13 January 2020

Adam Petrusek, PhD
Chair of the Committee for Lenka Procházková
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Dear Dr. Petrusek:

I was most gratified to be asked as an evaluator for Mgr. Lenka Procházková's doctoral thesis, "Exploring the Diversity of Snow Algae using a Polyphasic Approach." I just completed writing a major twenty-year update Review article with co-author Daniel Remias from Austria on "Snow and Glacial Algae" that will be published in the Journal of Phycology in April 2020. From writing this Review, I am very familiar with Lenka's research. Of her ten scientific papers on which her doctoral thesis is based, the first nine are in our Review, and the last one came out too late to be included.

First, I want to compliment Lenka for her professional achievements publishing ten manuscripts on her work with snow algae prior to getting her doctoral degree. All her research papers with highly respected co-workers illustrate the depth of her background, and her publications have appeared in very respected peer-reviewed journals. I see her as one of two broadly trained scientists (the other being Daniel Remias from Austria) to lead the future of snow and glacial algal research worldwide. Her research is polyphasic with backgrounds in systematics, distribution, life cycles, field ecology, physiological adaptations, electron microscopy, genomics, lipidomics, specialized techniques (high throughput sequencing, Raman micro-spectroscopy, and high-performance liquid chromatography), and the consequences of climate change and global warming. Lenka shows great self-confidence and has received excellent guidance and direction from her PhD advisor, Dr. Linda Nedbalová.

Second, I want to point out that Lenka has not only disseminated her research through the above-mentioned publications, but through very active attendance at professional meetings giving many oral talks and poster presentations. I met Lenka in October 2018 at the Second International Snow Algae Meeting (SAM) held in November 2018 in Potsdam, Germany. It was there I saw her give an oral presentation and had opportunities to discuss snow algal research with her.

Third, in reading Lenka's thesis it was like going through our Review accepted for publication in the Journal of Phycology because all the topics presented by Lenka are also in our Review. The main difference being our Review is a comprehensive update on both snow and glacial algae since the year 2000 and Lenka's thesis includes many historical papers on snow algae not in our Review. The way in which Lenka organized her thesis is very logical, easy to read and follow, and the flow of the reading is well organized as to how one might think of snow and glacial algae. I could not agree more in her opening section 1.1, "Why to work with snow algae?" The last sentence of unexpected surprises is certainly a highlight, as I was reminded of my own experiences seeing a wolf after doing field work in Washington State, having a night hawk land on my left shoulder at my field work site on Whiteface Mtn. Adirondacks, NY, and a snowshoe hair walk over my hiking boots while eating dinner in a lean-to at the same site in NY.

In the organization of her thesis, Lenka has very carefully incorporated her ten publications into the Table of Contents outline, 1.1-1.6, which is further evidence of her polyphasic background with snow algae. From here I will give comments by thesis page numbers of what I might have added or changed to what she presents, and I will not go into detail of what she has published in these peer-reviewed manuscripts. Before I do this, I want to reiterate that I have the highest praise for what she has accomplished to date as a graduate student.

Page 4, pH section at bottom, I would have added the two references below on optimal pH data from the lab correlated with field pH readings for the snow algae, *Cr. tughillensis*, *Cr. chenangoensis*, and *Cr. rosae* v. *psychrophila* (Hoham et al. 2007). Also, *Cr. rosae* v. *psychrophila* strains isolated from the Adirondacks, NY, appear to have adapted to increased acidity in the northeastern United States compared to strains isolated from the White Mtns, AZ, where the pH is less acidic (Hoham & Mohn 1985).

Hoham, R. W. & Mohn, W. W. 1985. The optimum pH of four strains of acidophilic snow algae in the genus *Chloromonas* (Chlorophyta) and possible effects of acid precipitation. *J. Phycol.* 21:603-9.

Hoham, R. W., Filbin, R. W., Frey, F. M., Pusack, T. J., Ryba, J. B., McDermott, P. D. & Fields, R. A. 2007. The optimum pH of the green snow algae, *Chloromonas tughillensis* and *Chloromonas chenangoensis*, from Upstate New York. *Arct. Antarct. Alp. Res.* 39:65-73.

Page 7, snow blooms, twice yellow algae are mentioned and an example is given in Fig. 1e on page 8. There are no yellow algae, and this has unfortunately been used in recent papers describing colors of snow algae. There are yellow-green algae (Xanthophyceae) where cells are green to greenish-yellow, but not yellow. The algae shown in Fig. 1e are golden brown (Chrysophyceae) due to the pigment, fucoxanthin, and not yellow like found on a banana peel. I suggest this be corrected in future mansucripts. All other colors given on p. 7 match with those shown on page 8, Fig. 1.

Page 9, at top, Lenka is first author (paper I) in what may be one of the most historic reexaminations of snow algae addressing red and orange cysts of the newly described genus, *Sanguina*, where red cysts were assigned to *S. nivaloides* and the rarer orange ones to *S. aurantia*. This was the first major attempt to address the problem of interpreting *Chlamydomonas nivalis*, the universal and ubiquitous snow alga to which most red cysts have been placed historically. The number of samples used indicates *Sanguina*'s worldwide distribution and suggests strongly that many previous if not most red snow samples placed in *Cd nivalis* most likely belong to *Sanguina*, even though it is not possible to go back to many original collections including the type material. For that reason most of these historic samples must retain the name *Cd. nivalis* to which they were assigned. Interestngly, molecular analyses reveal that *Sanguina* forms a single lineage within the green algal order, Chlamydomonadales, and is well separated from both *Chlamydomonas* and *Chloromonas.*

Page 9, at bottom, this color problem with yellow algae continues when it is stated that "Less frequently reported flagellates in snow are golden algae (Czosnowski 1948, Hindák 1969). A new species causing yellow blooms in snow was described as *Kremastochrysopsis austriaca* Remias, Procházková & R. A. Andersen." This is contradictory because in the first sentence they are called "golden algae" but in the second sentence "yellow algae." Again, these are chrysophytes, which are golden-brown, and not yellow.

Page 10, top, where it is stated that "Rarely reported flagellates in snow include euglenoids (Hoham & Blinn 1979), cryptophytes (Javornický & Hindák 1970) and dinophytes (Gerrath & Nicholls 1974)." Add (Moestrup et al. 2018) after (Gerrath & Nicholls 1974) for a dinophyte update.

Page 11, geographical distribution, for North America, Hoham & Blinn (1979) should be added because this distribution paper covers a large expanse in the American Southwest (Colorado, Utah, Arizona, and New Mexico) not covered in other references.

Page 11, *Cr. nivalis* v. *tatrae*, in your manuscript IV where it is discussed that this variety produces *Scotiella tatrae* like cysts as does *Cr. pichinchae* from Washington state (Hoham 1975). (I used variety here instead of the published subspecies before *tatrae* because I thought for algae variety is always used and not subspecies, which is used for animals). Since the former is in open exposures causing orange snow and the latter under tree canopies causing green snow, is this another example of a polyphyletic *Scotiella* like cyst as is the case for *Cr. nivalis* and the asexual cysts (formerly *Scotiella cryophila*) of *Cr. rosae* v. *psychrophila* and the *Scotiella psychrophila* K-1 cell type found in Austria?

Page 14, top paragraph, where *Chloromonas* and *Chlamydomonas* are spelled out but in the bottom of the same paragraph *C. nivalis* is used. This is confusing because there is both a *Chloromonas nivalis* and a *Chlamydomonas nivalis*. It would have been clearer throughout the thesis to have used when needed *Cr.* for *Chloromonas* and *Cd*. for *Chlamydomonas* as has been done in most publications with these snow algae. If *Chlainomonas* is included, then *Ch*. has been used.

Page 17, Fig. 2, what is the evidence that the cysts of *Cr. nivalis* are being wind distributed versus remaining lodged to ground cover and found basically in the same localities from year to year? Certainly, cysts can become air borne, but what percent of the total population is this? From my experience, populations of snow algae basically remain in the same localities year after year.

Page 18, line 7, add Hoham et al. (2006) to the list because in that publication nitrogen depletion combined with blue light enhances sexual reproduction in both *Cr. tughillensis* and *Cr. chenangoensis*. Or perhaps added as a separate statement since blue light is not mentioned in the paragraph.

Page 18, Fig. 3, upper right corner. The implication is that these cysts will be wind distributed and nothing is mentioned that most of them will remain on the soil or ground cover throughout the summer where they are covered by new snow in fall and winter. Then they germinate the following spring in essentially the same area as the previous year's population.

Page 18, Fig. 3, bottom, it is implied that meiosis or mitosis in these cysts occurs in the spring. Look at Hoham (1975) where meiosis is described in detail for *Cr. pichinchae* where many of the divisions occur in the fall after a hard freeze and dormancy period prior to new snow cover in the fall. To my knowledge no one else has ever followed how this happens by returning to the population site every month after snow melt until new snow covers the area.

Page 20, paragraph 2, food chains and food webs in snow are shown in detail at the microscopic level (Hoham & Duval 2001) and macroscopic level (Aitchison 2001) with figures. Both book chapters are in the Snow Ecology reference (2001) and would be good additions here.

Page 24, green snow in Antarctica with high protein content, it might be of interest that Bidigare et al (1993) reported that green snow in animal rookeries with high nutrient concentrations were probably protected from excess UV damage by producing apoproteins.

Page 25, bottom, it is stated that "The glycerolipid and fatty acid composition was similar between green and red blooms, which is consistent with the classical hypothesis that a high degree of fatty acid saturation is related to membrane stability at low temperatures (Bidigare et al. 1993)." I may be misinterpreting this statement, but in Bidigare et al. (1993), Table 3, the FA composition is very different between green (mostly saturated) and red snow (mostly unsaturated). I was involved in this study and the differences in FAs between the two snow colors became apparent when Bob Bidigare shipped samples from Antarctica to Texas on dry ice and the green samples did not survive because of the saturated fatty acids whereas the red cells did due to unsaturated FAs. The collection of new snow samples was repeated the following year and shipped using water ice.

Page 28, line 2, use of *Scotiella cryophila* Chodat K-1 infers that this is a valid species followed by a strain number K-1. All *Scotiella* and *Cryocystis* found in snow to date are resting stages in species of *Chloromonas*, and this is the case with *Scotiella cryophila*, which is the asexual resting stage of *Cr. rosae* v. *psychrophila* (Hoham et al. 2002). I realize that the *Scotiella cryophila* cysts found in Austria fall into a different clade than does *Cr. rosae* v. *psychrophila* from the Adirondacks, NY, and White Mtns., AZ, which implies this taxon is polyphyletic. However, the cysts in all localities are identical and the Austrian cysts should be referred to as *Scotiella cryophila* K-1 cell type to indicate it is not a valid species. It was good to see that Barcytė et al. (2019), EJP, called these cysts *Scotiella cryophila* K-1 cell type in their phylogenetic trees.

Page 32, paragraph 2, line 5, the parenthesis to the left of Lutz should be removed and again to the left of Lutz on Page 33, line 5.

Page 33, it is stated that "there is no existing laboratory strain of the most prominent snow and glacier algae such as *Sanguina nivaloides* or *Ancylonema nordenskiöldii* and *Mesotaenium berggrenii* (Williamson et al. 2019)." This will be one of the biggest challenges facing Lenka and other workers with snow and glacial algae to figure out the solution getting these algae in culture.

Pages 33-34, transcriptomes, the metagenomes are known for the two snow algae given here, *Chloromonas brevispina* UTEX SNO96 (J. A. Raymond 2014) and *Kremastochrysopsis austriaca* DR75b (Raymond & Remias 2019). The transcriptomes are known for three other snow algae, *Cr*. t*ughillensis* and *Cr. rosae* v. *psychrophila* (1000 genome project) and *Chromulina chionophilia* (K. Terpis, University of Rhode Island). All five of these taxa are discussed in our Review as indicated (Hoham & Remias, 2019, doi:1111/JPY.12952).

In summary, it is not clear to me if Lenka can incorporate any of my above suggestions into her PhD thesis at this late date. If so, they would be good additions. If not, this should not prevent her from receiving her PhD at Charles University. With great pleasure, I strongly support Lenka Procházková to receive the PhD for her very professional work and research on snow and glacial algae that she has presented.

Sincerely,

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USA