



OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Melissa Leibetseder

Thesis title: Latitudinal effect on mosquitoes' microbiota

Supervisor: Sonia M. Rodriguez-Ruano, Ph.D.

Referee: Mgr. Zoltán Füßy, Ph.D.

Referee's affiliation: Charles University, Prague

	Point scale ¹	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced length of the thesis parts (recommended length of the theoretical part is max. 1/3 of the total length), logical structure of the thesis	0-3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	1
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	1
Formal requirements – points in total		14
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1
Logic in the course of the experimental work	0-3	2
Completeness of the description of the used techniques	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3

¹ Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Quality of experimental data presentation	0-3	1
The use of up-to-date techniques	0-3	2
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	1
Practical requirements – points in total		18
POINTS IN TOTAL (MAX/AWARDED)		48
		32

Comments of the reviewer on the student and the thesis:

The presented thesis is formally acceptable and addresses a topic that is both scientifically contemporary and medically interesting. It reiterates an insight on *Wolbachia* being dominant symbionts of the mosquito *Cx. pipiens* in Europe. To my understanding, none of the geographical parameters considered here showed strong influence on alpha diversity. This could be addressed in follow-up studies, not only to determine how *Wolbachia* co-determine vector competence of host mosquitoes but also how they interact with each other, or alternatively if and how other components of the microbiota add to this picture. Still I would like to point out several issues.

My first concern regards the how the hypothesis of latitudinal structure of mosquitoes' microbiome is tested. This seems overly simplistic even if latitude would be "directly associated with temperature" – as claimed in the Abstract. As a consequence, few environmental parameters were considered besides geographic locality and habitat. Indeed, many parameters vary among the study's sampling localities that are found across the European continent. Maybe average temperature, humidity, time into the mosquito season, and other factors relevant to mosquito biology could have been recorded at the time of sampling to provide more metadata. Environmental samples could have been taken from nearby water bodies for comparison, rather than just being discussed at the very end of the study. Correlation of microbiota with vectored pathogens would also have been interesting. Potentially, there are procedural weaknesses, too. The read counts are not normalized by any quantitative standard or spike-in, though normalization has been shown repeatedly as crucial for elucidation of biological responses and removal of false positives. Normalization thus might be required for publication in an impacted journal. Curiously, pathogen reads were filtered out without providing evidence of these organisms being true contaminants (Methods chapter 3.7).

My second concern regards the visualization of results that does not allow a straightforward interpretation for neither the author nor the reader. For example, the two *Cx. pipiens* subspecies should be distinguishable by amplification of a 200- or 250-bp PCR product (see Material and Methods chapter 3.5). Figure 5 shows an example of subspecies PCR determination where specimen of both subspecies seem to be present, but band sizes are not marked and the author concluded that virtually all bands (93.2%) correspond to a single subspecies *Cx. pipiens pipiens* – so how does this follow from the 200- or 250-bp product detection? More importantly though, and being the critical result of the study, the figures 6-13 are not color-coded consistently and OTUs are not grouped by taxon, which hinders drawing any conclusion from the plots about taxonomic composition. Such grouping might have revealed that some bacterial groups are more frequently correlated with certain parameters.

There are some factual inaccuracies in the text. While viral, bacterial and protistan pathogens are mentioned as mosquito-borne, only viral and protistan examples are listed. Rarely still, mosquitoes also transmit roundworm parasites. On page 2, protist pathogens allegedly do not

replicate in mosquitoes, but it is common to know that *Plasmodium*, the causative agents of malaria, complete their sexual stage in mosquitoes and massively propagate before being able to infect their final mammalian hosts.

Lastly, chapter 3.1 of Material and Methods reads much like an introductory chapter and describes no employed material or methods.

In summary, the presented thesis is acceptable for a bachelor's degree at the Faculty of Science of the University of South Bohemia. The thesis documents that the candidate has had sufficient hands-on experience in molecular biology and biogeographic methods and hopefully she will strengthen her expertise in her forthcoming career.

Suggestions and questions, to which the student has to answer during the defense.

Mistakes, which the students should avoid in the future:

Please present some options for normalization of your data or discuss why you assume your data does not require normalization.

Is the *Wolbachia* interaction with *Cx. pipiens* possibly mutualistic or entirely parasitic and why? It is not prevalent in the sibling species *Cx. torrentium* and *restuans* that occupy similar habitats (Werblow et al., 2014; Muturi et al., 2016).

In light of *Wolbachia* being the dominant bacterial species in all your specimen, kindly discuss if the use of peptide nucleic acid (PNA) PCR clamps would be a solution to block the amplification of *Wolbachia* sequences and allow you to focus on the less abundant microbiota.

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade .²

In Prague date 15th July 2020

signature

² You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).