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Examination of the Doctor of Philosophy Thesis

presented by the candidate Dagmara Sirová entitled:

“Hunters or gardeners? Plant-microbe interactions in rootless carnivorous *Utricularia*”

The thesis submitted by **Dagmara Sirová** represents valuable new information resulting from the series of experiments focused on plant-microbe interaction and enzyme production in bladders of carnivorous plant of the genus *Utricularia*. I highly appreciate the fact that candidate chose the genus *Utricularia* as experimental material for her studies, particularly laborious work with collection of small volume of trap fluid.

The topic, aims and methods are in accordance with the latest standards and techniques, experiments are well planned and conducted and the amount of experimental work is adequate (or even exceed) the criteria for the PhD thesis in **Hydrobiology**.

There are eight main outcomes of the thesis published in eight papers. The first results from experimental investigation of enzyme production in the *Utricularia* bladders. The authors showed that the phosphatase had the highest enzymatic activity in the bladders. It seems that its activity is constitutive (i.e. is not induced by prey, as the authors concluded in their paper published in *Biologia*), what is in contrast with other carnivorous genera e.g. *Drosera*. On the contrary, the activities of other trap enzymes (including aminopeptidase) were low and even lower than in ambient water. Although it has been usually believed that nitrogen is the main element taken up from prey in carnivorous plants, the authors concluded that phosphorus uptake from prey is probably more important, at least in the genus *Utricularia*. In the second paper, the candidate found high nutrient concentration in trap fluid and suggests complex plant-microbe interaction, which is rather mutualistic than a simple predator-prey interaction, similar to mycorrhizal association in terrestrial plants. The results published in *Journal of Experimental Botany* were the most exciting for me. The fact that *Utricularia* plants supply photosynthetic carbon to the bladders and thus feed its microbial community, which helps in nutrient acquisition, brings a new insight into the carnivorous syndrome in plants. In general, it has been usually believed that carnivorous plants rather take up carbon from prey than exudate it into the traps to feed the microbial population. I evaluate this part as novel view on plant carnivory and very interesting. The next paper published in *Functional Plant Biology* revealed the chemical composition of exudates, which is composed of easily metabolized organic molecules (e.g. sugars), and the concentration is dependent on mineral nutrient supply. The fact that the amount of organic compounds in the fluid is dependent on light condition, provides convincing evidence in involvement of photosynthesis in this process. These results were submitted to *Fundamental and Applied Limnology* and I hope that they will be soon accepted.

The results from thesis were published in eight papers, some of them in journals with high impact factor (e.g. *New Phytologist*, *Journal of Experimental Botany*). Several tenths of SCI citations documents that the results were accepted by scientific community and high standard of the submitted thesis. Therefore, I have only some minor remarks:

- How can you explain the fact that carbon concentration in the filtered fluid determined by elemental analysis was the highest in old traps (paper II) and on the contrary, the concentration of organic compounds was the highest in young traps (paper VII)?
- You found contrasting growth effects of prey capture in two aquatic carnivorous plants (*Utricularia australis* and *Aldrovanda vesiculosa*, paper V). You explain no significant effect of prey addition on shoot biomass in *Utricularia* by the operation of a complex food web in their traps in contrast to *Aldrovanda*. It is not possible that positive response of *Aldrovanda* to feeding was caused by higher success in prey capture (55.5% vs. 19.1% of traps)? Moreover, *Aldrovanda* captured prey with higher tissue nutrient content.
- You found a significant rise in amino acid concentrations in *U stygia* traps from N-addition treatments, however the total concentration of amino acids is not denoted as significant in Table 2 (Paper VII).

In conclusion, I may say that the submitted thesis represents a valuable contribution to our knowledge about carnivorous plants of the genus *Utricularia*. The candidate showed adequate mastering in various methods. My comments are small and do not require correction in the thesis; they may be discussed and explained during the defense. The thesis shows good orientation of the candidate in recent literature as well as the capacity to discuss results with these data. Finally the candidate shows critical thinking and sound interpretation of results, as well as concise, brief and comprehensive conclusions of his work.

According to my opinion the thesis should be accepted as it stands.

In Bratislava, Slovakia, May 25., 2012



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Review of Dagmara Sirová Ph.D. Thesis “Hunters or gardeners? Plant-microbe interactions in rootless carnivorous *Utricularia*”

This study presents the main five aspects of the physiology of the *Utricularia* trap: extracellular enzyme measurements in the trap fluid, an evaluation of the commensal microbial community structure and function, stable isotope labeling to determine plant carbon allocation, and ion chromatography analyses of the composition of trap fluid. This study was financed by four grants. It should be emphasized that Ms. Sirová performed her PhD thesis in collaboration with various institutions including the Wetlands Ecology Laboratory, University of California, USA.

The PhD thesis contains seven papers and one manuscript that were submitted to journals; Ms. Sirová is coauthor of all of the material. What is important is that PhD student Sirová made an important contribution in all of these documents. Ms. Sirová is the first author of four of the documents presented. With the exception of the short note published in the *Carnivorous Plant Newsletter*, the papers were published in high-impact journals in the fields of plant physiology and general botany such as: *New Phytologist* (Impact Factor: 6.516), *Journal of Experimental Botany* (Impact Factor: 4.818) *Functional Plant Biology* (Impact Factor: 2.156), *Functional Plant Biology* (Impact Factor: 2.156), *Aquatic Botany* (Impact Factor: 2.087);. The articles, presented in her PhD thesis, were reviewed by independent reviewers of the journals and this situation puts me in a comfortable situation as I can focus on their merits.

However, in my opinion, the paper “Sirová D., Adamec L. & Vrba J. (2003) Enzymatic activities in traps of four aquatic species of the carnivorous genus *Utricularia*. New Phytologist 159 (3): 669-675” should be excluded from the dissertation because it contains results which were used to obtain another degree: thesis Bakalářská práce “Enzymatická aktivita v pastech 5 druhů rodu *Utricularia*.” But even when we exclude this paper, the other papers still present a strong degree of knowledge, which is a good basis for a PhD degree.

Recently, the ecological group of carnivorous plants has attracted the attention of specialists in various fields, e.g. ecological-plant physiology, embryology and cytology. Significant progress has especially been made in the case of members of the Lentibulariaceae family, which are one of the most intriguing carnivorous plants because of the greatly increased rates of nucleotide substitution across genomes, unusual changes in the structure of cytochrome *c* oxidase and also the fact that they present deep embryo simplification and the evolution of ultrasmall genomes.

While Ms. Sirová studied the trap commensal microbial community and their interactions with plant in depth, it should be mentioned that the idea of the occurrence of various organisms inside *Utricularia* traps which are not prey is not new. For example, Darwin was one of the first researchers who noticed that many living organisms are not killed by the plant in the traps of *Utricularia*: “*In all cases the bladders with decayed remains swarmed with living Algae of many kinds, Infusoria, and other low organisms, which evidently lived as intruders*” (Darwin, 1875. *Insectivorous Plants*: p. 405). Later, Richard (2001) suggested that the mutualistic relationships inside the traps for *Utricularia* growing in barren waters are more important than pure carnivory based exclusively on the predator-prey relationship. However, most authors who have analyzed *Utricularia* trap contents only counted and determined the microorganisms inside the traps and paid less attention to the trap physiology.

Ms. Sirowá along with her collaborators tried fill this void. They focused on the five main aspects of the physiology of the *Utricularia* trap (extracellular enzyme measurements in the trap fluid, the evaluation of commensal microbial community structure and function, stable isotope labeling to determine plant carbon allocation, and ion chromatography analyses of trap fluid composition).

In her thesis Ms Sirowá had three general aims to determine:

1. the chemical characteristics of the trap fluid
2. the characteristics of trap microbial communities
3. the role of trap age and mineral nutrient availability.

I think that all of these questions were answered.

Probably the greatest achievement of PhD student Sirowá is the statement that the plants supply photosynthetic carbon to their traps. Rootless *Utricularia* plants supply easily available organic C to the microbial community (mainly bacteria) thriving within the trap environment while later benefiting from its by-products. According to Sirowá, this phenomenon resembles the secretion of a wide variety of organic compounds by the plant's roots for the soil microbial community, and that this phenomenon may contribute to the great success of aquatic *Utricularia* in oligotrophic waters.

Despite these identifying the strong points, the duty of a reviewer is to indicate some of the weakness of a work. It is unfortunate that the PhD. dissertation/papers were limited to aquatic species, which are in the minority within the genus. I believe that the obtained results should not be generalized to other ecological groups of *Utricularia*. It is also unfortunate that Ms Sirowá did not fully discuss the role of algae in the *Utricularia* traps, especially because the role of these organisms has been conceived of differently, e.g. algae in *Utricularia* traps have been considered as prey by Peroutka et al. (2008). However, these minor drawbacks do not affect the total value of the research.

Conclusion

I think that the Ph.D. dissertation presented by Ms. Sirowá meets all of the requirements of a Ph.D. dissertation work.



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