**Methods**

**Ethical Statement**

Marine ecosystems are fragile therefore all procedures must be done with extensive precaution in order to reduce organismal stress and environmental impact. Collection methods were approved by Recursos Naturales of Puerto Rico (permit number). To reduce stress each tissue will be collected at each site as soon as possible. To reduce environmental impacts first the organisms will be removed from their respective environments.

**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 chosen 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. Once the specimens were physically separated from the environment, they were placed in a net bag to transport outside of the water. Once outside of the water, the specimens were placed in sea water to reduce harm. The individuals were measured, then prepared for gut tissue collection.

**Tissue Collection**

Sea urchin gut samples was 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 uL of company provided buffer and immediately placed in the -20 C freezer for long-term storage.

**DNA Sequencing**

DNA samples were thawed, aliquoted, and held on ice for transport at two different facilities for 16S rRNA and Cytochrome B sequencing.16S rRNA sequencing was at the Ponce School of Medicine core sequencing lab on Next Gen DNA sequencing machine. The Cytochrome B sequencing will be performed at University of Texas.

A PCR of the Cytochrome B was conducted in order 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 25uL for each sample was used. Following, is a table with the PCR ingredients.

**Bioinformatic Analysis**

*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/>).