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Possible links between white plague-like disease, scleractinian corals, and a cryptochirid gall crab

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ABSTRACT: White plague (WP) is a highly destructive coral disease that rapidly kills susceptible coral species by mass tissue lysis. The pathogen and underlying causes of this disease are not known. In this laboratory-based study, we examined a small coral-associated gall crab from the family Cryptochiridae in terms of a possible association with WP-like lesions. A series of experiments was conducted after observations that 2 scleractinian coral species, *Diploria labyrinthiformis* and *Pseudodiploria strigosa*, developed signs of WP-like disease within a laboratory holding aquarium and that small gall crabs were physically present in the center of each lesion. Using fragments of *D. labyrinthiformis*, a crab from one of the lesions was sequentially removed and placed, under controlled conditions, onto apparently healthy coral colonies, resulting in the development of similar lesions. Next-generation sequencing of the 16S rRNA gene was performed to profile the bacterial communities associated with the crab, lesions, and healthy corals. The microbiota of the crab and lesions were highly similar while that of apparently healthy colonies were significantly different. Significant differences were largely due to an increase in *Alphaproteobacteria* in crab and lesion communities. In particular, the *Roseobacter* clade had a higher relative abundance in the crab and WP-like lesions. This study suggests that the cryptochirid gall crab may be associated with development of WP-like lesions.

KEY WORDS: Coral disease · White plague · *Rhodobacteraceae* · Gall crab · *Diploria* · *Pseudodiploria*

INTRODUCTION

Coral diseases have been on the rise since they were first reported in 1965 (Squires 1965) and are responsible for a large portion of coral cover decline on tropical and sub-tropical reefs (Sutherland et al. 2004). One of the most destructive and widespread of coral diseases is white plague (WP). WP was originally described in 1977 (Dustan 1977) on reefs of the Florida Keys (USA). A WP epizootic event on these same reefs in 1995 was documented to kill 38% of the most susceptible (of 17 affected) coral species (Richardson et al. 1998a,b). As with the majority of coral diseases, WP is identified based on macroscopic phenotypic characteristics (Ainsworth et al. 2007), primarily a distinct, moving boundary between apparently healthy coral tissue and newly exposed (white) coral skeleton (Dustan 1977, Bythell et al. 2004). Three types of WP (Types I, II, and III) were defined based upon lesion rates, prevalence rates, and coral species affected (Richardson 1998, Richardson et al. 2001). In more recent years, investigators have been using the term WP-like, or white syndrome, when considering this coral disease, since to date no WP pathogen has been discovered (Bythell et al. 2004). As proposed by Bourne et al. (2015), the term WP (as well as 'white band' and 'white pox') is used to describe Caribbean diseases, while 'white

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syndrome' refers to Indo-Pacific white diseases. Studies of WP disease have included microbiological, histological, and cytological methods. Such studies have led to proposed WP pathogens that include *Aurantimonas coralicida* in the wider Caribbean (Denner et al. 2003) and *Thalassomonas loyana* in the Red Sea (Thompson et al. 2006). However, no single pathogen is consistently found in all WP lesions.

In studies of potential pathogens and mechanisms of coral disease, it is important to consider all aspects of the coral and its many associates. Corals live in association with a multitude of microorganisms including viruses, bacteria, archaea, fungi, protozoa, and the endosymbiotic alga *Symbiodinium*, which are all proposed to play roles in maintaining the health of the coral holobiont (Rohwer et al. 2002, Reshef et al. 2006, Rosenberg et al. 2007, Wegley et al. 2007). Corals also serve as habitat for many macroscopic creatures, including gall crabs. Some female gall crabs can spend their entire life on a single coral head (van der Meij 2014). Coral-dwelling crabs are among the most abundant macrofauna associated with corals (Stella et al. 2010), with up to 25% of coral colonies occupied by 1 or more of these crabs (Simon-Blecher & Achituv 1997). The interactions and dynamics between coral-dwelling gall crabs and the microbiota of the coral holobiont are entirely unknown, but are of interest since such coral-dwelling organisms have the potential to act as vectors of pathogenic microorganisms. Gall crabs in particular are suspect, since some males may be free living, moving from coral to coral in order to mate (Johnsson et al. 2006), although the life-history traits of gall crabs are sparsely studied and poorly understood. In this study, which was based on the observation that gall crabs were physically associated with WP-like lesions (Fig. 1), we conducted controlled laboratory experiments using aquaria to examine the connection between WP-like lesions on 2 WP-susceptible coral species, viz. *Pseudodiploria strigosa* and *Diploria labyrinthiformis*, and a cryptochirid gall crab. Laboratory experiments included coral–crab challenges supplemented with observations of lesion development and progression, and microbial assessment via 16S rRNA gene microbial profiling.

Fig. 1. Cryptochirid gall crab with associated white plague-like lesion on a fragment of the coral *Diploria labyrinthiformis*. Note the distinct boundary between the recently exposed white skeleton and apparently healthy tissue around the crab lesion as well as the outer skirt of the coral. Scale bar = ~5 mm

MATERIALS AND METHODS

Observations

Two *Pseudodiploria strigosa* colonies (approximately 25 cm^2) were collected from the Florida Keys National Marine Sanctuary (FKNMS) Coral Nursery in October 2013 and placed in a large (340 l) holding aquarium together with a number of other coral fragments that had been collected at various times and maintained in a healthy state since February 2011. No other colonies were collected at this time. Aquarium conditions were identical to those described by Pratte & Richardson (2014) (25°C, 34 ppt salinity, and a 12 h light:12 h dark cycle using metal-halide and fluorescent lighting). At no point were any of the coral fragments fed, although zooplankton could be seen when aquarium water was examined under a microscope. The holding aquarium contained small hermit crabs and snails purchased from an aquarium store in 2011 in order to maintain healthy algae levels. All other small/microscopic organisms within the aquarium originated from previously collected coral fragments. After 1 mo (November 2013), WP-like signs were observed on 1 of the 2 recently collected *P. strigosa* colonies. Visual examination revealed that 5 small (2 mm) crabs were on the surface of the afflicted colony and that in each case, the crab was in the center of a newly present lesion. These crabs may have been present on the *P. strigosa* colony at the time of the fragment collection, although they were not noted at that time. All 5 crabs were removed and either placed in 95% ethanol to preserve for identification purposes (n = 3), or crushed in RNAlater® (Life Technologies) for microbial analysis (n = 2). The affected *P. strigosa* colony was removed and placed in an isolation aquarium. This colony was not used

for further experimentation or analyses. In January 2014, a sixth crab resembling those found on the *P. strigosa* colony, and also associated with a WP-like lesion, was discovered on a separate, small (3 cm^2) *P. strigosa* fragment which had been maintained in the large holding aquarium prior to October 2013. Although the sixth crab resembled the 5 removed from the original *P. strigosa* colony, it cannot be certain whether this crab was associated with the same colony and migrated to a new fragment, or whether it was present prior to the introduction of the *P. strigosa* colony. The crab and associated small fragment were placed in a small (3.8 l) aquarium and used for further experimentation. Three months after the introduction of the original *P. strigosa* colony, WP-like lesions spread throughout the large holding aquarium for the next 6 mo, affecting roughly 30 to 50% of the *P. strigosa* fragments at any one time. The closely related species *Diploria labyrinthiformis* was also affected in similar proportions (see below).

Coral–crab challenges

Previously collected (November 2012 and October 2013) coral fragments all obtained from the FKNMS Coral Nursery were also maintained in the large holding aquarium at the time of disease outbreak. These fragments consisted of *D. labyrinthiformis* (~30 fragments), *Montastraea cavernosa* (~10 fragments), and *Siderastrea siderea* (~3 fragments), in addition to *P. strigosa* (~6 fragments). All fragments were approximately 3 cm^2 and had been previously set onto small cement pedestals. Some of these fragments had been maintained in this aquarium for over 2 yr, remaining apparently healthy and showing signs of growth. Prior to the disease outbreak, mortality of coral fragments of all 4 species was rare. Three previously collected *D. labyrinthiformis* fragments had been maintained in a smaller isolation aquarium (19 l) that had never been exposed to the same water or equipment as the large holding aquarium (in which WP-like lesions had been observed as described above) and had not come into contact with any diseased coral. These 3 fragments were considered, and are termed in this study, 'naïve.' One naïve fragment was placed in a small experimental aquarium (3.8 l) with a recirculating filter under the same lighting conditions as the 19 l aquarium. All experimental aquaria were sterilized using a combination of 5% bleach solutions followed by a 30 min wash in continuously flowing hot water (to remove bleach residue) and allowed to thoroughly dry before any experimentation. A small live gall crab was carefully removed using soft forceps, rinsed with sterile sea water, gently patted dry 3 times with Kimwipes™, and subsequently placed onto a naïve coral fragment to test for coral disease transmission. Observations for lesion initiation and development were made and recorded every 1 to 3 d. This experiment was repeated 3 times using the remaining naïve fragments. Lesions were allowed to progress until fragment mortality occurred (Table 1).

To compare WP-like lesion progression on fragments with and without the gall crab and to test for the possibility of water-borne transmissibility, 2 apparently healthy non-naïve *D. labyrinthiformis* fragments from the large holding aquarium, in which the original outbreak occurred, were placed in a small experimental aquarium (3.8 l) with a fine screen mesh (approximately 1 mm^2 mesh size) dividing the 2 fragments (i.e. the 2 fragments were placed in the same tank sharing the same water, with ample flow between them). The crab was rinsed as previously described and placed on 1 of the fragments on one side of the aquarium. Access to the other fragment was restricted by the screen that divided the aquarium. Accordingly, these fragments were termed 'screened' fragments. Observations were documented as above. This experiment was repeated 3 times, and all disease lesions were allowed to progress until fragment mortality.

To ensure that lesions and mortality were not due to aquarium conditions, 2 apparently healthy *D. labyrinthiformis* fragments (non-naïve) were maintained in separate identical small aquaria without crabs to serve as an experimental control (Table 1).

Metagenomic microbial analysis

Metagenomic analysis was conducted on coral skeleton, tissue, and surface mucopolysaccharide layer samples collected from 3 *D. labyrinthiformis* fragments with non-crab associated lesions from the large holding aquarium (termed 'disease lesion') and 3 *D. labyrinthiformis* fragments with lesions created by the experimental crab (termed 'crab lesion'). All fragments were different than those used in the crab challenge experiments. Samples were placed into 1 ml of RNAlater® (Life Technologies) and frozen at −80°C until further processing. Two crabs from the original observation were also crushed and preserved in 1 ml of RNAlater® (Life Technologies). Data for apparently healthy *D. labyrinthiformis* were used from a parallel study examining the microbiota of this coral species.

Table 1. Lesion progression for all *Diploria labyrinthiformis* fragments (each ca. 3 cm^2). Details of the experiments are provided in the 'Materials and methods'. t_0: day of crab introduction

Fragment ID	Days from t_0 to lesion formation	Days from t_0 to mortality or sampling
Coral–crab challenge		
Naïve fragment 1	20	53[a]
Naïve fragment 2	13	30[a]
Naïve fragment 3	13	22[a]
Screen experiment		
Crab fragment 1	3	7[a]
Screened fragment 1	4	8[a]
Crab fragment 2	6	14[a]
Screened fragment 2	11	22[a]
Crab fragment 3	9	24[a]
Screened fragment 3	12	28[a]
Metagenomics		
Apparently healthy 1	No lesion	No mortality
Apparently healthy 2	No lesion	No mortality
Apparently healthy 3	No lesion	No mortality
Disease lesion 1	Unknown[b]	Unknown[b]
Disease lesion 2	Unknown[b]	Unknown[b]
Disease lesion 3	Unknown[b]	Unknown[b]
Crab lesion 1	7	7[c]
Crab lesion 2	6	10[c]
Crab lesion 3	8	14[c]
Control		
Control 1	No lesion	No mortality
Control 2	No lesion	No mortality

[a]Days to mortality. [b]Sampled from large holding aquarium; lesion initiation date unknown. Lesion progression estimated at 1 cm wk^{-1}. [c]Days to sampling

All DNA extractions of mucus, tissue, and skeleton proceeded as described by Pratte et al. (2015) using the FastDNA™ Spin Kit for Soil (Qbiogene). DNA was quantified using the Qubit® 2.0 Fluorometer (Life Technologies) and pooled according to sample type. The V4 and V5 region of the 16S rRNA gene was amplified via PCR using primers F563/BSR926 (Claesson et al. 2010). PCR conditions were according to Pratte et al. (2015). All reactions were run in duplicate, and products were verified on a 1.8% TBE agarose gel and GelRed™ (Biotium). Pooled PCR products were then barcoded according to sample type and submitted to the DNA Sequencing Facility at Florida International University. Each sample type was given a separate barcode using the Ion Xpress RNA-Seq Barcode 01-16 Kit (Life Technologies) and run on the Ion 316 Chip v2 (Life Technologies). The Ion Torrent PGM (Life Technologies) performed 200 base-read sequencing using the Ion PGM Sequencing 200 Kit v2 (Life Technologies). Ion Torrent PGM sequences were filtered using Torrent Suite v4.2 software to remove polyclonal and low quality sequences. The total number of raw reads obtained was 2 837 430, with all samples having between 527 016 and 985 678 reads after quality control (see Table S1 in the Supplement at www.int-res.com/articles/suppl/d122p153_supp.pdf).

Data analysis

The .fastq files generated by the Ion Torrent server were imported into the Metagenomics (MG)-RAST server for analysis. This server is a public resource for the automatic phylogenetic and functional analysis of metagenomes (Meyer et al. 2008). All sequences are available on the MG-RAST public server under project number 12498. After importation, the sequences were screened for quality control a second time. Sequences of bacterial origin (as determined by MG-RAST) were sorted according to class and exported for further statistical analysis using PRIMER 6 software (Primer-E Ltd.). Only bacterial classes with a minimum of 5 reads were included in the analysis. Data were square root transformed, and cluster analysis was performed using Bray-Curtis similarity matrices (Clarke 1993, Clarke & Gorley 2001). Statistical significance between communities was tested using similarity profile analysis (SIMPROF, Clarke et al. 2008), and dissimilarity contributions were examined using a 1-way similarity percentages (SIMPER) analysis (Clarke 1993). Shannon-Wiener diversity indices (H' log e) were also calculated using PRIMER-E. Additionally, individual operational taxonomic units (OTUs) at the genus and species level were manually curated for OTUs of interest. Raw sequence data are available at NCBI's BioProject under Project ID PRKNA322416.

RESULTS

Observations

The crabs isolated from the original *Pseudodiploria strigosa* coral fragment were identified to be from the family Cryptochiridae (Kropp & Manning 1987). Genus and species could not be assigned, as the crabs were juveniles and distinguishing features were not yet developed. After removal of the crabs, tissue loss associated with the WP-like lesion slowly enlarged, taking nearly a year to kill the fragment. Lesions maintained the characteristics of

WP, with a distinct boundary of recently exposed skeleton at the remaining tissue margin. Other coral fragments (all ~3 cm^2) residing in the same large holding aquarium began showing signs of WP-like disease approximately 3 mo after the introduction of the initial *P. strigosa* fragment (discussed above). Within the holding tank, the majority of both *P. strigosa* and *Diploria labyrinthiformis* fragments eventually succumbed to the disease, whereas fragments of *Montastraea cavernosa* and *Siderastrea siderea* remained apparently healthy. Lesions progressed at highly variable rates, independent of species (*D. labyrinthiformis* or *P. strigosa*) (Table 1).

Coral–crab challenges

Each time the gall crab was placed onto a naïve *D. labyrinthiformis* fragment (n = 3 naïve fragments), a WP-like lesion developed directly under the crab (Fig. 1). Lesion initiation took place in 13 to 20 d, after which it took from 9 to 33 d to kill the fragment (Table 1). In the screened experiments where the crab did not have access to a fragment, but shared the same aquarium water, lesions resembling WP developed on both fragments over a period of days, with lesions developing first on the fragment containing the crab followed by the screened fragment. In each case (n = 3 experiments), the fragment containing the crab died first, followed by the fragment in the screened area of the aquarium. The 2 control coral fragments (non-naïve) maintained in an identical aquarium with no crab remained alive and healthy for 9 mo.

Bacterial communities

There was a clear pattern in the bacterial communities associated with the crab and WP-like lesions when compared with healthy coral, as shown in Fig. 2. SIMPROF indicated that the apparently healthy bacterial community was significantly different when compared to the rest of the samples ($p < 0.05$). This result was verified by SIMPER analysis. The bacterial communities of the disease lesion, crab lesion, and crab were similar (Fig. 2, Table 2), with cluster analysis revealing a similarity of 80%. In contrast, the apparently healthy bacterial community clustered independently (Fig. 3). SIMPER analysis indicated that for all lesion and crab communities, the *Alphaproteobacteria* was the most dissimilar class when compared to the apparently healthy bacterial community (Table 2). The apparently healthy community was composed of just 3.7% *Alphaproteobacteria*, while all other samples contained 25.9 to 35.1% *Alphaproteobacteria* (Fig. 2). *Bacilli* and *Clostridia* also contributed at least 15% each to the dissimilarities between apparently healthy and all other samples. *Bacilli* were reduced in both lesions (9.9 and 10.5%) compared to apparently healthy (19.0%), as were *Clostridia* (12.1 and 15.8% compared to 25.8%). The crab microbial community contained 1.4% *Fusobacteria*, compared to 0.5 to 0.9% for all other samples. *Gammaproteobacteria* were more abundant in the apparently healthy community (23.1%) when compared to all other communities (17.3–19.6%), as were *Planctomycetes* (2.9% compared to 0.4–0.6%). *Cyanobacteria* comprised a total of 2.2% of the population for the crab community, 1.1% for the crab lesion community, and 2.4% for the disease lesion community.

Comparisons within the data base at the genus and species levels revealed that *Kineococcus radiotolerans*, *Trichodesmium erythraeum*, *Lactobacillus vaginalis*, *Dethiobacter alkaliphilus*, and *Reinekea blandensis* were all more abundant in the apparently healthy bacterial community than in both bacterial communities associated with lesions. The genus *Vibrio* was present at 3.4% in the crab lesion, but only 0.3% in the crab and 0.1% in the disease lesion communities. The genera *Dinoroseobacter*, *Rhodobacter*,

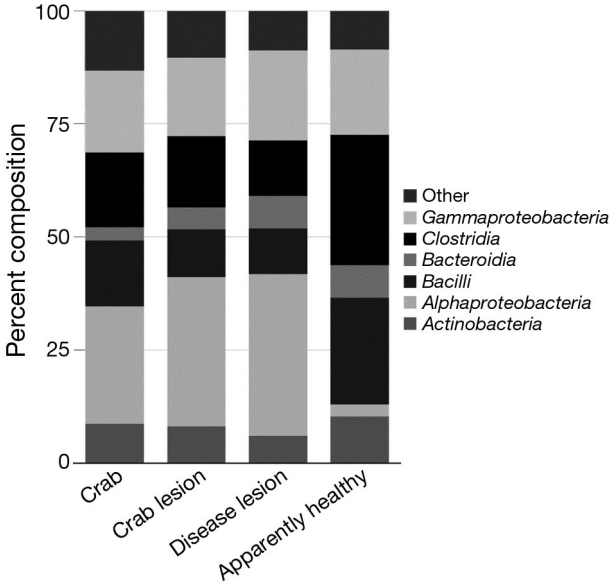

Fig. 2. Percent composition of bacterial communities associated with the cryptochirid gall crab (crab), lesions caused by the crab (crab lesion), lesions of white plague-like disease (disease lesion), and apparently healthy coral

Table 2. SIMPER analysis comparing 4 bacterial communities associated with a cryptochirid gall crab (crab), crab-exposed (crab lesion, CL), white plague-like (disease lesion, DL), and apparently healthy (AH) *Diploria labyrinthiformis*. Average dissimilarity is overall dissimilarity among samples, and the percent contribution of each bacterial class to the average dissimilarity shown is shown below. Classes contributing <2% not shown

	Crab vs. CL (%)	Crab vs. DL (%)	CL vs. DL (%)	Crab vs. AH (%)	CL vs. AH (%)	DL vs. AH (%)
Average dissimilarity	11.1	16.4	9.8	29.5	35.2	39.2
% dissimilarity by Class						
Actinobacteria	2.61	8.43	11.1	2.79	3.15	5.63
Alphaproteobacteria	32.3	28.6	11.0	39.6	43.3	41.9
Bacilli	17.9	14.2	3.3	15.5	18.6	17.7
Bacteroidia	8.30	12.7	11.6	7.3	3.5	<2
Clostridia	3.05	13.7	19.3	21.1	18.6	21.6
Deltaproteobacteria	7.95	10.1	7.71	<2	3.48	5.04
Flavobacteriia	4.33	<2	4.66	<2	<2	<2
Gammaproteobacteria	3.39	4.65	11.6	<2	<2	<2
Unclassified	10.3	<2	9.25	4.03	<2	<2

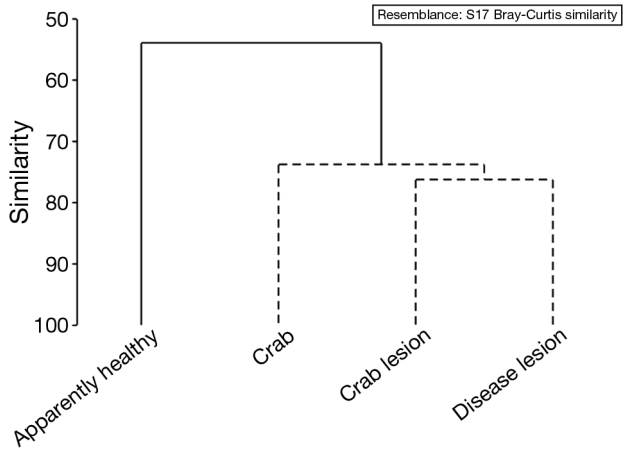

Fig. 3. Cluster analysis (based upon Bray-Curtis similarity) of bacterial communities associated with apparently healthy coral *Diploria labyrinthiformis* fragments, tissue loss lesions resembling white plague-like signs (disease lesion), lesions caused by the presence of a cryptochirid gall crab (crab lesion), and the bacterial community associated with the gall crab itself (crab). Solid lines indicate significant differences as revealed by SIMPROF analysis

Roseobacter, *Roseovarius*, and *Ruegeria* were responsible for the increase in *Alphaproteobacteria* in the lesion and crab samples, in particular due to an OTU similar to *Roseobacter denitrificans*.

DISCUSSION

Recently, the varying descriptions of WP-like and white syndrome lesions, diversity of potential pathogens associated with these lesions, and the ranges of coral species infected have been suggested to be a result of a variety of causations, rather than a single infectious agent (Ainsworth et al. 2007, Work et al. 2012, Cook et al. 2013, Kellogg et al. 2013, Bourne et al. 2015). In this paper, we demonstrate an association between WP-like lesions and the presence of a cryptochirid gall crab, which suggests yet another potential variety of WP-like disease causation. In this study, the gall crab-associated *Diploria labyrinthiformis* lesions appeared similar to those described for WP (Bythell et al. 2004) (Fig. 1), although categorization with previously described WP types (Types I, II, or III) could not be distinguished since different rates of lesion progression were observed (Table 1). The screened experiment demonstrated that the physical presence of the cryptochirid crab on a coral fragment was not necessary for the development of lesions, suggesting the potential of a water-borne pathogen; however, we also observed that the disease was more severe, in terms of lesion development and mortality rates, with direct contact between the crab and fragment as compared to exposure to the same water.

This study examined coral gall crabs. There are 2 classifications of coral-dwelling crabs, viz. the family Cryptochiridae (this study) and the families Trapeziidae and Tetraliidae that belong to the superfamily Trapezioidea (Pratchett et al. 2000, Stewart et al. 2006, 2013, Lai et al. 2009, Stier et al. 2010, McKeon et al. 2012). It is generally agreed that the Trapezioidea act to defend their coral hosts against predators and possibly slow disease progress (Pratchett 2001, Stier et al. 2010, McKeon et al. 2012, Pollock et al. 2013), while the ecology of the Cryptochiridae is less understood. It would be interesting for future studies to compare the interactions between coral-dwelling crabs and their associated coral colonies.

The composition of microbial communities was remarkably similar between the crab, crab lesion, and disease lesion communities (Figs. 2 & 3), suggesting that crabs may harbor a microbiota similar to that of diseased coral, or that the coral itself could have an impact on the crab microbiota. Further studies should examine the microbiota of cryptochirid crabs inhabiting healthy corals to determine whether the similarities in the coral and crab microbiota exist solely in a diseased state. The bacterial communities of the crab lesion and disease lesion were over 90% similar, indicating that the lesions associated with the presence of the crab were similar to those resembling WP. It cannot be determined how much of this similarity is due to opportunistic bacteria taking advantage of the release of nutrients produced by the lysing of coral tissue. It can be determined, however, that differences between *Alphaproteobacteria*, *Bacilli*, and *Clostridia* accounted for the large majority of dissimilarities detected between the apparently healthy and all other bacterial communities (Table 2). A higher abundance of *Cyanobacteria* was expected in association with the crab, as they are thought to cultivate cyanobacteria in their dwellings (Simon-Blecher et al. 1999). However, this did not appear to be the case in our study.

OTUs were further examined at the genus and species level. However, extreme caution must be used in interpretation, as the combination of short next generation sequencing reads and a limited M5NR MG-RAST database results in many OTUs with low e values and percent similarity (e.g. see Webster et al. 2010). The information for each OTU discussed is given in Table S2 in the Supplement. Despite these limitations, the data provided by MG-RAST at the genus and species levels can still prove useful. For instance, the genus *Vibrio* was examined carefully, as it has previously been associated with coral disease (Kushmaro et al. 2001, Ben-Haim et al. 2003) and bleaching (Bourne et al. 2008). *Vibrio* composed 3.4% of the community in the crab lesion, 0.3% in the crab community, and 0.1% in the disease lesion. In the crab lesion bacterial community, the relatively higher proportion of *Vibrio* was due to hits similar to *V. harveyi* and *V. parahaemolyticus. Vibrio* are commonly found in healthy corals (Kellogg et al. 2013), and at such low abundance, it is unlikely that a *Vibrio* species is the pathological agent in this case.

OTUs similar to *Kineococcus radiotolerans*, *Trichodesmium erythraeum*, *Lactobacillus vaginalis*, *Dethiobacter alkaliphilus*, and *Reinekea blandensis* were all more abundant in the apparently healthy bacterial community than both communities associated with lesions (Table S2 in the Supplement). They should be considered a part of the healthy *D. labyrinthiformis* holobiont community, with relative decreases in the abundance of these bacteria possibly indicating disease. In particular, OTUs similar to *R. blandensis*, *D. alkaliphilus*, and *L. vaginalis* comprised the majority (53.3%) of the apparently healthy bacterial community. The genera *Dinoroseobacter*, *Rhodobacter*, *Roseobacter*, *Roseovarius*, and *Ruegeria* were responsible for the increase in *Alphaproteobacteria* in the disease lesion, crab lesion, and crab bacterial communities. These genera, and the *Rhodobacteraceae* family overall, were not found in the apparently healthy community.

Previous WP studies have reported an increase in the *Rhodobacteraceae* family (Pantos et al. 2003, Pantos & Bythell 2006, Sunagawa et al. 2009, Kellogg et al. 2013, Roder et al. 2014a,b). In fact, *Rhodobacteraceae*, particularly the *Roseobacter* clade, has been reported for a variety of coral diseases, summarized by Mouchka et al. (2010). The majority of these studies attribute the abundance of *Rhodobacteraceae* in diseased samples to opportunistic heterotrophs, as this fits with the general *Rhodobacteraceae* life style (Buchan et al. 2005); however, the question of potential pathogenicity has not been resolved.

In summary, in this study we showed an association between the presence and development of WP-like lesions on corals and the presence of gall crabs. The mechanism by which the gall crab potentially caused the observed lesions is entirely unknown and could be due to abrasion, infection, or a combination of these. Further studies are needed to define the relationships between gall crabs, WP-like lesions, and the bacterial communities associated with each member.

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LITERATURE CITED

Ainsworth TD, Kramasky-Winter E, Loya Y, Hoegh-Guldberg O, Fine M (2007) Coral disease diagnostics: What's between a plague and a band? Appl Environ Microbiol 73:981–992

Ben-Haim Y, Zicherman-Keren M, Rosenberg E (2003) Temperature-regulated bleaching and lysis of the coral *Pocil-*

lopora damicornis by the novel pathogen *Vibrio coralliilyticus*. Appl Environ Microbiol 69:4236–4242

Bourne D, Iida Y, Uthicke S, Smith-Keune C (2008) Changes in coral-associated microbial communities during a bleaching event. ISME J 2:350–363

Bourne DG, Ainsworth TD, Pollock FJ, Willis BL (2015) Towards a better understanding of white syndromes and their causes on Indo-Pacific coral reefs. Coral Reefs 34: 233–242

Buchan A, Gonzalez JM, Moran MA (2005) Overview of the marine *Roseobacter* lineage. Appl Environ Microbiol 71: 5665–5677

Bythell J, Pantos O, Richardson L (2004) White plague, white band, and other 'white' diseases. In: Rosenberg E, Loya Y (eds) Coral health and disease. Springer-Verlag, Berlin, p 351–365

Claesson MJ, Wang Q, O'Sullivan O, Greene-Diniz R, Cole JR, Ross RP, O'Toole PW (2010) Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic Acids Res 38:e200

Clarke KR (1993) Non parametric multivariate analyses of changes in community structure. Aust J Ecol 18: 117–143

Clarke K, Gorley R (2001) PRIMER v6: user manual/tutorial. PRIMER-E, Plymouth

Clark KR, Somerfield PJ, Gorley RN (2008) Testing of null hypotheses in exploratory community analyses: similarity profiles and biota-environment linkage. J Exp Mar Biol Ecol 366:56–69

Cook GM, Rothenberger JP, Sikaroodi M, Gillevet PM, Peters EC, Jonas RB (2013) A comparison of culture-dependent and culture-independent techniques used to characterize bacterial communities on healthy and white plague-diseased corals of the *Montastraea annularis* species complex. Coral Reefs 32:375–388

Denner EB, Smith GW, Busse HJ, Schumann P and others (2003) *Aurantimonas coralicida* gen. nov., sp. nov., the causative agent of white plague type II on Caribbean scleractinian corals. Int J Syst Evol Microbiol 53: 1115–1122

Dustan P (1977) Vitality of reef coral populations off Key Largo, Florida: recruitment and mortality. Environ Geol 2:51–58

Johnsson R, Neves E, Franko GMO, da Silveira FL (2006) The association of two gall crabs (Brachyura: Cryptochiridae) with the reef-building coral *Siderastrea stellata* Verrill, 1868. Hydrobiologia 559:379–384

Kellogg CA, Piceno YM, Tom LM, DeSantis TZ, Gray MA, Zawada DG, Andersen GL (2013) Comparing bacterial community composition between healthy and white plague-like disease states in *Orbicella annularis* using PhyloChip™ G3 microarrays. PLOS ONE 8:e79801

Kropp RK, Manning RB (1987) The Atlantic gall crabs, Family Cryptochiridae (Crustacea, Decapoda, Brachyura). Smithsonian Institution Press, Washington, DC

Kushmaro A, Banin E, Loya Y, Stackebrandt E, Rosenberg E (2001) *Vibrio shiloi* sp. nov., the causative agent of bleaching of the coral *Oculina patagonica*. Int J Syst Evol Microbiol 51:1383–1388

Lai JC, Ahyong ST, Jeng M, Ng PK (2009) Are coral-dwelling crabs monophyletic? A phylogeny of the Trapezioidea (Crustacea: Decapoda: Brachyura). Invertebr Syst 23:402–408

McKeon CS, Stier AC, McIlroy SE, Bolker BM (2012) Multiple defender effects: synergistic coral defense by mutualist crustaceans. Oecologia 169:1095–1103

Meyer F, Paarmann D, D'Souza M, Olson R and others (2008) The metagenomics RAST server — a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 9:386

Mouchka ME, Hewson I, Harvell CD (2010) Coral-associated bacterial assemblages: current knowledge and the potential for climate-driven impacts. Integr Comp Biol 50:662–674

Pantos O, Bythell JC (2006) Bacterial community structure associated with white band disease in the elkhorn coral *Acropora palmata* determined using culture-independent 16S rRNA techniques. Dis Aquat Org 69: 79–88

Pantos O, Cooney RP, Le Tissier M, Barer MR, O'Donnell AG, Bythell JC (2003) The bacterial ecology of a plague-like disease affecting the Caribbean coral *Montastrea annularis*. Environ Microbiol 5:370–382

Pollock F, Katz S, Bourne D, Willis B (2013) *Cymo melanodactylus* crabs slow progression of white syndrome lesions on corals. Coral Reefs 32:43–48

Pratchett MS (2001) Influence of coral symbionts on feeding preferences of crown-of-thorns starfish *Acanthaster planci* in the western Pacific. Mar Ecol Prog Ser 214: 111–119

Pratchett M, Vytopil E, Parks P (2000) Coral crabs influence the feeding patterns of crown-of-thorns starfish. Coral Reefs 19:36

Pratte ZA, Richardson LL (2014) Impacts of temperature increase and acidification on thickness of the surface mucopolysaccharide layer of the Caribbean coral *Diploria* spp. Coral Reefs 33:487–496

Pratte ZA, Richardson LL, Mills DK (2015) Microbiota shifts in the surface mucopolysaccharide layer of corals transferred from natural to aquaria settings. J Invertebr Pathol 125:42–44

Reshef L, Koren O, Loya Y, Zilber-Rosenberg I, Rosenberg E (2006) The coral probiotic hypothesis. Environ Microbiol 8:2068–2073

Richardson LL (1998) Coral diseases: What is really known? Trends Ecol Evol 13:438–443

Richardson LL, Goldberg WM, Kuta KG, Aronson RB and others (1998a) Florida's mystery coral-killer identified. Nature 392:557–558

Richardson LL, Goldberg WM, Carlton RG, Halas J (1998b) Coral disease outbreak in the Florida Keys: plague type II. Rev Biol Trop 46:187–198

Richardson LL, Smith GW, Ritchie KB, Carlton RG (2001) Integrating microbiological, microsensor, molecular, and physiologic techniques in the study of coral disease pathogenesis. Hydrobiologia 460:71–89

Roder C, Arif C, Bayer T, Aranda M and others (2014a) Bacterial profiling of white plague disease in a comparative coral species framework. ISME J 8:31–39

Roder C, Arif C, Daniels C, Weil E, Voolstra CR (2014b) Bacterial profiling of white plague disease across corals and oceans indicates a conserved and distinct disease microbiome. Mol Ecol 23:965–974

Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser 243:1–10

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

Simon-Blecher N, Achituv Y (1997) Relationship between the coral pit crab *Cryptochirus coralliodytes* Heller and its host coral. J Exp Mar Biol Ecol 215:93–102

Simon-Blecher N, Chemedanov A, Eden N, Achituv Y (1999) Pit structure and trophic relationship of the coral pit crab *Cryptochirus coralliodytes*. Mar Biol 134:711–717

Squires DF (1965) Neoplasia in a coral? Science 148:503–505

Stella J, Jones G, Pratchett M (2010) Variation in the structure of epifaunal invertebrate assemblages among coral hosts. Coral Reefs 29:957–973

Stewart HL, Holbrook SJ, Schmitt RJ, Brooks AJ (2006) Symbiotic crabs maintain coral health by clearing sediments. Coral Reefs 25:609–615

Stewart HL, Price NN, Holbrook SJ, Schmitt RJ, Brooks AJ (2013) Determinants of the onset and strength of mutualistic interactions between branching corals and associate crabs. Mar Ecol Prog Ser 493:155–163

Stier A, McKeon C, Osenberg C, Shima J (2010) Guard crabs alleviate deleterious effects of vermetid snails on a branching coral. Coral Reefs 29:1019–1022

Sunagawa S, DeSantis TZ, Piceno YM, Brodie EL and others (2009) Bacterial diversity and white plague disease-associated community changes in the Caribbean coral *Montastraea faveolata*. ISME J 3:512–521

Sutherland KP, Porter JW, Torres C (2004) Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. Mar Ecol Prog Ser 266:273–302

Thompson FL, Barash Y, Sawabe T, Sharon G, Swings J, Rosenberg E (2006) *Thalassomonas loyana* sp. nov., a causative agent of the white plague-like disease of corals on the Eilat coral reef. Int J Syst Evol Microbiol 56: 365–368

van der Meij SET (2014) Host species, range extensions, and an observation of the mating system of Atlantic shallow-water gall crabs (Decapoda: Cryptochiridae). Bull Mar Sci 90:1001–1010

Webster NS, Taylor MW, Behnam F, Lücker S and others (2010) Deep sequencing reveals exceptional diversity and modes of transmission for bacterial sponge symbionts. Environ Microbiol 12:2070–2082

Wegley L, Edwards R, Rodriguez-Brito B, Liu H, Rohwer F (2007) Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. Environ Microbiol 9:2707–2719

Work TM, Russell R, Aeby GS (2012) Tissue loss (white syndrome) in the coral *Montipora capitata* is a dynamic disease with multiple host responses and potential causes. Proc R Soc Lond B Biol Sci 279:4334–4341

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