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Debating phylogenetic relationships of the scleractinian *Psammocora*: molecular and morphological evidences

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Abstract

The phylogenetic relationships of the scleractinian genus *Psammocora* with the other genera traditionally included in the family Siderastreidae and some Fungiidae are assessed based on combined skeletal and molecular data. *P. explanulata* differs from the other examined congeneric species (*P. contigua*, *P. digitata*, *P. nierstraszi*, *P. profundacella*, *P. superficialis*, and *P. stellata*) in possessing interstomatous septa between adult coralites, costae, and in having continuous buttress-like structures joining septal faces (i.e., fulturae) which typically occur in fungiids. These characters are shared with *Coscinaraea wellsi* but not with the remainder of the examined siderastreids (the congeneric *C. columna*, and *Anomastraea irregularis*, *Horastrea indica*, *Pseudosiderastrea tayamai*, *Siderastrea savignyana*) whose septa are interconnected by typical synapticulae. Most of the examined species form septa with distinct transverse groups of centers of calcification, a biomineralization pattern typical of the Robusta clade. The observations on skeletal structures corroborate the results of the ITS2 and 5.8S molecular phylogeny. *C. wellsi* and *P. explanulata* are phylogenetically very close to each other and show closer genetic affinity with the examined Fungiidae (*Halomitra pileus*, *Herpolitha limax*, *Fungia paumotensis*, and *Podabacia crustacea*) than with the other species in the genera *Psammocora* and *Coscinaraea*, or with any other siderastreid. Our results show that neither *Psammocora* nor *Coscinaraea* are monophyletic genera. The high genetic distances between the species of Siderastreidae, especially between *Pseudosiderastrea tayamai* and *Siderastrea savignyana* on one side and the other genera on the other, suggest a deep divergence in the phylogenetic structure of the family.

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Introduction

The Indo-Pacific coral genus *Psammocora* Dana, 1846 has a wide geographic distribution and it is commonly found in any reef environment, from seagrass beds and lagoons, to outer reef slopes down to 50 m depth. Most species may be cryptic even if some branching species can be locally abundant and form monospecific stands (Sheppard and Sheppard, 1991; Veron, 2000). Skeletal structures in *Psammocora* are complex and plastic, and some characteristic morphological features unique to the genus such as extrapolypal tentacles, and the intricate mesh of ento- and exosepta (Matthai, 1948a; Matthai, 1948b) have been largely overlooked in the literature thus leading to the use of confused terminology in descriptions by various authors.

Twenty-three extant *Psammocora* species have been described. Many of those have been put in synonymy (Veron and Pichon, 1976; Scheer and Pillai, 1983; Sheppard and Sheppard, 1991). A global revision of the genus, however, has never been undertaken. Eleven *Psammocora* species are listed in Cairns *et al*. (1999), and Veron (2000) retains twelve species. However species synonymies are not indicated or discussed in either publication.

Hypotheses about phylogenetic relationships of *Psammocora* and the resulting classification have changed dramatically over the last sixty years (Stolarski and Roniewicz, 2001). Vaughan and Wells (1943) considered *Psammocora* the only extant genus in the Thamnasteriidae, suborder Fungiida (Fig. 1a), based on the presence of synapticulothecal wall and simple trabeculae of septa. Alloiteau (1952) also placed *Psammocora, Stephanoseris* and *Plesioseris* in the family Thamnasteriidae. Wells (1956) still maintained *Psammocora* in the Thamnasteriidae, but placed the family in the Astrocoeniina (Fig. 1b). Chevalier and Beauvais (1987) brought back the Thamnasteriidae in the Fungiina but placed *Psammocora* in the new family Psammocoridae (Fig. 1c) along with *Stephanaria* Verrill, 1867, and *Plesioseris* Duncan 1884, both previously considered subgenera of *Psammocora*, and synonymised with it by Veron and Pichon (1976). Finally Veron (1995) based on skeletal features and on its "clear affinities" with *Coscinaraea* classified *Psammocora* in the Siderastreidae with *Siderastrea* Blainville, 1830, *Coscinaraea* Milne Edwards and Haime, 1848, *Anomastraea* Marenzeller, 1901, *Pseudosiderastrea* Yabe and Sugiyama, 1935, and *Horastrea* Pichon 1971 (Fig. 1d). These genera have been grouped together because of the common presence of synapticulae and the fusion of septa around the corallite fossa. A comprehensive treatment of the family skeletal structures, however, has never been published, and such characters have never been proved to be plesiomorphic. The genus *Craterastrea* Head, 1983 is poorly known and presently recorded from the Red Sea and Chagos only (Head, 1983; Sheppard, 1981; Sheppard, 1998; Sheppard and Sheppard, 1991). It was described as a siderastreid and has been later synonymised with the agariciid genus *Leptoseris* (Veron, 1995; Veron 2000; Inskipp and Gillett 2005).

Among the extant corals *Psammocora* bears structural similarities to *Coscinaraea* especially in the septa shape and corallites arrangement (Pandolfi, 1992; Veron, 2000), and to *Craterastrea*. *Coscinaraea columna* (Dana, 1846) and *Coscinaraea exesa* (Dana, 1846) were originally described as *Psammocora*, and the similarities between *Psammocora explanulata* Van der Horst, 1922, and *Coscinaraea wellsi* Veron and Pichon, 1980 are evident (Veron and Pichon, 1980; Veron, 2000). Morphologic affinities of the septa fusion patterns in *Psammocora* and in the fungiids *Lithophyllon* Rehberg, 1892 and *Polyphyllia* Blainville, 1830 have also been remarked (Veron and Pichon, 1976; Van der Horst, 1922).

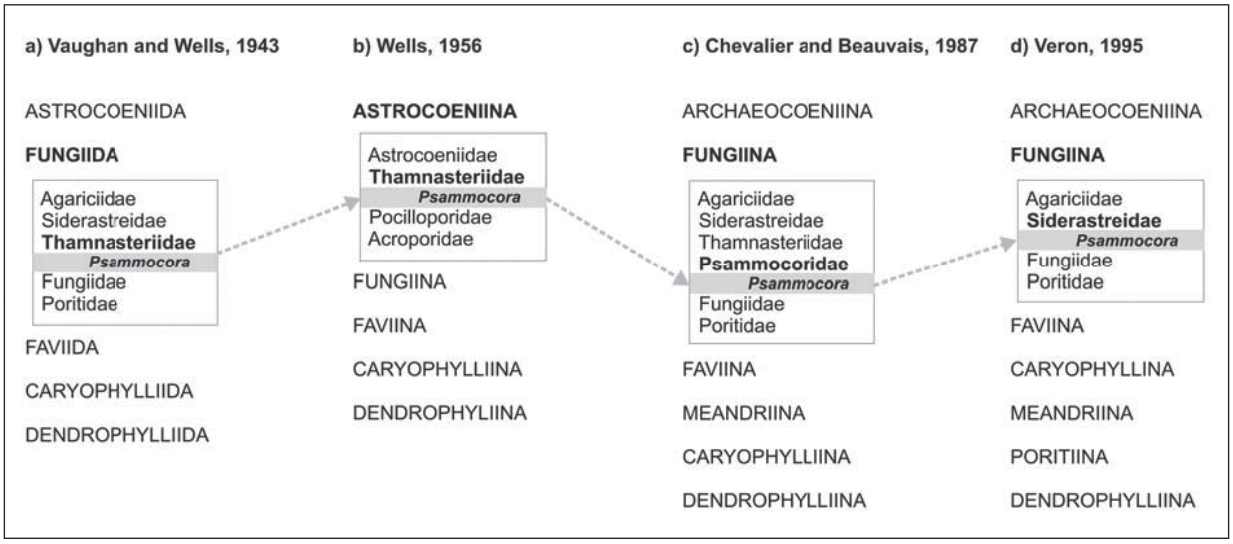


Fig. 1. Changes in classification of the genus *Psammocora*. Grey arrows indicate the change of position of the genus (in bold italic) within a family (in bold) and suborder (in bold capitals) according to a) Vaughan and Wells (1943), b) Wells (1956), c) Chevalier et Beauvais, 1987, d) Veron (1995). Modified from Stolarski and Roniewicz (2001).

The molecular phylogeny of the Fungiina has, so far, been only partially investigated. Studies based on mitochondrial DNA (Romano and Palumbi, 1996; Romano and Cairns, 2000) showed that the Fungiina are not monophyletic and that *Psammocora stellata* and *Coscinaraea* sp., cluster together with some of the Fungiidae. Chen *et al*. (2004) showed that based on ITS *Psammocora* and *Siderastrea* belong to distant clades, thus suggesting that the Siderastreidae *sensu* Veron (2000) are not monophyletic. However the phylogentic relationships within *Psammocora* and between it and the other genera in the family remain unknown.

In this paper we first provide a synthetic but comprehensive account of living and skeletal structures of the genus *Psammocora*, we then explore the morphologic and genetic affinities of seven *Psammocora* species. Finally we discuss the phylogenetic relationships of *Psammocora* with all the Siderastreidae and some Fungiidae based on the joint results of our morphological study and genetic investigations.

Material and methods

Species identification

Psammocora species were first identified following the descriptions and illustrations given by Pillai and Scheer (1976), Veron and Pichon (1976), Scheer and Pillai (1983), and Sheppard and Sheppard (1991). Identifications were then checked against the species original description and, when possible, type material was examined. Scleractinia collections and type material deposited at the Zoological Museum of Amsterdam (MZA), Naturalis in Leiden (RMNH), the Museum of Natural History in London (MNH), the National Museum of Natural History in Paris (MNHN), and the Museum of Tropical Queensland (MTQ), were examined. For the identification of *C. columna* (Dana, 1846), *Siderastrea savignyana* Milne Edwards and Haime 1850, *Anomastraea irregularis* Marenzeller, 1901, *Pseudosiderastrea tayamai* Yabe and Sugiyama, 1935, *Horastrea indica* Pichon, 1971, and *C. wellsi* Veron and Pichon, 1980, we referred to Veron and Pichon (1980), Carpenter *et al*. (1997), and to the species original descriptions. The type specimen of *Craterastrea levis* Head, 1983 (MNH 1981.4.1.4) was examined. However due to the species restricted geographic distribution (Head, 1983; Sheppard, 1991; Sheppard, 1998; Sheppard and Sheppard, 1991) and to its apparent rarity we did neither manage to retrieve any ethanol preserved material nor to collect it. Our data on the genus is therefore limited to the external skeletal structures. For the identification of the Fungiidae species *Halomitra pileus* (Linnaeus, 1758), *Podabacia crustacea* (Pallas, 1766), *Herpolitha limax* Esper, 1797, and *Fungia (Pleuractis) paumotensis* Stutchbury, 1833 we followed Hoeksema (1989).

Choice of Psammocora *species*

This work is part of a world revision of the genus for which a large set of specimens has been collected. For the purpose of the present analysis we included 7 *Psammocora* species (Table 1) for which the taxonomic status has been verified (registration code of the examined type material is given in parenthesis): *Psammocora contigua* (Esper, 1794) (SMF 5523), *Psammocora digitata* Milne Edwards and Haime, 1851 (MNHN 533), *Psammocora stellata* Verrill, 1864 (MNH 1870. 8.22.16), *Psammocora superficialis* Gardiner, 1898, *Psammocora profundacella* Gardiner, 1898, *Psammocora nierstraszi* Van der Horst, 1921 (ZMA Coel 1078), and *P. explanulata* (ZMA Coel 1071; ZMA Coel 1072; MNH 1937.11.25.17; MNH 1937.11.17.69).

Five *Psammocora* species (i.e., *P. obtusangula* (Lamarck, 1816) (MNHN 136), *P. haimeana* Milne Edwards and Haime, 1851 (MNHN 535), *Psammocora verrilli* Vaughan, 1907, *P. decussata* Yabe and Sugiyama, 1937, and *P. vaughani* Yabe and Sugiyama, 1936) have not been included in the analysis because of the following taxonomic reasons. The type specimen of *P. obtusangula* showed no substantial difference from the type of *P. contigua*. The taxonomic status of *P. haimeana*, *P. verrilli*, *P. decussata* and *P. vaughani* remains uncertain. The holotype of *P. verrilli* has not been studied by the authors yet, but in the illustration of the original description (Pl. XLIV, Figs. 1-1a) it shows striking similarities to *P. nierstraszi*. The holotypes *P. decussata* and *P. vaughani* deposited at the Institute of Geology and Paleontology, Tohoku University, Sendai (*P. decussata* IGPTU 61591; *P. vaughani* IGPTU 44975) could not be located (Nemoto Jun, 05/01/2006 *in litteris*). *P. decussata* is supposed to be an endemic species of Japan (Veron, 2000) but from the original illustration it is very similar to *P. contigua* (Yabe and Sugiyama, 1937, p. 428 Figs. 2-4). The corallite drawing and Figure 3 of the *P. vaughani* description in Veron (2000) (Vol 2, p. 157) depict a *Coscinaraea* sp. and certainly do not match either the original description or the clear illustrations in it (Yabe *et al*., 1936, Pl. XLI, Figs. 6 and 7). However the author

himself states that the specimens he identified as *P. vaughani* present "*Coscinaraea*-like" skeletal characters, and that he retained the species in the genus *Psammocora* only because of the small corallite size.

Sampling

Corals for this study were sampled while scuba diving between 2 and 30 m deep at different localities in Kuwait, Maldives, Mayotte, Reunion, Indonesia. Specimens of *P. stellata* from Costa Rica were collected and kindly provided by Jorge Cortes. Digital images of living corals were taken with a Nikon Coolpix 995 in an Ikelite underwater housing system. Coral specimens were collected, tagged and for each specimen 1 cm^2 was broken off the colony and preserved in absolute ethanol for molecular analysis. The remaining corallum was left for 48 hours in a 50% sodium hypochlorite at ambient temperature to remove all soft parts, rinsed in freshwater and dried for microscope observation. The *Psammocora* and Siderastreidae specimens studied for this work were deposited at the Museum of Natural History in Milan. The Fungiidae specimens belong to the GIS-LAY Mayotte Expedition Collection and are in Perpignan. Images of skeletons were taken with a Canon G5 camera through a Soligor B-52 Adapter Tube mounted on a Zeiss Stemi DV4 stereomicroscope. The list of sampled species and localities is in Table 1.

SEM preparation and analysis

Samples selected for microstructural analysis were ground with diamond suspension having grain sizes of 5 and 1 micrometers, and later polished with aluminium oxide (Buehler TOPOL 3 final polishing suspension with particle size 0.25 micrometres). After polishing, sections were rinsed in Milli-Q water, washed in an ultrasonic cleaner for 10 seconds, then etched for 10 seconds in 0.1% formic acid, then rinsed with Milli-Q water and air dried. After drying, the specimens were put on stubs with double sticky tape and sputter-coated with conductive platinum film. Polished and etched samples were investigated using SEM (Philips XL 20).

DNA extraction, amplification and sequencing

Total DNA extraction and purification was obtained using the DNAeasy® Tissue kit (QIAGEN, Qiagen Inc., Valencia, California, USA) reagents.

Table 1: List of the specimens collected and analysed for this study. For each specimen the species identification, locality, code and EMBL accession number are given respectively. In brackets the frequency of each haplotype, when higher than 1, is reported. CR = Costa Rica, I = Indonesia, K = Kuwait, M = Maldives, MAY = Mayotte, REU = Reunion.

Genus	*species*	Code/ source	EMBL accession number
Psammocora	*contigua*	I 110;	AM230604
		M 48; 49	AM230602; AM230603
		MAY 232; 254	AM230600; AM230601
	nierstraszi	M 43; 52; 53	AM230606; AM230607; AM230608
	profundacella	M 7; 9; 10; 18	AM230617; AM230618; AM230616; AM230619
		I 91	AM230615
	superficialis	K 118; 142	AM230614; AM230613
	stellata	CR 326; 328	AM230620; AM230621
	digitata	M 35	AM230610
		I 102, 97	AM230609 (2)
		M 16, 26	AM230609 (2)
	explanulata	MAY 447; 453	AM230611; AM230612
Coscinaraea	*columna*	K 117; 122	AM230598; AM230599
		M 41	AM230597
	wellsi	I 80; 108; 114	AM230622; AM230623 (2)
Horastrea	*indica*	REU 516; 518	AM230605 (2)
Siderastrea	*savignyana*	K 123; 144	AM230625 (2)
Pseudosiderastrea	*tayamai*	Chen *et al.* 2004	AY722790; AY722789
Anomastraea	*irregularis*	K 131; 124	AM231716; AM230624
Podabacia	*crustacea*	MAY 435; 456; 457	AM230626 (3)
Halomitra	*pileus*	MAY 442; 472	AM230630; AM230631
Fungia	*paumotensis*	MAY 440; 481	AM230627; AM230628
Herpolitha	*limax*	MAY 470; 473; 474; 480	AM230629 (4)

The concentration of the extracted solutions, was adjusted to 3 ng/µl, and used directly for PCR amplification of a ~ 400 bp fragments of rDNA, spanning a portion of the 5.8S gene, the entire ITS2 region and a portion of the 28S gene. Amplification was performed using the *5.8Sfor* primer (5'-TTT GAC GGT GGA TCT CTT GG-3') and the universal *ITS4* primer (5'-TCC TCC GCT TAT TGA TAT GC-3') (White *et al*., 1990). The *5.8Sfor* primer was specifically designed for Scleractinia by the authors on the basis of the homologue alignment of *P. profundacella*, *Lophelia pertusa*, *Madracis pharensis*, *M. senaria*, *Diploastrea heliopora*. A sequence of *Symbiodinum* was also included in order to avoid the amplification of the zooxanthella DNA. The obtained sequences were submitted to a BLAST query of the National Center for Biological Information (NCBI) sequence database, confirming their strict similarity to corals sequences and excluding the sequencing of zooxanthellae DNA.

Reactions were conducted in a 50 µl PCR mix consisting of 1X PCR Buffer, 2 mM $MgCl_2$, 0.4 µM of each primer, 0.1 mM of each dNTP, 2 U Taq DNA Polymerase (Sigma-Aldrich Co., St. Louis, Montana, USA) and 8 µl of DNA solution. The thermal cycle was the following: a first denaturation phase at 96°C for 2', followed by 30 cycles composed of three steps: (1) 10'' at 96°C; (2) 30'' at 50°C; (3) 4' at 72°C; finally, an extension phase at 72°C for 5'. The amplified samples were purified with commercial kits. Sequencing reactions were carried out using a dideoxy-dye-terminator method (CEQ™ DTCS-Quick Start kit, Beckman Coulter) and a Beckman Coulter CEQ™ apparatus. We used the reverse primer ITS4 for sequencing and the sequences were read with the Beckman Coulter CEQ™ software. Whenever the obtained sequences showed ambiguities, we performed a sequencing reactions using the forward 5.8Sfor primer.

Network building and distances calculation

The secondary structure of ITS2 has been inferred before alignment using the Mfold program (Zuker, 2003), which generates multiple free-energy diagrams and proposes a consensus model showing common stems, loops and bulges. Regions corresponding to conserved stems throughout the studied species have been used as consistent basis for the alignment of the more variable loop regions. Though structure reconstruction according to thermodynamic properties has been criticized in favour of analysis of covariation sites (Hershkovitz and Zimmer 1996; Gutell *et al*., 1994), it has been shown that, for Scleractinia at least, this approach provided comparable and reliable results (Goertzen *et al*., 2003; Chen *et al*., 2004). The secondary structure of the ITS2 was obtained for *P. crustacea*, *H. indica*, *P. explanulata*, *H. limax*, *H. pileus*, and *F. paumotensis*. The ITS2 sequences of the other species were excluded because of the scarce reliability of the sequence of the flanking 28S gene portion. This portion usually pairs with the flanking 5.8S portion to form the main loop of the structure.

Alignment was conducted with the software ClustalX (Thompson *et al.*, 1997) and then manually checked and adjusted with BioEdit 5.0.9 (Hall, 1999), according to the constrains evidenced by the ITS2 secondary structure. Identification of polymorphic and parsimony informative sites was conducted with DnaSP 3.52 software (Rozas and Rozas, 2001).

In order to infer phylogenetic relationships we considered a portion of 320 homologue positions, spanning part of the 5.8S gene (141 bp) and part of the ITS2 region (179 bp). We excluded from the analysis a portion of 12 bp in the ITS2 region because of long and not alignable insertions in *S. savignyana* and *P. tayamai*.

A sequence evolution model according to a maximum likelihood criterion was selected using Modeltest 3.06 (Posada and Crandall, 1998) to calculate the mean genetic distance between species (or groups) of interest.

Phylogeny was evaluated both by traditional and by networking approaches. Firstly, a phylogram was built, according to the Bayesian approach (Huelsenbeck *et al*., 2001), as implemented in the MrBayes program (Huelsenbeck and Ronquist 2001). Bayesian analysis was run starting four Markov chains from random trees and running them for 1300000 generations, with the first 1200000 generations (12000 trees) discarded. The analysis was run independently 4 times and monitored to ensure that standard deviation of split frequencies was <0.01.

A minimum spanning network of haplotypes was arranged with the software TCS 1.13 (Clement *et al.*, 2000), according to the statistical parsimony criterion proposed by Templeton *et al*. (1992, 1993) in order to evidence eventual ambiguities and reticulations.

Results

Morphology of Psammocora

Polyp morphology

Psammocora polyps are often expanded during the day (Fig 2A, C) though seldom obvious due to their small size and transparency. *In vivo* observation of the polyps

showed that the coenosarc is absent in all the species of *Psammocora*. When tentacles are extended their arrangement in concentric circles around the polyp mouth (Fig. 2A, C) is visible. A striking feature of *Psammocora* tentacles is their position with respect to the polyp gastrovascular cavity. The innermost circle of tentacles surrounds the polyp mouth and reaches its gastrovascular cavity directly, whereas the outer tentacle circles are connected indirectly to it via gastrovascular canals. The distinctive character of these "outer" tentacles herein termed extrapolypal (*sensu* Matthai, 1948b) was first noticed by Yonge (1930: p 40, "it is difficult often to distinguish to which of adjacent mouths particular tentacles rightly belong") and described by Matthai (1948b).

Skeletal structures

Septa organization in *Psammocora* bears unique characteristics among the Scleractinia which are directly related to the presence and organization of extrapolypal tentacles. In the calice two kinds of septa are found: the entosepta (bearing a tentacle) and the exosepta (*sensu* Chevalier, 1987). Entosepta and exosepta alternate around the fossa. Septa are connected by

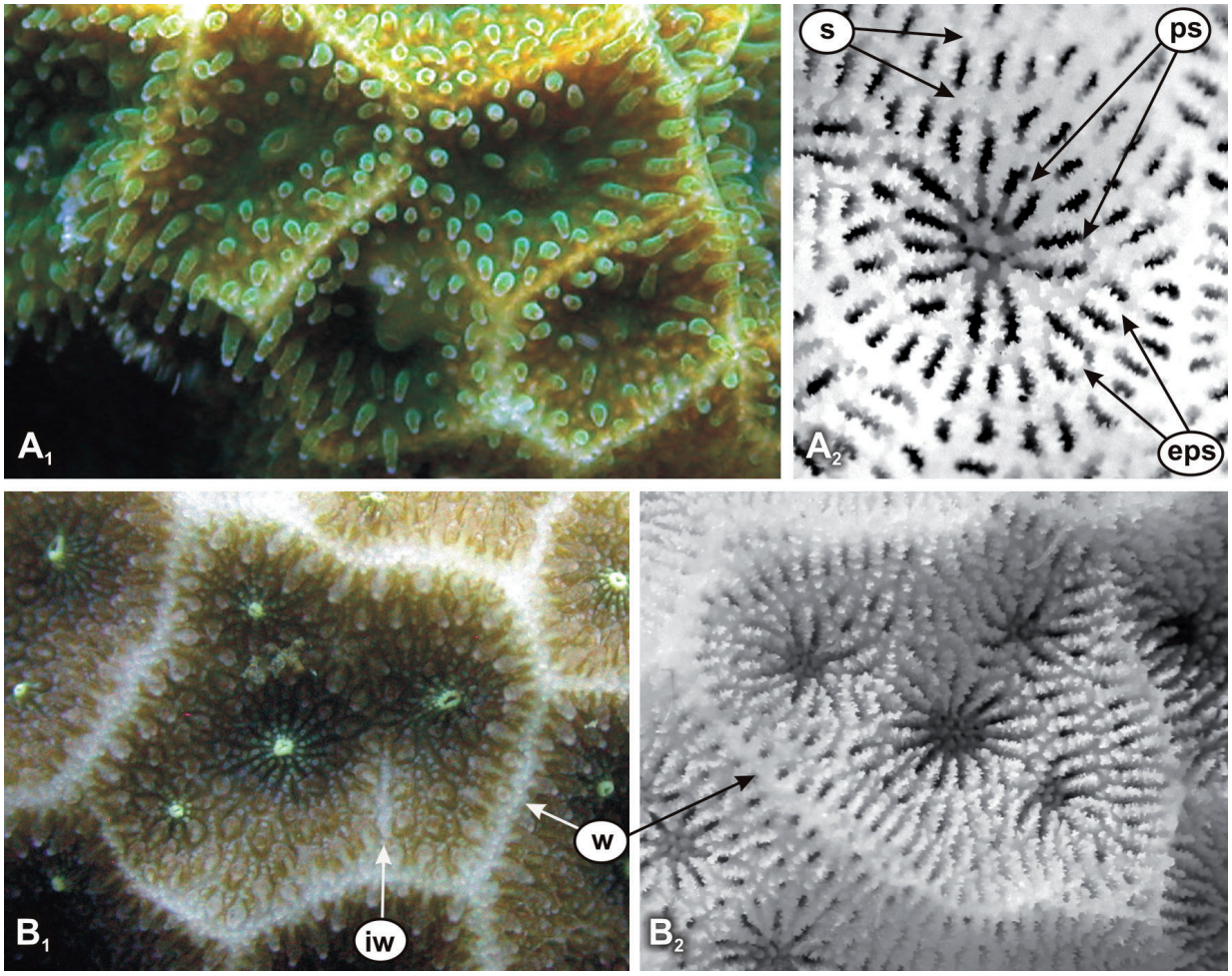


Fig. 2. Typical polyp and skeleton characters of *Psammocora*. (A) Extended polyps in a living colony of *P. profundacella* (M18) allow to see two to three concentric rows of white-tipped tentacles around each mouth. (B) A corallite of the same specimen after living tissue removal with petaloid septa (ps) around the calicular fossa, and concentric rows of enclosed petaloid septa (eps) corresponding to the concentric rows of tentacles visible in A. Synapticulae (s) joining the septa, and forming synapticular rings are also visible. (C) The *in vivo* picture of another *P. profundacella* colony (I91) shows series of polyps enclosed by a common wall (w). Note the outermost rows of tentacles surrounding all the four polyps within the wall, and the incipient wall (iw) starting to separate the corallite on the left from the others in the series. The intratentacular mode of budding is clearly visible. (D) Series of calices enclosed by a common wall (w) in another specimen (M10) after tissue removal.

crossbar-like rods called synapticulae (Vaughan and Wells, 1943). The exosepta furcate peripherally before reaching a first row of synapticulae delimiting the calice. At the furcation of an exoseptum a new entoseptum is formed. Between the first and the second row of synapticulae each of the two exosepta formed after the furcation can either divide again, or fuse with another exoseptum thus leading to the enclosure of the entoseptum found between them. Each enclosed entoseptum in the corallum corresponds to an extrapolypal tentacle in the living animal. The first circle of enclosed entosepta bears the second circle of tentacles visible *in vivo* around the polyp mouth (Fig. 2A, C). The reiteration of exosepta furcation and fusion processes, along with the formation of new enclosed entosepta, can occur from 2 to more than 10 times depending on the species. In *P. contigua*, *P. nierstraszi* series of enclosed entosepta are more numerous than in any other species (Fig. 3 A_1 and B_1 respectively) and the calices look immersed in a mesh of ento- and exosepta often erroneously referred to as the coenosteum. In *P. profundacella* (Fig. 3 C_1) two to five circles of enclosed entosepta can be easily seen around the calice. In *P. superficialis* (Fig. 3 D_1), *P. stellata* (Fig. 3 E_1) and *P. digitata* (Fig. 3 F_1) a maximum of 2 complete circles of enclosed entosepta usually form. In *P. explanulata* only one circle of enclosed entosepta, often incomplete, is found. Entosepta and enclosed entosepta can be thicker than the exosepta and reach the typical "petaloid" shape (well visible, for example, in Fig. $3D_1$, F_1, F_2). Petaloid septa in *Psammocora* are often exert above the colony surface. In less heavily calcified specimens although enclosed septa exist, they may not reach the typical petaloid shape and the different septa thickness is less evident. Septocostae are absent in all the *Psammocora* species we studied. In *P. explanulata* continuous septa going from one fossa to the other can be found (Fig. $3G_1$, Fig. $5B_2$). In this case, however, these septa are interstomatous septa in every respect similar to those found in the Fungiidae (*sensu* Hoeksema, 1989) and are not homologous with the septo-costae found in other coral families. In *P. explanulata* tentacular lobes very similar those found in some Fungiidae are also found.

Ridges composed of short series of minute granule/spines oriented transversally to the septal plane can be recognized at the growing septal edges of the *Psammocora* species we examined. In some species (e.g., *P. digitata*, *P. superficialis*, *P. stellata*, also *P. explanulata*) these transverse ridges form distinct septal paddles (Fig. $3D_{2,3}$, E_{2-3}, F_{2-3}, G_2). Microstructurally, the minute granule/spines correspond to centers of calcification (Centers of Rapid Accretion, *sensu* Stolarski 2003) as seen in transversally sectioned septa (Fig.$3D_4$, E_4, F_4).

The microstructural analysis of the skeletal elements connecting the septa in *Psammocora* confirms the presence of synapticulae (Fig. $5A_{1-2}$) in all the considered species but *P. explanulata* (Fig. $5B_{1-2}$). In the latter buttress-like structures develop below the septal edge (Fig. $5B_{1-3}$). These structures are arranged perpendicularly to the distal septal margins, and show arched zones of fibrous layers in longitudinal section (Fig. $5B_5$). In transverse section they exhibit ad-septal formation of the fibrous layers which join with a suture (Fig. $5B_4$).

The columella is present in all the examined *Psammocora* species, but the number of columellar processes, their size and disposition varies between species. *P. contigua*, *P. nierstraszi*, *P. superficialis*, and *P. digitata* all have a styliform columella (Fig. $3A_1$, B_1, D_1, F_1 respectively). *P. profundacella* and *P. stellata* present a composite columella made of one central process surrounded by 4 to 6 smaller processes. In *P. explanulata* the columellar processes are all of the same size, and in adult corallites their number ranges from 6 to 15.

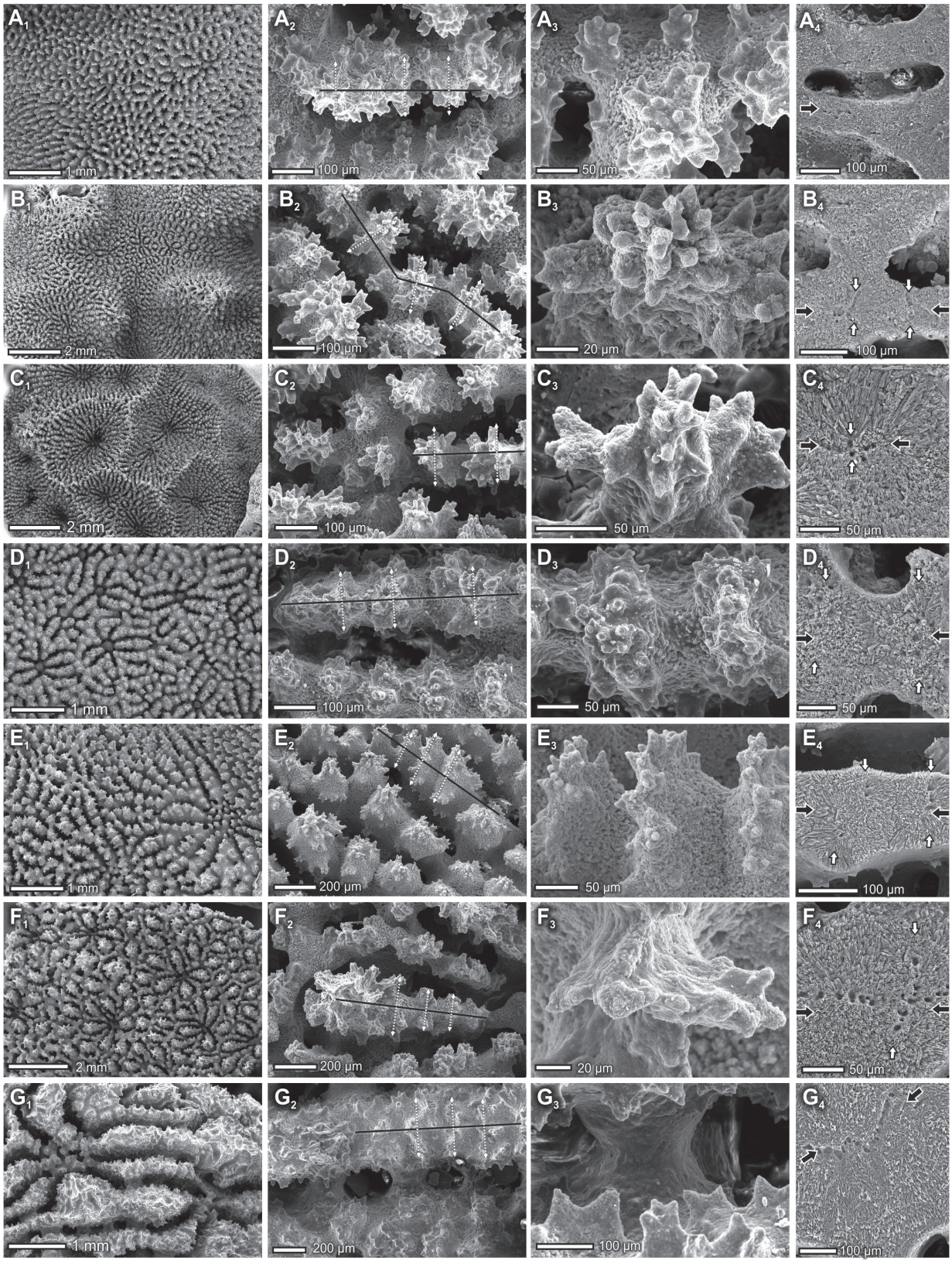
In all the examined species of *Psammocora* except *P. explanulata* colonies form an epithecal-synapticulothecal wall (for terminology see Roniewicz and Stolarski, 1999). In *P. explanulata*, however, the colony wall is septothecal.

Colony condition in *Psammocora* is ceriod (Fig. 2A, B) and the meandroid state can be reached via intratentacular budding (Fig. 2C, D) (Matthai, 1948a) and series of corallites can form. In some species corallites form series surrounded by so many concentric rows of synapticulae (i.e., *P. contigua*) that the exact boundaries of the wall can hardly be recognised (Fig. $3A_1$). In other species, i.e., *P. profundacella*, the shape, length and width of the series of corallites can be very variable even within the same colony However the wall boundaries are clearly recognisable (Fig. $3C_1$). Often a new a wall developing to separate corallites within the same series is visible (Fig. 2C). In *P. explanulata* series of corallites enclosed by a synapticulothecal wall never form.

P. explanulata differs from the remainder of the examined species in a number of skeletal structures summarized in Table 2.

Skeletal structures of the Siderastreidae

The septal fusion considered as typical of the Siderastreidae is present in all the examined species in the family (Fig. 4). The series of enclosed entosepta typical of *Psammocora*, however, were not found in any other


A1
1 mm
A2
100 µm
A3
50 µm
A4
100 µm
B1
2 mm
B2
100 µm
B3
20 µm
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C1
2 mm
C2
100 µm
C3
50 µm
C4
50 µm
D1
1 mm
D2
100 µm
D3
50 µm
D4
50 µm
E1
1 mm
E2
200 µm
E3
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E4
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F1
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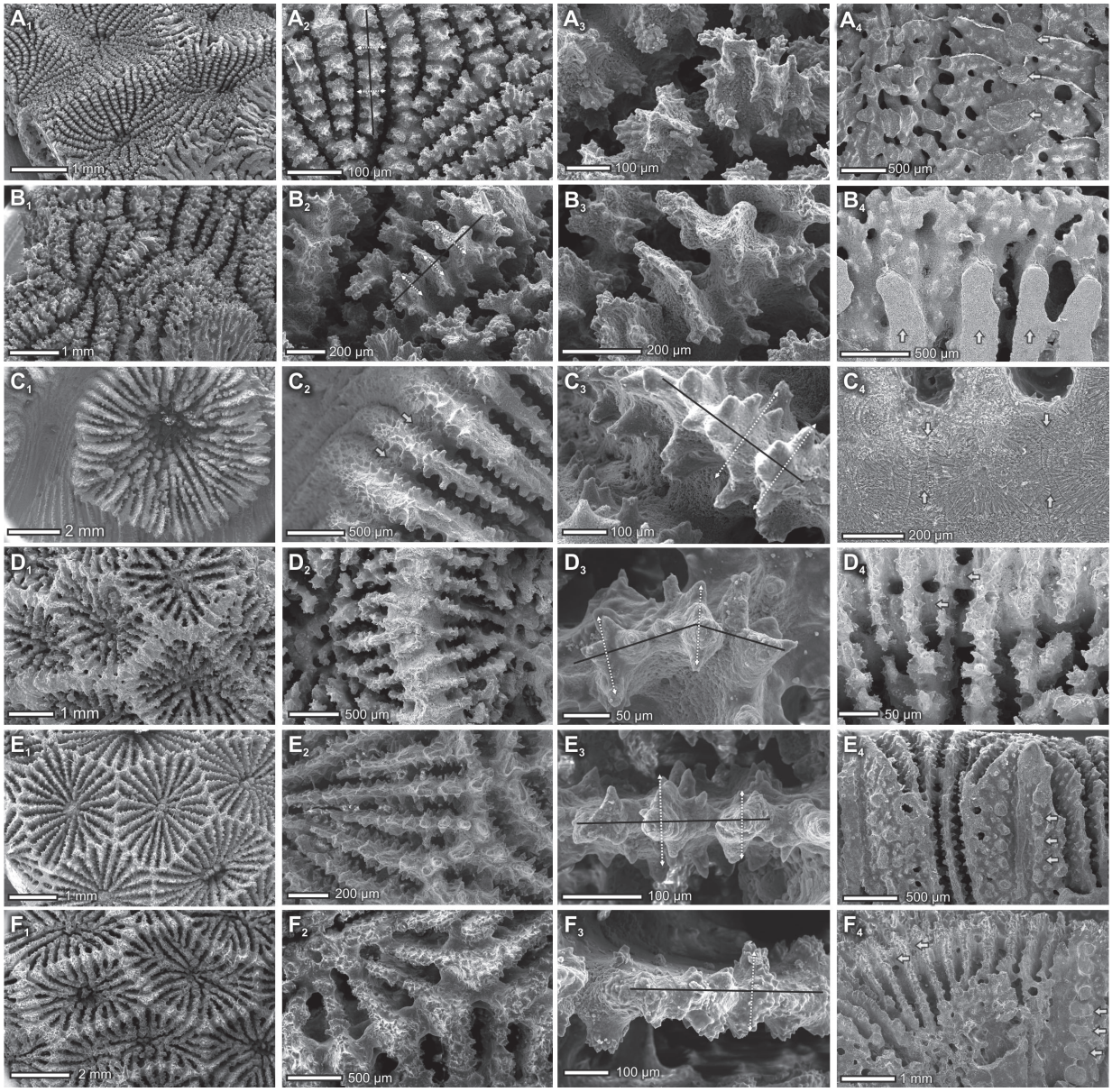

Fig. 4. Septal arrangement (subscripted with "1"), microarchitecture (subscripted with "2", and "3" [close-up of septal projection]) and septal junctions/wall structure (subscripted with "4") in the examined Siderastreidae (*sensu* Veron 2000): *Coscinaraea columna* (A), *Coscinaraea wellsi* (B), *Horastrea indica* (C), *Pseudosiderastrea tayamai* (D), *Siderastrea savignyana* (E), *Anomastraea irregularis* (F). Transverse groups of centers of calcification typical of the Robusta highlighted with white arrows whereas radial extension of septum with black line (marked in figure items subscripted with "2" and "4"). Main features of septal junctions (wall structure) marked with arrows: synapticulae (A_4, D_4, E_4, F_4), septotheca (C_4), and fulturae (B_4).

◀ *Fig. 3.* Septal arrangement (subscripted with "1"), microarchitecture (subscripted with "2", and "3" [close-up of septal projections]) and microstructure (subscripted with "4") in the examined *Psammocora* species: *P. contigua* (A), *P. nierstraszi* (B), *P. profundacella* (C), *P. superficialis* (D), *P. stellata* (E), *P. digitata* (F), *P. explanulata* (G). Basic biomineralization framework characterized by transverse groups of centers of calcification typical of Robusta (see Cuif et. al 2003) highlighted with white arrows whereas radial extension of septum with black lines/arrows in figure items subscripted with "2" and "4", respectively. Centers of calcification in all *Psammocora* species are ca. 20 µm in diameter but their arrangement at the growing septal edge is slightly different resulting in some variability in higher level micro-architectural units e.g, strongly bilaterally symmetrical "septal paddles" (D_3, E_3, F_3), or more star-like septal protrusions (A_3, B_3, C_3).

siderastreid but *C. wellsi* (Fig. 4B$_1$) which may have one circle of enclosed entosepta, often incomplete, as in *P. explanulata*. Petaloid septa like those described above in *Psammocora* form in *Coscinaraea*, *Craterastrea*, and are sometimes visible in *Horastrea* (Fig. 4A, B, C), but they never form in *Siderastrea*, *Pseudosiderastrea* and *Anomastraea* (Fig. 4D, E, F).

Synapticulae were observed in all the siderastreids (Fig. 4A$_4$, D$_4$, E$_4$, F$_4$) with the exception of *C. wellsi* which exhibits buttress-like structures joining the septa in all similar to those described for *P. explanulata* (Fig. 4B$_4$).

Striking differences of skeletal macrostructures were observed between *C. wellsi* (Fig. 4B) and the congeneric species *C. columna* (Fig. 4A). Though both species can form petaloid septa and interstomatous septa, they differ in the kind of structures joining the septa, in the nature of the wall and in the presence of tentacular lobes and costae (Table 2). Conversely, *C. wellsi* bears many similarities with *P. explanulata* (Table 2). The morphologic affinities between the two species concern the

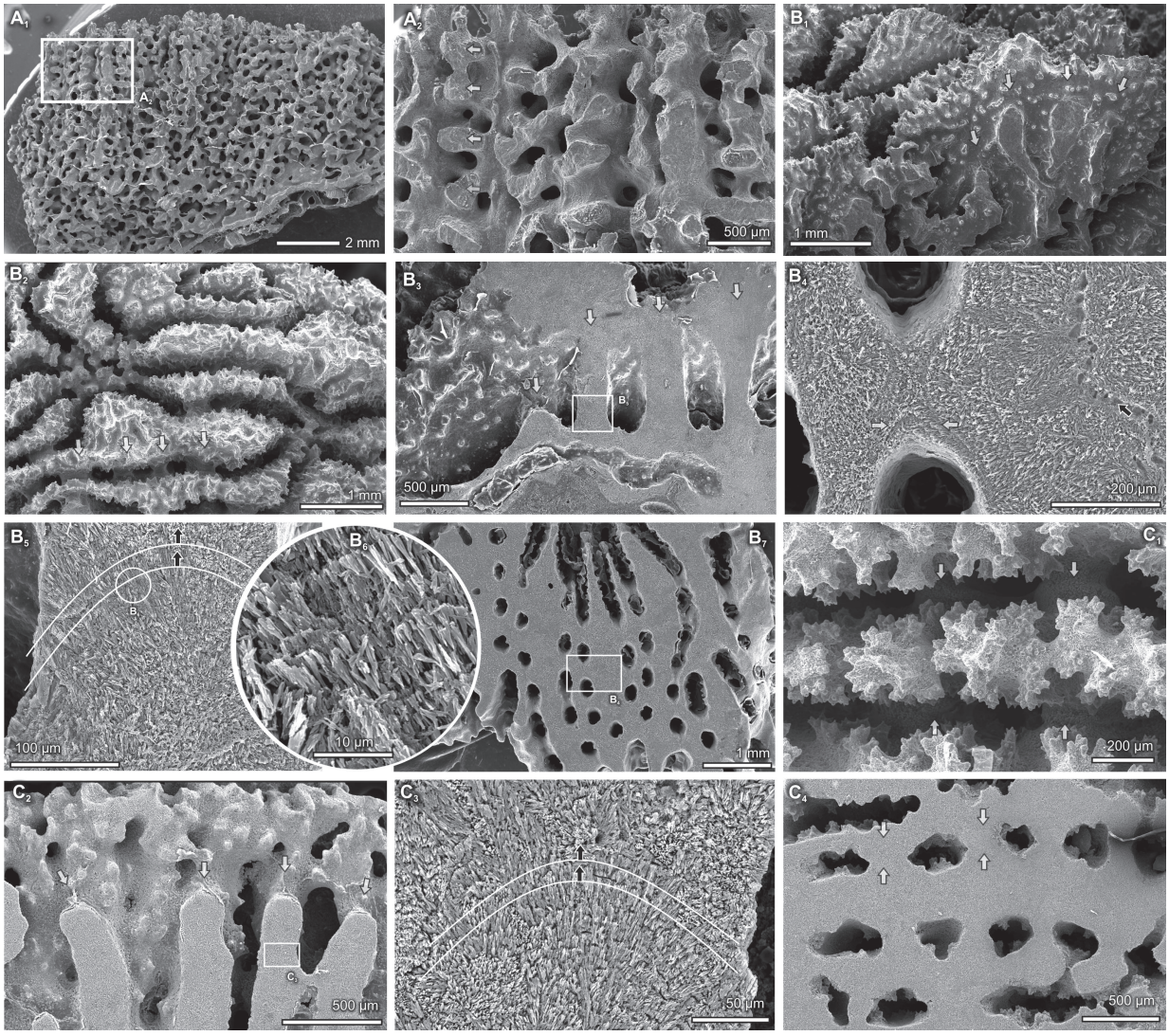

Fig. 5. Synapticulae as typically developed in *Psammocora* species (illustrated *P. digitata*, A) vs. fulturae in *P. explanulata* (B) and *Coscinaraea wellsi* (C). Fulturae are continuous bars (B$_1$, B$_3$, C$_2$; white arrows) vs. isolated from each other synapticulae (A$_2$; white arrows) and develop below septal edge (B$_1$, B$_2$, C$_2$) vs. developed at the same level synapticulae (A$_1$). Fulturae develop upwards the corallite as indicated by growth direction of the successive layers of fibers (B$_{5, 6}$, C$_3$) in longitudinal (B$_1$, C$_2$) or slightly oblique (B$_3$) sections but are formed from deposits growing from adjacent septa (white arrows in B$_4$; centers of calcification of midseptal zone marked with black arrow). Broken (A$_{1, 2}$-B$_{1, 2}$) and polished and etched skeletal sections (B$_{3\text{-}7}$, C$_{2\text{-}4}$).

presence in both of them of interstomatous septa, tentacular lobes, buttress-like structures joining the septa, and costae on a septothecal wall (Fig. 5B, C; Table 2).

Ridges composed of short spines/granules or well defined paddles are clearly discernible in the two species of *Coscinaraea* we examined (Fig. $4A_{2,3}$, $B_{2,3}$). In other genera, although granules do not form distinct septal paddles, there is tendency to form groups of spines/granules aligned transversely to the septal plane (Fig. $4C_3$, D_3, E_3, F_3).

Table 2. Skeletal characters in the examined Siderastreidae: 1) Multiple rows of enclosed septa surrounding the calicular fossa; 2) petaloid septa; 3) formation of series of calices enclosed by a common wall; 4) interstomatous septa; 5) tentacular lobes; 6) costae; 7) horizontal skeletal elements joining adjacent septa; 8) structure of the wall: + = present; - = absent; +/- = possibly present; s = synapticulae; f = fulturae; sew = septothecal wall; syw = synapticulothecal wall.

Genus	***species***	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Psammocora	*contigua*	+	+	+	-	-	-	s	syw
	nierstraszi	+	+	+	-	-	-	s	syw
	profundacella	+	+	+	-	-	-	s	syw
	superficialis	+	+	+	-	-	-	s	syw
	stellata	+	+	+	-	-	-	s	syw
	digitata	+	+	+	-	-	-	s	syw
	explanulata	+/-	+	-	+	+	+	f	sew
Coscinaraea	*wellsi*	+/-	+	-	+	+	+	f	sew
	columna	-	+/-	-	+	-	-	s	syw
Craterastrea	*levis*	-	+/-	-	-	-	+	s	sew
Horastrea	*indica*	-	+/-	+	-	-	+	s	sew
Siderastrea	*savignyana*	-	-	-	-	-	-	s	syw
Pseudosiderastrea	*tayamai*	-	-	-	-	-	-	s	syw
Anomastraea	*irregularis*	-	-	-	-	-	-	s	syw

The nature of the wall is not the same in all the Siderastreidae (Table 2). As described above for *Psammocora, Siderastrea*, *Pseudosiderastrea*, *Anomastraea* and *C. columna* also form epithecal-synapticulothecal wall. In *C. wellsi* and *H. indica* however, the colony wall has septothecal nature as in *P. explanulata*. The formation of series of calices enclosed by a common wall present in *Psammocora* is largely absent in the other siderastreids with the exception of *Horastrea*. This genus is unique among the siderastreids in forming plocoid instead of cerioid colonies.

The skeletal structures of *C. levis* resemble those of *C. columna* very closely. The species has been synonymised with the agariciid *Leptoseris* but the columella structure, septal ornamentation and synapticular pattern, very clearly illustrated and treated in the original description by Head (1983), leave no doubt on the fact that the species belongs to the Siderastreidae.

The molecular approach

The obtained electropherograms did not show any signal of intraspecific polymorphism ascribable to the presence of highly divergent gene copies within each sample genome, such as pseudogenes. In order to exclude this hypothesis, a detailed analysis of intraindividual variability would be required (Vollmer and Palumbi, 2004; Marquez *et al*., 2003). Yet, the presence of pseudogenes has been demonstrated only for the genus *Acropora* (Marquez *et al*., 2003), whose elevated polymorphism constitute an exception in Scleractinian genera rather than a common rule (Wei *et al*., 2006).

The secondary structure of the ITS2 of *H. indica*, *P. tayamai* and *P. crustacea* presented a type I structure with 4 domains (Chen *et al.*, 2004). Conversely *F. paumotensis*, *H. limax*, *P. explanulata*, and *P. contigua* fell into the type II model, with 5 domains (domain I appears divided in the two subdomains I_a and I_b). The conserved motif 5'-CRCGGYC-3' and its compensatory bases in stem II described for other family and genera (Chen *et al*., 2004) was also identified in our sequences. We also identified other conserved regions corresponding to stem IV (motif 5'-GGA -3' and its compensatory bases) and to the terminal stem of domain III (motif 5'- CG-CAC -3' and its compensatory bases) fixed in all the sequences we obtained except for *S. savignyana* and *P. tayamai*. Alignment was then adjusted imposing these constrains.

The complete alignment consisted of 76 variable positions, 66 of which were parsimony informative. When only sequences of *Psammocora*, *C. wellsi* and the Fungiidae were included, the number of variable sites was 32, of which 26 were parsimony informative.

The Statistical Parsimony analysis evidenced five distinct networks (Fig. 6), fixing a maximum value of 8 evolutionary steps allowed by a probability level of 0.95. The main network includes all the *Psammocora* specimens, the Fungiidae, and the Siderastreidae *C. wellsi* and *H. indica*. The other four networks separated from the main one are monospecific clades of the other Siderastreidae. Within the main clade, two principal subclades are evidenced. One subclade comprises *H. indica* and all the sequences of *Psammocora* with the exception of *P. explanulata*. The other connects all the Fungiidae sequences plus *P. explanulata* and *C. wellsi*. Three reticulations have been identified, though their

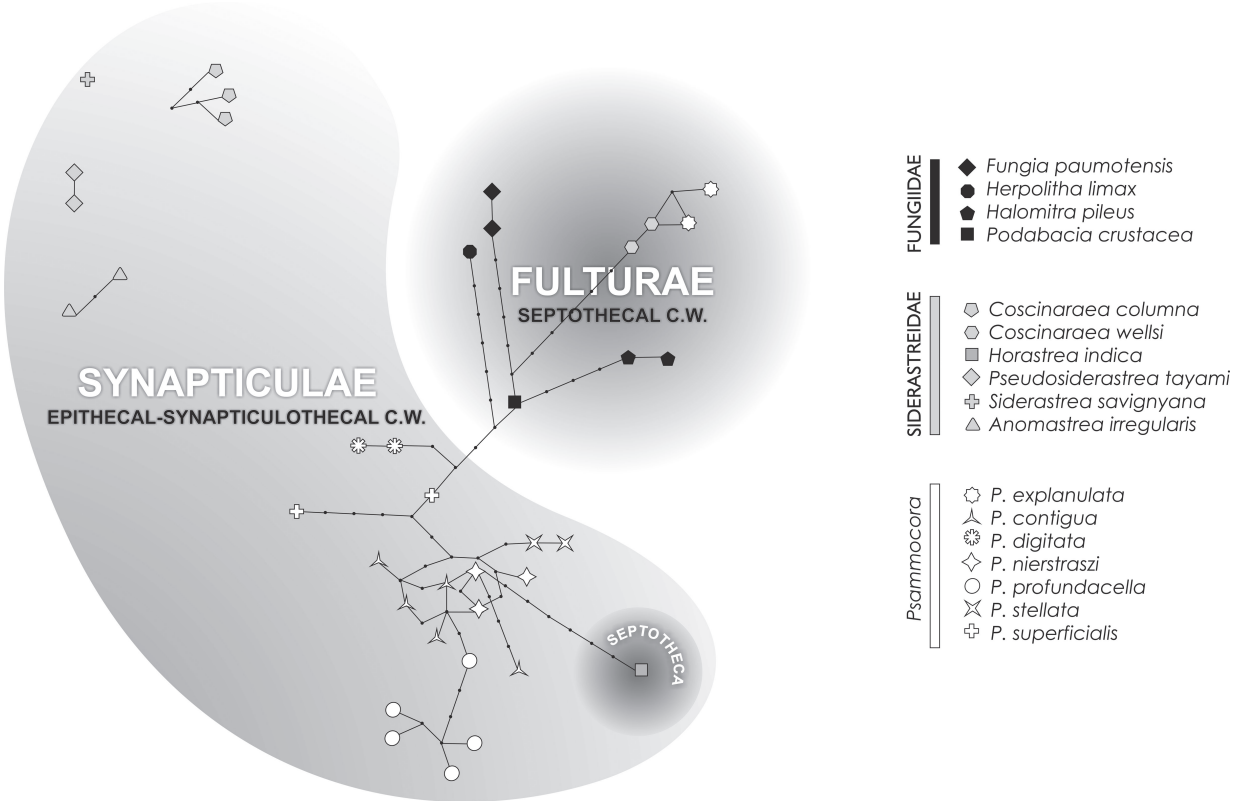

Fig. 6. Phylogenetic networks of ITS2 and 5.8S haplotypes of all the specimens listed in Table 1, calculated under a statistical parsimony criterion. Each polygon or circle in the network represents a haplotype. Each haplotype is univocally coded at species (shape) and family (colour) level since no haplotype is shared between species. One main clade including the haplotypes of all the examined *Psammocora* species (white filled shapes), *Horastrea indica* (grey filled square), *Coscinaraea wellsi* (grey filled hexagon) and of the Fungiidae (black filled shapes) is significantly disconnected from the other Siderastreidae (grey filled shapes). Grey shadows superimposed on the network indicate the nature of the colony wall (septothecal or epithecal-synapticulothecal c.w.) and the kind of structures connecting the septa (synapticulae or fulturae) found in the examined species. C.w. = colony wall.

impact on the overall topology is negligible.

The pairwise sequence divergences have been calculated on the basis of the Tamura and Nei evolutionary model (Tamura and Nei, 1993) accounting for Gamma correction of 0.3012 and equal base frequencies, as output by Modeltest software, and their values for each species estimated. Hereafter average distances for groups of interest are reported. The average distance of the genus *Psammocora* from the other Siderastreidae is 17.0 ± 17.3%, and from the Fungiidae is 4.0 ± 1.1%. The average interspecific distance within the genus *Psammocora* is 3.0 ± 1.6%, and within the genus *Coscinaraea* is 5.6 ± 0.6%. The average distance between *P. explanulata* and the other *Psammocora* species is 5.8 ± 0.8%, while the same species shows an average distance of 17.7 ± 20.6% from the Siderastreidae excluding *Psammocora*, and only of 4.0 ± 0.6% from the Fungiidae. Similarly the average distance of *C. wellsi* from *Psammocora* is 4.8 ± 1.4%, from the other Siderastreidae is 22.0 ± 22.1%, and from the Fungiidae is 3.6 ± 0.7%.

The phylogram, obtained according to a Bayesian approach, is reported in Figure 7. An eventual outgroup was not added, since it would introduce significant ambiguities and subjectivity in the alignment of the hypervariable region of ITS2. Consequently, the tree was midpoint rooted. Despite the phylogram adding information to the Statistical Parsimony network, the main patterns are conserved. In particular, 6 clades and subclades could be evidenced, congruent with the inferences obtained by Parsimony analysis. One of the main peculiarity here detected concerns the position of *C.*

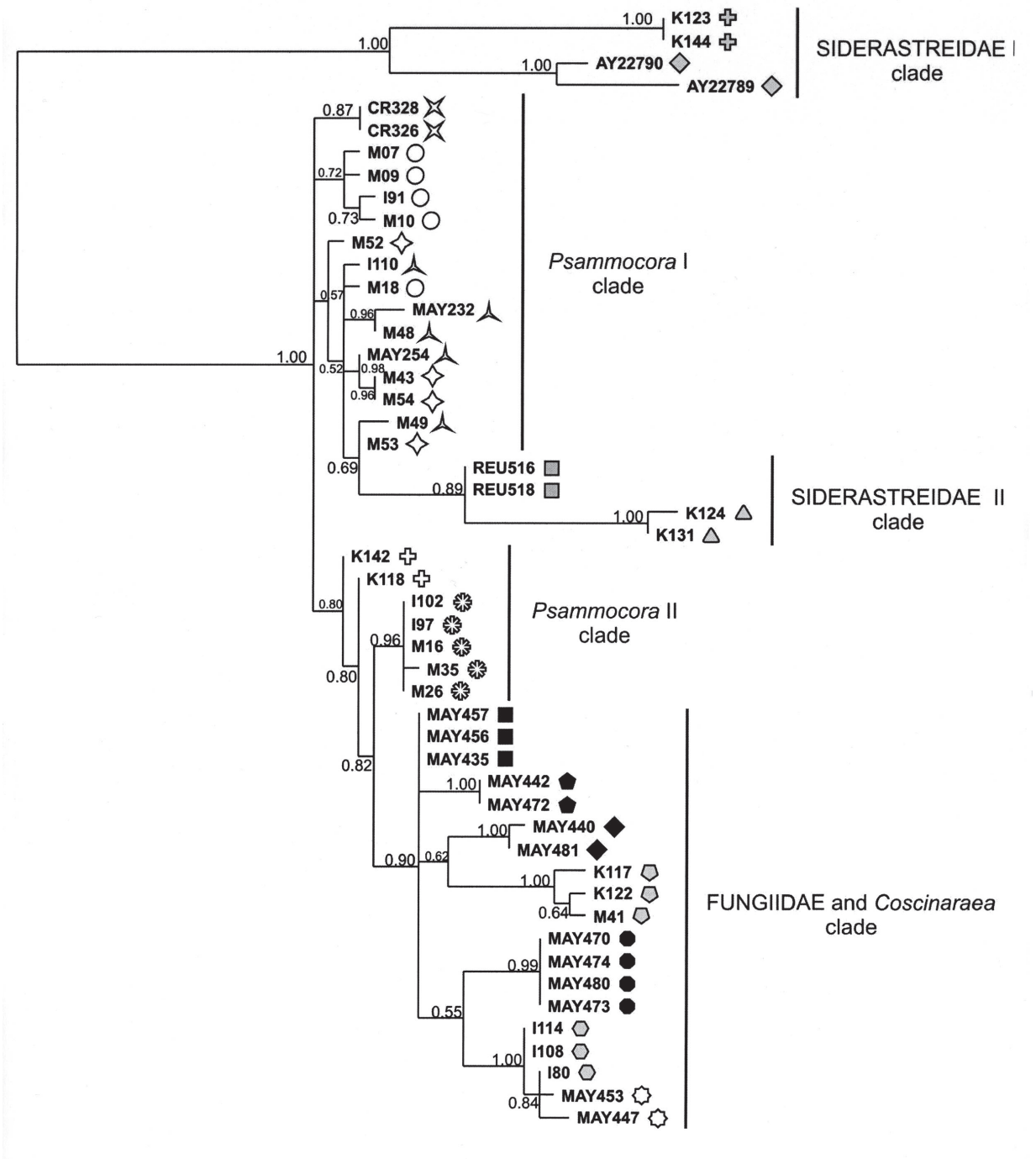

Fig. 7. Phylogenetic tree derived from Bayesian analysis of the partial 5.8S and ITS2 sequences of the studied Scleractinian species. Numbers at each node indicate the posterior nodal probabilities. Symbols explanation for each species as in Fig. 6.

columna, which appears affine to Fungidae, though the genus remains polyphyletic. Similarly, *A. irregularis* resulted affine to *H. indica*, yet it appears highly divergent from the main clades of the other Siderastreidae.

Discussion

Skeletal structures

The last fifty years of taxonomic history of Scleractinia point out the highly unstable nature of traditional, skeletal-based phylogenetic hypotheses. The case of *Psammocora* and the siderastreids is an excellent example of this (Fig. 1). Until recently the use of no other criteria than skeletal structures has resulted in arbitrary taxonomic frameworks. Moreover the proposed phylogenies were based on the most prominent skeletal components of the scleractinian corallum only, i.e. mostly septal features. Such biased perspective was, in turn, a possible reason for the underestimation of other skeletal structures as informative characters. In the peculiar case of the genus *Psammocora* even the septal features have been underestimated. The unique pattern of septa arrangement typical of the genus has been largely neglected in the last sixty years of published literature. The multiple rows of enclosed entosepta are missing in any other siderastreid genus including *Coscinaraea*, while they can be found in some Fungiidae. Such morphologic affinity between *Psammocora* and the Fungiidae seems to be corroborated by phylogenetic proximity as shown by the molecular results in our study. The lack of consideration of this macro-morphologic character has hence lead on the one hand to a biased evaluation of the structural affinities with the other siderastreid genera, while, on the other hand, it has also lead to the widespread use of incorrect terminology. The mesh of ento- and exosepta typical of *Psammocora* has been often erroneously referred to as the coenosteum while the extrapolypal tentacles above it as the coenenchyma (Milne Edwards and Haime, 1851; Klunzinger, 1879; Van der Horst; 1922; Hoffmeister, 1925; Yabe *et al*., 1937). Septocostae have also been recognised by various authors in *Psammocora* (Milne Edwards and Haime, 1851; Vaughan, 1907; Vaughan, 1918; Veron and Pichon, 1976; Veron, 2000) despite the absence of such structures in the genus.

Thecal structures were only superficially described in *Psammocora* species, and the structure of the colony/calice wall in *Psammocora* has not been mentioned at all in the original description of the genus (Dana, 1846). According to Milne. Edwards and Haime calices in *Psammocora* are "*sans muraille proprement dites*" ("without proper wall", 1851: 66), an opinion shared also by Vaughan and Wells (1943: 128) who stated that "corallite wall is absent, calicular boundaries sometimes marked by synapticular rings". Other authors have referred to the wall in *Psammocora* as "indistinct or wanting" (Verrill, 1866: 330) or "indistinct" (Veron, 2000: vol. 2, 144) without specifying its nature. Gardiner (1898: 358) described a "false wall formed by synapticula and trabeculae", and Chevalier and Beauvais (1987: 704) agreed with the former on the presence of a poorly defined and porous synapticular wall in *Psammocora*. Also thecal structures of the siderastreids have not been described thoroughly. Nonetheless the occurrence of synapticulothecal calicular wall was considered one of the diagnostic characters of the family (Wells 1956: F383).

The results of our study revealed significant differences between colony and calicular walls among typical *Psammocora* species and some siderastreids (Table 2) that seem to agree with the results of our molecular studies (Table 3 and Figure 6). Since the use of some of the morphological terms in modern scleractinian literature is often imprecise some key characteristics of these structures are discussed hereafter.

Colony wall (epitheca, complex wall structures, septotheca)

The colony wall and its distinction from the inter-calicular thecal structures have rarely been comprehensively treated. In the Scleractinia colony walls are most commonly comprised of epithecal composite structures: epitheca, the most external structure of the colony rim, is associated with other septa-connecting structures (epithecal-dissepimental, epithecal-trabeculothecal, epithecal-septothecal, epithecal-synapticulothecal walls; see Roniewicz and Stolarski, 1999). Growth acceleration of radial structures often results in the disappearance of the continuous epithecal cover and leads to the formation of other wall types, most typically septothecal.

In the *Psammocora* species we studied (with the exception of *P. explanulata*) colonies form epithecal-synapticulothecal wall. Conversely in *P. explanulata* the colony wall in adult colonies has a septothecal nature (early juvenile stages of colony wall formation have not been examined). The same septothecal wall develops also in the other taxa forming together with *P. explanulata* a clade in the molecular network shown in Figure 6 (i.e., fungiids and *C. wellsi*).

Calice thecal structures (synapticulae, fulturae, adtrabecular bars)

In many scleractinian taxa, especially those that form a porous skeleton, adjacent septa may join via crossbar-like rods called synapticulae (Vaughan and Wells, 1943:39). Synapticulae can be aligned across the adjacent interseptal spaces to form synapticular rings that in turn, if arranged one above the other, create a (inter)corallite wall called synapticulotheca. In the Fungiidae, Vaughan and Wells (1943) and Wells (1956) distinguished a synapticulothecal wall that could secondarily be thickened to form septotheca. A synapticulothecal wall was also recognised by the same authors in the Siderastreidae and in the Thamnasteriidae (that included *Psammocora*). Wells (1956: F385) mentioned that septal connections between of fungiid consisted of "compound synapticulae". Later Gill (1980) showed that the "simple" synapticulae widely occurring among scleractinians are not homologous to the "compound" synapticulae found in the Fungiidae. Hence, to avoid mixing up synapticular terminology, the same author coined the term "fulturae". Fulturae are continuous buttress-like structures occurring on septal faces developed below septal edge (see Gill, 1980: pl. 1:1 *Fungia*; Hoeksema, 1989: figs. 652 *Herpolitha*; 656 *Sandalolitha*; 664 *Lithophyllon*) which differ from the synapticulae, typically isolated from each other and developed often near the septal edge (Gill, 1977; 1980). Moreover, while synapticulae are commonly parallel to the trabeculae orientation, fulturae direction is mostly independent from that of septal trabeculae. Fulturae are arranged normally to distal septal margins and can be fused with similar structures on adjacent septa (Gill, 1980: 305). In longitudinal sections fulturae show fibrous layers arranged into arched zones (Gill, 1980: fig. 4a), which diverge from the "axial area of divergence".

The problem of homology between the synapticular structures became more complex as Roniewicz (1982) described vertically continuous, longitudinal interseptal structures on septal faces of the Jurassic coral *Thamnasteria concinna* (Goldfuss, 1826). According to Roniewicz (1982: 183) these structures, named adtrabecular bars, are "to a great extent, similar to fulturae" as they develop below the septal edge and are arranged normally to distal septal margins. However, adtrabecular bars differ from fulturae in the course of growth that follows that of particular septal trabeculae only, and in the consistent attachment to similar structures on adjacent septa.

Among the taxa examined in this paper, we distinguished a typical synapticulotheca in the corallites of all the *Psammocora* species (except *P. explanulata*), and all the siderastreids (except *Coscinarea wellsi*). The solid bars joining septa of *P. explanulata* and *C. wellsi* bear fulturae/adtrabecular bars characteristics: they develop below septal edge, are arranged normally to distal septal margins. They show arched zones of fibrous layers in longitudinal section, and exhibit ad-septal formation of their fibrous layers joining with a suture in the middle of the interseptal zone (Fig. 5B4) in transverse sections. The bar width in *P. explanulata* and *C. wellsi* is several times larger than the diameter of "calcification centers" on neighbouring septa (Fig. 5B4), hence their longitudinal extension follows the course of series of septal trabeculae. Arrangement of the solid bars in *P. explanulata* and *C. wellsi* resembles strikingly that of adtrabecular bars in the Jurassic *T. concinna*. In *Thamnasteria*, however, each adtrabecular bar is associated with a single septal trabecula. Noteworthy, these species all form thamnasteroid colonies, whose architecture may impose some constructional solutions of the solid bars different from that of solitary fungiid bearing typical fulturae. Conversely, even typical fungiid fulturae can occasionally follow the course of septal trabeculae (Gill, 1980: 304) and join with suture in interseptal zone. Consequently, there is no clear-cut distinction between adtrabecular bars and fulturae. We plan to investigate this problem comprehensively in a project that will focus on emergence of *Psammcora*-like structures among the fossil scleractinians. In this paper, however, we acknowledge that all mentioned buttress-like structures (fulturae *sensu lato*) share the same basic features (solid structure, regular spacing, development below the septal edge, arrangement normal to distal septal margin) whereas some structural differences may result from different colony specialization among the examined taxa. Possible homology between fulturae *sensu lato* is supported by our molecular results that group together fulturae-bearing fungiids, *Psammocora explanulata* and *Coscinarea wellsi* (Fig. 6).

Septal microarchitecture and microstructure

Recent molecular studies have challenged many long-held concepts concerning the evolution and systematic of scleractinian corals. One of the major outcomes of 28S rRNA and 16S rRNA studies is a phylogenetic hypothesis based on the occurrence of two major clades of Scleractinia: the Complexa and the Robusta clades, (in terminology by Kerr 2005) and few minor clades

(Romano and Palumbi, 1996; Romano and Cairns, 2000). Species of *Psammocora*, *Coscinaraea* and fungiids reveal affinity with the Robusta. Interestingly the Atlantic species *Siderastraea siderea* (the type species of the genus) is related to the Complexa clade (Romano and Cairns, 2000). Cuif *et al*. (2003) in a combined morpho-molecular study of 42 scleractinian species suggested that the occurrence of short series of calcification centers arranged transversally to the main septal course might be a skeleton-based synapomorphy of corals in the Robusta clade. Our survey of the microarchitectural and microstructural skeletal traits of *Psammocora* and of the Indo-Pacific siderastreids, not included in Cuif *et al*. (2003), also revealed that the formation of short series of calcification centers aligned transversely to the main course of septum is a prevailing septal biomineralization trait of these corals. In some of the specimens we examined, these transverse series were particularly distinct and expressed in septal microarchitecture as well defined paddles (i.e. *P. superficialis, P. digitata, P. explanulata*, and *Coscinaraea*) (Figs. 3: $D_{2\text{-}3}$, $E_{2\text{-}3}$, $F_{2\text{-}3}$; 4: $A_{2\text{-}3}$, $B_{2\text{-}3}$). All septal paddle-bearing species of *Psammocora* are positioned closely in the molecular network (Fig. 6). The possible taxonomic value of this character needs to be carefully evaluated in future quantitative studies of interspecific and intercolony variation of septal microarchitecture.

Phylogeny of *Psammocora* and the Siderastreidae

The utility of ITS2 marker for phylogenetic discrimination within the Scleractinia has been highly debated (van Oppen e*t al*., 2002; Vollmer and Palumbi, 2004; Chen *et al*., 2004), in parallel to its growing use (Hunter *et al*., 1997; Lopez and Knowlton, 1997; Medina *et al.*, 1997; Odorico and Miller, 1997; van Oppen *et al*., 2000, 2002; Diekmann *et al.*, 2001; Rodriguez-Lanetty and Hoegh-Guldberg, 2002; Chen *et al*., 2004). High levels of divergence within the genus *Acropora* and, more generally, in the taxa of the Complexa clade (Romano and Palumbi, 1996; Romano and Cairns, 2000) would prevent the use of ITS2 as a reliable marker due to retention of ancient lineages predating the origin of the species. Conversely the utility of ITS2 has been proved: 1) at the interfamiliar and intergeneric level when considering all the taxa included in the Robust clade (Diekmann *et al.*, 2001; Rodriguez-Lanetty and Hoegh-Guldberg, 2002; Chen *et al*., 2004), and 2) for the identification of the conserved region implied in the organization of the secondary structure of ITS2, regarded as an indispensable step for a reliable sequence alignment (Chen *et al*., 2004). Both conditions were met in the present study.

The minimum spanning network and the phylogram evidenced the presence of a wide range of divergence between and within the considered taxa. Some incongruences between the two phylogenetic representations are evidenced, mainly due to the different approaches they employ. However, common patterns could be easily evidenced. At the family level our results show that the Fungiidae are grouped in a monophylum though being paraphyletic to the genus *Coscinaraea* (*C. wellsi* according to both the approaches) and *P. explanulata*.

Conversely, the Siderastreidae are polyphyletic, highly divergent and characterised by six subclades, five of them significantly divergent according to Statistical Parsimony analysis. The high values of genetic distances detected between the species of this family confirm a deep phylogenetic structure of the Siderastreidae. In particular, the deepest divergence detected separates *P. tayamai* and *Siderastrea savignyana* from the remaining genera. This divergence was also suggested by Chen *et al*. (2004) and Romano and Cairns (2000), using respectively ITS2 on *P. tayamai* and *P. contigua*, and 28S on *Pseudosiderastrea* and *Siderastrea*. Conversely, analysis of the mtDNA 16S (Romano and Cairns, 2000; Le Goff-Vitry *et al*., 2004) grouped *P. stellata*, *P. contigua* and an unidentified specimen of *Coscinaraea* in a single clade with the Fungiidae, thus suggesting the phylogenetic affinity of these taxa. Both these inferences are congruent with our results. Their apparent contradiction is *de facto* due to their partial and non overlapping coverage of the species composition of the family Siderastreidae.

The phylogenetic relationships within the siderastreids showed that both *Psammocora* and *Coscinaraea* are not monophyletic and share a lineage at least in paraphyly with the Fungiidae, thus evidencing strong divergences within the two genera. The monophyletic status of both genera has never been investigated by means of molecular techniques before. Pandolfi (1992) while providing a cladistic biogeographic analysis of *Coscinaraea* also reconstructed the phylogeny of *Psammocora* and of the Siderastreidae using skeleton macrostructural characters. He concluded that *Coscinaraea* and *Psammocora* are monophyletic and closer to each other than to any other genus in the family. However, among the morphological characters he used, he did not include the microstructure of elements connecting septa, or other characters that proved useful in our study. Moreover, it is worth noting that the close phylogenetic

relationship between *Psammocora* and *Coscinaraea* evidenced in previous studies based on 16S mtDNA (Romano and Palumbi, 1996; Romano and Cairns, 2000; Le Goff-Vitry, 2004) is only in apparent contrast with our results. Such a close genetic affinity between the two genera was biased, since based in each cited study on the same unidentified *Coscinaraea* specimen (Genbank L76001) and a few *Psammocora* sequences. Moreover, the resolution power of mitochondrial markers is not sufficient to reveal phylogenetic differences at inter-specific level (Shearer *et al.,* 2002). The polyphyletic status of the two genera evidenced in our study could hence be explained by the application of a more variable molecular marker, a rDNA fragment, to a wider data set of species of the two genera.

The morphology based affinities within the siderastreids *sensu* Veron (2000), and between *Psammocora* and *Coscinaraea* in particular, are most unlikely to result from a real homology of the characters, and thus contrast with molecular data. Romano and Cairns (2000) were driven to the same conclusion for the suborder Fungiina, but failed to detect the same discrepancy within the families of this suborder. In the case of the Siderastreidae this was due to the incomplete coverage of species of the family. An indication of the potential polyphyly of the Siderastreidae was given in the work of Le Goff-Vitry *et al.* (2004) and in the combined morphological and molecular approach proposed by Kerr (2005). This last approach provided the fusion of the results of previous phylogenetic analysis based on different molecular markers and of morphology based phylogenies in a single supertree. Differently, our work provides a phylogenetic inference spanning most of the genera and species of the family using a single molecular marker and a combined morphological approach.

At the intrageneric level, the genus *Psammocora* showed a low divergence compared to the average interspecific distances detected for other genera of the Robusta clade (Chen *et al*., 2004), while *Coscinaraea* showed higher values of divergence. Within *Psammocora* the resolution at species level was only partially evidenced in our results. Specific clades could be distinguished unambiguously for *P. digitata*, and *P. stellata*. Conversely *P. contigua* and *P. nierstraszi* haplotypes were linked by reticulation and their relationship cannot be resolved. Intra-individual analysis is required to clarify whether this could indicate incomplete lineage sorting at species level or hybridization. The close phylogenetic relationship of *P. explanulata* and *C. wellsi* emerging from our study, however, matches the above mentioned skeletal structures affinity and suggests that the two species constitute a distinct lineage with stricter affinity with Fungiidae than with the family and genera they have been so far assigned to.

Therefore, the results of this study suggest the need for a revision of the systematic relationships within the genus *Psammocora*, *Coscinaraea* and between the families of the Fungiina.

Conclusions

As a result of the combined investigation of skeletal morphology and molecular phylogeny we can draw the following conclusions:

1. The genus *Psammocora* is not monophyletic. All the species we examined except *P. explanulata* share common macro and micro structural characters and result phylogenetically closely related. *P. explanulata* on the other hand differs from all the other species in the structure of the wall, presence of fulturae, costae and interstomatous septa between adult corallites.
2. The genus *Coscinaraea* is not monophyletic. The two species we examined present different macro and micro structural characters with respect to the structure of the wall, presence of fulturae and costae, and are genetically distant. *C. wellsi* is more closely related to the Fungiidae considered in this study than to the congeneric *C. columna*.
3. *C. wellsi* and *P. explanulata* are structurally and genetically closer to each other and to the Fungiidae than to any of the other Siderastreidae. The two species share macro and micro structural characters which are not found in any other of the Siderastreidae (i.e. interstomatous septa, tentacular lobes, costae, fulturae) and are phylogenetically very close to each other. Moreover their phylogeny shows stricter affinity with the Fungiidae than with *Psammocora*, *Coscinaraea*, or any other Siderastreidae.
4. The Siderastreidae *sensu* Veron (2000) are not monophyletic and deeply divergent. The genera *Psammocora* and *Coscinaraea* are closer to the Fungiidae than to the Siderastreidae. The genera *Siderastrea* and *Pseudosiderastrea* are those that share the least characters in common with the other genera in the family, and that are phylogenetically more distant from the others and closer between them.

Our study shows that care should be taken in inferring family level phylogenies from one or two species per

genus only until the monophyly of the traditionally recognised genera in the Scleractinia is proved. Moreover, it confirms that, despite the clear advances brought by the use of molecular techniques, comprehensive skeletal studies including previously neglected or misidentified macrostructural and microstructural characters can still provide new information and useful tools for the study of the phylogenetic relationships within the Scleractinia.

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