FACULTY OF SCIENCES

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To Prof. Dr. František Vácha
Dean of Faculty of Science
University of South Bohemia

DATE
30 October 2017

PAGE

OUR REFERENCE

Report PhD thesis M. Ekaterina Pushkareva

Dear Professor František Vácha,

In response to your letter, I hereby submit my evaluation report of the PhD thesis by Ekaterina Pushkareva entitled 'Ecology and diversity of microbial phototrophs in biological soil crusts of Polar Regions'. In summary, I found this a well-written, appropriately structured and interesting thesis, which consists of six papers already published or submitted to peer reviewed journals. As such, the thesis contains a comprehensive literature overview and another five primary research papers on the ecology, taxonomic composition and ecosystem functioning of phototrophic communities in biological soil crusts from both the Arctic and Antarctica. The work is novel and of relevance for polar microbiologists and I thereby recommend that the thesis is defended.

Below I have detailed my specific comments on the different chapters.

The introduction gives a concise overview on the distribution and ecological importance of BSCs in polar and other regions as well as the taxonomic composition of their photoautotrophic communities. Their role as ecosystem engineers for e.g. soil development and nitrogen and carbon cycling is subsequently discussed. The introduction ends with a description of the particular climatic and vegetation properties of the High Arctic and Antarctica, and an overview of BSC development in glacial forefields. Given the importance of the latter for the rest of the thesis, I would have appreciated a more in-depth summary of the differences in soil properties

(e.g., organic carbon and nitrogen content) along chronosequences and the different roles photo-autotrophs play in the successional stages.

The objectives of the thesis are clear and well-formulated. However, in my opinion some paragraphs in this part are in a way a repetition of parts of the introduction and could have been discussed in more detail there, i.e. methodologies to study the diversity of microbial phototrophs and the recent discovery of endemics in these communities (but see paper 1). But this is merely a technical issue.

The overview of the different papers is concise and well-structured. The focus is clear and the connection between the papers is well-explained.

The results and general discussion was in my opinion a little bit too short. While it focussed on the main findings of the thesis, a chapter on differences in community structure between Arctic and Antarctic soil crusts could have been included.

Also the perspectives were relatively short. I agree that a candidate should not give her/his future research agenda in a PhD thesis, but I expected to read at least some ideas about future potential research which will allow to further our knowledge on the diversity and ecosystem functioning of polar BSCs. However, this issue can be discussed during the defence.

Paper 1: A review of the ecology, ecophysiology and biodiversity of microalgae in Arctic soil crusts

This section provides an excellent overview on the taxonomic composition of BSCs in Arctic regions, the role of the most important environmental factors structuring these communities and adaptive properties enabling them to thrive in extreme polar conditions. The chapter also contains information on the ecological role BSCs play in polar environments. The issue of endemism is discussed in a final section. I expect that this paper will be a highly cited study. I have no further comments on this chapter.

Paper 2: Biodiversity and ecological classification of cryptogamic soil crusts in the vicinity of Petunia Bay, Svalbard

This paper is basically a general description of the biodiversity assessed using microscopy of the photoautotrophic organisms in BSCs. Based on macroscopic features and the diversity of cyanobacteria and microalgae, the BSCs in Petunia Bay (Svalbard) were classified into three main types. I am confident that the identifications were done properly and the taxa present are well illustrated. The section regarding changes in diversity and distribution of BSCs along an altitudinal gradient (p.62) was in my opinion too short and could have been illustrated with (a) figure(s) showing (i) the number of taxa versus altitude and (ii) and ordination or cluster diagram showing species turnover in the different samples.

Paper 3: Cyanobacterial community composition in Arctic soil crusts at different stages of development

In this chapters the cyanobacterial community composition in BSCs in four sites in the vicinity of Petunia Bay was studied using 454 sequencing and specific primers of the 16S rRNA gene. The samples were selected as to represent different stages of the development of BSCs. Multivariate analysis was applied to assess the importance of different soil environmental properties in explaining differences in community structure. The presence of particular OTUs is compared with BSCs from other polar regions. While the paper is rather descriptive, some interesting conclusions could be formulated. However, I have some problems with the suggested causal relation between the (significant) environmental variables and the distribution of cyanobacteria OTUs. More in particular it is also possible that the presence of other organisms like mosses and lichens are better competitors and have influenced the pH, rather than that pH has affected the cyanobacteria. This is issue can be discussed during the defence.

Paper 4: Nitrogen fixation and diurnal changes of photosynthetic activity in Arctic soil crusts at different development stage

This chapter is aimed at studying photosynthesis and nitrogen fixation in BSCs in Svalbard along a chronosequence. To achieve this field measurements of photosynthetic activity were combined with nitrogenase activity as assessed using acetylene-ethylene reduction assays, microscopic observations of biomass and measurements of environmental parameters (incl. temperature). I have no comments on this chapter.

Paper 5: Marked primary succession of cyanobacterial communities following glacier retreat in the High Arctic

In this chapter cyanobacterial community structure and abundance was studied in three glacier forefields in Svalbard. Multivariate analysis was used to identify those factors significantly structuring the communities. While some interesting results were obtained, for example that early communities are dominated by polar and alpine OTUs, I would like to discuss the relatively low sample size and the lack of replicates with the candidate. For example, only two BSCs in sites considered to be at a medium stage of development were included in the analysis. I would have also preferred to see a more complete comparison of the geographic range size of all the different OTUs, rather than a comparison based on only the most abundant OTUs. I would like to get more information during the defence on the criteria used to consider an OTU as an indicator OTU.

Paper 6: Cyanobacteria inhabiting biological soil crusts of a polar desert: Sør Rondane Mountains, Antarctica

In this chapter the biomass and community structure of cyanobacteria were studied using microscopy, a DNA fingerprinting technique and 454 pyrosequencing of parts of the 16 rRNA gene in BSCs from the Sør Rondane Mountains, East Antarctica. The paper is generally well written and contains some interesting results, but compared with the previous chapters, some smaller errors were present. For example on p.143, Obbels et al is erroneously cited. Also the statement that in Tytgat et al. (2016) and Obbels et al. (2016) most analyses were only conducted on the phylum level is not correct. In fact, the SIMPROF analyses in both publications were done on the OTU level. The map has a low resolution and the labels on the scale bar are

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hardly visible. While I agree that normalisation to a given sequence number is needed for direct comparisons of the OTU diversity between the samples, I found the number of sequences to which the samples were standardized relatively low (459). I wonder whether it wouldn't have been better to exclude the sample with a relatively low number of sequences from the analysis and standardize to a higher number of sequences. This can be discussed during the defence. I am unsure whether the significance in richness between the sites can be assessed based on such a small amount of samples.

I look forward to discuss the abovementioned issues and other aspects of the thesis during the defence on the 13th of November 2017.

Best regards,

Elie Verleyen

GHENT UNIVERSITY

Review of Ph.D. thesis

Student: **Ekaterina Pushkareva, M.Sc.**

Title: Ecology and diversity of microbial phototrophs in biological soil crusts of Polar Regions

Supervisor: doc. Ing. Josef Elster, CSc.

University of South Bohemia, České Budějovice, 2017, 169 p.

Biological soil crusts are complex communities of organisms living in the upper layer of soil that are crucial in the process of soil succession. Microbial phototrophs, i.e., cyanobacteria and microalgae, are important component of the crusts, because of their key role of primary producers. Moreover, they represent substantial sources of nitrogen due to the frequent presence of heterocytous cyanobacteria. Biological soil crusts are distributed in terrestrial environments worldwide. In polar regions, microbial phototrophs are among the first colonizers of newly deglaciated areas. Therefore, they are a valuable model for the study of biodiversity and ecological processes in extreme environments.

The Ph.D. thesis of Ekaterina Pushkareva deals with various aspects of diversity, ecology and ecophysiology of biological soil crusts both in the Arctic and in Antarctica. Despite the apparent ecological importance of these communities, our knowledge of all these aspects remains largely limited, and this thesis represents a significant milestone in this research area.

The thesis consists of one review and five original papers. The review and two papers were already published in high-quality impacted journals, one paper in a peer-reviewed without impact factor and two manuscripts are currently under review. The keynote participation of Ekaterina in completing the research and writing the manuscripts is evidenced by the fact that she is the first author of five of them. The core part of the thesis is supplemented by an introduction, objectives of the thesis, short overview of the papers and by conclusions that include a section discussing future perspectives of the research on this topic.

Since four of the publications included in the dissertation successfully underwent peer-review process, I can only conclude that the scientific quality of the thesis is outstanding.

The objectives are clearly outlined and the individual papers provide answers to the questions addressed. The methods used during field sampling and measurements and laboratory analyses are appropriate and correctly applied, leading to interesting and new results and achieving the objectives outlined for this thesis.

The review (Pushkareva et al. 2016, Polar Biology) provided the first comprehensive review of ecology, ecophysiology and biodiversity of cyanobacteria and microalgae in Arctic soil crusts. It represents a very good theoretical background for the following biodiversity and ecological studies that are included in this thesis.

In the second paper (Pushkareva et Elster 2013, Czech Polar Reports) the authors

describe the diversity of cyanobacteria and algae in biological soil crusts in the vicinity of Petunia Bay (central Svalbard). Based on light microscopy and fluorescence data, soil crusts were divided into three types. Such classification is a good starting point that enables a simple comparison of results from different regions.

The third study (Pushkareva et al. 2015, FEMS Microbiology Ecology) contains a first detailed assessment of cyanobacterial diversity in soil crusts in the High Arctic using high-throughput sequencing. In addition, the phylotype richness was assessed at different developmental stages, showing its maximum in mid-developed crusts.

In the fourth paper (Pushkareva et al. 2017, European Journal of Soil Biology), the patterns of nitrogen fixation and photosynthesis were studied in soil crusts in central Svalbard. The peak of nitrogenase activity was detected in poorly developed crusts and differences in the diurnal courses of photosynthesis among the community types. To my knowledge, this study represents the first quantification of these key processes in polar soil crusts.

The following manuscript (Pessi et al., submitted to Journal of Microbial Ecology) is focused on succession of cyanobacteria in soil crusts again in central Svalbard showing a relatively high community turnover that was explained by time since deglaciation and associated changes in environmental conditions

The last manuscript (Pushkareva et al., submitted to Systematic and Applied Microbiology) is dedicated to the assessment of diversity of soil crusts in Sør Rondane Mountains (continental Antarctica). The results of this study will also serve as baseline data in the experiment based on open top chambers that was established to evaluate the effect of climate change on soil communities.

Apart from the core chapters formed by individual papers, the thesis contains an introductory part that provides basic information about the diversity and ecology of soil crusts. The text is relatively short, but it contains the most important points. Moreover, a detailed review is given in the paper published in Polar Biology.

Overall, the thesis is a well-written and thorough piece of work based on the state of art methods and supported by extensive study of relevant scientific literature.

To conclude, the thesis fulfills all the criteria necessary for obtaining the Ph.D. degree, and it is my pleasure to recommend it for the public defense.

Questions:

1/ Major results of this thesis were obtained using high-throughput sequencing. Overall, the application of modern molecular techniques has opened new perspectives in the assessment of biodiversity. Could you comment on advantages and drawbacks of this approach in comparison with traditional ones that resulted from your work?

2/ The papers included in your thesis contain data from both from the Arctic and Antarctica. One of the open questions of polar microbiology is the incidence of endemism. Although the thesis is not focused on this topic, I would like to ask, if there are already some indications concerning this issue based on data available for microbial phototrophs from soil crusts?

3/ One of the aims of the Antarctic paper was to set baseline data for later assessment of changes connected with climatic changes? What are the supposed changes of soil crust communities? Are there any estimates how quickly will they react?

4/ For the assessment of nitrogenase activity of the crusts, you performed the acetylene-ethylene reduction assay. Why you have chosen this assay and what are advantages and disadvantages of other possible methods used for quantification of nitrogen fixation?

5/ Your thesis filled a lot of gaps in the research of diversity and ecology of biological soil crusts in polar regions, but also opened many new questions. What was the most surprising result for you and what would be the design of your next study on this topic if you could plan it without logistical and financial limits?

Prague, 8 November 2017

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