Peer Community In
Zoology

Cross-continents whitefly secondary symbiont revealed by metabarcoding

Yuval Gottlieb *based on peer reviews by* ***François Renoz***, ***Vincent Hervé*** *and 1 anonymous reviewer*

Laurence Mouton, Helene Henri, Rahim Romba, Zainab Belgaidi, Olivier Gnankine, Fabrice Vavre (2022) Analyses of symbiotic bacterial communities in the plant pest Bemisia tabaci reveal high prevalence of Candidatus Hemipteriphilus asiaticus on the African continent. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Zoology. https://doi.org/10.1101/2021.10.06.463217

Submitted: 11 October 2021, Recommended: 10 March 2022

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Whiteflies are serious global pests that feed on phloem sap of many agricultural crop plants. Like other phloem feeders, whiteflies rely on a primary-symbiont to supply their poor, sugar-based diet. Over time, the genomes of primary-symbionts become degraded, and they are either been replaced or complemented by co-hosted secondary-symbionts (McCutcheon and Moran 2012). In *Bemisia tabaci* species complex, the primary-symbiont is *Candidatus* Portiera aleyrodidarium, with seven secondary-symbionts that have been described to date. The prevalence and dynamics of these secondary-symbionts have been studied in various whitefly populations and genetic groups around the world, and certain combinations are determined under specific biotic and environmental factors (Zchori-Fein et al. 2014).

To understand the potential metabolic or other interactions of various secondary-symbionts with *Ca*. Portiera aleyrodidarium and the hosts, Mouton et al. used metabarcoding approach and diagnostic PCR confirmation, to describe symbiont compositions in a collection of whiteflies from eight populations with four genetic groups in Burkina Faso. They found that one of the previously recorded secondary-symbiont from Asian whitefly populations, *Candidatus* Hemipteriphilus asiaticus, is also found in the tested African whiteflies. The newly identified *Ca*. Hemipteriphilus asiaticus forms a different strain than the ones described in Asia, and is found in high prevalence in six of the tested populations and in three genetic groups. They also showed that Portiera densities are not affected by the presence of *Ca*. Hemipteriphilus asiaticus. The authors suggest that based on its high prevalence, *Ca*. Hemipteriphilus asiaticus may benefit certain whitefly populations, however, there is no attempt to test this assumption or to relate it to environmental factors, or to identify the source of introduction.

Mouton et al. bring new perspectives to the study of complex hemipteran symbioses, emphasizing the need to use both unbiased approaches such as metabarcoding, together with a priori methods such as PCR, in order to receive a complete description of symbiont population structures. Their findings are awaiting future screens for this secondary-symbiont, as well as its functional genomics and experimental manipulations to clarify its role. Discoveries on whitefly-symbionts delicate interactions are required to develop alternative control strategies for this worldly devastating pest.

References:

McCutcheon JP, Moran NA (2012) Extreme genome reduction in symbiotic bacteria. Nature Reviews Microbiology, 10, 13–26. https://doi.org/10.1038/nrmicro2670

Mouton L, Henri H, Romba R, Belgaidi Z, Gnankiné O, Vavre F (2022) Analyses of symbiotic bacterial communities in the plant pest *Bemisia tabaci* reveal high prevalence of *Candidatus* Hemipteriphilus asiaticus on the African continent. bioRxiv, 2021.10.06.463217, ver. 3 peer-reviewed and recommended by Peer Community in Zoology. https://doi.org/10.1101/2021.10.06.463217

Zchori-Fein E, Lahav T, Freilich S (2014) Variations in the identity and complexity of endosymbiont combinations in whitefly hosts. Frontiers in Microbiology, 5. https://doi.org/10.3389/fmicb.2014.00310

Reviews

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2021.10.06.463217
Version of the preprint: 1

Authors' reply, 11 February 2022

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Decision by Yuval Gottlieb, posted 11 December 2021

Asking for a revision for your preprint

Dear Laurence Mouton,
Your preprint has been reviewed by three reviewers.

We all agree that your findings regarding the under studied symbiont *Ca*. Hemipteriphilus asiaticus of *B. tabaci* in Africa, are interesting and important. They bring new perspectives to the study of hemipteran symbiosis, both methodology- and conceptually wise. However, it needs some improvement, both in analysis and in formatting before it can be recommended further.

Here are the main comments that need your attention:

1. Correction of the terminology of the method and the nomenclature of the symbionts.
2. Better defining of your sample material, and the sample size.
3. Reanalysis/classification of the data according to SILVA and the primers used.
4. Deposit the sequencing data in an accessible and accepted format (supplementary and SRA).
5. Improving the phylogeny and the symbiont consortium figures.

Additionally, please see all of the reviewers' comments and address them accordingly.

Looking forward seeing your revisions on the second round.

Sincerely,

Yuval

Reviewed by **François Renoz**, 10 December 2021

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Reviewed by **Vincent Hervé**, 07 December 2021

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Reviewed by anonymous reviewer 1, 08 December 2021

The manuscript by Mouton and colleagues explore the intracellular symbiotic community associated with Bemisia tabaci populations from Burkina Faso. The manuscript is well written clearly. Although the topic is very interesting, the study presents a few weaknesses that should be addressed before the manuscript is suitable for publication.

General comments:

The authors should use metabarcoding instead of metagenomics to avoid confusion.

Introduction:

The terms Primary symbiont (P-symbiont) and Secondary symbionts (S-symbiont) are more used with the meaning first and second than with their true meaning essential and not essential. This is particularly evident when the authors present nutritional symbiosis in cicadas and other auchenorrhyncha where the two symbionts (i.e. Sulcia and the co-symbiont) are both considered primary symbionts and are named co-primary symbionts. The authors should revise the introduction and the abstract to reflect that the terms primary and secondary are related to essentiality.

The description of the characteristics of symbiosis in the introduction is specific to hemipteran. For instance, not all primary symbionts and intracellular (e.g., *Ishikawaella*, *Tachikawaea gelatinosa*; see lines 48-49). Moreover, not all endosymbionts have an extremely reduced genome (e.g., Sodalis pierantonius, Sodalis-like symbiont of spittlebugs). The author could generalize their introduction or specify that they are talking about symbioses in hemipterans.

Results

In the section Bacterial community characterization, the authors should add a figure to summarise the bacterial diversity associated with the different samples. They should also report within the paragraph how many samples were analyzed and the standard error associated with the average number of reads (line 120) since results are reported before the material and methods.

Lines 138-139: The authors should reference table 2 when mentioning the newly designed primers for the first time

Discussion

Line 224: replace ”Since” with ”Since then”

Materials and Methods

In the section ”Sampling”, it is unclear how the DNA extraction was carried out or if the DNA was already extracted during the work reported in Henri et al. If the DNA was extracted for the current work, the authors should report the extraction protocol.

Line 308: Figure 1 should be Figure 2

In the section ”Characterization of the bacterial community”, the first paragraph should be split in two, separating the qPCR part from the metabarcoding part. In addition, the authors should also report exactly how many samples were analyzed using the metabarcoding approach and if the samples were pooled or not.

Line 375: ”Three hundred four” should be corrected to ”Three hundred and four”

The authors report some specific statistical analysis for which I do not have a sufficient background to comment on.