The bacterial microbiome of symbiotic and menthol-bleached polyps of *Galaxea fascicularis* in captivity

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ABSTRACT

Coral reefs support the livelihood of half a billion people but are at high risk of collapse due to the vulnerability of corals to climate change and local anthropogenic stressors. While understanding coral functioning is essential to guide conservation efforts, research is challenged by the complex nature of corals. They exist as metaorganisms (holobionts), constituted by the association between the (coral) animal host, its obligate endosymbiotic algae (Symbiodiniaceae), and other microorganisms comprising bacteria, viruses, archaea, fungi and other protists. Researchers therefore increasingly turn to model organisms to unravel holobiont complexity, dynamics, and how these determine the health and fitness of corals. The coral *Galaxea fascicularis* is an emerging model organism for coral symbiosis research with demonstrated suitability to aquarium rearing and reproduction, and to manipulation of the host-Symbiodiniaceae symbiosis ~~manipulation~~. However, little is known about the ~~how its~~ *G. fascicularis* microbiome ~~responds to~~ after long-term captivity and how it responds to menthol bleaching—the experimental removal of the Symbiodiniaceae which represents the first step in ~~the~~ coral-algal symbiosis manipulation ~~—remains unexplored~~. For this, we characterized the bacterial microbiome of symbiotic and menthol-bleached *G. fascicularis* originating from the Red Sea and South China Sea (Hong Kong) that were long-term aquarium-reared in separate facilities. We found that captive corals hosted a relatively simple microbiome composed of relatively few~~er~~ bacterial taxa, when compared to reports of ~~than typically found in~~ the microbiome of ~~wild~~ corals in the wild. ~~-~~Symbiotic ~~P~~polyps (clonal replicates) from the same colony had similar microbiomes, which were distinct from those of other colonies despite co-culturing in shared aquaria. ~~While t~~The response of the bacterial microbiome to menthol bleaching ~~differed~~ varied between the two facilities, warranting further investigation into the role of rearing conditions. Nevertheless, the changes in community composition observed in both instances appeared to be stochastic, and ~~microbiome destabilization and loss of structure emerged as a unifying response,~~ indicative of a dysbiotic state. Considering the importance of captivity and bleaching treatments for ~~holobiont~~ coral symbiosis research, our results—

although preliminary—contribute fundamental knowledge for the development of the Galaxea coral model for symbiosis research.

Keywords: coral, model organism, 16S, microbiome, holobiont, symbiosis, aquarium, ex situ

Introduction

By the end of this century, the livelihood of more than half a billion people will largely depend on the ability of corals to cope with changing ocean conditions (Hughes et al. 2017). Corals are vulnerable to climate change, pollution, and overfishing, which have caused the loss of half of the world's coral cover since the 1950s (Eddy et al. 2021; Eakin et al. 2022). As such, the reef structures they build and the multi-billion dollar ecosystem services they provide are at high risk of collapse (Costanza et al. 2014; van Hooidonk et al. 2016). Considering the high stakes, understanding coral functioning is essential to predict future scenarios and guide management and conservation efforts.

Corals exist as metaorganisms, or so-called holobionts (Rohwer et al. 2002), where complexity hinders our ability to unravel coral functioning and how it ultimately affects coral physiology and ecology (Rosenberg et al. 2007; Jaspers et al. 2019). Indeed, the coral holobiont comprises the animal host, obligate intracellular algal symbionts (Symbiodiniaceae), a rich and diverse bacterial community, together with other microorganisms such as archaea, fungi, viruses, and protists (Bourne et al. 2016; Pogoreutz et al. 2020). Even richer than their taxonomy is the potential diversity of relationships and interactions among members, and how these could contribute to holobiont health and resilience (Thompson et al. 2015; Pogoreutz et al. 2020).

Besides the coral host, the best understood member of the coral holobiont is the algal symbiont, as its photosynthesis-derived energy fuels the calcification process that builds reefs and allows corals to thrive in nutrient-poor waters (Muscatine and Porter 1977; Muscatine 1990). Bacteria are also involved in nutrient cycling and metabolism (Robbins et al. 2019; Tandon et al. 2020), as well as other essential physiological processes such as development (Webster et al. 2004; Tebben et al. 2015) and immunity (Certner and Vollmer 2018; Miura et al. 2019). Interestingly, bacteria also mediate host-Symbiodiniaceae dynamics through the mitigation of thermal and light stress on the algal symbiont (Motone et al. 2020; Connelly et al. 2022), and they produce antimicrobial agents from an organosulfur compound released by Symbiodiniaceae (Raina et al. 2016, 2017). A growing body of knowledge suggests a central role of tripartite interactions between bacteria, Symbiodiniaceae, and the coral host in nutrition, health and fitness (reviewed in Matthews et al. 2020). However, given the intricacy of players' diversity and their metabolic capacities, linking partner identity to function and holobiont phenotype proves particularly challenging.

Model organisms can help unravel holobiont complexity through manipulation. Comparison of different host-Symbiodiniaceae combinations in the sea anemone Aiptasia revealed that heat-tolerance of the symbiont is not linearly transferred to the host (Chakravarti et al. 2017; Gabay et al. 2019; Herrera et al. 2020). Thus complex mechanisms where holobiont properties cannot be predicted as the "sum of its parts" require a more holistic approach (Goulet et al. 2020). Such empirical testing of multi-partner interactions relies on the ability to study the holobiont upon experimental manipulation, namely by removing and/or adding members (Jaspers et al. 2019). Most of such studies in the field were conducted with non-calcifying species such as Aiptasia or the hydroid polyp *Hydra*, which are well established, tractable model organisms (Weis et al. 2008; Galliot 2012). Yet, while these models have proven instrumental for breakthrough discoveries in the cnidarian symbiosis field (e.g., Weis 2008; Murillo-Rincon et al. 2017; Pietschke et al. 2017; Gabay et al. 2018), they lack key features of reef-building corals such as calcification and the obligate endosymbiosis necessary to understand the ecology of coral functioning. To include these critical features, research with corals is irreplaceable (Puntin et al. 2022b).

The reef-building coral *Galaxea fascicularis* (Linnaeus, 1767) has been proposed as a model species for coral symbiosis research (Puntin et al. 2022a). This species is well represented in the literature, featured in studies that characterize the coral gastric cavity (Agostini et al. 2012; Zhou et al. 2020) and the calcification processes (Al-Horani et al. 2003, 2005, 2007), among others (Ferrier-Pagès et al. 1998; Niu et al. 2016; Miura et al. 2019). Introducing *G. fascicularis* as a model system, we previously demonstrated its ease of rearing in simplified

systems (closed, small volume), compatibility with *ex-situ* reproduction, effective removal of the algal symbiont through menthol bleaching, and subsequent reestablishment of the symbiosis with both cultured and environmental Symbiodiniaceae in adult individuals (Puntin et al. 2022a). This demonstrated the potential to experimentally produce a variety of coral-Symbiodiniaceae combinations to study symbiosis functioning and partner compatibility in a true reef-building coral. While recent coral probiotic approaches rapidly expand our knowledge on the functions of the bacterial fraction (Rosado et al. 2019; Peixoto et al. 2021), untangling the complexity in the holobiont requires detailed knowledge of the interrelationships that consider all three partners.

One of the main knowledge gap to advance mechanistic symbiosis research in the Galaxea model system is the effect of menthol bleaching of Symbiodiniaceae on the remaining coral microbiome. Menthol is becoming increasingly common in manipulatve experiments due to its efficacy while causing virtually no mortality (Wang et al. 2012; Matthews et al. 2015; Puntin et al. 2022a). To date, menthol bleaching has been used with a range of symbiotic cnidarians, including jellyfish (Röthig et al. 2021), anemones (Matthews et al. 2015; Dani et al. 2016), corallimorpharia (Lin et al. 2019), and nine species of reef-building corals (Wang et al. 2012, 2019; Puntin et al. 2022a; Scharfenstein et al. 2022; Chan et al. 2023). Yet, its impact on the bacterial fraction remains unknown. Another concerning aspect is the effect of long-term aquarium-rearing on the Galaxea bacterial microbiome, as captivity is known to affect coral microbiome composition (Kooperman et al. 2007; Pratte et al. 2015; Damjanovic et al. 2020). Since long-term aquarium rearing underpins maintenance of characterized clonal lineages, standardization, and reproducibility of model organism research, it is crucial to understand its impact on the host-associated microbiome. No studies so far have characterized the microbiome of long-term aquarium-reared individuals of this emerging model species. However, the bacterial community composition of wild *G. fascicularis* colonies have been investigated (e.g., Li et al. 2013; Cai et al. 2018b; Miura et al. 2019; Motone et al. 2020; Zhu et al. 2022), providing valuable baseline knowledge to gain insights into holobiont response to captivity. To address these knowledge gaps, we characterized the bacterial microbiome of symbiotic and menthol-bleached *G*. *fascicularis* polyps from the central Red Sea and the South China Sea that were maintained in two separate facilities for several months.

Materials and Methods

Coral collection and long-term aquarium rearing

Colonies of *Galaxea fascicularis* were collected from two locations: the Red Sea (hereafter referred as "Red Sea") and Hong Kong in the South China Sea (hereafter referred as "Hong Kong"). Red Sea colonies (n = 3) were collected from the central Saudi Arabian Red Sea at "Al Fahal" reef (N 22˚18.324' E 38˚57.930'), at 9-13 m depth in March 2019 (CITES permit 19-SA-000096-PD) and transported to the Ocean2100 aquarium facility at Justus Liebig University Giessen (Germany) (Schubert and Wilke 2018). In the aquarium system, light was provided by white and blue fluorescent lamps with a light:dark cycle of 12:12 h at 130-160 µmol photons m^{-2} s^{-1} to approximate light conditions at the collection site (Ziegler et al. 2015). Salinity was maintained around 35 and temperature at 26 °C. Colonies were fed daily with a combination of frozen copepods, Artemia, krill, and Mysis. Hong Kong colonies (n = 2) were collected from ≤ 5 m depth from Crescent Island (N 22° 31' 51.035", E 114° 18' 53.888) in June 2019 and transported to the University of Hong Kong (HKU), where they were maintained in a 500-L aquarium equipped with a filtration system and protein skimmer, and fed daily with Reef-Roids (Polyplab) and frozen artemia. Light intensity, salinity, and temperature conditions were consistent with those maintained in the Ocean2100 facility.

Menthol bleaching

At both locations, individual (clonal) polyps were mechanically isolated from their colony, mounted on coral glue (Red Sea colonies, JLU-Ocean2100; Grotech, Cora-Fix SuperFast) or attached to small ceramic tiles (Hong Kong colonies, HKU; Aron Alpha, GEL-10) (Fig. 1). After 10-14 days of healing, polyps were randomly divided between a 'symbiotic' and a 'bleached' group at each location. Both groups were maintained under the same conditions until healed, then the 'bleached' group was treated with menthol to chemically induce bleaching.

Menthol treatment was replicated at the two facilities and followed a protocol modified from Wang et al. (2012). Specifically, three days treatment in 0.38 mM menthol solution in filtered (1.2 µm) artificial seawater (FASW) was followed by one day rest and another day of menthol treatment. Menthol incubations lasted 8 h during the light period.

Bleaching was assessed in the Red Sea polyps by visual inspection under a fluorescence stereomicroscope (Leica MZ16 F) 10 days after the menthol treatment, when algal cells were not detectable in any polyp. At the same time point, Hong Kong polyps also appeared fully bleached under microscopic inspection (Olympus Optical, mod. CHK at 400×).

Post-bleaching rearing conditions

To prevent coral from Symbiodiniaceae exposure and symbiosis re-establishment, all polyps were kept in simplified (see below for details) systems with FASW (1.2 µm) after the menthol bleaching treatment. Here, polyps were fed daily with one small frozen adult Artemia each, followed by partial (~10 %) water change after 2-3 h. At both facilities, temperature, light, and salinity were maintained consistent with the long-term rearing conditions, while the setups differed. Specifically, at the Ocean2100 facility, the polyps were distributed among eight 5-L glass tanks (20 cm × 30 cm) (four per treatment), each equipped with a small pump (Resun SP-500) in a temperature-controlled water bath. At HKU, symbiotic and bleached polyps were maintained in separate 600 ml glass jars, each holding ~6 polyps and equipped with magnetic stir bars for water flow inside a Plant Growth Chamber (Panasonic MLR-352H-PA).

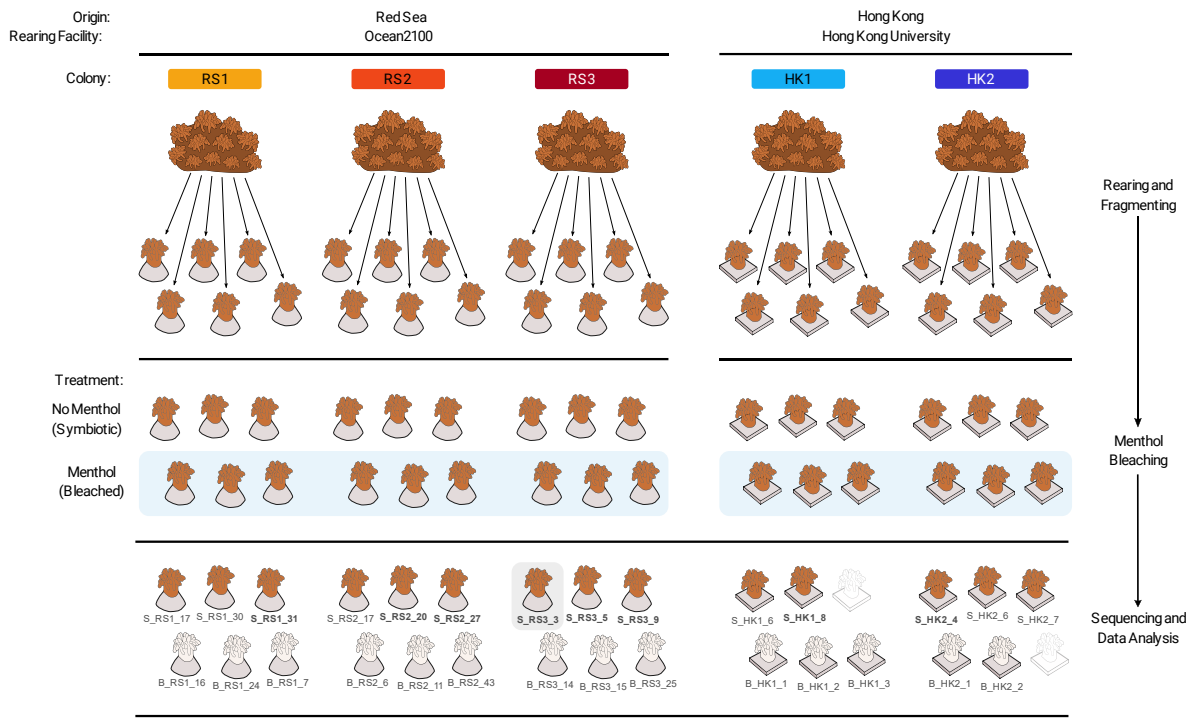

Figure 1 – Visual summary of the experimental design and data processing. Two samples (outlined polyps) were excluded from processing due to damage during shipping, while one sample (grey shaded) was omitted after rarefaction for alpha and beta diversity analysis owing to low sequencing depth. Symbiotic polyps with successful ITS2 sequencing are marked with their sample ID in bold.

Sampling for microbial analysis

16S rRNA amplicon sequencing was employed to characterize the bacterial communities of symbiotic and menthol-bleached polyps, and ITS2 amplicon sequencing for the Symbiodiniaceae communities of symbiotic polyps of *G. fascicularis* colonies from the two geographic locations (Fig. 1).

At both locations, three polyps per colony (5 colonies: RS1, RS2, RS3, HK1, HK2)~~(3 Red Sea, 2 Hong Kong)~~ per state (2 states: ~~here,~~ symbiotic~~, and~~ bleached) were sampled on the 13th day after the menthol treatment (n = 15 bleached and 15 symbiotic polyps). The polyps were rinsed with seawater, separated from the substrate, placed in sterile tubes, and stored at -80 °C. For transport, polyps were stored in RNAlater (R0901-100ML, Sigma-Aldrich, Hong Kong S.A.R.). Two samples from Hong Kong (1 symbiotic and 1 bleached) were damaged during shipping and therefore excluded from processing. All samples were processed together for DNA extraction (University of Derby's Aquatic Research Facility, UK) and subsequently sequenced in the same sequencing run (Bart's and the London Genome Centre, Queen Mary, University of London).

Bacterial and Symbiodiniaceae community analysis

DNA was extracted using the Qiagen DNeasy 96 Blood & Tissue kit. About 10 mg of coral tissue per sample was used as starting material. Extractions followed the user manual with centrifugations at working steps 10, 12, and 16 performed at 1500 *g* at doubled centrifugation times. Extracted DNA was sent to the sequencing facility for quality control, PCR, library preparation, and pair-end sequencing with Illumina MiSeq platform v3 (2 × 300 bp). The 16S rRNA gene region V5/V6 was amplified using the primers 784F and 1061R (Andersson et al. 2008), while Symbiodiniaceae ITS2 region was amplified using the primers SYM_VAR_5.8S2 and SYM_VAR (Hume et al. 2018). A contamination control consisting of pure RNAlater buffer was included in all steps.

Bacterial sequencing data were processed in Qiime2 (v.2021.11, Bolyen et al. 2019) and analyzed in R (v.4.1.0, R Core Team 2021). After primer removal, forward and reverse reads were truncated to 232 and 234 nt respectively, paired, dereplicated, quality checked, cleaned, and clustered to amplicon sequence variants (ASVs) using the denoise-paired method in DADA2 (Callahan et al. 2016). This resulted in a total of 138,620 sequences and 547 ASVs. ASVs were taxonomically assigned using a weighted classifier trained against the SILVA 138 database (99 % clustering, full length) (Yilmaz et al. 2013) with the classify-sklearn method from 'q2-feature-classifier' plug-in (Bokulich et al. 2018; Kaehler et al. 2019; Robeson et al. 2020). Then, sequences assigned to "mitochondria", "chloroplast", "Archaea", "Eukaryota", or "unknown" at the phylum level were removed. Sequences found in the control sample were considered potential lab contaminants and evaluated based on presence/absence across the coral samples and habitat description (e.g., known contaminants) of the closest BLASTn matches (GenBank) (for full details see https://zenodo.org/record/10551928~~7976283~~, "01_find_contaminants.R"). This led to the removal of five ASVs and their 18,990 sequences, and resulted in a final data set of 112,789 sequences and 515 ASVs across 28 samples (after excluding the contamination control).

Rarefaction and alpha and beta diversity calculations were performed with the R package 'phyloseq' (v.1.38.0, McMurdie and Holmes 2013), 'metagMisc' (v.0.0.4, Mikryukov 2023) and 'btools' (v.0.0.1, Battaglia 2022). Samples were rarefied to 2,690 sequences (based on 1,000 iterations of random subsampling without replacement), which caused the exclusion of one sample (symbiotic colony "RS3") due to low sequencing depth. Rarefaction curves showed that for most samples the number of ASVs plateaued before the rarefaction depth indicating that most of the diversity was captured and retained after rarefaction (Fig. S1). Alpha diversity was estimated through multiple indices chosen for their complementarity and comparability with previous studies (observed richness, Chao1, Shannon diversity, Simpson ~~evenness~~diversity, Pielou's evenness, and Faith's phylogenetic diversity). Differences in alpha diversity between symbiotic and bleached individuals were tested with t-tests or Mann-Whitney *U* tests depending on data distribution and variance. Beta diversity based on Bray-Curtis distances was visualized with non-metric multidimensional scaling (nMDS).

The contribution of host origin (Red Sea, Hong Kong) and symbiotic state (symbiotic, bleached) to microbiome community structure was assessed using multivariate homogeneity of groups dispersions analysis with the betadisper function (PERMDISP2), and one-way and two-way permutational multivariate analysis of variance (PERMANOVA) on Bray-Curtis distances with the adonis2 function in the 'vegan' R package (v.2.5.7,

Dixon 2003; Oksanen et al. 2020). Relative abundance bubble plots of bacterial community composition at bacterial family level and of core ASVs (occur in all groups by state and geographic origin), and the UpSet plot (Lex et al. 2014) were generated from non-rarefied data. The UpSet plot was created with the package 'UpSetR' (v.1.4.0, Conway et al. 2017). All other plots were created in 'ggplot2' (v.3.3.5, Wickham 2016).

For Symbiodiniaceae community analysis, raw ITS2 sequencing data were analyzed using the SymPortal workflow remote instance (Hume et al. 2019). ITS2 sequencing produced poor results and it was only possible to characterize eight of the symbiotic samples (6 Red Sea, 2 Hong Kong; see Fig. 1). Of these, two did not pass quality check (i.e., they had < 200 sequences/genus) and SymPortal could not predict ITS2 type profiles. Therefore, we report (post-MED) ITS2 sequences.

Results

Microbial diversity and richness were unaffected by menthol bleaching

When considering all colonies together, ~~A~~alpha diversity remained similar between symbiotic and menthol-bleached samples across all diversity and richness indices tested (incl. observed richness, Chao1, Shannon diversity, Simpson ~~evenness~~diversity, Pielou's evenness, and Faith's phylogenetic diversity, see Table S1, S2), and regardless of their origin ($P_{t\text{-test}}$ or $P_{Mann\text{-}Whitney\ U\text{-}test}$ > 0.05; Fig. 2~~1~~A). When considering each individual colony, alpha diversity ~~For one colony only (RS1), alpha diversity~~ was remarkably and consistently low in symbiotic RS1 polyps~~state~~, while in their bleached counterparts it was in range with the other colonies ~~when bleached~~ (Fig. 2~~1~~A, Fig. S2, Tab. S2). Within-colony difference between symbiotic and bleached polyps could only be tested for RS1 and RS2, and it was significant in RS1 across all alpha diversity indices tested (P_{Welch} < 0.05, Tab. S3).

Bacterial communities were generally uneven

A small number of ASVs dominated the bacterial communities, where the 3 and 9 most abundant ASVs accounted for > 25 % and > 50 % of the total number of sequences, respectively (Fig. 2~~1~~B). Evenness was on average lower among the symbiotic polyps. Specifically, in the symbiotic samples, the 5 (for Red Sea) and 4 (for Hong Kong) most abundant ASVs account for > 50 % of total reads, while in the bleached samples it took 7 (for Red Sea) and 12 (for Hong Kong) ASVs to pass the 50 % relative abundance threshold.

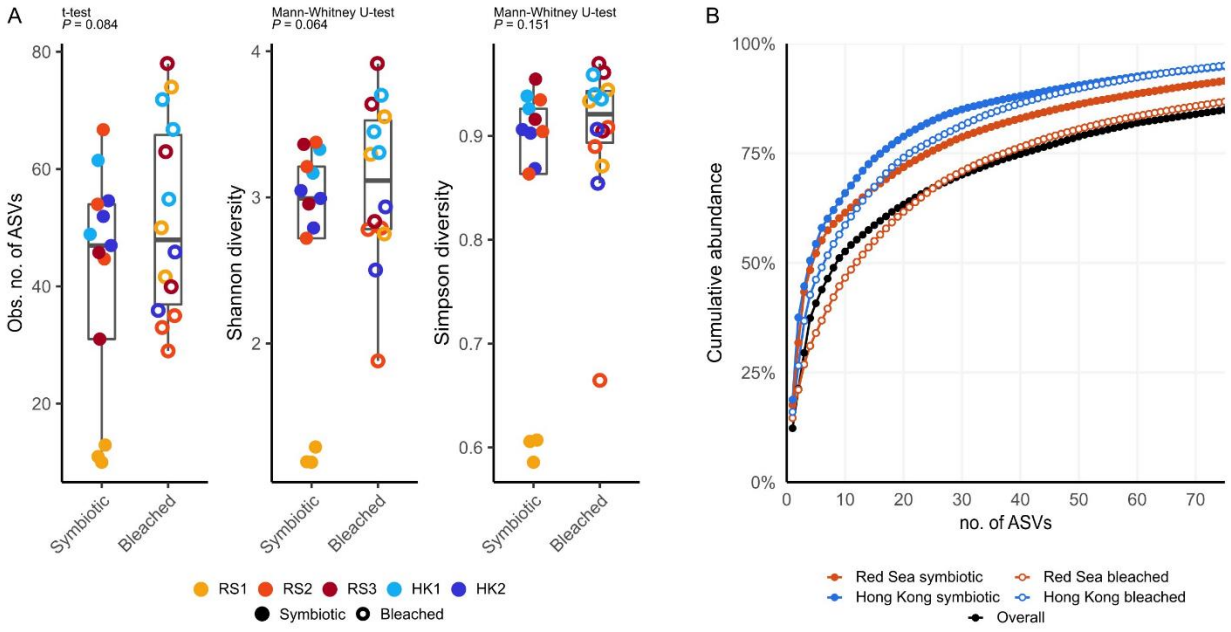

Figure 21 - Diversity and evenness of the bacterial communities of symbiotic and menthol-bleached polyps of *Galaxea fascicularis*. (A) Comparison of observed number of ASVs, and Shannon diversity index, and Simpson evenness diversity index between symbiotic and bleached samples; (B) ASV accumulation curve for the whole data set, and separated by symbiotic state and geographic origin.

Microbial community compositiondissimilarity patterns differed by geographic origin of colonies

Microbial communities of Red Sea samples showed significantly larger dissimilarities than those from Hong Kong, both within and between colonies (ANOVA on PERMDISP2, F = 118.7, P < 0.0001, Fig. 3A; Mann-Whitney U-test on pairwise Bray-Curtis distances, W = 102, P = 0.0001, Fig. 3B, Fig. S3).Microbial communities were significantly different between colonies from the Red Sea and Hong Kong (PERMANOVA, F = 5.46, P = 0.0003; Fig. 2A). However,

Mmicrobial communities of symbiotic Red Sea polyps clustered by colony, and Red Sea colonies appeared as different from each other as they were from those of Hong Kong colonies. Interestingly, O, and one symbiotic colony originating from the Red Sea did however share had a similar microbial community towith those from Hong Kong (Fig. 32A). Besides microbial community composition, colonies also differed in dispersion, where Red Sea microbial communities showed significantly larger dissimilarities than those from Hong Kong, both within and between colonies (ANOVA on PERMDISP2, F = 108.3, P < 0.0001, Fig. 2A; Mann-Whitney U-test on pairwise Bray-Curtis distances, W = 102, P = 0.0001, Fig. 2B).

Menthol-bleaching elicited a stochastic changesloss of structure in the microbial communities

Changes in community composition between symbiotic states differed for coral colonies from the two regions. For the Red Sea, there was no clear distinction in bacterial community composition between symbiotic and bleached polyps in terms of location and dispersion in the ordination space, when considering all colonies together (PERMANOVA, F = 0.76, PERMDISP2, F = 0.77, P > 0.05; Fig. 32C). For Hong Kong, the microbial communities of symbiotic and bleached polyps were significantly different (PERMANOVA, F = 4.0, P < 0.01; Fig. 32D), while the differnce ins dispersion was (just marginally) not statistically significant (PERMDISP2, F = 5.05 , P = 0.057). However, while symbiotic polyps clustered by colony for both Red Sea and Hong Kong (because of their similar microbial communities) (for both Red Sea and Hong Kong), bleached polyps showed no such clear grouping (Red Sea) or larger scattering compared to symbiotic polyps (Hong Kong), and collectively had a significantly higher within-colony dissimilarity ($P_{Mann\text{-}Whitney}$ = 0.0001, Fig. 32A,B, Fig. S3). This indicates inrandom changes a pattern of loss of structure in the communities of the menthol-bleached polyps.

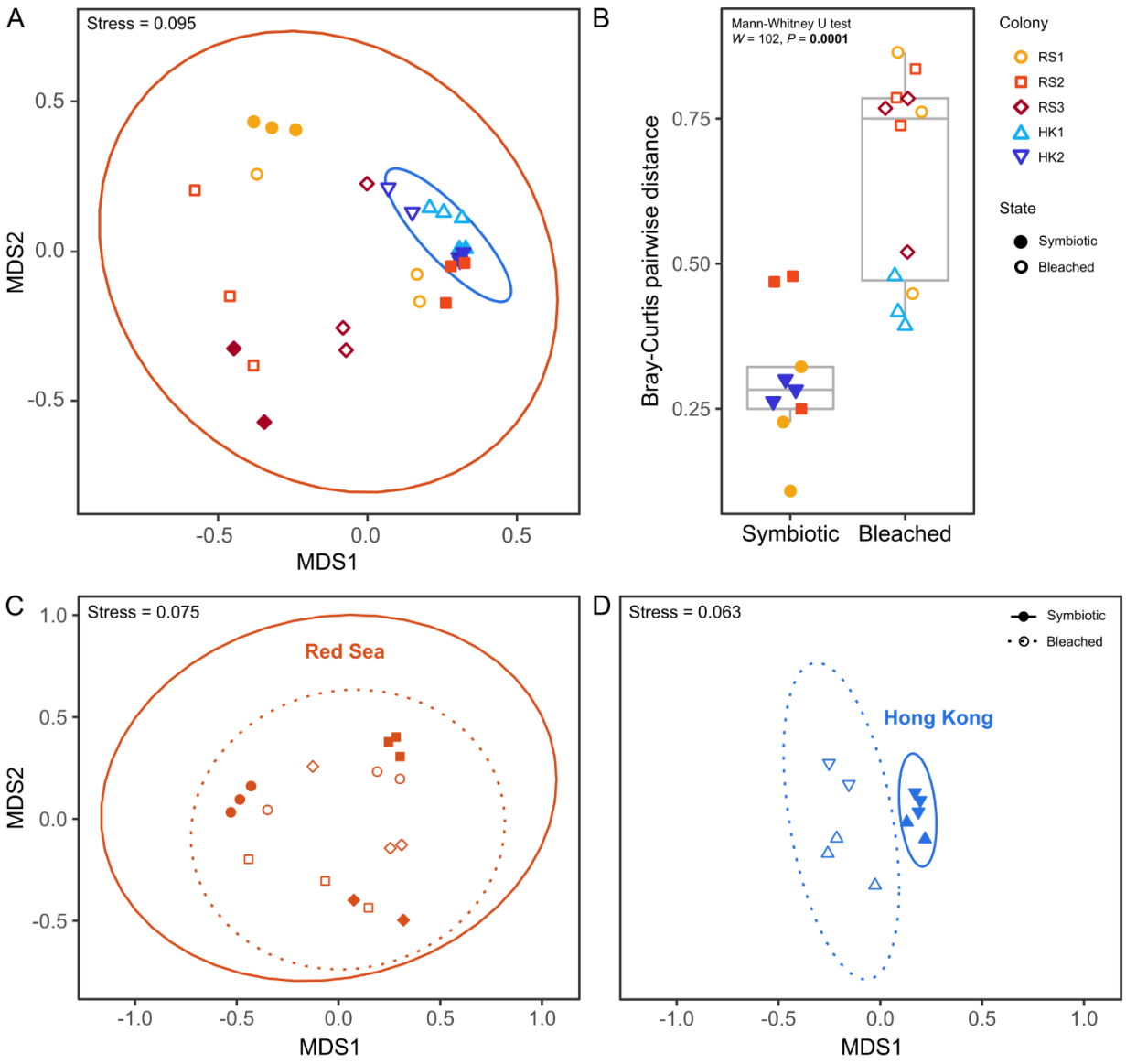

Figure ~~32~~ - Microbial community structure of *Galaxea fascicularis* from the Red Sea and Hong Kong. Non-metric multi-dimensional scaling (nMDS) plot of bacterial community composition based on Bray-Curtis dissimilarity for all polyps (A), Red Sea polyps (C), and Hong Kong polyps (D), and comparison of pairwise dissimilarity between symbiotic and bleached polyps of the same colony (only groups with $n = 3$ considered, but comparable results were found considering groups with $n < 3$, see Fig. S3) (B). Ellipses = 95 % confidence intervals (A, C, D); colors (A, D) or shapes (C, D) denote colony identity, filled symbols = symbiotic polyps, hollow symbols = bleached polyps.

Three bacterial families dominated most microbial communities

A total of 138 bacterial families were found across the 28 sampled polyps. Of these, 104 bacterial families occurred in symbiotic (74 in Red Sea, 66 in Hong Kong) and 109 in bleached (89 in Red Sea, 69 in Hong Kong) polyps. The three most abundant families *Rhodobacteraceae* (25.8 %), *Alteromonadaceae* (14.6 %), and *Moraxellaceae* (10.1 %) together represented > 50 % of all sequences, and the 11 most abundant families represented > 75 % of all sequences. Symbiotic colonies were largely dominated by members of the two bacterial families *Rhodobacteraceae* and *Alteromonadaceae*. Except for the two Red Sea colonies RS1 and RS3

that were dominated by *Moraxellaceae* and *Bacillaceae*, or that showed no consistently dominant family across individual polyps, respectively.

Bleached polyps were dominated by varying bacterial families, with inconsistent patterns between and within colonies and regions. In addition to the dominant families in symbiotic colonies, *Endozoicomonadaceae*, unclassified *Cellvibrionales*, and *Amoebophilaceae* became dominant in some bleached polyps. While members of the family *Endozoicomonadaceae* were the most abundant fraction in one bleached polyp (39 %; RS3), they were only present in 15 of the 28 polyps (7 symbiotic, 8 bleached), and with low relative abundance (mean 2.2 % in symbiotic, and 8.9 % in bleached). No single taxon (neither at ASV nor at bacterial familiy level) was significantly different in relative abundance between symbiotic and bleached polyps, regardless of whether samples from the Red Sea and Hong Kong were considered together or separately (Wilcoxon test with Benjamini-Hochman correction, all $P > 0.05$).

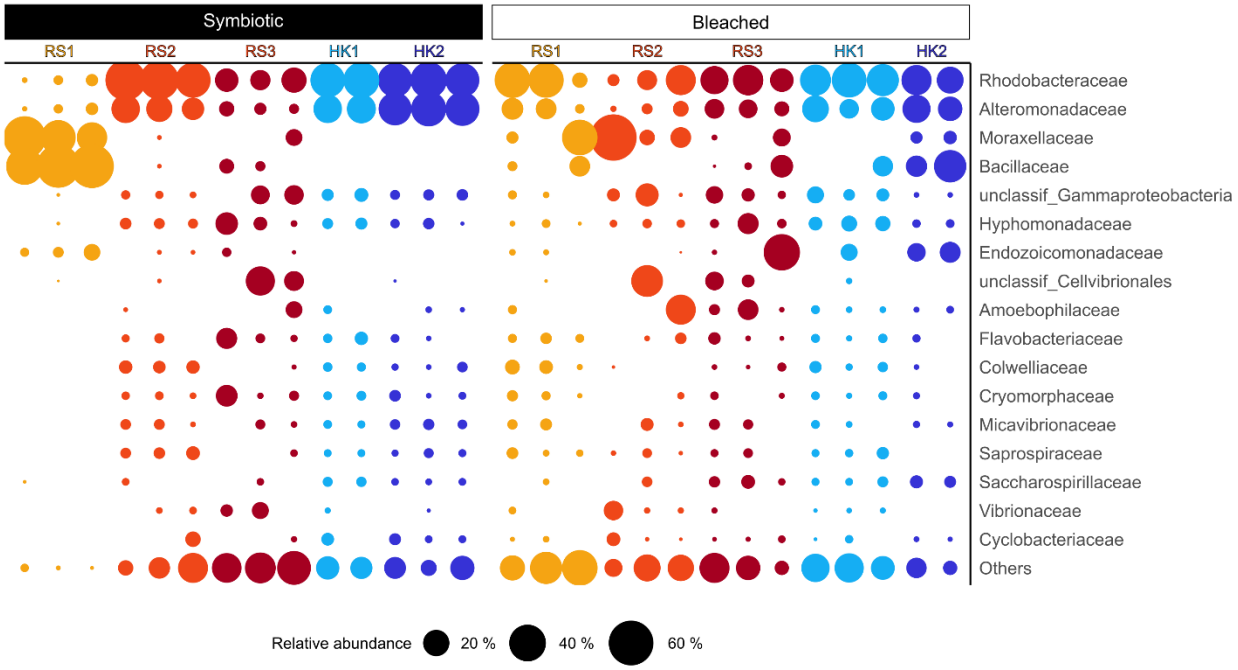

Figure 43 - Relative abundance of bacterial families in symbiotic and bleached *Galaxea fascicularis* polyps from the Red Sea and Hong Kong. Bubble size is proportional to the relative abundance per polyp of the 17 most abundant families, which account for 83.7 % of the total number of reads; all other 121 families that had a relative abundance < 1 % are grouped as 'Others'.

28 core ASVs were shared by all coral colonies and symbiotic states

Altogether, there were more exclusive than shared ASVs between experimental groups (Fig. 54A). Specifically, 33.4 % and 52.9 % of ASVs were exclusively found in symbiotic or bleached samples, respectively, while 5.4 % of ASVs (28) were found in all groups (symbiotic state and geographic origin) and were considered core taxa. Although no ASV was found in all samples (nor in all samples of the same experimental group, Tab. S34), three ASVs occurred in ≥ 70 % of samples, and five additional ASVs occurred in ≥ 60 % of samples (Fig. 54B). The sequences of these eight ASVs, which also corresponded to the most abundant of the 28 core ASVs, were BLAST-searched against the NCBI Nucleotide collection (Tab. 1), which revealed that most of the matching sequences were from samples associated with marine environments and/or organisms. Among these, the most abundant bacterial sequences belonged to the genera *Alteromonas*, *Ruegeria*, and *Nautella* (Fig. 54B, Tab. 1). Interestingly, two ASVs (ASV_001 and ASV_006), both assigned to the genus *Ruegeria*, matched with strains isolated from aquarium-reared *Galaxea fascicularis* from the South China Sea (collected in Hainan Island, China,

Zhou et al. 2020) and Japan (Miura et al. 2019). Further, three ASVs (ASV_006, ASV_018, ASV_020) matched with bacteria identified in *Acropora* spp. and *Pocillopora* spp. from the central Red Sea, amplified with the same primer set used for this study (Tab. 1).

All colonies of *Galaxea fascicularis* were dominated by *Cladocopium* spp. symbionts

Symbiodiniaceae composition was consistent in polyps from each colony and differed by region. *Cladocopium* spp. ITS2 sequences accounted for > 92 % of the sequences in all samples but one (RS2, 76 %) (Fig. S4). Of these, C1 was by large the dominant ITS2 sequence (~ 33 – 80 % relative abundance). Sequences C1b, C41, and C41f were exclusively and consistently found in polyps of one Red Sea colony (RS3), where they collectively accounted for ~ 26 % of reads. *Durusdinium* spp. sequences were found in only one colony from the Red Sea (RS2), where they accounted for 4 % and 24 % of the sequences, and of which the most abundant sequences were D1 and D4 (Fig. S4). One Red Sea sample (RS3) also hosted sequence A1 (genus *Symbiodinium*) at ~ 5 % abundance. In both Hong Kong colonies C1 was the dominant sequence (68 – 80 %), with C1c present in lower abundance (15 – 20 %) (Fig. S4).

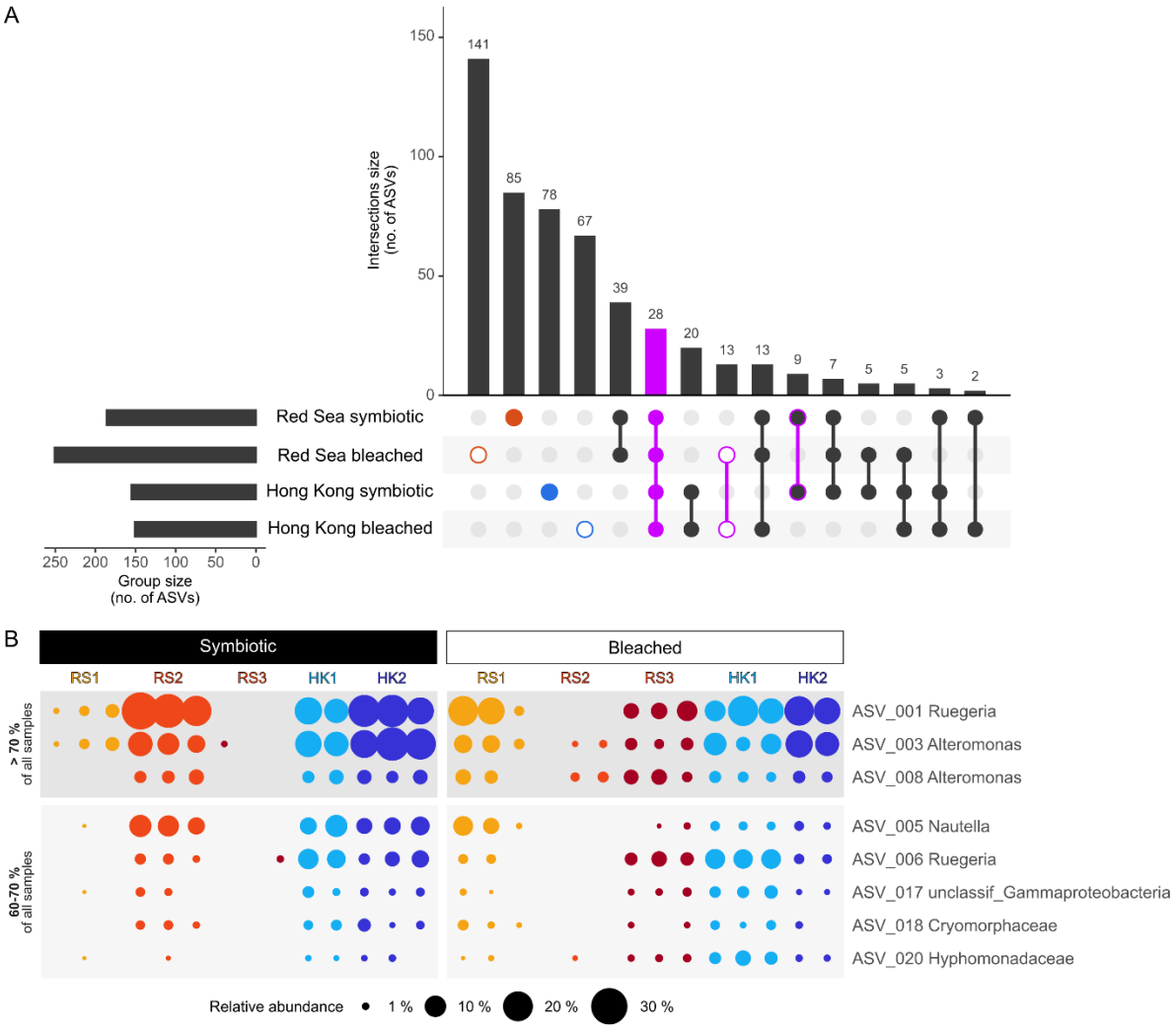

Figure 54 - Overview of total and core ASVs in *Galaxea fascicularis* symbiotic and menthol-bleached polyps from the Red Sea and Hong Kong. (A) UpSet diagram showing the number of observed ASVs per group

and per intersection. (B) Relative abundance of core bacterial ASVs by host origin and symbiotic state, considering only ASVs present in ≥ 70 % (top-darker grey block) and between 60 % and 70 % of samples (bottom-lighter grey block); bubble size is proportional to the relative abundance of ASVs in each sample.

Table 1 - Summary of NCBI BLASTn matches for the eight most common core ASVs (occur in both symbiotic and bleached samples from both Red Sea and Hong Kong, and in > 60 % of all samples) in order of abundance. For brevity, only the top three matches are reported (100 % query cover) with information on isolation source and location, where coral species are highlighted in bold. Taxonomy is reported as the lowest taxonomic level assigned by the Qiime2 classifier.

Taxonomy	ASV no.	GenBank Accession no.	Identity (%)	Isolation source (location)
Ruegeria	ASV_001	MK967091	100.00	Jellyfish, *Aurelia aurita* polyp (Kiel Bight, Baltic Sea)
		MT187950	100.00	***Galaxea fascicularis*** (Hainan, South China Sea)
		MT187656	100.00	***Galaxea fascicularis*** (Hainan, South China Sea)
Alteromonas	ASV_003	MT525287	100.00	Marine sediment (South Korea)
		MK967157	100.00	Artificial Seawater (Kiel Bight, Baltic Sea)
		MT515801	100.00	Marine sediment (South Korea)
Nautella	ASV_005	LC543501	100.00	Coastal seawater (Japan)
		MH556771	100.00	Ciliate, *Euplotes vannus* in Yantai Institute of Coastal Zone Research (Shandong, China)
		MK801649	100.00	Unspecified coral from South China Sea (Guangdong, China)
Ruegeria	ASV_006	MK736137	100.00	***Acropora hemprichii*** (Central Red Sea)
		MK736054	100.00	***Pocillopora verrucosa*** (Central Red Sea)
		MH807604	100.00	***Galaxea fascicularis*** (Aquarium, Japan)
Alteromonas	ASV_008	MT472680	100.00	Shrimp gut, *Penaeus vannamei* (India)
		MN704564	100.00	Undescribed source at marine molecular ecology lab (Zhejiang, China)
		MH556744	100.00	Ciliate, *Euplotes vannus* in Yantai Institute of Coastal Zone Research (Shandong, China)
unclassified *Gammaproteobacteria*	ASV_017	AB294979	99.61	Microbial mat at shallow submarine hot spring (Okinawa, Japan)
		KU629853	99.23	Seaweed, *Asparagopsis* sp. (Portugal)
		GU061986	99.23	Oceanic water (South China Sea)
unclassified *Cryomorphaceae*	ASV_018	KY374050	100.00	***Acropora hyacinthus*** (Central Red Sea)
		KF185498	99.60	Marine snow (Adriatic Sea)
		KU648372	95.65	Seawater (Gullmarsfjorden, Sweden)
unclassified *Hyphomonadaceae*	ASV_020	KY455482	100.00	***Acropora hemprichii*** (Central Red Sea)
		KY373570	100.00	***Acropora hyacinthus*** (Central Red Sea)
		CP017718	100.00	Algal culture, coral reef substrate (Palmyra Atoll, Pacific Ocean)

Discussion

We employed 16S rRNA gene amplicon sequencing to characterize the bacterial communities of symbiotic and menthol-bleached *G. fascicularis* polyps from the Red Sea and Hong Kong, and gained preliminary insights on the effect of long-term aquarium rearing on this emerging coral model system.

Menthol bleaching led to stochastic changes in the microbiome of~~changes in~~ Galaxea

Menthol bleaching was associated with changes in ~~led to a destabilization and loss of structure of~~ the bacterial communities that differed between individual polyps and~~,~~ produced~~ing~~ stochastic configurations. Unlike previous studies on Aiptasia that compared bleached and symbiotic individuals and found significantly different microbiomes (Röthig et al. 2016; Curtis et al. 2023), we could not identify a "signature of bleaching" as no bacterial taxa showed differential abundance between symbiotic states in our Galaxea colonies. This surprisingly included Symbiodiniaceae-associated bacteria that we were expecting to be reduced after the physical removal of Symbiodiniaceae (Fig. S5; Supplementary Materials and Methods). Such results could be an artifact of whole-tissue sampling in this study. As different coral compartments host distinct bacterial communities (Sweet et al. 2011; ~~D~~ Ainsworth et al. 2015; Apprill et al. 2016), changes at the level of the gastroderm, where Symbiodiniaceae and their bacterial communities are located, might have been masked. Alternatively, it might indicate that Symbiodiniaceae-associated bacteria only repesent a small proportion of the community in Galaxea, or that they were able to persist in the absence of Symbiodiniaceae.

The stochastic response to bleaching aligns well with the concept of an obligatory nature of the coral-algal symbiosis. As the coral depends on its algal symbiont for energy and other metabolic processes (Muscatine and Porter 1977; Muscatine 1990), the bleached state is not a stable alternative to the symbiotic state. During bleaching the weakened host becomes progressively unable to regulate its microbial community, leaving room for the establishment of opportunistic bacteria, producing novel and stochastic combinations (Zaneveld et al. 2017). In facultatively symbiotic cnidarians ~~,~~ (such as the anemone Aiptasia), symbiotic and bleached can constitute alternative stable states, and it is thus possible to identify distinct and characteristic symbiotic and bleached microbiome configurations (Röthig et al. 2016; Curtis et al. 2023).

In the nMDS-ordination space on microbial community composition (Fig. ~~2~~3), bleached Red Sea polyps moved towards the center of the plot to the other symbiotic colonies rather than spreading in any random direction. This, taken together with the observation that alpha diversity did not increase after bleaching (as otherwise expected with dysbiosis (Zaneveld et al. 2017)), points towards a "captivity" effect. Since the polyps were maintained together in filtered seawater in closed systems, their exposure to novel bacteria was limited, and bacteria shed by the other polyps likely constituted the predominant source of "novel" associates. In contrast, the response of the Hong Kong corals to menthol bleaching was directional and more uniform. ~~and we~~We hypothesize that this might reflect a new stable state of the tank water, rather than of the holobiont. While rearing conditions were largely replicated between facilities, feed type, tank volume and filtration systems differed. Feed can introduce bacteria into the system (Hartman et al. 2020), and uneaten portions could promote microbial growth. Such effects would have been amplified by the smaller volume of the containers used in Hong Kong compared to Red Sea. However, it should be noted that our experimental design did not allow us to directly test these hypotheses. We therefore suggest that future studies incorporate an adequately replicated "facility" factor in their design, as well as food and seawater samples ~~in their analysis~~ to better characterize the influence of rearing conditions on the host microbiome.

The microbiome of long-term aquarium-reared *Galaxea fascicularis*

The *G. fascicularis* polyps hosted simple bacterial microbiomes which were composed of a relatively small number of bacterial taxa (10-78 ASVs). Species richness in our aquarium-reared polyps was thus almost two orders of magnitude lower than those of wild *G. fascicularis* colonies sampled in the South China Sea (646-1,459 OTUs, Li et al. 2013) and those reported from a wide range of species and locations, which is typically in the order of 100s to 1000s of bacterial taxa (e.g., Ziegler et al. 2016; Hernandez-Agreda et al. 2018; Pollock et al. 2018; Galand et al. 2023). Due to the absence of direct comparison with wild colonies we are unable to draw conclusions on whether captivity caused a reduction in bacterial diversity. However, we hypothesize that captivity favours a streamlining of the microbiome, as ~~The reduced bacterial diversity likely resulted from captivity, where~~ stable and homogenous environmental conditions decrease both chances and need for the association with functionally and taxonomically diverse microbial partners. ~~–~~ In fact, ~~D~~decreases in metabolic diversity and species richness have consistently been reported for tropical reef-building corals reared in closed systems (Kooperman et al. 2007; Vega Thurber et al. 2009; Pratte et al. 2015; Damjanovic et al. 2020). The

same has also been reported for the anemone Aiptasia after only a few days of captivity (Hartman et al. 2020). ~~The observed~~Such effects may also have been exacerbated by the use of filtered seawater during the bleaching phase, which largely reduced the pool of available microbes (Dungan et al. 2021b). Additionally, as colony morphology is a major factor affecting coral microbial communities (Morrow et al. 2022), a loss of~~the decrease in~~ bacterial species ~~richness~~ might also be ascribed to reduced structural complexity, where single polyps have a simpler geometry with fewer micro-environments and ecological niches compared to larger colonies (Putnam et al. 2017).

Although some may see this reduction or simplification of the microbiome has a problem artefact associated with captive corals, simplified microbiomes ~~The reduction of microbial complexity~~ presents ~~the~~an opportunity to~~for~~ identify~~ing~~ essential associates and facilitat~~e~~ing the development of microbial manipulation protocols to unravel holobiont functioning (Jaspers et al. 2019; Puntin et al. 2022b). While the majority of studies report corals as hosting complex and rich microbial communities, the key functional players still remain elusive (Jaspers et al. 2019; Barreto et al. 2021). Culturing corals in sterile seawater may help to limit the horizontal acquisition of transient microbes and thus favor proliferation of core or stable members for detailed characterization (Dungan et al. 2021b). A simplified microbiome also facilitates further targeted or complete elimination of bacterial populations to produce gnotobiotic or axenic hosts. These could subsequently be re-inoculated to produce a range of host-bacteria combinations to test microbial functions and inter-partner dynamics (Fraune et al. 2015; Murillo-Rincon et al. 2017; Jaspers et al. 2019; Taubenheim et al. 2020). Reduced microbial complexity in captivity might therefore provide advantages for these specific experimental approaches with the Galaxea model.

Interestingly, the coral colonies tested here maintained distinct bacterial microbiomes even after long-term co-culturing, which supports a degree of host genotype effects controlling the microbiome composition as previously reported from Hydrozoan corals in the field (Dubé et al. 2021). Surprisingly, the microbiome of one Red Sea colony was highly similar to that of Hong Kong colonies, despite large differences in geographic and environmental origin (Wepfer et al. 2020). These colonies were also maintained in separate facilities, but rearing conditions were similar at both locations. In addition to the host phylogenetic basis of microbiome composition (Pollock et al. 2018), the similarity in environmental conditions may have induced convergence of microbial community composition (Dubé et al. 2021).

Besides host genotype, Symbiodiniaceae community composition observed herein could also explain differences in bacterial community composition between Red Sea colonies (Littman et al. 2010; Bernasconi et al. 2019). While only a small proportion of samples were successfully sequenced, we could identify patterns of Symbiodiniaceae-bacteria co-occurrence that warrant further investigation. We therefore recommend that future studies characterize a larger number of Galaxea holobionts at multiple locations across the species distribution range to explore links between host-Symbiodiniaceae-bacteria associations. This could elucidate the influence of each member on coral holobiont compositions and functioning.

Core bacterial associates of Galaxea

We defined core or stable microbial associates based on prevalence of ASVs across treatment groups and polyps. Notably, no taxa was present in 100 % of the samples, suggesting a certain degree of microbiome variability within this coral host species. Among the 28 core ASVs (occuring in all groups), the five most frequent and abundant ~~ones~~ were assigned to the genera *Alteromonas* and *Ruegeria*. Both *Alteromonas* and *Ruegeria* are common coral~~-s~~ associates reported from at least 20 other coral species, and sequences assigned to these two genera ranked 6th and 33rd most abundant in the Coral Microbial Database (Huggett and Apprill 2019).

Ruegeria spp. are commonly and consistently found in association with *G. fascicularis* in wild and aquarium-reared colonies, from Hong Kong to Japan and across seasons (Cai et al. 2018a, 2018b; Miura et al. 2019; Tang et al. 2020; Kitamura et al. 2021). Indeed, the two *Ruegeria* core ASVs had identical sequences with *Ruegeria* from *G. fascicularis* from Hainan and Japan that were maintained under aquarium conditions comparable to ours (Zhou et al. 2020, Miura et al. 2019). This shows that the Galaxea-*Ruegeria* association is highly conserved and therefore putatively biologically relevant. *Ruegeria* strains isolated from *G. fascicularis* were indeed previously identified as potential probiotics, through inhibitory activity towards the coral pathogen *Vibrio*

coralliilyticus (Kitamura et al. 2021). The ubiquitous and persistent Galaxea-*Ruegeria* association thus warrants attention in future investigations.

The potential role of *Alteromonas* spp. in the Galaxea holobiont functioning also deserves attention. Despite their high abundance and prevalence in this study (and in corals in general), the role of *Alteromonas* spp. remains controversial. They have been considered pathogenic, owing to their co-occurrence with coral diseases (e.g., Sunagawa et al. 2009), but also listed as candidate probiotic for their free radical scavenging abilities (Raina et al. 2009; Dungan et al. 2021a), which could be linked to their consistent association with Symbiodiniaceae (Lawson et al. 2018; Nitschke et al. 2020).

Bacteria in the family *Endozoicomonadaceae* are the most prominent members of coral microbiomes in a range of coral species (Morrow et al. 2012; Bayer et al. 2013; Neave et al. 2017; Pogoreutz et al. 2022), which have been investigated for their involvement in holobiont metabolism, for example in the C and S cycles (Neave et al. 2016; Ide et al. 2022). Yet, *Endozoicomonadaceae* were only present in approximately half of Galaxea polyps at between 0.06 and 39.12 % (mean 5.78 %) relative abundance. This is slightly lower than in wild *G. fascicularis* from Hong Kong (< 10 % relative abundance) in which, however, *Endozoicomonas* spp. were present in all samples (Cai et al. 2018a, 2018b). Such scarcity and inconsistent presence therefore suggests that *Endozoicomonas* spp. might not play an essential role in the *G. fascicularis* under captive conditions. If further proven, a lack of reliance on *Endozoicomonas* spp. in captivity could offer insights into which functions benefit from microbial help in the wild, hence highlight the role of this bacterial associate in the coral holobiont in the wider context.

Conclusions

Model organisms provide powerful tools for unraveling holobiont complexity. These models can be used to test hypotheses of functional relationships and inter-partner interactions through holobiont manipulation. To complement current cnidarian model systems such as Aiptasia and Hydra, we recently proposed the adoption of *Galaxea fascicularis* as a true coral model owing to its suitability to aquarium rearing and reproduction, and manipulation of its association with Symbiodiniaceae following menthol bleaching (Puntin et al. 2022a). However, how this bleaching treatment affected the bacterial microbiome remained to be explored.

In this study, we provided the first baseline assessment of the response of the Galaxea bacterial microbiome to menthol bleaching, and gain initial insights into the potential effects of long-term captivity in this coral species. OThe overall, response to menthol bleaching induced stochastic changes inwas a destabilization of the microbiome, indicating dysbiosis. However, captivity also affected Tthe response of the bacterial microbiome to bleaching, with differed nces observed between the two facilities, likely reflecting differences in rearing conditions, which remain to be addressed. Bacterial communities of the captive Galaxea colonies were composed of fewer taxa than reported for wild corals, which is in line with decreasing microbial diversity of many captive organisms. Captivity seemingly affected the bacterial microbi Nevertheless, symbiotic polyps originating from ome reducing its complexity, where different colonies maintained distinct community assemblies. This, and showed links to host and/or Symbiodiniaceae identity, which we recommend towarrant further investigatione.

A simplifiedThe observed microbiome simplification may could facilitate both characterization and manipulation of the microbiome, and it could guide the identification of essential ("core") members among the retained associates. In this regard, we identified *Ruegeria* spp. and *Alteromonas* spp. as candidate associates for further functional interrogation. In conclusion, our study contributes valuable information towards a better characterization of the Galaxea holobiont, as well as its continued development and establishment as a coral model system.

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Data, scripts, code, and supplementary information availability

Data are available online:
Raw sequencing data submitted to NCBI SRA BioProject PRJNA947274
Representative sequences (ASVs) submitted to NCBI GenBank Accession numbers OQ677536 to OQ677992
Additional data, scripts, and code are available online: https://doi.org/10.5281/zenodo.10551928 ~~https://doi.org/10.5281/zenodo.7976283~~ (Puntin 2024)

Conflict of interest disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article.

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References

Agostini S, Suzuki Y, Higuchi T, Casareto BE, Yoshinaga K, Nakano Y, Fujimura H (2012) Biological and chemical characteristics of the coral gastric cavity. Coral Reefs 31:147–156 http://link.springer.com/10.1007/s00338-011-0831-6

Ainsworth TD, Krause L, Bridge T, Torda G, Raina J-B, Zakrzewski M, Gates RD, Padilla-Gamiño JL, Spalding HL, Smith C, Woolsey ES, Bourne DG, Bongaerts P, Hoegh-Guldberg O, Leggat W (2015) The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. ISME J 9:2261–2274 https://www.nature.com/articles/ismej201539

Al-Horani FA, Al-Moghrabi SM, de Beer D (2003) Microsensor study of photosynthesis and calcification in the scleractinian coral, *Galaxea fascicularis*: active internal carbon cycle. J Exp Mar Bio Ecol 288:1–15 https://linkinghub.elsevier.com/retrieve/pii/S0022098102005786

Al-Horani FA, Ferdelman T, Al-Moghrabi SM, de Beer D (2005) Spatial distribution of calcification and photosynthesis in the scleractinian coral *Galaxea fascicularis*. Coral Reefs 24:173–180 http://link.springer.com/10.1007/s00338-004-0461-3

Al-Horani FA, Tambutté É, Allemand D (2007) Dark calcification and the daily rhythm of calcification in the scleractinian coral, Galaxea fascicularis. Coral Reefs 26:531–538 http://link.springer.com/10.1007/s00338-007-0250-x

Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyrén P, Engstrand L (2008) Comparative Analysis of Human Gut Microbiota by Barcoded Pyrosequencing. PLoS One 3:e2836 https://dx.plos.org/10.1371/journal.pone.0002836

Apprill A, Weber LG, Santoro AE (2016) Distinguishing between Microbial Habitats Unravels Ecological Complexity in Coral Microbiomes. mSystems 1:e00143-16 http://msystems.asm.org/lookup/doi/10.1128/mSystems.00143-16

Barreto MM, Ziegler M, Venn A, Tambutté E, Zoccola D, Tambutté S, Allemand D, Antony CP, Voolstra CR, Aranda M (2021) Effects of Ocean Acidification on Resident and Active Microbial Communities of Stylophora pistillata. Front Microbiol 12:1–13 https://www.frontiersin.org/articles/10.3389/fmicb.2021.707674/full

Battaglia T (2022) btools: A suite of R function for all types of microbial diversity analyses. https://rdrr.io/github/twbattaglia/btools/

Bayer T, Neave MJ, Alsheikh-Hussain A, Aranda M, Yum LK, Mincer T, Hughen K, Apprill A, Voolstra CR (2013) The Microbiome of the Red Sea Coral Stylophora pistillata Is Dominated by Tissue-Associated Endozoicomonas Bacteria. Appl Environ Microbiol 79:4759–4762 https://journals.asm.org/doi/10.1128/AEM.00695-13

Bernasconi R, Stat M, Koenders A, Huggett MJ (2019) Global Networks of Symbiodinium-Bacteria Within the Coral Holobiont. Microb Ecol 77:794–807 http://link.springer.com/10.1007/s00248-018-1255-4

Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Caporaso JG (2018) Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6:90 https://doi.org/10.1186/s40168-018-0470-z

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang K Bin, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik A V, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857 https://doi.org/10.1038/s41587-019-0209-9

Bourne DG, Morrow KM, Webster NS (2016) Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. Annu Rev Microbiol 70:317–340 http://www.annualreviews.org/doi/10.1146/annurev-micro-102215-095440

Cai L, Tian RM, Zhou G, Tong H, Wong YH, Zhang W, Chui APY, Xie JY, Qiu JW, Ang PO, Liu S, Huang H, Qian PY (2018a) Exploring coral microbiome assemblages in the South China Sea. Sci Rep 8:1–13 http://dx.doi.org/10.1038/s41598-018-20515-w

Cai L, Zhou G, Tong H, Tian R-M, Zhang W, Ding W, Liu S, Huang H, Qian P-Y (2018b) Season structures prokaryotic partners but not algal symbionts in subtropical hard corals. Appl Microbiol Biotechnol 102:4963–4973 http://link.springer.com/10.1007/s00253-018-8909-5

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583 https://www.nature.com/articles/nmeth.3869

Certner RH, Vollmer S V. (2018) Inhibiting bacterial quorum sensing arrests coral disease development and disease-associated microbes. Environ Microbiol 20:645–657 https://onlinelibrary.wiley.com/doi/full/10.1111/1462-2920.13991

Chakravarti LJ, Beltran VH, van Oppen MJH (2017) Rapid thermal adaptation in photosymbionts of reef-building corals. Glob Chang Biol 23:4675–4688 https://onlinelibrary.wiley.com/doi/10.1111/gcb.13702

Chan WY, Rudd D, Meyers L, Topa SH, van Oppen MJH (2023) Heat-evolved algal symbionts enhance bleaching tolerance of adult corals without trade-off against growth. bioRxiv https://www.biorxiv.org/content/10.1101/2023.06.17.545444v1

Connelly MT, McRae CJ, Liu P-J, Martin CE, Traylor-Knowles N (2022) Antibiotics Alter Pocillopora Coral-Symbiodiniaceae-Bacteria Interactions and Cause Microbial Dysbiosis During Heat Stress. Front Mar Sci 8:1–18 https://www.frontiersin.org/articles/10.3389/fmars.2021.814124/full

Conway JR, Lex A, Gehlenborg N (2017) UpSetR: an R package for the visualization of intersecting sets and their properties. Bioinformatics 33:2938–2940 https://doi.org/10.1093/bioinformatics/btx364

Costanza R, de Groot R, Sutton P, van der Ploeg S, Anderson SJ, Kubiszewski I, Farber S, Turner RK (2014) Changes in the global value of ecosystem services. Glob Environ Chang 26:152–158 http://dx.doi.org/10.1016/j.gloenvcha.2014.04.002

Curtis E, Moseley J, Racicot R, Wright RM (2023) Bacterial microbiome variation across symbiotic states and clonal lines in a cnidarian model. Front Mar Sci 10:1–9 https://www.frontiersin.org/articles/10.3389/fmars.2023.1113043/full

Damjanovic K, Blackall LL, Peplow LM, van Oppen MJH (2020) Assessment of bacterial community composition within and among Acropora loripes colonies in the wild and in captivity. Coral Reefs 39:1245–1255 https://link.springer.com/10.1007/s00338-020-01958-y

Dani V, Priouzeau F, Pagnotta S, Carette D, Laugier JP, Sabourault C (2016) Thermal and menthol stress induce different cellular events during sea anemone bleaching. Symbiosis 69:175–192 http://dx.doi.org/10.1007/s13199-016-0406-y

Dixon P (2003) VEGAN, a package of R functions for community ecology. J Veg Sci 14:927–930 https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1654-1103.2003.tb02228.x

Dubé CE, Ziegler M, Mercière A, Boissin E, Planes S, Bourmaud CA-F, Voolstra CR (2021) Naturally occurring fire coral clones demonstrate a genetic and environmental basis of microbiome composition. Nat Commun 12:6402 https://www.nature.com/articles/s41467-021-26543-x

Dungan AM, Bulach D, Lin H, van Oppen MJH, Blackall LL (2021a) Development of a free radical scavenging bacterial consortium to mitigate oxidative stress in cnidarians. Microb Biotechnol 14:2025–2040 https://onlinelibrary.wiley.com/doi/10.1111/1751-7915.13877

Dungan AM, van Oppen MJH, Blackall LL (2021b) Short-Term Exposure to Sterile Seawater Reduces Bacterial Community Diversity in the Sea Anemone, Exaiptasia diaphana. Front Mar Sci 7:1–10 https://www.frontiersin.org/articles/10.3389/fmars.2020.599314/full

Eakin CM, Devotta D, Heron S, Geiger E, De La Cour J, Gomez A, Skirving W, Baird A, Cantin N, Donner S, Gilmour J (2022) The 2014-17 Global Coral Bleaching Event: The Most Severe and Widespread Coral Reef Destruction. Res Sq 1–24 https://www.researchsquare.com/article/rs-1555992/v1

Eddy TD, Lam VWY, Reygondeau G, Cisneros-Montemayor AM, Greer K, Palomares MLD, Bruno JF, Ota Y, Cheung WWL (2021) Global decline in capacity of coral reefs to provide ecosystem services. One Earth 4:1278–1285 https://linkinghub.elsevier.com/retrieve/pii/S2590332221004747

Ferrier-Pagès C, Gattuso J, Cauwet G, Jaubert J, Allemand D (1998) Release of dissolved organic carbon and nitrogen by the zooxanthellate coral Galaxea fascicularis. Mar Ecol Prog Ser 172:265–274 http://www.int-res.com/abstracts/meps/v172/p265-274/

Fraune S, Anton-Erxleben F, Augustin R, Franzenburg S, Knop M, Schröder K, Willoweit-Ohl D, Bosch TCG (2015) Bacteria–bacteria interactions within the microbiota of the ancestral metazoan *Hydra* contribute to fungal resistance. ISME J 9:1543–1556 http://www.nature.com/articles/ismej2014239

Gabay Y, Parkinson JE, Wilkinson SP, Weis VM, Davy SK (2019) Inter-partner specificity limits the acquisition of thermotolerant symbionts in a model cnidarian-dinoflagellate symbiosis. ISME J 13:2489–2499 https://doi.org/10.1038/s41396-019-0429-5

Gabay Y, Weis VM, Davy SK (2018) Symbiont Identity Influences Patterns of Symbiosis Establishment, Host Growth, and Asexual Reproduction in a Model Cnidarian-Dinoflagellate Symbiosis. Biol Bull 234:1–10 http://www.journals.uchicago.edu/doi/10.1086/696365

Galand PE, Ruscheweyh H-J, Salazar G, Hochart C, Henry N, Hume BCC, Oliveira PH, Perdereau A, Labadie K, Belser C, Boissin E, Romac S, Poulain J, Bourdin G, Iwankow G, Moulin C, Armstrong EJ, Paz-García DA, Ziegler M, Agostini S, Banaigs B, Boss E, Bowler C, de Vargas C, Douville E, Flores M, Forcioli D, Furla P, Gilson E, Lombard F, Pesant S, Reynaud S, Thomas OP, Troublé R, Zoccola D, Voolstra CR, Thurber RV, Sunagawa S, Wincker P, Allemand D, Planes S (2023) Diversity of the Pacific Ocean coral reef microbiome. Nat Commun 14:3039 https://www.nature.com/articles/s41467-023-38500-x

Galliot B (2012) *Hydra*, a fruitful model system for 270 years. Int J Dev Biol 56:411–423 http://www.intjdevbiol.com/paper.php?doi=120086bg

Goulet TL, Erill I, Ascunce MS, Finley SJ, Javan GT (2020) Conceptualization of the Holobiont Paradigm as It Pertains to Corals. Front Physiol 11:1–8 https://www.frontiersin.org/article/10.3389/fphys.2020.566968/full

Hartman LM, van Oppen MJH, Blackall LL (2020) Microbiota characterization of Exaiptasia diaphana from the Great Barrier Reef. Anim Microbiome 2:10 https://animalmicrobiome.biomedcentral.com/articles/10.1186/s42523-020-00029-5

Hernandez-Agreda A, Leggat W, Bongaerts P, Herrera C, Ainsworth TD (2018) Rethinking the Coral Microbiome: Simplicity Exists within a Diverse Microbial Biosphere. MBio 9:1–14 https://journals.asm.org/doi/10.1128/mBio.00812-18

Herrera M, Klein SG, Schmidt-Roach S, Campana S, Cziesielski MJ, Chen JE, Duarte CM, Aranda M (2020) Unfamiliar partnerships limit cnidarian holobiont acclimation to warming. Glob Chang Biol 26:5539–5553 https://onlinelibrary.wiley.com/doi/10.1111/gcb.15263

van Hooidonk R, Maynard J, Tamelander J, Gove J, Ahmadia G, Raymundo L, Williams G, Heron SF, Planes S (2016) Local-scale projections of coral reef futures and implications of the Paris Agreement. Sci Rep 6:39666 http://www.nature.com/articles/srep39666

Huggett MJ, Apprill A (2019) Coral microbiome database: Integration of sequences reveals high diversity and relatedness of coral-associated microbes. Environ Microbiol Rep 11:372–385 https://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12686

Hughes TP, Barnes ML, Bellwood DR, Cinner JE, Cumming GS, Jackson JBC, Kleypas J, van de Leemput IA, Lough JM, Morrison TH, Palumbi SR, van Nes EH, Scheffer M (2017) Coral reefs in the Anthropocene. Nature 546:82–90 https://www.nature.com/articles/nature22901

Hume BCC, Smith EG, Ziegler M, Warrington HJM, Burt JA, LaJeunesse TC, Wiedenmann J, Voolstra CR (2019) SymPortal: A novel analytical framework and platform for coral algal symbiont next-generation sequencing ITS2 profiling. Mol Ecol Resour 19:1063–1080 https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13004

Hume BCC, Ziegler M, Poulain J, Pochon X, Romac S, Boissin E, de Vargas C, Planes S, Wincker P, Voolstra CR (2018) An improved primer set and amplification protocol with increased specificity and sensitivity targeting the *Symbiodinium* ITS2 region. PeerJ 6:e4816 https://peerj.com/articles/4816

Ide K, Nishikawa Y, Maruyama T, Tsukada Y, Kogawa M, Takeda H, Ito H, Wagatsuma R, Miyaoka R, Nakano Y, Kinjo K, Ito M, Hosokawa M, Yura K, Suda S, Takeyama H (2022) Targeted single-cell genomics reveals novel host adaptation strategies of the symbiotic bacteria Endozoicomonas in Acropora tenuis coral. Microbiome 10:1–16 https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-022-01395-9

Jaspers C, Fraune S, Arnold AE, Miller DJ, Bosch TCG, Voolstra CR (2019) Resolving structure and function of metaorganisms through a holistic framework combining reductionist and integrative approaches. Zoology 133:81–87 https://doi.org/10.1016/j.zool.2019.02.007

Kaehler BD, Bokulich N, McDonald D, Knight R, Caporaso JG, Huttley GA (2019) Species abundance information improves sequence taxonomy classification accuracy. Nat Commun 10:

Kitamura R, Miura N, Ito M, Takagi T, Yamashiro H, Nishikawa Y, Nishimura Y, Kobayashi K, Kataoka M (2021) Specific Detection of Coral-Associated Ruegeria, a Potential Probiotic Bacterium, in Corals and Subtropical Seawater. Mar Biotechnol 23:576–589 https://doi.org/10.1007/s10126-021-10047-2

Kooperman N, Ben-Dov E, Kramarsky-Winter E, Barak Z, Kushmaro A (2007) Coral mucus-associated bacterial communities from natural and aquarium environments. FEMS Microbiol Lett 276:106–113 https://academic.oup.com/femsle/article-lookup/doi/10.1111/j.1574-6968.2007.00921.x

Lawson CA, Raina J, Kahlke T, Seymour JR, Suggett DJ (2018) Defining the core microbiome of the symbiotic dinoflagellate, Symbiodinium. Environ Microbiol Rep 10:7–11 https://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12599

Lex A, Gehlenborg N, Strobelt H, Vuillemot R, Pfister H (2014) UpSet: Visualization of Intersecting Sets. IEEE Trans Vis Comput Graph 20:1983–1992 http://ieeexplore.ieee.org/document/6876017/

Li J, Chen Q, Zhang S, Huang H, Yang J, Tian X-P, Long L-J (2013) Highly Heterogeneous Bacterial Communities Associated with the South China Sea Reef Corals Porites lutea, Galaxea fascicularis and Acropora millepora. PLoS One 8:e71301 https://dx.plos.org/10.1371/journal.pone.0071301

Lin M-F, Takahashi S, Forêt S, Davy SK, Miller DJ (2019) Transcriptomic analyses highlight the likely metabolic consequences of colonization of a cnidarian host by native or non-native Symbiodinium species. Biol Open 8:1–11 https://journals.biologists.com/bio/article/doi/10.1242/bio.038281/259291/Transcriptomic-analyses-highlight-the-likely

Littman RA, Bourne DG, Willis BL (2010) Responses of coral-associated bacterial communities to heat stress differ with Symbiodinium type on the same coral host. Mol Ecol 19:1978–1990 https://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2010.04620.x

Matthews JL, Raina J, Kahlke T, Seymour JR, Oppen MJH, Suggett DJ (2020) Symbiodiniaceae-bacteria interactions: rethinking metabolite exchange in reef-building corals as multi-partner metabolic networks. Environ Microbiol 22:1675–1687 https://onlinelibrary.wiley.com/doi/10.1111/1462-2920.14918

Matthews JL, Sproles AE, Oakley CA, Grossman AR, Weis VM, Davy SK (2015) Menthol-induced bleaching rapidly and effectively provides experimental aposymbiotic sea anemones (*Aiptasia* sp.) for symbiosis investigations. J Exp Biol 219:306–310 http://jeb.biologists.org/cgi/doi/10.1242/jeb.128934

McMurdie PJ, Holmes S (2013) phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8:e61217 http://dx.plos.org/10.1371/journal.pone.0061217

Mikryukov V (2023) metagMisc: Miscellaneous functions for metagenomic analysis. https://rdrr.io/github/vmikk/metagMisc/

Miura N, Motone K, Takagi T, Aburaya S, Watanabe S, Aoki W, Ueda M (2019) Ruegeria sp. Strains Isolated from the Reef-Building Coral Galaxea fascicularis Inhibit Growth of the Temperature-Dependent Pathogen Vibrio coralliilyticus. Mar Biotechnol 21:1–8 http://link.springer.com/10.1007/s10126-018-9853-1

Morrow KM, Moss AG, Chadwick NE, Liles MR (2012) Bacterial Associates of Two Caribbean Coral Species Reveal Species-Specific Distribution and Geographic Variability. Appl Environ Microbiol 78:6438–6449 https://journals.asm.org/doi/10.1128/AEM.01162-12

Morrow KM, Pankey MS, Lesser MP (2022) Community structure of coral microbiomes is dependent on host morphology. Microbiome 10:113 https://doi.org/10.1186/s40168-022-01308-w

Motone K, Takagi T, Aburaya S, Miura N, Aoki W, Ueda M (2020) A Zeaxanthin-Producing Bacterium Isolated from the Algal Phycosphere Protects Coral Endosymbionts from Environmental Stress. MBio 11:1–13 https://journals.asm.org/doi/10.1128/mBio.01019-19

Murillo-Rincon AP, Klimovich A, Pemöller E, Taubenheim J, Mortzfeld B, Augustin R, Bosch TCG (2017) Spontaneous body contractions are modulated by the microbiome of Hydra. Sci Rep 7:15937 https://www.nature.com/articles/s41598-017-16191-x

Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. Ecosyst world 25:75–87

Muscatine L, Porter JW (1977) Reef Corals: Mutualistic Symbioses Adapted to Nutrient-Poor Environments. Bioscience 27:454–460 https://academic.oup.com/bioscience/article-lookup/doi/10.2307/1297526

Neave MJ, Apprill A, Ferrier-Pagès C, Voolstra CR (2016) Diversity and function of prevalent symbiotic marine bacteria in the genus Endozoicomonas. Appl Microbiol Biotechnol 100:8315–8324 http://dx.doi.org/10.1007/s00253-016-7777-0

Neave MJ, Rachmawati R, Xun L, Michell CT, Bourne DG, Apprill A, Voolstra CR (2017) Differential specificity between closely related corals and abundant Endozoicomonas endosymbionts across global scales. ISME J 11:186–200 http://dx.doi.org/10.1038/ismej.2016.95

Nitschke MR, Fidalgo C, Simões J, Brandão C, Alves A, Serôdio J, Frommlet JC (2020) Symbiolite formation: a powerful in vitro model to untangle the role of bacterial communities in the photosynthesis-induced formation of microbialites. ISME J 14:1533–1546 https://www.nature.com/articles/s41396-020-0629-z

Niu W, Huang H, Lin R, Chen C-H, Shen K-N, Hsiao C-D (2016) The complete mitogenome of the Galaxy Coral, *Galaxea fascicularis* (Cnidaria: Oculinidae). Mitochondrial DNA Part B 1:10–11 https://www.tandfonline.com/doi/full/10.1080/23802359.2015.1137796

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2020) vegan: Community Ecology Package. https://cran.r-project.org/package=vegan

Peixoto RS, Sweet M, Villela HDM, Cardoso P, Thomas T, Voolstra CR, Høj L, Bourne DG (2021) Coral Probiotics: Premise, Promise, Prospects. Annu Rev Anim Biosci 9:265–288 https://www.annualreviews.org/doi/10.1146/annurev-animal-090120-115444

Pietschke C, Treitz C, Forêt S, Schultze A, Künzel S, Tholey A, Bosch TCG, Fraune S (2017) Host modification of a bacterial quorum-sensing signal induces a phenotypic switch in bacterial symbionts. Proc Natl Acad Sci 114:E8488–E8497 https://pnas.org/doi/full/10.1073/pnas.1706879114

Pogoreutz C, Oakley CA, Rädecker N, Cárdenas A, Perna G, Xiang N, Peng L, Davy SK, Ngugi DK, Voolstra CR (2022) Coral holobiont cues prime Endozoicomonas for a symbiotic lifestyle. ISME J 16:1883–1895 https://www.nature.com/articles/s41396-022-01226-7

Pogoreutz C, Voolstra CR, Rädecker N, Weis V (2020) The coral holobiont highlights the dependence of cnidarian animal hosts on their associated microbes. Cellular dialogues in the holobiont. pp 91–118

Pollock FJ, McMinds R, Smith S, Bourne DG, Willis BL, Medina M, Thurber RV, Zaneveld JR (2018) Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. Nat Commun 9:4921 http://www.nature.com/articles/s41467-018-07275-x

Pratte ZA, Richardson LL, Mills DK (2015) Microbiota shifts in the surface mucopolysaccharide layer of corals transferred from natural to aquaria settings. J Invertebr Pathol 125: http://dx.doi.org/10.1016/j.jip.2014.12.009

Puntin G (2024) sPuntinG/Galaxea_Microbiome_PCI: Galaxea microbiome analysis 1st peer-review. https://doi.org/10.5281/zenodo.10551928

Puntin G, Craggs J, Hayden R, Engelhardt KE, McIlroy S, Sweet M, Baker DM, Ziegler M (2022a) The reef-building coral Galaxea fascicularis: a new model system for coral symbiosis research. Coral Reefs 1–33 https://doi.org/10.1101/2022.06.02.494472

Puntin G, Sweet M, Fraune S, Medina M, Sharp K, Weis VM, Ziegler M (2022b) Harnessing the Power of Model Organisms To Unravel Microbial Functions in the Coral Holobiont. Microbiol Mol Biol Rev https://journals.asm.org/doi/10.1128/mmbr.00053-22

Putnam HM, Barott KL, Ainsworth TD, Gates RD (2017) The Vulnerability and Resilience of Reef-Building Corals. Curr Biol 27:R528–R540 http://dx.doi.org/10.1016/j.cub.2017.04.047

R Core Team (2021) R: A Language and Environment for Statistical Computing. https://www.r-project.org/ https://www.r-project.org/

Raina J-B, Clode PL, Cheong S, Bougoure J, Kilburn MR, Reeder A, Forêt S, Stat M, Beltran V, Thomas-Hall P, Tapiolas D, Motti CM, Gong B, Pernice M, Marjo CE, Seymour JR, Willis BL, Bourne DG (2017) Subcellular tracking reveals the location of dimethylsulfoniopropionate in microalgae and visualises its uptake by marine bacteria. Elife 6:1–17 https://elifesciences.org/articles/23008

Raina J-B, Tapiolas D, Motti CA, Foret S, Seemann T, Tebben J, Willis BL, Bourne DG (2016) Isolation of an antimicrobial compound produced by bacteria associated with reef-building corals. PeerJ 4:e2275 https://peerj.com/articles/2275

Raina J-B, Tapiolas D, Willis BL, Bourne DG (2009) Coral-Associated Bacteria and Their Role in the Biogeochemical Cycling of Sulfur. Appl Environ Microbiol 75:3492–3501 https://journals.asm.org/doi/10.1128/AEM.02567-08

Robbins SJ, Singleton CM, Chan CX, Messer LF, Geers AU, Ying H, Baker A, Bell SC, Morrow KM, Ragan MA, Miller DJ, Forêt S, Ball E, Beeden R, Berumen M, Aranda M, Ravasi T, Bongaerts P, Hoegh-Guldberg O, Cooke I, Leggat B, Sprungala S, Fitzgerald A, Shang C, Lundgren P, Fyffe T, Rubino F, van Oppen M, Weynberg K, Robbins SJ, Singleton CM, Xin Chan C, Messer LF, Geers AU, Ying H, Baker A, Bell SC, Morrow KM, Ragan MA, Miller DJ, Foret S, Voolstra CR, Tyson GW, Bourne DG, Voolstra CR, Tyson GW, Bourne DG (2019) A genomic view of the reef-building coral *Porites lutea* and its microbial symbionts. Nat Microbiol 4:2090–2100 https://www.nature.com/articles/s41564-019-0532-4

Robeson MS, O\textquoterightRourke DR, Kaehler BD, Ziemski M, Dillon MR, Foster JT, Bokulich NA (2020) RESCRIPt: Reproducible sequence taxonomy reference database management for the masses. bioRxiv https://www.biorxiv.org/content/early/2020/10/05/2020.10.05.326504

Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser 243:1–10 http://www.int-res.com/abstracts/meps/v243/p1-10/

Rosado PM, Leite DCA, Duarte GAS, Chaloub RM, Jospin G, Nunes da Rocha U, P. Saraiva J, Dini-Andreote F, Eisen JA, Bourne DG, Peixoto RS (2019) Marine probiotics: increasing coral resistance to bleaching through microbiome manipulation. ISME J 13:921–936 http://www.nature.com/articles/s41396-018-0323-6

Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev Microbiol 5:355–362 https://www.nature.com/articles/nrmicro1635

Röthig T, Costa RM, Simona F, Baumgarten S, Torres AF, Radhakrishnan A, Aranda M, Voolstra CR (2016) Distinct bacterial communities associated with the coral model *Aiptasia* in aposymbiotic and symbiotic states with *Symbiodinium*. Front Mar Sci 3: http://journal.frontiersin.org/article/10.3389/fmars.2016.00234/full

Röthig T, Puntin G, Wong JCY, Burian A, McLeod W, Baker DM (2021) Holobiont nitrogen control and its potential for eutrophication resistance in an obligate photosymbiotic jellyfish. Microbiome 9:127 https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-021-01075-0

Scharfenstein HJ, Chan WY, Buerger P, Humphrey C, van Oppen MJH (2022) Evidence for de novo acquisition of microalgal symbionts by bleached adult corals. ISME J 16:1676–1679 https://www.nature.com/articles/s41396-022-01203-0

Schubert P, Wilke T (2018) Coral Microcosms: Challenges and Opportunities for Global Change Biology. Corals in a Changing World. InTech, pp 143–175 http://www.intechopen.com/books/corals-in-a-changing-world/coral-microcosms-challenges-and-opportunities-for-global-change-biology

Sunagawa S, DeSantis TZ, Piceno YM, Brodie EL, DeSalvo MK, Voolstra CR, Weil E, Andersen GL, Medina M (2009) Bacterial diversity and White Plague Disease-associated community changes in the Caribbean coral Montastraea faveolata. ISME J 3:512–521 http://www.nature.com/articles/ismej2008131

Sweet MJ, Croquer A, Bythell JC (2011) Bacterial assemblages differ between compartments within the coral holobiont. Coral Reefs 30:39–52 http://link.springer.com/10.1007/s00338-010-0695-1

Tandon K, Lu C-Y, Chiang P-W, Wada N, Yang S-H, Chan Y-F, Chen P-Y, Chang H-Y, Chiou Y-J, Chou M-S, Chen W-M, Tang S-L (2020) Comparative genomics: Dominant coral-bacterium Endozoicomonas acroporae metabolizes dimethylsulfoniopropionate (DMSP). ISME J 14:1290–1303 http://dx.doi.org/10.1038/s41396-020-0610-x

Tang K, Zhan W, Zhou Y, Xu T, Chen X, Wang W, Zeng Z, Wang Y, Wang X (2020) Antagonism between coral pathogen Vibrio coralliilyticus and other bacteria in the gastric cavity of scleractinian coral Galaxea fascicularis. Sci China Earth Sci 63:157–166

Taubenheim J, Willoweit-Ohl D, Knop M, Franzenburg S, He J, Bosch TCG, Fraune S (2020) Bacteria- and temperature-regulated peptides modulate β-catenin signaling in *Hydra*. Proc Natl Acad Sci U S A 117:21459–21468 www.pnas.org/cgi/doi/10.1073/pnas.2010945117

Tebben J, Motti CA, Siboni N, Tapiolas DM, Negri AP, Schupp PJ, Kitamura M, Hatta M, Steinberg PD, Harder T (2015) Chemical mediation of coral larval settlement by crustose coralline algae. Sci Rep 5:10803 http://www.nature.com/articles/srep10803

Thompson JR, Rivera HE, Closek CJ, Medina M (2015) Microbes in the coral holobiont: partners through evolution, development, and ecological interactions. Front Cell Infect Microbiol 4: http://journal.frontiersin.org/article/10.3389/fcimb.2014.00176/abstract

Vega Thurber R, Willner-Hall D, Rodriguez-Mueller B, Desnues C, Edwards RA, Angly F, Dinsdale E, Kelly L, Rohwer F (2009) Metagenomic analysis of stressed coral holobionts. Environ Microbiol 11:2148–2163

Wang J-T, Chen Y-Y, Tew KS, Meng P-J, Chen CA (2012) Physiological and biochemical performances of menthol-induced aposymbiotic corals. PLoS One 7:e46406 https://dx.plos.org/10.1371/journal.pone.0046406

Wang JT, Wang YT, Keshavmurthy S, Meng PJ, Chen CA (2019) The coral *Platygyra verweyi* exhibits local adaptation to long-term thermal stress through host-specific physiological and enzymatic response. Sci Rep 9:1–11 http://dx.doi.org/10.1038/s41598-019-49594-z

Webster NS, Smith LD, Heyward AJ, Watts JEM, Webb RI, Blackall LL, Negri AP (2004) Metamorphosis of a Scleractinian Coral in Response to Microbial Biofilms. Appl Environ Microbiol 70:1213–1221

Weis VM (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. J Exp Biol 211:3059–66 http://jeb.biologists.org/content/211/19/3059.abstract

Weis VM, Davy SK, Hoegh-Guldberg O, Rodriguez-Lanetty M, Pringle JR (2008) Cell biology in model systems as the key to understanding corals. Trends Ecol Evol 23:369–376 https://linkinghub.elsevier.com/retrieve/pii/S0169534708001614

Wickham H (2016) Ggplot2: Elegant graphics for data analysis. Springer International Publishing, Cham, Switzerland https://books.google.de/books?id=RTMFswEACAAJ

Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J, Ludwig W, Glöckner FO (2013) The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Res 42:D643–D648 https://doi.org/10.1093/nar/gkt1209

Zaneveld JR, McMinds R, Vega Thurber R (2017) Stress and stability: applying the Anna Karenina principle to animal microbiomes. Nat Microbiol 2:17121 http://www.nature.com/articles/nmicrobiol2017121

Zhou Y, Tang K, Wang P, Wang W, Wang Y, Wang X (2020) Identification of bacteria-derived urease in the coral gastric cavity. Sci China Earth Sci 63:1553–1563 https://link.springer.com/10.1007/s11430-020-9647-x

Zhu W, Zhu M, Liu X, Xia J, Wang H, Chen R, Li X (2022) Adaptive changes of coral Galaxea fascicularis holobiont in response to nearshore stress. Front Microbiol 13:1–15 https://www.frontiersin.org/articles/10.3389/fmicb.2022.1052776/full

Ziegler M, Roder C, Büchel C, Voolstra C (2015) Niche acclimatization in Red Sea corals is dependent on flexibility of host-symbiont association. Mar Ecol Prog Ser 533:149–161 http://www.int-res.com/abstracts/meps/v533/p149-161/

Ziegler M, Roik A, Porter A, Zubier K, Mudarris MS, Ormond R, Voolstra CR (2016) Coral microbial community dynamics in response to anthropogenic impacts near a major city in the central Red Sea. Mar Pollut Bull 105:629–640 https://linkinghub.elsevier.com/retrieve/pii/S0025326X15302496

The bacterial microbiome of symbiotic and menthol-bleached polyps of *Galaxea fascicularis* in captivity

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Supplementary Figures

Figure S1

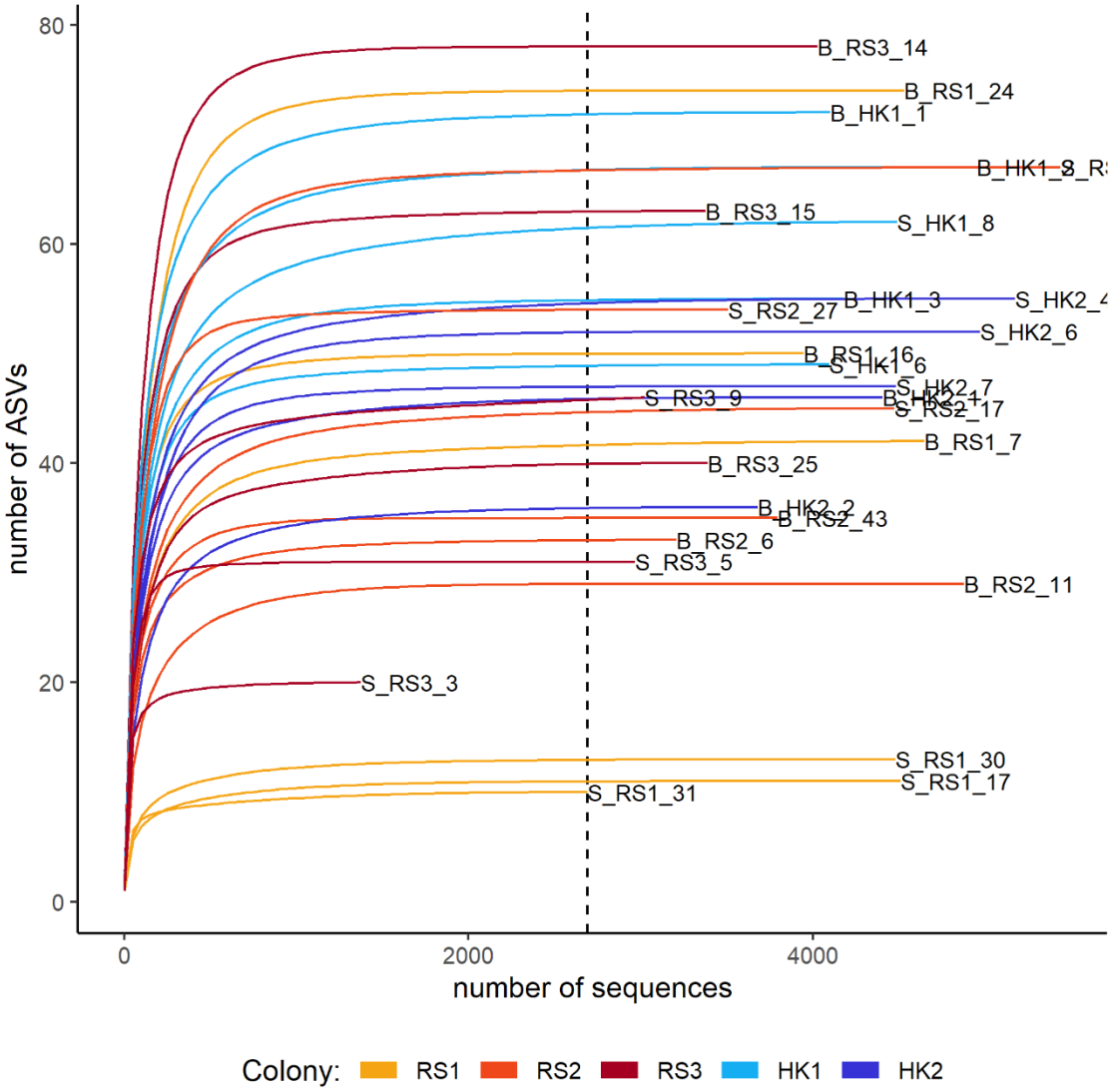

Figure S1 - Rarefaction curve. Dashed line indicates rarefaction depth applied (2,690).

Figure S2

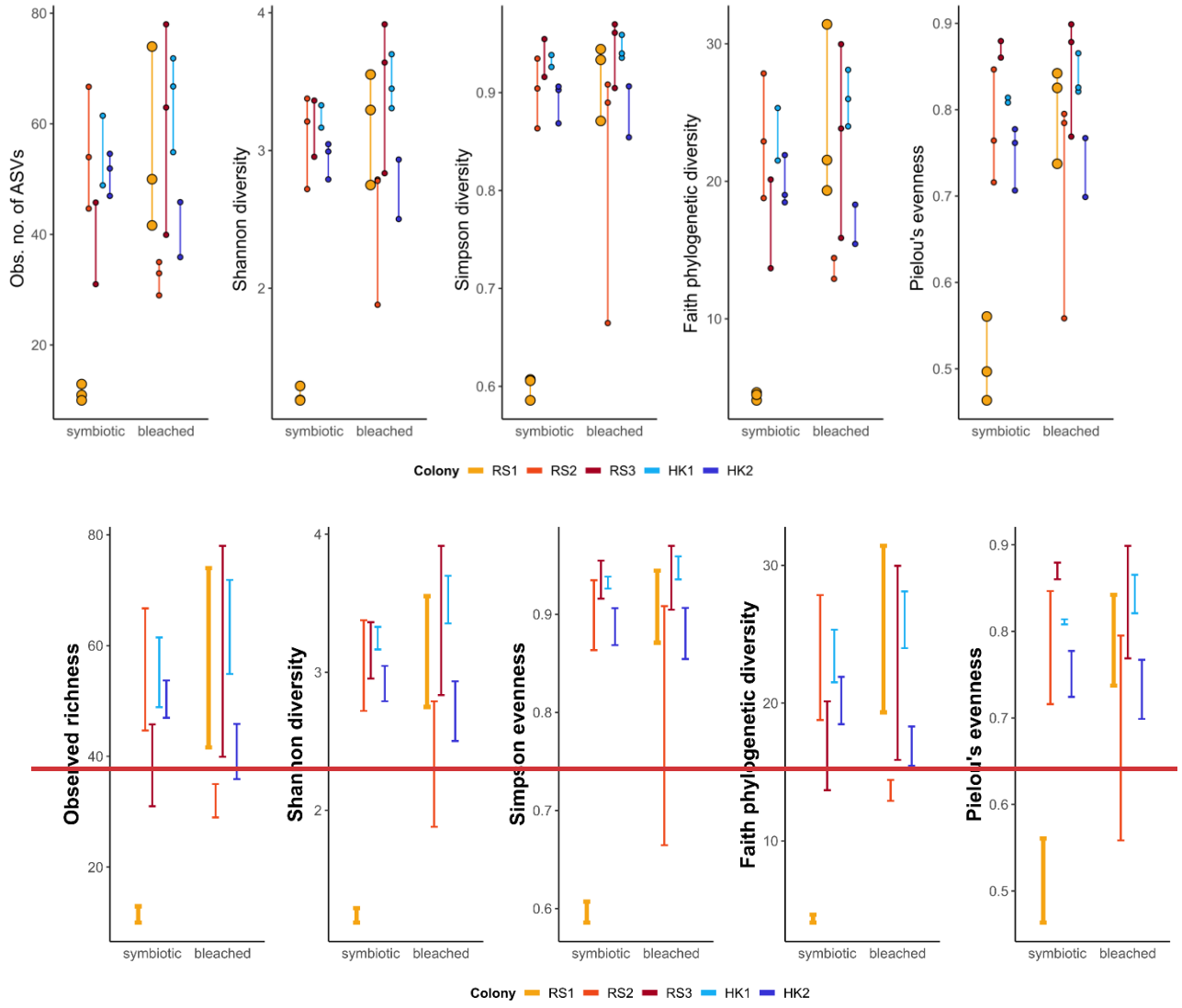

Figure S2 - Comparison of microbial diversity and richness between symbiotic and bleached colonies, displayed as raw data points and range~~95 % confidence interval~~s. Differences between symbiotic and bleached polyps could only be tested for RS1 and RS2 due to inadequate replication in the remaining colonies (results in Tab. S3). The difference between symbiotic states is significant for all tested indices in colony ~~Lack of overlap between intervals indicates significant difference ($p < 0.05$), which occurs in colony RS1 across all alpha diversity metrics.~~ RS1, which ~~intervals are~~is highlighted here with larger ~~line~~ points size for ease of identification.

Figure S3

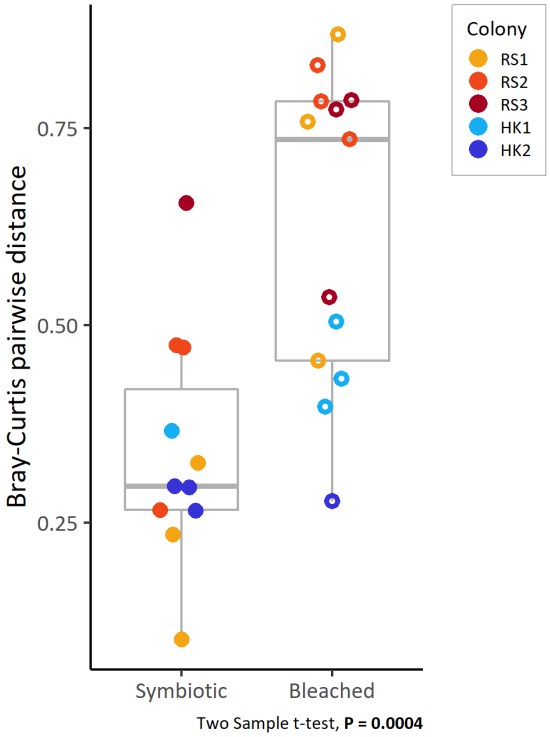

Figure S3 - Comparison of (within group) dissimilarity between symbiotic and bleached samples. Dissimilarity as pairwise distance (Bray-Curtis) between samples of the same colony, by symbiotic state. All groups considered (i.e., with *n* = 2 and *n* = 3).

Figure S4

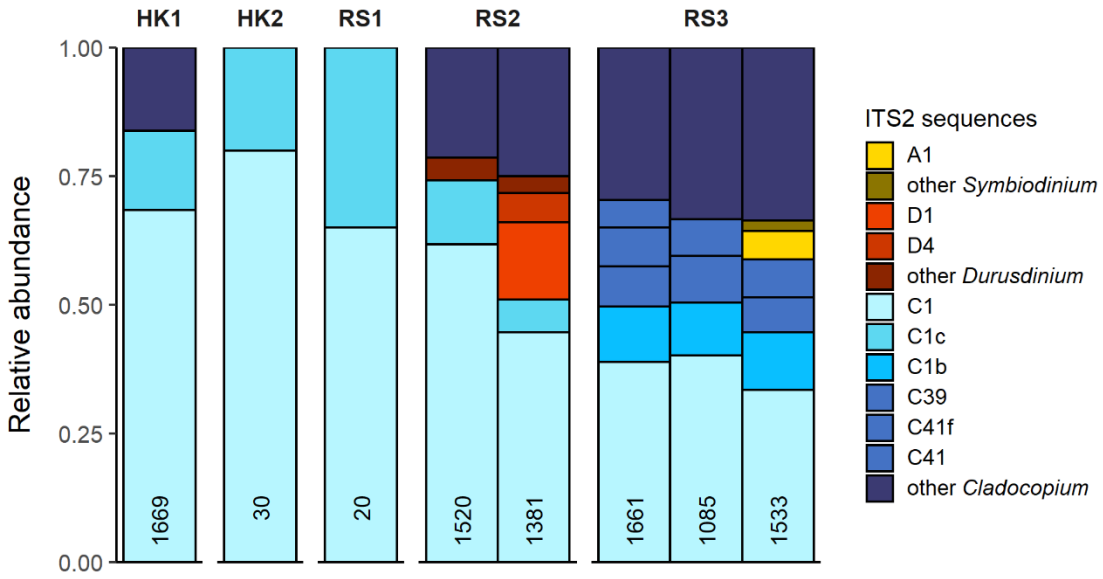

Figure S4 - Relative abundance of Symbiodiniaceae ITS2 sequences by individual symbiotic sample. Based on SymPortal (post-MED) ITS2 output. Sequences with relative abundances < 5 % (by sample) are grouped per genus as "other *". The total number of sequences per sample is indicated at the base of each column.

Figure S5

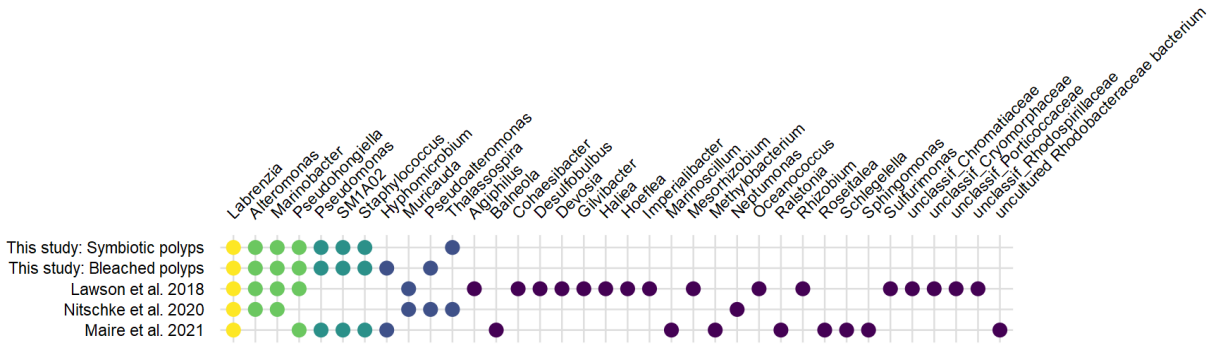

Figure S5 - Comparison between our findings ("This study") and other studies on Symbiodiniaceae-associated bacteria displayed as presence/absence of bacteria genera. Genera from this study that had no match with any of the other studies are omitted. Points are color-coded by number of intersections (co-occurrences). For details regarding the three studies considered, with specification of the respective data, please refer to the Supplementary Materials and Methods section.

Supplementary Tables

Table S1

Table S1 - Statistical testing of difference in community diversity and evenness between symbiotic (n = 13) and bleached (n = 14) colonies. The choice of the statistical test followed data inspection, where Shannon diversity, Simpson evenness, and Pielou's evenness showed non-normal distribution (qq-plots), while Simpson evenness also showed unequal variance (F-test, Bartlett test).

Alpha diversity metric	Test	Statistic	P
Observed richness	unpaired 2-samples t-test	1.417457	0.0843
Shannon diversity	unpaired 2-samples t-test	1.582024	0.0631
Simpson evenness	Welch t-test	1.45282	0.0816
Faith phylogenetic diversity	unpaired 2-samples t-test	1.446646	0.0802
Pielou's evenness	unpaired 2-samples t-test	1.44496	0.0804
Chao1	unpaired 2-samples t-test	1.396447	0.0874
Shannon diversity	Mann-Whitney U-test	113	0.1510
Simpson evenness	Mann-Whitney U-test	123	0.0639
Pielou's evenness	Mann-Whitney U-test	117	0.1100

Table S2

Table 2 - Summary of alpha diversity values by colony and symbiotic state.

State	Origin	Colony	n	Observed	Chao1	Shannon	Simpson	Pielou	Faith_PD
Symbiotic	Red Sea	RS1	3	11.301	11.302	1.222	0.600	0.507	4.394
Symbiotic	Red Sea	RS2	3	55.119	55.268	3.102	0.901	0.776	23.172
Symbiotic	Red Sea	RS3	2	38.386	38.758	3.159	0.935	0.870	16.902
Symbiotic	Hong Kong	HK1	2	55.174	55.519	3.247	0.932	0.811	23.419
Symbiotic	Hong Kong	HK2	3	51.158	51.310	2.943	0.892	0.748	19.793
Bleached	Red Sea	RS1	3	55.195	55.279	3.199	0.916	0.802	24.095
Bleached	Red Sea	RS2	3	32.320	32.322	2.483	0.821	0.713	13.914
Bleached	Red Sea	RS3	3	60.292	60.327	3.463	0.945	0.849	23.227
Bleached	Hong Kong	HK1	3	64.491	64.623	3.485	0.945	0.837	26.028
Bleached	Hong Kong	HK2	2	40.869	40.967	2.718	0.880	0.733	16.872

Table S3

Table S3 - Statistical testing of difference in community diversity and evenness between symbiotic (group2, n2) and bleached (group1, n1) polyps using the unequal variances unpaired t-test (Welch's test). Testing was limited to colonies RS1 and RS2 due to insufficient replication in the remaining colonies (n1 or n2 < 3).

Colony	Alpha diversity index	Group1	Group2	n1	n2	Statistic	df	P	
RS1	Observed	bleached	symbiotic	3	3	4.51	2.03	0.0445	*
RS1	Shannon	bleached	symbiotic	3	3	8.27	2.08	0.0127	*
RS1	Simpson	bleached	symbiotic	3	3	13.27	2.36	0.0028	**
RS1	Pielou	bleached	symbiotic	3	3	6.82	3.93	0.0026	**
RS1	Faith_PD	bleached	symbiotic	3	3	5.30	2.01	0.0335	*
RS2	Observed	bleached	symbiotic	3	3	-3.44	2.30	0.0613	
RS2	Shannon	bleached	symbiotic	3	3	-1.72	3.45	0.1720	
RS2	Simpson	bleached	symbiotic	3	3	-0.99	2.28	0.4170	
RS2	Pielou	bleached	symbiotic	3	3	-0.73	2.92	0.5200	
RS2	Faith_PD	bleached	symbiotic	3	3	-3.47	2.15	0.0668	

Table S~~3~~4

Table S~~3~~4 - Summary of taxonomy, abundance, and prevalence across the data set for the 28 core ASVs that occur across all groups (*state* × *origin*). 'Overall' (n = 28), 'Symbiotic' (n = 14), 'Bleached' (n = 14). Taxonomy is reported at the genus level unless otherwise indicated in parenthesis ("C" = Class, "F" = Family). Blocks indicate the 70 % and 60 % cut-offs of overall abundance across samples. ASV ids are ordered by their abundance across the whole data set. Note that the eight most abundant ASVs reported in this table are also the eight most abundant overall (regardless of which *state* × *origin* groups are considered). For the full table (515 ASVs, non-rarefied data) see: ~~https://doi.org/10.5281/zenodo.7976283~~https://doi.org/10.5281/zenodo.10551928 (~/out/Gfas_16S/core_mb/nonrarefied/ASV_occurrence_summary_all.csv)

Taxonomy (lowest taxonomic level when above genus)	ASV id	No. of samples found in			% of samples found in			abundance (reads count)			abundance (% of all reads)		
		Overall	Symbiotic	Bleached	Overall	Symbiotic	Bleached	Overall	Symbiotic	Bleached	Overall	Symbiotic	Bleached
Alteromonas	ASV_003	25	12	13	89.3	85.7	92.9	9281	5989	3292	8.2	10.8	5.8
Ruegeria	ASV_001	22	11	11	78.6	78.6	78.6	13836	8117	5719	12.3	14.6	10.0
Alteromonas	ASV_008	20	8	12	71.4	57.1	85.7	2838	1365	1473	2.5	2.5	2.6
Nautella	ASV_005	19	9	10	67.9	64.3	71.4	3883	2888	995	3.4	5.2	1.7
Ruegeria	ASV_006	19	9	10	67.9	64.3	71.4	3523	1668	1855	3.1	3.0	3.2
Gammaproteobacteria (C)	ASV_017	18	8	10	64.3	57.1	71.4	1130	503	627	1.0	0.9	1.1
Cryomorphaceae (F)	ASV_018	17	8	9	60.7	57.1	64.3	1114	659	455	1.0	1.2	0.8
Hyphomonadaceae (F)	ASV_020	17	6	11	60.7	42.9	78.6	951	174	777	0.8	0.3	1.4
Micavibrionaceae (F)	ASV_016	16	9	7	57.1	64.3	50.0	1150	638	512	1.0	1.1	0.9
Methylotenera	ASV_028	16	8	8	57.1	57.1	57.1	644	286	358	0.6	0.5	0.6
Oleibacter	ASV_013	15	7	8	53.6	50.0	57.1	1229	465	764	1.1	0.8	1.3
Colwelliaceae (F)	ASV_014	15	6	9	53.6	42.9	64.3	1225	532	693	1.1	1.0	1.2
Acinetobacter	ASV_011	13	7	6	46.4	50.0	42.9	1679	1214	465	1.5	2.2	0.8
Rhodobacteraceae (F)	ASV_025	13	7	6	46.4	50.0	42.9	759	560	199	0.7	1.0	0.3
Peredibacter	ASV_041	13	7	6	46.4	50.0	42.9	494	396	98	0.4	0.7	0.2
HTCC5015 (F: Arenicellaceae)	ASV_042	13	4	9	46.4	28.6	64.3	490	89	401	0.4	0.2	0.7
Algimonas	ASV_031	12	4	8	42.9	28.6	57.1	613	195	418	0.5	0.4	0.7
HIMB11 (F:Rhodobacteraceae)	ASV_022	11	3	8	39.3	21.4	57.1	902	152	750	0.8	0.3	1.3
Ruegeria	ASV_021	10	7	3	35.7	50.0	21.4	945	762	183	0.8	1.4	0.3
Vibrio	ASV_034	10	4	6	35.7	28.6	42.9	584	260	324	0.5	0.5	0.6
Flavobacteriaceae (F)	ASV_038	10	4	6	35.7	28.6	42.9	507	204	303	0.4	0.4	0.5
Labrenzia	ASV_055	10	3	7	35.7	21.4	50.0	359	68	291	0.3	0.1	0.5
Alteromonas	ASV_033	9	5	4	32.1	35.7	28.6	587	395	192	0.5	0.7	0.3
Micavibrionaceae (F)	ASV_054	8	4	4	28.6	28.6	28.6	382	202	180	0.3	0.4	0.3
Sphingomonadaceae (F)	ASV_050	7	3	4	25.0	21.4	28.6	404	235	169	0.4	0.4	0.3
Gammaproteobacteria (C)	ASV_059	7	4	3	25.0	28.6	21.4	293	177	116	0.3	0.3	0.2
Lewinella	ASV_062	6	2	4	21.4	14.3	28.6	260	46	214	0.2	0.1	0.4
Arenicella	ASV_117	5	3	2	17.9	21.4	14.3	89	69	20	0.1	0.1	0.0

Supplementary Materials and Methods

Symbiodiniaceae-associated bacterial taxa from other studies

We identified studies that characterized the bacterial communities associated with Symbiodiniaceae and compared these with the bacterial taxa found in our study, in either symbiotic or aposymbiotic (menthol bleached) samples. This way, we could preliminarily inspect for patterns of presence/absence of taxa between symbiotic states, on the assumption that Symbiodiniaceae-associated bacteria should be present in symbiotic corals and absent in bleached corals. This approach produced Figure S5 and incorporated the studies by Lawson et al. (2018), Nitschke et al. (2020), and Maire et al. (2021).

For more details, the formatted data and R scripts are available at: ~~https://doi.org/10.5281/zenodo.7976283~~https://doi.org/10.5281/zenodo.10551928

Lawson et al. 2018

Study summary

Characterized the bacterial communities associated with Symbiodiniaceae cultures, spanning 18 cultures across 5 genera (former clades) to define the core Symbiodiniaceae microbiome. Identified three OTUs, which were present in all cultures and corresponded to *Labrenzia*, *Marinobacter,* and *Chromatiaceae*.

Data considered

Supplementary material, Table S3, with description: **"**Operational taxonomic units (OTUs) defined as core members of the bacterial communities of *Symbiodinium* cultures and their corresponding GenBank accession numbers." This table reports the core taxa/ASVs overall (across all Symbiodiniaceae genera) and for each genus separately.

Extracted information and use

Information on bacteria taxonomy was extracted (from the article's Table S3) from the column "Taxonomic ID", which reports the bacterial taxonomy to the genus level when available, else to higher level preceded by "UC" (for "unclassified"). "UC " was replaced with "unclassif_" to match the naming system adopted in our study, and screened for matches. This resulted in four matches, all at the genus level.

Nitschke et al. 2020

Study summary

Study on symbiolites (photosynthesis-induced microbialites formed by calcifying co-cultures of Symbiodiniaceae and bacteria), with comparison of bacterial communities of symbiolites-producing (SP) and non-symbiolites producing (NP) Symbiodiniaceae cultures.

Data considered

Manuscript Table 2: "Bacterial isolates from symbiodiniacean cultures", with caption: "List of bacterial strains used in this study, including their taxonomic affiliation, GenBank accession numbers, and the Symbiodiniaceae strain of origin (ITS2 type). […]".

Bacteria were isolated from Symbiodiniaceae cultures belonging to strains (ITS2 types): A1, A2 (*Symbiodinium*), B1 (*Breviolum*), and C2 (*Cladocopium*).

Extracted information and use

The table reports taxonomy down to the species level (for all isolates). Genus level affiliation was extracted and, together with species names, cross checked with the data from our study. This resulted in 5 matches at the genus level and no matches at the species level.

Maire et al. 2021

Study summary

Characterized the bacterial communities associated with 11 Symbiodiniaceae strains spanning nine species and six genera, and distinguishing between intracellular, closely associated (on Symbiodiniaceae outer cell surface), and loosely associated bacterial communities.

Data considered

Supplementary "Dataset S3" corresponding to file "41396_2021_902_MOESM**4**_ESM.xlsx", with description: "Relative abundances of intracellular (A), closely associated (B), and loosely associated (C) core genera in all Symbiodiniaceae samples. A core genus is a genus that is present in every Symbiodiniaceae species within a given location.", which contains the phylogeny and abundance of the core genera for each location ("intracellular", "closely associated" and "loosely associated") for all 11 strains. With "core genera" corresponding to the genera found in all 11 Symbiodiniaceae strains for each location. The 11 Symbiodiniaceae strains (listed in Table S1, file "41396_2021_902_MOESM3_ESM.xlsx") belonged to the genera Symbiodinium, Breviolum, Cladocopium, Durusdinium, Fugacium, Gerakladium.

Extracted information and use

The names of the "core genera" for all locations (all three sheets: "A - Intracellular core genera", "B - Closely-assoc core genera", and "C - Loosely-assoc core genera") were extracted and crossed check with the data from our study, which resulted in 6 matches.

References

Lawson CA, Raina J, Kahlke T, Seymour JR, Suggett DJ (2018) Defining the core microbiome of the symbiotic dinoflagellate, Symbiodinium. Environ Microbiol Rep 10:7–11 https://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12599

Maire J, Girvan SK, Barkla SE, Perez-Gonzalez A, Suggett DJ, Blackall LL, van Oppen MJH (2021) Intracellular bacteria are common and taxonomically diverse in cultured and in hospite algal endosymbionts of coral reefs. ISME J 15:2028–2042 http://dx.doi.org/10.1038/s41396-021-00902-4

Nitschke MR, Fidalgo C, Simões J, Brandão C, Alves A, Serôdio J, Frommlet JC (2020) Symbiolite formation: a powerful in vitro model to untangle the role of bacterial communities in the photosynthesis-induced formation of microbialites. ISME J 14:1533–1546 https://www.nature.com/articles/s41396-020-0629-z

Puntin G (202~~43~~) sPuntinG/Galaxea_Microbiome_PCI: Galaxea microbiome analysis 1st peer-review. https://doi.org/10.5281/zenodo.10551928~~https://zenodo.org/record/7976283~~