Received: 10 January 2023 | Revised: 27 March 2023 | Accepted: 10 May 2023

DOI: 10.1111/maec.12757

RESEARCH ARTICLE

marine ecology WILEY

The phylum Chloroflexi and their SAR202 clade dominate the microbiome of two marine sponges living in extreme environmental conditions

Federica Maggioni[1] | Pierre-Louis Stenger[2] | Philippe Jourand[3] | Clarisse Majorel[1]

[1]ENTROPIE, IRD, University of New-Caledonia, University of La Reunion, CNRS, IFREMER, Labex Corail, Nouméa, France

[2]IAC, Institut Agronomique néo-Calédonien (IAC), Équipe Sol & Végétation (SolVeg), Nouméa, France

[3]IRD, UMR ENTROPIE, Saint Denis Cedex 9, France

Correspondence
Clarisse Majorel, 101 Promenade Roger Laroque, 98848 Noumea, New Caledonia, France.
Email: clarisse.majorel@ird.fr

Funding information
UMR ENTROPIE laboratory

Abstract

The capacity of marine sponges to cope with environmental changes is often attributed to the specific composition of their bacterial communities. In this study, we assessed the bacterial microbiome of two dominant sponges, *Rhabdastrella globostellata* (Rg) and *Hyrtios erectus* (He), living in the Bouraké lagoon (New Caledonia), where abiotic conditions daily fluctuate according to the tide. Sponge specimens, sediment and seawater samples were collected at 2–3 m depth. The bacterial communities were assessed using 16S rRNA metabarcoding, and variations between the two sponges were compared using PCA biplots. Chloroflexi was the dominant phyla in both He and Rg with an average relative abundance of 41.2% and 53.2%, respectively, while it was absent in sediment and seawater. Among the phylum Chloroflexi, SAR202 clade was dominant in both sponges, reaching an average relative abundance of 53.2% (He) and 78.7% (Rg). Principal component analysis (PCA) was used to identify the main variables driving the bacterial community structure in both sponges. The results indicated that the bacterial community structure in both sponges was strongly associated with Chloroflexi (70.9% of the phyla variance) and SAR202 clade (86.6% of the clade variance). The high relative abundance of the phylum Chloroflexi and the SAR202 clade observed in this study is the highest reported so far in the literature in marine sponges. Such a high relative abundance of these bacteria could suggest their involvement in the well-being of sponges in the extreme environmental conditions of Bouraké.

KEYWORDS
Bouraké lagoon, Chloroflexi, marine sponges, microbiome, New Caledonia, SAR202

1 | INTRODUCTION

Marine sponges are a diverse group of sessile, filter-feeding invertebrate animals (Gardères et al., 2016). They play a significant ecological role in coral reef ecosystems (Coppock et al., 2022) through their water filtering capacities and nutrient and organic matter recycling properties (Folkers & Rombouts, 2020). Among marine benthic organisms, demosponges may emerge as winners in future oceanic conditions induced by climate change (Bell et al., 2018). However, if some marine sponge species are less sensitive to elevated seawater temperature and ocean acidification, others appear to be negatively affected by the combined effects of these stressors (Bates & Bell, 2018; Bell et al., 2018).

The tolerance of sponges to environmental stresses may be attributed to their associated prokaryotic symbionts. Indeed, sponges are holobionts (i.e., host and symbiotic microbes) with various microorganisms associated with them (Thomas et al., 2016). Based on the abundance and diversity of microbes in tissues, sponges can be classified into high microbial abundance (HMA) or low microbial abundance (LMA; 10^5–10^6 bacteria per g of sponge wet weight; Hentschel et al., 2006). The symbionts are essential for the sponge's nutrition, immunity, defence and reproduction (Pita et al., 2018). In addition, the marine sponge microbiome plays a significant role in the biogeochemical cycling of key nutrients (C, N and P; Zhang et al., 2019). The sponge uptake of organic and inorganic nutrients is believed to occur through its symbiotic relationship, with reciprocal translocation of resources (Pita et al., 2018; Zhang et al., 2019). Thus, Dissolved Organic Carbon (DOC), a component of the Dissolved Organic Matter (DOM) pool, can contribute between 60% and 97% of the daily carbon intake of sponges (De Goeij et al., 2008; Hoer et al., 2018; McMurray et al., 2018), and associated bacteria are involved in this DOM uptake (Campana et al., 2021; Olinger et al., 2021). For example, phylum Chloroflexi dominates the microbiome of HMA sponges, with its clades SAR202, Caldilineae and Anaerolineae being the most important (Bayer et al., 2018). The bacterial phylum Chloroflexi has been observed to have common genomic features including central pathways in energy and carbon conversion, amino acid and fatty acid metabolism and respiration, suggesting that it plays a key role in marine sponge nutrition (Bayer et al., 2018).

The diversity of the sponge microbial community may contribute to the ecological adaptability and plasticity of the holobiont, allowing it to thrive even in disturbed environments (Bang et al., 2018). Thus, changes in the abundance of a particular species, phylum, or class in bacterial communities of marine sponges in coral reef ecosystems may indicate a response to external environmental factors and potential environmental adaptation (Webster & Reusch, 2017). In addition, specific environmental factors have been linked to changes in the microbial composition of marine sponges (Turon et al., 2019). For example, the structure of the microbial community of the marine sponge *Phyllospongia foliascens* (Pallas, 1766) changes with a shift from Bacteroidota to Cyanobacteriota when transferred from turbid inshore to oligotrophic offshore environments, suggesting that light may be driving the change (Luter et al., 2015). A similar shift in the bacterial community was observed in two marine sponges living in a natural volcanic CO_2 seep, *Coelocarteria singaporensis* (Carter, 1883) and *Cinachyra* sp. (Sollas, 1886), which host a higher relative abundance of photosynthetic microbes compared to control sites. The higher quantity of photosynthetic symbionts may provide them with a nutritional benefit under future climate scenarios (Morrow et al., 2015).

Overall, the fact that (i) the sponge microbiome plays a significant role in marine sponge nutrition, (ii) this microbiome can adapt to environmental changes and (iii) its variations in community structure reflect such changes (Ribes et al., 2016), suggest that sponge microbiomes also contribute to the ability of sponges themselves to cope with environmental changes. To test this hypothesis, our study assessed the bacterial diversity of two highly distributed marine sponges living in the natural laboratory of the Bouraké lagoon in the tropical archipelago of New Caledonia (Figure 1; Camp et al., 2017; Maggioni et al., 2021). The Bouraké lagoon is characterized by fluctuating conditions of acidification, warming and deoxygenation, where a well-diversified coral reef thrives (Camp et al., 2017; Maggioni et al., 2021). Environmental variability of dissolved oxygen, temperature and pH at Bouraké is related to the tidal cycle, with changes in a single day of up to 4.91 mg O2 L^{-1}, 6.50°C and 0.69 pH_T units respectively (Maggioni et al., 2021). These environmental conditions expose organisms to extreme conditions during low tide, with pH (7.2), dissolved oxygen (1.97 mg L^{-1}) and temperature 1–2°C higher than in adjacent reefs, whose standard lagoon environmental conditions are reported as pH 8.0 and 6.5 mg L^{-1} for the same period (Camp et al., 2017; Maggioni et al., 2021). Bouraké also has a nutrient-rich environment, and some chemical parameters, such as organic/inorganic carbon concentrations, are one to five times higher in the Bouraké lagoon than in adjacent reefs (Maggioni et al., 2021). Here we focused on two dominant marine sponge species belonging to the family Demospongiae: *Rhabdastrella globostellata* (Carter, 1883) and *Hyrtios erectus* (Keller, 1889). To determine their bacterial relative abundance and diversity we performed a descriptive 16S rRNA metabarcoding analysis. Our aim was to provide initial insight into the diversity of bacterial communities in Bouraké marine sponges, which may play an important role in the survival and adaptation of sponges in these extreme environmental conditions.

2 | MATERIALS AND METHODS

2.1 | Study site and sample collection

The study was carried out in the unique semi-enclosed reef lagoon of Bouraké, New Caledonia (South Pacific, Figure 1). We reported in Table S1 the main physical environmental parameter measured previously by Maggioni et al., 2021. Three samples (10 cm × 10 cm) of sponge *R.globostellata* (Carter, 1883) and *H. erectus* (Keller, 1889) were collected randomly from the outermost zone (sample code 1) to the innermost zone (sample code 3) of the Bouraké lagoon (Figure 1 and Figure S1, Table S2). In addition, three samples of seawater (1 L each) and the upper 15 cm of sediment (50 g each) near the sponges were collected. After collection, sediments and sponge samples were placed in independent plastic bags. All samples were transported to the laboratory (IRD, Nouméa, New Caledonia) and stored at 4°C. The sediment was frozen, then freeze-dried and preserved under vacuum, while the seawater was filtered (0.22 μM) and filters stored at −20°C. A small tissue fragment from each sponge specimen, previously washed three times with artificial seawater, was preserved in 70% ethanol for species identification. The remaining sponge tissue was frozen –at 80°C until DNA extraction.

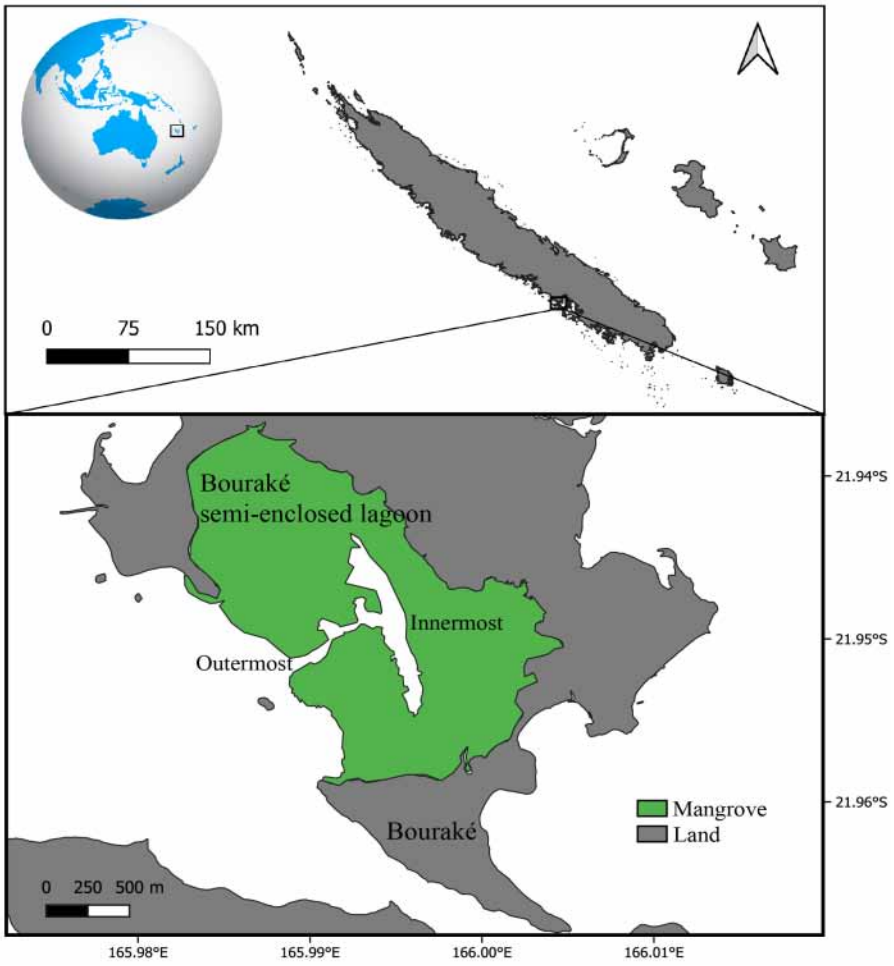

FIGURE 1 The Bouraké semi-enclosed lagoon study site. The top panel shows the location of the study sites in the Grande Terre of New Caledonia. At the bottom a zoom on the Bouraké semi-enclosed lagoon with the innermost and the outermost zone of the lagoon. Géorep New Caledonian database and QGIS software were used to build the figure (modified from Maggioni et al., 2021).

2.2 | DNA extraction and sequencing

DNA extractions were performed on (i) four aliquots of 1 g each taken from the freeze-dried sediment and then pooled ($n=3$), (ii) seawater filters ($n=3$) and (iii) four centimetres cores of the sponge choanosome ($n=3$ for each sponge species). The extractions were performed using the DNeasy PowerSoil® kit (Qiagen), following the manufacturer's instructions. The quality of total DNA was checked using a NanoDrop ND-2000 spectrophotometer (Thermofisher Scientific) and stored at −20°C until further analyses. Total DNA extracts were sent to GenoScreen (www.genoscreen.fr) for amplification, library construction and multiplexed sequencing. Given the complexity of the environmental conditions in the Bouraké lagoon, we chose to assess the microbial diversity using high-throughput sequencing (Next Generation Sequencing) of the V1V3 region of the 16S rDNA, which provides a better understanding of microbial diversity in complex microbiomes (Blanquer et al., 2016; Zheng et al., 2015). The partial V1–V3 region of the bacterial 16S rRNA gene sequences was then amplified using the specific primers 27F (5′-AGRGTTTGATCMTGGCTCAG-3′) modified from (Lane, 1991) and 519R (5′-GTNTTACNGCGGCKGCTG-3′) modified from (Lane et al., 1985), using an optimized and standardized amplicon library preparation protocol (Sheen, 1996; Metabiote®, GenoScreen). All the samples were sequenced in a MiSeq Illumina sequencer (Illumina) using a MiSeq reagent kit V3 (Illumina) and producing 2×300-bp long reads, according to the manufacturer's instructions.

2.3 | 16s rRNA gene sequencing analysis

Raw read quality was assessed with FastQC V. 0.11.9 (Andrews, 2010; Table S3), and reports were generated using MultiQC V. 1.6 (Ewels et al., 2016). Reads were cleaned and adaptors removed with Trimmomatic V. 0.39 (Bolger et al., 2014; Illuminaclip 2:30:10; leading 30, trailing 30, sliding window 26:30 and minlen 150). Cleaned read quality was checked with the same software (Table S2). Microbiome analysis was performed using the QIIME 2 framework V. 2021.4 (Bokulich et al., 2018). Dereplicated and trimmed sequences were imported into the framework as *PairedEndFastqManifestPhred33V2* format and denoised using the DADA2 plugin, based on the DADA2 V. 1.8 R library (Callahan et al., 2016), which removed singletons, chimeras and sequencing errors and processed the sequences into a table of exact amplicon sequence variants (ASVs; Callahan et al., 2017). All samples were rarefied according to the alpha-rarefaction tool with a maximum depth of 10,430 for the 'Observed number of features' (here, ASVs that are equivalent to species richness; DeSantis et al., 2006), Shannon entropy (richness and diversity that accounts for both

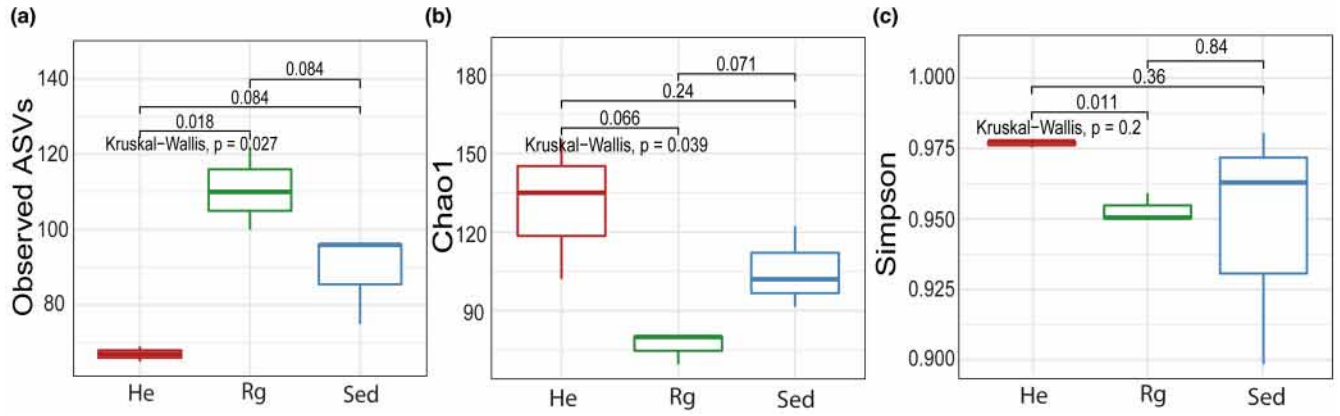

FIGURE 2 Alpha diversity index among the two sponges species *Rhabdastrella globostellata* (Rg), *Hyrtios erectus* (He) and sediment (Sed). (a) Observed ASVs, (b) Chao1, and (c) Simpson index.

abundance and evenness of taxa; Shannon & Weaver, 1949) and Faith PD (a measure of biodiversity that incorporates phylogenetic differences between species using the sum of the length of branches; Faith, 1992) give 2318 depth value for rarefaction by keeping all samples. A multiple sequence alignment was produced using MAFFT V. 7.310 (Katoh et al., 2002; Katoh & Standley, 2013), and a rooted phylogenetic tree relating the ASV sequences to one another was constructed using FastTree methods V. 2.1.10 (Price et al., 2009), According to Bokulich et al. (2018), generating a tree allows phylogenetic diversity analyses, including Faith's Phylogenetic Diversity (Faith PD) and weighted and unweighted UniFrac. In addition to counts of features per sample, these metrics require a rooted phylogenetic tree relating the features to one another. To characterize the diversity of this new and now rarefied dataset, we used the *q2*-diversity tool to produce a set of diversity indices, including the observed number of ASVs (same as above; DeSantis et al., 2006). Chao1 (the expected richness; Chao, 1984), Simpson evenness (diversity that accounts for the number of organisms and the number of species; Simpson, 1949), Pielou evenness (a measure of the relative evenness of species richness; Pielou, 1966), Shannon entropy (same as above; Shannon & Weaver, 1949) and Faith PD (Faith, 1992). The Bray–Curtis dissimilarity (Sørensen, 1948) and Jaccard similarity index (Jaccard, 1908) matrices were also calculated with the *q2-diversity* tool. Naive Bayes feature classifiers were trained using the *q2-feature-classifier* tool to assign taxonomy to the sequences (Bokulich et al., 2018).

For bacterial classifier training, the SILVA-138-SSURef-Full-Seqs QIIME pre-formatted database (*SILVA-138-SSURef-Full-Seqs.qza* as *DataSeq.qza* and *Silva-v138-full-length-seq-taxonomy.qza* as *RefTaxo.qza;* see https://github.com/mikerobeson/make_SILVA_db) was used, and for hooked V1V3 parts of the 16S region, we used the forward 27f sequence ('GTGCCAGCMGCCGCGGTAA') and the reverse 534r sequence ('GTNTTACNGCGGCKGCTG') on the *feature-classifier extract-reads* tool to limit the rate of bad assignments following Bokulich et al. (2018). All our raw sequences have been deposited in the Sequence Read Archive (SRA) under submission number SUB11051098 (BioProject IDRJNA804658), from SAMN25761711 to SAMN25761723 (9 samples). All scripts can be found at https://github.com/PLStenger/Rhabdastrella_globostellata_and_Hyrtios_erectus_microbiome.

2.4 | Statistical analysis

For the community diversity analysis, indices (see Section 2.3) were calculated with the R package *multcomp* V. 1.4–16 (Hothorn et al., 2008). For bacterial community composition, relative abundance was visualized through a barplot using the *ggplot2* R package V. 3.3.3 (Wickham et al., 2016). A Principal Component Analyses (PCA) biplot was used to explain variation within bacterial communities in both sponges using the R packages multcomp V. 1.4–16 (Hothorn et al., 2008), factoextra V. 1.0.7 (Kassambara & Mundt, 2020) and vegan V. 2.5–7 (Oksanen et al., 2019).

3 | RESULTS

3.1 | Bacterial community composition and diversity

The 16S rRNA gene V1–V3 region sequencing on the Illumina Miseq platform returned 299,637 reads. After filtering and quality control, 258,224 bacterial sequences were obtained, resulting in an average frequency of 28,691 ± 2900 sequence reads per sample (Table S3). We identified a total of 85,290 bacterial Amplicon Sequence Variants (ASVs) at 100% sequence similarity affiliated with 14 bacteria phyla. Rarefaction curves reached a plateau at 2318 sequences indicating that the sequencing effort was sufficient to cover most ASVs and to keep all samples (Figure S2). Seawater samples were not considered in the process because only two samples effectively worked during metabarcoding analysis; their relative bacterial abundance is reported in Table S4.

Significant differences were noted in the number of observed ASVs per species, which was almost twice as high in *R.globostellata*

as in *H. erectus* (Table S4, Figure 2a). We also found significant differences between the two sponges for the alpha index, but not for the Simpson evenness and the Faith PD (Figure S3). There was no difference in bacterial species richness between sponge species, as indicated by the Chao1 index, which showed the lowest average in *R. globostellata* (76.6 ± 6.1), and the highest in *H. erectus* (130.9 ± 26.9; Figure 2b, Table S4). *R. globostellata* had the lowest diversity, as indicated by a Simpson index of 0.95 ± 0.01 (Figure 2c, Table S4). Compared to both sponges, there were no significant differences in the alpha diversity index for sediments (Figure 2). In sediments, the observed ASVs and Chao1 index had average values of 89.0 ± 12.1 and 105.3 ± 15.6, respectively, and they were between the average values of the two sponge species (Table S4, Figure 2). Sediments only statistically differed from *R. globostellata* for the Faith PD diversity with average values of 7.28 ± 0.2 and 11.8 ± 1.1, respectively (Figure S3). The Simpson index in sediments (0.95 ± 0.04) was the same as for *R. globostellata* and close to that of *H. erectus* (Figure 2, Table S4).

The two sponge species had similar bacterial community compositions (Figure 3; Table S5). The microbiome of *R. globostellata* was composed by (average in descending order): Chloroflexi (53.2%), Proteobacteria (15.9%, with 14.7% Alphaproteobacteria and 0.1% Gammaproteobacteria), Cyanobacteria (10.6%), Acidobacteria (10.2%), Actinobacteria (8.2%), Dadabacteria (1.0%), Bdellovibrionota (0.5%), Patescibacteria (0.09%), Bacteroidota (0.02%) and Nitrospirota (0.02%; Figure 3; Table S5). Within the phylum Chloroflexi, SAR202 was the most abundant clade with an average of 78.7 ± 4.7% (Figure 3, Table S5), varying between 73.3% and 82.2% (Figure S4), followed by (average in descending order): TK30 (4.9%), TK10 (4.2%), TK17 (3.7%), SBR1031 (3.7%), JG30KFCM66 (3.2%), S085 (1.2%) and Caldilineales (0.5%). No significant differences were observed between the three-sponge samples in phyla and Chloroflexi clades (Figure S4, Table S5).

The bacterial community of *H. erectus* was composed by (average in descending order): Chloroflexi (41.2%), Proteobacteria (35.0%, with 39% Alphaproteobacteria and 0.1% Gammaproteobacteria),

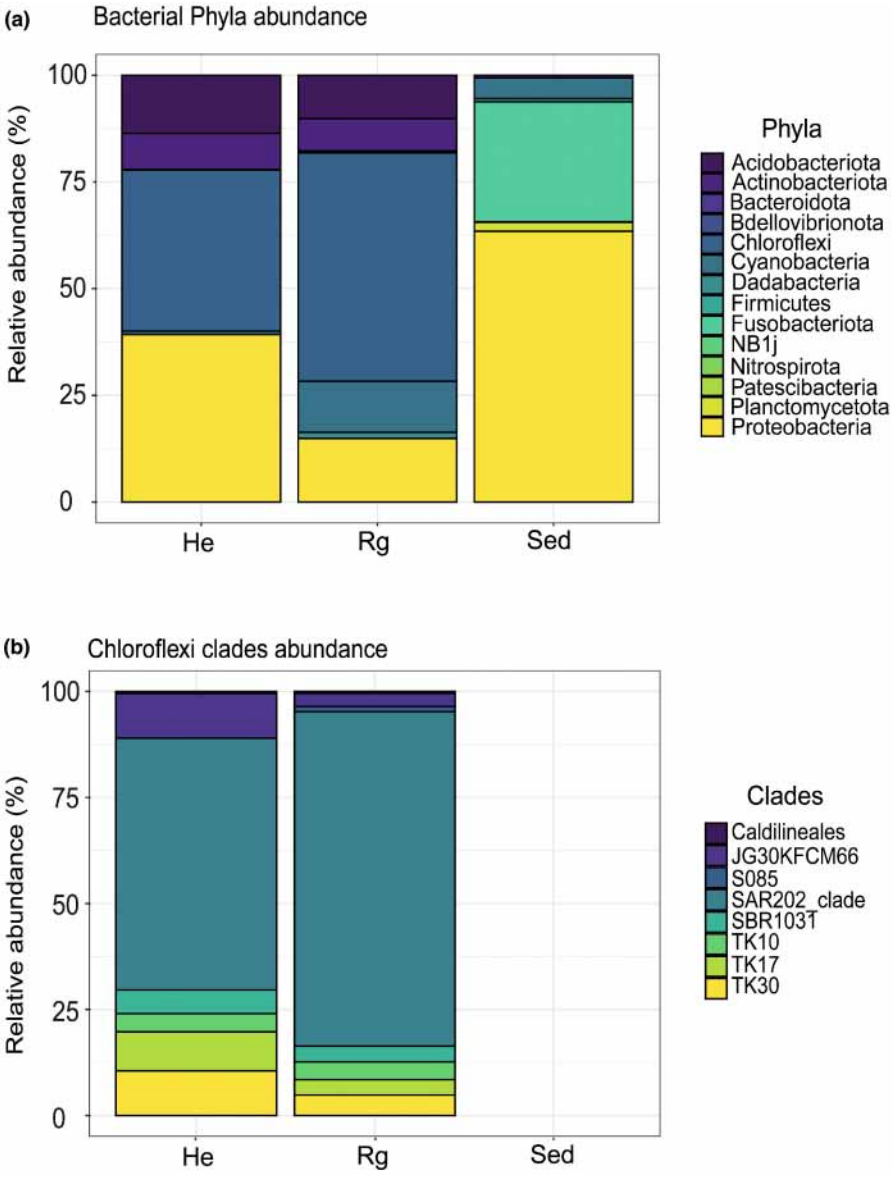

FIGURE 3 Relative abundance in percent of bacterial community composition based on 16S rRNA gene amplicon sequencing of two the sponges species *Rhabdastrella globostellata* (Rg_1, Rg_2, Rg_3), *Hyrtios erectus* (He_1, He_2, He_3), and sediments (Sed_1, Sed_2, Sed_3). (a) for Phyla, and (b) for Chloroflexi clades.

Acidobacteria (14.6%), Actinobacteria (8.1%), Cyanobacteria (0.9%), Bdellovibrionota (0.09%), Nitrospirota (0.04%; Figure 3; Table S5). Among Chloroflexi, SAR202 was the most abundant clade with an average of 57.3 ± 24.0% (Figure 3, Table S5), varying from 33.7% to 81.7% (Figure S4), followed by (average in descending order): JG30KFCM66 (10.84%), TK30 (10.80%), TK17 (10.0%), SBR1031 (5.8%), TK10 (4.7%) and Caldilineales (0.6%). Some differences were observed between the three-sponge samples, mainly in the Chloroflexi clades (Figure S4b, Table S5). The SAR202 clade was more than twice as high in He_3 (81.75) as in He_1 (33.66), while all other clades were almost twice as low in He_3 as in He_1 (Figure S4b; Table S5).

The bacterial community of sediment samples was composed of Proteobacteria (57.3%, with 60.7% Alphaproteobacteria and 2.7% Gammaproteobacteria), Fusobacteriota (35.2%), Cyanobacteria (4.1%), Planktomycetota (1.6%), Firmicutes (1.1%), Actinobacteria (0.7%) and Nitrospirota (0.03%). No Chloroflexi was found in sediments (Figure 3; Table S5).

The Venn diagrams show that the three *R. globostellata* individuals shared 64.8% of their microbiome (e.g., 162 AVSs), and the three *H. erectus* shared 46.9% of their microbiome (e.g., 147 AVSs), which may indicate that the microbiome of *R. globostellata* is less variable than that of *H. erectus* (Figure S5).

3.2 | Bacterial community structure

Variations in the relative abundance of the different phyla and Chloroflexi clades across the sponge samples contrasted with the more consistent richness estimates found in sediment samples (Figure 3). Principal Component Analysis (PCA) was used to study the structure of bacterial abundance of phyla and Chloroflexi clades in both sponge species (*R. globostellata* and *H. erectus*) and sediments. The PCA analysis shows 70.9% (53.1% + 17.8%) of the variance in phyla and 86.6% (65.2% + 21.4%) of the variance in Chloroflexi clades (Figure 4a,b). The results of the PCA analysis suggest that environmental drivers are likely influencing the bacterial community structure in both sponge species and sediments. The seawater sample was not considered because only two samples worked successfully during the metabarcoding analysis.

The first analysis was carried out at the phylum level (Figure 4a). The variation in the sponge samples is linked to the contributive arrow of Chloroflexi. The contributive arrows of the phyla Chloroflexi, Acidobacteriota, Actinobacteriota and Dadabacteriota point to the three samples of *R. globostellata*, which group together. In contrast, the three samples of *H. erectus* do not group together: sample He_3 is isolated from the other two samples, which are closer to *R. globostellata* (Figure 4a). The contributive arrows of Fusobacteria and Firmicutes point to sample Sed_1, while the arrows of Nitrospirota and NB1j point to sample Sed_2 (Figure 4a).

A second analysis was performed at the clade level in the phylum Chloroflexi, which was the most abundant phylum in the sponge microbial communities, but was not found in the sediment and seawater samples (Figure 3, Table S4). The distribution of Chloroflexi clades in the PCA representation differed between the two sponge species. The PCA results showed that the relative abundance of Chloroflexi clades SAR202 and S085 were strongly associated with *R. globostellata* samples, while the abundance of TK17 and JG30KFCM66 were associated with *H. erectus* samples (Figure 4b). No specific Chloroflexi clade was found to be driving the bacterial community structure of sample He_3, which had a different bacterial relative abundance (Table S4) compared to the other two *H. erectus* samples (Figure 4b). It is likely that other environmental factors are driving the bacterial community structure in this sample.

4 | DISCUSSION

In the present study, we analysed the bacterial community composition associated with two species of marine sponge living in the extreme environment of the Bouraké lagoon in New Caledonia. We found that the bacterial communities associated with the sponges were host-specific, and differed from those in marine sediments and seawater. The phylum Chloroflexi and the SAR202 clade were the most abundant in sponge samples (>41% and >57%, respectively), whereas, in sediments and seawater, the phylum Proteobacteria was the most abundant (>95%). These results confirm previous studies showing that Chloroflexi is host specific in sponges with high microbial abundance (HMA; Bayer et al., 2018; Hentschel et al., 2006; Pita et al., 2018). Since Chlorofexi and SAR202 have not been detected in water and sediment, their significant presence in sponges could result from vertical microbial transmission (sexual reproduction). This mechanism is known and well-documented in the establishment and maintenance of specific associations of spongy microbes on evolutionary timescales (Carrier et al., 2022; Schmitt et al., 2008; Sharp et al., 2007; Webster et al., 2010). However, while our results are in agreement with previous studies (Bayer et al., 2018; Busch et al., 2020), we report remarkably high levels of richness and relative abundance of the phylum Chloroflexi and SAR202 clade (up to 53.2% and 78.6% respectively) that have never been described before.

A recent study of microbial abundance in 63 marine sponge species reported an average abundance of Chloroflexi of 31.9 ± 5.3% (Bayer et al., 2018), while in the Sponge Microbiome Project, Chloroflexi sequences accounted for 20%–30% of the total microbiome in 19 sponge genera (Moitinho-Silva et al., 2017). Chloroflexi is one of the most abundant and diverse phyla associated with marine sponges (Bayer et al., 2018; Britstein et al., 2020; Moitinho-Silva et al., 2017). However, this is the first report of such a high abundance of this phylum, 53.2% in *R. globostellata* and 41.2% in *H. erectus*, while it was previously reported only at 20% in *H. erectus* and 45% in *R. globostellata* (Bayer et al., 2018; Steinert et al., 2016). The phylum Chloroflexi contains a high diversity of bacteria harbouring very diverse and various metabolic functions, ranging from anoxygenic photosynthesizers to obligate aerobic/anaerobic heterotrophs, thermophiles, halophiles, clades capable of reductive halogenation and even predators with gliding motility (Bayer

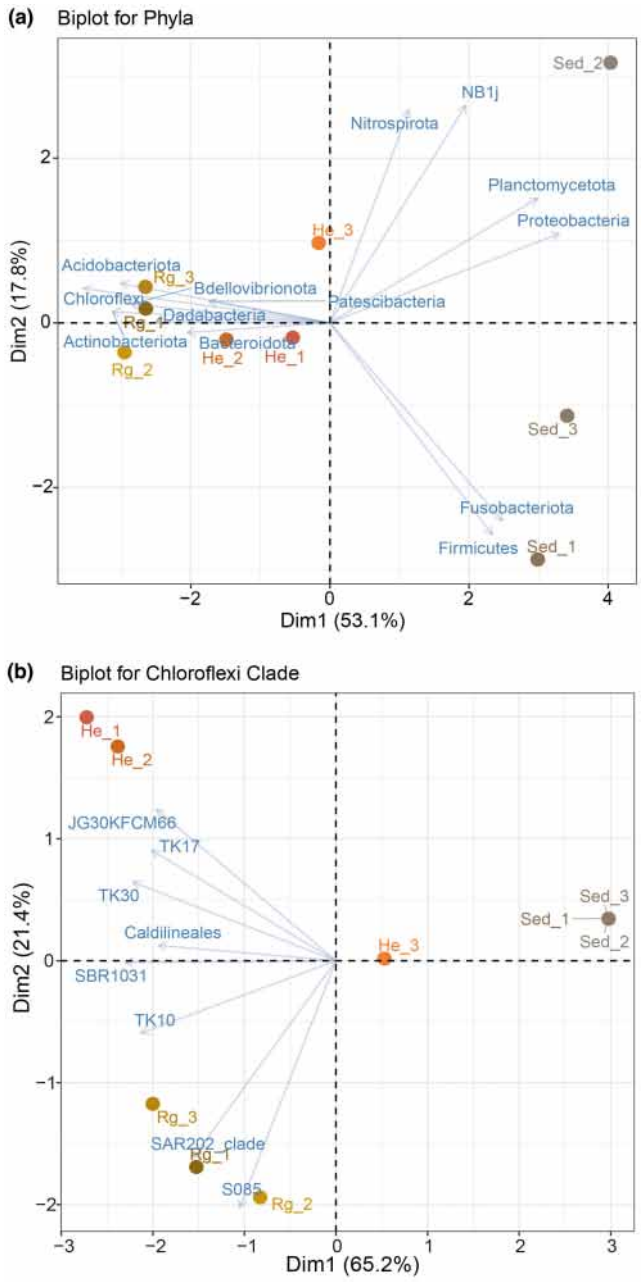

FIGURE 4 Principal component analysis on bacterial abundance in the two sponges *Rhabdastrella globostellata* (Rg_1, Rg_2, Rg_3), *Hyrtios erectus* (He_1, He_2, He_3), and sediments (Sed_1, Sed_2, Sed_3). (a) At the Phyla levels and (b) at Chloroflexi Clade levels.

et al., 2018). The importance of this phylum has been studied in deep-sea sponges where its recruitment in the holobiont suggests functional roles in the organic matter cycle and sponge adaptation to deep-sea environments (Busch et al., 2020; Campana et al., 2021; Landry et al., 2017). In addition, in shallow waters, members of Chloroflexi can convert inorganic carbon to organic carbon, which is possibly transferred to the host sponge and metabolized, giving a nutritional advantage to the host in this environment (Brück et al., 2010; Hardoim et al., 2012). In line with these studies, we hypothesize that the abundance of Chloroflexi in Bouraké sponges provides additional nutrients, including extra carbon sources that could help the sponge cope with specific environmental conditions of Bouraké and contribute to its fitness.

In the microbiome of the Bouraké sponges, SAR202 clade was the dominant representative of the phylum Chloroflexi. This dominance is similar to other sponge microbiomes described in the sponge microbiome project (Moitinho-Silva et al., 2017). However, in *R. globostellata*, SAR202 clade reached a higher average relative abundance (78.7%) than reported in other sponge microbiomes (e.g., 48% in Bayer et al., 2018 and 73% in Busch et al., 2020). Clade SAR202 is known to harbour genes contributing to sponge metabolisms such as glutamine synthesis, ammonia import, incorporation of sulfur and biosynthetic pathways for cofactor biosynthesis and vitamin (Bayer et al., 2018). Furthermore, SAR202 may play an important function in the degradation of recalcitrant organic matter (Bayer et al., 2018; Landry et al., 2017). Further studies are needed to better understand the role of Chloroflexi in shallow waters, but the prevalence of SAR202 may help the Bouraké sponges to uptake and recycle the abundant DOM present in the lagoon, thus providing the energy needed to cope with the extreme environmental conditions. This hypothesis is in agreement with a recent study showing that *R. globostellata* form Bouraké always uptake DOM also during extreme environmental conditions (Maggioni et al., 2023). In addition, we observed an increase in the relative abundance of SAR202 in the sponge *H. erectus* from the outer (He_1) to the inner (He_3) zone of the Bouraké lagoon. Although only three samples were analysed, and this result could be due to individual variability, the trend is similar to that of phylum Chloroflexi. Our results suggest that Chloroflexi, including SAR202 clade, could play an important role in the success of sponges under Bouraké's extreme environmental conditions, particularly in the innermost zone where the most extreme values and highest organic matter concentrations were reported (Maggioni et al., 2021).

Proteobacteria is only the second most abundant phylum in both *R. globostellata* (15.9%) and *H. erectus* (35%), while it is dominant in sediments (57.2%) and seawater (95%). This result is consistent with those described in the literature, where marine sponges harbour a relative abundance of Proteobacteria ranging from 23.6% to 80.3% (Cleary et al., 2018). Proteobacteria are involved in many metabolic processes, such as denitrification, DOM incorporation and ammonium assimilation (Hoffmann et al., 2009; Liu et al., 2020; Nelson & Carlson, 2012). For example, members of the class Alphaproteobacteria, which were the most abundant in the marine sponge samples of the Bouraké lagoon, have a role in the anaerobic oxidation of ammonium and nitrite (Woebken et al., 2007). The high abundance of Alphaproteobacteria, especially in *H. erectus*, could increase the incorporation of organic nitrogen and provide the sponge with the energy needed to cope with harsh environmental conditions.

Cyanobacteria was the third most abundant phylum in *R. globostellata*, with 10.6%, while in *H. erectus*, it represented only 0.9% of the total microbial abundance. The relative abundance of Cyanobacteria in *R. globostellata* has never been reported above 2.5% (Dat et al., 2018; Steinert et al., 2016). The high abundance

of Cyanobacteria in sponges can provide nutritional benefits, including nitrogen fixation, as nitrogen metabolism products can be transferred from the symbiotes to the sponge host in some species (Freeman & Thacker, 2011). This phylum could then help the sponge in the nitrogen cycle, with a mutual benefit: the supply of nutrients by the sponge and the production of secondary metabolites by the Cyanobacteria (Taylor et al., 2007). Although carbon metabolism in sponges is typically based on filter feeding of bacterioplankton, some species can obtain 50% of their carbon demand from Cyanobacteria photosymbionts (Cheshire & Wilkinson, 1991; Freeman & Thacker, 2011; Wilkinson, 1983). The higher abundance of Cyanobacteria in *R. globostellata* may lead to nutrient benefits and higher carbon fixation increasing energy to cope with the environmental conditions in the Bouraké lagoon.

The specific bacterial community of *R. globostellata* and *H. erectus*, with the dominance of Chloroflexi and SAR202 clade, is likely a response to the particular environmental conditions of the Bouraké lagoon. Therefore, we believe that the Bouraké sponge holobiont has reached an equilibrium and healthy state, which allows it to cope with the environmental changes of Bouraké lagoon (i.e. acclimatization to the extreme environmental parameters such as pH, DO and temperature). Furthermore, these new features could be transmitted to the next generation of sponges, resulting in holobiont adaptation.

5 | CONCLUSION

This study is the first to provide a taxonomic description of the bacterial communities of the sponges living in the Bouraké lagoon (New Caledonia, South West Pacific). Results show, for the first time, a high relative abundance of the phylum Chloroflexi and SAR202 clade in the sponges *R. globostellata* and *H. erectus* living in extreme environmental conditions. This work suggests that these microbial communities are host-specific and may provide sponges with the metabolic characteristics to thrive in the extreme environmental conditions of Bouraké, thus playing a key role in sponge survival and adaptation. Further analysis of a larger number of samples and comparisons with microbiomes of the same sponge species outside the Bouraké lagoon are needed to understand if this bacterial dominance is specific to the extreme conditions of the Bouraké lagoon or found in all the sponges of New Caledonia.

AUTHOR CONTRIBUTIONS

C.M. and F.M. conceived and designed the project; F.M. collected the data. C.M. and F.M. performed all the genetic analyses. F.M., C.M. and P-L.S. conducted the data analysis. F.M. and C.M. drafted the manuscript. All co-authors read and edited the final version of the manuscript.

ACKNOWLEDGMENTS

We wish to thank the reviewers for their constructive comments that greatly improved our manuscript. We wish to express our gratitude to the captains of the IRD (Institut de Recherche pour le Développement, Nouméa) vessels and the technical diving staff for fieldwork assistance. Special thanks to Dr. Riccardo Rodolfo-Metalpa for supporting the study and field sampling. We would also like to thank Dr. Claude Payri and Pr. Yves Letourneur for their general support, and Dr. Barbara Calcinai for her help in identifying the sponges. Thanks to Dr. Lydiane Mattio for the English revision. Federica Maggioni thanks the 'Ecole doctorale du Pacifique' of the University of New Caledonia for her Ph.D. fellowship (project REEF-ENGINE). We thank the 'Plateforme du vivant (PFV)' (IRD, Nouméa), the LAMA laboratory (IRD, Nouméa) and Genoscreen (Lille, France) for performing analyses and for the use of their instruments and facilities. We also thank the 'Province Sud' for sample collection permits (no. 3413-2019).

FUNDING INFORMATION

This work has been supported by the UMR ENTROPIE laboratory.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The dataset compiled and analysed in this study is available on the Sequence Read Archive (SRA) under submission number SUB11051098 (BioProject IDRJNA804658), from SAMN25761711 to SAMN25761723. The code used in this study is available at https://github.com/PLStenger/Rhabdastrella_globostellata_and_Hyrtios_erectus_microbiome.

ORCID

Clarisse Majorel https://orcid.org/0000-0002-0404-2692

REFERENCES

Andrews, S. (2010). FastQC: A quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Bang, C., Dagan, T., Deines, P., Dubilier, N., Duschl, W. J., Fraune, S., Hentschel, U., Hirt, H., Hülter, N., Lachnit, T., Picazo, D., Pita, L., Pogoreutz, C., Rädecker, N., Saad, M. M., Schmitz, R. A., Schulenburg, H., Voolstra, C. R., Weiland-Bräuer, N., … Bosch, T. C. G. (2018). Metaorganisms in extreme environments: Do microbes play a role in organismal adaptation? *Zoology*, *127*, 1–19.

Bates, T. E. M., & Bell, J. J. (2018). Responses of two temperate sponge species to ocean acidification. *New Zealand Journal of Marine and Freshwater Research*, *52*, 247–263. https://doi.org/10.1080/00288330.2017.1369132

Bayer, K., Jahn, M. T., Slaby, B. M., Moitinho-Silva, L., & Hentschel, U. (2018). Marine sponges as Chloroflexi hot spots: Genomic insights and high-resolution visualization of an abundant and diverse symbiotic clade. *MSystems*, *3*, e00150-18.

Bell, J. J., Bennett, H. M., Rovellini, A., & Webster, N. S. (2018). Sponges to be winners under near-future climate scenarios. *Bioscience*, *68*, 955–968. https://doi.org/10.1093/biosci/biy142

Blanquer, A., Uriz, M. J., Cebrian, E., & Galand, P. E. (2016). Snapshot of a bacterial microbiome shift during the early symptoms of a massive sponge die-off in the western Mediterranean. *Frontiers in Microbiology*, *7*, 752.

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., & Gregory Caporaso, J. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome*, *6*, 1–17.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*, 2114–2120.

Britstein, M., Cerrano, C., Burgsdorf, I., Zoccarato, L., Kenny, N. J., Riesgo, A., Lalzar, M., & Steindler, L. (2020). Sponge microbiome stability during environmental acquisition of highly specific photosymbionts. *Environmental Microbiology*, *22*, 3593–3607.

Brück, W. M., Brück, T. B., Self, W. T., Reed, J. K., Nitecki, S. S., & McCarthy, P. (2010). Comparison of the anaerobic microbiota of deep-water *Geodia* spp. and sandy sediments in the straits of Florida. *The ISME Journal*, *4*, 686–699.

Busch, K., Wurz, E., Rapp, H. T., Bayer, K., Franke, A., & Hentschel, U. (2020). Chloroflexi dominate the deep-sea golf ball sponges *Craniella zetlandica* and *Craniella infrequens* throughout different life stages. *Frontiers in Marine Science*, *7*, 674.

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*, *11*, 2639–2643. https://doi.org/10.1038/ismej.2017.119

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*, 581–583. https://doi.org/10.1038/nmeth.3869

Camp, E. F., Nitschke, M. R., Rodolfo-Metalpa, R., Houlbreque, F., Gardner, S. G., Smith, D. J., Zampighi, M., & Suggett, D. J. (2017). Reef-building corals thrive within hot-acidified and deoxygenated waters. *Scientific Reports*, *7*, 2434. https://doi.org/10.1038/s41598-017-02383-y

Campana, S., Busch, K., Hentschel, U., Muyzer, G., & de Goeij, J. M. (2021). DNA-stable isotope probing (DNA-SIP) identifies marine sponge-associated (DOM). *Environmental Microbiology*, *53*, 4489–4504. https://doi.org/10.1111/1462-2920.15642

Carrier, T. J., Maldonado, M., Schmittmann, L., Pita, L., Bosch, T. C. G., & Hentschel, U. (2022). Symbiont transmission in marine sponges: Reproduction, development, and metamorphosis. *BMC Biology*, *20*, 1–19.

Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, *11*, 265–270.

Cheshire, A. C., & Wilkinson, C. R. (1991). Modelling the photosynthetic production by sponges on Davies Reef, Great Barrier Reef. *Marine Biology*, *109*, 13–18.

Cleary, D. F. R., Pol, A. R. M., & de Voogd, N. J. (2018). Prokaryote composition and predicted metagenomic content of two *Cinachyrella* Morphospecies and water from west Papuan marine lakes. *FEMS Microbiology Ecology*, *94*, fix175. https://doi.org/10.1093/femsec/fix175

Coppock, A. G., Kingsford, M. J., Battershill, C. N., & Jones, G. P. (2022). Significance of fish–sponge interactions in coral reef ecosystems. *Coral Reefs*, *41*, 1285–1308.

Dat, T. T. H., Steinert, G., Cuc, N. T. K., Smidt, H., & Sipkema, D. (2018). Archaeal and bacterial diversity and community composition from 18 phylogenetically divergent sponge species in Vietnam. *PeerJ*, *6*, e4970.

De Goeij, J. M., Van Den Berg, H., van Oostveen, M. M., Epping, E. H. G., & van Duyl, F. C. (2008). Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. *Marine Ecology Progress Series*, *357*, 139–151.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., & Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, *72*, 5069–5072.

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*, 3047–3048.

Faith, D. P. (1992). Conservation evaluation and phylogenetic diversity. *Biological Conservation*, *61*, 1–10.

Folkers, M., & Rombouts, T. (2020). Sponges revealed: A synthesis of their overlooked ecological functions within aquatic ecosystems. In *YOUMARES 9-the oceans: Our research, our future* (pp. 181–193). Springer.

Freeman, C. J., & Thacker, R. W. (2011). Complex interactions between marine sponges and their symbiotic microbial communities. *Limnology and Oceanography*, *56*, 1577–1586.

Gardères, J., Domart-Coulon, I., Marie, A., Hamer, B., Batel, R., Müller, W. E. G., & Bourguet-Kondracki, M. L. (2016). Purification and partial characterization of a lectin protein complex, the clathrilectin, from the calcareous sponge *Clathrina clathrus*. 2. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *200*, 17–27.

Hardoim, C. C. P., Esteves, A. I. S., Pires, F. R., Gonçalves, J. M. S., Cox, C. J., Xavier, J. R., & Costa, R. (2012). Phylogenetically and spatially close marine sponges harbour divergent bacterial communities. *PLoS One*, *7*, e53029. https://doi.org/10.1371/journal.pone.0053029

Hentschel, U., Usher, K. M., & Taylor, M. W. (2006). Marine sponges as microbial fermenters. *FEMS Microbiology Ecology*, *55*, 167–177.

Hoer, D. R., Gibson, P. J., Tommerdahl, J. P., Lindquist, N. L., & Martens, C. S. (2018). Consumption of dissolved organic carbon by Caribbean reef sponges. *Limnology and Oceanography*, *63*, 337–351. https://doi.org/10.1002/lno.10634

Hoffmann, F., Radax, R., Woebken, D., Holtappels, M., Lavik, G., Rapp, H. T., Schläppy, M. L., Schleper, C., & Kuypers, M. M. M. (2009). Complex nitrogen cycling in the sponge *Geodia barretti*. *Environmental Microbiology*, *11*, 2228–2243.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal: Journal of Mathematical Methods in Biosciences*, *50*, 346–363.

Jaccard, P. (1908). Nouvelles recherches sur la distribution florale. *Bulletins – Société Vaudoise des Sciences Naturelles*, *44*, 223–270.

Kassambara, A., & Mundt, F. (2020). factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0. 7. https://CRAN.project.org/package=factoextra

Katoh, K., Misawa, K., Kuma, K. I., & Miyata, T. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, *30*, 3059–3066.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*, 772–780.

Landry, Z., Swan, B. K., Herndl, G. J., Stepanauskas, R., Giovannoni, S. J., Aluwihare, L., Santoro, A., & Northen, T. (2017). SAR202 genomes from the dark ocean predict pathways for the oxidation of recalcitrant dissolved organic matter. *MBio*, *8*, e00413-17.

Lane, D. J. (1991). 16S/23S rRNA sequencing. *Nucleic Acid Techniques in Bacterial Systematic*, *160*, 115–175. https://doi.org/10.1007/s00227-012-2133-0

Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L., & Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceedings of the National Academy of Sciences*, *82*, 6955–6959.

Liu, S., Baetge, N., Comstock, J., Opalk, K., Parsons, R., Halewood, E., English, C. J., Giovannoni, S., Bolaños, L. M., Nelson, C. E., Vergin, K., & Carlson, C. A. (2020). Stable isotope probing identifies bacterioplankton lineages capable of utilizing dissolved organic matter across a range of bioavailability. *Frontiers in Microbiology*, *11*, 1–27.

Luter, H. M., Widder, S., Botté, E. S., Abdul Wahab, M., Whalan, S., Moitinho-Silva, L., Thomas, T., & Webster, N. S. (2015). Biogeographic variation in the microbiome of the ecologically important sponge, *Carteriospongia foliascens*. *PeerJ*, *3*, e1435.

Maggioni, F., Bell, J. J., Pujo-Pay, M., Shaffer, M., Cerrano, C., Lemonnier, H., Letourneur, Y., & Rodolfo-Metalpa, R. (2023). Sponge organic matter recycling: Reduced detritus production under extreme environental conditions. *Marine Pollution Bulletin*, *190*, 114869.

Maggioni, F., Pujo-Pay, M., Aucan, J., Cerrano, C., Calcinai, B., Payri, C., Benzoni, F., Letourneur, Y., & Rodolfo-Metalpa, R. (2021). The Bouraké semi-enclosed lagoon (New Caledonia)- a natural laboratory to study the lifelong adaptation of a coral reef ecosystem to extreme environmental conditions. *Biogeosciences*, *18*, 5117–5140.

McMurray, S. E., Stubler, A. D., Erwin, P. M., Finelli, C. M., & Pawlik, J. R. (2018). A test of the sponge-loop hypothesis for emergent Caribbean reef sponges. *Marine Ecology Progress Series*, *588*, 1–14.

Moitinho-Silva, L., Nielsen, S., Amir, A., Gonzalez, A., Ackermann, G. L., Cerrano, C., Astudillo-Garcia, C., Easson, C., Sipkema, D., Liu, F., Steinert, G., Kotoulas, G., McCormack, G. P., Feng, G., Bell, J. J., Vicente, J., Björk, J. R., Montoya, J. M., Olson, J. B., … Thomas, T. (2017). The sponge microbiome project. *Gigascience*, *6*, 1–7.

Morrow, K. M., Bourne, D. G., Humphrey, C., Botté, E. S., Laffy, P., Zaneveld, J., Uthicke, S., Fabricius, K. E., & Webster, N. S. (2015). Natural volcanic CO_2 seeps reveal future trajectories for host-microbial associations in corals and sponges. *The ISME Journal*, *9*, 894–908. https://doi.org/10.1038/ismej.2014.188

Nelson, C. E., & Carlson, C. A. (2012). Tracking differential incorporation of dissolved organic carbon types among diverse lineages of Sargasso Sea bacterioplankton. *Environmental Microbiology*, *14*, 1500–1516.

Oksanen, J., Guillaume Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). Vegan: Community ecology package (version 2.5–6). *The Comprehensive R Archive Network*. https://cran.r-project.org/package=vegan

Olinger, L. K., Strangman, W. K., McMurray, S. E., & Pawlik, J. R. (2021). Sponges with microbial symbionts transform dissolved organic matter and take up organohalides. *Frontiers in Marine Science*, *8*, 1–13.

Pielou, E. C. (1966). The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology*, *13*, 131–144.

Pita, L., Rix, L., Slaby, B. M., Franke, A., & Hentschel, U. (2018). The sponge holobiont in a changing ocean: From microbes to ecosystems. *Microbiome*, *6*, 1–18. https://doi.org/10.1186/s40168-018-0428-1

Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). Fasttree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution*, *26*, 1641–1650.

Ribes, M., Calvo, E., Movilla, J., Logares, R., Coma, R., & Pelejero, C. (2016). Restructuring of the sponge microbiome favors tolerance to ocean acidification. *Environmental Microbiology Reports*, *8*, 536–544. https://doi.org/10.1111/1758-2229.12430

Schmitt, S., Angermeier, H., Schiller, R., Lindquist, N., & Hentschel, U. (2008). Molecular microbial diversity survey of sponge reproductive stages and mechanistic insights into vertical transmission of microbial symbionts. *Applied and Environmental Microbiology*, *74*, 7694–7708.

Shannon, C. E., & Weaver, W. (1949). The theory of mathematical communication. *International Business*, *3*, 31–32.

Sharp, K. H., Eam, B., Faulkner, D. J., & Haygood, M. G. (2007). Vertical transmission of diverse microbes in the tropical sponge *Corticium* sp. *Applied and Environmental Microbiology*, *73*, 622–629.

Sheen, J. (1996). Ca2+−dependent protein kinases and stress signal transduction in plants. *Science*, *274*, 1900–1902.

Simpson, E. (1949). Measurment of diversity. *Nature*, *163*, 688.

Sørensen, T. J. (1948). *A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons*. I kommission hos E. Munksgaard.

Steinert, G., Taylor, M. W., Deines, P., Simister, R. L., de Voogd, N. J., Hoggard, M., & Schupp, P. J. (2016). In four shallow and mesophotic tropical reef sponges from Guam the microbial community largely depends on host identity. *PeerJ*, *4*, e1936. https://doi.org/10.7717/peerj.1936

Taylor, M. W., Radax, R., Steger, D., & Wagner, M. (2007). Sponge-associated microorganisms: Evolution, ecology, and biotechnological potential. *Microbiology and Molecular Biology Reviews*, *71*, 295–347.

Thomas, T., Moitinho-Silva, L., Lurgi, M., Björk, J. R., Easson, C., Astudillo-García, C., Olson, J. B., Erwin, P. M., López-Legentil, S., Luter, H., Chaves-Fonnegra, A., Costa, R., Schupp, P. J., Steindler, L., Erpenbeck, D., Gilbert, J., Knight, R., Ackermann, G., Victor Lopez, J., … Webster, N. S. (2016). Diversity, structure and convergent evolution of the global sponge microbiome. *Nature Communications*, *7*, 1–12. https://doi.org/10.1038/ncomms11870

Turon, M., Cáliz, J., Triadó-Margarit, X., Casamayor, E. O., & Uriz, M. J. (2019). Sponges and their microbiomes show similar community metrics across impacted and well-preserved reefs. *Frontiers in Microbiology*, *10*, 1961–1974. https://doi.org/10.3389/fmicb.2019.01961

Webster, N. S., & Reusch, T. B. (2017). Microbial contributions to the persistence of coral reefs. *The ISME Journal*, *11*, 2167–2174. https://doi.org/10.1038/ismej.2017.66

Webster, N. S., Taylor, M. W., Behnam, F., Lücker, S., Rattei, T., Whalan, S., Horn, M., & Wagner, M. (2010). Deep sequencing reveals exceptional diversity and modes of transmission for bacterial sponge symbionts. *Environmental Microbiology*, *12*, 1–13. https://doi.org/10.1111/j.1462-2920.2009.02065.x

Wickham, H., Chang, W., & Wickham, M. H. (2016). Package 'ggplot2'. *Create elegant data visualisations using the grammar of graphics*. version 2.1, *2*, 1–189. http://ggplot2.tidyverse.org

Wilkinson, C. R. (1983). Net primary productivity in coral reef sponges: Published by: American Association for the Advancement of Science Stable. *Science*, *219*, 410–412. http://www.jstor.com/stable/1689940

Woebken, D., Fuchs, B. M., Kuypers, M. M., & Amann, R. (2007). Potential interactions of particle-associated anammox bacteria with bacterial and archaeal partners in the Namibian upwelling system. *Applied and Environmental Microbiology*, *73*, 4648–4657.

Zhang, F., Jonas, L., Lin, H., & Hill, R. T. (2019). Microbially mediated nutrient cycles in marine sponges. *FEMS Microbiology Ecology*, *75*, 1–14.

Zheng, W., Tsompana, M., Ruscitto, A., Sharma, A., Genco, R., Sun, Y., & Buck, M. J. (2015). An accurate and efficient experimental approach for characterization of the complex oral microbiota. *Microbiome*, *3*, 1–11.

SUPPORTING INFORMATION

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

How to cite this article: Maggioni, F., Stenger, P.-L., Jourand, P., & Majorel, C. (2023). The phylum Chloroflexi and their SAR202 clade dominate the microbiome of two marine sponges living in extreme environmental conditions. *Marine Ecology*, *00*, e12757. https://doi.org/10.1111/maec.12757