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A Stable Association of the Stress-Tolerant Zooxanthellae, *Symbiodinium* Clade D, with the Low-Temperature-Tolerant Coral, *Oulastrea crispata* (Scleractinia: Faviidae) in Subtropical Non-Reefal Coral Communities

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Chaolun Allen Chen, Katherine K. Lam, Yoshikatsu Nakano and Wan-Shen Tsai (2003) A stable association of the stress-tolerant zooxanthellae, *Symbiodinium* clade D, with the low-temperature-tolerant coral, *Oulastrea crispata* (Scleractinia: Faviidae) in subtropical non-reefal coral communities. *Zoological Studies* **42**(4): 540-550. We examined spatial and temporal zooxanthellae diversity in a low-temperature-tolerant coral, *Oulastrea crispata*, from 2 subtropical non-reefal coral communities, the Penghu Islands (the Pescadores), Taiwan and from Hong Kong, China using restriction fragment length polymorphism (RFLP) of partial nuclear small subunit ribosomal DNA (nssrDNA) and large subunit ribosomal DNA (nlsrDNA), together with phylogenetic analyses of DNA sequences. *Oulastrea crispata* occurs commonly on shallow reef depressions and on turbid bay bedrock inhabited by only a few other corals, and is a pioneer coral colonizing artificial substrates where environmental disturbance is high. This study demonstrates that the zooxanthellae associated with *O. crispata*, in Penghu and in Hong Kong belong to *Symbiodinium* clade D, a clade of zooxanthellae formally proposed to be stress tolerant in marginal habitats (Toller et al. 2001a). Analyses of zooxanthellae diversity showed no apparent symbiosis polymorphism on either a spatial or temporal scale, suggesting that the association with *Symbiodinium* clade D is stable in *O. crispata*. *Oulastrea crispata* possesses opportunistic life history traits, including a variety of reproductive strategies and physiological tolerances, enabling it to colonize a variety of substrata unfavorable to other corals. Our finding showed a stable association with a stress-tolerant symbiont which may provide a key to how *O. crispata* can achieve such physiological adaptability.
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Key words: Zooxanthellae, *Symbiodinium* clade D, *Oulastrea crispata*, Stress tolerance, Ribosomal DNA.

Reef-building corals associated with symbiotic intracellular dinoflagellates (zooxanthellae) dominate shallow, tropical benthic environments (Muller-Parker and D'Elia 1997). Zooxanthellae provide up to 95% of their photosynthetic products to the host corals, and these products contribute to host growth, reproduction, and maintenance. In return, the host corals offer the symbionts inorganic nutrients and protection (reviewed in Davies 1993). The evolutionary success of this host-symbiont relationship has allowed corals to prosper and to deposit calcium carbonate to build reefs in shallow, nutrient-poor tropical seas (reviewed in Muscatine and Porter 1977, Falkowski et al. 1984, Barnes and Chalker 1990, Muller-Parker and D'Elia 1997). With this capability of building 3-

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dimensional biological structures, tropical coral reefs are recognized as the home of the highest diversity of marine species anywhere on the Earth.

Despite the high biodiversity of tropical coral reefs and our extensive knowledge concerning the mutual physiological contributions between zooxanthellae and their symbiotic partners, our understanding of zooxanthellae diversity just began in the last 2 decades (reviewed in Trench 1997, Rowan 1998). Currently, we know that zooxanthellae in reef-building corals are members of the genus *Symbiodinium* (reviewed in Trench 1997, Rowan 1998). Early researchers considered that all zooxanthellae belonged to a single pandemic species, *Symbiodinium microadriaticum* (Freduenthal) (Taylor 1974). However, subsequent studies based on biochemical, morphological, karyotyping, infectivity, motility patterns, and DNA/DNA hybridization of cultured materials in the 1980s indicated that zooxanthellae comprise a heterogenous group of many species and strains (reviewed in Trench 1997, Rowan 1998). Molecular studies, including restriction fragment length polymorphisms (RFLPs) and DNA sequencing of nuclear small subunit ribosomal DNA (nssrDNA), nuclear large subunit rDNA (nlsrDNA), internal transcribed spacer (ITS) rDNA, and chloroplast large subunit (clsrDNA), have provided confirmation for this speculation (Rowan and Power 1991a b, Rowan and Knowlton 1995, Baker and Rowan 1997, Hunter et al. 1997, Rowan et al. 1997, Wilcox 1998, Carlos et al. 1999, Baillie et al. 2000, Yu et al. 2000, Baker 2001, LaJeunesse 2001, Toller et al. 2001a b, van Oppen et al. 2001, Burnett 2002, Santos et al. 2002, Chen et al. 2003). Those studies separate *Symbiodinium* into several clades. Three major clades (A, B, and C) have been consensually recognized among different research groups, but clades D and E might represent different clades according to the different genetic markers used by various investigators (Carlos et al. 1999, Baillie et al. 2000, LaJeunesse 2001, Loh et al. 2001, Rodriguez-Lanetty et al. 2001, Toller et al. 2001a b, van Oppen et al. 2001, Burnett 2002, Santos et al. 2002). Several studies have provided evidence that some coral species may harbor multiple clades of zooxanthellae, and diverse ecological roles for zooxanthellae may relate to different environmental factors (e.g., irradiance), despite confusion with the clade nomenclature of D and E. For example, Rowan and Knowlton (1995) and Rowan et al. (1997) found the sibling Caribbean coral species, *Montastrea annularis* and *M. faveolata*, associate with clades A, B, and C on an offshore reef of San Blas I., Panama, but *M. franksi* hosts only clade C. Clades A and B, or both, are predominant in shallow-water colonies or on colony tops (high irradiance), while clade C is predominant in deep-water colonies or on colony sides (low irradiance), and mixtures of clades A and/or B with C occur between these 2 extremes (Rowan and Knowlton 1995, Rowan et al. 1997). These results imply that coral may cope with environmental heterogeneity by varying the composition of their symbiotic populations (reviewed in Rowan 1998). Laterally in a survey of inshore reefs, a clade named *Symbiodinium* clade D was identified by analyses of nssrDNA, and was predominant in higher-irradiance habitats in *M. franksi* and 2 sibling species, while the other zooxanthellae observed was clade C. In contrast, offshore *M. franksi* mainly hosted clade C, but hosted clades A, B, C, and D in shallow waters and D and C in very deep water (Toller et al. 2001a). These observations combined with a few cases in Indo-Pacific corals (see discussion in Toller et al. 2001a) led to a hypothesis that clade D may be tolerant of stress.

If *Symbiodinium* clade D is a relatively stress-tolerant zooxanthellae as predicted by Toller et al. (2001a), we should be able to observe that corals living in habitats with extreme environmental conditions (e.g., high/low temperatures, high turbidity, high irradiance, etc.) may associate with *Symbiodinium* clade D. *Goniastrea aspera* colonies, a member of shallow-water communities of massive corals at Phuket, Thailand, harbored an apparently uniform population of *Symbiodinium* clade D, during several episodes of irradiance and thermal stress (Brown et al. 2002; reviewed in Douglas 2003). In this study, we examined spatial and temporal variations in zooxanthellae diversity in the zebracoral, *Oulastrea crispata*, occurring in 2 subtropical non-reefal communities. *Oulastrea crispata* is a member of the Faviidae. The distribution of *O. crispata* is restricted to the West Pacific with a range from Japan in the north to the Great Barrier Reef in the South (Veron 1993). *Oulastrea crispata* has been reported near the low tide mark and occurs on bare subtidal boulder surfaces in Hong Kong (Lam 2000a b). In the Penghu Is. in the middle of the Taiwan Strait and around islands of the Ryukyus Archipelago, *O. crispata* is common on shallow reef depressions and on turbid bay bedrock inhabited by only a few other corals (Nakano and Yamazato 1992). Several studies have suggested that *O. crispata* is resistant to adverse environmental conditions. This species is

tolerant of low water temperatures. It has been recorded from the shores of the Noto Peninsula, Japan, where winter water temperatures are usually between 7 and 10°C, and air temperatures are several degrees below freezing for about 20 d (Yajima et al. 1986). *Oulastrea crispata* is hermaphroditic and has an annual cycle of gametogenesis with an extended spawning period from July to Oct.; it releases planula in the resting period of the gametogenesis cycle in Hong Kong (Lam 2000a). In contrast, *O. crispata* in Okinawa is capable of releasing eggs, and both zooxanthellate sexual and asexual planula and, thus, is presumably both a broadcast spawner and a planula brooder (Nakano and Yamazato 1992). These opportunistic life history traits, including a variety of reproductive strategies and physiological tolerances to conditions unfavorable to other corals, provide *O. crispata* with the capability to colonize a variety of substrata. In this study, we examined the symbiont diversity in *Oulastrea crispata* from 2 non-reefal coral communities, Hong Kong and the Penghu Is., Taiwan, using polymerase chain reaction (PCR), restriction fragment length polymorphisms (RFLPs), and DNA sequencing of nssrDNA and nlsrDNA. While hosts with the above features may allow them to survive long cold winters, the nature of hosting stress-tolerant symbionts is a key to understanding how *O. crispata* achieves such physiological adaptability.

MATERIALS AND METHODS

Description of study sites

Field studies were conducted by the senior author in the Penghu Is., Taiwan, and by the 2nd author in Hong Kong (Fig. 1). The marine environments at both sites are influenced by monsoon systems. In summer, the warm South China Sea Current (SCSSC) driven by the southeast monsoon flows northwards from the South China Sea along the coast of China, and flows into the Taiwan Strait through the Penghu Channel. On the contrary, the cold fresh China coastal water (CCW) driven by the northeast monsoon influences the southern Chinese coast and enters the southern

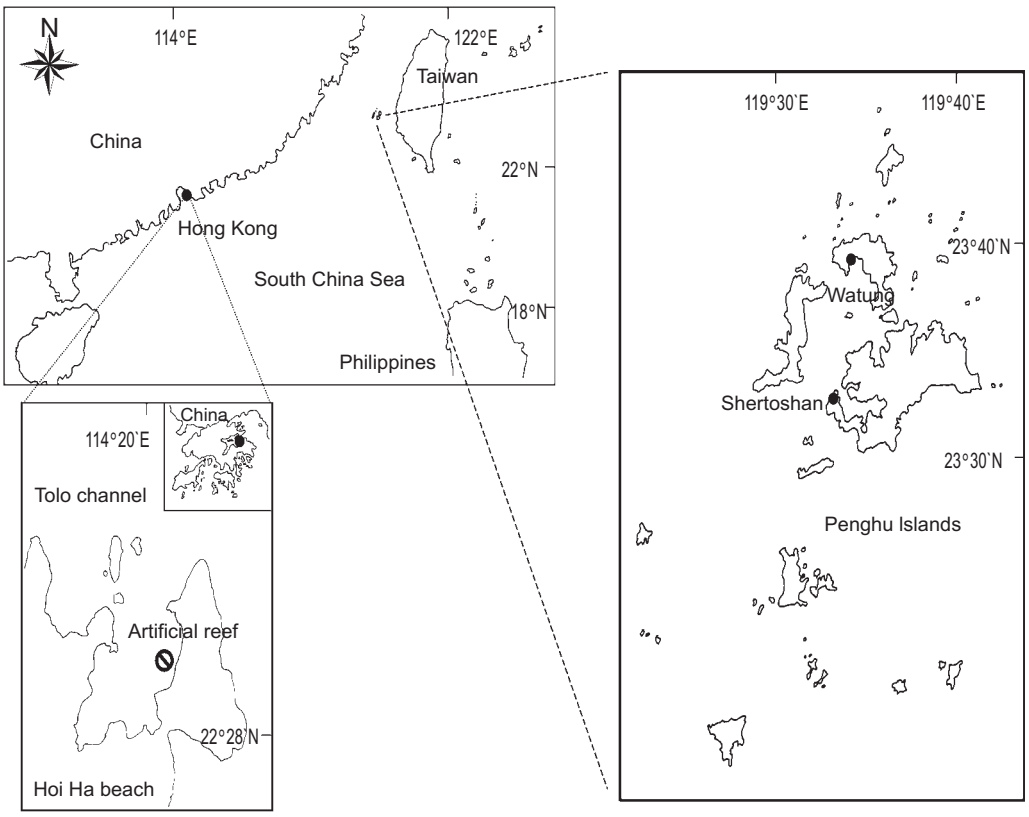

Fig. 1. Map of the Penghu Islands and Hong Kong showing the localities where *Oulastrea crispata* was sampled.

Taiwan Strait during winter. In addition, a weak side-branch of the Kuroshio Current flows in the Taiwan Strait up to the southern Penghu Is. in winter (for review, see Chen 1999). These complex ocean currents cause enormous fluctuations in environmental conditions at both sites (Fig. 2), e.g., minimum winter water temperatures at both sites (Chen 1999, Lam 2000a) are usually below the minimum temperature (18°C) for coral survival (reviewed in Veron 1995). Analyses of scleractinian distribution in Taiwan (Chen 1999) and species composition in Hong Kong (D. McCorry, pers. comm.) suggest that both sites can be categorized as subtropical non-reefal communities, providing ideal localities to examine the correlation between the existence of *Symbiodinium* clade D and extreme environmental conditions. In the Penghu Is., we sampled *O. crispata* from Watung and Shertoshan (Fig. 1). These 2 sites are shallow reef depressions where *O. crispata* occurs commonly near the low tide mark; colonies might even be exposed to the air during spring low tides. In Hong Kong, corals were collected from an artificial reef deployed within Hoi Ha Wan (Fig. 1, for a detailed description, see Lam 2000a), where the seabed is still covered by at least 0.5 m of loose mud on top of a hard substratum, and where there previously had been a coral community (Lam 2000a b).

Sampling strategies and the recording of water temperatures

In order to investigate temporal and spatial variations in zooxanthellae within *Oulastrea crispata*, we used 2 sampling strategies. For temporal variation, 20 colonies of *Oulastrea crispata* were sampled haphazardly from Watung, at 2-mon intervals between Mar. 2001 and Mar. 2002. A data log was deposited at the reef flat near the low tide mark to record *in situ* temperatures during the sampling period (Fig. 2). The data logger was set to collect temperature at 15-min intervals, and was retrieved for battery recharging at 90-d intervals. The temperature logger indicated that water temperatures in the area where *O. crispata* inhabited showed enormous fluctuations, ranging from 12°C in winter to 35°C in summer (Fig. 2). For spatial variation, an additional 20 colonies were sampled from Hoi Ha Wan, Hong Kong in May 2001, and Shertoshan in Nov. 2001, respectively.

Identification of zooxanthellae

Zooxanthellae were isolated and identified as described in Rowan and Powers (1991b) and

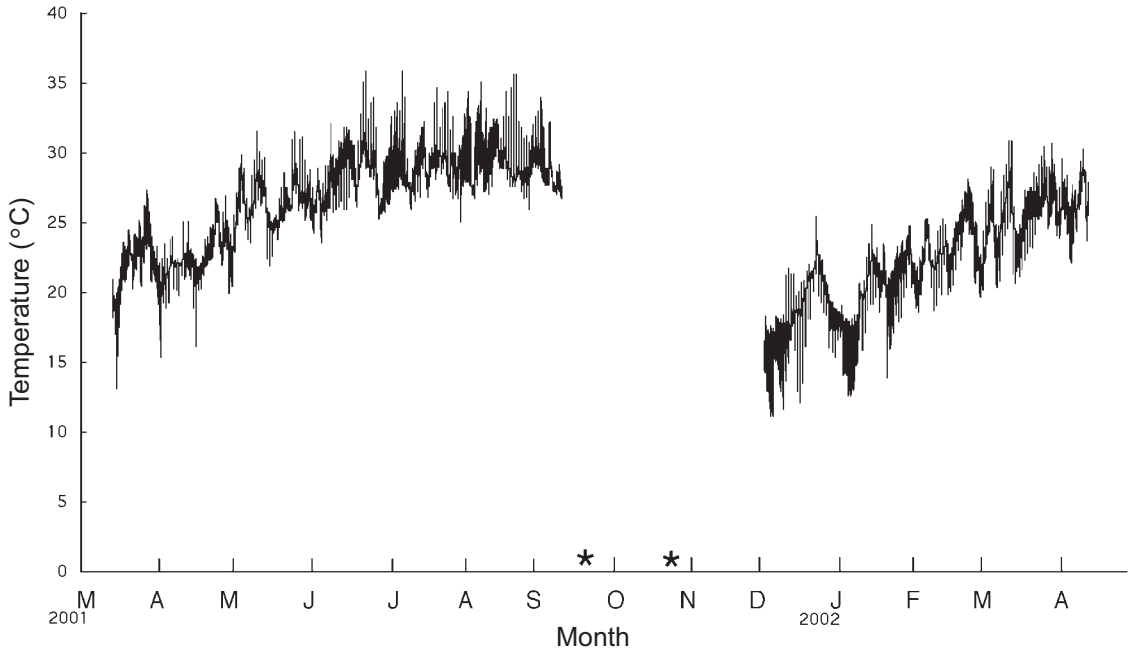

Fig. 2. In situ seawater temperatures recorded from March 2001 to April 2002 at Watung, the Penghu Islands. Sea water temperatures were not available between September 2001 and November 2001 due to malfunctioning of the data logger.

Rowan and Knowlton (1995), and DNA extraction followed the protocol described in Chen and Yu (2000) and Chen et al. (2000). Two sets of genetic markers, nssrDNA and nlsrDNA, were applied to assay zooxanthellae diversity. First, at the initial stage of this study, we used nssrDNA to visualize the classic genotypes defined by Rowan and Powers (1991a), Rowan and Knowlton (1995), and Toller et al. (2001a). nssrDNA was obtained by PCR amplification with a host-excluding primer pair (ss5z and ss3z), and then characterized by the restriction enzymes, *Sau3A* I and *Taq* I, which differentiate *Symbiodinium* clades A, B, C, and D/E (Rowan and Powers 1991a, Toller et al. 2001a). The *Symbiodinium* C nssrDNA gene and nlsrDNA gene from *Acropora palifera* (Chen et al. 2003) were amplified and digested with the same restriction enzymes to compare the RFLP patterns from *O. crispata*. Second, for the large-scale surveys of temporal and spatial samples, we amplified the 5'-end of nlsrDNA using the host-excluding primer pair, 5S: 5'-GCCGACCCGCTGAATTCAAGCATAT-3' and D23zoox: 5'-TGTGGCAYGTGACGCG-CAAGCTAAG-3', and then characterized it using the restriction enzyme *Rsa* I. All enzymes were purchased from MBI (Fermantas, Italy). PCR fragments of nssrDNA and nlsrDNA gene products were then cloned and sequenced as described in Chen et al. (2000). DNA sequences obtained from this study were deposited in GenBank with accession numbers AY051096 and AY051097 (nssrDNA) and AY139196 and AY139223 (nlsrDNA).

Phylogenetic analysis

For phylogenetic analysis, DNA sequences were initially aligned using CLUSTAL W 1.7 (Thompson et al. 1994), followed by manual editing using SeqApp 1.9 (Gilbert 1994), and using the Neighbor-joining algorithm (Saitou and Nei 1997) by PAUP 4.10Beta (Swofford 2002). We used only partial nssrDNA sequences (V2 and V4 domains) following the instructions of Rowan and Power (1991b), Rowan and Knowlton (1995), and Toller et al. (2001a), and the 5'-end of nlsrDNA sequences for phylogenetic reconstructions. Alignments of the DNA sequences are available from the senior author upon request. One thousand bootstrap replicates were performed to estimate the statistical support for each major clade. The nssrDNA and nlsrDNA sequences of published *Symbiodinium* clades A, B, C, and D were retrieved from GenBank for phylogenetic comparisons.

RESULTS

Of the 180 zooxanthellae extracts, PCR amplification of nssrDNA and nlsrDNA from symbionts from Hong Kong and Penghu Is. produced single amplicons of approximately 1600 bp for nssrDNA and 500 bp for nlsrDNA, respectively (data not shown). Restriction digestion of

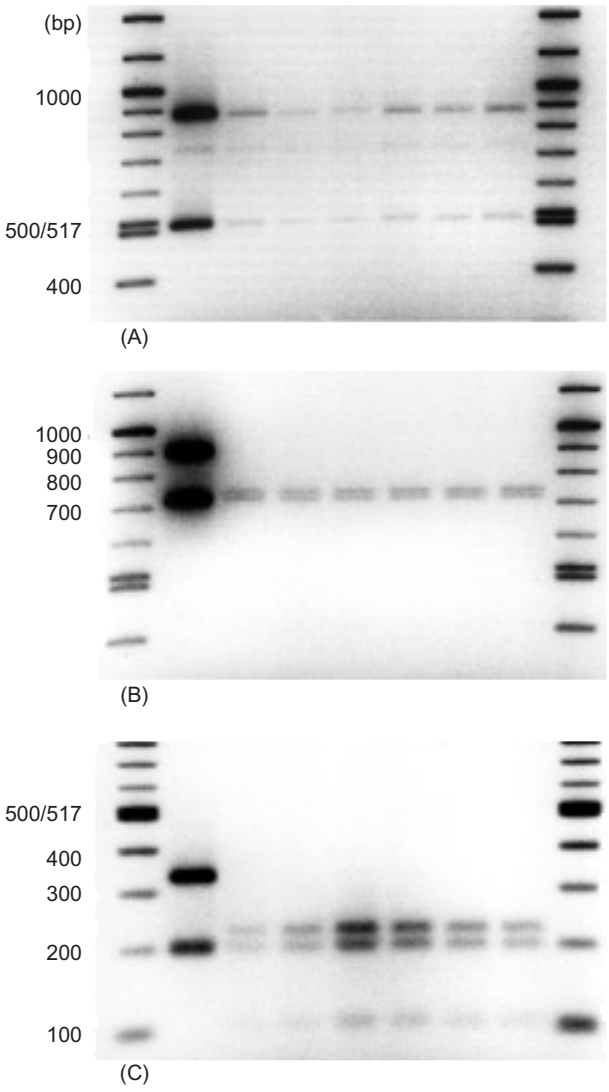

Fig. 3. RFLP genotypes of *Symbiodinium* clade D obtained from different colonies of *Oulastrea crispata* collected from Ho Ha Wan, Hong Kong (lanes 3-5) and Watung, the Penghu Islands (lanes 6-8). nssrDNA was amplified with host-excluding PCR primers and digested with *Sau3A* I (A) and with *Taq* I (B); nlsrDNA was amplified using primers designed in the present study and digested with *Rsa* I (C). Lane 2 was the RFLP genotype of *Symbiodinium* clade C obtained from *Acropora palifera*, and was used for comparisons. Lanes 1 and 9 contain DNA fragment size standards of a 100-bp DNA ladder.

nssrDNA by *Sau3A* I produced an RFLP profile consisting of 3 fragments of 900, 517, and 180 bp (Fig. 3a). In addition, *Taq* I produced an RFLP pattern with 2 fragments of 710 bp and one of 250 bp (Fig. 3b). Fragments smaller than 200 bp in length were not observed due to limitations of the agarose gels. nlsrDNA digested by *Rsa* I produced an RFLP profile consisting of 3 fragments of 220, 200, and 100 bp (Fig. 3c). These 3 digestion patterns correspond to the previously unassigned *Symbiodinium* clade D of Rowan and Power (1991a) and Baker (2001) or *Symbiodinium* E of Toller et al. (2001a) using these 2 rDNA fragment RFLPs. A Neighbor-joining analysis of 449 bp aligning the V2 and V4 regions of nssrDNA sequences placed zooxanthellae of *O. crispata* within the branch consisting of *Symbiodinium* clade D from the corals, *Montastrea faveolata*, *M. franksi*, and *Siderastrea siderea* (Toller et al. 2001a), with high bootstrap support (Fig. 4). As indicated by Toller et al. (2001a) that a dinoflagellate cultured from the sponge *Haliclona koremella*, formally assigned as *Symbiodinium* clade D (Carlos et al. 1999), was not similar to *Symbiodinium* clade D, we therefore concluded that zooxanthellae isolated from *O. crispata* belonged to *Symbiodinium* clade D. No other clades of zooxanthellae were detected throughout this survey.

In order to examine the spatial and temporal variations in zooxanthellae in *Oulastrea crispata*, we examined sequence divergence of the 5'-end of nlsrDNA. Divergence rates of the 5'-end of nlsrDNA appeared to be much faster than those of the V1-V4 regions of nssrDNA, and are thought to be suitable for exploring intracladal *Symbiodinium* phylogenetics (Loh et al. 2001, Santos et al. 2002). From the 65 taxa, 455 unambiguously aligned nucleotide sites were used in the phylogenetic analysis. A Neighbor-joining analysis showed that the zooxanthellae isolates of *O. crispata* collected from different localities (Hong Kong, Shertoshan, and Watung) or different periods of the year all

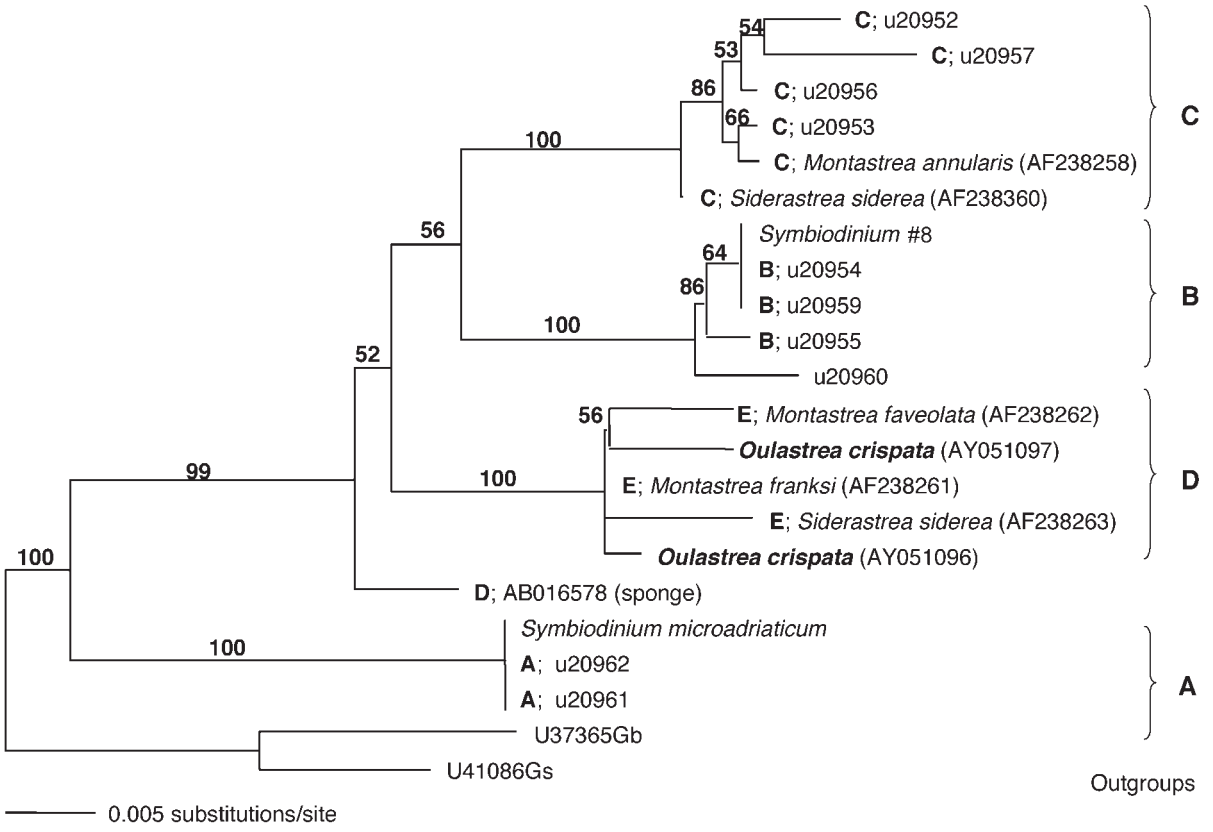

Fig. 4. Phylogenetic identification of zooxanthellae isolated from *Oulastrea crispata* inferred from partial nssrDNA sequences based on the method of Neighbor-joining (Saitou and Nei 1987). Sequences of *Symbiodinium microadriaticum*, *Symbiodinium* #8, and clades A, B, C, D, and D/E, were retrieved from GenBank following the accession numbers listed in Toller et al. (2001a). The dinoflagellates, *Gymnodinium beii* (Gb) and *G. simplex* (Gs), were used as outgroups for phylogenetic reconstruction. The numbers above each branch indicate the bootstrap values for 1000 replicates.

clustered with *Symbiodinium* clade D2 or E reported in GenBank (Fig. 3). No apparent differences were detected at either the spatial or temporal scales of *Symbiodinium* clade D divergence in *O. crispata*.

DISCUSSION

Stable association of *Oulastrea crispata* with *Symbiodinium* clade D*crispate* (Stable association of *Symbiodinium* clade D with *Oulastrea crispata*)

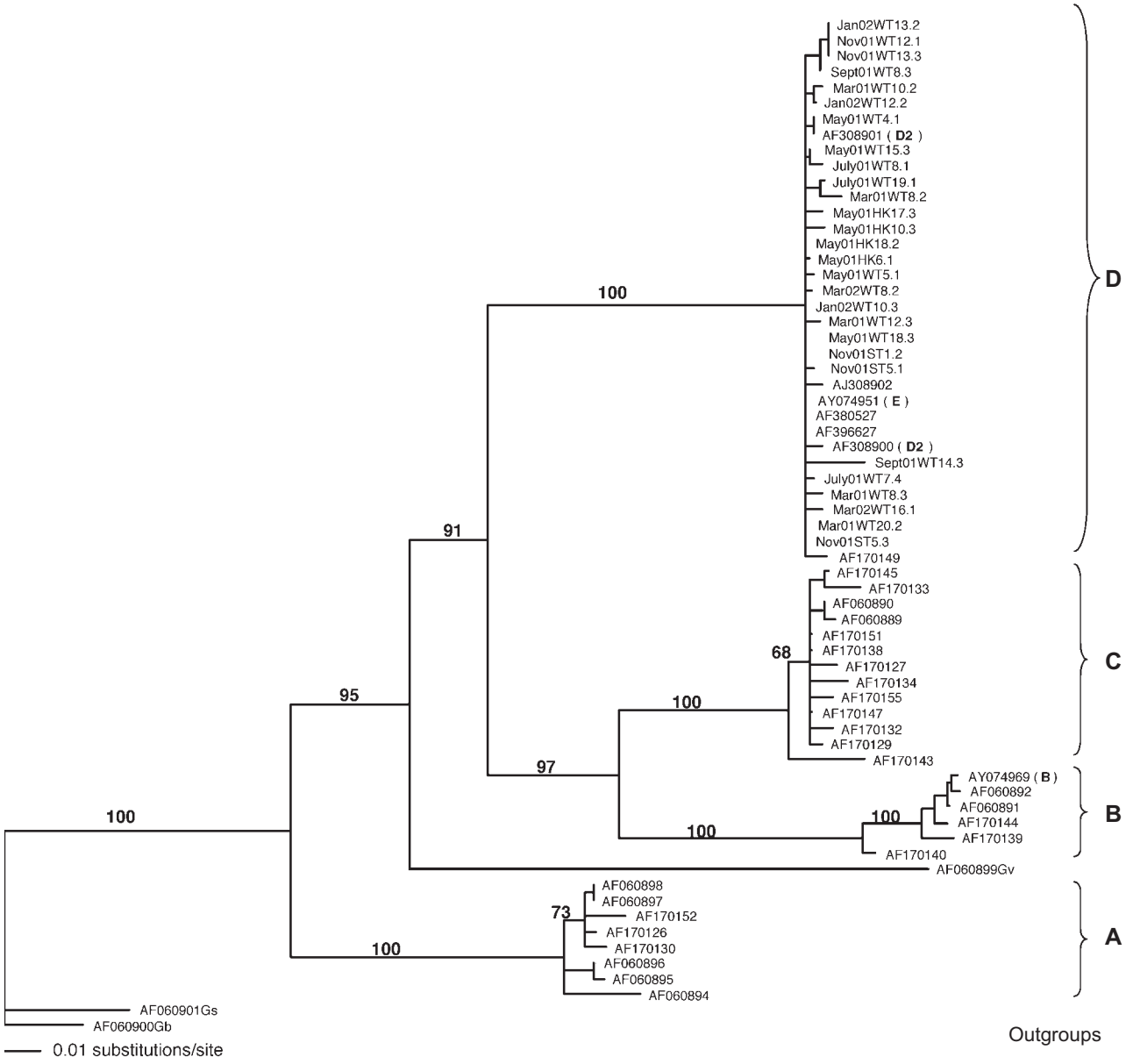

Fig. 5. Phylogenetic analysis of different spatial and temporal zooxanthellae isolates in *Oulastrea crispata* collected from Ho Ha Wan, Hong Kong (HK), and from Shertoshan (ST) and Watung (WT), the Penghu Islands. Phylogenetic reconstruction was inferred from partial nlsrDNA sequences based on the method of Neighbor-joining (Saitou and Nei 1987). Partial nlsrDNA sequences of clades A, B, C, and D, and a free-living dinoflagellate, *Gynodinium varian* (Gv), were retrieved from GenBank using their accession numbers. The dinoflagellates, *Gymnodinium beii* (Gb) and *G. simplex* (Gs), were used as outgroups for phylogenetic reconstruction. The numbers above each branch indicate the bootstrap values for 1000 replicates. Abbreviations of sequences are composed of the month, year, locality, and sample number. For example, "Jan02WT 13.2" represents the sequence obtained from sample 13 collected at Watung in January 2002.

The stable association with *Symbiodinium* D found in both Hong Kong and Penghu, *O. crispata* is unique among the Indo-Pacific scleractinian corals. Most of the Indo-Pacific and Caribbean scleractinian corals are associated with a single clade, *Symbiodinium* clade C (Darius et al. 2000, Chen et al. 2004). Some Indo-Pacific corals have indications of an association with *Symbiodinium* clade D, but these results were based on scattered geographic samplings and small numbers of observations (discussed in Toller et al. 2001a). Our survey of 52 scleractinian corals in southern Taiwan and the Penghu Is. identified 7 other coral species simultaneously associated with *Symbiodinium* clades C and D (Chen et al. 2004). Based on the ecological characteristics of *O. crispata*, we hypothesize that *O. crispata* collected from either high-latitude or tropical regions should also be associated with *Symbiodinium* clade D. Alternatively, *O. crispata* may show persistent polymorphic symbiosis of zooxanthellae as seen in *Seriatopra hystrix*, *Acropora longicythus*, and *Plesiastrea versipora*, collected from different geographic localities in the Indo-Pacific region (Loh et al. 2001, Rodriguez-Lanetty et al. 2001). Symbionts from Australian and Japanese *Ser. hystrix* were identified as *Symbiodinium* clade C, and Malaysian *Ser. hystrix* symbionts were *Symbiodinium* clade D (Loh et al. 2001). For *A. longicyathus*, 7 to 11 Australian and all Japanese and Malaysian colonies were associated with *Symbiodinium* C, but symbionts from the remaining Australian *A. longicyanthus* were *Symbiodinum* clade A (Loh et al. 2001). In *Plesiastrea versipora*, colonies collected from tropical and subtropical Australia contained symbionts belonging to *Symbiodinium* clade C, while *P. versipora* colonies at high-latitude sites contained *Symbiodinium* B (Rodriguez-Lanetty et al. 2001).

These studies suggest that geographically distinct varieties of symbionts within tissues of scleractinian corals are likely to be associated with algal physiological differences or correlated with differences in the dispersal range of the coral or symbiont propagules (Loh et al. 2001, Rodriguez-Lanetty et al. 2001). Further sampling of *O. crispata* from high-latitudinal Japan and tropical localities (Vietnam, the Spratly Is., and Pratas I.) are currently underway in order to examine the latitudinal diversity of symbiont associations in *O. crispata*.

Symbiodinium clade D in *Oulastrea crispata*: Stress tolerance or fidelity

Although speculative, our survey on zooxanthellae diversity within *O. crispata* tends to agree with the hypothesis that *Symbiodinium* clade D is more stress tolerant than other *Symbiodinium* clades. Previous studies of zooxanthellae diversity in scleractinian corals have revealed at least 4 clades of *Symbiodinium*, i.e., A, B, C, and D, which are associated with coral (reviewed in Rowan 1998, Baker 2001, Toller et al. 2001a b). In most cases, 1 species of coral hosts 1 species of zooxanthellae (Diekmann et al. 2002). However, studies on the Caribbean corals, the *Montastrea annularis* species complex, including *M. annularis*, *M. faveolata*, and *M. franksi*, have demonstrated a remarkable amount of diversity of symbiosis-host relationships (Rowan and Knowlton 1995, Rowan et al. 1997, Toller et al. 2001a). These results suggest that the diversity of coral and symbiont associations is not randomly distributed, and should be a complex assemblage of ecologically specialized units that are correlated to irradiance of the habitat (Rowan and Knowlton 1995, Rowan et al. 1997, Rowan 1998, Toller et al. 2001a). For example, on the offshore reef at Río Cartí of San Blas I., Panama, *M. annularis* and *M. faveolata* are associated with *Symbiodinium* clades A, B, and C at different depths or irradiances; *Symbiodinium* A or B is predominant at higher irradiance levels, but *M. franksi* hosts only C throughout nearly all of its depth range (Rowan and Knowlton 1995, Rowan et al. 1997). A subsequent survey at a coastal site at Río Cartí indicated that the *M. annularis* species complex predominantly hosted *Symbiodinium* clade D at locations with higher irradiance levels, instead of *Symbiodinium* A or B, as found at the offshore reef. In contrast, *Symbiodinium* clade D was distributed in very deep colonies of *M. franksi* at an offshore reef, at Cayos Limones. Apart from the high irradiance, other environmental stresses, such as fluctuations in temperature, salinity, nutrients, and sediments can affect the stability of coral-algal symbioses (Falkowski et al. 1993, Brown 1997, Wesseling et al. 1999). One possible scenario proposed to explain this different distribution pattern is that both sites are marginal habitats (Toller et al. 2001a). The coastal site at Río Cartí is near a large river where coral reefs are poorly developed or absent, and at the deep bottom of Cayos Limones, *M. franksi* colonies are not large and the reef itself disappears into the sediment. It has also been suggested that the *Symbiodinium* clade D represents a taxon of zooxanthellae 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. In other words, *Symbiodinium* clade D is rare or absent from other habitats not because it performs poorly in them, but because *Symbiodinium* clade A, B, and C are better adapted to those habitats and somehow exclude D (Toller et al. 2001a).

In contrast to the stress-tolerant hypothesis, we hypothesize that *Symbiodinium* clade D might represent a taxon of zooxanthellae competing for *O. crispata* (i.e., performing "best") in marginal habitats. First, *O. crispata* has a broad geographic distribution ranging from the tropical Indo-West-Pacific to high latitudes in Japan (Veron 1993), suggesting that *O. crispata* can cope with a wide range of ecological variations on a large geographic scale. Second, on a local scale, unlike most Indo-Pacific scleractinian corals that have restrictive distributions in clear and low-turbidity water, *O. crispata* can only be found in some extreme environments. *Oulastrea crispata* occurs commonly on shallow reef depressions and on turbid bay bedrocks inhabited by only a few other corals (this study, Nakano and Yamazato 1992). A temperature logger deposited in Wantung throughout the present study indicated that water temperatures in the area where *O. crispata* inhabited fluctuated enormously, ranging from 12°C in winter to 35°C in summer (Fig. 2). In addition, *O. crispata* is also a pioneer coral colonizing artificial substrates where environmental disturbance is high (Lam 2000b). In high latitudes of Japan, *O. crispata* can be found in habitats where winter temperatures are usually 7 to 10°C and air temperatures are several degrees below freezing for about 20 d each year (Yajima et al. 1986). These ecological characteristics suggest that association with a stress-tolerant symbiont, such as *Symbiodinium* clade D, may enhance the ability of *O. crispata* to survive in these harsh environments. Third, an association with *Symbiodinium* C could be found in other coral species at Watung (Chen et al. 2004), indicating that the availability of other clades of zooxanthellae is not a limiting factor for *O. crispata* forming symbiotic relationships with non-*Symbiodinium* clade D zooxanthellae. There is a possibility of host fidelity between *Symbiodinium* clade D and *O. crispata*. Further experiments on the physiology of both *O. crispata* and *Symbiodinium* clade D should be conducted to confirm these scenarios.

In conclusion, RFLP and phylogenetic analyses of nssrDNA and nlsrDNA genes demonstrated that the zooxanthellae associated with the zebra-coral, *Oulastrea crispata*, in Hong Kong and the Penghu Islands, Taiwan, belong to *Symbiodinium* clade D, a clade proposed to be tolerant of stress. Analyses of zooxanthellae diversity showed no apparent variation in *Symbiodinium* clade D on either a spatial or temporal scale, suggesting that the association with *Symbiodinium* clade D is stable in *O. crispata*. Possessing opportunistic life history traits, including a variety of reproductive strategies and physiological tolerances, provides *O. crispata* with the capability to colonize a variety of substrata unfavorable to other corals. In addition, the nature of hosting a stress-tolerant symbiont may also play a key role in *O. crispata*'s ability to achieve such physiological adaptability.

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REFERENCES

Baker AC. 2001. Reef corals bleach to survive change. Nature **411:** 765-766.

Baker AC, R Rowan. 1997. Diversity of symbiotic dinoflagellates (zooxanthellae) in scleractinian corals of the Caribbean and eastern Pacific. Proc. 8th Int. Coral Reef Symp. **2:** 1301-1306.

Baker AC, R Rowan, N Knowlton. 1997. Symbiosis ecology of two Caribbean acroporid corals. Proc. 8th Int. Coral Reef Symp. **2:** 1295-1300.

Ballie BK, CA Belda-Ballie, Y Maruyama. 2000. Conspecifity and Indo-Pacific distribution of *Symbiodinium* genotypes (Dinophyceae) from giant clams. J. Phycol. **36:** 1153-1161.

Barnes DJ, BE Chalker. 1990. Calcification and photosynthesis in reef-building corals and algae. *In* Z Dubinsky, ed. Ecosystems of the world. Vol. 25. Coral reefs. New York: Elsevier, pp. 109-131.

Brown BE. 1997. Coral bleaching: causes and consequences. Coral Reefs **16(Supplement):** S129-S138.

Brown BE, RP Dunne, MS Goodson, AE Douglas. 2002. Experience shapes the susceptibility of a reef coral to bleaching. Coral Reefs **21:** 119-126.

Burnett WJ. 2002. Longitudinal variation in algal symbionts (zooxanthellae) from the Indian Ocean zoanthid *Palythoa caesia*. Mar. Ecol. Prog. Ser. **234:** 105-109.

Carlos AA, BK Baillie, M Kawachi, T Maruyama. 1999. Phylogenetic position of *Symbiodinium* (Dinophyceae) isolates from tridacnids (Bivalvia), cardiids (Bivalvia), a

sponge (Porifera), a soft coral (Anthozoa), and a free-living strain. J. Phycol. **35:** 1054-1062.

Chen CA. 1999. Analysis of scleractinian distribution in Taiwan indicating a pattern congruent with sea surface temperature and currents: examples from *Acropora* and Faviidae. Zool. Stud. **38:** 119-129.

Chen CA, YW Yang, NV Wei, WS Tsai, LS Fang. 2004. Symbiont diversity in scleractinian corals from tropical reefs and subtropical non-reef communities in Taiwan. Coral Reefs (in press)

Chen CA, JK Yu. 2000. Universal primers for amplification of mitochondrial small subunit ribosomal RNA-encoding gene in scleractinian corals. Mar. Biotechnol. **2:** 146-153.

Chen CA, JK Yu, NV Wei. 2000. PCR strategies for amplification of the complete sequence of nuclear large subunit ribosomal RNA-encoding gene in corals. Mar. Biotechnol. **6:** 558-570.

Darius HT, C Dauga, PAD Grimont, E Chungue, PMV Martin. 2000. Diversity in symbiotic dinoflagellates (Pyrrhophyta) from seven scleractinian corals: restriction enzyme analysis of small subunit ribosomal RNA genes. J. Eukaryot. Microbiol. **45:** 619-627.

Davies PS. 1993. Endosymbiosis in marine cnidarians. *In* DM John, SJ Hawkins, JH Price, eds. Plant-animal interactions in the marine benthos. Oxford, UK: Clarendon Press, pp. 551-540.

Douglas AE. 2003. Coral bleaching--how and why? Mar. Pollut. Bull. **46:** 385-392.

Falkowski PG, Z Dubinsky, L Muscatine, JW Porter. 1984. Light and bioenergetics of a symbiotic coral. Bioscience **34:** 705-709.

Gilbert DC. 1994. SeqApp 1.9. A biological sequence editor and analysis program for Macintosh computers. Available from ftp.indiana.edu.

Hunter CL, CW Morden, CM Smith. 1997. The utility of ITS sequences in assessing relationships among zooxanthellae and corals. Proc. 8th Int. Coral Reef Symp. **2:** 1599-1602.

LaJeunesse TC. 2001. Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the internal transcribed spacer region: in search of a "species" level marker. J. Phycol. **37:** 866-880.

Lam KK. 2000a. Sexual reproduction of a low temperature tolerant coral, *Oulastrea crispata*, in Hong Kong, China. Mar. Ecol. Prog. Ser. **205:** 101-111.

Lam KK. 2000b. Early growth of a pioneer recruited coral *Oulastrea crispata* (Scleractinia, Faviidae) on PFA-concrete blocks in a marine park in Hong Kong, China. Mar. Ecol. Prog. Ser. **205:** 113-121.

Loh WKW, T Loi, D Carter, O Hoegh-Guldberg. 2001. Genetic variability of the symbiotic dinoflagellates from the wide ranging coral species, *Seriatopora hystrix* and *Acropora longicyathus*, in the Indo-West Pacific. Mar. Ecol. Prog. Ser. **222:** 97-107.

Muller-Parker G, C D'Elia. 1997. Interactions between corals and their symbiotic algae. *In* C Birkeland, ed. Life and death of coral reefs. New York: Chapman and Hall, pp. 96-113.

Muscatine L, JWP Poter. 1977. Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience **27:** 454-460

Nakano Y, K Yamazato. 1992. Ecological study of reproduction of *Oulastrea crispata* in Okinawa. Zool. Sci. **9:** 1292.

Rodriguez-Lanetty M, W Loh, D Carter, O Hoegh-Guldberg. 2001. Latitudinal variability in symbiont specificity within the widespread scleractinian coral *Plesiastrea versipora*. Mar. Biol. **138:** 1175-1181.

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

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

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

Rowan R, DA Powers. 1991a. Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). Mar. Ecol. Prog. Ser. **71:** 65-73.

Rowan R, DA Powers. 1991b. A molecular genetic classification of zooxanthellae and the evolution of animal-alga symbioses. Science **251:** 1348-1351.

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

Santos SR, DJ Taylor, RA III Kinizie, M Hidaka, K Sakai, MA Coffroth. 2002. Molecular phylogeny of symbiotic dinoflagellates inferred from partial chloroplast large subunit (23S)-rDNA sequences. Mol. Phylogenet. Evol. **23:** 97-111.

Swofford DL. 2002. PAUP 4.10b: phylogenetic analysis using parsimony (and other methods). Sunderland, MA: Sinauer Associates.

Taylor DL. 1974. Symbiotic marine algae: taxonomy and biological fitness. *In* WB Vernberg, ed. Symbiosis in the sea. Columbia, SC: Univ. of South Carolina Press, pp. 245-262.

Thompson JD, DG Higgins, TJ Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl. Acids Res. **22:** 4673-4680.

Toller WW, R Rowan, N Knowlton. 2001a. Zooxanthellae of the *Montastraea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* of different reefs and across depths. Biol. Bull. **201:** 348-359.

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

Trench R. 1997. Diversity of symbiotic dinoflagellates and the evolution of microalgal-invertebrate symbioses. Proc. 8th Int. Coral Reef Symp. **2:** 1275-1286.

Van Oppen MJH, FP Palstra, AMT Piqet, DJ Miller. 2001. Patterns of coral-dinoflagellate associations in *Acropora*: significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. Proc. Roy. Soc. Lond. B Bio. **268:** 1579-1767.

Veron JEN. 1995. Corals in space and time: the biogeography and evolution of the Scleractinia. Sydney, Australia: Univ. of New South Wales Press.

Veron JEN. 1993. Coral of Australia and the Indo-Pacific. Honolulu: Univ. of Hawaii Press.

Wesseling IA, J Uychiaoco, PM Alino, T Aurin, JE Vermaat. 1999. Damage and recovery of four Philippine corals from short-term sediment burial. Mar. Ecol. Prog. Ser. **176:** 11-15.

Wilcox TP. 1998. Large-subunit ribosomal RNA systematics of symbiotic dinoflagellates: morphology does not recapitulate phylogeny. Mol. Phylogenet. Evol. **10:** 436-448.

Yajima T, S Osamie, T Okamoto, S Yoshohiro, S Thutomu, M Masahiro. 1986. Ecological distribution of the reef coral, *Oulastrea crsipata* (Lamarck) at the shore region in the vicinity of Tsukumo Bay. Bull. Jpn. Sea Res Inst. Kanazawa Univ. **18:** 21-36.

Yu JK, TH Liao, LS Fang, WS Tsai, CA Chen. 2000. Do color patterns of *Pocillopora damicornis* correlate with zooxanthellae diversity? Coral Reefs **19:** 98-99.