Zooxanthellae of the Montastraea annularis Species Complex: Patterns of Distribution of Four Taxa of Symbiodinium on Different Reefs and Across Depths

W. W. Toller1,2, R. Rowan2,*, and N. Knowlton1,3

1Marine Biology Research Division 0202, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093-0202; 2University of Guam Marine Laboratory, Mangilao, Guam 96923; and 3Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama

Abstract. Corals of the Montastraea annularis complex host several different dinoflagellates in the genus Symbiodinium. Here we address two questions arising from our previous studies of these associations on an offshore reef. First, do the same taxa and patterns of association (Symbiodinium A and B found in higher irradiance habitats than Symbiodinium C) occur on an inshore reef? Second, does M. franksi at the limits of its depth range host only Symbiodinium C, as it does at intermediate depths? In both surveys, a new Symbiodinium taxon and different patterns of distribution (assayed by analyses of small ribosomal subunit RNA genes [srDNA]) were observed. Inshore, a taxon we name Symbiodinium E predominated in higher irradiance habitats in M. franksi and its two sibling species; the only other zooxanthella observed was Symbiodinium C. Offshore, M. franksi mainly hosted Symbiodinium C, but hosted Symbiodinium A, B, C, and E in shallow water and Symbiodinium E and C in very deep water. Symbiodinium E may be stress-tolerant. Observed srDNA heterogeneity within samples of Symbiodinium B, C, and E is interpreted as variation across copies within this multigene family. Experimental bleaching of Symbiodinium C supported this interpretation. Thus sequences from natural samples should be interpreted cautiously.

Introduction

Coral reefs are the most biologically diverse marine habitats. Underpinning this diversity are the reef-building corals themselves, which are obligate, mutualistic symbioses between coral animals and dinoflagellates (commonly called zooxanthellae). This partnership between heterotrophic hosts and photosynthetic symbionts allows corals to thrive in shallow, nutrient-poor tropical seas, and deposit calcium carbonate in amounts large enough to build reefs (reviewed in Muscatine and Porter, 1977; Falkowski et al., 1984; Barnes and Chalker, 1990; Muller-Parker and D’Elia, 1997).

Coral taxonomy at the species level, although occasionally frustrating (Knowlton and Jackson, 1994; Veron, 1995), has generally been sufficient to describe overall diversity and to define experimental subjects. This taxonomy seldom, however, has considered zooxanthellae, because it was widely assumed that one species of coral associates with only one species of zooxanthella—in other words, that host taxonomy identified both partners. Zooxanthellae are diverse (e.g., Schoenberg and Trench, 1980; Rowan, 1998), and it is now recognized that some species of corals associate with multiple species of zooxanthellae (Rowan and Knowlton, 1995; Rowan, 1998). Thus corals identified as members of the same species may not in fact be equivalent at the whole organism (holobiont) level, and the taxonomic identities of zooxanthellae may be as ecologically important as those of their hosts.

As far as is known, zooxanthellae in reef-building corals are members of the genus Symbiodinium (Rowan, 1998), which includes four species described as in vitro cultures (Freudenthal, 1962; Trench and Blank, 1987). Several other
cultured isolates of *Symbiodinium* have been named informally, but most members of the genus remain uncultured and undescribed (Rowan, 1998). Nevertheless, sequences and restriction fragment length polymorphism (RFLP) of genes that encode ribosomal RNA (rDNA) can be used to distinguish some taxa of *Symbiodinium* and to study ecological relationships among host, symbiont, and habitat diversity (Rowan and Powers, 1991a, b; Rowan and Knowlton, 1995; Rowan et al., 1997; Baker and Rowan, 1997; Hill and Wilcox, 1998; Darius et al., 1998; Baker, 1999). The present study uses genes that encode small ribosomal subunit RNA (srDNA).

Our earlier work concerned zooxanthellae of the sibling coral species *Montastraea annularis*, *M. faveolata*, and *M. franksi*, which are the dominant reef-building corals in the Western Atlantic (Goreau, 1959). On an offshore reef in the San Blas Islands of Panama, we found that both *M. annularis* and *M. faveolata* associate with three distinct taxa of *Symbiodinium* (A, B, and C, see Rowan and Knowlton, 1995; Rowan et al., 1997). *Symbiodinium* A and B, or both, are predominant in tissue exposed to high irradiance (shallower water or colony tops), *Symbiodinium* C is predominant in shaded tissue (deeper water or colony sides), and mixtures of *Symbiodinium* A and/or B with C occur between these extremes. Colonies of *M. franksi*, in contrast, were found to host only *Symbiodinium* C (Rowan and Knowlton, 1995); however, this coral species was not found at shallow depths on this reef. These observations led to two questions addressed here. First, do these symbiont taxa and patterns of association occur on other types of reef? Second, does the deeper distribution of *M. franksi* reflect an inability by this species to host those taxa of *Symbiodinium* with which *M. annularis* and *M. faveolata* associate in shallow water?

We also discuss some concerns about using srDNA to identify the *Symbiodinium* that we collected. Although srDNA was heterogeneous in samples of *Symbiodinium* B, C, and E, we found no evidence to suggest that the zooxanthellae in each of these samples were heterogeneous. We suspect that srDNA in these *Symbiodinium* is a heterogeneous multigene family, as is rDNA in some other dinoflagellates (Scholin et al., 1993; Scholin and Anderson, 1994, 1996). We discuss practical implications of this suspicion for the use of srDNA as a taxonomic character.

**Materials and Methods**

**Field collections and study sites**

Corals were identified in the field by colony-level characters (Weil and Knowlton, 1994). Apparently healthy colonies, separated from one another by >2 m, were sampled with hammer and 1/2-in (#12) steel hole punch, yielding a coral core with about 1.3 cm$^2$ of live colony surface. Cores were wrapped in aluminum foil and frozen in a cryogenic dry shipper (chilled with liquid nitrogen). Many colonies of

---

**Figure 1.** Collecting localities in the San Blas Archipelago (upper panel) and Bocas del Toro (lower panel), Republic of Panama (inset). Arrows with initials identify places where corals were sampled: AG, Aguadargana reef; RC, Rio Carti; CL, Cayos Limones; JP, Juan Point; CP, Cocos Point. Data from Aguadargana reef were reported previously (Rowan and Knowlton, 1995; Rowan et al., 1997).

*Montastraea annularis* and *M. faveolata* were sampled both on their tops and on their sides to obtain samples from relatively high- and low-irradiance tissues (respectively) within a colony (Rowan et al., 1997). Most colonies of *M. franksi* were sampled at only one location because their relatively flat morphologies made a distinction between colony top and side superfluous.

Coral colonies were sampled at three sites in the Republic of Panama (Fig. 1) between October 1997 and October 1998:

1. **Rio Carti, San Blas.** We sampled from a small coastal fringing reef adjacent to the mouth of a major river (Rio Carti Grande). During May to December, such nearshore sites are periodically subjected to heavy freshwater runoff and riverine sediments (Clifton et al., 1997; D'Croz et al., 1999). *Montastraea* species occur at Rio Carti from the barely subtidal to a depth of about 12 m, where hard substrate is replaced by soft-bottom sediments. We sampled the tops of all encountered colonies (*M. annularis*, $n = 4$; *M. faveolata*, $n = 20$; *M. franksi*, $n = 19$); 30 of these were also sampled on their sides.

2. **Cayos Limones, San Blas.** These reefs are located 15
km north of mainland Panama and are not strongly influenced by terrestrial runoff (D’Croz et al., 1999). We sampled from a relatively steep, leeward fringing reef that ends abruptly at depths between 35 and 40 m in soft sediments (see fig. 9 in Robertson and Glynn, 1977). On this reef, M. franksi is common below 8 m, and it is the dominant coral (with Agaricia lamarcki) below 15 m. We sampled M. franksi throughout its depth range (4 to 38 m, n = 78 colony tops).

(3) Bocas del Toro. Juan Point and Cocos Point reefs are located in the semienclosed lagoon of Bahia Almirante in the Province of Bocas del Toro. Like Rio Carti, these sites are affected by high rainfall and river outflow throughout much of the year. On many of the reefs in this area, M. franksi is the most abundant member of the M. annularis complex. We made a limited collection at depths of 1-15 m for comparative purposes, consisting of 1 top sample of M. annularis, 10 of M. franksi, and 3 of M. faveolata.

Observations of srDNA heterogeneity within samples of Symbiodinium C (see Results) prompted us to investigate the stability of these genotypes under stress. We identified 11 colonies of M. annularis (each colony consisting of a cluster of columns) that hosted heterogeneous RFLP genotypes of Symbiodinium C. After an initial sample, columns (one per colony) were transplanted from their natural habitat (ca. 10-14 m depth) to 1 m depth at either Cayos Limones (n = 4 transplants) or Aguadargana (n = 7 transplants) reefs (Fig. 1), where they bleached. Columns were sampled again after 4 days (Cayos Limones) or 40 days (Aguadargana). Transplants and determinations of zooxanthellae numbers were conducted as described in Toller et al. (2001). In the present study, however, we did not sample corals further (i.e., during zooxanthellar repopulation; see Toller et al., 2001).

Identification of zooxanthellae

Zooxanthellae were isolated and identified as described previously (Rowan and Powers, 1991b; Rowan and Knowlton, 1995). srDNAs were obtained by PCR amplification with a “host-excluding” primer pair (ss5 and ss3Z) or with universal primers (ss5 and ss3), and then characterized by restriction enzyme digestion. The host-excluding primer pair does not amplify known host srDNAs (Rowan and Powers, 1991b; unpubl. obs.), but does amplify srDNAs from a phylectic group that is much larger than Symbiodinium (McNally et al., 1994; Toller et al., 2001). All samples were assayed using host-excluding primers, and about one-third of them were also analyzed with universal primers. Data obtained from the two kinds of amplifications were always in agreement.

Every sample was analyzed by digesting amplified srDNA with Dpn II and with Tag I, which differentiate Symbiodinium A, B, and C by RFLP (Rowan and Powers, 1991a; Rowan and Knowlton, 1995; Rowan et al., 1997). RFLPs were diagnosed by comparison to genotype standards, which were obtained by PCR amplification from cloned srDNAs of Symbiodinium A, B, and C, all isolated from M. annularis (Rowan and Knowlton, 1995), and from Symbiodinium E (from M. faveolata, this study). These cloned genotype standards are denoted hereafter as A', B', C', and E'. We use the superscript zero to indicate srDNA clones, as opposed to taxa of Symbiodinium; clones obtained from different samples of the same taxon of Symbiodinium are distinguished by numbers (e.g., E'1 and E'2; see below).

Because universal PCR primers amplify coral host srDNA when it is present (Rowan and Powers, 1991b), a cloned srDNA from M. annularis (clone H'; see below) was used as an additional standard in RFLP analyses of these amplifications. Where RFLP analyses indicated mixtures of Symbiodinium A, B, C, or E in a sample, relative abundance (greater than or less than 50% of the total) was estimated by comparison to standard mixtures prepared from cloned srDNAs (Rowan and Knowlton, 1995; Rowan et al., 1997; see Fig. 4).

srDNA was cloned from three samples of Symbiodinium E; clone E'1 is from M. faveolata (from Rio Carti, 3 m depth), clone E'2 is from M. franksi (from Cayos Limones, 38 m), and clone E'3 is from the coral Siderastrea siderea (from Portobelo, Panama, 6 m). Amplified srDNAs (DNA for clone E'1 by universal PCR primers; DNAs for clones E'2 and E'3 by host-excluding PCR primers) were gel-purified, ligated into pGEM-T Easy Vector (Promega Corporation, Madison, WI), and then transformed into Escherichia coli according to manufacturer’s recommendations. From each ligation, 4-12 clones were characterized by amplifying srDNAs with host-excluding PCR primers and then digesting the PCR products with Dpn II, with Tag I, and with Hae III. Each cloned RFLP genotype was compared to the RFLP of its corresponding natural sample. srDNA of M. annularis was obtained with universal PCR primers from sperm DNA (Lopez et al., 1999) and cloned (clone H') as described above.

Clones E'1, E'2, and E'3 were sequenced completely, as were cloned genotype standards A', B', and C' (from which only partial sequences had been obtained previously; Rowan and Knowlton, 1995) and clone H'. Plasmids were prepared using QIAprep Spin Miniprep kits (Qiagen, Inc., Valencia, CA) according to manufacturer’s recommendations, and sequences were determined for both DNA strands using Big Dye Terminator sequencing kits (PE Corporation, Norwalk, CT) with vector sequencing primers T7 and M13-Reverse, and with srDNA sequencing primers 18F1 (5'-AGCTCGTAGTTGGGAGTC-3'), 18F2 (5'-TTAGTTGACTCAACACCGG-3'), 18R1 (5'-AGCTCAAATTAGCCGCAGGG-3'), 18R2 (5'-ATATACTCTTTGGAAGCTTG-3'), 18R3 (5'-AGCTTGATCAAAATAGCGCCGCAGGG-3'), and 18R4 (5'-AGCTGGAGTC-3'). Reactions were analyzed with an ABI
Identification of Symbiodinium E

Routine analyses of sDNAs with Dpn II and with Tag I revealed a zooxanthella in our surveys (see below) that was different from Symbiodinium A, B, and C (Fig. 2). We call this new RFLP genotype E (D has been assigned to a sponge symbiont [Carlos et al., 1999]). Cloned genotype E sDNAs (E^1, E^2, and E^3) from Montastraea faveolata, M. franksi, and Siderastrea siderea (respectively) were more than 99% similar in sequence to one another, and more than 96% similar to sDNAs of Symbiodinium A, B, and C that were cloned from M. annularis (genotype standards A*, B*, and C*). A neighbor-joining analysis of partial sDNA sequences (Fig. 3) places genotype E sDNAs within Symbiodinium (defined by cultured S. microadriaticum and Symbiodinium #8 [Rowan, 1992]).

373 sequencer (Applied Biosystems, Foster City, CA) and complete sequences were assembled using SeqEd software (Applied Biosystems). RFLP genotypes of cloned sDNAs were obtained from their sequences using Gene Construction Kit software (Textco, Inc., West Lebanon, NH). Note that we used only partial sDNA sequences in some analyses (Fig. 3); the full-length sDNA sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/; accession numbers AF238266-AF238258, AF238261-AF238263, and AF238267).

For phylogenetic analysis, we aligned partial sDNA sequences (Rowan and Powers, 1992) with Clustal X software (Thompson et al., 1997) and used neighbor-joining reconstruction (Saitou and Nei, 1987). The following sDNA sequences were obtained from GenBank: Symbiodinium microadriaticum (M88521), Symbiodinium #8 (M88509), Symbiodinium sp. PSP1-05 (AB016578), s11-2xba (U20961), s20-2xba (U20962), 37-4xba (U20959), 86-5xba (U20960), a12-5xba (U20954), a8-5xba (U20955), 175-5xba (U20952), 178-6xba (U20956), 33-6xba (U20958), a1-5xba (U20953), 178-8xba (U20957), Gymnodinium beii (U37366), Gyrodinium galaleanum (M88511), Gymnodinium simplex (M88512), and Polarella glacialis (AF099183). sDNA sequences from Symbiodinium C2 [clone C2^0.1 (AF238259) and clone C2^0.2 (AF238260)] are from Toller et al. (2001). A partial sequence of zooxanthella sDNA from the coral Montipora patula is from a previous study (Rowan, 1992).

To investigate sDNA variation within our samples of Symbiodinium in greater detail, we selected representative samples of each Symbiodinium taxon from each host coral species (M. annularis, M. faveolata, M. franksi) and made additional RFLP analyses. Different samples (from different colonies) of Symbiodinium A (n = 10), B (n = 12), C (n = 12), and E (n = 12) were analyzed with a panel of 12 restriction enzymes, used one at a time. These enzymes were Dpn II, Tag I, Alw I, BsrU I, Hae III, Hha I, Hinfl I, Mse I,Msp I, Nci I, Sau96 I, and Sty I. Samples of Symbiodinium E were investigated further with the enzymes Alu I, Bsp1286 I, Mae III, Mnl I, SfaN I, and Tsp45 I. We chose the latter enzymes based on RFLP differences among clones E^1, E^2, and E^3. All enzymes were purchased from New England Biolabs, Inc. (Beverly, MA) except for Mae III (Roche Diagnostics Corp., Indianapolis, IN).

Results

Figure 2. RFLP genotypes A, B, C, and E of Symbiodinium obtained from different colonies of Montastraea franksi. sDNAs were amplified with host-excluding PCR primers and digested with Dpn II (left) and with Tag I (right). Lane M contains DNA fragment size standards of (top to bottom) 1500 base pairs (bp), 1200 bp, and then 1000 bp to 100 bp in 100-bp increments.

Figure 3. Inferred phylogenetic relationships among sDNAs from different zooxanthellae. Partial sDNA sequences (Rowan and Powers, 1992) were grouped by the neighbor-joining method (Saitou and Nei, 1987). Symbiodinium microadriaticum and Symbiodinium #8 are cultured zooxanthellae (Rowan and Powers, 1992). A, B, and C (followed by GenBank accession numbers) are from Montastraea annularis (Rowan and Knowlton, 1995); three of these correspond to standard clones A*, B*, and C* (this study). Two sDNAs labeled C2 (hosts and clone numbers in parentheses) are from Toller et al. (2001). D (followed by GenBank accession number) is from a sponge (Carlos et al., 1999). sDNAs labeled E (host and clone numbers in parentheses) are from this study, except for that from the coral Montipora patula, which is from Rowan and Powers (1991a). The branch labeled R (to the left) indicates the root for this tree, obtained by including sDNA sequences from the dinoflagellates Gymnodinium beii, Gyrodinium galaleanum, Gymnodinium simplex, and Polarella glacialis (not shown).
Samples of zooxanthellae are from *Montastraea faveolata* (samples 1, 2) and *M. franksi* (sample 3), other lanes are clones E^0^ and C^0^ singly (1:0 and 0:1, respectively) and mixed together in molar ratios ranging from 8:1 to 1:8, to obtain standards. srDNAs were amplified with host-excluding PCR primers and then digested with *Dpn* II (top panel) and with *Taq* I (bottom panel). By visual inspection, samples 1-3 contain both *Symbiodinium* E and C, in ratios of about 4:1, 1:3, and 1:4, respectively. Lane M contains DNA size standards, as in Figure 2.

Distribution of different taxa of *Symbiodinium*

At Río Cartí, *M. franksi* was observed with only two taxa of zooxanthellae—*Symbiodinium* E and C—and the same two taxa were obtained from *M. faveolata* and *M. annularis* (Fig. 5) at this reef. *Symbiodinium* E was the predominant zooxanthella from all three *Montastraea* species: it occurred in 35 of 43 corals and was the only zooxanthella detected in 18 of these. In *M. franksi* and *M. faveolata*, *Symbiodinium* E was more common in higher irradiance habitats (colonies at 1-3 m depth, tops of colonies at 3-6 m depth) than in lower irradiance habitats (colony sides at 3-6 m depth and generally below 6 m). *Symbiodinium* C exhibited the converse pattern (Fig. 5). Samples from *M. annularis* (*n = 4*) showed the same top and side pattern of zooxanthellar distribution within colonies (Fig. 5), although our small sample size precludes an examination across depth. A zonation pattern was often observed in comparisons of tops and sides from the 16 doubly sampled colonies that had the two types of zooxanthellae. In 12 of these colonies, the ratio of *Symbiodinium* E:C decreased from top to side, in three there was no clear difference in the ratios, and in only one colony did the ratio increase from top to side.

At Cayos Limones, *M. franksi* associated primarily with *Symbiodinium* C (Fig. 6), which was the only taxon of zooxanthella observed between 6.5 and 33 m depth (*n = 53* colonies); this result is consistent with the previous study (Rowan and Knowlton, 1995) of *M. franksi* from depths
**ZOOXANTHELLAE IN DIFFERENT HABITATS**

between 6 and 11 m at Aguadargana, another nearby offshore reef (Fig. 1). However, in the shallowest and deepest colonies of *M. franksi*, different taxa of zooxanthellae were observed. Between 4 and 6 m, colonies contained, in order of decreasing frequency of occurrence, *Symbiodinium* B, C, A, and E. With the exception of *Symbiodinium* E in one colony, this distribution of taxa resembles that found in *M. annularis* at similar depths at Aguadargana reef (Rowan and Knowlton, 1995; Rowan et al., 1997). Samples from four of the six deepest colonies of *M. franksi* (35-38 m depth) contained *Symbiodinium* E only; the other two colonies contained *Symbiodinium* C only (Fig. 6). At both the shallow and deep extremes, colonies of *M. franksi* were relatively small, encrusting forms (<0.5 m diameter).

To find out if the congeners of *M. franksi* at Cayos Limones also host *Symbiodinium* E at their lower depth limits, we sampled the deepest colonies of *M. annularis* (*n* = 23) and *M. faveolata* (*n* = 5) that we could find. They were not very deep (12-17 m and 13-15 m, respectively), and like *M. franksi* at the same depths, contained *Symbiodinium* C only (not shown).

In our limited sample of corals from two reefs at Bocas del Toro (1-15 m depth), *M. franksi* was found with *Symbiodinium* E only (1 colony), with *Symbiodinium* E and C (4 colonies), with *Symbiodinium* C only (2 colonies), or with *Symbiodinium* A only (3 colonies). *M. faveolata* was found with *Symbiodinium* C only (2 colonies) or with *Symbiodinium* A only (1 colony). The single encountered colony of *M. annularis* contained *Symbiodinium* A. We did not observe *Symbiodinium* B in any of these samples.

Other diversity in zooxanthellar srDNAs

The routine RFLP analyses (with *Dpn* II and *Taq* I) reported above indicated that all samples of zooxanthellae in this study contained srDNAs of either *Symbiodinium* A, B, C, or E, or mixtures thereof, as defined by our standard, cloned srDNA genotypes (A<sup>0</sup>, B<sup>0</sup>, C<sup>0</sup>, E<sup>0</sup>-1). However, when zooxanthellal srDNAs were analyzed in greater detail (with additional restriction enzymes; see Methods and Materials), samples of *Symbiodinium* B, C, and E (but none of 10 tested samples of *Symbiodinium* A) were found to contain additional srDNAs that could not be attributed to genotypes A<sup>0</sup>, B<sup>0</sup>, C<sup>0</sup>, E<sup>0</sup>-1, or to host srDNA. These other srDNAs appeared as additional DNA fragments in restriction digests, as described below.

Twelve selected samples of *Symbiodinium* E and clones E<sup>0</sup>-1, E<sup>0</sup>-2, and E<sup>0</sup>-3 were all indistinguishable in digests with *Dpn* II (examples in Fig. 7, *Dpn* II panel) and with *Taq* I (not shown). In digests with *Mae* III, however, all of these samples had an additional DNA fragment in relatively low abundance (arrow in Fig. 7, *Mae* III panel) that was not part of the RFLP genotype of clones E<sup>0</sup>-1 and E<sup>0</sup>-2, but which was in the RFLP genotype of clone E<sup>0</sup>-3. Thus, these samples apparently contained at least two srDNAs—one defined in *Mae* III digests by clones E<sup>0</sup>-1 and E<sup>0</sup>-2, the other by clone E<sup>0</sup>-3. Similarly, an additional band in digests of sample srDNAs with *Mnl* I (arrow in Fig. 7, *Mnl* I panel) apparently represents the RFLP genotype of clone E<sup>0</sup>-1 (versus clones E<sup>0</sup>-2 and E<sup>0</sup>-3). Digestion of samples with *Alu* I also yielded an additional DNA fragment (arrow in Fig. 7, *Alu* I panel), and digestion of cloned srDNAs with *Alu* I showed that the genotype of clone E<sup>0</sup>-2 is unique. In all, additional bands like those shown in Figure 7 (arrows) were observed in 7 of 18 different restriction enzyme digests (other digests not shown) of the 12 tested samples of *Symbiodinium* E. Therefore, srDNA in these samples of *Symbiodinium* E was clearly heterogeneous. This heterogeneity did not, however, vary qualitatively nor quantitatively among the tested samples (e.g., Samples 1-3 in Fig. 7). Thus, clones E<sup>0</sup>-1, E<sup>0</sup>-2, and E<sup>0</sup>-3, which are different (Fig. 7; see also Fig. 3), were obtained from indistinguishable samples of zooxanthellae.

As with *Symbiodinium* E, srDNA heterogeneity was observed in all tested samples of *Symbiodinium* B. Two digests (out of 12) revealed heterogeneity—*Hlu* I and *Sty* I (examples in Fig. 8). In each of these, the additional fragments (arrows in Fig. 8) imply an srDNA with one restriction site gain relative to clone B<sup>0</sup>. Interestingly, a cloned srDNA from *Symbiodinium* B (*Symbiodinium* #8 isolated from a Hawaiian anemone [*Aiptasia pulchella*] Rowan and Powers, 1992) has both additional sites (S8 in Fig. 8; schematic genotype on the right), suggesting that samples of *Symbiodinium* B from other hosts may also exhibit srDNA heterogeneity. In our samples of *Symbiodinium* B from *Montastraea*, within-sample srDNA heterogeneity did not vary among the 12 tested samples (e.g., samples 1-4 in Fig. 8).

In the case of *Symbiodinium* C, srDNAs in all of 12 tested
samples were also heterogeneous. However, unlike *Symbiodinium* E and B (above), within-sample srDNA heterogeneity in *Symbiodinium* C varied both qualitatively (e.g., compare samples 3-5 in *Dpn* II panel, Fig. 9) and quantitatively (e.g., compare Samples 1-5 in *Hinf* I panel, Fig. 9) among samples. srDNA heterogeneity was observed in as few as one or as many as six different digests (examples in Fig. 9) among the 12 samples tested. That additional variation suggested that some or all samples might have contained more than one genotype of *Symbiodinium*.

We made two analyses that might have supported this hypothesis. First, because mixtures of *Symbiodinium* A, B, C, or E vary in proportion at different locations within a coral colony (Rowan and Knowlton, 1995; Rowan *et al.*, 1997; Results), we analyzed multiple samples from colonies of *M. annularis* in which *Symbiodinium* C had been observed previously. In 14 colonies (each consisting of a cluster of columns), we sampled one column on its top and on its side; srDNA genotypes were indistinguishable in every top-versus-side comparison (not shown). We also sampled the tops of one or two additional columns in 13 of these colonies, and again saw no differences in zooxanthellae RFLP genotype within any colony (not shown). Second, we speculated that if the additional srDNAs did represent distinct, co-occurring zooxanthellae, their relative abundance might change under stress (e.g., as in Rowan *et al.*, 1997). Transplantation of columns from deep to shallow
habitats resulted in bleaching of all columns, and effectively reduced zooxanthellar numbers (70% reduction on average). However, neither acute stress (5 days) nor prolonged stress (ca. 40 days) of zooxanthellae altered the RFLP genotypes that were observed (examples in Fig. 10)—the relative abundance of distinct sRDNA had not changed compared to samples taken prior to transplantation.

Discussion

Four taxa of Symbiodinium in the Montastraea annularis complex

Previous surveys of zooxanthellar diversity in Montastraea annularis, M. faveolata, and M. franci (Rowan and Knowlton, 1995; Rowan et al., 1997) are now shown to be incomplete. In surveys of additional habitats and depths, we found (i) a fourth taxon of Symbiodinium (E) that was not previously reported in these corals, (ii) differences in the distribution of zooxanthellae at offshore and coastal reefs, and (iii) multiple taxa of zooxanthellae in M. franci, which previously had been found to contain only Symbiodinium C.

Groups A, B, C, and E constitute the known diversity of coral-associated Symbiodinium (Rowan, 1998; this study), and M. annularis, M. faveolata, and M. franci all associate with at least one member of each of these groups. This is a remarkable amount of taxonomic diversity—at least 12 distinct symbioses—in what was previously (Knowlton et al., 1992; Rowan and Knowlton, 1995) regarded as one species of coral hosting one species of zooxanthella. Moreover, this diversity is not randomly distributed, suggesting that what was once viewed as a single quintessential generalist (Connell, 1978) is in fact a complex assemblage of ecologically more specialized entities.

Our observations from Cayos Limones now enable us to refute the speculation that M. franci associates exclusively with Symbiodinium C—this host coral can and does form symbioses with Symbiodinium A, B, and E. However, at this offshore reef, the latter host-zooxanthella combinations are observed only at the margins of this coral’s depth distribution (Fig. 6): shallow (Symbiodinium B > A > E) and very deep (Symbiodinium E; discussed further below). Other-
wise, M. franksi hosts Symbiodinium C throughout nearly all of its depth range (Fig. 6). Where colony growth is robust. Although in shallow water the distributions of zooxanthellae (mostly Symbiodinium A and B) are similar in M. franksi, M. faveolata, and M. annularis, the small size of M. franksi colonies in shallow water may reflect a relatively poor physiological fit between this coral host and these zooxanthellae.

The main question posed by our new results is why all three species in the Montastraea annularis species complex at a coastal site (Rio Carti) host predominantly Symbiodinium E at higher irradiance (Fig. 5), instead of Symbiodinium A or B, as found at offshore reefs (Rowan and Knowlton, 1995; Rowan et al., 1997; Fig. 6). One possible explanation is that this coastal site is characterized by environmental stress to which Symbiodinium E is more tolerant than are Symbiodinium A or B. High irradiance is a stress that may exacerbate (Brown, 1997) the many other kinds of stress found in nearshore environments (e.g., fluctuations in temperature, salinity, nutrients, sediments, and underwater irradiance; see Bowden, 1983; Kirk, 1994). All of these factors can affect the stability of coral-algal symbioses (Falkowski et al., 1993; Brown, 1997; Wesseling et al., 1999). In the San Blas Archipelago (Fig. 1), nearshore effects associated with freshwater runoff are limited to a relatively narrow coastal band and do not reach our offshore study sites at Aguadargana and Cayos Limones (D’Croz et al., 1999). Symbiodinium E was also common in Montastraea within a large coastal lagoon at Bocas del Toro, Panama (Fig. 1), an area of exceptionally high rainfall where water quality is also likely to be dominated by coastal effects.

A second (and perhaps related) question asks why Symbiodinium E was distributed differently at Cayos Limones, where it was common not at high irradiance but rather in the very deepest colonies of M. franksi (Fig. 6). Perhaps shallow and deep populations of Symbiodinium E are different species of zooxanthella, although we did not find any evidence to support this (see following section). Instead, we suggest that Symbiodinium E was actually not distributed so differently at these two sites. In both cases it was associated with marginal habitat: at great depth where M. franksi colonies are not large and where the reef itself disappears into sediment (Cayos Limones), and along the coast near a large river, where coral reefs are poorly developed or absent (Rio Carti). Bleaching-associated stress may be common in both habitats, due to occasional smothering by sediments in the former (e.g., Wesseling et al., 1999) and to near-shore conditions in the latter (see above). We propose that the Symbiodinium E we observed represents a taxon of zooxanthella that occurs in certain habitats not because it performs best in those habitats, but because it tolerates them, whereas Symbiodinium A, B, and C do not. According to this idea, Symbiodinium E is rare or absent from other habitats not because it performs poorly in them, but because Symbiodinium A, B, and C are better adapted to those habitats and somehow exclude it.

Anecdotal observations are consistent with our interpretation of Symbiodinium E as a stress-tolerant zooxanthella. We observed Symbiodinium E (diagnosed by Dpn II and Tag I digests of srDNA) in M. faveolata in the Bahamas (not shown), in four of seven colonies that were relatively unbleached during a natural bleaching event (D. Zawada, Scripps Institution of Oceanography, pers. comm.). We also found that Symbiodinium E— but not Symbiodinium B or C— was adept at repopulating severely bleached corals in experiments (Toller et al., 2001). These experimental results suggest that, in addition to tolerating stress, Symbiodinium E may also be good at colonizing corals whose zooxanthellar communities have been severely disrupted by stress.

Observations of zooxanthellae related to Symbiodinium E in other hosts and seas imply that this taxon, like the taxa Symbiodinium A, B, and C (Rowan, 1998), may represent a group (clade) of zooxanthellae. Those observations include the corals Montipora patula in Hawaii (Rowan and Powers, 1991a; Fig. 3), Acropora palifera in Australia (R. R., unpublished obs.), Pocillopora damicornis in the eastern Pacific (Baker, 1999), Goniatrea aspera in Thailand (A. Douglas, University of York, pers. comm.), and the giant clam Hipposus hippopus in Australia (R. R., unpublished obs.). In the context of our hypothesis that Symbiodinium E is stress tolerant in Montastraea, it is notable that G. aspera occurs on reef flats—an environment that is stressful for corals, and where coral bleaching events occur regularly (Brown et al., 2000). Similarly, in P. damicornis, Symbiodinium E was disproportionately common in unbleached colonies during an El Niño-related bleaching event (Symbiodinium D of Baker [1999] has an RFLP pattern that is indistinguishable from that of Symbiodinium E from Montastraea in three restriction enzyme digests; A. Baker, Wildlife Conservation Society, pers. comm.). These observations suggest that other members of the clade Symbiodinium E may also be stress tolerant.

The hypothesis that Symbiodinium E is a relatively stress-tolerant zooxanthella is based on circumstantial evidence, and should be tested in experiments in which environmental factors are controlled and physiological responses are measured. Descriptive studies of unmanipulated corals are, however, indispensable for framing realistic hypotheses in the first place.

Taxonomic interpretation of variation in zooxanthellar srDNA

We recognize the RFLP genotype E as a distinct taxon— Symbiodinium E—for the following reasons: (i) RFLP genotype E was common, and many samples contained only
this genotype (Fig. 5); (ii) the nonrandom distribution of RFLP genotype E (Figs. 5 and 6) strongly implies that it represents a distinct organism with distinct ecological attributes; and (iii) phylogenetic analyses of genotype E srDNAs place them within *Symbiodinium*, but distinct from srDNAs of genotypes A, B, and C (Fig. 3), which, by the same reasoning, represent distinct taxa of *Symbiodinium* (Rowan, 1998). In practice, these four taxa of *Symbiodinium* are readily identified by comparison to cloned srDNAs (RFLP genotypes A⁰, B⁰, C⁰, and E⁰⁰¹) digested with the enzymes *Dpn* II and *Taq* I.

By analyzing zooxanthellae srDNA with additional restriction enzymes, we found that samples containing srDNA of RFLP genotype B⁰, C⁰, or E⁰⁰¹ also contained at least one additional srDNA of a different RFLP genotype (examples in Figs. 7-9). What do these additional srDNAs represent, taxonomically? Like an srDNA in genotype C⁰ (Rowan and Knowlton, 1995), they appear to be from *Symbiodinium* (and not some other type of organism) because (i) they were distinguishable in fewer than one-half of different restriction digests, (ii) many of them seemed to represent simple, single restriction site changes compared to a cloned srDNA (not shown), and (iii) different srDNAs from samples of *Symbiodinium* E (Fig. 3) or of C⁰ (Rowan and Knowlton, 1995) differed relatively little in sequence.

Do these additional srDNAs represent distinct species or strains of *Symbiodinium*? In the case of *Symbiodinium* E and B, no evidence suggests that they do. Specifically, these srDNAs were not observed by themselves, nor did they vary in relative abundance from sample to sample (Figs. 7 and 8). This contrasts with observations on srDNAs of RFLP genotypes A⁰, B⁰, C⁰ and E⁰⁰¹, which occur alone, and also mix in a range of proportions (e.g., *Symbiodinium* C and E, Fig. 4; Rowan and Knowlton, 1995; Rowan et al., 1997).

Because srDNA is a multigene family in eukaryotes, srDNA heterogeneity (as seen within samples of *Symbiodinium* B and E) can reside in one organism—including dinoflagellates (Scholin et al., 1993; Scholin and Anderson, 1994, 1996)—among gene-family members (Hillis and Dixon, 1991). We favor this as an explanation for our data because it is parsimonious compared to the alternative of multiple strains of zooxanthellae that for some reason always co-occur in the same relative proportion. Testing this hypothesis requires the analysis either of one dinoflagellate (e.g., Yeung et al., 1996) or of a clonal culture (e.g., Scholin et al., 1993; Rowan et al., 1996).

Heterogeneity of srDNA within samples of *Symbiodinium* C was more intriguing because sample-to-sample variation was observed among colonies (Fig. 9). That observation suggested that different srDNAs within any one sample could represent different strains of *Symbiodinium*. If so, that sample-to-sample variation might also appear within one coral colony, either from place to place or time to time, especially before *versus* after an environmental change. We found no such variation (e.g., Fig. 10) in corals hosting *Symbiodinium* C, which again is consistent with the hypothesis that srDNA heterogeneity is a property of individual zooxanthellae. Different patterns of srDNA heterogeneity seen among samples of *Symbiodinium* C from different corals (Fig. 9) are different zooxanthellar genotypes, but we do not know if these differences are biologically significant (e.g., Scholin and Anderson, 1994, 1996).

Independent of its source, within-sample srDNA heterogeneity limits the information that can be obtained from srDNA sequences. This limitation is apparent in our analysis of *Symbiodinium* E. The sequence of clone E⁰⁰¹ implies that our RFLP analyses, using 18 enzymes (examples in Fig. 7), surveyed about 220 nucleotide positions (not shown). Heterogeneity was detected with seven enzymes, which implies a within-sample srDNA sequence diversity of about 3% (7 of 220 nucleotide positions). We do not know how this diversity is distributed; possibilities range from two srDNAs that differ at 7 of 220 positions (ca. 3% different srDNAs, similar to the difference between srDNAs of *Symbiodinium* A and B [Rowan and Powers, 1992]) to seven srDNAs that differ from one another at 1 of 220 positions (ca. 0.4% different srDNAs). Differences among srDNA clones E⁰⁰¹, E⁰², and E⁰³ fall within this range, and there is no reason to expect any cloned srDNA to represent our samples of *Symbiodinium* E with any greater precision. Moreover, the PCR creates chimeric DNA molecules when mixed templates are amplified, and many clones obtained from those PCR products will be artifacts (Bradley and Hillis, 1997; Wintzingerode et al., 1997; Darius et al., 1998).

Sequences of srDNAs obtained (as clones) from *Symbiodinium* in the *M. annularis* species complex are summarized in Figure 3. Because we have evidence for only four taxa—A, B, C, and E—the multiple branches within groups B, C, and E represent sequence variation within, not among, taxa. An exception to this statement is the pair of sequences labeled C2⁰⁰¹ and C2⁰², which came from an experimentally bleached *M. annularis* and from an unmanipulated colony of the coral *Siderastrea siderea*, respectively (see Toller et al., 2001). Ecological data and RFLP analyses strongly imply that C2⁰⁰¹ and C2⁰² represent a taxon (*Symbiodinium* C2) that is distinct from the taxon *Symbiodinium* C found commonly in unmanipulated *Montastraea* (Toller et al., 2001). We stress that this taxonomic difference could not be inferred from srDNA sequence data alone, given the levels of srDNA heterogeneity within samples of *Symbiodinium* C and C2 (Toller et al., 2001).

In conclusion, the problem of fully interpreting srDNA variation in natural samples of *Symbiodinium* is challenging. By themselves, srDNA sequence data contributed relatively little to understanding zooxanthellar diversity in *Montastraea*. RFLP data were much more informative, not the least because they revealed the informational limits of
srDNA sequences. Many samples of zooxanthellae from these species of coral contained more than one taxon of Symbiodinium (Figs. 4 and 5; Rowan et al., 1997), a phenomenon that would have been difficult to understand from srDNA sequences alone. RFLP data are easily obtained, at reasonable cost, from many samples of zooxanthellae, which allows ecological data to inform taxonomic decisions.

Acknowledgments

We thank the Kuna Nation and the Republic of Panama (Autoridad Nacional del Ambiente, Departamento de Cuan-entena Agropecuaria del Ministerio de Desarrollo Agropeucario, and Recursos Marinos) for permission to collect and export specimens. Many thanks to Javier Jara for tireless field assistance and to Juan Maté for help with the deep collections of M. franksi. Thanks to Ursula Anlauf and Suzanne Williams for advice. Thanks to Ralf Kersanach and David Kline for coral DNA and advice. David Zawada provided samples from the Bahamas. R. R. thanks Chris Hein and Uma Narayan for hospitality in California. This research was supported by the Andrew W. Mellon Foundation, the Smithsonian Tropical Research Institute, the Scripps Institution of Oceanography, and the National Institutes of Health.

Literature Cited

Baker, A. C. 1999. Symbiosis ecology of reef-building corals. Ph.D. dissertation, University of Miami. 120 pp.

Baker, A. C., and R. Rowan. 1997. Diversity of symbiotic dinoflagel-lates (zooxanthellae) in scleractinian corals of the Caribbean and Eastern Pacific. Proc. Eighth Int. Coral Reef Symp. 2: 1301–1306.

Barnes, D. J., and B. E. Chalker. 1999. Calcification and photosynthe-sis in reef-building corals and algae. Pp. 109–131 in Ecosystems of the World Vol. 25. Coral Reefs. Z. Dubinsky, ed. Elsevier, New York.

Bowden, K. F. 1983. Physical Oceanography of Coastal Waters. Ellis Horwood, Chichester, United Kingdom.

Bradley, R. D., and D. M. Hillis. 1997. Recombinant DNA sequences generated by PCR amplification. Mol. Biol. Evol. 14: 592–593.

Brown, B. E. 1997. Coral bleaching: causes and consequences. Coral Reefs 16: Suppl. S129–S138.

Brown, B. E., R. P. Dunne, M. S. Goodson, and A. E. Douglas. 2000. Bleaching patterns in reef corals. Nature 404: 142–143.

Carlos, A. A., B. B. Baille, M. Kawauchi, and T. Namibaya. 1999. Phylogenetic position of Symbiodinium (Dinophyceae) isolates from tridacnids (Bivalvia), cardinalis (Bivalvia), a sponge (Porifera), and a soft coral (Anthozoa), and a free-living strain. J. Phycol. 35: 1054–1062.

Clifton, K. E., K. Kim, and J. L. Wulff. 1997. A field guide to the reefs of Caribbean Panama with an emphasis on Western San Blas. Proc. Eighth Int. Coral Reef Symp. 1: 167–184.

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. Science 199: 1302–1310.

Darius, H. T., C. Dauga, P. A. D. Grimont, E. Chunge, and P. M. V. Martin. 1998. Diversity in symbiotic dinoflagellates (Pyrrhophyta) from seven scleractinian coral species: restriction enzyme analysis of small subunit ribosomal RNA genes. J. Eukaryot. Microbiol. 45: 619–627.

D’Croz, L., D. R. Robertson, and J. A. Martinez. 1999. Cross-shelf distribution of nutrients, plankton, and fish larvae in the San Blas Archipelago. Caribbean Panama. Rev. Biol. Trop. 47: 203–215.

Falkowski, P. G., Z. Dubinsky, L. Muscatine, and J. W. Porter. 1984. Light and the bioenergetics of a symbiotic coral. Bioscience 34: 705–709.

Falkowski, P. G., Z. Dubinsky, L. Muscatine, and L. R. McCluskey. 1993. Population control in symbiotic corals. Bioscience 43: 606–611.

Freudenthal, H. D. 1962. Symbiodinium gen. nov. and Symbiodinium microadriaticum sp. nov., a zooxanthella: taxonomy, life cycle, and morphology. J. Protozool. 9: 45–52.

Goreau, T. F. 1959. The ecology of Jamaican coral reefs I. Species composition and zonation. Ecology 40: 67–90.

Hill, M., and T. Wilcox. 1998. Unusual mode of symbiont repopulation after bleaching in Anthostegiella varians: acquisition of different zooxanthellae strains. Symbiosis 25: 279–289.

Hillis, D. M., and M. T. Dixon. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol. 66: 411–454.

Kirk, J. T. O. 1994. Light and Photosynthesis in Aquatic Ecosystems. 2nd ed. Cambridge University Press, Cambridge.

Knowlton, N., and J. B. C. Jackson. 1994. New taxonomy and niche partitioning on coral reefs: jack of all trades or master of some? Trends Ecol. Evol. 9: 7–9.

Knowlton, N., E. Weil, L. A. Weigt, and H. M. Guzmán. 1992. Sibling species in Montastrea annularis, coral bleaching, and the coral cli- mate record. Science 255: 330–333.

Lopez, J. V., R. Kersanach, S. A. Rehner, and N. Knowlton. 1999. Molecular determination of species boundaries in corals: genetic analysis of the Montastrea annularis complex using amplified fragment length polymorphisms and a microsatellite marker. Biol. Bull. 196: 80–93.

McNally, K. L., N. S. Govind, P. E. Thomé, and R. K. Trench. 1994. Small-subunit ribosomal RNA sequence analyses and a reconstruction of the inferred phylogeny among symbiotic dinoflagellates (Pyrrhophyta). J. Phycol. 30: 316–329.

Muller-Parker, G., and C. E. D’Elia. 1997. Interactions between corals and their symbiotic algae. Pp. 96–113 in Life and Death of Coral Reefs. C. Birkeland, ed. Chapman & Hall, New York.

Muscatine, L., and J. W. Porter. 1977. Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience 27: 454–460.

Robertson, D. R., and P. W. Glynn. 1977. Field guidebook to the reefs of San Blas Islands, Panama, Third Int. Symp. Coral Reefs. University of Miami, Florida. 15 pp.

Rowan, R. 1998. Diversity and ecology of zooxanthellae on coral reefs. J. Phycol. 34: 407–417.

Rowan, R., and N. Knowlton. 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. Proc. Natl. Acad. Sci. USA 92: 2850–2853.

Rowan, R., and D. A. Powers. 1991a. A molecular genetic classification of zooxanthellae and the evolution of animal-algal symbioses. Science 251: 1348–1351.

Rowan, R., and D. A. Powers. 1991b. Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). Mar. Ecol. Prog. Ser. 71: 65–73.

Rowan, R., and D. A. Powers. 1992. Ribosomal RNA sequences and the diversity of symbiotic dinoflagellates (zooxanthellae). Proc. Natl. Acad. Sci. USA 89: 3639–3643.

Rowan, R., S. M. Whitney, A. Fowler, and D. Yellowles. 1996. Rubisco in marine symbiotic dinoflagellates: form II enzymes in eu- karyotic oxygenic phototrophs encoded by a nuclear multigene family. Plant Cell 8: 539–553.

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

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406–425.

Schoenberg, D. A., and R. K. Trench. 1980. Genetic variation in Symbiodinium (= Gymnodinium) microadriaticum Freudenthal, and specificity in its symbiosis with marine invertebrates. III. Specificity and infectivity of Symbiodinium microadriaticum. Proc. R. Soc. Lond. B 207: 445–460.

Scholin, C. A., and D. M. Anderson. 1994. Identification of group- and strain-specific genetic markers for globally distributed Alexandrium (Dinophyceae). I. RFLP analysis of SSU rRNA genes. J. Phycol. 30: 744–754.

Scholin, C. A., and D. M. Anderson. 1996. LSU rDNA-based RFLP assays for discriminating species and strains of Alexandrium (Dinophyceae). J. Phycol. 32: 1022–1035.

Scholin, C. A., D. M. Anderson, and M. L. Sogin. 1993. Two distinct small-subunit ribosomal RNA genes in the North American toxic dinoflagellate Alexandrium fundyense (Dinophyceae). J. Phycol. 29: 209–216.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24: 4876–4882.

Toller, W. W., R. Rowan, and N. Knowlton. 2001. Repopulation of zooxanthellae in the Caribbean corals Montastraea annularis and M. faveolata following experimental and disease-associated bleaching. Biol. Bull. 201: 360–373.

Trench, R. K., and R. J. Blank. 1987. Symbiodinium microadriaticum Freudenthal, S. goreaei sp. nov., S. kawagutii sp. nov. and S. pilosum sp. nov.: gymnodinioid dinoflagellate symbionts of marine invertebrates. J. Phycol. 23: 469–481.

Veron, J. E. N. 1995. Corals in Space and Time: The Biogeography and Evolution of the Scleractinia. UNSW Press, Sydney, Australia.

Weil, E., and N. Knowlton. 1994. A multi-character analysis of the Caribbean coral Montastraea annularis (Ellis and Solander, 1786) and its two sibling species, M. faveolata (Ellis and Solander, 1786) and M. franksi (Gregory, 1895). Bull. Mar. Sci. 55: 151–175.

Wesseling, I., A. J. Uychiaoco, P. M. Alino, T. Aurin, and J. E. Vermaat. 1999. Damage and recovery of four Philippine corals from short-term sediment burial. Mar. Ecol. Prog. Ser. 176: 11–15.

Wintzingerode, F. v., U. B. Göbel, and E. Stackebrandt. 1997. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol. Rev. 21: 213–229.

Yeung, P. K. K., K. F. Kong, F. T. W. Wong, and J. T. Y. Wong. 1996. Sequence data for two large-subunit rRNA genes from an Asian strain of Alexandrium catenella. Appl. Environ. Microbiol. 62: 4199–4201.
Toller, Wesley, Rowan, R, and Knowlton, Nancy. 2001. "Zooxanthellae of the Montastrea annularis Species Complex: Patterns of Distribution of Four Taxa of Symbiodinium on Different Reefs and Across Depths." *The Biological bulletin* 201, 348–359. https://doi.org/10.2307/1543613.