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Patterns of bacteria-host associations suggest different ecological strategies between two reef building cold-water coral species

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Abstract :

Cold-water corals (CWC) are main ecosystem engineers of the deep sea, and their reefs constitute hot-spots of biodiversity. However, their ecology remains poorly understood, particularly, the nature of the holobiont formed by corals with their associated bacterial communities. Here, we analysed *Madrepora oculata* and *Lophelia pertusa* samples, collected from one location in a Mediterranean canyon in two different seasons (autumn and spring), in order to test for species specificity and temporal stability of the host-bacteria associations. The 16S rRNA sequencing revealed host-specific patterns of bacterial communities associated with *L. pertusa* and *M. oculata,* both in terms of community composition and diversity. All analyzed *M. oculata* polyps exhibited temporally and spatially similar bacterial communities dominated by haplotypes homologous to the known cnidarians-associated genus *Endozoicomonas*. In contrast, the bacterial communities associated with *L. pertusa* varied among polyps from the same colony, as well as among distinct colonies and between seasons. While the resilient consortium formed by *M. oculata* and its bacterial community fit the definition of holobiont, the versatility of the *L. pertusa* microbiome suggests that this association is more influenced by the environmental conditions or nutritional status. Our results thus highlight distinct host/microbes association strategies for these two closely related Scleractinians sharing the same habitat, suggesting distinct sensitivity to environmental change.

Highlights

► We examine the bacterial communities of two reef-building cold-water coral species from Mediterranean Sea ► The 16S rRNA sequencing revealed host-specific patterns of bacterial communities both in terms of community composition and diversity ► Our results highlight distinct host/microbes association strategies for these two closely related Scleractinians sharing the same habitat, suggesting distinct sensitivity to environmental change.

Keywords : Deep-sea corals, *Madrepora oculata*, *Lophelia pertusa*, bacterial communities, bacterial ecology, Mediterranean Sea

Introduction

The cold-water scleractinian corals (CWC) are main ecosystem engineers of the deep sea (Roberts et al., 2006). They form reef structures that provide niches and nursery grounds for a variety of species, including commercial and patrimonial species, which make these reefs hotspots of biodiversity at bathyal depth (Roberts et al., 2006). Deep water corals are restricted to cold temperatures (4-14°C) (Freiwald et al., 2004); nevertheless, they are able to colonize diverse environments from continental shelves, including in submarine canyons, or oceanic features like seamounts to mid-oceanic ridges. Despite the absence of photosynthetic symbiotic zooxanthellae, CWC are able to build reef structures equivalent to those found in tropical shallow-water environments (Freiwald et al., 2004). However, their growth rates are lower than for most shallow-water coral species (Brooke and Young, 2009; Lartaud et al., 2013; Orejas et al., 2011). Consequently, CWC structures are particularly vulnerable to direct human-induced activities (trawling, oil and gas exploitation, waste discharge), while they are also expected to be sensitive to climate change disturbances such as warming, acidification and deoxygenation (Levin and Le Bris, 2015). Likewise, the destruction of coral habitats results in inestimable losses for juveniles of commercially exploited species (Armstrong et al., 2014). Due to the constraints in accessing deep-sea habitats and the relatively recent efforts to study their ecology, there is a lack of understanding of the processes that underlies their resilience capacity to direct disturbance or environmental changes. This understanding is crucial not only to protect the corals but also to preserve the large number of associated species.

Among the 10 species of reef-building scleractinian CWC described (Freiwald and Roberts, 2005), *Lophelia pertusa* and *Madrepora oculata* are the most common and

occur frequently together in deep waters. Historically, *L. pertusa* was thought to dominate reef frameworks and, the more fragile *M. oculata* to form associated secondary reefs (Freiwald et al., 2002). However, their dominance pattern can alternate depending on geographical location (e.g., canyons of the Bay of Biscay and Mediterranean Sea; (Arnaud-Haond et al., 2015; Freiwald et al., 2009)). The key factors driving the respective abundance of these species could relies on distinct life strategies for *L. pertusa* and *M. oculata* (i.e., reproduction (Waller and Tyler, 2005)) or to different energy acquisition pathways as suggested by different growth patterns for individuals sharing the same *in situ* habitat (Lartaud et al., 2014).

Strong species-specific associations between coral and bacterial communities have been shown for tropical corals (Rohwer et al., 2002), which could be related to coral health (Reshef et al., 2006; Rosenberg et al., 2007). However, compared to tropical corals, research on CWC microbiomes is recent and still scarce. Recently, a symbiotic relationship was revealed between CWC and bacterial communities (Middelburg et al., 2015). Molecular survey have showed that living *L. pertusa* sampled at different locations harbored active bacterial communities (Yakimov et al., 2006) that were different from the surrounding sediments and water as well from dead coral branches (Neulinger et al., 2008; Schöttner et al., 2012, 2009; Yakimov et al., 2006). Similar results were reported for *M. oculata* from the North Atlantic Ocean (Hansson et al., 2009; Schöttner et al., 2012). However, the role of these microbes in the host metabolism and health is still unknown. Different bacteria likely have multiple roles in different communities and different parts of the corals. Bacteria observed on the tentacle ectoderm were different from the ones seen on the endoderm of the gastral cavity (Neulinger et al.,

2009). Skeleton and mucus bacteria communities appeared different (Schöttner et al., 2012, 2009) as well as those communities associated with mucus and polyps (Hansson et al., 2009). Interestingly, distinct bacterial communities were also observed between different color phenotypes of *L. pertusa* (Neulinger et al., 2008) or depending on the position of samples inside the reef (Schöttner et al., 2012). The large disparity in *L. pertusa* bacterial community composition across these studies and within colonies challenges the view of a specific bacteria-host association, even though the differences between *L. pertusa* and *M. oculata* bacteria (Hansson et al., 2009; Schöttner et al., 2012) suggest a species-specific association, similar to the one observed in tropical corals (Carlos et al., 2013). Because all the studies performed to date have relied on fingerprinting techniques (Schöttner et al., 2012, 2009) or on a limited number of cloned sequences (Hansson et al., 2009; Kellogg et al., 2009; Neulinger et al., 2008), the identity of potential coral-specific bacteria remains poorly resolved. In addition, *M. oculata*-associated bacteria have rarely been described in the Mediterranean Sea (Yakimov et al., 2006), where CWC grow at their upper temperature limit.

The main goal of this study was to test whether two different CWC species, *L. pertusa* and *M. oculata,* harbor species-specific bacterial communities while thriving in a single assemblage, and if this association was temporally variable. We reduced the possible sources of environmental variations by targeting colonies at a single location in the canyon Lacaze-Duthiers, a submarine canyon in the Gulf of Lion (northwestern Mediterranean Sea), characterized by extreme hydrodynamic events in winter transporting high fluxes of particulate material across the canyon (Canals et al., 2006). Each coral colony was identified by microsatellite genotyping and the bacterial

communities were described in detail by pyrosequencing the 16S rRNA gene. Likewise, we assessed the temporal variability of the coral-bacteria association from polyps tissue of colonies sampled during two different times of the year.

Materials and methods

Sampling and DNA extraction

Sampling was conducted using an underwater Remotely Operated Vehicle (ROV Super Achille) on the R/V Minibex Vessel (COMEX S.A.; Figure 1-A). The operations were conducted during two cruises to the Lacaze-Duthiers submarine canyon, off the coast of Banyuls sur Mer in the northwestern Mediterranean Sea (42°32.720 N; 03°25.260 W) in November 2010 and May 2011 (at ~520 m depth). During each cruise, healthy looking coral fragments were randomly collected from 4 colonies of *M. oculata* and 5 colonies of *L. pertusa* growing on a small area (80 x 30 m, Figure 1-B and C) located at the base of the western flank of the canyon. In particular, two colonies of *M. oculata* and *L. pertusa* growing together on the same substrate were sampled (M3 and L4). The colony fragments were collected sequentially with a net held by the gripper of the ROV and placed in separate polypropylene boxes, closed *in situ* to maintain the ambient bottom water temperature (~13°C) during the transport to the surface, and avoid cross contamination between samples as well as through the water column. On board, the CWC were dissected with sterilized instruments and gloves. The polyps corresponding to the basal and apical parts (separated by more than four polyps) of an intact fragment (Figure 1-D) were rapidly fixed separately in ethanol. An orange colony of *L. pertusa* was also

sampled during the second cruise to investigate the relationship between the colors of the polyps (classically white in this area) and the associated bacterial communities.

In the laboratory, the coral fragments were removed from ethanol, cut in two parts, and the polyp tissues were dissected under a binocular microscope. The DNA was extracted from the polyps with the CTAB method (Doyle and Doyle, 1987). Then, the DNA concentrations were measured by spectrophotometry (Nanodrop ND-1000, Thermo Fisher scientific Inc., MA, USA), and the quality was assessed by electrophoresis migration on a 1% agarose gel.

Microsatellite genotyping

The presence of clones in colony-replicated samples and the presence of different colonies within our samples was verified using 8 polymorphic microsatellite loci: c016, g028, g025, g016, C7 and C6 for *M. oculata* and D3, C44, A105, C126, C91, C61, C120, C142 and A5 for *L. pertusa* as previously described (Becheler et al., 2016). PCR was used to amplify the microsatellites loci, and the amplified products were diluted in formamide that contained the GENESCAN-350 (ROX; Applied Biosystem Ltd, Foster city, CA, USA) size standard. The size polymorphisms were screened using an ABI Prism 3130 DNA sequencer (Applied Biosystem). The DNA fragments were analyzed using the Genemapper software version 4.0 (Applied Biosystem). The clones present in the different samples were identified using Genclone 2.1 (Arnaud-Haond and Belkhir, 2007).

Sequencing of 16S rRNA bacterial genes

The V3 region of the bacterial 16S rRNA genes were amplified using the 27F AGRGTTTGATCMTGGCTCAG (Lane, 1995) and 519R GTNTTACNGCGGCKGCTG primers (Turner et al., 1999) with a single step and 28 cycles of polymerase chain reaction (PCR) using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA). The following PCR conditions were used: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. Following the PCR, all the amplicon products from the different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Pyrosequencing was performed with a Roche 454 FLX using commercially prepared Titanium reagents (Research and Testing Laboratory, Lubbock, TX).

Sequence data analyses

First, the quality of the sequences was controlled by removing all the reads that had a mismatch with the 16S rRNA primers, contained ambiguous nucleotides (N) or were < 300 bp long beyond the forward primer. A stringent quality trimming criteria was applied to remove reads that had ≥ 10% of bases with Phred values < 27. This procedure is recommended to ensure that when clustering at 97%, the influence of erroneous reads is minimized (Huse et al., 2010; Kunin et al., 2010). The sequences were then de-replicated and clustered at a 97% threshold using UCLUST (Edgar, 2010) for de novo OTU picking. The sequences from each operational taxonomic unit (OTU) were classified by comparing them with those from the Greengenes v.2011 database (DeSantis et al., 2006). The read quality filtering and length trimming, data set partitioning based on barcodes,

de-replication, clustering at 97% sequence identity and taxonomic classification were performed with PyroTagger (Kunin and Hugenholtz, 2010). Putatively chimeric sequences were identified as sequences having a best Blast alignment <90% of the trimmed read length to the reference database, >90% sequence identity to the best Blast match and OTU size ≤2.

To compare the bacterial communities in the diversity analysis, all the samples were randomly re-sampled to match the size of the sample containing the fewest sequences (n = 446). The Shannon diversity index (H',(Shannon, 1948)), the dominance index (D= 1-Simpson diversity index (Simpson, 1949)) and a cluster analysis were conducted using the PAST software on the re-sampled dataset (Hammer et al., 2001).

Phylogenetic tree, network analysis and statistics

A phylogenetic tree was constructed from the most relevant OTUs (> 5% of the sequences in all the dataset). All the sequences were deposited in the GenBank under Bioproject PRJNA296678 as well as by accession subnumber (SRP064006). The alignment and phylogenic analyses were performed with MEGA6 (Tamura et al., 2013) by including representative sequences from each OTU together with their best match from GenBank. The phylogenetic tree was based on a maximum likelihood calculation according to the generalized time-reversible model of nucleotide substitution with invariant sites and gamma distribution. The robustness of the inferred tree topology was evaluated by 1,000 bootstrap repetitions.

The associations between bacterial OTUs were characterized through MINE statistics by computing the maximal information coefficient (MIC) between each pair of

OTUs (Reshef et al., 2011). The MIC captures associations between data and provides a score that represents the strength of the relationship between data pairs. A matrix with MIC values > 0.3 was used with Cytoscape 3.1.1 to visualize the network of associations (Smoot et al., 2011). In these visualizations, the bacterial OTUs are represented as nodes connected by lines; the size of the nodes was proportional to the number of sequences, and the thickness of the lines was proportional to the MIC values. The force-directed layout was edge-weighted by the MIC values. The subnetworks of OTUs were defined with the HC-PIN hierarchical clustering algorithm (Wang et al., 2011). The OTUs were defined as species-specific when their representative sequences were found, at least, in all the duplicate samples of one species and not in the samples of the other species.

An analysis of similarity (ANOSIM) statistics was used to verify the significance of the dendrogram clustering by testing the hypothesis that bacterial communities from the same cluster were more similar in composition to each other than to communities in different clusters. A t-test was used to verify whether the differences in community diversity were significant, the dataset being parametric and the samples independent.

Results

Host microsatellite genotyping

The presence of genetically different colonies in our samples and the presence of clonal polyps within the basal and apical replicate samples of each branch were verified by microsatellite genotyping. In *M. oculata* and *L. pertusa,* the number of alleles per locus ranged from 2 to 6 (mean 3.8 ± 0.4 and 5.1 ± 0.2, respectively). All the sampled colonies were genetically different from each other on the basis of at least 7 microsatellite loci.

The basal and apical replicates corresponded to similar genotypes. The fragments of orange *L. pertusa* did not have a particular genetic signature compared to the white *L. pertusa* colonies.

Alpha diversity

A total of 60,188 bacterial 16S rRNA gene sequences remained from the coral samples after removing the poor quality reads, and a total of 1,110 OTUs were identified at a 97% sequence similarity cutoff. The Shannon diversity index (H') was significantly lower in *M. oculata* compared to *L. pertusa* (*t*-test, $p = 0.04$; Table 1). It varied from 0.0 to 1.4 in *M. oculata* (mean value of 0.8 ± 0.6) and from 0.4 to 2.7 in *L. pertusa* (mean 1.5 ± 1.0). The Simpson's D dominance index ranged from 0.4 to 1 for *M. oculata* (mean value 0.7 ± 0.2) and from 0.1 to 0.8 for *L. pertusa* (0.4 ± 0.3). The dominance index D was significantly higher in *M. oculata* compared to *L. pertusa* (*t*-test, $p = 0.03$).

Beta diversity

A comparison of the bacterial communities was conducted using the Bray-Curtis similarity index, and cluster analysis separated the coral samples into two main groups (Figure 2) that corresponded to the two host species. The bacterial community composition of *M. oculata* was significantly different from that of *L. pertusa* (ANOSIM, $p < 0.05$). Two *L. pertusa* communities (L2-A and B) were, however, separated from the other colonies of this species. For *M. oculata*, the similarity among bacterial communities was relatively high (> 60% similarity). The half of duplicate samples, i.e., the tissue within the apex and basal polyps of a single branch, grouped together for *M. oculata.* No

difference in community composition was detected between the two sampling dates (Figure 2; ANOSIM, $p > 0.05$). The clustering pattern for *L. pertusa* was different. Several communities were different from each other with similarity values as low as 4%. Although all the duplicate samples grouped together, we observed large differences in the community composition between polyps from the same branch and between different colonies (Figure 2). For *L. pertusa*, there was a specific temporal difference: all the samples from the spring 2011 grouped together and were separated from those from the autumn 2010 samples.

Bacterial community composition

All the *M. oculata* bacterial communities were dominated by OTUs belonging to the class *Gammaproteobacteria*, which represented 74-99% of the sequences (Figure 3). The singularity of the M1-A, M1-B and M3-B samples observed in the cluster analysis was due to a higher proportion of *Gammaproteobacteria* sequences in the bacterial community (96-99%). Other relevant OTUs belonged to *Bacteroidetes,* present mostly in sample M4 (9-16%), and *Alphaproteobacteria*, present in two *M. oculata* samples (12-16% of the sequences).

The dissimilarity observed among *L. pertusa* communities corresponded to the presence of different taxa in the different samples (Figure 3). The majority of the *Alphaproteobacteria* was observed in colony L1 (79 and 92% of the sequences in the basal and apical duplicates, respectively). The singularity of the L2 colony was illustrated by the presence of *Fusobacteria* and *Bacteroidetes* (26-42% and 31-37%, respectively). Among the *L. pertusa* corals, *Gammaproteobacteria* represented 6-78% of the sequences,

with higher proportions in colonies sampled during May 2011, including the orange phenotype. Less relevant bacterial groups, such as *Betaproteobacteria* and *Spirochaetes*, were found exclusively in *L. pertusa* colonies and represented 1 and 2% of the sequences, respectively.

Coral species-specific bacteria

An association network was constructed to identify the OTUs that were closely associated with each other. We identified three subnetworks with the HC-PIN hierarchical clustering algorithm (Figure 4). One network contained 9 closely associated OTUs only found in *M. oculata*. The other subnetwork contained a group of more closely related OTUs found in *L. pertusa*. A third group of associated OTUs contained those found in both coral species.

The main OTU from the *M. oculata* subnetwork (LDC-1; Figure 5) dominated all the *M. oculata* communities and represented 79 ± 17% of the sequences in the colonies (range from 55 to 99%). The second main OTU was LDC-3 and represented only 5 ± 6% of the sequences identified in *M. oculata*. These two species-specific OTUs belonged to the order *Gammaproteobacteria* and presented only 96% of similarity to *Endozoicomonas numazuensis* (GenBank accession NR11431), a facultative anaerobes that might ferment carbohydrates (Nishijima et al., 2013); Table S1 and Figure 6). The next relevant OTU, LDC-4, contained 4 ± 6% of the sequences obtained from *M. oculata* (Figure 5). The LDC-4 representative sequence had the highest similarity (93%) to an uncultured *Elizabethkingia* sp. (DQ917866) that belongs to the *Flavobacteriaceae* family (*Bacteriodetes*; Figure 6). Other bacteria specific to *M. oculata*, previously associated

with cnidarians (*i.e.,* LDC-2, 6, 8 and 13), were identified with lower sequence abundance (less than 2%) and belonged to the classes *Alphaproteobacteria* and *Epsilonproteobacteria*.

The second cluster was specific to *L. pertusa* samples and was composed of 15 weakly connected OTUs (Figure 4), which included the LDC-10 OTU found in all the *L. pertusa* samples. The sequence abundance of the LDC-10 OTU ranged from less than 1 to 83% and represented 31 ± 32% of all the *L. pertusa* sequences (Figure 5). This *L. pertusa*-specific OTU was classified as a *Gammaproteobacteria* with the highest homology (98%) to a black coral -associated bacteria clone (ctg_CGODA50,(Penn et al., 2006); Table S1, Figure 6). Other OTUs were detected only in *Lophelia* samples; however, they were not present in all the colonies. For example, the OTU LDC-9 represented 19±38% of the species sequences and belonged to *Alphaproteobacteria* (high homology, 97%) together with an uncultured bacterium (clone 6215-B73) identified in the gorgonian corals from the Aleutian Islands (Gray et al., 2011). However, the LDC-9 was detected with a high proportion only in one *L. pertusa* colony (between 79 to 92% of the total sample sequences of L1-A and L1-B, respectively). Interestingly, the OTU LDC-11 was only observed in the orange *Lophelia* colony (average of 23%). This OTU showed a high similarity (93%) with sequences previously associated with cnidarians identified as *Alphaproteobacteria* (clone P7-G09, (Bayer et al., 2013b)). Twelve other rare OTUs (< 2% abundance) were observed only in certain *L. pertusa* samples and together represented 15% of the total species-specific sequences.

Other relevant OTUs in CWC were shared between the two species. LDC-26 represented 8 ± 16% of the sequences in *L. pertusa* and 2 ± 4% in *M. oculata*. In addition,

it had the highest similarity (95%) to zebrafish digestive tract clone aab53e01 (*Cetobacterium somerae*, *Fusobacteria*), which has also previously been reported as associated with cnidarians. The LDC-18 represented 4 ± 8% of the *L. pertusa* sequences and 0 ± 1% of *M. oculata* and had a high similarity (90%) with an uncultured bacterium previously associated with deep seawater (*Bacteroidetes*).

Host associated bacteria

We tested whether the bacteria we amplified from coral polyps represented typical host-associated microbes or if they were similar to bacteria from other environments. We grouped the OTUs representing more than 5% of the sequences that had the best GenBank match (more than 82%) to sequences retrieved from a similar environment (associated to cnidarian, fishes found in the deep sea, in coastal shallow seawaters or from terrestrial origin, Figure 5). For *M. oculata*, 97% of the bacterial sequences were similar to those previously associated to cnidarians, while that was the case for only 67% of the *L. pertusa* sequences. The OTUs associated with deep sea environments (hydrothermal vents and surface sediments) represented less than 1% of the sequences in *M. oculata* and 3% in *L. pertusa*. In addition, *L. pertusa* had 14% of bacterial sequences that matched OTUs associated with samples from shallow marine environments, compared to 1% for *M. oculata*. Among the remaining sequences, 9 and 2% presented high homology with microbes associated with fishes in *L. pertusa* and *M. oculata*, respectively. Finally, 3% of sequences homologous to lineages from terrestrial samples were identified only in *L. pertusa* (Figure 5).

Finally, the 14 non species-specific OTUs matched OTUs previously found to be associated with samples from marine environments and fishes.

Phylogenetic analyses

No specific phylogenetic signal was observed between the bacterial communities of *M. oculata* and *L. pertusa* (Figure 6). However, among the *Gammaproteobacteria*, the species-specific OTUs were grouped according to the CWC species they originated from. Ten *L. pertusa* OTUs clustered together with LDC-10 and were separated from the main OTUs identified in *M. oculata* (LDC-1 and LDC-3). These *M. oculata Endozoicomonas* phylotypes were 95% similar to published sequences and grouped together in the phylogenetic tree (Figure 6). In contrast, the *Alphaproteobacteria* OTUs from both species clustered together in the phylogenetic tree.

Discussion

This first temporal characterization of bacterial communities associated with both *L. pertusa* and *M. oculata* in the Mediterranean Sea challenges previous results that showed intra- (Hansson et al., 2009) and inter- (Hansson et al., 2009; Schöttner et al., 2012) colony versatility of bacterial communities in *M. oculata versus* the spatial stability of *L. pertusa* (Schöttner et al., 2012). Indeed, our results show a low diversity in the temporally stable and species-specific communities associated with *M. oculata.* Instead, those bacterial communities associated with *L. pertusa* were more diverse and variable in space and time as well as possibly more susceptible to environmental variations. Our data support, however, previous findings based on DGGE (Hansson et al., 2009) and ARISA

(Schöttner et al., 2012) on the Northern Atlantic reefs that showed species-specific communities for *M. oculata*.

Our study on the corals from the northwestern Mediterranean deep-sea shows that *L. pertusa* and *M. oculata* collected at the same time at the same location had different associated bacteria, as observed for other CWCs (*e.g*. *Anthothela* sp. (Lawler et al., 2016)). The difference was observed in community composition, diversity of the community and specificity of the association. Overall, our results indicate that CWC bacterial communities are first structured by the host species and, thus, demonstrate that the earlier findings based on DGGE fingerprinting from the North Atlantic Ocean (Hansson et al., 2009) and based on ARISA from the Norwegian Sea (Schöttner et al., 2012) extend to the Mediterranean Sea. In *M. oculata*, however, our results showed that species-specific bacterial associations with colonies and polyps remained stable throughout the seasons, which contradicts the few existing data on *M. oculata* that suggest a within colony variability (Hansson et al., 2009; Schöttner et al., 2012). The stable community we report here might represent true host-associated bacteria because we directly targeted the bacteria from the tissue within the polyp, whereas earlier studies that amplified bacterial sequences from the mucus or from scraping branches could have detected opportunistic bacteria growing on the coral. In contrast, the bacterial communities associated with *L. pertusa* varied within the same sampling area, between polyps from the same colony and between seasons. This result also differs from earlier findings that showed that *L. pertusa* bacteria communities were more stable than those from *M. oculata* (Schöttner et al., 2012). However, our large amount of sequencing data

support the spatial variations reported earlier in *L. pertusa* (Hansson et al., 2009; Kellogg et al., 2009; Neulinger et al., 2008; Schöttner et al., 2009).

Our observation of the Lacaze-Duthiers submarine canyon probably reflects an essential difference in the microbe-host association strategy of both species. The high variability in the bacteria assemblages of *L. pertusa* compared with the higher specificity of *M. oculata* could reflect different ecological niches occupied by two species sharing the same habitat. This strategy was suggested for colonies from the European margins (Arnaud-Haond et al., 2015). Schöttner *et al*. attributed the variations in the bacterial community composition to both stochastic events and deterministic processes (Schöttner et al., 2012). The former corresponds to the random attachment of environmental bacteria to coral surfaces (Ritchie, 2006), and the latter reflects a selective regulation by the coral host (Rohwer and Kelley, 2004) and bacterial interactions with coral tissues (Rypien et al., 2010). For *L. pertusa*, we suggest that the lack of bacterial community stability in all the spatial scales, from intra to inter colony, might be because the bacterial communities detected in the polyps represent the microbiome of the coral gastrovascular cavity. We can, thus, hypothesize that the composition of the microbiome reflects the diet of the host as observed for other gut microbiomes (David et al., 2014; Miyake et al., 2015). In the hypothesis of a predominantly diet-shaped bacterial community, the stable *M. oculata* bacterial community might, then, indicate a narrow diet based on specific prey selected by the polyps. Conversely, the variable *L. pertusa* bacteria might reflect an opportunistic coral species that capture different prey depending on the location of the colony and the time of the year. Although both species rely predominantly on a zooplankton-based diet (Kiriakoulakis et al., 2005; Naumann et al., 2015), the enrichment in mono-unsaturated

fatty acids and $\delta^{15}N$ of *L. pertusa* tissues relative to *M. oculata* suggests possible different feeding strategies, different assimilation/storage efficiencies or different metabolisms between the two coral species (Kiriakoulakis et al., 2005).

In addition, *in situ* growth patterns were shown to differ between the two coral species on a seasonal basis (Lartaud et al., 2014). The winter dense water-cascading regime that characterize the northwestern Mediterranean canyons (Canals et al., 2006) was hypothesized as a potential cause of these changes that affect the growth of *M. oculata* but not that of *L. pertusa* (Lartaud et al., 2014). The differences in their specific microbiota further support the idea of *L. pertusa* as a more opportunistic species that is able to adapt its nutritional strategy. Instead, *M. oculata* growth would be perturbed if its specific prey disappears. Similarly, the thermal tolerance of *L. pertusa* is higher than that of *M. oculata,* which is highlighted by the decrease in respiration and calcification rate when *M. oculata* colonies change from 13 to 9 or 6°C (Naumann et al., 2014).

Alternatively, the bacteria we detected could also represent communities associated with the coral mucus. The mucus of *L. pertusa* and *M. oculata* have different C:N ratios and carbohydrate compositions (Wild et al., 2010, 2008), and *M. oculata* has an enormous potential for mucus production (Reitner, 2005). This could also lead to differences in the host-bacteria association because the coral mucus plays a significant role in the structuring of beneficial coral-associated microbia (Ritchie, 2006). However, this difference is insufficient to explain the variability of *L. pertusa* communities between two polyps of the same branch, separated by some centimeters.

Bacterial communities associated with *M. oculata* appeared to be more structured by the host species rather than by the temporal or spatial variability of the environmental

conditions, which is similar to the behavior of microbes associated with tropical corals (Knowlton and Rohwer, 2003). The *M. oculata* bacterial communities were dominated by two *Endozoicomonas* phylotypes that have been named *Spongiobacter* in earlier CWC studies (Hansson et al., 2009). We propose the use of *Endozoicomonas* because the previously mentioned *Spongiobacter* is based on its similarity to a GenBank sequence (AB205011) from a strain that has never been published. Conversely, the isolated *Endozoicomonas* strain and the genus itself were recently described (Neave et al., 2014; Nishijima et al., 2013; Pike et al., 2013). *Endozoicomonas* are aerobic or facultatively anaerobic bacteria that can perform carbohydrate fermentation (Neave et al., 2014; Nishijima et al., 2013; Pike et al., 2013) but their role in the host in still unknown.

Our sequences had only 95% sequence identity to the closest cultivated strain, *Endozoicomonas numazuensis*. Different phylotypes that belong to the genus *Endozoicomonas* were previously obtained from several cnidarian taxa, such as tropical coral species (Agostini et al., 2011; Ainsworth et al., 2015; Littman et al., 2009) and gorgonian (*Eunicella cavolini, E. verrucosa, Plexaura sp.* and *E. fusca*; (Bayer et al., 2013a; Pike et al., 2013; Ransome et al., 2014) but also from sponges in the Mediterranean Sea (Thiel et al., 2007). As suggested for other CWC(Hansson et al., 2009; Neulinger et al., 2008), we can assume a general class of coral-associated bacteria shared by both shallow- and deep-water corals, with ecological niches filled by different bacterial phylotypes in different host species. However, this close association between *Endozoicomonas* and coral or gorgonian could depend on the corals' health status (Bourne et al., 2008; Ransome et al., 2014). For example, healthy corals of *Acropora millepora* harbor high abundance of *Endozoicomonas* (*Spongiobacter*; more than 41%)

compared to bleached corals (less than 3%). This last result could also explain the variations in the dominance of these bacteria types observed in *Acropora sp*. as a function of the sampling sites (Littman et al., 2009). During our sampling, only healthy looking colonies of *M. oculata* were targeted. We can speculate that the dominance of this genus in *M. oculata* samples reveals the healthy status of the coral host.

In contrast, the bacterial communities associated with *L. pertusa* appeared more structured by variable spatial or temporal environmental factors as illustrated by the inter-samples, inter-season and inter-habitat differences. However, we identified one species-specific microbe that was always associated with *L. pertusa* polyps, i.e., LCD 10 [98% similarity to black coral clone ctg CGODA50 (Penn et al., 2006)]. It was present in different polyps, colonies, seasons, and colors but with a strong variation in abundance. This microbe has been reported to be associated with black coral in the seamounts of the Gulf of Alaska (Penn et al., 2006).

Interestingly, the bacterial communities observed in *L. pertusa* and *M. oculata* sampled in the Mediterranean Sea (at ca. 13°C) were not present in the colonies sampled at ca. 5°C in the NE Atlantic Ocean [e.g., Norwegian shelf (Wild et al., 2008), Ireland (Hansson et al., 2009; van Bleijswijk et al., 2015)] nor associated with the same species sampled at ca. 8°C in the northern Gulf of Mexico (Galkiewicz et al., 2011; Kellogg et al., 2009). Conversely, the most abundant clones previously associated with CWC were not detected in this study, except for bacteria of the genus *Endozoicomonas* (van Bleijswijk et al., 2015). For example, *L. pertusa*-specific mycoplasma-like bacteria, i.e., *Mycoplasma* spp. (Kellogg et al., 2009; Neulinger et al., 2008; van Bleijswijk et al., 2015) identified through culture-independent methods and other bacteria such as

Pseudoalteromonas spp., *Photobacterium* spp., uncultured *Achromobacter* sp. identified through culture-dependent methods (Galkiewicz et al., 2011) were not found in our sampling. None of the *Endozoicomonas* identified in *M. oculata* from our Mediterranean samples were previously identified associated with this species in the Atlantic Ocean (95% similarity to clone ME19, DQ917863 (Hansson et al., 2009)). However, *Endozoicomonas* sp. was previously identified in low proportion in mucus of *L. pertusa* sampled at ca. 8°C in the Logachev Mound province in NE Atlantic waters (van Bleijswijk et al., 2015). Accordingly, the coral associated bacterial assemblages could differ between the NE Atlantic Ocean and the Mediterranean Sea because they might have different temperature (and/or salinity) range tolerances. Other bacteria were identified after culturing them at 4°C (Galkiewicz et al., 2011), which lead to a methodological bias when compared to bacteria studied using molecular tools, explaining their absence in other studies (Kellogg et al., 2009; Neulinger et al., 2008). This specificity of the bacterial assemblages of the CWC from the Gulf of Lion could be related to local environmental conditions or host genetic specificity and requires further investigation throughout the Atlantic Ocean and the Mediterranean Sea.

Finally, we provide the first in-depth investigation, based on a mass characterization of rRNA 16S lineages, on the species-specific bacterial communities harbored by Mediterranean CWC in different seasons. Specific bacterial associations were identified for the first time, with a high spatial and temporal community stability harbored by *M. oculata,* whereas the microbial pattern of *L. pertusa* appeared more versatile and probably to be more influenced by environmental conditions. This result partially

contradicts previous findings based on classical methods and suggests different adaptive strategies of two species that share the same habitat. Interestingly, the dominance of the genus *Endozoicomonas* in *M. oculata* could also reflect a potential symbiosis between this genus and CWC, as observed in other cnidarians.

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Author contribution

A.L.M., F.L. and P.E.G. designed research; A.L.M., D.K., M.B. and S.A.H. performed research; A.L.M., D.K. and P.E.G. analyzed data; and A.L.M., D.K., F.L., S.A.H., N.L.B., and P.E.G. wrote the paper.

Additional Information

The authors declare no conflict of interest.

Data deposition: The data reported in this paper have been deposited in the NCBI database (accession no. SRP064006).

Figure legends

Figure 1. (A) Geographical location of the sampling site. The bathymetry was prepared using the GeoMapApp free software (www.geomapapp.org). (B) Coral colonies in the Lacaze Duthiers canyon and the arm of the ROV close to the sample (© UPMC LECOB / Chaire Fondation TOTAL). (C) Distribution of the colonies collected of the two species in the Lacaze Duthiers Canyon and (D) Sample of one colony with basal and apical polyps (© F. Lartaud, LECOB).

Figure 2. Dendrogram based on the Bray-Curtis diversity index showing the similarity between the bacterial community composition of *Madrepora oculata* and *Lophelia pertusa* in duplicates (apex and basal polyps in A and B, respectively) sampled in November 2010 (bold) and May-2011. The orange *L. pertusa* colony is indicated by "o".

Figure 3. Bacterial community composition of *Madrepora oculata* (M) and *Lophelia pertusa* (L) sampled in November-2010 and May-2011. The composition is based on average values >5% over all the duplicates of all the CWC. The orange *L. pertusa* colony was indicated by "o".

Figure 4. Association network of the cold-water scleractinian corals bacterial communities in which nodes correspond to the OTUs and edges correspond to the relationships calculated with the MINE statistics. The node size is proportional to the number of sequences contained in an OTU and the edge thickness to the weight of the relationship. Previous identifications were indicated by color [associated

with cnidarians (grey), in deep (blue) and shallow (light blue) marine environments, associated with fishes (red) and in terrestrial environment (brown)].

Figure 5. Frequencies of the bacterial OTUs in *Madrepora oculata* and *Lophelia pertusa* individuals in November-2010 and May-2011. The OTUs were classified by their previous identification [associated with cnidarians (light gray), in deep (dark grey) and shallow (white) marine environments, associated with fishes (squared) and in terrestrial environments (black circle)]. The node size is proportional to the number of sequences contained in an OTU. The orange *L. pertusa* colony was indicated by "o".

Figure 6. Maximum likelihood tree representing the relevant OTUs and their best matching sequences specific to *Madrepora oculata* (M), *Lophelia pertusa* (L) and unspecific sequences (LM). Bootstrap values higher than 50% and the EMBL accession number are specified. High microbial abundance sequences are in red.

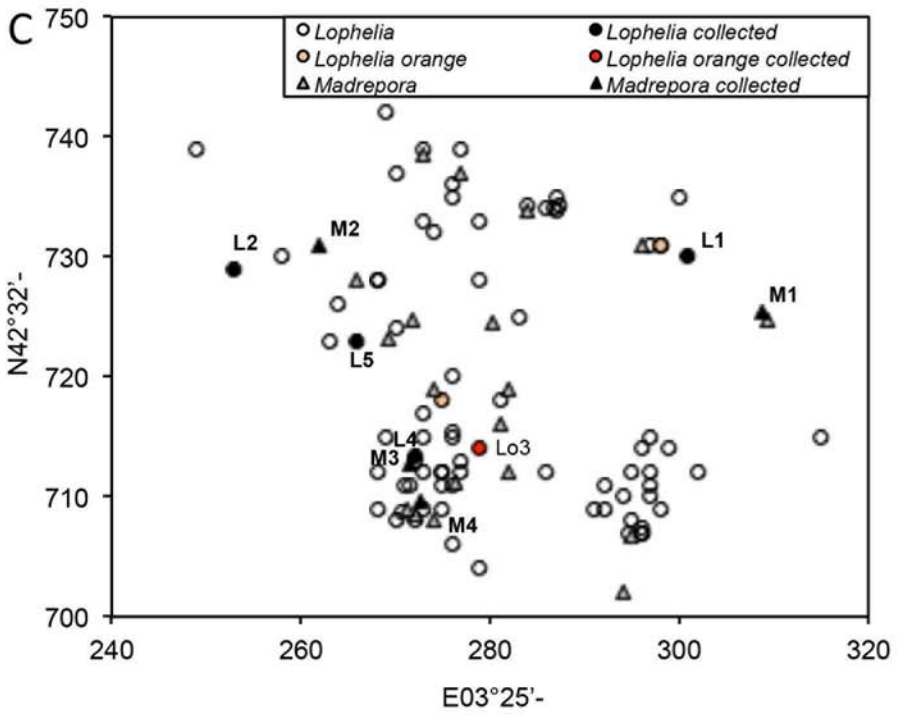
Table legend

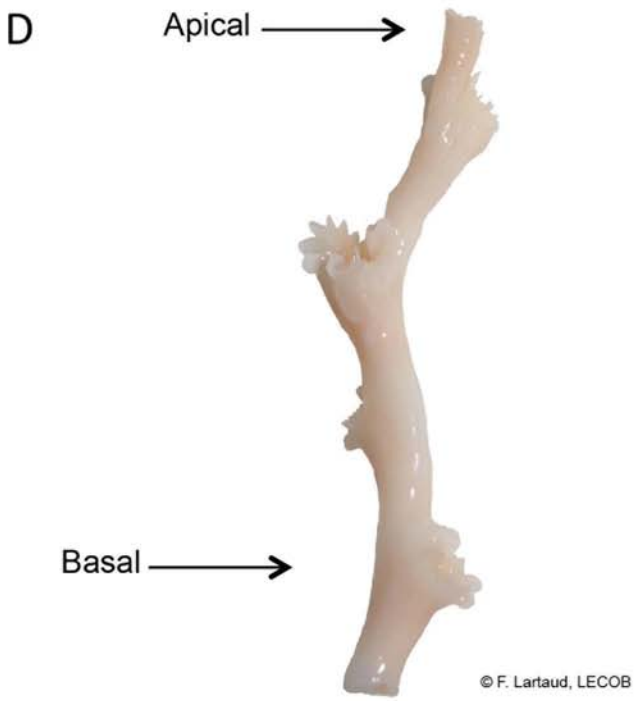
Table 1. Shannon (H') and dominance (D) indices and proportion of species-specific bacterial sequences and OTUs for each sample. Indices, proportion and OTUs numbers were calculated for each duplicate. Orange *Lophelia pertusa* was indicated y "o".

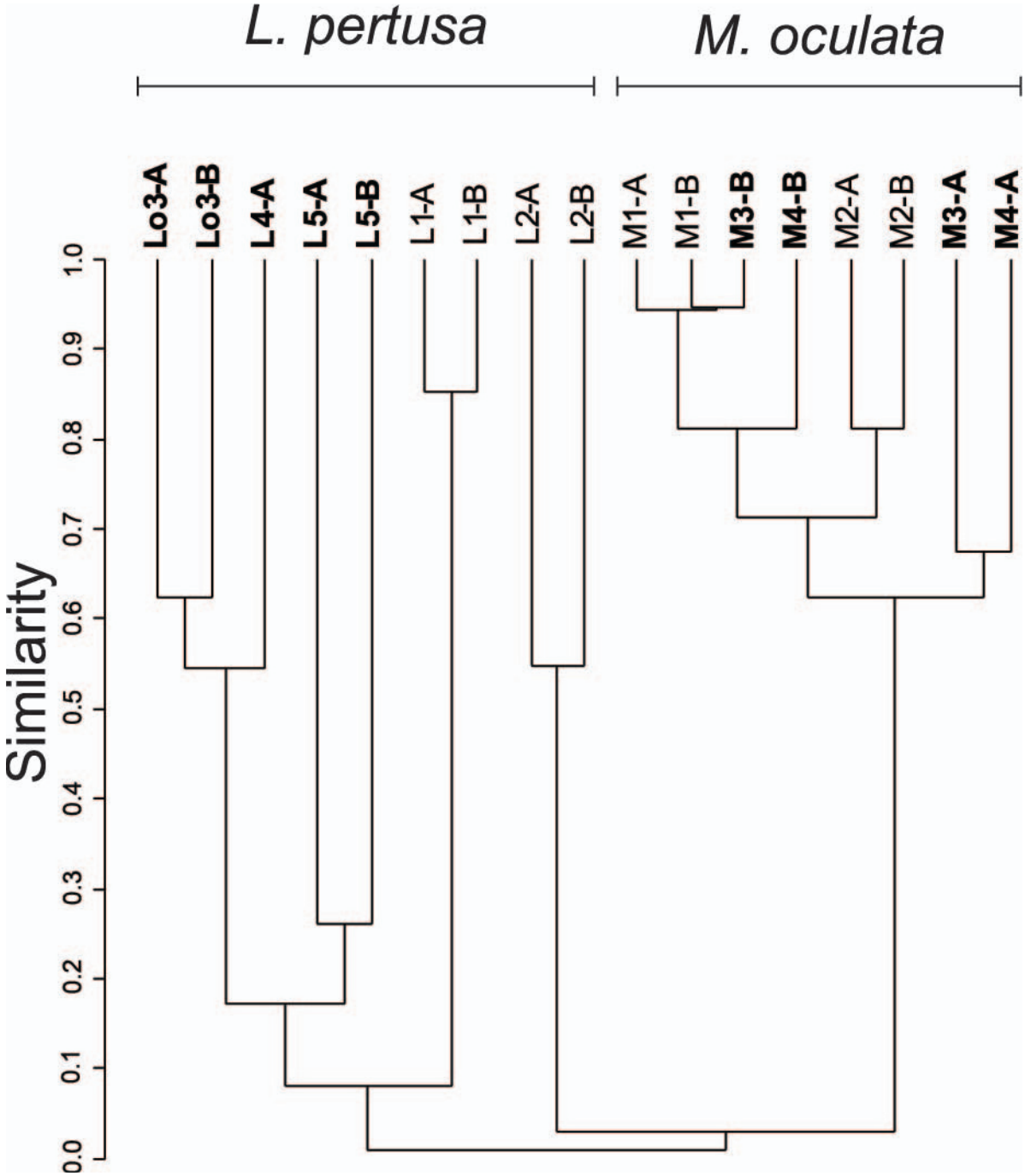
Species	Sampling date	Site	Sample	Sequences total	Species-specific OTUs	Species-specific sequences	OTUs total	H'	D
M. oculata	Nov-10	AZC3	M1-A	1899	1 (11.1)	1 (0.1)	2	0.0	1.0
			M1-B	2488	3 (33.3)	37 (1.5)	8	0.3	0.9
		A	M2-A	2907	4 (44.4)	599 (20.6)	15	1.1	0.5
			M2-B	2639	5 (55.6)	337 (12.8)	18	1.3	0.5
	May-11		M3-A	2572	4 (44.4)	661 (25.7)	8	1.3	0.4
			M3-B	5746	4 (44.4)	99 (1.7)	5	0.2	0.9
			M4-A	5773	7 (77.8)	2257 (39.1)	10	1.4	0.4
			M4-B	13500	6 (66.7)	1706 (12.6)	10	0.6	0.7
L. pertusa	Nov-10	AZC3	L1-A	4510	5 (11.1)	486 (10.8)	8	0.8	0.6
			L1-B	2821	1 (2.2)	30 (1.1)	4	0.4	0.8
		A	L2-A	992	8 (17.8)	124 (12.5)	24	2.6	0.1
			L2-B	5277	6 (13.3)	243 (4.6)	19	2.1	0.2
	May-11		Lo3-A	1222	4 (8.9)	257 (21.0)	7	0.6	0.6
			Lo3-B	2445	8 (17.8)	897 (36.7)	10	1.1	0.4
			L4-A	446	4 (8.9)	78 (17.5)	5	0.6	0.7
			L5-A	1440	12 (26.7)	526 (36.5)	20	2.5	0.1
			L5-B	3511	19 (42.2)	1587 (45.2)	28	2.7	0.1

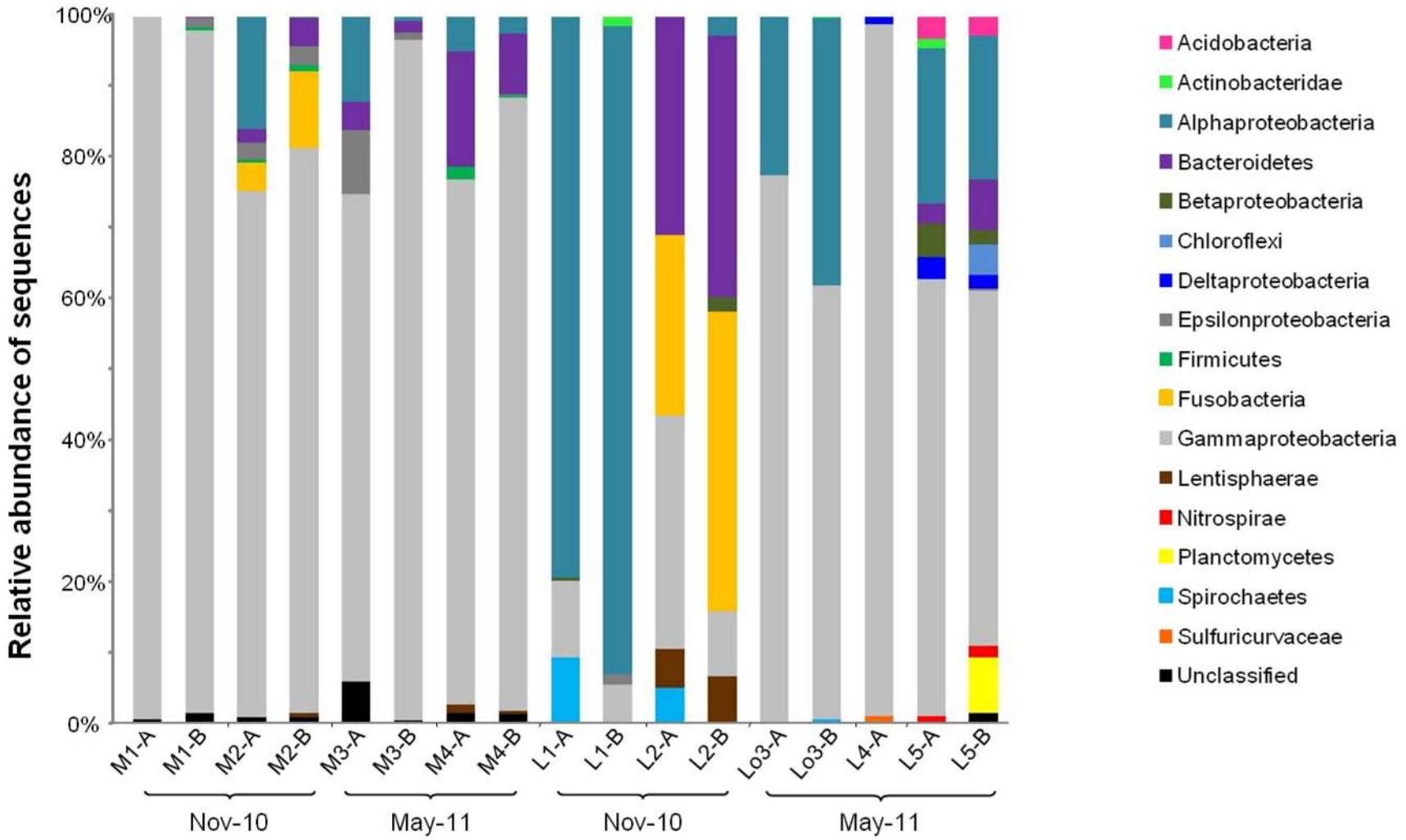
Highlights :

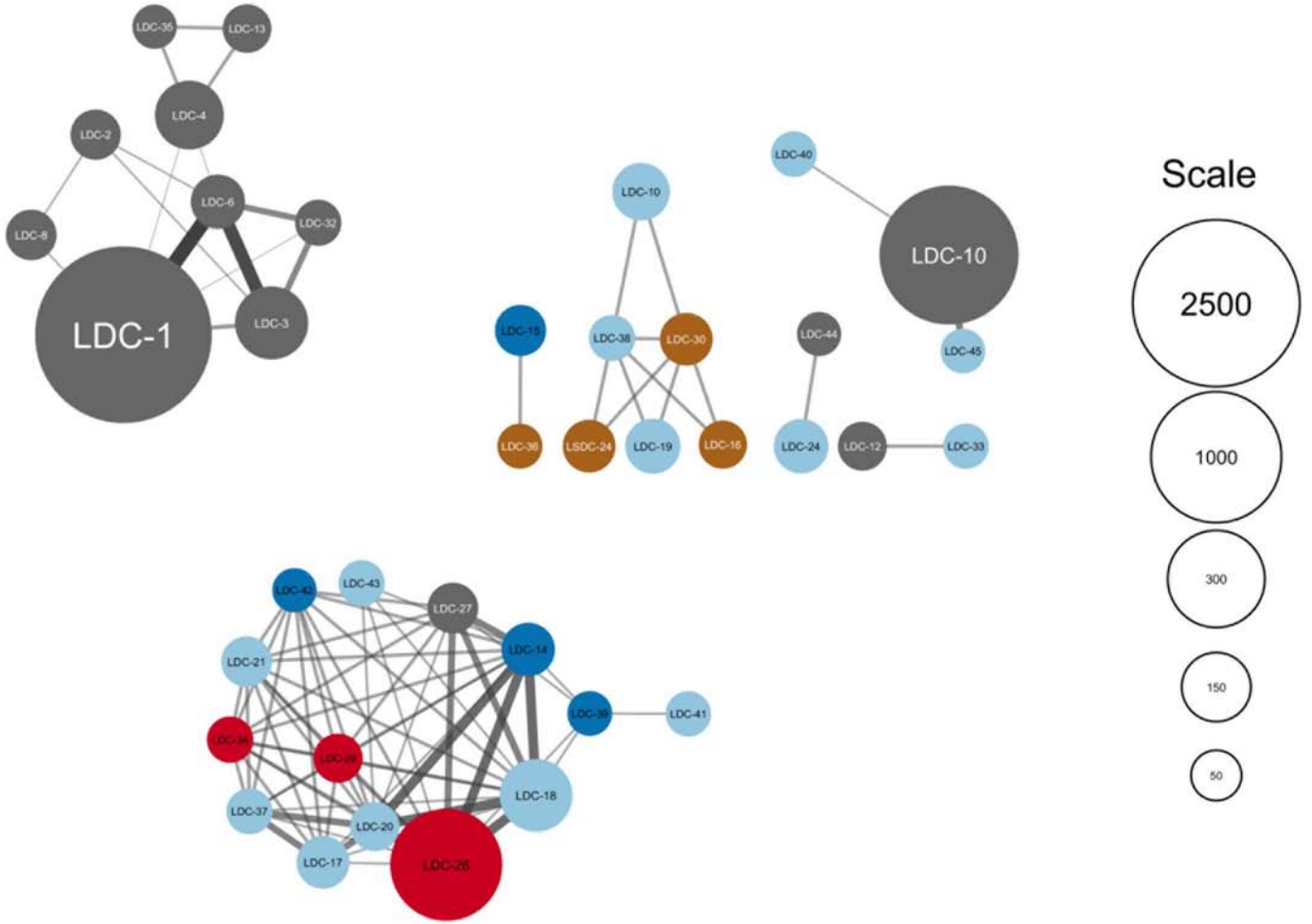
-We examine the bacterial communities of two reef-building cold-water coral species from Mediterranean Sea
- The 16S rRNA sequencing revealed host-specific patterns of bacterial communities both in terms of community composition and diversity
- Our results highlight distinct host/microbes association strategies for these two closely related Scleractinians sharing the same habitat, suggesting distinct sensitivity to environmental change.

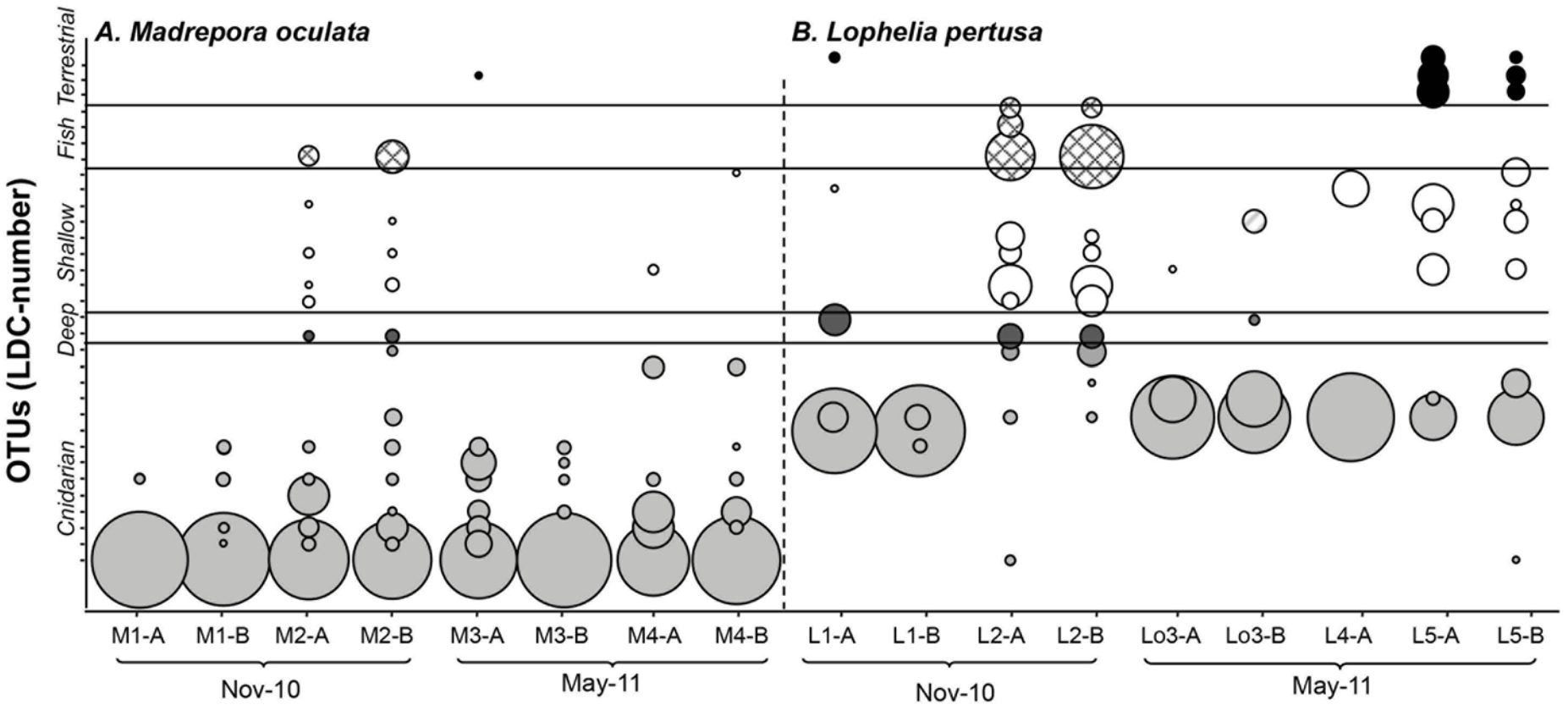












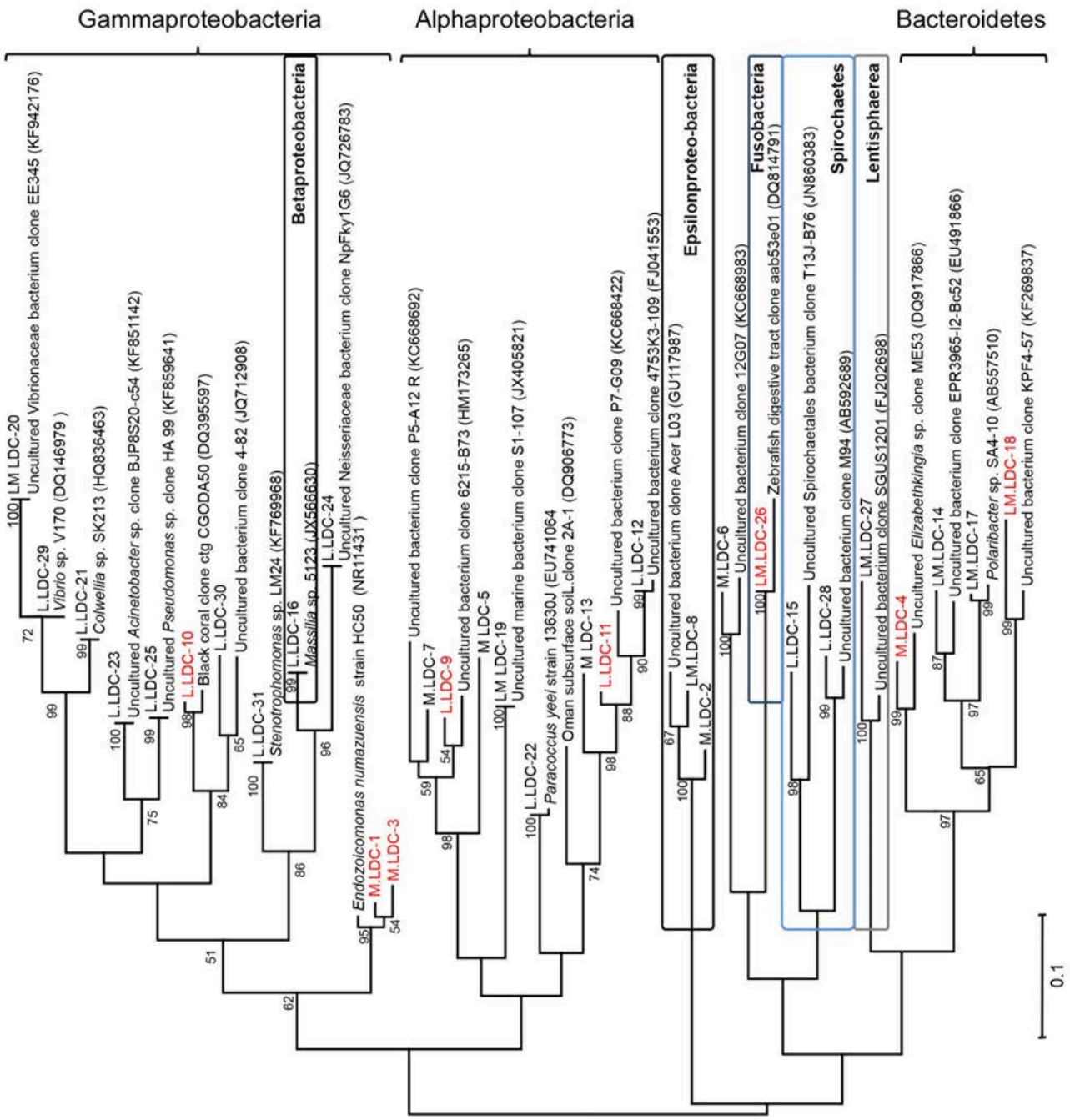


Table S1. Nearest relatives with their previous association for each of the OTUs presented at proportion higher than 5% in all the dataset, specific to *Madrepora ocululata* (M), to *Lophelia pertusa* (L) or present in the both CWC (LM).

OTU n°	CWC Host	Nearest relative, Genbank accession number	Homology (%)	Taxa	Associated to
L_LDC_9	L	Uncultured bacterium clone 6215-B73, HM173265	96	Alphaproteobacteria	Cnidarian
L_LDC_11	L	Uncultured bacterium clone P7-G09, KC668422	93	Alphaproteobacteria	Cnidarian
M_LDC_5	M	Uncultured bacterium clone P5-A12_R, KC668692	83	Alphaproteobacteria	Cnidarian
M_LDC_7	M	Uncultured bacterium clone 6215-B73, HM173265	97	Alphaproteobacteria	Cnidarian
M_LDC_13	M	Uncultured bacterium clone P7-G09, KC668422	87	Alphaproteobacteria	Cnidarian
L_LDC_22	L	*Paracoccus yeei* strain 13630J, EU741064	100	Alphaproteobacteria	Shallow marine
L_LDC_12	L	Uncultured bacterium clone 4753K3-109, FJ041553	99	Alphaproteobacteria	Cnidarian
LM_LDC_19	LM	Uncultured marine bacterium clone S1-107, JX405821	100	Alphaproteobacteria	Shallow marine
M_LDC_4	M	Uncultured *Elizabethkingia* sp. clone ME53, DQ917866	93	Bacteroidetes	Cnidarian
LM_LDC_18	LM	Uncultured bacterium clone KPF4-57, KF269837	90	Bacteroidetes	Shallow marine
LM_LDC_17	LM	*Polaribacter* sp.SA4-10 gene, AB557510	98	Bacteroidetes	Shallow marine
LM_LDC_14	LM	Uncultured bacterium clone EPR3965-I2-Bc52, EU491866	93	Bacteroidetes	Deep marine
L_LDC_16	L	*Massilia* sp. 5123, JX566630	100	Betaproteobacteria	Terrestrian
LM_LDC_8	LM	Uncultured bacterium clone Acer_L03, GU117987	94	Epsilonproteobacteria	Cnidarian
M_LDC_2	M	Uncultured bacterium clone Acer_L03, GU117987	91	Epsilonproteobacteria	Cnidarian
LM_LDC_26	LM	Zebrafish digestive tract clone aab53e01, DQ814791	97	Fusobacteria	Fish
M_LDC_1	M	*Endozoicomonas numazuensis strain HC50,* NR11431	96	Gammaproteobacteria	Cnidarian
L_LDC_10	L	Black coral clone ctg_CGODA50, DQ395597	98	Gammaproteobacteria	Cnidarian
M_LDC_3	M	*Endozoicomonas numazuensis strain HC50,* NR11431	95	Gammaproteobacteria	Cnidarian
L_LDC_25	L	Uncultured *Pseudomonas* sp. clone HA_99 , KF859641	100	Gammaproteobacteria	Shallow marine
L_LDC_31	L	*Stenotrophomonas* sp. LM24, KF769968	100	Gammaproteobacteria	Terrestrian
L_LDC_30	L	Uncultured bacterium clone 4-82, JQ712908	94	Gammaproteobacteria	Terrestrian
L_LDC_29	L	*Vibrio* sp. V170, DQ146979	100	Gammaproteobacteria	Fish
L_LDC_23	L	Uncultured *Acinetobacter* sp. clone BJP8S20-c54, KF851142	100	Gammaproteobacteria	Shallow marine
L_LDC_24	L	Uncultured Neisseriaceae bacterium clone NpFky1G6, JQ726783	100	Gammaproteobacteria	Shallow marine
LM_LDC_20	LM	Uncultured Vibrionaceae bacterium clone EE345, KF942176	100	Gammaproteobacteria	Shallow marine
L_LDC_21	L	*Colwellia* sp. SK213, HQ836463	100	Gammaproteobacteria	Shallow marine
LM_LDC_27	LM	Uncultured bacterium clone SGUS1201, FJ202698	100	Lentisphaerae	Cnidarian
L_LDC_15	L	Uncultured Spirochaetales bacterium clone T13J-B76, JN860383	83	Spirochaetes	Deep marine
L_LDC_28	L	Uncultured bacterium clone M94, AB592689	95	Spirochaetes	Fish
M_LDC_6	M	Uncultured bacterium clone 12G07, KC668983	93	Unclassified	Cnidarian