REVIEW OF PH.D. THESIS

Student:	**Mgr. Martina Pichrtová**
Title:	Stress resistance of polar hydro-terrestrial algae *Zygnema* spp. (Zygnematophyceae, Streptophyta)
Supervisor:	doc. RNDr. Yvonne Němcová, Ph.D.
Consultant:	doc. Ing. Josef Elster, CSc.

Polar hydro-terrestrial habitats are characterised by unstable extreme conditions with large variations of liquid water availability. Eukaryotic algae are a successful group of photoautotrophic organisms in this environment and together with cyanobacteria 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 Martina deals with various aspects of stress resistance of green filamentous algae *Zygnema* spp. that are common in polar wetlands both in the Arctic and in Antarctica. To date, stress resistance mechanisms in this genus remained largely underexplored, and this thesis represents a significant milestone in this research area.

The thesis consists of three papers that were already published in high-quality peer-reviewed journals. The keynote participation of Martina 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 and by a summary with concluding remarks.

Since all 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.

In the first paper (Pichrtová et al. 2013, Microbial Ecology), Arctic and Antarctic strains of *Zygnema* were shown to produce phenolics that protect cells from excessive UV radiation. Interestingly, these compounds are synthesised constitutively by *Zygnema*. Until now, their production was confirmed in few algal groups, so this paper is an important report of such stress adaptation. In addition, the variability of UV stress response among studied strains was demonstrated.

The second study (Pichrtová et al. 2014, FEMS Microbiology Ecology) focused on natural populations of *Zygnema* spp. from Svalbard. The production of resistant vegetative cells with specific morphological features called pre-akinetes was shown to play a key role in survival in Arctic meltwater pools. Furthermore, high osmotic stress tolerance in naturally dried cells suggested the importance of cell hardening for survival. Due to methodological and logistic obstacles, such field studies in polar regions are scarce. Therefore, this paper significantly contributes to our understanding of survival mechanisms of eukaryotic algae in extremely cold unstable habitats.

In the third study (Pichrtová et al. 2014, PLoS ONE), the role of pre-akinete hardening

in cell survival was tested in laboratory using a carefully planned experimental design. The authors also confirmed the role of nitrogen starvation in pre-akinete formation. This challenging aim yielded very interesting results and opened many new questions for future studies.

Moreover, the first and third paper provided a first insight into the molecular diversity of *Zygnema* in polar regions. The results showed a rather unexpected diversity and the first record of a *Zygnemopsis* from the Arctic. The report of such hidden diversity suggests that the application of polyphasic approach in ecophysiological studies is essential.

Apart from the core chapters, I have to stress that Martina succeeded to compile a comprehensive introduction that include all necessary points and represent a clearly arranged text for any interested reader. The concluding chapter comment the main results presented in the thesis and mention new challenges and questions in this research area. This part also contains some recommendations concerning the methodology of stress resistance tests in algae that are of general interest.

Overall, the thesis is a very well-written and thorough piece of work based both on field and laboratory 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/ You have recorded different responses of *Zygnema* strains to UV stress suggesting the presence of other resistance mechanisms. Could you summarise, which can be relevant for this genus?

2/ Do you think (generally) that the UV-resistance of algal strains can be affected by long-term laboratory cultivation in culture collections?

3/ *Zygnema* (and *Zygnemopsis*) are apparently well adapted to extreme conditions in polar wetlands? What about other Zygnematophyceae? Do you suppose that they share the same resistance mechanisms?

4/ Your data provided a first insight into the molecular diversity of polar *Zygnema* spp. Where you expect higher diversity: in the Arctic or in Antarctica?

5/ Could you explain in more detail, how you estimated the proportion of surviving cells in the laboratory experiment performed in the third study? Have you tested the optimal growth conditions (20 °C, light intensity 35 μmol/m^2/s)?

6/ The results of your laboratory experiment suggested the importance of nitrogen starvation in induction of pre-akinete formation. Do you have any evidence of decreasing nitrogen concentrations during the vegetation season in the Petuniabukta pools?

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