# A seascape approach to the microbial biogeography of mesophotic and deep-sea octocorals

Samuel A. Vohsen [1*] & Santiago Herrera[1#]

[1]Department of Biological Sciences, Lehigh University, Bethlehem, PA

[*]Corresponding Author: sav419@lehigh.edu

[#]Co-corresponding Author: santiago.herrera@lehigh.edu

**Competing Interests Statement**

The authors declare no conflicts of interest

**Abstract**:

Coral-associated microbiomes vary greatly between coral colonies and sampling locations with functional consequences on the coral host. However, the full extent of variability across the ranges of most coral species remains unknown, especially in octocorals and species from mesophotic and deep-sea habitats. Here we characterized the microbiomes of four octocoral species from mesophotic and deep-sea habitats in the northern Gulf of Mexico, *Muricea pendula*, *Swiftia exserta*, *Callogorgia delta*, and *Paramuricea biscaya* using 16S metabarcoding. We tested for microbiome differentiation between and within species, examining the influence of the coral's genotype and environmental factors that vary with depth (53-2224 m) and geographic location (over 680 m). Coral microbiomes were often dominated by a single amplicon sequence variant – ASV (>50% relative abundance) whose abundance varied across the range of the host coral. These included corallicolid apicomplexans, *Endozoicomonas*, members of the Mollicutes, and the BD1-7 clade of Spongiibacteraceae. Coral species, depth, and geographic location significantly affected microbial community composition, richness, and evenness, as well as the abundance of individual microbes. Differences in bottom temperature and surface primary productivity could explain part of the variation associated with depth and geographic location, respectively. However the coral genotype was confounded with depth so its effect could not be dissected. Altogether, this work demonstrates that the microbiomes of corals vary substantially across their ranges in ways that may have functional consequences, identifies important ecological drivers in mesophotic and deep-sea corals, and can inform restoration efforts.

## Introduction

Shallow-water, reef-building corals are well-known for associating with microalgae in the family Symbiodinaceae. Still, all corals, including those in the deep sea, form associations with diverse groups of bacteria[1], archaea[2, 3], unicellular eukaryotes[4], and viruses[5], collectively referred to as the coral microbiome. The coral microbiome resides throughout the coral, including skeleton[6, 7], tissues[8, 9], gastrovascular cavity[10], and even the mucus[1, 11]. Many members of the microbiome have proven important to the health of corals, either as pathogens[12] or providing sources of nutrition[13, 14].

Common coral associates include eukaryotic apicomplexans of the family Corallicolidae[15-19] and the bacterial genera *Mycoplasma*[20-25] and *Endozoicomonas*[1, 26-28]. These taxa are among the most-studied coral-associated microbes and exhibit many indications of a close relationship with their coral hosts. *Endozoicomonas* form dense aggregates in coral tissue[9, 29]. They are often dominant in individual microbiomes[29, 30] of soft corals of the subclass Octocorallia[27, 28, 31], hard corals of the order Scleractinia [1], and black corals of the order Antipatharia[32]. *Endozoicomonas* isolated from corals possess capabilities relevant to the coral host such as the ability to produce vitamins[33, 34], degrade dimethylsulfoniopropionate[35], cycle phosphorus[8], and degrade chitin[30]. *Endozoicomonas* responds to coral tissue extracts by activating genes involved in initiating symbiosis[33]. Similarly, corallicolid apicomplexans and *Mycoplasma* are commonly found in many coral species and reside within or on coral tissue[16, 24, 36]. Most apicomplexans[37] and *Mycoplasma* spp.[38] are parasites or pathogens. However, those found in corals have not yet been associated with evident pathologies or reduced fitness[39]. While these common coral associates have been the focus of extensive research, we have a limited understanding of the diversity of coral- microbe associations.

The compositions of coral microbiomes can vary substantially between and within coral species and across geographic locations[26, 40, 41]. The genotype of the host coral underlies some of this variation[40, 42, 43] as well as environmental factors such as water temperature[44], habitat type[45], and pollution levels[44, 46]. These factors can differ across the ranges of coral species, yet the scope of most studies is limited to a narrow selection of a species' known geographic or depth range. Further, most studies of microbiome variability have focused on shallow water corals, mainly scleractinians, and to a lesser degree on octocorals and antipatharian black corals[32, 41, 47]. In summary, the extent of coral microbiome variability is likely underestimated.

Variations in coral microbiomes can have functional implications. In shallow-water scleractinian species, certain microbial assemblages are associated with increased thermal tolerance[48-50]. Microbiome variation could be significant for mesophotic and deep-sea corals (those living deeper than 50 meters) because they often have wider depth and geographic ranges than shallow-water species[51].

Mesophotic and deep-sea corals support diverse and abundant biological communities in Earth's largest biome[52-56]. Most of these corals are heterotrophic because they live beyond the reach of sunlight sufficient for photosynthesis. Like shallow-water corals, mesophotic and deep-sea corals face human-induced environmental changes such as ocean warming, acidification, pollution, and ecosystem destruction [57-60]. In the mesophotic zone and deep sea, octocorals comprise approximately 70% of all coral species[61]. A comprehensive characterization of microbiome variation in octocorals across their geographic ranges is needed to understand the diversity and ecological roles of coral microbiomes in the mesophotic zone and the deep sea.

Mesophotic and deep-sea octocorals occur across a wide depth range down to at least 8,000m and worldwide along continental margins and seamounts[51, 62]. Light intensity, oxygen concentration, temperature, pressure, pH, salinity, food availability, and habitat structure vary with depth and geography. All these factors combine to strongly influence the ecology and evolution of marine animals[63-66]. Thus, factors that vary with depth and geographic location likely drive microbiome variation in mesophotic and deep-sea octocorals.

Here we test the hypotheses that the microbiomes of mesophotic and deep-sea octocorals vary significantly across their ranges and in correlation with environmental factors and host genotype. We characterized the microbiomes of the mesophotic octocorals *Muricea pendula* and *Swiftia exserta*, and the deep-sea octocorals *Callogorgia delta* and *Paramuricea biscaya* across their known depth and geographic ranges in the northern Gulf of Mexico (**Fig. 1**). All of these corals are dominant foundation species in their respective communities. At least three were impacted by the Deepwater Horizon oil spill[57, 58, 67, 68] and are the focus of restoration efforts. Understanding the geographic variation of their associated microbes is essential to understanding the ecology of coral ecosystems and can also inform restoration plans.

## Methods

### *Sample Collection*

Octocorals were sampled between 2009 and 2019 from multiple sites across their ranges in the northern Gulf of Mexico using Remotely Operated Vehicles (ROVs) or the human-occupied vehicle Alvin, and placed in temperature-insulated containers during multiple research cruises. Specimens of the mesophotic octocoral *Muricea pendula*, n = 140, were collected from 14 sites spanning depths between 53-117 m, and geographic distances between 34 and 690 kilometers. Specimens of the co-occurring mesophotic octocoral *Swiftia exserta*, n = 100, were collected from 10 sites spanning depths between 62-100 m, and geographic distances between 13 and 600 kilometers. Specimens of the upper-bathyal octocoral *Callogorgia delta*, n = 60, were collected from 6 sites spanning depths between 439-852 m and geographic distances between 14 and 350 kilometers. Finally, specimens of the lower-bathyal octocoral *Paramuricea biscaya*, n = 60, were collected from 6 sites spanning depths between 1370-2224 m, and geographic distances between 14 and 655 kilometers. Upon ROV recovery, subsamples from each colony were frozen with liquid nitrogen and/or in >95% ethanol and/or RNAlater. Details, including research cruise, time of collection, and preservation method for each sample, can be found in **Supplementary Table A**.

### *DNA purification*

Whole DNA was purified from *ca.* ten coral colonies from each sampling site using a modified salting-out procedure (dx.doi.org/10.17504/protocols.io.bypypvpw). In short, about 3mm$^3$ of coral tissue was digested in a lysis buffer with proteinase K and beta-mercaptoethanol, protein and cellular debris were precipitated with 7.5M ammonium acetate, and DNA was pelleted in 50% isopropanol, washed with 70% ethanol, and then resuspended in TE buffer. To standardize DNA concentrations and reduce the concentration of PCR inhibitors, DNA extract concentrations were measured on a Qubit 4.0 fluorometer with a qubit Broad Range assay and then diluted with TE buffer to 2ng/µl for *Swiftia exserta*, 20ng/µL for *Muricea pendula*, 10ng/µL for *Callogorgia delta*, and 10-40ng/µL for *Paramuricea biscaya*. DNA extracts of *C. delta* and *P. biscaya* that were less concentrated than these thresholds were not diluted. See **Supplementary Methods** for details.

*Microbial metabarcoding*

The hypervariable V1-V2 regions of the bacterial 16S rRNA gene were amplified following Vohsen et al.[15] using the universal primers 27F and 355R (see **Supplementary Methods** for details). PCR products were sent to the University of Illinois Chicago for library preparation and sequencing[69] on an Illumina Miseq platform with 250bp paired-end sequencing. Raw sequence data is available on the Sequence Read Archive (SRA) under BioProject ID PRJNA875098.

Raw sequence data were imported into *qiime2*[70] ver2021.2 and processed following Vohsen et al.[15] to create an amplicon sequence variant (ASV) table. In brief, reads were quality filtered, joined, checked for chimeras, and ASVs were determined with deblur and classified using a naïve-bayes classifier trained on the SILVA small subunit (SSU) ribosomal RNA database (Ref NR 99 release 132)[71]. Details can be found in the **Supplementary Methods**.

*Coral genotyping*

To investigate the influence of population structures of the host, Restriction site-Associated DNA sequence (RADseq) data were obtained from a subset of individuals as described by Bracco et al.[64] and Galaska et al.[65] In brief, DNA was sent to Floragenex Inc. (Eugene, OR) RAD for library preparation (PstI) and sequencing on an Illumina HiSeq platform. Reads were demultiplexed and quality filtered using the *process_radtags* program in Stacks v2.1[72]. Read clustering and single nucleotide polymorphism (SNP) calling were performed with DeNovoGBS v4.0.1[73] for *C. delta* and *P. biscaya*, and with the gstacks v2.1[72] after mapping to a genome assembly for *S. exserta* and *M. pendula*. We excluded SNP loci that: (1) had more than 10% of missing data, (2) had a minor allele frequency smaller than 0.01, or (3) were not biallelic. We also excluded individuals if they had missing data in >10% of the SNP loci for *S. exserta* and *M. pendula* and >30% for *C. delta* and *P. biscaya* or were identified as clones with R package poppr v2.8.6[74]. To reduce the dimensionality of the genetic data, a principal component analysis (PCA) was performed on the SNP data of each species separately (*S. exserta*, n = 54, 23,869 SNPs; *M. pendula*, n = 100, 9,611 SNPs; *C. delta*, n= 51, 14,013 SNPs, and *P. biscaya*, n = 58, 7,293 SNPs) using the R package ade4 v1.7[75]. The PCA axes were reduced to two dimensions using the uniform manifold approximation and projection method[76] using the R package *umap* v0.2.8. The two-dimensional coordinates were then used in statistical analyses to represent the genotype of each coral.

*Environmental Variables*

Publicly available satellite data and model-derived values for the Gulf of Mexico were obtained to evaluate the influence of environmental variables on microbial communities. The satellite-derived estimates of surface primary productivity ($mg/m^2d$), chlorophyll concentration ($mg/m^3$), inorganic suspended particulate matter concentration ($g/m^3$), and the particulate back-scattering coefficient at 443nm ($m^{-1}$) were obtained from the Copernicus-GlobColour Global Ocean Colour biogeochemical dataset. Model-derived values were obtained for the depth of the surface mixed layer (m), bottom seawater temperature (°C), bottom seawater oxygen concentration (mL/L), bottom seawater salinity, and bottom current magnitude (m/s) from the HYbrid Coordinate Ocean Model (HYCOM). Both datasets comprised monthly means from Jan 2011 to Dec 2018 at 4km grid resolution. The mean and standard deviation of monthly means for each variable were extracted at the sampling location of each coral colony. Only the mean bottom seawater temperature and surface primary productivity were retained for statistical

analyses. Other variables were excluded from the analyses because they were strongly correlated, or not substantially variable across sites, or were not justified by *a priori* hypotheses. See **Supplementary Methods** for further details.

*Statistical Analyses*

Rarefied microbial ASV richness was calculated for each coral colony using the *rarefy* function in the R package *vegan*[77] v2.5.7 using the minimum library size as the sampling depth according to Hurlbert[78]. Pielou's evenness was calculated for each individual using the function *evenness* in the R package *asbio*[79] v1.7 without including zeros. Microbial community richness and evenness were compared between coral species and across sites using paired two-tailed Wilcoxon rank-sum tests and a Bonferroni-adjusted α-value with the function *wilcox.test* in the base R package *stats* v4.1.2. In addition, the effects of depth and longitude on richness and evenness were evaluated for each species with a linear model using depth and longitude as predictors and richness or evenness as response variables independently.

Distance-based redundancy analysis[80] (dbRDA) was performed to evaluate the influence of several factors on microbial community compositions including host species, host genotype, depth, geography, bottom temperature, and surface primary productivity. Longitude was used to test for regional scale patterns across geography while sampling site was used as a finer scale variable. Models were constructed using the R command *dbrda* in the package *vegan* with Bray-Curtis dissimilarities. The significance of the models and their predictor variables were tested with the function *anova.cc*a using 9,999 permutations. A series of dbRDA models were constructed and evaluated to test several hypotheses regarding the influence of each variable. The details of each test are provided in **Supplementary Table F**. In addition, the diversity among *Endozoicomonas* and corallicolids associated with *S. exserta* and *M. pendula* were individually analyzed by applying the same dbRDA analyses described above. Relative abundances were re-calculated for all ASVs classified as *Endozoicomonas* or corallicolids as their relative abundance among each of those taxa. Coral samples without any representatives from the taxon of focus were removed.

Individual ASVs whose abundance differed along depth, longitude, and across sites were identified using Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC)[81] using the R packages *ancombc* v1.4.0 and *phyloseq* 1.38.0. Two separate ANCOM-BC analyses were conducted on each species individually: 1) using both depth and longitude as explanatory variables and 2) a global analysis using sampling sites as groups. ASVs were excluded from the analysis if they were present in fewer than five individual corals (half the colonies sampled at a single site). FDR was controlled using the Benjamini-Hochberg method. Since the global analysis using sampling sites as groups depends on which site was used as the reference, the analysis was repeated using each site as the reference. Only ASVs with a maximum q-value below $\alpha=0.05$ in all permutations were considered significantly different. In general, results were consistent regardless of the reference site. However, a few sites had much higher variation in microbial communities, resulting in many more significantly differential ASVs when used as the reference site. These analyses were repeated on the family level instead of ASVs to inform functional differences.

## Results

*Microbial communities are distinct among octocoral species*

Each coral species had a distinct microbial community in terms of composition and diversity (**Fig. 2A,B**; $R^2$ 43%, $p \leq 10^{-4}$). The microbiomes of *M. pendula* and *P. biscaya* had higher ASV richness and Pielou's evenness than *S. exserta* and *C. delta* (**Fig. 2C,D**; $p \leq 5.4 \times 10^{-6}$). The differences in richness and evenness between species appeared to be driven by the prevalence of a few ASVs that dominated the microbial community of individual colonies (**Fig. 2B**). The microbiomes of all four coral species were dominated by at least one ASV that represented more than 50% of the microbial community in at least one coral colony. A higher percentage of *S. exserta* (94%) and *C. delta* (85%) colonies hosted a dominant ASV compared to *P. biscaya* (70%) and *M. pendula* (38%). *S. exserta* hosted only two dominant ASVs: one classified as BD1-7 clade (Spongiibacteraceae) and another classified as *Mycoplasma*. Both *C. delta* and *P. biscaya* hosted three dominant ASVs. All three dominant ASVs in *C. delta* were recently described[36] as novel members of the class Mollicutes: two *Ca. Oceanoplasma* and one *Ca. Thalassoplasma*. The dominant ASVs in *P. biscaya* were classified as *Mycoplasma*, *Endozoicomonas*, and *Pseudoalteromonas*. *M. pendula* had the greatest diversity of dominant ASVs including two *Mycoplasma*, two *Marinoplasma*, an *Endozoicomonas*, an unclassified alphaproteobacterium, and a corallicolid apicomplexan (**Fig. 2B**). Dominant ASVs belonging to the same taxon were given short identifiers (ex: M1, M2, S2) to distinguish which coral species they were associated with (first letter of coral genus) and the rank of abundance (1 being most abundant).

*Factors shaping microbial community composition*

The microbial communities of all coral species differed significantly between sites. Differences between sites accounted for 33 to 59% of the variation within species (**Supp. Table F**) and were driven by significant trends across both depth ($p \leq 10^{-4}$) and longitude ($p \leq 0.0213$), which could simultaneously account for 11 to 17% and 3 to 12% of the variation, respectively, depending on the coral species. The only exception was *C. delta*, whose microbial community composition did not significantly correlate with longitude. After accounting for depth and longitude, the sampling site was still associated with a significant ($p \leq 0.012$) and substantial amount of additional variation (14-48%) for each coral species.

Several RDAs were conducted with and without depth and longitude to investigate whether bottom temperature, primary productivity, and host genotype contributed to the trends with depth and longitude. While excluding depth, host genotype was significantly correlated with microbial community composition in all species ($p \leq 0.0186$) except *S. exserta* as was bottom temperature ($p \leq 0.0261$) except in *P. biscaya* in which it was not tested due to low variation between sites (4.3-4.8°C). Similarly, surface primary productivity was a significant predictor ($p \leq 0.0114$) in all species except *S. exserta*. After including all variables, depth and longitude still explained a significant amount of variation ($p \leq 0.036$) except depth in *C. delta*.

*Microbiome patterns with depth*

Depth was correlated with microbiome diversity in various ways. In *Muricea pendula*, both richness (**Fig. 2E**; $p = 6 \times 10^{-6}$) and evenness (**Fig. 2F**; $p = 0.012$) decreased significantly with depth. The decrease in diversity in *M. pendula* was great enough that the average richness among colonies from the deepest sites was comparable to that observed in *S. exserta* and *C. delta*.

Conversely, both richness and evenness in *S. exserta* increased with depth ($p<6x10^{-6}$), as did richness in *C. delta* ($p<0.038$).

The relative abundances of multiple microbial taxa strongly correlated with depth including many dominant taxa (**Fig. 3A-D**). In *M. pendula*, *Endozoicomonas* ASV M1 increased in abundance with depth and so did the total abundance of all *Endozoicomonas* ASVs. Similarly, the abundance of the dominant corallicolid ASV M1 increased with depth in both *M. pendula* and *S. exserta*. This was true for the total abundance of all corallicolid ASVs as well (**Fig. 3B, Fig. S1A**). In contrast, other dominant ASVs decreased in abundance with depth: BD1-7 ASV S1 in *S. exserta*, *Ca. Marinoplasma* ASV M1 in *M. pendula*, and both *Ca. Oceanoplasma* ASV C1 and *Ca. Thalassoplasma* C1 in *C. delta*.

In addition, many other taxa that comprised a smaller proportion of the microbial community differed with depth (**Fig. 3E**). *M. pendula* hosted most of these and the majority decreased in abundance with depth. The most abundant of these non-dominant depth-decreasing taxa included photosynthetic groups such as Cyanobiaceae and chloroplasts but also ASVs in the families Spirochaetaceae, Helicobacteraceae, Flavobacteriaceae, Francisellaceae, Burkholderiaceae, and unclassified bacteria that clustered with Spirochaetia and Epsilonproteobacteria. Together with the dominant *Ca. Marinoplasma*, these taxa comprised a mean of 70% of the microbial community among colonies from the shallowest site (Sonnier Bank, 55 m) and decreased down to a mean total of 3% at the deepest site (Bright Bank, 116 m). Although many of these taxa were also detected in *S. exserta*, their abundances were either not significantly correlated with depth or increased with depth. In *C. delta*, the abundances of two sulfur-oxidizing taxa increased with depth: SUP05 and members of the novel family Thiobarbaceae.

In addition to microbial taxa differing in abundance with depth, their ASV compositions also differed. For example, in *S. exserta*, the composition of corallicolid ASVs differed across depth. (**Fig. S1K**, $p\leq10^{-4}$). Notably, *M. pendula* and *S. exserta* each hosted a unique plastotype at the deep site Elvers Bank (96 m). Similarly, the composition of *Endozoicomonas* ASVs in *M. pendula* differed with depth (**Fig. 3F**; $p\leq10^{-4}$). Similar transitions of ASVs within microbial taxa were also prevalent among the associates of *C. delta*. *Ca. Oceanoplasma* ASV C1 dominated the microbial community of most *C. delta* colonies from most sites except the deepest two sites (GC249 and GC290), where the closely related *Ca. Oceanoplasma* ASV C2 dominated some colonies (**Fig. 2B**). This pattern was also seen among *Endozoicomonas* and *Shewanella* associates of *C. delta* (**Fig. 3G,H**). A single common *Endozoicomonas* ASV was present in most colonies from all sites (dark gray), while other ASVs were mostly restricted between three depth zones. More strikingly, *Shewanella* communities between colonies from two depth zones (<637m and >794m) were completely non-overlapping, failing to share a single ASV.

*Microbiome patterns with geography*

Both richness and evenness were higher in eastern than western sites for both *M. pendula* and *S. exserta* ($p<0.0221$). Multiple microbes exhibited differences in abundance across the geographic range of their host coral on a regional scale along the east-west axis (**Fig. 4A-F**). *M. pendula* colonies from eastern sites had higher abundances of microbes that may be free-living such as ASVs classified as *Alteromonas*, *Pseudoalteromonas*, *Pseudomonas*, *Marinobacter*, *Ruegeria*, *Caedibacter*, Sphingomonadaceae, and photosynthetic taxa such as Cyanobiaceae but also microbes that may be symbiotic such as individual *Endozoicomonas* ASVs, corallicolids,

BD1-7, Spirochaetaceae, and the dominant *Mycoplasma* ASV M2. The mean total relative abundance of all ASVs that were more abundant in the east ranged between 0.8% and 26%, excluding the dominant *Mycoplasma* ASV M2 and dominant corallicolid plastotype. Some of these same ASVs and other closely related taxa were also more abundant in *S. exserta* in the east including *Pseudomonas, Pseudoalteromonas, Alteromonas, Ruegeria, Endozoicomonas,* Cyanobiaceae, BD1-7, and Spirochaetaceae (**Supplementary File 1**).

Other ASVs from the same microbial taxa were more abundant in *M. pendula* from western sites such as some classified as Cyanobiaceae, *Mycoplasma*, and *Endozoicomonas* (**Fig. 4C,D).** In addition, ASVs from other taxa were abundant in the west such as several *Ca. Marinoplasma* ASVs and photosynthetic taxa including several chloroplasts. Notably, two ASVs classified as *Prochloron* comprised up to a total of over 50% of the microbial community in some corals from adjacent sites in the west (**Fig. 4C**). Conversely, only two ASVs associated with *S. exserta* were significantly more abundant in the west including an unclassified gamma-proteobacterium and the dominant *Mycoplasma* ASV S1 which was completely absent from all samples from the three easternmost sites (**Fig. 4F**).

*Remaining Site Scale Geographic variation*

Many microbial taxa were not correlated with depth nor longitude but still differed in abundance across sites. In *P. biscaya*, there were no consistent patterns with depth nor longitude in the abundance of individual ASVs. However, there were substantial differences between sites (**Fig. 4G**). For instance, SUP05 had a high abundance in colonies from GC852, *Endozoicomonas* ASV P1 was abundant at DC673 and KC405, *Pseudoalteromonas* ASV P1 was abundant at MC297, and *Mycoplasma* ASV P1 was abundant at most sites but ranged from a mean of 0.1% to 74% across sites.

Microbes whose abundance differed between sites followed two general patterns. First, some microbes were present in most colonies from most sites but differed consistently in abundance across sites, such as the dominant *Mycoplasma* ASVs in *P. biscaya* (**Fig. 4G**), and ASVs classified as Gracilibacteria in *C. delta*. Second, many other microbes were only detected in colonies from a few sites or even a single site. This included a corallicolid only detected in *C. delta* colonies from VK826, GC234, and MC885 and another corallicolid only detected in colonies from GC234 (**Fig. S2G,H**). Other examples involved abundant ASVs such as three close relatives of *Endozoicomonas* in *M. pendula* at McNeil bank averaging a total relative abundance of 26% (**Fig. S2E**). Less abundant examples include potential parasites within Rickettsiales in all coral species.

**Discussion:**

*Differences Between Coral Species*

Coral species was the strongest factor differentiating microbial communities. Species differed markedly in which microbes they associated with and also the diversity of their microbial communities. This finding is consistent with previous coral studies[1, 27, 41, 82, 83]. All dominant ASVs were only abundant (over X%) in one coral species except some corallicolids which were found in both *S. exserta* and *M. pendula* where they totaled X% and Y% on average. The microbial taxa that dominated some coral species belonged to different phyla, such as BD1-7 in *S. exserta* and *Ca. Oceanoplasma* in *C. delta*, suggesting that their metabolic functions differ between coral species. However, some taxa such as *Endozoicomonas* and members of the

Mollicutes were associates of multiple species in this study and other studies suggesting that they may serve common functions across a wide range of coral species. The differences in community structure observed between these four coral species may reflect their unique ecologies. Differences in diversity have been proposed to reflect different ecological strategies[84]. Some corals may be microbial generalists that foster more diverse and flexible microbial communities, like those of *M. pendula* and *P. biscaya*, to respond to changing environmental conditions, while other corals associate with less diverse, more stable, and specialized communities like *S. exserta* and *C. delta*[83, 84] Alternatively, *S. exserta* and *C. delta* may have higher absolute abundances of their dominant associates reducing the richness and evenness of their communities. Measuring absolute microbial abundances would aid the interpretation of differences in richness and evenness between species.

*The Influence of Depth*

Depth strongly structured the microbial communities of all coral species investigated which exhibited differences in composition, diversity, and the abundance of specific microbial ASVs and families. The number and variety of taxon-specific patterns with depth reflect the likelihood that depth structures microbial communities through multiple mechanisms.

For instance, some variation in microbial communities may be due to temperature. Temperature decreases with ocean depth down to ~1500m in the Gulf of Mexico. Depth and temperature were correlated with community composition in all species shallower than this depth. Other studies have shown that shallow-water, scleractinian corals from locations with different temperature regimes host different microbial communities in both the Caribbean and the Red seas[48, 85]. Specifically, the stress of high temperatures has been shown to shape the microbial communities associated with corals[86], especially if they bleach[87-90]. Thus, for the coral species in our study, the microbiomes of colonies may differ across depth in correlation with differing thermal regimes because their host corals experience different frequencies or intensities of thermally induced stress. While temperature may be the underlying cause of some of the variation with depth, it does not appear to be the only factor since depth correlated with additional variation after accounting for temperature.

The genetic divergence of coral populations may further contribute to differentiation in microbial communities across depths. In the scleractinian *Pocillopora damicornis*, genetically distinct coral populations have been shown to harbor distinct microbial communities[43]. In our study, the genotype of coral hosts was significantly correlated with microbial community structure, but only when the correlates of depth and temperature were ignored. While the potential influence of host genotype is difficult to distinguish from correlates with depth, the turnover of the *Shewanella* community is consistent with the population genetic structuring of *C. delta*[63, 64]. Coral colonies from the deepest two sites were genetically distinct and hosted unique *Shewanella* communities. Thus, some of the differentiation in microbial communities across depth may be caused by genetic differentiation of the coral host.

Some depth patterns may reflect habitat type. For instance, members of the SUP05 cluster and the family Thiobarbaceae increased in abundance with depth in *C. delta*. These microbes are sulfur-oxidizers associated with chemosymbiotic fauna[91] and may reflect the greater prevalence of seeps along depth in the northern Gulf of Mexico. One of these microbes, SUP05, has been shown to correlate with markers of chemoautotrophic nutrition in corals[92] and thus may reflect an increase in chemoautotrophic food sources and a decrease in the abundance

of marine snow with depth. Other patterns with depth appear to be driven by different causes. For instance, the diversity of microbial associates of *M. pendula* decreased continuously with depth (Fig. 2E,F). The decrease in the abundance of bacterioplankton with depth could account for some of this. For example, the abundance of likely free-living bacteria found in *M. pendula*, such as *Serratia* and *Achromobacter*, decreased with depth. Further, potentially photosynthetic taxa associated with *M. pendula* predictably decreased with depth, likely reflecting decreasing light levels.

Altogether, depth structures microbial communities through multiple mechanisms, including changes in free-living microbial communities, decreasing light levels, differentiation of host corals, and transitions in habitat types.

Many microbial associates that may be symbiotic differed in abundance with depth, such as *Spirochaeta 2, Ca. Marinoplasma, Endozoicomonas, Mycoplasma, Ca. Oceanoplasma, Ca. Thalassoplasma,* and corallicolid apicomplexans. The reasons for this are unclear because many of these microbe's interactions with their coral hosts are not well characterized. Interestingly, corallicolids increased in abundance with depth in both *M. pendula* and *S. exserta,* but no depth pattern was found in corallicolids that infect *C. delta.* This is in contrast to previous studies that found a decrease in abundance along depth within the range of a shallow-water coral and across a depth range of 2 kilometers[15, 93]. These inconsistencies may be explained if corallicolids utilize intermediate host species like some other apicomplexans and if the distributions of these potential intermediate host species differ with depth in various ways. Some of the closest relatives of corallicolids are found in fish[94-96], opening the possibility that corallicolids may use fish as intermediate hosts and are limited by their depth ranges.

*Variation Across Geography*

In addition to variation across depth, we found that the microbial communities of all four species differed geographically. Corals from different sites differed strongly in microbial communities in all species. but nearby sites showed similarities creating regional-scale geographic variation.

*Regional scale geographic variation*

Regional scale variation was identified in both mesophotic coral species. Both *M. pendula* and *S. exserta* had higher diversity near the mouth of the Mississippi River, and many likely free-living microbial taxa were detected at higher abundances, such as *Alteromonas, Pseudoalteromonas, Pseudomonas,* and photosynthetic taxa. Their increased abundance may reflect the influence of the Mississippi River on bacterioplankton in the region. Further, there are marked longitudinal differences in surface primary productivity in the Northern Gulf of Mexico, higher in the area of influence of the Mississippi. Microbial communities of other shallow-water corals seem to be significantly influenced by surface primary productivity[97]. This is consistent with our observation that microbial communities of octocorals were significantly correlated with surface primary productivity.

However, taxa that are symbiotic, like *Endozoicomonas,* Mollicutes, and corallicolids also differed in abundance regionally consistent with other studies that found geographic variation in *Endozoicomonas* and *Mycoplasma* associates in shallow water corals[21, 26]. Some of the regional patterns in these potentially symbiotic taxa may result from limited dispersal since

some were restricted to nearby sites like *Mycoplasma* ASV S1, which was completely absent in all corals from the three easternmost sites.

*Site scale geographic variation*

For every coral species, microbial communities differed strongly between sites after accounting for depth and geographic region. This is likely due to differences in local conditions between sites that influence microbial community structure. For instance, the high abundance of the SUP05 cluster in *P. biscaya* at GC852 may reflect the presence of seeps nearby[98] (< 450 m). Similarly, the abundance of *Pseudoalteromonas* in *P. biscaya* at the Mississippi Canyon sites may reflect the lasting impact of the Deepwater Horizon oil spill[58, 68, 99]. Consistent deviations from depth trends at certain sites suggest that other factors in addition to depth play a role in determining the abundance of certain groups, such as the total corallicolid abundance in *M. pendula* at Roughtongue Reef which was higher than the trend with depth (Fig 3B). Conversely, corals from some subsets of sites displayed similar microbial communities despite being far apart and inhabiting different depths. For instance, *P. biscaya* from both KC405 and DC673 were dominated by *Endozoicomonas* ASV P1, but it comprised no more than 0.12% in any colony from any other site. These sites are the farthest apart and do not have overlapping depths. Interestingly, corals from these two populations are closely related[65] suggesting an influence of the coral host's genotype on microbiome composition.

Implications for restoration efforts

Characterizations of coral microbiomes based on samples from a single site or even a few may miss substantial variation across a species' range. This is important to consider in restoration plans that utilize collections from different locations. For example, a restoration plan could use this information to avoid inadvertently transmitting parasites to new sites through transplants or to avoid rearing infected and non-infected corals together in aquaria. Examples of geographic variation in potential parasites discovered in this work include corallicolids in *C. delta* at VK826, MC885, and GC234 and the unique corallicolids in *M. pendula* and *S. exserta* at Elvers Bank.

Conclusion:

This work characterizes the microbial communities of four dominant coral species in the Gulf of Mexico and demonstrates substantial variability across their ranges. These results provide promising targets to study the interactions between corals and their associated microbes. They also reveal substantial variability associated with depth and geography that could only be uncovered through extensive sampling. The numerous patterns associated with depth demonstrate that in addition to its ecological and evolutionary effects on corals, it also strongly structures their microbial communities.

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Competing Interests:

The authors declare no competing interests.

Data Availability Statement:

16S amplicon sequence data were submitted to the Sequence Read Archive (SRA) under Bioproject number PRJNA875098.

Figures:

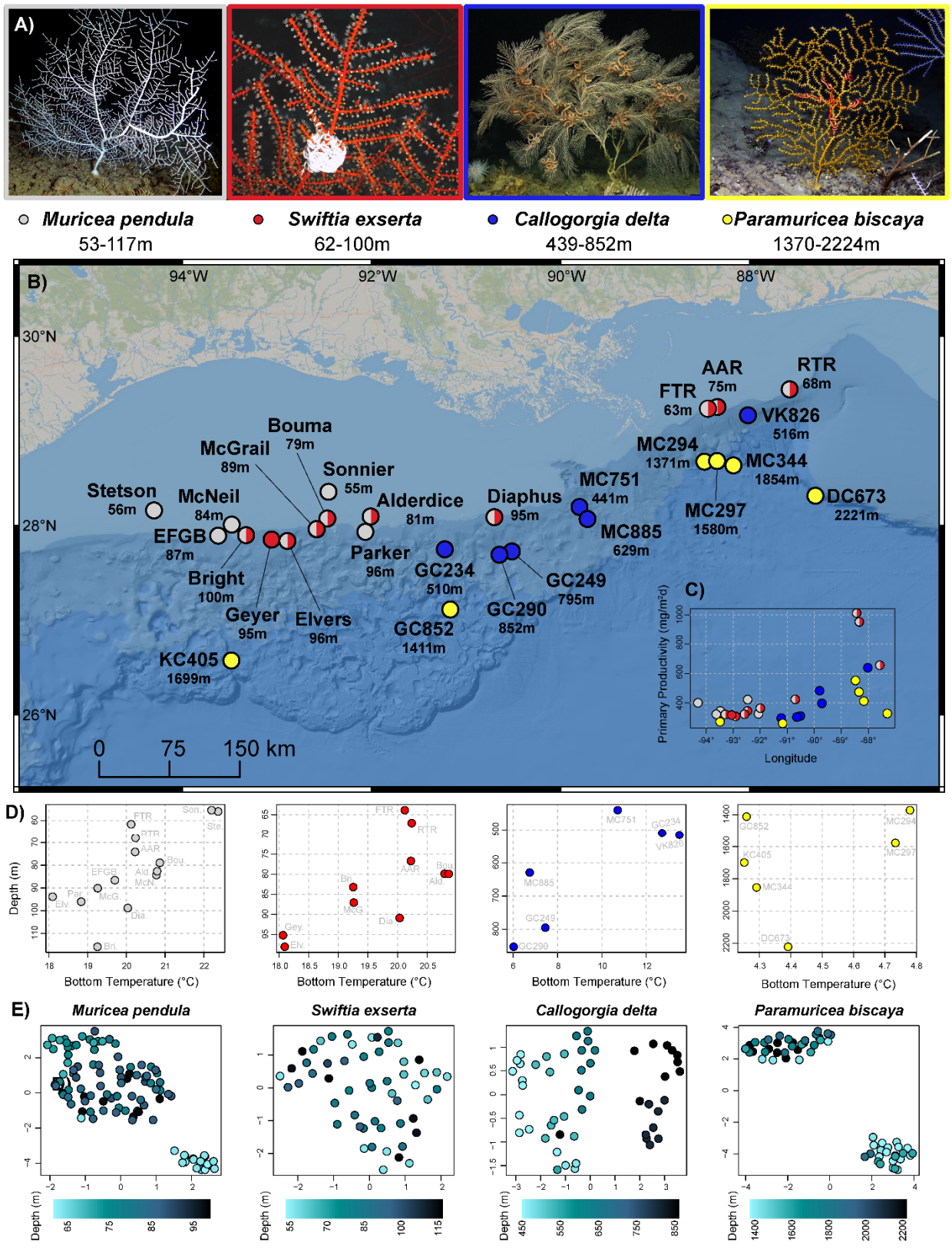

A)
Muricea pendula
53-117m
Swiftia exserta
62-100m
Callogorgia delta
439-852m
Paramuricea biscaya
1370-2224m
B)
94°W
92°W
90°W
88°W
30°N
28°N
26°N
AAR
75m
RTR
68m
FTR
63m
VK826
516m
MC294
1371m
MC344
1854m
MC751
441m
DC673
2221m
MC297
1580m
MC885
629m
Bouma
79m
McGrail
89m
Sonnier
55m
Stetson
56m
McNeil
84m
Alderdice
81m
Diaphus
95m
EFGB
87m
Parker
96m
GC234
510m
GC249
795m
Bright
100m
Geyer
95m
Elvers
96m
GC852
1411m
GC290
852m
KC405
1699m
0
75
150 km
C)
Primary Productivity (mg/m²d)
Longitude
D)
Depth (m)
Bottom Temperature (°C)
E)
Muricea pendula
Swiftia exserta
Callogorgia delta
Paramuricea biscaya
Depth (m)

Fig. 1: Sampling sites and their environmental and coral genotypic makeup. (A) In situ mages of the octocoral species in this study. (B) Geographic map displaying sampling locations in the Northern Gulf of Mexico (BOEM bathymetry). The mean seafloor depth of all samples from a site is reported next to each site name in meters. Sites are colored based on which coral species were sampled: light gray = *Muricea pendula*, red = *Swiftia exserta*, blue = *Callogorgia delta*, yellow = *Paramuricea biscaya*. Images of *C. delta* and *P. biscaya* courtesy of the ECOGIG consortium. (C) Primary productivity of each sampling site displayed across longitude and colored by coral species sampled. (D) Bottom seawater temperature for each sampling site displayed across depth for each coral species. (E) UMAP projections of the genetic background of coral colonies based on RADseq data. Each point represents the genotype of a coral colony and is colored by depth in meters.

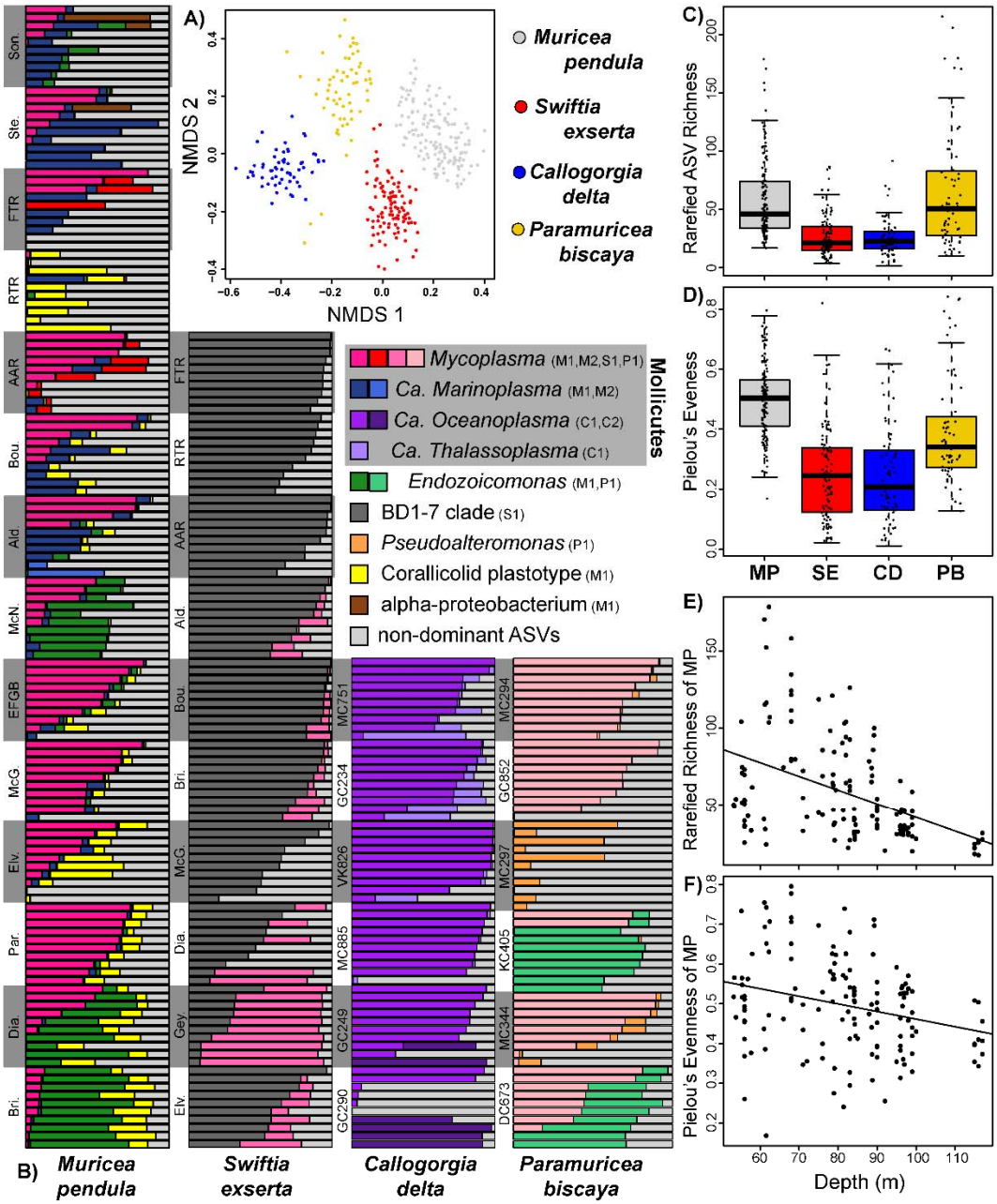



Fig. 2: Characterization of coral microbiomes. A) Non-metric multidimensional scaling plot of microbiome compositions using Bray-Curtis dissimilarities of ASV proportions. B) Barplots of dominant ASVs in each coral species. Each horizontal bar is an individual coral colony. Colonies are grouped by site which are sorted by depth. ASVs that comprised 50% or more in any sample are designated with different colors. All other ASVs are collapsed and shown in light gray. C) Rarefied ASV richness and D) Pielou's evenness between different coral species. MP = *Muricea pendula*, SE = *Swiftia exserta*, CD = *Callogorgia delta*, PB = *Paramuricea biscaya*. E) Rarefied ASV richness and F) Pielou's evenness in *Muricea pendula* with depth.

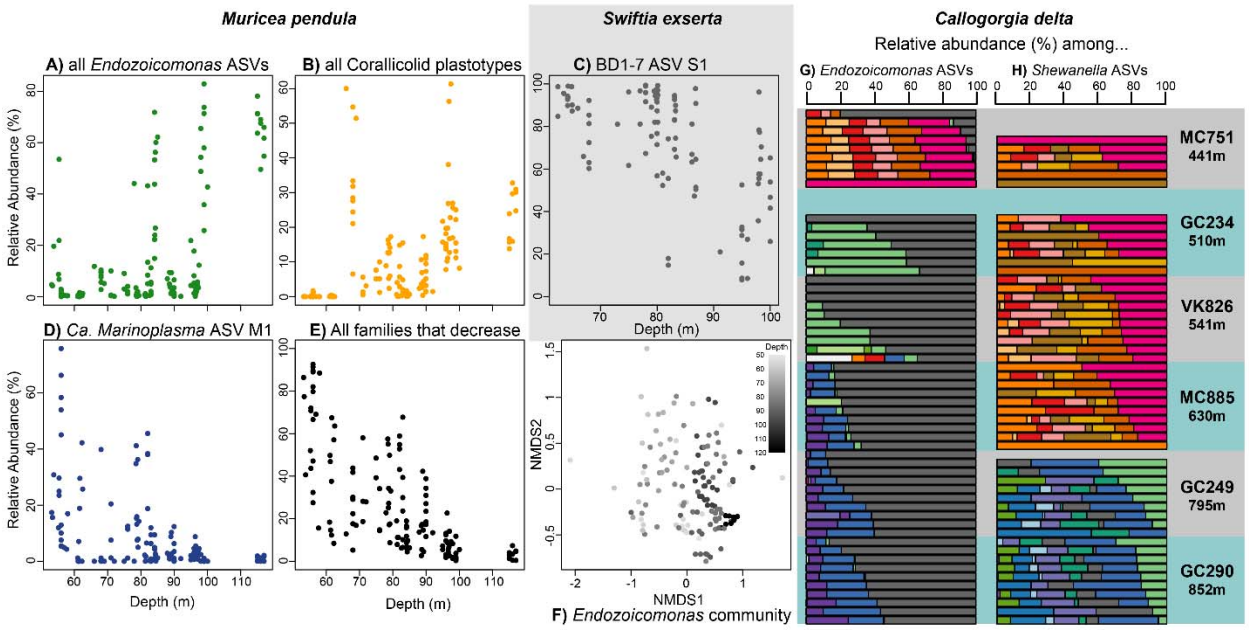
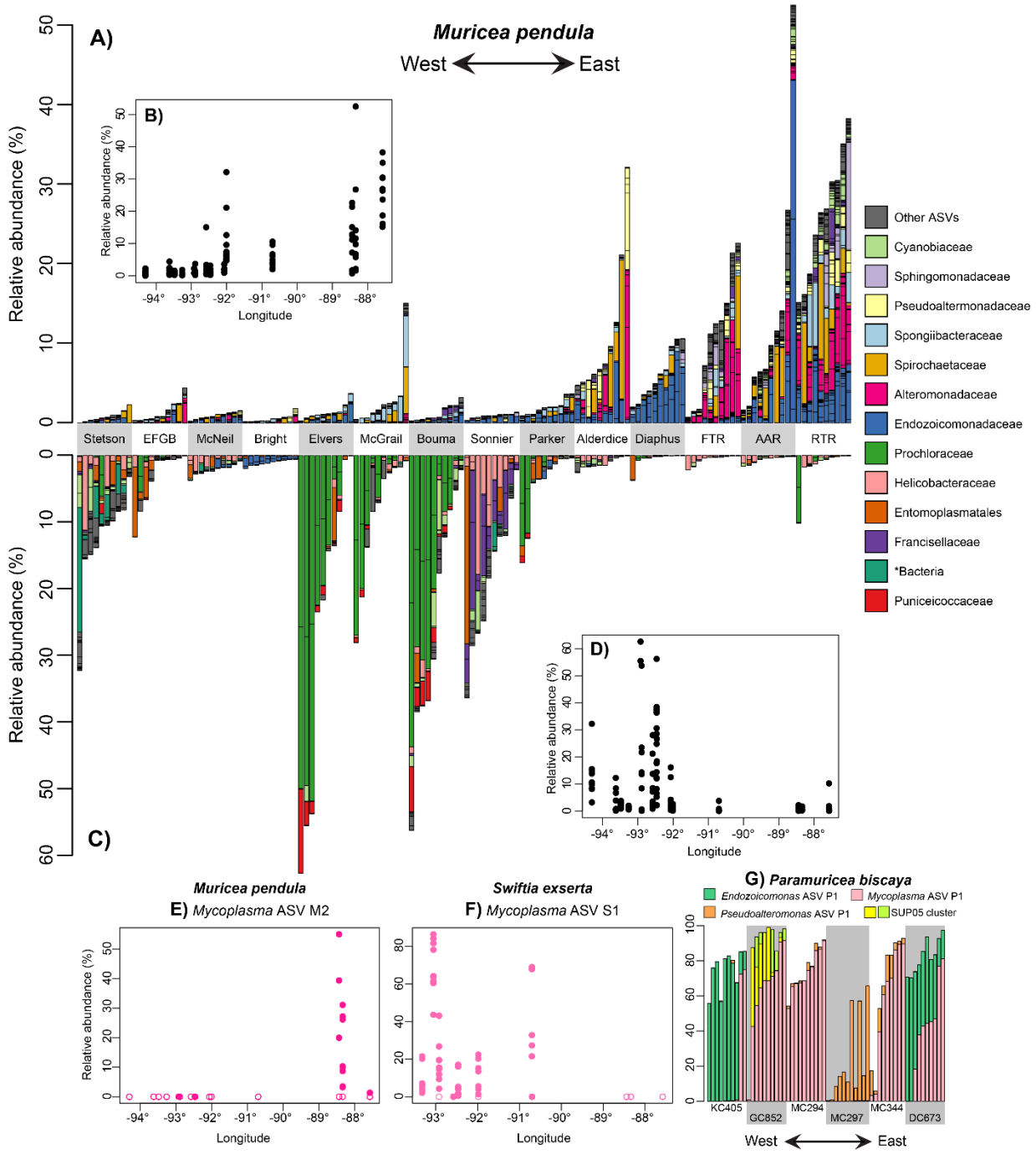



Fig. 3: Examples of depth patterns in coral microbiome composition. The relative abundances along depth in *Muricea pendula* of A) the sum of all *Endozoicomonas* ASVs, B) the sum of all corallicolid plastotypes, D) *Ca. Marinoplasma* ASV M1, E) the sum of all microbial families that decreased significantly with depth. F) NMDS ordination of *Endozoicomonas* communities in *M. pendula* showing patterns with depth. C) Relative abundance of BD1-7 ASV S1 with depth in *Swiftia exserta*. The community composition of G) *Endozoicomonas* and H) *Shewanella* communities in *Callogorgia delta*. Each horizontal bar is a single coral colony whose composition reflects the relative abundances of each ASV when restricted to only *Endozoicomonas* or *Shewanella* ASVs. Colors denote different ASVs. Colonies are arranged by site in order of increasing mean depth.


A)
Muricea pendula
West
East
Relative abundance (%)
B)
Relative abundance (%)
Longitude
Stetson
EFGB
McNeil
Bright
Elvers
McGrail
Bouma
Sonnier
Parker
Alderdice
Diaphus
FTR
AAR
RTR
Other ASVs
Cyanobiaceae
Sphingomonadaceae
Pseudoaltermonadaceae
Spongiibacteraceae
Spirochaetaceae
Alteromonadaceae
Endozoicomonadaceae
Prochloraceae
Helicobacteraceae
Entomoplasmatales
Francisellaceae
*Bacteria
Puniceicoccaceae
C)
D)
Muricea pendula
E) Mycoplasma ASV M2
Swiftia exserta
F) Mycoplasma ASV S1
G) Paramuricea biscaya
Endozoicomonas ASV P1
Mycoplasma ASV P1
Pseudoalteromonas ASV P1
SUP05 cluster
KC405
GC852
MC294
MC297
MC344
DC673
West
East

504

Fig. 4: Examples of geographic patterns in coral microbiome composition. Barplots of the relative abundance of microbial ASVs from *Muricea pendula* that differed significantly across longitude and were associated with A) eastern and C) western areas of its range in the Northern Gulf of Mexico. ASVs are colored by family or other taxonomic level detailed in the legend. Each coral colony is represented by a vertical bar, and colonies are grouped by site sorted from westernmost to easternmost starting from the left. The total relative abundances of these ASVs are shown in B) and D) plotted against longitude to better reflect relative distances between sites. The relative abundance of E) *Mycoplasma* ASV M1 in *M. pendula* and F) *Mycoplasma* ASV S1 in *S. exserta* along longitude. Samples where zero reads were detected are represented with open circles. G) Barplots showing the five most abundant ASVs that differed between sites in *P. biscaya.*

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