Review of the dissertation of Mgr. Nikola Polanská "Comparison and characterization of salivary proteins from *Sergentomyia* and *Phlebotomus* sand flies"

The work of Nikola Polanská fits into the long term research focus of the group of Prof. Petr Volf, which is the ecology, physiology and epidemiology of sand flies from the subfamily Phlebotominae. Members of this group are the vectors of several pathogenic agents, including viruses, bacteriae and protozoa. From medical point of view, the most important sand fly-transmitted pathogens are trypanosomatids of the genus *Leishmania.* Therefore the knowledge on how the sand flies facilitate the transmission and whether some of their proteins can be used as exposure markers or even vaccine targets, is of high importance.

The work of Nikola Polanská focuses on the to date neglected species *Sergentomyia schweizi* and its comparison with *Phlebotomus orientalis* and *P. perniciosus* – Old world vectors of leishmaniasis agents. Moreover, sialotranscriptomes of two lineages of *S. schweizi*, fed on different hosts for many generations, were compared in order to disclose, whether feeding on different host leads to changes in gene expression. The comparison was performed at several levels – transcriptomic, proteomic and functional, i.e. the activity of two major enzymes, responsible for blood feeding – apyrase and hyaluronidase, was evaluated by using recombinant enzymes. Finally, a detailed analysis of biogenic amines-binding proteins from YRP family across several sand fly species was performed and their specificity to various bioamines was addressed experimentally by using recombinant proteins.

In the introduction to the dissertation, author describes in details the ecology, behavior and medical importance of each of the three species, based on the available literature. In the following chapters, major salivary protein families of sand flies are described. The literature on their function and potential as exposure markers and/or vaccine targets is reviewed in each chapter in details and thoroughly.

The main article, where Nikola Polanská is the first author, shows that most proteins of *S. schweizi* are relatively distant from those of *Phlebotomus* and *Lutzomyia*, clustering at the base of phylogenetic tree, thus corroborating the phylogeny of phlebotomine group. This can be also due to an adaptation to different hosts (reptiles). **Question:** Is this usual in insect blood-feeding ectoparasites? In ticks, we observe much faster evolution of salivary multigenic protein families. The relationships among these proteins do not reflect actual tick evolutuion. What can be the reason for the difference between ticks and sand-flies in this specific aspect?

Very interesting is the analysis of two lineages of *S. schweizi*, fed either on mice or on geckos. Transcriptomic analysis revealed some genes that were up-regulated either in one or in the second lineage, however, these genes were incomplete and no functional prediction could be made. **Question:** Does author think that these incomplete mRNAs are really present in the transcriptome or is it simply an artefact of sequencing method?

Another observation was a significant difference in hyalouronidase activity between the two lineages, despite no difference in its amount or expression level. I suppose that there are more hyaluronidases in the genome of sand flies. It would be really interesting to see that such fast change in activity can be due to an adaptation to different host. This would show high adaptability of sand flies, which could have implications in epidemiology.

Question: How can be the difference in hyaluronidase activity explained? Is it due to differential expression of different variants of the enzyme or due to a mutation in the enzyme. I would like to see an alignment of the hyaluronidases from both lineages, if possible.

Concerning YRP family, the observation that the specificity of YRP towards different bioamines is species-specific is also intriguing, suggesting an adaptation to different vertebrate hosts. Notably, YRPs from *S. schweizi* did not bind serotonin and histamine like YRPs from *Phlebotomus* and *Lutzomyia* sand flies. **Question:** Is there any difference in biogenic amines used by mammals and reptiles? Or is it possible that reptilian bioamines play different role in their physiology and are not important in defense against the bite?

In other blood-feeding arthropods, such as ticks, the binding of bioamines is done by members of lipocalin superfamily, with thousands of members in this multigenic family. **Question:** Is there any resemblance between YRP and lipocalins either in mechanism of binding bioamines or in tertiary structure?

Part of the thesis deals with the usage of salivary proteins as exposure markers and vaccine targets. Exposure markers in animals and people are important for epidemiological surveillance. **Question**: What is the ratio of infected sand flies in whole population, I mean, what is the chance of getting bitten by sand flies in their area and what is the chance of getting infected when bitten? The search for vaccines against vectors (transmission blocking vaccines) is something like holy grail in disease vector research. In long term blood-feeders, this can make sense, considering that the transmission of some pathogens needs at least 24 hours of uninterrupted feeding and therefore the antibodies or cellular immunity can impair the feeding process. **Question**: Is it even possible to find such strong vaccine target in sand flies or mosquitoes that would completely block the transmission or the feeding itself? Please, try to speculate on this a bit, considering involved hosts' defense mechanisms.

Overall, the work of Nikola Polanská further expands the knowledge on the biology of sand flies as vectors and their interaction with their hosts. I believe that Nikola gained a lot of experience in the omic data analyses and also in functional analyses of proteins, including their expression in recombinant forms. This is very solid base for further research in this research area, where the combination of high-throughput approach and experimental functional characterization of interesting proteins can lead either to the discovery of exposure markers, vaccine targets or even novel drugs, such as anticoagulants or immunomodulatory substances.

The quality of the work of Nikola Polanská was acknowledged by publications in impacted journals and I have no doubt that she qualifies for obtaining Ph.D. title.

In České Budějovice 28.8.2020

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