Supervisor's evaluation of Ph.D. thesis by Mgr. Daniel Sojka – „Multienzyme proteolytic digestion of host blood in the gut of the soft tick *Ixodes ricinus*" –
(Petr Kopáček, Institute of Parasitology, Biology Center, Academy of Sciences of the Czech Republic)

Daniel Sojka has been working in my laboratory since 1998 when he started his bachelor diploma project. He has had a bad luck with me as a supervisor because I usually put on him quite unrealistic tasks such a searching for tick transferin or prophenoloxidase which in fact have not been described by anyone till nowadays. During his undergraduate studies he has cloned and sequenced the prophenoloxidase from the insect *Galleria mellonella* – and thus accomplished my Humboldt foundation project from stay in Berlin. Thereby Daniel started his PhD study with a handicap of having no results on ticks.
His first real contribution to our tick research was solving the practical problem of obtaining unfragmented genomic DNA and subsequently perform the Southern blot analysis to answer the question whether α_2-macroglobulin exists as a single copy gene in the soft tick *Ornithodoros moubata* (paper - Saravanan et al., Insect Biochemistry and Molecular Biology, 2003).
One of the most important contribution of Dan's work during the initial phase of our reverse genetics approach for studying tick molecules was the construction of high quality cDNA libraries from guts and salivary glands of the soft tick *O. moubata* and the hard tick *Ixodes ricinus.* Good, full-clone cDNA libraries had been painfully missing here for many years. Overcoming this problem in cooperation with Jindřich Chmelař helped also to the remarkable shift forward the molecular biology research in the laboratory of vector host-interaction headed by Dr. Jan Kopecký.
Part of this Dan's activity, especially the high throughput approach to screening cDNA libraries using membrane chips for non-abundant genes is reflected in the paper of Lenka Grunclová et al. (Biological Chemistry, 2006).
The core of Daniel's thesis is based on the research of the digestive enzymes in the hard tick gut. We have turned to this topics in the spring 2004, when it was clear that our soft tick colony is dying-off and we had to find some other object for our future tick research. The first stimulus came by appearance an EST in the GenBank coding for a partial sequence of an enzyme called "hemoglobinase" resulting from the work of our next door colleague Natalia Rudenko et al. By closer look we realized that tick digestion is fairly unknown at molecular level and lags far behind other blood-feeding parasites and disease vectors.
Daniel screened *I. ricinus* gut-specific cDNA library and isolated a full clone of the above mentioned enzyme which turned out to be an ortholog of legumain, a CD clan asparaginyl endopeptidases which has been later designated as IrAE. Dan has expressed the IrAE pro-enzyme in *E. coli,* which was soluble but not enzymatically active. That is why Daniel had to introduce in the lab a new eukaryotic expression system in the yeast *Pichia pastoris*. This was accomplished in co-operation with Jan Dvořák from Charles University in Prague. Jan Dvořák helped us also to start the fruitfull co-operation with the famous laboratory of prof. James McKerrow and Dr. Conor Caffrey at Sandler's Center for Basic Research in Parasitic Diseases, UCSF. Thank to the PhD. grant from czech Grant Agency, Daniel spent two months in 2005 in the laboratory of Conor Caffrey, where they finished the expression of IrAE in the large fermentor and characterized its biochemical properties by the extraordinary tools available there: peptide combinatorial library (Charles Craik), highly specific active-site based probes (Matthew Bogyo) or the schistosomal cystein peptidases (Mohhammed Sajid). Putting all the data together resulted in recent publishing a quite bulky story on *I. ricinus* legumain in the International Journal for Parasitology.

Beside this, Daniel designed degenerate primers, amplified specific products, screened the gut cDNA library and finally isolated genes of other tick digestive enzymes comprising a cathepsin D-like aspartic peptidase, papain-type cysteine peptidase - cathepsin B and L and finally the dipeptidyl peptidase I - cathepsin C. This set of tick gut enzymes matched almost excatly the digestive machinery of Shistosoma which in further stimulated our both-site co-operation with the Sandler's laboratory in San Francisco. Most of the above mentioned tick enzymes were also prepared as recombinant in *E. coli* (with substantial contribution of Zdeněk Franta).
Another lucky event in our tick research was the start a common project with the Institute of Organic Chemistry and Biochemistry in Prague, namely with Dr. Michael Mareš and Dr. Martin Horn. In the meantime, these guys performed a cutting edge substrate/inhibitor mapping of proteases present in the *I. ricinus* gut which corresponded to the same set of enzymes identified by the reverse genetics. The manuscript of Daniel (paper I) in his thesis is actually one possibility how to present the data gathered so far. However, it has still not been decided whether to emphasize more the biochemical part from Prague or the genetic data from our lab. Thus, it is quite possible that the manuscript will be finally splitted in two papers. One more biochemical and hopefully strong enough for publishing in Chemistry and Biology and the other biological – emphasizing its biological, evalutionary and comparative aspects may be appropriate for Trends in Parasitology. This issue should be definitely decided this fall during the second stay of Dan Sojka in Conor Caffrey's lab (this time covered from Sandler's budget).

During his PhD studies, Daniel Sojka many times proved his deep motivation and interest in many aspects of tick molecular biology and physiology. What I appreciate the most about him is that he was never afraid to enter the unexplored areas, ask the right and challenging questions and the endless courage to solve problems from all possible sites. Although it was sometime difficult to keep Daniel focused, he has definitely evolved to a very talented young scientist during his PhD studies. I can recommend Dan for a postdoc without any doubt to any top laboratory around the world, however I would be very happy if I can keep him to stay with our lab and topics since at this moment he seems quite indispensable for our future projects. I believe that he will gain his international experience by long-term stays in the co-operating laboratories (like that of Conor Caffrey) which will definitely be of mutual benefit. We are currently chasing for suitable grant supports to make this possible.

As a final note I would like to mention that Dan is also very talented musician and singer and I always wished to him to become rich and famous as a pop star and doing science as a hobby. However, for a further prospect of our laboratory it would be definitely much better if he stays a poor scientist like we all are and makes music for fun and joy.

Conclusion:
I am convinced that Mgr. Daniel Sojka fulfills all criteria requested by Biological Faculty University of South Bohemia in České Budějovice to be graduated as Ph.D.

In České Budějovice, June 26, 2007

Petr Kopáček
(supervisor)