



Jílové u Prahy, October 14, 2022

Reviewer's report on Ph.D. thesis of Mgr. Martina Lisnerová "Biodiversity of Myxozoa based on extensive screening of fish and environmental samples"

The Ph.D. thesis of Mgr. Martina Lisnerová is dedicated to Myxozoa, without any doubt one of the most interesting and, at the same time, seriously understudied lineages of Metazoa. Although the introductory part of the thesis is relatively short (17 pages) considering the scope of the doctoral project of the candidate and the range of methods used to fulfill its aims, it offers a valuable insight into this exciting group of animals. The text is sometimes difficult to follow (e.g., discoveries of novel protein variants are not ultrastructural findings, *Fredericella* is not a freshwater moss etc.) and would benefit from an additional round of proofreading, but this is not a serious issue. The aims of the thesis and a brief summary of the results are presented on five pages in a clear way.

The main part of the thesis consists of seven papers and manuscripts, five of which have been already published by international scientific journals with IF (IF 2.122-3.981). One manuscript has been submitted to a journal, and one was in preparation when the thesis was submitted. The candidate is the first author of three published papers as well as both unpublished manuscripts. The role of the candidate in each partial project has been specified in the thesis. In my opinion, the number and quality of included publications is more than adequate for a Ph.D. thesis. Among them, I find papers III, V, and the manuscript VII particularly interesting and valuable. I appreciate that the papers focus on various aspects of biology of Myxozoa, including morphological and molecular diversity (and environmental diversity), evolution, life cycles, host specificity etc. In fact, they not only contribute to intensively studied topics, but in some cases open completely new roads of research, the most important of which is development of an almost unbelievably sophisticated set of PCR primers for barcoding of environmental Myxosporea. I find the research on co-phylogeny of myxosporeans with their hosts and the immense number of Myxosporea isolates obtained from Chondrichthyes invaluable as well.

I have several questions/comments to the introductory chapter or included papers.

1. An important part of the thesis is based on the paper by Holzer et al. (2018), where the authors dated events in the evolution of Myxozoa and inferred the history of parasitism within this group. In 2021, Okamura & Gruhl (for citation see below) argued against some of the conclusions, and I believe that their arguments are worth of consideration. I do not intend to make the defendant responsible for a paper that she did not co-author (i.e., Holzer et al.). Yet, there is not a single reference to Okamura & Gruhl in the thesis. Could the defendant briefly evaluate the book chapter and discuss which arguments against Holzer et al. presented there are sound (if any)?

Okamura B & Gruhl A, 2021, Evolution, Origins and Diversification of Parasitic Cnidarians, in De Baets K & Huntley JW (eds.): The Evolution and Fossil Record of Parasitism. Springer, pp. 109-152.

2. According to Holzer et al. (2018), the most basal split within Myxosporea occurred some 540 (487-594) MYA and predated the split between Chondrichthyes and Osteichthyes by more than 150 MY (the latter was estimated to have occurred approximately 365 (304-427) MYA). However, the authors of the paper themselves follow their results of molecular dating only partially, because they say (in Conclusions) that “prehistoric cartilaginous fish became available approximately 450 MYA”, which clearly contradicts their molecular clock results. Indeed, 450, and not 365, MYA is a generally accepted split between Chondrichthyes and Osteichthyes. [Note that the oldest fossil believed to belong to the stem group of Chondrichthyes, *Shenacanthus*, which was described just a month ago, is ca. 436 MY old, i.e., out of the credibility interval estimated by Holzer et al.]. The defendant and the authors of the papers included in the thesis follow the timing of Myxozoa from Holzer et al., but not that of Vertebrata, so it seems they believe only a part of the analysis. If it is so, how would the defendant justify this approach? I think this should have been discussed in the thesis (on pages 6 and 7). Is it possible that the dating of evolutionary events within Myxozoa was biased as well in the analysis, just the other way? The sequences of Myxozoa form rather long branches. I am not an expert on molecular clock, but I tend to believe that long branches behave oddly in all situations.

3. In paper Paper III, Lisnerová et al. showed clearly that two of three principal lineages of Myxosporea split into subclades, one of which is in both cases specific for Chondrichthyes, while the other one is predominated by parasites of Osteichthyes. The authors believe that the ancient hosts of both “main” clades were Chondrichthyes and, in addition, the defendant states that “the inclusion of bony fish into the complex life cycles of Myxozoa took place several times independently”. I am not sure such claims are justified by the data. When we extrapolate the molecular dating by Holzer et al., the most basal split within both, oligochaete- and polychaete-infecting myxozoans, leading to chondrichthyes- and Osteichthyes-specific clades, might have occurred around 450 MYA (it should be later than some 495 MYA, but not earlier than some 400 MYA), which nicely corresponds to the split between Chondrichthyes and Osteichthyes. I believe that this can be explained by an alternative scenario, in which the ancestral myxosporeans parasitized stem gnathostomates and diversified there into the so-called oligochaete- and polychaete-infecting clades prior the split of the host lineage into Chondrichthyes and Osteichthyes. When the split occurred, it caused both, oligochaete- and polychaete-infecting myxozoans, to split into Chondrichthyes- and Osteichthyes-specific subclades. Such a scenario does not include several independent ancient host switches of Myxosporea between Chondrichthyes and Teleostei. Neither it includes Chondrichthyes as older hosts of Myxosporea than Osteichthyes. I would like the candidate to comment on this.

4. The most interesting result of the paper VII (in my opinion) is the discrepancy between Sanger and Illumina sequencing results of fish tissues. Since the manuscript was in preparation when the thesis was submitted, I expect the discussion has been expanded since (I think it should be). How much of the new diversity of myxosporeans detected by Illumina sequencing of fish tissue might be attributed to PCR or sequencing errors? My impression is that the authors of the manuscript believe that almost none, but, on the other hand, Illumina metabarcoding coupled with PCR is notorious to introduce artificial OTUs due to amplification/sequencing errors.

Just a note - the PCR kit used to amplify the SSU rRNA gene of myxosporeans in this project contains DNA polymerase that was shown to be slightly more prone to amplification errors (but significantly more, if I interpret the results well) than several other DNA polymerases (Nagai S et al., 2022, Metabarcoding and Metagenomics 6: e77704; the paper was probably published after the thesis was submitted).

5. This is more a comment than a question, but the candidate is free to react. The authors of the papers included in the thesis described dozens of new myxozoan species, which is a valuable contribution to the known diversity of this group. Unfortunately, the International Code of Zoological Nomenclature Articles 16.4. and 16.4.1., which are considered of crucial importance, were violated probably in all cases: "Every new specific ... name published after 1999 ... must be accompanied in the original publication by the explicit fixation of a holotype, or syntypes". In addition, Article 73.2.1.1. defines that "only those specimens expressly indicated by the author as those upon which the new taxon is based ... are syntypes" (holotype has similar requirements, see Art. 73.1.1.; hapantotype cannot be designated, because Myxozoa are not protists; I am aware that isolated DNA can be in some cases designated as a type). Maybe this comment seems to be purely pedantic and useless, but since the newly described species lack correctly designated types, their names might be deemed unavailable (by legalists), and anybody can "describe" the species again just on the basis of the original papers and name them differently (or use the originally proposed names). "Anyone" obviously includes the authors of the originally proposed names.

Conclusion

I am convinced that the thesis contributes significantly to the understanding the diversity and evolution of Myxosporea as a whole, and I certainly recommend it to be successfully defended.

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