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Review of the master thesis by **Bc. Martina Hrabcová** entitled **"Biology, life cycle and phylogeny of Malacosporeans in fish and Bryozoans"**.

The submitted thesis aims to study the natural history of malacosporean Myxozoa at several different levels. The student aimed to study i) malacosporean life cycles using newly devised transmission experiments, ii) to screen potential hosts for infection using a variety methods, iii) reconstruct phylogenetic relationship between newly found representatives and earlier studies, and (finally) iv) to investigate malacosporean infection in marine Bryozoans. Majority of the aims are very complementary, only the last aim seems a bit ad hoc, given that it was based on samples from a single locality.

The study managed to fulfil most if not all of its aims. Despite the cohabitation experiment failed to trace transmissions between successive hosts, it provided novel methodology by establishing an approach for successful mid-term maintaining of infection free bryozoan colonies in captivity. Screening of environmental fish host samples provided new host records as well as evidence for several new malacosporean species confirmed molecularly. Molecular screening of the marine sample set did not reveal presence of malacosporeans, but showed presence of the of the other myxozoan class.

The thesis is written in excellent English, the text is mostly clear and it reads very well, with only a few exceptions of typing errors (e.g. Proteinase K was probably used to digest proteins, not DNA on Page 21.). The quality of the graphical outputs is very good either. Only in Fig.1., it is not clear where is the parasite located inside Bryozoan body, an indication should have been added to the picture as was done in Figs. 4. or 5.

A few critical remarks and questions (**in bold** - to be answered during defence):

The student analysed a large collection of biological material. It is not clear, whether the whole sampling and wetlab part of the research was performed solely by the student, or whether the material was accumulated and analysed over a longer period of time. This is not a criticism of potential lack of work. The cohabitation experiment, DNA sequencing and computational analyses provide more than enough evidence of the numerous activities carried out by the student.

Dissection of PCR bands from gels was used. If taxon specific primers were used, were there multiple bands present yet?

Why not use mitochondrial genes to supplement SSU rDNA in clades (Figs. 11-13) with low support?

Models used in the phylogenetic analysis were not tested? A model including invariant sites (GTR+G+I) was used in Bayesian analysis, why not also in RAxML?

No Malacosporea were detected in marine Bryozoa, but origin of the detected Myxosporea was explained in the Discussion as accidental consumption with food particles. If Malacosporea were present

in the ecosystem would they not enter Bryozoan bodies in a similar way? Or do you expect Malacosporea to be present in the ecosystem at a much smaller scale?

In conclusion, I enjoyed reading the thesis and expect its successful defence. I propose an excellent grade, but I will make a final decision depending on the student's thesis presentation during defence.

Jan Štefka.

In České Budějovice, 2015-05-25.

Evaluation of Master Thesis of Martina Hrabcová
"Biology, life cycle and phylogeny of malacosporeans in fish and bryozoans"

The thesis is in English. It is unusual and I think it should be appreciated. Moreover, it is in *good* English. As a non-native speaker, I am somewhat disqualified from evaluating the grammar and overall quality of the language, but, although I found a few formulations which *I* would never use, I claim that the thesis is very well written.
The fact that abbreviation "*i.e.*" is consistently put in italics, but "e.g." is not made me nervous.

The first (review) part of the thesis is not only well written, but also informative and interesting, it was a pleasure to read it. Remaining chapters seemed a bit more "messy" to me, they were slightly more difficult for me to understand; this may be my own problem, but I believe these chapters could be better structured and/or arranged. Nonetheless, after re-reading of several paragraphs and revisiting several tables, everything was (generally) clear.
In Tab. III, the links to two following tables are wrong (II and III instead of IV and V). Below the Tab. III, the tables IV and V are also erroneously referred to as Table III. Cypriniformes and Perciformes are NOT families (page 32).

Aims of the thesis were spread over several topics, some aims were achieved, some tasks proved difficult to be solved: malacosporeans seem to be tricky and elusive creatures. I want to stress out that I am convinced the author of the thesis did her best and *e.g.* the failed transmissions experiments are not her fault, they just reflect the difficulties to handle malacosporea and still can serve as a basis for future experiments. The difficulties, as well as more easily achieved results, are discussed thoroughly in the thesis.

I have several questions, which are partly real questions based on pure interest of mine and partly hidden criticism:

1) I suppose fish-released malacospores are homologous to myxospores, whereas bryozoan-released malacospores to actinospores. It seems the types of malacospores differ in their cell numbers (pp. 5 – 6). Do typical myxospores and actionospores differ in similar way? Are there any other differences in the two malacospore types? Especially such that can be homologized to typical myxo- / actino-spores?
2) Some methods questions… During the sterile bryozoa cultivation, the medium was aerated. Was the air filtered or sterilized in some other way?
   According to chapter 3.5.1., DNA was isolated from 200 mg of bryozoans: how many zooids it approximately is?
   When cultured, could the bryozoans be fed with e.g. *Paramecium* or yeasts? These organisms seem easy to be obtained to me, I would try them…
3) Was it only *P. repens* cultivated? Maybe *F. sultana* would be easier to maintain for longer time as it is a "species that can survive the winter without production of statoblasts" (page 13). Or not? Did you try to culture this (or other) species?
4) The cohabitation experiment 1 failed presumably because the bryozoans were healthy despite the fact that they were from a "positive locality". Were they PCR- / microscopy-checked for malacosporeans prior to the experiment? Or only fish from the pond were examined?
5) Concerning the trees: woudn't a myxosporean sequence (or a number of them) be a better outgroup than *Hydra*? I also think "Novel lineage" and Malacosporea sp. 1 and 2 are not "weaker supported lineages" (page 36): they are isolated lineages of SINGLE sequences, thus no support can be defined for them. Is that so?

6) In chapter 1.2.2., transformation of sac-stages into worm-stages is described. If this transformation is possible, is it really necessary to assume that worm-stages were lost in some malacosporeans or in *B. plumatellae*-sac "species"? Maybe the worm-stages were just not found because the organism was observed in its sac-phase?
7) Why did you expect sac formation in riverine habitats and worms in static waters (page 43)? Are these stages released from the bryozoan, or malacospores only? Then sacs / worms wouldn't influence the chance or spores to spread…
   You state that the strict occurrence of different forms of *B. plumatellae* (worm / sac) in different hosts is a fact supporting "two species interpretation" (page 43). In my opinion, this fact is expected for the "facultative polymorphism in different bryozoans" as well. And therefore support neither alternative. Do I miss something?

Despite my questions, some of which try to point to some problematic statements, the thesis of Martina Hrabcová is a high-quality one and I strongly recommend to accept it for the defense.

In České Budějovice, 25. 5. 2015

Mgr. Martin Kostka, Ph.D.