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Diversity of the Symbiotic Alga *Symbiodinium* in Tanzanian Scleractinian Corals

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Abstract—With the current increase in frequency of coral bleaching events, knowledge on the genetic diversity of symbiotic algae in the genus *Symbiodinium* harboured by reef-building corals is important to understand how coral reefs will respond to global climate change. This study was undertaken as very little is known about the subject in reef-building corals in Tanzania and yielded information on the genetic diversity of *Symbiodinium* in 66 scleractinian coral species from 19 common genera. The internal transcribed spacer two (ITS-2) region in nuclear ribosomal DNA (rDNA) was used to identify the symbiont types following polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and sequencing. ITS-2 *Symbiodinium* types from clades A, C and D, previously reported in corals from other regions, were detected. While ITS-2 sequences from *Symbiodinium* clade C varied significantly, those from clades D and A did not. Most reef-building corals were found to be poor at forming symbioses with multiple symbiotic algae, indicating low adaptability to environmental change. This study followed ten years after the 1998 coral bleaching event and thus provided information needed for studies on temporal changes in *Symbiodinium* diversity in Tanzanian corals, especially in relation to large-scale bleaching events.

INTRODUCTION

Reef-building corals (Scleractinia) are known to host unicellular photosynthetic algal (dinoflagellate) symbionts in the genus *Symbiodinium*, commonly called zooxanthellae, within the vacuoles of their gastrodermal cells. These dinoflagellates are now known to play an important role in both the physiology and ecology of coral reefs through their contribution to the host's nutrition and capacity to deposit calcium carbonate and build reefs in shallow, oligotrophic marine environments (Muscatine, 1990). Unfortunately, the past few decades have witnessed an increase in episodes of coral bleaching which is associated with a breakdown in the coral-dinoflagellate relationship (Hoegh-Guldberg, 1999). This is induced by stresses resulting from exposure to high temperatures and insolation (Douglas, 2003). It results in the disappearance of colour from the corals (bleaching), either due to degradation of the algal pigments and/or partial to total loss of the host's *Symbiodinium* population (Hoegh-Guldberg, 1999; Douglas, 2003).

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Growing physiological and ecological evidence shows that various *Symbiodinium* types render corals different levels of resistance to coral bleaching (Kinzie *et al*., 2001; Sampayo *et al*., 2008). Therefore, symbiosis recombination is a potentially important mechanism by which corals adapt to changing climate (Rowan, 2004). Thus, knowledge on the genetic diversity of *Symbiodinium* harboured by reef corals within a particular region could help in predicting the severity of bleaching events. Although Tanzania is amongst the countries blessed with a high diversity of reef-building corals (>250 species; Obura *et al*., 2004), very little is known about the associated diversity of *Symbiodinium*. Tanzanian material was considered in two studies undertaken by Macdonald *et al*. (2008) and LaJeunesse *et al*. (2010). In the former, symbionts types were examined in only a single coral species, *Stylophora pistillata*, collected at two sites in the Tanzanian coast. LaJeunesse *et al*. (2010), on the other hand, surveyed numerous host taxa but only at sites around the island of Zanzibar. More comprehensive work was needed and, in this study, the genetic diversity of *Symbiodinium* types was assessed in 66 reef-building coral species collected at five locations along the Tanzanian coast.

The ITS-2 genetic marker provides enough resolution to differentiate *Symbiodinium* types that are ecologically separated, while being sufficiently conserved to enable comparison within communities (LaJeunesse, 2001, 2003). The resulting ITS-2 diversity represents the natural biological variations, which in turn represent differences in function (LaJeunesse, 2001). This is probably the reason that the current classification of *Symbiodinium* is largely based on this region of the rDNA (Ulstrup *et al*., 2007; Goulet *et al*., 2008). It was the marker employed in this study.

Figure 1. Map of Tanzanian coast with sampling sites for this study.

MATERIALS and METHODS

Sampling

Samples of reef-building corals were collected at five locations along the Tanzanian coast (Figure 1). Coral fragments were collected at Changuu on Zanzibar in April 2008 and on mainland and Mafia reefs (Tanga mjini, Dar es Salaam, Mafia, and Mtwara) in August 2008. Small (1-3 cm), healthy coral fragments were collected at 1-7 m by snorkelling or SCUBA diving on reefs where coral species diversity was high. Juveniles were excluded to avoid spurious results introduced by the horizontal zooxanthellar transmission strategy used by a large number of coral species in Tanzania while young (Coffroth *et al*., 2001). In this process, zooxanthellar acquisition is initially non-selective but ontogenic changes result in a shift to dominant zooxanthellar strains (Coffroth *et al*., 2001; Stat *et al*., 2008). Photographs were taken of each parent colony before breaking a small fragment for the sample. The photographs were compared with those in Veron (2000) for identification. The coral fragments were preserved in a 20% dimethyl sulfoxide (DMSO), 0.25 M ethylenediaminetetraacetic acid (EDTA) and saturated sodium chloride (NaCl) solution. The samples were shipped to Pennsylvania State University for analysis.

DNA extraction and Polymerase Chain Reaction

DNA extractions were performed on the fragments using a non-toxic protocol modified from LaJeunesse *et al.* (2003). A small piece of skeleton and tissue was combined with glass beads (Ceroglass, Columbia USA) and 600 µl of a cell lysis solution (0.2 M Tris, 2 mM EDTA, 0.7% SDS, pH 7.6), and shaken at high speed using a BioSpec beadbeater. The extract was then incubated with proteinase K (20 mg/ml) at 65°C for 1 h. After incubation, proteins were precipitated from the solution using ammonium acetate (9 M) and the sample was stored at -20°C overnight. The frozen extract was centrifuged (10 000 g for 15 min), and the supernatant was removed and placed in a new tube. The DNA was precipitated from the solution with 100% isopropanol, and again centrifuged (10 000 g for 5 min). The DNA pellet was then washed with 70% ETOH, air dried, re-suspended in 75 µl of distilled water and stored at -20°C.

Polymerase Chain Reaction (PCR) amplification of *Symbiodinium* DNA targeted the internal transcribed spacer two (ITS-2) region of the ribosomal DNA (rDNA) (LaJeunesse, 2001). The forward primer (ITSintfor2, 5'GAATTGCAGA ACTCCGTG 3') with GC clamp, and the reverse primer (ITSintrev2, 5' GGGATCCAT ATGCTTAAGTT CAGCGGGT 3') designed by LaJeunesse and Trench (2000), were used for PCR amplification under the following cycling conditions: a denaturing step of 4 min at 94°C, 30 cycles at 94°C for 60 s, 57°C for 60 s, and 72°C for 60 s, with an extension step of 5 min at 72°C. The PCR products were viewed on 1.5% agarose gels stained with ethidium bromide.

DGGE analysis and sequencing of the PCR products

The amplified ITS-2 products were mixed with gel loading dye (Biorad DCode Dye Solution) at a ratio of 1:1 before being loaded on the DGGE gels, 10 µl of each sample being loaded into wells in 8%, 1 mm thick acrylamide gels (16 cm) with an internal gradient of 45 to 90% denaturants (formamide and urea). One of the wells was loaded with a marker (PCR product of already known symbiont type). Gels were run at 100 V for 15 hours, after which they were stained for 20-30 min using SYBR Green (Amresco, diluted ten times from stock) and visualized using a UV-Doc digital camera gel imaging system. Gels were scored, based on unique banding profiles which included the entire complexity of bands present in the profile. Dominant bands in the DGGE gel profiles were carefully cut out and diluted overnight in 500 µl dH2O and re-amplified on the following day using ITS2 with no GC clamp. The products of the re-amplified excised bands were purified using an UltraClean PCR purification Kit (Molecular Biology Laboratories, USA). Purified DNA products were sequenced using forward (ITSintfor2) and reverse (ITS2reverse) primers in separate runs at the Pennsylvania State University Science Facility. Sequences were then identified using sequences published in gene banks.

RESULTS

Many of the DGGE profiles were complex due to the presence of several minor bands (Figure 2), a finding reported in similar studies (Silverstein *et al*., 2011). Therefore, it was not easy to directly determine the number of *Symbiodinium* types in each sample. After careful excision of the bands and re-amplification of the PCR products, it was found that most of the minor bands were attributable to heteroduplexes resulting from intragenomic variations (Thornhill *et al*., 2007; Sampayo *et al*., 2009).

Of the 505 samples analysed, 22 distinct ITS-2 *Symbiodinium* types in three clades (A, C, and D) (Figure 3) were widely distributed with in the 66 coral species examined (Table 1) within 19 genera (Figure 4). Clade C was the most frequently encountered algal type in all of the coral species sampled. Further analysis revealed that ITS-2 *Symbiodinium* C3u was the most common as it was found in ten (*Acropora, Pavona, Echinopora, Favites, Goniastrea, Platygyra, Fungia, Galaxea,*

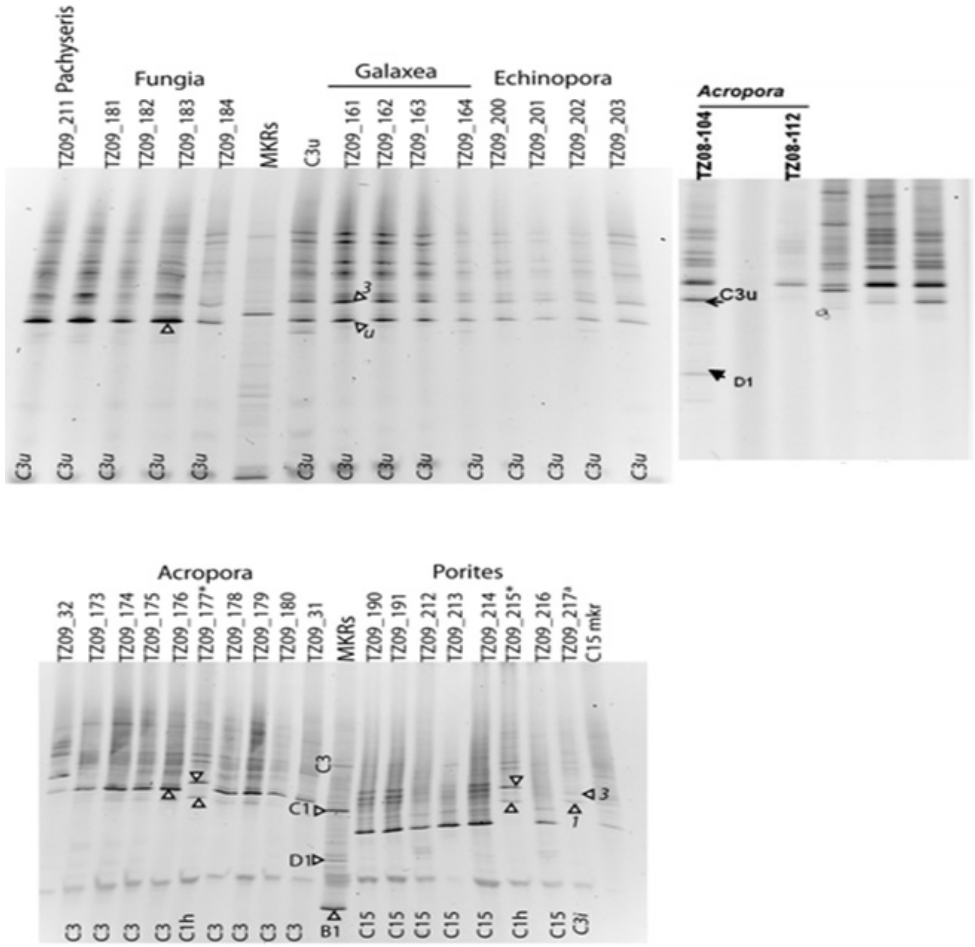

Figure 2. PCR-DGGE profiles of *Symbiodinium* types found in reef-building corals along the Tanzanian coast. The clade type is denoted by letters in uppercase and the ITS-2 DGGE fingerprint type by a number followed by letters in lower case.

Echinophyllia, and *Oxypora*) of the 19 coral genera. C3z was the second most common ITS-2 *Symbiodinium* and was found in the genera *Acropora, Goniastrea, Platygyra* and *Montastraea* (Table 1). C1b-s was also found to be a generalist, inhabiting the genera *Leptastrea, Plesiastrea* and *Goniopora*. Unlike clades C and D, *Symbiodinium* clade A (A15 and A16) were detected only in the fire coral, *Millepora*. Most of the *Symbiodinium* ITS-2 types detected manifested a high degree of generic specificity. For example, C17, C1b, C105, C15, C1h and A15 were found respectively only in the genera *Montipora, Plesiastrea, Stylophora, Porites, Pocillopora* and *Millepora*.

The majority of coral colonies harboured a single *Symbiodinium* type (Figure 1). However, a few colonies had multiple symbionts, e.g. *Acropora* TZO8-104 (Figure 2). Of the 19 coral genera studied, eight (42%) had the ability to establish symbioses with multiple symbionts (Figure 3). Analysis at the species level revealed that 31% of the corals had multiple *Symbiodinium* types, the genus *Acropora* accommodating the greatest number (types C3u, C3z, C109a, C109b, C115, and D1a; Figure 4).

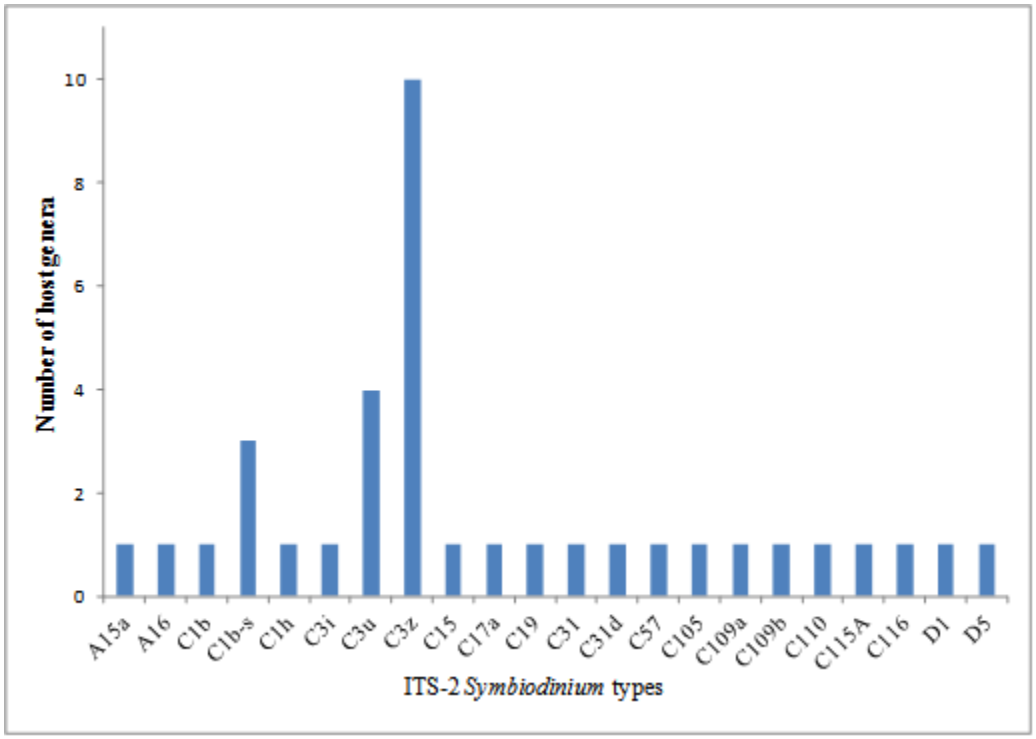

Figure 3. Diversity and prevalence ITS-2 *Symbiodinium* types in Tanzanian reef-building corals. The clade type is denoted by letters in uppercase and the ITS-2 DGGE fingerprint type by a number followed by letters in lower case.

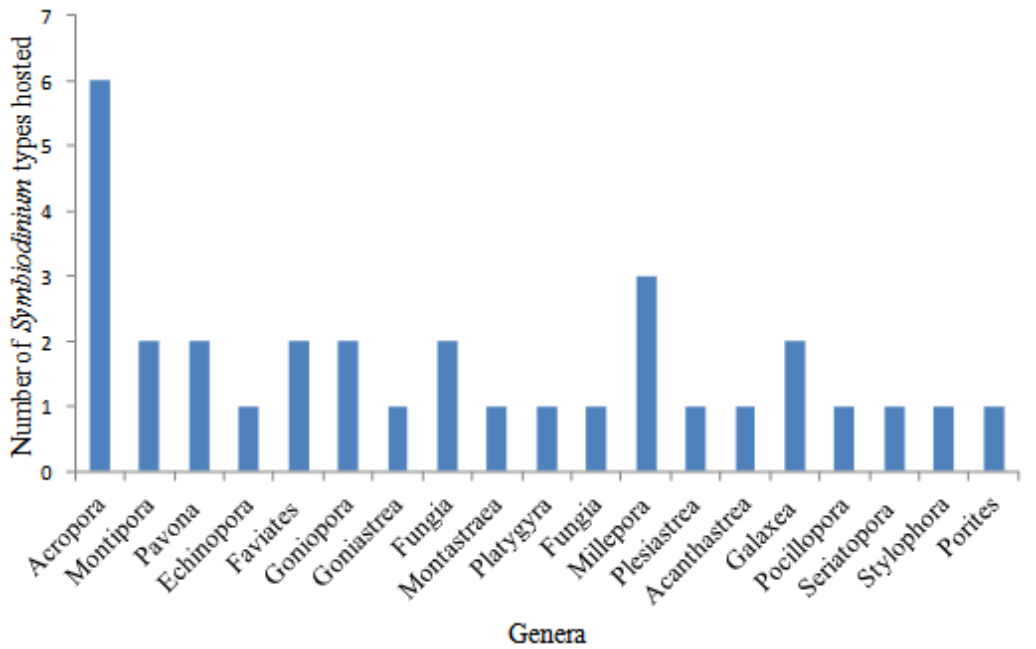

Figure 4. Diversity and ITS-2 *Symbiodinium* types hosted by different of reef-building coral genera along the Tanzanian coast.

DISCUSSION

Overview of coral symbioses in Tanzania

This is the first detailed study of the genetic diversity of *Symbiodinium* in reef-building corals along the Tanzanian coast. Unlike earlier, more limited studies (Macdonald *et al*., 2008; LaJeunesse *et al*., 2010), a wider range of coral species has now been examined on geographically separated reefs. Generally, *Symbiodinium* types in Tanzanian corals did not differ from those in other parts of the world. Phylotypes A to D occur regularly in scleractinian corals (Baker, 2003) and representatives of three (A, C and D) were detected in the 66 species studied, comprising a substantial proportion of the estimated 250 species of scleractinian coral found in Tanzania (Obura *et al*., 2004). The predominance of clade C in Tanzanian corals was consistent with previous studies undertaken on the Great Barrier Reef (LaJeunesse *et al*., 2003), in the Caribbean (Goulet *et al*., 2008) and Hawaii (LaJeunesse *et al*., 2004). The detection of *Symbiodinium* clades A and D respectively in colonies of *Millepora* sp. and *Seriatopora* hystrix indicated that non-C symbioses are nevertheless present in Tanzanian reef-building corals.

Most of the *Symbiodinium* types detected in the study were similar to those encountered by LaJeunesse *et al*. (2010) at Zanzibar. This similarity in Tanzanian coral-*Symbiodinium* symbioses may be attributable to their symbiont acquisition strategy. In most corals, symbionts are acquired through inherited or vertical transmission (Stat *et al*., 2008). This strategy ensures persistence in specificity in coral-*Symbiodinium* symbioses, especially in coral populations that share the same ancestors (Stat *et al*., 2008), and seems the likely mechanism along the Tanzanian coast and its islands. Corals species that acquire symbionts horizontally, or from the environment, may have a greater diversity of *Symbiodinium* in their tissues (Stat *et al*., 2008, Silverstein *et al*, 2011). The data in this study infer that this was not the case.

Greater symbiont diversity was encountered by Visram and Douglas (2006) along the Kenyan coast where clades A, C and D were found in seven scleractinian species. This is surprising, considering the proximity of the Kenyan and Tanzanian reefs; those in southern Kenya (Mombasa) and northern Tanzania (Tanga) are <100 km apart. There were other discrepancies between the results, e.g. Visram and Douglas (2004) found clades A and D associated with *Acropora* species, unlike the present study where it was primarily associated with clade C (Table 1). Similarly, *Galaxea fascicularis* was associated with clade D in Kenya but C3u in Tanzania (Table 1). Thus, further regional studies are needed to establish whether these contradictions reflect local environmental differences on Kenya and Tanzanian reefs, or methodological differences.

Potential for symbiont shuffling in Tanzanian reef-building corals

Coral species that host multiple symbionts have the ability to shuffle or regulate the abundance of their symbiont communities to survive environmental change (Berkelmanns & van Oppen, 2006). Previous studies have shown that this ability is limited to a few coral species (LaJeunesse *et al*., 2003; Thornhill *et al*., 2006) and, according to estimates by Goulet (2006), only 23% of reef-building coral species are able to host multiple *Symbiodinium* clades. Unlike Goulet's (2006) study which was conducted to cladal level, this study analysed the symbionts to the subcladal level, generally referred to as the species level (LaJeunesse *et al*., 2004). Relatively few Tanzanian corals hosted multiple clades in the present study, indicating that they would have a commensurately low adaptability to environmental change. It is convenient, though, to consider *Symbiodinium* types at species level, since types of the same clade are now known to manifest differences in environmental tolerance (Sampayo *et al*., 2008).

Most corals species that hosted multiple symbionts in the present study were found to accommodate more than one *Symbiodinium* species from the same clade (Table 1).

Table 1. *Symbiodinium* diversity in reef-building coral hosts along the Tanzanian coast.

Genera	Species	Tanga	Dar es Salaam	Zanzibar	Mafia	Mtwara
Acropora	*Acropora appressa*	C3u(6)		C3z(4), C109a (2),D1(1)		
	Acropora formosa	C3u(6)	C3(6)	C3u(4), D1(2)	C3(6), D1(2)	C3(6)
	Acropora gemmifera	C3u(6)		C109b (1)		
	Acropora glauca	C3u(6)		C3z (2)		
	Acropora granulosa	C3u(6)		C109b (1)		
	Acropora hemprichi	C3u(6)	C3(6),D1(2)	C3(6)	C3(6), D1a(2)	C3(6)
	Acropora latistella	C3u(6)		C3z (4)		
	Acropora loripes	C3u(6)		C3z (1), C3u (2)		
	Acropora lutkeni	C3u(4)		C3z (4), C109b(1)		
	Acropora retusa			C3z(3), C109a-c (2)		
	Acropora samoensis			C3z (5),C109b (4)		
	Acropora secale	C3u(6)	C3u(6)	C3u (5), C109a (3)		
	Acropora solitaryensis		C3u(6)	C3z (1)		
	Acropora subulata	C3u(6)		C109a (1)		
	Acropora tenuis			C3 (1), D1 (1)		
	Acropora valida			C115a (1), C3z (1)		
Montipora	*Montipora monasteriata*	C17(4)	C17(4)	C31 (1)		
	Montipora peltiformis			C110 (1)		
	Montipora tuberculosa	C17(2)	C17(2)	C31d (1)	C17(2)	C17(2)
	Montipora undata	C17(4)	C17(4)	C17a (1)	C17(4)	C17(4)
Pavona	*Pavona explanulata*			C3u (1) C3u (1)		
Fungia	*Fungia granulosa*			C3u (2)		
Millepora	*Millepora platyphylla*	A15c(6), C57a (1)	C57a (8)	C57a (5),	A15c (8)	A15c (8)
	Millepora sp.	A15c (6)	A15c (6)	A15c(7),C57(2), A16(2)		C57a (2)
Acanthastrea	*Acanthastrea echinata*			C3u (1)		
	Acanthastrea hemprichii			C3u (1)		
Galaxea	*Galaxea astreata*	C3u (4)		C3u (4)		
	Galaxea fascicularis	C3u (6)		C3u (2)	C3u (8)	C3u (8)
Echinophyllia	*Echinophyllia aspera*			C3 (4)		
	Echinophyllia echinata	C3u		C3u (1)		
Oxypora	*Oxypora lacera*			C3u (5)		
Pocillopora	*Pocillopora damicornis*	C1h (22)		C1h (36)	C1h (24)	C1h (30)
	Pocillopora elegans	C1h (1)		C1c (3)		
	Pocillopora eydouxi	C1h (1)		C1h (30)		
	Pocillopora verrucosa	C1h (22)		C1h (36)	C1h (24)	C1h (30)
Seriatopora	*Seriatopora hystrix*	D1 (24)		D1 (4)		
	Stylophora pistillata	C105a (6)	C105a (6)	C105a (10)	C105a (7)	C105a (8)
Goniopora	*Goniopora columna*			C1b-s (1)		
Porites	*Porites cylindrica*	C15 (6)	C15 (6)	C15 (10)	C15 (8)	C15 (8)
	Porites lobata			C15 (10)		
Porites	*Porites lutea*	C15 (2)	C15 (2)	C15 (10)	C15 (8)	C15 (8)
	Porites profundus			C15 (10)		
	Porites rus	C15 (6)	C105a (6)	C15 (10)	C15 (8)	C15 (8)

The identifier for each symbiont refers to the evolutionarily divergent clade (uppercase letter) and the ITS-DGGE fingerprint type, including the designations of one or more dominant intragenomic sequences in the ribosomal array (numbers and lowercase letters). Numerals in parentheses indicate the number of colonies in which a particular symbiont was found. Two or more types separated by a forward slash indicate that the symbionts co-occurred in the sample.

Of the material studied, 31% of the coral species in 42% of the genera formed multiple symbioses. Most of these were in the genus *Acropora*, amongst the most susceptible to bleaching (Marshall & Baird, 2000). A polymorphic association of this nature with *Acropora* was also found in Western Australia where Acropora established symbiosis with *Symbiodinium* clades C40 and D1a in some locations (Silverstein *et al*., 2011). However, the contribution of this to bleaching resistance may be insignificant in the face of environmental stress (LaJeunesse *et al*., 2009) as both the host and symbionts must cope with environmental disturbances.

Thermal intolerance of Seriatopora

Seriatopora was one of the genera most affected by the 1998 bleaching event (Muhando & Mohammed, 2002; Obura *et al*., 2004; McClanahan *et al*., 2007). While its current distribution extends southward to higher latitudes where temperatures are lower (Obura *et al*., 2004), it was found to be associated with a single thermo-tolerant *Symbiodinium* type (D1) along the entire Tanzanian coast (Table 1). Although there is no data on the *Symbiodinium* types that *Seriatopora* was associated with prior to the 1998 bleaching event, if one considers the genus' symbiont acquisition strategy and the number of years that have passed since the bleaching event, the genus probably hosted its present symbiont. This is probable because *Seriatopora* employs vertical transmission to acquire its symbionts (Loh *et al*., 2001). Furthermore, even where bleaching has caused the acquisition of foreign *Symbiodinium* types, the regular clades that occurred before bleaching had returned after two years (Thornhill *et al*., 2006). It is thus probable that D1 was the dominant symbiont of Seriatopora before the 1998 bleaching event, yet this coral genus suffered high mortality. Associations between *Symbiodinium* D1 and *Seriatopora* are clearly not particularly resistant to bleaching and this may be due to the sensitivity of the animal. Thus, both the animal and symbiont are responsible for holobiont thermo-tolerance.

CONCLUSION

The findings of this study contribute to our knowledge of the global distribution of *Symbiodinium*. The data also provide baseline information for future studies on possible changes in Tanzanian *Symbiodinium* distribution as a result of current and future trends in climate change. Based on previous studies on coral-symbiont relationships, the potential for changes in the cladal abundance of their symbionts as a survival strategy to perturbation is relatively high in some Tanzanian coral genera. Thermal intolerance of the genus *Seriatopora*, which was found to form a symbiosis with the thermo-tolerant *Symbiodinium* type D1, showed that functional tolerance to environmental change depends on both the host and symbiont.

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