

Master thesis of Bc. Matěj Kučera “Influence of dietary components and redox enzymes on intestinal microbiota proliferation in the tick *Ixodes ricinus*”

(Supervisor’s evaluation)

Matěj Kučera has joined our lab five years ago and since his very beginning, he is under the expert guidance of MSc. Jan Perner. Matěj’s topic has been generally focused on the redox homeostasis in the tick gut. In his Bachelor thesis (defended in 2012), Matěj identified and characterized *I. ricinus* gene coding for NADPH dual oxidase (DUOX). This hydrogen peroxide producing enzyme is known to play a key role in the fruit fly midgut epithelial immunity. In mosquitoes, DUOX determines the vector capacity via affecting the balance between commensal microflora and transmitted pathogens. The maintenance of redox homeostasis in the gut of blood feeding parasites is challenged by the enormous surplus of the pro-oxidative heme received from the blood meal.

The topic of Matěj Kučera’s Master thesis addresses two important aspects in frame of our current major research project “The role of hemoglobin in tick metabolism and transmission of tick-borne pathogens”, namely (i) redox balance in the gut of ticks fed on hemoglobin-rich and hemoglobin-poor diet; (ii) the effect of hemoglobin on the proliferation of commensal microflora and *Borrelia* spirochetes in the tick midgut.

To experimentally address these questions, Matěj had to master the demanding technique of *in vitro* membrane feeding of adult *I. ricinus* females. Moreover, for the future transmission experiments, it is absolutely necessary to adapt the membrane feeding also *I. ricinus* nymphs. We have loaded this difficult task to Matěj, asking him to adjust the method of membrane feeding for nymphs. I originally thought, that achievement of this goal would be satisfactory enough for a full-value diploma thesis. Matěj displayed an extraordinary inventory potential and enthusiasm to fulfill this task. By a systematic optimization of membrane preparations, scent formulations and feeding condition, he has finally implemented the nymphal feeding as a routine method in our lab. Although it really means a milestone for our research, this part of Matěj’s work is rather hidden as a supplement to his thesis.

In his experimental work, Matěj achieved some remarkable results that, if independently reproduced and verified, will provide a basis for very interesting findings and publications. I will just briefly highlight some of his most striking results:

- (i) The hemoglobin uptake strongly suppressed the tick midgut microflora compared to serum-fed ticks.
- (ii) Tick faeces are almost aseptic.
- (iii) Host complement does not play a significant role in regulation of tick intestinal microbiota.
- (iv) The number of *Borrelia* spirochets in the tick gut is in reciprocal relationship to the symbiotic microbiota.
- (v) RNAi silencing of DUOX results in high proliferation of intestinal microbiota and clearly impairs tick feeding and reproduction.

These results were achieved by a careful application of the most advanced and demanding methods we use in our tick research –*in vitro* feeding, tick micro-dissections, RNA isolations, qRT-PCR analysis, RNA interference and many others, which Matěj definitely proved to master.

Matěj decided to write his thesis in English. He read quite a lot literature and often introduced me the papers I had not seen before. He also has a gift of ‘easy-writing’, which sometimes resulted in rather extensive and talkative style, especially in the discussion part.

I was happy to see Matěj working in the lab together with Jan Perner and Jitka Konvičková. I only regret that Matěj does not seem to continue the research he has started. However, the doors of our labs remain open after he explores the life of the antipodes at the opposite site of the Globe.

I am fully convinced that Matěj Kučera’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)

Jan Perner (co-supervisor)