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Ciliate communities consistently associated with coral diseases

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ABSTRACT

Incidences of coral disease are increasing. Most studies which focus on diseases in these organisms routinely assess variations in bacterial associates. However, other microorganism groups such as viruses, fungi and protozoa are only recently starting to receive attention. This study aimed at assessing the diversity of ciliates associated with coral diseases over a wide geographical range. Here we show that a wide variety of ciliates are associated with all nine coral diseases assessed. Many of these ciliates such as *Trochilia petrani* and *Glauconema trihymene* feed on the bacteria which are likely colonizing the bare skeleton exposed by the advancing disease lesion or the necrotic tissue itself. Others such as *Pseudokeronopsis* and *Licnophora macfarlandi* are common predators of other protozoans and will be attracted by the increase in other ciliate species to the lesion interface. However, a few ciliate species (namely *Varistrombidium kielum*, *Philaster lucinda*, *Philaster guamense*, a *Euplotes* sp., a *Trachelotractus* sp. and a *Condylostoma* sp.) appear to harbor symbiotic algae, potentially from the coral themselves, a result which may indicate that they play some role in the disease pathology at the very least. Although, from this study alone we are not able to discern what roles any of these ciliates play in disease causation, the consistent presence of such communities with disease lesion interfaces warrants further investigation.

1. Introduction

Historically, most coral diseases (specifically those showing aspects of tissue loss) have been associated with numerous pathogenic bacteria (Ben-Haim and Rosenberg, 2002; Cervino et al., 2008; Frias-Lopez et al., 2003; Kushmaro et al., 2001; Luna et al., 2010; Patterson et al., 2002; Richardson et al., 1998; Sussman et al., 2008). However, attention is now turning to other microorganisms such as fungi, viruses and ciliates (Katz et al., 2014; Sweet and Bythell, 2012; Sweet et al., 2014). The first coral disease associated with ciliates for example was Skeleton-Eroding Band (SEB), which was initially described in 2001 (Antonius and Lipscomb, 2001). Characterized by a speckled black band associated with the lesion interface, the disease is thought to be caused by the folliculinid ciliate, *Halofolliculina corallasia* (Winkler et al., 2004). Three years later another coral disease Brown Band Disease (BrB) was described and associated with the ciliate *Philaster guamense*, at the time described as *Porpostoma guamense* (Lobban et al., 2011; Willis et al., 2004). Over in the Caribbean, two years after BrB was first described ciliates similar to those associated with SEB (*Halofolliculina*) were reported affecting over 26 Caribbean reef-building coral species, and the term Caribbean Ciliate Infection (CCI) was coined (Croquer et al., 2006a). Six years later still and White Syndrome (WS), the most prevalent disease sign around the world has also been shown to have a diverse ciliate community associated with the lesion interface (Sweet and Bythell, 2012). More recently, White Band Disease (WBD), White Plague, Brown Jelly Syndrome and another BrB-like syndrome in the Caribbean have all been described as having ciliates associated with the disease signs (Randall et al., 2014; Sweet et al., 2014). Although there are an increasing number of studies linking different ciliate species to specific coral disease states, there are currently no published studies which highlight exactly how such ciliates cause disease. Furthermore, there have been no controlled inoculation experiments identifying if indeed these proposed pathogens are the primary causal agents. This has led to the general belief that many if not all these coral associated ciliates are opportunistic, eating the dead and dying tissue caused by another as yet unknown pathogenic agent. In fact, two recent studies have highlighted that this is actually likely the case, at least for WBD in the Caribbean and WS in the Indo-Pacific (Sweet and Bythell, 2015; Sweet et al., 2014). However, regardless of the specific role of ciliates (which remains to be determined), a first step in understanding their importance in coral disease would be to assess the community associated with different disease states globally. Here, we therefore aim to provide an initial baseline assessment of the ciliate communities associated with nine dominant coral diseases located in both the Indo-Pacific and the Caribbean. Although every care was taken to ensure that a representative sample was taken, we by no means guarantee that every ciliate species has been described here in this study and it remains highly likely

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that more species will be associated with these diseases as further studies assess for them.

2. Methods

2.1. Coral samples

Any coral disease which was present during the time of sampling was collected. Coral from seven Genera were sampled (*Acropora cervicornis*, *Acropora muricata*, *Acropora aspera*, *Pocillopora damicornis*, *Colpophyllia natans*, *Orbicella annularis*, *Porites lutea*, *Goniopora djiboutiensis* and *Diploria strigosa*), from nine geographical locations (Venezuela, Colombia, Australia, the Solomon Islands, Fiji, La Reunion, Moyonette, South Africa and the Maldives) (Table 1). The diseases included; White Syndrome (WS), White Plague Disease (WPD), White Band Disease (WBD), *Porites* White Patch Syndrome (PWPS), Caribbean Ciliate Infection (CCI), Skeletal Eroding Band (SEB), Caribbean Yellow Band Disease (YBD), Brown Band Disease (BrB) and Black Band Disease (BBD) (Fig. 1). Signs associated with each disease were characterized as outlined by the Global Coral Disease Database (coraldisease.org/diseases). Any coral with signs of disease were tagged and monitored in situ to assess disease progression before sampling. Coral fragments were collected only from tagged corals with signs of progression. Fragments (which included the disease lesion interface and 1 cm of healthy tissue) were transported to the field laboratories and the ciliate community assessed under light microscopy. Single cell isolates (minimum of three per ciliate species) were taken for both morphological and genetic identification as described below. In addition, coral fragments containing only apparently healthy tissue were collected from the same colony as that of the disease tissue fragment. Apparently healthy fragments were similarly assessed for the presence of ciliates.

2.2. Identification of ciliate diversity

2.2.1. Microscopic observation and characterization of the dominant ciliates

Microscopic and behavioral observations of associated ciliate species were made using an Olympus SZX7 binocular microscope and Olympus LG-PS2 fiber-optic light source immediately after collecting the coral samples from the field. During assessment of the ciliate community, any corals not being immediately assessed were stored in separate aquaria with free flowing filtered sea water until they were placed under the microscopes. All corals were analyzed within 2 h from initial sampling. Still images were captured using a QImaging Micropublisher 3.3 camera and Q-Capture v6 imaging software. Higher magnification images were obtained using an Olympus BX51 compound microscope. The images were compared to morphological descriptions in previous studies (Carey, 1992; Croquer et al., 2006b; Lee et al., 2000; Page et al., 2008; Shimano et al., 2008; Song, 2000; Sweet and Bythell, 2012), alongside the use of a dichotomous key in the 'Illustrated Guide to the Protozoa' (Lee et al., 2000). Morphological characteristics provided a further means of distinguishing ciliate morphotypes to confirm that our sequence data (see below) matched previously identified protozoan species. Features such as kinetosomal make-up and oral infraciliary structures such as the AZM (Adoral Zone of Membranelles) are highly conserved and together with organelle distribution, size, shape and color can be routinely used for distinguishing genera (Lee et al., 2000). A minimum of 30 min was spent assessing the ciliate community associated with each sample. Where possible, replicate samples of the disease were assessed from the same location and the same coral species. Unfortunately, standardization of samples was impossible as sampling was opportunistic and depended on the coral disease encountered. This resulted in an uneven representation of the different coral diseases assessed and the number and species of corals sampled (Table 1). Due to

Table 1
Illustrates the common disease name, the location sampled, the number of replicates, which coral species were sampled and the ciliates present on the coral diseases reported in this study.

Disease name	Location sampled	Number of corals sampled and from which species	Ciliates species present
White Syndrome (WS)	Australia, Solomons, Fiji, Maldives, UK Aquaria	N = 40 *Acropora muricata* (15) *Acropora aspera* (15) *Pocillopora damicornis* (10)	*Diophrys* sp., *Holosticha diademata*, *Varistrombidium kielum*, *Protocruzia adherens*, *Trochilioides recta*, *Uronema heteromarinum*, *Philaster lucinda*, *Philaster guamense*, *Trochilia petrani*, *Glauconema trihymene*, *Litonotus pictus*, *Euplotes* sp., *Aspidisca* sp., *Pseudokeronopsis* sp., *Hartmannula derouxi*, *Licnophora macfarlandi*, *Dysteria derouxi*, *Hemigastrostyla enigmatica*, *Chaenea vorax*, *Acineta* sp.
Brown Band Disease (BRB)	Australia	N = 4 *Acropora muricata*	*Diophrys* sp., *Varistrombidium kielum*, *Philaster lucinda*, *Philaster guamense*, *Glauconema trihymene*, *Euplotes* sp., *Pseudokeronopsis* sp., *Holosticha diademata*
Porites White Patch Syndrome (PWPS)	Moyonette, Reunion, South Africa	N = 9 *Porites lutea*	*Holosticha diademata*, *Varistrombidium kielum*, *Uronema heteromarinum*, *Philaster lucinda*, *Dysteria derouxi*, *Paracineta limbata*
White Plaque (WP)	Venezuela, Columbia	N = 8 *Colpophyllia natans* (4) *Orbicella annularis* (4)	*Holosticha diademata*, *Varistrombidium kielum*, *Protocruzia adherens*, *Trochilioides recta*, *Uronema heteromarinum*, *Philaster lucinda*, *Licnophora macfarlandi*, *Anteholosticha* sp., *Cryptocaryon* sp., *Dysteria derouxi*, *Paracineta limbata*, *Chaenea vorax*, *Acineta* sp., *Suctoria* sp.
White Band (WB)	Venezuela, Columbia	N = 8 *Acropora cervicornis*	*Varistrombidium kielum*, *Protocruzia adherens*, *Trochilioides recta*, *Philaster lucinda*, *Trochilia petrani*, *Glauconema trihymene*, *Pseudokeronopsis* sp., *Licnophora macfarlandi*, *Anteholosticha* sp., *Dysteria derouxi*, *Paracineta limbata*, *Trachelotractus* sp., *Chaenea vorax*
Skeletal Eroding Band (SEB)	Moyonette, Reunion, South Africa	N = 4 *Acropora muricata*	*Holosticha diademata*, *Varistrombidium kielum*, *Trochilioides recta*, *Philaster lucinda*, *Licnophora macfarlandi*, *Halofolliculina corallasia*, *Dysteria derouxi*, *Paracineta limbata*, *Chaenea vorax*, *Condylostoma* sp.
Caribbean Ciliate Infections (CCI)	Venezuela	N = 3 *Acropora cervicornis*	*Philaster lucinda*, *Halofolliculina corallasia*, *Suctoria* sp.
Black Band Disease	Venezuela, Moyonette, Maldives, Reunion, South Africa	N = 18 *Diploria strigosa* (4) *Diploria* sp. (2) *Orbicella annularis* (3) *Colpophyllia natans* (2) *Acropora muricata* (3) *Porites lutea* (2) *Goniopora djiboutiensis* (2)	*Holosticha diademata*, *Protocruzia adherens*, *Philaster lucinda*, *Chaenea vorax*, *Suctoria* sp.
Caribbean Yellow Band Disease	Venezuela	N = 8 *Montastraea annularis*	*Holosticha diademata*, *Protocruzia adherens*, *Trochilioides recta*, *Suctoria* sp.