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## **The review of the Mgr. thesis by Bc. Hynek Mazanec:**

### Molecular and morphological characterisation of digeneans of the family Strigeidae Railliet, 1919 from Iceland.

I strongly appreciate the combination of a classic morphological descriptions with a modern molecular analyses. This study is an excellent example of such an approach: it applies molecular and morphological procedures to identify larval and adult stages of trematodes of the family Strigeidae in Iceland.

The work is logically arranged, clearly written and testifies to the careful study of the literature and the critical approach to the results obtained. It is the result of the excellent work of the student as well as good job of his supervisor and consultant. In fact, I have only few minor critical remarks and questions. In the following text I have marked them in bold.

Hynek Mazanec processed the material provided by Karl Skírnisson (University of Iceland) and by colleagues from the laboratory of Helminthology. While the first intermediate hosts (freshwater snails) were alive, the definitive hosts (water birds) were frozen. **In my view, this should be mentioned in Materials and Methods. Can the freezing of the material before its fixation affect the morphology of trematodes?**

Hynek Mazanec explored representative material of flukes of the family Strigeidae from all over Iceland. He described species of the genera *Apatemon*, *Australapatemon* and *Cotylurus*, including novel lineages that probably represent new species. Characterisation based on phylogenetic analyses of two markers (mitochondrial *cox1* and nuclear 28S) in combination with morphological features led to distinction of seven lineages in Iceland.

**To obtain the idea of abundance of these species would be useful to provide at least brief information about other helminths found in the material examined. Is such information available? Can be presented in the defence?**

One of the conclusions concerns the first intermediate hosts of strigeids: *Radix balthica* was detected as the main first intermediate host in freshwater in Iceland. **Is this fact influenced by the abundance of this fresh-water snail in Iceland? According the literature, *Galba truncatula* should be present in some of**

**sampled localities as well. Could this species theoretically serve as first intermediate host of strigeids as well?**

Very valuable result is at least partial elucidation of life cycles of strigeid flukes in Iceland.

**Could a parasitological examination of the leeks bring a more detailed description of some of them? Do you plan it?**

The biogeography of Iceland is controversial. Some authors believe that all Icelandic life originated after postglacial dispersal events (tabula rasa hypothesis), but others assume that some organisms survived the last glaciation in ice-free refugia. **Can your research contribute to supporting one of these two hypotheses?**

The diploma thesis under consideration undoubtedly meets the criteria of the Faculty of Science. I recommend that it be awarded the title of Mgr.



České Budějovice May 19 2018

Doc. RNDr. Oleg Ditrich, CSc

**Opponent's Opinion on the Master Thesis of Bc. Hynek Mazanec "Molecular and morphological characterisation of digeneans of the family Strigeidae Railliet, 1919 from Iceland".**

In his Master's Thesis, Hynek Mazanec identified Icelandic samples including larval stages and adult trematodes belonging to the family Strigeidae, by the use of molecular and morphological techniques. Such studies, where adult and larval stages are linked, add to our understanding of the life cycles of parasites and extend our knowledge on trematode distribution and biodiversity.

The introduction of the work is adequate and provides a comprehensive overview of the topic. The objectives are clear and concise, although not phrased as regularly, and fulfilled as explained in the results, which are divided into adequate sections. The results are comprehensively summarized, complemented by several tables, detailed drawings and phylogenetic trees. I only miss the inclusion of a table with sequences divergence, which would have allowed a deeper discussion of some aspects, but in general, all remarkable aspects presented in the results are considered in the discussion and conclusions. Taxonomic evaluation is written with detail and in general very well. It is a huge effort to measure and describe the morphology of cercariae and adults, and compare morphometric data of the available literature. I consider the work successful both in terms of the formal aspect and the main content. The text is in general well written, with correct English, and not abundant typing errors.

I have the following comments or queries for the submitted work:

- 1) Some concepts are presented in the Introduction but never appear again in the text, e.g. existence of mesocercariae. Generally, mesocercarial stages exist when an additional paratenic host is included in the life cycle of the parasite, turning to be a four-host cycle. However, it is not a frequent condition and thus in my opinion, it is not necessary to include this unless important to later discuss the results. Thus, did you find any mesocercariae?
- 2) The study includes different methods, including SEM. Since the author mentions that samples were collected by his colleagues (by the way, nice gesture), I would like to know if he did the rest of techniques.
- 3) Generally, Materials and Methods are well described and supported by tables, but I miss some information in some parts; for example, in relation to ML and BI analyses (e.g. on what number of generations was based BI calculation? what number of replications was used to calculate bootstrap support in ML?) or used primers (the use of internal primers is not mentioned in the text (p. 11)).
- 4) The description of molecular results is well done and describes the relationship and matching of the produced sequences with published sequences. However, similarity/divergence values between sequences are missing in some comparisons (e.g. *Australapatemon burti* and *A. burti* LIN 1 from Gordy et al 2017). I would have appreciated that the author includes a table with pairwise nucleotide sequence comparisons for the aligned and published sequences, as this would help in the understanding of the relationships between certain sequences. In relation to this point, some other questions arise:

What variability exists between sequences from one species, e.g. *A. gracilis*, and is this in concordance with published results?

Gordy et al. 2017 suggested that the lineage identified as *Australapatemon burti* LIN 1 could comprise more than one species. Have you compared the divergence within lineages of, for example, *Apatemon* or *Australapatemon* and compared it to the intra-lineage variation published from *cox1* and 28S sequences (data given in Gordy et al. 2017)? What do you think is happening that would explain such intra-lineage variation?

- 5) As mentioned above, taxonomic evaluation is well done, but there is a lack of uniformity in the presentation of morphological results, i.e. why metacercariae are not included in the descriptions? Therefore, I miss some descriptions, or at least the explanation why not all stages were included. Moreover, the type of cercariae (furcocercous cercariae) should be mentioned in the taxonomic description, not later in remarks.
- 6) Only 5 septae are drawn in *A. minor* while in the text it says there are 6 (p. 41). What is the correct number?
- 7) Representative photomicrographs of live sporocysts and cercariae should have been included, as the author mentions this material in Materials and Methods (p. 10).
- 8) Prevalence data are missing.
- 9) The author claims that metrical data of his material of adult *A. minor* and *A. burti* overlap, and therefore, more specimens are needed to compare these species. Does this mean the identification has to be confirmed? Or is this identification based on the morphology of larval stages?
- 10) The author suggests that leeches collected in Lake Mývatn by Lindegaard (1979) could be possibly infected with *Australapatemon* species. As there are no trematode identifications in this paper, probably the author means that *Australapatemon* species could use leeches present in this lake as hosts, although never recorded, but it should be reworded to avoid later confusions. Related to this, were leeches found or dissected during the collection of the author's material?
- 11) Formally, the work is written without error, with only a few misspellings and minor mistakes (for example, p. 6 "mitochondrial c oxidase subunit 1", instead of "mitochondrial cytochrome c oxidase subunit 1"; p. 7 "Collecting" instead of "Collection"; p. 12 "echinoderm mitochondrial code" instead of "echinoderm and flatworm mitochondrial code"; p. 62 "bulbocercous" instead of "bulbocauda"; lineage 3 is called *Australapatemon* sp. 'Lin. 1I' (p. 20), but this full name is missing in the figures), only in the table;...).

In spite of the comments made, I consider the Master Thesis of Hynek Mazanec to be a valuable work, demonstrating the importance of the presented results as well as the student's acquired skills. The scope of the work is perfectly suited to the requirements of Master's degree and therefore, I fully recommend it for the defense.

České Budějovice, 20.05.2018  
Ana Born Torrijos, Ph.D.

