Exploring the Stony Coral Tissue Loss Disease Bacterial Pathobiome

RUNNING TITLE: SCTLD Bacterial Pathobiome

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ABSTRACT A devastating novel coral disease outbreak, referred to as Stony Coral Tissue Loss Disease (SCTLD), was first described in 2014. It is thought to have originated offshore of Miami-Dade County, FL, but has persisted and spread, affecting new reefs along the Florida Reef Tract and reefs of at least 8 other Caribbean jurisdictions. We investigated the microbial communities of clinically normal and diseased specimens of five species of affected corals using targeted 16S ribosomal DNA sequencing (Illumina MiSeq). Fifty-nine bacterial sequences were identified using contrast analysis that had enriched abundance in diseased coral host microbiomes relative to the microbiomes of clinically normal hosts. Several sequences from known bacterial pathogens were identified in this group. Additionally, we identified fifty-three bacterial species that had differentially elevated numbers in clinically normal coral host samples relative to samples from diseased host corals. The bacterial consortia composing the clinically normal and diseased coral microbiomes were clearly distinct taxonomically. Predicted functional profiles based on taxonomy, however, were found to be quite similar. This indicates a high level of functional redundancy among diseased and clinically normal microbiome members. Further examination of the direct sequencing data revealed that while some bacteria were differentially distributed according to disease status, others were not. Fifty-one bacterial species were found in both diseased and clinically normal coral host samples and not differentially abundant in either disease state. These still may be important in explaining the presentation of disease.

IMPORTANCE Determining causation is a management top priority to guide control and intervention strategies for the SCTLD outbreak. Towards this goal we examined bacterial taxa that were differentially elevated in numbers in diseased corals as compared to clinically normal

corals at Looe Key, FL in August 2018. Many of the bacterial species we detected are known to be pathogenic to humans, animals, and (or) plants, and some of these have been found associated with diseased corals in other studies. Microbes that were present (or conspicuous by their absence) in both diseased as well as clinically normal corals were also examined because "healthy" corals from a diseased location such as Looe Key may have been exposed but may not have been showing overt disease at the time of sampling. Although untangling of causation is not possible currently, certain bacterial cliques and excess nutrients appear to be potential risk factors in SCTLD pathology.

Stony coral tissue loss disease (SCTLD) is a newly emerged lethal disease that has been affecting scleractinian corals along the Florida Reef Tract (FRT) since 2014. With white plague-like signs (https://nmsfloridakeys.blob.core.windows.net/floridakeys-prod/media/docs/20181002-stony-coral-tissue-loss-disease-case-definition.pdf), this disease outbreak was first recorded at Virginia Key near Miami (1, 2). By the winter of 2015, the disease had spread to the northern area of the Upper Keys within the Florida Keys National Marine Sanctuary. In 2016 and 2017 the disease spread north through Martin County and south through the Upper Keys (2). Between 2014-2015, SCTLD was found by W. F. Precht et al. (1) to spread at a rate of 2.5-5 km per month, but this rate of disease advancement apparently increased to 8-22 km per month in 2017-2018 as noted by K. Neely (3). In 2018, researchers were observing corals infected with SCTLD in the Middle Keys, and by December of 2019 corals infected by SCTLD were found well south of Key West and into the Marquesas Keys. Though originating in Florida, signs of SCTLD were recognized in Jamaica in 2017 and the disease now has been reportedly observed or suspected in the Mexican Caribbean, St. Maarten, the U.S. Virgin Islands, the Dominican Republic, the Turks and Caicos, Belize, the Netherlands Antilles, Puerto Rico, and the Bahamas (2).

SCTLD has been found to affect more than 20 of the 45 Caribbean reef-building coral species (stony corals), does not seem to be self-limiting as in the case of some other diseases, and does not seem to slow in cooler seasons (2). The affected species include four of seven Caribbean species that are listed as threatened (Federal Register 2006, 2014) under the Endangered Species Act of 1973 and have been further categorized as either highly susceptible, intermediately susceptible, presumed susceptible, and low susceptible species. The disease

54 causes rapid mortality among affected coral species, with a high rate of transmission and it has
55 affected a large geographic range over the more than six years it has been spreading (2). It is
56 interesting to note that neither *Acropora palmata* nor *A. cervicornis* show susceptibility to
57 SCTLD. As most reported coral diseases have low prevalence (<5%) and are not considered
58 contagious (1), this multi-year outbreak is somewhat of a unique phenomenon. The gross
59 morphological characteristics of the disease can vary among sites and species. Tissue loss often
60 appears basally, peripherally or both on a colony and spreads upward leaving intact white
61 skeleton (indicative of the rapid tissue loss) before algal colonization follows within 3-7 days.
62 Tissue loss lesions can also present as patches within intact tissue that increase in size, often
63 fusing into larger denuded areas. Histologically, the lesions appear first along the basal body
64 wall then progress upward to envelop the rest of the polyp (J. H. Landsberg, unpublished data;
65 https://nmsfloridakeys.blob.core.windows.net/floridakeys-prod/media/docs/20181002-stony-
66 coral-tissue-loss-disease-case-definition.pdf).

67 Causative agents for coral reef declines appear to be diverse and complex. Risk factors
68 include water pollution, habitat destruction, overfishing, invasive species, global climate
69 change, and coral diseases (4, 5). This outbreak has precipitated increased monitoring and
70 research efforts on SCTLD including its etiology, and transmission dynamics, along with
71 intervention actions to halt or minimize impacts. Interventions including treatment of diseased
72 colonies with specific antibiotics have been shown to retard or stop the tissue loss suggesting
73 bacterial involvement in the disease etiology (6-11). The similar disease presentation and
74 response to treatment across many coral species suggests that the same causative agent(s) are
75 at work with limited variation among coral hosts.

76 Metagenomic sequencing of the 16S rRNA gene has been widely used to study the
77 bacterial composition of multiple and varied sample types, most notably perhaps, the human
78 microbiome (12-15). Increasingly however, metagenomic studies have been made on an ever
79 broadening array of environmental microbial consortia including those associated with plants,
80 animals, water, and sediment (16-19) as well as those associated with corals (20-25).

81 The aim of this study was to identify possible causative agents for SCTLD that have been
82 affecting the Florida Reef Tract since 2014. We report here the bacterial consortia associated
83 with five SCTLD-susceptible coral species (FIG 1) collected from Looe Key, FL in 2018 during an
84 active outbreak. Direct, high throughput 16S amplicon sequencing (Illumina MiSeq) was used to
85 determine if or how microbial communities differ between clinically normal and diseased
86 corals.

87

88 **RESULTS**

89 We performed 16S sequencing of the bacterial consortia associated with diseased and
90 clinically normal specimens of five important species of Caribbean stony corals affected by
91 SCTLD to identify potential causative agents. A total of 10,081,496 sequence reads passed
92 quality filtering with 6,410,207 of these classified by the One Codex platform at the species
93 level and 1,546 total bacterial species were identified in the diseased coral samples. Bacterial
94 species detected in the clinically normal coral samples totaled 1,981. After trimming
95 uninformative species classifications with the lowest read assignments (lower 0.25%), 1,119
96 and 1,407 species classifications remained from the diseased and clinically normal samples,
97 respectively. Remaining sequence assignments for all libraries numbered minimally 96 to 1,961

98 depending on the host sample (Table 2). Thus, the classifications retained for further analyses
99 were those that were observed a reasonable number of times.

100 **Diversity analyses.** We examined the distribution of genetic diversity within samples
101 (alpha diversity) as well as among disease states (beta diversity). Alpha diversity measures
102 (Simpson; FIG 2) were somewhat variable for some species and (or) disease state replicates, but
103 these variations were not statistically significant among the coral host microbiomes that were
104 tested regardless of host species and (or) disease status. The Mann-Whitney test yielded a p-
105 value of 0.84211 for the comparison of diseased versus clinically normal alpha diversity values,
106 and evaluation of differences in alpha diversity among coral species sampled using Kruskal-
107 Wallis testing yielded a p-value of 0.50768. In contrast, beta diversity analysis of Jenson-
108 Shannon divergence measures using ANOSIM demonstrated that the microbes associated with
109 the diseased and clinically normal states were of quite different composition (FIG 3).

110 **Distribution of bacterial taxa across coral species and according to disease state.** The
111 distribution across host corals of the most commonly identified bacterial species are shown in
112 Tables 3 and 4. Two species of *Achromobacter* and three species of *Fulvivirga* were common
113 among the clinically normal coral microbiomes (Table 3) and *Achromobacter xylosoxidans* was
114 universally present. Two species of Arcobacter were common among the diseased coral
115 microbiomes with *Arcobacter bivalviorum* being a member of all diseased microbiome libraries
116 as were *Algicola bacteriolytica* and *Clostridioides difficile.* Contrast analysis using edgeR
117 revealed fifty-nine bacterial species that were enriched in abundance in diseased coral host
118 samples relative to clinically normal hosts and are shown in Table 5. Fifty-three bacterial
119 species had differentially elevated numbers in clinically normal coral host samples relative to

120 diseased host corals (Table 6), while fifty-one bacterial species were found in both diseased and
121 clinically normal coral host samples and not differentially abundant in either disease state
122 (Table 7). All the taxa listed in Table 3 were found to be present in clinically normal as well as
123 diseased samples (Table 7) except for *Fulvivirga kasyanovil*, found only in clinically normal
124 samples (Table 6). All the taxa listed in Table 4 were found to be among those elevated in
125 diseased coral samples (Table 5) except for *Arcobacter sp.* UDC415 and "*Candidatus*
126 Amoebophilus asiaticus" that were present in both clinically normal and diseased coral samples
127 (Table 7). Some bacteria known from other studies to be generally abundant and associated
128 with a wide variety of corals and other marine invertebrates (22) were rare in the Looe Key
129 coral microbiomes. *Ruegeria spp.*, for example, were relatively low in abundance and restricted
130 to the clinically normal samples (Table 6). *Endozoicomonas* species abundances in clinically
131 normal coral samples were found to be 0% in *C. natans* and *D. labyrinthiformis*, 0.186% in *D.*
132 *stokesii*, 0.013% in *O. annularis*, and 1.604% in *P. strigosa*. *Endozoicomonas* in diseased coral
133 samples was only detected in *O. annularis* at an abundance of 0.582%.

134 **Inferred expression of major gene groupings.** The inferred expression levels of the
135 Kyoto Encyclopedia of Genes and Genomes (KEGG) major gene groupings including amino acid
136 metabolism, biosynthesis of secondary metabolites, carbohydrate metabolism, energy
137 metabolism, glycan biosynthesis and metabolism, lipid metabolism, metabolism of cofactors
138 and vitamins, metabolism of amino acids, metabolism of terpenoids and polyketides,
139 nucleotide metabolism, and xenobiotics biodegradation and metabolism were similar
140 regardless of disease status and microbiome composition (FIG 4). Thus, major functionalities of

141 the members of the clinically normal coral microbiomes are also present in equivalent numbers
142 in the diseased coral microbiomes.

143

144 **DISCUSSION**

145 Stony Coral Tissue Loss Disease (SCTLD) is one of the more severe problems to confront Florida
146 and Caribbean reefs in the 21st century, potentially causing significant loss to ecosystem
147 services and impacting economies. This outbreak is unusual due to its extended duration,
148 apparent highly contagious nature, range of coral species affected, and rate of progression
149 (https://floridakeys.noaa.gov/coral-disease/). Based on reports that amoxicillin can arrest tissue
150 loss progression in several species, a number of researchers have focused on microbiome
151 analyses to identify potential causative agents. J. L. Meyer et al. (25) recently reported
152 identifying five amplicon sequence variants that were higher in abundance in diseased as
153 opposed to healthy specimens of *Montastraea cavernosa*, *Orbicella faveolata*, *Diploria*
154 *labyrinthiformis* and *Dicocoenia stokesii*. The identified amplicon sequence variants were found
155 to be from an unclassified genus of Flavobacteriales, sequences identified as *Fusibacter*
156 (Clostridiales), *Planktotalea* (Rhodobacterales), *Algicola* (Alteromonadales), and *Vibrio*
157 (Vibrionales). The authors also identified opportunistic or saprophytic colonizers including
158 *Epsilonbacteraeota, Patescibacteria, Clostridiales, Bacteroidetes*, and *Rhodobacterales* that
159 were also found to be enriched in SCTLD disease lesions.

160 **Bacterial taxa found to be differentially elevated in abundance according to disease**
161 **state.** The taxonomic classifications we report here were performed at the species level using a
162 closed reference database and a classification first approach, whereas those of J. L. Meyer et al.

163 (25) and more recently those of S. M. Rosales et al. (26) were obtained using a cluster first
164 approach. Although both strategies are widely used, each has pros and cons (27). Nevertheless,
165 many of the findings of the two studies have general similarities regarding taxa identified as
166 differentially elevated in numbers in diseased host corals. Sequence classifications that we
167 obtained from the clinically normal coral samples were found to represent mostly bacterial
168 species often found in coastal marine environments. In contrast, we found that several
169 differentially abundant sequence classifications obtained from the diseased coral samples
170 relate to bacteria with known pathogenic potential. Particularly notable are those that are
171 common in specimens with active disease signs across the five coral species studied and that
172 are relatively high in abundance. These include, but are not limited to, *Clostridioides difficile,*
173 *Romboutsia lituseburensis, Arcobacter bivalviorum, Algicola bacteriolytica, Vibrio sp.* r24*,*
174 *Shimia aquaeponti, Burkholderia gladioli, and Pseudoalteromonas haloplanktis* (Tables 4 and
175 5). In fact, *Clostridioides difficile, Arcobacter bivalviorum*, and *Algicola bacteriolytica* were found
176 without exception in every diseased coral specimen. *Burkholderia gladioli*, usually a plant
177 pathogen, produces several inhibitory and (or) toxic substances, among them gladiolin,
178 bongkrek acid, enaxyloxin, and toxoflavin which can be fatal to humans. It is unknown if these
179 toxins affect corals and (or) lower vertebrates. The presence of *Clostridioides difficile*,
180 *Romboutsia lituseburensis* and other species often associated with soil, human, and animal gut
181 consortia may implicate surface runoff and (or) fecal contamination as a contributing factor to
182 SCTLD.

183 **Bacterial taxa found not to be differentially elevated in abundance according to**
184 **disease state.** In addition to the bacterial species found to be differentially elevated in

185 abundance according to disease state, some prominent species' numbers were found to be
186 relatively constant regardless of disease state, and therefore their function(s) as microbiome
187 members are important to consider. *Achromobacter xylosoxidans, Achromobacter denitrificans,*
188 *Prosthecochloris vibrioformis,* and *"Candidatus* Amoebophilus asiaticus*"* were in this class as
189 were several species of *Halodesulfovibrio*. *Achromobacter xylosoxidans* has been identified as
190 an amoeba-resistant bacterium (ARB) in several studies (28, 29). Many free-living amoebas
191 including *Acanthamoeba, Dictyostelium, Hartmannella*, and *Naegleria* can harbor bacteria (30,
192 31). ARBs have defense mechanisms that allow them to avoid ingestion or to survive in
193 amoeba and other protists intracellularly and multiply. They may even persist through the
194 resting cyst stage of the amoebae. Bacteria that can survive within amoebae gain protection
195 from environmental insults from their amoeboid host (28, 32, 33). Because the survival
196 mechanisms possessed by ARBs are similar to traits required for survival during infection of
197 higher eukaryotes, ARBs are often found to be pathogenic (28, 29, 34-37).
198 "*Candidatus* Amoebophilus asiaticus" has been found widely in Caribbean corals (22)
199 and was found to be among the dominant members of both the clinically normal and diseased
200 Looe Key coral microbiomes sampled. Although it is an obligate endosymbiont of
201 *Acanthamoeba* of the T4 grouping, it has not been possible to transfer it to other amoeba to
202 date (38). This potentially indicates that this microbe is not widely amoeba-compatible or that
203 its primary host may be something other than amoebae. Analysis of the genome of "*Ca.*
204 Amoebophilus asiaticus" (39) revealed that it shares eukaryotic domains that are significantly
205 enriched in the genomes of other intracellular parasitic bacteria (including chlamydiae*,*
206 *Legionella pneumophila*, *Rickettsia bellii, Francisella tularensis, and Mycobacterium avium*).

207 Thus, these diverse bacteria appear to exploit common mechanisms for interaction with their
208 hosts. It is not clear if *"Ca.* Amoebophilus asiaticus" is internal to coral-associated free-living
209 amoebae, or if the bacterium infects coral amoebocytes (40). If internal, the parasitic nature of
210 this microbe might predispose corals to biotic and abiotic disease. If other amoeba-resistant
211 bacteria that are present in high abundance (i.e. *Achromobacter sp.* as shown by amoeba co-
212 culture) are also intracellular, the combination could be further debilitating. Amoebae are
213 considered "training grounds" for bacterial pathogens and "*Ca.* Amoebophilus asiaticus" has an
214 impressive number of insertion sequences in its genome (39) as does *Achromobacter* (41-43)
215 making horizontal gene transfer and dissemination of virulence elements very possible in the
216 confinement of intracellular space.
217 *Endozoicomonas,* another taxon that is known to be endosymbiotic, can be dominant in
218 many coral and marine invertebrate species (44-47) achieving abundances as high as 85% (40,
219 44). *Endozoicomonas* is known to be capable of intracellular as well as free-living lifestyles, is
220 sensitive to dysbiosis of the microbiome, and is diminished in intracellular numbers under
221 unfavorable conditions. *Endozoicomonas spp.* are generally thought to be associated with
222 healthy hosts (22, 48), but exhibit multiple relationships with their hosts, transitioning through
223 symbiotic, mutualistic, and parasitic roles opportunistically (46). This endosymbiont has been
224 thought to provide for metabolism of dimethylsulfoniopropionate (DMSP; (49-53) but genes
225 for processing of DMSP were not identified in sequenced species (45, 46). Recently, however, K.
226 Tandon et al. (54) have identified a dominant coral bacterium, *Endozoicomonas acroporae* that
227 does metabolize DMSP. Other than DMSP processing, this endosymbiont is thought to provide
228 benefit to the host including nutritional functions (55), antimicrobial functions (56), and

229 functions associated with algal symbiont interactions (44, 57). Interestingly however,
230 *Endozoicomonas* can also be a pathogen for fish (58). The finding of low *Endozoicomonas*
231 abundances (less than 1.6%) in the bacterial consortia of multiple coral species at Looe Key
232 regardless of disease state may be an indication that the "clinically normal" corals were
233 stressed and (or) infected but not showing overt disease at the time of sampling. Disease and
234 mortality continued unabated throughout 2018 as SCTLD moved southward along the Florida
235 reef tract.

236 **Potential nutrient influence on affected coral microbiomes.** Sewage contamination and
237 surface runoff has long been recognized as problematic for coral health (5). V. N. Bednarz et al.
238 (59) found that diazotroph-derived nitrogen assimilation by scleractinian corals was dependent
239 on their metabolic status. More recently, T. Lachnit et al. (60) found that coral microbiome
240 exposure to nutrient-rich conditions leads to dysbiosis and disease development. Another study
241 has examined the impacts of land-based sources of pollution on the microbiota of Southeast
242 Florida coral reefs and found that the major influence was from sewage outfalls adjacent to
243 reef tracts, but that runoff from inlet outfalls was substantial as well (61). B. E. Lapointe et al.
244 (62) have recently reported on a three-decade study of nitrogen enrichment at Looe Key and
245 found a connection with reef decline. Nutrient levels can also modulate parasitic infections as
246 shown by J. G. Klinges et al. (63) who have recently identified a marine invertebrate-associated
247 Rickettsiales parasite that the authors found is encouraged by nutrient enrichment to overgrow
248 and weaken and (or) kill the host coral cells it infests.

249 Excess nutrient levels that have been identified along the Florida Reef Tract may also
250 drive another adverse process in stony Caribbean corals that may explain SCTLD pathology.

251 *Prosthecochloris vibrioformis*, found in great abundance in all our coral specimens, is a strictly
252 anaerobic green sulfur endolithic bacterium that has nitrogen assimilation and reduction
253 pathways and produces proline in nitrogen rich environments (64). Proline has been found to
254 be essential for the growth of *C. difficile* as well as other pathogens (65). *C. difficile* is a strict
255 anaerobe that would be expected to locate in coral anoxic zones such as in the skeleton or at
256 the skeletal-tissue interface. *C. difficile* produces toxins and is also known to produce para-
257 cresol (p-cresol), a compound that is inhibitory to many Gram-negative bacteria that is thought
258 to play a role in the ability of *C. difficile* to colonize tissues (66). An abundant species that we
259 found ubiquitous in our coral specimens, *Achromobacter xylosoxidans,* can degrade p-cresol via
260 p-cresol methylhydroxylase (67) unlike many other Gram- negative bacteria and would be
261 immune to the effects of p-cresol. Additionally, although *Achromobacter xylosoxidans* is
262 generally classified as aerobic, it can function anaerobically via denitrification (41). *Clostridia*
263 have been found to be present in high abundance in coral skeleton and *Prosthecochloris*
264 *vibrioformis* is dominant in stony corals due to the density of their skeletons providing a very
265 anaerobic environment (64). Thus, it is possible that excess nutrients fuel *Prosthecochloris*
266 *vibrioformis* growth and nitrogen fixation that subsequently supports *Clostridioides difficile*
267 colonization, growth, and coral tissue damage via toxin production. The production of toxins at
268 the tissue-skeletal interface may explain the observation that SCTLD pathology first affects the
269 basal body wall of the coral polyps.

270 **Taxonomic redundancy.** Finally, we tested to what degree members of the clinically
271 normal and diseased coral microbiomes had similar inferred functionality profiles. Beta
272 diversity analysis demonstrated that the microbes associated with the two disease states were

273 quite different. The inferred expression of major gene groupings for these two groups,
274 however, was similar regardless of disease status and microbiome composition. This result is
275 similar to that found in other complex microbiomes including the human gut (13) and the algae,
276 *Ulva* (17, 68) and reveals the redundancy of metabolic potential in coral-associated bacteria
277 and the fluidity of specific bacterial species membership in the microbiomes of clinically normal
278 and diseased stony corals.

279 **Conclusion.** While disease outbreaks are not uncommon, this event is unique due to its
280 large geographic range, extended duration, rapid progression, high rates of mortality and the
281 number of species affected. The disease is thought to be caused by bacteria and can be
282 transmitted to other corals through direct contact and water circulation (11). This study reveals
283 the bacterial component of SCTLD specifically at a place and a time associated with a disease
284 that is moving rapidly both geographically and temporally (69). Our inferences are made based
285 on the abundance of bacterial sequences in diseased versus clinically normal states. Abundance
286 is not an unequivocal measure of causality, however. It is possible that some rare, but
287 influential bacterium somehow triggers remodeling of the coral microbiome (70, 71) given the
288 variety of interactions among coral-associated bacteria (72). The redundancy of the microbiome
289 members functional repertoire observed in this study as well as others (73) suggests that the
290 makeup of the coral microbiome is a rather fluid thing influenced by space, time and abiotic
291 factors. The dysbiosis generated from transient membership changes and microbiome
292 remodeling in response to environmental circumstances may result in bacterial cliques being
293 formed that collectively exhibit properties that have pathological implications for the host.

294 Unfortunately, it is not possible to pinpoint a specific causative agent for SCTLD at this
295 juncture. It is possible, however, to offer some hypotheses regarding the SCTLD pathogenesis. It
296 is tempting to conjecture, given the evidence presented here for example, that microbial
297 endoparasites (such as "*Ca.* Amoebophilus asiaticus") may predispose Caribbean corals to
298 infection by opportunistic pathogens normally held at bay by a healthy microbiome including
299 beneficial endosymbionts such as *Endozoicomonas* or that *Clostridioides difficile* introduced by
300 terrestrial or surface runoff and fueled by excess nutrients may reside in the anoxic zone at the
301 skeletal-tissue interface and produce the histopathological effects observed with SCTLD.

302 There may not be a single specific pathogen that causes SCTLD, but rather a
303 polymicrobial mixture of bacteria each adding their "special touch" to the disease process and
304 not necessarily being consistent in composition from one location to another or over time. We
305 have identified a relatively short list of bacteria associated with SCTLD pathology. Our findings
306 combined with those of prior and ongoing studies should help to guide the search for the
307 causative agent(s) of this disease and their isolation into culture for detailed study. Proteomic
308 and (or) metabolomic approaches may help to identify universal pathogenic mechanisms that
309 operate regardless of the exact microbiome composition.

310

311 **MATERIALS AND METHODS**

312 Five coral species (FIG 1) were targeted for collection on August 26, 2018 from two
313 closely situated sites (separated by only 444 meters) at Looe Key, within the Florida Keys
314 National Marine Sanctuary, (Table 1) under permit number FKNMS-2017-100, expiration date

315 December 31, 2018. Duplicate specimens from actively diseased and clinically normal coral
316 colonies were collected from *Orbicella annularis* (OANN), *Diploria labyrinthiformis* (DLAB),
317 *Colpophyllia natans* (CNAT), *Pseudodiploria strigosa* (PSTR), and *Dichocoenia stokesii* (DSTO)
318 except for *D. labyrinthiformis* for which we had only one clinically normal specimen (n=19 total
319 specimens). Coral specimens were collected in the 2-5 meter depth range on scuba using
320 hammer and clean chisels to collect fragments. Fragments were placed in zippered plastic bags
321 for transport to the surface. Each of the coral specimens was placed into a separate 5-gal
322 bucket filled with local natural seawater and covered with a lid. These samples were
323 transported (approximately 1.5 h) to the Everglades National Park, Florida Bay Interagency
324 Science Center (FBISC) in Key Largo, Florida for subsampling and processing. Samples were
325 processed for metagenomic sequencing of bacterial suspensions from coral tissue
326 homogenates. A subsample (approximately 3.25 cm^2) that included tissue and skeleton from
327 the disease margin of affected specimens or clinically normal specimens was aseptically
328 removed and homogenized in a pre-sterilized mortar and pestle by grinding the tissue section
329 in 2 mL of filter-sterilized artificial seawater (Instant Ocean Spectrum Brands, Blacksburg, VA)
330 into a slurry. The homogenates were transferred into 2 mL sterile microcentrifuge tubes and
331 centrifuged at 180 x *g* for 10 min to remove cellular debris. The supernatants (1 mL) containing
332 bacteria were then recovered, bacteria were pelleted at 10,000 x g for 5 min, and the pellets
333 were resuspended in 800 µL DNA/RNA Shield (Zymo Research, Irvine, CA) and frozen until DNA
334 was extracted and used for direct 16S amplicon sequencing.

335 **DNA extraction, amplification, and sequencing.** Genomic DNA from bacterial
336 suspensions prepared from tissue homogenates was extracted and purified using the Zymo

337 *Quick*-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research, Irvine, CA). All extracted DNA was
338 stored in the supplied kit elution buffer at -20°C until polymerase chain reaction (PCR) was
339 performed. Negative controls included kit reagent and field blanks. Positive controls were
340 provided by extracts of a ZymoBIOMICS Microbial Community Standard (D6300; Zymo
341 Research, Irvine, CA). Bacteria were characterized using PCR that targeted the V3 and V4
342 portion of the 16S rRNA gene to amplify that gene segment for subsequent Illumina MiSeq
343 analysis. Amplicons were produced in two steps, first using rRNA-specific primers (see below) to
344 generate a high concentration of input template followed by less efficient fusion primers that
345 incorporate exogenous sequencing adapters. Oligonucleotide primers for the V3-V4 16S rRNA
346 gene region (74, 75) were used to create a single amplicon of ~460 base pairs (bp). The primers
347 for the first amplification reaction were 16S Forward (5' – CCTACGGGNGGCWGCAG – 3') and
348 16S Reverse (5' – GACTACHVGGGTATCTAATCC – 3') (74, 76). Cycling parameters were: initial
349 denaturation for 3 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at
350 72°C, followed by a final extension at 72°C for 7 min. Amplicon size was confirmed by agarose
351 gel electrophoresis using 5 µL of the reaction product. PCR products were cleaned with the
352 Qiagen PCR Purification Kit (Valencia, CA) and quantified using a Qubit fluorometer (dsDNA HS
353 Assay Kit, ThermoFisher Scientific, Grand Island, NY). Samples were diluted in 10 mM Tris buffer
354 (pH 8.5) to a final concentration of 5 ng/µL. Using the 16S rRNA primers modified with the
355 sequencing adaptors specified in Illumina's 16S Metagenomic Sequencing Library Preparation
356 (CT #: 15044223 Rev. B), amplicon libraries were prepared following the manufacturer's
357 protocol. Each sample was indexed with Illumina's Nextera XT multiplex library indices, which
358 incorporates two distinct 8 bp sequences on each end of the fragment. Libraries were

359 quantified fluorometrically, as above. DNA size distribution was determined with an Agilent
360 2100 Bioanalyzer using the Agilent DNA 1000 Kit (Santa Clara, CA). The combined pool of
361 indexed libraries was diluted to 4 nM using 10 mM Tris pH 8.5. A final 10 pM preparation was
362 created with a 15% PhiX control spike and analyzed on a MiSeq 600 v3 cartridge. DNA
363 sequences (16S) derived from the bacterial consortia of clinically normal and diseased coral
364 samples as well as reagent and field blanks were treated identically.

365 **Sequence analysis.** Sequences were classified taxonomically using the One Codex
366 (https://www.onecodex.com/) pipeline (27, 77) by uploading machine-processed, quality
367 filtered, and de-multiplexed FASTQ files to the One Codex web site for processing against the
368 curated targeted database available there. Classification assignments were then downloaded
369 from the One Codex web site as Microsoft Excel files. Low-abundance, non-informative
370 classifications were removed from all data sets by trimming taxonomic assignments of
371 sequencing reads from the bacterial component of each coral host to exclude those whose
372 frequency was less than 0.25% of the total classification assignments associated with that coral
373 host. Remaining classifications were then combined to obtain master lists of bacterial species
374 found in clinically normal and diseased corals and ranked according to the number of reads
375 classified to each member. Excel pivot tables were used to examine the distribution of
376 identified bacterial species across coral species.

377 **Statistical analysis, diversity, and functional profiling inferred from bacterial**
378 **taxonomy.** Sequence classifications filtered as described above were also imported into
379 MicrobiomeAnalyst (78) for further analyses including alpha and beta diversity to examine
380 within and among sample diversity respectively, statistical analysis of bacterial association with

381 disease state, and inference of biochemical pathways expressed in the consortia. Classifications
382 additionally were filtered to remove those with a prevalence of less than 20% across samples
383 and features with low variance (less than 10% based on the interquartile range). Data were
384 transformed using the centered log ratio (CLR) method (79). Alpha diversity was calculated
385 using the Simpson Index expressed as the compliment (ranging between zero and one where
386 larger is more diverse) and tested for significance using Mann-Whitney or Kruskal-Wallis tests
387 as appropriate. Beta diversity was examined using Analysis of Group Similarities (80) of Jenson-
388 Shannon Divergence measures and visualized by NMDS (Nonmetric Multidimensional Scaling).
389 Differences in the bacterial community composition between diseased and clinically normal
390 corals were examined using the edgeR (81) method with an adjusted p value of 0.01. Finally,
391 gene expression was inferred using the Tax4Fun module (82) of the MicrobiomeAnalyst
392 package. The KEGG KO assignments that were generated were then assigned to major
393 functional groupings and visualized with the MicrobiomeAnalyst Shotgun Data Profiling
394 routines.

395 **Data availability.** Raw sequences are available in the National Center for Biotechnology
396 Information (NCBI) Sequence Read Archive (SRA) under project number PRJNA625928,
397 accession numbers SAMN14596532 to SAMN14596550.

398

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656

Table 1. Site number, latitude, longitude, Scientific Name, Abbreviation, Common Name, Status (CN = Clinically Normal, D = Diseased), Depth of Collection (meters), and sample identification of 19 corals collected from Looe Key, Florida.

Site	Latitude	Longitude	Scientific Name	Abbreviation	Common Name	Status	Depth (m)	Sample ID
1	N 24° 32' 40.812''	W 81° 24' 30.96''	*Colpophyllia natans*	CNAT	boulder brain coral	D	6.71	CNAT_DisBgF
2	N 24° 32' 44.988''	W 81° 24' 15.84''				D	7.01	CNAT_DisBgBBUnl
2	N 24° 32' 44.988''	W 81° 24' 15.84''				CN	6.40	CNATHC-8
2	N 24° 32' 44.988''	W 81° 24' 15.84''				CN	5.18	CNATHC-9
1	N 24° 32' 40.812''	W 81° 24' 30.96''	*Dichocoenia stokesii*	DSTO	elliptical star coral	D	5.49	DSTO_DisBgJ
1	N 24° 32' 40.812''	W 81° 24' 30.96''				D	5.49	DSTO_DisBgL
1	N 24° 32' 40.812''	W 81° 24' 30.96''				CN	5.49	DSTOHC-1
1	N 24° 32' 40.812''	W 81° 24' 30.96''				CN	5.49	DSTOHC-3
1	N 24° 32' 40.812''	W 81° 24' 30.96''	*Diploria labyrinthiformis*	DLAB	grooved brain coral	D	6.40	DLAB_DisBgA
2	N 24° 32' 44.988''	W 81° 24' 15.84''				D	5.79	DLAB_DisBgE
2	N 24° 32' 44.988''	W 81° 24' 15.84''				CN	7.01	DLABHC-5
1	N 24° 32' 40.812''	W 81° 24' 30.96''	*Orbicella annularis*	OANN	lobed star coral	D	7.01	OANN_DisUNLB
1	N 24° 32' 40.812''	W 81° 24' 30.96''				D	7.01	OANN_DisBgD
2	N 24° 32' 44.988''	W 81° 24' 15.84''				CN	5.49	OANNHC-6
2	N 24° 32' 44.988''	W 81° 24' 15.84''				CN	5.79	OANNHC-7
1	N 24° 32' 40.812''	W 81° 24' 30.96''	*Pseudodiploria strigosa*	PSTR	symmetrical brain coral	D	5.49	PSTR_DisBgH
1	N 24° 32' 40.812''	W 81° 24' 30.96''				D	5.18	PSTR_DisBgI
1	N 24° 32' 40.812''	W 81° 24' 30.96''				CN	5.49	PSTRHC-2
1	N 24° 32' 40.812''	W 81° 24' 30.96''				CN	5.49	PSTRHC-4

Table 2. Total numbers of classified reads for each library type, reads trimmed (0.25% of total bacterial reads classified for each coral host species), and classified reads retained for analysis. CNAT = *Colpophyllia natans*: DLAB = *Diploria labyrinthiformis*; DSTO = *Dichocoenia stokesii*; OANN = *Orbicella annularis*; and PSTR = *Pseudodiploria strigosa*.

		Host Coral				
Library Type	**Sequence Reads**	**CNAT**	**DLAB**	**DSTO**	**OANN**	**PSTR**
Diseased	Total	420,249	66,222	204,950	784,580	226,004
	Trimmed	< 1,051	< 166	< 512	< 1,961	< 565
	Retained	419,198	66,056	204,438	782,619	225,438
Clinically Normal	Total	462,966	85,138	77,018	38,349	121,738
	Trimmed	< 1,157	< 213	< 193	< 96	< 304
	Retained	461,808	84,925	76,825	38,253	121434

Table 3. Distribution of classified bacterial sequences across clinically normal host coral species. CNAT = *Colpophyllia natans*: DLAB = *Diploria labyrinthiformis*; DSTO = *Dichocoenia stokesii*; OANN = *Orbicella annularis*; and PSTR = *Pseudodiploria strigosa*.

Microbe	CNAT	DLAB	DSTO	OANN	PSTR	Species Affected
Achromobacter xylosoxidans	X	X	X	X	X	5
Achromobacter denitrificans	X	X		X	X	4
***"Candidatus* Amoebophilus asiaticus"**	X	X		X	X	4
Fulvivirga imtechensis	X	X		X	X	4
Fulvivirga kasyanovii	X	X		X	X	4
Fabibacter misakiensis		X		X	X	3
Fulvivirga lutimaris	X	X		X		3
Nitrincola lacisaponensis	X		X		X	3
Rhodospirillum rubrum	X	X		X		3
Sinorhizobium fredii	X		X		X	3
171 More- found on one or two hosts						

Table 4. Distribution of classified bacterial sequences across diseased coral hosts. CNAT = *Colpophyllia natans*: DLAB = *Diploria labyrinthiformis*; DSTO = *Dichocoenia stokesii*; OANN = *Orbicella annularis*; and PSTR = *Pseudodiploria strigosa*.

Microbe	CNAT	DLAB	DSTO	OANN	PSTR	Species Affected
Algicola bacteriolytica	X	X	X	X	X	5
Arcobacter bivalviorum	X	X	X	X	X	5
Clostridioides difficile	X	X	X	X	X	5
Arcobacter sp. UDC415		X	X	X	X	4
***"Candidatus* Amoebophilus asiaticus"**	X	X	X	X		4
Pseudofulvibacter geojedonensis		X	X		X	3
Romboutsia lituseburensis	X		X		X	3
Shimia aquaeponti	X		X	X		3
Vibrio sp. r24	X		X		X	3
95 More- found on one or two hosts						

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Table 5. Bacterial species enriched in diseased coral hosts as determined by edgeR contrast analysis. Designations are predicated at a false discovery rate (FDR) < 0.01. Shown are the logarithm of 2-fold change (log2FC), the logarithm of counts per million reads (log_2CPM), the Benjamini-Hochberg (B-H) adjusted P value (Pvalue), and the FDR. The table is sorted from highest to lowest abundance (log_2CPM).

Bacterial Species	log2FC	Log_2CPM	Pvalue	FDR
Clostridioides difficile	5.832	16.425	3.160E-06	9.071E-06
Arcobacter bivalviorum	11.320	16.378	1.253E-11	2.042E-09
Tepidibacter mesophilus	8.471	13.556	1.669E-06	5.440E-06
Algicola bacteriolytica	4.113	13.416	1.801E-03	2.718E-03
Shimia aquaeponti	8.262	13.341	1.943E-07	1.131E-06
Vibrio sp. r24	8.190	13.263	1.752E-08	2.561E-07
Marinilabilia nitratireducens	7.999	13.082	3.786E-07	1.870E-06
Burkholderia gladioli	7.912	12.996	4.535E-07	2.174E-06
Pseudofulvibacter geojedonensis	7.795	12.885	2.781E-06	8.243E-06
Marinifilum fragile	7.756	12.845	1.299E-06	4.504E-06
Staphylococcus epidermidis	3.957	12.802	4.630E-03	6.799E-03
Vibrio nigripulchritudo	7.559	12.638	3.033E-08	3.166E-07
Pseudahrensia aquimaris	7.477	12.577	1.191E-05	2.520E-05
Tenacibaculum litopenaei	7.367	12.464	2.712E-06	8.185E-06
Abyssivirga alkaniphila	4.250	12.215	2.063E-03	3.084E-03
Amphritea japonica	7.080	12.169	5.726E-08	5.490E-07
Romboutsia lituseburensis	7.009	12.094	1.885E-08	2.561E-07
Vibrio alginolyticus	6.990	12.070	2.569E-09	6.978E-08
Carboxylicivirga taeanensis	6.870	11.979	6.971E-06	1.623E-05
Meridianimaribacter flavus	6.601	11.713	1.571E-06	5.226E-06
Rhizobium sp. Trp 3	6.397	11.519	4.939E-06	1.258E-05
Vibrio harveyi	6.314	11.409	2.097E-09	6.836E-08
Arcobacter molluscorum	6.236	11.364	4.615E-06	1.194E-05
Mesorhizobium sp.	6.210	11.341	9.035E-06	2.045E-05

Yersinia enterocolitica	6.165	11.286	5.160E-07	2.336E-06
Shimia isoporae	6.062	11.193	1.990E-06	6.360E-06
Natronoflexus pectinivorans	5.979	11.117	6.027E-06	1.489E-05
Vibrio parahaemolyticus	5.961	11.072	4.088E-09	9.519E-08
Fusibacter paucivorans	5.876	11.019	4.533E-06	1.192E-05
Eubacterium tenue	5.801	10.948	3.972E-06	1.061E-05
Aestuariibacter halophilus	5.789	10.941	1.074E-05	2.399E-05
Lentibacter algarum	5.586	10.749	8.574E-06	1.968E-05
Clostridium thermosuccinogenes	5.576	10.732	2.431E-06	7.621E-06
Pseudoalteromonas haloplanktis	5.541	10.685	7.903E-08	6.134E-07
Mesorhizobium loti	5.431	10.593	8.378E-07	3.339E-06
Oceanirhabdus sediminicola	5.420	10.593	1.106E-05	2.403E-05
Thalassolituus oleivorans	5.405	10.553	3.108E-08	3.166E-07
Ketogulonicigenium vulgare	5.362	10.521	1.754E-07	1.077E-06
Tissierella creatinini	5.257	10.443	1.833E-05	3.727E-05
Vibrio vulnificus	5.169	10.334	2.574E-08	2.997E-07
Alkaliphilus peptidifermentans	5.054	10.241	5.114E-07	2.336E-06
Roseovarius aestuarii	4.988	10.185	9.409E-07	3.573E-06
Bythopirellula goksoyri	4.962	10.180	3.012E-05	5.644E-05
Tissierella praeacuta	4.791	10.023	2.896E-05	5.489E-05
Myroides indicus	4.649	9.901	4.199E-05	7.690E-05
Tindallia magadiensis	4.619	9.870	1.852E-05	3.727E-05
Marinifilum albidiflavum	4.582	9.837	1.589E-05	3.278E-05
Epibacterium multivorans	4.538	9.796	1.096E-05	2.403E-05
Rhizobium tropici	4.513	9.774	1.125E-05	2.412E-05
Cohaesibacter gelatinilyticus	4.399	9.684	4.486E-05	8.124E-05
Amphritea ceti	4.285	9.572	6.391E-06	1.551E-05
Hoeflea phototrophica	4.195	9.521	2.868E-04	4.583E-04
Cohaesibacter haloalkalitolerans	4.186	9.505	8.503E-05	1.444E-04

Pseudoteredinibacter isoporae	4.144	9.473	1.133E-04	1.903E-04
Vibrio cholerae	4.140	9.464	5.581E-05	9.996E-05
Clostridium scindens	4.040	9.388	2.032E-04	3.280E-04
Sunxiuqinia elliptica	3.992	9.352	3.222E-04	5.098E-04
Streptomyces lazureus	3.931	9.297	1.627E-04	2.652E-04
Kordiimonas gwangyangensis	3.665	9.089	3.531E-04	5.533E-04

Table 6. Bacterial species enriched in clinically normal coral hosts as determined by edgeR contrast analysis. Designations are predicated at a false discovery rate (FDR) < 0.01. Shown are the logarithm of 2-fold change (log2FC), the logarithm of counts per million reads (log_2CPM), the Benjamini-Hochberg (B-H) adjusted P value (Pvalue), and the FDR. The table is sorted from highest to lowest abundance (log_2CPM).

Bacterial Species	log2FC	Log_2CPM	Pvalue	FDR
Heliothrix oregonensis	-10.362	15.430	6.492E-08	5.569E-07
Aureivirga marina	-10.051	15.121	2.288E-07	1.243E-06
Blastocatella fastidiosa	-3.996	14.774	5.076E-03	7.388E-03
Chloroflexus aurantiacus	-3.283	13.728	4.550E-03	6.742E-03
Mycoplasma mycoides	-4.034	13.702	1.188E-03	1.809E-03
Spirosoma navajo	-8.304	13.383	2.275E-08	2.852E-07
Muricauda lutaonensis	-7.559	12.652	9.644E-07	3.573E-06
Iamia majanohamensis	-7.537	12.632	1.785E-07	1.077E-06
Streptococcus sanguinis	-7.125	12.227	9.605E-07	3.573E-06
Candidatus Solibacter usitatus	-6.976	12.083	3.459E-07	1.762E-06
Spirochaeta halophila	-6.873	11.983	2.153E-07	1.210E-06
Bacillus licheniformis	-6.848	11.958	1.708E-07	1.077E-06
Klebsiella pneumoniae	-6.813	11.925	8.615E-09	1.560E-07
Vibrio sp. Cl G9	-6.470	11.595	3.172E-06	9.071E-06
Marinoscillum furvescens	-6.464	11.591	7.350E-07	3.153E-06
Dictyoglomus turgidum	-6.451	11.575	6.448E-08	5.569E-07
Candidatus Phytoplasma fraxini	-6.442	11.567	5.657E-09	1.153E-07
Fulvivirga kasyanovii	-6.435	11.563	1.025E-07	7.596E-07
Candidatus Liberibacter asiaticus	-6.419	11.545	1.214E-08	1.979E-07
Aeromonas caviae	-6.406	11.533	1.586E-07	1.077E-06
Aciditerrimonas ferrireducens	-6.282	11.421	2.604E-06	8.009E-06
Arthrobacter davidanieli	-6.194	11.331	6.928E-07	3.052E-06
Labilibacter aurantiacus	-5.993	11.144	6.794E-06	1.605E-05
Tamlana agarivorans	-5.991	11.140	1.557E-06	5.226E-06

Oenococcus oeni	-5.984	11.133	7.049E-08	5.745E-07
Algisphaera agarilytica	-5.923	11.079	8.398E-07	3.339E-06
Eionea nigra	-5.829	10.990	6.472E-06	1.551E-05
Ilumatobacter fluminis	-5.552	10.737	3.232E-06	9.082E-06
Sinorhizobium medicae	-5.397	10.586	1.837E-10	1.497E-08
Pseudomonas marincola	-5.360	10.558	9.923E-07	3.594E-06
Gloeobacter violaceus	-5.269	10.472	2.584E-07	1.359E-06
Pseudomonas putida	-5.245	10.452	1.275E-06	4.504E-06
Moraxella catarrhalis	-5.109	10.326	7.350E-10	3.993E-08
Bartonella bacilliformis	-5.068	10.290	1.052E-09	4.288E-08
Bdellovibrio bacteriovorus	-4.794	10.052	1.997E-05	3.970E-05
Pectobacterium atrosepticum	-4.780	10.040	1.274E-05	2.662E-05
Sulfobacillus thermosulfidooxidans	-4.777	10.036	8.062E-07	3.339E-06
Serratia liquefaciens	-4.732	9.998	3.617E-06	9.955E-06
Haloferula chungangensis	-4.553	9.845	2.867E-05	5.489E-05
Roseivirga marina	-4.509	9.807	5.499E-06	1.379E-05
Thioprofundum lithotrophicum	-4.437	9.743	1.379E-07	9.776E-07
Aridibacter kavangonensis	-4.347	9.671	3.665E-06	9.955E-06
Coxiella burnetii	-4.084	9.456	4.122E-05	7.635E-05
Gimesia maris	-4.078	9.452	2.079E-05	4.084E-05
Thalassotalea agarivorans	-4.006	9.393	6.572E-05	1.152E-04
Roseivirga spongicola	-3.970	9.365	2.736E-05	5.309E-05
Lawsonella clevelandensis	-3.882	9.296	8.244E-05	1.415E-04
Spiribacter curvatus	-3.762	9.203	7.087E-05	1.229E-04
Ruegeria intermedia	-3.647	9.113	5.797E-05	1.027E-04
Lewinella cohaerens	-3.603	9.083	1.482E-04	2.441E-04
Ekhidna lutea	-3.299	8.862	1.211E-04	2.015E-04
Gluconobacter oxydans	-3.248	8.824	5.105E-04	7.849E-04
Portibacter lacus	-3.118	8.736	3.608E-04	5.601E-04

Table 7. Bacterial species present in both diseased and clinically normal coral host samples. Designations are predicated at a false discovery rate (FDR) < 0.01. Shown are the logarithm of 2-fold change (log2FC), the logarithm of counts per million reads (log_2CPM), the Benjamini-Hochberg (B-H) adjusted P value (Pvalue), and the false discovery rate (FDR). The table is sorted from highest to lowest abundance (log_2CPM).

Bacterial Species	log2FC	logCPM	Pvalue	FDR
Prosthecochloris vibrioformis	-0.204	16.350	8.764E-01	8.929E-01
"Candidatus Amoebophilus asiaticus*"*	-2.296	16.193	7.091E-02	9.713E-02
Arcobacter sp. UDC415	2.027	15.337	1.499E-01	1.894E-01
Arcobacter nitrofigilis	1.707	14.929	3.458E-01	3.969E-01
Rhodospirillum rubrum	-2.420	14.804	7.877E-02	1.061E-01
Achromobacter xylosoxidans	-1.509	14.608	1.575E-01	1.975E-01
Aquimarina muelleri	1.041	13.905	5.184E-01	5.788E-01
Roseivirga seohaensis	-0.235	13.847	8.593E-01	8.865E-01
Halodesulfovibrio spirochaetisodalis	2.843	13.371	6.094E-02	8.563E-02
Tepidicaulis marinus	3.848	13.261	7.222E-03	1.042E-02
Candidatus Pelagibacter ubique	-1.488	13.253	2.255E-01	2.723E-01
Fulvivirga imtechensis	-1.281	12.590	2.811E-01	3.344E-01
Halodesulfovibrio oceani	-0.620	12.524	6.381E-01	6.843E-01
Halodesulfovibrio marinisediminis	-2.349	12.495	8.180E-02	1.093E-01
Achromobacter denitrificans	-1.600	12.418	1.092E-01	1.435E-01
Pectobacterium carotovorum	0.404	12.300	7.613E-01	7.955E-01
Pirellula sp.	0.468	12.241	6.948E-01	7.402E-01
Pseudovibrio denitrificans	2.105	12.177	1.152E-01	1.467E-01
Aquisalinus flavus	1.354	12.177	3.023E-01	3.530E-01
Alteromonas macleodii	-0.096	12.151	9.073E-01	9.073E-01
Wukongibacter baidiensis	-0.132	12.088	9.050E-01	9.073E-01
Kiloniella spongiae	1.650	12.028	2.665E-01	3.194E-01
Clostridium botulinum	-0.555	11.918	5.812E-01	6.428E-01
Nitrincola lacisaponensis	-1.234	11.911	1.692E-01	2.074E-01

Rubritalea tangerina	1.993	11.909	8.334E-02	1.104E-01
Azospirillum brasilense	-2.278	11.752	4.922E-02	7.013E-02
Thalassobius mediterraneus	1.691	11.707	1.601E-01	1.987E-01
Achromobacter marplatensis	-1.708	11.609	7.195E-02	9.774E-02
Pseudoalteromonas tetraodonis	1.576	11.576	4.948E-02	7.013E-02
Microcystis aeruginosa	-0.183	11.557	8.340E-01	8.659E-01
Fulvivirga lutimaris	-1.187	11.513	3.032E-01	3.530E-01
Sinorhizobium fredii	-0.496	11.482	5.837E-01	6.428E-01
Fabibacter misakiensis	-0.160	11.468	8.690E-01	8.908E-01
Riemerella anatipestifer	0.478	11.461	7.176E-01	7.595E-01
Prosthecochloris indica	0.906	11.447	4.719E-01	5.305E-01
Mesoplasma florum	-1.018	11.403	3.309E-01	3.826E-01
Amphiplicatus metriothermophilus	0.592	11.367	6.276E-01	6.774E-01
Halodesulfovibrio aestuarii	1.369	11.365	3.006E-01	3.530E-01
Hoeflea halophila	-0.880	11.325	4.411E-01	4.993E-01
Aeromonas salmonicida	0.162	11.313	8.865E-01	8.975E-01
Anaplasma platys	-1.428	11.310	1.609E-01	1.987E-01
Phyllobacterium leguminum	2.050	11.304	1.137E-01	1.467E-01
Thermanaerovibrio acidaminovorans	-2.019	11.214	6.707E-02	9.264E-02
Pelagibius litoralis	-1.730	11.175	1.149E-01	1.467E-01
Mycoplasma gallisepticum	-1.600	11.071	6.322E-02	8.808E-02
Woeseia oceani	0.591	11.061	6.016E-01	6.537E-01
Bartonella henselae	-0.495	10.981	5.985E-01	6.537E-01
Lentisphaera profundi	1.572	10.951	1.150E-01	1.467E-01
Natranaerovirga hydrolytica	1.088	10.929	3.718E-01	4.238E-01
Acinetobacter radioresistens	-0.387	10.845	7.250E-01	7.624E-01
Thermosynechococcus elongatus	1.385	10.806	1.772E-01	2.155E-01

FIG 1. Representative samples of five coral species affected by Stony Coral Tissue Loss Disease (SCTLD) at Looe Key, FL in August 2018.

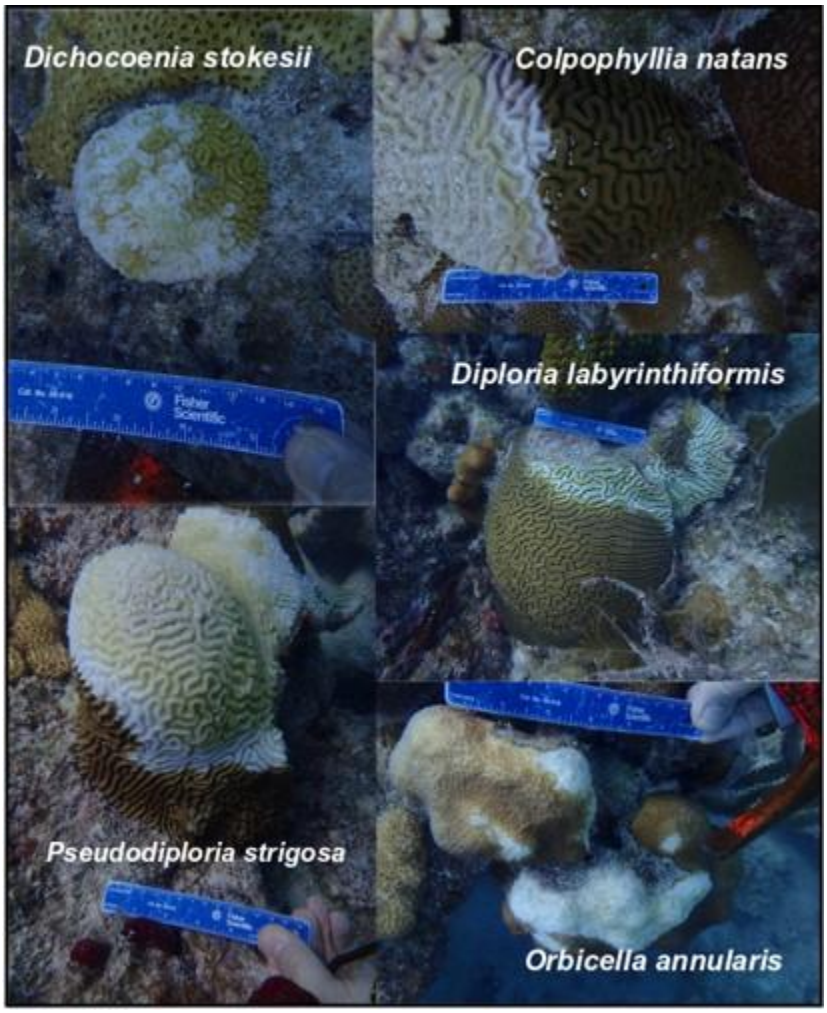

FIG 2. Alpha diversity as measured by the Simpson index was generally similar among the microbiomes of all five coral hosts species regardless of health status.

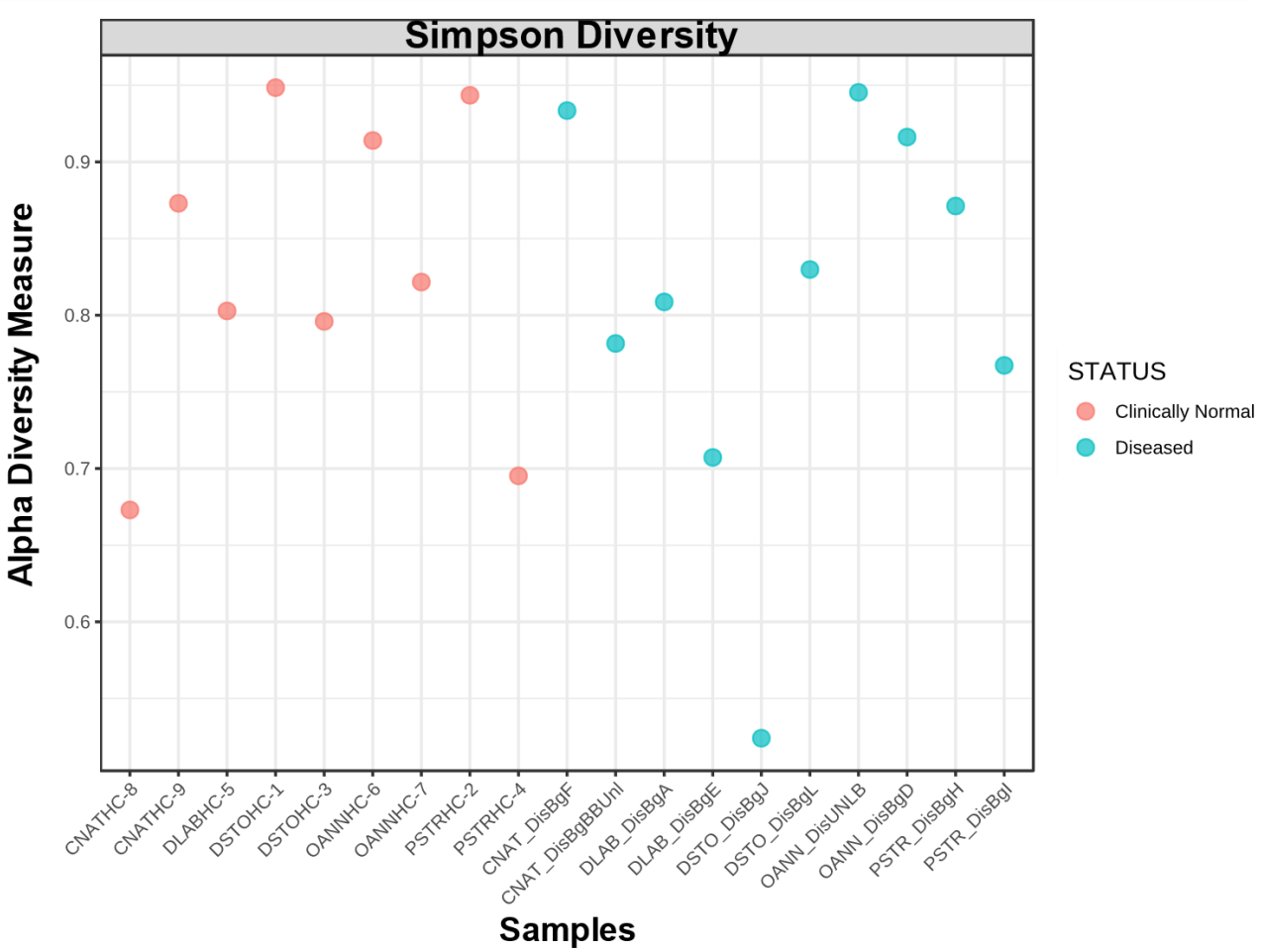

FIG 3. Nonmetric multidimensional scaling visualization of Jenson-Shannon Divergence measures using ANOSIM demonstrated that the microbiome taxa of five stony coral species affected by SCTLD are distinct between clinically normal and diseased colonies.

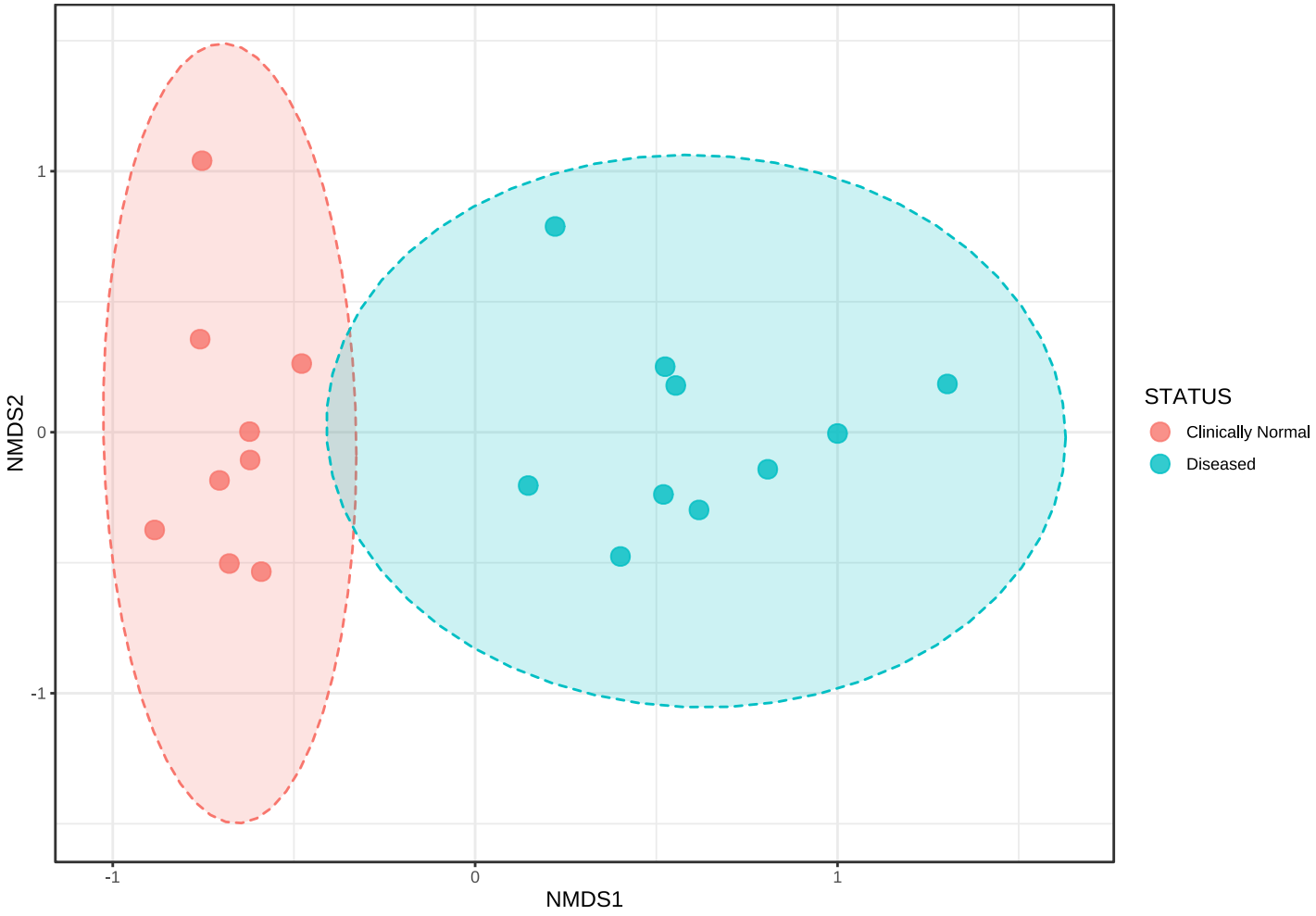
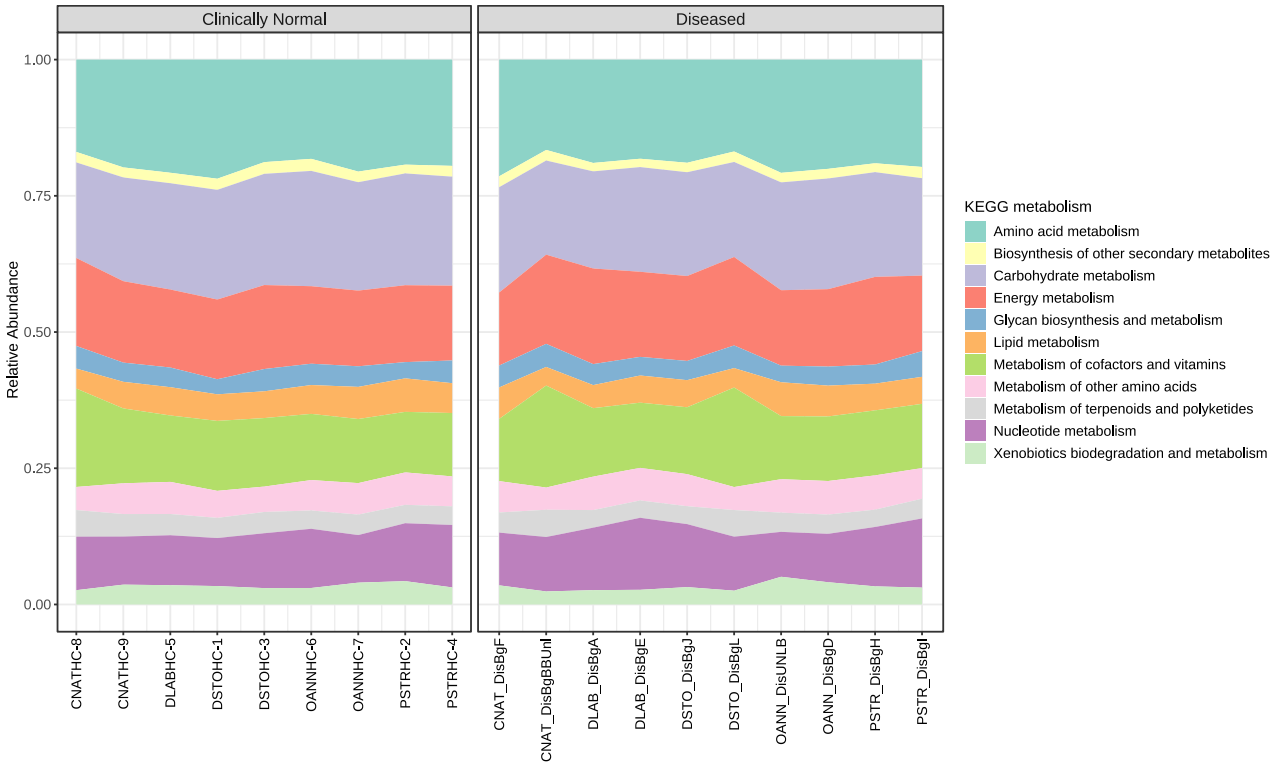
FIG 4. KEGG metabolism comparison of clinically normal and diseased coral microbiomes. Gene transcription was inferred using Tax4Fun.