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Review of the PhD thesis of Mgr. Vojtěch Taraška (2024): Biosystematic and chorological study of *Dactylorhiza maculata* agg. in Central Europe

The reviewed thesis written by V. Taraška presents an elaborative study of the taxonomically complicated *Dactylorhiza maculata* complex, shedding more light on its variability in terms of morphology, cytotype diversity and its link to ecology of included taxa. The thesis is 122 pages long, written in English and divided into five chapters: chapter 1 encompasses general introduction and aims of the thesis, in chapters 2 to 4, results published in three scientific publications are presented and discussed along with the description of the used methods, while the chapter 5 contains a summary of the outcomes of the thesis. The structure of the thesis is well thought and meets the formal requirements.

The author uses clear and well explanatory language throughout the thesis, proving that he is very well oriented in the topic. This is apparently supported by the fact that he has been focusing on the studied complex since his bachelor study. He cites literature that is up-to-date and relevant to the topic, with outstanding number of almost 290 references in the list.

In the first chapter, V. Taraška systematically introduces the family Orchidaceae, followed by the parts about the genus and the target complex. These texts almost flawlessly prove author's erudition regarding the complexity of the studied group. At the end of this chapter, aims of the thesis are stated. While the connection of the aims to the topic of conservation biology are mentioned, these links could be more explicitly incorporated in the formulation of the aims, which would emphasize the general potential importance of the thesis. Also, formulation of objectives in the form of questions or hypotheses could make them more straightforward and easier for evaluation of their accomplishment.

The results obtained by the author and his colleagues were summarized in three articles published in international peer-reviewed journals: *Plant Systematics and Evolution*, *Folia Geobotanica*, and *Preslia*. In first two, V. Taraška (et al. 2021, 2024) is listed as the first author, while he is a co-author of the third article (Kaplan et al. 2017) that represents a compilation of reports of distributional data on diverse genera of vascular plants in the Czech Republic. In the published studies, V. Taraška with his team managed to sample a respectable number of populations within Central Europe despite rarity of the studied taxa. I want to appreciate the thorough treatment regarding the taxonomy and nomenclature of the studied objects, as well as the effort of producing a determination key and distribution maps, which will be of great interest to field botanists

and conservation biologists. Speaking of downsides, I believe that some parts of discussion could benefit from setting the results in a little broader context. For example, it would be interesting to learn if the cytogeographical patterns so far observed in *Dactylorhiza maculata* agg. are known in any other polyploid complexes outside *Dactylorhiza*. Similarly, if we know any other groups that embody such a great variability and complicated taxonomy which may be shaped by the same combination of factors. The reader could inquire if there are known cases of resolved phylogenies in groups with such extent of complexity and if so, what methods were used to unravel them.

In the concluding chapter, V. Taraška summarizes the results and comments its potential application in nature conservation. He confirms that the evolutionary history of *D. maculata* complex remains largely unresolved, despite several attempts to do so with use of various molecular markers, involving even rather advanced RADseq method. Yet, this thesis represents a very important stepping stone for researchers that will continue in this effort. Importantly, I am convinced that the outcomes of this thesis do not carry message only for the conservationists or rather limited group of botanists that are interested in biosystematics of *Dactylorhiza*. There are several findings which could possibly be presented as additional “take home” messages for broader audience and it is important to highlight them. For example, the author confronts his findings with the studies recently published by other working groups, by which he points out the problematics of attempting to resolve large-scale phylogenies of complex groups without detailed knowledge of the individual sublineages within them. This is a problem that should be considered when working with any other recently diversified and/or polyploid complex. Moreover, it emphasizes the significance of integrative approach (application of multiple types of data) in biosystematics studies, which should be considered a standard, even though it is not always feasible for one working group. In this regard, a more detailed elaboration on future prospects would be appreciated – the author could have briefly mentioned what would be the next expected checkpoints in the process of revealing the taxonomic diversity and evolutionary history of the target complex.

I present these few critical comments (some of them rather only reflecting personal preferences) only as an advice and I do not consider any of the commented issues to be a major mistake. On the contrary, I consider the thesis of the PhD candidate to be generally well-written with the scientific quality definitely meeting the international standard. The included publications could be used as good examples of studies that bring novel and crucial insights into the spatial structure of variability within taxonomically complicated groups even without using molecular data. Vojtěch Taraška demonstrates that he is an expert on the *D. maculata* complex in the context of Central European populations and his findings could play an irreplaceable role in the future elucidation of evolution and taxonomy of this extraordinary genus. **Taking all into account, I consider the presented PhD thesis to be definitely suitable for defence and it is my pleasure to recommend that Vojtěch Taraška should be awarded the degree “philosophiae doctor” after successful defence of the thesis.**

Below, I list specific questions addressed to the author that could be worth discussing after the presentation of his thesis:

- 1) In the study presented in chapter 2, you reported considerable proportions of DNA-triploid individuals in mixed populations with diploids and/or tetraploids, all belonging to *D. maculata* subsp. *fuchsii*. In general, triploids in diverse lineages of vascular plants were often found to be sterile. Have you observed any signs that would indicate that the odd-ploidy individuals in the studied complex are less viable or infertile (including rare hexaploids observed among other taxa in chapter 3)?
- 2) On p. 39 you report that you observed progressively partial endoreplication (PPE) in the studied samples of *D. maculata* subsp. *fuchsii*. Was the proportion of endoreplicated genome stable among the studied populations/taxa? Did you observe any taxon-specificity regarding the extent of PPE? What is generally known about PPE in the genus *Dactylorhiza* or in some of the higher taxonomic ranks that it is classified into (e.g., subtribe Orchidinae)?
- 3) I am not sure if I understand the recalculation of DAPI- and PI-stained samples described at p. 58. What was the purpose of this procedure? How was the selection of 14 individuals to be analyzed with both fluorochromes made? Wouldn't the ratio for recalculation be different for individual taxa?
- 4) In the study presented in chapter 3 (p. 53) you encountered several populations that could not be unequivocally classified (referred to as 'agg'). Were all the individuals within these populations of transitional nature (not classifiable due to intermediate values of their characters) or rather a mix of individuals that could separately be attributed to one of the two (or more) taxa? What does it suggest about the origin of these populations?
- 5) The last question is rather speculative: if you had a large budget and plenty of spare time, what steps would you take to resolve evolution and biogeography of *D. maculata* agg. as effectively as possible in light of the currently available methodological approaches?

in Martin (Slovakia), 2.4.2024



Mgr. Adam Kantor, PhD.

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