



Bacterial Communities in Coral Offspring Vary Between *in situ* and *ex situ* Spawning Environments

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Examining the bacterial communities of offspring is key to understanding the establishment of coral-bacteria associations. Although high sensitivity to the environment is expected, previous studies have only examined bacterial communities of coral offspring in *ex situ* (laboratory) environments, not in *in situ* (field) environments. Here, we examined and compared the effect of *ex situ* and *in situ* environments on bacterial communities of newly released offspring (eggs and larvae) and their maternal colonies in two phylogenetically distant coral species with different reproductive modes: *Dipsastraea speciosa* (Scleractinia; spawner) and *Heliopora coerulea* (Octocorallia; brooder). Our results demonstrated that the spawning environments do not affect the bacterial composition in maternal colonies, but influence that of the offspring (eggs of *D. speciosa* and larvae of *H. coerulea*). Dominant bacterial operational taxonomic units (OTUs) varied between *in situ* and *ex situ* environments in the eggs of *D. speciosa*. The composition of bacterial communities among larvae of *H. coerulea* samples was more diverse in *in situ* environments than in *ex situ* environments. This study provides the first information on *in situ* bacterial communities in coral eggs and larvae and highlights their sensitivities to the local environment. Future studies must take into consideration the influence of *ex situ* environments on bacterial communities in coral offspring.

Keywords: bacterial communities, coral offspring, sampling method, alpha diversity, beta diversity

INTRODUCTION

Resident bacterial communities are known to profoundly influence the physiology and health of both flora and fauna (Thompson et al., 2015; Wang et al., 2017; Douglas, 2019; van Oppen and Blackall, 2019; Apprill, 2020). Likewise, in reef-building corals, recent studies have found that coral-associated bacteria play important functional roles, such as protection against pathogens and the provision of nutrients (carbon, nitrogen, and sulfur). For example, diazotrophs that inhabit within the coral tissue or skeleton (Rohwer et al., 2002; Radecker et al., 2015) provide fixed nitrogen products that are crucially limiting resources in oligotrophic coral reef waters, sustaining their high productivity (Lesser et al., 2007).

Despite the importance of coral-bacteria associations, we know very little about the mechanisms relating to the establishment. Studying microbial communities in coral offspring is key to understanding the establishment of the coral-microbe association, but nevertheless, the majority of knowledge comes from studies on coral endosymbiotic algae (Symbiodiniaceae) in coral offspring, closely related to the reproductive mode of coral host. Most brooding corals release larvae with endosymbiotic algae, whereas spawning corals release eggs without those (Bright and Bulgheresi, 2010; Hartmann et al., 2017). However, such information has not been studied well in coral-bacteria associations.

Previous studies have examined coral-associated bacteria in coral offspring (eggs and larvae) in *ex situ* (laboratory) environments, not in *in situ* (field) environments (Supplementary Table 1). This difference could lead to there being a bias of real bacterial communities in coral offspring as a few studies have demonstrated that environmental conditions are an important factor that affects the bacterial communities in coral offspring (Lema et al., 2016; Leite et al., 2017; Zhou et al., 2017; Bernasconi et al., 2019; Damjanovic et al., 2020a). In adult corals, there was a study recently published showing the difference in bacterial communities between *ex situ* and *in situ* environments (Damjanovic et al., 2020b). However, no such *ex situ* and *in situ* comparisons were studied for the bacterial communities in coral offspring, in spite of that bacteria and coral offspring are sensitive to the environment (Chan et al., 2019; Epstein et al., 2019).

Here we examined the effect of *in situ* and *ex situ* environments on bacterial communities of coral offspring and maternal corals in two coral species with different reproductive modes; *Dipsastraea speciosa* (Scleractinia; spawner) and *Heliopora coerulea* (Octocorallia; brooder). Our results indicate that bacterial communities vary between *in situ* and *ex situ* environments in coral offspring, but not in maternal corals. The results highlight the sensitivity of bacterial communities in coral offspring to the environment and bring to light a potential bias introduced by customary *ex situ* sampling methods that may affect studies on bacteria associated with coral offspring.

MATERIALS AND METHODS

We examined bacterial communities of offspring (eggs or larvae) and maternal corals in two coral species with different reproductive modes, *D. speciosa* (spawner) and *H. coerulea* (brooder, branching morphotype; Yasuda et al., 2014). *D. speciosa* is hermaphroditic spawner that releases buoyant egg-sperm bundles into the water column for external fertilization during annual spawning events (Lin and Nozawa, 2017). *H. coerulea* is a gonochoric brooder, which releases planula larvae in early summer, that is neutral to negative buoyancy (Babcock, 1990; Harii et al., 2002). Larvae of *H. coerulea* are brooded for up to 2 weeks inside polyps and then moved to the surface of maternal colonies for “surface brooding” for ≈ 4 days before detaching for dispersal (Babcock, 1990; Harii and Kayanne, 2003). Two sampling methods were employed in this study; *ex*

situ (laboratory) sampling and *in situ* (field) sampling (Table 1). Sampling was conducted at Lyudao (Green Island), Taiwan in April and May 2018 (Figure 1A).

Ex situ Sampling

Seawater used in the experiment was collected from Gongguan and stored in a 1,500-L tank at the beginning of the experiment (April 26; 4 days before the full moon). The seawater stock tank was placed in the hallway of the Green Island Marine Research Station (GIMRS) in the shade, and seawater was aerated with air stones. Seawater from the stock tank was used for coral rearing for the *ex situ* sampling.

For *D. speciosa*, one fragment (≈ 10 cm in diameter) with mature pigmented eggs (violet gray) was collected from five colonies using a hammer and a chisel at 3–8 m depth in Gongguan, Lyudao on April 27 (3 days before the full moon; Figure 1A). The fragments were kept in a Gongguan harbor (≈ 3 m depth) for 1 day to allow for recovery and then transferred to GIMRS (Figure 1A). The five fragments were placed separately in 20-L rearing tanks with aerated filtered seawater (100 μm) in the laboratory. Seawater temperature was maintained at $\approx 26^\circ\text{C}$ by an air-conditioner. The rearing tanks were kept under fluorescent light (6,000 lux) during daytime (6:00–18:00) and kept in darkness during nighttime (18:00–6:00) to induce spawning (Lin et al., 2021). A quarter of the seawater in the rearing tank was changed every 3 days with filtered seawater (100 μm) from the seawater stock tank.

The spawning of *D. speciosa* fragments was monitored daily by using a plastic cup placed upside down on the top of each fragment. When fragments spawned at $\approx 22:00$, egg-sperm bundles were released from polyps, floated to the water surface, and gradually disintegrated into eggs and sperm. As eggs have strong buoyancy, eggs were trapped inside the reversed cup, while sperm dispersed into the water. Spawning of *D. speciosa* occurred on May 1 and 2 [1 and 2 days after the full moon (AFM)] and spawned eggs (≈ 200 eggs) were collected at 7:00 the following morning (≈ 9 h after spawning). Egg samples from 2 fragments were collected on the first day (sample ID: ds2l and ds4l) and others on the second day. After spawning, a small tissue sample (≈ 1 cm in diameter) was collected from each maternal coral (fragment).

For *H. coerulea*, one branch (2 cm in diameter, 5–8 cm in length) that had many brooded larvae partially emerge from polyps on the branch surface was collected from each of the five female colonies using a hammer and a chisel at 5–8 m depth at Daibaisa, Lyudao on May 1 (1 day AFM; Figure 1A). Each branch was kept in a zip-lock bag with seawater, transferred to GIMRS, and placed in a 3-L rearing tank with aerated filtered seawater (100 μm). Seawater from the 1,500-L seawater tank was used in the rearing tank, and filtered seawater was changed every 1–2 days. During the rearing period, larvae of *H. coerulea* gradually appeared, detached from polyps, and drifted into the water column (Harii and Kayanne, 2003). Ten to fifteen larvae from each branch that were released on May 5 (5 days AFM) were collected in the rearing tank by a dropper. After collecting larvae, a small tissue sample (≈ 1 cm in diameter) was collected from each branch using a nipper as a sample of the maternal

TABLE 1 | Summary of samples used in the present study.

Species	Reproductive mode	Taxon (subclass/order/family)	Sampling method	Sample name	
				Offspring	Mother
<i>Dipsastraea speciosa</i>	Spawner	Hexacorallia/Scleractinia/Marulinidae	<i>In situ</i>	ds1f, ds2f, ds3f, ds4f, ds5f	DS1F, DS2F, DS3F, DS4F, DS5F
			<i>Ex situ</i>	ds1l, ds2l, ds3l, ds4l, ds5l	DS1L, DS2L, DS3L, DS4L, DS5L
<i>Heliopora coerulea</i>	Brooder	Octocorallia/Helioporacea/Helioporidae	<i>In situ</i>	hc1f, hc2f, hc3f, hc4f, hc5f	HC1F, HC2F, HC3F, HC4F, HC5F
			<i>Ex situ</i>	hc1l, hc2l, hc3l, hc4l, hc5l	HC1L, HC2L, HC3L, HC4L, HC5L

Abbreviations in sample names denote as follows; the first 2 characters represent coral species (DS for *Dipsastraea speciosa*, HC for *Heliopora coerulea*); the number (1–5) is replicate colony number; the last character is sampling method [F for the *in situ* (field) samples and L for the *ex situ* (laboratory) samples]; capital letters for maternal coral samples and small letters for offspring samples.

colony. A 1-L seawater sample was collected from the 1,500-L seawater tank for reference of environmental microbes for the *ex situ* samples of both species on May 1 (1 day AFM).

In situ Sampling

The spawning timing of *D. speciosa* is well-documented at the study location and was expected between 21:00 and 22:00 around 5–7 days AFM from April to June (Lin and Nozawa, 2017). Colonies of *D. speciosa* that had matured pigmented eggs (violet gray) were selected by visually examining small fragments of colonies broken off using a hammer and a chisel at 3–7 m depth at Gongguan on May 3 (3 days AFM). A gamete trap consisting of a funnel and a 50 ml falcon tube was positioned on each of the 5 selected colonies (Figure 1B). Spawning of the colonies was monitored daily from 21:00 to 22:00 using scuba from May 3 (3 days AFM) and was observed from 21:00 to 22:00 on May 5 (5 days AFM). Egg-sperm bundles were released from colonies, floated toward the water surface, and were captured by the gamete trap (Figure 1B). Spawning gametes in the falcon tube were collected at 22:00 on the spawning night. A tissue sample was taken from the five colonies using a hammer and a chisel and kept in a zip-lock bag with seawater. A 1-L seawater sample was also collected as a sample of environmental bacteria at 3-m depth.

For *H. coerulea*, larvae loosely attached to the colony surface were collected *in situ* from five colonies at 1–3 m depth in Gongguan on May 5 (5 days AFM; Figure 1B). Larvae from each colony were collected using a dropper and kept in a 15-ml falcon tube. A tissue sample from each colony was collected using a hammer and a chisel and kept in a zip bag with seawater. One-liter seawater sample was collected as a sample of environmental bacteria at 3-m depth.

Total DNA Extraction and Amplification

All *ex situ* and *in situ* samples of gametes, larvae, and maternal coral tissue were washed twice with filtered seawater (0.22 μm), and the sperms were filtered out during washing, then coral samples were stored in 99% ethanol at –20°C at GIMRS. The seawater samples were filtered through cellulose acetate membranes with 0.2-μm pores (Aventec, Tokyo, Japan) and the membranes were stored at –20°C. All coral samples were washed with tris-EDTA (TE) buffer (10 mM Tris-HCl and 1 mM

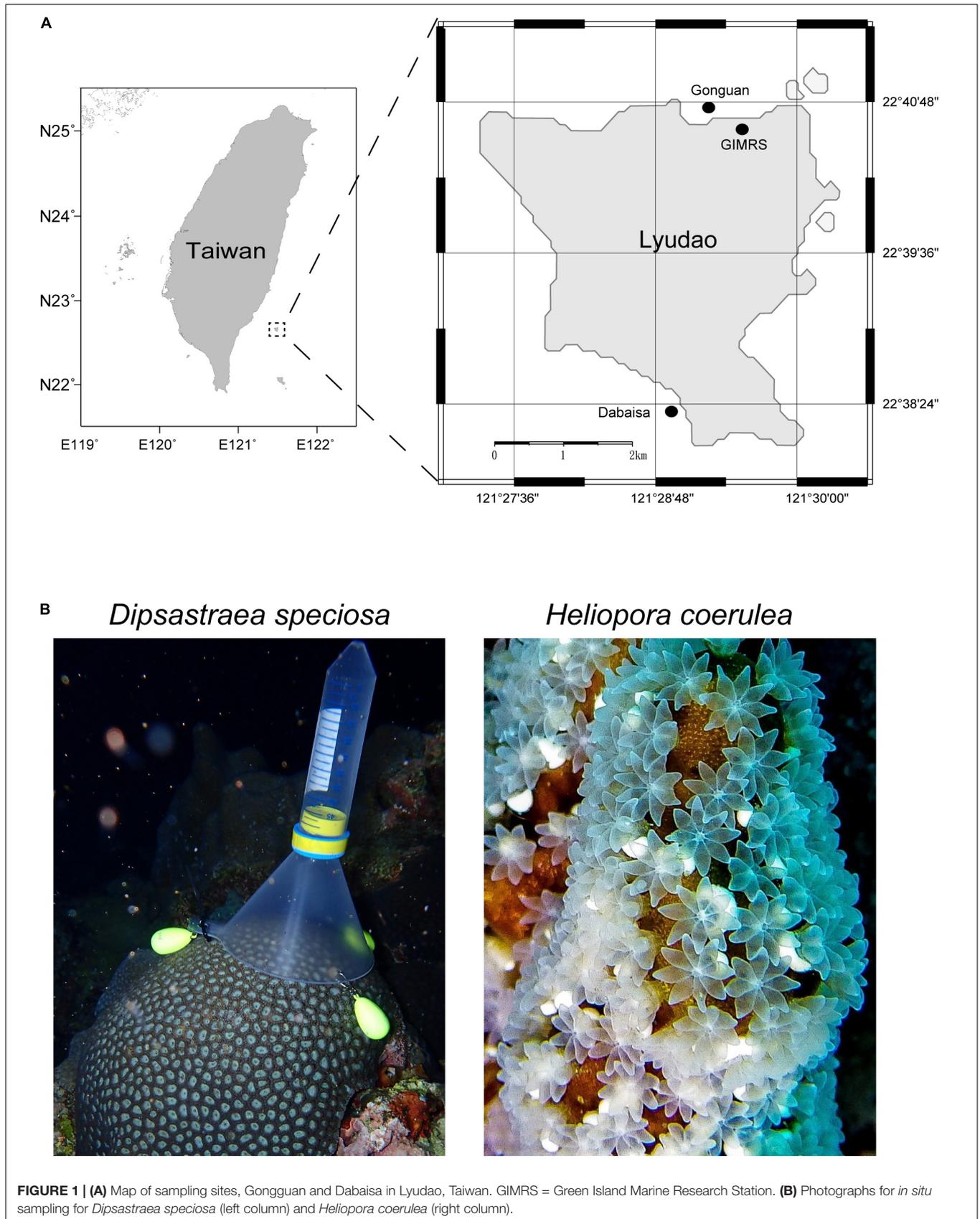
EDTA, pH 8) before DNA extraction. Egg and larval samples were frozen in liquid nitrogen and then homogenized using a sterile plastic pestle in 1.5 ml tubes. Coral tissue samples that contained skeletons were grounded using a sterile stainless mortar and pestle with liquid nitrogen. The homogenized tissues and membranes of seawater samples were suspended in TE buffer for total DNA extraction using the modified traditional cetyltrimethylammonium bromide (CTAB) method (Wilson, 2001; Hong et al., 2009).

Amplification of the 16S ribosomal RNA gene was performed by PCR using a pair of universal bacterial primers: 968F (5'-AACGCGAAGAACCCTTAC-3') and Uni1391R (5'-ACGGGCGGTGWGTRC-3') for the bacterial V6–V8 hypervariable regions (Nubel et al., 1996; Jorgensen et al., 2012). The PCR was performed in 50 μl reaction volumes, consisting of 2.5 U TaKaRa Ex Taq (Takara Bio, Otsu, Japan), 1X TaKaRa Ex Taq buffer, 0.2 mM deoxynucleotide triphosphate mixture (dNTP), 0.2 mM of each primer, and more than 20 ng of total DNA. The thermocycler was set to an initial step of 94°C for 3 min, 30 cycles of 94°C for 30 s, 55°C for 10 s, 72°C for 15 s, and a final extension of 72°C for 5 min. Target DNA bands (≈420 bp) were examined on 1.5% agarose gel after electrophoresis and eluted using QIAEX II Gel Extraction Kit (Qiagen, Valencia, CA, United States).

To tag each of the bacterial V6–V8 amplicon with a unique barcode sequence, each tag primer was designed with four overhanging nucleotides at 5' ends of the common primers (Supplementary Table 2). The tagging reaction was performed with a 5-cycle PCR, each cycle was run at 94°C for 30 s, 56°C for 10 s, and 72°C for 20 s with the modified primers. End products were purified by the same gel elution method described above, and DNA concentration was determined with the Qubit dsDNA HS assay (Invitrogen, Carlsbad, CA, United States).

Sequencing and Data Processing

We pooled all V6–V8 amplicons of coral and seawater samples equally for 2 × 250 pair-end reads in Illumina NovaSeq paired-end sequencing (BIOTools Co., Taipei, Taiwan). High-quality reads that were produced after raw reads were merged, sorted, and trimmed using mothur (Schloss et al., 2009) based on the following criteria: (1) reads of 350–500 bp long, (2) average



quality score > 27, (3) homopolymer length < 8 bp, and (4) reads with any ambiguous base (N) removed. Then the four base tags and primer sequences were removed.

For the operational taxonomic unit (OTU) analysis, quality-filtered reads were pooled together and OTUs were assigned at 97% identity with the UPARSE pipeline (Edgar, 2013). In UPARSE, de-replication was performed and singletons were excluded (options: `-derep_prefix` and `-minsize 2`). Each OTU was classified with a bootstrap value set to 0.8 using a classifier (Wang et al., 2007) against the SILVA database (release 128) implemented in mothur. OTUs that were not assigned to a *Bacteria* domain or those that were assigned to Chloroplast were removed in subsequent analyses.

Data Analyses

Alpha-diversity and beta-diversity of bacterial communities were calculated after rarefying to an even 8,000 sequence depth in each sample by USEARCH (v10; options: `-otutab_norm`). For alpha-diversity, the Shannon index, Chao1 richness, and Species Evenness were calculated. Kruskal-Wallis tests were conducted to detect significant differences in the indexes of each coral species. Comparisons were made between the *in situ* and *ex situ* samples of eggs, larvae, and maternal colonies and between offspring (eggs or larvae) and maternal colonies within the *in situ* or *ex situ* samples. Statistical analyses were performed using R ver. 3.6.2 (R Core Team, 2019).

For beta-diversity, relative abundances of bacterial families (Supplementary Figure 1) and OTUs were calculated for individual samples (Chen et al., 2011), and then the Bray-Curtis distance was estimated between samples (Bray and Curtis, 1957). Based on the Bray-Curtis distance, the non-metric multidimensional scaling (nMDS) analysis and the agglomerative hierarchical clustering (CLUSTER) with complete-linkage were performed using the Primer 6 software (PRIMER-E, Lutton, Ivybridge, United Kingdom). nMDS plot with 95% CIs around each group was visualized with the “`stat_ellipse`” function in R. Significant variations between *in situ* and *ex situ* samples were examined using Analysis of Similarity (ANOSIM) in the Primer 6 software.

Data Availability Statement

The data sets generated for this study can be found in the NCBI SRA PRJNA706097.

RESULTS

The data set of bacterial communities consisted of 2,361,933 reads from a total of 40 samples of offspring and maternal corals and three seawater samples after quality control and removal of no-*Bacteria* and *Chloroplast* OTUs. The number of reads per sample ranged between 8,277 and 179,418, with an average of 54,928 reads per sample, representing 6,601 OTUs. The data set was rarefied to the lowest number reads (8,000 reads) across samples, resulting in 5,181 OTUs for the analysis (Supplementary Figure 2 and Supplementary Table 3).

Alpha diversity of bacterial communities was compared using the Shannon, Chao1, and Evenness indices (Figure 2). There was no significant variation in alpha diversity of bacterial communities between *in situ* and the *ex situ* samples in maternal corals and offspring of each coral species (Kruskal-Wallis tests; $p > 0.05$). Although there was a tendency of higher alpha diversity in offspring than maternal colonies in *D. speciosa* and the opposite in *H. coerulea*, these were not statistically significant (Kruskal-Wallis tests; $p > 0.05$).

Beta diversity of bacterial communities was grouped into three clusters; maternal corals, offspring, and seawater samples (ANOSIM, $R = 0.56$, $p < 0.001$; Figure 3A). The bacterial communities of maternal corals were clustered by species (2-way crossed ANOSIM, $R = 0.97$, $p < 0.001$), except for sample “DS4F” (Figure 3A), whereas the bacterial communities of offspring were primarily grouped by the *in situ* and *ex situ* sampling environments (2-way crossed ANOSIM, $R = 0.71$, $p < 0.001$). The nMDS analysis indicated similar patterns, with a wide distribution of *in situ* samples of offspring, especially for *H. coerulea* larvae (Figure 3B).

The top 30 OTUs (> 0.09% in cumulative relative abundance) of bacteria in the maternal corals and offspring were compared between the *in situ* and *ex situ* samples for each species (Figure 4 and Supplementary Table 4). *D. speciosa* had three OTU clusters, Groups 1–3 (Figure 4A and Supplementary Table 4A): The maternal coral samples were dominated by OTU Group 2. However, the offspring samples were dominated by OTU Group 1 for the *in situ* samples, and OTU Group 3 for the *ex situ* samples. Dominant bacterial OTUs in Group 2 were almost absent in both offspring and seawater samples, whereas some dominant OTUs in the offspring samples were detected in maternal corals.

In *H. coerulea*, there were four OTU clusters, Groups 4–7 (Figure 4B and Supplementary Table 4B). The maternal corals were dominated almost evenly by OTUs in Groups 4, 5, and 7, irrespective of sampling environments. The OTUs dominated in maternal corals were also dominant in offspring, except for sample “hc2f.” The offspring samples were dominated by OTUs in all four OTU groups: OTUs in Group 6 were more abundant in *in situ* samples, whereas OTUs in Group 4 were more common in *ex situ* samples (Figure 4B). Four OTUs were detected in offspring samples of both coral species; OTU1 and OTU5 in *in situ* samples and OTU12 and OTU18 in *ex situ* samples (Figure 4).

DISCUSSION

This study examined the effect of *in situ* and *ex situ* environments on bacterial communities of both offspring (eggs and larvae) and maternal corals in two phylogenetically distant coral species, with different reproductive modes; *D. speciosa* and *H. coerulea*. Our results demonstrate that spawning environments influence the composition of bacterial communities in offspring, but not in maternal corals. These findings are in agreement with previous studies that conclude bacterial communities in coral offspring are sensitive to local environments (Apprill et al., 2009; Zhou et al., 2017; Chan et al., 2019; Epstein et al., 2019) and highlight

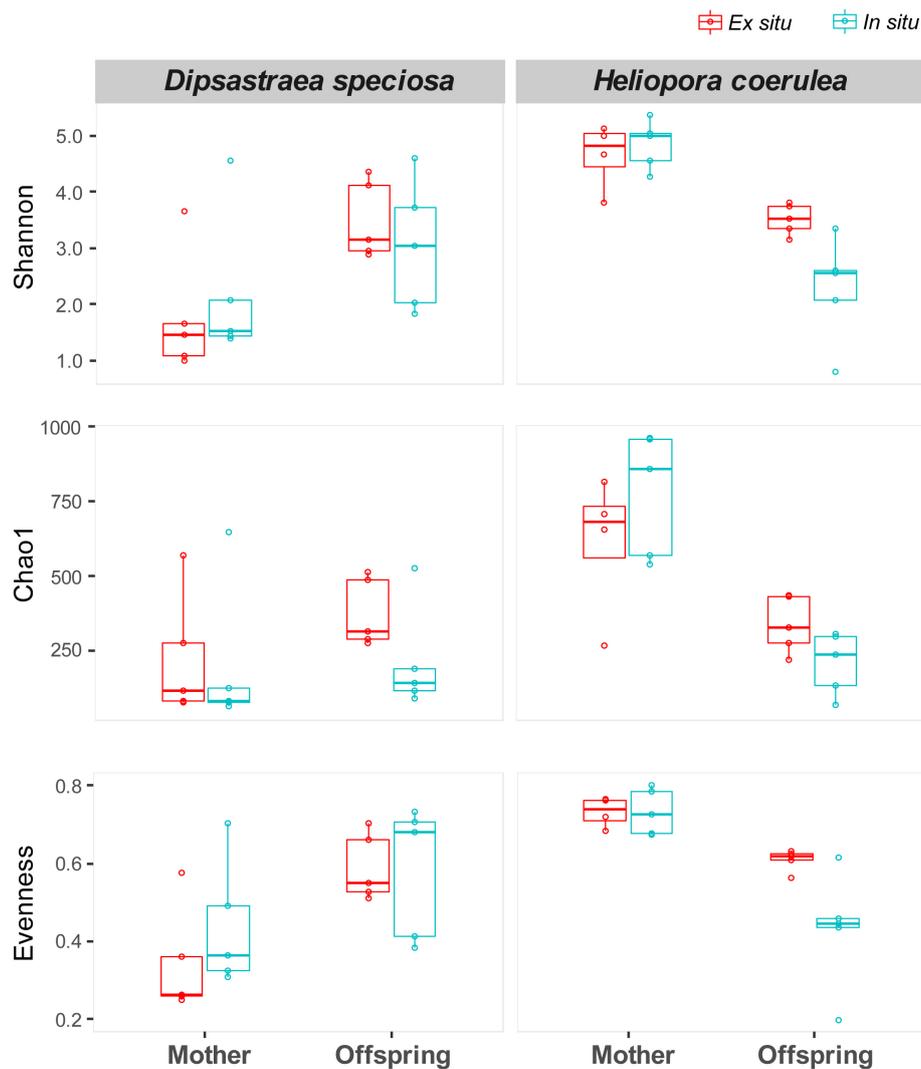


FIGURE 2 | Alpha-diversity indices of bacterial communities (Shannon diversity, Chao 1 richness, and evenness) for the *in situ* (cyan) and *ex situ* samples (red) of maternal corals and offspring (eggs or larvae) in *Dipsastraea speciosa* and *Heliopora coerulea*. No significant variation was detected between the *in situ* and *ex situ* samples of maternal corals and offspring and between maternal corals and offspring within the *in situ* and *ex situ* samples of each species (Kruskal-Wallis test; $p > 0.05$).

that sensitivity is higher than bacterial communities of their maternal colonies.

Alpha Diversity and the Winnow Process Hypothesis

While the alpha-diversity of bacterial communities was similar between the *in situ* and *ex situ* environments, they showed different trends between offspring and maternal corals in the two coral species: Alpha-diversity tended to be higher in offspring compared to maternal corals in *D. speciosa*, whereas this tendency was opposite in *H. coerulea*. The result for the spawning coral *D. speciosa* supports the “winnow process” hypothesis which proposes that alpha-diversity of bacterial communities is initially higher in early life stages and declines in later life

(Littman et al., 2009). However, such a hypothesis could not explain the opposite trend of bacterial community for brooding coral, *H. coerulea*. Although Damjanovic et al. (2020a) reported higher alpha-diversity of bacterial communities in larvae than in maternal corals in brooding scleractinian coral, *Pocillopora acuta*, two of the five sample pairs showed similar alpha-diversity between them. Therefore, the currently limited information does not support the winnow process hypothesis for brooding corals.

Bacterial Communities in Eggs of *Dipsastraea speciosa*

The dominant bacterial OTUs (the top 30 OTUs) in eggs of *D. speciosa* were different between *in situ* and the *ex situ* environments and inconsistent with their maternal colonies. The

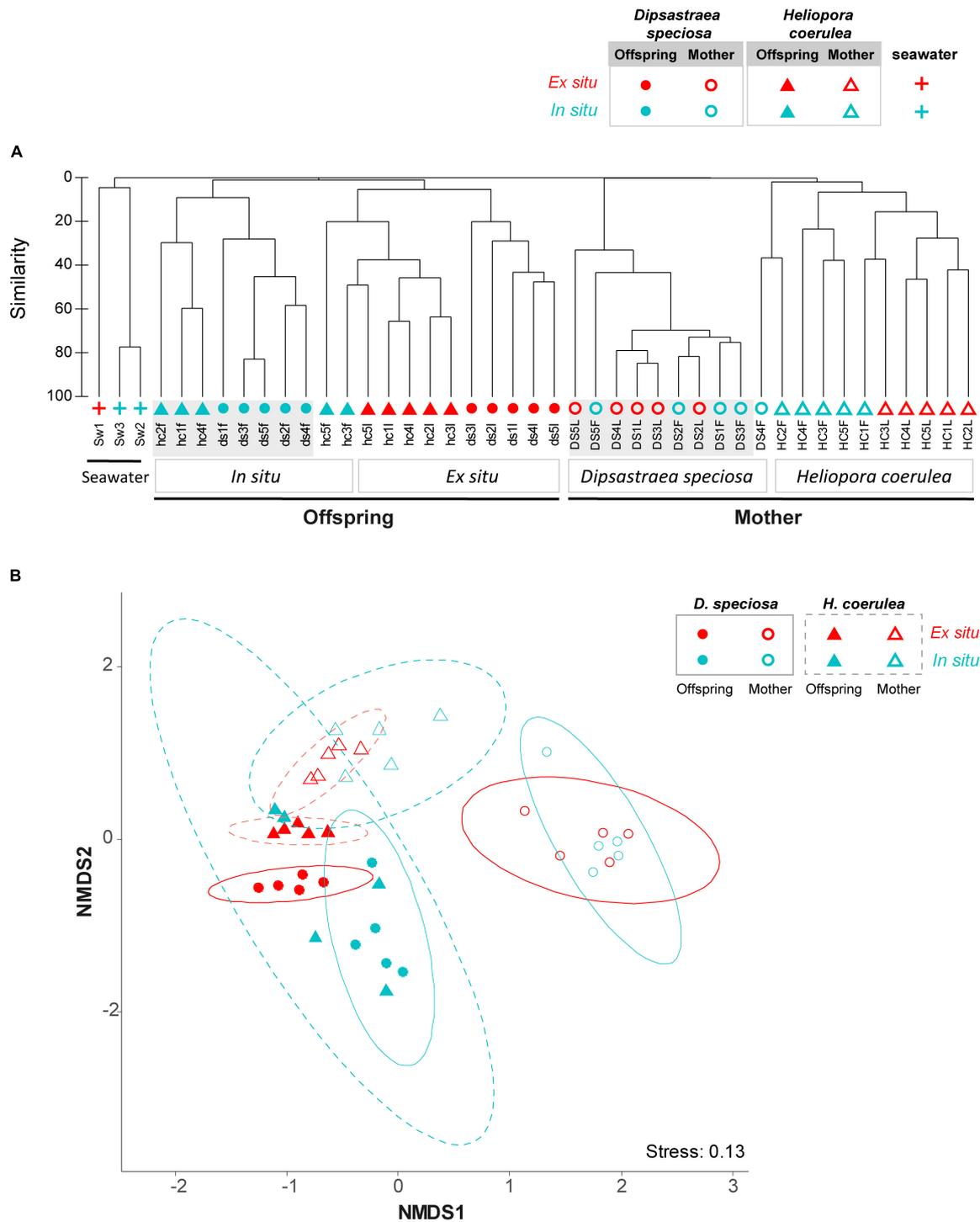


FIGURE 3 | Bacterial community composition in **(A)** clustering and **(B)** nMDS plots. The symbols and colors denote; *Dipsastraea speciosa* (circles), *Heliopora coerulea* (triangles), and seawater (crosses); offspring (filled symbols) and maternal corals (blank symbols); *in situ* (cyan) and *ex situ* (red) environments. 95% CI ellipses are also shown in the nMDS plot for *D. speciosa* (solid line) and *H. coerulea* (dashed line). Abbreviations in sample names denote as follows; the first 2 characters represent coral species (DS for *D. speciosa*, HC for *H. coerulea*); the number (1–5) is replicate colony number; the last character is sampling method [F for the *in situ* (field) samples and L for the *ex situ* (laboratory) samples]; capital letters for maternal coral samples and small letters for offspring samples. nMDS, non-metric multidimensional scaling.

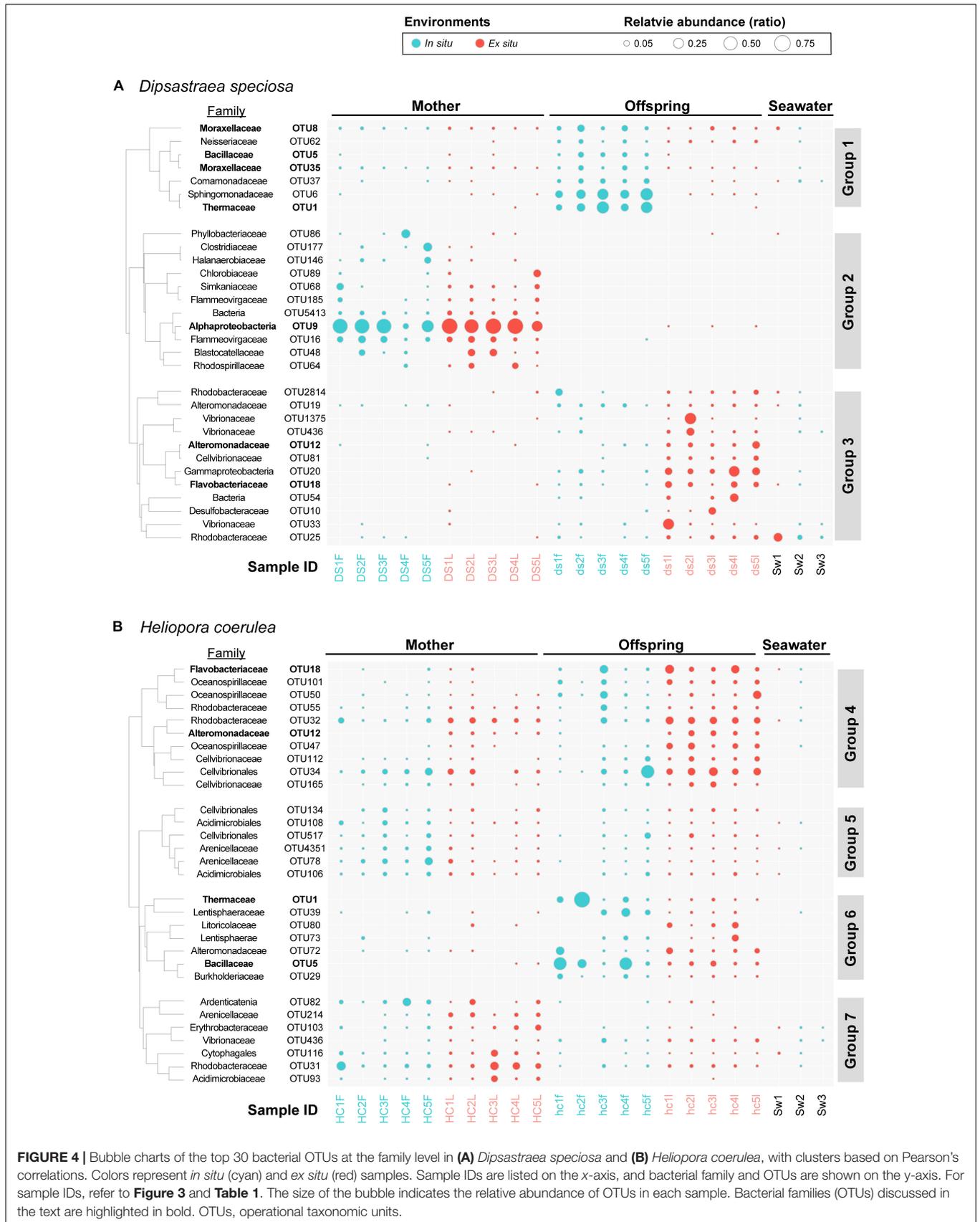


FIGURE 4 | Bubble charts of the top 30 bacterial OTUs at the family level in **(A) *Dipsastraea speciosa*** and **(B) *Heliopora coerulea***, with clusters based on Pearson's correlations. Colors represent *in situ* (cyan) and *ex situ* (red) samples. Sample IDs are listed on the x-axis, and bacterial family and OTUs are shown on the y-axis. For sample IDs, refer to **Figure 3** and **Table 1**. The size of the bubble indicates the relative abundance of OTUs in each sample. Bacterial families (OTUs) discussed in the text are highlighted in bold. OTUs, operational taxonomic units.

same dominant bacterial OTUs between *in situ* and *ex situ* mother samples (Group 2 in **Figure 4A**) indicate that *ex situ* maternal colonies were not under stress, because many studies have shown that the bacterial communities in adult coral were changed under stress (Bourne et al., 2008). However, unlike maternal colonies, this stability was absent in the offspring samples, and the dominant bacterial group in the mother was not vertically transmitted to their offspring.

The dominant bacteria in *in situ* and *ex situ* offspring (Groups 1 and 3 in **Figure 4A**) can be detected in both maternal colonies and seawater samples, so that it is difficult to suggest that these groups were vertically transmitted from mother or horizontally transmitted from environments. Interestingly, both Group 1 and Group 3 dominant bacteria can be detected in *in situ* and *ex situ* offspring samples, but with different relative abundance.

In *ex situ* offspring samples, we had similar results in *D. speciosa* offspring with previous studies. Many of the dominant bacteria in *ex situ* samples (Group 3 in **Figure 4A**) have been previously reported from other studies which collected offspring samples under *ex situ* conditions; e.g., *Rhodobacteraceae*, *Alteromonadaceae*, *Vibrionaceae*, and *Flavobacteriaceae* (Apprill et al., 2009, 2012; Lema et al., 2016; Zhou et al., 2017; Bernasconi et al., 2019; Chan et al., 2019; Damjanovic et al., 2019a, 2020a). Of these, *Rhodobacteraceae* and *Alteromonadaceae* have been detected in eggs of other spawning scleractinian coral species (Apprill et al., 2009; Zhou et al., 2017; Bernasconi et al., 2019; Damjanovic et al., 2019a).

In contrast, the majority of dominant bacteria detected in *in situ* offspring samples in the present study were newly recorded in bacterial communities in the eggs of spawning scleractinian coral species [e.g., *Anoxybacillus* (OTU5; family *Bacillaceae*), *Meiothermus* (OTU1; family *Thermaceae*), and *Enhydrobacter* (OTU35; family *Moraxellaceae*)], except *Acinetobacter* (OTU8; family *Moraxellaceae*). *Acinetobacter* was reported as core bacteria and suggested vertically transmitted from mother to offspring through the mucus in coral *Mussismilia hispida* (Leite et al., 2017). This finding indicates the bacteria group might be horizontally transmitted from the *in situ* environments of this study but more likely have existed already in other studies but was previously not noted due to low relative abundance in *ex situ* samples.

The variance in relative abundance between *in situ* and *ex situ* offspring samples may come from that the *ex situ* environment had smaller water volume and water exchange rate. Among *ex situ* offspring-dominated bacteria (Group 3), *Vibrionaceae*, *Alteromonadaceae*, and *Rhodobacteraceae* have been reported to be uptaken from the environment by coral recruits (Lema et al., 2016; Damjanovic et al., 2019a,b). In addition, *Alteromonas* (OTU19; belonged to Family *Alteromonadaceae*) was reported that it was released from maternal colony to seawater with offspring when spawning, and the offspring would uptake the bacteria from seawater (Ceh et al., 2013). Therefore, smaller water volume and exchange rate of the *ex situ* environment may increase the probability of the bacteria in seawater being uptaken by coral offspring. On the other hand, the two *in situ* dominated bacterial OTUs, *Acinetobacter* (OTU8) and *Enhydrobacter* (OTU35), that appeared in all the sample pairs of

D. speciosa from both *in situ* and *ex situ* environments indicate that some bacteria are also vertically transmitted via mucus-covered egg-sperm bundles.

Bacterial Communities in Larvae of *Heliopora coerulea*

Brooding coral *H. coerulea* shared more common dominant bacteria between maternal colonies and their offspring samples compared to spawning coral *D. speciosa* (**Figure 4**). This may be due to the longer incubation time of brooder offspring developing to larvae inside mother than that of spawner offspring. However, in previous studies, only a small number of bacterial strains were shared between mother and their offspring in brooding scleractinian coral *Pocillopora damicornis* and *P. acuta* (Epstein et al., 2019; Damjanovic et al., 2020a). These dissimilarities are most likely caused by the super-dominant (>90% relative abundance) bacteria (taxa) in maternal colonies that were not dominant in the offspring of *P. damicornis* and *P. acuta*. *H. coerulea* is phylogenetically distant (Class Octocorallia) from scleractinians (Class Hexacorallia) and the phylogenetic difference might affect these results. The other possibility may come from the difference in contact time between maternal corals and offspring: Larvae of *H. coerulea* have a longer brooding (contact) time including the unique “surface brooding” (Babcock, 1990; Harii and Kayanne, 2003) compared to eggs of *D. speciosa* and larvae of *P. damicornis* and *P. acuta* (Epstein et al., 2019; Damjanovic et al., 2020a).

Although the majority of the dominant bacteria in larvae of *H. coerulea* was consensus with their maternal colonies and may be vertically transmitted from maternal corals (irrespective of *in situ* or *ex situ* environments), the spawning environment also affected the beta diversity of bacterial communities in the larvae. The larvae from the *in situ* environment had more diverse bacterial communities among samples than those from the *ex situ* environment (**Figure 3B**). The higher beta diversity of bacterial communities in the offspring from the *in situ* environment may indicate the prevalence of horizontal transmission of bacteria from environment to coral offspring and flexibility of bacterial communities in coral offspring in nature. Previous studies demonstrated that coral recruits under different planulation environments had different bacterial communities in four *Acropora* species and *Pocillopora damicornis* (Chan et al., 2019; Damjanovic et al., 2019b; Epstein et al., 2019). Our results further demonstrate that the spawning environment affects bacterial communities in coral offspring before the planulation stage.

CONCLUSION AND IMPLICATION

This study provides the first report on *in situ* bacterial communities from coral offspring (eggs of the scleractinian coral, *D. speciosa*, and larvae of the octocoral, *H. coerulea*). We demonstrated that different environments influence bacterial communities in coral offspring, but not in maternal colonies, highlighting the sensitivity of bacterial communities to local environments for offspring compared to maternal corals.

Bacterial communities between maternal colonies and offspring were more similar in *H. coerulea* than in *D. speciosa*, which suggest a higher ratio of vertically transmitted bacteria in *H. coerulea*, probably due to the longer contact time between offspring and maternal corals. Our findings on the sensitivity of bacterial communities in coral offspring to the local environment call attention to future studies when bacterial communities in coral offspring are sampled from *ex situ* environments.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/>, SRA PRJNA706097.

AUTHOR CONTRIBUTIONS

YN and J-HS contributed to the study design and wrote the manuscript. C-HL, YN, C-LF, VDHD, and AJM performed the coral sampling. J-HS and C-HL performed the experiment. J-HS

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analyzed the data. YN and AJM contribute to the manuscript revision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.796514/full#supplementary-material>

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