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Written assessment and review – PhD candidate Mgr. Adam Kantor – study programme "Botany" – August 2023

Dear PhD committee, dear PhD candidate.

The submitted Ph.D. thesis comprises three main parts: (1) Some general chapters introducing the key topics of the PhD-thesis, *Cardamine*, the study system, biogeography of the study area, polyploidization and hybrid evolution, technological and methodological innovation in biosystematics phylogenetics with special reference to HybSeq). (2) Three case studies (all as first author, two published in international peer-reviewed Journals – *Frontiers in Plant Sciences & Annals of Botany*, one submitted to *Taxon* and in a review process). (3) A conclusion summarizing the PhD in the light of up to date research. Of particular focus is the plant genus Cardamine, a widely distributed Brassicaceae that is studied mainly in SW Asia and the Balkan Peninsula that also are evidenced as important refugia for diploid and allopolyploid plant evolution during the Pleistocene. Moreover, the use of up-to-date technology, especially, HybSeq, was crucial for some of the conclusions drawn.

Kantor`s thesis addresses highly relevant core questions in plant evolutionary and biosystematics biology: Mainly how does evolution (morphological and biogeographical) accompanied with allopatric and/or polyploid formation interact with diversification and speciation. He has applied mostly up-to-date methods (morphometrical, genome size measurements, HybSeq with appropriate phylogenetic/phylogenomical pipelines, etc...), has achieved an excellent sampling strategy, has teamed up with national and international collaborators and also has obtained a very good overview on relevant theory and literature.

Kantor`s thesis is very well written, well communicated with illustrations and as mentioned before already communicated to the international audience. To name very positive aspects as well as some critical of the thesis: The general part of the thesis is very, is well addressing theory, state of knowledge in relation of the study system. Most of illustrations in all parts and tables show that the candidate has worked very carefully and scientifically sound. Maybe it is according to the rules, but I missed the supplementary information from the published studies as part of the thesis. I missed also a wider discussion that relate to some fundamental aspects in plant systematics, especially in the light of NGS data. I also would like to point out some critical questions below.

Adam Kantor submitted a PhD thesis, which is very good because of the following reasons: First, it addresses some fundamental questions in botany/plant systematics, mainly related to allopatric and hybrid evolution. His integrative approach gives new insights and interesting perspectives and raises new questions related to diploid and polyploid speciation. Second, the introduction and summary/conclusion of his PhD-thesis is accompanied by three scientific articles ("papers") of which two are already published and one are in a well advanced manuscript stage already submitted to a peer reviewed international journal. Finally yet importantly, the scientific quality is clearly at an international level. All this shows that. Overall, I consider the PhD-thesis suitable to be defended and it clearly fulfils all scientific criteria expected at this education level and career stage.

Some general and specific questions in detail worth to be discussed

ad 4.1 – The "ITS 1 & 2", the internal transcribed spacer regions of the nuclear DNA is frequently used in systematics despite difficulties also discussed in the general introduction. What is your opinion for its future application as "DNA-Barcode" in plants? You also compare phylogenies obtained from closed PCR and Hyb-Seq, but not direct sequencing. Would you recommend establishing despite difficulties ITS as a standard barcode?

I found this study very interesting in particular as the genomic data were explored widely and the integrative approach allowed great conclusions including allopolyploid origin. While NGS technology and bioinformatics is without any doubt bringing great advance and discoveries, still many species and species complexes are not so well understood and the dependence of lab technology is difficult for many research institutions. Which advancement do you expect for "getting the job done" to describe many unknown species worldwide with limited effort given. Where did NGS technology contribute and will it contribute in the future? Will Hyb-Seq be easily globally available in the future?

ad 4.2 – You included modelling environmental niches in your study. The *Cardamine* species studied, however, are depending on wet habitats. Such conditions are not directly related to climate variables. What would be needed for improving modelling ecological niches of species in "special" habitats aside the biome related climax vegetation? For example, you had only 27 occurrences for *C. penzesii*. How do limited exact (!) occurrence data potentially influence such modelled results?

I still see the support for describing a new species ("cf. *uliginosa*") as phylogenetic data show that this taxon is close to *C. acris*. How can you rule out this?

ad 4.3. – Congratulations that you managed also some revisionary work as part of you PhD including the description of a new species. As in the former study the thorough morphometrical analysis is extremely useful for such an endeavour. *Cardamine acris* is a variable species (also in genome size), what is your main argument that the new species is "not only" geographical variant of *acris* or "just" a subspecies?

with	regards