

Opponent review of the bachelor degree thesis of Amila Kličić

"Microbiota of Arctic mosquitoes. "

As the title suggests, the presented thesis aims to describe Arctic mosquitoes' microbiome's composition and dynamics. I like the topic very much and find it attractive, with possible further application in pest control and epidemiology. In general, I find the aims to be met using appropriate methodology. At least to some extent, the author showed the ability to compose a scientific text. On the other hand, I also found a wealth of small-to-bigger issues decreasing the quality of the text. The thesis has the usual structure (apart from the Annotation listing only the aims) and sufficient length. It is written in English. I detected many articles and commas missing, some meaningless phrases, but the general language level is satisfactory as far as I can tell.

Unfortunately, it is not the case for the presentation of results. I am missing a more detailed legend in most of the figures. The Figures are also barely readable without magnification (or I am just going old 😊?). Bigger font and Figure layout should be used. Moreover, Figures in the Introduction lack numbering and any legend at all!

The Discussion is one block of text three-and-half pages long. It needs to be structured into paragraphs! On the other hand, it is maybe the best part of the thesis. The author shows her critical thinking, insight into the problematics, and possible application of the results.

Please find my specific comments below. I would like to hear the answer to the bold ones during the presentation.

1. I think the first pages of the thesis should not be numbered. Having 'the page one' twice in the document is confusing.
2. Page 4(1st) - Abstract. Mosquito one of the tiniest organisms in the world? Maybe seen through the elephant eyes, but how about the bacteria and protists forming the mosquito microbiota?
3. 16S gene sequencing method – I think a 16S metabarcoding would be more fitting.
4. Page 1(2nd). "*Their importance in ecosystems is great, especially in aquatic ecology.*" I am neither ecologist nor an entomologist, but there are surely more recent sources considering recent methodological progress and big-data studies. I suppose there other environments where Insects play a more crucial role. Also, further down the page, the term "human enemy" is maybe a bit strong.
5. Chapter 1.1. The unusuality of insect exoskeleton is true, when viewed from the vertebrate/mammal/primate/human point of view, but given the total numbers, it is rather us being very unusual.
6. The same chapter: I thought the insect body size is limited by oxygen concentration in the atmosphere rather than the exoskeleton constraint? Or am I wrong here?
7. The first picture describing the morphology of the adult mosquito is very general and describes the anatomy of the adult insect. Which I am sure everybody relevant knows.
8. Page 4(2nd). I'd say the health threats are the pathogens transmitted by mosquitoes rather than the mosquitoes themselves.
9. Not sure why almost 3/4 page of Introduction is dedicated to malaria? How relevant is it to the presented thesis?
10. Again, not sure the wide ecological valence of mosquitoes is caused by their body size, which is not so small for insects anyway.

11. While the first part of Introduction (1.1 and 1.2) is not that great (to say the least), the remaining part (1.3 and 1.4) is very good. Almost as if somebody else wrote it (which I hope is not the case!). However, I miss a brief introduction of methods used for microbiome studies.
12. Aims should be listed after the Introduction!
13. Chapter 2.1 Author should explain what the following places listed in the thesis: Nordaustlandet, Kvitøya are. It is not up to the reader to look it up. Further in the text, she mentions three sampling sites, whereas, as apparent from Table 1., there were five? Although not mandatory, it is usual to add GPS coordinates or a map of sampling sites.
14. Chapter 2.3 I think the Method chapter is not the place to describe the principle of PCR? It is a commonly known mechanism (at least among Faculty of Sciences students and employees) and does not need the introduction anyway.
15. **Please explain how to screen for a recent blood-meal with oligos specific for mosquito COI? What do the results tell us?**
16. **Figure 2. There are bands of different sizes than 300 bp on the gel. What are those products?**
17. Figure 3. Taxonomic profiles are barely readable. A more detailed legend of the Figure description would be welcome. For example, samples have unique codes from which the reader can only guess the sampling year, but not the sampling site and the sampling date.
18. Figures 4-6. Again, the legend of the graph is barely readable. An increase in font size would be welcome.
19. **Figure 5. Does it make sense to compare the alpha-diversity of individual sampling dates? From Fig 6. it is apparent that the sampling site also has an effect on the diversity (and the same applies for Fig 6., only vice versa). Wouldn't some multivariate analysis taking in account both variables (sampling site and day) be more appropriate?**
20. **The author also mentions that: "*differences by year and date show differences in mosquito microbiome based on temperature and weather conditions.*" Wouldn't it be more appropriate to use those actual values instead of date and year?**
21. **In Discussion, the author states that the microbiome has a great impact on disease transmission. Hence its knowledge is crucial for preventing transmitting these diseases. However, how can we manipulate the microbiome composition to affect the level of transmission? What is the practical output?**
22. **At the end of Discussion author asks a question: "What will happen when, with the ongoing climate change, *Aedes nigripes* spreads further than the Arctic and occupy other habitats and various different regions?". Maybe I do not understand the point, but where else could it spread? Down to the south? Or up north? What is more arctic than Arctics?**

I think the thesis has all the essential features and deserves to be awarded the BSc title. On the other hand, I think it could be much better. It is by no means excellent, but I reserve my right to suggest the final grade after the Presentation.

In České Budějovice January 22nd

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