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**Opinion on the dissertation of MSc. Michał May entitled: Plasticity
of nutrition strategies in mixotrophic orchids as an intermediate stage in
evolution of mycoheterotrophy**

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Faculty of Biology

Institute of Environment
Sciences

European Community

Centre of Excellence

The doctoral dissertation of MSc. Michał May entitled “Plasticity of nutrition strategies in mixotrophic orchids as an intermediate stage in the evolution of mycoheterotrophy” was carried out under the supervision of prof. Marc-Andre Selosse. The supervisor is engaged in research and teaching students in France, China, Poland and several other countries where young people are provided with the possibility to hear his lectures and practical demonstration of symbiosis during symbiosis conferences. Indeed he is one of the most influential symbiosis scientists world-wide.

The dissertation consists of ten chapters including an abstract, preface, introduction, main research questions, separate chapter including five published papers, methods, general discussion, unpublished paper including suggestions that came out of the work done, final discussion and conclusions, supplementary information and references.

The focal point of the dissertation are the nutrition strategies of orchids, including autotrophy, mixotrophy and mycoheterotrophy. As those strategies differ in the dependency of the plants on carbon sources from photosynthesis and fungi, the researchers looked into the genetic content of plastid DNA and changes in transcriptomes. The dissertation includes five papers published in journals such as Mitochondrial DNA Part B, Genome Biology and Evolution, in Mycorrhiza and Frontiers in Plant Sciences. This is rather exceptional for polish standards, where in the vast majority of cases, up to three papers become a part of a doctoral dissertation. Also, the coauthors are usually from Poland and not as many as in the present case. This is an advantage of being under the supervision of such experienced and internationally acclaimed scientists.

On the other hand, it is hard to understand the PhD student's actual involvement in the published investigations. According to contribution statements,

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MSc Michał May was the first author in three cases and involved in manuscript preparation in 4 papers. His work included material collection, DNA isolation, data analysis, fungal barcoding, transcriptomic analysis, bioinformatics processing, assembling, and annotating the plastid genomes.

This means that he was involved in all possible activities that are necessary for a scientist involved in the modern work of a taxonomist, ecologist and mycologist. All the published articles are exciting. I highly recommend these reports to my students. As an academic teacher, I can see an excellent work plan. I can easily see that the supervisor and the student have made excellent use of the available opportunities related to a wide network of collaborators.. This involvement was essential for the success of the MAESTRO project granted to the supervisor by the National Science Center, although, the fact the Supervisor was the grant holder should be emphasized. As for the evaluation of the dissertation, I would instead focus on the parts that have not been published so far, hoping that in these parts, I will be able to better assess the work done by the PhD student. The introduction shows an excellent knowledge of the doctoral student and very accurate citations. However, in some places, there are imperfections in citing (e.g. not according to the date of publications, see page 18).

Table 1 should be carefully revised, after van der Heijden et al. 2015, but I recommend taking a few moments to rethink and improve this table, especially regarding characteristic structures. The way it is shown is, in my opinion, oversimplified. Most importantly, peletons can be found in AM, orchid and ericoid mycorrhiza. In addition, nonmycorrhizal plants are never devoid of symbiotic fungi and are often parasitized by, e.g. *Rhizoctonia* strains. Almost in all plants, DSEs are present. I would be interested to hear the definition of mycorrhizal symbiosis vs endophytic fungi symbiosis. According to Araujo et al. 2008 "*Endophytes are microorganisms that inhabit the interior of plant tissues, causing no harm to the host and that do not develop external structures, excluding in this way nodulating bacteria and mycorrhizal fungi*". Nowadays, we know more about plant symbionts, and it would be to state that there is a plant that does not contain any fungi. Plants are perceived as holobionts. Even *in vitro*, plants are always assisted by hidden symbiotic fungi that, if lost, leave the host in deficiency of nutrients or/and protection against pathogens. What is missing in the introduction is a part that was included in the supplementary material and summarized the information on photosynthesis (page 160-164). Although the photosynthesis part could be shorter, but what concerns the non-photosynthetic functionality of the chloroplasts (page 164) is of great importance in the case of this dissertation. I do not understand the rationale in this case, as usually, data that is not crucial for the

dissertation is placed in the supplementary material, . The introduction is very well written and very informative. Understanding the role of non-photosynthetic chloroplasts would add to the general understanding and role of the organelle in the plant. This is because the main finding of the dissertation is that the change from the autotrophy to mycoheterotrophy is not associated with the loss of activity of photosynthesis related genes. However, there are many differences in gene expression profiles between autotrophic and mycoheterotrophic species. The most important changes were associated with remodeling of the plant metabolism; with function loss rather than metabolic alteration. As shown in the 6th unpublished paper, the reliable protocol for sequencing and bioinformatics pipeline that includes combining multiple sequencing approaches in a hybrid assembly process has been presented. This part, in my opinion, is also a valuable step as it can be applied in future to understand evolutionary mechanisms leading to such flexibility in adaptation of orchids and other plants to diverse contexts. It was the right choice to use the *Arabidopsis* model for the obvious reasons (model plant).

Methodology (chapter 6) is the repetition of the information given in the published papers, and it contains relatively unimportant information that could be have been moved to the Supplementary material.

All five manuscripts were followed by elegantly written general discussion (Ch. 7), which I highly grade. It shows that the PhD student is a mature scientist and that he is aware of the need of further studies. There is also the final discussion (chapter 9) that provided us with the technical obstacles encountered by the researchers during the PhD study and possibilities for future research. The PhD student seems to realize what a unique opportunity he had to deal with such an important issue and appreciates the opportunities that have been created for him by the supervisor. I hope it has taught him the humility that every researcher should have. I hope that the exceptional cooperation with many good scientists will not result in the inability to act when it is necessary to show independence.

All the activities mentioned above and the results show that the candidate did a lot during research and was well-prepared to discuss these data. He seems to be a well-prepared scientist for future carrier and should be welcome in any European or US laboratory. I hope that his experience and knowledge will be not lost.

In my opinion, the thesis of MSc. Michał May fulfils all the requirements of a PhD thesis and thus can be considered for a doctorate. I fully support the acceptance of the dissertation in its current form and I would strongly support further steps towards doctoral thesis acceptance. I also believe that this dissertation stands out and deserves consideration for the award of the

distinction. It does not only provide a new insights into the subject of orchid symbiosis but also gives us critical discussion of the molecular methodology and presents an original line of problem solving.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'K. Turnau', written in a cursive style.

Kraków, 29.05.2022

Prof. dr hab. Katarzyna Turnau