

Worldwide biogeography of *Symbiodinium* in tropical octocorals

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ABSTRACT: Although octocorals are important components of coral reefs, most research on the genetic diversity of symbiotic zooxanthellae (*Symbiodinium* spp.) has focused on scleractinian (stony) corals. For both groups, most geographic comparisons have occurred within the same ocean or only included a few geographic sites. We characterized the genetic diversity of *Symbiodinium* in tropical octocorals in 15 geographic areas throughout the world. By combining octocoral samples analyzed in this study with published data, the cladal identities of zooxanthellae in 117 octocoral species were identified and a global analysis was performed. For 63 octocoral species, the *Symbiodinium* type was also identified. The vast majority (87.2%) of octocorals hosted a single zooxanthella clade. Most octocorals also hosted specific *Symbiodinium* types. A biogeographic pattern emerged. Octocorals from 4 geographic areas in the Indo-west Pacific hosted *Symbiodinium* C. In the Great Barrier Reef, 11 octocoral species (24.4%) hosted *Symbiodinium* D, 3 species hosted *Symbiodinium* G, and 1 species each hosted *Symbiodinium* A and B. Red Sea octocorals hosted predominantly *Symbiodinium* C, with *Symbiodinium* A occurring in 3 species. In Hawaii, 2 octocorals hosted *Symbiodinium* C and 1 hosted *Symbiodinium* A. In Bermuda, only clade B was found. In the Caribbean, unlike in the Indo-Pacific, the dominant *Symbiodinium* was B, predominantly type B1, with *Symbiodinium* C occurring in a few species. Understanding the similarities and differences between octocoral and scleractinian coral symbioses with zooxanthellae may enable predictions of which symbioses will survive in periods of environmental change.

KEY WORDS: *Symbiodinium* · Zooxanthellae · Octocorals · Corals · GIS · Clade · RFLP · Biogeography

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INTRODUCTION

On coral reefs, the mutualism between cnidarians (e.g. octocorals, corals, sea anemones) and their symbiotic dinoflagellates (zooxanthellae) creates the framework of the ecosystem. To address ecological and evolutionary questions concerning these symbioses, it is imperative to first identify the partners involved. Zooxanthella identification in the past was hindered by the lack of distinguishing morphological characteristics. Several time-consuming techniques, requiring a great deal of expertise, were employed for algal identification. Examples include culturing and/or identifying zooxanthellae based on their motile form (Trench 1997) and

using antigenic features (Kinzie & Chee 1982) and cell architecture (Blank 1987). These techniques lead to the differentiation of only a small number of *Symbiodinium* species.

Knowledge of the diversification and classification of zooxanthellae has grown tremendously since the initial application of molecular techniques (Rowan & Powers 1991a). Based on sequence comparison of small (ssRNA) and large (lsRNA) subunit ribosomal RNA, zooxanthellae are currently divided into 8 clades (reviewed by Pochon et al. 2006, Stat et al. 2006) although Tchernov et al. (2004) distinguished between 13 clades based on genetic distance. Each clade includes multiple zooxanthella species (Rowan 1998).

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Studies of other DNA regions such as internal transcribed spacer (ITS) regions of nuclear DNA (Baillie et al. 2000, LaJeunesse 2001, van Oppen et al. 2001), the 23S rDNA of chloroplasts (Santos et al. 2002, Pochon et al. 2006), *psbA* minicircle (Barbrook et al. 2006), mitochondrial sequences (Takabayashi et al. 2004), and microsatellites (Santos et al. 2004, Magalon et al. 2006) support the cladal relationships based on ssRNA and lsRNA and provide within-clade resolution. Most reports have identified *Symbiodinium* at the clade level.

Within-clade comparison of zooxanthellae is complicated by the fact that researchers, using different parts of the genome for the within-clade division, create their own nomenclature with minimal cross-reference to other sub-cladal nomenclature (LaJeunesse 2001, van Oppen et al. 2001, Santos et al. 2003a). Consequently, a single within-clade nomenclature does not exist, and 2 identities can be given to the same zooxanthella type. For example, *Symbiodinium* named B1 (LaJeunesse 2002) is equivalent to B184 (Kirk et al. 2005). In addition, prior to within-clade classification, it is important to initially compare hosts and distinguish zooxanthellae at the clade level.

One can ask questions about zooxanthellae from the individual symbiont level (Goulet & Coffroth 2003a,b), the symbiont population level (e.g. Santos et al. 2003b, Magalon et al. 2006), the broad clade level, and all levels in between, with each level providing important information about the symbioses. Clade-level comparisons are informative. For example, comparisons of *Symbiodinium* clades within cnidarian species have been used to elucidate between- and within-species biogeographic patterns (LaJeunesse & Trench 2000, Loh et al. 2001, Rodriguez-Lanetty et al. 2001, Baker 2003) and intraspecific physiological differences (Little et al. 2004, Rowan 2004, Goulet et al. 2005, Berkelmans & van Oppen 2006, LaJeunesse et al. 2007). Since this study is a broad geographic study, the clade level is the most appropriate level for comparison. Furthermore, cladal distinction allows comparison to previously published studies.

Most studies characterizing the genetic identity of zooxanthellae have concentrated on scleractinian corals (reviewed by Baker 2003, Stat et al. 2006), even though octocorals are major components of the benthos on many reefs (Goldberg 1973, Kinzie 1973, 1974, Muzik 1982, Lasker & Coffroth 1983, Sánchez et al. 1997, 2003, Fabricius & Alderslade 2001). Research determining zooxanthella clade identity in octocorals has focused on juveniles (Coffroth et al. 2001) or on a few octocoral species and/or one geographic area (Rowan & Powers 1991a, LaJeunesse 2001, 2002, Santos et al. 2001, 2002, 2003b, 2004, Goulet & Coffroth 2003a,b, Barneah et al. 2004). One study sampled mul-

iple geographic locations in the Caribbean, reporting on zooxanthella clades in 35 octocoral species (Goulet & Coffroth 2004).

Biogeographic studies of zooxanthella clades in scleractinian corals, octocorals, or zoanthids have predominantly compared different sites within the same ocean (Loh et al. 2001, Rodriguez-Lanetty et al. 2001, Burnett 2002, Rodriguez-Lanetty & Hoegh-Guldberg 2003, Goulet & Coffroth 2004). Three studies compared zooxanthella clades in hosts from 1 site in the Caribbean versus the Great Barrier Reef in the Indo-west Pacific; 2 of these studies were on scleractinian corals (Baker & Rowan 1997, LaJeunesse et al. 2003) and 1 on 34 species of zooxanthellate octocorals (van Oppen et al. 2005). The objective of this study was to perform a comprehensive worldwide biogeographic comparison of *Symbiodinium* clades in tropical octocorals.

MATERIALS AND METHODS

Sampling scheme. We identified zooxanthella clades in octocoral species that were collected from various locations throughout the tropics (reference 1 in Table 1). Our data were combined with those reported in the literature for a total data set of 117 octocoral species (Table 1). Since samples were collected while surveying the reefs, sample sizes varied. The combined data set included samples from numerous reef sites from 15 geographic locations that were identified according to the country or location: Australia, Bahamas, Barbados, Belize, Bermuda, Fiji, Florida Keys, Guam, Hawaii, Israel, Japan, Mexico, Panama, Dry Tortugas, and US Virgin Islands (Table 2). These geographic locations were in the Atlantic, Caribbean, Pacific, Red Sea, and Indo-west Pacific.

For each octocoral that we sampled, 6 cm of octocoral 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 microcentrifuge tubes in 95% ethanol. We used 0.5 × 0.5 cm of octocoral tissue for DNA extraction.

DNA analysis. DNA was extracted from each piece of tissue using a phenol chloroform protocol as described by Goulet & Coffroth (2003a). We amplified the ssRNA with a 'universal' primer (ss5) and a zooxanthella-biased primer, ss3Z (Rowan & Powers 1991b) following the protocol described by Goulet & Coffroth (2003a). We digested the resulting PCR product with the restriction enzymes *TaqI* (MBI Fermentas) and *DpnII* (New England Biolabs). We ran the product on a 2% w/v agarose (Eastman Kodak) gel, which was stained 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.

Table 1. Identity of *Symbiodinium* clades in 117 host octocoral species sampled throughout the tropics. *Symbiodinium* type is based on the nomenclature of LaJeunesse (2001). Au: Australia, Ba: Bahamas, Bb: Barbados, Be: Bermuda, Bz: Belize, Fj: Fiji, Fl: Florida Keys, Gu: Guam, Ha: Hawaii, Is: Israel, Ja: Japan, Me: Mexico, Pa: Panama, To: Dry Tortugas, VI: US Virgin Islands. Clades or types co-occurring in the same colony are denoted with the symbol '&'. (N): number of colonies sampled at each location; N: total number of colonies sampled for each octocoral species

Family	Octocoral host		Zooxanthella		Location (N)	N	Source
	Genus	Species	Clade	'Type'			
Alcyoniidae	<i>Cladiella</i>	<i>pachyclados</i>	C		Is	3	11
		<i>tuberculoides</i>	C		Is	3	11
		spp.	C,D		Au (2), Fj (1)	3	1, 18
	<i>Klyxum</i>	spp.	C,D	C64	Au	3	12, 18
	<i>Lobophytum</i>	<i>compactum</i>	C	C1:3a	Au	7	3
		spp.	C	C1, C3j	Au (12), Fj (4), Gu (1)	17	1, 8, 12, 18
	<i>Rhytisma</i>	<i>fulvum fulvum</i> spp.	C		Au (2), Is (18)	20	1, 11
			C	C1, C1:1	Au	3	3, 18
	<i>Sarcophyton</i>	<i>ehrenbergi glaucum trocheliophorum</i> spp.	C		Au	1	17
			C		Gu (4), Is (4)	8	1, 11
			C		Gu (5), Is (5)	10	1, 11
			C	C1, C1:3a, C3j, C65, C71a	Au (26), Fj (6), Ja (1)	33	1, 3, 8, 12, 18
	<i>Sinularia</i>	<i>abrupta erecta flexibilis gardineri gyrosa leptoclados lochmodes maxima polydactyla querciformis</i> spp.	C		Ha	7	1
			C		Fj	3	1
			C	C1:3a	Au	8	3
			C		Is	3	11
			C		Fj	3	1
			C		Is	3	11
			C		Ja	1	17
			C		Gu	1	1
C			C1:3a	Au (6), Gu (2), Is (3)	11	1, 3, 11	
C			C1, C1c, C3j, C65	Fj (6), Is (3)	9	1, 11	
C		Au (18), Fj (14), Gu (4), Ja (1)	37	1, 8, 9, 12, 17, 18			
Anthothelidae	<i>Alertigorgia</i>	<i>orientalis</i>	C,D		Au	2	18
	<i>Erythropodium</i>	<i>caribaeorum</i>	B,C	B1, C1,C3	Bb (8), Bz (5), Me (1), Pa (21)	35	2, 7, 21
			C	C1	Au	1	12
Briareidae	<i>Briareum</i>	<i>asbestinum stechei violacea</i> spp.	B,C	B1, B2, B19, B19a, B33, C1	Ba (4), Bb (2), Bz (4), Fl (38), Me (1), Pa (38), To (8), VI (3)	98	2, 6, 9, 18, 19, 21
			D		Au	2	18
			C		Au	2	18
			C	C1, C1:1, C1:2, C3, C23	Au	22	1, 3, 8, 12
Clavularidae	<i>Clavularia</i>	<i>koellikeri</i>	D	D3	Au	6	3
		sp.	D	D3	Au	4	12, 18
Ellisellidae	<i>Junceella</i>	<i>fragilis</i>	G		Au	3	18
		spp.	C		Au (2), Gu (1)	3	1
Gorgoniidae	<i>Gorgonia</i>	<i>flabellum</i>	B	B1	Bb (1), Me (1), Pa (4), VI (1)	7	2, 7, 14, 19, 21
		<i>mariae</i>	B	B1	Ba (1), Bb (2), Me (2), Pa (4)	9	2, 7, 18, 21
		<i>ventalina</i>	B	B1	Bb (3), FL (48), Me (1), Pa (3)	55	2, 15, 16, 21
		spp.	B	B1	Ba (1), Fl (11), Pa (1)	13	2, 6, 9, 14
	<i>Hicksonella</i>	<i>expansa</i>	C	C65	Au	1	12

Table 1. (continued)

Family	Octocoral host		Zooxanthella		Location (N)	N	Source	
	Genus	Species	Clade	'Type'				
Gorgoniidae	<i>Leptogorgia</i>	sp.	B		Fl	2	14	
(continued)	<i>Pinnigorgia</i>	<i>flava</i>	C	C1:1&C1:2	Au	2	3	
	<i>Plexaurella</i>	<i>dichotoma</i>	C		Me (2), Pa (2)	4	2, 18	
		<i>grisea</i>	B,C		Me (1), Pa (1)	2	2, 18	
		<i>nutans</i>	B,C	B1, B1a	Ba (1), Bb (1), Me (2), Pa (3)	7	2, 7, 18, 21	
		sp.	B,C	B1, B1L, C2r	Bb (2), Fl (4), Pa (2), VI (1)	9	2, 14, 21	
	<i>Pseudopterogorgia</i>	<i>acerosa</i>	B	B1	Fl	2	9	
		<i>americana</i>	B	B1, B1i	Bb (3), Bz (2), Fl (10), Me (2)	17	2, 6, 7, 9, 21	
		<i>bipinnata</i>	B	B1	Ba (93), Pa (2), VI (1)	96	2, 15, 21	
		<i>elisabethae</i>	B	B1	Ba	767	2, 6, 9, 10, 15	
		<i>kallos</i> <i>rigida</i> sp.	B B B	B1 B1 B1	Me Me Bb	1 1 4	7 7 21	
	<i>Pterogorgia</i>	<i>anceps</i>	B	B1	Me (2), Pa (4)	6	2, 7, 18	
		<i>citrina</i>	B	B1, B1m	Bb (1), Me (1), VI (1)	3	18, 21	
	<i>Rumphella</i>	spp.	C	C1	Au	2	12, 18	
Helioporidae	<i>Heliopora</i>	<i>coerulea</i>	C	C1	Au	5	12, 18	
Ifalukellidae	<i>Plumigorgia</i>	<i>schoboti</i> sp.	C C	 C1	 Au	 1 1	 18 12	
Isididae	<i>Isis</i>	<i>hippuris</i> spp.	D C	 C3, C23	 Au	 1 2	 18 8, 12	
Metithaetae	<i>Asterionella</i>	sp.	C		Gu	4	1	
Nephtheidae	<i>Capnella</i>	<i>lacerthliensis</i>	C		Au	2	1	
		spp.	A,C,D		Au	2	18	
	<i>Lemnalia</i>	spp.	C	C1:2	Au (4), Is (5)	9	1, 3, 18	
	<i>Litophyton</i>	<i>arboreum</i>	A		Is	10	1, 11	
	<i>Nephthea</i>	spp.	A,B,C,D	B1, B1n, B36, C1, C1:2, D1a	Au (8), Is (3)	11	3, 8, 11	
		<i>Paralemnalia</i>	<i>digitiformis</i>	C	C1:2, C64	Au	3	3
			<i>eburnea</i>	C		Is	3	11
			<i>thyrsoidea</i> spp.	C C	 C1:2	 Au (4), Is (3) Au	 7 2	 3, 11 18
	<i>Stereonephthya</i>	<i>cundabiluensis</i>	A	A9	Is	6	11, 20	
		spp.	C,G	C15	Au (2), Fj (3)	5	1, 3, 18	
Plexauridae	<i>Eunicea</i>	<i>asperula</i>	B		Me (1), Pa (1)	2	2, 18	
		<i>calyculata</i>	B		Me	1	18	
		<i>clavigera</i>	B&C	B1, C1	Me	2	7	
		<i>colombiensis</i>	B		Pa	2	2	
		<i>fusca</i>	B		Pa	1	2	
		<i>laciniata</i>	B	B1	Me (2), Pa (5)	7	2, 7, 18	
		<i>mammosa</i>	B	B1, B9	Ba (2), Me (1), Pa (7)	10	2, 7	
		<i>pallida</i>	B		Pa	1	2	
		<i>succinea</i>	B		Me	2	18	
		<i>tayrona</i>	B		Pa	2	2	
		<i>tourneforti</i>	B,C,B&C	B1, C1	Ba (1), Be (1), Me (3), Pa (5)	10	1, 2, 7, 18	
		spp.	B	B1	Ba (3), Fl (14), Me (2), Pa (4)	23	2, 6, 9, 18, 14	

Table 1. (continued)

Family	Octocoral host		Zooxanthella		Location (N)	N	Source	
	Genus	Species	Clade	'Type'				
Plexauridae (continued)	<i>Euplexaura</i>	<i>nuttingi</i>	G		Au	1	18	
	<i>Muricea</i>	<i>atlantica</i>		B		Pa	2	2
		<i>elongata</i>		B	B1	Bb (1), Pa (1)	2	2, 21
		<i>laxa</i>		B		Be (1), Pa (4)	5	2
		<i>muricata</i>		B	B1	Me (3), Pa (4)	7	2, 7, 18
		<i>uniden</i>		B		Pa	1	2
		sp.		B	B1	Bb (2), Fl (6)	8	9, 14, 21
	<i>Muriceopsis</i>	<i>flavida</i>		B		Me (1), Pa (4)	5	2, 18
		<i>urabensis</i>		B		Pa	1	2
		spp.		B		Ba (3), Pa (3)	6	2
	<i>Plexaura</i>	<i>flexuosa</i>		B	B1, B1b	Ba (3), Bb (1), Bz (1), Fl (35), Me (2), Pa (8)	50	2, 6, 7, 9, 21
		<i>homomalla</i>		B,C,B&C	B1, B1a, C1	Ba (3), Bb (1), Fl (2), Me (3), Pa (46)	55	2, 6, 7, 18, 19, 21
		<i>kuna</i>		B	B1	Ba (25), Bz (1), Fl (20), Pa (105), VI (8)	159	2, 4, 5, 6, 9
		<i>Pseudoplexaura</i>	<i>flagellosa</i>	B	B1, B8	Ba (2), Be (1), Fl (3), Me (1), Pa (2)	9	1, 2, 7
		<i>porosa</i>	B		Ba (1), Be (1), Bz (6), Fl (1), Pa (7)	16	1, 2	
		<i>wagenaari</i>	B	B1	Fl (2), Me (2), Pa (1)	5	2, 7, 18	
		spp.	B	B1, B1L	Ba (2), Bb (1), Fl (1), Pa (3), VI (1)	8	2, 9, 21	
Subergorgiidae	<i>Subergorgia</i>	<i>suberosa</i>	C		Gu	1	1	
Tubiporidae	<i>Tubipora</i>	<i>musica</i>	C,D	C1b	Au (3), Is (1)	4	1, 12, 18	
Xeniidae	<i>Anthelia</i>	<i>edmondsoni</i>	A		Ha	8	1	
		<i>glauca</i>	C		Is	3	11	
		spp.	C	C64, C84a	Au	4	1, 3, 12	
	<i>Asterospicularia</i>	<i>laurae</i>	C	C15	Au	2	3	
	<i>Cespitularia</i>	spp.	C	C15, C1q	Au	9	1, 3, 18	
	<i>Efflatounaria</i>	sp.	C	C1c, C1:3a	Au	7	3	
	<i>Heteroxenia</i>	<i>fuscescens</i>	C		Is	13	1, 11	
		spp.	C	C15, C15e, C64	Au	6	3, 12	
	<i>Sarcothelia</i>	sp.	C	C15b	Ha	2	13	
	<i>Sympodium</i>	sp.	D		Au	1	18	
	<i>Xenia</i>	<i>farauensis</i>	C		Is	3	11	
<i>macrospiculata</i>		C		Is	3	11		
<i>umbellata</i>		C		Is	3	11		
spp.		C	C1k, C15, C64	Au (17), Is (11)	28	1, 2, 3, 17, 18		

Sources: 1. This study; 2. Goulet & Coffroth 2004; 3. T. L. Goulet et al. unpubl.; 4. Rowan & Powers 1991a; 5. Rowan & Powers 1991b; 6. Santos et al. 2001; 7. LaJeunesse 2002; 8. LaJeunesse et al. 2003; 9. Santos et al. 2003a; 10. Santos et al. 2003b; 11. Barneah et al. 2004; 12. LaJeunesse et al. 2004a; 13. LaJeunesse et al. 2004b; 14. Pochon et al. 2004; 15. Santos et al. 2004; 16. Kirk et al. 2005; 17. Strychar et al. 2005; 18. van Oppen et al. 2005; 19. Banaszak et al. 2006; 20. Barneah et al. 2007; 21. Searchable Database of *Symbiodinium* Diversity—Geographic and Ecological Diversity (SD2-GED) (www.auburn.edu/~santosr/sd2_ged.htm)

Table 2. Sampling of octocoral species throughout the tropics. In each geographic area, the number of sites and the number of octocoral genera sampled are given. Total N: total number of colonies sampled in each country

Area	No. of sites	No. of genera	Total N
Australia	27	31	222
Bahamas	17	8	812
Barbados	5	9	34
Belize	5	5	19
Bermuda	1	4	5
Fiji	1	5	40
Florida Keys	16	9	195
Guam	5	6	24
Hawaii	1	3	17
Israel	1	13	112
Japan	2	2	3
Mexico	3	11	44
Panama	9	11	305
Tortugas	1	1	8
US Virgin Islands	2	7	16

We determined the molecular weight of the RFLP bands by either (1) scanning negatives of the RFLP gels into a computer and using the program NCSA GelReader 2.0.5 (NCSA, University of Illinois) or (2) obtaining and analyzing a digital image of the gel using the KODAK Electrophoresis Documentation and Analysis System (EDAS).

Data analysis. We combined our data with what is currently known in the literature to generate a data set of *Symbiodinium* clade identities in 117 tropical octocoral species (see references in Table 1). We only used data obtained from field-collected samples and not from zooxanthella cultures. Culturing is highly selective, and the zooxanthellae in culture may not represent the dominant *Symbiodinium* clade found within a host species (Goulet & Coffroth 1997, Santos et al. 2001, LaJeunesse 2002). If a report did not specify the sample size, we conservatively assigned an N of 1 to those data.

If an octocoral was not identified to the species level, we listed the genus and a 'sp.' notation. To be conservative, we did not treat each genus level report as a different species. Instead, we combined these reports, listing all such reports as 'spp.' Such an approach may have underestimated the number of species hosting a single *Symbiodinium* clade; for example, if in 3 reports about species within a given genus, 2 documented clade B and 1 documented clade C, we reported this as spp. hosting multiple clades ('B&C'). Octocorals were classified as hosting multiple *Symbiodinium* clades if individuals from that octocoral species hosted 2 or more clades either at different depths on the same reef, on different reefs, in different geographic locations, or within the same colony.

When available, we incorporated data on *Symbiodinium* types within zooxanthella clades. However, *Symbiodinium* types are distinguished using different parts of the genome: ITS1 and ITS2 (e.g. LaJeunesse 2001), ITS2 (van Oppen et al. 2001), and 23S rDNA of chloroplasts (Santos et al. 2003a). Furthermore, researchers have used different nomenclature for within-clade distinction (LaJeunesse 2001, van Oppen et al. 2001, Santos et al. 2003a). We chose the nomenclature of LaJeunesse (2001), since most biogeographic within-clade data in octocorals use this nomenclature. For 3 types published by other researchers we were able to 'translate' because an equivalent was provided. Type B184 is the same as LaJeunesse type B1 (Kirk et al. 2005). Sub-clade C1 and C2 used by van Oppen et al. (2001) are equivalent to LaJeunesse type C1 and C3i, respectively (LaJeunesse et al. 2003). If we could not identify the type according to LaJeunesse's nomenclature, we did not list it.

GIS data analysis. We entered data in tabular format to create a Geographic Information System (GIS) map using ArcMap (version 8.3, ESRI). The GIS included a world map as the primary base layer. A geographic coordinate system was used for the spatial component of the samples. The latitude and longitude coordinates of the samples were converted to decimal format. The data were plotted to reveal any spatial patterns resulting from clade distributions. Feature map creation of specific octocoral attributes involved querying the main coral database and sub-setting the queried features as an individual layer. Once a sub-layer containing any specific coral attribute was created, these data were then joined with the world map and global reference layer to produce a spatial distribution map. The subset process was repeated for all additional queried features. Within each of the 15 geographic areas, multiple sites were sampled (Table 2). Due to close geographic proximity, for the map presentation (see Figs. 1 & 2), we pooled the data from all sites into one geographic location. Since the sites from the Great Barrier Reef (Australia) were from different latitudes, we grouped sites in close proximity, but we still delineated 3 sites within the Great Barrier Reef. Of the 117 octocoral species, 47 species were sampled from more than 1 geographic location enabling latitudinal and longitudinal geographic comparison of *Symbiodinium* clades in conspecifics.

RESULTS

By combining our data with those found in other sources, we were able to compare the *Symbiodinium* clade identity in 117 species of octocorals belonging to 46 genera and 15 families (Fig. 1, Table 1). These octo-

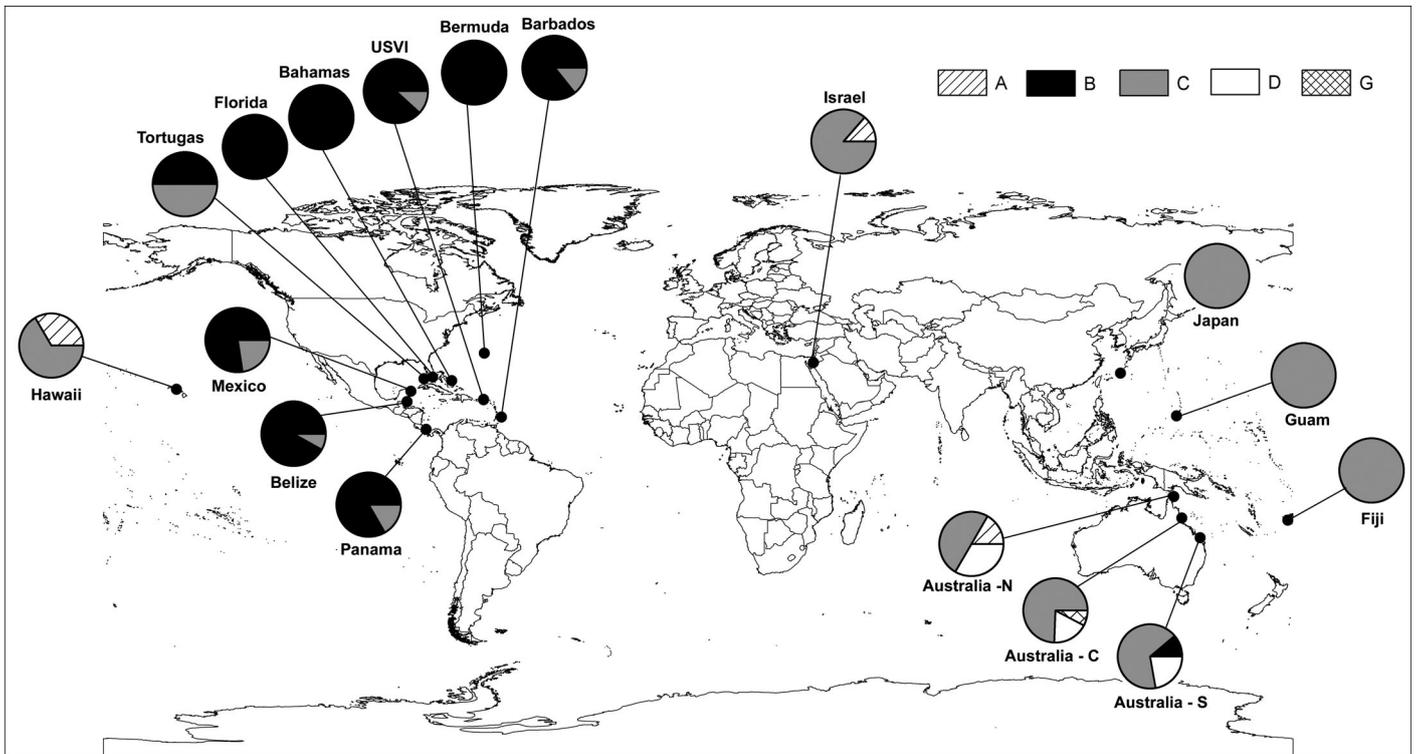


Fig. 1. Global distribution of *Symbiodinium* clades in tropical octocorals. Pie charts represent the percent of each clade at each geographic location. Number of sites sampled at each geographic location, number of genera sampled, and total number of samples are listed in Table 2. USVI: US Virgin Islands

corals were collected from reefs near 15 locations worldwide (Table 2). Because many of the samples were collected while surveying reefs, sample sizes per species ranged from 1 to 767 (Table 1).

The majority of the octocoral species sampled (87.2%) harbored a single detectable *Symbiodinium* clade (Table 1, Fig. 1). For 63 octocoral species, within-clade data are available using the nomenclature of LaJeunesse (2001; Table 1). In total, 13 *Symbiodinium* B types were found in octocorals, with type B1 being the dominant type, occurring in 31 of 32 species for which the B type is known. Within *Symbiodinium* C, 18 types were found in 35 species for which the C type is known. In 15 of these 35 species, *Symbiodinium* C1 occurred. Only 2 types were reported from clade D (D1a and D3). Of the 15 species that hosted multiple zooxanthella clades, 8 species hosted *Symbiodinium* B and C, with 3 species hosting *Symbiodinium* B and C simultaneously within the same colony; 4 species hosted C and D; 1 species hosted C and G; 1 species hosted A, C, and D; and 1 species hosted zooxanthellae from clades A, B, C, or D.

To determine if cladal specificity occurred at the family level, we compared zooxanthella clades in 5 families in which 10 or more species were sampled. In

the family Alcyoniidae, all 23 species sampled hosted *Symbiodinium* C, with 2 species also hosting *Symbiodinium* D. For 8 of the species, the *Symbiodinium* type is known, and some of these types (C3j, C71a) were found exclusively in the Alcyoniidae. In the family Gorgoniidae, 17 of 21 species hosted *Symbiodinium* B, with 3 of these also hosting *Symbiodinium* C. Of these 17 species, within-clade identity is known for 15 species, all of which hosted type B1 with some hosting additional B types (Table 1). The remaining 4 gorgoniid species hosted *Symbiodinium* C. In the 11 Nephtheidae species, 6 hosted only *Symbiodinium* C, 2 hosted *Symbiodinium* A, and 3 hosted multiple zooxanthella clades (A, B, C, D, or G; Table 1). We had within-clade data for 6 Nephtheidae species. Four of these species hosted clade C1:2. In 29 species sampled in the Plexauridae, 25 species hosted only clade B zooxanthellae, and 3 species hosted clades B and C. *Euplexaura nitnigi* was the only plexaurid sampled from the Indo-west Pacific, and it hosted only clade G (Table 1). *Symbiodinium* type was known for 14 plexaurid species. All 14 species hosted type B1, and 5 of these had an additional B type. For the 3 species hosting *Symbiodinium* C, type C1 occurred in all of them. In the 14 Xenidiidae species, 12 species hosted *Symbio-*

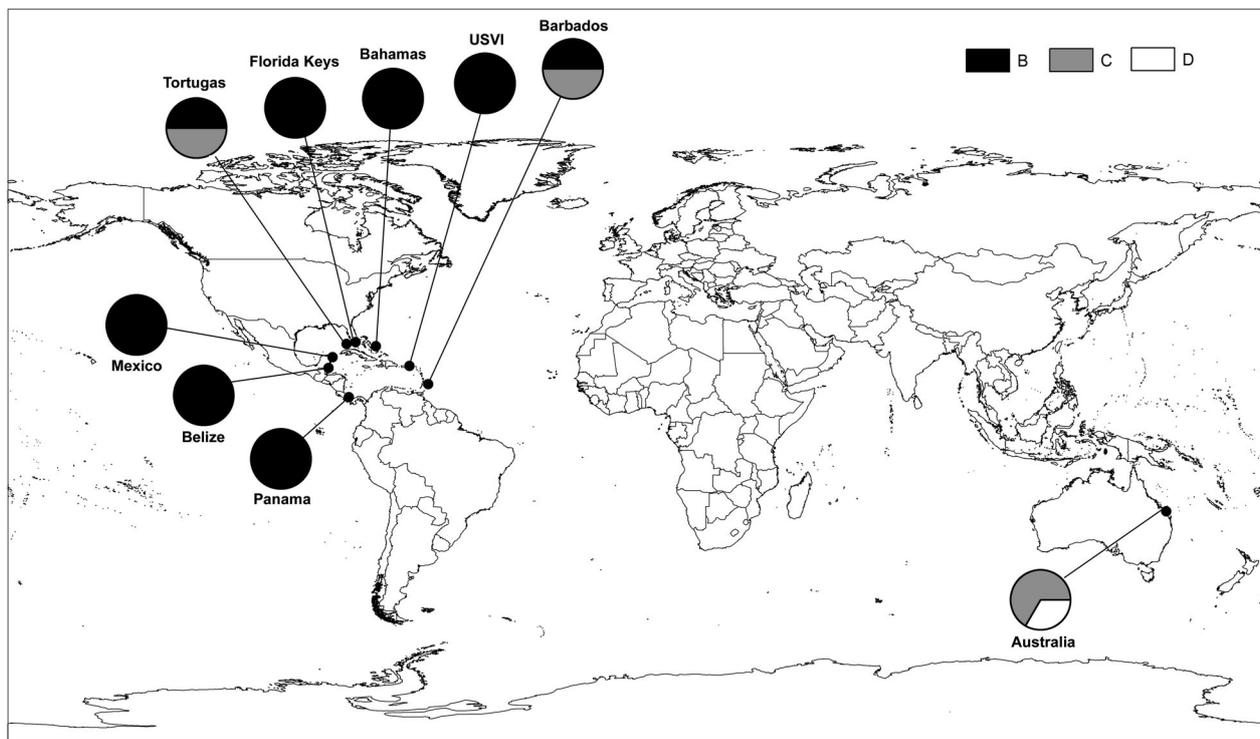


Fig. 2. Distribution of *Symbiodinium* clades in the family Briaridae. USVI: US Virgin Islands

dinium C while *Anthelia edmondsoni* hosted clade A and *Sympodium* sp. hosted clade D zooxanthellae (Table 1). *Symbiodinium* types C1k, C1q, and C84a have been reported only from the Xenidae (LaJeunesse et al. 2004, T. L. Goulet et al. unpubl.).

Comparing zooxanthella clades in octocorals throughout the tropics revealed a bio-geographic pattern (Fig. 1, Table 1). Octocorals in multiple sites in the Indo-west Pacific (Great Barrier Reef [GBR], Guam, Fiji, and Japan), Red Sea (Israel), and Pacific (Hawaii) hosted predominantly clade C zooxanthellae (Fig. 1). *Symbiodinium* A was found in octocorals sampled from Hawaii, Israel, and the GBR, while *Symbiodinium* D and G were only found in GBR octocorals (Fig. 1). Octocorals in the Caribbean and Bermuda hosted predominantly *Symbiodinium* B.

Certain *Symbiodinium* types were distributed globally while others exhibited a limited distribution (Table 1). *Symbiodinium* type B1 was very common in octocoral species in Bermuda and the Caribbean for which type data are available. *Symbiodinium* type C1 and C3 were found both in the Caribbean and the Indo-west Pacific, but in the Caribbean these are the only C types, while the Indo-west Pacific has numerous C types.

In total, 47 octocoral species were sampled from 2 or more geographic locations (Table 1). In the majority

(76.6%) of these species, conspecifics hosted the same zooxanthella clade in all different geographic locations, both in latitudinal and longitudinal comparisons (Table 1). For example, *Sinularia polydactyla* hosts *Symbiodinium* C in Australia, Guam, and Israel. *Gorgonia mariae* hosts *Symbiodinium* B, type B1, in the Bahamas, Barbados, Mexico, and Panama. We compared the zooxanthella clades in the family Briaridae from samples obtained in the Caribbean and the Indo-west Pacific. We found that Briaridae in the Caribbean hosted predominantly *Symbiodinium* B (92.8%) with only 7 of 98 colonies hosting *Symbiodinium* C (Fig. 2). Briaridae colonies sampled in the Indo-west Pacific hosted *Symbiodinium* C with *Briarium stechei* only hosting *Symbiodinium* D (Fig. 2).

DISCUSSION

Octocorals are an important constituent of many coral reefs, and yet few studies have focused on *Symbiodinium* clade identity in octocorals (Goulet & Cofroth 2003a,b, 2004, Santos et al. 2003b, 2004, Barneah et al. 2004, van Oppen et al. 2005). The data compiled in this study enabled a worldwide biogeographic synthesis of *Symbiodinium* clades in tropical octocoral species.

Specificity of *Symbiodinium* clades in octocorals

Most (87.2%) of the 117 zooxanthellate octocoral species sampled worldwide hosted a single detectable *Symbiodinium* clade. Although for some of the octocoral species only small sample sizes are currently available, the samples are probably a true representation of the clades present in these species. This conclusion is based on multiple lines of evidence. First, octocorals hosting multiple clades were identified as such with sample sizes of 2 to 3 individuals (Table 1). This detection concurs with the fact that in scleractinian corals, sample sizes of 5 or less detect 87.5% of the scleractinian coral species hosting multiple clades (Goulet 2007). Furthermore, increasing sampling effort does not change the finding that the vast majority of octocorals host a single zooxanthella clade (Baker & Romanski 2007). Second, the clade identity of zooxanthellae in octocoral species is compiled from multiple studies conducted on different reefs and different geographic locations (see Table 1 for references). The vast majority of scleractinian coral species also host a single *Symbiodinium* clade (LaJeunesse 2002, Baker 2003, Goulet 2006, 2007). Of the 12.8% of octocoral species that hosted more than 1 zooxanthella clade, only *Eunicea clavigera*, *E. tournerforti*, and *Plexaura homomalla* hosted multiple clades simultaneously within the same colony. Octocorals hosting a single zooxanthella clade may not be able to change that clade even if new environmental conditions arise (Goulet 2006).

Hosting only one *Symbiodinium* clade demonstrates a degree of specificity. Furthermore, octocoral taxonomic affinity exists between an octocoral family and zooxanthella clade. For example, all 23 species sampled from the family Alcyoniidae, from 6 geographic locations, host *Symbiodinium* C with 2 species also hosting *Symbiodinium* D (Table 1). In the 21 species sampled from the Gorgoniidae, 18 species were sampled from up to 5 sites throughout the Caribbean, and 17 of them hosted *Symbiodinium* B (Table 1). Our results concur with Goulet & Coffroth's (2004) description of host taxonomic affinity in both octocorals and scleractinian corals within the Caribbean. Specificity may also occur at the sub-cladal level. For example, the Alcyoniidae was the only family to host *Symbiodinium* type C3j. In scleractinian corals, pocilloporids have the same *Symbiodinium* type (C1c) throughout their geographic range in the GBR, Hawaii, and eastern Pacific (LaJeunesse et al. 2003).

Cladal specificity in certain species has been attributed to the mode of symbiont transmission. In a study in the Red Sea, all octocoral species that had maternal symbiont transmission of zooxanthellae (brooders with vertical transmission) hosted *Symbiodinium* A while the species with environmental acquisition (horizontal

transmission) hosted *Symbiodinium* C (Barneah et al. 2004). For 2 of the 3 genera that brood, *Nephthea* and *Stereonephthya*, we now have clade identity data for samples from the GBR and Fiji. Unlike the samples collected in the Red Sea that only associated with clade A symbionts, *Nephthea* in the GBR hosts *Symbiodinium* B, C, or D (van Oppen et al. 2005, T. L. Goulet et al. unpubl.), and *Stereonephthya* hosts *Symbiodinium* C or G in the GBR (van Oppen et al. 2005, T. L. Goulet et al. unpubl.) and *Symbiodinium* C in Fiji (current study). Mode of transmission may therefore not explain specific *Symbiodinium* in octocorals. Lack of correlation between transmission mode and zooxanthellae was also observed in *Montipora* spp. and *Acropora* spp. in Australia and Indonesia (van Oppen 2004).

Biogeography of *Symbiodinium* clades in octocorals

Zooxanthellae in octocorals exhibit a worldwide biogeographic pattern. For example, octocorals from the Indo-west Pacific hosted predominantly clade C, which is also the dominant clade in scleractinian corals of that region (LaJeunesse et al. 2004a). In octocorals, the GBR displayed the highest *Symbiodinium* variability (A, B, C, D, and G) compared to anywhere else currently sampled in the Indo-west Pacific. In Fiji, Guam, and Japan, octocorals hosted only clade C (Fig. 1, Table 1). Rodriguez-Lanetty et al. (2001) also found higher cladal variability in Australian samples of the scleractinian coral *Plesiastrea versipora*.

Symbiodinium A zooxanthellae occurred in 1 octocoral species in the GBR (van Oppen et al. 2005). Clade A also associated with 3 octocoral species sampled in the Red Sea (Barneah et al. 2004, this study). In Hawaii, *Symbiodinium* A occurs in 1 octocoral species (LaJeunesse et al. 2004b, this study) out of the 4 zooxanthellate octocoral species (Fabricius & Alderslade 2001). In the GBR, *Symbiodinium* B was represented in 1 species, and *Symbiodinium* G was found in 3 octocoral species that have previously been assumed to be azooxanthellate (van Oppen et al. 2005).

Based on octocoral samples collected from the GBR, van Oppen et al. (2005) concluded that *Symbiodinium* D is a common clade in octocorals in the Indo-west Pacific. Some types of *Symbiodinium* D have been implicated as being 'heat tolerant' (Rowan 2004, Berkelmans & van Oppen 2006, LaJeunesse et al. 2007) and therefore of potential importance during climate change. The current study shows that although clade D zooxanthellae occur in 24.4% of GBR octocorals (11 of 45 species sampled), it has not been documented in octocorals in the other 4 sites sampled in the Indo-west Pacific and the Red Sea. Furthermore, in a study conducted during a bleaching event in the GBR,

bleached and unbleached colonies of octocoral species hosted the same *Symbiodinium* clade and types within a clade, and *Symbiodinium* type did not explain bleaching susceptibility among species (T. L. Goulet et al. unpubl.). In octocorals, therefore, species survivorship in changing environmental conditions may rely on aspects of the symbioses other than the zooxanthella clade present. For example, bleaching susceptibility may be a consequence of differences in host tolerance (Loya et al. 2001, Brown et al. 2002, Bhagooli & Hidaka 2004)

Similar to octocorals in the Indo-west Pacific, 1 *Symbiodinium* clade was most prevalent in Caribbean octocorals. Unlike in the Indo-west Pacific where octocorals hosted predominantly clade C, the majority of the 48 Caribbean octocoral species and all Bermuda octocorals hosted clade B zooxanthellae. In the Caribbean, most octocorals (83.3%) exclusively hosted clade B while 8 species hosted clade B, clade C, or both simultaneously. In the Caribbean, only 1 of the octocoral species sampled, *Plexaurella dichotoma*, hosted *Symbiodinium* C exclusively. In contrast, the dominant *Symbiodinium* clade of scleractinian corals in the Caribbean is clade C (see Goulet & Coffroth 2004 for references). In addition, Caribbean scleractinians host clades A and D, neither of which has been documented in Caribbean octocorals. The occurrence of clade A and B zooxanthellae in the Caribbean may have evolved in the Plio-Pleistocene transition (3.5 to 1.5 million years ago) when cooler temperatures and increased seasonality may have occurred (Baker 2003, LaJeunesse et al. 2003). Why clade B became the dominant clade in octocorals, and clade A is not found at all within Caribbean octocorals, remains to be determined.

Corals found over a wide geographic range, either latitudinal or longitudinal, may host different zooxanthella clades over their range, aiding survival in the different environments (LaJeunesse & Trench 2000, Loh et al. 2001, Rodriguez-Lanetty et al. 2001). Our study includes data on the *Symbiodinium* clade(s) in 7 octocoral species across a latitudinal range in the West-Pacific. Most of these octocorals (5 of 7) did not display a latitudinal difference in zooxanthellae, hosting only *Symbiodinium* C from samples as far apart as Japan and Australia. On the other hand, the genus *Stereonephthya* hosted *Symbiodinium* C and G in Australia, *Symbiodinium* C to the north in Fiji, and *Symbiodinium* A in the Red Sea. The scleractinian coral *Plesiastrea versipora* also exhibits variability in Australian samples, hosting *Symbiodinium* B and C (Rodriguez-Lanetty et al. 2001) although it only hosts *Symbiodinium* C in Japan (Rodriguez-Lanetty & Hoegh-Guldberg 2003).

Our study also included longitudinal data on 10 octocoral species that were sampled both in the Red Sea

(Israel) and in the GBR, Fiji, or Guam. Eight of the species hosted only *Symbiodinium* C across this wide range. The exceptions were *Nephthea* spp., which hosted clade A in the Red Sea and *Symbiodinium* B, C, and D in the GBR, and *Tubipora musica*, which hosted clade C in the Red Sea and *Symbiodinium* C and D in the GBR. Longitudinal variability has been reported for the zoanthid *Palythoa caesia*, which hosts only *Symbiodinium* C in the west of the Indian Ocean, while in the eastern Indian Ocean it occurs with either *Symbiodinium* C or D (Burnett 2002).

***Symbiodinium* types in octocorals**

At the sub-clade level, some *Symbiodinium* types were widespread while others were confined to a specific geographic location. *Symbiodinium* types C1 and C3 inhabited octocorals in the Caribbean and Indo-west Pacific. Types C1 and C3 also associate with multiple scleractinian coral species on reefs in the Caribbean and Indo-west Pacific (LaJeunesse 2002, LaJeunesse et al. 2003, 2004a) and are considered to be generalists and ancestral to other, more specialized, C types (LaJeunesse et al. 2003, 2004a, LaJeunesse 2004). B1 was ubiquitous throughout the Caribbean and Bermuda in octocorals, while the other B types were restricted to particular octocoral species (LaJeunesse 2002, 2004). Certain scleractinian coral species also form symbioses with highly specific *Symbiodinium* types (LaJeunesse et al. 2003, 2004a).

Although there are more scleractinian coral species in the Indo-Pacific compared to the Caribbean, the diversity of *Symbiodinium* types is higher in the Caribbean compared to the Indo-Pacific (LaJeunesse et al. 2003, 2004a). In octocorals, as in soritid foraminiferans (Pochon et al. 2004), this is not the case. The symbioses of Caribbean octocorals is dominated by types B1 and C1 (for the few C symbioses). In the Indo-west Pacific, there are many C types in symbioses with octocorals. Why octocorals differ from scleractinian corals requires further investigation and may prove pivotal in differential survivorship as climate change continues.

Taxonomic versus geographic differences in *Symbiodinium* clades in octocorals

Differences in zooxanthella clades found in octocorals in the different geographic locations may be a result of geographic or taxonomic differences. It is difficult to uncouple the two, since taxonomically different hosts exist in the different major geographic areas. In the Caribbean, 2 families (Plexauridae and Gorgoniidae) dominate (Sánchez et al. 2003), whereas in the

Indo-Pacific, 23 different families occur (Fabricius & Alderslade 2001). The geographic difference in octocoral family diversity may explain the difference in *Symbiodinium* clade and type diversity in octocorals from these areas (van Oppen et al. 2005).

Very few species are cosmopolitan, making global comparisons difficult. The family Briareidae is represented from 8 locations in the Caribbean, and from the GBR in the Indo-west Pacific (Fig. 2). Briareids are capable of associating with *Symbiodinium* C in both oceans, but the proportion of colonies that do so differ between the oceans. The majority of Briareidae sampled in the Caribbean hosted *Symbiodinium* B (92.8%), while in the GBR they hosted *Symbiodinium* C and D (Fig. 2). In addition, the Gorgoniidae in the Caribbean hosted only clade B while in Australia they hosted only clade C. On the other hand, Anthothelidae associated with *Symbiodinium* C in the Caribbean and in the GBR even though *Symbiodinium* C is not a common clade in octocorals in the Caribbean. The 1 representative of the Plexauridae from the Indo-west Pacific, *Euplexaura nuttingi* sampled in the GBR, was the only plexaurid to host *Symbiodinium* G. In the Xenidiidae, *Symbiodinium* A was restricted to the 1 species collected from Hawaii. Additional comparisons between cosmopolitan families will aid in determining the influence of geography versus phylogeny in these symbioses.

The importance of *Symbiodinium* clade-level resolution for understanding cnidarian-algal symbioses

Using differences in DNA between different *Symbiodinium* has enabled researchers to address previously inaccessible questions about cnidarian-zooxanthella symbioses. These questions are from broad biogeographic analyses (e.g. this study) to differences between individual *Symbiodinium* genotypes (Goulet & Coffroth 2003a,b) with the full range of resolution in between. For every level, there are appropriate molecular techniques, with new techniques being adopted and modified. Differences in DNA encoding for the ssRNA categorized *Symbiodinium* into clades (Rowan & Powers 1991a). Other parts of the genome such as lsRNA (Baker & Rowan 1997, Pochon et al. 2004), the 23S rDNA of chloroplasts (Santos et al. 2002, Pochon et al. 2006), *psbA* minicircle (Barbrook et al. 2006), and mitochondrial sequences (Takabayashi et al. 2004) have confirmed the clade distinction.

Attempts have been made to find clade-level physiological differences between *Symbiodinium*. Comparison of *Symbiodinium* physiology within a clade from different host species revealed as much variability

within a clade as between clades (Savage et al. 2002, Tchernov et al. 2004). On the other hand, if 2 different *Symbiodinium* clades are compared within the same host species, physiological differences emerge. For example, clade D zooxanthellae are more tolerant of elevated seawater temperatures and/or irradiance compared to clade C zooxanthellae in the scleractinian corals *Pocillopora* spp. (Rowan 2004, LaJeunesse et al. 2007) and *Acropora millepora* (Berkelmans & van Oppen 2006). Clade A zooxanthellae withstand elevated seawater temperatures better than clade B zooxanthellae in the sea anemone *Aiptasia pallida* (Goulet et al. 2005). Juveniles of the scleractinian corals *Acropora millepora* and *A. tenuis* grow at different rates if they are inhabited by clade C or D (Little et al. 2004). Therefore, although it appears that one cannot generalize about physiologies of *Symbiodinium* within a given clade, in a particular host-symbiont genotypic combination, the physiology differs depending on the zooxanthella clade involved (Goulet et al. 2005).

The ability to change symbionts has also been documented at the clade level. Cnidarians that are capable of hosting multiple zooxanthella clades may, in response to environmental conditions, change the proportion of each clade (symbiont shuffling). For example, *Acropora millepora* shuffles between clades C and D (Berkelmans & van Oppen 2006). The use of new molecular techniques such as real-time PCR has increased our detection of clades within cnidarian species capable of hosting multiple clades (Mieog et al. 2007). It still remains to be determined whether cells detected using molecular techniques are identifying zooxanthellae in symbiosis, or zooxanthella contaminants found externally and/or not in symbiosis (Loram et al. 2007). The clade-level resolution, although broad, provides important information on cnidarian-zooxanthella symbioses. However, the examples above deal with scleractinian corals and sea anemones pointing to the paucity of data on octocorals. The current study contributes to our knowledge of zooxanthella clades in octocorals by addressing the fundamental question of zooxanthella clade identity in octocorals in different geographic locations.

CONCLUSIONS

Understanding the symbioses between octocorals and zooxanthellae is crucial to understanding coral reefs. Octocorals associate with 5 *Symbiodinium* clades (A, B, C, D, and G) while clade F found in scleractinians (Baker 2003) has not been documented in octocorals to date. In the Indo-west Pacific, Red Sea, and Hawaii, octocoral species predominantly associate with *Symbiodinium* C, which is also the dominant

zooxanthella clade in Indo-west Pacific scleractinian corals. In the Caribbean and Bermuda, octocorals predominantly associate with *Symbiodinium* B, which is not the dominant clade found in scleractinian corals. Deciphering similarities and differences between the symbioses of octocorals and scleractinian corals and their zooxanthellae may aid in predicting coral species survivorship vis-à-vis global climate change.

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