



Sponge-associated microbes in the twilight zone of Curaçao

D. F. R. Cleary¹ · D. J. van Bendegom² · N. C. M. Gomes¹ · N. J. de Voogd^{3,4}

Received: 27 September 2023 / Accepted: 20 April 2024
© The Author(s) 2024

Abstract

Mesophotic reefs, located in the "Twilight Zone" of the ocean, are coral reefs that exist in relatively deep waters ranging from approximately 30 to 150 m below the surface. These reefs are situated just beyond the reach of conventional SCUBA diving and are typically explored using advanced diving techniques or with the aid of submersibles. In the present study, we used a state-of-the-art submersible to sample 26 sponge specimens belonging to 11 species. High (HMA) or low (LMA) microbial abundance status was assigned to species based on TEM imagery. Prokaryotic communities associated with these sponges were, furthermore, assessed using high-throughput sequencing. Proteobacteria, Chloroflexi, Actinobacteriota, and Acidobacteriota were the most abundant phyla overall. HMA/LMA status proved to be a highly significant predictor of prokaryotic composition. HMA sponges also tended to be more diverse in terms of richness and evenness than LMA sponges. 14 predictor-classes were identified using an exploratory technique based on machine learning including classes within the phyla Chloroflexi (e.g., Dehalococcoidia and JG30-KF-CM66) and Acidobacteriota (Thermoanaerobaculia and Subgroups 11 and 21). Previous studies have demonstrated the prevalence of the HMA/LMA dichotomy in shallow waters and, recently, the deep sea. Our results demonstrate its prevalence in the mesophotic realm.

Keywords 16S · Bacteria · Caribbean · Composition · Curaçao · Porifera

Non-technical summary: Mesophotic reefs are typically just outside the reach of normal SCUBA diving. In the present study, a submersible was used to explore sponges inhabiting the mesophotic reefs of Curaçao. TEM imagery and next-generation sequencing were subsequently used to study prokaryotic composition and to assess to what extent the sponges sampled fit within the HMA/LMA dichotomy.

✉ D. F. R. Cleary
cleary@ua.pt

✉ N. J. de Voogd
n.j.de.voogd@biology.leidenuniv.nl

¹ Department of Biology & CESAM, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal

² Department of Biology, University of Amsterdam, Amsterdam, Netherlands

³ Naturalis Biodiversity Center, Leiden, The Netherlands

⁴ Institute of Biology (IBL), Leiden University, Leiden, the Netherlands

1 Introduction

Mesophotic reefs encompass a depth range of approximately 30 to 150 m below the surface of the ocean. They are diverse and can be found in tropical and subtropical regions worldwide, particularly in areas where the ocean floor slopes steeply. Although they receive less light than shallower reefs, mesophotic reefs still support an array of photosynthetic organisms that provide the foundation of the mesophotic food web in addition to receiving energy from productive surface waters (Keesing et al. 2012; Kramer et al. 2022). Mesophotic reefs also often contain unique and diverse marine life adapted to the lower light levels found at these depths (Lesser et al. 2019). In a global study, Lesser et al. (2019) found evidence of a break at 60 m for multiple taxa marking a transition from shallow-water to mesophotic communities. The mesophotic zone also serves as a refuge for species that are threatened in shallower waters, and may contain previously unknown species (Rocha et al. 2018). Despite their potential ecological significance, mesophotic reefs remain understudied and poorly understood. Because they are difficult to access, much of what we know about these reefs comes from surveys using remotely operated vehicles, submersibles, technical divers or dredging (Bongaerts

et al. 2015; Glasl et al. 2017; Tamir et al. 2019; Ferrier-Pagès et al. 2022; Cleary et al. 2023a).

Mesophotic reefs have received increasing attention in recent years due to their potential importance as refuges for marine life (Bridge et al. 2013; Rocha et al. 2018; Bongaerts and Smith 2019). They are often thought of as a buffer zone for shallower reefs, acting as a source of coral larvae and other organisms that can help repopulate damaged or destroyed shallow reefs. They also provide habitats for a range of marine species, including corals, crustaceans, and sponges, many of which are unique to these deeper waters (Bongaerts et al. 2013; Estronza et al. 2017; Macartney et al. 2021). For example, in a recent study, 31 sponge species were discovered and thirteen were described from the mesophotic zone of Bonaire and Klein Curaçao using the manned 'Curasub' submersible (van Soest et al. 2014). The majority of the listed sponge species appear to be restricted to the mesophotic reef zone.

Species inhabiting both shallow-water and mesophotic zones may also differ in key traits. In the Mediterranean, Idan et al. (2020), for example, showed that shallow-water and mesophotic populations of the sponge species *Chondrosia reniformis* and *Axinella polypoides* both adapted their reproduction to changes in temperature and depth. In shallow waters, both species showed seasonality, indicating that temperature affected their reproductive activities. However, in the mesophotic zone where the temperature changes are much smaller, neither species demonstrated seasonality. *Chondrosia reniformis* had lower fecundity in the mesophotic zone and likely invested more in asexual reproduction by budding. On the other hand, *A. polypoides* in the mesophotic zone had enhanced and continuous asynchronous sexual reproduction throughout the year. Sponges found in mesophotic zones also tend to have higher proportions of heterotrophic as opposed to phototrophic symbionts, which can absorb dissolved organic matter from the water column rather than relying on light energy (Olson and Kellogg 2010; Olson and Gao 2013; Morrow et al. 2016; Steinert et al. 2016; Macartney et al. 2021; Cleary et al. 2023a).

Sponges and their microbial symbionts have been widely studied as models for host-microbe interactions in the marine environment (Taylor et al. 2007; Hentschel et al. 2012; Cleary et al. 2013, 2015, 2018, 2019a, b; Thomas et al. 2016; Swierts et al. 2018; Busch et al. 2022). Earlier studies showed marked differences in microbial densities among different sponge species and initially referred to sponges with high microbial densities as 'bacterial' sponges and those with low microbial densities as 'non-symbiont' sponges, which later became 'high microbial abundance' (HMA) and 'low microbial abundance' (LMA) sponges. The differences in microbial densities between both groups can be substantial varying from 10^5 – 10^6 microbial cells/g of tissue in LMA sponges

to 10^8 – 10^{10} microbial cells/g of tissue in HMA sponges (Hentschel et al. 2006).

Sponge microbiome studies also showed that in addition to differing in abundance, bacterial communities of LMA sponges were less diverse and compositionally more similar to bacterial communities found in seawater (Thacker and Freeman 2012; Blanquer et al. 2013; Moitinho-Silva et al. 2014; Bayer et al. 2014). This led to speculation that these communities were mainly, or exclusively, derived from seawater due to the pumping activities of the sponges and that there was no resident bacterial community to speak of. This was contrasted with the diverse bacterial communities found in HMA species and their marked differences with the bacterioplankton and bacterial communities from LMA sponges (Schmitt et al. 2011; Erwin et al. 2015; Chaib De Mares et al. 2017; Cleary et al. 2019a; Cleary et al. 2020; 2021). Subsequent studies, however, have shown that LMA species may host bacterial communities that are similar to, but not identical to those found in water. Multi-taxon studies have also shown that there can be considerable compositional variation among LMA species (Cleary et al. 2020; 2021; Busch et al. 2022).

HMA species, in contrast to LMA species, tend to house less variable bacterial communities with less variation within and among species than found among LMA species. Several studies have, furthermore, noted the pronounced over-representation of certain phyla in HMA sponges (Bayer et al. 2014; Cleary et al. 2015; de Voogd et al. 2015, 2019; Polónia et al. 2015; Moitinho-Silva et al. 2017). These include the phyla Chloroflexi, Poribacteria, Actinobacteriota, Acidobacteriota, PAUC34f, and Gemmatimonadota (Bayer et al. 2014; Moitinho-Silva et al. 2017). These phyla are much less prevalent, or even absent, in typical LMA species, which instead are usually dominated by Proteobacteria, or cyanobacteria in the case of cyanosponges such as *Lamellodysidea herbacea* and *Terpios hoshinota* (Podell et al. 2020; Rützler and Muzik 1993). Finally, it should be noted that various studies have found LMA and HMA sponges to differ in other traits, particularly in relation to morphology and physiology. For example, LMA sponges tend to pump greater volumes of water and have larger aquiferous canals and choanocyte chambers than HMA sponges whereas HMA sponges tend to have higher mesohyl densities. Certain polyketide synthase genes have also only been found in HMA sponges (Vacelet and Donadey 1977; Weisz et al. 2007; Hochmuth et al. 2010). Despite the above, it is not always possible to clearly assign HMA or LMA status based on electron microscopy, microbial composition based on 16S rRNA gene sequencing, or morphological traits.

Although well-studied in shallow waters, fewer studies have assessed the prevalence of HMA and LMA sponges in deeper waters. Most of the sponges, which have been

assigned HMA or LMA status (e.g., Gloeckner et al. 2014; Moitinho-Silva et al. 2017), thus far, are shallow-water species with a few extending to the mesophotic zone such as *Cinachyrella kuekenthali* or true deep-sea species such as *Geodia barretti*, although its status appears to have been only based on amplicon sequencing data (Radax et al. 2012). Recently, Busch et al. (2022) also provided evidence of the HMA/LMA dichotomy in the deep sea in addition to identifying a novel type of LMA community in glass sponges. However, the deep sea, in contrast to the mesophotic zone, is truly dark and although the deep sea appears even less accessible than the waters up to 150 m, more studies have been carried out on the microbiomes of deep sea sponges (Jackson et al. 2013; Kennedy et al. 2014; Steinert et al. 2016; Tian et al. 2016; Busch et al. 2022; Steffen et al. 2022; Garritano et al. 2023) than those of mesophotic sponges (e.g., Steinert et al. 2016; Indraningrat et al. 2022). Studies of sponge microbiomes in the mesophotic zone have, furthermore, focused on single or limited sets of species and the impact of depth on microbiome composition. For instance, specimens of Giant Barrel sponges (*Xestospongia muta*) in the mesophotic zone were enriched with Chloroflexi as opposed to Cyanobacteria in shallower waters (Cleary et al. 2023a). To the best of our knowledge, no study has, hitherto, compared the microbiomes of multiple sponge species only from the mesophotic zone. Our study, thus, complements previous studies of the prevalence of the HMA/LMA dichotomy in shallow and deep-sea environments.

In the present study, 26 sponge samples were collected using a submersible at two sites off the islands of Curaçao and Klein Curaçao along depths ranging from 90 to 188 m (note a single sample was collected at 13 m depth). Here, we provide the first in-depth assessment of the prokaryotic communities of 11 sponge species from the mesophotic zone of Curaçao. Most of these sponges have, furthermore, only been recorded from the mesophotic zone (van Soest et al. 2014). The main hypothesis of the present study was to test if the HMA/LMA dichotomy holds for mesophotic zone sponges. HMA or LMA status was assigned to species based on TEM imaging and HMA/LMA status was used as a predictor for variation in sponge-associated prokaryotic composition. In addition to the above, we used an exploratory technique based on machine learning to identify taxonomic indicators of HMA or LMA status.

2 Material and methods

2.1 Sample collection and sites

Curaçao is a small country in the Caribbean, approximately 65 kms from the Venezuelan coast, with a surface

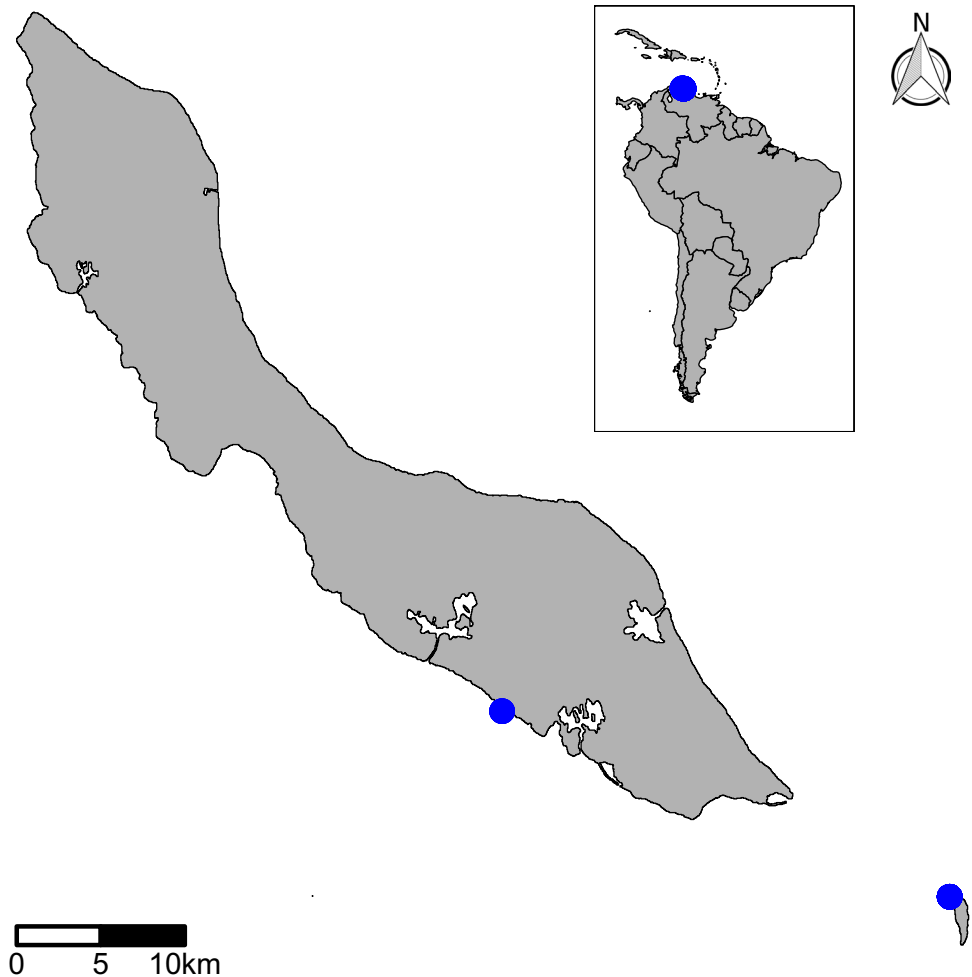
area of ~444 km². It consists of the main island of Curaçao and a small, uninhabited island, Klein Curaçao. In the present study, samples were collected off the southern coast of the main island of Curaçao and off of Klein Curaçao with the Curasub submersible from August 29th till October 31st, 2018 (Fig. 1 and Supplementary Data 1). All samples, but one, were collected between 90 and 188 m depth; a single specimen of *Xestospongia muta* was collected at 13 m depth. The Curasub (Fig. 2) was operated from either the support vessel RV Chapman or directly from the substation at the Curaçao Sea Aquarium. Sponges were visually located and photographed with a GoPro black 7 or Olympus G6 from inside the Curasub. Samples were collected using manipulator arms. The sponges were placed into a basket in front of the Curasub during the dive. Upon surfacing, sponge samples were transferred from the collecting basket to buckets filled with seawater. In the laboratory on board of RV Chapman, the sponges were photographed and a cylindrical section of approximately 1 by 2 cm was cut from each specimen containing both the ectosome and choanosome, and placed in a vial containing 96% alcohol or fixed in a mixture of 0,5% formalin, 1,25% glutaraldehyde in 0.22 uM filtered sea water and transferred to a saline buffer after 48 h. Care was taken to include the surface and interior of each sponge segment. The vials were shipped to the Netherlands and stored at -20 °C until DNA extraction. The sponges were identified by Nicole de Voogd using classical morphological characters. Voucher specimens have been deposited in the sponge collection of Naturalis Biodiversity Center (as RMNH POR#).

The species investigated in the present study belonged to 5 orders and 11 species. The species sampled were *Ageles flabelliformis* (Carter, 1883), *Phakellia folium* Schmidt, 1870, *Acanthella cubensis* (Alcolado, 1984), *Neopetrosia ovata* van Soest et al. 2014, *Petrosia* sp., *Petrosia* aff. *weinbergi* van Soest, 1980, *Xestospongia muta* (Schmidt, 1870), *Calthropella (Pachataxa) lithistina* (Schmidt, 1880), *Cinachyrella kuekenthali* (Uliczka, 1929), *Coralistes typus* Schmidt, 1870, and *Neophrissospongia jorgeorum* Dias, Kelly & Pinheiro, 2023 (Fig. 2). Between 1 and 3 samples were collected per species.

2.2 Transmission electron microscopy

Several pieces of fixed sponge material were cut into approximately 1 × 1 × 2 mm pieces. These were rinsed three times with 0.1 M sodium cacodylate buffer, fixed in 2% osmium tetroxide in the dark for two hours, and rinsed again three times with 0.1 M sodium cacodylate buffer. Subsequently, the material was stained in the dark for an hour in 3% uranyl acetate (aqueous solution). The

Fig. 1 Map of study area showing location of sample sites (blue circles). The inset shows the location of Curaçao with respect to South America



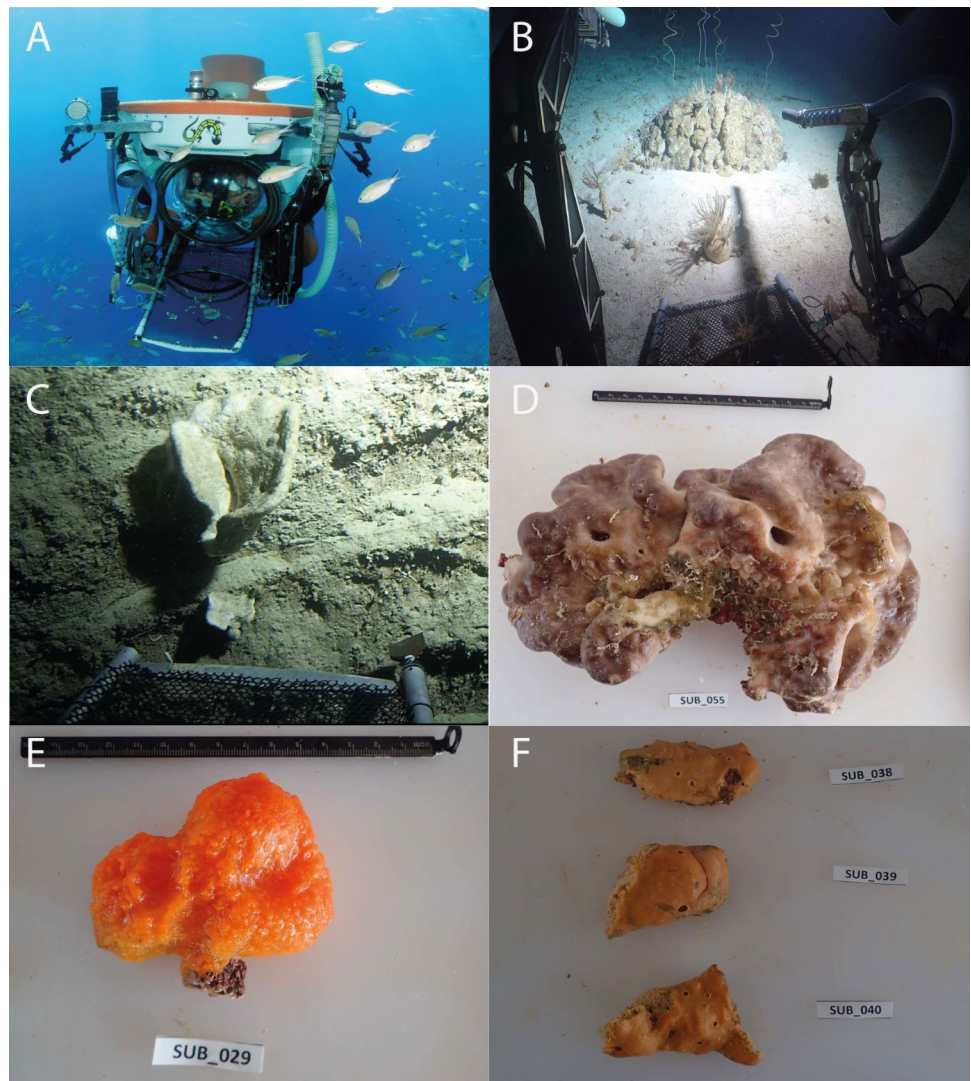
samples were dehydrated in a series of ethanol concentrations (30%, 50%, 70%, 96%, and twice in absolute ethanol). After dehydration, the material was placed into acetonitrile twice for 15 min and then in a 1 to 1 epon 812 acetonitrile solution overnight. The next day, the epon: acetonitrile solution was refreshed and the mixture was centrifuged slowly for 3 h at a speed of ~ 2 RMP for three hours. Thereafter, the samples were placed twice in a vacuum for 20 min and afterwards in a stove at 60 °C for 48 h. After the embedding process, three separate samples were sectioned into thin slices (80 nm) with a microtome and post-stained for 20 min with uranyl acetate and for 15 min with lead citrate. The samples were imaged using transmission electron microscopy (JEM 1400 Plus, JEOL Ltd., Tokyo, Japan).

2.3 DNA extraction and next-generation sequencing analysis

DNA was extracted using the Qiagen DNeasy Powersoil extraction kit (Qiagen, Venlo, the Netherlands). Approximately 250 mg of tissue was used from each sponge

sample; tissue was taken from all sides of the specimen (outside to core, and if applicable top, middle and bottom of the sample). The manufacturer's protocol was followed with the exception of the initial vortexing step, which was carried out using the Qiagen TissueLyser II (Qiagen NV, Hilden, Germany). Sponge tissue was cut into small pieces using sterilised tweezers and scalpel blades, and transferred to PowerBead Pro tubes containing ceramic and silica beads of different sizes. An extraction blank, in which no tissue was added to the PowerBead Pro tubes, was also included. The library preparation was conducted using a two-step PCR protocol for all samples in addition to two negative controls (mQ water instead of template DNA) and the extraction blank. For the first PCR, the V3-V4 regions of the 16S rRNA gene were targeted and amplified using the primers 314F/ S-D-Bact-0785-a-A-21 (5'-CCTACGGGNGGCWGCAG-3'/5'-GACTACHVGGG TATCTAATCC-3'; Klindworth et al. 2013) with added 5' Nextera transposase adaptors using the KAPA HiFi Hot-Start Ready Mix PCR Kit with a T100 Thermal Cycler (Bio-Rad, Hercules, CA, United States). The following PCR conditions were used: initial denaturation at 95 °C

Fig. 2 **A.** View of the submersible “Curasub”, **B.** view from the interior of submarine at a depth of 120 m, **C.** *Calthropella lithistina* attached to a steep wall, **D.** specimen of *Neophrissospongia jorgeorum* after collection, **E.** specimen of *Acanthella cubensis* after collection, and **F.** fragments of 3 individuals of *Petrosia* aff. *weinbergi* after collection



for 3 min, 30 cycles of denaturation at 98 °C for 20 s, annealing at 55 °C for 30 s, followed by extension at 72 °C for 30 s. The final extension was carried out at 72 °C for 1 min. PCR success was confirmed on an E-Gel™ (agarose gels at 2%), and the absence of amplification was validated for the negative controls and the extraction blank. PCR products were then cleaned with NucleoMag NGS-Beads (bead volume at 0.9 times the total volume of the sample, Macherey Nagel, Düren, Germany) using the VP 407AM-N 96 Pin Magnetic Bead Extractor stamp (V&P Scientific, San Diego, CA, United States). For the second PCR, the cleaned PCR products (1 µL each) were amplified and labelled using the MiSeq Nextera XT DNA library preparation kit (Illumina, San Diego, CA, United States) with the same thermal cycling scheme limited to 8 cycles. PCR products were then analysed with the Fragment Analyser Agilent 5300 using the DNF-910–33 dsDNA Reagent Kit (35–1,500 bp) protocol (Agilent Technologies, Santa Clara, CA, United States) to confirm successful labelling

of the DNA fragments. Negative controls and extraction blanks remained negative after this step. Pooling at equimolar concentration was performed with QIAgility 2 (Qiagen, Hilden, Germany). The final pool was then cleaned with NucleoMag NGSBeads, eluted in Milli-Q and the DNA concentration was verified using Tapestation 4150 (Kit HSD 5000, Agilent Technologies, Santa Clara, CA, United States). Paired-end sequence reads were generated with an Illumina MiSeq v3 PE300 platform at BaseClear B.V. (Leiden, Netherlands). FASTQ read sequence files were generated using bcl2fastq version 2.20 (Illumina). Initial quality assessment was based on data passing the Illumina Chastity filtering. Subsequently, reads containing PhiX control signal were removed using an in-house filtering protocol. In addition to this, reads containing (partial) adapters were clipped (up to a minimum read length of 50 bps). The second quality assessment was based on the remaining reads using the FASTQC quality control tool version 0.11.8. The DNA sequences generated in

this study can be downloaded from NCBI BioProject Id: PRJNA715750.

2.4 16S rRNA gene sequencing analysis

The 16S rRNA amplicon libraries were analysed using QIIME2 (version 2019.7; Bolyen et al. 2019). Raw data were imported yielding a demultiplexed ‘qza’ data file (artifact). The DADA2 plugin (Callahan, et al. 2016) in QIIME 2 was subsequently used to trim sequences (final length 400 nt). The DADA2 analysis yielded output archives containing an OTU (at a 100% similarity threshold, also known as amplicon sequence variant or ‘ASV’) table, denoising stats, and a fasta file of representative sequences. The feature-classifier plugin with the extract-reads method was then used with the *i*-sequences argument set to *silva-138-99-seqs.qza*. This was followed by the feature-classifier plugin with the fit-classifier-naive-bayes method and the *i*-reference-taxonomy method set to *silva-138-99-tax.qza*. Both *silva-138* files can be obtained from <https://docs.qiime2.org/2020.8/data-resources/?highlight=silva>. The feature-classifier plugin was then used with the classify-sklearn method and the *i*-reads argument was set to the representative sequences file generated by the DADA2 analysis to produce a table with taxonomic assignments for all OTUs. Finally, mitochondria, chloroplasts, and Eukaryota were filtered out using the QIIME 2 taxa plugin with the filter-table method. All OTUs unclassified at Domain and Phylum level were also removed. The final dataset consisted of 333005 sequences and 4094 OTUs. The OTU and taxonomy tables were later merged in R 4.1.2 (R Core Team 2021). Supplementary Data 2 contains all OTU counts per sample and taxonomic assignments of all OTUs. The 50 most abundant OTUs overall, thus including all samples, are presented in Supplementary Data 3.

2.5 BLAST

Accession numbers of closely related organisms to selected OTUs were obtained using NCBI Basic Local Alignment Search Tool (BLAST) (<https://www.ncbi.nlm.nih.gov/books/NBK62051/>). In the present study, the sequence similarities ranged from 87.82% to 100% (Supplementary data 3).

2.6 Statistical analyses

The OTU counts table was used in R to compare diversity and higher taxon abundance among sponge host species. Diversity indices, namely, rarefied richness, evenness, Shannon's H' and Fisher's alpha, were obtained using the rarefy and diversity functions from the vegan 2.6–4 (Oksanen et al. 2022) package in R. Variation in prokaryotic composition

among sponge host species was visualised with Principal Coordinates Analysis (PCO). For the PCO, a Bray–Curtis distance matrix was first obtained using the phyloseq 1.44 package (McMurdie and Holmes 2013) whereby the count data were rarefied using the rarefy_even_depth function with the sample.size argument set to the minimum sample size (12206 in the present study) and subsequently \log_{10} transformed. The ordinate function in phyloseq was used to run the PCO analysis and generate data for the ordination. A heatmap was produced showing variation in OTU abundance using the pheatmap function from the pheatmap 1.0.12 package (Kolde 2019).

Sponge species were assigned HMA or LMA status based on a qualitative assessment of TEM images. We tested if HMA/LMA status was a significant predictor of prokaryotic composition using the adonis function from the vegan 2.6–4 package in R. We subsequently used the multipatt function from the indicpecies package in R (De Cáceres and Legendre 2009) and an exploratory technique based on machine learning, Boruta (Kursa et al. 2010), to identify bacterial classes, which distinguished between HMA and LMA sponges. Prior to the Boruta analysis, highly correlated classes (>0.8) were removed from the data frames. The Boruta function from the Boruta package in R (Kursa and Rudnicki 2010) was then used with the microbial abundance status (HMA versus LMA) as response variable and bacterial class as feature (predictive variable). The doTrace argument in the Boruta function was set to 2 and the maxRuns argument set to 1000; other arguments used default values. A more detailed description of the Boruta method can be found in Cleary et al. (2023b).

3 Results

In the present study, 10 out of the 11 sponge species were investigated using TEM imaging to visualise microbial density (Fig. 3). The species *N. ovata*, *C. lithistina*, *C. typus*, *Petrosia* aff. *weinbergi*, *Petrosia* sp. and *N. jorgeorum* contained dense and morphologically diverse microorganisms indicative of HMA status (Supplementary Fig. 1). We did not investigate *X. muta* because multiple specimens have already been investigated using TEM and its HMA status is well established. In contrast to the above, the species *A. cubensis*, *P. folium*, and *C. kuekenthali*, contained relatively low microbial densities indicative of LMA status (Supplementary Fig. 2). Although *A. flabelliformis* contained relatively large bacterial cells, the density of these cells was low, which also indicates LMA status (Supplementary Fig. 2). As evident in Fig. 3, HMA and LMA sponges separated along the first axis of a PCO ordination and HMA status was a highly significant predictor of prokaryotic composition (adonis: $F=4.529$, $P<0.001$, $R^2=0.751$).

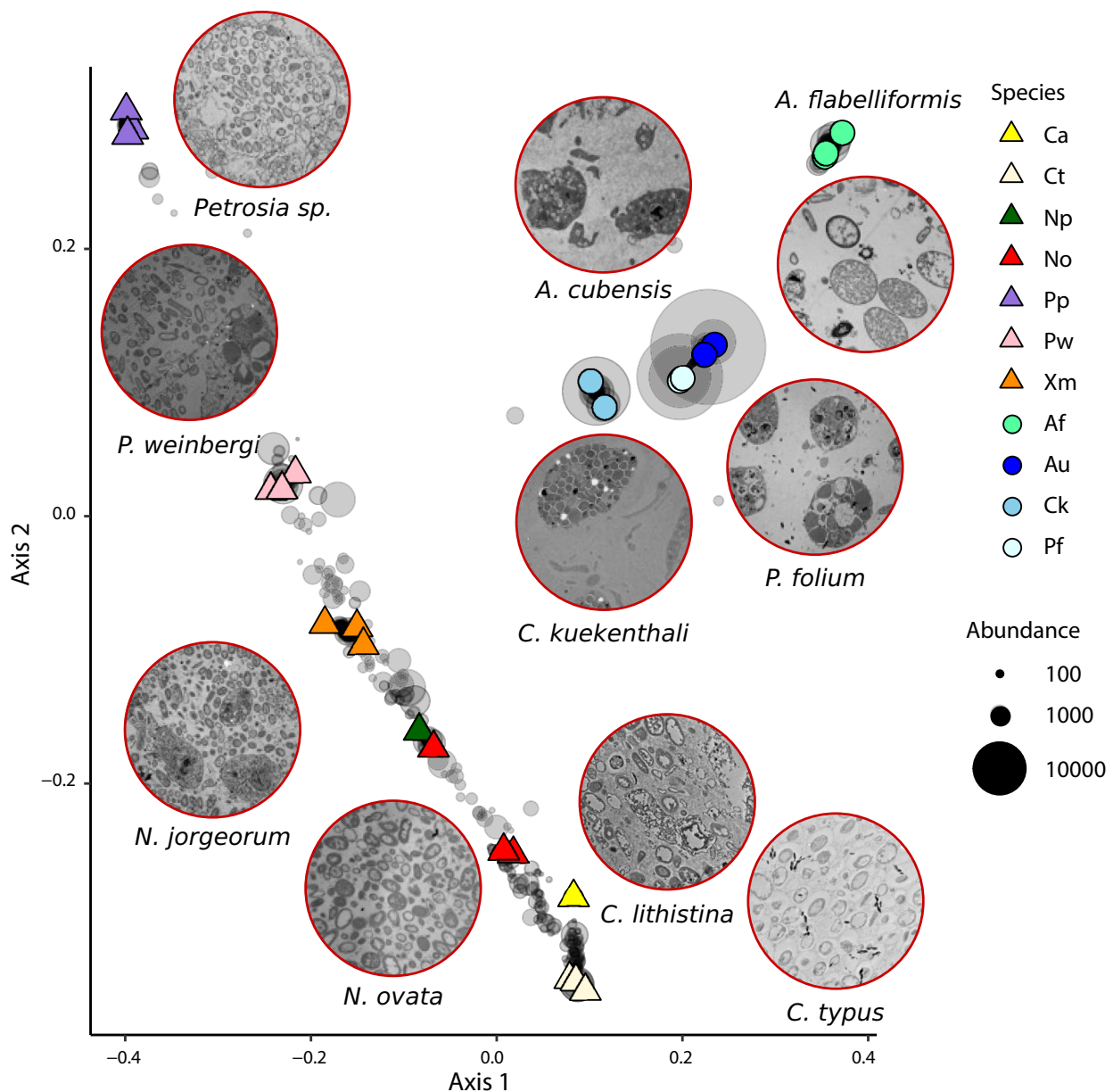


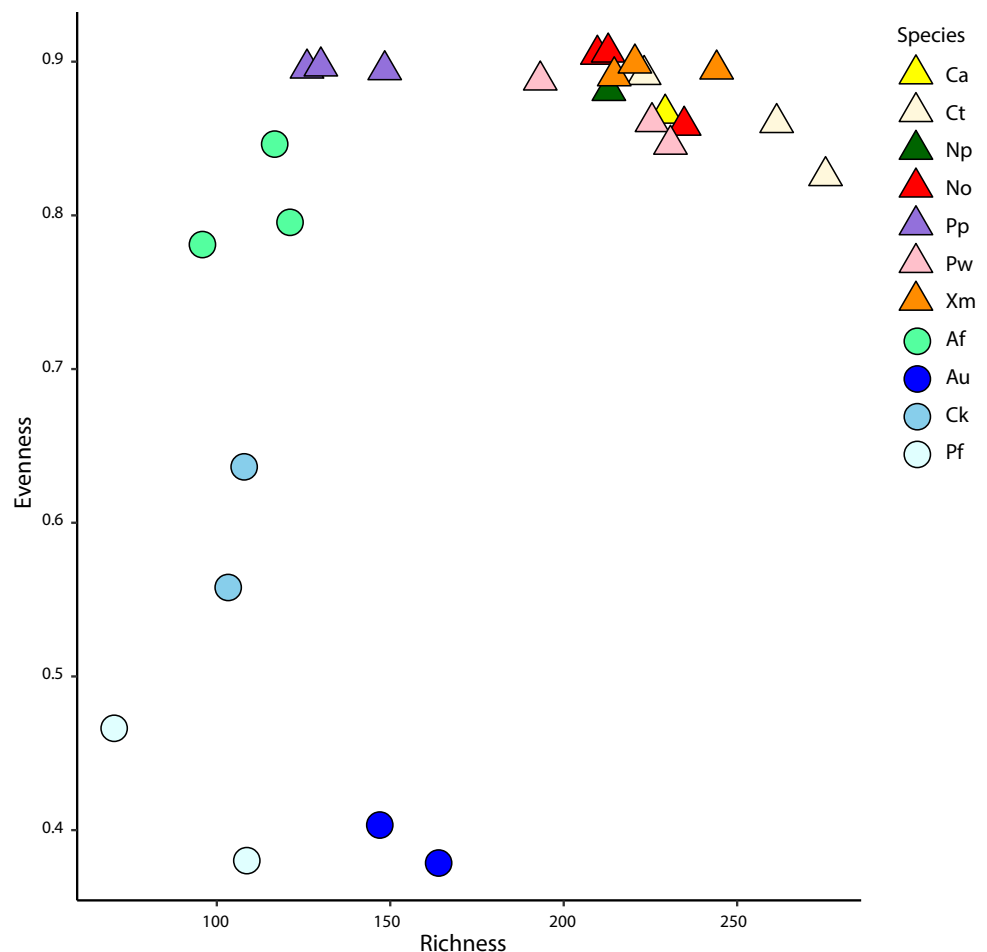
Fig. 3 Ordination showing the first two axes of the principal coordinates analysis (PCO) of OTU composition. Symbols are colour-coded and represent samples from different sponge host species as shown in the legend on the right side of the figure. Grey symbols represent weighted averages scores for OTUs. The symbol size is proportional to abundance (number of sequence reads). The legend symbols represent the following sponge species: Af: *Agelas flabelliformis*, Pf:

Phakellia folium, Au: *Acanthella cubensis*, No: *Neopetrosia ovata*, Pp: *Petrosia* sp., Pw: *Petrosia* aff. *weinbergi*, Xm: *Xestospongia muta*, Ca: *Calthropella lithistina*, Ck: *Cinachyrella kuekenthali*, Ct: *Coralistes typus*, and Np: *Neophrissospongia jorgeorum*. TEM images of all species, with the exception of *X. muta*, are placed adjacent to the sponge host species

LMA sponge species were also less diverse in terms of richness and evenness than HMA sponge species although the difference was less pronounced for *A. flabelliformis* (Fig. 4). The most abundant phyla across all samples were Proteobacteria (99256 sequences, 967 OTUs), Chloroflexi (76186 sequences, 879 OTUs), Actinobacteriota (43541 sequences, 306 OTUs), and Acidobacteriota (27074 sequences, 423 OTUs) (Supplementary Data 2). In terms of

bacterial class abundance (Fig. 5), all HMA species, with the exception of *N. jorgeorum*, were dominated by Dehalococcoidia (Chloroflexi). 98.8% of all sequences classified as Dehalococcoidia, furthermore, belonged to the SAR202 clade. The LMA sponge species were more variable as pertains to Class relative abundance. *Agelas flabelliformis*, for example, was similar to the HMA sponge species with respect to class representation. *Cinachyrella kuekenthali*, in

Fig. 4 Variation in richness and evenness among sponge species samples: The legend symbols represent the following sponge species: Af: *Agelas flabelliformis*, Pf: *Phakellia folium*, Au: *Acanthella cubensis*, No: *Neopetrosia ovata*, Pp: *Petrosia* sp., Pw: *Petrosia* aff. *weinbergi*, Xm: *Xestospongia muta*, Ca: *Calthropella* sp., Ck: *Cinachyrella kuekenthali*, Ct: *Corallistes typus*, and Np: *Neophrissospongia jorgeorum*



contrast, was dominated by Acidimicrobiia members followed by Gammaproteobacteria and Dadabacteriia. Both *A. cubensis* and *P. folium* were dominated by Gammaproteobacteria, with this most pronounced in *A. cubensis*. *Phakellia folium*, in turn, had the greatest Nitrospiria component.

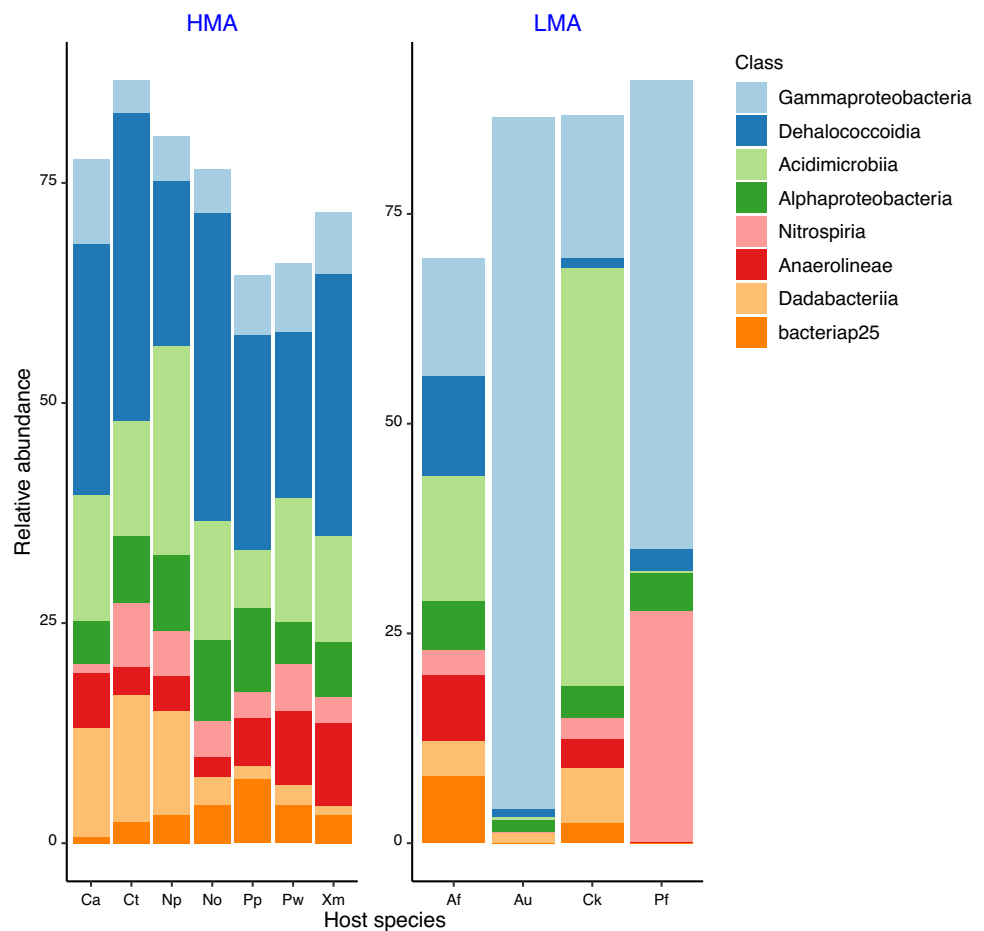
A heatmap of the 50 most abundant OTUs (representing 37.6% of all sequences) is shown in Fig. 6 including dendrograms of host species and OTUs based on hierarchical clustering. All host sponge species clustered according to HMA/LMA status. Supplementary Data 3 contains a list of these OTUs including taxonomic assignments and the results of the BLAST analyses. Nine OTUs, classified as Acidobacteriota, Spirochaetota, Alphaproteobacteria, SAR202 clade, and Nitrosococcales were enriched in HMA sponge species and formed a cluster in Fig. 6. All of these OTUs had high sequence similarities (> 99%) to organisms detected across a range of sponge species (Supplementary Data 3) including *Aplysina cauliformis*, *Astrosclera willeyana*, *Ircinia oros*, *Ircinia strobilina*, and *Xestospongia testudinaria*.

Other sets of OTUs were restricted to specific host sponge species as shown in Fig. 6. In the HMA sponge

species, four OTUs, for example, were restricted to *Petrosia* aff. *weinbergi*, which had high sequence similarities to organisms detected in one coral species (*Porites lutea*), and three sponge species (*X. muta*, *Ircinia felix*, and *I. strobilina*) (Supplementary Data 3). Five OTUs, classified as Actinobacteriota, Dadabacteria, and Nitrospirota were restricted to the lithistid sponges *C. lithistina* and *C. typus*. and had high sequence similarities (> 99%) to organisms detected in the sponges *Characella* sp., *Corallistes* sp., *Desmacidon* sp., and *Geodia barretti*.

In the LMA sponge species, seven OTUs, classified as Acidobacteriota, Actinobacteriota, Dadabacteria, Entotheonellaeota and Myxococcota, were restricted to *A. flabelliformis*. These had high sequence similarities to organisms obtained from host sponges identified as *Astrosclera willeyana*, *Geodia pachydermata*, *Agelas dilatata*, *Xestospongia exigua*, and *Isops phlegraei*. Three OTUs, classified as Gammaproteobacteria and Nitrospirota, were restricted to *P. folium*. and were similar to organisms obtained from the sponge species

Fig. 5 Stacked barplots of the mean relative abundances of the eight most abundant bacterial classes. The x-axis codes represent the following sponge species: Af: *Agelas flabelliformis*, Pf: *Phakellia folium*, Au: *Acanthella cubensis*, No: *Neopetrosia ovata*, Pp: *Petrosia* sp., Pw: *Petrosia* aff. *weinbergi*, Xm: *Xestospongia muta*, Ca: *Calthropella* sp., Ck: *Cinachyrella kuekenthali*, Ct: *Coralistes typus*, and Np: *Neophrissospongia jorgeorum*. The bars are colour-coded according to the bacterial Class as indicated by the legend



Axinella verrucosa and *Axinella corrugata* in addition to an organism obtained from a grass carp pond in China, but with a sequence similarity of only 88.3%. Four OTUs, classified as Gammaproteobacteria, genus *Nitrosopumilus*, and *Bdellovibrionota*, were restricted to *A. cubensis*. and were similar to organisms obtained from the sponge *Raspailia topsenti* and the coral *Favia* sp. (Fig. 6 and Supplementary Data 3).

The Boruta analysis yielded 14 significant class-level predictors of which eight with the greatest importance values are presented in Fig. 7. The rest of the significant class-level predictors are presented in Supplementary Fig. 3. Results of the Boruta and IndVal ('indicspecies') analyses are provided in Supplementary Data 4 and 5. Six of the eight class-level predictors were also significant using the IndVal approach as outlined in the methods section, the exceptions being Dehalococcoidia and the BD2-11 terrestrial group. In contrast, Subgroup 21, Subgroup 11, JG30-KF-CM66, and Thermoanaerobaculia, with the greatest importance values in the Boruta analysis, were all highly significant ($P < 0.001$) predictors based on the IndVal analysis. All presented class-level predictors, with the exception of *Bdellovibrionota*, were more abundant in HMA sponge species.

4 Discussion

The results of the present study using TEM imagery and next-generation sequencing clearly demonstrate the prevalence of the HMA/LMA dichotomy in sponges inhabiting the mesophotic zone of Curaçao. We, furthermore, identified pronounced variation in diversity, and higher taxon abundance, in addition to identifying closely related organisms to the abundant OTUs in selected sponge species. Our results also showed that HMA or LMA status based on TEM imagery is a highly significant predictor of prokaryotic composition. For example, the first axis of the PCO ordination separated sponge species identified as HMA using TEM imagery in addition to the known HMA *X. muta* (Gloeckner et al. 2014) from species identified as LMA, namely *Phakellia folium*, *A. cubensis*, *C. kuekenthali*, and *A. flabelliformis*. In addition to the above, the HMA species also had higher richness and evenness than the LMA sponge species although this difference was less pronounced for *A. flabelliformis*. We previously showed (Cleary et al. 2020, 2021) that evenness was consistently higher in HMA than in LMA species. The HMA species identified in the present study also shared OTUs closely related to organisms detected in

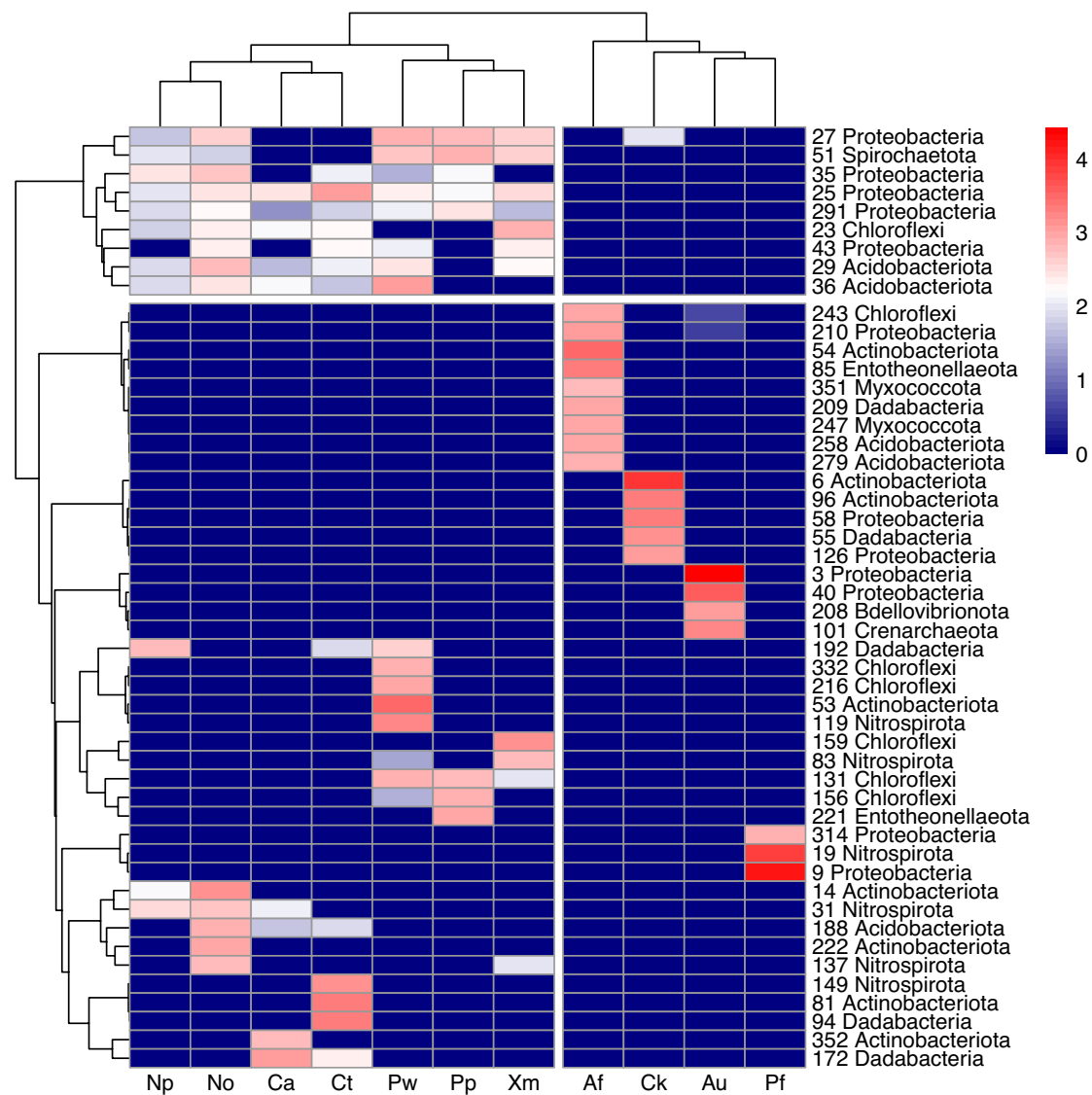


Fig. 6 Heatmap of the 50 most abundant OTUs. Dendrograms are based on hierarchical clustering of sponge host species and OTUs. The x-axis codes represent the following sponge species: Af: *Agelas flabelliformis*, Pf: *Phakellia folium*, Au: *Acanthella cubensis*, No: *Neopetrosia ovata*, Pp: *Petrosia* sp., Pw: *Petrosia* aff. *weinbergi*, Xm:

Xestospongia muta, Ca: *Calthropella*, Ck: *Cinachyrella kuekenhali*, Ct: *Corallistes typus*, and Np: *Neophrissospongia jorgeorum*. Abundance is shown on a log₁₀ scale as indicated by the legend at the right of the figure

known HMA sponge species such as *Aplysina cauliformis*, *Ircinia* spp., *Astrosclera willeyana*, and *Xestospongia testudinaria* (Karlińska-Batres and Wörheide 2013; Gloeckner et al. 2014; Cleary et al. 2019a). All of these characteristics strongly indicate HMA status, as confirmed using TEM imagery for the species *N. ovata*, *Petrosia* sp., *Petrosia* aff. *weinbergi*, *C. lithistina*, *C. typus* and *N. jorgeorum*.

Neopetrosia ovata, *Petrosia* sp. and *Petrosia* aff. *weinbergi* belong to the family Petrosiidae (order Haplosclerida) and several other species belonging to this family have been identified as HMA sponges (e.g., *Petrosia ficiformis*, *Petrosia elephantotus*, *Xestospongia muta*,

and *Xestospongia testudinaria*; Gloeckner et al. 2014; Moitinho-Silva et al. 2017). Although sponges belonging to other families (Chalinidae, Callyspongiidae, Niphatiidae) within the order Haplosclerida appear to exclusively consist of LMA species, the Petrosiidae appears to only consist of HMA sponges. Sponges within this family are often large, massive and conspicuous as opposed to the soft bodied representatives of other haplosclerid families.

The species *C. lithistina* (Family Geodiidae), *N. jorgeorum*, and *C. typus* (both Family: Corallistidae) all belong to the order Tetractinellida. All three species, furthermore, shared several abundant taxa including OTUs

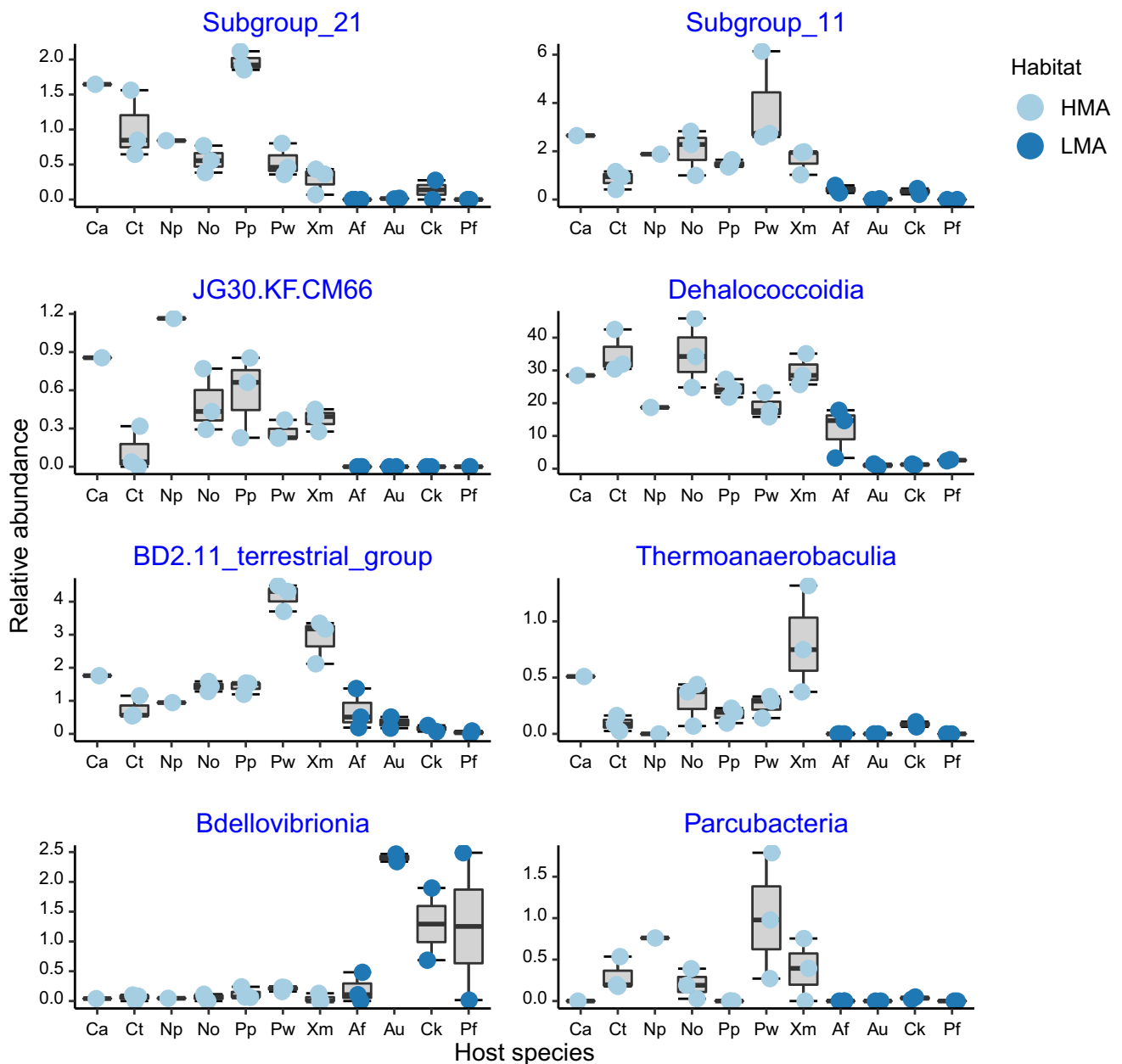


Fig. 7 Relative abundances of bacterial classes, which differentiated between HMA and LMA sponge species in order of importance. The x-axis codes represent the following sponge species: Af: *Agelas flabelliformis*, Pf: *Phakellia folium*, Au: *Acanthella cubensis*,

No: *Neopetrosia ovata*, Pp: *Petrosia* sp., Pw: *Petrosia* aff. *weinbergi*, Xm: *Xestospongia muta*, Ca: *Calthropella* sp., Ck: *Cinachyrella kuekenthali*, Ct: *Corallistes typus*, and Np: *Neophrissospongia jorgeorum*

classified as Chloroflexi and Acidobacteriota, which are both HMA indicator taxa (Moitinho-Silva et al. 2017). In comparison to other host species, the tetractinellids, including *C. kuekenthali*, had relatively high abundances of OTUs classified as Dadabacteria although each species had a distinct abundant dadabacterial OTU (see Fig. 6). Marine pelagic members of the phylum Dadabacteria were shown to have smaller genome sizes, lower nitrogen content and the genomic potential to degrade microbial DOM,

particularly peptidoglycans and phospholipids (Graham and Tully 2021). Graham and Tully (2021) also identified distinct clades with the potential for (or lack thereof) photoheterotrophy via proteorhodopsin.

Samples of *C. lithistina*, *C. typus*, and *N. jorgeorum* clustered close to the petrosid *N. ovata* but were distinct from samples of the other petrosid species. Likewise in Taiwan, tetractinellid species sampled from caves housed prokaryotic communities with high evenness and representation of HMA

indicator taxa, but which were compositionally distinct from prokaryotic communities of the petrosid *Xestospongia testudinaria* sampled from nearby coral reef habitat (Cleary et al. 2024). Recently, three new species belonging to the genus *Neopetrosia*, including *N. ovata*, were described from mesophotic reefs of Bonaire and Klein Curaçao (van Soest et al. 2014). These species coexist sympatrically with the giant barrel sponge *X. muta*, and all belong to the family Petrosiidae differing in spicule size and shape among other traits. We collected our specimens from the same location as the type material.

Dehalococcoidia abundance was particularly high in HMA sponge species and varied from $18.9 \pm 3.84\%$ in *Petrosia* aff. *weinbergi* to $34.97 \pm 10.56\%$ in *N. ovata* and was much lower in LMA sponge species. Most OTUs classified as Dehalococcoidia belonged to the SAR202 clade (98.8%). Landry et al. (2017) suggested that SAR202 members obtained from the dark ocean were involved in the oxidation of relatively recalcitrant to persistent refractory organic compounds. Likewise, Saw et al. (2020) showed that the genomes of SAR202 MAGs at varying depths encoded for enzymes involved in the oxidation of compounds resistant to degradation due to chiral complexity. In sponges, Bayer et al. (2018) also suggested that sponge-associated Chloroflexi, including SAR202 members, were involved in the degradation of labile and recalcitrant DOM. Taken together, these results, and the high relative abundance of SAR202 in the present study, suggest a potentially important role in the degradation of recalcitrant DOM compounds in mesophotic HMA sponges and possibly a greater dependence on DOM for sponges in the mesophotic zone than in the shallow-water realm, although this requires further validation. It is, furthermore, of interest to note that all sponge species, including the LMA sponges albeit in lower abundances, harboured Dehalococcoidia members in contrast to shallow-water studies (Cleary et al. 2020, 2021). Cyanobacterial abundance, in contrast, was only $0.21 \pm 0.52\%$ across all species and six of the seven HMA species had no recorded cyanobacterial sequences.

In contrast to the above-mentioned HMA species, the sponge species *P. folium*, *A. cubensis* and *C. kuekenthali* had relatively low evenness. *Phakellia folium* and *A. cubensis* also had very high abundances of small subsets of OTUs, which had relatively low sequence similarities to organisms detected in corals, other sponge species and a grass carp pond. These characteristics suggest LMA status. This same pattern has been previously observed in other LMA sponge species where we showed that they were dominated by small subsets of highly abundant OTUs in addition to large numbers of OTUs shared with seawater (Cleary et al. 2019a, 2020, 2021). This contrasts with typical HMA species, which shared large numbers of OTUs, which were often rare or absent in seawater. The LMA species also hosted

abundant taxa with low sequence similarities to sequences in the NCBI nucleotide database with each LMA species hosting its own unique small subset of OTUs.

LMA species have previously been characterised by the dominance of Proteobacteria, Cyanobacteria and/or Bacteroidetes (Moitinho-Silva et al. 2017). In contrast, however, to *P. folium* and *A. cubensis*, which were dominated by Gammaproteobacteria, *C. kuekenthali* was dominated by Acidimicrobiia (Actinobacteriota), which is considered a HMA indicator taxon, and Dadabacteriia. In a screening study of antimicrobial and anticancer activity in marine sponges, Proteobacteria and Actinobacteriota (or Actinomycetota) were shown to be the most prominent producers of bioactive compounds (Gavriilidou et al. 2021). Actinobacterial members have also been shown to be prominent producers of biosurfactants (Freitas-Silva et al. 2022) and potentially play a role in promoting heavy metal tolerance in their host sponge (Bauvais et al. 2015). Importantly, species of *Cinachyrella* and the related *Paratetilla* are often found in areas subject to sediment deposition and river plumes as are various HMA species such as *X. testudinaria* (Cleary and de Voogd 2007; de Voogd et al. 2009; Moura et al. 2016), which should promote a microbiome enriched in genes involved in heavy metal tolerance and DOM utilisation.

The most abundant OTUs in *P. folium* were classified as the gammaproteobacterial UBA10353 marine group and genus *Nitrospira* (Nitrospirota). The Nitrospirota phylum includes nitrite-to-nitrate oxidisers and commamox oxidisers, which oxidise ammonia to nitrate (Daims et al. 2016). The abundance of *Nitrospira* members in *P. folium* suggests a potentially important role of its sponge-associated prokaryotic community in nitrification. Pajares et al. (2020) previously showed that the tropical Mexican Pacific oxygen minimum zone was dominated by SAR11, SAR406, SAR324, SAR202, Thiomicrospirales, Nitrospinales and the UBA10353 marine group. In contrast, the euphotic zone was dominated by Synechococcales, Flavobacteriales, Verucomicrobiales, Rhodobacterales, SAR86, and Cellvibrionales. Rust et al. (2020) previously identified a bin in the sponge *Mycale hentscheli*, affiliated with the UBA10353 marine group. They noted that multiple members of the sponge microbiome, including the UBA10353 bin contributed to the chemical diversity of the sponge. UBA10353 marine group members have also been recorded in a deep sea Fe–Mn crust biofilm (Bergo et al. 2021) and an oxygen minimum zone in the Andaman Sea (Guo et al. 2022).

Despite housing a prokaryotic community similar in certain aspects to HMA sponge species (e.g., in terms of diversity and class abundance), the sponge *A. flabelliformis*, clustered with the LMA species in the PCO ordination, and did not share any abundant OTUs with the HMA sponge species. Instead, it housed nine abundant OTUs of which only two were shared with another sponge species (*A. cubensis*).

Although Gloeckner et al. (2014) assigned species of *Agelas*, including *A. citrina*, *A. dilatata*, *A. dispar*, *A. wiedenmayeri*, *A. cervicornis*, *A. conifera*, and *A. oroides*, HMA status, they did report that these species contained less dense microbial communities compared to other HMA species. The TEM images of this species clearly showed large bacterial cells, but these were not densely packed as shown in the images of the HMA species. Cleary et al. (2019a, b) also previously remarked that species of *Agelas* sampled in Thailand and Taiwan clustered in between HMA and LMA species in an ordination. The results of the present study, thus, confirm previous studies suggesting that species of *Agelas* house prokaryotic consortia distinct from both HMA and LMA species.

We were able to identify a number of class-based predictors of HMA/LMA status. The Dehalococcoidia and JG30-KF-CM66 are both classes within the phylum Chloroflexi, which has been identified by various studies as an important indicator phylum of HMA status (Schmitt et al. 2011; Moitinho-Silva et al. 2017). Sequences belonging to the the BD2-11 terrestrial group have previously been recorded across a range of biotopes including marine sediments, sponges, and soils (Hanada and Sekiguchi 2014). The BD2-11 terrestrial group is also a class of the Gemmatimonadota phylum (according to the SILVA database used), which has been recognised as an important indicator phylum of HMA status (Moitinho-Silva et al. 2017). Members of the recently described Thermoanaerobaculia class have previously been implicated in sulfur cycling (Flieder et al. 2021). The Thermoanaerobaculia, Subgroup 11, and Subgroup 21 clades all belong to the Acidobacteriota phylum, which has previously been identified as a HMA indicator phylum (Moitinho-Silva et al. 2017). Acidobacteriota members are important components of the soil bacterial community where they have been shown to be heterotrophic, often oligotrophic and sensitive to shifts in pH (Fierer et al. 2007, 2012; Jones et al. 2009). They have also previously been suggested to be important indicators of wetland restoration and trophic status (Hartman et al. 2008). Sponge-specific clusters were also shown to be ‘particularly well represented’ among certain bacterial taxa including the Acidobacteriota, in addition to Chloroflexi, Cyanobacteria, Poribacteria, and Betaproteobacteria (Simister et al. 2012). In the sponge *Rhopaloeides odorabile*, Acidobacteriota (and Chloroflexi) members also appeared to be sensitive to elevated temperature under which conditions they were replaced by Proteobacteria, Bacteroidetes and Firmicutes (Webster et al. 2008). Future work should focus on trying to unravel the role that Acidobacteriota members play in marine sponges and the nature of their association with HMA sponges in particular.

5 Conclusion

The results from TEM imagery and compositional analysis align with respect to HMA/LMA status. In addition, this study supports previous research highlighting the prevalence of small subsets of highly abundant OTUs, and concomitantly lower evenness, in LMA sponge species. Taken together, our results demonstrate that the HMA/LMA dichotomy extends to sponges inhabiting the mesophotic zone. Although samples of the LMA sponge species clustered together, suggesting compositional similarity, it should be noted that there were marked differences in the relative abundances of the dominant classes of LMA species with Gammaproteobacteria dominating the prokaryotic communities of *P. folium* and *A. cubensis*, whereas Acidimicrobiia (previously identified as a HMA indicator taxon; Moitinho-Silva et al. 2017) dominated the communities of *A. flabelliformis* and *C. kuekenthali*.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13199-024-00992-6>.

Acknowledgements This work was made possible with the help of Kristin Methlagl and Tamara Schwent of “Uncharted Blue”, Luc Hardy (Sagax entertainment), Joost Engelberts, Jochem Salemink, Siebren Hodes, Bas Zwartepoorte (Bureau Voorlichting), and Edwin van Huis (Naturalis). A special thanks goes to Adriaan “Dutch” Schriers for giving us the opportunity to use the ‘Curasub’ submarine and to the crew of the RV ‘Chapman’ and the submarine pilots Barbara van Bebber, Bruce Brant, and Jordy Stolk for making the expedition run smoothly. Carole Baldwin and Dave Kline (Smithsonian Institution) are thanked for their help in the field. This work is part of the research programme NWO-VIDI with project number 16.161.301 by the Netherlands Organization for Scientific Research (NWO). This work was also supported by European Funds through COMPETE [FCOMP-01-0124-FEDER-008657] and by National Funds through the Portuguese Foundation for Science and Technology (FCT) within the LESS CORAL [PTDC/AAC-AMB/115304/2009] project. We also acknowledge financial support to CESAM from FCT/MCTES (UIDP/50017/2020+ UIDB/50017/2020 + LA/P/0094/2020), through national funds. We thank Martin van der Plas and Rob Langelaan for their support in the lab.

Funding Open access funding provided by FCTIFCCN (b-on).

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Bauvais C, Zirah S, Piette L, Chaspoul F, Domart-Coulon I, Chapon V, Gallice P, Rebuffat S, Pérez T, Bourguet-Kondracki ML (2015) Sponging up metals: bacteria associated with the marine sponge *Spongia officinalis*. *Mar Environ Res* 104:20–30. <https://doi.org/10.1016/j.marenvres.2014.12.005>
- Bayer K, Kamke J, Hentschel U (2014) Quantification of bacterial and archaeal symbionts in high and low microbial abundance sponges using real-time PCR. *FEMS Microbiol Ecol* 89:679–690
- Bayer K, Jahn MT, Slaby BM, 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(6):e00150-18. <https://doi.org/10.1128/mSystems.00150-18>
- Bergo NM, Bendia AG, Ferreira JCN, Murton BJ, Brandini FP, Pelizari VH (2021) Microbial diversity of deep-sea ferromanganese crust field in the Rio Grande Rise, southwestern Atlantic Ocean. *Microb Ecol* 82(2):344–355. <https://doi.org/10.1007/s00248-020-01670-y>
- Blanquer A, Uriz MJ, Galand PE (2013) Removing environmental sources of variation to gain insight on symbionts vs. transient microbes in high and low microbial abundance sponges. *Environ Microbiol* 15(11):3008–19. <https://doi.org/10.1111/1462-2920.12261>
- 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 et al (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>
- Bongaerts P, Frade PR, Ogier JJ, Hay KB, van Bleijswijk J, Englebert N, Vermeij MJ, Bak RP, Visser PM, Hoegh-Guldberg O (2013) Sharing the slope: depth partitioning of agariciid corals and associated *Symbiodinium* across shallow and mesophotic habitats (2–60 m) on a Caribbean reef. *BMC Evol Biol* 13:205. <https://doi.org/10.1186/1471-2148-13-205>
- Bongaerts P, Frade PR, Hay KB, Englebert N, Latijnhouwers KR, Bak RP, Vermeij MJ, Hoegh-Guldberg O (2015) Deep down on a Caribbean reef: lower mesophotic depths harbor a specialized coral-endosymbiont community. *Sci Rep* 5:7652. <https://doi.org/10.1038/srep07652>
- Bongaerts P, Smith TB (2019) Beyond the “Deep Reef Refuge” hypothesis: A conceptual framework to characterize persistence at depth. In: Loya Y, Puglise KA, Bridge T (eds.), *Mesophotic coral ecosystems*, pp. 881–895. Springer. https://doi.org/10.1007/978-3-319-92735-0_45
- Bridge TC, Hoey AS, Campbell SJ, Muttaqin E, Rudi E, Fadli N, Baird AH (2013) Depth-dependent mortality of reef corals following a severe bleaching event: implications for thermal refuges and population recovery. *F100Res* 2:187. <https://doi.org/10.12688/f1000research.2-187.v3>
- Busch K, Slaby BM, Bach W, Boetius A, Clefsen I, Colaço A, Creemers M, Cristobo J, Federwisch L, Franke A, Gavriilidou A, Hethke A, Kenchington E, Mienis F, Mills S, Riesgo A, Ríos P, Roberts EM, Sipkema D, Pita L, Schupp PJ, Xavier J, Rapp HT, Hentschel U (2022) Biodiversity, environmental drivers, and sustainability of the global deep-sea sponge microbiome. *Nat Commun* 13(1):5160. <https://doi.org/10.1038/s41467-022-32684-4>
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 13(7):581–583. <https://doi.org/10.1038/nmeth.3869>
- Chaib De Mares M, Sipkema D, Huang S, Bunk B, Overmann J, van Elsas JD (2017) Host specificity for bacterial, archaeal and fungal communities determined for high- and low-microbial abundance sponge species in two genera. *Front Microbiol* 8:2560. <https://doi.org/10.3389/fmicb.2017.02560>
- Cleary DFR, de Voogd NJ (2007) Environmental determination of sponge assemblages in the Spermonde Archipelago, Indonesia. *J Mar Biol Assoc UK* 87:1669–1676
- Cleary DFR, Becking LE, Pires ACC, de Voogd NJ, Egas C, Gomes NCM (2013) Habitat and host related variation in sponge bacterial communities in Indonesian coral reefs and marine lakes. *FEMS Microbiol Ecol* 85:465–482
- Cleary DFR, de Voogd NJ, Polónia ARM, Freitas R, Gomes NCM (2015) Composition and predictive functional analysis of bacterial communities in seawater, sediment and sponges in an Indonesian coral reef environment. *Microb Ecol* 70:889–903. <https://doi.org/10.1007/s00248-015-0632-5>
- Cleary DFR, Polónia ARM, Becking LE, de Voogd NJ, Purwanto GH, Gomes NCM (2018) Compositional analysis of bacterial communities in seawater, sediment and high and low microbial abundance sponges in the Misool coral reef system, Indonesia. *Mar Biodivers* 48:1889–1901. <https://doi.org/10.1007/s12526-017-0697-0>
- Cleary DFR, Polónia ARM, Huang YM, Gomes NCM, de Voogd NJ (2019a) A comparison of prokaryote communities inhabiting sponges, bacterial mats, sediment and seawater in Southeast Asian coral reefs. *FEMS Microbiol Ecol* 95:fiz169. <https://doi.org/10.1093/femsec/fiz169>
- Cleary DFR, Swierts T, Coelho FJRC, Polónia ARM, Huang YM, Ferreira MRS, Putschakarn S, Carvalheiro L, van der Ent E, Ueng JP, Gomes NCM, de Voogd NJ (2019b) The sponge microbiome within the greater coral reef microbial metacommunity. *Nat Commun* 10:1644. <https://doi.org/10.1038/s41467-019-09537-8>
- Cleary DFR, Polónia ARM, Reijnen BT, Berumen ML, de Voogd NJ (2020) Prokaryote communities inhabiting endemic and newly discovered sponges and octocorals from the Red Sea. *Microb Ecol* 80:103–119. <https://doi.org/10.1007/s00248-019-01465-w>
- Cleary DFR, Polónia ARM, de Voogd NJ (2021) Composition and diversity of Prokaryotic communities sampled from sponges and soft corals in Maldivian waters. *Mar Ecol* 42:e12638. <https://doi.org/10.1111/maec.12638>
- Cleary DFR, de Voogd NJ, Stuij T, Swierts T, Oliveira V, Polónia ARM, Louvado A, Gomes NCM, Coelho FJRC (2023a) A study of sponge symbionts from different light habitats. *Microb Ecol* 86:2819–2837. <https://doi.org/10.1007/s00248-023-02267-x>
- Cleary DFR, Oliveira V, Gomes NCM, Bialecki A, de Voogd NJ (2023b) A comparison of free-living and sponge-associated prokaryotic communities with a focus on calcareous sponges. *FEMS Microbiol Ecol* 99(3):fiad014. <https://doi.org/10.1093/femsec/fiad014>
- Cleary DFR, Huang YM, Polónia ARM, van der Plas M, Gomes NCM, de Voogd NJ (2024) Sponges and their prokaryotic communities sampled from a remote karst ecosystem. *Mar Biodivers* 54:8. <https://doi.org/10.1007/s12526-023-01387-4>
- Daims H, Lückner S, Wagner M (2016) A new perspective on microbes formerly known as nitrite-oxidizing bacteria. *Trends Microbiol* 24:699–712
- De Cáceres M, Legendre P (2009) Associations between species and groups of sites: indices and statistical inference. *Ecology* 90:3566–3574. <https://doi.org/10.1890/08-1823.1>
- de Voogd NJ, Becking LE, Cleary DFR (2009) Sponge community composition in the Derawan Islands, NE Kalimantan, Indonesia. *Mar Ecol Prog Ser* 396:219–230. <https://doi.org/10.3354/meps08349>
- de Voogd NJ, Cleary DFR, Polónia ARM, Gomes NCM (2015) Bacterial communities of four different biotopes and their functional genomic nitrogen signature from the thousand-island reef

- complex, West-Java, Indonesia. *FEMS Microbiol Ecol* 91:fiv019. <https://doi.org/10.1093/femsec/fiv019>
- de Voogd NJ, Gauvin-Bialecki A, Polónia ARM, Cleary DFR (2019) Assessing the bacterial communities of sponges inhabiting the remote western Indian Ocean island of Mayotte. *Mar Ecol* 39(6):e12517. <https://doi.org/10.1111/maec.12517>
- Erwin PM, Coma R, López-Sendino P, Serrano E, Ribes M (2015) Stable symbionts across the HMA-LMA dichotomy: low seasonal and interannual variation in sponge-associated bacteria from taxonomically diverse hosts. *FEMS Microbiol Ecol* 91(10):fiv115. <https://doi.org/10.1093/femsec/fiv115>
- Estronza AMG, Alfaro M, Schizas NV (2017) Morphological and genetic species diversity in ostracods (Crustacea: Oligostraca) from Caribbean reefs. *Mar Biodiv* 47:37–53. <https://doi.org/10.1007/s12526-016-0525-y>
- Ferrier-Pagès C, Bednarz V, Grover R, Benayahu Y, Maguer JF, Rottier C, Wiedenmann J, Fine M (2022) Symbiotic stony and soft corals: Is their host-algae relationship really mutualistic at lower mesophotic reefs? *Limnol Oceanogr* 67:261–271. <https://doi.org/10.1002/lno.11990>
- Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. *Ecology* 88(6):1354–1364. <https://doi.org/10.1890/05-1839>
- Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J* 6(5):1007–1017. <https://doi.org/10.1038/ismej.2011.159>
- Flieder M, Buongiorno J, Herbold CW, Hausmann B, Rattei T, Lloyd KG, Loy A, Wasmund K (2021) Novel taxa of Acidobacteriota implicated in seafloor sulfur cycling. *ISME J* 15(11):3159–3180. <https://doi.org/10.1038/s41396-021-00992-0>
- Freitas-Silva J, de Oliveira BFR, Dias GR, de Carvalho MM, Laport MS (2022) Unravelling the sponge microbiome as a promising source of biosurfactants. *Crit Rev Microbiol* 49(1):101–116. <https://doi.org/10.1080/1040841X.2022.2037507>
- Garritano AN, Majzoub ME, Ribeiro B, Damasceno T, Modolon F, Messias C, Vilela C, Duarte G, Hill L, Peixoto R, Thomas T (2023) Species-specific relationships between deep sea sponges and their symbiotic Nitrosopumilaceae. *ISME J* 17(9):1517–1519. <https://doi.org/10.1038/s41396-023-01439-4>
- Gavriilidou A, Mackenzie TA, Sánchez P, Tormo JR, Ingham C, Smidt H, Sipkema D (2021) Bioactivity screening and gene-trait matching across marine sponge-associated Bacteria. *Mar Drugs* 19(2):75. <https://doi.org/10.3390/md19020075>
- Glasl B, Bongaerts P, Elisabeth NH, Hoegh-Guldberg O, Herndl GJ, Frade PR (2017) Microbiome variation in corals with distinct depth distribution ranges across a shallow-mesophotic gradient (15–85 m). *Coral Reefs* 36(2):447–452. <https://doi.org/10.1007/s00338-016-1517-x>
- Gloekner V, Wehr M, Moitinho-Silva L, Gernert C, Schupp P, Pawlik JR, Lindquist NL, Erpenbeck D, Wörheide G, Hentschel U (2014) The HMA-LMA dichotomy revisited: an electron microscopical survey of 56 sponge species. *Biol Bull* 227(1):78–88. <https://doi.org/10.1086/BBLv227n1p78>
- Graham ED, Tully BJ (2021) Marine Dadabacteria exhibit genome streamlining and phototrophy-driven niche partitioning. *ISME J* 15(4):1248–1256. <https://doi.org/10.1038/s41396-020-00834-5>
- Guo R, Ma X, Zhang J, Liu C, Thu CA, Win TN, Aung NL, Win HS, Naing S, Li H, Zhou F, Wang P (2022) Microbial community structures and important taxa across oxygen gradients in the Andaman Sea and eastern Bay of Bengal epipelagic waters. *Front Microbiol* 13:1041521. <https://doi.org/10.3389/fmicb.2022.1041521>
- Hanada S, Sekiguchi Y (2014) The phylum Gemmatimonadetes. In: *The Prokaryotes* 4th ed. (Eds: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F). Springer, Berlin/Heidelberg, Germany, 11:677–681
- Hartman WH, Richardson CJ, Vilgalys R, Bruland GL (2008) Environmental and anthropogenic controls over bacterial communities in wetland soils. *Proc Natl Acad Sci USA* 105(46):17842–17847. <https://doi.org/10.1073/pnas.0808254105>
- Hentschel U, Usher KM, Taylor MW (2006) Marine sponges as microbial fermenters. *FEMS Microbiol Ecol* 55:167–177
- Hentschel U, Piel J, Degnan SM, Taylor MW (2012) Genomic insights into the marine sponge microbiome. *Nat Rev Microbiol* 10:641–654. <https://doi.org/10.1038/nrmicro2839>
- Hochmuth T, Niederkrüger H, Gernert C, Siegl A, Taudien S, Platzer M, Crews P, Hentschel U, Piel J (2010) Linking chemical and microbial diversity in marine sponges: possible role for poribacteria as producers of methyl-branched fatty acids. *ChemBioChem* 11:2572–2578. <https://doi.org/10.1002/cbic.201000510>
- Idan T, Goren L, Shefer S, Brickner I, Ilan M (2020) Does depth matter? Reproduction pattern plasticity in two common sponge species found in both mesophotic and shallow waters. *Front Mar Sci* 7:610565. <https://doi.org/10.3389/fmars.2020.610565>
- Indraningrat AAG, Steinert G, Becking LE, Mueller B, de Goeij JM, Smidt H, Sipkema D (2022) Sponge holobionts shift their prokaryotic communities and antimicrobial activity from shallow to lower mesophotic depths. *Antonie Van Leeuwenhoek* 115(10):1265–1283. <https://doi.org/10.1007/s10482-022-01770-4>
- Jackson SA, Flemer B, McCann A, Kennedy J, Morrissey JP, O’Gara F, Dobson AD (2013) Archaea appear to dominate the microbiome of *Inflatella pellicula* deep sea sponges. *PLoS One* 8(12):e84438. <https://doi.org/10.1371/journal.pone.0084438>
- Jones RT, Robeson MS, Lauber CL, Hamady M, Knight R, Fierer N (2009) A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *ISME J* 3(4):442–453. <https://doi.org/10.1038/ismej.2008.127>
- Karlińska-Batres K, Wörheide G (2013) Phylogenetic diversity and community structure of the symbionts associated with the coralline sponge *Astrosclera willeyana* of the Great Barrier Reef. *Microb Ecol* 65(3):740–752. <https://doi.org/10.1007/s00248-013-0212-5>
- Keesing JK, Usher KM, Fromont J (2012) First record of photosynthetic cyanobacterial symbionts from mesophotic temperate sponges. *Mar Freshw Res* 63:403–408. <https://doi.org/10.1071/MF11216>
- Kennedy J, Flemer B, Jackson SA, Morrissey JP, O’Gara F, Dobson AD (2014) Evidence of a putative deep sea specific microbiome in marine sponges. *PLoS One* 9(3):e91092. <https://doi.org/10.1371/journal.pone.0091092>
- Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 41:e1–e1. <https://doi.org/10.1093/nar/gks808>
- Kolde R (2019) pheatmap: Pretty Heatmaps. R package version 1.0.12. <https://CRAN.R-project.org/package=pheatmap>
- Kramer N, Tamir R, Ben-Zvi O, Jacques SL, Loya Y, Wangpraseurt D (2022) Efficient light-harvesting of mesophotic corals is facilitated by coral optical traits. *Funct Ecol* 36:406–418. <https://doi.org/10.1111/1365-2435.13948>
- Kursa MB, Rudnicki WR (2010) Feature selection with the Boruta package. *J Stat Softw* 36:1–13. <https://doi.org/10.18637/jss.v036.i11>
- Kursa MB, Jankowski A, Rudnicki W (2010) Boruta. A system for feature selection. *Fundam Inform* 101:271–285. <https://doi.org/10.3233/FI-2010-288>
- Landry Z, Swan BK, Herndl GJ, Stepanauskas R, Giovannoni SJ (2017) SAR202 Genomes from the Dark Ocean Predict Pathways

- for the oxidation of recalcitrant dissolved organic matter. *mBio* 8(2):e00413-17. <https://doi.org/10.1128/mBio.00413-17>
- Lesser MP, Slattery M, Laverick JH, Macartney KJ, Bridge TC (2019) Global community breaks at 60 m on mesophotic coral reefs. *Glob Ecol Biogeogr* 28(10):1403–1416. <https://doi.org/10.1111/geb.12940>
- Macartney KJ, Slattery M, Lesser MP (2021) Trophic ecology of Caribbean sponges in the mesophotic zone. *Limnol Oceanogr* 66:1113–1124. <https://doi.org/10.1002/lno.11668>
- McMurdie PJ, Holmes S (2013) phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8:e61217
- Moitinho-Silva L, Bayer K, Cannistraci CV, Giles EC, Ryu T, Seridi L, Ravasi T, Hentschel U (2014) Specificity and transcriptional activity of microbiota associated with low and high microbial abundance sponges from the Red Sea. *Mol Ecol* 23(6):1348–1363. <https://doi.org/10.1111/mec.12365>
- Moitinho-Silva L, Steinert G, Nielsen S, Haroim CCP, Wu Y-C, McCormack GP, López-Legentil S, Marchant R, Webster N, Thomas T, Hentschel U (2017) Predicting the HMA-LMA status in marine sponges by machine learning. *Front Microbiol* 8:752. <https://doi.org/10.3389/fmicb.2017.00752>
- Morrow KM, Fiore CL, Lesser MP (2016) Environmental drivers of microbial community shifts in the giant barrel sponge, *Xestospongia muta*, over a shallow to mesophotic depth gradient. *Environ Microbiol* 18(6):2025–2038. <https://doi.org/10.1111/1462-2920.13226>
- Moura RL, Amado-Filho GM, Moraes FC, Brasileiro PS, Salomon PS, Mahiques MM, Bastos AC, Almeida MG, Silva JM Jr, Araujo BF, Brito FP, Rangel TP, Oliveira BC, Bahia RG, Paranhos RP, Dias RJ, Siegle E, Figueiredo AG Jr, Pereira RC, Leal CV, Hajdu E, Asp NE, Gregoracci GB, Neumann-Leitão S, Yager PL, Francini-Filho RB, Fróes A, Campeão M, Silva BS, Moreira AP, Oliveira L, Soares AC, Araujo L, Oliveira NL, Teixeira JB, Valle RA, Thompson CC, Rezende CE, Thompson FL (2016) An extensive reef system at the Amazon River mouth. *Sci Adv* 2(4):e1501252. <https://doi.org/10.1126/sciadv.1501252>
- Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Stevens MHH, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, and de Caceres M, Durand S, and Evangelista HBA, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill MO, Lahti L, McGlenn D, Ouellette MH, Ribeiro Cunha E, Smith T, Stier A, ter Braak CJF, Weedon J (2022) *Vegan: Community Ecology Package*. R package version 2.6–4
- Olson JB, Gao X (2013) Characterizing the bacterial associates of three Caribbean sponges along a gradient from shallow to mesophotic depths. *FEMS Microbiol Ecol* 85(1):74–84. <https://doi.org/10.1111/1574-6941.1111574-6941>
- Olson JB, Kellogg CA (2010) Microbial ecology of corals, sponges, and algae in mesophotic coral environments. *FEMS Microbiol Ecol* 73(1):17–30. <https://doi.org/10.1111/j.1574-6941.2010.00862.x>
- Pajares S, Varona-Cordero F, Hernández-Becerril DU (2020) Spatial distribution patterns of bacterioplankton in the oxygen minimum zone of the tropical Mexican Pacific. *Microb Ecol* 80(3):519–536. <https://doi.org/10.1007/s00248-020-01508-7>
- Podell S, Blanton JM, Oliver A, Schorn MA, Agarwal V, Biggs JS, Moore BS, Allen EE (2020) A genomic view of trophic and metabolic diversity in clade-specific *Lamellodysidea* sponge microbiomes. *Microbiome* 8(1):97. <https://doi.org/10.1186/s40168-020-00877-y>
- Polónia ARM, Cleary DFR, Freitas R, de Voogd NJ, Gomes NCM (2015) The putative functional ecology and distribution of archaeal communities in an Indonesian coral reef environment. *Mol Ecol* 24:409–423. <https://doi.org/10.1111/mec.13024>
- R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>
- Radax R, Rattei T, Lanzen A, Bayer C, Rapp HT, Urich T, Schleper C (2012) Metatranscriptomics of the marine sponge *Geodia barretti*: tackling phylogeny and function of its microbial community. *Environ Microbiol* 14(5):1308–1324. <https://doi.org/10.1111/j.1462-2920.2012.02714.x>
- Rocha LA, Pinheiro HT, Shepherd B, Papastamatiou YP, Luiz OJ, Pyle RL, Bongaerts P (2018) Mesophotic coral ecosystems are threatened and ecologically distinct from shallow water reefs. *Science* 361(6399):281. <https://doi.org/10.1126/science.aag1614>
- Rust M, Helfrich EJM, Freeman MF, Nanudorn P, Field CM, Rückert C, Kündig T, Page MJ, Webb VL, Kalinowski J, Sunagawa S, Piel J (2020) A multiproductor microbiome generates chemical diversity in the marine sponge *Mycale hentscheli*. *Proc Natl Acad Sci USA* 117(17):9508–9518. <https://doi.org/10.1073/pnas.1919245117>
- Rützler K, Muzik K (1993) *Terpios hoshinota*, a new cyanobacteriosponge threatening Pacific reefs. *Sci Mar* 57:395–403
- Saw JHW, Nunoura T, Hirai M, Takaki Y, Parsons R, Michelsen M, Longnecker K, Kujawinski EB, Stepanauskas R, Landry Z, Carlson CA, Giovannoni SJ (2020) Pangenomics analysis reveals diversification of enzyme families and niche specialization in globally abundant SAR202 Bacteria. *mBio* 11(1):e02975-19. <https://doi.org/10.1128/mBio.02975-19>
- Schmitt S, Deines P, Behnam F, Wagner M, Taylor MW (2011) Chloroflexi bacteria are more diverse, abundant, and similar in high than in low microbial abundance sponges. *FEMS Microbiol Ecol* 78(3):497–510. <https://doi.org/10.1111/j.1574-6941.2011.01179.x>
- Simister RL, Deines P, Botté ES, Webster NS, Taylor MW (2012) Sponge-specific clusters revisited: a comprehensive phylogeny of sponge-associated microorganisms. *Environ Microbiol* 14(2):517–524. <https://doi.org/10.1111/j.1462-2920.2011.02664.x>
- Steffen K, Indraningrat AAG, Erngren I, Haglöf J, Becking LE, Smidt H, Yashayaev I, Kenchington E, Pettersson C, Cárdenas P, Sipkema D (2022) Oceanographic setting influences the prokaryotic community and metabolome in deep-sea sponges. *Sci Rep* 12(1):3356. <https://doi.org/10.1038/s41598-022-07292-3>
- Steinert G, Taylor MW, Deines P, Simister RL, de Voogd NJ, Hoggard M, Schupp PJ (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>
- Swierts T, Cleary DFR, de Voogd NJ (2018) Biogeography of prokaryotic communities in closely related giant barrel sponges across the Indo-Pacific. *FEMS Microbiol Ecol* 94:fy194. <https://doi.org/10.1093/femsec/fy194>
- Tamir R, Eyal G, Kramer N, Laverick JH, Loya Y (2019) Light environment drives the shallow-to-mesophotic coral community transition. *Ecosphere* 10(9):e02839. <https://doi.org/10.1002/ecs2.2839>
- Taylor MW, Radax R, Steger D, Wagner M (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiol Mol Biol Rev* 71:295–347. <https://doi.org/10.1128/MMBR.00040-06>
- Thacker RW, Freeman CJ (2012) Sponge-microbe symbioses: recent advances and new directions. *Adv Mar Biol* 62:57–111

- Thomas T, Moitinho-Silva L, Lurgi M, Björk JR, Easson C, Astudillo-García C, Olson JB, Erwin PM, López-Legentil S, Luter H, Chaves-Fonnegra A, Costa R, Schupp PJ, Steindler L, Erpenbeck D, Gilbert J, Knight R, Ackermann G, Victor Lopez J, Taylor MW, Thacker RW, Montoya JM, Hentschel U, Webster NS (2016) Diversity, structure and convergent evolution of the global sponge microbiome. *Nat Commun* 7:11870. <https://doi.org/10.1038/ncomms11870>
- Tian RM, Sun J, Cai L, Zhang WP, Zhou GW, Qiu JW, Qian PY (2016) The deep-sea glass sponge *Lophophysema eversa* harbours potential symbionts responsible for the nutrient conversions of carbon, nitrogen and sulfur. *Environ Microbiol* 18(8):2481–2494. <https://doi.org/10.1111/1462-2920.13161>
- Vacelet J, Donadey C (1977) Electron microscope study of the association between some sponges and bacteria. *J Exp Mar Biol Ecol* 30:301–314. [https://doi.org/10.1016/0022-0981\(77\)90038-7](https://doi.org/10.1016/0022-0981(77)90038-7)
- van Soest RWM, Meesters EH, Becking LE (2014) Deep-water sponges (Porifera) from Bonaire and Klein Curaçao, Southern Caribbean. *Zootaxa* 3878(5):401–443
- Webster NS, Cobb RE, Negri AP (2008) Temperature thresholds for bacterial symbiosis with a sponge. *ISME J* 2(8):830–842. <https://doi.org/10.1038/ismej.2008.42>
- Weisz JB, Hentschel U, Lindquist N, Martens CS (2007) Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. *Mar Biol* 152:475–483

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.