

Master thesis of Bc. Jitka Konvičková “Factors regulating the expression and activity of digestive enzymes in the tick *Ixodes ricinus*”

(Supervisor's evaluation)

Jitka Konvičková has appeared in our lab long time ago already as a student of the high school (Biskupské gymnásium) and then she continued as a student of the Faculty of Science where she achieved Bachelor degree in 2013. From the very beginning, Jitka's topics has been focused on tick digestive peptidases. She is a co-author of one paper describing the dynamics of the tick hemoglobinolytic machinery (published in *Parasites&Vectors* by Franta et al., 2010). Her contributions were acknowledged in other papers published by our group (Franta et al., 2011 – *Int. J. Parasitology*; Sojka et al., 2013 *Trends in Parasitology* and Perner et al., 2015 – submitted paper). Beside her own work, she has also supervised the scientific projects of two high school students, namely Markéta Jirsová and Gabriela Slípková in the frame of the project Open Science.

In her Master thesis, Jitka first addressed a simple question - What happens to the tick digestive machinery in virgin *I. ricinus* females? Her data conclusively demonstrated that not only rapid engorgement but also blood digestion is triggered only in fertilized females. Another simple experiment of her refuted the long-term dogma that *I. ricinus* female mate mainly during their on-host feeding. Jitka has demonstrated, that the vast majority females has been fertilized already before attachment to the host. Although these data may seem to be trivial, they are novel, rather surprising and, to my opinion, worth to be published.

The main core of Jitka's Master thesis covers one important aspect of our current major project – “The role of hemoglobin in tick metabolism and transmission of tick-borne pathogens” . Using a tricky method of *in vitro* membrane feeding, Jitka fed adult *I. ricinus* females on the whole blood and hemoglobin-depleted serum and compared the expression of nine digestive peptidases comprising isoforms of cathepsin B, L, D, C and legumains in dependence on the hemoglobin presence/absence in tick diet. Her data suggest that in most cases, the hemoglobinolytic multi-enzyme system is quite similar to the enzymatic network used for digestion of other serum proteins. She found one interesting exception from this concept, namely the expression of cathepsin B, the most abundant digestive peptidase, seems to be significantly up-regulated in blood-fed compared to serum-fed ticks. In addition, Jitka also exploited the quantitative real-time PCR to provide exact data on tissue expression profiles of the above mentioned peptidase.

Last but not least, Jitka examined the hypothesis that some of the enzyme isoforms function as early and late stage digestive peptidases in the course of on-host-feeding and off-host digestion, respectively. In order to prove it, she tested whether RNA interference may be used as a method for functional assessment of enzymes expressed in fully engorged females and demonstrated the necessity of re-injection of dsRNA to assure complete RNAi silencing.

During all the years Jitka have been working in our lab, she has mastered a large portfolio of methods and experimental skills required from an expert in molecular biology.

Jitka was brave enough to write down her Master thesis in English, and to my opinion she did it quite well. Certainly, more writing skills will be needed before she will be able to write the engaging introduction and valuable discussion, but I have no doubt that she is much more advanced than I ever was in her age. I would like to keep Jitka in our lab at least until she finishes her first-author publication and would be happy if she decides to continue and further develop her scientific career.

I am fully convinced that Jitka Konvičková's Master thesis fulfills all criteria requested by Faculty of Science, University of South Bohemia in České Budějovice to be awarded by a Master degree.

In České Budějovice, April 30, 2015



Petr Kopáček (supervisor)