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Niche partitioning of closely related symbiotic dinoflagellates

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Abstract

Reef-building corals are fundamental to the most diverse marine ecosystems, yet a detailed understanding of the processes involved in the establishment, persistence and ecology of the coral–dinoflagellate association remains largely unknown. This study explores symbiont diversity in relation to habitat by employing a broad-scale sampling regime using ITS2 and denaturing gradient gel electrophoresis. Samples from *Pocillopora damicornis*, *Stylophora pistillata* and *Seriatopora hystrix* all harboured host-specific clade C symbiont types at Heron Island (Great Barrier Reef, Australia). While *Ser. hystrix* associated with a single symbiont profile along its entire depth distribution, both *P. damicornis* and *Sty. pistillata* associated with multiple symbiont profiles that showed a strong zonation with depth. It is shown that, with an increased sampling effort, previously identified 'rare' symbiont types within this group of host species are in fact environmental specialists. A multivariate approach was used to expand on the common distinction of symbionts by a single genetic identity. It shows merit in its capacity not only to include all the variability present within the marker region but also to reliably represent ecological diversification of symbionts. Furthermore, the cohesive species concept is explored to explain how niche partitioning may drive diversification of closely related symbiont lineages. This study provides thus evidence that closely related symbionts are ecologically distinct and fulfil their own niche within the ecosystem provided by the host and external environment.

Keywords: ecology, *Pocillopora*, *Seriatopora*, *Stylophora*, *Symbiodinium*-ITS2, zonation

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Introduction

Reef-building corals form a mutualistic relationship with unicellular dinoflagellates (genus *Symbiodinium*) and this symbiosis underpins the success of tropical reef building systems worldwide. Despite the persistence of coral reef systems over geological time, there has been a dramatic and unprecedented decline of coral community composition and diversity over the last 30 years. Approximately 30% of reefs have already been damaged and a further 60% of coral reefs are predicted to be lost within the next 30 years (Hughes *et al*. 2003). A detailed understanding, however, of the processes involved in the establishment, persistence, and ecology of the coral–dinoflagellate association remains largely unknown.

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Initially, reef-building corals were thought to associate with a single symbiont, *Symbiodinium microadriaticum* (Freudenthal 1962) but additional species were later described based on differences in morphological ultrastructure (Blank & Trench 1985; Trench & Blank 1987), cell size (LaJeunesse 2001), isozyme profiles (Schoenberg & Trench 1980), and photosynthetic efficiency (Chang *et al*. 1983; Iglesias-Prieto & Trench 1994, 1997a). However, the description of *Symbiodinium* species is complex due to the lack of clear morphological features, but the introduction of molecular techniques has assisted the differentiation of *Symbiodinium* types, with thus far eight major clades have been identified. Their phylogeny is supported by various molecular markers, e.g. 18S rDNA (Rowan & Powers 1991), 28S rDNA (Loh *et al*. 2001), 23S chloroplast large subunit (Santos *et al*. 2002) and mitochondrial DNA (Takabayashi *et al*. 2004). Different clades have been shown to exhibit distinct geographical distributions at

both large (Baker 1999; LaJeunesse & Trench 2000; Loh *et al*. 2001; Rodriguez-Lanetty *et al*. 2001; Burnett 2002) and small scales (van Oppen *et al*. 2001; Rodriguez-Lanetty *et al*. 2003) as well as showing intraspecific depth zonation (Rowan & Knowlton 1995; Baker *et al*. 1997; Rowan *et al*. 1997). While the cladal classification has highlighted patterns of symbiont ecology, physiology and evolution, it may be limited because similar function of symbionts can evolve across different clades due to convergent evolution. This limitation is highlighted by reports that different clades do not shape the occurrence of corals along reefs (Diekman *et al*. 2002; Savage *et al*. 2002) or that members of the same clade display highly variable physiologies both *in situ* and in culture (Iglesias-Prieto & Trench 1997a, b; Warner *et al*. 1999; Tchernov *et al*. 2004).

To expand on the cladal system, the highly variable internal transcribed spacer 2 (ITS2) region of the rDNA was posed as a higher resolution (Hunter *et al*. 1997) or species-specific marker by showing that it could easily distinguish between formerly described *Symbiodinium* species (LaJeunesse 2001). A rapid expansion of the cladal system into subcladal types (e.g. C1, C2, C3, etc.) followed, and to date, approximately 155 ITS2 types have been classified that include generalist as well as specialist symbiont types (LaJeunesse 2002; LaJeunesse *et al*. 2003, 2004a). Studies thus far have focused on symbiont diversity, geography, phylogeny and have advanced our understanding of host–symbiont associations by covering multiple taxa and families of hosts. However, the power of the ITS region to elucidate ecologically relevant patterns within coral–dinoflagellate associations remains to be fully tested due to limited sample sizes, direct correlation with environmental factors, local symbiont availability and host ranges. In the latter case, efforts to examine the effect of the environment on symbiont distributions can be confounded due to lack of similar host species across different habitats. To date, very few studies using more variable DNA markers have focused on fine-scale variability of symbionts within and between populations of a single host using large-scale sampling designs (Santos *et al*. 2003; Ulstrup & van Oppen 2003). Thus, given the vast availability of differential habitats, the full range of intraspecific host–symbiont associations on the subcladal level may not be recognized. Furthermore, while some level of functional differentiation has been linked to the cladal level, such as the temperature tolerance of clade D (Baker *et al*. 2004; Rowan 2004; Berkelmans & Van Oppen 2006), knowledge on functional differences between subclades is mostly lacking. Consequently, exploring how closely related subcladal symbiont types vary ecologically or functionally has the potential to greatly enhance our understanding of what drives host–symbiont associations.

Coral species of the ubiquitous family Pocilloporidae have been shown to associate with a variety of symbiont ITS2 types (LaJeunesse *et al*. 2004a, b; LaJeunesse 2005); however, work to date has involved limited sample sizes per depth and/or geographical location (ranging from $n = 1–5$). To assess whether these sample sizes accurately describe environmental distributions of host–symbiont combinations, and to minimize the effect of host population divergence, a comprehensive assessment across the entire depth range of three species of pocilloporid corals was undertaken at Heron Island on the southern Great Barrier Reef (GBR) in Australia. The ITS2 in combination with denaturing gradient gel electrophoresis (DGGE; *sensu* LaJeunesse 2002) has shown that symbiont profiles obtained from pocilloporid corals are often comprised of multiple bands (LaJeunesse *et al*. 2004a, b; LaJeunesse 2005). This complexity in the profile can indicate that multiple symbionts are present within a single host, and hence, we extend traditional analyses of *Symbiodinium* DGGE profiles to those published in microbial studies. DGGE has been employed in a variety of studies in combination with rDNA marker regions (Muyzer 1999) to study community diversity (Wang *et al*. 2004), temporal dynamics (Stamper *et al*. 2003), succession (Sekiguchi *et al*. 2002), niche diversification (Palys *et al*. 1997), and identification of functional groups of microbes (Duineveld *et al*. 2001). Furthermore, the application of alternate species concepts has not been fully explored for many unicellular eukaryotes, including *Symbiodinium*, and it is expected that these systems will benefit greatly from established concepts for bacterial systems (Moreira & Lopéz-García 2002; Finlay & Fenchel 2004). The cohesion species concept (Templeton 1989) in particular has received attention because it explains how speciation can occur as a result of ecological diversification in asexual organisms such as bacteria (Cohan 2002).

Thus, the main objectives of this study were: (i) to explore diversification of symbionts in relation to habitat, (ii) to investigate whether multiple symbionts co-occur within a single host, and (iii) to assess the appropriateness of alternate analysis and species concepts to explain observed patterns in the distribution of *Symbiodinium*.

Materials and methods

Study location, sample collection and preparation

Samples of *Stylophora pistillata* ($n = 290$), *Pocillopora damicornis* ($n = 146$) and *Seriatopora hystrix* ($n = 84$) were collected in October 2004 and 2005 from Harry's Bommie (23°27.625′S, 151°55.759′E), Tenements II (23°25.983′S, 151°55.756′E) and 4th Point (23°25.911′S; 151°59.330′E) at Heron Reef (Heron Island) on the southern GBR, Australia (Fig. 1). At each site, fragments of ~3 cm² were collected from the uppermost part of colonies of the three species from three depths (3–5 m, 9–11 m and 17–19 m). All colonies were collected at least

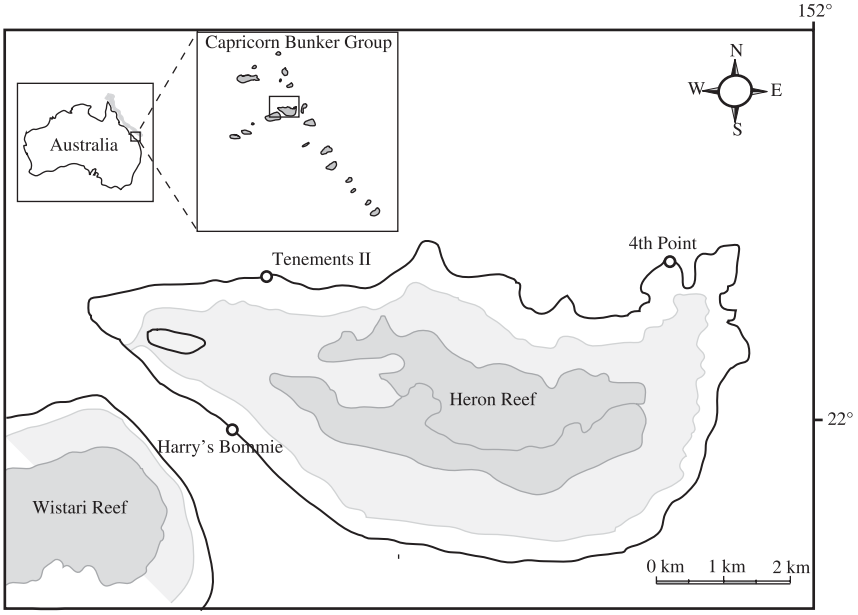

Fig. 1 Heron and Wistari Reef, showing the location of the three sampling sites: Harry's Bommie, Tenements II and 4th Point. Inset: position of the Capricorn Bunker Group on the southern Great Barrier Reef and of Heron Island within this group.

Table 1 ITS2 types identified within each symbiont profile and number of colonies with each profile per coral species for each depth and location on Heron Island. Sp, *Stylophora pistillata*; Pd, *Pocillopora damicornis*; Sh, *Seriatopora hystrix*. Pd4 and Pd 5 contain both C33 and C33a but in different proportions (see also Fig. 2), while Pd4* and Pd5* have either variant in isolation. New ITS2 types with their respective GenBank Accession numbers are as follows: *1, EF541145; *2, EF541146; *3, EF541147; *4, EF541148; *5, EF541149

Site	Depth	Sp1	Sp2	Sp3	Sp4	Pd1	Pd2	Pd3	Pd4	Pd4*	Pd5	Pd5*	Sh1
Harry's Bommie	3–5 m	10	5	16	0	9	0	0	16	2	1	0	10
	9–11 m	4	1	22	11	0	0	0	19	1	0	0	14
	17–19 m	0	1	15	24	0	0	0	13	1	0	1	10
Tenements II	3–5 m	12	16	7	2	6	12	1	1	0	0	0	18
	9–11 m	8	1	14	12	6	6	3	1	0	2	0	14
	17–19 m	2	1	6	22	3	1	0	8	0	8	1	18
4th Point	3–5 m	9	5	19	1	1	18	2	3	0	0	0	—
	9–11 m	—	—	—	—	—	—	—	—	—	—	—	—
	17–19 m	8	0	14	22	—	—	—	—	—	—		—
ITS2 types identified		C35*1 C35a*2	C1 C78	C8 C8a	C79	C42 C1 C1b C1c	C42 C42a C1 C1b C1c	C42 C42a C42b C1 C1b C1c	C33a C33	C33a*3	C33 C33a	C33*4	C3 C3t*5

4 m apart to avoid sampling possible clones formed by fragmentation. Due to weather limitations, sampling at 4th Point was limited to *pistillata* (3–5 m, 16–18 m) and *P. damicornis* (3–5 m) (see Table 1 for detailed sample information). To test for symbiont zonation within a colony, fragments were collected at three depths at Harry's Bommie and Tenements II from the top ('high light') and the bottom most shaded ('low light') parts of colonies *pistillata* ($n = 60$) and *P. damicornis* ($n = 30$). All collected fragments were temporarily housed in flow-through aquaria, and the tissue processed and stored as outlined by Loh *et al*. (2001). DNA was extracted using a QIAGEN Plant Mini Kit with inclusion of all optional additional steps.

ITS2 PCR and DGGE

The ITS2 rDNA region was amplified using the primer sequences of LaJeunesse (2002) under the following polymerase chain reaction (PCR) conditions: 0.5–1.0 μL of

DNA, 1.5 μL 10× PCR buffer (Invitrogen), 1.0 μL 50 mM $MgCl_2$, 0.7 μL 10 mM dNTPs, 0.5 μL 20 mM ITSintfor2, 0.5 μL 20 mM ITS2Clamp, 0.15 μL of Platinum *Taq* DNA Polymerase (Invitrogen) and distilled water added to a total volume of 30 μL per reaction. The cycling conditions were: 5 min at 94 °C; 30 cycles at 45 s 94 °C, 45 s 60 °C and 60 s at 72 °C, with a prolonged final extension of 20 min at 72 °C to reduce the formation of so-called heteroduplexes (Janse *et al*. 2004). This PCR protocol yielded ITS2–DGGE profiles not significantly different from those produced by a touch-down protocol or when DNA was first cycled without the GC-clamped primer and then with the ITSClamp primer (to determine whether the GC-rich end preferentially binds to certain sequences) (E.M. Sampayo unpublished). PCR products were checked on 2% agarose gels stained with ethidium bromide.

Amplified ITS2 products were run on a Biorad DCode System using DGGE following a modification of methods described by LaJeunesse (2002) due to use of a different electrophoresis system. Denaturing gradients, running time and voltage were modified for optimal resolution of the samples used in this study: 8% polyacrylamide gels with an internal gradient of 30–60% denaturants (urea and formamide) were poured following the manufacturer's instructions (Biorad Laboratories). Wells were rinsed with unpolymerized acrylamide prior to loading to minimize sample detention after which 8–12 μL of the PCR product was loaded using 2× DGGE loading dye (2 mL DCode dye solution, 7 mL glycerol and 1 mL dH_2O) in a 1:1 ratio. Gels were run at 100 V for 12 h, stained for 20–35 min using SYBR green (Amresco, 10 000 × diluted in 0.5× TAE) and photographed using a digital camera fitted with a SYBR green filter.

Sequencing

To classify profiles and provide a means of comparison to published data (e.g. LaJeunesse *et al*. 2003, 2004a, b) prominent bands were excised from the DGGE profiles. In most profiles, multiple dominant bands were excised to check whether they represented real symbiont types or heteroduplexes. Excised bands were stored overnight at 4 °C in 500 μL of distilled water, after which 1 μL was used as template for PCR using the ITSintfor2 and ITS2 reverse primer without the GC-clamp (LaJeunesse 2002). Cycling conditions were: 94 °C for 3 min; 38 cycles at 94 °C for 30 s, 52 °C for 45 s, 72 °C for 45 s, and a final extension of 10 min at 72 °C. Generally, direct sequencing of purified PCR products (High Pure PCR purification kit, Roche Laboratories) yielded ambiguous sequences, and consequently, excised bands were cloned using a pGEM T-Easy vector kit (Promega) and TOPO One Shot competent cells (Invitrogen). As cloning may introduce artefacts due to PCR error by the cloning in of chimeras and heteroduplexes (Speksnijder *et al*. 2001), the motility of inserts was checked on DGGE gels to ensure that the 'desired' fragment was inserted. Only those clones that migrated to the same position as the original excised band were grown and purified using an UltraClean Mini Plasmid Prep Kit (Mo Bio Laboratories). Multiple clones were sequenced from excised bands from three to four individuals sharing the same profile to ensure that comigration of nonsimilar sequences did not occur (Sekiguchi *et al*. 2001). Sequencing was undertaken at the Australian Genome Research Facility using vector-specific forward and reverse primers in separate runs. Sequences were aligned using SEQMAN (Lasergene), checked manually and the consensus sequence blasted (http://www.ncbi.nlm.nih.gov/BLAST/).

Statistical analysis

Each individual colony was assigned a symbiont profile, and in the rare occasion of a mixed profile the most dominant symbiont (based on brightness on the gel) was assigned to that individual. After each colony was assigned a particular symbiont profile, a nested analysis of similarity (ANOSIM) (depth nested within site) was calculated using Bray-Curtis distance to determine the effect of depth and/or site on symbiont distribution within a host species. The analysis was done separately for each host as no overlap of symbionts occurred between species.

Samples containing multiple bands within each symbiont profile may be the result of heterogeneity within the ribosomal array and/or mixed occurrence of symbionts. As such, all the bands visible within each profile were scored to determine similarity between profiles encompassing the full genetic diversity present within the sample. Representative samples for each profile were loaded onto a single gel and all visible bands within a profile were scored with inclusion of heteroduplexes (that are equally characteristic of a profile) (ALPHAEASE software). Chromatograms of each profile were visually checked and baseline subtractions performed within lanes to adjust for differences in loading concentrations of DNA. The resultant assignment scores were assimilated into a presence/absence matrix and analysed in a similar fashion to AFLP (e.g. Hodkinson *et al*. 2000) or DGGE data used for microbial community analysis (Sekiguchi *et al*. 2002; Stamper *et al*. 2003). A multidimensional scaling (MDS) plot was constructed from the presence/absence matrix using Bray-Curtis dissimilarity, where similarity was only assigning to a joined presence and not a joined absence of bands (Quinn & Keough 2002). All multivariate statistics were done using the software package PRIMER (version 5.2.1) (Clarke 1993).

Finally, sequences obtained from excised dominant bands of each symbiont profile were aligned using Clustal X (version 1.83) (Thompson *et al*. 1997). An unrooted sequence network was generated using the algorithm developed by

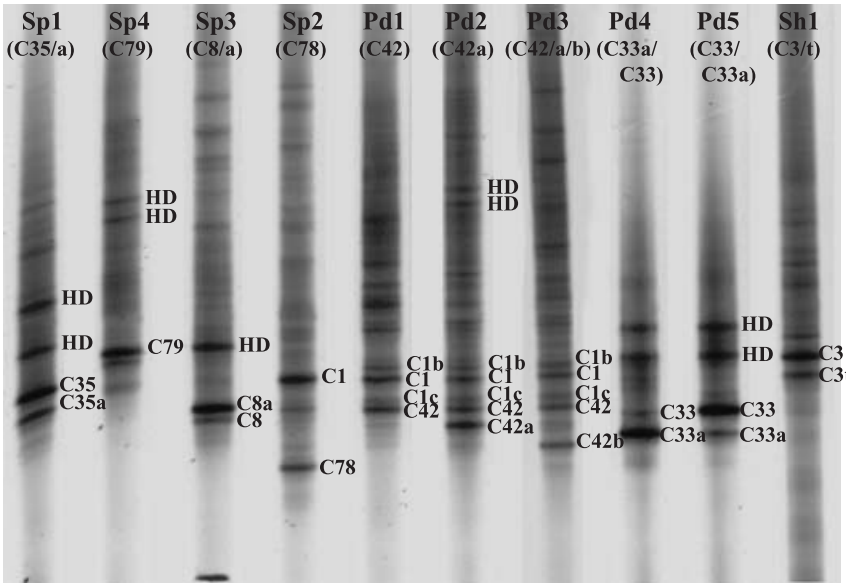

Fig. 2 ITS2-DGGE gel showing symbiont profiles found in *Stylophora pistillata* (Sp), *Pocillopora damicornis* (Pd), and *Seriatopora hystrix* (Sh). Profiles are numbered according to species and order found, i.e. Sp1, *Sty. pistillata* profile 1; Sp2, *Sty. pistillata* profile 2. Below each profile is the designation as per LaJeunesse *et al*. (2003), where the profile is characterized by the bottom most dominant band(s). Identified sequences and heteroduplexes (HD) are shown next to bands (note that this is not exhaustive and many bands remain unidentified). The bottom-most band visible in profile Sp3 is a pseudogene with a deletion of the complete ITS2 region.

Templeton *et al*. (1992) with the program TCS (version 1.21) (Clement *et al*. 2001) treating gaps as a fifth character state and a 90% connection limit between haplotypes.

Results

With the exception of *Seriatopora hystrix*, which harbours only a single symbiont profile (hereafter named Sh1), multiple profiles were identified in *Stylophora pistillata* (four profiles: Sp1, Sp2, Sp3, Sp4) and *Pocillopora damicornis* colonies (seven profiles: Pd1, Pd2, Pd3, Pd4, Pd4*, Pd5, and Pd5*) (Fig. 2). In total, 10 different symbiont profiles were obtained that showed no 'cross-over' between host species and thus symbionts are highly host species-specific. Generally a single profile was detected, however on rare occasions mixed profiles were observed whereby Sp4 contained faint bands at the same position as Sp1, Sp1 contained faint bands of Sp2, and Pd2 contained bands of profile Pd4/5. In all instances, the additional bands were faint compared to the 'normal' profile found in that particular individual. Furthermore, of the 90 colonies analysed for symbiont differentiation between the upper vs. the lower part of the colony, only three *pistillata* colonies and one *P. damicornis* colony showed a difference (4% of colonies), suggesting that each colony generally associates with a single symbiont profile. The *P. damicornis* colony had profile Pd4 in the top 'high-light' part and profile Pd5 in the shaded part of the colony. The three *pistillata* colonies had Sp3 +4/Sp4, Sp3/Sp4, and Sp2/Sp3 in the 'high-light'/shaded parts of each colony respectively.

Symbiont diversity and depth

Symbiont diversity within *Sty. pistillata* did not differ significantly between sites ($R = -0.211$, $P = 0.850$), but did show a strong relationship with depth ($R = 0.137$, $P = 0.001$). The influence of depth was evident at all sites, however, with subtle differences in abundances ($n = 291$, Table 1, Fig. 3a–d). 41–75% (accumulative) of the colonies found in the shallows associated with Sp1 or Sp2. At the immediate and deep depths Sp1 occurred at lower density while Sp2 was scarce and only found in four colonies at the intermediate and deep depths. Sp4 was predominantly found in the deep (50–71%), whereas Sp3 was almost equal in abundance across all depths. At Harry's Bommie and 4th Point, patterns of symbiont distribution were similar, while at Tenements II, a higher relative abundance of Sp2 in the shallow (43% vs. 16%) was observed. Interestingly, the relative abundance of Sp3 at Tenements II is also lower and Sp2 appears to have occupied this space at this particular site and depth.

Similarly, the distribution of symbionts associated with *P. damicornis* showed no significant effect of site ($R = 0.533$, $P = 0.100$), yet differed significantly with depth ($R = 0.069$, $P = 0.006$) ($n = 146$, Table 1, Fig. 3e–h). Harry's Bommie is almost completely dominated by Pd4 with 64% (shallow) to 100% (intermediate) of all colonies harbouring this profile. At both 4th Point and Tenements II, Pd1 and Pd2 predominate in the shallows (respectively 85% and 79% cumulative of these two profiles) and Pd4 and Pd5 are more abundant in the deep (80% cumulative at Tenements II). Overall, Pd4 and Pd5 are commonly found in the deep while Pd1 and Pd2 predominate in shallower areas, and both Pd3 and Pd5* are rare (Table 1).

ITS2-D66E profile complexity

Symbiont profiles are generally characterized by the (single) most dominant band in the profile, and the type designated according to sequence identity (e.g. LaJeunesse

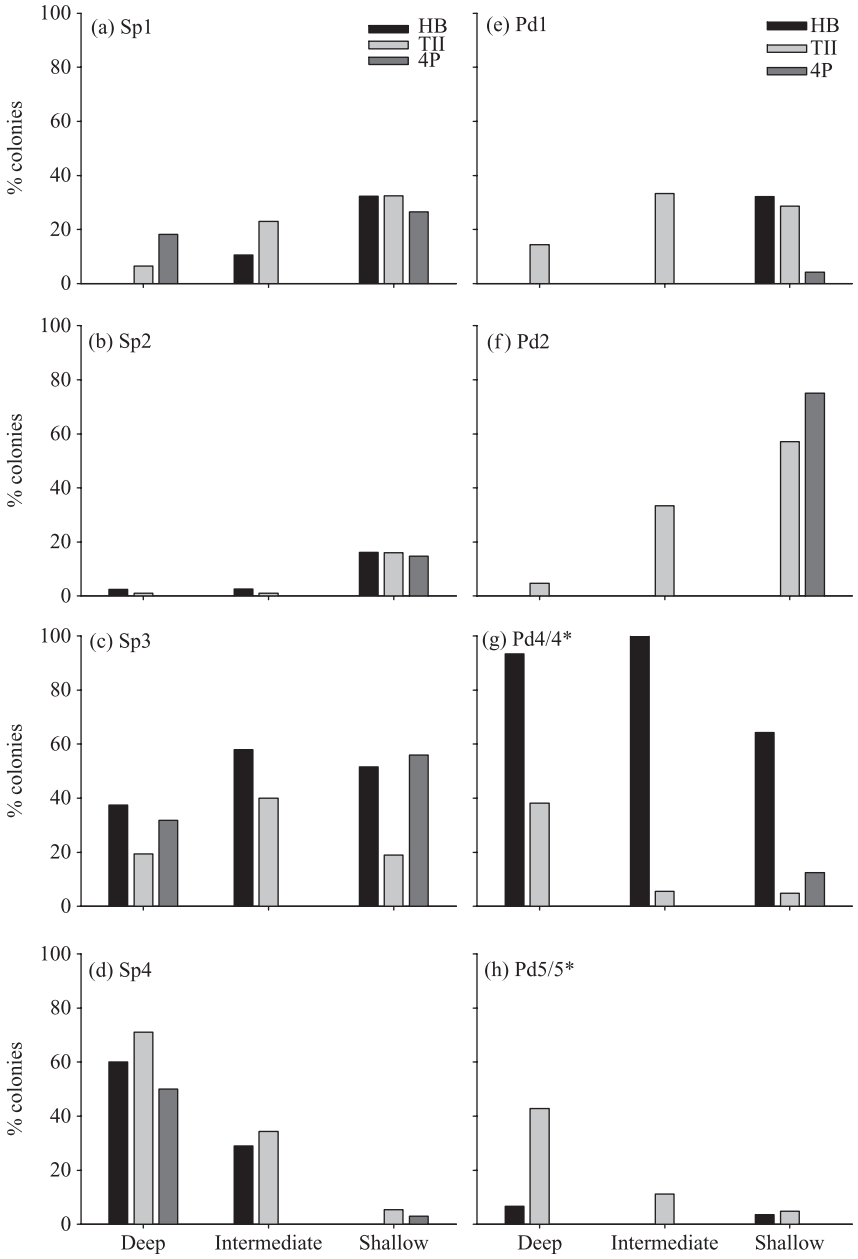

Fig. 3 Depth zonation of symbionts found in *Stylophora pistillata* (a–d) and *Pocillopora damicornis* (e–h). Bars represent relative frequency of each profile at each collection site and depth (sample sizes in Table 1). Pd3 is not included because it is rare (see Table 1) while Pd4/Pd4* and Pd5/5* are represented as groups. Depths: shallow (3–5 m); intermediate (9–11 m); deep (17–19 m). HB, Harry's Bommie; TII, Tenements II, 4P, 4th Point. Note that at 4th Point, no data was collected at the intermediate and deep depths for *P. damicornis* for *S. pistillata* at the intermediate depth.

2002). However, the isolation of multiple bands from each profile revealed many previously classified symbiont types present in a single profile (Fig. 2), suggesting that classification based on the most dominant band may underestimate genetic diversity. Profiles ranged from very complex, containing many ITS2 types (e.g. Pd1, Pd2), to simple containing few types (e.g. Sp1, Pd4/5) (Fig. 2, Table 1). Although each profile was unique, host-specific band brightness did vary in some profiles (i.e. Sp1, Pd4/Pd5). This variation was not only highly consistent between samples but also reproducible with duplicate PCR amplifications of the same sample. The dominant sequences in profiles Pd4 and Pd5 (C33 and C33a) were generally found co-occurring and designated Pd4 where C33a was 'dominant' and Pd5 where C33 was dominant. However, on rare occasions C33a was found in isolation (in four out of 78 colonies) and consequently designated Pd4* (Table 1). Additionally, in two deep *P. damicornis* samples, profile Pd5* was found with the sequence variant C33 in isolation (not shown in Fig. 2). The most complex situation involved C42/C42a/C42b with individual colonies containing combinations of bands present in this profile (Fig. 2 and Table 1).

All identified sequence variants belonged to clade C and differed from each other from 1 to 10 base-pair changes in the ITS2 and partial 5.8S (Fig. 4). The sequence network

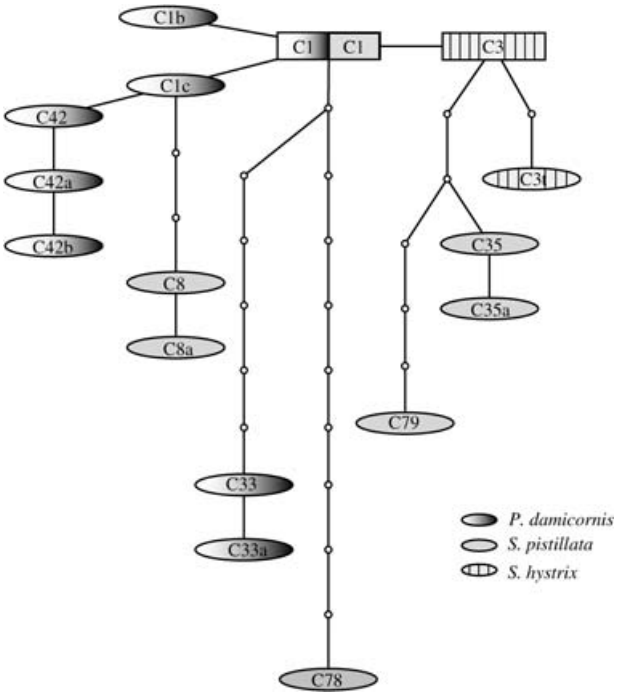

Fig. 4 Unrooted sequence network (TCS) showing the position of ITS2 types in relation to ancestral types C1 and C3 (square boxes). Connections represent single base-pair changes with 90% connection support and internodal branch-lengths do not represent distance. Different colouration represents the host species in which each ITS2 variant was identified.

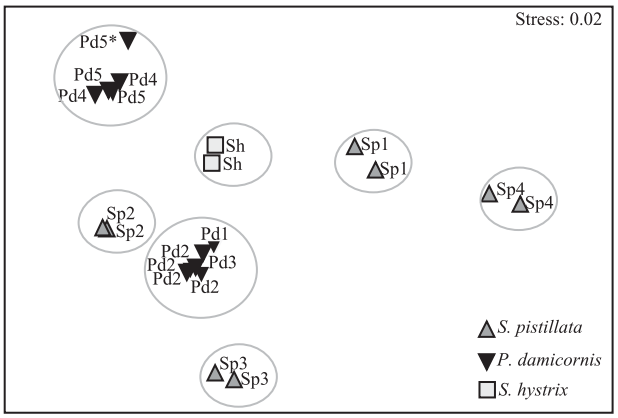

Fig. 5 MDS plot constructed using all bands visible in symbiont profiles from *Stylophora pistillata*, *Pocillopora damicornis*, and *Seriatopora hystrix* at Heron Island (Great Barrier Reef, Australia). Multiple samples were entered for each profile to incorporate fluctuations in banding profiles (usually in the upper fainter bands). Despite these fluctuations or the lack of a single closely related variant from the profile (e.g. Pd4/5 or Pd1/2/3) these profiles are more similar to each other than any of the other profiles and are grouped with a thin line. Profile designations correspond with those in Fig. 2.

suggests that symbionts from these species of pocilloporid corals have evolved from the previously identified ancestral C-types, C1 and C3 (LaJeunesse 2005). *P. damicornis* symbionts (i.e. the C42 and C33 lineage) diverged from C1, whereas *S. hystrix* contained variants C3 and C3t. In *S. pistillata*, divergence did not originate from only one of the ancestral type and colonies harboured symbionts that have diverged from both ancestral types (C1 and C3). Profile Sp2 highlights that highly divergent variants can be present (C1 and C78) within a single colony (Fig. 4). Thus, while it was beyond the scope and objectives of this study to exhaustively sequence all the bands in a profile, the data indicate that profiles consist of a multitude of variants within a closely related clade C cluster. The occurrence of these multiple sequence variants was the underlying reason to score profiles using all visible bands. Scoring bands into a presence/absence matrix showed that closely related profiles with minor variation in the profile (e.g. Pd1, Pd2, Pd3) remained tightly grouped, i.e. they are more similar to each other than to any of the other profiles (Fig. 5). Interestingly, the MDS plot identifies the same groupings as the sequence network (Fig. 4) and suggests that with inclusion of all the genetic variability present in a single sample, each profile maintains its species-specific nature as well as individual divergence. The use of a multivariate approach on these profiles shows merit in the ability to identify functional groups of symbionts while at the same time accounting for the presence of multiple genetic variants within a single sample.

Discussion

The use of the genetic species concept to delineate functionally different groups of symbionts is problematic because genetic differences per sé do not confer functional differences. Ideally, genetic variants should be substantiated with ecological, physiological, or morphological characteristics in order to be classified as functionally distinct ecotypes or strains. The present study has uncovered that specific host–symbiont combinations correlate to distinct niches as defined by a depth gradient, therefore representing a defined unit of selection that fulfils a function within the ecosystem. While host-specificity has been previously documented (LaJeunesse *et al*. 2004b), this study showed that distinct niche partitioning occurs between closely related clade C symbionts and that multiple symbionts within this clade may be present in a coral colony.

Symbiont niche diversification

Symbiont strategies were highly diverse and closely related symbiont types are either depth-generalists or -specialists.

Seriatopora hystrix harbours a single generalist symbiont across its entire depth range and at all locations. This symbiont has a wide environmental range while symbionts found in *Stylophora pistillata* and *Pocillopora damicornis*, show a clear preference for depth. The depth zonation of *S. pistillata* and *P. damicornis* symbionts mirrors patterns found on the cladal level in *Montastraea annularis* and *Montastraea franski* where depth zonation of symbionts occurs as a response to light (Rowan & Knowlton 1995; Rowan *et al*. 1997). In general, deep reef areas appear to sustain less diverse symbiont communities than shallow areas, which is likely due to more variable conditions found in the shallow reef zone. At the collection sites, temperature fluctuations are approximately twice as high in the shallows, and the light environment in the deep is more homogeneous (E.M. Sampayo, unpublished data). Thus, the depth zonation of the subcladal types found in this study suggests that the symbionts are adapted to different environmental parameters and as such provide an avenue for the host to extend its depth range (Iglesias-Prieto & Trench 1997b). Directed sampling within a single reef zone may further uncover patterns of niche partitioning between the multiple 'shallow' symbionts.

To date, *S. pistillata* has been reported to harbour the generalist symbiont type C1 in shallow water and the specialist C8/a in deep water (LaJeunesse *et al*. 2003), while C79 is reported as rare in the Western Pacific (LaJeunesse 2005). However, we have shown that the C8/a profile (Sp3) appears to be a depth generalist in *S. pistillata* and have further identified C79 (Sp4) as a 'deep water' specialist. Additionally, the ancestral C1 variant is always found in conjunction with C78 (in Sp2) and forms a highly host-specific profile. Profile C35/a (Sp1) is commonly found in *S. pistillata* colonies (up to 32%) in the shallow but had thus far only been reported in a single colony of *Ser. hystrix* (LaJeunesse 2005). Furthermore, variability between sites is important because if, for example, Harry's Bommie had exclusively been sampled, the preference of Pd4/Pd5 and Pd1, Pd2 (Table 1) to the deep and shallow reef zones, respectively, would have remained undetermined. Thus, this study highlights the importance of ample within and between site replication to accurately establish ecological patterns of symbiont diversity within a coral host.

The findings from this study are also particularly interesting given that community-wide surveys at the cladal level have yet to show a distinct functional role of the symbionts within the ecosystem. Our data demonstrate that subcladal differences are ecologically relevant and as such are likely to contribute significantly to the performance of the holobiont (host plus symbiont). This may be particularly important in the Indo-Pacific where clade C is dominant in most environments and host taxa (Baker *et al*. 1997; van Oppen *et al*. 2001; LaJeunesse *et al*. 2003). Subcladal differences have proven valuable to explain zonation or geographical distribution of host species (LaJeunesse *et al*. 2003; Iglesias-Prieto *et al*. 2004; LaJeunesse *et al*. 2004a), yet many studies on adaptive advantages of different symbiont types still focus their attention at the cladal level (e.g. Baker *et al*. 2004; Berkelmans & van Oppen 2006). Nonetheless, there is growing support to include subcladal differences in efforts to distinguish functionally relevant features of the holobiont (Iglesias-Prieto & Trench 1997a, b; Tchernov *et al*. 2004; Warner *et al*. 2006), and our results support the usefulness of the ITS2 region to uncover ecologically relevant patterns that may otherwise be overlooked at the cladal level.

Mixed symbiont communities

Although the methodology used in this study (ITS2-DGGE) has recently received some criticism (van Oppen & Gates 2006; Apprill & Gates 2007), our study supports continued use of the ITS2 region in combination with DGGE. Limitations of the technique, such as heteroduplex formation during PCR amplification (Speksnijder *et al*. 2001) or stacking of bands at a single position in the gel (Sekiguchi *et al*. 2001), have long been acknowledged and can be partially overcome by either repeated sequencing of bands at similar position in the gel and, or the application of more community-based multivariate analysis (Sekiguchi *et al*. 2002; Stamper *et al*. 2003; Wang *et al*. 2004). Exhaustive cloning and sequencing within a sample has been suggested as a potential alternative to determine the full complexity of symbiont diversity (Apprill & Gates 2007); however, this approach may not prove to be time- or cost-effective with large sample sets. Furthermore, the practice of cloning the rDNA has been cautioned because it may lead to erroneous interpretation of phylogenetic relationships if the dominant rDNA repeat is not correctly identified (Baker 2003; LaJeunesse *et al*. 2004a; van Oppen & Gates 2006). Heterogeneity of the rDNA regions has been reported for other organisms (Buckler *et al*. 1997) as well as *Symbiodinium* (Baille *et al*. 2000; Toller *et al*. 2001; LaJeunesse *et al*. 2003) but it generally appears to be limited as the rDNA is constantly homogenized through concerted evolution. As such, the use of the rDNA has seen continued support due to its ability to uncover a vast array of diversity and to differentiate 'species' or ecotypes, not only in *Symbiodinium* (e.g. LaJeunesse 2005; van Oppen *et al*. 2001) but also in mixed communities of other unicellular organisms (DeLong & Pace 2001; Ferris *et al*. 2003; Stoeck & Epstein 2003; Johnson *et al*. 2006).

Profiles from the coral species in this study commonly contained two or three dominant bands within a single individual colony, which represent closely related sequences such as C42a and C42b, or highly diverged sequence variants such as C1 and C78 (Fig. 4). At present, *Symbiodinium*

ITS2 profiles containing multiple codominant bands are considered to originate from a single symbiont type based on: (i) the presence of high sequence similarity of duplex bands (heterogeneity within the rDNA); (ii) the lack of variability in band brightness, and/or (iii) sequence variants are not found in isolation (LaJeunesse 2002; LaJeunesse *et al*. 2004a). However, the lack of evidence to support the coexistence of closely related symbionts may be due to a combination of limited band identification within complex profiles and low sample sizes within single host species. This study shows that closely related sequences such as C33 and C33a may not necessarily be codominant copies within the ribosomal array. The possibility exists that the two variants co-occur within the same host because profiles show variations in band brightness (consistent across different samples and multiple PCRs of the same sample) as well as the independent expression of one variant in the absence of the other (Pd4*, Pd5*). Similarly, the complex profiles Pd1, Pd2 and Pd3 contain many closely related sequence variants (C42/C42a/C42b and C1/C1b/C1c, Fig. 2) and vary in not only band presence or absence between samples but also show a difference in abundance between sites. Contrastingly, Sp1 (C35 and C35a) showed some variation in band brightness but neither variant was observed to occur in isolation and as such these sequences are more likely to represent codominant copies within the rDNA of a single organism. Concerted evolution purges diversity from the ribosomal array on a population level within a single species. This may not occur at the same rate between different species and may explain why some contain codominant copies within the rDNA while others have been fully homogenized. The finding that closely related sequence variants, while occupying the same niche, not only vary in abundance but also occur in isolation would suggest that they are likely to represent population-level differences within an ecotype (or species) rather than intragenomic of the rDNA.

Corals appear to be highly specific with respect to their symbiotic partner (LaJeunesse 2005) and the majority of coral species on the GBR associate with a single *Symbiodinium* clade C (Goulet 2006). Polymorphic symbioses are generally reported at the cladal level (e.g. Ulstrup & van Oppen 2003; Little *et al*. 2004; Warner *et al*. 2006) and very rarely have types of the same clade been found to inhabit a single coral species or a single individual (LaJeunesse *et al*. 2003; Dove *et al*. 2006; Thornhill *et al*. 2006). However, the coexistence of multiple symbiont types within pocilloporid corals may be more common than originally thought. This study shows that mixed symbionts of a single clade can be present inside a host colony (i.e. Sp1 with background of Sp2), and on rare occasions, differences are found between the top and bottom part of a single colony. The complexity of ITS2–DGGE profiles identified in this study and previous studies (e.g. LaJeunesse *et al*. 2004a) highlights the importance of identifying the origins of sequence variability the merit of ecological approaches and community-based analysis to expand our understanding of the role of subcladal symbiont types. Variability due to heterogeneity of the ribosomal array of a single species is generally largely contained within that lineage, which is supported by the fact that multivariate analysis, which includes all the genetic variability present within a sample, showed little or no overlap between the different profiles. This finding, coupled with the identification of multiple, closely related symbiont types within an individual host, challenges current opinion and suggests that complex ITS2–DGGE profiles may sometimes represent a mixed community rather than a single symbiont type. The fact that closely related symbiont types not only exhibit niche diversification but potentially coexist within individual hosts indicates that the low sequence divergence within a single clade represents ecological adaptation.

Species concepts and ecology

The cohesion species concept defines a species as a group of organisms whose divergence is capped by one or more forces of cohesion, and therefore comfortably encompasses other species concepts such as the ecological species concept (van Valen 1976) and the evolutionary species concept (Wiley 1978). Although this species concept was originally developed for all organisms regardless of their level of recombination (Templeton 1989), the cohesion species concept has been proposed to be highly applicable to bacterial systematics because it can accommodate their asexual nature (Cohan 2002). A potent force of cohesion for organisms that undergo low levels of recombination is natural selection that purges genetic diversity from the group in periodic selection events (Templeton 1989). New species are formed when a mutant or strain is able to inhabit a new ecological niche, after which it is no longer subject to the periodic selection events of its 'ancestral' population. Thus, the 'nascent' and 'ancestral' populations no longer interact and can be considered separate species as the force of cohesion no longer caps their respective divergence (Cohan 2001, 2002).

This cohesion species concept may also provide a framework in which coexistence and population dynamics of closely related *Symbiodinium* types can be interpreted. It is highly conducive to *Symbiodinium* because the single-celled dinoflagellates are maintained in high densities within their host tissues and are presumably in a vegetative state during the majority of the symbiotic life stage. In this vegetative state, symbionts are not only haploid but also reproduce largely asexually (Pfiester & Anderson 1987; Santos & Coffroth 2003), and thus recombination may be low while the symbionts are inside the host tissues. Although recombination has been shown to occur in cultured

Symbiodinium (LaJeunesse 2001; Goulet & Coffroth 2003; Santos *et al*. 2003), it possibly only occurs outside the host tissues during the free-living stage of the life cycle. For the host species in this study, which maternally inherit symbionts, levels of recombination may be further reduced as high levels of isolation are imposed on the symbionts over host generations. Maternally transmitting symbionts is suggested as a mechanism that ensures the persistence of a successful symbiosis adapted to the local environment (Loh *et al*. 2001); however, it simultaneously promotes formation of multiple, closely related sequence variants originating from a common (ancestral) type due to increased isolation. Placing closely related variants in sequence networks helps with the identification of clusters that have been shown to overlap with ecological differentiation (Palys *et al*. 1997). Strains within such a cluster still suffer from each other's selection events (competition) and as such represent population level differences within an ecotype. This would be especially applicable to *P. damicornis* profiles, whereby the slight differences in abundance observed between sites may be due to differential competitive effort or local prevalence of each strain.

While in higher organisms competitive exclusion requires both reproductive isolation and ecological divergence, predominantly clonal unicellular organisms such as *Symbiodinium* spp. may show analogies to other single-celled organisms (eukaryotes and prokaryotes) in which ecological differentiation is sufficient to cause divergence. Within the framework that the cohesive species concept provides, it is easily conceivable that multiple symbionts diverge by utilizing a different resource on a spatial or temporal scale within the host (Ramsing *et al*. 2000). Especially morphological plasticity as a result of environmental adaptation of the coral can create a wide range of macro- and microenvironments for the intracellular symbionts. Inhabiting such microniches within the host allow multiple symbiont types to escape each other's selection events after which divergence takes place independently for each ecotype. However, the occurrence of mixed types in relation to internal colony light gradients was not commonly found in this study. This may be the result of host factors such as small colony size or skeletal features that 'equalize' internal light levels (Enriquez *et al*. 2005). On the other hand, the finding that multiple, closely related symbionts form distinct evolutionary lineages that are depth- and host-specific suggests that they confer an advantage to their host, allowing them to successfully inhabit a specific environment.

In conclusion, we have shown that the integration of alternate speciation concepts and analysis show great merit in scleractinian host–symbiont studies, and emphasize that sampling regimes should focus on the role of symbionts within their functional habitat. To date, many sequence variants have been identified within clade C that are believed to represent a rapid diversification from ancestral taxa rather than originating from intragenomic or artefactual sequence variants (LaJeunesse 2001, 2005; Rodriguez-Lanetty 2003). This study substantiates these findings on a functional level and provides evidence that closely related symbionts are not only ecologically distinct but also fulfil their own niche within the ecosystem provided by the host in combination with the external environment. Increased sample size and site replication further reveal that host–symbiont associations are not necessarily constrained to a single combination. As such, this subcladal symbiont variability is likely to play an important role in the host and should feature in future investigations into the evolutionary dynamics and flexibility of host–symbiont symbiont associations over space and time.

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References

Apprill AM, Gates RD (2007) Recognizing diversity in coral symbiotic dinoflagellate communities. *Molecular Ecology*, online first: doi: 10.1111/j.1365–294X.2006.03214.x.

Baille BK, Belda-Baille CA, Maruyama T (2000) Conspecificity and Indo-Pacific distribution of symbiodinium genotypes (Dinophyceae) from giant clams. *Journal of Phycology*, **36**, 1153–1161.

Baker AC (1999) *The symbiosis ecology of reef-building corals*. PhD Thesis, Rosenthiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida.

Baker AC, Rowan R, Knowlton N (1997) Symbiosis ecology of two Caribbean acroporid corals. *Proceedings 8th International Coral Reef Symposium, Panama*, **2**, 1295–1300.

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Corals' adaptive response to climate change. *Nature*, **430**, 741–741.

Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for corals reefs in an era of climate change. *Proceeding of the Royal Society of London. Series B, Biological Sciences*, **273**, 2305–2312.

Blank RJ, Trench RK (1985) Speciation and symbiotic dinoflagellates. *Science*, **229**, 656–658.

Buckler SE, Ippolito A, Holtsford TP (1997) The evolution of ribosomal DNA: divergent paralogues and phylogenetic implications. *Genetics*, **145**, 821–832.

Burnett WJ (2002) Longitudinal variation in algal symbionts (zooxanthellae) from the Indian Ocean zoanthid *Palythoa caesia*. *Marine Ecology Progress Series*, **234**, 105–109.

Chang SS, Prezelin BB, Trench RK (1983) Mechanisms of photoadaptation in three strains of the symbiotic dinoflagellate *Symbiodinium microadriaticum*. *Marine Biology*, **76**, 219–229.

Clarke KR (1993) Non-parametric multivariate analysis of changes in community structure. *Australian Journal of Ecology*, **18**, 117–143.

Clement M, Posada D, Crandall KA (2001) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9** (10), 1657–1659.

Cohan FM (2001) Bacterial species and speciation. *Systematic Biology*, **50**, 513–524.

Cohan FM (2002) What are bacterial species? *Annual Reviews in Microbiology*, **56**, 457–487.

DeLong EF, Pace NR (2001) Environmental diversity of bacteria and archaea. *Systematic Biology*, **50** (4), 470–478.

Diekmann OE, Bak RPM, Tonk L, Stam WT, Olsen JL (2002) No habitat correlation of zooxanthellae in the coral genus *Madracis* on a Curacao reef. *Marine Ecology Progress Series*, **227**, 221–232.

Dove S, Ortiz JC, Enriquez S *et al*. (2006) Response of holosymbiont pigments from the scleractinian coral *Montipora monasteriata* to short-term heat stress. *Limnology and Oceanography*, **51** (2), 1149–1158.

Duineveld BM, Kowalchuk GA, Keijzer A, van Elsas JD, van Veen JA (2001) Analysis of bacterial communities in the rhizosphere of *Chrysanthemum* via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. *Applied and Environmental Microbiology*, **67** (1), 172–178.

Enriquez S, Mendez ER, Iglesias-Prieto R (2005) Multiple scattering on coral skeletons enhances light absorption by symbiotic algae. *Limnology and Oceanography*, **50**, 1025–1032.

Ferris MJ, Kuhl M, Wieland A, Ward DM (2003) Cyanobacterial ecotypes in different optical microenvironments of a 68 °C hot spring mat community revealed by 16S–23S rRNA Internal Transcribed Spacer region variation. *Applied and Environmental Microbiology*, **69** (5), 2893–2898.

Finlay BJ, Fenchel T (2004) Cosmopolitan metapopulations of free-living microbial eukaryotes. *Protist*, **155**, 237–244.

Freudenthal HD (1962) *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov, a zooxanthella: taxonomy, life cycle and morphology. *Journal of Protozoology*, **9**, 45–53.

Goulet TL (2006) Most corals may not change their symbionts. *Marine Ecology Progress Series*, **321**, 1–7.

Goulet TL, Coffroth MA (2003) Genetic composition of zooxanthellae between and within colonies of the octocoral *Plexaura kuna*, based on small subunit rDNA and multilocus DNA fingerprinting. *Marine Biology*, **142**, 233–239.

Hodkinson TR, Renvoize SA, Chonghaile GN, Stapleton CMA, Chase MW (2000) A comparison of ITS nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (Bambusoideae, Poaceae). *Journal of Plant Research*, **113**, 259–269.

Hughes TP, Baird AH, Bellwood DR *et al*. (2003) Climate change, human impacts, and the resilience of coral reefs. *Science*, **301**, 929–933.

Hunter CL, Morden CW, Smith CM (1997) The utility of ITS sequences in assessing relationships among zooxanthellae and corals. *Proceedings of the 8th international Coral Reef Symposium. Panama*, **2**, 1599–1602.

Iglesias-Prieto R, Beltran VH, LaJeunesse TC, Reyes-Bonilla H, Thome PE (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the Eastern Pacific. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **271**, 1757–1763.

Iglesias-Prieto R, Trench RK (1994) Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. *Marine Ecology Progress Series*, **113**, 163–175.

Iglesias-Prieto R, Trench RK (1997a) Acclimation and adaptation to irradiance in symbiotic dinoflagellates. II. Response of chlorophyll-protein complexes to different photon-flux densities. *Marine Biology*, **130**, 23–33.

Iglesias-Prieto R, Trench RK (1997b) Photoadaptation, photoacclimation and niche diversification in invertebrate-dinoflagellate symbiosis. *Proceedings of the 8th International Coral Reef Symposium, Panama*, **2**, 1319–1324.

Janse I, Bok J, Zwart G (2004) A simple remedy against artifactual double bands in denaturing gradient gel electrophoresis. *Journal of Microbiological Methods*, **57**, 279–281.

Johnson ZI, Zinser ER, Coe A, McNulty NP, Woodward EMS, Chisholm SW (2006) Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. *Science*, **311**, 1737–1740.

LaJeunesse TC (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a 'species' level marker. *Journal of Phycology*, **37** (5), 866–880.

LaJeunesse TC (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine Biology*, **141** (2), 387–400.

LaJeunesse TC (2005) Species radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. *Molecular Biology and Evolution*, **22** (3), 570–581.

LaJeunesse TC, Bhagooli R, Hidaka M *et al*. (2004a) Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. *Marine Ecology Progress Series*, **284**, 147–161.

LaJeunesse TC, Loh WKW, van Woesik R, Hoegh-Guldberg O, Schmidt GW, Fitt WK (2003) Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. *Limnology and Oceanography*, **48** (5), 2046–2054.

LaJeunesse TC, Thornhill DJ, Cox EF, Stanton FG, Fitt WK, Schmidt GW (2004b) High diversity and host specificity observed among symbiotic dinoflagellates in reef coral communities from Hawaii. *Coral Reefs*, **23** (4), 596–603.

LaJeunesse TC, Trench RK (2000) Biogeography of two species of *Symbiodinium* (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *Biological Bulletin*, **199** (2), 126–134.

Little AF, van Oppen MJH, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. *Nature*, **304**, 1492–1494.

Loh WKW, Loi T, Carter D, Hoegh-Guldberg O (2001) Genetic variability of the symbiotic dinoflagellates from the wide ranging coral species *Seriatopora hystrix* and *Acropora longicyathus* in the Indo-West Pacific. *Marine Ecology Progress Series*, **222**, 97–107.

Moreira D, López-García P (2002) The molecular ecology of microbial eukaryotes unveils a hidden world. *Trends in Microbiology*, **10** (1), 31–38.

Muyzer GM (1999) DGGE/TGGE: a method for identifying genes from natural ecosystems. *Current Opinion in Microbiology*, **2** (3), 317–322.

van Oppen MJH, Gates RD (2006) Conservation genetics and the resilience of reef-building corals. *Molecular Ecology*, **15** (13), 3863–3854.

van Oppen MJH, Palstra FP, Piquet AMT, Miller D (2001) Patterns of coral–dinoflagellate associations in *Acropora*: significance of local availability and physiology of *Symbiodinium* strains and host–symbiont selectivity. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **268**, 1759–1767.

Palys T, Cohan FM, Nakamura LK (1997) Discovery and classification of ecological diversity in the bacterial world: the role of DNA sequence data. *International Journal of Systematic Bacteriology*, **47**, 1145–1156.

Pfiester LA, Anderson DM (1987) Dinoflagellate reproduction. In: *The Biology of Dinoflagellates* (ed. Taylor FRJ), pp. 611–648. Blackwell Scientific Publications, London.

Quinn JP, Keough MJ (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge, UK.

Ramsing NB, Ferris MJ, Ward DM (2000) Highly ordered vertical structure of *Synechococcus* populations within the one-millimeter-thick photic zone of a hot spring cyanobacterial mat. *Applied and Environmental Microbiology*, **66**, 1038–1049.

Rodriguez-Lanetty M (2003) Evolving lineages of *Symbiodinium*-like dinoflagellates based on ITS1 rDNA. *Molecular Phylogenetics and Evolution*, **28** (1), 152–168.

Rodriguez-Lanetty M, Chang SJ, Song JI (2003) Specificity of two temperate dinoflagellate–anthozoan associations from the north-western Pacific Ocean. *Marine Biology*, **143** (6), 1193–1199.

Rodriguez-Lanetty M, Loh W, Carter D, Hoegh-Guldberg O (2001) Latitudinal variability in symbiont specificity within the widespread scleractinian coral *Plesiastrea versipora*. *Marine Biology*, **138** (6), 1175–1181.

Rowan R (2004) Coral bleaching — thermal adaptation in reef coral symbionts. *Nature*, **430**, 742–742.

Rowan R, Knowlton N (1995) Intraspecific diversity and ecological zonation in coral algal symbiosis. *Proceedings of the National Academy of Sciences, USA*, **92** (7), 2850–2853.

Rowan R, Knowlton N, Baker A, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, **388**, 265–269.

Rowan R, Powers DA (1991) A molecular genetic classification of zooxanthellae and the evolution of animal-algal symbioses. *Science*, **251**, 1348–1351.

Santos SR, Coffroth MA (2003) Molecular genetic evidence that dinoflagellates belonging to the genus *Symbiodinium* are haploid. *Biological Bulletin*, **204**, 10–20.

Santos SR, Gutierrez-Rodriguez C, Lasker HR, Coffroth MA (2003) *Symbiodinium* sp. associations in the gorgonian *Pseudopterogorgia elisabethae* in the Bahamas: high levels of genetic variability and population structure in symbiotic dinoflagellates. *Marine Biology*, **143** (1), 111–120.

Santos SR, Taylor DJ, Kinzie RA, Sakai K, Coffroth MA (2002) Evolution of length variation and heteroplasmy in the chloroplast rDNA of symbiotic dinoflagellates (*Symbiodinium*, Dinophyta) and a novel insertion in the universal core region of the large subunit rDNA. *Phycologia*, **41** (4), 311–318.

Savage AM, Trapido-Rosenthal H, Douglas AE (2002) On the functional significance of molecular variation in *Symbiodinium*, the symbiotic algae of Cnidaria: photosynthetic response to irradiance. *Marine Ecology Progress Series*, **244**, 27–37.

Schoenberg DA, Trench RK (1980) Genetic variation in *Symbiodinium* (=*Gymniodinium*) *microadriaticum* Freudenthal, and its specificity in its symbiosis with marine invertebrates. I. Isoenzyme and soluble protein patterns of axenic cultures of *Symbiodinium microadriaticum*. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **207**, 405–427.

Sekiguchi H, Tomioka N, Nakahara T, Uchiyama H (2001) A single band does not always represent single bacterial strains in denaturing gradient gel electrophoresis analysis. *Biotechnology Letters*, **23** (15), 1205–1208.

Sekiguchi H, Watanabe M, Nakahara T, Xu B, Uchiyama H (2002) Succession of bacterial community structure along the Changjiang River determined by denaturing gradient gel electrophoresis and clone library analysis. *Applied and Environmental Microbiology*, **68** (10), 5142–5150.

Speksnijder A, Kowalchuk GA, De Jong S, Kline E, Stephen JR, Laanbroek HJ (2001) Microvariation artifacts introduced by PCR and cloning of closely related 16S rRNA gene sequences. *Applied and Environmental Microbiology*, **67** (1), 469–472.

Stamper DM, Walch M, Jacobs RN (2003) Bacterial population changes in a membrane bioreactor for graywater treatment monitored by denaturing gradient gel electrophoretic analysis of 16S rRNA gene fragments. *Applied and Environmental Microbiology*, **69** (2), 852–860.

Stoeck T, Epstein S (2003) Novel eukaryotic lineages inferred from small-subunit rRNA analyses of oxygen-depleted marine environments. *Applied and Environmental Microbiology*, **69** (5), 2657–2663.

Takabayashi M, Santos SR, Cook CB (2004) Mitochondrial DNA phylogeny of the symbiotic dinoflagellates (*Symbiodinium*, Dinophyta). *Journal of Phycology*, **40** (1), 160–164.

Tchernov D, Gorbunov MY, de Vargas C *et al*. (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences, USA*, **101** (37), 13531–13535.

Templeton AR (1989) The meaning of species and speciation: a genetic perspective. In: *Speciation and its Consequences* (eds Otte D, Endler JA), pp. 3–27. Sinauer Associates, Sunderland, Massachusetts.

Templeton AR (1994) The role of molecular genetics in speciation. In: *Molecular Ecology and Evolution: Approaches and Applications* (eds Schierwater B, Streit B, Wagner GP, DeSalle R), pp. 455–477. Birkhäuser Verslag, Basel, Switzerland.

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. 111. Cladogram estimation. *Genetics*, **132**, 619–633.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **24**, 4876–4882.

Thornhill DJ, LaJeunesse TC, Kemp DW, Fitt WK, Schmidt GW (2006) Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Marine Biology*, **148**, 711–722.

Toller WW, Rowan R, Knowlton N (2001) Zooxanthellae of the *Montastraea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. *Biological Bulletin*, **201**, 248–359.

Trench RK, Blank RJ (1987) *Symbiodinium microadriaticum* Freudenthal, *S. goreauii* sp. nov., *S. kawagutii* sp. nov. and *S. pilosum* sp. nov. gymnodinioid dinoflagellate symbionts of marine invertebrates. *Journal of Phycology*, **23**, 469–481.

Ulstrup KE, van Oppen MJH (2003) Geographic and habitat partitioning of genetically distinct zooxanthellae (*Symbiodinium*)

in *Acropora* corals on the Great Barrier Reef. *Molecular Ecology*, **12**, 3477–3484.

van Valen L (1976) Ecological species, multispecies, and oaks. *Taxon*, **25**, 233–239.

Wang Q, Deeds JR, Place AR, Belas R (2004) Dinoflagellate community analysis of a fish kill using denaturant gradient gel electrophoresis. *Harmful Algae*, **4** (1), 151–162.

Warner M, Fitt WK, Schmidt GW (1999) Damage to photosystem II in dinoflagellates: a determinant of coral bleaching. *Proceedings of the National Academy of Sciences, USA*, **96**, 8007–8012.

Warner ME, LaJeunesse TC, Robison JE, Thur RM (2006) The ecological distribution and comparative photobiology of symbiotic dinoflagellates from reef corals in Belize: Potential implications for coral bleaching. *Limnology and Oceanography*, **51** (4), 1887–1897.

Wiley EO (1978) The evolutionary species concept reconsidered. *Systematic Zoology*, **27** (1), 17–26.

Eugenia Sampayo is interested in the evolutionary ecology of Symbiodinium and the role these symbiotic dinoflagellates play within their host such as determining range margins or the response to environmental disturbance. This study was part of her PhD thesis entitled 'Diversity and Ecology of Symbiodinium in Pocilloporid corals' at the University of Queensland. Lorenzo Franceschinis was involved in this study as part of his undergraduate degree and he has a general interest in the marine environment and functional genomics. Ove Hoegh-Guldberg is director of the Centre for Marine Studies and his research focuses on the effects of global climate change on reef ecosystems. Sophie Dove is interested in the photobiology and heat sensitivity of corals. Her research is directed to understand the molecular and biochemical processes underlying coral productivity and growth under different environmental conditions.