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REPORT

A mechanism of transmission and factors affecting coral susceptibility to *Halofolliculina* sp. infection

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Abstract Anecdotal evidence collected since 2004 suggests that infections caused by ciliates in the genus *Halofolliculina* may be related to coral mortality in more than 25 scleractinian species in the Caribbean. However, the relationship between the presence of ciliates and coral mortality has not yet been firmly established. Field and laboratory manipulations were used to test if ciliate infections harm corals, if ciliates are able to infect healthy colonies, and if coral susceptibility to ciliate infection depends on temperature, depth, distance to an infected colony, and the presence of injuries. Ciliate infections were always characterized by a visually detectable front of ciliates located on recently exposed coral skeletons. These infections altered the normal structure of the colony by causing tissue mortality (0.8 ± 0.95 cm month^{-1}, mean $\pm$ *SD*) and by delaying or preventing recovery from injuries. Under laboratory conditions, ciliates transmitted directly and horizontally from infected to healthy hosts, and coral susceptibility to ciliate infections increased with the presence of injuries. After invasion, the ciliate population grew, rapidly and after 8 d, produced tissue mortality on 32% of newly infected hosts. Thus, our results support the existence of a new Caribbean coral syndrome that is associated with tissue mortality, is infectious, and transmits directly and horizontally. Even though the role of ciliates in the development of lesions on coral tissues remains unclear, their presence is by far the most conspicuous sign of this syndrome; thus, we propose to name this condition Caribbean ciliate infection (CCI).

Keywords Caribbean ciliate infection · Coral reefs · Diseases · Syndromes

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Introduction

The rapid emergence of coral diseases during the past three decades has been linked to natural and anthropogenic factors (Peters 1997; Cook et al. 1998; Harvell et al. 1999; Bruno et al. 2003, 2007). This is particularly true in the Caribbean, where diseases have also been linked to the rapid decline of coral reef communities (Bruckner et al. 1997; Aronson and Precht 2001; Nugues 2002). Temporal and spatial variability of diseases affecting coral reef organisms have been correlated with temperature stress, water pollution, sedimentation, coral cover, and depth (Edmunds 1991; Bruckner and Bruckner 1997; Acosta 2001; Antonius and Lipscomb 2001; Richardson and Kuta 2003; Voss and Richardson 2006; Bruno et al. 2007). Nevertheless, the effect of these parameters on coral susceptibility to infections, and most importantly, on the mechanisms of disease transmission are still poorly understood (Richardson 1998a; Ben-Haim et al. 1999; Cervino et al. 2004).

Because coral diseases are relatively new within epidemiological research, the use of standardized terminology is under discussion (Lesser et al. 2007; Work et al. 2008). For this study, the following definitions are relevant: (1) a "disease" is an interruption, cessation, or disorder of body functions, systems, or organs regardless of etiology (i.e., cause of disease), (2) a "syndrome" is often used as synonymous of disease (Work et al. 2008); however, some scientists prefer to use it for a disease of unknown etiology (Lesser et al.

2007); (3) an "infection" is the invasion and multiplication of a microorganism in or on a host (Peters 1997); an infection, however, does not necessarily impair host body functions and is not therefore synonymous of disease (Sutherland et al. 2004); (4) an "infectious disease" is one that can be transmitted from one host to another (Sutherland et al. 2004); (5) an "infectious pathogen" can consume the host in the same way as a predator; nevertheless, predators do not reproduce on or in their prey as infectious pathogens do; and (6) "virulence" is the capacity of a pathogen to cause disease in the individual host (Peters 1997; Sutherland et al. 2004).

The study of coral diseases in the field provides important temporal and spatial information for several epidemiological parameters (i.e., prevalence, incidence, and rate of mortality). Determining the pathology of coral diseases, the relationship between putative pathogens and clinical signs (i.e., any detectable expression of the disease that can be objectively observed on the host), the mechanisms affecting pathogen dispersion, and the environmental factors affecting their virulence, however, requires additional experimental manipulative approaches (Aeby and Santavy 2006). For instance, spatial patterns produced by black band disease (BBD) were correlated with nutrient concentration and temperature (Kuta and Richardson 2002), but additional laboratory experiments were required to show how temperature affects BBD virulence (Antonius 1985; Voss and Richardson 2006).

Only a few protozoans have been proposed as possible etiological agents of coral diseases (Peters 1997; Cerrano et al. 2000; Antonius and Lipscomb 2001) and/or direct consumers of coral tissues (Cooper et al. 2007). For example, skeletal eroding band (SEB) syndrome is related to a species of ciliate in the genus *Halofolliculina*, which affects corals in the Indo-Pacific and the Red Sea (Willis et al. 2004; Winkler et al. 2004; Page and Willis 2008). SEB had not, however, been reported in the Caribbean (Antonius and Lipscomb 2001) until recently, when Cróquer et al. (2006a, b) found a folliculinid ciliate interacting with over 25 scleractinian coral species. Ciliates examined from different localities in the Caribbean seem to belong to a unique species (Cróquer et al. 2006a) that has yet to be named, whereas ciliates affecting Indo-Pacific corals are classified as *Halofolliculina corallasia* (Antonius and Lipscomb 2001). Thus, due to the apparent differences in etiology, the present study does not refer to the Caribbean *Halofolliculina* infection as SEB.

Despite the presumptive differences between Caribbean and Indo-Pacific ciliate species, both produce similar clinical signs on their hosts: a dark cluster of sessile ciliates located on the recently exposed coral skeleton (Antonius and Lipscomb 2001; Cróquer et al. 2006b). Moreover, ciliates from both regions have a lorica and a free-living phase that moves toward the living tissue, penetrates it, and attaches itself to the coral skeleton as the front rapidly advances (Antonius and Lipscomb 2001; Cróquer et al. 2006b). The susceptibility of corals to SEB in the Indo-Pacific has been correlated with depth, pollution, and temperature (Willis et al. 2004; Winkler et al. 2004). In the Indo-Pacific, SEB is more prevalent than any other coral disease (Page and Willis 2008) and is more common in polluted and warmer waters (Antonius and Lipscomb 2001; Willis et al. 2004; Winkler et al. 2004). In contrast, the prevalence of the Caribbean ciliates appears to be higher in oceanic versus coastal human-influenced reefs (Cróquer et al. 2006a). SEB has been shown to produce tissue mortality in the Red Sea and Great Barrier Reef corals, with rates of tissue mortality varying from 0.1 to 1 mm d^{-1} for the former (Antonius and Lipscomb 2001) and from 2 to 3 mm d^{-1} for the latter (Page and Willis 2008). Despite the potential negative effects that the Caribbean ciliate might have on coral reefs, neither the effect of ciliates on corals nor the effect of environmental parameters on host susceptibility has ever been tested or demonstrated.

In this study, field and laboratory manipulations were used to determine (1) whether ciliate infections have a negative effect on coral hosts, (2) whether ciliate infection transmits to healthy colonies (as one of the possible mechanisms of transmission), (3) whether ciliates reproduce on recently infected hosts, and (4) how environmental factors affect the dynamics of ciliate infections (see Table 1 for specific hypotheses and associated laboratory and field experiments).

Materials and methods

Manipulative and experimental approach

Field and laboratory experiments were conducted at the Smithsonian Institute's (STRI) Bocas Research Station in Bocas del Toro (BDT), Panama. At this site, *Halofolliculina* sp. infects 2% of the coral colonies ($N = 23{,}849$) and is highly prevalent (29%) among populations of *Agaricia tenuifolia* (Cróquer et al. 2006b). *A. tenuifolia* was used in the experiments because it is one of the dominant corals in terms of both cover and density (Guzmán and Guevara 1999) and because it is prone to being affected by ciliates (Cróquer et al. 2006b). In order to reduce stress and facilitate collection, transportation, and manipulation, colonies were collected at STRI Point Reef, Isla Colon (09°20′58″ N; 82°15′48″ W), which is close to the research station (≈500 m).

Each colony used in the laboratory and/or field experiments was manually collected and brought to the laboratory in individual plastic bags filled with sea water. Colonies

Table 1 Experiments to test each of the four hypotheses postulated in this study

Sequence of the study	Hypothesis	Experiment title	Supported (yes) versus not supported (no)
1. Effect of ciliate infections on their hosts	H1 (a) Infections will produce tissue mortality and/or (b) will decrease the capacity of corals to regenerate tissues	*Field experiment 1* Effect of ciliate infections on tissue mortality	H1a: Yes
		Field experiment 2 Effect of ciliate infections on tissue regeneration	H1b: Yes
2. Transmission and development of ciliate infections	H2 (a) Ciliates will transmit directly from an infected (ciliate source) to a healthy colonies coral	*Lab. experiment 1* Mechanism of transmission	H2a: Yes
	H3 (a) After host invasion, population of ciliates will grow over the newly infected host, (b) will produce similar signs to those diseases colonies in the field	*Lab. experiment 2* (a) Growth of the population of ciliates on recently infected host and (b) signs of recently infected host	H3a: Yes H3b: Yes
3. Effect of environmental factors on the epizootiology of ciliate infections	H4 Coral susceptibility to ciliate infections will increase with (a) temperature, (b) with depth, (c) with the presence of injuries on the healthy colonies colonies and/or (d) with the proximity to infected colonies	*Field experiment 3* Effect of depth, injuries, and distance on host susceptibility to ciliate infections	H4a × H4c: Yes H4b: No H4c: Yes H4c × H4d: Yes
		Lab. experiment 1 Effect of temp., injuries, and distance on host susceptibility to ciliate infections	

were examined with a stereoscope and sorted as: (1) "healthy" when corals had no ciliates (all healthy colonies were assumed to be susceptible to ciliate infections) and (2) "ciliate-infected" colonies, which were those showing the typical clinical signs (i.e., clusters of sessile ciliates lying between living tissues and recently-exposed coral skeleton) of ciliate infection. Colonies were separated according to their condition into two groups of three tanks each, one group for infected individuals and one for healthy individuals. For experiments in the field, colonies were transported back to the reef site in individual bags and deployed on pedestals according to their treatment. Colonies used for laboratory experiments were initially acclimated for 48 h and then deployed on pedestals in individual aquaria according to their treatment. Three experiments were conducted in the field and two in the laboratory, each arranged in a fully orthogonal design (see Table 1).

Field experiment 1

In order to determine whether the presence of ciliates had an effect on coral tissue mortality, 30 colonies infected with ciliates at each of two depth intervals (3–5 and 9–13 m) were evaluated for 19 consecutive days. In order to evaluate the possible effect of manipulation on the mortality rate of infected colonies, 30 healthy colonies were used as procedural controls at each depth interval. Infected and control colonies were photographed from two opposite sides at the beginning of experiment 1. After 19 d, all colonies were photographed again using the same two opposite sides and from the same distance. For each side, the rate of tissue mortality was estimated by comparing the position of the ciliates on both sides at the beginning and at the end of the experiment. This tissue mortality, averaged for both sides of the colony, was used as the response variable. A fixed two-factor permutation ANOVA (PERMANOVA) was used to measure the effect of the presence of ciliates, depth, and their possible interaction, on the rate of tissue mortality (cm month^{-1}). This permutation test was used because the data was not normally distributed.

Field experiment 2

In this experiment, the capacity to regenerate tissue was compared between infected and healthy colonies. In order to induce ciliate infection in the field, a fragment from each of 35 healthy colonies was broken off and the injured colonies were returned to the field and set up on individual PVC pedestals close to (<10 cm) a naturally occurring infected colony for 19 days. All experimental colonies had a single lesion (1–2 cm^2) on their base that was used as the initial size of each injury. After 19 days, ten of these experimental colonies became infected by ciliates and 25 remained healthy. The amount of tissue regeneration on healthy and

infected colonies was measured on day 19 using the following categories: (1) 0–25%; (2) 26–50%; (3) 51–75%; and (4) 76–100%. The effect of ciliate infections on tissue regeneration was evaluated with an R × C contingency table using a χ^2 test.

Field experiment 3

A fully orthogonal four-factor design with two levels for each factor was used to test whether a source of ciliate infection (presence = treatment; absence = control), depth (3–5 m, 9–13 m), injury (presence, absence), and distance from the source of infection (2.5, 10 cm) had an effect on coral susceptibility to infection (i.e., frequency of infection for each treatment). For this experiment, the source of infection for each of the treatments consisted of colonies infected with ciliates. Each combination of levels had five replicates consisting of PVC pedestals with two colonies attached to each end of the structure (see Fig. 1a). Experimental units were deployed randomly in the reef at each depth interval and were situated at least 2.5 m away from any naturally occurring or experimental colony (infected or healthy).

Injuries were artificially inflicted only on healthy colonies by scraping off ≈1 cm^2 of their tissues in the center of each using a dissection needle (Fig. 1a). After 10 d, healthy colonies were removed from the field and transported to the laboratory in individual plastic bags filled with sea water. Once in the laboratory, they were examined using a stereoscope to determine whether or not they had become infected; if infected, we counted all of the ciliates per colony.

A classification tree analysis was used to evaluate whether these four factors affected the probability that a healthy colony would become infected. This technique ordinates a categorical variable in a tree, which is based on repeated splitting of the data (De'ath and Fabricius 2000). Splitting was based on the proportion of infected and uninfected colonies at each level of a particular treatment. If the factor being considered (e.g., depth) affected coral susceptibility, the data were partitioned into levels (e.g., 3–5 and 9–13 m) and classified according to the frequency of infection. This analysis also arranges the different explanatory variables in order of importance, with the first splitting of the tree representing the most important factor determining coral susceptibility, the second split representing the second most important factor, and so on. Leaves denote treatments that had no effect on the frequency distribution of infected and non-infected colonies.

Laboratory experiment 1

This experiment tested one of the possible mechanisms of transmission and the effect of temperature, artificial injuries in healthy corals, and distance from the source of the infection, on host susceptibility. For this, a fully orthogonal three-factor design with two levels for each factor was used to test the effect of (1) temperature (26 ± 1 and 31 ± 1°C, mean ± *SD*), (2) injuries (presence–absence), and (3) distance from infection source (2 and 10 cm). Experimental units consisted of plastic aquaria (25 × 10 × 15 cm) each holding a pair of colonies (one infected and one healthy) arranged on top of a PVC pedestal. Distance and lesion treatments followed the same protocol as for field experiment 1. Each combination of levels was replicated five times with five pairs of healthy colonies as controls. In order to determine if ciliates transmit directly and horizontally, we compared the probability of healthy colonies becoming infected when exposed to an infected colony versus a healthy colony under each set of temperature and injury conditions. Colonies bearing ciliates were assumed to be the source of infection because the water in all of the aquaria was mechanically filtered and UV treated prior to and during the course of the experiment.

Aquaria had continuous air flow, and water (filtered and treated with UV) was changed every 48 h. After 10 d, the frequency of infections and the density of ciliates in all colonies that became infected during the experiment were quantified using a stereoscope. A fixed three-factor

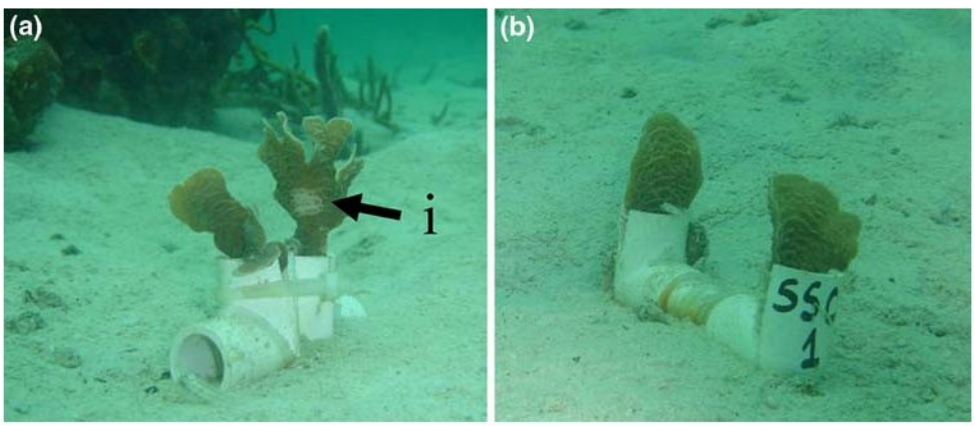

Fig. 1 Experimental settings used for field experiments. (**a**) Distance of 2.5 cm between infected and injured healthy colony (i = injury); (**b**) distance of 10 cm between infected and non-injured healthy colony

permutation ANOVA and a classification tree analysis were used to examine the effect of temperature, distance, injuries, and their possible interactions, on ciliate density and host susceptibility, respectively.

Laboratory experiment 2

In this experiment, the 36 healthy colonies that became infected during field experiment 3 (16 from the shallow habitat and 20 from the deeper habitat) were used to quantify the population growth of ciliates on recently infected colonies and to determine whether this growth could be affected by the original depth of the colony and temperature. The initial number of ciliates found in these recently infected colonies (a maximum of 10 d after infection) was counted using a stereoscope. Initial population sizes ranged from 3 to 14 ciliates per colony. Colonies were placed individually into aquaria, and half were kept at 26°C and the other half at 30°C. After 8 d, the number of ciliates on each colony was counted again. Ten healthy colonies were used as controls for each of the four treatments (2 depths × 2 temperatures). The effect of temperature and depth on the population growth of ciliates was measured with a permutation analysis of variance for two fixed factors ($n = 8$ colonies for each level, for a total of 32). As the initial number of ciliates differed among infected colonies, the population growth of ciliates was estimated as the percent change in the numbers of ciliates at the end of the experiment.

Results

Field experiment 1

Ciliate-infected colonies lost tissue at a rate ten times higher than healthy control colonies (0.79 ± 0.08 cm month^{-1}; $n = 56$ versus 0.08 ± 0.06 cm month^{-1}; mean $\pm$ *SD*, $n = 60$, respectively; Fig. 2, *df* 1, 125; $P < 0.01$; Table 2). Moreover, 90% of the infected colonies ($n = 56$) experienced tissue mortality; in the 10% remaining colonies, no tissue mortality was recorded despite the presence of the infection. Tissue mortality was similar in both shallow and deeper habitats (Table 2).

Field experiment 2

Healthy colonies had a higher capacity to recover from artificial injuries than colonies infected by ciliates ($\chi^2 = 121.55$; $df = 3$; $P < 0.01$). While 84% of the healthy colonies regenerated 75–100% of the injuries, only 11% of ciliate-infected colonies were successful in regenerating and recovering tissues to the same degree (Fig. 3).

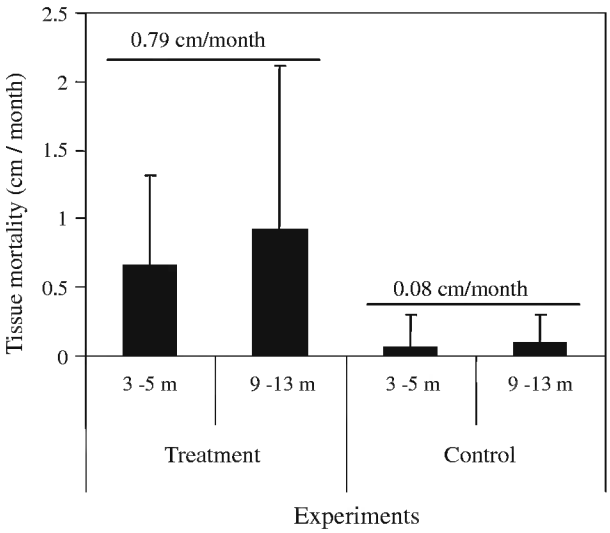

Fig. 2 Tissue mortality rates (cm month^{-1}) for colonies infected by ciliates (treatment) and for healthy colonies (control) in shallow (3–5 m) and deep (9–12 m) zones of the reef. Lines over the bars denote the groups that do not differ statistically (Treatment: ciliate infected colonies and Control: healthy colonies). The numbers on these lines denote the mean value for each group

Table 2 Analysis of variance for tissue mortality (cm month^{-1}) in field experiment 2: Type I sum of squares, fixed factors

Source	*df*	SS	MS	*F*-ratio	*P*-value
A: Depth	1	0.114	0.114	0.320	0.572
B: Infected versus not infected	1	14.621	14.621	41.01	**<0.001**
A × B	1	0.317	0.317	0.89	0.347
Residual	125	44.560	0.356		
Total	128	59.612			

Bold number denotes statistical significance

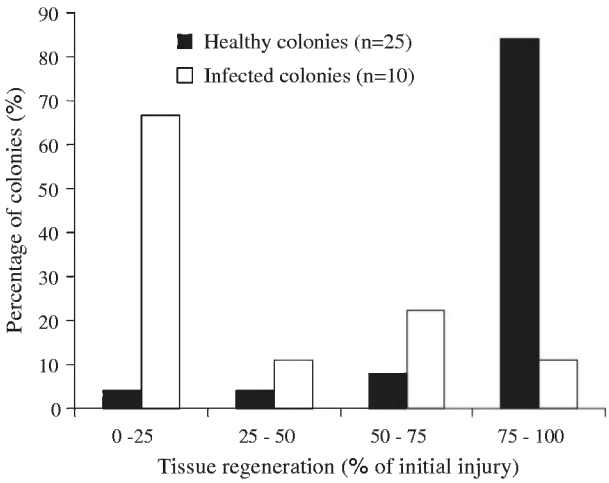

Fig. 3 Tissue regeneration from injuries (percentage surface area regenerated with respect to the initial area of lesion) in *Agaricia tenuifolia* colonies infected or not infected by the ciliate *Halofolliculina* sp.

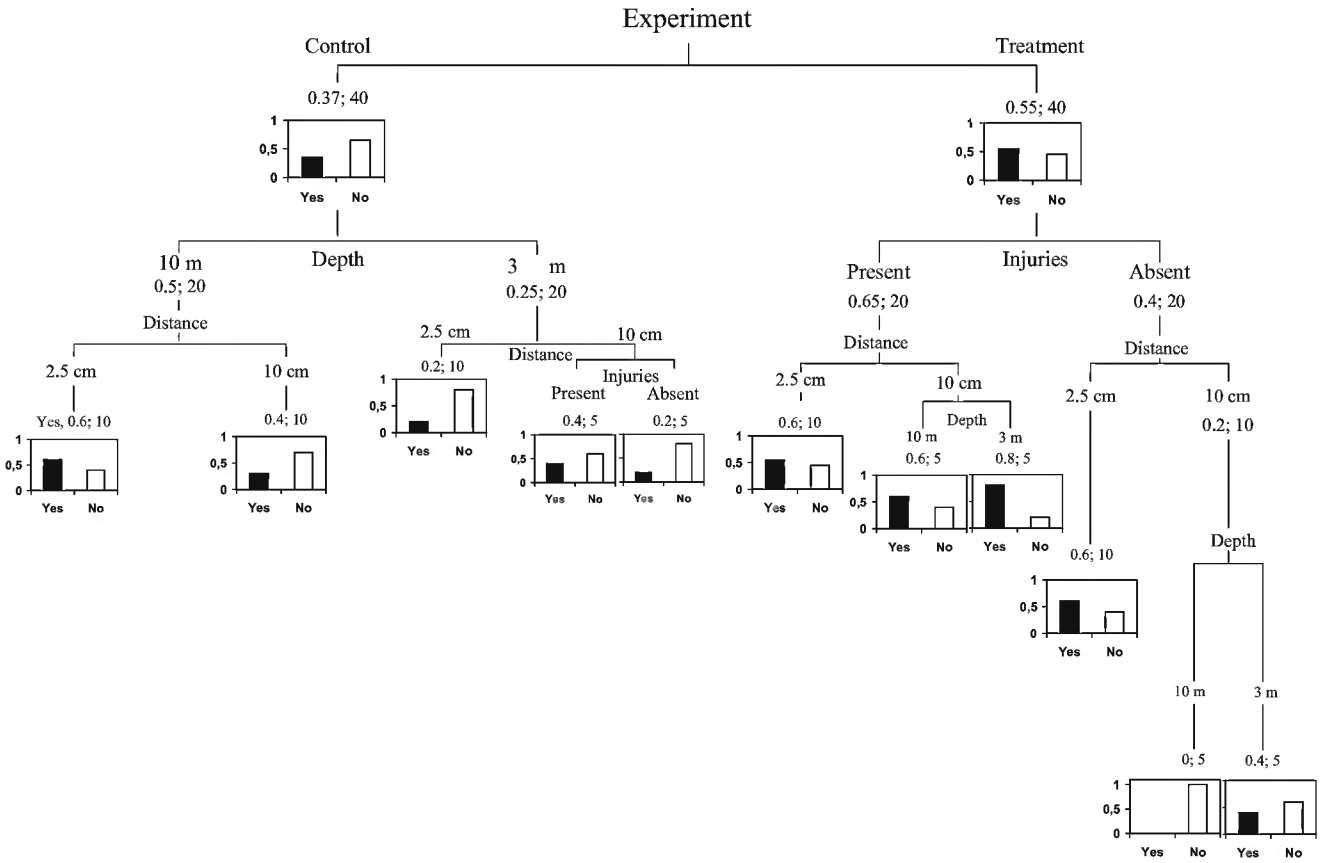

Fig. 4 Classification tree for the probability of infection of coral colonies during field experiment 3. The factors in the experiment were: ciliate infection, depth, distance to source of infection, and the presence of injuries on healthy colonies. The first number on top of each split represents the proportion of infected colonies, and the second number is the total number of colonies subjected to this treatment

Field experiment 3

In the field, the most important factor affecting host susceptibility to ciliate infection was the presence of an infected colony nearby, which acted as a source of ciliates (i.e., 55% of the 40 healthy colonies located at 2 or 10 cm away from an infected colony became infected; Fig. 4). However, 37% of the 40 control colonies also became infected, indicating either that ciliates can reach colonies located more than 2.5 m away from an infected coral or that additional sources of ciliates exist. For these control colonies, depth was the most important factor affecting the probability of infection, as deeper colonies were twice as susceptible as shallower ones (50% vs. 25%, respectively; $n = 20$).

For healthy colonies exposed to infected corals, the presence of injuries increased the probability of ciliate infection compared to colonies with no injuries (65% vs. 40%, respectively; $n = 20$). For healthy colonies with no injuries, the distance from an infected colony was the most important factor affecting susceptibility. While 60% of the healthy colonies with no injuries separated from infected corals by 2.5 cm developed ciliate infections, only 20% of those separated by 10 cm became infected (Fig. 4). In contrast, for injured corals, susceptibility to ciliate infections was similar regardless of the distance from an infected colony (60% and 70% for those at 2.5 cm and 10 cm, respectively; Fig. 4). Coral susceptibility to ciliate infections was similar in both shallow and deeper habitats.

Laboratory experiment 1

After 10 d of exposure to infected colonies, 86% of healthy colonies ($n = 40$) became infected by ciliates, whereas none of the control colonies were infected. Host susceptibility to infection was enhanced by the presence of injuries, as 80% of injured corals became infected ($n = 20$) versus only 35% ($n = 20$) of non-injured corals (Fig. 5). For injured colonies, temperature was the most important factor affecting host susceptibility: 90% of the colonies ($n = 10$) kept in 30°C water became infected compared to only 70% of the colonies ($n = 10$) kept in 26°C water. For non-injured corals, the distance to a ciliate source was the most important factor affecting susceptibility (40% vs. 30% for 2.5 and 10 cm away from infected colonies, respectively; Fig. 5). Although this is only a 10% difference, the proximity to the source of infection also increased the probability of infec-

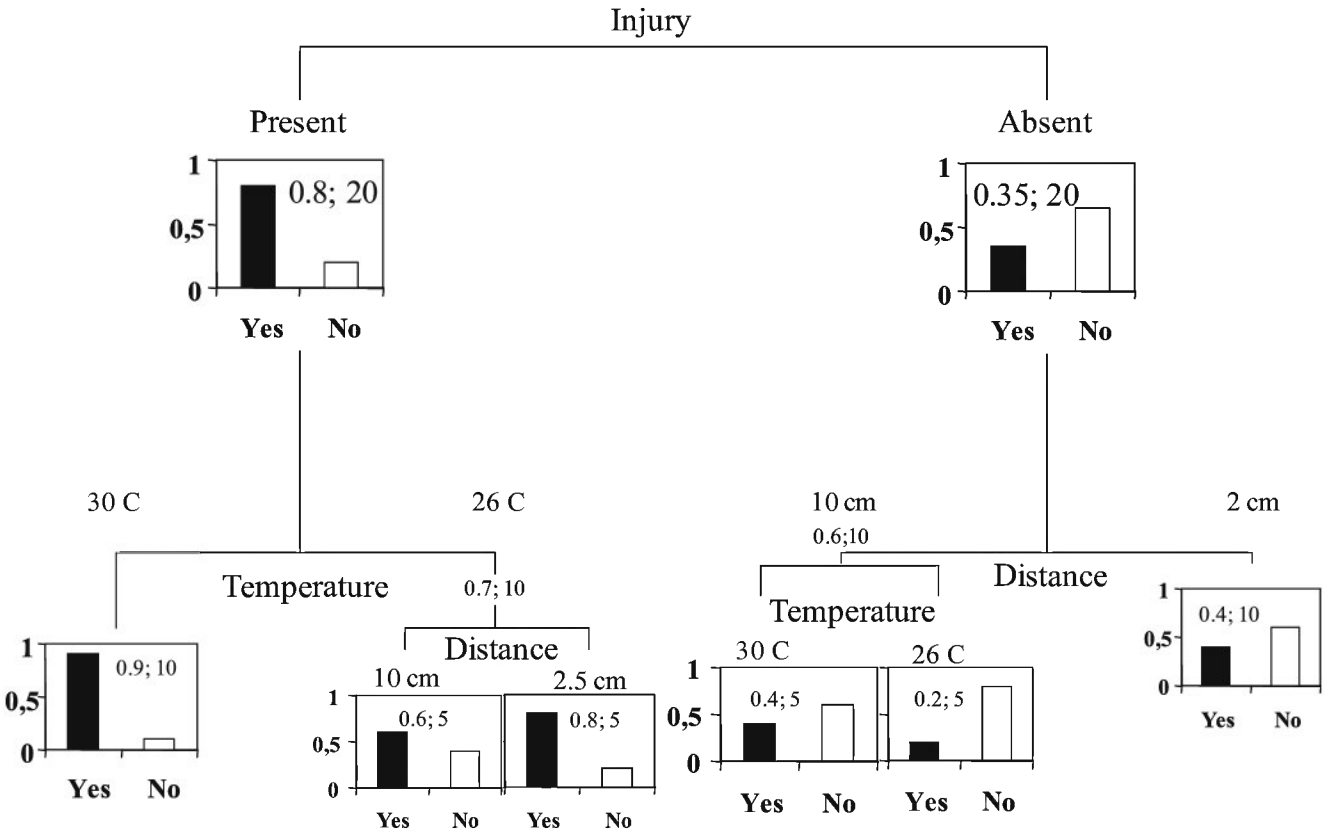

Fig. 5 Classification tree for the probability of infection of colonies in laboratory experiment 1 subjected to two temperatures (26°C and 31°C), two distances (2.5 and 10 cm) between healthy and infected colonies, and the presence–absence of injuries on healthy colonies. The first number on top of each split represents the proportion of infected colonies, and the second number is the total number of colonies subjected to this treatment

tion for non-injured colonies in field experiment 1. For those colonies that became infected, the density of ciliates increased from 0.1 to 33.9 ± 43.7 ciliates cm^{-2} in 10 d (mean ± *SD*; min six ciliates cm^{-2}; max 170 ciliates cm^{-2}; Fig. 6). None of the environmental factors tested had an effect on the density of ciliates ($P > 0.05$).

Laboratory experiment 2

The population of ciliates grew continuously on their hosts during the course of the experiment. After 8 d, the number of ciliates per colony increased by 357%, from an average of seven ciliates per $colony^{-1}$ on the first day to up to 50 ciliates per $colony^{-1}$ (mean of 32 ciliates per $colony^{-1}$) on day 8. Population growth of ciliates was higher for hosts collected from deeper habitats than for hosts taken from shallower ones (i.e., an increase of 486% vs. 196%, respectively; Fig. 7, Table 3). Against expectations, temperature had no effect on the growth of the ciliate populations (Table 3). After 8 d, ciliate infections produced tissue mortality on 32% of the infected hosts ($N = 36$, Fig. 6). During this experiment, only two out of the 36 colonies tested were able to recover from infections by growing over the ciliate band with a new tissue.

Discussion

This study shows that (1) experimentally infected colonies exhibited characteristic signs of naturally infected colonies; those signs consisted of a front of ciliates of *Halofolliculina* sp. located between live tissue and recently exposed coral skeleton; (2) the presence of this ciliate front altered the normal tissue structure of their hosts by causing tissue mortality and by delaying recovery from injuries; (3) ciliates were able to invade a healthy colony from an infected colony, and after this invasion, (4) ciliate populations grew on this recently infected host and produced tissue mortality after 8 days. Considering that a disease is an alteration of the normal structure of any body part manifested by a characteristic set of clinical signs of known or unknown causes (Work and Aeby 2006) and that an infection is the invasion and multiplication of a microorganism on or in its host (Peters 1997), these results support the proposal that *Halofolliculina* sp. is an infectious organism strongly related to a new disease in Caribbean corals.

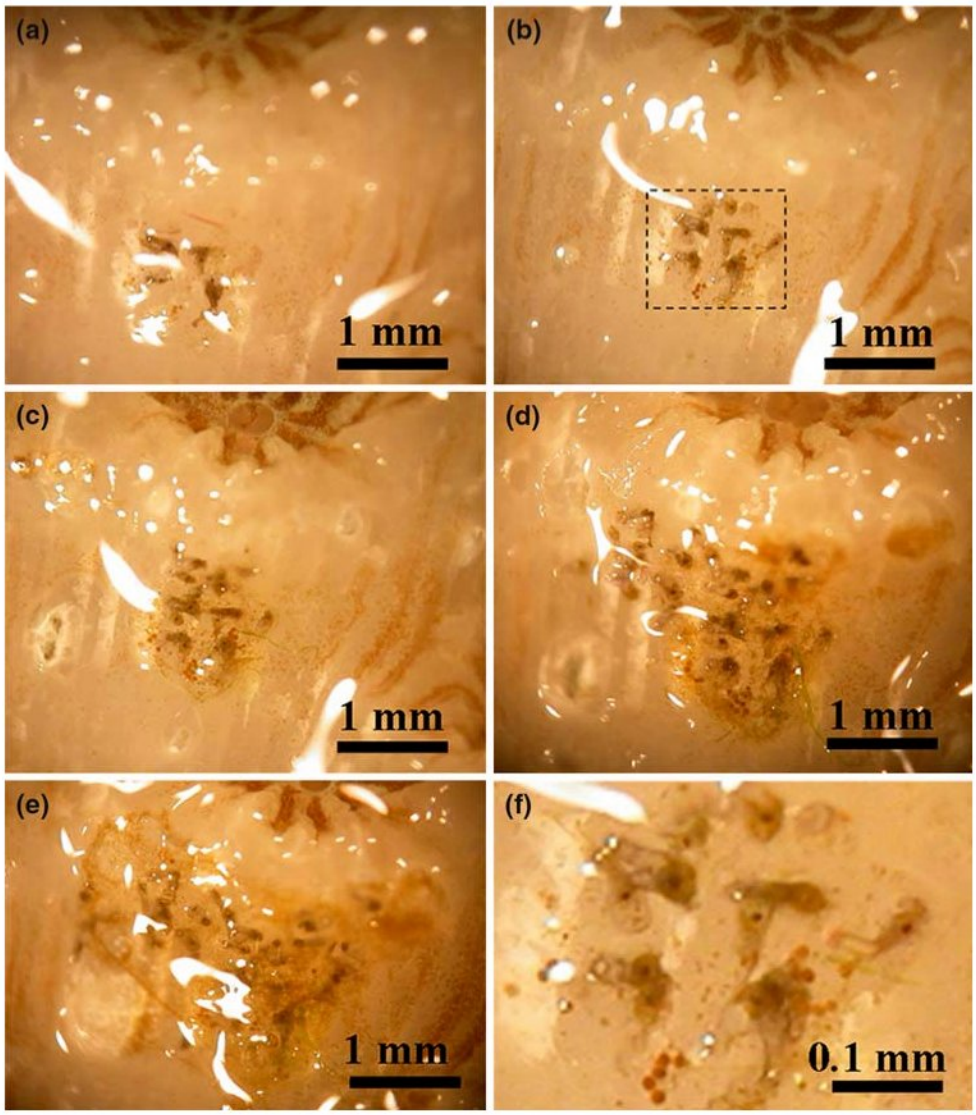

Fig. 6 Increase in lesion size from collection to the 8th day of the experiment for one of the healthy colonies that became infected after 10 d in the field. (**a**) Day of collection in the field; (**b**) day 2; (**c**) day 4; (**d**) day 6; (**e**) day 8; and close-up of the ciliate aggregation illustrated in (**b**)

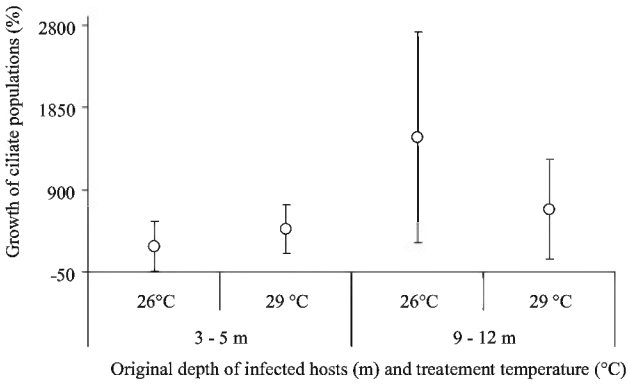

Fig. 7 Increase in the size of ciliate populations, as a percentage of the initial number of ciliates (mean ± *SD*; $n = 8$ for each treatment), in colonies of *Agaricia tenuifolia* infected with *Halofolliculina* sp. Colonies were extracted from shallow (3–5 m) and deep (9–12 m) zones of the reef and then subjected to low (26°C) and high (29°C) seawater temperatures for 8 d

Naming coral diseases is controversial because the etiology for most of them remains unknown. Diseases are often named after the most conspicuous morphological feature of lesions (Work and Aeby 2006); we propose, therefore, to name the clinical signs described in this study as Caribbean ciliate infection (CCI). It remains unclear, however,

Table 3 Analysis of variance for the effect of original depth of the host (depth) and temperature on the growth of ciliate populations after 8 d of experiment (ciliates cm^{-2}) in laboratory experiment 2: Type I sum of squares, fixed factors

Source	*df*	SS	MS	*F*-ratio	*P*-value
A: Depth	1	99,815.946	99,815.946	46.158	**0.010**
B: Temperature	1	35,996.066	35,996.066	16.646	0.167
A × B	1	65,356.110	65,356.110	30.223	0.063
Residual	28	605,495.269	21,624.831		
Total	31	806,663.392			

Bold number denotes statistical significance

whether ciliates are the primary pathogens, opportunistic invaders of coral tissue, or members of a consortium of pathogenic microorganisms. Therefore, we propose that CCI should be referred to as syndrome, as proposed by Lesser et al. (2007), rather than as a disease.

Effects of ciliate infection disease on coral hosts

Ciliate infections probably produced the tissue mortality observed in our experiments, supporting the premise that infections have negative effects on corals. Nevertheless, two alternative explanations are also possible. Tissue mortality could be produced by a consortium of microbial organisms, including ciliates (1), or ciliates could invade dead tissue previously destroyed by another pathogen (2). In the latter scenario, ciliates would be opportunistic organisms that invade recently denuded skeleton, which is supported by the higher rate of infection observed on injured corals. However, the higher infection rate observed on injured corals does not exclude *Halofolliculina* sp. as a pathogen, because injuries have also been shown to increase the probability of infection by pathogens such as *Phormidium corallyticum* in BBD (Aeby and Santavy 2006).

None of the colonies that became infected during the experiments showed signs of any other diseases described for corals. While this fact does not exclude the possibility that ciliates could be opportunistic, it is clear that 32% of the colonies that became infected with ciliates had dead tissue in areas where ciliates reproduced and grew during the experiments (Fig. 6). Antonius and Lipscomb (2001) found that the virulence of the congener ciliate *H. corallasia* remained identical before and after treating infected colonies with antibiotics and suggested that *H. corallasia* was the causative agent of SEB. However, a more recent study of SEB epizootiology suggested that *H. corallasia* was not the causative agent of SEB, because the infection by ciliates did not lead to tissue loss of their host (Page and Willis 2008). In contrast, the results of the present study demonstrate ciliate-associated coral tissue mortality and suggest that the presence of *Halofolliculina* sp. is at least among the potential causes of CCI.

Tissue mortality on colonies of *Agaricia tenuifolia* infected by *Halofolliculina* sp. occurred at a rate that was two to three times faster (0.79 ± 0.08 cm month^{-1}, mean $\pm$ *SD*) than those caused by other Caribbean coral diseases, such as yellow band disease (YBD) and dark spot disease (DSD, 0.4–0.5 cm month^{-1}; Cervino et al. 2001; Bruno et al. 2003; Bruckner and Bruckner 2006). Nevertheless, diseases such as BBD and white plague disease (WPD) can kill coral tissues faster than ciliate infections, i.e., 1.5–2.5 and 18–21 cm month^{-1}, respectively (Kuta and Richardson 1996, 1997; Richardson 1998b; Nugues 2002; Borger and Steiner 2005). In this study, the rate of tissue mortality caused by ciliates was ten-fold slower than the rate measured for SEB at the Great Barrier Reef (6–9 cm month^{-1}; Page and Willis 2008) but faster than that reported for the Red Sea (0.4–0.5 cm month^{-1}; Antonius and Lipscomb 2001). Thus, *Halofolliculina* sp. infections produced tissue mortality as fast as the most virulent Caribbean coral diseases (i.e., WPD, BBD, DSD, and YBD sensu Weil 2004). The high rate of tissue mortality together with the wide range of hosts affected by this ciliate (>50% of scleractinian corals from the Caribbean) suggests that these infections could have a significant negative effect on Caribbean reefs.

Direct and horizontal transmission of ciliates

Under laboratory conditions, healthy corals deployed in the vicinity of infected colonies became infected, whereas pairs of healthy colonies (i.e., controls) did not. Because individual systems with UV filtered water were used, the ciliates that invaded healthy corals probably came from infected colonies. This finding supports the premise that *Halofolliculina* sp. is infectious and that it can be transmitted horizontally and directly. Although the means of locomotion of the infectious phase of *Halofolliculina* sp. remains unclear, it might reach healthy colonies by water transport (swimming and/or passive current transport) and/or by crawling. Recently, Page and Willis (2008) observed that healthy colonies held in unfiltered water in the absence of infected colonies were successfully colonized by ciliates, which supports the idea that this organism can be transmitted through water. In both cases (water-borne or crawling), propagation would depend on the distance between healthy and infected corals and on oceanographic conditions at small spatial scales, such as the direction and intensity of currents.

The results of field experiment 3 and laboratory experiment 1 partially support the hypothesis that host susceptibility increases with proximity to the source of infection. This evidence is considered partial because the effect of artificial injuries on healthy colonies seemed to be more important for susceptibility than distance from an infected colony. The presence of injuries on healthy colonies increased their probability of becoming infected regardless of the distance from an infected colony. However, healthy colonies with no injuries located 2 cm from an infected colony had a greater probability of infection than those 10 cm away from the source of infection. Moreover, the control colonies in field experiment 3, which were deployed >2.5 m from an infected colony, showed lower susceptibility than colonies deployed at <10 cm from an infected colony.

Alternatively, infections on control colonies situated >2.5 m from an infected colony could also be produced by

infection mechanisms other than those suggested by laboratory experiment 1 (i.e., water born and/or crawling). For other diseases, vectors and reservoirs can increase coral susceptibility. For example, the presence of the Caribbean fire worm *Hermodice carunculata*, the butterflyfish *Chaetodon capistratus*, and the Pacific butterflyfish *Chaetodon multicinctus* increased the probabilities of infection of corals with specific diseases (Aeby 1998, 2002; Sussman et al. 2003; Aeby and Santavy 2006). Considering the effect of injuries on coral susceptibility to ciliate infection, it is possible that predation by snails on corals could increase coral susceptibility to these infections. Interestingly, Caribbean ciliates have been observed on the shells of corallivorous snails (S. Rodriguez, pers. obs.), suggesting that the injuries caused during predation could facilitate ciliate establishment. Thus, although our results support a direct form of transmission, other possible mechanisms, such as those in which vectors, predation, and/or reservoirs could be involved, can not be discarded.

Healthy colonies with injuries were more prone to infection by ciliates than uninjured ones, both in laboratory and field experiments, which agrees with results from two other studies, one on BBD (Antonius 1985; Aeby and Santavy 2006) and the other on SEB (Page and Willis 2008). It is likely that the motile infectious phases of ciliates opportunistically settle on recently denuded skeleton, attracted perhaps by the release of zooxanthellae from degrading or damaged coral tissues (as suggested by Page and Willis 2008) or by the availability of newly exposed substrate. Ciliates could also be attracted by chemical signals from degrading tissues. Another possibility is that injured corals have fewer resources available for defense than healthy colonies. Corals have an energy budget that is partitioned for processes such as growth, reproduction, tissue regeneration, and defense (Mullen et al. 2004). Amongst these processes, regeneration is suggested as a priority for corals (e.g. Oren et al. 1997; Nagelkerken et al. 1999). Even though our results support the premise that injuries increase the corals' susceptibility to ciliate infection, the mechanisms that explain this process are unknown and must be investigated using more specific hypotheses.

Environmental factors affecting ciliate infection dynamics

A relationship between temperature and the virulence of diseases has been recorded for many marine invertebrate pathogens, including ciliate infections in oysters (Cook et al. 1998); BBD, YBD, aspergillosis, white plague type II, bacterial bleaching, and DSD in corals; and ciliate infections in Mediterranean gorgonians (Edmunds 1991; Cerrano et al. 2000; Alker et al. 2001; Frias-Lopez et al. 2002; Kuta and Richardson 2002; Riegl 2002; Boyett et al. 2007). However, in the present study, temperature only increased susceptibility to ciliate infections in injured colonies. Temperature might also affect coral susceptibility indirectly by promoting the formation of wounds on corals as a result of bleaching or by facilitating the invasion of other pathogens (Lesser et al. 2007), which in turn might produce damage to coral tissues, thereby favoring infection by ciliates.

In conclusion, this study provides evidence that the ciliate *Halofolliculina* sp. constitutes a new infectious coral syndrome for Caribbean corals. Sessile ciliates settled between living tissues and recently exposed coral skeletons visually characterize this condition, and their presence alters the normal body functions of the host by producing tissue mortality and delaying and or reducing tissue regeneration. Ciliates transmit to healthy hosts directly and horizontally, although we cannot disregard other possible mechanisms of transmission (e.g., vector-mediated). Effective transmission of these protozoans is enhanced by the presence of injuries in healthy corals. The high prevalence and widespread distribution of ciliate infection in Caribbean coral reefs (Cróquer et al. 2006a), its rapid transmission, and the fast rates of tissue mortality on infected hosts suggest that CCI represents another problem for Caribbean corals. The mechanisms producing tissue mortality and the role of ciliates (alone or in a microbial consortium) in the development of coral pathogenesis must be further investigated.

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