FACULTY OF SCIENCES

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OUR REFERENCE

Concerns: Report on the habilitation thesis 'Diversity, ecology and ecophysiology of algae and cyanobacteria from extreme environments' by Dr. Linda Nedbalová

Dear members of the habilitation commission,

Please find below my evaluation report of the habilitation thesis by Dr. Nedbalová.

The thesis consists of three parts, which are all related to the diversity, ecology and ecophysiology of eukaryotic algae and Cyanobacteria in extreme habitats from both polar and temperate regions, namely Antarctic lakes, snow habitats and acidic lakes. While these environments are evidently different in their ecology and biotic communities, the three parts are clearly interlinked and the rationale for combining them into the thesis are well-described in the general introduction. All parts contain at least 8 separate chapters and clearly reflect the significant contribution Dr. Nedbalová made to these different research fields. In general, I highly appreciate the multitude of methods and techniques used to study these ecosystems. Below I have given my general appreciation on the different chapters.

Part I: Lakes on James Ross Island (Antarctica)

This part consists of 8 chapters and gives a broad, but also detailed view on the physical and chemical limnology of lakes in James Ross Island (Antarctic Peninsula) and their diversity and ecology of eukaryotic and prokaryotic algae. As a whole, the work on these lakes by Dr. Nedbalová has changed the region from being one of the least studied ones, to one of the best studied lake districts in the Antarctic Peninsula. As such, the lakes in James Ross Island were 'put on the map' and the data generated provided a highly needed contribution to similar studies

on lakes further to the North (i.e. Livingston Island and Signy Island) and those in East Antarctica further to the South (i.e., the McMurdo Dry Valleys in Victoria Land and the coastal Oases in East Antarctica).

In the first chapter, the study region is described and a summary is given on the different lake types present. While this is merely a descriptive chapter, I highly appreciate this type of studies on Antarctic lakes because they provide the necessary baseline data on the limnological diversity of lacustrine ecosystems. This is needed, given that (i) the Antarctic Peninsula appears to be one of the most rapidly warming regions on Earth, and (ii) polar lakes respond quickly to climate-induced environmental changes. As such the study provides the data for future limnological investigations of the region and has set the scene for explaining beta diversity patterns in biota, ranging from Bacteria, microeukaryotes and invertebrates, and link those to differences in environmental properties.

The second chapter provides a view on the relation between temperature and dissolved organic carbon content, and the abundance of aerobic anoxygenic phototrophic bacteria in the lakes. It is the first study on the abundance of this group of organisms in Antarctic lakes.

In the third chapter, the presence of the largest freshwater invertebrate in Antarctica (*Branchinecta gaini*) is reported in the lakes of James Ross Island. In some lakes, this species appeared to have attained relatively large population sizes. This is intriguing, given that previous paleolimnological studies have revealed its present during the Mid to Late Holocene climate optimum in the region, but also that it likely disappeared during the Late Holocene (Björck et al. 1996). This chapter thus calls for new paleolimnological studies on the lakes in order to assess whether the presence of *B. gaini* is the result of a recent recolonization event, potentially in response to climate warming as revealed in ice cores (Mulvaney et al. 2012) and lake sediment cores (Sterken et al. 2012) from the region. It also provides the rationale for new phylogenetic studies of this taxon in order to identify its population genetic structure and potential source region. The latter can be other lakes in the Antarctic Peninsula but also in Sub-Antarctic South Georgia or even Southern South America.

The following seven chapters (except chapter 7 and 9) are taxonomic studies of phototrophic microbes belonging to different phyla, including diatoms, green algae and cyanobacteria. The fruitful collaboration between Dr. Nedbalová and taxonomic experts in these different groups resulted in the discovery and description of a number of species new to science. This added new and additional evidence to the debate on potential endemism among microorganisms in Antarctica. This is not only interesting from a scientific point of view as it calls for a revision of the so-called ubiquity hypothesis among microbes (Baas-Becking 1934), but also in the light of protecting Antarctic habitats in times of increased anthropogenic influence and climate changes (Chown et al. 2017).

In the 4th chapter, two marker genes are used to study the two strains of planktonic green algae obtained from the lakes. This is combined with (i) microscopic observations of the strains, (ii) the analysis of their fatty acid composition, and (iii) growth experiments to reveal their temperature tolerance. An interesting result is the relatively high concentration of PUFA's in these strains, making them potential candidates for biotechnological applications at low temperatures.

In chapter 5 differences in the diatom communities in the lakes and streams in James Ross Island are related to environmental properties and put into a wider context of the bioregionalisation of the diatom flora in the Antarctic Realm. In addition, a transfer function for conductivity was developed. One critical note on this transfer function however is its relatively low jack-knifed R^2. This might be related to the relatively short gradient in conductivity. However, this clearly doesn't undermine the general quality of this chapter. More in particular, I strongly believe this chapter forms an important contribution to the knowledge on the diatom flora of the Antarctic, because the region appeared to be situated at the boundary between the two main biogeographic regions generally identified based on the distribution of macroscopic organisms, namely Maritime Antarctica and Continental Antarctica (Chown & Convey 2007). The occurrence of a number of species previously believed to be confined to Continental Antarctica is intriguing. Importantly, the most recent taxonomic insights were used for developing this dataset. I am therefore convinced that this dataset will provide new insights into biogeographic patterns in diatoms in the Antarctic Realm, once integrated with existing datasets from other Antarctic regions (e.g. Sterken et al. 2016, Tavernier et al. 2014).

In the 6th chapter three new Cyanobacteria species are described based on both morphological analysis and sequencing of their 16S rRNA genes. As mentioned above, this provides new evidence for the existence of endemic microbial species in Antarctica. Interestingly, the three species might even be endemic to Maritime Antarctica, suggesting that the general biogeographic scheme recognized in macroscopic organisms (Terauds et al. 2012) might be also present in Cyanobacteria. This is important given that Cyanobacteria form the base of the food-web in most Antarctic lakes.

In the 7th chapter several chemical identification techniques were used to study the presence of unusual molecular species of N-acylphosphatidylethanolamines. I am not expert in this discipline, so I am unable to assess the contribution of this paper to this field of research. The chapter is published in Phytochemistry which is a Q1 journal in plant sciences (34/212), so I assume it is an important paper.

In chapter 8 the unusual occurrence of a branched filamentous green algae belonging to the genus *Hazenia* is reported from the lakes in James Ross Island. It was brought into culture and described as a new species to science. This is an interesting result and calls for more detailed studies of chlorophytes in Antarctica. Importantly, this can add new evidence on the existence of endemic taxa within the Chlorophyta as was previously also detected in coccal green algae

(De Wever et al. 2009). The unusual ecology of the species is quite interesting, because it occurred in the littoral zone of the lakes; a habitat which is generally dominated by Cyanobacteria in Antarctic lakes but not by green algae.

In chapter 9, the occurrence of calcite precipitating microbial mats is reported. The microbial mats were studied using thin section analysis and microscopy. The chemical composition of the calcium carbonate spicules was also studied. This is the first report of such a community and therefore interesting. Particularly the preservation of these spicules in lake sediments might hold an interesting application in paleolimnological studies of Maritime Antarctic lakes. More in particular, these spicules might be a proxy that can be used to detect the presence of these communities, which will provide additional information obtained from the analysis of traditional proxies, such as diatoms and fossil pigments.

In the final chapter of part I, a *Monoraphidium* species isolated from Antarctica was grown in an outdoor medium-sized bioreactor under early winter conditions in Central Europe. This experiment revealed the potential of cold-adapted species isolated from polar regions for biotechnological applications. The high concentration of specific PUFAs make the strain even more promising for such applications.

Part II: Snow algae

The first chapter is a review of green algae occurring snow, which was published as a chapter in the book 'Algae and Cyanobacteria in Extreme Environments'. An overview of the different life forms and their ecology and ecophysiology is given. It ends with a summary of their geographic distribution. While this is not a paper published in an international journal, I believe it forms an important contribution to the topic. Testimony for that is that the chapter is cited on the Wikipedia page regarding snow algae.

Chapters 2 to 4 provide inventories of snow algal communities from different regions, including the Bulgarian Vitosha Mountains, parts of the Andes in Ecuador and the Giant Mountains in Czech Republic. While these studies are merely descriptive, they proved to be valuable. For example, five new species for Bulgaria were reported in chapter 2. In chapter 4 different species are linked to specific environmental conditions, including nutrient concentrations, light conditions and temperature.

In chapter 5 the PUFA content in *Chloromonas brevispina* isolated from a snow field in a Bohemian forest was studied. It revealed the presence of short and medium-chain PUFAs and as such added new data to the knowledge on the presence of PUFAs in chlorophytes. This is important, given that most studies are/were focussed on the widely studied genus *Chlamydomonas*. The relatively high content of PUFAs (>75%) suggests that the production of these lipids might be involved in the adaptation of this organism to live at low temperatures. This evidently calls for further research.

In chapter 6, a new method is presented for the rapid screening of the total lipid content of algae and identifying the different forms of natural TAGs without a preceding purification or separation step. I am not an expert in the field of lipidomics, so I cannot really assess the contribution of this chapter to this research field. However, I noticed the paper was cited in a review regarding lipidomics in archaebacteria, bacteria, yeast, fungi, algae, plants, and animals.

In chapter 7 a polyphasic approach involving microscopy, DNA sequencing, and lipid and pigment analysis was used to study a snow algae previously described as *Scotiella tatrae*. However, the taxon appeared to be closely related to *Chloromonas nivalis* and was therefore transferred to the latter taxon as a subspecies. Despite this, *S. tatrae* appeared to differ in astaxanthin production and the number of aplanozygote cell wall flanges compared with *C. nivalis*. The study highlighted the need for polyphasic approaches to reveal the true diversity of microbial communities in cold environments.

Chapter 8 is an ecophysiological study on the temperature dependence of the photosynthesis and thylakoid lipid composition in a *Chlamydomonas nivalis* species. Together with chapters 9 and 10, which are on the identification of esters in the pigment astaxanthin, these topics are out of my field of expertise. Hence, I cannot really assess the importance of these studies. However, the three chapters were published in high quality journals within this discipline, namely FEMS Microbiology Ecology, and Phytochemistry, which are ranked as Q2 and Q1 journals in Web of Science, respectively.

Chapter 11 provides a proof of concept to use a portable Raman spectrometer to detect the presence of carotenoids (mainly astaxanthin) and hence photosynthetic algae in snow fields. The results obtained using the portable device were tested against data obtained using a laboratory Raman spectrometer and microscopy screenings. The chapter was published in the journal Astrobiology as this approach might be potentially used to study the presence of photosynthetic organisms on other planets.

Part III: acidic habitats

In chapter 1, the biology and physical and chemical limnology of an extremely acidic lake in Czech Republic are described. This involved measurements and sampling of the water column at a 6-weeks' time interval during an entire year. A multitude of abiotic and biotic variables were analysed, which makes the dataset very interesting. Although these kind of studies are merely descriptive, they are very useful case studies of how highly impacted man-made lakes can evolve into extreme and unique habitats, characterized by a particular flora and fauna.

The second chapter is a follow-up of the first one and was aimed at studying the dominant phytoplankton species present in Lake Hromnice, as well as L. Plesné. It involved microscopy and DNA sequencing, which revealed the presence of a new species in the latter lake. The

dominant phytoplankton taxa appeared to be chlorophytes belonging to the genus *Coccomyxa*. Interestingly, the capability of *Coccomyxa* to live in acid habitats evolved within different *Coccomyxa* species. The study clearly highlights the lack of taxonomic data within this genus.

In chapter 3 a microbial red algae isolated from a coal spoil heap was studied using DNA sequencing and morphology. The sequence data were compared with taxa found in extremely acidic geothermal environments elsewhere. The study is interesting for at least three reasons. First, red algae remain poorly studied in terrestrial and lacustrine systems worldwide. This is because they are often absent in these ecosystems, with the exception of acidic environments and fast flowing streams. Most red algae studied to date are typically marine. Second, the occurrence of the *Galdieria* species in this environment is interesting, because it was so far only reported from acidic geothermal habitats. Third, given the discontinuous geographic distribution of these environments, the *Galdieria* species can be used as a model system to assess the genetic structure of microorganisms in function of geographic distance. The phylogenetic tree (Fig.6) is in this respect interesting as it clearly shows the existence of different lineages being present on the different continents. It is therefore a nice example of potential dispersal limitation in microorganisms, as was similarly observed based on the diatom data in part I.

In chapter 4 species specific differences in phosphatase activity in dinoflagellates and variations between three acidified lakes are reported. I particularly appreciated the multivariate analysis which revealed the importance of Al and pH in explaining these differences. However, this also highlighted the need for additional data regarding e.g. trophic status of the species, grazing and light availability.

Chapter 5 is a classical study aimed at assessing the response of benthic diatoms to sewage pollution on mountain streams. Benthic diatoms are ideally suited for this purpose and widely used as biological indicators (Smol et al. 2010). The replacement of natural assemblages by pollution tolerant species that are often found in downstream regions is worrying. Hence, the study highlighted the potential influence of even small scale human activities on the water quality of these ecosystems. This is important, because the effect of these activities is often neglected.

In chapter 6, the results from an interesting long-term monitoring dataset from a German lake are presented. The lake became acidified in the 1980's and only slowly recovered afterwards. A forest die-back reversed this recovery. The dataset is impressive as it contains data on both the abiotic and biotic conditions in the lake since the early 1980s until 2012. The shift in phytoplankton abundance in response to changes in P concentrations and availability is interesting, as well as the shift in zooplankton community structure. As such, it is a nice study on the effect of acid rain deposition and catchment-scale processes on the functioning of lake ecosystems.

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Chapter 7 is related to the previous chapter and integrated monitoring data obtained from eight glacial lakes during a 12 year's period. The study combines biological data and measurements of abiotic conditions. The data revealed the partial recovery in 50% of the sites studied. The response of the lakes after decreased acid rain deposition depended on the Al concentration, both directly as indirectly. The food webs appeared to be still completely different compared to the pre-impact situation. I really appreciated the parts regarding food web effects on the recovery rate of the lakes and the effect of dispersal limitation on the recolonization potential of zooplankton. The study also highlighted that recovery not allows follows a predictable trajectory, but that lake specific differences in e.g. food web complexity largely influence the response of the lakes.

Chapter 8 is a descriptive study on the limnology of two Polish lakes with a pH of ~5. As with the other similar studies in this work, the datasets are interesting because they combine measurements of different variables (both biotic and abiotic). The results obtained were compared with existing paleolimnological records based on diatoms and cladocerans remains. The presence of a population of *D. pulex* in one of these mountain lakes is interesting, given that this species (flock) is generally restricted to low altitude water bodies. The study therefore calls for developing molecular phylogenies of this taxon.

In summary, this is a very interesting piece of work, which consists of 29 chapters published as a book chapter or in peer-reviewed journals, of which the majority are ranked as Q1 or Q2 in Web of Science. Most papers cover different disciplines and the microorganisms are often studied using a polyphasic approach involving microscopy, DNA sequencing and metabolomics. While covering a wide range of habitats, including lakes in the Antarctic Peninsula, snow fields in European and South America, and acidic lakes in Central Europe, the work was well-focussed. Each separate part contained a concise, yet complete description of the habitats and study region. The main focus of the work was put on the diversity, ecology and ecophysiology of photosynthetic algae such as Cyanobacteria, diatoms and green algae. Interestingly, the taxonomic inventories of these organisms resulted in the discovery and description of species new to science in all groups studied, underscoring the unique nature of these habitats. As such, it provides an interesting contribution to the long-standing debate on potential endemism in microbial organisms. The presented work is by any standard adequate for an associate professor position.

Sincerely yours,

Prof. Dr. Elie Verleyen

24/04/2018

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