

Review to the PhD thesis by Jana Steinová:

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

I'm honoured to be able to review such a good PhD thesis. The structure and clarity of the thesis will be perhaps appreciated by most readers. It has a long and well written introduction followed by four interesting papers.

In the role of the opponent, however, I must be critical and beforehand I apologize for a lot of criticism.

Let's start with a slight misleading in the title. The red-fruited *Cladonia* species, appearing in the thesis title, are only a very thin wire linking the four including papers as they are rather marginally employed in the papers 1 and 4.

#### **Comments/questions to the introduction:**

(1) You write that the lichen thallus can be maintained for several thousand years (without any reference). — I believe you, but has it ever been proved?

(2) You state that lichens are currently understood to be meta-organisms hosting many diverse cohabitants which contribute in different ways to the prosperity of the holobiont. — Has it ever been proved that some cohabitant, different from myco- and fycobiont, contributed to the prosperity of the lichen?

(3) You listed a lot of vegetative morphological characters that are used in phenotype-based species delimitation of lichens. — That is not truth for many lichens. Which lichens did you dismiss when writing this passage?

(4) You write: speciation should be regarded as a process and not as a single event in time.  
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I have quite a different opinion. Could you figure out some theoretical situation, when speciation is a single event in time?

(5) I'm happy to see the text about "modern" species delimitation methods: single-locus (e.g. SpedStem) and multi-loci (e.g. BP&P). — In my opinion, these methods are only a temporary fashion that hardly has an additional value to phylogenetic analyses. Could you explain how do these methods help to delimit species?

(6) I appreciate the holistic approach in the thesis, but some important aspects seem to be missing. Within the chapter on factors shaping diversity of lichen symbionts, there is no discussion about factors shaping mycobiont diversity. These factors could be hard to find within your model group, if most of the species co-occur in the same habitats and have very similar ecology. Could you suggest any drivers of their diversity?

## Papers 1 and 3

In my opinion, both papers represent very nice biodiversity studies. I do not have any questions regarding their content, because Jana did not play the principal role in their writing.

## Paper 2

I consider it an interesting and important study providing phylogenies of two DNA loci and mapping some phenotypic data on terminals of the phylogenetic trees. However, the data interpretation and taxonomic conclusion is, in my opinion, too buck-passing, not saying anything.

First, I have some questions:

- (1) You have the phrase "species delimitation" in the title, but the paper only little deals with it. Why you did not go further behind single-gene phylogenetic analyses? You had a possibility to test if your four species scenario (or any other scenario) is supported or not. You could do it by inferring a species tree as a hypothesis and test your hypothetic species delimitation by BP&P or another method.
- (2) You counted 31 parsimony-informative characters in both sequenced loci. Does it involve also outgroups? If so, how variable are your ingroups?
- (3) Did you count the differences in sequence data between sister clades? I wonder how many mutations make differences between clades (e.g. 1a and 4a in the B-tubuline phylogeny).

Second, I try to propose a scenario which is perhaps more simple and plausible than your complicated interpretations. I'll explain my point of view (the two-species-scenario) in following steps:

- (1) phylogenies of the two loci are really incongruent, but
- (2) ITS phylogeny shows quite reasonable tree with one supported lineage including only non-soresdiate lichens and two lineages with only soresdiate lichens,
- (3) the two lineages with soresdiate lichens have unresolved relationship in the ITS tree and they can form together a single species.
- (4) the two "traditional" soresdiate species and the two non-soresdiate species do not form any reasonable clade in any of the single-locus trees (unlike written in the paper, *C. diversa* is not an exception; see Figure 2B), and
- (5) both "traditional" species pairs are hardly recognized by phenotype data, so
- (6) we have a good reason to recognize two species only: (a) *C. deformis* + *C. pleurota*, and (b) *C. coccifera* + *C. diversa*,
- (7) why the data in B-tubulin show such a complicated pattern? Simply because it still does not show any species-level pattern. The supported clades are (in my opinion) only groups of sequences within one of the two well-recognised species.
- (8) There is still a chance that there is more than two species within the pool of sampled specimens, but we do not have any unambiguous molecular data to support it, so
- (9) if we like to recognize between the morphotypes (e.g. to recognize traditional species *C. diversa* from *C. coccifera*), we perhaps should use an infrageneric level for them.

If you do not agree with this scenario, your arguments are much appreciated.

#### **Paper 4**

A very interesting paper showing a pattern in photobiont selectivity between sorediate and non-sorediate populations of the *C. coccifera* group. I have few comments and questions:

(1) You call the sorediate specimens as "asexual" (also in the abstract and then throughout the text), but it is probably wrong or at least it was not tested. Could you provide data that sorediate species have lower production of apothecia (and pycnidia) than non-sorediate species?

(2) Further: could you provide any data that the production of spores in apothecia of sorediate species is reduced? Or do these spores show any characters of lowered viability?

(3) An interesting result could be but was not discussed: *Asterochloris* associated with sorediate specimens group with *Asterochloris* from numerous non-sorediate species and vice-versa. Paying attention to this fact would avoid misleading by the readers towards a biased causality that "photobiont identity supports formation of vegetative diaspores" or that "different photobionts are intentionally selected by sorediate vs. non-sorediate mycobionts".

Best wishes and looking forward to see You at the excellent defence,

9 August 2018,

Jan Vondrák