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Evaluation of the PhD. thesis

„Complexity of lichen symbiosis“

by Ivana Černajová

At the beginning the thesis is summarized to include the introduction, objectives, main results, conclusions and references. This synthesized part refers to and is followed by the core of the PhD thesis, series of four published papers in relevant WOS-journals, as well as one manuscript standing before submission. Ivana is the main author of all the papers and it is worth pointing out, that apart from designing and writing the manuscripts, she was also largely involved in the practical lab work and evaluation of the results. All the papers are co-authored by her supervisor Pavel Škaloud, and three of them – in addition – co-authored also by the colleagues from *‚alma mater‘* University – Jana Steinová, Jana Schmidtová and Zuzana Škvorová, as well as foreign collaborators – Ulf Schiefelbein (twice) and Francesco Dal Grande. Practically all the papers present some important novelties beyond the fields of lichenology and algology. Alternative methodological approaches can serve as a good background and inspiration for the research of further scientists. The main results are highlighted in abstracts and conclusions of each paper.

Formally the work is almost excellent, written straightforwardly, with illustrative photodocumentation and practically without typo-errors (not considering the last, not yet sumbitted manuscript :-)).

Statements followed by questions:

In Paper 1 surprising omnipresence of associated yeast fungi was demonstrated in terricolous (in part saxicolous) cup-lichens (the genus *Cladonia*). Routinely putting them into culture is an achievement in itself. Molecular data (supported by isolated cultures) also showed that the majority of the discovered yeasts belonged to the family Microsporomycetaceae, previously known mainly as vascular plant-associated fungi. The most abundant yeast member was described as a new genus and species: ***Lichenozyma pisutiana***. The naming and ‚showing‘ of the fungus (previously in Genbank as an unknown member of Cystobasidiomycetes) was a meaningful step, facilitating subsequent workers to recognise and discover the same and related species in a wider range of lichen groups (? solely) growing in other habitats. As was already noted by Ivana (p. 30 in her PhD. thesis), this species was subsequently found also in crustose lichens, epiphytic and saxicolous members of Lecanoraceae (Mark et al. 2020, Cometto et al. 2022).

I have two broader questions in relation to the paper 1:

i) ***Can you imagine that basidiomycetous yeast species have even a wider ecological range of their hosts and their habitats. Have you or anybody else checked their presence in the cortex of (semi-)aquatic / sea-shore lichens?***

ii) ***Do you agree with the arguments by Li and coathors (Li et al. 2020, Studies in Mycology 96: 78-79, who proposed to put Lichenozyma into synonymy Microsporomyces) about the incongruence of the datasets used, the choise of outgroups and missing relevant sequences in your phylogeny? Do you mean, can the genera in Cyphobasidiales be distinguished by other means than by molecular data?***

In Paper 2, you demonstrated the co-dispersal of basidiomycetous yeasts with soredia on the basis of the results of soredia cultivation experiments.

iii) ***Theoretically, could lichen-inhabiting basidiomycetous yeasts also be dispersed by invertebrates or even large animals (not considering the dispersal of soredia on the body surface)? Would it be feasible to design an experiment to test whether viable yeasts persist in pellets after passing the ingested lichen cortex through the diggestive tract of animals?***

I am targeting this question because of Ivana's earlier experience with experiments on lichen-feeding snails.

A small, by-side remark:

In introductory part, at the end of p. 16, it is stated: „Consequently, large monophyletic mycobiont groups (families, orders, subclasses or even classes) are conservative in their choice of photobiont type or even photobiont genera (e.g., *Trebouxia* in Umbilicariomycetidae, *Nostoc* in Collematineae, *Trentepohlia* in Graphidaceae; Rambold et al. 1998, Miadlikowska et al. 2006)."

Recent papers show that *Apatococcus* specifically may occur in *Fuscidea* (Umbilicariomycetidae) (Zahradníková et al. 2017, Protist 168) and within the genus *Graphis* it is demonstrated that more species of both *Printzina* and *Trentepohlia* can occur (Hametner et al. 2014, Phycol. Res. 62). It may have ben intended to refer to Collemataceae (and not to Collematineae), as Placynthiaceae and Coccocarpiaceae also belong within Collematineae and their species often contain other cyanobacteria than *Nostoc*.

Final assessment:

Ivana completed her doctorate under supervision of the respected personality in science, Pavel Škaloud, who through his enthusiasm and his head full of ideas can stimulate the interest of both starting and advanced students and who can also direct the work properly towards the right goal. The main credit now however must be given to Ivana who is also enthusiastic and her mind is full of ideas too. She is also creative, tenacious and independent.

For me this is an impressively wide-ranging thesis that succintly reflects its title – ***Complexity of lichen symbiosis***. It encompasses various aspects of the symbiotic nature of lichenized fungi, shedding further light on the hitherto poorly known inhabitants and exhabitants of the micro-ecosystem called ***lichen***, and their spreading. It reveals the identities of additional associated basidiomycetous yeasts, considerably extends the list of known symbiotic algae of border-line lichens, and also started to reduce the long list of Cladonia species. The implementation of routine culture techniques, precisely designed experiments, numerous and high quality data, sound conclusions, these are all high scientific attributes of Ivana's work and a good prerequisite for her further scientific career.

I declare no conflicts and at the same time warmly **recommend this thesis for the defence**!

Zdeněk Palice