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Review article

Evolution of the soritids-*Symbiodinium* symbiosis

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Abstract

Coral reef ecosystems worldwide gather a myriad of invertebrates, including sponges, jellyfishes, anemones, corals, and mollusks that are hosts to a diverse group of dinoflagellates of the genus *Symbiodinium*. Among protists, *Symbiodinium* endosymbionts have been reported in ciliates and large soritid foraminifera. Recent molecular phylogenetic studies on the symbionts of soritids have revealed an extraordinary diversity of *Symbiodinium* lineages, most of which are specifically associated with this relatively small group of foraminifera. Additional ecological and evolutionary studies have shown that (1) the specificity between soritids and *Symbiodinium* is greater than previously thought and can also be found at a lower taxonomic level within Soritinae, (2) the diversity of soritid-specific *Symbiodinium* spp. is much greater in the Indo-Pacific than in Western Atlantic, hence correlates positively with the distribution of soritid diversity, (3) soritid symbionts did not present faster evolutionary rates compared to the Metazoan symbionts, suggesting that other factors such as the predominantly vertical transmission of symbionts and/or biogeographic isolation may be responsible for the host-symbiont specificity and diversity observed in Soritinae. Additionally, a relaxed molecular clock method was applied on 105 *Symbiodinium* rDNA sequences to estimate when the various clades diversified from each other. Our results suggested that the genus originated in early Eocene, and that the majority of extant lineages diversified since mid-Miocene, about 15 million years ago. Here we review the history of our research on soritid-*Symbiodinium* relationship and discuss its future perspectives.

Keywords: Molecular phylogeny, rDNA, foraminifera, Soritacea, *Symbiodinium*, "zooxanthellae"

1. Introduction

Symbiosis involving the dinoflagellates in the genus *Symbiodinium* and numerous marine organisms including representatives of the Protista, Porifera, Cnidaria and Mollusca, is undoubtedly the most important ecological association of present coral reef ecosystems. Although observed in marine environment since the 19th Century (Brandt, 1881; Klebs, 1884; Chatton, 1923; Hovasse, 1924; Kawaguti, 1944; McLaughlin and Zahl, 1959), the genus *Symbiodinium* has only been formally described in the 1960s (Freudenthal, 1962). Until the 1970s, all symbiotic dinoflagellates were classified as members of a single pandemic species, *Symbiodinium microadriaticum* Freudenthal, adapted to life in a symbiotic state (Taylor, 1971, 1974).

However, beginning in the mid-1970s, evidence drawn independently from behavioral, morphological, biochemical, and physiological approaches (e.g. Kinzie, 1974; Leutenegger, 1977; Schoenberg and Trench, 1980a,b,c; Chang and Trench, 1982; Iglesias-Prieto et al., 1992) began to accumulate and revealed that these dinoflagellates were in fact characterized by a high degree of taxonomic diversity. The molecular revolution that followed confirmed the existence of a tremendous level of diversity within the genus *Symbiodinium* and provided new tools for the investigation of its taxonomy, ecology and specificity (reviewed by Baker, 2003; Coffroth and Santos, 2005).

Although the possibility of algal symbiosis in some foraminifera has been widely accepted since the 1920s (Cushman, 1922; Myers, 1943; Jepps, 1956), it is only since the mid-1960s that a real explosion of interest in foram-algal symbiosis took place, especially in larger foraminifera (Hedley, 1964; Lee et al., 1979; Lee and McEnery, 1983;

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Leutenegger, 1977, 1984; Lee and Hallock, 1987; ter Kuile et al., 1987). The outcome showed that today's larger foraminifera are host for an amazing diversity of algal groups, including dinoflagellates, chlorophytes, rhodophytes, chrysophytes and diatoms (see Lee and Anderson, 1991), suggesting that symbiosis has been a key element in the ecological success of foraminifera throughout geological times.

Dinoflagellates endosymbionts are only found in a relatively small group of planktonic and benthic foraminifera. In planktonic species such as *Globigerinoides rubber*, *G. conglobatus*, *G. sacculifer* and *Orbulina universa*, the symbiotic dinoflagellates have been morphologically and genetically identified as *Gymnodinium beii* (Spero, 1987; Gast and Caron, 1996). Recent molecular data, however, showed that these symbionts form a monophyletic group closely related to the *Symbiodinium* species complex, which deserves the creation of a new dinoflagellate genus (Shaked and de Vargas, 2006). In larger benthic foraminifera, only representatives of the sub-family Soritinae (Miliolida, Soritidae) are host to dinoflagellates of the genus *Symbiodinium*. Soritinae are miliolid foraminifera common in tropical and subtropical shallow waters and characterized by large imperforate, discoid test with annular chambers, reaching up to 15 mm in diameter. The presence of *Symbiodinium* dinoflagellates in soritid forams were first observed morphologically (Ross, 1972; Müller-Merz and Lee, 1976; Leutenegger, 1977; McEnery and Lee, 1981; Lee and Lawrence, 1990), and later confirmed in SSU rDNA-based molecular studies (Langer and Lipps, 1995; Lee et al., 1995). Below, we review the molecular data gathered since 2001, on phylogeny, biogeography, and diversity, of soritid-*Symbiodinium* symbiotic relationship.

2. *Symbiodinium* Phylogeny and Classification

Over the last two decades, the analysis of various molecules has been used to elucidate the degree of genetic diversity within *Symbiodinium* (reviewed in Coffroth and Santos, 2005). For a long time, the use of SSU and LSU nuclear ribosomal (nrDNA) genes has dominated such investigations, leading to a molecular classification constituted of a number of lineages or clades that can be considered as subgenera of the genus *Symbiodinium*. Prior to the analysis of soritid symbionts, the phylogenetic classification of the genus *Symbiodinium* contained five lineages called clades A, B, C, D, and E, which were identified by analyses of cnidarian and molluscan hosts (Rowan and Powers 1991; Carlos et al., 1999; LaJeunesse and Trench, 2000). In comparison to the metazoan hosts, a detailed phylogenetic investigation of the soritid symbionts revealed that they were exceptionally diverse. Although some of them clustered within clade C with cnidarian symbionts, the majority of them belonged to six independent symbiont 'groups' (called Fr1-Fr6) that were not usually found in other *Symbiodinium*-bearing hosts (Pawlowski et al., 2001). The specificity of these unusual symbiont 'groups' for soritid foraminifera was later confirmed by comparing the symbionts identity of 157 forams and 110 neighboring corals obtained from 12 localities in Guam, Micronesia (Pochon et al., 2001). For the sake of taxonomic clarity, the symbiont 'groups' Fr2-Fr5 were assigned to a single clade F (Pochon et al., 2001; LaJeunesse, 2001) and referred to as F2-F5 (Pochon et al., 2006), while the remaining two, Fr1 and Fr6, were renamed clade H (Pochon et al., 2004) and clade G (Pochon et al., 2001), respectively. Consequently, it is now accepted that the genus *Symbiodinium* contains at least eight lineages or clades A through H. The soritid symbionts are classified in five out of the eight *Symbiodinium* clades.

Within each of these clades, a great number of *Symbiodinium* strains or 'species' can be identified by using more variable molecules such as chloroplast 23S-rDNA (Santos et al., 2002a,b), nuclear ITS regions (LaJeunesse, 2001, 2005; Rodriguez-Lanetty, 2003; Fabricius et al., 2004; Van Oppen et al., 2001, 2005a), or microsatellite flanking regions (Santos et al., 2004). However, deciding which level of molecular variability best differentiates the species status in the genus *Symbiodinium* is still debated (Coffroth and Santos, 2005).

The phylogenetic validity of the *Symbiodinium* clades was recently challenged by applying independent phylogenetic analyses on the chloroplast gene coding for the ribosomal large subunit 23S (cp23S-rDNA) Domain V (Santos et al., 2002a), the plastid-encoded *psbA* (Takishita et al., 2003), and the mitochondrial-encoded *cox1* (Takabayashi et al., 2004). All three studies produced topologies that were not statistically different from those generated from nuclear rDNA providing the first set of evidence supporting the published major clades of *Symbiodinium*. All of these studies, however, did not include the *Symbiodinium* types found in soritid foraminifera and, thus, have restricted the analyses to a portion of the currently known diversity of the genus. To correct this, we have applied detailed analyses involving cp23S-rDNA and nr28S-rDNA genes, including all known *Symbiodinium* lineages (Pochon et al., 2006). The nr28S and cp23S phylogenetic trees constructed by using various models produced very similar topologies (Fig. 1). In most analyses, the branching order of clades was extremely well conserved between both genes, the main difference being the position of clade E, which was found either branching as the sister group to the assemblage comprising clades B, C, F, and H (cp23S tree), or next to the clade A as sister to other clades (nr28S tree). The concatenation of both genes (1420 base pairs) produced a highly resolved topology i.e. with strong bootstrap support (BS), mostly congruent with the nr28S tree (Pochon et al., 2006). Taken together, our

Table 1. *Symbiodinium* clades, their host range and geographic distribution.

Symbiodinium clade	Host range	Geographic distribution	Selected references
A	Common in cnidarians and molluscs; Absent in foraminifera	Dominant: Caribbean Sea, Red Sea; Subordinate: Indo-Pacific regions	Rowan and Powers (1991) Baker and Rowan (1997) Barneah et al. (2004)
B	Common in cnidarians, particularly in gorgonian corals; Absent in foraminifera	Dominant: Caribbean Sea; Subordinate: Indo-Pacific regions	Goulet and Coffroth (1997, 2003) Lewis and Coffroth (2004) Santos et al. (2003, 2004)
C	Wide range, including foraminifera	Dominant: Indo-Pacific regions Red Sea; Subordinate: Caribbean Sea	van Oppen (2004) Pochon et al. (2004) LaJeunesse (2001, 2005)
D	Subclade D2 common in corals; Subclade D1 present in a sponge (*Haliclona koremella*) and in foraminifera	Dominant: Indo-Pacific regions Subordinate: Caribbean Sea	Carlos et al. (1999) Baker et al. (2004) Rowan (2004) Garcia et al. (2006)
E	Limited range, observed in the anemone *Anthopleura elegantissima*; Also detected free-living; Absent in foraminifera	Indo-Pacific regions	Wilcox (1998) LaJeunesse and Trench (2000) Gou et al. (2003) Santos (2004)
F	Specific to foraminifera Rarely in corals	Dominant: Indo-Pacific regions Subordinate: Caribbean Sea	Pochon et al. (2001, 2006) Rodriguez-Lanetty et al. (2003) LaJeunesse et al. (2004)
G	Present in foraminifera, bioeroding sponges, octocorals, scleractinian corals	Pacific Ocean	van Oppen et al. (2005a,b) Schoenberg and Loh (2005) Pochon et al. (2001, 2006)
H	Specific to foraminifera	Dominant: Caribbean Sea Subordinate: Indo-Pacific regions	Pochon et al. (2004, 2006)

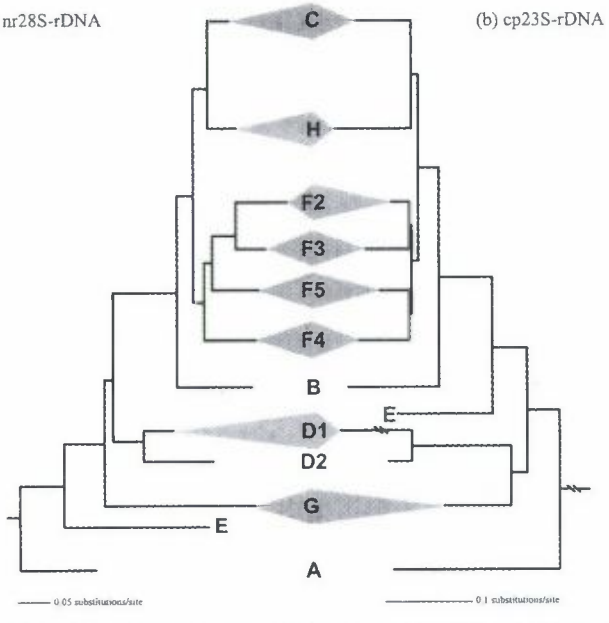

Figure 1. Phylogenetic representations of the genus *Symbiodinium* based on (a) the nuclear 28S ribosomal gene and (b) the chloroplastic 23S ribosomal gene (modified from Pochon et al., 2006). The phylograms are rooted using the dinoflagellate *Gymnodinium simplex* (data not shown). The *Symbiodinium* clades and subclades are indicated with letters A to H. Soritid symbionts have representatives in five out of the eight *Symbiodinium* clades (grey areas).

results provided a basis for establishing the molecular classification of the genus *Symbiodinium*. Host ranges and geographic distributions of the eight *Symbiodinium* lineages are summarized in Table 1.

3. Timescale and Rates of *Symbiodinium* Evolution

Evolutionary rates

Dinoflagellates in the genus *Symbiodinium* are endosymbionts of thousands of host metazoan species distributed in at least three phyla: the Porifera, the Cnidaria, and the Mollusca. Therefore, the finding of such a great diversity of *Symbiodinium* genotypes (clades C, D, F, G, and H) in a single foraminiferal sub-family (Soritinae) is quite remarkable. Particularly, because the first appearance of the Soritinae in the fossil record dates back to about 25 to 30 million years ago (MYA) (Haynes, 1981; Lee et al., 1997), which is much less than the history of scleractinian corals symbiosis. Furthermore, it has been shown that the acquisition of *Symbiodinium* by the ancestor of Soritinae foraminifera was a key innovation that promoted important morphological and ecological adaptations within this group of protists (Hallock, 1985; Richardson, 2001). Therefore, if

the acquisition of *Symbiodinium* dinoflagellates triggered the evolution of soritids, it is reasonable to ask whether faster evolutionary rates could explain the high diversity of *Symbiodinium* observed in these foraminiferan hosts.

The differences in evolutionary rates between all *Symbiodinium* lineages and within two different genes (nr28S and cp23S) were estimated by relative rate (RRT) tests (Pochon et al., 2006). Results did not show a consistent acceleration in evolutionary rates for all clades involving foraminiferal symbionts. For example, in the nuclear data set, only clade F showed high evolutionary rates together with clade B, which to date has never been detected in foraminifera. In the chloroplastic set, clade A and G showed slightly slower evolutionary rates than the other clades with P values sometimes lower than the 0.05 significance level. The outcome of the RRT tests suggests that among many others alternative factors, the mode of vertical transmission of symbionts across foraminiferal generations by multiple fission (Fujita et al., 2000) and/or population bottlenecks resulting from ecological and physiological isolation (LaJeunesse, 2005), may explain the specificity as well as the important diversifications of foraminiferal symbionts.

Evolutionary timescale

The timing of *Symbiodinium* evolution has been limited by lack of preservation of symbionts in the fossil record. Despite a rich fossil record, the origin of Scleractinia has remained shrouded in controversy (Stanley and Fautin, 2001), and determining which coral taxa in the fossil record were 'zooxanthellate' was always problematic (Stanley, 2003). Stanley and Swart (1995) claimed that late Triassic scleractinian corals inhabiting shallow-water complexes of the Thetys were predominantly 'zooxanthellate', like their living counterparts from present day reefs. Several other studies recognized the existence of 'zooxanthellate' (z-like) corals prior to the Cretaceous-Tertiary (K-T) boundary (Rosen and Turnsek, 1989; Rosen, 2000; Kiessling and Baron-Szabo, 2004), with no discussion on the real nature of these Mesozoic 'zooxanthellae'. Were they *Symbiodinium* spp. 'zooxanthellae' or other types of photosymbionts that were replaced later during the Cenozoic Era?

A standard molecular clock on a LSU rDNA data set, recently suggested that the ancestor of the *Symbiodinium* species complex evolved during the K-T boundary (65 MYA) in warm tropical waters (Tchernov et al., 2004), which corresponds to a major transition time from the extinct Mesozoic rudist-based reefs, to the modern scleractinian-dominated reefs.

By applying a relaxed molecular clock method to our chloroplastic and nuclear rDNA data sets, and by calibrating them with the first fossil appearance of the Soritinae foraminifera, we recently proposed that the first radiation event within the genus *Symbiodinium* may, in fact, have occurred some 50 MYA, i.e., at the beginning of Eocene (Pochon et al., 2006). Our results suggested that the emergence of the genus *Symbiodinium* spp. was in fact facilitated by the cooler seasonal global climate, lower sea level, and global coastlines increase that took place during the Eocene time, which promoted regional differences and biodiversity (Scortese, 1997; Bice et al., 2000).

We can speculate that this divergence was somehow related to the large Eocene radiation of scleractinian corals, during which many modern coral families appeared (Wood, 1999; Kiessling and Baron-Szabo, 2004). Although we cannot exclude that some members of *Symbiodinium* clade A originated already in Paleocene and could descend from the symbionts that passed through the K-T boundary, we hypothesize (Fig. 2) that *Symbiodinium* clade A was the first to diversify approximately 50 MYA, followed by the radiation of clades E, G, and D around 40 MYA, corresponding to the formation of the Antarctic Circumpolar Current (ACC), which increased Antarctic ice sheets and ocean fertility (Zachos et al., 2001; John et al., 2003).

Symbiodinium clade B appeared in the early Oligocene following the cooling and rapid expansion of Antarctic continental ice-sheets, which persisted until the latter part of the Oligocene (26 to 27 MYA), when a warming trend reduced the extent of Antarctic ice (Zachos et al., 2001). The late Oligocene to early Miocene period coincides with the appearance of the Soritinae foraminifera in the fossil record (Haynes, 1981; Loeblich and Tappan, 1987), and with the diversification of the ancestors of the *Symbiodinium* clades F, H, and C.

From this point until the middle Miocene (15 MYA), global ice volume remained low and bottom water temperature trended slightly higher (Wright et al., 1991; Miller et al., 1991), with a warm phase peak in the late middle Miocene climatic optimum (17 to 15 MYA), followed by a gradual cooling and reestablishment of a major ice-sheet on Antarctica by 10 MYA (Flower and Kennett 1995; Böhme, 2003). Around 13 MYA the Tethys Ocean closed and the Isthmus of Panama started to uplift, inducing the isolation of marine populations in various ocean basins (John et al., 2003). These compounding factors apparently favored the spectacular explosion of symbionts types that emerged in almost all *Symbiodinium* clades during the mid to late Miocene time (see Fig. 2).

The Pliocene-Pleistocene transition (3–4 MYA) saw the onset of Northern Hemisphere glaciations that began with the final closure of the Central American Isthmus (Coates and Obando, 1996). This geological event produced drastic changes in ocean circulation patterns that ultimately led to major fluctuations in sea-surface temperatures (Haq et al., 1987; Heinze and Crowley, 1997), and is probably responsible for the biogeographic break observed nowadays between Indo-Pacific and Caribbean *Symbiodinium* populations (Baker and Rowan, 1997; Baker, 2003; Pochon

et al., 2004; LaJeunesse, 2005).

The juxtaposition of the clocked *Symbiodinium* tree (Fig. 2) with the long-term patterns in Cenozoic global climate (Zachos et al., 2001) reveals that the major diversifications of this genus occurred during global cooling periods: the origination of *Symbiodinium* clades A, B, D, E, and G during the Eocene cooling, and the massive radiation that took place in all lineages since mid-Miocene. It is remarkable (Fig. 2) that none of extant symbionts, except those harboured by soritid foraminifera and the *Symbiodinium* clade A (and to a lesser extent clade B), diverged during the period between late Oligocene warming and mid-Miocene climatic optimum (25–15 MYA). This conforms to present day observations that periods of global warming negatively affect corals symbioses (Hughes et al., 2003).

Although recent studies show that corals can overcome this problem by adapting to more 'thermally tolerant' symbionts (Baker et al., 2004; Rowan, 2004), the evolutionary history of *Symbiodinium* suggests that long term increase of water temperature may significantly reduce *Symbiodinium* diversity, constituting a serious threat for the survival and diversity of coral-reef ecosystems. Interestingly, it has been observed that the Cenozoic reef carbonate production (mainly determined by reef abundance) goes up when climate cools (W. Kissling, pers. comm.). Furthermore, Pomar et al. (2006) recently showed that mid to late Miocene scleractinian corals started to build a more rigid framework in the shallow-water euphotic zone up to sea level, corresponding to a change from aragonite-inhibiting to aragonite-facilitating episodes. Our 'cooling hypothesis' may provide a simple explanation for these reef-climate paradoxes.

The interpretations described above have to be taken with some cautions. Although we used relaxed molecular clock techniques, widely applied in recent evolutionary studies, their accuracy remains disputable (Bromhan, 2006). A number of intrinsic factors may have influenced the general picture shown in Fig. 2. First, since the symbionts are not preserved in the fossil record, we disposed of only two dates to calibrate our tree: the acquisition of *Symbiodinium* in soritid foraminifera (25–30 MYA) and the closure of the Isthmus of Panama (3–4 MYA) separating Indo-Pacific and Caribbean *Symbiodinium* 'types' in clade H. Similarly, LaJeunesse (2005) successfully used the estimates of the final closure of the Isthmus and other biotic factors to calculate a molecular clock for the many 'types' found within clade C by using the highly variable ITS2 rDNA region. His results suggested that each ocean possesses a diverse clade C assemblage that appears to have independently radiated by successive bursts through host specialization and allopatric differentiation since at least 6–9 MYA.

Second, and as already underlined above, the 28S rDNA gene seems to be far too conserved for the resolution of the within clades diversifications. As a result, the within-clades branches are artificially stretched in a stair-like manner, which is unlikely to have occurred. Finally, we cannot exclude the possibility that other lineages existed in the past and simply disappeared during the Cenozoic, especially because extinction can be shown to be almost equal to origination on average for all marine organisms when excluding the so called 'pull of the recent' effect (Jablonski et al., 2003; Kissling, 2005).

Consequently, additional investigations using other genes and techniques are needed to explore further the timing of evolutionary history of the genus *Symbiodinium*.

4. *Symbiodinium* Biogeography

The phylogeography of the genus *Symbiodinium* has been extensively studied since the mid 1990s (reviewed in Baker, 2003). Early studies explored the distribution of the phylogenetic clades within and between oceans or across various latitudinal gradients, and rapidly provided evidence for some biogeographic patterns in different parts of the world (Rowan, 1996; Baker and Rowan, 1997; Baker, 1999; Baillie et al., 2000; Loh et al., 2001; Rodriguez-Lanetty et al., 2001, 2003; Burnett, 2002; Goulet and Coffroth, 2004). For example, in scleractinian corals from both the Indo-Pacific and the Caribbean, the members of *Symbiodinium* A and B have been found more commonly at higher latitudes, while clade C was more abundant in tropical latitudes. Even more striking is the biogeographic break existing between both regions, with a much bigger proportion of the different *Symbiodinium* clades (especially A and B) in Caribbean corals than in their Indo-Pacific counterparts (Fig. 3a).

By contrast, *Symbiodinium* C is by far the most common and diversified clade throughout the Indo-Pacific (LaJeunesse, 2005), although members from clade D are now detected with increasing frequencies, especially in reefs that have suffered from bleaching episodes (Baker et al., 2004; Rowan, 2004).

With increasing number of studies, it became clear that the phylogenetic level of clade is not sufficient for deciphering fine scale biogeographic distribution in the genus *Symbiodinium*. Since each clade contains many symbiont 'types' with various physiological and ecological adaptations, several studies used more variable markers for the biogeographic observations (LaJeunesse and Trench, 2000; Lajeunesse, 2001, 2005; Rodriguez-Lanetty and Hoegh-Guldberg, 2002; LaJeunesse et al., 2003, 2004; Santos et al., 2003, 2004; Van Oppen et al., 2005b). For instance, Santos et al. (2003) sampled 575 specimens of *Pseudopterogorgia elisabethae* along a ~450 km transect in the Bahamas and by using the allele size variation at two microsatellite loci, unveiled a striking biogeographic structure in what was believed to be different populations of

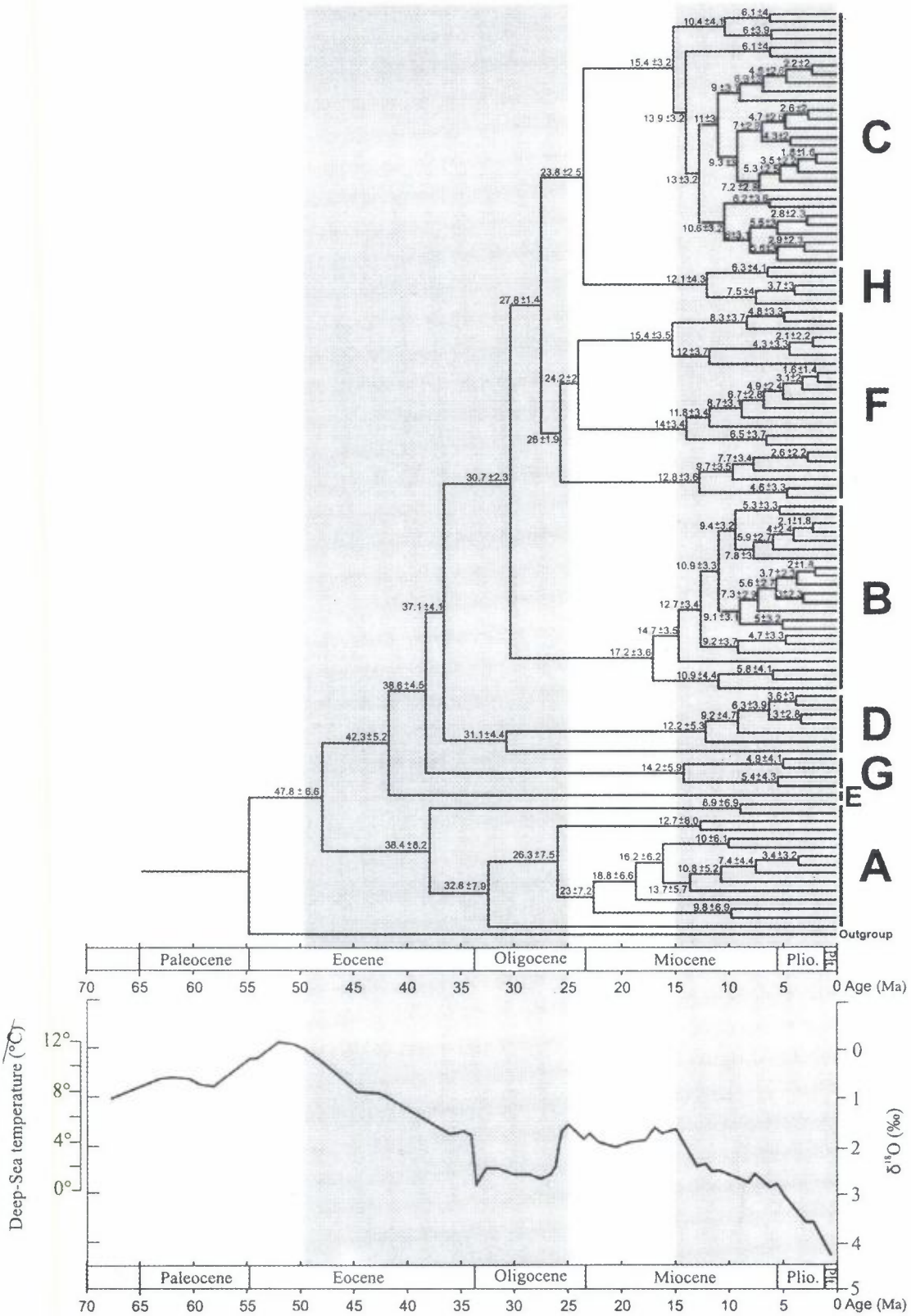

Figure 2. Chronogram obtained from 105 partial LSU (nr28S) *Symbiodinium* sequences acquired from GenBank as well as from our unpublished data base, with ages inferred from the Bayesian rate autocorrelation method using two nodes under palaeontological constraints. Numbers at nodes correspond to the standard deviations around divergence ages. The *Symbiodinium* clades and subclades are indicated with letters A to H. Below the chronogram is the deep-sea temperature curve derived from oxygen isotope analysis (Zachos et al., 2001). Major diversifications in the genus *Symbiodinium* occur during global cooling periods of the Cenozoic (grey areas).

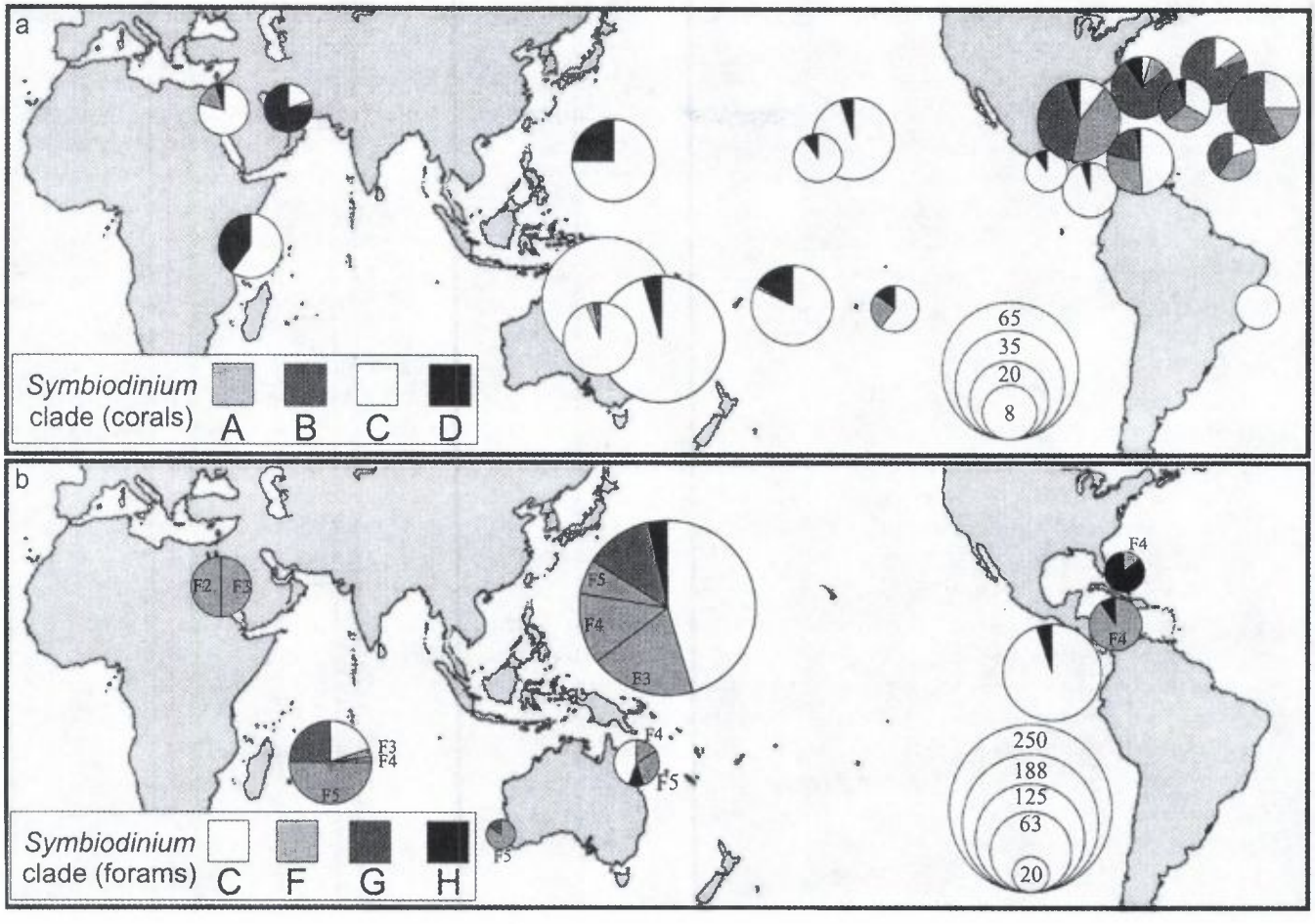

Figure 3. Global distribution of *Symbiodinium* dinoflagellates in scleractinian corals versus soritid foraminifera. (a) Pie charts, modified from Baker (2003), reflect the distribution of the clades A, B, C, and D among species of coral host sampled, with the diameter of the pie chart approximately proportional to the square root of the number of species sampled (see inset scale). (b) Pie charts reflect the distribution of clades C, F, G, and H among soritid foraminifera, with the diameter of the pie chart proportional to the number of soritid specimens analysed.

a *Symbiodinium* 'species', suggesting that many more studies are needed until we clearly understand the biogeographic structures in the genus *Symbiodinium*.

The biogeography of soritid symbionts is still in its early stage. Pochon et al. (2004) first compared the biogeographic distribution of soritid symbionts between the Pacific Ocean and the Caribbean Sea. Genetic examination, using restriction fragment length polymorphism (RFLP) analyses, was conducted on 61 foraminiferal individuals from the Caribbean and 82 from the eastern Pacific to rapidly assess which clade of *Symbiodinium* they harbored. By gathering this information together with our previous data on the *Symbiodinium* diversity in soritid foraminifera collected from five main regions worldwide (Red Sea, Indian Ocean, Micronesia, Western Australia, and Eastern Australia), we have obtained the following indication: a biogeographic break in *Symbiodinium* spp. distributions, analogous to that demonstrated for cnidarian symbionts, is clearly evident between Indo-Pacific and Caribbean soritids (Fig. 3b). Clade H and sub-clade F4 in clade F dominate 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 (Pochon et al., 2004). Compared to the Indo-Pacific, the diversity of symbionts in the Caribbean/Atlantic soritids is relatively low. Among seven divergent symbiont groups (clade C, clade F comprising the divergent sub-clades F2–F5, clade H, and clade G) identified in our previous study (Pochon et al., 2006), only two clades (F4, H) are found in the Caribbean soritids. This is in sharp contrast with Caribbean corals, which have been found to

associate with 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 foraminiferal populations from the western Atlantic may be primarily 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 (Langer and Hottinger, 2000).

5. Host-Symbiont Specificity and Coevolution

The specificity of the *Symbiodinium* harbored by soritids raises the question of a possible coevolution between foraminiferal hosts and their symbionts. Coevolution, i.e. the process of reciprocal evolutionary change between interacting species driven by natural selection, is one of the major processes organizing the Earth's biodiversity. Originally perceived as a unidirectional and almost static phenomenon, in which one species evolves strictly in response to the evolution of its partner (Darwin, 1859), coevolution is now seen as a very powerful and dynamic process in which interspecific interactions can be highly divergent across both narrow and broad geographic scales, thereby fuelling continuing coevolution of taxa (Thomson, 1994; Thompson and Cunningham, 2002).

In *Symbiodinium*, coevolutionary processes have been largely understudied, mostly because the markers used in most investigations (i.e SSU and LSU rDNA) did not provide any support for them. Two attempts to use more variable ITS1 rDNA marker to explore a possible host-symbionts coevolution in scleractinian corals (Van Oppen et al., 2001) and octocorals (Van Oppen et al., 2005a) did not detect any evidence for it. It may be possible, however, that speciation in coral-algal symbiosis is driven by short-term coevolutionary processes during periods of relative stability (LaJeunesse, 2002, 2005), which may become evident in forthcoming studies using new molecular markers (Coffroth and Santos, 2005).

The existence of numerous *Symbiodinium* lineages that associate specifically with soritids suggest that the foraminifera participate in driving the evolution of this dinoflagellate group. In an attempt to address this issue Garcia-Cuetos et. al. (2006) examined the relation between *Symbiodinium* and its foraminiferal hosts at a lower taxonomic level by genetically identifying both host specimens and their symbionts. They identified 22 phylotypes of Soritinae, among which 15 were associated with a single geographic locality and 14 possessed a single 'group' of *Symbiodinium*. Therefore, the majority of phylotypes showed a strict specificity for a unique *Symbiodinium* 'group' in a single locality. This strong host-symbiont specificity was considered to be the combined result of a selective recognition mechanism, vertical transmission of symbionts, and biogeographic isolation.

The comparison of the *Symbiodinium* and the Soritinae trees (28S versus 18S rDNA, respectively) shows no patterns of coevolution *sensu stricto* between foraminifera and their symbionts (Pochon, personal observations). Some members of the *Symbiodinium* clades C and F are well represented in all three genera of Soritinae, suggesting various degrees of flexibility in the foram-algal relationship. However, when analyzing the ITS2 rDNA 'types' of all the symbiont 'groups', a different picture is emerging. A surprisingly high degree of connectivity exists between some specific ITS2 ribotypes and specific soritid phylotypes (Pochon et al., in preparation). This suggests that coevolutionary processes might be present in restricted area, where strong reciprocal selection creates coevolutionary hotspots (see Thompson, 1994). The fact that these hotspots are implanted in a broader matrix of coevolutionary coldspots where local selection is nonreciprocal (Thompson, 2005), may contribute to the existence of few 'generalist' and many 'specialist' *Symbiodinium* ribotypes worldwide (LaJeunesse, 2005). Understanding the coevolutionary dynamics of these mutualistic symbioses is probably one of the biggest challenges for future ecological studies on *Symbiodinium* dinoflagellates.

6. Conclusions and Perspectives

Molecular phylogenetic studies of symbiotic dinoflagellates in soritid foraminifera have considerably broaden our knowledge on the diversity, specificity, biogeography, and evolution of the genus *Symbiodinium*, in a way that was still inconceivable prior to the year 2001. These studies have also challenged traditional views of foram-algal evolution and systematics. First, the initial hypothesis that soritid hosts continually exchange symbionts with their cnidarian neighbors (Lee et al., 1997) has been invalidated by the finding of several *Symbiodinium* lineages specifically associated with soritid foraminifera at various taxonomic ranks, and also by the absence of the widespread *Symbiodinium* A and B in these organisms. Second, the classical view of Soritinae phylogeny based on the increasing morphological complexity of their skeleton was not supported by molecular data (Garcia et al., 2006). Instead, their morphology may have primarily been shaped by the different ecological environments that each genus has exploited throughout geological times (Pochon et al., in prep.). Third, the morphological features used to distinguish the different species existing in Soritinae do not seem reliable regarding the important cryptic diversity, especially in the genus *Sorites*.

Molecular investigations on the soritids and their symbionts, however, are still at their early stages and several aspects of their ecology and evolution await further development. First, a detailed analysis of the foram-algal dynamics in space and time is needed. A study in progress based on a one-year survey that was carried out on the Island of Guam between 2002 and 2003 (Pochon et al., in prep.) will hopefully provide useful information on the diversity and specificity of the ITS2 ribotypes found in different soritid phylotypes, with an emphasis on both depths gradients (ecology) and time constraints (seasonality). It will also explore the soritid reproduction cycles through which the transmission of symbionts occurs.

Second, the biogeography of soritids and their symbionts is still in its infancy. A much more extensive sampling must be done before we can accurately draw a precise mapping of their worldwide distribution and relationships. New molecular markers such as chloroplastic and mitochondrial genes, as well as the use of microsatellite loci may be better adapted than classical nrDNAs for deciphering fine scale biogeographical networks, and may be necessary in the investigations of coevolutionary processes.

Third, no study has yet considered physiological experiments on soritid symbionts to test whether they show thermo-tolerance or if they are better adapted to high light environments than other typically metazoan symbionts. Our molecular data showed that some soritid symbionts are closely related to symbiont 'types' that are suspected to be thermo-tolerant, such as C15 and D2 (Baker et al., 2004; LaJeunesse, 2005; Pochon et al., 2004). Soritid foraminifera have never been reported to bleach and they seem to be not affected at all in reefs that are suffering sever bleaching (Hoenhegger, pers. comm.; Pochon, pers. obs.). However, physiological evidence is needed to confirm these observations.

Finally, one of the most crucial gaps is the lack of data on free-living *Symbiodinium* in the water column or in the sediment. Knowing if all or only a small proportion of *Symbiodinium* 'types' detected to date *in situ* can also survive outside the hosts would greatly benefit our knowledge on the ecology and adaptability of many host-symbiont relationships. Such information is necessary to understand the complex dynamics of reef ecosystems and to be able to build predictions on how these fragile habitats will respond to increasing environmental changes.

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