Supplemental Data: Environmental populations of symbiotic dinoflagellates in the genus *Symbiodinium* can initiate symbioses with reef cnidarians

Mary Alice Coffroth, Cynthia F. Lewis, Scott R. Santos, Jessica L. Weaver

Supplemental Experimental Procedures

Detection of *Symbiodinium* in the water column. To determine if *Symbiodinium* were present in the water column above the reef, we utilized newly settled octocoral polyps (referred to here as recruits) (*Briareum* sp.) as "symbiont samplers". Prior to the formation of a mouth, planulae and recruits do not contain algal symbionts; however, once a mouth is present, these polyps readily take up *Symbiodinium* from the surrounding water [S1, S2]. Three-day-old asymbiotic *Briareum* sp. larvae were placed into containers with mesh covers and a suitable substrate (dead gorgonian branches, cleaned in fresh water and air-dried) and suspended 20 m above the reef (5 m below the surface). The larvae metamorphosed to polyps within 5 to 7 days and over a period of three months, 57 polyps were collected from the arrays and the *Symbiodinium* within the polyp characterized as described below.

Isolation of dinoflagellates from the benthos. Natural and artificial substrates, in various proximities to the benthos, were surveyed for the presence of "dinoflagellate-like" organisms at several sites in the Florida Keys (Table S1). Reef rubble (i.e., coral skeletons and sea urchin tests) was collected periodically from the KOA and Craig sites. Plexiglas settlement plates, attached to cinder blocks (sites KOA and GRS) or attached to the bottom (site TN), were removed and sampled approximately monthly. Time in the

field ranged from 2.5 weeks to over one year. Upon collection, the surface of the substrate was sprayed with 0.2 μm filtered seawater (FSW), the resultant wash spun at 800 rpm for 5 minutes and the pellet examined microscopically for cells that visually resembled dinoflagellates. If dinoflagellate-like cells were observed, approximately 15 μl of the pellet suspension was added to 5-10 dram vials containing 1.0 ml of f/2 culture media [S3] and maintained under a 14h/10h light-dark cycle at 27 ^{o}C. When growth of the isolates was observed, an aliquot was examined microscopically. Any isolates that resembled dinoflagellates were transferred to 50 ml flasks with 30 ml f/2 medium. To reduce the level of contaminating microorganisms, cultures that contained dinoflagellate-like isolates were also treated with antibiotics [S4]. Cultures were transferred to fresh media approximately monthly.

Molecular characterization of *Symbiodinium*. DNA was extracted [S1] from the recruits and from those benthic isolate cultures that, upon microscopic examination, resembled dinoflagellates and amplified with the polymerase chain reaction (PCR) using dinoflagellate-biased 18S rDNA primers ss5 and ss3z [S5]. Those samples that produced a PCR product were subjected to restriction fragment length polymorphism (RFLP) analysis using digestion with the endonuclease *Taq* I. Cladal identity was established by comparisons to restriction digests of cloned standards. To further verify that the isolates cultured from the benthos were *Symbiodinium,* these isolates were amplified using primers for a hypervariable region of Domain V in the chloroplast 23S rDNA gene (cp-type), that preferentially amplify *Symbiodinium* to the exclusion of other dinoflagellates [S6].

The placement of cultured isolates within the genus of *Symbiodinium* was determined by phylogenetic analysis of the entire internal transcribed spacer (ITS) region of the rDNA [S7]. The products were cloned (as needed) according to manufacture's instructions (TOPO cloning kit, Invitrogen) and sequenced by Roswell Park Cancer Institute DNA Sequencing Laboratory. Ambiguities in the resulting sequences were corrected by comparison to the complement DNA strand in SEQUENCHER v4.2 (Gene Codes Corporation). Finished sequences were aligned manually using SE-AL v2.0a11 (available at http://evolve.zoo.ox.ac.uk/). Maximum parsimony analyses were performed in PAUP*4.0 [S8] using 10 random sequence additions with tree-bisection-reconnection (TBR), gaps treated as missing data and branch support assessed by bootstrap analysis of 1000 replicates. The outclade group for the clade A tree was the symbiont of *Zoanthus sociatus* (*S. pilosum*) and for clade B, *S. muscatinei*.

Symbiont uptake experiments. Molecular evidence demonstrated that a subset of dinoflagellates isolated from the benthos was closely related to *Symbiodinium* (Fig. 1). To establish that these "*Symbiodinium*-like" dinoflagellates represent an environmental population of symbionts, we followed uptake of these dinoflagellates by asymbiotic recruits of two octocoral species, *Briareum* sp. and *Pseudopterogorgia elisabethae*. These surface brooding octocorals produce asymbiotic larvae that acquire symbiotic algae upon metamorphosis [S2]. Developing embryos were collected from the surface of female colonies and placed in containers with 500 ml of FSW and a suitable substrate. Within 3-5 days, the planulae settled and metamorphosed into asymbiotic polyps. Six isolate cultures (Table S3) that amplified with the cp23S-rDNA chloroplast primers were

randomly-selected and used to inoculate the recruits (2-3 replicates per culture). Water was changed every third day followed by addition of 500 cells/ml (final concentration) of the appropriate culture. Two or three containers of recruits were maintained in FSW without the addition of algae as a negative control. Additionally three containers with *Pseudopterogorgia elisabethae* polyps were inoculated with a *Symbiodinium* culture isolated from *P. elisabethae* (SSPe) to verify uptake. Polyps were sampled approximately weekly and preserved in 95% ethanol. Polyps were subjected to cp-type analysis to verify that the culture used to inoculate the polyps had actually been taken up and the ITS region was sequenced in a subset of these as described above.

Table S1 Locations in the Middle Florida Keys where natural and artificial substrates were collected and surveyed for the presence of dinoflagellate-like organisms.

Habitat	Site name	Depth
Mixed grassbed and hard bottom, Florida Bay	KOA	2 m
Shallow hardbottom, ocean side, Long Key, FL	Craig	2 m
Grassbed, ocean side, Long Key, FL	GRS	4 m
Hardbottom site near Tennessee Reef, FL	TN	6 m

Table S2. A list of isolates resembling dinoflagellates that were brought into culture.

Isolate number[1]	Clade[1]	Cp-type[1]	Current Clade[2]	Current cp-type[2]	Source[3]
4404	B	184	B	184	GRS
4405	ND	184+194			GRS
4425	ND	ND[4]			TN
4427	ND	ND			TN
4431	B	184+194			CRAIG
4433	ND	ND			CRAIG
4434	ND	ND			CRAIG
4436	ND	ND			CRAIG
4440	ND	184			CRAIG
4447	ND	184+194			GRS
4456	B	184			GRS
4477	ND	ND			KOA
4562	A	184+194	A	194	GRS
4624	ND	ND			KOA
4664	ND	ND			KOA
4701	ND	ND			GRS
4705	ND	ND			GRS
5175	B	184+194			KOA
5176	A	184+194	A	<194	KOA

5177	A	194	A	<194	KOA
5182	A	194+206	A		KOA
5184	A	<194	A	<194	KOA
5186	A	194	A	<194	KOA
5188	B	184+194			KOA
5191	B	194			KOA
5192	B	184+194			KOA
5194	A	ND	A	194	KOA
5870	A	ND	A	<194	GRS
BZ-20	B	178	No amp	No amp	CRAIG
BZ-20 float	ND	ND			CRAIG
TN4B	B	184+194	No amp	No amp	TN

[1]The isolate number, clade and cp-type (based on hypervariable region of Domain V of chloroplast 23S rDNA) that were initially determined are given first in the table.

[2]The current clade and cp-type are given for those isolates that are currently viable.

[3]See Table S1 for source locations and abbreviations.

[4]Some of the cultures were lost over time or overgrown by non-symbiotic dinoflagellates and other protists. ND (not determined) indicates the clade and /or cp-type were not determined prior to loss of the culture.

Table S3 Summary of symbiont types recovered from uptake experiments.

Symbiont types are based on size variation in the hypervariable region of Domain V of chloroplast 23S rDNA (cp-type). These symbionts (A194 and B184) have been recovered from newly settled gorgonian polyps and are commonly found in many reef-building corals [Coffroth, unpubl. data, S10]. Octocoral species used in each experiment are as indicated. Values given as the percent of the total number of polyps sampled. 'Mixture' indicates polyps that harbored symbiont types in addition to either B184 or A194. Those treatments where the predominant symbiont type within the polyps matches the isolate inoculum are in bold-face. It should also be noted that molecular analyses of those polyps maintained in filtered seawater (FSW, control) detected the presence of other members of *Symbiodinium* Clades A and B that were distinct from the environmental isolates. As the polyps were maintained in FSW, these contaminants are most likely due to symbionts on the surface of the planulae, perhaps trapped in the surface mucus layer, which then infected the host.

Host species: ***Briareum* sp**.

Isolate	Cp-type	B184	A194	B184+A194	Mixture	No amp	Total
4562	**A194**	10%	**77%**	13%	-	0	31
5196	**A194**	21%	**74%**	25%	-	0	47
Control		35%	3%	10%	24%	28%	29

Host species: ***Pseudopterogorgia elisabethae***

Isolate	Cp-type	B184	A194	B184+A194	No amp	Total

4404	**B184**	**70%**			30%	10
4562	**A194**	12.5%	**62.5%**		25%	16
5177	A194	75%			25%	8
5184	A194	62.5%			37.5%	8
5196	**A194**		**91%**	6%	3%	33
SSPe	**B184**	**75%**	25%			8
Control		17%			**83%**	6

Table S4 Source and reference for sequences used in Figures 1 and 2.

Accession number	Name of Isolate	Host/Reference
Clade A isolates		
AF333507	*Symbiodinium* sp. ITS2 type A3	*Hippopus hippopus* [S10]
AY074983	*Symbiodinium microadriaticum* ITS2 type A1	*Cassiopeia xamachana* [11]
AF184949	*Symbiodinium* sp. P082-2	*Amphisorus hemprichii* [S12]
AF184948	*Symbiodinium* sp. HA3-5	free-living isolate [S12]
AF427468	*Symbiodinium pilosum* ITS2 type A2	*Zoanthus* sociatus [S13]
Clade B isolates		
AY074978	*Symbiodinium* sp. ITS2 type B1	*Aiptasia* sp. Bermuda [S11]
EF026100	Culture 571	*Briareum* sp. (polyp) [S14]
AF499784	*Symbiodinium* sp. ITS2 type B7	*Madracis* sp. [S15]
AY239361	*Symbiodinium* sp. ITS2 type B11	No host given [S16]
AF333510	*Symbiodinium muscatinei* ITS2 type B4	*Anthopleura elagantissima* [S16]

Table S5 Pairwise uncorrected (i.e., *p*) genetic distances of Clade A *Symbiodinium* sequences in Figure 1a. The environmental isolates are shown in bold face. The source and reference for the non-environmental isolates are given in Table S4. Genetic distances were calculated in PAUP*4.0 [S8]

	A1	**4562**	**5196**	A3	PO82-2	**5176**	**5182**	HA3-5	**5184**
4562	0.000[1]	--							
5196	0.0000	0.0023	--						
A3	0.0251	0.0302	0.0244	--					
PO82-2	0.1650	0.1687	0.1740	0.1605	--				
5176	0.1660	0.1712	0.1718	0.1609	0.0104	--			
5182	0.1313	0.1634	0.1610	0.1186	0.0071	0.0000	--		
HA3-5	0.1709	0.1735	0.1739	0.1656	0.0125	0.0021	0.0024	--	
5184	0.1758	0.1755	0.1761	0.1702	0.0146	0.0042	0.0024	0.0021	--
A2	0.1453	0.1722	0.1712	0.1734	0.1865	0.1800	0.1621	0.1843	0.1889

[1]Isolate 4562 and A1 appear to be identical because the sequences do not have complete overlap. 4562, 5196 and A1 share identical sequences where they overlap (the region used for this analysis), but of those three, only 4562 and 5196 are completely overlapping. Across the overlapping region, 4562 and 5196 have a single bp that is different (about 10 bp into the alignment), which accounts for 0.2% difference in genetic distance.

Table S6 Pairwise uncorrected (i.e., *p*) genetic distances of Clade B *Symbiodinium* sequences in Figure 1b. The environmental isolate is shown in bold face.

	4404	571	B7	B1	B11
571	0.00000	--			
B7	0.01248	0.01210	--		
B1	0.00808	0.00806	0.00403	--	
B11	0.01608	0.01606	0.02021	0.01612	--
B4	0.05539	0.05574	0.06846	0.06432	0.06464

Supplemental References

S1. Coffroth, M.A., Santos, S.R., and Goulet, T.L. (2001). Early ontogenetic expression of specificity in a cnidarian-algal symbiosis. Mar. Ecol. Prog. Ser. 222**,** 85-96.

S2. Kinzie, R. A. (1974). Experimental infection of aposymbiotic gorgonian polyps with zooxanthellae. J. Exp. Mar. Biol. Ecol. 15, 335-345.

S3. Guillard, R. R. L., and Ryther, J. H. (1962). Studies of marine planktonic diatoms. *I. cycotella nana* hustedl, and *Deronula cofervacea* (cleve) gran. Can. J. Microbiol. 8, 229-39.

S4. Polne-Fuller, M. (1991). A novel technique for preparation of axenic cultures of *Symbiodinium* (Pyrrophyta) through selective digestion by amoebae. J. Phycol. 27, 552-554.

S5. Rowan, R., and Powers, D. A. (1991). A molecular genetic identification of zooxanthellae and the evolution of animal-algal symbioses. Science 251, 1348-1351.

S6. Santos, S. R., Taylor, D. J., and Coffroth, M. A. (2001). Genetic comparisons of freshly isolated versus cultured symbiotic dinoflagellates: Implications for extrapolating to the intact symbiosis. J. Phycol. 37, 900-912.

S7. Santos, S. R., Gutierrez-Rodriguez, C., and Coffroth, M. A. (2003). Phylogenetic identification of symbiotic dinoflagellates via length heteroplasmy in domain V of chloroplast large subunit (cp23S)-ribosomal DNA sequences. Mar. Biotech. 5, 130-140.

S8. Swofford, D. L. (2000). PAUP*, Phylogenetic Analysis Using Parsimony (*and other methods), Version 4.0b10, (Sinauer, Sunderland, Mass).

S9. LaJeunesse T.C. (2002). Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Mar Biol 141**,** 387-400

S10. LaJeunesse, T. C. (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.

S.11 Genbank submission by B. K. Baillie, A. A. Carlos, C. A. Belda-Baillie, T. Maruyama. Phylogenetic analysis of genus *Symbiodinium* using nITS DNA sequences.

S12. Savage, A. M., Goodson, M. S., Visram, S., Trapido, R. H., Wiedenmann, J., and Douglas, A. E. (2002). Molecular diversity of symbiotic algae at the latitudinal

margins of their distribution: dinoflagellates of the genus *Symbiodinium* in corals and anemones. Mar. Ecol. Prog. Ser. 244**,** 17-26.

S13. Santos, S.R., Taylor, D. J., Kinzie, R. A., Hidaka, M., Sakai, K., and Coffroth, M. A. (2002). Molecular phylogeny of symbiotic dinoflagellates inferred from partial chloroplast large subunit (23S)-rDNA sequences. Mol. Phylogenet. Evol. 23, 97-111.

S14. Genbank submission by S. R. Santos

S15. Diekmann , O. E., Olsen, J. L., Stam, W. T., and Bak, R. P. M. (2003). Genetic variation within *Symbiodinium* clade B from the coral genus *Madracis* in the Caribbean (Netherlands Antilles). Coral Reefs 22, 29-33.

S16. LaJeunesse**,** T. C., Loh, W. K. W., van Woesik, R., Hoegh-Guldberg, O., Schmidt, G. W., and Fitt, W. K. (2003). Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. Limnol. Oceanogr. 48, 2046-2054.