

Innsbruck, 22.07.2023

Review of the PhD thesis of Mgr. Adam Kantor: Evolution of members of the genus *Cardamine* from the Anatolia-Caucasus region and the Balkan Peninsula

Using an array of complementary methods, ranging from flow cytometry for genome size and ploidy-level estimation, partly complemented by cytogenetic analyses, over phylogenetic and phylogenomic inference of relationships among the study taxa, to multivariate morphometrics and partly environmental niche modelling, A. Kantor disentangled relationships among different groups of hygrophytic *Cardamine* species from south-west Asia and the Balkan Peninsula. Besides shedding light on evolutionary relationships that partly contradict traditional taxonomic views based on morphology, A. Kantor and co-authors established novel approaches in analyses of NGS HybSeq data to resolve the origin of polyploid lineages, which is certainly of great general interest.

In comprehensive introductory chapters, the author presents the study genus *Cardamine*, with a special focus on the hygrophytic species that were traditionally included in the *C. amara*, *C. pratensis* and *C. raphanifolia* species complexes. The introduction to study taxa is followed by a review of the biogeographic and phylogenetic studies important for understanding diversification patterns that contributed to high diversity of the study area, which ranged from south-eastern Europe (the Balkan Peninsula) to western Asia (Anatolia, Caucasus, Iranian Highlands). A special attention is given to Balkan-Anatolian biogeographic connections and migration routes across Asia Minor. Finally, polyploidisation, one of the most important diversification processes in flowering plants, and its importance in evolution of *Cardamine* is presented. Linked to the introduction, the two main aims and explicit hypotheses to be tested are presented. The first specific aim deals with inference of phylogenetic patterns within the study group, leading to revised taxonomy, whereas the second aim is more general, dealing with exploration of polyploid and reticulate evolution in the study group. The introduction is followed by detailed description of methods, indicating that candidate has a good knowledge of integrative approaches, which are nowadays state-of-the-art in evolutionary biodiversity research. It is important to point out that A. Kantor was involved in all processes, ranging from field work over lab work and in-depth data analyses to manuscript preparations.

Apart from the introductory chapters, the thesis is composed of two scientific papers and a manuscript, which is certainly suitable for submission to a scientific journal. Adam Kantor is the first author in two of them and a shared first author in one, and in all cases his contribution to the studies is significant. One of the papers have been published in *Frontiers in Plant Sciences* and the other in *Annals of*

Botany, which are both high-impact journals in the field of plant sciences and evolutionary research. In addition, it is obvious from bibliography of A. Kantor, that he presented results of his scientific as well as floristic activities at different conferences and/or in national journals, which additionally illustrates his quality as a scientist.

The two published papers and the manuscript included in the PhD thesis explore and disentangle relationships in different groups of closely related *Cardamine* species. (1) In the first paper the Greek stenoendemic (to Pindos) *Cardamine barbaraeoides* is in focus. Using an array of complementary methods, including GISH and a novel approach based on HybSeq data that allows identification of sub-genomes inherited from different progenitors, the authors inferred an allotetraploid origin of the focal species, with parental lineages including *C. amara/C. lazica* and *C. acris/C. anatolica* (the last described as a new species in the third paper). It is of high importance that authors developed the novel approach for NGS data analyses, which will surely be helpful to disentangle origins of other polyploid species. (2) In the second paper the authors disentangle relationships among the south-west Asian (Anatolian, Caucasian and Iranian Highlands) species of the study group, using similar approaches as in the first paper, but including also environmental niche modelling. This study is of high significance, as such in-depth studies of the biota from this part of the world are largely lacking and the paper brings important aspects about processes that led to high diversity of this area and will for sure be important reference for future research in this part of the world. Besides highlighting the importance of Black Sea and Caspian Sea regions as glacial refugia, the study also indicates that polyploidisation was not an important evolutionary driver in the study group (with *C. wiedemanniana* being an exception), which stands in contrast to more northern, European areas. (3) In the third study (manuscript), the populations from north-western Anatolia (the Uludağ Mts) traditionally assigned to *C. uliginosa* are in focus. Again, using an integrative approach including multiple analyses the authors show that these populations form an independent evolutionary lineage, which is more closely related to the Balkan endemic *C. acris* than to the western Asian *C. uliginosa*, leading to the description of a new species *C. anatolica*. I was pleased to see that the study ended with a comprehensive taxonomic treatment of the new species including an identification key for all studied taxa.

In summary, I find the thesis well written and the conclusions justified and supported by the results. For the reasons outlined above I am absolutely convinced that the PhD thesis of the candidate Adam Kantor meets the international standards (grade: passed), and it is therefore a pleasure to recommend that he should be awarded the scientific-academic degree "philosophiae doctor" after successful defence of the thesis.

Yours sincerely,

(Dr. Božo Frajman)