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Molecular Evidence for Host—Symbiont Specificity in Soritid Foraminifera

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Symbiosis between the dinoflagellate genus *Symbiodinium* and various invertebrates and protists is an ubiquitous phenomenon in shallow tropical and subtropical waters. Molecular studies undertaken on cnidarian symbionts revealed the presence of several distinctive lineages or subgeneric clades of *Symbiodinium* whose taxonomic level provides limited information about the specificity between invertebrate hosts and their symbionts. This contrasts with the finding of several *Symbiodinium* clades being present almost exclusively in foraminifera and belonging to the subfamily Soritinae. To test whether such specificity also exists at a lower taxonomic level within Soritinae, we obtained the SSU rDNA sequences from 159 soritid individuals collected in nine localities worldwide and representing all known morphospecies of this subfamily. For each individual, the symbionts were determined either by sequencing or by RFLP analysis. We distinguished 22 phylotypes of Soritinae in relation with a number of symbiont "groups" corresponding to 3 clades and 5 subclades of *Symbiodinium*. Among the 22 soritid phylotypes, 14 show strict symbiont specificity and only one was found to be a host for more than two "groups" of *Symbiodinium*. It is suggested that the strong host—symbiont specificity observed in Soritinae is a combined effect of a selective recognition mechanism, vertical transmission of symbionts, and biogeographical isolation.

Key words: *Foraminifera*; molecular phylogeny; rDNA; Soritacea; *Symbiodinium*; symbiosis.

Introduction

Symbiotic relationships between many invertebrate hosts and the dinoflagellates of the genus *Symbiodinium* are fundamental for the development, productivity, and survival of coral reef ecosystems worldwide. *Symbiodinium* dinoflagellates are present in hundreds of corals and other marine invertebrates, including sponges, medusae, and bivalves. Among protists, *Symbiodinium* has been identified in large benthic foraminifera belonging to the subfamily Soritinae (Lee and Lawrence 1990; Leutenegger 1977, 1984) and reported recently in ciliates (Lobban et al. 2002).

Soritinae are miliolid foraminifera common in tropical and subtropical shallow waters and characterized by a large imperforate, discoid test with annular chambers, reaching up to 15 mm in diameter. These protists present, in some area, a spectacular carbonate production rate of approxi-

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mately 5 kg $CaCO_3$ m^{-2} $year^{-1}$ (Fujita et al. 2000). They belong to the superfamily Soritacea, which also includes the Archaiasinae and Peneroplidae, bearing chlorophyte and rhodophyte symbionts, respectively (Richardson 2001). To date, Soritinae comprise six morphologically described species classified in three genera: *Sorites*, *Amphisorus* and *Marginopora* (Gudmundsson 1994; Lee et al. 2004). All species share a similar general morphology and differ by external features like the thickness of the test, the shape, and number of chamberlets, and the form and distribution pattern of peripheral apertures (Gudmundsson 1994). However, despite these test differences, it is often difficult to distinguish one species from another, especially in the case of young individuals.

For a long time, the increase of morphological complexity of the test, from the frail *Sorites* to the more solid *Amphisorus* and to the extremely robust *Marginopora*, was thought to reveal the evolutionary path of the Soritinae (Gudmundsson 1994; Lehmann 1961). However, a molecular study based on the SSU rRNA gene sequences (Holzmann et al. 2001) provided new insights into the phylogeny and diversity of this group. According to this study, the Soritinae are divided into two clades: one clade is composed of *Marginopora vertebralis* and a radiation of *Sorites orbiculus* and *S. marginalis*; the second clade comprises *Amphisorus hemprichii*, *M. kudakajimaensis*, and an unknown *Sorites*-like species (Holzmann et al. 2001). Most spectacularly, this study revealed an exceptionally high genetic diversity of Soritinae, particularly within the genus *Sorites*, which seriously questioned the validity of morphological criteria for species identification in this subfamily.

Algal symbiosis is a recurrent phenomenon among larger foraminifera that seems to have occurred independently in most miliolid lineages (Hallock 1999). Yet, Soritacea is the only superfamily where endosymbiosis is an all-pervading feature (Hallock 1999; Lee and Anderson 1991) that has been considered to have driven the evolution and morphological diversification of the superfamily (Hallock 1985, 1987).

The first SSU rDNA-based molecular studies of dinoflagellate symbionts of Soritinae confirmed that they belonged to the *Symbiodinium* species complex (Langer and Lipps 1995; Lee et al. 1995) and suggested that they share a common evolutionary history with the symbionts present in corals and other invertebrates. However, further molecular studies based on more variable LSU and ITS rDNA regions show that the soritid symbionts are exceptionally diverse and that the majority of them belong to symbiont "groups" that are not usually found in other *Symbiodinium*-bearing hosts (Pawlowski et al. 2001; Pochon et al. 2001). Six independent "groups" (Fr1—Fr6) specific to Soritinae were identified (Pawlowski et al. 2001). Four of them (Fr2—Fr5) were later grouped into a single clade F and referred to as subclades F2—F5 (Pochon et al. 2005), while the remaining two, Fr1 and Fr6, were renamed clade H (Pochon et al. 2004) and clade G (Pochon et al. 2001), respectively. Distinction of these "groups" was recently confirmed by analysis of chloroplastic 23S rDNA sequences (Pochon et al. 2005). Only one lineage (C) was found to be commonly shared by soritids and other invertebrates, but even in this case, the analysis of the highly variable ITS2 rDNA region showed that the foraminiferal symbionts belong to a well-defined subclade (Pochon et al. 2004). Although some recent studies report the presence of some soritid-specific "groups" (F2 and G) in some invertebrates (Rodriguez-Lanetty et al. 2002; Schoenberg and Loh 2005; van Oppen et al. 2005a, b), these occurrences remain rather exceptional.

To better understand this unusual host—symbiont specificity observed in Soritinae, we examined the relation between *Symbiodinium* and its foraminiferal hosts at a lower taxonomic level. Because the morphological criteria for identification of soritid species appeared to be not very reliable to describe the diversity of Soritinae, we sequenced all 159 examined specimens and distinguished the main phylogenetic lineages. We determined the "group" of *Symbiodinium* for each specimen by using RFLP or by sequencing. Our results show a surprisingly high level of specificity between soritid phylotypes and their symbionts. Possible geographical and reproductive causes of this specificity are discussed.

Results

Symbiodinium LSU rDNA Phylogeny

Phylogenetic analysis of 103 LSU rDNA sequences of *Symbiodinium* allowed us to identify 8 different clades: A, B, C, D, E, F, G, and H (Fig. 1). Two of them, D and F, are subdivided into 2 and 4 subclades, respectively. Most of the clades and subclades are supported by high bootstrap values and posterior probabilities, referred to here as node support (NS). Soritid symbionts are present in clades C, D, F, G, and

H, dominating the last three clades (F, G, and H). Phylogenetic relationships within the *Symbiodinium*-complex are relatively stable. In all analyses, the clade A branches at the base of the tree followed by clade E, and the radiation of clades B, F, H, and C is always present in the same order. There is a very weak NS for the monophyly of clade F and the relationships between the F subclades vary. In the ML and MrBayes trees, subclade F4 branches at the base, followed by subclade F5 and the sister subclades F2 and F3. In the MP tree, the subclade F5 branches at the base, followed by subclade F4 and the other two subclades. Moreover, the position of clades D and G is not resolved. Compared to the ML tree, both clades have swapped their positions in MP analysis and their relation is unresolved in the MrBayes tree.

Soritinae SSU rDNA Phylogeny

Phylogenetic analysis of 33 SSU rDNA sequences that almost cover the total length of the gene revealed four main clades of Soritinae, strongly supported in all analyses (Fig. 2). The first clade groups together 7 sequences of *A. hemprichii* and one sequence of *M. kudakajimaensis*. The second clade comprises two sequences of an unidentified soritid from Elat. The third clade contains 4 sequences of *M. vertebralis*; whereas, the forth clade includes 19 sequences of *Sorites* spp. The first and the second clade group together with very strong NS (100%). The other two clades also form a cluster but with weaker NS (73—96%). The relationships within the clades of *Amphisorus* and *Marginopora* are relatively well resolved, whereas the large radiation of *Sorites* spp. is weakly resolved.

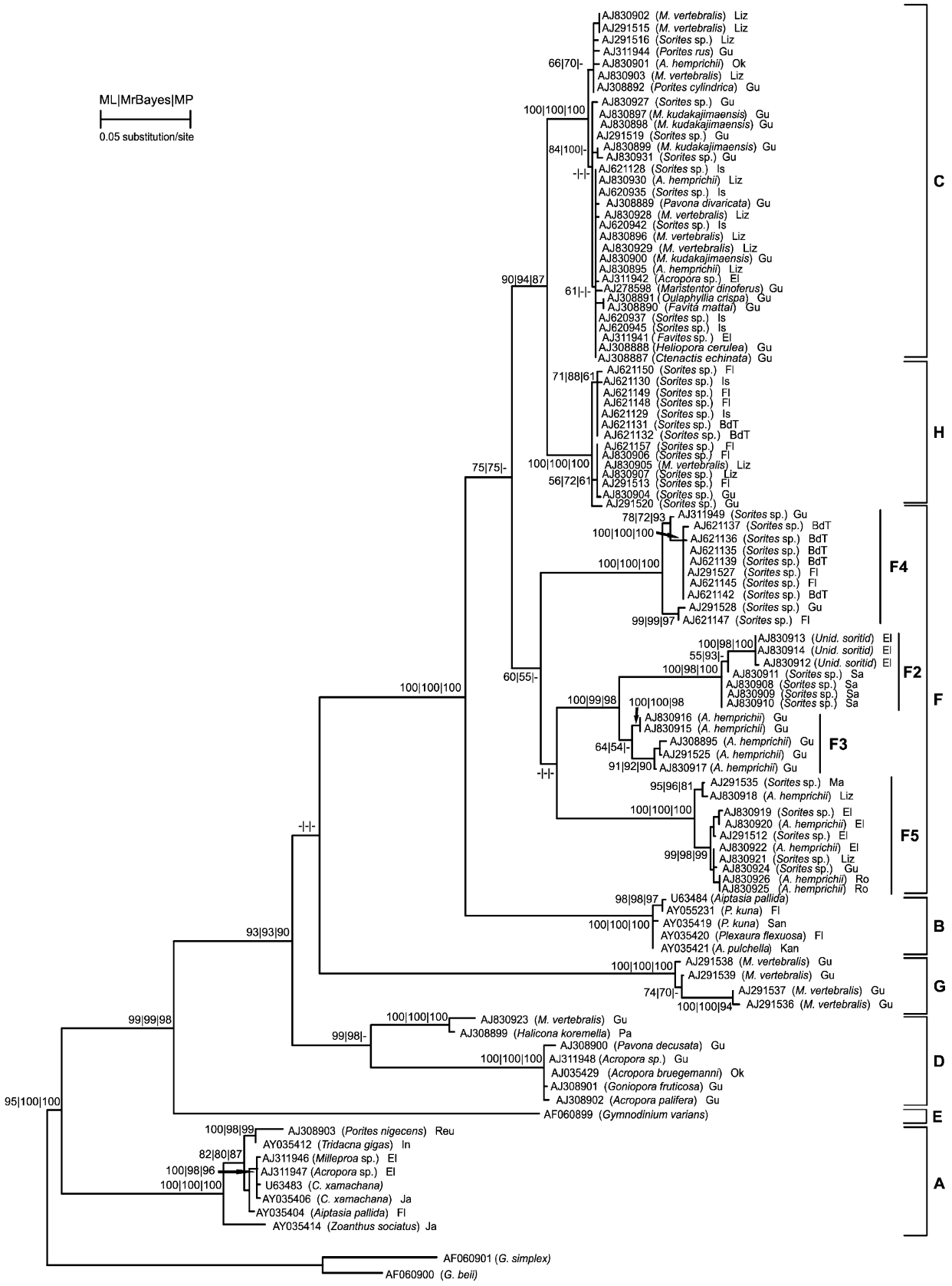
To increase the number of sequences for each clade, additional 126 partial (s6f-s14RF) SSU rDNA sequences were obtained, resulting in a total of 159 sequences of Soritinae (Fig. 3). Phylogenetic analysis of these sequences reveals a very similar general topology as the tree based on the almost total length of the SSU rDNA sequences. The relations among the four clades are also similar, though their NS are much weaker and the branching order within the clade of *Sorites* sp. is slightly different. Moreover, within each of theses clades, between 1 and 12 subclades or phylotypes are revealed (Fig. 3). Based on biogeographical observations, we applied a genetic distance greater than 0.3% to differentiate the phylotypes. We obtained 22 phylotypes of Soritinae (including between 1 and 27 sequences), 15 of which (68.2%) are associated with a single geographic locality. All phylotypes are listed in Table 1.

Discussion

Symbiodinium Phylogeny

The general topology of the *Symbiodinium* tree matches the results obtained in previous studies (reviewed in Baker 2003 and Pochon et al. 2005). The basal position of clade A, followed by clade E and the sister group relations between clades C and H are well established (Fig. 1). Yet, the branching order between other clades is more variable. For example, clade G appears in most studies as a sister group of lineage D (Pawlowski et al. 2001; Pochon et al. 2001, 2005); whereas, we found clade G to be an independent branch between clades D and B with inconsistent statistical support. Likewise, the position of the lineage B as a sister clade of F as previously reported (Pawlowski et al. 2001; Pochon et al. 2001) differs from our results where clade B appears as a sister group to clades F+H+C (100% NS), in agreement with Pochon et al. (2005).

Until now, the symbionts of Soritinae were found in four main clades (C, F, G, and H). Here, we report the first occurrence of the *Symbiodinium* "group" D1 in foraminifera. This "group" was represented to this point by a single sequence or "type" of the PSP1-05 cultured symbiont from a Palauan sponge (Carlos et al. 1999). These authors questioned, however, whether this *Symbiodinium* "type" is a symbiont of the sponge or a free-living strain that contaminated the culture media. Another recent study reported a *Symbiodinium* "type" D1 in the scleractinian coral *Pocillopora verrucosa* from eastern Pacific reefs (Iglesias-Prieto et al. 2004). In fact, ITS sequence comparisons of this reported "type" reveal that it is a *Symbiodinium* "type" belonging to subclade D2, sensu Pochon et al. (2001) (data not shown). New data show that the *Symbiodinium* "group" D1 is quite common in *M. vertebralis* from shallow waters in Guam (Pochon et al., unpubl. observations), suggesting that this *Symbiodinium* subclade may represent another foram-specific "group". Interestingly, *Symbiodinium* clade G or subclade D1 have so far been found in association with *M. vertebralis*, with the exception of one G "type" housed in a *Sorites* sp. (Fig. 1). *Symbiodinium* members from clade G are,


ML|MrBayes|MP
0.05 substitution/site
AJ830902 (M. vertebralis) Liz
AJ291515 (M. vertebralis) Liz
AJ291516 (Sorites sp.) Liz
AJ311944 (Porites rus) Gu
AJ830901 (A. hemprichii) Ok
AJ830903 (M. vertebralis) Liz
AJ308892 (Porites cylindrica) Gu
AJ830927 (Sorites sp.) Gu
AJ830897 (M. kudakajimaensis) Gu
AJ830898 (M. kudakajimaensis) Gu
AJ291519 (Sorites sp.) Gu
AJ830899 (M. kudakajimaensis) Gu
AJ830931 (Sorites sp.) Gu
AJ621128 (Sorites sp.) Is
AJ830930 (A. hemprichii) Liz
AJ620935 (Sorites sp.) Is
AJ308889 (Pavona divaricata) Gu
AJ830928 (M. vertebralis) Liz
AJ620942 (Sorites sp.) Is
AJ830896 (M. vertebralis) Liz
AJ830929 (M. vertebralis) Liz
AJ830900 (M. kudakajimaensis) Gu
AJ830895 (A. hemprichii) Liz
AJ311942 (Acropora sp.) El
AJ278598 (Maristentor dinoferus) Gu
AJ308891 (Oulaphyllia crispa) Gu
AJ308890 (Favia mattai) Gu
AJ620937 (Sorites sp.) Is
AJ620945 (Sorites sp.) Is
AJ311941 (Favites sp.) El
AJ308888 (Heliopora cerulea) Gu
AJ308887 (Ctenactis echinata) Gu
AJ621150 (Sorites sp.) Fl
AJ621130 (Sorites sp.) Is
AJ621149 (Sorites sp.) Fl
AJ621148 (Sorites sp.) Fl
AJ621129 (Sorites sp.) Is
AJ621131 (Sorites sp.) BdT
AJ621132 (Sorites sp.) BdT
AJ621157 (Sorites sp.) Fl
AJ830906 (Sorites sp.) Fl
AJ830905 (M. vertebralis) Liz
AJ830907 (Sorites sp.) Liz
AJ291513 (Sorites sp.) Fl
AJ830904 (Sorites sp.) Gu
AJ291520 (Sorites sp.) Gu
AJ311949 (Sorites sp.) Gu
AJ621137 (Sorites sp.) BdT
AJ621136 (Sorites sp.) BdT
AJ621135 (Sorites sp.) BdT
AJ621139 (Sorites sp.) BdT
AJ291527 (Sorites sp.) Fl
AJ621145 (Sorites sp.) Fl
AJ621142 (Sorites sp.) BdT
AJ291528 (Sorites sp.) Gu
AJ621147 (Sorites sp.) Fl
AJ830913 (Unid. soritid) El
AJ830914 (Unid. soritid) El
AJ830912 (Unid. soritid) El
AJ830911 (Sorites sp.) Sa
AJ830908 (Sorites sp.) Sa
AJ830909 (Sorites sp.) Sa
AJ830910 (Sorites sp.) Sa
AJ830916 (A. hemprichii) Gu
AJ830915 (A. hemprichii) Gu
AJ308895 (A. hemprichii) Gu
AJ291525 (A. hemprichii) Gu
AJ830917 (A. hemprichii) Gu
AJ291535 (Sorites sp.) Ma
AJ830918 (A. hemprichii) Liz
AJ830919 (Sorites sp.) El
AJ830920 (A. hemprichii) El
AJ291512 (Sorites sp.) El
AJ830922 (A. hemprichii) El
AJ830921 (Sorites sp.) Liz
AJ830924 (Sorites sp.) Gu
AJ830926 (A. hemprichii) Ro
AJ830925 (A. hemprichii) Ro
U63484 (Aiptasia pallida)
AY055231 (P. kuna) Fl
AY035419 (P. kuna) San
AY035420 (Plexaura flexuosa) Fl
AY035421 (A. pulchella) Kan
AJ291538 (M. vertebralis) Gu
AJ291539 (M. vertebralis) Gu
AJ291537 (M. vertebralis) Gu
AJ291536 (M. vertebralis) Gu
AJ830923 (M. vertebralis) Gu
AJ308899 (Halicona koremella) Pa
AJ308900 (Pavona decusata) Gu
AJ311948 (Acropora sp.) Gu
AJ035429 (Acropora bruegemanni) Ok
AJ308901 (Goniopora fruticosa) Gu
AJ308902 (Acropora palifera) Gu
AF060899 (Gymnodinium varians)
AJ308903 (Porites nigecens) Reu
AY035412 (Tridacna gigas) In
AJ311946 (Millepora sp.) El
AJ311947 (Acropora sp.) El
U63483 (C. xamachana)
AY035406 (C. xamachana) Ja
AY035404 (Aiptasia pallida) Fl
AY035414 (Zoanthus sociatus) Ja
AF060901 (G. simplex)
AF060900 (G. beii)
C
H
F4
F2
F3
F5
F
B
G
D
E
A

however, also able to establish symbiotic relationships with some sponges (Schoenberg and Loh 2005), octocorals (van Oppen et al. 2005b) and corals (van Oppen et al. 2005a), which raises questions concerning the degrees of host—symbiont specificity among these partnerships. *Symbiodinium* clades G and D are genetically closely related, and subclade D2 may have opportunistic properties that would make it ideal for environmental recruiting since it is commonly found in recovering reefs after bleaching (Baker 2003; Baker et al. 2004; Rowan 2004). Further studies are required to determine whether clade G and subclade D1 represent new opportunistic "groups" of symbionts or are more specific to foraminifera and some other invertebrate hosts.

Our study also reveals the presence of two distinct phylotypes in the subclade F2. To this day, the symbionts belonging to this subclade were represented by isolates from the Gulf of Elat (Pawlowski et al. 2001). Our data show that this subclade is also present in another locality of the Red Sea (Safaga); however, the phylotype identified in Safaga differs slightly from the one found in Elat. Compared with relatively large geographical distribution of other phylotypes, such high diversity of *Symbiodinium* in a relatively restricted area is quite unusual, but can be explained by a rapid differentiation of symbionts in the Gulf of Elat, which is a particularly enclosed environment (Karako-Lampert et al. 2004). The Gulf has different geophysical properties that make it a distinctive environment from the other locations in the Red Sea. First, it is separated from the Red Sea and the Indian Ocean by two shallow straits, Babu el Mandeb and Tiran. Second, the winter temperature (averaging 20 °C) is considered the limiting minimum for the development of coral reefs (Wood 1999). Finally, due to the extreme aridity in the area, the absence of fresh water input, and the high degree of evaporation, its salinity can rise to 40.5‰ (Reiss and Hottinger 1984). All those particular elements influence the development of a singular reef community and unusual symbiotic associations (Karako-Lampert et al. 2004).

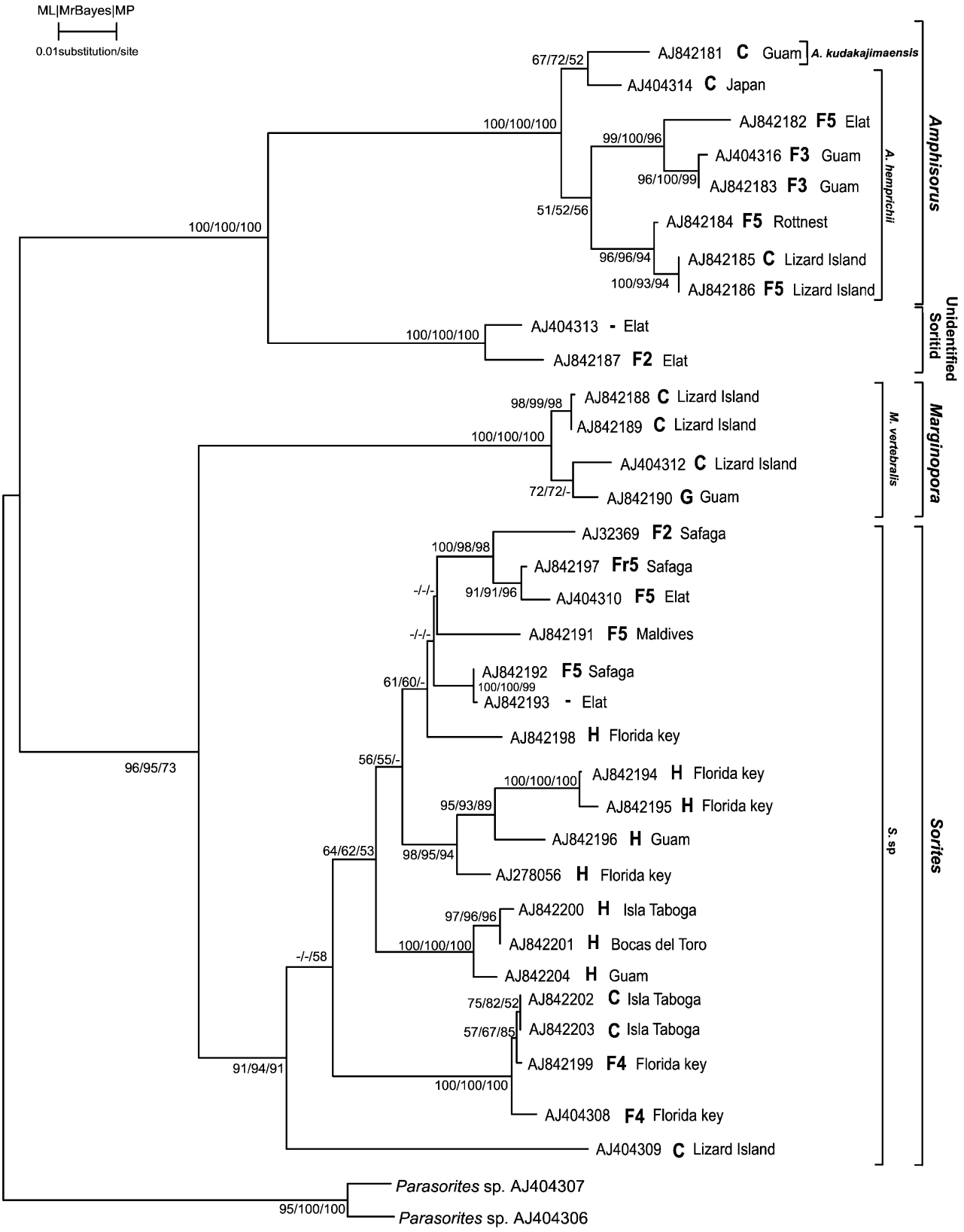
Soritinae Taxonomy

Phylogenetic analyses of our data confirmed the findings of the first molecular study of Soritinae, which was based on the same gene (SSU rDNA) but used shorter and less numerous sequences (Holzmann et al. 2001). The topology of our tree (Fig. 2) is almost identical to that published by Holzmann et al. (2001), but the support for all main clades increased considerably. In particular, there is strong support in our analyses for the clade of *Sorites* sp., which was weakly supported in the previous analysis. Both studies clearly indicate that there is an urgent need for a taxonomic revision of the Soritinae in at least three aspects discussed below.

First, the taxonomic status of *M. kudakajimaensis* Gudmundsson (1994) needs to be revised. This species was placed in the genus *Marginopora* based on the presence of a median skeleton similar to the one found in *M. vertebralis* (Gudmundsson 1994). However, the molecular analyses of all specimens morphologically identified as *M. kudakajimaensis*, including the paratypes of this species (Holzmann et al. 2001) indicate consistently that this species is closely related to *A. hemprichii*. The analysis of complete sequences places it as a sister group to *A. hemprichii* (Fig. 2). Both species share a similar elongated to irregular shape of peripheral apertures and slightly rounded chamber sutures (Gudmundsson 1994). Based on these morphological features and undisputable molecular phylogenetic data, we propose that *M. kudakajimaensis* should be moved from the genus *Marginopora* to *Amphisorus*.

Second, a new species of Soritinae present in the Gulf of Elat needs to be described. This species appeared in our analyses as a new clade of sequences branching as a sister group to

Figure 1. Phylogenetic reconstitution of *Symbiodinium* species complex inferred from partial large subunit rDNA based on the maximum likelihood method. The tree includes 103 sequences (73 soritid sequences and 23 marine invertebrate sequences). The *Symbiodinium* clades and subclades are designated by the letters A—H. For each sequence, the accession number is followed in parentheses by the name of the host and by an abbreviation indicating the geographical origin (Liz: Lizard Island, Gu: Guam, Is: Isla Taboga, El: Elat, Fl: Florida keys, BdT: Bocas del Toro, Sa: Safaga, Ma: Maldives, Ro: Rottnest, San: San Blas Islands, Kan: Kaneohe Bay, Pa: Palau, Ok: Okinawa, Reu: Reunion Islands, In: Indo-Pacific, Ja: Jamaica). The numbers at nodes correspond to bootstrap values in ML and MP analyses and posterior probabilities in MrBayes analyses. The values inferior to 50 are replaced by a dash (-).


ML|MrBayes|MP
0.01substitution/site
AJ842181 C Guam
A. kudakajimaensis
AJ404314 C Japan
AJ842182 F5 Elat
AJ404316 F3 Guam
AJ842183 F3 Guam
AJ842184 F5 Rottnest
AJ842185 C Lizard Island
AJ842186 F5 Lizard Island
A. hemprichii
Amphisorus
AJ404313 - Elat
AJ842187 F2 Elat
Unidentified Soritid
AJ842188 C Lizard Island
AJ842189 C Lizard Island
AJ404312 C Lizard Island
AJ842190 G Guam
M. vertebralis
Marginopora
AJ32369 F2 Safaga
AJ842197 Fr5 Safaga
AJ404310 F5 Elat
AJ842191 F5 Maldives
AJ842192 F5 Safaga
AJ842193 - Elat
AJ842198 H Florida key
AJ842194 H Florida key
AJ842195 H Florida key
AJ842196 H Guam
AJ278056 H Florida key
AJ842200 H Isla Taboga
AJ842201 H Bocas del Toro
AJ842204 H Guam
AJ842202 C Isla Taboga
AJ842203 C Isla Taboga
AJ842199 F4 Florida key
AJ404308 F4 Florida key
AJ404309 C Lizard Island
S. sp
Sorites
Parasorites sp. AJ404307
Parasorites sp. AJ404306
67/72/52
100/100/100
99/100/96
96/100/99
51/52/56
100/100/100
96/96/94
100/93/94
100/100/100
98/99/98
100/100/100
72/72/-
100/98/98
-/-/-
91/91/96
-/-/-
61/60/-
100/100/99
96/95/73
56/55/-
100/100/100
95/93/89
64/62/53
98/95/94
97/96/96
100/100/100
-/-/58
75/82/52
57/67/85
91/94/91
100/100/100
95/100/100

Amphisorus (Fig. 2). Two of these specimens, identified as *Sorites* sp. were already studied in Holzmann et al. (2001). The authors noticed that the specimens had a thinner test and more delicate appearance than other *Sorites*, with a diameter not exceeding 3 mm. The SEM analysis undertaken on one specimen showed that the apertures had elongated to irregular shapes and somewhat rounded chamber sutures, similar to *A. hemprichii* (Holzmann et al. 2001). Our analysis identified another four specimens of this species isolated in the same locality, suggesting the presence of a new endemic species of soritid in the Gulf of Elat.

Third, a profound morphological revision of the genus *Sorites* is needed. As far as we know, only two recent species of this genus have been formally described (*S. orbiculus* and *S. marginalis*). This contrasts with a great number of highly divergent phylotypes revealed by our study. By using a sequence divergence greater than 0.3%, 12 distinct phylotypes of *Sorites* can be identified (Fig. 3). The morphological features used to distinguish the two *Sorites* species do not seem reliable regarding their phylogenetic position (Holzmann et al. 2001). More detailed morphometric analysis is necessary, in order to describe the molecular phylotypes of this genus.

Host—symbiont Specificity

Our data confirm the specificity of relationships between *Symbiodinium* and Soritinae observed in previous studies. At the level of *Symbiodinium* clades, this specificity may not be as strict as suggested by our first analyses (Pawlowski et al. 2001; Pochon et al. 2001), which is in agreement with the idea that the "clade-level" taxonomy provides limited information from an ecological standpoint (LaJeunesse 2005). Since then, single occurrences of foraminifer-specific *Symbiodinium* "groups" were reported in other invertebrates (LaJeunesse 2001; Rodriguez-Lanetty et al. 2003; Schoenberg and Loh 2005; van Oppen et al. 2005a, b). However, these reports remain exceptional. Our study, by identifying the symbiont of subclade D1 in one of the *M. vertebralis* isolates, widens the soritid's symbionts repertory. So far, no soritids have been found to host the symbionts of clades A or B, and conversely, none of the invertebrate hosts were found to bear the soritid-specific symbionts of clade H and subclades F3, F4, and F5.

In addition, our analyses provide clear evidence that the host—symbiont specificity does not concern only the Soritinae as a group but it can also be observed at lower taxonomic levels. The phylogenetic analyses of 159 Soritinae revealed the existence of at least 22 molecular phylotypes, which are found in relationship with 8 *Symbiodinium* "groups" corresponding to 3 clades and 5 subclades. Among the 22 phylotypes of Soritinae recognized, 14 phylotypes possess a single "group" of symbionts, 7 phylotypes have been found with 2 "groups" of symbionts, and only one phylotype was found to bear three different "groups" of *Symbiodinium* (Table 1). While it is true that the sample sizes of the different Soritinae phylotypes might have an effect on the observed specificity (Fig. 3), the majority of phylotypes show a strict specificity for a unique *Symbiodinium* "group" in a single locality. In phylotypes formed by few sequences, e.g. AmpII or SorIV, the actual diversity might not have been revealed. Yet some other phylotypes, for instance MarII or SorXII, show some specificity. Despite their numerous sequences (14 and 27 sequences, respectively), they only harbor two different "groups". Nevertheless, the phylotype MarI (13 sequences) was found in relationship with as many as three different *Symbiodinium* "groups". Our results are consistent with the general finding that hosts are more specific than symbionts (Baker 2003). The majority of soritid phylotypes appears to be very selective in the choice of their symbiont "group", while the symbionts are often present in more than one soritid phylotype. For instance, of the three *Sorites* sp. phylotypes found in the Red Sea, one of them houses only the F2 "group", while the two others bear the F5 "group". This is a tangible example of host specificity since other "groups" of *Symbiodinium* (clade C in this case) are found in neighboring corals (data not shown).

Figure 2. Phylogenetic reconstitution of Soritinae inferred from almost total small subunit rDNA based on the maximum likelihood method. The tree includes 33 sequences. For each sequence, the accession number is followed by the clade or subclade of symbiont harbored by the specimen and by its geographical origin. The numbers at nodes correspond to bootstrap values in ML and MP analyses and posterior probabilities in MrBayes analyses. The values inferior to 50 are replaced by a dash (-).

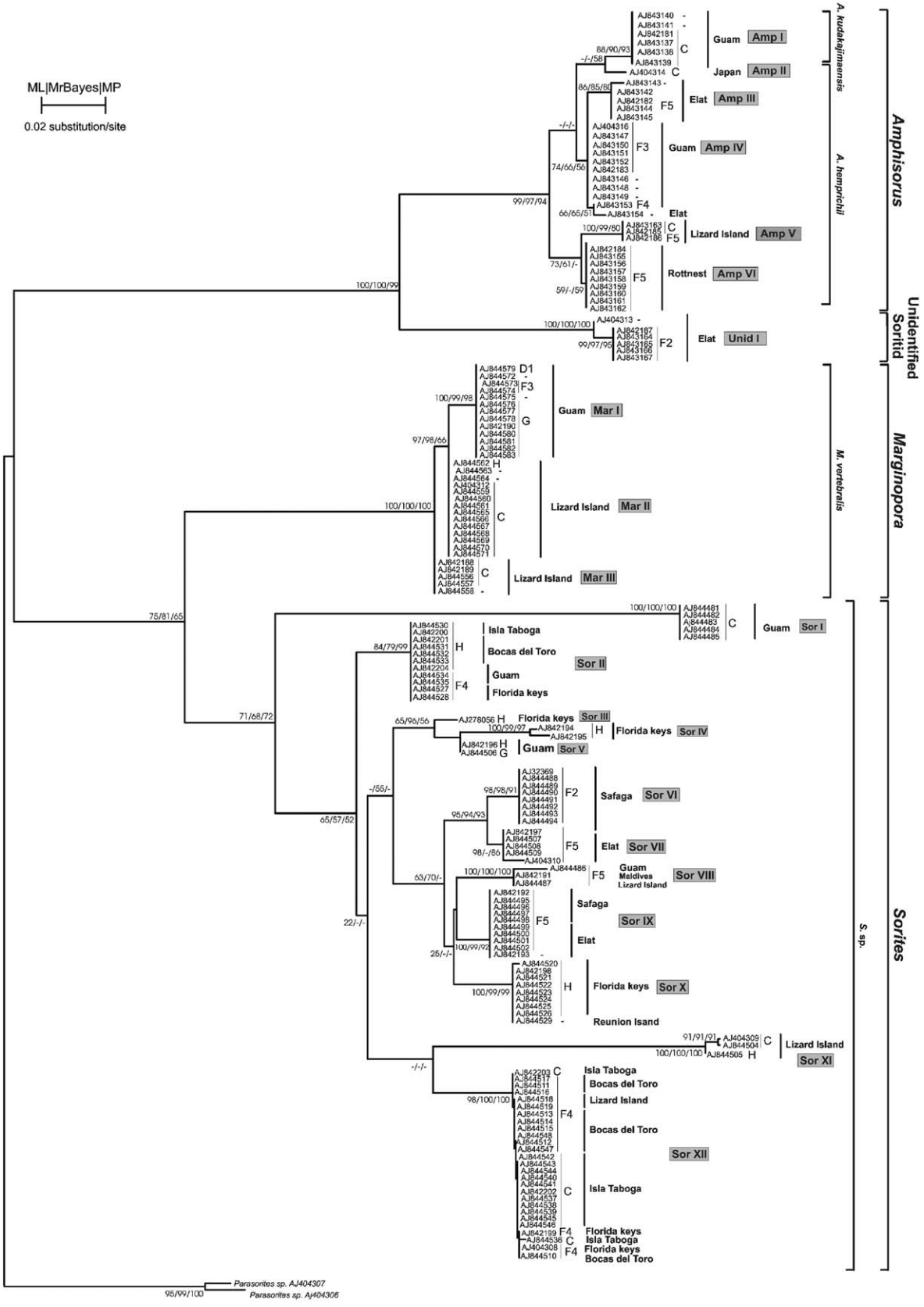

ML|MrBayes|MP
0.02 substitution/site
A. kudakajimaensis
A. hemprichii
Amphisorus
Unidentified Soritid
Marginopora
M. vertebralis
Sorites
S. sp.
Guam Amp I
Japan Amp II
Elat Amp III
Guam Amp IV
Elat
Lizard Island Amp V
Rottnest Amp VI
Elat Unid I
Guam Mar I
Lizard Island Mar II
Lizard Island Mar III
Guam Sor I
Isla Taboga
Bocas del Toro
Guam
Florida keys
Sor II
Florida keys Sor III
Florida keys Sor IV
Guam Sor V
Safaga Sor VI
Elat Sor VII
Guam
Maldives
Lizard Island Sor VIII
Safaga
Sor IX
Elat
Florida keys Sor X
Reunion Isand
Lizard Island
Sor XI
Isla Taboga
Bocas del Toro
Lizard Island
Bocas del Toro
Sor XII
Isla Taboga
Florida keys
Isla Taboga
Florida keys
Bocas del Toro
Parasorites sp. AJ404307
Parasorites sp. Aj404306

Table 1. List of soritid phylotypes and the corresponding symbionts.

Soritid phylotype			Sequence number	Locality	*Symbiodinium* clades/ subclades
1	Amp I	*M. kudakajimaensis*	6	Guam	C
2	Amp II	*A. hemprichii*	1	Okinawa	C
3	Amp III	*A. hemprichii*	5	Elat	F5
4	Amp IV	*A. hemprichii*	10	Guam, Elat	F3/F4
5	Amp V	*A. hemprichii*	3	Lizard Island	C/F5
6	Amp VI	*A. hemprichii*	9	Rottnest	F5
7	Unid I	*Unidentified soritid*	6	Elat	F2
8	Mar I	*M. vertebralis*	13	Guam	F3/D/G
9	Mar II	*M. vertebralis*	14	Lizard Island	C/H
10	Mar III	*M. vertebralis*	5	Lizard Island (30 m)	C
11	Sor I	*Sorites* sp.	5	Guam	C
12	Sor II	*Sorites* sp.	11	Panama, Florida Keys, Guam	F4/H
13	Sor III	*Sorites* sp.	1	Florida Keys	H
14	Sor IV	*Sorites* sp.	2	Florida Keys	H
15	Sor V	*Sorites* sp.	2	Guam	G/H
16	Sor VI	*Sorites* sp.	8	Safaga	F2
17	Sor VII	*Sorites* sp.	5	Safaga, Elat	F5
18	Sor VIII	*Sorites* sp.	3	Guam, Maldives , Lizard Island	F5
19	Sor IX	*Sorites* sp.	10	Safaga, Elat	F5
20	Sor X	*Sorites* sp.	9	Florida Keys, Reunion	H
21	Sor XI	*Sorites* sp.	3	Lizard Island	C/H
22	Sor XII	*Sorites* sp.	27	Panama, Florida Keys, Lizard Island	C/F4

Moreover, a narrow biogeographical partitioning of the host was observed. Out of the 22 soritid phylotypes, 15 are clearly related to a single locality. Except for AmpIV (Fig. 3), all *Amphisorus* phylotypes and all *Marginopora* are present only in one area, while only half of the *Sorites* phylotypes are restricted to a specific region.

Interestingly, five out of eight soritid phylotypes with more than one "group" of symbionts were found in Guam or on Lizard Island, while the three remaining phylotypes have at least a representative in one of these two localities. This is in agreement with the fact that almost all "groups" of symbionts, except F2, have been found in soritids from this region of the Indo-Pacific. The evolutionary history of the symbionts may have been shaped by biogeographical constraints, with a high diversity of "groups" throughout the Pacific and few "groups" in more extreme environments, such as the Caribbean, the Red Sea, and the Southern Indian Ocean.

To explain such specificity between *Symbiodinium* and Soritinae, two different hypotheses can be proposed. The first one is based on the

Figure 3. Phylogenetic reconstitution of Soritinae inferred from partial small subunit rDNA based on the maximum likelihood method. The tree includes 159 soritid sequences. For each sequence, the accession number is followed by the clade or subclade of symbiont harbored by the specimen and by the geographical origin of the specimen. The numbers at nodes correspond to bootstrap values in ML and MP analyses and posterior probabilities in MrBayes analyses. The values inferior to 50 are replaced by a dash (-). A gray box encircling the name of the phylotypes are positioned in front of the group of corresponding sequences. Each name of phylotype corresponds to the first letters of the genus it belongs to followed by a roman numeral attributed to it.

mechanism of host—symbiont recognition as a selective factor. To settle as endosymbionts, the dinoflagellates must avoid initial digestion by their foraminiferal host (Lee and Anderson 1991). Only the *Symbiodinium* cells recognized by a soritid phylotype would be allowed to cross the digestion barrier and enter into the foraminiferal cytoplasm. However, if this hypothesis is correct, we would expect that the phylogenetically related soritid are always adapted to the same symbionts, which is not always the case.

The second hypothesis is based on predominantly vertical acquisition of symbionts in Soritinae. Like most of the foraminifera, soritids have a complex life cycle with sexual and asexual generations. In some species, the alternation of generations is regular; while in others, the life cycle is dominated by an asexual phase. Because the symbionts do not need to leave the mother cells when reproducing by multiple fission, this can easily create a local specificity in asexually reproducing species. The comparison of population dynamics in soritids with single and multiple "types" of symbionts can provide an interesting test of this hypothesis (Pochon et al., unpubl. observations).

It is possible that the strong host—symbiont specificity observed in Soritinae is a combined effect of both a selective recognition mechanism and vertical transmission. Additionally, the environmental impact most probably plays a significant role in the specificity and diversity of the forams-*Symbiodinium* pairings. A geographic pattern in distribution of some symbionts, such as the absence of the *Symbiodinium* clade C in the Caribbean population of *Sorites* (phylotype 22) and its presence in the population of the same phylotype on the other side of the Isthmus of Panama (Pochon et al. 2004), suggests that these differential symbiotic associations have evolved in response to the different environments each region has experienced over the last 3—4 million years.

Finally, when comparing the *Symbiodinium* and the Soritinae trees, it is clear that there is no coevolution sensu stricto. Yet, coevolution should not be completely discarded. If we consider the geographic mosaic theory of coevolution (Thompson 1994; Thompson and Cunningham 2002), interspecific interactions at the population level are influenced by the ecosystem in which the interaction occurs. Factors, such as the natural environment, the genetic and demographic structure of populations, and the community context, may shape the specificity of the foram—symbiont interactions. Therefore, coevolution might be present in restricted areas where strong reciprocal selection creates coevolutionary hotspots. More detailed study of the impact of environmental factors on the distribution of the symbionts and their hosts will provide better insight into the specificity of their relationships.

Direction for Future Research within Foraminifera—*Symbiodinium* Symbiosis

In summary, our study confirms the specificity of relationship between soritid foraminifera and *Symbiodinium* dinoflagellates. By analyzing 159 SSU sequences obtained from soritid individuals collected worldwide, we not only broaden our knowledge of the Soritinae taxonomy and diversity, but we also provide the first comparison of patterns between foram—algal symbiosis and the host phylogeny. Furthermore, we show that the host—symbiont specificity does not concern only the Soritinae as a group but that it can also be observed at a lower taxonomic level. We conclude that this high specificity is probably driven by selective recognition mechanisms, vertical transmission of symbionts, and biogeographical isolation. Yet, neither strict coevolution nor clear biogeographic patterns have been revealed. This might be due to the phylogenetic analysis of Soritinae being is based on fairly conserved SSU rDNA sequences. Even if informative at the genus level, this gene appears as inappropriate for resolution at the species level, especially for the genus *Sorites*. It would be of great interest to apply a similar host—symbiont investigation using more variable molecules, such as the ITS rDNA regions. Such an approach may be useful for comparing closely related holosymbiont taxa as well as for examining finer scale patterns within and among host species across biogeographic regions.

Finally, several studies have shown that a given host can harbor more that one *Symbiodinium* genotype simultaneously (Baillie et al. 2000; Rowan 1998; Santos et al. 2004; van Oppen et al. 2005b). In the present study, the observation of multiple *Symbiodinium* genotypes was scarce (data not shown), suggesting the presence of a unique *Symbiodinium* strain per foraminifer. Yet, determining which molecule(s) best differentiates the species level within the genus *Symbiodinium* remains unresolved. It is currently considered that the ITS resolution either approximates the species

level or gathers closely related species (Coffroth and Santos 2005; LaJeunesse 2001, 2005). The exploration of ITS2 resolution of foraminiferal symbionts will hopefully determine whether a given host cell preferentially harbors a single or multiple *Symbiodinium* genotypes simultaneously.

Methods

Sampling, DNA extraction, PCR amplification, Sequencing and RFLP: The soritids analyzed in this study were collected worldwide between 1999 and 2003. The collection includes specimens from the Red Sea (Safaga and Elat), the Southern Indian Ocean (Reunion Island and Rottnest), the Western Indian Ocean (Maldives), the Eastern Pacific Ocean (Guam, Lizard Island-Great Barrier Reef), the Western Pacific Ocean (Panama), and the Caribbean Sea (Panama and Florida).

After morphological identification, the specimens were cleaned with small paintbrushes in several changes of 0.2 μm filtered sea water. Once cleaned, their tests were broken into fragments. One of them was transferred to a guanidine DNA extraction buffer and the others were preserved on micropalaeontological slides for further morphological investigation. The extractions were processed as described in Pochon et al. (2001). PCR amplifications were performed in a total volume of 50 μl with an amplification profile consisting of 40 cycles of 30 s at 94 °C, 45 s at 52 °C, and 120 s at 72 °C, followed by 5 min at 72 °C for final extension. The PCR products were purified using High Pure PCR Purification Kit (Roche), and then sequenced directly with an ABI PRISM Big Dye Terminator Cycle Sequencing Kit using either an ABI 377 or ABI 3100 DNA sequencer (Perkin-Elmer), according to the manufacturer's instructions.

PCR amplifications of the foraminifer SSU rDNA were performed with a set of three pairs of primers: sA10/LyS1, s6F/s14RF, and s14F3/s17. The primer sequences are given in Holzmann et al. (2001), except for the primer LyS1 (5′-CTCCAACTATCTCCATCGA-3′). By overlapping the three fragments, a total SSU sequence was obtained for 24 specimens. PCR amplifications of the dinoflagellate rDNA were performed as described in Pawlowski et al. (2001).

The PCR-based Restriction Fragment Length Polymorphism (RFLP) method was applied to PCR products amplified with ITS-DINO and L-0 using HindIII (Roche diagnostics) (specific site: AAGCTT) as the restriction enzyme. The digestion mix contained 8 μl distilled water, 2 μl B incubation buffer and 0.5 HindIII restriction enzyme and 10 μl PCR product. Samples were incubated at 37 °C for a minimum of 4 h.

Phylogenetic Analyses: Three data sets of sequences were analysed. All three sets were first aligned using Clustal X (Thompson et al. 1997) and then improved manually using BioEdit 5.0.9 sequence alignment software (Hall 1999). The first set comprised 103 sequences of *Symbiodinium* LSU rDNA, including 73 sequences from soritids and 30 sequences from marine invertebrates. *Gymnodinium simplex* and *Gymnodinium beii* were used as outgroup following Wilcox (1998). The second set contained almost complete (A10-s17) SSU rDNA sequences for 33 soritids. The third set was composed of 159 partial (s6F-s14RF) SSU rDNA sequences of Soritinae. Two sequences of *Parasorites* were chosen as outgroup in the two latter data sets.

Modeltest, implemented in the PAUP* 4.0.b10 software (Swofford 2002), identified the general time reversible (GTR) model (Lanave et al. 1984) as the best model for our analyses, taking into account a proportion of invariant sites (I) and a gamma distribution shape parameter (γ). Using these settings, a tree was reconstructed with the PhyML software (Guidon and Gascuel 2003) using the maximum likelihood (ML) method (Felsenstein 1981). The reliability of internal branches was assessed using the non-parametric bootstrap method with 1000 replicates. Then, a Bayesian method was used to infer phylogeny, using the program MrBayes (Huelsenbeck and Ronquist 2001). One out of every ten trees was sampled for a million generations with kappa and DNA substitution parameters estimated during the research. After excluding the first sampled trees categorized as the "burn-in period" (6,000, 3,000, and 12,000 excluded trees in Figs 1—3, respectively), a consensus tree was constructed using PAUP* (Swofford 2002). Finally, each data set was analyzed with the maximum-parsimony (MP) method (Farris 1970) as implemented in PAUP*. The MP trees were obtained via stepwise addition and then swapped using the tree-bisection-reconnection (TBR) algorithm. Characters were equally weighted and aligned gaps were considered as a fifth base. The reliability of the internal branches was estimated by using the bootstrap method with 1000 replicates (Felsenstein 1985).

Accession numbers of the new sequences are from AJ843137 to AJ843167, AJ844556—AJ844583, AJ844481—AJ844548, and from AJ842181 to AJ842204.

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References

Baillie BK, Belda-Baillie CA, Maruyama T (2000) Conspecificity and Indo-Pacific distribution of *Symbiodinium* genotypes (Dinophyceae) from giant clams. J Phycol **36**: 1153—1161

Baker AC (2003) Flexibility and specificity in coral—algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. Annu Rev Ecol Evol Syst **34**: 661—689

Baker AC, Starger CJ, McClanahan TM, Glynn PW (2004) Corals' adaptative response to climate change. Nature **430**: 741

Carlos AA, Baillie BK, Kawachi M, Maruyama T (1999) Phylogenetic position of *Symbiodinium* (Dinophyceae) isolates from tridacnids (Bivalvia), cardiids (Bivalvia), a sponge (Porifera), a soft coral (Anthozoa), and a free-living strain. J Phycol **35**: 1054—1062

Coffroth MA, Santos SR (2005) Genetic diversity of symbiotic dinoflagellates in the genus *Symbiodinium*. Protist **156**: 19—34

Farris JS (1970) Methods in computing Wagner trees. Syst Zool **19**: 83—92

Felsenstein J (1981) Evolutionary trees from DNA sequences: maximum likelihood approach. J Mol Evol **17**: 368—376

Felsenstein J (1985) Confidence limits on phylogenetics: an approach using the bootstrap. Evolution **39**: 783—791

Fujita K, Nishi H, Saito T (2000) Population dynamics of *Marginopora kudakajimaensis* Gudmundsson (Foraminifera: Soritidae) in the Ryukyu Islands, the subtropical northwest Pacific. Mar Micropaleontol **38**: 267—284

Gudmundsson G (1994) Phylogeny, ontogeny and systematics of Recent Soritacea Eherenberg 1839 (Foraminiferida). Micropaleontology **40**: 101—155

Guidon S, Gascuel O (2003) A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol **52**: 696—704

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser **41**: 95—98

Hallock P (1985) Why are larger foraminifera large? Paleobiology **11**: 195—208

Hallock P (1987) Fluctuation of the Tropic Resource Continuum: a factor of Global Diversity Cycles. Paleoceanography **2**: 457—471

Hallock P (1999) Symbiont-Bearing Foraminifera. In Sen Gupta BK (ed) Modern Foraminifera. Kluwer, Dordrecht, The Netherlands, pp 123—139

Holzmann M, Hohenegger J, Hallock P, Piller WE, Pawlowski J (2001) Molecular phylogeny of larger foraminiera. Mar Micropaleontol **43**: 57—74

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics **17**: 754—755

Iglesias-Prieto R, Beltrán VH, LaJeuness TC, Reyes-Bonilla H, Thomé PE (2004) Different algal symbionts explain the vertical distribution of dominant reef corals of the eastern Pacific. Proc R Soc London B **271**: 1757—1763

Karako-Lampert S, Katcoff DJ, Achituv Y, Dubinsky Z, Stambler N (2004) Do clades of symbiotic dinoflagellates in scleractinian corals of the Gulf of Eilat (Red Sea) differ from those of other coral reefs? J Exp Mar Biol Ecol **311**: 301—314

LaJeunesse TC (2001) Investigating the biodiversity, ecology and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS

region: in search of a "species" level marker. J Phycol **37**: 866—880

LaJeunesse TC (2005) "Species" radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene—Pliocene transition. Mol Biol Evol **22**: 570—581

Lanave C, Preparata G, Saccone C, Serio G (1984) A new method for calculating evolutionary substitution rates. J Mol Evol **20**: 86—93

Langer MR, Lipps JH (1995) Phylogenetic incongruence between dinoflagellate endosymbionts (*Symbiodinium*) and their host foraminifera (*Sorites*): small subunit ribosomal RNA gene sequence evidence. Mar Micropaleontol **26**: 179—186

Lee JJ, Anderson R (1991) Symbiosis in Foraminifera. In Lee JJ, Anderson R (eds) Biology of Foraminifera. Academic Press, London, pp 157—222

Lee JJ, Lawrence C (1990) Endosymbiotic Dinoflagellates from the Larger Foraminifera *Amphisorus hemprichii* and *Sorites marginalis*. In Nardon P, Grenier AM, Margulis L, Smith DC (eds) Endocytobiology IV. Inst Nat Rech Agronom, Paris, pp 221—223

Lee JJ, Burnham B, Cevasco ME (2004) A new modern soritid foraminifer, *Amphisorus saurensis* n. sp., from the Lizard Island Group (Great Barrier Reef, Australia). Micropaleontology **50**: 357—368

Lee JJ, Wray CG, Lawrence C (1995) Could foraminiferal zooxanthellae be derived from environmental pools contributed to by different coelenterate hosts? Acta Protozool **34**: 75—85

Lehmann R (1961) Strukturanalyse einiger Gattungen der Subfamilie Orbitolitinae. Ecolgae Geologicae Helvetiae **54**: 597—667

Leutenegger S (1977) Ultrastructure and motility of dinophyceans symbiotic with larger, imperforated foraminifera. Mar Biol **44**: 157—164

Leutenegger S (1984) Symbiosis in benthic foraminifera: specificity and host adaptations. J Foraminiferal Res **14**: 16—35

Lobban CS, Schefter M, Simpson AGB, Pochon X, Pawlowski J, Foissner W (2002) *Maristentor dinoferus* n.gen. n.sp., a giant heterotrich ciliate (Spirotrichea: Heterotrichida) with zooxanthellae, from coral reefs on Guam, Mariana Islands. Mar Biol **140**: 411—423

Pawlowski J, Holzmann M, Fahrni J, Pochon X, Lee JJ (2001) Molecular identification of algal endosymbionts in large miliolid foraminifera: 2. Dinoflagellates. J Eukaryot Microbiol **48**: 368—373

Pochon X, LaJeunesse TC, Pawlowski J (2004) Biogeographic partitioning and host specialization among foraminiferan dinoflagellate symbionts (*Symbiodinium*; Dinophyta). Mar Biol **146**: 17—27

Pochon X, Montoya JI, Stadelmann B, Pawlowski J (2005) Molecular phylogeny, evolutionary rates and divergence timing of the symbiotic dinoflagellate genus *Symbiodinium*. Mol Phylogenet Evol, in press

Pochon X, Pawlowski J, Zaninetti L, Rowan R (2001) High genetic diversity and relative specificity among *Symbiodinium*-like endosymbiotic dinoflagellates in soritid foraminiferans. Mar Biol **139**: 1069—1078

Richardson SL (2001) Endosymbiont change as a key innovation in the adaptive radiation of Soritida (Foraminifera). Paleobiology **27**: 262—289

Reiss ZS, Hottinger L (eds) (1984) The Gulf of Aqaba-Ecological Micropaleontology. Springer-Verlag, Berlin

Rodriguez-Lanetty M, Cha HR, Song JI (2002) Genetic diversity of symbiotic dinoflagellates associated with anthozoans from Korean waters. Proc 9th Int Coral Reef Symp, vol. 1, pp 163—166

Rodriguez-Lanetty M, Chang SJ, Song JI (2003) Specificity of two temperate dinoflagellate—anthozoan associations from the north-western Pacific Ocean. Mar Biol **143**: 1193—1199

Rowan R (1998) Diversity and ecology of zooxanthellae on coral reefs. J Phycol **34**: 407—417

Rowan R (2004) Thermal adaptation in reef coral symbionts. Nature **430**: 742

Santos SR, Shearer TL, Hannes AR, Coffroth MA (2004) Fine-scale diversity and specificity in the most prevalent lineage of symbiotic dinoflagellates (*Symbiodinium*, Dinophyceae) of the Caribbean. Mol Ecol **13**: 459—469

Schoenberg CHL, Loh W (2005) Molecular identity of the unique symbiotic dinoflagellates found in the bioeroding demosponge *Cliona orientalis*. Mar Ecol Prog Ser **299**: 157—166

Swofford DL (2002) PAUP* 4.0: Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence

alignment aided by quality analysis tools. Nucleic Acids Res **24**: 4876—4882

Thompson JN (1994) The Geographic Mosaic Theory of Coevolutionary Process. In Thompson JN (ed) The Coevolutionary Process. The University of Chicago Press, Chicago, pp 219—238

Thompson JN, Cunningham BM (2002) Geographic structure and dynamics of coevolutionary selection. Nature **417**: 735—738

van Oppen MJH, Mahiny AJ, Done TJ (2005a) Geographic distribution of zooxanthella groups in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. Coral Reefs, in press

van Oppen MJH, Mioeg JC, Sanchez CA, Fabricius KE (2005b) Diversity of algal endosymbionts (zooxanthellae) in octocorals: the role of geography and the host relationships. Mol Ecol **14**: 2403—2417

Wilcox TP (1998) Large subunit ribosomal RNA systematics of symbiotic dinoflagellates: morphology does not recapitulate phylogeny. Mol Phylogenet Evol **10**: 436—448

Wood R (1999) Reef Evolution, 1st Ed. Oxford University Press, New York