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Incidence and identity of photosynthetic symbionts in Caribbean coral reef sponge assemblages

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Marine sponges are abundant and diverse components of coral reefs and commonly harbour photosynthetic symbionts in these environments. The most prevalent symbiont is the cyanobacterium, *Synechococcus spongiarum*, isolated from taxonomically diverse hosts from geographically distant regions. We combined analyses of chlorophyll-*a* (chl-*a*) concentrations with line-intercept transect surveys to assess the abundance and diversity of reef sponges hosting photosymbionts on Caribbean coral reefs in the Bocas del Toro Archipelago, Panamá. To identify symbionts, we designed PCR primers that specifically amplify a fragment of the 16S ribosomal RNA gene from *S. spongiarum* and used these primers to screen potential host sponges for the presence of this symbiont. Chlorophyll-*a* data divided the sponge community into two disparate groups, species with high (>125 μg/g, N=20) and low (<50 μg/g, N=38) chl-*a* concentrations. Only two species exhibited intermediate (50–125 μg/g) chl-*a* concentrations; these species represented hosts with reduced symbiont populations, including bleached *Xestospongia muta* and the mangrove form of *Chondrilla nucula* (*C. nucula* f. *hermatypica*). Sponges with high and intermediate chl-*a* concentrations accounted for over one-third of the species diversity and abundance of sponges in these communities. Most (85%) of these sponges harboured *S. spongiarum*. Molecular phylogenies reveal that *S. spongiarum* represents a sponge-specific *Synechococcus* lineage, distinct from free-living cyanobacteria. The prevalence of sponge–photosymbiont associations and dominance of symbiont communities by *S. spongiarum* suggest a major role of this cyanobacterium in sponge ecology and primary productivity on coral reefs.

INTRODUCTION

Coral reef invertebrates commonly associate with photosynthetic symbionts that contribute autotrophic nutrients to host metabolism, thereby enhancing growth rates and competitive abilities in the demanding reef environment. Reef-building corals responsible for the formation and persistence of the reef habitat depend on supplemental nutrition from symbiotic algal populations (Muscatine & Porter, 1977; Stanley, 2006). Invertebrate–photosymbiont relationships among coral reefs encompass diverse host taxa, including sea anemones (Muller-Parker & Davy, 2001), didemnid ascidians (Hirose et al., 1996), tridacnid clams (Baillie et al., 2000), and marine demosponges (Rützler, 1990). In addition to the increased fitness of intact holobionts, the prevalence of these symbioses in reef environments represents a major source of primary productivity (Hatcher, 1990). Understanding the impact of these associations on host ecology, symbiont evolution and reef ecosystem functioning has immediate implications for the conservation of tropical invertebrates and preservation of coral reef habitats.

Marine sponges comprise an abundant component of the coral reef benthos and frequently harbour photosynthetic symbionts in reef environments (Diaz & Rützler, 2001). Among Caribbean and Great Barrier Reef sponge communities, over one-third of the species host symbiotic cyanobacteria (Wilkinson, 1987). In Zanzibar (West Indian Ocean), reef sponges hosting photosynthetic symbionts account for 85% of intertidal and 64% of subtidal species (Steindler et al., 2002). Similar to other invertebrate–photosymbiont relationships, symbionts may provide their host sponges with supplemental nutrition (Arillo et al., 1993), while host sponges may act as a refuge from heavy grazing pressures.

Diverse photosynthetic symbionts inhabit marine sponges, including dinoflagellates (Schönberg & Loh, 2005) and filamentous algae (Carballo & Ávila, 2004); however, the most prevalent symbionts are filamentous and single-celled cyanobacteria. *Oscillatoria spongeliae* is a filamentous cyanobacterium commonly associated with Indo-Pacific reef sponges (Hinde et al., 1994; Thacker & Starnes, 2003; Ridley et al., 2005; Thacker, 2005) and inhabits at least three Caribbean species (Thacker et al., 2007). *Synechococcus spongiarum* is a single-celled cyanobacterium commonly associated with Caribbean, Mediterranean and Indo-Pacific reef sponges (Rützler, 1990; Usher et al., 2004a,b; Steindler et al., 2005; Thacker, 2005). Molecular characterization of sponge-associated cyanobacteria revealed that *O. spongeliae* represents a specialist symbiont, with genetically-distinct populations inhabiting specific host species (Thacker & Starnes, 2003; Thacker et al., 2007), while *S. spongiarum* appears to represent a generalist symbiont, exhibiting little genetic divergence based on host taxonomy or collection

Table 1. *Taxonomy, collection location, and PCR-screening of sponge samples used to assess the specificity of the new primer pair SYN180F and SYN1230R, designed to amplify partial 16S rRNA gene sequences from the sponge symbiont* Synechococcus spongiarum. *Citations following species names indicate the authority source; citations following undescribed species indicate genus-level authority.*

Order	Family	Species	Region	No.	PCR
Verongida	Aplysinidae	*Aiolochroia crassa* (Hyatt, 1875)	Bahamas	4	-
			Bermuda	2	+
		Aplysina aerophoba Nardo, 1843	Mediterranean	2	+
		Aplysina cauliformis (Carter, 1882)	Bahamas	3	+
		Aplysina fistularis (Pallas, 1766)	Bahamas	3	+
			Bermuda	2	+
		Aplysina fulva (Pallas, 1766)	Bahamas	3	+
		Verongula gigantea (Hyatt, 1875)	Bahamas	4	+
		Verongula rigida (Esper, 1794)	Bahamas	4	+
	Ianthellidae	*Ianthella basta* (Pallas, 1766)	Guam	3	-
	Pseudoceratinidae	*Pseudoceratina arabica* (Keller, 1889)	Palau	4	+
Dendroceratida	Darwinellidae	*Aplysilla longispina* George & Wilson, 1919	Bermuda	2	-
		Chelonaplysilla erecta (Row, 1911)	Bermuda	1	-
		Chelonaplysilla sp. de Laubenfels, 1948	Palau	1	-
	Dictyodendrillidae	*Dictyodendrilla nux* (de Laubenfels, 1950)	Bermuda	1	-
Dictyoceratida	Thorectidae	*Dactylospongia metachromia* (de Laubenfels, 1954)	Palau	2	-
		Smenospongia aurea (Hyatt, 1877)	Bahamas	3	+
Chondrosida	Chondrillidae	*Chondrilla* cf. *nucula* Schmidt, 1862	Bermuda	2	+
Haplosclerida	Petrosiidae	*Neopetrosia exigua* (Kirkpatrick, 1900)	Palau	3	+

+, amplification; -, no amplification.

location (Steindler et al., 2005). Although the global distribution of *S. spongiarum* has been well documented, few studies have investigated the local abundance of these symbionts among reef sponge communities (Rützler, 1990).

In addition to providing supplemental nutrition, symbiotic cyanobacteria may play multiple ecologically relevant roles, benefiting their hosts through the production of secondary metabolites (Flatt et al., 2005), the provision of fixed nitrogen (Wilkinson & Fay, 1979), and the reduction of UV-exposure and oxidative stress (Yahel et al., 2003). As a result, cyanobacterial symbionts constitute a significant component of the ecological success and evolutionary history of coral reef sponge communities.

Our study examined the local abundance and diversity of sponges hosting photosymbionts and the prevalence of *S. spongiarum* among Caribbean coral reef sponges. Specifically, we assessed the incidence, photosynthetic activity and symbiont identity of Caribbean sponge–photosymbiont associations in the Bocas del Toro Archipelago, Panamá, using line-intercept transect surveys, chlorophyll-*a* quantification and PCR-amplification. We designed PCR primers to amplify partial 16S ribosomal RNA (rRNA) gene sequences from *S. spongiarum*, thereby allowing for the rapid detection of specific cyanobacterial symbionts in coral reef sponges.

MATERIALS AND METHODS

Primer design and specificity

The oligonucleotide forward primer SYN180F (5'-TAA TAC CCC ATA TGC CGA GAG GTG AAA CGA ATT TCG CCT GGG G-3') and reverse primer SYN1230R (5'-GAG TAG CGA TCT TGC AAA AGT TAG CTA ATC TCG TAA ACC GTG G-3') were designed based on previously characterized cyanobacterial gene sequences (Steindler et al., 2005; Thacker, 2005) to amplify a fragment of the 16S rRNA subunit from the sponge symbiont *Synechococcus spongiarum*. To assess the specificity of the new primer pair, whole genomic extracts from 49 previously collected sponge samples (representing 17 species; Table 1) were screened for the presence of *S. spongiarum* using PCR amplification with SYN180F and SYN1230R. Whole genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega); genomic extracts were cleaned using the Wizard DNA Clean-Up System (Promega). The total PCR reaction volume was 50 µl, including 25 pmol of each primer, 10 nmol of each dNTP, 1X MasterTaq PCR Buffer (Eppendorf), and 1× TaqMaster additive (Eppendorf). Thermocycler conditions consisted of an initial denaturing time of 4 min at 94°C, followed by the addition of 0.5 units MasterTaq DNA polymerase (Eppendorf), then 35 cycles of 0.5 min at 94°C, 0.5 min at 60°C, and 1 min at 72°C, with a final extension time of 2 min at 72°C. The PCR products were visualized on a 1.5% agarose gel with ethidium bromide staining. The PCR products were cloned using the pGEM-T Easy Vector System (Promega); plasmids were harvested using the QIAprep Spin Miniprep Kit (Qiagen). Forward and reverse sequencing reactions were performed at the University of Alabama at Birmingham Center for AIDS Research DNA Sequencing Core Facility. At least one symbiont sequence was recovered from each sponge species that screened positive for *S. spongiarum*. Forward and reverse sequences were compared using Sequencher (Gene Codes) to ensure the accuracy of sequencing results, yielding a final consensus sequence. Consensus sequences were aligned in

Table 2. *Taxonomy of sponge samples collected from coral reefs and mangroves in the Bocas del Toro Archipelago, Panama. Traditional Linnaean taxonomy and phylogenetic clades (G1, G2, G3, G4) are shown. Citations following species names indicate the authority source; citations following undescribed species indicate genus-level authority.*

Clade	Order	Family	Species
Homoscleromorpha	Homosclerophorida	Plakinidae	*Plakinastrella onkodes* BL (Uliczka, 1929)
			Plakinastrella onkodes ML (Uliczka, 1929)
			Plakinastrella onkodes TN (Uliczka, 1929)
Demospongiae			
Keratosa (G1)	Dendroceratida	Darwinellidae	*Chelonaplysilla erecta* (Row, 1911)
	Dictyoceratida	Dysideidae	*Dysidea etheria* de Laubenfels, 1936
		Ircinidae	*Ircinia campana* (Lamarck, 1814)
			Ircinia felix (Duchassaing & Michelotti, 1864)
			Ircinia sp. FT Nardo, 1833
			Ircinia sp. GO Nardo, 1833
			Ircinia sp. PC Nardo, 1833
			Ircinia sp. RA Nardo, 1833
			Ircinia strobilina (Lamarck, 1816)
		Spongiidae	*Spongia (Spongia) pertusa* Hyatt, 1877
			Spongia (Spongia) tubulifera Lamarck, 1813
		Thorectidae	*Hyrtios proteus* Duchassaing & Michelotti, 1864
Myxospongiae (G2)	Chondrosida	Chondrillidae	*Chondrilla nucula* f. *hermatypica* Duran & Rützler, 2006
			Chondrilla nucula f. *mangle* Duran & Rützler, 2006
			Chondrosia reniformis Nardo, 1847
	Verongida	Aplysinidae	*Aiolochroia crassa* (Hyatt, 1875)
			Aplysina cauliformis (Carter, 1882)
			Aplysina fulva (Pallas, 1766)
			Aplysina lacunosa (Pallas, 1766)
			Verongula reiswigi Alcolado, 1984
			Verongula rigida (Esper, 1794)
(G3)	Haplosclerida	Callyspongiidae	*Callyspongia (Cladochalina) vaginalis* (Lamarck, 1814)
		Chalinidae	*Haliclona (Halichoclona) vansoesti* de Weerdt et al., 1999
			Haliclona (Reniera) mucifibrosa de Weerdt et al., 1991
			Haliclona walentinae Diaz et al., 2007
		Niphatidae	*Amphimedon compressa* Duchassaing & Michelotti, 1864
			Amphimedon erina (de Laubenfels, 1936)
			Niphates caycedoi (Zea & van Soest, 1986)
			Niphates erecta Duchassaing & Michelotti, 1864
		Petrosiidae	*Neopetrosia carbonaria* (Lamarck, 1814)
			Neopetrosia subtriangularis (Duchassaing, 1850)
			Xestospongia bocatorensis Diaz et al., 2007
			Xestospongia muta (Schmidt, 1870)
			Xestospongia proxima (Duchassaing & Michelotti, 1864)
			Xestospongia rosariensis Zea & Rützler, 1983
			Xestospongia sp. de Laubenfels, 1932
(G4)	Astrophorida	Geodiidae	*Erylus formosus* Sollas, 1886
	Hadromerida	Clionaidae	*Cliona delitrix* Pang, 1973
			Cliona varians (Duchassaing & Michelotti, 1864)
		Placospongiidae	*Placospongia intermedia* Sollas, 1888
		Spirastrellidae	*Spirastrella coccinea* (Duchassaing & Michelotti, 1864)
		Suberitidae	*Suberites aurantiacus* (Duchassaing & Michelotti, 1864)
		Tethyidae	*Tectitethya crypta* (de Laubenfels, 1949)
	Halichondrida	Axinellidae	*Dragmacidon reticulatum* (Ridley & Dendy, 1886)
		Dictyonellidae	*Scopalina ruetzleri* (Wiedenmayer, 1977)
		Halichondriidae	*Ciocalypta* sp. (Bowerbank, 1862)
			Halichondria (Halichondria) melanodocia de Laubenfels, 1936
	Poecilosclerida	Coelosphaeridae	*Lissodendoryx colombiensis* (Zea & van Soest, 1986)
		Crambeidae	*Monanchora arbuscula* (Duchassaing & Michelotti, 1864)
		Iotrochotidae	*Iotrochota birotulata* (Higgin, 1876)
		Microcionidae	*Clathria (Thalysias) schoenus* (de Laubenfels, 1936)
		Mycalidae	*Mycale (Arenochalina) laxissima* (Duchassaing & Michelotti, 1864)
			Mycale (Mycale) laevis (Carter, 1882)
		Raspailiidae	*Ectyoplasia ferox* (Duchassaing & Michelotti, 1864)
		Tedaniidae	*Tedania ignis* (Duchassaing & Michelotti, 1864)
	Spirophorida	Tetillidae	*Cinachyrella alloclada* (Uliczka, 1929)
			Cinachyrella sp. Wilson, 1925

Clustal X (Thompson et al., 1997) using default settings. Neighbour-joining phylogenies were constructed using MEGA 3.1 (Kumar et al., 2004). Top BLASTn GenBank matches and cultured cyanobacteria sequences were used for outgroup comparison.

Sponge collection and identification

Sponge samples were collected by SCUBA diving from shallow (<15 m) coral reefs at the Smithsonian Tropical Research Institute's Bocas del Toro Research Station, Bocas del Toro, Panamá, in the summer of 2005. Samples were processed in three parts: (1) one piece was processed immediately for chl-*a* concentration (see below); (2) one piece was preserved in RNAlater (Ambion) for subsequent genetic analyses; and (3) one piece was preserved in 70% ethanol for morphological identification.

Sponge species and morphotypes were identified using gross morphological characters (e.g. colour, growth form, texture, consistency, etc.) and spicule dimensions in conjunction with local species keys and records (Diaz, 2005). Full taxonomic affiliations and currently accepted species names were obtained from the World Porifera Database (van Soest et al., 2005). The *Chondrilla nucula* species complex was divided into two sub-specific groups, corresponding to the reef form (*C. nucula* f. *hermatypica*) and mangrove form (*C. nucula* f. *mangle*; Duran & Rützler, 2006). For a phylogenetic context, supra-ordinal groupings were based on molecular studies that divide demosponges into four monophyletic clades (G1, G2, G3 and G4; Borchiellini et al., 2004; Boury-Esnault, 2006).

*Chlorophyll-*a *quantification*

Chlorophyll-*a* concentrations were determined for 264 sponge samples (representing 60 species; Table 2). Freshly collected sponge tissue was separated into ectosome and choanosome and approximately 0.25 g of each tissue fraction were separately extracted in 10 ml of 90% acetone. Samples were wrapped in foil to prevent photodegradation of chlorophyll and held overnight at 4°C. Following extraction, 1.5 ml of solution was centrifuged to concentrate suspended particles. Subsequently, 1.0 ml of supernatant was transferred to spectrophotometric cuvettes and absorbances were quantified at 750 nm, 664 nm, 647 nm, and 630 nm. Chlorophyll-*a* concentrations were estimated using the equations of Parsons et al. (1984) and standardized by extracted sponge mass.

PCR screening

Whole genomic DNA was extracted from 48 sponge samples (representing 46 species; Table 2) as described above. For PCR-screening of genomic extracts, three separate reaction conditions and primer sets were used to test for: (1) the presence of sponge DNA, an indication of successful extraction; (2) the presence of cyanobacteria DNA, to survey for all cyanobacteria in sponges; and (3) the presence of the sponge symbiont *S. spongiarum* DNA. For the first set of reactions, the sponge-specific oligonucleotide primers SP58bF and SP28cR (Thacker & Starnes, 2003) were used to amplify a segment of the sponge nuclear ribosomal RNA gene corresponding to the entire second internal transcribed spacer (ITS-2) region and the 5' end of the 28S subunit. If this amplification was not successful, the sample was dropped from subsequent analyses. For the second set of reactions, the cyanobacteria-specific oligonucleotide primers CYA359F and CYA781R (Nübel et al., 1997) were used to amplify a partial segment of the 16S rRNA gene (approximately 420 bp), following the thermocycler conditions described above. For the third set of reactions, the *S. spongiarum*-specific primers SYN180F and SYN1230R were used to amplify a partial segment of the 16S rRNA gene (approximately 1000 bp).

Reef and sponge community transects

Twelve 50-m line-intercept transects were conducted at five reefs in the Bocas del Toro Archipelago at depths of 1.5, 3, 4.5 and 6 m. Recorded at each metre mark were: (1) the substrate or organism at the mark, to assess sponge abundance on the reef benthos; and (2) the closest sponge species to the mark, to assess species abundances within reef sponge assemblages. Taxa that could not be immediately identified in the field were photographed and sampled for subsequent laboratory identification. A randomized block analysis of variance (ANOVA) was used to test the effects of location and depth on the percentage of sponges with high and intermediate chl-*a* concentrations (termed photosynthetically active species). The percentage abundance of photosynthetically active species was compared to that of non-photosynthetically active species by a one-sample *t*-test, with a null hypothesis that these two groups are equally abundant (i.e. that 50% of the sponges are photosynthetically active) within local assemblages.

RESULTS

Recovered 16S rRNA gene sequences (GenBank Accession numbers EF656438 to EF656451) were most similar to those of the sponge symbiont *Synechococcus spongiarum* (Usher et al., 2004b) and related sponge-associated *Synechococcus* sequences. A molecular phylogenetic analysis revealed a *Synechococcus* lineage recovered exclusively from marine sponges and clearly differentiated from all free-living cyanobacteria (Figure 1). Sponge-specific *Synechococcus* sequences formed a well-supported monophyletic clade with group 6 cyanobacteria (Honda et al., 1999; Robertson et al., 2001), but comprised a distinct lineage within this clade, consistent with previous phylogenetic analyses (Steindler et al., 2005; Thacker, 2005). All PCR amplicons sequenced in this study belonged to the sponge-specific *Synechococcus* clade, indicating that the SYN primer pair specifically targets 16S rRNA genes from *S. spongiarum* cyanobacterial symbionts.

Chlorophyll-*a* concentrations of coral reef sponges exhibited a wide range of values and divided the sponge community into two major groups: species with high (>125 μg/g) and low (<50 μg/g) chl-*a* concentrations. Chl-*a* concentrations ranged from near 0 (*Erylus formosus*, 0.78 ±1.16 μg/g) to over 400 μg/g (*Haliclona walentinae*, 422.94 ±9.72 μg/g; Figure 2). Indicative of hosting photosynthetic symbionts, high chl-*a* sponges encompassed 20 species (33%) and averaged 206.90 ±22.12 μg/g chl-*a*. Low chl-*a* sponges encompassed 38 species (63%) and averaged 11.61 ±7.74 μg/g chl-*a*. Only

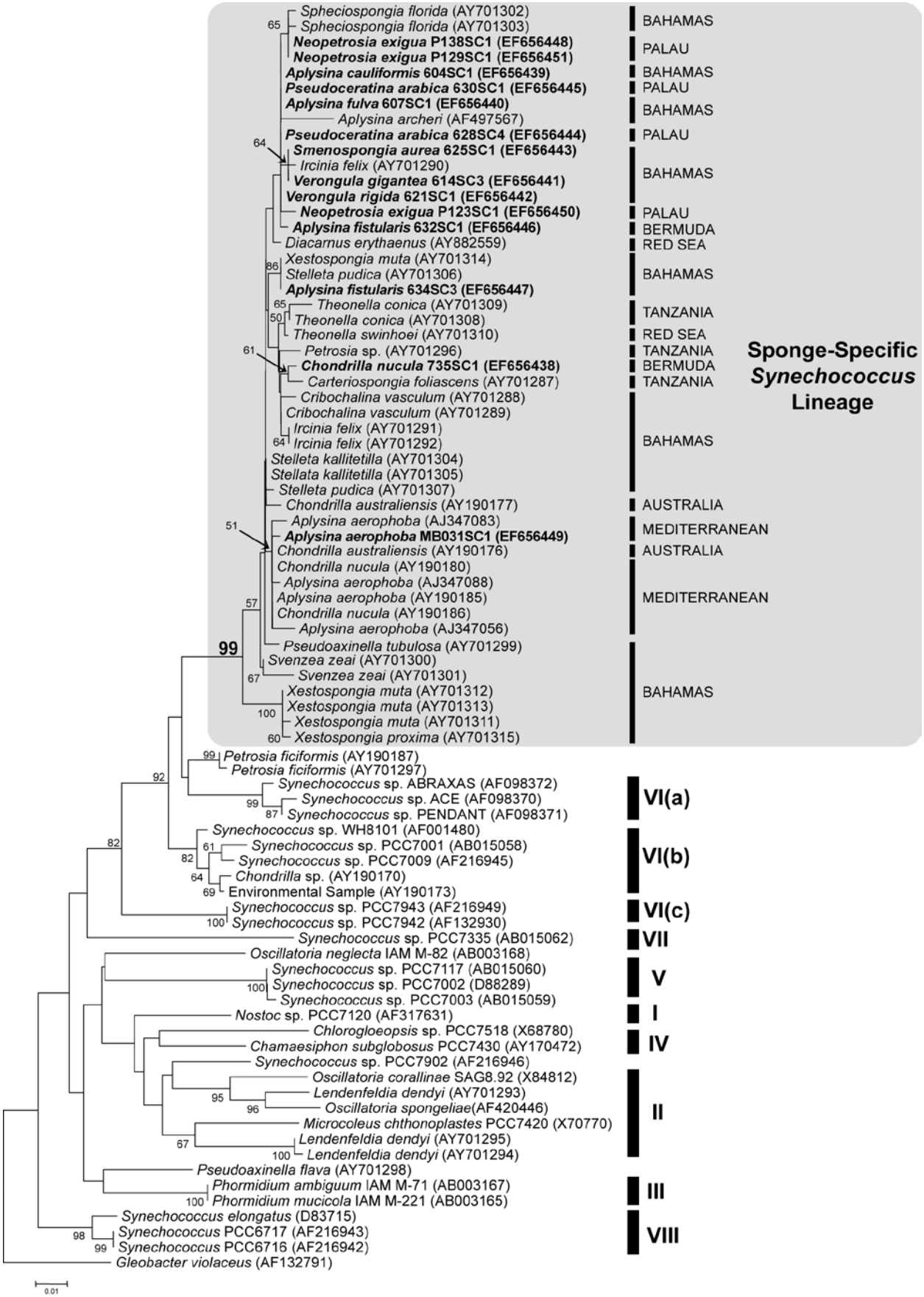

Figure 1. Neighbour-joining phylogeny of 16S rRNA gene sequences from sponge-associated cyanobacteria with representatives from the seven major cyanobacterial lineages (Honda et al., 1999; Robertson et al., 2001). Labels on terminal nodes refer to the host sponge species or the cyanobacterial strain followed by the GenBank accession number in parentheses; sequences from this study are indicated in bold. Numbers on nodes refer to percentage bootstrap support after 1000 replicates. Thick black bars on the right indicate the phylogenetic grouping of each sequence. The grey box highlights the sponge-specific *Synechococcus spongiarum* clade. Thin black lines and capital labels indicate sponge collection location. Scale bar represents 0.01 substitutions per site.

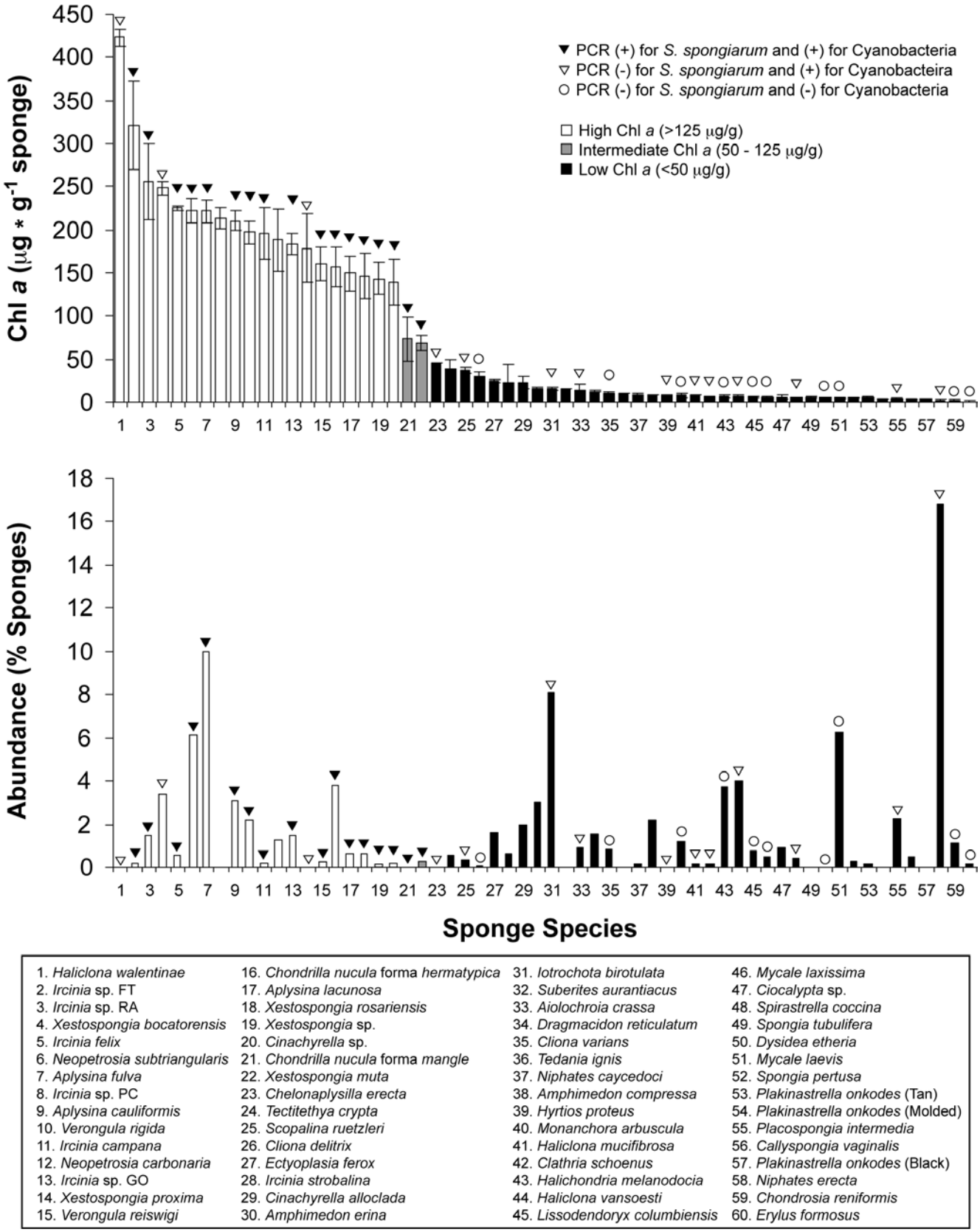

Figure 2. Chlorophyll-*a* (chl-*a*) concentrations and abundance of Panamanian coral reef sponge species. Light bars indicate species with high (>125 μg/g) chl-*a* concentrations, grey bars indicate species with intermediate (50–125 μg/g) chl-*a* concentrations, and black bars indicate species with low (<50 μg/g) chl-*a* concentrations. Abundance is depicted as a percentage of the sponge community. PCR-screening for the presence of cyanobacteria and the sponge symbiont *Synechococcus spongiarum* is overlaid on chl-*a* and abundance data: dark triangles indicate species positive for cyanobacteria and *S. spongiarum;* open triangles indicate species positive for cyanobacteria and negative for *S. spongiarum*; and open circles indicate species negative for cyanobacteria and *S. spongiarum*. Species listed as numbers on x-axes refer to the boxed key.

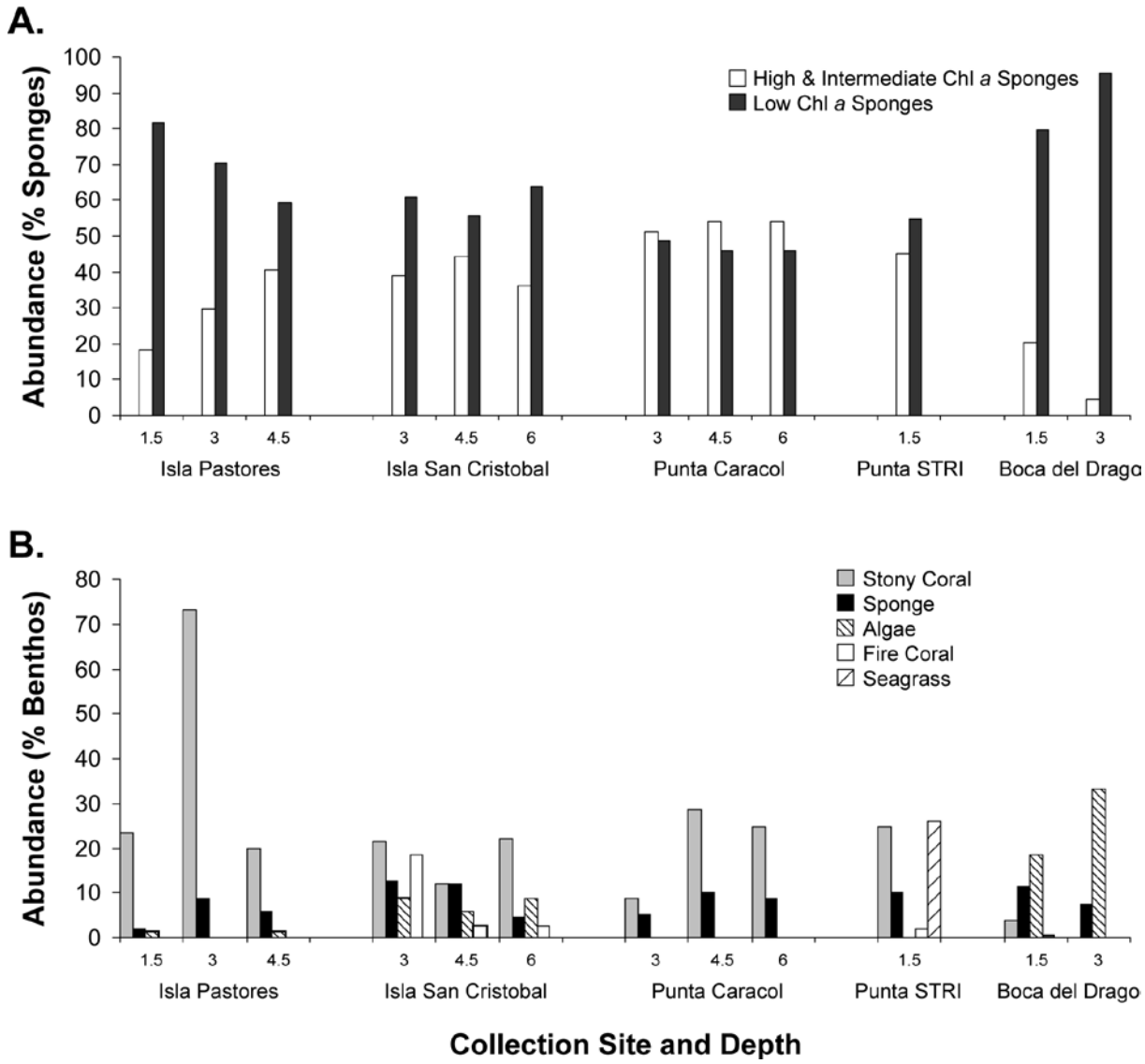

Figure 3. Percentage of photosynthetically active sponges and abundance of the five dominant invertebrate groups at five reef sites and four depths (1.5, 3, 4.5, and 6 m). (A) Filled bars represent percentage of sponges with high (>125 μg/g) and intermediate (50–125 μg/g) chl-*a* concentrations, while open bars represent percentage of sponges with low (<50 μg/g) chl-*a* concentrations; (B) bars represent abundance of the five dominant reef invertebrate groups: stony corals (grey); sponges (black); algae (right hatches); fire coral (open); and sea grass (left hatches).

two species exhibited intermediate chl-*a* concentrations (50–125 μg/g), both representing sponges with reduced symbiont populations: partially bleached *Xestospongia muta* (68.77 ±8.31 μg/g) and the mangrove form of *Chondrilla nucula* (*C. nucula* f. *mangle*; 73.35 ±25.44 μg/g).

The PCR screening revealed that the majority of reef sponges have some association with cyanobacteria and that most high chl-*a* sponges host *S. spongiarum* (Figure 2). Sponge-specific primers yielded successful amplification in 89% (N=41) of the 46 species initially screened. These 41 species were subsequently investigated with cyanobacteria-specific primers, which yielded amplification in 76% (N=31) of the 41 sponge species investigated, including all (N=18) of the high chl-*a* species investigated, both intermediate chl-*a* species and 52% (N=11) of the 21 low chl-*a* species investigated. Primers specific to *S. spongiarum* yielded amplification in 42% (N=17) of the 41 sponge species investigated, including 83% (N=15) of the 18 high chl-*a* species investigated. Both intermediate chl-*a* sponge species were positive for *S. spongiarum*, supporting the claim that these species host cyanobacterial symbionts, although in low abundance. No amplification of *S. spongiarum* was seen in sponges that exhibited low chl-*a* concentrations (0 of 21 species investigated).

Sponges were abundant members of the reef communities, second only to corals, and averaged 8.23% of the total benthos (range 2.00–12.67%) and 16.95% of the live benthos (range 4.48–31.58%). Six species dominated the sponge community: *Niphates erecta* (16.23%), *Aplysina fulva* (10.01%), *Iotrochota birotulata* (8.06%), *Mycale laevis* (6.23%), *Neopetrosia subtriangularis* (6.06%), and *Haliclona vansoesti* (4.00%), collectively accounting for half (50.58%) of the total sponge abundance (Figure 2). Two dominant reef sponges had high chl-*a* concentrations and harboured *S. spongiarum* (*A. fulva* and *N. subtriangularis*). The percentage of photosynthetically active sponges (those species with high and intermediate chl-*a* concentrations) varied significantly across sites (F=10.181, df=4, P=0.023), but

Table 3. *Taxonomic relationships of reef sponge species exhibiting high chlorophyll-*a *concentrations and harbouring the symbiotic cyanobacterium,* Synechococcus spongiarum.

Clade	Order	Family	Species	High chl-*a*	SYN (+)
Homoscleromorpha	Homosclerophorida	Plakinidae	3	–	–
Demospongiae					
Keratosa (G1)	Dendroceratida	Darwinellidae	1	–	–
	Dictyoceratida	Dysideidae	1	–	–
		Irciniidae	7	6 (85%)	5 (71%)
		Spongiidae	2	–	–
		Thorectidae	1	–	–
Myxospongiae (G2)	Chondrosida	Chondrillidae	3	2 (67%)	2 (67%)
	Verongida	Aplysinidae	6	5 (83%)	5 (83%)
(G3)	Haplosclerida	Callyspongiidae	1	–	–
		Chalinidae	3	1 (33%)	–
		Niphatidae	4	–	–
		Petrosiidae	7	7 (100%)	4 (57%)
(G4)	Astrophorida	Geodiidae	1	–	–
	Hadromerida	Clionaidae	2	–	–
		Placospongiidae	1	–	–
		Spirastrellidae	1	–	–
		Suberitidae	1	–	–
		Tethyidae	1	–	–
	Halichondrida	Axinellidae	1	–	–
		Dictyonellidae	1	–	–
		Halichondriidae	2	–	–
	Poecilosclerida	Coelosphaeridae	1	–	–
		Crambeidae	1	–	–
		Iotrochotidae	1	–	–
		Microcionidae	1	–	–
		Mycalidae	2	–	–
		Raspailiidae	1	–	–
		Tedaniidae	1	–	–
	Spirophorida	Tetillidae	2	1 (50%)	1 (50%)

not across depth (F=1.220, df=3, P=0.411). Within reef sponge assemblages, photosynthetically active species accounted for over one-third of the observed abundance (36.62%, range 4.35–54.11%; Figure 3), but non-photosynthetic species were significantly more abundant (t=3.013, df=11, P=0.012). Collection sites varied in the diversity and abundance of benthic invertebrates; however, no clear trends were detected between reef community composition (coral cover, algae presence, etc.) and the prevalence of sponge species with high chl-*a* concentrations (Figure 3).

Taxonomic diversity of coral reef sponge communities spanned 29 families and ten orders; however, species hosting photosynthetic symbionts were confined to six sponge families, representing five orders and all four supra-ordinal phylogenetic clades (Table 3). Interestingly, the three most speciose families present in these communities exhibited a high incidence of species with high chl-*a* concentrations (83–100%) and species hosting *S. spongiarum* (57–83%; Table 3).

DISCUSSION

Photosymbionts were common among the coral reef sponge assemblages investigated, accounting for over one-third of local sponge diversity and abundance. These data are consistent with previous surveys of Caribbean sponge populations (Wilkinson, 1987) and suggest that hosting photosymbionts is a common strategy among sponges. Furthermore, the cyanobacterium *Synechococcus spongiarum* dominated the symbiont communities that inhabit reef sponges and was identified in 85% of the sponge species with high and intermediate chl-*a* concentrations. The prevalence of *S. spongiarum* within these reef sponge assemblages suggests that these symbionts may contribute significantly to overall coral reef productivity.

Essential to the study of sponge–cyanobacteria symbioses is the ability to accurately delineate mutualistic symbionts from transient cyanobacteria. In addition to sponge-specific associates, cyanobacteria isolated from sponge tissue may represent transient species, either selectively filtered and consumed by the sponge or simply passing through the sponge aquiferous system (Pile et al., 1996). Additionally, cyanobacteria may inhabit epibiotic fouling communities on the sponge surface or represent pathogenic species that overgrow host sponges (Rützler, 1988). Indeed, several sponges in our study that exhibited low chl-*a* concentrations were positive for PCR-screening of cyanobacteria. In contrast, amplification with *S. spongiarum* primers clearly indicated the presence of this symbiotic cyanobacterium, as all sequenced amplicons from this primer set belonged to the sponge-specific *Synechococcus* clade. Detection of *S.*

spongiarum using specific primers will facilitate the study of sponge–cyanobacteria associations by allowing for rapid and accurate identification of this symbiont.

Synechococcus spongiarum is a common sponge cyanobacterial symbiont that has been reported from at least 25 host sponge species from tropical and temperate reefs. Previous studies have reported that this symbiont inhabits diverse sponge hosts and is common among reef sponges (Usher et al., 2004a; Steindler et al., 2005). Our data suggest that *S. spongiarum* is the dominant photosymbiont within Caribbean coral reef sponge communities and accounts for the majority of the primary productivity in these sponges. In fact, *S. spongiarum* was not detected in only three of the 18 high chl-*a* species screened. Two of these species, *Haliclona walentinae* and *Xestospongia bocatorensis*, host the filamentous sponge symbiont, *Oscillatoria spongeliae* (Thacker et al., 2007). Although the remaining species, *Xestospongia proxima*, may host distinct symbiont populations with unique gene sequences not recoverable with the current primer set, Steindler et al. (2005) reported *S. spongiarum* from this species (Figure 1). In addition, for specimens that did not show positive screens, sponge metabolites may have interfered with DNA extractions and inhibited PCR amplifications. Interestingly, the species *Cliona varians* (formerly *Anthosigmella varians*), known to harbour symbiotic zooxanthellae (Rützler, 1990; Hill & Wilcox, 1998), did not exhibit the high chl-*a* concentrations characteristic of sponge–cyanobacteria symbioses, suggesting a comparatively low abundance or low photosynthetic activity of dinoflagellate symbiont populations in these sponges.

If photosynthetic symbionts can provide an alternative source of carbon to sponges, why do not all sponges host them? In our surveys, a significant majority of sponges were non-photosynthetic. Hosting photosynthetic symbionts may involve physiological costs that are not yet well described for sponges, including competition between symbiont and host cells for limited nutrients and host control of symbiont cell division and population size (Smith, 1991). In addition, although sponges may offer protection from grazing by generalist herbivores to their symbionts, they may also attract specialist predators. For example, the opisthobranch mollusc *Tylodina perversa* prefers to consume tissues of the sponge *Aplysina aerophoba* that contain high concentrations of cyanobacteria (Becerro et al., 2003). Likewise, gastropterid molluscs associated with *Dysidea granulosa* seem to forage on this sponge's symbiotic cyanobacteria (Becerro et al., 2006). Despite these costs, many species of photosynthetic sponges were more abundant than their non-photosynthetic counterparts (Figure 2).

The taxonomic affiliations of sponges hosting *S. spongiarum* suggest that multiple symbiont colonization events have occurred over evolutionary time. Although species hosting cyanobacterial symbionts spanned five sponge orders and all four supra-ordinal phylogenetic clades, the 22 high and intermediate chl-*a* species were confined to six families. Most species within these families hosted photosymbionts (79%), commonly *S. spongiarum* (61%). Notably, the three most speciose sponge families on the reefs were included in this group, which could suggest that this symbiotic relationship has promoted host speciation. Alternatively, closely related hosts within these families may be more susceptible to symbiont colonization. Indeed, it may be possible for symbionts to better persist over evolutionary time-scales in multiple, closely related species rather than in a single, but abundant, species.

The prevalence of photosymbionts in coral reef sponges and the dominance of symbiont communities by *S. spongarium* indicate that this cyanobacterium plays a major role in reef primary productivity. The phylogeny of *S. spongiarum* reveals that these symbionts comprise a distinct lineage of cyanobacteria specific to marine sponges and suggests that these symbionts are specifically adapted to their host sponges. Since sponges are among the most basal metazoans (Medina et al., 2001) and cyanobacteria represent an ancient lineage of bacteria (Tomitani et al., 2006), resolving their host–symbiont interactions may reveal fundamental insights into the evolution and maintenance of ecologically successful animal–bacteria symbioses.

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