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Prof. RNDr. Jiří Neustupa
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Re: report on the dissertation of Mgr. Ivana Černajová

To the chair of the examining committee:

I have read the thesis of Mgr. Ivana Černajová and enclose my report. I have no bias or conflict of interest to report.

Overall the thesis was a pleasure to read. Mgr. Černajová has clearly invested a lot of work in the five papers/chapters included in the thesis and exhibits a sufficient understanding of the background, methods and theory of their field. Overall, the thesis is concerned with symbiotic surveys in the lichen system, broadly defined. Two of them concentrate on surveys of algal symbionts in seashore habitats, one on surveys of basidiomycete yeasts in *Cladonia*, one on trying to find the cause (including possible accessory symbionts) for an unusual morphotype in *Cladonia* and one on laboratory culturing of canonical symbionts, with observations on accessory symbionts. Perhaps the results of the thesis that will have the biggest and longest impact on the field are the discovery of the predominance of ulvophyceean symbionts in seashore lichens, at a scale not previously documented.

Overall the thesis is well written and professional. The candidate clearly has a good grasp on the construction of scientific articles, and I found all of the five chapters easy to follow. Unfortunately the English was somewhat problematic and clearly had not been thoroughly edited by anybody who speaks English as a mother tongue, but it was seldom a problem for understanding the content. One of the deficiencies I have slightly less understanding for is the high frequency of spelling errors. This is not a result of speaking English as a second language, as misspellings include many Latin names and even the family name of one of Mgr. Černajová's co-authors, twice. I have placed comments on the spelling mistakes that I noticed on a single read of the thesis, but it requires a careful follow-up check to clean it up.

I have a few areas of specific criticism of the scientific content. One concerns the use of citations. My attention was first drawn to the candidate's citations of some of my own work to support claims or statements that I did not make, such as that basidiomycete yeasts are obligate symbionts of lichens. I have found this to be the case of citations of other works as well, including crediting authors with statements or work they did not say or do. This is not fatal, and can be corrected, but it suggests that the candidate has not paid close attention to the statements and claims in scientific papers, is treating them loosely, and/or has not distinguished these from hearsay or secondary impressions about those papers. It is also worrisome because I cannot assess the validity of every cited statement, and it calls into question other citations. This is a bad habit and must be nipped in the bud.

Another area I would like to address, and follow up on with the candidate at the exam, is whether they feel that their methods were really appropriate for the questions asked. I was confused by the acknowledgement, on the one hand, about the limitations in application of amplicon barcoding and Sanger sequencing, and the length to which the candidate goes to try to read into the meaning of the results, on the other. This applies to both the study on yeasts and the two studies on algae. I would have appreciated a much more robust discussion of the known room for error and the propensity of these methods to miss organismal components for which primers do not bind, and what that means specifically for the reliability of the candidate's results.

Along the same lines, I was surprised to see the primer combination used for ITS the yeast study, as this was one of the less successful combinations in my experience. How much a priori testing was there of primer combinations, and might the used primer combination have to do with low detection rate?

Finally, I could not help but notice the difference in the way yeasts and algae were treated in the different discovery studies; yeasts were treated with skepticism vis-à-vis whether they were symbionts, but if cells had chloroplasts, they were automatically called symbionts. I want to explore with the candidate the current mainstream thinking on symbiosis theory and goods and services exchange in microbial organisms; what the expectations are of a symbiont or a symbiosis; and whether symbionts check a box or perform a function. Expect me to ask a lot about function at the defense.

As I said above, none of these shortcomings are fatal; to the contrary, the candidate clearly did a very good job overall on the thesis and gained the kind of experience one would expect in our field for a Ph.D. candidate. In summary, I think the dissertation of Ivana Černajová fully deserves to be recommended for the thesis defence. Please accept the annotated PDF of the thesis with ~155 comments meant to help the candidate in making revisions.

Best regards,

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