

Opponent review of the bachelor thesis

Author: Amelie Klein

Title: Phylogenetic and seasonal patterns in the microbiome of mosquito vector

The bachelor thesis of Amelie Klein deals with several important aspects of mosquito *Culex pipiens* which can have important implications as potential vector for viruses. In actual circumstances the main aims are very important. The thesis is really nicely written, I learned a lot about microbiomes of mosquitos and about mosquitos in general. In this manner the introduction very nicely introduces to the main goals and focus of the thesis. The methods are clearly written and Amelie used up to date methods (i.e. next generation sequencing) which is the gold standard in recent microbiological studies. She used proper bioinformatics pipelines which are recommended for such datasets focused on amplicon sequencing. There are several minor typos (i.e. italic font for genus and species names).

Results are presented in very detailed graphs (mostly tables and bar-charts). Amelie compared two microbiomes of mosquito in Spain and Slovakia as well as different mosquito forms and seasonal patterns. Since the differences in microbiome between different species and forms can be expected I think the most interesting results were obtained from the seasonal patterns, which clearly shows the important aspect of environmental factors (temperature, humidity).

The discussion is in the most parts clearly written, sometimes it was maybe difficult to identify when Amelie speaks about for example statistics including/excluding Wolbachia (i.e. alpha diversity indexes). The conclusions are clear and sound.

I have several questions and suggestions for the author:

1. Did you tried ordination diagrams (PCoA or NMDS) to see the distribution of different microbiomes between different locations, forms and season?
2. How did you judge that the mosquito microbiome between different locations/forms/season is not different. Which statistical test you used? Did the test include Wolbachia or was done without?
3. I think your analyses did not catch the important player which you mentioned in the introduction, the viruses which mosquito transfer from host to host. How would you extend the experiment to see if the microbiome affects viral transmission? Which method you would use?
4. You presented basically two was of diversity results (with and without Wolbachia). Both show dramatically different results. Which approach you would recommend?

5. There are quite nice bioinformatics pipelines which help "translate" 16S amplicon data to functional bacterial guilds (i.e. FAPROTAX, TAXFORFUN or PICRUST). Did you think about these algorithms to see differences in functional potential of mosquito's microbiomes?
6. How many OTUs you were able to taxonomically assign to genus level? How many reads per sample you have? How was the variance in sequence number?

Formal recommendations:

I would recommend to present the detailed (sample by sample) data in supplement (e.g. figure 5).

I would prefer to present legend with taxons not OTU number and if you present it in color to use better colors (not the default excel).

Overall I think the quality bachelor thesis of Amelie Klein is high the defense.

Doc. Ing. Jiri Barta, Ph.D.