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# A deep-sea slant on the molecular phylogeny of the Scleractinia

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**Abstract**

*Lophelia pertusa* and *Madrepora oculata* are azooxanthellate corals with nearly cosmopolitan distributions. They form cold-water reefs in the upper bathyal zone on continental margins and offshore banks [A.D. Rogers, Int. Rev. Hydrobiol. 84 (1999) 315]. *Lophelia* is classified in the family Caryophylliidae and *Madrepora* in the family Oculinidae, both on the basis of skeletal morphology. Recent molecular studies of the scleractinians have given a new insight into the evolutionary history of this group. This study was aimed at clarifying the phylogenetic relationships of *Lophelia* and *Madrepora*, through the analysis of partial sequences of the mitochondrial 16S rDNA. Sequences were obtained for samples of *L. pertusa* collected in the northeast Atlantic and off Brazil, *M. oculata*, four other deep-sea and eight tropical coral species from the Réunion island in the Indian Ocean. The sequences were aligned with 69 homologous sequences of Scleractinia. Maximum parsimony and Bayesian analyses support previously published molecular topologies. The two specimens of *L. pertusa* grouped with two caryophylliids, confirming the existing classification of the species, but the large genetic distance between the two *Lophelia* samples suggests that these populations are genetically isolated from one another. *M. oculata* did not cluster with oculinids, but formed a monotypic clade lying between the families Pocilloporidae and Caryophyliidae. Phylogenetic analysis also suggested cryptic speciation within the tropical taxa *Pocillopora meandriana* and possibly *Acropora humilis*.


*Keywords:* Scleractinia; Deep-sea corals; Phylogeny; *Lophelia*; *Madrepora*; Mitochondrial 16S rDNA; Indian ocean

## 1. Introduction

Colonial scleractinians are the main reef-building organisms of the planet, and sustain amongst the most species rich marine ecosystems. Among the 1314 currently known valid scleractinian species, 656 are zooxanthellate and 669 azooxanthellate (Cairns, 1999). If shallow-water corals are fairly well studied, the less accessible, deep-water fauna is still poorly known and new azooxanthellate genera are still being described (Cairns, 1999). Recent studies have shown that cold-water corals can form deep-water reefs associated with a diversity of fauna comparable to that harboured by tropical reefs (Rogers, 1999). Deep-sea corals have been reported worldwide on the shelf break and upper bathyal zone on the continental margins (Rogers, 1999). The main azooxanthellate reef-builders are the species *Lophelia pertusa*, *Goniocorella dumosa*, *Oculina varicosa*, and *Solensmilia variabilis*; other species, such as *Madrepora oculata*, *Desmophyllum dianthus*, *Dendrophyllia cornigera*, *S. variabilis*, and *Enallopsammia* spp. also contribute to the formation of these frameworks (Rogers, 1999). The systematics and distribution of these corals are important in terms of the distribution of diversity associated with the reefs they form, but are poorly understood (Rogers, 1999).

The advent of molecular approaches has considerably improved the understanding of the evolutionary relationships among scleractinians (Chen et al., 1995; Romano and Cairns, 2000; Romano and Palumbi, 1996; Veron et al., 1996). In particular, Romano and Palumbi (1996) showed that Scleractinia are divided into two main lineages that do not correspond to morphologically based suborders, and did not support morphological

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hypotheses about relationships among families and suborders. The suborder Caryophylliina and particularly the family Caryophylliidae, into which the species *L. pertusa* is placed, are not classified by a well-defined set of morphological characters. Romano and Cairns (2000) showed, through molecular data analysis, that representatives of the family Caryophylliidae were found throughout the phylogenetic tree of the Scleractinia, suggesting that this suborder was polyphyletic. They concluded that the use of a combination of morphological characters to define this family and its subfamilies has led to a poor resolution of the evolutionary relationships of its constituent genera and species. The family Oculinidae, into which *M. oculata* is placed, has a poor fossil record (Veron, 1995) and Romano and Cairns (2000) topology did not support its monophyletic status.

This study is based on phylogenetic analysis of partial sequences of the mitochondrial 16S ribosomal RNA encoding gene, which was shown to be informative for phylogenetic investigations of scleractinian corals (Romano and Cairns, 2000). It aimed at confirming the systematic classification of the deep-water corals *L. pertusa* and *M. oculata* by molecular phylogenetic comparisons with a range of new and previously published scleractinian coral sequences, mainly taken from the studies of Romano, Palumbi, and Cairns. New sequences comprise a range of tropical, shallow-water reef-forming corals and a range of azooxanthellate deep-sea species including the reef-builders *L. pertusa*, *M. oculata*, and *Dendrophyllia alternata* and the solitary corals *Fungicyathus marenzelleri*, *Caryophyllia ambrosia*, and *Flabellum angulare*.

## 2. Materials and methods

Specimens of deep-sea corals were collected by a variety of methods during research cruises. The tropical ones were sampled in Réunion Island by Jean-Pascal Quod and Lionel Bigot of ARVAM (Agence pour la Recherche et la Valorisation Marines, Saint-Denis, Ile de la Réunion, France) and identified by Professor Gérard Faure and Michel Pichon of Ecole Pratique des Hautes Etudes (Perpignan, France). For molecular analysis, the pieces of coral were kept in tanks containing seawater and tissue pieces were extracted from the coral colonies using a knife and immediately placed into 95% ethanol. See Appendix A for details of sample locations and collecting methods.

A preliminary study was carried out to identify useful primers for the consistent amplification of the mitochondrial 16S rRNA region for *L. pertusa*. DNA was extracted from the ethanol-preserved tissue using a high salt extraction protocol. Pieces of tissue were homogenised with 639 μl of extraction buffer (containing 600 μl of TNE, 15 μl of proteinase K, and 24 μl of 20% SDS) and incubated at 55 °C until completely dissolved. Three hundred microliters of 6 M NaCl were then added. This solution was mixed on a rotator for 20 min. The precipitate was then pelleted by centrifugation at 9875*g* for 20 min and the supernatent drawn off by pipette. Six hundred microliters of chloroform were added to the supernatant. This solution was mixed for 1 min by inversion and then underwent centrifugation at 9875*g* for 1 min. The resulting aqueous phase was drawn off and mixed with 750 μl of ice-cold isopropanol, followed by centrifugation at 9875*g* for 15 min. The pellet was washed with 70% ice-cold ethanol, subsequently dried and resuspended in TE (pH 8.0; 10 mM Tris–HCl, 1 mM EDTA).

Universal primers were used for PCR amplification of partial sequences of the 16S rRNA encoding gene:

16Sar (5′-CGCCTGTTTATCAAAAACAT-3′),

16Sbr (5′-CCGGTTTGAACTCAGATCATG-3′) (Palumbi et al., 1991).

The PCR solution contained: 5 μl of 10× PCR buffer (containing 1 mM Tris–HCl, KCl, pH 8.3), 5 μl of 3 mM $MgCl_2$, 4 μl of 0.2 mM dNTP, 5 μl of "Q-solution," 0.5 μl of *Taq* Polymerase (all reagents from Qiagen, Crawley, West Sussex, UK), 37.5 pmol of each primer and 1 ng of DNA template. An initial denaturation step of 95 °C for 4 min was performed before adding the *Taq* polymerase. Amplification was then carried out over 35 cycles of 1 min at 95 °C, 1 min at 55 °C, 1 min at 72 °C, followed by a 7 min extension step at 72 °C. PCR was performed in a Perkin–Elmer 480 thermocycler.

The PCR products were then separated on a 1% agarose gel, subsequently extracted from the gel and purified using the QIAquick Gel extraction kit (Qiagen). Cycle sequencing reactions were performed, using BigDye cycle sequencing kit (PE Applied Biosystems, Warrington, Cheshire, UK) according to the manufacturer's instructions and with 6 ng of amplified DNA. The sequencing reaction products were purified using Qiagen DyeEx Spin kits and sequences were detected on an ABI 377 automated sequencer. The samples were sequenced in both directions. A Blast search (basic local alignment search tool) was conducted on GenBank to ensure that the resulting DNA sequence data was homologous to partial 16S rRNA sequences for corals. PCR amplification and subsequent sequencing using the primers 16Sar and 16Sbr was inconsistent and subject to frequent failures (see Section 3). The sequences of two individuals were visualised using Chromas Version 1.62 (McCarthy, 1997) and aligned using Clustal X Version 1.5b (Thompson et al., 1997) to give a consensus sequence for *L. pertusa*.

Using this consensus sequence, internal primers were designed, using the programme Primer 3 (Rozen and Skaletsky, 1998):

LP16SF (5′-TTGACCGGTATGAATGGTGT-3′),
LP16SR (5′-TCCCCAGGGTAACTTTTATC-3′).

These primers gave consistent amplification and sequencing reactions for *L. pertusa* and additional coral material collected in the tropics by MLGV (see Appendix A). Subsequently, DNA was extracted using Qiagen QIAquick DNA extraction kits according to the manufacturer's instructions. Whenever possible, two individuals were amplified and sequenced for each species. The PCR solution contained: 2 μl of 10× PCR buffer, 4 μl of "Q-solution," 2 μl of 3 mM $MgCl_2$, 1.6 μl of 0.2 mM dNTP, 0.2 μl of *Taq* Polymerase (all reagents from Qiagen), 10 pmol of each primer and 2 ng of DNA template. The following PCR conditions were used: 95 °C for 5 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min and a final extension step of 72 °C for 10 min. The PCR was performed using Hybaid PCR Expess Thermal Cycler. The PCR products were then purified using Qiagen QIAquick spin columns. A cycle sequencing reaction was carried out using DYEnamic ET terminator reagent premix (Amersham Pharmacia, Little Chalfont, Buckinghamshire, UK) and 5 μl of amplified DNA. The reaction was performed using a PTC-0225 DNA Engine Tetrad (NJ Reasearch). The products were purified using an ethanol precipitation method according to the manufacturer's instructions (Amersham Pharmacia). A MegaBACE 500 (Amersham Pharmacia) automated sequencer was used for the visualisation of labelled DNA fragments resulting from the cycle sequencing reaction. The samples were sequenced in both directions for sequence checking. Base calling was checked as previously and one consensus sequence was obtained for each species.

### 2.1. Sequence analysis

Sequences were obtained for 15 scleractinian species, distributed in nine families. A Blast search was performed on GenBank and the matching homologous coral sequences were retained for subsequent alignment. These included 69 previously published sequences (France et al., 1996; Romano and Cairns, 2000; Romano and Palumbi, 1996) to give a total of 85 sequences representing 62 genera, distributed in 20 families (Appendix B). *Hydra vulgaris* (Cunningham and Buss, 1993) was used as an out-group.

The sequences were aligned using the multiple sequence alignment program PRRN Version 3.1.0b for Unix (Gotoh, 1996). This algorithm uses a double nested iterative strategy with randomization that optimizes the weighted sums-of-pairs with affine gap penalties; the weights and the alignment are thus simultaneously optimized (Notredame, 2001). Phylogenies were constructed using PAUP* Portable version 4.0b10 for Unix (Swofford, 1993). The data were analysed using maximum parsimony and minimum evolution criteria. For the second method, two models of evolution were specified, using the estimations provided by the program Modeltest (Posada and Crandall, 1998): the Hasegawa–Kishino–Yano nucleotide substitution model (Hasegawa et al., 1985) with a gamma distribution and the transversion model (Rodriguez et al., 1990) with a gamma distribution, according to the Likelihood Ratio Test criterion and the AIC (Akaike Information Criterion) (Akaike, 1974), respectively. Five hundred random replicates were used for all heuristic searches. The support of groupings was estimated using 1000 bootstrap replicates and the fast stepwise option. A Bayesian analysis was performed using the program MrBayes Version 3 (Huelsenbeck and Ronquist, 2001), setting the likelihood model according to Modeltest estimations. Trees were displayed using the software TreeView Version 1.6.0 (Page, 1996).

Pairwise genetic distances were calculated between all 85 sequences using the F84 model (Felsenstein and Churchill, 1996; Kishino and Hasegawa, 1989), with a gamma distribution and a coefficient of variation of 1.2657. This was done using DNADIST Version 3.6a2.1, from PHYLIP package (Felsenstein, 1990).

## 3. Results

Only two samples of *L. pertusa* were of a high enough quality for analysis following amplification with primers 16Sar and 16Sbr (Palumbi et al., 1991). The occurrence of secondary products resulted in high "background noise" and low levels of signal strength from sequencing reactions. Multiple amplification products were also reported in previous studies (Romano and Cairns, 2000; Romano and Palumbi, 1996). The two sequences were 565 bp long and aligned with other scleractinian 16S mt DNA sequences. The newly designed internal primers, LP16SF and LP16SR, gave consistent PCR amplifications with product ranging from 227 to 465 bp in length.

The parsimony analysis was based on 267 parsimony-informative characters. The bootstrap 50% majority-rule consensus tree was 987 steps long and showed a consistency index of 0.5866.

The tree resulting from Bayesian analysis is shown in Fig. 1. This tree showed a similar topology to maximum parsimony and maximum likelihood trees but had a better resolution. *L. pertusa* from the northeastern and the southwestern Atlantic cluster together with *Caryophyllia* spp. *M. oculata* does not cluster with other members of the family Oculinidae. Specimens of *Acropora humilis* from Réunion and Guam do not cluster together, though this topology has a low probability of partition (0.12). Specimens of *Pocillopora meandriana* from the Pacific and Indian Oceans do not group together, with a high probability of partition (0.95).

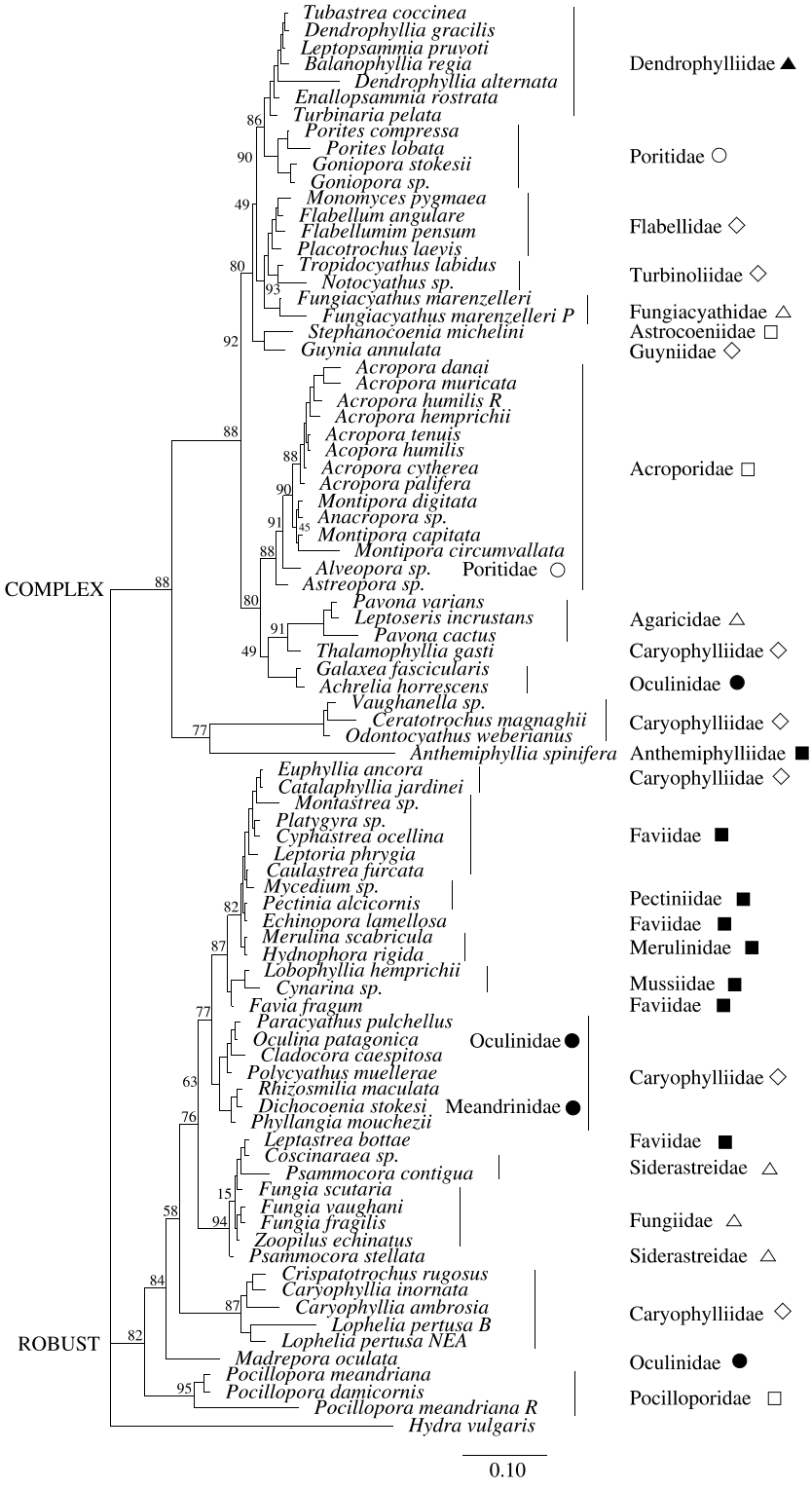


Fig. 1. Phylogram resulting from the Bayesian analysis using the transversion model with a gamma shape parameter (0.6242). Probabilities of the partitions, expressed in percentages, are shown at node labels. The scale unit is the mean of the posterior probability density. A capital letter by the species indicates the origin of the sample when necessary (*Acropora humilis* R—Réunion Island; *Lophelia pertusa* B—off Brazil [22°41.3′S, 40°27.3′W]; *Lophelia pertusa* N E A—North East Atlantic; *Fungiacyathus marenzelleri* P—Porcupine Seabight; *Pocillopora meandriana* R—Réunion Island). Symbols represent morphological suborders: □, Archaeocoeniina; △, Fungiina; ■, Faviina; ◇, Caryophylliina; ●, Meandriina; ○, Poritiina; and ▲, Dendrophylliina.

## 4. Discussion

The obtained topology showed distinct "robust" and "complex" clades, as described by Romano and collaborators (Romano and Cairns, 2000; Romano and Palumbi, 1996, 1997). Romano and Palumbi (1996) defined "robust" corals as having solid, heavily calcified skeletons resulting from the solid construction of corallite walls and forming massive or plate-like structures. The "complex" corals have less heavily calcified skeletons, resulting from the porous construction of the corallite walls showing a light, complex architecture.

*Lophelia pertusa* is placed, according to morphological characters, into the suborder Caryophylliina. According to Wells (1956), this suborder is the most successful of all scleractinian groups in adaptation to extreme environments and species generally occur in deep water, are azooxanthellate and often solitary (Cairns, 1990). It is defined by a combination of shared ancestral characters (Alloiteau, 1952; Chevalier and Beauvais, 1987; Roniewicz and Morycowa, 1993; Wells, 1956). Although most of the families from this suborder are found in the "complex" clade, representatives of the family Caryophylliidae are found throughout the scleractinian phylogenetic tree. This suggests that the suborder Caryophylliina and the family Caryophylliidae are not monophyletic, which supports the hypothesis formulated by Romano and Cairns (2000).

The family Caryophylliidae is divided into six subfamilies: the Thecocyathinae, Caryophylliinae, Turbinoliinae, Desmophyllinae, Parasmiliinae, and Eusmiliinae (Wells, 1956). The present topology globally supports the observations made by Romano and Cairns (2000) concerning the subfamily groupings and their relationships to other scleractinians.

The inclusion of one additional representative of the subfamily Desmophyllinae reveals that the representatives of this subfamily do not group together. *Thalamophyllia* groups with representatives of the Agariciidae, among the "complex" corals, as shown by Romano and Cairns (2000), whereas the two specimens of *L. pertusa* form a sister clade to the group consisting of the genera *Caryophyllia* and *Crispatotrochus*, among the "robust corals." These two genera are in the subfamily Caryophylliinae and were described as forming a well-supported clade by Romano and Cairns (2000). Other representatives of the subfamily Caryophylliinae are found throughout the topology, in the "robust" clade as well as in the "complex" clade.

The tree branches separating *L. pertusa* specimens collected in the northeast Atlantic and off Brazil are extremely long and the genetic distance separating them is of 6.96%. For congeneric species of the genus *Acropora*, the lowest genetic distances (0%) are recorded among specimens collected in the Pacific (*Acropora cytherea*, *A. humilis*, and *Acropora tenuis*) and the highest one (2.96%) is between a specimen of *Acropora palifera* collected in the Pacific Ocean and one of *Acropora muricata*, from the Indian Ocean. A very high genetic distance (7.90%) is reported between the specimen of *D. alternata* collected off Brazil and the one of *Dendrophyllia gracilis*, collected in the Bahamas. The genetic distance reported between the two *Lophelia* samples indicates a high level of genetic differentiation and suggests that eastern and western Atlantic populations have been genetically isolated for a considerable time, or may even represent separate species. This agrees with the conclusions of recent molecular studies showing that several invertebrates from the eastern Atlantic/Mediterranean and Brazilian coastal areas are amphi-Atlantic species complexes (e.g., sponges, Lazoski et al., 2001; molluscs *Octopus vulgaris*, Söller et al., 2000).

This phylogenetic analysis does not support the current classification of the deep-sea framework-building coral *M. oculata* in family Oculinidae (Wells, 1956). This family, along with the family Meandrinidae, were traditionally placed within the suborder Faviina (Wells, 1956). They now constitute the suborder Meandriina (Veron, 1995). In the present analysis, the representatives of the Oculinidae and Meandrinidae do not group together, but seem to be closely related to caryophyliids (see also Romano and Cairns, 2000). The family Oculinidae consists of two subfamilies (Wells, 1956), the Oculininae, which is represented in the present analysis by the genera *Oculina* and *Madrepora*, and the Galaxeinae by the genera *Achrelia* and *Galaxea*. The genera *Achrelia* and *Galaxea* group together among the "complex" corals, which is consistent with previous molecular analyses (Romano and Cairns, 2000). *Madrepora* and *Oculina* are both found in the "robust" clade, but *M. oculata* occurs in a monotypic grouping lying between the Caryophylliinae and the Pocilloporiidae. As such, this species may form a new family or even higher systematic grouping within the "robust" clade.

As with previous molecular phylogenetic analyses, the present tree indicates other problematic areas in the current classification of corals. At the suborder level, the monophyletic origin of the Archaeocoeniina is not supported. The families Astrocoeniidae and Acroporidae, both in the "complex" clade, group apart from the family Pocilloporidae, in the "robust" clade, which supports the results of Romano and Cairns (2000).

The family Acroporidae is described as the most speciose of all scleractinians (Wells, 1956) and shows unique features related to reproductive behaviour (Babcock et al., 1986). The evolutionary relationships within this family are still poorly known. Fukami et al. (2000), using the mitochondrial genes cytochrome *b* and ATPase 6 sequences, suggested a monophyletic origin to the genus *Acropora* and showed a close relationship between the genera *Montipora* and *Anacropora*. The present topology supports to this hypothesis. Moreover,

they found a significant divergence between the subgenera *Isopora* and *Acropora* and proposed that the two subgenera are classified as independent genera. In the present analysis, the probability of the partition between the species *Acropora palifera*, from the subgenus *Isopora*, and the representatives of the genus *Acropora* is high (0.88).

The monophyletic origin of the suborder Fungiina, was debated on several occasions: by Roniewicz and Morycowa (1993) on the basis of micro-structural characters, by Veron et al. (1996), using 28S rDNA sequences, and Romano and Cairns (2000), who used mitochondrial 16S DNA sequences. The present study does not support the monophyly of the Fungiina. As in Romano and Cairns (2000) topology, the families Siderastreidae and Fungiidae group together in the "robust" clade, the families Agaricidae and Fungicyathidae are found in the "complex" clade. The position of the genus *Fungiacyathus*, outside the Fungiidae, is consistent with the revision made on a morphological basis by Chevalier and Beauvais (1987), who created a new family for this genus, traditionally placed in the Fungiidae, and with the topology presented by Romano and Palumbi (1996). The genus *Psammocora* was first placed in the family Siderastreidae by Veron (1986), on the basis of skeletal characters; this was supported by Romano and Cairns topology (2000). The present study shows its placement within the Fungiina clade.

In the present topology, all the families from the suborder Faviina, except from the family Anthemiphylliidae, (Faviidae, Pectiniidae, Merulinidae, and Mussidae) are grouped within the "robust" clade, which supports a monophyletic origin of these families, as hypothesized by Veron et al. (1996) and Romano and Cairns (2000). The family Anthemiphyllidae was found in Romano and Cairns (2000) topology on a separate basal branch of the polytomy. It is found in the "complex" clade in the present study. Representatives of the family Faviidae all group in the clade Faviina, except for the two genera *Cladocora* and *Lepastrea*. *Cladocora* groups with caryophyllids in the "robust" clade. *Lepastrea* groups with the families Siderastreidae and Fungiidae. These observations support those of Romano and Cairns (2000), who suggested a re-examination of the taxonomic status for these genera.

The suborders Poritiina and Dendrophylliina are found among the "complex" corals and are closely related, as in Romano and Cairns (2000) topology. Veron et al. (1996) first hypothesized a close relationship between the family Poritidae, traditionally placed within the suborder Fungiina, and the family Dendrophylliidae. In the family Poritidae, the genera *Porites* and *Goniopora* group together, whereas the genus *Alveopora* groups with *Astreopora*, from the family Acroporidae. The affinities of *Alveopora* with acroporids have been already suggested on the basis of morphological (Veron et al., 1996) and molecular data (Romano and Cairns, 2000).

At the species level, the current analysis also revealed some potential misidentifications of tropical shallow-water corals. The sample of *P. meandriana* collected in Réunion Island does not form a cluster with the sample sequenced by Romano and Cairns (2000) and collected in Hawaii. The genetic distance between these two specimens (13.78%) is not in the range of values reported for congeneric species, even considering the generally high genetic distances between specimens collected in Réunion Island and in the western Atlantic or the Pacific Ocean. The highest genetic distance recorded between congeneric species for specimens collected in the Pacific and Indian Oceans is of 5.10%, for the genus *Montipora*. Moreover, the probability of partition between the specimen of *P. meandriana* (Réunion Island) and the cluster formed by *P. meandriana* and *Pocillopora damicornis* is high (0.95). This suggests that one of these specimens was misidentified or that this species is a complex.

Likewise, the sample of *A. humilis* collected in Réunion Island does not form a cluster with the one sequenced by Romano and Cairns (2000), collected in Guam. However, the genetic distance between these two samples is only 0.76%. The low probability of partition between these two groups (0.12) suggests that the resolution of the current analysis is not sufficient. Higher resolution molecular tools would be required to perform an investigation of this group. McMillan et al. (1991) showed differentiation among closely related species of *Acropora* using highly repetitive DNA sequences and Van Oppen et al. (2001) examined molecular relationships across 28 species of *Acropora* using a nuclear intron and the mtDNA putative control region.

## 5. Conclusions

Although based on only one molecular marker: partial sequences of the mitochondrial 16S ribosomal RNA encoding gene, this study allowed to place deep-sea corals into the context of recent studies on the phylogeny of Scleractinia. It supports the conclusions of previous molecular analyses: Scleractinians group in two major clades, only three morphological suborders appear as monophyletic: the Faviina, Poritiina, and Dendrophyliina and most morphological families have a monophyletic origin, except from the Faviidae, Caryophyliidae, Poritidae, and Oculinidae. This analysis has revealed the phylogenetic relationships of the deep-water corals *L. pertusa* and *M. oculata* with other scleractinians and has confirmed the taxonomic status

of a specimen collected off Brazil morphologically identified as *L. pertusa*. The high genetic distances reported between specimens collected off Brazil and samples from the same species (in the case of *L. pertusa*) or congeneric species (for *Dendrophyllia*) sampled in the northern Atlantic, suggest the existence of cryptic species among the still poorly known azooxanthellate corals. Cryptic speciation might also occur among shallow-water coral species, as shown through the analysis of specimens collected in the Indian Ocean. Misidentification of some of the samples is another possibility; this would confirm the idea that the morphological characters traditionally used for defining scleractinians are not sufficient for confident identification of species.

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## Appendix A

List of the species collected, including the sample location and collecting method

| Species | Sample location | Collection method | Cruise/collector |
|---|---|---|---|
| *Lophelia pertusa* | Rockall Trough | Agassiz trawl | RRS Discovery cruise 248 |
| | Galicia Bank | Agassiz trawl | Pelagia OMEX 98 |
| | Brazilian slope (22°41.3′S, 40°27.3′W) | ROV | Gardline Surveys Ltd |
| *Dendrophyllia alternata* | Brazilian slope (Campos Basin) (23°48.8′S, 41°41.3W) | Trawl | R.V. Prof. W. Besnard |
| *Madrepora oculata* | Porcupine Seabight | OTSB trawl | RRS Discovery cruise 260 |
| *Fungiacyathus marenzelleri* | Porcupine Seabight | OTSB trawl | RRS Discovery cruise 260 |
| *Caryophyllia ambrosia* | Porcupine Seabight | OTSB trawl | RRS Discovery cruise 260 |
| *Flabellum angulare* | Porcupine Seabight | OTSB trawl | RRS Discovery cruise 260 |
| *Pocillopora meandriana* | Réunion Island | Hand | J.P. Quod and L. Bigot |
| *Montipora circumvallata* | Réunion Island | Hand | J.P. Quod and L. Bigot |
| *Acropora danai* | Réunion Island | Hand | J.P. Quod and L. Bigot |
| *Acropora hemprichii* | Réunion Island | Hand | J.P. Quod and L. Bigot |
| *Acropora humilis* | Réunion Island | Hand | J.P. Quod and L. Bigot |
| *Acropora muricata* | Réunion Island | Hand | J.P. Quod and L. Bigot |
| *Psammocora contigua* | Réunion Island | Hand | J.P. Quod and L. Bigot |
| *Pavona cactus* | Réunion Island | Hand | J.P. Quod and L. Bigot |
| *Porites lobata* | Réunion Island | Hand | J.P. Quod and L. Bigot |

## Appendix B

List of the scleractinian species used in the phylogenetic analysis, including their sources and accession numbers

| SUBORDER<br>Family<br>Genera | Source | GenBank Accession Nos. |
|---|---|---|
| ARCHAEOCOENIINA | | |
| Astrocoeniidae | | |
| *Stephanocoenia michelini* | Romano and Cairns (2000) | AF265581 |
| Pocilloporidae | | |
| *Pocillopora damicorinis* | Romano and Cairns (2000) | L76019 |
| *Pocillopora meandriana* | Romano and Cairns, 2000 | L76018 |
| *Pocillopora meandriana* | Réunion Island | AF550373 |
| Acroporidae | | |
| *Montipora capitata* | Romano and Cairns (2000) | L76015 |
| *Montipora digitata* | Romano and Cairns (2000) | L75993 |
| *Montipora circumvallata* | Réunion Island | AF550368 |
| *Anacropora sp.* | Romano and Cairns (2000) | L75992 |
| *Acropora cytherea* | Romano and Cairns (2000) | L75995 |
| *Acropora hemprichii* | Réunion Island | AF550359 |
| *Acropora humilis* | Romano and Cairns (2000) | L75996 |
| | Réunion Island | AF550360 |
| *Acropora muricata* | Réunion Island | AF550361 |
| *Acropora palifera* | Romano and Cairns (2000) | AF265593 |
| *Acropora danai* | Réunion Island | AF550358 |
| *Acropora tenuis* | Van Oppen et al., 2002 | AF338425 |
| *Astreopora* sp. | Romano and Cairns (2000) | AF2665591 |
| FUNGIINA | | |
| Siderastreidae | | |
| *Psammocora contigua* | Réunion Island | AF550371 |
| *Psammocora stellata* | Romano and Cairns (2000) | L76021 |
| *Coscinaraea* sp. | Romano and Cairns (2000) | L76001 |
| Agariciidae | | |
| *Pavona cactus* | Réunion Island | AF550370 |
| *Pavona varians* | Romano and Cairns (2000) | L76016 |
| *Leptoseris incrustans* | Romano and Cairns (2000) | L76012 |
| Fungiidae | | |
| *Fungia scutaria* | Romano and Cairns (2000) | L76005 |
| *Fungia fragilis* | Romano and Cairns (2000) | L75998 |
| *Fungia vaughani* | Romano and Cairns (2000) | L75999 |
| *Zooplius echinatus* | Romano and Cairns (2000) | L76024 |
| Fungiacyathidae | | |
| *Fungiacyathus marenzelleri* | Romano and Cairns (2000) | L76004 |
| | Porcupine Seabight | AF550364 |
| FAVIINA | | |
| Pectinidae | | |
| *Pectinia alcicornis* | Romano and Cairns (2000) | L76017 |
| *Mycedium* sp. | Romano and Cairns (2000) | AF265608 |
| Mussidae | | |
| *Lobophyllia hemprichii* | Romano and Cairns (2000) | L76013 |

**Appendix B** (*continued*)

| SUBORDER<br>Family<br>Genera | Source | GenBank Accession Nos. |
|---|---|---|
| *Cynarina* sp. | Romano and Cairns (2000) | AF265613 |
| Merulinidae | | |
| *Hydnophora rigida* | Romano and Cairns (2000) | L76009 |
| *Merulina scabricula* | Romano and Cairns (2000) | L76014 |
| Anthemiphyllidae | | |
| *Anthemiphyllia spinifera* | Romano and Cairns (2000) | AF265596 |
| Faviidae | | |
| *Caulastrea furcata* | Romano and Cairns (2000) | L75997 |
| *Cyphastrea ocellina* | Romano and Cairns (2000) | L76132 |
| *Echinopora lamellosa* | Romano and Cairns (2000) | L76003 |
| *Lepastrea bottae* | Romano and Cairns (2000) | L76010 |
| *Leptoria phrygia* | Romano and Cairns (2000) | L76011 |
| *Montastrea* sp. | Romano and Cairns (2000) | AF265610 |
| *Platygyra* sp. | Romano and Cairns (2000) | AF265611 |
| *Cladocora caespitosa* | Romano and Cairns (2000) | AF265612 |
| *Favia fragum* | France et al. (1996) | U40295 |
| CARYOPHYLLIINA | | |
| Caryophylliidae | | |
| *Catalaphyllia jardinei* | Romano and Cairns (2000) | L76000 |
| *Euphyllia ancora* | Romano and Cairns (2000) | L76002 |
| *Rhizomsmilia maculata* | Romano and Cairns (2000) | AF265602 |
| *Thalamophyllia gasti* | Romano and Cairns (2000) | AF265590 |
| *Caryophyllia inornata* | Romano and Cairns (2000) | AF265599 |
| *Caryophyllia ambrosia* | Porcupine Seabight | AF550362 |
| *Phyllangia mouchezii* | Romano and Cairns (2000) | AF265605 |
| *Polycyathus muellerae* | Romano and Cairns (2000) | AF265606 |
| *Paracyathus pulchellus* | Romano and Cairns (2000) | AF265603 |
| *Crispatotrochus rugosus* | Romano and Cairns (2000) | AF265600 |
| *Odontocyathus weberianus* | Romano and Cairns (2000) | AF265594 |
| *Vaughanella* sp. | Romano and Cairns (2000) | AF265595 |
| *Ceratotrochus magnaghii* | Romano and Cairns (2000) | AF265597 |
| *Lophelia pertusa* | North East Atlantic | AF550367 |
| | Brazil | AF550365 |
| Flabellidae | | |
| *Flabellum impensum* | Romano and Cairns (2000) | AF265582 |
| *Flabellum angulare* | Porcupine Seabight | AF550363 |
| *Monomyces pygmaea* | Romano and Cairns (2000) | AF265583 |
| *Platotrochus laevis* | Romano and Cairns (2000) | AF265604 |
| Turbinoliidae | | |
| *Tropidocyathus labidus* | Romano and Cairns (2000) | AF265585 |
| *Notocyathus* sp. | Romano and Cairns (2000) | AF265584 |
| Guyniidae | | |
| *Guynia annulata* | Romano and Cairns (2000) | AF265580 |
| MEANDRIINA | | |
| Oculinidae | | |
| *Galaxea fascicularis* | Romano and Cairns (2000) | L76006 |
| *Achrelia horrescens* | Romano and Cairns (2000) | L75994 |

**Appendix B** (*continued*)

| SUBORDER<br>Family<br>Genera | Source | GenBank Accession Nos. |
|---|---|---|
| *Oculina patagonica* | Romano and Cairns (2000) | AF265601 |
| *Madrepora oculata* | Porcupine Seabight | AF550369 |
| Meandrinidae | | |
| *Dichocoenia stokesi* | Romano and Cairns (2000) | AF265607 |
| PORITIINA | | |
| Poritidae | | |
| *Porites compressa* | Romano and Cairns (2000) | L76020 |
| *Porites lobata* | Réunion Island | AF550372 |
| *Goniopora stokesii* | Romano and Cairns (2000) | L76008 |
| *Goniopora* sp. | Romano and Cairns (2000) | L76007 |
| *Alveopora* sp. | Romano and Cairns (2000) | AF265592 |
| DENDROPHYLLIINA | | |
| Dendrophylliidae | | |
| *Turbinaria pelata* | Romano and Cairns (2000) | L76023 |
| *Tubastrea coccinea* | Romano and Cairns (2000) | L76022 |
| *Dendrophyllia gracilis* | Romano and Cairns (2000) | AF265588 |
| *Dendrophyllia alternata* | Brazil | AF550366 |
| *Balanophyllia regia* | Romano and Cairns (2000) | AF265587 |
| *Leptopsammia pruvoti* | Romano and Cairns (2000) | AF265579 |
| *Enallopsammia rostrata* | France et al. (1996) | U40294 |

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