Characterization of microbiome diversity in mosquito species and *Wolbachia* infections across *Culex* species in Wooster, Ohio

Tisa Tuladhar, and Dr. Ferdinand Nanfack Minkeu; Department of Biochemistry and Molecular Biology, The College of Wooster, Ohio

Abstract

Mosquitoes are major vectors of diseases like West Nile virus. Their microbiomes, especially *Wolbachia*, influence disease transmission. This study characterized *Wolbachia* infections in *Culex* mosquitoes from Wooster, Ohio, using qPCR, strain-specific PCR, and sequencing. Results showed 100% infection rate with *Wolbachia*, with most mosquitoes displaying moderate to high infection intensity. The *wAlbB* strain was most prevalent (80%), while *wAlbA* occurred in 40% of samples and 35% had dual infections. Phylogenetic analysis linked local strains to those in *Aedes albopictus*, suggesting cross-species conservation. Microbiome analysis revealed dominance by *Pseudomonas* and *Bacteroides*, and a large portion of unclassified taxa. These findings support the potential for *Wolbachia*-based vector control and emphasize the need for further studies on interactions with pathogens like West Nile virus.

Background and Significance

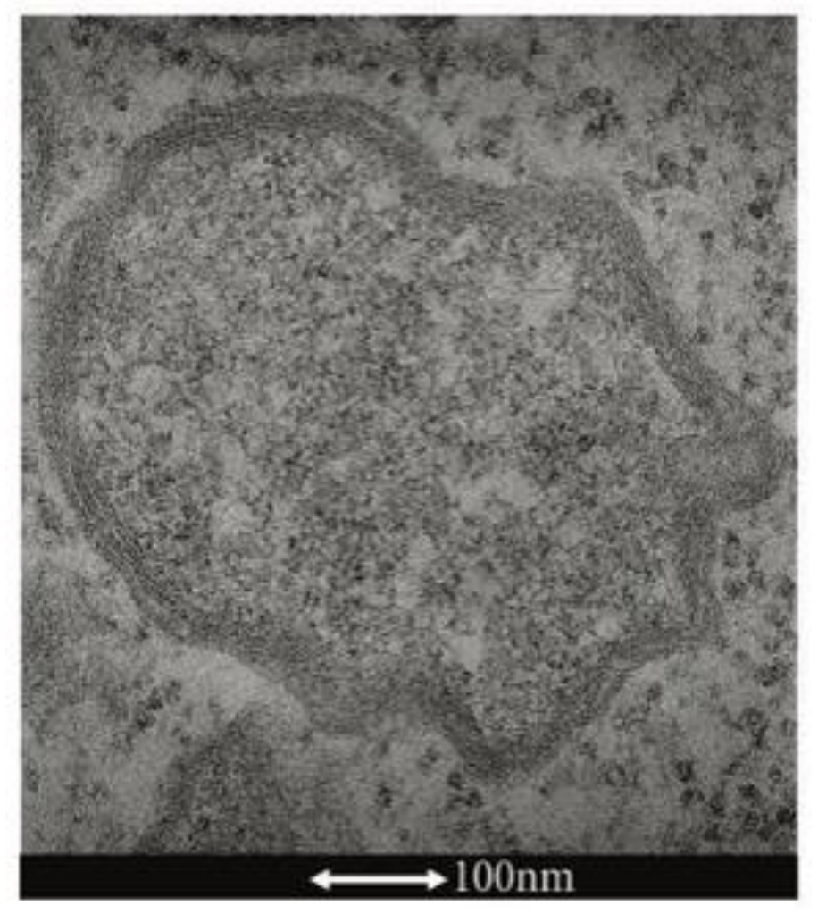

Figure 1: Microscopic image of *Wolbachia*

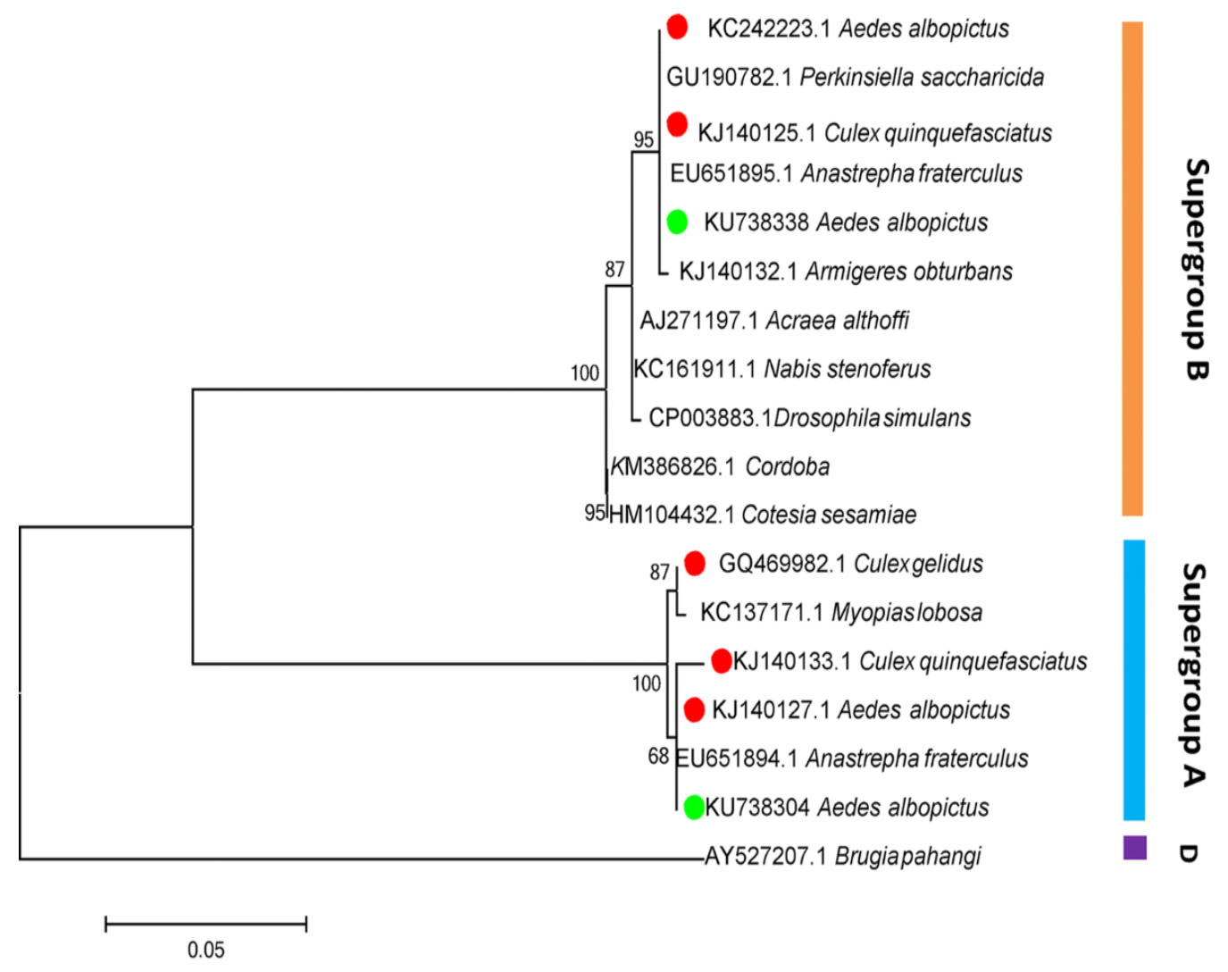

Figure 2: Phylogenetic relationships of *Wolbachia* strains infecting diverse insect hosts.

- The microbiome of mosquitoes, especially their gut bacteria, plays a vital role in influencing their ability to transmit pathogens. Certain bacteria like *Serratia* can enhance virus replication, while others inhibit it.[1]
- *Wolbachia* strains vary across species and environments. Some strains (*wAlbA*, *wAlbB*) can co-infect a single mosquito and have different effects on reproduction and immunity.[2,3]

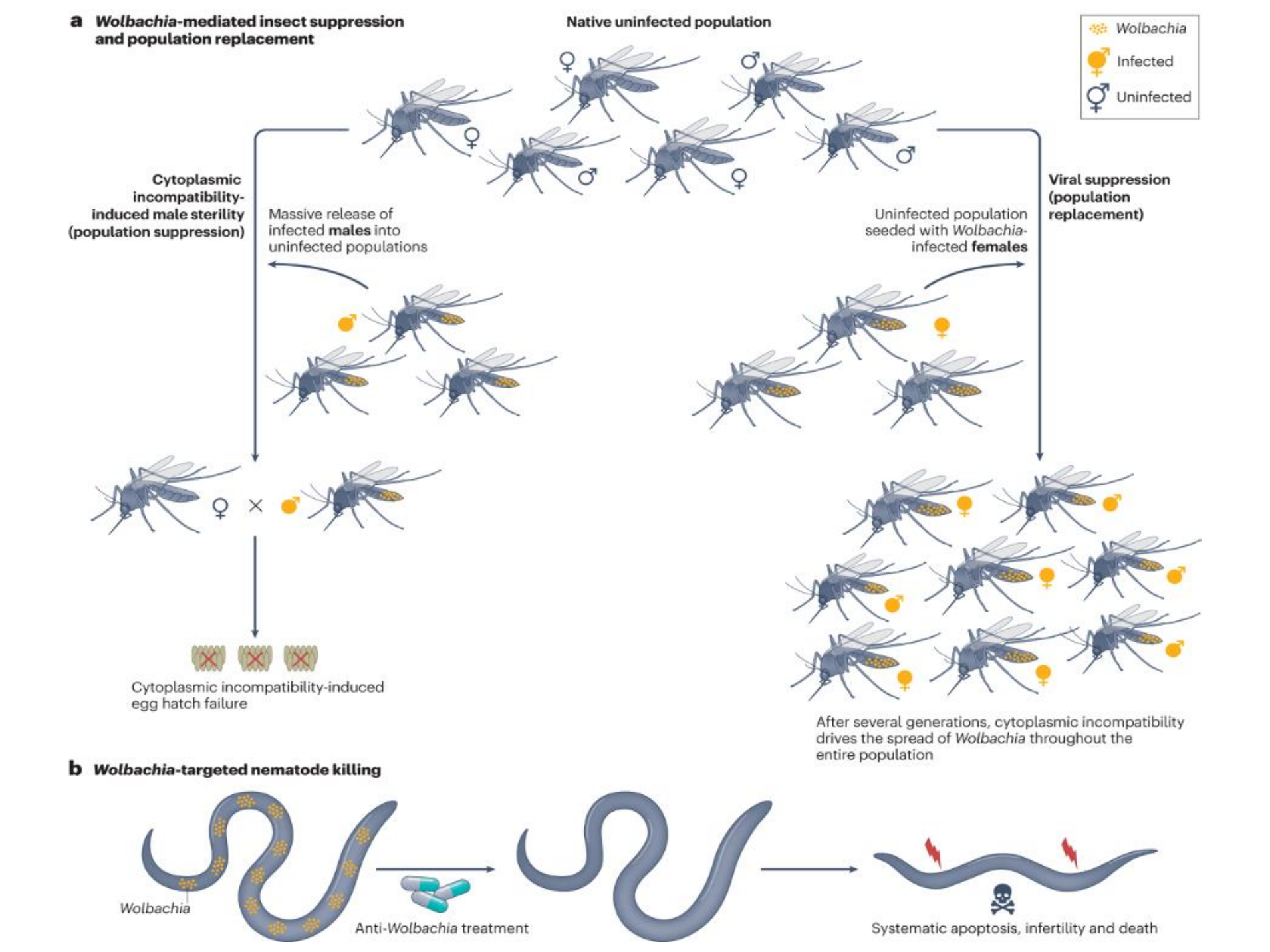

Figure 3: Potential application of *Wolbachia* in insect pest control: (a) Insect suppression through cytoplasmic incompatibility and (b) nematode killing via anti-*Wolbachia* treatment.

Hypothesis & Research Objectives

- Quantify *Wolbachia* infection intensity in Culex mosquito populations.
- Determine the abundance of different *Wolbachia* strains (*wsp*, *wAlbA*, and *wAlbB*) in Culex mosquitoes.
- Analyze the genetic diversity and phylogenetic relationships of *Wolbachia* strains.

Hypothesis: Culex mosquitoes from Wooster, Ohio, will exhibit moderate *Wolbachia* infection intensity and reduced microbiome diversity, with strain-specific variations in *Wolbachia* prevalence and genetic diversity.

Methods

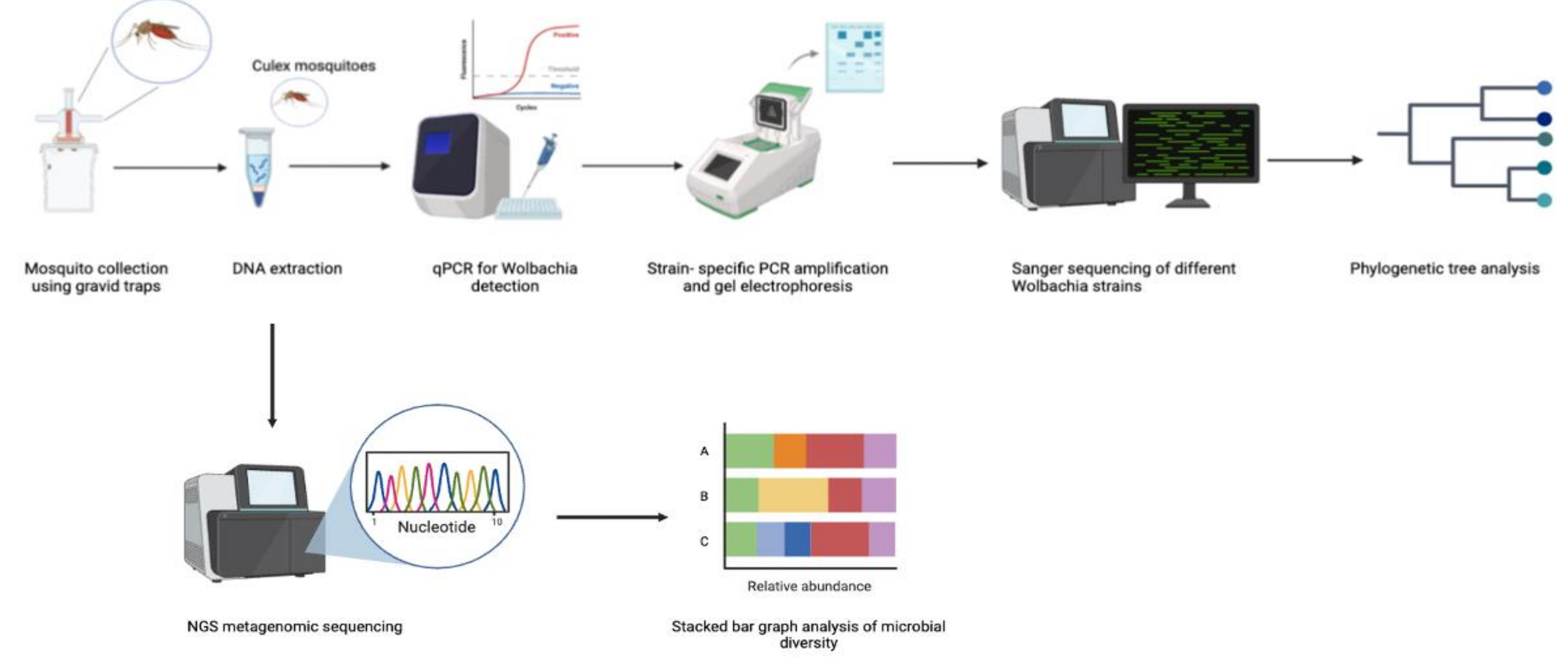

Figure 4: Workflow for *Wolbachia* strain characterization and microbial diversity analysis in *Culex* mosquitoes.

Results

Variation of *Wolbachia* Loads

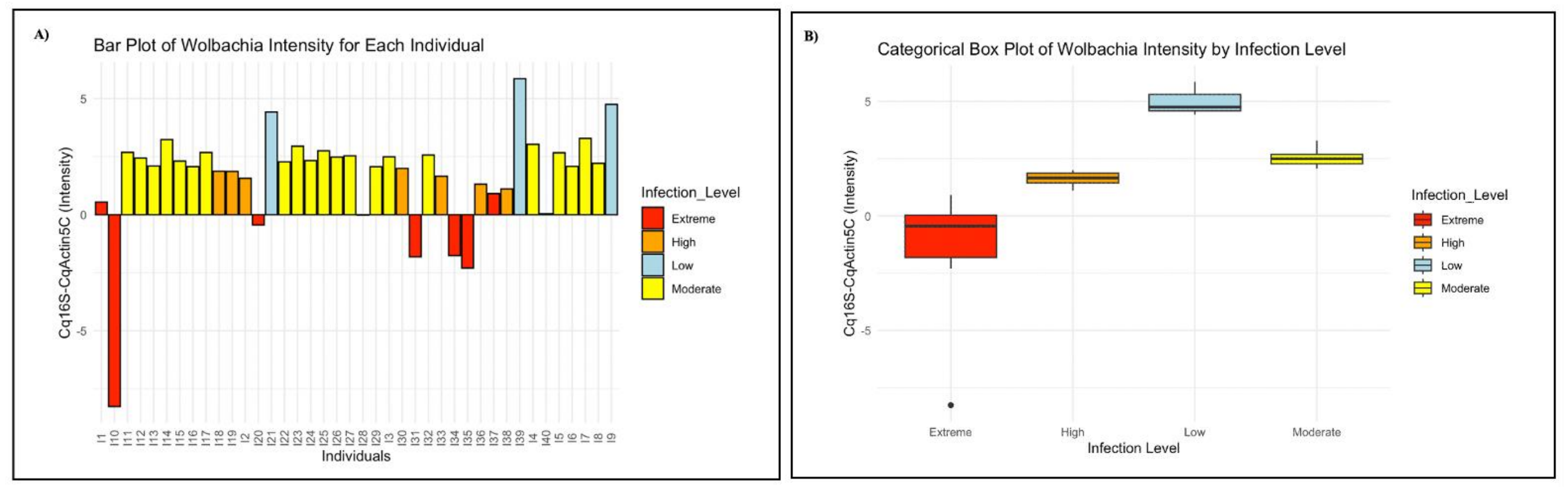

Figure 5: Level of intensity of Wolbachia infections. A) Bar plot showing the level of intensity of Wolbachia infections i.e. ΔCq (Cq16S-CqActin5C) in each sample. **B)** Box plot plot showing the level of intensity of Wolbachia infections i.e. ΔCq (Cq16S-CqActin5C) in each sample. This plot groups individuals into categories based on their Wolbachia infection levels (Low, Moderate, High, Extreme). It shows the spread and variability of infection intensities within each category.

- All *Culex* mosquitoes are infected with *Wolbachia*.
- Most mosquitoes show moderate levels of infection.

Genetic diversity and phylogenetic relationship of *Wolbachia* strains

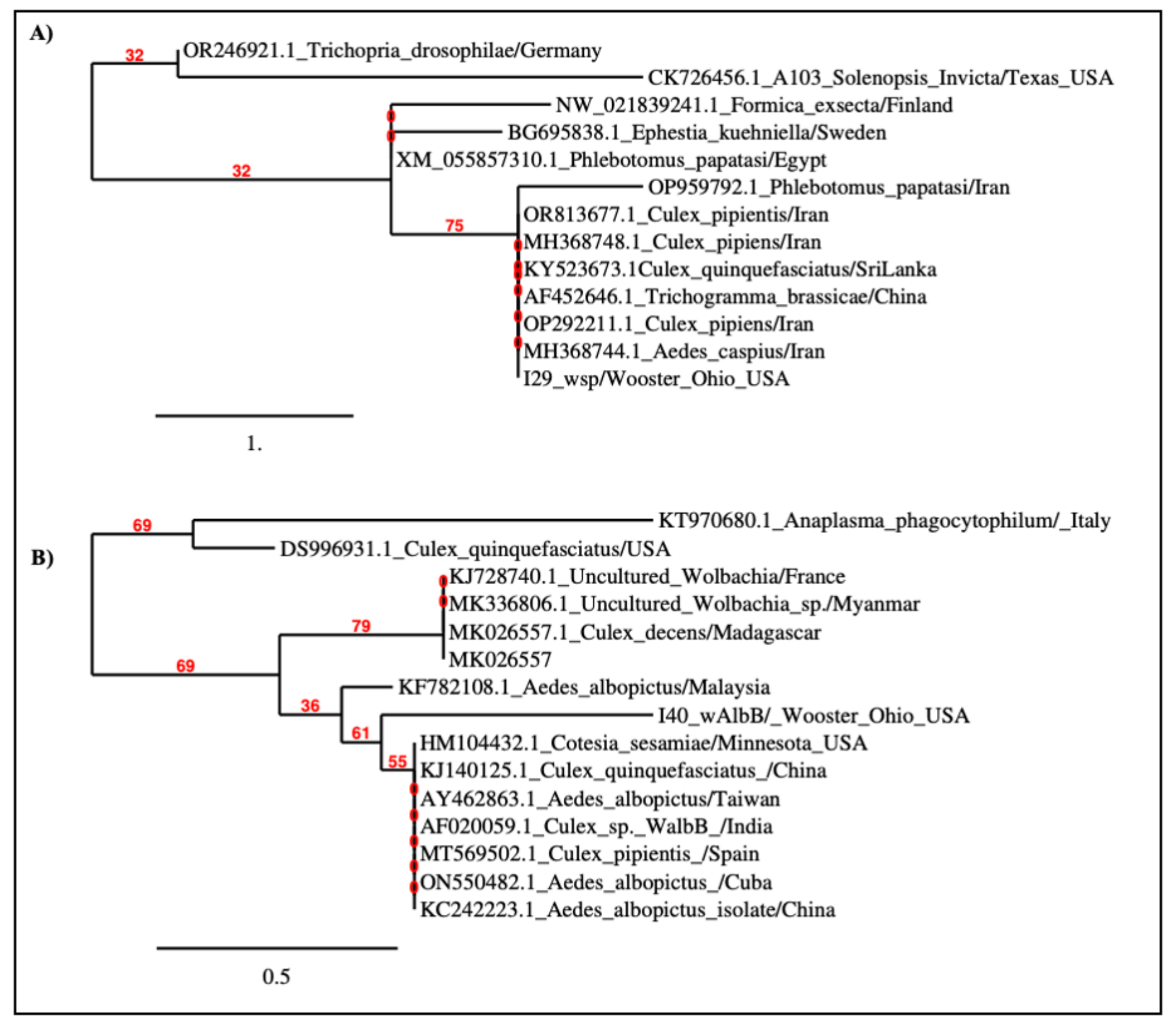

Figure 6: Phylogenetic analysis of *Wolbachia* strains based on *wsp* and *wAlbB* sequences. **(A)** Maximum Likelihood phylogenetic tree of *wsp* sequences, shows I29_*wsp/*Wooster_Ohio_USA clusters with *Wolbachia* strains from *Culex pipiens* and *Aedes caspius*, suggesting a close evolutionary relationship. Bootstrap values are indicated in red. **(B)** Maximum Likelihood phylogenetic tree of *wAlbB* sequences, indicating its placement within *Wolbachia* supergroup B. The sample I40_*wAlbB/*Wooster_Ohio_USA clusters with *Wolbachia* strains from *Aedes albopictus* collected from China, Cuba, Malaysia, and Taiwan, emphasizing the geographical relatedness of these strains. Bootstrap support values are displayed in red. The scale bars indicate genetic distance.

- Sequencing revealed the presence of diverse strains, including wAlbA and wAlbB, in *Culex* mosquitoes.
- Strains grouped into separate clades, indicating varied evolutionary origins and host associations.

Wolbachia abundance in *Culex* Mosquitoes

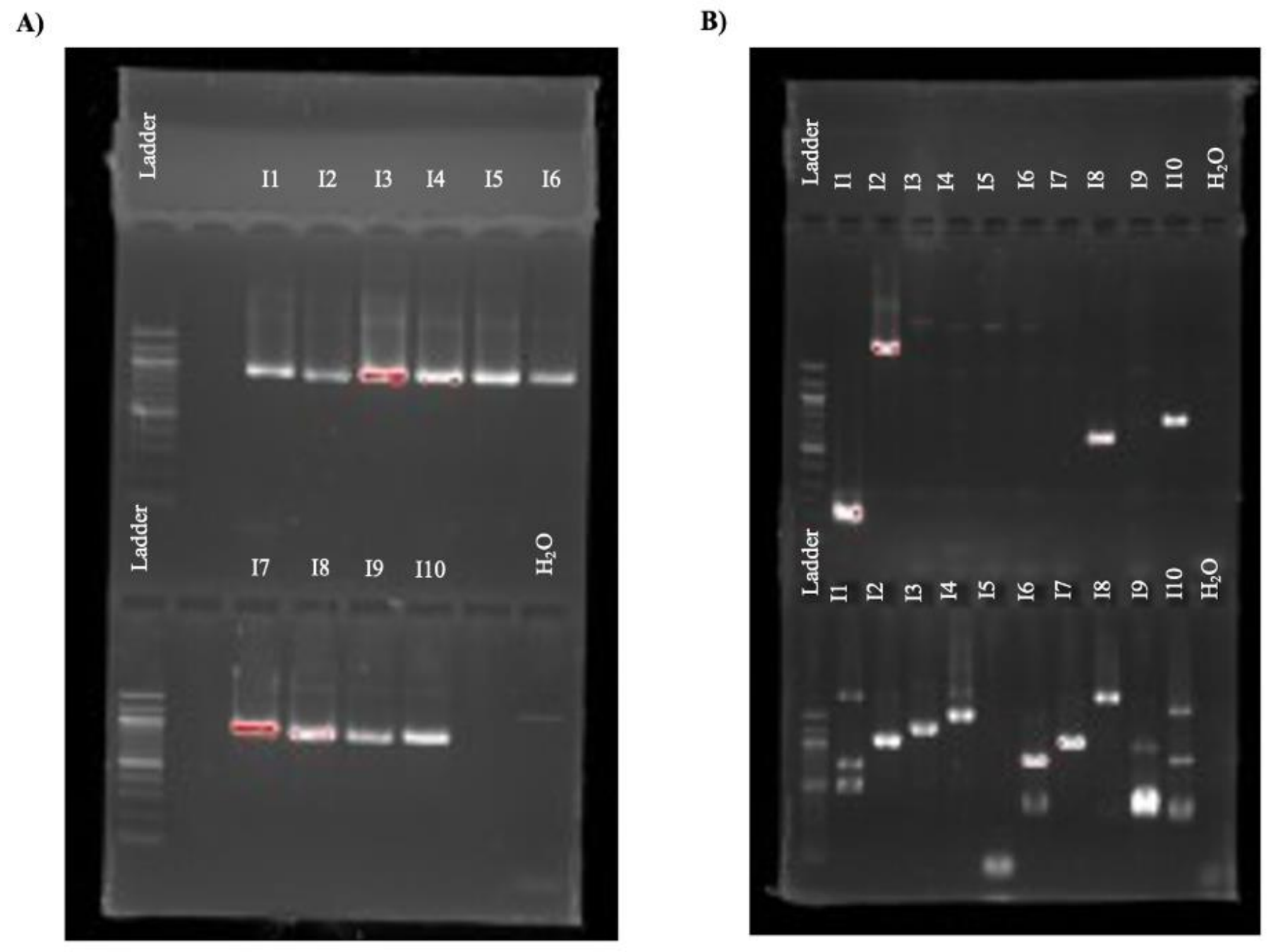

Figure 7: Gel electrophoresis of *wsp* **(A)**, *wAlbA*, and *wAlbB* **(B)** gene amplification products from *Culex* mosquito samples.

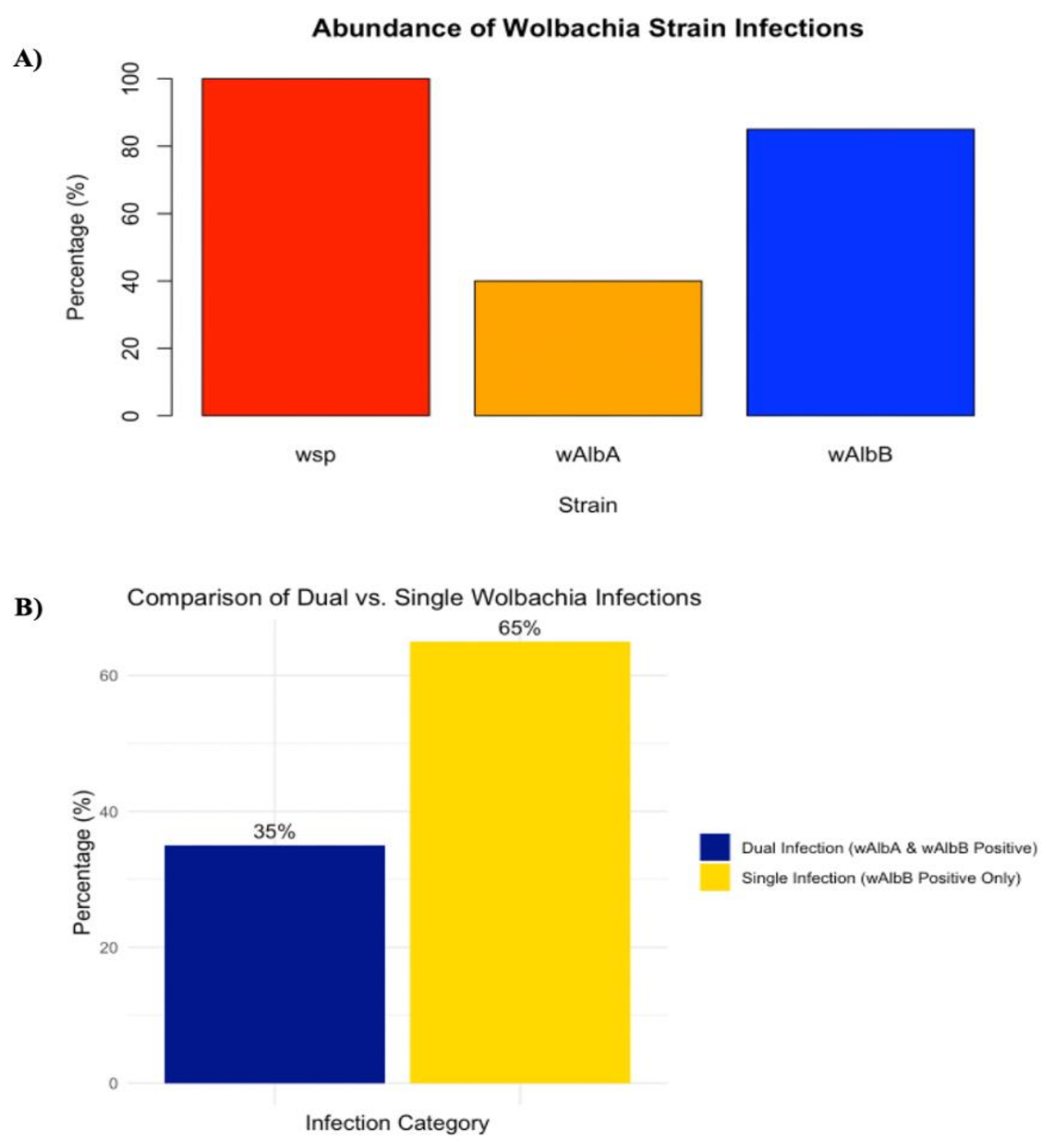

Figure 8: Gel electrophoresis of *wsp* **(A)**, *wAlbA*, and *wAlbB* **(B)** gene amplification products from *Culex* mosquito samples.

- A majority of *Culex* individuals tested positive, suggesting stable natural infections.
- qPCR results showed differing levels of *Wolbachia*, with some mosquitoes carrying significantly higher loads.

Abundance of Microbial Diversity

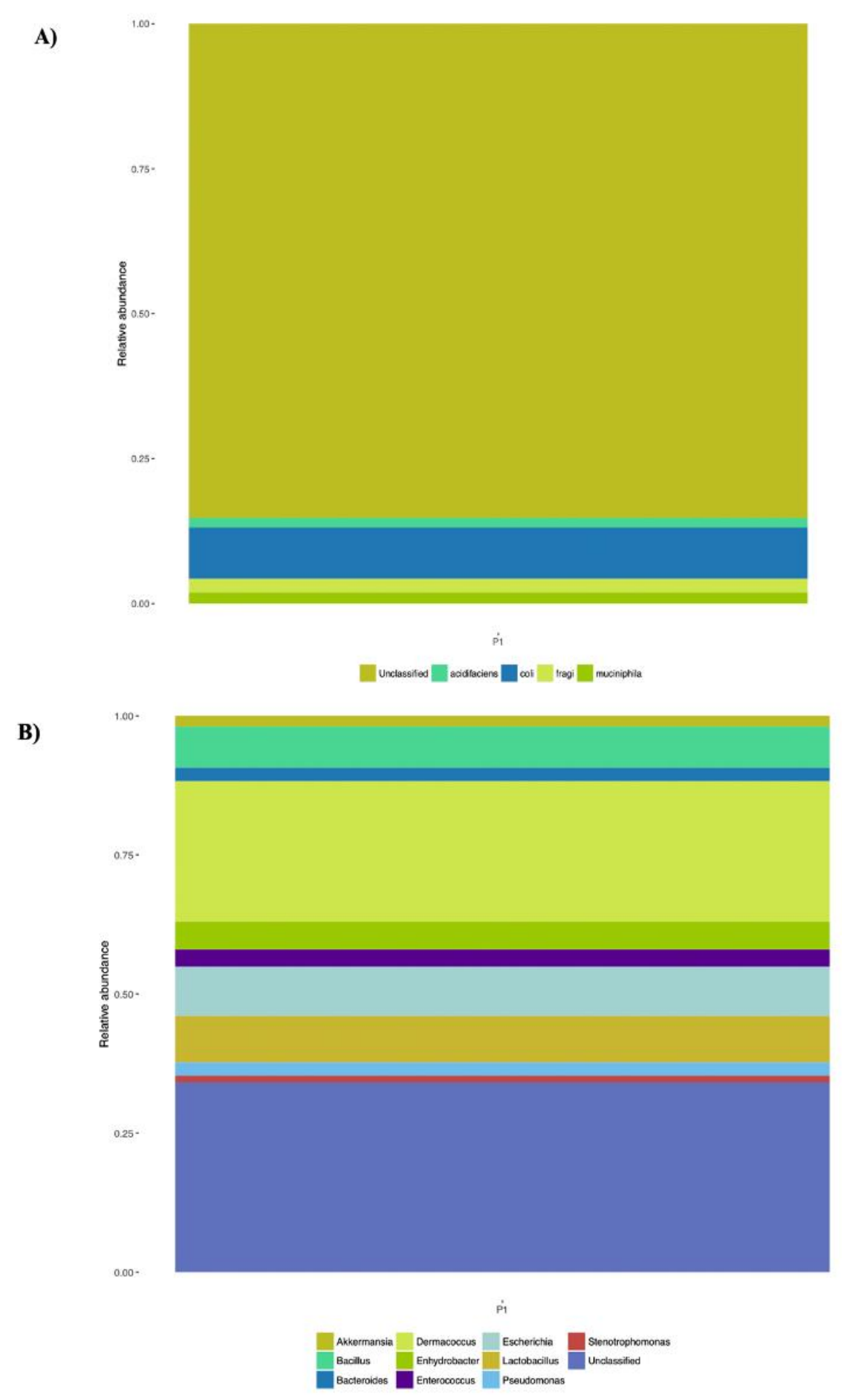

Figure 9: Stacked bar chart of microbial abundances at A) species level and **B)** genus level, calculated as probability of total 16S rRNA reads within each group.

- Genera such as *Serratia*, *Pseudomonas*, and *Enterobacter* were dominant microbiome.
- Lack of Wolbachia detected.

Conclusion

- *Wolbachia* is a stable and widespread symbiont in *Culex* populations in Wooster, Ohio, hence it is an effective option for local biocontrol strategies.
- The dominance of the *wAlbB* strain suggests it may offer a reproductive advantage, influencing long-term population dynamics.[4]
- Differences between lab-reared and field mosquitoes show the importance of natural environments in maintaining microbial diversity and symbiont transmission.[5]
- This study adds to the evidence that using *Wolbachia* and the mosquito microbiome might play an important part in reducing mosquito-borne disease transmission.

Future Research

- Investigate if *Wolbachia*-infected *Culex* mosquitoes reduce West Nile virus transmission.
- Study how seasonal and environmental changes affect infection rates and microbiome diversity.
- Explore interactions between *Wolbachia* and key microbes like *Pseudomonas* and *Bacteroides*.
- Assess how dual infections (*wAlbA + wAlbB*) influence mosquito fitness and reproduction.
- Study how different *Wolbachia* strains and bacterial densities impact mosquito reproduction and pathogen-blocking ability.
- Examine whether *Wolbachia* impacts insecticide resistance in *Culex* populations.

Acknowledgements

- I would like to thank my advisor, Dr. Ferdinand Nanfack Minkeu, for his guidance and support. I would want to thank Copeland Funding.
- I also want to thank the professors at Wooster, my family, my lab mates, and my friends for their support.

References

1. Shi, H., Yu, X., & Cheng, G. (2023). Impact of the microbiome on mosquito-borne diseases. *Protein & Cell*, *14*(10), 743–761. https://doi.org/10.1093/procel/pwad021. Covacin & Barker, 2006; Foster et al., 2008)
2. Covacin, C., & Barker, S. C. (2006). Supergroup F Wolbachia bacteria parasitise lice (Insecta: Phthiraptera). *Parasitology Research*, *100*(3), 479–485. https://doi.org/10.1007/s00436-006-0309-6
3. Foster, J. M., Kumar, S., Ford, L., Johnston, K. L., Ben, R., Graeff-Teixeira, C., & Taylor, M. J. (2008). Absence of Wolbachia endobacteria in the non-filariid nematodes Angiostrongylus cantonensis and A. costaricensis. *Parasites & Vectors*, *1*(1), 31. https://doi.org/10.1186/1756-3305-1-31
4. Anders, K. L., Indriani, C., Ahmad, R. A., Tantowijoyo, W., Arguni, E., Andari, B., Jewell, N. P., Rances, E., O'Neill, S. L., Simmons, C. P., & Utarini, A. (2018). The AWED trial (Applying Wolbachia to Eliminate Dengue) to assess the efficacy of Wolbachia-infected mosquito deployments to reduce dengue incidence in Yogyakarta, Indonesia: Study protocol for a cluster randomised controlled trial. *Trials*, *19*(1), 302. https://doi.org/10.1186/s13063-018-2670-z
5. Hughes, G. L., Dodson, B. L., Johnson, R. M., Murdock, C. C., Tsujimoto, H., Suzuki, Y., Patt, A. A., Cui, L., Nossa, C. W., Barry, R. M., Sakamoto, J. M., Hornett, E. A., & Rasgon, J. L. (2014). Native microbiome impedes vertical transmission of Wolbachia in Anopheles mosquitoes. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(34), 12498–12503. https://doi.org/10.1073/pnas.1408888111