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Bacteria of the genus *Endozoicomonas* dominate the microbiome of the Mediterranean gorgonian coral *Eunicella cavolini*

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ABSTRACT: Forming dense beds that provide the structural basis of a distinct ecosystem, the gorgonian *Eunicella cavolini* (Octocorallia) is an important species in the Mediterranean Sea. Despite the importance and prevalence of this temperate gorgonian, little is known about its microbial assemblage, although bacteria are well known to be important to hard and soft coral functioning. Here, we used massively parallel pyrosequencing of 16S rRNA genes to determine the composition and relative abundances of bacteria associated with *E. cavolini* collected from different depths at a site on the French Mediterranean coast. We found that whereas the bacterial assemblages of *E. cavolini* were distinct and less diverse than those of the surrounding water column, the water depth did not affect the bacterial assemblages of this gorgonian. Our data show that *E. cavolini*'s microbiome contains only a few shared species and that it is highly dominated by bacteria from the genus *Endozoicomonas*, a *Gammaproteobacteria* that is frequently found to associate with marine invertebrates.

KEY WORDS: Microbial communities · Gorgonian · *Eunicella cavolini* · 16S tag sequencing

INTRODUCTION

Associations between marine invertebrates and prokaryotes have commonly been observed. Some of the best studied examples are from the benthic fauna found near hot vents and cold seeps, where mussels and tubeworms live in tight symbiotic association with bacteria and take advantage of the energy produced by the bacteria (see Dubilier et al. 2008 for review). Other marine host species rely on heterotrophic bacteria to metabolize otherwise inaccessible organic compounds. For instance, at whale fall sites, the *Osedax* worm lives in symbiosis with bacteria of the order *Oceanospirillales.* These bacteria are thought to break down organic compounds in whale bone, providing nutrition to the worm (Rouse et al. 2004). In addition to nutritional interactions, such partnerships can facilitate other beneficial processes, as in the squid *Euprymna scolopes*, which hosts *Vibrio fisheri* bacteria in a specialized light organ that provides nightly camouflage from predators (McFall-Ngai 2008).

Prokaryotes also play important roles in the functioning of scleractinian tropical reef corals. Species-specific coral–bacterial associations exist across distant locations for some coral species (Rohwer et al. 2002, Sharp et al. 2012), but not for others (Morrow et al. 2012). Microbes provide benefits to corals, such as nitrogen fixation (Williams et al. 1987, Shashar et al. 1994, Rohwer et al. 2002, Lesser et al. 2004), antibi-

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otic production (Ritchie 2006), mucus recycling and food supply (Ferrier-Pages et al. 2000, Wild et al. 2004). However, microbes can also be agents of coral diseases when environmental conditions are not optimal (Frias-Lopez et al. 2002, Pantos et al. 2003, Bourne & Munn 2005). In studies of tropical octocorallia (Gorgonacea), the focus has been on antimicrobial activity (Cimino et al. 1984, Roussis et al. 2001, Couch et al. 2008) or microbial diseases, particularly those that affect sea fans (Bruno et al. 2007, Mydlarz et al. 2008, Rivest et al. 2010).

In comparison with tropical anthozoans, there are only a few studies on the microbial associations of temperate anthozoans, such as Mediterranean gorgonians (Rivière et al. 2010). These gorgonians are among the most representative and structurally important species of the Mediterranean coastal benthos (Gili & Ros 1985). The diversity and richness of the habitats they form are often compared to those of tropical coral reefs (Ballesteros 2006). Gorgonians can develop very dense populations (Weinbauer & Velimirov 1995) and therefore play an important role as ecosystem engineers (Jones et al. 1994) by providing biomass and structural complexity (Harmelin 1995). They are also important drivers of energy and matter from the planktonic to the benthic ecosystem (Gili & Coma 1998). Like many of the ~20 described gorgonian species that inhabit the Mediterranean Sea (Carpine & Grasshoff 1975), *Eunicella cavolini* (Koch, 1887) occurs from the surface to 100 m depth (Gili et al. 1989, Harmelin 1995). It is one of the most abundant structural species within the rocky sublittoral zone (Gili & Ros 1985, Harmelin 1995, Ballesteros 2006). Mediterranean gorgonians exhibit slow growth rates and low natural mortality rates (Harmelin 1995, Gili & Coma 1998, Linares et al. 2007, Bramanti et al. 2009); they are, however, especially vulnerable to anthropogenic disturbances and climatic anomalies (Garrabou & Harmelin 2002, Santangelo et al. 2007, Linares & Doak 2010), which have increasingly induced mass mortality events (Linares et al. 2005, Coma et al. 2006, Garrabou et al. 2009). These mortality events often occur during summer when the temperature is abnormally high and when there is strong stratification, inducing nutrient depletion and pathogen colonization (Cerrano et al. 2000). A *Vibrio* strain with thermo-dependent virulence was isolated from diseased colonies of *Paramuricea clavata* (Gorgonacea), suggesting that pathogenic bacteria play a role in gorgonian mortality events (Bally & Garrabou 2007).

Despite the increasing threats to gorgonians and their importance to the Mediterranean benthic ecosystem, the composition of the microbial communities living in association with *Eunicella cavolini* remains completely unknown. We therefore sought to study the bacterial assemblage found in healthy colonies of *E. cavolini*, to elucidate whether the community composition changes with depth, and to discover whether *E. cavolini* has a clearly defined core microbiome. These data will serve as a reference and baseline to further investigate changes in the composition and functions of microbiota resulting from elevated temperatures, disease, and other environmental stressors.

MATERIALS AND METHODS

Sampling and DNA extraction

Eunicella cavolini individuals were sampled in the Bay of Villefranche-sur-Mer on the French Mediterranean coast (43° 41′ 10″ N, 7° 19′ 0″ E) in June 2010. Samples were collected using SCUBA from 3 depths (24, 30, and 41 m, n = 3 at each depth). This zone is characterized as oligotrophic with low concentrations of inorganic and organic nutrients (Bustillos-Guzmán et al. 1995, Lacroix & Nival 1998). The maximum depth at the study site was 80 m. The gorgonian colonies were found to be attached to the rocky substrate. The sampled individuals were separated by several meters (>3 m). There were no signs of disease, necrosis, or mortality in the samples or surrounding colonies during the months around the collection time. At each sampling depth, several branches from 3 different colonies were cut with clean scissors, individually enclosed in sterile plastic bags and brought back to the laboratory. The gorgonians were then rinsed with 0.2 µm filtered seawater and flash-frozen in liquid nitrogen until DNA extraction. We named the 3 samples collected at 24 m G24A, G24B, and G24C, those at 30 m G30A, G30B, G30C and those at 41 m G41A, G41B, G41C. To identify bacterial communities that were also found in the surrounding seawater, we collected seawater in the vicinity of the sampled colonies. We named these 3 samples W24, W30, and W41, according to the depth at which they were sampled. To isolate the bacteria in the seawater samples, we filtered 2 to 3 l samples from each depth under low pressure on 0.2 µm Isopore 25 mm filters (Millipore). The filters were subsequently flash-frozen for downstream analyses. DNA was extracted from the gorgonian samples by crushing whole tissue samples in liquid nitrogen to a powder

and then processing 500 mg of each powder sample with a commercially available extraction kit (MoBio) according to Rohwer et al. (2002). The same kit was also used to extract DNA from one half of each filter.

PCR amplification of 16S rDNA

To obtain an amplicon of a size suitable for 454 sequencing, the 27F (5′-CGT ATC GCC TCC CTC GCG CCA TCA GNN NNN NNN tcAGA GTT TGA TCC TGG CTC AG-3′) and 338R (5′-CTA TGC GCC TTG CCA GCC CGC TCA GcaTG CTG CCT CCC GTA GGA GT-3′) primers were used to amplify variable regions 1 and 2 of the 16S rRNA gene. Both primers contained Roche 454 pyrosequencing adaptors (underlined above) for use in the library construction, as well as a 2-bp linker sequence (lowercase) to prevent tag or adapter influence on the polymerase chain reaction (PCR) (Berry et al. 2011). The forward primer also contained a sample-specific barcode sequence (indicated as N). PCR was performed using a Qiagen Multiplex PCR kit with 2 µM of each primer adjusted to a final volume of 25 µl with RNAse-free water. For the gorgonian samples, 2 ng DNA were used as the template. The DNA extracted from the filters was below the detection limit for the photometer used, but a 1:10 dilution of the extraction was sufficient to yield ample PCR product, whereas a negative control did not amplify. The temperature cycling for amplification was as follows: 1 cycle at 95°C for 15 min; 30 cycles at 94°C for 45 sec, 60°C for 30 sec and 72°C for 40 sec; and 1 final extension at 72°C for 10 min. All reactions were performed in duplicate. The PCR products were quantified using a Qubit photometer (Invitrogen) and then pooled in equal amounts after purification with a Qiagen PCR purification kit. Sequencing was performed using the Roche 454 FLX using titanium chemistry.

Full-length 16S sequences of *Endozoicomonas* were obtained via PCR from 3 samples (G30B, G30C, G41A) that had large percentages of this bacteria (as determined by 454 sequencing analysis) using the primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′). The PCR product was cloned using a Qiagen PCR Cloning Kit and sequenced in both directions on a capillary sequencer. Thirty clones were sequenced, 20 of which originated from *Endozoicomonas*.

Data analysis

We used mothur (version 1.16.1), an open-source software developed for microbial ecology data analysis, for most steps (Schloss et al. 2009). Sequence reads were split according to barcodes and quality trimmed to an average quality of 27 in a 50 bp window. These reads were aligned against the SILVA alignment database (release 108; Pruesse et al. 2007), and we removed any sequences that did not cover variable regions 1 and 2 (SILVA alignment positions 1044 to 5436). To reduce sequencing noise, a pre-clustering step as implemented in mothur (1 bp difference) was performed (Huse et al. 2010). Chimeric sequences were removed using UCHIME as implemented in mothur (Edgar et al. 2011). Sequences are available in the NCBI Sequence Read Archive under accession number SRA050215.1.

Beta diversity based on the Yue and Clayton theta measure for dissimilarity (Yue & Clayton 2005) was calculated in mothur based on a dataset subsampled to the minimal number of reads in any sample (2580). In addition ANOSIM analyses were used to test for differences between sample groups based on the Yue-Clayton theta, which were calculated with the anosim command in the vegan package (Oksanen et al. 2012), within the R Statistical Package (R Core Team 2012). As an alternative beta diversity measure weighted UniFrac (Lozupone & Knight 2005) was calculated with mothur, also based on the subsampled dataset.

The association of single operational taxonomic units (OTU) to sample groups was calculated using the indicspecies package (De Cáceres & Legendre 2009), also within the R Statistical Package (R Core Team 2012). We performed 2 tests with different partitions of the data, the first splitting the samples into water and gorgonians and the second grouping just the gorgonian samples by depth.

Full-length clone sequences were vector- and quality-trimmed with CodonCode Aligner 3.7.1, aligned to the SILVA database (release 108) using the SINA aligner available on the SILVA website (Pruesse et al. 2007) and subsequently imported into ARB version 5.1 (Ludwig et al. 2004). The sequences are available in Genbank under accession numbers JQ691564 to JQ691583. With these full-length sequences as well as related sequences available in SILVA, a maximum likelihood phylogenetic tree was generated using PhyML (Guindon & Gascuel 2003), as implemented in ARB, with a GTR model and 1000 bootstraps. Additionally, a neighborhood-joining tree with 1000 bootstraps was calculated in MEGA version 5 (Tamura et al. 2011) using the Jukes Cantor correction.

Table 1. *Eunicella cavolini*. Gorgonian coral and water samples taken in Bay of Villefranche-sur-Mer on the French Mediterranean coast: depth of collection, number of sequences and operational taxonomic units at the 0.03 difference level (OTUs), and alpha diversity measures

Sample code	Depth (m)	No. of sequences	No. of OTUs	Inverse Simpson index	Shannon index	Chao1	Shannon evenness
Water samples							
W24	24	29812	1711	35.44	4.87	2911.0	0.65
W30	30	30765	1199	19.01	4.23	2039.0	0.60
W41	41	34859	1734	31.09	4.68	3676.7	0.63
***E. cavolini* samples**							
G24A	24	7900	785	16.14	4.43	1199.6	0.67
G24B	24	13427	119	1.32	0.79	162.3	0.17
G24C	24	2580	136	3.39	2.21	232.6	0.45
G30A	30	18256	704	5.64	3.44	1176.1	0.52
G30B	30	5555	147	1.73	1.29	233.0	0.26
G30C	30	4424	156	1.65	1.38	217.2	0.27
G41A	41	10028	132	1.15	0.52	254.2	0.11
G41B	41	3315	100	2.46	1.52	151.8	0.33
G41C	41	3448	413	5.07	3.61	638.0	0.60

RESULTS

We produced 528 977 reads from 12 samples, 3 water samples and 3 gorgonian colonies collected from 3 depths (24, 30, and 41 m, n = 9, Table 1). Of these reads, 164 369 were used for analysis after quality trimming and chimera detection (water samples: 95 436 reads, length 228.9 ± 0.05 bp, average ± SE; gorgonian samples: 68 933 reads, length 242.9 ± 0.04 bp). The water samples were thus represented by more sequences in our library with about a 4-fold higher number on average (Table 1). We clustered all sequences at the 0.03 difference level to obtain OTUs. The bacterial community of the seawater was much more diverse than that in the gorgonian tissues (inverse Simpson index, Mann-Whitney *U* test between all 3 water samples and all 9 gorgoninan samples, p = 0.0091). The difference in diversity is caused by both the number of OTUs and the evenness. Observed counts of OTUs, as well as the Chao 1 estimate of species richness (Chao 1984), are higher in the water samples, as is the evenness (Table 1). The much higher diversity was also reflected in rarefaction curves for the different samples. Although the number of sequences was much higher for the water samples, their curves did not reach a plateau (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m479p075_supp.pdf). This indicates that more sequencing would be necessary to capture the complete diversity of the bacteria present. The diversity data for the gorgonian samples was significantly lower than for water, but not homogeneous within the gorgonian samples. In each of the groups of replicates, one had a higher diversity as compared with the others. These were G24A, G30A and G41C (Table 1).

We were interested in the differences in the bacterial communities between the water and gorgonian samples, as well as among the gorgonian samples from different depths. We compared the community structure of the samples using an OTU-based approach. We used the Yue and Clayton measure of dissimilarity (Yue & Clayton 2005) to calculate a distance matrix, and then generated a UPGMA tree and a heatmap based on this matrix (Fig. 1). The water samples were clearly separated from all gorgonian samples, indicating that the bacterial communities were considerably different (ANOSIM p = 0.006). Within the gorgonian samples, no structuring according to sampling depth was apparent (ANOSIM p = 0.99). Furthermore, the replicate samples from each depth did not cluster together. The G24A sample, which exhibited much higher diversity than all other gorgonian samples, was far removed from the remaining gorgonian samples in the tree (Fig. 1). Interestingly, G30A and G41C (the other 2 samples with high diversity) were not far removed from the rest, but close to each other (Fig. S2 in the Supplement).

In addition to the analysis based on OTU clustering, we generated a tree with all sequences to analyze differences in community composition between samples. Here, we used weighted Unifrac (Lozupone & Knight 2005) to calculate a distance matrix between all samples, which was then reduced in complexity through principal coordinate analysis (PCoA).

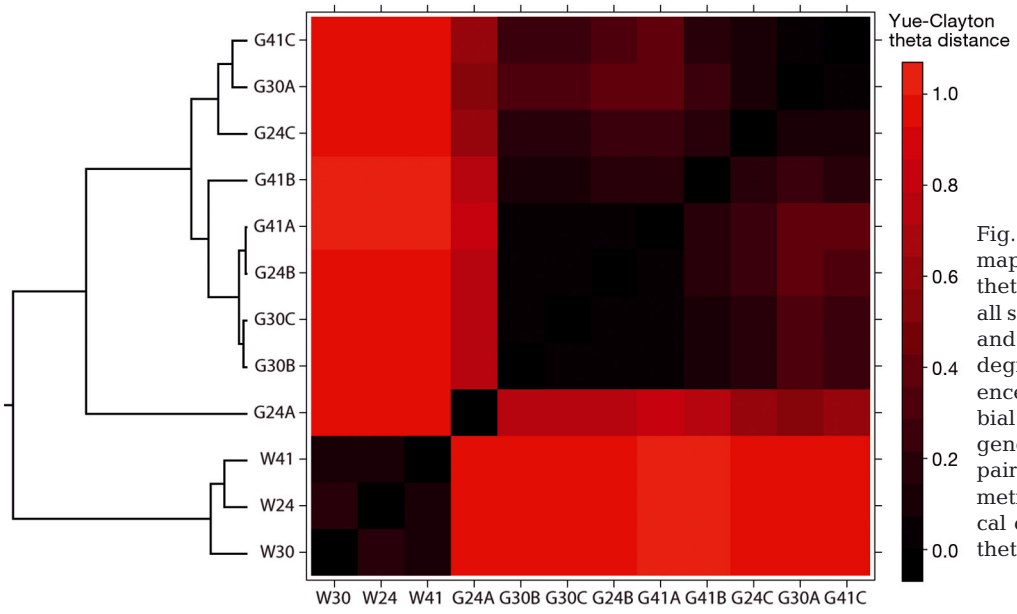

Fig. 1. *Eunicella cavolini.* Heatmap and tree of the Yue-Clayton theta distance measure between all samples. The intensity of black and red shading indicates the degree of similarity and difference, respectively, in the microbial communities. The tree was generated using the unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering of the Yue-Clayton theta values based on subsampled OTU counts

The result confirmed that although the gorgonian samples were clearly distinct from the water samples, they did not cluster by water depth (Fig. S2 in the Supplement). Additionally, they did not form a tight group as the water samples did, suggesting that they contained more variable assemblages of bacteria.

To determine the identities of the bacteria present in the gorgonians, we classified all 16S sequences against the SILVA database (release 108; Pruesse et al. 2007). On the level of phyla, *Proteobacteria* were the dominant group, accounting for 60 to 93% of all tags in the gorgonian samples (Fig. 2A). In the water samples, *Proteobacteria* accounted for about one-third of all sequences, with *Cyanobacteria* and *Bacteroidetes* being the other major groups. Within *Proteobacteria*, *Gammaproteobacteria* was the dominant group among the gorgonians, representing 76% of all *Proteobacteria*. When looking at the relative contributions on the genus level, it becomes apparent that one genus within the *Gammaproteobacteria*, *Endozoicomonas,* was the most dominant group in the gorgonian bacterial community (Fig. 2B). While mostly absent from the seawater, *Endozoicomonas* accounted for 10 to 60% of the sequence reads from the gorgonian samples, with an average of 42%. The sample with the lowest abundance of *Endozoicomonas* was G24A, the 'outlier' in the set, which instead was comprised of a high concentration of sequences from the genus *Ruegeria*, which had low abundance in or was absent from all other samples.

We also deduced the dominance of *Endozoicomonas* from the OTU data. Only 7 OTUs at the 0.03 difference level were present in all of the *Eunicella cavolini* samples tested (Table 2). These 7 OTUs, however, accounted for 64% of all reads in the 9 gorgonian samples, with OTU3 accounting for 61% of these reads. We classified OTU3 as *Endozoicomonas*. We also assigned 13 other OTUs to this genus; however, they contained <6 reads.

To identify additional OTUs that were significantly associated with the gorgonians, we used the statistical package indicspecies (De Cáceres & Legendre 2009). Indicspecies tests the associations of species patterns with sample groups. Testing all gorgonian samples as one group against all water samples, we found that 786 OTUs were significantly associated ($p < 0.05$) with the water samples (data not shown), while 8 OTUs were significantly associated with the gorgonian group (Table S1 in the Supplement). Of these 8, the OTU with the highest number of reads was again OTU3. The second highest was OTU41, part of the genus *Halieda* and also in the *Gammaproteobacteria* group. All other OTUs had total read numbers of <130 and were assigned to the genera *Elizabethkingia*, *Arthrobacter*, *Stenotrophomonas*, *Acidovorax*, an unclassified deltaproteobacterium, as well as one unclassified OTU. Four OTUs that were present in all gorgonian samples (Table 2) were not significantly associated with the gorgonians as compared to water (OTUs 1, 2, 41 and 111).

Grouping the gorgonians by the different depths, we found only a few OTUs that were significantly associated with a particular depth. Two OTUs, one classified as *Aquimarina* and the other unclassified but a member of *Myxococcales*, were found only

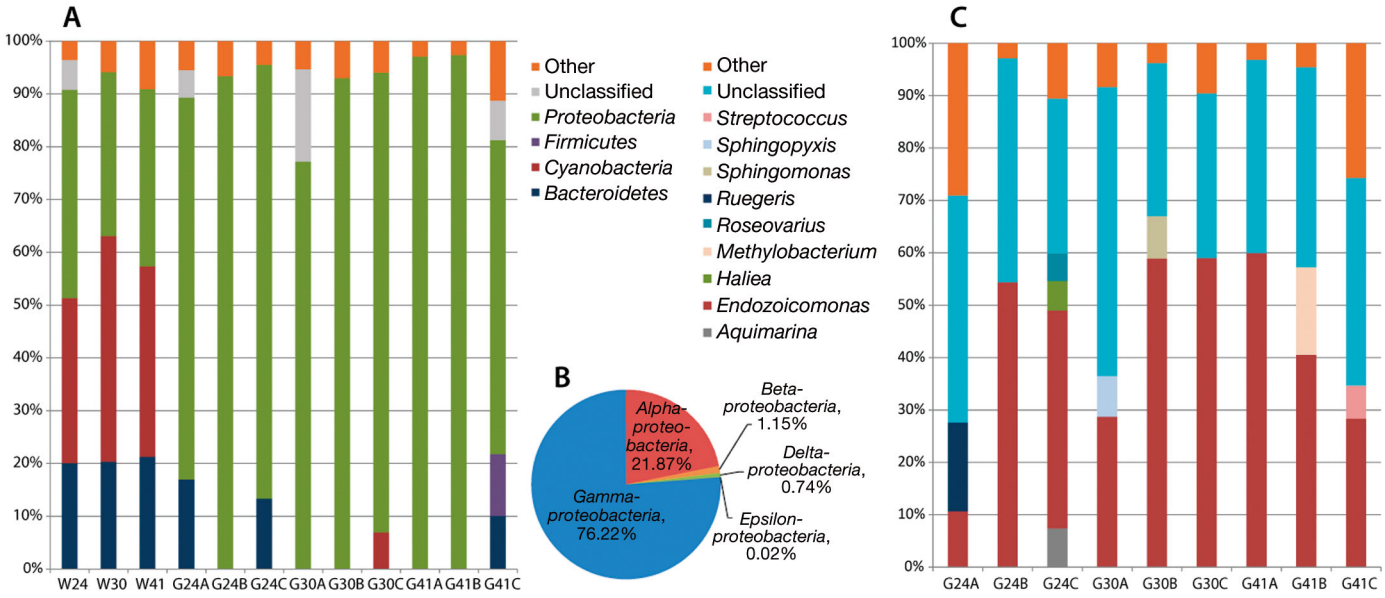

Fig. 2. *Eunicella cavolini.* Bacterial community compositions of water and gorgonian samples (indicated by the letters W and G, respectively, in the sample codes). (A) Relative contributions to samples on the phylum level. (B) Average composition of the *Proteobacteria* phylum in the gorgonian samples. (C) Relative contributions on the genus level in the gorgonian samples. Phyla in (A) and genera in (B) contributing less than 5% of the reads have been grouped together in the 'other' category

Table 2. *Eunicella cavolini*. Summary of operational taxonomic units (OTUs) that were present in all samples of the gorgonian tested. For each OTU, the number of reads in each sample, the sum of reads for all samples and the percentage of all reads are shown. The lower panel shows the taxonomic assignment of the OTUs. Bootstrap values of less than 100 are shown in parentheses

	Sample code									Sum	% of all reads
	G24A	G24B	G24C	G30A	G30B	G30C	G41A	G41B	G41C		
OTU1	7	148	10	35	78	97	4	9	4	392	0.57
OTU2	24	157	18	56	78	148	9	2	2	494	0.72
OTU3	1311	11662	1355	7492	4187	3435	9330	1998	1519	42289	61.35
OTU41	61	48	150	419	17	83	20	2	48	848	1.23
OTU111	9	34	7	11	4	49	28	6	13	161	0.23
OTU124	10	55	7	7	5	7	12	3	6	112	0.16
OTU215	6	23	6	2	6	12	11	10	5	81	0.12

Taxonomic assignment of OTU (bootstrap value)

OTU1	*Cyanobacteria*; *Cyanobacteria*; Family_II; GpIIa; unclassified
OTU2	*Cyanobacteria*; *Cyanobacteria*; Family_II; GpIIa; unclassified
OTU3	*Proteobacteria*; *Gammaproteobacteria*; *Oceanospirillales*; *Hahellaceae*(99); *Endozoicomonas*(68)
OTU41	*Proteobacteria*; *Gammaproteobacteria*; *Alteromonadales*; *Alteromonadaceae*(99); *Haliea*(98)
OTU111	*Actinobacteria*; *Actinobacteria*; *Actinomycetales*; *Propionibacteriaceae*; *Propionibacterium*
OTU124	*Actinobacteria*; *Actinobacteria*; *Actinomycetales*; *Micrococcaceae*; *Arthrobacter*
OTU215	*Bacteroidetes*; *Flavobacteria*; *Flavobacteriales*; *Flavobacteriaceae*; *Elizabethkingia*

among the gorgonians at the 24 m depth (Table S1). The group of samples from 30 m contained an *Endozoicomonas* OTU that was not found among the other gorgonians, and the samples from 41 m were associated with one OTU that could not be assigned in the SILVA taxonomy database. However, none of these OTUs was very abundant, with <200 reads per sample for *Aquimarina* and <20 for the other groups.

We sequenced the full-length 16S rRNA gene of the *Endozoicomonas* bacteria from *Eunicella cavolini* to analyze their phylogenetic position. Phylogenetic analysis revealed that they belong to the order

Oceanospirillales and the family *Hahellaceae*, and most bacteria closely related to them are associated with marine invertebrates (Zielinski et al. 2009, Jensen et al. 2010). The closest relatives in the database were isolated from a Caribbean gorgonian, *Gorgonia ventalina* (98.3% identity) (Sunagawa et al. 2010) and the arctic gorgonian *Alcyonium antarcticum* (93.3% identity) (Webster & Bourne 2007). Most other closely related bacteria were associated with either soft corals of the order Alcyonacea, such as the gorgonians, or scleractinian corals (Fig. S3 in the Supplement).

DISCUSSION

Although the sample set for this study was rather small, we produced, for the first time, a large dataset of 16S reads from the bacterial community of *Eunicella cavolini* to understand the microbiology of a gorgonian from the Mediterranean Sea. We found that its bacterial community differs from that of the surrounding water and is less diverse. We therefore suggest that temperate octocorals exhibit a specific microbiome that is distinct from that of the surrounding water, as do tropical scleractinian corals (Rohwer et al. 2002).

We found between 100 and 785 OTUs at the 0.03 difference level in the gorgonians. The differences in OTUs between the individual samples were not dependent on the number of reads analyzed. For example, while we analyzed 7900 reads from sample G24A, which clustered into 785 OTUs, sample G24B had 13427 reads, but contained only 119 OTUs. To compare the diversity in *Eunicella cavolini* to other gorgonians, we reanalyzed sequences produced by Sunagawa et al. (2010) from the Caribbean coral *Gorgonia ventalina* using the same methods described above. The value for the Shannon diversity for this tropical gorgonian, 3.09, was in the same range as the values for *E. cavolini* (Table 1), with the caveat that the studies used different regions of the 16S gene. For the bacterial community of scleractinian corals similar values from about 3 to 6 for the Shannon diversity index are reported (Bourne & Munn 2005, Chen et al. 2011).

We sampled gorgonians from 3 different depths to determine whether their associated bacteria also differed, which might suggest that the bacteria were influenced by abiotic factors in the host's habitat. Alternatively, if the gorgonian host provided a stable environment to the bacteria that did not change with water depth, we would expect similar bacterial species to colonize host individuals. We found that while the bacterial community composition was more varied than that of the water, it did not vary according to depth. This pattern was independent of whether our comparisons took into account presence and/or abundance of OTUs. *Eunicella cavolini* is normally found from 20 to 100 m, and there may be greater changes in bacterial assemblages as the depth increases beyond 41 m; however, based on our results, we conclude that water depth is not a major factor in driving and structuring bacterial community composition in gorgonian corals.

The large variability between the samples was also reflected in the bacterial taxa shared among all individual *Eunicella cavolini*, with only 7 of 2067 OTUs found in all gorgonian samples. Although this overlap was quite small, one OTU was not only present in all samples, but made up a very large percentage of all sequences analyzed. The dominance of this OTU, classified as the genus *Endozoicomonas*, is the main feature that is common between all gorgonian samples we analyzed. The 6 other shared OTUs accounted for <2% of all reads. These bacteria may play specific roles but are unlikely to provide basic functions that would require the symbiont to be present in large numbers. Furthermore, as we sampled in only one location, we cannot say whether these OTUs were present in samples taken from other parts of the Mediterranean.

One aim was to determine whether a core microbiome for a habitat or host existed. The core bacterial taxa contained in such a microbiome could be assumed to carry out vital functions for the host. Here, we defined the core microbiome by shared membership between samples.

We found that 7 OTUs were shared among all host individuals, with 6 of low abundance. The core microbiome of *Eunicella cavolini* may thus be restricted to the genus *Endozoicomonas*. Additional sampling that covers a larger geographical and temporal range would be required to determine whether a core microbiome truly exists. However, it is likely that the most abundant taxa are also the most persistent, as was found in a time series study in the North Sea (Gilbert et al. 2009).

The genus *Endozoicomonas* belongs to the family *Hahellaceae* and to the order *Oceanospirillales*, a group of heterotrophic, aerobic marine bacteria that are able to break down a wide range of organic compounds (Brenner et al. 2005). The *Endozoicomonas* species investigated here are part of a branch of the bacterial phylogenetic tree whose other members are also associated with marine invertebrates (Fig. S3 in

the Supplement). *Endozoicomonas* have been found in sponges (some under the name *Spongiobacter*) (Thiel et al. 2007, Mohamed et al. 2008, Nishijima et al. 2013), bivalves (Zielinski et al. 2009, Jensen et al. 2010), ascidians (Martínez-García et al. 2007), a nudibranch (Kurahashi & Yokota 2007), polychaetes (Goffredi et al. 2007), sea anemones (Schuett et al. 2007, Du et al. 2010), starfish (Choi et al. 2010), scleractinian corals (Bourne & Munn 2005, Bourne et al. 2008, Hansson et al. 2009, Littman et al. 2009, Raina et al. 2009, Sunagawa et al. 2010, Yang et al. 2010, Speck & Donachie 2012), and different tropical gorgonians and other soft corals (Webster & Bourne 2007, Sunagawa et al. 2010). In the Caribbean gorgonian *Gorgonia ventalina*, the most common bacteria are *Endozoicomonas*. These bacteria were the most closely related to the bacteria from *Eunicella cavolini* in our phylogenetic tree of the *Encozoicomonas* (Fig. S3 in the Supplement; see also Sunagawa et al. 2010). The fact that the 2 closest bacterial relatives in Fig. S3 originate from gorgonians, but from very different habitats and geographic ranges, suggests an evolutionarily old association between the gorgonian hosts and the *Endozoicomonas*. If the association were of more recent origin, it would be expected that the *Endozoicomonas* in *G. ventalina* would be more closely related to those of other Caribbean species, such as the scleractinian corals found there. While we report on a sample of limited geographic range, we expect the dominance of the *Endozoicomonas* in the microbiology of *E. cavolini* to be present across the range of the species, given that it seems to be a feature even in related and geographically distant gorgonian species.

While *Endozoicomonas* bacteria have been shown to be intranuclear parasites in *Bathymodiolus* mussels (Zielinski et al. 2009), they seem to form symbiotic associations in all other documented cases, although the basis of the symbiosis is not known for most examples. One exception is the polychaete *Osedax*, which lives on bones at whale fall sites. Symbiotic *Endozoicomonas* in *Osedax* appear to break down compounds in the bones and provide nutrition to their host (Goffredi et al. 2005). *Endozoicomonas* have also been implicated in sulfur cycling in corals, as they are able to metabolize dimethylsulfoniopropionate (DMSP; Raina et al. 2009, 2010). DMSP is produced by photosynthetic algae, which provide food for the filter-feeding *Eunicella cavolini*. It is thus probable that *Endozoicomonas* also provide DMSP degradation to temperate gorgonians.

Measured by their widespread abundance in marine invertebrates and their dominance in the structure of the microbiomes of species such as *Eunicella cavolini*, bacteria of the *Endozoicomonas* group likely have an important ecological function. As this association is so widespread among hosts of different phyla, it either arose multiple times throughout evolutionary history or, more likely, is very old.

In summary, we examined associations between temperate anthozoans and bacteria in this study. Our data can provide a baseline for further investigations of changes in the composition and functions of microbiota.

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