Molecular Ecology (2004) **13**, 459–469 doi: 10.1046/j.1365-294X.2004.02058.x

Fine-scale diversity and specificity in the most prevalent lineage of symbiotic dinoflagellates (*Symbiodinium*, Dinophyceae) of the Caribbean

S. R. SANTOS,* T. L. SHEARER,* A. R. HANNES and M. A. COFFROTH
Department of Biological Science, State University of New York at Buffalo, Buffalo, NY, 14260, USA

Abstract

The success of coral reefs is due to obligate mutualistic symbioses involving invertebrates and photosynthetic dinoflagellate symbionts belonging to the genus *Symbiodinium*. In the Caribbean, the vast majority of octocorals and other invertebrate hosts associate with *Symbiodinium* clade B, and more selectively, with a single lineage of this clade, *Symbiodinium* B1/B184. Although B1/B184 represents the most prevalent *Symbiodinium* in the Caribbean, there is little evidence supporting fine-scale diversity and host–alga specificity within this lineage. We explored simultaneously the questions of diversity and specificity in *Symbiodinium* B1/B184 by sequencing the flanking regions of two polymorphic microsatellites from a series of *Symbiodinium* clade B cultures along with *Symbiodinium* B1/B184 populations of the octocorals *Pseudopterogorgia elisabethae*, *P. bipinnata* and *Gorgonia ventalina*. Seven unique sequence variants were identified based on concatenation of the two loci. Phylogenetic analyses of these variants, which we refer to as phylotypes, recognized five as belonging to B1/B184, thus providing the first evidence of distinct taxa within this *Symbiodinium* lineage. Furthermore, sympatric *P. elisabethae* and *P. bipinnata* at San Salvador in the Bahamas were found to harbour distinct *Symbiodinium* B1/B184 phylotypes, demonstrating unequivocally the existence of fine-scale specificity between Caribbean octocorals and these algae. Taken together, this study exemplifies the complex nature of *Symbiodinium* biodiversity and specificity.

Keywords: coral, endosymbiosis, flanking region, microsatellite, population structure, zooxanthellae

Received 4 June 2003; revision received 26 September 2003; accepted 15 October 2003

Introduction

The success of coral reefs, considered to be one of the most biodiverse ecosystems in the world, is due in large part to obligate mutualistic symbioses involving invertebrates and photosynthetic dinoflagellate symbionts (Hallock 2001). These algae, referred to commonly as zooxanthellae and belonging predominantly to the genus *Symbiodinium*, establish relationships with various and numerous invertebrate hosts, including foraminiferans, sponges, scleractinian corals, anemones, octocorals, zoanthids, hydrocorals, mollusks (Glynn 1996) and ciliates (Lobban *et al*. 2002). Dinoflagellate symbionts play a significant role in host nutrition and physiology by translocating enough photosynthetically fixed carbon to meet the hosts' respiratory demands (Falkowski *et al*. 1984; Muscatine *et al*. 1984) and facilitating the assimilation and conservation of nitrogen (Lewis & Smith 1971; Burris 1983; Ambariyanto & Hoegh–Guldberg 1996). For the scleractinian corals, whose skeletons comprise the physical structure of reefs, calcification rate is also influenced by the presence of *Symbiodinium* (Pearse & Muscatine 1971; Barnes & Chalker 1990).

It was once thought that there was only a single pandemic species of symbiotic dinoflagellate, *S. microadriaticum* Freudenthal (Taylor 1974). Over the last three decades, however, behavioural, infectivity, molecular, physiological and ultrastructural studies have revised this notion (reviewed by Santos *et al*. 2001). For example, genetic analysis of nuclear small subunit ribosomal DNA (18S-rDNA) in *Symbiodinium* revealed levels of variability comparable to that

Correspondence and present address: S. R. Santos, Department of Biochemistry and Molecular Biophysics, University of Arizona, Tucson, AZ, 85721, USA. Fax: (520) 621 3709; E-mail: srsantos@email.arizona.edu
*S. R. S. and T. L. S. contributed equally to this work.

© 2004 Blackwell Publishing Ltd

of orders of free-living dinoflagellates (Rowan & Powers 1992). This has led to the development of a classification framework for members of the *Symbiodinium* based on nuclear ribosomal (nrDNA) genes and identifies algal populations and isolates as belonging to one of several large groups, or clades [i.e. *Symbiodinium* clades A, B, C (Rowan & Powers 1991), D (Carlos *et al*. 1999), E (*S. californium*; LaJeunesse & Trench 2000; LaJeunesse 2001) and F (LaJeunesse 2001)], with each clade comprised probably of many species (Rowan 1998).

In the Caribbean, octocorals are the most conspicuous reef macrofauna, with up to 40 species co-occurring in a single area (Sánchez *et al*. 2003). Like many other invertebrate hosts, these cnidarians acquire their symbionts anew at each generation from the immediate environment (Kinzie 1970). Under natural conditions, newly metamorphosed polyps of Caribbean octocorals acquire rapidly and can harbour simultaneously *Symbiodinium* belonging to different clades (Coffroth *et al*. 2001). In contrast, juveniles and adults of the same octocoral species harbour algal populations belonging to *Symbiodinium* clade B (Goulet 1999; Coffroth *et al*. 2001). Taken together, this demonstrates an ontogenic expression of selectivity for *Symbiodinium* clade B in Caribbean octocorals (Coffroth *et al*. 2001). Furthermore, although diversity exists within Caribbean *Symbiodinium* clade B (LaJeunesse 2001, 2002; Santos *et al*. 2003a), the vast majority of octocorals associate specifically with a single lineage based on internal transcribed spacer (ITS) sequence variation (LaJeunesse 2002). This lineage, designated type B1 by LaJeunesse (2001) and analogous to chloroplast large subunit ribosomal DNA (cp23S-rDNA) genotype B184 of Santos *et al*. (2003a) (unpublished data), is also found in other invertebrate hosts and ultimately represents the most prevalent *Symbiodinium* in the Caribbean (LaJeunesse 2002). However, it remains unknown whether fine-scale diversity and host–alga specificity exists within *Symbiodinium* B1 / B184.

We address these issues by investigating the diversity and specificity of Caribbean *Symbiodinium* B1/B184 populations using microsatellites. Microsatellites are segments of DNA in which a specific mono- to hexanucleotide motif is repeated in an array. The success of these markers as versatile and accessible tools for population genetic studies stems from the polymorphic nature of the array; changes in length, generated typically by polymerase slippage during DNA replication, can be detected easily by gel electrophoresis and unique length variants (i.e. alleles) scored. Although useful for discerning genetic individuals, absolute allele size may not be a reliable indicator of phylogenetic relationships (Orti *et al*. 1997) due to size homoplasy, a situation where alleles identical in state (i.e. size) are not identical by descent (reviewed by Estoup *et al*. 2002). Alternatively, mutations in the flanking regions surrounding repeat arrays have proved informative for inferring relationships among alleles, populations and species (Streelman *et al*. 1998; Markova *et al*. 2000; Rossetto *et al*. 2002). In the current study, we apply this latter approach to describe genetic diversity within the *Symbiodinium* B1/B184 lineage and to evaluate levels of host–alga specificity. Alleles from the polymorphic dinucleotide microsatellites CA4.86 and CA6.38 (Santos & Coffroth 2003; Santos *et al*. 2003b) were sequenced from a series of *Symbiodinium* clade B cultures in addition to *Symbiodinium* B1/B184 populations of the octocorals *Pseudopterogorgia elisabethae*, *P. bipinnata* and *Gorgonia ventalina* from sites in the Caribbean. Using phylogenetically informative characters in the flanking regions of alleles from both loci, we provide the first evidence that *Symbiodinium* B1/B184 is comprised of distinct taxa and demonstrate unequivocally the existence of fine-scale specificity between Caribbean octocorals and these algae.

Materials and methods

Symbiodinium *cultures*

The nucleotide variation present at CA4.86 and CA6.38 was appraised initially by sequencing alleles from 11 cultures of *Symbiodinium* clade B, obtained from a variety of hosts and different geographical locations (Table 1). These isolates represent the 10 two-locus microsatellite genotypes identified from culture by Santos & Coffroth (2003). The most common genotype recovered from culture by Santos & Coffroth (2003) was 191/106 (this convention indicates the allele size (base pairs: bp) of CA4.86 and CA6.38, respectively, and is followed throughout this work when describing *Symbiodinium* two-locus genotypes) and isolates obtained from *Briareum asbestinum* (Florida Keys) and *Plexaura kuna* (San Blas Islands, Panama) polyps were chosen specifically to assess variability within this genotype. Cultures were maintained as described in Santos *et al*. (2001).

Symbiodinium *populations of* P. elisabethae

Geographic differentiation of *Symbiodinium* populations within a single host species was explored by sequence analysis of the algal symbionts from *P. elisabethae* collected across the Bahamas (Fig. 1). *Symbiodinium* from these field-collected samples exhibit allele size variability that is indicative of population-level variation at the microsatellite loci used in this analysis (Santos *et al*. 2003b). Sequences were obtained from alleles that span the size classes observed at CA4.86 and CA6.38 (Santos *et al*. 2003b; Table 1); however, to detect sequence differentiation within the most frequently recovered allele from each locus, a representative was sequenced from the northwestern (i.e. SC or GR), middle (i.e. LS or CI) and southeastern (i.e. HC, RC or RR) Bahamas (Table 1) sites which span a distance of 10–100 s of kilometres (Fig. 1). Thus, in total, five and eight

© 2004 Blackwell Publishing Ltd, *Molecular Ecology*, 13, 459–469

Table 1 Information about the *Symbiodinium* cultures and *Symbiodinium* populations of *Pseudopterogorgia elisabethae*, *P. bipinnata* and *Gorgonia ventalina* used in this study. All samples belonged to *Symbiodinium* clade B. With the exception of culture #579, a *Symbiodinium* phylotype designation was assigned only to samples in which both loci were sequenced

	Host	Sample designation	Collection location	*Symbiodinium* cp23S-rDNA genotype	*Symbiodinium* phylotype	Locus CA4.86 allele size (bp) (Accession no.)	Locus CA6.38 allele size (bp) (Accession no.)
Culture	*Aiptasia pallida*	FLAp2	Long Key, Florida	B184	P5	179 (AY264293)	102 (AY264294)
	Briareum asbestinum (polyp)[a]	579	Long Key, Florida	B223	P7	187 (AF474168)	'null'
	B. asbestinum (polyp)[a]	1140	Long Key, Florida	B184	P4	191 (AY264295)	106 (AY264296)
	B. asbestinum (polyp)[a]	1394	Long Key, Florida	B184	P4	191 (AY264297)	104 (AY264298)
	Plexaura kuna (polyp)[a]	Pk208	San Blas Islands, Panama	B184	P4	183 (AY264299)	100 (AY264300)
	P. kuna (polyp)[a]	Pk702	San Blas Islands, Panama	B211	P6	193 (AF474167)	98 (AF474171)
	P. kuna (polyp)[a]	Pk704	San Blas Islands, Panama	B184	P4	193 (AF474166)	104 (AF474170)
	P. kuna (polyp)[a]	Pk706	San Blas Islands, Panama	B184	P4	191 (AY264301)	106 (AY264302)
	P. kuna	Pk807	Florida Keys	B184	P4	193 (AY264303)	100 (AY264304)
	Pocillopora damicornis	Pd	Hawaii	B184	P5	179 (AY264305)	100 (AY264306)
	Pseudopterogorgia elisabethae	SSPe	Riding Rock, Bahamas	B184	P1	193 (AY264307)	112 (AY264308)
In hospite	*Gorgonia ventalina*	Gv42I	East Sambos, Florida	B184	P3	199 (AY264309)	112 (AY264310)
	G. ventalina	Gv737D	Pickles Reef, Florida	B184	P3	197 (AY264311)	108 (AY264312)
	G. ventalina	Gv738D	Pickles Reef, Florida	B184	P3	199 (AY264313)	110 (AY264314)
	Pseudopterogorgia bipinnata	Pb1pr	Pillar Reef, Bahamas	B184	P4	191 (AY264315)	100 (AY264316)
	P. bipinnata	Pb5pr	Pillar Reef, Bahamas	B184	P4	191 (AY264317)	100 (AY264318)
	P. bipinnata	Pb38rr	Riding Rock, Bahamas	B184	P4	187 (AY264319)	104 (AY264320)
	P. bipinnata	Pb43rr	Riding Rock, Bahamas	B184	P4	191 (AY264321)	100 (AY264322)
	Pseudopterogorgia elisabethae	Pe13	Hog Cay, Bahamas	B184		195[b]	98 (AY264323)
	P. elisabethae	Pe20	Cat Island, Bahamas	B184	P2	195 (AY264326)	96 (AY264327)
	P. elisabethae	Pe22	Sweetings Cay, Bahamas	B184	P2	191 (AY264324)	98 (AY264325)
	P. elisabethae	Pe236	Rum Cay, Bahamas	B184		195[b]	118 (AY264328)
	P. elisabethae	Pe257	Rum Cay, Bahamas	B184		193[b]	122 (AY264329)
	P. elisabethae	Pe286	Little San Salvador, Bahamas	B184	P1	193 (AY264330)	118 (AY264331)
	P. elisabethae	Pe504	Gorda Rock, Bahamas	B184	P1	193 (AY264332)	118 (AY264333)
	P. elisabethae	Pe644	Riding Rock, Bahamas	B184	P1	193 (AY264334)	112 (AY264335)

[a]A polyp is defined as a newly settled and metamorphosed planula.
[b]Allele not sequenced.

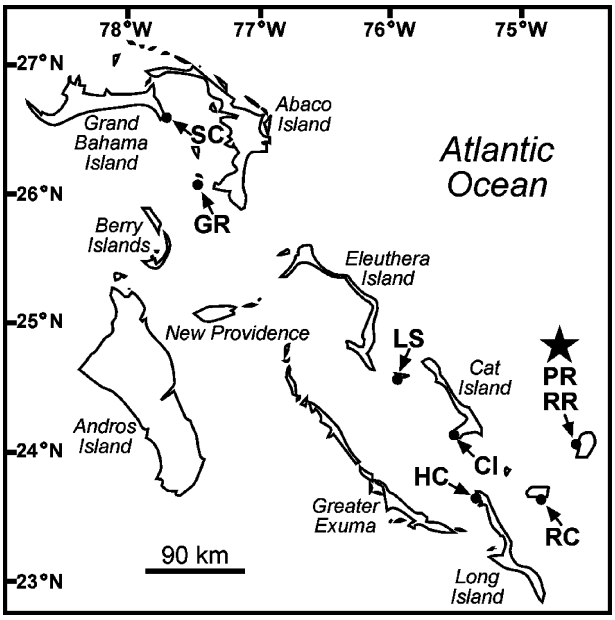

Fig. 1 Map of the Bahamas depicting sampling sites of *Pseudopterogorgia elisabethae*. Filled star denotes sites where *P. bipinnata* was also sampled. Two-letter abbreviations are as follows: (SC), Sweetings Cay 26°34′ N, 77°48′ W; (GR), Gorda Rock 26°07′ N, 77°33′ W; (LS), Little San Salvador 24°35′ N, 75°58′ W; (CI), Cat Island 24°09′ N, 75°32′ W; (HC), Hog Cay 23°37′ N, 75°21′ W; (RC), Rum Cay 23°37′ N, 74°52′ W; (PR), Pillar Reef and (RR), Riding Rock (24°04′ N, 74°32.5′ W) (adapted from Santos *et al*. 2003b).

alleles from CA4.86 and CA6.38, respectively, were sequenced from the *Symbiodinium* populations of *P. elisabethae* (Table 1).

Symbiodinium *populations of other Caribbean octocorals*

The *Symbiodinium* populations of two other Caribbean octocorals were also sampled in this study to evaluate the existence of host–alga specificity at two geographical / taxonomic levels: (1) between sympatric congeners and (2) between allopatric noncongeners. To assess the *Symbiodinium* of a closely related octocoral species occupying the same reef, the symbiont populations of *P. elisabethae* were compared to that of *P. bipinnata* collected from identical locations in the Bahamas — Pillar Reef (n = 46) and Riding Rock (n = 47) on San Salvador (filled star in Fig. 1). For the second comparison, the *Symbiodinium* populations of *G. ventalina* sampled from the Florida Keys were screened for these loci. Like *P. elisabethae*, planulae of these octocorals are asymbiotic and become infected with *Symbiodinium* from the environment following settlement. For these *Symbiodinium* populations, the size range of microsatellite alleles for both loci was determined and at least one allele from each of the recovered size classes was selected for sequencing (Table 1).

DNA extraction, PCR, cloning and sequencing

Total nucleic acids from *Symbiodinium* cultures *P. bipinnata* and *G. ventalina* were extracted and quantified according to the methods of Coffroth *et al*. (1992) while nucleic acids from *P. elisabethae* were isolated as described in Santos *et al*. (2003b). Phylogenetic identity of *Symbiodinium* cultures and populations *in hospite* was assessed by cp23S-rDNA genotyping (Santos *et al*. 2003a). Microsatellite amplification and allele detection was conducted as described in Santos & Coffroth (2003). For every CA4.86 and CA6.38 allele listed in Table 1, three clones were sequenced in both directions using the methods outlined in Santos *et al*. (2002b). Forward and reverse sequences for a clone were aligned using SEQUENCHER 4.1 (GENECODES) and ambiguities corrected by comparison to the complement strand. Sequences were deposited in GenBank under Accession nos. listed in Table 1.

Phylogenetic analyses

For each sample, the numerically dominant sequence recovered from both CA4.86 and CA6.38 was concatenated, the nucleotides comprising the repeat array removed and the remaining flanking regions used to define the genetic variant of each *Symbiodinium* culture or population *in hospite*. Sequences were aligned manually using Se-Al v2.0a11 (http://evolve.zoo.ox.ac.uk/) and a collection of representative variants deposited into TreeBase (http://www.treebase.org) under study Accession no. S952 and matrix Accession no. M1578. To assess the phylogenetic signal of the alignment, the skewness of tree length distributions (g_1) was calculated on the basis of 10^6 randomly sampled parsimony trees (Hillis & Huelsenbeck 1992). In addition, a permutation tail probability (PTP) analysis (Fu & Murphy 1999) of 10 000 replicates was used to examine the level of homoplasy in the data set. The g_1-test and PTP analysis were conducted with PAUP* version 4.0b10 (Swofford 2002).

Phylogenetic analyses were performed using multiple algorithms and treated insertion–deletions (indels) as missing data. Thus, relationships among the *Symbiodinium* isolates are inferred solely on substitutions in the microsatellite flanking regions. Maximum parsimony (MP) reconstruction utilized character optimization with accelerated transformation (ACCTRAN), 10 repetitions of random sequence additions, starting trees obtained by stepwise addition, and branches swapped by tree-bisection–reconnection (TBR). Neighbour-joining (NJ), maximum likelihood (ML) and Bayesian analyses were conducted under the Jukes & Cantor (1969) model of DNA evolution as selected by hierarchical likelihood ratio tests (hLRTs) in MODELTEST version 3.06 (Posada & Crandall 1998). Branch supports in the MP, NJ and ML trees were estimated by bootstrap analysis of 1000 replicates in PAUP*. The Bayesian analysis was

performed with MRBAYES version 3.0b4 (Huelsenbeck & Ronquist 2001) and employed the procedures outlined by Leaché & Reeder (2002). Culture Pk702 (*Symbiodinium* B211) was used as the outgroup in all analyses because MP phylogenies based on cp23S-rDNA place this isolate at a basal position within *Symbiodinium* clade B (Santos *et al*. 2002a, 2003a).

Results

Variation in the flanking regions of microsatellite alleles from Symbiodinium *cultures*

Cp23S-rDNA genotyping identifies six lineages in clade B of *Symbiodinium* (Santos *et al*. 2003a). Three of these (*Symbiodinium* B184, B211 and B223) are represented by the cultured isolates in Table 1. For the *Symbiodinium* B184 and B211 cultures, an allele was recovered from both loci. In the case of culture 579, a member of *Symbiodinium* B223, an allele was amplified from CA4.86 but CA6.38 possessed a 'null' (i.e. none amplifying) allele (Table 1). Recovery of only a single allele per locus is consistent with *Symbiodinium* being haploid in the vegetative life stage (Santos & Coffroth 2003). Allele sizes for CA4.86 and CA6.38 from the cultures ranged between 179–193 bp and 98–112 bp, respectively (Table 1).

A total of 21 alleles (n = 11 from CA4.86 and n = 10 from CA6.38) were sampled from the 11 *Symbiodinium* cultures. For each allele, the flanking regions sequenced from three clones were found to be identical. This lack of variability between clones suggests that a single sequence type was present at each locus. Five distinct variants, referred to hereafter as phylotypes (abbreviated as P_, where _ is a number), were identified from the cultures following concatenation of the microsatellite loci sequences (Table 1). *Symbiodinium* B184 isolates belonged to one of three phylotypes (Table 1 and Fig. 2). All *Symbiodinium* B184 cultures isolated from newly settled polyps of *B. asbestinum* (Florida Keys) and polyp and adult *P. kuna* (Panama and Florida Keys, respectively) were of the same phylotype (P4). Similarly, a phylotype (P5) was shared by the *Aiptasia pallida* (Florida Keys) and *Pocillopora damicornis* (Hawaii) B184 cultures. The phylotype of culture SSPe, obtained from a *P. elisabethae* colony at Riding Rock, San Salvador, was distinct to this culture (P1). Unique phylotypes were also recovered from the *Symbiodinium* B211 and B223 cultures (P6 and P7, respectively). Corrected pairwise distances ranged between 0.8 and 7.8% for all isolates and 0.8–2.0% for within *Symbiodinium* B184 (Table 2).

Flanking regions of microsatellite alleles from Symbiodinium *populations of* P. elisabethae

P. elisabethae in the Bahamas is known to associate with *Symbiodinium* B184 populations (Santos *et al*. 2003a) that exhibit strong genetic structure (Santos *et al*. 2003b). The sequencing of microsatellite alleles from these populations revealed that *P. elisabethae* harbours one of two distinct *Symbiodinium* B184 phylotypes (P1 and P2; Table 1). Of the five CA4.86 alleles sampled, the majority of sequenced clones (14 of 15; 93%) possessed flanking regions identical to culture SSPe, regardless of allele size (representing variation due to number of repeats) or sampling location (Table 1). At locus CA6.38, alleles belonged to one of two distinct groups. For the five alleles in the size range of 112–122 bp, flanking regions from 14 of 15 clones were indistinguishable from culture SSPe [*P. elisabethae* (RR), Fig. 2]. Concatenating the numerically dominant sequence from CA4.86 (see above) with the sequence from the 112–122 bp alleles of CA6.38 suggests that these *P. elisabethae* colonies are associating with the P1 phylotype characterized previously from culture SSPe (Fig. 2). On the other hand, for the 96 and 98 bp alleles that were sampled (n = 3 alleles; Table 1), all nine clones were identical and possessed a 13 bp indel

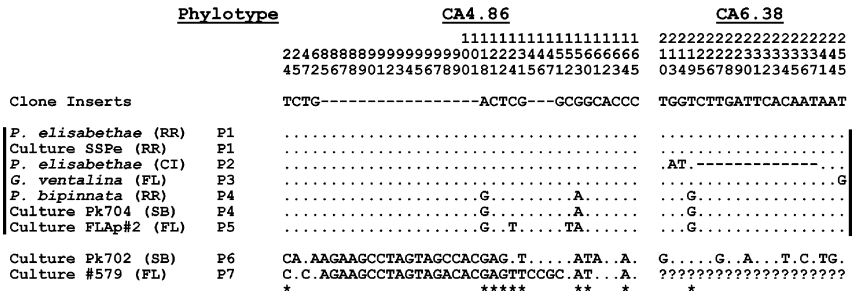

Fig. 2 Sequence variation among phylotypes of *Symbiodinium* clade B for the concatenated flanking regions of microsatellite loci CA4.86 and CA6.38 (length = 277 bp). Names bracketed by vertical lines belong to *Symbiodinium* B1/B184. Cultures Pk702 and 579 belong to *Symbiodinium* B211 and B223, respectively. Only those positions differing from the clone inserts used in primer construction are shown. The number at the top (read vertically downwards) designates site position. A dot (.) indicates a base pair identical to that of the bacterial clones while a letter signifies a change in the nucleotide. Dashes (–) indicate gaps introduced into the alignment. A question mark (?) represents a nucleotide of unknown identity. An asterisk (*) marks those sites that are parsimony informative.

Table 2 Absolute (below diagonal) and Jukes–Cantor (JC) corrected (above diagonal) pairwise distances of *Symbiodinium* clade B phylotypes based on the concatenated flanking regions of microsatellite loci CA4.86 and CA6.38. CI = Cat Island, Bahamas; FL = Florida Keys; HI = Hawaii; RR = Riding Rock, Bahamas; SB = San Blas Islands, Panama

	(P1)	(P2)	(P3)	(P4)	(P5)	(P6)	(P7)
(P1) *Pseudopterogorgia elisabethae* (RR) and culture SSPe (RR)	—	0.0082	0.0039	0.0118	0.0197	0.0735	0.0689
(P2) *P. elisabethae* (CI)	2	—	0.0124	0.0208	0.0293	0.0686	0.0689
(P3) *Gorgonia ventalina* (FL)	1	3	—	0.0157	0.0237	0.0778	0.0689
(P4) *Pseudopterogorgia bipinnata* (RR) and culture Pk704 (SB)	3	5	4	—	0.0078	0.0692	0.0559
(P5) Cultures FLAp2 (FL) and Pd (HI)	5	7	6	4	—	0.0778	0.0559
(P6) Culture Pk702 (SB)	18	16	19	17	19	—	0.0390
(P7) Culture 579 (FL)	11	11	11	9	9	7	—

as well as two substitutions relative to culture SSPe [*P. elisabethae* (CI), Fig. 2]. Along with these unique mutations, the 96 bp and 98 bp alleles possessed a thymine residue (position 219 of Fig. 2) characteristic for the CA6.38 locus of the *Symbiodinium* culture and other populations from *P. elisabethae*. Combining the sequence of the 96 and 98 bp alleles from CA6.38 with the CA4.86 sequence recovered from the same samples represents the P2 phylotype (Fig. 2) harboured by the other *P. elisabethae* colonies. In addition, one clone from Pe286 at Little San Salvador had flanking regions matching the CA4.86 allele of culture FLAp 2, while a CA6.38 clone from the same sample possessed a guanine residue (position 219 of Fig. 2) characteristic of cultures FLAp 2 or Pk704.

Flanking regions of microsatellite alleles from Symbiodinium *populations of* P. bipinnata *and* G. ventalina

Cp23S-rDNA analysis placed the *Symbiodinium* populations of *P. bipinnata* at Pillar Reef and Riding Rock within lineage B184. Microsatellite analysis of the 93 colonies revealed that the majority (89 of 93; 95.7%) had a two-locus genotype of 191/100. In contrast, the numerically dominant *Symbiodinium* genotype of *P. elisabethae* colonies (91 of 93; 97.8%) at Pillar Reef and Riding Rock is 193/112 (confirmed by the microsatellite analysis of an additional 25 colonies from each site in 2002), with the remaining two colonies being 191/112 (Santos *et al*. 2003b). Furthermore, to date, *Symbiodinium* populations of genotype 191/100 have not been found in association with *P. elisabethae* of the Bahamas (Santos *et al*. 2003b). One colony each of *P. bipinnata* at Pillar Reef and Riding Rock was 191/100, 104 while another at Pillar Reef was 187,191/104; because these three colonies possess two alleles either at CA4.86 or CA6.38, it suggests that two *Symbiodinium* B184 genotypes reside within them (Santos & Coffroth 2003). The remaining *P. bipinnata* colony was 187/104 (at Riding Rock), another two-locus genotype which has not been found in association with *P. elisabethae* (Santos *et al*. 2003b).

The flanking regions of the four CA4.86 alleles sampled from *P. bipinnata* (n = 12 clones sequenced) were found to be identical to *Symbiodinium* B184 cultures from *B. asbestinum* and *P. kuna* (culture Pk704, Fig. 2). Similarly, at CA6.38, 11 of 12 clones had the guanine residue (position 219, Fig. 2) diagnostic of these same cultures while one CA6.38 clone from a Riding Rock *P. bipinnata* had the thymine residue characteristic of *Symbiodinium* associated with *P. elisabethae*. Taken together, *P. bipinnata* harbours a *Symbiodinium* B184 phylotype (P4) distinct from the phylotype of sympatric *P. elisabethae* (P1) but identical to cultures isolated from *B. asbestinum* and *P. kuna* at different geographical locations.

From the sampling of *G. ventalina* colonies, the microsatellite flanking regions for eight of nine clones from the three CA4.86 alleles were identical to culture SSPe (one clone had flanking regions matching culture Pk704). Although nine of nine clones from the three CA6.38 alleles had the thymine residue characteristic of *P. elisabethae Symbiodinium* populations, the same clones possessed a guanine residue at position 255 (Fig. 2). Thus, this substitution distinguishes the *Symbiodinium* phylotypes of *P. elisabethae* (P1 and P2) from that of *G. ventalina* (P3).

Phylogenetic analysis

Significant phylogenetic signal (mean = 38.15, SD = 2.28, $g_1 = -1.62$, $P < 0.01$) relative to homoplasy (PTP: $P < 10^{-4}$) was present in the concatenated microsatellite flanking regions, implying the appropriateness of the sequences for inferring relationships among the *Symbiodinium* phylotypes. *Symbiodinium* identified as B184 by cp23S-rDNA analysis formed a lineage with strong (97–100%) support that excluded culture 579, the sole *Symbiodinium* B223 isolate included in the analysis (Fig. 3). Within the B184 lineage, two distinct groups are apparent. One group is comprised of the *Symbiodinium* culture and populations *in hospite* from *P. elisabethae* as well as the algal symbionts of *G. ventalina*, while the other group consists of the *Symbiodinium* populations of *P. bipinnata* and cultures from cnidarian host of various species and geographical regions (Fig. 3). The two

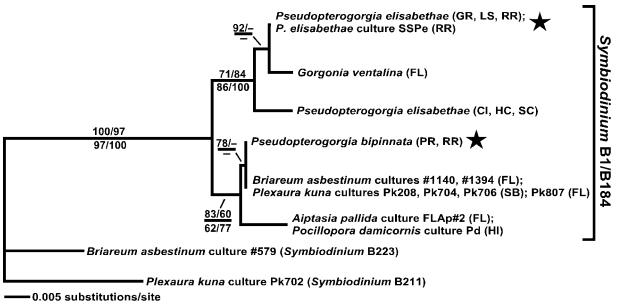

Fig. 3 Phylogenetic relationships inferred for *Symbiodinium* clade B from the concatenated flanking regions of microsatellite loci CA4.86 and CA6.38. Samples belonging to *Symbiodinium* B1/B184 are bracketed at the right. Two-letter abbreviations of geographical location (in parentheses) are from Fig. 1 and Table 2. Filled stars denote *Symbiodinium* populations sampled from *Pseudopterogorgia elisabethae* and *P. bipinnata* at San Salvador, Bahamas (see text for details). The length of the most parsimonious tree was 28 steps with a consistency index (CI) of 0.96, homoplasy index (HI) of 0.04 and retention index (RI) of 0.92. Maximum likelihood (ML) tree (–ln L = 547.39). Values above vertical line are bootstrap support as percentages of 1000 resamplings for neighbour-joining (NJ, left) and maximum parsimony (MP, right) analyses, respectively. Values below vertical line are bootstrap support as percentages of 1000 resamplings for maximum likelihood (ML, left) and Bayesian posterior probabilities from 15 000 trees (right), respectively.

groups exhibit moderate (60–83%) to strong (71–100%) support while relationships among the phylotypes within each group are tenuous and unresolved (Fig. 3).

Discussion

This is the first study to examine the fine-scale phylogenetics of the most prevalent dinoflagellate symbionts in the Caribbean, *Symbiodinium* B1/B184. Using DNA substitutions in the flanking regions of two microsatellites, we report that *Symbiodinium* B1/B184 is comprised of distinct taxa. This discovery brings critical new insight to the diversity, biogeography and specificity of this prominent lineage of *Symbiodinium* and extends our knowledge of these enigmatic dinoflagellates.

Symbiodinium *diversity and the species concept*

As stated earlier, it is now well accepted that *Symbiodinium* clades represent species complexes. The designation of a *Symbiodinium* species has relied traditionally on a morphological (including cytological attributes) species concept (Trench & Blank 1987; Trench & Thinh 1995). It should be noted, however, that diagnostic morphological characters can be reduced or not readily apparent depending on life history, stage in the cell cycle or specifics of cell preparation (Trench & Blank 1987) and that evolutionarily cohesive groups within symbiotic dinoflagellates are not necessarily delineated by morphology (Wilcox 1998). Given this, a phylogenetic species concept has been proposed as more appropriate for classifying *Symbiodinium* (LaJeunesse 2001). This idea finds support in the correspondence of morphological and functional characteristics with phylogenetic groupings based on ITS sequences, suggesting that ITS diversity may in fact reflect species level differences in some *Symbiodinium* groups (LaJeunesse 2001). At the same time, LaJeunesse (2001) also pointed out that ITS sequence divergence might not be an appropriate unit of measure for the designation of species in all *Symbiodinium* groups. For *Symbiodinium* B1/B184, this appears to be the case for the following reasons. First, the identical ITS type of *Symbiodinium* B1 does not reflect biologically relevant functional characteristics that are apparent at the level of phylotypes, such as fine-scale specificity between algae and host (Fig. 3 and below). Second, given the slower evolutionary rate of ITS genes in *Symbiodinium* clade B relative to orthologous genes in clades A and C (Santos *et al*. 2002a), estimates of divergence and phylogenetic relationships based on this molecule should be considered conservative. The fact that isolates of *Symbiodinium* B1 have identical ITS regions, yet belong to phylotypes divergent by up to 2% (Table 2 and Fig. 3), illustrates this point. Taken together, we conclude that the *Symbiodinium* B1 ITS type represents a taxonomic level above that of a 'species' while phylotypes (i.e. sequence variants in the regions flanking these microsatellite arrays) fulfil more effectively the criteria of the phylogenetic species concept (reviewed by Luckow 1995) for this group. In this case, the sequence variation in these microsatellite flanking regions corresponds to differences among 'species', while within a phylotype, size variation between microsatellite alleles (due to changes in repeat number) corresponds to population-level differences. Although additional work is needed to calibrate molecular sequence divergence within a taxonomic framework, such information will be particularly useful in *Symbiodinium* biology, especially if evolutionary patterns similar to those reported here for *Symbiodinium* B1/B184 are observed in other symbiotic dinoflagellate ITS types.

Biogeography of Symbiodinium*: distribution of phylotypes vs. genotypes*

In this study, we found that *Symbiodinium* B184 phylotypes were distributed widely. For example, the *Symbiodinium* P1 and P2 phylotypes associated with *P. elisabethae* were found across the Bahamas. This is consistent with previous findings of identical cp23S-rDNA (Santos *et al*. 2002a) and ITS (Baillie *et al*. 2000; LaJeunesse 2001; Santos *et al*. 2001; LaJeunesse 2002) sequences from *Symbiodinium* populations of widespread hosts and implies dispersal of these symbiotic algae via intrinsic motility (Fitt *et al*. 1981; Fitt &

Trench 1983; Crafts & Tuliszewski 1995), oceanic currents (Loh *et al*. 2001; Santos *et al*. 2003b), 'hitchhiking' within the alimentary system of host predators (Augustine & Muller-Parker 1998) or other mechanisms. In contrast, the *Symbiodinium* B184 genotypes (defined as a unique combination of microsatellite allele sizes at loci CA4.86 and CA6.38) of *P. elisabethae* exhibit striking population differentiation, suggesting retention within localized populations (Santos *et al*. 2003b). Although these patterns may be confined to the *Symbiodinium* populations of *P. elisabethae*, discrepancies in geographical distributions have been documented for these dinoflagellates (Rodriguez-Lanetty 2003) and we feel that future work, using fine-scale genetic markers such as microsatellites, will find it a common feature of algal-invertebrate symbioses. Thus, we present the following hypothesis to address the inconsistency in biogeography of *Symbiodinium* flanking region phylotypes and microsatellite genotypes. We propose that *Symbiodinium* phylotypes become widespread geographically by mechanisms such as those described earlier. Within a phylotype, genotypic differentiation between reefs occurs over time due to the high evolutionary rate of microsatellite repeat arrays relative to the surrounding flanking regions (Schlotterer 2000). Recombination, for which there is growing evidence for in *Symbiodinium* (reviewed in LaJeunesse 2001; Santos *et al*. 2003b; but see Rodriguez-Lanetty 2003), would also play a role by erasing molecular divergence between phylotype populations while potentially creating novel genotypes. Ultimately, if there is little movement of genotypes between reefs, acquisition of symbionts at each host generation from the immediate environment and specificity between partners, near or complete fixation of a *Symbiodinium* genotype in a host population would occur eventually. Furthermore, the process would be facilitated on a given reef if selective advantages were conveyed by a particular *Symbiodinium* genotype to individuals of a host species.

Fine-scale specificity between Caribbean octocorals and members of Symbiodinium *B1/B184*

It has been shown that microsatellite PCR primers for the *Symbiodinium* B184 populations of *P. kuna* and *P. elisabethae* have a strong affinity to the algal populations from which they were designed (Santos & Coffroth 2003). This implied genetic differentiation between seemingly identical *Symbiodinium* populations and suggests fine-scale host–algal specificity in octocorals (Santos & Coffroth 2003). These ideas are supported and extended by the data presented here. Microsatellite genotyping of the field-derived algal populations in this study revealed distinct differences in allele frequencies between the octocoral species. Of the 15 alleles observed from both loci, only one allele size class at each locus (i.e. 191 bp at CA4.86 and 112 bp at CA6.38) is shared either between *G. ventilina*, *P. bipinnata* or *P. elisabethae*. Thus, alleles from the loci appear to be partitioned by octocoral species according to size with little to no overlap among the three hosts (Table 3).

The fact that *P. elisabethae* colonies were found to harbour one of two closely related *Symbiodinium* phylotypes across the Bahamas (Figs 2 and 3) is indicative of an active recognition process between partners. Furthermore, the specificity exhibited between particular *Symbiodinium* B184 phylotypes and *P. elisabethae* or *P. bipinnata* truly exemplified this recognition phenomenon. Because the two host species are sympatric at Riding Rock and Pillar Reef, San Salvador (filled star in Fig. 1), they are exposed to a common environmental pool of *Symbiodinium* following

Table 3 Summary of alleles recovered from *Symbiodinium* microsatellite loci CA4.86 and CA6.38 of the octocorals *Gorgonia ventilina*, *Pseudopterogorgia bipinnata* and *P. elisabethae*

		Octocoral species		
	Allele size (bp)	*G. ventilina*	*P. bipinnata*	*P. elisabethae*
Locus CA4.86	187		X	
	191		X	X
	193			X
	195			X
	197	X		
	199	X		
Locus CA6.38	96			X
	98			X
	100		X	
	104		X	
	108	X		
	110	X		
	112	X		X
	118			X
	122			X

settlement. If specificity between host and algal phylotype did not exist, it would be expected that colonies would randomly take up *Symbiodinium* phylotypes or a common phylotype might exist in both octocorals. In addition, these *P. elisabethae* colonies, and probably those of *P. bipinnata*, belong to different size/age classes (Lasker *et al*. 2003) representing multiple generations of host reproduction and concomitant *Symbiodinium* infection events. In spite of this, fine-scale specificity, independent of environmental influences or chance historical events, is maintained at a local level between colonies of these host species and particular B184 phylotypes (filled stars in Fig. 3).

Specificity between a *Symbiodinium* B184 phylotype and a species of octocoral, however, does not appear to be perfect. As evident from the sequencing of clones, all three octocorals also appear to associate with phylotypes that are distinct from the numerically dominant phylotype *in hospite*. This is consistent with multiple algal types being present at low levels within a given octocoral host (Coffroth *et al*. 2001; Goulet & Coffroth 2003; Santos & Coffroth 2003). Due to the presence of these 'cryptic' populations, a *Symbiodinium* culture may not necessarily reflect the dominant algae of a particular host life stage (Coffroth *et al*. 2001), species or individual (Santos *et al*. 2001; LaJeunesse 2002; Santos *et al*. 2003c). Thus, although the symbionts of *B. asbestinum* and *P. kuna* appear to be identical to the phylotype found in *P. bipinnata* (P4), this is probably an artefact because these cultures were isolated from polyps, and the CA4.86 and CA6.38 primer sets typically fail to amplify alleles from adult *B. asbestinum* and *P. kuna Symbiodinium* populations *in hospite* (Santos & Coffroth 2003; unpublished data). Similarly, the phylotype (P5) cultured from *A. pallida* (FLAp 2) and *P. damicornis* (Pd) is not representative of these host species (Santos *et al*. 2001) and probably represents a cryptic symbiont. For the octocorals, these cryptic phylotypes are probably relicts of the initial symbiont uptake by the asymbiotic planulae; however, they may also represent *Symbiodinium* populations acquired secondarily by the adult host (Lewis & Coffroth unpublished data). In either case, how these cryptic populations affect their host remains to be elucidated.

Acknowledgements

We are grateful to D. Brancato and J. Weaver for maintaining the *Symbiodinium* cultures and the Bahamas Department of Fisheries, the Florida Keys National Marine Sanctuary and the Republic of Panama for permission to collect and export samples from the Bahamas, Florida and Panama, respectively. We would also like to thank Dr C. Gutiérrez-Rodríguez for access to *Pseudopterogorgia elisabethae* samples, Dr H. R. Lasker and M. S. Barbeitos for help in collections of *P. elisabethae* and *P. bipinnata*, Dr R. A. Kinzie III for access to additional *Symbiodinium* cultures, the staff and scientists of the Don Gerace Research Center, the Smithsonian Tropical Research Institute and the Keys Marine Laboratory for technical support, the crew of the R/V *Sea Ray* for logistical support during *P. elisabethae* collections and the Kuna Nation for permission to work in the San Blas Islands, Panama. We acknowledge Dr T. C. LaJeunesse for PCR–DGGE analyses, which helped to confirm that *Symbiodinium* ITS type B1 and cp23S-rDNA genotype B184 are analogous, and Dr D. Harvell and J. Ward for providing the *G. ventalina* samples. The comments of Dr M. Rodriguez-Lanetty and two anonymous reviewers improved the manuscript; they have our gratitude. Sherwood SCUBA is gratefully acknowledged for donating SCUBA equipment used in this study. This research was supported by a National Science Foundation (NSF) Minority Graduate Fellowship (SRS), NSF grants OCE-95–30057 and OCE-99–07319 (MAC) and grants from the New York State Sea Grant Program (R/XG-9) and the National Undersea Research Center at the Caribbean Marine Research Center (CMRC-99–3301 and 99-NRHL-01–01C) (MAC and HRL).

References

Ambariyanto, Hoegh–Guldberg O (1996) Nutrient enrichment and the ultrastructure of zooxanthellae from the giant clam *Tridacna maxima*. *Marine Biology*, **125**, 359–363.

Augustine L, Muller-Parker G (1998) Selective predation by the mosshead sculpin *Clinocottus globiceps* on the sea anemone *Anthopleura elegantissima* and its two algal symbionts. *Limnology and Oceanography*, **43**, 711–715.

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

Barnes DJ, Chalker BE (1990) Calcification and photosynthesis in reef-building corals and algae. In: *Ecosystems of the World 25: Coral Reefs* (ed. Dubinsky Z), pp. 109–131. Elsevier, New York, NY, USA.

Burris RH (1983) Uptake and assimilation of $^{15}NH_4$ by a variety of corals. *Marine Biology*, **75**, 151–155.

Carlos AA, Baillie BK, Kawachi M, Maruyama T (1999) Phylogenetic position of *Symbiodinium* (Dinophyceae) isolates from Tridacnids (Bivalvia), Cardiids (Bivalvia), a sponge (Porifera), a soft coral (Anthozoa), and a free-living strain. *Journal of Phycology*, **35**, 1054–1062.

Coffroth MA, Lasker HR, Diamond ME, Bruenn JA, Bermingham E (1992) DNA fingerprinting of a gorgonian coral: a method for detecting clonal structure in a vegetative species. *Marine Biology*, **114**, 317–325.

Coffroth MA, Santos SR, Goulet TL (2001) Early ontogenetic expression of specificity in a cnidarian–algal symbiosis. *Marine Ecology Progressive Series*, **222**, 85–96.

Crafts CB, Tuliszewski JR (1995) Motility rhythms in cultured zooxanthellae isolated from the scyphomedusa *Linuche unguiculata*. *Bulletin of Marine Science*, **56**, 822–825.

Estoup A, Jarne P, Cornuet JM (2002) Homoplasy and mutation model at microsatellite loci and their consequences for population genetic analysis. *Molecular Ecology*, **11**, 1591–1604.

Falkowski PG, Dubinsky Z, Muscatine L, Porter JW (1984) Light and the bioenergetics of a symbiotic coral. *Bioscience*, **34**, 705–709.

Fitt WK, Chang SS, Trench RK (1981) Motility patterns of different strains of the symbiotic dinoflagellate *Symbiodinium* (= *Gymnodinium*) *microadriaticum* (Freudenthal) in culture. *Bulletin of Marine Science*, **31**, 436–443.

Fitt WK, Trench RK (1983) The relation of diel patterns of cell division to diel patterns of motility in the symbiotic dinoflagellate

Symbiodinium microadriaticum Freudenthal in culture. *New Phytologist*, **94**, 421–432.

Fu J, Murphy RW (1999) Discriminating and locating covariance: an application of permutation tail probability (PTP) analyses. *Systematic Biology*, **48**, 380–395.

Glynn PW (1996) Coral reef bleaching: facts, hypotheses and implications. *Global Change Biology*, **2**, 495–509.

Goulet TL (1999) *Temporal and spatial stability of zooxanthellae in octocorals*. PhD Dissertation, State University of New York at Buffalo, New York.

Goulet TL, Coffroth MA (2003) Genetic composition of zooxanthellae between and within colonies of the octocoral *Plexaura kuna*, based on small subunit rDNA and multilocus DNA fingerprinting. *Marine Biology*, **142**, 233–239.

Hallock P (2001) Coral reefs, carbonate sediments, nutrients and global change. In: *The History and Sedimentology of Ancient Reef Systems* (ed. Stanley GD Jr), pp. 387–427. Kluwer Academic Publishing, New York, NY, USA.

Hillis DM, Huelsenbeck JP (1992) Signal, noise, and reliability in molecular phylogenetic analysis. *Journal of Heredity*, **83**, 189–195.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Jukes TH, Cantor C (1969) Evolution of protein molecules. In: *Mammalian Protein Metabolism* (ed. Munro MN), pp. 21–132. Academic Press, New York, NY, USA.

Kinzie RA III (1970) *The ecology of the gorgonians (Cnidaria: Octocorallia) of Discovery Bay, Jamaica*. PhD Dissertation, Yale University, New Haven, Connecticut.

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

LaJeunesse TC (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine Biology*, **141**, 387–400.

LaJeunesse TC, Trench RK (2000) Biogeography of two species of *Symbiodiniu*m (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *Biology Bulletin*, **199**, 126–134.

Lasker HR, Boller ML, Castanaro J, Sánchez JA (2003) Determinate growth and modularity in a gorgonian coral. *Biology Bulletin*, **205**, 319–330.

Leaché AD, Reeder TW (2002) Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. *Systematic Biology*, **51**, 44–68.

Lewis DH, Smith DC (1971) The autotrophic nutrition of symbiotic marine coelenterates with special references to hermatypic corals. I. Movement of photosynthetic products between the symbionts. *Proceedings of the Royal Society of London Series B*, **178**, 111–129.

Lobban CS, Schefter M, Simpson AGB, Pochon X, Pawlowski J, Foissner W (2002) *Maristentor dinoferus* n. gen., n. sp., a giant heterotrich ciliate (Spirotrichea: Heterotrichida) with zooxanthellae, from coral reefs on Guam, Mariana Islands. *Marine Biology*, **140**, 411–423.

Loh WKW, Loi T, Carter D, Hoegh-Guldberg O (2001) Genetic variability of the symbiotic dinoflagellates from the wide ranging coral species *Seriatopora hystrix* and *Acropora longicyathus* in the Indo-West Pacific. *Marine Ecology Progressive Series*, **222**, 97–107.

Luckow M (1995) Species concepts: assumptions, methods and applications. *Systematic Botany*, **20**, 589–605.

Markova KD, Nekrutenko A, Baker RJ (2000) Evolution of microsatellite alleles in four species of mice (genus *Apodemus*). *Journal of Molecular Evolution*, **51**, 166–172.

Muscatine L, Falkowski PG, Porter JW, Dubinsky Z (1984) Fate of photosynthetically-fixed carbon in light and shade-adapted colonies of the symbiotic coral, *Stylophora pistillata*. *Proceedings of the Royal Society of London Series B*, **222**, 181–202.

Orti G, Pearse DE, Avise JC (1997) Phylogenetic assessment of length variation at a microsatellite locus. *Proceedings of the National Academy of Sciences USA*, **94**, 10745–10749.

Pearse VB, Muscatine L (1971) Role of symbiotic algae (zooxanthellae) in coral calcification. *Biology Bulletin*, **141**, 350–363.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Rodriguez-Lanetty M (2003) Evolving lineages of *Symbiodinium*-like dinoflagellates based on ITS1 rDNA. *Molecular Phylogenetics and Evolution*, **28**, 152–168.

Rossetto M, McNally J, Henry RJ (2002) Evaluating the potential of SSR flanking regions for examining taxonomic relationships in the Vitaceae. *Theoretical and Applied Genetics*, **104**, 61–66.

Rowan R (1998) Diversity and ecology of zooxanthellae on coral reefs. *Journal of Phycology*, **34**, 407–417.

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

Rowan R, Powers DA (1992) Ribosomal RNA sequences and the diversity of symbiotic dinoflagellates (zooxanthellae). *Proceedings of the National Academy of Sciences USA*, **89**, 3639–3643.

Sánchez JA, McFadden CS, France SC, Lasker HR (2003) Molecular phylogenetic analyses of shallow-water Caribbean octocorals. *Marine Biology*, **142**, 975–987.

Santos SR, Coffroth MA (2003) Molecular genetic evidence that dinoflagellates belonging to the genus *Symbiodinium* Freudenthal are haploid. *Biology Bulletin*, **204**, 10–20.

Santos SR, Gutiérrez-Rodríguez C, Coffroth MA (2003a) Phylogenetic identification of symbiotic dinoflagellates via length heteroplasmy in domain V of chloroplast large subunit (cp23S)-rDNA sequences. *Marine Biotechnology*, **5**, 130–140.

Santos SR, Gutiérrez-Rodríguez C, Lasker HR, Coffroth MA (2003b) *Symbiodinium* sp. associations in the gorgonian *Pseudopterogorgia elisabethae* in the Bahamas: high levels of genetic variability and population structure in symbiotic dinoflagellates. *Marine Biology*, **143**, 111–120.

Santos SR, Kinzie RA III, Sakai K, Coffroth MA (2003c) Molecular characterization of nuclear small subunit (18S)-rDNA pseudogenes in a symbiotic dinoflagellate (*Symbiodinium*, Dinophyta). *Journal of Eukaryotic Microbiology*, **50**, 408–412.

Santos SR, Taylor DJ, Coffroth MA (2001) Genetic comparisons of freshly isolated vs. cultured symbiotic dinoflagellates: implications for extrapolating to the intact symbiosis. *Journal of Phycology*, **37**, 900–912.

Santos SR, Taylor DJ, Kinzie RA III, Hidaka M, Sakai K, Coffroth MA (2002a) Molecular phylogeny of symbiotic dinoflagellates inferred from partial chloroplast large subunit (23S)-rDNA sequences. *Molecular Phylogenetics and Evolution*, **23**, 97–111.

Santos SR, Taylor DJ, Kinzie RA III, Sakai K, Coffroth MA (2002b) Evolution of length variation and heteroplasmy in the chloroplast rDNA of symbiotic dinoflagellates (*Symbiodinium*, Dinophyta) and a novel insertion in the universal core region of the large subunit rDNA. *Phycologia*, **41**, 311–318.

Schlotterer C (2000) Evolutionary dynamics of microsatellite DNA. *Chromosoma*, **109**, 365–371.

Streelman JT, Zardoya R, Meyer A, Karl SA (1998) Multilocus phylogeny of chichlid fishes (Pisces: Perciformes): evolutionary comparison of microsatellite and single-copy nuclear loci. *Molecular Biology and Evolution*, **15**, 798–808.

Swofford DL (2002) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4.0b10, Sinauer Associates, Sunderland, MA, USA.

Taylor DL (1974) Symbiotic marine algae: taxonomy and biological fitness. In: *Symbiosis in the Sea* (ed. Vernberg WB), pp. 245–262. University of South Carolina Press, Colombia, SC, USA.

Trench RK, Blank RJ (1987) *Symbiodinium microadriaticum* Freudenthal, *S. goreauii* sp. nov., *S. kawagutti* sp. nov. and *S. pilosum* sp. nov. gymnodinioid dinoflagellate symbionts of marine invertebrates. *Journal of Phycology*, **23**, 469–481.

Trench RK, Thinh LV (1995) *Gymnodinium linucheae* sp. nov.: the dinoflagellate symbiont of the jellyfish *Linuche unguiculata*. *European Journal of Phycology*, **30**, 149–154.

Wilcox TP (1998) Large-subunit ribosomal RNA systematics of symbiotic dinoflagellates: morphology does not recapitulate phylogeny. *Molecular Phylogenetics and Evolution*, **10**, 436–448.

S. R. Santos and T. L. Shearer are population and evolutionary biologists who use genetic methods to address questions relating to dinoflagellate symbionts and their invertebrate hosts. A. R. Hannes is a collaborator with similar interests in these symbiotic systems. The laboratory of M. A. Coffroth explores issues ranging from conservation to population genetics in the marine environment using molecular tools and techniques.
