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Committee for PhD studies in Parasitology,
Faculty of Science,
University of South Bohemia,
Czech Republic

Evaluation report of PhD Thesis "Bioinformatics and functional characterization of taxonomically-restricted genes in Myxozoa", by Mr. Jiri Kyslik

Dear PhD committee members,

In his dissertation, Mr. Kyslik addresses an open topic in evo-devo of taxonomically restricted genes, which were suggested to be of great importance for morphological specification and species-specific adaptive processes. Jiri focused on myxozoans, a large group of endoparasites that has recently been placed within the Cnidaria Phylum. Cnidarians, dated to about 700 mya, are characterized by their unique stinging capsules termed cnidocysts, which are considered as phylum-specific novelties. Although myxozoans have lost genetic and structural complexity, they have retained the cnidarian cnidocysts, also known as polar capsules.

The first (published) paper focused on the diversity and evolution of myxozoan minicollagen genes. In free-living cnidarians, these genes encode the key components of the capsule and tubule wall, and provide the capsule its strength and elasticity. Jiri findings reveal a new type of minicollagen that is specific to Myxozoa. His detailed analysis indicated that despite the simplification process they underwent, the repertoire of myxozoan minicollagen genes was not reduced. To gain more data, he generated two transcriptomes of myxozoan species, which he analyzed using a novel bioinformatics pipeline he developed.

In the second (submitted) paper, the expression kinetics and localization of the minicollagens were analyzed. The findings demonstrated that minicollagen expression was restricted to the sporogenic phase, when the capsule is formed. Additionally, Jiri's pioneering work shows, for the first time, the specific localization of the newly identified minicollagen, using light and electron microscope immunohistochemistry. The results strengthen the evolutionary importance of minicollagens as the main building blocks of the myxozoan capsule.

In the third (in-preparation) paper, Jiri performed comparative transcriptomics of developmental stages of the myxozoan *Sphaerospora molnari*. Among the genes that were found, minicollagens and other known cnidocyst genes were expressed at a similar stage, supporting the results of his two

papers. Interestingly, stage-specific expression of orphan taxonomically restricted genes was identified. To my understanding, this is the first myxozoan transcriptomic profiling of different developmental stages.

The thesis is well written and reads fluently. I appreciate the breadth and depth of the introduction, in which Jiri reviewed all the topics that are relevant to his research. I am impressed with the amount of background reading and his ability to summarize the main points and ideas in a clear and logical way. Jiri's research combines bioinformatic tools with experimental work. He has displayed remarkable productivity, in terms of mastering diverse techniques, collecting and analyzing a great amount of data, and presenting his results and conclusions through coherent writing. His findings shed new light on Myxozoa taxonomically restricted genes and provide new data for further research.

The research work has already resulted in one scientific publication in a leading journal, a second paper is under review and the third paper is in preparation. I am sure that the second and the third papers merit publication in a respectable journal.

I conclude that the quality of the work in this thesis is worthy of being defended.

Yours sincerely,



Prof. Tamar Lotan

Head, Marine Biology Department