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**Review & overall assessment of the Ph.D. thesis submitted by Eva Holá, “(A)sexual life of liverworts” at the University of South Bohemia in České Budějovice, Faculty of Science, in 2015.**

The submitted Ph.D. thesis is a compendium comprising several chapters, a general introduction and three already published manuscripts. Finally there is a short conclusion.

The overall level of the thesis is a very good standard. The outcome of the thesis is based on intense field work followed by time-consuming microscopy and lab work. The applicant clearly demonstrated her ability to collect data in the field and in the lab, to select and use appropriate methods (for both ecological and molecular data) and to put the results in the broader context and framework of dispersal ecology of bryophytes. I especially appreciate following aspect of the thesis: (1) Author studied dispersal of liverworts using diverse methodological approaches (classical propagule quantification, observation of sex-specific pattern of vegetative/generative dispersal, and indirect evidence based on molecular techniques). (2) The thesis properly analysed microsatellite data and addressed aspects of both generative and vegetative dispersal.

There is a nice Introduction summarizing bryophyte life-cycle, reproduction modes and particularity of dioicous species. Last part of the Introduction reviews consequences of genetic diversity in bryophytes and its connection to reproduction mode in bryophytes. Conclusion at the end of the thesis is rather short and is structured in paper-by-paper manner instead of uniting all the concepts, approaches, results and discussion.

All three paper included in the thesis are already published or accepted for publication in international journals and underwent through journal's review process. Therefore my questions are either general or they are concerning the third paper of the thesis, which uses molecular data to estimate dispersal pattern and whose analyses and interpretations I am most familiar with.

**Specific questions:**

- (1) How did you select species to study? Is there any connection among selected species except of the fact that all of them are liverworts? Could you draw any general (not a species-specific) conclusion based on your results except of rather trivial statements ‘asexual propagules are formed in large quantities’? Or maybe this is not trivial either...

- (2) The first paper (gemma production in *Lophozia*) lacks clear hypotheses formulation at the end of Introduction. Could you formulate the tested hypotheses at least now? Or do you think that this paper is more or less only descriptive and does not require hypothesis testing?
- (3) On page 85 you write about direct and indirect approaches for studying plant dispersal. Could you generally review all theoretically available methods that are used in dispersal ecology (not just in bryophytes) and summarize their merits and drawbacks? Especially (but not solely), could you focus on direct and indirect approaches connected with molecular methods?
- (4) In paper III you tested linkage disequilibrium among microsatellite loci to distinguish between sexual and asexual reproduction within a population. Could you explain the theoretical concept behind the used test?
- (5) On page 76 you described selection of  $R_{ST}$ -like against  $F_{ST}$ -like method for AMOVA calculations. However, the description is not straightforward. Could you clarify it now and also explain both models? Do you think that (statistical) comparison of results based on both methods could provide an insight to the types of mutations and also bring support for 'somatic mutations'? How?
- (6) Could you provide more information about microsatellite development process? I want to see number of obtained reads, median read length, number of SSRs identified, number of loci tested etc. Did you use multiplex PCR for loci amplification? How did you assess reliability of your data? Did you any repeated analyses?
- (7) When assessing clonal dispersal in Paper III you employed  $P_{sex}$  calculations to estimate probability that the same multilocus genotype (MLG) emerged randomly from independent recombination process. However, were genotypes with  $P_{sex}$  values higher than 0.05 treated as identical when calculating distances of clonal dispersal (Fig. 6)?
- (8) In paper III you used rarefaction to get comparable estimates of observed allele numbers for samples with uneven sizes. However, the difference between large and small populations was not exactly tested (there is only speculation that 'larger populations tend to have bigger pool of genotypes', page 82). Anyway, are they statistically different? Finally, have you observed any unique alleles for CZ, FI or even for a single population?

Minor remarks (Paper III; what a pity it's already accepted...):

- (1) Missing reference for software Multilocus v 1.3 (page 76)
- (2) Population in Tables 1 and 3 should be in the same order. Add number of samples analysed from each population to Table 1. I suggest combining Fig. 3 with Table 4 and adding a calculation of  $N/N_g$ .
- (3) In Table 3 there are AMOVA results based on  $R_{ST}$ -like or  $F_{ST}$ -like approach? Add explanation what is  $F_{CT}$ ,  $F_{SC}$ , and  $F_{ST}$ .
- (4) Have you tried testing correlation between  $N_g$ ,  $N_a$  etc. with number of observed patches with perianths (page 80, above Table 4) and with linkage disequilibrium (page 84)?

- (5) What do you mean with 'moderate levels of genetic diversity' (page 82)?
- (6) What is the affinity of *Crossocalyx hellerianus* to conifer wood (see first paragraph on page 83)? From information provided within Paper III it is not clear whether low proportion of decaying conifer wood in CZ is very important reason for lower abundance of *C. hellerianus* or not...
- (7) Confusing caption of S4 Fig. One assumes three graphs (A, B and C).
- (8) Instead of providing (or in addition to) S1 Table of pairwise  $R_{ST}$  values it will be useful to see NJ tree or PCoA based on this matrix to graphically illustrate relationships among populations.
- (9) It will be nice to provide additional information about spatial autocorrelation results (e.g., as S2 Table) including number of pairs, mean distance, exact value of kinship coefficient and its SE etc. per distance class. Also S3 Table with allelic frequencies per population and region will be nice.

The study of Eva Holá brings valuable insight to the reproduction and dispersal of dioicous liverworts. It fulfils the criteria necessary for obtaining Ph.D. degree at the University of South Bohemia in České Budějovice. I consider it suitable for defence.

Praha, 14<sup>th</sup> September 2015



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## Evaluation Report

### Dissertation by Eva Holá: (A)sexual Life of Liverworts

The Dissertation by Eva Holá includes a comprehensive set of investigations on reproduction, vegetative growth and population genetics of liverworts. The title of the thesis is short but describes the scope of the conducted research well.

Eva Holá has produced a great amount of original data on a challenging group of plants, liverworts, with unique reproduction patterns. She has then successfully analysed and processed the data into a general introduction section and three scientific papers, which form a nice ensemble of integrated research on liverworts. Two papers have already been published and the third paper is accepted for publication, all in international peer-reviewed journals. Although there are several collaborators listed, Eva Holá is always the first author, who has had the prime responsibility of the work.

The whole work shows that Eva Holá masters a wide range of ecological, molecular and data analysis methods, and is able to formulate the findings in a lucid manner. The thesis provides important new insights into the biology of liverworts, thus providing novel scientific knowledge. Eva Holá has been able to complete a challenging and biologically important project, and she has certainly shown ability to plan and execute scientific research in an appropriate and also independent manner.

I warmly recommend the thesis to be defended.

I have a few specific questions I wish the Candidate to answer:

- 1) In the studied liverwort species, which are the decisive factors leading to either sexual or asexual reproduction? Which are the decisive factors influencing the relative and absolute frequencies of gemma and spore production and germination? Are there differences among the studied species or even among genotypes in these reproductive traits?
- 2) What are the roles of spores vs. gemmae in dispersal and what is the dispersal capacity over short and longer distances in the studied liverwort species? Considering the dispersal capacity and the present genetic variability, how critical would habitat fragmentation be for the evolutionary future of the studied liverworts?
- 3) How were the nine used microsatellites selected after sequencing the SSR-enriched library: were there other candidate microsatellites, were the used microsatellites selected based on the type and length of the repeat, and were other microsatellites tested?

In Helsinki, August 25, 2015

A handwritten signature in black ink, appearing to read 'Helena Korpelainen'.

Dr. Helena Korpelainen  
Professor

