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ORIGINAL ARTICLE

Symbiodinium (internal transcribed spacer 2) diversity in the coral host *Agaricia lamarcki* (Cnidaria: Scleractinia) between shallow and mesophotic reefs in the Northern Caribbean (20–70 m)

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Keywords
Coral reefs; operational taxonomic units (OTUs); *Symbiodinium trenchii*; symbiont zonation; symbiosis.

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Abstract

This study investigated differences in *Symbiodinium* diversity in the scleractinian coral species *Agaricia lamarcki* between shallow (20–25 m) and mesophotic (50–70 m) depths in the Northern Caribbean. Corals were sampled in each of four shallow sites (20–25 m; n = 18) and three mesophotic sites (50–70 m; n = 18) from Mona Island (Puerto Rico) and the US Virgin Islands during a mesophotic exploratory cruise and from the La Parguera shelf edge, off Southwestern Puerto Rico. *Symbiodinium* diversity was assessed using internal transcribed spacer 2 sequences clustered into operational taxonomic units (OTUs). Clustering resulted in eight clade C OTUs and one clade D OTU. Of these, there were three common *Symbiodinium* OTUs consisting of C3 and D1a.*N*14 in shallow reefs and C11.N4 in mesophotic reefs. Statistical tests (permutational multivariate analysis of variance and analysis of similarity) showed significant differences between clade C *Symbiodinium* OTUs in *A. lamarcki* colonies located at shallow and mesophotic depths, indicating symbiont zonation. *Symbiodinium* diversity in *A. lamarcki* from the Northern Caribbean is comparable to previous reports in the Southern Caribbean for this species. This is the first report of the thermal tolerant species *Symbiodinium trenchii* (D1a) in *A. lamarcki*.

Introduction

Reef-building corals are energetically dependent on their photosynthetic symbionts (genus *Symbiodinium*) and the exponential decrease in light with increasing depth is one of the most important factors driving their distributions over wide depth ranges (Rowan *et al.* 1997; Lesser *et al.* 2009, 2010; Cooper *et al.* 2011). In this respect, depth zonation of genetically distinct *Symbiodinium* genotypes (symbiont zonation) has been mostly attributed to the differential photoacclimation responses of the different symbiont genotypes to decreasing light levels with increasing depth (Rowan & Knowlton 1995; Iglesias-Prieto *et al.* 2004; Frade *et al.* 2008). While there are a growing number of studies investigating *Symbiodinium* diversity in corals along mesophotic depth gradients (Chan *et al.* 2009; Lesser *et al.* 2010; Cooper *et al.* 2011; van Oppen *et al.* 2011a; Bongaerts *et al.* 2013, 2015a,b; Pochon *et al.* 2015; Ziegler *et al.* 2015), the role of symbiont zonation and its influence on the depth distributions in corals is understudied, especially on an ecosystem-wide scale.

In the Caribbean, the most extensive surveys of *Symbiodinium* diversity in mesophotic corals have been carried out in Curaçao (Bongaerts *et al.* 2013, 2015a,b). These results show that symbiont zonation is common on a reef-wide scale, a dominant trait in species with the widest

depth ranges, and is more common in broadcast spawning species (Bongaerts *et al.* 2015a). An assessment of the lower mesophotic community (60–100 m) revealed a specialized coral community composed of *Agaricia grahamae*, *Agaricia undata* and *Madracis pharensis* (90 m), all associating with putative 'deep specialist' *Symbiodinium* types (Bongaerts *et al.* 2015b). An earlier study by Bongaerts *et al.* (2013) used internal transcribed spacer 2 (ITS2) denaturing gradient gel electrophoresis (DGGE) profiles to examine five *Agaricia* species and their *Symbiodinium* associations in Curaçao (2–60 m). Four of the five species showed distinct depth distributions and specific symbiont associations, whereas *Agaricia lamarcki* was the only species showing symbiont zonation (Bongaerts *et al.* 2013). Although a 'deep specialist' symbiont association is not a universal mechanism to survive at mesophotic depths (Chan *et al.* 2009; Bongaerts *et al.* 2010), these studies highlight the important role of symbiont zonation and its influence on the depth distributions of some coral species (Bongaerts *et al.* 2013, 2015a,b). Because some coral species can have geographic differences in symbiont associations (LaJeunesse *et al.* 2004; Garren *et al.* 2006; Jones *et al.* 2008; Stat *et al.* 2008; Finney *et al.* 2010), local assessments of *Symbiodinium* diversity are needed to further understand coral–symbiont associations on a broader geographic scale.

The coral species *Agaricia lamarcki* inhabits cryptic areas on shallow reefs (10–40 m) and is also one of the most abundant species inhabiting mesophotic reefs in the Caribbean (Bongaerts *et al.* 2013; Sherman *et al.* 2013), thus representing the ideal species to examine *Symbiodinium* diversity across a wide depth range. In this study, *Symbiodinium* ITS2 operational taxonomic units (OTUs) were constructed to investigate (i) differences in *Symbiodinium* diversity in *A. lamarcki* from shallow (20–25 m) and mesophotic depths (50–70 m) from Mona Island and La Parguera in Puerto Rico, and St. Croix and St. Thomas in the US Virgin Islands (USVI) and (ii) establish if the pattern of symbiont zonation in *A. lamarcki* is comparable to previous studies in the Southern Caribbean (Curaçao, Bongaerts *et al.* 2013, 2015a,b).

Material and Methods

Sample collection

Agaricia lamarcki (n = 36) samples were collected in 2010 in each of four shallow sites (20–25 m; n = 18) and three mesophotic sites (50–70 m; n = 18) from Mona Island (Puerto Rico) and St. Croix, USVI, during a mesophotic exploratory cruise and from the La Parguera shelf edge, located off Southwestern Puerto Rico. There were no mesophotic samples for comparison from St. Thomas USVI. Study sites are described in Sherman *et al.* (2013). Shallow-water colonies were collected with SCUBA-NITROX while trimix-rebreather systems were used to collect mesophotic colonies. Coral specimens were identified by Dr Ernesto Weil and preserved in 100% ethanol. Specimens were cataloged in a database by colony number, date and location, and deposited in the Museum of Marine Invertebrates at Magueyes Marine Laboratories, University of Puerto Rico, Mayagüez.

DNA extraction, PCR, cloning and sequencing

Total genomic DNA (gDNA) was extracted using a modified Cetyl-trimethylammonium bromide protocol after Dempster *et al.* (1999). The ITS2 region was PCR amplified using standard thermal cycle conditions using the *Symbiodinium* specific primers, ITSintfor2 (5′-GAATTGCAGAACTCCGTG-3′) and ITS2 reverse (5′-GGATCCATATGCTTAAGTTCAGCGGGT-3′) (LaJeunesse 2002). PCR products for the *Symbiodinium* ITS2 region were purified using QIAquick® PCR Purification Kit (Qiagen, Valencia, CA, USA), ligated overnight into the pGEM® T-Easy Cloning Vector, transformed into JM109 competent cells (Promega, Madison, WI, USA) and grown overnight on selective Luria broth media (ampicillin 50 $\mu g \cdot ml^{-1}$, isopropyl-β-D-thio-galactopyranoside (IPTG) 0.1 mM, 5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside (X-GAL) 50 $\mu g \cdot ml^{-1}$). ITS2 clones were sequenced with M13 primers at the High-Throughput Genomics Center (hts-eq.org; Seattle, WA, USA).

Sequence analysis and OTUs

Symbiodinium ITS2 sequences were edited using CODONCODE ALIGNER (v. 4.2.7, CodonCode Corporation, www.codoncode.com) and aligned to closely related ITS2 sequences from GenBank. MOTHUR (v. 1.29; Schloss *et al.* 2009) was used to generate a distance matrix with each gap treated as a mutation and subsequently clustered into OTUs using a 97% sequence similarity threshold with the furthest neighbor algorithm. OTUs represented by a single sequence were not considered in downstream analyses and omitted from the data set. Novel sequences representing an OTU were named after their closest ITS2 sequence followed by a capital italic *N* (to represent 'novel'), with the next available integer in GenBank (*i.e.* C3b.*N*9) used to identify novel *Symbiodinium* ITS2 sequences in *Agaricia* spp. from Curaçao (Bongaerts *et al.* 2013).

Statistical analyses of *Symbiodinium* OTUs

The frequency of *Symbiodinium* OTUs in each colony was square root transformed and statistical analyses

performed using the software Paleontological Statistics (PAST v. 3.0; Hammer *et al.* 2001). Because of limited sampling, location was not considered. Instead, samples were pooled across locations for statistical analyses. To test for symbiont zonation of *Symbiodinium* OTUs by depth, a permutational multivariate analysis of variance (PERMANOVA; Anderson 2005, 2006) was performed with depth as a factor and colonies as the replicate. Analysis of similarity (ANOSIM) among *Symbiodinium* OTUs was also performed using the Bray–Curtis co-efficient of similarity matrix to identify differences between shallow and mesophotic *Symbiodinium* OTUs occurring in *Agaricia lamarcki* (Clarke 1993). Minimum-spanning networks for representative OTUs (ancestral sequences) were inferred in NETWORK v. 4.6 with the full median joining algorithm (Bandelt *et al.* 1999) and illustrated with Network PUBLISHER v. 1.3 (Fluxus-engineering.com).

Results

A total of 235 *Symbiodinium* ITS2 sequences was examined among 36 colonies of *Agaricia lamarcki* from shallow (n = 18 colonies) and mesophotic depths (n = 18 colonies) (Table 1). Briefly, 29 colonies hosted clade C

Table 1. Sampling summary by location and depth, number of cloned ITS2 sequences for each *Agaricia lamarcki* sample, and the distribution of sequences among *Symbiodinium* operational taxonomic units (OTUs).

colony number (id)/depth	no. clones	1-C3	2-C11.N4	3-C3d	4-C3b.*N*9	5-C11.*N*10	6-C.*N*11	7-C11.*N*12	8-C.*N*13	9-D1a.*N*14
shallow samples										
122-Cane Bay, St. Croix (20–25 m)	9	7					2			
123-Cane Bay, St. Croix (20–25 m)	7									7
16-Cane Bay, St. Croix (20–25 m)	6	3	2		1					
2-Mona Island, PR (20–25 m) [a]	8	6		1						
3-Mona Island, PR (20–25 m)	10	9		1						
5-Mona Island, PR (20–25 m)	11	4	3	2		1			1	
79-Mona Island, PR (20–25 m)	2									2
80-Mona Island, PR (20–25 m)	4									3
81-Mona Island, PR (20–25 m)	4									4
21-St. Thomas (20–25 m) [a]	8	4		2					1	
22-St. Thomas (20–25 m) [a]	7	2								4
24-St. Thomas (20–25 m)	3	3								
25-St. Thomas (20–25 m)	9	5		1	3					
34-La Parguera, PR (20–25 m)	9									9
33-La Parguera, PR (20–25 m)	3	3								
26-La Parguera, PR (20–25 m)	9	9								
28-La Parguera, PR (20–25 m)	11	9	1	1						
76-La Parguera, PR (20–25 m)	3	3								
mesophotic samples										
120-Cane Bay, St. Croix (67 m)	10		9					1		
121-Cane Bay, St. Croix (67 m)	8		7					1		
17-Cane Bay, St. Croix (55 m) [a]	8	5	1					1		
19-Cane Bay, St. Croix (67 m)	8	7			1					
10-Mona Island, PR (67 m)	6	3	3							
6-Mona Island, PR (67 m)	3	3								
7-Mona Island, PR (50 m)	10	3	7							
78-Mona Island, PR (50 m)	3		3							
82-Mona Island, PR (50 m)	2	1	1							
84-Mona Island, PR (72 m)	9	2	7							
6A-La Parguera, PR (60 m) [a]	5	2	1			1				
43-La Parguera, PR (60 m) [a]	6		5							
44-La Parguera, PR (60 m)	2	1				1				
46-La Parguera, PR (67 m)	8		8							
48-La Parguera, PR (55 m)	6	3	3							
4A-La Parguera, PR (60 m)	6	1	4				1			
41-La Parguera, PR (60 m)	3	1				2				
3A-La Parguera, PR (70 m)	9		9							

[a]OTUs represented by a single sequence were not considered in downstream analyses and omitted from the data set.

Symbiodinium and five colonies hosted clade D, while one colony from shallow reefs of St. Thomas USVI hosted both clades C and D simultaneously. The prevalence of *Symbiodinium* OTUs recovered in *A. lamarcki* colonies located at shallow and mesophotic depths is presented for each location (Fig. 1). Clustering ITS2 sequences resulted in nine *Symbiodinium* OTUs, eight in clade C and one in clade D (Table 1). The most common *Symbiodinium* OTUs (*i.e.* containing the majority of sequences) in *A. lamarcki* across all sampling locations were C3 OTU1, C3d OTU3 and D1a.*N*14 OTU9 in shallow waters and C11.N4 OTU2 at mesophotic depths (Table 2, Fig. 1). Statistical tests (PERMANOVA, P = 0.0001, Pseudo-F = 9.65, df = 1; ANOSIM, P = 0.0001, R = 0.33) showed significant differences between clade C *Symbiodinium* OTUs (*i.e.* C3 OTU1 and C11.N4 OTU2) located at shallow and mesophotic depths, indicating symbiont zonation in *A. lamarcki*. The minimum spanning network illustrates *Symbiodinium* C3 OTU1 as the inferred ancestral (ITS2) sequence among *Symbiodinium* OTUs recovered (Fig. 2). The most common clade C OTUs (C3, C3d and C11.N4) were identical to their reference sequences in GenBank (Table 2). The C11.N4 OTU2 consisted of C11 (40%, LaJeunesse 2002) and C11.N4 sequences (60%, Bongaerts *et al.* 2013). The C3b.*N*9 OTU4 sequence is 1 bp different from its

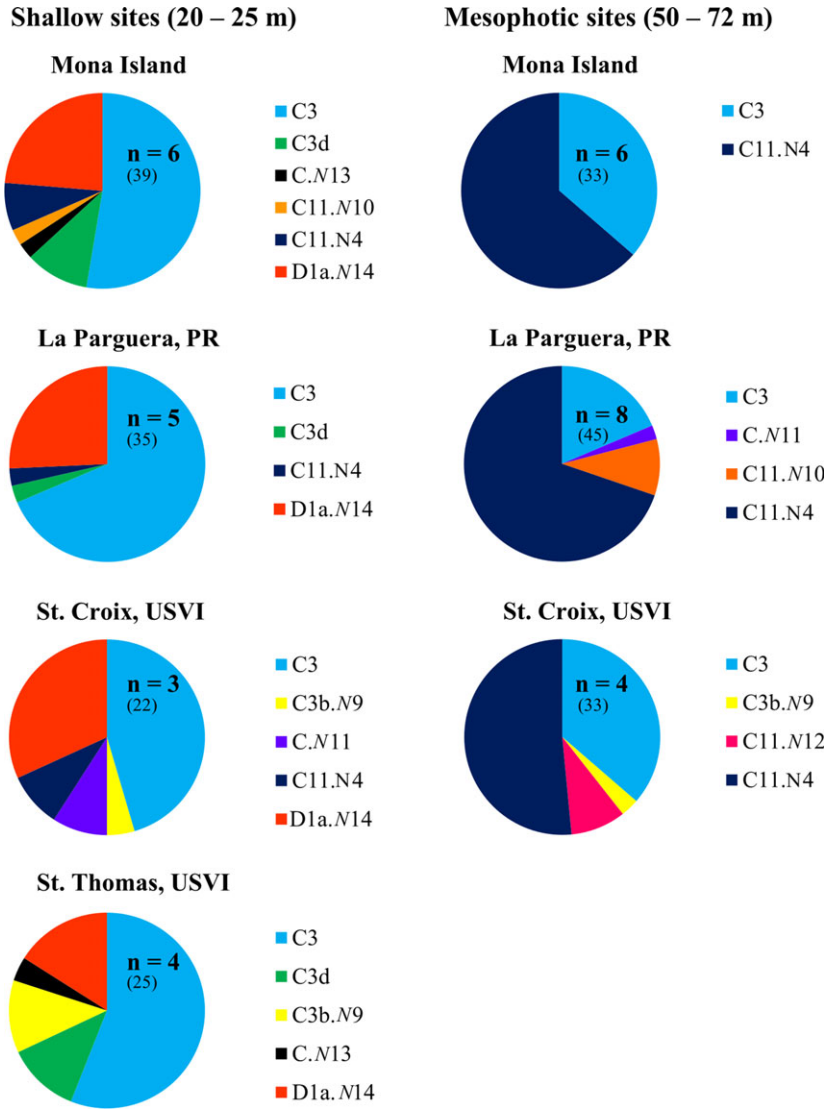

Fig. 1. The prevalence of all *Symbiodinium* operational taxonomic units occurring in *Agaricia lamarcki* colonies located at shallow and mesophotic depths for each location. The value n represents the number of colonies sampled in each location and the value in parentheses represents the number of cloned sequences. USVI = US Virgin Islands.

Table 2. *Symbiodinium* internal transcribed spacer 2 (ITS2) operational taxonomic unit (OTU) representatives (rep), the number of sequences (seqs) clustered in each OTU, GenBank accession numbers and ITS2 sequence references for novel OTU sequences.

OTU number	OTU-rep	no. seqs/OTU	GenBank accession no.	references
1	C3	99	100% match	LaJeunesse (2002) (AF499789)
2	C11.N4	74	100% match	Bongaerts *et al.* (2013) (KF551188)
3	C3d	8	100% match	LaJeunesse (2002) (AF499792)
4	C3b.*N*9[a]	5	KP109696	LaJeunesse (2002) (AF499791)
5	C11.*N*10[a]	5	KP109697	Bongaerts *et al.* (2013) (KF551188)
6	C.*N*11[a]	3	KP109698	Thornhill *et al.* (2007) (EU074889)
7	C11.*N*12[a]	3	KP109699	LaJeunesse (2002) (AF499800)
8	C.*N*13[a]	2	KP109700	Barbrook *et al.* (2014) (HG515026)
9	D1a.*N*14[a]	29	KP109701	LaJeunesse *et al.* (2014) (KJ019889)

[a]*N* refers to novel sequences recovered in this study.

reference sequence (C3b, LaJeunesse 2002). The C11.*N*10 OTU5 sequence is characterized by a 1-bp deletion and a 2-bp insertion, while C11.*N*12 OTU7 contains an 8-bp deletion from its reference sequence (C11, LaJeunesse 2002). The C.*N*11 OTU6 is 1 bp different compared to a C1 sequence (culture 152, LaJeunesse 2002), considered to be a PCR chimera (LaJeunesse 2002). Because of this, the C1 sequence (*Symbiodinium goreaui*, LaJeunesse 2002) was incorporated into the network to illustrate the relationship among the C.*N*11, C3 and C1 sequences (Fig. 2). The C.*N*13 OTU8 has a 4-bp deletion and 1-bp difference compared to the C3 reference sequence (LaJeunesse 2002) (Table 2). The D1a.*N*14 OTU9 consists of D1a (42%, LaJeunesse *et al.* 2014) and D1 sequences (58%, LaJeunesse 2002) and is characterized by a single (1 bp deletion) difference compared to its reference sequence (*Symbiodinium trenchii*, LaJeunesse *et al.* 2014). Novel ITS2 sequences representative of OTUs were submitted to GenBank (Table 2).

Discussion

The ITS2 region has been the most widely employed phylogenetic marker in the exploration of coral–*Symbiodinium* diversity along shallow and mesophotic depth gradients (Chan *et al.* 2009; Bongaerts *et al.* 2010, 2011, 2013, 2015a,b; Lesser *et al.* 2010; Pochon *et al.* 2015; Ziegler *et al.* 2015). Notwithstanding this, the multi-copy nature of the ITS2 region with numerous intragenomic variants has invoked considerable debate regarding the interpretation of *Symbiodinium* diversity and species delineation (Apprill & Gates 2007; Thornhill *et al.* 2007, 2010; Correa & Baker 2009; Sampayo *et al.* 2009; Stat *et al.* 2011, 2013). Therefore, to provide a conservative yet meaningful interpretation of these data, this study considers *Symbiodinium* ITS2 diversity in the context of OTUs. While the OTU approach does not completely alleviate the problems associated with intra-genomic variation and PCR artifacts, it does reduce the overall complexity (Sneath & Sokal 1973; Thornhill *et al.* 2007; Correa & Baker 2009; Stat *et al.* 2013), and a 97% sequence similarity has gained recent support as the most informative threshold for studies of *Symbiodinium* using the ITS2 gene (Arif *et al.* 2014; Stat *et al.* 2015). In this regard, the C11.N4 sequence reported in *Agaricia lamarcki* from Curaçao (Bongaerts *et al.* 2013) differs by only a single (1 bp) deletion from its reference C11 sequence (LaJeunesse 2002). As such, the C11.N4/C11 OTU2 (inclusive of C11) is interpreted here as *Symbiodinium* C11 (LaJeunesse 2002). Likewise, the novel D1a.*N*14 OTU9 is also characterized by a single (1 bp deletion) difference and therefore is interpreted as *Symbiodinium trenchii* (*sensu* D1a, or D1–4; but see LaJeunesse *et al.* 2014). Furthermore, *S. trenchii* is the only clade D species inhabiting Atlantic/Caribbean corals (Pettay *et al.* 2015). In addition, some of the novel clade C OTUs with few sequences are characterized by substantial differences compared to novel sequences identified in DGGE ITS2 profiles in *Agaricia lamarcki* in Curacao (Bongaerts *et al.* 2013; Table 2, Fig. 2). Although these novel sequences likely represent intra-genomic variants of a single rDNA lineage (Thornhill *et al.* 2007; Bongaerts *et al.* 2013), it cannot be discounted they may represent a mix of distinct background symbionts (Stat *et al.* 2011; Bongaerts *et al.* 2013). However, the taxonomic and ecologic significance of this diversity remains uncertain owing to a lack of consensus in the interpretation of ITS2 diversity (Correa & Baker 2009; LaJeunesse & Thornhill 2011; Stat *et al.* 2011).

Results of this study indicate that *Agaricia lamarcki* associates with a different *Symbiodinium* community across its depth distribution in the Northern Caribbean. Furthermore, this is the first report of the thermal-tolerant *S. trenchii* (LaJeunesse *et al.* 2014) recovered in shallow-water colonies of *A. lamarcki.*

The *Symbiodinium* OTUs C3 OTU1 and C11.N4 OTU2 were present in *Agaricia lamarcki* colonies located at shallow and mesophotic depths; however C11.N4 OTU2 is most abundant at mesophotic depths, indicating that *A. lamarcki* associates with C11.N4, previously described

as a putative 'deep specialist '(Curaçao, Bongaerts *et al.* 2013). These data are consistent with the C11.N4/C11 DGGE profile recovered by Bongaerts *et al.* (2013). The *Symbiodinium* types C11.N4/C11 also occur in the deep-water species *Agaricia grahamae* and *Agaricia undata* (Bongaerts *et al.* 2015a,b). Noteworthily, *Symbiodinium* C3d (Bongaerts *et al.* 2013) was also present (C3d OTU3) in a few shallow-water colonies of *A. lamarcki* from Mona Island, La Parguera and St. Croix (Fig. 1). These data parallel *Symbiodinium* diversity recovered in *A. lamarcki* from the Southern Caribbean where two different DGGE ITS2 profiles were observed, C3/C3d or C3/C11 in shallow reefs, transitioning to exclusively the C3/C11 profile at mesophotic depths (Bongaerts *et al.* 2013; Fig. 1). The most common or dominant *Symbiodinium* ITS2 sequences representing OTUs in this study are identical to those found in other coral species, including other *Agaricia* spp. (C3), *Montastraea cavernosa* and *A. lamarcki* (C3d) (Lesser *et al.* 2010; Bongaerts *et al.* 2013; Barbrook *et al.* 2014), as well as *Scolymia cubensis*, *Mussa angulosa*, *Mycetophyllia ferox* and *Mycetophyllia lamarckiana* (C11) (LaJeunesse 2002; Finney *et al.* 2010), and more recently *A. lamarcki*, *A. undata* and *A. grahamae* (C11.N4/C11) (Bongaerts *et al.* 2013, 2015a,b).

Depth zonation of genetically distinct *Symbiodinium* among reef-building corals has long been considered one of the most important factors driving the distribution of corals with wide depth distributions (Rowan & Knowlton 1995). A number of recent studies have reported host–symbiont depth specialization (Sampayo *et al.* 2007; Lesser *et al.* 2010; Bongaerts *et al.* 2013; Pochon *et al.* 2015), but a concise explanation as to which symbiotic partner is driving their depth distributions has not yet been provided. Furthermore, why some coral species with wide depth distributions exhibit symbiont zonation and others do not is presently unclear (Bongaerts *et al.* 2015a; Pochon *et al.* 2015). Conversely, a recent study combining ecologic, physiologic and molecular data examined

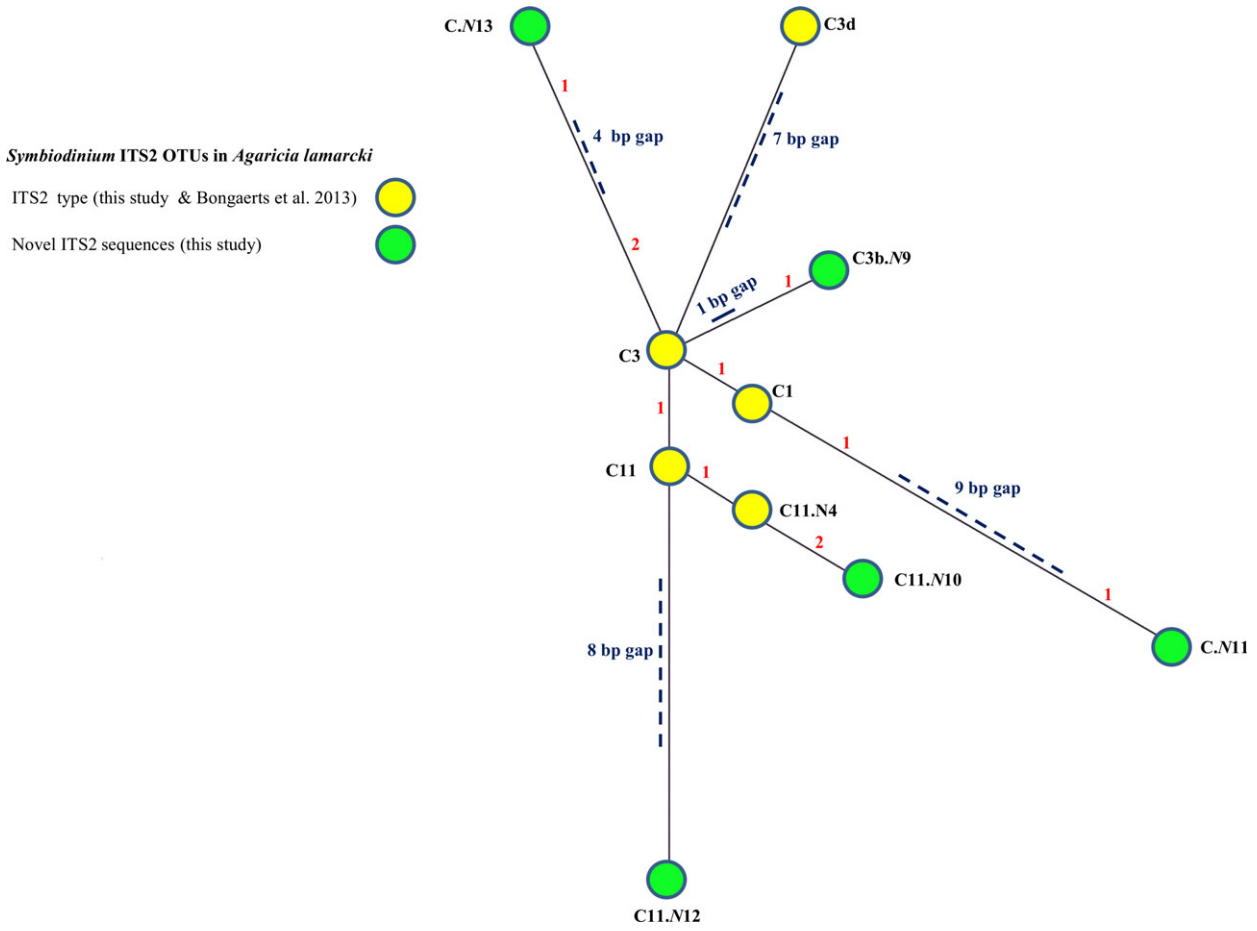

Fig. 2. Minimum spanning network of *Symbiodinium* clade C operational taxonomic units (OTUs) in the coral host *Agaricia lamarcki*. Gaps are indicated by blue dashes and mutations are indicated in red. ITS2 = internal transcribed spacer 2.

photoacclimation among four coral genera (*Porites*, *Leptoseris*, *Pachyseris* and *Podabacia*) and their symbionts along a mesophotic depth gradient in the Red Sea (Ziegler *et al.* 2015). Overall, general photoacclimation strategies were common among all coral host–symbiont combinations, such that both symbiont cell densities and photoprotective pigments to light harvesting pigment ratios significantly decreased with depth (Ziegler *et al.* 2015). Interestingly, the authors reported that the coral hosts have an effect on the photosynthetic pigment composition of their symbionts. For example, in a comparison of *Symbiodinium* type C1 in *Podabacia* and *Pachyseris*, the ß-carotene: chl *a*, peridinin: chl *a* and diadinoxanthin: chl *a* ratios significantly differed over depth among host species. Ziegler *et al.* (2015) concluded that depth acclimation in corals is facilitated by symbiont physiology (*e.g. Symbiodinium* pigment composition and cell densities) which in turn is host-specific, as demonstrated by their analysis of different coral species associating with the same symbiont type. Their study underpins the importance of the ecologic and physiologic interactions of both corals and their *Symbiodinium* types to better leverage an understanding of the drivers in their distributions along mesophotic gradients (Ziegler *et al.* 2015)

This is the first report of physiologically tolerant *Symbiodinium trenchii* (*e.g.* D1a.*N*14 OTU9 this study) associating with *Agaricia lamarcki* in shallow reefs. *Symbiodinium trenchii* is endemic to the Indo-Pacific Ocean and new evidence shows that this species has quickly spread to corals throughout the Greater Caribbean on ecologic timescales (Pettay *et al.* 2015). The authors revealed that Atlantic/Caribbean populations of *S. trenchii* have low genetic diversity and several widespread and genetically similar clones compared to Indo-Pacific populations (Pettay *et al.* 2015). Physiologically tolerant clade D *Symbiodinium* have received considerable attention for their potential role in mitigating the effects of changing ocean conditions (*e.g.* thermal anomalies, ocean acidification and pollution) related to climate change and human activities. While corals hosting *S. trenchii* may be afforded some resilience to various environmental disturbances (Rowan 2004; Berkelmans & van Oppen 2006; Abrego *et al.* 2008; van Oppen *et al.* 2011b), there is a tradeoff between increased survival and reduced growth (calcification) at the ecosystem scale and this has raised concerns over the long-term productivity and reef-building capacity among Caribbean coral reef communities (Ortiz *et al.* 2013; LaJeunesse *et al.* 2014; Pettay *et al.* 2015). Nonetheless, the presence of *S. trenchii* in *A. lamarcki* could be a result of selection and or acclimation to three high thermal anomalies between 1998 and 2003, when bleaching was more intense at intermediate depths (10–30 m). In these cases, *A. lamarcki* bleached and recovered with only a few colonies showing partial mortality. During the 2005 intense bleaching event, *A. lamarcki* bleached but was resistant to mortality, in contrast with the other agaricids (*i.e. Undaria* spp.) who suffered high mortality at these depths (Weil *et al.* 2009). Nonetheless, the finding that *A. lamarcki* can associate with *S. trenchii* in shallow reefs may impart some resilience during future environmental disturbance.

Conclusions

Collectively, the ecology of coral–symbiont assemblages from mesophotic studies establishes how the symbiont community is distributed in *Agaricia lamarcki* over its depth range across a larger biogeographic range in the Caribbean. Overall, *Symbiodinium* diversity and the pattern of depth zonation in *A. lamarcki* sampled from Mona Island, La Parguera and the USVI is similar to that found in *A. lamarcki* in the Southern Caribbean (Bongaerts *et al.* 2013). Continuing research into the biology and ecology of mesophotic ecosystems is essential to understanding adaptation and their roles as potential refugia in an era of rapid global environmental degradation This work serves as an important baseline study in the northern Caribbean for future resilience assessments.

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