**Revision of the bachelor thesis of Jan Kotál : Towards the functional characterization of a "cysteine rich" protein family member from *Ixodes ricinus***

**Supervisor: Michail Kotsyfakis PhD.**, Institute of Parasitology, BC ASCR, České Budějovice, Czech Republic, EU

**Revised by: Daniel Sojka PhD.**, Institute of Parasitology, BC ASCR, České Budějovice, Czech Republic, EU

The work presented by Jan Kotál is thematically very attractive and is in full agreement with the most nowadays approach to study vector - host interactions by functional and biochemical characterization of tick saliva components. It is a great pleasure to see the progress in topics initially studied here at the institute of Parasitology by prof. Kopecký and now strongly pushed forward by Jan's supervisor Dr. Kotsyfakis. As already mentioned in the thesis introduction, the herein studied gene/ 11.5 kDa MW protein tagged as contig-763 and further described as cysteine rich /cyrich- (evoking Zurich, Swiss capital?), was initially described among 1006 singletons from a *I. ricinus* salivary gland project performed by the co-supervisor of this thesis Dr. Chmelař and published in 2008 in BMC Genomics Journal. Despite the traditional concept of Bc. theses, the aims of this work are not indicated in the introduction chapter. However, they are clearly visible from the chapter concept: (i) biochemical characterization: preparation and purification of the recombinantly expressed contig-763 protein + performing protease inhibitory screening assays; (ii) preparation of contig-763 dsRNA, performing the RNAi knock-down and looking for changes in the phenotype markers of ticks. I have happily agreed to work out the revision of this thesis, my comments are following:

**Formal issues:** The thesis is in general very properly prepared, it has optimal length, great concept of chapters and is lacking general formal mistakes, however, some less important could be found:

E. C. Table 3: marking the pH of buffers is not united within the table, pH8 x pH 7.8, methods: spacing in between numbers and units differs. I also would prefer spacing not only at the end of paragraphs but also at their beginning, figure 16. headline should be percentage, not percentage, 2.4.1.- culture was incubated at 37°C, 4 hours, shaking-RPM???, etc..

**Content:** Although author has not obtained positive results, he confirms to learn a broad spectrum of experimental methods that could be sufficient for a level-up Ms/Mgr. degree thesis. I personally admire the level of English language and the concept of chapters. Also in line with my subjective opinion of Bc. theses at the Faculty of Sciences, the in-detail method description is very suitable as this thesis serves as the first real resume of laboratory experiments and protocols in student's career. I have two scientific remarks: From the short introduction I do not understand why this cysteine rich protein should have protease inhibitory activity. Is it structurally and functionally predicted or is that just a speculation. Secondly I have to admit, that phenotyping of RNAi experiments should be done only after the expressional decrease of the protein is confirmed, at any level of expression –mRNA, protein or activity if possible.

**Evaluation:** I recommend this work to be defended with a high classification mark with the final decision depending on the oral presentation of this work.

**Questions to author:**

1. RNAi –with all my respect- the RNAi is not proved to work - no decrease of mRNA is visible in figure 9. from three tissues: guts, salivary glands and ovaries. As I have already said It would be optional to work out the RNAi protocol first before evaluating phenotype, because with these results you can not convince me to believe statement in chapter 4 „ silencing does not lead to any changes in tick's mortality, feeding length, size or egg laying ability". No wonder the phenotype marker changes are fuzzy, however I agree with the fact that egg mass value is also defined by the seasonal factors as seen from figure 8. One reason could be that you use the same PCR primers for dsRNA construction and for RT-PCR knock down control. What would be the optimal strategy to improve the monitoring resolution by selecting another primer pair for RT-PCR? Could you suggest a method to test the RNAi knock down on protein level when you have a purified recombinant protein?
2. Could you explain more deeply what is the reason to test your gene product for peptidase inhibitory activity?
3. If you will follow with this project in your Ms. degree, what would be your future plans. Could you briefly introduce us 2 - 3 main goals?

In České Budějovice, 9. 6. 2011 worked out by

**RNDr. Daniel Sojka, PhD.**