

## Reviewer comments to Milan Kotlínek PhD theses: **Factors limiting the distribution of the mycoheterotrophic plants in fragmented landscape**

Milan submitted theses that are exceptional from several points of view: First, the topic is inherently interdisciplinary. It uses ecology, phylogeny, demography, plant science. Second, it uses rich assortments of techniques, as methods of field ecology, molecular techniques, and modelling. Third, the topic of theses by Milan is highly actual and connects basic research and practical questions of nature protection on a group of organisms that are particularly vulnerable to contemporary changes in habitat quality, size and isolation. Although final papers are a collective enterprise of several researchers specialized in their fields, Milan mastered during his study several techniques and his contribution spans from planning experiments, field sampling, molecular techniques to manuscript writing. The thesis consists of a general Introduction, summary of results, 6 papers, 5 published and one manuscript, Milan is the first author of four papers. His contribution to individual papers varies from 16 to 52 %. Papers were published in very good journals and are a combination of research papers and reviews. I take the liberty and recommend the theses for public defense.

Questions for the defense:

The first three papers are not exactly matching the topic of the theses, but I appreciate this topical and stylistic expansion. I have a question to the first paper, the last paragraph of the discussion. I am curious why the occurrence of rhizoctonia-associated species in *Neottia* complicates the simple scenario where the ancestor of the tribe Neottieae is considered mycoheterotrophic. I understand that reversal from mycoheterotrophy to autotrophy is not very probable but the three other possible cases (i) mycoheterotrophy is common for the whole genus, (ii) mycoheterotrophy evolved only in a few related species of a genus or (iii) is common for several related genera is only a matter of our artificial delimitation of genus – or not?

Have you considered publishing contributions on biological flora in a framework of Biological flora of Central Europe published in *Flora and Perspectives of Ecology, Evolution and Systematics*?

Nearly all remaining papers heavily rely on the method of seed germination in the field. Plenty of seeds in small frames put into the soil and controlled after one or several years whether protocorms start to develop. I like this method as it provides a nice way how to catch a rare moment when an orchid seed encounters symbiotic fungi and starts to grow. It enables assessment of fungal identity and fungal distribution in soil although abiotic conditions are inevitably involved. It is interesting that in any of the papers this method is discussed although it might have its limitations. Could you discuss pros and cons of this technique?

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