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Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean

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Abstract

The specific identity of endosymbiotic dinoflagellates (*Symbiodinium* spp.) from most zooxanthellate corals is unknown. In a survey of symbiotic cnidarians from the southern Great Barrier Reef (GBR), 23 symbiont types were identified from 86 host species representing 40 genera. A majority (>85%) of these symbionts belong to a single phylogenetic clade or subgenus (''C'') composed of closely related (as assessed by sequence data from the internal transcribed spacer region and the ribosomal large subunit gene), yet ecologically and physiologically distinct, types. A few prevalent symbiont types, or generalists, dominate the coral community of the southern GBR, whereas many rare and/or specific symbionts, or specialists, are found uniquely within certain host taxa. The comparison of symbiont diversity between southern GBR and Caribbean reefs shows an inverse relationship between coral diversity and symbiont diversity, perhaps as a consequence of more-rapid diversification of Caribbean symbionts. Among clade C types, generalists C1 and C3 are common to both Caribbean and southern GBR symbiont assemblages, whereas the rest are regionally endemic. Possibly because of environmental changes in the Caribbean after geographic isolation through the Quaternary period, a high proportion of Caribbean fauna associate with symbiont taxa from two other distantly related *Symbiodinium* clades (A and B) that rarely occur in Pacific hosts. The resilience of *Porites* spp. and the resistance of *Montipora digitata* to thermal stress and bleaching are partially explained by their association with a thermally tolerant symbiont type, whereas the indiscriminant widespread bleaching and death among certain Pacific corals, during El Niño Southern Oscillation events, are influenced by associations with symbionts possessing higher sensitivity to thermal stress.

Cnidarians such as hard corals, soft corals, sea fans, and anemones are the principal faunal constituents of benthic coral reef communities. They also appear to display the greatest sensitivity to environmental stress and are, therefore, indicators of reef health (Hoegh-Guldberg 1999). Most tropical cnidarians are dependent on the primary productivity of endosymbiotic dinoflagellates, *Symbiodinium* spp., for their survival (Muscatine and Porter 1977). Damage to the cellular processes that maintain these intimate associations leads to partner decoupling or ''bleaching'' (Hoegh-Guldberg 1999), a phenomenon that, when severe enough, results in the dramatic decline of reef ecosystem communities (Glynn 1993; Hoegh-Guldberg 1999; Loya et al. 2001).

Cnidarian-symbiont associations have different susceptibilities to environmental stress (Warner et al. 1996). The physiology of a specific symbiont may determine the tolerance of certain corals to thermal stress and resistance to bleaching (Warner et al. 1996, 1999; Iglesias-Prieto and

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Trench 1997; Rowan et al. 1997). Some investigators have hypothesized that the formation of different partner combinations or novel symbioses could be mechanisms by which corals and other cnidarian symbioses respond or ''adapt'' to global climate change (Rowan and Powers 1991; Buddemeier and Fautin 1993; Baker 2001). However, this hypothesis assumes that symbiotic associations are highly flexible and adapt rapidly to environmental change (Hoegh-Guldberg et al. 2002).

The development and testing of ecological hypotheses related to any natural system requires the identification of the organisms that are of ecological significance. Until recently, inexact methods for assessing the identity of *Symbiodinium* spp. impeded the assessment of their diversity and biogeography and, as a consequence, limited our understanding of host-symbiont specificity (Trench 1997). Molecular methods and the use of genetic markers have enhanced our understanding of symbiont systematics, ecology, and evolution (Schoenberg and Trench 1980*a*; Rowan and Powers 1991; LaJeunesse 2001, 2002; Pochon et al. 2001). Clearly, these dinoflagellates are genetically and ecologically diverse, have distinctive biogeographic and reefwide distributions, and many show high host specificity (Schoenberg and Trench 1980*b*; Rowan et al. 1997; Trench 1997; Baker 1999; Loh et al. 2001; LaJeunesse 2002). In spite of the crucial role they and their hosts play in the biological processes of coral reef ecosystems, progress toward the understanding of *Symbiodinium* spp. diversity, their ecology, and their biogeographical distribution has been gradual.

Here, we assess general *Symbiodinium* diversity on the southern Great Barrier Reef (GBR, Heron Island), from sampling 86 cnidarian host species (mostly reef-building corals) collected along transects in shallow (1–3 m) and middepth (8–10 m) habitats. We categorize symbionts as nonspecific generalists (found in numerous host taxa) or host specialists (associating with specific cnidarian genera or species). Although we do not exhaustively determine the full range of symbionts with which a particular host associates, patterns of partner combinations did emerge that may partially explain bleaching resilience. Finally, we compared the diversity of southern GBR *Symbiodinium* spp. with that of Caribbean reefs (LaJeunesse 2002). Our results revealed large differences between Caribbean and Pacific coral populations in terms of the taxonomic and phylogenetic diversity of their dinoflagellate symbionts.

Materials and methods

Individuals of a coral species inhabiting a particular environment and biogeographic region usually possess the same symbiont type (LaJeunesse and Trench 2000; LaJeunesse 2002), with some exceptions (Loh et al. 2001). Repetitive sampling from one host taxa in a reef zone typically yields the presence of a single symbiont type (Baker et al. 1997). In a majority of cases, it appears that host identity is most critical for locating a certain symbiont type (at the resolution of internal transcribed spacer 2 [ITS2] analyses); the importance of environmental influences being secondary (LaJeunesse 2002, unpubl. data). Therefore, an emphasis was placed not on replication of samples but rather on sampling from a diverse range of hosts. In February and March 2002, symbiotic invertebrates were collected by SCUBA or snorkel at several reef transect sites on and near Heron Island reef of the southern GBR. Collections were made from shallow (1–3 m) and deeper (>8 m) reef sites, to obtain corals living under different irradiances. Host taxa distributed at both depths were collected to identify possible ''polymorphisms.'' Sampling colonies from deep and shallow habitats is an efficient way of identifying coral species that associate with more than one symbiont (Baker 1999). Host fragments representative of the entire colony were collected and processed to separate symbionts from host tissues, as described elsewhere (Loh et al. 2001; LaJeunesse 2002). The resulting algal pellet was preserved in 20% dimethyl sulfoxide and 0.25 M ethylene diaminetetraacetic acid (EDTA) in NaCl-saturated water (Seutin et al. 1991).

The Wizard DNA prep protocol (Promega) was used to extract nucleic acids. Between 20 and 40 mg of material was placed into 1.5-ml microcentrifuge tubes with 250 μg 0.5-mm glass beads and 600 μl nuclei lysis buffer (Promega) and bead beaten for 140 s at a maximum speed in a Biospec Mini-Beadbeater. The lysate was then incubated with 0.1 mg ml^{-1} proteinase K for 1 h at 65°C, followed by incubation with 6 μg ml^{-1} RNase at 37°C for 10 min. Protein precipitation buffer (250 μl) (Promega) was then added and the extract incubated on ice for 10–15 min. After centrifugation for 5 min at 15,000 g, 600μl of supernatant was transferred to a second 1.5-ml tube that contained 700 μl isopropanol 100% and 50 μl NaAc (3 mol L^{-1} [pH 5.6]). After an incubation on ice for 10 min, the precipitated DNA was centrifuged and the pellet washed with 70% ethanol. The DNA was centrifuged again for 5 min, dried, and resuspended in 95 μl H_2O and 5 μl 10× Tris-EDTA.

Sequencing of the 28S rRNA gene or ribosomal large subunit gene (LSUrDNA) enabled the determination of the phylogenetic relatedness and the level of relative evolutionary divergence between symbionts. The D1/D2 domains of the LSUrDNA gene were amplified from *Symbiodinium* spp. using primers 5′-CCT CAG TAA TGG CGA ATG AAC A-3′ and 5′-CCT TGG TCC GTG TTT CAA GA-3′, as described by Loh et al. (2001). Polymerase chain reaction (PCR) fragments were either sequenced directly or cloned and then sequenced from both 5′ and 3′ ends using a Perkin Elmer 373A DNA automated sequencer (at the Australian Genome Research Facility at the University of Queensland). Partial LSU sequences (450 bases) were aligned manually. Type A7 from *Millepora platyphylla* was used as an outgroup. A genuswide phylogeny was constructed under maximum parsimony using PAUP 4.0b8 software under default settings but with gaps and deletions assessed as a fifth character state (Swofford 1993). A bootstrap resampling (500 replicates) was conducted to assess relative branch support.

Denaturing-gradient gel electrophoresis (DGGE), a rapidly evolving DNA marker that effectively allows the molecular discrimination of *Symbiodinium* spp., was used to analyze the ITS2 of nuclear ribosomal RNA genes (LaJeunesse 2001, 2002). PCR-DGGE analyses of the ITS2 region were conducted using the forward primer, ''ITSintfor2'' (5′-GAATTGCAGA ACTCCGTG-3′) (LaJeunesse and

Trench 2000), which anneals to a "*Symbiodinium*-conserved" region in the middle of the 5.8S ribosomal gene and the highly conserved reverse primer that anneals to the LSU "ITS2CLAMP" (5′-CGCCCGCCGC GCCCCGCGCC CGT CCCGCCG CCCCCGCCC GGGATCCATA TGCTTAAG TT CAGCGGGT-3′), an ITS-reverse universal primer modified with a 39-bp GC clamp (underlined) (LaJeunesse and Trench 2000). A "touchdown" amplification protocol with annealing conditions 10°C above the final annealing temperature of 52°C was used to ensure PCR specificity. The annealing temperature was decreased by 0.5°C after each of 20 cycles. Once the annealing temperature reached 52°C, it was maintained at that setting for another 20 cycles. Samples were loaded onto an 8% polyacrylamide denaturing gradient gel (45%–80% urea-formamide gradient; 100% consists of 7 mol L^{-1} urea and 40% deionized formamide) and separated by electrophoresis for 9.5 h at 150 V at a constant temperature of 60°C (LaJeunesse 2002). The gel was stained with Sybr Green (Molecular Probes) for 25 min according to the manufacturer's specifications and photographed using 667 Polaroid film.

The ribosomal tandem array in many *Symbiodinium* spp. (and probably in most eukaryotes) is not completely homogenized. When uniformity is perceived, in reality it is a matter of dominance in which a high proportion of the repeats all share the same nucleotide sequence. Sometimes two or more codominant sequences are present within a genome. The presence of codominants was first visualized via PCR-DGGE. Multiple bands were presumed to originate from the same genome when they repeatedly cooccurred at the same relative intensity. Mutual origin from a single genome was further confirmed after sequences were compared. Most variants observed in *Symbiodinium* were distinguished by a single base substitution or insertion/deletion (indel). Apparently, the mechanisms of concerted evolution or biased gene conversion rarely permit the existence of divergent variants for very long. The conversion from one dominant sequence to another proceeds incrementally and may take hundreds of thousands of years or perhaps more than a million years (LaJeunesse unpubl. data).

For the identification of a new symbiont type, prominent diagnostic bands from denaturing gels were excised, reamplified, and sequenced as described elsewhere (LaJeunesse 2002). ITS2 sequences were aligned manually using Sequence Navigator version 1.0 software (ABI). Cladistic analyses, using maximum parsimony and maximum-likelihood, were performed on aligned data sets using PAUP 4.0b8 software with the default settings (Swofford 1993). Under maximum parsimony, sequence gaps were designated as a fifth character state. A bootstrap resampling was done for 500 replicates to assess relative branch support.

Results

Analyses by PCR-DGGE of ITS2 variants enabled the identification of 23 distinctive *Symbiodinium* spp. (1 clade A, 1 clade B, 20 clade C, and 1 type clade D) inhabiting the cnidarian fauna sampled from shallow (<3 m) and middepth (10 m) reef environments near Heron Island (Web Appendix 1 at http://www.aslo.org/lo/toc/vol_48/issue_5/2046al.pdf). Among the 24 coral taxa sampled from both deep and shallow environments, 9 species (37.5%)—*Stylophora pistillata, Acropora tenuis, Porites annae, Pavona varians, Lobophyllia corymbosa, Cyphastrea serailia, Goniastrea australensis, Merulina ampliata,* and *Echinopora lamellosa*—contained different symbiont types in each environment. These data confirm the occurrence of multiple symbiont partnerships (polymorphic symbioses) existing over the depth distribution of some GBR corals (Baker 1999).

Figure 1, panels a and b, shows the signature profiles of each symbiont type classified from clade C. As was described in greater detail in *Materials and methods,* the occurrence of multiple bands was often due to intragenomic variations within the ribosomal multigene array of a single symbiont's genome (Buckler et al. 1997). Bands diagnostic of a particular symbiont type were sequenced and given an alphanumeric designation. In cases where two or more variants cooccurred intragenomically, heteroduplexes were usually produced from the mismatching of these variants during the PCR process. Because these contain mispaired bases, they are less stable and cease migrating higher in the gel under lower denaturant concentrations (Fig. 1a,b). Bands that cooccurred repeatedly with the same relative intensities, which are usually distinguished by a 1-bp substitution or indel, were considered to be codominant intragenomic variants and diagnostic of a single symbiont entity or type. In few cases was the presence of more than one symbiont type detected (LaJeunesse 2002).

Figures 2 and 3 are phylogenetic reconstructions based on sequence data from the LSU and the ITS2 region, respectively. The LSU genuswide phylogeny in Fig. 2 indicates that most (87%) of the symbiont types identified in the cnidarian fauna from Heron Island are classified within the subgeneric epithet: clade C (LaJeunesse 2001). Ninety-three percent of host species associate with clade C *Symbiodinium* (Web Appendix 1). The soft octocoral *Nephthea* spp. collected from 3 m contained the only symbionts identified from clade B that were indistinguishable from the B1 symbiont that dominates Caribbean fauna (LaJeunesse 2002). A deeper *Nephthea* sp. colony hosted type D1a. Several scleractinian hosts, including *Goniastrea favulus, Echinopora hirsutissima,* and *Hydnophora microconos* also possessed type D1a at depths >3 m. The fire coral, *M. platyphylla,* contained symbiont A7 and was the only species found to host *Symbiodinium* from clade A. However, two scleractinians, *Acropora longicyathus* (Loh et al. 2001) and *Pocillopora damicornis* (Loh unpubl. data), sometimes hosted a *Symbiodinium* sp. in clade A (type A9/A9a in *A. longicyathus;* Loh and LaJeunesse unpubl. data).

The ITS2 phylogeny presented in Fig. 3 shows that types C3, C1, and C21 were the most prevalent among host genera, whereas numerous others were host specific and/or rare. This phylogeny includes paralog sequences of intragenomic variants that are diagnostic of a particular symbiont. For example, deeper dwelling or shaded colonies of *S. pistillata* contained a symbiont type denoted by the presence of two codominant variants in their genomes, designated C8 and C8a. Symbiont types that are designated C1 followed by a lowercase letter (e.g., C1b) indicate that their genomes con-

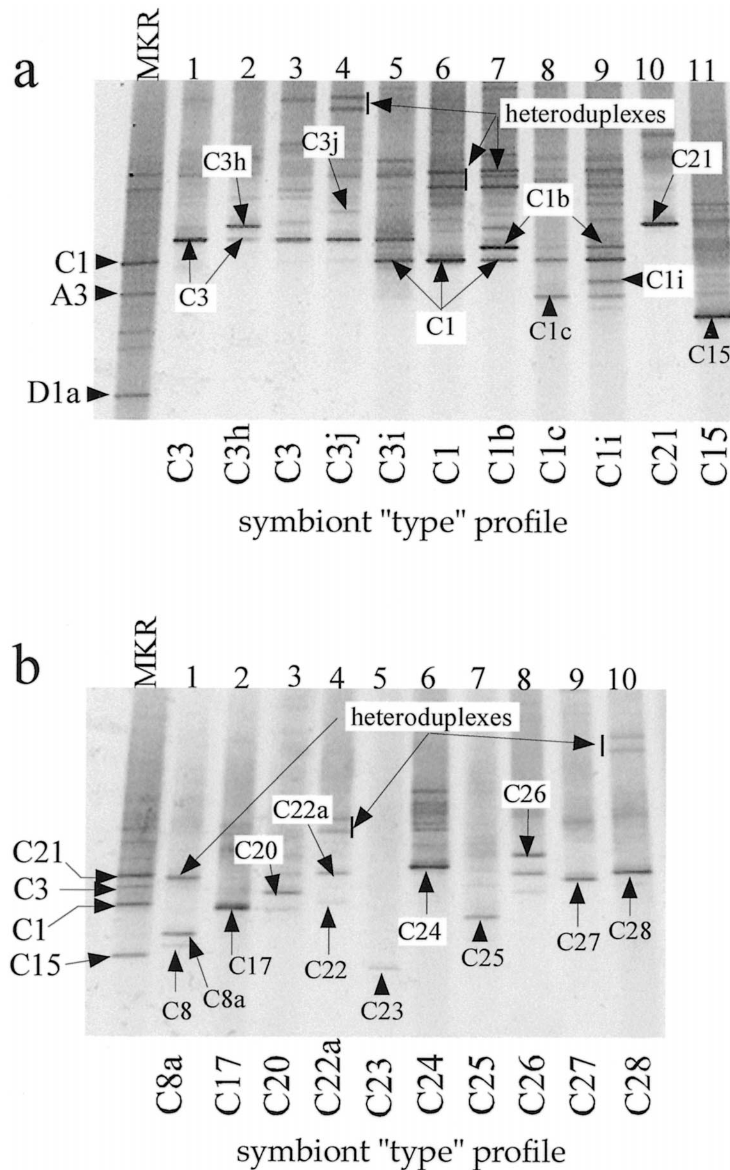

Fig. 1. PCR-DGGE profiles of lineage C types observed in hosts from Heron Island reefs. Presented as negative images, they show the identity of diagnostic bands excised from the gel and sequenced for phylogenetic analysis. The alphanumeric name of each symbiont type is given below the corresponding profile; uppercase letters indicate lineage (clade), numbers represent ITS type, and lowercase letters denote a characteristic rDNA paralog, when one was identified. (a) Standards (MKR) in lane 1 are pooled PCR amplifications of C1, A3, and D1a; (b) C21, C3, C1, and C15 PCR products were combined to create a second set of standards. Examples of heteroduplexes are indicated.

tain the C1 sequence and a codominant sequence, C1b. The symbiont is therefore designated by the band that distinguishes it from a C1 symbiont (*see Materials and methods*).

The most common symbiont types from each clade (B1, C1, C3, and D1a) were found in both the Caribbean and Pacific (A3 is found in tridacnid clams from the Pacific (Baillie et al. 2000) (Fig. 4). A majority of types consisting of less common, host-specialized, and/or rare symbionts, appeared to be endemic to each province. Symbioses involving *Symbiodinium* spp. from clades A and B were substantially more numerous in the Caribbean. Approximately half of the host genera from the Caribbean associated with type B1 over all, or part, of their depth distributions (LaJeunesse 2002). In contrast, type B1 occurred rarely in symbioses from the GBR (Fig. 4).

A more complete analysis of total host diversity from the Caribbean (50 genera, ~75 species) uncovered 50% more symbionts (35 types) than were found in the 40 genera (86 species) surveyed at Heron Island (23 types) (Fig. 4). Realistically, continued sampling of greater host diversity from the GBR, especially from unsampled genera and other regions of the Indo-Pacific, will uncover more symbiont di-

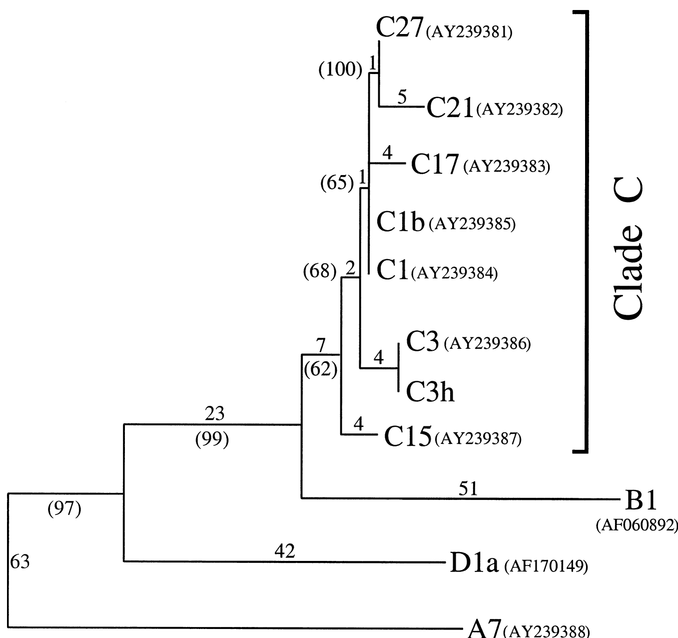

Fig. 2. A genuswide phylogenetic reconstruction of partial LSUrDNA sequences (D1/D2 domains) from different symbionts detected in the cnidarian fauna from the southern GBR. Although *Symbiodinium* spp. in clades A, B, and D were represented, the majority (87%) of the identified symbiont types were from clade C. Informative base-pair differences and indels from a maximum-parsimony analysis of an alignment of 448 characters are presented above branch segments. Numbers in brackets are bootstrap values for 500 replicates. Corresponding GenBank accession numbers are provided next to each symbiont type.

versity. On the basis of previous studies, it is expected that most new types will fall within clade C (Baker 1999). The findings made here are most likely representative of the entire Capricorn bunker group, given that preliminary analyses of corals from One Tree Island (40 km southeast of Heron Island) have demonstrated that the symbiont types found at Heron Island occur in the same species of coral at this location (Bui et al. unpubl. data).

Discussion

Knowledge of symbiont diversity—their biogeographic distribution, physiological limitations, and degree of specialization—have major implications for understanding larger scale issues such as the adaptability of zooxanthellate corals to climate change. The potential for the recombination of different partners and natural selection of host populations associating with more tolerant symbionts may serve to create communities of holosymbionts suited to changed environments (Buddemeier and Fautin 1993; Iglesias-Prieto and Trench 1997). Substantiation that these adjustments to environmental change are realistic depends on first surveying the diversity of symbionts and how they associate with the diversity of hosts over local, regional, and global scales.

The phylogeny of *Symbiodinium* can be divided into several clades or subgenera, each of which contains an as-yet-undetermined number of genetically distinct ecological "types" or species. Viewed from the molecular genetic classification of ITS2 "type," the prevalence of particular symbionts across host taxa in both the western Pacific and Caribbean provinces follows a classical Fisher log-normal distribution that is characterized by several very common types and many host-specific and/or rare types (LaJeunesse 2002). However, the assemblage of symbionts within the southern GBR coral community is notably different than those of the Caribbean because of the scarcity of *Symbiodinium* types originating from clades A and B. These findings support earlier suppositions that the prevalence of clade C symbionts in eastern Pacific corals is characteristic of the entire Pacific province (Baker and Rowan 1997) and confirms the preliminary findings of Loh et al. (1998) from One Tree Island (GBR), Baker (1999) from Lizard Island (GBR), and LaJeunesse et al. (unpubl. data) from Hawaii and Okinawa, Japan.

Host specificity and physiological differences among clade "C" Symbiodinium *spp.*—It remains to be determined how functional differences relate to genetic differences among *Symbiodinium* spp. Clearly, distinct ecological niches accompany the numerous, but closely related, symbiont types or species identified within clade C (Fig. 3). In the Pacific, types C1, C3, and C21 occupy tissues from a wide range of host taxa at various depths and are viewed as generalists. In contrast, specific symbionts have ecological distributions that are limited to specific host tissues, so these are viewed as specialists. The symbionts from *Montipora* spp. (C17), *Turbinaria* spp. (C22a), *S. pistillata* at 10 m (C8a), *P. varians* at 10 m (C27), and nonscleractinians (e.g., anemone [C25], zoanthid [C20], and alcyonacian soft corals [C3j]) have distinctive ecological distributions, in the sense that certain host tissues constitute a particular habitat (LaJeunesse 2002). The distribution of specific types are also influenced by irradiance; shallow-dwelling *S. pistillata* contain the symbiont generalist, C1, whereas deeper colonies appear to harbor the specialist, C8a. The specific symbiont C1c from Pocilloporid corals has a similar biogeographic distribution to its host, ranging from Heron island in the southern GBR to Lizard Island (north-central GBR; Baker and LaJeunesse unpubl. data), Hawaii (LaJeunesse et al. unpubl. data), and the eastern Pacific (Baker and LaJeunesse unpubl. data). *Symbiodinium* species that associate selectively with a specific host, sometimes at certain depths, and over wide biogeographic ranges must be adapted to that host's intracellular environment and are, therefore, functionally different from others that share very similar rDNA-ITS sequences.

Substantial differences in thermal tolerance among clade C *Symbiodinium* spp. may influence the bleaching susceptibility of certain coral taxa. The "bleaching-resistant" *Montipora digitata* was found paired with type C15, a unique match that distinguishes this coral from most montiporid symbioses that are sensitive to thermal stress. In a heating experiment conducted on Heron Island in February and March of 2002, type C15 within *Porites cylindrica* displayed greater photosynthetic stability under thermal stress than types C8a and C1 present in *S. pistillata* (Hoegh-Guldberg et al. unpubl. data). Symbiont C15 is found in many *Porites*

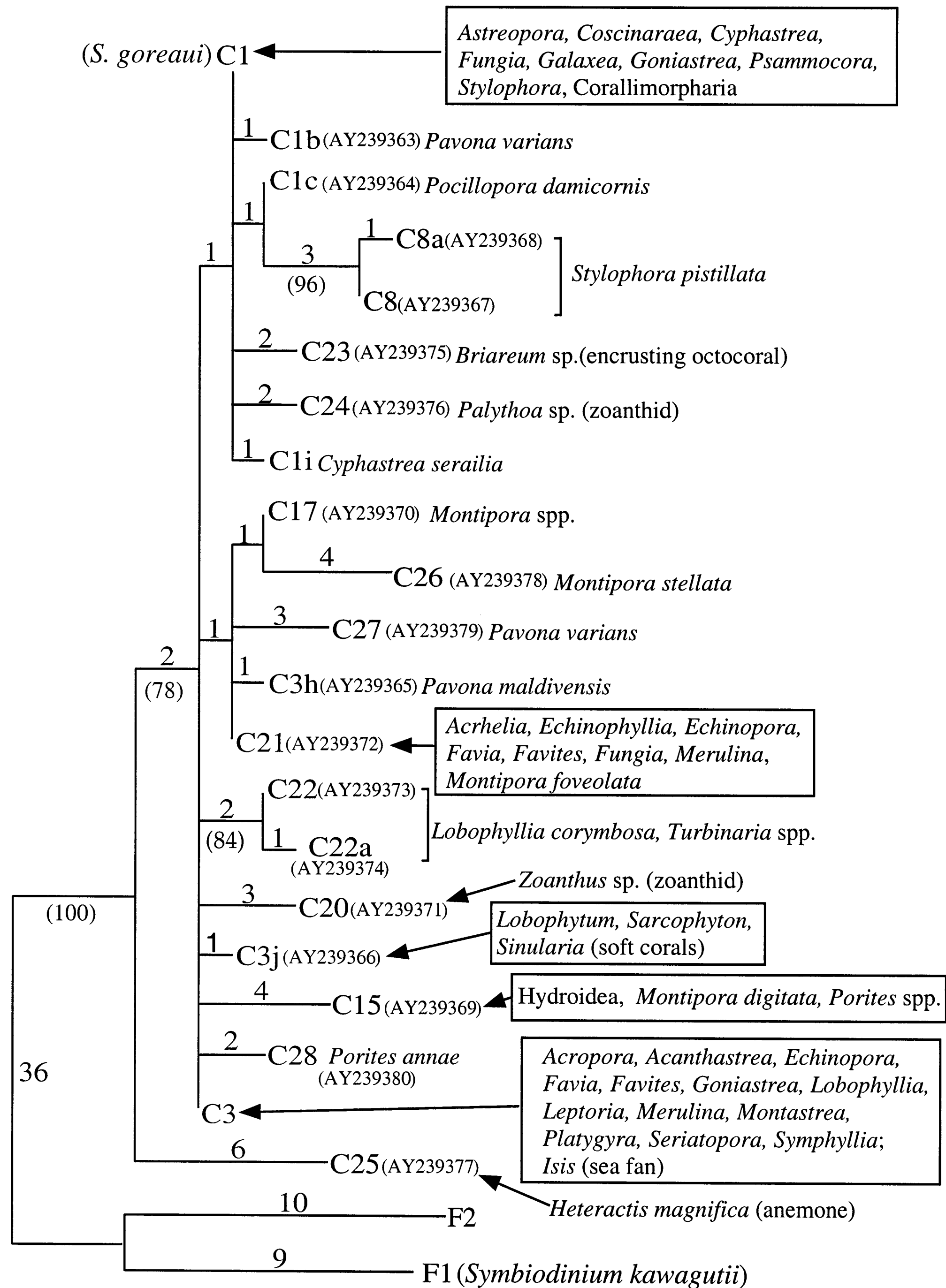

Fig. 3. Phylogenetic reconstruction of ITS2 genes from lineage C types based on an alignment of 301 characters using maximum parsimony. Symbiont types that were distinguished by as little as a single base substitution or that contained a distinctive paralog occupy distinct niches corresponding with host identity and less with depth. Therefore, although there is little divergence among clade C symbionts, there are recognizable ecotypes with distinct physiological adaptations to specific host tissues. Some symbionts exhibit a wide host range (generalists), but many are specialized to a specific coral genus or species. Types F1 (*S. kawagutii*) and F2 were used as outgroups. Gaps were assessed as a fifth character state. Numbers above branch segments indicate informative base-pair differences. Numbers in brackets are bootstrap values for 500 replicates. Corresponding GenBank accession numbers are provided next to each symbiont type.

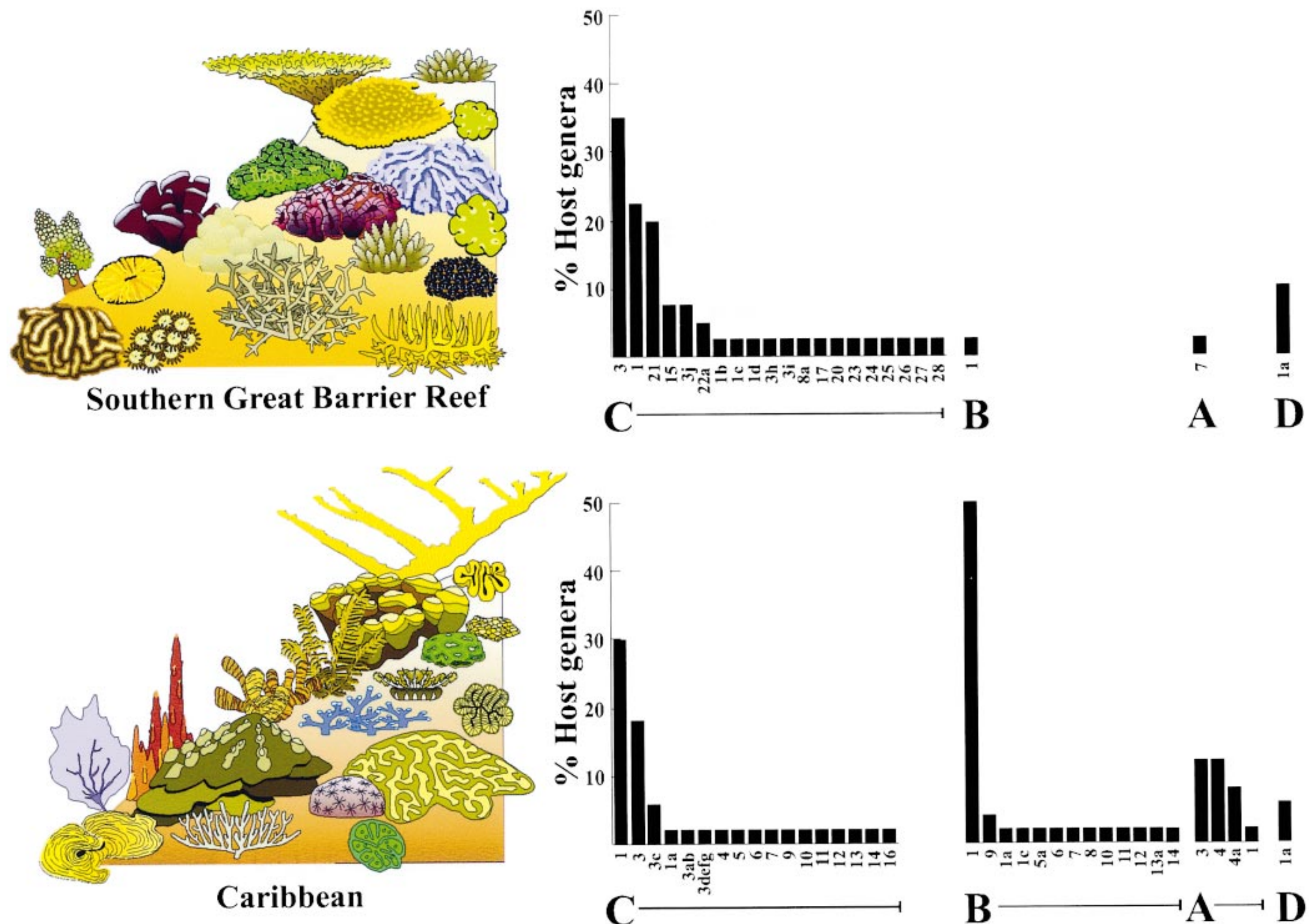

Fig. 4. Comparison of zooxanthellae (*Symbiodinium* spp.) communities from the southern GBR (upper panels) with the Caribbean (lower panels). The graphs separate symbiont types by clade and compare the prevalence of each type by showing the relative number of host genera with which each associates (given as % of host genera). Each community assemblage contains several ecologically common host generalists, usually occurring both provinces, and the existence of numerous host-specific and/or rare endemic types (LaJeunesse 2002). On the GBR, and perhaps indicative of the entire Pacific (Baker 1999), coral symbioses involving *Symbiodinium* spp. from clades A and B are scarce.

spp. taxa throughout the Indo-Pacific, including Hawaii (LaJeunesse et al. unpubl. data) and Kenya (Baker and LaJeunesse unpubl. data), and partially explains why this coral group tends to be resilient to thermal anomalies and bleaching episodes (Brown and Suharsono 1990; Jokiel and Coles 1990; Hoegh-Guldberg and Salvat 1995).

Specialization for a particular host environment and differences in thermal tolerance underscore the fact that, although sequence divergence between clade C symbionts is low, this lineage contains many species that are ecologically and physiologically distinct. These observations reaffirm that closely related organisms can be physiologically different, and genetic divergence does not always correlate with physiological divergence (Moore et al. 1998). Comparative studies that reveal more about the nature of these physiological and genetic relationships are imperative.

Latitude and stress history effecting shifts in symbiont communities—The species-rich and ecologically dominant acroporids sampled at Heron Island all contained symbiont type C3 (except *A. tenuis,* which had C3i at 10 m). These observations are different from the findings of Van Oppen et al. (2001) who, in characterizing the ITS of symbionts from *Acropora* spp. colonies on near-shore and midshelf reefs from lower latitudes on the central GBR, detected several other symbionts. The differences discussed below highlight the influence of latitude, and possibly bleaching history, on the distribution and diversity of symbiont taxa in certain corals.

Water temperatures on inshore reefs along the GBR during the 1998 El Niño Southern Oscillation event far exceeded those experienced by offshore reef sites (Skirving and Guinotte 2001) and resulted in more severe inshore bleaching (Berkelmanns and Oliver 1999). In 2001, Van Oppen et al. reported *Symbiodinium* from clade D (Baker 2001) in 60% of the acroporids sampled at their near-shore site. These particular *Symbiodinium* spp. are commonly found in recently bleached recovering corals (Baker 2001) or are associated

with certain scleractinia from marginal habitats (Toller et al. 2001, published as clade E). Type C1 was identified more frequently among colonies from near-shore versus midshelf reefs. (Type C1 is equivalent to subclade C1 in Van Oppen et al. [2001]; note that each subclade identified by Van Oppen et al. is equivalent to a particular ITS2 ''type;'' [LaJeunesse 2002], sequencing from cloned PCR products [Speksnijder et al 2001; Diekmann et al. unpubl. data] combined with the confounding effect of intragenomic variation [LaJeunesse 2002], probably resulted in microvariation, real and/or artifact, that produced sequence clusters in their phylogeny.) At their midshelf reef site, where less bleaching occurred, *Acropora* spp. associated predominantly with symbiont type C3i (equivalent to subclade C2; Van Oppen et al. 2001). The flexibility that acroporids show in associating with different *Symbiodinium* at regional scales (Loh et al. 2001; Van Oppen et al. 2001) indicates that their symbioses are strongly regulated by prevailing environmental conditions and that partner recombination driven by physical factors is a plausible acclimatory mechanism. Furthermore, the lack of symbiont diversity associated with the acroporid corals in a given region may explain previous widespread, indiscriminant bleaching (90%–100%) and the death of this group during sudden periods of environmental stress (Brown and Suharsono 1990; Hoegh-Guldberg and Salvat 1995).

Coral physiology (Loya et al. 2001), combined with symbiont physiology (Warner et al. 1999), determines an association's bleaching susceptibility. It must be emphasized that the full range of potential symbiont partners and the extent to which a particular association expands the environmental tolerance of a coral is unknown. Ultimately, determining the full ecological significance of symbiont diversity, host-symbiont specificity, coral physiology, and differing susceptibilities of individual colonies within a coral species is central to predicting how endosymbiotic reef animals from different biogeographic regions may respond to climactic change and increases in sea surface temperature.

Greater relative symbiont diversity in the Caribbean—At the phylogenetic level of clade, Pacific hosts associate predominantly with *Symbiodinium* spp. from clade C (Baker and Rowan 1997; Loh et al. 1998; Baker 1999; LaJeunesse et al. unpubl. data). This contrasts with the Caribbean where symbioses involving *Symbiodinium* spp. from clades A and especially B are more common (Fig. 4) (Baker and Rowan 1997; LaJeunesse 2002). Nonetheless, the diversity of clade C types will ultimately be greater in the Pacific (Baker and LaJeunesse unpubl. data; LaJeunesse et al. unpubl. data). Obviously, more sampling is required of the tremendous host species diversity characteristic of the Indo-Pacific, but, unless considerably more symbiont types are found in the reefs around Heron Island, it would appear that the ratio of symbiont to host diversity is greater on a Caribbean reef system than that in the southern GBR.

The greater proportion of symbioses involving *Symbiodinium* spp. from different clades in Caribbean hosts is probably explained by geological events and differences in paleoenvironments over the last 3–4 million yr. The uplift of the Central American Isthmus, 3.1–3.5 million yr ago (Ma) (Coates and Obando 1996), isolated, and thereby substantially reduced, the dispersal capability and effective population sizes within Caribbean reef communities. ''Caribbean symbioses'' involving symbionts from clade A and B probably evolved during the environmental perturbations and extinctions that accompanied the Plio-Pleistocene transition (3.5–1.5 Ma). During this time, the Caribbean neotropics experienced a dramatic increase in faunal turnover and diversification (Jackson 1994; Budd 2000). Evidently, the environmental changes and associated extinctions of the late Pliocene in the Caribbean were less severe in the tropical Pacific (Vermeij 1987), where coral reef assemblages appeared to remain stable during this time (Veron and Kelley 1988). Molecular rate estimates on time of divergence have indicated that many specialized Caribbean symbionts radiated after the coral extinctions of 1.6–0.8 Ma, whereas more of their counterparts from the GBR date further back, to the separation of the Atlantic and Pacific (LaJeunesse unpubl. data).

The compounding factors of past environmental variance and geographic isolation have probably shaped the evolution of symbiont diversity, resulting in the present dissimilarity of community assemblages between regions. Host diversity (habitat diversity) and abundance (habitat availability) may also be factors that influence *Symbiodinium* spp. diversity and the relative proportion of generalists versus specialists.

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