

Review report on PhD thesis entitled

'Diversity and phylogeny of symbiotic partners in zeorin-containing red-fruited Cladonia species'

by

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Jana Steinová submitted a PhD thesis which encompasses several aspects of the lichen symbiosis, using the zeorin-containing red-fruited *Cladonia* species as model group. The concept of the lichen symbiosis has suffered important changes during the last decades, adding more relevance to the, often neglected in the past, photosynthetic partners and including the study of other organisms associated to the symbiosis like bacterial communities. Jana's thesis takes into consideration this new perspective on the lichen symbiosis and using a well-known group of lichen-forming fungi (although not taxonomically well understood) and proposes an holistic research which ranges from the molecular-based species delimitation of the fungal partners, the characterization of the diversity of photosynthetic partners associated and the study of the microbial communities typically found on the surface of these species including state-of-the-art microscopic techniques.

Main goals of this doctoral dissertation were: 1) to test using molecular markers the current species delimitation of the taxonomically difficult group of red-fruited *Cladonia* species, 2) to disentangle the puzzling taxonomy to the photobiont genus *Asterochloris*, typically associated with *Cladonia* species; 3) to test whether reproduction and dispersal strategies shape the diversity of photobionts associated to the red-fruited *Cladonia* group; 4) to shed light on the effect of abiotic variables on the diversity and abundance of microbial communities associated with different lichens, including *Cladonia* species.

First chapter introduces the main questions of the thesis and provides an overview on key questions addressed in this PhD as changes on the concept of lichen symbiosis, the species delimitation problem in lichen-forming fungi, intertwining the general ideas about species concepts in biology with the problems found in lichens as well as short review on the main methods using molecular data to delimit species. Objectives are summarized in

chapter 2 and conclusions of the research are given in chapter 3. Finally, four papers, three of them already published and one submitted are found in chapter 5.

Questions for the defense

- Paper 1: The presence of a higher amount of bacteria in senescent areas than in healthy ones and an apparent higher diversity of bacterial phyla regarding healthy areas, which show a predominance of Alphaproteobacteria, seem to point an active control of bacterial communities carried out by the lichen symbiosis. Which mechanisms do you think the fungus or the whole system could use to control their associated populations of bacteria?
- Paper 2: What are the chances for an ecological-driven morphological pattern in this group of species? Have you or other researchers tried this hypothesis? Do you think that another symbiont may be blurring the taxonomy of this group?
- Paper 2: Some of the species analysed have wide distribution ranges out of the area studied, do you think that expanding the taxa sampling could improve the species delimitation or, on the contrary, would add more noise?
- Paper 3: Differences in genetic diversity among *Asterochloris* species is striking. Do you think that the observed variability correspond to actual intraspecific variability or it may be pointing to the presence of higher diversity not identifiable with the used morphological tools?
- Paper 3: Most of the characters used to delimit species in *Asterochloris* are only useful associated with the frequency in which they appear in certain species and when all are used together. Do you think a similar approach could be used to delimit species in another difficult group as the red-fruited *Cladonia*? or the problem there goes beyond such a 'simple' solution?
- Paper 4: The higher specificity towards their symbionts by asexually reproduced species is attributed to differences in size and quantity of propagules, what about the very different conditions photobionts suffer in a non-corticate structure like soredia vs a more protected one like granules, perhaps more species are able to live in the likely milder conditions found within the granules.
- Paper 4: Do the species with lower specificity (asexual ones) have smaller distribution ranges or narrower environmental niches?

Conclusion

To summarize, it is worth to note that: 1) the thesis is very well written without any major shortcomings, 2) the author has carried out a critical review of the literature available on the topics treated and demonstrates along the manuscript that she masters it, 3) during the term of his PhD thesis candidate Jana Steinová has clearly acquired proved skills that



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will help her to continue a career in science such as the identification of lichen forming fungi species (i.e. *Cladonia*, a well-known taxonomically difficult genus), useful technical skills such the tools to carry out phylogenetic analyses and molecular based species delimitation, microbiological skills such microscopic techniques for bacterial in-situ visualization, and 4) Jana has worked in several renowned research teams during her PhD, showing the ability to work in group.

According to my point of view this thesis manuscript meets the standard requirements imposed on a dissertation in the field and its quality fulfills the criteria necessary for obtaining the PhD degree, so I clearly recommend its acceptance.

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