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Heat-induced shift in coral microbiome reveals several members of the Rhodobacteraceae family as indicator species for thermal stress in *Porites lutea*

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Abstract

The coral holobiont is a complex ecosystem consisting of coral animals and a highly diverse consortium of associated microorganisms including algae, fungi, and bacteria. Several studies have highlighted the importance of coral-associated bacteria and their potential roles in promoting the host fitness and survival. Recently, dynamics of coral-associated microbiomes have been demonstrated to be linked to patterns of coral heat tolerance. Here, we examined the effect of elevated seawater temperature on the structure and diversity of bacterial populations associated with *Porites lutea*, using full-length 16S rRNA sequences obtained from Pacific Biosciences circular consensus sequencing. We observed a significant increase in alpha diversity indices and a distinct shift in microbiome composition during thermal stress. There was a marked decline in the apparent relative abundance of Gammaproteobacteria family Endozoicomonadaceae after *P. lutea* had been exposed to elevated seawater temperature. Concomitantly, the bacterial community structure shifted toward the predominance of Alphaproteobacteria family Rhodobacteraceae. Interestingly, we did not observe an increase in relative abundance of *Vibrio*-related sequences in our heat-stressed samples even though the appearance of *Vibrio* spp. has often been detected in parallel with the increase in the relative abundance of Rhodobacteraceae during thermal bleaching in other coral species. The ability of full-length 16S rRNA sequences in resolving taxonomic uncertainty of associated bacteria at a species level enabled us to identify 24 robust indicator bacterial species for thermally stressed corals. It is worth noting that the majority of those indicator species were members of the family Rhodobacteraceae. The comparison of bacterial community structure and diversity between corals in ambient water temperature and thermally stressed corals may provide a better understanding on how bacteria symbionts contribute to the resilience of their coral hosts to ocean warming.

KEYWORDS
coral bleaching, coral microbiome, coral-associated bacteria, Endozoicomonas, heat stress, PacBio sequencing, *Porites lutea*, Rhodobacteraceae, thermal bleaching, thermal stress

1 | INTRODUCTION

Coral reefs are among the most productive and biologically diverse ecosystems on Earth (Connell, 1978). They are estimated to cover only 0.1%–0.5% of the ocean floor (Copper, 1994; Spalding & Grenfell, 1997); nevertheless, coral reefs harbor almost a third of all marine fish species and support the productivity of ~25% of marine fisheries (Moberg & Folke, 1999). The coral "holobiont" is a complex ecological unit consisting of coral animals and a suite of associated symbionts including the dinoflagellate Symbiodiniaceae, fungi and bacteria (Bourne & Munn, 2005; Koren & Rosenberg, 2006; Pantos et al., 2003; Rohwer, Breitbart, Jara, Azam, & Knowlton, 2001; Rohwer, Seguritan, Azam, & Knowlton, 2002; Rosenberg, Koren, Reshef, Efrony, & Zilber-Rosenberg, 2007). A number of studies have shown that corals harbor a dynamic and highly diverse consortium of microbes that have coevolved with their hosts and contributed important functions to the holobionts (Ainsworth et al., 2015; Bourne, Morrow, & Webster, 2016; Bourne & Munn, 2005; Krediet, Ritchie, Paul, & Teplitski, 2013; Rohwer et al., 2001, 2002; Rosenberg & Loya, 2013; Thompson, Rivera, Closek, & Medina, 2014). Coral-associated bacteria appear to be involved in the provisioning and cycling of carbon, nitrogen and sulfur in coral reefs (Cardini et al., 2015; Ceh et al., 2013; Chimetto et al., 2008; Lema, Willis, & Bourne, 2012; Lesser et al., 2007; Rädecker, Pogoreutz, Voolstra, Wiedenmann, & Wild, 2015; Raina, Tapiolas, Willis, & Bourne, 2009; Siboni, Ben-Dov, Sivan, & Kushmaro, 2008). In addition, members of the coral microbiome may have beneficial roles in protecting their hosts against pathogenic and opportunistic bacteria by preventing their colonization through the production of antibacterial compounds or through physical occupation of otherwise available niches (Kelman, Kashman, Rosenberg, Kushmaro, & Loya, 2006; Koh, 1997; Krediet et al., 2013; Reshef, Koren, Loya, Zilber-Rosenberg, & Rosenberg, 2006; Ritchie & Smith, 2004).

Thermally induced coral bleaching has resulted in significant decline in live coral cover at regional scales (Brown, 1997; Bruno & Selig, 2007; Fitt, Brown, Warner, & Dunne, 2001; Gardner et al., 2019; Gardner, Côté, Gill, Grant, & Watkinson, 2003; Hughes et al., 2018; Jones, 2008). Earlier attempts to understand the coral host responses to ocean warming have mainly focused on the partnership between corals and their photosynthetic symbionts (Baker, 2003; Boulotte et al., 2016; Fautin & Buddemeier, 2004; Howells et al., 2012; Hume et al., 2016; Keshavmurthy et al., 2014; Rowan, 2004). Adaptive mechanisms to elevated water temperature such as Symbiodiniaceae "shuffling" (an adjustment in relative abundance of Symbiodiniaceae type in-hospite) and Symbiodiniaceae "switching" (an acquisition of new Symbiodiniaceae types from the surrounding environment) have been proposed (Baker, 2003; Baker, Starger, Mcclanahan, & Glynn, 2004; Berkelmans & van Oppen, 2006; Boulotte et al., 2016; Fautin & Buddemeier, 2004; Jones, Berkelmans, Oppen, Mieog, & Sinclair, 2008; Tamar, 2006). The roles of bacterial consortium in the coral holobiont symbioses during thermal stress have largely been overlooked until recently. A study by Gilbert, Hill, Doblin, and Ralph (2012) examined the response of a coral holobiont to thermal stress when the bacterial community was treated with antibiotics and demonstrated that a disruption to the microbial consortium diminished the resilience of the holobiont. Intact microbial communities offered coral holobionts resilience to thermal stress and increased the rate of recovery after bleaching events (Gilbert et al., 2012). More recently, dynamics of coral-associated microbiomes have been demonstrated to be linked to patterns of coral heat tolerance (Ziegler, Seneca, Yum, Palumbi, & Voolstra, 2017). Ziegler et al. (2017) showed that the composition of *Acropora hyacinthus*-associated bacterial community was different across thermally variable habitats and adapted to the new environment when corals were reciprocally transplanted. Subsequent exposure of those transplanted corals to thermal stress conditions changed the bacterial community of heat-sensitive corals from a more stable, cooler environment, whereas heat-tolerant corals from a highly variable, warmer environment harbored a stable bacterial community (Ziegler et al., 2017).

Branching coral species, especially acroporids, are generally fast-growing and known to be more susceptible to thermal bleaching compared to massive *Porites* corals, which are slow-growing and more "stress-tolerant" (Darling, Alvarez-Filip, Oliver, Mcclanahan, & Côté, 2012; Loya et al., 2001; Pisapia et al., 2016). Shifts in coral-associated microbiomes during bleaching events have been documented mostly in *Acropora* species: *Acropora millepora* and *Acropora tenuis* from the Great Barrier Reef, *Acropora millepora* from Fiji and *Acropora muricata* in Nan-wan, Taiwan (Bourne, Iida, Uthicke, & Smith-Keune, 2008; Grottoli et al., 2018; Lee, Davy, Tang, & Kench, 2016, 2017; Littman, Willis, Pfeffer, & Bourne, 2009). Here, we investigated the dynamics of bacterial communities associated with a massive stony coral *Porites lutea*, a dominant reef-builder widely distributed in the Indo-Pacific, under short-term thermal stress. Our survey of *P. lutea* microbiomes utilized a long-read PacBio SMRT sequencing technology to capture full-length 16S rRNA sequences in order to examine the shifts in coral-associated bacterial community structure and diversity. Full-length 16S rRNA sequences have a benefit of spanning all hypervariable regions, enabling high-resolution taxonomic classification of bacterial species (Pootakham et al., 2017). The investigation of coral-microbial interactions and their dynamics during environmental stress will help further our understanding of the involvement of bacterial symbionts in the acclimatization and resilience of coral holobiont to climate change.

2 | MATERIALS AND METHODS

2.1 | Sample collection and DNA extraction

Five *P. lutea* colonies were collected from the Maiton Island in the Andaman Sea (7°45′42.5″N 98°28′51.3″E) at the depth of 7–10 m and immediately placed in containers with aerated seawater. We chose colonies that were at least 10 m apart from each other to reduce the possibility of collecting clonal colonies. *P. lutea* colonies were transported back to shore (Phuket Marine Biological Center) within an hour of collection and transferred to flow-through aquaria (transparent glass tanks with the dimension of 120 × 25 × 25 cm, holding approximately 75 L), which circulated seawater pumped from the reef to the Phuket Marine Biological Center Research Station. Each colony was fragmented into four nubbins using a hammer and chisel and acclimated for 14 days in flow-through aquaria at ambient temperature (29°C) with a 12-hr light–dark cycle and irradiance of 150 μmol m^{-2} s^{-1}. Two nubbins from each colony were placed in the "control" aquarium, and the other two were placed in the "heated" aquarium. The position of ten nubbins (two from each of the five colonies) in the aquarium was randomized. During the experiment, one aquarium was maintained at the ambient water temperature while the other aquarium was exposed to a heat treatment, which involved an incremental ramping of seawater temperature at a rate of 0.5°C/day for six consecutive days using one 100-W submersible aquarium heater (Eheim). From day 7 to day 13, the temperature was maintained at 34°C in the "heat treatment" aquarium (Figure 1a). Coral nubbins were removed from the aquaria for sampling 6 (D6C and D6H) and 13 (D13C and D13H) days after the onset of the thermal stress treatment (Figure 1b). Since the coral nubbins in the control and heated aquaria were from the same parent colonies (one fragment from each colony in each tank), changes in the microbiome were due to treatment effects and not genetic differences between colonies. It is worth noting that we used one aquarium for each condition (control and heated); hence, there was no technical replicate in this study. However, we closely monitored the temperature in both aquaria to ensure that it remained stable in the control aquarium (29°C) and was steadily increasing until it reached 34°C in the heated aquarium (Figure 1a).

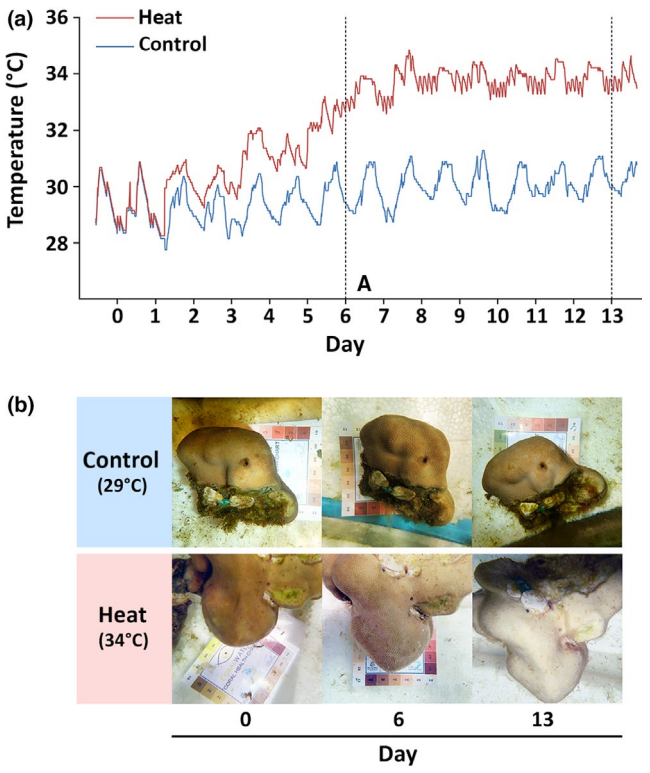

FIGURE 1 Temperature logs and physiological response of *Porites lutea* subjected to a heat treatment. (a) Records of daily water temperatures in control (blue line) and heated (red line) aquaria during the 13-day experiment. Vertical dotted lines indicate the days on which the samples were collected (day 6 and day 13). (b) Pictures of representative coral fragments from the control (~29°C) and heated (~34°C) aquaria taken prior to (day 0) and following the imposition of short-term thermal stress (day 6 and day 13)

Coral tissues were collected using scalpel blades, placed in sterile screw-capped tubes, and immediately frozen in liquid nitrogen. DNA extraction was performed using the CTAB protocol reported in Pootakham et al. (2017). DNA samples were quantified using the NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific) and diluted to 50 ng/μl for PCR amplification.

2.2 | 16S rRNA amplification and PacBio sequencing

To obtain full-length 16S rRNA amplicons, we carried out the PCR using 50 ng of genomic DNA template and two bacterial-specific primers 27F (5′-AGAGTTTGATCMTGGCTCAG) and 1492R (5′-TACGGYTACCTTGTTACGACTT). To multiplex amplicons from nine samples into one SMRTbell library, we used 27F and 1492R primers tailed with 16-base PacBio barcodes at their 5' ends (https://github.com/PacificBiosciences/Bioinformatics-Training/blob/master/barcoding/pacbio_384_barcodes.fasta). Amplification reactions were carried out using barcoded forward (27F) and reverse (1492R) primers in a final volume of 20 μl consisting of 0.4 U of Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific), 0.2 mM dNTPs, 1× Phusion HF Buffer, 0.1 μM of each primer and 1.5 mM $MgCl_2$. The PCR conditions used were the same as those reported in Pootakham et al. (2017). Since we were unable to amplify 16S rRNA fragments from samples D13C-5 and D13H-5 due to their low DNA quality, the number of independent data points for each condition is as follows: D6C n = 5; D6H n = 5; D13C n = 4; and D13H n = 4. PCR products were subsequently purified with Agencourt AMPure XP magnetic beads (Beckman Coulter) and eluted in 20 μl elution buffer (EB). The Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific) was used to quantify the purified PCR products. Barcoded 16S amplicons were multiplexed in equimolar concentrations, and a total of 500 ng of pooled DNA was used for library preparation. SMRTbell libraries were constructed (each containing amplicons from nine samples) and sequenced on a PacBio RSII system using the P6-C4 chemistry with a 6-hr movie time (Pacific Biosciences).

2.3 | 16S rRNA sequence data analysis

To obtain the circular consensus sequences (CCS) with a minimum of five full passes, PacBio raw reads were demultiplexed and processed using the RS-ReadsOfInsert protocol (SMRT Analysis software version 2.3). Consensus 16S rRNA sequences that were shorter than 1,000 nt (likely generated as a result of partial amplification) were removed from further analyses. Based on the CCS read length distribution (Figure A1), we arbitrarily chose 1,000 nt as a cutoff to avoid using sequences that contained nonoverlapping hypervariable regions for downstream analyses since they might affect the taxonomic classification as well as the diversity index calculation. Both strands of the consensus sequences were screened for the presence of chimeric sequences using the abundance-based algorithm in UCHIME (Edgar, 2010) and a reference dataset from Ribosomal Database Project (RDP) (Cole et al., 2014). Filtered sequences were analyzed as previously described in Pootakham et al. (2017) using QIIME software version 1.9.1 (Caporaso et al., 2010). Sequences classified as unknown or chloroplasts were removed prior to subsequent analyses. Alpha and beta diversity analyses were performed using the QIIME pipeline based on sequence data that had been clustered into OTUs at a 97% similarity level. The OTU table was rarefied to an even depth of 5,652 sequences per sample corresponding to the lowest number of sequences present in a sample. Taxonomic classification was assigned to the representative sequence of each OTU using the RDP classifier retrained toward the Greengenes database V13.8 (DeSantis et al., 2006). Since we had full-length 16S rRNA sequence information, "unassigned" OTUs were subsequently classified based on their representative sequences using local sequence alignment (BLAST search) against the nt database. If the representative sequences of the OTUs returned the best hits with known taxonomy, those OTUs were classified accordingly. However, if the representative sequences of the OTUs returned "uncultured environmental sample" as their best hits, those OTUs remained categorized as "unassigned." We further investigated the nature of "unassigned" sequences by constructing the maximum-likelihood phylogenetic tree using MEGA7 software (Kumar, Stecher, & Tamura, 2016). The clustering of the sequences was tested by a bootstrap approach using 1,000 replications. Comparison between bacterial communities associated with *P. lutea* samples in this study (maintained in the aquarium) and those associated with *P. lutea* samples collected in situ from the same site (MT1-MT3 samples from Pootakham et al. (2017) and MT-Mar samples from Pootakham et al. (2018)) were analyzed as mentioned above using QIIME software version 1.9.1 (Caporaso et al., 2010).

White's nonparametric *t* test was used to test for differences in relative abundance of associated bacteria between samples collected in control and heated aquaria (White, Nagarajan, & Pop, 2009). To compare bacterial community compositions, we applied a nonparametric permutational multivariate analysis of variance (PERMANOVA) using the weighted UniFrac distance matrix as implemented in the vegan function Adonis (permutation = 1,000) (Lozupone & Knight, 2005; Oksanen, Blanchet, Kindt, Legendre, & Minchin, 2013). The UniFrac method measures the distance between communities based on the lineages they contain (Lozupone & Knight, 2005). The representative OTU sequences were aligned to generate a phylogenetic tree using the command *pick_open_reference_otus.py* (http://qiime.org/scripts/pick_open_reference_otus.html). We used the weighted version of the UniFrac metric, which accounts for the relative abundance of each of the taxa within the communities (Lozupone & Knight, 2005).

2.4 | Core microbiome and indicator species identification

For the identification of *P. lutea* core microbiome members and the identification of indicator species, we employed the information from the full-length 16S rRNA sequences and followed the analysis pipeline reported in Pootakham et al. (2017). Members of the *P. lutea* core microbiome were identified at 100% sample coverage (the minimal percentage of all samples in which an OTU must be present to be considered part of the core microbiome) based on previous studies (Lawler et al., 2016; van de Water et al., 2016; Hadaidi et al., 2017; Pootakham et al., 2017).

2.5 | Performance comparison between full-length 16S rRNA and short hypervariable fragments

To compare the performance of short hypervariable regions (V3-V4 and V5-V6) and full-length 16S rRNA sequences in resolving taxonomic classification, we obtained the V3-V4 and V5-V6 sequences in silico from their respective full-length reads using 5′-ACTCCTACGGGAGGCAGCAG and 5′-CTACCAGGGTATCTAATC for the V3-V4 region and 5′-GGATTAGATACCCTGGTAGTCC and 5′-CTCACGRCACGAGCTGACG for the V5-V6 region. The extracted in silico V3-V4 and V5-V6 fragments and their counterpart full-length sequences were aligned to the RDP type strain reference sequences using BLAST (e-value cutoff of 10^{-10}). Any sequences that yielded a minimum of two best hits from different species with identical e-values, aligned sequences, and bit-scores were categorized as "ineffective" for the species-level classification. For classification at the genus level, any query sequence that had two or more best hits belonging to different genera was considered "unassigned."

3 | RESULTS

3.1 | Evaluation of coral-associated bacterial diversity during thermal stress using full-length 16S rRNA gene sequences

The degree of bleaching was monitored using the Coral Health Chart from Coral Watch (https://coralwatch-old.org/web/guest/coral-health-chart). Coral samples that had experienced thermal stress were partially bleached (color scale: E3) after six days, and pigments were completely lost 13 days after the onset of heat treatment (color scale: E1; Figure 1b). Coral samples that were maintained in the control aquarium at 29°C remained pigmented throughout the

TABLE 1 Number of circular consensus sequence (CCS) reads, OTUs, and alpha diversity indices of *Porites lutea* microbiome under thermal stress. D6C and D13C indicate that samples were taken from the control aquarium on day 6 and day 13, respectively. D6H and D13H indicate that samples were taken from the heated aquarium on day 6 and day 13, respectively

Condition	Sample	Number of CCS reads	Number of processed reads[a]	Number of OTUs	Chao1	Simpson	Shannon	Good's coverage
D6C	D6C-1	18,213	13,317	768	989	0.9053	5.6155	0.9805
	D6C-2	21,716	15,085	211	335	0.6844	2.4380	0.9928
	D6C-3	11,873	8,582	172	430	0.7662	2.9071	0.9878
	D6C-4	35,302	26,091	391	552	0.8109	3.7021	0.9937
	D6C-5	29,280	21,091	996	1,249	0.9261	6.0606	0.9845
D6H	D6H-1	22,349	15,984	948	1,331	0.9397	6.4742	0.9769
	D6H-2	16,886	11,476	746	1,108	0.9293	5.9003	0.9695
	D6H-3	11,952	8,463	1,072	1,751	0.9783	7.4745	0.9330
	D6H-4	13,447	9,438	573	772	0.9274	5.9100	0.9782
	D6H-5	23,381	15,547	914	1,334	0.8657	5.5290	0.9742
D13C	D13C-1	21,138	15,480	234	417	0.7780	3.5362	0.9935
	D13C-2	16,216	12,188	408	676	0.7915	3.6422	0.9833
	D13C-3	26,510	16,924	1,046	1,322	0.8877	5.2677	0.9750
	D13C-4	18,187	11,459	816	1,328	0.8103	4.5242	0.9588
D13H	D13H-1	27,513	19,834	1,602	2,144	0.9661	7.7104	0.9688
	D13H-2	21,953	14,759	1,187	1,725	0.9712	7.2585	0.9648
	D13H-3	10,342	5,652	829	1,507	0.9108	6.8358	0.8563
	D13H-4	10,996	7,580	772	1,104	0.9713	7.0518	0.9557
	Total:	357,254	248,950					

[a]After removal of chimeras and reads shorter than 1,000 bases.

experiment (color scale: E4-E5) and did not show any sign of bleaching. Full-length 16S rRNA genes were amplified from coral-associated bacteria and sequenced using the PacBio platform. A total of 1,416,896 PacBio reads totaling 29.5 Gb were obtained from 16 SMRT cells. Polymerase reads were assembled and demultiplexed into 357,254 circular consensus sequencing (CCS) reads with a minimum of five full passes (Table 1). After removing 16S rRNA CCS reads shorter than 1,000 nucleotides and chimeric sequences, we obtained a total of 248,950 reads. The number of processed reads ranged from 5,652 to 26,091 per sample, with an average of 13,831 reads/sample (Table 1).

A series of alpha diversity indices were calculated for each sample in order to evaluate the diversity of coral-associated bacterial communities present within the samples. A wide range in numbers of operational taxonomic units (OTUs) at a 3% dissimilarity level was observed, with the lowest number of OTUs (172) associated with D6C-3 sample and the highest number of OTUs (1,602) associated with D13H-1 sample (Table 1). Good's coverage indices along with the rarefaction curves that have plateaued off for most samples indicated that sufficient sampling has been achieved to capture the total diversity of the bacterial communities under investigation (Table 1, Figure A2). The Shannon indices ranged from 2.43 (D6C-2) to 7.71 (D13H-1), similar to those reported for *P. lutea* from the South China Sea, Indo-Pacific, and West Indian Ocean (Li et al., 2013; McKew et al., 2012; Sere et al., 2016; Shannon, 1948). A significant difference between the Shannon indices associated with samples from control and heated aquaria was observed on day 13 (D13C vs. D13H samples; Mann–Whitney *U* test, *p*-value = .02857). The Simpson diversity indices ranged from 0.6844 (D6C2) to 0.9783 (D6H-3), and significant differences in the values of Simpson diversity indices were observed between samples in the control and heated treatment groups on both day 6 and day 13 (Mann–Whitney *U* test; D6C vs. D6H, *p*-value = .03175; D13C vs. D13H, *p*-value = .02857). The values of both Shannon and Simpson diversity indices were significantly higher when coral samples were subjected to thermal stress.

To evaluate the diversity of bacterial community present among samples, the beta diversity indices were calculated (Figure 2). Principal coordinates analysis (PCoA) of weighted UniFrac distances based on RDP results demonstrated that control and heat-treated coral samples harbored distinct bacterial communities, and this distinction became more evident after an extended period of thermal stress (PERMANOVA; D6C vs. D6H, pseudo-F = 8.372, p = .007; D13C vs. D13H, pseudo-F = 17.492, p = .032; D6H vs. D13H pseudo-F = 2.872, p = .037; Figure 2).

3.2 | Structure and composition of coral-associated microbiomes under thermal stress

Using a confidence threshold of 80%, 220,698 out of 284,950 filtered reads from all 18 samples were assigned based on the RDP

classifier (Wang, Garrity, Tiedje, & Cole, 2007). CCS reads were classified into 22 phyla (15 known and 7 candidate phyla), 60 classes, and 122 orders (Table S1). Proteobacteria represented a ubiquitous and dominant taxon in all *P. lutea* samples irrespective of the heat treatment (Figure 3). Microbial communities associated with *P. lutea* also consisted of members of Cyanobacteria and Bacteroidetes. We compared bacterial communities associated with *P. lutea* samples in the control aquarium with previously reported communities associated with *P. lutea* samples collected in situ from the same sampling site (Pootakham et al., 2017, 2018) and noticed that bacterial communities associated with coral samples that had been brought into aquaria harbored a significantly higher proportion of Cyanobacteria (Figure A3; White's nonparametric *t* test, *p*-value = .013).

We observed a significant alteration in the composition of coral-associated microbiomes 6 days (D6C vs. D6H; PERMANOVA, pseudo-*F* = 1.906, *p* = .018) and 13 days (D13C vs. D13H; PERMANOVA, pseudo-*F* = 2.389, *p* = .024) after the samples had been exposed to elevated water temperature (Figure 3). Interestingly, a substantial proportion (~12%) of the OTUs present in the microbiomes associated with heat-stressed corals could not be assigned based on the RDP classifier. The full-length nature of the 16S rRNA sequence data allowed us to further assign taxonomy by aligning the representative sequence of each OTU to the Genbank nt database using BLAST. Approximately 50% of the unassigned sequences matched unclassified bacterial sequences obtained from uncultured environmental samples (labeled as "unassigned" in Figure 3), while the other 40% and 10% of the previously unassigned sequences (based on RDP classifier) matched sequences belonging to Bacteroidetes and Proteobacteria phyla, respectively. In addition, we constructed the phylogenetic tree to investigate the relationship of the top four most abundant unassigned OTUs (OTU8, OTU67, OTU9974, and OTU12304) with known sequences. While OTU8 and OTU9974 appeared to be related to a member of Flammeovirgaceae and Planctomycetales (Pla4 lineage), respectively, OTU67 and OTU12304 did not seem to be related to any bacterial sequences with known classification (Figure A4).

Compared to samples maintained at ambient temperature, Gammaproteobacteria family Endozoicomonadaceae became significantly less abundant when corals experienced a thermal stress (White's nonparametric *t* test, $p < .01$; Figure 4). On the contrary, Alphaproteobacteria appeared to be dominant in the bacterial communities associated with corals in the heated aquarium. More specifically, Alphaproteobacteria family Rhodobacteraceae increased in their relative abundances significantly after *P. lutea* experienced a thermal stress (White's nonparametric *t* test, $p < .01$; Figure 4). Additionally, the proportions of Alteromonadaceae and Saprospiraceae present in the heat-treated samples increased significantly compared to those present in the control samples (White's nonparametric *t* test, $p < .05$; Figure 4).

3.3 | Identification of the *P. lutea* core microbiome

Previous studies have reported the association of similar bacterial populations with the same coral species inhabiting different geographic locations (Bourne & Munn, 2005; Frias-Lopez, Zerkle, Bonheyo, & Fouke, 2002; Hong, Yu, Chen, Chiang, & Tang, 2009; Ritchie & Smith, 2004; Rohwer et al., 2001, 2002; Rohwer & Kelley, 2004), suggesting that corals hosted species-specific microbiome regardless of the environmental factors. Even though the composition of bacterial communities associated with untreated and heat-stressed corals appeared to be different, we identified a number of conserved bacterial species consistently present in 100% of the samples (regardless of their relative abundances in the communities). According to our stringent criterion, a total of ten species representing four orders and three phyla were identified as putative members of the core microbiome (Figure 5). The majority of core microbiome candidates were Proteobacteria, with Gammaproteobacteria (five species) and Alphaproteobacteria (three species) constituting 47.4% and 8.4% of the core microbiome

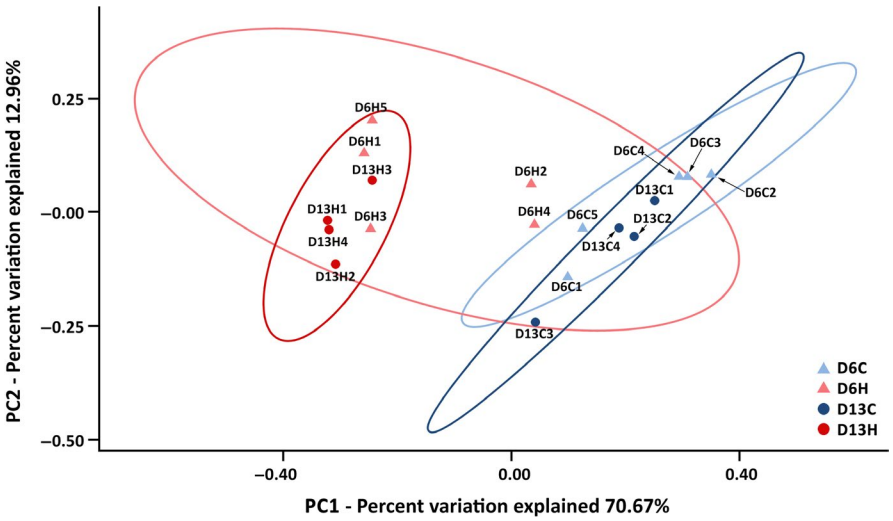

FIGURE 2 Beta diversity estimates of *Porites lutea*-associated microbiomes during a thermal stress. A principal coordinates analysis (PCoA) plot of coral-associated bacterial communities during a thermal stress based on weighted UniFrac distances. Blue symbols indicate samples from the control aquarium (~29°C), and red symbols indicate samples from the heated aquarium (~34°C)

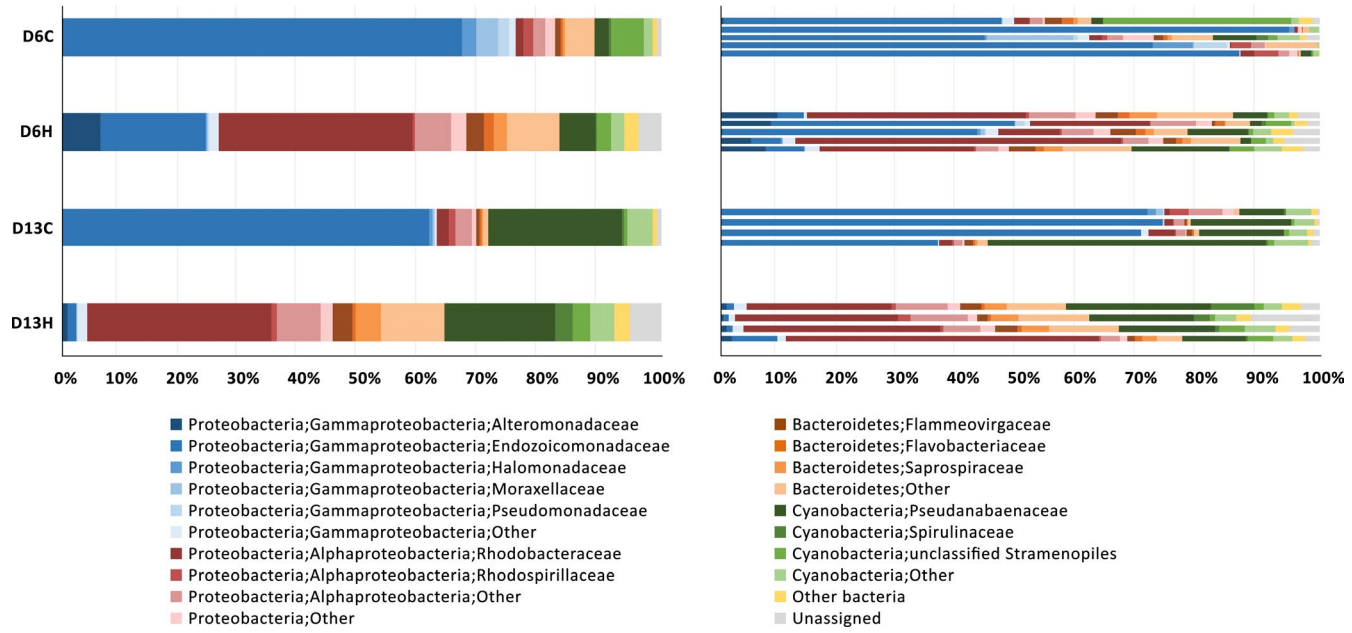

FIGURE 3 Dynamics of coral-associated bacteria during a thermal stress. Bar charts displaying the composition of bacterial communities associated with *Porites lutea* in control and heated aquaria at a group level (left) and an individual colony level (right). Taxonomic classification of OTUs present in each sample at the phylum/class/family levels was based on the Greengenes database. Eleven most abundant families were depicted, and the remaining taxa were grouped together under "others."

population, respectively. *Endozoicomonas elysicola* (25.32%), *Endozoicomonas euniceicola* (11.23%), and *Prochlorothrix hollandica* (10.03%) were the top three most abundant species, while *Pseudoruegeria aquimaris* (0.78%), *Kistimonas asteriae* (1.71%), and *Tropicibacter phthalicicus* (2.51%) were among the least abundant species in the core microbiome (Figure 5). We also compared the data from this study to those from previous reports (Pootakham et al., 2017, 2018) and found one bacterial species, *Endozoicomonas elysicola*, to be exceptionally persistent in all 30 *P. lutea* colonies investigated across different geographical locations and seawater temperatures. Most of the species identified as putative members of *P. lutea* core microbiome were not present in high abundance. In fact, only three members had a relative abundance higher than 10% within the whole community while five had a relative abundance between 1% and 6%, and the remaining was found to have lower than 1% relative abundance within the community.

3.4 | Indicator species associated with heat-stressed corals

We examined the microbial communities from both untreated and heat-stressed samples to see whether any specific bacterial species were significantly associated with corals that had been exposed to elevated water temperature. We applied the indicator value (INDVAL) method to analyze our data for the presence of indicator species for control and heated conditions (De Caceres & Legendre, 2009). A complete list of bacteria that were identified as indicator species for the control or heat-stressed corals on either day 6 or day 13 is provided in Table S2. Bacterial species that were identified as indicator species (for either control or heat-stressed condition) on both day 6 and day 13 are considered strong indicator species and listed in Table 2.

All six indicator species for corals maintained at ambient temperature (D6C and D13C) belonged to Gammaproteobacteria family Endozoicomonadaceae, consistent with the information that members of this family were significantly more abundant in the microbiomes associated with corals from control aquarium (Figure 4 and Table 2). Interestingly, with the exception of *Rhodomonas salina* and *Eionea nigra*, all indicator species for coral microbiomes that had experienced a thermal stress (D6H and D13H) were members of Alphaproteobacteria family Rhodobacteraceae, with several species detected exclusively in either D6H or D13H samples (species with an index value of 1; Table 2).

3.5 | Taxonomic classification at species resolution using full-length 16S rRNA sequence information

Classifying the taxonomy of 16S rRNA sequences based on short hypervariable fragments of the 16S rRNA gene can be difficult. In this study, we adopted the PacBio CCS technology to obtain full-length 16S rRNA sequences covering all hypervariable regions of the 16S rRNA genes. In order to demonstrate the benefit of utilizing full-length sequences in 16S rRNA gene-based community surveys, we extracted the commonly used V3-V4 and V5-V6 regions from the full-length reads and aligned both of the in silico simulated 420-nucleotide V3-V4 and 255-nucleotide V5-V6 amplicons and their respective full-length counterparts against reference sequences in the RDP (Cole et al., 2014). A much higher proportion of full-length 16 rRNA sequences could be classified at the species level compared to those of V3-V4 and V5-V6 fragments (Figure 6).

We were able to assign taxonomic classification at the species level for 99.8%, 86.2%, and 82.0% for the full-length 16S rRNA sequences, V3-V4 hypervariable fragments, and V5-V6 hypervariable fragments, respectively. Furthermore, we assigned taxonomy (at the genus level) to each in silico (V3-V4 and V5-V6) amplicon extracted from the full-length sequence and compared taxonomic profiles obtained from short amplicons to those obtained from the full-length 16S sequences (Table S3). Taxonomic classification based on hypervariable V3-V4 or V5-V6 regions yielded different microbiome profiles from the classification based on full-length 16S rRNA sequences. For example, *Roseovarius* was one of the two most prevalent genera associated with heat-stressed corals (D6H and D13H) when using full-length 16S rRNA sequences for classification (Table S3). However, when V5-V6 fragments were used instead of their full-length counterparts, *Pseudoruegeria* was erroneously represented as the most abundant genus affiliated with heat-stressed microbiomes (when the taxonomic classification was performed with full-length 16S data, the relative abundance of *Pseudoruegeria* in the heat-stressed microbiomes was <2%; Table S3). The proportion of unassigned sequences was also considerably higher when partial 16S fragments were used instead of their full-length counterparts, suggesting that the commonly used hypervariable V3-V4 and V5-V6 regions were identical among several bacterial 16S rRNA sequences in the reference database (Table S3).

4 | DISCUSSION

4.1 | Thermal stress affects the structure and diversity of coral-associated microbiomes

A diverse group of bacteria are known to associate with corals, and dynamics of bacterial community have recently been shown to be linked to patterns of heat tolerance in their hosts (Ziegler et al., 2017). Here, we investigated the effects of elevated water temperature on the structure and diversity of microbiomes associated with *P. lutea* using full-length 16 rRNA gene sequences. We employed the aquarium system since it allowed us to control other environmental factors (i.e., the only difference between controlled and heated aquaria was the water temperature). The microbiomes of corals have been known to shift their compositions when brought into aquaria. Kooperman, Ben-Dov, Kramarsky-Winter, Barak, and Kushmaro (2007) previously reported a decrease in relative abundance of Cyanobacteria in *Fungia granulosa* that had been maintained in the aquarium (Kooperman et al., 2007). Our observation with *P. lutea* revealed the opposite trend (Figure A3), with Cyanobacteria being more abundant in aquarium samples. The relative abundance of Cyanobacteria appeared to increase with the amount of time that *P. lutea* samples had been maintained in the aquarium (White's nonparametric *t* test; D6 vs. D13, *p*-value = .023; Figure 3). However, the shift in the relative abundance of Cyanobacteria was independent of an increase in water temperature since it was observed in both control and heat-treated samples (White's nonparametric *t* test; D6C vs. D6H and D13C vs. D13H, *p*-value > .05).

At an average sequencing depth of 13,831 reads per sample, the rarefaction curves have plateaued off for most samples, suggesting that sufficient sampling has been achieved to capture the total diversity of bacterial communities under investigation. Simpson and Shannon diversity estimates revealed that thermal stress treatments had significantly higher bacterial alpha diversity compared to the controls (Mann–Whitney *U* test; D6C vs. D6H, *p*-value = .03175; D13C vs. D13H, *p*-value = .02857), similar to previous observations in *Acropora millepora* (Bourne et al., 2008), *Acropora muricata* (Lee, Davy, Tang, & Kench, 2016; Shiu et al., 2017), and *Pocillopora damicornis* (Tout et al., 2015). A number of studies have provided emerging evidence that environmental stressors including elevated water temperature led to an increase in alpha diversity within coral-associated microbiomes (Bourne et al., 2008; Lee et al., 2016; McDevitt-Irwin, Baum, Garren, & Vega Thurber, 2017; Meron et al., 2011; Shiu et al., 2017; Tout et al., 2015). Meron et al. (2011) demonstrated that corals exposed to environment with lower pH exhibited higher microbial diversity (Meron et al., 2011). Corals experiencing a high level of anthropogenic disturbance have also been shown to harbor microbiomes with higher diversity than those farther away from the disturbance (Morrow, Moss, Chadwick, & Liles, 2012). Furthermore, a major coral microbiome restructuring and a marked increase in microbial diversity were evident when *F. granulosa* corals were exposed to long-term high salinity (Röthig, Ochsenkühn, Roik, Merwe, & Voolstra, 2016).

Besides an increase in the alpha diversity observed, the community composition also changed dramatically especially after 13 days of heat treatment (Figure 2). There was a notable increase in the

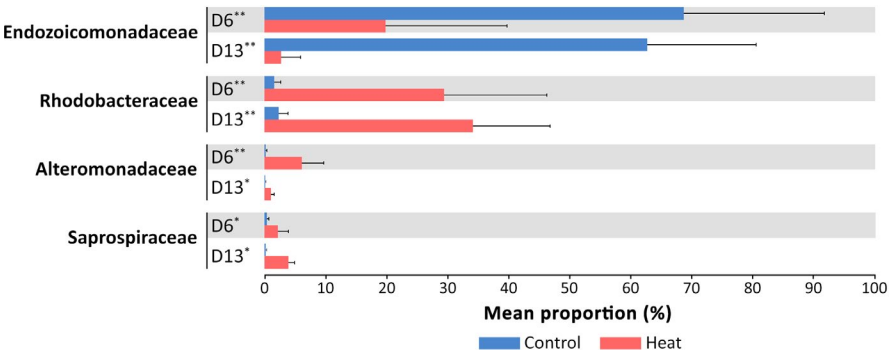

FIGURE 4 Changes in relative abundance of coral-associated bacteria under thermal stress. A bar chart displaying average proportions of four bacterial families, the relative abundance of which was significantly different between control and heat treatment (White's nonparametric *t* test). A single asterisk (*) indicates a significance level of $p < .05$, and a double asterisk (**) indicates a significance level of $p < .01$

Phylum	Class	Order/Family	Genus/species	Average ubiquity (%)			Average abundance (%)	D6C	D6H	D13C	D13H
				This study ($n = 5$)	2017[a] ($n = 18$)	2018[b] ($n = 7$)					
Bacillariophyta	Bacillariophyceae	Naviculales/Phaeodactylaceae	*Phaeodactylum tricornutum*	100	55.56	42.11	3.16				
Cyanobacteria	no class	Synechococcales/Pseudanabaenaceae	*Prochlorothrix hollandica*	100	-	26.32	10.03				
Proteobacteria	Alphaproteobacteria	Rhodobacterales/Rhodobacteraceae	*Pseudoruegeria aquimaris*	100	27.78	52.63	0.78				
			Roseovarius halotolerans	100	-	15.79	5.09				
			Tropicibacter phthalicicus	100	72.22	47.37	2.51				
	Gammaproteobacteria	Oceanospirillales/Endozoicomonadaceae	*Endozoicomonas elysicola**	100	100	100	25.32				
			*Endozoicomonas euniceicola**	100	100	95	11.23				
			*Endozoicomonas montiporae**	100	94	100	5.43				
			*Endozoicomonas numazuensis**	100	100	95	3.67				
			*Kistimonas asteriae**	100	94	94	1.71				

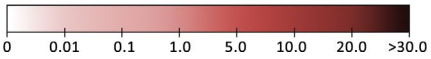

FIGURE 5 *Porites lutea* core microbiome. A list of bacterial species present in *P. lutea* core microbiome, their average ubiquity (the percentage of all *P. lutea* samples in which the species was detected) in this and previous studies [2017[a] refers to a study by Pootakham et al. (2017), and 2018[b] refers to a study by Pootakham et al. (2018)], and their average relative abundance across all conditions. The heat map shows the average abundance of the species under each condition. The asterisk (*) indicates species that have previously been identified as members of *P. lutea* core microbiome. The dash (-) indicates that the sequences affiliated with that particular species were not detected

relative abundance of Alphaproteobacteria and a simultaneous decline in the relative abundance of Gammaproteobacteria during thermal stress (Figure 3). A clear shift in Gammaproteobacteria community was observed in conjunction with the reduction in Endozoicomonadaceae abundance, which became more pronounced after 13 days of heat treatment (Figure 4). Similar to our results, a marked decline in the proportion of Endozoicomonadaceae in the coral-associated microbiome during thermal stress has been reported in *P. damicornis* (Tout et al., 2015) as well as in *P. lutea* (Pootakham et al., 2018). Endozoicomonadaceae has been reported to inhabit both coral mucus and coral tissue of several scleractinian species including *Acropora cervicornis*, *P. astreoides*, and *P. furcate* (Ainsworth et al., 2015; Bayer et al., 2013; Chu & Vollmer, 2016). Fluorescent in situ hybridization (FISH) experiments conducted by Bayer et al. (2013) showed that members of the Endozoicomonadaceae family (genus *Endozoicomonas*) resided in close proximity to Symbiodiniaceae cells within the coral endoderm, suggesting an intimate association with their coral hosts. Researchers have proposed *Endozoicomonas* to be beneficial symbionts (McDevitt-Irwin et al., 2017), playing an important role in sulfur recycling (Raina et al., 2009) and protein/carbohydrate transport for the host (Neave, Michell, Apprill, & Voolstra, 2017). The decline in relative abundance of these potentially symbiotic taxa was also induced by other environmental stressors besides elevated water temperature. Previous studies have found that the relative occurrence of sequences associated with Endozoicomonadaceae decreased in *Acropora* and *Porites* corals in response to ocean acidification (Morrow et al., 2015) and in *Porites* and *Paramuricea* corals in response to diseases (Meyer, Paul, & Teplitski, 2014; Vezzulli, Pezzati, Huete-Stauffer, Pruzzo, & Cerrano, 2013).

The structural abundance of dominant microbiome members under normal condition did not seem to be maintained under thermal stress. While there was a significant reduction in the relative abundance of Endozoicomonadaceae following the exposure to thermal stress, we observed a concomitant increase in the relative abundance of Rhodobacteraceae, Alteromonadaceae, and Saprospiraceae (Figures 3 and 4). Ziegler et al. (2017) also noticed a higher proportion of Rhodobacteraceae in the bacterial communities associated with the highly variable pool (with a temperature range of 25–35°C) compared to the moderately variable pool (with temperature range of 26–32°C). A dramatic community shift during thermal stress, which primarily involved an increase in the relative abundance of Rhodobacterales, was also observed in *P. damicornis* (Tout et al., 2015). Similarly, a study by van de Water et al. (2018) showed that members of Rhodobacteraceae (genera *Rhodovulum* and *Dinoroseobacter*) became more abundant in *Montipora aequituberculata*-associated microbiome after an extended period of elevated water temperature treatment (van de Water et al., 2018). Patterns of increased Rhodobacterales-affiliated sequences in the tissue layer have also been reported when *A. muricata* corals were exposed to high temperature for three days (Lee, Davy, Tang, & Kench, 2017). The order Rhodobacterales appears to be opportunistic bacteria, flourishing and increasing their relative abundance when coral hosts experience biotic (e.g., white band and white plaque diseases) and abiotic (e.g., thermal stress and ocean acidification) stress during which there may be open niche space (Cardenas, Rodriguez, Pizarro, Cadavid, & Arevalo-Ferro, 2012; Meron et al., 2011; Roder et al., 2014; Welsh et al., 2017). Interestingly, even though Rhodobacteraceae became more abundant in coral-associated communities under thermal stress (Tout et al., 2015; Lee et al., 2017; van de Water et al., 2018) and low pH (Meron et al., 2011), Grottoli et al. (2018) observed a decrease in sequences affiliated with this bacterial family when coral hosts were simultaneously exposed to both elevated seawater temperature and ocean acidification (Grottoli et al., 2018).

Several studies have reported an increase in relative abundance of Rhodobacteraceae in the microbiomes of diseased corals (Kellogg et al., 2013; Pollock, Wada, Torda, Willis, & Bourne, 2016; Sunagawa et al., 2009). Using PhyloChip™ G3, Kellogg et al. (2013) showed that a number of Rhodobacteraceae OTUs were consistently and significantly enriched in white plaque samples of *Orbicella faveolata*, similar to the work reported by Sunagawa et al. (2009) using an earlier generation PhyloChip™ (Kellogg et al., 2013; Sunagawa et al., 2009). Rhodobacteraceae OTUs were also represented in greater

TABLE 2 A list of indicator species significantly ($p < .05$) associated with corals under normal (D6C, D13C) or elevated temperature (D6H, D13H) as revealed by the indicator value (INDVAL) analysis

				INDVAL (*p*-value)			
Phylum	**Class**	**Family**	**Indicator species**	**D6C**	**D13C**	**D6H**	**D13H**
Cryptophyta	Cryptophyceae	Pyrenomonadaceae	*Rhodomonas salina*	–	–	0.800 (.048)	0.991 (.026)
Proteobacteria	Alphaproteobacteria	Rhodobacteraceae	*Actibacterium mucosum*	–	–	0.973 (.008)	0.987 (.031)
			Aestuariivita boseongensis	–	–	0.984 (.013)	0.811 (.033)
			Aliiroseovarius crassostreae	–	–	0.980 (.008)	0.964 (.022)
			Celeribacter neptunius	–	–	0.958 (.010)	0.927 (.029)
			Confluentimicrobium lipolyticum	–	–	0.990 (.009)	0.955 (.035)
			Donghicola eburneus	–	–	0.994 (.011)	0.997 (.036)
			Leisingera aquimarina	–	–	1.000 (.008)	0.989 (.032)
			Lutimaribacter pacificus	–	–	0.800 (.038)	1.000 (.029)
			Marivita litorea	–	–	0.947 (.011)	1.000 (.030)
			Planktomarina temperata	–	–	0.911 (.019)	0.957 (.031)
			Ponticoccus lacteus	–	–	0.986 (.031)	0.976 (.034)
			Poseidonocella sedimentorum	–	–	0.769 (.046)	0.893 (.048)
			Pseudoruegeria aquimaris	–	–	0.868 (.017)	0.917 (.035)
			Pseudoruegeria lutimaris	–	–	1.000 (.005)	0.937 (.026)
			Roseovarius halotolerans	–	–	0.982 (.005)	0.973 (.028)
			Roseovarius pacificus	–	–	0.986 (.009)	0.977 (.040)
			Ruegeria atlantica	–	–	0.913 (.026)	0.978 (.028)
			Ruegeria pomeroyi	–	–	0.978 (.013)	0.989 (.031)
			Sulfitobacter geojensis	–	–	0.800 (.044)	1.000 (.024)
			Thalassobius mediterraneus	–	–	0.800 (.046)	0.984 (.028)
			Thalassococcus lentus	–	–	0.968 (.014)	0.865 (.029)
			Tropicibacter phthalicicus	–	–	0.988 (.011)	0.958 (.038)
	Gammaproteobacteria	Cellvibrionaceae	*Eionea nigra*	–	–	0.983 (.042)	0.941 (.035)
		Endozoicomonadaceae	*Endozoicomonas elysicola*	0.828 (.014)	0.981 (.030)	–	–
			Endozoicomonas euniceicola	0.880 (.011)	0.970 (.023)	–	–
			Endozoicomonas gorgoniicola	0.866 (.007)	0.982 (.020)	–	–
			Endozoicomonas montiporae	0.846 (.008)	0.972 (.031)	–	–
			Endozoicomonas numazuensis	0.786 (.047)	0.986 (.031)	–	–
			Kistimonas asteriae	0.858 (.026)	0.980 (.048)	–	–

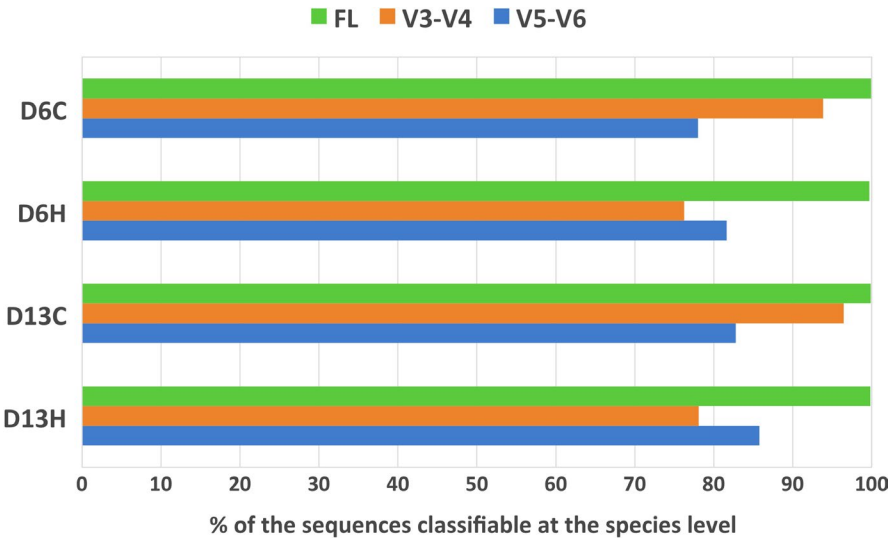

FIGURE 6 Performance comparison between full-length 16S rRNA and hypervariable fragments (V3-V4 or V5-V6). A bar graph illustrating proportions of sequence reads from each treatment that are classifiable at the species level using full-length (FL; green) or partial 16S rRNA gene fragments (V3-V4, orange; V5-V6, blue).

proportions in diseased samples of *Diploria strigosa* and *Siderastrea siderea* compared to the samples from healthy colonies (Cardenas et al., 2012). Rhodobacterales are fast-growing taxa, exhibiting a quick response to increasing availability of amino acids and other resources (Mayali, Weber, Mabery, & Pett-Ridge, 2014) that could be made available from diseased cells damaged by pathogens such as *Vibrio* spp (Welsh et al., 2017). The appearance of *Vibrio*-related sequences has been observed in parallel with the increase in the relative abundance of Rhodobacteraceae during thermal bleaching in *A. millepora* (Bourne et al., 2008), *A. muricata* (Lee et al., 2016), *A. gemmifera* (Gardner et al., 2019), and *P. damicornis* (Tout et al., 2015). Members of the genus *Vibrio* are known for their roles as opportunistic or pathogenic bacteria associated with white band, yellow blotch/band disease, and white-syndrome-like diseases (Barneah, Ben-Dov, Kramarsky-Winter, & Kushmaro, 2007; Cervino et al., 2004; Gil-Agudelo, Fonseca, Weil, Garzon-Ferreira, & Smith, 2007; Gil-Agudelo, Smith, & Weil, 2006; Luna, Bongiorni, Gili, Biavasco, & Danovaro, 2010; Richie & Smith, 1998; Ritchie, 1994; Rosenberg & Falkovitz, 2004; Sussman, Willis, Victor, & Bourne, 2008). Interestingly, despite the similarity in the bacterial profile shifts associated with diseases and thermal stress, we did not observe an increase in relative abundance of *Vibrio*-related sequences in our heat-treated samples. Indeed, we hardly detected any *Vibrio* OTUs in our samples across treatments and time periods. The absence of *Vibrio* species in both healthy and thermally bleached *P. lutea* colonies was consistent with previous study (Pootakham et al., 2017, 2018).

4.2 | *Porites lutea* core microbiome

The concept of core microbiome has recently been introduced in coral-microbial studies (Ainsworth et al., 2015; Chu & Vollmer, 2016; Hernandez-Agreda, Leggat, Bongaerts, & Ainsworth, 2016; Lawler et al., 2016; Robertson, Haltli, Mccauley, Overy, & Kerr, 2016; van de Water et al., 2016; Hernandez-Agreda, Gates, & Ainsworth, 2017; Sweet & Bulling, 2017), and it is characterized as the stable populations consistently present across complex microbial assemblages (Shade & Handelsman, 2012). Members of a core microbiome are likely to be important for the development, health, and functioning of their hosts (Ley, Turnbaugh, Klein, & Gordon, 2006; Round & Mazmanian, 2009). We stringently defined the members of the *P. lutea* core microbiome as bacterial species that were present in 100% of all samples based on the percentages of sample coverage previously used in coral microbiome studies (Lawler et al., 2016; van de Water et al., 2016). With full-length 16S rRNA sequence data, we were able to achieve the taxonomic classification of the core microbiome members at the species resolution. Most of *P. lutea* core microbiome members belonged to Alphaproteobacteria family Rhodobacteraceae and Gammaproteobacteria family Endozoicomonadaceae (Figure 5). Rhodobacterales are thought to be among the most abundant and diverse bacteria in marine environments, accounting for a quarter of the total bacteria present in coastal and polar regions (Wagner-Dobler & Biebl, 2006). They are functionally diverse and involved in a range of biological activities from the production of antibiotic compounds to the reduction of trace metals (Brinkhoff et al., 2004). They also contribute to ocean carbon and sulfur cycling through the oxidation of carbon monoxide and the production of dimethylsulfide (Wagner-Dobler & Biebl, 2006).

Another prominent taxon that constituted the majority of *P. lutea* core microbiome was the family Endozoicomonadaceae, which has been reported as dominant members of the core microbiomes in *P. furcate*, *A. cervicornis*, *P. verrucosa*, *P. astreoides*, and *Stylophora pistillata* (Chu & Vollmer, 2016; Glasl, Webster, & Bourne, 2017). Notably, all five Endozoicomonadaceae species designated as core microbiome members in this study have previously been identified in the *P. lutea* core microbiome (Pootakham et al., 2017, 2018). Moreover, *E. elysicola* was identified in all 30 *P. lutea* samples collected from different sampling sites, during different seasons and under varying temperature conditions (Figure 5). Members of the family Endozoicomonadaceae, especially genus *Endozoicomonas*, are ubiquitous and highly abundant in the marine

environment. While they do not appear to be common in deep-sea corals, members of this family are frequently found associated with tropical and temperate coral species (Cardenas et al., 2012; La Rivière, Garrabou, & Bally, 2015; Lee et al., 2012; Morrow et al., 2012; Sunagawa et al., 2009). Based on their widespread abundance in marine invertebrates and their dominance in the microbiomes of several coral species (Chu & Vollmer, 2016; Glasl et al., 2017), *Endozoicomonas* species are likely to play important biological and ecological roles in coral holobionts. *Endozoicomonas* has been detected on the surface mucus layer and in coral skeleton of *Acropora* and *Porites* hosts, and it has been suggested that members of this taxon may be involved in biofilm production that promotes surface colonization of other bacteria (Jessen et al., 2013; Speck & Donachie, 2012). The *Endozoicomonas* genomes encoded a complete set of putative proteins in the synthetic pathways for various amino acids including alanine, cysteine, glycine, lysine, methionine, serine, and threonine, raising the possibility that the microbial symbionts, especially members of the core microbiome, may provide essential amino acids that cannot be synthesized by the host (Neave et al., 2017; Price et al., 2014).

4.3 | Members of the Rhodobacteraceae family are indicator species for thermal stress in *Porites lutea*

To determine whether specific microbial taxa were significantly associated with corals under thermal stress, we applied an indicator species analysis (INDVAL) to identify representative or indicative bacterial species for elevated water temperature (De Caceres & Legendre, 2009). Interestingly, the microbial communities of heat-stressed corals were characterized primarily (59 out of 81 species) by a group of bacterial indicator species belonging to 19 different Rhodobacteraceae genera (Table 2; Table S2). On the contrary, nearly all indicator species for normal water temperature were Endozoicomonadaceae (genera *Endozoicomonas* and *Kistimonas*). Members of Rhodobacteraceae have previously been reported as indicator taxa for polluted environment and sites impacted by sedimentation and local sewage or municipal wastewater (Ziegler et al., 2016). Several OTUs with sequences related to Rhodobacteraceae have also been identified as indicative microbial taxa for diseased coral tissues (Roder et al., 2014; Sere et al., 2013; Sunagawa et al., 2009). The results presented in this work suggested that members of Rhodobacteraceae family were robust indicator species for thermally stressed *P. lutea* colonies.

Scleractinian corals are among the largest producers of DMSP (Broadbent & Jones, 2004; Broadbent, Jones, & Jones, 2002), a compound that plays a role in alleviating cellular oxidative stress as DMSP and its breakdown products (dimethylsulfide [DMS] and dimethylsulfoxide [DMSO]) can readily scavenge hydroxyl radicals and other reactive oxygen species (Sunda, Kieber, Kiene, & Huntsman, 2002; Yoch, 2002). Interestingly, Garren et al. (2014) found that coral hosts under thermal stress exude mucus that was richer in DMSP, which appeared to trigger heightened pathogen response, suggesting that pathogenic (e.g., *Vibrio coralliilyticus*) or opportunistic (e.g., Rhodobacteraceae) bacteria may use this chemical cue to target stressed hosts (Garren et al., 2014). *Ruegeria pomeroyi*, one of the indicator species for heat-stressed *P. lutea*, was capable of metabolizing DMSP utilizing both the demethylation pathway (releasing methanethiol) and the cleavage pathway (producing DMS) (Reisch et al., 2013). The ability to utilize DMSP exuded by stressed corals may allow *R. pomeroyi* to thrive and expand its niches within the coral holobiont especially during thermal stress. Another indicator species for thermal stressed microbiome was *Aliiroseovarius crassostreae*, a marine Rhodobacter that has been shown to be a causative agent of *Roseovarius* oyster disease, which resulted in high mortality rates in juvenile eastern oysters in the United States (Boettcher, Geaghan, Maloy, & Barber, 2005). To this point, there has been no report linking *R. pomeroyi* or *A. crassastreae* to disease conditions in *P. lutea*. Future studies are required to determine whether these two indicator species are capable of inducing signs of coral diseases.

4.4 | Full-length 16S rRNA sequences enable taxonomical classification at the species resolution

Classifying the taxonomy of 16S rRNA sequences based on short hypervariable fragments of the 16S rRNA gene can be challenging. Our results showed that the number of "unassigned" sequences was remarkably higher when partial 16S fragments were used instead of the full-length sequences (Table S3). Taxonomical classification at the genus level yielded noticeably different microbiome profiles when short fragments encompassing hypervariable regions, especially the V5-V6 regions, were used instead of the full-length 16S rRNA sequences (Table S3). Misclassification of partial 16S rRNA sequences (V3-V4 or V5-V6 regions) can affect the accuracy of taxonomic profiles as well as bacterial community's estimates of species richness (Youssef et al., 2009). Youssef et al. (2009) compared the OTU estimates for each simulated hypervariable region to those for full-length or nearly full-length 16S rRNA sequences at different taxonomic cutoffs and demonstrated that the number of OTUs and species richness could be either underestimated or overestimated, depending on the hypervariable sequences used (Youssef et al., 2009). The bias in species richness estimates stemming from the use of partial 16S sequences can be explained by the percentages of conserved, variable, and hypervariable nucleotide positions in the sequences used to perform the analyses (Youssef et al., 2009).

5 | CONCLUSIONS

Emerging evidence suggests that environmental stressors, especially the climate change-associated pulse warming events, can induce changes in the coral microbiomes (Ainsworth & Gates, 2016; Bourne et al., 2008; Gardner et al., 2019; Grottoli et al., 2018; Hoegh-Guldberg et al., 2007; Lee et al., 2016, 2017; Littman et al., 2009; Wang et al., 2018; Zhou et al., 2017; Ziegler et al., 2017). Several studies have highlighted the significance of coral-associated microorganisms and their roles in fitness and survival of the

host animals (Ainsworth et al., 2015; Bourne et al., 2008; Gilbert et al., 2012; Lesser, Mazel, Gorbunov, & Falkowski, 2004; Shieh & Lin, 1992; Tout et al., 2015). Our work examined the effect of thermal stress on the composition and structure of bacterial communities associated with *P. lutea*, one of the dominant reef-builders widely distributed across the Indo-West Pacific, including the Gulf of Thailand and Andaman Sea (Yeemin et al., 2009). We observed a significant increase alpha diversity indices and a prominent shift in microbiome composition during thermal stress. This is one of the first coral-associated microbiome studies that utilized the long-read PacBio SMRT sequencing technology to capture full-length 16S rRNA gene, which allowed over 99.8% of the sequences to be classified at the species level. The ability to achieve species-resolution classification enabled us to identify members of *P. lutea* core microbiome and a group of indicator species associated with thermal stress. The knowledge on how thermal stress impacts the diversity and composition of coral microbiomes may help us gain a better understanding of how these associated bacteria contribute to the acclimatization and resilience of the coral holobiont to thermal bleaching.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

WP and ST conceptualized the study and acquired funding. WP, TY, LP, NJ, and ST carried out investigation. WP, WM, CS, CN, and WK carried out formal analysis. WM and WK carried out visualization experiments. WP wrote the original draft of the manuscript. WP, WM, and WK reviewed and edited the manuscript.

ETHICAL APPROVAL

None required.

DATA AVAILABILITY STATEMENT

The PacBio 16S rRNA sequence data were deposited in the GenBank NCBI Sequence Read Archive (SRA) database under the accession number PRJNA429083.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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APPENDIX

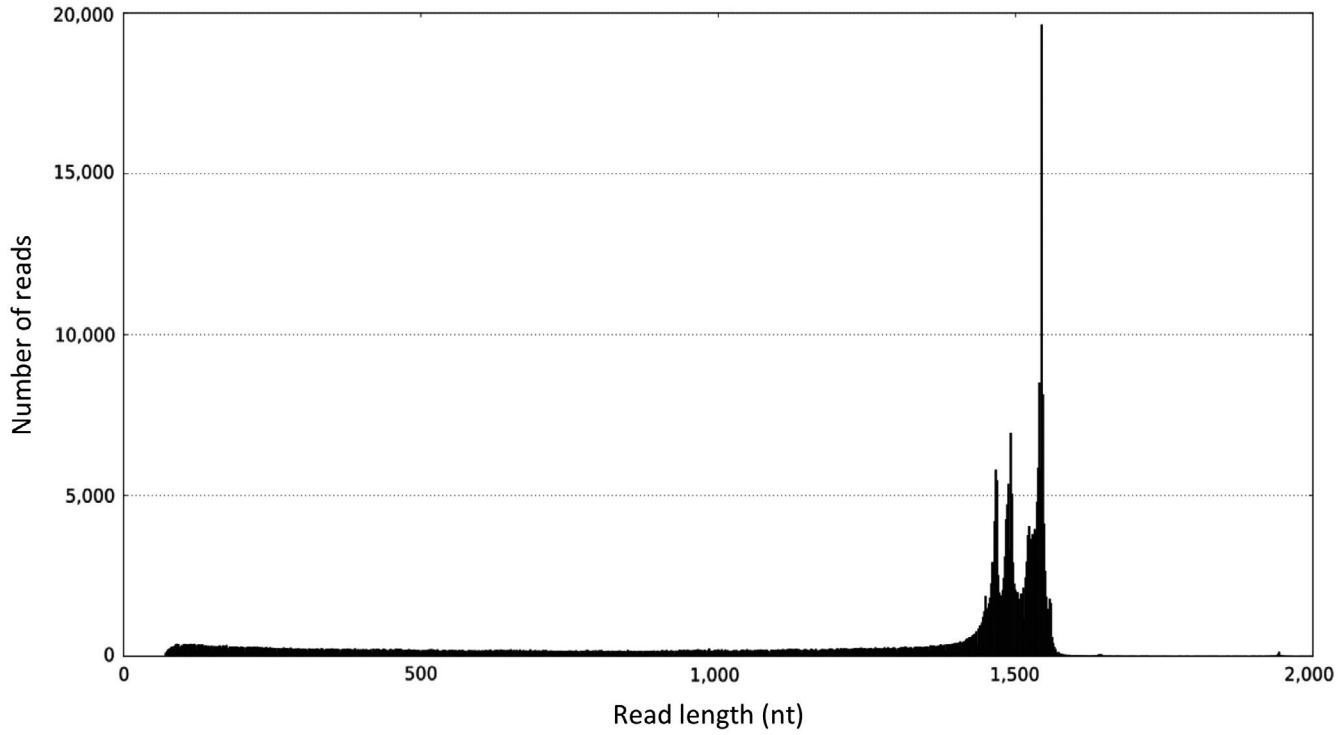

FIGURE A1 A histogram showing CCS read length distribution of 16S rRNA amplicons

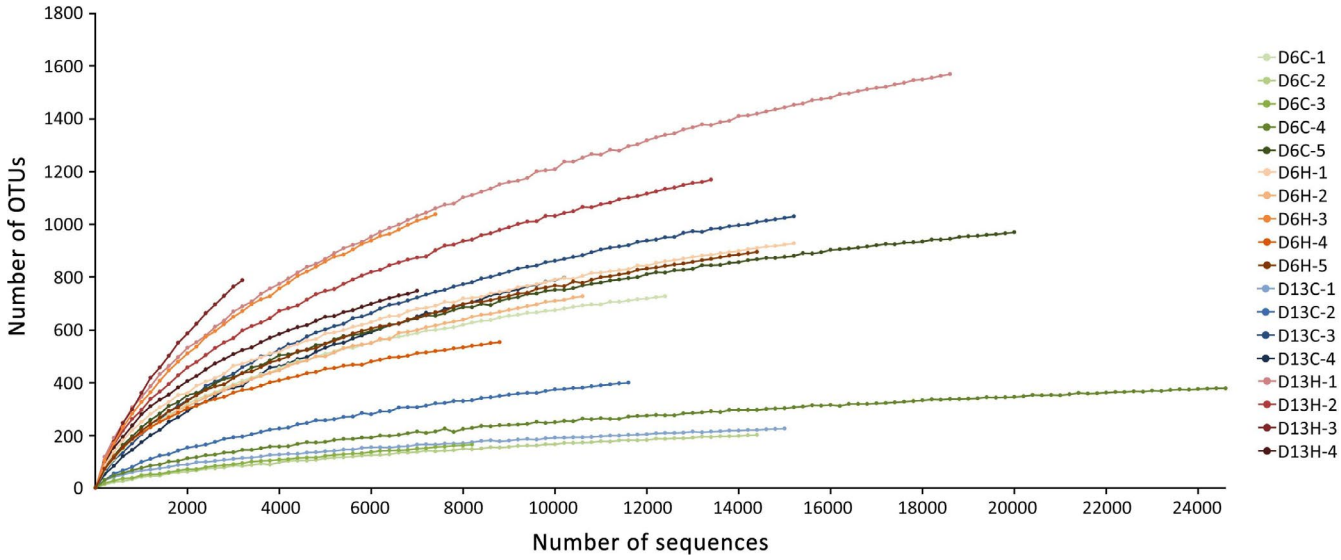

FIGURE A2 Rarefaction curves of OTUs for the coral-associated bacterial community samples from control and heated aquaria

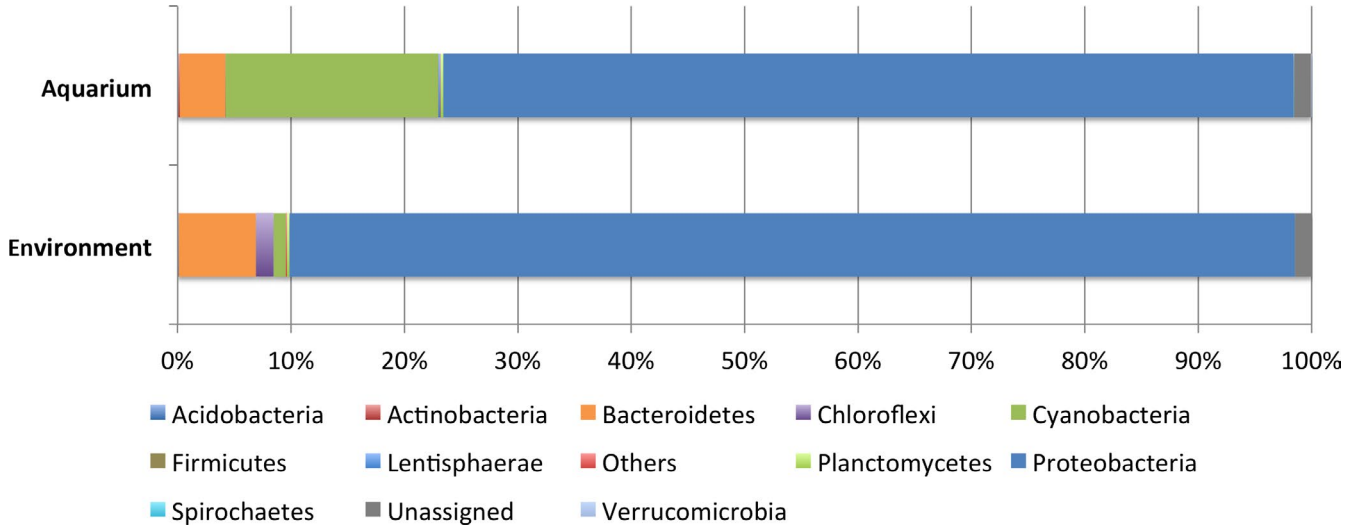

FIGURE A3 A bar chart showing the composition of bacterial communities associated with *P. lutea* in this study (aquarium) and those associated with *P. lutea* collected directly from the field (environment). Taxonomic classification of OTUs present in each sample at the phylum level was based on the Greengenes database

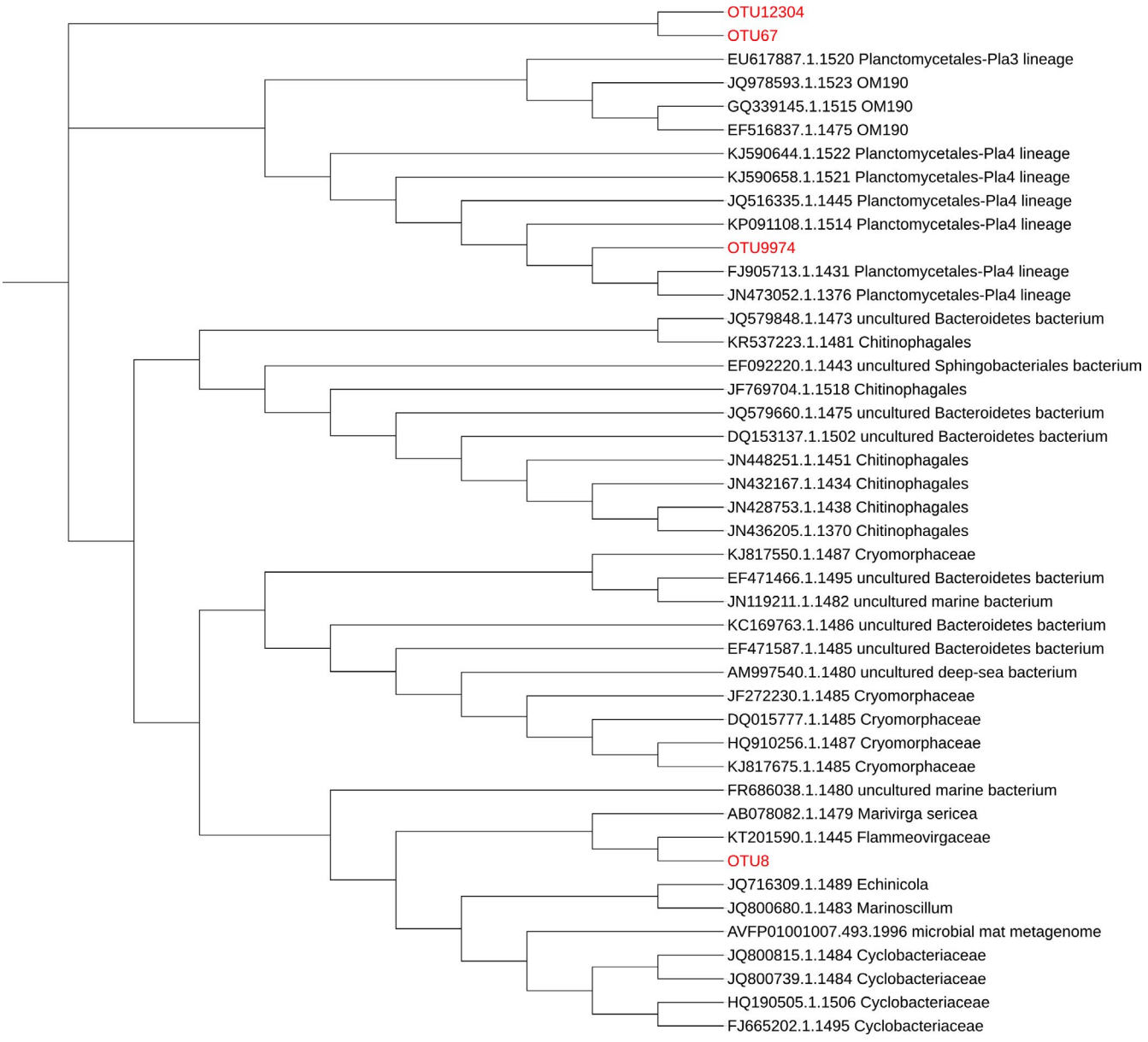

FIGURE A4 A maximum-likelihood phylogenetic tree generated using MEGA7, showing the relationship of four "unassigned" OTUs with known bacterial sequences. The clustering of the sequences was tested by a bootstrap approach using 1,000 replications