



Přírodovědecká fakulta
Faculty of Science
Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

STATEMENT OF THE DIPLOMA THESIS REVIEWER

Name of the student: Bc. Helena Mondeková, BSc

Thesis title: Functional analysis of fibrinogen-related proteins (FREPs, Ixoderins) of the tick *Ixodes ricinus* and their function in pathogen transmission

Supervisor: RNDr. Ondřej Hajdušek, Ph.D.

Reviewer: Doc. MVDr. Pavel Široký, Ph.D.

Reviewer's affiliation: Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1. 612 42 Brno

	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	3
quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
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	3
Formal requirements – points in total		20
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of 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

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

Completeness of the description of the used techniques	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	3
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	3
Formal requirements – points in total		27

POINTS IN TOTAL (MAX/AWARDED)	48	47
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Suggestions and questions, to which the student has to answer during the defense:

Very nice work dealing with important topic, related to vectorial capability of ticks. Do author know about pathogenicity of some transmitted agents to their tick vector? Has uninfected and infected ticks, respectively, comparably the same fitness, survival rates, feeding capability?

Author mentions that males of *I. ricinus* are not feeding. It is general trait of the genus *Ixodes* or to all ixodid ticks?

Which other tick species of veterinary and medical importance occur in the Czech Republic, and what is their distribution pattern?

Eventual mistakes, which the students should avoid in the future:

Author used full justification of the text throughout the thesis except references, which are left justified. Just for feeling, better it should be unified. Nevertheless, it is just nit-picking note to otherwise very nice work.

Eventual additional comments of the reviewer on the student and the thesis:

Nice reading, fluent text, directly focusing to aims of the study, not too long. I like it!

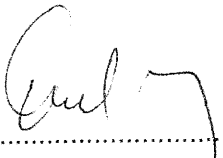
Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense

In Brno date 14.1.2016



 signature



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STATEMENT OF THE BACHELOR/DIPLOMA* THESIS REVIEWER

Name of the student: Helena Mondekova

Thesis title: Functional Analysis of fibrinogen-related proteins (FREPs, Ixoderins) of the tick *Ixodes ricinus* and their function in pathogen transmission

Supervisor: RNDr. Ondrej Hajdusek Ph.D.

Reviewer: Ryan O. M. Rego Ph.D.

Reviewer's affiliation: Institute of Parasitology, BC ASCR, Ceske Budejovice

	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	3
quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	2
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	3
Formal requirements – points in total		19
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of 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

* Choose one

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

Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	3
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	3
Formal requirements – points in total		25
POINTS IN TOTAL (MAX/AWARDED)	48	44

Suggestions and questions, to which the student has to answer during the defense:

As an opponent for the thesis of Helena Mondelkova, titled “ Functional analysis of fibrinogen-related proteins (FREPs, Ixoderins) of the tick *Ixodes ricinus* and their function in pathogen transmission”, I was able to gauge that the project done was of very good quality and the scientific writing was up to standard. The introduction was well researched and the data was presented well. The results definitely add to the growing knowledge of tick immunity and the function of the proteins that are involved. Given the data obtained, I can see that this work could be publishable and have thus tried to provide criticism that is constructive both for this work and for the student in her future endeavors. I would like if Miss Mondekova could address the following questions that I have during her defense:

1. Section 2.3 – Second paragraph. There has not been any report I know of to differentiate between encapsulation that does occur in ticks and nodulation that may or may not occur in ticks (what is considered as nodulation as seen by Ceraul and colleagues, 2002 does not involve any melanization like in insects and is yet to be proven). Even these authors used the term encapsulation/nodulation. Is there any such published report of actual nodulation in ticks as that which is seen in insects?

2. Section 2.5 – Third paragraph – The *Borrelia* chromosome is a constant 910kbp or less. More importantly, it is incorrect to state a minimum number, for the plasmids present in *Borrelia*. For example, the genome of *Borrelia finlandensis* SV1 consists of only 10 plasmids. Also the total plasmid content together can be lower than 533kbp. Please comment.

3. Section 3.1.4 It has not been mentioned in the thesis, the growth conditions for the *B. afzelii* strain used as well as the *C. albicans*. Please do this at the defense. Is there a strain number / designation for the *C. albicans* used in this project?

4. Section 3.2.2.2 It would have been more valuable to have a group of ticks that were sampled at a later time point post-feeding rather than immediately after feeding. Any effect on borrelia/bacterial load may be more pronounced over time given the growth rate of bacteria in vivo (2weeks at least). In my opinion the extremely short/absent time frame for looking at total borrelia/bacterial load after feeding makes this experiment not as useful as it could be. An example of this is Chou et al., 2015 (Nature). Please comment.

5. Section 3.2.6 – This section was confusingly written and is the only full section that has possible mistakes that I would like the candidate to address:

a) Why were there different temperatures/incubation times used for the incubation of *B. afzelii* and *C. albicans* with the hemolymph?

b) Shouldn't the order, in which the ALEXA anti-rabbit antibodies were used, be the opposite of what is written in the thesis, based on the figure provided? (Also although not stated, I expect the ALEXA 594 to be an anti-rabbit antibody)

c) I have never come across the term cover slide and I believe that the term cover slip is being used confusingly when describing this method. I would appreciate if a graphical abstract (hand-drawn) or a simple description (flow-chart) showing the setup of where a microscope slide was used and when was a coverslip used during the phagocytic assays, would be presented.

d) How were the *Candida* cells visualized using the fluorescence microscope? I do not see any fluorophore/antibody used for their staining.

e) There appears to be either a third section 3.2.6.3 missing because the first paragraph on page 28 which talks about how the phagocytic index was measured, mentions only how *Borrelia* phagocytosis was measured. If this method was used for both, then the section should have a title for eg. 'Deducing the phagocytic index". Since this paragraph follows the phagocytosis assay section using *C. albicans*, I am assuming there is a section head missing as well as missing information on *C. albicans* phagocytic index measurement. Please comment.

6. Figure 6, the y-axis for Ixoderin C is different to graphs for the other two Ixoderins in the same figure.

7. Page 41, Last sentence. It would be more apt to just say there is a significant difference in the bacterial numbers. Using the word 'interestingly' suggests something unique, yet Figure 9B had already made the reader expect that an average weight difference of 2mg between the two sexes of ticks would lead to an increased bacterial load.

8. Page 46 – While discussing the transmission experiment the candidate suggests that there is a decrease in the number of *B. afzelii* transmitted between the *gfp* and triple KD groups (found not significant – Figure 11). As there is no difference in the number of *Borrelia* between the two groups (Figure 10), does the candidate believe the triple KD has caused this reduction? Could it not be more likely that the number of infected ticks feeding (do not forget the effect of feeding by other uninfected ticks at the same time) on each mouse within the two groups and the number of spirochetes within each tick, could also lead to such results?

9. The references section has two papers (first author de la Fuente, J.), which are not alphabetically in their order and are on page 51 after the authors with surnames beginning with K.

Eventual mistakes, which the students should avoid in the future:

There were some spelling errors/ grammatical mistakes throughout the thesis which did not diminish the strength of the work. I understand that English is a second language but the use of the articles (a, an, the) was absent in a lot of instances where they were needed or used incorrectly. I would suggest that for any future work written in English, the student should have it corrected by a native speaker of the English language.

Eventual additional comments of the reviewer on the student and the thesis:

If the student can confirm, during her defense, that the RNAi work done on the ticks within this project was carried out by her, then I would like to change my points awarded from 2 to 3 for the "Experimental difficulty of the thesis".

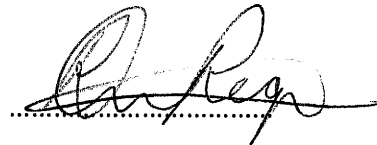
Conclusion:

In conclusion, I

r e c o m m e n d / ~~do not recommend~~*

the thesis for the defense and I suggest the grade **A**.²

In **České Budějovice** date **13.1.2016**



signature

² You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted.