Advisor opinion on Mgr. thesis of Bc. Veronika Dornakova

"Identification and characterization of histidine-rich peptides from hard ticks *Ixodes ricinus* and *Ixodes scapularis*"

Work presented by Veronika Dornakova was conducted in Laboratory of molecular biology of vectors and pathogens and represents an extension of long term research of our laboratory on analysis of differentially expressed tick genes in response to pathogen invasion or blood feeding. Presented work as well is connected to Veronika's Bc. project that dealt with isolation and partial characterization of novel histidine-rich protein from *Ixodes ricinus* (ricinus) with presumable antimicrobial activity that was predicted on the basis of structural and sequential similarity to previously described related proteins from *Rhipicephalus microplus* (microplusin) and *Amblyomma hebraeum* (hebraein).

Achievement of the highlighted project aims required employment of wide variety of techniques from molecular biology, microbiology and biochemistry that Veronika readily learned and applied. Her interest to the studied subject resulted in more than impressive literature review presented in Mgr. thesis. The use of the degenerate PCR primers designed on the basis of known sequences of microplusin and hebraein led to discovery of 9 transcripts that represent, most probably, a new multigenic protein family (HM-proteins) with unknown functions. It was obvious that the time frame of Mgr. project wasn't sufficient for analysis and characterization of the whole group, that included transcripts from *Ixodes ricinus* and *Ixodes scapularis*. Phylogenetic analysis showed that newly discovered HM-like proteins are distant from previously analyzed ricinusin but clusters together with original microplusin and hebraein sequences. From this point all following work was done simultaneously on two representatives of different protein families. Tests conducted with purified recombinants or shortened His-rich synthetic HM-peptide with purpose to detect the presence of antimicrobial or proteinase inhibition activity failed overcoming presumption of a role of histidine residues in antimicrobial activity. This might be a preliminary confirmation of the fact that HM-like homologues from tick species other than original might not possess the same antimicrobial activity, which might be connected to a tick vectorial capabily as well. It might point on the possible existence of synergetic compounds, necessary for establishment of proper activity or that experiments weren't conducted under the optimal conditions. Anyway, the results achieved are valuable and interesting and represent a wide field for following researches on both protein families that requires more time and human power.

Being enormously energetic, enthusiastic and emotional person Veronika conducted incredible amount of work that, sometime, according to my opinion, was unnecessary. My

own opinion is that being too opened and acceptable of others advices or a lead Veronica was rather weak in keeping the main direction of her project. I think that reasonable decreasing of Veronika's energy to a healthy level, keeping patience and persistence in her research that sometime were in deficit, being reasonable, thoughtful and first of all independent in choosing of her way in research will definitely be very profitable for Veronika in her further work, as self-believe and healthy self-confidence are the major (but not the only) requirements for independent scientist.

I would like to recommend the thesis presented by Veronika Dornakova to defense.

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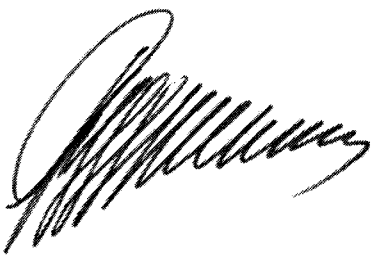

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