A review of the diploma thesis of Tereza Šamšulová

Green microalgae in polar lakes: diversity, biogeography and methodological comparison

Tereza investigated a diversity of the green algae of polar lakes using culture Sanger sequencing and amplicon sequencing (ITS2 and 18S rRNA markers). She gathered a huge dataset of strains and environmental samples. She applied common methods to less studied organisms and environments. Tereza found that Arctic green algae had higher diversity than Antarctic. The diversity of ITS2 revealed a relatively high number of putative endemic taxa. No identical sequences were found in the culture Sanger sequencing and amplicon sequencing. Tereza's work provides a significant step forward to our understanding of microbial diversity and distribution. I hope that it will be published soon.

Some specific comments and questions are listed below.

- The introduction provides quiet comprehensive overview of global patterns in microbial diversity with a focus on green algae and methods. Some important aspect are missing: P 24, there are several other methods of strain isolation such as flow cytometry sorting. P 25, you could mention third generation sequencing.
- P 31. What was the length of the PCR products using each of your primer sets? You mention that assembled sequences were between 220 to 400 bp. Were the resulted sequences of such significantly different lengths? Ribosomal RNA tends to have insertion and deletion but not that long. In addition, you mention that the minimal sequence length was 220bp. This is confusing.
- P 32. A technical note. All R packages and programs need to be cited including their version so that the analyses could be repeated.
- Fig. 15. Some Svalbard samples were mixed with Antarctic. Did these localities have anything in common? Were the same Svalbard localities close to Antarctic in the ITS analysis?
- P 49. In the 18S analyses, incertae sedis were rare, in the ITS quite frequent. Why is that? What could they be? Is the database, you searched, so incomplete?
- P 57. It should be noted that mentioned species are ubiquitous as the variability of the molecular markers allow. Deeper sequencing using whole-genome or RADseq could uncover some geographical differentiation.
- Studies comparing cultured material with environmental sequencing are indeed rare. Nevertheless, they often show that cultured taxa diversity overlaps only partly with the environmental sequencing.

Altogether, Tereza's thesis represent an impressive piece of work from both experimental and data presentation point of view, so I can happily recommend it for the defense.

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