Patterns of septal biomineralization in Scleractinia compared with their 28S rRNA phylogeny: a dual approach for a new taxonomic framework

JEAN-PIERRE CUIF, GUILLAUME LECOINTRE, CHRISTINE PERRIN, ANNIE TILLIER & SIMON TILLIER

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A molecular phylogeny of the Scleractinia is reconstructed from approximately 700 nucleotides of the 5′end of the 28S rDNA obtained from 40 species. A comparison of molecular phylogenic trees with biomineralization patterns of coral septa suggests that at least five clades are corroborated by both types of data. Agaricidae and Dendrophylliidae are found to be monophyletic, that is supported by microstructural data. Conversely, Faviidae and Caryophylliidae are found to be paraphyletic: Cladocora should be excluded from the faviids, whereas Eusmilia should be excluded from the caryophylliids. The conclusion is also supported by the positions, sizes and shapes of centres of calcification. The traditional Guyniidae are diphyletic, corroborating Stolarski's hypothesis 'A'. Some results from our most parsimonious trees are not strongly statistically supported but corroborated by other molecular studies and microstructural observations. For example, in the scleractinian phylogenetic tree, there are several lines of evidence (including those from our data) to distinguish a Faviidae–Mussidae lineage and a Dendrophylliidae–Agaricidae–Poritidae–Siderastreidae lineage. From a methodological standpoint, our results suggest that co-ordinated studies creating links between biomineralization patterns and molecular phylogeny may provide an efficient working approach for a re-examination of scleractinian classification. This goal is important because in the evolutionary scheme proposed by Wells that presently remains the basic framework in coral studies, patterns of septal microstructures are involved. Validating from molecular phylogenies a given microstructural character state as a potential synapomorphy for a clade is the only way to include fossils in the coral classification, an approach that should allow the unity of coral classification to be maintained up to the origin of the phylum in the Triassic times.

Jean-Pierre Cuif, Faculté des Sciences, Bât. 504, Géologie F-91405 Orsay-Cedex, France.
Guillaume Lecointre, Annie Tillier & Simon Tillier, Service de Systématique Moléculaire, Muséum National d'Histoire Naturelle, 43 rue Cuvier, 75231 Paris Cedex 05, France. E-mail: lecointr@mnhn.fr
Christine Perrin, Laboratoire de Paléontologie (CNRS UMR 8569) Muséum National d'Histoire Naturelle, 8 rue Buffon, 75231 Paris Cedex 05, France.

Introduction

Since the beginning of coral studies, the classification of Scleractinia has been based on their calcareous skeletons observed at various scales, from overall morphology to microstructural patterns of the skeleton. Attempts to include characteristics of the polyps in supra-specific taxa (i.e. Duerden 1902; Matthai 1914) have not been continued. The taxonomic scheme based on skeletal characters and proposed by Vaughan & Wells (1943), later refined by Wells (1956), remains the common working framework among corallists dealing with both extant and fossil species.

Since Wells (1956), various attempts have been made to offer alternative views to the Wells global evolutionary proposal. Among them, Roniewicz & Morycowa (1993) and Veron (1995) are the only ones to present an 'evolutionary tree' that can formally be compared with that of Wells. Mastering the understatement style, Veron ironically quoted: 'Curiously, these two trees have little in common' (Veron *et al.* 1996: 8). In fact they are so different that their comparison fully demonstrates the need for a new approach to coral global taxonomy. Veron lucidly pointed out the origin of this result. The two evolutionary schemes are based on

completely different sets of characters, without any attempt to introduce any co-ordination between them. Roniewicz & Morycowa (1993) based their opinions on skeletal architectural features, as they were dealing with fossils studied mostly by using transverse sections. On the other hand, Veron (1995) based his attempt on external morphology and *in situ* observations of extant corals. From a methodological standpoint, the complete discrepancy between the two proposals can be considered as a positive conclusion. There is a need for a third independent data set to provide us with a tree which will allow us to understand why the two previous sets of characters are discordant. This will maximize the chances of assessing the phylogenetic value of skeletal characters; the ultimate aim being to use this knowledge when fossils will be included in the matrix of characters for phylogenetic analysis. This strategy is based on the methodological point of view according to which the reliability of phylogenetic inferences must come from corroboration (or repeatability) from multiple independent data sets (Hillis 1995; Miyamoto & Fitch 1995; Lecointre & Deleporte 2000; Chen *et al*. in press). Independence and naturalness of partitions must be thought of in terms of differences in the properties of the homoplasy accumulated in each partition.

Molecular phylogenies can help in providing us with such an independent tree. Partial Scleractinian molecular phylogenies already exist (Chen *et al*. 1995; Veron *et al*. 1996; Romano & Palumbi 1996; Romano & Cairns 2000) and initiated the 'molecular revolution' (Stolarski & Roniewicz 2001) of scleractinian classification. In addition to obvious disparity with recent morphology-based evolutionary schemes, discrepancies are also noticeable among conclusions of these early molecular studies. Differences in taxonomic sampling and tree reconstruction methods are probably responsible for apparent discrepancies among these first molecular trees. Moreover, and as it has long been recognized (Veron 1986), numerous genera gather extant species of doubtful biological validity. In order to determine the consistency between the present classification and any new taxonomic approach, a very careful selection of the species to be sampled is needed in order to preserve a coherent nomenclatural framework. Ideally, whenever possible, type species of generic names should be used; and nominotypic taxa of families should be included; and the various characters used for each species should be observed from a single specimen.

In the skeletal architecture of corals, improvement in our understanding of the biomineralization process led to a re-examination of the two basic concepts that have grounded the common view of coral microstructure for decades, and which underlie Wells' major scheme (Wells 1956, fig. 237): the 'trabecular organization' of septa (Ogilvie 1896) and the 'spherulitic crystallization process' (Bryan & Hill 1941). With respect to the progress that has been made during the last three decades in our understanding of how organisms control the formation of their mineralized hard parts, it appears that the monocrystalline structure of coral fibre or the trabecular microstructural model can no longer be accepted. Preparative methods based on the organic matrix concept (Towe 1972) allow new insights into the fine-scale organization of coral skeleton units for both fibres and centres of calcification. First among the investigators who were dealing with coral biomineralization, Johnston (1980) was able to present electron microscope pictures of the organic meshwork that controls aragonite crystallization, showing the obvious inadequacy of the spherulitic crystallization concept. As a consequence, the trabecular model of septal microstructure, based on the radial disposition of fibres, also appears in need of re-examination (Cuif *et al*. 1996; Cuif & Dauphin 1998; Cuif & Perrin 1999).

Here we report a new attempt to compare the Wells evolutionary tree with a double series of data: patterns of skeletal biomineralization and results from rDNA sequencing, both acquired from soft tissues and skeletons belonging to the same specimens.

Materials and methods

Materials

In order to create a reliable working framework that avoids sampling bias such as collecting potentially polyphyletic families using the wrong species, the following rules were observed during the collection of specimens (Table 1): (1) As far as possible, special effort was made to select the involved species among the key species in the present coral taxonomy, i.e. genus type species, with priority to genera that define families; (2) Although precision about the geographical origin of type specimens is frequently lacking, specimens representative of species were collected in geographical areas that, as far as possible, correspond to the type locality or initial indications (i.e. La Guadeloupe for 'Caribbean Sea' or 'West Indies', etc.).

Although selecting specimens with high taxonomic value was a key point in the field part of this work, this rigor also brings difficulties. Such difficulties can be illustrated by Acroporidae, the richest family among extant corals. Type specimens of the type species of *Acropora*, *Millepora muricata* Linnaeus, 1758: 792, were long lost, and uncertainty even exists on the biogeographical area of the species. Veron (1986: 128) proposed the 'West Indies', whereas Chevalier (1987: 688) indicated 'Ile d'Amboine (Indonésie)'. The question was recently solved by designation of a neotype from the reef faunas of Banda Island (Indonesia) by Veron & Wallace (1993). Difficulties in accessing the type locality explain why the key species for Acroporidae was not included in the present study. Unfortunately, no other data are available on this species because *Acropora muricata* was not involved in

Table 1 Taxonomic sampling, geographical origins and GenBank accession numbers.

Family	Name	Place	Collector	Year	Accession number
Agaricidae	*Agaricia undata* (Ellis & Sol)	Guadalupe Island	D. Lamy & J. P. Cuif	1998	AF549214
	Pavona varians (Verrill, 1864)	Moorea Island	M. Adjeroud & J.P. Cuif	1994	AF549254
	Leptoseris sp.	Moorea Island	M. Adjeroud & J.P. Cuif	1994	AF549215
Caryophylliidae	*Caryophyllia smithii* (Stockes & Broderip, 1828)	Pantelleria, siculotunisian detroit	H. Zibrowius	1997	AF549216
	Desmophyllum cristagalli (Milne-Edwards & Haime, 1848)	Strait of Sicily	H. Zibrowius	1997	AF549217
	Eusmilia fastigiata (Pallas, 1766)	Guadalupe Island	D. Lamy & J.P. Cuif	1998	AF549218
Dendrophylliidae	*Tubastrea micrantha* (Ehrenberg, 1834)	New Caledonia	P. Laboute	1997	AF549219
	Balanophyllia regia (Gasse, 1860)	France, Marseille, grotte du Figuier	H. Zibrowius	1997	AF549221
	Balanophyllia europaea (Risso, 1826)	France, Cassis, calanque du port Miou	H. Zibrowius	1997	AF549220
	Astroides calycularis (Pallas, 1766)	Pantellaria, strait of Sicily	H. Zibrowius	1997	AF549248
Faviidae	*Favia fragum* (Esper, 1795)	Guadalupe Island	D. Lamy & J.P. Cuif	1998	AF549222
	Favia stelligera (Dana, 1846)	Moorea Island	M. Adjeroud & J.P. Cuif	1994	AF549223
	Caulastrea furcata (Dana, 1848)	New Caledonia, banc Gail	P. Laboute	1997	AF549224
	Cladocora caespitosa (Lamarck, 1816)	France, Cassis, calanque du port Miou	H. Zibrowius	1997	AF549225
	Cladocora arbuscula (Lesueur, 1820)	Guadalupe Island	D. Lamy & J.P. Cuif	1998	AF549226
	Diploastrea heliopora (Lamarck, 1816)	New Caledonia, Utoé pente externe	P. Laboute	1997	AF549227
	Leptoria phrygia (Lamarck, 1806)	New Caledonia, Utoé pente externe	P. Laboute	1997	AF549228
	Montastrea annularis (Ellis & Sol., 1786)	Guadalupe Island	D. Lamy & J.P. Cuif	1999	AF549229
	Montastrea curta (Dana, 1846)	Moorea Island	M. Adjeroud & J.P. Cuif	1994	AF549230
	Platygyra daedalea (Ellis & Sol., 1786)	New Caledonia, Utoé pente externe	P. Laboute	1997	AF549231
	Leptastrea sp.	Moorea Island	M. Adjeroud & J.P. Cuif	1994	AF549232
Guyniidae	*Guynia annulata* (Duncan, 1873)	France, Cassis, grotte des Trémies	H. Zibrowius	1997	AF549233
	Stenocyathus vermiformis (Pourtalès, 1868)	Strait of Sicily, CS96-138	H. Zibrowius	1997	AF549247
Meandrinidae	*Meandrina pectinata* (Lamarck, 1801)	Guadalupe Island	D. Lamy & J.P. Cuif	1999	AF549234
	Dichocoenia stockesi (Milne-Edwards & Haime, 1848)	Guadalupe Island	D. Lamy & J.P. Cuif	1998	AF549235
	Dendrogyra cylindrus (Ehrenberg, 1834)	Guadalupe Island	D. Lamy & J.P. Cuif	1999	AF549249
Mussidae	*Mussa angulosa* (Pallas, 1776)	Guadalupe Island	D. Lamy & J.P. Cuif	1998	AF549236
	Lobophyllia corymbosa (Forskal, 1775)	Guadalupe Island	D. Lamy & J.P. Cuif	1998	AF549237
	Echinophyllia aspera (Ellis & Sol., 1786)	New Caledonia, Utoé pente externe	P. Laboute	1997	AF549241
	Manicina areolata (Ellis & Sol., 1786)	Guadalupe Island	D. Lamy & J.P. Cuif	1999	AF549255
	Mycetophyllia lamarckiana (Milne-Edwards & Haime, 1849)	Guadalupe Island	D. Lamy & J.P. Cuif	1998	AF549238
	Blastomussa merleti (Wells, 1961)	New Caledonia	P. Laboute	1997	AF549239
Oculinidae	*Oculina diffusa* (Lamarck, 1816)	Guadalupe Island	D. Lamy & J.P. Cuif	1998	AF549240
Pocilloporidae	*Pocillopora verrucosa* (Ellis & Sol., 1786)	Moorea Island	M. Adjeroud & J.P. Cuif	1994	AF549252
	Madracis pharensis (Heller, 1868)	France, Cassis, calanque du port Miou	H. Zibrowius	1997	AF549242
	Madracis decactis (Lyman, 1859)	Guadalupe Island	D. Lamy & J.P. Cuif	1998	AF549243
	Stylophora pistillata (Esper, 1797)	Aquarium of Monaco	J.P. Cuif	1998	AF549253
Poritiidae	*Porites porites* (Pallas, 1786)	Guadalupe Island	D. Lamy & J.P. Cuif	1998	AF549244
Rhizangiidae	*Phyllangia mouchezii* (Lacaze-Duthier, 1897)	France, Marseille, grotte du Figuier	H. Zibrowius	1997	AF549245
Siderastreidae	*Siderastrea radians* (Pallas, 1766)	Guadalupe Island	D. Lamy & J.P. Cuif	1998	AF549246
Outgroups	*Corynactis viridis*	France, Roscoff	M. Manuel	1998	AF549251
	Actinia sulcata	France, Roscoff	M. Manuel	1998	AF549250

the extensive molecular study by Romano & Cairns (2000). One could object that one could use another species (it is also the case for Fungiidae, Flabellidae). This criterion was followed only when external studies established the monophyly of the family. For example, *Dendrophyllia ramea* was lacking but replaced by other dendrophylliid species because Cairns (2001) established the monophyly of the Dendrophylliidae.

Forty species were involved in this study. They originated from three major reef areas: Caribbean archipelago (La Guadeloupe), Tuamotu (Moorea Island) and New Caledonia (Table 1). According to Wells' classification (Wells 1956), these species belong to 35 genera and to 12 families (Agariciidae, Caryophylliidae, Dendrophylliidae, Faviidae, Guyniidae, Meandrinidae, Mussidae, Oculinidae, Pocilloporidae, Poritidae, Rhizangiidae, Siderastreidae) and to four over the six superfamilies recognized by Wells (Agariciicae, Poriticae, Faviicae, Caryophylliicae). Molecular phylogenies were rooted on 28S sequences of a sea anemone (*Actinia*) and a corallimorpharian (*Corynactis*). Biological samples involved in the present study are registered under the numbers 504-4-409-1/01–1/06 within the collection of J.-P. Cuif, located at the Department of Geology, University of Paris XI (Orsay, France). A number

of specimens were from the collection of H. Zybrowius, Laboratory of Marine Biology, Endoume, rue Batterie des Lions (Marseille, France).

Biomineralization patterns

Coral species were recognized using morphological features, septal morphologies and corresponding septal ultrastructures. In a first step, improved morphological descriptions of septal growing edges were made, using scanning electron microscope observation of distal parts of the septa. Polished surfaces were then prepared by grinding the upper parts of the septa (sometimes after resin embedding), with water dispersing alumine powders or diamond spray; the ground surface being broadly perpendicular to the septal growth direction. The polished surfaces were etched using light formic acid (0.1%) with the addition of 3% glutaraldehyde (mean etching time: 45 s) or 2% formaldehyde solution followed by light acetic acid (1%) etching (20 s). The etching of polished surfaces reveals traces of successive micron-scaled growth steps that remain invisible on direct observation of the septa, even when using the scanning electron microscope; fibres appearing then as elongated and homogenous crystal-like units. Remnants of the fibre-producing biomineralization process consist of organic-rich and organic-depleted zones that were created within fibres by their incremental growth process, resulting in a differential sensibility to etching agents. Thus, a very clear observation of fibre growth patterns can be obtained, as well as a description of the positions and arrangements of calcification centres (Cuif & Dauphin 1998).

Molecular phylogeny

As the purpose of the study was to reassess both the monophyly and the relative positions of families (some of which date back to the early Mesozoic times), the 5′ end of the 28S rDNA appeared suitable. Romano & Cairns (2000) have already published a scleractinian neighbour joining tree from 28S sequences. However, these sequences cannot be mixed with the present ones for two reasons. First, the sequences on which Romano & Cairns's tree was based were 222 bp long, which is very short and may explain the lack of resolution of the corresponding 28S tree (Romano & Cairns 2000: 1056). The maximum sequence length for some of their taxa was 306 bp, while our sequences are more than 700 bp long: mixing both data would introduce a huge number of question marks into the matrix. Second, we consider that the reliability of the clades comes from corroboration through several independent studies rather than from total evidence approaches.

Sequencing techniques. Most of the tissues were fixed in 70% ethanol (Table 1). DNA extraction and washing were performed following the method of Winnepenninckx *et al.* (1993). Tissues were powdered in liquid nitrogen using a mortar and pestle, and then suspended in a CTAB solution at 60 °C. Some of them were directly incubated in the CTAB solution overnight. Total genomic DNA was precipitated by the addition of two-thirds of the total volume of isopropanol and stored at –20 °C overnight. After centrifugation, the pellet was washed, dried and resuspended in sterile ultrapure water. The DNA concentration was evaluated with a spectrophotometer. The polymerase chain reactions (PCR; Mullis & Faloona 1987; Saiki *et al.* 1988) were performed using the following universal primers generating double-stranded templates of portions of the 5′ end of the 28S rDNA (including the C1, D1, C2, and D2 domains, about 750 bp in total length). PCR was performed in a 50 µL volume using 0.3 µg of template DNA, with (final concentrations) 5% DMSO, 0.165 mM each dNTP, 1.5 U *Taq* DNA (Biotaq, Quantum Bioprobe), 20 mM Tris-HCl, pH 8.55, 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 150 µg/mL BSA, and 30 pmol of each of the two primers. The primer sequences were:

C1′ 5′-ACC CGC TGA ATT TAA GCA T-3′ located in the C1 domain, forward.

C2 5′-TGA ACT CTC TCT TCA AAG TTC TTT TC-3′ located in the C2 domain, reverse.

C2′ 5′-GAA AAG AAC TTT GRA RAG AGA GT-3′ located in the C2 domain, forward.

D2MAD 5′-TCG GAT GGA CCC ATA TGA-3′ located in the 3′ end of the D2 domain, reverse.

The primer 'D2MAD' was especially designed for scleractinians in order to avoid the amplification of resident zooxanthellae. Temperature cycles were performed using a Biometra trioblock. Thermal cycling was denaturation 94 °C 2 min, annealing temperature (55 °C) 2 min, extension 72 °C 2 min, then 30 × (94 °C 30 s, 55 °C 30 s, 72 °C 30 s). The 5′ end of the 28S rDNA was amplified either from the C1′–D2MAD (750 bp), C2′-D2MAD (400 bp), or C1′C2 (350 bp). The PCR products, which were always opened in a separate room under a special hood, were determined by electrophoresis in 1.5% agarose-BET and TBE buffer (Sambrook *et al.* 1989), and visualized with molecular weight marker XIV (Boehringer Mannheim). To remove active primers and nucleotides, the PCR products were purified from the agarose gel using the QIAEX II gel extraction kit (Qiagen) or directly treated with Exonuclease (5 U/5 µL PCR product) and Shrimp alcaline phosphatase (0.5 µL/5 µL PCR product) (purification kit Amersham). Most of the purified PCR templates were directly sequenced using the Thermosequenase cycle sequencing kit (Amersham). Direct sequencing was performed with the same primers used for the amplification. For four species (*Leptastrea* sp., *Leptoseris* sp., *Pavona varians*, *Pocillopora verrucosa*), the PCR products were cloned in the phagemidic PCRscript TM SK(+) vector using the PCRscript TM SK(+) cloning kit (Stratagene) following the procedure recommended by the manufacturer. This kit has a unique SrfI

site in the multiple cloning site (MCS) of the vector. The ligation is performed in the presence of SrfI and ligase; SrfI reopens religated vectors, and then maintains a high steady-state concentration of opened vector DNA, consequently increasing the ligation efficiency. A classical white/blue selection (Sambrook *et al*. 1989) was used for screening recombinant clones. Four white colonies per cloning were picked and grown overnight in L-broth at 37 °C. The phagemidic DNA was then extracted (Sambrook *et al*. 1989). For each colony, the size of the insert was determined by digestion of the recombinant phagemidic DNA with BssHII and electrophoresed in a 1% agarose gel (as described above). Sequencing on microplates was performed with the T7 sequencing kit from Pharmacia, using the method of terminator dideoxynucleotides (Sanger *et al*. 1977). Each colony was sequenced with external vector primers KS and T3, and at least two colonies per cloning were sequenced.

Data analysis. Sequences were read and entered twice using the computer package MUST (Philippe 1993), and aligned using the facilities of ED, within MUST. The total 28S data matrix contains 702 positions, including 340 variable and 225 positions informative for parsimony. In a first approach, insertions and deletions were analysed as such (one indel counting as one character whatever the number of indels in the neighbouring sites). A very short insertion of three bases is observed in the D1 domain of *Pavona*, *Agaricia* and *Leptoseris* (112–115). The situation is more complex in the D2 domain. Short unambiguous insertions are observed in the D2 domain of *Manicina* (362–363), *Mussa* and *Mycetophyllia* (389–390), *Pavona*, *Agaricia* and *Leptoseris* (607–608). A single long unambiguous insertion is observed in the D2 domain for *Corynactis* (449–454). These situations are easy to handle. Three more complex stretches exhibit indels of variable sizes. The region 496–512 is from 17 (in *Favia fragum*) to 10 nucleotides long, depending on the species. The shorter regions 536–542 and 573–588 also show length variations through the taxa. Consequently, three strategies were used: (1) data matrices were analysed with these three stretches aligned by eye, (2) after the removal of all positions exhibiting indels (in the latter case, the data matrix contains 601 positions), (3) the alignment was also performed via direct optimization using POY version 2 (Gladstein & Wheeler 2000) with various gap costs ranging from 2 to 9, in order to evaluate the impact of options in alignments on the stability of the clades discussed in this paper.

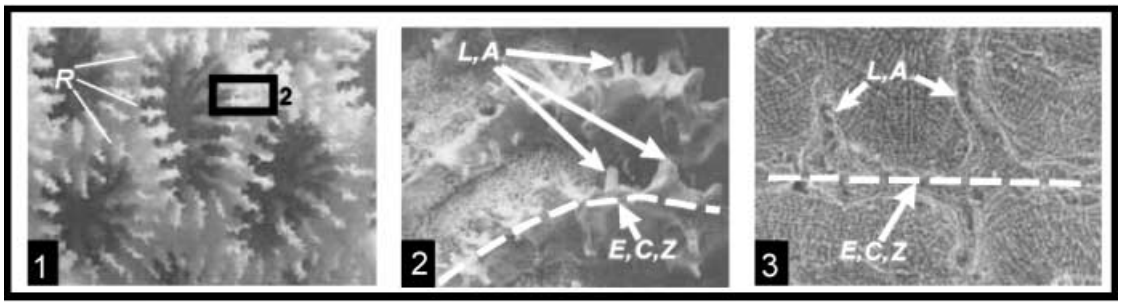
Using the complete manual alignment, relative transitional saturataion was examined using the COMP-MAT program of MUST, by plotting pairwise transitional differences against pairwise transversional differences. MUST includes the neighbour joining method (Saitou & Nei 1987) and allows very fast bootstrap analyses with this tree construction method using the NJBOOT program. These methods were used to determine differences in results between the neighbour joining and parsimony methods, a strategy useful not for getting the final tree, but to help in diagnosing the instability of long branches (see below). Bootstrap proportions from NJBOOT are generally slightly higher than those obtained from a bootstrap parsimony approach (data not shown). The main phylogenetic analyses were performed with PAUP 3.1.1 (Swofford 1993) and PAUP* version 4.0b2a for Macintosh (Swofford 1999). Characters were unordered. Heuristic searches were performed with the whole species set (42 species). Full heuristic searches with 100 random addition sequences followed by tree bisetion reconnexion (TBR) swapping were conducted. Bootstrap analyses (Felsenstein 1985) were performed with PAUP, using heuristic searches and 1000 iterations.

Absolute saturation tests were performed using COMP-MAT of MUST and PAUP 3.1.1. The pairwise numbers of differences were plotted against pairwise numbers of inferred substitutions in the most parsimonious tree (Philippe *et al*. 1994), for transitions and transversions separately (Lavoué *et al*. 2000). This allowed us to determine the absolute transversional saturation that would not have been detected by the relative saturation test described above. This was performed using ACCTRAN and DELTRAN optimizations in order to determine the impact of optimization on the plot. This impact has a null or negligible impact on the correlation of the two matrices.

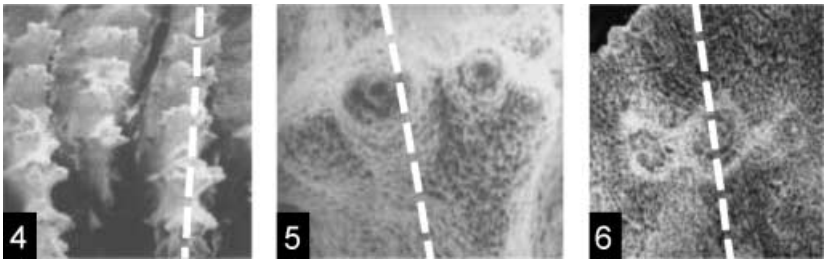
Results

The two-step secretory process of coral septa

Among these 40 species that represent the widest assemblage ever studied at an ultrastructural level, the formation of septal structures appears to follow a common two-step process [for a review see Cuif & Sorauf (2001)], with considerable differences in the development and organizational specificity of the two phases. The first step produces the initial septal framework at the upper edge of the septa, and is followed by an increase in septal thickness through the addition of lateral fibrous tissue. Actually, coral septa are complex three-dimensional structures not only because of the two different secretory processes that occur in the specialized zones of the ectoderm, but also as both secretory zones are growing upward, by the progressive overlapping of the upper part of the septum (i.e. the initial framework) by the fibrous tissue produced during the second biomineralizing step. Therefore, structures that are made visible on transverse sections of septa, although they only show a bi-dimensional view of this three-dimensional structure, must be interpreted by taking into account the time-based production of the two main septal calcareous structures. This is exemplified here, in septa of *Favia stelligera* (Figs 1–3): (a) The median part of the section of the septal structure (Fig. 3, white line), corresponds to the section of



Figs 1–3 *Favia stelligera*, from corallite morphology to biomineralization patterns. Figures 1–3 exemplify the basis of microstructural analysis of coral skeletons. The usual observation of corallite morphology (Fig. 1) or septal micromorphology (Fig. 2) is complemented by an analysis of the internal organization of calcareous units that results in the two-step biomineralization process. In *Favia stelligera* the early calcification zone (Fig. 2: ECZ) is continuous and rather straight, but produces numerous additional lateral axes (Fig. 2: LA) on which fibres develop during the second step of the septal growth. Figure 3 shows how the microstructural analysis provides us with fine-scaled and very informative data about septal growth. The correspondence between molecular data and the microstructural analysis opens the way to create a link between these two taxonomic approaches of coral skeletons. Figure 1: view of the calicinal surface (4×). Figure 2: morphology of the growing edge of the septa: lateral axes are well visible. Figure 3: polished and etched section showing global microstructural patterns. Etching makes the retrieval of the two-step growth process easier. It also allows more precise comparisons of biomineralization patterns between species.



Figs 4–6 *Favia fragum*. Upper view of the growing edge of the septa (Fig. 4: ×15); morphological view of a septal paddle (transversal group of calcification centres; Fig. 5: ×70); centres of calcification on a polished and etched section (Fig. 6: ×80).

the three-dimensional framework that was initially built, and is still visible at the growing edge of the septum (Fig. 2). Note the presence of the lateral axis (LA), perpendicular to the mediosepta plan. (b) On each side of the septal framework, but with frequently asymmetrical development, progressive embedding of the initial septal framework occurs, by incremental growth of fibres (Fig. 3). With respect to taxonomic practice, micromorphological observation of growing edges and study of etched transversal surfaces result in completely different evaluations of septal characteristics. The various granules, spines, etc., that have long been described as ornamental elements of septum sides (and therefore considered as secondary in importance), appear to be much more significant that previously admitted, as they are actually the parts of the initial framework that remain visible until they become completely covered by the second step fibrous tissue. Thus, taking into account the two-step formation of septa leads to a process-based evaluation of septal characteristics, that gives prominent value to the spatial arrangement of centres of calcification controlling the micromorphology of the initial septal framework.

Consistency of biomineralization patterns with presently admitted coral families

With respect to the family level in the Wells taxonomic proposal, study of spatial repartition and specific features of centres of calcification shows various situations. However, in some families, biomineralization patterns exhibit specific dispositions that could be accepted as synapomorphies for the family.

Comparison of ultrastructural patterns among species of the family Faviidae leads to recognition of various organizations of the initial septal framework. In the monotypic species for the family, *Favia fragum* (also type species of the genus), septa are characterized by short series of centres of calcification, well visible at the growing edge of the septa where they often build flat paddles that are transversally orientated with respect to the septal plan (Figs 4, 5; see also Cuif & Perrin 1999). This micromorphology is clearly recognizable through observation of biomineralization patterns. Centres of calcification that correspond to the growing tips of the septal framework paddles may be recognized on polished and etched sections, with global orientation transversal to the mediosepta plan (Fig. 6, white line). These well-defined

biomineralization units are separated by short septal zones in which centres occur in the median septal plan. This biomineralization pattern is shared by several traditional faviid genera, presently gathered in the family Faviidae. For instance, *Caulastrea furcata* (Fig. 7), *Leptoria phrygia*, *Platygyra daedalea*, *Manicina areolata* (Fig. 8) exhibit very comparable septal structures. Whatever the proportion between the radial lines of centres and transversal series that build paddles, the repetition of the paddle-producing growth process leads to the formation of a vertically orientated septal architecture (i.e. in correspondence with the growth direction).

In contrast, some other members of the family Faviidae *sensu* Wells, i.e. in *Cladocora caespitosa*, septa are built on the basis of a single linear series of centres of calcification, isodiametric (about 20 µm in diameter) and regularly spaced (Figs 9–11), and arranged in a purely radial alignment (no indication of transversal series of centres). This suggests that *Cladocora* does not belong to the Faviidae.

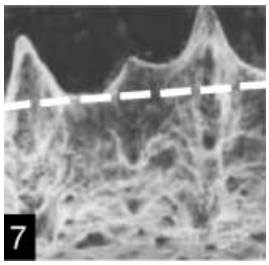


Fig. 7 Oblique view of the septal growing edge in *Caulastrea furcata*. Note the morphological similarity with *Favia fragum* (×60).

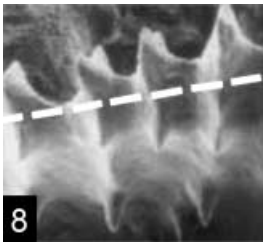


Fig. 8 The septal growing edge of *Manicina areolata*, another Faviid coral, with typical production of series of structural units repeated in a vertical arrangement (×40).

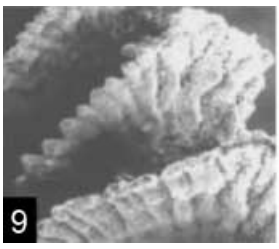
In members of the family Mussidae (*sensu* Wells 1956), septa are morphologically characterized by the 'mussoid teeth' (Fig. 12). At the growing edge of septa, centres of calcification are densely packed: the mussoid tooth is built by a multilayered arrangement of centres of calcification (Fig. 13). However, linear continuity is maintained between teeth because centres exist in the lower parts of the septal growth line. Conversely, in *Diploastrea heliopora*, the strong septal teeth (Fig. 14) that are usually assimilated to mussoid teeth, appear to be made by closely patched centres of calcification, with no continuity between the separated patches. This results in the very different profile of the septal teeth between true mussids and *Diploastrea heliopora*, suggesting an isolated taxonomic position for this monospecific genus.

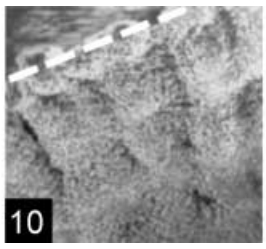
In representatives of Agaricidae *sensu* Wells, centres of calcification are densely arranged in a single straight line. This feature is consistent with the linear and smooth profile of the growing edge of septa. However, lateral crests that are synchronically produced on both sides of the septa with rather regular alternation are typical for this family. When such structures are in contact with the observation surface, zig zag lines or branching patterns can be observed in the septal framework.

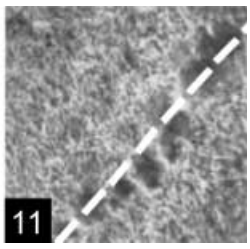
In the four species of Dendrophylliidae studied here, calcification centres are very distinct with respect to their size and spatial arrangement. Centres appear to be less than 4–5 µm in diameter (they reach up to 25–30 µm in other families studied here), and are irregularly grouped in small clusters (Figs 18, 19) instead of being simply circular patches as in other families (Figs 5, 11).

28S-based phylogeny

Relative saturation plots exhibited a good correlation between pairwise transitional differences and pairwise transversional differences ($r = 0.83$), with no plateau. Absolute saturation plots exhibited no mutational saturation for transversions, but clearly exhibited a plateau for transitions (plots available upon request). Saturation was reached for taxa with long branches such as *Favia fragum*, *Manicina*, *Pocillopora* and *Stylophora*. We do not favour the complete removal of transitions because homoplasy is not homogeneously spread across the molecular trees (Philippe *et al*. 1996), a feature that partly



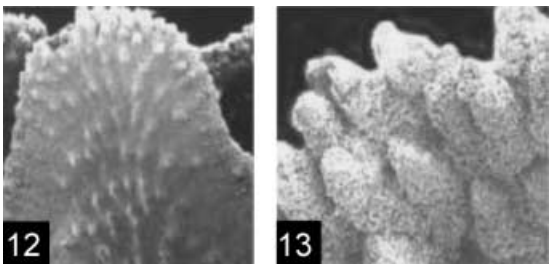




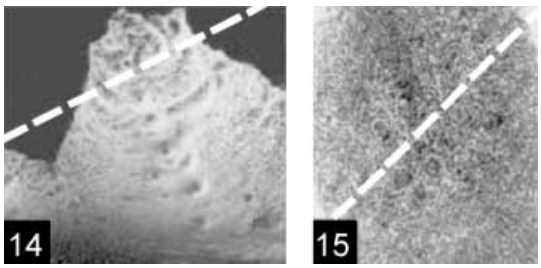
Figs 9–11 *Cladocora caespitosa*. Calicinal surface (Fig. 8: ×10); lateral view of septa (Fig. 9: ×20); growing edge of a septum (Fig. 10: ×90); linear arrangement of centres of calcification in a polished and etched section (Fig. 11: ×80).

Table 2 Stability of clades of interest across alignments involving different gap costs. The first column of results gives the presence of clades in the strict consensus of the 40 equiparsimonious trees obtained from the manual alignment using ED of MUST (Philippe 1993). The second gives the same type of data for the same alignment, from which stretches of indels have been removed (see Materials and methods). The next columns give the same kind of data from direct optimization (Gladstein & Wheeler 2000) with increasing gap cost (2, 3, 4 … until 9) in the alignment. Analyses of trees show that clades A and B are not recovered in direct optimization because of unstable branching of two faviid long branches *Favia fragum*, *Manicina areolata*, and the basal attraction of the long blanch *Blastomussa merleti*.

Clade	Name	All manual 40 (Fig. 20)	Removed 346 (Fig. 21)	2 9	3 1	4 3	5 1	6 25	7 13	8 14	9 2	Interpretation
Taxa with transversal centres	A	no	yes	no	no	no	no	no	no	no	no	
A minus *Blastomussa, Manicina, F. fragum*	A′	yes	no	no	yes	yes	yes	yes	no	no	yes	Clade A minus three long branches
F + G + *Guynia* + *Siderastrea* + *Porites*	B	yes	yes	yes	no	no	no	no	no	no	no	Complex corals
Eusmilia with Meandrinidae	C	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	Paraphyly of the Caryophylliidae
Cladocora with *Oculina*	D	yes	yes	no	yes	yes	yes	yes	yes	yes	yes	Paraphyly of the Faviidae
Stenocyathus with other Caryophylliidae	E	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	Polyphylies of traditional guyniids and caryophylliids
Agaricidae	F	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	Monophyly of the Agaricidae
Dendrophylliidae	G	yes	yes	yes	yes	yes	yes	yes	yes	no	no	Monophyly of the Dendrophylliidae
B minus *Porites*	H	yes	no	yes	yes	no	yes	yes	no	no	no	
Pocilloporidae with *Porites*	I	no	no	no	no	yes	no	yes	yes	yes	no	
C + *Leptastrea*	J	no	yes	no	yes	yes	yes	yes	yes	yes	yes	Paraphyly of the Faviidae
Pocilloporidae	K	no	no	yes	yes	yes	yes	yes	yes	yes	yes	Monophyly of the 4 Pocilloporidae



Figs 12, 13 *Lobophyllia corymbosa*, a representative of the family Mussidae, showing the fan system organization of the 'mussoid tooth' (Fig. 12: ×12) with a multilayered organization of centres of calcification at the top (Fig. 13: ×75).



Figs 14, 15 In contrast to previous species, the septal tooth of *Diploastrea heliopora* exhibits an axial arrangement of centres of calcification, gathered within a circular zone at the distal part of each conical tooth. (Fig. 14: ×18; Fig. 15: ×40).

explains why homoplasy may increase phylogenetic structure (Philippe *et al*. 1996; Källersjö *et al*. 1999; Wenzel & Siddall 1999; Sennblad & Bremer 2000). Removing homoplasy is also removing signal. We therefore prefer to use absolute saturation plots exclusively for identifying those taxa responsible for an excess of homoplasy (homoplasy providing the plateau) and then to modulate the phylogenetic interpretations concerning these taxa. The reliability of their position in trees will be far weaker whatever the statistical robustness associated. Following this strategy, we know in advance that we will not have to give much credit to the interrelationships of taxa responsible for saturation.

Using random addition sequences (100 iterations), the matrix with indels (702 positions) yielded 346 equiparsimonious trees of 1046 steps [consistency index (CI) = 0.502, retention index (RI) = 0.626; strict consensus in Fig. 20]. The matrix without indels (601 positions) yielded (under the same conditions) 40 equiparsimonious trees of 741 steps (CI = 0.471, RI = 0.626; strict consensus in Fig. 21). In Figs 20 and 21 the following clades are noticeable: the Agaricidae (F), the Dendrophylliidae (G), clade C (the Meandrinidae with *Eusmilia* and *Phyllangia*), clade D (*Cladocora* with *Oculina*, showing the paraphyly of the Faviidae), clade E, i.e. the Caryophylliidae excluding *Eusmilia* and including *Stenocyathus* (Guyniidae). Our data support the paraphylies of the Caryophylliidae (clade C), the Faviidae (clade D), and the traditional Guyniidae also called Guynioidea (Stolarski 2000) (clade E), with high bootstrap supports and whatever the gap cost in alignments (Table 2). The paraphylies of the Mussidae and the Pocilloporidae are not reliable because of possible long branch attraction artefacts (see Discussion). The paraphyly of the Meandrinidae is not robustly supported: within clade C the

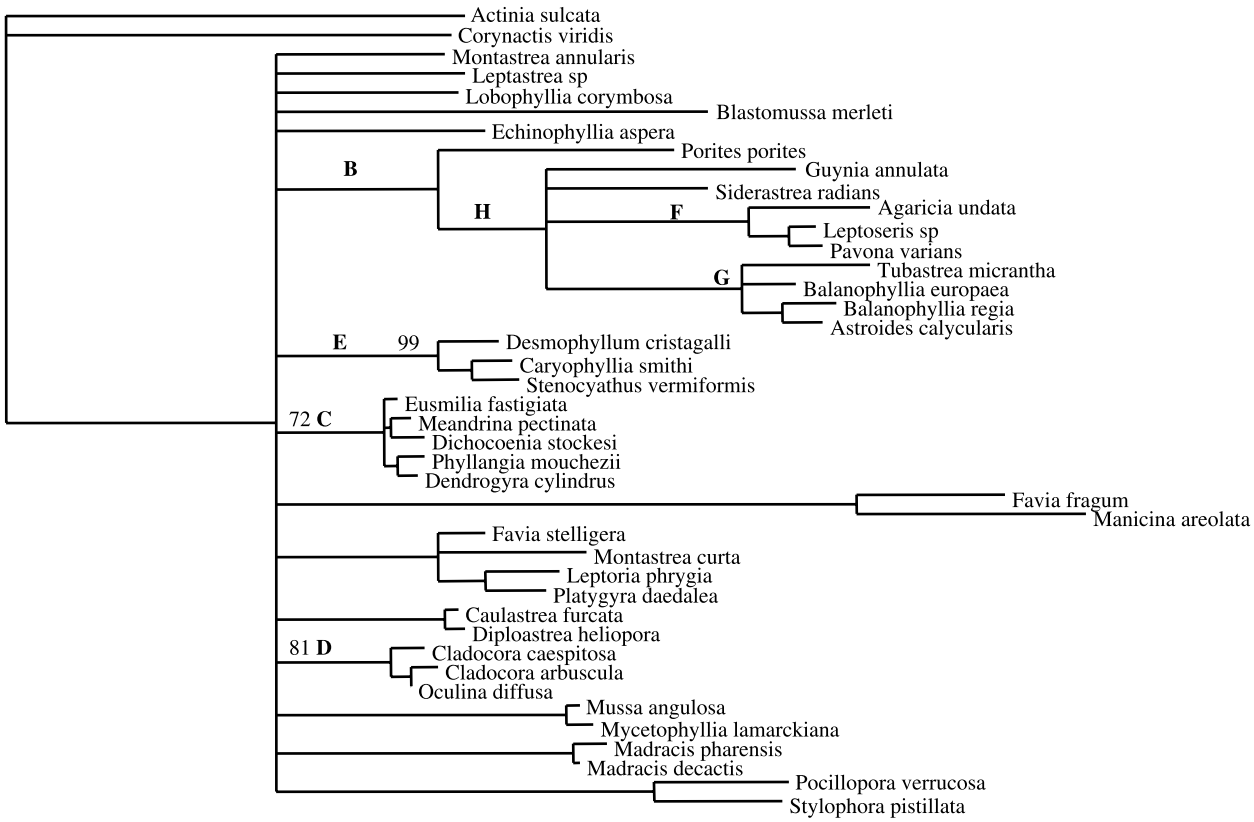


Fig. 20 Strict consensus of 346 most parsimonious trees of 1046 steps (consistency index = 0.502, retention index = 0.626) from the matrix including indels (702 characters). The branch length is proportional to the number of steps, the numbers above internal branches are bootstrap proportions calculated from 1000 iterations. Only bootstrap proportions over 70% are shown.

branch length of the node grouping *Phyllangia* and *Dendrogyra* is very short; *Phyllangia* and *Eusmilia* could be external to the three present meandrinid genera with a very few extra steps.

In Figs 20 and 21, two other nodes require attention, not because of their present statistical support, but in relation to other molecular studies and corroboration from microstructural characters. Clade A (Faviidae–Mussidae lineage), if we leave aside the groupings of saturated sequences possibly due to long branch attraction (*Favia fragum*, *Manicina*, *Blastomussa*, *Pocillopora*, *Stylophora*), is found in four other molecular studies and is corroborated by microstructural features (see discussion). Another clade of interest is clade H, corresponding to clade B minus *Porites*, as *Porites* is the most basal within clade B and sometimes groups with the monophyletic Pocilloporidae (monophyletic Pocilloporidae is clade K, Table 2).

Discussion

To date, no formal consensus exists concerning the position of the various biomineralization patterns of Scleractinia with respect to global evolution of the order (Roniewicz & Morycowa 1993). This study, which first aimed to determine the extent to which potential synapomorphies suggested by biomineralization patterns are corroborated by molecular trees, provides us with the additional opportunity to suggest global relationships between microstructural clusters.

Tree reconstruction artefacts

Five taxa (*Favia fragum*, *Manicina areolata*, *Pocillopora verrucosa*, *Stylophora pistillata* and, to a lesser extent, *Blastomussa merleti*) exhibit longer branches (Fig. 20), which are not attracted towards the outgroups in the most parsimonious trees (Figs 20, 21). From both matrices, a neighbour joining tree clusters these five taxa together or puts them paraphyletic at the bottom of the tree (trees not shown), showing the sensitivity of that method to basal long branch attraction artefacts (Philippe & Adoutte 1998: 47; Philippe & Laurent 1998; Philippe & Forterre 1999). It was previously noticed that this method enables taxa to be clustered on the sole basis of symplesiomorphic nucleotides, even when no long branch attraction at all could be suspected (Leclerc *et al.* 1998). Here our empirical case shows a higher sensitivity of the neighbour joining method to basal attractions of long branches with the

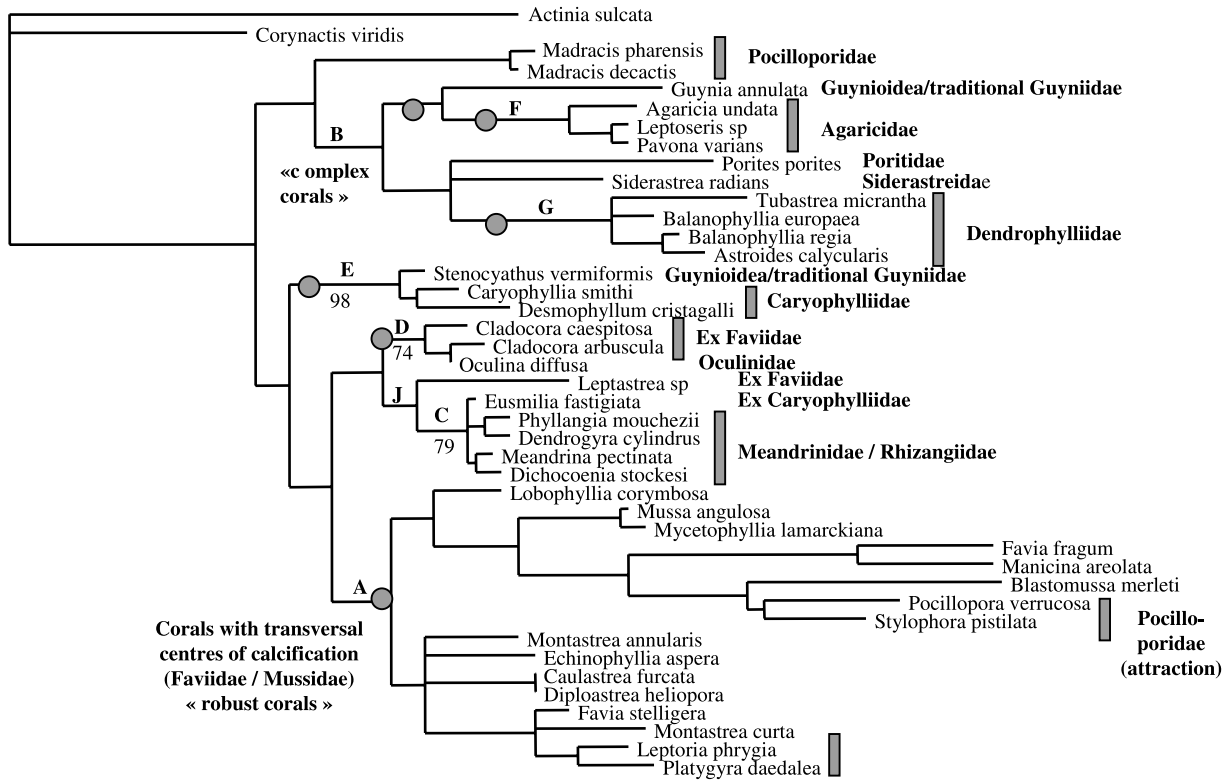


Fig. 21 Strict consensus of 40 most parsimonious trees of 741 steps (consistency index = 0.471, retention index = 0.626) from the matrix excluding indels (601 characters). The branch length is proportional to the number of steps, the numbers above internal branches are bootstrap proportions calculated from 1000 iterations. Only bootstrap proportions over 70% are shown. Circles are clades that can be supported by biomineralization patterns of coral septa.

outgroups. In the most parsimonious trees, these five taxa do form a clade that is not basal, but always illustrates artefactual groupings of fast evolving taxa (Felsenstein 1978). This clade suggests the paraphyly of the Pocilloporidae. This paraphyly is not reliable, the clade *Pocillopora* + *Stylophora* possibly being attracted towards *Favia fragum* because of common long branches. Monophyletic Pocilloporidae (*Pocillopora* + *Stylophora* as the sister group of *Madracis*) are found among parsimonious trees a single step longer. Table 2 helps us to understand the impact of these five taxa. Among different alignments of different gap costs, the monophyly of the Pocilloporidae (clade K) is always found, and it is never possible to recover clade A because *Blastomussa*, *Manicina* and *Favia fragum* are moving across the tree, a topological instability that prevents any conclusion about the relationships of these three taxa. When clade A′ is considered (A minus these three), its stability across different gap costs is far better. Moreover, these three taxa lead to an underestimation of the reliability of clade B, which is never recovered from alignments with different gap costs in direct optimization. This is due to the fact that in gaps costs 4, 7, 8, 9, *Manicina* and *Favia fragum* come within clade B with no stable position, which explains why in Table 2 clade B is never recovered as monophyletic. With gaps costs 4, 5, 6, 7, 8, *Porites* is no longer in clade B as the most basal, but becomes the sister group of the Pocilloporidae. The monophyly of clade H (which is B minus *Porites*) is recovered in gap costs 2, 3, 5, 6, exactly when *Favia fragum* and *Manicina* do not branch within clade B.

Four molecular studies: comparisons

In the studies of Chen *et al.* (1995), Veron *et al.* (1996) and Romano & Cairns (2000) based on shorter sequences of 28S rDNA, the same main dichotomy was found: the Faviidae–Mussidae lineage (our clade A), and the Poritidae–Dendrophylliidae lineage (our clade B). Interestingly, Romano & Palumbi (1996), then Romano & Cairns (2000), found these same groupings from a different source of data, the mitochondrial 16S rDNA. Comparing these studies with ours, there are four common families branched between lineage A and lineage B, of which the interrelationships never meet the complete taxonomic congruence: the Pocilloporidae, the Meandrinidae, the Siderastreidae, and the Caryophylliidae.

From our Table 2 it is impossible to say whether the Poritidae are the sister group of the Pocilloporidae (clade I) or the sister

group of clade H (the rest of clade B). In Veron *et al*. (1996), Pocilloporidae were found to be more closely related to a group corresponding to our clade A. However, the statistical support was weak. In the studies of Romano & Palumbi (1996) and Romano & Cairns (2000), the representative of the Pocilloporidae was also found to be closer to the Faviidae–Mussidae (clade A) than to the Dendrophylliidae–Poritidae, with a better statistical support. Our data suggest that neither the paraphyly of the Pocilloporidae nor the relationships of its components (*Madracis*, *Stylophora*, *Pocillopora*) are supported. Nevertheless, it has to be noticed that the monophyly of the Pocilloporidae is recovered across direct alignments with different gap costs (Table 2). Moreover, in Fig. 21, there is a risk that the relationships shown for *Pocillopora* and *Stylophora* are due to an attraction towards *Favia fragum* and *Manicina* because of common higher rates of change. We are therefore inclined to disregard the apparent contradiction between the present position of *Madracis* and the positions of Pocilloporidae in the studies of Romano & Palumbi (1996) and Romano & Cairns (2000), and to take as unreliable the apparent paraphyly of the Pocilloporidae.

There is an apparent contradiction between the relationships of the Meandrinidae in the studies of Veron *et al*. (1996) and Romano & Cairns (2000) as closer to the Mussidae–Faviidae group (like here), and the relationships of the 'Meandriina' in the tree of Romano & Palumbi (1996) as closer to the Poritidae–Dendrophylliidae group, named 'complex corals'. Our study exhibits poor statistical support for the node grouping the Meandrinidae with clade A. However, that node was recovered by Romano & Cairns (2000) from 68 16S mtDNA sequences and 45 shorter 28S sequences. One must keep in mind that the meandrinid species chosen in Romano & Palumbi (1996) were all different (even the genera were different from those used in other studies). This weakens the evaluation of taxonomic congruence, at least because neither the homogeneity of the genus *Meandrina* nor the monophyly of the Meandriina are well established. This is the reason why we have chosen to sample type species of each genus. Congruence is far better when the genera chosen are the same, such as between the 16S tree of Romano & Cairns (2000) and the present study.

There is a rather strong contradiction between the relationships found for the Siderastreidae in Veron *et al*. (1996) (as the sister group of the clade Pocilloporidae + Meandrinidae + clade A with a good support) or in Romano & Cairns (2000) (closer to clade A) and the relationships of the Siderastreidae found here (within clade B, closely related to the Dendrophylliidae and Agaricidae). This contradiction must be further evaluated using species common to both data sets, as the genera sampled were not the same in the three studies [*Pseudosiderastrea* in Veron *et al*. (1996) and in the 28S data of Romano & Cairns (2000), *Psammocora* and *Coscinarea* in Romano & Cairns's 16S tree, and *Siderastrea* here], suggesting polyphyly of the Siderastreidae. This polyphyly may be supported by the fact that in Romano & Cairns's trees, the Siderastreidae are inside (16S) or outside (28S) the 'robust' corals according to the genus (and the gene) used.

Romano & Palumbi (1996) obtained good statistical support for the Caryophylliina closely related to the Faviidae. Their two caryophylliinan genera (not the same as ours) are even closer to the Faviidae than their representative of the Mussidae. In our trees, the Mussidae appear closer to the Faviidae than the Caryophylliidae are, but the statistical support is weak and the studied genera were different. Interestingly, the genera sampled here allowed us to show with high support that the Caryophylliidae are polyphyletic, a polyphyly already clearly shown by the two trees of Romano & Cairns (2000).

Comparison of biomineralization patterns and molecular trees

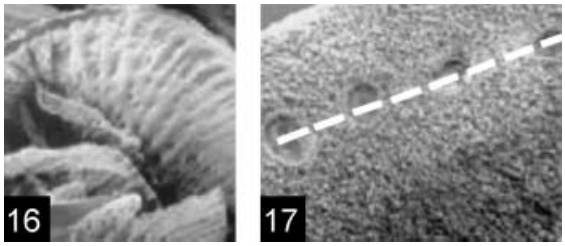
The molecular data included the Faviidae–Mussidae lineage in clade A and suggested excluding *Cladocora* and *Leptastrea* from the Faviidae (Table 2, lines D and J). In *Favia fragum*, the type species of the type genus of the Faviidae, septal growing edges are well characterized from both morphological and microstructural standpoints. Regularly arranged growth units at the septal edge (Figs 11, 12) are produced by short series of calcification centres that exhibit a transversal arrangement with respect to the radial direction of the septal plan. Superposition of these short series of growth increments results in a vertically arranged ornamentation of the septal sides, not found in *Agaricia* and *Pavona*, in which the septal sides typically bear horizontal series of spines or granules. The presence of transversal series of centres is also typically observed in *Caulastrea furcata* (Fig. 14) and *Manicina areolata* (Fig. 15). With this respect, the fact that molecular results separate faviid/mussid corals from agariciids at opposite major branchings of the tree (Fig. 21) supports this microstructure-based interpretation. It corresponds to the grouping in clade A (Fig. 21) of corals with a transversal arrangement of calcification centres, although it is clear that some members of clade A exhibit unstable branchings because of their long branch (Table 2, see below). Conversely, the septal microstructure in another faviid, *Cladocora caespitosa*, differs from that of typical faviids: centres of calcification arranged in linear series of round-shaped spots, with no indication of structure transverse to the radial plan. It is striking that the molecular results excluded *Cladocora* from faviids (clade D), here corresponding to biomineralization patterns.

In clade H (Fig. 20 only, Agaricidae, Dendrophylliidae, Siderastreidae, *Guynia*), molecular trees show the Agaricidae as monophyletic (F). As recalled above, species belonging to *Agaricia*, *Leptoseris* and *Pavona* are characterized by the septal sides bearing parallel lines of granules or spines. This

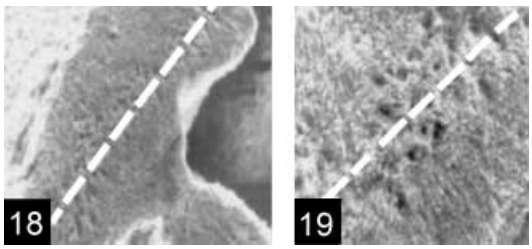
morphological pattern corresponds to the presence of a continuous line of densely packed centres of calcification, a biomineralization feature that was very frequent among coral septa during the early radiation of Scleractinia in the Triassic period (Cuif 1980), and is also observed in very conservative corals such as guyniids (Stolarski 1997, 2000). Interestingly, the molecular data suggest a sister group relationship of *Guynia annulata* with the *Agaricia*, *Leptoseris*, *Pavona* clade (Fig. 21, collapsed in Fig. 20), suggesting that such a pattern may not be symplesiomorphic, but synapomorphic of the clade grouping F and *Guynia* (and possibly some fossils from the early radiation). Interestingly, in parallel, the traditional guyniids appear polyphyletic, as the other guyniid representative, *Stenocyathus*, is the sister group of some Caryophylliidae (*Caryophyllia* and *Desmophyllum*) within clade E. This result corroborates Stolarski's hypothesis 'A' (Stolarski 2000), according to which different patterns of septal calcification centres and wall structures strongly divide traditional guyniids (*sensu* the Guynioidea), with Stenocyathidae within a clade with the Caryophylliidae and the Flabellidae, excluding the Guyniidae *sensu stricto* (*Guynia*). Although poorly supported under the bootstrap criterion, the most parsimonious tree shows the monophyly of the family Dendrophylliidae (G), the value of which also being recognized on the basis of a very distinct biomineralization pattern. This clade is also stable across alignments (Table 2). Septa are built by clusters of small centres of calcification, less than 4–5 μm in diameter, a very distinctive feature with respect to all other studied species (Figs 6, 7: *Balanophyllia europaea*).

With respect to the traditional classification, clade C (*Eusmilia*, *Phyllangia* and the Meandrinidae) clearly appears heterogeneous. *Meandrina pectinata*, *Dendrogyra cylindrus* and *Dichocoenia stockesi* have long been recognized as members of the same family, Meandrinidae, but the present study does not support the monophyly of this family or the family Dendrogyridae in its classical extension, as was proposed by Alloiteau (1952). *Eusmila fastigiata* is traditionally considered a caryophyllid, whereas *Phyllangia mouchezii* belongs to the Rhizangiidae. Nevertheless, in both species the round-shaped septal growth edges produce circular and clearly distinct centres of calcification that can be retrieved by etching polished surfaces (Figs 16, 17). Thus, in spite of its low bootstrap support for this unexpected clade C, this clade was always found whatever the alignment (Table 2) and could be considered as a reasonable working hypothesis.

The major interest of clade D (*Cladocora caespitosa*, *Cladocora arbuscula* and *Oculina diffusa*) is to suggest that Faviidae is a paraphyletic unit from which the genus *Cladocora* should be excluded, a result fully corroborated by biomineralization patterns. In *Cladocora caespitosa*, septa are built on the basis of a single linear series of centres of calcification, isodiametric (about 20 μm in diameter) and regularly spaced (Figs 9–11).



Figs 16, 17 *Eusmilia fastigiata* exhibits well-defined, circular and separated centres of calcification, a very distinctive feature from the very small and linearly and closely arranged centres of Caryophyllids (Fig. 16: ×12; Fig. 17: ×45).



Figs 18, 19 *Balanophyllia europaea*: typical feature for dendrophylliid corals: tiny centres of calcification arranged in irregular clusters (Fig. 18: ×25; Fig. 19: ×75).

No indication of any transversal structure is visible. This biomineralization pattern strongly differs from those observed in typical Faviidae, and especially *Favia fragum* (cf. Figs 4–6).

Clade E (the Caryophylliidae excluding *Eusmilia* and including the guyniid *Stenocyathus*) offers another example of three species belonging to a family that is partly supported by both molecular and microstructural patterns. It must be noticed that clade E is recovered whatever the alignment (Table 2). From the biomineralization standpoint, the common feature of the three species gathered in clade E is the presence of a medioseptal continuous line of centres of calcification, resulting in a septal microstructure that was called diffus trabecula by Schindewolf (1942). Such a microstructural pattern is commonly found among non-zooxanthellate corals that are frequently also deep sea living organisms. Particularly abundant among Triassic corals, continuity of the median calcification line appeared as a primitive shared character, that could have been preserved in different lineages. Molecular data grouping species with those characters suggested that they might have arisen several times by convergence. As suggested by previous observations, the evolutionary process probably leads to differences in the biochemical composition of calcification matrices among the centres of calcification (Cuif & Dauphin 1998) but microstructural observation of the global geometric features of the calcification line does not

permit recognition of these differences. In spite of this similarity in geometrical arrangement of calcification centres, the alternation of the production of the lateral axis generally leads to a distinct separation among septa of agariciids and caryophylliids for instance, a feature that corroborates the presently recovered clade E. As already mentioned above, clade E also corroborates Stolarski's hypothesis 'A' (Stolarski 2000), according to which different patterns of septal calcification centres and wall structures suggest that the Stenocyathidae are more closely related to the Caryophylliidae and the Flabellidae than to Guyniidae such as *Guynia*. More precisely, in Stolarski's hypothesis 'A', the Stenocyathidae is the sister group of the Flabellidae, the two families being within a clade shared with the Caryophylliidae, clearly excluding *Guynia*. For the moment, in the absence of representatives of the Flabellidae in our molecular study, the closest extant sister group of the Stenocyathidae has not yet been tested from molecular data.

Conclusion

A comparison of biomineralization patterns of coral septa with molecular phylogenic trees leads to the conclusion that at least five clades are corroborated by both types of data: D, E, F, G, *Guynia* + agaricids, and, to a certain extent, clade A (Fig. 21) if we hold as artefactual the unstable branching patterns of the long branches *Favia fragum*, *Manicina areolata*, *Blastomussa merleti* (Fig. 20, Table 2), and the attraction they provoke on *Stylophora* and *Pocillopora*. Thus, molecular clades give the value of synapomorphies to similar tri-dimensional arrangements of centres of calcification. Although some substantial changes among some of the presently recognized families are suggested by the results of the present study, a rather consistent response is obtained between molecular and microstructural groupings for many of them.

Much more important appear to be the changes that are suggested by the relationships between biomineralization/molecular clades compared with the respective positions of families in the Wells evolutionary tree. The inadequacy of the widely used Wells global taxonomic and evolutionary scheme has long been suggested on the basis of microstructural analysis of Triassic corals (Cuif 1980). In a remarkable series of papers that culminates in the Stolarski & Roniewicz (2001) synthesis, the phylogenetic interest of some extant corals with archaic (i.e. Permian-like) microstructures was emphasized (Stolarski 1995, 1996; Roniewicz & Stolarski 1999). The positions of *Guynia* and *Stenocyathus* given by our molecular analyses bring strong support to Stolarski's hypothesis 'A' (Stolarski 2000: 30). The importance of these results cannot be overemphasized: they considerably highlight the hypothesis of a phylogenetic relationship between Permian and Mesozoic coral faunas, a conclusion that completely changes our view of the overall coral evolution.

Since Heackel's (1896) diphyletic theory, we are familiar with the idea of a complete separation between palaeozoic corals (Tetracorals, 'Rugosa'), and the 'Hexacorals' that arose during the Triassic period. In spite of changes in the corallite development of Permian corals that were shown by Schindewolf (1942), the possibility of relationships between Permian and Triassic corals was not accepted. Evidence of Permian-like microstructures in Triassic corals from Dolomites (*Zardinophyllum*; Montanarro-Gallitelli 1975), and among the various members of the Pachythecalidae family (Cuif 1975), were not more influential.

Stolarski's results, corroborated by our present molecular study, clearly establish not only that some Permian corals may have survived the faunistic crisis that marks the end of the Palaeozoic, but that they have also continued to exist until today. Therefore, the main result of the present study might be that it suggests a way to create a consistent working framework to elaborate a new global scheme for coral classification and global evolutionary analysis. That framework is in two steps. In the first step, study of extant corals allows precise evaluation of skeleton-based characters using soft tissue-based methods (including molecular ones). This should allow us to assess the relevance of skeletal criteria that could be used in the second step, the purely palaeontological approach. By contrast to the attempts using a single type of character, this two-step process should allow us to benefit from the important amount of information from fossil corals, and maintain a common global scheme for the whole period during which Scleractinia have evolved.

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Figs 1–19 Examples of biomineralization patterns resulting from the arrangement of calcification centres and the tri-dimensional growth orientation of fibres (in all pictures, the white lines indicate the radial direction).