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Symbiodinium diversity in mesophotic coral communities on the Great Barrier Reef: a first assessment

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ABSTRACT: Despite a growing interest in mesophotic coral ecosystems (MCEs), information on the photosynthetic endosymbionts (genus *Symbiodinium*) associated with scleractinian corals inhabiting deep reef ecosystems is sparse. Here, the deep-water *Symbiodinium* diversity is assessed from 10 different coral genera at a depth range of 45 to 70 m on the Great Barrier Reef (GBR), Australia. *Symbiodinium* identity was established using denaturing gradient gel electrophoresis (DGGE) fingerprinting of the internal transcribed spacer region 2 (ITS2) of the ribosomal DNA. Except for the novel *Symbiodinium* type C131 (found in *Porites*), all *Symbiodinium* types have previously been identified in shallow reef corals across the Pacific. Specimens of *Seriatopora*, *Montipora,* and *Porites* harboured similar symbionts as reported in shallow water (e.g. C3n, C3n-hh, C15, and C17), thus adhering to patterns of host-specificity across a wide depth range. However, several other *Symbiodinium* types were found to transcend previously established patterns of host-specificity at mesophotic depths. For example, 'host-specialist' types C3i and C3k (previously only reported in *Acropora* spp.) were found here to associate with a range of different genera (*Leptoseris*, *Pachyseris*, *Fungia*, and *Echinophyllia*). Although limited in sample size, this preliminary survey indicates that mesophotic habitats on the GBR may not represent an isolated community in terms of *Symbiodinium* diversity, which has significant relevance to their potential to act as refugia. Moreover, the present study identifies the need to examine symbiont diversity across broad environmental ranges (including MCEs) in order to gain an accurate understanding of symbiosis specificity and distribution range of specific coral-*Symbiodinium* associations.

KEY WORDS: *Symbiodinium* · Mesophotic · Deep reefs · Coral · ITS2 · DGGE · Great Barrier Reef

INTRODUCTION

The successful establishment and persistence of coral reef ecosystems in tropical nutrient-poor waters is made possible by the symbiosis between cnidarians and dinoflagellates of the genus *Symbiodinium* (Muscatine et al. 1981). Given the important role that *Symbiodinium* plays in the success of coral reef ecosystems, it is not surprising that this group has received much attention, and the genetic characteri-

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zation of *Symbiodinium* into cladal (e.g. Rowan & Powers 1991, Baker & Rowan 1997) and subcladal types (e.g. van Oppen et al. 2001, LaJeunesse 2002) has greatly enhanced our understanding of the symbiosis. Studies of *Symbiodinium* diversity on broad latitudinal or longitudinal gradients have shown distinct biogeographical patterns (e.g. LaJeunesse et al. 2003, 2010, Silverstein et al. 2011), whereas more local or species-specific studies have highlighted ecological zonation, physiological diversification, and host-specificity of distinct symbionts (e.g. Rowan & Knowlton 1995, LaJeunesse et al. 2003, 2004, Iglesias-Prieto et al. 2004, Sampayo et al. 2007, Frade et al. 2008a).

Community-wide shifts in symbiont diversity occur with increasing depth (LaJeunesse 2002, LaJeunesse et al. 2003, 2004, 2010), but this observation is likely to be, in part, the result of host community composition changes over depth. Nonetheless, studies focusing on single coral species show a similar pattern, and depth zonation of symbionts has been shown to occur on a cladal level in a number of Caribbean coral species such as *Acropora cervicornis, Montastraea annularis, M. franski, M. faveolata, Stephanocoenia intersepta,* and *Porites asteroides* (Rowan & Knowlton 1995, Baker et al. 1997, Toller et al. 2001, Iglesias-Prieto et al. 2004, Warner et al. 2006). Symbiont depth zonation is further observed using taxonomic identification at the subcladal, or 'type', level (LaJeunesse 2002) in species such as *Madracis pharensis* (Frade et al. 2008a) or *Montastraea cavernosa* in the Caribbean (Warner et al. 2006), and *Pocillopora damicornis, Seriatopora hystrix,* or *Stylophora pistillata* in the Indo-Pacific (Sampayo et al. 2007, Bongaerts et al. 2010a). Most studies of *Symbiodinium* diversity have been limited to shallow waters of less than 30 m and do not cover the full bathymetric range of coral species (but see Lesser et al. 2010, Cooper et al. 2011a, van Oppen et al. 2011). As a result, limited information is available on the *Symbiodinium* from mesophotic (>30 m) coral communities and the extent to which these communities overlap with adjacent shallow water coral communities (Bongaerts et al. 2010b).

Mesophotic coral ecosystems (MCEs) may potentially play an important role in the overall resilience of reef ecosystems by functioning as refugia during periods of stress (Glynn 1996, Hughes & Tanner 2000) and representing subsequent sources of propagules for recovering shallow reef habitats (Lesser et al. 2009, Bongaerts et al. 2010b, van Oppen et al. 2011). However, habitat-related differences in *Symbiodinium* diversity may affect coral population connectivity (Bongaerts et al. 2010a, Frade et al. 2010) if host and symbiont lineages are coupled through co-evolutionary processes (Loh et al. 2001, Sampayo et al. 2007, Frade et al. 2008a). Given the prevalence of niche specialization and ecological zonation of *Symbiodinium* (LaJeunesse et al. 2003, 2004, Finney et al. 2010), it is expected that extensive differences in *Symbiodinium* diversity occur between shallow and deep habitats.

Here we provide an initial assessment of *Symbiodinium* diversity associated with the mesophotic coral communities that occur at depths ranging from ~45 to 70 m on the Great Barrier Reef (GBR) (Bridge et al. 2011a,b,c). Specimens (n = 45) collected on a research expedition (Webster et al. 2008) were used to assess symbiont diversity from MCEs using denaturing gradient gel electrophoresis (DGGE) fingerprinting of the internal transcribed spacer region 2 (ITS2) of the ribosomal DNA. *Symbiodinium* diversity was compared to previously published information on related coral taxa from shallower GBR locations to provide a first indication of whether mesophotic corals associate with a distinct deep-water *Symbiodinium* community and whether known patterns of host-symbiont specificity are maintained across broad bathymetric ranges.

MATERIALS AND METHODS

A total of 45 coral colonies were collected from 45 to 70 m depth using rock dredge sampling during an exploratory cruise on the RV 'Southern Surveyor' (September to October 2007). Samples were collected from 3 locations (Fig. 1): (1) Ribbon Reefs (northern GBR: 15° 38′ S, 145° 80′ E; 15° 49′ S, 145° 82′ E), (2) Noggin Pass (northern GBR: 17° 09′ S, 146° 57′ E; 17° 10′ S; 146° 57′ E), and (3) Hydrographers Passage (central GBR: 19° 69′ S. 150° 23′ E). A large fragment of each colony was stored in 95% ethanol, and the remainder was bleached and deposited at the Queensland Museum (Townsville, Australia) for further taxonomic identification.

The specimen samples preserved in ethanol were drained and the supernatant was rinsed using a 3-fold wash step using DNAB and centrifugation at 25 000 × *g*. DNA was extracted using a QIAGEN Plant Mini Kit with inclusion of all optional steps suggested by the manufacturer. Because DNA yield of some samples was low, all samples were amplified in a nested-PCR approach (Gou et al. 2003, Hirose et al. 2008, Yamashita et al. 2010). The complete ITS

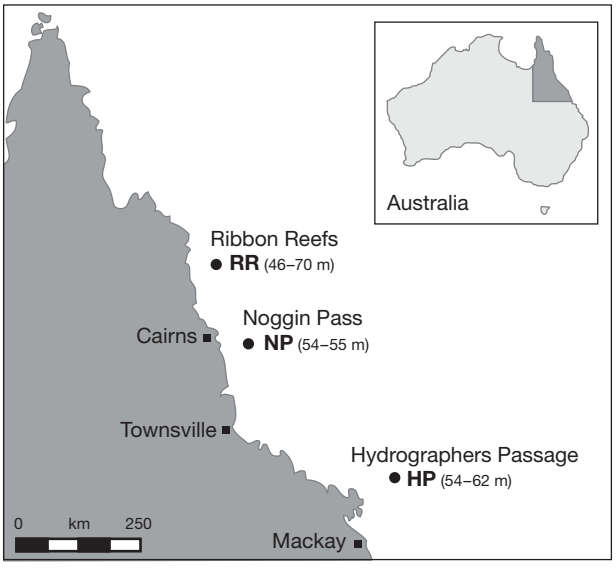

Fig. 1. Locations (Ribbon Reefs, Noggin Pass and Hydrographers Passage) and sampled depth ranges

region was amplified using the ZITSUPM13/ZITSDNM13 primer set (Santos et al. 2001) and, after normalizing concentrations through dilutions, served as a template for a PCR using the ITSintfor2/ITS2Clamp primer set (LaJeunesse 2002). The first PCR was done using ~10 ng template DNA, 1 µl 10× PCR buffer (Invitrogen), 0.5 µl 50 mM $MgCl_2$, 0.35 µl 10 mM dNTPs, 0.35 µl 10 mM ZITSUPM13, 0.35 µl 10 mM ZITSDNM13, 0.07 µl of Platinum *Taq* DNA Polymerase (Invitrogen), and dH_20 water to a total volume of 10 µl per reaction following a cycling protocol of 1 × 94°C (2 min), 35× [30 s at 94°C, 30 s at 60°C, 45 s at 72°C], 1 × 72°C (10 min). The second PCR, for DGGE, was done using 1.0 µl of the primary PCR amplicons, 2 µl 10× PCR buffer (Invitrogen), 1.0 µl 50 mM $MgCl_2$, 0.7 µl 10 mM dNTPs, 0.7 µl 10 mM ITSintfor2, 0.7 µl 10 mM ITS2Clamp, 0.15 µl of Platinum *Taq* DNA Polymerase (Invitrogen), and dH_20 water to a total volume of 20 µl per reaction following a touchdown PCR cycling protocol described in LaJeunesse (2002).

Amplified ITS2 fragments were run on a CBScientific Denaturing Gradient Gel Electrophoresis (DGGE) system using the conditions described in Sampayo et al. (2009) with each sample run at least 3 times from different PCR amplifications on different gels to ensure consistency of profiles. Additionally, comparisons were run between the 'nested' PCR procedure and direct amplifications using the ITS2-GC primers for several representative samples (from the genera *Acropora*, *Galaxea*, and *Montipora*), and the nested reaction did not produce profiles different from direct amplifications. To determine the symbiont type, dominant bands were excised from 2 to 4 samples of each characteristic profile, eluted overnight in 100 µl dH_2O, after which 1 µl of template was used to re-amplify the DNA using the non-GC clamped primers ITSintfor2 and ITS2reverse (cf. LaJeunesse 2002).

Successful PCR re-amplifications from excised DGGE bands were sequenced using ABI BigDye Terminator chemistry at the Australian Genome Research Facility (Brisbane, Australia). All obtained ITS2 rDNA sequences were aligned and chromatograms were visually checked (Codoncode Aligner) prior to being blasted on GenBank (http://www.ncbi.nlm.nih.gov/BLAST/). For comparative purposes, the *Symbiodinium* GBR database (Tonk et al. unpubl. data) was used to extract information on: (1) the occurrence and host species of observed *Symbiodinium* types on shallower sections of the GBR, and (2) the different types of *Symbiodinium* associated with the studied coral genera in shallow GBR waters. Phylogenetic analysis of sequences was performed using maximum parsimony and maximum likelihood in PAUP (vs. 4.0b10; Swofford 2003) under the delayed transition setting, using indels as a 5th character state and calculation of bootstrap support values based on 1000 replicates.

RESULTS

Identification of coral specimens

Coral collections from the RV 'Southern Surveyor' research cruise to the northern and central GBR retrieved samples from at least 10 genera belonging to 7 major coral families: the Acroporidae, Agariciidae, Fungiidae, Oculinidae, Pectinidae, Pocilloporidae, and Poritidae (Fig. 2, Table 1). Within these families, most specimens (n = 31) could be identified to the species level: *Acropora elegans*, *Leptoseris hawaiiensis*, *Pachyseris speciosa*, *Fungia* cf. *danai*, *Galaxea astreata*, *Echinophyllia aspera,* and *Seriatopora hystrix* (Table 1) (Bridge et al. 2011c). The remaining specimens (n = 14) could only be identified down to genus level and may represent either known species with a notably distinct appearance at mesophotic depths (preventing accurate identification based on morphological characteristics), or they may represent currently un-described species within the genus (Wallace & Muir pers. comm.).

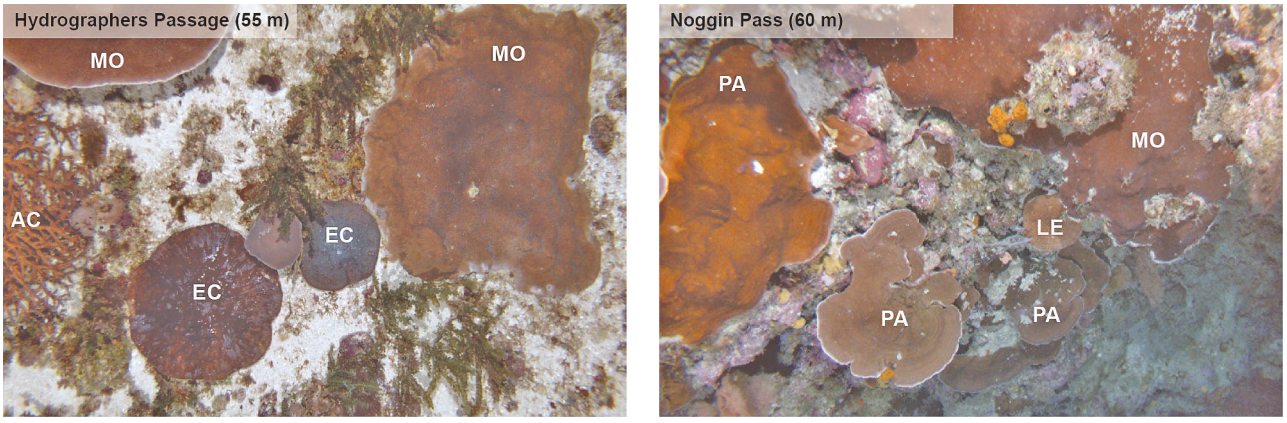

Fig. 2. AUV footage from sampled reefs: (left) Hydrographers Passage at 55 m depth with colonies of *Acropora* spp. (AC), *Montipora* sp. (MO) and Echinophyllia (EC); (right) Noggin Pass at 60 m depth, with colonies of *Leptoseris* sp. (LE), *Pachyseris* sp. (PA) and *Montipora* sp. (MO)

Table 1. *Symbiodinium* ITS2 subcladal types identified on the Great Barrier Reef (GBR) for the genera and species sampled in this study. Types in **bold** were found for the same coral genus in previous studies. Type C3h contains intragenomic variants C3 and C21. RR: Ribbon Reefs; HP: Hydrographers Passage; NP: Noggin Pass

Genus (family)/species	This study		Previous studies[a]		
	Location (depth)	Mesophotic (45–70 m)	Deep (20–30 m)	Intermediate (10–20 m)	Shallow (0–10 m)
Acropora (Acroporidae)			–	C1, **C3**, C3k, D1	C1, C21, **C3**, C3h, C3i, C3k, A, D
Acropora elegans	RR (46–48 m)	**C3** (n = 3)	–	–	–
Montipora (Acroporidae)			–	C26a, C61	**C3**, C15, **C17**, C21, C26, C26a, C31, C73
Montipora spp.	RR (70 m)	**C3** (n = 2)			
	HP (54–55 m)	**C17** (n = 4)			
	NP (54–62 m)	**C3** (n = 1)			
Leptoseris (Agariciidae)			–	C3h, C21	C1
Leptoseris hawaiiensis	RR (70 m)	C3i (C1/C3) (n = 5)	–	–	–
Pachyseris (Agariciidae)			–	C3h, C21	C3h
Pachyseris speciosa	NP (54 –62 m)	C3k (n = 8)	–	C3h, C21	–
Pavona (Agariciidae)			–	C3h, C21	C1, C1b, C3h, C27
Pavona spp.	RR (49–59 m)	C3 (n = 1), C3k (n = 1)	–		
Fungia (Fungiidae)			–	C1, C3h	C1, C21, C3h
Fungia cf. *danai*	HP (54–55 m)	C3i (C1/C3) (n = 1), C3k (n = 2)	–	–	–
Galaxea (Oculinidae)			–	C1, D1a	C1, C21, D1a
Galaxea astreata	HP (54–55 m)	C3 (n = 3)	–	C1, D1a	C1
Echinophyllia (Pectinidae)			–	C1, C3h	C3, C3h, C3ha, C21
Echinophyllia aspera	NP (54–62 m)	C3k (n = 7)	–	–	C3h, C3ha, C21
Seriatopora (Pocilloporidae)			C120, **C3n-t**, C3-ff	C3, **C3n-t**	**C3n-t**, C120, C120a, C1m-aa
Seriatopora hystrix	RR (46–48 m)	**C3n** (n = 1)	C120, **C3n-t**, C3-ff	C3, **C3n-t**	**C3n-t**, C120, C120a, C1m-aa
	HP (54–55 m)	C3n-hh (n = 1)			
Porites (Poritiidae)			**C15**	**C15**, C28, C60	–
Porites spp.	RR (49–70 m)	**C15** (n = 3), C131 (n = 1)	–		
	HP (54–55 m)	*C131* (n = 1)	–		

[a]Loh et al. 2001, van Oppen et al. 2001, 2005, LaJeunesse et al. 2003, 2004, Ulstrup & van Oppen 2003, Fabricius et al. 2004, van Oppen 2004, Ulstrup et al. 2006, 2008, Sampayo et al. 2007, Jones et al. 2008, Stat et al. 2008, Bongaerts et al. 2010a

Mesophotic *Symbiodinium* ITS2 diversity

A total of 7 previously described *Symbiodinium* ITS2 types, i.e. C3, C3i, C3k, C3n, C3n-hh, C15, and C17, were recovered, as well as one new symbiont type, C131 (GenBank #JF320826; Figs. 3 & 4). Four of the 8 total identified *Symbiodinium* types consistently contained 2 to 3 co-dominant ITS2 sequences within a singe ITS2-DGGE fingerprint (C3i, C3k, C3n, C3n-hh; Figs. 3 & 4) across samples and thus likely represent intra-genomic variants within the rDNA of a single symbiont (LaJeunesse 2002, LaJeunesse et al. 2004, Thornhill et al. 2007, Sampayo et al. 2009). In the case of *Symbiodinium* type C3i, it may be possible that a mixed *Symbiodinium* community is present consisting of *Symbiodinium* type C1, C3, and/or C3i (containing both C1 and C3 in its genome), since some variability was seen in relative brightness of the respective DGGE bands between samples (see also discussion in LaJeunesse et al. 2004 for type C3h; Sampayo et al. 2007 for type C33a). However, further investigation into this specific symbiont type with more samples would be necessary to confirm this observation.

Symbiont type C3 was found in *Acropora elegans* (n = 3), *Montipora* spp. (n = 3), *Pavona* sp. (n = 1), and *Galaxea* (n = 3), whereas type C3i was observed in *Leptoseris hawaiiensis* (n = 5) and *Fungia* cf. *danai* (n = 1). Type C3k was identified in *Pachyseris speciosa* (n = 8), *Pavona sp.* (n = 1), *Fungia* cf. *danai* (n = 2), and *Echinophyllia aspera* (n = 7). The remaining *Symbiodinium* types were each only observed in a single species, with C3n (n = 1) and C3n-hh (n = 1) found in association with *Seriatopora hystrix*, C17 with *Montipora* sp. (n = 4), and C15 (n = 3), and C131 (n = 2) in association with *Porites* spp. Although the sensitivity of ITS2-DGGE limits detection of *Symbiodinium* present in low abundances (Mieog et al. 2007), it does provides an accurate representation of the dominant symbiont present within a coral colony (LaJeunesse et al. 2003, Frade et al. 2008a, Sampayo et al. 2009).

Shallow versus mesophotic *Symbiodinium* ITS2 community diversity

Except for the novel type C131, all *Symbiodinium* types observed at mesophotic depths in the present study have been reported previously in shallow sections of the GBR (Fig. 4). *Symbiodinium* types C3n, C15, and C17 were observed at mesophotic depths in the respective host genera *Seriatopora*, *Porites*, and *Montipora*, and have previously been observed for these same genera in shallow sections of the GBR (Table 1). Similarly, *Symbiodinium* type C3 was found in association with *Acropora* and *Montipora* at mesophotic depths, and has been reported in these same genera in shallow waters. However, C3 was also observed in deep-water *Galaxea* and *Pavona*, whereas in shallow water these host genera have only been reported in association with symbionts

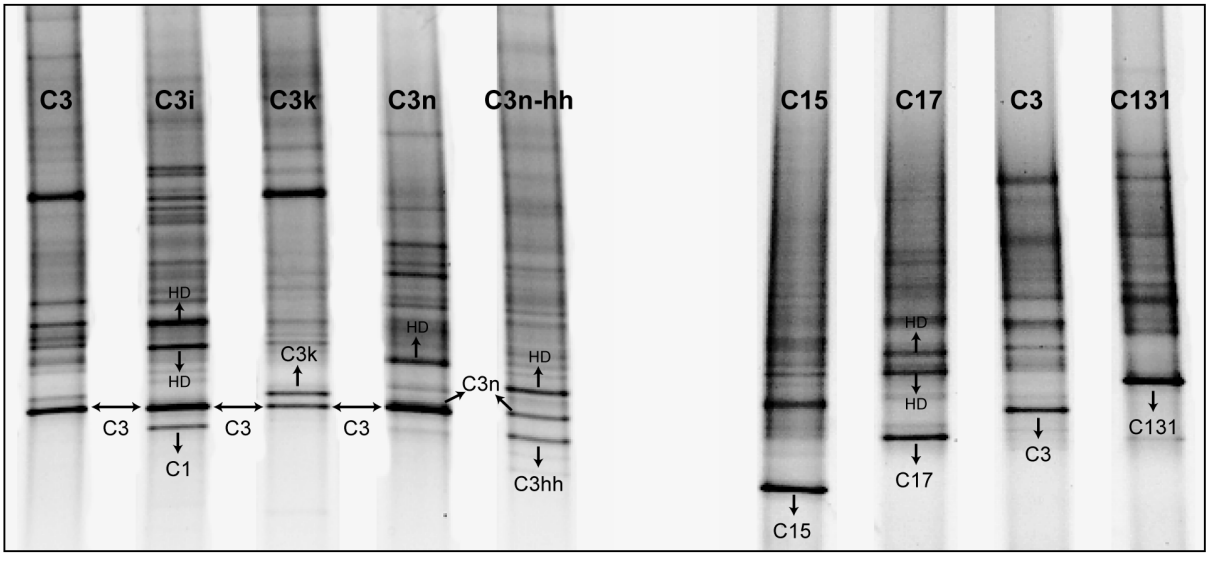

Fig. 3. Denaturing gradient gel electrophoresis gels of *Symbiodinium* ITS2 rDNA showing the 8 distinct *Symbiodinium* profiles observed in this study: (left) C3, C3i, C3k, C3n, and C3n-hh; (right) C15, C17, C3, and C131. Characteristic sequences used to identify each symbiont type are shown adjacent to bands in the gel image (note that C3 and C3n co-migrate to the same position; see also Sampayo et al. 2007). HD = heteroduplex

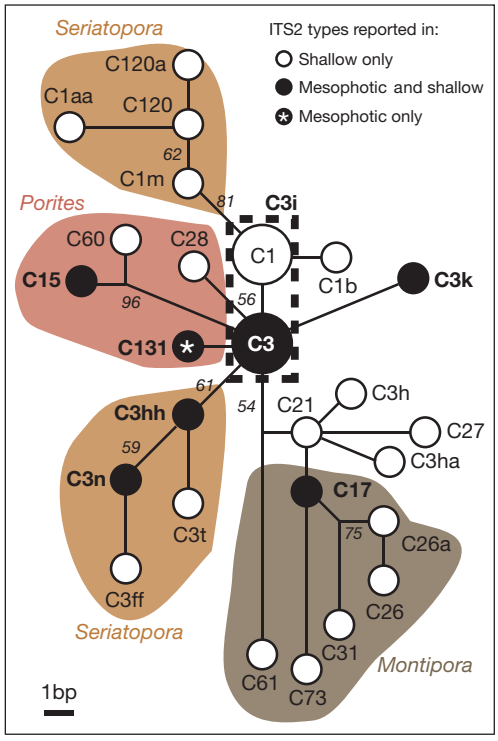

Fig. 4. Most parsimonious (MP) unrooted tree of ITS2 *Symbiodinium* types identified in mesophotic (>30 m) and shallow (<30 m) corals on the Great Barrier Reef (GBR) for the genera sampled in this study. Ancestral types C1 and C3 are represented by larger circles for illustrative purposes only and circle size is not representative of frequency. *Symbiodinium* C3i is characterized by both the C1 and C3 sequence (indicated by a dashed box). Branch distance is represented by length of connections, and MP bootstrap values higher than 50 are shown. Symbiont types depicted in white have been described only in shallow water, whereas those in black have been observed in both shallow and mesophotic coral colonies. The novel symbiont type (C131) is depicted in black with an asterisk and was only found in mesophotic corals in this study. Shaded areas group *Symbiodinium* types that are host-specific (species or genus)

such as C1 and D1a (*Galaxea*), and C1, C1b, C21, C27, and C3h (*Pavona*) (Table 1). *Symbiodinium* types C3i and C3k were found at mesophotic depths in respectively 2 (*Leptoseris* and *Fungia*) and 4 different host genera (*Pachyseris*, *Pavona*, *Fungia,* and *Echinophyllia*), but have not been reported in these host genera at shallow depths, where these genera are found instead in association with types such as C1, C3h, and C21 (Table 1). Lastly, *Symbiodinium* type C131 (identified in deep-water *Porites*) has not been previously reported but is closely related to C3 as opposed to the C15 radiation (that includes C15b through C15m) with which *Porites* spp. commonly associates at shallow depths (Fig. 4).

DISCUSSION

Lack of a differentiated deep-water *Symbiodinium* community

The present study represents a first assessment of *Symbiodinium* diversity at mesophotic depths of the GBR, and, although limited in sample size, demonstrates a lack of a differentiated mesophotic symbiont community. Except for the novel *Symbiodinium* type C131 encountered in 2 *Porites* specimens, all symbiont types observed across the 10 different genera in this study have been reported previously in shallow-water habitats of the GBR. The pandemic (e.g. LaJeunesse 2005) and host-generalist *Symbiodinium* type C3 that occurs across a wide range of host genera at both shallow and intermediate (<20 m) depths on the GBR (LaJeunesse et al. 2003, 2004) was also found in mesophotic samples of *Acropora elegans*, *Montipora* spp., *Pavona* spp., and *Galaxea astreata*. *Symbiodinium* types C3i, C3k, C3n, and C3n-hh are all closely related to the C3 type (still contain the C3 sequence as a co-dominant repeat within the ribosomal array; Fig. 3) and are frequently reported in shallow-water specimens of *Acropora* spp. (C3i and C3k; LaJeunesse et al. 2003, 2004) and *Seriatopora hystrix* (C3n and C3n-hh; Sampayo et al. 2007, Stat et al. 2008, Bongaerts et al. 2010a, Wicks et al. 2010), respectively. Although putative 'deep-specialist' *Symbiodinium* types have been identified in species such as *Madracis pharensis* (40 to 60 m), *M. formosa* (40 to 60 m) (Frade et al. 2008a,b), and *Montastraea cavernosa* (46 to 91 m) in the Caribbean (Lesser et al. 2010), our results demonstrate, across a range of coral genera, that association with such specialists is not a prerequisite to survive under the extreme, low-light conditions encountered in MCEs.

Instead, several *Symbiodinium* types commonly found in shallow sections of the GBR appear to have bathymetric distribution ranges that extend well into the mesophotic zone, with *Symbiodinium* C3 and C3i occurring down to 70 m and types C3k, C3n, C3-hh, C15 and C17 observed at depths over 50 m. Although these represent the largest bathymetric ranges reported for *Symbiodinium* types on the GBR, similarly large depth ranges (>50 m) for certain *Symbiodinium* types (e.g., C1b, C1c, C3, and C27) have been observed in other parts of the world (LaJeunesse 2005, Chan et al. 2009, Cooper et al. 2011a). In fact, a recent study by Wagner et al. (2011) reveals the presence of several *Symbiodinium* types common to shallow-water (including C15) in several species of Antipatharia ('black corals') at extreme depths rang-

ing from 100 to 400 m. The occurrence of the 7 *Symbiodinium* types identified here across depth ranges of 50 m on the GBR supports the notion that certain *Symbiodinium* types can have very broad bathymetric distribution ranges (Wagner et al. 2011); however, this, of course, may be dependent on the specific host they associate with.

Novel host-*Symbiodinium* associations at mesophotic depths

Although the mesophotic *Symbiodinium* community showed little indication of differentiation in terms of ITS2 diversity when compared to shallow-water habitats, more than half of the observed combinations of host species and *Symbiodinium* types represented novel partnerships. For example, *Symbiodinium* type C3 occurs across a wide range of different genera in shallow water (e.g. LaJeunesse et al. 2003, 2004), but was here identified for the first time on the GBR in association with species in the genera *Pavona* and *Galaxea* (Table 1). *Pavona* spp. do associate with the closely related C3h on shallow habitats of the GBR, and both genera are found in symbiosis with C3u, which is a generalist in the Andaman Sea near Thailand (LaJeunesse et al. 2010). Similarly, *Symbiodinium* types C3i and C3k were found previously only in association with shallow-water *Acropora* spp. (LaJeunesse et al. 2003, 2004), but are observed here at mesophotic depths across 5 different genera (Table 1). Thus, the apparent lack of differentiation observed for the *Symbiodinium* community in terms of species diversity in the mesophotic habitat can be seen in a different light when considering both symbiotic partners (the holobiont). This observation highlights the importance of integrative approaches looking at both symbiotic partners to determine community similarity, but also demonstrates that the distribution range of *Symbiodinium* types can be strongly dependent on the host species they are associated with and vice versa.

In contrast to the novel host-symbiont partnerships, specimens of the genera *Porites, Montipora,* and *Seriatopora* associated with the same genus-specific lineages of *Symbiodinium* as in shallow water (Table 1). *Porites* and *Montipora* associated with *Symbiodinium* types C15 and C17, respectively, that are also common to shallow-water specimens of these species across the Indo-Pacific Ocean (LaJeunesse 2005, LaJeunesse et al. 2003, 2004, 2010). However, *Seriatopora hystrix* was found at mesophotic depths in association with C3n, which is in line with a depth zonation reported for the northern GBR where *S. hystrix* associates with C120 in the shallow (<7 m) and C3n-t in deeper water (>24 m) (Bongaerts et al. 2010a). However, C3n-t is commonly observed in shallow water on the southern GBR (Sampayo et al. 2007, Stat et al. 2008) and the closely related C3n-hh (observed here in *S. hystrix* at ~55 m) has recently been reported in shallow water at Lord Howe Island (Wicks et al. 2010). Potentially, these host-symbiont associations thrive under specific environmental conditions such as lower light, increased heterotrophic resources, or distinct temperature regimes, which are not unique to deeper water and can also be found in shallow water at higher latitudes or in marginal habitats.

Factors driving the distribution of host-*Symbiodinium* associations

Bathymetric zonation patterns of *Symbiodinium* are often equated to partitioning across a light gradient (Iglesias-Prieto et al. 2004, Finney et al. 2010), and clear photo-physiological trade-offs have been described for coral species hosting divergent symbiont types in relation to light availability. For example, in the Caribbean, *Madracis pharensis* exhibits a shift from *Symbiodinium* type B7 to B15 over depth (Frade et al. 2008a). B7 has a competitive advantage in high-light due to the presence of specific photoprotective pathways, and B15 is advantageous under low-light due to its larger and highly pigmented cells (Frade et al. 2008b,c). Similarly, *Seriatopora hystrix* in Western Australia exhibits a cladal symbiont shift over depth (from clade D to C), where hosting clade D offered a competitive advantage in high-irradiance environments with greater energy stores in the form of storage lipids, but at increased metabolic cost over depth (Cooper et al. 2011a,b, van Oppen et al. 2011). Nonetheless, the finding that certain *Symbiodinium* types can occur across a broad range of light conditions ranging from near the surface to mesophotic depths, indicates that other environmental factors may be equally as important in driving the distribution of host-*Symbiodinium* associations. Although direct evidence is lacking, bathymetric differences in temperature regimes (Winters et al. 2009, Cooper et al. 2011a), light spectra (Mass et al. 2010), and/or increased availability of heterotrophic resources (Lesser et al. 2010) are likely to be equally important in the distribution host-*Symbiodinium* associations and may explain some of the observed overlap between shallow and deep communities.

Regional and latitudinal variation in environmental variables are known to drive geographic distribution patterns of host-*Symbiodinium* associations in shallow water (LaJeunesse et al. 2010) and may be equally important in driving spatial variation at mesophotic depths. Although the differences in *Symbiodinium* types observed for *Montipora*, *Seriatopora,* and *Porites* at different locations (Table 1) are not sufficiently replicated here to distinguish potential effects of depth, location, or inter-specific differences, there are likely to be differences reflective of distinct local environmental conditions. In particular, Hydrographers Passage appears distinct from the other 2 locations as it experiences strong currents and high chlorophyll concentrations in the water column, which would result in higher nutrient concentrations as well as limit the availability of light at depth (Bridge et al. 2011a,b). Additionally, localized upwelling affects temperature and nutrient availability in deeper water (Wolanski & Pickard 1983), and has been observed previously on the Ribbon Reefs (Bongaerts et al. 2010a, Bongaerts et al. unpubl. data). Nonetheless, future studies are needed to assess how specific localized and latitudinal features may affect host-*Symbiodinium* diversity on mesophotic reefs.

Host-symbiont specificity and symbiont acquisition mode

The observation of previously identified 'host-specialists' *Symbiodinium* types C3i and C3k across 5 different genera other than *Acropora* at mesophotic depths, highlights the importance of surveying *Symbiodinium* diversity across a broad range of environments to get a better understanding of specificity in host-symbiotic associations, and the environmental tolerance ranges associated with each partnership. Nonetheless, the observation that several host-specific *Symbiodinium* lineages are also present at mesophotic depths indicates that host-specificity can be maintained across large depth ranges. Although the present study clearly demonstrates that our knowledge on host-specificity and distribution ranges is likely to change as increased information becomes available, the extensive studies and widespread maintenance of specificity observed in *Symbiodinium* types associated with *Seriatopora*, *Montipora,* and *Porites* (extending here into mesophotic depths, types C3n, C15, and C17) support the idea that host specificity is strengthened through the process of vertical symbiont acquisition (symbionts transferred maternally) utilized by these particular coral species (Loh et al. 2001, van Oppen 2004, Sampayo et al. 2007, Stat et al. 2008). In contrast, the coral genera found here to associate with host-generalist *Symbiodinium* types (C3, C3i, and C3k), i.e. *Acropora*, *Leptoseris*, *Pachyseris*, *Pavona*, *Fungia*, *Galaxea*, and *Echinophyllia*, are all broadcast spawners (release sperm/egg bundles into the water column) that transfer symbionts horizontally (symbionts acquired from the environment).

Given the strong differences in prevailing reproductive modes in the Caribbean versus the Indo-Pacific (Richmond & Hunter 1990), we hypothesize that there may be differences in the extent of specialization of the *Symbiodinium* community to deep-water habitats in these different regions of the world, as the vast majority of Indo-Pacific corals are broadcast spawners. In contrast, a brooding reproductive mode (generally but not exclusively coupled to vertical symbiont acquisition) predominates in the Caribbean, and appears to be the exclusive mode of reproduction in Caribbean 'deep-specialist' coral species (Bongaerts et al. 2010b). However, the extent to which prevailing coral reproduction modes affect *Symbiodinium* diversity on mesophotic reefs can only be assessed as increased information becomes available from these understudied habitats.

CONCLUSIONS

The ability of deep reefs to act as a source of propagules for their shallow-water counterparts largely depends on the genetic similarity of corals and their associated *Symbiodinium* at their bathymetric extremes. In this first assessment, there was little indication of a differentiated deep-water *Symbiodinium* community, with the majority of corals associating with pandemic *Symbiodinium* types also commonly found in shallow water. Novel host-symbiont associations were only observed in species with a horizontal symbiont acquisition mode, whereas species with a vertical symbiont acquisition mode harbored previously described host-specific lineages of *Symbiodinium* (with the exception of the novel symbiont C131). These observations indicate that mesophotic reefs can harbor coral-*Symbiodinium* combinations similar to those in shallow water, which appears hopeful with regards to the ability of deep reefs to act as a source for recruits in shallow water. Nonetheless, it must be noted that the coral host and associated *Symbiodinium* may still be differentiated on the population level (Barshis et al. 2010, Bongaerts et al. 2010a, Finney et al. 2010). As we move forward with

research on MCEs and their potential role as refugia, integrative molecular approaches looking at both *Symbiodinium* and host population structure will be important to gain a better understanding of how different life history strategies (e.g. reproductive and symbiont acquisition modes) affect levels of connectivity between shallow and deep habitats. Finally, the present study demonstrates the importance of examining symbiont diversity across broad environmental ranges (including mesophotic habitats), in order to improve our understanding of specificity in the coral-algal symbiosis.

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