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ĂProkaryotic abundance and community structure in mucus of reef- building coralsò

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ĂWie jede Blüte welkt und jede Jugend

Dem Alter weicht, blüht jede Lebensstufe,

Blüht jede Weisheit auch und jede Tugend

Zu ihrer Zeit und darf nicht ewig dauern.

Es muß das Herz bei jedem Lebensrufe

Bereit zum Abschied sein und Neubeginne,

Um sich in Tapferkeit und ohne Trauern

In andre, neue Bindungen zu geben.

Und jedem Anfang wohnt ein Zauber inne,

Der uns beschützt und der uns hilft, zu leben.ñ

Hermann Hesse (1941): Die Stufen

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Abstract

Bacterial and archaeal communities inhabit the surface mucus layer of the reef-building corals *Montastraea annularis, Porites astreoides* and *Siderastrea siderea.* To determine the diversity, spatial distribution and host specificity of mucus-inhabiting prokaryotes, terminal restriction fragment length polymorphism (T-RFLP) of the 16S rDNA was used to investigate the archaeal and bacterial community composition. Additionally, coral mucus release was measured and prokaryotic abundance determined for several coral species. In general, bacterial and archaeal communities associated with coral mucus were distinctly different from those of the ambient water and nearby sediments. Bacterial communities in coral mucus were more diverse (H', 3.8-4.0 OTUs) than archaeal communities (H', 2.7-2.9). Mucus of *M. annularis* showed the highest diversity of bacterial communities (H' 4.0) whereas mucus of *S. siderea* showed the highest diversity of archaeal communities (H', 2.9). The bacterial community of the mucus layer was found to be host species-specific, while archaeal communities were not. Moreover, bacterial communities within coral species changed with spatial distance of the coral colonies while archaeal communities of the mucus layer of corals did not exhibit spatial variability. The prokaryotic abundance varied among coral species and was related to the thickness of the mucus layer. Corals with a thinner mucus layer (on average 70 µm for *P. astreoides*) harbored less prokaryotes per mucus volume (2.1 x 10^6 cells ml^{-1}) than corals with thicker layers (220 µm for *M. cavernosa*; 3.23x 10^6 cells ml^{-1}). Coral species showed different dynamics of mucus release over time. Exposing coral heads to air resulted in initially high mucus release rates in *Montastrea cavernosa, Diploria labyrinthiformis* and *Colpophyllia natans,* decreasing rapidly over time. *Porites astreoides* showed a gradual decrease of mucus production and *Diploria strigosa* released mucus at rather constant rates. Taken together, this study demonstrates that bacterial communities associated with coral mucus show interspecific and spatial variability, while archaeal communities do not exhibit coral host-specificity and spatial variability.

1. Introduction

The coral holobiont includes the coral itself, zooxanthellae, fungi, endolitihic algae, Archaea and Bacteria (Rohwer et al. 2002; Kellogg 2004). The coral host epithelium is separated from the seawater environment through a thin layer of mucus in which microbes are embedded acting as a barrier to the ambient water and potential environmental stress (Herndl and Velimirov 1986). Overall corals invest 20 to 45% of their daily photosynthetic production to generate mucus, produced by secretory cells in the coral's ectoderm (Crossland 1987, Edmunds and Davies 1986, Brown and Bythell 2005). Zooxanthellae play an important role in mucus production, transferring photosynthetically fixed carbon to the coral which is partially released as mucus (Muscatine et al. 1981). Mucus is a lipopolysaccharide-protein complex (Brown and Bythell 2005) consisting of several carbohydrates (C6 sugars, C5 sugars, desoxysugars, amino sugars), lipids and inorganic nutrients (Wild et al. 2005; Ducklow and Mitchell 1979b). There is evidence for a species-specific composition of mucus, for instance *Fungia* contained significantly more fucose than the other corals (Wild et al. 2005; 2010). Corals produce and release continuously mucus and nearly half of the released mucus dissolves in the water column and plays a role as microbial substrate in reef waters (Wild et al. 2004).

Mucus has several functions in corals: it protects the coral against sedimentation, desiccation during air-exposure at low tide, functions as sunscreen through UV-absorbing compounds and plays a role in the ciliary feeding of corals (Brown and Bythell 2005, Wild et al. 2004, Cross et al. 1984). Mucus serves also as a growth medium for cooperative or commensal microorganisms preventing colonization of the mucus by pathogens (Brown and Bythell 2005). The nutrient-rich mucus provides potential niches for microbes but the actual ecological role of bacterial and archaeal communities in mucus is still poorly known (Paul et al. 1986; Herndl and Velimirov 1986; Rosenberg et al. 2007). Siboni et al. (2008) suggest that ammonia-oxidizing Archaea are important for the nitrogen cycle in the coral. Rowher et al. (2001) suggest that Bacteria are involved in nitrogen cycling too. Bacteria can also acquire nutrients for the coral and act as a protective shield against pathogens by producing antimicrobial substances (Rohwer et al. 2002; Wegley et al. 2007).

Climate change, pollution and overfishing are affecting the interactions between corals and microbes and can lead to coral diseases such as the white- and black-band disease or coral bleaching (Rosenberg et al. 2007). Bacterial communities inhabiting the mucus of healthy

corals are distinctly different from those of moribund or diseased corals (Littman et al. 2010; Gil-Agudelo et al. 2007; Cárdenas et al. 2011). It has been estimated that over the past 30 years about 30% of coral reefs have been lost worldwide (Harvell et al. 2007). Thus, to better understand the environmental factors leading to coral-diseases, we also need better knowledge on the dynamics of the microbial communities inhabiting the coral mucus layer.

Previous studies showed that coral associated archaeal- and bacterial communities are diverse and differ from those of the surrounding seawater (Rohwer et al. 2002; Kellogg 2004; Klaus et al. 2005; Tremblay et al. 2011). Our knowledge on the archaeal communities in corals, however, is rather limited. The few studies available suggest that archaeal communities in the mucus and tissue do not vary among different coral species and the community composition remains rather stable over large geographical distances (Wegley et al. 2004; Kellogg 2004; Siboni 2012; Klaus et al. 2005). Several studies indicate that bacterial communities are common in the mucus, tissue and skeleton of corals. Mucus and tissue have species-specific bacterial communities differing from those of the surrounding seawater (Tremblay et al. 2011, Rohwer et al. 2002). Some studies showed that species-specific bacterial communities do not change with spatial distance (Ritchie and Smith 1997, Rohwer et al. 2002, Rohwer et al. 2001), while other studies found that bacterial communities do change over geographical scales (McKew et al. 2012; Kvennefors et al. 2010).

The present study analyzes the prokaryotic community composition of three coral species, *Montastrea annularis, Porites astreoides* and *Siderastrea siderea*. All three corals are important and common Caribbean reef-building corals. *Montastrea annularis* has different morphotypes but this study focuses on the typical columnar shape. Individual colonies reach a diameter of up to 3 m and the surface is covered with distinctive corallites (Humann 1993). *Porites astreoides* has a plate-like morphology in shallower waters and appears as rounded heads and domes in deeper waters with a yellow-brown or yellow-green color (Humann 1993). *Siderastrea siderea* has a hemispherical boulder shape, reaches a diameter of 1.8 m with a brownish color (Humann 1993).

The aim of this study is to compare prokaryotic abundances and community structure in the surface mucus layer of *M. annularis*, *P. astreoides* and *S. siderea*. The community structure was assessed at different sites and depths off the Caribbean island off Curaçao (Netherlands Antilles).

2. Materials and Methods

Study site

Fieldwork was conducted on Curaçao, an island in the southern Caribbean, from mid May to end June 2012 (FIG. 1). Fieldwork activities were based at CARMABI (Caribbean Research and Management of Biodiversity) Foundation in Piscaderabaai near Willemstad. The fringing reef is characterized by a gradual slope to 10-15 m depth and a drop off to 50-60 m (Bak 1977). Three sampling sites were chosen, Buoy One, about 500 m west of CARMABI, Snake Bay and Vaersen Bay (see FIG. 2).

FIG. 1 Curaçao and the Caribbean sea (Frade et al. 2008, S. 692)

Coral mucus collection:

Sterile cotton swabs were used to collect the mucus from healthy, light-exposed corals. The samples were taken by SCUBA diving and by gently rolling the swabs over the surface of the coral colony (covering ca. 9 cm^2). Swabs were kept in 2 ml tubes, which were brought to the surface and placed in a cooling box, filled with seawater to maintain in situ temperature conditions. Some corals were removed with hammer and chisel and were transported in a plastic bag which, in turn, was placed in a cooling box. Upon arrival at the CARMABI station, the coral heads were placed in an aquarium with running seawater. Mucus samples were taken after one week from these incubated corals. Sediment samples were taken from the vicinity of the sampled corals by filling 2 ml tubes with sediment. For comparison, water was collected with a plastic cylinder

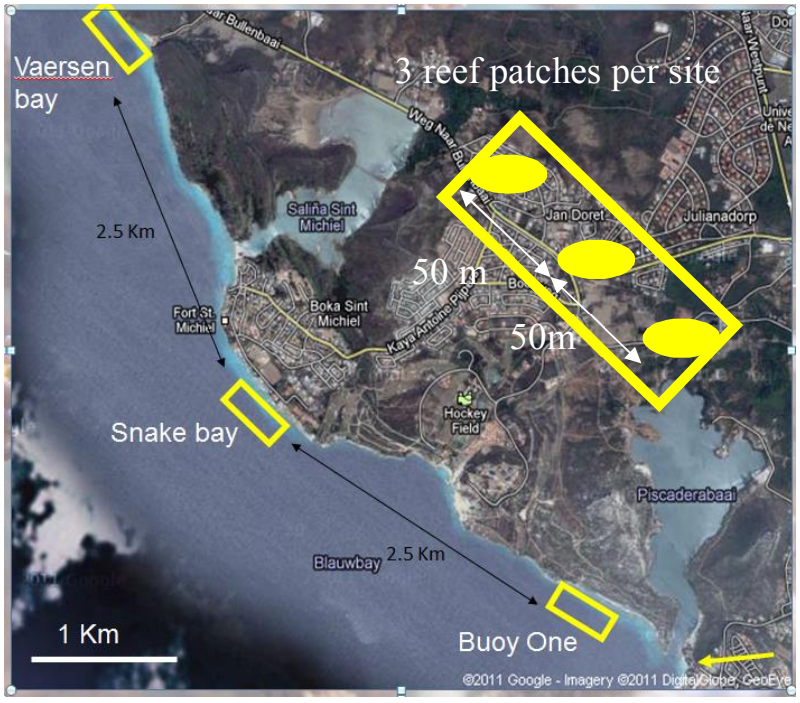


FIG. 2 Curaçao and the three dive sites (Google 2011)

(5 l) by plugging the open ends with silicone stoppers. For characterizing the prokaryotic community composition of the ambient water, 2 l of water was filtered through a 0.2 µm polycarbonate filter (Millipore). Samples were flash-frozen in liquid nitrogen and stored at -20°C within 2 h after collection (Guppy and Bythell 2006). Samples were transported back to the home lab (University of Vienna) in a biofreezer and stored at -80°C.

For the present study, we report the results of mucus samples collected at the same sites as described above by Pedro Frade in 2011, using the same method as mentioned above. Mucus from three different coral species, *Porites astreoides (*Poritidae*), Siderastrea siderea (*Siderastreidae*) Montastraea annularis (*Faviidae*)*, and reference samples from sediment and ambient water were taken at the three sites (Buoy One, Snake Bay and Vaersen Bay) and at two depths (5 and 15 m). The site Buoy One is 2.5 km apart from Snake Bay and 5 km apart from Vaersen Bay. At each site three patches were sampled, each 50 m away from each other (see FIG. 2).

Nucleic acid extraction

DNA was extracted from the swabs (containing prokaryotes from the coral mucus layer), the ambient water and the sediment using a FastDNA spin kit for soil (MP Biomedicals). Cells were mechanically lysed by bead beating with a Fast Prep Instrument for 40 s at a speed setting of 6.0. The DNA was eluted with 80 µl of DES (DNase/pyrogen-free water).

Archaeal 16S rDNA amplification

Archaeal 16S rRNA genes were amplified from the DNA extracts using the Archaea specific 21F primer (5'-TTCCGGTTGATCCYGCCGGA-3'; DeLong 1992) and the 915R primer (5'-GTGCTGCCCCGCCAATTCCT-'3; Stahl & Amann 1991). Fluorescent FAM and VIC labels were linked to the 5'-end of forward and reverse primer, respectively. One PCR reaction (50 µl) contained 23.2 µl of Sigma water, 5 µl of 10x Dream taq buffer (Fermentas), 2.5 mM $MgCl_2$, 0.2 µM dNTP's, 5 U BSA, 0.25 µM of each primer, 0.06 U of Dream taq polymerase (Fermentas, 5 U/µl) and 6-8 µl of the template DNA. A touchdown-PCR protocol was applied as follows: 1 cycle at 95°C for 4 min; 20 cycles at 95°C for 1 min, held at 66°-55°C for 45 s, and at 72°C for 90 s; 20 cycles at 95°C for 1 min, at 55°C for 45 s and at 72°C for 1 min and 1 cycle at 72°C for 10 min and held at 4°C.

Bacterial 16S rDNA amplification

The bacterial 16S rRNA gene was amplified for the same samples using the Bacteria specific Primer 27F-FAM primer (5'-AGAGTTTGATCMTGGCTCAG-3') and the universal 1492R-VIC primer (5'TACGGYTACCTTGTTACGACTT-3'; Amann et. al 1995). One PCR reaction (50 µl) contained 36.2 µl of Sigma water, 5 µl of 10x Dream taq buffer (includes 20 mM $MgCl_2$), 0.2 µM dNTP's, 5 U BSA, 0.25 µM of each primer, 0.06 U of Dream taq polymerase (Fermentas, 5 U/µl) and 2 µl of the template DNA. The PCR program was 1 cycle at 95°C for 4 min, 32 cycles at 95°C for 1 min, at 55°C for 45 s, at 72°C for 90 s, 1 cycle at 72°C for 10 min and held at 4°C.

Terminal-restriction fragment length polymorphism (T-RFLP) analysis

The PCR products of the DNA extracts of the mucus, sediment and ambient water were checked on 1.5% agarose gels run at 100 V for 30 min and stained for 20 min in a TBE-Buffer SYBR Gold bath. For each sample, three PCR replicates were pooled and purified with the PCR-Extract Mini Kit from Prime. PCR products were further digested with 0.2 µl of restriction enzyme 20 U *Hha*I (New England BioLabs), 0.2 µl of 100x BSA (New England BioLabs) and 2 µl of 10x buffer (New England BioLabs) at 37°C for 720 min on an Eppendorfer Mastercycler Pro S. The enzymatic reaction was thermally terminated at 65C° for 20 min and cooled down to 4°C. The DNA concentration for each sample was measured spectrophotometrically (Nanodrop). Thereafter, 200 ng of each sample was mixed with 10 µl HiDi formamide and 0.4 µl LIZ, a size-marker (Genescan 1200), and denatured at 95°C for 3 min (Moeseneder et al. 2001). Samples were sequenced with a 16x capillary sequencer (Applied Biosystems) and the output was visualized with PeakScanner and then transferred into the software GelCompare. The presence of T-RFLP peaks was scored using the GelCompare software. The electropherograms were standardized by the size marker and to avoid scoring primers and restriction fragments larger than the size marker, peaks smaller than 50 bp and larger than 1200 bp were eliminated from the data set. Background noise was calculated by the Wiener cut-off scale and background scale and discarded from peak selection. For the archaeal community, the minimum profiling for peaks to be scored was 3% of peak height, and the shoulder sensitivity 2. For the bacterial community, the minimum profiling was 0.4% of peak height and shoulder sensitivity 2. In this study, the percentage of minimum profiling was chosen in a way that the total number of operational taxonomic units (OTUs) identified by GelCompare would be in the same range of the number of OTUs identified manually when using the Peak Scanner software.

First, band classes were automatically created according to existing peaks. Then, a band class was deleted if located less than 1 bp away from another band class. Further, a new band class was created to match any peak located more than 3 bp away from its nearest band class and bands were assigned automatically to new band classes. The outcome, a presence/absence matrix including each band class (OTU) and each sample was transferred into Excel, into the program Primer6 (Clarke and Gorley 2006), into EstimateS (Colwell 2005; Version 7.5.2) and into SPSS (Version 15) for further analysis.

Statistical analyses related to prokaryotic community composition

The distribution of OTUs and their rank frequency distribution were calculated using Excel. Beta-diversity was calculated after Whittaker (1960): $\beta = \alpha / \gamma$. Here gamma-diversity is the total OTU richness of coral species or sites and alpha-diversity is the average OTU number of colonies from each species or sites. The richness and the Shannon-Wiener diversity index were calculated using Primer6. To evaluate the dissimilarity between the samples, the Jaccard coefficient was calculated using the program Primer6. Analysis of Similarity (ANOSIM) was used to test whether microbial communities differ significantly in their composition (presence/absence) between coral species, sites, reef patches and depths using the Jaccard coefficient as similarity index between samples. The MDS ordination plots showed a profiling of the community structure. Rarefaction curves were calculated by EstimateS (Statistical Estimation of Species Richness and Shared Species from Samples, Colwell 2005). One-way analysis of Variance (ANOVA) was testing the homogeneity in the number of OTU averages using SPSS Version 15.0 (SPSS Inc.). Spearman rank correlation coefficient was used to compare VIC- and FAM-derived matrices and to test the significance of the correlation between spatial (geographical) distances and community distances (based on Jaccard similarity). The spatial distance matrix was calculated for each pair of samples originating from each site and reef patch and for community distances, presence/absence matrices were used. Canonical correspondence analysis (CCA) was performed with XLSTAT from Microsoft Excel. CCA can detect relationships between species and environmental patterns by weighted averaging ordination, which arranges the species along environmental patterns (Ter Braack 1986). Prior to the CCA test, presence/absence matrices were transformed into Hellinger matrices to give less weight to rare species (Legendre and Gallagher 2001; Ramette 2007).

The Hellinger transformation is:

$$y'_{ij} = \sqrt{\frac{y_{ij}}{y_{i+}}}$$

Where y_{ij} is the species value for site *i* and species *j* and y_{i+} represents the sum of all species values for site *i*.

Measuring mucus secretion rates

For measuring mucus secretion rates five coral species, *Colpophyllia natans, Porites astreoides, Diploria labyrinthiformis, Montastrea cavernosa* and *Diploria strigosa* were collected and brought back to the boat in a plastic bags where they were placed in a cooler filled with ambient water. Upon return to the CARMABI station, coral heads were exposed upside down to air and the mucus dripping off the coral head was collected after 30 sec, 1 min, 2 min and 5 min. The dripping mucus was collected in Greiner tubes (Wild et al. 2005; 2010).

To evaluate the abundance of prokaryotic cells, 100-200 µl (10x diluted) of homogenized mucus were placed on filters and fixed with 2% formaldehyde for 4 h. Thereafter, filters were flash-frozen with liquid nitrogen for 10 min and then stored at -80°C. Filters were stained with DAPI (1µg/ml) and hybridized in the dark on ice for 3 min. Filters were then washed in 80% ethanol, rinsed with Milli-Q water, dried and mounted with 1x PBS (0.5 µg/ml), VectaShield (1 µg/ml) and Citifluor (5.5 µg/ml) on a microscope slide. Filters were kept frozen at -20°C until analyses. Enumerating the prokaryotic cells was performed on an epifluorescence microscope (Zeiss Axiovision), at 1250x magnification (Herndl 2007). The microscope was equipped with a 120 W metal halid lamp (HXP 120C) and appropriate filter sets for DAPI, Alexa488 and transmission light. Minimum counts were 200 cells or 20 squares with an area of 0.01 mm².

Statistical analyses of prokaryotic abundance

Cell abundance per ml and abundance as percentage were calculated in Excel. The amount of mucus was measured and the total volume of mucus released was normalized to time to obtain mucus release rates. To determine cell abundances, counts of three filters were averaged. To compute the cell abundance as percentage, first the percentages of each filter time series of one coral were calculated and then, the mean values for each time point of all three filters were calculated.

3. Results

Number of archaeal and bacterial OTUs, richness and diversity indices

Archaeal 16S rRNA gene fragments were found in 81% of all samples: 80% in *M. annularis*, 88% in *P. astreoides*, 66% in *S. siderea* and they were always present in sediment and ambient water samples. Bacterial 16S rRNA gene fragments were present in all samples (Table 1). The Spearman rank correlation, applied to Jaccard similarity matrices generated from presence/absence data of OTUs, showed that the FAM and the VIC datasets for both Archaea (Roh=0.5; P=0.001) and Bacteria (Roh=0.473; P=0.001) were positively correlated (VIC results are presented in the Supplementary Information).

Overall, coral mucus harbored a lower number of archaeal OTUs than bacterial OTUs (based on T-RFLP). In total, 48 FAM-labelled archaeal OTUs were detected in the coral mucus: 39 OTUs in mucus of *M. annularis*, 34 OTUs in mucus of *P. astreoides* and 28 OTUs in mucus of *S. siderea* (FIG. 3; the total number of OTUs does not match with the sum of OTUs because of shared OTUs). A total of 131 bacterial OTUs associated with coral mucus were detected: 81 OTUs in the mucus of *M. annularis*, 90 bacterial OTUs in the mucus of *P. astreoides* and 92 OTUs in *S. siderea* mucus (FIG. 4). The total number of OTUs in the sediment and ambient water was 24 and 13 OTUs for Archaea, respectively, and 38 and 25 OTUs for Bacteria, respectively. The average number of archaeal OTUs was not significantly different among coral species and the sediment and ambient water community (ANOVA, $F_{4, 160}$=0.492; P=0.741). Also, the number of bacterial OTUs inhabiting coral mucus was not significantly different from that of the ambient water and sediments (ANOVA, $F_{3, 173}$=1; P=0.383). However, the number of sediment bacterial OTUs was significantly higher than that of the ambient water (Tukey HSD test, p=<0.05).

Archaeal OTU richness of coral mucus did not vary between sites. The number of archaeal OTUs was lower in Vaersen Bay (27 OTUs) than in Snake Bay (36 OTUs) and Buoy One (37 OTUs) (the number of OTUs does not overlap with the sum of OTUs because of shared OTUs; Table 2). The average number of archaeal OTUs per colony varied between 6 and 8 OTUs per site and species. Bacterial OTU richness was higher in Buoy One (114 OTUs) than in Snake Bay (80 OTUs) and Vaersen Bay (69 OTUs). The number of bacterial OTUs in the mucus layer of the coral species differed between sites. The mucus layer of colonies of *M. annularis* harbored the highest number of bacterial OTUs in Snake Bay (51 OTUs) followed by Buoy One (33 OTUs) and Vaersen Bay (27 OTUs). *S. siderea* exhibited the highest OTU

richness in Buoy One (70 OTUs) followed by Snake Bay (53 OTUs) and Vaersen Bay (41 OTUs). Snake Bay exhibited the lowest average number of bacterial OTUs inhabiting the mucus per colony, varying from 4 to 6, while the average number of OTUs in Buoy One varied from 10 to 13 (Table 2). No significant difference was found between archaeal OTUs among species and sites (ANOVA, $F_{2,138}$=1.371; P=0.257) while a highly significant difference among species and sites was found for bacterial OTUs (ANOVA, $F_{2,147}$=10.8; P=0.001). Post Hoc test Tukey-HSD showed that there is a significant difference between Vaersen Bay, which had a lower number of OTUs (4-6 OTUs) and the other two sites ($P_{\text{Buoy One}}$=0.001; $P_{\text{Snake bay}}$=0.02), but no difference between Buoy One (10-13 OTUs) and Snake Bay (8-11 OTUs; P=0.105).

Generally, bacterial communities were more diverse (H′, 3.8-4.0) than archaeal communities (H′, 2.7-2.9). Archaeal communities of the ambient water and sediments exhibited a similar diversity to the communities in the mucus (H′, 2.3-2.7). However, ambient water and sediment bacterial communities were less diverse than bacterial mucus communities (H′, 3.0-3.1) (Table 2). The beta-diversity was calculated to determine the intraspecific variability in bacterial and archaeal community composition and between sites. Beta-diversity was higher in archaeal than in bacterial communities and higher in the sediments and ambient water than in the mucus of the corals (Table 1). The beta-diversity for archaeal communities in coral mucus collected at Buoy One was 0.19, in Snake Bay 0.17 and in Vaersen Bay 0.22. Bacterial communities exhibited a beta-diversity at Buoy One of 0.1, at Snake Bay 0.1 and at Vaersen Bay 0.1.

The Chao2 richness index for archaeal communities, estimating the expected number of OTUs in a given community, was about 33-150% higher than the observed OTU richness. For the archaeal community, Chao2 was highest in *P. astreoides* mucus (86 OTUs). The Chao2 richness index for bacterial communities was about 61-77% higher than the observed richness of all three coral species (Table 1; VIC results are given in Tables S1-S2).

Table 1. Richness of archaeal and bacterial communities inhabiting the mucus layer of the three coral species, the adjacent sediment and the ambient water. The percentage of successful PCRs, the total number of samples and total richness is given as number of OTUs, Chao2 Estimator, Shannon diversity Index (H`) and β-diversity.

species for archeal communities	successful PCR (%)	total sample nr.	Richness	Chao2 Estimator	Shannon Index (H`)	β-diversity
M. annularis	80%	60	39	52	2.916	0.2
P. astreoides	88%	60	34	86	2.725	0.2
S. siderea	66%	61	28	43	2.724	0.2
sediment	100%	19	24		2.736	0.2
water	100%	8	13		2.328	0.4
species for bacterial communities						
M. annularis	100%	52	81	130	3.759	0.1
P. astreoides	100%	54	90	146	3.895	0.1
S. siderea	100%	52	92	134	4.036	0.1
sediment	100%	18	38		3.062	0.4
water	100%	7	25		3.104	0.2

Table 2. Total richness (total number of OTUs) and colony richness (average number of OTUs per sampled colony, ±SD) of archaeal and bacterial communities for each species and site.

sites	Buoy One		Snake Bay		Vaersen Bay	
Archaeal community	**Total richness**	**Colony richness**	**Total richness**	**Colony richness**	**Total richness**	**Colony richness**
M. annularis	34	8 ±5	30	6±3	17	6±3
P. astreoides	23	6±3	30	6±4	19	6±3
S.siderea	23	7±3	16	7±3	21	6±3
Total	37		36		27	
Bacterial community						
M. annularis	33	10±6	51	11±7	27	4±3
P. astreoides	14	13±7	48	8±4	32	5±4
S. siderea	70	10±6	53	8±7	41	6±7
Total	114		80		69	

Distribution and relative abundance of individual OTUs

The numbers of unique and shared archaeal and bacterial OTUs inhabiting the mucus layer of the three coral species are shown in FIG. 3 and 4. In the archaeal community, 17 out of 48 OTUs (or 35%) were unique to the mucus layer of the specific coral species (FIG. 3). Remarkably, the same percentage of unique bacterial OTUs were obtained as for Archaea (FIG. 4). For the bacterial community, 46 out of 131 OTUs were specific to the mucus layer of the coral species (FIG. 4). *M. annularis* showed the highest archaeal richness with 39 different OTUs. Of these, 9 OTUs were unique to *M. annularis* mucus, 22 OTUs were shared with the other two coral species, 6 OTUs were shared with *P. astreoides* and 2 OTUs with *S.*

siderea (FIG. 3). For the bacterial communities, *S. siderea* mucus harbored the highest number of OTUs. Fifteen OTUs were unique to *S. siderea* mucus, 47 were shared with the mucus layer of the other two coral species and 10 and 13 OTUs were shared with mucus layer of the *M. annularis* and *P. astreoids,* respectively (FIG. 4).

The distribution of individual archaeal and bacterial OTUs across coral species and of the ambient water and sediment is given in FIGS. 5 and 6. In the mucus layer of the coral species, two archaeal OTUs (55 and 56-bp) and one bacterial OTU (562 bp) were present in all the colonies. In archaeal communities, 23-32% of the OTUs were present in 30 % of all corals (FIG. S1). In the sediment archaeal community, 75% of the OTUs and in the ambient water 88% of the OTUs were also found in at least one mucus sample of a coral species. The mucus layer of *M. annularis* shared 38% of the archaeal OTUs with the sediment community and 64% with the ambient water community. The mucus layer of *P. astreoides* shared 47% of archaeal OTUs with the sediment community and 79% with the ambient water. The mucus layer of *S. siderea* shared 57% of the archaeal OTUs with the sediment habitat and 71% with the ambient water community. In bacterial communities, nearly half of the OTUs were just present in one or two colonies (FIG. S2). The sediment bacterial community shared 92% of the OTUs with at least one mucus sample of a coral species. In the ambient water, 88% of bacterial OTUs were shared with at least one mucus sample of a coral species. Overall, the mucus layers of the three coral species shared 30-38% of bacterial OTUs with the sediment community and 21-24% with the ambient water. (VIC results are shown in FIGS. S5-S8).

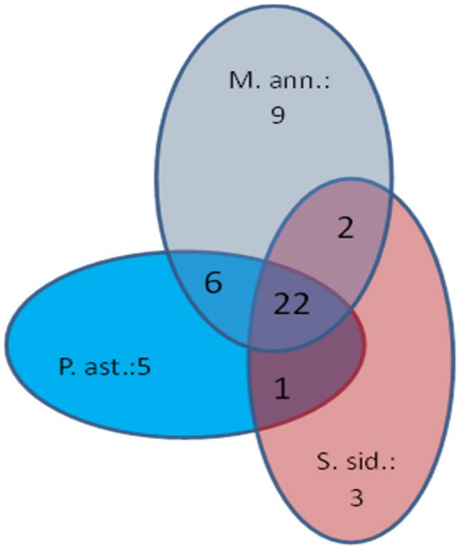


FIG. 3 Distribution of Archaea. Unique, shared and ubiquitously present OTUs.

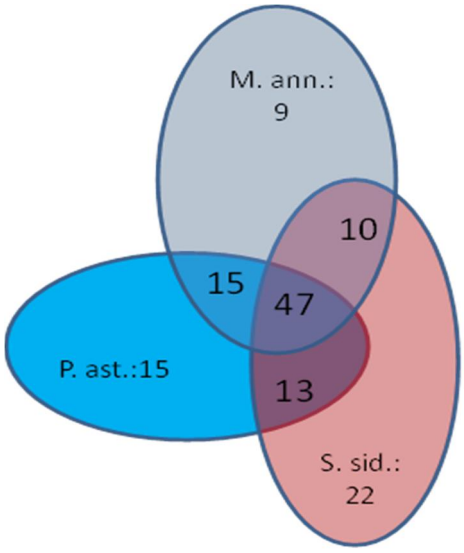


FIG. 4 Distribution of Bacteria. Unique, shared and ubiquitously present OTUs.

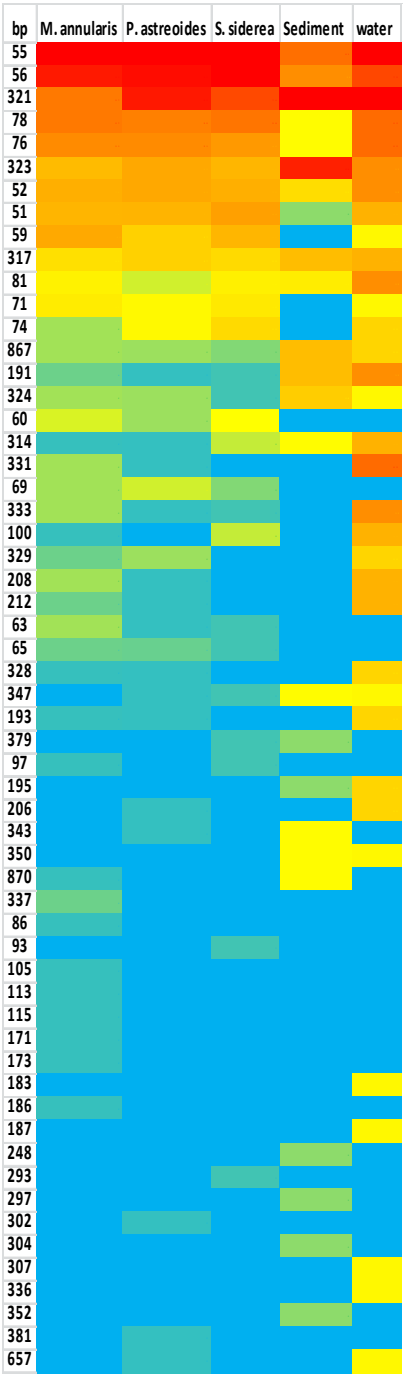
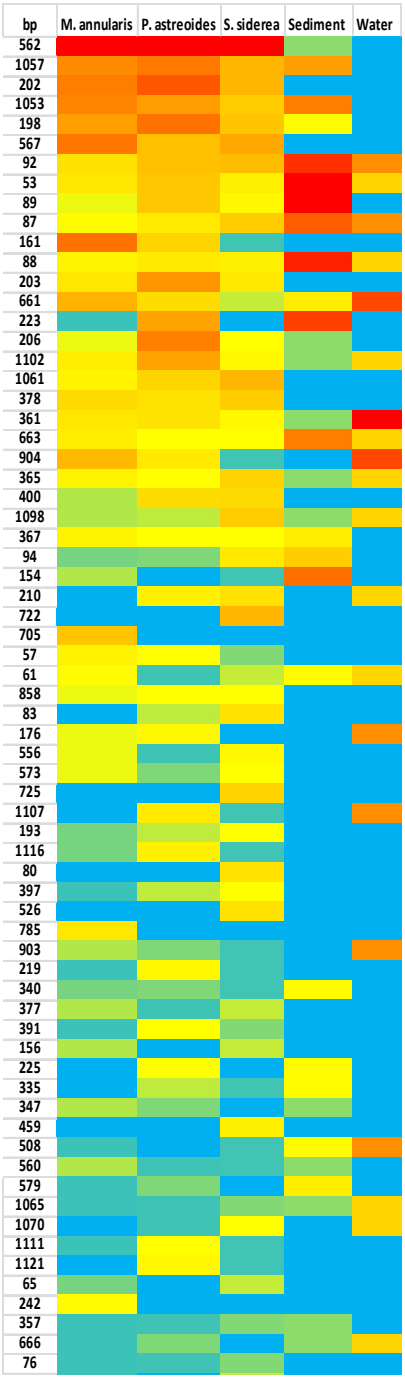


FIG. 5 Distribution of archaeal OTUs across coral species and habitats. Color code indicates the percentage of colonies where the specific OTU was present.



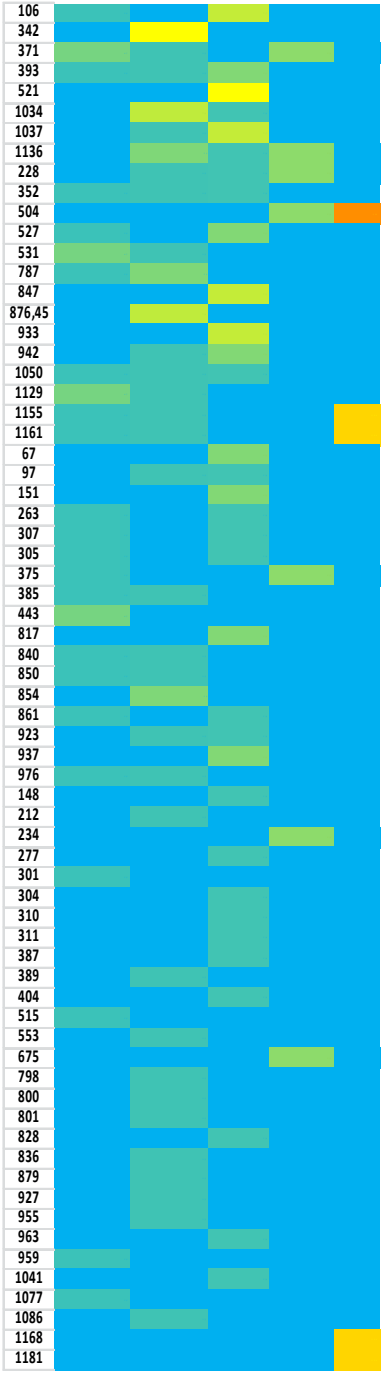
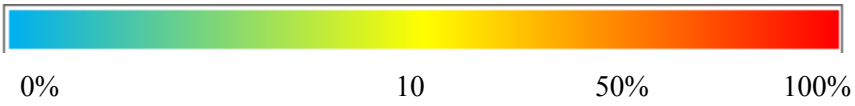


FIG. 6 Distribution of bacterial OTUs across coral species and habitats. Color code indicates the percentage of colonies where the specific OTU was present.



Community structure of Archaea and Bacteria

The archaeal communities inhabiting the mucus layer of the three coral species were similar (FIG. 5). Analysis of similarity (ANOSIM), based on Jaccard distances between pairs of samples, showed that archaeal communities did not differ between the three coral species (n=141; R=-0.002; P=0.56). Non-metric multidimensional scaling (NMDS) and hierarchical cluster analysis (FIG. S3), applied to a Jaccard similarity matrix of the archaeal community showed no clear separation based on host species. There were differences in the community structure of mucus-associated Archaea between sites (R=0.027; P=0.01), however, analyses on single coral species showed no differences between sites (*M. annularis*: n=48; R=0.016; P=0.67; *P. astreoides*: n=53; R=0.01; P=0.3; *S. siderea*: n=40; R=0.041; P=0.16). Analyzing all coral species together, a depth stratification of the archaeal community composition in the mucus layers was detectable (R=0.034; P=0.005). The same type of analyses with single coral species, however, showed no difference between 5 and 15 m depth (*M. annularis*: R=0.036; P=0.09; *P. astreoides*: R=0.029; P=0.1; *S. siderea*: R=0.043; P=0.12). The lack of depth and site related trends in the archaeal community composition might be due to the low sample number for individual coral species.

Archaeal mucus communities were different from the archaeal communities in sediments and ambient waters (ANOVA: sediment: n=18; P=0.001; water: n=8; P=0.003). Corals kept in aquaria showed no differences in archaeal community structure to corals from the field (*M. annularis*: R=-0.02; P=0.47; *P. astreoides* R=-0.1; P=0.65; *S. siderea* R=0.1; P=0.2). Canonical correspondence analysis (CCA) depicted that 9.3% of the archaeal community composition was influenced by the parameters coral species, site and depth. CCA was also made without Hellinger transformation, and with arcsine transformation, arriving at a similar explanatory power of the variables (9.8%; arcsine: 9.4%) as with the Hellinger transformation.

In contrast to the archaeal mucus community, the bacterial mucus community differed significantly among the coral species (ANOSIM, R=0.115; P=0.001). Bacterial communities associated with the mucus layers of *M. annularis* (R=0.155; P=0.001) and *P. astreoides* (R=0.303; P=0.001) differed among sites, while the mucus bacterial community of *S. siderea* differed significantly only between Buoy One and Snake Bay (R=0.09; P=0.02). Bacterial communities associated with the mucus layers of *P. astreoides* (R=0.379; P=0.001) and *S. siderea* did not vary significantly with depths (R=0.661; P=0.001), while the mucus associated bacterial community of *M. annularis* from 5 m was different from that at 15 m

depth (R=0.067; P=0.008). The mucus associated bacterial communities from the three coral species were significantly different from communities in the adjacent sediments and ambient water (sediment: P=0.001; ambient water: P=0.001). NMDS plots and cluster analyses revealed a cluster of the mucus associated bacterial communities of the three species without a clear separation between the coral species (see FIG. S3). CCA results indicated that 10% of the variation in the mucus associated bacterial community was explained by the parameters coral species, sites and depths. CCA results from the presence/absence matrix and arcsine transformation of bacterial community were similar to Hellinger transformation (11.3%; arcsine: 10.6%). VIC results are given in Tables S3-S6.

Community composition *vs.* spatial distances

Spearman correlation was applied to determine whether there was a correlation between spatial distance and community dissimilarities. In mucus associated archaeal communities, *M. annularis* (R=0.006; P=0.41), *P. astreoides* (R=-0.005; P=0.54) and *S. siderea* (R=0.082; P=0.053) showed no correlation between community dissimilarity and spatial distance. Also, sediment archaeal community dissimilarity was not related to spatial distance (R=0.027; P=0.36). The archaeal community changes in the ambient water, however, correlated with spatial distance between samples (R=0.469; P=0.03).

In contrast to the archaeal community, the mucus associated bacterial communities of *M. annularis* (R=0.188; P=0.001) and *P. astreoides* (R=0.28; P=0.001) changed significantly with spatial distance while for the mucus community composition of *S. siderea*, no correlation to spatial distance was detected (R=0.035; P=0.19), supporting the results of the ANOSIM, which also did not indicate differences between sites. The bacterial community dissimilarity in sediments and ambient also did not correlate with spatial distance. VIC results are shown in Table S6.

Mucus release and abundance of prokaryotes

Prokaryotic abundance in released mucus varied between 3.7 x 10^6 cells ml^{-1} and 2.1 x 10^6 cells ml^{-1}. Mucus release rates of different coral species were measured and the abundance of prokaryotes determined using DAPI-staining and epifluorescence microscopy. Mucus release rates decreased rapidly over time in *M. cavernosa, D. labyrinthiformis* and *C. natans* with mucus release rates dropping within 1 min to 15-34% of the mucus released within the first 30 s, 6-16% after 2 min and 4-11% after 5 min. The abundance of mucus associated prokaryotes decreased gradually to 54-70% of the prokaryotic abundance contained in the mucus released within 30 s after 1 min, to 31-50% after 2 min and to 24-37% after 5 min (FIGS. 9-14). *D. strigosa* maintained a stable mucus production at the beginning with 120% of the prokaryotic abundance in mucus after 30 s detected after 1 min, 20% after 2 min and 16% after 5 min and kept the highest relative abundance of prokaryotes until 2 min (FIGS. 15-16). Mucus release of *S. siderea* decreased quickly as did prokaryotic abundance in the released mucus (FIGS. 17-18). *P. astreoides* released large amounts of mucus at the beginning and thereafter, mucus release rates were maintained at a rather constant rate (FIGS. 19-20). Prokaryotic abundance of *P. astreoides* was also decreasing rapidly and remained stable at $\approx$ 1x10^5 cells ml^{-1}. Highest prokaryotic abundance was detected in the mucus produced by *C. natans* (3.7 x 10^6 cells ml^{-1}), followed by *M. cavernosa* (3.2 x 10^6 cells ml^{-1}) and *D. labyrinthiformis* (3.1 x 10^6 cells ml^{-1}) and *P. astreoides* mucus had the lowest prokaryotic abundance (2.1 x 10^6 cells ml^{-1}). Overall, each coral species exhibited its own dynamics in mucus release. Generally, however, mucus production and mucus associated prokaryotic abundance decreased over time when the corals were stressed by exposing them to air.

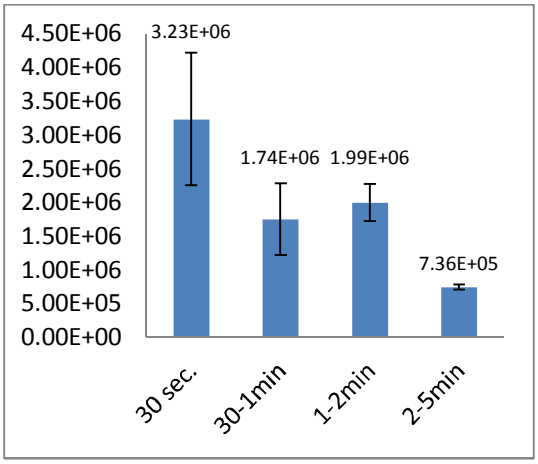


FIG. 7 *M. cavernosa.* Prokaryotic abundance (mean ± SD) per mucus volume (ml^{-1}) measured over time during air exposure.

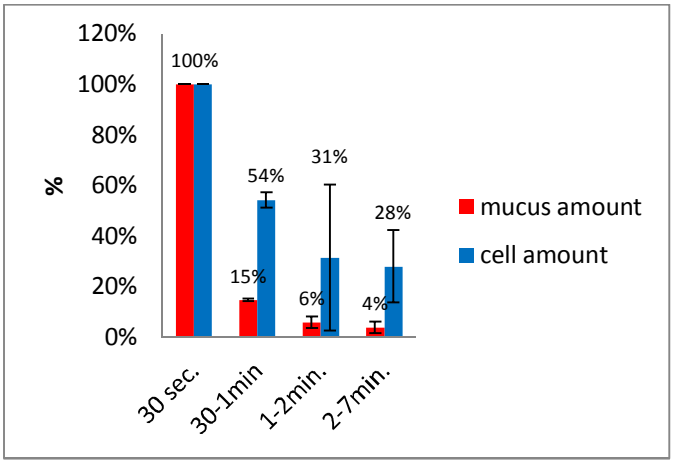


FIG. 8 *M. cavernosa.* Mucus volume released and cell abundance in mucus relative to the mucus release and cell abundance measured during the first 30 sec of air exposure.

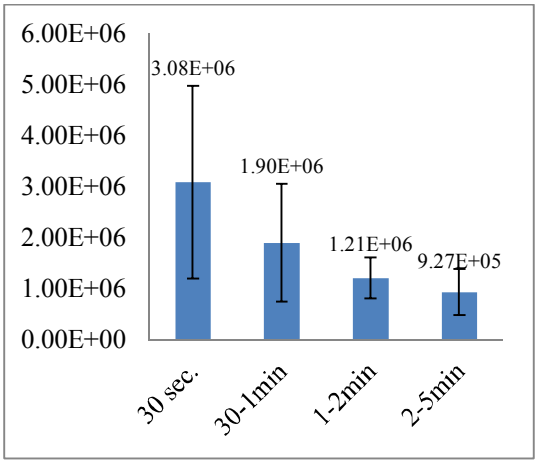


FIG. 9 *D. labyrinthiformis.* Prokaryotic abundance (mean ± SD) per mucus volume (ml^{-1}) measured over time during air exposure.

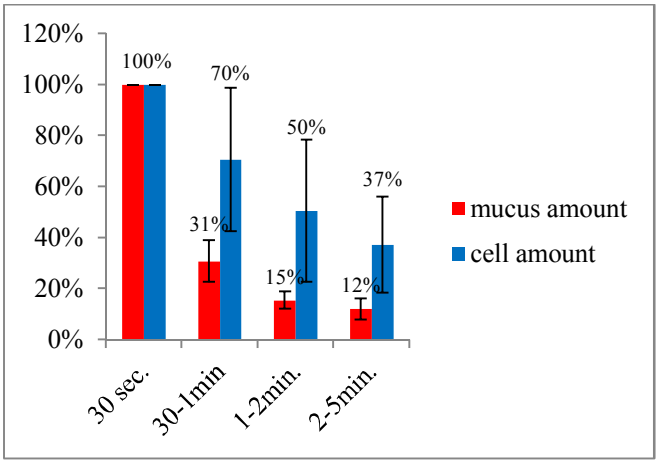


FIG. 10 *D. labyrinthiformis.* Mucus volume released and cell abundance in mucus relative to the mucus release and cell abundance measured during the first 30 sec of air exposure.

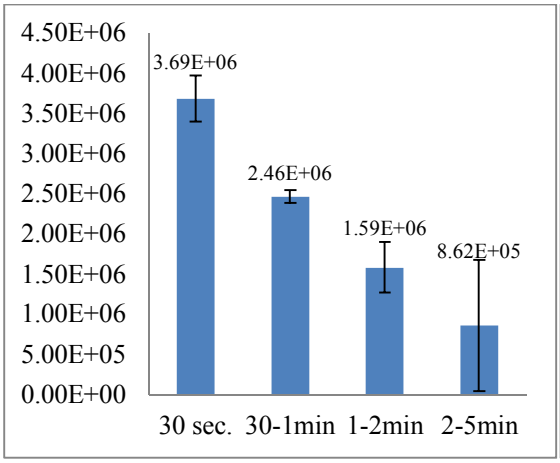


FIG. *11 C. natans.* Prokaryotic abundance (mean ± SD) per mucus volume (ml^{-1}) measured over time during air exposure.

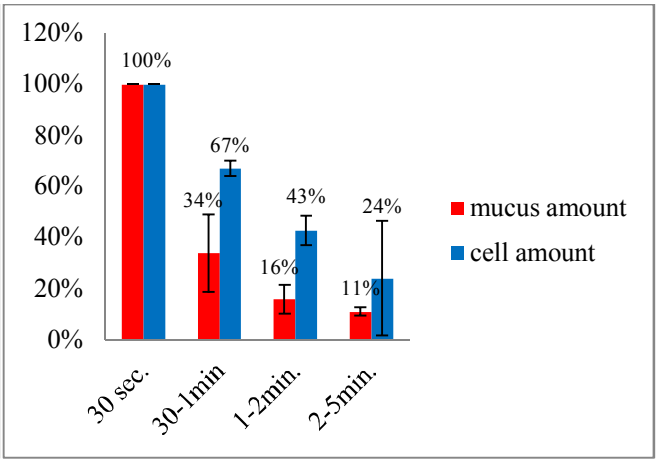


FIG. 12 *C. natans.* Mucus volume released and cell abundance in mucus relative to the mucus release and cell abundance measured during the first 30 sec of air exposure.

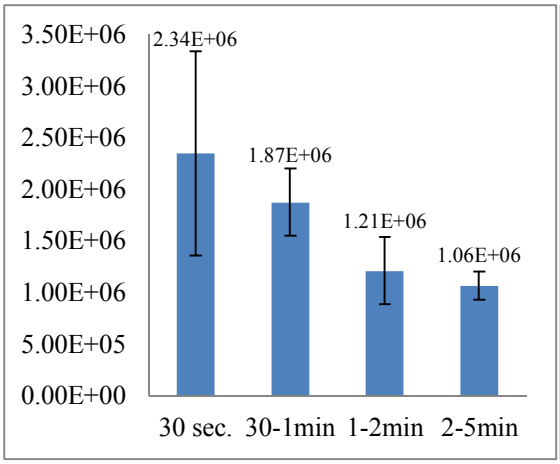


FIG. 13 *D. strigosa.* Prokaryotic abundance (mean ± SD) per mucus volume (ml^{-1}) measured over time during air exposure.

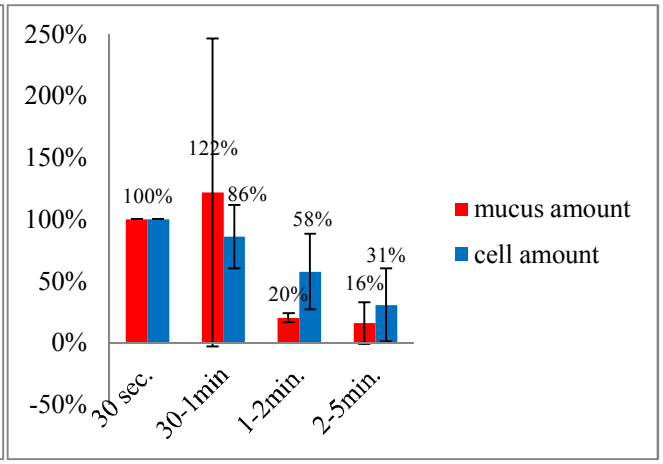


FIG. 14 *D. strigosa.* Mucus volume released and cell abundance in mucus relative to the mucus release and cell abundance measured during the first 30 sec of air exposure.

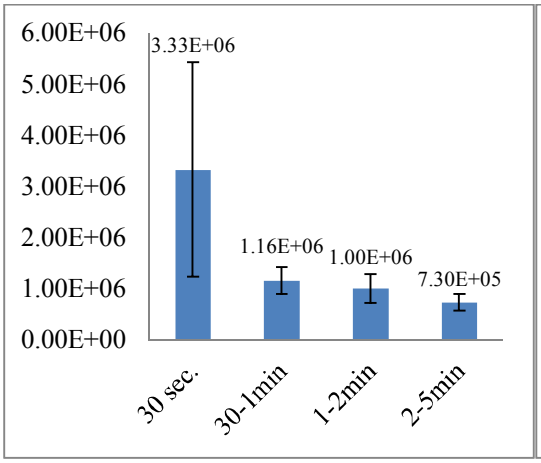


FIG. 15 *S. siderea.* Prokaryotic abundance (mean ± SD) per mucus volume (ml^{-1}) measured over time during air exposure.

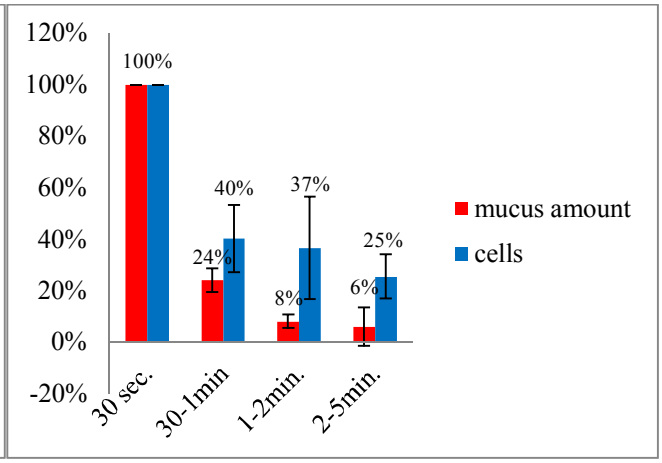


FIG. 16 *S. siderea.* Mucus volume released and cell abundance in mucus relative to the mucus release and cell abundance measured during the first 30 sec of air exposure.

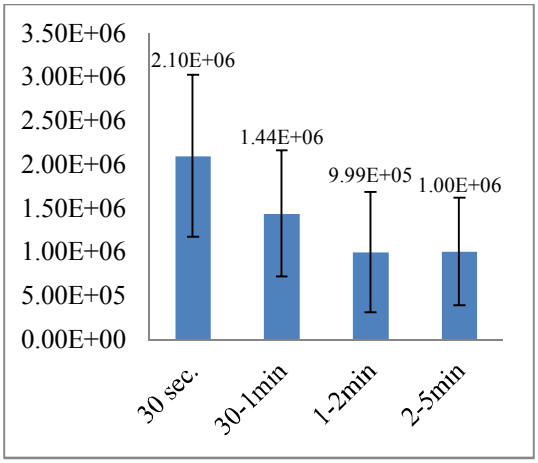


FIG. 17 *P. astreoides.* Prokaryotic abundance (mean ± SD) per mucus volume (ml^{-1}) measured over time during air exposure.

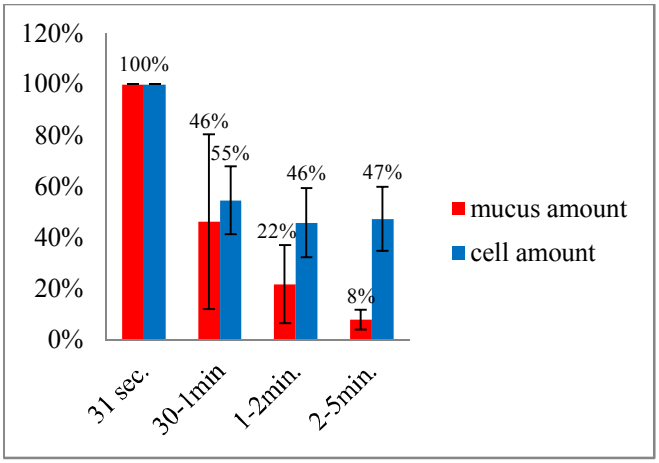


FIG. 18 *P. astreoides.* Mucus volume released and cell abundance in mucus relative to the mucus release and cell abundance measured during the first 30 sec of air exposure.

4. Discussion

General considerations about the detection of bacterial and archaeal communities in mucus

Previous studies and present results suggest that Archaea are not as abundant as Bacteria in coral colonies (Wegley et al. 2004; Kellogg 2004). In this study, archaeal communities were present in at least 86% of coral mucus samples, whereas Bacteria were detected in all mucus samples. Wegley et al. (2004) detected Archaea communities in the tissue of 50% of *P. astreoides* colonies and in 67% of *M. annularis* colonies, while Kellogg (2004) did not detect any Archaea in the surface layer of *P. astreoides*. Even if Archaea are occuring in apparently low abundance, they might play an important role in the nitrogen cycle for the coral (Siboni et al. 2008).

For T-RFLP analysis the software Gelcompare was used. The number of OTUs finally obtained depends on the thresholds set in the electropherogram and on the bin width. For Archaea the threshold for minimum profiling was 3% of peak height, which resulted in lower numbers of mucus associated OTUs (*M. annularis*: 39 OTUs; *P. astreoides*: 34 OTUs; *S. siderea*: 28 OTUs) than the setting of the threshold of 0.5% (*M. annularis*: 210 OTUs; *P. astreoides*: 60 OTUs; *S. siderea*: 185 OTUs). Gelcompare calculates the individual peak heights in relation to the highest peak within a sample. For Archaea in coral mucus, the number of major peaks per samples was low. Thus, even small peaks were included in the analysis. In mucus associated bacterial communities, the OTU numbers also changed with the setting of the threshold of 0.4% (*M. annularis*: 81 OTUs; *P. astreoides*: 90 OTUs; *S. siderea*: 92 OTUs) and 1% (*M. annularis*: 49 OTUs; *P. astreoides*: 56 OTUs; *S. siderea*: 59 OTUs). In this study, the percentage of minimum profiling was chosen in a way that the total number of OTUs identified by the Gelcompare software is in the same range of the number of peaks identified manually when using the Peak Scanner software.

Interspecific differences of archaeal and bacterial communities associated with coral mucus, in sediments and ambient water

In general, bacterial communities in coral mucus are characterized by a larger number of OTUs (total number of OTUs: 131; in mucus of *M. annularis:* 81 OTUs; in *P. astreoides:* 90 OTUs; in *S. siderea:* 92 OTUs) than archaeal communities (total number of OTUs: 48 OTUs; *M. annularis:* 39 OTUs; *P. astreoides:* 34 OTUs; *S. siderea:* 28 OTUs). Siboni et al. (2012) found a total of 87 archaeal OTUs in the mucus layer of corals (*Acanthastrea* sp.: 26 OTUs;

Favia sp.: 31 OTUs; *F. granulosa*: 30 OTUs). Klaus (2005) detected 90 bacterial OTUs in *M. annularis* and 77 OTUs in *S. siderea*, while Daniels et al. (2011) detected 47 bacterial OTUs in *M. annularis* mucus. No differences in the number of archaeal and bacterial OTUs were found in the mucus layers among the different coral species tested in this study (Table 1). Differences in the number of mucus-associated bacterial OTUs were found, however, among different sites, caused by a significant difference in the bacterial community composition of the coral mucus between Vaersen Bay and the other two sites (Table 2). Siboni et al. (2008) found interspecific differences in mucus-associated archaeal communities in corals. McKew et al. (2012), and Guppy and Bytell (2006) reported similar differences in the number of bacterial OTUs between geographic sites and no differences in the mucus layers among coral species. Klaus et al. (2005) pointed out that pollution and environmental conditions might have led to the observed differences among sites.

In general, the Shannon-Wiener Diversity Index was lower for archaeal communities than for mucus-associated bacterial communities ($H'_{archaea}$=2.7-2.9; $H'_{bacteria}$=3.1-4.2). Similarly, archaeal diversity in surface mucus of corals from Heron Island (Great Barrier Reef), from the Gulf of Eliat (Red Sea), and from the Caribbean was also lower than that of Bacteria for the same sites (Siboni et al. 2008; Wegley et al. 2004). Bacterial diversity in coral mucus layers of the Great Barrier Reef was lower than in this study, while the bacterial diversity obtained from corals of the Gulf of Eilat was similar to the that determined in the present study (Kvennefors et al. 2010; Kooperman et al. 2007).

In the present study, coral mucus samples yielded more diverse bacterial 16S rDNA sequences than ambient water or sediment samples. Potentially, this could be attributed to the higher prokaryotic abundance in the mucus than in the ambient water and sediments. However, a rough estimate shows that extracted samples of the mucus contained about 10^4-10^5 cells in total, ambient water contained 10^7-10^8 cells and sediment samples contained 10^6-10^7 cells per sample. Hence, there is no relation between prokaryotic abundance and bacterial diversity. Higher diversity of Bacteria in mucus might be due to a higher number of rare (low abundance) species in the ambient water, not detectable by T-RFLP. Due to the low beta-diversity of Bacteria in coral mucus (Table 1), it seems that every colony has its own microbial composition. Therefore, every colony can harbor different OTUs which may be not detectable by T-RFLP analysis in the ambient water and sediments, due to their low abundance in these habitats.

The present study shows that the bacterial and archaeal diversity and richness in the mucus were similar among the coral species (Table 1), while the composition of the prokaryotic

communities differed among the coral species. Archaeal communities in coral mucus exhibited a higher beta-diversity than bacterial communities. Hence, archaeal communities have a higher evenness than bacterial communities.

Mucus is consisting of several carbohydrates, lipids and inorganic nutrients (Wild et al. 2005; Ducklow and Mitchell 1979b) and there is evidence for a species-specific chemical composition of mucus, such as *Fungia* containing significantly more fucose than other corals (Wild et al. 2005; 2010). Potentially, these interspecific differences in the substrate composition of the mucus layer among corals are reflected in the interspecific bacterial community composition.

Distribution and relative abundance of individual OTUs

About 35% unique OTUs were detected in archaeal and bacterial communities, and 65% of the OTUs were shared at least between two coral species (FIGS. 3-4). Numbers of unique, shared and ubiquitous OTUs (FIGS. 3-4) showed that bacterial and archaeal communities are influenced by different factors. In archaeal communities, *M. annularis* has the highest number of unique OTUs and *S. siderea* the lowest. In contrast, in bacterial communities, *M. annularis* has the lowest number of unique OTUs and *S. siderea* the highest. Almost half of the bacterial and archaeal OTUs were specific for individual coral colonies (FIGS. S1-S2). This suggests that each colony has a distinct prokaryotic community composition. About 64-79% of the archaeal OTUs and 20-24% of the bacterial OTUs of coral mucus were also found in the archaeal and bacterial community in the ambient water (FIGS. 5-6). In the study of Kellogg (2004), 75% of the sequences of the archaeal community of scleractinian corals were closley related those of the ambient water. Despite this overlap in the OTUs found in coral mucus layers and ambient waters, distinct clusters of mucus harboring Bacteria and ambient water Bacteria were detectable (ANOSIM: $p<0,05$). Our findings corroborate with other studies showing that corals harbor bacterial communities distinctly different from those of the ambient water (Gil-Agudelo et al. 2007; Klaus et al. 2005; Rohwer et al. 2001; 2002).

Community structure of Archaea and Bacteria

Previous studies and the present study show that Archaea are not host species-specific (Wegley et al. 2004; Kellogg 2004; Siboni et al. 2012;) and that there is no signficant difference in archaeal community composition between sites. Other studies show that even over a large spatial distance same coral genera of the Red Sea, the Great Barrier Reef and the Virgin Islands exhibit a high similarity in their archaeal communities (up to 100%) (Siboni et

al. 2008; Kellogg 2004). Depth is no variable determining the variation in archaeal communities. Overall, it appears that Archaea form a general coral-archaeal symbiotic association, not varying between sites, depths and corals (Siboni et al. 2008). Mucus from corals held in an aquarium showed also no difference in their archaeal community structure. Kooperman et al. (2007) incubated corals from the Red Sea in artificial seawater. After three weeks, the bacterial community of these aquarium corals was significantly different from that of field corals with a lower number of OTUs inhabiting the mucus layer. Kooperman et al. (2007) suggest that corals can adapt their bacterial communities to different environmental conditions. The present study suggests that environmental conditions have only little effect on archaeal communities in coral mucus layers.

In contrast to Archaea, bacterial communities inhabiting coral mucus are coral species-specific (Ducklow and Mitchell 1979a; Rohwer et al. 2002). Ritchie (2006) and Shnit-Orland and Kushmaro (2009) showed that coral-derived Bacteria growing on the mucus exhibit antimicrobial activity and that the mucus itself has antibacterial properties. These authors suggest that different coral species can "select" their own microbial community. It is still unknown how and from where the corals acquire their specific bacterial community. At the moment two different hypotheses exist. Sunagawa et al. (2010), using next generation sequencing, showed that corals acquire rare members of the bacterial community from the ambient water. The second hypothesis is that corals use vertical transmission. Sharp et al. (2012) showed that *P. astreoides* transmitted bacterial cells from the adult colonies to larvae. Previous studies showed that some coral species have the same bacterial community over a large spatial distance (Ritchie and Smith 1997; Rohwer et al. 2001; 2002), while others and the present study indicate that bacterial community compositions in coral mucus varies in space (McKew et al. 2012; Kvennefors et al. 2010). In this study, mucus bacterial communities varied over spatial distances of only a few kilometers.

Depth gradient is relating to other gradients such as light and nutrients, which affect the coral physiological state (Olson and Kellogg 2010). Bacterial communities of *P. astreoides* and *S. siderea* showed no differences between depth layers, while *M. annularis* showed a difference between 5 and 15 m. Klaus et al. (2005) sampled tissue of *M. annularis* and *D. strigosa* off Curaçao at four sample sites, one site being Snake Bay also sampled in the present study. These authors showed that the bacterial community of *M. annularis* did not vary spatially but that of *D. strigosa* varied between sampling sites. Kvennefors et al. (2010) suggested that

spatial differences in mucus bacterial community composition are a response to changes in environmental parameters.

Spatial variation in the archaeal and bacterial community

We tested the hypothesis that communities are changing over distance. Archaeal communities of corals and sediment samples did not vary over distance, while bacterial communities of *M. annularis* and *P. astreoides* varied spatially. The Baas-Becking hypothesis states that "everything is everywhere, but the environment selects" (Baas-Becking 1934). Hughes Martiny et al. (2006) found that increasing spatial distance leads to a decrease in community similarity. The present study suggests that archaeal communities on coral mucus are not influenced by environmental variations, but have, instead, a more general and homogeneous distribution over space. Bacterial communities of *M. annularis* and *P. astreoides* appear to be more affected by local variations in environmental parameters.

Dynamics of mucus release, cell abundance and mucus thickness

In general, every coral species had its own dynamics of mucus release. *M. cavernosa, D. labyrinthiformis*, *C. natans* and *S. siderea* exhibited a rapid decrease in mucus release over time while *D. strigosa* and *P. astreoides* kept the mucus production stable for some time (FIGS. 9-20). It is well known that stressed corals produce more mucus than under non-stress conditions (Gottfried and Roman 1983) and that mucus production varies among coral species (Coffroth 1990, present study). Ritchie and Smith (2004) report that the abundance of cultivable Bacteria in coral mucus is about 100 times higher than in the adjacent waters.

The thickness of the mucus layer was measured in *M. cavernosa*, *D. labyrinthiformis*, *D. strigosa*, *S. siderea* and *P. astreoides* (established by Jatkar et al. 2010). The thickest mucus layer was measured in *M. cavernosa* with 220 µm and in *S. siderea* with 160 µm. The mucus layer of these two coral species also had a higher prokaryotic abundance during the first 30 s of mucus release. *D. labyrinthiformis* had a thickness of 100 µm and a prokaryotic abundance of 3.1×10^6 ml^{-1}. *D. strigosa* (80 µm) and *P. astreoides* (70 µm) had the thinnest mucus layers and the lowest prokaryotic abundances. Ducklow and Mitchell (1979b) suggest that every coral has its own mucus composition and different rates of mucus release, which affects the abundance of bacteria in the mucus. Thus, there is a relation between the thickness of the mucus layer and,prokaryotic abundance probably related to slower removal rates of mucus which, in turn, allow longer residence time of prokaryotes in the mucus.

Conclusions

The prokaryotic communities inhabiting the mucus layer of three different coral species, *Montastrea annularis*, *Porites astreoides* and *Siderastrea siderea,* were investigated in three reef locations off Curaçao. We found archaeal and bacterial OTUs inhabiting the mucus layer distinctly different from the community composition in the ambient water and sediments. Overall richness of bacterial OTUs did not vary much among the coral species, however compositional differential were apparent. Bacterial communities in coral mucus are host specific and differ between sites. In contrast, archaeal communities do not exhibit interspecific differences in composition between species, sites or depths. Species-specific bacterial composition may be driven by interspecific variations in the biochemical composition of the mucus but this is still rather uncertain and should be the target of further research. Under a rapidly changing climate, the balance between the coral host and its microbes can be rapidly disturbed. It is therefore important to understand the roles of microbes on corals.

5. Acknowledgements

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Supplementary Information

Figures based on the analysis of FAM Primer

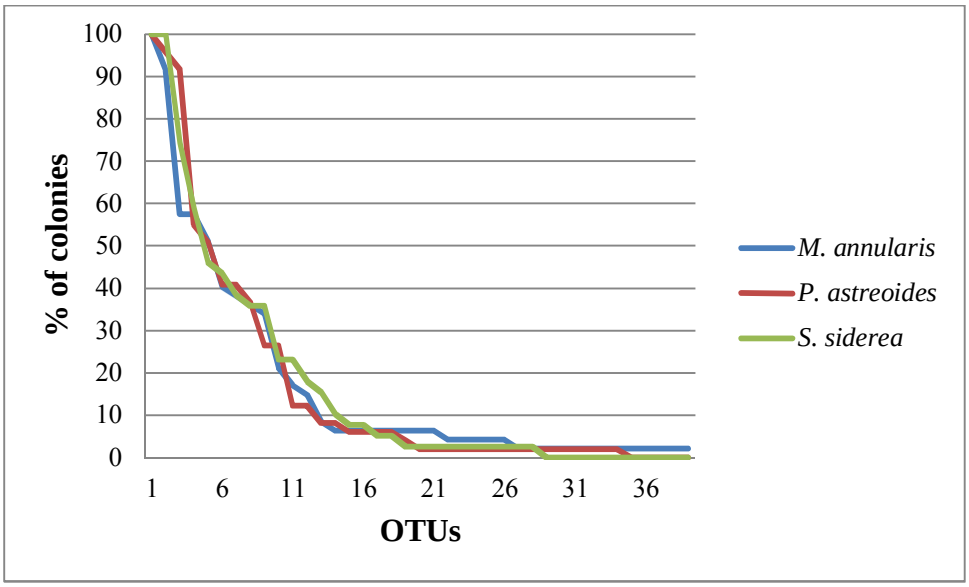


FIG. S1 Rank frequency distribution of mucus associated archaeal OTUs in colonies of *M. annularis, P. astreoides* and *S. siderea*

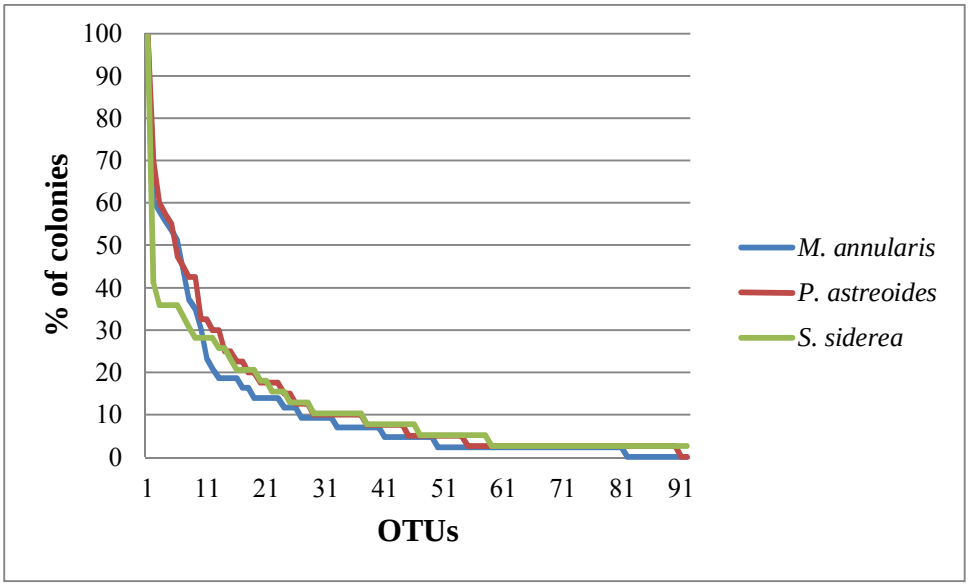


FIG S2 Rank frequency distribution of bacterial OTUs in colonies of *M. annularis, P. astreoides* and *S. siderea*

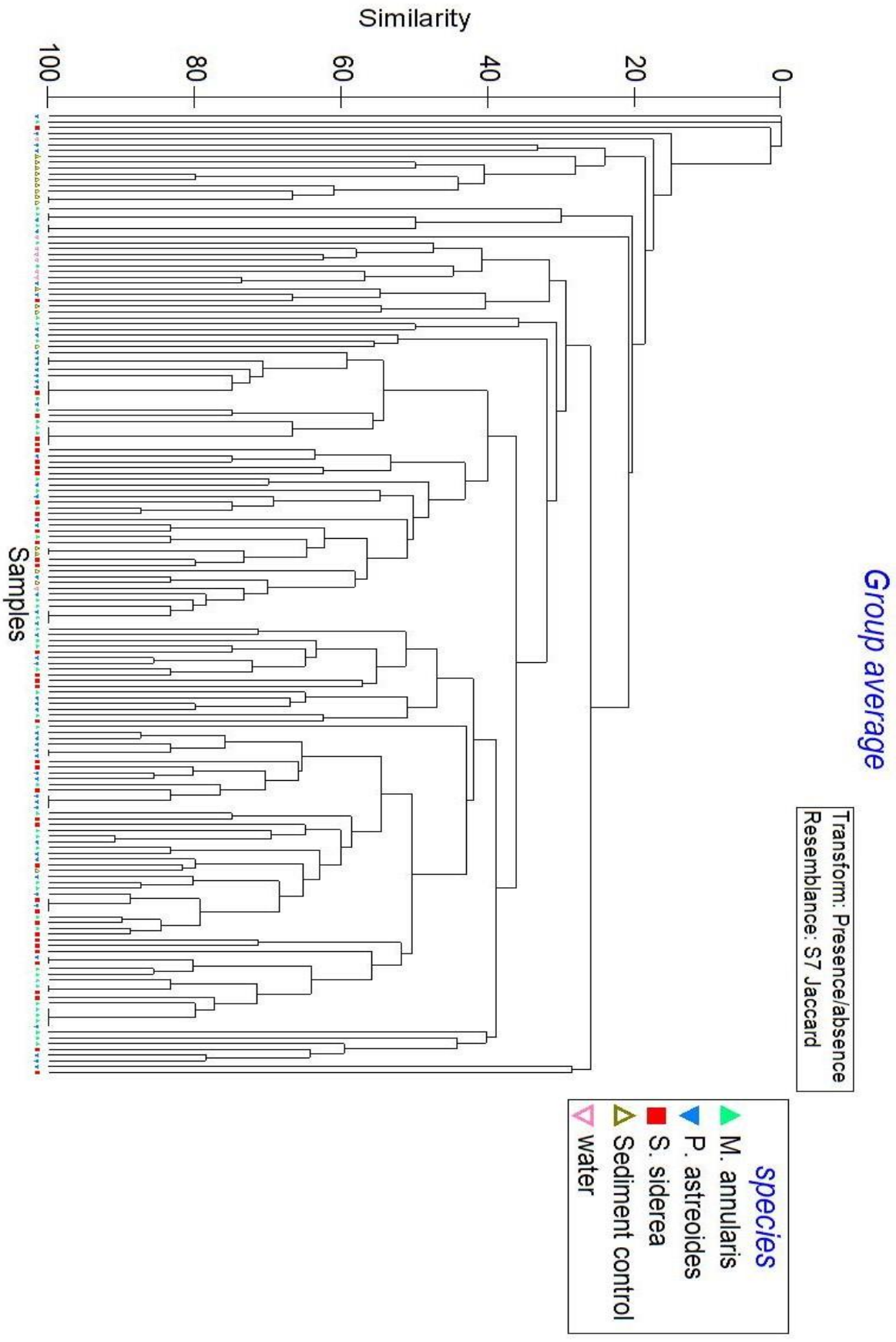


FIG. S3 Hierarchical cluster of similarity of the mucus associated archaeal communities of the three coral species and adjacent sediments and ambient water

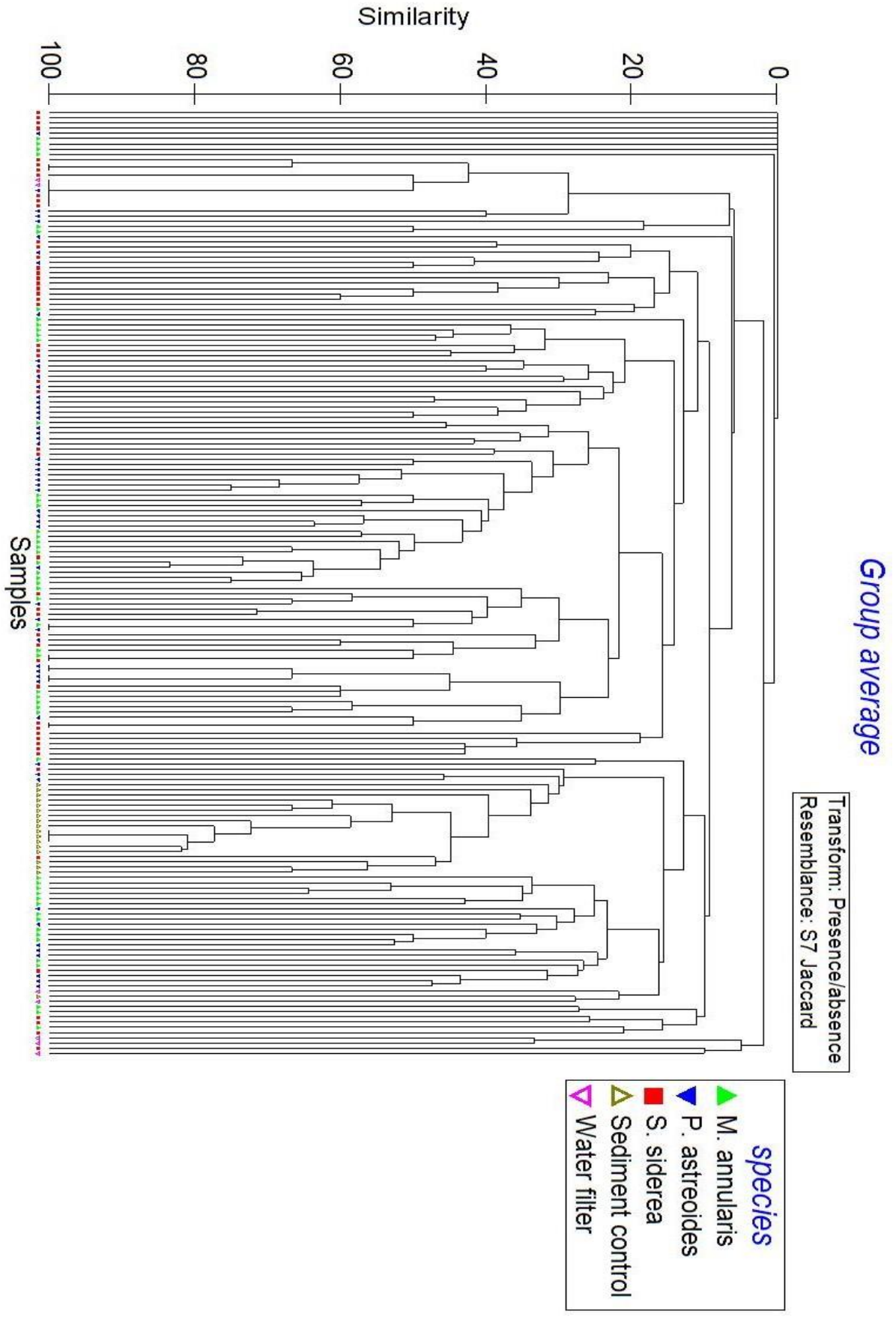


FIG. S4 Hierarchical cluster of similarity of the mucus associated bacterial communities of the three coral species and adjacent sediments and ambient water

Tables based on the analysis of the VIC Primer:

Table S1 Description of archaeal and bacterial communities inhabiting the mucus layer of *M. annularis*, *P. astreoides* and *S. siderea* and the adjacent sediment and the ambient water. The percentage of successful PCRs, total number of samples and total richness is given as total number of OTUs Chao2 Estimator and Shannon diversity Index (H`).

species for archeal communities	successful PCR (%)	total sample nr.	Richness	Chao2 Estimator	Shannon Index (H`)
M. annularis	80%	60	32	43	2.822
P. astreoides	88%	60	35	80	2.836
S. siderea	66%	61	32	41	2.88
sediment	100%	19	26		2.836
water	100%	8	39		3.421
species for bacterial communities					
M. annularis	100%	52	52	95	3.631
P. astreoides	100%	54	54	130	3.823
S. siderea	100%	52	52	149	3.837
sediment	100%	18	18		3.642
water	100%	7	7		3.336

Table S2 Total richness (total number of OTUs) and colony richness (average number of OTUs) of archaeal and bacterial communities for each species and site based on VIC Primer

site	Buoy One		Snake Bay		Vaersen Bay	
Archaeal community	Total richness	Colony richness	Total richness	Colony richness	Total richness	Colony richness
M. annularis	23	5±4	19	5±3	24	7±3
P. astreoides	21	5±3	28	6±4	19	6±3
S.siderea	26	6±4	18	5±3	23	7±5
Bacterial community						
M. annularis	56	18±7	54	16±7	42	12±6
P. astreoides	65	20±6	69	19±9	54	14±7
S. siderea	70	16±6	62	12±8	49	10±9

Table S 3 Analysis of similarity (ANOSIM) indicates whether the mucus associated archaeal- and bacterial communities associated with coral mucus differ among corals and the ambient water and the sediment.

Archaeal community	*P. astreoides*	*S. siderea*	water control	sediment control	all
M. annularis	p=0.45; R=-0.001	p=0.91; R=-0.02	p=0.001; R=0.428	p=0.001; R=0.57	p=0.001; R=0.188
P. astreoides		p=0.97; R=-0.32	p=0.002; R=0.497	p=0.001; R=0.531	
S. siderea	p=0.97; R=-0.32		p=0.001; R=0.6	p=0.001; R=0.623	
Bacterial community					
M. annularis	p=0.001; R=0.122	p=0.001; R=0.097	p=0.18; R=0.104	p=0.04; R=0.132	p=0.001; R=0.085
P. astreoides		p=0.001; R=0.122	p=0.31; R=0.061	p=0.14; R=0.072	
S. siderea	p=0.001; R=0.122		p=0.7; R=-0.076	p=0.81; R=-0.064	

Table S4 Analysis of similarity (ANOSIM) shows whether mucus associated archaeal- and bacterial communities of corals differ between the three sites (B.O.= Buoy One; S.B.=SnakeBay; V.B.=Vaersen Bay).

Archaeal community	B.O.-S.B.	B.O.-V.B.	V.B.-S.B.	all
M. annularis	p=0.13; R=0.043	p=0.06; R=0.083	P=0.12; R=0.041	P=0.006;R=0.079
P. astreoides	p=0.3; R=0.012	P=0.006; R=0.136	P=0.02; R=0.081	P=0.09; R=0.06
S. siderea	p=0.1; R=0.063	P=0.18; R=0.048	P=0.08; R=0.082	P=0.09; R=0.06
Bacterial community				
M. annularis	p=0.34; R=0,009	p=0.024; R=0.1	P=0.03; R=0.1	P=0.025;R=0.066
P. astreoides	p=0.05; R=0.055	P=0.002; R=0.13	P=0.18; R=0.01	P=0.27; R=0.06
S. siderea	p=0.01; R=0.104	P=0.02; R=0.093	P=0.04; R=0.081	P=0.006; R=0.094

Table S5 Analysis of similarity indicates whether archaeal- and bacterial communities in the coral mucus corals differ between 5 and 15 meters.

Archaeal community	Difference between 5 and 15m
M. annularis	p=0.40; R=0
P. astreoides	p=0.83; R=-0.022
S.siderea	p=0.37; R=0.007
Bacterial community	
M. annularis	p=0.02; R=0.06
P. astreoides	p=0.38; R=0.004
S. siderea	p=0.08; R=0.032

Table S6 Spearman`s correlation show if community matrix varies over distance. Community matrix = Presence/Absence Matrix; Distance matrix = calculated out of the distance of the three sites and patches;

Archaeal community	significance value p	Rho
M. annularis	0.089	0.058
P. astreoides	0.028	0.076
S.siderea	0.016	0.108
sediment control	0.09	0.106
Bacterial community		
M. annularis	0.02	0.075
P. astreoides	0.066	0.066
S. siderea	0.167	0.033
sediment control	0.021	0.215

Figures based on the analysis of VIC Primer

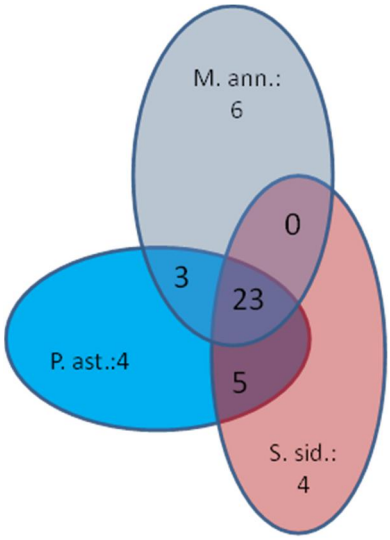


FIG. S5 Unique, shared and ubiquitous OTUs represented in the archaeal community based on VIC Primer

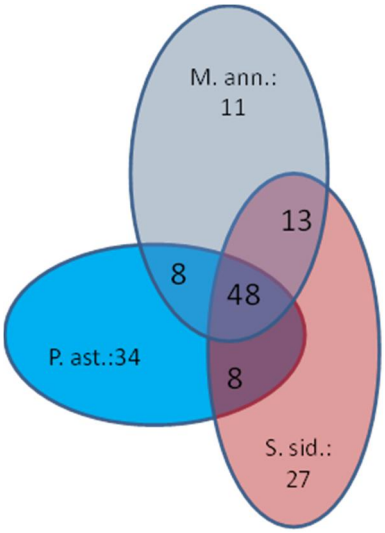


FIG. S6 Unique, shared and ubiquitous OTUs represented in the bacterial community based on VIC Primer

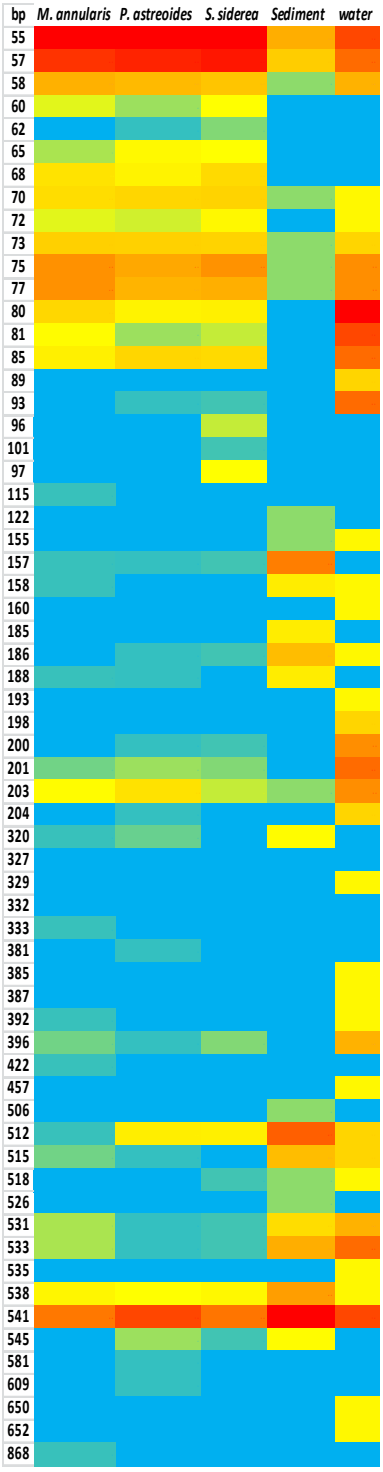


FIG. S7 Archaeal OTU distribution across species and habitats, based on VIC Primer. Color code indicates the percentage of colonies where the specific OTU was present.

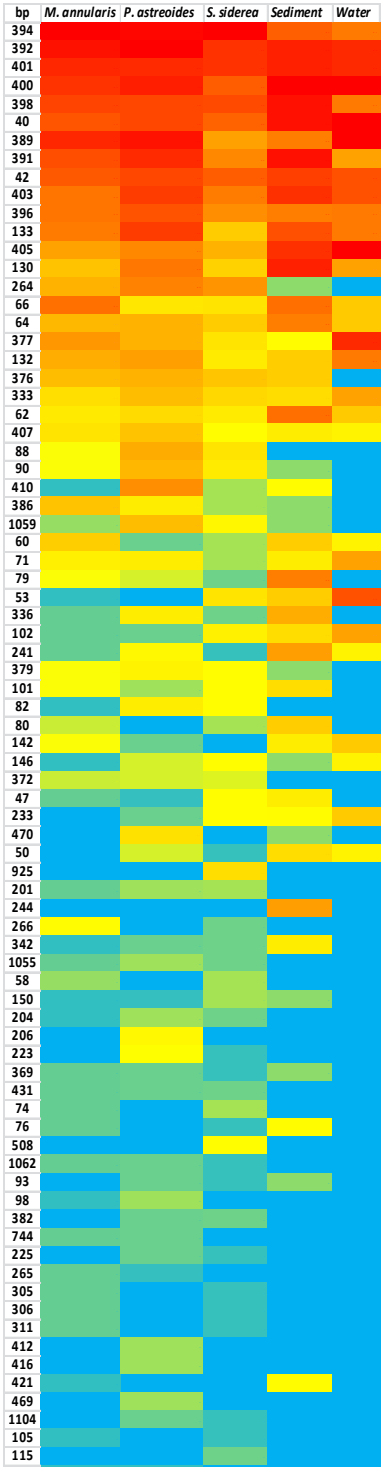
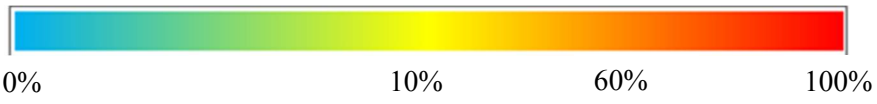


FIG. S8 Bacterial OTU distribution across species and habitats, based on VIC Primer. Color code indicates the percentage of colonies where the specific OTU was present.



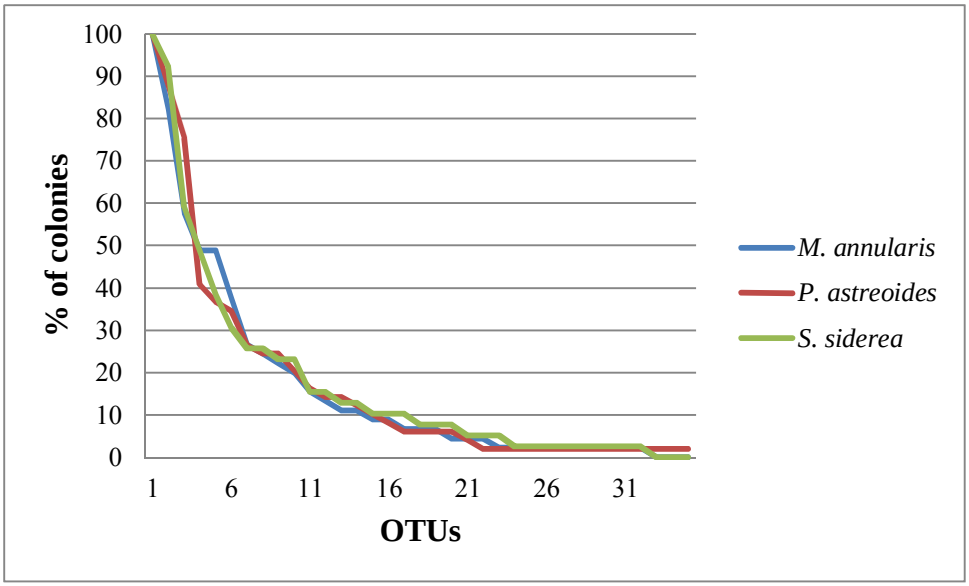


FIG S9 Rank frequency distribution of mucus associated archaeal OTUs in colonies of *M. annularis, P. astreoides* and *S. siderea*

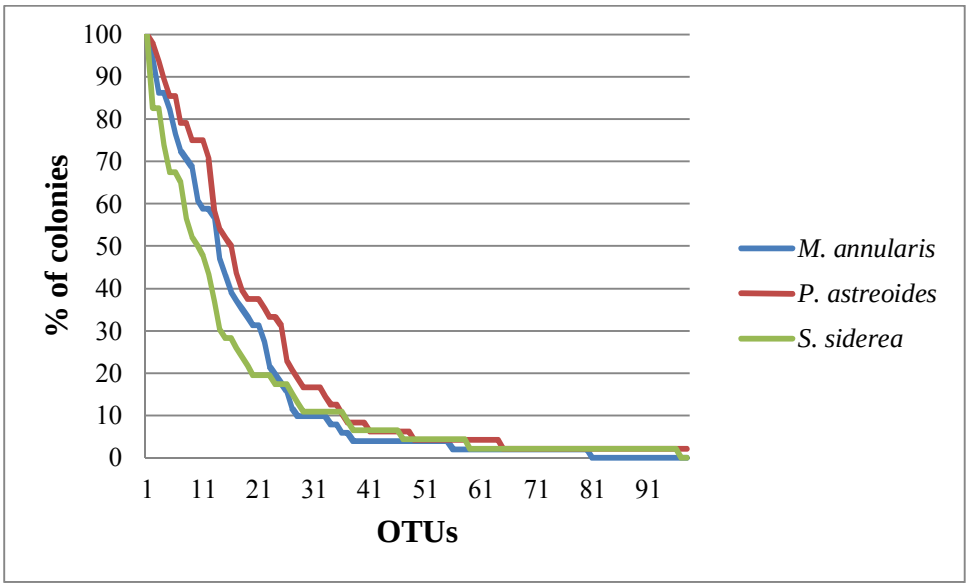


FIG S10 Rank frequency distribution of mucus associated bacterial OTUs in colonies of *M. annularis, P. astreoides* and *S. siderea*

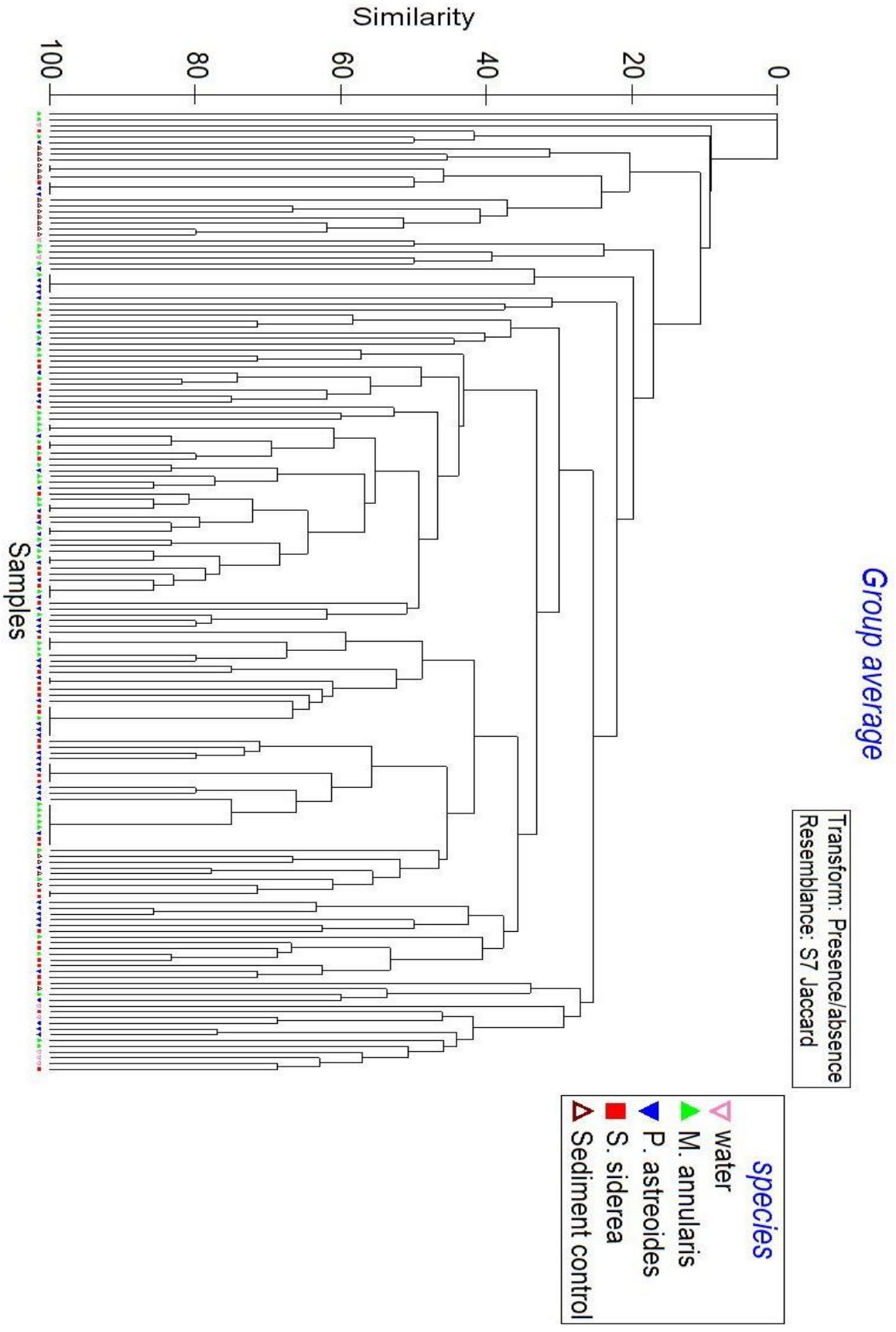


Fig. S1 Hierarchical cluster of similarity of the mucus associated archaeal communities of the three coral species and adjacent sediment and ambient water.

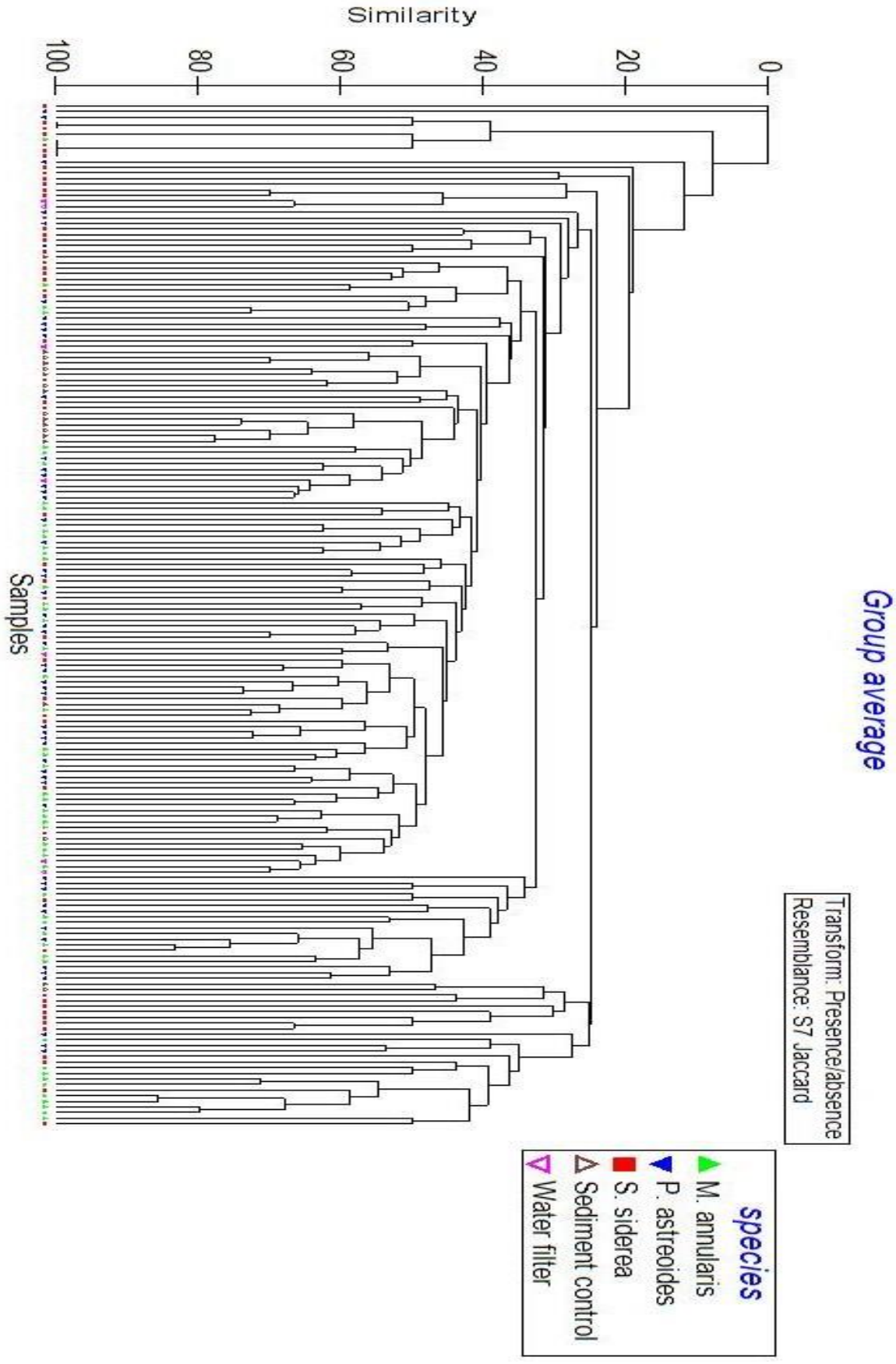


Fig. S12 Hierarchical cluster of similarity of the mucus associated bacterial communities of the three coral species and adjacent sediment and water samples.

Deutsche Zusammenfassung

Bakterielle und archaeale Gemeinschaften besiedeln die Mucusschicht (Schleimschicht) von riffbildenden Korallen *Montastraea annularis, Porites astreoides* und *Siderastrea siderea*. Diese Arbeit beschäftigt sich mit der Diversität, räumliche Verteilung und Wirtspezifität von Prokaryoten, welche den Mucus bevölkern. Mit der Methode terminale Restriktionsfragmentlängenpolymorphismus (T-RFLP) von 16S rDNA wurde die Zusammensetzung der Archaea- und Bakteriengemeinschaft am Mucus erforscht. Weiters wurde die Mucus Abgabe über eine Zeitreihe von Luft-exponierten Korallen gemessen und die Abundanz der Prokaryoten gezählt und berechnet. Generell unterschieden sich die Bakterien- und Archaeagemeinschaften des Mucus von denen der Umgebungswasser und des Sedimentes. Bakterielle Gemeinschaften hatten eine höhere Diversität (H‘, 3,8-4,0 OTUs) als Archaeagemeinschaften (H‘, 2,7-2,9). Mucus von *M. annularis* hatte die höchste bakterielle Diversität (H‘, 4,0), während der Mucus von *S. siderea* die höchste archaeale Diversität aufwies (H‘, 2,9). Bakterielle Gemeinschaften wiesen eine Wirtspezifität auf während archaeale Gemeinschaften keine Spezialisierung auf bestimmte Korallenarten zeigten. Bakterielle Gemeinschaften variierten über die Entfernung (über eine Distanz von Kilometern), während archaeale Gemeinschaften sich über eine geographische Distanz nicht veränderten. Die Prokaryotenabundanz änderte sich zwischen den verschiedenen Arten und stand im Zusammenhang mit der Dicke der Mucusschicht. Korallen mit einer dünneren Mucusschicht (70 µm im Durchschnitt für *P. astreoides*) haben eine geringere Abundanz von Prokaryoten per Mucus Volumen (2,1x 10^6 cells ml^{-1}) als Korallen mit einer dickeren Mucusschicht (220 µm für *M. cavernosa*; 3,2x 10^6 cells ml^{-1}). Generell zeigten verschiedene Korallenarten unterschiedliche Dynamiken von Mucus-Abgabe. *Montastrea cavernosa, Diploria labyrinthiformis* und *Colpophyllia natans* zeigten eine schnelle Abnahme der Mucusproduktion, während die Abundanz der Prokaryoten graduell abnahm. *Porites astreoides* zeigte einen graduelle Abnahme der Mucusproduktion und *Diploria strigosa* zeigte eine stabile Mucusproduktion während der Luftexposition. Diese Studie demonstriert, dass bakterielle Gemeinschaften, welche mit der Mucusschicht von Korallen assoziiert sind, interspezifische und spatiale Variation aufwiesen, während Archaea-Gemeinschaften sich nicht zwischen verschiedenen Arten oder Orten unterscheiden.

Curriculum Vitae

1. Education

1993-1996:	Primary school, Innsbruck
1996-1997:	Primary school, Villach
1997-2005:	BG&BRG St. Martin, Villach, focus on languages (English, Italian und Latin)
Final examination:	14/06/2005

2. Studies

2005-2013:	Diploma study of Biology/Ecology with specialization in marine biology at the University of Vienna
2005-dato:	Journalism at the University of Vienna
2013:	Diploma thesis at the Department of Marine Biology, University of Vienna
Diploma title:	Prokaryotic abundance and community structure in mucus of reef building corals

Marine and biological field courses:

2010:	Biology and systematics of neotropical amphibians, French Guyana
2010:	Marine biological field course, Piran, Slovenia
2010:	Marine biological field course on the Mediterranean fauna and flora; Centre for Marine Research, Rovinj, Croatia
2010/2011:	Advanced marine ecology course at the Royal Netherlands Institute for Sea Research (NIOZ) Texel, Netherlands
2012:	Fieldwork at CARMABI, to collect samples/data for diploma thesis, Curaçao, Netherlands Antilles

3. Additional work experience

2003:	3 weeks internship at a veterinarian, Mag. Nina Winkler in Velden, Austria
2004-2011:	internship at a shop of traditional costumes in Villach, Austria

4. Personal skills

Languages:	German (first language), English (fluent), Italian (school)
Computer skills:	MS Office, Primer6, SPSS, EstimateS, Past