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Symbiont specificity and bleaching susceptibility among soft corals in the 1998 Great Barrier Reef mass coral bleaching event

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Abstract Considerable variability in bleaching was observed within and among soft coral taxa in the order Alcyonacea (Octocorallia: Cnidaria) on the central Great Barrier Reef (GBR, latitude 18.2°–19.0°S, longitude 146.4°–147.3°E) during the 1998 mass coral bleaching event. In April 1998, during a period of high sea surface temperatures, tissue samples were taken from bleached and unbleached colonies representative of 17 soft coral genera. The genetic identities of intracellular dinoflagellates (*Symbiodinium* spp.) in these samples were analyzed using PCR-denaturing gradient gel electrophoresis fingerprinting analysis of the internal transcribed spacer regions 1 and 2. Alcyonaceans from the GBR exhibited a high level of symbiont specificity for *Symbiodinium* types mostly in clade C. A rare clade D type (*D3*) was associated only with *Clavularia koellikeri*, while *Nephthea* sp. hosted symbionts in clade B (*B1n* and *B36*). Homogenous *Symbiodinium* clade populations were detected in all but one colony. Colonies that appeared bleached possessed symbiont types that were genetically indistinguishable from those in non-bleached conspecifics. These data suggest that parameters other than the resident endosymbionts such as host identity and colony acclimatization are important in determining bleaching susceptibility among soft corals.

Introduction

Exposure to high sea temperature, high levels of photosynthetically active radiation and ultraviolet light (UV), as well as sedimentation and low salinity, can disrupt intracellular symbioses that coral reef cnidarians have with dinoflagellates in the genus *Symbiodinium*, also known as zooxanthellae (Fitt et al. 2001; Douglas 2003). This disruption can lead to the loss of zooxanthellae, a phenomenon termed coral bleaching (reviewed in Coles and Brown 2003). The extent to which hosts lose their populations of symbiotic dinoflagellates varies among species (Hoegh-Guldberg and Salvat 1995; Fabricius 1999; Loya et al. 2001; Douglas 2003), within species (Lasker et al. 1984; Porter et al. 1989; Glynn 1990; Berkelmans and Oliver 1999; Ulstrup et al. 2006; LaJeunesse et al. 2007), and sometimes between regions of a colony (Rowan and Knowlton 1995; Rowan et al. 1997). This variability has been attributed to the thermal tolerance of the symbiont (Berkelmans and van Oppen 2006; Ulstrup et al. 2006), differences in host tolerance (Loya et al. 2001; Brown et al. 2002b; Bhagooli and Hidaka 2004; D'Croz and Maté 2004), or particular host–symbiont combinations (holobionts) (Rowan 2004; Goulet et al. 2005).

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Episodes of mass coral bleaching, involving the relatively sudden and widespread symbiont reduction in host tissues, have increased in frequency and intensity over the past few decades (reviewed in Coles and Brown 2003; Wilkinson 2004). In 1998, mass coral bleaching occurred across the Indo-Pacific and was one of the worst on record (Hoegh-Guldberg 1999). For the Great Barrier Reef (GBR), mild bleaching was initially recorded in late January 1998. By April, 87% of inshore reefs and 28% of offshore reefs exhibited bleaching (Berkelmans and Oliver 1999). The high temperatures of 1998 affected not only scleractinian corals but also severely impacted many soft coral species (Fabricius 1999).

Octocorals or soft corals are important constituents of coral reef ecosystems (Goldberg 1973; Kinzie 1973; Kinzie 1974; Muzik 1982; Lasker and Coffroth 1983; Sánchez et al. 1997; Fabricius and Alderslade 2001; Sánchez et al. 2003). Few studies, however, have examined how they and their symbionts differentially respond to environmental stress (Fabricius 1999; Strychar et al. 2005). To assess the relative sensitivities of zooxanthellate octocorals to thermal stress, communities were surveyed at numerous reefs in the central GBR system during the 1998 sea surface heating event. Samples of bleached and unbleached tissues were collected for genetic analyses of resident *Symbiodinium* populations. This work was undertaken to determine whether differences in bleaching susceptibility between species, among individuals of a species, and within colonies could be attributed to different endosymbiotic dinoflagellates.

Materials and methods

Soft coral collections

In 1998, bleaching on the GBR started in late January, and by April it was severe in many locations (Berkelmans and Oliver 1999). During the height of the bleaching event, on April 6–13, 1998, video transects and collection of tissue samples from alcyonacean colonies were conducted on nine reefs in the central section of the GBR (Fig. 1). These reefs represented inshore (Phillips, Pandora, Fantome, and Great Palms Island) and offshore (Lodestone, John Brewer, Bramble, Faraday, Myrmidon) reefs.

On each of the reefs, three to four belt-transects (0.6 × 50 m) were video-recorded at 2 m and 5–6 m depth following Abdo et al. (2003). Alcyonacean and scleractinian corals underneath 5 fixed points on 40 stops of the video per transect (200 points per transect) were identified to genus level where possible, pooling some morphologically similar genera that were indistinguishable on video (e.g., *Efflatounaria* and *Cespitularia*). The bleaching extent of each colony was categorized as "not bleached," "partially bleached" (either parts of the colony bleached, or the whole colony clearly paler than typical for that genus), and "totally bleached" (Hoegh-Guldberg and Salvat 1995). Video surveys were complemented by visual surveys of the extent of bleaching in each genus along each of the transects (Fabricius, unpublished data), and the two independent estimates showed a high level of agreement. Bleaching estimates for *Stereonephthya* and members of the family

Fig. 1 Map of central Great Barrier Reef with locations of nine sampled reefs. Tissue samples were collected from 17 alcyonacean genera, April 6–13, 1998, at the height of the bleaching event
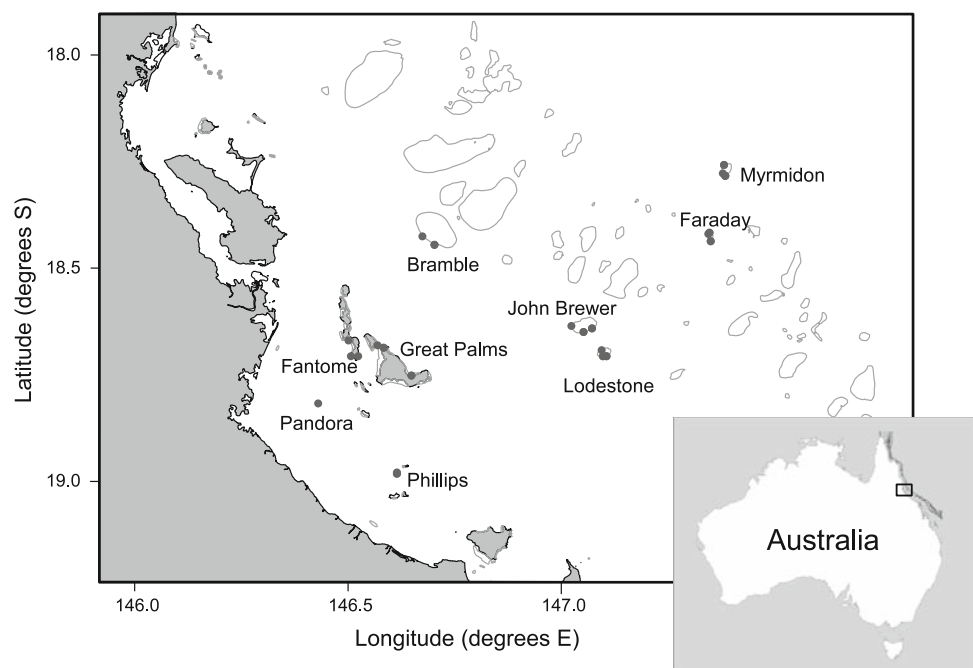
Xeniidae were afflicted with a degree of uncertainty due to their natural light pigmentation, an issue that did not affect any other genera. A reduction in zooxanthella densities in bleached colonies was confirmed with zooxanthella counts (K. Michalek-Wagner, unpublished data).

For DNA analysis, 88 colonies, belonging to 20 species in 17 genera (7 families) of alcyonaceans, were sampled at the beginning of the video transects on inner- and outer-shelf reefs at both 2 and 5–6 m depth. Within sites, colonies were collected (Table 1) at least 2 m apart to avoid sampling clone mates. Bleached colonies were found for 12 of these genera. Colonies were sampled by removing 1–2 cm^2 of coral tissue from the tip (capitulum) in branching species, or an equivalent amount of tissue from encrusting forms. Since different areas of a colony may host different *Symbiodinium* (Rowan et al. 1997), for colonies belonging to 12 branching species in 10 genera, samples were also collected from the colony bases (stems) (Table 2) for within-colony *Symbiodinium* comparison. Tissue samples were placed in 1.5-ml microcentrifuge tubes and preserved in 95% ethanol. Since small sample sizes per cnidarian species are sufficient to detect *Symbiodinium* variability in cnidarians (Goulet 2007), and larger octocoral sample sizes

do not uncover additional *Symbiodinium* variability (Baker and Romanski 2007), we opted to sample more representative genera with smaller samples sizes as opposed to focusing on only a few alcyonacean species.

PCR-DGGE and sequencing

A 0.5-cm^2 piece of colony tissue was placed in a 1.5-ml microcentrifuge tube and DNA was extracted and re-suspended following the protocol of Goulet and Coffroth (2003a). The internal transcribed spacer (ITS) regions 1 and 2 were amplified from each extract using the respective forward and reverse primer sets, "ITS 1 clamp" (CGCCC GCCGC GCCCCGCGCC CGTCCCGCCG CCCCCGCCC GGGATCCGTT TCCGTAGGTG AACCTGC) and "ITSint-rev2" (TTC ACG GAG TTC TGC AAT) (LaJeunesse et al. 2008); and "ITS 2 clamp" and "ITSintfor2" (LaJeunesse and Trench 2000) with the touch-down thermal cycle as described in LaJeunesse et al. (2003). Products from these PCR reactions were electrophoresed on denaturing gradient gels (50–90% urea-formamide for ITS 1 and 45–80% for ITS 2) using a CBScientific system (Del Mar, CA, USA).

Table 1 The *Symbiodinium* type found in Alcyonacean species sampled during the 1998 mass bleaching event (April 1998) on the Great Barrier Reef

Host taxon		Rank	*Symbiodinium* "type" in colonies (*n*)	
Family	Species		Bleached	Unbleached
Clavulariidae	*Clavularia koellikeri* (6)	L	D3 (1)	D3 (5)
Alcyoniidae	*Sinularia cf polydactyla* (6)	M	C1:3a (2)	C1:3a (4)
	Sinularia flexibilis (8)	M	C1:3a (2)	C1:3a (6)
	Sarcophyton sp. (10)	M	C1:3a (4)	C1:3a (6)
	Lobophytum compactum (7)	M	C1:3a (3)	C1:3a (4)
	Rhytisma sp. (1)	L	n/s	C1:1 (1)
Nephtheidae	*Nephthea* sp. (5)	M	B1n (2); B36 (1)	B1n (1), C1:2&B36 (1)
	Stereonephthya sp. (1)	L	n/s	C15 (1)
	Lemnalia sp. (3)	L	n/s	C1:2 (3)
	Paralemnalia digitiformis (3)	L	C1:2 (1)	C1:2 (1), C64 (1)
	Paralemnalia thyrsoides (4)	L	C1:2 (1)	C1:2 (3)
Xeniidae	*Xenia* sp. (3)	H	C64 (1)	C64 (1), C15 (1)
	Heteroxenia sp. (1)	H	n/s	C64 (1)
	Cespitularia sp. (6)	H	C15 (4)	C15 (1), C1q (1)
	Efflatounaria sp. (7)	H	C1c (2), C1:3a (1)	C1c (4)
	Anthelia sp. (1)	H	C84a (1)	n/s
	Asterospicularia laurae (2)	M	n/s	C15 (2)
Briareidae	*Briareum* sp.1 (7)	L	C1:1a (4), C1:2 (1)	C1:1a (2)
	Briareum sp. 2 (6)	L	C1:1a (1)	C1:1a (5)
Gorgoniidae	*Pinnigorgia flava* (1)	L	C1:1&C1:2 (1)	n/s

Bleaching susceptibility of an Alcyonacean species was ranked as low (L), medium (M) or high (H) based on the video transects, field observations, and published literature (Fabricius 1999; Strychar et al. 2005). Types co-occurring in the same colony are denoted with the symbol "&". *n/s* no sample, *n* number of colonies

Table 2 Alcyonacean colonies that were sampled both at the tip (or capitulum) and the stem (base) of the colony during the 1998 mass bleaching event on the Great Barrier Reef

Host taxon		*Symbiodinium* "type" in samples (*n*)			
		Tip		Stem	
Family	Species	Bl	U	Bl	U
Alcyoniidae	*Sinularia cf polydactyla*	C1:3a (1)	C1:3a (1)	C1:3a (1)	C1:3a (3)
	Sinularia flexibilis		C1:3a (3)	C1:3a (2)	C1:3a (3)
	Sarcophyton sp.	C1:3a (3)	C1:3a (3)	C1:3a (1)	C1:3a (3)
	Lobophytum compactum	C1:3a (2)	C1:3a (3)	C1:3a (3)	C1:3a (3)
Nephtheidae	*Nephthea* sp.	B1n (2); B36 (1)	B1n (1), C1:2&B36(1)	n/s	B1 (1)
	Lemnalia sp.	n/s	C1:2 (2)	n/s	C1:2 (2)
	Paralemnalia digitiformis	n/s	C1:2 (1), C64 (1)	n/s	C1:2 (1), C64 (1)
	Paralemnalia thyrsoides	C1:2 (1)	C1:2 (3)	n/s	C1:2 (2)
Xeniidae	*Cespitularia* sp.	C15 (2)	n/s	C15 (3)	C15 (1), C1q (1)
	Efflatounaria sp.	C1c (2)	C1c (4)	C1c (1), C1:3a (1)	n/s
	Asterospicularia laurae	n/s	C15 (1)	n/s	C15 (2)
Gorgoniidae	*Pinnigorgia flava*	C1:1 (1)	C1:1 (1)	C1:2 (1)	n/s

Bl bleached, *U* unbleached, *n* number of samples, *n/s* no sample

As described by LaJeunesse (2002) and LaJeunesse et al. (2003), prominent bands in the lower half of the fingerprint were excised, reamplified, and directly sequenced. The production of PCR-denaturing gradient gel electrophoresis (DGGE) generated rDNA fingerprints, followed by the excision and sequencing of the most diagnostic (brightest) bands, introduces a critical step for filtering out rare functional and non-functional variants. Bacterial cloning is known to retrieve numerous functional intragenomic variants, pseudogenes, and PCR artifacts that often deviate from sequences generated by direct sequencing or from the sequencing of dominant bands found in PCR-DGGE profiles (Thornhill et al. 2007). For this reason, PCR-DGGE analysis of rDNA was used to identify the sequence of the numerically dominant copy (or copies) that characterizes the genome. These fingerprints are often dominated by one or several codominant variants whose sequences usually differ by one or two base changes. This technique also identifies the existence of mixed symbiont populations present in a host (LaJeunesse 2002; Thornhill et al. 2006b).

Phylogenetic analyses

Sequences were aligned manually using the software Sequence Navigator version 1.0 (ABI, Division of Perkin Elmer, Foster City, CA, USA). Base substitutions in the ITS rDNA of *Symbiodinium* clade C are far from saturation (LaJeunesse 2004); therefore, a maximum parsimony (under heuristic search mode) analysis was conducted using PAUP* (Swofford 2000). Maximum parsimony does not assume a particular model of molecular evolution and allows for use of informative sequence gaps and insertions (one entire gap or deletion, indel, is scored as a fifth character state). Applications of neighbor-joining, maximum likelihood, and Bayesian inference methods, which do not take into account indels, have consistently yielded similar phylogenetic reconstructions using these datasets (LaJeunesse 2004).

Results

In many octocoral taxa, completely bleached colonies were found adjacent to conspecifics that appeared only partially bleached or unbleached. The video transects showed that across all taxa, on average 43% of inshore colonies and 21% of offshore colonies, were partially or totally bleached. The percentage of bleached colonies differed substantially among taxa during the height of the bleaching event. For example, 72% of *Lobophytum* colonies, and 85% of *Sinularia flexibilis* colonies were partially or totally bleached (Table 1, and online supplementary material). In contrast, <10% of colonies of *Briareum* spp., *Rhytisma fulvum*, *Lemnalia* spp., and *Paralemnalia* spp. were partially or totally bleached. Observations showed that Xeniidae tended to die within a few days after the onset of bleaching and were therefore likely to be under-represented in the video data, while bleached Alcyoniidae were quite long-lived and potentially over-represented in the data (Fabricius 1999). Our estimates of bleaching susceptibility (Table 1) were

based on these and other published observations as well as the video records.

Separate PCR-DGGE fingerprint profiles for both ITS regions 1 and 2 were employed to characterize *Symbiodinium* populations in bleached and normally pigmented soft coral colonies. The combined analysis improved resolution of closely related types and discriminated between profiles where dominant bands of unlike sequences happened to comigrate (e.g., *C15* and *B1n*, Fig. 2). For some symbionts, the sequences of the diagnostic ITS 1 band possessed more base substitutions than its ITS 2 counterpart and vice versa (Figs. 2, 3).

The combined analysis of the ITS 1 and ITS 2 regions using rDNA-DGGE yielded 12 genetically distinctive *Symbiodinium* types consisting of two clade B types, nine clade C, and only one clade D type (Table 1, Fig. 2). In this subclade nomenclature, the capital letter designates the clade, and the numbers and lower case letters indicate the dominant
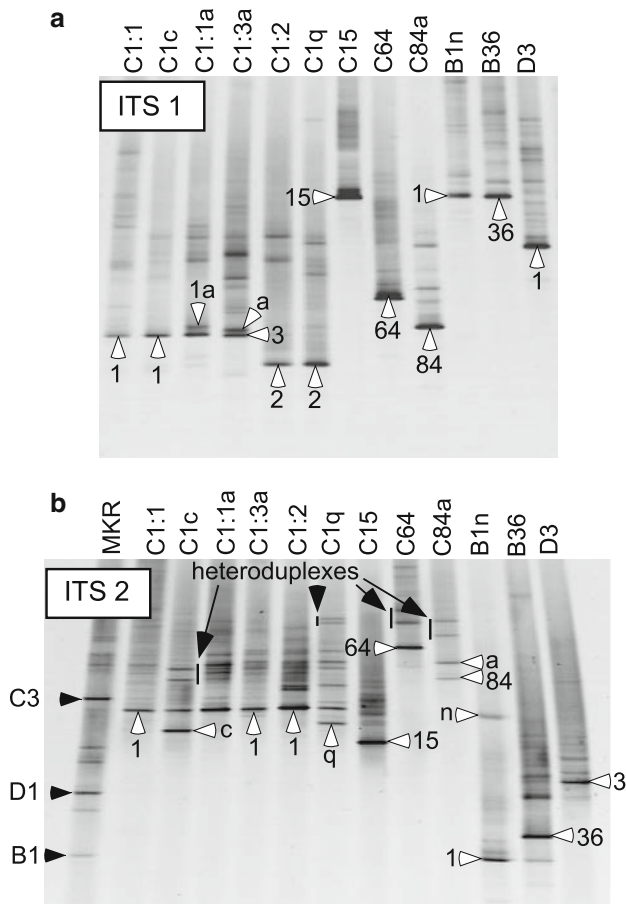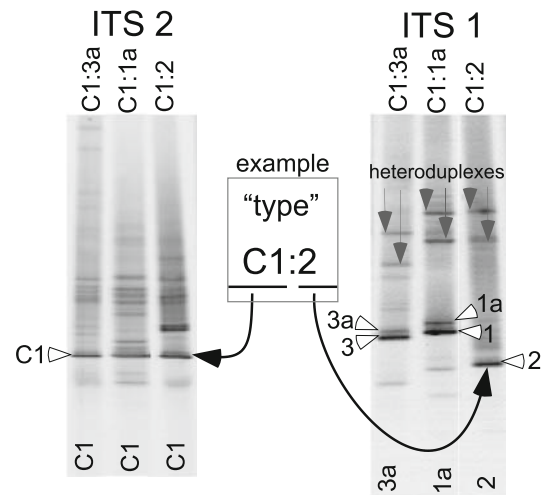

Fig. 3 Guide to the expanded alphanumeric taxonomy of genetically distinctive *Symbiodinium* populations. In this example, three slightly different yet conservatively classified *C1* types, as determined by PCR-DGGE fingerprinting analysis of the ITS 2, are clearly distinguished by three different fingerprint profiles using this same analysis on the ITS 1. The prominent band in the *middle lane* of the ITS 1 analysis contains the analogous ancestral *C1* sequence and is designated 1 the presence of a second diagnostic band is given the lowercase letter *a*

intragenomic ITS sequence characteristic of an ecologically distinct *Symbiodinium* sp. In several notable cases (*C1:1a* and *C1:3a*; *C1:2* and *C1q*; *B1n* and *B36;* see Table 1 for hosts), subtle yet repeatable differences existed among ITS 2 fingerprints that contained the *C1* sequence as the dominant diagnostic band (Figs. 2, 3). The ITS 1 profiles of these samples, however, were distinguishable because the sequences of the diagnostic band, or bands (i.e. the dominant sequence variant, or variants, in the genome), were divergent (Fig. 5). In keeping with the current nomenclature (sensu LaJeunesse 2002), for situations where the ITS 1 enhanced genetic resolution among *Symbiodinium* exhibiting ecological differences (i.e., host specificity), a colon followed by a second numeric was placed after the ITS 2 designation. Therefore, while the genera *Sinularia*, *Briareum* and *Paralemnalia* hosted *Symbiodinium* containing the same dominant ITS 2 sequence, ITS 1 fingerprints showed with greater clarity that their symbiont populations were genetically different and highly specific (Fig. 4). More importantly, the divergent sequences of the diagnostic band from ITS 1 fingerprints allowed ecologically distinct populations to be phylogenetically differentiated (see below).

Alcyonaceans in the GBR exhibited high host–symbiont specificity (Table 1, Fig. 4). Within clade C, certain *Symbiodinium* types were specific to particular soft coral lineages. For example, type *C1:3a* was found in the four genera (5 species) sampled from the family Alcyoniidae while a closely related type, *C1:1a*, occurred only in *Briareum* spp. colonies (Table 1, Fig. 4). Indicative of the consistency of

Fig. 2 *Symbiodinium* types found within octocorals from the Great Barrier Reef. PCR-denaturing gradient gel electrophoresis (*DGGE*) fingerprinting analysis of the internal transcribed spacer (*ITS*) regions 1 (**a**) and 2 (**b**). *Open arrowheads* indicate the prominent bands diagnostic of a particular profile that are excised and sequenced for subsequent phylogenetic reconstructions
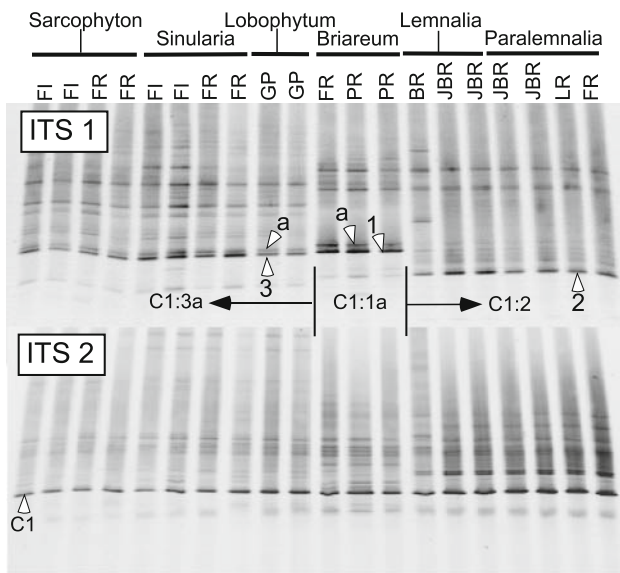
Fig. 4 The *Symbiodinium* type(s) found within specific octocoral host taxa. A comparison of PCR-DGGE fingerprinting analysis of the ITS regions 1 and 2. *FI* Fantome Island, *FR* Faraday Reef, *GP* Great Palms, *PR* Phillips Reef, *BR* Bramble Reef, *JBR* John Brewer Reef, *LR* Lodestone Reef

Fig. 5 Phylogenetic reconstructions using maximum parsimony of clade C *Symbiodinium* spp. obtained from Great Barrier Reef Alcyonaceans. **a** Comparison between reconstructions based on ITS 1 and ITS 2 sequences of diagnostic bands excised from PCR-DGGE fingerprints. Each terminal branch represents a distinct symbiont population. **b** Phylogeny based on a combination of ITS 1 and ITS 2 data. Host genera of colonies from which each symbiont were identified are listed

these relationships, the same *Symbiodinium* type was found in colonies of the same species from reefs separated by distances of up to 50 km (Fig. 4). Colonies of a given species hosted the same *Symbiodinium* type in bleached and unbleached colonies (Table 1), and different parts of a colony hosted the same symbiont population (Table 2). For example, both bleached and unbleached *Clavularia koellikeri* possessed *Symbiodinium* type *D3*.

Variability in symbiont type from colony to colony was observed in seven genera. These included *Paralemnalia* (*C1:2* or *C64*), *Xenia* (*C15* or *C64*), *Cespitularia* (*C15* or *C1q*), and *Efflatounaria* (*C1c* or *C1:3a*) with one symbiont usually being more prevalent among host individuals than the other (Table 1). *Nephthea* sp. hosted two *Symbiodinium* types belonging to clade B (*B1n* and *B36*). A mixed symbiont population consisting of two types from different clades (*B36* and *C1:2*) was found in one colony of *Nephthea* sp. (Table 1).

Phylogenetic reconstructions of clade C diversity based on ITS 1 and ITS 2 sequences of diagnostic bands from PCR-DGGE fingerprints were essentially identical, but differed in their overall resolution (Fig. 5a). This is mainly due to situations where certain ITS 2 fingerprints, while distinctive from each other, contained the *C1* sequence as the diagnostic band (Fig. 3). While these ITS 2 profiles possessed fixed differences in faint band patterns, none were deemed bright enough to define the profile (as discussed above). For example, during the electrophoresis of a weak PCR reaction, these faint bands may not always be resolved in the resulting fingerprint. In contrast, the ITS 1 profiles produced diagnostic bands that differed from each other by
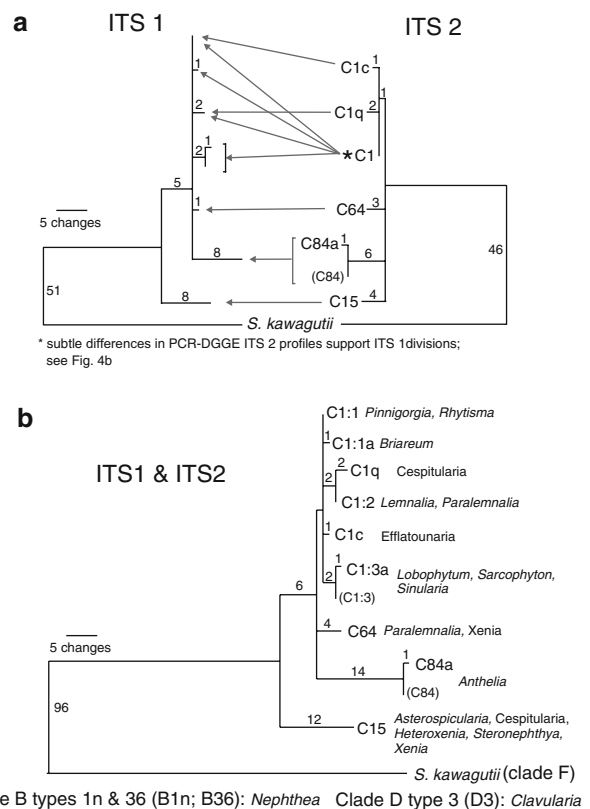
up to several base changes (Fig. 5a). These findings show that there are cases where the ITS 1 region is more suitable for characterizing ecologically different populations of *Symbiodinium*, because it has evolved more rapidly than the ITS 2 (cf. LaJeunesse et al. 2008). As a result of this, the ITS 1 phylogeny contained several additional terminal nodes (Fig. 5a). Branch lengths (number of changes) between the most divergent types (e.g., *C84a* and *C15*) were also greater for the ITS 1 (Fig. 5a). A single phylogenetic reconstruction (maximum parsimony) was derived from the incorporation of both spacer region sequences (Fig. 5b). Nomenclature incorporating the resolution of PCR-DGGE on both regions (cf. Fig. 3) was added to this tree, as well as a listing of the host genera where each type was found.

Discussion and conclusions

If global warming continues as projected, symbiotic associations involving cnidaria and their dinoflagellate symbionts will experience increasing stress, potentially leading to

severe reductions in these communities (Hoegh-Guldberg 1999). Many experimental investigations have simulated high seawater temperature conditions to learn how cnidarians and their endosymbiotic *Symbiodinium* clades respond to stress (Warner et al. 1996; Rowan 2004; Tchernov et al. 2004; Goulet et al. 2005; Strychar et al. 2005; Berkelmans and van Oppen 2006; Anthony et al. 2007; Ferrier-Pagès et al. 2007). Few investigations have been conducted during a natural stress event (Glynn et al. 2001; LaJeunesse et al. 2007). This study investigated the bleaching patterns of an understudied yet ecologically important group of symbiotic cnidarians common to many Indo-Pacific reefs and is one of few studies to biopsy symbiotic cnidarians of any kind during a major stress event (Glynn et al. 2001).

Over large geographic areas, many cnidarian-algal symbioses exhibit varying levels of specificity and different partner combinations (LaJeunesse et al. 2004a). Due possibly to environmental conditions over the last five to seven million years (LaJeunesse 2004), the majority of octocorals and scleractinian corals in the Pacific host *Symbiodinium* belonging to clade C (Goulet 2006; Goulet 2007). Indeed, concurring with the current study, clade C *Symbiodinium* spp. dominate the soft coral communities on the central GBR (LaJeunesse et al. 2003, 2004a; Strychar et al. 2005; van Oppen et al. 2005b; Goulet et al. 2008). In the current study, only *C. koellikeri* is associated with clade D, while *Nephthea* sp. was the only one that was associated with symbionts from different clades (B and C).

Alcyonaceans from the GBR in general exhibit strong specificity for particular types of *Symbiodinium* within a given clade. A thorough analysis of diversity, utilizing PCR-DGGE fingerprinting of both the ITS 1 and ITS 2, identified many *Symbiodinium* populations that existed in certain soft corals, but not in others (Fig. 5). In general, many of the *Symbiodinium* spp. characterized from soft corals do not occur in scleractinian corals, anemones, and zoanthids (LaJeunesse et al. 2003, 2004a). Only two *Symbiodinium* spp. identified in the current analyses are known to exist in distantly related hosts. Type *C15*, found in *Stereonephthya* sp., and three Xeniidae species (*Xenia* sp., *Cespitularia* sp., and *Asterospicularia laurae*) is known to occur in scleractinians (e.g., *Porites* spp.) and in species of Hydrozoa and Foraminifera from the GBR (LaJeunesse et al. 2003; LaJeunesse 2004; Pochon et al. 2004). *Symbiodinium C1c*, found only in *Efflatounaria* sp. in the current study, is common among pocilloporid corals in the GBR (LaJeunesse et al. 2003).

It appears that the alcyonacean host–symbiont combinations have remained stable through time. Genera in the family Xeniidae that hosted *C64* or *C15* in our 1998 study hosted these same symbionts when surveyed in 2003 (LaJeunesse et al. 2004a). *Clavularia koellikeri,* which harbored *Symbiodinium D3,* was the only cnidarian found with

this symbiont in 2003 (LaJeunesse et al. 2004a). Although sample sizes were small for some soft coral species, the spatial consistency from reef to reef (Fig. 4) and temporal stability (LaJeunesse et al. 2004a), combined with the finding that *Symbiodinium* variability in cnidarians can be detected with less than five samples (Goulet 2007), and that increasing within species samples sizes does not reveal additional *Symbiodinium* variability in octocorals (Baker and Romanski 2007), we believe that these findings are indicative of the *Symbiodinium* populations found within the sampled octocoral hosts in the GBR.

Despite exposure to severe thermal stress during the bleaching event of 1998, symbionts in the putatively stress-tolerant clade D subgenus occurred in only one host species. Six colonies of *C. koellikeri* collected from three different reefs contained *Symbiodinium D3*. Including *Clavularia*, other stress-resistant octocorals from the GBR, *Briareum* and *Isis hippuris*, are known to associate with clade D *Symbiodinium* (van Oppen et al. 2005b), although the current study found only clade C in *Briareum* spp. Investigations into the genetic diversity of clade D show that it contains a number of types or "species" with markedly different host distributions (cf. LaJeunesse et al. 2004b). As in the case of *D3* in *C. koellikeri*, some appear to be host-specific and have a geographically limited distribution. *Symbiodinium D1a* described in the Caribbean as a postbleaching, stress-tolerant, opportunist (Baker 2001; Toller et al. 2001; Thornhill et al. 2006a), occurs on the GBR and elsewhere (LaJeunesse et al. 2003, 2004a); its presence, however, was not detected among the soft corals surveyed in the present study.

Susceptibility to stress differs widely among symbiotic cnidarians and among individual colonies of a species (Lasker et al. 1984; Fisk and Done 1985; Brown and Suharsono 1990; Williams and Bunkley-Williams 1990; Hoegh-Guldberg and Salvat 1995; Marshall and Baird 2000; Loya et al. 2001). This variability can also extend to areas within colonies of a given species, whereby some areas bleach while other portions look normal (Rowan and Knowlton 1995; Rowan et al. 1997). Variation in bleaching responses may be attributed to differences in physiology among *Symbiodinium* spp. (Rowan 2004; Goulet et al. 2005; Berkelmans and van Oppen 2006; Ulstrup et al. 2006; Warner et al. 2006; LaJeunesse et al. 2007). Therefore, colonies with thermally tolerant symbionts may bleach less than those with more sensitive resident populations.

Bleaching among alcyonaceans on the central GBR was variable from species to species. Certain species and/or groups of soft coral responded differently to stress than others. In the present study, the octocoral families Briareidae, Clavulariidae, Gorgonidae, and Nephtheidae exhibited low bleaching susceptibilities. The Alcyoniidae, with the exception

of *Rhytisma*, were moderately susceptible to bleaching, as was described by Marshall and Baird (2000) for *Sarcophyton* sp. (although they classified *Sinularia* sp. and *Lobophytom* sp. as highly susceptible). Furthermore, the alcyoniid *Sarcophyton ehrenbergi* was found to be tolerant to elevated temperatures under experimental manipulation (Strychar et al. 2005) highlighting the need to reconcile field observations with laboratory findings and the importance of a comparison among multiple species. Previously, *S. ehrenbergi* was compared to one other alcyoniid, *Sinularia* sp. and *Xenia* sp. (Strychar et al. 2005). The majority of the Xeniidae sampled exhibited high bleaching susceptibility. Consistent with these observations, Strychar et al. (2005) found that *Xenia* sp. was more sensitive to elevated temperatures than bleaching-susceptible scleractinian corals in the genus *Acropora*. While these families and genera of soft coral displayed relatively high specificity for different *Symbiodinium* spp., which may explain differences in beaching, data from the current study suggest that many factors are important in determining bleaching susceptibility for a given alcyonacean group.

The dominant resident symbiont type did not explain the variable bleaching among colonies of the same alcyonacean species and the occurrence of bleached and unbleached sections within colonies resulting from the thermal stress in 1998. Bleached colonies occurred beside normally pigmented conspecifics with the same symbiont type. Bleached and unbleached colonies hosting the same symbiont type were also found in three scleractinian coral species (*Stylophora pistillata*, *Acropora millepora*, and *A. tenuis*) several months following the 2002 bleaching event on the GBR (van Oppen et al. 2005a).

In cases of partial colony bleaching, there was no indication that different *Symbiodinium* populations explained the occurrence of bleached or unbleached tissues. This was not surprising since most soft corals appear to possess homogenous symbiont populations that remain stable over many years (LaJeunesse 2002; Goulet and Coffroth 2003a, b, 2004; LaJeunesse et al. 2003; Santos et al. 2003; LaJeunesse et al. 2004a; Strychar et al. 2005). Symbiont homogeneity within a colony has been reported for *Sarcophyton ehrenbergi* and *Sinularia* sp. in the GBR (Strychar et al. 2005), *Plexaura kuna* in the Caribbean (Goulet and Coffroth 2003a), and in bleached and unbleached sides of the scleractinian coral *Goniastrea aspera* in Thailand (Brown et al. 2002b).

Differential tolerance to stress among cnidarian species, irrespective of the *Symbiodinium* they host, may explain differences in bleaching (Gates and Edmunds 1999; Loya et al. 2001; Bhagooli and Hidaka 2004). Coupled with differences in life history, such as colony growth rate, these between-species differences may explain species survivorship and abundance on a reef. For example, *S. flexibilis*

maintained its dominance of some inshore reefs in the GBR following the 1998 bleaching event due to successful asexual propagation (Bastidas et al. 2001). Furthermore, contrasting microhabitat properties (especially through shading from neighboring corals, and differences in the water flow regime) or genetic differences among individual colonies of the same species may also influence bleaching patterns (Edmunds 1994; D'Croz and Maté 2004). Finally, acclimatization can also influence bleaching susceptibility (Brown et al. 2002a; D'Croz and Maté 2004). In Thailand, the sides of *G. aspera* colonies that were either sun-exposed or shaded during emersion at low tide contained different levels of host heat shock proteins and the antioxidant-enzyme copper zinc superoxidase dismutase (Brown et al. 2002a). The potential for physiological differences among alcyonaceans, and how different taxa may respond differently to stressors, requires further examination (Strychar et al. 2005).

The determinants of severe bleaching among alcyonacean corals appear to be complex. Symbiont genetic identity, while important for some host taxa, could not explain the variability in bleaching response within and between many soft coral species. Since specificity and stability in cnidarian–dinoflagellate symbioses are common (Rodriguez-Lanetty et al. 2003, 2004; LaJeunesse et al. 2005; Goulet 2006; Thornhill et al. 2006b), especially among octocorals (Goulet and Coffroth 2003b, 2004; Santos et al. 2003; Kirk et al. 2005; Goulet et al. 2008), rapid environmental changes may not lead to new symbiotic combinations in alcyonaceans. The physiological resiliency of host and symbiont under their current range of partner combinations may limit their potential for surviving acute environmental stress.

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