A review of Ph.D. Theses *Microevolutionary processes in selected genera of the* Rosaceae *family* traits by Lenka Macková

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The Thesis under review deals with the microevolutionary processes in two genera (*Prunus*, *Cotoneaster*) of family *Rosaceae*, which are known for extensive hybridization and in *Cotoneaster* also by presence of apomixis (apospory). Generally, family *Rosaceae* represents biosystematically one of the most complex groups of higher plants and especially in the apple tribe phenomena like hybridization, polyploidization and apomixis makes phylogenetic or taxonomic studies quite challenging.

Author significantly contributed to the knowledge of processes shaping the evolution in the family. The role of hybridization in homoploid and hetreroploid crosses was studied on example of *Prunus* species and author provided new insights in gene-flow between wild and crop species, demonstrating "creative and destructive" evolutionary force of hybridization. The role of apomixis, polyploidy and hybridization was studied on example of genus *Cotoneaster* and author discovered high variation of different reproduction strategies.

The Thesis is structured into the Introduction section and the Case studied section, the letter presents three case studies.

The introduction section is well-written overview on the studied topic; author provides detailed but targeted information on major evolutionary forces in *Rosaceae*, their impact on classification and gives additional information on its social importance as a crop species. Author focused on evolutionary processes in selected genera, highlighted important facts such as repeated polyploidization events, unequal distribution of hybridization among clades within *Rosaceae* and presented important cases of heteroploid or crop-to-wild hybridization in the family, emphasizing the major findings in other genera of the family (surprisingly, only *Sorbus* is not described in detail).

Second major part, Cases studied chapter can be divided in 2 thematic sections, Cases studies I+II focusing on biosystematic consequences of hybridization in *Prunus*, and Cases study III focusing on consequences of variation of reproduction modes in *Cotoneaster*.

Case studies I and II present studies (published in two papers) on hybrids of rare species *Prunus fruticosa* with crop and wild relatives (*P. avium, P. cerasus*). Author found formation of fertile hybrids between tetraploids and sterile triploid hybrids in heteroploid crosses and described the putative gene-flow in populations. Fertility (formation of functional or degenerated megaspores) was further confirmed by cyto-embryological analyses.

Morphometric analyses revealed that the leaf-shape variation represents a continuum. All the findings are underlined by flow-cytometric analyses on large sample set (111 individuals in case study I and 761 individuals in case study II). Study also revealed the destructive impact of introgressive hybridization on natural population of threatened species *P. fruticosa*. In my opinion, this study (two papers) is good example of excellent biosystematic study employing varying techniques and providing new insights in role of homoploid vs. heteroploid hybridization. The study is not important just for understanding the microevolutionary processes in *Prunus*, but it has general impact on understanding of gene-flow in evolution of *Rosaceae* or other species.

Case study III provides insights into reproduction modes of *Cotoneaster integerrimus* agg. Large sample set (503 individuals from 119 populations) were analysed by flow-cytometry. Study revealed that ploidy variation is in Western Carpathians limited to tetraploid and pentaploid levels, which is in contrary to previous studies. It is important novel finding (based on reliable measurements), which could be a good starting point for further taxonomic revision of the *C. integerrimus* complex. However, this finding is crucial for understanding the taxonomy of the group; I personally consider the most important outcome the discovery of variation in reproduction modes. It perfectly fits to previous studies in other aposporous rosacean genera (*Crataegus*, *Rubus*) providing broader insights on reproduction strategies in *Rosaceae*, or plants in general. Especially interesting is detection of rarely present haploid parthenogenesis in *Cotoneaster*. Such findings have to be taken into consideration for any modern taxonomic treatments and especially in *Cotoneaster* such information was lacking. This study brings novel findings which are crucial for further taxonomic concepts in the genus.

Questions

1) Homoploid introgressive hybridization can be directed by ecological/environmental conditions (e.g. *Salix*, *Helianthus* and many more examples). Can be (e.g. just superficially) seen such differentiation in hybrids *C.* ×*eminens*, *C.* ×*mohacsyana* and their parental species?

2) Recent investigation on some cultural plants lead to distinguishing them as hybrids, the best examples are *Malus* ×*domestica* or *Prunus* ×*subhirtella* (both taxa were previously considered as species), for other taxa are indication of putative hybrid origin too (e.g. *Prunus serrulata*, *Prunus sargentii;* at least some of their cultivars). For *Prunus cerasus* has already been known it is of past hybrid origin (similarly to *P. domestica*), your study showed extensive gene-flow to its parent species, having nearly no reproduction barriers. My question is what is your opinion on taxon *P. cerasus* as a species? Are there some arguments favouring the sour cherry as species or hybrid (i.e. *Prunus* ×*cerasus*)?

3) In the *Cotoneaster* study, out of 5440 seeds only 1114 seeds developed fully having embryo and endosperm. Thus, 79,5 % dropped out from the analyses. What is your idea on such high rate of undeveloped seeds? Is there some evidence that such seeds are e.g. from unpollinated "sexual" embryo sacs, or unpollinated "pseudogamous" embryo sacs? What is the usual pattern of ratio developed vs. undeveloped seeds in fruits?

4) The fruit colour is considered one of the most important characteristics in the *Cotoneaster* taxonomy (cf. the Fryer and Hylmö Monograph). You have found that this characteristic is unreliable in *C. integerrimus* agg. My question is to what extent it is general phenomenon. Do you have some quantification of colour variation? Is it regional or general phenomenon? And finally, what do you think about species delimitations based on fruit colour in other species complexes (cf. Fryer and Hylmö Monograph of 2009).

Conclusion

Personally, I have read this Thesis with great interest and pleasure, I consider findings of the PhD candidate presented in this Thesis highly interesting and I appreciate that author well described important evolutionary phenomena in studied genera. Although no molecular techniques were employed in presented studies, the Thesis represents excellent biosystematic study bringing novel insights into evolutionary processes in *Rosaceae* plants. Generally, the Ph.D. Thesis is characterised by scientific originality, author's effort employing various scientific and statistical techniques and experiments, and well presentation of results in 3 papers. The study significantly contributes to the field of reticulate evolution research.

It shows that the author has great ability for doing the scientific research. This is all confirmed by 3 published papers in respected journals in the field. I therefore recommend the thesis for the defence.

In Olomouc, 30 June 2020.

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