**Methods**

**Ethical Statement**

*D. antillarum* habituates in fragile marine ecosystems and the procedures described here were done with extensive precaution to reduce organismal stress and environmental impact. The collection methods were approved by Recursos Naturales of Puerto Rico (permit number) and these included to collect tissue outside of the marine environment and as quickly as possible.

**Urchin Collection**

Sea urchins were collected across 8 different locations across the municipalities of Puerto Rico representing: North, East, South and West regions. The municipalities were chosen based on accessibility and these were Ceiba, Culebra, Guanica, Guayama, Isabella, Luquillo, Ponce and Rincon. The specimens chosen were of either sex or any age. A diving knife was used to carefully separate *D. antillarum* from the environment where it was found by scooping the specimens from the place that they were found. This was only done on urchins that were easy to remove. Once the specimens were physically separated from the environment, they were placed in a diving bag to transport outside of the water. Once outside of the water, the specimens were placed in sea water to reduce harm until the individuals were measured and prepared for gut tissue collection.

**Tissue Collection**

Sea urchin gut samples were collected at each site to avoid overstressing the animal, potentially risk contamination, or even potentially change microbiome composition. Once the individuals were measured their spines were cut out and an incision was made in the test surrounding the peristomial membrane using ethanol sterilized scissors dissecting around the mouth. The peristomial membrane, along with the nested mouth (Artistotle’s Lantern) was lifted from the animal and the gut was collected using ethanol sterilized tweezers. The samples were placed in a 1.5mL tube and held on ice while in immediate transition for storage in a -20 C freezer.

**DNA Extraction**

The Sigma-Aldrich GenElute Stool DNA Isolation Kit was used to isolate DNA from the samples according to the manufacturer’s protocol. The DNA was eluted into 50 µL of company provided buffer and immediately placed in the -20 ˚C freezer for long-term storage.

**DNA Sequencing**

*16S V4 Region*

DNA samples were thawed, aliquoted, and held on ice for transport to the sequencing facility. The16S rRNA sequencing was conducted at the Ponce School of Medicine core sequencing lab on an Illumina MiSeq Next Gen DNA sequencing machine. This machine generated raw paired dual index files that were used to conduct further bioinformatic analysis.

*Cytochrome B*

A PCR of the Cytochrome B was conducted to replicate and be able to sequence the region. The sequence of twenty-seven different species of sea urchin mitochondrial DNA sequences (Chunxia 2016, Bronstein 2019) was aligned to find regions were primers could be constructed for the Cytochrome B region. The DNA region of the gene was found to be mostly conserved around the 14,988 – 16,070 Bp region in the mitochondrial DNA alignments. The forward primer used was the position 14,968 - 14,987 bps. The annealing temperature of this primer was 47.4˚C. The reverse primer used was the position 16,071-16,093 bps. The annealing temperature of this primer was 52.4˚C. The final annealing temperature was 47.4˚C. A PCR was conducted using BioLabs Q5 High0Fidelity DNA Polymerase. A total reaction of 25µL for each sample was used. Following, is a table with the PCR ingredients.

**Bioinformatic Analysis**

*Raw Data*

The sample data along with commands used for this study can be found at (<https://github.com/mercadocapote/diadema_ajmc2020>). The 16S sample data files are the V4 region of the 16S rRNA gene that were sequenced using Illumina MiSeq machine which generated paired sample files with dual index. Additionally, there was sample metadata files generated by the researchers at the site of collection which described location, size, and habitat of each specimen. The metadata sample files are in the .tsv format.

*QIIME2*

The QIIME2 microbiome bioinformatics platform was used to perform the microbiome analysis of the 16S rRNA samples. All of the information pertaining the installation procedure can be found at <https://docs.qiime2.org>. QIIME2 was installed as a virtual machine using the latest Virtual Box Image from the QIIME2 website (<https://docs.qiime2.org/2020.2/install/>) and using the latest Virtual Box Version (<https://www.virtualbox.org/>).

*Geneious Prime*