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Contents

Front cover: Collecting biopsies from corals living off the coast of Zanzibar, Tanzania. Reef-building corals and many other tropical cnidarians depend on dinoflagellate endosymbionts (*Symbiodinium* spp.) for their survival and growth. Harbouring a particular symbiont can result in major differences in stress tolerance among individual host colonies. The genetic analysis of symbionts in corals acquired from different habitats and regions around the world offers definitive answers regarding the extent to which partner combinations can change in response to long-standing environmental conditions and provides clearer insight into how these systems are likely to respond to global warming (see pp. 785–800 in this issue). Photo credit: Ove Hoegh-Guldberg, Global Change Institute, The University of Queensland.

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SPECIAL PAPER

Long-standing environmental conditions, geographic isolation and host–symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus *Symbiodinium*

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ABSTRACT

Aim This study examines the importance of geographic proximity, host life history and regional and local differences in environment (temperature and water clarity) in driving the ecological and evolutionary processes underpinning the global patterns of diversity and distribution of symbiotic dinoflagellates. By comparing and contrasting coral–algal symbioses from isolated regions with differing environmental conditions, we may assess the potential of coral communities to respond to significant changes in climate.

Location Indian Ocean.

Methods Community assemblages of obligate symbiotic invertebrates were sampled at numerous sites from two regions, the north-eastern Indian Ocean (Andaman Sea, western Thailand) and the western Indian Ocean (Zanzibar, Tanzania). Molecular genetic methods, including denaturing gradient gel electrophoresis analysis of the ribosomal internal transcribed spacers, DNA sequencing and microsatellite genotyping, were used to characterize the 'species' diversity and evolutionary relationships of symbiotic dinoflagellates (genus *Symbiodinium*). Host–symbiont specificity, geographic isolation and local and regional environmental factors were evaluated in terms of their importance in governing the distribution and prevalence of certain symbiont taxa.

Results Host-generalist symbionts (*C3u* and *D1-4*, formerly *D1a* now designated *Symbiodinium trenchi*) frequently occurred alone and sometimes together in hosts with horizontal modes of symbiont acquisition. However, the majority of *Symbiodinium* diversity consisted of apparently host-specific 'species'. Clade C *Symbiodinium* were diverse and dominated host assemblages from sites sampled in the western Indian Ocean, a pattern analogous to symbiont communities on the Great Barrier Reef with similar environmental conditions. Clade D *Symbiodinium* were diverse and occurred frequently in hosts from the north-eastern Indian Ocean, especially at inshore locations, where temperatures are warmer, water turbidity is high and large tidal exchanges commonly expose coral populations to aerial desiccation.

Main conclusions Regional and local differences in cnidarian–algal combinations indicate that these symbioses are ecologically and evolutionarily responsive and can thrive under various environmental conditions. The high temperatures and turbid conditions of the north-eastern Indian Ocean partly

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explain the ecological success of Clade D *Symbiodinium* relative to Clade C. Phylogenetic, ecological and population genetic data further indicate that Clade D has undergone an adaptive radiation, especially in regions around Southeast Asia, during the Pleistocene.

Keywords

Adaptive radiation, Andaman Sea, dinoflagellate endosymbionts, Great Barrier Reef, Indian Ocean, reef corals, *Symbiodinium,* Zanzibar.

INTRODUCTION

Endosymbiotic dinoflagellates in the genus *Symbiodinium* are among the most abundant and important of all microbial eukaryotes present in coral reef ecosystems (Trench, 1993). Their mutualistic associations with reef-building and many reef-dwelling cnidarians explain the ecological dominance of this animal phylum in shallow, tropical, marine environments over geological time-scales (Wood, 1998). However, the existence of healthy coral reef ecosystems is threatened by the apparent sensitivity of most coral-algal symbioses to rising sea surface temperatures (SSTs) (Glynn, 1993; Brown, 1997; Hughes *et al.*, 2003). Investigating how symbiotic organisms respond ecologically and evolutionarily to different environmental conditions may indicate whether corals, and the reefs that they build, can persist under the environmental changes caused by rapid global warming.

Until recently, little was known about the diversity and ecology of *Symbiodinium* (Trench, 1993), but advances in molecular genetic characterizations of these morphologically similar microbes have revealed significant genetic divergence and diversity (Baker, 2003; Coffroth & Santos, 2005; LaJeunesse, 2005). The molecular genetic classification of *Symbiodinium* into functionally distinct evolutionary entities (using alpha-numeric designations equivalent to 'species'), grouped within eight divergent phylogenetic 'clades' (A to H), has greatly improved awareness of their ecology and evolution (LaJeunesse, 2002; Pochon *et al.*, 2004, 2007; Rodriguez-Lanetty *et al.*, 2004; Thornhill *et al.*, 2006a,b, 2008; Sampayo *et al.*, 2007, 2008, 2009; Frade *et al.*, 2008; Goulet *et al.*, 2008).

An important question of coral–algal symbiosis research focuses on how different physiological adaptations among *Symbiodinium* species influence the response of the host to episodes of stress (Berkelmans & van Oppen, 2006; Suggett *et al.*, 2008). Corals and other symbiotic cnidarians harbouring physiologically 'sensitive' symbionts appear to be more susceptible to bleaching, a condition that involves the disassociation of the symbioses when exposed to stressors such as prolonged warming and high solar irradiation, or some combination of these factors (Fitt *et al.*, 2001). Regional and world-wide bleaching events have caused mass mortalities across entire coral communities (Brown, 1997). Corals in many central and western Indian Ocean locations were severely affected by the 1997–98 El Niño–Southern Oscillation (ENSO) event (Hoegh-Guldberg, 1999), during which 80–90% of live coral cover was lost (Wilkinson, 2000). Although some areas have since shown significant recovery (Sheppard *et al.*, 2008), it is uncertain how these communities may respond to future thermal stress events. Current projections indicate that bleaching events are likely to increase in frequency and severity, with the possibility that coral-dominated reefs may become scarce within the next 30–50 years (Hoegh-Guldberg, 1999; Hoegh-Guldberg *et al.*, 2007).

Despite perceptions that coral–algal symbioses are sensitive to changes in their environment, coral communities develop and grow in regions throughout the tropics and subtropics where environmental factors including average annual temperatures and seasonal changes in water clarity and temperature vary substantially (Sheppard, 1983; Coles, 1997; Brown, 2007). There is some indication that different regional environments significantly influence the ecology and evolution of coral–algal symbioses (Loh *et al.*, 2001; LaJeunesse *et al.*, 2004a; LaJeunesse, 2005). The predominance of thermally tolerant host–symbiont combinations may explain in part why coral communities in the Andaman Sea, north-eastern Indian Ocean, appear adapted to high SSTs (Brown, 2007). This may also partially explain why corals living in this warm and relatively turbid coastal region have not experienced extensive bleaching since 1995, despite recent occurrences of temperature anomalies exceeding the seasonal summer maxima (Brown, 1997, 2007).

The 'species' diversity, ecology and biogeography of dinoflagellate symbionts are relatively undescribed for much of the Indian Ocean (but see Burnett, 2002; Baker *et al.*, 2004; McClanahan *et al.*, 2005; Visram & Douglas, 2006; MacDonald *et al.*, 2008). Given the projected increases in SST expected to affect the Indian Ocean region (Sheppard, 2003), there is a critical need to obtain information on community-wide patterns of host–symbiont associations from different habitats and regions, as differences in symbioses may partially explain how coral reef communities respond to environmental change. This investigation thus examines the 'species' diversity of *Symbiodinium* and their host distributions from opposite regions of the Indian Ocean that were differentially impacted by bleaching and mortality. Diversity from these regions was then compared with previous data gathered on symbioses from

the central Great Barrier Reef (GBR) to determine how geographic proximity and host life history, in combination with local and regional environments, influence the ecological dominance and evolutionary success among different groups of *Symbiodinium*.

MATERIALS AND METHODS

Regional environmental conditions

General environmental parameters were assessed in each region where samples were collected. Chlorophyll *a* (Chl *a*, a proxy for water clarity) and SST data from January 2003 to December 2007 were acquired from the Giovanni online data system (http://gdata1.sci.gsfc.nasa.gov/daac-bin/G3/gui.cgi?instance_id=ocean_month; downloaded 4 August 2008), which was developed and is maintained by the NASA Goddard Earth Sciences (GES) Data and Information Services Center (DISC). MODIS/Aqua monthly Chl *a* and SST values averaged over a 24 × 24 km grid were acquired from reflectance measurements taken from positions in the southern Andaman Sea just below Phuket, Thailand, immediately east of Zanzibar, and to the east of the central GBR, Australia. These values were averaged for each month from January 2003 to December 2007 and plotted with standard deviation error values.

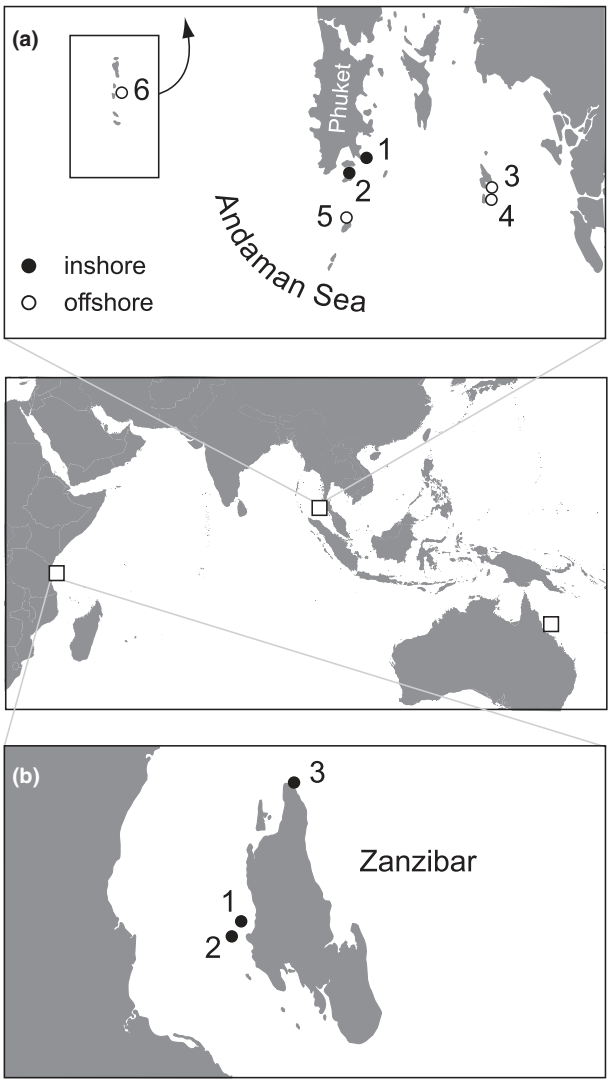

Figure 1 Indo-Pacific locations of *Symbiodinium* collections (open squares). (a) The north-eastern Indian Ocean, Andaman Sea, comprising inshore sites (1, Cape Panwa; 2, Koh Hae) and offshore sites (3, Koh Phi Phi Don; 4, Koh Phi Phi Lae; 5, Koh Racha; 6, the Similan Islands) (Thailand). (b) The western Indian Ocean, Zanzibar, Tanzania, comprising two leeward sites (1, Changuu Island; 2, Bawe Island) and one windward site (3, Banda Kuu).

Sample collections

Invertebrates harbouring *Symbiodinium* were sampled by free diving or scuba on reefs at six locations in the north-eastern Indian Ocean (Andaman Sea, Thailand) in late February 2007 (Fig. 1a). Similar collections were made in mid-April 2007 from several locations around the island of Zanzibar (Tanzania) in the western Indian Ocean (Fig. 1b). A diverse range of host taxa was sampled, including hard corals, soft corals, anemones, zoanthids, corallimorphs and tridacnid clams (Fig. 2a).

Collections were made with the goal of sampling the greatest *Symbiodinium* diversity possible across the widest range of host species. Thus, few samples were taken per host species and the present study does not attempt to quantify the complete

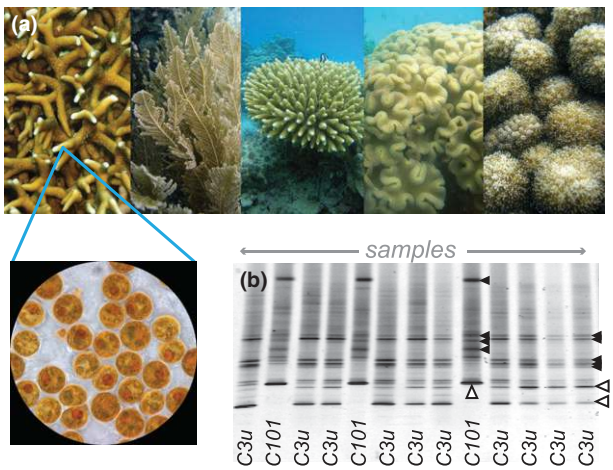

Figure 2 (a) Various reef cnidarians, including (pictured from left to right) *Millepora* sp., *Aglaophenia cupressina*, *Acropora retusa*, *Sacrophyton* sp. and *Galaxea astreata*, were sampled for analysis of *Symbiodinium* (inset) diversity. Polymerase chain reaction (PCR) amplifications of the internal transcribed spacer 2 (ITS2) region were electrophoresed on denaturing gradient gels (b) to produce diagnostic fingerprints of *Symbiodinium* exhibiting distinct ecological and/or geographic distributions (e.g. *C3u* and *C101*; i.e. 'species'). Bands in the lower portion of each profile are homoduplexes of co-abundant (dominant) intra-genomic variants and were excised (white arrowheads) for direct sequencing. Bands in the upper part of a profile are heteroduplexes that form during PCR (black arrowheads).

symbiont diversity associated with a particular species of host. Differences in host diversity between various reef habitats and locations made sampling from a proportional number of species at each site difficult, or impossible. In addition, inshore and intertidal locations potentially limited the amount of symbiont diversity recovered due to lower levels of host diversity in these areas.

Host taxa collected in Thailand were identified in the field before a small fragment, oral disc or tentacle was removed. Hosts sampled in Zanzibar were initially photographed using a Canon underwater digital camera. Most samples could be identified to the species level using this method. All specimens were preserved in 20% dimethyl sulphoxide (DMSO), 0.25 M ethylenediaminetetraacetic acid (EDTA) in NaCl-saturated water (Seutin *et al.*, 1991) and eventually stored at −20°C until further processing.

DNA extractions and denaturing gradient gel electrophoresis fingerprinting of the internal transcribed spacer region

Nucleic acids were extracted using the Wizard DNA prep protocol by Promega (Madison, WI, USA). Small fragments containing approximately 20–30 mm^2 of tissue surface area were placed into 1.5-mL microcentrifuge tubes with 250–350 μg of 0.5 mm glass beads and 600 μL of Nuclei Lysis Buffer (Promega) and bead-beaten for 1 min at 1200 *g* in a Biospec Beadbeater. The lysate was then incubated with 0.1 mg mL^{-1} proteinase K for 1–2 h at 65°C, after which Protein Precipitation Buffer (250 μL) was added and the extract chilled on ice for 15–20 min (or frozen overnight at −20°C). After centrifugation for 5 min at 15 000 *g*, 500–550 μL of supernatant was transferred to a second 1.5-mL tube containing 700 μL of 100% isopropanol and 25 μL of sodium acetate (NaOAc; 3 M, pH 5.6). These tubes were stored overnight at −20°C, after which precipitated DNA was centrifuged (15 000 *g*) for 5 min, the isopropanol supernatant removed and the pellet washed with 70% ethanol (EtOH) then centrifuged again for 5 min, dried, and resuspended in 80 μL of DNase-free H_2O.

Of the molecular genetic markers employed for the study of *Symbiodinium* diversity (Coffroth & Santos, 2005), denaturing gradient gel electrophoresis fingerprinting of the internal transcribed spacer region (ITS-DGGE) best resolves *Symbiodinium* profiles that represent independently evolving, ecologically distinct lineages (de Queiroz, 2007; Sampayo *et al.*, 2009). This technique generates a profile involving one or more bands that correspond to the most numerically common sequence variant(s) in the genome of a particular symbiont (Thornhill *et al.*, 2007). The precision and reproducibility of this method standardizes the analysis of *Symbiodinium* diversity, allowing for direct comparisons among host communities throughout the world.

For each sample, the ITS2 region was amplified using primers 'ITS2 clamp' and 'ITSintfor 2' (LaJeunesse & Trench, 2000) with the touch-down thermal cycle profile given in LaJeunesse (2002). The ITS1 region was also amplified for a subset of samples (cf. Goulet *et al.*, 2008). Products from these polymerase chain reactions (PCRs) were subject to electrophoresis for 15–16 h on denaturing gradient gels (45–80% for ITS2, 50–90% for ITS1) using a CBS Scientific system (Del Mar, CA, USA). Gels were stained with 1 × SYBR Green (Molecular Probes, Eugene, OR, USA) for 30 min, and were photographed using a Fotodyne (Hartland, WI, USA) imaging system.

Sequencing and assigning taxonomic designators

Prominent bands of each characteristic ITS-DGGE fingerprint were excised, eluted overnight in DNase-free H_2O, reamplified using the reverse primer without the GC-rich clamp (52°C annealing for 40 cycles) and directly sequenced. Bands excised for characterization of a symbiont type were not only those that were dominant but also those mainly present in the lower portions of the gel to minimize sequencing of heteroduplexes that run higher in the gel due to lower melting characteristics (Myers *et al.*, 1989). The sequences of diagnostic bands, and accompanying ITS2-DGGE fingerprints, were compared with each other and with symbionts characterized previously from other regions.

A specific alpha-numeric designation was given to each new fingerprint found. The capital letter refers to the clade, which is followed by a number that pertains to a new unique ITS sequence diagnostic of the fingerprint profile. In situations where two or three co-abundant variants exist in the genome (often within one or two base changes of each other), lower case letters were assigned to the evolutionarily derived sequence(s). For example, *C107-a* and *C106-a* are distinct unrelated fingerprints representing symbionts found in different hosts and are characterized by two dominant bands that correspond to abundant intra-genomic ITS2 sequence variants present in their ribosomal arrays. Owing to the high diversity of Clade C *Symbiodinium* species identified to date, the alpha-numeric nomenclature for symbiont profiles containing the ancestral *C3* or *C1* sequence now requires a doubling of alphabetic characters (e.g. 'aa', 'bb', 'cc', etc…) to name new symbiont fingerprints.

For Clade D ITS2-DGGE fingerprints, a novel and more precise nomenclature was adopted wherein each dominant sequence from a characteristic profile was assigned a separate and unique number. For example, *D1* refers to a specific profile with one dominant band (because there is only one homoduplex and therefore no heteroduplexes). In comparison, *D1-4* refers to a different but closely related Clade D *Symbiodinium* whose genome contains abundant copies of the '1' sequence but also contains a second co-abundant '4' sequence. Because of the sequence similarity between '1' and '4', they produce two heteroduplexes that comprise the upper bands of the fingerprint profile.

Community comparisons

Previously published data from the central GBR (LaJeunesse *et al.*, 2004a) were included for the purposes of comparing

symbiont diversity from the Indian Ocean with that of the western Pacific. This dataset comprised 351 samples representing 72 host genera in approximately 28 cnidarian families and two molluscs (Tridacnidae, Glaucidae) collected from two mid-shelf sites and one inshore site.

Symbiont community assemblages from each collection site were compared using the software package PRIMER v.6.0 (Plymouth Marine Lab, Lutton, Ivybridge, UK). A similarity dendrogram was generated from a cluster analysis of the diversity characterized at each site in each region. The presence of a particular symbiont was assigned a value of 1, while its absence was assigned a value of 0. Calculated similarity between sites was based on zero-adjusted Bray–Curtis similarity coefficients (Bray & Curtis, 1957).

The prevalence of Clade D *Symbiodinium* was calculated for the western and north-eastern Indian Ocean and the central GBR by averaging the percentage of samples in which Clade D was detected. Error bars were calculated from the standard deviation among sites within a region. A pairwise Student's *t*-test was used to determine the relative significance of Clade D prevalence between regions.

Phylogenetic analyses

Phylogenetic reconstructions based on dominant ribosomal repeat sequences derived from the ITS-DGGE fingerprinting method (Fig. 2b) were conducted using maximum parsimony (MP) under the default settings of PAUP* 4.0b10 (Swofford, 2000). Informative sequence gaps were included as a fifth character state, delayed-transformation (DELTRAN) was chosen for character state optimization, and no model of molecular evolution was assumed. A representative sequence from Clade H (Pochon *et al.*, 2004) was used as an outgroup to root the phylogeny. To assess the statistical significance of internal branching, a thousand bootstrap replicates were performed. Posterior probabilities were also calculated using MRBAYES v.3.0b4 (Huelsenbeck & Ronquist, 2001). Two million generations were analysed under the general time-reversible (GTR) model of sequence evolution, beginning with an unspecified tree topology and no defined prior probabilities. The posterior probabilities were calculated after removing the first 500,000 generations of 'burn-in'.

Microsatellite analyses

The extent of genetic isolation among closely related Clade D *Symbiodinium* was assessed using microsatellites. Five additional samples of *D1-4-6*, 4 harboured by *Montipora* collected from Oahu, Hawaii (LaJeunesse *et al.*, 2004b), and one from a *Pocillopora damicornis* colony in the display aquarium at the Hetzel Union Building at Pennsylvania State University, were included in these analyses. The DNA of each sample was diluted to 50 ng μL^{-1} prior to amplification of each microsatellite locus according to PCR conditions optimized by Pettay & LaJeunesse (2009). Fragments were analysed on an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with a 500-bp standard (LIZ-labelled) at the Pennsylvania State University Nucleic Acid Facility. Sizes were visually analysed using GENEMARKER v.1.51 (SoftGenetics, State College, PA, USA) and each sample was assigned to a multi-locus genotype. For the few samples with mixed genotypes, the dominant allele was used in assembly of the multi-locus genotype. Identical genotypes (clones) were then removed from the dataset before genetic similarity and statistical analyses were conducted.

A pairwise, individual-by-individual, genetic distance matrix was generated using the software package GENALEX (v.6.1; Peakall & Smouse, 2006). For each pairwise comparison, loci with the same state were assigned a value of 0, while loci that differed were given a value of 1. These values were then calculated across multiple loci.

To determine genetic differentiation between Clade D types, a pairwise genetic distance matrix was generated using GENALEX. The genetic distances within and between these populations were calculated and an analysis of molecular variance (AMOVA) was conducted. A Φ_{PT} value (a haploid equivalent to F_{ST}) was calculated for both the entire dataset and all pairwise comparisons between populations. To determine whether the Φ_{PT} values were significantly different from zero, a permutation procedure was utilized in GENALEX using 9999 permutations, and using $\alpha = 0.05$ after sequential Bonferroni correction for multiple comparisons (Rice, 1989). To graphically represent the genetic distance between types, a principal coordinates analysis (PCoA) was conducted using GENALEX and a three dimensional graph generated using SIGMAPLOT 2001 (v.7.0; SPSS Inc., Chicago, IL, USA). To further investigate the possibility of genetic differentiation among representatives of the same *Symbiodinium* type that originate from different host taxa, an AMOVA was conducted for the *D5* samples defined by host genus origin (*Goniopora*, *Montipora* and *Pocillopora*), using the same methods described above.

RESULTS

Regional environmental differences

Average monthly SSTs and Chl *a* concentrations (from 2003 to 2007) were markedly higher in the Andaman Sea than in the western Indian Ocean (Zanzibar) and central GBR (Australia) (Fig. 3a,b), which is consistent with historical trends (Hoegh-Guldberg, 1999; Brown & Phongsuwan, 2004). The warmest periods of the year in Zanzibar and the central GBR are 2°C cooler than the maximum temperatures in the Andaman Sea, where seawater temperatures remain well above 28°C throughout the year (Fig. 3a). The western Indian Ocean near Zanzibar and the central GBR have relatively low Chl *a* concentrations (indicative of high water clarity) throughout much of the year compared to the southern Andaman Sea (Fig. 3b). Extinction coefficients for light penetration measured *in situ* at various sites in the eastern and central Indian Ocean confirm these satellite data (Dunne & Brown, 1996).

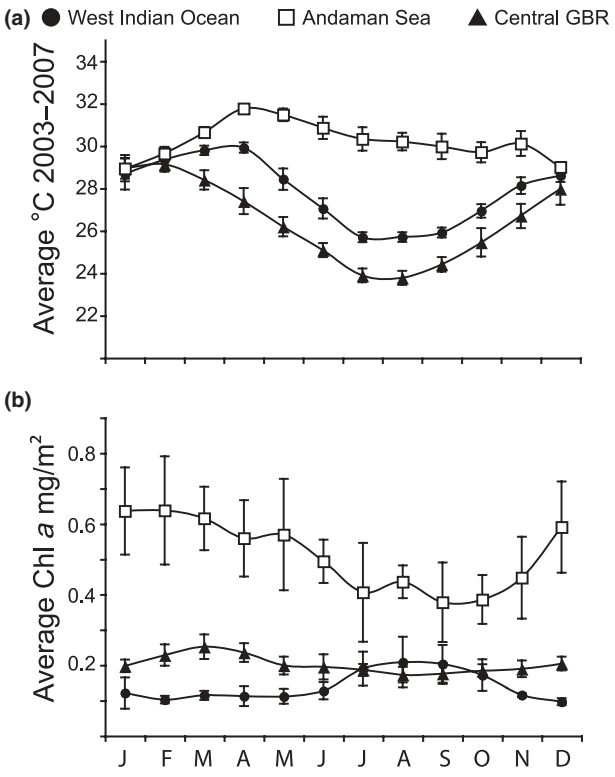

Figure 3 (a) Monthly averages of sea surface temperatures (SSTs) and (b) chlorophyll *a* (Chl *a*) concentrations for the Andaman Sea, the outer coast of Zanzibar and the central Great Barrier Reef (GBR), Australia. Average monthly satellite measurements from January 2003 to December 2007 were acquired from the Giovanni online data system, which is maintained by the NASA Goddard Earth Sciences Data and Information Services Center. Each point represents the monthly average ± SD.

Symbiodinium diversity, ecology and biogeography

A total of 36 distinct ITS2 *Symbiodinium* fingerprints were characterized from a total of 565 samples, representing 56 host genera in 25 families, collected at six sites in the north-eastern Indian Ocean (see Appendix S1 in Supporting Information). Forty-seven ITS2 *Symbiodinium* fingerprint types were characterized from 376 samples representing 70 host genera in 30 families, collected at three sites in the western Indian Ocean (Appendix S2). The genomes of these dinoflagellates contained one to three numerically dominant sequences that, when amplified using PCR and analysed with DGGE, produced characteristic fingerprints. Reproducible fingerprint profiles were characterized through the sequencing of diagnostic bands from two or three representative samples (such as those shown in Fig. 2b). Identical sequences were always retrieved from analogous bands in samples with the same fingerprint. For complex fingerprints (e.g. *C3u*), bands in the upper part of the profile were identified as heteroduplexes that form during the PCR of a genome where the ribosomal array is dominated by two or more similar sequence variants (Fig. 2b; cf. LaJeunesse, 2002). (See Materials and Methods for further details about the naming of *Symbiodinium* based on their ITS2 fingerprint profiles.) GenBank accession numbers for the ITS2 sequence data used to characterize the *Symbiodinium* diversity in this study are provided in Appendix S3.

Much of the diversity described in both regions consisted of apparently host-specific (observed only in samples from one host genus) and/or possibly rare types (found in only one or two samples). 'Species' diversity in Clade C was considerably higher in Zanzibar host communities (39 types) than in Thailand (23 types), while diversity in Clade D was higher in Thailand (11, vs. 4 from Zanzibar; Fig. 4a,b). Members of Clade A *Symbiodinium* were identified in fire corals in the genus *Millepora* from Zanzibar (Fig. 4a, Appendix S2) and in giant clams (*Tridacna*) from Thailand. The intertidal anemone *Anthopleura* sp., from Thailand, harboured a new Clade F sequence, while a member of Clade G (*G1*) was identified in samples from several scleractinians collected in the intertidal zone and was especially common among colonies in *Coeloseris mayeri*.

The host generalist *C3u* was the dominant detectable symbiont in many corals with open systems of symbiont acquisition in both the western and north-eastern Indian Ocean (Fig. 4a,b; Appendices S1 & S2). *Symbiodinium D1-4* (formerly *D1a*; LaJeunesse, 2002), designated *Symbiodinium trenchi* hereafter (LaJeunesse *et al.*, 2005), was also associated with coral species with open systems of symbiont acquisition and was especially common among colonies from the Andaman Sea. Several additional 'species' occurred in both regions, but most of the *Symbiodinium* diversity characterized was unique to one region (Fig. 4a,b).

Data presented in Appendices S1 & S2 show a range of host–symbiont specificity and variation among partner combinations. One potentially important observation is that corals in the genus *Montipora* from Thailand mostly harboured *Symbiodinium C15* or *D5* while the *Montipora* species in Zanzibar were associated with *Montipora*-specific types.

Community similarities and phylogenetic relationships

Similarity analyses of community assemblages indicated that symbiont diversity from the central GBR was distinct from regions in the Indian Ocean (Fig. 5). While more similar to each other relative to sites on the GBR, about 70% of the symbiont diversity described in the north-eastern and western Indian ocean was different. Collection sites within each region possessed the greatest overlap in diversity, but relative similarities were clearly influenced by environmental conditions characteristic of each site. For example, while Koh Racha was closer to Koh Hae, its symbiont diversity was similar to the Similan Islands farther to the north-west (Figs 1 & 5). Both Koh Racha and the Similan Islands are exposed to clearer outer shelf waters. The diversity of the Cape Panwa community was unusual because it was the only site where specimens were collected inter-tidally and where a number of unusual *Symbiodinum* were found (e.g. *C119*, *D8*, *F3* and *G1*). Lastly, the mid-shelf sites at the Feather and Rib reefs had similar

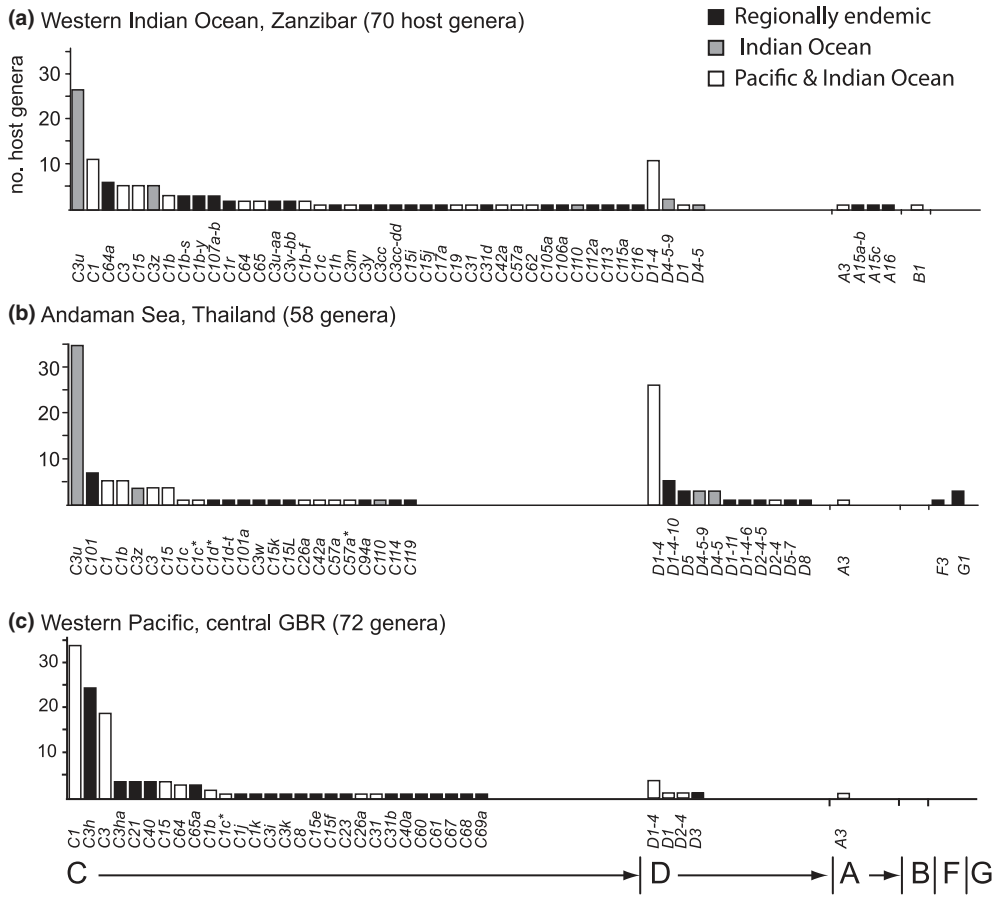

Figure 4 *Symbiodinium* type diversity showing the total number of host genera that were associated with each particular symbiont based on analysis of samples from (a) Zanzibar, Tanzania, (b) the Andaman Sea, Thailand, and (c) the central Great Barrier Reef (GBR), Australia (from LaJeunesse *et al.*, 2004a).

symbiont compositions relative to the inshore site (Fig. 5; see LaJeunesse *et al.*, 2004a, for further details).

Approximately 75% of the Clade C *Symbiodinum* 'species' characterized were unique to the Indian Ocean and many of these were regionally endemic (Figs 5 & 6). However, a quarter of the diversity was identified previously from surveys of Pacific coral reef communities (12 out of 49 Clade C types) and usually originated from the same host taxa.

Symbiodinium genomes may possess multiple co-dominant ITS sequence variants that usually differ by one or two base changes. Therefore, the ribosomal array for many *Symbiodinium* is more accurately characterized by multiple co-abundant sequence variants visualized as multiple bands in ITS-DGGE profiles (Fig. 2b). For example, the profile of *C3u* contained two common sequences (i.e. bands), while *C101* possessed a single numerically abundant sequence in its genome (see Fig. 2b). Therefore, many *Symbiodinium* species were characterized by multiple sequences in the phylogenies depicted in Figs 6 and 7. Unrooted Clade D polytomies based on ITS1 and ITS2 sequences from DGGE fingerprinting (Fig. 7a,b) were complementary, and their combined information improved resolution among members of this closely related symbiont group.

Prevalence and diversity of Clade D *Symbiodinium* in the Indian Ocean

Clade D *Symbiodinium* were significantly more frequent among hosts collected from warm and turbid sites of the north-eastern Indian Ocean in comparison with the western Indian Ocean or the central GBR (Fig. 8). Particular host taxa frequently associated with specific Clade D types were *Goniopora*, *Montipora*, *Oulastrea* and *Pocillopora* (scleractinians), as well as other cnidarians such as *Heliopora* (blue corals) and various corallimorphs. Of the Clade D types detected, *S. trenchi* (*D1-4*) had the widest host range and was the most common symbiont in samples where more than one symbiont was detected (81%, $n = 37$ of 46 in Thailand and 76%, $n = 13$ of 17 in Tanzania).

Finding reef communities in the Andaman Sea with high host diversity and a high diversity and frequency of Clade D *Symbiodinium* prompted more detailed geographic analyses of this group's distribution in the region. The symbioses from 12 common scleractinian genera living on 'inshore reefs' versus 'offshore reefs' were compared to determine the extent to which local environmental factors influenced the prevalence of symbioses involving symbionts from this group (Fig. 9). For

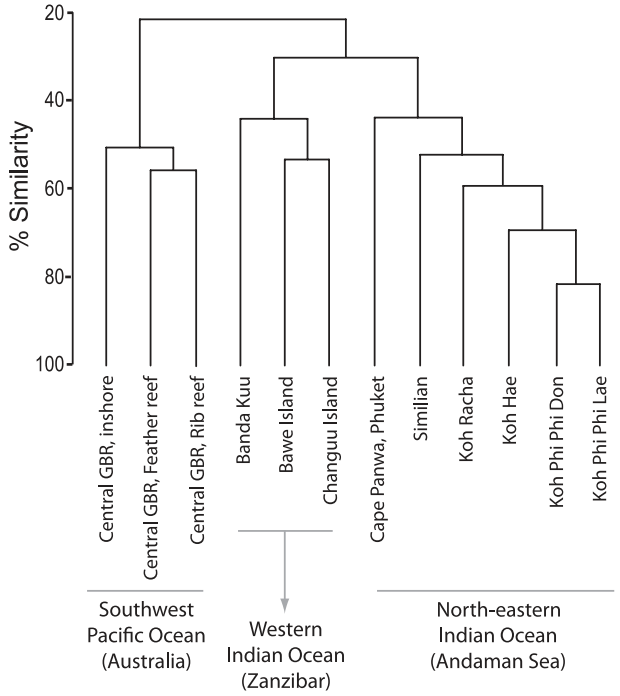

Figure 5 Dendrogram reconstructed from group-average linking based on Bray–Curtis similarity from presence/absence data of *Symbiodinium* diversity at sites in the Andaman Sea, western Indian Ocean (Zanzibar), and the central Great Barrier Reef (GBR), Australia.

some coral species, Clade D *Symbiodinium* occurred at higher frequencies among inshore colonies especially in those species with horizontal modes of symbiont acquisition (e.g. *Favites*, *Platygyra*, *Symphyllia*), while among other species there was no detectable environmental influence on the distribution of Clade D *Symbiodinium* (e.g. *Acropora*; *Porites*, Fig. 9).

Inter-colony variability in the dominant resident symbiont ranged widely among host taxa. For example, seven different symbiont types were found associating with colonies in the genus *Pocillopora* (three Clade D types and four Clade C types) while only *Symbiodinium D8* associated with the intertidal coral *Oulastrea crispata* (Fig. 9). Sample sizes of inshore and offshore colonies were not always proportional, and therefore further sampling may be required to determine with greater certainty how different environments influence the presence of certain partner combinations.

Based on the analysis of eight microsatellite loci, unique haploid genotypes were identified in four of four *D2-4-5* samples, three of four *D8* samples, five of seven *D1-4-6* samples, 15 of 16 *D5* samples and 27 of 31 *S. trenchi* (*D1-4*) samples. Background genotypes were identified in six of 62 samples analysed. PCoA based on the genetic distances between genotypes generated five clusters, each directly corresponding to a particular ITS-DGGE fingerprint (Fig. 10; *x*-, *y*- and *z*-axes account for approximately 70% of the genetic variability). Statistical analyses of Φ_{PT} values indicated that significant population structuring exists between symbiont types (Appendix S4). Genotypes of *S. trenchi* from samples originating from the western (Zanzibar) and north-eastern (Thailand) Indian Ocean clustered together and could not be differentiated with the sample sizes analysed (Φ_{PT} 0.057). The genotypes of *D5*, from the Andaman Sea, clustered together but exhibited some substructure between genotypes collected from *Goniopora* versus *Pocillopora* (there was some overlap in genetic similarity among the *D5* genotypes found in *Goniopora* and *Montipora*, Appendix S5).

DISCUSSION

Knowledge of the ecology and biogeography of symbiotic dinoflagellates is crucial for deducing the biological foundations of cnidarian–algal symbioses. As *Symbiodinium* 'species' diversity and their distributions are characterized from more regions, novel and recurring patterns are revealed that both reinforce and broaden concepts of how symbiotic corals respond ecologically and evolve to cope with various environmental conditions. Understanding these processes may clarify the extent to which reef corals can respond to current and future trends in global warming.

Factors that influence regional differences in symbiont diversity and ecological dominance

The diverse coral assemblages of some Indian Ocean reef ecosystems contain many symbioses involving Clade D *Symbiodinium*. Previous surveys of Indo-Pacific corals from cooler, less turbid environments rarely reported finding this symbiont group (e.g. LaJeunesse, 2002; LaJeunesse *et al.*, 2003, 2004a,b; Chen *et al.*, 2005; McClanahan *et al.*, 2005; Visram & Douglas, 2006). Recent investigations at sites with extreme temperatures and/or high turbidity found Clade D *Symbiodinium* in great abundance (Fabricius *et al.*, 2004; Rowan, 2004; Garren *et al.*, 2006; Lien *et al.*, 2007; Mostafavi *et al.*, 2007; LaJeunesse *et al.*, 2008). However, most of these studies focused on particular host species often living in marginal habitats. Preliminary experimental and ecological data indicate that *Pocillopora* species and *Acropora millepora* Clade 'D' holobionts have a greater tolerance of thermal stress and/or photoacclimatize rapidly to changes in irradiance, relative to Clade C holobionts (Iglesias-Prieto *et al.*, 2004; Rowan, 2004; Berkelmans & van Oppen, 2006; LaJeunesse *et al.*, 2007). These physiological characterizations are consistent with the ecological and biogeographic distributions of Clade D *Symbiodinium* 'species' (relative to Clade C) observed in the present study.

Regional and local environments within the Indian Ocean have probably influenced the relative dominance of two host-generalist symbionts, *Symbiodinium C3u* and *S. trenchi* (*D1-4*). While *C3u* was dominant among reef cnidarians in both regions, high temperatures and turbidity may account for the greater prevalence of *S. trenchi* in the Andaman Sea (Fig. 8). Among locations within this region of Southeast Asia, *S. trenchi* was especially prevalent at inshore sites where turbidity and tidal cycles are greatest (Fig. 9; Dunne & Brown, 1996).

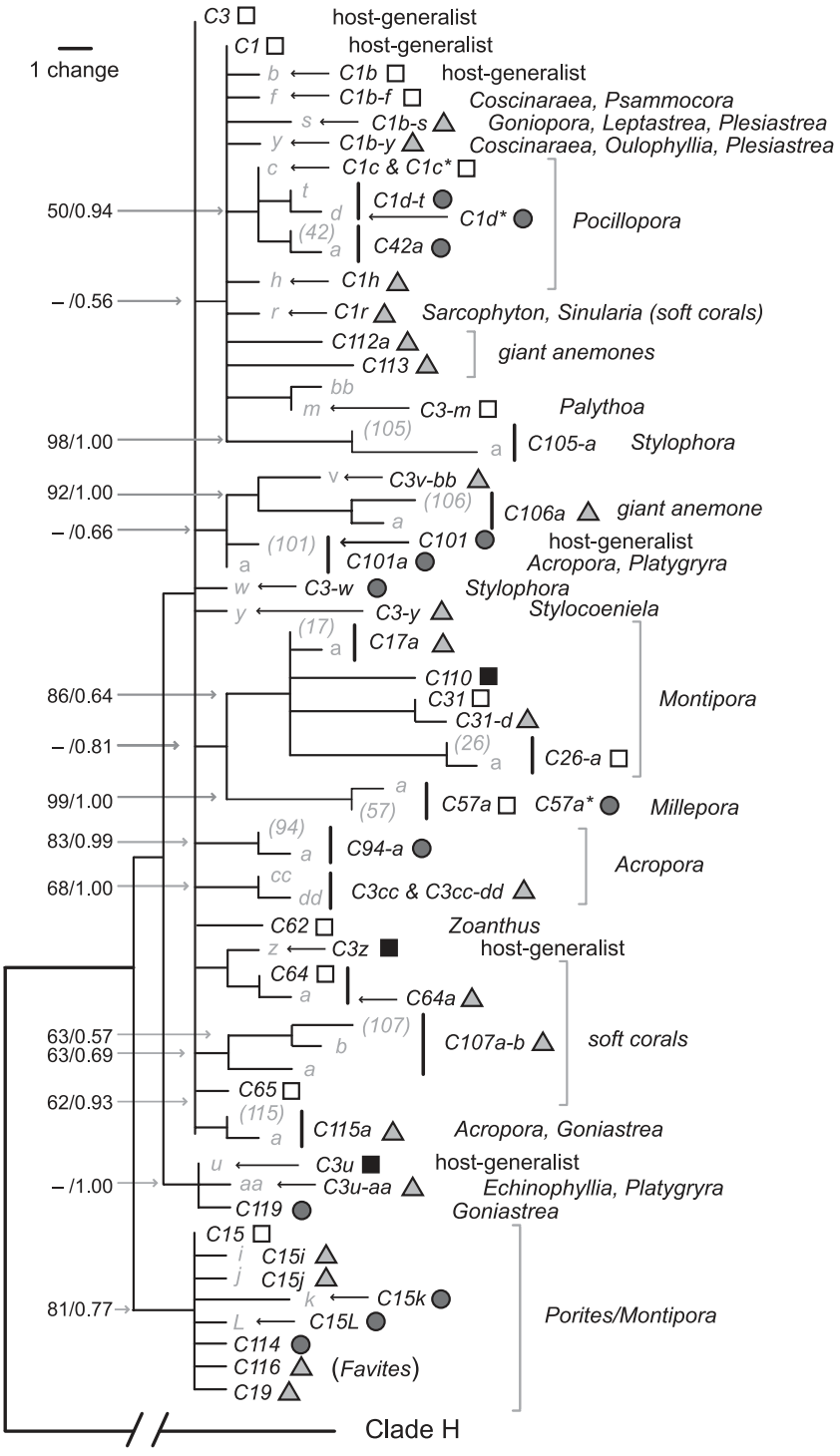

Figure 6 Phylogenetic reconstruction inferring evolutionary relationships among Clade C *Symbiodinium* using maximum parsimony and based on internal transcribed spacer 2 (ITS2) sequence data. Light grey triangles designate *Symbiodinium* observed in hosts surveyed from Zanzibar. Dark grey circles designate types found only in the Andaman Sea, Thailand. Black squares designate those *Symbiodinium* types found in both regions, while white squares identify types that are also known to occur in the Pacific. Numbers separated by a forward slash mark to the left of the phylogeny are bootstrap values based on a thousand replicates (first number) and posterior probabilities (second number) for internal branch support. The corresponding host origins are provided to the right. *Symbiodinium* that associated with five or more host genera were designated 'host-generalists'.

Both generalists occurred almost exclusively in coral species that must acquire symbionts from the environment during early development (e.g. Fig. 9). Approximately 80–90% of Indo-Pacific corals broadcast spawn to produce aposymbiotic larvae that depend on horizontal symbiont acquisition (Richmond & Hunter, 1990). Not only does this explain the ecological success of host-generalist *Symbiodinium* in many Indo-Pacific reef communities, it further suggests the possibility that, in just a few generations, a coral community can become dominated by a different symbiont if it is compatible with coral species being recruited to the community (LaJeunesse *et al.*, 2004a).

The similarity of average monthly temperatures and water clarity in the western Indian Ocean and the central GBR may explain the ecological dominance of Clade C *Symbiodinium* in both regions (Fig. 4a,c). Closer inspection of the diversity

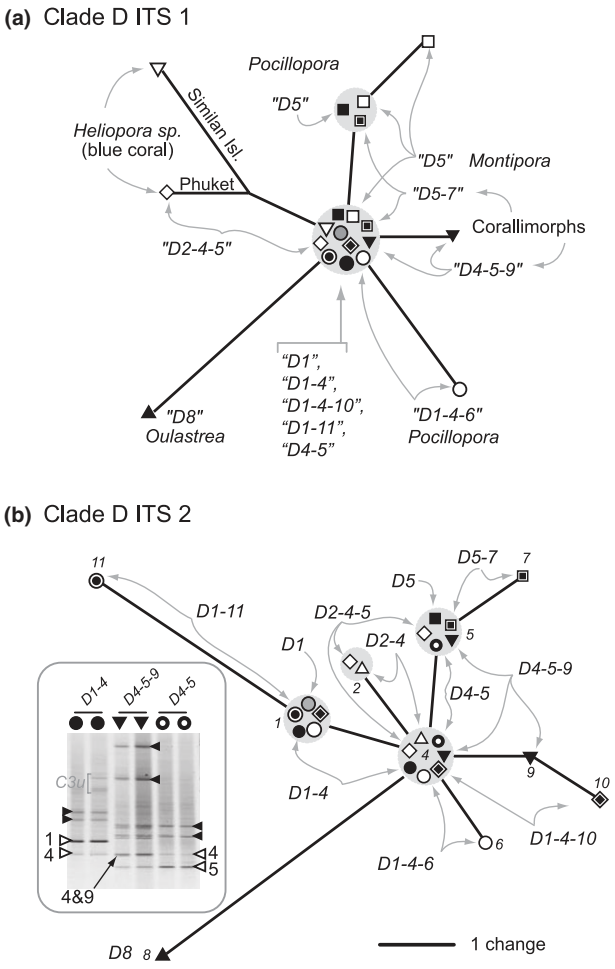

Figure 7 Phylogenetic relationships among closely related Clade D *Symbiodinium* based on intra-genomically dominant (a) internal transcribed spacer 1 (ITS1) and (b) ITS2 sequences acquired using ribosomal DNA (rDNA) denaturing gradient gel electrophoresis (DGGE) fingerprinting (inset). Many possess ancestral sequences that are still dominant in the ribosomal array as well as a second or third co-dominant sequence that distinguishes one symbiont type from another. To illustrate the occurrence of shared dominant sequences, symbols corresponding to sequences derived from particular ITS2-DGGE fingerprints were used in each phylogeny (see text for further details). The numerical nomenclature in quotations for the ITS1 was based on diagnostic sequences from ITS2-DGGE profiles. The inset depicts three different Clade D *Symbiodinium*. The presence of a third co-dominant sequence '9', which co-migrates with sequence '4', explains the four additional heteroduplexes in the *D4-5-9* fingerprint. The open and closed arrowheads designate homoduplexes and heteroduplexes, respectively.

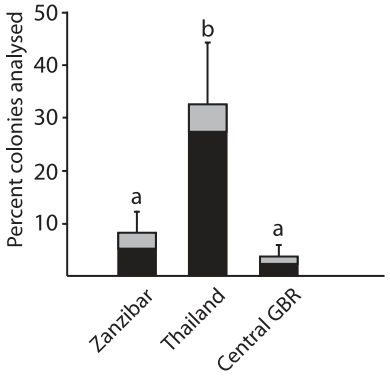

Figure 8 The frequency (i.e. prevalence) of clade D *Symbiodinium* in samples from geographically separated host communities across the Indo-Pacific: Zanzibar, Thailand and the central Great Barrier Reef (GBR), Australia. Grey portions denote the proportion of samples that contained mixtures of at least two symbionts usually involving *Symbiodinium trenchi* (*D1-4*) and 'species' from Clade C. The greater frequency of Clade D in the Andaman Sea was influenced by the high diversity of host-specific Clade D 'species'. Error bars were generated from standard deviations calculated from proportions of colonies found harbouring Clade D *Symbiodinium* from three to six study sites within each region (the lower-case letters designate significance,*$P < 0.05$; based on pairwise Student's *t*-tests).

within Clade C, however, shows that there is very little similarity in 'species' composition between the Pacific and Indian Oceans (Figs 4a–c & 5). Despite the environmental differences and geographic separation of western and north-eastern regions, the overlap of Clade C diversity is greater within the Indian Ocean (Figs 5 & 6). These diverse symbiont assemblages probably evolved after the formation of a biogeographic barrier between the Indian and Pacific Oceans approximately 1–3 Ma (Benzie, 1999).

Even within the Indian Ocean, most of the Clade C diversity characterized in each region appears to have evolved independently (Fig. 6). While there is some measure of overlap in *Symbiodinium* diversity between the Andaman Sea and the western Indian Ocean, especially among host-generalists, the phylogeny in Fig. 6 suggests that the independent evolution of many host-specific *Symbiodinium* has produced 'species' assemblages endemic to each region. These patterns further indicate that many host-specialist symbionts have limited dispersal capabilities, a possibility that could be tested by population genetic analyses (Santos *et al.*, 2003). These patterns indicate that regional environments influence the relative ecological success of a *Symbiodinium* clade, but that the degree of isolation and ecological specialization substantially influences the evolution of 'species' diversity within a clade.

Regional environments appear to influence host–symbiont specificity and ecological dominance among Clade C *Symbiodinium*. For example, *Symbiodinium C15*, commonly found in *Porites* throughout much of the Indo-Pacific (LaJeunesse, 2005), was also harboured by numerous *Montipora* species sampled from the Andaman Sea (Fig. 9). The conditions in this region may favour *C15*, which appears to be thermally tolerant (Fisher, 2006). A different group of *Montipora*-specific *Symbiodinium* was associated with *Montipora* from the milder environments of the western Indian Ocean (e.g. *C17a*, *C31d*; Fig. 6). These particular symbioses appear to be sensitive to environmental

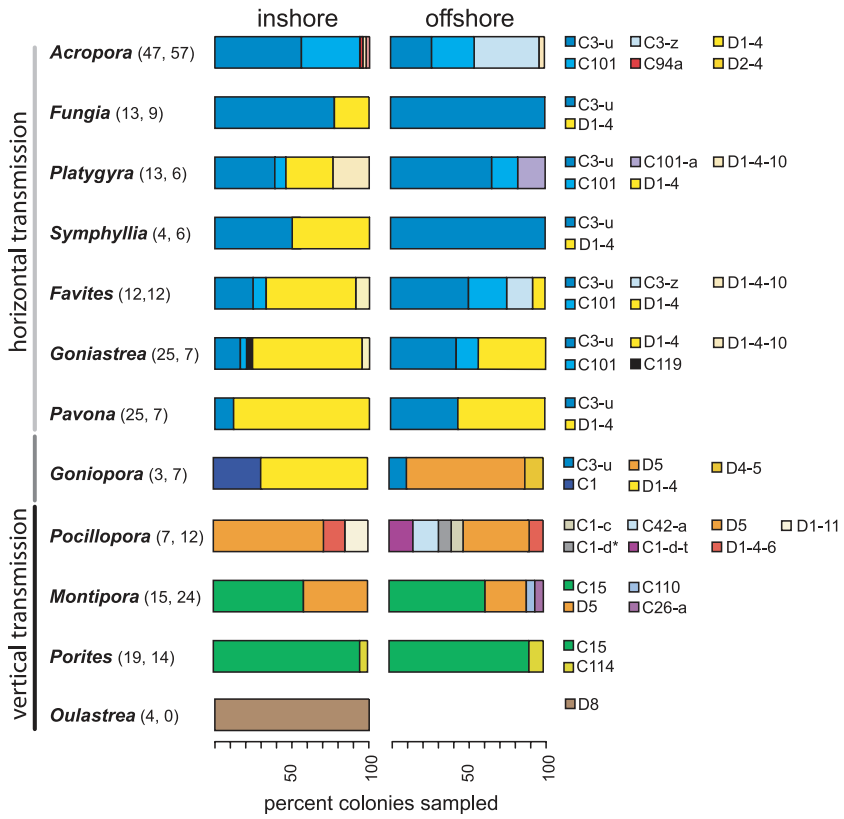

Figure 9 The influences of 'inshore' influenced 'offshore' environments on the distribution and frequency (i.e. prevalence) of various *Symbiodinium* types among different colonies of common coral genera. Shades of blue, purple and green correspond to symbionts in Clade C while shades of yellow and brown refer to different symbionts in Clade D. Numbers in parentheses beside genus names indicate the number of colonies analysed from inshore and offshore sites, respectively. The mode of symbiont acquisition, either from the parent (vertical) or from the environment (horizontal), is indicated to the left of the coral genus names.

stress (Baird & Marshall, 2002; Dove *et al.*, 2006), which is probably why so few examples were found in the Andaman Sea (Fig. 9; Appendices S1 & S2).

In addition to influencing the relative dominance of *S. trenchi* (*D1-4*), local environmental factors (inshore versus offshore) also affected the presence or absence of associations involving different Clade C *Symbiodinium* (Fig. 9). For example, *Symbiodinium C3z* was absent from populations of *Acropora* species found on inshore reef environments (Fig. 9). At the inshore site of Cape Panwa, photosynthetically active radiation (PAR) diminishes to 1% by 11.5 m whereas PAR does not reach 1% until depths of 26 m at the offshore site of Koh Racha (Dunne & Brown, 1996). The inshore–offshore zonation of *Acropora* harbouring *C3z* suggests that the ecological distribution of certain partner combinations also depends on physiological differences among genetically similar *Symbiodinium* 'species' (e.g. Ulstrup & van Oppen, 2003; Berkelmans & van Oppen, 2006). Virtually unstudied, the functional diversity found within various clades of *Symbiodinium* is potentially high (e.g. Savage *et al.*, 2002; Tchernov *et al.*, 2004; Sampayo *et al.*, 2008; Thornhill *et al.*, 2008) and may include species important to coral communities during times of major climate change.

Collectively, these observations emphasize that regional and local environmental factors, in combination with ecological specialization and isolation, drive the biogeographic complexity of host–symbiont partnerships (e.g. LaJeunesse, 2005; van Oppen *et al.*, 2005b). Both biotic and abiotic interactions create regional and local mosaics of differing co-evolved host–symbiont associations. This biological complexity may be of critical importance in the response of these symbioses to global warming (see below).

Resolving 'species' diversity among Clade D *Symbiodinium*

Most Clade D *Symbiodinium* 'species' differ by only a few base changes in the most common ITS1-5.8S-ITS2 sequence variants of each genome (Fig. 7a,b). Concerted evolution, involving genetic recombination and generational turnover, 'homogenizes' the ribosomal array so that one or a few sequence variants are numerically dominant in the genomes of individuals comprising a population (Dover, 1982). As such, minor, albeit fixed, differences in these ITS sequences delimit ecologically distinct *Symbiodinium* (operational taxonomic units, or OTUs; e.g. LaJeunesse, 2002; Pochon *et al.*, 2004, 2007; Rodriguez-Lanetty *et al.*, 2004; Thornhill *et al.*, 2006b, 2008; Sampayo *et al.*, 2007, 2008; Frade *et al.*, 2008; Goulet *et al.*, 2008). Slight differences in ITS sequences are usually attributed to inter-individual variation (Litaker *et al.*, 2007; Alverson, 2008). However, much of this previously reported variation among samples is probably due to intra-genomic variation where, during the process of sequencing from bacterially cloned ribosomal DNA (rDNA), rare sequence variants, sometimes numerous in the genome, are haphazardly recovered (Thornhill *et al.*, 2007).

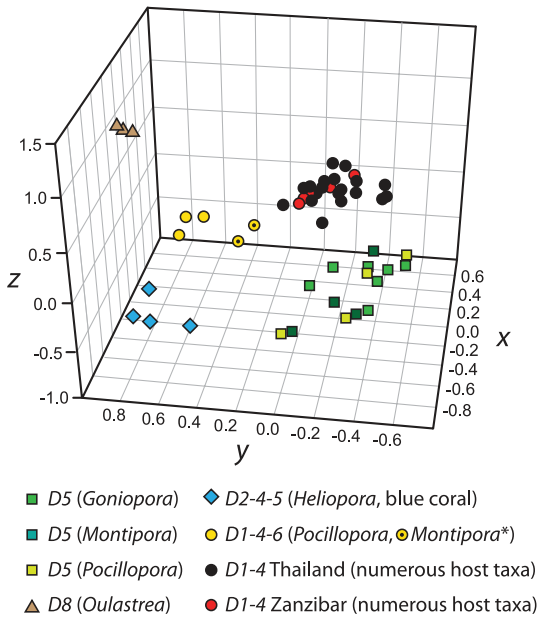

Figure 10 Three-dimensional principal coordinates analysis with axes *x*, *y* and *z* explaining 33, 22 and 15% of the variation (totalling 70%), respectively, in the genetic distances of multilocus genotypes (eight microsatellite loci) from 54 unique clones representing five ecologically distinct Clade D *Symbiodinium*. The ordination analysis indicates that populations of *Symbiodinium D5*, *D8*, *D2-4-5*, *D1-4-6* and *Symbiodinium trenchi* (*D1-4*, formerly *D1a*) are genetically isolated (see Appendices S4 and S5). Samples of *D1-4-6* from *Montipora* ('*') were collected in Oahu, Hawaii (LaJeunesse *et al.*, 2004b).

Multi-locus genotyping of Clade D *Symbiodinium* provides further empirical evidence that populations exhibiting different ecological distributions and ITS-DGGE profiles, no matter how similar in sequence, appear to be reproductively isolated (Fig. 10, Appendix S4; Pettay & LaJeunesse, 2009). *D2-4-5* associated with the blue coral, *Heliopora*, *D8* from *Oulastrea crispata*, *D1-4-6* from *Pocillopora*, and *D5* from *Goniopora*, *Montipora* and *Pocillopora* were all well differentiated (based on AMOVA and PCoA of individual genotypes, Fig. 10, Appendix S4). Several other Clade D *Symbiodinium* appear specific to coral species whose distributions extend to subtropical and temperate high-latitude environments, including *Pocillopora* from the eastern Pacific (LaJeunesse *et al.*, 2008) and *Oulastrea crispata* in the northwest Pacific (Lien *et al.*, 2007). The correspondence between genetic and ecological differentiation indicates that specialization to particular host taxa is extremely important in the evolution of *Symbiodinium* (LaJeunesse, 2005) and is consistent with the fundamental evolutionary concept that ecological specialization leads to reproductive isolation and speciation (Futuyma & Moreno, 1988; Schluter, 2001; de Queiroz, 2007).

Symbiodinum trenchi (*D1-4*) was ecologically distinguished from other Clade D members as the only host-generalist symbiont associating with various broadcast-spawning corals. Population genetic data differentiated it from other Clade D *Symbiodinium* (Fig. 10, Appendix S4), but could not distinguish western and north-eastern populations, indicating that gene flow and/or dispersal among populations of *S. trenchi* may occur to some degree across the Indian Ocean. LaJeunesse *et al.* (2005) provisionally named *S. trenchi* in their comparative analysis of dinoflagellate genome sizes. Aspects of *S. trenchi*'s ecology were recently described during the course of a major bleaching event in the Caribbean (LaJeunesse *et al.*, 2009). Formal naming of eukaryotic microbial species traditionally relies on morphology (e.g. Trench & Blank, 1987; Hansen & Daugbjerg, 2009); however, evidence that satisfies phylogenetic, ecological and population genetic species concepts (de Queiroz, 2007) distinguishes *S. trenchi* from other Clade D *Symbiodinium*. Such molecular ecological analyses provide a comprehensive and realistic approach for delimiting species boundaries among morphologically similar eukaryotic microbes.

The evolution of coral–algal symbioses and environmental change: past and present

The preliminary characterizations of *Symbiodinium* 'species' diversity from different regions across the Indo-Pacific suggest that the combination of persistent high temperatures and variable light conditions have facilitated the ecological success and evolutionary radiation of Clade D, especially in the Andaman Sea. This region is part of a massive warm water zone encompassing Southeast Asia, Indonesia and northern Australia. The shelf waters bounded by the Indonesian archipelagos and Asia are comparatively shallow and have undergone repeated ocean level transgressions and regressions since the beginning of the Pleistocene (Potts, 1983; Voris, 2000). Vast and extremely shallow lagoon-like habitats were created during repeated glacial and interglacial periods, creating environments similar to the inshore habitats of the Andaman Sea, and would have facilitated the ecological success and evolutionary radiation of Clade D. Given the limited, albeit fixed, sequence divergence among the these *Symbiodinium*, relative to those in Clades C and B, rough molecular clock estimates of ITS2 evolution (one base conversion in the dominant ITS2 per 0.75–1.3 Myr; LaJeunesse, 2005) place the timing of this group's radiation during the Pleistocene (2.6 Ma to 12,000 years ago).

Coral communities on the GBR have suffered from severe bleaching and mortality, with over 60% of corals experiencing bleaching in 1998 and 2002 (Berkelmans & Oliver, 1999; Berkelmans *et al.*, 2004), but despite these disturbances, post-bleaching surveys of diversity indicate that the prevalence of Clade D *Symbiodinium* among host populations has remained low in the GBR (Figs 4c and 8; LaJeunesse *et al.*, 2003, 2004a; van Oppen *et al.*, 2005a; Goulet *et al.*, 2008; Sampayo *et al.*, 2008; Stat *et al.*, 2008) as well as in most regions of the Indian Ocean (Figs 4a and 8; McClanahan *et al.*, 2005; Visram & Douglas, 2006; MacDonald *et al.*, 2008). One probable explanation is that water temperature and turbidity are historically

low in these areas (conditions that are not favourable to the proliferation of *S. trenchi*), and hence few corals have associations with stress-tolerant *Symbiodinium* (Figs 4 & 8). Without the existence of pre-bleaching baseline data for locations in the Persian Gulf and along the equatorial coast of Kenya, western Indian Ocean (Baker *et al.*, 2004; Sotka & Thacker, 2005), it is more probable that the prevalence of Clade D in these regions is due to naturally occurring associations resulting from persistent environmental conditions.

The current pace of temperature change is thought to be orders of magnitude more rapid than the fastest glacial–interglacial shifts that occurred during the Pleistocene (Hoegh-Guldberg *et al.*, 2007). Barring the long-range dispersal and rapid establishment of thermally tolerant symbionts and/or corals, the degree to which coral reef ecosystems can cope with this rate of change may depend on the region's present composition of corals and their co-evolved symbioses. Coral communities with diverse symbiotic combinations, such as those of the Andaman Sea, may be more resilient than others to climate change. Stress-tolerant associations, already present at high frequencies, may increase further due to symbiont 'shuffling' (i.e. competition) involving background populations of certain 'species' of symbiont and/or differential mortality of sensitive host–symbiont combinations (Berkelmans & van Oppen, 2006; Jones *et al.*, 2008; Sampayo *et al.*, 2008; LaJeunesse *et al.*, 2009). However, coral communities in regions with historically milder environmental conditions may not possess enough thermally tolerant symbioses to support a functioning ecosystem under recurring episodes of severe stress and natural selection.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 *Symbiodinium* diversity in host assemblages from reefs in the Andaman Sea, north-eastern Indian Ocean (Thailand).

Appendix S2 *Symbiodinium* diversity in host assemblages from reefs in the western Indian Ocean (Zanzibar, Tanzania).

Appendix S3 GenBank accession numbers for the sequences of diagnostic bands from denaturing gradient gel electrophoresis fingerprinting of the internal transcribed spacer region 2 (ITS2-DGGE) characterized from the Indian Ocean.

Appendix S4 Pairwise comparisons of Φ_{PT} values (a haploid equivalent to F_{ST}) among Clade D *Symbiodinium* populations differentiated by denaturing gradient gel electrophoresis fingerprinting of the internal transcribed spacer region (ITS-DGGE).

Appendix S5 Pairwise comparisons of Φ_{PT} values (a haploid equivalent to F_{ST}) among *Symbiodinium D5* populations originating from *Montipora*, *Goniopora* and *Pocillopora*, respectively.

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BIOSKETCH

The research interests of **Todd C. LaJeunesse** (and his students and colleagues) include examining the relative influence of biotic and abiotic factors, including climate change, on the co-evolution of animal–microbe mutualisms. Central to this work is the development of molecular approaches for the accurate and precise delimitation of ecologically relevant species diversity of microbial eukaryotes. For more information see: http://homes.bio.psu.edu/people/faculty/lajeunesse/index.html.

Author contributions: T.C.L., B.B., O.H.-G. and W.K.F conceived the project; T.C.L., N.P., D.O.O., O.H.-G. and W.K.F. identified, collected and processed samples; T.C.L., D.T.P. and E.M.S. collected and analysed molecular-genetic data; and T.C.L. led the writing.

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Appendix S1 *Symbiodinium* diversity in host assemblages from reefs in the Andaman Sea, north-eastern Indian Ocean (Thailand). The identifier for each symbiont refers to the evolutionarily divergent clade (uppercase letter) the ITS-DGGE fingerprint type including the designations of one or more dominant intragenomic sequences in the ribosomal array (numbers and lowercase letters). Numerals in parentheses indicate the number of colonies in which a particular symbiont was found. Two or more types separated by a forward slash indicate that the symbionts co-occurred in the sample. Depth ranges of host collection are given in parentheses for each study site. The asterisk next to *C1c* and *C57a* indicates that the fingerprint of this symbiont is characterized by the 'c' or 'a' band only and lacks the more ancestral 'C1' or 'C57' band, respectively.

Host Family Genus	Species	Cape Panwa (0-5m)	Hae (2-6m)	Phiphi Don (2-6m)	Phiphi Lae (6-12m)	Racha (2-5m)	Similan Islands (2-12m)
Acroporiidae							
Acropora	*Acropora aspera*	C3u (3), D1-4 (1)		C3u (1)		C3z (1)	
	Acropora austera	C3u (3)	C101 (2)	C3u (1)	C3u (1)	C3z (1)	C3z (1)
	Acropora cerealis						C101 (1)
	Acropora clatharata					C3z (1)	C3z (1)
	Acropora cytherea						C3z (1)
	Acropora danai						C3z (1)
	Acropora digitifera	C3u (2), D1-4 (1)					
	Acropora divaricata		C101 (1)	C101 (1)	C101 (1)		
	Acropora elseyi					C3z (1)	
	Acropora florida		C101 (1)	C101 (1)	C101 (1)	C3z (1)	
	Acropora formosa	C3u (3)	C101 (1)	C3u (1)	C3u (1)	C3z (1)	C101 (1)
	Acropora gemmifera					C3z (1)	C3z (1)
	Acropora grandis		C101 (1)	C101 (1)			
	Acropora horrida					C3z (2)	
	Acropora humilis	C3u (3)	C101 (1)			C101 (1)	C3z (1)
	Acropora hyacinthus	C3u (2), C101 (1)	C94a (1)	C3u (1)	C3u (1)	C101a (1)	
	Acropora microphthalma		C101 (1)				
	Acropora millepora						C101 (1)
	Acropora monticulosa					C101 (1)	
	Acropora nasuta	C101 (1)	C101 (1)	C3u (1)			
	Acropora nobilis	C3u (1), C101 (1)	C101 (1)			C3z (1)	C101 (1)
	Acropora palifera		C101 (1)				
	Acropora pulchra	D2-4 (1)					
	Acropora robusta						C3z (1)
	Acropora rudis						C3z (1)
	Acropora samoensis	C3u (1)	C101 (1)	C3u (1)		C101 (1)	
	Acropora secale	C3u (2)	C101 (1)	C101 (1)		C3z (1)	
	Acropora solitaryensis	C3u (2)		C3u (1)			

	Acropora sp.			C3u (3)		C3z (3)	
	Acropora specifera	C3u (2)					
	Acropora subulata		C101 (1)	C3u (1)	D1-4 (1)		C3z (1)
	Acropora tenuis	C3u (1)	C101 (1)	C101 (1)		C3z (1)	C3u (1)
	Acropora valida	C3u (1)		C3u (1), C101 (1)	D1-4 (1)	C3z (1)	
Astreopora	*Astreopora myriophthalma*	D1-4 (1)		D1-4 (1)	D1-4 (1)	C1b (1)	
Montipora	*Montipora angulata*					C15k (1)	C15 (1)
	Montipora caliculata			D-5 (1)		C15 (1)	C110 (1)
	Montipora aequituberculata		D-5 (1)				C15 (1)
	Montipora digitata	C15 (5)	C15 (1)	C15 (1)		C15 (1)	
	Montipora efflorescens			D5 (1)	D5 (1)	C15 (1)	
	Montipora hispida		C15 (1)	C15 (1)		D-5/C15 (1)	D-5 (1)
	Montipora mollis						C15 (1)
	Montipora sp.	C15 (2), D5 (4)	D5 (1)	C15 (1)		C15 (3)	C26a (1)
	Montipora tuberculosa	D5 (1)		C15 (1)		C15 (1)	C15 (1)
	Montipora verrucosa					D5 (1)	
Actiniidae							
Anemonia	*Anemonia* sp.				C3 (1)		
Anthopleura	*Anthopleura* sp.	F3 (3)					
Agariciidae							
Coeloseris	*Coeloseris mayeri*	D1-4 (3), D1-4/G1 (3)	D1-4 (1)	D1-4 (1)		D1-4 (1)	C3u (1)
Gardineroseris	*Gardineroseris planulata*	C3u (1)		C109a (1)	C3u (1)	C3u (1)	D1-4 (1)
Leptoseris	*Leptoseris mycetoseroides*			C1b (1)	C1b/D1-4 (1)		C3u (1)
Pachyseris	*Pachyseris rugosa*		C3u (1)				C3u (1)
	Pachyseris sp.		D1-4 (1)				
	Pachyseris speciosa			C3u (1)	D1-4 (2)		
Pavona	*Pavona decussata*	D1-4 (4)	D1-4 (1)	D1-4 (1)			
	Pavona explanulata		D1-4 (1)	D1-4 (1)	D1-4 (1), C3u (1)		D1-4 (1)
	Pavona minuta				C3u (1)		
	Pavona duerdeni						C3u (1),
	Pavona varians	D1-4/C3u (1)	C3u (1)	D1-4 (1)	C3u (1), D1-4 (1)	D1-4/C3u (1)	C3u/D1-4 (1)
	Pavona venosa			C3u (1)			
Alcyonidae							
Cladiella	*Cladiella sp.*	D4-5-9 (2)		D4-5-9 (1)			
Sarcophyton	*Sarcophyton sp.*		C1 (1)				
Sinularia	*Sinularia sp.*				C1(1)		C1(1)
Caryophylliidae							
Euphyllia	*Euphyllia ancora*				C3u (1)		
	Euphyllia glabrescens		C1b (1)		C1b (1)		
Plerogyra	*Plerogyra sinuosa*			C3u/D1-4 (1)	C3u/D1-4 (1)		
Corallimorpharia							
Corallimorpharia	*Corallimorpharia* sp.		D4-5 (1)	D5-7 (1)	D4-5-9 (2)	D4-5-9 (2)	D5 (1)
Dendrophylliidae							

Turbinaria	*Turbinaria frondens*	C3u (4), C3u/D1-4 (1), D1-4/C3u (1)					
Euphyliidae							
Physogyra	*Physogyra lichtonsteini*			D-1-4/C3u (1)	C3u/D1-4 (1)	C3u (1)	C3u (1)
Faviidae							
Cyphastrea	*Cyphastrea serailia*			C3u (1)	C3u/D1-4 (1)		
	Cyphastrea sp.					C3u (1)	C3u (1)
Diploastrea	*Diploastrea heliopora*	C3u/D1-4 (1), D1-4 (2)		D1-4 (1)	D1-4 (1)	C3u (1)	
Echinopora	*Echinopora gemmacea*			D1-4/C3u (1)	C3u (1)		
	Echinopora horrida	C3u (1)					
	Echinopora lamellosa	C3u (1)	C3u (1)	C3u (1)			C3u (1)
	Echinopora sp.						C3u (1)
Favia	*Favia favus*				C3u (1)		
	Favia lizardensis			C3u (1)			
	Favia pallida	C3u (2)		C3u (1)			
	Favia sp.				C3u (1)	D1-4 (1)	C101 (1)
	Favia speciosa	C3u (1)		C3u (1)			
	Favia stelligera			C101 (1)		C3z (1)	C101 (1)
Favites	*Favites abdita*	C101 (1), D1-4 (5), D1-4-10 (1), D1-4/C3u (1)		C3u (1)	C101/C3u (1)	C3z (1)	C3z/C3u (1)
	Favites halicora			C3u (1)		C101 (1)	
	Favites pentagona	C3u (2)		C3u (1)	C3u (1), D1-4 (1)	C3u (1)	
Goniastrea	*Goniastrea aspera*	D1-4 (19), C119/D1-4 (1)					
	Goniastrea edwardsi			C3u (1)		C3z (1)	
	Goniastrea favulus	D1-4-10/C3u (1)					
	Goniastrea pectinata	C3u (2), D1-4 (2)		C3u (1)	C3u/D-1-4 (1)		
	Goniastrea retiformis	D1-4 (2), D1-4/C3u (1)	C101 (1)		D-1-4 (1)	D1-4 (1)	
Leptastrea	*Leptastrea pruinosa*	D1-4 (2)	D1-4 (1)				C3u/D1-4 (1)
	Leptastrea sp.			D1-4 (1)		D1-4 (1)	
Leptoria	*Leptoria phrygia*			C3u (1)		C3u (1)	C101 (1)
Montastraea	*Montastraea curta*	C3u (1), D1-4/C3u (1)		C3u (1)	C101 (1)		
	Montastraea valenciennesi	C3u (2), D1-4-10/G-1 (1)					C3u/D1-4 (1)
Oulastrea	*Oulastrea* sp.	D8 (3), D8/D1-4 (1)					
Platygyra	*Platygyra daedalea*	D1-4 (3), C3u (1), C3u/D1-4 (1)		C3u (1)	C3u (1)		
	Platygyra lamellina					C101a (1)	
	Platygyra pini	D1-4(1), D1-4-10(1)	C3u (1)		C3u(1)		
	Platygyra sinensis	C3u (1), D1-4-10 (2), D1-4 (1)	C101 (1)	C3u (1)		C101 (1)	
	Platygyra verweyi	C3u (1)					
Fungiidae							
Fungia	*Fungia danai*		C3u (1)				C3u (1)

	Fungia echinata	C3u/D1-4 (1), D1-4 (1)	C3u (2)	C3u (1)		C3u (1)	C3u (1)
	Fungia fungites	C3u (2), C3u/C15(1), D1-4 (1)	C3u (1)	C3u (1)		C3u (1)	C3u (1)
	Fungia granulosa		C3u (1)		C3u (1)		
	Fungia paumotensis			C3u (1)	C3u (1)	C3u (1)	
	Fungia repanda		C3u (1)		C3u (1)		
Herpolitha	*Herpolitha limax*	C3u (1), C3u/D1-4 (1)	C3u (1)	C3u/D1-4 (1)			C3u (1)
Lithophyllon	*Lithophyllon undulatum*				C3u (1)		
Podabacia	*Podabacia crustacea*	D1-4 (3)	D1-4 (1)				
Polyphyllia	*Polyphyllia talpina*				C3u (2)		
Gorgonacea							
Gorgonian	*Gorgonia* sp.				D1-4 (1)		
Helioporidae							
Heliopora	*Heliopora coerulea*		D2-4-5 (1)	D2-4-5 (1)	D2-4-5 (1)	D2-4-5 (1)	D-5-?
Merulinidae							
Hydnophora	*Hydnophora exesa*	C3u (2)	C3u/C15 (1)	C3u (1)	C3u (1)		
	Hydnophora microconos	C3u (2)		C3u (1)	C3u (1)	C3u (1)	C3u (1)
	Hydnophora rigida	C3u (3)		C3u (1)			C3u (1)
Merulina	*Merulina ampliata*	C3u/D1-4 (1), D1-4/C3u (1)	C3u (1)		C3u(1)		C3u/C3z (1)
	Merulina scabricula		C3u (1)	C3u (1)	C3u (1)		C3u (1)
Milleporidae							
Millepora	*Millepora platyphylla*		C57a (2)	C57a (1)	C57a (1)		C57a* (1)
	Millepora tenella						C57a (1)
	Millepora sp.		C57a (1)			C57a (3), C57a* (1)	
Mussidae							
Australomussa	*Australomussa rowleyensis*			C3u (1)	C3u (1)		
Lobophyllia	*Lobophyllia hemprichii*	D1-4 (1)	C3u (1)	D1-4 (1)	D1-4/C3u (1)		
Symphyllia	*Symphyllia agaricia*			C3u (1)	C3u (1)		
	Symphyllia radians	C3u (1), D1-4 (2)	C3u (1)	C3u (1)	C3u (1)		C3u (1)
	Symphyllia recta			C3u (1)			
Nephtheidae							
Nephthea	*Nephthea* sp.				C1 (1)	C3 (1)	
Oculinidae							
Galaxea	*Galaxea astreata*			D-1-4/C3u (1)	C1b (1)		
	Galaxea fascicularis	C3u (4)	C3u (1)	C3u (1)	C3u (1)	C3u (1)	C3u (1)
Pectiniidae							
Echinophyllia	*Echinophyllia aspera*	C3u (4)	C3u (1)	C3u (1)	C3u (1)		
Mycedium	*Mycedium elephantotus*	C3u (2)	C3u (1)		C3u (1)		
Oxypora	*Oxypora lacera*			C3u (1)			
Pectinia	*Pectinia alcicornis*	C3u (2)			C3u (1)		
	Pectinia lactuca			C3u (1)			
	Pectinia paeonia		C3u (1)				
Pocilloporidae							

Pocillopora	*Pocillopora damicornis*	D5 (1), D1-4-6 (1)	D5 (2)	D5 (1)	D5 (1)	D5 (1)	
	Pocillopora eydouxi	D1-11 (1)	D5 (1)		C42a (1)		C42a (1)
	Pocillopora meandrina			D-5 (1)	C1d* (1)		C1d-t (1)
	Pocillopora verrucosa	D5 (1)		D1-4-6 (1)		C1c (1)	C1d-t (1)
Stylophora	*Stylophora pistillata*						C3w (1)
Poritidae							
Goniopora	*Goniopora columna*			D1-4 (2)			
	Goniopora djiboutiensis		D1-4 (1)				
	Goniopora fruticosa		D1-4 (1)	D5 (1)	D5 (1)		D4-5 (1)
	Goniopora sp.		C1 (1)	D5 (1)	D5 (2)		C3u (1)
Porites	*Porites annae*		C15 (1)			C15 (1)	
	Porites cylindrica			C15 (1)			C15 (1)
	Porites lobata		C15 (2)	C15 (1)		C15 (1)	C15 (1)
	Porites lutea	C15 (10)	C15 (1)	C15 (1)		C15 (1)	C15 (1)
	Porites nigrescens	C15L (1)	C15 (1)	C15 (1)			
	Porites rus		C15 (2)	C15 (2)	C15 (1)		C15 (1)
	Porites sp.			C15 (1)	C15 (1)		
	Porites stephensoni	C114 (1)		C15 (1)		C114 (1)	C15 (1)
Siderastreidae							
Psammocora	*Psammocora contigua*	C1b (1), D-1-4 (1)		C1b (1)		C1c* (1)	
	Psammocora digitata	C3 (1)	C3u (1)			C3u (1)	
	Psammocora profundacella					C1b (1)	
Stichodactyliidae							
Heteractis	*Heteractis magnifica*		C3 (1)				
	Heteractis sp.				C3 (1)		
Tridacniidae							
Tridacna	*Tridacna crocea*					D1-4 (1); A3 (1)	
Zoanthidae							
Palythoa	*Palythoa* sp.	D4-5/C3u (1)	C1 (1), D1-4 (1)				

Appendix S2 *Symbiodinium* diversity in host assemblages from reefs in the western Indian Ocean (Zanzibar, Tanzania). The identifier for each symbiont refers to the evolutionarily divergent clade (uppercase letter) the ITS-DGGE fingerprint *type* including the designations of one or more dominant intragenomic sequences in the ribosomal array (numbers and lowercase letters). Numerals in parentheses indicate the number of colonies in which a particular symbiont was found. Two or more types separated by a forward slash indicate that the symbionts co-occurred in the sample. Depth ranges of host collection are given in parentheses for each study site.

Host Family / Genus	Species	Banda Kuu (3-12m)	Bawe (2-6m)	Changuu (2-6m)
Acroporiidae				
Acropora	*Acropora appressa*	C109a (1), C109a/D1-4 (1)	C3 (1)	
	Acropora gemmifera			C109b (1)
	Acropora glauca		C3z (2)	
	Acropora granulosa			C109b (1)
	Acropora latistella		C3z (2)	C3z (1)
	Acropora loripes		C3z (1)	
	Acropora lutkeni		C3z (1)	C109b(1), C3(2)
	Acropora retusa	C3 (1)	C109a-c (1)	
	Acropora samoensis		C109b (1), C3z (1)	C109a-c (1), C109b (1), C3z (1)
	Acropora secale	C109a (1), C3 (2), C3u (1)		
	Acropora solitaryensis	C3z (1)		
	Acropora sp.	C109a (2), C3 (1), C3u (1)		C109a-c (1), C3 (1), C3z (1)
	Acropora sp.A	C109a-b (1), C115a (1)	C3z (1)	C3z (7)
	Acropora subulata		C109a (1)	
	Acropora tenuis			C3 (1), D1 (1)
	Acropora valida	C115a (1)	C3z (1)	
Astreopora	*Astreopora myriophthalma*		C1(1), C1b (1)	
Montipora	*Montipora monasteriata*	C31 (1)		
	Montipora peltiformis	C110 (1)		
	Montipora sp.	C17a (1)		
	Montipora tuberculosa	C31d (1)		
	Montipora undata	C17a (1)		
Actiniidae				
Anemonia	*Anemonia manjano*	C1 (1)	C1 (1)	C1 (1)
Actinodiscidae				
Actinodiscus	*Actinodiscus nummiforme*		C1 (1)	C1 (1)
Rhodactis	*Rhodactis mussoides*		C3u(1)	C3u (3)
	Rhodactis rhodostoma		D-4-5-9 (1)	D-4-5-9 (6)
Agariciidae				
Gardineroseris	*Gardineroseris planulata*		C3u/D1-4 (1)	

Leptoseris	*Leptoseris mycetoseroides*			C3u (2)
Pachyseris	*Pachyseris rugosa*	C3u (1)		
	Pachyseris sp.		C3u (2)	
	Pachyseris speciosa			C3u (1)
Pavona	*Pavona explanulata*	C3u (1)	C3u (1)	
	Pavona maldivensis	C3u (1)		
	Pavona varians	C1b (1)		C3u (2)
Aglaopheniidae				
Aglaophenia	*Aglaophenia cupressina*			A3 (4)
Aiptasiidae				
Aiptasia	*Aiptasia* sp.		B1 (1)	B1 (1)
Alcyonidae				
Alcyonium	*Alcyonium* sp.		C107a-b (1)	
Lobophytum	*Lobophytum* sp.	C107a-b (1), C65 (1)		
Rhytisma	*Rhytisma* sp.	C64a (1)		
Sarcophyton	*Sarcophyton* sp.	C106 (4)		C1r (2)
Sinularia	*Sinularia* sp.	C1 (2), C65 (1)		C1r (2), C65 (1)
Caryophylliidae				
Plerogyra	*Plerogyra sinuosa*	C3u(2)	C3u(1), C3u/D1-4 (1)	
Corallimorpharia				
Corallimorpharia	*Corallimorpharia* sp.	D-4-5-9 (1)	C1 (1)	
Dendrophylliidae				
Turbinaria	*Turbinaria reniformis*	C1b (1)		
Discosomatidae				
Discosoma	*Discosoma nummiformis*		C1 (1)	C1 (1)
	Discosoma sp.		D1-4 (1)	
	Discosoma unguja		C1/D1-4 (1), D1-4 (1)	C3v (2), D1-4 (1), D4-5-9(1)
Euphyliidae				
Physogyra	*Physogyra lichtonsteini*		C3u/D1-4 (1)	C3u (1), C3u/D1-4 (1)
Faviidae				
Diploastrea	*Diploastrea heliopora*	C3u/D1-4 (1)		
Echinopora	*Echinopora gemmacea*		C3u (1)	
	Echinopora hirsutissima			C3u (2)
	Echinopora lamellosa			C3u (3)
	Echinopora robusta	C3u (1)	C3u/C19 (1)	
	Echinopora sp.	C1 (1)		C3u (1)
Favia	*Favia helianthoides*			C3u (1)
Favites	*Favites abdita*	C3 (2)		
	Favites chinensis	C3u (1)		
	Favites halicora	C3u/C116 (1)		
	Favites sp.			D1-4/C3u (1)
	Favites stylifera	C3u (1)		
	Favites vasta	C3u (3)		

Goniastrea	*Goniastrea aspera*	C3u (1)		
	Goniastrea columella	C3u (1)		
	Goniastrea favulus			C3z (1)
	Goniastrea peresi	C1 (2), C115a (1)		
	Goniastrea sp.	C15 (1)		
Leptastrea	*Leptastrea purpurea*	C1b-s (1)		
Montastraea	*Montastraea magonistercasa*		C3z (1)	
Oulophyllia	*Oulophyllia crispa*	C1b-y (1), C3u (2)		
Platygyra	*Platygyra contorta*	C3u (1)		
	Platygyra daedalea		C3z (1)	
	Platygyra deformis			C3u (1)
	Platygyra sinensis	C3u-aa (1), C3u (2)		C3u (1)
	Platygyra sp.			C3u (2), C3z (1)
Plesiastrea	*Plesiastrea versipora*	C1b-s (1), C1b-y (1)		
Fungiidae				
Fungia	*Cycloseris* sp.	C1 (2)		
	Fungia fungites			C3u (1)
	Fungia granulosa	C3u (2)		
	Fungia sp.			C3u (4), C3u/D1-4 (1)
Heliofungia	*Heliofungia* sp.			C3u (1)
Herpolitha	*Herpolitha limax*	C3u (2)		
Podabacia	*Podabacia crustacea*		C3u (1)	
Merulinidae				
Hydnophora	*Hydnophora exesa*	C3 (1), C3u (2)		C3u(1)
Merulina	*Merulina ampliata*	C3z (1)		
Milleporidae				
Millepora	*Millepora platyphylla*	C57a (2)		
	Millepora sp.	A15c (1)		A15c (2), A15a-b (2), A16 (1), C57a (1)
Mussiidae				
Acanthastrea	*Acanthastrea echinata*	C3u (1)		
	Acanthastrea hemprichii	C3u (1)		
Lobophyllia	*Lobophyllia hemprichii*		C3u (2)	C3u (4)
	Lobophyllia sp.		D1-4/C3u (1)	
Symphyllia	*Scolymia* sp.	C3u (1)		
	Symphyllia recta	C3u (1)		
	Symphyllia sp.	C3u (2)		C3u (1)
Nephtheidae				
Lemnalia	*Lemnalia* sp.	C64a (5)		C107a-b (1)
Paralemnalia	*Paralemnalia* sp.	C64a (1)		
Oculinidae				
Galaxea	*Galaxea astreata*			C3u (4)
	Galaxea fascicularis	C3u (2)		C3u (1)
	Galaxea sp.			C3u (1), D1-4 (1)

Pectiniidae				
Echinophyllia	*Echinophyllia aspera*	C3 (1)		C3u (1)
	Echinophyllia echinata	C3u-aa (1)		
Mycedium	*Mycedium elephantotus*	C3u (3)	C3u/D1-4 (1)	
	Mycedium sp.	C3u (1)	C3u (1)	
Oxypora	*Oxypora lacera*	C3u (1)	C3u (1)	
	Oxypora sp.		C3u (1)	
Pectinia	*Pectinia africanum*	C3u(1)		
Platyhelminthes				
Platyhelminthes	*Platyhelminthes* sp.		C64 (1)	
Pocilloporidae				
Pocillopora	*Pocillopora damicornis*	C1h (1)	C1h (1)	C1h (2)
	Pocillopora elegans			C1c (1)
	Pocillopora eydouxi	C1h (1)	C1h (1)	C1h (1), C42a (1)
	Pocillopora	C1h (1)		
	Pocillopora sp.			C1h (2)
	Pocillopora verrucosa	C1h (1), C1c (1)	C1h (1)	C1h (5)
Seriatopora	*Seriatopora hystrix*			D1 (4)
Stylocoeniela	*Stylocoeniela amrata*			C3y (1)
Stylophora	*Stylophora pistillata*		C105a (1)	C105a (5), C105a/C19 (1)
Poritidae				
Alveopora	*Alveopora* sp.	C64 (1)		
Goniopora	*Goniopora columna*	C1b-s (1)		
	Goniopora sp.	C1b-s (1), C3u (1), C15 (1)		
Porites	*Porites cylindrica*	C15 (2), C15j (1)		C15 (4), C15j (2)
	Porites lobata		C15 (2)	C15 (3), C15i (1)
	Porites lutea	C15 (3)	C15 (1)	C15 (4), C15i (1)
	Porites profundus	C15 (1)		C15j (3)
	Porites rus	C15 (1)	C15 (2)	C15 (7)
	Porites sp.	C15j (1), C1 (1)		C15 (2)
Ricordeidae				
Ricordea	*Ricordea juma*		C1 (2)	
Siderastreidae				
Coscinaraea	*Coscinaraea columna*	C1b-f (1), C3u (1)		
	Coscinaraea crassa	C1b-f (1)		
	Coscinaraea monile	C1b-y (1)		
	Coscinaraea sp.	C1b-y (1)		
Psammocora	*Psammocora haimeana*	C1b-f (1)		
	Psammocora profundacella		C1 (1)	C1 (1)
Stichodactyliidae				
Heteractis	*Heteractis aurora*		C113 (2)	
	Heteractis crispa	C106 (1)		C3m (1)
	Heteractis magnifica	C112a (1)		C112a (5)

Tridacniidae				
Tridacna	*Tridacna maxima*		C1 (4)	C1 (1)
	Tridacna sp.	C1 (1)		
Tubiporidae				
Tubipora	*Tubipora musica*	D4-5 (2)		
Xeniidae				
Anthelia	*Anthelia* sp.	C64a (1)		
Cespitularia	*Cespitularia* sp.	C64a (1)		
Efflatounaria	*Effloutonaria* sp.	C64a (1)		
Xenia	*Xenia sp.*	C64a (2)		
Zoanthidae				
Palythoa	*Palythoa* sp.	C1b (3)		C3u (1), C15 (1)
Zoanthus	*Zoanthus* sp.		C62 (1)	C62 (1), C3v (1)

Appendix S3 GenBank accession numbers for ITS 2 sequences derived from ITS-DGGE fingerprinting of *Symbiodinium* from Zanzibar (Z), in the western Indian Ocean, and the Andaman Sea (A), in the north-eastern Indian Ocean. The host genus names and region(s) where each symbiont was found are provided.

***Symbiodinium* 'type'**	**(Co-) dominant rDNA sequences in 'type' profile**	**GenBank accession number**	**Host genus (geographic location)**
A3	A3	AF333507	*Aglaophenia* (Z)
A15a-b	A15 A15a A15b	EU792884 EU792885 EU792886	*Millepora* (Z)
A15c	A15 A15c	EU792884 EU782887	*Millepora* (Z)
A16	A16	EU792888	*Millepora* (Z)
B1	B1	AF333511	*Aiptasia* (Z)
C1	C1	AF333515	*Actinodiscus* (Z), *Anemonia* (Z), *Astreopora* (Z), *Corallimorpharia* (Z), *Discosoma* (Z), *Echinopora* (Z), *Fungia* (Z), *Goniastrea* (Z), *Goniopora* (A), *Nephthea* (A), *Palythoa* (A), *Porites* (Z), *Psammacora* (Z), *Ricordia* (Z), *Sarcophyton* (A), *Sinularia* (A, Z), *Tridacnea* (Z)
C1b	C1 C1b	AF333515 AY239363	*Astreopora* (A, Z), *Euphyllia* (A), *Galaxea* (A), *Leptoseris* (A), *Palythoa* (Z), *Pavona* (Z), *Psammacora* (A), *Turbinaria* (Z)
C1b-f	1 1b 1f	AF333515 AY239363 AY258490	*Coscinarea* (Z), *Psammacora* (Z)
C1b-s	1 1b 1s	AF333515 AY239363 GU111865	*Goniopora* (Z), *Leptastrea* (Z), *Plesiastrea* (Z)
C1b-y	1 1b 1y	AF333515 AY239363	*Coscinarea* (Z), *Oulophyllia* (Z), *Plesiastrea* (Z)
C1c	1 1c	AF333515 AY239364	*Pocillopora* (A, Z)
*C1c**	1c	AY239364	*Psammacora* (A)
*C1d**	1d	GU111885	*Pocillopora* (A)
C1d-t	1 1d 1t	AF333515 AY258488 GU111867	*Pocillopora* (A)
C1h	1 1h	AF333515 AY258473	*Pocillopora* (Z)
C1r	1 1r	AF333515 GU111866	*Sacrophyton*, (Z), *Sinularia* (Z)
C3	3	AF499789	*Acropora* (Z), *Anemonia* (A), *Echinophyllia* (Z), *Favites* (Z), *Heteractis* (A, Z), *Hydnophora* (Z), *Nephthea* (A)

C3m	3 3m	AF499789 AY258497	*Palythoa* (Z)
C3u	3 3u	AF499789 GU111879	*Acropora* (A, Z), *Acanthastrea* (Z), *Australomussa* (A), *Coeloseris* (A), *Coscinarea* (Z), *Cyphastrea* (A), *Diploastrea* (A, Z), *Echinophyllia* (A, Z), *Echinopora* (A, Z), *Euphyllia* (A), *Favia* (A, Z), *Favites* (A, Z), *Fungia* (A, Z), *Galaxea* (A, Z), *Gardinoseris* (A, Z), *Goniastrea* (A, Z), *Goniopora* (A, Z), *Heliofungia* (Z), *Herpolitha* (A, Z), *Hydnophora* (A, Z), *Leptastrea* (A), *Leptoria* (A), *Leptoseris* (A, Z), *Litophyllon* (A), *Lobophyllia* (A, Z), *Merulina* (A), *Montastrea* (A), *Mycedium* (A, Z), *Oulophyllia* (Z), *Oxypora* (A, Z), *Pachyseris* (A, Z), *Pachyseris* (Z). *Pavona* (A, Z), *Pectinia* (A, Z), *Physiogyra* (A, Z), *Platygyra* (A, Z), *Plerogyra* (A, Z), *Pocillopora* (A), *Podabacia* (Z), *Polyphyllia* (A), *Psammacora* (A), *Rhodactis* (Z), *Symphyllia* (A, Z), *Turbinaria* (A)
C3u-aa	3 3u 3aa	AF499789 GU111879 GU111880	*Echinophyllia* (Z), *Platygyra* (Z)
C3v-bb	3 3v 3bb	AF499789 GU111868 GU111901	*Discosoma* (Z), *Zoanthus* (Z)
C3w	3 3w	AF499789 GU111869	*Stylophora* (A)
C3y	3 3y	AF499789 GU111870	*Stylocoeniela* (Z)
C3z	3 3z	AF499789 GU111884	*Acropora* (A, Z), *Favia* (A), *Favites* (A), *Goniastrea* (A, Z), *Merulina* (Z), *Montastraea* (Z), *Platygyra* (Z)
C3-cc	3 3cc	AF499789 GU111904	*Acropora* (Z)
C3-cc-dd	3 3cc 3dd	AF499789 GU111904 GU111905	*Acropora* (Z)
C15	15	AY239369	*Montipora* (A), *Porites* (A, Z), *Goniastrea* (Z), *Goniopora* (Z), *Palythoa* (Z)
C15i	15 15i	AY239369 GU111871	*Porites* (Z)
C15j	15 15j	AY239369 GU111872	*Porites* (Z)
C15k	15 15k	AY239369 GU111873	*Montipora* (A)
C15L	15 15L	AY239369 GU111874	*Porites* (A)
C17a	17 17a	AY239369 GU111875	*Montipora* (Z)
C26a	26 26a	AY239378 AY258501	*Montipora* (A)
C31	31	AY258496	*Montipora* (Z)
C31d	31	AY258496/GU111893	*Montipora* (Z)

	31d	GU111894	
C42a	1	AF333515	*Pocillopora* (A, Z)
	42	AY765402	
	42a	FJ529656	
C57a	57	AY589761	*Millepora* (A, Z)
	57a	GU111883	
*C57a**	57a	GU111883	*Millepora* (A)
C62	62	AY589766	*Zoanthus* (Z)
C64	64	AY589768	*Alveopora* (Z), *Platyhelminthes* (Z)
C64a	64	AY589768	*Anthelia* (Z), *Cespitularia* (Z), *Efflatounaria* (Z), *Lemnalia* (Z), *Paralemnalia* (Z), *Rhytisma* (Z), *Xenia* (Z)
	64a		
C65	65	AY589769	*Lobophytum* (Z), *Sinularia* (Z)
C94a	94	GU111876	*Acropora* (A)
	94a	GU111877	
C101	101	GU111881	*Acropora* (A), *Favia* (A), *Favites* (A), *Goniastrea* (A), *Leptoria* (A), *Montastraea* (A), *Platygyra* (A)
C101a	101	GU111881	*Acropora* (A), *Platygyra* (A)
	101a	GU111882	
C105a	105	GU111886	*Stylophora* (Z)
	105a	GU111887	
C106	106	GU111888	*Heteractis* (Z)
	106a	GU111903	
C107a-b	107	GU111889	*Alcyonum* (Z), *Lemnalia* (Z), *Lobophytum* (Z), *Sarcophyton* (Z)
	107a	GU111890	
	107b	GU111891	
C110	110	GU111892	*Montipora* (A, Z)
C112a	112	GU111895	*Heteractis* (Z)
	112a		
C113	113	GU111896	*Heteractis* (Z)
C114	114	GU111897	*Porites* (A)
C115a	115	GU111898	*Acropora* (Z), *Goniastrea* (Z)
	115a	GU111902	
C116	116	GU111899	*Favites*
C119	119	GU111900	*Goniastrea* (A)
D1	D1	EU449061	*Acropora* (Z), *Seriatopora* (Z)
D1-4 (D1a)	1	EU449061	*Acropora* (A), *Astreopora* (A), *Coeloseris* (A), *Diploastrea* (A), *Discosoma* (Z), *Echinopora* (A), *Favia* (A), *Favites* (A, Z), *Fungia* (A), *Galaxea* (A, Z), *Gardinoseris* (A), *Goniastrea* (A), *Goniopora* (A), *Leptastrea* (A), *Lobophyllia* (A, Z), *Merulina* (A), *Montastraea* (A), *Pachyseris* (A), *Palythoa* (A), *Pavona* (A), *Physogyra* (A), *Platygyra* (A), *Podabacia* (A), *Psammacora* (A), *Symphyllia* (A), *Tridacna* (A), *Turbinaria* (A)
	4 (a)	AF499802	
D1-4-6	1	EU449061	*Pocillopora* (A)
	4	AF499802	
	6	EU812742	
D1-4-10	1	EU449061	*Favites* (A), *Goniastrea* (A), *Montastraea* (A), *Platygyra* (A)
	4	AF499802	
	10	EU812741	
D1-11	1	EU449061	*Pocillopora* (A)

	11	EU812740	
D2-4	2	AY686649	*Acropora* (A)
	4	AF499802	
D5	5	EU812743	*Corallimorpharia* (A), *Goniopora* (A), *Gorgonian* (A), *Montipora* (A), *Pocillopora* (A)
D2-4-5	2	AY686649	*Heliopora* (A)
	4	AF499802	
	5	EU812743	
D4-5-9	4	AF499802	*Palythoa* (A), *Corallimorpharia* (Z), *Discosoma* (Z), *Rhodactis* (Z)
	5	EU812743	
	9	EU812746	
D4-5	4	AF499802	*Corallimorpharia* (A), *Goniopora* (A), *Tubipora* (Z)
	5	EU812743	
D5-7	5	EU812743	*Corallimorpharia* (A)
	7	EU812747	
D8	8	EU812748	*Oulastrea* (A)
F3	3	AM748566	*Anthopleura* (A)
G1	1		*Coeloseris mayeri* (A)

Appendix S4 Pairwise comparisons of genetic dissimilarity among Clade D *Symbiodinium* populations differentiated by their ITS-DGGE fingerprints. PhiPT (Φ_{PT}) values were calculated as the proportion of the variance (genetic) among genotypes in a population, relative to the total variance in both populations combined. These values are analogous to F_{st} and based on haploid data (Peakall & Smouse, 2006). Most values are significant ($P < 0.05$, bold) except for the comparison between *D1-4* populations and certain comparisons involving the limited sample size of *D8*.

	D1-4 (Thailand)	*D1-4* (Zanzibar)	*D1-4-6*	*D2-4-5*	*D5*	*D8*
D1-4 (Thai)	–	0.057	**0.411**	**0.643**	**0.423**	**0.722**
D1-4 (Zan)		–	**0.293**	**0.562**	**0.391**	0.717
D1-4-6			–	**0.324**	**0.334**	**0.472**
D2-4-5				–	0.400	0.612
D5					–	**0.517**
D8						–

Appendix S5 Pairwise comparisons of genetic dissimilarity among *D5 Symbiodinium* populations distributed to different host taxa. The populations of *D5* associated with *Montipora* and *Pocillopora* were statistically different ($P < 0.05$). Higher sample numbers would probably increase the significance of genetic differentiation between populations the associating with *Pocillopora* versus *Goniopora*.

	D5 (Montipora)	*D5 (Goniopora)*	*D5 (Pocillopora)*
D5 (Montipora)	–	0.090	**0.262**
D5 (Goniopora)		–	0.443
D5 (Pocillopora)			–