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RESEARCH ARTICLE

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Biogeographic partitioning and host specialization among foraminiferan dinoflagellate symbionts (*Symbiodinium*; Dinophyta)

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Abstract Large discoidal soritid foraminiferans (Soritinae) are abundant in coral reef ecosystems. As with the many cnidarian invertebrates that inhabit these systems, they also depend on symbiotic dinoflagellates (*Symbiodinium*) for their growth and survival. Several particular *Symbiodinium* sub-genera or clades inhabit these soritids. One of these groups, referred to as clade C, dominates corals and their relatives throughout the tropical Indo-Pacific. In contrast, the distributions of *Symbiodinium* spp. from clades A, B, and C are more evenly apportioned across Caribbean invertebrate communities. To explore the possibility that a similar biogeographic break exists in the symbionts harbored by soritids, we surveyed the *Symbiodinium* spp. from the soritid genus *Sorites*, collected from the Pacific and Caribbean coasts of Panama as well as from Florida. Characterization of *Symbiodinium* obtained from foraminiferal and cnidarian samples was conducted using restriction fragment length polymorphism and phylogenetic analyses of the nuclear internal transcribed spacer region 2 (ITS 2) and a portion of the large subunit ribosomal DNA sequences. A distinctive biogeographic break between the kinds of symbionts found in *Sorites* from the East Pacific and Caribbean was clearly evident. Differences between cnidarian and foraminferan symbioses in each ocean may be explained by the subjection of Caribbean communities to severer environmental conditions during the early Quarternary. Caribbean *Sorites* spp. harbored symbionts described from clade F (specifically sub-clade Fr4) and clade H (formally referred to as Fr1), while *Sorites* spp. from the eastern Pacific were dominated by a single *Symbiodinium* haplotype in clade C. An ITS 2 phylogeny determined that most clade C "types" recovered from Indo-Pacific soritids form a monophyletic sub-lineage with other clade C symbionts typically found in Pacific corals from the genus *Porites*. The existence of multiple *Symbiodinium* lineages at various taxonomic levels associated specifically with soritids indicates that symbioses with these hosts are important in driving *Symbiodinium* spp. evolution.

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Introduction

Dinoflagellates of the genus *Symbiodinium*, and commonly referred to as "zooxanthellae", occur as endosymbionts in a wide variety of marine invertebrates and protists (Taylor 1974; Trench 1993). These symbionts are essential components of coral reef ecosystems; their presence contributes significantly to the productivity, survival, and success of their hosts (Muscatine and Porter 1977). Contrary to the original paradigm of a single unique species, *Symbiodinium microadriaticum* (Freudenthal 1962), studies based on a variety of morphological, biochemical, and physiological data have demonstrated that the genus *Symbiodinium* is a diverse assemblage (Schoenberg and Trench 1980a, 1980b, 1980c; Chang et al. 1983). Recent molecular studies have expanded our appreciation of the diversity, ecology, and evolution within the genus *Symbiodinium*. Molecular phylogenies constructed with nuclear (Rowan and Powers 1991; Wilcox 1998; Carlos et al. 1999; LaJeunesse 2001; Savage et al. 2002) and chloroplastic (Santos et al. 2002) rRNA genes on *Symbiodinium*, collected

from numerous cnidarian and several molluscan hosts, revealed five divergent lineages or clades referred to as A, B, C, D, and E (Baker 2003). Similar investigations of *Symbiodinium* spp. associated with soritid foraminiferans (sub-family Soritinae) discovered a diversity of "types", most of which were divergent from those found in cnidarians. Large subunit (LSU) rDNA sequence phylogenies warranted the erection of two more clades, F (sub-clades Fr2–Fr5) and G (Fr6), and identified a distinct phylotype fairly divergent from C and F clades, called Fr1 (Pawlowski et al. 2001; Pochon et al. 2001). Soritid foraminiferans also harbor *Symbiodinium* from clade C.

Use of the internal transcribed spacer (ITS) region as a genetic marker in biodiversity and host–symbiont specificity surveys has proven useful in distinguishing symbionts that differ markedly in their ecological distribution, physiological fitness, and/or host infectability within each of these major clades (LaJeunesse 2001, 2002; LaJeunesse et al. 2003). ITS 2 analyses of *Symbiodinium* spp. obtained from a wide diversity of cnidarian and several molluscan hosts from the Pacific Ocean and the Caribbean Sea have revealed the existence of numerous "types" or "species" within each major phylogenetic clade. Clade C dominates Indo-Pacific host communities (Baker 2003, unpublished data; LaJeunesse et al. 2003, 2004), and with over 100 ecologically distinct "types" now described, it is the most diverse and prevalent symbiont group among cnidarian host taxa worldwide (LaJeunesse 2002; LaJeunesse et al. 2003, 2004, unpublished data). In the Caribbean, its dominance in the host community is shared with clade B (Baker 2003; LaJeunesse et al. 2003).

Ultimately, the unique symbiont community diversity in western Atlantic reefs probably evolved in response to environmental conditions and isolation resulting from the final closure of the Central American Seaway (3.1–3.5 million years ago; Coates and Obando 1996), and the onset of Northern Hemisphere glaciation that marked the Pliocene–Pleistocene transition (Baker and Rowan 1997; LaJeunesse et al. 2003; Baker 2003). The rise of the Central American Isthmus (Woodring 1966) and associated modifications in oceanic circulation patterns (Haug and Tiedmann 1998) resulted in severe paleoclimatic changes in the western Atlantic. Evidence on tonnoidean gastropods from late Pliocene fossil records indicates that seaways still operated, at least intermittently during interglacial periods of high sea level, to connect the Atlantic and Pacific Oceans, suggesting that complete cessation of water exchanges took place in the early to middle Pleistocene (Beu 2001). Since that time, the Isthmus of Panama has been an absolute barrier to marine organisms.

The evolution of symbioses involving algal endosymbionts probably led to the adaptive radiation (morphological diversification) of the foraminiferal superfamily Soritaceae (Lee et al. 1979; Hallock 1985). The acquisition of *Symbiodinium* dinoflagellates was a key innovation that promoted important ecological and morphological changes observable today among members of the sub-family Soritinae (Richardson 2001). These *Symbiodinium*-bearing foraminiferans diverged from their closest *Chlorophytes*-bearing relatives between 25 and 30 million years ago (Haynes 1981; Loeblich and Tappan 1987). While it is recognized that acquisition of *Symbiodinium* favored the evolution and adaptation of Soritinae members to coral reef ecosystems, the possibility that these foraminiferal hosts have promoted the evolution of certain *Symbiodinium* lineages is still under examination.

This study investigates the diversity of *Symbiodinium* spp. inhabiting soritid foraminifera in the genus *Sorites* from both sides of the Isthmus of Panama as well as in Florida. Genetic examination, using restriction fragment length polymorphism (RFLP) analyses, was conducted on 61 foraminiferan individuals from the Caribbean and 82 from the eastern Pacific to rapidly assess which clade of *Symbiodinium* spp. they harbor. A total of 128 randomly sampled cnidarians from each region was also sampled to test if any harbored what are now generally believed to be soritid-specific *Symbiodinium* spp. and, in addition, to verify earlier studies reporting differences in Caribbean versus Pacific algal–invertebrate symbioses (Baker and Rowan 1997; LaJeunesse et al. 2003). For a subset of those *Sorites* collected in each region (13 from Florida, 14 from Panama/Caribbean and 15 from Panama/Pacific), the ITS 2 and partial LSU rDNA of *Symbiodinium* with different RFLP patterns were sequenced. Additionally, 30 ITS 2 rDNA sequences of clade C *Symbiodinium* obtained from Indo-Pacific Soritinae were compared to all ITS 2 C "types" characterized to date.

Materials and methods

Sample collections, DNA extraction, PCR (polymerase chain reaction) amplification and purification

Foraminiferal and cnidarian samples were collected between August and September 2001, from a total of 13 sites in the 3 following locations: Isla Taboga (eastern Pacific, coast of Panama), Bocas del Toro (Caribbean Sea, coast of Panama), and Florida Keys (Atlantic Ocean), as shown in Fig. 1. All soritid foraminifera ($N = 320$) collected within those locations belonged exclusively to the genus *Sorites*, known to be circumtropical, cosmopolitan, and characterized by a greater range in water depth distribution than the genera *Amphisorus* and *Marginopora* (Pochon, personal observations), which are restricted to the Indo-Pacific Oceans (Langer and Hottinger 2000). Cnidarian hosts represented 12 genera in 8 families of scleractinian corals, 5 genera in 2 families of octocorals, and 1 genus of the hydrozoan fire coral (see "Supplementary electronic material"). DNA extractions, PCR amplifications, and purifications were processed as described in Pochon et al. (2001).

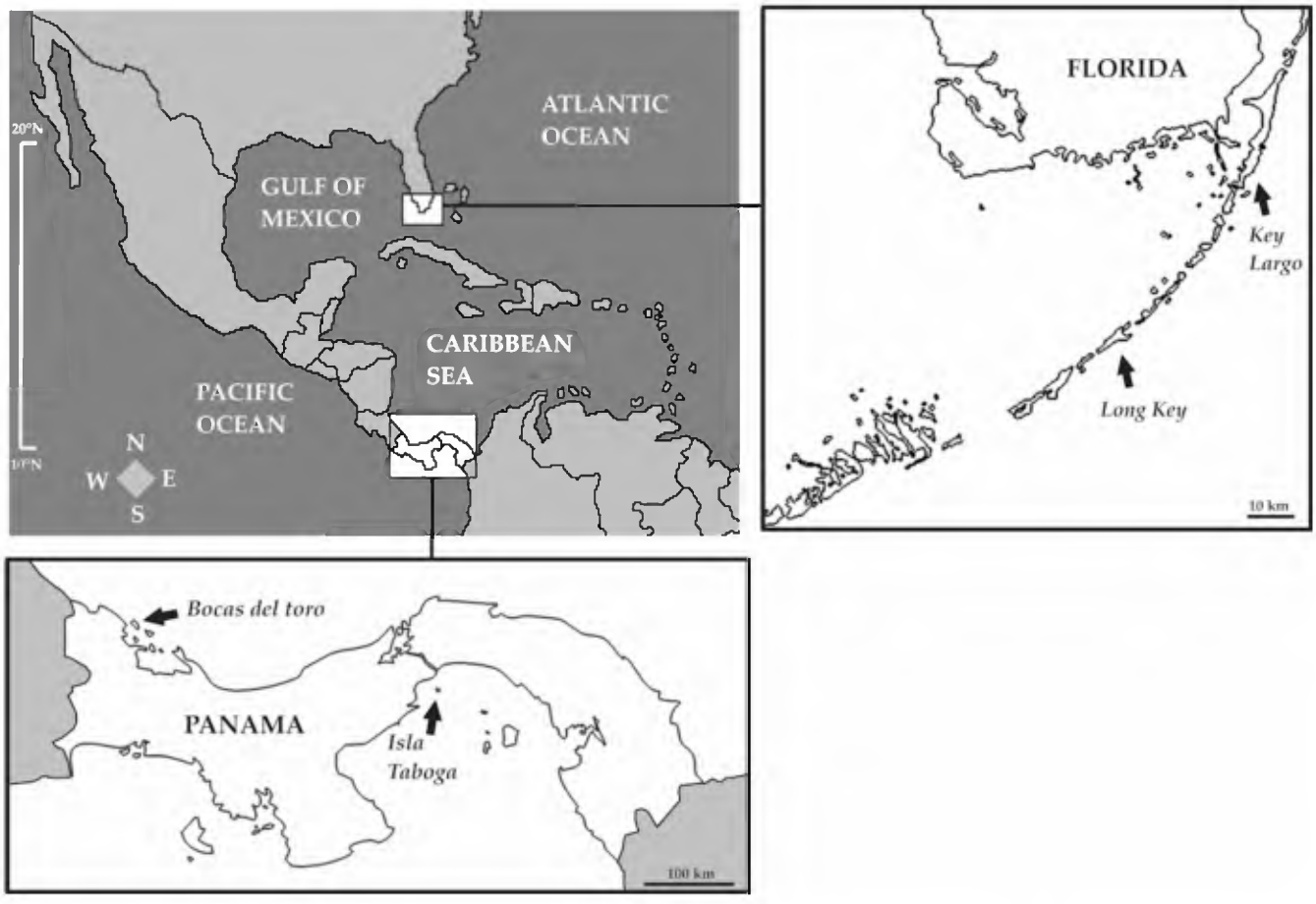


Fig. 1 Maps of sampling locations. The map of Florida shows two locations, each one including several sampling sites, with two sites in Key Largo (Key Largo Bay, Pickles Reef) and two sites in Long Key (Tenessy Reef and Foram Farm, Keys Marine Laboratory). The map of Panama shows two locations, with five sampling sites in Bocas del Toro (Cayo Nancy, Hospital Point, Sandfly Bay, Coral Key, and Mangrove Inn) and two sites in Isla Taboga (Taboga Reef 1 and 2)

RFLP analyses

RFLP analyses were conducted on *Symbiodinium* PCR products obtained from the partial nuclear 28S rDNA. The restriction enzyme *Hind*III (A↓AGCTT) was used on 143 foraminiferal and 128 cnidarian samples following Pochon et al. (2001). The latter enzyme provided nearly identical RFLP patterns between clade C and phylotype Fr1, a fact that could negatively affect interpretation of the results. Consequently, we used the *Taq*I enzyme (T↓CGA) on 105 foraminiferal and 64 cnidarian samples belonging to clades C and phylotype Fr1 to further differentiate between these "types".

Sequencing and phylogenetic analyses

A total of 44 foraminiferal samples were chosen for phylogenetic analyses of their symbionts. These samples corresponded to 42 out of the 143 foraminiferal samples displaying different *Symbiodinium* RFLP patterns and collected in this study (13 from Florida, 14 from Panama/Caribbean, and 15 from Panama/Pacific) and 2 samples collected in Florida in July 1997 and July 1998, respectively, FL 836 and FL 751a (cf. Pawlowski et al. 2001). All samples were sequenced directly in both directions with ABI PRISM Big Dye Terminator Cycle Sequencing Kit using the ABI 377 DNA sequencer (Perkin-Elmer), according to the instructions of the manufacturers.

Two data sets of phylogenetic analyses were conducted. In the first set, we analyzed the concatenated ITS 2 and partial LSU rDNA of the 44 samples outlined above. Four additional sequences of *Symbiodinium* clade G, also referred to as phylotype Fr6 [GenBank AJ291536–AJ291539], were chosen as the outgroup, following Pochon et al. (2001). All sequences were aligned by using Clustal X (Thompson et al. 1994) and further improved manually by using BioEdit 5.0.9 sequence alignment software (Hall 1999). Tree reconstruction was conducted by using the program PAUP* (ver. 4.0 beta 10) and inferred by using the maximum-likelihood (ML) method (Felsenstein 1981). Two likelihood approaches were used for reconstructing phylogenetic relationships. First, the MODELTEST program (Posada and Crandall 1998) identified the general time-reversible (GTR) model (Lanave et al. 1984) as the best model for our ML analyses, taking into

account a proportion of invariant sites (*I*), and with a gamma distribution shape parameter (*G*) calculated from the data set. Using the likelihood settings recommended by MODELTEST, an ML tree was reconstructed using a heuristic search and random addition of sequences in PAUP*. Second, using the best fit model from MODELTEST, Bayesian-likelihood analysis for the estimation of phylogeny was assessed using the program MrBayes (Huelsenbeck and Ronquist 2001). One out of every ten trees was sampled for 1,000,000 generations with kappa and DNA substitution parameters estimated during the search. The consensus tree was computed (PAUP* ver. 4.0b10, Swofford 2002) on the last 99,500 sampled trees, excluding the 5,000 first generations identified as the "burn-in period". Starting trees for ML analysis were obtained via stepwise addition, and then swapped using the tree-bissection-reconnection (TBR) algorithm. The reliability of internal branches was assessed using the bootstrap method with 100 replicates (Felsenstein 1985). Host species names, collection sites and dates, GenBank accession numbers, and length of all sequences included in our analyses are given in Table 1. Each DNA extraction received a DNA collection identification number that also appears in Table 1. The alignment is available from TreeBase (http://www.treebase.org) as a Nexus file (study accession number S1125, matrix accession number M1928).

In the second set of phylogenetic analyses, we compared 30 ITS 2 rDNA sequences of clade C *Symbiodinium* obtained from foraminiferal hosts collected in the Gulf of Elat, Guam, the Great Barrier Reef (GBR), and the eastern Pacific (Panama) to 100 ITS 2 rDNA sequences of clade C *Symbiodinium* obtained from cnidarian and molluscan hosts, which include all ITS 2 C "types" known to date (LaJeunesse 2001, 2002; LaJeunesse et al. 2003; LaJeunesse, unpublished data). Fr1

Table 1 List of host species, collection localities and dates, reference numbers used in Fig. 2, as well as symbiont sequences' length and corresponding accession numbers in GenBank. DNA extract refers to the DNA collection identification numbers (see "Materials and methods") (*PC* Panama/Pacific;*PP* Panama/Caribbean; *FL* Florida;*LSU* large subunit)

Host species	Collection site	Date	Reference no.	DNA extract	LSU length	GenBank no.
Sorites sp.	Bocas del Toro, PC	Sep 2001	PC 251	1653(X)	1,025 nt	AJ621131
Sorites sp.	Bocas del Toro, PC		PC 256	1659(X)	1,025 nt	AJ621132
Sorites sp.	Bocas del Toro, PC		PC 259	1662(X)	1,025 nt	AJ621133
Sorites sp.	Bocas del Toro, PC		PC 263	1666(X)	1,025 nt	AJ621134
Sorites sp.	Isla Taboga, PP		PP 27	1382(X)	1,025 nt	AJ621129
Sorites sp.	Isla Taboga, PP		PP 38	1393(X)	1,025 nt	AJ621130
Sorites sp.	Florida Keys, FL	Aug 2001	FL 34	1286(X)	1,025 nt	AJ621148
Sorites sp.	Florida Keys, FL		FL 36	1288(X)	1,024 nt	AJ621149
Sorites sp.	Florida Keys, FL		FL 71	1331(X)	1,028 nt	AJ621150
Sorites sp.	Florida Keys, FL		FL 75	1335(X)	1,028 nt	AJ621151
Sorites sp.	Florida Keys, FL	Jul 1998	FL 751a	751a(J)	1,032 nt	AJ291513
Sorites sp.	Florida Keys, FL	Aug 2001	FL 12	1256(X)	1,032 nt	AJ621152
Sorites sp.	Florida Keys, FL		FL 30	1282(X)	1,032 nt	AJ621153
Sorites sp.	Florida Keys, FL		FL 31	1283(X)	1,032 nt	AJ621154
Sorites sp.	Florida Keys, FL		FL 73	1333(X)	1,032 nt	AJ621155
Sorites sp.	Florida Keys, FL		FL 13	1259(X)	1,032 nt	AJ621156
Sorites sp.	Florida Keys, FL		FL 79	1351(X)	1,032 nt	AJ621157
Sorites sp.	Isla Taboga, PP	Sep 2001	PP 1	1354(X)	1,055 nt	AJ620934
Sorites sp.	Isla Taboga, PP		PP 2	1356(X)	1,054 nt	AJ620935
Sorites sp.	Isla Taboga, PP		PP 3	1358(X)	1,054 nt	AJ620936
Sorites sp.	Isla Taboga, PP		PP 26	1381(X)	1,054 nt	AJ620937
Sorites sp.	Isla Taboga, PP		PP 32	1387(X)	1,054 nt	AJ620938
Sorites sp.	Isla Taboga, PP		PP 39	1394(X)	1,054 nt	AJ620939
Sorites sp.	Isla Taboga, PP		PP 51	1406(X)	1,054 nt	AJ620940
Sorites sp.	Isla Taboga, PP		PP 73	1428(X)	1,054 nt	AJ620941
Sorites sp.	Isla Taboga, PP		PP 80	1435(X)	1,053 nt	AJ620942
Sorites sp.	Isla Taboga, PP		PP 88	1443(X)	1,055 nt	AJ620943
Sorites sp.	Isla Taboga, PP		PP 100	1455(X)	1,054 nt	AJ620944
Sorites sp.	Isla Taboga, PP		PP 111	1466(X)	1,053 nt	AJ620945
Sorites sp.	Isla Taboga, PP		PP 124	1479(X)	1,054 nt	AJ621128
Sorites sp.	Bocas del Toro, PC		PC 227	1628(X)	1,043 nt	AJ621135
Sorites sp.	Bocas del Toro, PC		PC 229	1630(X)	1,043 nt	AJ621136
Sorites sp.	Bocas del Toro, PC		PC 230	1632(X)	1,042 nt	AJ621137
Sorites sp.	Bocas del Toro, PC		PC 234	1636(X)	1,042 nt	AJ621138
Sorites sp.	Bocas del Toro, PC		PC 244	1646(X)	1,042 nt	AJ621139
Sorites sp.	Bocas del Toro, PC		PC 247	1649(X)	1,041 nt	AJ621140
Sorites sp.	Bocas del Toro, PC		PC 253	1656(X)	1,043 nt	AJ621141
Sorites sp.	Bocas del Toro, PC		PC 257	1660(X)	1,041 nt	AJ621142
Sorites sp.	Bocas del Toro, PC		PC 258	1661(X)	1,041 nt	AJ621143
Sorites sp.	Bocas del Toro, PC		PC 261	1664(X)	1,042 nt	AJ621144
Sorites sp.	Florida Keys, FL	Aug 2001	FL 74	1334(X)	1,042 nt	AJ621145
Sorites sp.	Florida Keys, FL	Jul 1997	FL 836	836(J)	1,042 nt	AJ291527
Sorites sp.	Florida Keys, FL	Aug 2001	FL 77	1349(X)	1,040 nt	AJ621146
Sorites sp.	Florida Keys, FL		FL 78	1350(X)	1,040 nt	AJ621147

sequences were used as outgroup. Phylogenetic analyses of this data set were inferred by applying the maximum-parsimony (MP) method (Farris 1970), using the heuristic search option with random addition of sequences (100 replicates) and a branch-swapping algorithm (TBR). Characters were equally weighted and alignment gaps were treated as a fifth base. The data set was also analyzed by applying the Bayesian method (as described above) and the ML method using the program PhyML (Guindon and Gascuel 2003). GenBank accession numbers of each foraminiferal symbiont "type" are: C90 (AJ620934–AJ620945; AJ621128), C91 (AJ291517–AJ291519; AJ621542–AJ621543), C92 (AJ291514), C93 (AJ621533–AJ621536), C94 (AJ621537–AJ621539), C15 (AJ291516; AJ621540–AJ621541), and C19 (AJ291515). (TreeBase matrix accession number M1927).

Statistical analyses of topological congruency

Tests of topological congruency were conducted on a complete *Symbiodinium* data set of partial 28S rDNA (see Fig. 2a), to statistically examine if the foraminiferal symbionts referred to as phylotype Fr1 are members of a distinct *Symbiodinium* clade. The ML phylogenetic tree was constructed under the best fit model of DNA evolution (TrN+G; $-\ln L = 5{,}919.62051$) determined by hierarchical likelihood ratio tests in MODELTEST v3.06 and used stepwise addition and branch swapping by TBR. SH tests (Shimodaira and Hasegawa 1999) were applied by using a RELL bootstrap of 10,000 replicates, as implemented in PAUP* (ver 4.0b8). For the SH tests, ML trees, with (L_1) and without (L_2) constraints, were constructed using the heuristic search option under the parameters obtained with MODELTEST for this particular data set (TreeBase matrix accession number M1929).

Results

RFLP analyses

RFLP analysis of 28S rDNA revealed that the 143 foraminiferal samples included in our study contained *Symbiodinium* spp. belonging to clade C or phylotypes Fr1 and Fr4 (see Table 2). Eastern Pacific foraminiferal samples were found in association with a great majority of clade C *Symbiodinium* ($N=80$) and a small number of phylotype Fr1 ($N=2$). In contrast, Caribbean foraminiferal samples ($N=61$) harbored only the two phylotypes, Fr1 or Fr4. Phylotype Fr4 dominated foraminiferal samples from Bocas del Toro ($N=39$), while phylotype Fr1 dominated in the Florida Keys ($N=22$). No mixed genotypes were detected.

RFLP analyses on 128 randomly sampled cnidarians from each region revealed the presence of *Symbiodinium* clades A, B, C, or D (D2 sensu Pochon et al. 2001). Eastern Pacific cnidarians harbored exclusively clade C *Symbiodinium*, whereas Caribbean cnidarians were found in association with clade A, B, C, or D (data not shown). Detailed lists of the 143 foraminiferal and 128 cnidarian hosts analyzed in this study (Appendices 1, 2), including sampling period, sampling localities and specific sites, DNA reference numbers, and corresponding collection identification numbers, species names, sampling depth, and *Symbiodinium* phylotypes are available as Electronic Supplementary Material.

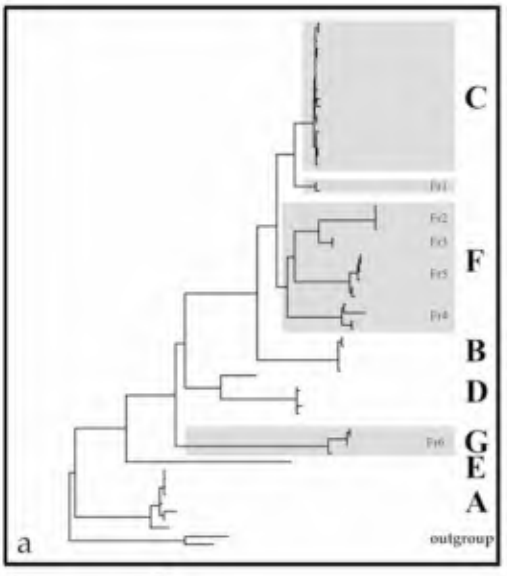
Phylogenetic analyses

In the first set of phylogenetic analyses of concatenated ITS 2 and 28S rDNA, the length of the sequences ranged from 1,024 bp (sample FL 36, phylotype Fr1) to 1,055 bp (samples PP 88 and PP 1, clade C), as shown in Table 1. The observed variation in the size of the sequences is due to an indel of 30 bp, shared by all members of the Fr1 *Symbiodinium* group, starting at position 683 downstream from the 5′-end of the LSU rDNA. The G+C content of the entire region is approximately 50%, which is consistent with the G+C content of the ITS region previously reported within clade B (Santos et al. 2001).

Symbiodinium spp. that associate with soritid foraminifera are represented by six divergent phylotypes, Fr1–Fr6, and several representatives within clade C. The phylotypes or sub-clades Fr2–Fr5 were recently assigned to clade F, and Fr6 is synonymous with clade G, while the phylotype Fr1 forms an independent lineage between clades C and F (Fig. 2a).

Phylogenetic relations between foraminiferal symbionts from the eastern Pacific, the Caribbean Sea, and Florida are presented in Fig. 2b. Both ML and Bayesian analyses resulted in highly supported topologies. The sub-clade Fr4 shows a basal position to clade C and phylotype Fr1 with, respectively, 13.59% and 13.55% of mean divergence. Phylotype Fr1 is highly supported, and exhibits a wider diversity than previously reported. The genetic divergence within phylotype Fr1 ranges from 0.097% to 1.98%, and the mean genetic divergence between phylotype Fr1 and clade C *Symbiodinium* reaches 6.77%. Furthermore, SH tests revealed significant differences in topologies constraining phylotype Fr1 within clade C ($\delta = L_1 - L_2 = -19.82736$; $P = 0.0124$; log-likelihood (L_1) $= -5{,}939.44787$) or within clade F ($\delta = L_1 - L_2 = -110.83772$; $P = 0.000$; log-likelihood (L_1) $= -6{,}030.45823$). These observations favor the creation of an additional *Symbiodinium* clade designation (clade H), which will replace the term phylotype Fr1. Clade C *Symbiodinium* from Isla Taboga is composed of 13 very closely related sequences, the only observed differences among sites being constituted by four deletions in the LSU region.

In the second set of phylogenetic analyses, each distinctive ITS 2 rDNA sequence of clade C *Symbiodinium* from foraminiferal hosts was aligned and compared with 100 ITS 2 rDNA sequences representing different



Isla Taboga, Pacific Ocean

Bocas del Toro, Caribbean Sea

Florida Keys, Atlantic Ocean

◀

Fig. 2a, b Phylogenetic trees of *Symbiodinium* dinoflagellates from nuclear ribosomal DNA sequences. **a** Phylogram based on partial LSU rDNA showing the complete phylogeny of *Symbiodinium* dinoflagellates inhabiting soritid foraminiferans and other invertebrates (modified from Pochon et al. 2001). Soritid foraminiferal symbionts branch within clade C and form six "foraminiferan-specific" phylotypes referred to as Fr1–Fr6 (*grey areas*). **b** Maximum-likelihood phylogram based on ITS 2 and partial LSU rDNA sequences, obtained from 44 foraminiferal samples (*Sorites* sp.) collected in Isla Taboga, Bocas del Toro, and Florida Keys. *Symbiodinium* sequence names correspond to the reference number shown in Table 1. *Numbers at nodes* are the bootstrap values obtained with ML and the Bayesian posterior probabilities, respectively. *Letters above branches* correspond to the main *Symbiodinium* clade designations. *Names below branches* correspond to the *Symbiodinium* phylotypes described in Pochon et al. (2001). The phylogram is rooted using four outgroup sequences from clade G

"types" of clade C *Symbiodinium* found in cnidarian and molluscan hosts (Fig. 3a). Phylogenetic analyses were based on 366 aligned sites (with gaps included). The MP, ML, and Bayesian analyses showed congruent results (data not shown). The detailed phylogram displayed in Fig. 3b shows that soritid *Symbiodinium* spp. from this clade form two distinct clusters. The first cluster groups seven sequences from the GBR and represents two distinct ITS 2 rDNA "types". The second cluster groups 23 sequences from the GBR, Guam, and the Gulf of Elat and forms a monophyletic sub-clade with other *Symbiodinium* spp. that generally associate with coral hosts in the genus *Porites*. Emerging from the base of this lineage, C90 is the "type" found to be most prevalent in eastern Pacific soritids (see Fig. 2b). This "type" has not been found in the cnidarian hosts from that region (Baker and LaJeunesse, unpublished data) and may be regionally endemic to this part of the Pacific. The other soritid clade C "types" identified from different biogeographic regions (C91, C19, and C92) are dispersed throughout this sub-clade phylogeny, with the more derived forms branching among the radiation stemming from the C15 "type".

Discussion

The biogeographic partitioning between East Pacific and Caribbean symbioses

A biogeographic break in *Symbiodinium* spp. distributions, analogous to that demonstrated for cnidarian symbionts (Baker and Rowan 1997), was clearly evident between East Pacific and Caribbean *Sorites* spp. (Fig. 2). Clade H (formally sub-clade Fr1; Pawlowski et al. 2001; but elevated here to clade status) together with sub-clade Fr4 in clade F dominates the community of Caribbean soritids. Members in both symbiont groups also occur in the Indo-Pacific (Pawlowski et al. 2001; Pochon et al. 2001), but are divergent from their Caribbean counterparts (data not shown). East Pacific soritids associate with a single "type" from clade C, as suggested by the analyses of ITS sequence data, while none of the Caribbean soritids investigated were found in association with clade C members. The split between East Pacific and Caribbean soritid symbiont communities is almost complete, except for two specimens from clade H found in the eastern Pacific that were genetically identical to some Caribbean *Symbiodinium* spp. of this "type". Given a relatively high sequence divergence within clade H, it is rather unlikely that the identical sequences found on both sides of the Isthmus of Panama result from long-lasting genetic stasis. It is more probable that the recent dispersal of symbionts or their foraminiferan hosts by birds or with water ballast (Proctor and Malone 1965; Hewitt et al. 2004) is responsible for this finding.

Compared to the Indo-Pacific, the diversity of symbionts in the Caribbean/Atlantic soritids is relatively low. Among seven divergent lineages (clade H, clade F comprising the divergent sub-clades Fr2–Fr5, and clade G, originally Fr6) identified in our previous study (Pochon et al. 2001), only two lineages are found in the Caribbean soritids. This is in contrast with Caribbean corals, which have been found to associate with a relatively high symbiont diversity and several endemic lineages belonging to *Symbiodinium* clades A, B, C, and D (Baker 2003; LaJeunesse et al. 2003). The narrower phylogenetic symbiont diversity found in the western Atlantic is partially explained by the presence of only one soritid genus (*Sorites*) in the Caribbean/Atlantic region. Two other genera of Soritinae: *Amphisorus* and *Marginopora*, are commonly found throughout the Indo-Pacific, but are absent in the Caribbean. Like many other marine organisms, the larger foraminiferans exhibit higher diversities in the Indo-Pacific region than in the central Pacific, Gulf of Elat (Red Sea), and Caribbean/Atlantic regions (Renema et al. 2001). Nutrient concentrations are among several environmental factors that influence the diversity and distribution of large foraminiferan species over space and time (Hallock 1988). The absence of a deep-water euphotic foraminiferal assemblage in the Caribbean is correlated to relatively high nutrient levels. The more stable nutrient conditions in oligotrophic regions of the Indo-Pacific allow the occurrence of specialized shallow/deep water

Table 2 Total number of foraminiferal samples analyzed in the three investigated locations and corresponding proportion of *Symbiodinium* clade C and/or phylotypes Fr1 and Fr4

Location	No. of samples	*Symbiodinium* C	*Symbiodinium* Fr1	*Symbiodinium* Fr4
Isla Taboga, eastern Pacific	82	80	2	0
Bocas del Toro and Florida Keys, Caribbean	61	0	23	38

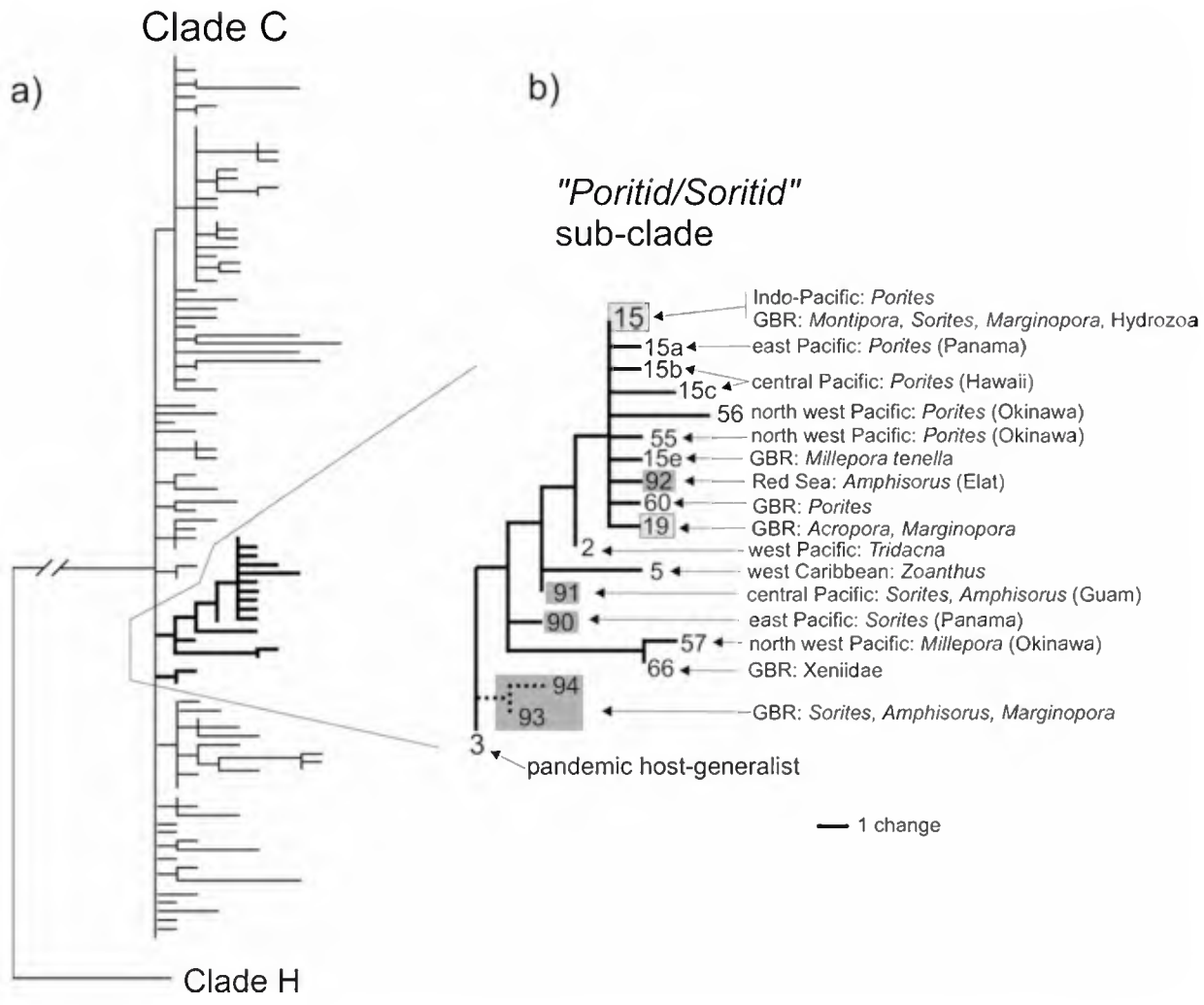


Fig. 3a, b Phylogenetic relationships of clade C *Symbiodinium* spp. and the localization of Indo-Pacific soritid symbionts. Among the great diversity of clade C *Symbiodinium* surveyed, those that associate with soritid foraminiferans belong to two sub-lineages diverging from the generalist *Symbiodinium* "type" C3. **a** The *inset* is a maximum-parsimony phylogram based on ITS 2 haplotypes depicting the radiation of over 100 clade C *Symbiodinium* spp. identified from a wide diversity of hosts from the Indo-Pacific and Caribbean (LaJeunesse 2002; LaJeunesse et al. 2003, 2004, unpublished data). *Bold face lines* indicate the phylogenetic position of the sub-lineages presented in detail. **b** *Alpha-numeric "types" in dark-grey shaded squares* were found in soritid hosts from the Indo-Pacific regions, whereas those with *light-grey shaded squares* were the *Symbiodinium* "types" found in soritid and cnidarian hosts. *Doted lines* group the two "types" from the first cluster; *plain lines* group the *Symbiodinium* "types" from the second cluster

foraminiferal species (Hallock 1987), which may have influenced the genetic diversity and diversification of soritid foraminifera and their respective symbionts.

There are a number of possibilities that may explain the biogeographic differences observed between Caribbean and East Pacific soritid symbiont communities. The unusual prevalence of sub-clade Fr4 and clade H "types" in the Caribbean may be a consequence of isolation and the onset of Northern Hemisphere glaciations that began with the final closure of the Central American Isthmus. This major geological event produced drastic changes in ocean circulation patterns that ultimately led to major fluctuations in sea-surface temperatures beginning during the Pliocene–Pleistocene transition, 3–4 million years ago (Haq et al. 1987; Heinze and Crowley 1997). The observed genetic divergence between the many "types" within clade H is consistent with an adaptive radiation following mass extinctions observed for Caribbean invertebrate communities, including corals, mollusks, and foraminifera during the early Pliocene (Jackson et al. 1993; Budd et al. 1996; Cheetham and Jackson 1996; Richardson 2001). A similar shift in cnidarian symbioses may have occurred at this time involving clade B *Symbiodinium* (Baker 2003; LaJeunesse et al. 2003). The extreme isolation and marginal environmental conditions of the eastern Pacific may also explain why *Sorites* from this region are dominated by a single "type" (C90) (cf. Glynn and Ault 2000). The community assemblage of this region comprises mostly of Indo-Pacific migrants with some endemics and few relict species related to western Atlantic ancestors (Glynn and Ault 2000). Like many eastern Pacific reef-building coral species, the *Sorites* and their

symbionts have likely proliferated from founder populations originating from elsewhere in the Pacific.

Our limited sampling from the Caribbean/Atlantic found no examples of *Sorites* harboring clade C *Symbiodinium*. In several other regions of the Indo-Pacific, associations with *Sorites* and clade C have been documented (Pochon et al. 2001). There is the possibility that these combinations existed at one time in the Caribbean, but were subsequently replaced by symbioses with members of clades F and H during the climatic and environmental changes of the early Pliocene and/or Pliocene–Pleistocene transition (Jackson et al. 1993). A distinct Caribbean community had been established by the late Miocene–early Pliocene (6–3 million years ago), through the origin and rapid diversification of both corals and benthic foraminifera (Collins et al. 1996). Sub-clade Fr4 and clade H may have diverged a long time before these events, evolving along with the radiation of soritids during the late Oligocene (Rodriguez-Lanetty 2003; LaJeunesse, unpublished data), and have persisted through numerous climatic upheavals.

Eastern Pacific soritid symbionts belong to a specific *Symbiodinium* C sub-clade

Host specialization is probably important in driving *Symbiodinium* spp. speciation (LaJeunesse et al. 2004; LaJeunesse, unpublished data). The existence of numerous host-specific *Symbiodinium* lineages that associate with soritids indicate that these hosts are important participants in driving the evolution of this dinoflagellate group. This is evident by: (1) the presence of many divergent symbiont lineages that are specific to soritid foraminifera and (2) the existence, at a lower taxonomic scale, of two sub-clades within clade C. As indicated by major differences in phylogeographic patterns between the Pacific and Caribbean, foraminiferan symbioses appear to have undergone dramatic shifts similar to their cnidarian neighbors in response to major environmental change.

Clade C is the only major *Symbiodinium* lineage known to associate consistently with invertebrates, foraminifera, and other protists (Pochon et al. 2001; Lobban et al. 2002). Most of the approximately 100 clade C symbiont "types" or haplotypes distinguished by PCR-DGGE ITS 2 fingerprinting and sequence data possess distinctive ecological, environmental, and geographic distributions. Many demonstrate specificity for certain host taxa and/or have particular depth distributions (LaJeunesse et al. 2003). In earlier studies, clade C *Symbiodinium* spp. originating from soritids appeared to be indiscriminately dispersed throughout the phylogeny of this clade based on analysis of LSU rRNA gene sequences (Pochon et al. 2001). However, re-analysis of clade C *Symbiodinium* with ITS 2 data sets derived from invertebrate collections from all over the world places sequences obtained from foraminiferal samples in two distinct clusters (Fig. 3a). The first one, composed by the foraminiferal *Symbiodinium* "types" C93 and C94, has diverged independently from the generalist "type" C3 (Fig. 3b). The second cluster forms a monophyletic sub-clade of *Symbiodinium* spp. that commonly associates with corals of the genus *Porites*. In light of these new data, this sub-clade also appears to have co-evolutionary relations with large soritid foraminiferans.

The most prevalent and widely distributed "type" from this poritid/soritid-dominated clade C sub-lineage is "type" C15, a thermally tolerant *Symbiodinium* sp. (UNESCO sponsored workshop on coral bleaching, Heron Island, Australia 2001; Hoegh-Guldberg, unpublished data). It is widely distributed in *Porites* spp. throughout the Indo-Pacific, but also has a host range that is more generalized than other members of this dinoflagellate group that have thusfar been found in associations with the coral *Montipora digitata*, the hydroid *Aglaophenia* sp., and now foraminifera from the GBR. C15 is ancestral to a number of rare derived variants that are more restricted in host and geographic ranges (Fig. 3). While half of the "types" radiating from C15 associate with various species of *Porites*, others are found in host taxa including *Millepora*, *Acropora*, and also soritid foraminifera.

While this sub-clade is common to the Indo-Pacific, the finding of C5 in the western Caribbean "grey" *Zoanthus* sp. indicates that its divergence probably began before the biogeographic barrier between the Pacific and Atlantic formed. Specialization to a particular resource and/or habitat often leads to speciation (Futuyma and Moreno 1988). Phylogenetic diversification in clade C suggests a recurring pattern, whereby numerous host-specific ("host-specialists") and/or regionally endemic "types" have evolved from a small number of ancestral host-generalists (Fig. 3). Periodically certain host-specialists must spread to new host taxa and become geographically more widespread (Piercey-Normore and DePriest 2001). The evolution of the C15 sub-clade, important to all Indo-Pacific *Porites*, may have started in the foraminifera. The basal position of "type" C90 from the eastern Pacific and "type" C91 in *Sorites* from Guam in this sub-clade suggests that soritid foraminifera contribute to the evolution of symbionts that shift to cnidarian hosts. Such interpretation is further substantiated by the recent finding of a clade F "type" in the high latitudinal coral *Alveopora japonica* (Rodriguez-Lanetty et al. 2003a, 2003b). The *Symbiodinium* sub-clades Fr2–Fr5 comprised in clade F were once thought to be exclusive to soritid foraminifera (Pawlowski et al. 2001). The finding of sub-clade Fr2 in *A. japonica* living along the coast of South Korea further suggests that host shifts can occur between foraminiferans and cnidarian *Symbiodinium*.

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