Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Rudolf Nechvile

Thesis title: Cathepsin L of Sphaerospora molnari – localization, function and diagnostic tools
Supervisor: **Mgr. Astrid Holzer, Ph.D.**

Referee: RNDr. Zdeněk Franta, Ph.D.
Referee`s affiliation: UCH, USB

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages)**, balanced length of the thesis parts** (recommended length of the theoretical part is max. 1⁄3 of the total length)**, logical structure of the thesis**	0-3	2,5
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	1
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	3
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	1,5
Formal requirements – points in total		16
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	3

[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		22
POINTS IN TOTAL (MAX/AWARDED)	**48**	**(38)[2]**

Comments of the reviewer on the student and the thesis:

Rudolf Nechvile in his bachelor thesis entitled *Cathepsin L of Sphaerospora molnari – localization, function and diagnostic tools* describes the establishment of lab approaches to study the expression profile of cathepsin L –like cysteine peptidase from Myxosoan parasite *Sphaerospora molnari*. The thesis has 31 pages and is divided into 7 chapters (introduction, aims, materials and methods, results, discussion, conclusion and references). The introduction in in my eyes very short (has only two pages) and does not really introduce the reader to the topic. I am missing better introduction to the parasite life cycle, I would also appreciate showing expression profile of Cath L, and information about other isoforms as well as other *S. molnari* peptidases. All this would help the reader to dive better into the topic. The thesis contains several typos and the figure numbering at the end of results does not correspond (there are same number for different figures). The discussion is quite well written, and deals with all findings presented in the thesis.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

1: In introduction you state that the cathepsin L may be a prime vaccine candidate against the parasite. Can you comment on how many other isoforms of cath L are present and how conservative are to each other?

2: You mention that cath L play important digestive role in other parasite (eg fasciola), and that this can be also case for *S. molnari*. I do agree with this, however it is important to say that in fasciola and some other blood feeding parasites (ticks, schistosomes) it is not a single enzyme plying the role, but rather well orchestrated cascade of various peptidases (e.g., cath L, B, C, D, legumain). Is there anything known for *S. molnari*? Do you have the information which other enzymes may be involved in blood digestion?

3: You use qPCR to monitor the expression profile of Cath L. I am missing better information about the procedure. What was the design of primers and probes? Were these specific enough to amplify only one CathL isoform? What did you use as an interplate control? I would also recommend running samples in triplicates instead of duplicates, as this can be sometimes hard to evaluate. Have you tried to repeat the runs with higher amount of DNA as your Ct values are quite low (28-37). Did you run each fish DNA independently or did you pool all DNA into one sample? You data shows very high variation.

4: Can you comment on why did you do the relative quantification instead of absolute one? This could be maybe more suitable for your setup. What does the relative parasite number mean?

[2] Enter the number of points awarded.

How did you get it?

5: Can you show the SDS PAGE gel corresponding to the WB in figure 1 and 2? From this figure 1 it is very hard to evaluate the quality of the antibodies.

6: In flow cytometry experiments as well as in immunofluorescence labeling you show some CathL signal for naïve fish, but there is no signal in WB experiments. Can you comment on this?

7: Are you sure about the specificity of signals in Figure 9? It seems to me more like and artefact. I am also missing any scale bar and bright field pictures to better evaluate the results. Can you show these during your defense?

8: I know that the preparation of the recombinant Cath L was not a part of your thesis, but do you know its purity? Did you run a sample on SDS PAGE? Why I ask is because you use the recombinant protein for both WB as well as elisa experiments. Especifically in Elisa, it is important to know the protein purity and concentration as this can strongly influence your results.

9: in the discussion part you state that *S. molnari* can secrete the CathL to aid feeding. Can you go in more details here?

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I will suggest the grade based on candidate's performance during presentation and answers to my questions

In **České Budějovice** date 09.09.2022

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signature