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# Taxonomic Annotation of Near-Coral Seawater Microbiota in Kilifi, Kenya

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**TAXONOMIC ANNOTATION OF NEAR-CORAL  
SEAWATER MICROBIOTA IN KILIFI, KENYA**

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SIT Tanzania and Kenya Spring 2021

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## ABSTRACT

The general objective of this study was to analyze the microbiome of seawater above a coral reef in Kilifi, Kenya. Specific objectives included establishing a baseline microbiota profile, classifying the identified organisms at various taxonomic levels, and conjecturing about reef health from the presence or absence of bioindicator species including *Vibrio* bacteria. Sequenced 16S rRNA gene sequences from seawater samples at Kuruwitu Conservancy in Kilifi, Kenya were taxonomically classified by exact matching employing the Dada2 software package and the naïve Bayesian classifier method with 97% similarity cut off. The seawater microbiota contained mostly *Proteobacteria* (73.28%), followed by *Bacteroidetes* (14.08%) and *Cyanobacteria* (4.47%). The *Cyanobacteria* levels were low compared to what has previously been observed of seawater from diseased and degraded reefs in Japan and Curacao, possibly indicating the health of the Kuruwitu reef. The presence of disease-causing *Vibrio* may be of concern, but since there is no known “healthy range” for *Vibrio* more research and monitoring are needed to draw conclusions. It is recommended that seawater sampling and genomic based taxonomic analysis be repeated and coupled with reef health monitoring in order to correlate changes in the holobiont to subsequent inclines or declines in reef health.

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## 1.0 INTRODUCTION

50% of corals have already died in the past 20 years, and it is predicted that 90% will be dead by the year 2050 (Vince 2020). This is due to a variety of factors, but the six biggest threats to coral reefs are overfishing/destructive fishing, watershed pollution, marine pollution, coastal development, thermal stress, and ocean acidification (Wear 2016). Coral reefs are a keystone species, meaning they have a disproportionately high impact on the ecosystem and can be a deciding factor in whether an entire ecosystem survives or not (NOAA n.d.). For example, 25% of ocean fish rely on coral reefs (NOAA n.d.). Additionally, over ½ billion people rely on coral reefs for food, income, and protection (NOAA n.d.). The economic value of coral reefs is estimated to be tens of billions of USD, underscoring not only their ecological significance, but also the consequential role they play in human livelihoods (NOAA n.d.). If corals continue to die at their current rate, the future of human survival, as well as the survival of millions of other species is in grave danger.

Corals are invertebrates, but they are closely linked to a symbiotic consortium of a variety of microorganisms, known as their microbiome or holobiont (van Oppen & Blackall 2019). Recently, research has suggested that the health of all organisms greatly depends on their microbiome, and corals are no different (van Oppen & Blackall 2019). The coral microbiome is made up of dinoflagellates, bacteria, fungi, viruses, and archaea (van Oppen & Blackall 2019). The role of dinoflagellates in coral survival has been widely studied, and it is known that they are vital for acquiring and recycling nutrients, since corals are only able to obtain a small portion of their nutrients through heterotrophic feeding (Vanwonderghem & Webster 2020). Organisms in the microbiome also play an important role in vitamin and amino acid synthesis, and pathogen

control, although these processes are less well studied (Vanwonderghem & Webster 2020). There are likely other important roles played by the microbiome that have yet to be discovered at all.

Microorganisms are consequential to maintaining the coral ecosystem under environmental stress, but they can also lead to coral's demise (Vanwonderghem & Webster 2020). Two of the main things killing corals, bleaching and disease, can be directly linked to changes in the holobiont. Bleaching occurs due to higher than normal ocean temperatures which causes a breakdown in the symbiotic relationship of corals and symbiodiniaceae endosymbionts, thus starving the corals because they receive less algal photosynthate (van Oppen & Blackall 2019). When a state of dysbiosis is caused by climate change or other factors (i.e. pollution), opportunistic pathogens can emerge in the microbiome, causing diseases (Vanwonderghem & Webster 2020). Thus, understanding how the coral holobiont gets disturbed by climate change and how it can potentially be maintained, returned to a previous state, or beneficially altered could play an important role in sustaining corals during this period of rapid decline.

The microbiome of seawater, in particular, has been found to be the best diagnostic indicator for inferring shifts in surrounding environment. It is five times more responsive to changes in the environment than host-associated microbiomes, and at least one study suggests that up to 56% of observed compositional variation in the seawater microbiome can be directly explained by environmental parameters. Thus, it has been recommended that microbial sampling of seawater be integrated immediately into reef health monitoring initiatives (Glasl et. al 2019).



## **1.1 PROBLEM STATEMENT**

No one has been able to stop corals from dying at an alarming rate around the world. The Indian Ocean is home to unique coral ecosystems that could go extinct within the next 50 years. Metagenomics is a fast-growing field of coral reef research that has the potential to reveal key information relevant to saving corals, yet there have been few genomic studies on coral in the Indian Ocean, and only one (yet to be published) in Kenya, from which some of the data in this study is drawn.

There is no established baseline of the community genomic profile for a healthy reef in Kenya, and thus genomic comparisons cannot be used to determine if one of the many reefs in the area is headed for disaster, even though changes in the genomic profile have been found to be an early indicator which could allow for intervention. Additionally, it is unknown if Kenyan corals' microbiomes makes them more or less perceptible to bleaching, disease, climate change and other issues affecting corals, which could be used by researchers cross breeding corals to create resilient strains, and those experimenting with the use of Beneficial Microorganisms for Corals (BMCs) to re-stabilize coral microbiomes after bleaching events.

## **1.2 RESEARCH QUESTION**

What organisms exist in the microbiome of seawater above a coral reef in Kilifi, Kenya? What levels do they exist at, and what does their presence indicate about the health of the reef?

## **1.3 OBJECTIVES**

### **1.3.1 General Objective**

1. Taxonomically classify the organisms that make up the microbiome of seawater above a coral reef in Kilifi, Kenya.

### 1.3.2 Specific Objectives

1. Adapt the Dada2 software tutorial to analyze the quality profile, filter, trim, denoise, learn the error rate, construct an ASV table, and then assign taxonomy to the sequences contained in SRR10416015 and SRR10416016.
2. Record a baseline microbiome genomic profile for the seawater surrounding the coral reef at Kuruwitu Conservancy by documenting the holobiont makeup at the kingdom, phylum, class, order, family, and genus levels. This can be used in the future to determine if the microbiome has been destabilized.
3. Determine if the presence of, or amount of bioindicator species including *Vibrio*, *Flavobacterium*, *Synechococcaeae*, and *Cyanobacteria* indicate anything about the health of the coral reef and surrounding environment, such as if there is nutrient over-enrichment or dangerously high seawater temperatures.

### 1.4 LITERATURE REVIEW

Distinct microbiome community profiles correlate to different bleaching susceptibilities (Gardner et. al 2019). Higher bacterial diversity, species richness, and community evenness were observed in bleaching resistant corals in the Seychelles compared to bleaching susceptible corals (Gardner et. al 2019). Interestingly, observed species richness in the microbiomes of corals in the Great Barrier Reef *increased* during a bleaching event, suggesting it is not simply species richness of the microbiome which can indicate coral health (Bourne et. al 2007). Rather, changes in the coral microbiome appear to be a much better indicator. Changes in the microbiomes of corals in the Great Barrier Reef occurred before visual signs of bleaching, suggesting that monitoring of coral microbiomes can be used as an early warning sign of bleaching (Bourne et.

al 2007). The bleached corals had increased levels of *Vibrio* bacteria (now 17% of clones) and lower levels of *Spongio* bacteria (down to 3% of clones from 41% pre-bleaching) (Bourne et. al 2007). The microbiomes of these specific corals returned to normal post-bleaching, and the corals also made full recoveries, which indicates the importance of restoring the normal coral holobiont after it has been disturbed and shows coral's ability to heal (Bourne et. al 2007).

Researchers studying coral reefs in Japan also found an inverse correlation between coral cover and heterotrophic microbe presence (including *Vibrio* bacteria) (Meirelles et. al 2018). They found reads corresponding to heterotrophs to be between 78.1% to 92.1%, and the percentage of reads corresponding to potential coral pathogens to be between 6.9% and 18.2% (Meirelles et. al 2018). Further, they found 87% of reads to be bacteria, and of those *Proteobacteria* reads were the most abundant (>48.5% at all sites), followed by *Cyanobacteria* (>7.1% at all sites), and then *Bacteroidetes* (>6.1% at all sites) (Meirelles et. al 2018). The Ishigaki reefs the researchers took samples from are classified as “degraded” by reef health standards, so their microbial profiles offer insight into what may be aspects of unhealthy coral holobionts (Meirelles et. al 2018). Frias-Lopez et. al (2002) analyzed 16S rRNA data from seawater above BBD-infected *M. annularis*, *M. cavernosa*, and *D. strigosa* coral colonies in Curacao, Netherlands Antilles. They found the microbiome profile to be composed mainly of *Proteobacteria* (37-60%), followed by *Cyanobacteria* (30-43%), and then CFBs (3-14%) (Frias-Lopez et. al 2002). *Planctomycetales*, *Firmicutes*, and *Chloroplasts* also were present in smaller numbers (Frias-Lopez et. al 2002). This data also provides an insight into an unhealthy coral holobiont.

## 2.0 METHODS

Because there has been such limited coral genomic research so far in the Western Indian Ocean, this study focuses on a coral reef in Kilifi, Kenya. The reef is located in the Kuruwitu Conservancy ( $-3.809^{\circ}$ ,  $29.829^{\circ}$ ), which is managed by the Kuruwitu Conservation & Welfare Association. Kuruwitu Conservancy was established in 2005 as the first Locally Managed Marine Area (LMMA) in Kenya, and comprises a 30 hectare Marine Protected Area (MPA) that is a no-take zone (“About Us” 2017). This maximum level of protection hopefully allows the reef to thrive with minimal human influence, thus making it a good candidate for establishing a “natural” baseline.



Figure 1. Location of Kuruwitu Conservancy in Kilifi County, Kenya. Source: Google Maps

The duplicate 16S rRNA sequence reads analyzed in this paper (SRR10416015 and SRR104106016) were collected from seawater above the reef at Kuruwitu with methods described by Wambua et. al (2019). Fastq files of the sequence reads were obtained from the

publicly available Sequence Read Archive of the NCBI. The Dada2 software package was used in the R programming language to analyze and taxonomically classify the 16S rRNA sequences contained in the fastq files. First, the quality of the reads, or how accurately the base was assigned at each location was plotted so that the sequences could be trimmed appropriately to exclude sections before or after which the quality was low.

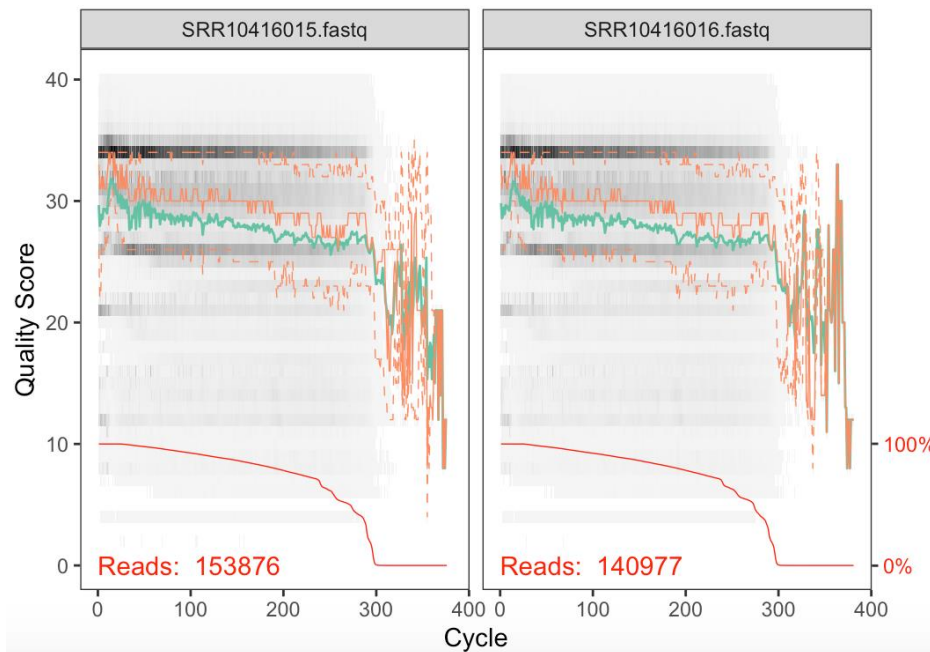


Figure 2 Quality profile reads for two seawater samples taken above coral reefs in Kuruwitu Conservancy

Because the quality of the reads drastically deteriorated after 300, the max length was set to 298.

Next, the Dada2 software was used to estimate the error rates using machine learning.

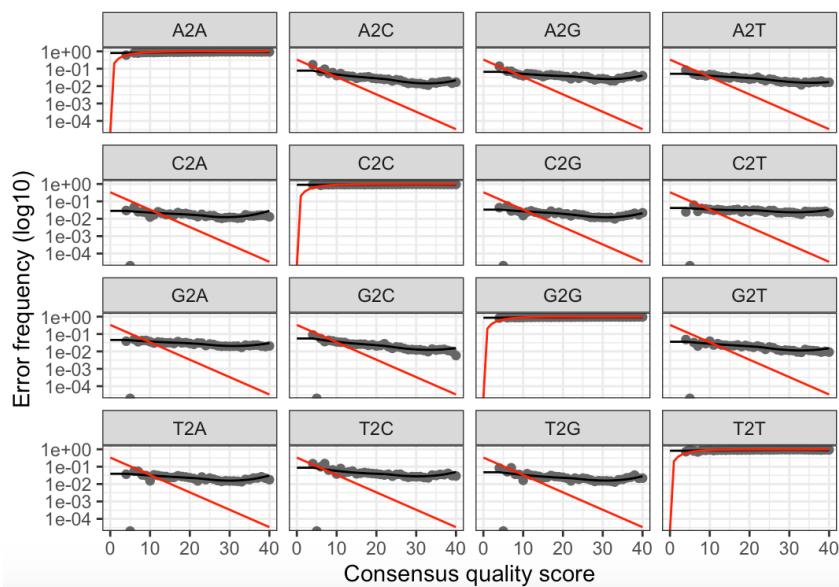


Figure 3 Visualization of error rates

Then, Dada2 was used to determine the number of true sequence variants in the sample. An Amplicon Sequence Variant (ASV) table was constructed. Chimeras were removed, and then taxonomy was assigned to the sequence variants using the naïve Bayesian classifier method. Species were assigned if there are 100% matches to sequenced reference strains using the Silva species assignment reference database.

The sequence data was sorted and visualized at the kingdom, phylum, class, order, family, and genus level in order of most to least common group of organisms present. This data was then compared to 16S rRNA data collected from coral seawater in other locations including Florida, USA and Curacao, Netherlands Antilles which had been previously analyzed and published on.

### 3.0 RESULTS

The duplicate seawater samples yielded 153,876 and 140,977 sequences, 152,343 and 139,831 filtered sequences, 130,095 and 119,369 denoised sequences, and 119,544 and 109,678 non-chimeric sequences, respectively. From these, 1023 ribosomal sequence variants (RSVs) were deduced.

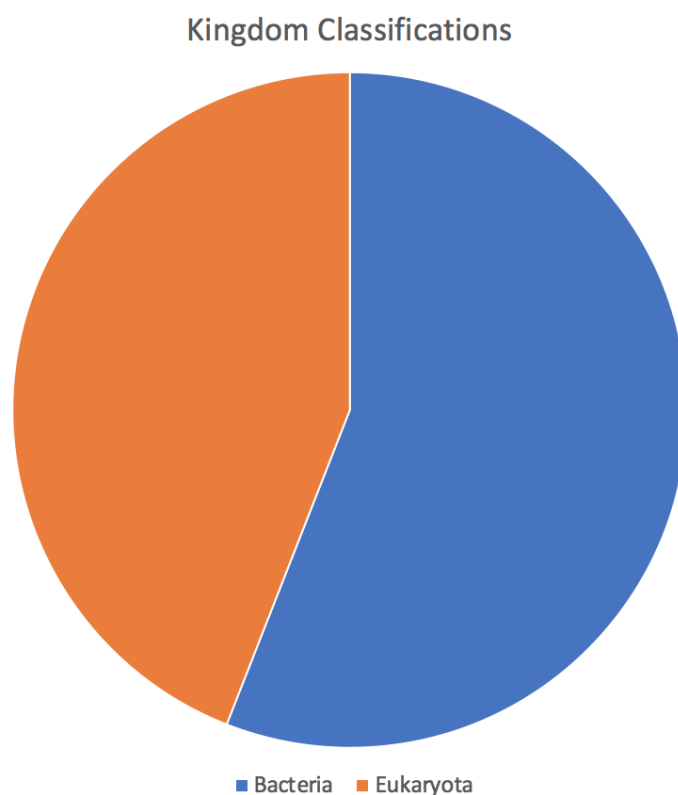


Figure 4 Kingdom classifications of Kuruwitu reef seawater

The breakdown of the 206,271 sequences identified to the kingdom level is shown in Figure 4. The identified microorganisms of the holobiont of the Kenyan reef water consisted of 55.9% bacteria and 44.1% eukaryote (Figure 4).

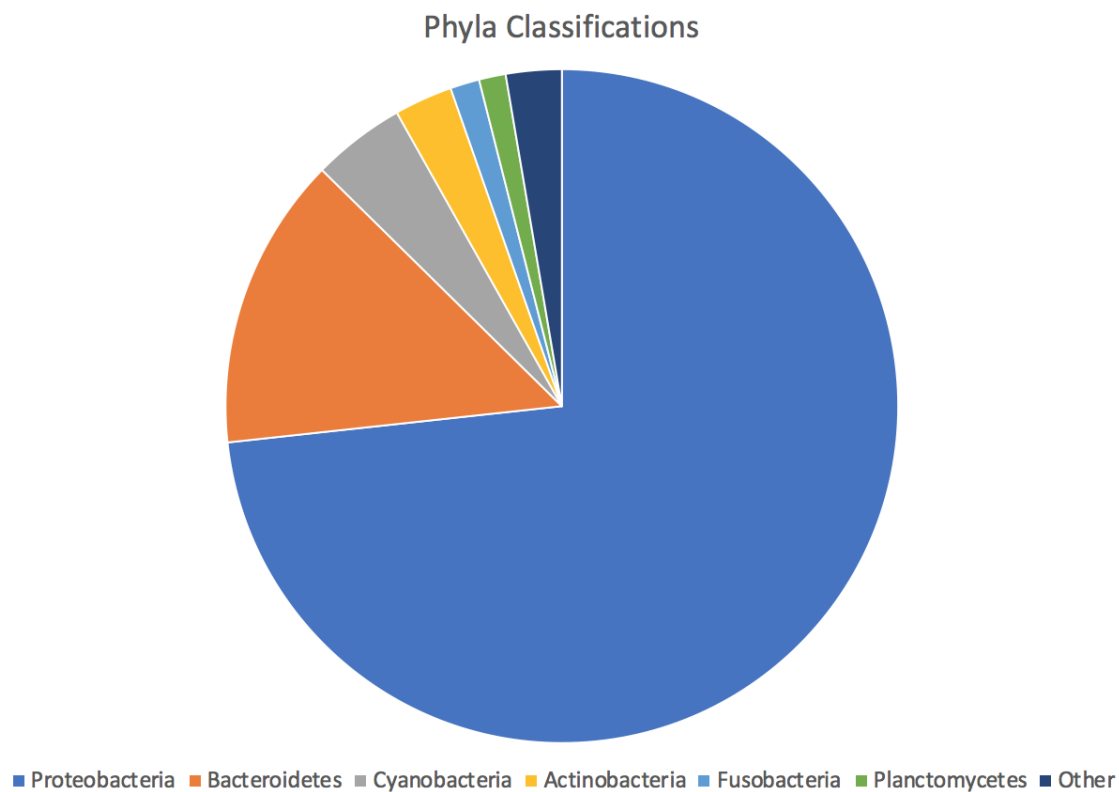


Figure 5 Phyla classifications of microorganisms in Kuruwitu reef seawater

The breakdown of the 114,729 sequences identified at the phylum level is displayed by Figure 5. It shows the identified components of the microbiome were made up of 73.3% *Proteobacteria*, 14.1% *Bacteroidetes*, 4.5% *Cyanobacteria*, 2.8% *Actinobacteria*, 1.4% *Fusobacteria*, 1.3% *Planctomycetes* and 2.7% *Other* (see appendix Table 1).



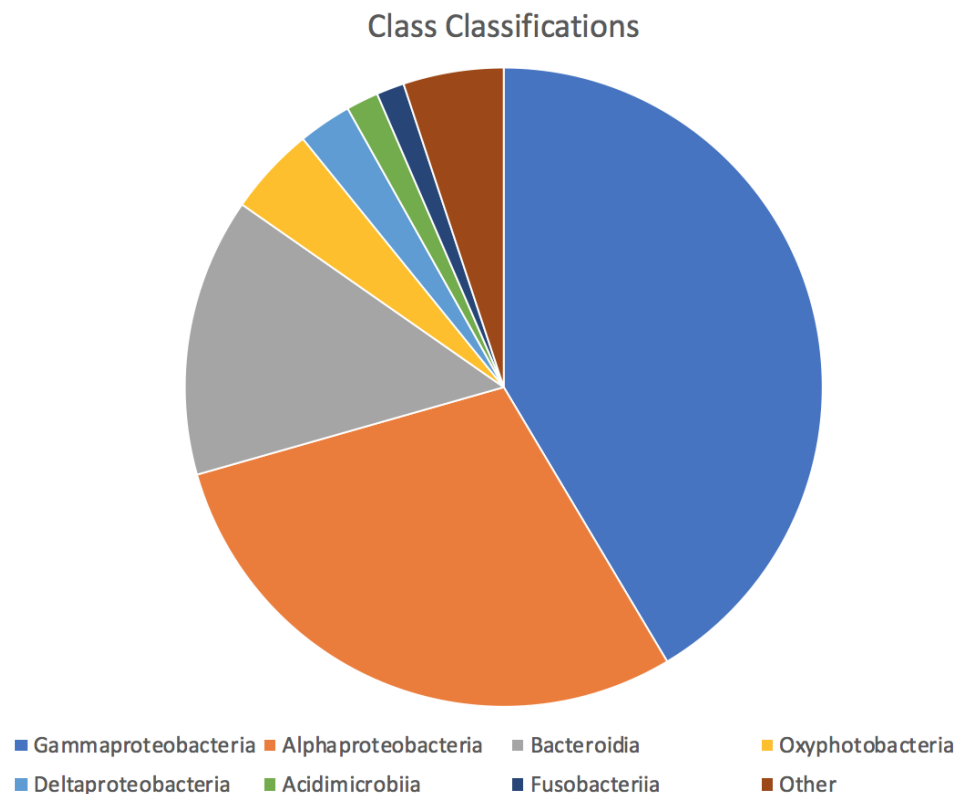


Figure 6 Class classifications of microorganisms in Kuruwitu reef seawater

The breakdown of the 114,471 unique sequences identified to the class level is displayed by Figure 6. It shows that 41.4% were *Gammaproteobacteria*, 29.2% were *Alphaproteobacteria*, 14.1% were *Bacteroidia*, 4.5% were *Oxyphotobacteria*, 2.7% were *Deltaproteobacteria*, 1.6% were *Acidimicrobiia*, 1.4% were *Fusobacteriia*, and 5.1% were Other (see appendix Table 2).

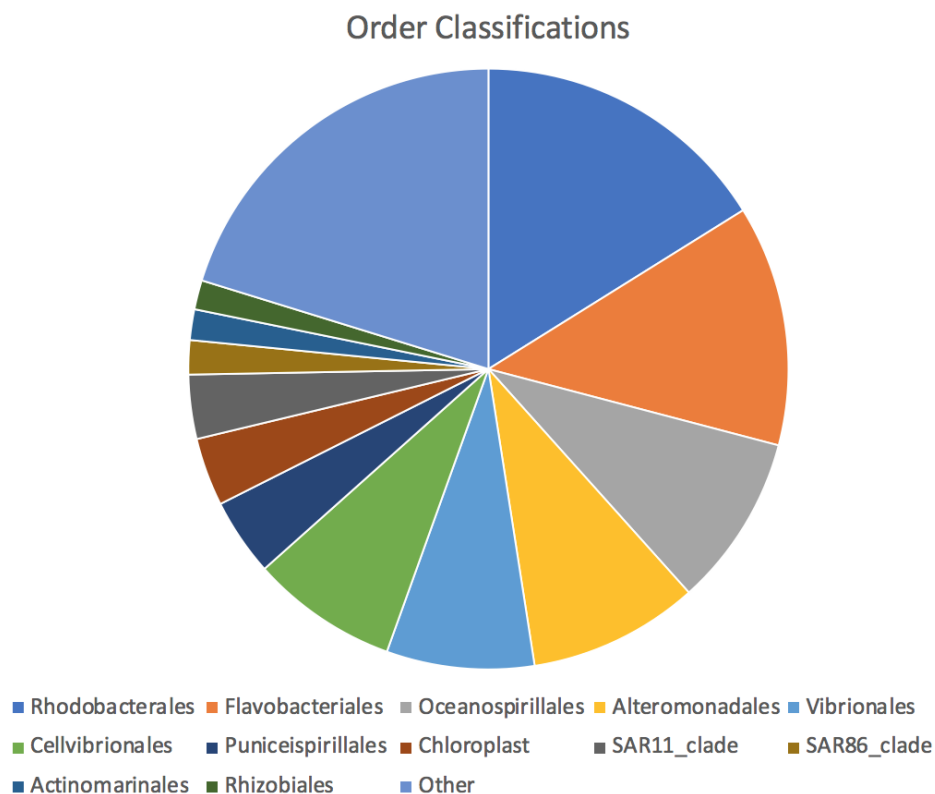


Figure 7. Order classifications of microorganisms in Kuruwitu reef seawater

The breakdown of the 109,466 unique sequences identified to the order level of classification is displayed by Figure 7. It shows 16.2% were *Rhodobacterales*, 13.0% were *Flavobacteriales*, 9.3% were *Oceanospirillales*, 9.2% were *Alteromonadales*, 8.0% were *Vibrionales*, 7.9% were *Cellvibrionales*, 4.2% were *Puniceispirillales*, 3.7% were *Chloroplast*, 3.5% were *SAR11\_clade*, 1.8% were *SAR86\_clade*, 1.6% were *Actinomarinales*, 1.6% were *Rhizobiales*, and 20.2% were Other (see appendix Table 3).

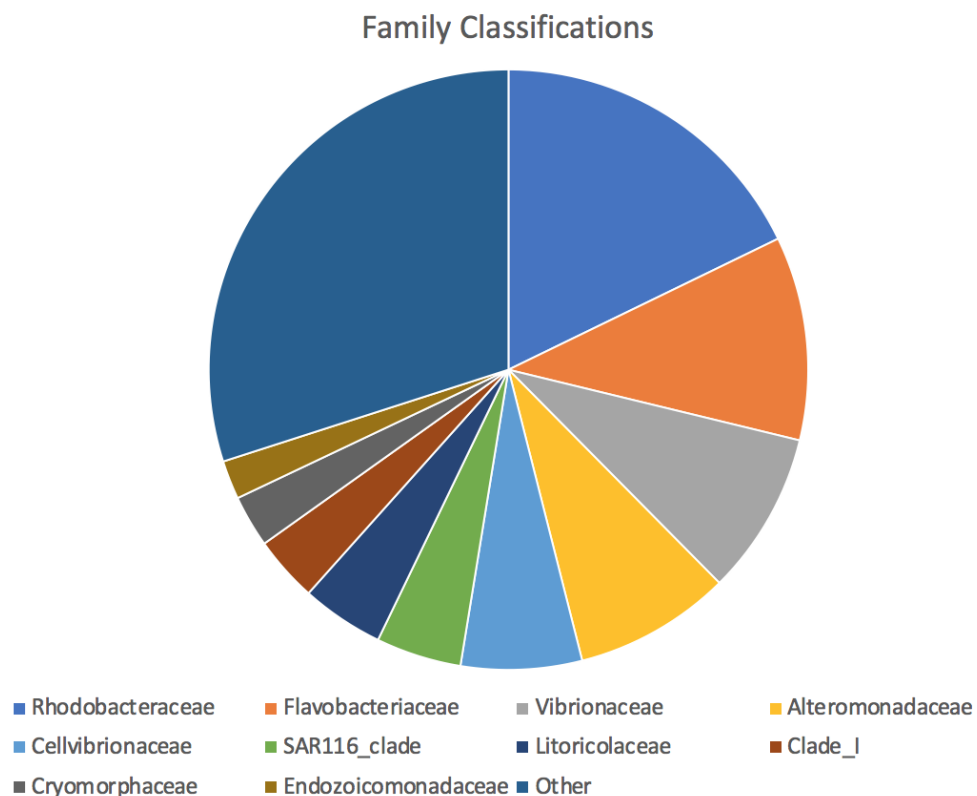


Figure 8 Family classifications of microorganisms in Kuruwitu reef seawater

The breakdown of the 99,151 unique sequences identified to the family level is displayed by

Figure 8. It shows 17.8% were *Rhodobacteraceae*, 11.0% were *Flavobacteriaceae*, 8.8% were *Vibrionaceae*, 8.4% were *Alteromonadaceae*, 6.5% were *Cellvibrionaceae*, 4.6% were *SAR116\_clade*, 4.5% were *Litoricolaceae*, 3.5% were *Clade\_I*, 2.8% were *Cryomorphaceae*, 2.1% were *Endozoicomonadaceae*, and 30% were *Other* (see appendix Table 4).

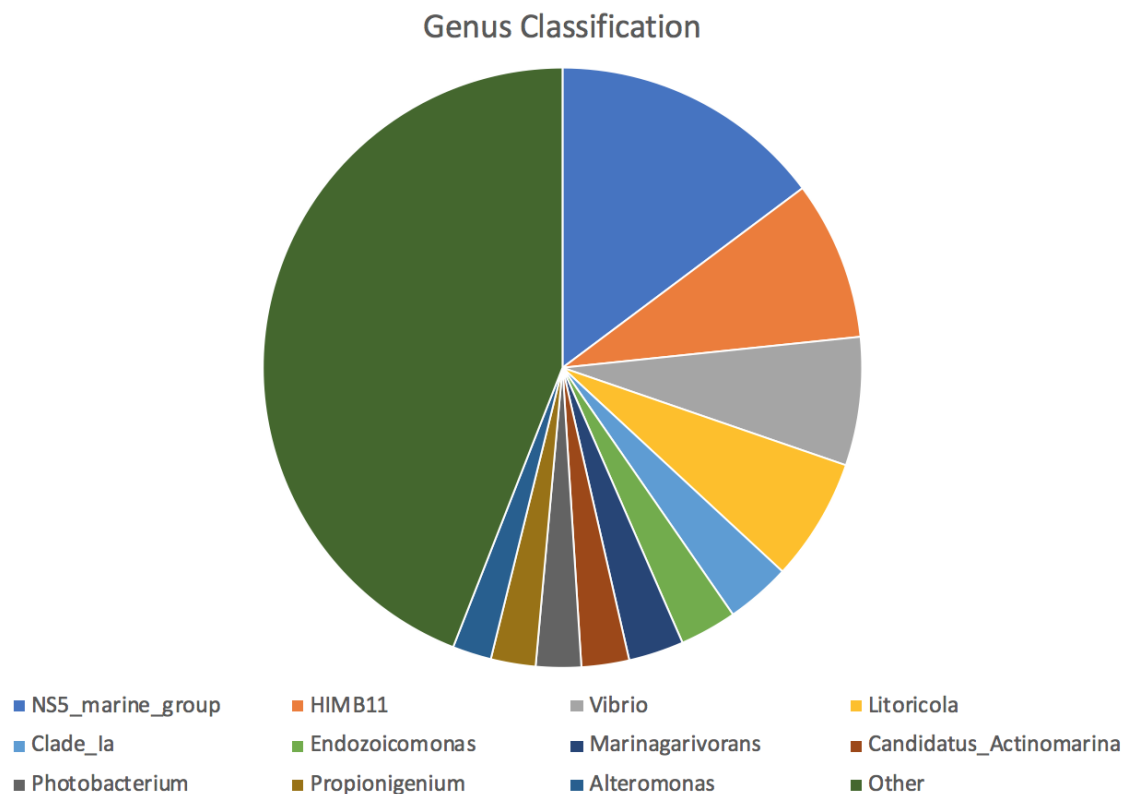


Figure 9 Genus classifications of microorganisms in Kuruwitu reef seawater.

Figure 9 shows the breakdown of the 66,848 unique sequences identified to the genus level. Of these, 14.8% were *NS5\_marine\_group*, 8.6% were *HIMB11*, 6.9% were *Vibrio*, 6.6% were *Litoricola*, 3.5% were *Clade\_Ia*, 3.1% were *Endozoicomonas*, 3.0% were *Marinagarivorans*, 2.6% were *Candidatus\_Actinomarina*, 2.4% were *Photobacterium*, 2.4% were *Propionigenium*, 2.1% were *Alteromonas*, and 44.1% were Other (see appendix Table 5) (Figure 9). The presence of *Vibrio* is specifically important to note.

## 4.0 DISCUSSION

The data collected established a baseline microbial community profile for the seawater above the coral reef at Kuruwitu Conservancy. This is useful for monitoring changes over time and establishing correlations between specific holobiont changes and reef health. At this time, looking specifically at particular groups of organisms that were identified allows one to conjecture about the current health of the reef. Previous research has indicated potential bioindicator species that are correlated with abiotic stressors (Laas et. al 2002). *Rhodobacteraceae*, *Cryomorphaceae*, *Synechococcaeae*, *Vibrio* and *Flavobacterium* are usually correlated with unusually high water temperatures (Laas et. al 2002). *Vibrio* were the third most common genus in the Kenyan seawater and accounted for 6.92% of the identified organisms. *Synechococcus\_CC9902* were also detected, but only accounted for a small (0.53%) percentage of organisms. The relatively large *Vibrio* presence could be of concern, since members of the *Vibrio* genus are agents of disease in corals (Munn 2015). The YB1 *Vibrio* species strain was found to cause temperature-dependent bleaching and tissue loss in *Pocillopora damicornis* off the coast of Zanzibar, Tanzania, which is located less than 100 miles from the study site in Kilifi, Kenya (Munn 2015).

However, during a bleaching event on the Great Barrier Reef, *Vibrio* accounted for 17% of clones, suggesting the percentage of *Vibrio* present at the Kuruwitu reef (6.92%) could not yet be causing bleaching (Bourne et. al 2007) Additionally, multiple species of *Vibrio* have also been found in healthy corals (Munn 2015). Thus, because there is no baseline for what a healthy level of *Vibrio* is for Kenyan reefs, it is more important to monitor the *Vibrio* levels in the future and watch for any increase, which could be an indicator of seawater that has become dangerously warm for the reef.

Between the degraded Ishigaki reefs in Japan, BBD diseased reefs in Curacao, Netherlands Antilles, and the Kuruwitu reef in Kenya, the Kenyan reef had the lowest percentage of *Cyanobacteria* (4.47%, compared to 7.12% in Japan and 30-43% in Curacao). *Cyanobacteria*, or blue-green algae are important to coral reefs as they provide nitrogen through nitrogen fixation and are grazed on by coral organisms (Charpy et. al 2012). However, *Cyanobacteria* can form pathogenic microbial consortia with other microbes on corals tissues and thus kill corals, and *Cyanobacteria* blooms can slow restocking of the adult coral populations (Charpy et. al 2012). Additionally, *Cyanobacteria* mats kill scleractinian corals (stony corals) by poisoning them, and multiple coral diseases including black band disease (BBD) are caused by *Cyanobacteria* (Charpy et. al 2012). The much higher level of *Cyanobacteria* (30-43%) in the BBD infected Curacaoian reefs supports the hypothesis that *Cyanobacteria* cause BBD.

Clearly there is a balance of *Cyanobacteria* to be maintained for a healthy reef system, but the acceptable range has yet to be extensively studied or documented, particularly in Kenya. The low levels of *Cyanobacteria* observed in the Kuruwitu Reef are likely a sign of good health, but it is impossible to know with no baseline of a “healthy reef” to compare to. Like with *Vibrio*, monitoring for any changes in *Cyanobacteria* will be an important factor in using microbial analysis to monitor the reef health of Kuruwitu going forward.

Another notable difference between the Kenyan 16S rRNA sequence data and the 16S rRNA sequence data from Curacao and Japan is the higher percentage of *Proteobacteria* in Kenya (73%, vs. 37-60% in Curacao and at least 48% for each site in Japan) (Frias Lopez et. al 2002) (Meirelles et. al 2018). No studies could be found on what a healthy percentage of

*Proteobacteria* is for coral reef seawater, but hopefully the health of the Kuruwitu reef and the *Proteobacteria* levels can be monitored in the future to establish such a range.

## 5.0 CONCLUSION AND RECOMMENDATIONS

The holobiont of seawater above the coral reef at Kuruwitu Conservancy is made up mostly by *Proteobacteria* (73.28%), followed by *Bacteroidetes* (14.4%), and then *Cyanobacteria* (4.47%). Kuruwitu had lower levels of *Cyanobacteria* than seawater taken from reefs in Curacao and Japan, which, coupled with previous research on the harm too much *Cyanobacteria* can do to a reef, appears to be a sign of health. The presence of *Vibrio* bacteria (6.91%) is somewhat of concern since *Vibrio* are known to be causative agents of disease and bleaching in corals, but the *Vibrio* levels were still far below those observed during a bleaching event on the Great Barrier Reef (17%), so it is impossible to determine if the *Vibrio* observed are of detriment to the reef (Bourne et. al 2007).

It is recommended that seawater collection and 16S rRNA microbiome analysis should be completed at regular intervals in the future, as well as during any unusual events such as coral bleaching or disease spread. This should be done in combination with recording data that indicates reef health such as coral cover, fish abundance and diversity, fleshy macroalgal index, coral recruitment, and so on so that correlations can be drawn between microbiome makeup and reef health. More research should also be done on treating coral reefs with microorganisms that have been depleted during, for example, a bleaching event to see if artificially restoring the holobiont is a useful technique for helping coral recovery.



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## 7.0 APPENDIX

Phylum	Number of reads	Percentage
Proteobacteria	84074	73.281
Bacteroidetes	16155	14.081
Cyanobacteria	5133	4.474
Actinobacteria	3206	2.794
Fusobacteria	1614	1.407
Planctomycetes	1472	1.283
Euglenozoa	934	0.814
Verrucomicrobia	645	0.562
Epsilonbacteraeota	517	0.451
Lentisphaerae	441	0.384
Firmicutes	334	0.291
Marinimicrobia_ (SAR406_clade)	50	0.044
Gemmatimonadetes	45	0.039
Acidobacteria	32	0.028
Patescibacteria	22	0.019
Spirochaetes	17	0.015
Tenericutes	13	0.011
Fibrobacteres	10	0.009
Dadabacteria	9	0.008
Omnitrophicaeota	6	0.005
Total	114729	100

Table 1. Full Phylum Breakdown

Class	Number of reads	Percentage
Gammaproteobacteria	47430	41.434
Alphaproteobacteria	33371	29.152
Bacteroidia	16139	14.099
Oxyphotobacteria	5121	4.474
Deltaproteobacteria	3095	2.704
Acidimicrobiia	1875	1.638
Fusobacteriia	1614	1.410
Actinobacteria	1262	1.102

Euglenida	934	0.816
Phycisphaerae	909	0.794
Verrucomicrobiae	645	0.563
Campylobacteria	517	0.452
Oligosphaeria	441	0.385
vadinHA49	417	0.364
Clostridia	290	0.253
Planctomycetacia	131	0.114
Thermoleophilia	69	0.060
Gemmatimonadetes	45	0.039
Bacilli	42	0.037
Saccharimonadia	22	0.019
Spirochaetia	17	0.015
Rhodothermia	16	0.014
Blastocatellia_ (Subgroup_4)	14	0.012
Mollicutes	13	0.011
Fibrobacteria	10	0.009
Dadabacteriia	9	0.008
Holophagae	9	0.008
Subgroup_6	9	0.008
Melainabacteria	3	0.003
Erysipelotrichia	2	0.002
Total	114471	100

Table 2. Full Class Breakdown

Order	Number of reads	Percentage
Rhodobacterales	17661	16.134
Flavobacteriales	14184	12.957
Oceanospirillales	10142	9.265
Alteromonadales	10049	9.180
Vibrionales	8714	7.960
Cellvibrionales	8668	7.918
Puniceispirillales	4566	4.171
Chloroplast	4015	3.668
SAR11_clade	3778	3.451
SAR86_clade	2014	1.840

Actinomarinales	1807	1.651
Rhizobiales	1724	1.575
Fusobacteriales	1614	1.474
Bdellovibrionales	1375	1.256
Pseudomonadales	1345	1.229
Betaproteobacteriales	1191	1.088
Bacteroidales	1060	0.968
Caulobacterales	927	0.847
Phycisphaerales	909	0.830
Propionibacteriales	891	0.814
Sphingomonadales	824	0.753
Micavibrionales	773	0.706
Salinisphaerales	752	0.687
Desulfobacterales	719	0.657
Aphagea	712	0.650
Synechococcales	704	0.643
Rhodospirillales	683	0.624
Oligoflexales	642	0.586
Parvibaculales	616	0.563
Cytophagales	598	0.546
Campylobacterales	517	0.472
Rickettsiales	462	0.422
Arenicellales	461	0.421
P.palmC41	433	0.396
Xanthomonadales	373	0.341
Verrucomicrobiales	360	0.329
Pseudonocardiales	357	0.326
Steroidobacterales	342	0.312
Clostridiales	290	0.265
Opitutales	261	0.238
Chitinophagales	192	0.175
Nostocales	159	0.145
Desulfovibrionales	149	0.136
Pirellulales	131	0.120
Gammaproteobacteria _Incertae_Sedis	130	0.119
Reyranellales	122	0.111
Sneathiellales	114	0.104
Myxococcales	109	0.100

Ectothiorhodospirales	103	0.094
KI89A_clade	78	0.071
Microtrichales	68	0.062
Limnotrichales	63	0.058
Gemmatimonadales	45	0.041
Aeromonadales	42	0.038
Lactobacillales	42	0.038
HOC36	37	0.034
OM182_clade	36	0.033
SAR324_clade (Marine_group_B)	36	0.033
Francisellales	32	0.029
Thalassobaculales	30	0.027
Nitrosococcales	29	0.026
NB1-j	27	0.025
Pedosphaerales	24	0.022
Saccharimonadales	22	0.020
Spirochaetales	17	0.016
Rhodothermales	16	0.015
Coxiellales	15	0.014
Corynebacteriales	14	0.013
Mycoplasmatales	13	0.012
Fibrobacterales	10	0.009
Acanthopleuribacteriales	9	0.008
Dadabacteriales	9	0.008
Sphingobacteriales	9	0.008
Gastranaerophilales	3	0.003
Enterobacteriales	2	0.002
Erysipelotrichales	2	0.002
Total	109466	100

Table 3. Full Order Breakdown

Family	Number of reads	Percentage
Rhodobacteraceae	17661	17.812
Flavobacteriaceae	10882	10.975
Vibrionaceae	8714	8.789
Alteromonadaceae	8393	8.465
Cellvibrionaceae	6470	6.525

SAR116_clade	4566	4.605
Litoricolaceae	4419	4.457
Clade_I	3504	3.534
Cryomorphaceae	2779	2.803
Endozoicomonadaceae	2069	2.087
Actinomarinaceae	1720	1.735
Fusobacteriaceae	1614	1.628
Haliaceae	1336	1.347
Bacteriovoracaceae	1298	1.309
Pseudomonadaceae	1247	1.258
Pseudoalteromonadaceae	1025	1.03
Burkholderiaceae	935	0.943
Phycisphaeraceae	909	0.917
Rhizobiaceae	907	0.915
Saccharospirillaceae	897	0.905
Nocardiodaceae	846	0.853
Sphingomonadaceae	824	0.831
Desulfobulbaceae	719	0.725
Aphagea_fa	712	0.718
Cyanobiaceae	704	0.710
Micavibrionaceae	694	0.700
Oligoflexaceae	633	0.638
Hyphomonadaceae	621	0.626
Marinomonadaceae	564	0.569
AEGEAN- 169_marine_group	538	0.543
Marinifilaceae	537	0.542
Cyclobacteriaceae	522	0.526
Arcobacteraceae	517	0.521
Methyloligellaceae	501	0.505
Solimonadaceae	501	0.505
Arenicellaceae	461	0.465
Alcanivoracaceae	444	0.448
S25-593	437	0.441
OCS116_clade	431	0.435
Xanthomonadaceae	373	0.376
Rubritaleaceae	360	0.363
Pseudonocardiaceae	357	0.360
Woeseiaceae	342	0.345

Colwelliaceae	331	0.334
Nitrincolaceae	331	0.334
Caulobacteraceae	306	0.309
NS9_marine_group	299	0.302
Clade_IV	274	0.276
Puniceicoccaceae	261	0.263
Methylophilaceae	239	0.241
Shewanellaceae	201	0.203
PS1_clade	174	0.175
Crocinitomicaceae	149	0.150
Desulfovibrionaceae	149	0.150
Saprospiraceae	139	0.140
Spongiibacteraceae	139	0.140
Pirellulaceae	131	0.132
Unknown_Family	130	0.131
Kangiellaceae	127	0.128
Lachnospiraceae	122	0.123
Reyranellaceae	122	0.123
Sneathiellaceae	114	0.115
Ectothiorhodospiraceae	103	0.104
Moraxellaceae	98	0.099
Family_XII	94	0.095
Brii41	78	0.079
Bdellovibrionaceae	77	0.078
Cyanobacteriaceae	70	0.071
Microtrichaceae	68	0.069
Clostridiaceae_1	66	0.067
Limnotrichaceae	63	0.064
Chitinophagaceae	53	0.053
Phormidiaceae	51	0.051
Gemmatimonadaceae	45	0.045
Propionibacteriaceae	45	0.045
Flammeovirgaceae	44	0.044
Aeromonadaceae	42	0.042
Streptococcaceae	42	0.042
Francisellaceae	32	0.032
Haliangiaceae	31	0.031
Bacteroidetes_BD2-2	30	0.030
Methylophagaceae	29	0.029



Paraspirulinaceae	26	0.026
Rickettsiaceae	25	0.025
Pedosphaeraceae	24	0.024
Prolixibacteraceae	19	0.019
Tannerellaceae	19	0.019
EC94	17	0.017
Spirochaetaceae	17	0.017
Rhodothermaceae	16	0.016
Coxiellaceae	15	0.015
Corynebacteriaceae	14	0.014
Mycoplasmataceae	13	0.013
Xenococcaceae	12	0.012
Parvibaculaceae	11	0.011
Magnetospiraceae	10	0.010
0319-6G20	9	0.009
Acanthopleuribacteraceae	9	0.009
NS11-12_marine_group	9	0.009
Enterobacteriaceae	2	0.002
Erysipelotrichaceae	2	0.002
Total	99151	100

Table 4. Full Family Breakdown

Genus	Number of reads	Percentage
NS5_marine_group	9887	14.791
HIMB11	5722	8.560
Vibrio	4624	6.917
Litoricola	4419	6.611
Clade_Ia	2332	3.489
Endozoicomonas	2055	3.074
Marinagarivorans	1992	2.980
Candidatus_Actinomarina	1720	2.573
Photobacterium	1631	2.440
Propionigenium	1614	2.415
Alteromonas	1403	2.099
Nioella	1278	1.912
Pseudomonas	1247	1.865
Tateyamaria	1207	1.806

Clade_Ib	1172	1.753
XY-R5	1072	1.604
Agaribacter	1047	1.566
Mameliella	992	1.484
Candidatus_Endobugula	972	1.454
Nocardioides	846	1.266
Peredibacter	833	1.246
Erythrobacter	804	1.203
Aestuariibacter	741	1.109
Distigma	712	1.065
Limnobacter	658	0.984
Marinomonas	564	0.844
Algimonas	551	0.824
Arcobacter	517	0.773
Methyloceanibacter	501	0.749
Algicola	498	0.745
Pseudobacteriovorax	463	0.693
Alcanivorax	444	0.664
Reinekea	435	0.651
Pseudoalteromonas	423	0.633
Catenococcus	401	0.600
Desulfotalea	398	0.595
OM60(NOR5)_clade	385	0.576
Ruegeria	375	0.561
Rubritalea	360	0.539
Pseudonocardia	357	0.534
Synechococcus_CC9902	356	0.533
Pseudohalaea	354	0.530
Prochlorococcus_MIT9313	348	0.521
Woeseia	342	0.512
Pseudoxanthomonas	336	0.503
Thalassotalea	331	0.495
Polaribacter_4	323	0.483
Brevundimonas	306	0.458
Panacagrimonas	280	0.419
NS2b_marine_group	277	0.414
Halobacteriovorax	270	0.404
Arenicella	255	0.381
Methylophilus	239	0.358

Candidatus_Puniceispirillum	232	0.347
Brucella	231	0.346
HTCC5015	206	0.308
Maritimibacter	206	0.308
Ferrimonas	201	0.301
Curvibacter	132	0.197
NS10_marine_group	128	0.191
Paraglaciecola	128	0.191
Reyranella	122	0.183
Rubripirellula	120	0.180
Allorhizobium- Neorhizobium- Pararhizobium-Rhizobium	118	0.177
Kangiella	116	0.174
Ferrovibrio	114	0.171
Halodesulfovibrio	111	0.166
Marinifilum	110	0.165
Fabibacter	105	0.157
Psychrosphaera	104	0.156
Lentimonas	99	0.148
Marinoscillum	97	0.145
Coraliomargarita	92	0.138
Hoeflea	78	0.117
Lewinella	78	0.117
Fusibacter	74	0.111
Roseobacter_clade_ NAC11-7_lineage	71	0.106
Hyphomonas	70	0.105
Symphothece_PCC-7002	70	0.105
NS4_marine_group	69	0.103
Moraxella	68	0.102
Sva0996_marine_group	68	0.102
Fluviicola	64	0.096
Limnothrix	63	0.094
Amphritea	60	0.090
Crocinitomix	59	0.088
Bdellovibrio	55	0.082
Glaciecola	55	0.082
Sediminibacterium	53	0.079
Tenacibaculum	52	0.078

Trichodesmium_IMS101	51	0.076
Lachnoclostridium_12	48	0.072
Kordia	47	0.070
Cutibacterium	45	0.067
MWH- UniP1_aquatic_group	45	0.067
Aureibacter	44	0.066
Flammeovirga	44	0.066
Verruc-01	44	0.066
Fulvivirga	43	0.064
Oceanococcus	43	0.064
Aeromonas	42	0.063
Streptococcus	42	0.063
Desulfovibrio	38	0.057
Lysobacter	37	0.055
Aquimarina	36	0.054
Halioglobus	36	0.054
Psychroflexus	34	0.051
Silvanigrella	34	0.051
Clostridium_sensu_ stricto_1	33	0.049
Haliangium	31	0.046
Marine_Methylotrophic Group_3	29	0.043
Catenovulum	28	0.042
Winogradskyella	28	0.042
Spirulina_DRTO-55.2	26	0.039
Candidatus_Arcanobacter	25	0.037
Aquibacter	24	0.036
SM1A02	24	0.036
Epulopiscium	23	0.034
Mangrovimonas	23	0.034
Actibacter	22	0.033
OM27_clade	22	0.033
Sphingopyxis	20	0.030
Macellibacteroides	19	0.028
Ekhidna	18	0.027
Spirochaeta_2	17	0.025
Coxiella	15	0.022
Corynebacterium_1	14	0.021

Kistimonas	14	0.021
Reichenbachiella	14	0.021
Candidatus_Bacilloplasma	13	0.019
Chroococciopsis_ PCC-6712	12	0.018
Nonlabens	12	0.018
Aliikangiella	11	0.016
Acanthopleuribacter	9	0.013
Francisella	7	0.010
Rubidimonas	6	0.009
Spongiimicrobium	4	0.006
Turicibacter	2	0.003
Total	66846	100

Table 5. Full Genus Breakdown