Laboratory colonization by *Dirofilaria immitis* alters the microbiome of female *Aedes aegypti* mosquitoes

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Research

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Abstract

Background: The ability of blood feeding arthropods to successfully acquire and transmit pathogens of medical and veterinary importance has been shown to be interfered or enhanced by the arthropod's native microbiome. Mosquitoes transmit bacteria, viruses, protozoan and filarial nematodes, majority of which contributes to the 17% of infectious disease cases worldwide. *Dirofilaria immitis*, a mosquito transmitted filarial nematodes of dogs and cats, is vectored by several mosquito species including *Aedes aegypti*.

Methods: In this study, we investigated the impact of *D. immitis* colonization on the microbiome of laboratory reared female *A. aegypti*. Metagenomic analysis of the V3-V4 variable region of the microbial 16SRNA was used for identification of the microbial differences down to species level.

Results: We generated a total of 1068 OTUs representing 16 phyla, 181 genera and 271 bacterial species. Overall, in order of abundance, Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes were the most represented phylum with *D. immitis* infected mosquitoes having more of Proteobacteria (71%) than uninfected mosquitoes (56.9%). An interesting finding in this study is the detection of *Klebsiella oxytoca* in relatively similar abundance in infected and uninfected mosquitoes, suggesting a possible endosymbiotic relationship. It has been previously shown to indirectly compete for nutrients with fungi on the domestic housefly eggs and larva. While *D. immitis* colonization has no effect on the overall species richness, we identified significant differences in the composition of selected bacteria genus and phylum between the two groups. We also reported distinct compositional and phylogenetic differences in the individual bacteria species when commonly identified bacteria were compared.

Conclusions: In conclusion, this is the first study to the best of our knowledge to understand the impact of a filarial infection on the microbiome of its mosquito vector. Further studies is required to identify bacteria species that could play an important role in the mosquito biology. While the microbiome composition of *A. aegypti* mosquito have been previously reported, our study shows that in an effort to establish itself, a filarial nematode modifies and alters the overall microbial diversity within its mosquito host.

Background

Study organisms: The mosquito used for investigation was the *A. aegypti,* Liverpool Blackeye strain, a highly susceptible mosquito strain to *D. immitis* used predominately in research for *Aedes sp.* (Buxton & Mullen 1981). Although *A. aegypti* is just one of 28 different mosquito species reported to vector *D. immitis*, the relative expertise on this particular species limited us to its use as our study organism.

Mosquito maintenance: *Aedes aegypti*, originally obtained from the Filariasis Research Reagent Resource Center (FR3) (Michalaski et al. 2011), were raised under standard laboratory conditions – 27 °C, 80+5% relative humidity, and a 12:12-hour light diurnal cycle (Dharmarajan et al. 2019). Adult female mosquitoes, five days post emergence were blood fed using artificial membrane feeder. One day prior to membrane feeding 31 female mosquitoes were transferred to ~500 mL plastic containers with mesh tops (henceforth "cages"). Females were starved of sugar for 12 hours and deprived of water for four hours

prior to blood feeding. Mosquitoes in each cage (31 each) were allowed to feed for two hours or until repletion on a Parafilm membrane stretched over an inverted water-jacketed glass membrane feeder maintained at 40^{o}C. Each feeder was filled with 200 uL of dog blood infected or uninfected with *D. immitis*. The amount of microfilariae fed to each mosquito group was determined as previously reported (Dharmarajan et al., 2019). The dog blood was obtained from FR3.

Dirofilaria immitis detection

Prior to screening for *D. immitis* infection from mosquitoes, DNA from individual mosquito samples was extracted using a commercially available kit (QIAGEN, DNeasy Blood & Tissue Kit) and quality was confirmed using a nanodrop machine. All blood fed (infected and uninfected blood) mosquito samples were screened for *D. immitis* infection irrespective of whether they were fed on infected or uninfected blood by amplifying the COI gene (656bp) of the *D. immitis* mitochondrial DNA (Murata *et al.,* 2003). Briefly, a 25µl reaction was set up comprising 1µl each of the COI-Forward (5'–TGATTGGTGGTTTTGGTAA–3') and COI-Reverse (5'–ATAAGTACGAGTATCAATATC–3') primers, 12.5µl of 2X mastermix (New England Biolabs), 2.5 µl DNA template and 8.5 µl of nuclease free water. For each cycle that was run, a *D. immitis* infected blood sample and nuclease free water were simultaneously included as positive and negative controls respectively. The PCR cycle comprises of an initial denaturation step at 94^{0}C for 5 minutes and 40 cycles of 94^{0}C for 1 minute, annealing at 50^{0}C for 2 minutes and extension at 72^{0}C for 3 minutes. A final extension at 72^{0}C for 5 minutes and an infinite hold at 4^{0}C.

Confirmation of amplification was done by loading the PCR products in a SYBR safe stained gel. Briefly, 2% gel was made by autoclaving a solution of 1X TAE buffer and molecular grade agar. SYBR safe stain (1 µl SYBR safe: 10 mL TAE buffer) was added to the agar solution, poured into a precast gel tray and allowed to cool. To load the samples onto the gel, 6 µl of PCR product was mixed with 4 µl of 6X dye and pipetted into the wells. Lastly, 5 µl of a low molecular weight DNA ladder was loaded onto the gel and the gel could run for 45 minutes at 100 V. Amplified PCR products were viewed using a Chemidoc gel imager (Supplementary Figure 1).

16S rRNA library preparation and sequencing

Six individual mosquito genomic DNA were pooled to make one biological replicate and five biological replicates each of *D. immitis* infected and uninfected pools were prepared for metagenomic analysis. The hypervariable V1-V3 region of the 16S rRNA gene was PCR amplified using the forward primer 27F; **5'-AGRGTTTGATCMTGGCTCAG-3'**, and reverse primer 519R; **5' – GTNTTACNGCGGCKGCTG - 3'** as outlined by the 16S Illumina's MiSeq protocol (www.mrdnalab.com, Shallowater, TX, USA). Briefly, PCR was performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 min, followed by 30-35 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. After amplification, PCR products were electrophoresed in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple

samples are pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples are purified using calibrated Ampure XP beads. Then the pooled and purified PCR product is used to prepare Illumina DNA library. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer's guidelines.

Sequence analysis

Sequence analysis was done using the Quantitative Insights into Microbial Ecology (QIIME 2), unless stated otherwise. Briefly, processing of raw fastq files were demultiplexed. The Atacama soil microbiome pipeline was incorporated for quality control of demultiplexed paired-end reads (Supplementary Figure 2) using the DADA2 plugin as previously described (Callahan et al., 2016). Sequence alignment and subsequent construction of phylogenetic tree from representative sequences was done using the MAFFT v7 and FasTree v2.1 plugin (Price at al., 2010). Operational taxonomic assignment was done using the qiime2 feature-classifier plugin v7.0 which was previously trained against the **SILVA 132 database** preclustered at 99%. Tables representing operational taxonomic units (OTUs) and representative taxonomy were exported from R and used for diversity metric analysis using the Microbiome Analyst web-based interface (Chong et al., 2020; Dhariwal et al., 2017). Raw data from this analysis were submitted deposited and assigned the GenBank BioProject number **#PRJNA606536**.

Alpha diversity

To establish whether alpha diversity differs across mosquito samples, reads were transformed and low abundance OTUs were filtered from the datasets. The Observed OTU metric was used to estimate specie richness by identifying unique OTUs present across the tick groups, while the Shannon index were used to estimate both richness and evenness. The Mann-Whitney/Kruskal-Wallis was used to determine statistical significance of alpha diversity.

Beta diversity

To compare the differences in the microbiome between mosquito groups, based on measures of distance or dissimilarity, dissimilarity matrix were generated from log-transformed sequence data and ordination of the plots were visualized using both the Principal Coordinates Analysis (PCoA) and the Nonmetric Multidimensional Scaling (NMDS). The matrix used in calculating beta diversity includes the Bray_Curtis and unweighted UniFrac distance matrix. The Permutational MANOVA (PERMANOVA) was used to test for statistical significance of the dissimilarity measures.

Methods

D. immitis effectively colonizes A. aegypti mosquitos under laboratory conditions

A total of 31 mosquitoes were artificially infected to generate *D. immitis* infected mosquito. PCR analysis and confirmation of infection status using gel electrophoresis revealed an infection prevalence of 87% (Supplementary data, Fig 1).

Microbiome composition

Analysis of the demultiplexed paired-end-reads generated a total of 602502 reads which ranges from 31861 to 110235, and an average of 54758 reads. Mosquitoes infected with *D. immitis* had the highest number of reads (382714) compared to uninfected mosquitoes with a total of 219788 reads. Taxonomic classification using the SILVA 132 (99% OTUs full-length sequences) reference database identified 268 operational taxonomic units (OTUs), 11 phyla, 16 genera, and 136 species (Supplementary Figure 2 and Table 1).

D. immitis infection alters relative abundance of bacteria taxa in A. aegypti. Taxonomic assignment was done against the SILVA database to observe for the difference in microbiome composition and relative abundance of bacteria species. Both infected and uninfected mosquitoes possess similar composition of bacteria taxa with differences observed in the relative abundances of specific bacteria species. The phylum Proteobacteria and Bacteroidetes were present in higher abundance in both mosquito groups. Mosquitoes infected with *D. immitis* had relatively higher abundance of Proteobacteria (71%) with lower amount of Bacteroidetes (27%), while uninfected mosquitoes have lower amount of Proteobacteria (56.9%) and a higher abundance of Bacteroidetes (37%) (Fig. 1). Among the detected bacteria genera, the genus *Klebsiella* were detected at relatively similar abundances in both infected (36.3%) and uninfected (34.6%) mosquitoes (Fig 2A). The genera *Ochrobactrum*, *Sphingobacterium*, and *Pseudomonas* were present exclusively in uninfected mosquitoes albeit at an abundance of 4.4%, 3.6% and 3% respectively (Fig 2). The genera *Enterobacter* (26.1%) and *Elizabethkingia* (9.4%) were in higher abundance in *D. immitis* infected mosquitoes (Fig 2A). *Enterobacter hormaechei* (24.4%) and *Elizabethkingia meningoseptica* (9.4%) were more abundant in infected mosquitoes, while *Chryseobacterium indolgenes* (27.4%), and *Grimontella senegalensis* (3.9%), were more abundant in uninfected mosquitoes (Fig.2B). Additional information on the relative bacteria distribution in individual mosquito can be found in the Supplementary figures 4, 5, 6, and 7.

D. immitis infection affects species diversity in A. aegypti. Following demultiplexing of paired end reads and quality control by removing chimera, we normalized OTU counts for individual biological replicates and a rarefaction curve was generated at a depth of 20000 (Fig. 3). Adequate depth coverage was reached as evidenced by the individual curves plateauing out on the rarefaction curve. Our results indicated that phylogenetic diversity, estimated using Shannon index and number of observed OTUs, was reduced in infected mosquitoes compared against uninfected mosquitoes (Fig. 4A and B). Surprisingly, both metrics of alpha diversity used showed no significance when the Kruskal-Wallis test was applied (Observed OTUs: p-value: 0.053173; (Mann-Whitney) statistic: 3; Shannon Index; p-value: 0.30952; (Mann-Whitney) statistic: 7). We also visualized the similarities and differences in the microbial composition of infected and uninfected mosquitoes by carrying out Principal Components Analysis (PCoA) of the unweighted UniFrac and Bray Curtis and distance matrixes (Fig. 5C and D). Figures 5C and 5D shows distinct clustering of *D. immitis* infected mosquito replicates with no outliers. Beta diversity was significantly different when *D. immitis* infected mosquito was compared to those uninfected using the

Unweighted UniFrac distance matrix (PERMANOVA, F-value: 1.4043; R-squared: 0.14932; p-value < 0.286).

Community profiling and correlation analysis

To assess the extent to which highly abundant bacteria phylum and genus were represented in *R. microplus* ticks, we used a combination of pattern correlation and heat map analysis. A very strong positive correlation was seen between the genus *Ralstonia*, *Francisella, Pantoea, Elizabethkingia, Wolbachia, Herbaspirillum,* and *Achromobacter* and *D. immitis* infected mosquitoes (Figure 6A). Heat map analysis and phylogenetic tree of the highly represented and dominant bacteria genera also showed the above identified genera to be well represented in more than one of the *D. immitis* infected mosquito (Supplementary Figure 8, and 9).

To explore how top taxa differs between both infected and uninfected mosquitoes, classical univariate statistical comparisons analysis was applied to identify for phylum and genus that exhibit significant differences (Mann-Whitney test) in their composition. The phylum Actinobacteria (p; 0.036145, FDR; 0.1440), and Firmicutes (p; 0.005556, FDR; 0.14401) has significantly higher abundance in the uninfected mosquitoes (Figure 6B and C). Our analysis also shows that the *D. immitis* mosquito has significantly higher abundance of the genera *Elizabethkingia* (p; 0.01508, FDR; 0.175), and *Wolbachia* (p; 0.011925, FDR; 0.11448), while *Pseudomonas* (p; 0.015873, FDR; 0.12698) was much abundant in the uninfected mosquito (Figure 7A, B, and C).

Results

D. immitis effectively colonizes A. aegypti mosquitos under laboratory conditions

A total of 31 mosquitoes were artificially infected to generate *D. immitis* infected mosquito. PCR analysis and confirmation of infection status using gel electrophoresis revealed an infection prevalence of 87% (Supplementary data, Fig 1).

Microbiome composition

Analysis of the demultiplexed paired-end-reads generated a total of 602502 reads which ranges from 31861 to 110235, and an average of 54758 reads. Mosquitoes infected with *D. immitis* had the highest number of reads (382714) compared to uninfected mosquitoes with a total of 219788 reads. Taxonomic classification using the SILVA 132 (99% OTUs full-length sequences) reference database identified 268 operational taxonomic units (OTUs), 11 phyla, 16 genera, and 136 species (Supplementary Figure 2 and Table 1).

D. immitis infection alters relative abundance of bacteria taxa in A. aegypti. Taxonomic assignment was done against the SILVA database to observe for the difference in microbiome composition and relative abundance of bacteria species. Both infected and uninfected mosquitoes possess similar composition of bacteria taxa with differences observed in the relative abundances of specific bacteria species. The

phylum Proteobacteria and Bacteroidetes were present in higher abundance in both mosquito groups. Mosquitoes infected with *D. immitis* had relatively higher abundance of Proteobacteria (71%) with lower amount of Bacteroidetes (27%), while uninfected mosquitoes have lower amount of Proteobacteria (56.9%) and a higher abundance of Bacteroidetes (37%) (Fig. 1). Among the detected bacteria genera, the genus *Klebsiella* were detected at relatively similar abundances in both infected (36.3%) and uninfected (34.6%) mosquitoes (Fig 2). The genera *Ochrobactrum*, *Sphingobacterium*, and *Pseudomonas* were present exclusively in uninfected mosquitoes albeit at an abundance of 4.4%, 3.6% and 3% respectively (Fig 2). The genera *Enterobacter* (26.1%) and *Elizabethkingia* (9.4%) were in higher abundance in *D. immitis* infected mosquitoes (Fig 2). Except for *Klebsiella oxytoca*, which was present in similar abundance for infected (36%) and uninfected (34%) mosquitoes (Fig. 3). *Enterobacter hormaechei* (24.4%) and *Elizabethkingia meningoseptica* (9.4%) were more abundant in infected mosquitoes, while *Chryseobacterium indolgenes* (27.4%), *Grimontella senegalensis* (3.9%), and *Ochrobactrum spp* (4.4%) were more abundant in uninfected mosquitoes (Fig.3). Additional information on the relative distribution of individual bacteria taxa can be found in the figure 4 and 5 (Phylum and class) and tables 3-6 (order to species), under the supplementary section.

D. immitis infection affects species diversity in A. aegypti. Following demultiplexing of paired end reads and quality control by removing chimera, we normalized OTU counts for individual biological replicates and a rarefaction curve was generated at a depth of 2000 (Fig. 4A). Adequate depth coverage was reached as evidenced by the individual curves plateauing out on the rarefaction curve. Our results indicated that phylogenetic diversity, estimated using Faith's phylogenetic distance and number of observed OTUs, was reduced in infected mosquitoes compared against uninfected mosquitoes (Fig. 4B and C). We also visualized the similarities and differences in the microbial composition of infected and uninfected mosquitoes by carrying out Principal Components Analysis on the Bray Curtis and the unweighted UniFrac distance matrixes (Fig. 4D and E). Figures 4D and E shows distinct clustering of *D. immitis* infected mosquito replicates with no outliers.

Discussion

To the best of our understanding, this study is the first to utilize 16S metagenomic analysis to understand the significance of a filarial nematode on the microbiome of a mosquito vector under laboratory conditions. This technique offers the advantage of detecting both culturable and unculturable pathogenic and non-pathogenic microbes from a given DNA or RNA. As previously reported from previous metagenome studies on mosquitoes and similar arthropods or insects (Mancini *et al.,* 2018; Coon *et al.,* 2014; Coatsworth *et al.,* 2018), Figure 1A shows that the phylum Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes was detected from the mosquito groups tested. Similar studies have also identified the phylum Proteobacteria as one of the dominant phylum in the microbiome of *A. aegypti* mosquitoes (Ramirez *et al.,* 2012; Audsley *et al.,* 2017). We observed an inverse relationship in the abundances of the phylum Proteobacteria and Bacteroidetes in our study with *D. immitis* infected mosquitoes having a higher abundance of the phylum Proteobacteria when compared to the uninfected mosquitoes and vice-versa (Supplementary data, figure 4). The Proteobacteria group are the largest

phylum found in different environments, plants and animals (http://www.bacterio.net/; Degli and Martinez, 2017) with members ranging from free living commensals to pathogenic microbes. We found that the genus *Klebsiella* was present in relatively similar abundances in both infected and uninfected mosquitoes. Although no known function has been associated to this group in insects or arthropods, *Klebsiella* belongs to a class of gram Proteobacteria known as Gammaproteobacteria with previous detection in field and laboratory raised *Culex quinquefasciatus*, *A. albopictus*, and *A. aegypti* (Gonçalves *et al.,* 2019; Yadav *et al.,* 2015; Wu *et al.,* 2019).

We also identified the genus *Enterobacter* in *D. immitis* infected mosquito at a relative abundance of approximately 20 folds of the uninfected mosquitoes was also observed (Figure 2). This bacteria genus has been detected in similar microbiome studies of *A. aegypti* where they have been associated with their role in blood meal digestion due to their hemolytic activities (Coon *et al.,* 2016) which could explain why the genus *Enterobacter* have been commonly associated with females of other mosquito species (Minard *et al.,* 2013). The role(s) *D. immitis* colonization and infection density plays in increasing the abundance of *Enterobacter* was beyond the scope of this study, an elegant study by Cirimotich and colleagues showed the inhibition of *Plasmodium* infection in *Anopheles* (*A*) *gambiae* mosquitoes mediated by a bacterium designated as *Enterobacter* Esp_Z. The inhibition was due to the production of reactive oxygen species (ROS) by the bacteria. A pro-pathogen role was also recently associated with the *Enterobacter* genus, as it was shown that they produces Enhancins or Enhancins-like proteins, which facilitate pathogen colonization by degrading the peritrophic matrix (Wu et al., 2019). If bacteria such as *Enterobacter* can also blocks *D. immitis* colonization by inducing ROS production will be interesting to see. *Enterobacter hormaechei* is another bacterium that demonstrated increased abundance in *D. immitis* infection as shown in Figure 3. The genus *Enterobacter* have been proposed to aid in blood digestion in hematophagous insects due to their hemolytic activities (Gaio *et al.,* 2011). Several reports have also identified different mosquito refractoriness or susceptibility to pathogen infection in the presence of different *Enterobacter* species. Cirimotich et al. (2011) reported refractoriness of *Anopheles* (*A*) *gambiae* to *Plasmodium* infection in the presence of an *Enterobacter* species. Another species of *Enterobacter* (*E. cloacae*) was also reported to express a mucin degrading Enhancin protein that breaks down the mucin component of the *A. aegypti* peritrophic matrix (Wu *et al.,* 2019) although this wasn't shown to facilitate Dengue virus infection. Another bacteria genus that also increased with the presence of *D. immitis* infection is *Elizabethkingia*. Previous report has identified this genus from the midgut of laboratory reared (Gonçalves *et al.,* 2019) and field collected (Audsley at al., 2017) *A. aegypti*. These studies did not associate any known role to this bacterium. Bacteria in the genus *Chryseobacterium*, *Ochrobactrum*, *Sphingobacterium*, and *Pseudomonas* were all present in higher abundance in uninfected mosquito group. The detection of *Chryseobacterium* from our study agrees with similar detection from previous report in laboratory reared mosquitoes (Chen *et al.,* 2016; Wang *et al.,* 2011). *Pseudomonas*, a Gram-negative, Gammaproteobacteria was previously shown to have reduced abundance in *Wolbachia* positive *A. aegypti* mosquitoes. A similar observation was also made from our study where the abundance of *Pseudomonas* was inversely correlated with the presence of *D. immitis* infection in the mosquito as shown in Figure 2. Bahia et al. (2014) reported blocking of the *Plasmodium* parasite by *Pseudomonas*

putida isolated from *A. gambiae.* Unexpectedly, only few bacteria species were differentially altered in *D. immitis* infected and uninfected mosquitoes. *Klebsiella* (*K.*) *oxytoca,* a Gammaproteobacteria belonging to the genus *Klebsiella*, was found at relatively similar abundance in infected and uninfected mosquitoes (Fig 3). A study reported the detection of *K. oxytoca* from laboratory reared and field collected *C. quinquefasciatus* and *A. aegypti* (Gonçalves *et al.,* 2019). The maintenance of a bacteria by arthropods in both natural and artificial conditions could indicate an important role played by the bacteria. Another studies reported that *K. oxytoca* reverse radiation induced loss of copulatory maintenance in male *Ceratitis capitate* (Ami *et al.,* 2009). Another study on the fungicidal effects of bacterial colonies found on the domestic housefly eggs revealed that fungal growth is inhibited by the presence of *K. oxytoca* by producing antifungal metabolites and nutrient depletion (Lam et al., 2009). Put together, we are proposing *K. oxytoca* as an important bacteria *A. aegypti* with a likely endosymbiotic role, though further studies are still required to understand the specific roles of *K. oxytoca* in the mosquito biology including how it is maintained in the mosquito. Another interesting data from this study was the inverse correlation between *D. immitis* infection and microbial diversity and richness. In mosquitoes, the innate immune response is activated in the presence of invading pathogens as it was previously shown that activation of the toll pathway and production of reactive oxygen species have been reported in mosquitoes challenged with *Plasmodium* (Molina-Cruz *et al.,* 2008) and filarial nematodes (Edgerton *et al.,* 2020). These immune effectors while countering pathogen, could in extension disrupt the normal mosquito microbiome community which could explain the overall reduction in the microbial richness observed from this study as seen in Figure 4B and C. Our observation of reduced microbial richness in infected mosquitoes was in contrast to what was reported in a previous study which shows a more diverse microbiome in *Plasmodium*-infected *Anopheles* mosquitoes (Bassene *et al.,* 2018). While our current study did not factor the effect blood meal could have on the overall outcome of the microbial richness, quiet a number of studies have reported reduction in the bacterial diversity following experimental feeding on host blood in *A. aegypti* (Muturi et al., 2019), *An. gambiae* (Wang et al., 2011).

Conclusion

This study fills a knowledge gap on the interaction between a mosquito vector and a filarial pathogen of veterinary significance. We were able to show that while some bacterial species were found to be present in both mosquito groups, the relative abundances of individual species changes with the infection status, with infected mosquitoes presenting a reduced microbial richness. This indicates a likely consequence of the nematode in altering favoring or inhibiting the growth of members of the bacterial community. Ongoing studies focuses on the shift in the distribution of culturable bacteria taxa in infected and uninfected mosquitoes, while also comparing the effects of *D. immitis* colonization on the microbial diversity of different tissues of the *A. aegypti* mosquito.

Declarations

Ethics Approval and Consent to participate:

Mosquitoes were blood-fed using an artificial membrane feeder. The protocol for the laboratory was approved by the Institutional Biosafety Committee.

Consent of Publication:

All authors read and approved the manuscript for publication.

Availability of data and material:

The datasets supporting the conclusion of this article are included within the article and its additional files. Raw data are available from the corresponding author upon request.

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Author contributions:

Conceived and designed the experiments: GD, SK

Performed the experiments: ASA, EN, AG, MCH, KW, SMN

Analyzed the data: ASA, GD, SK

Contributed reagents/materials/analysis tools: GD, SK

Wrote the paper: ASA, EN, AG, GD, SK

All authors have read and approved the manuscript

Competing Interests:

The authors have declared that no competing interests exist.

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Figures

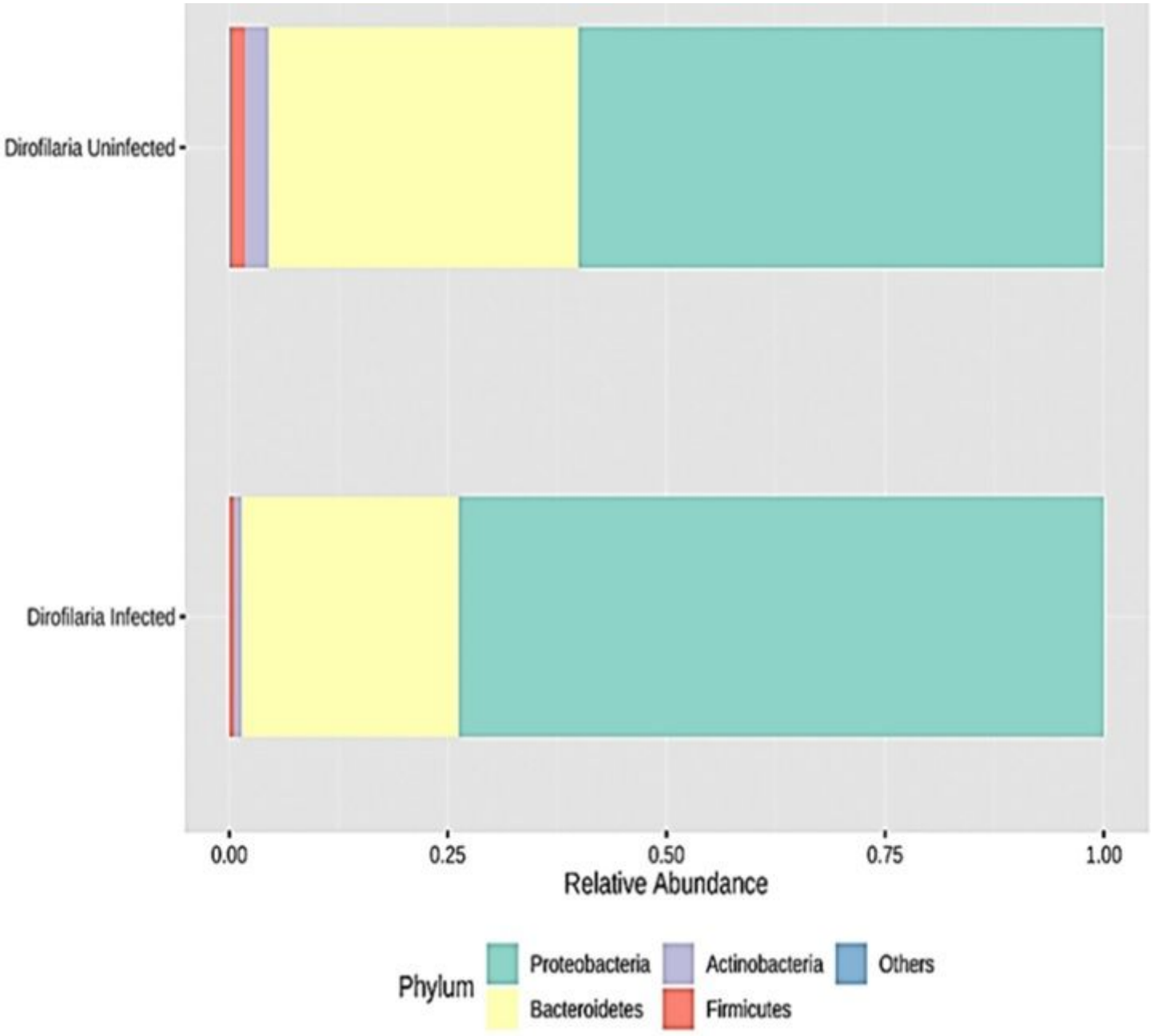

Figure 1

Relative distribution of bacteria at phylum taxonomic level in D. immitis infected and uninfected Aedes aegypti mosquitoes. Bacterial taxa with less than 1% abundances were grouped as others.

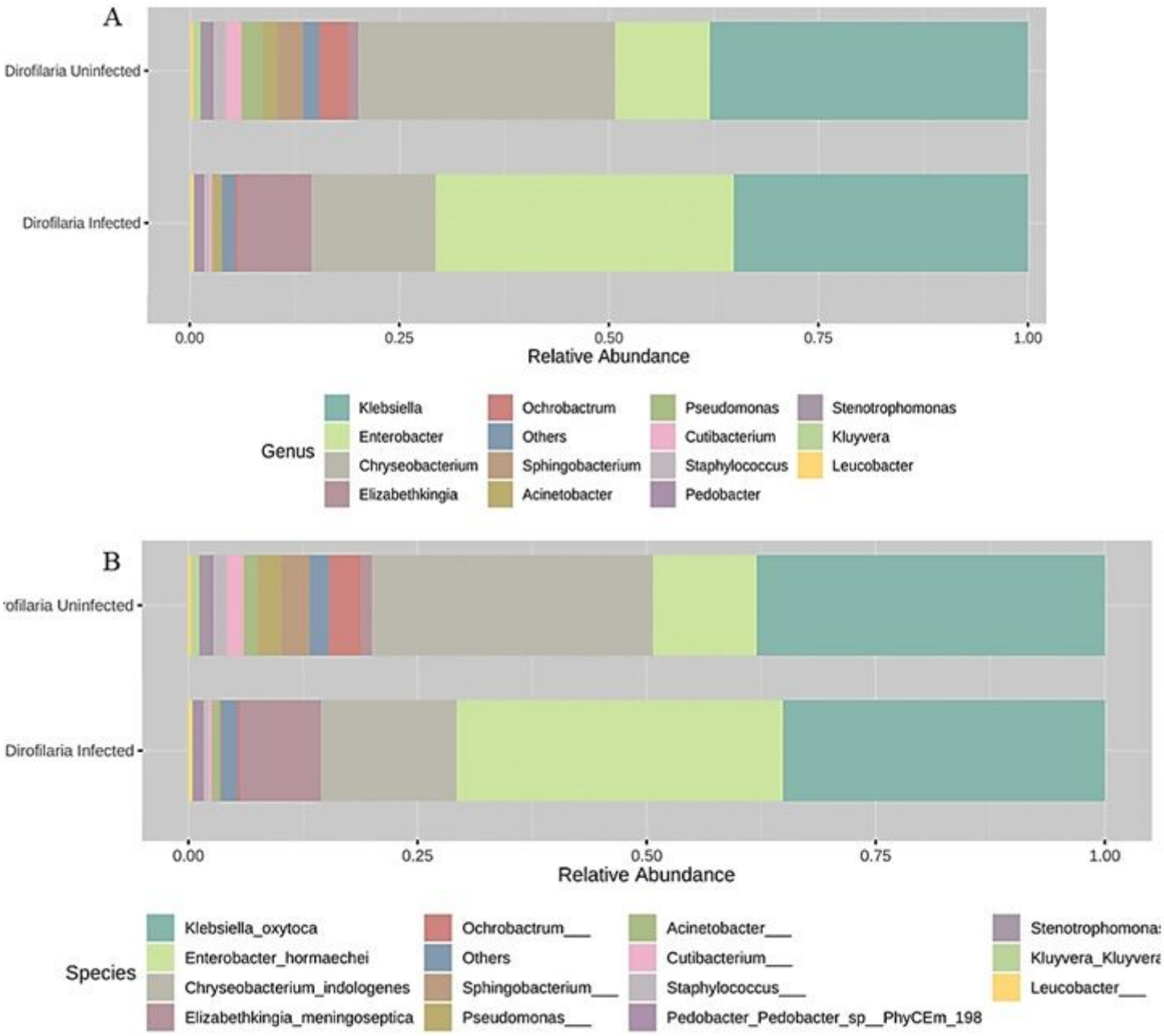

Figure 2

Relative distribution of bacteria at A) genus and B) species taxonomic level in D. immitis infected and uninfected Aedes aegypti mosquitoes. Bacterial taxa with less than 1% abundances were grouped as others.

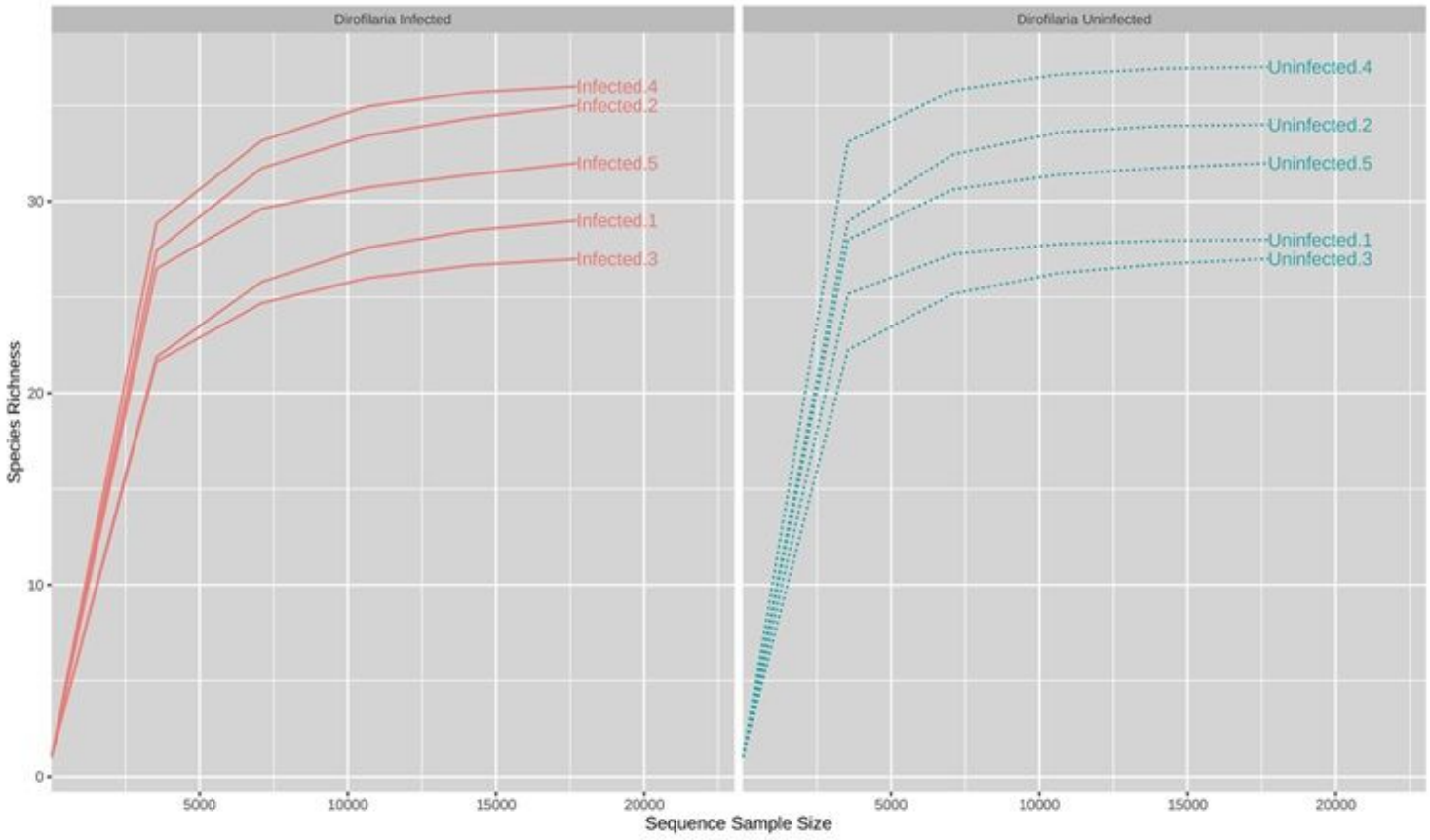

Figure 3

Rarefaction analysis of biological replicates rarefied at a sequenced depth of 20000. Curves from each samples was allowed to reach a plateau so as to prevent trade off of rarely represented bacterial OTUs.

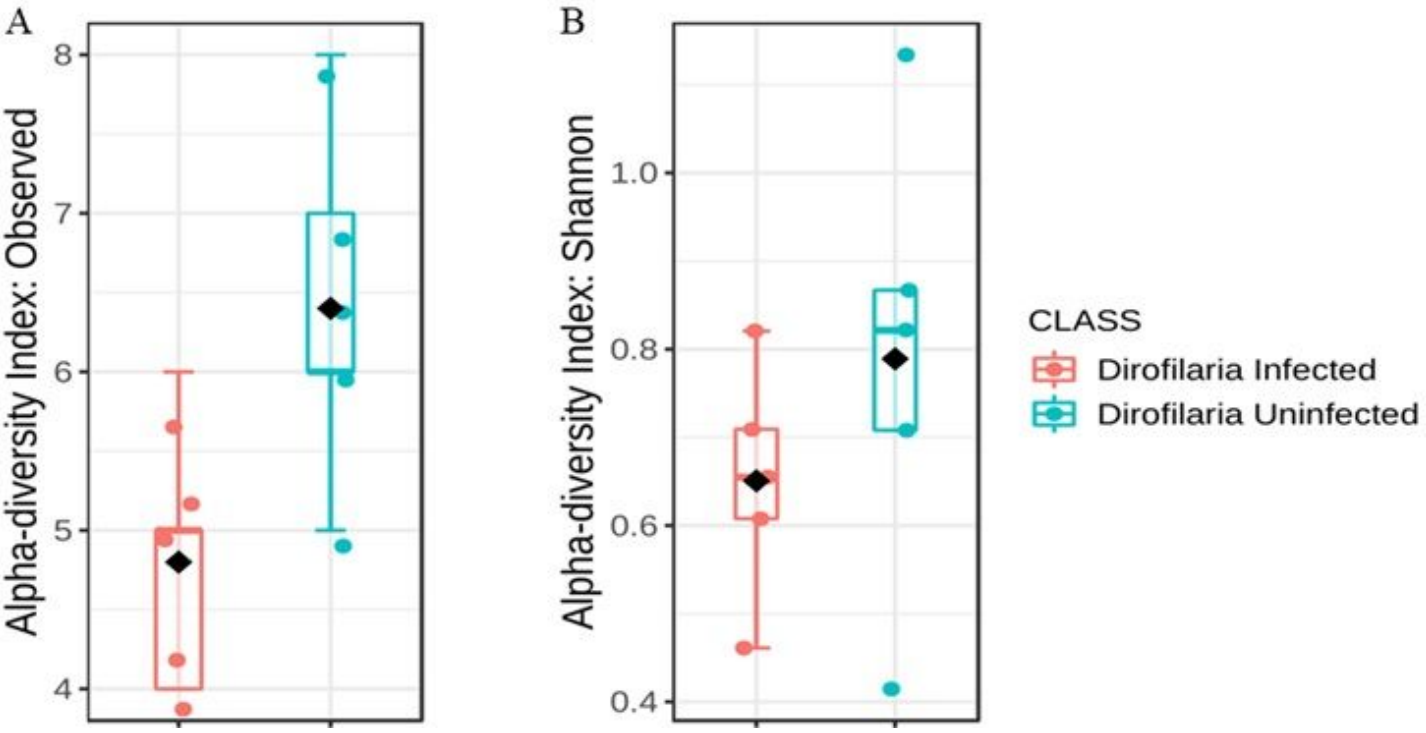

Figure 4

A), Alpha diversity analysis. A), Observed OTUs (Kruskal-Wallis, p=0.053173) distance and B), Shannon diversity index (Kruskal-Wallis, p=0.30952) were both used as metrics to measure alpha diversity. Principal coordinate analysis (PCoA) of D), Bray_Curtis and E), Unweighted UniFrac distance matrixes as measures of beta diversity.

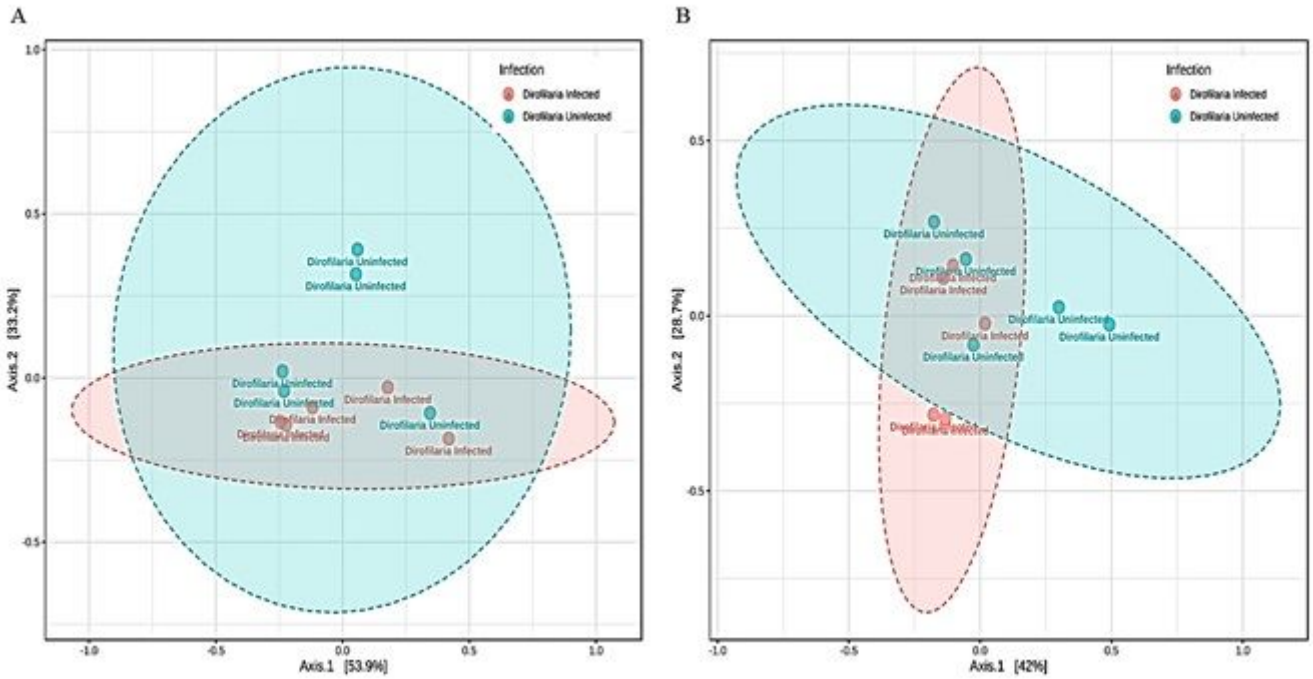

Figure 5

Principal coordinate analysis (PCoA) of A), unweighted UniFrac ($p<0.05$) and B), Bray_Curtis ($p<0.05$) distance matrixes as measures of beta diversity. Statistical significance was estimated by PERMANOVA.

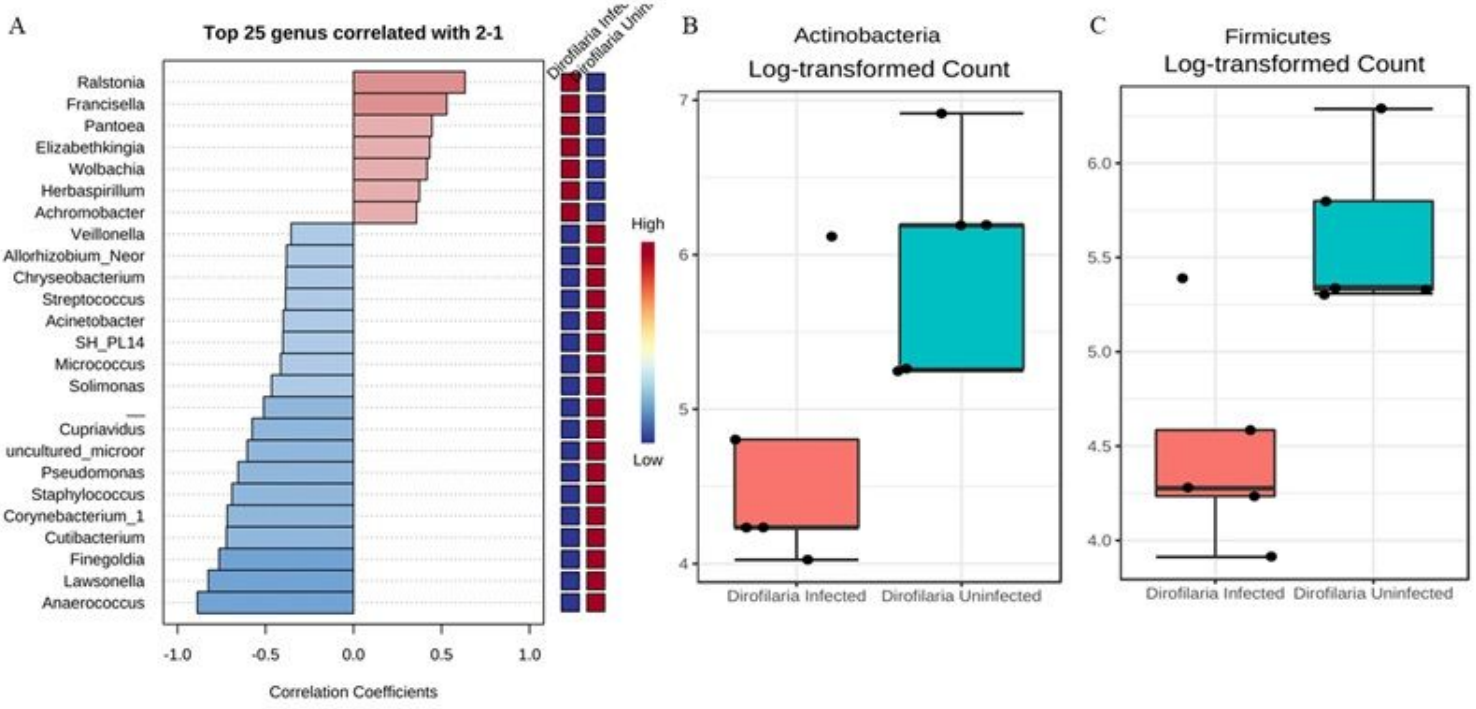

Figure 6

Pattern correlation analysis and significant abundance analysis of top taxa identified in between D. immitis infected and uninfected mosquito. (A) SparCC correlation of top 25 bacteria Genus showing bacteria with strong positive correlation to those mosquito that were D. immitis infected. Box plots analysis of bacteria that shows significant differences in their abundances between infected and uninfected mosquito in the phylum B), Actinobacteria (p = 0.036145), and C) Firmicutes (p = 0.005556).

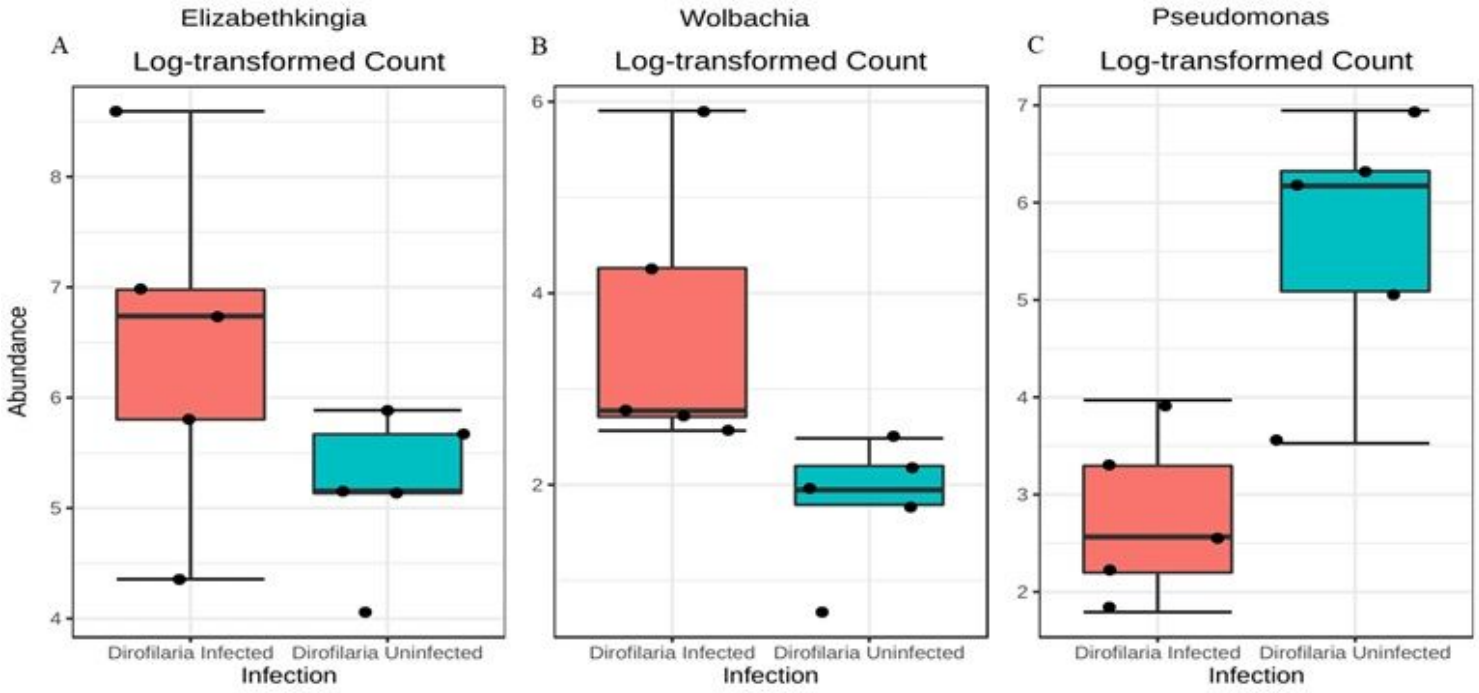

Figure 7

Box plots analysis of bacteria that shows significant differences in their abundances between infected and uninfected mosquito in the genus A), Elizabethkingia (p = 0.01508), B), Wolbachia, (p = 0.011925) C) Pseudomonas (p = 0.015873).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- MosquitoD.immitisMethods.png
- SupplementaryMaterials.pdf