Vol. 444: 117–132, 2012
doi: 10.3354/meps09413

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published January 10

Patterns of *Symbiodinium* distribution in three giant clam species across the biodiverse Bird's Head region of Indonesia

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ABSTRACT: The formation and persistence of modern coral reefs depends largely on organisms that host dinoflagellate algal symbionts of the genus *Symbiodinium*. There are important ecological and physiological differences among *Symbiodinium* types, and many host species are able to associate with multiple types, which may facilitate adaptation to local environmental change. Using denaturing gradient gel electrophoresis (DGGE) and sequencing of internal transcribed spacer-2 (ITS2) ribosomal DNA, we identified 11 *Symbiodinium* types belonging to clades A, C, and D in 250 host animals from 3 *Tridacna* species in eastern Indonesia. Individuals with multiple symbiont types were common: 42% of all clams had symbionts from multiple clades and 15% of all clams had multiple types from a single clade. *T. crocea* associated more often with clade C symbionts and less frequently with clade D symbionts. *T. squamosa* associated more frequently with clade D and less often with clade C symbionts. *T. maxima* did not preferentially associate with a particular *Symbiodinium* clade, but sample sizes were low. We used both satellite sea surface temperature and *in situ* recordings to characterize the thermal environment in the study area. Clams with clade C and D symbionts were located in areas with higher mean temperatures, while clams with clade A symbionts were in cooler areas. This is consistent with previous research indicating that clade C and D types may be more heat-tolerant than clade A. These results support the hypothesis that giant clams can associate with different symbiont types based on local environmental conditions.

KEY WORDS: *Symbiodinium* · *Tridacna* spp. · Thermal tolerance · Climate change · Coral reef · Indonesia

INTRODUCTION

Numerous marine invertebrates, including many species of corals, foraminiferans, protists, anemones, jellyfish, and some mollusks have a mutualistic symbiosis with a diverse group of dinoflagellates of the genus *Symbiodinium* (Baker 2003). Extensive phylogenetic investigations based on small subunit (SSU) and large subunit (LSU) nuclear ribosomal genes have led to the currently recognized phylogeny of 9

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distinctive clades (A to I) in this dinoflagellate genus (Pochon & Gates 2010). Additional subcladal diversity has also been revealed through the use of highly variable markers such as nuclear rDNA internal transcribed spacer (ITS) regions (LaJeunesse 2001, 2002, van Oppen et al. 2001, 2005a,b, Rodriguez-Lanetty & Hoegh-Guldberg 2003), and other markers (Santos et al. 2004, Barbrook et al. 2006, Magalon et al. 2007, Kirk et al. 2009).

Different types of *Symbiodinium* in symbiosis exhibit different ecological and physiological patterns, such as depth zonation patterns, seasonal relative abundance changes, as well as sensitivity to light and temperature. For example, consistent patterns of depth zonation among *Symbiodinium* types have been demonstrated in numerous coral and other invertebrate hosts. *Montastraea annularis* and *M. faveolata*, 2 dominant Caribbean coral species, contain members of at least 4 *Symbiodinium* clades, of which at least 3 show predictable depth distributions (Rowan & Knowlton 1995, Rowan et al. 1997, Toller et al. 2001a). A survey of the distribution of *Symbiodinium* in a variety of Caribbean invertebrate hosts also found depth zonation to be an emergent property of patterns in symbiont distribution (LaJeunesse 2002). In the tropical western Atlantic (Caribbean), *Symbiodinium* clade A typically occurs in shallow reef corals and is noted for an ability to produce UV-protective mycosporine-like amino acids (MAAs) in culture, which has been highlighted as a potential reason for this (Banaszak et al. 2000, 2006). Symbionts in clade A have also been suggested to be opportunistic, and have appeared after disturbances such as disease (Toller et al. 2001b). However, in the Pacific, clade A is relatively rare in scleractinian corals (Baker et al. 1997, LaJeunesse 2002) and has only been found at relatively high latitudes (reviewed in Baker 2003). Seasonal changes in total algal density and the relative abundance of multiple *Symbiodinium* types in hosts have also been reported in corals (Chen et al. 2005). Thermal sensitivity also varies between *Symbiodinium* clades (Rowan et al. 1997, Glynn et al. 2001, Berkelmans & van Oppen 2006, Jones et al. 2008) and even between types within a single clade (Tchernov et al. 2004, Sampayo et al. 2008).

Many coral species are able to host multiple *Symbiodinium* types, sometimes even within the same colony (Rowan & Knowlton 1995, Apprill & Gates 2007, Baker & Romanski 2007). It is now clear that the bleaching resistance of individual coral colonies is due, at least in part, to the type(s) of symbiont it hosts (Rowan et al. 1997, Glynn et al. 2001, Baker et al. 2004, Berkelmans & van Oppen 2006, Jones et al. 2008, LaJeunesse et al. 2009; but see also Baird et al. 2009). However, relatively little research has been done on the role of symbiont diversity in giant clams, and consequently it is difficult to draw conclusions as to the applicability of these results to this group.

Giant clams (genus *Tridacna*) are one of only a few mollusk taxa that maintain symbioses with *Symbiodinium*. Unlike corals, which host *Symbiodinium* as intracellular symbionts in the endodermal cell layer, symbionts of clams are intercellular and are maintained in the hemal sinuses of hypertrophied siphon (Trench et al. 1981). Previous studies have identified giant clam symbionts belonging to clades A, C, and D (reviewed in Baker 2003). Individual host clams can associate with symbionts from multiple clades simultaneously (Baillie et al. 2000a, Carlos et al. 2000), and a preliminary laboratory bleaching study showed that *T. gigas* and *T. derasa* individuals with clade A and clade C symbionts responded differently to elevated temperatures (Sison 2003). Clams with clade A symbionts were more susceptible to bleaching following exposure to elevated water temperature, compared to clams hosting clade C symbionts, in agreement with the results of Rowan et al. (1997) for corals. However, results of a follow-up experiment indicate that clade A symbionts in giant clams respond better to low light levels, compared to clade C symbionts (Sison 2003), which is the reverse of that found by Rowan & Knowlton (1995) and Rowan et al. (1997) for corals. Replicated plots with controlled environmental conditions indicate that different symbiont communities result in differential survivorship and growth rates in tridacnid clams (Belda-Baillie et al. 1999), demonstrating that different symbiont types can have a substantial impact on their giant-clam hosts. Taken together, these results indicate that clams, like corals, host different symbionts that vary in their environmental optima. However, as results with clade A indicate (Sison 2003), it is not clear whether physiological characteristics associated with symbionts in one host (such as corals) translate to other hosts (such as clams), in part because significant diversity within clades precludes their direct comparison at this taxonomic level (Savage et al. 2002).

Previous studies on the association between *Symbiodinium* and giant clams have been conducted under laboratory conditions, but almost nothing is known about this symbiosis in the ocean. To begin to fill this gap, we conducted the present study, and aimed to (1) describe *Symbiodinium* diversity in nat-

ural populations of 3 species of giant clams (*Tridacna crocea*, *T. maxima*, and *T. squamosa*), and (2) determine the relationships between *Symbiodinium* type, host species, host genotype, and the thermal environment. The present study was conducted in the Bird's Head region of eastern Indonesia because this region is a biodiversity hotspot in the Coral Triangle (Allen & Erdmann 2009, Veron et al. 2009) and is the focus of multiple conservation efforts by local government and international non-profit organizations. The present study takes advantage of existing data sets (e.g. clam tissue collections, *in situ* data loggers, and remotely sensed sea surface temperature [SST] data) and combines them in a preliminary investigation of *Symbiodinium* diversity in giant-clam hosts.

MATERIALS AND METHODS

Sampling

Giant clams were sampled at 12 locations in the study area between 2 April 2005 and 22 November 2006 (Table 1). *Symbiodinium* are located in specialized tubes within the clam mantle tissue (Norton et al. 1992). Samples were collected non-lethally by clipping a piece of mantle tissue using a hemostat and sharp scissors and this was then preserved in 95% ethanol. Samples were collected at depths typical for the locations of each species on the reef: *Tridacna crocea* from 0 to 1 m, *T. maxima* from 1 to 4 m, and *T. squamosa* from 2 to 10 m.

Table 1. *Tridacna* spp. Giant-clam collections. Map labels correspond to Fig. 1

Map label	Population	Locality	Date collected (dd/mm/yy)	n
T. crocea				
Adoki	Biak	Adoki Village	3/4/05	14
24		Owi	5/4/05	13
Fak Fak	Fak Fak	Tuburwasa	4/5/06	16
Nabire	Nabire		15/2/06	18
9	Dampier Straight	Kri	4/7/05	14
12		Jefman Island	10/7/05	14
15	Misool	Waaf	22/11/05	18
Kumur	Teluk Cenderawasih	Pulau Kumbur	18/2/06	21
23		Tridacna Atoll	10/9/05	21
Yapen	Yapen		4/6–4/8/05	12
			Total:	161
T. maxima				
24	Biak	Pulau Rasba	12/4/05	3
24		Owi	5/4/05	10
9	Dampier Straight	Kri	4/7/05	2
12		Jefman Island	10/7/05	1
14	Misool	Nampale	21/11/05	4
15		Waaf	22/11/05	2
Kumur	Teluk Cenderawasih	Pulau Kumbar	18/2/06	1
Yapen	Yapen	Serui Fish Market	4/6 & 4/8/05	5
			Total:	28
T. squamosa				
24	Biak	Pulau Rasba	12/4/05	7
24		Owi	5/4/05	2
Nabire	Nabire		15/2/06	7
5	Dampier Straight	Teluk Mayalibit	8/7/05	3
3		Alyui Bay	5/7/05	1
12		Jefman Island	10/7/05	3
14	Misool	Nampale	21/11/05	16
Kumur	Teluk Cenderawasih	Pulau Kumbur	18/2/06	16
Yapen	Yapen	Teluk Kananroi	12/2/06	5
Yapen		Serui Fish Market	8/4/05	1
			Total:	61

Symbiodinium identification

Genomic DNA was extracted from each tissue sample using 10% Chelex (Walsh et al. 1991). A portion of the 5.8S, the entire ITS2 region, and a portion of the 28S rDNA were amplified using the primers 'ITSintfor2' and 'ITS2CLAMP' (LaJeunesse 2002). PCR amplifications were performed in a total volume of 25 µl, with a thermocycling profile consisting of 35 cycles of 30 s at 94°C, 30 s at 60°C, and 45 s at 72°C, followed by a final extension at 72°C for 3 min.

Successful *Symbiodinium* ITS2 amplicons were subsequently analyzed using denaturing gradient gel electrophoresis (DGGE) using a CBS Scientific System with denaturing gradient gels (35 to 75% formamide, 8% polyacrylamide denaturing gradient; 100% consisting of 7 M urea and 40% deionized formamide) for approximately 16 h at 90 V. Representative DGGE bands and unique profiles were excised from each gel and gel stabs were PCR-amplified using the same primers, except for removing the GC clamp from the reverse primer, which is unnecessary for sequencing (Apprill & Gates 2007). PCR amplifications were performed in a total volume of 10 µl,

with a thermocycling profile consisting of 20 cycles of 30 s at 94°C, 45 s at 60 to 50°C (decreasing 0.5°C each cycle), and 30 s at 72°C, followed by a final extension at 72°C for 10 min (Apprill & Gates 2007). PCR products were prepared for sequencing following Barber & Boyce (2006). Forward and reverse strands were sequenced on an ABI 377 or an ABI 3730 automated sequencer with Big Dye (Applied Biosystems) terminator chemistry.

The sequences' chromatographs were manually checked and aligned with Sequencher 4.5 (Gene Codes). Each sequence type was compared against named sequences in the GenBank database using the BLAST search tool (Altschul et al. 1990). Sequences that did not have a positive match (100% sequence identity) were named for the closest match with the base position of the non-matching base identified. For example, C15-31 in the present study is identical to the previously identified type C15 except at base 31. Note that single-nucleotide differences can be the result of PCR error, highly unlikely in this example as C15-31 was found in 96 sequences, yet could explain the 5 singleton sequences in the data set. These are reported for completeness, but should be verified in additional individuals. Note also that DGGE analysis of multi-copy rDNA, including the ITS2 region, cannot reliably estimate relative densities of multiple symbiont types; therefore we could not perform any analyses related to symbiont density with each host.

Comparisons between observed and expected *Symbiodinium* distributions were made using chi-squared tests of independence. Expected values were calculated based on observed frequencies of symbiont clades in each species and/or environment.

Phylogenetic analysis of host clams

Approximately 500 bp of the mitochondrial cytochrome *c* oxidase subunit-1 gene (COI) was PCR-amplified from 10% Chelex DNA extractions (Walsh et al. 1991) using *Tridacna*-specific primers (*T. crocea*: Tridacna1F and Tridacna3R, DeBoer et al. 2008; *T. maxima*: MaximaF3 [GTT TAG RGT RAT AAT YCG AAC AG] and HCO-2198, Folmer et al. 1994; and *T. squamosa:* SquaF3 [CAT CGT TTA GAG TAA TAA TTC G] and SquaR1 [ATG TAT AAA CAA AAC AGG ATC]). The hot-start thermocycling profile began with 15 s at 94°C, then 38 cycles of 30 s at 94°C, 30 s at 50°C, and 45 s at 72°C, and a final extension of 72°C for 3 min. PCR products were prepared for sequencing following Barber et al. (2006). Forward and reverse strands were sequenced on an ABI 377 or an ABI 3730 automated sequencer using Big Dye terminator chemistry. Sequences were proofread and aligned in Sequencher 4.5. Protein translations were confirmed in MacClade 4.05 (Sinauer). A COI gene tree was constructed using the neighbor-joining method as implemented in MEGA 4.0 (Tamura et al. 2007) with support based on 1000 bootstrap replicates.

Study area and temperature data

The Bird's Head region of Indonesia is comprised of 2 ecologically distinct areas (Donnelly et al. 2003). Southern Raja Ampat reef communities are strongly structured by cool-water upwellings and dominated by soft corals and benthic filter feeders, while the northern reefs are more hard-coral-dominated and have less upwelling influence. Temperature data was obtained from the study area using 2 methods, both intended to generally characterize the thermal environment in these 2 distinct areas.

First, we used the georeferenced NASA Moderate Resolution Imaging Spectroradiometer (MODIS) SST data (11 micron daytime at 9 km resolution) downloaded from http://oceancolor.gsfc.nasa.gov. For each clam collection locality, we obtained mean SST data during the month of collection, the month preceding collection, the year of collection, and over the entire MODIS mission to date (1 January 2002 to 2 January 2008). Relationships between temperature variables were assessed using Spearman's correlations in SPSS. We used unpaired *t*-tests for groups with unequal variances to determine if mean temperatures differed for clams with and without each symbiont clade.

We also obtained temperature information from *in situ* Hobo v.2 and Tidbit data loggers (Onset Computer) placed in the study area. Loggers deployed at depths of 3 m at 24 locations recorded temperature every 15 min between September 2005 and January 2009 (Fig. 1, Table 2). Data from all loggers located in NE Raja Ampat (n = 7) and data from loggers in SW Raja Ampat (n = 17) were averaged to summarize data for these 2 ecologically distinct areas. We calculated the mean, minimum, and maximum temperature recorded at each logger. Variation in temperature is expected to have a significant effect on short-term acclimatization and longer-term adaptation of hosts (McClanahan et al. 2007), and therefore, characterization of the data included measures of skewness and kurtosis.

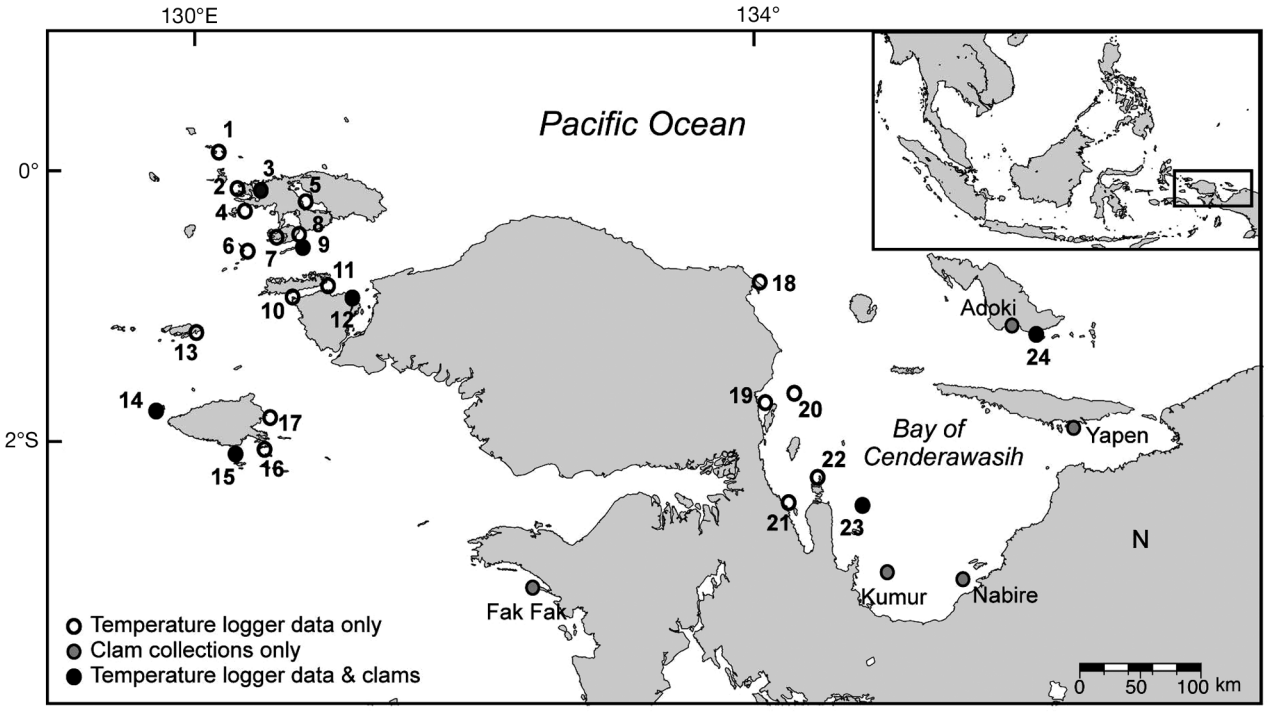

Fig. 1. *Tridacna* spp. Raja Ampat, Indonesia with locations of *in situ* temperature loggers and clam sampling sites. Site labels correspond to 'Map label' in Tables 1 & 2

Table 2. Location and temperature statistics for data loggers. SW RA includes SW Raja Ampat; N RA includes NE Raja Ampat and the Bay of Cenderawasih. Map labels correspond to Fig. 1. Total number of days for which a logger collected data was used in the 'weighted average' calculation since the amount of data available from each logger varied. *$p < 0.05$ between regions

Region	Map label	Location	No. of days with data	Temperature (°C) Avg.	Min.	Max.	Skew	Kurtosis	GPS coordinates
SW RA	1	Wayag	973	28.96	27.4	30.4	−0.34	0.12	00°09.845′ N, 130°00.644′ E
	2	Kawe Rocks (Roibe)	739	28.55	26.2	30.1	−0.40	0.12	00°06.547′ S, 130°11.943′ E
	3	Alyui Bay Cendana	372	28.62	27.4	29.7	−0.34	0.38	00°11.318′ S, 130°15.246′ E
	4	Batang Pele	585	28.85	27.2	30.8	−0.46	−0.17	00°16.700′ S, 130°13.758′ E
	5	Mayalibit Bay	937	30.04	27.0	32.9	−0.60	−0.32	00°17.848′ S, 130°48.490′ E
	6	Fam Group (Melissa's Garden)	243	28.87	27.5	30.7	−0.44	0.29	00°35.390′ S, 130°18.909′ E
	7	Arborek Manta aggregation site	912	28.89	26.4	30.8	−0.38	0.35	00°33.737′ S, 130°32.495′ E
	8	Mike's Point	665	29.15	27.9	31.5	−0.11	−0.13	00°30.941′ S, 130°40.348′ E
	9	Kri EcoResort	839	28.89	26.4	30.6	−0.18	−0.06	00°33.334′ S, 130°40.664′ E
	10	West Selat Sagewin	269	28.47	24.6	30.1	−1.26	2.32	00°57.019′ S, 130°39.756′ E
	11	Sagewin Strait	852	29.08	26.1	31.0	−0.48	0.49	00°53.552′ S, 130°55.664′ E
	12	Jefman Island, Sorong Bay	897	29.39	26.4	31.5	−0.36	0.83	00°55.641′ S, 131°07.408′ E
	13	Kofiau Group	1048	28.76	21.4	31.6	−1.26	3.18	01°15.864′ S, 129°40.789′ E
	14	Nampale mangrove channels	606	28.60	24.7	30.3	−1.08	0.73	01°47.873′ S, 129°38.570′ E
	15	Waaf Island	655	28.58	26.1	30.9	−0.89	−0.60	02°08.936′ S, 130°13.283′ E
	16	Fiabacet Rocks	679	28.84	26.5	31.6	−0.87	−0.05	02°13.333′ S, 130°29.543′ E
	17	Inner Misool Karst Bay	678	28.87	25.9	31.0	−0.76	−0.30	01°58.928′ S, 130°27.574′ E
		Weighted average		**28.96**	**26.1**	**31.0**	**−0.59**	**0.43**	
N RA	18	Pulau Lemon, Manokwari	1155	29.54	27.1	31.1	−0.66	2.43	00°53.395′ S, 134°04.782′ E
	19	Rumberpon	1135	29.56	28.1	31.0	−0.36	0.27	01°44.227′ S, 134°12.146′ E
	20	Pulau Nusambier	628	29.61	22.2	31.6	0.16	1.41	01°58.820′ S, 134°41.723′ E
	21	Pulau Yop	1128	29.55	27.0	31.6	−1.06	1.32	02°30.427′ S, 134°22.670′ E
	22	Pulau Roon	504	29.63	28.4	31.2	−0.21	0.35	02°16.840′ S, 134°33.787′ E
	23	Tridacna Atoll	628	29.76	28.4	31.2	−0.09	−0.13	02°29.691′ S, 134°58.997′ E
	24	Pulau Owi, Biak	86	29.44	28.4	30.6	−0.46	0.53	01°14.870′ S, 136°11.225′ E
		Weighted average		**29.59***	**27.0**	**31.3**	**−0.47**	**1.07**	

RESULTS

Symbiodinium identifications

Positive PCR amplifications and scoreable DGGE profiles were obtained for a total of 250 giant clams, including 161 *Tridacna crocea*, 28 *T. maxima*, and 61 *T. squamosa*. The observed profiles were typically characterized by 1 or 2 distinctive bands, corresponding to a specific DNA fingerprint of the ITS2 rDNA region (Fig. 2).

In total, 11 *Symbiodinium* ITS2 sequence types were identified during this survey (Table 3). The clade C symbiont C15-31 was the most common, found in 160 individual clams. All symbiont types were observed in all 3 host species, with the exception of sequences obtained from only a single individual ('singleton' types), and type A3-223, which was only observed in 10 of 161 *Tridacna crocea* individuals (Table 3).

Mixed *Symbiodinium* communities were commonly detected within single host individuals. Symbionts from multiple clades were detected in 106 of 250 samples (42%), including 48 of 161 *Tridacna crocea* (30%), 49 of 61 *T. squamosa* (80%), and 9 of 28 *T. maxima* (32%). Intra-clade *Symbiodinium* diversity within a single host clam was detected in 38 of 250 (15%) total samples. Table 4 lists the number of hosts in which each *Symbiodinium* clade combination was identified.

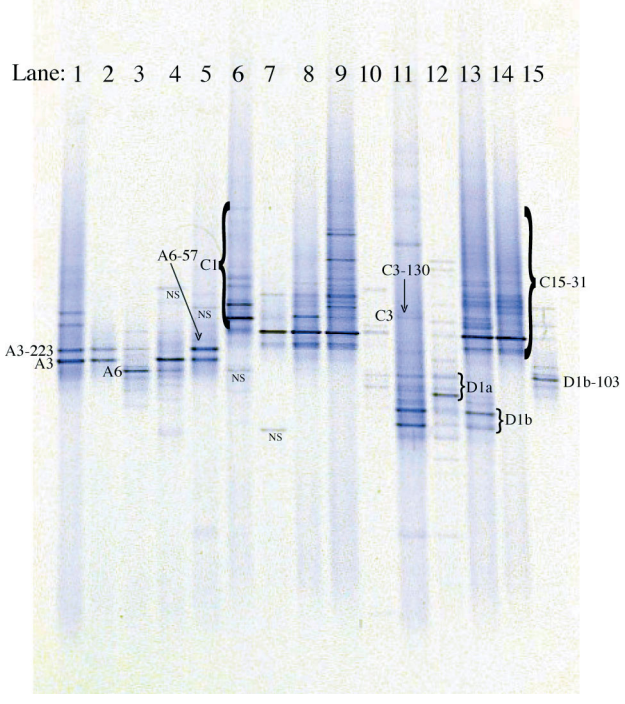

Fig. 2. *Symbiodinium* in *Tridacna* spp. DGGE gel showing representative samples with each ITS2 sequence identified. Faint bands labeled 'NS' failed sequencing and were not scored. DGGE run on a CBS Scientific System with denaturing gradient gels (35–75% formamide, 8% polyacrylamide denaturing gradient; 100% consisting of 7 M urea and 40% deionized formamide) for approximately 16 h at 90 V. Host animal for Lanes 1–10 and 12–14 is *T. crocea*, Lane 11 is *T. squamosa*, and Lane 15 is *T. maxima*. Complete scores for each lane: Lane 1: C1, A3-223, A3; Lane 2: A3-223, A3; Lane 3: A3-223, A6; Lane 4: A3, A6; Lane 5: A6-57, A3; Lane 6: C1; Lane 7: C15-31; Lane 8: C15-31; Lane 9: C15-31; Lane 10: C15-31, D1a; Lane 11: C3-103; C3, D1a, D1b; Lane 12: C15-31, D1a; Lane 13: C14-31, D1b; Lane 14: C15-31; Lane 15: C15-31, D1b-103

Host diversity

A total of 113 COI haplotypes were identified during this survey (Fig. 3). Phylogenetic structure within host species was apparent, with distinct clades present in *Tridacna crocea*, and in *T. maxima*, but not in *T. squamosa* (Fig. 3). *T. crocea* and *T. squamosa* were inferred as sister groups and the most derived, with *T. maxima* basal to those species, consistent with the accepted *Tridacna* phylogeny (Schneider & Foighil 1999). *T. maxima* and *T. crocea* species clades each contained 2 well-supported lineages.

Host-symbiont specificity

In terms of simple presence/absence, host specificity for *Symbiodinium* was low, as individuals from all 3 host species associated with multiple symbiont types in clades A, C, and D (Fig. 3). However, the relative percentages of each symbiont clade, and some symbiont types, differed markedly between host species. Host species identity and *Symbiodinium* clade were not independent (χ^2 = 62.84, df = 4, p = 7.34×10^{-13}; Fig. 3). *Tridacna crocea* individuals associated more with clade C symbionts and less frequently with clade D symbionts than would be expected if symbiont types were distributed evenly among host species. No difference was detected between observed and expected symbiont types in *T. maxima* individuals, although sample sizes were small. *T. squamosa* individuals hosted fewer clade C and more clade D symbionts than would be expected by chance. Other host–symbiont combinations were observed in frequencies that would be expected if associations were random.

Table 3. *Symbiodinium* in *Tridacna* spp. *Symbiodinium* ITS2 types identified in giant-clam hosts. Sequences with a match (100% sequence identity) to GenBank sequences were named according to LaJeunesse (2002). Sequences that did not have a positive match were named for the closest match with the base position of the non-matching base identified. For example, C15-31 in the present study is identical to the previously identified type C15 (GenBank accession no. AF195157.1) except at base 31. Number of host clams found with the symbiont type is based on DGGE profile. Number of sequences is those recovered from sampled clams. GenBank accession number is of a representative of each type

Symbiodinium type	Tridacnid host	No. of host clams with symbiont	No. of sequences	Sequence length in bp (excluding primers)	GenBank accession no.
C15-31	All 3 spp.	160	96	287	HQ896362
A6	All 3 spp.	76	32	255	HQ896367
D1b / D5	All 3 spp.	65	32	285	HQ896365
D1a	All 3 spp.	46	22	285	HQ896364
A3	All 3 spp.	30	23	255	HQ896366
C1	All 3 spp.	11	18	287	HQ896370
A3-223	*T. crocea*	10	8	255	HQ896363
A6-57	*T. crocea*	1	1	255	HQ896371
C3	*T. squamosa*	1	1	287	HQ896369
C3-130	*T. squamosa*	1	1	287	HQ896368
D1b-103	*T. maxima*	1	1	285	HQ896373

Table 4. *Symbiodinium* in *Tridacna* spp. Number of hosts in which each *Symbiodinium* clade combination was identified. Multiple-letter columns (e.g. AA, CC) indicate that the clam hosts multiple types of *Symbiodinium* within that clade

Host species	*Symbiodinium* clade combination									
	A	AA	C	CA	CAD	CC	CD	D	DA	Total
T. crocea	23	5	83	35	2		9	2	2	161
T. maxima	7		8	3		1	6	3		28
T. squamosa	2		4	2	2	1	11	5	34	61
Total	32	5	95	40	4	2	26	10	36	250

Host and symbionts interact intimately in this system given their physical association and reliance on each other for nutrients. Therefore, host-symbiont genotype pairings might be non-random. A test for an association between host genotype (clade) and symbiont clade was only performed in *Tridacna crocea*, due to small sample sizes for the other *Tridacna* species. The 2 clades of *T. crocea* were not distributed randomly across the study area (92% of individuals in one *T. crocea* clade were located in the NE part of the study area; Fig. 4). When we controlled for location (NE Raja Ampat vs. SW Raja Ampat), host clade was not correlated with the presence of any symbiont type (Pearson partial correlations, all $r < 0.13$, $df = 158$, all $p > 0.125$).

Temperature and symbiont distribution

For clam collection sites with *in situ* loggers in place prior to clam collection (Fig. 1: Sites 9, 12, 14, and 24), we compared MODIS and *in situ* temperature data. Average monthly temperatures from remotely sensed MODIS data (sea surface at 9 km resolution) were warmer than those obtained from *in situ* loggers at 3 m depth ($p < 2.5 \times 10^{-11}$, paired *t*-test). However, average temperature calculated from the MODIS satellite and *in situ* loggers were positively correlated ($r = 0.663$).

MODIS SST variables (mean temperature during month of collection, mean temperature during month preceding collection, mean temperature during year of collection, and mean temperature over entire MODIS mission) were all positively correlated (all Spearman's $r > 0.64$, all $p < 0.01$). Clams with clade A symbionts were typically located in areas with lower temperature than clams without clade A symbionts, regardless of the timeframe over which temperature was averaged (Mann-Whitney test: all $t > 3.3$, all $p \leq 0.008$; Fig. 5). Clams with clade C symbionts were typically located in higher-temperature areas, compared to those without clade C symbionts, regardless of the timeframe over which temperature was averaged (Mann-Whitney test: all $t > 2.2$, all $p \leq 0.02$; Fig. 5). Clams with clade D symbionts were typically located in areas with higher temperatures in the month of collection and month prior to collection (Mann-Whitney test: both $t > 4.2$, both $p < 0.001$; Fig. 5), but there was no difference between the 2 groups for temperature averaged across all years or in the year of collection (both $t < 1.23$, both $p > 0.22$). These results remain unchanged if each host species is analyzed independently (data not shown). These results remain unchanged when using sequential Bonferroni-corrected p-values to account for multiple tests on the data, except that the comparison of temperatures during the month of collection is not significant for clams with and without clade C (Mann Whitney $p = 0.019$; sequential Bonferroni-corrected $p = 0.017$).

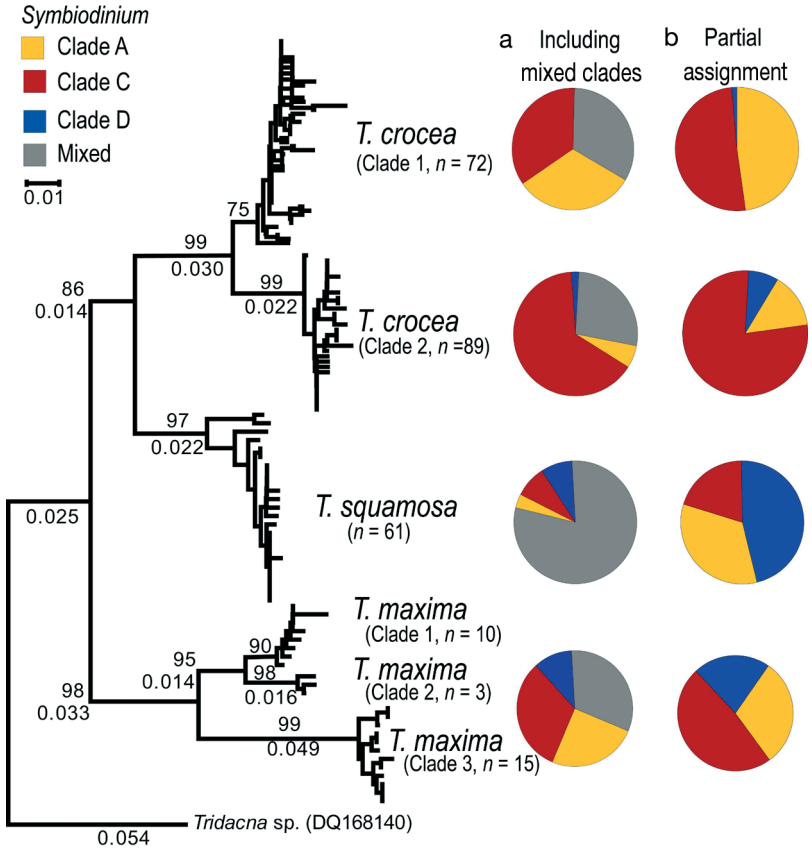

Fig. 3. *Symbiodinium* in *Tridacna* spp. Neighbor-joining tree of all host *Tridacna* COI sequences showing major clades within each species. Bootstrap support (1000 replicates) is shown above branches, and branch length is shown below branches (branches shorter than 0.01 are not labeled). The outgroup used is identified as *Tridacna* sp. (GenBank accession no. DQ168140). Multiple divergent clades are apparent for *T. crocea* (as previously reported: DeBoer et al. 2008, Kochzius & Nuryanto 2008) and *T. maxima*. (a) Percentage of individuals in each species (or clade for *T. crocea*) with each *Symbiodinium* clade (A, C, D, or multiple 'mixed' clades). (b) Percentage of individuals in each species (or clade for *T. crocea*) with each *Symbiodinium* clade in which hosts containing multiple 'mixed' clades are partially attributed to each symbiont clade (e.g. hosts containing both clades A and C are counted as 0.5 individuals for clades A and C)

Not all temperature loggers collected data over the entire time period from September 2005 to January 2009 (Table 2). Therefore, data from all loggers located in NE Raja Ampat (n = 7) and data from all loggers in SW Raja Ampat (n = 17) was averaged to summarize the thermal environment for these 2 ecologically distinct areas. Average water temperature was significantly higher in NE Raja Ampat than in SW Raja Ampat (p = 0.001, *t*-test; Table 2).

Because of higher average temperatures in NE Raja Ampat, a greater number of host clams were predicted to contain heat-tolerant clade C and D *Symbiodinium* within this area, compared to areas in the SW, which were predicted to host members of clade A relatively more frequently. Host clam location (NE vs. SW Raja Ampat) and symbiont clade are not independent (χ^2 = 46.57, df = 2, p < 7.72 × 10^{-11}). This result remains unchanged if each host species is analyzed independently (data not shown). Within the warmer waters of NE Raja Ampat, fewer host clams were observed to harbor clade A than what was expected and more host clams associated with clade C than was expected (Fig. 4). In the SW Raja Ampat region, more host clams associated with clade A than was expected. Hosts with clade D symbionts were found at similar frequencies in both regions.

Temperature variability itself may influence symbiont community composition. Tests for an association between temperature variability (measured by skewness and kurtosis) used only the 5 populations for which temperature data and >10 samples of *Tridacna crocea* were available (sample sizes were too low in other host species). Neither skew nor kurtosis was significantly correlated with the percentage of any symbiont clade (Spearman's correlation, all r < 0.301, all p > 0.05).

DISCUSSION

Symbiont diversity in tridacnid clams from Indonesia

The most common symbiont recorded in our study was *Symbiodinium* C15-31 (Table 3). This subtype has not been recorded to date, but is closely related to C15, which is common in certain scleractinian corals throughout the Indo-Pacific, particularly *Porites* and *Montipora* (LaJeunesse 2005). C15 is also found in various alcyonaceans (Goulet et al. 2008), some zoanthids (Reimer & Todd 2009), and occasionally in hydrozoans, e.g. *Millepora* (LaJeunesse 2005). It would appear that tridacnid clams, at least in Indonesia, associate with a novel type of C15 that may be specific to these hosts.

The second most common symbiont (A6) has been recorded in *Tridacna* from the Philippines (Baillie et al. 2000b) and Japan (LaJeunesse et al. 2004). It has

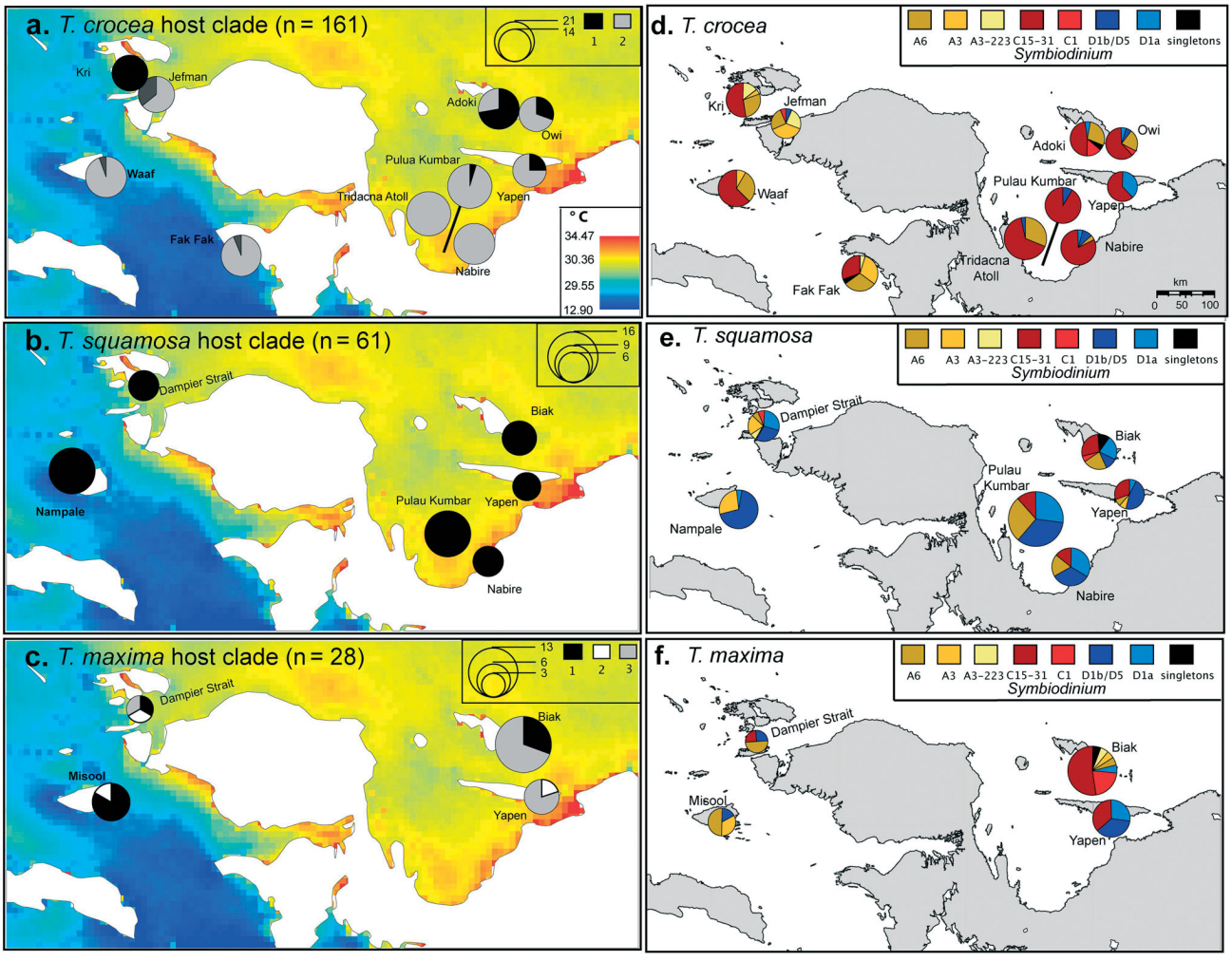

Fig. 4. *Symbiodinium* in *Tridacna* spp. (a–c) Frequency of each COI clade for (a) *T. crocea*, (b) *T. squamosa*, and (c) *T. maxima*. Host clades are defined based on a neighbor-joining tree of COI sequence shown in Fig. 3. Circle size in each key indicates number of host clams sampled in each population. Note that sample sizes differ in panels a, b and c. Underlying color shows remotely sensed mean sea surface temperature over the entire MODIS mission (1 Jan 2002 to 2 Jan 2008). (d–f) Frequency of each *Symbiodinium* type in surveyed populations of (d) *T. crocea*, (e) *T. squamosa*, and (f) *T. maxima*. Hosts containing multiple *Symbiodinium* types are scored as contributing equally (50%) to each type, as explained in the legend of Fig. 3

not been recorded in other hosts and consequently also appears to be specific to tridacnid clams.

The D1b/D5 subtype has also not been recorded to date. Although D1b has been previously identified (GenBank accession nos. EU449056 and EU449060), no information on its host origin is reported. D5 is rare but very taxonomically widespread, having been found in corallimorphs, alcyonaceans, scleractinians, helioporaceans, and zoanthids in the Indian Ocean (LaJeunesse et al. 2010).

D1a is a very common symbiont on coral reefs worldwide and has been recorded from a wide variety of scleractinian corals (LaJeunesse et al. 2004, 2009, 2010, Thornhill et al. 2006, Silverstein et al. 2011). It has also been found in zoanthids (Reimer & Todd 2009), corallimorphs, alcyonaceans, helioporaceans, and gorgonians, as well as *Tridacna* in the NE Indian Ocean (LaJeunesse et al. 2010).

A3 is common in Caribbean scleractinian corals and the scyphozoan *Cassiopeia*, and is also found in zoanthids, the hydrocoral *Millepora*, and the anemone *Condylactis* (LaJeunesse 2002). In the Indo-Pacific it has only been recorded from *Tridacna* in the Andaman Sea, and in the hydrozoan *Aglaophenia* in the western Indian Ocean (LaJeunesse et al. 2010). A3-223 is closely related to A3, but has not been previously recorded.

C1 is one of the most common *Symbiodinium* subtypes worldwide and is both geographically cosmopolitan and phylogenetically widespread among

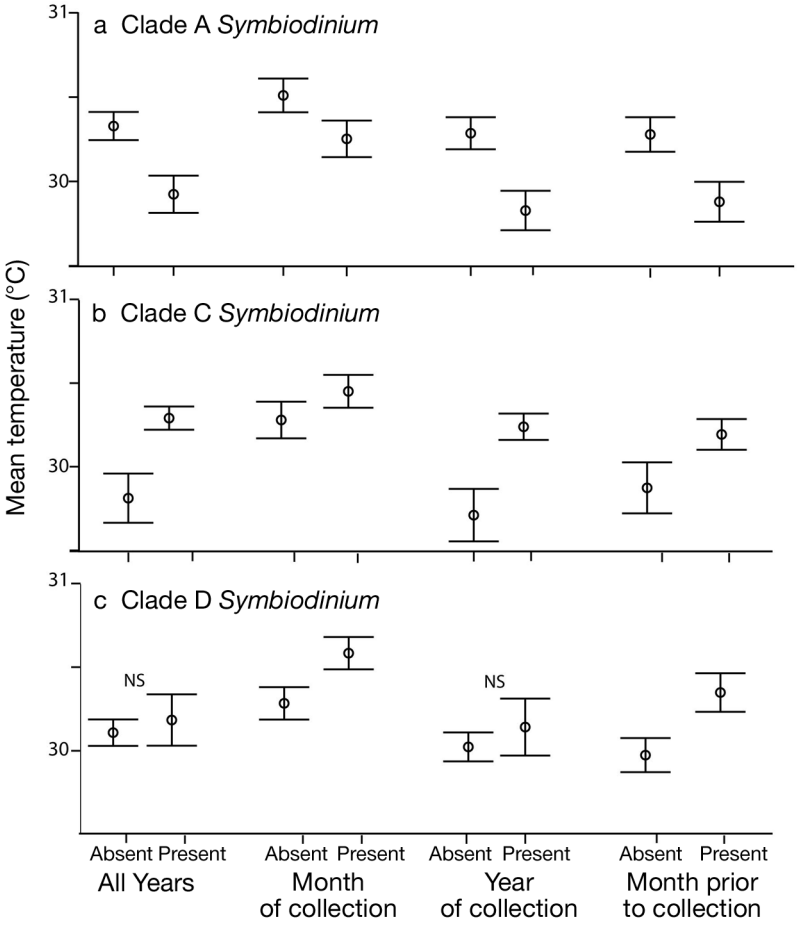

Fig. 5. *Symbiodinium* in *Tridacna* spp. Comparison of mean (± 95% CI) sea surface temperature (SST) measurements from MODIS using unpaired *t*-test for groups with unequal variances for clams with and without *Symbiodinium* (a) clade A, (b) clade C, and (c) clade D. All comparisons are significant ($p < 0.05$), except as indicated by NS ($p > 0.05$). 'All years' corresponds to mean SST over the entire MODIS mission (1 Jan 2002 to 2 Jan 2008)

different hosts. In addition to being common in scleractinian corals worldwide (LaJeunesse 2005), in the Indian Ocean it has also been found in anemones, corallimorphs, zoanthids, alcyonaceans, and also *Tridacna* (LaJeunesse et al. 2010).

The remaining *Symbiodinium* subtypes detected in the present study (A6-57, C15-135, C3, C3-130, and D1b-103) were all found in just 1 host clam. All of these symbionts are novel types that have not been recorded elsewhere, with the exception of C3, which is similar to C1 in the range of hosts with which it associates, and the geographic range over which it occurs.

The overall distribution of symbionts reveals a complex symbiotic picture. While *Tridacna* shows a high degree of flexibility in their associations (with at least 11 different symbionts in 3 clades of *Symbiodinium*), most clams appear to be dominated by symbionts that appear to be specific to these hosts (C15-31 and/or A6). However, at least 88 clams in the present study contained symbionts (D1a, A3, C1, and C3) that are very common in other hosts elsewhere. This suggests that clams do have the ability to associate with other symbionts that have a broad phylogenetic and geographic distribution. One of these symbionts (D1a) is associated with thermotolerance in scleractinian corals (see 'Temperature effects on host *Symbiodinium* community'), and its presence in *Tridacna* on warm Indonesian reefs may indicate higher potential for these hosts to survive at high temperatures.

Much work has been done on the environmental drivers of symbiont diversity in different hosts, particularly corals (Baker 2003, Stat et al. 2006). At the clade level, distributional differences were first reported between *Symbiodinium* in the Caribbean, with clades A and B common in shallow water and clade C more often found in deeper water (Rowan & Knowlton 1995, Rowan et al. 1997, Baker 2001, LaJeunesse 2002). In the Indo-Pacific, the focus has shifted to clades C and D and their distribution relative to temperature and bleaching history, since clades A and B are relatively uncommon (Baker 2003). Studies on bleached reefs have indicated that clade D increases as a result of bleaching (Glynn et al. 2001, Baker et al. 2004, Berkelmans & van Oppen 2006, Jones et al. 2008, LaJeunesse et al. 2009), but long-term environmental history, particularly high temperature with low temperature variability, is also likely to play an important role in determining the relative abundance of clade D (Baker et al. 2004, Fabricius et al. 2004, McClanahan et al. 2007, LaJeunesse et al. 2010, Oliver & Palumbi 2011), although over large scales these pattern may be obscured by other biogeographic factors (Oliver & Palumbi 2009). Clade-level diversity in *Symbiodinium* has been reported in clams previously (Rowan et al. 1996, Rowan 1998, Baillie et al. 2000a, 2000b, Sison 2003), but the distribution of this diversity over ecological or biogeographic scales has not been reported.

Mixed *Symbiodinium* communities in clam hosts

In the present study, many host individuals (42%) simultaneously harbored *Symbiodinium* from multiple clades. The ecological significance of heterogeneous symbiont infections is not yet known, but it has been suggested that mixed symbiont communities may have an adaptive advantage during periods of environmental disturbance such as bleaching (Buddemeier & Fautin 1993, Rowan 1998). Previous studies employing variable molecular markers have shown that giant clams can harbor a mixture of *Symbiodinium* types (Rowan & Powers 1991, Baillie et al. 2000a, as well as the present study). The bulk of recent research on *Symbiodinium* diversity is based on DGGE analysis of ITS2 rDNA, which has been suggested to be the most broadly applicable and appropriate marker for characterizing *Symbiodinium* diversity (LaJeunesse 2001, Correa et al. 2009). Future applications of more sensitive molecular methods that are designed to identify *Symbiodinium* in low abundance (such as quantitative PCR) may reveal additional symbiont diversity within individual clams (Mieog et al. 2007, Correa et al. 2009).

It is important to note that the *Symbiodinium* diversity in giant clams described here should be considered a minimum estimate for 3 reasons. First, rare types present at low abundance (up to 10 to 20% of the total symbiont population, depending on type) may not be detected by DGGE (LaJeunesse et al. 2008). Recent development of quantitative PCR primers for *Symbiodinium* enable sensitive detection of symbionts present in very low numbers, more so than is possible using PCR-DGGE (Mieog et al. 2007, Correa et al. 2009). Application of these techniques to giant clam-algal symbioses will increase our understanding of the utility of diverse symbionts in this system. Second, we analyzed only a portion of the total host mantle tissue and therefore additional symbiont types in the larger tissue area may have been missed. Our sampling method involved clipping a small (1 cm^2) piece of tissue from the outer edge of the mantle. It is possible that other tissues, deeper within the clam or toward the central siphons, might be subject to different environmental conditions (e.g. lower light levels) that could favor different symbiont types. Although to date no studies have investigated spatial variability in *Symbiodinium* within individual giant clams, the evidence demonstrating that individuals can routinely host multiple symbiont types (Baillie et al. 2000b, Carlos et al. 2000, Ishikura et al. 2004, present study) suggests that this is possible. If different symbionts are not evenly distributed throughout clam tissues, then the fact that individual hosts were partially biopsied, rather than sacrificed in their entirety, suggests that symbiont diversity within individuals may be higher than reported here. Finally, populations of *Tridacna* were not exhaustively sampled throughout the study area, and were sampled over a small extent of their geographic range. Therefore these results should not necessarily be interpreted as being representative of the total *Symbiodinium* diversity present in giant clams at all locations.

It is also important to note that different *Symbiodinium* types affect other physiological host traits including growth rate, reproduction, and health, and therefore hosts may face trade-offs when switching symbiont types (e.g. Little et al. 2004). It remains unknown how these traits are affected by *Symbiodinium* in giant clam hosts.

Host–symbiont specificity

Giant clams do not maternally transfer symbionts to their eggs (Fitt & Trench 1981); rather, clam veligers acquire free-living *Symbiodinium* from their environment. Such 'horizontal acquisition' of symbionts allows individual clams the flexibility to potentially establish symbioses with new or multiple types of free-living *Symbiodinium* from the environment. This method of symbiont acquisition could result in an initial non-selective uptake of symbionts from the environment (Coffroth et al. 2010), followed by 'winnowing' of suitable symbionts in early ontogeny, as in the coral *Fungia* (Dunn & Weis 2009). Alternatively, hosts could preferentially select symbiont strains at the infection stage as seen in squid (Nishiguchi 2002).

Our results suggest that giant clams routinely host different types of *Symbiodinium*, and that the symbiont type(s) present depend on the clam species in question and the thermal environment in which it is found. Our results indicate that *Tridacna crocea* individuals associate more often with clade C and less so with clade A symbionts. *T. maxima* associate less often with only clade A symbionts than expected, but more often with clade C only and mixed-clade symbionts. *T. squamosa* associates more often with mixed clades than expected. Some of this variation could be explained by habitat differences between species, particularly depth, which has been associated with symbiont community structure in previous studies (reviewed in Baker 2003). *T. crocea* and *T. maxima* inhabit shallow waters (0.5 to 7 m), and *T. squamosa* is found slightly deeper (<15 m). It is possible that the

wider depth range of *T. squamosa* predisposes it to hosting more diverse symbiont assemblages as a result of more variable environmental conditions. Because the present study did not record the exact depth for each sample collected, it is impossible for us to test this hypothesis directly. However, given the habitat differences among species, and the temperature stresses associated with these differences, the variation in symbiont communities between species is consistent with the hypothesis of holobiont adaptation to environmental conditions. However, because the 3 giant clam species studied here have not been sampled across their entire range, the differences in *Symbiodinium* communities between host species reported here should be regarded as location-specific pending further study. It should not be assumed that these associations are representative of the full range of associations in which these clam species are involved.

Evolutionary history may account for some variation in symbiont community between host species, but the exact relationship is unclear from our results. *Tridacna crocea* and *T. squamosa* are sister species, with *T. maxima* basal to them (Schneider & Foighil 1999). Additional phylogenetic structure is apparent within host species, with distinct COI clades present in *T. crocea* (DeBoer et al. 2008, Kochzius & Nuryanto 2008) and *T. maxima*, but not in *T. squamosa* (Fig. 3). However, when controlling for collection location (NE Raja Ampat vs. SW Raja Ampat), host clade was not correlated with the presence of any symbiont type (Pearson partial correlations, all $r < 0.13$, $df = 158$, all $p > 0.125$).

Temperature effects on host *Symbiodinium* community

We took advantage of 2 existing data sources to obtain information on temperature in the study area: NASA's SST data from the MODIS satellite and a collection on *in situ* loggers in the Bird's Head region. Temperature data from both sources are positively correlated, but MODIS SSTs were significantly higher than those recorded by *in situ* loggers. We do not see this result as problematic for several reasons: (1) this result is not surprising given the differences between the 2 methods in depth (surface vs. 3 m) and resolution (9 km for MODIS and 0 km for *in situ* loggers); (2) the conclusions of this study rely entirely on the MODIS satellite data, not on a combination of data from loggers and MODIS. The *in situ* logger data were only used to obtain measures of temperature variability in the study area, which cannot be directly calculated from the MODIS data. Neither of the measures of temperature variability was significantly associated with *Symbiodinium* distribution in giant clams (see 'Results'), but the analysis is included for completeness as coral researchers have previously found an association between thermal variability and symbiont distribution (e.g. McClanahan et al. 2007). It is important to note that the power of these tests in the present study was small with only 5 sites with long-term data included. Ideally, future studies focused on determining the absolute relationships between giant clams, *Symbiodinium*, and the thermal environment should rely on temperature data obtained from loggers in direct proximity to study animals.

Our results indicate that SST history plays a role in explaining the distribution of *Symbiodinium* in the present study of giant clams, but we emphasize that this study was geographically restricted, so the apparent specificity reported here should be regarded as location-specific pending further study. Clams hosting clade A symbionts were more common in areas with lower mean temperatures in the month of collection, the month prior to collection, the year of collection, and over all years of MODIS temperature collection (1 January 2002 to 2 January 2008; Fig. 4). In contrast, clams with clade C and D symbionts were more common in areas with higher mean temperatures, regardless of the time period over which temperature was averaged. The presence of clade D symbionts in giant clams appears to be more closely tied to short-term temperature history, as clams with clade D symbionts were from areas with higher temperatures during the month of collection and the month prior to collection, but there was no difference in mean temperatures over the entire year of collection or the entire temperature-data time series (Fig. 5). This implies that clade D may be a transient symbiont clade whose distribution is determined by recent environmental history, as has been suggested in other recent studies (Baker et al. 2004, Berkelmans & van Oppen 2006, Thornhill et al. 2006, LaJeunesse et al. 2009).

Cumulative temperature data from the MODIS satellite document that the waters of NE Raja Ampat are warmer, on average, than waters in SW Raja Ampat (Fig. 4). Giant clams located in the warmer waters of NE Raja Ampat were predicted to have a higher proportion of clade C and D *Symbiodinium* types, thought to be thermally tolerant types based on previous studies in clams (Sison 2003) and corals (reviewed in Baker 2003). The results reported here

are consistent with this prediction; clams in NE Raja Ampat associated more often with clade C symbionts than expected, and clams in SW Raja Ampat associated more frequently with clade A types. This result is consistent with previous studies in corals, reporting temperature influences on symbiont community structure in some species, most notably when coral hosts bleach in response to high temperatures (Rowan et al. 1997, Glynn et al. 2001, Kinzie et al. 2001, Baker 2003, Douglas 2003).

Little experimental research has been conducted on bleaching in giant clams. Preliminary studies show that *Tridacna gigas* and *T. derasa* with clade A symbionts (A6 and A3a identified by PCR-DGGE of ITS2) succumbed to bleaching after 6 d of exposure to an elevated temperature of 33.5°C, whereas clams with clade C symbionts (C2) did not (Sison 2003). Clams containing clade A symbionts that were exposed to elevated temperature experienced severe declines in symbiont densities, leading to significant reductions in their photosynthetic rates, tissue weight, and host respiration. In contrast, the same 2 clam species with clade C symbionts did not show significant changes in algal density, photosynthetic rates, tissue weight, or respiration rates when maintained at 33.5°C. Photosynthetic measurements, using respirometry, on cultured *Symbiodinium* revealed that the maximum photosynthetic capacities of clade A types were negatively affected by short-term exposure to elevated temperature of 35°C, whereas the clade C *Symbiodinium* were not (Sison 2003). These experimental results are consistent with our current finding that giant clams from warmer waters (e.g. NE Raja Ampat) are more likely to associate with thermally tolerant symbionts.

Potential use of clams in reef-restoration efforts

Many invertebrate hosts routinely release intact *Symbiodinium* cells as a means of regulating symbiont density within the host (Muscatine & Pool 1979, Hoegh-Guldberg & Smith 1989, Baghdasarian & Muscatine 2000, Yamashita et al. 2011). Clams in natural conditions expel high numbers of intact symbionts in their feces at rates of 4.9×10^5 cells d^{-1} (5.6 cm shell length; Maruyama & Heslinga 1997, Buck et al. 2002). These rates are much higher than corals, which are an order of magnitude lower (specific expulsion rates of $1–20 \times 10^4$ cells d^{-1}; Hoegh-Guldberg & Smith 1989). Since clams represent relatively high-density aggregations of symbionts, with high release rates, it is possible that high densities of clams could contribute significantly to the local availability of symbionts on reefs. Tridacnid clams have been successfully farmed and transplanted around the Philippines for over 2 decades (Gomez & Mingoa-Licuanan 2006), and can be raised with different symbionts depending on the inoculates supplied at the larval stage (Fitt & Trench 1981, Fitt 1985, Molea & Munro 1994). Farmed clams raised with particular symbiont types (such as *Symbiodinium* D1a, which has been shown to be thermotolerant in corals and which may behave similarly in clams as seen in the present study) could therefore be used as 'incubators' to assist in the recovery of reefs devastated by bleaching events. By increasing the availability of thermotolerant symbionts, clams could help increase the local resilience of reefs to bleaching. Many questions remain (longevity of thermotolerant symbionts in clams following outplanting, acquisition of symbionts expelled by corals, and their longevity in corals if acquired), but given the paucity of management strategies to increase bleaching resistance in the face of warming ocean temperatures, such ideas may be worth testing in the field.

Acknowledgements. We thank MENRISTEK and LIPI for permitting our research program across Indonesia under permits 1187/SU/KS/2006 and 04239/SU.3/KS/2006; the Cenderawasih National Park Authority, BKSDA Papua, and DKP Raja Ampat for permissions to collect samples in Papua; and PHKA for permission to export samples under CITES export permits 09114/IV/SATS-LLN/2005 and 07219/IV/SATS-LN/2006. D. Pada and C. Rotinsulu of Conservation International Indonesia Marine Program provided logistical support and collected samples in the field. C. J. Starger provided assistance collecting molecular data, and E. J. Sbrocco assisted with analysis of satellite data. This research was funded by grants to P.H.B. from Conservation International (through the David and Lucile Packard Foundation Ecosystem Based Management of the Bird's Head Seascape project and the Walton Family Foundation's Bird's Head Seascape initiative) and the National Science Foundation (Biological Oceanography, OCE-0349177) with additional support from a grant from The American Museum of Natural History to T.S.D. Also, T.S.D. was partially supported during writing of this manuscript by NSF PIRE Award OISE-0730256 to P.H.B. and K. E. Carpenter. A.C.B. was supported by the Wildlife Conservation Society (Tiffany & Co. Foundation), the Lenfest Ocean Program, and a Pew Fellowship in Marine Conservation.

LITERATURE CITED

Allen GR, Erdmann MV (2009) Reef fishes of the Bird's Head Peninsula, West Papua, Indonesia. Check List 5:587–628 Available at: http://www.checklist.org.br/getpdf?SL032-09

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Apprill AM, Gates RD (2007) Recognizing diversity in coral symbiotic dinoflagellate communities. Mol Ecol 16: 1127–1134

Baghdasarian G, Muscatine L (2000) Preferential expulsion of dividing algal cells as a mechanism for regulating algal-cnidarian symbiosis. Biol Bull (Woods Hole) 199: 278–286

Baillie BK, Belda-Baillie CA, Maruyama T (2000a) Conspecificity and Indo-Pacific distribution of *Symbiodinium* genotypes (Dinophyceae) from giant clams. J Phycol 36: 1153–1161

Baillie BK, Belda-Baillie CA, Silvestre V, Sison M, Gomez AV, Gomez ED, Monje V (2000b) Genetic variation in *Symbiodinium* isolates from giant clams based on random-amplified-polymorphic DNA (RAPD) patterns. Mar Biol 136:829–836

Baird A, Bhagooli R, Ralph P, Takahashi S (2009) Coral bleaching: the role of the host. Trends Ecol Evol 24:16–20

Baker AC (2001) Reef corals bleach to survive change. Nature 411:765–766

Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium.* Annu Rev Ecol Evol Syst 34:661–689

Baker AC, Romanski AM (2007) Multiple symbiotic partnerships are common in scleractinian corals, but not in octocorals: comment on Goulet (2006). Mar Ecol Prog Ser 335:237–242

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

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Corals' adaptive response to climate change. Nature 430: 741

Banaszak AT, LaJeunesse TC, Trench RK (2000) The synthesis of mycosporine-like amino acids (MAAs) by cultured, symbiotic dinoflagellates. J Exp Mar Biol Ecol 249: 219–233

Banaszak AT, Santos MG, LaJeunesse TC, Lesser MP (2006) The distribution of mycosporine-like amino acids (MAAs) and the phylogenetic identity of symbiotic dinoflagellates in cnidarian hosts from the Mexican Caribbean. J Exp Mar Biol Ecol 337:131–146

Barber P, Boyce SL (2006) Estimating diversity of Indo-Pacific coral reef stomatopods through DNA barcoding of stomatopod larvae. Proc R Soc Lond B Biol Sci 273: 2053–2061

Barber PH, Erdmann MV, Palumbi SR (2006) Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. Evolution 60:1825–1839

Barbrook AC, Visram S, Douglas AE, Howe CJ (2006) Molecular diversity of dinoflagellate symbionts of cnidaria: the *psbA* minicircle of *Symbiodinium.* Protist 157: 159–171

Belda-Baillie CA, Sison M, Silvestre V, Villamor K, Monje V, Gomez ED, Baillie BK (1999) Evidence for changing symbiotic algae in juvenile tridacnids. J Exp Mar Biol Ecol 241:207–221

Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. Proc R Soc Lond B Biol Sci 273:2305–2312

Buck BH, Rosenthal H, Saint-Paul U (2002) Effect of increased irradiance and thermal stress on the symbiosis of *Symbiodinium microadriaticum* and *Tridacna gigas.* Aquat Living Resour 15:107–117

Buddemeier RW, Fautin DG (1993) Coral bleaching as an adaptive mechanism: a testable hypothesis. Bioscience 43:320–326

Carlos AA, Baillie BK, Maruyama T (2000) Diversity of dinoflagellate symbionts (zooxanthellae) in a host individual. Mar Ecol Prog Ser 195:93–100

Chen CA, Wang AT, Fang LS, Yang YW (2005) Fluctuating algal symbiont communities in *Acropora palifera* (Scleractinia: Acroporidae) from Taiwan. Mar Ecol Prog Ser 295:113–121

Coffroth MA, Poland DM, Petrou EL, Brazeau DA, Holmberg JC (2010) Environmental symbiont acquisition may not be the solution to warming seas for reef-building corals. PLoS One 5:e13258

Correa AM, McDonald MD, Baker AC (2009) Development of clade-specific *Symbiodinium* primers for quantitative PCR (qPCR) and their application to detecting clade D symbionts in Caribbean corals. Mar Biol 156:2403–2411

DeBoer TS, Subia MD, Ambariyanto, Erdmann MV, Kovitvongsa K, Barber PH (2008) Phylogeography and limited genetic connectivity in the endangered boring giant clam across the coral triangle. Conserv Biol 22: 1255–1266

Donnelly R, Neville D, Mous P (2003) Report on a rapid ecological assessment of the Raja Ampat islands, Papua, eastern Indonesia held October 30–November 22, 2002, Bali The Nature Conservancy–Southeast Asia Center for Marine Protected Areas, Sanur, Bali. www.reefbase.org/resource_center/publication/pub_22900.aspx

Douglas AE (2003) Coral bleaching — how and why? Mar Pollut Bull 46:385–392

Dunn SR, Weis VM (2009) Apoptosis as a post-phagocytic winnowing mechanism in a coral-dinoflagellate mutualism. Environ Microbiol 11:268–276

Fabricius KE, Mieog JC, Colin PL, Idip D, Van Oppen MJH (2004) Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. Mol Ecol 13:2445–2458

Fitt WK (1985) The effect of different strains of the zooxanthellae *S. microadriaticum* on growth and survival of their coelenterate and molluscan hosts. Proc 5th Int Coral Reef Symp, Tahiti 6:131–136

Fitt WK, Trench RK (1981) Spawning, development and acquisition of zooxanthellae by *Tridacna squamosa* (Mollusca, Bivalvia). Biol Bull (Woods Hole) 161:213–235

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Glynn PW, Mate JL, Baker AC, Calderon MO (2001) Coral bleaching and mortality in Panama and Ecuador during the 1997-1998 El Niño-Southern Oscillation event: spatial/temporal patterns and comparisons with the 1982–1983 event. Bull Mar Sci 69:79–109

Gomez ED, Mingoa-Licuanan SS (2006) Achievements and lessons learned in restocking giant clams in the Philippines. Fish Res 80:46–52

Goulet TL, LaJeunesse TC, Fabricius K (2008) Symbiont specificity and bleaching susceptibility among soft corals in the 1998 Great Barrier Reef mass coral bleaching event. Mar Biol 154:795–804

Hoegh-Guldberg O, Smith GJ (1989) The effect of sudden changes in temperature, light and salinity on the popula-

tion density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. J Exp Mar Biol Ecol 129:279–303

Ishikura M, Hagiwara K, Takishita K, Haga M, Iwai K, Maruyama T (2004) Isolation of new *Symbiodinium* strains from tridacnid giant clam (*Tridacna crocea*) and sea slug (*Pteraeolidia ianthina*) using culture medium containing giant clam tissue homogenate. Mar Biotechnol 6:378–385

Jones AM, Berkelmans R, van Oppen MJH, Mieog JC, Sinclair W (2008) A community change in algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proc R Soc Lond B Biol Sci 275:1359–1365

Kinzie RA, Takayama M, Santos SR, Coffroth MA (2001) The adaptive bleaching hypothesis: experimental tests of critical assumptions. Biol Bull (Woods Hole) 200:51–58

Kirk NL, Andras JP, Harvell CD, Santos SR, Coffroth MA (2009) Population structure of *Symbiodinium* sp. associated with the common sea fan, *Gorgonia ventalina*, in the Florida Keys across distance, depth, and time. Mar Biol 156:1609–1623

Kochzius M, Nuryanto A (2008) Strong genetic population structure in the boring giant clam, *Tridacna* crocea, across the Indo-Malay Archipelago: implications related to evolutionary processes and connectivity. Mol Ecol 17: 3775–3787

LaJeunesse TC (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a 'species' level marker. J Phycol 37:866–880

LaJeunesse TC (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Mar Biol 141:387–400

LaJeunesse TC (2005) 'Species' radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene–Pliocene transition. Mol Biol Evol 22:570–581

LaJeunesse TC, Bhagooli R, Hidaka M, DeVantier L and others (2004) Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. Mar Ecol Prog Ser 284:147–161

LaJeunesse TC, Reyes-Bonilla H, Warner ME, Wills M, Schmidt GW, Fitt WK (2008) Specificity and stability in high latitude eastern Pacific coral-algal symbioses. Limnol Oceanogr 53:719–727

LaJeunesse TC, Smith RT, Finney J, Oxenford H (2009) Outbreak and persistence of opportunistic symbiotic dinoflagellates during the 2005 Caribbean mass coral bleaching event. Proc R Soc Lond B Biol Sci 276:4139–4148

LaJeunesse TC, Pettay T, Phongsuwan N, Brown B, Obura D, Hoegh-Guldberg O, Fitt WK (2010) Long-standing environmental conditions, geographic isolation and host-symbiont influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus *Symbiodinium.* J Biogeogr 37:785–800

Little AF, van Oppen MJH, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. Science 304:1492–1494

Magalon H, Flot JF, Baudry E (2007) Molecular identification of symbiotic dinoflagellates in Pacific corals in the genus *Pocillopora.* Coral Reefs 26:551–558

Maruyama T, Heslinga GA (1997) Faecal discharge of zooxanthellae in the giant clams *Tridacna derasa*, with reference to their in situ growth rate. Mar Biol 127:473–477

McClanahan TR, Ateweberhan M, Ruiz Sebastian C, Graham NAJ, Wilson SK, Bruggemann JH, Guillaume MMM (2007) Predictability of coral bleaching from synoptic satellite and in situ temperature observations. Coral Reefs 26:695–701

Mieog JC, van Oppen MJH, Cantin NC, Stam WT, Olsen JL (2007) Real-time PCR reveals a high incidence of *Symbiodinium* clade D at low levels in four scleractinian corals across the Great Barrier Reef: implications for symbiont shuffling. Coral Reefs 26:449–457

Molea T, Munro PE (1994) Influence of symbiont strain on early growth of tridacnids. Asian Fish Sci 7:91–102

Muscatine L, Pool RR (1979) Regulation of numbers of intracellular algae. Proc R Soc Lond B Biol Sci 204:131–139

Nishiguchi MK (2002) Host–symbiont recognition in the environmentally transmitted sepiolid squid–*Vibrio* mutualism. Microb Ecol 44:10–18

Norton JH, Shepherd MA, Long HM, Fitt WK (1992) The zooxanthellal tubular system in the giant clam. Biol Bull (Woods Hole) 183:503–506

Oliver T, Palumbi S (2009) Distributions of stress-resistant coral symbionts match environmental patterns at local but not regional scales. Mar Ecol Prog Ser 378:93–103

Oliver TA, Palumbi SR (2011) Many corals host thermally resistant symbionts in high-temperature habitat. Coral Reefs 30:241–250

Pochon XP, Gates RD (2010) A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawai'i. Mol Phylogenet Evol 56:492–497

Reimer JD, Todd PA (2009) Preliminary molecular examination of zooxanthellate zoanthids (Hexacorallia: Zoantharia) and associated zooxanthellae (*Symbiodinium* spp.) diversity in Singapore. Raffles Bull Zool Suppl 22: 103–120

Rodriguez-Lanetty M, Hoegh-Guldberg O (2003) Symbiont diversity within the widespread scleractinian coral *Plesiastrea versipora*, across the northwestern Pacific. Mar Biol 143:501–509

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

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

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

Rowan R, Whitney SM, Fowler A, Yellowlees D (1996) Rubisco in marine symbiotic dinoflagellates: form II enzymes in eukaryotic oxygenic phototrophs encoded by a nuclear multigene family. Plant Cell 8:539–553

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

Sampayo EM, Ridgway T, Bongaerts P, Hoegh-Guldberg O (2008) Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. Proc Natl Acad Sci USA 105:10444–10449

Santos SR, Shearer TL, Hannes AR, Coffroth MA (2004) Fine-scale diversity and specificity in the most prevalent lineage of symbiotic dinoflagellates (*Symbiodinium*, Dinophyceae) of the Caribbean. Mol Ecol 13:459–469

Savage AM, Goodson MS, Visram S, Trapido-Rosenthal H, Wiedenmann J, Douglas AE (2002) Molecular diversity of symbiotic algae at the latitudinal margins of their distribution: dinoflagellates of the genus *Symbiodinium*

in corals and sea anemones. Mar Ecol Prog Ser 244:17–26
Schneider JA, Foighil DO (1999) Phylogeny of giant clams (Cardiidae: Tridacninae) based on partial mitochondrial 16S rDNA gene sequences. Mol Phylogenet Evol 13: 59–66
Silverstein RN, Correa AMS, LaJeunesse TC, Baker AC (2011) Novel algal symbiont (*Symbiodinium* spp.) diversity in reef corals of western Australia. Mar Ecol Prog Ser 422:63–75
Sison M (2003) The effects of zooxanthellae clades A and C on giant clam host, in particular to its early growth, survival, recruitment and response to thermal stress. Master's thesis, University of Philippines, Diliman, Quezon City
Stat M, Carter D, Hoegh-Guldberg O (2006) The evolutionary history of *Symbiodinium* and scleractinian hosts—symbiosis, diversity, and the effect of climate change. Perspect Plant Ecol Evol Syst 8:23–43
Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
Tchernov D, Gorbunov MY, de Vargas C, Narayan Yadav S, Milligan AJ, Häggblom M, Falkowski PG (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proc Natl Acad Sci USA 101:13531–13535
Thornhill DJ, LaJeunesse TC, Kemp DW, Fitt WK, Schmidt GW (2006) Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. Mar Biol 148:711–722
Toller WW, Rowan R, Knowlton N (2001a) Repopulation of zooxanthellae in the Caribbean corals *Montastraea annularis* and *M. faveolata* following experimental and disease-associated bleaching. Biol Bull (Woods Hole) 201:360–373
Toller WW, Rowan R, Knowlton N (2001b) Zooxanthellae of the *Montastraea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. Biol Bull (Woods Hole) 201: 348–359
Trench RK, Wethey DS, Porter JW (1981) Observations on the symbiosis with zooxanthellae among the Tridacnidae (Mollusca, Bivalvia). Biol Bull (Woods Hole) 161:180–198
van Oppen MJH, Palstra FP, Piquet AMT, Miller DJ (2001) Patterns of coral-dinoflagellate associations in *Acropora*: significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. Proc R Soc Lond B Biol Sci 268:1759–1767
van Oppen MJH, Mahiny AJ, Done TJ (2005a) Geographic distribution of zooxanthella types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. Coral Reefs 24:482–487
van Oppen MJH, Mieog JC, Sanchez CA, Fabricius KE (2005b) Diversity of algal endosymbionts (zooxanthellae) in octocorals: the roles of geography and host relationships. Mol Ecol 14:2403–2417
Veron JEN, Devantier LM, Turak E, Green AL, Kininmonth S, Stafford-Smith M, Peterson N (2009) Delineating the coral triangle. Galaxea J Coral Reef Stud 11:91–100
Walsh PS, Metzger DA, Higuchi R (1991) Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513
Yamashita H, Suzuki G, Hayashibara T, Koike K (2011) Do corals select zooxanthellae by alternative discharge? Mar Biol 158:87–100

Editorial responsibility: Matthias Seaman, Oldendorf/Luhe, Germany

Submitted: March 29, 2010; Accepted: September 11, 2011
Proofs received from author(s): December 1, 2011