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REPORT

# The microbiomes of two Singaporean corals show site-specific differentiation and variability that correlates with the seasonal monsoons

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**Abstract** Corals host abundant microbial communities, or microbiomes, that play essential roles in the function of the coral holobiont. We examined the mucus microbiome in corals within the port of Singapore, where corals persist despite intense anthropogenic impacts. The coral mucus microbiomes of *Pectinia paeonia* and *Platygyra sinensis* at three reef sites were tracked by 16S rRNA gene amplicon sequencing from January 2019 to January 2020. Both coral species displayed spatial and temporal differences in microbiome composition, suggesting site specificity and seasonality in microbiome composition consistent with the monsoons. The temporal shifts in relative abundance of dominant taxa were different between the two coral species. Nonetheless, Proteobacteria was the most abundant phylum in both coral species and was reduced during the southwest (SW) monsoon, while Cyanobacteria and Crenarchaeota increased. The presumptive beneficial endosymbiont *Endozoicomonas* was only associated with corals at the reef site located the farthest from the Singapore mainland. The coral microbiomes reflected seasonal changes, while the seawater displayed distinct temporal microbial compositions and site-specific differentiation within all sampling dates. The persistence of coral reefs within the port of Singapore highlights the adaptive ability of corals to respond to stressful environments, and this study provides further evidence that a flexible microbiome could be an important part of the strategy employed by corals to remain resilient.

**Keywords** Urban reef · Mucus microbiome · Microbiology · *Pectinia paeonia* · *Platygyra sinensis*

## Introduction

Corals host a diverse microbial consortium, or microbiome, consisting of dinoflagellate *Symbiodiniaceae*, Bacteria, Archaea, viruses and fungi (Blackall et al. 2015; Bourne et al. 2016). The coral microbiome is suggested to contribute a number of essential functions for its host, including nutrient cycling, the breakdown of dimethylsulfoniopropionate (DMSP) and immune responses (Bourne et al. 2016; Moynihan et al. 2022; Peixoto et al. 2021). As corals experience global declines, particularly due to climate change, there is increasing value in understanding the extent to which the microbiome supports coral health, or if microbiome manipulation, through inoculation with presumptively beneficial bacteria, could provide a mechanism of enhancing coral resilience to environmental stressors (van Oppen et al. 2017; Webster et al. 2018; Voolstra and Ziegler 2020; Peixoto et al. 2021).

Coral microbiomes are species-specific (Pollock et al. 2018; Speare et al. 2020), although intraspecific variation can result from biogeographic differences (Klaus et al. 2007; Ziegler et al. 2017; Wainwright et al. 2019; Deignan and McDougald 2022) and environmental perturbations (Klaus et al. 2007; Ziegler et al. 2016, 2019; Leite et al. 2018; Claar et al. 2020). Within a coral, the microbiome

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can differ by microhabitat of the tissue, skeleton, gut cavity or mucus (Rohwer et al. 2002; Pollock et al. 2018) The mucus microbiome displays comparatively greater responsiveness to environmental changes than the tissue or skeleton but has been shown to shift in succession with the tissue microbiome to thermal stress (Lee et al. 2015; Pollock et al. 2018; Marchioro et al. 2020). Microbiome stability was first suggested to aid corals during stress exposure (Grottoli et al. 2018; Hadaidi et al. 2017), but more recently, the microbiome flexibility hypothesis proposed that the ability of the microbiome to respond to changes in the environment enhances coral resilience (Reshef et al. 2006; Voolstra and Ziegler 2020).

While the rapid response of the microbiome to environmental stress has been well-documented, long-term patterns in microbiome composition are less well-characterized. On tropical reefs, long-term monitoring has shown temporal stability in microbiome composition (Epstein et al. 2019a; Dunphy et al. 2019), with seasonal changes correlating to a small extent with rainfall (Li et al. 2014). On temperate reefs with contrasting summer and winter conditions, seasonal shifts in microbiome composition are more common (Koren and Rosenberg 2006; Roder et al. 2015; Sharp et al. 2017; Palladino et al. 2021; Yu et al. 2021), although this pattern is not universally observed (van de Water et al. 2018). Understanding the natural fluctuations in coral microbiomes will help better diagnose stress in corals and can serve as a baseline of the microbial community before attempts at inoculation to improve coral resilience.

The port of Singapore is a highly urbanized environment with most reefs constrained to < 10 m depths due to high sedimentation rates and subsequent low light attenuation (Bauman et al. 2015; Guest et al. 2016; Sin et al. 2016; Chou et al. 2019). Singaporean waters are a confluence of regional waters merging between the Andaman Sea via the Malacca Strait to the west and the South China Sea to the east, with daily tidal currents mixing local waters (Sin et al. 2016). Singapore experiences two monsoon periods, a northeast (NE) monsoon from December to mid-March and a southwest (SW) monsoon from June through September, separated by two inter-monsoon periods (Sin et al. 2016) The monsoons influence local water currents by increasing the westward flow of water, particularly during the northeast Monsoon (Sin et al. 2016). The seasonality of the two monsoons results in a bimodal pattern for many water quality parameters in Singapore including temperature, salinity, light attenuation, dissolved organic matter (DOM), dissolved inorganic nitrogen (DIN) and phosphate (Chénard et al. 2019; Tanzil et al. 2019; Martin et al. 2021). The SW monsoon is generally characterized by higher seawater temperatures and nutrients than the NE monsoon. Seasonal changes in surface seawater microbial communities have been detected in the Singapore Straits consistent with the monsoons (Chénard et al. 2019). However, no studies have looked for seasonal patterns in coral microbiome compositions within Singapore.

Differences in coral microbiome community composition have been detected for the corals *Pocillopora acuta* and *Merulina ampliata* across reefs within Singapore (Wainwright et al. 2019; Fong et al. 2020; Deignan and McDougald 2022), but it is unknown if other coral species follow the same biogeographic pattern of microbiome differentiation or if the spatial differentiation is maintained over time. In this study, we monitored the mucus microbiomes of two coral species, *Pectinia paeonia* and *Platygyra sinensis*. We sampled coral colonies on seven occasions encompassing both monsoon seasons at three different reef sites to track the spatial and temporal composition of the microbial communities to better understand how coral microbiomes are adapted to the urban reef environments of Singapore.

## Methods

Six colonies each of *Pectinia* and *Platygyra* were identified morphologically and selected at random from Pulau Hantu (1°13′34″ N, 103°44′46″ E), Kusu Island (1°13′32″ N, 103°51′35″ E), and Pulau Satumu, or Raffles Lighthouse (1°09′39″ N, 103°44′26″ E; see Supplementary Information SI Fig. 1). All colonies were marked with color-coded tags for repeatable sampling. *Pectinia* and *Platygyra* were selected for this study because they were readily abundant on the study reefs and consistently produced sufficient mucus to allow for collection without resorting to destructive sampling methods. From January 2019, each reef was visited every 2 months until January 2020 (Fig. 1). Each sampling event was split over two consecutive days. For coral microbiome collection, mucus was sampled from the colony surface by agitating ~ 5 $cm^2$ with a 5-mL syringe. The syringes were placed upright on ice for transport to the laboratory, where the mucus was centrifuged for 20 min at 13,000 RCF and stored at − 80 °C. Additionally, triplicate 1 L seawater samples were collected at each site 0.5 m above the reef benthos and placed on ice for transport to the laboratory for filtration through a 0.2 µm cellulose acetate filter (Sartorius Stedim Biotech). All filters were immediately stored at − 80 °C. Approximately, 10 mL of filtered seawater was collected from each site in an acid cleaned falcon tube and flash frozen in a liquid nitrogen dry shipper for nutrient analysis on a SEAL AA3 autoanalyzer at the Asian School of the Environment at Nanyang Technological University. Seawater temperature was recorded via HOBO data loggers deployed on the reef flat at each site (Jenny Fong, National University of Singapore).

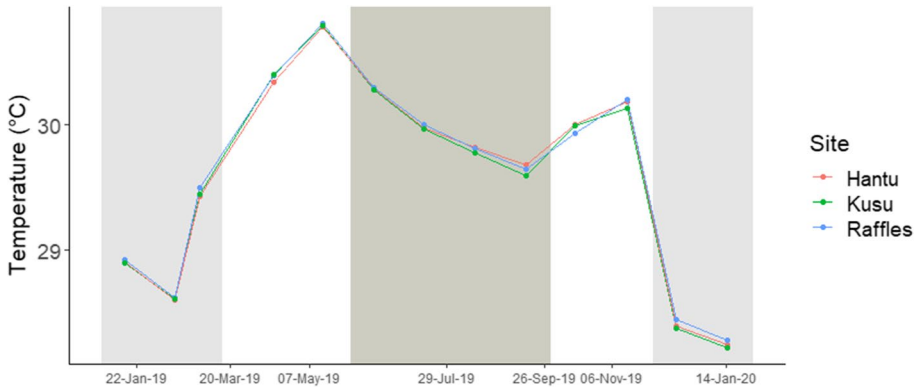


**Fig. 1** Monthly mean seawater temperature at each reef site with the sampling dates on the x-axis. The northwest monsoon is indicated by the light gray color block, and the southwest monsoon is indicated by the dark gray color block

## Coral genotyping

Each coral colony (18 *Pectinia* and 18 *Platygyra*) from the three reef sites was included in the molecular analysis. DNA was extracted from the coral mucus samples with the Qiagen DNeasy PowerBiofilm Kit followed by the Zymo Genomic DNA Clean & Concentrator™ Kit. DNA purity was measured spectrophotometrically with NanoDrop (Thermo Scientific) and quantified with Qubit™ 2.0 fluorometer. Two molecular markers were selected for species identification, the mitochondrial noncoding intergenic region (hereon referred to as IGR) and nuclear ribosomal DNA containing internal transcribed spacers 1 and 2 and 5.8S rDNA (hereon referred to as ITS). IGR were amplified with Faviidae specific primers MNC1f (5′-GAGCTG GGCTTCTTTAGAGTG-3′) and MNCIr (5′-GTGAGA CTCGAACTCACTTTTC-3′) in a total PCR reaction volume of 50 µL, with 50 ng of genomic DNA, 1 X KAPA HiFi HotStart ReadyMix kit (KAPA BIOSYSTEMS) and 0.2 µM of forward and reverse primers. The PCR amplification cycle, modified from Huang et al. (2009), consisted of 5 min at 95 °C for 1 cycle, followed by 35 cycles of 94 °C for 45 s, 50 °C for 45 s and 72 °C for 1.5 min, with a final extension of 72 °C for 5 min. Coral-specific forward primer A18S (Takabayashi et al. 1998) and universal primer ITS-4 were used for amplification of ITS in a total PCR reaction volume of 50 µL, with 25 ng of genomic DNA, 1 X KAPA HiFi HotStart ReadyMix kit (KAPA BIOSYSTEMS) and 0.2 µM of forward and reverse primers. The amplification conditions were set at 5 min at 94 °C for 1 cycle, followed by 30 cycles of 95 °C for 60 s, 55 °C for 60 s and 72 °C for 60 s, with a final extension at 72 °C for 10 min. PCR products were purified with Zymo Clean-up and Concentrator kit and sent for DNA sequencing via Applied Biosystems 3730xl DNA Analyzer after processing with BigDye Terminator v3.1 Cycle Sequencing Kit. Sequences were edited, aligned and concatenated using Geneious Prime. The coral ITS is known to have intra-colonial (van Oppen et al. 2000, 2002; Fukami et al. 2004; Lam et al. 2006) and intragenomic variation (van Oppen et al. 2000; Lam et al. 2006; Huang et al. 2011). Therefore, three to six clonal sequences were subsequently aligned at 50% threshold for each ambiguous sample to derive a consensus sequence with no ambiguities. IGR and ITS sequences were trimmed after alignment to exclude any missing data and the final length for analysis was 928 bp and 586 bp for *Pectinia*, 764 bp and 674 bp for *Platygyra*, respectively. Identity of coral sequences were confirmed via BLAST. Sequence reads were uploaded to GenBank under Accession numbers OL913748-65 (*Pectinia* ITS), OL913769-86 (*Platygyra* ITS), OM154196-213 (*Pectinia* IGR), and OM154178-95 (*Platygyra* IGR).

Phylogenetic analysis was processed with MEGA 10.2.0 (Kumar et al. 2018) including one representative GenBank sequence for *P. paeonia* (Accession numbers: HQ203681.1, HQ203385.1) and *P. sinensis* (Accession numbers: FJ345534.1, HQ203393.1) along with two other species for each genus (Accession numbers: HQ203678.1, HQ203680.1, HQ203382.1, HQ203384.1, HQ203682.1, HQ203684.1, HQ203386.1, HE648534.1). To prevent ambiguous alignment of the IGR (Huang et al. 2011), the concatenated IGR and ITS sequences were aligned separately for *Pectinia* and *Platygyra* with their outgroup *Mycedium elephantotus* (Accession numbers: HQ203675.1, HQ203377.1) and *Leptoria phrygia* (Accession numbers: FJ345529.1, HQ203365.1), respectively, generating two matrices of 1401 and 1264 characters each. Phylogenetic relationships were reconstructed using maximum likelihood analyses (ML) on Tamura-Nei model (1993) with 1000 bootstrap replicates to determine internal branch support.

Genetic diversity among sites was calculated using DnaSP v 6.0 (Rozas et al. 2017). Genetic differences among and between populations were analyzed by computing pairwise FST and analyses of molecular variance (AMOVA) implemented in Arlequin version 3.0 (Excoffier et al. 2005). Considering that ITS was present in multiple copies in several samples and can sometimes have higher intra- than inter-colonial variation (Lam et al. 2006), only IGR was used for population genetic analysis.

### Spatial and temporal microbiome analysis

DNA extraction from coral mucus and seawater was conducted using the Qiagen DNeasy PowerBiofilm Kit followed by the Zymo Genomic DNA Clean & Concentrator™ Kit for prokaryotic community analysis. Extracted DNA was stored at − 20 °C. For examining the prokaryotic portion of the microbiome, PCR was run with 10 µL HotStarTaq Plus Master Mix (Qiagen), 1 µL each of 10 µM forward and reverse primers, 4 µL of RNAse-Free water, 1 µL of 100% dimethyl sulfoxide (DMSO), 1 µL of 200 ng/µL BSA, and 2 µL template DNA (5 ng/µL). The 515F (Parada) and 806R (Apprill) primers were used to amplify the V4 region of the 16S rRNA gene (Caporaso et al. 2011; Apprill et al. 2015; Parada et al. 2016). Triplicate PCR reactions were run using the following conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 53 °C for 40 s, and 72 °C for 1 min and a final extension of 10 min at 72 °C. Triplicate samples were pooled, and a bead clean-up was done using Agencourt Ampure XP (Beckman Coulter) to isolate the targeted V4 region. Finally, all samples were quantified using a Qubit 2.0 fluorometer and quality checked on an Agilent 2200 TapeStation before library preparation and amplicon sequencing on an Illumina MiSeq platform at the Singapore Centre for Environmental Life Sciences Engineering (Nanyang Technological University). The samples were multiplexed and run on three consecutive runs to ensure sufficient sampling depth across all samples. Raw sequencing reads were uploaded to the NCBI Sequence Read Archive (SRA) under BioProject PRJNA791535.

Amplicon sequencing data were processed using Dada2 version 1.16 (Callahan et al. 2016) to generate amplicon sequence variants (ASVs) for each sample replicate. Firstly, forward and reverse sequence reads were merged by sample to combine the data output from the three sequencing runs into a single set of forward and reverse sequences per sample. Sequence reads were trimmed to 240 for the forward read and 210 for the reverse read, then filtered with an expected error rate of 2. Error learning algorithms were applied to the forward and reverse reads before reads were merged, and chimeric sequences were removed (SI Table 1). Contaminating sequence reads based on comparison with blank extractions were removed using the *decontam* package (Davis et al. 2018). Taxonomy was assigned to the genus level using the built in Bayesian classifier *assignTaxonomy* based on the SILVA SSU r138.1 database. Sequences identified as mitochondria, chloroplast or unassigned to a Domain were removed. Finally, rarefaction curves were used to assess samples which reached their ASV maximum (SI Fig. 2). Samples were then rarefied to 29,184 sequence reads per sample to account for variation in sequencing depths for the calculation of diversity metrics (Weiss et al. 2017). Alpha diversity metrics, including observed richness, Chao1, Shannon diversity (H), and inverse Simpson, were calculated using *phyloseq* (McMurdie and Holmes 2013) and compared by site and date for each species with Kruskal–Wallis tests following Shapiro–Wilk tests for normality. Follow-up Dunn tests with Bonferroni corrections were used for pairwise comparisons.

To examine any patterns within the coral-associated prokaryotic communities, non-metric multidimensional scaling (nMDS) plots were created using Bray–Curtis similarity matrices of square root transformed data with *vegan* and plotted with *ggplot2* in R v3.4.3. Permutational multivariate analyses of variance (PERMANOVA) were performed using the *adonis2* function to examine differences in community composition in the samples. The full experimental design was tested for species, site, and date and modeled for repeated measures to account for resampling of the same coral colonies over time. PERMANOVA was also run for each species and the seawater by site and date. Significance values were based on 9999 permutations, with a Bonferroni correction for all pairwise comparisons. Permutational multivariate analysis of dispersion (PERMDISP) was used to test for homogeneity of dispersion among sites for *Pectinia* and *Platygyra* using *betadisper*. Two factor PERMANOVA was employed to test for within-site temporal differentiation for *Pectinia* and *Platygyra*, and within-date site differentiation for the seawater samples.

To further examine site differentiation, an indicator species analysis was applied to *Pectinia* and *Platygyra* using the *multipatt* function in the *indicspecies* package based on a point biserial correlation coefficient function and 9999 permutations (De Cáceres and Legendre 2009). This analysis identifies indicator species, or in this case ASVs, based on both ASV relative abundance and fidelity to determine ASVs associated with a given group or location. Indicator ASV analysis was also applied to *Pectinia* and within-site for *Platygyra* for the peak of the NE monsoon (January 2019 and January 2020) and the SW monsoon (July 2020). Results focused on ASVs identified as highly significant ($p < 0.01$). Alluvial plots of indicator ASV taxonomy were created using RAWGraphs (Mauri et al. 2017). Seasonal changes in the coral microbiomes were further examined by correlating microbiome composition with environmental changes. The *envfit* function from *vegan* was used to correlate environmental parameters with sampling date for *Pectinia* and the seawater and for sampling date within-site for *Platygyra*. The environmental parameters tested included nitrate, nitrite, ammonia, phosphate, silicate, and mean monthly seawater temperature. Only significant correlations are reported for each group ($P < 0.05$).

## Results

### Coral phylogenetics

Phylogenetic analysis was unable to draw clear species distinction due to paraphyletic clades in both corals. *Pectinia* and *Platygyra* samples both generated poorly supported clades with *Pectinia lactuca and P. alcicornis* (Fig. 2a), and *Platygyra daedalea* and *P. acuta* nested within each (Fig. 2b), respectively. This could be due to low interspecific distances within mt IGR in some corals, especially *Platygyra* spp. (Huang et al. 2009) and high intraspecific variability of the ITS rDNA (Takabayashi et al. 1998; Vollmer and Palumbi 2004; Wei et al. 2006). Although the interspecific distance has been shown to be high for *Platygyra* (Wei et al. 2006), our un-concatenated ITS dataset was also unable to yield a resolved tree. Further, our *Pectinia* samples, which were morphologically identified with high confidence as *Pectinia paeonia,* were the most different from the *P. paeonia* sequence obtained from GenBank and genetically closest

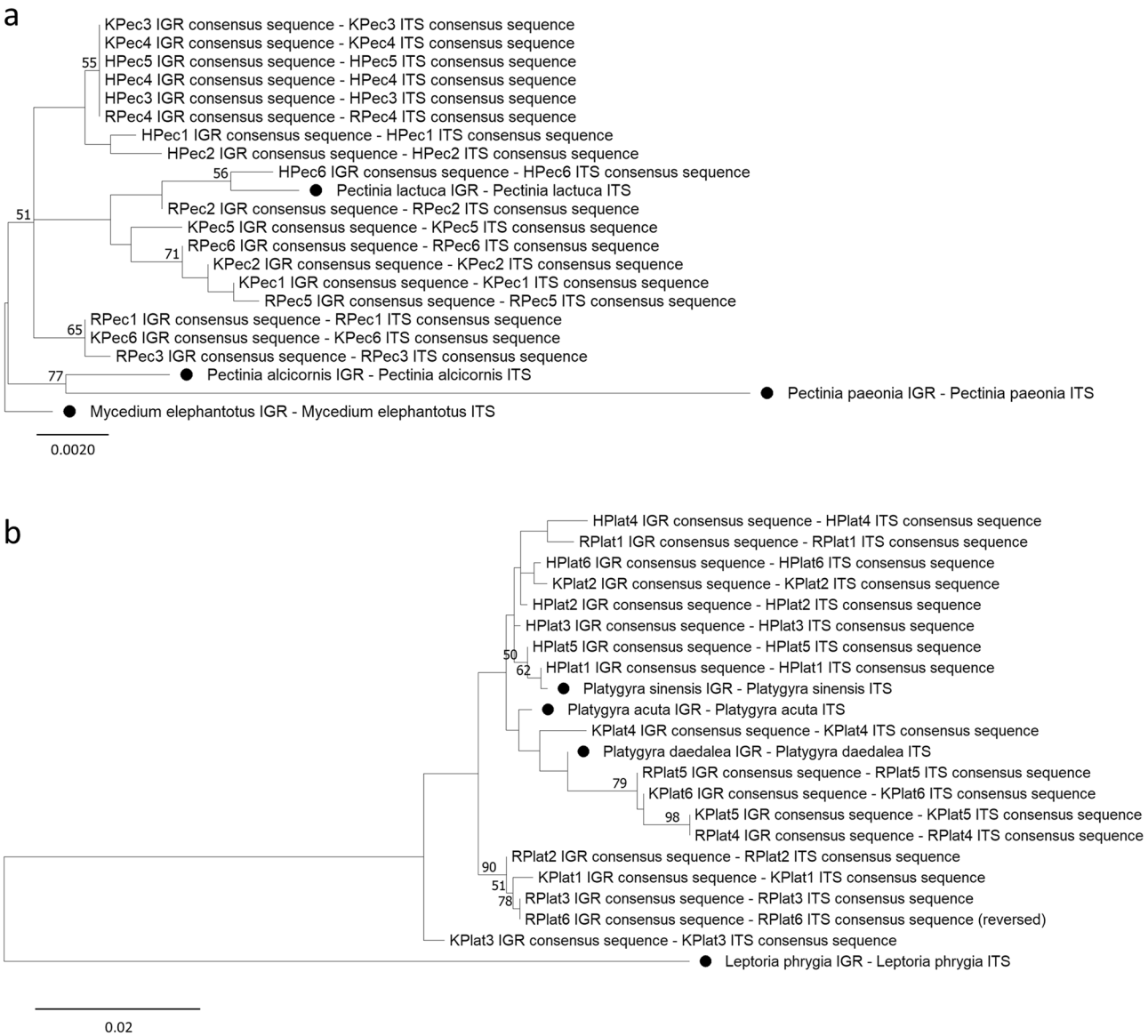


**Fig. 2** **a** Maximum likelihood tree for *Pectinia* generated with concatenated mt IGR and ITS rDNA sequences, including one representative sequence of *Pectinia paeonia* and two *Pectinia* species from GenBank (black circles), with *Mycedium elephantotus* as outgroup. **b** Maximum likelihood tree for *Platygyra* generated with concatenated mt IGR and ITS rDNA sequences, including one representative sequence of *Platygyra sinensis* and two *Platygyra* species from GenBank (black circles), with *Leptoria phrygia* as outgroup. Specimen names starting with 'H' indicate Hantu, 'K' from Kusu, and 'R' from Raffles. Distances calculated with Tamura-Nei Neighbor-Joining tree with 1000 bootstraps. Bootstrap values > 50% are given above branches

to *P. lactuca*, with most of the difference residing in ITS rDNA. However, the specimens used for this study were closely related to each other with variability within *Pectinia* at 1.1% and within *Platygyra* at 3.9%. Therefore, we continue to refer to each species as *P. paeonia* and *Platygyra sinensis* given the morphological identifications and the poor species distinction arising from the low resolution of the IGR and ITS genes.

There were five *Pectinia* and six *Platygyra* haplotypes. Haplotype diversity for both *Pectinia* and *Platygyra* were high at all reef sites (0.600–0.800) and among sites (0.614, 0.797), and nucleotide diversity was low (0.001–0.004). For both coral species, Kusu had the highest haplotype diversity, followed by Raffles then Hantu (SI Tables 2 and 3). Both coral species had one haplotype shared across all three reef sites. Kusu and Hantu each contained a unique *Pectinia* and *Platygyra* haplotype. AMOVA analysis revealed a well-connected population indicating that the different reef sites are not genetically differentiated. *Pectinia* and *Platygyra* both had higher within population (101.82, 88.99%) than among population (− 1.82, 11.01%) variation and non-significant ($P > 0.05$) F values between sites (SI Tables 4–9). While the results are consistent with expectation (Tay et al. 2015, 2016), higher sample numbers would be necessary to resolve species distinctions and population connectivity for both coral species.

### Spatial and temporal microbiome analysis

In total, 125 samples of each *Pectinia* and *Platygyra* and 63 Seawater samples were processed for microbiome analysis. Across all samples, 42,442 unique ASVs were identified (23,306 for *Pectinia*; 25,913 for *Platygyra*; 4725 for seawater). There were no differences in any alpha diversity metrics by site or date for *Pectinia* (SI Table 10). For *Platygyra*, there were no differences in richness, Chao 1, or Shannon diversity by site; however, the inverse Simpson diversity was lower at Hantu compared to Kusu and Raffles. When examining the *Platygyra* samples by date, the richness and Shannon diversity were lower in July compared to the January timepoints (SI Table 11). Overall, *Platygyra* had a higher alpha diversity than *Pectinia* for all metrics ($P < 0.0001$; SI Table 12). For the seawater, there were no differences in alpha diversity metrics by site. However, there were inconsistent differences by date (SI Table 13). For example, richness was higher in September, March had a lower Shannon diversity, and the inverse Simpson was lower in March and November, particularly compared to January and July.

The microbiome compositions for *Pectinia*, *Platygyra* and the seawater were significantly different (PERMANOVA; Pseudo-$F$: 43.383, $P = 0.0001$; SI Table 14) and were therefore examined separately. Both *Pectinia* and *Platygyra* displayed site (*Pectinia*: Pseudo-$F = 3.7226$, $P = 0.0001$; *Platygyra*: Pseudo-$F = 3.559$, $P = 0.0001$) and temporal differentiation (*Pectinia*: Pseudo-$F = 1.9297$, $P = 0.0001$; *Platygyra*: Pseudo-$F = 2.5$, $P = 0.0001$). Site differentiation was detected among all three reef sites for both coral species (Fig. 3a, b; SI Table 15). PERMDISP did not detect any significant differences in dispersion for *Pectinia* (Pseudo-$F = 0.4053$, $P = 0.6665$), but there was a significant difference for *Platygyra* (Pseudo-$F = 19.53$, $P = 0.0001$), with Hantu being distinct from both Kusu and Raffles ($P = 0.0001$). Proteobacteria was the most abundant phylum in both coral microbiomes, but *Pectinia* had an overall higher abundance than *Platygyra* (Fig. 3c, d). In *Pectinia*, this was dominated by members of the order Rhodospirillales (SI Table 16), while in *Platygyra,* the more abundant orders within the Proteobacteria included SAR11 clade, Pseudomonadales, Rhodobacterales, Rhodospirillales, and Rhizobiales (SI Table 17). The second most abundant phylum for *Pectinia* was Bacteroidota, which was highest at Hantu, and was dominated by order Flavobacteriales. For *Platygyra*, the second most abundant phylum was Cyanobacteria, which was highest at Raffles, and dominated by both Synechococcales and Cyanobacteriales. Indicator species analysis for *Pectinia* found 46 indicator ASVs for Hantu, 34 for Kusu and 33 for Raffles (Fig. 4; SI Table 18). For *Platygyra*, there were 62 indicator ASVs for Hantu, 73 for Kusu, and 39 for Raffles (Fig. 5; SI Table 19) with the greatest number of different taxa identified at Kusu. Both *Pectinia* and *Platygyra* at Raffles had members of Endozoicomonadaceae identified as indicator ASVs, however the proportion was higher for *Platygyra*. *Pectinia* contained potentially pathogenic Simkaniaceae as indicator ASVs from Hantu and Raffles. Members of Terasakiellaceae were identified for both *Pectinia* and *Platygyra* as indicator ASVs at Hantu and to a lesser extent at Kusu but was not identified for either coral species at Raffles.

For *Platygyra*, there was a significant interaction between site and date in the two-factor PERMANOVA, indicating temporal differentiation within each site (Pseudo-$F = 1.1238$, $P = 0.0026$). However, this within-site temporal effect was not observed for *Pectinia* (Pseudo-$F = 0.8833$, $P = 0.9983$). For *Pectinia* and within-site for *Playgyra*, January timepoints showed the greatest differentiation from July, suggesting that the coral microbiomes change consistently with the peak of the monsoon seasons (Fig. 6; SI Table 20). Temporal shifts in the dominant phyla were not always consistent across sites (Fig. 7). However, general trends in the relative abundance of the dominant phyla included increases in Proteobacteria, Bacteroidota, Actinobacteria, Planctomycetota, and Verrucomicrobiota during the NE monsoon (peak in January) and increases in Cyanobacteria, Crenarchaeota and Firmicutes during the SW monsoon (peak in July). Indicator species analysis revealed a low number of ASVs representative of the monsoon peaks in January and July (SI Table 21),

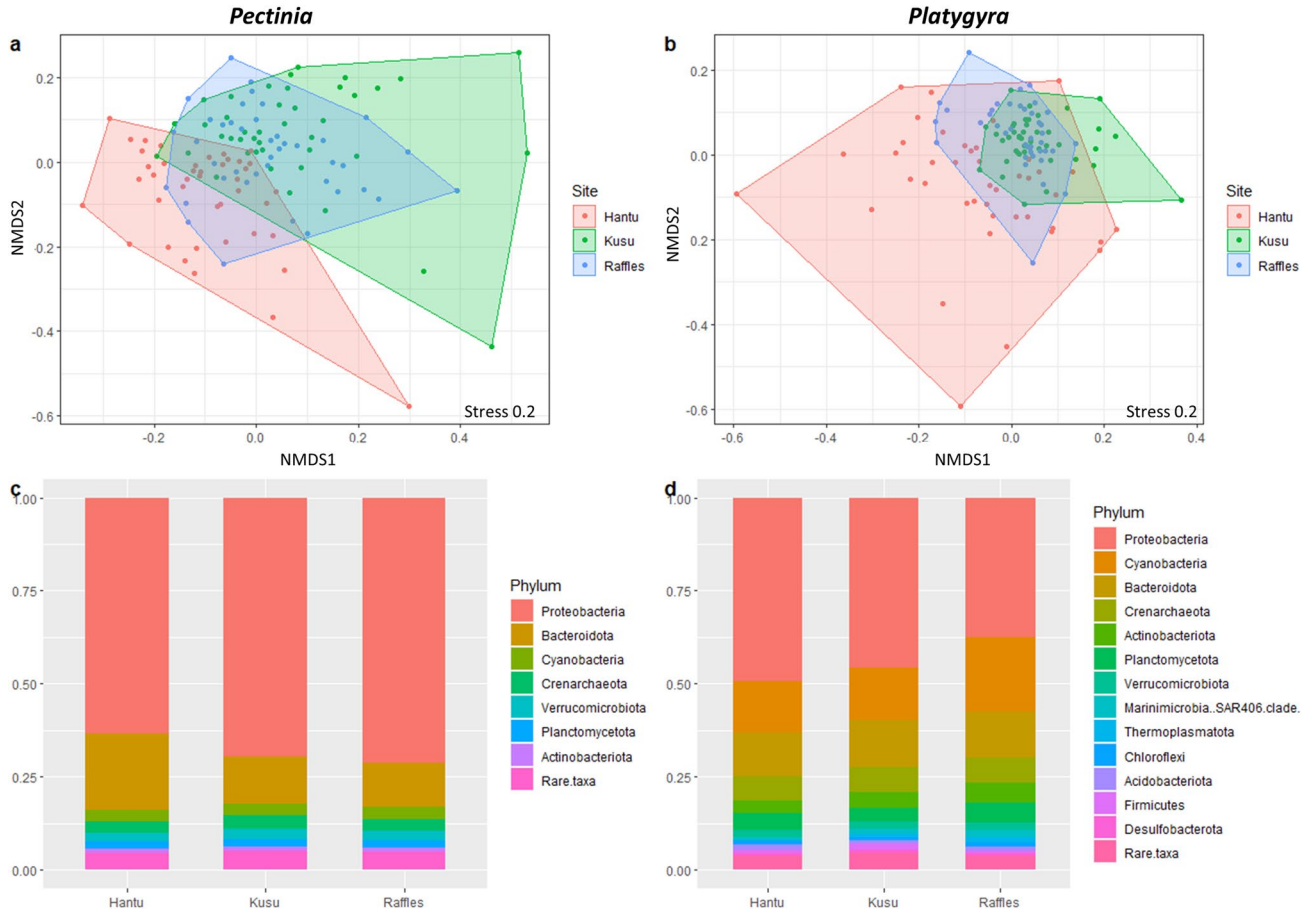


**Fig. 3** nMDS plots of all **a** *Pectina* and **b** *Platygyra* samples grouped by reef site. Differences among sites are reflected in the relative abundance of phyla within each site for **c** *Pectina* and **d** *Platygyra*. Phyla with a relative abundance < 0.01 were grouped as Rare taxa

suggesting that the temporal changes in microbiome composition were the result of a shift in the relative abundance of the dominant taxa rather than the induction of new microbial members in the community.

As for the corals, seawater microbiomes were dominated by Proteobacteria and Cyanobacteria. Cyanobacteria abundance was lowest in January, inversely correlated to an increase in Proteobacteria (Fig. 8b). Crenarchaeota and Thermoplasmatota increased with the SW monsoon season. The seawater microbiome composition did not differ by site (Pseudo-$F = 0.8091$, $P = 0.7467$) but displayed strong temporal differentiation for all sampling dates (Pseudo-$F = 11.483$, $P = 0.0001$; SI Table 22). Two-factor PERMANOVA for date and site found significant differentiation by site within each sampling date (Pseudo-$F = 1.5841$, $P = 0.0006$; Fig. 8a).

Seawater nutrients varied throughout the year, which was consistent with the changing seasons (SI Fig. 3). The *envfit* analysis for *Pectinia* found no correlation of environmental parameters with sampling date ($P = 0.3031$). For *Platygyra*, microbiome differences between sampling dates were correlated with mean monthly temperature at Hantu (Fig. 6b), while at Kusu, differences were correlated with nitrate, nitrite, ammonia, phosphate and mean monthly temperature (Fig. 6c). Nitrite, ammonia, phosphate and mean monthly temperature were correlated with microbiome differences at Raffles (Fig. 6d). Mean monthly seawater temperature was associated with months during the SW monsoon, along with nitrite and phosphate at Kusu and Raffles and nitrite at Kusu. Ammonia was correlated with sampling dates during the NE monsoon at Kusu and Raffles. The seawater microbiome composition was significantly correlated with all environmental parameters. Notably, mean monthly temperature and phosphate were correlated with the SW monsoon, while ammonia and silicate were indicative of the NE monsoon (Fig. 8a).

## Discussion

### Spatial and temporal variability in coral microbiomes

The mucus microbiomes of *Pectinia* and *Platygyra* exhibited spatial and temporal differentiation in prokaryotic

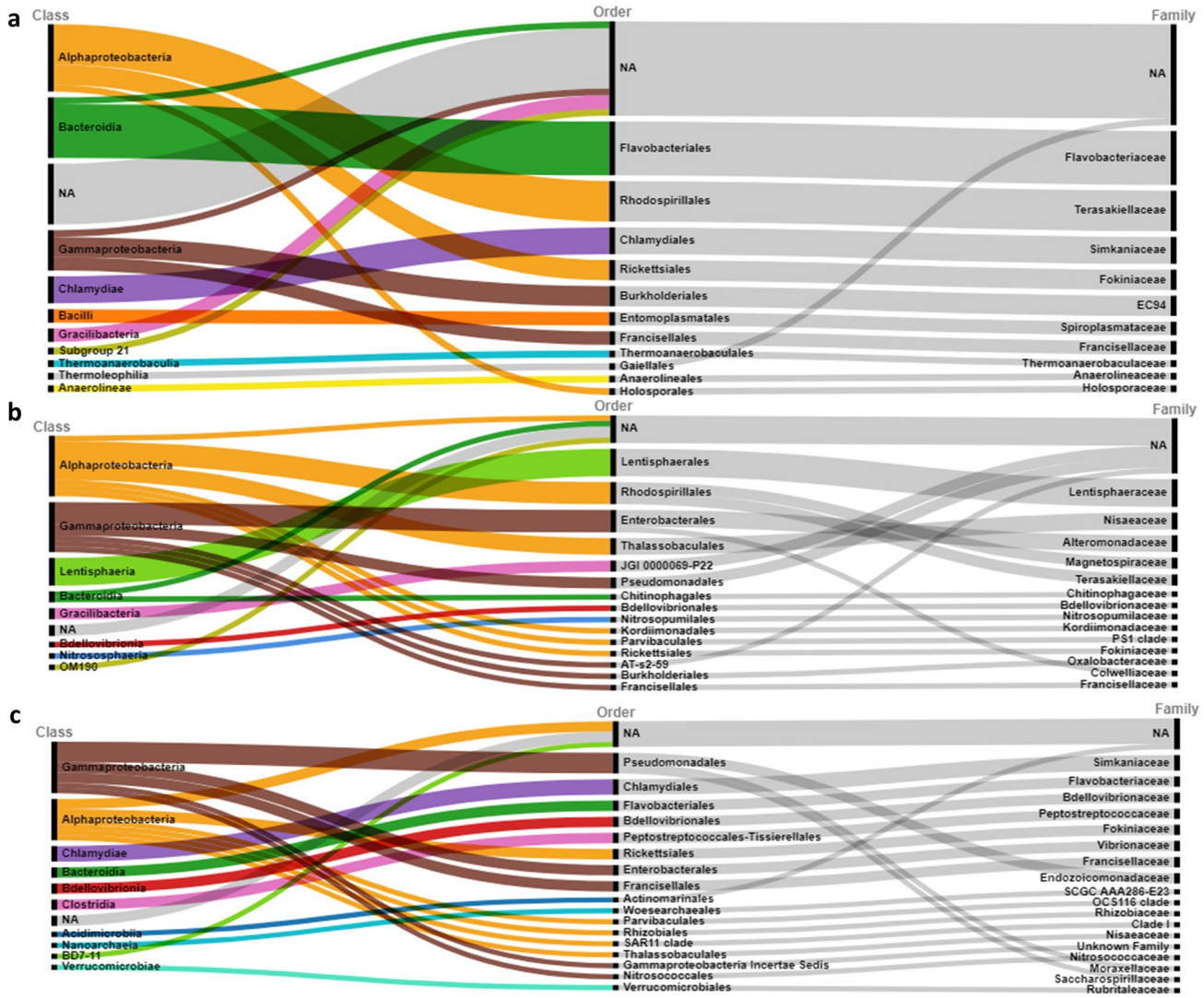


**Fig. 4** Taxonomic breakdown of indicator ASVs for *Pectinia* at **a** Hantu, **b** Kusu, and **c** Raffles. Alluvial plots are scaled in proportion to the number of indicator ASVs identified for each site

community composition. Genotyping analysis of the two coral species gave assurance that the site differentiation did not arise by genetic differentiation, as was previously observed in *Acropora tenuis* (Glasl et al. 2019a) and *Millepora* corals (Dubé et al. 2021). Both *Pectinia* and *Platygyra* had the highest haplotype diversity at Kusu, which is hypothesized to receive more external larval input than the other sites (Tay et al. 2015; Afiq-Rosli et al. 2021). However, both corals are broadcast spawning hermaphrodites (Baird et al. 2009) and displayed high genetic connectivity consistent with expectations for marine broadcast spawners in Singapore (Tay et al. 2015, 2016).

The temporal variation in coral mucus microbiomes was consistent with the monsoon seasons, with sampling that occurred at the peak of each monsoon (January and July) displaying the greatest overall differentiation. *Pectinia* microbiomes collectively displayed significant temporal shifts; however, those temporal shifts were not significantly associated with any of the environmental parameters measured. *Platygyra* displayed more specificity in its response to seasonal changes, experiencing significant within-site temporal changes at each of the three reefs and association with specific environmental parameters. Thus, *Platygyra* reflected a greater degree of environmental responsiveness, while *Pectinia* reflected more stability.

Microbiome flexibility is proposed as a key mechanism for coral adaptive resilience to environmental changes (Voolstra and Ziegler 2020). The mucus fraction of the coral is more responsive to environmental changes than the tissue or skeletal fractions (Pollock et al. 2018; Marchioro et al. 2020), but it still maintains coral host specificity (Pollock et al. 2018) and can shift in succession with

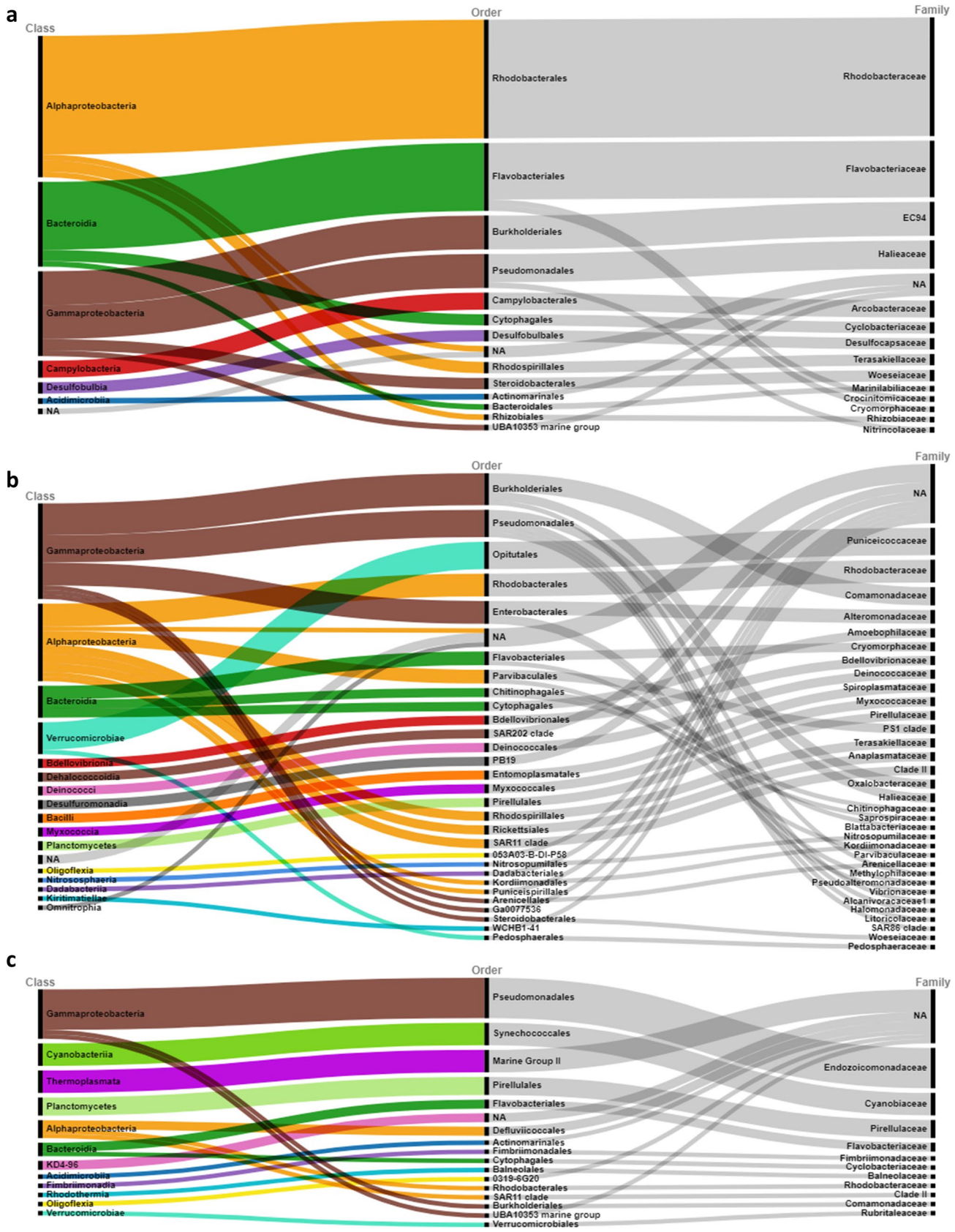


**Fig. 5** Taxonomic breakdown of indicator ASVs for *Platygyra* at **a** Hantu, **b** Kusu, and **c** Raffles. Alluvial plots are scaled in proportion to the number of indicator ASVs identified for each site

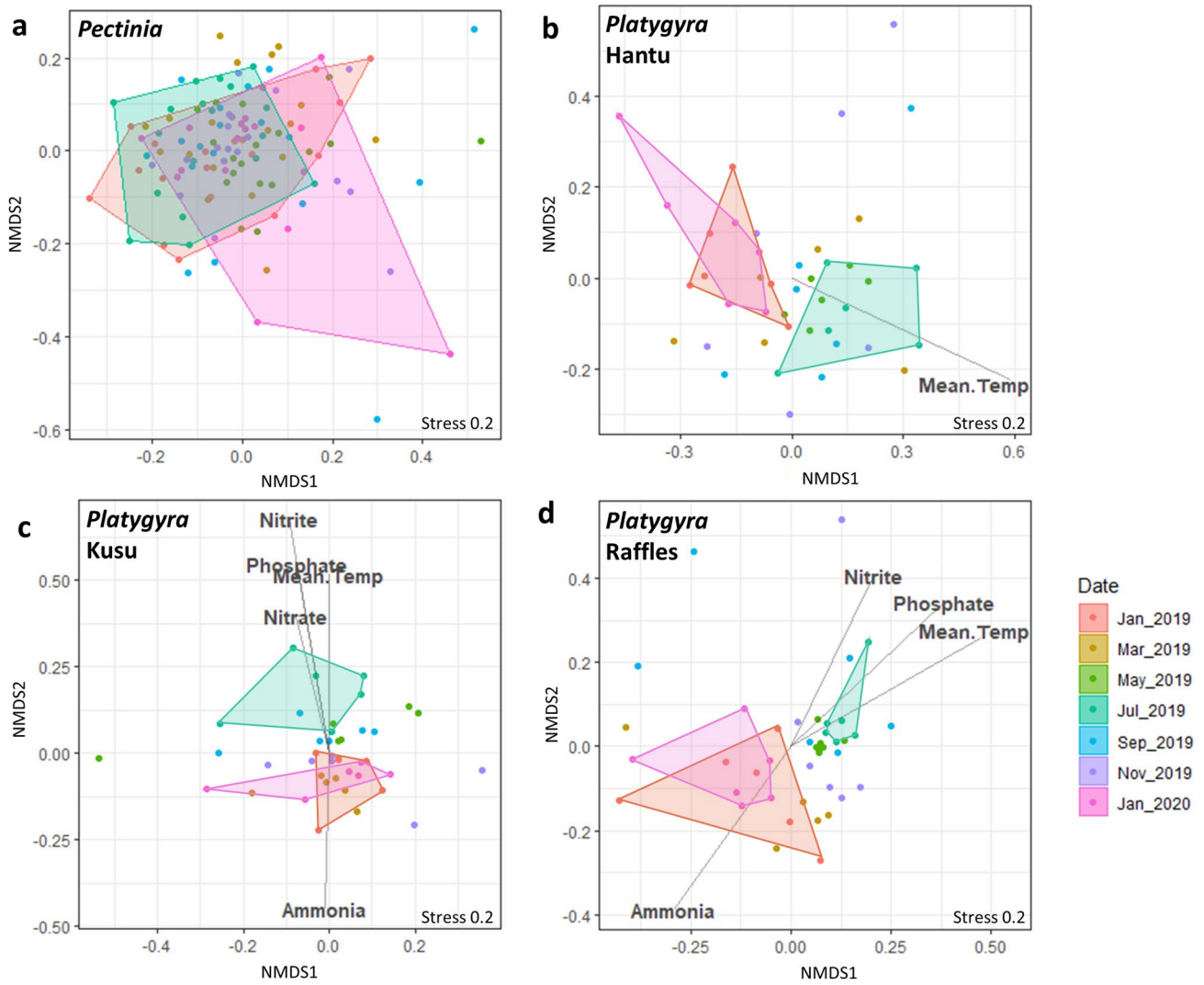


**Fig. 6** nMDS plots colored by sampling date with January (2019, 2020) and July timepoints outlined for **a** all *Pectinia* samples, and *Platygyra* samples from **b** Hantu, **c** Kusu, and **d** Raffles. Directional vectors represent the significant environmental parameters based on *envfit* analysis

the tissue microbiome in response to environmental stress (Lee et al. 2015) The mucus microbiome contributes to coral health by creating a selective environment, which supports a distinct microbial community and acts as a first line of defense against pathogens (Rohwer et al. 2002; Shnit-Orland and Kushmaro 2009; Glasl et al. 2016). Therefore, we suggest that the mucus communities presented here reflect a valuable indicator of the impacts of environmental conditions on the coral holobionts. *Pectinia* and *Platygyra* mucus microbiomes conformed to both spatial and temporal environmental fluctuations. Both species are common on shallow impacted reefs in the Pacific (Dikou and Woesik 2006; Browne et al. 2014; Ng et al. 2016; Chow et al. 2019), and a flexible microbiome could be one of their mechanisms to tolerate these harsh environments. In particular, *Platygyra* is a slow-growing, massive coral (Darling et al. 2012) and grows in large colonies (frequently > 50 cm diameter) across Singapore, a trait associated with high microbiome flexibility (Ziegler et al. 2019). Correspondingly, this species showed a high degree of specificity in fluctuations of its microbiome to the environment, reflecting within site temporal differentiation correlated with specific seawater parameters. The microbiome of *Platygyra* was also characterized as having a higher diversity than *Pectinia*. *Pectinia* is a fast-growing coral (Ng and Chou 2014), which has been associated with lower microbiome flexibility. The adaptive microbial community, particularly of *Platygyra*, could also be a contributing factor in the resilience of this species to tolerating biotic stressors present within impacted reef systems, like regular competitive interaction with algae (Fong and Todd 2021). However, in Singapore, *Pectinia* has been reported

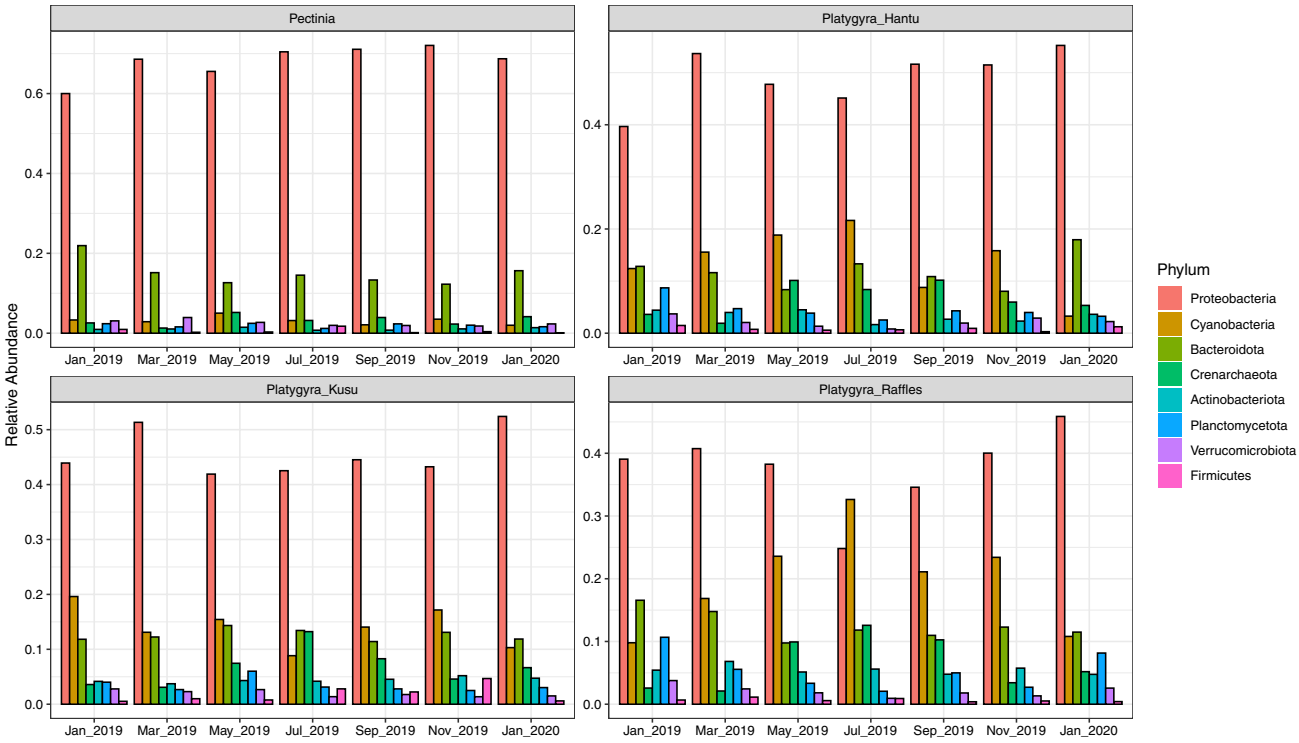


**Fig. 7** Relative abundance of the eight most abundant phyla at each sampling date for *Pectinia* samples combined from all sites and for *Platygyra* samples separated by site (Hantu, Kusu, and Raffles)

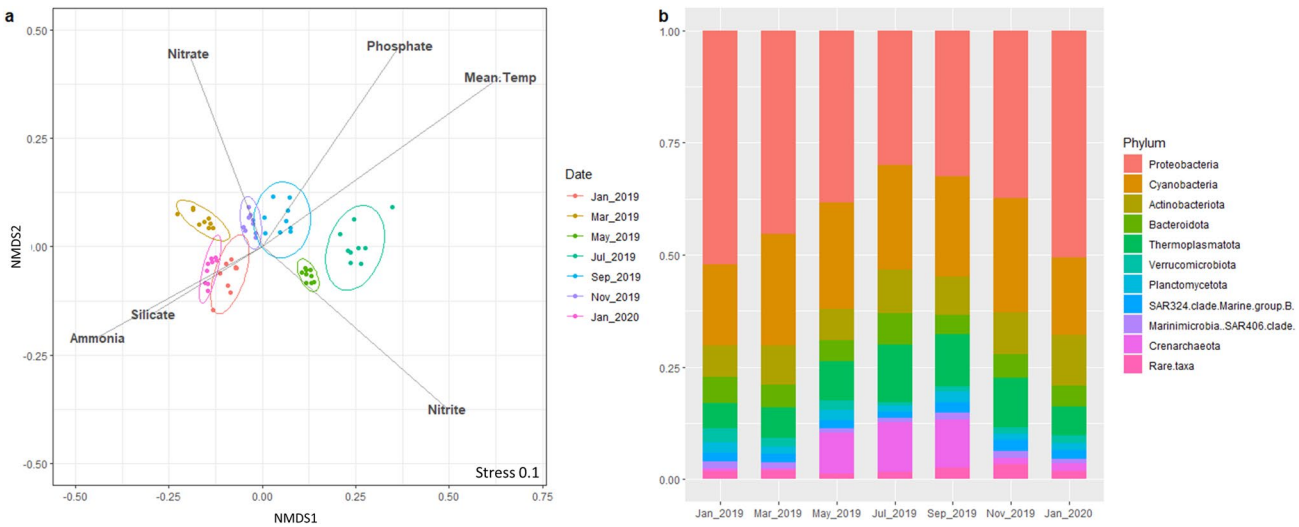


**Fig. 8** **a** nMDS plot of all seawater samples colored by sampling date with ellipses representing 95% confidence intervals. Directional vectors represent the significant environmental parameters based on *envfit* analysis. **b** Temporal differences are reflected in the relative abundance of phyla in seawater at each sampling date. Phyla with a relative abundance < 0.01 were grouped as Rare taxa

to have higher resilience to bleaching than *Platygyra* (Ng et al. 2020).

Of the three reefs examined in this study, Raffles is located farthest from the mainland of Singapore, ~ 14 km. For this reason, it is thought to experience reduced anthropogenic impacts compared to Hantu or Kusu, which are located ~ 7.5 km and 5 km away from the mainland, respectively. Indicator species analyses supports this assertion. *Endozoicomonas* is considered a beneficial coral endosymbiont, occupying over 90% of bacterial relative

abundance in some corals (Bayer et al. 2013; Epstein et al. 2019b; Maher et al. 2020). However, Endozoicomonaceae are frequently reduced or outcompeted under environmental or anthropogenic stress (Roder et al. 2015; Ziegler et al. 2016; McDevitt-Irwin et al. 2017). Coral microbiomes from Singapore have been reported to contain a low abundance or absence of Endozoicomonaceae, even in coral species with high Endozoicomonaceae abundance elsewhere (Wainwright et al. 2019; Fong et al. 2020; Deignan and McDougald 2022; Moynihan et al. 2022). In this study, *Endozoicomonas* were only detected as indicator ASVs at Raffles. Conversely, members of Terasakiellaceae, not included as indicator species for Raffles, have been shown to become enriched by moderate heat stress (Li et al. 2021), suggesting they could be an indicator of higher levels of environmental stress experienced by corals at Hantu and Kusu. Flavobacteriales, which have been associated with algae-dominated degraded reefs (Kelly et al. 2014) and heat stressed corals (Gajigan et al. 2017), were found as indicator ASVs present in noticeably higher abundance at Hantu.

### Spatial and temporal variability in seawater microbiomes

In addition to spatial differentiation, coral microbiomes shifted in relative abundance of the dominant taxa in response to environmental changes associated with the peak of the seasonal monsoons. During the SW monsoon in Singapore, dissolved inorganic nitrogen (DIN), phosphate and silicate are elevated (Sin et al. 2016; Chénard et al. 2019; Tanzil et al. 2019) and the nutrient parameters measured in this study were consistent with previous observations. Seawater temperatures peak during the inter-monsoon periods (Sin et al. 2016), suggesting that seawater temperatures alone are not enough to drive the greatest shifts in coral microbiomes. At both Raffles and Kusu, phosphate, ammonia and nitrite were correlated with seasonal monsoon differences in *Platygyra* microbiomes, with the additional parameter of nitrate correlating with the SW monsoon at Kusu. Differences in coral microbiomes between Kusu and Raffles were previously linked with their location on either the windward or leeward side of the islands (Wainwright et al. 2019), but this is the first study to correlate those differences with site-specific microbiome responses to environmental parameters associated with the seasonal monsoons.

The temporal shift in seawater microbes was more tightly linked to environmental parameters than those observed for the corals, as previously reported for Porites (Glasl et al. 2019b). The seawater maintained a distinct microbial community at each sampling timepoint and were correlated with all environmental parameters measured. Previously, Chenard et al. (2019) found that DIN, phosphate and silicate were correlated with differences in surface seawater microbial communities from the Singapore Straits during the SW monsoon. The seawater samples from this study were collected directly above the reef benthos, suggesting perhaps a greater interaction between environmental parameters and the benthic community in shaping the seawater microbiome. However, higher Cyanobacteria and Crenarchaeota relative abundances were detected in both corals and the seawater during the SW monsoon, showing some consistency in the effect of environmental changes on these systems. Increased sampling intervals could help establish whether there is a predictive link between changes in the seawater microbial communities and changes in the coral mucus microbiomes.

## Conclusion

Corals around Singapore survive in a highly urbanized reef environment, yet they persist in the face of this anthropogenic disturbance. One source of their resilience could be a flexible microbiome, which was particularly evident for *Platygyra*. Further research is needed to investigate the functional shifts in the microbiome in response to seasonal changes and the subsequent impact on coral host health. As targeted microbiome manipulation grows in popularity as a potential mechanism for enhancing coral resilience (Epstein et al. 2019c; Rosado et al. 2019; Santoro et al. 2021), it is important to understand the spatial variability and temporal shifts in coral microbiomes to ensure that any manipulation is deployed in accordance with the natural microbiome fluctuations.

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**Declarations**

**Conflict of interest** The authors have no relevant financial or non-financial interests to disclose.

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## References

Afiq-Rosli L, Wainwright BJ, Gajanur AR, Lee AC, Ooi SK, Chou LM, Huang D (2021) Barriers and corridors of gene flow in an urbanized tropical reef system. Evol Appl 14:2502–2515

Apprill A, McNally S, Parsons R, Weber L (2015) Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol 75:129–137

Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. Annu Rev Ecol Evol Syst 40:551–571

Bauman AG, Guest JR, Dunshea G, Low J, Todd PA, Steinberg PD (2015) Coral Settlement on a Highly Disturbed Equatorial Reef System. PLoS ONE 10:e0127874

Bayer T, Neave MJ, Alsheikh-Hussain A, Aranda M, Yum LK, Mincer T, Hughen K, Apprill A, Voolstra CR (2013) The Microbiome of the Red Sea Coral Stylophora pistillata Is Dominated by Tissue-Associated Endozoicomonas Bacteria. Appl Environ Microbiol 2013:79

Blackall LL, Wilson B, van Oppen MJH (2015) Coral—the world's most diverse symbiotic ecosystem. Mol Ecol 24:5330–5347

Bourne DG, Morrow KM, Webster NS (2016) Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. Annu Rev Microbiol 70:317–340

Browne NK, Precht E, Last KS, Todd PA (2014) Photo-physiological costs associated with acute sediment stress events in three nearshore turbid water corals. Mar Ecol Prog Ser 502:129–143

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci 108:4516–4522

Chénard C, Wijaya W, Vaulot D, dos Santos AL, Martin P, Kaur A, Lauro FM (2019) Temporal and spatial dynamics of Bacteria, Archaea and protists in equatorial coastal waters. Sci Rep 9:16390

Chou LM, Huang D, Tan KS, Toh TC, Goh BPL, Tun K (2019) Singapore. In: Sheppard CRC (ed) World Seas: An Environmental Evaluation, vol II. The Indian Ocean to the Pacific. Academic Press, London, pp 539–558

Chow GSE, Chan YKS, Jain SS, Huang D (2019) Light limitation selects for depth generalists in urbanised reef coral communities. Mar Environ Res 147:101–112

Claar DC, McDevitt-Irwin JM, Garren M, Thurber RV, Gates RD, Baum JK (2020) Increased diversity and concordant shifts in community structure of coral-associated *Symbiodiniaceae* and bacteria subjected to chronic human disturbance. Mol Ecol. https://doi.org/10.1111/mec.15494

Darling ES, Alvarez-Filip L, Oliver TA, McClanahan TR, Côté IM (2012) Evaluating life-history strategies of reef corals from species traits. Ecol Lett 15:1378–1386

Davis NM, Proctor D, Holmes SP, Relman DA, Callahan BJ (2018) Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 6:226

De Cáceres M, Legendre P (2009) Associations between species and groups of sites: indices and statistical inference. Ecology 90:3566–3574

Deignan LK, McDougald D (2022) Differential Response of the Microbiome of Pocillopora acuta to Reciprocal Transplantation Within Singapore. Microb Ecol 83:608–618

Dikou A, van Woesik R (2006) Survival under chronic stress from sediment load: Spatial patterns of hard coral communities in the southern islands of Singapore. Mar Pollut Bull 52:7–21

Dubé CE, Ziegler M, Mercière A, Boissin E, Planes S, Bourmaud CAF, Voolstra CR (2021) Naturally occurring fire coral clones demonstrate a genetic and environmental basis of microbiome composition. Nat Com 12:1–12

Dunphy CM, Gouhier TC, Chu ND, Vollmer SV (2019) Structure and stability of the coral microbiome in space and time. Sci Rep 9:6785

Epstein HE, Smith HA, Cantin NE, Mocellin VJL, Torda G, van Oppen MJH (2019a) Temporal Variation in the Microbiome of Acropora Coral Species Does Not Reflect Seasonality. Front Microbiol 10:1775

Epstein HE, Torda G, van Oppen MJH (2019b) Relative stability of the Pocillopora acuta microbiome throughout a thermal stress event. Coral Reefs 38:373–386

Epstein HE, Smith HA, Torda G, van Oppen MJH (2019c) Microbiome engineering: enhancing climate resilience in corals. Front Ecol Environ 17:100–108

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Fong J, Todd PA (2021) Spatio-temporal dynamics of coral–macroalgal interactions and their impacts on coral growth on urbanised reefs. Mar Pollut Bull 172:112849

Fong J, Deignan LK, Bauman AG, Steinberg PD, McDougald D, Todd PA (2020) Contact and Water-Mediated Effects of Macroalgae on the Physiology and Microbiome of Three Indo-Pacific Coral Species. Front Mar Sci 6:831

Fukami H, Budd AF, Levitan DR, Jara J, Kersanach R, Knowlton N (2004) Geographic differences in species boundaries among members of the *Montastraea annularis* complex based on molecular and morphological markers. Evolution 58:324–337

Gajigan AP, Diaz LA, Conaco C (2017) Resilience of the prokaryotic microbial community of *Acropora digitifera* to elevated temperature. Microbiologyopen 6:e00478

Glasl B, Herndl G, Frade P (2016) The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. ISME J 10:2280–2292

Glasl B, Smith CE, Bourne DG, Webster NS (2019a) Disentangling the effect of host-genotype and environment on the microbiome of the coral *Acropora tenuis*. PeerJ 7:e6377

Glasl B, Bourne DG, Frade PR, Thomas T, Schaffelke B, Webster NS (2019b) Microbial indicators of environmental perturbations in coral reef ecosystems. Microbiome 7:94

Grottoli AG, Dalcin Martins P, Wilkins MJ, Johnston MD, Warner ME, Cai WJ, Melman TF, Hoadley KD, Pettay DT, Levas S, Schoepf V (2018) Coral physiology and microbiome dynamics under combined warming and ocean acidification. PLoS ONE 13:e0191156

Guest JR, Tun K, Low J, Vergés A, Marzinelli EM, Campbell AH, Bauman AG, Feary DA, Chou LM, Steinberg PD (2016) 27 yrs of benthic and coral community dynamics on turbid, highly urbanised reefs off Singapore. Sci Rep 6:36260

Hadaidi G, Röthig T, Yum LK, Ziegler M, Arif C, Roder C, Burt J, Voolstra CR (2017) Stable mucus-associated bacterial

communities in bleached and healthy corals of Porites lobata from the Arabian Seas. Sci Rep 7:45362
Huang D, Meier R, Todd PA, Chou LM (2009) More evidence for pervasive paraphyly in scleractinian corals: systematic study of Southeast Asian Faviidae (Cnidaria; Scleractinia) based on molecular and morphological data. Mol Phylogenet Evol 50:102–116
Huang D, Licuanan WY, Baird AH, Fukami H (2011) Cleaning up the "Bigmessidae": Molecular phylogeny of scleractinian corals from Faviidae, Merulinidae. Pectiniidae and Trachyphylliidae BMC Evol Biol 11:37
Kelly LW, Williams GJ, Barott KL, Carlson CA, Dinsdale EA, Edwards RA et al (2014) Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors. Proc Nat Acad Sci 111:10227–10232
Klaus JS, Janse I, Heikoop JM, Sanford RA, Fouke BW (2007) Coral microbial communities, zooxanthellae and mucus along gradients of seawater depth and coastal pollution. Environ Microbiol 9:1291–1305
Koren O, Rosenberg E (2006) Bacteria associated with mucus and tissues of the coral *Oculina patagonica* in summer and winter. Appl Environ Microbiol 72:5254–5259
Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547
Lam KK, Loo AH, Todd PA, Chew FT, Chou LM (2006) Existence of intra-colonial paralogues of the ribosomal internal transcribed spacer (ITS) impedes studies of intracolonial genetic variation in the scleractinian coral *Diploastrea heliopora* (Lamark 1816). Raffles Bull Zool 54:485–489
Lee STM, Davy SK, Tang SL, Fan TY, Kench PS (2015) Successive shifts in the microbial community of the surface mucus layer and tissues of the coral *Acropora muricata* under thermal stress. FEMS Microbiol Ecol 91:fiv142
Leite DCA, Salles JF, Calderon EN, Castro CB, Bianchini A, Marques JA, van Elsas JD, Peixoto RS (2018) Coral bacterial-core abundance and network complexity as proxies for anthropogenic pollution. Front Microbiol 9:833
Li J, Chen Q, Long LJ, Dong JD, Yang J, Zhang S (2014) Bacterial dynamics within the mucus, tissue and skeleton of the coral Porites lutea during different seasons. Sci Rep 4:1–8
Li J, Long L, Zou Y, Zhang S (2021) Microbial community and transcriptional responses to increased temperatures in coral *Pocillopora damicornis* holobiont. Environ Microbiol 23:826–843
Maher RL, Schmeltzer ER, Meiling S, McMinds R, Ezzat L, Shantz AA, Adam TC, Schmitt RJ, Holbrook SJ, Burkepile DE, Vega Thurber R (2020) Coral microbiomes demonstrate flexibility and resilience through a reduction in community diversity following a thermal stress event. Front Ecol Evol 8:555698
Marchioro GM, Glasl B, Engelen AH, Serrão EA, Bourne DG, Webster NS, Frade PR (2020) Microbiome dynamics in the tissue and mucus of acroporid corals differ in relation to host and environmental parameters. PeerJ 8:e9644
Martin P, Sanwlani N, Lee TWQ, Wong JMC, Chang KYW, Wong EWS, Liew SC (2021) Dissolved organic matter from tropical peatlands reduces shelf sea light availability in the Singapore Strait, Southeast Asia. Mar Ecol Prog Ser 672:89–109
Mauri M, Elli T, Caviglia G, Uboldi G, Azzi M (2017) RAWGraphs: A visualisation platform to create open outputs. In Proceedings of the 12th Biannual Conference on Italian SIGCHI Chapter New York, NY,USA:ACM. 28:1–28:5
McDevitt-Irwin JM, Baum JK, Garren M, Vega Thurber RL (2017) Responses of Coral-Associated Bacterial Communities to Local and Global Stressors. Front Mar Sci 4:262
McMurdie PJ, Holmes S (2013) Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8:e61217
Moynihan MA, Goodkin NF, Morgan KM, Kho PYY, dos Santos AL, Lauro FM, Baker DM, Martin P (2022) Coral-associated nitrogen fixation rates and diazotrophic diversity on a nutrient-replete equatorial reef. ISME J 16:233–246
Ng CSL, Chou LM (2014) Rearing juvenile 'corals of opportunity' in in situ nurseries–A reef rehabilitation approach for sediment-impacted environments. Mar Biol Res 10:833–838
Ng CSL, Toh TC, Chou LM (2016) Coral restoration in Singapore's sediment-challenged sea. Reg Stud Mar Sci 8:422–429
Ng CSL, Huang D, Toh KB, Sam SQ, Kikuzawa YP, Toh TC et al (2020) Responses of urban reef corals during the 2016 mass bleaching event. Mar Pollut Bull 154:111111
van Oppen MJH, Wörheide G, Takabayashi M (2000) Nuclear markers in evolutionary and population genetic studies of scleractinian corals and sponges. Proc 9th Int Coral Reef Symp 1:131–138
Palladino G, Biagi E, Rampelli S, Musella M, D'Amico F, Turroni S, Brigidi P, Luna GM, Candela M (2021) Seasonal changes in microbial communities associated with the jewel anemone *Corynactis viridis*. Front Mar Sci 8:627585
Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol 18:1403–1414
Peixoto RS, Sweet M, Villela HDM, Cardoso P, Thomas T, Voolstra CR, Høj L, Bourne DG (2021) Coral probiotics: Premise, promise, prospects. Annu Rev Anim Biosci 9:265–288
Pollock JF, McMinds R, Smith S, Bourne DG, Willis BL, Medina M, Vega Thurber R, Zaneveld JR (2018) Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. Nat Commun 9:4921
Reshef L, Koren O, Loya Y, Zilber-Rosenberg I, Rosenberg E (2006) The coral probiotic hypothesis. Environ Microbiol 8:2068–2073
Roder C, Bayer T, Aranda M, Kruse M, Voolstra CR (2015) Microbiome structure of the fungid coral *Ctenactis echinata* aligns with environmental differences. Mol Ecol 24:3501–3511
Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser 243:1–10
Rosado PM, Leite DCA, Duarte GAS, Chaloub RM, Jospin G, da Rocha UN, Saraiva JP, Dini-Andreote F, Eisen JA, Bourne DG, Peixoto RS (2019) Marine probiotics: increasing coral resistance to bleaching through microbiome manipulation. ISME J 13:921–936
Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol 34:3299–3302
Santoro EP, Borges RM, Espinoza JL et al (2021) Coral microbiome manipulation elicits metabolic and genetic restructuring to mitigate heat stress and evade mortality. Sci Advan 7:eabg3088
Sharp KH, Pratte ZA, Kerwin AH, Rotjan RD, Stewart FJ (2017) Season, but not symbiont state, drives microbiome structure in the temperate coral *Astrangia Poculata*. Microbiome 5:120
Shnit-Orland M, Kushmaro A (2009) Coral mucus-associated bacteria: a possible first line of defense. FEMS Microbiol Ecol 67:371–380
Sin TM, Ang HW, Buurman J, Lee AC, Leong YL, Ooi SK, Steinberg P, Teo SLM (2016) The urban marine environment of Singapore. Reg Stud Mar Sci 8:331–339
Speare L, Davies SW, Balmonte JP, Baumann J, Castillo KD (2020) Patterns of environmental variability influence coral-associated bacterial and algal communities on the Mesoamerican Barrier Reef. Mol Ecol 29:13

Takabayashi M, Carter DA, Ward S, Hoegh-Guldberg O (1998) Inter- and intra-specific variability in ribosomal DNA sequence in the internal transcribed spacer region of corals. Proc Aust Coral Reef Soc 75th Ann Conf 241–248

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526

Tanzil JTI, Goodkin NF, Sin TM, Chen ML, Fabbro GN, Boyle EA, Lee AC, Toh KB (2019) Multi-colony coral skeletal Ba/Ca from Singapore's turbid urban reefs: Relationship with contemporaneous in-situ seawater parameters. Geochim Cosmochim Acta 250:191–208

Tay YC, Noreen AME, Suharsono CLM, Todd PA (2015) Genetic connectivity of the broadcast spawning reef coral *Platygyra sinensis* on impacted reefs, and the description of new microsatellite markers. Coral Reefs 34:301–311

Tay YC, Chng MW, Sew WW, Rheindt FE, Tun KPP, Meier R (2016) Beyond the coral triangle: high genetic diversity and near panmixia in Singapore's populations of the broadcast spawning sea star *Protoreaster nodosus*. R Soc Open Sci 3:160253

van de Water JAJM, Voolstra CR, Rottier C, Cocito S, Peirano A, Allemand D, Ferrier-Pagès C (2018) Seasonal Stability in the Microbiomes of Temperate Gorgonians and the Red Coral *Corallium rubrum* Across the Mediterranean Sea. Microb Ecol 75:274–288

van Oppen MJ, Willis BL, Van Rheede T, Miller DJ (2002) Spawning times, reproductive compatibilities and genetic structuring in the Acropora aspera group: evidence for natural hybridization and semi-permeable species boundaries in corals. Mol Ecol 11:1363–1376

van Oppen MJH, Gates RD, Blackall LL et al (2017) Shifting paradigms in restoration of the world's coral reefs. Glob Chang Biol 23:3437–3448

Vollmer SV, Palumbi SR (2004) Testing the utility of internally transcribed spacer sequences in coral phylogenetics. Mol Ecol 13:2763–2772

Voolstra CR, Ziegler M (2020) Adapting with Microbial Help: Microbiome Flexibility Facilitates Rapid Responses to Environmental Change. BioEssay 42:e2000004

Wainwright BJ, Afiq-Rosli L, Zahn GL, Huang D (2019) Characterisation of coral-associated bacterial communities in an urbanised marine environment shows strong divergence over small spatial scales. Coral Reefs 38:1097–1106

Webster NS, Wagner M, Negri AP (2018) Microbial conservation in the Anthropocene. Environ Microbiol 20:1925–1928

Wei NWV, Wallace CC, Dai CF, Ruby K, Pillay M, Chen CA (2006) Analyses of the ribosomal Internal Transcribed Spacers (ITS) and 5.8 S gene indicate that extremely high rDNA heterogeneity is a unique feature in the scleractinian coral genus A*cropora* (Scleractinia; Acroporidae). Zool Stud Taipei 45:404

Weiss S, Xu ZZ, Peddada S, Amir A, Bittinger K, Gonzalez A, Lozupone C, Zaneveld JR, Vázquez-Baeza Y, Birmingham A, Hyde ER, Knight R (2017) Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 5:27

Yu X, Yu K, Liao Z, Chen B, Deng C, Yu J, Yao Q, Qin Z, Liang J (2021) Seasonal fluctuations in symbiotic bacteria and their role in environmental adaptation of the scleractinian coral *Acropora pruinosa* in high-latitude coral reef area of the South China Sea. Sci Total Environ 792:148438

Ziegler M, Roik A, Porter A, Zubier K, Mudarris MS, Ormond R, Voolstra CR (2016) Coral microbial community dynamics in response to anthropogenic impacts near a major city in the central Red Sea. Mar Pollut Bull 105:629–640

Ziegler M, Seneca F, Yum L, Palumbi SR, Voolstra CR (2017) Bacterial community dynamics are linked to patterns of coral heat tolerance. Nat Commun 8:14213

Ziegler M, Grupstra CG, Barreto MM, Eaton M, BaOmar J, Zubier K, Al-Sofyani A, Turki AJ, Ormond R, Voolstra CR (2019) Coral bacterial community structure responds to environmental change in a host-specific manner. Nat Commun 10:3092

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