Symbiodinium and Giant Clams (Genus: *Tridacna*): Patterns of Distribution Across Three Host Species in the Biodiverse Bird's Head Region of Indonesia

Timery S. DeBoer
Boston University, Biology Department, 5 Cummington St, Boston, MA 02215, USA
tsdeboer@bu.edu

Ambariyanto
Diponegoro University, Faculty of Fisheries and Marine Sciences, Kampus Tembalang, Semarang, Indonesia
ambariyanto@telkom.net

Mark V. Erdmann
Conservation International, Indonesia Marine Program Jl. Dr. Muwardi No. 17 Bali, Indonesia
mverdmann@gmail.com

Andrew C. Baker
Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA
abaker@rsmas.miami.edu

Abdul Hamid A. Toha
State University of Papua, Faculty of Animal Science, Fisheries and Marine Sciences, Manokwari-West Papua, Indonesia
hamid.toha@unipa.ac.id,

Paul H. Barber
University of California, Los Angeles, Dept. of Ecology and Evolutionary Biology 621 Charles E. Young Dr. South, Los Angeles, CA 90095
paulbarber@ucla.edu

Abstract

Reef corals, other marine invertebrates, and protists are hosts to a group of exceptionally diverse dinoflagellate symbionts in the genus *Symbiodinium*. Numerous studies have documented ecologically-important differences among *Symbiodinium* types in depth zonation, photoadaptation to different irradiance levels, heat tolerance, and susceptibility to bleaching. Many host species are able to maintain associations with multiple symbiont types simultaneously, which may permit rapid adaptation to local environmental change. This study focused on *Symbiodinium* diversity in giant clams (genus *Tridacna*) from the biodiverse Bird's Head region in eastern Indonesia. We identified 12 unique *Symbiodinium* types in 250 host individuals from three Tridacnid species, based on denaturing gradient gel electrophoresis (DGGE) and sequencing of internal transcribed spacer-2 (ITS-2) rDNA. All types were from Clades A, C, and D and were detected in each of the three host species. Individuals with multiple symbionts from different clades were common (42% of all individuals). Symbiont type and host species were significantly associated. *T. crocea* had more individuals with only Clade C symbionts and fewer individuals with mixed clade symbionts than expected. *T. maxima* had fewer individuals with only Clade A symbionts than expected, but more Clade C only and mixed clade symbionts. *T. squamosa* had more individuals with mixed clades than expected. A total of 45 data loggers recorded water temperature at 3 meters within the study area. Giant clams sampled from the warmer waters of the Bay of Cenderawasih had a greater proportion of Clade C symbionts and fewer Clade A symbionts than expected. This is consistent with

previous research on Tridacnid symbionts that showed Clade C types to be more heat tolerant than Clade A. Our results are consistent with those reported for coral hosts and point to the possibility that giant clams may associate with different symbiont types based on local environmental conditions. Further research is necessary to understand the implications of climate change on internationally-protected giant clams and their symbionts.

1. Introduction

Reef corals, other marine invertebrates, and protists are hosts to a group of exceptionally diverse dinoflagellate symbionts in the genus *Symbiodinium*. Extensive phylogenetic investigations, based primarily on the analyses of small subunit (SSU) and large subunit (LSU) nuclear ribosomal (nrDNA) genes, have led to the currently recognized phylogeny of eight distinctive groups in the genus *Symbiodinium*, referred to as clades A, B, C, D, E, F, G and H [*1, 2*]. Further diversity within these clades has been revealed through the use of highly variable markers such as polymorphic microsatellites [*3, 4*], the plastid-coding psbA minicircle [*5*], and nrDNA Internal transcribed spacer regions [*6-11*]. Appreciable differences in the ecology and physiology of these 'types' has been identified in numerous studies [*12-18*].

In many species, single host individuals may contain more than one taxon in residence at any one time [*19, 20*]. Experiments on corals have shown that the greater resistance to high temperatures and irradiation of certain zooxanthellae taxa is indicative that resistance to bleaching is a property of the zooxanthellae and not the animal [*21*]. Therefore, the particular type of zooxanthellae in symbiosis with an animal host could have a major influence on the stability and properties of the resulting association.

Giant clams (genus *Tridacna*) are among the few mollusks to maintain symbioses with *Symbiodinium*. Previous studies using SSU sequences or RAPD patterns have identified giant clam symbionts belonging to Clades A, C, and D [*22*]. Single host individuals commonly associate with symbionts from multiple clades simultaneously [*23, 24*]. To date, no study has focused on identifying *Symbiodinium* from giant clams using the highly variable marker ITS-2. The bulk of recent research on *Symbiodinium* diversity is based on DGGE analysis of ITS-2 rDNA, which is suggested to be the most broadly applicable and appropriate marker for examining and describing *Symbiodinium* diversity [*6*]. And no previous study has attempted to relate symbiont genotype to host clam genotype.

Here, denaturing gradient gel electrophoresis (DGGE) was used to investigate diversity in *Symbiodinium* ITS-2 from giant clams collected from the Bird's Head region of eastern Indonesia. This geographic region is a biodiversity hotspot in the Coral Triangle and is subject to intense conservation efforts by local government and international non-profit organizations. In order to assess the degree of host-symbiont specificity in this system, the cytochrome c oxidase I (COI) marker was used to examine the diversity of giant clam hosts. This study, based on *Symbiodinium* samples isolated from three *Tridacna* species, revealed a diverse assemblage of symbionts and their hosts. The distribution of *Symbiodinium* types discovered during this survey is related to both host species and water temperature.

2. Materials and methods

2.1 Sampling

Giant clams were sampled at 14 locations across the study area between April 2, 2005 and November 22, 2006 (Table 2). All samples were obtained non-destructively by clipping a piece of mantle tissue, which was stored in 95% ethanol. Zooxanthellae are located in

specialized tubes within the clam mantle tissue [*25*]. Because only a portion of the mantle was analyzed from each individual, this sampling strategy resulted in a minimum estimate of symbiont diversity in each individual clam host.

2.2 *Symbiodinium* identification

Whole genomic DNA was extracted using 10% Chelex [*26*]. A portion of the 5.8S, entire internal transcribed spacer 2 region (ITS2) and portion of the 28S rDNA were amplified using the primers 'ITSintfor2' and 'ITS2CLAMP' [*7*]. PCR amplifications were performed in a total volume of 25 ul, with a thermocycling profile consisting of 35 cycles of 30s at 94°C, 30s at 60°C, and 45s at 72°C, followed by a final extension at 72°C for 3 min.

Successful *Symbiodinium* ITS-2 amplicons were analyzed by DGGE using a CBS Scientific System (Del Mar, CA, USA) with denaturing gradient gels (45–80% formamide, 8% polyacrylamide denaturing gradient; 100% consisting of 7 M urea and 40% deionized formamide) for approximately 12 h at 150 volts. Representative DGGE bands and unique profiles (as described by LaJeunesse [*7*]) were excised from each gel and submitted for sequencing to identify symbiont types; and, subsequently, to detect and control for methodological artifacts and intragenomic variation. Direct sequences of these bands were obtained after gel excision and PCR re-amplification following LaJeunesse [*7*], replacing the reverse primer with 'ITS2rev', which lacks the GC clamp [*27*]. The touchdown thermocycling profile consisted of 20 cycles of 30 s at 94°C, 45s at 60-50°C (decreasing 0.5°C each cycle), and 30s at 72°C, followed by a final extension at 72°C for 10 min [*27*]. Sequences were edited in Sequencher 4.5 (Gene Codes Corp., Ann Arbor, MI).

Identical sequences were grouped in individual contigs in Sequencher 4.5. A representative sequence from each contig was uploaded into NCBI BLAST and identified if 100% similar to an existing sequence and named following the alphanumeric system of LaJeunesse [e.g., *7, 28, 29*]. *Symbiodinium* type names start with a letter corresponding to the Symbiodinium clade and followed by a number designating its unique identity within the clade. If no sequence in the database matched 100%, the sequence was named for the closest match with non-matching bases identified (e.g. C15-31 matched type C15 except at base 31).

We identified the symbiont clade present in each host clam. For many individuals, symbiont types from multiple clades were identified. Clams with mixed clades were assigned a equal fraction of each symbiont clade type, since DGGE data cannot reliably estimate relative densities of each type. For example, individuals with Clade A and C symbionts were designated as 50% Clade A and 50% Clade C in all analyses, unless otherwise specified.

2.3 Phylogenetic analysis of host clams

Approximately 500bp of the mitochondrial cytochrome *c* oxidase subunit-1 gene (CO1) was amplified from 10% Chelex DNA extractions [*30*] via polymerase-chain reaction (PCR) with *Tridacna*-specific primers [*31*]. Hotstart thermocycling parameters were: 80°C hold, initial denaturation at 94°C/15 sec, and 38 cycles of 94°C/30 sec, 50°C/30 sec, 72°C/45 sec, and a final extension of 72°C/3 min. PCR products were prepared for sequencing following Barber et al [*32*]. Forward and reverse stands were sequenced on an ABI 377 or an ABI 3730 automated sequencer using Big Dye (Applied Biosystems, Foster City, CA) terminator chemistry. Sequences were proofread and aligned in Sequencher 4.5. Protein translations were confirmed in MacClade 4.05 [*33*]. A CO1 genetree was constructed using the neighbor-joining method as implemented in PAUP* 4.0b10 [*34*].

2.4 Temperature data

A total of 24 Hobo v.2 and Tidbit data loggers (Onset Computer Corp.), placed at 3 meter depth, recorded temperature every 15 minutes between Sept 2005 and Feb 2007 (Fig. 1; Table 1). Data from all loggers located within the Bay of Cenderawasih (n = 7) and data from all loggers outside the bay around Raja Ampat (n = 17) were averaged to summarize data for these two ecologically-distinct areas.

3. Results

3.1 *Symbiodinium* identifications

Positive PCR amplifications and scoreable DGGE profiles were obtained for a total of 250 *Tridacna* hosts, including 161 *T. crocea*, 28 *T. maxima*, and 61 *T. squamosa*. The observed profiles were typically characterized by a few distinctive bands, corresponding to a specific fingerprint of the ITS-2 rDNA region.

In total, 12 unique *Symbiodinium* ITS-2 sequence types were identified during this survey. Their host distribution, abundance and corresponding number of sequences obtained for type verification, as well as the sequences size and GenBank accession numbers, are shown in Table 3. The most common type is a Clade C symbiont with an ITS-2 sequence identical to GenBank Accession number AF195157.1 previously isolated from a *Tridacna* host. This sequence differs from LaJeunesse's C15 sequence at a single base. Hereafter, this sequence will be called C15-31. All symbiont types were observed in all three host species, with the exception of sequences obtained from only a single individual, and type A3-223 which was only observed in *T. crocea* (Table 3).

Often, mixed *Symbiodinium* genotypes were detected within a single host specimen. Symbionts from multiple clades were detected in 106 (42%) hosts, including 49 (80%) *T. squamosa*, 9 (32%) *T. maxima*, and 48 (30%) *T. crocea*. Intra-clade diversity within a single host was detected in 38 (15%) of all host clams.

3.2 Host diversity

In order to assess the degree of specificity between the Tridacnid clams and their symbionts, we sequenced the mtDNA gene cytochrome *c* oxidase I (COI) of all host clams. A total of 113 unique haplotypes were found during this survey (Fig. 2).

Genetic structure within species is apparent, with distinct clades present in *T. crocea* (as previously reported [*31*, *35*]), and in *T. maxima* (Fig. 2). Genetic structure is not apparent in *T. squamosa* sequences, but sample sizes for this species are low.

3.3 Host-symbiont specificity

The proportion of *Symbiodinium* types within each host species, and within each clade for *T. crocea* is represented by pie charts in Figure 2. At the level of host species, specificity is low; individuals from all 3 host species contain Type A, C, and D symbionts. However, the relative percentages of each symbiont type differ markedly between host species. Host species identity and symbiont clade are not independent (chi-square test for independence, p < 7.34E-13, Figure 2). *T. crocea* individuals have more individuals with Clade C symbionts and fewer Clade D symbionts than expected. We did not detect a difference between observed and expected symbiont types in *T. maxima* individuals, although sample sizes are small. *T.*

squamosa individuals hosted fewer Clade C and more Clade D symbionts, than expected by chance.

Considering individuals where we did detect mixed symbiont clades, Clade D did not randomly associate with the other two observed clades (chi-squared test of independence, $p < 1.3E-05$). If mixed, Clade D symbionts appear more often with Clade A types. This result is not dependent on host species; Clade D is found more often with Clade A in both *T. crocea* and *T. squamosa* hosts (both $p < 2.7E-09$). There were too few mixed-clade *T. maxima* individuals to test for an association within that host species.

Host and symbiont interact intimately in this system. Therefore, host-symbiont genotype pairings may also be non-random. Due to small sample sizes for other clades, we were only able to test for an association between host clade and symbiont clade in *T. crocea.* Symbiont-host associations at the clade level are not independent ($p = 9E-06$). Black clade *T. crocea* more commonly have Clade A only symbionts than expected. Blue clade *T. crocea* occur less with Clade A and more often with Clade C symbionts than expected. However, this result appears to be an artifact of the spatial distribution of host clades themselves. At individual locations where both host clades are common (Adoki, Owi, Jefman, and Yapen), associations between host clades and symbionts do not differ from expected values (all $p > 0.50$, see Figure 3).

3.4 Temperature and symbiont distribution

A total of 24 data loggers, placed at 3 meter depth, recorded temperature every 15 minutes between July 2005 and November 2008. Not all loggers collected data over this entire time period. Therefore, data from all loggers located within the Bay of Cenderawasih ($n = 7$) and data from all loggers outside the bay around Raja Ampat ($n = 17$) was averaged to summarize the thermal environment for these two ecologically-distinct areas. Average water temperature is significantly higher inside the Bay of Cenderawasih compared to outside ($p = 0.005$, t-test; Table 1).

Because of higher average temperatures inside the bay, we predicted that a greater number of host clams would contain heat-tolerant Clade C and D *Symbiodinium* within the bay, compared to areas outside. Host clam location (e.g. inside bay or outside bay) and symbiont type are not independent ($p < 7.72E-11$, chi-squared test for independence). This result remains consistent if each host species is analyzed independently (data not shown). Host clams sampled within the Bay of Cenderawasih had fewer Clade A and more Clade C symbionts than expected (Figure 3).

4. Discussion

High proportion of hosts with mixed *Symbiodinium* clades

Early work based on restriction fragment length polymorphism (RFLP) data from the small subunit ribosomal RNA found that most of the zooxanthellae populations sampled in giant clams are of a single genetic population [*12*]. However, studies employing other, more variable, molecular markers have shown giant clams can harbor a mixture *Symbiodinium* types [*23, 36-38*]. The bulk of recent research on *Symbiodinium* diversity is based on DGGE analysis of ITS-2 rDNA, which is suggested to be the most broadly applicable and appropriate marker for examining and describing *Symbiodinium* diversity [*6*]. In this study, many host individuals (42%) harbored *Symbiodinium* from multiple clades. The ecological significance of polymorphic symbiosis is not known, but it has been suggested that polymorphic symbiosis

may have an adaptive advantage during periods of environmental perturbations such as bleaching [*39*].

It is important to note that the symbiont diversity described here should be considered a minimum estimate for two reasons. First, rare types present at 5-10% of the total symbiont population may not be detected by DGGE [*40*]. Second, only a portion of host mantle tissue was analyzed for symbionts and other types may occur throughout the large tissue area.

Host-symbiont specificity

Giant clams do not maternally transfer symbionts to their eggs [*41*]; clam veligers acquire their zooxanthellae from the water column through filter-feeding. Such 'horizontal acquisition' of symbionts allows individual clams the potential to establish symbiosis with any zooxanthellae genotypes present in the water column. Whether or not giant clams 'select' specific types of zooxanthellae remains unknown.

The results of our study suggest that giant clam hosts do not associate randomly with different zooxanthellae. Instead, *T. crocea* individuals have more individuals with only Clade C symbionts and fewer individuals with mixed clade symbionts than expected. *T. maxima* has fewer individuals with only Clade A symbionts than expected, but more Clade C only and mixed clade symbionts. *T. squamosa* has more individuals with mixed clades than expected. Some of this variation could be explained by small habitat differences between species, particularly depth. Symbiont community structure is associated with depth in numerous studies [*reviewed in 22*]. *T. crocea* and *T. maxima* inhabit shallow waters (0.5 m - 7 m), and *T. squamosa* is found slightly deeper (< 15 m). Depth data was not collected for individuals collected for this study. Future sampling efforts should record depth of the host clam to determine what effect this variable has on determining symbiont community structure.

However, we did find within-host-clade specificity in *T crocea.* Individuals of the same species should occur at similar depths. The two host clades, as determined by COI sequence, do not associate with the same symbiont types in equal frequency. Black clade individuals associate with Clade A only, Clade C only, and mixed clade *Symbiodinium* in equal proportions, but blue clade *T. crocea* are more commonly associated with Clade C symbionts. These results suggest that there may be beneficial host-symbiont genotype combinations in this system. However, this result may be an artifact of the spatial distribution of host clades themselves. At individual locations where both host clades are common (Adoki, Owi, Jefman, and Yapen), associations between host clade and symbiont clade do not differ from expected values (all $p > 0.50$, see Figure 3). To better understand host-clade and symbiont clade specificity, more sampling is required at sites containing equal proportions of each clade to avoid site-specific effects confounding the results.

The effect of temperature on *Symbiodinium* types in giant clams

Long-term water temperature data collected within the study area has documented that the Bay of Cenderawasih is warmer, on average, than areas outside the bay in Raja Ampat. Temperature is known to influence symbiont community structure in corals, most notably when coral hosts bleach in response to high temperatures [*22, 42-44*]. Based on these well-supported results for corals, we predicted that giant clams located in the warmer waters of the bay would have a higher proportion of thermally-tolerant Clade C and D *Symbiodinium* types. Our results are consistent with this prediction- clams within the bay associated more often with Clade C symbionts than expected, and clams outside the bay associated more frequently with Clade A types.

Little experimental research has been done on bleaching in giant clams. Preliminary studies conducted at the University of Philippines Marine Science Institute show that *T. gigas* and *T. derasa* with Clade A symbionts succumb to bleaching after 6 days of exposure to elevated temperature of 33.5ºC, whereas the clams with Clade C symbionts resisted bleaching at the same temperature [*45*]. The algal populations of individuals in both species with Clade A symbionts were severely reduced in response to elevated temperature, which led to the significant reduction in their photosynthetic rates, tissue weight and CZAR. The respiration rates of the associations also increased significantly. In contrast, *T. gigas* and *T. derasa* with Clade C symbionts, on the other hand, did not show any significant change in algal density, photosynthetic rates, tissue weight, calculated CZAR, or increase in respiration rates when maintained at 33.5ºC. Photosynthetic measurements on cultured zooxanthellae species revealed that the maximum photosynthetic capacities of Clade A zooxanthellae types were negatively affected by short-term exposure to elevated temperature of 35ºC whereas the Clade C zooxanthellae species were not [*45*].

Future research

How and why do multiple symbiont types persist within a single host? The answer to this question may be especially interesting for giant clam hosts, which have a specialized zooxanthellae tube system [*46*] to house symbionts that generate microhabitats for different symbionts. The advantages that a host gains from its association with different zooxanthellae types may depend on the extent of variation among different symbiont types. Hosts that have a wider range of symbiont 'tolerance' may have an adaptive advantage during periods of rapid environmental change. Future research into the distribution of multiple types within a single individual is necessary. In addition, ecological experiments to test host fitness with single versus mixed symbiont types would also contribute to our understanding of this system.

Many coral reef species may be able to survive temperature increases if hosts are able to swap their algal symbionts for more thermally tolerant varieties [*47*]. Buddemeier and Fautin [*48*] proposed the “adaptive bleaching hypothesis (ABH)”, which predicts that changing combinations of hosts and symbiotic algae have the potential to create new ‘ecospecies’ with different environmental tolerances. Disturbances, such as environmental change, bleaching, or disease might provide an opportunity for new opportunistic partnerships to become established [*49, 50, 39, 51*]. Reciprocal depth transplants and controlled temperature experiments with simultaneous monitoring of symbiont communities are necessary to test the adaptive bleaching hypothesis in giant clams.

Literature Cited

1. M. A. Coffroth, S. R. Santos, *Protist* **156**, 19 (Jun, 2005).
2. M. Stat, D. Carter, O. Hoegh-Guldberg, *Perspectives in Plant Ecology Evolution and Systematics* **8**, 23 (2006).
3. H. Magalon, J.-F. Flot, E. Baudry, *Coral Reefs* **26**, 551 (2007).
4. S. R. Santos, T. L. Shearer, A. R. Hannes, M. A. Coffroth, *Molecular Ecology* **13**, 459 (Feb, 2004).
5. A. C. Barbrook, S. Visram, A. E. Douglas, C. J. Howe, *Protist* **157**, 159 (Jun, 2006).
6. T. C. LaJeunesse, *Journal of Phycology* **37**, 866 (Oct, 2001).
7. T. C. LaJeunesse, *Marine Biology* **141**, 387 (Aug, 2002).
8. M. Rodriguez-Lanetty, O. Hoegh-Guldberg, *Marine Biology* **143**, 501 (Sep, 2003).
9. M. J. H. van Oppen, A. J. Mahiny, T. J. Done, *Coral Reefs* **24**, 482 (Nov, 2005).

10. M. J. H. Van Oppen, J. C. Mieog, C. A. Sanchez, K. E. Fabricius, *Molecular Ecology* **14**, 2403 (Jul, 2005).
11. M. J. H. van Oppen, F. P. Palstra, A. M. T. Piquet, D. J. Miller, *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**, 2617 (Dec, 2001).
12. A. C. Baker, R. Rowan, N. Knowlton, *Proceedings of the 8th International Coral Reef Symposium* **2**, 1295 (1997).
13. C. A. Belda-Baillie *et al.*, *Journal of Experimental Marine Biology and Ecology* **241**, 207 (Aug, 1999).
14. A. A. Carlos, B. K. Baillie, M. Kawachi, T. Maruyama, *Journal of Phycology* **35**, 1054 (Oct, 1999).
15. C. A. Chen, A. T. Wang, L. S. Fang, Y. W. Yang, *Marine Ecology-Progress Series* **295**, 113 (2005).
16. S. Karako-Lampert, D. J. Katcoff, Y. Achituv, Z. Dubinsky, N. Stambler, *Journal of Experimental Marine Biology and Ecology* **311**, 301 (Nov, 2004).
17. R. Rowan, N. Knowlton, *Proceedings of the National Academy of Sciences of the United States of America* **92**, 2850 (Mar, 1995).
18. R. Rowan, N. Knowlton, A. Baker, J. Jara, *Nature* **388**, 265 (Jul, 1997).
19. A. C. Baker, A. M. Romanski, *Marine Ecology-Progress Series* **335**, 237 (2007).
20. R. a. K. Rowan, N, *Proceedings of the National Academy of Sciences* **92**, 2850 (1995).
21. W. K. Fitt, M. E. Warner, *Biological Bulletin* **189**, 298 (Dec, 1995).
22. A. C. Baker, *Annual Reviews in Ecology, Evolution, and Systematics* **34**, 661 (2003).
23. B. K. Baillie, C. A. Belda-Baillie, T. Maruyama, *Journal of Phycology* **36**, 1153 (Dec, 2000).
24. A. A. Carlos, B. K. Baillie, T. Maruyama, *Marine Ecology-Progress Series* **195**, 93 (2000).
25. J. H. Norton, H. C. Prior, B. Baillie, D. Yellowlees, *Journal of Invertebrate Pathology* **66**, 307 (Nov, 1995).
26. P. S. Walsh, D. A. Metzger, R. Higuchi, *Biotechniques* **10**, 506 (Apr, 1991).
27. A. M. Apprill, R. D. Gates, *Molecular Ecology* **16**, 1127 (Mar, 2007).
28. T. C. LaJeunesse, *Molecular Biology and Evolution* **22**, 1158 (Apr, 2005).
29. T. C. LaJeunesse, S. Lee, S. Bush, J. F. Bruno, *Coral Reefs* **24**, 157 (Mar, 2005).
30. P. S. Walsh, D. A. Metzger, R. Higuchi, *BioTechniques* **10**, 506 (1991).
31. T. S. DeBoer *et al.*, *Conservation Biology* **22**, 1255 (Oct, 2008).
32. P. H. Barber, M. V. Erdmann, S. R. Palumbi, *Evolution* **60**, 1825 (Sep, 2006).
33. W. P. Maddison, D. R. Maddison. (Sinauer, Sunderland, MA, 2002).
34. D. L. Swofford. (Sinauer, Sunderland, MA, 2002).
35. M. Kochzius, A. Nuryanto, *Molecular Ecology* **17**, 3775 (Sep, 2008).
36. B. K. Baillie *et al.*, *Marine Biology* **136**, 829 (Jun, 2000).
37. B. K. Baillie, V. Monje, V. Silvestre, M. Sison, C. A. Belda-Baillie, *Proceedings of the Royal Society of London Series B-Biological Sciences* **265**, 1949 (Oct, 1998).
38. R. Rowan, S. M. Whitney, A. Fowler, D. Yellowlees, *Plant Cell* **8**, 539 (Mar, 1996).
39. R. Rowan, *Journal of Phycology* **34**, 407 (Jun, 1998).
40. D. J. Thornhill, M. W. Daniel, T. C. LaJeunesse, G. W. Schmidt, W. K. Fitt, *Journal of Experimental Marine Biology and Ecology* **338**, 50 (Oct, 2006).
41. W. K. Fitt, R. K. Trench, *Biological Bulletin* **161**, 213 (1981).
42. A. E. Douglas, *Marine Pollution Bulletin* **46**, 385 (Apr, 2003).
43. O. Hoegh-Guldberg, *Marine and Freshwater Research* **50**, 839 (1999).

44. R. A. Kinzie, M. Takayama, S. R. Santos, M. A. Coffroth, *Biological Bulletin* **200**, 51 (Feb, 2001).
45. M. Sison. (University of Philippines, Diliman).
46. J. H. Norton, M. A. Shepherd, H. M. Long, W. K. Fitt, *Biological Bulletin* **183**, 503 (Dec, 1992).
47. A. C. Baker, *Nature* **411**, 765 (Jun, 2001).
48. R. W. Buddemeier, D. G. Fautin, *Bioscience* **43**, 320 (May, 1993).
49. A. C. Baker, *Nature* **411**, 765 (Jun, 2001).
50. A. C. Baker, *Nature* **415**, 602 (Feb, 2002).
51. W. W. Toller, R. Rowan, N. Knowlton, *Biological Bulletin* **201**, 348 (Dec, 2001).

Figures & Tables

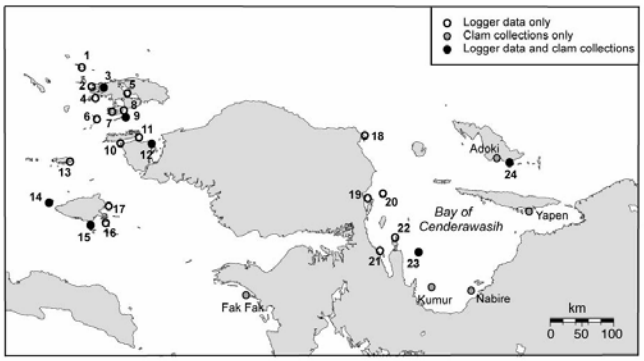

Figure 1. Map of Raja Ampat, Indonesia showing location of temperature loggers and clam sampling sites. Numbers correspond to locality information in Table 1.

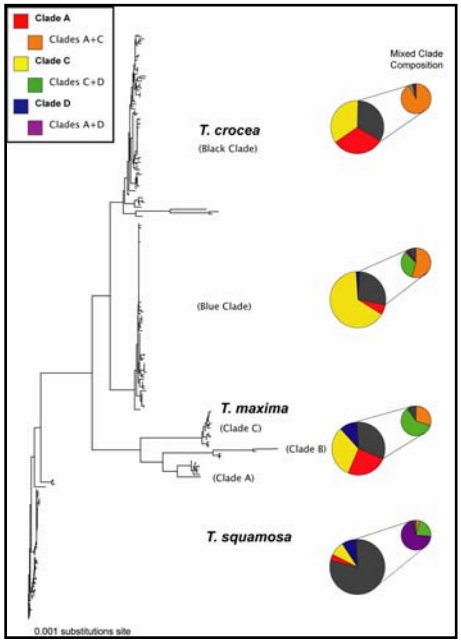

Figure 2. Neighbor-joining tree of all *Tridacna* COI sequences showing major clades within each species. Large circles represent symbiont communities in each host species or host clade. Smaller circles represent the composition of 'mixed' clade individuals.

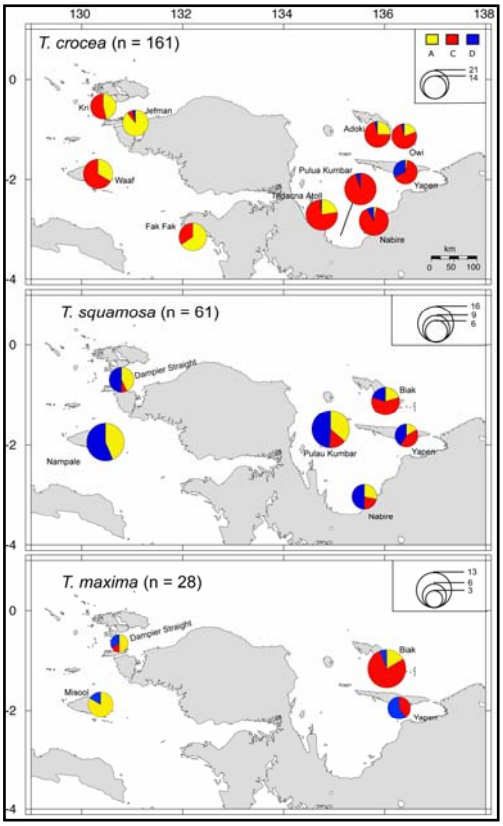

Figure 3. Relative frequency of each symbiont clade in each host species. Hosts with 'mixed' clade symbionts are scored as having a percentage of each clade, as explained in methods.

Table 1. Location and summary of data from temperature loggers. Data is summarized for Raja Ampat and Bay of Cenerawasih loggers. See Figure 1 for map of locations.

Region	Map #	Location	Avg	Min	Max	# days with data	GPS Coordinates
RA	1	Wayag	28.957	27.37	30.37	956	N 00°09.845' E 130°00.644'
RA	2	Kawe rocks (Roibe)	28.464	26.15	29.94	726	S 00°06.547' E 130°11.943'
RA	3	Alyui Bay Cendana	28.615	27.36	28.74	368	S 00°11.318' E 130°15.246'
RA	4	Batang Pele (Suaka Margasatwa Laut	28.836	27.18	30.62	702	S 00°16.700' E 130°13.758'
RA	5	Mayalibit SW Bay 3m	30.707	27.99	34.12	1036	S 00°17.848' E 130°48.490'
RA	6	Fam Group Melissa's Garden	28.870	27.54	30.68	239	S 00°35.390' E 130°18.909'
RA	7	Arborek Manta aggregation site	28.884	27.03	30.07	897	S 00°33.737' E 130°32.495'
RA	8	Mike's Point	29.149	27.94	31.48	652	S 00°30.941' E 130°40.348'
RA	9	Kri EcoResort	28.885	26.40	30.62	824	S 00°33.334' E 130°40.664'
RA	10	West Selat Sagewin	28.470	24.55	30.07	264	S 00°57.019' E 130°39.756'
RA	11	Sagewin Strait 3m	29.077	26.12	31.03	839	S 00°53.552' E 130°55.664'
RA	12	Jefman Island, Sorong Bay, 3m	29.306	26.18	31.54	972	S 00°55.641' E 131°07.408'
RA	13	Kofiau Group	29.474	25.52	30.93	1037	S 01°15.864' E 129°40.789'
RA	14	Nampale mangrove channels	28.600	24.73	30.27	597	S 01°47.873' E 129°38.570'
RA	15	SE Waaf Island (SE Misool)	28.602	26.06	30.90	631	S 02°08.936' E 130°13.283'
RA	16	Fiabacet Rocks	28.829	26.48	31.13	668	S 02°13.333' E 130°29.543'
RA	17	Inner Misool Karst Bay 1	28.848	25.94	30.95	667	S 01°58.928' E 130°27.574'
		AVERAGE	28.975	26.50	30.79		
		WEIGHTED AVERAGE	29.079	26.55	30.97		
Bay	18	Pulau Lemon, Manokwari	29.551	27.10	31.13	1137	S 00°53.395' E 134°04.782'
Bay	19	Rumberpon, Teluk Cenderawasih	29.514	28.12	30.85	615	S 01°44.227' E 134°12.146'
Bay	20	Pulau Nusambier, TC	29.609	28.37	31.59	617	S 01°58.820' E 134°41.723'
Bay	21	Pulau Yop, Teluk Cenderawasih	29.774	28.15	31.56	1113	S 02°30.427' E 134°22.670'
Bay	22	Pulau Roon, T. Cenderawasih	29.630	28.36	31.16	496	S 02°16.840' E 134°33.787'
Bay	23	Tridacna Atoll, T. Cenderawasih	29.765	28.42	31.20	505	S 02°29.691' E 134°58.997'
Bay	24	Pulau Owi, BIAK	29.430	28.40	30.63	143	S 01°14.870' E 136°11.225'
		AVERAGE	29.610	28.13	31.16		
		WEIGHTED AVERAGE	29.635	27.98	31.25		

Table 2. Collection locality, date, and sample sizes for giant clam tissue collections.

Host species	Region	Locality	Date Collected	*n*
T. crocea	Biak	Adoki Village	4/3/2005	14
		Owi	4/5/2005	13
	Fakfak	Tuburwasa	5/4/2006	16
	Nabire		2/15/2006	18
	Dampier Straight	Kri	7/4/2005	14
		Jefman Island	7/10/2005	14
	Misool	Waaf	11/22/2005	18
	Teluk Cenderawisih	Pulau Kumbur	2/18/2006	21
		Tridacna Atoll	9/10/2005	21
	Yapen		4/06 - 4/8/05	12
			Total T. crocea	161
T. maxima	Biak	Pulau Rasba	4/12/2005	3
		Owi	4/5/2005	10
	Dampier Straight	Kri	7/4/2005	2
		Jefman Island	7/10/2005	1
	Misool	Nampale	11/21/2005	4
		Waaf	11/22/2005	2
	Teluk Cenderawisih	Pulau Kumbar	2/18/2006	1
	Yapen	Serui Fish Market	4/6 & 4/08/05	5
			Total T. maxima	28
T. squamosa	Biak	Pulau Rasba	4/12/2005	7
		Owi	4/5/2005	2
	Nabire		2/15/2006	7
	Dampier Straight	Teluk Mayalibit	7/8/2005	3
		Alyui Bay	7/5/2005	1
		Jefman Island	7/10/2005	3
	Misool	Nampale	11/21/2005	16
	Teluk Cenderawisih	Pulau Kumbur	2/18/2006	16
	Yapen	Teluk Kananroi	2/12/2006	5
		Serui Fish Market	4/8/2005	1
			Total T. squamosa	61

Table 3. *Symbiodinium* ITS-2 types identified in giant clam hosts. Types are named following LaJeunesse [*7*]. Types that differ from a previously-identified symbiont type by one base are named with the unique base identified (e.g. C15– 31). Tridacnid hosts: c= *Tridacna crocea;* m= *T. maxima;* s= *T. squamosa*.

Type Name	Tridacnid host	Type Abundance	Number of Sequences	Sequence Length*	Representative Sequence
C15-31	c,m,s	160	96	287	Tri1-17
A6	c,m,s	76	32	255	Tri3-2
D1b / D5	c,m,s	65	32	285	Tri2-9
D1a	c,m,s	46	22	285	Tri2-3a
A3	c,m,s	30	23	255	Tri2-19
C1	c,m,s	11	18	287	Tri4-1
A3-223	c	10	8	255	Tri2-1
A6-57	c	1	1	255	Tri6-7
C-15&135	c	1	1	287	Tri6-11
C3	s	1	1	287	Tri3-20
C3-130	s	1	1	287	Tri3-19
D1b-103	m	1	1	285	Tri11-7