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Review of doctoral dissertation of Michał May

Plastyczność strategii odżywiania storczyków miksotroficznych jako stadium pośredniego w ewolucji mykoheterotrofii

Plasticity of nutrition strategies in mixotrophic orchids as an intermediate stage in evolution of mycoheterotrophy

The main part of doctoral thesis of mgr Michał May entitled *Plastyczność strategii odżywiania storczyków miksotroficznych jako stadium pośredniego w ewolucji mykoheterotrofii* (*Plasticity of nutrition strategies in mixotrophic orchids as an intermediate stage in evolution of mycoheterotrophy*) are five publications in English, published in three consecutive years (2019-2021):

1. **May M**; Novotná A; Minasiewicz J; Selosse M-A; Jąkałski M. 2019. The complete chloroplast genome sequence of *Dactylorhiza majalis* (Rchb.) P.F. Hunt et Summerh. (Orchidaceae). MITOCHONDRIAL DNA PART B, VOL. 4, NO. 2, 2821–2823.
2. Lallemand F; **May M**; Anna; Jąkałski M. 2019. The complete chloroplast genome sequence of *Platanthera chlorantha* (Orchidaceae). MITOCHONDRIAL DNA PART B., VOL. 4, NO. 2, 2683–2684.
3. Lallemand F; Logacheva M; Le Clainche I; Bérard A; Zheleznaia E; **May M**; Jąkałski M; Delannoy É; Le Paslier M-C; Selosse M-A. 2019. Thirteen new plastid genomes from mixotrophic and autotrophic species provide insights into heterotrophy evolution in

Neottieae orchids. GENOME BIOLOGY AND EVOLUTION Volume 11, Issue 9: 2457–2467.

4. **May M**; Jąkowski M; Novotná A; Diemel J; Ayasse M; Lallemand F; Figura T; Minasiwicz J; Selosse M-A. 2020. Three-year pot culture of *Epipactis helleborine* reveals autotrophic survival, without mycorrhizal networks, in a mixotrophic species. MYCORRHIZA 30:51–61.
5. Jąkowski M, Minasiwicz J, Caius J, **May M**, Selosse M-A, Delannoy É. 2021. The Genomic Impact of Mycoheterotrophy in Orchids. FRONTIERS IN PLANT SCIENCE 12: 632033.

In the case of two publications M. May is the first and corresponding author. The declarations of all authors clearly document important contribution of M. May to creating the publications. His participation concerns all stages of studies – from conceptual work, through collection of material to analyses, laboratory and statistical analyses, and at the end preparation of manuscripts and their reviewing and editing.

The fundamental part of PhD of M. May's comprises scientific articles published in reputable journals. They were reviewed by at least ten reviewers, thus I restricted to point out these findings, which are the most important for science development and the most interesting in my opinion. The first two publication (*The complete chloroplast genome sequence of Platanthera chlorantha...* and *The complete chloroplast genome sequence of Dactylorhiza majalis...*) were published as announcements. Despite their short form, they provide important input into explanation of evolutionary aspects of orchids. They document complete chloroplast genome sequences of *Platanthera chlorantha* and *Dactylorhiza majalis*. In the case of the studies on the first species the data enrich knowledge in this topic, since only one plastome of Platantherans are known. In the case of *Dactylorhiza majalis*, the species belonging to taxonomically difficult and problematic genus with high frequency of hybridization, polyploidization and introgression between its representatives, it is the first fully sequenced plastome for this genus. Obtained results are useful in identification of *Dactylorhiza* species, in the analyses of genetic diversity of Orchidaceae, thus are important from phylogenetical point of view. In this moment I have a questions to PhD student: Results in these publications show 134 genes, of which 113 are unique for both taxons studied. In which way this identity could be explained? The next question concerns these results, which document that the phylogenetic relationships between *D. majalis* and other members of the Orchidaceae showed its clustering together with representatives of the subtribe Orchidinae.

What about phylogenetic position of this species documented by other authors in former studies?

In the third article, being part of dissertation of M. May (*Thirteen new plastid genomes from mixotrophic and autotrophic species provide insights into heterotrophy evolution in Neottieae orchids*), published in *Genome Biology and Evolution*, authors tested how gene content and selective pressure are related to nutrition type and carried out a tentative plastomebased phylogenetic reconstruction of Neottieae. Thirteen species were chosen for this study, mainly from *Epipactis* and *Cephalanthera* genus. They represented all six Neottieae genera and 9 of them were mixotrophic ones. Such representation enabled wider and more precise, than in earlier studies, documentation of genetic basis of heterotrophy evolution in Neottieae. One of the most important and interesting findings of this study was to document that photosynthesis-related genes did not experience selection relaxation in mixotrophic species compared with their autotrophic relatives. Moreover, authors reported even evidence for selection intensification for some of them and explain this by their importance for fruit formation. The obtained results in this publication were basis of suggestion that an evolutionary radiation at the beginning of Neottieae history took place, which may be linked to mixotrophy emergence.

The next paper (*Three-year pot culture of Epipactis helleborine reveals autotrophic survival, without mycorrhizal networks, in a mixotrophic species*) presents results of very interesting experiment. Under the same conditions, in common garden, individuals of three *E. helleborine* subspecies were cultured without mycorrhizal network. The aim of this experiment was to characterize the mycorrhizal associates of transplanted plants and the level of autotrophy. Using, respectively, metabarcoding methods to identify the fungal community in roots and isotopic and N abundance to characterize the autotrophy level, researchers found evidence of autotrophic survival of the mixotrophic *E. helleborine*. M. May together with co-authors found clear domination of rhizoctonias from three rhizoctonia families – fungus typical for autotrophic orchids. Their abundance was importantly higher than that noted for other species studied in this context, although the last studies were conducted on plants, which grew in situ. Moreover, researchers found that the 1-year-cultivated individuals were characterized by more abundant ectomycorrhizal fungi than those cultivated for 3 years. What about hypothetical results, when plants would be cultivated longer? It should be also accented that some fungus groups were common, but their abundance varied between plants. This result generates a question – would this variation be maintained in the case of the increase of sample size? Summarizing, the most valuable results of this interesting study concern plasticity of

mixotrophy. Authors suggest that this phenomenon may be plastic not only ecologically, but also evolutionary. In my opinion, presented experimental studies should be further developed (with increasing sample size and at longer time), since they give important information due to evolutionary point of view, explaining evolution of trophic in plants, especially in orchids.

In the last article (*The Genomic Impact of Mycoheterotrophy in Orchids*) M. May and co-authors used RNA-sequencing in three parts of *Neottia nidus-avis* and *Epipogium aphyllum* individuals (flowers, stems, and roots or rhizomes), which were sampled from natural conditions. Authors identified expressed gene sets in each case. Then compared gene sets of the three mycoheterotrophic orchids to that of three autotrophic orchid species (from literature data) in order to highlight the gene losses and gains associated with the switch to mycoheterotrophy in orchids. Additionally, authors identified genes that are differentially expressed between the three investigated tissues. Results of the studies evidence that switch to mycoheterotrophy is not connected with novel genes, but exclusively with loss of genes related to photosynthesis. Moreover, this switch does not involve new pathways and functions. Important results are also these, which document the differentiation of genes expressed between three organs analysed as well as organ-specific genes. It is interesting that the highest numbers of differentially expressed genes were noted between under- and aboveground organs and the most organ-specific genes were found in the mycorrhizal root/rhizome. It is reflected in numerous metabolic functions. I would like to pay attention to well-constructed and very informative figures, which clearly illustrate pathways differentially expressed between organs in *E. aphyllum* and *N. nidus-avis* and show comparison of distribution of pathways between different organs of mycoheterotrophic orchids and autotrophic non-orchid plants.

Additionally, M. May's includes in his thesis the manuscript entitled *Practices for reliable hybrid de novo assembly of plant transcriptomes*. It has methodological character and is evaluation of multiple methodical approaches to develop a set of practices for effective, reliable, and efficient assembly of plant transcriptomes in high-throughput pipelines. *Arabidopsis thaliana* was used as a model species with using Illumina and Oxford Nanopore MinIon sequencing data. I hope it will be soon available for readers, since it present valuable information.

Aside from above mentioned publications M. May's Phd thesis contains other chapters, which present important information. The first of them are Abstract and Preface, where we can found background and motivation of presented dissertation, the scope of dissertation and Acknowledgements. I would like to pay a special attention to the next

chapter, Introduction, constructed from few detailed subchapters, which well introduce to the study problems. In this part M. May, using rich literature sources, shows the diversity of nutrition strategies among plants, characterizes the common phenomenon in nature - mycorrhiza, its types as well as its role for plant trophic strategies. It is worth to accent that author pays close attention to Orchidaceae family in the context of presented problems and shows its importance as a model object for explaining plant nutrition strategies. Then, M. May presents research questions and aims of his study. Both general and specific goals are precisely and clearly formulated.

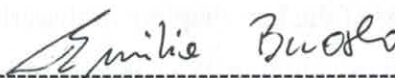
Although in each publication used methods were detailly described, in chapter number six M. May presents shortly main of them and addresses particular techniques to a given scientific problem. I am impressed by their diversity. These are advanced techniques, including DNA and RNA sequencing, stable isotope analyses or in silico data processing. All of them were adequately used and enabled to achieve scientific goals. Diversity of applied methods translates into quantity and quality of presented results. Moreover, this skill is a good start point to develop of farther studies.

Two of the last chapters of dissertation are Discussion and Final discussion (Chapters 7 and 9). In my opinion, the contents of both parts could be presented as one, eventually divided into two subchapters. In contrast to Discussions in particular articles they don't touch detailed results. First of them is summary rather. In this part M. May mainly summarizes the most important results and points out these of them, which are novel or have the greatest importance for development of knowledge about mechanisms of trophy strategies in plants, especially orchids, and their evolution. It seems that in this part some information from earlier chapters are repeated. The Final discussion concerns largely obstacles and opportunities of research. In this place M. May described backstage of research process and showed the difficulties connected with some procedures. Main of them are scarcity of plant material and problem with preparation of appropriate samples for DNA and RNA extraction (e.g., without various soil-related compounds) or prevention of degradation of RNA. On the one hand, Final discussion includes valuable information, which can be used by people conducting similar studies. On the other hand, sometimes it gets the impression that some results may be unreliable, e.g. due to limited sample size. Moreover, I do not fully agree with M. May's statement that the conservation status of orchids limits the sample size. Some of them are relatively common in Poland (*D. majalis*, *E. helleborine*), that taking a slightly larger sample for research does not pose a threat to the preservation of their population. Finally, I would like to draw attention to the chapter, in which the doctoral student presents perspectives for further

research. This proves his scientific maturity and awareness of knowledge gaps in the field of interest to him.

Final evaluation statement

In conclusion, I highly appreciate Mr. May's dissertation. The research issues undertaken by the PhD student are part of the current issues in the field of broadly understood plant evolution with the use of modern research tools. Mr. May's doctoral thesis provides valuable, new data for science, broadening the knowledge of the mechanisms of shaping plant trophies. The importance of the results obtained, their mature interpretation, and perfect mastery of the research methods are sufficient grounds to consider Mr. May an experienced researcher. Therefore, with full conviction and pleasure, I strongly recommend to the Council of the Faculty of Biology of the University of Gdańsk to accept M. May's doctoral dissertation and admit its author to public defense. The dissertation meets all the conditions for doctoral dissertations.



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