Opponent's review of the doctoral thesis „Origin, inheritance and ecological significance of apomixis in the genus *Hieracium* s.str.: The role of genetic and epigenetic mechanisms" presented by Jan Pinc

The doctoral thesis of Mgr. Jan Pinc "Origin, inheritance and ecological significance of apomixis in the genus *Hieracium* s.str.: The role of genetic and epigenetic mechanisms" consists of 206 pages. The work is divided into two chapters. Chapter I includes a very detailed and nicely written introduction, focused on sexual and asexual reproduction, apomixis and the genus *Hieracium*, which was used throughout the work as a model group suitable for testing various hypotheses. Chapter II consists of four case studies, one of which has already been published in the prestigious American Journal of Botany, the other is in print in Plant Systematics and Evolution, and the remaining two are manuscripts prepared or already submitted for review. Of the four articles submitted for defense, the candidate is the first author in three cases and there is therefore no doubt about his fundamental role in individual publications.

The thesis is written well, formatted consistently, without major mistakes. The comprehensive introduction reviews the current state of the knowledge about the importance of hybridization, polyploidy and epigenetic mechanisms in selected species of the genus *Hieracium* using up-to-date literature. In addition, the quality of elaboration of individual works is quite high, does not differ between published and unpublished works, the level of knowledge is also at a high level, methodological approaches are correct and and the discussion points to the author's ability to incorporate his results into current knowledge.

The first case study evaluated the origins and variation of two triploid taxa (*Hieracium pallidiflorum*, *H. picroides*) presumably derived from the same diploid parental pair (*H. intybaceum*, *H. prenanthoides*). Based on suite of approaches ranging from morphological, phylogenetic (three unlinked molecular markers) and cytogenetic analyses (in situ hybridization) to genome size screening and genome skimming the authors proved multiple origins of hybridogenous apomicts and their allopolyploid origin. I especially appreciate the variety of methods used in this work, which led to very interesting results that can be used in the classification of individual species. Demonstration of incongruence between rapidly evolving DNA such as satellite DNA or transposable elements and phylogenetic patterns is very interesting mainly because some studies discuss the utility of these molecular markers in phylegenetic studies. In contrast, this study shows that repetitive DNA may not correspond to phylogenetic structure.

The second case study is focused on a very interesting issue of neopolyploiodization. Extensive experimentation has shown that neopolyploidization in *Hieracium* s. str. is relatively rare, and it occurs, regardless of the origin of individual plants, i.e. whether or not they are hybrids.

The third case study aims to explain geographic parthenogenesis distribution pattern in *Hieracium alpinum*. The results show that biparental inbreeding can only partially explain the reduced colonization ability of the diploid sexually reproducing individuals of *Hieracium alpinum*.

I was looking forward to the fourth and last article on epigenetics. Although the whole work has the role of genetic and epigenetic mechanisms in the title, I expected to learn here how epigenetic mechanisms allow genetically invariable apomictic lineages of *Hieracium alpinum* to increase the evolutionary potential. Unfortunately, this article is only methodological and in no way contributes to the understanding of epigenetics in relation to the distribution of apomectik lineages in space. I am not saying that it is poorly processed or non-interesting, it just deviates from the overall concept of the dissertation and if it wasn't here, basically nothing would have happened.

Nevertheless, the above-mentioned comments do not significantly decrease the quality of the presented thesis and I consider the thesis suitable for the award of a Ph.D. degree. In order to complement the view on the studied topic I would like the candidate to respond to the following questions:

1) You wrote that *"Nevertheless, once formed, neopolyploids can significantly contribute to the production of polyploid progeny and thus stabilize new polyploid lineages."*
Is this visible in the field? You got eight neopolyploids out of 3739 analysed individuals in culture. What is the probability that neopolyploids will spread successfully in natural conditions?

2) I am not a *Hieracium* expert at all, but I wonder if some neopolyploids in culture were morphologically the same as the species/individuals found in nature? Have you observed this or the progeny were morphologically completely different from what is in nature?

3) You wrote that *"One experimental hybrid from the H. intybaceum × H. alpinum cross contained diploid and tetraploid tissues in the leaves, which was confirmed by three independent cytometrical analyses."*
Should this be a product of endoreduplicfation. If not, how do you explain the presence of chimeric plant with two ploidy levels?

4) You wrote that *"The hexaploid offspring may have originated from a fusion of two unreduced gametes (3n+3n). However hexaploid and possibly triploid cytotypes could be also explained by double fertilisation of reduced gametes (2n+2n+2n or n+n+n), i.e. by polyspermy."*
Polyspermy in *Hieracium*, this is very strong statement. Do you have any other proof of double fertilisation in Asteraceae?

5) You wrote that *"Our results suggest that biparental inbreeding could to some extent explain a lowered colonizing ability of sexual diploid of H. alpinum, but the GP pattern observed is also likely to be influenced by other processes and their interactions."*
Which other processes could explain GP pattern observed in *Hieracium alpinum*?

6) What would happen if you will do inbred crosses for several generations? Is there any possibility that inbreeding depression will express after several generations of selfing?

7) You wrote that *"Therefore, both, biparental inbreeding and limited number of S-alleles in a small founder population might increase the risk of non-compatible crosses which will ultimately reduce the reproductive output (i.e. fertile seeds)."*
How do you differentiate between inbreeding depression and incompatible mating? Which of the two processes is more important in your view? Could the low number of S loci in small populations be the cause of any or low inbreeding found in your study compared to the situation in the field?

8) How is epigenetic variation related to fitness? Is there any possibility that majority of responses are due to phenotypic plasticity?

Conclusion: The applicant has clearly demonstrated her scientific skills. The thesis entitled „Origin, inheritance and ecological significance of apomixis in the genus *Hieracium* s.str.: The role of genetic and epigenetic mechanisms" by Jan Pinc fulfils the requirements expected of a dissertation thesis. I therefore recommend the thesis for the defence.

Prague, 15 September 2020 Bohumil Mandák