteria and OD1 candidate phylum seem to be also very common in *L. baicalensis*.

Significant changes in composition of the bacterial community were detected in sponges with BRS. Species diversity appeared higher in the diseased sponge group, which seems to be a recurrent observation in marine sponges [22, 40, 74] and corals [75]. This higher diversity is likely due to the emergence of new opportunistic species benefiting from sponge loss of health and resulting tissue degradation. However, the change detected in the bacterial community of diseased sponges can also be explained by the depletion of several OTUs which are common in healthy specimens.

Previous studies have shown that uncultured Oscillatoriales are often associated with marine sponge and coral diseases [21, 26, 40, 76], these cyanobacteria being highly represented in diseased *L. baicalensis*. Some detailed investigations revealed several putative pathogens within this order. Species of the *Hormosilla* genus were involved in mangrove sponge disease (MSD) [48] and detected in necrosis of *Callyspongia* (*Euplaccella*) aff. *biru* [40]. The filament-forming *Limnothrix*, *Plectonema*, and *Leptolyngbya* were detected in sponge orange band disease [26]. The red-pigmented *Leptolyngbya* spp. appeared on lesions of the *Aplysina* red band syndrome [77], and some unclassified Oscillatoriales were also found forming a white veil on three dictyoceratid sponges [49]. The results obtained in this study show that Oscillatoriales are important components of a complex assemblage of opportunistic bacteria and can be used as a marker for BRS. However, a significant increase of their abundance is not necessarily equivalent to a demonstration of their precise role as a primary pathogenic agent. Further studies are thus required to prove their pathogenic function.

Cyanobacteria predominance has already been observed in bleached and diseased Baikal sponges [54, 78]. In these recent studies, *Synechococcus* spp. were shown to be highly abundant (> 80%) in diseased sponges. This contrasts with our findings which reveal that *Synechococcus* OTU (98–99%

similarity with *S. rubescens* AM709629 and *Synechococcus* sp. from bleached Baikal sponge JQ272733)—which is also common in lake water—was considerably less represented both in BRS (0.3%) and in healthy sponges (1.7%) (no significant difference was observed between the two sponge categories, $p = 0.22$; $U = 79.00$). However, the earlier reports listed above were based on the analysis of a single sponge individual, using clone library sequencing [54], so it is rather hard to believe that their findings accurately reflect the existing high variability between disease symptoms across Lake Baikal sites, sea-seasons, and years.

Analysis of nucleotide similarity in sequences that were substantially detected in diseased sponges revealed a variety of aerobic and anaerobic bacteria belonging to the families Rhodobacteraceae (e.g., 99% EU641680, EU376256), Sphingomonadaceae (98% KC157048), Burkholderiaceae (99% AB826334), Comamonadaceae (99% GU454909, KX771629), Nitrosomonadaceae (98% HQ595214), Xanthomonadaceae (99% EU64 068 2), and Verrucomicrobiaceae (98% AJ401106), which are known to be involved in the biodegradation of a wide range of carbohydrates and biopolymers [79–83]. Among them, some Rhodobacteraceae may be associated with sponge and coral diseases [4, 40, 42, 84]. Moreover, genera such as *Flavobacterium* (96% NR_112662), *Flexibacter* (80% KP997194) and *Acidovorax* (96% KF769125), which are known to be fish or plant pathogens [85–88], were recorded in abundance in diseased *L. baicalensis*.

All observed changes in the microbial community of diseased sponges have been attributed to three main observations: proliferation of common OTUs (12%), depletion of species-specific OTUs (7% of the bacterial community), and appearance of new OTUs (7%) that may be acquired from ambient water or present in minimal quantities.

Thus, the change in the bacterial community which has been detected in diseased *L. baicalensis* is undoubtedly much more complex than a simple case of emerging pathogens. Indeed, it appears to include a high number of opportunistic and proliferating bacterial species, some of which likely become pathogenic, leading to disease progression when they proliferate in the sponge body. The results of this study have allowed to detect the consequences of events that lead to changes in the sponge-associated bacterial community and the progression of BRS. The initial cause of BRS and of these changes in the bacterial community remains unclear, and a better understanding of the process will require greater inter-disciplinary as well as an integrative approach to detect the triggers of disease outbreak in Baikal sponges.

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Legends

Figures

Fig. 1 a Healthy sponge *Lubomirskia baicalensis* are often inhabited by crustacean *Brandtia parasitica*. b Sponge with signs of Brown Rot Syndrome

Fig. 2 Location of sampling sites in Lake Baikal

Fig. 3 Principal component analysis (PCA) based on Pearson correlation matrix of relative OTU abundance shows differences in bacterial communities. Sponge-associated bacterial communities of BRS and healthy sponges are clearly separated

Fig. 4 Composition of major eubacterial taxa in *L. baicalensis* and lake water assessed using 16S rRNA gene pyrosequencing. Relative abundances of taxa are expressed in percentage of total 16S rRNA gene reads. Bacteroidetes and Proteobacteria prevail in sponge-associated bacterial communities of healthy and BRS sponges whereas Actinobacteria dominate in the water sample. Sampling sites L, OV, and T; Healthy sponges H; Brown Rot Syndrome sponges BRS; Unaffected area of BRS sponge H*. The group “other” includes taxa that account for less than 1% of reads

Fig. 5 The most abundant bacterial taxa identified in BRS sponges. The mean values of relative abundance of 16S rRNA gene reads in OTUs were found significantly higher in the group of diseased sponges in comparison with the group of healthy sponges

Tables

Table 1 Sponge samples

Table 2 The water chemical properties in sampling sites

Table 3 Richness and diversity indices of the sponge-associated bacterial communities obtained from 16S rRNA gene pyrosequencing

Fig. 1

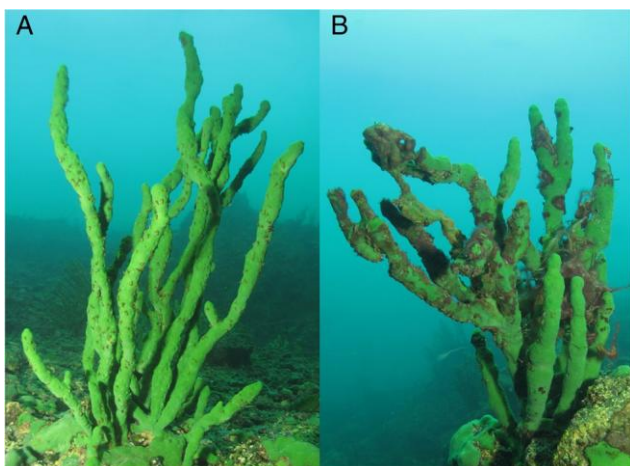


Fig. 2

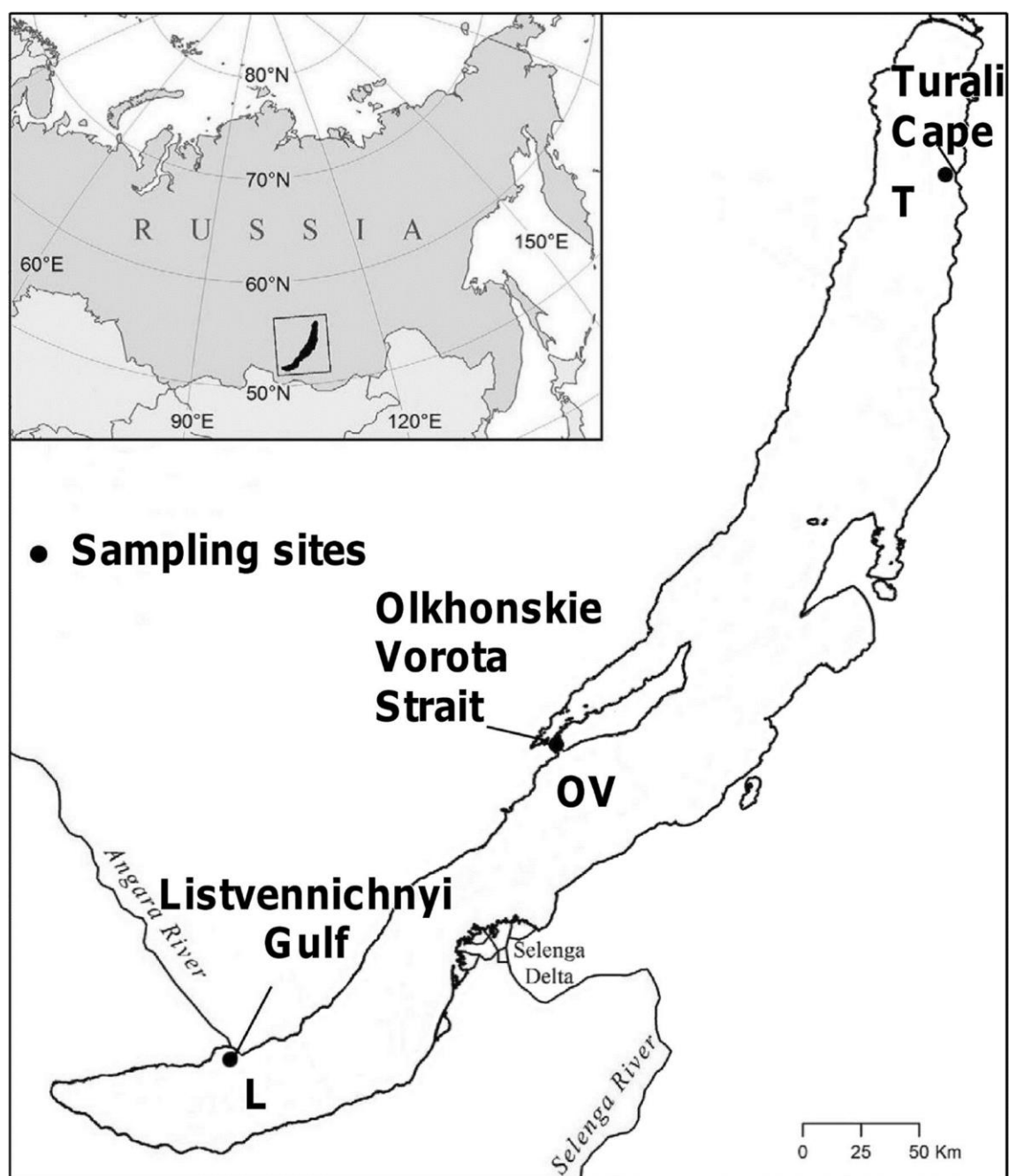


Fig. 3

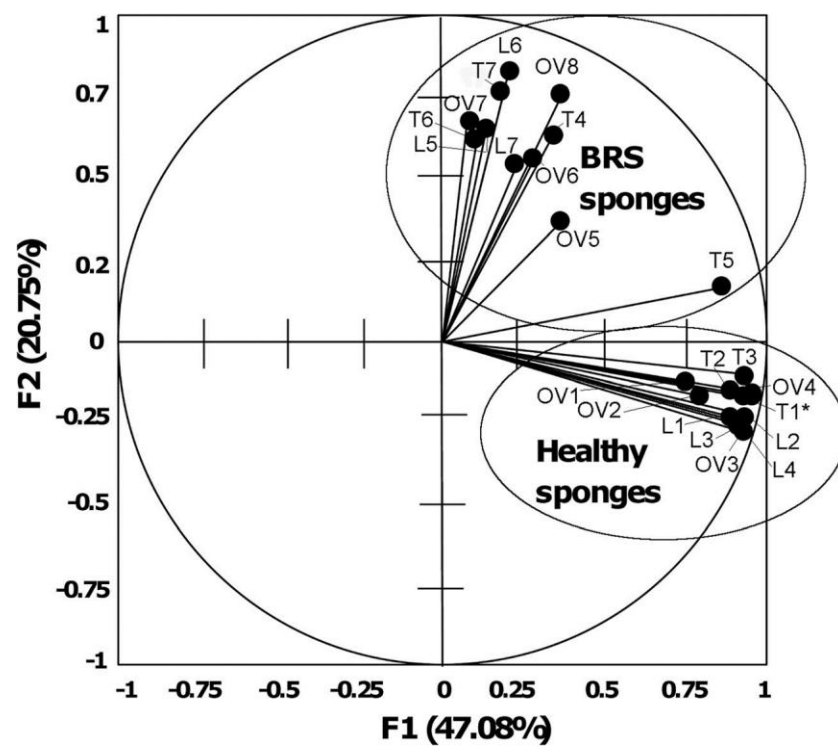


Fig. 4

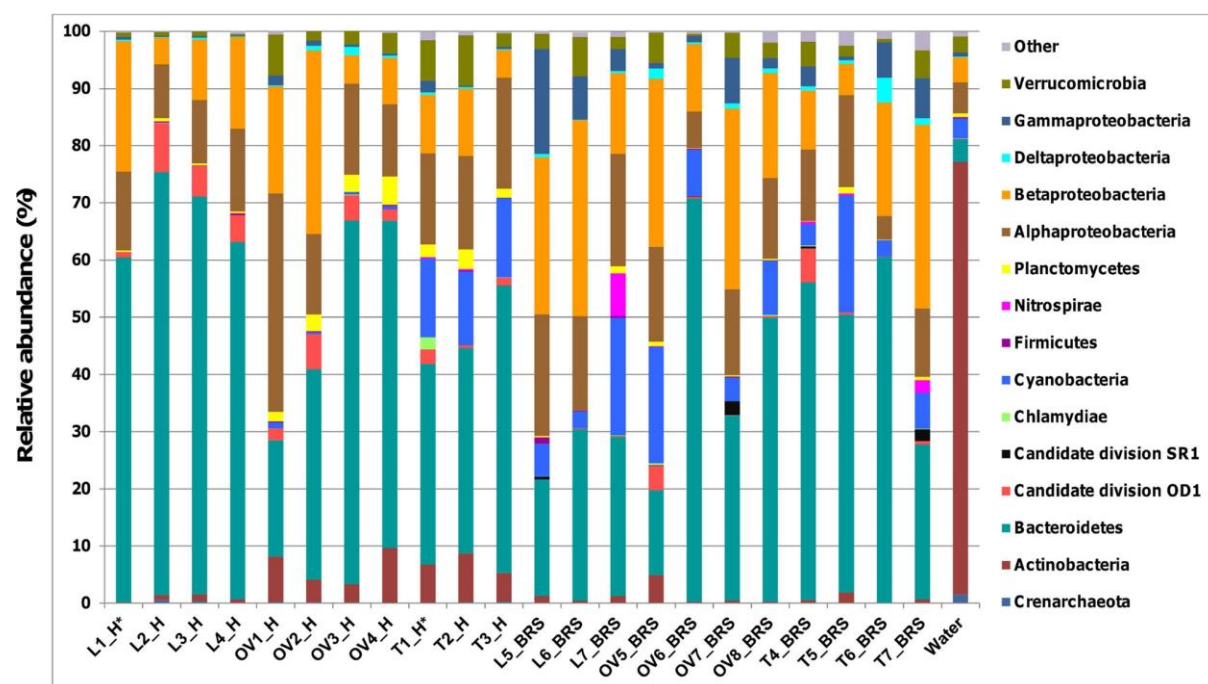


Fig. 5

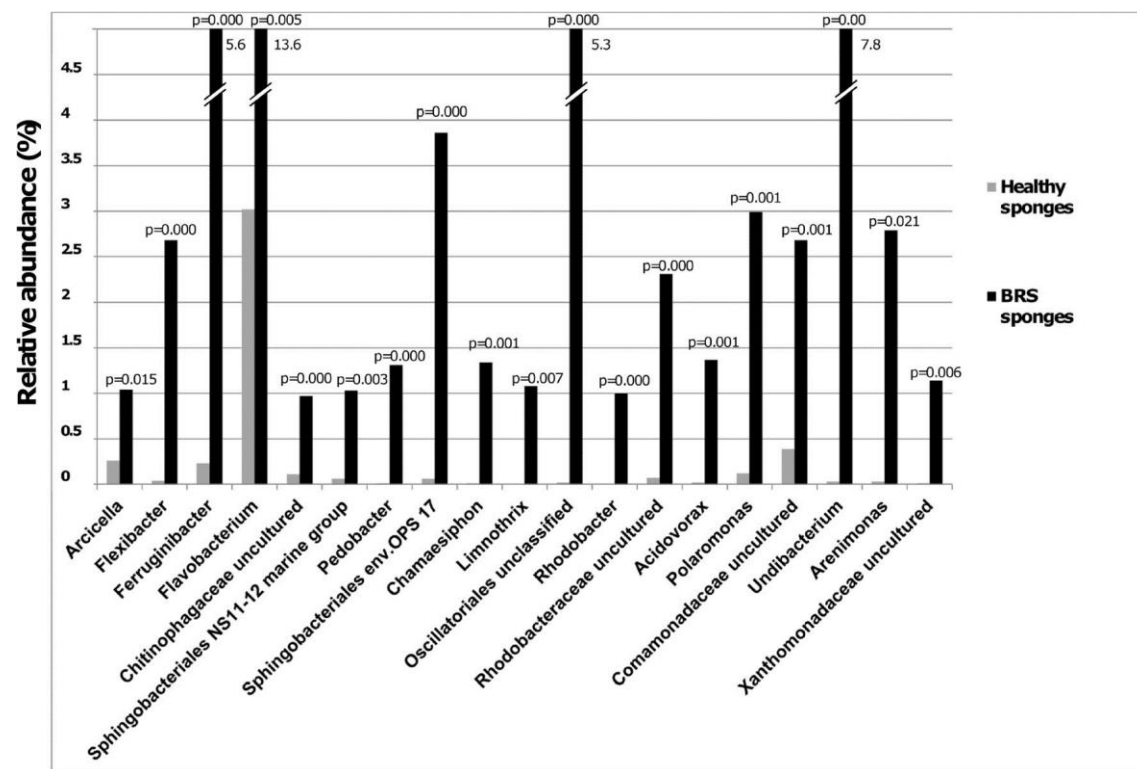


Table 1

Sample	Morph	Sampling sites	Status	Depth (m)
L1_H	Branched	Listvennichnyi Gulf	UAD	12
L2_H	Branched	Listvennichnyi Gulf	healthy	12
L3_H	Branched	Listvennichnyi Gulf	healthy	17
L4_H	Branched	Listvennichnyi Gulf	healthy	17
L5_BRS	Branched	Listvennichnyi Gulf	BRS	7
L6_BRS	Branched	Listvennichnyi Gulf	BRS	7
L7_BRS	Branched	Listvennichnyi Gulf	BRS	17
OV1_H	Branched	Olkhonskie Vorota Strait	healthy	11
OV2_H	Branched	Olkhonskie Vorota Strait	healthy	13
OV3_H	Branched	Olkhonskie Vorota Strait	healthy	13
OV4_H	Branched	Olkhonskie Vorota Strait	healthy	5
OV5_BRS	Branched	Olkhonskie Vorota Strait	BRS	11
OV6_BRS	Branched	Olkhonskie Vorota Strait	BRS	5
OV7_BRS	Branched	Olkhonskie Vorota Strait	BRS	10
OV8_BRS	Branched	Olkhonskie Vorota Strait	BRS	11
T1_H*	Encrusting	Turali Cape	UAD	5
T2_H	Branched	Turali Cape	healthy	20
T3_H	Branched	Turali Cape	healthy	20
T4_BRS	Encrusting	Turali Cape	BRS	5
T5_BRS	Branched	Turali Cape	BRS	20
T6_BRS	Branched	Turali Cape	BRS	20
T7_BRS	Branched	Turali Cape	BRS	20

UAD unaffected area of diseased individual, *BRS* Brown Rot Syndrome sponges

Table 2

Site	T (°C)	pH	O ₂ (mg/L)	NH ₄ ⁺ (mg/L)	NO ₂ ⁻ (mg/L)	NO ₃ ⁻ (mg/L)	Mineral P (mg/L)	Organic (mg/L)	Total P (mg/L)
L	3	7.8 ± 0.1	12.2 ± 0.3	0.030 ± 0.02	0.003 ± 0.00	0.400 ± 0.05	0.006 ± 0.00	0.006 ± 0.00	0.012 ± 0.00
L, near bottom, 5-m depth	3	7.2 ± 0.1	NA	0.560 ± 0.47	0.055 ± 0.01	0.690 ± 0.07	0.025 ± 0.03	0.024 ± 0.02	0.049 ± 0.05
OV	3	8.1 ± 0.1	13.9 ± 0.5	0.010 ± 0.00	0.020 ± 0.01	0.180 ± 0.04	0.005 ± 0.02	0.003 ± 0.00	0.008 ± 0.00
T	3	7.9 ± 0.0	12.7 ± 0.14	0.030 ± 0.00	0.003 ± 0.00	0.310 ± 0.01	0.008 ± 0.00	0.001 ± 0.00	0.009 ± 0.00
Background water, surface	3–6	7.9–8.3	9–14	< 0.005	< 0.002	0.50	0.016	NA	NA

NA not analyzed

Table 3

Samples, healthy sponges	L1_H	L2_H	L3_H	L4_H	OV1_H	OV2_H	OV3_H	OV4_H	T1_H*	T2_H	T3_H	Water
Chao 1	139.5	94.9	108.1	153	147.1	146.8	178.5	176.9	211.6	209.2	186.0	189.5
Shannon	2.0	1.6	1.7	1.8	3.2	2.6	2.1	2.6	3.4	3.2	2.9	2.5
ACE	161.6	109.1	112.8	163.2	173.3	161.9	198.9	207.8	221.7	262.7	196.1	190.8
OTUs at 0.03	119	87	95	89	101	109	121	126	169	136	155	144
Samples, BRS sponges	L5_ BRS	L6_ BRS	L7_ BRS	OV5_ BRS	OV6_ BRS	OV7_ BRS	OV8_ BRS	T4_ BRS	T5_ BRS	T6_ BRS	T7_ BRS	
Chao 1	142.7	155.9	157.0	162.6	177.1	224.0	209.7	212.4	273.5	187.1	293.1	
Shannon	3.7	3.4	3.5	3.5	2.9	3.4	3.9	3.1	3.7	3.1	4.2	
ACE	148.2	166.3	160.2	214.4	173.1	271.6	227.6	251.2	294.6	196.7	312.6	
OTUs at 0.03	142	151	148	132	109	171	172	149	252	151	254	