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Dear Professor Fiala,

I am pleased to provide the following review and comments on the PhD thesis submitted by your candidate, Mr. Jiří Kyslík: *Bioinformatics and functional characterization of taxonomically-restricted genes in Myxozoa,* which comprises an introduction and statement of objectives, and results presented as three research papers.

Mr Kyslík's research has expanded our knowledge of myxozoan parasites using combined 'omics analytical approaches, microscopy and immunochemistry. He studied these parasite both specifically - in the case of two problematic parasites of European fishes *Myxidium lieberkuhni* and *Sphaerospora molnari*, and more generally in providing context of myxozoans as a group of highly specialized and reduced Cnidaria, by his work on taxonomically-restricted genes. For two paper, he focused on minicollagens, which are a core structural component of the titular cnidarian stinging organelles, the cnidae. And for the third paper he explored more general "pathogenicity-related" myxozoan genes.

I recommend that the thesis be defended as planned. The document is well developed and the candidate Jiří Kyslík has demonstrated a sufficient body of work for a PhD.

On the attached pages, I have provided specific comments and questions about the thesis, to form the basis of further discussions. I have also made comments/corrections directly in the thesis pdf, which I have supplied together with this letter "Kysilik_Jiri_2022_DP_SAcomments".

I look forward to hearing Mr. Kyslík's presentation of his work, and to discuss specific aspects of it with him and the other committee members.

Sincerely,

Dr. Stephen Atkinson

Atkinson - Detailed review and comments.

Part 1 - Introduction

The Introduction covers the existing literature on the specific topics that are researched in this thesis. There is some text that is not relevant to the thesis topics and could be deleted (regarding myxozoan development in the invertebrate hots - see my question (below) for more details). Figure 1, reproduced from a published paper, has more details about the myxozoan life cycle than are needed for this thesis – however the figure does serve to illustrate the general complexity of the myxozoan life cycles and illustrates a core theme of the research – which is that although myxozoans have simplified many aspects of their biology compared with free-living Cnidaria, these parasites have layers of complexity we are still discovering (as Mr. Kyslík demonstrates in this thesis).

I appreciate the effort that must be needed to write a technical summary in a language other than your mother-tongue. The English in the Introduction is acceptable for a thesis. I have added comments when I don't understand something (words or grammar) – I have not correcting the English in every sentence.

One part of the introduction that is missing some detail is in the section of TRGs – the taxonomically-restricted genes. Specifically, I found myself wanting to know more of the methodology as to how a researcher discovers TRGs. I have put this is a question (listed below). I would welcome discussion on this topic by Mr. Kyslík as TRGs are core to this thesis.

Questions for the candidate:

Q. RE part 1.3 Myxozoan life cycle and cellular development. I understand why you review the myxozoan invasion process and sporogony in its vertebrate host, but why do you also review development in invertebrates and Bryozoa (this does not seem relevant to your study).
[You could delete from half way down pg 6 the rest of 1.3, including figure 2 (which is not original anyway).]

Q. pg 8 near bottom, regarding myxozoan nematocysts. Please explain what you mean by "Nevertheless, some hidden morphology is questionable (Okamura et al. 2015). "
- Are myxozoan polar capsules really all the same?

Q. pg13 middle, you say "In the context of body plan formation, the pivotal signaling pathways (Hox-like, Runx, Wnt pathway, Hedgehog pathway) appears to be absent from the myxozoan genomes (Chang et al. 2015)."
- Do you consider this statement to be correct, given the most recent genome data (e.g. Guo 2022)?
- If these pathways are non-functional in Myxozoa - can you suggest any other genetic mechanisms for body plan organization in Myxozoa?

All about TRGs: Q. bottom of pg 13 "genes lacking homology in other organisms"
- Given that TRGs are core to this thesis, and that one of the principal definitions of TRGs is a lack of homology with genes from other sequenced taxa, please give more information on precisely how you calculate homology - what threshold? what approaches? structural/folding considerations? or just sequence? nucleotide sequence? AA sequence? Specific database searches?
- Could many supposed "TRGs" just be distant relatives of well-known genes in other taxa (but are categorized as TRGs because they fall outside the similarity score?

- Or are TRGs defined by their ability to code for a truly unique character?
Discuss.

P_a_p_e_r_ _I_._:_ _K_y_s_l_ík_,_ _J_._, Kosakyan, A., Nenarokov, S., Holzer, A.S., Fiala, I. (2021) The myxozoan minicollagen gene repertoire was not simplified by the parasitic lifestyle: computational identification of a novel myxozoan minicollagen gene. BMC Genomics 22: doi.org/10.1186/s12864-021-07515-3. [IF= 3.9]
JK executed the bioinformatics pipelines, performed data analyses, and drafted the manuscript.

This paper is published, and is very well written. The writing and figures are of a high standard.

Questions for the candidate:

I see that you have a hypothesis that nematocyst diversity is linked with minicollagen diversity, and that this is not supported by myxo data: myxozoa have multiple minicollagens but only a single type of nematocyst. I would like you to consider if the diversity in the genome is actually present in the organisms, just not only the myxospore stage (that you investigated). i.e. could this hidden diversity be in actinospore stage? How could you test a hypothesis that the minicollagen diversity in the genome indicates nematocyst diversity across the organisms entire life history?
Q. Describe your PCR approach to determine close spatial relationship of Ncol 4 and 5 on the genome [pg34 last para].

Q. could there be even more myxozoan-specific minicollagens to be discovered? Can you suggest any methods, other than transcriptomics/genomics that might reveal novel minicollagens?

P_a_p_e_r_ _I_I_._:_ _K_y_s_l_ík_,_ _J_._, Vancová, M., Bartošová-Sojková, P., Lövy, A., Holzer, A.S., Fiala, I. Expressional profiling and cellular localization of myxozoan minicollagens demonstrate their involvement in polar capsule formation during sporogenesis. Manuscript submitted to International Journal for Parasitology.
JK carried out all experiments, analysed gene expression and immunostainings, and drafted the manuscript.

This paper is under review (published in bioarxiv). It is well written. The English is good and the figures of a high standard. This paper showcases several areas of technical expertise that the candidate learned during his PhD studies: bioinformatics, PCR, qPCR, antibody/protein work and electron microscopy.

Questions for the candidate:

Please explain the final sentence of the Abstract "Furthermore, our findings have practical implications as minicollagens are useful targets for a more accurate identification of the developmental phase of myxozoan parasites." Specifically, why do we want to know more about the developmental phase? (I do not understand this importance)

From 2.6: how did you determine this peptide should be used as an antigen? Was the process of ordering this antibody straightforward (did everything work as expected, or did you have some challenges?)

From 3.4: What was the earliest sporogonic stage that you observed labelling in? (i.e. did you only see

recognizable capsules were labelled with the gold nanoparticles, or did you see labelling of parts of earlier stages?) Also, please speculate on identity of the crystal-like structures "Autofluorescence of crystal-like structures (Supplementary Fig. S4A-B) was observed in the pre-sporogonic stages under epifluorescence (Supplementary Fig. S4A-B)." Look at Fig S4 and discuss.

P_a_p_e_r_ _l_l_l_._:_ _ Wiśniewska, M., Alama-Bermejo G., **K_y_s_l_ík_ _J_.**, Kolísko, M., Holzer A.S., Kosakyan A. Game of genes: Exploring the major parasitic strategies in myxozoans, case study: *Sphaerospora molnari*. Manuscript in advanced preparation.
JK contributed to taxonomically-restricted genes identification, visualization, and edited the manuscript.

This paper is in draft form, and the candidate is a contributing author. The English is understandable, but needs editing prior to submission to a journal. I have commented within the thesis PDF where I either did not understand the meaning, or I suggested another word in some cases (but not all).

Questions for the candidate:

Pg 70 Introduction. The paper states "The main aspect of the success of Myxozoa probably lies in their complex life cycle, while all other groups are monoxenous and are not parasitic throughout their whole life cycle. "
Why would a multi-host life cycle allow a parasite to be more "successful" than one with only a single host? Justify your statement.

Pg 73. Can you provide any further information about the genome reference? (Is it going to be published soon?) I suggest you give a brief summary of this genome resource (where the biological material is from, how it was sequenced and assembled).

How did you normalize each transcriptome/gene set before doing the expression comparisons?

Table 2. Please comment on the difference in "Total predicted proteins" between the genome and the de novo transcriptome (why such a large difference?)

Please explain what is shown in Figure 3 – what is important/significant here?

Figure 4 is very complex – will it even be readable when published? It may be better for the reader if you simply state the main metabolic pathways that you discovered (unless this type of diagram will be already familiar to the reader?)
Similarly, Figure 8 has a lot of small text – it might only be readable if available online at high resolution... so maybe summarize its main data in text form in the published paper.

The Discussion needs a final Conclusion/summary paragraph to remind the reader of the most significant findings of the study.
