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Dear Professors Hypša and Nováková,

I have reviewed Ms. Rihova's thesis and found that it meets my expectations for a PhD thesis. My review focuses on some bigger picture areas in which Ms. Rihova could improve the thesis through additional synthesis of her findings and placing them in a broader context.

My comment are primarily to do with the introduction and the two in-progress chapters. The two published chapters have gone through peer-review and I do not have much meaningful input. The few things I thought could use some improvement are as follows.

I'd like to see a little more discussion of the expected features of a primary symbiont genome. She leans heavily on genome size and GC content, but there are other features which should at least be mentioned. Some mention of the evolutionary processes that lead to the canonical features of a symbiont genome would demonstrate her understanding of how the larger forces of reduced effective population size, genetic drift, and Muller's ratchet shape the genomes of obligate symbionts. The introductory chapters and material are largely drawn from reviews, and it would be better to cite the primary literature, especially more recent findings from newly analyzed symbiont genomes, including intermediate states of symbiont evolution. I also thought she could expand on the discussion of symbiont replacement in Auchenorrhyncha more, as they provide another example of extensive symbiont replacement.

At the end of the introduction, it would be good for her to speculate on why blood feeding lice have such variable symbionts. What aspect(s) of their physiology or natural history could underly this phenomenon? Are there clues from the available genomes of lice that indicate differences in their digestive system or immune system that may underlie the variation in symbionts? I see this as an exercise in synthesizing what she's done in the broader context of louse symbiosis and biology.

For the second in-progress chapter, I think it's important to explicitly state what criteria she is using to determine if bacteria are secondary symbionts. How can she leverage her data to differentiate between symbiotic bacteria, commensals, or parasitic bacteria?

In the conclusion, it would be useful to have a discussion of the limits of her approaches to studying symbionts: what can we say from genome sequencing, and what can we not? What other approaches could be combined with sequencing to further our understanding of these systems?

To reiterate, I think her thesis is very well written and sufficient for a PhD. I look forward to her defense.

Sincerely,

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