Evaluation of Master thesis from Michaela Konečná.

Hereby I provide a review on the diploma thesis of Michaela Konečná. As written by the author: "In the present study, the reproductive modes, pollen viability and length in most taxa of the genus *Ficaria* and intertaxa homoploid crossing and intrataxa (interpopulation) outcrossing between/within selected taxa of the genus Ficaria were assessed using a combination of pollen exclusion bags, pollen viability analysis, morphometric analysis of pollen length, genome size estimation and experimental crosses."

As is clear from the author's statement, she did a lot of work and challenged herself with the project's complexity and writing in English. I highly appreciate the language choice, as it is rare to see English theses on the Master's level. Nonetheless, I must admit that sometimes it was tough for me to follow the text, feeling that the author does not understand many presented topics and struggles with the writing.

The author measured hundreds of pollen grains, did many experimental crossings, analyzed a lot of data. Still, I am missing a general understanding of evolution and biological thinking, which stays behind the work done. For example, she states: "The results also demonstrated that the absence of occurrence of autonomous apomixis and autonomous selfing and high pollen viability do not act as sufficient prezygotic barriers to prevent hybridization between most taxa of the genus *Ficaria*." How could the absence of apomixis or high pollen viability act as a prezygotic barrier? Or you state, that "germination has the strongest effect on the postzygotic barrier." Germination cannot affect the postzygotic barrier while it is its manifestation…

Bellow, I will pick up a few comments that came into my mind while reading the thesis and ask several questions. At the end of the review, I present some more recommendations, which could help with Michaela's scientific writing in the future.

In the Introduction, I am missing a lot of information about the topic (e.g., the morphology of the studied taxa), many of the information and terms suddenly appear in the Discussion instead of the Introduction, many terms are not explained at all (e.g., Sturge's Rule, geitonogamy, xenogamy).

One of the questions asked is: "What is the variability of pollen viability in the studied taxa? Does the pollen viability relate to hybrid or polyploid origins?" But as the origin is unknown, and you don't study it here, you can't relate your findings to the taxa origin.

Do not present data from other authors in your Results, especially if not published yet. Instead, limit the findings and focus on what you have done. Keep consistent information provided (e.g., in the results part, you suddenly talk about longitude and latitude not mentioned before or after).

Discussion is written chaotically, with off-topic information, and without move to the goal. Please, for future, avoid all the speculations in the Discussion (e.g., you state: "This study provides the first evidence for the potential production of unreduced gametes in the genus Ficaria," but in another part of the thesis, you write: "No unreduced male gametes formed by cultivated offspring from pollination treatments were recorded.", it is opposite to each other, plus you did not test it at all).

Double-check the references to be present in both text and the list of references. And show that you can understand papers you read – referred papers often do not state anything about the referred topic (e.g., "In general, hybrids display a mosaic parent-like, and novel trait rather than intermediate

ones in the first filial and especially in the subsequent generations (Rieseberg & Ellstrand 1993, Rieseberg 1995; Rieseberg et al. 1999; Mallet 2005, Abbott et al. 2013)." From the referred papers, only two talk about mosaic characters).

Don't write until the last moment; try to have some time with the thesis/manuscripts aside and rereading it later. That would help detect at least some errors and typos.

I have several questions:

1. You talk about "*Ranunculus ficaria* L. as the only species originally considered in the genus" (*Ficaria* or *Ranunculus*?). Can you explain how related it is to your taxa? By the way, referring to the paper from Sell in 1994, you say that "later more taxa were described," referring to publications from much earlier (1958, 1961, and 1962).

2. Šiková, in her master thesis (2014), addressed similar questions on the same taxa, although she states different results than the presented thesis. For example, the pollen viability of the taxa shown in both studies was about 20 % higher in the case of Šiková. Also, the pollen size varied considerably. Could you explain why your pollen grains are much bigger?

	Šiková:	Konečná:
FC 2x	34 μm	43 μm
3x hybrid	36 μm	48 μm
FVV 4x	38 μm	48 μm

3. You state that the pollen size increased with the ploidy level of the taxon. The tiny pollen of diploids could cause a strong correlation between pollen size and genome size. If you analyze it only for polyploids, is the correlation still there?

4. As one of the crossing experiments, you used isolation of the flowers without any treatment to test for selfing. But considering proterandry in *Ficaria*, could not the result be affected by that? How could you overcome/test it?

5. Can you explain how do you understand the term mosaic parent-like?

When reading the thesis repeatedly, I cannot recommend a better mark than D. I have the feeling that the author lost herself in the topic and that the experimental part was fragmentary. I will be happy to improve the mark based on the defense.

I have some more recommendations.

Simplification of the text is needed. To avoid overusing the terms again and again, especially if once explained. For example, use instead of "intrataxa (interpopulation) outcrosses" the term 'crossing' and for the "intertaxa homoploid crosses" the term 'hybridization'. You will simplify the text to better flow. As you did only homoploid hybridization, there is no need to repeat "homoploid crossing"

48times in the text. The same is true for many terms in the brackets, which you repeatedly write, e.g., achenes (seeds).

In the Introduction, you correctly use the term "pollen size", but throughout the rest of the thesis, "pollen length" is wrongly used.

4th objective of the thesis is very difficult to follow; the goals should be short and straightforward; you don't have to mention all the subspecies used in variable crossings here, as you will explain it later in the Methods.

In Tables 3 and 4, I am missing information on how many individuals were used (was it one individual per population?) and the percentage of well-developed seeds.

Table 2 is redundant. When there are zero developed seeds for testing the presence of apomixis, you do not need to put the zeros in the table. When you compare this table to Table 3, you have a different number of flowers tested (e.g., subsp. *calthifolia:* 35 vs. 21 for apomixis and 34 vs. 18 for selfing).

In sum, shorten the text to what you have done and found and how it relates to the current knowledge; avoid speculations, references to unpublished data, repetition of the same topics, references to what is not related to your work. Shortening and sharping the text would help the reader to follow. Differentiation of the Introduction and Result sections in the identical/similar sub-chapters as in the Discussion could help. Do not repeat the data from Results in the Discussion; refer to Tables and Figures instead. Some of the results appear for the first time in the Discussion, which should not be the case. Significant parts of the Discussion should be moved to the Introduction. Present only your data and how do they relate to the current knowledge. Everything else is redundant. And the last – read, read, read.

In Brno 19. 08. 2021   Mgr. Petra Šarhanová Ph.D.