

### **Evaluation of the thesis manuscript of Mrs Magdalena Jancarova to obtain a Ph.D. degree**

First of all, I sincerely apologize that I could not be present in person on the day of the defense of Mrs Jancarova's thesis as I am organizing at the same period a meeting between all the partners of a project I am coordinating.

The PhD project of Mrs Magdalena Jancarova aimed to study biotic and abiotic factors affecting the transmission of various micro-organisms (namely viruses, parasites and gregarines) by several species of Phlebotomine sand flies (Diptera: Psychodidae). This very ambitious project can be divided into four separate studies and led to three publications in internationally recognized, peer-reviewed journals (all classified in the first quartile for their discipline of interest). A fourth paper, containing a substantial amount of results, is in preparation and will be published, without any doubt, in a near future even if some complementary experiments are likely necessary.

The introduction is very well written and pleasant to read. I would have appreciated more specific chapters on *Leishmania* and gregarines (for example) but, very understandably, Mrs Jancarova made several editorial choices given the broad range of the topics studied during her thesis. The chapters on viruses transmitted by sand flies and gut microbiome (in particular) are rather synthetic but exhaustive, giving strong evidences that Mrs Jancarova is able to extract, from scientific literature, all the information relevant to her project.

The questions raised by Mrs Jancarova throughout her manuscript are quite original and of first importance to understand more precisely the role of various factors to the dissemination and persistence of the microorganisms transmitted by sand flies. The quality of the work presented in the manuscript, at the frontiers of so many different topics, such as viruses, parasites, extrinsic (temperature) and intrinsic (gut microbiome, tissue barriers) factors, is to put to the credit of Mrs Jancarova and place her in a very favorable position for future projects on the transmission of pathogens by blood sucking insect vectors. Indeed, as it is rather frequent, the questions asked in each given discipline are often different but can be easily transferred to other topics. A striking example in the manuscript is the way "virologist" and "parasitologist" may study the immune response and the gut microbiome of infected insect vectors.

In general, the experiments are very well designed, and performed in a rigorous manner. Mrs Jancarova used very diverse technics (going from molecular biology, virology, entomology microscopy) to test her different hypothesis, giving strong evidence that she is a very capable experimentalist.

Overall, the data obtained are convincing, presented in good quality illustrations and rigorously interpreted with appropriate statistical analyses. The first part of the result chapter concerning the "Experimental infection of sand flies by Massilia virus and viral transmission by co-feeding on sugar meal", is the less accomplished and raised numerous comments and questions. This chapter presents a huge amount of data that are not always easy to follow and could have been described in more details.

- 1- The legend of figure 2 could have been more detailed and mentioned the representation of, what I believe are, fitted lines. In general, I did not find throughout the text (and in the

material and methods) how these curves are calculated and the purpose to use such a representation.

- 2- What are the typical Ct values obtained by RT-qPCR and is there any cut-off value under which the sample is considered negative?
- 3- How the physical titers (i.e. "number of viral particles") obtained by RT-qPCR and presented in Tables 2, S1 and S2 are calculated? Moreover, can we correlate this number to an infectious viral particles titer?
- 4- This correlation is particularly important as it could help to define the minimal dose of infectious viral particles needed to establish a successful infection in a given sand fly species after a sugar meal. On the same way, I am curious to know if Mrs Magdalena Jancarova performs a specific experiment to answer this point.
- 5- Has the stability of Massilia viral particles been specifically assessed (depending of the temperature and time for example)?
- 6- The detection by RT-qPCR of MASV (presence or absence of the virus) in sugar meal is quite variable through the time (Tables 1 and 2). What are the hypotheses that may explain such pattern?
- 7- In Table 2, how can we explain the decrease of the infection rate of *P. perniciosus* female and male infected by "co-feeding on sugar" from day 13 to 21? What are the consequences of these data on the dynamic of transmission and persistence of MASV in the field?
- 8- Is there any obvious explanation (immunity, ...) about the variability of the infection rate and number of virions observed between the tested sand fly species and in particular: *P. perniciosus*, *P. argentipes* and *L. longipalopis* (Figure 3, 4 and S1; table S1 and S2)?
- 9- Without any consideration of reagents or infrastructures availability, what experiment would you design to dissect more precisely the mechanism of infection and transmission of MASV through "co-feeding on sugar"?

Despite these comments and questions, it is important to note that the main finding of this work, i.e. the transmission of Massilia virus from infected to uninfected sandflies through a shared sugar meal, is supported by the data presented, quite novel and really exciting. It opens new avenues to investigate transmission and persistence in the field of Phleboviruses (such as Toscana virus) in particular and arboviruses in general.

The chapter "Summary and conclusions" is synthetic and well written. Overall, Mrs Magdalena is able to formulate hypotheses, design the appropriate experiments and give perspectives to her study. She certainly demonstrates a significant degree of scientific autonomy.

In conclusion, Mrs Magdalena Jancarova acquired the necessary level to obtain a Ph.D. degree and I thus emit, without any reserve, a very favorable opinion for the defense of her thesis in order to award her the Ph.D title.

Maxime Ratinier



I also noted some minor errata throughout the Introduction chapter:

1. p4, line 6: it seems that the percentages of individual have been inverted compared to the PFU titers.
2. p4, third paragraph: change “achieve” to “reach”
3. p4 last paragraph: “virus” to “viruses”
4. p5 first paragraph: “more virus then” to “more virus than”
5. p5 first paragraph: “non-transmitted” to “non-transmitter”
6. p5 last paragraph: “not included in this text” to “not included in this manuscript”
7. p9: “thermodynamic” to “thermodynamic”
8. p9: “Bunyaviridae” to “Phenuiviridae” (newly established by ICTV)
9. p14: “genom” to “genome”
10. p18: “posses” to “possess”