Evaluation of Ph.D. Thesis of Nikola Polanská

During 2012, I had the oportunity to spend 3 months at Dr. Petr Volf's laboratory (Chales University in Prague) for a fruitful research stay, for my own PhD, where I gained great knowledge about insect saliva from one of the best European research groups on sand flies. Since then, I continued my research focused on sand fly salivary proteins and I expanded it to the study of mosquito salivary proteins at the National Institutes of Health (Maryland, US). During my time at Dr. Petr Volf's laboratory, I met Nikola Polanska. Therefore, it is my pleasure today to serve as a reviewer of her PhD Thesis on sand fly saliva.

ABSTRACT

The abstract is concise and summarizes the main findings of the four articles presented in this Thesis.

INTRODUCTION

The introduciton is 33 pages long and covers well the literature on sand fly biology and salivary proteins including both old, historic articles and the most updated literature. The PhD candidate did a great work compiling all this information. As a general comment, I believe a section on taxonomy of sand flies would have been helpful, as many comparisons of sand fly salivary proteins are done according to the sand flies' taxonomy.

Specific comments/questions:

- Page 12: "Differences in sand fly saliva composition were found between various populations, between colonies of the same species that originated from distant localities, and even among long-term maintained colonies".
  The expression of salivary genes seems to be modulated by environmental conditions as well (Coutinho-Abreu et al. 2010, Coutinho-Abreu et al. 2011, Coutinho-Abreu and Ramalho-Ortigao 2011). These authors detected an increased expression for most *Phlebotomus papatasi* salivary genes, coinciding with a marked limitation in the availability of sugary foods due to drought and an increase in cutaneous leishmaniais cases in the area. In addition, the availability of the blood supply source can modulate the expression pattern of salivary genes. This work suggests that modulation of the composition of salivary proteins may play a role in *Leishmania* transmission. However, more studies are needed to further test this hypotesis.
  What is your opinion on these studies in relation with the salivary gene expression differences seen in the two lineages of *Sergentomyia schwetzi* adapted to blood fed in different hosts? Could these studies be a confirmation in the field of your recent findings at transcriptomic level of isolated sand flies forced to feed on different blood sources in the laboratory?

- Page 13: "The adaptation to possibly lower amounts of ADP in avian and reptile blood could be a reason for the low anti-clotting and apyrase activities in *Culex quinquefasciatus* (Ribeiro, 2000) and *Dipetalogaster maxima* (Ribeiro et al., 1998), two arthropods that prefer to blood feed on birds and lizards, respectively".
  Culex quinquefasciatus mosquitoes are traditionally considered bird-feeders that later adapted to mammalian blood-feeding. It is true that the apyrase activity in *C. quinquefasciatus* is low, most propably due to the traditional preference to blood feed on birds. However, recent work

has described a *C. quinquefasciatus* D7 protein that has functionally diverged to bind and scavenge ATP and ADP which may compensate for the low salivary apyrase activity (Martin-Martin et al. 2020). Blocking the action of ADP, an important platelet aggregation mediator seems to be an evolutionary advantage for *C. quinquefasciatus* mosquito blood feeding on mammals.

- Page 17: "All three recombinant YRPs from *L. longipalpis* showed a different antigenicity for different hosts. The recombinant protein LJM17 was recognized by a broad spectrum of hosts, namely: chicken (Soares et al., 2013), dogs, foxes and humans (Teixeira et al., 2010). On the other hand, the protein LJM11 was only antigenic in dogs and humans, whereas the third YRP (LJM111) showed a very low antigenicity in humans (Teixeira et al., 2010)".
  Do you think that the differences in antigenicity might be a strategy for the insect to evade the host immune system to retain the biological functionality of these proteins?

- What is your opinion on glycosylation of salivary proteins? From the last 10 years work, it is becoming clear that the system utilized to produce the salivary recombinant proteins matter. For some proteins, glycosylation is essencial for the their activity (I. e. *Lutzomyia longipalpis* hyaluronidase – LuloHya, (Martin-Martin et al. 2018)) and for many proteins, glycosylation seems to be essential for using salivary proteins as markers of exposure. Even a recent publication on the glycome of *L. longipalpis* salivary proteins supports the specificity of glycosylation patterns in insects (Mondragon-Shem et al. 2020).

- Page 18: Regarding the immunochromatographic test, how similar is the YRP used in the ICT from *P. perniciosus* to *P. orientalis* and *S. schwetzi?*.

  Would the SP03B-ICT be helpful as a marker of exposure to *S. schwetzi*? Have you tested the ICT test with sera from geckos and mice used to maintain the two lineages of *S. schwetzi?*

  Do the ICT tests cross-reacts among sand fly species? If antibodies against *P. perniciosus* and *P. orientalis*-YRP crossreacted, and taking into account that these two sand fly species do not overlap geographically: Could the SP03B-ICT be used in geographically distant places such as Ethiopia as a marker of *P. orientalis*?.

  Does the SP03B-ICT ccrossreact with other blood feeding arthropod bites, such as mosquitoes, ticks, bitting midges?

- Page 20: "Since the *P. orientalis* lufaxin is almost half the length of homologues in other sand flies (Vlkova et al., 2014), it has not been included in any of the phylogenetic analysis published so far. However, since *P. orientalis* lufaxin is highly related to the lufaxin of *P. perniciosus* with a 88 % sequence similarity and other lufaxins from Larroussius sand flies cluster together (Coutinho-Abreu and Valenzuela, 2018), it can be assumed that also the lufaxin from *P. orientalis* will cluster with the lufaxins of other Larroussius species (Vlkova et al., 2014)."

Even if Lufaxin-like from *P. orientalis* is half the size of the characterized FXa inhibitor from *L. longipalpis*. It would be intersting to test if the inhibition of FXa activiy is mantained in *P. orientalis*. If lufaxin-like from *P. orientalis* remains active, it could be helpful in investigating the point of contact with FXa or the active site.

OBJECTIVES

There is a general goal and three well specified and clear objectives, one per research topic covered in this Thesis.

ARTICLES

Nikola Polanska is presenting 4 original research articles in her PhD Thesis. All of them have been already critically evaluated by experts on the topic during the peer-review process as part of the journal publishing procedure. Overall, the hypothesis were coherent, both the experimental design and methodology were appropriate and conclussions were well sustained based on the results shown. I have a few comments/questions/suggestions.

ARTICLE #1: *Sergentomyia schwetzi*: Salivary gland transcriptome, proteome and enzymatic activities in two lineages adapted to different blood sources. Polanska N, Ishemgulova A, Volfova V, Flegontov P, Votypka J, Yurchenko V, Volf P. *PLoS One*. 2020;15(3), e0230537. doi:10.1371/journal.pone.0230537

This manuscript describes the salivary gland transcriptome and proteome of *Sergentomyia schwetzi*, and it is the first sialotranscriptome of a *Sergentomyia* species. Besides, it is the first published sand fly sialome using Illumina technology, which results in a much higher number of transcripts sequenced compared to the transcriptomes done by sequencing phage cDNA libraries.

- As a future experiment, a reverse approach regarding the blood feeding of the two different sand fly lineages could be implemented to confirm your findings. From the G-M colony of *S. schwetzi* fed on geckos, sand flies could be separated, and their diet changed to see if over generations (~40 as the ones stated in the manuscript) there is a reversion of the changes observed. A similar approach could be done with other sand flies to confirm the findings.

- Have you tested the endonuclease activity of the salivary gland extract of the two lineages of *S. schwetzi*?

- Have you tested the factor Xa inhibitory activity of the salivary gland extract of the two lineages of *S. schwetzi*?

- Apart from salivary genes, could the difference in blood source have changed the expression levels of other genes, such as the ones involved in immune responses?

- Do the two lineages have the same vector competence? If you wanted to perform an experiment to test this hypothesis, how would you do it?

- Do the two lineages have the same fitness? What experiments would you do to test the fitness cost of the sand fly lineages?

ARTICLE #2: Amine-binding properties of salivary yellow-related proteins in phlebotomine sand flies. Sumova P, Sima M, Kalouskova B, Polanska N, Vanek O, Oliveira F, Valenzuela JG, Volf P. Insect Biochemistry and Molecular Biology. 2019; 115, 103245. doi:10.1016/j.ibmb.2019.103245

This manuscript used the novel microscale thermophoresis technique to gain binding information on *L. longipalpis, P. pernicious and P. orientalis* yellow related proteins and showed that this technique is comparable with isothermal titration calorimetry.

ARTICLE #3: Interactions between host biogenic amines and sand fly salivary yellow-related proteins. Spitzova T, Sumova P, Volfova V, Polanska N, Poctova L, Volf P. Parasites & Vectors. 2020;13(1), 237. doi:10.1186/s13071-020-04105-2

This manuscript used the novel microscale thermophoresis technique to gain binding information on *P. argentipes* and *S. schwetzi* yellow related proteins. Moreover, they investigate the role of histamine, serotonin, and anti-saliva antibodies in the blood meal in sand fly fitness studying oviposition and mortality.

- What is your opinion on the finding that *S. schwetzi* YRP did bind the biogenic amines serotonin and histamine. Are the amino acids involved in the YRP-binding site present in *S. schwetzi* YRP?

- Is the electrostatic potential of *S. schwetzi* YRP surface known? It might provide useful information about the lack of binding.

ARTICLE #4: The recombinant protein rSP03B is a valid antigen for screening dog exposure to *Phlebotomus perniciosus* across foci of canine leishmaniasis. Kostalova T, Lestinova T, Maia C, Sumova P, Vlkova M, Willen L, Polanska N, Fiorentino E, Scalone A, Oliva G, Veronesi F, Cristóvão JM, Courtenay O, Campino L, Gradoni L, Gramiccia M, Volf P. Medical and Veterinary Entomology. 2017;31(1), 88-93. doi:10.1111/mve.12192

This work validates the use of SP03B as a reliable marker of dog exposure in the field.

SUMMARY AND CONCLUSIONS

This section is 5 pages long and the author clearly summarizes the main findings and nicely connects the 4 papers presented in this Thesis.

REFERENCES

There are 250 references on the bibliografic list which denotes a great insight of the PhD candidate into the specific research topic. The list of references covers well the current knowledge on sand fly saliva and it is up to date.

GENERAL REVIEW

The PhD thesis of Mrs. Polanska is a comprehensive and well written piece of work, with great findings on the characterization of salivary proteins at transcriptomic, proteomic and biochemical levels. In

addition to the basic science contribution, applied science to the field has been demostrated in this Thesis with the use of a salivary protein as a marker of sand fly exposure of dogs in a foci of canine leishmaniosis. With a detailed introduction on the topic, four papers published in high impact journals already evaluated at the peer-review process prior publishing (one impressive paper as a first author and three papers as a co-author), I believe this PhD Thesis meets all requirements of a high standard thesis.

Place: Rockville, Maryland, United States of America.

Date: August 10th, 2020.

Signature:

***Ines Martin Martin**, PhD*
Laboratory of Malaria and Vector Research
National Institute of Allergy and Infectious Diseases
National Institutes of Health
12735 Twinbrook Parkway TW3/2W09
Rockville 20852, Maryland
Telephone: (240) 627-3412
Email: ines.martin-martin@nih.gov

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