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FEATURE ARTICLE

Unexpected diversity of *Endozoicomonas* in deep-sea corals

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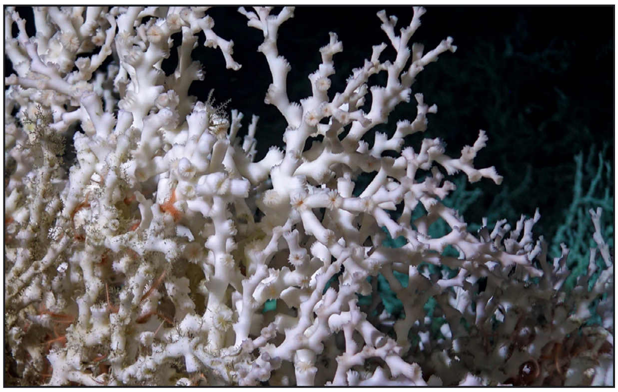
ABSTRACT: The deep ocean hosts a large diversity of azooxanthellate cold-water corals whose associated microbiomes remain to be described. While the bacterial genus *Endozoicomonas* has been widely identified as a dominant associate of tropical and temperate corals, it has rarely been detected in deep-sea corals. Determining microbial baselines for these cold-water corals is a critical first step to understanding the ecosystem services their microbiomes contribute, while providing a benchmark against which to measure responses to environmental change or anthropogenic effects. Samples of *Acanthogorgia aspera*, *A. spissa*, *Desmophyllum dianthus*, and *D. pertusum* (*Lophelia pertusa*) were collected from western Atlantic sites off the US east coast and from the northeastern Gulf of Mexico. Microbiomes were characterized by 16S rRNA gene amplicon surveys. Although *D. dianthus* and *D. pertusum* have recently been combined into a single genus due to their genetic similarity, their microbiomes were significantly different. The *Acanthogorgia* spp. were collected from submarine canyons in different regions, but their microbiomes were extremely similar and dominated by *Endozoicomonas*. This is the first report of coral microbiomes dominated by *Endozoicomonas* occurring below 1000 m, at temperatures near 4°C. *D. pertusum* from 2 Atlantic sites were also dominated by distinct *Endozoicomonas*, unlike *D. pertusum* from other sites described in previous studies, including the Gulf of Mexico, the Mediterranean Sea and a Norwegian fjord.

KEY WORDS: Coral · Deep-sea coral · Microbiome · Bacteria · *Lophelia pertusa* · Biodiversity



Microbiomes of deep-sea coral *Desmophyllum pertusum* (*Lophelia pertusa*) at Richardson Ridge are dominated by *Endozoicomonas*.

Photo: Erik Cordes, Chief Scientist of the DEEP SEARCH program, BOEM, USGS, NOAA OER. © Woods Hole Oceanographic Institution

1. INTRODUCTION

There are more species of corals in the deep ocean than there are in shallow waters (Roberts & Hirshfield 2004, Roberts et al. 2009). This incredible diversity of cold-water corals, including both calcifying scleractinians and soft octocorals, serves a fundamental role in creating 3-dimensional structure in the deep sea (Roberts et al. 2006). This structure provides the foundation for biodiversity hot spots that support a large variety of invertebrates as well as economically important fish species (Buhl-Mortensen & Mortensen 2004, 2005, Stone 2006, Cordes et al. 2008). However, the technical difficulty and expense

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of accessing this environment means that relatively little is known about these cold-water coral ecosystems and the basic biology of their keystone species, including age, growth rates, reproduction, and microbiome compositions.

Cold-water corals are azooxanthellate (Stanley & Cairns 1988) and therefore their prokaryotic symbionts are hypothesized to play an even larger role in nutrient cycling and waste management than prokaryotic symbionts do in the nutrition of zooxanthellate corals (Neulinger et al. 2008, Kellogg 2019). The best microbially characterized cold-water coral is *Desmophyllum pertusum* (*Lophelia pertusa*), a complex mound-building species that is particularly abundant in the North Atlantic (Yakimov et al. 2006, Neulinger et al. 2008, 2009, Hansson et al. 2009, Kellogg et al. 2009, 2017, Schöttner et al. 2009, Galkiewicz et al. 2011, 2012, Weinbauer et al. 2012, van Bleijswijk et al. 2015, Meistertzheim et al. 2016, Chapron et al. 2020, Galand et al. 2020). The bacterial communities of a handful of other deep-sea scleractinian corals have also been characterized: *Dendrophyllia* sp. (Röthig et al. 2017b), *Eguchipsammia fistula* (Röthig et al. 2017a,b), *Madrepora oculata* (Hansson et al. 2009, Meistertzheim et al. 2016), and *Rhizotrochus typus* (Röthig et al. 2017b). More recently, baseline descriptions of the bacterial communities of a number of deep-sea octocorals have been published (Gray et al. 2011, Kellogg et al. 2016, Lawler et al. 2016, Goldsmith et al. 2018, Weiler et al. 2018).

While there have been increasing numbers of reports describing *Endozoicomonas*-dominated microbiomes in tropical scleractinian corals (Morrow et al. 2012, Speck & Donachie 2012, Bayer et al. 2013b, Roder et al. 2015, Apprill et al. 2016, Gignoux-Wolfsohn et al. 2017, Brener-Raffalli et al. 2018, Pogoreutz et al. 2018, Camp et al. 2020) as well as tropical and temperate octocorals (Bayer et al. 2013a, Vezzulli et al. 2013, van de Water et al. 2017, McCauley et al. 2020, Reigel et al. 2020), this bacterial genus has been notably scarce in deep-sea cold-water corals. An analysis that included 6 deep-sea octocoral species found *Endozoicomonas* to be rare in *Anthothela* spp. and undetectable in the other species (Kellogg 2019). An exception is *M. oculata*, whose microbiome is dominated by *Endozoicomonas* in both the Mediterranean and the Atlantic (Rockall Bank), although not by the same phylotypes (Hansson et al. 2009, Meistertzheim et al. 2016, Galand et al. 2018). These collections occurred over a depth range of 520 to 781 m, with Atlantic site temperatures of 7.6–9.0°C and Mediterranean site temperatures of ~13°C. Further, the 3 studies that identified *Endozoicomonas* as dominating the *M. oculata* microbiome also examined *D. pertusum* collected from the same sites at the same time via the same methods and found few or no *Endozoicomonas* (Hansson et al. 2009, Meistertzheim et al. 2016, Galand et al. 2018). Prior studies of *D. pertusum* from the Mediterranean (Yakimov et al. 2006), a Norwegian fjord (Neulinger et al. 2008), Rockall Bank (van Bleijswijk et al. 2015), and Gulf of Mexico/western Atlantic (Kellogg et al. 2009, 2017, Galkiewicz et al. 2011) have either not detected *Endozoicomonas* or found it to be extremely rare (<1.5% relative abundance).

Cataloging microbial baselines for cold-water coral species is a critical first step to understanding and predicting the ecosystem services their microbiomes contribute, as well as providing a benchmark against which to measure changes in response to environmental change or anthropogenic impacts. The specific objectives of this work were to (1) determine if microbiome data would corroborate the taxonomic amalgamation of *D. dianthus* and *D. pertusum* into a single genus; (2) compare corals that share a habitat type/depth zone in submarine canyons (*D. dianthus*, *Acanthogorgia aspera*, and *A. spissa*); and (3) establish benchmark bacterial community data for *D. dianthus*, *A. aspera*, and *A. spissa*. This resulted in the unexpected discovery that both *Acanthogorgia* spp. and some *D. pertusum* microbiomes were dominated by *Endozoicomonas*.

2. MATERIALS AND METHODS

2.1. Sample sites and collections

Deep-sea coral samples were collected for microbial analysis during 4 research cruises between the years 2013 and 2019 via a combination of crewed undersea vehicles and remotely operated vehicles (ROVs) (Table 1, Fig. 1). Samples from Norfolk Canyon were collected in 2013 on the NOAA ship 'Ronald H. Brown' using the ROV 'Jason II' (RB-13-03-HBH Deepwater Canyons). Samples from the West Florida sites Many Mounds and Okeanos Ridge were collected in 2017 on the NOAA ship 'Nancy Foster' using the ROV 'Odysseus' (NF1708 Southeast Florida Deep Coral Initiative). The 2018 and 2019 collections were part of the DEEP SEARCH program and were conducted on the RV 'Atlantis' using the human-occupied vehicle (HOV) 'Alvin' (AT41) and the NOAA ship 'Ronald H. Brown' using the ROV 'Jason II' (RB1903), respectively.

Table 1. Sample collection sites and associated environmental data for coral collections. ND: not determined; dates are given as mo/d/yr

Coral	Site	Sample ID	Date	Depth (m)	Latitude (°N)	Longitude (°W)	Temp (°C)	Salinity
Acanthogorgia aspera	Norfolk Canyon	685Q2[a]	5/11/13	1328	37.04984	74.51377	4.1	35.0
Acanthogorgia aspera	Norfolk Canyon	685Q3	5/11/13	1336	37.04989	74.51357	4.2	35.0
Acanthogorgia aspera	Norfolk Canyon	685Q4[a]	5/11/13	1312	37.05002	74.51246	4.1	35.0
Acanthogorgia aspera	Norfolk Canyon	685Q5	5/11/13	1311	37.04991	74.51234	4.2	35.0
Acanthogorgia aspera	Cape Lookout	RB1903-J2-1135-Q3[a]	4/25/19	944	33.91891	75.83344	4.5	35.0
Acanthogorgia spissa	Pamlico Canyon	RB1903-J2-1132-Q1	4/22/19	1476	34.93145	75.15013	3.9	35.0
Acanthogorgia spissa	Pamlico Canyon	RB1903-J2-1132-Q2	4/22/19	1476	34.93145	75.15013	3.9	35.0
Acanthogorgia spissa	Pamlico Canyon	RB1903-J2-1132-Q5	4/22/19	1476	34.93145	75.15013	3.9	35.0
Acanthogorgia spissa	Pamlico Canyon	RB1903-J2-1132-Q6	4/22/19	1402	34.93160	75.15148	4.0	35.0
Desmophyllum dianthus	Blake Escarpment	AT41-A4964-Q1	8/25/18	1216	31.32269	77.24234	4.3	35.0
Desmophyllum dianthus	Blake Deep	RB1903-J2-1131-Q4	4/18/19	1321	31.28760	77.23677	4.1	35.0
Desmophyllum dianthus	Blake Deep	RB1903-J2-1131-Q6	4/18/19	1320	31.28767	77.23660	4.2	35.0
Desmophyllum dianthus	Blake Deep	RB1903-J2-1131-Q8	4/18/19	1321	31.28762	77.23677	4.1	35.0
Desmophyllum dianthus	Pamlico Canyon	RB1903-J2-1132-Q3	4/22/19	1567	34.93077	75.15035	3.9	35.0
Desmophyllum dianthus	Pamlico Canyon	RB1903-J2-1132-Q4	4/22/19	1567	34.93077	75.15035	3.9	35.0
Desmophyllum dianthus	Pamlico Canyon	RB1903-J2-1132-Q8	4/22/19	1567	34.93077	75.15035	3.9	35.0
Desmophyllum pertusum	Richardson Ridge	AT41-A4962-Q2	8/23/18	695	32.00998	77.39507	9.2	35.2
Desmophyllum pertusum	Richardson Ridge	AT41-A4963-Q2	8/24/18	827	31.98494	77.41471	5.0	35.1
Desmophyllum pertusum	Richardson Ridge	AT41-A4963-Q3	8/24/18	789	31.98449	77.41393	6.8	35.1
Desmophyllum pertusum	Richardson Ridge	AT41-A4963-Q8	8/24/18	684	31.98459	77.41106	10.9	35.4
Desmophyllum pertusum	Richardson Ridge	AT41-A4963-Q10	8/24/18	685	31.98450	77.41122	10.9	35.4
Desmophyllum pertusum	Cape Fear Coral	AT41-A4968-Q1	8/29/18	381	33.57256	76.46505	7.0	35.5
Desmophyllum pertusum	Cape Fear Coral	AT41-A4968-Q2	8/29/18	460	33.57551	76.46792	7.7	35.2
Desmophyllum pertusum	Pea Island Seep	RB1903-J2-1133-Q3	4/23/19	296	34.67360	75.79777	11.4	35.5
Desmophyllum pertusum	Many Mounds	NF1708-10-01	8/19/17	480	26.20755	84.72610	ND	ND
Desmophyllum pertusum	Many Mounds	NF1708-10-08	8/19/17	496	26.20576	84.72679	ND	ND
Desmophyllum pertusum	Many Mounds	NF1708-11-04	8/19/17	432	26.20725	84.71101	ND	ND
Desmophyllum pertusum	Okeanos Ridge	NF1708-12-01	8/20/17	521	25.66988	84.58431	7.1	35.0

[a]These samples were removed from the study due to low numbers of sequences

Five *Acanthogorgia aspera* were collected from Norfolk Canyon. These small yellow gorgonians were commonly encountered in this canyon and were collected along a long steep wall. Finding *A. aspera* in Norfolk Canyon was a northward range extension for this species because it was previously only known from the Gulf of Mexico and south of Cape Hatteras (Brooke et al. 2017). The *A. aspera* collected from Cape Lookout was growing on a boulder in an area of scattered boulders with a few coral colonies growing on them, including bamboo and black corals. *A. spissa* occurring in Pamlico Canyon were collected along the northern steep canyon wall from terrace overhangs commonly also populated by *Desmophyllum dianthus*. With the exception of the Cape Lookout collection at 944 m depth, *Acanthogorgia* spp. were collected below 1300 m (Table 1).

The *D. dianthus* colony from Blake Escarpment was growing on the skeleton of a dead bamboo coral. The 3 *D. dianthus* collected at Blake Deep were from a rock overhang at the top of a ridge, also populated by anemones, *Anthomastus* sp., black coral, and bamboo corals. Pamlico Canyon *D. dianthus* colonies were also from a ledge underhang community, likewise populated by *Solenosmilia variabilis*, *Acanthogorgia* sp., and brisingid sea stars. These solitary cup corals were all collected below 1200 m (Table 1).

The Richardson Ridge site is part of a *D. pertusum* mound complex that covers an area over 100 km^2 (Stetson et al. 1962). This large area of linear mounds of *D. pertusum* exists below the Gulf Stream (Stetson et al. 1962, Legeckis 1979, Popenoe 1994), deeper than most *D. pertusum* habitats known for this area (Table 1), and is subject to high current speeds. Corals in this area experience a variable temperature range (Table 1). The Cape Fear coral mound site was also subject to strong currents and can be impacted by Gulf Stream meanders (Bane & Brooks 1979). A single colony of *D. pertusum* was found growing at the shallow Pea Island seep site on authigenic carbonate substrate. The Gulf of Mexico/West Florida Slope collections of *D. pertusum* were conducted at 2 sites: Many Mounds and Okeanos Ridge (Fig. 1). The Many Mounds site was characterized by dense ag-

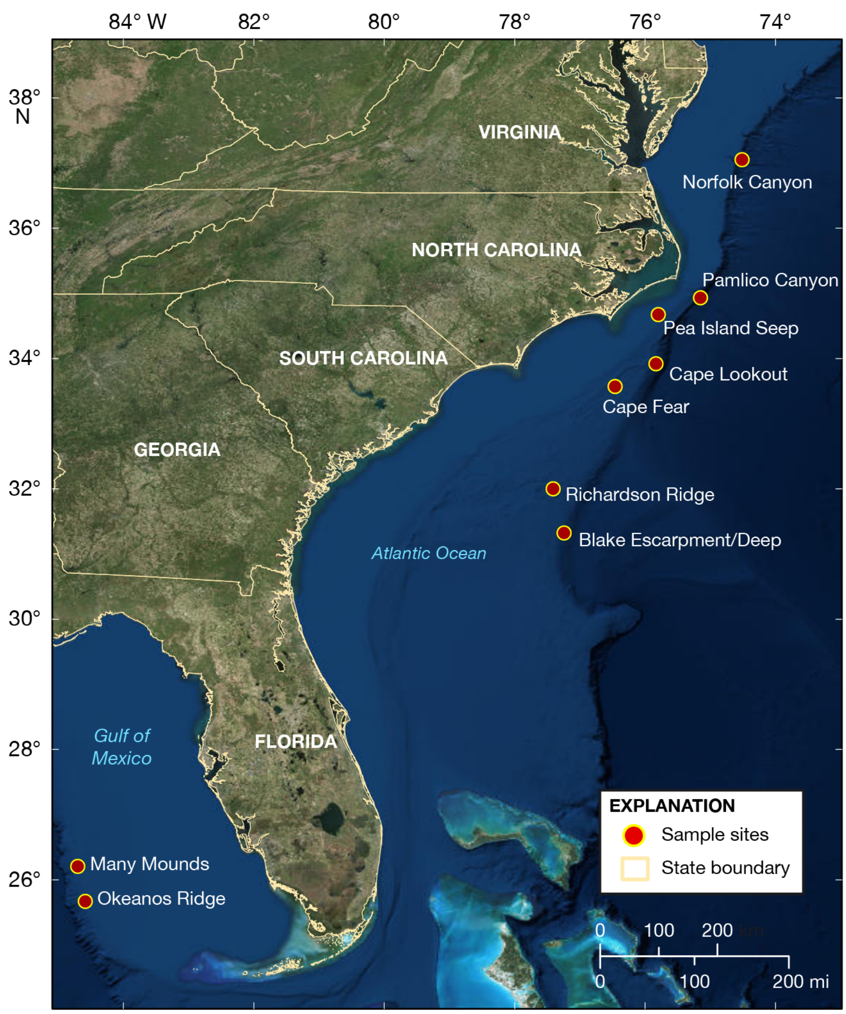

Fig. 1. Sampling site locations. See Table 1 for additional sampling site metadata. Base-map credit: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

gregations of *D. pertusum* mixed with scattered sponges, soft corals and octocorals (Ross et al. 2017). Western Atlantic and Gulf of Mexico *D. pertusum* colonies are predominantly the white color morph; however, sample NF1708-10-08 was a rare orange colony. The Okeanos Ridge *D. pertusum* was collected from a wall slope.

Subsamples of coral colonies were collected using the respective vehicle's manipulator arm to remove a branch (or in the case of *D. dianthus*, the entire cup coral). Samples were placed into individual, thermally insulated containers that had been precleaned (washed with freshwater, interiors wiped with 100% ethanol to remove any biofilms or particulates from prior collections), filled with freshwater, and sent down sealed. When opened to receive a coral collection, the freshwater was replaced by seawater local to the collection site due to density differences. The containers were then sealed at depth to prevent microbial contamination from other sample collections or passing through different water masses during vehicle recovery. At the conclusion of the dive, containers were brought into a cold room or laboratory and samples were removed using ethanol-sterilized forceps. All corals were lightly rinsed with sterile 1× phosphate-buffered saline (PBS) to remove any loosely associated surface microbes. For octocorals (*A. aspera*, *A. spissa*), branches that had not been in contact with the manipulator claw were cut off using ethanol-sterilized shears and transferred to sterile tubes. For the stony corals (*D. dianthus*, *D. pertusum*), samples were placed into sterile aluminum weigh boats and a flame-sterilized hammer was used to break open the calyces to expose polyp tissue, which was transferred to sterile tubes. All samples were preserved with RNAlater (Invitrogen), stored for 24 h at 4°C to allow the preservative to infiltrate the tissues, and then moved to −20°C until processing. Subsamples of the corals collected for microbial analyses were shared with researchers conducting population genetics/taxonomy studies, allowing for accurate host identification of octocorals and the possibility of interpreting microbiome trends against coral genotypes (Goldsmith et al. 2018).

2.2. Nucleic acid extraction and sequencing

Prior to extraction, coral samples were rinsed with 0.2 µm-filtered and autoclaved 1× PBS to remove excess salts from RNAlater (Invitrogen). Coral pieces were placed into sterile microcentrifuge tubes using flame-sterilized forceps, 2 ml of the sterile 1× PBS was added to the tube, which was then inverted 3 times and then centrifuged at 4000 × *g* (30 s). The coral samples were then weighed using a sterile technique and approximately 0.1–0.2 g sample^{-1} were used for extraction. Extractions were done in duplicate for each coral using the DNeasy PowerBiofilm kit (Qiagen), and replicates were combined after the final step. The manufacturer's protocol was followed with the exception that a FastPrep (MP Biomedicals) was used on setting 5 (~3100 rpm) for homogenization in place of a PowerLyzer (Qiagen). Kit blanks (extractions with no sample added) were processed at the same time as the samples. DNA was quantified using a Qubit dsDNA High Sensitivity (HS) assay (Invitrogen), and extractions were diluted to approximately 30 ng µl^{-1}, and submitted for sequencing.

Polymerase chain reaction (PCR) amplification and sequencing were performed by the RTSF Genomics Core at Michigan State University. The V3-V4 regions of the 16S rRNA gene were amplified with the primers 341F (5′-CCT ACG GGA GGC AGC AG-3′) (Herlemann et al. 2011) and 806R (5′-GGA CTA CHV GGG TWT CTA AT-3′) (Caporaso et al. 2011). One µl of genomic DNA was added to 7.5 µl of 2× Dream *Taq* Master Mix (Thermo Scientific) and 6.4 µl of a 0.5 µM primer mix. Amplification conditions were an initial melting period of 2 min at 95°C, followed by 30 cycles of 95°C for 40 s, 50°C for 30 s, and 72°C for 60 s, and a final anneal step at 72°C for 7 min. After PCR, the output of all reactions was batch normalized using an Invitrogen SequalPrep DNA Normalization Plate, and all material recovered from the plate was pooled. The pooled material was cleaned up and concentrated using AmpureXP magnetic beads (Beckman Coulter Life Sciences). The pool was quality controlled and quantified using a combination of Qubit dsDNA HS, Agilent 4200 TapeStation HS DNA1000, and Kapa Illumina Library Quantification qPCR assays. After quality control, this pool was loaded onto an Illumina MiSeq v2 Standard flow cell, and sequencing was carried out in a 2 × 250 bp paired end format using a MiSeq v2 500 cycle reagent cartridge. Custom sequencing and index primers complementary to the 341F/806R sequences used for preparing the libraries were added to appropriate wells of the reagent cartridge. Base calling was done by Illumina Real Time Analysis (RTA) v1.18.54, and output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.19.1. Sequence data are available from the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA699458 (submission SUB9027826) and are also available online as a US Geological Survey data release (https://doi.org/10.5066/P9Z1HPKR).

2.3. Data curation and analysis

Data were imported into QIIME2 (version 2020.8) and sorted into amplicon sequence variants (ASVs) using the DADA2 pipeline with the parameters: --p-trim-left-f 50 --p-trim-left-r 50 --p-trunc-len-f 225 and --p-trunc-len-r 225 (Callahan et al. 2016, Bolyen et al. 2019). Taxonomy was assigned with the SILVA reference database trained for the V3-V4 region (silva-138-99) (Quast et al. 2013). ASVs identifying as mitochondria, chloroplasts, or not assigned to the domains 'Bacteria' or 'Archaea' were removed. All samples were rarefied to 11 778 sequences, removing 3 samples from the dataset that did not contain this number of sequences (Table 1). The QIIME2 diversity plugin was used to compute all alpha diversity metrics (observed ASVs, Shannon diversity index, Pielou's evenness, Faith's phylogenetic diversity) and a permutational multivariate ANOVA (PERMANOVA) for each alpha diversity metric (Shannon 1948, Kruskal & Wallis 1952, Pielou 1966, Faith 1992, DeSantis et al. 2006). The QIIME2 diversity plugin was also used to calculate all beta diversity metrics (Bray-Curtis dissimilarity, Jaccard similarity index, and weighted and unweighted Unifrac distances) (Jaccard 1908, Bray & Curtis 1957, Lozupone & Knight 2005, Lozupone et al. 2007). For each beta diversity metric, an analysis of similarities (ANOSIM) and a resemblance-based permutation test (PERMDISP) were conducted (Clarke 1993, Anderson 2001, Anderson & Walsh 2013). A second analysis was conducted with only those samples associated with scleractinian corals (*D. dianthus* and *D. pertusum*), including all diversity indices and statistical analyses listed above.

2.4. *Endozoicomonas* analysis

All sequences within the genus *Endozoicomonas* were identified for further analysis. Sequences were aligned in MEGA X (Kumar et al. 2018, Stecher et al. 2020) using ClustalW (Thompson et al. 1994), with the addition of relevant sequences from NCBI's SRA and Nucleotide databases. An evolutionary tree of the 20 most common *Endozoicomonas* sequences was constructed using the maximum likelihood method (Tamura & Nei 1993), with a bootstrap value of 500.

3. RESULTS

After quality control, the number of sequences per sample ranged from 494 to 722 149, with an average of 216 210 and a standard deviation of ±216 213. After rarefaction to 11 778 sequences, 3 samples were removed due to low numbers of sequences (Table 1), as was the extraction kit blank.

3.1. Alpha diversity metrics

Desmophyllum dianthus had a significantly higher number of observed ASVs than *D. pertusum* and *Acanthogorgia spissa*, but not *A. aspera* (Fig. 2, Table 2), likely due to the small number of *A. aspera* samples that were successfully sequenced (n = 2). Although Shannon diversity and Pielou's evenness were not significantly different between species, Faith's phylogenetic diversity was significantly different when comparing *D. dianthus* to all other coral species (Table 2). When examined by site location, *D. dianthus* demonstrated higher numbers of observed sequences in 2 of the 3 locations (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m673p001_supp.pdf).

3.2. Beta diversity metrics

In a principal coordinate analysis (PCoA) based upon Bray-Curtis dissimilarities of all corals, there was a clear differentiation between the octocorals and scleractinians, in spite of *Acanthogorgia* spp. and

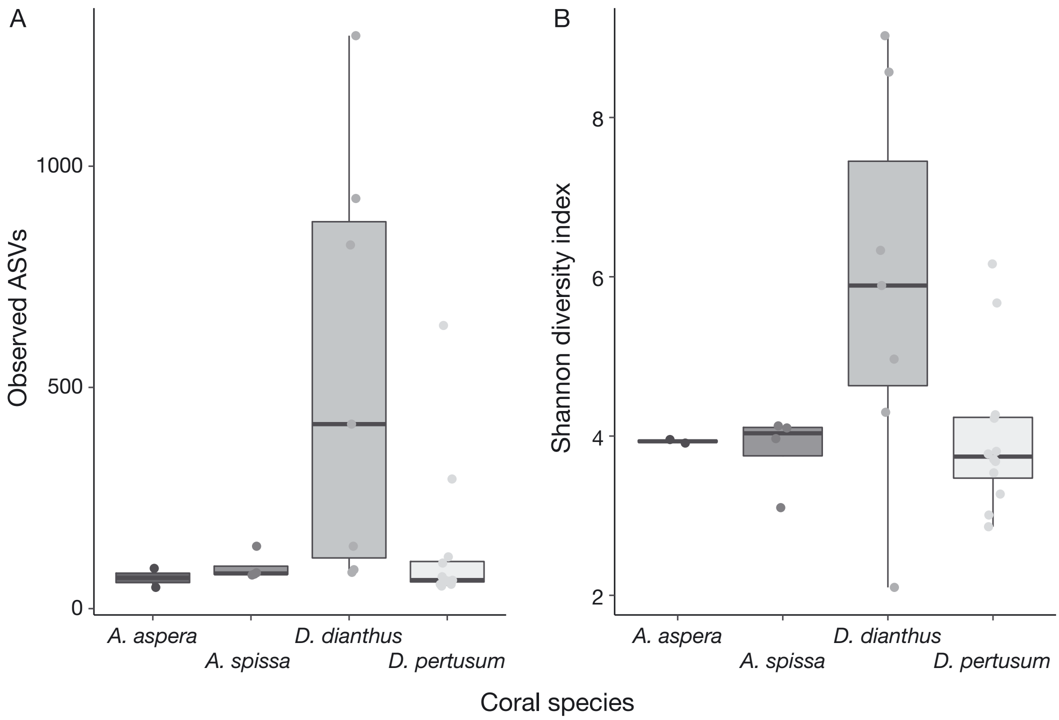

Fig. 2. (A) Observed amplicon sequence variants (ASVs) and (B) Shannon diversity indices for *Acanthogorgia aspera*, *A. spissa*, *Desmophyllum dianthus*, and *D. pertusum*. All collection sites are pooled (see Fig. S1 for site-specific diversity metrics). Boxplots represent median, 25 to 75% interquartile range and non-outlier range; points beyond the whiskers are outliers

Table 2. Pairwise alpha and beta diversity statistics for *Acanthogorgia aspera*, *A. spissa*, *Desmophyllum dianthus*, and *D. pertusum*. The number of observed amplicon sequence variants (ASVs) and Faith's phylogenetic diversity were significantly different between species (p = 0.029 and 0.017, respectively), while Shannon diversity and Pielou's evenness were not (p = 0.114 and 0.362, respectively), so no pairwise comparisons are given. Beta-diversity statistics are based upon Bray-Curtis dissimilarity. Both an analysis of similarities (ANOSIM; p = 0.001, Global r = 0.74) and dispersion (PERMDISP; p = 0.082) were significantly different between species. All pairwise p-values are adjusted, and significant values of p < 0.1 are indicated with *

Species 1	Species 2	Observed ASVs	Faith's phylogenetic diversity	ANOSIM		PERMDISP
		p	p	p	r	p
A. aspera	*A. spissa*	0.643	0.532	0.123	0.607	0.148
A. aspera	*D. dianthus*	0.286	0.081*	0.040*	0.964	0.355
A. aspera	*D. pertusum*	0.557	0.855	0.030*	0.979	0.346
A. spissa	*D. dianthus*	0.088*	0.042*	0.002*	0.778	0.024*
A. spissa	*D. pertusum*	0.337	0.628	0.002*	0.865	0.036*
D. dianthus	*D. pertusum*	0.052*	0.007*	0.002*	0.648	0.346

D. dianthus having been collected from similar habitats and depths. Both *Acanthogorgia* species clustered tightly together with the exception of 1 sample from Norfolk Canyon that still clustered relatively close (Fig. 3A). Three groupings of *D. pertusum* were observed, one of which clustered tightly with *D. dianthus*. Similar patterns were seen according to Jaccard similarity indices and unweighted Unifrac distances, but not weighted Unifrac distances (Fig. S2). This indicates that the observed patterns are driven by the equal importance of shared rare and dominant microbial taxa, rather than by abundance (dominance) of specific taxa. While Bray-Curtis does factor in abundance, it weights the abundance of shared species more, which explains the tighter clustering of *Acanthogorgia* spp. in Fig. 3A compared to the clusters in Fig. S2. Upon the independent analysis of *D. pertusum* and *D. dianthus*, each species clustered separately except for 1 sample from each species (Fig. 3B). In this analysis, *D. pertusum* samples collected from the Gulf of Mexico sites (Many Mounds and Okeanos Ridge) also clustered apart from other *D. pertusum* samples.

Microbial community structure (ANOSIM) and dispersion (PERMDISP) were analyzed between all coral species using Bray-Curtis dissimilarities. Dispersion was only significantly different between *A. spissa* and each scleractinian coral (*D. pertusum* and *D. dianthus*; Table 2). Microbial community structure was significantly different between all coral species except for *A. aspera* and *A. spissa*, with the high r-values indicating that the communities were very distinct between species (Table 2).

3.3. Community and *Endozoicomonas* analysis

Community analysis at the class level indicated a substantial percentage of *Gammaproteobacteria* (Fig. S3). Upon further taxonomic evaluation, the majority of these *Gammaproteobacteria* were unexpected *Endozoicomonas* spp. Richardson Ridge and Cape Fear *D. pertusum* were dominated (13–88 %) by ASVs belonging to the genus *Endozoicomonas*, while those collected from other sites had very few (0–3 %) *Endozoicomonas* (Fig. 4). Microbial communities from *D. dianthus* did not contain more than 0.13 % *Endozoicomonas* ASVs for any of the sampling sites. Both *Acanthogorgia* species also contained a large proportion of *Endozoicomonas* ASVs,

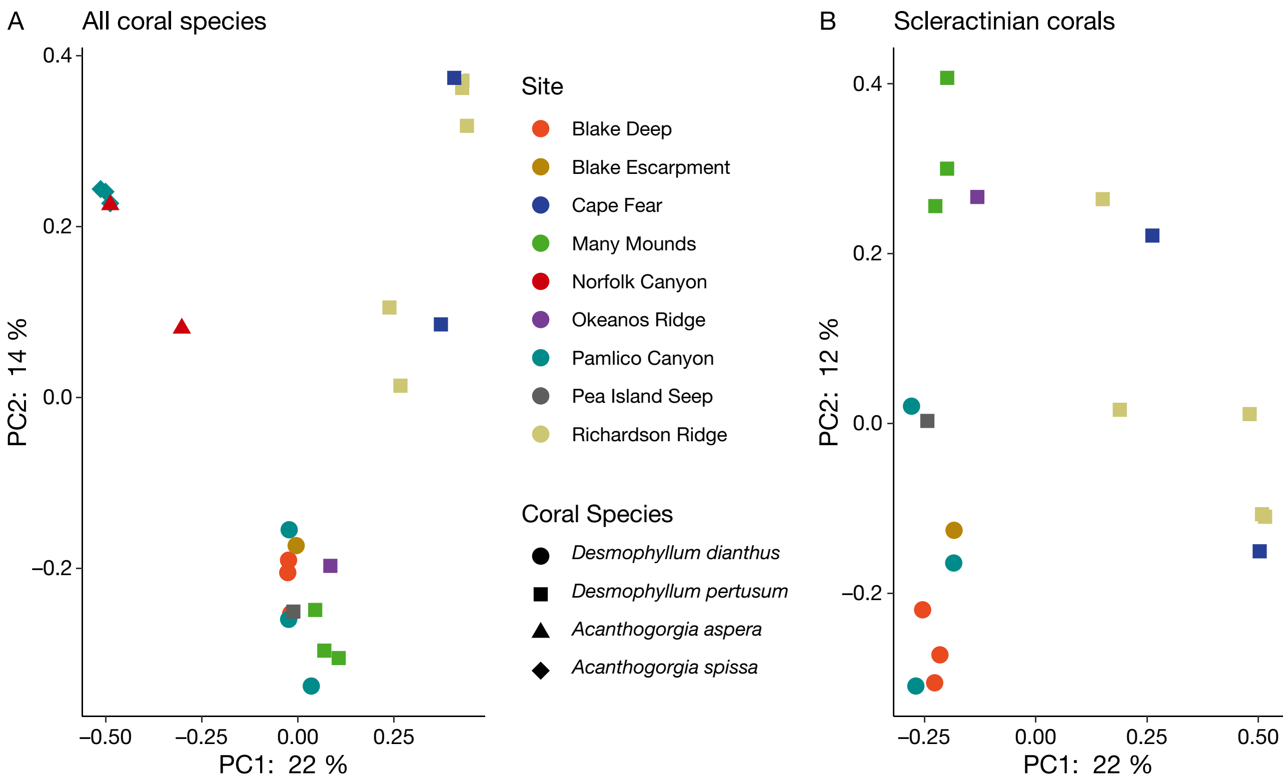

Fig. 3. Principal coordinates analysis (PCoA) based upon a Bray-Curtis dissimilarity matrix for 4 deep-sea coral species collected from 9 different sites. (A) All coral species; (B) only scleractinian coral species (Bray-Curtis dissimilarity matrices were calculated independently)

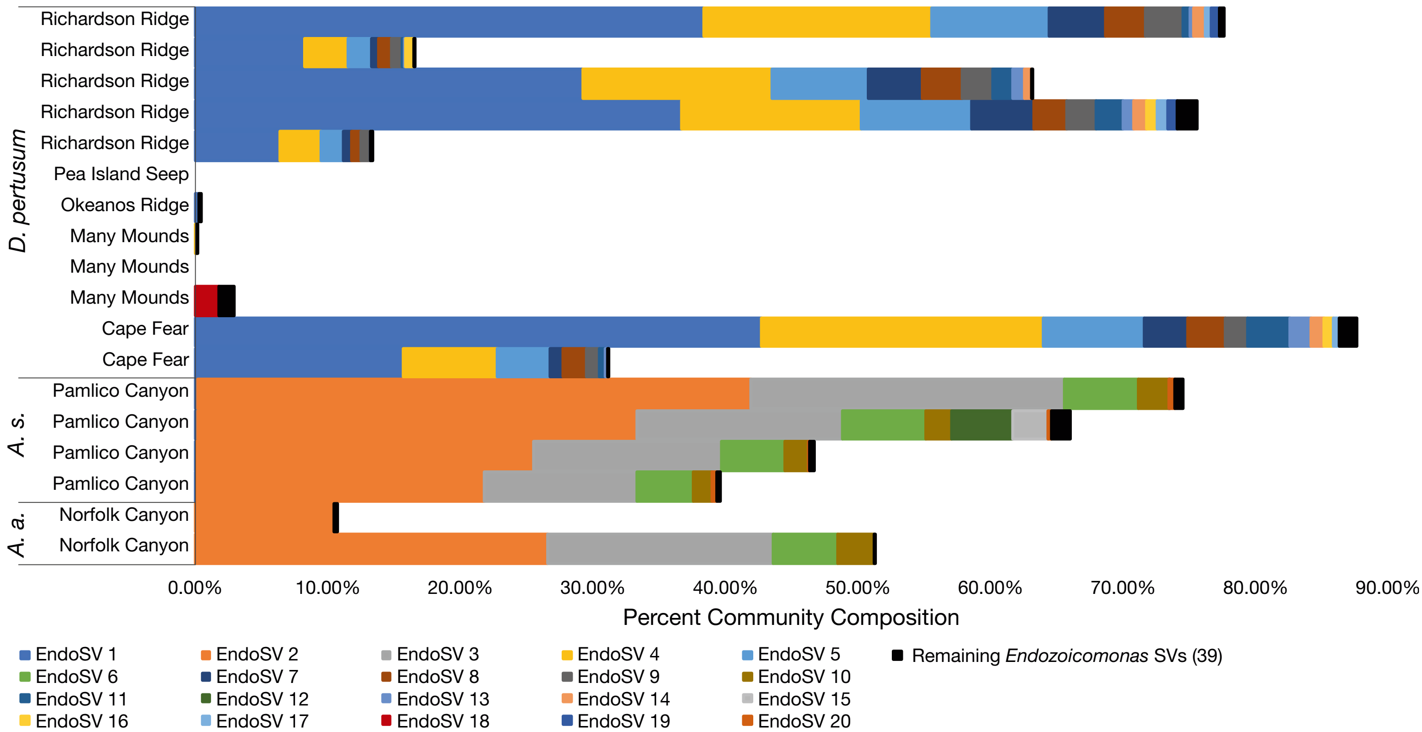

Fig. 4. Percent community composition of all *Endozoicomonas* amplicon sequence variants (ASVs) for 3 deep-water coral species: *Acanthogorgia aspera* (*A.a.*), *A. spissa* (*A.s.*), and *Desmophyllum pertusum*. The top 20 most abundant ASVs are given independently, with the remaining 39 combined

ranging from 11 to 75% (Fig. 4). Interestingly, the 2 *Acanthogorgia* species shared many *Endozoicomonas* ASVs, despite being collected from different regions. A phylogenetic tree was created to determine phylogenetic relatedness of the *Endozoicomonas* ASVs. The *Endozoicomonas* ASVs clustered into 2 main branches, those from *D. pertusum* and those from both *Acanthogorgia* species (Fig. 5).

4. DISCUSSION

Compared to tropical or even temperate corals, there have been relatively few microbiome studies of cold-water corals from the deep ocean. Deep-sea octocoral microbiomes show variation in their most abundant taxa: *Paragorgia arborea* is dominated by *Tenericutes* (Weiler et al. 2018), *Anthothela grandiflora* is dominated by a combination of *Gammaproteobacteria* and *Spirochaetes* (Lawler et al. 2016), *Primnoa resedaeformis* and *Paramuricea placomus* are both dominated by *Proteobacteria*, but vary in whether *Alpha*- or *Gammaproteobacteria* are more abundant (Kellogg et al. 2016, Goldsmith et al. 2018). In contrast, deep-sea stony corals including *Eguchipsammia fistula* in the Red Sea, and *Desmophyllum pertusum* and *Madrepora oculata* in the Mediterranean, all are dominated by *Gammaproteobacteria* (Meistertzheim et al. 2016, Röthig et al. 2017b). We found both *Acanthogorgia* spp., as well as *D. pertusum*, to be dominated by *Gammaproteobacteria*, but *D. dianthus* had a variable mixture of *Alpha*- and *Gammaproteobacteria* (Fig. S3).

4.1. Dissimilarity between *D. dianthus* and *D. pertusum* microbiomes

The bacterial communities associated with cup coral *D. dianthus* had significantly more variability and richness compared to the community associated with *D. pertusum* (Fig. 2, Fig. S1). While a few colonies of the 2 species grouped together (Fig. 3, Fig. S2), the 2 species hosted significantly different microbiomes (Table 2, Fig. S3). Interestingly, the single *D. pertusum* sample from the Pea Island Seep site, which might be predicted to have an unusual microbiome due to the shallow depth, relatively high temperature (11.4°C), and influence of seep conditions, was both an outlier from the other *D. pertusum* samples and closest to *D. dianthus* samples (Fig. 3).

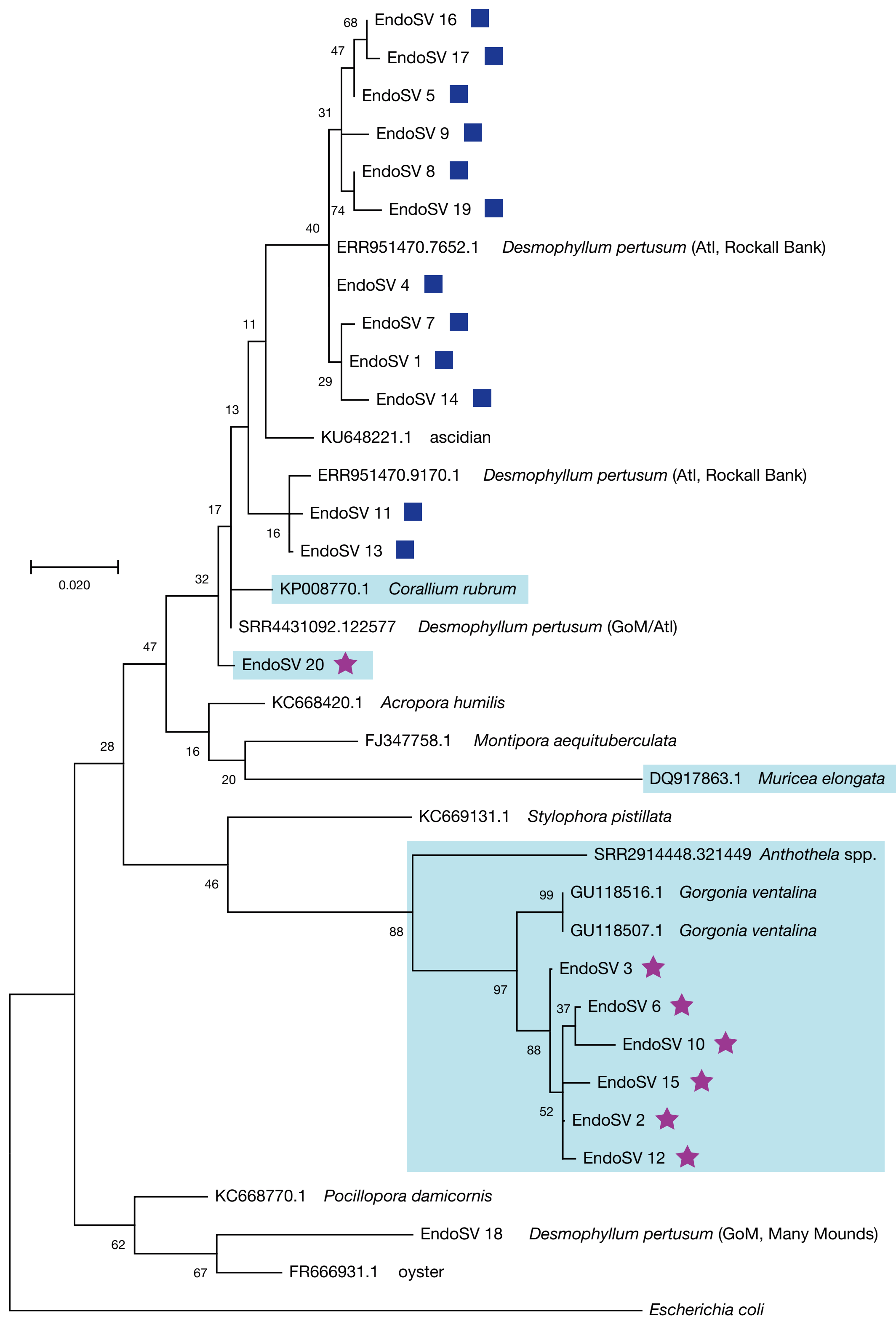

Fig. 5. Maximum likelihood evolutionary tree of the top 20 most abundant *Endozoicomonas* amplicon sequence variants (EndoSV 1–20) detected in *Acanthogorgia* spp. and *Desmophyllum pertusum* with similar sequences from the NCBI nucleotide (https://www.ncbi.nlm.nih.gov/nuccore) or sequence read archive (SRA) databases (https://trace.ncbi.nlm.nih.gov/Traces/sra/) (accession numbers are shown). Bootstrap values are given at each node (500 repetitions). *Escherichia coli* was used as an outgroup. Blue squares indicate *Endozoicomonas* amplicon sequence variants only found in *D. pertusum* samples from Richardson Ridge and Cape Fear sites. Purple stars indicate *Endozoicomonas* amplicon sequence variants only found in *Acanthogorgia* spp. samples. Aqua boxes indicate *Endozoicomonas* genotypes identified from octocorals. Atl: Atlantic; GoM: Gulf of Mexico

There was some expectation that *D. dianthus* and *D. pertusum* might have similar microbiomes because the 2 corals have been reclassified into a single genus based on genetic similarity (Addamo et al. 2016). Prior work on tropical scleractinians has shown conservation of bacterial communities at the genus level (Littman et al. 2009) or that coral microbiome composition tracks host phylogeny (Sunagawa et al. 2010, Pollock et al. 2018). However, we typically encountered these 2 corals in different depth zones (Table 1) and different habitats, which likely has some influence on their microbiomes (Pantos et al. 2015, Hernandez-Agreda et al. 2016). Moreover, recent research on *D. pertusum* has indicated that diet may have a strong influence on the microbiome (Galand et al. 2020) and a comparison between *D. pertusum* and *D. dianthus* showed differences in ingestion rates of different sized prey (Tsounis et al. 2010).

An additional hypothesis to consider is that of differing 'microbiome flexibility' between *D. pertusum* and *D. dianthus* (Ziegler et al. 2019). A reciprocal transplant experiment with 2 tropical stony corals found that one (*Acropora hemprichii*) had a flexible microbiome that varied between experimental sites, while the other (*Pocillopora verrucosa*) maintained a stable microbiome, indicating the existence of different host–microbiome adaptation strategies (Ziegler et al. 2019, Voolstra & Ziegler 2020). In the Mediterranean, microbiome comparisons between *D. pertusum* and *M. oculata* revealed that the microbiome of *M. oculata* was stable across seasons and during a reciprocal transplant experiment, while that of *D. pertusum* was more clearly influenced by environmental conditions (Meistertzheim et al. 2016, Chapron et al. 2020). Further, multiple prior studies of *D. pertusum* have provided evidence that the bacterial community of this coral exhibits variability across different geographic sites (Neulinger et al. 2008, Kellogg et al. 2009, 2017, Schöttner et al. 2009) and the clustering patterns observed in our current study are consistent with *D. pertusum* having a more flexible microbiome than *D. dianthus* (Fig. 3B).

4.2. Similarity between *Acanthogorgia* spp. microbiomes

In contrast, the microbiomes of the 2 *Acanthogorgia* species clustered very tightly, with 1 exception, and were not statistically distinguishable (Fig. 3, Table 2). With the exception of 1 Norfolk Canyon *A. aspera*, the *Acanthogorgia* spp. microbiomes were dominated (40–75%) by *Endozoicomonas* (Fig. 4). Conserved core ASVs (present in all 6 samples analyzed) included 2 *Endozoicomonas* phylotypes and 4 *Shewanella* phylotypes. The 2 *A. aspera* samples had higher relative abundance of class *Bacteroidia* compared to *A. spissa* (Fig. S3). Previous work found that the microbiomes of 2 species of the cold-water octocoral genus *Anthothela* also clustered together and were statistically indistinguishable (Lawler et al. 2016). The only prior knowledge for *Acanthogorgia*-associated bacteria comes from a study that screened a variety of deep-sea corals in the Gulf of Mexico for the presence of chemoautotrophic bacteria from the SUP05 cluster (Vohsen et al. preprint https://www.biorxiv.org/content/10.1101/2020.02.27.968453v1). That study detected genus-specific SUP05 phylotypes at high abundance (>10% relative abundance) in *A. aspera* using primers that targeted the V1-V2 region of the 16S rRNA gene (Vohsen et al. preprint https://www.biorxiv.org/content/10.1101/2020.02.27.968453v1). We detected 2 SUP05 cluster phylotypes at low abundance in our *A. spissa* samples but not in any of the *A. aspera* samples. This could be a methodological issue (i.e. differential detection by V3-V4 primers compared to V1-V2) or a biogeographic difference between microbiomes of Atlantic corals and Gulf of Mexico corals. In support of it being a biogeographic difference, we detected 3 SUP05 cluster phylotypes occurring in 4 to 6 *D. pertusum* samples: 1 phylotype was only present in Gulf of Mexico samples (Many Mounds and Okeanos Ridge), and the other 2 had higher numbers of sequence reads in Gulf of Mexico samples compared to Atlantic samples (Cape Fear and Richardson Ridge).

4.3. Dominance of *Endozoicomonas* in *Acanthogorgia* spp. and site-specific *D. pertusum*

There were clear differences between the *Endozoicomonas* community in *D. pertusum* compared to *Acanthogorgia* spp. (Figs. 4 & 5). Host-specificity of *Endozoicomonas* genotypes has been observed in both shallow-water scleractinians and octocorals (Lee et al. 2012, La Rivière et al. 2015, Neave et al. 2017b, van de Water et al. 2017). There was substantial overlap of *Endozoicomonas* genotypes between *A. aspera* and *A. spissa* (as has been seen in sister species of *Eunicella* (van de Water et al. 2017)); however, EndoSV 20 was only present in *A. spissa* samples (Fig. 4). Studies of Mediterranean octocorals found evidence of codivergence between *Endozoicomonas* genotypes and their hosts (La Rivière et al. 2015, van de Water et al. 2017). Examination of rare *Endozoico-*

monas genotypes from *D. pertusum* and *Anthothela* spp. found that they clustered with scleractinian and octocoral sequences, respectively, further suggesting influence of host phylogeny (Pollock et al. 2018, Kellogg 2019). In this study, all but one of the major *Endozoicomonas* genotypes from *Acanthogorgia* spp. formed a clade that clustered closely with other octocorals, *Gorgonia ventalina* and *Anthothela* spp. (Fig. 5). One rare genotype (0.09–0.42% in *A. spissa* samples), EndoSV 20, fell outside the main octocoral cluster (Fig. 5). This variation may be evidence of the influence of both the control and local adaptation of the host (Neave et al. 2016).

A biogeographic study of *Endozoicomonas* genotypes in the widely distributed coral species *Stylophora pistillata* and *P. verrucosa* found that *S. pistillata* had geographically distinct *Endozoicomonas* communities (Neave et al. 2017b), similar to what we observed here in *D. pertusum*. The *Endozoicomonas* genotypes from *D. pertusum* at Richardson Ridge and Cape Fear sites formed 2 main clades, each including a sequence from *D. pertusum* from Rockall Bank (van Bleijswijk et al. 2015), on the eastern side of the Atlantic (Fig. 5). These groups were separate from an *Endozoicomonas* genotype that was detected in *D. pertusum* samples from multiple Gulf of Mexico sites as well as a western Atlantic site off Cape Canaveral, Florida (Kellogg et al. 2017) and from EndoSV 18 from *D. pertusum* in the Gulf of Mexico, the Many Mounds site (Fig. 5). In the case of *S. pistillata* and *P. verrucosa*, Neave et al. (2017b) hypothesized that the difference could be due to reproductive strategy; as a brooder, *S. pistillata* could more strictly control its microbiome by vertical transmission, resulting in geographic structuring. This hypothesis does not hold for *D. pertusum*, which is a broadcast spawner (Brooke & Järnegren 2013). However, there is prior evidence for differentiation of *D. pertusum* microbiomes based on geographic region, including differences between the Gulf of Mexico and western Atlantic (Neulinger et al. 2008, Kellogg et al. 2017).

4.4. What is driving the unusual *D. pertusum* microbiomes at Richardson Ridge/Cape Fear sites?

Shallow-water studies of both scleractinians and octocorals suggest that reductions of *Endozoicomonas* abundance in hosts that are typically dominated by them are linked to unfavorable environmental conditions, such as excessive nutrients, temperature stress (hot or cold), or lower pH (Vezzulli et al. 2013, Morrow et al. 2015, Roder et al. 2015, Neave et al. 2016, van de Water et al. 2017, Maher et al. 2019, Ziegler et al. 2019, Shiu et al. 2020). However, we do not know of any prior examples where coral microbiomes that typically do not host large numbers of *Endozoicomonas* have been found to shift to being dominated by them. In general, environmental disturbances that affect the metabolism and physiology of the coral host also change the microbiome (Vega Thurber et al. 2009, Lee et al. 2017). However, as mentioned in Section 4.1, the amount of 'microbiome flexibility' or restructuring of the bacterial composition under environmental change varies depending on the coral host (Ziegler et al. 2019). This microbiome flexibility is hypothesized to be a rapid adaptation that provides the coral host with a more beneficial bacterial community to improve coral fitness under the new conditions (Reshef et al. 2006, Voolstra & Ziegler 2020). The Richardson Ridge and Cape Fear sites on the Blake Plateau are affected by the Gulf Stream (Stetson et al. 1962, Bane & Brooks 1979, Legeckis 1979, Popenoe 1994), which can translate into extreme variability in water temperature, salinity, nutrients, and current speeds (e.g. Mienis et al. 2014). These factors have been shown to affect coral microbiomes in tropical systems (Guppy & Bythell 2006, Littman et al. 2009, Zaneveld et al. 2016, Lee et al. 2017). The species makeup of a microbial community following disturbance may be explained by the environmental preferences and competitive abilities of the particular microbes (Maher et al. 2019, Ziegler et al. 2019). However, the majority of coral-associated *Endozoicomonas* appear to be sensitive to temperature stress outside the optimal range of 15–30°C (Kellogg 2019, Shiu et al. 2020), implying that these *D. pertusum* phylotypes may be particularly unusual in their compatibility with rapid shifts in temperature.

The functional diversity revealed from coral-associated *Endozoicomonas* genomes that have been sequenced underscores the complexity of their co-diversification with their hosts (Ding et al. 2016, Neave et al. 2017a, Tandon et al. 2020). This diversity may indicate that different strains of *Endozoicomonas* can provide unique ecosystem services to their coral host, such as carbohydrate cycling, amino acid synthesis, or production of vitamins and cofactors (Neave et al. 2017a).

4.5. Conclusions

Two cold-water scleractinian corals, *Desmophyllum dianthus* and *D. pertusum* (*Lophelia pertusa*) that have been combined into a single genus, had significantly different bacterial microbiomes. Benchmark bacterial microbiomes for 2 deep-sea octocorals, *Acanthogorgia aspera* and *A. spissa*, were found to be statistically indistinguishable and dominated by *Endozoicomonas*. This is the first report of coral microbiomes dominated by *Endozoicomonas* occurring below 1000 m, at temperatures of 3.9–4.5°C. Distinct and diverse genotypes of *Endozoicomonas* unexpectedly dominated the microbiome of *D. pertusum* at Richardson Ridge and Cape Fear sites. All prior studies of *D. pertusum* in other regions found this genus to be rare or absent, even when present in neighboring coral *Madrepora oculata*. The unusual microbiomes at these sites may be linked to the extreme variability experienced by these corals due to interactions with the Gulf Stream. Future research directions could include further characterization of these unusual *Endozoicomonas* genotypes by cultivation and comparative genomics to understand their tolerance of cold temperatures and potentially unique metabolic capabilities.

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