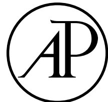
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# Evolving lineages of *Symbiodinium*-like dinoflagellates based on ITS1 rDNA

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**Abstract**

*Symbiodinium*-like dinoflagellates have been shown to be a diverse group of endosymbionts that associate mutualistically with many kinds of coral reef dwellers, including cnidarians, molluscs, and protists. A high number of genetically ITS types of symbionts have been reported to date. However, whether these recently identified *Symbiodinium* ITS types indeed represent independent evolutionary lineages is still unsettled. Here I tested the null hypothesis that certain group of symbionts sampled from different geographical locations are derived from a single evolutionary lineage using a nested clade analysis (NCA). I analyzed a total of 174 ITS1 sequences from GenBank and pooled them into 74 ITS1 distinct haplotypes. Using these haplotypes, the statistical parsimony criterion produced 23 independent network trees, each one corresponding to a genetically independent evolving lineage. Some of these lineages revealed certain degree of specificity with some host groups at least at the phylum level. Within the previously described 28S-rDNA phylotype *A*, five ITS1 lineages were resolved. Phylotypes *B* and *C* resolved each in two ITS1 lineages. The highest ITS1 symbiont diversity was observed within the phylotype *F*, in which 11 lineages were resolved. Moreover, most of these lineages were associated uniquely with protist hosts from the group of foraminiferans. Here it is suggested that this high genetic diversity of endosymbionts associated with foraminiferans is linked with the evolution of soritacean foraminifera, which seems to have been driven by endosymbiosis. Lastly, the absence of genetic recombination presented in this study, suggest a lack of hybridisation at least among the major 28S-rDNA phylotypes within *Symbiodinium*-like dinoflagellates. This supports highly the idea that these phylotypes are indeed independent evolutionary units, which should be considered at least as different species. Whether they belong to the same genus or to different higher taxa still needs to be revised.
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## 1. Introduction

*Symbiodinium*-like dinoflagellates have been shown to be a diverse group of endosymbionts that associate mutualistically with many kinds of coral reef dwellers, including cnidarians, molluscs, and protists (Baillie et al., 2000a; Baker, 1999; LaJeunesse, 2001; Pawlowski et al., 2001; Pochon et al., 2001; Rowan, 1998; Trench, 1993). These photosynthetic microalgae have played a key role in the evolutionary success of their hosts in shallow, tropical benthic environments (Muller-Parker and D'Elia, 1997; Stanley and Swart, 1995). Although only a few species of this genus have been formally described (Banaszak et al., 1993; Freudenthal, 1962; Trench and Blank, 1987), evidence of morphological, biochemical, and especially genetic differences have contributed to substantiate the notion that this genus is indeed a multi-species complex (Baker, 1999; Banaszak et al., 1993; LaJeunesse, 2001; Pawlowski et al., 2001; Pochon et al., 2001; Rowan and Powers, 1991a,b; Schoenberg and Trench, 1980a,b; Trench and Blank, 1987; Wilcox, 1998). Early phylogenetic analyses of the highly conserved small subunit (SSU) of nuclear ribosomal DNA revealed divergent lineages within *Symbiodinium*-like dinoflagellates (McNally et al., 1994; Rowan and Powers, 1991a,b). The same genetic lineages, originally referred as clades or phylotypes, were also supported by the evidence from a more variable region within the large subunit (LSU) of nuclear ribosomal DNA (Baker, 1999; Wilcox, 1998). More recently, phylogenetic analyses of organellar DNA sequences

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based on the chloroplast large subunit (23S—domain V) rDNA region have corroborated the phylogeny previously inferred from nuclear DNA regions (Santos et al., 2002). Based on all these genetic markers, seven major phylotypes or genetic lineages have been identified and labelled as clades *A*, *B*, *C*, *D*, *E*, *F*, and *G* (Baker, 1999; Carlos et al., 1999; Pawlowski et al., 2001; Pochon et al., 2001; Rowan and Powers, 1991a,b).

In attempting to improve the phylogenetic resolution within these lineages, LaJeunesse (2001) used the internal transcribed spacers (ITS) of nuclear ribosomal DNA to detect genetic variability within 47 *Symbiodinium* isolates cultured from 34 different host species. It appears that not only the non-coding ITS rDNA region exhibits greater phylogenetic resolution than those of 18S and 28S rDNA within this group (Baillie et al., 2000a; LaJeunesse, 2001; Santos et al., 2001; Van Oppen et al., 2001), but also it seems to act like a single-copy gene (Baillie et al., 2000a), which makes it stand out as a very useful genetic marker for phylogenetic studies of the *Symbiodinium*-like dinoflagellates. LaJeunesse (2001)'s results show the existence of a remarkable genetic diversity within six of the major phylotypes (*A–F*), which resulted in the identification of 17 ITS types from parsimony phylogenetic trees. Some of these ITS groups corresponded morphologically and physiologically with some of the few taxonomically described species of this genus, *Symbiodinium microadriaticum*, *Symbiodinium pilosum*, *Symbiodinium. goreauii*, and *Symbiodinium kawagutii*. Moreover, other studies have also found multiple *Symbiodinium* ITS1 types within certain host species living in particular geographical areas (Baillie et al., 2000a; Santos et al., 2001; Van Oppen et al., 2001).

Whether or not these recently identified *Symbiodinium* ITS types represent actual independent evolutionary lineages is still unclear. To date, the traditional phylogenetic methods applied to reconstruct the genealogical history of the genetic variation within *Symbiodinium*-like dinoflagellates do not provide the needed statistical power to straddle the interface between intra- and interspecific evolution of this group. And, it is precisely at this interface that the process of speciation occurs (Templeton, 1989). By constructing a network haplotype tree and converting it into a hierarchical set of nested branches or clades using the analytical procedure known as nested clade analysis (NCA), it is possible to test the null hypothesis that certain group of organisms sampled from different geographical locations are derived from a single evolutionary lineage. This approach is based on the operational concept of cohesion species of Templeton (2001), which defines an evolutionary lineage as a reproducing population with sufficient historical continuity to have its own evolutionary trajectories and tendencies.

In this study, network haplotype trees are constructed using 174 ITS1 rDNA sequences of *Symbiodinium*-like dinoflagellates obtained from GenBank database, which were reported from an extensive number of hosts and geographical locations (Baillie et al., 1999, 2000a; Hunter et al., 1997; LaJeunesse, 2001; Lobban et al., 2002; Pawlowski et al., 2001; Pochon et al., 2001; Rodriguez-Lanetty and Hoegh-Guldberg, 2002; Rodriguez-Lanetty et al., 2002; Santos et al., 2001; Takabayashi et al., 1998; Van Oppen et al., 2001). Subsequently, nested clade analyses are applied to detect phylogeographical patterns within the resolved networks or lineages. This study focuses only on ITS1 rDNA data, as there is currently more information available in GenBank about this region compared to the ITS2 region. Furthermore, recombination analyses using two different approaches were performed to evaluate the degree of genetic recombination within the *Symbiodinium* ITS1 data, which is a critical aspect that has been overlooked in previous phylogenetic analyses. This is extremely relevant as high levels of genetic recombination could distort and misrepresent the actual evolutionary history of the gene under study, by generating mosaic genes, where different regions have different phylogenetic histories. With these analyses, I aimed to: (1) resolve the evolutionary lineages within *Symbiodinium*-like dinoflagellates using ITS1 haplotypes identified from 74 host species all around the world; (2) elucidate any geographical pattern within these evolutionary lineages using statistical analyses; and (3) determine whether there is any association of these lineages with host species.

## 2. Material and methods

### 2.1. Search of ITS1 sequences, alignment and haplotype network estimation

Sequences of ITS1 rDNA from *Symbiodinium*-like dinoflagellates were searched directly in GenBank database (web site at http://www.ncbi.nlm.nih.gov) on April 2002. The searches were also performed applying the procedure of FASTA search in GenBank using ITS1 *Symbiodinium* sequences previously identified as 28S-rDNA-phylotypes *A*, *B*, *C*, *D*, *E*, *F*, and *G* as input files (Baillie et al., 2000a; LaJeunesse, 2001; Pawlowski et al., 2001; Pochon et al., 2001; Rodriguez-Lanetty and Hoegh-Guldberg, 2002; Rodriguez-Lanetty et al., 2002; Santos et al., 2001; Van Oppen et al., 2001). This also allowed retrieving *Symbiodinium* sequences stored in GenBank under different taxonomic names. The retrieved ITS1 sequences were aligned together using Clustal X (Thompson et al., 1994). Nevertheless, separate alignments of ITS1 sequences within 28S-rDNA phylotypes were also performed to check for any discrepancy within the full alignment. The algorithm of Templeton et al. (1992) was used to estimate the hap-

Table 1
ITS1 haplotypes of *Symbiodinium*-like dinoflagellates identified from all the sequences found in GenBank database

| Phylo-types (28SrDNA) | Identified ITS1 haplotypes[m] | Other similar sequences in GenBank database[m] | Hosts and Geographical locations |
|---|---|---|---|
| A | AF333504[f] | AF333505[f], AJ311947[h] | *Condylactis gigantea* (Jamaica), *Cassiopeia xamachana* (Florida), *C. frondosa* (Jamaica), *C. andromeda* (Israel), *Stylophora pistillata* (Israel), *Acropora* sp (Israel) |
| | AJ311946[h] | | *Millepora* sp (Israel) |
| | AF333506[f] | AF184942[c] | *Zoanthus* sp (Australia), *Z. sociatus* (Jamaica), *Bartholomea annulata* (Barbados), *Meandrina meandrites* (Jamaica), *Heliopora* sp (Enewetak), *Gorgonia ventalina* (Bermuda, Jamaica, Puerto Rico), *Corculum cardissa* (Palau), *Tridacna gigas* (Australia) |
| | AF184941[c] | | *Sarcophyton glaucon* (Australia) |
| | AF333507[f] | AF183575[c], AF184943–5[c], AF184947[c], AF186050–69[c], AF195143[d], AF195145–51[d] | *Hippopus porcellanus* (Philippines), *Hippopus hippopus* (Philippines, Palau), *Tridacna maxima* (Enewetak, Philippines, Palau), *T. squamosa* (Philippines, Palau, Australia), *T. derasa* (Philippines, Palau), *T. gigas* (Philippines, Palau), *T. crocea* (Philippines, Okinawa, Australia), *Fragum unedo* (Palau), *Corculum monstrosum* (Palau), *C. cardissa* (Palau), *Cassiopeia mertensii* (Hawaii), *Mastigias* sp (Palau) |
| | AF333508[f] | | *Tridacna squamosa* (Palau) |
| | AF333509[f] | | *Linuche unguiculata* (Bermuda), *Plexaura homomalla* (Bahamas) |
| | AF184949[c] | | *Amphisorus hemprichii* (Palau) |
| | AF184948[c] | | Free-living symbiont (Hawaii) |
| B | AF333511[f] | AF360550–1[i], AF360554[i], AF360556–8[i], AF360563[i], AF360565–7[i], AF360571–3[i], AF184940[c], Syd-1[k], AF195152[d] | *Pseudoterogorgia americana* (Florida), *P. elisabethae* (Bahamas), *P. bipinnata* (Jamaica), *Plexaura kuna* (Virgin Is, Florida, Bahamas, Panama), *P. flexuosa* (Florida), *Eunicea* sp (Florida), *Pocillopora dammicornis* (Hawaii), *Porites evermanni* (Hawaii), *Plesiastrea versipora* (Australia), *Oculina diffusa* (Bermuda), *Aiptasia pulchella* (Hawaii), *A. tagetes* (Bermuda, Puerto Rico), *A. pallida* (Florida), *Lebrunia danae* (Jamaica), *Cassiopeia* sp (Australia), *C. xamachana* (Jamaica), *Corbulifera* sp (Palau) |
| | AF360555[i] | | *Plexaura kuna* (Panama) |
| | AF333512[f] | AF360564[i], AF360574[i] | *Aiptasia pallida* (Florida), *A. pulchella* (Hawaii), *Plexaura flexuosa* (Florida) |
| | AF333513[f] | | *Oculina diffusa* (Bermuda) |
| | AF333514[f] | AF360575[i] | *Dichotomia* sp (Bahamas), *Plexaura kuna* (Panama) |
| | AF360560[i] | | *Briareum asbestinum* (Florida) |
| | AF360562[i] | | *Plexaura kuna* (Panama) |
| | AF360552[i] | | *P. kuna* (Panama) |
| | AF360569[i] | | *P. homomalla* (Panama) |
| | AF360553[i] | | *P. kuna* (Panama) |
| | AF333510[f] | | *Anthopleura elegantissima* (Western Coast of USA) |
| C | AF333515[f] | AY186566–7[k], AY186569[k], AF195144[d], AY186627[j], AF380533[g], AF380545[g], AF380555–6[g], AF380558[g], AF380563[g], AJ311941–2[h] | *Rhodactis Heteractis lucida* (Jamaica), *Heteractis* sp (Korea), *Discosoma sanctithomae*, *Plesiastrea versipora* (Japan), *Acropora sarmentosa* (Australia), *A. longicyathus* (Australia),*Corculum cardissa* (Palau), *Marginopora vertebralis* (Israel), *Sorites* sp (Israel) |
| | AY186560[k] | AY186561–3[k], | *Plesiastrea versipora* (Japan) |
| | AY186565[k] | | *P. versipora* (Japan) |
| | AF038913[b] | AF038915[b] | *Heliofungia actinoformis* (Australia) |
| | AJ278598[l] | | *Maristentor marianus* (Guam) |
| | AY186570[k] | | *Plesiastrea versipora* (Japan) |
| | AY186568[k] | | *P. versipora* (Japan) |
| | AY186564[k] | | *P. versipora* (Japan) |
| | AY186628[j] | | *Heteractis* sp (Korea) |
| | AJ311943[h] | | *Lobophyllia* sp (Guam) |
| | AF038916[b] | AF038917[b] | *Stylophora pistillata* (Australia) |
| | AF038910[b] | AF038911–2[b] | *Goniopora tenuidens* (Australia) |
| | AY186571[k] | | *Plesiastrea versipora* (Japan) |
| | AF380531[g] | AF380544[g], AF380549[g], | *Acropora aspera* (Australia), *A. millepora* (Australia), *A. gemmifera* (Australia), *A. longicyathus* (Australia), *A. cuneata* (Australia), *A. florida* (Australia) |

Table 1 (*continued*)

| Phylo-types (28SrDNA) | Identified ITS1 haplotypes[m] | Other similar sequences in GenBank database[m] | Hosts and Geographical locations |
|---|---|---|---|
| | | AF380557[g], AF380559–62[g] | |
| | AF380534[g] | | *A. gemmifera* (Australia) |
| | AF380536[g] | | *A. cuneata* (Australia) |
| | AF380554[g] | | *A. millepora* (Australia) |
| | AF380543[g] | | *A. vallida* (Australia) |
| | AF380546[g] | | *A. latistella* (Australia) |
| | AF380553[g] | | *A. millepora* (Australia) |
| | AF380547[g] | | *A. latistella* (Australia) |
| | AJ291519[e] | AJ291517–8[e] | *Sorites* sp (Guam) |
| | AF333518[f] | AF195153–7[d] | *Hippopus hippopus* (Palau), *H. porcellanus* (Palau), *Tridacna deresa* (Palua), *T. gigas* (Palau), *T. crocea* (Palau) |
| | AJ291515[e] | | *Marginopora vertebralis* (Australia) |
| | AJ291516[e] | | *Sorites* sp (Australia) |
| | AJ291514[e] | | *Amphisorus hemprichii* (Israel) |
| | AJ311944[h] | | *Porites rus* (Guam) |
| | AF180127[a] | AF180128[a] | *Porites lobata* (Hawaii), *Porites compressa* (Hawaii) |
| | AF180125[a] | | *Porites evermanni* (Hawaii) |
| | AF180126[a] | | *Porites evermanni* (Hawaii) |
| | AF180129[a] | | *Porites porites* (Florida) |
| D | AF334660[f] | | *Montastrea faveolata* (Caribbean) |
| E | AF334659[f] | | *Anthopleura elegantissima* (Western Coast of USA) |
| F | AJ291520[e] | | *Sorites* sp (Guam) |
| | AJ291513[e] | | *Sorites* sp (Florida) |
| | AJ291521[e] | AJ291522–4[e] | *Sorites* sp (Israel), *Amphisorus hemprichii* (Israel) |
| | AY186626[j] | | *Alveopora japonica* (Korea) |
| | AJ291525[h] | | *M. kudakajimaensis* (Guam) |
| | AJ291533[e] | | *Amphisorus hemprichii* (Israel) |
| | AJ291534[e] | | *Sorites* sp (Israel) |
| | AJ291512[e] | | *Sorites* sp (Israel) |
| | AJ291532[e] | | *Amphisorus hemprichii* (Israel) |
| | AJ291530[e] | AJ311945[h], AJ291531[e] | *Amphisorus hemprichii* (Israel) |
| | AF360576[i] | | *Sinularia* sp (Guam) |
| | AF333516[f] | AJ291529[e] | *Meandrina meandrites* (Jamaica),*Sorites* sp (Guam) |
| | AJ291535[e] | | *Amphisorus hemprichii* (Maldives) |
| | AF333517[f] | AF184946[c], AF360577[i] | *Montipora verrucosa* (Hawaii, Australia) |
| | AF180130[a] | | *Porites astreoides* (Florida) |
| | AJ291526[e] | | *Sorites* sp (Guam) |
| | AJ291527[e] | | *Sorites* sp (Florida) |
| | AJ291528[e] | | *Sorites* sp (Guam) |
| | AJ311949[h] | | *Sorites* sp (Guam) |
| G | AJ291536[h] | AJ291537[h] | *Marginopora vertebralis* (Guam), *M. kudakajimaensis* (Guam) |
| | AJ291538[h] | AJ291539[h] | *Marginopora vertebralis* (Guam), *M. kudakajimaensis* (Guam) |

Each haplotypes has been labelled with an accession number from one of the sequences belonging to each particular haplotype. Information of host species and geographical locations are also shown.

[a] Hunter et al. (1997).
[b] Takabayashi et al. (1998).
[c] Baillie et al. (1999).
[d] Baillie et al. (2000a).
[e] Pawlowski et al. (2001).
[f] LaJeunesse (2001).
[g] Van Oppen et al. (2001).
[h] Pochon et al. (2001).
[i] Santos et al. (2001).
[j] Rodriguez-Lanetty et al. (2002).
[k] Rodriguez-Lanetty and Hoegh-Guldberg (2002).
[l] Lobban et al. (2002) (submitted in GenBank by Pochon and Pawlowski, 2000).
[m] Sequences are labelled using their GenBank accession numbers. Haplotypes were identified in the alignment when gaps were treated as missing data.

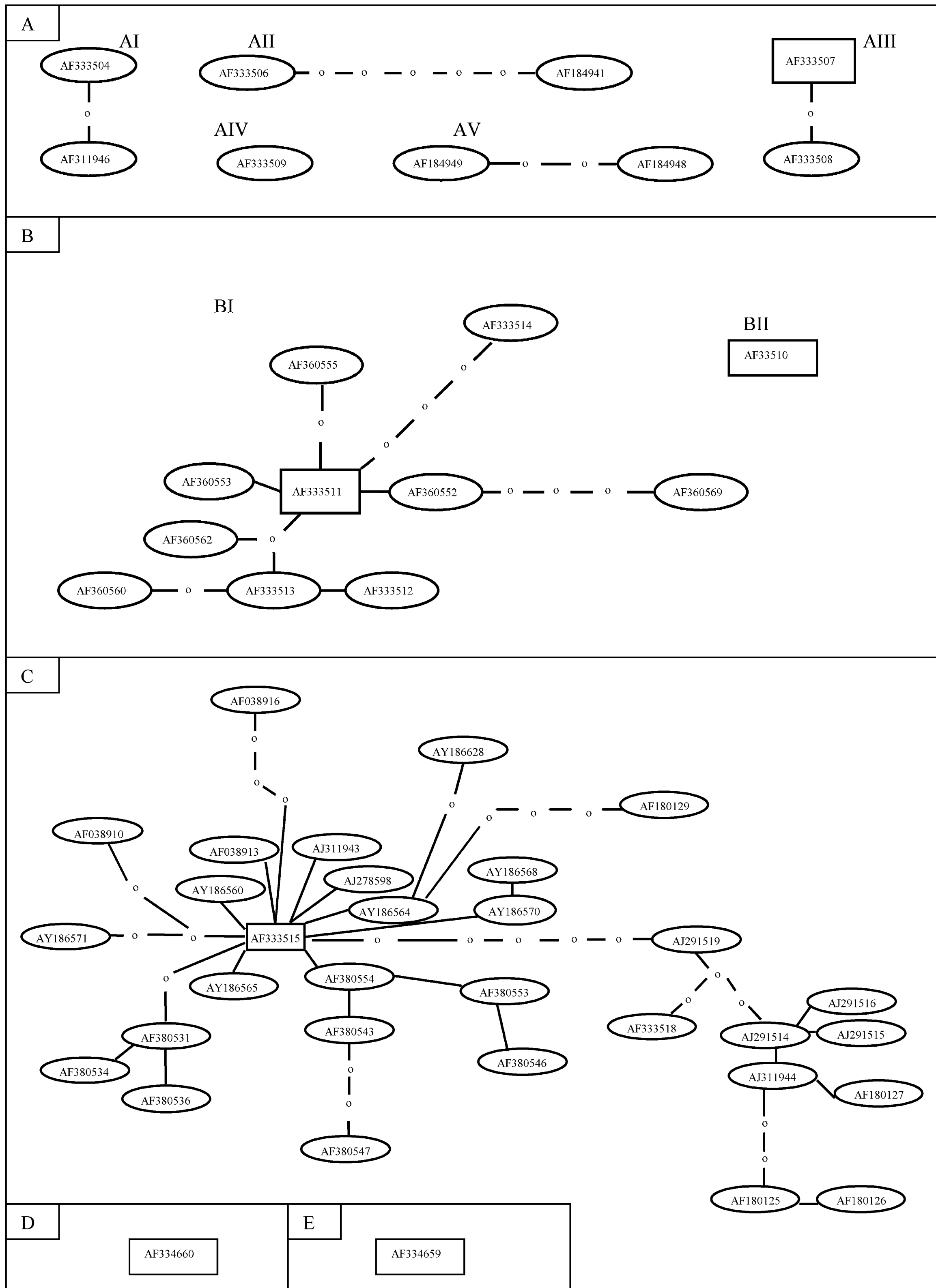


Fig. 1. Unrooted ITS1 rDNA haplotype networks from *Symbiodinium*-like dinoflagellates in 28S-rDNA phylotype *A* (a); *B* (b); *C* (c); *D* (d); *E* (e); *F* (f); and *G* (g). Zeros (0) within the networks, represent missing haplotypes not found in the field. The lines connecting haplotypes represent one mutation difference. In (f), the original clade labelling from Pochon et al. (2001) is indicated between brackets.

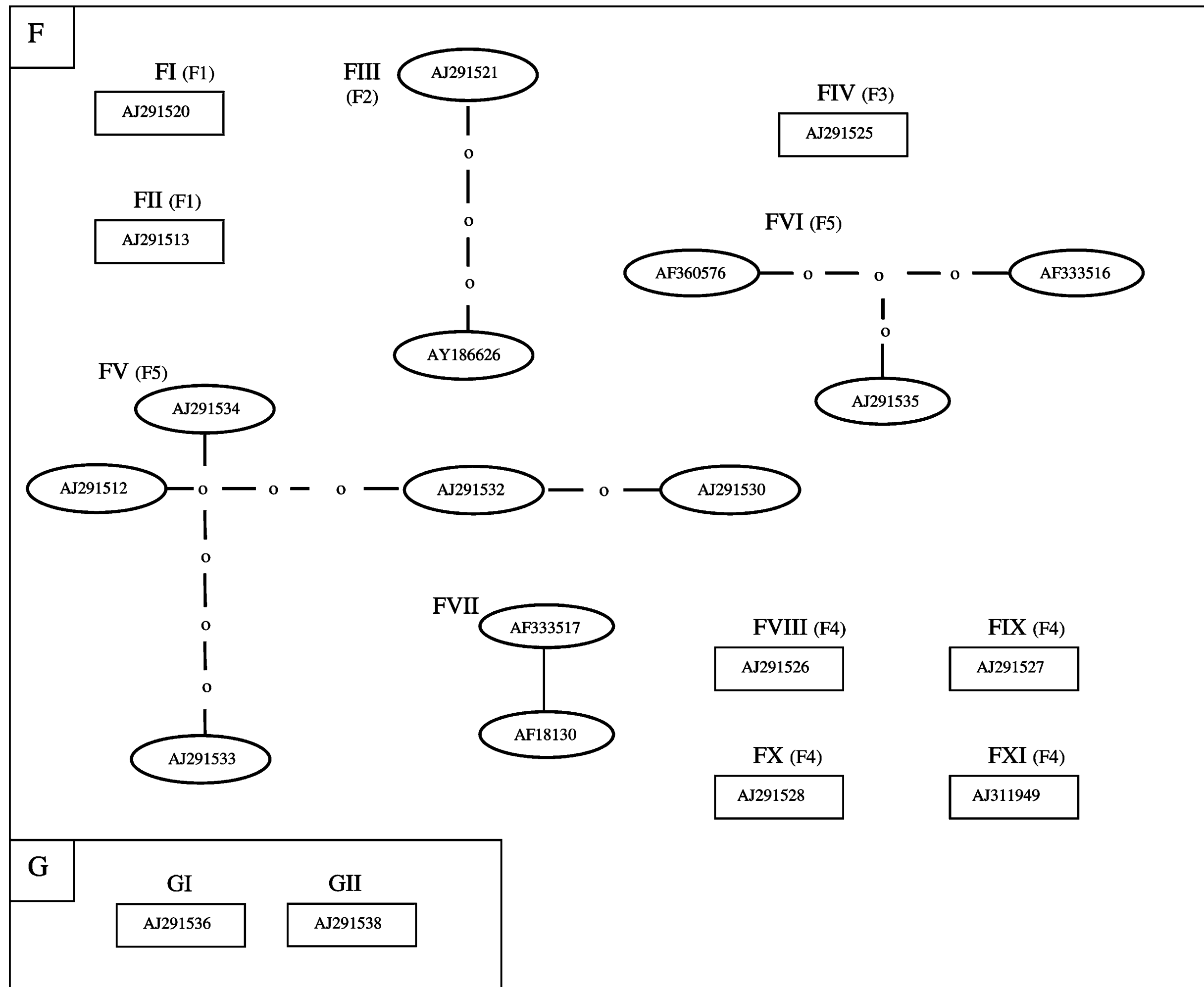


Fig. 1. (*continued*)

lotype network trees. This method not only estimates the unrooted haplotype tree but also provides a 95% plausible set for all haplotype linkages within the unrooted tree. The gaps within the alignment were treated either as missing data or included as fifth character (state), and then comparisons were brought into the results when differences were detected. To construct the haplotype networks, the TCS 1.13 software developed by Clement et al. (2000) was used. The program collapses sequences into haplotypes and calculates the frequencies of the haplotypes in the sample. These frequencies are used to estimate haplotype outgroup probabilities, which correlate with haplotype age. However, to avoid statistical artefacts due to differences of sampling effort in some of the geographical locations from the distinct studies from which the ITS1 data came from, repeated identical sequences from same geographical location (reported from the same author) were removed from the data set, and only those identical sequences (reported by the same author) from different locations were included and kept for the subsequent statistical analyses.

### 2.2. Nested clade analysis and test for geographical and host associations

Once the 95% plausible set was estimated, a nested design was drawn on top of the haplotype trees, using Templeton and Sing (1993)'s algorithm. For that, haplotypes (0-step clades) separated by a single mutation were nested into 1-step clades, carrying on from the tips to the interior of the network. The resulting clades were grouped into two-step clades (under the same principle), and so on, until the final level of nesting encompassed the entire tree. Afterwards, an exact permutational contingency analysis of categorical variation was implemented in a nested fashion. For each step level, the associations between clades and geographical locations, and clades and host groups were tested. Because the number of samples per host species was not adequate to perform meaningful statistical analyses, host species were grouped into higher taxonomic categories (as shown in Table 2), and used them as the categorical variable in the clade—host association tests. The contingency tests were performed using the software

Chiperm 1.2 (available at the Crandall lab web site at http://bioag.byu.edu/zoology/crandall_lab/programs.htm) on the clades with more than one haplotype, sample location (for geographical association cases) and host group (for host association cases), following the algorithm given by Templeton and Sing (1993).

### 2.3. Recombination analysis

Evidence of recombination among the ITS1 haplotypes within and between the resolved network trees was explored using two softwares (RETICULATE and GENECONV) that use different methods for detecting DNA recombination. RETICULATE (Jakobsen and Esteal, 1996), which is a compatibility method, defines two sites as compatible when their evolutionary history is congruent with the same tree, not requiring the phylogeny of the sequences to be known (Posada, 2002). A large number of non-compatible sites is then indicative of recombination. This program not only identifies the existence of recombination, but also tells the amount of recombination detected. On the other hand, GENECONV (Sawyer, 1999) is a nucleotide substitution distribution method that examines the sequences either for a significant clustering of substitutions or for a fit to an expected statistical distribution (Posada, 2002). GENECONV assigns global permutation $P$ values based on BLAST-like global scores (10,000 replicates) that are considered as evidence of recombination if these values are smaller than 0.05 (Sawyer, 1989).

## 3. Results

### 3.1. Resolved networks

A total of 174 ITS1 sequences (~198–229 bp) from *Symbiodinium*-like dinoflagellates were found out in GenBank database, and from them 74 different haplotypes were identified. Table 1 shows these haplotypes and all the ITS1 sequences belonging to each one of them, including information from the hosts and geographical locations. Those haplotypes represented by more than one GenBank sequence were named using a randomly chosen one of the accession numbers from the group of sequences (see Table 1).

The phylogenetic relationship among all the 74 ITS1 haplotypes is shown in Fig. 1. The parsimony criterion resolved 23 separate ITS1 network trees within the seven previously described 28SrDNA phylotypes (*A–G*), although some of them were represented by a single haplotype. The connections between these network trees could not be ascertained since the number of mutation separating them was higher than the maximum number of mutational connections between pairs of sequences justified by the parsimony criterion with a probability of 0.95. The ITS 1 haplotypes within the 28S-rDNA phylotypes *A* were resolved in five separate networks (Fig. 1A). The ITS1 haplotypes from phylotype *B* resolved in two networks (Fig. 1B), and those from phylotype *C* grouped together in a single network (Fig. 1C). The highest number of ITS1 haplotypes within networks was observed within networks BI and C, with 10 and 31 haplotypes, respectively. Phylotype *D* and *E* resolved separately in single-haplotype networks, as only one ITS1 sequence from each of the two phylotype was present in the data set (Figs. 1D–E). The highest number of networks was found within phylotype *F*, in which 11 network trees were resolved (Fig. 1F). Although, a higher number of networks (15 in total) were resolved in this phylotype when gaps within the alignment were included as a fifth state. Nevertheless, since the relatively high number of gaps in this group could overestimate the actual number of ITS1 lineages within this phylotype, a more conservative estimate of lineage diversity treating the gaps as missing data was more reasonable. Moreover, most of these *F* networks were composed only by a single haplotype (Fig. 1F). Lastly, the phylotype *G* resolved in two single-haplotype networks (Fig. 1G).

The pairwise distance within and among the 23 ITS1 network trees is shown in Table 3. The genetic distance within network trees having more than one haplotype ranged between 0.04 and 2.21%, and among the trees it stretched between 2.47 and 44.07%. The ITS1 networks within the phylotype *A* showed a low genetic variation, which ranged between 2.47 and 15.51%. Networks BI and BII also revealed a low genetic differentiation between them (4.26%), but both were remarkably different to all the networks belonging to phylotype *A* (over 32%). These two networks, on the other hand, revealed a higher relatedness with the network C (difference ~21.5%) and with the networks from phylotype *F* (less than 23%). Likewise, network C also showed a higher similarity with networks from phylotype *F* (less than 20% different). The networks D, E, GI, and GII were found to encompass the most distinct ITS1 haplotypes from *Symbiodinium*-like dinoflagellates, revealing genetic differences over 34% when compared to the other networks. However, GI and GII showed to be highly related, showing a pairwise difference of 2.77%.

### 3.2. Geographical associations

The number and frequency of haplotypes within networks from phylotype *A* was too small to perform any meaningful statistical analysis. However, it can be said in terms of geographical distribution that networks AI and AII are widely distributed, as their ranges go from the Caribbean Sea to the Red Sea, and from the Caribbean Sea to the West Pacific Ocean, respectively (Table 2). On the other hand, ITS1 haplotypes from

Table 2
Geographical distribution and host groups of ITS1 *Symbiodinium* networks

| ITS1 networks | Geographical distribution | Host group |
|---|---|---|
| AI | East and West Caribbean Sea–Red Sea | Actinaria–Scleractinia–Scyphozoa |
| AII | East and West Caribbean Sea–Australian Eastern Seaboard–North Western Pacific Ocean | Actinaria–Zoanthiniaria–Scleractinia–Octocorallia–Bivalvia |
| AIII | North Western and Central Pacific Ocean–Australian Eastern Seaboard–Red Sea | Scyphozoa–Bivalvia |
| AIV | West Caribbean Sea | Octocorallia–Scyphozoa |
| AV | North Western and Central Pacific Ocean | Foraminifera |
| BI | East and West Caribbean Sea–North Western and Central Pacific Ocean–Australian Eastern Seaboard | Actinaria–Scleractinia–Octocorallia–Scyphozoa–Hydrozoa |
| BII | North Eastern Pacific Ocean (California–Oregon, USA) | Actinaria (*Anthopleura elegantissima*) |
| C | East and West Caribbean Sea–North Western and Central Pacific Ocean–Australian Eastern Seaboard–Red Sea | Actinaria–Scleractinia–Corallimorpharia–Bivalvia–Foraminifera–Ciliata |
| D | Caribbean Sea | Scleractinia |
| E | Eastern Pacific Ocean (California, USA) | Actinaria (*Anthopleura elegantissima*) |
| FI | North Western Pacific Ocean | Foraminifera |
| FII | West Caribbean Sea | Foraminifera |
| FIII | North Western Pacific Ocean–Red Sea | Scleractinia–Foraminifera |
| FIV | North Western Pacific Ocean | Foraminifera |
| FV | Red Sea | Foraminifera |
| FVI | East Caribbean Sea–North Western Pacific Ocean–Indic Ocean | Scleractinia–Octocorallia–Foraminifera |
| FVII | West Caribbean Sea–Central Pacific Ocean–Australian Eastern Seaboard | Scleractinia |
| FVIII | North Western Pacific Ocean | Foraminifera |
| FIX | West Caribbean Sea | Foraminifera |
| FX | North Western Pacific Ocean | Foraminifera |
| FXI | North Western Pacific Ocean | Foraminifera |
| GI | North Western Pacific Ocean | Foraminifera |
| GII | North Western Pacific Ocean | Foraminifera |

network AIII and AV have only been found within the Red Sea and West/Central Pacific Ocean, and the haplotype from network IV uniquely in the Caribbean Sea (Table 2).

Nested clade analyses could not be performed on networks BII, D, E, FI–II, FIV, FVIII–FXI, GI, and GII due to the fact that only one haplotype was present in each of these networks. The nested clade structure of networks BI and C are shown in Figs. 2A–C, and the statistical contingency results of the association between geography and haplotype distribution within the nested clades are given in Table 4. From network BI, clades 1–4, 1–6, 2–2, 2–3, and 3–1, and from network C, clades 1–1, 1–14, 1–15, 2–1, 2–6, 3–1, 3–2, and 4–1 contained more than a geographical location and a haplotype to thus be able to perform contingency analyses. As seen in Table 4, no significant association was detected between geography and haplotype distribution in network BI. Similar results were observed in most of the clades within network C, except in the last nested clade (4–1), in which a significant association was detected. The results in network BI show that this network represents a single evolving lineages, and their haplotype members are widely distributed geographically, ranging from the Caribbean Sea to the West/Central Pacific Ocean (see Table 2). With regard to network C, the results show a geographical association in the distribution of haplotypes (Table 4), where ITS1 haplotypes from clade 3–1 are differently geographically distributed to those in clade 3–2 (Fig. 2C). This suggests that these two clades within network C, which are actually separated by five mutations (see Figs. 1C and 2C), might represent two different evolving lineages.

With regard to the networks within group *F*, only the network FIII and FVI presented the minimum needed number of haplotypes and geographical locations to be able to perform a contingency analysis on the nested clade structure shown in Fig. 2B. The results showed a lack of significant association between geography and haplotype distribution within these two networks (Table 4). Haplotypes from network FVI, along with FVII, are actually widely geographically distributed, ranging from the Caribbean Sea to Indo-Pacific Ocean, and network FIII from the North Western Pacific Ocean to the Red Sea. Some of the other *F* networks, however, show clearly restricted geographical distribution (Table 2). The haplotypes within networks FI, FIV, FVIII, and FX–XI have been found only within the north western Pacific Ocean (Guam I.), network FII and FIX within the West Caribbean Sea (Florida coast), and network FV within the Red Sea (Israel coast). Although all the networks within phylotype *F* seem to represent independent evolving lineages, more samples are required especially from the networks represented by single

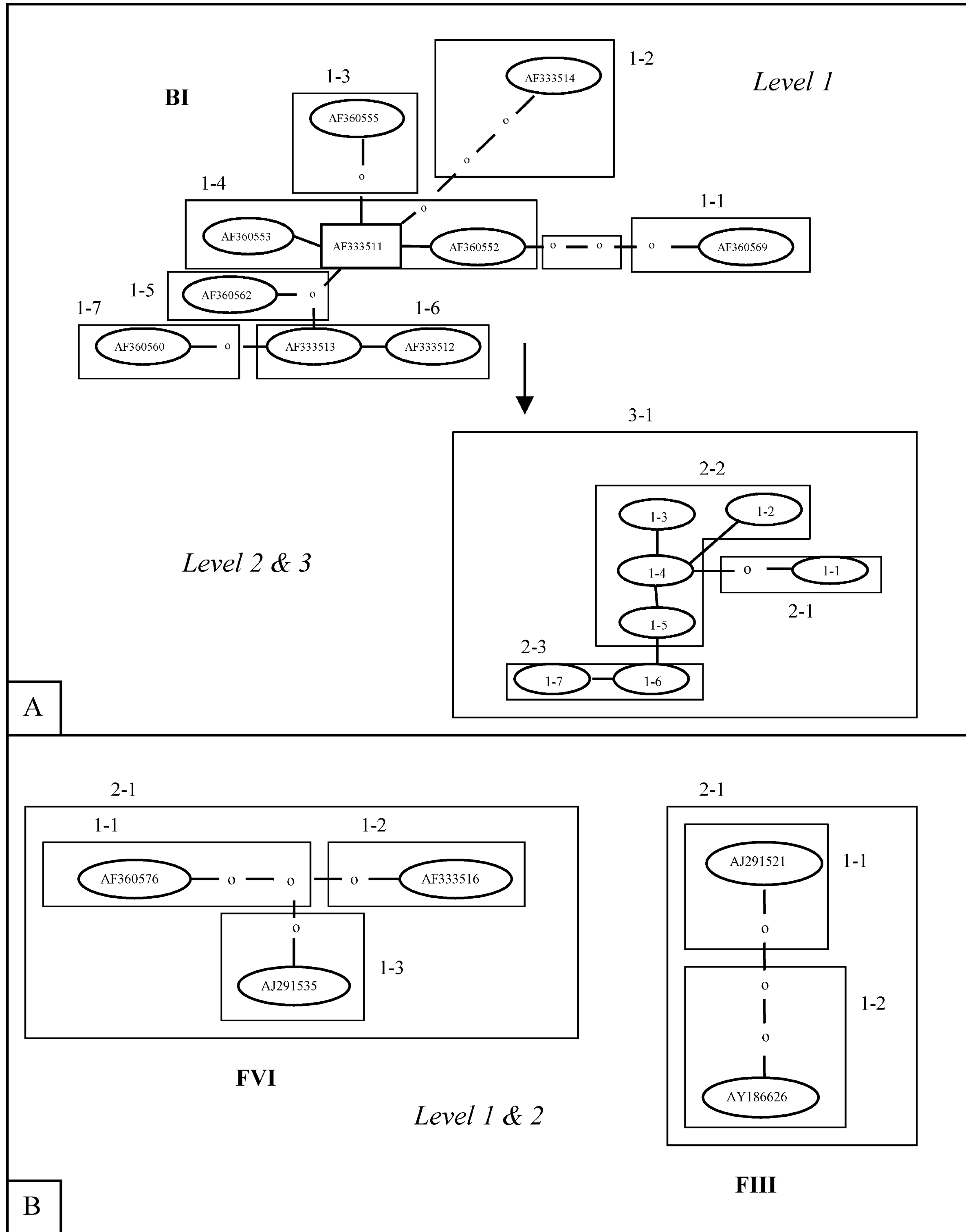


Fig. 2. Clade nesting of the ITS1 rDNA haplotype networks. (A) Network BI; (B) network FIII and FVI; and (C) network C. Zeros (0) within networks, represent missing haplotypes not found within the field. The lines connecting haplotypes represent one mutation difference. Within each network, haplotypes separated by only a mutation are grouped within squares composing the first step of the clade nesting. Then, the subclades from level 1 separated by only a mutation are grouped to compose the second step of the nesting structure (level 2). This procedure goes on until the entire network in grouped in one nested clade.

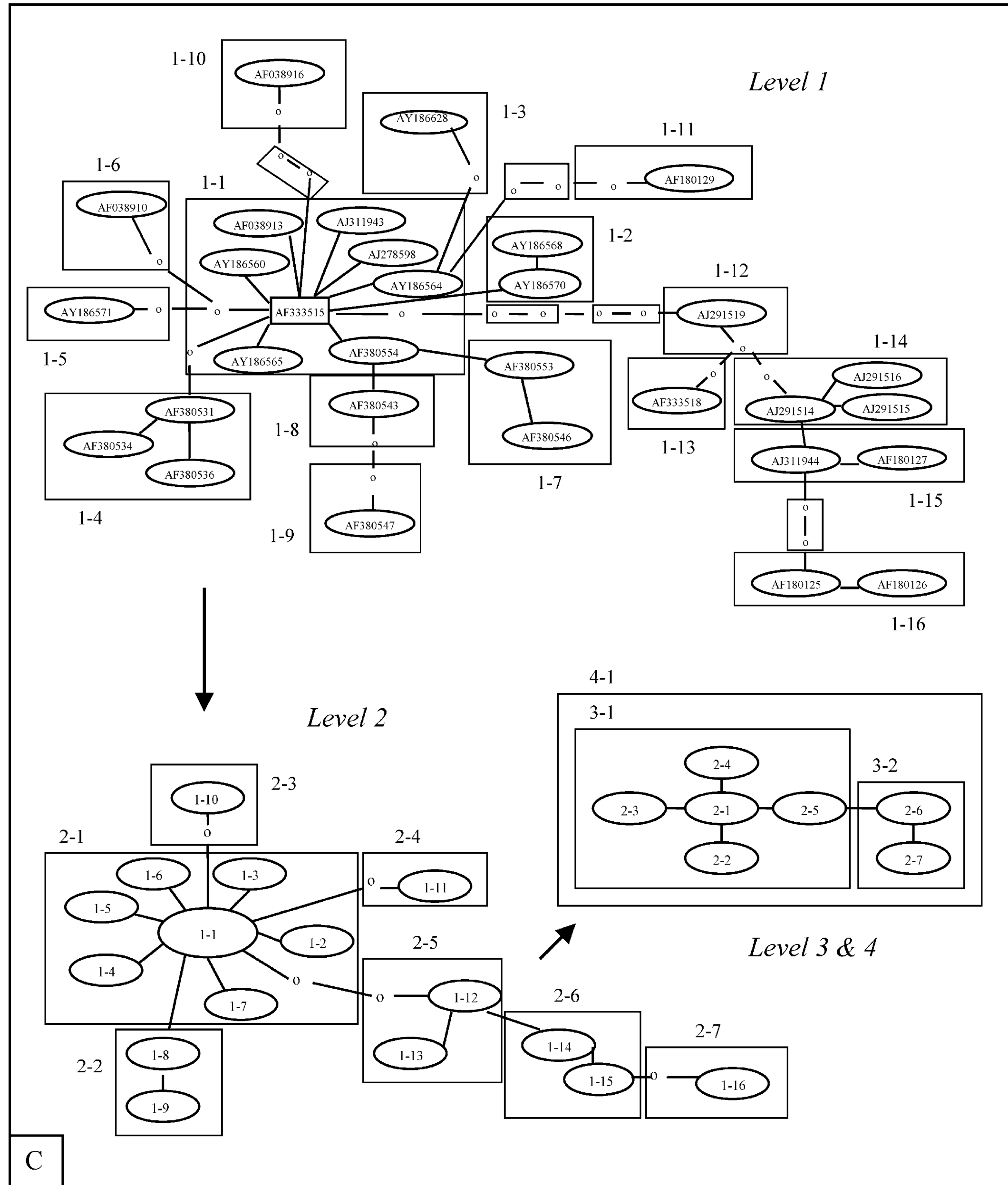


Fig. 2. (*continued*)

haplotypes to understand the genealogy of this phylotype.

### 3.3. Host and ITS1 Symbiodinium haplotype associations

As with the geographical association tests, the small number of haplotypes within the networks from phylotype *A* does not allow to perform any meaningful contingency statistical analysis between host group and haplotype distribution. However, from Table 2, it can be noticed that all the hosts harboring Symbiodinium ITS1 haplotypes from network AI and AIV belong to the phylum cnidaria, and those harboring haplotypes from network AV belong to the group of foraminiferans (Phylum Sarcodina). On the other hand, the haplotype from network AII and AIII are harbored by a number of host species from the phyla cnidaria and mollusca (Tables 1 and 2).

The association results between host group and haplotype distribution from network BI shown in Table 4, reveals a lack of host–symbiont ITS1 type association. However, as seen in Table 2, all the hosts harboring haplotypes from this network belong exclusively to the phylum Cnidaria. Network BII, represented by a single haplotype, has only been reported so far in symbionts associated with the anemone, *Anthopleura elegantissima* (Cnidaria: Actinaria). Within network C, no significant association between host group and ITS1 symbionts was

Table 3
Genetic differentiation within and among networks of *Symbiodinium*-ITS1 haplotypes

| Networks | AI | AII | AIII | AIV | AV | BI | BII | C | D | E | FI | FII | FIII | FIV | FV | FVI | FVII | FVIII | FIX | FX | FXI | GI | GII |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AI | **0.20** | 14.33 | 3.68 | 2.47 | 15.51 | 34.74 | 32.81 | 30.62 | 41.11 | 39.53 | 32.41 | 30.04 | 30.04 | 34.78 | 30.66 | 32.51 | 32.54 | 32.51 | 31.92 | 31.92 | 31.13 | 43.08 | 42.69 |
| AII | | **0.79** | 14.12 | 13.83 | 13.24 | 35.33 | 34.39 | 32.61 | 44.27 | 43.21 | 34.12 | 33.73 | 33.54 | 36.23 | 35.25 | 35.67 | 35.70 | 35.57 | 34.78 | 35.44 | 33.47 | 43.35 | 43.74 |
| AIII | | | **0.04** | 4.37 | 14.05 | 34.32 | 32.39 | 31.67 | 41.11 | 39.13 | 32.79 | 31.20 | 31.60 | 34.76 | 32.39 | 34.07 | 34.50 | 32.39 | 32.00 | 31.60 | 31.60 | 43.46 | 43.06 |
| AIV | | | | **0.00** | 15.02 | 36.32 | 34.39 | 31.79 | 41.50 | 39.53 | 33.20 | 31.23 | 31.62 | 35.57 | 31.90 | 33.70 | 33.73 | 33.60 | 33.20 | 32.81 | 32.02 | 43.87 | 43.48 |
| AV | | | | | **1.19** | 34.90 | 33.79 | 33.98 | 43.28 | 42.29 | 33.99 | 31.82 | 34.74 | 36.96 | 35.46 | 37.06 | 37.48 | 34.58 | 33.40 | 33.79 | 33.40 | 44.07 | 44.07 |
| BI | | | | | | **0.78** | 4.26 | 21.76 | 38.75 | 36.76 | 20.58 | 19.53 | 23.05 | 21.98 | 18.21 | 17.16 | 17.94 | 19.81 | 19.41 | 18.23 | 19.41 | 35.33 | 35.24 |
| BII | | | | | | | **0.00** | 21.37 | 37.94 | 36.76 | 20.95 | 19.76 | 21.19 | 20.95 | 18.35 | 17.89 | 18.71 | 20.55 | 20.55 | 18.97 | 19.76 | 35.57 | 35.57 |
| C | | | | | | | | **2.21** | 40.97 | 39.21 | 20.02 | 14.93 | 15.68 | 18.35 | 17.03 | 16.98 | 16.99 | 13.36 | 13.15 | 14.31 | 13.21 | 37.47 | 37.09 |
| D | | | | | | | | | **0.00** | 30.83 | 36.76 | 41.11 | 38.97 | 38.34 | 36.59 | 37.75 | 38.21 | 39.92 | 39.13 | 38.34 | 37.94 | 33.60 | 32.41 |
| E | | | | | | | | | | **0.00** | 41.11 | 38.74 | 38.58 | 38.74 | 38.51 | 38.44 | 39.39 | 38.34 | 37.55 | 39.13 | 37.94 | 34.78 | 32.81 |
| FI | | | | | | | | | | | **0.00** | 20.55 | 16.44 | 16.60 | 18.24 | 17.98 | 19.50 | 18.18 | 17.79 | 17.00 | 17.39 | 35.57 | 35.57 |
| FII | | | | | | | | | | | | **0.00** | 18.74 | 17.39 | 15.70 | 15.71 | 15.94 | 17.00 | 15.81 | 17.00 | 15.42 | 38.34 | 37.94 |
| FIII | | | | | | | | | | | | | **0.63** | 15.26 | 15.79 | 15.67 | 15.73 | 17.63 | 17.63 | 18.02 | 16.84 | 38.18 | 38.18 |
| FIV | | | | | | | | | | | | | | **0.00** | 15.64 | 16.30 | 15.42 | 17.79 | 18.18 | 19.37 | 17.79 | 35.97 | 35.57 |
| FV | | | | | | | | | | | | | | | **1.47** | 4.36 | 3.86 | 12.76 | 12.37 | 10.95 | 10.22 | 37.61 | 36.82 |
| FVI | | | | | | | | | | | | | | | | **1.12** | 4.81 | 13.44 | 13.14 | 11.96 | 10.97 | 36.17 | 35.38 |
| FVII | | | | | | | | | | | | | | | | | **1.58** | 12.91 | 13.18 | 12.78 | 11.20 | 37.02 | 36.23 |
| FVIII | | | | | | | | | | | | | | | | | | **0.00** | 4.74 | 5.93 | 4.35 | 36.76 | 36.36 |
| FIX | | | | | | | | | | | | | | | | | | | **0.00** | 3.56 | 2.77 | 36.36 | 35.97 |
| FX | | | | | | | | | | | | | | | | | | | | **0.00** | 2.77 | 37.55 | 37.55 |
| FXI | | | | | | | | | | | | | | | | | | | | | **0.00** | 37.55 | 37.15 |
| GI | | | | | | | | | | | | | | | | | | | | | | **0.00** | 2.77 |
| GII | | | | | | | | | | | | | | | | | | | | | | | **0.00** |

The average percentage of pairwise differences of sequences between networks is shown above diagonal, and within networks is indicated by diagonal elements, in bold. The Pairwise distance method was used for the calculations.

Table 4
Nested exact contingency analysis of geographical regions and host groups with clades of the ITS1 rDNA haplotypes in networks BI, C, and FIII and FVI from *Symbiodinium*-like dinoflagellates

| Clade | Network BI | | | | Clade | Network C | | | | Clade | Network FIII | | Clade | Network FVI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Geography | | Host | | | Geography | | Host | | | Geography/Host[b] | | | Geography/Host[b] | |
| | $\chi^2$ | $P$ | $\chi^2$ | $P$ | | $\chi^2$ | $P$ | $\chi^2$ | $P$ | | $\chi^2$ | $P$ | | $\chi^2$ | $P$ |
| 1–4 | 4.61 | 0.67 | 1.37 | 1.00 | 1–1 | 11.48 | 0.96 | 5.83 | 1.00 | 2–1 | 3.00 | 0.34 | 2–1 | 5.00 | 0.86 |
| 1–6 | 0.44 | 1.00 | 4.00 | 0.49 | 1–14 | 3.00 | 1.00 | [a] | [a] | | | | | | |
| 2–2 | 4.09 | 1.00 | 2.14 | 1.00 | 1–15 | 0.22 | 1.00 | [a] | [a] | | | | | | |
| 2–3 | 0.31 | 1.00 | 1.88 | 1.00 | 2–1 | 19.70 | 0.39 | 13.91 | 0.61 | | | | | | |
| 3–1 | 6.71 | 0.35 | 3.77 | 0.71 | 2–6 | 6.00 | 0.18 | 6.00 | 0.10 | | | | | | |
| | | | | | 3–1 | 41.13 | 0.07 | 13.97 | 0.37 | | | | | | |
| | | | | | 3–2 | 2.67 | 0.70 | 1.60 | 0.46 | | | | | | |
| | | | | | 4–1 | 20.61 | 0.001* | 5.08 | 0.17 | | | | | | |

The nested design is given in Fig. 2.
[a] No host variation within clade, therefore the contingency analysis could not be performed.
[b] Statistical results from host and geography tests were the same.
* Significant at $P < 0.05$.

found (Table 4). In fact, symbionts from this network showed to associate with a wide range of hosts, including members of different phyla (Table 2).

With regard to networks FIII and FVI, no significant association between haplotypes and host groups was found (Table 4). Hosts from these two networks were represented by cnidarians (scleractinian and Octocorallia) and foraminiferan species (Tables 1 and 2). Network FVII was the only group of ITS1 symbionts within phylotype *F* represented uniquely by scleractinian hosts (Table 2). On the other hand, all the ITS1 haplotype symbionts from the other eight *F* networks, including the two networks from phylotype *G*, were hosted exclusively by protists, foraminiferan species (Tables 1 and 2).

### 3.4. Recombination

GENECONVS did not detect recombination among the haplotypes within any of the resolved network groups shown in Fig. 1. However, RETICULATE detected significant recombination within group *F* (Table 5). This could have been an artefact from the software when many blocks of gaps are present in the alignment, which was the case of the sequence alignment from phylotype *F* (see alignments at http://home.ewha.ac.kr/~jisong/). A substantial shortcoming of RETICULATE when many blocks of gaps are present is that the sites where there are gaps tend to be highly compatible and outside those regions have low compatibility, so the clustering is actually an effect of where there are gaps in the alignment or not (Jakobsen, RETICULATE programmer, personal communication). This is why conclusions about the presence of recombination should not be derived on the basis of a single method (Posada, 2002; Posada and Crandal, 2002). *P* values, by RETICULATE for the network groups B and C, are not given in Table 5 as the number of parsimony sites and incompatible pairwise comparisons within them are extremely small to perform any meaningful statistical analysis, which, to some extent, suggests a lack of recombination among those haplotypes. In general, the lack of recombination found within the ITS1 data set validates that the phylogenetic results presented in this study have not been misrepresented or distorted by genetic recombining effects.

## 4. Discussion

Phylogenetic analyses of ITS1 haplotypes performed using a statistical parsimony criterion (Clement et al., 2000) indicated that *Symbiodinium*-like dinoflagellates are represented by diverse groups of genetic lineages. Particularly, the results showed that at least 23 evolving molecular lineages, comprised of 74 ITS1 haplotypes,

Table 5
Results (*P* values) of recombination among ITS1 haplotypes within the 28S rDNA-symbiont phylotypes

| Method | Phylotype *A* | Phylotype *B* | Phylotype *C* | Phylotype *F* | Phylotype *G* |
|---|---|---|---|---|---|
| RETICULATE | 0.15 | — | — | 0.01* | 0.10 |
| GENECONVS | 0.32 (2.65) | 0.68 (1.44) | 0.45 (2.81) | 0.29 (4.16) | 0.12 (0.97) |

Phylotypes *D* and *E* are not shown, as only an ITS1 sequence from each of these phylotypes was included in the data set. *P* values, by RETICULATE, for the network groups *B* and *C* are not given as the number of parsimony sites and incompatible pairwise comparisons within them were extremely small to perform any meaningful statistical analysis.

Maximum BLAST-like scores given by RETICULATE are shown between brackets.

* Significant at $P < 0.05$.

are present within this group of symbiotic dinoflagellates. However, more genetic lineages must exist worldwide, as for instance, some recent results based on ITS2 DNA profiles show high symbiont diversity within the Caribbean Sea (LaJeunesse, 2002). Most of the ITS1 lineages resolved here belonged to the 28S-rDNA phylotypes *A* and *F*. Nevertheless, though phylotypes *B* and *C* resolved in only three ITS1 networks, they encompassed the higher haplotype diversity, with 11 and 31 haplotypes, respectively. We cannot ignore, however, the fact that these two symbiont phylotypes (*B* and *C*) have been found within the most worldwide studied host groups (Scleractinia and Octocorallia), which means that the actual symbiont haplotype diversity from other phylotypes might have not been fully explored, and perhaps currently underestimated.

Some of the resolved ITS1 lineages are consistent with some of the ITS groups distinguished by LaJeunesse (2001) within the previously identified 18S and 28S rDNA phylotype *A*. The networks AI, AII, and AIV, showing an average pairwise difference of 10.21%, match with the groups of *Symbiodinium microadriaticum* (A1), *S. pilosum* (= *Symbiodinium meandrinae*, A2), and *Symbiodinium* (= *Gymnodinium*) *linucheae* (A4), respectively reported by LaJeunesse (2001). The two previously described subspecies *S. microadriaticum*, subsp. *microadriaticum* (A1), and subsp. *condylactis* (A1.1) (= *S. cariborum sensu* Banaszak et al., 1993) by Blank and Huss (1989), belong to the single evolving lineage, network AI, exhibiting a remarkably low ITS1 pairwise difference between them (0.2%), in comparison to the average genetic difference of 11.06% among all the networks within phylotype *A*. Network AIII, included the haplotypes from the two ITS types, A3 and A5, described by LaJeunesse (2001), and the ITS subgroup A3 described by Baillie et al. (1999, 2000a). It is worth highlighting that the symbionts from this network (AIII) were harbored by a high number of molluscan host species including the clam, *Corculum cardissa*. Originally, it was thought that this clam species associates specifically with a particular symbiont species, described as *Symbiodinium corculorum* by Banaszak et al. (1993). Afterwards, LaJeunesse (2001) proposed that this symbiont species is a synonymy of *S. pilosum* (= *S. meandrinae*), since no ITS rDNA and morphological differences were detected between those symbionts extracted from *C. cardissa* and those belonging to the species, *S. pilosum*. However, the current study shows that *C. cardissa* associates with more than one ITS1 *Symbiodinium* group (lineage), as symbionts in network AII (that includes *S. pilosum*) and AIII were hosted by this clam species. It is likely that those distinct symbionts associated with some individual hosts from *C. cardissa*, and described by Banaszak et al. (1993) as *S. corculorum* might be in fact the ones included in network AIII. Further morphological and genetic revisions within this *Symbiodinium* group are needed to confirm whether or not the morphological described species, *S. corculorum* is a synonymy of *S. pilosum*, as recently suggested by LaJeunesse (2001).

Although the five evolutionary ITS1 lineages within phylotype *A* are well differentiated, the overall haplotype diversity within these lineages is remarkably low compared to lineages BI and C (Figs. 1B–C), which suggest that they are stable evolutionary units with low rate of divergence. While statistical association tests between hosts and symbiont haplotypes could not be performed within group *A* because of the small number of samples, certain degree of association at higher host taxa level was noticed (see Table 2). For instance, networks AI and AIV were represented by symbionts only harbored by cnidarian hosts, whereas symbionts in networks AV have been found uniquely in association with foraminiferan (Protists) hosts. These results from the faster evolving ITS1 contrast with data on 18S rDNA within phylotype *A*, which showed a lack of specificity of host–endosymbiont associations irrespective of taxonomic level (Rowan and Powers, 1991a,b). LaJeunesse (2002) has also shown evidence, at the level of ITS2 resolution, of specificity between some host–phylotype *A* symbiont partners, and that these specific associations occur over wide geographic ranges within the Caribbean Sea.

Ten out of the ITS1 haplotypes from phylotypes *B* showed to be closely related and resolved in one single network tree (BI), whilst the other haplotype (AF333510) revealed to be slightly different (4.26%) and resolved in a separate network (BII). This last haplotype within network BII belonged to the recently classified endosymbiont, *Symbiodinium muscatinei* (*sensu* LaJeu-

nesse and Trench, 2000), which seems to be uniquely associated with the host anemone, *Anthopleura elegantissima*. The results from this study support this newly classified endosymbiont species. Additionally, LaJeunesse (2001) distinguished three ITS subgroups within phylotype *B*, including sequences from *Symbiodinium pulchrorum* and *S. bermudense* (*sensu* Banaszak et al., 1993), while Santos et al. (2001) distinguished two subgroups. However, the genetic pairwise difference among their sequences, which all are included within network BI (Fig. 1B), was merely of 0.78%. This variation is very small compared to differences among all other ITS1 networks. Similar analyses (not shown here) on ITS2 region within this group also confirm this result. Additionally, the fact that no significant geographical association was found in the distribution of the haplotypes within network BI suggests that all the 10 haplotypes within this network, including the subgroups identified by LaJeunesse (2001) and Santos et al. (2001), belong to a single molecular evolutionary lineage. This group of symbionts also seems to be specific to cnidarian hosts, as shown in Table 2.

ITS1 haplotypes from the 28S rDNA phylotype *C* were also highly related phylogenetically among each other, resulting in a single network tree (Fig. 1C). However, the distribution of the 31 haplotypes within the network *C* did reveal geographical association, indicating that this phylotype might be, at least, represented by two evolving ITS1 lineages. As seen in Table 4, a significant association between geography and haplotypes in nested clade 4–1 was detected (Fig. 2C). The sub-clade 3–2 is represented mostly by haplotypes found in the North Western Pacific (Guam-Palau Is.) and Central Pacific Ocean (Hawaii Is.), while sub-clade 3–1 is represented by haplotypes from all around the world, including the Caribbean Sea and the Western Pacific Ocean, especially from the Coral Sea (Eastern Australian Seaboard). This result is consistent with the two ITS types in phylotype *C* identified previously by LaJeunesse (2001). The subclade 3–1 includes the ITS type C1 from LaJeunesse (2001), representing the only valid symbiont species described so far for the 28S-rDNA phylotype *C*, *S. goreauii* (LaJeunesse, 2001; Trench and Blank, 1987; Trench, 2000). Moreover, within this sub-clade the previous subgroups of ITS1 C1, C2, and C3 described by Van Oppen et al. (2001) were also encompassed. On the other hand, the sub-clade 3–2 includes the ITS type C2 from LaJeunesse (2001). Additionally, recent evidence from a large geographical scale study of ITS2 symbiont diversity within the Caribbean Sea indicates that the ITS type C2 is absent from this region (LaJeunesse, 2002), which support the finding that this ITS lineage (C2) is restricted at least within the central and north Western Pacific Ocean. Endosymbionts within network *C* are mainly associated with cnidarians and molluscans, however, a few symbiont haplotypes, from sub-clade 3–2 (type C2, *sensu* LaJeunesse, 2001) were found in association with protist (Foraminifera and Ciliata) hosts.

Network *E* was only represented by a single ITS1 haplotype (AF334659), and found to be highly different compared to all the other networks (over 32%). This result corresponds and supports the proposed separate species of *S. californium* described by Banaszak et al. (1993). Network *D* was also represented by a single ITS1 haplotype (AF334660), and showed to be, as network *E*, strikingly different to the other ITS1 networks (over 32%). Recent evidence based on the 18S rDNA region (Burnett, 2002) and the 28S rDNA region (Loh et al., 2001) suggests that this phylotype is abundant and diverse within the Indian Ocean and the South China Sea. However, more samples are still required to better understand the evolution of ITS1 haplotypes within these two phylotypes.

The diversity of ITS1 endosymbionts within phylotype *F*, which are mostly associated with soritacean foraminiferan, is indeed exceptional compared to other *Symbiodinium* reported from other hosts. Pochon et al. (2001) and Pawlowski et al. (2001) already recognized this fact based on their results from the 28S rDNA region, finding six very distinct groups of endosymbionts living uniquely within foraminiferans. In the present study, it was shown that the diversity of endosymbionts within soritacean is even greater, based on the ITS1 rDNA region, detecting the existence of apparently 11 molecular lineages of endosymbionts within phylotype *F*. The pairwise difference among these networks was in average $13.9 \pm 4.9\%$. And only the ITS1 networks from FVIII to FXI, which belong to the 28S-rDNA phylotype F4 (*sensu* Pochon et al., 2001), showed among them pairwise differences lower than 6%. The current data show that three of the ITS1 lineages within phylotype *F* have also been found in other marine invertebrate hosts. As for instance, network FVII, which includes the ITS type F1 ( = *S. kawagutii*, *sensu* LaJeunesse, 2001), contained symbiont haplotypes only found with scleractinian hosts. In fact, the scleractinian *Montipora verrucosa* both in the Coral Sea (Eastern Australian Seaboard) and Central Pacific Ocean (Hawaii Is.) seems to associate exclusively with symbionts from this ITS1 lineage. The other two networks are FIII and FVI, which also include symbiont haplotypes found in scleractinian and octocorallian hosts. With regard to the phylotype *G*, foraminiferan hosts also harbored the two resolved ITS1 lineages within this group.

This high diversity of endosymbionts found in foraminiferan hosts is coherent with the idea, suggested by Lee and Hallock (1987), that the evolution of soritacean foraminifera has been driven by endosymbiosis within these shallow-water benthic organisms. Richardson (2001) has recently shown evidence suggesting that the acquisition of new endosymbionts, changing from

chlorophyte to dinophyte, in soritacean facilitated a change in habitat from an epifaunal, free-living mode of life to one of living attached to phytal and non-phytal substrata. Furthermore, subsequent interplay between the newly acquired endosymbionts and the following change in the ecology of the group might have resulted in the subsequent concerted and rapid diversification of both endosymbionts and hosts. This rationalisation could explain why the three genera of Soratinae, originated 25 million years ago from Archaisinae (Haynes, 1981), harbor a highly diversified group of *Symbiodinium*-like dinoflagellates, while in cnidarians, which are known to have possessed endosymbionts since the Triassic period (Stanley and Swart, 1995), only a few number of endosymbiont phylotypes have been detected. Although, based on the conserved 18S rDNA (Lee et al., 1995), dinoflagellate-bearing soritid foraminifera seem to have originally acquired their symbionts from an environmental pool contributed to by cnidarians in their habitat probably millions of years ago, it appears that afterwards natural selection have acted on the foraminifera-endosymbiont association as single unit.

### *4.1. Recombination within Symbiodinium-like dinoflagellates*

The lack of recombination found in ITS1 sequences from *Symbiodinium*-like dinoflagellates within the major 28S-rDNA phylotypes could be the result of an absence of sexual reproduction within these organisms. However, it could also be the consequence of the concerted evolution of the ITS1 rDNA variants within the lineages (i.e., resolved networks) from the major phylotypes, even in presence of sexual reproduction. The low genetic variation (less than 4%) and lack of non-compatible sites of ITS1 sequences within the resolved networks might be the result of genetic homogenisation occurred through a process known as molecular drive (Elder and Tuner, 1995; Hillis and Dixon, 1991), which results in concerted evolution. However, on the other hand, the lack of recombination among the lineages within certain phylotypes (*A*, *B*, and *C*), in which the genetic difference is above 13%, suggest indeed an absence of hybridisation among these lineages. It is worth stating at this point that the situation within phylotype *F*, in terms of recombination is not that clear, as significant recombination was detected by one of the software programs used. Although it seems likely to be due to a statistical artefact based of the number gaps within the alignment, further analyses should be done on this group.

While sexual reproduction has been reported in dinoflagellates (Pfiester and Anderson, 1987), there has been little evidence of this in S*ymbiodinium*-like dinoflagellates (Baillie et al., 2000b; LaJeunesse, 2001). The results presented here show molecular evidence suggesting that at least very sporadic events of sexual recombination might be taking place within these organisms. This is consistent with the long-standing suggestions of the absence of sexual reproduction in *Symbiodinium* based on observations of cultured algae (Schoenberg and Trench, 1980a; Trench, 1987).

To date, there is still a debate upon which circumstances either sexuality or asexuality is favored by natural selection. Some recent experimental (Chippindale et al., 2001) and theoretical (Sa Martins and Moss de Oliveira, 1998) evidence show that sexual reproduction, and subsequent genetic recombination facilitate adaptive evolution, however some other theoretical studies support the conclusion that genome mixing is a risky endeavor, as recombination breaks up favorable gene combination that have increased in frequency under the action of natural selection (see Otto and Lenormand, 2002 for review). Moreover, asexual organisms can produce twice as many offspring as sexual organisms, so that the ratio of asexual to sexual offspring should initially double each generation, resulting in what has been called a "twofold cost" for sexual organisms (West et al., 1999). Additionally, it has been well documented that sexual reproduction effort is remarkably high (Stearns and Hoeksrea, 2000). Given to these costs, natural selection appears to have favored asexual reproduction in many wild populations of micro-organisms, which might also hold true for *Symbiodinium*-like dinoflagellates, as suggested in this study. Nevertheless, asexuality may make it difficult for a population to evolve in response to rapid changing environmental conditions (Hurst and Peck, 1996). And this is a critical issue under the current scenario of global climate changes occurring in our planet, which are currently threatening coral reefs.

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