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## Review of PhD thesis entitled "*Reproduction and hybridization in ferns*" by Ondřej HORNYCH

### *Overall assessment*

The thesis focuses on several facets of reproduction pathways and interspecific hybridization in ferns. This is because we have still rather limited knowledge about both mechanisms / processes, although both play an important role in speciation in this group of plants.

Specifically, the candidate and his collaborators have examined two different life stages in the life cycle of ferns, i.e. spores and gametophytes. Spore abortion index was studied in detail in three groups of plants – sexual, asexual – apomictic and interspecific hybrids and methodological guidelines were proposed how to accurately and efficiently use this parameter when studying fertility in ferns. Antheridogene production and susceptibility were studied at the level of gametophyte, as this fern hormonal system is involved in spatiotemporal sex expression in homosporous ferns and potentially also in interspecific competition. Finally, the candidate and his collaborators quantified frequency and direction of interspecific hybridization in the *Dryopteris carthusiana* complex. To achieve the aims, the candidate used various methodological approaches like, spore measurements and cultivation, cultivation of gametophytes, flow cytometry and sequencing of plastid loci.

The thesis includes four chapters; one introductory chapter, which shortly characterizes particular aspects of three different life stages in *Pteridophytes*, following by three chapters representing three papers published in renowned journals here ordered chronologically: Plant Systematics and Evolution, American Journal of Botany and New Phytologist. The candidate was the first author on all three papers. Overall, the thesis is very-well written, in comprehensive way, and is logically structured. The results of the thesis are original, definitely widen our knowledge about various aspects of hybridization and reproduction of ferns, but also point out to further hidden aspects of secret life of ferns which should be still explored.

Despite of several unclear issues summarized below, which are, however, rather the results of my curiosity than the weaker points of the study, I feel that the PhD work fully meets the high standards for a doctoral thesis at the University of South Bohemia in České Budějovice, and I have no hesitation to recommend Ondřej for a doctorate award.

A handwritten signature in blue ink that reads "Patrik Mráz".

Prague, February 4, 2021

Patrik Mráz

Because the results of this thesis have already been critically reviewed and published in very good international journals, I would rather focus on a few general or missing aspects of the papers / Introductory chapter. I hope these and other questions prepared for general discussion will stimulate the discussion with the candidate, members of examination committee and audience, as well.

1. How can we distinguish between polyploid hybrids and polyploid apomicts, given the fact that the latter ones are often (or ? solely) of hybrid origin? How the tested groups were defined for the purpose of spore abortion index study?

2. It is stated that antheridiogens released from almost mature archegonia can stimulate formation of antheridia of neighbour not yet differentiated gametophytes transforming them to unisexual, i.e. male individuals. At the same time, antheridiogens should stimulate germination of spores in a dark. What was the fate of the gametophytes arisen from these spores?

3. It is not clear to me how apomicts could suppress sexuals using AG system (see very simple but clear statement on p. 73), if antheridiogenes are produced solely by archegonia-producing gametophytes, but these are missing in apogamous ferns ... Should I read this statement that only sexual-apomictic hybrids are able to do and the statement is thus very simplistic and in fact not correct? If so, how important is this putative mechanism of interspecific competition given the frequency of sexual-apomictic hybrids?