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Coral microbiome in estuary coral community of Pearl River Estuary: insights into variation in coral holobiont adaptability to low-salinity conditions

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Abstract

Background Low salinity is a crucial environmental stressor that affects estuarine coral ecosystems considerably. However, few studies have focused on the effects of low-salinity conditions on coral-associated microorganisms and the adaptability of coral holobionts.

Methods We explored the community structure of coral symbiotic Symbiodiniaceae and associated bacteria in low-salinity conditions using samples of six coral species from the Pearl River Estuary and analyzed the adaptability of coral holobionts in estuaries.

Results The symbiotic Symbiodiniaceae of all six studied coral species were dominated by *Cladocopium,* but, the Symbiodiniaceae subclades differed among these coral species. Some coral species (e.g., *Acropora solitaryensis*) had a high diversity of symbiotic Symbiodiniaceae but low Symbiodiniaceae density, with different adaptability to low-salinity stress in the Pearl River Estuary. Other coral species (e.g., *Plesiastrea versipora*) potentially increased their resistance by associating with specific Symbiodiniaceae subclades and with high Symbiodiniaceae density under low-salinity stress. The microbiome associated with the coral species were dominated by *Proteobacteria*, *Chloroflexi*, and *Bacteroidetes*; however, its diversity and composition varied among coral species. Some coral species (e.g., *Acropora solitaryensis*) had a high diversity of associated bacteria, with different adaptability owing to low-salinity stress. Other coral species (e.g., *Plesiastrea versipora*) potentially increased their resistance by having minority bacterial dominance under low-salinity stress.

Conclusions High Symbiodiniaceae density and high bacterial diversity may be conducive to increase the tolerance of coral holobiont to low-salinity environments. Different coral species have distinct ways of adapting to low-salinity stress, and this difference is mainly through the dynamic regulation of the coral microbiome by corals.

Keywords Coral holobionts, Associated bacteria, Symbiotic Symbiodiniaceae, Low-salinity stress, Microbial community variation

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Introduction

Coral reefs, known as the "rainforests of the ocean," are distributed in 110 countries and regions worldwide, accounting for approximately 0.17–0.5% of the global ocean and providing habitat for approximately 30% of marine organisms [1, 2]. The association between coral hosts and their microbiome is the basis for coral growth and reef development [3]. Coral holobionts include cnidarian polyps, bacteria, Symbiodiniaceae, archaea, fungi, and other microorganisms [4, 5]. Symbiodiniaceae are critical microorganisms in coral holobionts that use light energy for photosynthesis and provide fixed organic carbon to the coral host [6]. Symbiodiniaceae can utilize the metabolic waste produced by the coral host to replenish its nitrogen, phosphorus, and other vital nutrients [7]. Community-associated bacterial communities play key roles in coral holobionts, including nitrogen [8, 9], carbon [10], sulfur [11, 12], phosphorus fixation [13], intrametallic homeostasis [13], tissue repair [13], and antibiotic production [14]. Many coral-associated bacteria also protect corals from invasion by pathogens and other exogenous bacteria by secreting antibiotics [14, 15].

Corals are generally considered stenohaline, with limited ability to regulate osmotic pressure and adapt to or survive salinity changes [16]. Salinity is a critical environmental factor affecting their growth and distribution, and the suitable salinity range for growth is generally 32–40‰ [17]. Changes in salinity due to climate change may affect the physiology and metabolism of corals [18–20]. The corals have poor ability to regulate cellular osmotic pressure [21]. A short-term decline in salinity leads to significant changes in cellular respiration and photosynthesis in the coral symbiont Symbiodiniaceae [22]. Freshwater runoff is a major cause of coral mortality in estuarine coral reefs, particularly in those close to major river systems [22].

Environmental changes caused by global warming and destructive anthropogenic activity exacerbate damage to coral reef ecosystems and predispose coral holobionts to dysbiosis [23, 24], leading to high susceptibility to infections by opportunistic pathogens and coral mortality. Estuarine ecosystems are characterized by complex and variable environments, high biodiversity and productivity, and a high degree of human disturbance [25]. Global climate change has led to an unusual increase in extreme rainfall events in multiple regions [26–28], resulting in marine organisms frequently experiencing low-salinity stress in estuarine regions. Low salinity is one of the causes of coral bleaching. Many studies have explored the effects of low salinity on the physiological performance of the early stages of coral; for example, low salinity decreases coral larval recruitment and growth rates [29–31]. Low salinity may affect the physiological and biochemical processes, cellular changes, microbiome alterations, as well as the reproductive and survival capabilities of corals [32–34]. Low salinity stress can disrupt the symbiotic relationship within the coral holobiont, influencing the adaptability and stability of the coral holobiont [22]. In conclusion, Low-salinity stress can severely impact the growth, reproduction, photosynthesis, and respiration of corals, impair the normal functions of cells, and pose a serious threat to the health of corals [22, 35].

The sea section of the Pearl River Estuary in Guangdong represents the estuary of the Pearl River, the second largest river in China in annual runoff. Facing the South China Sea, the Pearl River Estuary is a typhoon-prone area and is susceptible to storm surges and flooding outbreaks, resulting in a large influx of freshwater into the estuary [36]. Corals in the Pearl River Estuary are affected by the year-round influx of freshwater. In the summer of 2022, record-breaking pre-flood rainfall in South China was lasted from May to June, resulting in a severe coral bleaching event in the coral communities of the Wanshan Islands in the Pearl River Estuary [37]. However, the effects of low-salinity stress on coral holobionts in the Wanshan Islands, and corals' adaptability to low-salinity environments, are not well studied.

In this study, we aimed to answer two fundamental questions: are there differences in coral microorganisms in the coral communities of the Pearl River Estuary, and how well are the coral holobionts adapted to low salinity. We tested two hypotheses: (1) the compositions of coral microorganisms in the coral communities of the Pearl River Estuary vary in low-salinity conditions when compared with high salinity conditions, and (2) the physiological parameters of corals influence coral adaptability to low-salinity. To answer the two questions, we collected local water quality parameters and the coral symbiotic Symbiodiniaceae density, chlorophyll a (Chl a) content, and microbial diversity throughout the Pearl River Estuary. The findings of this study enhance the understanding of changes in estuarine coral reef communities and facilitate the conservation of estuarine corals and further research.

Experimental procedures

Study area and coral sample collection

The study area was located in the Wanshan Islands, Pearl River Estuary, China (Fig. 1). The Pearl River is the largest river in southern China and comprises Lingdingyang, Jidianmen, Mudianmen, and Huangmaohai. A large amount of freshwater from the Pearl River flows into the South China Sea, resulting in the salinity of seawater in the Pearl River Estuary being lower than the average salinity of seawater. Furthermore, rainstorms decrease seawater salinity in the Pearl River Estuary. In this study, after the

rainstorm, two islands, Pingzhou and Miaowan, from the Pearl River Estuary were used for coral sample collection (Fig. 1). Water parameters, including the seawater surface temperature (SST; °C), surface water dissolved oxygen content (DO; mg/L), and salinity (Sal), were measured around the two islands.

In the Wanshan Islands, the dominant coral genera are, for example, *Plesiastrea*, *Platygyra*, *Porites*, *Montipora*, and *Acropora* [38]. In this study, 36 samples of six representative coral species, *Platygyra daedalea*, *Plesiastrea versipora*, *Acropora solitaryensis*, *Montipora peltiformis*, *Echinophyllia aspera*, and *Galaxea fascicularis*, were collected from around the two islands at water depths of 3–6 m. Among these samples, there are six samples for each coral species. The coral samples were obtained by scuba divers using hammers and chisels. The coral samples were cleaned using artificial sterile seawater (salinity: 35‰) to ensure they were not disturbed by free-living Symbiodiniaceae and fungi. A portion of the coral samples were 20–50 cm^{-2} in size, and Symbiodiniaceae density and chlorophyll a (Chl a) content were determined. The remaining coral samples were cut and placed in 5 mL freezing tubes, immediately snap-frozen, and stored in liquid nitrogen for subsequent experiments. The data related to corals in normal salinity were extracted from existing studies and reanalyzed [39–46]. Relevant data comprised Symbiodiniaceae density, Chl a content, symbiotic Symbiodiniaceae and coral-associated bacteria composition, and diversity of coral-associated bacteria. The data from under normal salinity were compared with those from low-salinity conditions, and their differences were observed.

Coral symbiotic Symbiodiniaceae

Measurement of coral symbiotic Symbiodiniaceae density and Chl a content

Coral tissue was removed using a Waterpik™ (3–5 kgf cm^{-2}) containing 0.22 μm filtered seawater, and the volume of the initial slurry was measured in a graduated cylinder. The slurry was homogenized and resampled into four 3 mL aliquots. The slurry samples were centrifuged for 5 min (6,500 $r{\cdot}min^{-1}$). After discarding the supernatant, the Symbiodiniaceae at the bottom were preserved in 1 mL 5% formaldehyde at 6 °C for 2–4 h before proceeding to the next step of the analysis. Repeated counts of Symbiodiniaceae densities were performed using hemocytometry ($n = 8–12$). The coral surface area was determined based on the correlation between the weight of the aluminum foil and the surface area [47, 48]. In detail, the aluminum foil was pressed to cover the surface of the coral skeleton, and the foil from the covered area was cut and weighted. A 10 × 10 cm piece of aluminum foil was used to confirm the weight of the foil per square centimeter. The surface area of the coral skeleton was calculated using the weight of the foil covering the surface of the coral skeleton and the weight of the foil per square centimeter. Next, 2 mL of the algal solutions were collected thrice from the same sample bottle in 2 mL centrifuge tubes and centrifuged for 5 min (4,000 $r{\cdot}min^{-1}$). The supernatant was slowly removed, 1.5 mL acetone solution was added to the precipitate, and these samples were stored at 4 ℃ for 24 h. The extracted solutions were centrifuged for 5 min (4,000 $r{\cdot}min^{-1}$), and 200 μL of the supernatant was collected to determine the absorbance

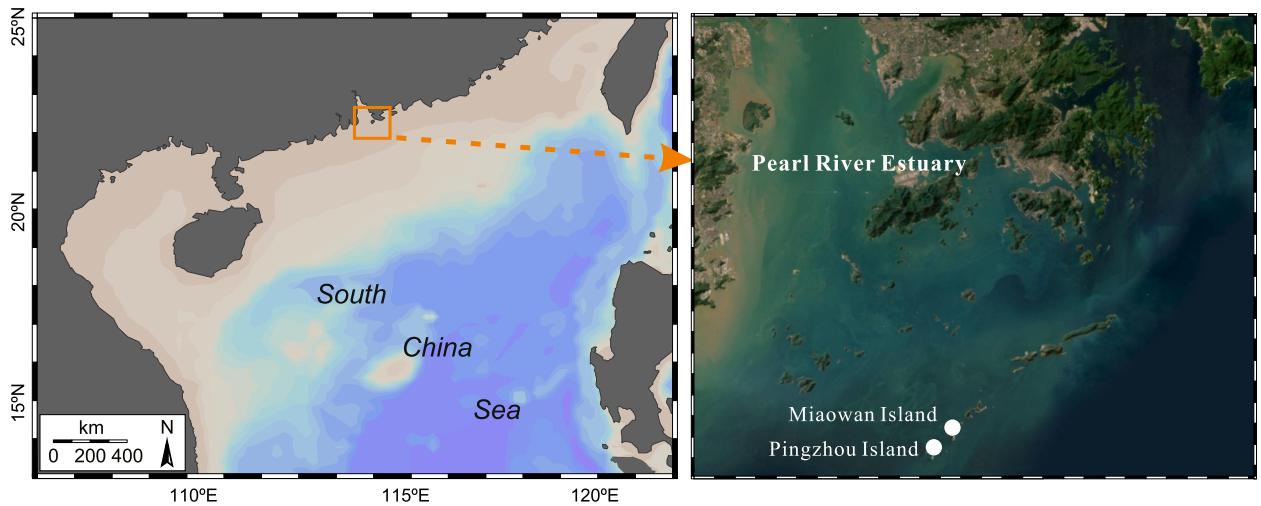

Fig. 1 Sampling site information. Coral samples and local water environmental parameters were collected in two islands of Pingzhou and Miaowan, from the Wanshan Islands, Pearl River Estuary, China

by using a spectrophotometer at wavelengths of 750, 664, 647, and 663 nm. The Mann–Whitney U test was used to analyze the significance of differences between groups ($p < 0.05$).

Total DNA extraction, Polymerase Chain Reaction (PCR) amplification, and Illumina MiSeq sequencing of coral symbiotic Symbiodiniaceae

The total DNA of coral holobionts was extracted as follows: ~ 50 mg of coral tissue and mucus was sampled, and genomic DNA was extracted using the DNeasy® Plant Kit per the manufacturer's instructions. The extracted DNA samples were used as PCR templates after the examination of quality and purity. The quality and concentration of DNA were determined using 1.0% agarose gel electrophoresis and a NanoDrop2000 spectrophotometer (Thermo Scientific, United States) and stored at −80 ℃ for further use. PCR amplification of the Symbiodiniaceae ITS2 region of rDNA was performed using the primers F:5′-GAATTGCAGAACTCCGTG-3′ and R:5′-GGGATCCATATATGCTTAAGTTCAGCGGGGT-3′, with a six-nucleotide barcode unique to each sample [49, 50], and a 50 μL reaction volume PCR amplification was performed. The reaction volume contained ~ 50 ng DNA, 25 μL 2 × Taq Platinum Polymerase Chain Reaction Master Mix (Tiangen, China), 200 nM of each primer, and ddH2O added to achieve the final volume. The reaction was conducted for 5 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 51 °C, and 30 s at 72 °C, with a final extension for 5 min at 72 °C, using an ABI GeneAmp® 9700 thermal cycler [51]. Triplicate PCR products were collected from each sample, purified, and quantified using the AxyPrep DNA Gel Extraction Kit and QuantiFluor™ ST Fluorescence Quantification System for sizes between 301 and 340 bp. Purified amplicons were pooled in equimolar amounts and subjected to paired-end sequencing on an Illumina MiSeq platform according to standard protocols (2 × 300).

Analysis of α-diversity and community composition of coral symbiotic Symbiodiniaceae

Strict quality control and sequence filtering were performed to ensure the accuracy of the results. After short and low-quality sequences were removed by the sequencing company, full-length ITS2 rDNA fragments were obtained by applying the paired-end-sequence amalgamation (PEAR) tool to merge overlapping PE reads to generate ITS2 sequences [52]. After identifying a unique sequence, ITS2 tags were demultiplexed into all samples using the QIIME 2 platform [53]. BLASTn was used to compare all ASV sequences with the ITS2 database [54, 55]. The resulting symbiont ITS2 Symbiodiniaceae subclade counts were combined for downstream statistical analyses. The symbiotic Symbiodiniaceae H′ diversity index was calculated using the R software environment (version 4.2.3) and Bray–Curtis to assess the α-diversity in the symbiotic Symbiodiniaceae composition among coral species. Kruskal–Wallis analysis was performed on the α-diversity data based on different corals. A phylogenetic tree of the Symbiodiniaceae was constructed in MEGA 6 by using the maximum likelihood method (based on the Kimura 2-parameter model) [44, 56]. Raw reads were deposited into the NCBI Sequence Read Archive database (Accession Number: PRJNA1086834).

Coral-associated bacteria

Total DNA extraction, PCR amplification, and Illumina MiSeq sequencing of coral-associated bacteria

Approximately 50 mg of coral tissue and mucus was cut with scissors, and the total genomic DNA was extracted from the coral samples by using the TIANamp Marine Animal DNA Kit per the manufacturer's instructions. The quality and concentration of DNA were determined using 1.0% agarose gel electrophoresis and a NanoDrop2000 spectrophotometer (Thermo Scientific, United States) and stored at −80 ℃ for further use. The total genomic DNA that fulfilled the conditions $1.8 < 260/280 < 2.0$ and $260/230 > 2.0$ was used as a template. Upstream primers carrying Barcode sequences 338F (5'-ACTCCTACGGGG AGGCAGCAG-3') and 806R (5'-GGACTACHVGGG TWTCTAAT-3') were used with the ABI GeneAmp® 9700 Thermal Cycler for the PCR amplification of the V3–V4 region of the bacterial 16S rRNA gene [48, 57]. An ABI GeneAmp 9700 thermal cycler (Thermo Fisher Scientific, Waltham, MA, United States) was used as a PCR reaction system, and the reactions were conducted under the following conditions: 3 min at 95°C, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 10 min. PCRs were run in triplicate per sample, which were conducted using a 20 μL reaction volume of TransGen AP221-02 (TransGen Biotech, Beijing, China) containing: 4 μL 5 × FastPfu Buffer (TransGen Biotech, Beijing, China), 2 μL 2.5 mM dNTPs, 0.8 μL (5 μm) forward primer, 0.8 μL (5 μm) reverse primer, 0.4 μL FastPfu DNA Polymerase (TransGen Biotech, Beijing, China), 0.2 μL BSA and 10 ng template DNA; the final volume was adjusted to 20 μL using ddH_2O [55]. Next, 2 μL amplified product was collected, and product integrity was determined using 2% agarose gel electrophoresis at a voltage of 115 V for 45 min to confirm whether the fragment size was between 420 and 460 bp. PCR products were purified using PCR Clean-up Kit according to the manufacturer's instructions (Yuhua, China), and Qubit 4.0 (Thermo Fisher Scientific, USA) was used

to detect and quantify the recovered products. The purified PCR products were constructed using NEXTFLEX ® Rapid DNA-SeqKit: (1) split-link (2) Use magnetic bead screening to remove split-self-connected segments; (3) Enrichment of library templates by PCR amplification; (4) The PCR products were recovered by magnetic beads to obtain the final library. Sequencing was performed using the umimaNextseq2000 platform (Majorbio Biopharm Technology Company, Shanghai, China). Raw reads were deposited into the NCBI Sequence Read Archive database (Accession Number: PRJNA1086806).

Analysis of α-diversity and community composition of associated bacteria

Paired reads of the bacterial 16S rRNA gene output from the Illumina MiSeq platform were merged with overlapping PE reads by using PEAR software to obtain the complete 16S rRNA gene V3–V4 region sequence, which allowed for a maximum mismatch ratio of 0.2. The complete 16S rRNA gene V3–V4 region was homogenized to remove average quality scores of < 20 and bases of < 50 bp. Sequence orientation was based on primer sequences with valid sequences from barcode-identified samples. Non-repetitive sequences were extracted using Uparse. Single sequences and chimeras without repeats were removed, and based on the valid ASV data, they were processed and denoised. Next, species were classified to obtain the ASVs of bacterial samples for the assessment and statistical analysis of bacterial diversity and community composition. The SILVA v138 database was used for the identification of bacterial species. Rarefaction curves construction as well as alpha diversity indices were calculated on rarified data set (30,000 sequences for bacteria) using the Mothur (v1.30.2). Alpha diversity indices were compared with Kruskal Wallis test using the stats package in R (v3.3.1). The bacterial community composition was visualized by NMDS based on Bray–Curtis distance, using the Vegan package in R software (version 4.2.3). PERMANOVA was conducted to test for statistically significant differences in community composition among groups [58].

Results

Water parameters of Wanshan Islands, Pearl River Estuary

The water parameters of the Wanshan Islands in the Pearl River Estuary showed that in July 2022, the SST ranged from 29.44 ± 0.07 °C to 29.72 ± 0.08 °C, DO ranged from 6.65 ± 1.46 to 7.84 ± 0.56 mg/L, and salinity ranged from 23.26 ± 0.05‰ to 24.83 ± 1.03‰. Owing to heavy rainfall in the upper reaches of the Pearl River over several days, a large amount of freshwater was imported into the Pearl River Estuary, resulting in low-salinity conditions. In this study, the average salinity of the sampling sites was 24.4 ± 1.1‰, which is much lower than the normal salinity level (30.61–32.39‰) in the Pearl River Estuary [59], indicating that corals are subjected to low-salinity conditions in the Wanshan Islands.

Coral symbiotic Symbiodiniaceae in Wanshan Islands, Pearl River Estuary

Density and Chl a content of coral symbiotic Symbiodiniaceae

The Symbiodiniaceae density significantly differed among the six coral species (Fig. 2A, $p < 0.05$, Table S1). The symbiotic Symbiodiniaceae density assessment results showed that *P. daedalea* had the highest Symbiodiniaceae density ($6.91 \pm 2.07 \times 10^6$ cells/cm^2), and *E. aspera* had the lowest density ($3.31 \pm 0.88 \times 10^6$ cells/cm^2). Thus, the densities of other Symbiodiniaceae coral species were between those of *P. daedalea* and *E. aspera*.

Moreover, there was a significant difference in Chl a content among these coral species ($p < 0.05$). *G. fasciculatis* exhibited the highest Chl a content (14.88 ± 2.19 μg/cm^2), which was much higher than those of the other coral species (Fig. 2B). The lowest Chl a content was 4.55 ± 1.56 μg/cm^2 for *A. solitaryensis*, *M. peltiformis*, *P. versipora*, *P. daedalea*, and *E. aspera*. The Chl a content of *E. aspera* was between that of *A. solitaryensis* and *G. fascicularis*.

Diversity of coral symbiotic Symbiodiniaceae

In this study, 1,535,930 Symbiodiniaceae ITS2 reads were obtained in low-salinity conditions to fulfill the analysis criteria after rigorous screening and quality control. Of the Symbiodiniaceae ASVs, 1,070 valid ASVs were obtained for diversity and community analyses.

The Ace, Chao, Shannon, and Simpson indices for the symbiotic coral Symbiodiniaceae are shown in Fig. 3 and Table S2. The symbiotic Symbiodiniaceae of *E. aspera* had the highest Shannon index (2.09 ± 0.05), and that of *P. daedalea* was the lowest (1.27 ± 0.22). Overall, significant differences were observed in the diversity indices among the six coral species ($p < 0.05$).

Composition of coral symbiotic Symbiodiniaceae

As shown in the composition diagram (Fig. 4), *Cladocopium* was the dominant Symbiodiniaceae among the six coral species (relative abundance > 97%, Table S3). Among them, *A. solitaryensis*, *P. daedalea*, and *G. fascicularis* had similar compositions to Symbiodiniaceae, and the abundance of the dominant *Cladocopium* C1 ranged from 80.4% to 84.5%. The compositions of *P. versipora*, *E. aspera*, and *M. peltiformis* differed from those of *A. solitaryensis*, *P. daedalea*, and *G. fascicularis*. *P.*

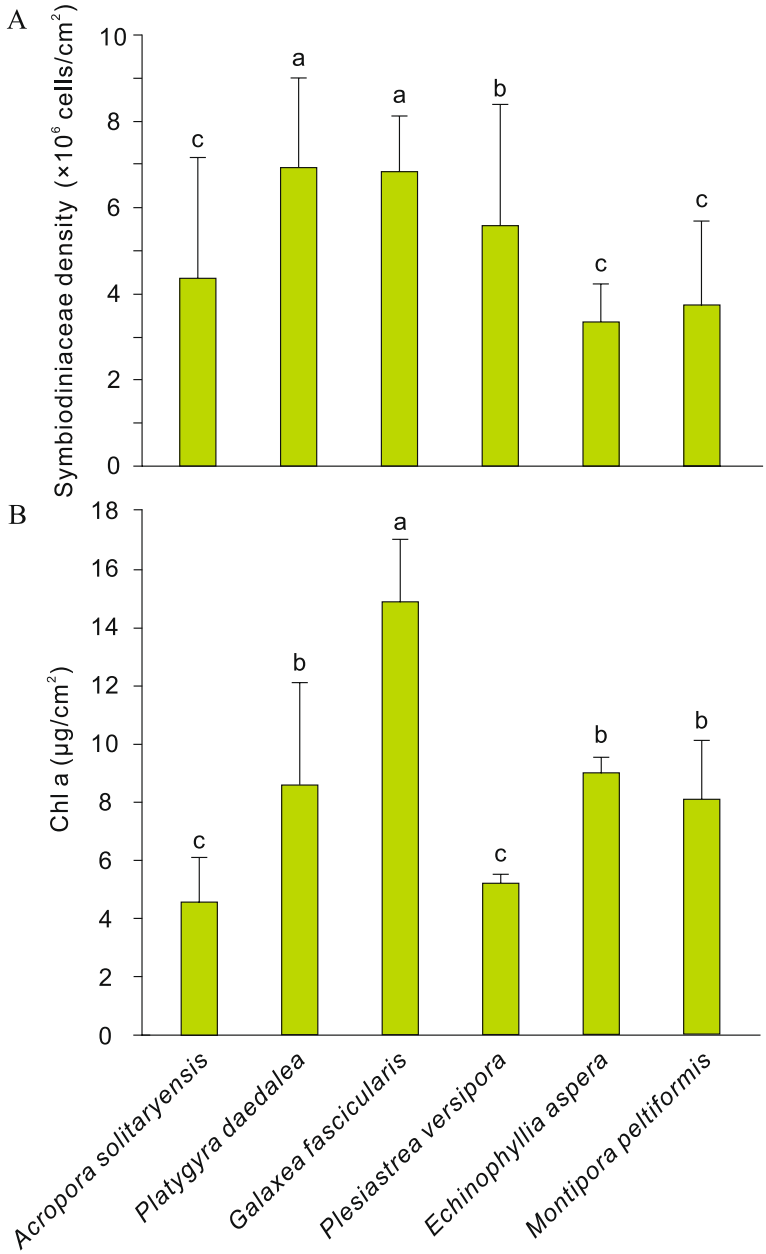

Fig. 2 Symbiodiniaceae density (**a**) and Chl a content (**b**) of six coral species from the Wanshan Islands, Pearl River Estuary. 'a', 'b' and 'c' represent the differences between different corals in Symbiodiniaceae density and Chl a conten, respectively (Mann–Whitney U test, $P < 0.05$)

versipora had the highest percentage of *Cladocopium* C1, and the abundance of *Cladocopium* C1 was lower than that of *A. solitaryensis*, *P. daedalea*, and *G. fascicularis*. *Cladocopium* Cspc, *Cladocopium* C44, and *Cladocopium* C1p were highly abundant (> 5%). The symbiotic Symbiodiniaceae of *E. aspera* had the highest abundance of *Cladocopium* C18 (49.7%), followed by *Cladocopium* C1 (26.4%) and *Cladocopium* C1p, *Cladocopium* C16 (> 5%). *Cladocopium* C3d was dominant in *M. peltiformis* (79.8%). *Cladocopium* C21.11 and *Cladocopium* Cspc also showed a high abundance (> 5%).

A phylogenetic tree of the dominant Symbiodiniaceae subclades in low-salinity conditions was constructed (Fig. 5). Three major groups were suggested for the development of the symbiotic Symbiodiniaceae in six coral species from the Pearl River Estuary. *Cladocopium* C16, *Cladocopium* C21.11, and *Cladocopium* Cspc had close phylogenetic relationships, and the phylogenetic

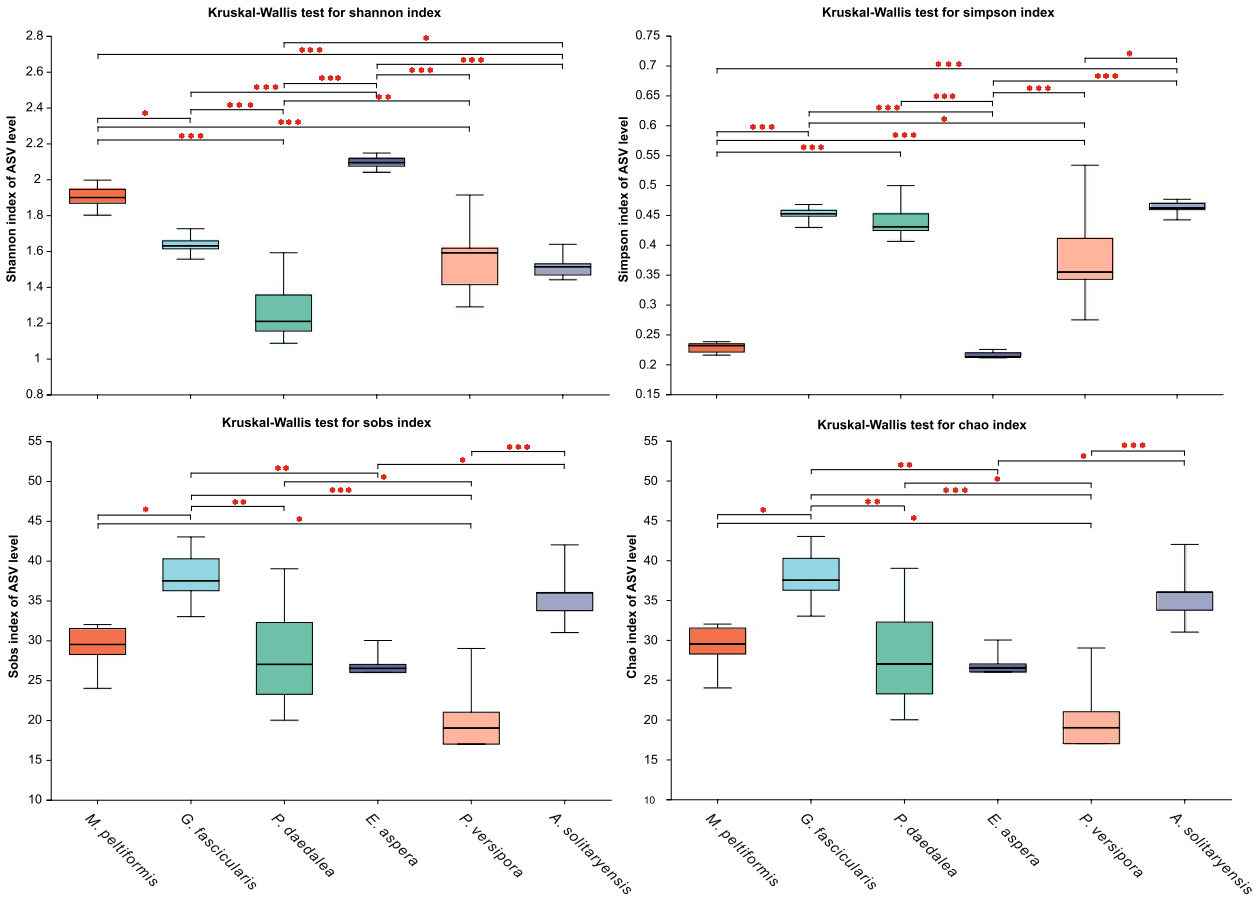

Fig. 3 Diversity indices of coral symbiotic Symbiodiniaceae of six coral species from the Wanshan Islands, Pearl River Estuary. The statistical differences were calculated by the Kruskal–Wallis H Test followed by pairwise testing using the Mann–Whitney U test (*: $0.01 < p < 0.05$; **: $0.001 < p < 0.05$; ***: $p < 0.001$)

relationships of *Cladocopium* C65a, *Cladocopium* C1p, and *Cladocopium* C72 were closer than those of *Cladocopium* C44, *Cladocopium* C1ca, and *Cladocopium* C1. The symbiotic Symbiodiniaceae composition of *M. peltiformis*, including *Cladocopium* C3.14, *Cladocopium* C21.11, *Cladocopium* Cspc, and *Cladocopium* C3d, showed closer phylogenetic relationships. The symbiotic Symbiodiniaceae composition of *A. solitaryensis*, including *Cladocopium* Cspc, *Cladocopium* C72, *Cladocopium* C1p, *Cladocopium* C1p.C45, *Cladocopium* C1ca, *Cladocopium* C1, and *Cladocopium* C78a, showed closer phylogenetic relationships.

Diversity and composition of coral-associated bacteria in Wanshan Islands, Pearl River Estuary

Diversity of coral-associated bacteria

In this study, 1,425,904 reads were obtained from the six coral species after rigorous screening and quality control (Table S4). After excluding archaea, chloroplasts, and mitochondrial sequences, in total, 16,112 valid bacterial ASVs were identified. Per these bacterial ASVs, the Shannon index showed that *A. solitaryensis* had a relatively low level of associated bacterial diversity and that *P. versipora* and *G. fascicularis* had higher levels of associated bacterial diversity (Fig. 6). The Shannon index also showed a significant difference in bacterial community diversity between *P. versipora* and *A. solitaryensis* ($p < 0.001$). The bacterial community diversity of *P. versipora* and *M. peltiformis* also significantly differed ($p < 0.05$). The Simpson diversity index was the opposite of that of the Shannon diversity index. The Ace index statistics showed that *P. versipora* and *G. fascicularis* had the highest levels of total ASVs (Fig. 6), and *A. solitaryensis* had the lowest levels of total bacterial species, which differed from those of *P. versipora* ($p < 0.05$). In normal salinity, the Sobs index and Shannon index of *M. peltiformis* and *Platygyra* were lower than in low-salt conditions. Furthermore, at normal salinity, the Simpson index of *M. peltiformis* and *Platygyra* was higher than that of

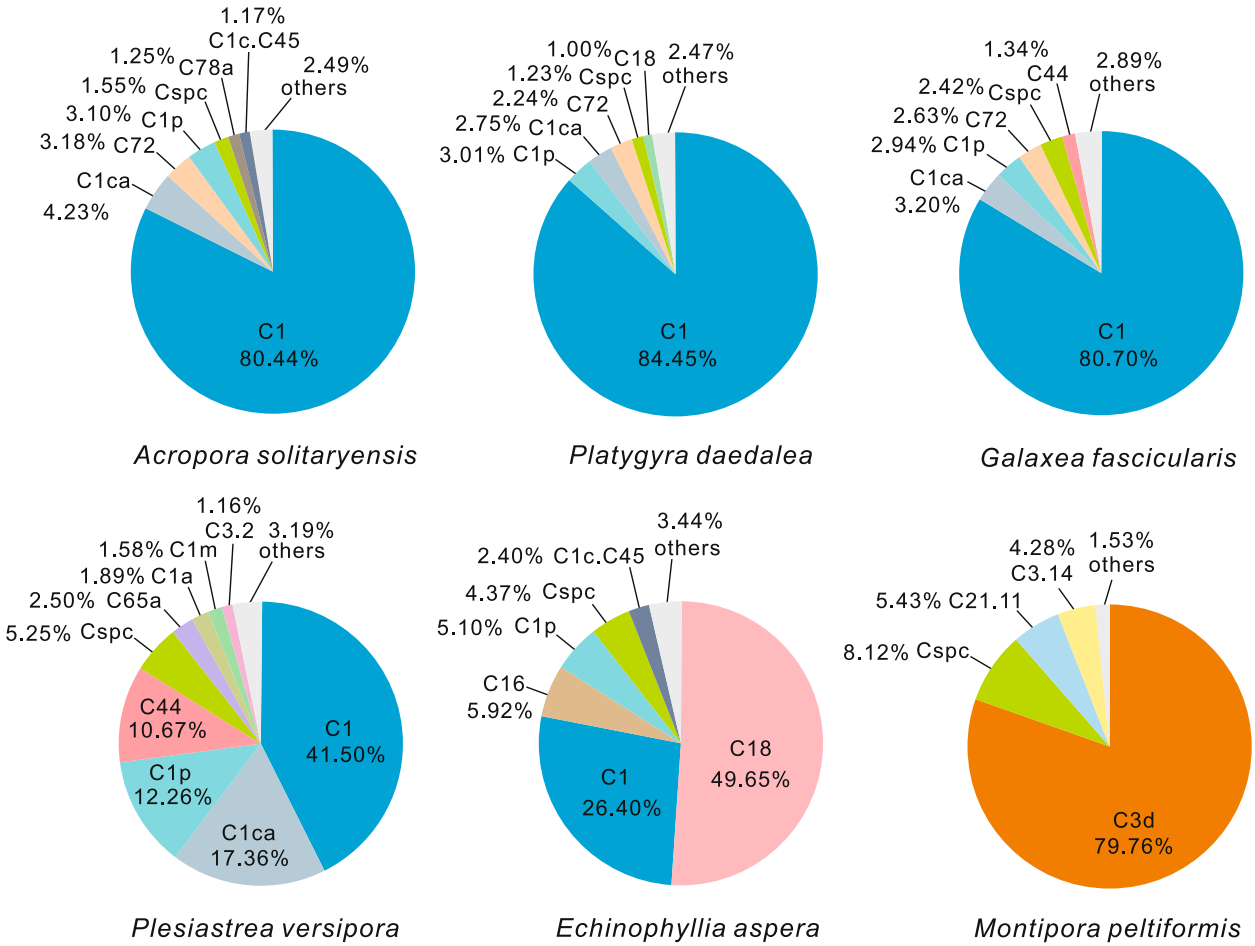

Fig. 4 Composition of symbiotic Symbiodiniaceae in six coral species from the Wanshan Islands, Pearl River Estuary. The Symbiodiniaceae subclades such as C1, C44, and Cspc,were be classified as the *Cladocopium*

low-salinity conditions. These results indicated increased bacterial diversity in corals in low-salinity conditions.

According to the NMDS analysis (Fig. 7), the community composition of the bacteria associated with the six coral species suggested that *E. aspera* differed significantly ($p<0.05$) from the other coral species in associated bacterial community composition. The NMDS analysis also suggested that the associated bacteria community of *P. versipora* differed significantly from those of the other coral species ($p<0.05$). *A. solitaryensis*, *M. peltiformis*, *P. daedalea*, and *G. fascicularis* had similar bacterial communities.

Composition of coral-associated bacteria

At the phylum level, the bacteria associated with *A. solitaryensis* and *P. daedalea* were dominated by Proteobacteria (Fig. 8, Table S5). For *P. versipora* and *E. aspera*, Chloroflexi were the dominant bacteria; other bacteria, such as Proteobacteria, Bacteroidota, and Actinomycetota, also had relatively high abundances. In the cases of *G. fascicularis* and *M. peltiformis*, the dominant bacteria were Proteobacteria, Bacteroidota, and Firmicutes. The bacterial composition of corals in normal salinity differs from that in low-salinity conditions. The bacteria associated with *M. peltiformis* were dominated by Cyanobacteria, with relative abundances of 92.15% at normal salinity. In normal conditions, the relative abundances of γ-proteobacteria (20%), α-proteobacteria (12%), and Cyanobacteria (39%) in the associated bacteria community of *G. fascicularis* were relatively high. The dominant bacteria in both low-salt conditions is Proteobacteria, and the dominant bacteria in normal salinity is Cyanobacteria. For *Platygyra*, Proteobacteria was the dominant phylum, with 71.87% in normal salinity, followed by Firmicutes. This suggests that different corals are affected by low salinity to different degrees and that low-salinity conditions affect the composition of some coral symbiotic bacteria.

Fig. 5 Phylogenetic tree of coral symbiotic Symbiodiniaceae of six coral species from the Wanshan Islands, Pearl River Estuary

At the genus level, we selected the top 30 bacteria in terms of total abundance for plotting. Among these six coral species had high abundances of *Vibrio* (relative abundance: 4.2 ± 4.8%) and Rhodobacteraceae (relative abundance: 3.9 ± 2.6%, Fig. 9, Table S6). *G. fascicularis* had high abundances of *Pseudomonas* (relative abundance: 4.6 ± 2.4%), *Photobacterium* (relative abundance: 4.5 ± 2.4%), and *Pseudoalteromonas* (relative abundance: 4.5 ± 2.4%). *Ruegeria* was observed in *P. daedalea* and *M. peltiformis*, with relative abundances of 2.9 ± 1.9% and 1.0 ± 1.2%, respectively. At the genus level, in normal salinity, the bacteria associated with *M. peltiformis* were dominated by *Synechococcus* CC9902 (89.16%). The dominant genus in *Platygyra* was *Endozoicomonas* (52.30%). The results demonstrated that the dominant genus composition of corals differed significantly between normal salinity and low-salinity conditions (Mann–Whitney U test, $p < 0.05$).

Discussion

Adaptation of coral-Symbiodiniaceae holobionts to the low-salinity in the Pearl River Estuary

Owing to the influences of short-term rainstorms and massive freshwater input, low-salinity conditions endure in the Pearl River Estuary. Low-salinity conditions have significant negative impacts on coral-Symbiodiniaceae holobionts, and coral symbiotic Symbiodiniaceae density decreases in low salinity [22, 60]. The symbiotic Symbiodiniaceae density reflects differences in the adaptability of coral species in low-salinity conditions. For example, *G. fascicularis* can normally survive more than 60 d when exposed to 20‰ salinity [61]; however, the growth rate of *Platygyra acuta* slows when the salinity is < 26‰ and stops at salinities < 22‰ [30]. In low-salinity conditions, Liu et al. [60] found that the Symbiodiniaceae density and Chl a content of *Porites lutea* decreased significantly when the surrounding seawater salinity was < 30‰, and

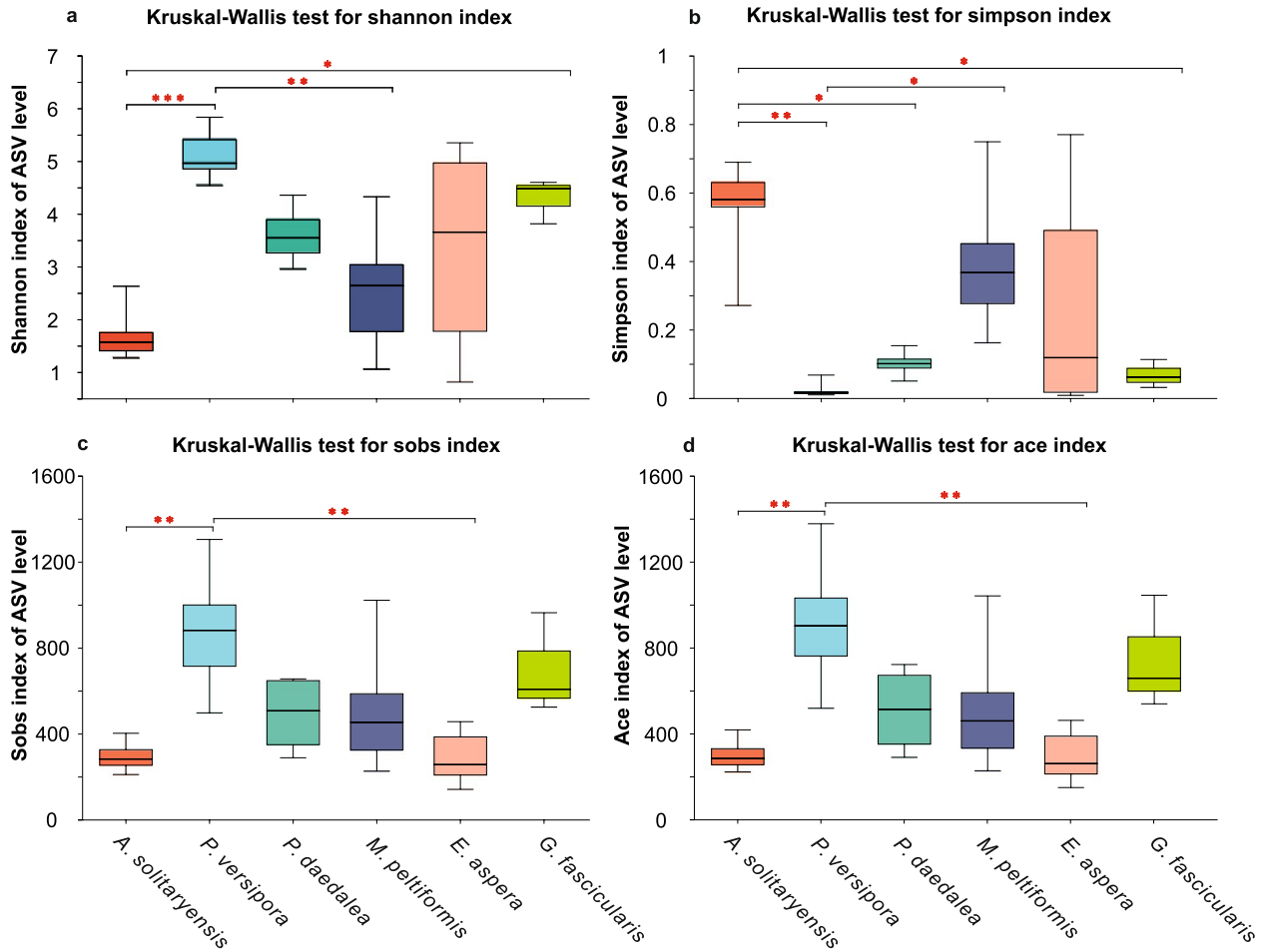

Fig. 6 Diversity indices of coral-associated bacteria of six coral species from the Wanshan Islands, Pearl River Estuary. The statistical differences were calculated by the Kruskal–Wallis H Test followed by pairwise testing using the Mann–Whitney U test (*: $0.01 < p < 0.05$; **: $0.001 < p < 0.05$; ***: $p < 0.001$)

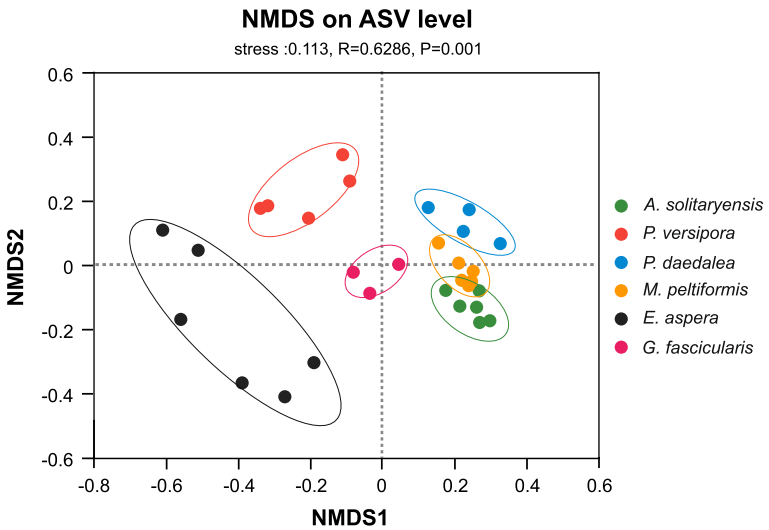

Fig. 7 NMDS analysis of associated bacteria of six coral species from the Wanshan Islands, Pearl River Estuary

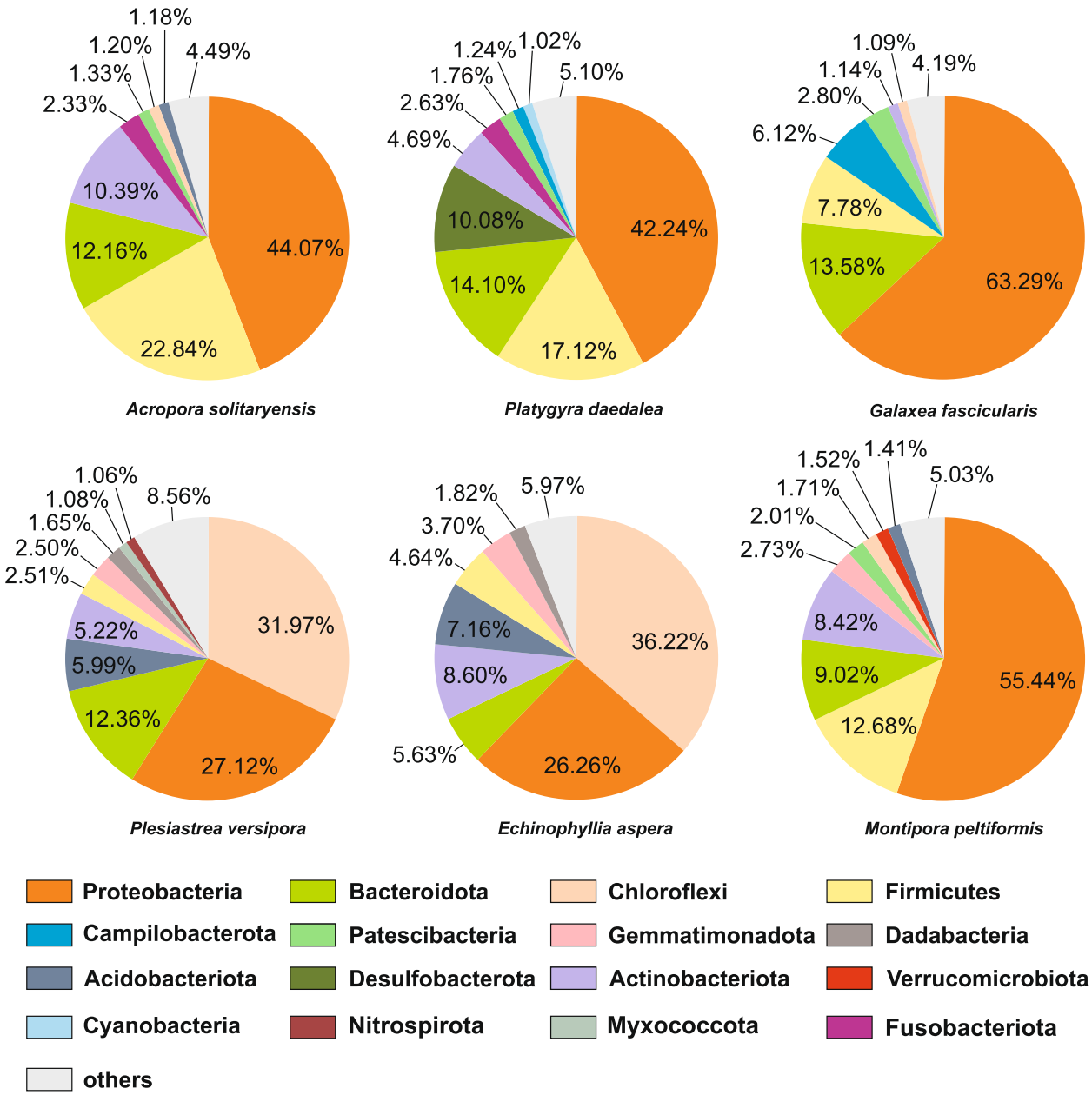

Fig. 8 Composition of coral-associated bacteria at the phylum level in the Wanshan Islands, Pearl River Estuary

the Symbiodiniaceae density and Chl a content decreased dramatically when the salinity decreased from 30‰ to 20‰ until the corals bleached. Similarly, in this study, the density and Chl a content of coral symbiotic Symbiodiniaceae differed significantly in the low-salinity conditions of the estuary, with some corals showing high Symbiodiniaceae densities and Chl a content, such as *G. fascicularis*; other corals showed low Symbiodiniaceae densities, such as *E. aspera*. The Symbiodiniaceae density of *P. versipora* was 6.8×10^6 cells/cm^2 [39], and that of *M. peltiformis* was 4×10^6 cells/cm^2 at normal salinity [40]. Both corals exhibited a decrease in Symbiodiniaceae density in low-salinity conditions. The Chl a content of *A. solitaryensis* was 15.21 μg/cm^2, and that of *M. peltiformis* was approximately 11 μg/cm^2 at normal salinity [41, 42]. In this study, the Chl a content of *A. solitaryensis* was 2.43 μg/cm^2, and that of *M. peltiformis* was 8.09 μg/cm^2 in low-salinity conditions. Both corals showed decreases in Chl a content. These findings suggest that low-salinity conditions affect Symbiodiniaceae density and Chl a

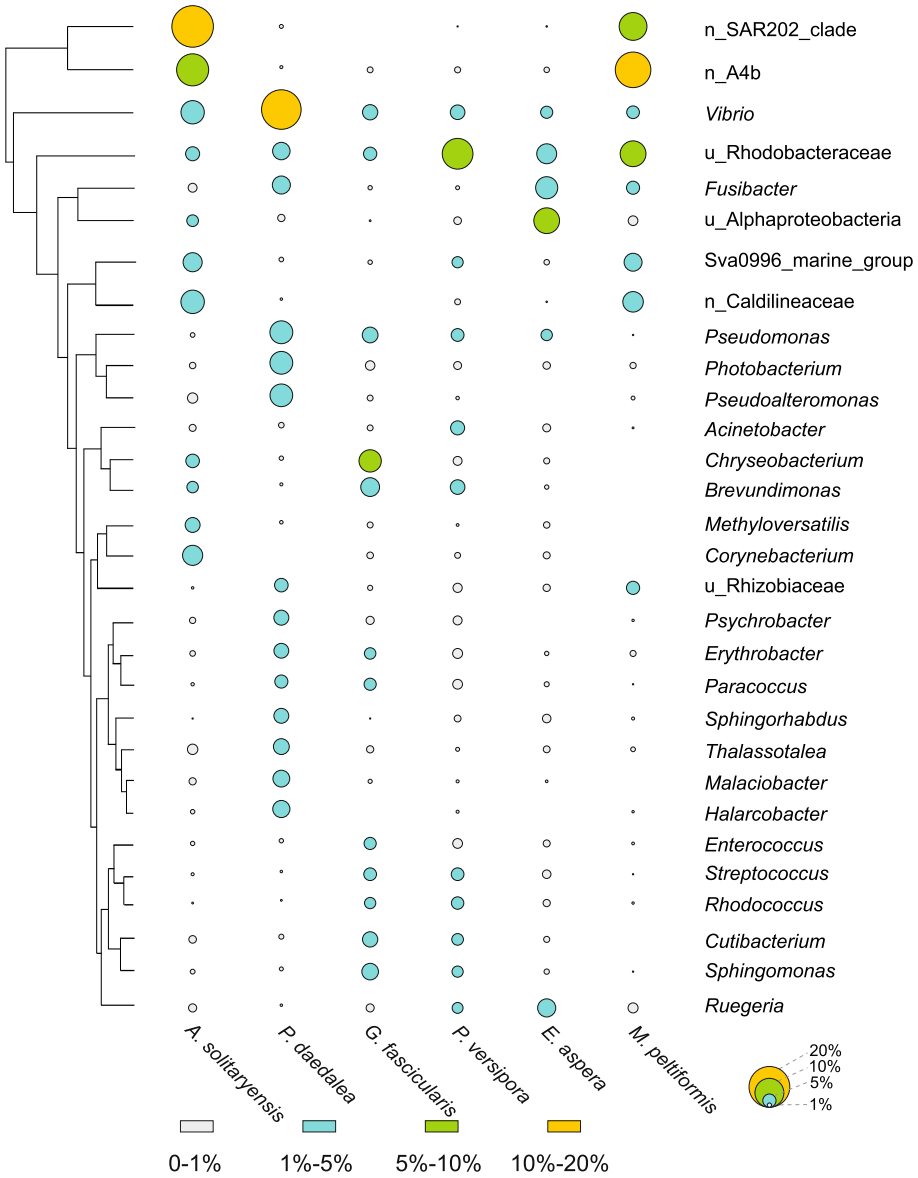

Fig. 9 Associated bacterial composition at the genus level of six coral species in the Wanshan Islands, Pearl River Estuary

content, and differences in Symbiodiniaceae density and Chl a content could be responsible for the different adaptations of corals to low-salinity conditions.

Environmental factors drive various types of and different environmental influences in Symbiodiniaceae coral holobionts [62, 63]. Studies have found that the dominant symbiotic Symbiodiniaceae is closely related to the tolerance of coral-Symbiodiniaceae holobionts to environmental stress [64–66]. Symbiodiniaceae subclades confer different physiological characteristics and environmental tolerances to coral holobionts [65, 67, 68]. In this study, the dominant symbiotic Symbiodiniaceae subclades in all corals were *Cladocopium* C1, excluding *M. peltiformis* and *E. aspera*. Some corals with *Cladocopium* C1-dominant species had a lower percentage of *Cladocopium* C1 than corals in normal salinity conditions, for example, *P. versipora* with 75% in normal salinity and 41.5% in this experiment [43], and some coral *Cladocopium* C1 percentage gaps do not vary much, for example, *G. fascicularis*, with approximately 80% in normal and low-salinity conditions [43]. *Cladocopium* C1 is a subclade of Symbiodiniaceae with high photosynthetic efficiency [69]. Under the low-salinity stress, corals may establish a symbiotic relationship with highly photosynthetically

efficient Symbiodiniaceae to maintain the functioning of the calcium carbonate secretion-storage system [70]. However, some of the dominant symbiotic Symbiodiniaceae subclades change from corals between the normal salinity and low-salinity conditions. In normal conditions, for example, the dominant symbiotic Symbiodiniaceae subclades of the *M. peltiformis* were *Cladocopium* C3d, followed by *Cladocopium* C2r [44]. In low-salinity conditions, the dominant symbiotic Symbiodiniaceae subclades were *Cladocopium* C3d, followed by *Cladocopium* Cspc. Notably, some corals were symbiotic with different symbiotic Symbiodiniaceae subclades when experienced low-salinity stress. For example, *Cladocopium* Cspc and C3d were detected from *M. peltiformis* in low salinity conditions, but *Cladocopium* C3d and C2r were detected in normal salinity conditions. Research has observed that *M. peltiformis* has high susceptibility to environmental influences [45]. The flexible changes in the composition of symbiotic Symbiodiniaceae subclades may be conducive *M. peltiformis* to survive and adapt to low-salinity stress.

Coral symbiotic Symbiodiniaceae types are typically determined by environmental selection and co-evolution [71]. The results of this study showed that among the six coral species, some of them differed significantly in their Symbiodiniaceae diversity and composition. Coral holobionts can regulate physiological and ecological characteristics (e.g., coral host stress, Symbiodiniaceae densities, Symbiodiniaceae subclades, and bacterial communities) to adapt to external environmental changes [72, 73]. The results suggest that different corals adapt to low salinity by regulating their symbiotic relationship with Symbiodiniaceae. Based on the phylogenetic tree of Symbiodiniaceae, two typical symbiotic Symbiodiniaceae species selected by corals survived in low-salinity conditions. For example, the Symbiodiniaceae subclades of *M. peltiformis* belong to similar branches of the phylogenetic tree, and those of *A. solitaryensis* are distributed throughout the phylogenetic tree. This suggests that symbiotic Symbiodiniaceae may be regulated by corals in low-salinity conditions. Some coral species establish symbiotic relationships with specific Symbiodiniaceae subclades (e.g., *Cladocopium* C1) to increase their photosynthetic rate, and other coral species establish symbiotic relationships with multiple Symbiodiniaceae subclades to enhance their Symbiodiniaceae diversity and tolerance to low-salinity conditions.

Adaptation of coral-bacterial holobionts to low-salinity Pearl River Estuary condition

Salinity is a key factor influencing the diversity and composition of bacterial communities in coastal corals [74, 75]. Microbiome dynamics are linked to coral environmental tolerance [76]. And a high diversity of the bacterial community may contribute to niche complementation and/or functional redundancy [23, 77]. In our study, six studied coral species suggested that the bacterial communities associated with each of them showed significant differences in diversity and composition. Specifically, relatively high bacterial diversity was detected in the *P. versipora*, while relatively low bacterial diversity in the *A. solitaryensis*.

Compared with normal salinity environment, bacterial composition of different corals widely changed in low-salinity environment [45, 46]. For example, *M. peltiformis* showed distinct changes in bacterial composition. At the phylum level, the bacteria associated with *M. peltiformis* were dominated by Cyanobacteria, with relative abundances of 92.15% in normal salinity environment. At the genus level, the bacteria associated with *M. peltiformis* were dominated by *Synechococcus* CC9902 (89.16%) [45]. However, in low-salinity conditions, the dominant ASVs were Proteobacteria at the phylum level. At the genus level, u_Rhodobacteraceae were the dominant bacteria. *Synechococcus* spp. is conducive coral holobiont to nitrogen fixation [78] and photosynthesis [79], indicating their potential roles in the hologenomic nutrient cycling of corals and the health state of coral hosts. Rhodobacteraceae may also enhance the resilience of corals by absorbing DMSP from Symbiodiniaceae and producing antibacterial compounds against pathogens [80]. Evidently, low salinity can affect changes in the bacterial composition of corals, which further impacts the health of coral holobionts [45]. In contrast, the bacterial composition of *G. fascicularis* was relatively in stable. Under normal-salinity conditions, the relative abundances of γ-Proteobacteria, α-Proteobacteria, and Cyanobacteria of *G. fascicularis* were 20%, 12%, and 39% respectively, remaining at a relatively high level [46]. Under low-salinity environment, Proteobacteria becomes were also the dominant bacteria, which is similar to the bacterial composition under normal conditions. This indicates a potential variation between the stability of the microbiome and the susceptibility to bleaching among coral species. The bacterial diversity might be important factors in coral adaptability to low-salinity conditions. Some coral species may survive better than others in low-salinity conditions by maintaining a high level of bacterial diversity. Coral microbiome with high diversity may have high physiological and ecological acclimatization to low-salinity conditions [81].

Community-associated bacterial communities have high flexibility and diverse functions and are closely related to the health of coral symbiotic functionaries. For

example, Proteobacteria and Bacteroidetes have a high tolerance to salinity stress [82]. In this study, bacteria from Proteobacteria and Bacteroidetes tended to dominate the coral-associated communities, and their insensitivity to salinity allowed these bacteria to survive better than other bacteria in low-salinity conditions. Rhodobacteraceae were highly abundant in all six coral species, and their ability to grow through photosynthesis, metabolize CO_2, and fix nitrogen plays an important role in the carbon and nitrogen cycles of marine ecosystems [83].

In this study, additional differences were observed in the relative abundances of bacterial phyla and genera, reflecting varying adaptability among coral species in low-salinity conditions. Coral-associated bacteria may be related to coral growth, nutrient metabolism, the immune system, antioxidant capacity, resilience, and tolerance [84, 85]. In our study, the coral microbial communities in low-salinity areas revealed that *Pseudoalteromonas* was extremely abundant in some corals, such as *P. daedalea*. *Pseudoalteromonas* has been shown to have significant antagonistic effects on various pathogens [86]. In this study, *Pseudoalteromonas* and *Vibrio* were detected in the composition of coral-associated bacteria. *Pseudoalteromonas* may be conducive to inhibit the growth and reproduction of *Vibrio* via antagonism, potentially decreasing the risk of disease in corals [87]. Furthermore, a high abundance of *Ruegeria* was found in *P. daedalea* and *M. peltiformis*. *Ruegeria* is a potential probiotic coral that produces antibiotics that can inhibit the growth of *Vibrio* [88]. High abundance of *Vibrio* in low-salinity conditions may interfere with the associated relationship between corals and bacteria [89, 90]; the antagonistic effect of *Pseudoalteromonas* and the antibiotics produced by *Ruegeria* could be conducive to inhibiting the growth of *Vibrio*, maintaining the stability of the relationship and the mutual cooperation between corals and bacteria. Under low-salinity stress, the increased abundance of specific bacterial taxa may contribute to the health and survival of corals [91, 92].

Conclusions

Different coral species have different adaptations to low-salinity stress by regulating the diversity and composition of Symbiodiniaceae and bacteria. Some coral species improved their adaptation to low-salinity stress in the Pearl River Estuary based on a high diversity of symbiotic Symbiodiniaceae and associated bacteria. In contrast, some other coral species may increase their resistance by associating with specific Symbiodiniaceae subclades, with high Symbiodiniaceae density, or minority bacterial dominance under low-salinity stress.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-04013-4.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.
Supplementary Material 6.

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Authors' contributions
We have taken appropriate measures to ensure the accuracy and reliability of the data and have followed the principles of scientific integrity in data analysis and result presentation. Z.Q. and K.Y. conceived the research; Z.Q., B.C., X.Y. and Y.Z. contributed the materials; M.L., K.G., Z.L. and R.M. performed all experiments; L.W. and L.X. identified coral species; M.L., K.G., Z.Q., and K.Y. wrote the manuscript; all authors edited and approved the manuscript. The authors declare that they have no conflict of interest.

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Data availability
The data used in this paper are available from supporting information (SI), and the raw sequences were deposited in the NCBI Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/, accession number: Symbiodiniaceae: PRJNA1086834; bacteria: PRJNA1086806).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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