

Supervisor's evaluation of Ph.D. thesis by RNDr. Zdeněk Franta – „Blood meal digestion in the hard tick *Ixodes ricinus*” –

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It is almost unbelievable that Zdeněk Franta is around in my lab for more than decade since he joined us sometime in 2001 as a bachelor undergraduate. He has finished his undergraduate studies in 2005 with Master diploma focused on the molecules from the soft tick *Ornithodoros moubata* which used to be our previous model until our colony was exterminated by infection with *Chryseobacterium indologenes*. Description of this unlucky event was the subject of the first Zdeněk's co-authorship paper published in 2006.

The beginning of his PhD. study matched well with the new era of our laboratory when we completely turned our focus to the reverse genomics research on the common hard tick *Ixodes ricinus* which presents the most serious threat which drives mushroom pickers out of our woods.

The main topics of Zdeněk PhD. thesis followed the research line coined in our lab by Daniel Sojka – namely the tick digestive machinery. The scientific question behind this research is simple – How a tick can engorge hundred-times its own weight and process all the host blood so effectively into eggs? Certainly, this amazing physiological feature of ticks may conceal a key to an efficient tick control. The data on tick digestion known before we started to work on this subject were rather scattered and inconsistent. We decided to map the tick digestive system in one species at a well-defined stage of feeding – namely the semi-engorged *I. ricinus* females. Zdeněk has significantly participated in all our papers published on this subject since 2007. Three papers subsequently describe the tick digestive apparatus as a cascade or network of cysteine and aspartic peptidases. The “genetic” paper by Sojka et al., (Parasite & Vectors, 2008, cited already 24-times) identified the genes coding for the digestive enzymes in the *I. ricinus* gut-specific cDNA. This work was continued by the biochemical and proteomic analysis of the hemoglobinolytic cascade of enzymes present in the tick gut extract. It is fair to admit, that this work published in quite prestigious journal Chemistry & Biology (Cell Press) was mainly carried out by our friends at Inst. of Organic Chemistry and Biochemistry in Prague (laboratory of Dr. Michael Mareš). The third, Zdeněk Franta's first author paper (Parasites & Vectors, 2010) describes the dynamics of expression and activities of digestive enzyme in the course of tick feeding on the host. This general description of tick digestive system is further supported by detailed characterization of individual enzymes – namely legumain (Sojka et al., 2007), Cathepsin L (Franta et al., 2011) and a manuscript on Cathepsin D (Sojka et al., under review in JBC). These multi-authors papers made in co-operation with laboratory of Michael Mareš in Prague and James McKerrow and Connor Caffrey lab at Sandler Center, UCSF are quite heavily stuffed with data and demonstrate well to those who takes the trouble to read them, why our lab is always below the average in the number of publications. Zdeněk also significantly contributed to Ondra Hajdušek's glamorous ferritin 2 paper, since iron metabolism in ticks is tightly linked with blood digestion. Besides this, Zdeněk Franta is also involved in our tick immunity research focused on complement-like molecules in ticks. All together, the name of Zdeněk Franta appears on 10 published papers which have been already 100-times cited by others.

These numbers witness about a fact I wish to emphasize. Without any exaggeration, Zdeněk Franta has long been and still is the most reliable, universal and therefore the most helpful member of our team. Among others, he has mastered the tricks how to get good quality RNA from any tick tissue which is the basic prerequisite for any successful follow-up experiments. Therefore almost all mRNA tissue profiling presented in our papers was performed by Zdeněk. Later, he has pioneered in our lab the method of quantitative real time PCR and

gained expertise in expression and purification of recombinant proteins. Last, I was really excited, when Zdeněk recently succeeded in cloning the whole molecule of tick alpha-2-macroglobulin (about 5000 bp) for its active expression and dreamed-of crystallization.

During his PhD. study, Zdeněk have had the chance to present his results at several well recognized conferences. From those, I would mentioned his contribution at the Insect Science Conference in Tucson, 2006 and his oral presentations at ICOPA in Melbourne, 2010 and recently on TTP7-conference, in Zaragoza, 2011. I was very happy that we were able to support Zdeněk to attend the famous 6-weeks Parasitology course at MBL Woods Hole, in 2007. He also stayed two times in the laboratory of Connor Caffrey at UCSF (3 months in 2008 and 6 weeks in 2009), which allowed him to perform the work which was mainly reflected in his cathepsin L paper. This all makes Zdeněk Franta's CV quite remarkable which will for sure open him the door of the best laboratories for a postdoc. Honestly, I would like to keep Zdeněk here as long as possible. On the other hand, I am well aware that he needs to meet another demanding boss for further development of his scientific career.

Conclusion:

I am fully convinced that Zdeněk Franta's thesis fulfills all criteria requested by Faculty of Science, University of South Bohemia in České Budějovice to be awarded by a title Ph.D. in Parasitology.

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Petr Kopáček
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