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Biogeography of Two Species of *Symbiodinium* (Freudenthal) Inhabiting the Intertidal Sea Anemone *Anthopleura elegantissima* (Brandt)

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Abstract. We have analyzed the genetic profiles of dinoflagellate populations obtained from the Pacific coast sea anemone *Anthopleura elegantissima* (Brandt) at collection sites from Washington to California. Genetic differences within the symbiont populations of California anemones have been uncovered by restriction length polymorphism (RFLP) analysis of the small subunit (SSU) and large subunit (LSU) ribosomal RNA genes, and by denaturing gradient gel electrophoresis (DGGE) of the internal transcribed spacer region 2 (ITS 2). The existence of two *Symbiodinium* species is substantiated by sequence analysis of the variable regions V1, V2, and V3 of the SSUrDNA, which also establishes their phylogenetic relatedness to other members of the genus *Symbiodinium.* Anemones from Washington and Oregon harbor a single dinoflagellate species, for which we propose the name *S. muscatinei* sp. nov. At these northern locations, *S. muscatinei* either exists alone or co-occurs with the *Chlorella*-like green algal symbiont. Our results indicate that *S. muscatinei* co-occurs with a second dinoflagellate, *S. californium,* in mixed populations in central and southern California. We suggest that the geographic distribution of these dinoflagellates is related to the temperature cline created by latitude.

Introduction

Intertidal anemones of the genus *Anthopleura* are abundant along the Pacific coast of North America (Hand, 1955). *A. elegantissima,* the most common and wide ranging species, is distributed along the rocky intertidal from Alaska to central Baja California (Hand, 1955; Francis, 1979; McFadden *et al.,* 1997). Throughout its geographical range this species harbors intracellular dinoflagellates of the genus *Symbiodinium.* Anemones in regions north of California may also host a *Chlorella*-like (Chlorophyta) alga alone or in mixed populations with the dinoflagellate (Muscatine, 1971). Whether a particular algal symbiont occurs in an anemone, and whether it is present alone or in mixed populations are phenomena believed to be influenced by physical parameters. Relative sensitivities to light and temperature appear to be most significant in regulating the distribution of algal populations. Anemones in warm, bright habitats typically possess the dinoflagellate symbiont, whereas anemones in cool, shadier habitats contain the green alga (Secord, 1995; Saunders and Muller-Parker, 1997).

Environmental parameters have also been implicated in modulating *Symbiodinium* populations in some tropical symbioses. The Caribbean reef-building corals *Montastraea annularis* and *M. faveolata* either host a single algal taxon or have mixed algal populations with patterns of distribution and relative abundance that may be in response to irradiance and temperature (Rowan and Knowlton, 1995; Rowan *et al.,* 1997). Continued analyses of *Symbiodinium* populations in scleractinians are resolving greater diversity within individual hosts than previously recognized (Baker and Rowan, 1997; Baker *et al.,* 1997; Baker, 1999). Restriction fragment length polymorphism (RFLP) analysis of the large subunit (LSU) ribosomal DNA from 107 Pacific and Caribbean corals (Baker, 1999) identified 69 species that harbored a single algal taxon; 13 other species harbored more than one *Symbiodinium* taxon, but not in the same colony, and 25 species sometimes harbored more than one algal taxon within the same colony. Depth (and thus reduced light) was

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Abbreviations: DGGE, denaturing gradient gel electrophoresis; ITS 2, internal transcribed spacer region 2; LSU, large subunit; RFLP, restriction fragment length polymorphism; SSU, small subunit.

regarded as the most significant factor regulating the distribution of a particular alga.

Previous studies conducted on algae isolated from *A. elegantissima* collected in Washington and California showed marked differences in mitotic indices (Wilkerson *et al.,* 1983) and carbon translocation (Shick and Dykens, 1984; Verde and McCloskey, 1996). The possibility that these differences reflect different *Symbiodinium* species has been suggested (Verde and McCloskey, 1996), but has not been empirically tested. A limited genetic study was conducted by Rowan and Powers (1991) on symbiont populations collected from Pacific Grove, California. Their RFLP and partial small subunit (SSU) ribosomal DNA sequence analyses from cloned amplification products detected only one dinoflagellate taxon belonging to lineage B *Symbiodinium* (*sensu* Rowan and Powers, 1991). It is now recognized that analyses based on the conserved SSUrRNA gene underestimate genetic diversity (Rowan, 1998). Furthermore, sampling from a single collection site would not necessarily identify symbiont diversity in a host with a range of thousands of kilometers.

The extensive geographic range of *A. elegantissima,* its occupancy of diverse intertidal habitats, its occurrence in two different growth forms—solitary and clonal—which may represent two distinct species (McFadden *et al.,* 1997), and its ability to harbor symbionts from different algal divisions suggest that *A. elegantissima* may harbor more than one taxon of symbiotic dinoflagellate. This possibility is strengthened by the finding that different *Symbiodinium* spp. are adapted to different regimes of light (Iglesias-Prieto and Trench, 1994, 1997b) and temperature (Warner *et al.,* 1996). To test the hypothesis that different dinoflagellate taxa may co-occur in *Anthopleura,* anemones were collected from intertidal habitats along a latitudinal gradient ranging from Puget Sound in Washington to San Diego in Southern California. A genetic examination, using traditional RFLP analyses of the SSUrDNA and LSUrDNA, and analyses of partial SSUrDNA sequences were conducted on *Symbiodinium* populations isolated from these anemones. Denaturing gradient gel electrophoresis (DGGE; Myers *et al.,* 1985; Abrams and Stanton, 1992) is a technique frequently used to characterize and compare genetic diversity in complex microbial assemblages from samples collected over spatial and temporal scales (Muyzer *et al.,* 1993; Muyzer and Smalla, 1998). This technique was used here to analyze the variable internal transcribed spacer region 2 (ITS 2), and thus to visualize and identify the occurrence of more than one algal taxon within a host.

Materials and Methods

Anemone collections

Specimens of symbiotic *Anthopleura elegantissima* were collected from rocky intertidal locations along the Washington, Oregon, and California coastlines. Between February 1997 and August 1998, collections were conducted at Anaco Beach, Fidalgo Island, Washington (48°29′; 122°42′); Coos Bay, Charleston, Oregon (43°34′; 124°33′); Carmel, California (36°55′; 121°92′); Cayucos, California (35°44′; 120°88′); Ellwood Beach and Campus Point, Santa Barbara, California (34°43′; 119°83′); and Swami's Beach, Cardiff-by-the-Sea (Encinitas), California (33°04′; 117°29′). Symbiotic *A. xanthogrammica* were collected only from Cayucos. Individual anemones were collected from a range of habitats, from exposed upper intertidal to shaded lower intertidal locations. Aposymbiotic *A. elegantissima* were obtained from drainage sluice-ways beneath the wet lab facilities of the Marine Science Institute of the University of California, Santa Barbara, California.

Isolation of algal cells from tissues of host anemones

Oral discs and tentacles from anemones were macerated in a Tenbroek tissue grinder. This step was followed by a 5-min centrifugation at about 800 × *g* in a Dynac II benchtop centrifuge. The pellets containing the algal cells were resuspended and further homogenized with a Tissue Tearor (Model 985-370) to dissociate most of the remaining animal cellular debris. After a second centrifugation, the algal pellets were resuspended in Millipore-filtered (porosity 0.22 μm) seawater, centrifuged, and resuspended two or three times to remove most of the animal debris from the algal cell preparation.

The cultured *Symbiodinium californium* (#383, Banaszak *et al.,* 1993) was originally isolated, in 1989, from a solitary form of *A. elegantissima* by using a technique developed by Polne-Fuller (1991). The isolate was grown in 1 l of ASP-8A (Blank, 1987) for 2 months at 17°C, illuminated by banks of VitaLite fluorescent tubes delivering 80 μmol quanta $m^{-2}s^{-1}$ photosynthetically active radiation on a 14:10 (light:dark) photoperiod. Algae were harvested by centrifugation at 9000 × *g* in a Sorvall RC-5B centrifuge.

DNA extraction, amplification, RFLP

Symbiodinium populations were isolated from 64 specimens of *A. elegantissima* (37 clonal and 27 solitary) and three of *A. xanthogrammica.* DNA was extracted from about 25 mg of algal material by using a proteinase digestion and spin-column separation protocol described in the QIAamp Tissue kit (Qiagen Corporation, Santa Clarita, CA). From the spin-column eluate, 1 μl of product was used as a template to amplify the small-subunit ribosomal RNA gene (SSUrDNA) and part of the large subunit ribosomal RNA gene (LSUrDNA) (Lenaers *et al.,* 1989). SSUrDNA was amplified on a Perkin-Elmer thermal cycler 2400 using primers of Rowan and Powers (1991), and under the following conditions: an initial denaturing step of 3 min at 92°C followed by 35 cycles of 30 s at 92°C, 40 s at 52°C,

and 30 s at 72°C, followed by a single cycle of 5 min at 72°C. A fragment corresponding to a region between 28 bp and 929 bp of the *Prorocentrum micans* LSU rRNA gene containing the variable portions D1–D3 (Lenaers *et al.*, 1989; Wilcox, 1998) was amplified using primers described by Wilcox (1998) with the same protocol used for the SSUrDNA, but with an optimal annealing temperature of 48°C.

Restriction digests were performed by incubating amplified products with 1–5 units of Taq I (New England BioLabs, Beverly, MA) at 65°C for 3 to 5 h or with 1–5 units of Dpn II (New England BioLabs) for 4 to 5 h at 37°C. Products of the digests were separated by electrophoresis in a 2.5% high-melt agarose gel at a constant 70 V for 3 h.

Denaturing gradient gel electrophoresis (DGGE)

Primers for polymerase chain reaction (PCR)-DGGE analyses were designed to amplify the variable internal transcribed spacer region II (ITS 2), producing a fragment size of 330–360 bp. ITS and 5.8S rDNA sequence data (unpublished) from cultured *Symbiodinium* spp. isolated from various cnidarian and molluscan hosts were compared to identify conserved regions. An internal primer "ITSintfor2" (5′GAATTGCAGAACTCCGTG-3′) was designed from this analysis, and it anneals to a conserved region of the 5.8S rDNA. Primer ITS-Reverse (Coleman *et al.*, 1994) was modified with a 40-bp GC clamp (Sheffield *et al.*, 1989) and is referred to as "ITS2CLAMP" (5′CGCCCGCCG-CGCCCCGCGCCCGTCCCGCCGCCCCCGCCCGGGA-TCCATATGCTTAAGTTCAGCGGGT-3′). A "touchdown" amplification (Don *et al.*, 1991) protocol was used to ensure specificity. Initial annealing conditions began 10°C above the final annealing temperature of 52°C. Every two cycles, the annealing temperature was decreased one degree. After 20 cycles the annealing temperature was held, and remained at 52°C for another 15 cycles. Reaction products were loaded onto an 8% acrylamide denaturing gradient gel (a 40% to 75% gradient, 100% consists of 7 *M* urea and 40% deionized formamide). PCR products were loaded on the gel with a 2% Ficoll loading buffer (2% Ficoll-400, 10 m*M* Tris-HCl pH 7.8, 1 m*M* EDTA, 1% bromophenol blue) and separated by electrophoresis for 22 h at 90 V at a constant temperature of 60°C. The gel was then stained in a 1X TAE and 5 μg/ml ethidium bromide solution for 15 min, washed in deionized water for 15 min and photographed.

DNA sequencing, alignment, and analysis

Partial sequences of the SSUrDNA and the ITS spacer (ITS 1 and 2 and 5.8 rRNA gene) were obtained from direct cycle sequencing of amplified products. Cycle sequencing of amplified SSUrDNA products was accomplished using Rowan and Power's (1991) forward primer and an internal primer (Anderson *et al.*, 1993), which permitted sequence reads spanning the V1, V2, and part of the V3 variable regions (Sogin and Gunderson, 1987; as described in Rowan and Knowlton, 1995). Reagents were supplied and reaction conditions specified in the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA). Reaction products were analyzed on an Applied Biosystems 310 Genetic Analyzer (Division of Perkin Elmer, Foster City, CA). The resulting chromatograms were checked and edited using Sequence Navigator, version 1.0, software (ABI, Division of Perkin Elmer, Foster City, CA). Sequence composites of the SSUrDNA were assembled and, including gaps, totaled 375 nucleotides. Partial sequences from the two algal types identified in our RFLP and DGGE analyses were then aligned by eye with published *Symbiodinium* sequences obtained from Genbank; they included *S. corculorum, S. microadriaticum, S. pilosum, S. pulchrorum, Gymnodinium varians, G. simplex, Porocentrum micans,* and four *Symbiodinium* spp. symbiotic with the coral *Montastraea annularis.* Cladistic analyses, by the method of parsimony, were conducted on the aligned data set using PAUP 4.0b4 software under default settings (Swofford, 1993). A bootstrap analysis was conducted on the most parsimonious tree to assess relative support for each branch (Felsenstein, 1985).

Results

Anemones collected from high and low intertidal habitats at each study site contained the same *Symbiodinium* populations. We found no indication, based on our molecular analyses, that the *Symbiodinium* populations in *A. elegantissima* are influenced by the local environmental differences within the littoral zone. However, we found significant differences between the algal populations in anemones collected from Washington and Oregon, and those collected at lower latitudes in California. Figure 1 summarizes the findings from our RFLP and DGGE analyses that detected a single genetic entity in northern anemones and at least two genetic entities in southern anemones.

Taq I digests of the SSUrDNA amplified from northern populations of *Symbiodinium* exhibited a characteristic "clade B" restriction pattern (Rowan and Power, 1991) (Fig. 1A, lane 1). The same analysis conducted on southern populations produced a mixed pattern consisting of the "clade B" type with a second undescribed pattern (Fig. 1C, lane 1). We have identified this latter pattern as diagnostic of a second algal type. It is identical to the restriction pattern of cultured *S. californium,* whose SSUrDNA does not yield a classical "clade A, B, or C" pattern (Fig. 1C, lane 2) (*cf.* RFLP type "T6" in Darius *et al.*, 1998). This new pattern results from the loss of a restriction site at approximately position 1500 and the gain of a restriction site at position 870 of the amplified 1785 bp product, as determined from the entire SSUrDNA sequence of *S. californium* (GenBank

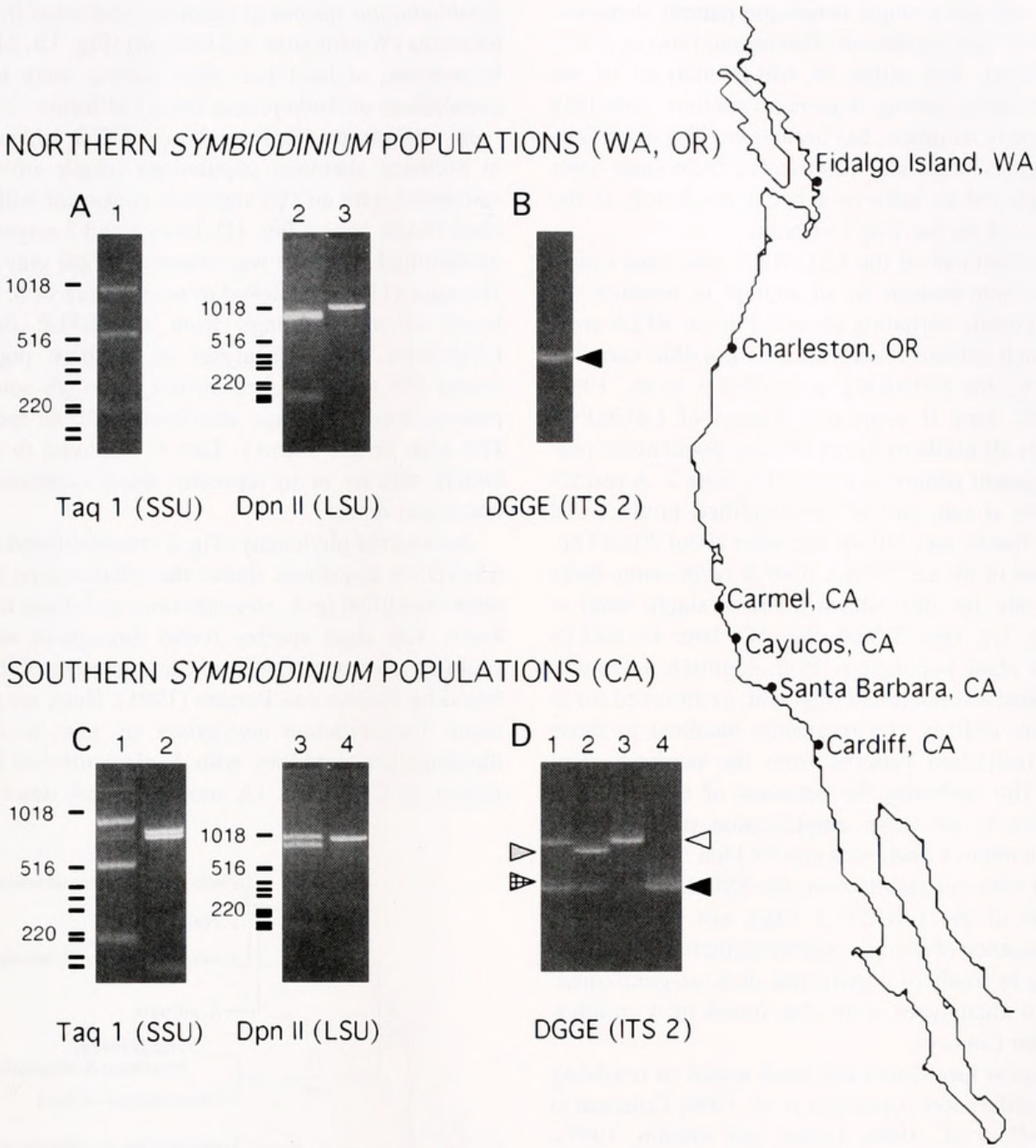

Figure 1. Pacific coastline of North America depicting collection sites and genetic analyses of northern and southern *Symbiodinium* populations. ITS 2, internal transcribed spacer region 2; LSU, large subunit; SSU, small subunit. (A) RFLP analysis of the SSUrDNA and LSUrDNA from northern algal populations. Lane 1, Taq 1 digest of SSUrDNA indicative of lineage B *Symbiodinium* (Rowan and Powers, 1991); lane 2, Dpn II digest of the D1–D3 variable region of the LSUrRNA gene; and for comparison, in lane 3, Dpn II digest of the same region from *S. californium* (#383). (B) DGGE gel of the ITS 2 depicting a single ITS signature (black arrow). (C) RFLP analysis of the ribosomal repeat from southern algal populations; lane 1, Taq 1 digest of SSUrDNA showing the lineage B *Symbiodinium* co-occurring with a second pattern not belonging to any of the described "clades" (*sensu* Rowan and Powers, 1991); lane 2, Taq 1 digest of SSUrDNA of #383, a pattern identical to the undescribed RFLP profile in lane 1. Lanes 3 and 4 are Dpn II digest on LSUrRNA gene amplified from natural populations and the cultured clonal isolate #383 respectively; indicates more than one algal taxon in southern populations. (D) DGGE gel showing three representative profiles of ITS 2 signatures from natural populations, lanes 1, 2, and 4; and for comparison, lane 3 depicts the ITS signature for #383 (white arrow). (See text for further explanation.)

accession #AF225965). The poor amplification of the SSUrDNA from *S. californium* in mixed populations may explain why the smallest fragment (130 bp) appears to be absent from RFLPs on natural samples (Fig. 1C, lane 1). Dpn II restriction digests of SSUrDNA from both freshly isolated northern and southern algal samples and cultured *S.*

californium produced a single restriction pattern characteristic of "clade B" *Symbiodinium* (Rowan and Powers, 1991) (data not shown). The utility of RFLP analyses of the SSUrDNA in distinguishing sequence variation, especially among congeneric members, has limitations. The more variable gene regions and more informative techniques were therefore employed to achieve a better resolution of the variation detected by the Taq 1 enzyme.

An 850-bp fragment of the LSUrRNA gene was examined by restriction analysis in an attempt to measure the extent of the genetic varitation observed in our RFLP analysis of the small subunit and to uncover possible variation not resolved by the SSUrRNA gene (Baker *et al.,* 1997; Wilcox, 1998). Dpn II restriction digests of LSUrDNA amplified from all northern *Symbiodinium* populations produced the fragment pattern in Figure 1A, lane 2. A restriction site exists at one end of the amplified product and produces two bands, one 740 bp, the other about 70–80 bp. A Dpn II digest of the LSUrDNA from *S. californium* lacks a restriction site for this enzyme, and a single band is depicted (Fig. 1A, lane 3, and also 1C, lane 4). RFLPs conducted on algal populations from southern anemones always contained a nondigested fragment, as observed for *S. californium,* as well as two fragments identical to those observed in restriction patterns from the northern algal populations. This indicates the presence of two different gene sequences in the same amplification product—one with and one without a restriction site for Dpn II. These data are consistent with our results from the SSUrDNA digests. Taq 1 digests of the LSUrDNA (data not shown) also showed the presence of a single type in northern populations and two types in southern populations of *A. elegantissima.* The same two algal types were also found in *A. xanthogrammica* from Cayucos.

The ITS region has historically been useful in resolving species-level differences (Gonzalez *et al.,* 1990; Coleman *et al.,* 1994; Goff *et al.,* 1994; Larsen and Medlin, 1997). Among dinoflagellates, it has been used to resolve differences between closely related species within the genus *Alexandrium* (Adachi *et al.,* 1996) and to assess intraspecific variation in *Gymnodinium catenatum* (Adachi *et al.,* 1997). Hunter *et al.* (1997), in their preliminary study, reported that the ITS is potentially a good marker for interspecific comparisons between *Symbiodinium* taxa. In this study, a region encompassing the ITS 2 and a portion of the 5.8S was chosen for DGGE analyses.

DGGE separates amplification products by differences in sequence composition and nucleotide order. The results are therefore a qualitative assessment of the entire sequence. Application of DGGE has permitted the rapid assessment of complex microbial populations and identification of specific microbes from marine, aquatic, and terrestrial samples (Muyzer and Smalla, 1998). This analysis, applied to *Symbiodinium* populations, has verified the existence of a single *Symbiodinium* species in anemones collected from northern locations (Washington and Oregon) (Fig. 1B, black arrow). In contrast, at least two algal species were identified in populations of *Anthopleura* from California.

In southern populations, the algal ITS "signature" found in northern anemone populations (black arrow) is often associated with an ITS signature consistent with *S. californium* (white arrow; Fig. 1D, lanes 1 and 3 respectively). An unidentified ITS type was observed (light gray arrow; Fig. 1D, lane 2) but is believed to be a variant of *S. californium* based on our findings from the RFLP data on the LSUrDNA. In our analyses of southern populations, a fourth ITS type (hatched arrow), although sometimes appearing faint, is always associated with the most common ITS type (black arrow). This is believed to either be a DGGE artifact or to represent fixed variation within the ribosomal repeat.

An inferred phylogeny (Fig. 2) reconstructed from partial SSUrDNA sequences shows the relationships between the algae identified in *A. elegantissima* and those from tropical hosts. The algal species found throughout northern and southern anemone populations is a member of the B lineage found by Rowan and Powers (1991). Here, we propose the name *Symbiodinium muscatinei* sp. nov. to refer to the dinoflagellate symbiotic with *A. elegantissima* from Washington to California. (A morphological description must

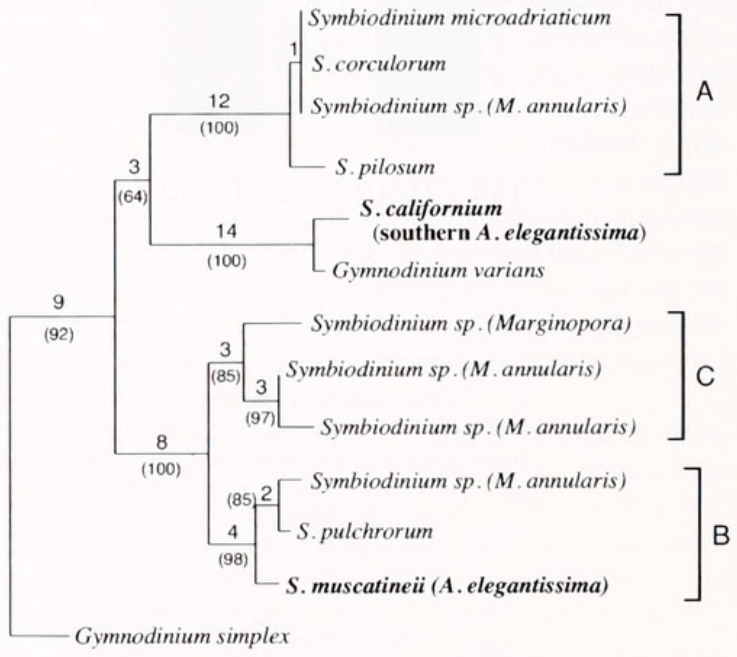

Figure 2. Phylogenetic reconstruction of the *Symbiodinium* lineage by the method of parsimony using a 375-bp composite of the SSUrRNA gene spanning the V1, V2, and V3 variable region. *S. californium* is closely related to *Gymnodinium varians* and forms a separate lineage. *S. muscatinei* groups with members of the B lineage and is probably the same taxa identified in the study by Rowan and Powers (1991). Numerals above branch segments indicate number of base pair changes; numbers in parentheses below show bootstrap consistency based on 1000 replicates. For simplicity, the out group, *Prorocentrum micans,* is not shown.

await achieving axenic culture, but the partial sequence of the SSUrDNA has been given the GenBank accession numbers AF228362 and AF228363 for the V1, and V2 and V3 regions of the SSUrDNA, respectively.) *S. californium,* identified in anemones from California only, is closely related to *Gymnodinium varians*; together they form a lineage separate from the described "clades A, B, and C" (bracketed). Sequence differences between the SSUrDNA from *S. muscatinei* and *S. californium* are significantly greater than differences observed between described species. The entire ITS region was sequenced from each alga and compared (data not shown). No reliable alignment was possible due to extreme sequence divergence. The ITS sequence of *S. muscatinei* was aligned and compared with sequences from several other lineage B *Symbiodinium.* Results indicated a level of divergence many times greater (12%) than interspecific variation observed among other dinoflagellates (Adachi *et al.,* 1997). These data collectively indicate the presence of two distinct dinoflagellate species.

Animal DNA isolated from aposymbiotic *A. elegantissima* was used to determine whether host material would be a source of contamination in freshly isolated algal samples. Amplification was never achieved with the primers and reaction conditions used for the amplification of LSUrDNA and SSUrDNA. However, the ITS 2 primers designed for DGGE amplified host DNA, but only rarely when algal DNA was present.

Cloned *S. californium* (#383) served as a control, so we could ascertain the presence of pseudogenes or natural variation in ribosomal repeats within a single genome. Ribosomal pseudogenes have been observed in some dinoflagellates (Scholin *et al.,* 1993; Adachi *et al.,* 1996). Although none have been reported in *Symbiodinium,* their presence cannot be discounted. The DGGE analysis on *S. californium* (#383) repeatedly identified a single type with no obvious intragenomic variation.

Discussion

The molecular methods employed in this study demonstrate that there are two distantly related species of *Symbiodinium* in populations of *Anthopleura elegantissima* along the Pacific coast of the United States. Evidence based on RFLP, DGGE, and sequence analysis indicates the presence of a single *Symbiodinium* species, designated here as *S. muscatinei,* in northern populations. This is consistent with previous reports that many hosts harbor a single population of algae (Schoenberg and Trench, 1980a; Baker and Rowan, 1997; Bythell *et al.,* 1997; Billinghurst *et al.,* 1997; Stochaj and Grossman, 1997). Our analyses also show that anemones from southern latitudes in California harbor a mixed dinoflagellate population consisting of two congeneric species: *S. muscatinei* identified from northern anemones, and *S. californium.*

It has been recognized for some time that some invertebrate taxa may simultaneously harbor more than one algal taxon (Muscatine, 1971; Trench and Winsor, 1987). The coexistence of two or more *Symbiodinium* taxa in the same host was first described by Rowan and Knowlton (1995) in the Caribbean reef building corals *Montastrea annularis* and *M. faveolata.* Since then, more than one *Symbiodinium* taxon has been identified in populations of certain other coral species. Furthermore, some individual colonies have been shown to harbor mixed symbiont taxa (Baker and Rowan, 1997; Darius *et al.,* 1998; Baker, 1999; Carlos *et al.,* 1999; Banaszak *et al.,* 2000). From the data of Baker (1999), about 23% of the total coral taxa sampled may have mixed symbiont populations, indicating that the presence of more than one symbiont simultaneously is not uncommon.

Environmental parameters have been hypothesized to regulate the distribution and population dynamics of each symbiont in hosts that harbor more than one algal species (Rowan *et al.,* 1997; Baker, 1999). The patterns of these distributions are specific and correlate closely with environmental gradients. *Symbiodinium* species examined in culture and *in hospite* show species-specific physiological adaptations to photosynthetically active radiation and temperature (Chang *et al.,* 1983, Iglesias-Prieto and Trench, 1994, 1997a, b; Warner *et al.,* 1996, 1999). Algal species better adapted for a particular environment will out-compete those less suited (Schoenberg and Trench, 1980b; Rowan *et al.,* 1997; Saunders and Muller-Parker, 1997).

Previous studies have focused primarily on the reef-wide distribution of symbioses involving more than one algal taxon, but very little is known about the biogeography of algal symbionts and about how the distribution of algal species in geographically widespread hosts may be influenced by differences in environment. Ultimately, the problem revolves around the determination of the species of algae involved in the associations. The difficulty in delineating species has been a long-standing problem for oceanographers studying phytoplankton biogeography (Round, 1981). With regard to *Symbiodinium,* one example is the coral *Plesiastrea versipora,* which has an unusually broad latitudinal distribution along the east coast of Australia. On the tropical Great Barrier Reef it forms a symbiosis with *Symbiodinium* sp. of the C lineage, while in cooler temperate waters off Sydney it harbors a *Symbiodinium* sp. from the B lineage (Baker, 1999).

Temperature and irradiance are the most significant environmental variables that change predictably with latitude. Within the tropics, among coral species with more than one algal taxon, the algae exhibit ecological zonation that correlates with irradiance (Rowan and Knowlton, 1995; Baker, 1999). Irradiance in temperate regions is less consistent and may not be an important parameter regulating the distribution of *S. californium.* When compared to California, Washington and Oregon have longer periods of daylight during

the summer, but experience shorter periods in the winter. Assessment of the influence of light as an environmental factor that regulates algal distribution is further complicated because anemones act to control irradiance levels impinging on the algae by covering their surfaces with fragments of rock and shell (Dykens and Shick, 1984).

Temperature is an environmental factor that regulates species distributions along the coast of California (Newman, 1979), and it probably governs the distribution of *S. californium*. Collection sites from Oregon and Washington routinely experience colder temperatures than locations in California (Fig. 3), particularly in winter. In addition, northern anemones experience greater annual temperature fluctuations, which may also be of selective importance. Low temperature extremes, like high temperatures, may result in the loss of symbionts from hosts (Muscatine *et al.*, 1991). Temperature as a selective force is supported by the observation that, in northern anemone populations that harbor *S. muscatinei* and the green Chlorella-like alga, symbiont growth rates are more strongly affected by temperature than by irradiance (Saunders and Muller-Parker, 1997). Temperature changes associated with increased latitude may also influence the competitive balance between *S. californium* and *S. muscatinei* by affecting their relative growth rates. We also suggest the possibility that low temperatures found in the north exclude *S. californium*; in the south, *S. californium* and *S. muscatinei* coexist because the latter species may have a wider temperature tolerance. Because attempts to culture *S. muscatinei* were unsuccessful, controlled physiological comparisons were not possible.

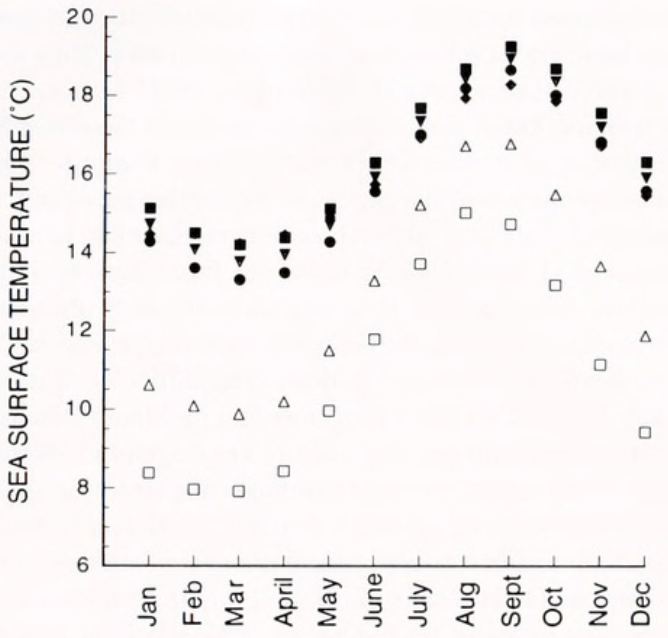

Figure 3. Average coastal sea surface temperature data from the Comprehensive Ocean-Atmosphere Data Set (COADS) monthly climatology records (1946–1989). Graph compares the seasonal fluctuation of sea surface temperature in degrees Celsius at collection sites in Washington (open square), Oregon (open triangle), and from central and southern California (Carmel, solid circle; Cayucos, solid triangle; Santa Barbara, solid square; Cardiff-by-the-Sea (Encinitas), solid diamond). The locations are shown on the map in Figure 1. The southern locations in California have similar seasonal temperatures and are always 1° to 6°C warmer than northern locations depending on the time of year. The range in temperature fluctuation between March (lowest yearly temperature) and September (highest) is greater for Washington and Oregon (*ca.* 7°C) than for locations in California (*ca.* 4°–5°C).

The nonrandom sorting or specificity of algal-invertebrate symbioses is contingent on the interplay of multiple factors (Trench *et al.*, 1981; Trench, 1988, 1997), and is not expressed as one alga for one host (Schoenberg and Trench, 1980b). Cnidarian hosts are symbiotic with selectively few microalgal taxa (Trench, 1997), yet they are exposed to hundreds and possibly thousands of "free-living" microalgal species. The "complementariness of the dynamically interacting attributes" (Dubos and Kessler, 1963) of both symbiont and host substantially limits possible symbiotic combinations. The extent to which a host shows specificity for one or more algal species depends ultimately on the poorly understood cellular and molecular processes that may take place during initial intracellular contact, and possibly also after the association is established (Colley and Trench, 1983; Trench, 1993). In those hosts harboring more than one algal taxon, symbiont distributions are strongly influenced by changes in the physical environment over both local (Rowan and Knowlton, 1995; Secord, 1995) and geographic sales (Baker, 1999). Because the influence of environment on host-symbiont dynamics can be variously interpreted, further experimental analyses are needed to explain the mechanisms that result in these observed patterns (Saunders and Muller-Parker, 1997). For example, it is unclear whether changes in the physical environment have an intrinsic or extrinsic effect on the biology of the symbiosis. Changes in the environment might modify the physiological integration of the symbiotic partners so that they are no longer compatible. Finally, differential changes in symbiont growth rates may, and can, cause competitive exclusion of one algal species over another (Provasoli *et al.*, 1968). Progress in elucidating these mechanisms should provide a more accurate description of host symbiont interactions and specificity.

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