

Running title: Bacteriophages in black band disease

## Novel T4 bacteriophages associated with black band disease in corals.

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### ***Originality-Significance Statement***

**Identify the key aspects of originality and significance that place the work within the top 10% of current research in environmental microbiology.**

Research on marine diseases has focused on bacteria as etiological agents, whereas the potential role of viruses has had scant attention. In this study, we examine the diversity of hitherto unexplored T4-bacteriophages associated with black band disease (BBD), a ubiquitous coral disease impacting a wide range of scleractinian coral species. Overall, the bacteriophage community showed mainly novel bacteriophage sequences. We discovered 3 candidate T4-bacteriophage operational taxonomic units (OTUs) that are dominant in BBD-lesions. Our results suggest that viruses may be involved in the pathogenicity of BBD and should be considered as a member of the BBD microbial consortium. Since we present the first assessment of the bacteriophage diversity associated with BBD, our research considerably advances understanding of the role of viruses in the etiology of this polymicrobial coral disease.

## Summary

Research into causative agents underlying coral disease have focused primarily on bacteria, whereas potential roles of viruses have been largely unaddressed. Bacteriophages may contribute to diseases through the lysogenic introduction of virulence genes into bacteria, or prevent diseases through lysis of bacterial pathogens. To identify candidate phages that may influence the pathogenicity of black band disease (BBD), communities of bacteria (16S rRNA) and T4-bacteriophages (*gp23*) were simultaneously profiled with amplicon sequencing among BBD-lesions and healthy-coral-tissue of *Montipora hispida*, as well as seawater (study site: the central Great Barrier Reef). Bacterial community compositions were distinct among BBD-lesions, healthy-coral-tissue, and seawater samples, as observed in previous studies. Surprisingly, however, viral beta diversities based on both operational taxonomic unit (OTU)-compositions and overall viral community compositions did not differ statistically between the BBD-lesions and healthy-coral-tissue. Nonetheless, relative abundances of three bacteriophage OTUs, affiliated to Cyanophage PRSM6 and *Prochlorococcus* phages P-SSM2, were significantly higher in BBD-lesions than in healthy tissue. In addition, 32 *gp23* OTUs showed nucleotide similarities to existing CRISPR-Cas spacers in BBD associated cyanobacterial genomes. These OTUs associated with our BBD samples suggest the presence of bacteriophages that infect members of the cyanobacteria-dominated BBD community, and thus have potential roles in BBD pathogenicity.

**Keywords:** viruses, coral, cyanobacteria, cyanophage, 16S rRNA, *gp23*

## Introduction

Over the last few decades, diseases have increasingly contributed to reef coral mortality across the globe (Bourne *et al.*, 2009; Rosenberg *et al.*, 2007; Willis *et al.*, 2004), however, for the majority of coral diseases described to date, knowledge of pathogens is still limited (Harvell *et al.*, 2007; Sheridan *et al.*, 2013). Coral disease diagnostics and mitigation strategies are urgently required (Pollock *et al.*, 2011), because coral diseases are predicted to increase as environmental conditions continue to change, such as increasing seawater temperatures (Bruno *et al.*, 2007; Maynard *et al.*, 2015; Selig *et al.*, 2006), decreasing water quality (Sutherland *et al.*, 2004), and increasing severity of anthropogenic impacts (Aeby *et al.*, 2011; Lamb *et al.*, 2014; Lamb and Willis, 2011). However, such strategies can only be developed effectively if disease etiologies are fully understood. While most coral disease studies have focused on the role of bacteria in pathogenesis, the influence of viruses on coral health may be substantial (van Oppen, 2009; Vega-Thurber and Correa, 2011), but has received little attention (Cervino *et al.*, 2004; Davy *et al.*, 2006; Soffer *et al.*, 2014).

Black band disease (BBD) is observed on coral reefs worldwide and affects a wide range of coral species (Page and Willis, 2006; Sutherland *et al.*, 2004). A suite of microorganisms has been identified in close association with the disease, including the filamentous cyanobacterium species *Roseofilum reptotaenium*, sulphate-reducing *Desulfovibrio* bacteria, *Cytophaga*, Alphaproteobacteria, various heterotrophic bacteria (Cooney *et al.*, 2002; Miller and Richardson, 2011; Sato *et al.*, 2010), and unique archaea (Sato *et al.*, 2013). Among these members of the BBD microbial community, filamentous

cyanobacteria, and *Desulfovibrio* bacteria have been recognised as the main biological drivers of the disease in particular as the former dominate the lesion biomass and the latter produce toxic sulphide (Brownell and Richardson, 2014; Stanic *et al.*, 2011). Our understanding of microbial dynamics within BBD-lesions has progressed considerably over the last few decades, especially due to bacterium-centred research (Sato *et al.*, 2016), but identification of the agent(s) responsible for the onset of the disease, strategies for managing and mitigating the disease, and a potential role for viruses in BBD have yet to be determined.

Viruses are typically present in high densities (up to  $10^{10}$  viruses  $g^{-1}$  wet weight) within cyanobacteria-dominated, photosynthetic microbial mats (Carreira *et al.*, 2015a; Carreira *et al.*, 2015b) and hypersaline mats (Pacton *et al.*, 2014; de Wit *et al.*, 2015), both of which have stratified microenvironments similar to those observed within the BBD mat (Glas *et al.*, 2012). Recent detection of viruses in metagenomic and metatranscriptomic analyses of the BBD microbial community (Arotsker *et al.*, 2016; Sato *et al.*, 2017) indicates that certain bacteriophages (or simply ‘phages’) may be associated with BBD. If phages infect and lyse the major bacterial taxa in the BBD mat, this could reduce the impact of the polymicrobial disease, e.g., through natural processes or as phage therapy (Efrony *et al.*, 2007; 2009). Conversely, lysis of BBD-associated bacteria may additionally fuel the progression of the disease as organic matter released from lysed bacteria promotes the growth of other bacterial members, in a similar way compared to the viral shunt (Wilhelm and Suttle, 1999, Vega-Thurber *et al.*, 2017). Phages are also capable of lysogenic conversion, which may contribute to pathogenesis of the disease through the introduction

of new genetic information such as virulence genes into the genome of the pathogen (Buerger *et al.*, 2016b). This virally-mediated pathogenicity inducing mechanism has been suggested for the coral pathogen *Vibrio coralliilyticus* that causes some types of white syndromes (Weynberg *et al.*, 2015). Relevance of bacteriophages in BBD pathogenesis is further suggested by the genomes of BBD-associated cyanobacteria harbouring CRISPR-Cas systems, which defend against bacteriophage infections (Buerger *et al.*, 2016b). However, the phage community of BBD has not been described in detail and thus their possible role in BBD aetiology has not been identified.

While a wide range of bacterial species are present in the BBD lesion, we focused our study on BBD-associated cyanobacteria and cyanophages, as cyanobacteria dominate the lesion in biomass (Rützler *et al.*, 1983; Rützler and Santavy, 1983). To date, a large part of cyanophage diversity has been found within the T4-bacteriophages belonging to the family *Myoviridae* in the order *Caudovirales* (reviewed in Shestakov and Karbysheva, 2015; Saffermann *et al.*, 1983; Comeau and Krisch, 2008). A conserved gene that codes for the major capsid protein (*gp23*) is shared by T4-bacteriophages, making it a suitable marker gene for taxon identification and diversity estimation (Ackermann and Krisch, 1997; Filée *et al.*, 2005). Therefore, we compared T4-type bacteriophage communities among samples of BBD-lesions and healthy tissue from the coral *Montipora hispida*, as well as seawater control samples using amplicon sequencing of *gp23* genes. In parallel, partial 16S rRNA gene amplicon sequencing was performed to profile corresponding bacterial communities, which were compared one another and against bacterial communities previously reported from each sample environment.

## Results

### Bacterial community composition based on the 16S rRNA gene

Bacterial 16S rRNA genes were taxonomically assigned by searching against the Greengenes database using the software package Quantitative Insights Into Microbial Ecology (QIIME). Bacterial communities associated with BBD-lesions, healthy coral tissue, and seawater samples formed separate, significantly distinct ( $p < 0.05$ ) clusters on an MDS plot based on OTU relative abundance (Fig. 1A, Table S1). In addition, bacterial sequences associated with BBD-lesions and healthy tissues had relatively low alpha diversity (OTU-diversity in an individual sample; calculated as Chao1-index) in comparison to seawater bacterial communities (Fig. S1). OTUs assigned to cyanobacteria of the genus *Oscillatoria* (*Roseofilum*), sulphate-reducing bacteria of the genus *Desulfovibrio*,  $\epsilon$ -proteobacteria *Arcobacter*, and  $\gamma$ -proteobacteria *Thalassomonas* had significantly higher relative abundances in BBD-lesions compared to healthy coral tissue (Fig. 2A, Table 1, a link to the complete OTU table available in supplementary material Table S2). Healthy coral tissue samples showed higher relative abundances of bacterial sequences associated with Endozoicimonaceae (Class:  $\gamma$ -proteobacteria) than BBD-lesions. Seawater samples had high relative abundance of OTUs associated with Actinobacteria, OCS155, Synechococcaceae and Pelagibacteraceae (Fig. 2A, Table 1).

BBD-lesions

### T4 bacteriophage community composition based on *gp23*

Bacteriophage taxonomy was assigned to *gp23* sequences using MetaVir by BLAST

search (e-value <  $10^{-7}$ ) against the viral RefSeq database and bacteriophage community diversity was analysed with QIIME. Significant BLAST hits to *gp23* reads were returned for  $89 \pm 9\%$  (mean  $\pm$  SD) in BBD-lesions,  $88 \pm 11\%$  in healthy coral tissue and  $98 \pm 2\%$  in seawater samples. Nevertheless, many sequences were novel, indicated by the nucleotide identity of 56.5% on average ( $n = 19 * 10^6$  sequences) shared over 389 bases with any publically available sequences. Healthy tissue- and BBD lesion-associated bacteriophage communities had lower alpha diversity (Chao1-index) compared to seawater communities (Fig. S1). Community compositions based on *gp23* OTUs relative abundance were not significantly distinct between BBD-lesions and healthy tissues (p-adjusted > 0.133, Table S1), but both were significantly distinct from seawater (Fig. 1B, adjusted p-values < 0.005, Table S1). BBD-lesions However, taxonomic affiliations of *gp23* sequences using MetaVir (Fig. 2B) indicated that the most abundant taxon group in BBD was Cyanophage PRSM6 (average relative abundance in BBD samples: 28.86%), whereas the most abundant in healthy coral tissue was *Pelagibacter* phage HTVC008M (average relative abundance in healthy tissue samples: 26.21%). Comparisons of relative OTU abundances among sample types identified three OTUs that were significantly more abundant in BBD samples than in healthy tissues; Cyanophage PRSM6 denovo17715 and 25920, and Prochlorococcus phage P-SSM2 denovo17862 (Table 1). Several OTUs in healthy coral tissues showed higher relative abundance than BBD-lesions, such as Cyanophage PRSM6 denovo21074, Cyanophage PRSM1 denovo7224 and *Pelagibacter* phage HTVC008M denovo10335, as well as an unclassified OTU denovo5392 (Table 1). Five of the CRISPR-Cas spacer sequences of *Geitlerinema* sp. showed nucleotide similarities to 32 *gp23* OTU sequences (average bit score of 17.9, Table S2). The



matching OTUs were present mainly in seawater samples at a low abundance, on average found in approximately 2 of the 13 seawater samples. Only one out of the matching OTUs was present in three BBD samples at a respective OTU count of one. The spacer sequence NoG7\_3 showed the most similarities with 26 OTU sequences and is potentially targeting a more conserved region of the *gp23* region. None of the *R. reptotaenium* AO1 spacer sequences showed similarities to the current T4 bacteriophage data set.

## Discussion

### Bacterial communities

In agreement with previous studies (Casamatta *et al.*, 2012; Cooney *et al.*, 2002; Arotsker *et al.*, 2015; Klaus *et al.*, 2011), bacterial taxa known to associate with BBD, such as *Desulfovibrio* and *Oscillatoria* (*Roseofilum*) cyanobacteria, were significantly more prevalent in BBD compared to healthy tissue samples (Fig. 2A, adjusted p-values in Table 1). Seawater samples were enriched for bacterial taxa typically found in plankton and seawater samples (Table 1, reference taxa found in Houghton, 2015; Hunter-Cevera *et al.*, 2016; West *et al.*, 2016) and healthy coral colonies generally had significantly higher occurrence of Endozoicimonaceae (Table 1), known coral bacterial symbionts associated with healthy coral tissue (Apprill *et al.*, 2012; Bayer *et al.*, 2013; Morrow *et al.*, 2012; Neave *et al.*, 2017). However, these data must be interpreted with caution due to the methodological caveats (e.g., potential degradation of rare taxa during sample process and difficulties to compare seawater samples with coral samples as they were collected in different years and preserved with different methods; see caveats sections in experimental procedures, see section methodological caveats). Nevertheless, bacterial taxa associated

with the respective sample types in this study were also seen in previous studies (see references mentioned above). Therefore, we are confident that our analyses of the BBD bacteriophage diversity are ecologically relevant to respective sample environments and hypotheses regarding bacteriophage-hosts interactions are reasonable.

### **Bacteriophage communities - BBD-lesions versus healthy coral tissue**

We have detected sequences of the T4-bacteriophage major capsid protein *gp23* that are associated with BBD-lesions in comparison to ones associated with healthy coral tissue (Table1, Fig. 2B). Surprisingly, however, overall bacteriophage communities associated with BBD-lesions and healthy coral tissues were not as distinct as expected, given the observed differences in the bacterial communities (Fig. 1B). We can only speculate about why a significant overall difference was not detected between the T4 bacteriophage communities of BBD and healthy coral tissue samples. In terms of bacteriophage-host associations, bacteriophages range from being generalists that are able to infect a wider range of bacteria strains, to specialists that are able to infect only a specific strain of bacteria (Flores *et al.*, 2011; Koskella and Meaden, 2013). For instance, *Prochlorococcus* phage P-SSP7 only infects a high light-adapted *Prochlorococcus* strain (Sullivan *et al.*, 2003), in contrast to several other cyanophages (Sullivan *et al.*, 2003) and *Synechococcus* phage Syn9 (Weigele *et al.*, 2007) that have a wider host range and can infect many *Prochlorococcus* and *Synechococcus* strains. In our case, overlapping phage community patterns can be explained to a certain extent if common generalist phages occurred in both BBD and healthy tissue despite the presence of specialist phages in each environment. Microhabitats may also have varied slightly among samples of the same type

during sample collection. Environmental conditions such as temperature, UV-light, salinity and nutrients are known to affect the abundance of certain viruses (reviewed in Mojica and Brussaard, 2014). Anoxia and sulphidic conditions within BBD-lesions may have inactivated and degraded bacteriophages to variable extents, which could have contributed to the detected community compositions within sample types (Fig. 1). Lastly, it is possible that the genetic marker *gp23* does not sufficiently cover the taxonomic range of the bacteriophage communities present in our samples, and therefore may not have allowed identifying a major difference between the communities in BBD-lesions and healthy coral tissue.

### **Potential hosts of T4 bacteriophages**

Nevertheless, three OTUs of bacteriophage sequences showed significantly higher relative abundance in BBD-lesions than in healthy coral tissue (three OTUs, e.g. Cyanophage PRSM6 denovoOTU25920; Table 1, Fig. 2A). Accordingly, the most abundant bacteriophage taxon within the BBD community was Cyanophage PRSM6 (Fig. 2B). Bacteriophage abundances can be dependent on the abundance of their bacterial host, and environmental conditions that affect the growth and abundance of their host bacteria (Chibani-Chennoufi *et al.*, 2004; Chow *et al.*, 2013). Highly abundant bacteriophage sequences in BBD samples may therefore represent phages that are able to infect bacteria that are associated with BBD-lesions. Although this has to be verified further with viral isolation and inoculation tests, these cyanophages could infect BBD-associated cyanobacteria that dominate the lesion biomass (Rützler *et al.*, 1983; Rützler and Santavy, 1983), such as the cyanobacterium *R. reptotaenium* (Casamatta *et al.*, 2012). The

presence of bacteriophages capable of infecting BBD-associated cyanobacteria within BBD-lesions is supported by recent studies. Complex CRISPR-Cas systems, which defend against bacteriophage infections, and prophage signatures that contain putative virulence factors have been detected in the genomes of the BBD-associated filamentous cyanobacteria, *R. reptotaenium* AO1 and *Geitlerinema* sp. BBD\_1991 (Buerger *et al.*, 2016; Den Uyl *et al.*, 2016). Accordingly, 5 of the spacer sequences of *Geitlerinema* sp. BBD\_1991 showed nucleotide similarities to 32 *gp23* OTU sequences, pointing towards a relation between the current *gp23* sequences and the spacers of the BBD cyanobacterial CRISPR-Cas system. While the spacer-OTU matches were not exact in order to prove that the sequence is a target of the cyanobacterial CRISPR-Cas system, the corresponding OTUs were detected only in low abundance and mainly present in seawater samples, not in BBD or healthy coral tissue (Table S2). Moreover, high-throughput shotgun sequencing recovered a relatively higher proportion of particular cyanophage sequences in a metatranscriptomic data set from samples of fully developed BBD-lesions compared to the pre-disease 'cyanobacterial patch' stage (Sato *et al.*, 2017). Most of these BBD metatranscriptomic viral reads were identical to photosynthetic gene D1 sequences of cyanobacteria dominating the BBD lesion, inferring a phage-cyanobacterial host association (Sato *et al.*, 2017). The complementary results of these recent studies corroborate that the dominant BBD cyanobacteria are likely to be a target of BBD-associated bacteriophages, such as the novel bacteriophage sequences detected in this study. Thus, further studies may reveal more bacterium-bacteriophage pairings that characteristically occur within the BBD lesion. The presence of bacteriophages can shape and accelerate the evolution of pathogenic bacteria in biofilms by enforcing a strong

selection pressure (Davies *et al.* 2016) and result in an arms race between the bacteriophage community and the bacteria (Stern and Sorek, 2011). It is therefore possible that BBD associated bacteriophages can influence and/or may have influenced the evolution of the BBD-associated cyanobacteria and their potential role in the BBD pathogenicity.

### **Concluding remarks**

This study provides the first estimate of the bacteriophage diversity associated with BBD disease in comparison to those associated with coral tissue and seawater. We identified a number of bacteriophage OTUs abundant in BBD-lesions, which potentially infect BBD-associated bacteria. Viral infection of BBD-associated bacteria could contribute to increased BBD pathogenicity through lysogeny and the integration of virulence genes into the BBD bacterial host genomes. Conversely, it could also reduce the abundance of BBD associated bacteria and mitigate disease virulence by lysing their hosts, which is the concept implemented in a phage therapy (Efrony *et al.*, 2007; 2009). However, BBD-associated bacteriophages may also indirectly fuel BBD virulence through the lysis of BBD associated bacteria including the cyanobacteria and release of organic matter within the lesion, which may promote the growth other bacteria within BBD-lesions that are harmful to coral tissue. Further research is required to test the above mentioned hypotheses, and future studies should focus on retrieving whole virome (viral metagenome) data sets associated with BBD using a method targeted towards viruses, such as density-gradient separation of viral particles, as well as on cultivating disease-associated bacteriophages in order to determine their characteristics, functions and host ranges.

## Experimental Procedures

### Coral tissue sampling and DNA extractions

An opportunistic set of lesion samples from BBD-infected colonies and tissue samples from healthy coral colonies of the coral *Montipora hispida* were collected from reefs surrounding Orpheus Island (18.6376 S, 146.4982 E) at depths of 3 - 5 m during 2007 and 2008 (BBD lesion n = 10 samples; healthy tissue n = 4 samples; each sample from a different coral colony; Table S3). While BBD samples were collected as the disease transitioned from low prevalence in winter months to high prevalence in summer months, healthy coral tissue samples were collected at the end of the summer sampling period. BBD-lesions with underlying coral skeleton and tissue from healthy corals were removed with sterile razor blades (20-50 mg per sample), preserved in 100% ethanol and stored at -20°C until further processing. Prior to DNA extraction, ethanol was evaporated from samples for 10 min at 30°C. In 2013, whole DNA content of coral tissues and BBD-lesions (~20 mg) was extracted for T4-bacteriophage and bacterial community analyses with a Mo-Bio Power Plant Pro kit (cat. no. 13400-50), according to the manufacturer's recommendations with the following modifications. Samples were crushed and lysed by bead beating in Power Plant Pro kit solution PD1 (450 µL), PD2 (50 µL) and RNase A (3 µL, 25 mg/mL) for 60 seconds at max speed (BioSpec 1001, Mini-Beadbeater-96) to disrupt cells and to remove RNA-contamination. Proteinase K (15 µL, 20 mg/mL) was subsequently added to all samples, incubated for 1 hour at 56°C, 10 min at 65°C, and bead beaten again for 1 min at max speed to fully lyse the tissue and open virus capsid proteins that contain the target DNA. DNA was eluted from columns with 50 µL TE,

incubation time 2 x 5 min.

### **Seawater sampling and DNA extractions**

Seawater samples were collected as controls between 2012 and 2015 near healthy and BBD-affected corals to supplement this project (seawater BBD n = 8; seawater healthy-coral n = 6; Table S3). For each sample, approximately 20 L of seawater were collected in five 4 L container bags less than 5 cm above the coral surface. Water samples were stored in carboys on ice water and in the dark, and transported to the Australian Institute of Marine Science (AIMS) before being stored at 4°C. The following day all seawater samples were filtered through 0.22 µm (Sterivex, GV, Cat no. SVGV010RS, Lot. No. 412H6407; Billerica, MA, USA) to capture bacterial communities for DNA extraction. The loaded filters were stored at -20°C until further processing. Bacteria cells captured on Sterivex filters were disrupted by replacing seawater with a lysis buffer (1M Tris-HCl, 0.5M EDTA, 25.6 g Sucrose in 100 mL MiliQ) and subsequent incubation for 24 h. Disrupted cells (500 µL) were taken for DNA extraction with a Mo-Bio Power Plant Pro kit according to the previously described conditions. The remaining virus communities in the seawater filtrate, after removal of bacteria with Sterivex filters, were concentrated to approximately 200 mL by tangential flow filtration (TFF, filter cassette: 10 kDa) with subsequent 50 mL back-flush. Viral DNA in remaining seawater samples was extracted for T4-bacteriophage analyses with a Mo-Bio Power Plant Pro kit, using the methods described above (volume for DNA extractions: 20 µL of viral concentrate at  $\sim 2 \times 10^6 - 10^7$  viral particles mL<sup>-1</sup>).

### **Methodological caveats**

Since the DNA extractions were conducted up to six years after sample collection, it is possible that some of the DNA has degraded over time although samples were preserved and stored in the conditions mentioned above. This may have resulted in an underestimation of the true diversity and failed to capture rare, low abundance taxa in our samples. It is also possible that the different preservation methods of seawater, coral, and BBD tissue samples influenced the respective detected bacterial and bacteriophage community compositions (ethanol preservation for disease and tissue samples compared to freezing or refrigeration of seawater samples). In addition, seawater samples were collected over different years after BBD-lesions and coral tissue samples were collected, and are therefore difficult to directly compare one another. In order to examine the potential caveats of our data set, we compared the OTU compositions in our samples to those previously analysed in relevant projects (e.g. Cooney *et al.*, 2002; Houghton, 2015; Apprill *et al.*, 2012). Since our bacterial analyses reflect bacterial taxa previously described from the corresponding sample groups, we are confident that possible sample-preparation biases were small and that our analyses are reasonable and meaningful when the above mentioned caveats are taken into account.

### **Polymerase chain reaction (PCR)**

The bacteria 16S rRNA gene V3 and V4 regions were amplified with PCR primers Bakt\_341F and Bakt\_805R (Herlemann *et al.*, 2011; Klindworth *et al.*, 2013), and the T4 bacteriophage gp23 capsid protein gene was amplified with the primer pair MZIA6 and MZIA1bis (Filée *et al.*, 2005), both with an expected amplicon size of ~500 bp (Table S4). A touchdown PCR protocol was used to increase specificity during amplification of the



marker genes with a MyTaq polymerase (Bioline, BIO-25041, for cycle details see Table S5). The amplified 16S rRNA and T4 *gp23* PCR products were loaded on an agarose gel (TBE, 1.5 %) and amplicons in the range of 250 - 600 bp were excised and purified with a Qiaquick Gel Purification kit (Qiagen). Illumina sequencing adapters were added to amplicons in a second PCR that used the locus specific primers with the Illumina overhang adapters in 15 amplification cycles (Table S5). All libraries were normalised to a volume of 20  $\mu\text{L}$  at a DNA concentration of 5  $\text{ng } \mu\text{L}^{-1}$  before next generation sequencing with Illumina Miseq (2x300 bp read, v3 chemistry) performed at Ramaciotti Centre, UNSW, Australia. Paired-end sequences were merged with the software PEAR 0.9.5 (Zhang *et al.*, 2014) (average merged reads proportions: 92.2%). Merged reads with a mean phred score below 30 and length below 75 bases were removed (~31% of the total reads). Samples with a low merged proportion of < 50% or less were not considered for further analyses because of potential technical biases and under-representation of associated communities. Further, primer sequences and duplicates were removed from data sets with Fastx v0.0.13 ([http://hannonlab.cshl.edu/fastx\\_toolkit/](http://hannonlab.cshl.edu/fastx_toolkit/)) to account for PCR amplification bias and subsequently used to compare biodiversity among samples (bioinformatics pipeline code available through github, links in Table S2). An average number of ~45,000 reads per sample were left after quality control and dereplication (Table S3).

### **Bacterial community composition based on 16S rRNA gene sequences**

Bacterial biodiversity among and within samples was analysed using the software package QIIME version 1.9.1 (Zuris *et al.*, 2014), (bioinformatics pipeline in Table S2). In brief, sequences with a minimum of 97% sequence similarity were grouped into *de novo*

operational taxon units (OTUs) with UCLUST, aligned with Python Nearest Alignment Space Termination (PyNAST, Caporaso *et al.*, 2010), identified taxonomically with the Greengenes 16S rRNA database (version gg\_13\_8; DeSantis *et al.*, 2006) and visualised as boxplots. Alpha diversity (i.e. diversity of OTUs within an individual sample) was measured with Chao1 index and compared among different sample types. Beta diversity (i.e. diversity of community compositions among different samples) was assessed with Bray-Curtis distances calculated from the relative OTU abundance and visualised in a multidimensional scaling plot (MDS). In addition, bacterial OTUs with a taxonomic assignment to eukaryotic chloroplasts were not considered in the bacterial analysis (links to OTU tables and assigned taxonomy available in Table S2).

#### **T4 bacteriophage community composition based on *gp23* gene sequences**

Bacteriophage *gp23* sequences were analysed in QIIME with the built-in *de novo* OTU picking algorithm to compare respective alpha and beta diversities. Sequence similarity cut-offs may not accurately reflect viral taxonomic boundaries, such as boundaries among species or other taxonomic levels (Erko and Ebers, 2006). In addition, sequence similarity cut-offs are not well established for virus communities, as species boundaries between viruses are less defined compared to bacteria and genetic substitution can occur frequently. Based on tests across UCLUST clustering cut-offs (45 ~ 97% sequence similarities, Fig. S2), showing consistent separation patterns among sample types from 85% to 97%, a 97% sequence similarity cut-off, consistent with bacterial analyses, was chosen for the further OTU analyses to retain detection sensitivity to community differences. OTU sequences were aligned in QIIME with the algorithm of Multiple

Alignment using Fast Fourier Transform (MAFFT), and alpha-rarefaction curves were calculated with Chao1 index. Bray-Curtis distances were calculated from the relative OTU abundance, and overall community differences were visualised in an MDS plot. *Gp23* sequences were taxonomically identified using MetaVir (Roux *et al.*, 2011) with a BLASTx with a  $10^{-7}$  e-value threshold against the viral RefSeq database from the 05.01.2015 (Roux *et al.*, 2011; Fig. 2B). Links to raw data, OTU tables, and assigned taxonomy available in Table S2. OTU sequences that showed statistically different abundance between healthy coral tissue and BBD-lesions were submitted to the NCBI (accession numbers in Table 1). In addition, the *gp23* sequences were compared to a CRISPR-Cas spacer data set of the BBD associated cyanobacteria *Geitlerinema* sp. and *R. reptotaenium* (Buerger *et al.*, 2016b). This step may reveal bacteriophages that previously infected the cyanobacteria, and that spacers potentially contribute to a resistance against bacteriophage infections.

### Statistical tests

Beta diversities based on the relative OTU abundance for bacteria (16S rRNA) and T4-bacteriophage (*gp23*) were calculated in form of Bray-Curtis distance matrices and were compared among the 4 sample types (BBD, Healthy-tissue, Seawater-BBD, Seawater-Control) with a one-way PERMANOVA, permutations  $n = 9999$  with Bonferroni sequentially corrected p-values (Fig. 1). Relative abundances of OTUs for 16S rRNA and *gp23* sequences were compared between BBD and Healthy-tissue sample types with the DESeq2 package, available through R and the QIIME script `differential_abundance.py` (Fig. 2, DESeq2 output tables available Table S2). As recommended in the QIIME

workflow, we removed OTU singletons and low depth OTUs below a total of 1000 sequences across samples before the DESeq analysis in order to exclude potential sequencing errors.

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