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Microbial community associated with Baicalian crustacea near gas-oil deepwater discharges

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ABSTRACT. Using scanning electron microscopy we investigated the epibiotic associations of peritrichial sessile ciliates of the *Lagenophrys* (Ciliophora: Lagenopryidae) and prokaryotes of various morphologies on the surface of endemic amphipods *Macropereiopus florii* (Dybowsky 1874) and ostracods *Candona* (Candonidae). We noted biofouling and mineralization in the areas of formation of a bacterial biofilm, which includes Fe, P, and other elements. High-throughput sequencing on the Illumina MiSeq platform showed the presence of such bacterial phyla as *Actinobacteria*, *Gracilibacteria*, *Cyanobacteria*, *Proteobacteria*, *Bacteroidetes* in the ostracod microbiome, of which the latter two were dominant. Sequence analysis of the 16S rRNA gene fragment showed epibiotic microorganisms that are related to the colorless sulfur bacteria *Thiothrix* sp. More than 50% of all sequences were attributed to endosymbiont bacteria of the genus *Rickettsia*, and the presence of endosymbionts of the genus *Ca. Cardinium* was shown for the first time for Baikal ostracods.

Keywords: epibiosys, methane seep, Baikal, crustacea, microbial ecology

Symbioses play a key role in life and evolution on our planet. New symbiotic associations of aquatic ecosystems can significantly expand the capabilities of partners involved in these relationships. This leads to important ecological changes in habitats, contributes to biodiversity and high productivity (Cavanaugh et al., 2005; Gast et al., 2009). In the zones affected by deep-sea hydrothermal springs and methane seeps, as well as in coastal zones saturated with organic matter, invertebrates live, which often are in symbiosis with chemolithoautotrophic bacteria. In such communities, animals associated with prokaryotes, or symbiotrophs, are dominant in the overwhelming majority of cases (Carman and Dobbs, 1997; Van Dover et al., 2002).

Epibiotic organisms, such as prokaryotes, protozoa, microscopic algae and fungi, can enter into mutually beneficial relationships with basibionts, but in most cases these associations are determined as commensalism. Nevertheless, some studies suggest an indirect effect of epibionts on their crustacean hosts (Wahl, 2008).

Methane and oil seeps, mud volcanoes and outcrops of highly mineralized fluids existing on the bottom of Lake Baikal are associated with biological communities of various compositions. They include a wide range of organisms from bacteria and microscopic fungi to invertebrates (Namsaraev et al., 2002; Zemskaya et al., 2012).

The aim of our research was to study the probable ways of involving carbon of different origin (for example, methane) and other biogenic elements in food webs using integral methods (electron microscopy, high-throughput sequencing). One of the mechanisms responsible for the entrainment of methane carbon may be symbiotic relationships between aquatic invertebrates and microbial communities that include methanotrophs (Goffredi et al., 2008).

In the area of the hydrothermal spring in Frolikha Bay, we were the first to find epibiotic associations of ciliates *Lagenophrys* sp. and prokaryotes on the surface of the cuticle of Baikal endemic amphipods (Khalzov et al., 2018). In the areas of the Goloustnoye methane seep, samples of ostracods *Candona* sp. were taken, on the surface of which shells of *Lagenophrys* sp. were also found, forming a consortium with microorganisms. The study using scanning electron microscopy showed different morphology of cells of microorganisms, and also revealed the presence of a special mineral substance on which bacteria are located.

In this study bacterial fouling of ostracod valve was mainly located near ciliate loricae. The area of the observed bacterial biofilms occupied ~12% of the valve surface. The density of bacteria in the biofilm formed by these bacteria was approximately 20 cells per 100 μm^2 . The surface of the ostracod valves had many objects of non-biological nature: mineral particles and crystals of

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various morphology localized between bacterial cells. Elemental analysis of mineral substance revealed the presence of Si, Al, P, S, Ca, and Fe.

High-throughput sequencing on the Illumina MiSeq platform showed the presence of such bacterial phyla as *Actinobacteria*, *Gracilibacteria*, *Cyanobacteria*, *Proteobacteria*, *Bacteroidetes* in the ostracod microbiome, of which the latter two were dominant. The main part of the bacteria associated with ostracods was represented by the phylum *Proteobacteria* (77%), among which representatives of the class *Alphaproteobacteria* dominated (54%). Most of the sequences of this class were assigned to the genus *Rickettsia* (50%).

The *Gammaproteobacteria* class accounted for 22% of the total number of sequences. Only 11% of all sequences belonged to the family *Methylomonaceae*, and about 7% were members of the *Thiotrichaceae* family, resembling the sulfur bacteria of the *Thiothrix* genus.

Representatives of the phylum *Bacteroidetes* accounted for 22% of the total number of OTUs, 17% were attributed to the order *Flavobacteriales*. Among them, the majority of representatives belonged to the unclassified, only a small part (0.8%) belonged to the *Flavobacteraceae* family.

For the first time in the microbiome of Baikal ostracods, they were found in sequences belonging to endosymbionts of the orders *Rickettsiales* and *Holosporales*. It is quite probable that the representatives of *Rickettsia* we identified may be endosymbionts of ostracods. The presence of rickettsia in freshwater ostracods has been shown for the first time. Representatives of the genus *Cardinium* for Baikal species of crustaceans were also not previously noted.

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