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Thesis Review for Joel Brown, University of South Bohemia

Evaluation of the thesis

The microbiome is one of the most critical research areas in contemporary entomology. Microbial symbionts are often integral to insect biology, and can influence processes directly relevant to human welfare, such as disease transmission, pollination, and pest pressure on crops. Joel's dissertation makes important contributions to the study of insect microbiomes, and bears on other fields as well, including the ecology of more "traditional" kinds of communities.

One of these contributions is shown in Chapter 2, wherein a case is made for how and why microbiomes can be incorporated into metacommunity ecology, using insects and their symbionts as an example. This link is a good example of the interdisciplinary nature of the thesis, and demonstrates the value of (historically neglected) microbial communities to ecological theory. I think that unique features of host-associated communities could be explored more deeply. For example, host-associated microbiomes, unlike say soil bacteria or a population of trees, occupy a habitat that is under selection. This concept is explored in the "ecosystem on a leash" model. Further, microbial populations can evolve within the lifetime of individual hosts.

Another contribution is in demonstrating the importance of ecological factors to structuring insect microbiomes, in two different study systems. Key findings are that ontogeny can have enormous influence on microbiome assembly, and that captivity has strong impacts on the microbiome of *Drosophila*, a commonly lab-reared group. These findings were likely aided by controlling for the impact of diet, which often influences insect microbiomes. To someone setting out to explore the microbiomes of an unstudied group of insects, I would point them to these papers as a guide. Of course, this broad approach cannot answer all of the relevant questions. For example, it would be valuable to know where these microbes are within the host body, how abundant they are (i.e. number of cells), and how their activities do or do not affect host physiology and ecology. However, this work provides a solid understanding of microbiome compsition and dynamics, which will inform future finer-scale and mechanistic studies.

A perhaps underemphasized value of the thesis is that it advances the biology of *Drosophila* and triatomine insects. These are key groups. *Drosophila* is a longstanding model system, and one increasingly studied in the wild and beyond just *D. melanogaster*. Triatomines are a major disease vector. The new questions arising from this work will undoubtedly be taken up by other biologists studying these insects. For example, how does highly reduced microbiome diversity impact the relevance of laboratory-based studies of *Drosophila*? Why does *Dietzia* come to dominate triatomines (of some species) and does it influence vector competence?

Two of the chapters are published, and two are unpublished but not far from being submission-ready. There is a bit of a tension in Chapter 3: much of the context and justification is in the altitudinal gradient question, yet the effect of altitude turned out to fairly minimal, and the paper focuses on the much stronger effect of lab rearing. In Chapter 4 as well, which uses transplants, the effect of altitude is also minimal. One idea is to discuss the lack of significant altitudinal variation in more depth, bringing in more of the natural history of the hosts and microbes. For example, would we expect local adaptation in these host populations? Do the traps/cages (or natural food substrates), or *Drosophila* behavior, allow for buffering of environmental temperatures? What about the thermal niches of the microbes—are they perhaps able to grow similarly well across the altitudinal gradient? Another suggestion is that Chapter 3 and 4 would be strong as one combined paper, although they could also stand alone.

Lastly, a general and minor recommendation is to explore the older literature in more depth. For example, there is one comment that could be taken to suggest that insect microbiome research began in 25 years ago. Yet it began much earlier, only using microscopy and culturing instead of sequencing. For example, in triatomines, the history of microbial symbiosis research goes back to the 1930s. Likewise, there are relevant studies of fly-microbe interactions from the 1970s and 1980s and probably earlier. These findings are still valuable, and are often complementary to insights gained by sequencing.

Overall, the thesis is an impressive body of work, significantly advancing our understanding of insect microbiomes, and I conclude that the thesis can be accepted without hesitation. I look forward to seeing Joel's future contributions to the field.

Questions

1. Animals can gain new functions through symbiosis, as discussed in the thesis, but they can also do so through horizontal gene transfer (HGT) from microbes. From the host's perspective, what are the costs and benefits of symbiosis versus HGT? What do we know (if anything) about HGT in *Drosophila* and triatomines? And would it be possible to incorporate dispersal of microbial genes, in addition to dispersal of microbial and host organisms, into the metacommunity framework outlined in Chapter 2?

2. A number of environmental and host factors are described that shape variation in microbiome diversity and composition. What do you think is the functional significance of such variation—in other words, how might this variation feed back to affect hosts, or their environments? Is it possible that there is high functional redundancy, or alternatively, that many of the microbes don't do anything functionally relevant to hosts in the first place? If you were to address these questions with future research, how would you go about it?

3. In the work on *Drosophila* and triatomines, bacterial richness is generally quite low—or at least, extremely uneven. Communities are often dominated by just a handful of taxa, or in some cases, one. Why are insect-associated microbiomes (with the notable exception of termites) so much less diverse than human or cow gut microbiomes?

4. The method used to characterize microbiomes in this research involves amplicon sequencing of the 16S rRNA gene, and analysis of 97% operational taxonomic units. How might measuring absolute abundance of microbes change the picture, if at all? For example, would you expect the described changes in microbiome structure over *Triatoma* development, or between lab-reared and wild *Drosophila*, to be paralleled by changes in absolute abundance? Similarly, would you expect greater taxonomic resolution (i.e. beyond 97% OTUs) to change the described patterns at all? What method(s) would you use to achieve greater resolution in future work?

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Opponent Review of the Ph.D. Thesis

Title: Microbiomes in the context of insect communities

Author: Joel J. Brown

Opponent: Pavel Drozd

Topic and objectives

Author research topic is relevant and belongs to one of the current trends in insect ecology. Furthermore, the knowledge about the coevolution of insects and microorganisms and the role of microbiome associations in insect ecology could be useful for many applications (e.g., biological control, epidemiology, pharmacy etc.). In my opinion, the thesis represents a fresh insight into the problematic of insect microbiome from the community level point of view. The aims of the research are clearly formulated with respect to the current state of knowledge.

Structure

The thesis is based on two published papers (review and research article) and two manuscripts (research articles), all with the major contribution of J. J. Brown (first author). Chapter Introduction is well written and describes the structure of the thesis, brings a general overview of the topic and clearly explains the objectives. I appreciate that the author prepared and published the review firstly to create the framework for his consequent research.

Methodology

The thesis is based on the high standards of research design and analyses. The author is familiar with both methodologies of observation studies and experiments, and thus his arguments are well supported by relatively large datasets and sophisticated analyses.

Results and Conclusions

Results are presented in a clear and logical format using appropriate analytical tools.

Comments and questions to the author:

- The author mentioned that life stage is the key factor affecting the microbiome composition. Is there a reverse effect of microbiome on life stage? Are there any tendencies of the microbiome to manipulate with some life history traits, which brings some benefits for the microbiome?
- Hammer et al. (2019) provided cases of wild invertebrates which do not have a resident microbiome, yet their fitness does not appear to suffer. Could you explain the general ecological traits of taxa or guilds for which we can expect the absence of a resident microbiome? Is there any pattern?
- Characteristics of obligate and facultative symbionts are defined from the "host point of view" („*necessary for host survival*"). Do you think that we can also classify obligate symbionts as bacterial symbionts which are not able to survive without the host (it means that the host is necessary for microbe survival)?

- In the review (pg. 31), you mentioned that *Wolbachia* cannot survive outside the host. Does it mean "outside the insects"? Li et al. 2017 (also cited in your thesis) found out that *Wolbachia* can survive in plant leaves about 50 days.
- I would be careful using diversity indices for metabarcoding data. The concept of Shannon diversity is based on the proportion of species, but the real proportion could differ from the proportion of reads which could be affected by the methodology (contrary to using Bray Curtis). Thus, the index could be used only for samples obtained by the same methodology. In my opinion, a better option is to use log of species richness instead of Shannon index, particularly for a high number of OTUs.

Overall assessment

The presented dissertation of Joel Brown definitely fulfils the requirements of the PhD dissertation. The author proved high theoretical and methodological background. He is able to formulate hypotheses, design the research according to the hypotheses, and present the results. I recommend the dissertation to be defended and I have no doubt that the author will successfully defend his work.

In Ostrava May 27th 2021

doc. Mgr. Pavel Drozd, Ph.D.

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