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As a reviewer, I submit my review of the thesis entitled “Evolution and Phylogeny of Mesozoa” submitted by PhD candidate Marie Drábková for the obtention of the PhD degree from the *University of South Bohemia*.

The PhD thesis submitted by the candidate summarizes the work done to resolve the dicyemid and orthonectid position in the tree of life and to elucidate aspects of the dicyemid life cycle through population genetic studies. These research questions are very relevant and ambitious, and advances on both aspects will be of great interest to the scientific community. The structure of the thesis consists of an introduction, four chapters presented as manuscript (some of which are already published) and a final section summarizing the results and exposing future perspectives.

In general, I found the work very complete and clear evidence of the candidate’s development as researcher. Also, it should be noted that the scientific production of the candidate during her PhD research is noteworthy with two published manuscripts as first author, one published manuscript as second author and a manuscript under preparation in a very advanced state.

The **title** “Evolution and Phylogeny of Mesozoa” might appear somehow broad and perhaps a more focussed title would have been desirable. Yet, the fact that the knowledge of the evolution and phylogeny of the taxa of study is so scarce could justify such broad title as we are now moving towards knowing more about the subject.

The PhD thesis manuscript starts with an **introduction** including a very detailed review of each of the groups of study, Dicyemida and Orthonectida, including their morphological characteristics, reproductive strategies, lifestyle, geographic distribution, as well as their diversity and distribution. This thorough literature analysis could constitute a review article by itself, and it will be a very useful resource for researchers who start working in any of those groups. In this section I did miss a figure including the current consensus on the phylogenetic tree of the groups mentioned to be used as a resource when going through the text.

The section **aims and scope** of the thesis sets the framework where the thesis is framed, with the general questions to be solved. At this stage and after the thorough literature review, I would have appreciated to see one or a few

hypotheses to be tested throughout the work and to finalize the manuscript with the thesis statement.

The core of the thesis is composed of four chapters that are very complete and self-sustained pieces of work, while constituting a very important part of the overall aims of the thesis.

Chapter I consists of a very comprehensive phylogenomic study, for which new transcriptomes and a genome of dicyemid species were generated. The analyses presented are well designed and performed and reveal important outcomes. Yet, one of the main questions, the monophyly of mesozoans seems unresolved and here is a question for the candidate:

You seem quite confident that Mesozoa are monophyletic, but your results do not fully support that hypothesis.

- *In your opinion, what are the main arguments that support monophyly of Mesozoa?*
- *If you had more time/resources, what extra analyses would you do for a future study to further support this hypothesis?*

Chapter II consists of a population genetics study on a dicyemid parasite and its host, for which newly generated and already available mitochondrial sequences were analysed. The study is based on an impressive dataset and the analyses are well performed. **Chapter III** consists of a intrapopulation genetics study on a dicyemid parasite using newly generated microsatellite markers revealing important information about the reproduction of the parasite. It has been reported that in some cases, mitochondrial sequences and microsatellites provide a limited signal compared to analyses based on a large number of SNP markers. Thus, where is a question for the candidate:

Do you think that, using a large number of nuclear markers such as for example thousands of SNPs discovered and genotyped using restriction site associated sequencing or low coverage Whole Genome Sequencing, the results would have been different?

- *If so, why?*
- *If not, why?*

Chapter IV consists of a review of current knowledge gaps in cephalopod and their parasites and on identifying topics that should be addressed in the future. This work is very interesting and a good reference for researchers in the field. Perhaps it would have been interesting to have this work as the first chapter, setting the group for the following. Considering this review work, here is my question for the candidate:

Given the current knowledge gaps and developments in the field of genetics

- *Which do you think will be the greatest advances in better understanding cephalopods and their parasites?*
- *Which do you think are the main obstacles for increasing the knowledge in cephalopods and their parasites?*

The final section, **summary and future directions**, is quite short but this is expected given that extensive discussion have been presented in each chapter and that chapter IV includes an important section on future directions.

To finalize, I would like to emphasize the impressive quality of this work and to clarify that my comments should be seen as constructive criticisms hopefully useful for the candidate's future endeavours and that by no means indicate low quality of the thesis. In fact, I found this work excellent in view of its innovative nature, degree of detail in the writing and relevance of the results to the scientific community. Thus I strongly recommend the thesis to be defended

Yours sincerely,

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