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Bacterial communities and species-specific associations with the mucus of Brazilian coral species

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We investigated the existence of species-specific associations between Brazilian coral species and bacteria. Pyrosequencing of the V3 region of the 16S rDNA was used to analyze the taxonomic composition of bacterial communities associated with the mucus of four coral species (*Madracis decactis, Mussismilia hispida, Palythoa caribaeorum,* and *Tubastraea coccinea*) in two seasons (winter and summer), which were compared with the surrounding water and sediment. The microbial communities found in samples of mucus, water, and sediment differed according to the composition and relative frequency of OTUs. The coral mucus community seemed to be more stable and resistant to seasonal variations, compared to the water and sediment communities. There was no influence of geographic location on the composition of the communities. The sediment community was extremely diverse and might act as a "seed bank" for the entire environment. Species-specific OTUs were found in *P. caribaeorum, T. coccinea,* and *M. hispida.*

T he association between microorganisms and reef–building corals, also called the coral holobiont, has been studied due to its influence on coral physiology and health. The microorganisms associated with corals can play a role in the host's health by providing a food source[1], protecting the host against pathogenic bacteria by the production of antibacterial compounds, and occupying specific niches[2–4]. Studies have shown that environmental changes, such as a decrease in pH or a rise in temperature and organic matter, can impact the composition of the coral holobiont[5–7]. Based on these observations, Reshef et al.[8] suggested the probiotic hypothesis: changes in microbial communities under different environmental conditions allow a rapid and versatile adaptation of the coral holobiont.

Despite the importance of microbial communities to coral health, there are few studies that have focused on the discovering of species-specific associations between corals and bacteria. Species-specific bacterial associations have been found in other animals, such as sponges, hydra, and colonial hydroids[9–11], but the existence of these associations in corals is still controversial. Rohwer et al.[12] found a ribotype of the Gammaproteobacteria group, closely associated to the coral *Porites astreoides.* Reis et al.[13] and de Castro et al.[14] suggested bacterial species-specific associations in the mucus of *Mussismilia braziliensis* and *Mussismilia hispida.* However, neither of these studies used the microbiota of sediment or of other coral species to confirm the possibility. Kvennefors et al.[15] found species- and site-specific associations in the bacterial communities of two coral species from the Australian Great Barrier Reef. Ceh et al.[16] analyzed the microbial communities associated with the tissues of three coral species, and compared them with the surrounding water and sediment, finding no species-specific associations. Lema et al.[17] assessed the diversity of the *nifH* gene, which encodes a bacterial dinitrogenase, in the tissue and mucus of three coral species from the Australian Great Barrier Reef. The sequences found in the mucus showed a great diversity, and no specific associations with either coral species or sampling site. The authors suggested that this finding was due to the ephemeral nature of the mucus. However, they also found that the dominant nifH sequence differed among the tissues of the coral species studied.

The existence of species-specific interactions between bacteria and corals is still uncertain. Few studies have characterized the coral bacterial community composition in a sufficiently statistically robust manner to support it (which means, with replicates, multiple coral species, sampling of the surrounding environment, and a sample size large enough to find rare bacterial species). The aim of this work was to investigate the existence of species-specific associations between Brazilian coral species and bacteria. Pyrosequencing of the V3 region of the 16S

rRNA gene was used to analyze the taxonomic composition of bacterial communities associated with the mucus of four coral species (*Madracis decactis*, *Mussismilia hispida*, *Palythoa caribaeorum*, and *Tubastraea coccinea*) found along the southeast Brazilian coast, during two seasons (winter and summer). The results were compared with the surrounding environment (water and sediment). Our hypothesis is that different coral species harbor distinct mucus microbial communities.

Results

Taxonomic composition and community analyses. A total of 25,035 OTUs were found in all the samples, of which more than 43% were singletons (Table 1). The Good's coverage ratio ranged between 83 and 98%. A greater diversity (H') of OTUs was found in the sediment samples (5.7 ± 0.9). The water samples presented the lowest diversity (3.5 ± 0.5), while the mucus samples were more diverse, with values ranging from 1.2 to 5.8.

In all the samples, most of the OTUs were classified as Proteobacteria (Figure 1). A higher proportion of Cyanobacteria sequences were found in water samples, and Actinobacteria and Acidobacteria sequences were more frequent in sediment samples. However, no substantial differences of phylum distribution were observed among the habitats, seasons, sampling sites, or coral species. Among the

Proteobacteria, Alphaproteobacteria dominated in the water samples, while Gammaproteobacteria dominated in the samples of sediment and the mucus of *P. caribaeorum*, *T. coccinea*, and *M. hispida*. A higher proportion of Betaproteobacteria was found in the mucus samples of the scleractinian corals *M. hispida* (1.2%), *M. decactis* (1.6%), and *T. coccinea* (2.8%) than in the other environments (Water = 0.53%, Sediment = 0.51%, and *P. caribaeorum* = 0.53%).

The analysis of similarities (ANOSIM) indicated that the OTU composition was significantly different among the communities found in the three habitats (mucus, water, and sediment, P = 0.0001).

The n-MDS was performed using Bray-Curtis similarities between the samples (Figure 2; stress value = 0.1554). In Figure 2, there is a clear grouping of the samples from water and of the samples from sediment. According to Figures 2 and 3, and the ANOSIM analysis (Table 3), samples of *P. caribaeorum* mucus were the most dissimilar, and there was no clear differentiation between the mucus samples from the Scleractinian coral species.

The ANOSIM analysis showed that there were no significant differences between the mucus communities in the two seasons (comparison of the summer and winter mucus samples gave P-values of 0.329 (*M. hispida*), 0.3303 (*M. decactis*), 0.3345 (*P. caribaeorum*), and 0.6665 (*T. coccinea*)). In the case of the water and sediment samples, the differences between the summer and winter were significant (P = 0.0004 and 0.0011, respectively). In our study, the

Table 1 | Sample information

| Sample	Habitat	Coral species	Season	Number of reads	Number of OTUs	Good's coverage ratio	Shannon index (H')
BD12A	Water	*M. decactis*	Winter	26122	1467	97.28%	4.11
BD12M	Mucus	*M. decactis*	Winter	3508	677	88.28%	4.744
BD12S	Sediment	*M. decactis*	Winter	41722	5200	93.75%	6.506
BD14A	Water	*M. decactis*	Winter	11911	775	96.55%	3.501
BD14M	Mucus	*M. decactis*	Winter	6595	899	92.27%	4.584
BD14S	Sediment	*M. decactis*	Winter	27542	3623	93.25%	6.072
BD22A	Water	*M. decactis*	Summer	35164	1516	97.65%	3.964
BD22M	Mucus	*M. decactis*	Summer	23931	3155	92.62%	5.761
BD24A	Water	*M. decactis*	Summer	24865	1090	97.61%	3.555
BD24S	Sediment	*M. decactis*	Summer	20895	3430	91.05%	6.409
BT12A	Water	*T. coccinea*	Winter	16672	1068	96.88%	3.433
BT12M	Mucus	*T. coccinea*	Winter	15069	1605	94.28%	4.793
BT12S	Sediment	*T. coccinea*	Winter	5434	891	90.60%	4.973
BT14A	Water	*T. coccinea*	Winter	31259	1037	98.58%	3.26
BT14M	Mucus	*T. coccinea*	Winter	15538	1173	96.09%	4.178
BT14S	Sediment	*T. coccinea*	Winter	6065	1207	88.77%	5.52
BT22A	Water	*T. coccinea*	Summer	18878	986	97.18%	3.376
BT22M	Mucus	*T. coccinea*	Summer	9874	1399	91.79%	5.063
BT22S	Sediment	*T. coccinea*	Summer	12661	1848	91.53%	4.566
BT24A	Water	*T. coccinea*	Summer	24438	1143	97.50%	3.672
BT24M	Mucus	*T. coccinea*	Summer	16295	2845	90.71%	6.002
BT24S	Sediment	*T. coccinea*	Summer	12640	3246	85.26%	6.957
SM12A	Water	*M. hispida*	Winter	25212	843	98.30%	3.203
SM12M	Mucus	*M. hispida*	Winter	6315	1014	91.24%	5.143
SM14M	Mucus	*M. hispida*	Winter	6550	1286	88.76%	5.341
SM14S	Sediment	*M. hispida*	Winter	16921	1257	96.28%	3.886
SM22A	Water	*M. hispida*	Summer	23455	689	98.50%	2.595
SM22M	Mucus	*M. hispida*	Summer	13831	1276	94.94%	4.392
SM22S	Sediment	*M. hispida*	Summer	8565	2382	83.79%	6.706
SM24A	Water	*M. hispida*	Summer	16956	1014	96.76%	3.467
SM24M	Mucus	*M. hispida*	Summer	20676	3032	92.55%	5.933
SM24S	Sediment	*M. hispida*	Summer	14721	2286	91.30%	5.037
SP12M	Mucus	*P. caribaeorum*	Winter	3726	241	95.89%	1.306
SP14A	Water	*P. caribaeorum*	Winter	3546	670	88.47%	4.824
SP14M	Mucus	*P. caribaeorum*	Winter	28080	1830	96.60%	3.562
SP14S	Sediment	*P. caribaeorum*	Winter	17378	2336	93.03%	5.777
SP22M	Mucus	*P. caribaeorum*	Summer	7487	1274	89.35%	4.454
SP22S	Sediment	*P. caribaeorum*	Summer	13420	3161	86.38%	6.728
SP24A	Water	*P. caribaeorum*	Summer	37184	1342	98.10%	3.253
SP24M	Mucus	*P. caribaeorum*	Summer	10313	1582	90.61%	4.441
SP24S	Sediment	*P. caribaeorum*	Summer	13957	3234	86.92%	6.759
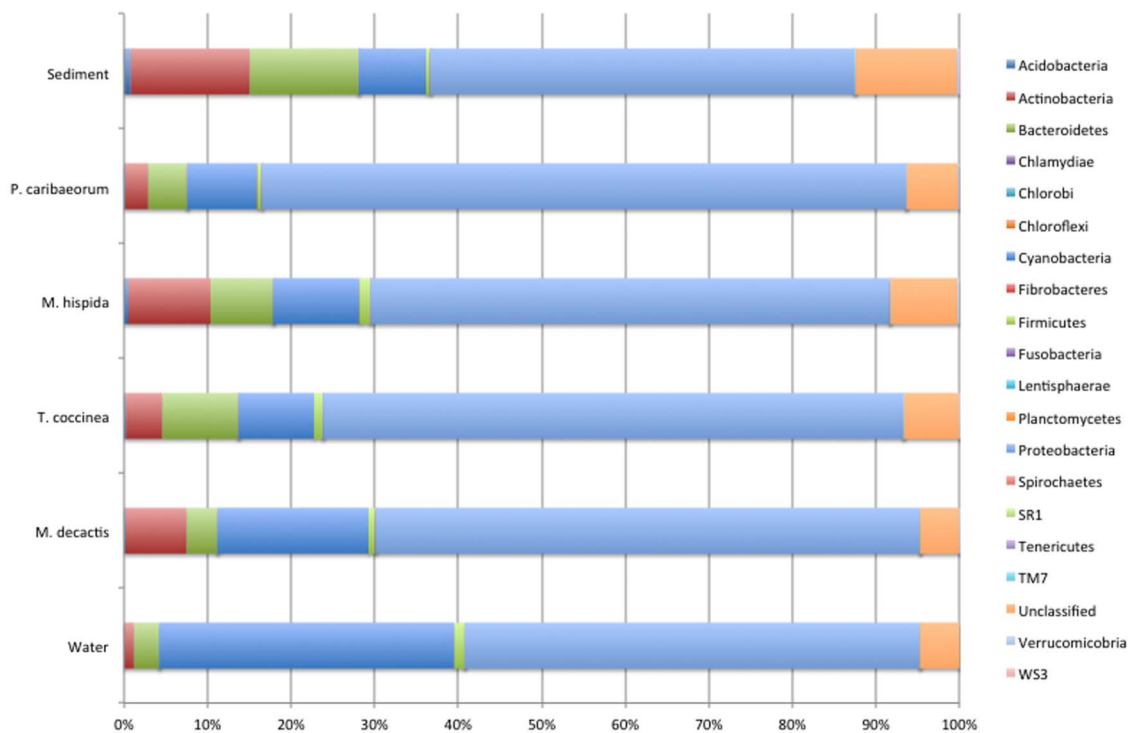
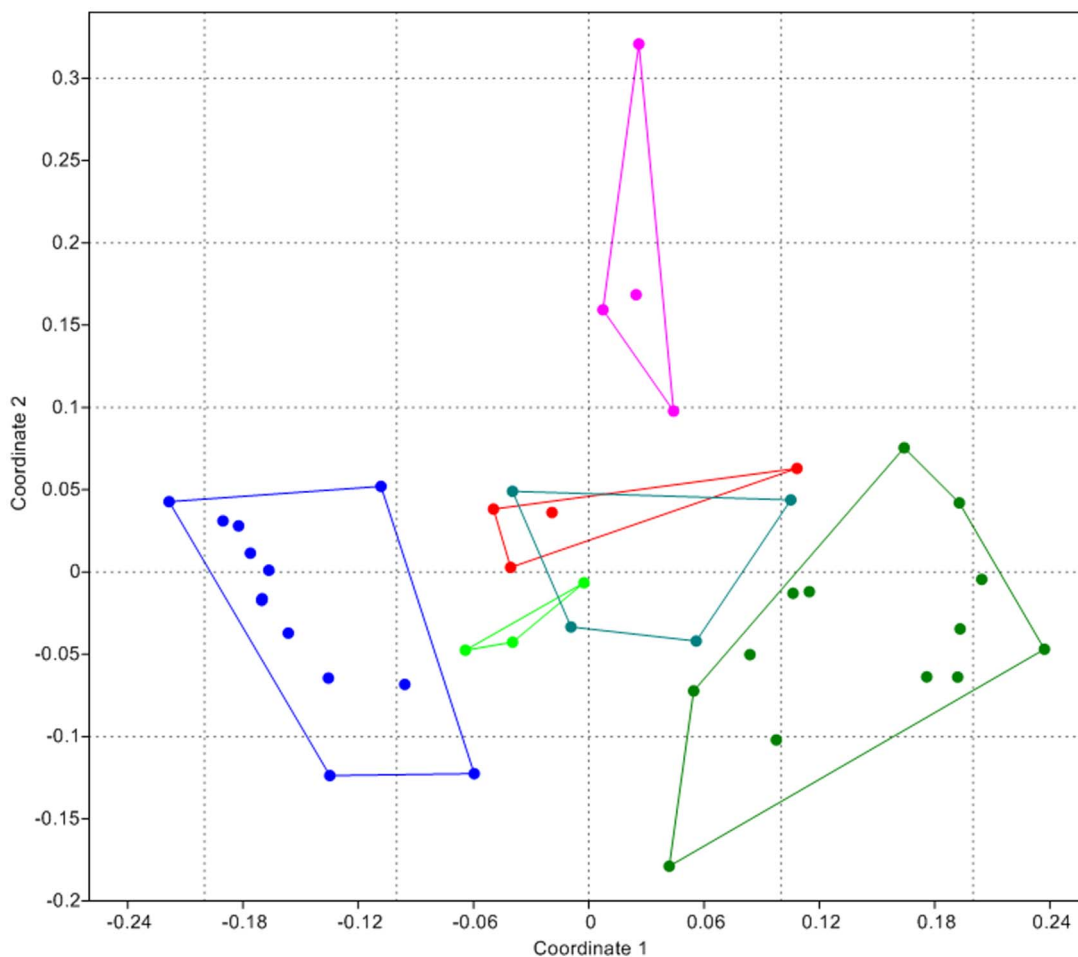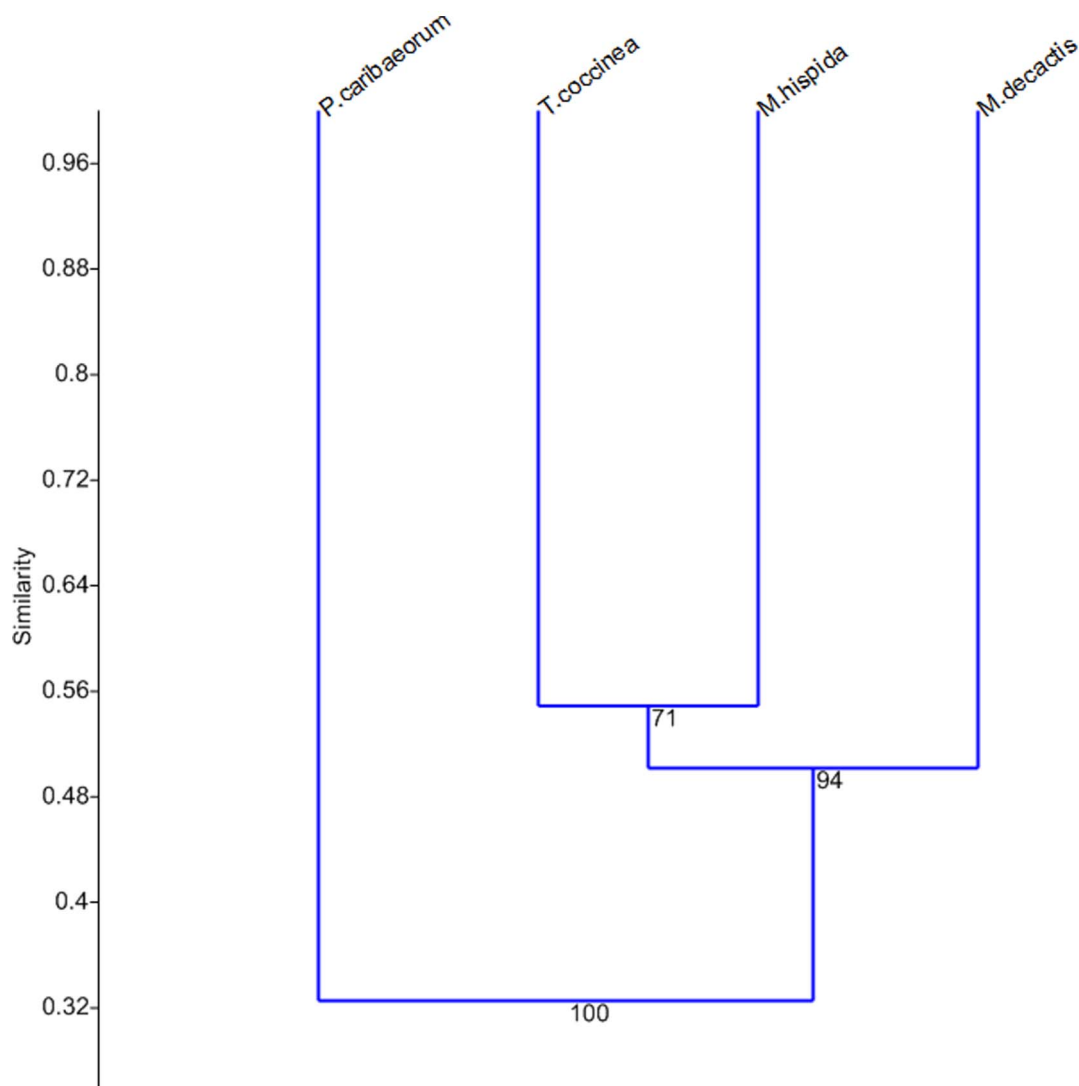Figure 1 | Average of phylum relative abundance in each habitat.

Figure 2 | Nonmetric multidimensional scaling (nMDS) plots of the first 2 dimensions based on Bray-Curtis dissimilarities for water (dark blue), sediment (dark green), *M. hispida* mucus (light blue), *M. decactis* (light green), *T. coccinea* (red), and *P. caribaeorum* (pink). The stress value is 0.1554.

Figure 3 | UPGMA cluster analysis of the mucus communities of the four coral species studied. The UPGMA clustering was based on Bray-Curtis dissimilarities using the mean of the relative abundance of OTUs for each coral species. Bootstrap values (in%) for 1000 replicates are given at the nodes.

communities of water and sediment did not differ significantly between the two sampling sites (P = 0.1749 and 0.1723, respectively). The difference between the sampling sites was significant for the samples of mucus (P = 0.0124), however the difference was not significant (P = 0.0808) when the samples of Palythoa were excluded from the analysis.

Most abundant OTUs for each habitat. The SIMPER analysis highlighted the OTUs primarily responsible for the observed differences between the habitats studied, using the relative abundances of the OTUs in each habitat. The five main results for each habitat are presented in Table S1, and their relative abundances are given in Table S2 (supplementary material). The OTU 23763, which was mainly associated with the water samples, was classified to the GPIIA group of Cyanobacteria, which includes the genera *Prochlorococcus* and *Synechococcus* that contribute most to the primary production in oligotrophic oceans[18].

Some of the OTUs most related with *M. decactis* presented similarity with bacterial genera often associated with the degradation of organic pollutants. These included OTU 6997, which showed similarity with *Rhodococcus erythropolis*[19], OTU 23270, related to *Sphingomonas*[20], and OTU 19915, classified as *Brachybacterium*[21]. In addition, OTU 6481, attributed to the *Acinetobacter* genus, has been found in great abundance in bleached corals[22].

The OTUs of *M. hispida* mucus, identified by SIMPER as important for distinction of the microbial community, were only found in the colonies of this species, but in low abundance. None showed similarity with previously described microorganisms. However, four of them (14290, 20636, 25133, and 23022) showed greater similarity in databases with sequences of 16S rDNA libraries of Brazilian coral.

The OTU 7634 dominated in the communities of *P. caribaeorum* mucus, representing around 40% of all reads found in these samples. This OTU and another two, also identified by SIMPER analysis, were classified as Gammaproteobacteria, and showed similarity to sequences of 16S rDNA libraries of the soft coral *Sinularia* sp subjected to thermal stress.

One of the OTUs of *T. coccinea*, 17001, was classified in the genus *Tenacibaculum* and showed similarity with the sequence of 16S libraries of corals affected by black-band disease. The OTU 9209, classified as Alphaproteobacteria, showed similarity with a sequence of 16S from the Scleractian cold-water coral *Lophelia pertusa*. The OTU 16320 was classified in the genus *Haliagium*, a marine myxobacterium isolated in Japan that has known antifungal and antibiotic properties[23].

The OTU 16689, associated with sediment, was classified to the nitrogen-fixing genus *Mesorhizobium*[24], and the OTU 20185 was classified as a Gammaproteobacterium, which showed 100% similarity with a sequence from a 16S library of bleached *Siderastrea stellata*.

Table 2 | Information about species-specific OTUs

OTU	RDP Classifier [bootstrap value]	blastn (identity)	Description (GENBANK)	Title (GENBANK)	Authors (GENBANK)	Coral mucus
14290	Alphaproteobacteria[88%]	HQ837587 (98%)	uncultured bacterium; coral reef	Bacterial communities associated with three endemic *Mussismilia* species in disturbed coral reef environment from Abrolhos Bank, Brazil	Castro, A. and Kruger, R.	*M. hispida*
21841	Alphaproteobacteria[92%]	FJ628227.1 (97%)	uncultured bacterium; brackish water from anoxic fjord	Phylogenetic diversity of transition and anoxic zone bacterial communities within a near-shore anoxic basin: Nitinat Lake	Schmidtova, J., et al.	*M. hispida*
23022	Chromatiales[55%]	FJ216075 (99%)	uncultured gamma proteobacterium; coral	Archaea, bacteria, and algal plastids associated with the reef-building corals *Siderastrea stellata* and *Mussismilia hispida* from Buzios, South Atlantic Ocean, Brazil	Lins-de-Barros, M.M., et al.	*M. hispida*
6336	Gammaproteobacteria[99%]	JF268381.1 (94%)	uncultured bacterium; New Zealand cold seep	Microbial communities of marine methane seeps at Hikurangi Margin (New Zealand)	Ruff, S.E., et al.	*T. coccinea*
15129	Alphaproteobacteria[51%]	FJ041420.1 (98%)	uncultured bacterium; Lophelia pertusa (scleractinian coral)	Culture-independent characterization of bacterial communities associated with the cold-water coral Lophelia pertusa in the northeastern Gulf of Mexico	Kellogg, C.A., et al.	*T. coccinea*
11074	Alphaproteobacteria[59%]	FJ041420.1 (97%)	uncultured bacterium; Lophelia pertusa (scleractinian coral)	Culture-independent characterization of bacterial communities associated with the cold-water coral Lophelia pertusa in the northeastern Gulf of Mexico	Kellogg, C.A., et al.	*T. coccinea*
9266	Gammaproteobacteria[89%]	AB611839.1 (94%)	uncultured gamma proteobacterium; polyp gastric cavity	Biological and chemical characteristics of the coral gastric cavity	Agostini, S., et al.	*P. caribaeorum*
3573	Gammaproteobacteria[97%]	JF721992.1 (93%)	bacterium HQE2; Styela clava	Isolation and phylogenetic analysis of bacteria from the Styela clava	Miao, T., et al.	*P. caribaeorum*

In addition to these results, genera present in at least two coral species, identified from mucus libraries, included *Achromobacter* (Betaproteobacteria), *Acidovorax* (Betaproteobacteria), *Agarivorans* (Gammaproteobacteria), *Faecalibacterium* (Firmicutes), *Fibrobacter* (Fibrobacter), Flammeovirga (Bacteroidetes), *Marinobacter* (Gammaproteobacteria), *Marmoricola* (Actinobacteria), *Psychrobacter* (Gammaproteobacteria), *Serratia* (Gammaproteobacteria), and *Zunongwangia* (Bacteroidetes). *Marmoricola* and *Psychrobacter* were found in the four coral species.

Species-specific OTUs. We found species-specific OTUs in *P. caribaeorum*, *T. coccinea*, and *M. hispida*. All these OTUs were present in low abundance (between 20 and 190 reads, around 0.02% of the total reads for each coral).

The OTUs 23022, 14290, and 21841 were only found in three of the four colonies of *M. hispida*. The OTUs 6336, 11074, and 15129 were only found in the four colonies of *T. coccinea*. The OTUs 9266 and 3573 were found in the four colonies of *P. caribaeorum*. Table 2 presents information about these OTUs.

Most of the genera that were found associated with mucus are aerobic and heterotrophic, and are commonly found in a wide range of hosts. These include *Acinetobacter*, *Brachybacterium*, *Escherichia*, *Janthinobacterium*, *Pseudomonas*, *Sphingomonas*, and *Tenacibaculum*. However, some of the species-specific OTUs related to the Scleractinian corals were classified to autotrophic bacterial groups, such as Alphaproteobacteria and Chromatiales (purple sulfur bacteria).

Discussion

The bacterial communities found in the three habitats (mucus, water, and sediment) were significantly different, which is in agreement with most of the studies published to date[25,26]. Some bacterial groups and OTUs were most related to a determined habitat. For example, the OTUs attributed to Betaproteobacteria, a group that includes the *Janthinobacterium* genus, were found to be most associated with the mucus of *M. hispida*, *M. decactis*, and *P. caribaeorum*. *Janthinobacterium*, previously belonging to the Chromobacterium genus, is a gram-negative violacein-producing Betaproteobacterium found in soil, water, and some animal surfaces[27,28]. Members of this family have already been found in the mucus of corals, and Kooperman *et al.*[29] suggested that they can be an obligate symbiont of *Fungia granulosa*.

Our results indicate not only that the microbial community of the mucus was distinct from the surrounding environment, but also that it was more stable throughout the seasons than the communities of the water column and the sediment. We suggest that the community of the mucus is less influenced by physical factors, such as temperature, compared to the communities in the surrounding environment. Our results differ from those obtained by Ceh *et al.*[16], who studied the microbial communities of three coral species in Australia and concluded that seasonal changes were more important in determining the microbiota than the coral species and the spatial separation of coral. We believe that the stability found in the mucus community in our study can be attributed to the antimicrobial properties of the mucus. Studies have demonstrated the bacterial growth inhibitory activity of the mucus of several coral species[2,30], and we have observed this property for the mucus of the coral species studied in this work (unpublished data). As suggested by Kvennefors *et al.*[15], host and microbial factors can be responsible for the selection and maintenance of the bacteria that can inhabit the coral tissues.

The results also indicated that host phylogeny and genetic factors might be more important in determining the bacterial community than the presence of the zooxantellae (found in association with *P. caribaeorum*, *M. hispida*, and *M. decactis*, but not *T. coccinea*), since we observed that the bacterial community of the zoanthid *P. caribaeorum* was different to the communities of the other species

Table 3 | P-values derived from ANOSIM pairwise comparisons of bacterial community composition values using Bray-Curtis values. The values highlighted in bold are statistically significant (P < 0.05)

	Water	M. decactis	T. coccinea	M. hispida	P. caribaeorum	Sediment
Water	0	**0.0017**	**0.0008**	**0.0004**	**0.0005**	**0.0001**
M. decactis	**0.0017**	0	0.0893	0.171	**0.0278**	**0.0052**
T. coccinea	**0.0008**	0.0893	0	0.1748	**0.031**	**0.0008**
M. hispida	**0.0004**	0.171	0.1748	0	**0.0311**	**0.0345**
P. caribaeorum	**0.0005**	**0.0278**	**0.031**	**0.0311**	0	**0.0007**
Sediment	**0.0001**	**0.0052**	**0.0008**	**0.0345**	**0.0007**	0

studied. This supports the notion of the existence of Scleractinian-specific associations[31].

We have found OTUs that seem to be specific for each coral species. The species-specific OTUs of *P. caribaeorum* are related to the *Endozoicomonas* genus, which belongs to the order Oceanospirillales. *Endozoicomonas* and Oceanospirillales bacteria have been frequently found associated with corals, especially of the *Porites* spp. genus[32]. They dominate the community of *Porites astreoides*[33], and the species *Endozoicomonas montiporae* was first isolated from the coral *Montipora aequituberculata*[34]. The role of these bacteria and the characteristics that allow their widespread association with marine organisms are still unknown[32].

One of the most interesting species-specific OTUs was 23022, only found in the mucus of *M. hispida* and classified to the order Chromatiales, a group of microaerophilic or anaerobic, photosynthetic, and sulfur-oxidizing bacteria[35]. The major hits of this OTU in the databases are with sequences of 16S libraries of *Mussismilia* species, which is strong evidence for the specificity of this OTU to *M. hispida*. In corals, phototrophic sulfur-oxidizing bacteria are usually found in the microbial consortia that cause black band disease. This is a major disease affecting corals, and is characterized by degradation of the tissue of the animal by the action of hydrogen sulfide (H2S) produced by sulfate-reducing bacteria[36], such as those found in sediment. Childress & Girguis[37] suggested that the H2S detoxification function of sulfur-oxidizing bacteria in the symbiotic relationships with marine animals is not as important as the inorganic carbon fixing function. Nevertheless, we believe that H2S detoxification may be the main function required in a relationship between zooxanthellate corals and sulfur-oxidizing bacteria, since algae endosymbionts are responsible for production of most of the energy used by the corals. Thus, our hypothesis is that the presence (at low density) of phototrophic sulfur-oxidizing bacteria, such as those characterized by the OTU 23022, could assist in the balance of sulfur compounds in the coral tissues, protecting the host against the action of H2S. Raina *et al.*[38,39] have suggested the role of sulfur compounds in determining the bacterial communities associated with corals. Based on these observations, we believe that more studies are needed to investigate the role of sulfur compounds in the coral holobiont, in order to elucidate their importance in species-specific associations between bacteria and corals.

We emphasize the importance of including coral reef sediments in future studies of coral microbiota. As observed in this study as well as by Schöttner *et al.*[25,26], sediment presented the most diverse bacterial community among the coral reef habitats studied. We observed that mucus shared more OTUs with sediment than with water, suggesting that sediments might act as a seed bank of bacteria that could colonize the coral surfaces. Furthermore, sediment can also provide a reservoir of opportunistic coral pathogens, so that the study of this habitat could aid in understanding bacterial dynamics in coral reef environments.

We are aware that it is not possible to affirm that the species-specific OTUs found in this study represent mucus residents, because (as discussed by Lema *et al.*[16]) the ephemeral nature of the coral mucus could preclude strong bacterial associations. Hence, many

of these bacteria may actually inhabit the coral tissues, which could explain their low density in the mucus samples.

Methods

Sample collection. The samples were obtained during the winter of 2010 (June) and the summer of 2011 (January), at two locations: São Sebastião Channel (S 23°51′467 W 45°25′867) and Búzios Island (S 23°48′157 W 45°07′181) in São Paulo State, Brazil. The sea surface temperature was around 20°C in the winter and 28°C in the summer at the two locations. The water depth was 9 m at São Sebastião Channel and 11 m at Búzios Island. The samples of *Mussismilia hispida* (Scleractinia) and Palythoa caribaeorum (Zoantharia) were collected from the São Sebastião Channel, and the samples of Tubastraea coccinea (Scleractinia) and Madracis decactis (Scleractinia) were collected from Búzios Island.

From each coral colony (apparently healthy) we collected 10 ml of mucus, 1 liter of surrounding water directly above the colony, and 50 ml of sediment directly below the colony. Four colonies of each coral species were sampled, spaced around 10 m from each other. Water samples were collected in sterile plastic bottles and first filtered through sterile filter paper to remove debris and then vacuum filtered through a 0.22 micrometer Millipore membrane, which was used for the DNA isolation. Sediment samples were collected in sterile 50 ml centrifugation tubes. The mucus samples were collected using sterile syringes. All the samples were stored on ice during transport to the laboratory, and frozen at −20°C until DNA isolation was performed.

DNA isolation and 16S library construction and pyrosequencing. Total DNA of all samples was isolated using the Wizard Genomic DNA Purification Kit (Promega), according to the manufacturer's instructions for Gram-positive bacteria, using a pellet of the environmental sample (or a membrane in the case of water samples, instead of the pellet of cells). The average yield was around 10 ng/µL of DNA for each sample.

The amplification of the V3 region of the 16S rDNA was performed according to Clingenpeel *et al.*[40], using the primers 338F (ACTCCTACGGGAGGCAGCAG) and 533R (TTACCGCGGCTGCTGGCAC)[41]. The amplification reactions (final volume 25 µL) employed 3–10 ng of DNA, 1U AccuPrime Pfx® DNA polymerase (Invitrogen), AccuPrime Pfx Reaction Mix® 1X (Invitrogen), and 0.4 mM of each primer. Amplification was performed under the following conditions: initial denaturation at 94°C for 1 min, and 25 cycles at 94°C for 30 seconds, 62°C for 1 min, and 72°C for 1 min. After these 25 cycles, a further five cycles were performed, using the same conditions, in order to add the specific barcodes from each sample and the adapters A and B (reaction with final volume of 50 µL). The amplification products were purified with the GFX PCR and DNA Gel Band Purification Kit (GE Healthcare), quantified using the Qubit Kit (Invitrogen), and the quality (integrity and presence of a unique band) was confirmed by 1% agarose gel electrophoresis. An equimolar mixture of the samples from each library (summer and winter) was employed in order to obtain 5000 ng of PCR product in total, with a minimum concentration of 20 ng/µl. The winter library was composed of 20 samples: 6 of water, 8 of mucus, and 6 of sediment. The summer library was composed of 21 samples: 7 of water, 7 of mucus, and 7 of sediment. The amplicons were sequenced with the 454 technology, a GS FLX Roche Company platform.

Sequence processing and statistical analysis. The initial processing of the sequences was carried out with the package QIIME (http://qiime.org/). The sequences were screened according to quality (minimum of 25) and size (between 150–240 bp). No ambiguous bases or mismatches were admitted in the sequence of the primers, and we checked for the existence of chimeras. The sequences with similarity greater than or equal to 97% were grouped into operational taxonomic units (OTUs), and the taxonomic classification was performed using the RDP (Ribosomal Database Project: http://rdp.cme.msu.edu/classifier/classifier.jsp) classification threshold of 80%. Rarefaction curves were also constructed with the QIIME package. The Good's coverage ratio (C) was calculated according to the formula $C = 1 - ni/N$, where ni is the number of OTUs that were sampled only one time (singletons) and N is the total number of sequences in the sample. The Shannon index (H) was calculated according to the formula $H = -\Sigma\, pi\, \ln pi$, where pi is the proportion of OTU i relative to the total number of OTUs.

The community analysis was performed according to Laverock *et al.*[42] and Theis *et al.*[43]. Multidimensional scaling (n-MDS) analysis was used to obtain the

community similarities (Bray-Curtis values), employing the relative abundances of OTUs in each sample. Analysis of similarities (ANOSIM) was used to test the hypothesis that bacterial communities from the same habitat were more similar to each other than to communities from different habitats. Similarity percentage (SIMPER) analysis was used to identify the most abundant OTUs for each habitat, employing the relative abundances of OTUs in each sample. The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering was based on Bray-Curtis dissimilarities using the average between the relative abundances of OTUs of all the samples from each coral species. Bootstrapping with 1000 resamplings was performed to determine the robustness of the clustering. All these analyses were performed with the statistical software PAST (http://folk.uio.no/ohammer/past/).

We have considered as species-specific OTUs those found in only one coral species and in at least three of the four colonies studied, since they are not necessarily a symbiont found in all the individuals of the species.

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Author contributions

L.M.M.O., C.C. and T.T.T. conceived the work. C.C. performed the experiments and wrote the manuscript. All authors reviewed the final manuscript.

Additional information

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