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**Review & overall assessment of the Ph.D. thesis (field: Botany) submitted by Mgr. Jaroslav Zahradniček “Phylogeny and evolutionary trends in the subtribe Hieraciinae” at the Charles University Prag in 2017.**

*Overall assessment*

The submitted Ph.D. thesis comprises two main parts: (1) General chapters introducing and summarizing the PhD-thesis and (2) four case studies (‘papers’) within the studied topic. The first chapter provides a comprehensive and informative scientific overview and positions the conducted research well in a general and international scientific context within botany. The case studies in the second part are framed as separate scientific articles; two (out of four) are first authored by Mr. Zahradniček, one of these is included as a manuscript; another two are co-authored by the candidate. Three of these are already published in peer-reviewed international scientific journals (Annals of Botany, Taxon, Botanical Journal of the Linnean Society). An author contribution statement clarifies that despite the PhD-thesis includes contributions from many authors, Zahradniček contributed substantial parts of idea, development of research, methodology data and collection, production of results and manuscript preparation.

Zahradniček’s thesis deals with subjects relevant in taxonomy and biosystematics, evolutionary biology of plants (including breeding system and hybrid evolution), and phylogeography. His research touches also fundamental questions, especially related to the understanding of the diversification of recently evolved taxa as well as evolutionary significance of genome size and hybrid evolution. Study objects from the family of Asteraceae are the genera *Hieracium* (a highly diversified plant group with extensive hybrid evolution and apomictic breeding system), *Andryala* (a rather small genus with radiated diploids in the Mediterranean and adjacent biomes), and *Schlagintweitia intybacea* (= *Hieracium intybaceum*) which is because of its evolutionary isolated position another lineage within the Hieraciinae. The applicant has applied a variety of methods in his studies, i.e., molecular phylogenetic ones, genome size measurements and chromosome counting, breeding system analyses, statistical analyses related to habitat properties and also genetic fingerprinting and phylogeographical methodology. Plant material was samples in many parts of Europe and the excellent sampling has been the base for carrying out the studies successfully.

Jaroslav Zahradniček’s submitted PhD-thesis expands botanical (in particular biosystematical and evolutionary) knowledge: Genome sizes and chromosome numbers of many taxa studied have been published the first time. Taxonomical ‘difficult’ groups are now better understood, and our knowledge of the significance of polyploidy in connection with asexual (agamic) reproduction has been expanded. As three of the four articles building the core of his theses are already published in peer-reviewed and international scientific journals the overall good quality of research is already documented. His collaborations mainly at the national, but also

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at the international level are documented and his establishment as a botanist within the scientific community has already started. Overall I consider his PhD-thesis suitable to be defended and it fulfills all criteria expected.

*Specific questions to the defendant (unordered)*

In your first paper (published 2009) you did conclude that "phylogeny is the most important factor explaining genome size variation". This is puzzling as genome size variation might respond to processes (which are discussed), but not by phylogeny/relationship per se. How has been and in future could science be expanded towards a better understanding of genome sizes (and genome evolution) in *Hieracium* (but also in general) nowadays?

Phylogenetic approaches have difficulties with (interspecific) hybridization and allopolyploid evolution. How does the fact that your research has shown that also 'basic' species in *Hieracium* are of hybrid origin change the view of this taxonomical concept? How did you evidence this hybrid origin exactly?

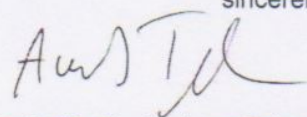
You present two studies/papers dealing with the mainly Western Mediterranean *Andryala*, which (mainly?) geographically radiated since the Pliocene. You concluded that genome size variation found might be a result of random processes ('genetic drift', 'neutral evolution'). Genetic drift is indeed a process changing allele frequencies in (small) populations randomly. Genome size changes are, however, not simple changes in allele frequencies (which would not lead to a loss/gain of parts of a genome). Could you explain how you imagine "neutral genome drift" at a genomic level could result in genome size changes nevertheless?

The phylogeography of *Hieracium intybaceum*, which has been evidence as a separate lineage in Hieraciinae for several millions of years (right?), did evidence a rather weak phylogeographical pattern in Central Europe. You concluded that glacial refugia might have been "in the southern part of the distribution range and occasionally in the Western Alps". Could you explain the evidence? Are there any alternative scenarios?

The study groups are "complex and difficult ones" from a taxonomical point of view and you (mostly) avoid drawing any taxonomical conclusions derived from your molecular data. Do you see severe conflicts between the existing taxonomy, better said taxonomical views, of *Hieracium* (but also in *Andryala*) and your results? Could you discuss briefly if the taxonomical concept in *Hieracium* should be completely reconsidered?

*Hieracium* is known for (allo-)polyploid evolution combined with substantial apomictic breeding systems in polyploids. How much are changes in genome sizes involved in speciation/diversification in this genus? I would like to hear your opinion to this point.

sincerely,



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