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Examiner Report:

PhD Thesis: Evolution and Phylogeny of Mesozoa

Author: Marie Drábková

I am pleased to write a thesis review on Marie Drábková's dissertation. The thesis is written in English with good clarity of language. It is free from significant factual or formal errors. The thesis has 169 pages and consists of six major sections – an introduction, four research chapters, and a summary. Three of the four research chapters have Marie as first and corresponding author and have already been published in peer-reviewed journals.

As the thesis title suggests, the parasitic groups Dyciemia and Orthonectida (Mesozoa) are the focus of this thesis. In the introduction, the author summarizes the current knowledge of these organisms. This summary clearly demonstrates how limited our knowledge of these fascinating organisms is, as it is even unclear whether these two groups of parasites indeed form a monophyletic group that would then be called Mesozoa. This fundamental lack of knowledge validates the scientific value of Marie Drábková's research. In the first research chapter (unpublished), Marie uses state of the art phylogenomic analyses to resolve their phylogenetic position within the Lophotrochozoa. The main phylogenomic dataset is built from ± 1000 genes, and while I might opt for a slightly different approach to dataset assembly, it is a useful resource in its own right for the community. The author also uses several advanced approaches to resolve and pinpoint the most correct position of the long branching Dyciemiids and Orthonectids. In the second research chapter, the author analyses the population structure of host *Sepia officinalis* and its Dyciemiid parasite in the Mediterranean Sea. The COXI gene is used for these analyses, which reveal several sub-populations of the host, as well as a certain level of co-divergence between the populations of parasite and host. In the third chapter, the author studies the population structure of *Dyciema moschatum* within its host using eight novel microsatellites. The results show non-clonal populations of the parasite, suggesting the existence of sexual reproduction within the host. The fourth chapter is mostly a review of cephalopod parasites, including a section on Dyciemiid parasites.

The evaluated PhD thesis is clearly of very high quality, as all of its research chapters are either published or in the review process. It is notable that Marie is first and corresponding author on three of the four research chapters. The introduction and summary sections are well written and informative for the reader; the introduction section provides a truly great overview of the studied organisms for readers unfamiliar with the topic. It is very important to state that Marie Drábková has evidently mastered a wide array of research methods and approaches used to study evolution, population genetics, and phylogenetics,

and was able to publish the results in international peer-reviewed journals. Overall, I am very impressed by the thesis, and I am pleased to recommend it for acceptance at the University of Southern Bohemia and award Marie Drábková a PhD degree.



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Comments and questions to the author:

Since my expertise is in phylogenomics and the first chapter is “under review”, I will be mostly asking about chapter 1.

1. Very minor error: On page 17 the author states that phylogenomics has been useful to resolve several uncertain aspects of the tree of life, including monophyly of excavates citing Hampl et al. 2009 paper. While this statement is generally correct, the monophyly of excavates is still unresolved and the taxon Excavata, as defined, is today considered by most not to be monophyletic. This is truly a minor error, just to clarify for the author.
2. Regarding the monophyly of dyciemids and orthonectids:
 - a. Looking at Figure 1. in chapter 1., the dyciemids and orthonectids both form extremely long branches; therefore, LBA between the two groups as a cause of their monophyly immediately comes to mind. Also, when one of these two groups is removed from the analyses (taxa removal trees alt02 and alt03) the position of the other groups appears to change? Would that possibly also imply that their monophyly is artificial?
 - b. What type/kind of error is LBA? Does the fact that “shorter” datasets from genes preselected to be less prone to LBA (C50BLR) also suggest the mesozoa monophyly is a result of LBA?
3. How did the author establish burn-in 1500 generations for phylobayes? Did they look at likelihood stability over time or is the 1500 more of an arbitrarily chosen value?
4. On page 17, the author states that manual assembly of single gene datasets is impossible. Is it truly so? Are there solid arguments for manual ortholog selection? Also, was any significant number of single gene trees used for the concatenation inspected manually?
5. Did the authors consider serial removal of fast evolving and/or heterotacheous sites to test the stability of support for crucial nodes? I think this would be especially interesting for the contrasting less and more complex ML models used by the authors (raxml vs IqTree with C60).