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Vážený pán Mgr. Zdeněk Kaplan, PhD. predseda komisie Přírodovědecká fakulta UK 128 00 Praha 2, ČR

Váš list značky / zo dňa	Naša značka	Vybavuje / tel.	Košice 6. 3. 2022
VEC: zaslanie oponentsk	ého posudku		
Vážený pán predseda,			
na základe Vašej žiados Šrámkovej v doktorskom		sielam oponentský posudo Botanika.	k na prácu Mgr. Gabriel y
		S pozdravo prof. RNDr	om ⁻ . Pavol Mártonfi, PhD.

Reviewer's opinion on the work of Ph.D.

Author: Mgr. Gabriela Šrámková

Evolution of the genus Arabidopsis in its centre of diversity, / Evoluce v centru diversity rodu Arabidopsis.

The presented work has the character of basic botanical research using the most modern methods in the evaluation of intraspecific variability and phylogenetic relationships of the species *Arabidopsis halleri* and the species complex *Arabidopsis arenosa*. It has an impact on a broader understanding of evolutionary processes and the spatial distribution of genetic diversity in these complexes. From this point of view, the work is highly topical. The work brings a lot of knowledge that has not been known or preliminarily studied. The objectives of the work were defined appropriately and were fulfilled in the submitted work.

Study plant samples for the dissertation were collected over a large area across the entire distributional range of the *Arabidopsis arenosa* complex and *Arabidopsis halleri* in Europe and to include all previously recognized European species and subspecies of the complexes as well as non-European subspecies of *Arabidopsis halleri* was also included by sampling populations spanning most of its distribution in Japan. The methods for solving the presented goals were chosen according to the needs. The ploidy level of all individuals was assessed by flow cytometry, complemented with chromosome counts and homoploid genome size diversity analyzes for some accession and to reveal phylogenetic relationships, several molecular methods were used (among them AFLP - amplified fragment lenth polymorphism, SSR - nuclear micosatelite markers, non-coding chloroplasts DNA sequences, single / low-copy gene sequencing, double-digest RADseq - restriction associated DNA sequencing and also WGS - whole genome sequencing). Several multivariate morphometric analysis methods were used in the standard way to evaluate the data structure.

The dissertation is divided into Part A - General chapters and Part B - Case studies. The general chapters consist of 36 pages, which are processed at a very good level. In the literature survey the author deals with general questions of biodiversity centers and speciation, as well as the genus *Arabidopsis* with a special focus on the studied species or complexes *Arabidopsis halleri* and *Arabidopsis arenosa*. Objectives are classified into three groups, they are formulated very precisely and were fulfilled in the submitted dissertation. The Materials and methods section is written briefly, but all details on the wide range of laboratory methods used can be found in the relevant case studies. The key results and discussion are appropriate to the scope of Part A, they are written clearly and with regard to the expected results of the study. Conclusions and future prospects highlight the need for further study and emphasize the need for a new taxonomic concept for *Arabidopsis arenosa*, and another challenge for the study is hybridization between the *Arabidopsis arenosa* and *Arabidopsis lyrata* complexes. References are sorted neatly and I did not find missing or redundant items among them. Part B - Case studies contains a total of 5 papers, of which 4 are published in renowned scientific journals and one is manuscript. It can be said that together all these works exceed the content and

quality requirements for dissertations. Three of the already published works are from the Q1 quartile and one from the Q2 quartile according to the Scimago journal rank. Two works (one from Q1 - Botanical Journal of Linnean Society and one from Q2 - Plant Systematics and Evolution) are those where the doctoral student is the first author with a share of 45% and 80% and the other two works are still 35% or 30%, respectively. On one from the works it is 10%. In total, however, the doctoral student registers 8 scientific papers in SCI journals. According to the SCOPUS database, it records 37 citations without self-citations and reaches h-index 3. In the WOS database the data were confusing in my opinion (I was not looking for a reason why) and therefore I do not list them.

I consider the identification of three major genetic lineages within Europe for *Arabidopsis halleri*, which are strongly correlated with major geographical barriers in the Central European mountain systems, to be the most valuable results achieved in this work. Their more detailed study made it possible to define 5 subgroups (Western Alpine, Eastern Alpine, Hercynian, Western Carpathian, South-Eastern Carpathian) and also to find morphological deviations of the lines and to create a new classification of *Arabidopsis halleri*. For *Arabidopsis arenosa* species complex, the most interesting results are concerning the discovery of 3 cytotypes and five diploid and five tetraploid genetic lineages and their uncorrelation with the current taxonomic concept of this species complex. This is also a good idea for further taxonomic work in this complex.

I have the following questions for the author about the submitted work:

- 1. The species *Arabidopsis halleri* is well known as a hyperaccumulator of heavy metals (Zn, Cd), which the author also states on p. 8. There are detailed studies on this, which also deal with, for example, population differentiation for Zn tolerance, or multiple origin of metallicolous population of this taxon (Pauwels et al. 2006, Pauwels et al. 2005). Did the author find that the relationship between individual genetic lines (or subspecies) and the ability to hyperaccumulate would ever be examined? If so, have there been any differences? If not, is it possible to compare data from different papers from geographically different regions?
- 2. The author deals extensively with specific subspecies of studied taxa and in a short chapter 1.2.1 (p. 7) she also deals with the approach to the understanding of this concept and in various works also the relationship between the understanding of genetic lines and the concept of the term subspecies. However, when defining a subspecies, it uses mostly zoological work (often devoted to mammals) to explain the term, e. g. Taylor et al. 2017a, Taylor et al. 2017b, Martien et al. 2017, Padial et al. 2010. However, definitions of species "tailored" mainly to mammals (e. g. Mayr in various works, etc.) in the past have shown that they are often unusable in plants due to a wide range of plant "species": linneons, jordanons (autogamous microspecies), apomictic microspecies and frequent hybridization between species, polyploidization and the like. Can the author justify why the cited "zoological" definitions of the subspecies should be currently applicable to plants as well? Or does she consider only a group of allogamous species when defining a subspecies? And although in different parts of the work she explains the relationships between subspecies and genetic lines, I still conclude the third question at this point: what does the author consider a subspecies in her work and what is a genetic line and what is the relationship between them?

3. The genus *Arabidopsis* (as *Cardaminopsis*) was in relatively recent times also processed in Květena ČR 3 (Měsíček et al. 1992) and in Flóra Slovenska V/4 (Měsíček et Goliašová 2002). However, the author of dissertation follows the concepts adopted in these works only partially and her taxonomic conception of the two studied taxa is different. I ask the author to explain why this is so.

The work, as already stated, is the work of basic scientific research and brings either results that are already and will be used in science in the future in the form of results already published or brings new data that are a springboard for further work on knowledge of taxonomic structure and evolutionary processes in the genus *Arabidopsis*. Based on the above, it can be concluded that the objectives of the work defined in the assignment of the final work were met in full.

Conclusion

The submitted dissertation Mgr. Gabriela Šrámková meets the criteria for a dissertation and on that basis I propose to award her the academic title of "philosophiae doctor" in the doctoral study program Botany.

Košice 6 March 2022

Prof. RNDr. Pavol Mártonfi, PhD.