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High genetic diversity and relative specificity among *Symbiodinium*-like endosymbiotic dinoflagellates in soritid foraminiferans

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Abstract Soritids are large calcareous foraminiferans abundant in Indo-Pacific coral reefs. Soritids are known to host endosymbionts morphologically and genetically similar to *Symbiodinium*-like dinoflagellates commonly found in corals and other marine invertebrates. In order to examine the phylogenetic relationships between symbionts present in foraminiferal and coelenterate hosts, we used DNA sequencing and PCR-based RFLP methods to analyse 157 foraminiferal and 110 coral DNA samples from 12 localities in Guam (Micronesia) collected in July 1999 and December 1999. Ribosomal DNA sequences were obtained for 14 foraminiferal and 12 coral samples. Sequence analyses allowed identification of six different *Symbiodinium* phylotypes among soritids and two phylotypes among examined coelenterates. A single phylotype, previously described as lineage C, was common in foraminiferans and corals. The PCR-based RFLP analysis of 157 foraminiferal and 110 coral samples shows that lineage C dominates coral symbiont communities, accounting for 78% in both sampling periods. On the other hand, our data show that lineages C and Fr6 dominated foraminiferal symbiont communities in July 1999 versus a clear predominance of lineages C and Fr3 in November 1999. The other three phylotypes present in soritids (Fr1, Fr4, Fr5) were uncommon and seem to occur seasonally. The phylogenetic analysis of the present data indicates relative specificity of soritid symbionts compared to other *Symbiodinium*-like dinoflagellates, but the causes of this specificity are not yet understood.

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Introduction

Soritids are miliolid foraminiferans belonging to the subfamily Soritinae, characterised by large porcellaneous discoidal tests of about 1 cm in diameter that are very common on coral reefs (Allen and Steene 1996). The Soritinae comprises three genera (*Sorites*, *Amphisorus*, *Marginopora*) and about six Recent species, for which taxonomic identification based on morphological features is difficult (Gudmundsson 1994). Soritids are particularly abundant in the Indo-Pacific, where they play an important role in biogeochemical mineral cycling (Murray 1991). The genus *Sorites* is also common in the Caribbean, while the distribution of *Amphisorus* and *Marginopora* is restricted to the Indo-Pacific region (Langer and Hottinger 2000).

Compared to the other extant families of large miliolid foraminiferans, Archaiasinae, Peneroplidae and Alveolinidae, which are hosts respectively to chlorophytes, rhodophytes and diatoms, Soritinae bear dinoflagellates as symbionts (Lee and Anderson 1991). Symbiotic association seems to have been essential for the successful adaptation of soritids to oligotrophic environments. The photosynthetic activity of symbionts provides their foraminiferal hosts with the energy necessary for survival and growth in oligotrophic environments (Hallock 1999). Several experimental studies demonstrated that algal symbiosis enhances calcification, contributing to the exceptional growth of foraminiferal tests (Lee and McEnery 1983; ter Kuile 1991). Soritids present, in some areas, a spectacular

carbonate production rate of approximately 5 kg $CaCO_3$ m^{-2} $year^{-1}$ (Fujita et al. 2000).

Morphological and ultrastructural studies of cultured isolates and in situ preparations tentatively identified soritid symbionts as belonging to the genus *Symbiodinium* (Müller-Merz and Lee 1976; Leutenegger 1977; McEnery and Lee 1981; Lee and Lawrence 1990). Endosymbiotic dinoflagellates belonging to the genus *Symbiodinium* are found in a wide variety of coelenterate hosts (reviewed by Trench 1987, 1992, 1993). *Symbiodinium microadriaticum* (Freudenthal 1962) was initially believed to be the only species involved. However, numerous subsequent morphological, biochemical, physiological and behavioural studies have led to the taxonomic description of several new *Symbiodinium* species (Schoenberg and Trench 1980a,b,c; Chang et al. 1983). In total, all symbiotic dinoflagellates represent at least five genera classified in three orders (Banaszak et al. 1993; Fensome 1993; Gast and Caron 1996).

Recent molecular genetic studies have provided new tools for the identification and classification of symbiotic dinoflagellates belonging to the *Symbiodinium* species complex. SSU (small subunit) rRNA gene sequences and RFLP (restriction fragment length polymorphism) patterns were used to assess the genetic diversity of *Symbiodinium*-like zooxanthellae cultured in vitro (Rowan and Powers 1992) and sampled in their natural environment (Rowan and Powers 1991a,b). Both RFLP and sequence data show the presence of three well-distinguished groups of *Symbiodinium*, called lineages A, B and C (Rowan and Powers 1991a,b, 1992). Genetic identification of these phylotypes and their phylogenetic relationships with other dinoflagellate genera was confirmed by subsequent molecular studies based on LSU (large subunit) rDNA sequences (McNally et al. 1994; Zardoya et al. 1995; Wilcox 1998; reviewed in Rowan 1998). Intraspecific diversity and polymorphism of zooxanthellae in corals led to the identification of symbiont zonation and the discovery of a possible impact on coral bleaching (Rowan and Knowlton 1995; Rowan et al. 1997; Baker 2001). More recent studies have reported two additional molecular phylotypes of *Symbiodinium*. Carlos et al. (1999), in a study based on SSU rDNA analyses, mentioned a "type D" obtained from the Palauan sponge *Haliclona koremella*. Baker (1999) pointed out another type D based on LSU rDNA sequences.

The molecular identification of soritid symbionts was first attempted by analysis of SSU rDNA obtained from *Amphisorus hemprichii*, *Marginopora kudakajimaensis* (Lee et al. 1995) and *Sorites orbiculus* (Langer and Lipps 1995). Analysis of these sequences confirmed the taxonomic status of foraminiferal zooxanthellae as belonging to the *Symbiodinium* species complex. However, because SSU rRNA genes evolve at a slow rate, the molecule provides only limited information about the phylogenetic relationships of foraminiferal symbionts. A recent study based on much more variable ITS (internal transcribed spacer) and LSU rDNA sequences, revealed unexpectedly high molecular diversity between soritid symbionts, suggesting that some of them are not related to the *Symbiodinium*-like symbionts found in coelenterates (Pawlowski et al. 2001b). This study, however, was based on a limited number of samples and lacked information on the phylotypes of the corals from the localities where the soritids were collected. Here, we test the hypothesis of the specificity of soritid symbionts by analysing DNA samples obtained from 157 soritid and 110 coral isolates collected within the same localities. In complement, we present a global picture of all *Symbiodinium* molecular types based on partial LSU rDNA sequences, including representatives of the A, B, C, D and E molecular types compared to the seven phylotypes found in soritids.

Materials and methods

Collection

We analysed 208 specimens of soritids and 152 coral samples collected in Guam in July and December 1999. Foraminiferans and corals were collected within the same localities. Additionally, soritids were collected from the Great Barrier Reef (Lizard Island), the southern Indian Ocean (Réunion Island), the Red Sea (Safaga, Elat), the western Indian Ocean (Maldives) and the Caribbean Sea (Florida). One soritid foraminifer with its symbionts is shown in Fig. 1.

DNA extraction, PCR amplification and sequencing

DNA of foraminiferans and their symbionts was extracted either by using DOC lysis buffer (as described in Holzmann and Pawlowski 1996) or the DNeasy Plant minikit (Qiagen). All specimens, except very small ones, were broken, and only fragments of the

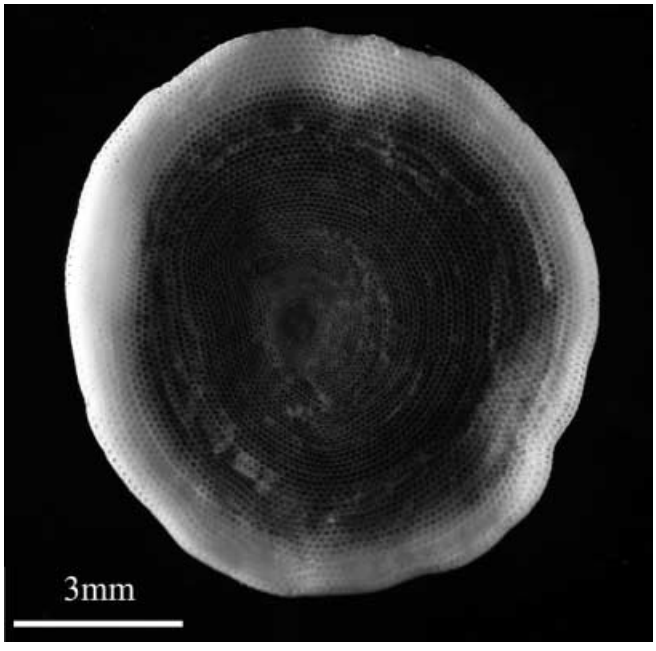

Fig. 1 One specimen of soritid foraminifer (*Marginopora vertebralis*). Symbionts (*Symbiodinium* sp.) are restricted to the inner part of their foraminiferal host (*dark zone*) avoiding the external digesting area (*light zone*)

tests were taken for extraction. The remaining parts of the tests were preserved for SEM study of foraminiferal morphology. Identification of foraminiferans was performed by analysing external structures of the test skeleton (chamberlet arrangements and apertures), following Gudmunsson (1994). DNA of coral symbionts was extracted from cell lysates using procedures described in Rowan and Powers (1991a). DNA of *Maristentor* symbionts was extracted by using DOC lysis buffer. The sponge symbionts, previously designated as type D (PSP1–05) by Carlos et al. (1999), were provided by MBI (Marine Biotechnology Institute, Kamaishi-shi, Japan) from their culture. DNA of the sponge symbionts was extracted in guanidine lysis buffer, precipitated with isopropanol and dissolved in distilled water.

PCR (polymerase chain reaction) amplifications were performed in a total volume of 50 µl, with an amplification profile consisting of 40 cycles of 30 s at 94°C, 30 s at 50°C and 120 s at 72°C, followed by 5 min at 72°C for final extension. The amplified PCR products were purified using the High Pure PCR purification kit (Roche Diagnostics). PCR products were sequenced directly with the ABI PRISM Big Dye Terminator Cycle sequencing kit using ABI 377 DNA sequencer (Perkin-Elmer), all according to the instructions of the manufacturers. Some amplified PCR products were purified using the High Pure PCR purification kit, then ligated into the pGEM-T vector system (Promega) and cloned into XL-2 ultracompetent cells (Stratagene).

PCR amplifications were performed by using the dinoflagellate-specific primer S_DINO (5′CGCTCCTACCGATTGAGTGA) situated at the 3′-end of the SSU rDNA and the universal primer L_O [5′GCTATCCTGAG(AG)GAAACTTCG] situated about 900 nucleotides (nt) downstream of the 5′-end of the LSU rDNA. The amplified fragment includes the 3′-end of the SSU rDNA (about 100 nt), the whole ITS region (ITS1 + 5.8 S + ITS2) and the 5′-end of the LSU rDNA (about 900 nt). Its total length ranged from 1572 to 1647 nt. Alternatively, the specific *Symbiodinium*

Table 1 List of host species, collection localities and dates, as well as symbiont DNA sequence accession numbers in GenBank. *Numbers in parentheses* correspond to different hosts of the same species (see also Fig. 2). *DNA extract* refers to the DNA collection identification number (see "Materials and methods")

Host species	Collection site	Date	DNA extract	Accession number
Marginopora vertebralis (1)	Lizard Island	Jul 97	490(J)	AJ291531
Sorites sp. (2)	Lizard Island	Jul 97	489(J)	AJ291516
Amphisorus hemprichii (3)	Elat, Israel	Apr 99	1366(J)	AJ291514
Porites rus	Guam, Micronesia	Jul 99	1675a(J)	AJ311944
Porites cylindrica	Guam, Micronesia	Dec 99	8(X)	AJ308892
Pavona divaricata	Guam, Micronesia	Dec 99	60(X)	AJ308889
Marginopora vertebralis (4)	Elat, Israel	Apr 99	9b(J)	AJ311941
Sorites sp. (5)	Elat, Israel	Apr 99	5b(J)	AJ311942
Lobophyllia sp.	Guam, Micronesia	Jul 99	1673(J)	AJ311943
Acropora sp. (1)	Réunion Island	Aug 00	806(X)	AJ308893
Amphisorus hemprichii (6)	Réunion Island	Aug 00	32f(X)	AJ308894
Sorites sp. (7)	Guam, Micronesia	Jul 99	1690(J)	AJ291518
Sorites sp. (8)	Guam, Micronesia	Jul 99	1650(J)	AJ291517
Sorites sp. (9)	Guam, Micronesia	Jul 99	1591(J)	AJ291519
Maristentor dinoferus	Guam, Micronesia	Dec 99	S2(X)	AJ278598
Favia matthai	Guam, Micronesia	Dec 99	329(X)	AJ308890
Oulaphyllia crispa	Guam, Micronesia	Dec 99	328(X)	AJ308891
Heliopora cerulea	Guam, Micronesia	Dec 99	50(X)	AJ308888
Ctenactis echinata	Guam, Micronesia	Dec 99	458(X)	AJ308887
Sorites sp. (10)	Florida Keys	Jul 98	751(J)	AJ291513
Sorites sp. (11)	Guam, Micronesia	Jul 99	1678(J)	AJ291520
Sorites sp. (12)	Elat, Israel	Apr 99	1318(J)	AJ291522
Sorites sp. (13)	Elat, Israel	Apr 99	1334(J)	AJ291521
M. kudakajimaensis (14)	Guam, Micronesia	Dec 99	188c1(X)	AJ308895
M. kudakajimaensis (15)	Guam, Micronesia	Jul 99	1635(J)	AJ291525
M. kudakajimaensis	Guam, Micronesia	Dec 99	188c2(X)	AJ308896
M. kudakajimaensis	Guam, Micronesia	Dec 99	188c3(X)	AJ308897
Sorites sp. (16)	Florida Keys	Jul 98	836(J)	AJ291527
Sorites sp. (17)	Guam, Micronesia	Jul 99	1631(J)	AJ291528
Sorites sp. (18)	Guam, Micronesia	Jul 99	1681(J)	AJ311949
Sorites sp. (19)	Guam, Micronesia	Jul 99	1679(J)	AJ291526
Amphisorus hemprichii (20)	Maldives	Oct 97	650(J)	AJ291535
Amphisorus hemprichii (21)	Réunion Island	Aug 00	33f(X)	AJ308898
Amphisorus hemprichii (22)	Elat, Israel	Apr 99	1360(J)	AJ311945
Sorites sp. (23)	Elat, Israel	Apr 99	1305(J)	AJ291512
Sorites sp. (24)	Guam, Micronesia	Jul 99	1593(J)	AJ291529
Haliclona koremella	Palau, Micronesia	Sep 97	PSP1_05	AJ308899
Goniopora fruticosa	Guam, Micronesia	Dec 99	342(X)	AJ308901
Acropora sp. (2)	Guam, Micronesia	Dec 99	1655(J)	AJ311948
Pavona decusata	Guam, Micronesia	Dec 99	63(X)	AJ308900
Acropora palifera	Guam, Micronesia	Dec 99	542(X)	AJ308902
M. kudakajimaensis (25)	Guam, Micronesia	Jul 99	1582(J)	AJ291537
Marginopora vertebralis (26)	Guam, Micronesia	Jul 99	1643(J)	AJ291536
M. kudakajimaensisis (27)	Guam, Micronesia	Jul 99	1584(J)	AJ291539
Marginopora vertebralis (28)	Guam, Micronesia	Jul 99	1645(J)	AJ291538
Millepora sp.	Elat, Israel	Apr 99	20b(J)	AJ311946
Acropora sp. (3)	Elat, Israel	Apr 99	3b(J)	AJ311947
Porites nigrecens	Réunion Island	Aug 00	807(X)	AJ308903

primer ITS-DINO (5′GTGAATTGCAGAACTCC), situated in the ITS region, was used for amplification and sequencing. The PCR products amplified with ITS-DINO included the 3′-end of the 5.8 S region, the whole ITS2 region and the 5′-end of the LSU rDNA. Detailed data on collection localities and dates, as well as DNA sequence accession numbers in GenBank are given in Table 1. Each DNA extraction received a DNA collection identification number that also appears in Table 1.

RFLP

A total of 157 foraminiferal and 110 coral samples collected in Guam were analysed by the PCR-based RFLP method. The 110 coral samples represented 23 genera in 10 scleractinian coral families (G. Pauley, personal observations). The RFLP was applied to PCR products amplified with primers ITS-DINO and L_O, and then digested with the restriction enzyme *Hin*dIII (Roche Diagnostics). The digestion mix contained 8 µl of distilled water, 2 µl of B incubation buffer and 0.5 µl of *Hin*dIII to which 10 µl of PCR product was added and incubated at 37°C overnight. Samples containing composite genotypes were amplified by PCR, cloned and sequenced.

Sequence analysis

The sequences were aligned manually by using GDE 2.2 software (Larsen et al. 1993). Phylogenetic analyses were performed on a fragment of 506 unambiguously aligned sites located in the LSU rDNA, for which a large number of sequences of *Symbiodinium*-like dinoflagellates from corals and other hosts were available from GenBank (Baker et al. 1997; Wilcox 1998; Baker 1999). Three methods were used for sequence analysis: (1) the neighbour-joining (NJ) method (Saitou and Nei 1987) applied to distances corrected for multiple hits and for unequal transition and transversion rates, and using Kimura's two-parameter model (Kimura 1980); (2) the maximum-likelihood (ML) method as implemented in the fast DNAm1 program (Olsen et al. 1994); and (3) the maximum-parsimony (MP) method (Farris 1970). The reliability of internal branches in the NJ and ML trees was assessed by 1000 and 100 bootstrap replicates, respectively. The PHYLO_WIN program (Galtier and Gouy 1996) was used for distance computations, tree building and bootstrapping.

Results

Phylogenetic analyses

During analyses 16 sequences of foraminiferal symbionts and 12 sequences of coral symbionts collected in Guam were compared to 14 sequences of soritid symbionts from other localities and 20 sequences of *Symbiodinium*-like dinoflagellates, representing 5 cultured species and 15 isolates from corals and other marine invertebrates, including the symbionts of a ciliate (*Maristentor dinoferus*) and a sponge (*Haliclona koremella*). The sequences of *Gymnodinium simplex* and *Gymnodinium beii*, the latter one isolated from the planktonic foraminifer *Orbulina universa* (Spero 1987), were used as the outgroup, following Wilcox (1998). Phylogenetic analysis of the 62 sequences allows differentiation of 12 distinct phylotypes among the *Symbiodinium* species complex (Fig. 2). The sequences of soritid symbionts from Guam cluster in six phylotypes. Five of them (Fr1, Fr3, Fr4, Fr5, Fr6) include only foraminiferal symbionts, while one, lineage C, also contains symbionts from other hosts. The coral symbionts from Guam cluster within two phylotypes, C and D2. Among five other phylotypes distinguished in our analyses one was found exclusively in soritids from the Gulf of Elat (Fr2), and four include the cultured or collected symbionts of corals and other invertebrates from different localities (A, B, D, E). Phylotypes D1 and E contain single sequences of, respectively, PSP1-05-cultured symbionts from a Palauan sponge (Carlos et al. 1999) and *Gymnodinium varians*, the taxonomic status of which is controversial (Wilcox 1998). Because of notable genetic distances separating the different types (from 5% to 35%) and low divergence within the clades (about 1%), each of them can most probably be considered a separate species.

The phylogenetic relationships within the *Symbiodinium* species complex are congruent with previous studies (Rowan 1998; Pawlowski et al. 2001b). Lineage A branches next to the outgroup in the basal part of the tree, followed by phylotype E and a radiation of the other phylotypes. Within this radiation, the foraminiferal phylotype Fr6 arises as the sister group [50% (ML) bootstrap support] to lineages D1 and D2, with 24.21% and 26.71% of divergence, respectively. Lineages D1 and D2 differ by 13.69% of sequence divergence. Lineage B emerges as the sister group [37% (ML) bootstrap support] to phylotypes Fr2, Fr3, Fr4 and Fr5. These associations are supported by very low bootstrap values and change depending on the phylogenetic model used. For instance, in NJ analysis using the pairwise gap removal option, type B appears as a sister taxon to lineage C and Fr1–Fr5 (data not shown). Preliminary statistical work (data not shown) favours the creation of clade F that includes the phylotypes Fr2, Fr3, Fr4 and Fr5, as well as the creation of clade G that includes the phylotype Fr6. This more convenient clade nomenclature (A, B, C, D, E, F, G) will be described in detail in a consensus presently being prepared (T.C. LaJeunesse, personal communication). Nevertheless, NJ with global gap removal, MP (data not shown) and ML analyses provide congruent topologies to that shown in Fig. 2.

RFLP

Our RFLP data confirm the distinction of phylotypes inferred from analysis of LSU rDNA sequences (Fig. 3a). *Hin*dIII appeared to be an efficient restriction enzyme to distinguish all phylotypes of foraminiferal and coelenterate symbionts. As shown in Fig. 3a, eight different patterns can be detected, of which seven are characteristic for foraminiferal symbionts. Identical patterns appear for lineages A, D1 and Fr6 (the latter three phylotypes do not possess the *Hin*dIII restriction site). No data are available for lineage B. Coral symbionts of lineages A, B and C are more efficiently distinguished by using the restriction enzyme *Taq*I, as previously described (Baker et al. 1997; Billinghurst et al. 1997; Darius et al. 1998; Loh et al. 1998; Rowan 1998; Carlos et al. 1999); however, our attempts show that this enzyme is inappropriate to discriminate all described phylotypes of foraminiferal symbionts. Indeed, *Taq*I produced RFLP patterns

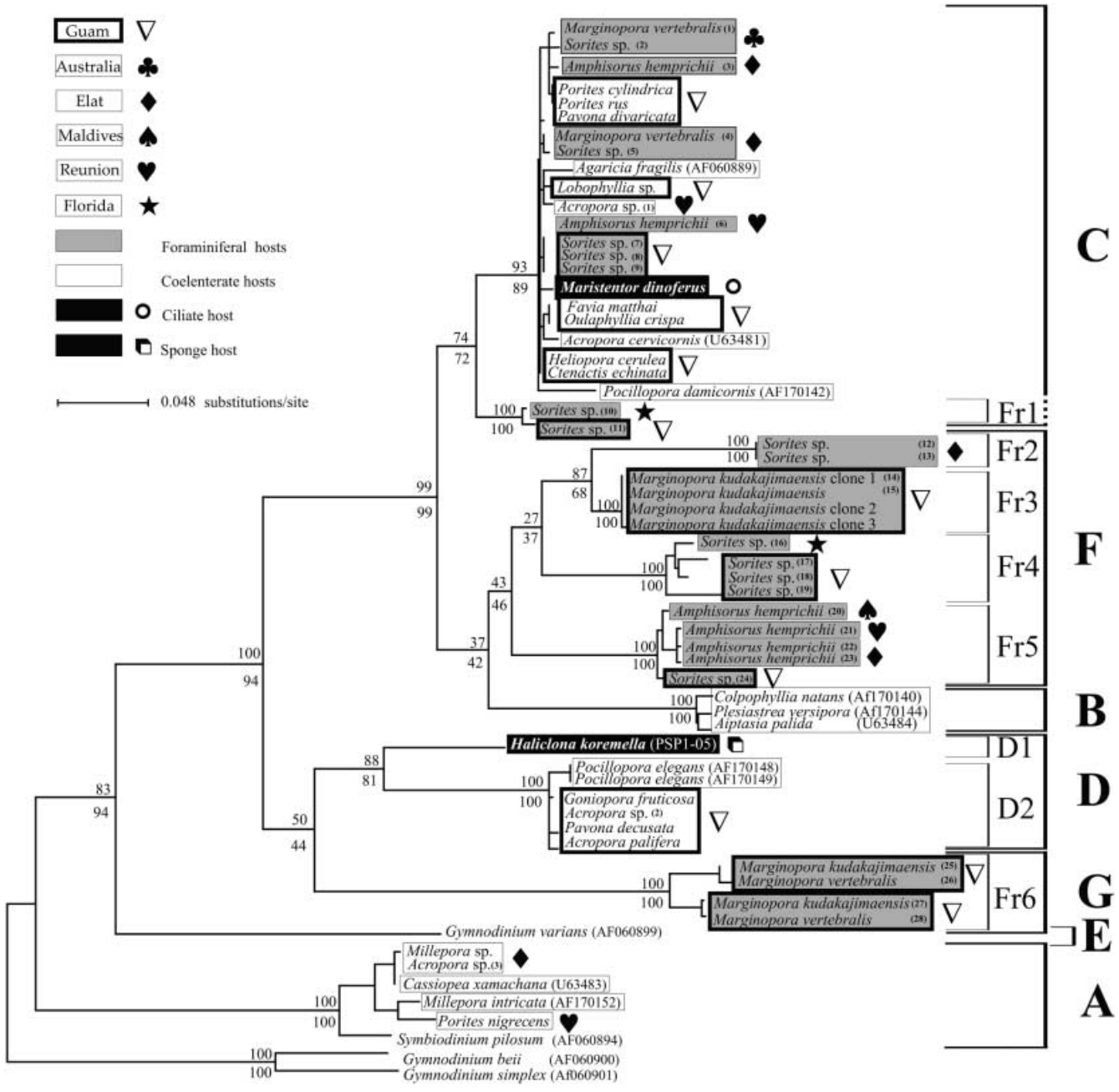

Fig. 2 Phylogenetic reconstruction of the *Symbiodinium* species complex inferred from partial large subunit rDNA, based on the method of maximum-likelihood. The tree includes 30 sequences of soritid symbionts (*grey*), and 28 sequences of coelenterate symbionts (*white*), including a ciliate's (*Maristentor dinoferus*) symbionts (*black with symbol*) and a sponge's (*Haliclona koremella*) symbionts (*black with symbol*) (*Fr1–Fr6* phylotypes of foraminiferal symbionts; *A–D* phylotypes of coelenterate symbionts). Lineage C groups foraminiferal and coelenterate symbionts. Lineage F includes foraminiferal phylotypes Fr2–Fr5. Lineage D includes phylotypes D1 and D2. Lineage G includes the foraminiferal phylotype Fr6. Taxa names followed by accession numbers were obtained from GenBank. Different sampling localities are represented by *different symbols* (see key). The *numbers above and below branches* correspond to bootstrap values obtained with ML and NJ analyses, respectively. *Numbers in parentheses* correspond to different hosts of the same species (see also Table 1)

including four to six bands, some of them being identical among distinct phylotypes (data not shown).

Additionally, 157 foraminiferal and 110 coral samples from two expeditions in Guam (July and November 1999) were analysed by RFLP analysis. Coral symbionts from both sampling periods revealed two consistent RFLP patterns, C and D2, with mean occurrences of 78% and 22%, respectively. Foraminiferal symbiont data brought to light remarkable differences between July and December 1999. In July, the 65 foraminiferans analysed revealed six phylotypes at various densities: C (27.69%), Fr1 (1.50%), Fr3 (9.20%), Fr4 (23.07%), Fr5 (9.20%) and Fr6 (29.23%). In December (92 foraminiferans analysed), only three phylotypes were detected,

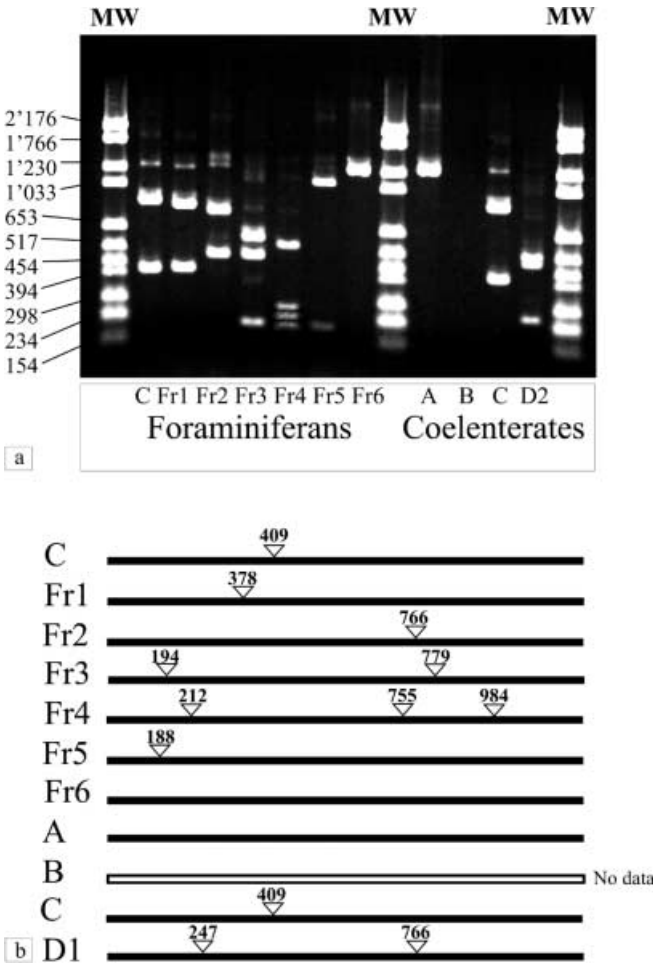

Fig. 3 **a** Specific-lineage RFLP patterns obtained with *Hin*dIII (AAGCTT). Uncut amplification products of ribosomal DNA are situated between partial 5.8 S and partial large subunits, representing 1299 base pairs. Fragment size was determined by molecular weight (*MW*) marker. **b**. Position of *Hin*dIII restriction site(s) for each phylotype (phylotype abbreviations, see legend Fig. 2)

with a clear predominance of lineage C (75.27%), followed by phylotypes Fr3 (23.65%) and Fr6 (1 individual). The various densities of coral and foraminiferal phylotypes are presented in Fig. 4. Two composite genotypes were apparent among foraminiferans, and we succeeded in cloning and sequencing four distinct phylotypes in these foraminiferal samples (data not shown). No composite genotypes were found in corals. In total, 208 foraminiferal and 152 coral samples were processed by PCR, and we found a respective amplification mismatch of 24.51% and 27.63%. Detailed data on collection localities, sampling depth and date and symbiont molecular phylotypes are given in Table 2.

Foraminiferal host morphology and symbiont phylotypes

On the basis of Gudmunsson (1994), we examined and identified the 157 foraminiferal hosts by scanning electron microscopy (SEM) (data not shown). Among the 157 forminiferans studied, we identified 57 *Sorites* sp., 53 *Amphisorus hemprichii*, 30 *Marginopora kudakajimaensis* and 17 *Marginopora vertebralis*.

Only three *Symbiodinium* phylotypes (C, Fr3, Fr6) were found in association with *A. hemprichii*, *M. kudakajimaensis* and *M. vertebralis*, whereas *Sorites* sp. could harbour one of the five following phylotypes: C, Fr1, Fr3, Fr4, or Fr5. Detailed lists of the 157 foraminiferal and 110 coral hosts from Guam (Appendix 1, 2), including sampling period, sampling localities, species names, sampling depth and corresponding *Symbiodinium* phylotypes are available as supplementary electronic material at http://dx.doi.org/10.1007/s002270100674.

Discussion

All soritid symbionts belong to the *Symbiodinium* species complex

Phylogenetic analysis of our data shows that all isolates of Soritinae examined in this study contain, exclusively, symbionts belonging to the *Symbiodinium* species complex. This is in agreement with morphological and ultrastructural studies describing symbionts of Soritinae as typical *Symbiodinium*-like zooxanthellae (Müller-Merz and Lee 1976; Leutenegger 1977; McEnery and Lee 1981). The present data also confirm previous molecular studies, which identify foraminiferal symbionts as belonging to the genus *Symbiodinium*, based on SSU (Langer and Lipps 1995; Lee et al. 1995) and LSU rDNA (Pawlowski et al. 2001b) sequence analyses. We have not found evidence of the presence of any other types of symbiotic dinoflagellates in Soritinae. The coexistence of two different genera of dinoflagellates, *Symbiodinium* and *Amphidinium*, has been reported within the soritid *Amphisorus hemprichii* (Lee et al. 1997). Although our PCR primers were designed to amplify a broad range of dinoflagellate lineages, including *Amphidinium*, no sequence corresponding to this genus was detected in our isolates.

The relationships within *Symbiodinium* inferred from our data are congruent with previous rDNA-based phylogenies of this genus (Rowan 1998; Wilcox 1998). The position of foraminiferal symbionts, within lineage C and next to lineages B, C and D, is in agreement with our previous study (Pawlowski et al. 2001b). Although we have examined a large number of isolates, we have not found any soritid symbionts branching within or next to lineage A. We have found this phylotype in several scleractinian corals collected at Elat and Réunion Island, but never among foraminiferans collected within the same sampling areas. Two studies based on SSU rDNA report lineage A in the foraminiferal host *A. hemprichii*. The first one shows foraminiferal symbionts from Elat closely related to the lineage A symbionts from a Jamaican jellyfish, *Cassiopea xamachana* (Lee

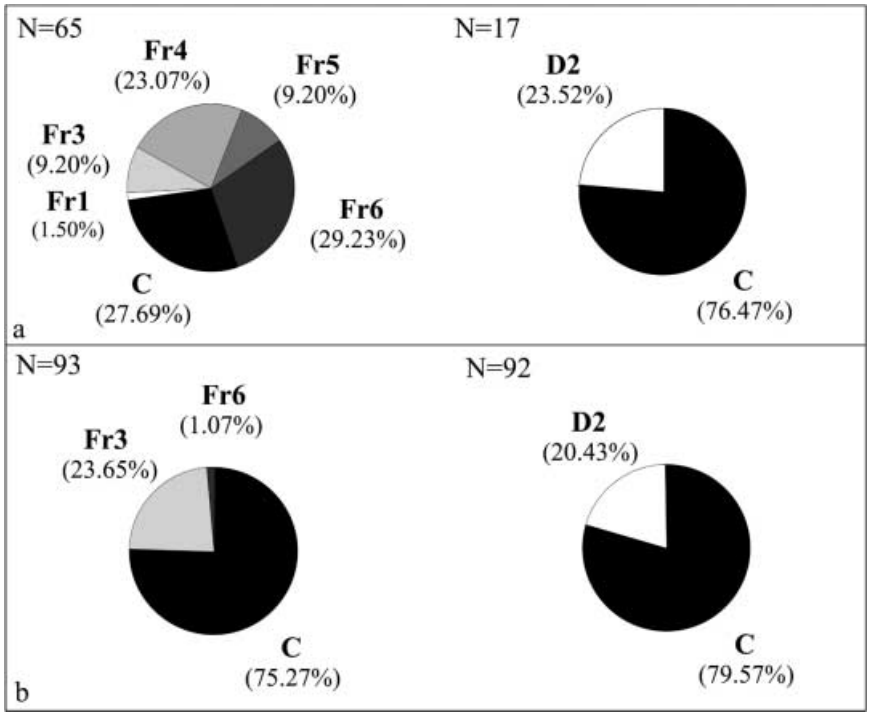

Fig. 4a, b Diagrams representing the various densities of: **a** foraminiferal ($n=65$) and coral ($n=17$) phylotypes sampled in Guam in July 1999; **b** foraminiferal ($n=93$) and coral ($n=92$) phylotypes sampled in Guam in December 1999

Table 2 List of Guam localities, sampling depth and period, as well as number of individuals with identified foraminiferal and coral symbiont phylotypes

Localities	Depth	Period	Foraminifer						Coral	
			C	Fr1	Fr3	Fr4	Fr5	Fr6	C	D1
Pago Bay	Shallow	Jul 99	7	–	2	2	–	2	–	–
Pago Bay	Shallow	Dec 99	11	–	5	–	–	–	15	2
Cocos Lagoon	Shallow	Dec 99	3	–	2	–	–	–	5	3
Bile Bay	3 m	Dec 99	3	–	–	–	–	–	2	1
Bile Bay	8 m	Dec 99	3	–	–	–	–	–	2	1
Bile Bay	15 m	Dec 99	4	–	3	–	–	–	9	1
Neye	8 m	Dec 99	1	–	1	–	–	–	2	–
Neye	20 m	Dec 99	–	–	1	–	–	–	1	–
Orote	10 m	Dec 99	4	–	–	–	–	–	2	–
Orote	20 m	Dec 99	4	–	–	–	–	–	1	–
Orote	35 m	Dec 99	1	–	4	–	–	–	5	–
Harbour	5 m	Jul 99	–	–	–	3	–	–	–	–
Harbour	20 m	Jul 99	–	1	–	1	–	–	7	4
Harbour	Shallow	Dec 99	16	–	–	–	–	–	10	5
Luminao	Shallow	Jul 99	2	–	–	–	2	2	–	–
Piti	Shallow	Jul 99	–	–	–	4	–	–	–	–
Tumon	Shallow	Dec 99	–	–	–	–	–	–	2	–
Gun Beach	Shallow	Dec 99	3	–	–	–	–	1	4	–
Gun Beach	5 m	Dec 99	4	–	–	–	–	–	2	2
Gun Beach	43 m	Dec 99	–	–	3	–	–	–	5	1
Double Reef	Shallow	Jul 99	5	–	3	5	4	15	2	–
Double Reef	Shallow	Dec 99	3	–	–	–	–	–	1	2
Double Reef	10 m	Dec 99	2	–	2	–	–	–	2	–
Double Reef	20 m	Dec 99	2	–	–	–	–	–	1	–
Double Reef	37 m	Dec 99	2	–	1	–	–	–	1	–
Ritidian	Shallow	Jul 99	4	–	1	–	–	–	4	–
Ritidian	Shallow	Dec 99	3	–	–	–	–	–	1	2

et al. 1995; reviewed in Rowan 1998). The second one shows similar results in connection with an *Amphisorus* specimen collected in Palau (Carlos et al. 1999). Both studies, however, were based on *Symbiodinium* cells isolated and cultivated in the laboratory. The fact that some enriched seawater media can favour the growth of lineage A strains versus other phylotypes (Carlos et al. 1999) may explain the incongruence between their find-

ings and our present data obtained from freshly isolated material.

Soritid symbionts are highly diverse and relatively specific

The most striking particularity of symbionts observed in Soritinae is their exceptional diversity compared to *Symbiodinium* reported from other hosts. It is paradoxical that three genera of Soritinae, originating about 25 million years ago from Archaisinae (Haynes 1981), bear at least seven genetically different phylotypes of *Symbiodinium*, while in the coelenterate taxa, which are known to have possessed endosymbionts since the Triassic period, i.e. for at least 240 million years (Veron 1995), only four molecular phylotypes have been detected. Two hypotheses can be proposed to explain the origin of soritid symbionts. The first one would suggest that the earliest Soritinae acquired their symbionts from some coelenterates, such as scleractinian corals. The fact that all molecular phylotypes of *Symbiodinium* present in Soritinae are either identical to coral symbionts (C) or appear as their sister groups (C/Fr1, B/Fr2-Fr5, D/Fr6) favours this hypothesis. An alternative hypothesis would be that soritid symbionts originated from some free-living zooxanthellae. This hypothesis is in agreement with the concept that foraminiferans can easily acquire symbionts from environmental pools (Lee et al. 1995). Very little is known, however, about the distribution of free-living *Symbiodinium*, and, as far as we know, there is no evidence for the presence of *Symbiodinium*-like symbionts in the natural environment.

High diversity of soritid symbionts may be partly due to their relative specificity. Among the seven zooxanthellae phylotypes housed by soritids, six are specific for this group (lineage C, which accumulates symbionts from coelenterates and protozoans, being the only exception). Such host–symbiont specificity is unusual among zooxanthellate hosts. Until now, the same lineages of *Symbiodinium* have been found in various groups of animal hosts, including scleractinian corals, sea anemones and molluscs (McLaughlin and Zahl 1966; Blank and Trench 1986; Trench 1987). A newly discovered ciliate species, *Maristentor dinoferus*, also bears the same *Symbiodinium* genotype (C) found in corals and other coelenterates (Lobban et al., in press). To our knowledge, foraminiferans appear to be the only group possessing their own specific, *Symbiodinium*-like zooxanthellae.

No matter where soritid symbionts originated, their acquisition by foraminiferans seems to have been followed by isolation and independent evolution. One of the factors that could maintain the ''foraminiferal–algal'' specificity is the asexual reproduction of foraminiferal specimens (termed ''schizogony''). During this type of reproduction, the algal cells are vertically transmitted from asexually reproducing adults to offspring. Schizogony may be a way to maintain only one *Symbiodinium* type in the cell. Although reproduction in soritid foraminiferans is known to be primarily asexual (Ross 1972), sexual reproduction cannot be excluded. The foraminiferal symbionts are certainly released into the environment periodically. The fact that different genera of Soritinae from the same localities tend to share the same symbionts suggests that free symbiont transmission occurs among foraminiferans. Our results indicate that transmission of symbionts (except lineage C) does not occur between foraminiferans and other hosts.

The specificity of foraminiferal symbionts is also surprising given the well-known predisposition of foraminiferans to enter endosymbiotic relationships with a wide range of diverse algal phylotypes (Lee and Anderson 1991). Flexibility in the acceptance of different potential foraminiferal endosymbionts was considered to increase the chances of adaptation to a broader range of environmental parameters (Lee et al. 1997). The fact that different groups of foraminiferans harbour very different phylotypes of symbionts may not be contradictory with the specificity of the host–symbiont relationship. A recent study of chlorophyte symbionts of Archaiasinae, a sister group to Soritinae, showed that all members of this subfamily bear specific types of *Chlamydomonas*-like algae (Pawlowski et al. 2001a).

Soritid symbionts in space and time

Our study of a large number of soritid samples from Guam and other regions reveals some temporal patterns in the distribution of soritid symbionts. The RFLP analyses of the 157 foraminiferal and 110 coral samples collected in Guam show a predominance of lineage C. This finding is consistent with previous studies of several different East Pacific coral species (Rowan and Powers 1991a; Baker and Rowan 1997; Loh et al. 1998). According to our data, lineage C, which harbours symbionts from the 3 genera of soritids, 20 genera of scleractinian corals, 1 ciliate and unidentified anemones (data not shown) from Guam, appears to be the predominant phylotype in this region. The abundance of other lineages, however, seems to vary depending on the season. Six phylotypes (C, Fr1, Fr3, Fr4, Fr5, Fr6) were found in specimens collected in July, while only three phylotypes (C, Fr3, Fr6) were found in December. One can speculate that this difference is due to a seasonality of ''free-living'' *Symbiodinium*-like zooxanthellae, under the hypothesis that foraminiferans can acquire their symbionts from environmental pools. Such seasonal variability in other dinoflagellate populations has been evidenced in long-term studies by several authors (Turquet et al. 1998; Chinain et al. 1999a,b). Alternatively, each foraminiferal specimen may contain several lineages of symbionts, the abundance of which varies depending on the season. Combining the RFLP method, cloning and PCR with primers specific for different phylotypes can test this second hypothesis. The analysis of RFLP-based composite genotypes and possible

cryptic phylotypes in foraminiferans is actually in progress in our laboratory. A 1-year survey is needed to determine with more clarity whether seasonality exists in foraminiferal symbiont communities, and whether one "dominant" type is replaced by other types within a short period.

Although some phylotypes were only found within specific locations, such as Elat (Fr2) and Guam (Fr3, Fr6), there is no evidence of biogeographical structure in their distributions. This is underlined by the fact that some foraminiferans collected at very distant locations (Florida and Guam) can harbour closely related *Symbiodinium* phylotypes (see Fr1 and Fr4). T.C. LaJeunesse (personal communication) has found that some *Symbiodinium* isolates from the Caribbean possess identical ITS sequences to isolates from the Red Sea and to those from the West Pacific, indicating that some *Symbiodinium* populations may have extensive biogeographic distributions.

Our results are preliminary and address a set of topical questions concerning the ecology and evolution of *Symbiodinium*-like dinoflagellates. Much work remains before we can answer these questions. We accept the challenges ahead and look forward to the results of future studies.

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