Response to Reviewers

The authors express their sincere gratitude to the reviewers for their insightful and constructive comments, which have significantly enhanced the manuscript. Authors respond to each comment (in the red font, pls see below) and have made tracked changes to the script.

Reviewer Comments:

Reviewer 2

a. Title (Lines 1–3): The title is long and does not clearly highlight novelty. I would include a clearer novelty focus, for example: 'highlighting its role in virus transmission' to make the title more impactful.
Ans. We appreciate the reviewer's suggestion regarding improving the title. The authors carefully evaluated several alternative titles incorporating explicit novelty elements (e.g., emphasizing virus transmission). However, doing so resulted in overly long or overly specific titles that compromised clarity and overall balance. Therefore, we opted to retain the current title, which concisely captures the core scope (prevalence and distribution) and the key finding (dominance of Arsenophonus in Asia II-1) without overextending its focus. We believe this represents a balanced and accurate reflection of the study.

b. Abstract (Lines 16–42): No clear statement of novelty, and I believe it overclaims Arsenophonus' role. I would include a clear novelty line, like: 'This is the first study linking symbiont distribution with cryptic species in Pakistan. I would also write more cautiously: 'suggesting a potential role' instead of direct claims about virus transmission.

Ans. We thank the reviewer for this constructive suggestion. We would like to clarify that the original abstract did not intend to overstate the role of Arsenophonus, but rather to relate its high prevalence to its previously established association with virus transmission. Nevertheless, to improve clarity and avoid any perception of overclaiming, we have revised the wording in the abstract. Please see Line 56 and 58 in the revised script.

c. Lines 53–57: Weak problem statement for Pakistan. That should be like this: "There is limited data linking symbionts and virus dynamics in Pakistan."
Ans. Authors have revised the text to address the comment. Please see Line 72-78 of the revised script.

d. Lines 63–66: No clear research gap explanation. I would suggest you write something like: 'Despite known roles of symbionts, their distribution and epidemiological role in Pakistan remain unclear.'

Ans. Authors appreciate this comment. However, instead of writing this in the above-mentioned lines, we have already mentioned it at Lines 99-102 of the initially submitted manuscript. In the revised script, it can be found at Lines 126-133.

e. Lines 97–102: No clear hypothesis or research question. Please include a hypothesis in a measurable or directional way, like: 'We hypothesize that Arsenophonus dominance is linked with Asia II-1 and virus spread.'
Ans. Authors addressed this comment by modifying the last paragraph of the Introduction section. Please see Lines 126-133 in the revised script.

f. Lines 110–112: It is an old sampling (2012–2014) and is not justified. Please add the justification. Do these data provide baseline information for long-term trends? Do they take this long to analyze after data collection? Or any other reason?
Ans. We thank the reviewer for this observation. Although sampling was conducted in 2012–2014, we retained these data because they provide a critical historical baseline for *Bemisia tabaci* cryptic species and their secondary endosymbionts in Pakistan during the peak cotton leaf curl disease epidemic. This pre-2015 dataset was collected prior to the documented northward expansion of MEAM1 and major shifts in insecticide use, and is therefore essential for future longitudinal studies tracking temporal changes in vector–symbiont prevalence.
The interval between collection and publication reflects the scale of the work. Additionally, progress was further delayed by the retirement of Supervisor, Dr Rob Briddon, and funding constraints to publish this work.

g. Lines 131–139: Is there any limitation of the PCR method? I bet there are many limitations. PCR may miss low-level infections; advanced methods could improve detection. Yes?
Ans. PCR remains foundational but is insufficient for low-titer or complex infections. For higher sensitivity and comprehensive detection, metagenomics or whole-genome sequencing can capture co-infections and novel variants that targeted PCR entirely misses. However, for large-scale diversity screening across numerous samples, conventional PCR remains cost-effective and widely adopted for endosymbiont detection. Metagenomics or whole-genome sequencing may be prioritized in future studies where resources permit.

h. Lines 161–168: It is better to include details such as test values and degrees of freedom for clarity.
Ans. Degree of freedom (k-1) has been mentioned in the revised script at Line 202.

i. Lines 169–172: I believe it is a weak method (Pearson correlation between binary data). Justify it. This method may not fully represent ecological interactions. Don't we want to see interactions?

Ans. Authors agreed to the reviewer that Pearson correlation is inappropriate to infer ecological interactions. But appropriate for mapping co-occurrence patterns across host genetic backgrounds and geography. Our results "Network edges" represent statistical co-occurrence (Pearson/φ correlation) and do not imply ecological interaction.

j. Lines 24–25 vs 176–178: I find an inconsistency in the number of species (3 vs 6). Is it like six species were identified, but only the major ones were analyzed further?
Ans. Reviewer made a highly useful query. As two species, Asia II-5 and Asia II-7, didn't yield PCR amplicon, so this study included four species in the subsequent analyses. Please see Lines 42 and 218-220 in the revised script.

k. Lines 185–186: Sample imbalance (very few Asia I samples). Any justifications? I would be very cautious because an unequal sample size may affect comparisons.
Ans. The authors thank the reviewer for this observation. We agree that the sample size for Asia I is smaller compared to the other cryptic species. However, it is important to clarify that this study was designed as a field-based survey intended to capture the natural distribution of *B. tabaci* populations, rather than as a balanced experimental comparison. Consequently, the sampling was constrained by the actual availability and prevalence of the cryptic species within the surveyed regions.
We are mindful that unequal sample sizes can affect statistical comparisons; therefore, we have interpreted the data for the less frequent species with caution and have avoided over-generalizing the results for Asia I.

l. Lines 194–197: Exclusion of species is not properly explained. Were those excluded due to low sample size or no amplification?
Ans. This has been clarified in the revised version of the script. Please see Lines 219-220.

m. Lines 230–231: Only p-values given, no full stats. I always prefer full statistical details (f, df, p value), which also increase transparency. Journals may have different rules.
Ans. Thank you for this valuable feedback. We completely agree that reporting full statistical details—F-values, degrees of freedom (df), and exact p-values—is essential for transparency, reproducibility, and scientific rigor. However, this analysis was conducted long ago, and we failed to retrieve/recall the data. We recognize this as a limitation and fully acknowledge that journal requirements and reviewer expectations increasingly favor comprehensive statistical reporting. Authors will keep this point in their mind for the next manuscripts.

n. Lines 251–256: We most of the time think correlation is causation or overinterpret correlations as biological interaction. Please present this as a co-occurrence rather than direct interaction.
Ans. Authors agreed to the reviewer and made modification in the paragraph. Please see lines 306 and 309 in the revised script.

o. Lines 329–333: I believe Arsenophonus' role is overstated. I would tone it down. It may just contribute to transmission rather than to the strong claims your paper makes
Ans. We appreciate the reviewer's perspective regarding the emphasis placed on *Arsenophonus*. However, we would like to clarify that our discussion of its role is not intended to overstate its importance, but rather to interpret our findings in the context of established literature.

p. Lines 295–300: Weak explanation for low Rickettsia. I would include environmental or ecological reasons behind this.
Ans. Authors added ecological and environmental reasoning to the paragraph. Please see Lines 352-359 of the revised script.

q. Lines 360–363: Can we go over ecological context (climate, cropping, and pesticides)? Do environmental factors influence symbiont distribution?
Ans. Authors revised the discussion section as per suggestion. Please see lines 440--449.

r. For the entire Discussion section. I would include a paragraph in which the paper describes the limitations, obstacles, or factors that might affect the experiment. For example, the study is limited by an old sample, the use of PCR, and a lack of functional validation.
Ans. We thank the reviewer for this valuable suggestion. Rather than introducing a separate paragraph, the limitations of the study have been addressed at relevant points within the Discussion to maintain narrative coherence. We believe this integrated approach avoids redundancy while clearly conveying the study limitations.

s. Conclusion (Lines 369–374): Are we too general? And slightly overclaiming? I would probably write like this: 'These findings provide a baseline and require further functional studies.'
Ans, Change has been made. Lines 458-463.