The Mouton et al. study provides a complete picture of the bacterial symbiotic community associated with the *B. tabaci* complex. For this they used a metagenomic approach complemented by a more specific diagnostic on a wider range of samples. The authors point out in particular the presence of *Hemipteriphilus*, a secondary symbiont recently detected in Asian populations and whose biological significance in *B. tabaci* remains unknown. I really enjoyed reading this study. It is very well written and the ideas are clearly established. The weakness of the study is that it is quite descriptive and we are left wanting more. But it has the merit of being technically sound and of establishing new perspectives. I have no major comments to make and I find the study suitable for publication. I do, however, have some minor comments and suggestions.

I find it inappropriate to see in the same idea "facultative" symbiont (L61) and "co-obligate" symbiont ("S-symbionts that complement the metabolic network" L64). For me it is not the same "symbiotic object", not the same evolutionary history. Or it should be clearly mentioned that there are secondary or additional symbionts in *Bemisia* and that some are co-obligate partners. But don't use the term facultative (co-obligate symbionts are additional but not facultative in essence). The terms "secondary" and "facultative" are often used as synonyms. But in my opinion, it would be more relevant to use "secondary" for "additional" and "facultative" when these additional symbionts are not mandatory for the development of the host.

Concerning *Hemipteriphilus*, I think it is a pity that the authors did not try to determine the tissue and cellular tropism of this symbiont. Indeed, since the biological significance of the presence of this bacterium is still unknown in *Bemisia*, determining whether it is present in bacteriocytes and its location in relation to the obligate symbiont *Portiera* could provide valuable information on its status. Indeed, several studies show that in di-symbiotic systems where symbionts complement each other metabolically, the bacteria show a very close physical proximity in the bacteriome with highly nested bacteriocytes [1–7] (and sometimes a nested location with a symbiont living in another symbiont [8]). In my opinion the *Bemisia* model is super interesting to tackle these developmental aspects. If the authors still have samples of whole individuals, it would be worthwhile to examine them (at least for the MED-Q1 biotype).

A comment for further works: I also find that analyzing the density of the obligate symbiont in relation to the presence of additional symbionts is a very good idea. This is an aspect that is missing in many studies and that allows to eventually show the impact of the additional symbiont on the ancestral primary symbiont. Such data along with a fine analysis of tissue tropism by FISH during host development could shed light on many aspects in these multipartner interactions.

L285: delete "clearly": the word is already used L278.

L313: "Mediterranean" and not "Meditteranean"

The authors used Greengenes. I don't have a problem with that (but is that database still updated?). Otherwise there are other databases like kraken2 and SILVA.

I would have appreciated a presentation of the metagenomic results such as a table summarizing the sequencing data, the taxonomic assignment of the identified bacteria, a graph showing the proportion of reads for each identified taxa, the relative abundance of bacterial taxa in the form of a heat map. I think this data can be put in Supplemental Material (which I don't see here).

References

- 1. Wu D, Daugherty SC, Aken SEV, et al (2006) Metabolic Complementarity and Genomics of the Dual Bacterial Symbiosis of Sharpshooters. PLOS Biol 4:e188. https://doi.org/10.1371/journal.pbio.0040188
- 2. Mao M, Yang X, Poff K, Bennett G (2017) Comparative Genomics of the Dual-Obligate Symbionts from the Treehopper, Entylia carinata (Hemiptera: Membracidae), Provide Insight into the Origins and Evolution of an Ancient Symbiosis. Genome Biol Evol 9:1803–1815. https://doi.org/10.1093/gbe/evx134
- 3. Manzano-Marín A, Szabó G, Simon J-C, et al (2017) Happens in the best of subfamilies: establishment and repeated replacements of co-obligate secondary endosymbionts within Lachninae aphids. Environ Microbiol 19:393–408. https://doi.org/10.1111/1462-2920.13633
- 4. Manzano-Marín A, D'acier AC, Clamens A-L, et al (2020) Serial horizontal transfer of vitamin-biosynthetic genes enables the establishment of new nutritional symbionts in aphids' di-symbiotic systems. ISME J 14:259–273. https://doi.org/10.1038/s41396-019-0533-6
- 5. Koga R, Bennett GM, Cryan JR, Moran NA (2013) Evolutionary replacement of obligate symbionts in an ancient and diverse insect lineage. Environ Microbiol 15:2073–2081. https://doi.org/10.1111/1462-2920.12121
- 6. Bennett GM, Moran NA (2013) Small, Smaller, Smallest: The Origins and Evolution of Ancient Dual Symbioses in a Phloem-Feeding Insect. Genome Biol Evol 5:1675–1688. https://doi.org/10.1093/gbe/evt118
- 7. Van Leuven JT, Meister RC, Simon C, McCutcheon JP (2014) Sympatric speciation in a bacterial endosymbiont results in two genomes with the functionality of one. Cell 158:1270–1280. https://doi.org/10.1016/j.cell.2014.07.047
- 8. Husnik F, McCutcheon JP (2016) Repeated replacement of an intrabacterial symbiont in the tripartite nested mealybug symbiosis. Proc Natl Acad Sci 113:E5416–E5424. https://doi.org/10.1073/pnas.1603910113