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Review of the PhD thesis by Jakub Těšitel, entitled "Biology of the Root-Parasitic Rhinanthoid Orobanchaceae", defence on 2 September 2011, České Budějovice

Supervisor: Prof. Jan Lepš Advisor: Dr. Milan Štech

The thesis counts 104 densely printed pages and consists of a concise general introduction of four pages including references, and seven individual papers, of which five have already been published as articles in international peer-reviewed journals with a good to excellent ranking. One paper is a review of the literature, while the six other papers describe experimental work and form a showcase of the large variation in techniques the candidate has mastered: DNA sequencing, phylogenetic analyses, carbon isotope analysis, microscopy and morphometrics. The candidate has also published an earlier peer-reviewed paper, Těšitel & Štech 2007, which does not form part of this PhD thesis (why not, was it his MSc thesis?), although it would have fitted well with the other papers on systematics, it is cited several times. For paper 7, the candidate is last author, not first, which in my view is unusual for a PhD thesis. The thesis shows that the candidate has established several collaborations, both within the University of South Bohemia and in foreign universities (Sheffield, Montpellier), which have greatly aided in expanding his knowledge of the different facets of his subject.

In general, the English is very good, although unfortunately a number of typing errors have managed to survive the publishing process undetected.

The variety of the subjects and species studied within the Rhinanthoid Orobanchaceae makes it difficult to turn the different papers into a single coherent work. I can see why the paper on the overall phylogeny and evolution of the whole group was put first, but it would have been more logical to continue with the other two papers that deal with systematics and biogeography, which are now placed at the end of the thesis as paper 6 and 7. A second section could then have consisted of the purely physiological papers 2-4, which could have been preceded by paper 5, which forms a bridge between physiology and evolution, so basically in reverse order than in the thesis, but with a more logical transition from the first part to the second, without the rupture that is now present between paper 1 and 2. It may not be customary for a PhD thesis in the Czech system to have a general discussion at the end, but it would have been very useful, certainly in this case, to provide an overview of how exactly the candidate's work as a whole has contributed to the field and how the different elements of the research come together in the big picture.

Five out of the seven papers have been through the process of peer review, and the candidate has thus proven that he is capable, with or without foreign co-authors, of producing good-quality science. I was involved in the review process for two of the papers in this thesis, one as a guest editor and another as external reviewer, and in both cases the initial comments of the reviewers were quite positive and the revisions to the point. The resulting papers are of good quality, and it would not be appropriate to review them again in detail, and I will therefore limit my specific remarks to the remaining unpublished papers, 5 and 7. It is not indicated whether these have been or will be submitted to a peer-reviewed journal, I hope that this will still happen, they would be useful contributions to the overall body of knowledge on Rhinanthoid Orobanchaceae.

The title of paper 5 is a bold statement ("Leaf excretory glands play a key role..."), which in the main text turns out to be a rather tempting and plausible, but unproven hypothesis. Since more work is definitely needed to indeed prove this key role, it would be wiser to tone down on the certainty of the title.

In paper 7, figure 1, the difference between black and grey is difficult to see, it would be better to use different symbols as well for the three types. It makes not much sense to use total number of internodes as a separate variable, since it is completely explained by the two other variables, vegetative and intercalary nodes, unless there is a negative correlation between these two latter variables, which should be tested.

Overall, this thesis and especially the individual articles that have been published, are of an excellent quality and a promising start of a scientific career. I should also mention that the candidate has made a very good impression four years ago at the biennial meeting of the European Society of Evolutionary Biology in Uppsala, where he was originally presenting a poster, but was asked to give a talk in place of a speaker that could not come. He managed, despite the very short preparation time, to deliver a good presentation on the subject of his poster, seasonal variation in *Melampyrum*, which has not been included in his thesis. He has also taken the initiative to organise the second International Symposium on the Biology of non-weedy Orobanchaceae, which took place in August 2008 in České Budějovice, where researchers on all aspects of the biology of these hemiparasites, from ecology and physiology to systematics, were united. The candidate's thesis is a worthy witness of the scientific value of this kind of cross-disciplinary meetings.

#### Questions

1 – Since a general discussion is missing from the thesis, I would like to ask the candidate to evaluate his contribution to the knowledge and understanding of the evolution and physiology of the Rhinanthoid Orobanchaceae, and which gaps he still sees, in both his own work and the overall knowledge. He should also elaborate on how to fill these gaps, and which priority he would attach to each of them.

2 – Paper 5 proposes a interesting, yet untested hypothesis on the role of the excretory glands in the evolution of holoparasitism in the Rhinanthoid Orobanchaceae. Elaborate on how their precise role can be tested and quantified, how these structures could have evolved, and if these glands (or analogous structures) can also be found in other clades in the Orobanchaceae.

3 – Discuss the two hypotheses you propose on the evolution of the tetraploid *Odontites* at the end of paper 7 in the light of your own results, whether polyploidisation is more or less likely to have preceded ecological differentiation, and how these hypotheses could be tested.

4 – Discuss the evolution of seasonal variation and its role in the diversification of this clade.

Renate A. Wesselingh 26 August 2011

# Evaluation of the Ph.D. thesis by

# **RNDr. Jakub Těšitel**

### entitled

# "Biology of the Root-Parasitic Rhinanthoid Orobanchaceae"

Parasitic plants, in spite of their peculiar mode of life, have not been favourite objects for detail research till now, and, therefore, much information on their evolution and functioning is still lacking. The thesis of Dr. Těšitel represent quite valuable attempt to fill the existing gap.

The thesis is presented as an assemblage of seven separate scientific papers. Most of them have been published recently in renowned botanical journals. That means that their scientific value was sufficiently evaluated during peer review process and it would be rather superfluous to evaluate each of them once more. Instead, I would like to present some general comments and conclusion.

1) The scientific scope of Dr. Těšitel, exposed in his thesis, is admirable - from problems of biogeography, ecology, and physiology to molecular taxonomy of parasitic plants of Rhinanthoid group of the *Orobanchaceae* family. On the other hand, the papers presented in the thesis are not always sufficiently compatible, that means they are not all focused on solving of closely related specific problems. Three of the included papers are oriented to some important aspects of taxonomy or biogeography of the selected plant group. The remaining four papers are devoted to physiological problems, mainly to importance of heterotrophic carbon gain in hemiparasitic species. Nevertheless, both the groups of studies are excellent, based on modern conceptual and methodical approach. After my opinion, it could be sufficient to present only one half of the papers as a thesis. In this case it could be easily framed the subgroup of selected papers by a more extensive Introduction, and also by lacking general discussion and conclusion.

2) As stated by the author in the Introduction, the physiological and evolutionary view on Rhinanthoid parasitic plants should be integrated by paper No. 5, presenting a synthesis of available data on leaf excretory glands (including some newly found and very interesting data on their ultrastructure). Unfortunately, I consider the paper as preliminary only, describing the glands mainly from structural (anatomical) point of view and some of its conclusions are too speculative. The most important problem - mechanism of their function (particularly, mechanism of the putative active, metabolic energy driven water transport) remains, most probably, still unsolved. Am I right?

3) There are some incorrect terms or expressions, mainly in the unpublished manuscripts included in the thesis (e.g., *weight* instead of mass, *light intensity* ...). Their number is small. I am also not sure, if the "Rhinanthoid Orobanchaceae" is a correct term (within the rigid taxonomical hierarchy) for the group of the studied plant.

#### Conclusion

The reviewed thesis is well written and the presented research is quite interesting and valuable. The minor problems mentioned above can be discussed during the oral presentation. The thesis is acceptable in the present form, and, therefore, I recommend awarding RNDr. Jakub Těšitel with the Ph.D. degree.

Brno, August 8, 2011.

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Prof. RNDr. Jan Gloser Masaryk University, Faculty of Science, Brno