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To whom it may concern:

I write to summarize my assessment of the work and impact that comprises Filip Husník's PhD thesis, from my perspective as his PhD advisor.

When Filip started his work in my lab, in mealybugs, we knew a number of things about sap-feeding insects and their interactions with endosymbiotic bacteria. We knew that these endosymbionts could have very small genomes that encoded few genes. We knew that sap-feeding insects sometimes acquired bacterial genes through horizontal gene transfer (HGT), but the functions of these genes were unclear. We knew that endosymbiont replacement seemed to happen occasionally, but that it was somewhat rare and unknown in mealybugs.

Filip's first project was to explore the role that HGT might be playing in the *P. citri* symbiosis. This was an especially good system to ask this question, because *Tremblaya* had such a small genome. If any endosymbiont needed help from HGTs, it would be *Tremblaya*. Using a combination of transcriptomics and genomics, Filip showed that at least 22 genes of bacterial origin were on the mealybug genome and that these genes were overexpressed in the insect tissue that housed the endosymbionts. Remarkably, he showed that many of these genes seemed to complement genes missing on the endosymbiont genomes. This work was eventually published with Filip as first author (Husník et al., 2013, *Cell* 153:1567-1578), and has had significant impact. As of today, it has been cited 135 times, in part because it was on the vanguard of a series of papers from numerous systems showing that endosymbionts are supported by HGT on host genomes.

Filip's next major contribution from his PhD work involved expanding his studies beyond *P. citri*. Previous work had shown that the intra-*Tremblaya* symbionts might not have been monophyletic (that is, they did not arise from a single infection using single gene trees), but the support values on the trees were always low, making firm conclusions difficult. Filip performed a large genomic survey of five additional mealybug species, completing nine new endosymbiont genomes and five new insect draft genomes. This work showed that the intra-*Tremblaya* endosymbionts had likely been replaced several times over the course of mealybug evolution. But in each case, no matter if the replaced endosymbionts were new or old, they had lost genes that rendered them reliant on both the pre-existing endosymbiont (*Tremblaya*), as well as the HGTs on the insect genome. Filip was also first author on this work (Husník and McCutcheon, 2016, *Proc Natl Acad Sci USA* 113:E5416-E5424), which I think will make a similar impact to his previous paper because he has shown that endosymbiont replacement is possible, even common, in the most intricate and co-dependent endosymbioses.

Overall, Filip's work has helped define the tempo and mechanisms that build endosymbiotic relationships. His work has uncovered parallels between insect endosymbionts and organelles that were largely unknown before his work. Given this, I recommend that Filip's thesis is appropriate to move forward for a PhD defense.

Sincerely,

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