



18/10/2022

To: Whom It May Concern

This letter provides an assessment and a review with regards to the PhD thesis of Martina Lisnerová, entitled "Biodiversity of Myxozoa based on extensive screening of fish and environmental samples". I am delighted to have had the chance to read Martina's work with interest, I would recommend her thesis as a proof for PhD qualification without hesitation. The particular strength of Martina's work is the broad and consistent application of well-tested and reliable data collection and analysis methods to ask novel questions regarding macroevolutionary patterns among myxozoan parasites. Martina draws on existing database resources and supplements them with new data to show patterns of recent and ancient myxozoan radiations in teleost and elasmobranch hosts, and demonstrates the feasibility of characterising myxozoan diversity using eDNA sampling.

The thesis consists of three data chapters comprising seven published papers/manuscripts, which is an impressive achievement. In addition, four chapters introducing and summarising the new findings are provided. I enjoyed reading the work presented, which was done to a professional quality with minor problems in style, writing and clarity.

I have four questions, the first and last more philosophical, one system specific and one technical question.

1. **On semantics of selection for low virulence:** On page 1 of the thesis, you state that "*the co-existence of myxozoans with their hosts has long been adaptive*" – does this conversely suggest that a scenario exists where a host-parasite relationship is **not** "adaptive"? Could you explain what you mean by "adaptive" in this situation? In the following paragraphs you refer to myxozoans that cause disease and virulence in their hosts. Could you debate, by relating the case of myxozoans to other metazoan parasites, the idea that host-parasite evolutionary trajectories do not have to tend towards low virulence to be considered "adaptive".
2. **On myxozoan radiations:** Your paper on *M. pseudodispar* shows fantastic modern parasitological and taxonomic work to delve deeper into species concepts in derived taxa. You refrain from speculating why *M. pseudodispar* amongst so many myxobolids has undergone such a spectacular radiation. Could you provide some thoughts on why *M. pseudodispar* has been so successful, when many other very similar myxobolids have not radiated to the same degree.



3. **On eDNA:** It is a very nice idea to enrich myxozoan spores from sediments before DNA extraction. This certainly will increase the success rate of detection, and it is clear that with further sampling, your work has huge potential to improve our understanding of myxozoan diversity. Could you comment on two very technical points: 1) Focussing on sediment sampling can incorporate spores recently released from hosts, but also spores stored in “seed banks” in the sediment, as well as DNA from non-viable, old spores, potentially bound and stabilised by silica. Did you consider sampling water instead/in addition, and if so, would you expect the overlap between host and environmentally detected OTUs to change? 2) The pre-amplification with general 18S primers (ERIB) is an unusual step in eDNA analyses. What biases could result from this and do you see any evidence for artifacts that could be attributed to the pre-amplification?
4. **On theories of cospeciation:** The work presented here underlines in many cases the observed alignment of host-parasite associations, with inferred strong co-phylogenetic signal in many clades. Could you discuss how this finding adds to the modern theories of cospeciation in host-parasite systems and given unlimited resources, what research direction would you pursue to establish myxozoans as the new “text book” example of host-parasite cospeciation?

I regret I cannot attend the examination in person, and would like to give my congratulations on excellent research to Martina, with warmest wishes for her future career and life.

Sincerely,



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