REVIEW OF PH.D. THESIS

Student: **Daria Tashyreva, M.Sc.**

Title: Production of dormant stages and stress resistance of polar cyanobacteria

Supervisor: doc. Ing. Josef Elster, CSc.

University of South Bohemia, České Budějovice, 2015, 146 p.

Polar habitats are often characterised by unstable extreme conditions with large variations of liquid water availability and temperature. Filamentous cyanobacteria are a successful group of photoautotrophic organisms in this environment and together with eukaryotic algae they play there a key role as primary producers. They have to be resistant to the extreme conditions and therefore provide a valuable model for the study of adaptive strategies required to survive in such cold and fluctuating habitat.

The Ph.D. thesis of Daria deals with various aspects of stress resistance of polar cyanobacteria, a group that is common in a wide range of habitats both in the Arctic and in Antarctica. Despite its ecological importance, stress resistance mechanisms including production of dormant stages remained largely underexplored in this group, and this thesis represents a significant milestone in this research area.

The thesis consists of one book chapter and three papers. Except for one paper that is currently under revision, they were already published in high-quality peer-reviewed journals and one monograph. The keynote participation of Daria in completing the research and writing the manuscripts is evidenced by the fact that she is the first author of all of them. The core part of the thesis is supplemented by an introduction, aims and tasks, summary and by conclusions that include a section discussing future prospects of the topic.

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

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

The book chapter (Tashyreva et Elster 2012, Springer) provided the first comprehensive review of production of dormant stages and biochemical mechanisms of stress resistance in polar cyanobacteria. It represents a very good theoretical background for the following laboratory experiments and field study.

In the second paper (Tashyreva et al. 2013, PLoS ONE) the authors present a newly developed staining protocol for multiparameter assessment of cell activity in *Phormidium*. The second main benefit of this study is the introduction of a clear classification of cells according to their metabolic status. Such classification will enable a better comparison of results of future studies based on this methodology.

In the third study (Tashyreva et Elster 2015, Frontiers in Microbiology), the role of nitrogen starvation in desiccation tolerance of three *Microcoleus* strains was tested in

laboratory using a carefully planned experimental design. The authors showed that the tolerance to complete desiccation is not a condition for the success of cyanobacteria in polar environments and suggested the importance of stress avoidance. This study highly benefited from the novel staining protocol published in the previous paper.

The fourth paper (Tashyreva et Elster, Microbial Ecology, in revision) is a field study describing the annual cycle of two *Phormidium* populations in shallow seepages in Svalbard. To my knowledge, this study documented for the first time a surprisingly high ratio of viable cells in overwintering communities and a constant metabolic activity throughout the vegetative season, which are probably the key factors ensuring the success of these cyanobacteria in such habitats. Again, this study benefited from the single-cell level evaluation of activity that was developed and published by Daria.

Apart from the core chapters, I have to stress that Daria succeeded to compile a comprehensive introduction that include all necessary points and represent a clearly arranged text for any interested reader. The aims of the thesis are clearly stated and the concluding chapters comment the main results presented in the thesis and mention new challenges and questions in this research area.

Overall, the thesis is a very well-written and thorough piece of work based both on laboratory and field experiments 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/ The multiparameter staining protocol developed in the frame of this thesis could represent a very powerful tool in ecological studies in wider context. Could you comment on its possible use when working with other cyanobacterial genera or eukaryotic algae?

2/ Polar strains of *Phormidium* and *Microcoleus* are apparently well adapted to extreme conditions in polar wetlands. What about strains from other regions? Do you suppose that they share the same stress resistance mechanisms?

3/ Do you think (generally) that the freezing and desiccation tolerance of cyanobacterial strains can be affected by long-term laboratory cultivation in culture collections?

4/ The results of your laboratory experiments suggested the key role of nitrogen starvation in induction of stress tolerance. However, the field study in Petuniabukta has not provided any evidence of its importance. Can you discuss the possible role of other environmental factors?

5/ Your thesis filled a lot of gaps in ecology and ecophysiology of polar cyanobacteria, but also opened many new questions. What was the most surprising result for you and what would be your next laboratory experiment/field study on this topic?

Prague, 20 February 2016

Nedbalová

.....

Linda Nedbalová
Department of Ecology
Faculty of Science, Charles University in Prague

UNIVERSITÉ LAVAL

CEN : Centre d'études nordiques
Centre for Northern Studies

20 February 2016

Professor Jan Kucera,
Head of the Committee for
PhD Studies in Botany,
Faculty of Science,
University of Southern Bohemia,
370 05 České Budějovice,
Czech Republic.

Dear Dr. Kucera,

Examination report for the PhD thesis "Production of Dormant Stages and Stress Resistance of Polar Cyanobacteria" by Daria Tashyreva

In response to your letter of 22 January 2016, I hereby submit my report on the above thesis. In summary, this is a logically structured, well written thesis that combines literature synthesis, laboratory experiments and field observations. Three of the chapters are already published in the international scientific literature and the fourth is currently under review. The work addresses an important issue in polar microbial ecology, and it is original and to a high standard. I hereby recommend that the thesis be defended.

Detailed comments:
This thesis focuses on the question of microbial dormancy, which is of broad interest in microbiology. The subject is of special relevance to polar ecosystems where organisms face severe variations in temperature, light and water availability. The thesis is logically separated into five sections, and my examination notes on each section are as follows.

Section 1. Introduction. This introductory chapter of the thesis is a very good review and synthesis of the extensive literature on stress and adaptation in polar environments, the biological characteristics of dormancy, and the responses of polar cyanobacteria to extreme conditions. I like the way this culminates in a clear statement of six gaps in understanding, followed by the objectives, results and conclusions from the thesis research, which targeted those gaps, and prospectives for subsequent research.

I will be interested to discuss at the defense the concept of dormancy, which seems to be defined in somewhat contradictory terms in the literature; e.g., "...a reversible state of the low metabolic activity..." (p. 13) versus "...when the conditions cannot support even the minimal rate of metabolism" (also p. 13).

It may also be interesting to consider the concept of "scout strategy" in the microbiological literature (e.g., Epstein, S. S. 2009 Microbial awakenings. Nature 457, 1083, and associated correspondence).

It seems that a comparison between *Nostoc* and *Phormidium* could generate important insights into the different physiological strategies for dormancy. Also, there are observations on dormant (but not specialized) cells of *Microcystis* in lake sediments in the harmful bloom literature that could be useful to compare.

p.28: The thesis does not include an explicit statement of hypotheses that were tested, but many of the questions and objectives could be formulated as hypotheses. This is an aspect to be discussed at the defense.

In general, the thesis is very well presented, with excellent English language text, figures, and tables, with only rare typographical errors. A few minor corrections are μmol m^{-2} s^{-1} (pp 2, 27, 124); ref 176 is incomplete here and is cited in different ways in the different chapters; check also other citations from Whitton and Potts; ref. 98 is incomplete, along with other chapters from this book (the editors should be given: B. J. Fuller, N. Lane & E. E. Benson); ref. 20: check year; the letters 'SE' sometimes turn up in the citations, and it looks to be a software transfer error; ref. 142: *Azotobacter* (italics).

Section 2. Production of dormant stages and stress resistance of polar cyanobacteria. This synthesis has been published in a well-known book series on life in extreme environments, and draws attention to the lack of understanding about dormancy despite its critical importance for cyanobacterial success in the polar regions.

Section 3. A novel staining protocol for multiparameter assessment of cell heterogeneity in *Phormidium* populations (cyanobacteria) employing fluorescent dyes. This presents the work by the candidate to optimize multiple staining protocols (and microscopy filter block combinations) to test dormancy in mat-forming cyanobacteria, which play a major role in many polar and alpine aquatic ecosystems. This section of the research has been published in the well-cited international journal *PLoS ONE.* Some consideration might be given to other approaches to test cell heterogeneity, and the strengths and weaknesses of the staining approaches used here.
Tab. 2 p. 92: what does ± mean?

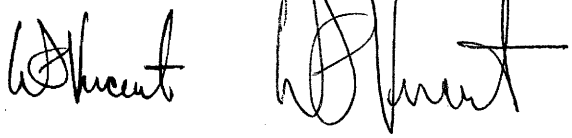
Section 4. Effect of nitrogen starvation on desiccation tolerance of Arctic *Microcoleus* strains (cyanobacteria). This has been published in the Nature Publishing Group journal *Frontiers in Microbiology.* This examines how tolerance of water stress is affected by temperature and nitrogen depletion. It would be useful to see the growth curve in the minus-N BG11 treatment; was growth zero and how did pigments change over the 2-3 weeks? Figure 2: fluorescence microscopy is not always noted; what did the cell autofluorescence look like under N starvation by comparison with the others? The results in the –N treatment in Fig. 3 are extremely interesting. Is it likely that there could be N-limitation in all microbial mat environments? (see:

Varin, T., Lovejoy, C., Jungblut, A.D., Vincent, W.F. and Corbeil, J. 2010. Metagenomic profiling of Arctic microbial mat communities as nutrient scavenging and recycling systems. Limnology and Oceanography 55: 1901–1911; there may also be information of relevance in the companion paper: Varin, T, Lovejoy, C., Jungblut, A.D., Vincent, W.F., and Corbeil, J. 2012. Metagenomic analysis of stress genes in microbial mat communities from extreme Arctic and Antarctic environments. Applied and Environmental Microbiology 78: 549-559).

Section 5. Annual cycles of two cyanobacterial mat populations in hydro-terrestrial habitats of the High Arctic. This is under review in the international journal *Microbial Ecology*, and presents very interesting new data on the high winter survival rates of *Phormidium* in an Arctic seep environment. The work is well integrated with the literature, and part of its strength is the combination of methods applied. Tab. 1 p130: it would be best to delete the final decimal digit in these results since they have no meaning, given the size of the SD values. The data for nitrite and phosphate (more correctly termed 'soluble reactive phosphorus') could be removed from the table and just referred to in the text, for example as: 'Nitrite and SRP concentrations were below the limits of detection (10 μg N or P l^{-1}) on all dates of sampling.' Fig. 7: it would be useful to see the pigment autofluorescence in this post-freezing sample, as in Fig. 6.

I look forward to discussing these and many other aspects of the candidate's excellent work during the defense on 29 February 2016.

Yours sincerely



Warwick F. Vincent, PhD, FRSC, hon. FRSNZ
Scientific Director, Centre for Northern Studies
Canada Research Chair in Aquatic Ecosystem Studies
Professor of Biology