

**Review of Andrea Veselá's Doctoral Thesis "External conditions, seed characteristics and other factors affecting seed germination of alpine species"**

Reviewer: Jiří Doležal, Institute of Botany, Czech Academy of Sciences

Andrea Veselá's work examines the influence of changing temperature and moisture conditions on germination behavior in selected species of alpine plants from Europe and the Himalayas and further explores how germination success is affected by key seed properties such as seed mass, carbohydrate and nitrogen content, as well as plant maternal effects and the duration of cold stratification, and finally how species abundance is related to germination success and failure. The submitted thesis consists of four studies, one of which has been published in journal *Flora* and all others have been submitted to peer-reviewed journals. Andrea Veselá is the first author of all four studies. The individual case studies are preceded by Introduction on various aspects of germination in the life cycle of plants, ecological consequences of germination and overview of candidate's own research.

Overall, the published and submitted papers are of high quality, clearly demonstrating the talent and skills of Andrea Veselá in the scientific research. This includes setting up experiments, field collection of seed material, data analysis and writing articles, everything required to conduct independently successful scientific work. There is therefore no doubt that the thesis and the candidate's contribution to it meet the international requirements of the PhD dissertation.

In terms of scope, this work is one of the most impressive initiatives in the germination ecology of plants I have encountered. I appreciate the author's efforts to present state-of-the-art concepts in plant germination ecology in the Introduction. In spite of that, the Introduction as well as some drafts (Study #3 and #4) would clearly deserve further adjustment and deeper thoughts, as there are some typos, factual inconsistencies, and incorrect referencing, which I tried to list below among "Specific remarks".

As the Reviewer is asked to define a few questions for which the Candidate needs some time to prepare, I would ask the Candidate to prepare to respond to the following queries:

1. Seeds germinated much better when exposed to warmer and wetter conditions and germinated much less when exposed to cold and dry conditions. While well demonstrated experimentally in all four case studies, these results are not very surprising. An interesting finding is that seeds from wild plants differed in germination success from conspecific seeds from plants grown in the garden, suggesting the importance of the parental environment for the fate of the seed. It is often a specific environment that plays a decisive role in whether the seeds germinate and makes it all the way to adulthood, and therefore one may wonder why none of these experimental studies on seeds germinated under controlled conditions were followed by field "verification" experiments by sowing seeds in native conditions. *Could the candidate walk the audience through the germination success of alpine plants in the wild (not details), and speculate about possible advantages and disadvantages of controlled and field experiments.*
2. Study #3 on the germination of Himalayan *Impatiens* gives the impression that it is written in a hurry because it contains inconsistencies and typos, etc., which need to be corrected and reformulated. It would be appreciated to see a list of focal species directly in the main text (in a table or on a phylogenetic tree) to better understand the main points. *Could the candidate walk*

- the audience through each species / clades etc to find out how successful their germination is in relation to the variables tested (prec, temp, seed mass) and speculate how their habitat preferences will translate into similarities or differences in their germination reactions.*
3. The main conclusion of study #3 is that "predicted climate change represented by a shorter stratification period followed by warmer temperature will negatively affect species' germination. Germination of the Impatiens species will, therefore, probably be negatively affected by climate change forcing the species to migrate to higher altitudes". *Could the candidate walk the audience through the rationale of these predictions and if these predictions concern all 14 studied taxa or the response will be rather species-specific. Could the candidate speculate what would be other limiting factors (e.g. habitat versus dispersal limitation, sink versus source limitation) of alpine species upward migration in response to the negative effects of global climate change on plant performance including seed germination.*
  4. Study #4 is better than the previous study in that it did not hide the focal species, but the table, when the species are listed, comes too late (like Table 3) after all the results have been submitted. The authors concluded that "in common species, the proportions of germinated seeds were significantly higher during cold stratification than those of rare species". However, this result (Fig. 1A) seems to be strongly driven by only three species such as *Luzula* spp, while the rest does not seem to differ in germination success during cold stratification. *Could the candidate provide more details about this key finding and explain why the general conclusion is made on differences between common and rare species if the germination pattern is driven by only few of them. Some clues as to why *Luzula campestris* germinates well and *L. spicata* so poorly? Could the candidate walk the audience through the mechanisms that make rare species rare and common species common if not differences in germination?*

#### Specific remarks

**Pg11 Pa2:** for germination

**Pg11:** the seeds ARE sucking water rapidly

**Pg13 incorrect citation:** Mendoza et al. 2009 is used to backup the claim that alpine species are more limited by seedling establishment than species with lowland distribution. However, study by Mendoza et al. 2009 is about habitat degradation and its affect on recruitment limitation and its components (seed limitation versus establishment limitation) of woody plant communities in a Mediterranean landscape.

**Pg 13 inappropriate citation:** Körner 2007 is a paper the use of 'altitude' in ecological research In the context of climate change, not about alpine environments as the most vulnerable system

**Pg14 Pa2:** Alpine species are exposed TO high temperature

**Pg15:** "The species response to different moisture conditions is studied less frequently than response to temperature since it is more difficult to control it."

*I'd rather say the opposite, but maybe I'm wrong. You can support this statement with a few examples.*



**Pg15:** "Several recent studies have shown that the number of germinated seeds, and the germination speed decreases with decreasing moisture (e.g., Wen et al. 2015; Ruhl et al. 2015)."

*This is not completely true. There are many experimental studies from drylands that were exploring the importance of rain for seed germination.*

**Pg15:** "To our knowledge, only one such study exists for alpine environments (Orsenigo et al. 2015), in which a decrease in germination in warm, dry conditions was found."

*Again, this is not true. There are many studies on germination of alpine plants such as Tudela-Isanta, M, Fernández-Pascual, E, Wijayasinghe, M, et al. Habitat-related seed germination traits in alpine habitats. *Ecol Evol.* 2018; 8: 150– 161, see cited literature.*

**Pg16:** Study BY Bauk et al. (2017)...

**Pg16:** This knowledge is important especially in alpine regions, since there are considered as the most vulnerable system to climate changes (Körner 2007)....cite rather a book of Ch. Körner from 2003.

**Pg16** by environmentally induced changes

**Pg17** I used field-collected and garden-cultivated SEEDS in my research and compared the results

**Pg17** Positive effect of seed mass on germination was demonstrated in studies of (Navarro and

**Pg17** Seed mass is not changed

**Pg17** ...missing word: have to control for species phylogenetic relationships.

**Pg17** The comparison of rare and common *Cirsium* species in northern Bohemia, Czech Republic has shown that...

*why are you talking about common species if this paragraph is about introducing the phylogeny??? Comparing rare and common species is ok, but this is unrelated to possible phylogenetic differences that you wished to introduced in this paragraph, right?*

**Pg20** be consistent, you use both behaviour and behavior, but better to stick with one

**Pg22 Pa1** What is the importance of changes in temperature and moisture between sites

**Pg23** Phenotypic plasticity allows species TO cope

**Pg23** species of genus *Impatiens* coming FROM an elevation gradient

**Pg54** as hypothesized by Flégrová and Krahulec (1999)...instead of as hypothesized by (Flégrová and Krahulec 1999), also in line with Flégrová and Krahulec (1999).

**Pg95** title: maybe "elevation" is missing from the title. i.e. Study 3: SEED MASS AND PLANT ORIGIN INTERACT TO DETERMINE ALPINE SPECIES GERMINATION PATTERNS ALONG AN Elevation GRADIENT

Pg96 Pa2 Recent studies, for instance, found that populations from the warmest conditions **have shown** the highest germination

Pg96 revise Cavieres and Arroyo, 2000).

Pg96 make sentences separate ... seed mass (Wang et al., 2009; Wang et al., 2012; Liu et al., 2013; Hradilová et al., 2019; Rees et al., 2001) hypothesised

Pg97 rephrase: It is based on seven species and showed that more closely-related species had more similar germination behaviour and the authors (Carta, Hanson and Muller, 2016a) point out that this subject requires further attention. Such knowledge is likely to increase our understanding of the evolution of the requirements for species' germination

Pg98 elevational instead of evelational gradients

Pg125 Table 2. The results of comparing rare and common congeners in total proportions of germinated seeds and in detailed categories **OF** light/temperature conditions

Pg126 Fig. 1. Proportions of germinated seeds **IN/OF** common and rare congeners

Pg133 However, with the use **OF** fresh seeds,

Pg134 what kind of MSMT project funded this research, be specific and add grant number

According to my experience and opinion the thesis of **Andrea Veselá** fulfils all formal requirements (respecting length, structure, style, publications, content and quality) of a PhD dissertation. **Andrea Veselá** clearly demonstrated her ability to carry out an independent scientific research, and present its results to an international scientific community, and also, to draw from her results conclusions that can have direct consequences for alpine plant responses to ongoing climate changes. The thesis provides a body of work that is a good example of how much field work, controlled experiments and theory in ecology can contribute to the field when it is well thought out, conducted, analyzed and interpreted. The novel results will not only serve as valuable tests of existing hypotheses but should also go on to inspire new work in plant germination ecology both experimental and theoretical.

Yours sincerely,

České Budějovice, 15.09.2020

Jiří Doležal