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Metagenomics and Biodiversity of Sphagnum Bogs

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Abstract—Biodiversity of sphagnum bogs is one of the richest and less studied, while these ecosystems are among the top ones in ecological, conservation, and economic value. Recent studies focused on the prokaryotic consortia associated with sphagnum mosses, and revealed the factors that maintain sustainability and productivity of bog ecosystems. High-throughput sequencing technologies provided insight into functional diversity of moss microbial communities (microbiomes), and helped to identify the biochemical pathways and gene families that facilitate the spectrum of adaptive strategies and largely foster the very successful colonization of the Northern hemisphere by sphagnum mosses. Rich and valuable information obtained on microbiomes of peat bogs sets off the paucity of evidence on their eukaryotic diversity. Prospects and expectations of reliable assessment of taxonomic profiles, relative abundance of taxa, and hidden biodiversity of microscopic eukaryotes in sphagnum bog ecosystems are briefly outlined in the context of today's metagenomics.

Keywords: biodiversity, metagenomics, sphagnum, bogs

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Bogs play an important role in natural ecosystems. *Sphagnum* is a cosmopolitan genus of mosses that forms the environment of most bogs and is the most abundant in the boreal zone of the Northern hemisphere. Sphagnum mosses are capable of the cation type of tissue metabolism and thus create a specific acidic environment [1]. Such an environment prevents rapid decay of organic matter, and carbon compounds consequently accumulate as peat. Peat of the Northern hemisphere forms a global system of carbon fixation, and stores to about one third of its global stock [2], which exceeds two- to threefold its reserves in all tropical forests of the planet [3].

Approximately 4 million km^2 of the Earth's surface is covered with peatlands, and more than half of them are in Russia. Peat bogs play a vital role in global exchange of greenhouse gases and other important components of the atmosphere (CH_4, CO, N_2O, NH_3, H_2S, etc.), and act as a global environmental factor in the formation of hydrological and even climatic characteristics of ecosystems (e.g., see [4–6]). As is well known, peat has economic value as fuel critical in many regions, isolation material, natural absorbent, and antiseptic. The United States, the European Union, and Japan are now the main consumers of *Sphagnum*, and import large amounts of dried moss for household purposes. Techniques of *Sphagnum* cultivation are being developed in Canada and the EU, particularly, to provide for its renewable resource in view of the likely depletion of peat stocks, as the rate of peat growth in nature is extremely low, 0.75–1.00 cm per year.

It is important to realize the value of sphagnum bogs as not only an important economic resource but also a unique reservoir for biodiversity and its conservation. For instance, peat depositions of pollen of higher plants and spores of mosses and ferns are an important information source for stratigraphic dating of climatic changes over the last glacial period. Pollen record and plant relics are used in paleoecological studies to reconstruct successions of landscapes and plant communities in the context of climatic changes [7, 8], and provide a source of archeological evidence to study human development of the landscape over the Holocene to the present [9, 10].

Consortia of prokaryotic microorganisms (microbiomes) associated with sphagnum mosses were recently studied in detail to uncover the factors that maintain sustainability and productivity of host plant populations [11–13]. Earlier studies that used differential electrophoresis showed that microbiomes of individual *Sphagnum* species are highly specific and differ in biodiversity indices regardless of their geographical locations [14].

The advent of high-throughput sequencing (HTS) heralded the era of new opportunities and paved the emergence of a new scientific field, metagenomics. HTS and state-of-the-art PCR-based methods made it possible to target individual DNA markers or complete operons in total environmental DNA (eDNA

metabarcoding). These data can be used for fast and accurate taxonomic profiling of communities, including detection of hidden biodiversity and novel groups, and for studying functional groups of genes and gene families within the total gene pool. Recent data obtained with HTS corroborate earlier findings, and support high taxonomic diversity of the *Sphagnum* microbiome [15, 16].

Hyaline cells and stems of *Sphagnum* plants were shown to support various communities of methanotrophic bacteria involved in the efficient cycle of methane absorption and metabolism as a mechanism of carbon fixation and storage in bog ecosystems [17−20].

In the absence of the root system in mosses, bacterial communities play an important role in supplying organic metabolites to the host plants, maintaining their general viability and protection from adverse conditions [21]. In support of this notion, the species specificity of the microbiome and the spectrum of ecological groups of bacteria are known to directly correlate with the profile of secondary metabolites produced by the host plants [15] and be determined by such abiotic factors as acidity and nutrient richness of the environment [16]. The core of the *Sphagnum* microbiome is not only species specific but is transferred through plant generations within spore capsules [22], demonstrating a vivid example of an evolutionarily stable relic metacommunity.

Large-scale genomic reconstructions of the microbiome revealed the functional diversity of *Sphagnum* microbial consortia, as well as biochemical pathways and gene families that sustain their diverse adaptive strategies [23]. The mere presence of some of the observed functional gene groups indicates that plants and microorganisms closely interplay in processes of nitrogen fixation, nutrients absorption, and developing resistance to oxidative stress and desiccation. *Sphagnum* microbial communities differ from those of peat soils and higher plants, particularly, in having higher presence of bacterial genetic systems involved in respiration (their ratio correlates with the vertical oxygen gradient [24]) and cell motility [25], which is of apparent functional significance for sustaining surface communities of the phyllosphere. Higher relative abundances are also observed for various systems of cell stress response, especially the oxidative stress, and for biosynthesis pathways of iron-binding proteins (siderophores) that are characteristic of oligotrophic and ombrotrophic communities, and are known agents of antagonistic microbial interactions [26].

Generally, discoveries of these gene families provided a much deeper insight into functioning of the prokaryotic communities of sphagnum mosses. Here, the abundance of mobile elements in the microbiome that are typical for symbiotic bacterial communities [27, 28] indicates plausible symbiosis between bacteria and mosses. High presence of systems involved in oxidative stress response and nitrogen fixation and absorption [15, 16, 29] most likely facilitates the very successful colonization of vast areas by sphagnum mosses. Biochemical pathways of nitrogen fixation and absorption considerably overrepresent systems of its catabolism, thus providing biological grounds for the paramount role of sphagnum bogs in fixing and depositing organic matter on the global scale. Apart from describing a high functional genetic diversity of the *Sphagnum* microbiome, the mentioned studies indicate that many bacterial taxa have not yet been discovered in these communities by traditional methods of microbiology.

In the view of important findings reported for the *Sphagnum* microbiome, the apparent need is to study eukaryotic consortia of *Sphagnum* as well. Conventional sampling and microscopy approaches (e.g., see [30]) demonstrated a substantial portion of unicellular eukaryotes and microscopic animals of various taxonomic rank to remain unknown to or undescribed by systematicists. Based on collected evidence, this particular sphagnum bog in Germany is now protected by law as a valuable national biodiversity reservoir. Microscopic studies of such biotopes are laborious, time consuming, and require collective effort of many taxonomists, which consequently makes oriented projects long-term and difficult to accomplish. Clearly, today's metagenomics offers apt means to describe eukaryotic diversity. The metagenomic approach can be used to not only catalogue the known taxa but to uncover hidden biodiversity of unicellular eukaryotes, microscopic animals, plants, and fungi. Many publications focus on studies of marine eukaryotic diversity, including benthic, meiobenthic, and plankton communities, employing HTS generation of short barcodes within the 18S rRNA (variable regions V4 and V9) and 28S rRNA (variable regions D1, D2, and D3) genes or ribosomal internal transcribed spacers (ITS) (e.g., see [31−35]). Paradoxically, communities of the rift zones in deep see are studied better than are far easier to reach communities of bogs.

Ribosomal DNA markers have been used to characterize eukaryotic species abundance and spatial patterning of communities in various biotopes. Some works employed HTS approaches for large-scale generation of conventional barcodes (cytochrome *c* oxidase I, COI) to profile freshwater communities at the species level [36]. However, high variability of traditional markers often precludes a reliable identification of higher-rank taxa, and impairs computer inferences [37−39]. In this context, it seems promising to generate vast metagenomic datasets not only for conventional barcodes but also for extended or overlapping amplicons (whole genome shotgun). This approach will likely overcome the known technical limitations and allow to obtain reliable information on the taxonomic composition, relative abundance of individual eukaryotic groups, and to uncover hidden biodiversity of bog biotopes.

Another advantage of the whole-genome shortgun is the possibility to generate primary genomic data for a broad range of microeukaryotic taxa from bog ecosystems. Whole genome sequences of such organisms can hardly be expected in the near future. To the more, metagenomic data is a promising source of discovering yet unknown taxonomic groups.

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