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### Evaluation Report for the Ph.D. Thesis

submitted to Charles University in Prague

Faculty of Science, Department of Botany

**Title:** Origin, inheritance and ecological significance of apomixis in the genus *Hieracium* s.str.: The role of genetic and epigenetic mechanisms.

**Author:** Mgr. Jan Pinc

**Supervisor:** doc. Mgr. Patrik Mráz, Ph.D.

Dear Dr. Tomas Fer, Chair of the Board and Thesis Committee,

I appreciate the invitation to review the Ph.D. thesis of Mgr. Jan Pinc. I declare I have no conflicts of interest in the evaluation process of the Ph.D. Thesis and submit herein my review report.

Jan Pinc prepared his Doctoral Dissertation within the study programme *Botany* at the Faculty of Science, Charles University. The thesis contents 207 pages, structured in an Abstract, two chapters and the CV of Mgr. Jan Pinc. Chapter I includes an introduction to the problem, a description of the state of the art in the genus *Hieracium* s. str. used here as experimental material, the objectives of the thesis with a brief description of results and a list of cited references. Chapter II includes four papers in international peer-reviewed journals, two accepted or published and two practically ready to be submitted to specialized journals. The results of the Thesis are noteworthy in different ways. Beside the research findings detailed below, during the development of the Thesis, Mgr. Jan Pinc carried out different academic activities of high relevance to his professional formation, including the supervision of bachelor thesis, involvement in teaching, international research visits and field trips, participation in conferences and secured research funds. The scientific cooperation with different international institutions and scientists, including local partners (Institute of Botany of the Czech Academy of Sciences, Průhonice), regional partners (Technical University of Munich, Germany) and overseas partners (Commonwealth Scientific and Industrial Research Organization, Australia) is also to be highlighted.

The dissertation describes reproductive and ecological aspects of apomixis in species of the genus *Hieracium* s. str., with a focus on the role of genetic and epigenetic attributes in the origin and maintenance of biodiversity within the genus. Using experimental hybridization of sexual diploid cytotypes and a combination of biological traits including morphology, allelic variation and genome size, crucial data is presented which supporting a hybrid origin of apomictic polyploid cytotypes and the plausible dynamics of diploid and polyploid cytotypes in natural populations of *H. pallidiflorum* and

*H. picroides* (Introduction, Paper I). The study further presents a detailed evaluation of the reproductive development and performance of hybrids between eight sexual diploid *Hieracium* species using both morphology, embryology, flow cytometry, and seed set. Such results clearly indicate that post-hybridization neopolyploidization is rare, but when it happens, the neopolyploids produces more polyploids in a frequency higher than the parentals (Introduction, Paper II). Likewise, similar approaches are used to understand the role of inbreeding and interspecific competition in *H. alpinum* (Introduction, Paper III). All these studies present valuable information which is framed within a metapopulation concept to provide a better understanding of the observed pattern of distribution of sexual and apomictic cytotypes, a widely known phenomenon called geographic parthenogenesis. Finally, since epigenetic signals are expected to play an important role in adaptation of clonal (apomictic) lineages, an assessment of alternative protocols for analysis of DNA methylation changes is carried out. The results describe the best methodology for experimental induction of stable DNA demethylation in *H. alpinum* (Introduction, Paper IV).

#### Questions to the author:

- Considering the complications raised using the three mentioned molecular markers (*ETS*, *trnT-trnL*, *gsh1*; Paper I), would not have been convenient the use of omics approaches? How do you see such technologies in the resolution of evolutionary questions, particularly in *Hieracium* spp.?
- There is a possibility that hybrids might have arisen through independent polytopic hybridizations among distinct parental genotypes or residual sexuality in already established hybrids (Paper I). Do you think this could be experimentally tested somehow?
- The neopolyploids evaluated (Paper II) seem to form similar amounts of reduced and unreduced male and female gametes. The frequency of gamete abortion is similar between parents and hybrids during megasporogenesis but not at later stages, in which ovule abortion is dramatically higher in hybrids. Do you have a possible explanation for this observation?
- It is widely accepted that unreduced female gametes are more effective than unreduced pollen in the formation of neopolyploids. Yet, you did not observe unreduced ovules (Paper II). Do you think the polyploids in your plant model derive from unreduced gametes? What could be the role of polyspermy in the formation of natural polyploids?
- Chimeras usually have tissues with different ploidies. In the current analyses (Paper II), small portions of tissue were evaluated separately and were found with two ploidies which may suggest the occurrence of intermixed diploid and tetraploid cells. How this can be explained?
- Regarding your experiments on interspecific competition between *Hieracium alpinum* and *Nardus stricta* (Paper III), an extra experiment including apomictic polyploids of *H. alpinum* was not considered because of the allopatric distribution of both diploid and triploid cytotypes. However, such analysis may have benefit understanding the possible outcomes in cases of neopolyploids arising in diploid population. There were other reasons than the natural distribution of cytotypes to not consider such experiment?
- The results of biparental inbreeding (Paper III) shows no significant difference between seed set under inbreeding-outbreeding treatments. Do you have an explanation or hypothesis regarding the standard compatibility systems and S-alleles that might explain this observation? Besides possible effects of dominance, do you think that the high diversity observed among diploids could neglect biparental inbreeding?
- In the analyses of DNA methylation (Paper IV), the percentage of mC do not correlate to genome size but rather to the percentage of mC in the untreated plants. However, all plants in this experiment come from seeds of the same parent individual (an apomictic triploid genotype) and

hence, they are expected to have the same life history. If this is correct, how can those results be explained?

#### Notes to the author

- For the paper II particularly, but also for the thesis in general, there are two recent reviews dealing with the specific conditions surrounding the establishment of neopolyploids in apomicts, the stabilization of a lineage and the dynamics of cytotypes in natural and experimental populations from which the presentation and discussion could benefit substantially. These are Hojsgaard 2018, *Frontiers in Plant Science* 9:230; and Hojsgaard and Hoerandl 2019, *Frontiers in Plant Science* 10:358.
- Some sentences in the text are ambiguous or confusing. For example, when referring to the formation of selfed progeny when used as pollen donor. Since these plants are hermaphrodites, selfed progeny would arise from self-pollination.
- There are a number of studies dealing with the formation of neopolyploids after experimental crossing and under natural conditions. So, is not completely clear why you focus only on the work by Considine et al. (2012). For example, Ramsey and Schemske (1998), a paper that you cite in other parts of the Thesis, is a well-known review collecting information on neopolyploids from many studies and summarizing most relevant aspects about neopolyploids biology.
- A few references cited in the text are missing in the reference lists (e.g. Mráz et al. 2020). The Genome Collisions Hypothesis was presented by Carman (2007), it is a book chapter.
- Discussed fitness traits due to inbreeding effects are different to those presented in the results.

#### Overall review

The dissertation of Mgr. Jan Pinc presents a significant collection of valuable and interesting results. The submitted thesis provides ample evidence of the expertise acquired by the candidate regarding the application of the scientific method, the use of different experimental techniques, the analytical evaluation and the interpretation of complex and original results, as well as its presentation using a scientific writing style. Following my evaluation of the present Ph.D. thesis, I conclude that it satisfies the requirements for a Ph.D. thesis in the field of Botany. I have not assessed the thesis for plagiarism.

I recommend this Thesis for a dissertation and to award the candidate with the Ph.D. degree after successful defence of the Thesis.

Sincerely,



D. Hojsgaard

September 14<sup>th</sup>, 2020