#Project title: Microbiota associated with echinoid eggs and the implications for maternal provisioning
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#What: Computational pipeline for the V3/V4 region of the 16S rRNA gene (i.e., phylogenetic marker for bacteria) QIIME2 (v 2019.1), SILVA (v 132), and Amplicon Sequence Variants (ASVs)

```
### STEP 1: IMPORT RAW FILES INTO QIIME2 ###
qiime tools import \
  --type 'SampleData[PairedEndSequencesWithQuality]' \
  --input-path
  --input-format CasavaOneEightSingleLanePerSampleDirFmt \
  --output-path


### STEP 2: TRIM PRIMERS OFF RAW READS #
qiime cutadapt trim-paired \
  --i-demultiplexed-sequences
  --p-front-f CCTACGGGNGGCWGCAG \
  --p-front-r GACTACHVGGGTATCTAATCC \
  --p-error-rate 0 \
  --o-trimmed-sequences


### STEP 3: JOIN/MERGE PAIRED-ENDS ###
qiime vsearch join-pairs \
  --i-demultiplexed-seqs
  --o-joined-sequences
  --p-minovlen 20 \
  --p-maxdiffs 10 \
  --p-minmergelen 350 \
  --p-maxmergelen 550 \
  --p-allowmergestagger \
  --p-truncqual 10 \
  --p-minlen 100 \
  --p-qmax 41 \
  --p-qmaxout 41
```

```
### STEP 4: QUALITY CONTROL PAIRED-END READS ###
qiime quality-filter q-score-joined \
  --i-demux
  --o-filtered-sequences
  --o-filter-stats
  --p-quality-window 5 \
  --p-min-quality 25



### STEP 5: REIMPORT FILTERED FILES WITH MODIFIED NAMES ###
          #NOTE: THIS IS OPTIONAL
qiime tools export \
  --input-path
  --output-path

qiime tools import \
  --type 'SampleData[SequencesWithQuality]' \
  --input-path
  --output-path
  --input-format SingleEndFastqManifestPhred33



### STEP 6: DENOISE PROCESSED READS ###
qiime deblur denoise-16S \
  --i-demultiplexed-seqs
  --p-trim-length 400 \
  --o-representative-sequences
  --o-table
  --p-sample-stats \
  --o-stats



### STEP 7: CREATE PHYLOGENY ###
#ALIGNMENT OF REPRESENTATIVE SEQUENCES
qiime alignment mafft \
  --i-sequences
  --o-alignment

#MASK HIGHLY VARIABLE NOISY POSITIONS IN ALIGNMENT
qiime alignment mask \
  --i-alignment
  --o-masked-alignment
```

```
#CREATE PHYLOGENY WITH FASTTREE
qiime phylogeny fasttree \
  --i-alignment
  --o-tree

#ROOT PHYLOGENY AT MIDPOINT
qiime phylogeny midpoint-root \
  --i-tree
  --o-rooted-tree



### STEP 8: ASSIGN TAXONOMY WITH TRAINED CLASSIFIER ###
qiime feature-classifier classify-sklearn \
  --i-classifier silva-132-99-classifier.qza \
  --i-reads
  --o-classification
  --p-confidence
  --p-read-orientation same



### STEP 9: FILTER ARCHAEA ###
qiime taxa filter-table \
  --i-table
  --i-taxonomy
  --p-exclude Archaea \
  --p-mode contains \
  --o-filtered-table



### STEP 10: FILTER FEATURES FROM DNA SAMPLES ###
#IDENTIFY FEATURES IN DNA KIT BLANKS
qiime tools export \
  --input-path
  --output-path

biom convert \
  -i
  -o
  --to-tsv

#FILTER FEATURES FOUND IN DNA KIT BLANKS
qiime feature-table filter-features \
```

```
  --i-table
  --m-metadata-file
  --o-filtered-table



### STEP 11: RAREIFY TABLE ###
qiime feature-table rarefy \
  --i-table
  --p-sampling-depth 812 \
  --p-with-replacement \
  --o-rarefied-table



# NOTE: ALL STEPS ABOVE ARE PART OF PRE-PROCESSING. BELOW IS TAILORED TO THE DATASET AND APPROACH OF INTEREST AND, HERE, IS SPECIFIC TO THIS DATASET #



### FIGURE 1: Community similarity of egg-associated microbiota based on membership and composition ###
        #NOTE: DATA FROM THESE COMMANDS ALSO GENERATED THE DATA FOR Figure 3 and 5
#beta-diversity, unweighted UniFrac
qiime diversity beta-phylogenetic \
  --i-table
  --i-phylogeny
  --p-metric unweighted_unifrac \
  --p-variance-adjusted \
  --o-distance-matrix

qiime diversity pcoa \
  --i-distance-matrix
  --o-pcoa

qiime tools export \
  --input-path
  --output-path

# export "ordination.txt" and change all '-' in sample labels to '.' and then make q1-mapping file that cooresponds with q2-metadata file. Import both q1-compatible files #

# in QIIME1 #
make_2d_plots.py \
```

```
  -i
  -o
  -m
  -b
  --ellipsoid_opacity
  --scree

qiime diversity beta-group-significance \
  --i-distance-matrix
  --m-metadata-file
  --m-metadata-column
  --p-method permanova \
  --p-pairwise \
  --o-visualization


#beta-diversity, weighted UniFrac
qiime diversity beta-phylogenetic \
  --i-table
  --i-phylogeny
  --p-metric weighted_unifrac \
  --p-variance-adjusted \
  --o-distance-matrix

qiime diversity pcoa \
  --i-distance-matrix
  --o-pcoa

qiime tools export \
  --input-path
  --output-path

# export "ordination.txt" and change all '-' in sample labels to '.' and then make q1-mapping file
that cooresponds with q2-metadata file. Import both q1-compatible files #

# in q1 #
make_2d_plots.py \
  -i
  -o
  -m
  -b
  --ellipsoid_opacity
  --scree

qiime diversity beta-group-significance \
  --i-distance-matrix
```

```
  --m-metadata-file
  --m-metadata-column
  --p-method permanova \
  --p-pairwise \
  --o-visualization



#beta-diversity, weighted UniFrac, beta rarefaction
qiime feature-table group \
 --i-table
 --p-axis sample \
 --m-metadata-file
 --m-metadata-column Species \
 --p-mode sum \
 --o-grouped-table


qiime diversity beta-rarefaction \
  --i-table
  --p-metric weighted_unifrac \
  --p-clustering-method upgma \
  --m-metadata-file
  --p-sampling-depth 2436 \
  --i-phylogeny
  --o-visualization



 ### FIGURE 2: Diversity of the bacterial communities associated with echinoid eggs ###
          #NOTE: DATA FROM THESE COMMANDS ALSO GENERATED THE DATA FOR
Figure 4, 6, S2, S3, S4
#alpha-diversity, faith
qiime diversity alpha-phylogenetic \
  --i-table
  --i-phylogeny
  --p-metric faith_pd \
  --output-dir

qiime tools export \
  --input-path
  --output-path

#alpha-diversity, observedOTUs
qiime diversity alpha \
  --i-table
```

```
  --p-metric observed_otus \
  --output-dir

qiime tools export \
  --input-path
  --output-path

#alpha-diversity, McintoshE
qiime diversity alpha \
  --i-table
  --p-metric mcintosh_e \
  --output-dir

qiime tools export \
  --input-path
  --output-path

#alpha-diversity, McintoshD
qiime diversity alpha \
  --i-table
  --p-metric mcintosh_d \
  --output-dir

qiime tools export \
  --input-path
  --output-path



### Figure S1: Alpha rarefaction curve for the bacterial community associated with the ten
echinoid species ###
qiime diversity alpha-rarefaction \
  --i-table
  --p-max-depth 812 \
  --i-phylogeny
  --m-metadata-file
  --output-dir

qiime tools export \
  --input-path
  --output-path
```