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Review of the PhD thesis of Petr Vít: Evolutionary and conservation consequences of interspecific hybridization in rare plant species

Using flow cytometry, mostly complemented with morphometric analyses of several morphological characters, and partly with molecular data (microsatellites used in the study of *Sorbus*), P. Vít studied hybridisation among species from four different plant genera, mostly focusing on the geographic area of the Czech Republic. He used genome size to identify "pure" species as well as the hybrids between them, explored their spatial distribution and ecological preferences and discussed the measures necessary for their conservation.

In comprehensive introductory chapters, the author reviews our present knowledge about different evolutionary consequences of hybridisation among (closely related) taxa, how humans can enhance hybridisation, as well as different methods that can be used to identify hybrids in natural populations. As a next point he presents the four taxa under investigation and concludes with the aims of his thesis and future directions. Apart from the introduction, the thesis is composed of four scientific papers (P. Vít being the first author in two of them), three of them published in Preslia and one accepted in Plos One. Even if the number of articles (especially those with P. Vít as the first author) included in the thesis is moderate and they are mostly published in a single, however good quality journal Preslia, it is obvious from his bibliography that P. Vít has been involved in several different studies and has published his results, as a co-author, in different international journals, which additionally illustrates his quality as a scientist.

The importance of hybridization, both hetero- as well as homoploid hybrid formation, in plant evolution has long been acknowledged and the theoretical backgrounds of different modes of hybrid formation have been tested experimentally or studied in natural systems. Hybridisation can be important in the formation of new evolutionary lineages (e.g., species), but can also have completely opposite effect, blurring the boundaries between species and in a long run even lead to the homogenisation of gene pools of hybridising taxa. Especially taxa with narrow distributions can be strongly affected by gene flow with closely related, but widely distributed congeners. This is thus an important issue in plant conservation and identifying hybrids and separating them from the "pure" lineages is an important point when designating conservation areas on the microscale as well as defining management strategies.

Defining hybridisation and identifying hybrids in nature is not an easy task and several reports about hybridisation in nature rely solely on intuition and somehow intermediate appearance of putative hybrids. The number of studies addressing this issue using well-defined methodology is limited, and I am happy to see that P. Vít and his colleagues successfully overcame this problem by using genome size measured by flow cytometry. The methods used comprise extensive field work in order to obtain the plant material (up to 2086 individuals sampled in the study of *Cerastium*!), DAPI/PI flow cytometry of high number of samples,

chromosome counts in the study of *Sorbus*, as well as comprehensive statistical analyses of different morphological characters, applying both classical, distance-based morphometrics, as well as geometric morphometrics which has been rarely used in plant taxonomy and thus greatly increases the value of the papers. Molecular methods (microsatellites) have only been used in the study of *Sorbus* and I find it a bit unfortunate that they have not been applied in any of the other three studies, especially in the case of *Cerastium*, as this would certainly be of an added value. Still, the overall quality of the research and the results obtained by P. Vít and co-workers and presented in his thesis can be assessed as highly relevant and all presented studies are well-done and yield strong, convincing results.

In the study of *Cerastium alsinifolium* (Paper I) extensive hybridisation with widely distributed *C. arvense* has been detected, presenting a serious nature conservation problem. Authors, in the paper, mention some preliminary results of molecular studies, but present no data. I am curious if at this stage the author has advanced with the analyses of the data and if it would be possible to see the results, even if at the preliminary stage? *Cerastium alsinifolium* is presumably more closely related to *C. alpinum* rather than *C. arvense*. I find it a pity that no plants of *C. alpinum* were included in the morphometric analyses and especially in the identification key. Can the candidate list some characters which can reliably be used to distinguish *C. alsinifolium* (and the hybrids) from *C. alpinum*? The authors interpreted the hybrids with higher genome size as F1 hybrids formed through unreduced gamets of *C. arvense* (octoploid, not hexaploid as stated on p. 20 of the thesis, however correctly on p. 30) and reduced gametes of *C. alsinifolium*. Could it be that some hexadecaploid plants of *C. arvense* were also present in the population, but were not detected due to the relatively small sample of *C. arvense* (as compared to the hybrids and *C. alsinifolium*). In our more extensive screening of *C. arvense* we detected such mixed populations on the Balkan Peninsula and this might be alternative explanation of the observed pattern in the study of P. Vít et al.

In the study of *Sorbus* (Paper II), the author lists *Sorbus torminalis*, *S. aucuparia* and *S. chamaemespilus* as diploid sexual, and taxa from the *S. aria* group as tetraploids, being involved in hybridisation and establishment of new hybrid (apomictic) taxa (P. 14). Aren't there also diploid populations of *S. aria* that can be involved in hybridisation or are they not present in Czech Republic? And a more philosophical question: what is the author's opinion about describing any small, geographically limited, apomictic population as a new species, even if it can presumably be recognised only by experts? Many such taxa have e.g., been described from the Czech Republic and Great Britain, but the rest of Europe is quite neglected in this respect.

In the study of *Nymphaea* (Paper III) the hybridisation detected between *N. alba* and *N. candida* is much rarer than assumed previously. Are the few occurrences of the hybrid *N.* x *borealis* in the author's opinion presumably results of *in situ* hybridisation or some long-distance dispersal?

In the last study of *Diphasiastrum* (Paper IV) the authors detected extensive hybridisation between different taxa in the Czech Republic and concluded that taxonomic recognition of hybrids is not grounded. Having this in mind, how "real" are these species in the candidate's opinion and is hybridisation between them purely result of human activities or a common process in undisturbed areas as well?

I find the thesis well written and the conclusions justified and supported by the results. For the reasons outlined above, and also taking into consideration the impressive publication list of P. Vít, I am absolutely convinced that the candidate meets the goals of the thesis, 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,

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(Dr. Božo Frajman)