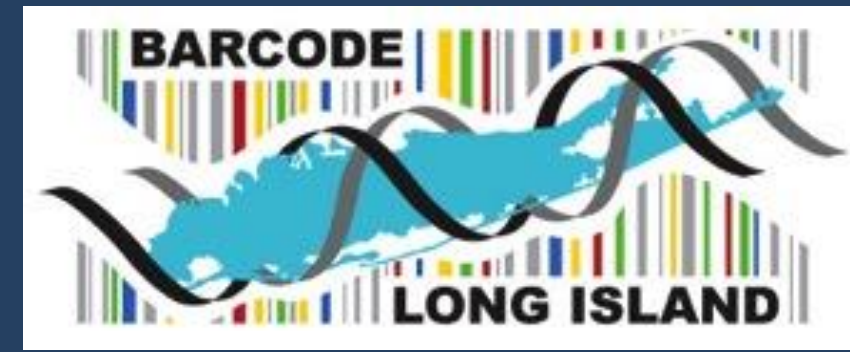


# Analyzing and Comparing the Microbiome Compositions of *Aedes albopictus* and *Culex sp.* Mosquitoes



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## Abstract

Mosquitoes are responsible for the transmission of illnesses which plague mankind every year. Within these vectors lie bacteria, both harmful and beneficial, that vary in identity and abundance, making up what is called a microbiome. The impact of these microbiomes on the ability of mosquitoes to transmit diseases is of interest to the scientific community as this relationship may be crucial to combating disease. To investigate this relationship locally, the microbiomes of *Aedes albopictus* and *Culex sp.* mosquitoes were mapped using Illumina sequencing and Jupyter Notebook programming. Furthermore, to test for a correlation between mosquito species and particular bacteria presence, statistical tests were conducted to compare the bacteria identified in *Aedes albopictus*, a species rarely reported to carry West Nile virus (WNV), to the bacteria identified in *Culex sp.*, which are frequently reported carriers of WNV. This disparity in WNV transmission makes understanding the differences between these two species' microbiomes of interest for possible human health significance. A two tailed T-test for statistical correlation yielded a total of 6 p-values below 0.05. This data suggests little difference in the microbiome compositions of the different mosquito species, suggesting mosquito species has little impact on microbiome composition. Currently, further samples are undergoing analysis to be included into the experimental data pool increasing the results validity.

## Introduction

- The gut of the mosquito is the home to hundreds of bacteria that live off of the mosquito. Some bacteria are harmful to the mosquito while others help the mosquito (Weiss & Aksoy, 2011).
- It is believed that the microbiomes of the *Culex sp.* and *Aedes albopictus* differ but the question being researched is what bacteria will be different and how will it affect the mosquitoes? (Akorli, Gendrin, Pels, Yeboah-Manu, Christophides, & Wilson, 2016).
- The bacteria in the midgut of the mosquitoes may have an effect on the ability for the mosquitoes to carry or transmit viruses (Hedge, Rasgon, & Hughes, 2015).
- How do the gut microbiomes of *Culex sp.* and *Aedes albopictus* differ?



Figure 1 and 2: Close up image of an *Aedes albopictus* (left) and *Culex sp.* (right) sample.

## Materials & Methods

- Sample Collection:** Samples were received from SCDOH
- Microbiome DNA Extraction:** A MoBio Powersoil procedure was used for DNA isolation and extraction. PCR procedures were used to amplify the 16S rRNA gene. Gel electrophoresis was used to confirm amplification. DNA was then sent to CSHL for Illumina sequencing. Coding then began to identify the abundance of each bacteria. T- tests were conducted to assess the validity of the experiment.

**Table 1:** Table provided by SCDOH with collection data

Date	Trap Site	Species	Non-Blooded	Gravid	Total	Y	X
10/6/2016	BHG Old Town	PRE		2	6	8	40.93237 -73.09349
10/6/2016	BHG Canaan	PRE		1	3	4	40.78219 -73.02198
10/26/2016	ISS Lake	ALB		1	0	1	40.69793 -73.31506
10/26/2016	ISS Champlin Creek	ALB		3	0	3	40.74306 -73.20108
10/26/2016	BNS Imola	ALB		5	0	5	40.68621 -73.39713

**Table 2:** Shows the related taxa with p-values below 0.05. This table demonstrates the weakness of the p-values calculated and the T-test results.

Taxa Level	Phylum	Class	Order	Family	Genus
Name	Firmicutes	Bacilli	Lactobacillales	Unspecified	Unspecified
p-value	0.1694	0.2316	0.0385	0.029	0.029
Name	Proteobacteria	Epsilonproteobacteria	Campylobacteriales	Camplobacteraceae	Arcobacter
p-value	0.3261	0.0165	0.0165	0.1704	0.2328
Name	Proteobacteria	Gammaproteobacteria	Alteromonadales	Colwelliaceae	Unspecified
p-value	0.3261	0.1614	0.018	0.1771	0.1771

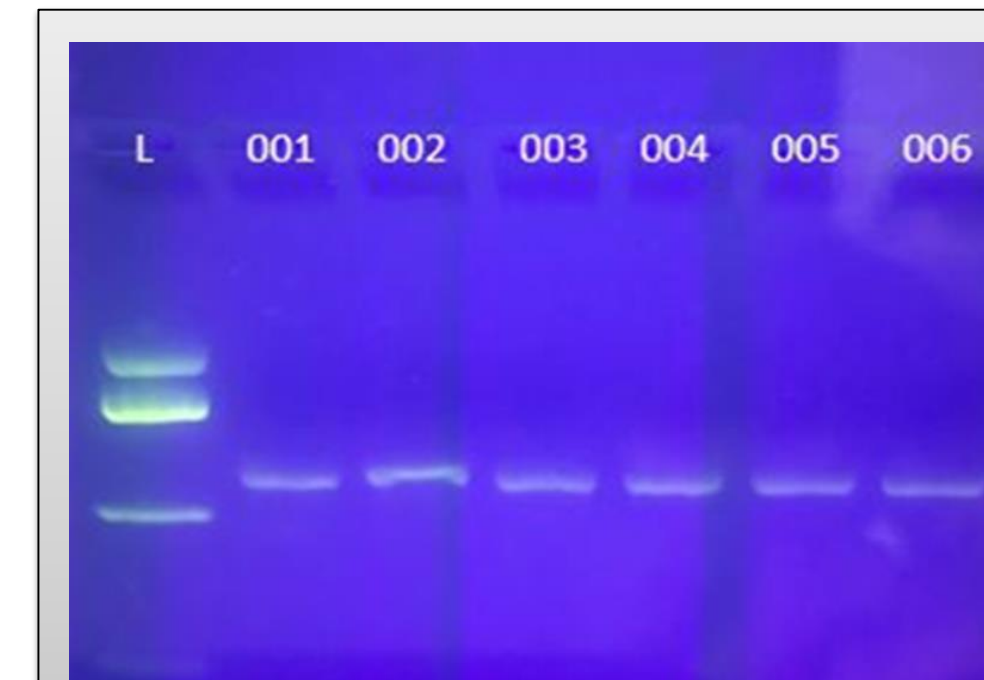
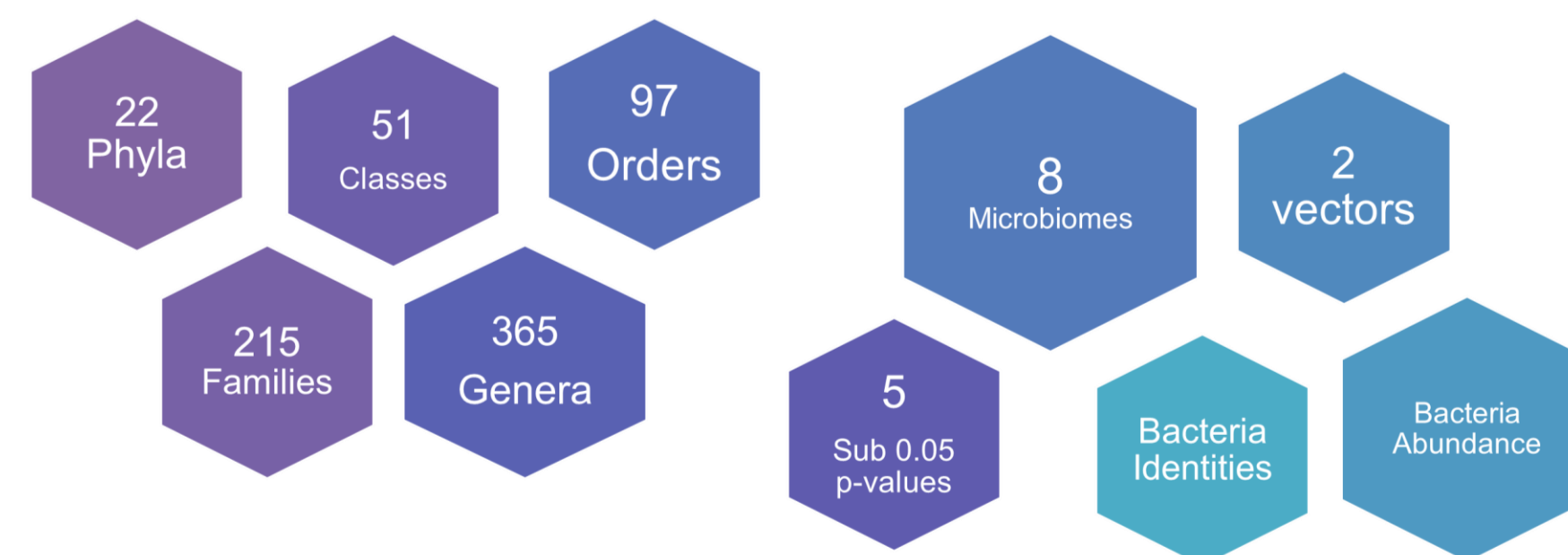


Figure 3: Electrophoresis Gel confirming presence of 16S rRNA gene.

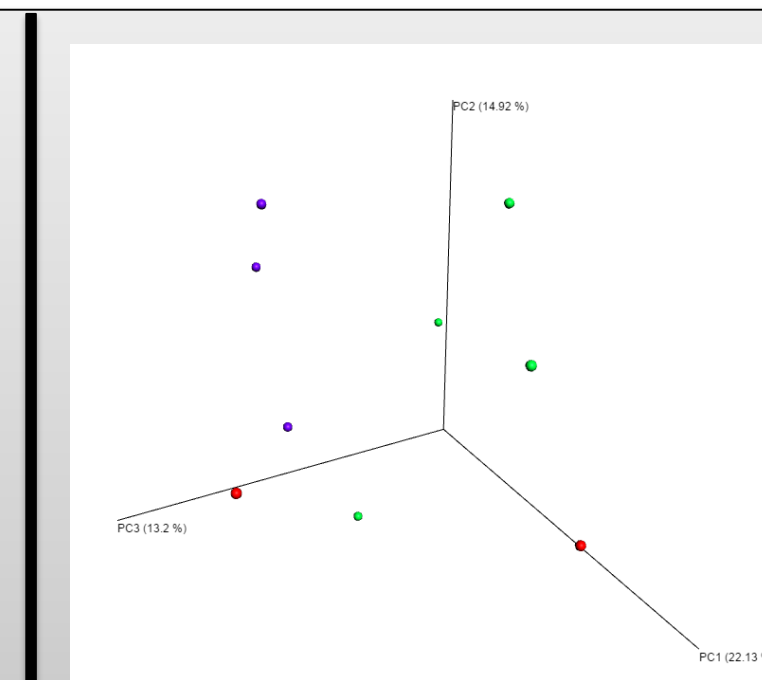


Figure 4: Scatter plot showing relationship of each sample to another.

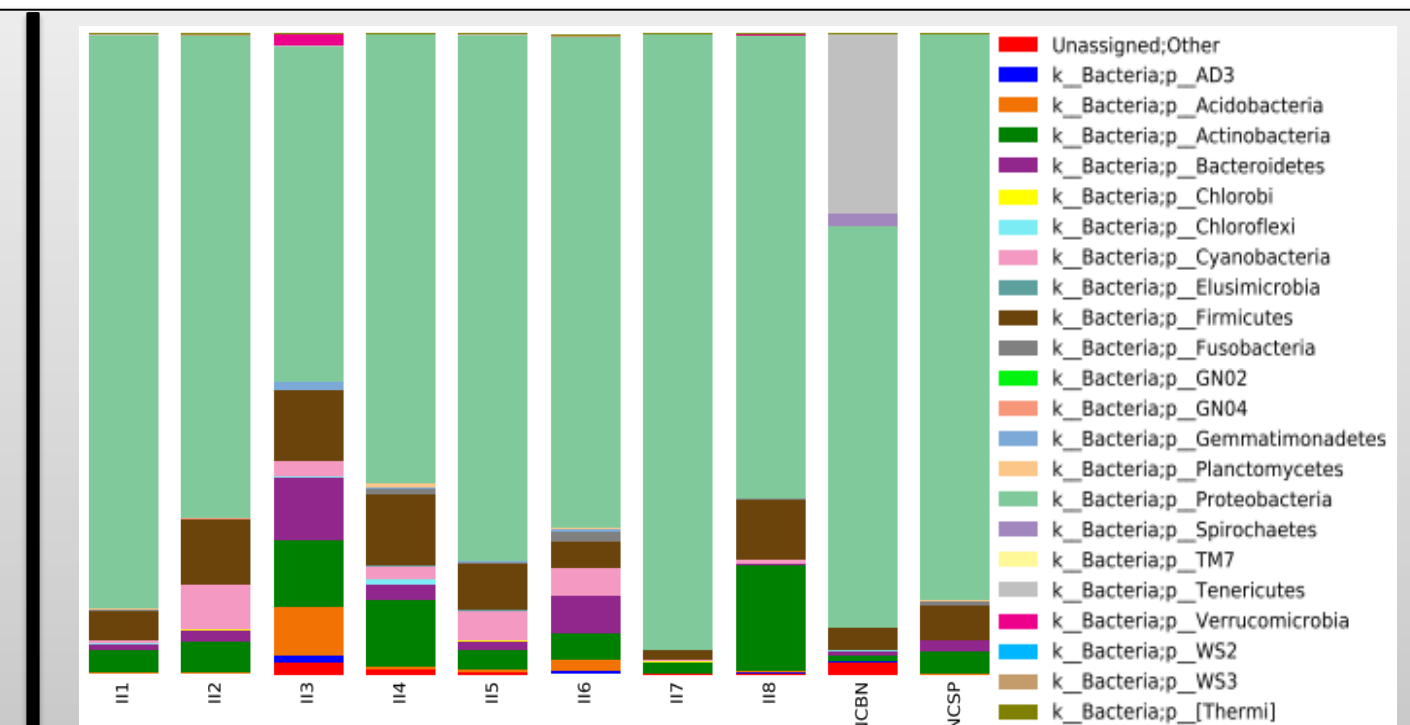


Figure 5: Phyla barplot of bacteria identified in the midgut of each mosquito. I11-I14 = *Aedes albopictus* I15-I18 = *Culex sp.*

## Discussion

- Of the 20 samples collected, 8 were used in successful DNA extractions and PCR amplifications that were sequenced.
- Several interesting Phyla of bacteria were discovered amidst the samples:
  - Proteobacteria:** They include a wide variety of pathogens, such as *Rhizobiales*, *Rickettsiales*, *E. coli*. Others are free living (nonparasitic), and include many of the bacteria responsible for nitrogen fixation. Are gram negative. Most abundant phylum of bacteria in all of the samples accounting for 76.6% of the total bacteria sequenced.
  - Wolbachia:** Genus of *Proteobacteria*. It is one of the world's most common parasitic microbes and is possibly the most common reproductive parasite in the biosphere. Its interactions with its hosts have evolved to be mutualistic rather than parasitic. Some host species cannot reproduce, or even survive, without *Wolbachia* infection. Found present heavily in samples I11 (54.4%), I17(13.5%), and I18 (36.4%).
- Wolbachia* was identified by other researchers to potentially possess anti-pathogenic qualities when inhabiting mosquitoes (Wiess & Askoy, 2011).
- No blatant distinction exists in the midgut composition between species when analyzing the bacteria based on phylum. In almost every instance, when a certain phyla is present in one sample, it is present in all samples in a similar quantity regardless of special difference.
- Current analysis does not support our initial hypothesis that the microbiomes would differ between species; however, further analysis will continue in order to assess this hypothesis.
- Future research is being conducted with 10 new samples increased sample size will increase the validity of the T-tests.

## References

Akorli, J., Gendrin, M., Pels, N. A. P., Yeboah-Manu, D., Christophides, G. K., & Wilson, M. D. (2016). Seasonality and locality affect the diversity of anopheles gambiae and anopheles coluzzii midgut microbiota from Ghana. *PLoS one*, 11(6), from e0157529. <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0157529>

Barcode Long Island. Web. 6 Nov. 2015. Retrieved from: <http://www.barcodeil.org/>.

Hedge, S., Rasgon, J. L., & Hughes, G. L. (2015, September). The microbiome modulates arbovirus transmission in mosquitoes. Retrieved November 9, 2016, from <https://www.researchgate.net/>

PowerSoil DNA Isolation Kit [Instruction manual]. (n.d.).

Weiss, B., & Aksoy, S. (2011). Microbiome influences on insect host vector competence. *Trends in parasitology*, 27(11), 514-522. from <http://www.sciencedirect.com/science/article/pii/S1471492211000833>

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