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Symbiodinium diversity among host clionaid sponges from Caribbean and Pacific reefs: Evidence of heteroplasmy and putative host-specific symbiont lineages

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ABSTRACT

Among the Porifera, symbiosis with *Symbiodinium* spp. (i.e., zooxanthellae) is largely restricted to members of the family Clionaidae. We surveyed the diversity of zooxanthellae associated with sponges from the Caribbean and greater Indo-Pacific regions using chloroplast large subunit (cp23S) domain V sequences. We provide the first report of Clade C *Symbiodinium* harbored by a sponge (*Cliona caesia*), and the first report of Clade A *Symbiodinium* from an Indo-Pacific sponge (*C. jullieni*). Clade A zooxanthellae were also identified in sponges from the Caribbean, which has been reported previously. Sponges that we examined from the Florida Keys all harbored Clade G *Symbiodinium* as did *C. orientalis* from the Indo-Pacific, which also supports earlier work with sponges. Two distinct Clade G lineages were identified in our phylogenetic analysis; *Symbiodinium* extracted from clionaid sponges formed a monophyletic group sister to *Symbiodinium* found in foraminiferans. Truncated and 'normal' length variants of 23S rDNA sequences were detected simultaneously in all three morphotypes of *C. varians* providing the first evidence of chloroplast-based heteroplasmy in a sponge. None of the other sponge species examined showed evidence of heteroplasmy. As in previous work, length variation in cp23S domain V sequences was found to correspond in a highly precise manner to finer resolution of phylogenetic topology among *Symbiodinium* clades. On a global scale, existing data indicate that members of the family Clionaidae that host zooxanthellae can form symbiotic associations with at least four *Symbiodinium* clades. The majority of sponge hosts appear to harbor only one cladal type of symbiont, but some species can harbor more than one clade of zooxanthellae concurrently. The observed differences in the number of partners harbored by sponges raise important questions about the degree of coevolutionary integration and specificity of these symbioses. Although our sample sizes are small, we propose that one of the Clade G lineages identified in this study is comprised of sponge-specialist zooxanthellae. These zooxanthellae are common in Caribbean sponges, but additional work in other geographic regions is necessary to test this idea. Sponges from the Indo-Pacific region harbor zooxanthellae from Clades A, C, and G, but more sponges from this region should be examined.

1. Introduction

Among the Dinokaryota, the dinoflagellate genus *Symbiodinium* is notable for its mutualistic intrazoic lifestyle (Freudenthal, 1962), and its singular ecological importance providing energy to support coral reefs as one of the most diverse communities on the planet (Muscatine and Porter, 1977). Members of the genus *Symbiodinium* (i.e., zooxanthellae) have long been known to associate with an impressive number of invertebrate hosts (reviewed in Trench 1987, 1997). Due to a lack of taxonomically informative characteristics, however, the diversity within the genus *Symbiodinium* remained largely unappreciated until the early 1980s when several papers out of the Trench laboratory began to reveal significant differences among 'strains' of zooxanthellae (Schoenberg and Trench, 1980a–c). Subsequent application of molecular techniques continues to uncover an astonishing diversity among zooxanthellae isolated from different invertebrate hosts (e.g., Rowan and Powers, 1991; LaJeunesse, 2001, 2002; Pochon et al., 2001, 2004; Coffroth and Santos, 2005; Sampayo et al., 2009). An understanding of the evolutionary relationships among the nine recognized 'Clades' (A–I) is emerging (e.g., LaJeunesse, 2001, 2002; Pochon et al., 2006; Pochon and Gates, 2010). While a significant portion of the diversity has been uncovered, it is clear that additional surveys are likely to reveal further zooxanthella diversity. Non-cnidarian hosts are particularly important targets for this type of exploration (e.g., Pochon et al., 2001, 2004, 2006; Goulet et al., 2008).

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On tropical coral reefs, sponges belonging to the Clionaidae perform extraordinarily important roles in the structure and function of reefs (e.g., Rützler, 1975; Rose and Risk, 1985; Risk et al., 1995; Holmes, 1997; Holmes et al., 2000). They contribute as much as 30% of the sediments in the reef environment with boring rates often between 2 and 20 kg m^{-2} yr^{-1} (Fütterer, 1974; see Table 4 in Schönberg, 2002). Although *Symbiodinium* is found in a variety of sponges (Garson et al., 1998, 1999; Carlos et al., 1999; Scalera-Liaci et al., 1999), the symbiosis is most common in sponges of the Clionaidae (e.g., Sará and Liaci, 1964; Rützler, 1990), The symbiotic association is important because, among other reasons, the intracellular zooxanthellae appear to enhance boring and growth rates of the sponge through their photosynthetic activity (Rosell and Uriz, 1992; Hill, 1996; Schönberg, 2006). Some clionaids are among the most aggressive competitors for space, and many of these sponges are zooxanthellate bioeroders (Suchanek et al., 1983; Alcolado, 1990; Alvarez et al., 1990, Diaz et al., 1990, Schmahl, 1990; Hill, 1998; Schönberg and Wilkinson, 2001; Zea and Weil, 2003; Chaves-Fonnegra and Zea, 2007; Chaves-Fonnegra et al., 2007).

Despite the importance of clionaid-based bioerosion of coral reefs, and the role zooxanthella symbionts play in sponge activity, we know relatively little about the nature of the association or the zooxanthella partners involved (Schönberg and Suwa, 2007; Schönberg et al., 2008). Early morphological work found interesting differences among zooxanthellae harbored by different species of sponge (e.g., Vacelet, 1981; Rützler, 1990). Schönberg and Loh (2005) were among the first to apply molecular tools to identify zooxanthella partners in clionaid sponges, and they found Clade G zooxanthellae in disparate Indo-Pacific populations of *Cliona orientalis*. Recent work by Granados et al. (2008) found Clades A, B and G zooxanthellae in five clionaid sponges from the Caribbean region (*C. tenuis*, *C. aprica*, *C. caribbaea*, *C. laticavicola* and *C. varians*). Schönberg and Loh (2005) used 28S rDNA in their analysis of *C. orientalis*; Granados et al. (2008) used 18S rDNA, ITS, and Domain V cp23S rDNA sequences in their analysis. In the latter study, different molecular markers were used on different species for phylogenetic analysis (e.g., cp23S rDNA sequences were used to place zooxanthellae from only one sponge, *C. varians*, in a phylogenetic context).

Our goal was to characterize zooxanthella diversity in several species from a variety of tropical and sub-tropical habitats. We focused our efforts on chloroplast large subunit (cp23S rDNA) domain V so that we could provide a detailed comparison of sponge-*Symbiodinium* in the context of recent phylogenetic work that has been done with this molecular marker (Santos et al., 2002b, 2003; Pochon et al., 2006; Granados et al., 2008). We also compared zooxanthella partner identities in the Florida Keys with those reported from other parts of the Caribbean. Finally, we were intereseted in assessing whether the same type of zooxanthellae was recovered in the habitat generalist *C. varians* regardless of the depth of occurrence.

2. Methods and materials

Sponge samples were collected using SCUBA or snorkeling from locations shown in Table 1. Samples from the Florida Keys were transported to the Mote Tropical Research Laboratory (Summerland Key, FL) where they were frozen in liquid nitrogen or stored in 75% ethanol. DNA was isolated using a modified CTAB protocol (Doyle and Doyle 1987; Cullings 1992) whereby ≈100 mg of sponge tissue was ground in 350 µl of CTAB buffer. Once the sponge tissue was ground, another 350 µl of CTAB buffer was added with 20 µl of proteinase K (20 mg/ml). Tubes were incubated at 65 °C for 1 h. An equal volume of 24:1 chloroform:isoamyl alcohol was then added, tubes were inverted several times and allowed to sit for 5 min before centrifugation for 2 min at maximum speed (≈16 rcf). The aqueous phase was placed in a new tube, mixed with an equal volume of isopropanol, and placed at −20 °C overnight. The solution was then spun at 16 rcf for 15 min; the resulting pellet was washed three times in 70% ethanol. A final wash of 95% ethanol was performed before the pellet was allowed to dry at which point it was re-suspended in a 10 mM Tris HCl, pH 8.5 buffer.

Samples from non-Floridian sponges were obtained on SCUBA or snorkel and preserved in 70% ethanol or DNA preservative (20% DMSO in 0.25 M EDTA, pH 8.0 saturated with NaCl). DNA was extracted using phenol, choloroform, and isoamyl alcohol following the protocol given in Loh et al. (2001) including slight modifications listed in Schönberg and Loh (2005). Extracted DNA from Floridian and Indo-Pacific sponges were then treated identically (see below).

We employed the Zhang et al. (2000) primers to amplify domain V of the cp23S rDNA molecule. Genomic DNA from a single host was used in each PCR reaction. PCR products were cloned using the TOPO® TA Cloning Kit after gel purification (Qiagen, MinElute Kit). Insert-positive colonies were selected from each sponge representative; appropriately sized inserts were identified using PCR with vector primers. Clones (n = 15–25) from each species were screened with the restriction enzyme *Bsu36I*. Unique RFLPs were sequenced in both directions for all species using M13 forward and reverse primers at Virginia Commonwealth University's Nucleic Acid Research Facility. We obtained zooxanthella sequences with one or more nucleotide differences from several of the cloned pool of PCR product obtained from a given sponge species, and each of these cp23S rDNA variants were included in our phylogenetic analysis.

Table 1
Depth and collection locale for all sponge species used in this study. Accession numbers for sequences obtained in this study are also included. FLK = Florida Keys, USA; HER = Heron Island, Australia (°N; °W); OKI = Okinawa, Japan; CBC = Carrie Bow Cay, Belize; NCAL = Récife Senez, New Caledonia; PIMB = Peel Island, Moreton Bay, Australia; LPB = Little Pioneer Bay, Orpheus Island, Australia.

Species	Depth (m)	Location	Lat/long	Accession numbers
Cervicornia cuspidifera	12	FLK	24°32.89′N; 81°25.40′W	GU219488-9
Cliona caesia	12	HER	23°26.21′S; 81°26.53′E	GU219509
Cliona caesia	0.4	OKI	26°16.42′N; 151°55.80′E	GU219510
Cliona caribbaea	10	FLK	24°32.94′N; 81°22.77′W	GU219511-13
Cliona caribbaea	?	CBC	16°48.89′N; 88°4.77′W	GU219506-7
Cliona jullieni	2	NCAL	19°48.48′S; 165°35.81′E	GU219508
Cliona orientalis	2–3	PIMB	27°29.16′S; 153°21.07′E	GU219514-5
	2–3	LPB	18°35.81′S; 146°29.37′E	GU219516
Cliona varians forma incrustans	10	FLK	24°32.94′N; 81°22.77′W	GU219495-7
Cliona varians forma rigida	15	FLK	24°32.94′N; 81°22.77′W	GU219498-9 and GU219500
Cliona varians forma varians	<1	FLK	24°40.97′N; 81°26.53′W	GU219490-1 and GU219494
Unidentified Cliona	10	FLK	24°32.94′N; 81°22.77′W	GU219492-3
Unidentified species (non-clionaid)	10	FLK	24°32.94′N; 81°22.77′W	GU219501 and GU219504