# Fluctuating algal symbiont communities in *Acropora palifera* (Scleractinia: Acroporidae) from Taiwan

## Chaolun Allen Chen[1,2,*], Jih-Terng Wang[3], Lee-Shing Fang[4], Ya-Wen Yang[1]

[1]Research Center for Biodiversity, Academia Sinica, Nankang, Taipei 115, Taiwan, ROC
[2]Institute of Oceanography, National Taiwan University, Taipei 106, Taiwan, ROC
[3]Department of Food Science and Technology, Tajen Institute of Technology, Yanpu, Pingtung 907, Taiwan, ROC
[4]National Museum of Marine Biology and Aquarium, Hengchun, Pingtung 946, Taiwan, ROC

ABSTRACT: Seasonal dynamics of algal symbiont communities from the reef flat-dwelling coral *Acropora palifera* was monitored from January 2000 to July 2001 at Tantzei Bay, Kenting National Park, southern Taiwan. Zooxanthellae density and pigment contents monitored throughout the 18 mo sampling period indicated that no significant bleaching occurred in *A. palifera*. Molecular phylotyping showed that 2 distinct symbionts, Phylotypes C and D, were associated with *A. palifera*, either individually or simultaneously. Population surveys throughout the bimonthly sampling period demonstrated that the symbiont community within *A. palifera* was highly dynamic with significant fluctuations; a drop in the Phylotype D percentage occurred in May, which was correlated with a rise in seawater temperature in the late spring/early summer at the sampled reef. Direct tracking of tagged corals provided evidence that seasonal fluctuations in the algal symbiont communities not only involve changes in zooxanthellae densities and pigment contents, but also a reshuffling of different *Symbiodinium* phylotypes. Our study highlights that stress tolerance among phylotypes urgently needs to be re-evaluated through a better understanding of the physiological traits of symbionts.

KEY WORDS: Symbiont community · Phylotype · Stress tolerance · nlsrDNA RFLPs · Symbiont reshuffling

## INTRODUCTION

Coral bleaching, described as the loss of symbiotic dinoflagellates (also known as zooxanthellae) and algal pigments, or both, has been a major global concern for the health of reef corals (Brown 1997a,b, Hoegh-Guldberg 1999, Fitt et al. 2001, Glynn et al. 2001, Coles & Brown 2003, Douglas 2003, Hughes et al. 2003). Bleaching has long been regarded to be deleterious in corals; it can lead to reduced fecundity and growth rates, impaired healing after mechanical damage, increased vulnerability to disease infection, and worst of all, mass mortality (reviewed in Brown 1997a,b, Douglas 2003). The mass coral bleaching event of 1998 is considered the most severe on record, and was pri-

marily attributed to abnormally high seawater temperatures (reviewed in Hoegh-Guldberg 1999, Hughes et al. 2003). In addition, solar radiation stress also plays an important role in triggering coral bleaching (Brown 1997a,b, Coles & Brown 2003).

The loss of symbiotic dinoflagellates involves changes in the zooxanthellae density in the tissues of reef corals. It has been shown that the density of zooxanthellae associated with coral tissues is not necessarily constant throughout the year (Stimson 1997, Brown et al. 1999, Fagoonee et al. 1999, Fitt et al. 2000, Warner et al. 2002). A study of *Pocillopora damicornis* in Hawaii indicated that symbiotic algal density responded to seasonal differences in environmental parameters with a positive correlation between the

concentration of dissolved $NO_3$ and algal density, and a negative correlation between algal density and solar radiation (Stimson 1997). A 5 yr monitoring study of symbiotic algal densities in the coral *Acropora formosa* in Mauritius showed a correlation between density and nitrate concentrations (Fagoonee et al. 1999). Fitt et al. (2000), who studied 5 species of reef-building corals at 3 different depths in the Bahamas for 4 yr, indicated that annual fluctuations occurred in tissue mass and symbiotic algal densities. These results show that corals from all depths exhibit bleaching every year, regardless of the color changes visible to human eyes. It was concluded and hypothesized that these patterns are driven by the effects of seasonal changes in light and temperature on algal and animal physiology, and should be applicable to all tropical reef-building corals worldwide (Fitt et al. 2000).

Despite the seasonal dynamics of symbiotic algal densities and their correlations with environmental parameters being well documented in reef-building corals, such changes involving fluctuating algal symbiont diversities within a single colony or population of reef corals are not yet well understood. Symbiotic algae are mainly of the genus *Symbiodinium.* It is now known that *Sybmiodinium* is highly diverse and is currently recognized, from molecular evidence, as consisting of 7 major phylogenetic clades (phylotypes) (A to G, reviewed in Baker 2003a,b). Among them, Phylotypes A, B, C, D, and F are known to be associated with scleractinian corals (Rowan & Powers 1991, Rowan & Knowlton 1995, LaJeunesse 2001, Loh et al. 2001, Santos et al. 2001, Toller et al. 2001a, Baker 2003a,b, Chen et al. 2003, 2004). Recent studies have demonstrated not only the occurrence of multiple phylotypes within coral hosts, but also diverse ecological factors driving the ecological zonation of different phylotypes among coral colonies and even over the surface of individual coral colonies (Rowan & Knowlton 1995, Rowan et al. 1997, Baker 2001, Toller et al. 2001a,b, Chen et al. 2003, 2004). *Symbiodinium* diversity is thus thought to be a mechanism by which corals adapt to changing environmental conditions (Buddemeier & Fautin 1993, Rowan et al. 1997, Baker 2001, 2003a,b).

In this study, the seasonal dynamics of *Symbiodinium* phylotypes were investigated over an 18 mo period in *Acropora palifera* on a reef in Kenting National Park, southern Taiwan. *A. palifera* occurs on a reef flat where disturbances (e.g. wave action, temperature fluctuations, etc.) are high. Our previous study showed that *A. palifera* is associated with 2 distinct *Symbiodinium* phylotypes, C and D, either individually or simultaneously (Chen et al. 2004). Population-wide surveys during bimonthly samplings and the direct tracking of symbionts within individual coral colonies demonstrated that the symbiont community within *A. palifera* is highly dynamic, not only involving changes in algal densities but also changes in the compositions of *Symbiodinium* Phylotypes C and D.

## MATERIALS AND METHODS

**Study site and collection of corals.** Colonies of *Acropora palifera* were sampled bimonthly from a reef flat (~1 to 2 m in depth) at Tantzei Bay (121° 28′ E, 22° 35′ N), Kenting National Park, southern Taiwan, from January 2000 to July 2001. *A. palifera* in this area grows into encrusting plate forms, probably due to the high frequency of disturbances, including strong waves, turbidity, and exposure to the air during spring low tides. Seawater temperatures were converted from data logs deposited by Dr. T.-Y. Fan, National Museum of Marine Biology and Aquarium at the Tiaoshi reef, 2 km north of our sampling site. Temperature was recorded at 60 min intervals. Our previous survey indicated that *A. palifera* in Tantzei Bay is associated with 2 diverse phylotypes of *Symbiodinium*, either individually or simultaneously (Chen et al. 2005). It was documented that spatial variations may occur within an individual colony when corals host diverse phylotypes (Rowan et al. 1997). In order to examine the spatial variability in *Symbiodinium* phylotypes within individual colonies of *A. palifera*, 5 colonies were collected in January 2000, and different parts (margin and center) of each colony were subsampled for molecular phylotyping. For the population survey, >25 colonies were randomly sampled by scuba diving along a 50 m transect line laid on the reef flat. In addition, 20 colonies were tagged in March 2000 to ensure subsequent collection from the same colony. A yellow plastic tag was nailed close to each coral colony. This yellow tag helped us to retrieve the same colony by snorkeling. However, 12 tags were lost due to several typhoons which hit the Kenting National Park area during the sampling period. A piece of coral skeleton (about 5 cm in diameter) was chiseled off from each colony, placed in individual pre-labeled plastic bags, and immediately transported to the laboratory in an insulated cooler.

**Monitoring of coral bleaching and determination of densities and chlorophyll species of zooxanthellae.** In order to monitor bleaching, seasonal changes in zooxanthellae density and chlorophyll species were examined. Freshly collected pieces of coral were airbrushed with filtered sweater (0.45 µm) modified from the method described in Rowan (1991). The samples were airbrushed completely, until they turned white, to ensure the majority of algae were extracted. The resulting homogenized solution was subsampled twice (15 ml) for chlorophyll $a$ and $c_2$ determinations. A

smsall aliquot (ca. 1 ml) was preserved with 10% (w/v) formalin for the zooxanthellae count. Numbers of zooxanthellae were calculated from replicate (n = 5) hemocytometer counts. The other aliquot was preserved in 70% ethanol for later molecular phylotyping. Chlorophyll samples were centrifuged to collect the algae and were frozen overnight to help break the cells. Acetone was added to the frozen pellet, and the mixture was homogenized in a small glass tissue grinder, then allowed to develop overnight at –20°C. Absorbances were read on a spectrophotometer. Chl *a* and chl *c*$_2$ contents were calculated using the equations of Jeffery & Humphrey (1975). The sample of *Acropora palifera* was nearly flat; thus, surface area was determined by covering the sample with aluminum foil and standardizing the densities, and chl *a* and chl *c*$_2$. Data from January and March 2000 were not used in the statistical analyses due to a problem of acetone treatment.

**Molecular phylotyping.** Molecular phylotypes defined by Rowan (1991), Rowan & Knowlton (1995), and Chen et al. (2003, 2004) were used to classify the zooxanthellae associated with *Acropora palifera*. DNA extraction and phylotyping were modified from methods described by Chen et al. (2003, 2004). The brown cell pellets were washed 3 to 5 times with 10 ml aliquots of ZB (ZB: 0.4 M NaCl, 10 mM EDTA, 20 mM Tris-HCl, pH 7.6, 8 mM DTT, 0.05% Tween 20, 0.05% Triton 100), and once in DNA isolation buffer (DNAB: 0.4 M NaCl, 50 mM EDTA, pH 8.0). After that, cells were resuspended in 0.75 ml of DNAB, containing 1% (w/v) sodium dodecyl sulfate (SDS), and heated to 65°C for 30 min. Proteinase K (Promega) was then added to a final concentration of 0.5 mg ml$^{-1}$, prior to incubation at 50°C for at least 6 h. Subsequently, samples were extracted with phenol/chloroform, and DNA was precipitated with absolute ethanol and 0.3 M sodium acetate overnight at –20°C. Precipitated DNA was resuspended in 100 µl aliquots of water and stored at –20°C. Two sets of genetic markers, nuclear smallsubunit ribosomal DNA (nssrDNA) and nuclear largesubunit DNA (nlsrDNA), were used to assay the zooxanthellae phylotypes. nssrDNA was obtained by PCR amplification with a host-excluding primer pair (ss5z: 5′-GCAGTTATAR TTTATTTGAT GGTYRCTGCT AC-3′, and ss3z: 5′-AGCACTGCGT CAGTCCGAAT AATTCACCGG-3), and then characterized using the restriction enzymes, *Sau3A* I and *Taq* I, which differentiate 4 of the *Symbiodinium* phylotypes, A, B, C, and D (Baker 2003a). The 5′-end of the nlsrDNA was amplified using a host-excluding primer pair (5S: 5′-GCC-GACCCGCTGAATTCAAGCATAT-3′, and D23zoox: 5′-TGTGGCAYGT GACGCGCAAGCTAAG-3′). The PCR was performed in a PC-9606 thermal sequencer (Corbett Research), using the following thermal cycles:

1 cycle at 95°C (3 min), 50°C (1 min), and 72°C (2 min); 4 cycles at 94°C (30 s), 50°C (1 min), and 72°C (2 min); and 25 cycles at 94°C (30 s), 57°C (1 min), and 72°C (2 min). The amplification reaction was conducted with 50 to 200 ng of a DNA template and MBI *Taq* polymerase (Fermantas), in a 50 µl reaction volume using a buffer supplied with the enzyme under conditions recommended by the manufacturer. The PCR products were electrophoresed in a 1% agarose (FMC Bioproduct) gel, using 1× TAE buffer to assess the yield. PCR products were then characterized using the restriction enzyme, *Rsa* I (Chen et al. 2003, 2004). All enzymes were purchased from MBI (Fermantas).

**Statistical analyses.** Statistical analyses were performed using Statview 5.1. Non-parametric statistics was adopted since data were not normally distributed. The significance of zooxanthellae densities, chl *a*, and chl *c*$_2$, measured over the duration of the study, were determined by the Kruskal-Wallis test. The significance of seasonal fluctuations and pairwise comparisons of phylotypic compositions in the bimonthly population survey were examined using Friedman's test and a chi-square test, respectively.

## RESULTS

No visual bleaching occurred in *Acropora palifera* during the sampling period. This was supported by the zooxanthellae densities and pigment contents (Fig. 1), which were within a range of values for an unbleached status of zooxanthellae densities (Brown 1997a,b, Fagoonee et al. 1999, Fitt et al. 2000). Algal densities fluctuated significantly over time ($F_{7,267}$ = 7.05, p < 0.001, Fig. 1A). Similar patterns were also observed for the pigments; chl *a* ($F_{7,267}$ = 10.89, p < 0.001) and chl *c*$_2$ ($F_{7,267}$ = 10.6, p < 0.001) (Fig. 1B).

Molecular phylotyping using PCR and restriction fragment length polymorphisms (RFLPs) of nssrDNA and nlsrDNA clearly indicated that *Symbiodinium* Phylotypes C and D were the 2 phylotypes associated with *Acropora palifera*. Among the 328 isolates, 3 types of associations, Phylotype C, Phylotype D, and a mixture of Phylotypes C and D, were found in the population of *A. palifera* off the reef flat in Kenting National Park, southern Taiwan (Figs. 2 & 3). Molecular cloning and DNA sequencing confirmed the coexistence of the 2 phylotypes in *A. palifera* (Chen et al. 2004).

Five colonies were collected to examine the spatial variability in *Symbiodinium* phylotypes within an individual colony. Two colonies contained only Phylotype C, and 1 colony contained only Phylotype D. The other 2 colonies, which contained both Phylotypes C and D, showed a uniform distribution throughout the colony. *Acropora palifera* associated solely with Phylotype D,
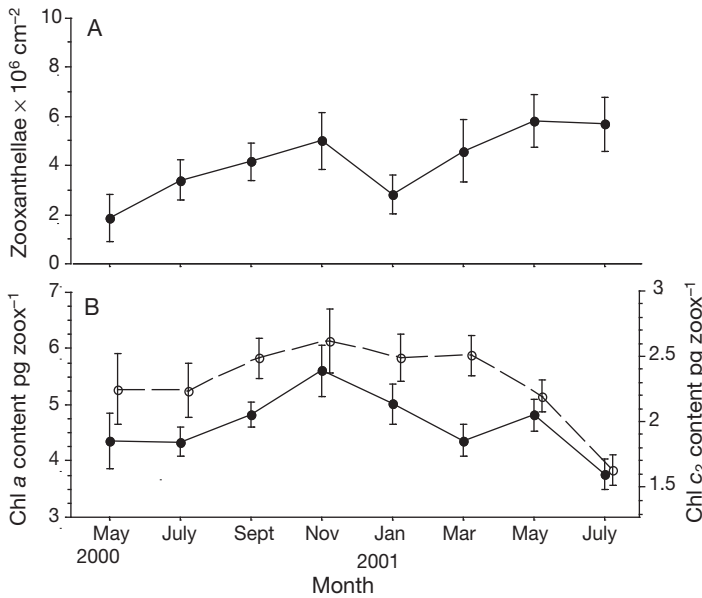
Fig. 1 *Acropora palifera*. (A) Density of zooxanthellae, (B) chl *a* (●), and chl *c*$_2$ (O) in relation to time of year. Data are expressed as the mean ± 95% confidence interval

and this was the major type of symbiosis found in the bimonthly sampling, ranging from 33.3% in May 2001 to 80.65% in July 2000. The association with only Phylotype C peaked in both May 2000 (40%) and 2001 (36.67%). Low frequencies of colonies with a mixture of Phylotypes C and D were observed throughout the study period, ranging from 3.23% in July 2000 to 23.68% in March 2001 (Fig. 2). The seasonal fluctuation in *Symbiodinium* compositions was significant over time (Friedman's test, $\chi^2 = 12.95$, p < 0.05) and variable in pairwise comparisons (Table 1). The highest significant difference was found for the comparison between May of both 2000 and 2001, and other months ($\chi^2$-test, p < 0.001). This was reflected in the composition of Phylotype D, which dropped significantly in May of both 2000 and 2001 (Fig. 3). Tracking of tagged colonies provided the first evidence that the compositions of the symbiont community can completely shift from a single phylotype to a mixture of phylotypes and vice versa (Fig. 4). This fluctuation in the symbiont community differed among individual hosts; i.e. in some colonies, symbiont phylotypes fluctuated greatly among months. For example, Colony no. 2 hosted Phylotype C in March 2000, then started to incorporate Phylotype D in July 2000, and shifted only to D in September 2000. Following these shifts, colony no 2. returned to solely hosting Phylotype C in November 2000. In 2001, the symbiont community in this colony also fluctuated dramatically. In contrast, some colonies retained the same phylotype throughout the entire sampling period (Colony no. 6, Fig. 4).

## DISCUSSION

The data sets collected in this study indicated that zooxanthellae densities and pigment contents of *Acropora palifera* varied temporally. There is a consensus that zooxanthellae density in reef-building corals averages $1 \times 10^6$ cells cm$^{-2}$, but considerable variations have been reported (Brown 1997a,b, Stimson 1997, Brown et al. 1999, Fagoonee et al. 1999, Hoegh-Guldberg 1999, Fitt et al. 2000). In contrast, bleached corals have densities of less than $0.5 \times 10^6$ cells cm$^{-2}$ (Fagoonee et al. 1999, Fitt et al. 2000). The zooxanthellae densities in *A. palifera* were within this range and showed no visual signs of bleaching during the sampling period. No visible signs of coral bleaching were observed in other scleractinian corals in the same area during the sampling period. It is well-documented that algal density fluctuations are directly influenced by changing solar radiation levels and seawater temperatures (Brown et al. 1999, Fitt et al. 2000). Brown et al. (1999) studied 4 Indo-Pacific massive corals and showed that algal densities had a weak correlation to solar radiation, which declined to minimum values at the time of year when sea surface temperatures reached the annual maximum. A similar temperature effect was also observed in the 5 yr monitoring of 5 Caribbean corals from 2 depth zones, in which the lowest values of algal densities and pigment contents usually occurred during the late summer–fall period (Fitt et al. 2000). Although the algal densities and pigment contents of *A. palifera* were variable among the bimonthly samplings, they did not fit the annual pattern of densities being low during the summer–fall and high in the winter–spring period. This observation might be limited due to a short sampling period (1.5 vs. ~4 to 5 yr) in which seasonal patterns do not readily appear.
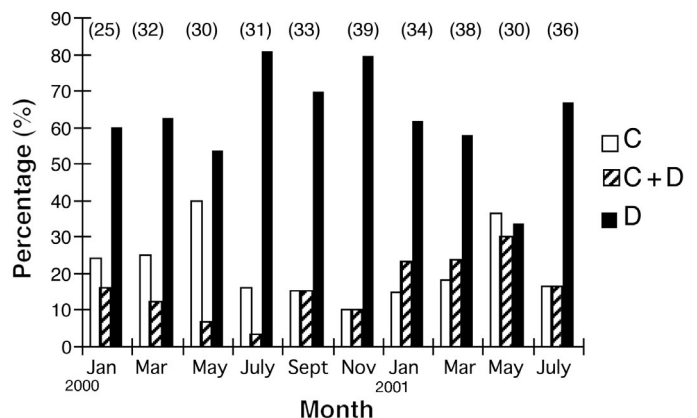


Fig. 2 *Acropora palifera*. Composition of *Symbiodinium* phylotypes in relation to time of year. Sample size for each time of year is indicated in parentheses above the bars

Table 1. Pairwise $\chi^2$-test of monthly variation of *Symbiodinium* phylotype composition based on Fig. 1. ns: not significant; *p < 0.05; **0.05 < p < 0.001; ***p < 0.001

|        | Jan 00 | Mar 00 | May 00 | Jul 00 | Sep 00 | Nov 00 | Jan 01 | Mar 01 | May 01 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Jan 00 |        |        |        |        |        |        |        |        |        |
| Mar 00 | 0.62[ns] |      |        |        |        |        |        |        |        |
| May 00 | 7.96* | 5.48[ns] |      |        |        |        |        |        |        |
| Jul 00 | 13.62** | 9.90** | 17.74*** |    |        |        |        |        |        |
| Sep 00 | 2.8[ns] | 3.31[ns] | 16.62*** | 8.83* |    |        |        |        |        |
| Nov 00 | 9.74* | 8.66* | 23.65*** | 5.17[ns] | 2.54[ns] |  |        |        |        |
| Jan 01 | 3.71[ns] | 6.48* | 21.38*** | 18.89** | 2.58[ns] | 8.80* |   |        |        |
| Mar 01 | 2.49[ns] | 5.27[ns] | 17.89*** | 20.26** | 3.48[ns] | 11.26** | 0.40[ns] | |        |
| May 01 | 14.87*** | 18.89*** | 19.07*** | 50.62*** | 27.60*** | 44.40*** | 18.82*** | 14.10*** | |
| Jul 01 | 1.61[ns] | 2.56[ns] | 15.08*** | 11.15* | 0.31[ns] | 4.60[ns] | 1.51[ns] | 1.87[ns] | 22.56*** |

The seasonal dynamics of algal densities reported in previous studies and our own observations indicate that there is a natural annual pattern of changes in algal–coral symbioses. Our study presents the first evidence that the symbiont community within coral colonies is dynamic, not only as revealed by fluctuating zooxanthellae densities and other parameters but also involving changes in *Symbiodinium* phylotypes. Symbiotic community change with no visible signs of coral bleaching has been postulated (reviewed in Baker 2003a). This change may involve 2 levels of shifting in host corals: (1) between phylotypes and (2) among different variants within a phylotype. Five distinct phylotypes (A, B, C, D, and F) have been identified as being associated with reef-building corals in the Caribbean, the Indian Ocean, and the Pacific Ocean using conserved nuclear ribosomal-coding genes, nssrDNA, and nlsrDNA (reviewed in Baker 2003a). Multiple phylotypes of *Symbiodinium* within single individuals and/or colonies have also been documented in a number of reef-building corals. However, most studies focused on single-time (snapshot) sampling for bathymetric distribution, biogeography, and depth-related patterns of zonation (reviewed in Baker 2003a); therefore, long-term changes in symbiont communities have never been detected. Our results suggest that snapshot sampling might underestimate symbiont diversity, especial for those coral hosts harboring more than 1 phylotype of *Symbiodinium*. In addition, symbiotic community changes may involve a shifting of different symbionts within phylotypes. For example, several tropical Pacific scleractinians exhibit patterns of depth zonation with 2 variants of Phylotype C using a rapidly evolving region of the ribosomal gene, the internal transcribed spacer (ITS) (LaJeunesse et al. 2002). Whether these 2 variants of Phylotype C also involve temporal variation within a single coral colony as seen at the phylotypic level is worthy of further investigation.

Several ecological surveys and experiments have implied that there are stress-tolerant differences (to temperature, irradiation, etc.) among *Symbiodinium* phylotypes (Rowan & Knowlton 1995, Rowan et al. 1997, Baker 2001 2003a,b, 2004, Loh et al. 2001, Rodriguez-Lanetty et al. 2001, Toller et al. 2001a,b, Savage et al. 2002, Chen et al. 2003, 2004, Knowlton & Rohwer 2003, Rowan 2004). For example, Phylotype D was first recognized as an opportunistic symbiont that plays an important role in recently bleached corals in the process of recovering their steady-state symbiont communities, or corals occurring in stressed and marginal habitats (Rowan et al. 1997, Baker 2001, 2003a,b, Glynn et al. 2001, Toller et al. 2001a,b, Chen et al. 2003, 2004, Fabricius et al. 2004, Little et al. 2004). Phylotype D, on the contrary, is the dominant *Symbiodinium* (>60%) as-
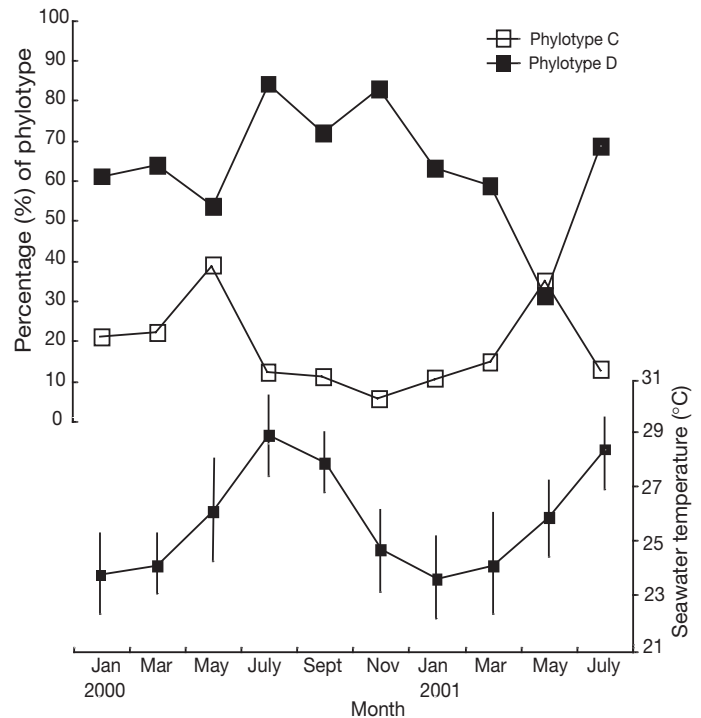


Fig. 3 *Acropora palifera*. Percentage of Phylotypes C and D and seawater temperature in relation to time of year
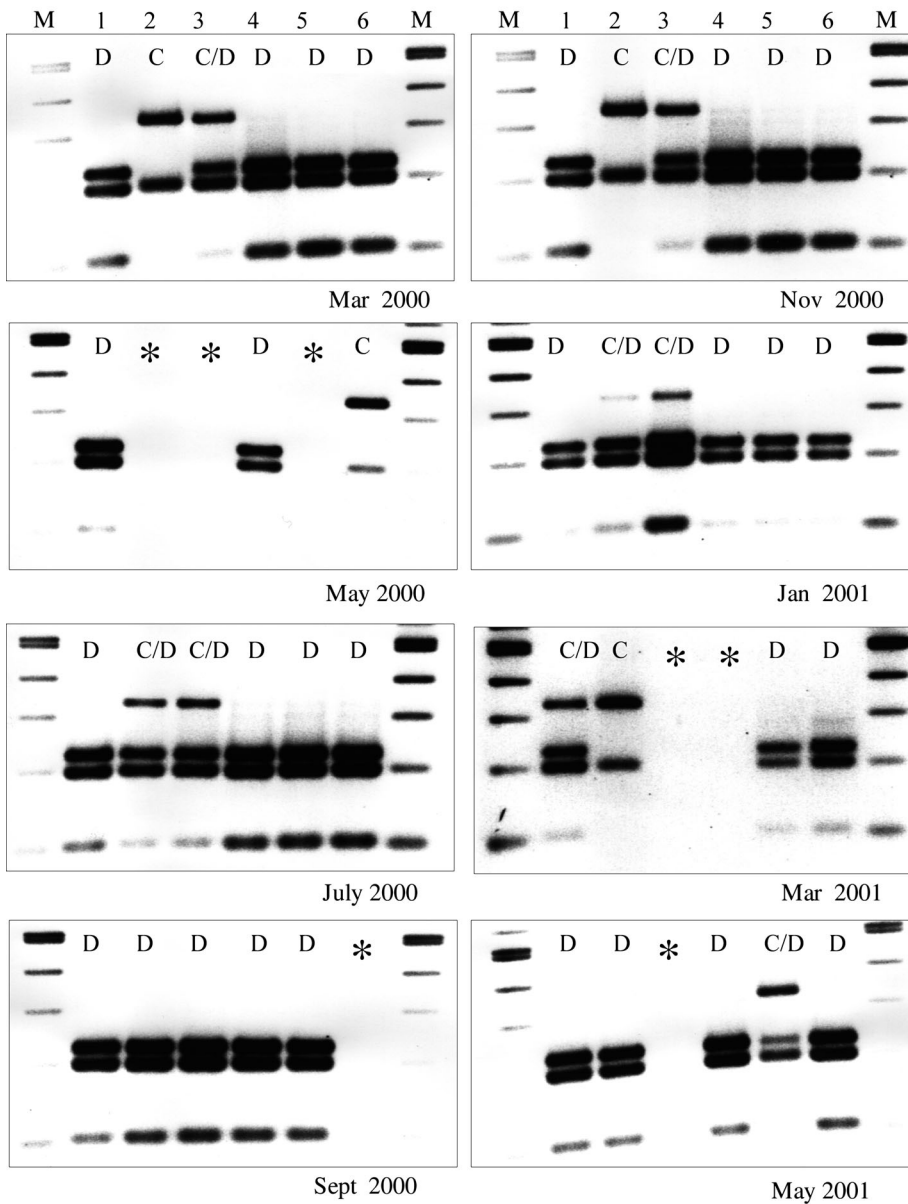
Fig. 4. *Acropora palifera*. RFLP phylotyping of zooxanthellae freshly isolated from several examples of tagged corals throughout the sampling period. PCR products of nuclear large-subunit ribosomal DNA (nlsrDNA) were digested with *Rsa* I. Lanes at both ends of the gels labeled with M are DNA fragment size standards of a 100 bp DNA ladder. Each lane (1 to 6) on the gel represents the tagged colony subsampled in that month. For example, Colony no. 1 hosts Phylotype D from March 2000 to January 2001, but hosts Phylotypes C and D in March 2001, and then changes back to Phylotype D in May 2001. *PCR amplifications were unsuccessful due to the poor quality of the DNA extraction

sociated with *Acropora palifera* in the shallow water of this reef flat where environmental disturbances are high. Even with the shuffling symbiont communities observed in individual colonies, from Phylotype D to C and C/D or vice versa, our data imply that instead of being an opportunistic symbiont, Phylotype D shows a certain fidelity in its relationship with *A. palifera*. This view of the physiological potential of Phylotype D was also suggested by the stable association of Phylotype D with the low temperature-tolerant coral *Oulastrea crispata* in subtropical nonreefal coral communities (Chen et al. 2003). Further surveys of symbiont diversity in *O. crispata* from temperate Japan to the tropical Gulf of Thailand showed that Phylotype D was as-

sociated with this widely distributed coral across the West Pacific (Lein et al. unpubl. data). In addition, Phylotype D was also found in stable associations mixed with Phylotype C in *Euphyllia* spp. at the reef edge of 2 different coral communities in the Taiwan Strait (Chen et al. 2004). Taken together, although having a higher potential for stress tolerance, Phylotype D may represent the 'best-performing' *Symbiodinium* for corals living in frequently disturbed environments. Nevertheless, this scenario should be corroborated by studying more coral species co-occurring in the same environment before a firm conclusion can be stated.

Recent studies have taken the further step of suggesting that Phylotype D is a heat-tolerant *Symbiodi-*

*nium* (Glynn et al. 2001, Baker 2003a,b, 2004, Ulstrup & van Oppen 2003, Fabricius et al. 2004, Little et al. 2004, Rowan 2004). Glynn et al. (2001) showed that Far-Eastern Pacific corals hosting Phylotype D symbionts had suffered less from the 1998 coral bleaching event than those coral associated with other symbiont phylotypes. *Acropora millepora* was associated with Phylotype D in the warmer inshore reef, and with Phylotype C in the mid-shelf reefs of the Great Barrier Reef (van Oppen et al. 2001). Baker (2004) found a much higher proportion of Phylotype D in corals that had survived the severe bleaching in the Arabian Gulf than in coral from the Red Sea; the former has greater temperature fluctuations and higher salinities. Fabricius et al. (2004) showed that corals associated with Phylotype D did not suffer coral mortality during the 1998 coral-bleaching event in the Palauan reefs. Thermal experiments showed that *Pocillopora verrucosa* hosting Phylotype D increased photoprotection after exposure to high temperatures, indicating that Phylotype D is a high-temperature specialist (Rowan 2004). The symbiont communities in *A. palifera* fluctuated greatly with Phylotype D, significantly dropping in late spring/early summer when seawater temperatures rose sharply (Fig. 3). Two scenarios for examining the heat-tolerance of Phylotype D could explain these fluctuations of symbiont communities. First, an increase of Phylotype D was observed in summer (July), which corresponded to the highest water temperature in the area. The proportion of Phylotype D remained steady (unchanged) through the whole autumn and early winter, with a significant drop in May of both years. The opposite was true for the distribution of the symbiont from Clade C, which decreased in summer and was stable in winter (Fig. 3). This suggests that either Clade C symbionts are heat-sensitive or Clade D symbionts are heat-tolerant or both. In contrast, *A. palifera* occurs in the reef flat off Tantzei Bay where corals experience wide-ranging temperature fluctuations daily and monthly. If Phylotype D is specifically heat-tolerant, it would be reasonable to predict that not only would Phylotype D be the major *Symbiodinium*, but that it should also be maintained in a stable association with *A. palifera* living in the shallow reef flat that is constantly exposed to high temperatures during low tides, as seen in *Goniastrea aspera* inhabiting a similar habitat in Phuket, Thailand (Brown et al. 2000, 2002). *G. aspera* colonies harbor an apparently uniform *Symbiodinium* community of Phylotype D. However, the stability of the association was not what we observed for *A. palifera* in the bimonthly sampling period. The negative correlation indicated that physiological constraints other than heat operated on the fluctuation of Phylotype D in *A. palifera.* Experiments conducted with 2 Caribbean *Montastrea* species showed that

Phylotype D is relatively stress-tolerant to changing light levels (Toller et al. 2001b). These results and the discovery of Phylotype D in association with corals inhabiting either shallow water or the reef edge in deep water of both the Caribbean and the Indo-Pacific coral reefs (Toller et al. 2001a, Chen et al. 2004) suggests that the physiological performance of both Phylotype D and the holobionts (the whole-animal–dinoflagellate association) cannot be simply explained by heat-tolerance, and other factors (e.g. irradiation, sedimentation etc.) should also simultaneously be considered (Toller et al. 2001a,b).

The significant fluctuation in *Symbiodinium* composition in *Acropora palifera* during late spring/early summer might be related to differences in physiological properties between Phylotypes C and D, and their contribution to the holobionts, although there is little experimental evidence available to justify this hypothesis. Little et al. (2004) monitored the growth of juveniles of 2 *Acropora* species infected with Phylotypes C and D, respectively. It was shown that juveniles infected with Phylotype C grew ~2 to 3 times faster than those infected with Phylotype D, indicating that algal endosymbionts significantly contribute to the physiological attributes of the coral holobiont (Little et al. 2004). Although a PCR-RFLP survey of tagged *A. palifera* clearly distinguished Phylotypes C and D, and their mixture in agarose gels, the PCR technique may fail to detect the existence of a phylotype when its relative density is extremely low. Nevertheless, shuffling the symbiont communities from Phylotype D-dominant to Phylotype C-dominant in *A. palifera* may still be regarded as a contribution to a better physiological performance of holobionts in the face of seasonal fluctuations of environmental conditions, including temperature and irradiation etc. Determining whether this fluctuation is related to differences of acclimation of physiological performances between Phylotypes C and D, and their interaction with the coral host requires further confirmation.

In conclusion, our study reveals that the regulation of the symbiotic community within coral hosts is dynamic, and furthermore, may routinely involve changes between different *Symbiodinium* phylotypes. In addition, significant changes of symbiont compositions during seawater temperature rises in late spring/early summer also highlight that the scenario of stress tolerance among phylotypes urgently needs to be re-evaluated by understanding the physiological traits of symbionts.

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