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The genetic identity of dinoflagellate symbionts in Caribbean octocorals

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Abstract Many cnidarians (e.g., corals, octocorals, sea anemones) maintain a symbiosis with dinoflagellates (zooxanthellae). Zooxanthellae are grouped into clades, with studies focusing on scleractinian corals. We characterized zooxanthellae in 35 species of Caribbean octocorals. Most Caribbean octocoral species (88.6%) hosted clade B zooxanthellae, 8.6% hosted clade C, and one species (2.9%) hosted clades B and C. *Erythropodium caribaeorum* harbored clade C and a unique RFLP pattern, which, when sequenced, fell within clade C. Five octocoral species displayed no zooxanthella cladal variation with depth. Nine of the ten octocoral species sampled throughout the Caribbean exhibited no regional zooxanthella cladal differences. The exception, *Briareum asbestinum*, had some colonies from the Dry Tortugas exhibiting the *E. caribaeorum* RFLP pattern while elsewhere hosting clade B. In the Caribbean, octocorals show more symbiont specificity at the cladal level than scleractinian corals. Both octocorals and scleractinian corals, however, exhibited taxonomic affinity between zooxanthella clade and host suborder.

Keywords Zooxanthella · Clade · Symbiosis · Octocoral · Coral · RFLP

Introduction

The cornerstone of the coral reef ecosystem is the symbiosis between cnidarians (e.g., corals, octocorals, sea anemones) and unicellular dinoflagellates commonly called zooxanthellae. Studies of zooxanthella symbioses have previously been hampered by the difficulty of identifying the algae. Past techniques relied on culturing and/or identifying zooxanthellae based on their free-swimming form (Trench 1997), antigenic features (Kinzie and Chee 1982), and cell architecture (Blank 1987), among others. These techniques were time-consuming, required a great deal of expertise, and resulted in the differentiation of only a small number of zooxanthella species. Molecular techniques amplifying zooxanthella DNA encoding for the small and large subunit ribosomal RNA (ssRNA and lsRNA) have enabled researchers to divide zooxanthellae into groups known as clades (Rowan 1998). Each clade (also known as types (Diekmann et al. 2002), or phylotypes (LaJeunesse 2001)) includes multiple zooxanthella species (Rowan 1998). Researchers have also used other DNA regions such as internal transcribed spacer (ITS) regions (LaJeunesse 2001) and chloroplast large subunit (23S)-rDNA sequences (Santos et al. 2002). Although these DNA sequences and/or techniques may provide within-clade resolution, the zooxanthellae still cluster in the cladal division (Santos et al. 2002).

In the past decade, researchers have identified the zooxanthellae in 240 different cnidarian host species, the majority of which have been scleractinian corals. For a few scleractinian species in the Caribbean, coral colonies were compared over depth (Baker 1999; Baker and Rowan 1997; Baker et al. 1997; Billingham et al. 1997; Rowan and Knowlton 1995; Rowan et al. 1997) or over geographic location (Diekmann et al. 2002).

Although octocorals are a major component of the benthos on many reefs in the Caribbean (Goldberg 1973; Kinzie 1974; Kinzie 1973; Lasker and Coffroth 1983; Muzik 1982; Sanchez et al. 1997), most studies

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examining the zooxanthella-coral symbiosis have focused on scleractinian corals (Lajeunesse et al. 2003; Trench 1997; Trench 1993). Previously, the zooxanthella clades in juvenile gorgonians (<10 cm) of 10 species of octocorals were identified (Coffroth et al. 2001). Recently, the zooxanthella cladal identity in several adult octocoral species has been reported (Goulet and Coffroth 2003a; LaJeunesse 2001, 2002; Rowan and Powers 1991a; Santos et al. 2001, 2002), although most reports are based on a sample size of one individual per species, and/or from one geographic location. In the current study, we identified the zooxanthellae in adult Caribbean octocorals. First, we determined the cladal identity of zooxanthellae in 35 Caribbean octocoral species. Second, for five representative octocoral species, we examined if zooxanthella clades varied in conspecifics with depth. Third, we examined zooxanthella cladal variability within ten octocoral species from six different locations throughout the Caribbean. Finally, we compared our findings to the data available for scleractinian corals in the Caribbean.

Methods

Sampling scheme

We identified the zooxanthella clades in 35 octocoral species from the Caribbean (Bahamas, Belize, Florida Keys, Panama, and US Virgin Islands) and from four octocoral species from Bermuda (Table 1). In the Caribbean, 2–10 different reefs were sampled at each location (Bahamas, 4 reefs; Belize, 3 reefs; Florida Keys, 10 reefs; Panama, 5 reefs; and US Virgin Islands, 2 reefs). In Bermuda, samples came from one reef.

To determine if conspecifics host the same zooxanthella clades at different depths, colonies were sampled from shallow (<3 m) and deep (>10 m) water. We focused on five species, *Erythropodium caribaeorum*, *Briareum asbestinum*, *Plexaura kuna*, *P. homomalla*, and *P. flexuosa*. We chose these octocorals since they represent members of the suborders Holaxonia and Scleraxonia and are abundant on Caribbean reefs. To determine if conspecifics hosted the same zooxanthella clade at different geographic locations, 10 octocoral species, from six genera, were sampled at a minimum of two geographic locations (Table 1).

For each octocoral sampled, six cm of coral tissue was removed from the tip, in branching species, or an equivalent amount of tissue from encrusting forms. Tissue samples were preserved in 1.5-ml eppendorf tubes in 95% ethanol. We used 0.5×0.5 cm of octocoral tissue for DNA extraction. In addition, 4 cm of tissue were dried for octocoral identification. Octocoral species identifications were made based on external characteristics, sclerite analysis, and species description using Bayer (1961).

DNA Analysis

We extracted DNA following the protocol of Goulet and Coffroth (2003a). We amplified the ssRNA with PCR using a “universal” primer (ss5) and a zooxanthella-biased primer, ss3Z (Rowan and Powers 1991b). We used 10 ng of DNA in a 30- μ l PCR reaction volume, with 1X PCR buffer (Perkin-Elmer/Cetus, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl₂, 0.001% (wt/vol) gelatin), 100 μ m of each dNTP, 0.2 μ M of each primer, and 1 unit *Taq* polymerase. The PCR profile consisted of 2.5 min at 94 °C; 1 min at 56 °C; 2 min at 72 °C followed by 30 cycles of 1 min at 94 °C; 1 min at 56 °C; 2.5 min at 72 °C; and ending with 8 min at 72 °C. We digested the resulting PCR product with either the restriction enzyme *Taq* I (MBI Fermentas) or *Dpn* II (New England Biolabs). We then ran the product on a 2% w/v agarose (Eastman Kodak Co.) gel and stained it with ethidium bromide for UV visualization. RFLP genotypes of cloned ssRNA genes were run as standards on each gel, in addition to 100-bp ladders. We scanned negatives of the RFLP gels into a computer and determined the molecular weight of the RFLP bands using the program NCSA GelReader 2.0.5 (NCSA, University of Illinois).

Two unknown RFLP patterns found in *Erythropodium caribaeorum* (EC2 and EC4) were characterized by cloning the PCR product (Promega pGEM-T Vector System), performing cycle sequencing (Perkin-Elmer, ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit), and sequencing the resulting PCR product (Guelph Molecular Supercentre, University of Guelph). For comparison with other available sequences, we used the internal primers ss2.5 and ss2.3 (Wilcox 1997) to amplify the 600-bp variable region of the ssRNA that includes the variable domains V2-V4 (Sadler et al. 1992). In order to sequence the entire 1700-bp PCR product of the EC2 pattern, we used the internal primers ss2.5 and ss2.3 (Wilcox 1997), in addition to ss5M and ss5E (T. Shearer, unpubl. data). We then aligned the sequence with other available zooxanthella sequences using SeqApp (1.9a169). Using PAUP, we generated the most parsimonious tree using a heuristic search with 500 bootstrap replicates (Swofford 1998) and calculated a Jukes-Cantor distance matrix of the zooxanthellae in the *Symbiodinium* group C (Swofford 1998).

We compared our findings to those published for scleractinian corals in the Caribbean (Baker and Rowan 1997; Baker et al. 1997; Billingham et al. 1997; Diekmann et al. 2002; LaJeunesse 2002; Rowan and Knowlton 1995; Rowan et al. 1997; Rowan and Powers 1991b; Toller et al. 2001b; Wilcox 1997). To determine if taxonomic affinity existed between the scleractinian host families and the zooxanthella groups found within them, we conducted a Chi-square, goodness-of-fit test (Zar 1984).

Table 1 The identity of zooxanthella clades in 35 host octocoral species sampled throughout the Caribbean and Bermuda. *N* denotes the total number of colonies sampled for each octocoral species from each location. *B* Bahamas; *Be* Bermuda; *Bz* Belize; *F* Florida Keys; *P* Panama; *U* U.S. Virgin Islands

Family	Genus	Species	Location (<i>N</i>)	Zooxanthella clade	
Anthothelidae	<i>Erythropodium</i>	<i>caribaeorum</i>	Bz (5), P (21)	C	
Briareidae	<i>Briareum</i>	<i>asbestinum</i>	B(4),Bz (6), F(19),P(38)	B(62) or C(5)	
Gorgoniidae	<i>Gorgonia</i>	<i>flabellum</i>	P (3)	B	
		<i>mariae</i>	P (4)	B	
		<i>ventalina</i>	P (3)	B	
		sp.	B (1), P (1)	B	
	<i>Pseudopterogorgia</i>	<i>americana</i>	Bz (2)	B	
		<i>bipinnata</i>	P (2)	B	
		<i>elisabethae</i>	B (23)	B	
		<i>anceps</i>	P (4)	B	
		<i>asperula</i>	P (1)	B	
		<i>colombiensis</i>	P (2)	B	
	Plexauridae	<i>Pterogorgia</i>	<i>laciniata</i>	P (5)	B
			<i>mammosa</i>	P (7)	B
		<i>Eunicea</i>	<i>pallida</i>	P (1)	B
			<i>tayrona</i>	P (2)	B
<i>turnerforti</i>			B (1), P (5)	B	
sp.			B (3)	B	
<i>Muricea</i>			<i>atlantica</i>	P (2)	B
			<i>elongata</i>	P (1)	B
			<i>laxa</i>	Be (1), P(4)	B
			<i>muricata</i>	P (4)	B
<i>Muriceopsis</i>			<i>flavida</i>	P (4)	B
			<i>urabensis</i>	P (1)	B
			sp.	B (3), P (3)	B
<i>Plexaura</i>			<i>flexuosa</i>	B(3), Bz(2), F(36), P(8)	B
			<i>homomalla</i>	B(2), F(2), P(45)	B
			<i>kuna</i>	B(19),F(16), P(29),U(6)	B
	<i>Plexaurella</i>	<i>dichotoma</i>	P (2)	C	
		<i>grisea</i>	P (1)	C	
		<i>nutans</i>	P (3)	B	
<i>Pseudoplexaura</i>	<i>flagellosa</i>	B (1), F (3), P(2)	B		
	<i>porosa</i>	B(1), Bz(6), F(1), P(7)	B		
	sp.	B (2), P (3)	B		
	<i>wagenaari</i>	F (2), P (1)	B		

Results

Throughout the Caribbean, 31 out of the 35 octocoral species sampled hosted clade B zooxanthellae (Fig. 1, Table 1). Two of the three octocoral species sampled from the genus *Plexaurella* (Plexauridae) hosted clade C zooxanthellae (Fig. 1, Table 1). We did not find clade A, D or E zooxanthellae in any of the Caribbean octocoral species sampled. In some samples of the octocorals *Plexaura kuna*, *P. flexuosa*, and *Pseudopterogorgia elisabethae*, when the PCR product was digested with the restriction enzyme *Taq* I, in addition to the clade B RFLP pattern, we found a novel RFLP pattern containing the three fragments: 740-, 649-, and 340-bp long (e.g., Fig. 1, Pst e lane). When sequenced, we found that this RFLP pattern aligned most closely (BLAST search, 95% base sharing) with the apicomplexan *Sarcocystis aucheniae*. In addition, with *Taq* I, several *P. flexuosa* samples exhibited an extra band. This band could be

explained by an extra restriction site in clade B zooxanthellae. When digested with *Dpn* II, *P. flexuosa* samples exhibited a characteristic clade B RFLP pattern (Fig. 1).

Zooxanthellae sampled from *E. caribaeorum* (Anthothelidae) showed three distinct ssRNA-RFLP patterns. One banding pattern matched the banding pattern of clade C zooxanthellae. Two additional unique zooxanthella RFLP patterns (EC1 and EC2) were observed when the ssRNA was digested with the restriction enzyme *Dpn* II (Fig. 1). Each *E. caribaeorum* colony sampled displayed one of the unique patterns along with a typical clade C pattern. The unique patterns, however, were never found together in the same *E. caribaeorum* or alone. Sequencing the cloned PCR products determined that the unknown zooxanthella clade in *E. caribaeorum* most closely aligned with clade C zooxanthellae.

Most *Briareum asbestinum* sampled (62 of 67) hosted clade B zooxanthellae, but in the Dry Tortugas (Florida Keys), four out of the five samples had the typical clade

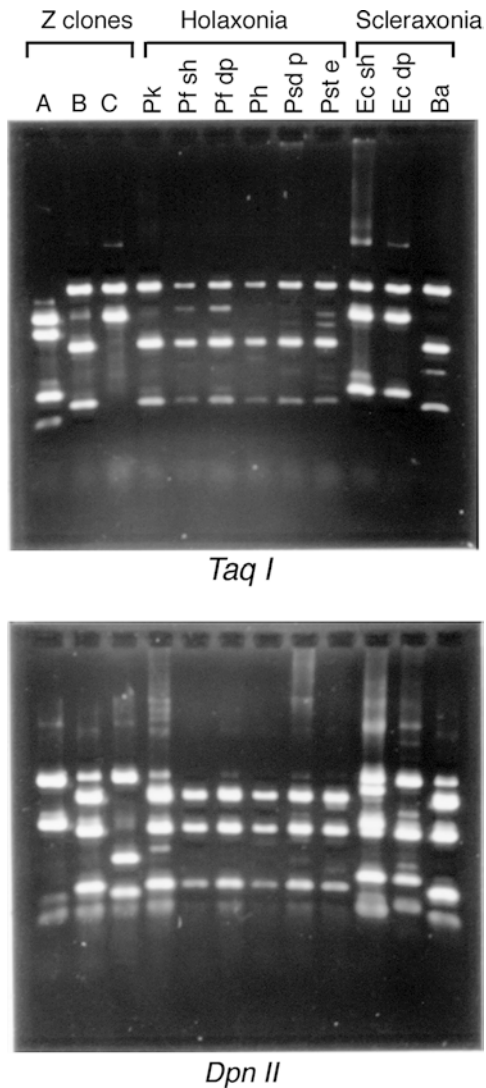


Fig. 1 Restriction fragment length polymorphism (RFLP) gels of zooxanthella DNA encoding for the small subunit ribosomal RNA. The PCR-amplified product was digested with the restriction enzymes *Taq* I (upper gel) and *Dpn* II (lower gel). Lanes from left to right are of: standards for clades A, B, and C (Z clones A, B, C); zooxanthellae from the Caribbean octocorals *Plexaura kuna* (Pk), *P. flexuosa* (Pf) from shallow (sh) and deep (dp), *P. homomalla* (Ph), *Pseudoplexaura porosa* (Psd p), *Pseudopterogorgia elisabethae* (Pst e), *Erythropodium caribaeorum* (Ec) from shallow and deep, and *Briareum asbestinum* (Ba)

C pattern and an RFLP pattern identical to the EC2 pattern found in *Erythropodium caribaeorum*.

Examination of zooxanthellae in conspecific hosts at different depths revealed that the zooxanthella clade did not change as a function of depth (Table 2). *Plexaura flexuosa*, *P. homomalla*, *P. kuna*, and *Briareum asbestinum* all harbored clade B zooxanthellae irrespective of depth. *Erythropodium caribaeorum* hosted clade C zooxanthellae and the two variants of clade C both in shallow and deep water with no observable depth zonation.

In nine of the ten octocoral species surveyed from different geographic areas within the Caribbean, con-

Table 2 Zooxanthella clades (Z) found in conspecifics of five different octocoral species sampled from shallow (<3 m) and deep (>10 m). N denotes the total number of colonies sampled for each octocoral. *Briareum asbestinum*, *Erythropodium caribaeorum*, *Plexaura homomalla*, and *Plexaura kuna* were sampled in the San Blas Islands, Panama. Samples of *Plexaura flexuosa* were obtained from the Florida Keys

Species	Shallow (<3 m)		Deep (>10 m)	
	Z	N	Z	N
<i>Briareum asbestinum</i>	B	15	B	12
<i>Erythropodium caribaeorum</i>	C	8	C	11
<i>Plexaura kuna</i>	B	6	B	9
<i>Plexaura homomalla</i>	B	15	B	15
<i>Plexaura flexuosa</i>	B	18	B	18

specifics hosted the same zooxanthellae (Table 1). For example, *Eunicea tournerforti* hosted clade B zooxanthellae from sites as far apart as Bermuda and Panama. The one exception was *Briareum asbestinum* that hosted clade B zooxanthellae in the Bahamas, Belize, Panama, and most sites in the Florida Keys. In the Dry Tortugas (Florida Keys), one of the five colonies sampled hosted clade B zooxanthellae, while the other four colonies hosted simultaneously clade C and one of the unique clade C variants (EC2) isolated from *E. caribaeorum*.

Zooxanthella cladal identities have been determined for 43 scleractinian coral species in the Caribbean (Table 3). Of these scleractinian coral species, 30.2% host clade C zooxanthellae, 23.3% host clade B, 2.3% host clade A and 44.2% host multiple zooxanthella clades (Table 3) (Baker 1999; Baker and Rowan 1997; Baker et al. 1997; Billingham et al. 1997; Diekmann et al. 2002; LaJeunesse 2002; Rowan and Knowlton 1995; Rowan et al. 1997; Rowan and Powers 1991b; Toller et al. 2001b; Wilcox 1997). There was a significant taxonomic affinity between scleractinian suborders in the Caribbean and the zooxanthella clade hosted (comparison of scleractinian coral species in four categories, those hosting clade A, B, C, and multiple clades; $X^2 = 28.3$, $df = 3$, $p < 0.001$).

Discussion

Among the Caribbean octocoral species examined in this study, the vast majority (88.6%) hosted only clade B zooxanthellae. In contrast, in scleractinian corals, the most common zooxanthella clade found in the Caribbean is clade C, but clades A and B also occur frequently (Baker 1999, 2001; Baker and Rowan 1997; Baker et al. 1997; Billingham et al. 1997; Darius et al. 1998; LaJeunesse 2002; Rowan and Powers 1991a; Rowan and Powers 1991b; Rowan and Powers 1992; Toller et al.

Table 3 Literature summary of cladal identity of zooxanthellae (Z) in 44 scleractinian coral species from the Caribbean. *Denotes scleractinian coral species that were sampled both in shallow and deep waters

Suborder	Family	Genus	Species	Z clade	Source			
Astrocoeniina	Acroporidae	<i>Acropora</i>	<i>cervicornis</i> *	A, C	1,2,3,4,5			
			<i>palmata</i>	A	1,2,3,4,5			
	Astrocoeniidae	<i>Stephanocoenia</i>	<i>melanosticta</i> *	A, D, C	2,4,5,6			
			<i>decactis</i> *	B	2,4,5,6			
	Pocilloporidae			<i>formosa</i> *	B	6		
				<i>mirabilis</i> *	B	1,2,6		
				<i>pharensis</i> *	B	6		
				<i>senaria</i> *	B	6		
				<i>meandrites</i>	B	2,4,5		
				<i>fastigata</i>	B, C	2,5		
				<i>Cladocora</i>	B	5		
				<i>natans</i> *	B, C	2,4,5		
Caryophylliida	Caryophylliidae	<i>Eusmilia</i>	<i>fastigata</i>	B, C	2,5			
			<i>Cladocora</i>	B	5			
Faviida	Faviidae		<i>Colpophyllia</i>	B, C	2,4,5			
			<i>Diploria</i>	B, C	2,4,5			
			<i>Diploria</i>	B, C	2,4,5,7			
			<i>Diploria</i>	B, C	2,4,5,7			
			<i>Favia</i>	B, C	1,2,5			
			<i>Manicina</i>	B, C	5			
			<i>Montastraea</i>	A, B, C, D	2,4,8,9,10			
			<i>cavernosa</i> *	C	2,5,6,8,11			
			<i>faveolata</i> *	A, B, C, D	2,5,8,9,10,11			
			<i>franksi</i> *	A, B, C, D	6,10			
			Meandrinidae		<i>Dendrogyra</i>	<i>cylindrus</i>	B	5
						<i>Dichocoenia</i>	B	5
Mussidae			<i>Isophyllia</i>	C	5			
			<i>Isophyllastrea</i>	B or C	2,4,5,6			
			<i>Mycetophyllia</i>	C	5,6			
			<i>ferox</i>	C	2			
			<i>lamarckiana</i>	C	5			
Fungiida	Agariciidae		<i>Scolymia</i>	C	5			
			<i>Agaricia</i>	C	2,5,6			
			<i>danae</i>	C	2			
			<i>fragilis</i>	C	5,12			
			<i>humilis</i>	C, D	5			
			<i>lamarcki</i>	C	2,6			
			<i>temuifolia</i>	C	2,5			
			<i>Leptoseris</i>	C	2,5,6			
			Poritidae		<i>Porites</i>	<i>cucullata</i>	C	2,5,6
						<i>asteroides</i> *	A, B, C	2,4,5
						<i>colonensis</i>	A or C	2,4,5
						<i>divaricata</i>	C	2,5
<i>furcata</i>	A, B, C	2,4,5						
Siderastreidae		<i>Siderastrea</i>	<i>porites</i>	C	2,4			
			<i>radians</i>	B	5			
			<i>siderea</i> *	C, D	2,4,5			

Source: 1. Rowan and Powers 1991a; 2. Baker and Rowan 1997; 3. Baker et al. 1997; 4. Baker 1999; 5. LaJeunesse 2002; 6. Diekmann et al. 2002; 7. Wilcox 1997; 8. Rowan and Knowlton 1995; 9. Rowan et al. 1997; 10. clade E reported by Toller et al (2002) is the same as clade D, see Baker (2003) for discussion; 11. Billinghamurst et al. 1997; 12. Wilcox 1998

2001b; Wilcox 1997). Like Caribbean octocorals species, the majority of Caribbean scleractinian coral species sampled (55.8%) host a single numerically dominant zooxanthella clade (Baker 1999; Baker and Rowan 1997; Baker et al. 1997; Billinghamurst et al. 1997; Darius et al. 1998; Diekmann et al. 2002; LaJeunesse 2002; Rowan and Powers 1991a; Rowan and Powers 1991b, 1992; Wilcox 1997).

In the current study, only *Briareum asbestinum* hosted two zooxanthella clades, although these clades did not occur simultaneously within a single colony. In the 44.2% of Caribbean scleractinian coral species that host multiple zooxanthella clades, the majority host a single numerically dominant zooxanthella clade at a time (Baker 1999; Baker and Rowan 1997; Baker et al. 1997; Rowan and Knowlton 1995; Rowan et al. 1997; Wilcox

1997). In these cases, variation in the zooxanthella clades hosted within a coral species is a function of depth. Depth zonation of zooxanthella clades has been attributed to differences in light level preferences among the different zooxanthella clades (Rowan 1998; Rowan and Knowlton 1995; Rowan et al. 1997). In these corals, zooxanthellae in clades A and B inhabit shallow water colonies, while clade C zooxanthellae are found in deeper water colonies, or in shaded areas of the same individual coral colony (Baker 1999; Rowan and Knowlton 1995; Rowan et al. 1997; Toller et al. 2001b). For example, *Montastraea annularis* and *M. faveolata* host four zooxanthella clades, and the distribution of these zooxanthella clades follows light and depth gradients (LaJeunesse 2002; Rowan and Knowlton 1995; Rowan et al. 1997; Toller et al. 2001a).

Since 97.1% of Caribbean octocoral species examined hosted a single algal clade, there was no zooxanthella cladal variation as a function of depth in those octocoral species. Lack of cladal variation with depth has also been documented for *Montastraea cavernosa* (Billingham et al. 1997) and five species of the scleractinian *Madracis* (Diekmann et al. 2002). *Erythropodium caribaeorum* and the Dry Tortugas population of *B. asbestinum* also did not display cladal variation with depth; however, one of the two clade C zooxanthella variants co-occurred with clade C zooxanthellae. Since each of the unique RFLP patterns always co-occurred with the RFLP pattern of clade C, it is possible that these patterns represent pseudogenes or are the result of a gene duplication event. Further research will elucidate this matter.

Unlike in the current study, LaJeunesse (2002) found two clades in a single representative of *Eunicea turnerforti* and *Plexaura homomalla* in the Yucatan Peninsula, although in the four *P. homomalla* individuals sampled in Lee Stocking Island, Bahamas, he only found one clade, which is in agreement with our findings. We sampled six *E. turnerforti* from two geographic locations and 49 *P. homomalla* from three geographic locations and found no cladal variability. Since the clades in question are clades B and C, they can be easily distinguished using ssRFLP. A larger sample size in the Yucatan Peninsula is necessary to determine if two clades are indeed common in the two species in question at this site.

From our study, we conclude that in the Caribbean, there is a strong specificity between octocoral hosts and their zooxanthella clade. Although other cnidarians on the same reef harbor a range of zooxanthella clades, the vast majority of octocorals harbored only clade B. Furthermore, each octocoral species harbored the same zooxanthella clade regardless of sampling location (Table 1). For example, *Plexaura kuna* sampled in the San Blas Islands, Panama, the Bahamas, Florida Keys, and the US Virgin Islands all had clade B zooxanthellae. *Pseudoplexaura flagellosa* had clade B zooxanthellae in locations spanning from Bermuda to Panama. Conversely, geographic variation has been documented in zooxanthellae in scleractinian species in the Indo-Pacific (Loh et al. 2001; Rodriguez-Lanetty et al. 2001; van Oppen et al. 2001). The only exception to the trend in Caribbean octocorals was *Briareum asbestinum*, which hosted clade B zooxanthellae in all locations except for colonies sampled from the Dry Tortugas, Florida Keys. This difference may be attributed to the fact that the *B. asbestinum* population in the Dry Tortugas is genetically distinct from other *B. asbestinum* populations (D. Brazeau; personal communication.). Therefore, the difference in zooxanthella clade in *B. asbestinum* from the Dry Tortugas may be due to the presence of a cryptic sibling species of *B. asbestinum* at this site. Thus, our results can be interpreted as either both species are present with each harboring a different zooxanthella clade or that *B. asbestinum* is absent and the cryptic sibling species may

be able to host both zooxanthella clades B and C, non-simultaneously.

Initial surveys of zooxanthella diversity among coral hosts found no taxonomic affinity between hosts and symbionts; closely related hosts had different zooxanthella clades while taxonomically distant hosts had zooxanthella symbionts belonging to the same clade (Rowan and Powers 1991a). This conclusion, however, was based on a comparison of host species over a wide geographic range. When the comparison is limited to coral hosts from the Caribbean region, coral species within a suborder tend to harbor zooxanthellae from the same clade (Chi-square, goodness-of-fit test, $p < 0.001$, Table 3). For example, in the suborder Fungiida, the majority of coral species sampled host clade C zooxanthellae (Table 3). Among Caribbean octocorals, most species hosted only clade B zooxanthellae. In the Plexauridae, the only two species that host clade C belong to the genus *Plexaurella*. The other two octocoral species that host clade C belong to the suborder Holaxonia. More octocorals and corals from throughout the world need to be examined to determine if a host/symbiont pattern exists within a geographic area.

The results reported here also suggest that octocorals that harbor clade C zooxanthellae may be more susceptible to bleaching. Few octocoral species are reported to bleach, however, nearly all octocoral species known to host clade C zooxanthellae bleach, while only three octocoral species that harbor clade B have been reported to visibly bleach. Of the five octocorals reported to bleach (Lang et al. 1992; Lasker et al. 1984; Williams and Bunkley-Williams 1990), we now know that *Erythropodium caribaeorum* and *Plexaurella dichotoma* harbor clade C. *P. nutans* and *Eunicea* sp. have clade B, and *Briareum asbestinum* harbors clades B or C. One assumption in making these comparisons is that hosts do not change their symbionts from year to year. Goulet and Coffroth (2003b) demonstrated that this is the case for *Plexaura kuna*. We collected the survey samples over more than a decade (1988–2002). In each case, octocorals belonging to the same species all exhibited the same zooxanthella clade. Therefore, the zooxanthella clade found within a host remains the same over a long time and the assumption appears justified, at least for octocorals.

The finding of cladal specificity within Caribbean octocorals does not mean that these symbionts lack variation. Using molecular techniques that have more resolving power than that of ssrDNA, variation has been detected both between octocoral species at the cladal level (LaJeunesse 2002; Santos et al. 2002, 2003a, 2004) and within species at the population level (Goulet and Coffroth 2003b; Santos et al. 2003b).

In summary, within Caribbean cnidarians, four major zooxanthella clades have been identified. Octocorals almost exclusively hosted zooxanthellae belonging to clade B, while scleractinian corals host all four major zooxanthella clades. In addition, octocorals showed no depth-related changes in zooxanthella clades, as has

been reported for some scleractinian corals (Baker 1999; Baker et al. 1997; Rowan 1998; Rowan and Knowlton 1995; Rowan et al. 1997). Finally, the zooxanthella clade within most of the octocoral species does not vary within the Caribbean, with the exception of the clades within *B. asbestinum*. The zooxanthella cladal affinity in octocorals is noteworthy given that the octocorals sampled inhabit different depths and environments (Goldberg 1973; Kinzie 1973; Lasker and Coffroth 1983). It remains to be determined why Caribbean octocorals display such specificity for clade B zooxanthellae while scleractinian corals host all four zooxanthella clades.

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