Exploring the Stony Coral Tissue Loss Disease Bacterial Pathobiome

RUNNING TITLE: SCTL D 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

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

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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

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lesions appear first along the basal body wall then progress upward to envelop the rest of the polyp (J. H. Landsberg, unpublished data; 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

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

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

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

RESULTS

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

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

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

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

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

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

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

DISCUSSION

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

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

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

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

abundance according to disease state, some prominent species' numbers were found to be relatively constant regardless of disease state, and therefore their function(s) as microbiome members are important to consider. *Achromobacter xylosoxidans*, *Achromobacter denitrificans*, *Prosthecochloris vibrioformis*, and "*Candidatus* Amoebophilus asiaticus" were in this class as were several species of *Halodesulfovibrio*. *Achromobacter xylosoxidans* has been identified as an amoeba-resistant bacterium (ARB) in several studies (28, 29). Many free-living amoebas including *Acanthamoeba*, *Dictyostelium*, *Hartmannella*, and *Naegleria* can harbor bacteria (30, 31). ARBs have defense mechanisms that allow them to avoid ingestion or to survive in amoeba and other protists intracellularly and multiply. They may even persist through the resting cyst stage of the amoebae. Bacteria that can survive within amoebae gain protection from environmental insults from their amoeboid host (28, 32, 33). Because the survival mechanisms possessed by ARBs are similar to traits required for survival during infection of higher eukaryotes, ARBs are often found to be pathogenic (28, 29, 34-37).

"*Candidatus* Amoebophilus asiaticus" has been found widely in Caribbean corals (22) and was found to be among the dominant members of both the clinically normal and diseased Looe Key coral microbiomes sampled. Although it is an obligate endosymbiont of *Acanthamoeba* of the T4 grouping, it has not been possible to transfer it to other amoeba to date (38). This potentially indicates that this microbe is not widely amoeba-compatible or that its primary host may be something other than amoebae. Analysis of the genome of "*Ca.* Amoebophilus asiaticus" (39) revealed that it shares eukaryotic domains that are significantly enriched in the genomes of other intracellular parasitic bacteria (including chlamydiae, *Legionella pneumophila*, *Rickettsia bellii*, *Francisella tularensis*, and *Mycobacterium avium*).

Thus, these diverse bacteria appear to exploit common mechanisms for interaction with their hosts. It is not clear if “*Ca. Amoebophilus asiaticus*” is internal to coral-associated free-living amoebae, or if the bacterium infects coral amoebocytes (40). If internal, the parasitic nature of this microbe might predispose corals to biotic and abiotic disease. If other amoeba-resistant bacteria that are present in high abundance (i.e. *Achromobacter* sp. as shown by amoeba co-culture) are also intracellular, the combination could be further debilitating. Amoebae are considered “training grounds” for bacterial pathogens and “*Ca. Amoebophilus asiaticus*” has an impressive number of insertion sequences in its genome (39) as does *Achromobacter* (41-43) making horizontal gene transfer and dissemination of virulence elements very possible in the confinement of intracellular space.

Endozoicomonas, another taxon that is known to be endosymbiotic, can be dominant in many coral and marine invertebrate species (44-47) achieving abundances as high as 85% (40, 44). *Endozoicomonas* is known to be capable of intracellular as well as free-living lifestyles, is sensitive to dysbiosis of the microbiome, and is diminished in intracellular numbers under unfavorable conditions. *Endozoicomonas* spp. are generally thought to be associated with healthy hosts (22, 48), but exhibit multiple relationships with their hosts, transitioning through symbiotic, mutualistic, and parasitic roles opportunistically (46). This endosymbiont has been thought to provide for metabolism of dimethylsulfoniopropionate (DMSP; (49-53) but genes for processing of DMSP were not identified in sequenced species (45, 46). Recently, however, K. Tandon et al. (54) have identified a dominant coral bacterium, *Endozoicomonas acroporae* that does metabolize DMSP. Other than DMSP processing, this endosymbiont is thought to provide benefit to the host including nutritional functions (55), antimicrobial functions (56), and

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

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

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

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

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

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

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

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

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

MATERIALS AND METHODS

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

December 31, 2018. Duplicate specimens from actively diseased and clinically normal coral colonies were collected from *Orbicella annularis* (OANN), *Diploria labyrinthiformis* (DLAB), *Colpophyllia natans* (CNAT), *Pseudodiploria strigosa* (PSTR), and *Dichocoenia stokesii* (DSTO) except for *D. labyrinthiformis* for which we had only one clinically normal specimen (n=19 total specimens). Coral specimens were collected in the 2-5 meter depth range on scuba using hammer and clean chisels to collect fragments. Fragments were placed in zippered plastic bags for transport to the surface. Each of the coral specimens was placed into a separate 5-gal bucket filled with local natural seawater and covered with a lid. These samples were transported (approximately 1.5 h) to the Everglades National Park, Florida Bay Interagency Science Center (FBISC) in Key Largo, Florida for subsampling and processing. Samples were processed for metagenomic sequencing of bacterial suspensions from coral tissue homogenates. A subsample (approximately 3.25 cm²) that included tissue and skeleton from the disease margin of affected specimens or clinically normal specimens was aseptically removed and homogenized in a pre-sterilized mortar and pestle by grinding the tissue section in 2 mL of filter-sterilized artificial seawater (Instant Ocean Spectrum Brands, Blacksburg, VA) into a slurry. The homogenates were transferred into 2 mL sterile microcentrifuge tubes and centrifuged at 180 x g for 10 min to remove cellular debris. The supernatants (1 mL) containing bacteria were then recovered, bacteria were pelleted at 10,000 x g for 5 min, and the pellets were resuspended in 800 µL DNA/RNA Shield (Zymo Research, Irvine, CA) and frozen until DNA was extracted and used for direct 16S amplicon sequencing.

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

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

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

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

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

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

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

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407

408 Roles in this research were equally shared by five scientists. W.B.S provided conceptualization,
409 data curation, formal analysis, investigation, methodology, validation, visualization and writing
410 the original draft manuscript. D.D.I. provided data curation, investigation, resources, writing –
411 review and editing. K.M.B. provided funding acquisition, investigation, project administration,
412 resources, supervision and writing – review and editing. C.M.W. provided funding acquisition,
413 investigation, project administration, resources, and writing in review and editing. A.B. provided
414 project administration and resources. K.N. provided resources and writing – review and editing.

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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"	<i>Colpophyllia natans</i>	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"	<i>Dichocoenia stokesii</i>	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"	<i>Diploria labyrinthiformis</i>	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"	<i>Orbicella annularis</i>	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"	<i>Pseudodiploria strigosa</i>	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	121,434

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
<i>Achromobacter xylosoxidans</i>	X	X	X	X	X	5
<i>Achromobacter denitrificans</i>	X	X		X	X	4
" <i>Candidatus Amoebophilus asiaticus</i> "	X	X		X	X	4
<i>Fulvivirga imtechensis</i>	X	X		X	X	4
<i>Fulvivirga kasyanovii</i>	X	X		X	X	4
<i>Fabibacter misakiensis</i>		X		X	X	3
<i>Fulvivirga lutimaris</i>	X	X		X		3
<i>Nitrincola lacisaponensis</i>	X		X		X	3
<i>Rhodospirillum rubrum</i>	X	X		X		3
<i>Sinorhizobium fredii</i>	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
<i>Algicola bacteriolytica</i>	X	X	X	X	X	5
<i>Arcobacter bivalviorum</i>	X	X	X	X	X	5
<i>Clostridioides difficile</i>	X	X	X	X	X	5
<i>Arcobacter</i> sp. UDC415		X	X	X	X	4
" <i>Candidatus</i> Amoebophilus asiaticus"	X	X	X	X		4
<i>Pseudofulvibacter geojedonensis</i>		X	X		X	3
<i>Romboutsia lituseburensis</i>	X		X		X	3
<i>Shimia aquaeponi</i>	X		X	X		3
<i>Vibrio</i> sp. r24	X		X		X	3
95 More- found on one or two hosts						

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 (log2CPM), the Benjamini-Hochberg (B-H) adjusted P value (Pvalue), and the FDR. The table is sorted from highest to lowest abundance (log2CPM).

Bacterial Species	log2FC	Log2CPM	Pvalue	FDR
<i>Clostridioides difficile</i>	5.832	16.425	3.160E-06	9.071E-06
<i>Arcobacter bivalviorum</i>	11.320	16.378	1.253E-11	2.042E-09
<i>Tepidibacter mesophilus</i>	8.471	13.556	1.669E-06	5.440E-06
<i>Algicola bacteriolytica</i>	4.113	13.416	1.801E-03	2.718E-03
<i>Shimia aquaeponti</i>	8.262	13.341	1.943E-07	1.131E-06
<i>Vibrio</i> sp. r24	8.190	13.263	1.752E-08	2.561E-07
<i>Marinilabilia nitratreducens</i>	7.999	13.082	3.786E-07	1.870E-06
<i>Burkholderia gladioli</i>	7.912	12.996	4.535E-07	2.174E-06
<i>Pseudofulvibacter geojedonensis</i>	7.795	12.885	2.781E-06	8.243E-06
<i>Marinifilum fragile</i>	7.756	12.845	1.299E-06	4.504E-06
<i>Staphylococcus epidermidis</i>	3.957	12.802	4.630E-03	6.799E-03
<i>Vibrio nigripulchritudo</i>	7.559	12.638	3.033E-08	3.166E-07
<i>Pseudahrensia aquimaris</i>	7.477	12.577	1.191E-05	2.520E-05
<i>Tenacibaculum litopenaei</i>	7.367	12.464	2.712E-06	8.185E-06
<i>Abyssivirga alkaniphila</i>	4.250	12.215	2.063E-03	3.084E-03
<i>Amphritea japonica</i>	7.080	12.169	5.726E-08	5.490E-07
<i>Romboutsia lituseburensis</i>	7.009	12.094	1.885E-08	2.561E-07
<i>Vibrio alginolyticus</i>	6.990	12.070	2.569E-09	6.978E-08
<i>Carboxylicivirga taeanensis</i>	6.870	11.979	6.971E-06	1.623E-05
<i>Meridianimaribacter flavus</i>	6.601	11.713	1.571E-06	5.226E-06
<i>Rhizobium</i> sp. Trp 3	6.397	11.519	4.939E-06	1.258E-05
<i>Vibrio harveyi</i>	6.314	11.409	2.097E-09	6.836E-08
<i>Arcobacter molluscorum</i>	6.236	11.364	4.615E-06	1.194E-05
<i>Mesorhizobium</i> sp.	6.210	11.341	9.035E-06	2.045E-05

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

<i>Pseudoteredinibacter isopora</i>	4.144	9.473	1.133E-04	1.903E-04
<i>Vibrio cholerae</i>	4.140	9.464	5.581E-05	9.996E-05
<i>Clostridium scindens</i>	4.040	9.388	2.032E-04	3.280E-04
<i>Sunxiuqinia elliptica</i>	3.992	9.352	3.222E-04	5.098E-04
<i>Streptomyces lazareus</i>	3.931	9.297	1.627E-04	2.652E-04
<i>Kordiimonas gwangyangensis</i>	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 (log₂CPM), the Benjamini-Hochberg (B-H) adjusted P value (Pvalue), and the FDR. The table is sorted from highest to lowest abundance (log₂CPM).

Bacterial Species	log ₂ FC	Log ₂ CPM	Pvalue	FDR
<i>Heliobacter oregonensis</i>	-10.362	15.430	6.492E-08	5.569E-07
<i>Aureivirga marina</i>	-10.051	15.121	2.288E-07	1.243E-06
<i>Blastocatella fastidiosa</i>	-3.996	14.774	5.076E-03	7.388E-03
<i>Chloroflexus aurantiacus</i>	-3.283	13.728	4.550E-03	6.742E-03
<i>Mycoplasma mycoides</i>	-4.034	13.702	1.188E-03	1.809E-03
<i>Spirosoma navajo</i>	-8.304	13.383	2.275E-08	2.852E-07
<i>Muricauda lutaonensis</i>	-7.559	12.652	9.644E-07	3.573E-06
<i>Iamia majanohamensis</i>	-7.537	12.632	1.785E-07	1.077E-06
<i>Streptococcus sanguinis</i>	-7.125	12.227	9.605E-07	3.573E-06
<i>Candidatus Solibacter usitatus</i>	-6.976	12.083	3.459E-07	1.762E-06
<i>Spirochaeta halophila</i>	-6.873	11.983	2.153E-07	1.210E-06
<i>Bacillus licheniformis</i>	-6.848	11.958	1.708E-07	1.077E-06
<i>Klebsiella pneumoniae</i>	-6.813	11.925	8.615E-09	1.560E-07
<i>Vibrio</i> sp. Cl G9	-6.470	11.595	3.172E-06	9.071E-06
<i>Marinoscillum furvescens</i>	-6.464	11.591	7.350E-07	3.153E-06
<i>Dictyoglomus turgidum</i>	-6.451	11.575	6.448E-08	5.569E-07
<i>Candidatus Phytoplasma fraxini</i>	-6.442	11.567	5.657E-09	1.153E-07
<i>Fulvivirga kasyanovii</i>	-6.435	11.563	1.025E-07	7.596E-07
<i>Candidatus Liberibacter asiaticus</i>	-6.419	11.545	1.214E-08	1.979E-07
<i>Aeromonas caviae</i>	-6.406	11.533	1.586E-07	1.077E-06
<i>Aciditerrimonas ferrireducens</i>	-6.282	11.421	2.604E-06	8.009E-06
<i>Arthrobacter davidanieli</i>	-6.194	11.331	6.928E-07	3.052E-06
<i>Labilibacter aurantiacus</i>	-5.993	11.144	6.794E-06	1.605E-05
<i>Tamlana agarivorans</i>	-5.991	11.140	1.557E-06	5.226E-06

<i>Oenococcus oeni</i>	-5.984	11.133	7.049E-08	5.745E-07
<i>Algisphaera agarilytica</i>	-5.923	11.079	8.398E-07	3.339E-06
<i>Eionea nigra</i>	-5.829	10.990	6.472E-06	1.551E-05
<i>Ilumatobacter fluminis</i>	-5.552	10.737	3.232E-06	9.082E-06
<i>Sinorhizobium medicae</i>	-5.397	10.586	1.837E-10	1.497E-08
<i>Pseudomonas marincola</i>	-5.360	10.558	9.923E-07	3.594E-06
<i>Gloeobacter violaceus</i>	-5.269	10.472	2.584E-07	1.359E-06
<i>Pseudomonas putida</i>	-5.245	10.452	1.275E-06	4.504E-06
<i>Moraxella catarrhalis</i>	-5.109	10.326	7.350E-10	3.993E-08
<i>Bartonella bacilliformis</i>	-5.068	10.290	1.052E-09	4.288E-08
<i>Bdellovibrio bacteriovorus</i>	-4.794	10.052	1.997E-05	3.970E-05
<i>Pectobacterium atrosepticum</i>	-4.780	10.040	1.274E-05	2.662E-05
<i>Sulfobacillus thermosulfidooxidans</i>	-4.777	10.036	8.062E-07	3.339E-06
<i>Serratia liquefaciens</i>	-4.732	9.998	3.617E-06	9.955E-06
<i>Haloferula chungangensis</i>	-4.553	9.845	2.867E-05	5.489E-05
<i>Roseivirga marina</i>	-4.509	9.807	5.499E-06	1.379E-05
<i>Thiopropfundum lithotrophicum</i>	-4.437	9.743	1.379E-07	9.776E-07
<i>Aridibacter kavangonensis</i>	-4.347	9.671	3.665E-06	9.955E-06
<i>Coxiella burnetii</i>	-4.084	9.456	4.122E-05	7.635E-05
<i>Gimesia maris</i>	-4.078	9.452	2.079E-05	4.084E-05
<i>Thalassotalea agarivorans</i>	-4.006	9.393	6.572E-05	1.152E-04
<i>Roseivirga spongicola</i>	-3.970	9.365	2.736E-05	5.309E-05
<i>Lawsonella clevelandensis</i>	-3.882	9.296	8.244E-05	1.415E-04
<i>Spiribacter curvatus</i>	-3.762	9.203	7.087E-05	1.229E-04
<i>Ruegeria intermedia</i>	-3.647	9.113	5.797E-05	1.027E-04
<i>Lewinella cohaerens</i>	-3.603	9.083	1.482E-04	2.441E-04
<i>Ekhidna lutea</i>	-3.299	8.862	1.211E-04	2.015E-04
<i>Gluconobacter oxydans</i>	-3.248	8.824	5.105E-04	7.849E-04
<i>Portibacter lacus</i>	-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 (log2CPM), the Benjamini-Hochberg (B-H) adjusted P value (Pvalue), and the false discovery rate (FDR). The table is sorted from highest to lowest abundance (log2CPM).

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

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