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Coralline demosponges—a geobiological portrait

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Abstract. The polyphyletic coralline demosponges possess a calcareous basal skeleton of 4 major morphotypes. Each has its own phylogenetic history, with different mechanisms of formation. One extant taxon of each skeletal type has been investigated, and its biochemical (e.g., intracrystalline organic matrix proteins), geochemical (e.g., stable isotopes), and histological properties described in detail.

The thalamid *Vaceletia* shows similarities in its skeletal features to extinct archaeocyathid sponges due to the presence of special Ca^{2+} waste deposit chambers in the lower part of the skeleton. In our opinion this type is phylogenetically the most important one because it represents one possible evolutionary way of Ca^{2+} detoxification and illustrates one function of basic biomineralization (Ca^{2+}-detoxification). More sophisticated biomineralization processes are developed in the agelasid *Ceratoporella*, the "chaetetid" hadromerid sponge *Spirastrella* (*Acanthochaetetes*) *wellsi*, and the "stromatoporoid" agelasid *Astrosclera willeyana*. Each of these taxa shows a distinct process of formation with a unique composition of its intracrystalline organic matrix and geochemical features, here characterized in detail. A model of phylogenetic relationships and grades of development is proposed.

The first metazoans with $CaCO_3$ biomineralization were the worm-like Cloudinidae from the late Sinian, which form a tube with a foliated structure. However, the taphonomy-controlled mode of basal skeleton formation in Archaeocyatha and Vaceletidae is the most ancient type of biologically-controlled metazoan biomineralization. In general, basal skeletons of coralline sponges represent the simplest biologically controlled mineralization, intermediate between biologically induced type (e.g., organomineralization) and the fully enzymatically-controlled mineralization of higher Metazoa.

Key words : *Astrosclera*, biomineralization, *Ceratoporella*, coralline demosponges, Porifera, *Spirastrella* (*Acanthochaetetes*), *Vaceletia*

Introduction

Sponges are active filter-feeding organisms, with their evolutionary history beginning in the early Proterozoic, based on sponge-specific fossil organic molecules (biomarkers) (McCaffrey *et al*., 1994). The first mineralized sponge remains (spicules) are known from the late Proterozoic (Sinian) of China (Steiner *et al*., 1993) and Namibia (Reitner, unpublished data). Calcified basal skeletons occur first in the Tommotian with archaeocyathid sponges (e.g., Reitner *et al*., 1997b). Poriferan calcareous basal skeletons are formed in most cases in equilibrium with ambient seawater, based on stable isotope signals (Reitner, 1992). The lack of neuronal systems, muscular cells, and organs places the Phylum Porifera towards the phylogenetic base of the Metazoa. Coralline sponges or "sclerosponges" are a polyphyletic grouping of pinacophoran sponges (Demospongiae and Calcarea) (Reitner, 1992 ; Reitner and Mehl, 1996), able to construct a secondary basal skeleton of high-Mg calcite or aragonite (Reitner, 1992 ; Vacelet, 1977, 1979, 1985). They significantly contributed to reef formation since the beginning of the Phanerozoic (e.g., stromatoporoid reefs of the Ordovician to Devonian). Replaced in their reef-building function by scleractinian corals in modern reefs, living representatives of the coralline sponges are nearly all restricted to cryptic niches of coral reefs or deep forereef areas (Hartman and Goreau, 1975 ; Reitner, 1993). Beginning in the Mid-Cretaceous, hermatypic corals became more and more dominant as frame builders of reefs with the rapid co-evolution of coralline red algae. Since then, coralline sponges are restricted to cryptic niches and deep reef areas with low or absent light.

On the generic level, coralline sponges such as *Ceratoporella, Acanthochaetetes, Astrosclera* and *Vaceletia* are regarded as "living fossils", due to their occupation of the same ecological niches for hundreds of million years. Furthermore, they show the same basal skeleton characteristics as their fossil relatives (e.g., Reitner, 1992 ; Wörheide, 1998). In this study, histological and biochemical characteristics related to basal skeleton formation of these four extant coralline sponges are presented. *Vaceletia* is an example of a thalamid or sphinctozoid grade of skeletal organisation, with the earliest representative of this genus known from the Middle Triassic (Reitner, 1992). Morphological similarity to the extinct group of Cambrian Archaeocyathida is profound. The relationship of organic macromolecules to biomineralization was also investigated for the chaetetid *Spirastrella* (*Acanthochaetetes*) *wellsi*, the stromatoporoid *Astrosclera willeyana*, and the crust-type *Ceratoporella nicholsoni*. It has been shown in our previous studies that biomineralization processes in coralline demosponges are relatively simple but extremely conservative (Bergbauer *et al*., 1996 ; Reitner, 1987 ; Reitner and Engeser, 1985 ; Reitner and Gautret, 1996 ; Wörheide *et al*., 1997a, b ; Wörheide, 1998).

Based on our now extended and new data we are able to postulate evolutionary trends in coralline demosponge biocalcification, from taphonomy-controlled biomineralization in *Vaceletia* to very complex biomineralization with an initial intracellular formation of basal skeleton elements in *Astrosclera*.

Material and Methods

A detailed description of material studied and methods used has been given in Reitner (1992, 1993), Reitner *et al*. (1997b), Wörheide *et al*. (1997a, b), Wörheide (1998) and Reitner *et al*. (2000). See these papers for details.

Geobiology of coralline sponges

Bacteria in coralline sponge tissues

Many sponges possess various amounts of heterotrophic and phototrophic bacteria, but although various attempts have been made, it is extremely difficult to cultivate these bacteria using classical methods. To overcome this, a new molecular-biological method was used to determine bacteria in sponges : FISH = Fluorescence In Situ Hybridization (Manz *et al*., 2000 ; Schumann-Kindel *et al*., 1997). In all enrichment cultures with lactate as sole carbon source, which were inoculated with tissue from the two demosponges *Chondrosia reniformis* and *Petrosia ficiformis*, bacteria could be detected by *in situ* hybridization using highly specific probes (Manz *et al*., 1998) for sulfate-reducing bacteria (SRB) of the *Desulfobacter*- and *Desulfovibrio*-group. There is a distinct population of facultative and strict anaerobic bacteria within the selected sponges. Due to the different enrichment cultures of the sponge-associated anaerobic bacteria it is possible to characterize the organisms involved, both physiologically and phylogenetically. Examination with SRB-specific probes revealed that there is no dominance of a specific sulfate-reducing organism. The cultivated sulfate reducing organisms cluster in the *Desulfobacter*- and *Desulfovibrio*-group of the delta-*Proteobacteria*.

A great number of bacteria display a remarkable metabolic potential. These bacteria could be assigned to the alpha-, gamma- and delta-subclass of *Proteobacteria*. Coralline sponges, except *S.* (*Acanthochaetetes*) contain bacteria which constitute 50% and more of the biomass of the sponge individual. This figure is based on the number of bacteria *versus* number of sponge cells per area unit in different anatomical parts of the sponge (ectosome, choanosome). Other sponges have a lower amount of bacteria (10-20% of the sponge biomass). This observation, therefore, is of particular interest because some sponges can be regarded as "bacteria containers". The majority of sponge-related bacteria are symbiotic and sponge specific (i.e., not common in the ambient water outside the sponge, Wilkinson, 1984). Most published papers deal with phototrophic cyanobacteria in sponges and their role within the reef community (e.g., Sarà, 1971 ; Vacelet, 1971 ; Wilkinson, 1978c ; Wilkinson and Fay, 1979). The role of enormous numbers of heterotrophic bacteria is until now barely known (Santavy *et al*., 1990 ; Vacelet, 1970, 1971, 1975 ; Wilkinson, 1978a ; Wilkinson, 1978a, b, c). Heterotrophic bacteria in sponges are concentrated within the intercellular mesohyl. They are enriched within mesohyl zones of the choanosomal layers. *Vaceletia crypta* shows a high density of mesohyl bacteria (Reitner, 1993 ; Vacelet, 1977). Based on their phenotypic characteristics and physiological properties, the bacteria determined closely resemble those of the gamma-*Proteobacteria*. Most forms observed show a gram-negative cell wall structure. Many of these bacteria are related to the taxa *Vibrio* and *Aeromonas*. Nearly the same bacterial populations were detected in *Astrosclera willeyana* and within "lithistid" demosponges. All of these sponges have prominently developed mesohyl spaces with a network of thin fibers [probably bacterial exo-polymeric substances (EPS)] (Wörheide, 1998), inbetween the bacteria are located, and such sponges are characterized by small choanocyte chambers (*Astrosclera willeyana* 10-15 μm, *Vaceletia crypta* 15-20 μm, *Geodia* sp. 5-10 μm), as well as with a decreased abundance of choanocyte chambers per area unit. Within *S.* (*Acanthochaetetes*) *wellsi*, symbiotic bacteria observed are small (<1 μm) and rarely ovoid. Choanocyte chambers here are very large with a diameter of 40-50 μm. Endolithic heterotrophic cyanobacteria of the *Plectonema* group are common in the calcareous basal skeleton (Reitner, 1993).

Little is known about the function of heterotrophic bacteria in coralline sponges and sponges in general, in contrast to that of phototrophic ones (Wilkinson, 1978a, b ; Wilkinson and Fay, 1979 ; Wilkinson and Trott, 1983). Bacteria known in *Ceratoporella nicholsoni* are able to ferment sucrose and fucose, but are unable to ferment glucose, as is typical for most aeromonads. In all observed coralline sponges from around Lizard Island large amounts of fucose and galactose were detected, indicating fermentative processes (Reitner, 1992, 1993). An important observation here is that bacteria take up dissolved amino acids (Wilkinson and Garrone, 1980) and they are also able to degrade sponge collagen (Wilkinson *et al*., 1978). Reiswig (1981) observed that dissolved

organic carbon (DOC) produced by bacteria must be an important nutrient source for the sponges. The fermentative metabolism of the symbionts and DOC provided allows sponges to survive ecological crises when pumping rates are decreased. Santavy *et al*. (1990) have postulated that anaerobic zones may enhance calcification of the basal skeleton of *Ceratoporella* by maintaining acidic environments, and Wörheide (1998) postulated similar anaerobic micro-environments for *Astrosclera*, thereby facilitating biocalcification by increasing pH and alkalinity. The uptake of symbiotic bacteria by archaeocytes for digestion was frequently observed in *Astrosclera* (Wörheide, 1998).

In conclusion, the natural products of symbiotic bacteria in sponges play primary roles as : a nutrient source, a control of metabolic processes, a way to eliminate metabolic waste, and a way to perhaps enhance calcification. Our new results have shown that mesohyl bacteria may also enhance the formation and structure of sponge specific membrane lipids. Typical biomarkers of demosponges are special carbonic acids ("demospongic acids"). Precursors of these fatty acids are formed by sponge-related bacteria (Thiel *et al*., 1999). Wörheide (1998) observed in *Astrosclera willeyana* that mobile cells in the sponge (bacteriocytes) take up bacteria with subsequent phagocytosis. Non-phagocytized remains of these bacteria are some exo-polymeric substances (EPS) which are concentrated in large vacuoles of waste cells. Most of the EPS are acidic polysaccharides and able to bind divalent cations. Therefore, Wörheide (1998) postulated that *Astrosclera* uses the EPS remains in large vacuoles to trigger initial nucleation of $CaCO_3$ crystals.

Pyrite in sponges—Product of Sulfate reducing bacteria

The distinct population of anaerobic sulfate-reducing bacteria (SRB) of the delta-*Proteobacteria* is responsible for pyrite formation. Apart from these, other anaerobic bacteria with different morphotypes can also be enriched. Gram positive *Clostridia* (typical proteolytic or saccharolytic bacteria) are observed in histological sections in a few cases. These organisms generally play a significant role in the degradation of organic material. After death of a sponge, the inner part of the sponge tissue can become anoxic if the specimen is located in a container situation (e.g., in enclosed spaces, borings, sediment ; see below). Only this taphonomic supposition allows the complete preservation of (soft) sponge skeletons, which only have a very low fossilization potential. Apparently, after death sponge-related bacteria are no longer controlled by the active substances produced by the sponge (like certain antibiotics), and very rapid growth of these sponge bacteria begins, as observed in artificial decaying experiments. The microbial population oxidizes the organic carbon of tissue to CO_2, and sulfide is then formed by degradation of organic S-compounds as well as by the reduction of sulfate. This degradation can also decrease the redox potential in remnants of the sponge to −400 mV (in experiments). Under these conditions, it is possible for sulfide to rapidly precipitate as FeS. If higher concentrations of sulfide and iron are present, FeS_2 would be precipitated. Bacterial sulfate reduction significantly increases carbonate alkalinity which controls calcification events. We have measured high values of alkalinity (10-40 meq/l) in artificial sponge decaying cultures. Additionally, ammonification processes complete the degradation process, also increasing alkalinity. Sulfate reduction and ammonification together increase general alkalinity and favor special taphonomic calcification events that are characteristic for many fossil sponge occurrences, mainly in autochthonous spiculites and mud mounds. Pyritization can go hand in hand with calcification. Degradation of organic matter leads to the formation of NH_3 and CO_2 due to (based on) the total oxidation of organic matter. As a result, the carbonate alkalinity, mainly HCO_3^-, increases which is important for taphonomically controlled $CaCO_3$ formation. Organic acids are oxidized to CO_2 which is partly precipitated as calcium carbonate, provided that sufficient concentrations of cations are present. Therefore, the pH may slowly increase. Such mineralizing events in sponge tissues are restricted to sponges located in small caverns (e.g., boring cavities of excavating sponges), semi-closed pockets of sediment, specimens within thick spicule mats, or restricted to sponges with a rigid skeleton combined with thick organic tissue (rigid Hexactinellids, lithistids, sponges with a basal skeleton). We have never observed calcification in sponges without these protective features. First results show that the bacteria studied are linked to the *Vibrio*-group of gamma-*Proteobacteria*. The cultivated sulfate-reducing organisms can be assigned to the *Desulfobacter*- and *Desulfovibrio*-group of the delta-*Proteobacteria* (Schumann-Kindel *et al*., 1997).

Principles of biomineralization of coralline sponge basal skeleton

According to Lowenstam (1981) and Lowenstam and Weiner (1989), biomineralization is by definition a biological process which is controlled by organisms. Thus, skeletons are formed that are integral, functional parts of the organisms. Organomineralization (Defarge and Trichet, 1995 ; Reitner *et al*., 1995, 1997a ; Trichet and Defarge, 1995) is the term used to describe mineralization processes that involve organic molecules or particles, whether linked to living organisms or not. Thus, organomineralization is not necessarily linked to living micro-organisms or metazoans, but may occur anywhere in non-living, re-organized macromolecular films or aggregates (e.g., Reitner *et al*., 1997a, 2000) (Figure 1).

Organic macromolecules involved in organo- and biomineralization are polyanionic polymeres of dividing chains composed of monomers such as amino acids and sugars (e.g., Marsh, 1994). Their substantial participation in biomineralization is indicated by the fact that organic macromolecules can comprise up to 5% of the total mass of a biomineral. As a consequence of molecular size and abundance of polar groups, organic macromolecules of a biomineral comprise "soluble organic matrices" (SOM), water soluble after dissolving the mineral by EDTA (ethylenediamine-tetraacetate), and "insoluble organic matrices" (IOM). IOM substances are more or less neutrally charged, to a high degree polymerized, and important as frame building matrices (like collagen, cellulose, chitin, or silk

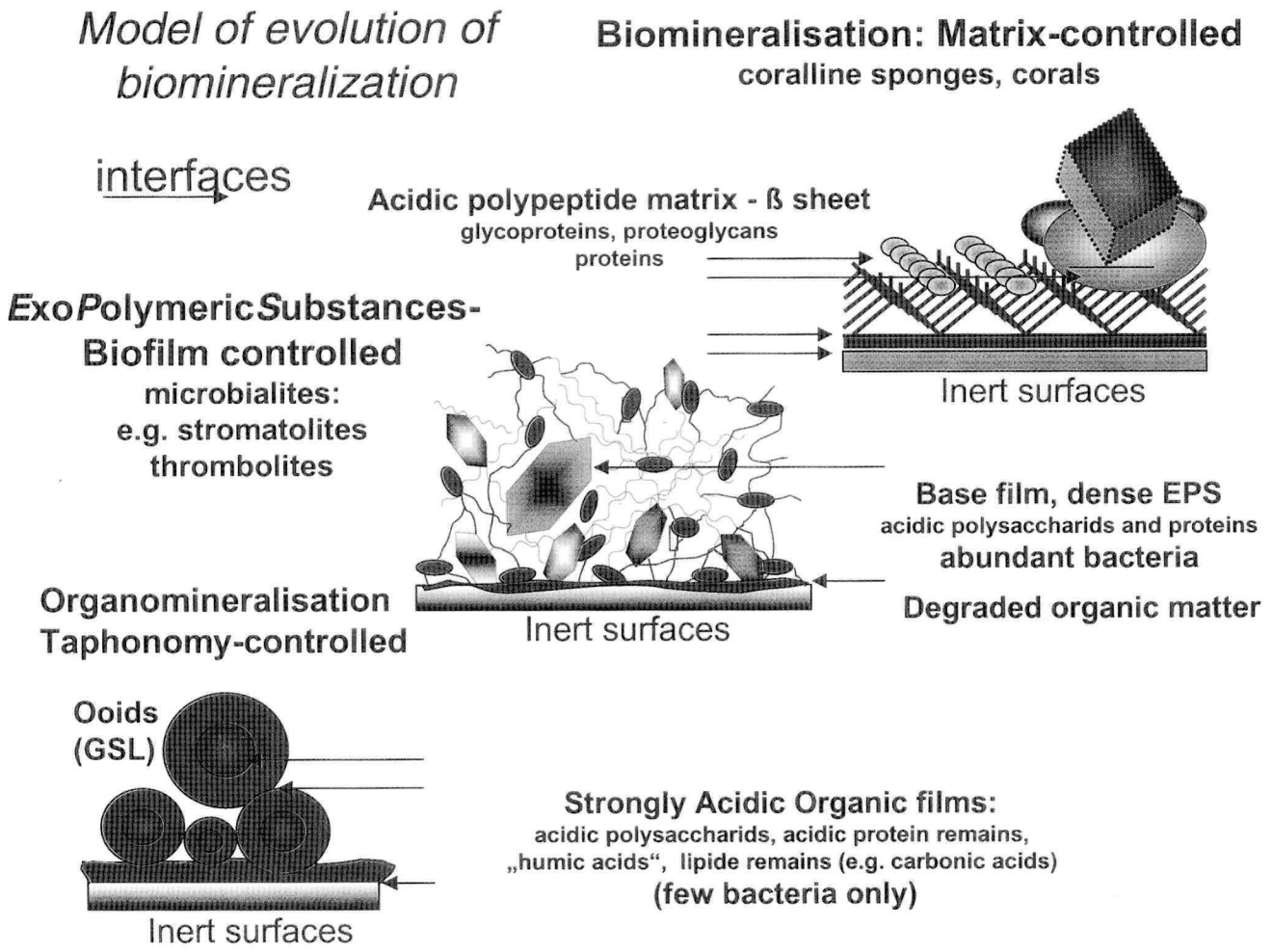


Figure 1. Model for evolution of biomineralization from organomineralization (taphonomy-controlled) of Great Salt lake Ooids via an exo-polymeric substance (EPS) to a biofilm-controlled calcification in microbialites to matrix-mediated mineralization of coralline sponges.

proteins). Rigid mineralized surfaces, serving as adsorption/binding sites for SOM, may be considered analogous to insoluble matrices.

SOM molecules are often highly acidic, due to abundant free, negatively-charged carboxylate groups. Here, molecules typically are rich in the amino acids aspartic acid (asp) and/or glutamic acid (glu). With their two carboxylate groups these amino acids remain negatively charged after peptide bonding, in contrast to amino acids with aliphatic, basic, or aromatic residues. SOM usually exhibit relatively low mean molecular weights of 10-30 kD. Also common are saccharide groups which are covalently (glycosidic) bonded with acidic, protein-forming glycoproteins. These have free carboxylate- and/or sulfate groups which also are proton donors. Further acidic macromolecules are acidic proteoglycanes, glycosaminoglycans and proteins, (glycosaminoglycane=Uronic acid+aminosugars), and simple polysaccharids.

The crucial first process of biomineral precipitation is the formation of seed crystals (nucleation). Nucleation occurs either in a homogenic or heterogenic form (Sigg and Stumm, 1989). Homogenic nucleation is observed only under artificial conditions in extremely pure solutions and is not realized in natural environments. In contrast, heterogenic nucleation is ubiquitous in nature and happens spontaneously in the presence of very small extraneous particles, such as molecules, ions, or foreign atoms within supersaturated solutions. These extraneous particles have a catalytic effect by decreasing the activation energy of crystal forming ions. This process is most successful when nucleation surfaces of extraneous particles are closely similar to the newly forming seed crystal (Sigg and Stumm, 1989). In organo- and biomineralization, heterogenous crystal nucleation is provided by acidic organic macromolecules that attract and bind divalent cations such as Ca^{2+}, Mg^{2+} and Sr^{2+}, or by their free carboxylate and/or sulfate groups. Acidic SOM, when adsorbed to an insoluble organic matrix, have been shown to induce $CaCO_3$ mineral formation. To the contrary,

dissolved acidic SOM strongly inhibit mineral precipitation. This is explained by Addadi and Weiner (1985, 1989) utilizing a model that postulates the formation of a flat molecular monolayer of acidic macromolecules in polypeptide ligature chains on the surface of an (inert) IOM substance (Figure 1). The chains are linked by hydrogen bonding and are arranged on the neutral, non-reactive IOM surface in a folded secondary structure as β-sheets (Addadi *et al*., 1990 ; Addadi and Weiner, 1989 ; Boskey, 1996 ; Worms and Weiner, 1986). The negatively charged COO^- -groups of the β-sheets are responsible for binding of Ca^{2+} from the liquid phase. If the COO^- groups have definite distances, the Ca^{2+} ions form an initial crystal plane, e.g. the 001 plane, perpendicular to the C-axis of $CaCO_3$ crystals. Carboxylate groups and complexed Ca^{2+} ions form an interface between the acidic organic macromolecule and the inorganic crystal. The distances between the vertical side chains of the β-sheets (COO^--groups) thus determine the calcium carbonate mineral formed (4,99Å = calcite, 4,96Å = aragonite, 4,13Å = vaterite) (Addadi and Weiner, 1989 ; Wheeler and Sikes, 1989). In certain cases the carboxylate groups of asp and glu in the β-sheets are arranged distally (far from the insoluble/framebuildung matrix) in one plane.

Very important for crystal growth are the vertical side chains of β-sheets, constructed of glycosidic-linked acidic saccharide-groups, mostly oligosaccharides. These sugars have structurally disorganized, negatively charged sulfate groups. These sulfate groups are considered to be responsible for creation of a Ca^{2+} flux towards the β-sheet, whereas nucleation is caused by binding at carboxylate groups. However, crystal growth is also limited or controlled by these very large and acidic macromolecules (Addadi and Weiner, 1985, 1989 ; Wheeler and Sikes, 1989).

Beside the function of enzymes like calmodulin and membrane bound Ca^{2+}-ATPase, which directly transports calcium to loci where it is used as a physiological control factor (Degens, 1979), $CaCO_3$ biomineral formation can be useful for the organism because this process allows the elimination of a cell-toxic surplus of Ca^{2+}.

Extremely acidic macromolecules, predominantly glycoproteins, inhibit precipitation by Ca^{2+} binding until the saturation of their acidic groups. These very acidic compounds are able to serve as inhibitors of any mineralization, or else, only allow the crystal to grow in selected directions. In coralline demosponges, these acidic macromolecules are enriched in mucus substances in areas of active calcification (see case studies below, and Reitner and Gautret, 1996 ; Wörheide, 1998). Most successful crystal formation takes place in the presence of weak to medium acidic glycoproteins in mucus, optimally with asp and glu concentrations of 25-30%. This phenomenon was also observed during formation of high-Mg calcite in the sponge *Spirastrella* (*Acanthochaetetes*) *wellsi* (Reitner and Gautret, 1996). The inhibition potential of very acidic macromolecules, however, decreases rapidly when most of the negatively charged valences are neutralized. Then, the now only weak acidic mucus starts to mineralize rapidly.

Evolutionary trends of biomineralization in coralline sponges

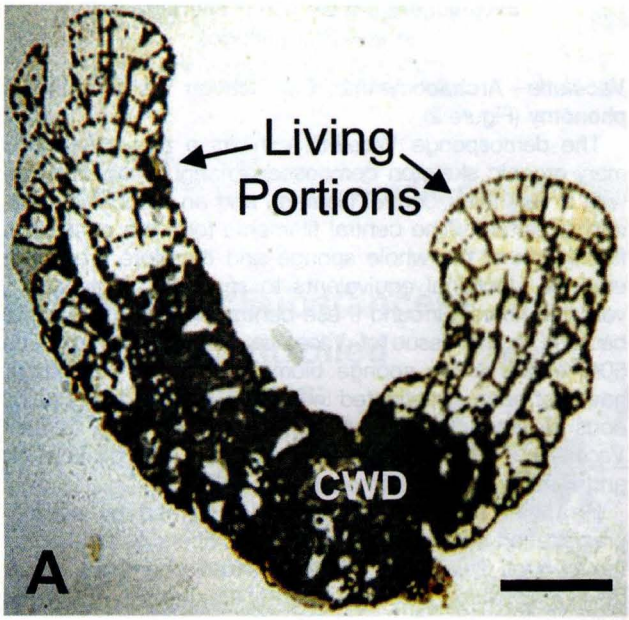
***Vaceletia*—Archaeocyatha : Calcification via controlled taphonomy** (Figure 2)

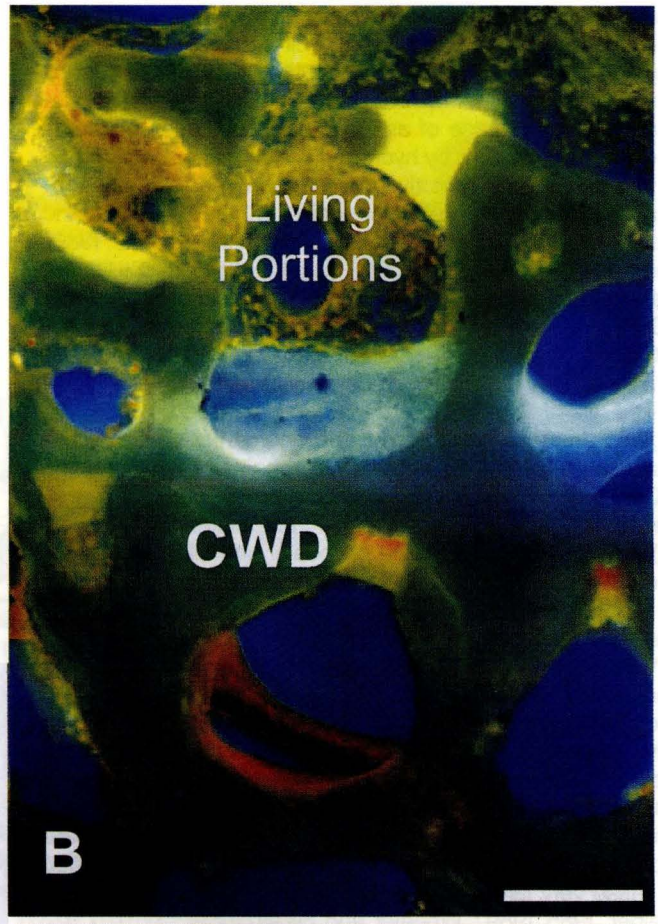
The demosponge *Vaceletia* exhibits a non-spicular, primary organic skeleton composed of irregular organic fibers with a very thick central filament, and an aragonitic secondary skeleton. The central filaments form the constructive framework of the whole sponge and therefore are considered as functional equivalents to spicules. A network of very thin fibers surround these central filaments. Symbiotic bacteria in the tissue of *Vaceletia* comprise approximately 50% of the entire sponge biomass. So far, the bacteria have not been investigated in detail. Two modes of calcareous skeleton formation take place within the tissue of *Vaceletia* (Reitner, 1992 ; Reitner *et al*., 1997b ; Wörheide and Reitner, 1996 ; Wörheide *et al*., 1996).

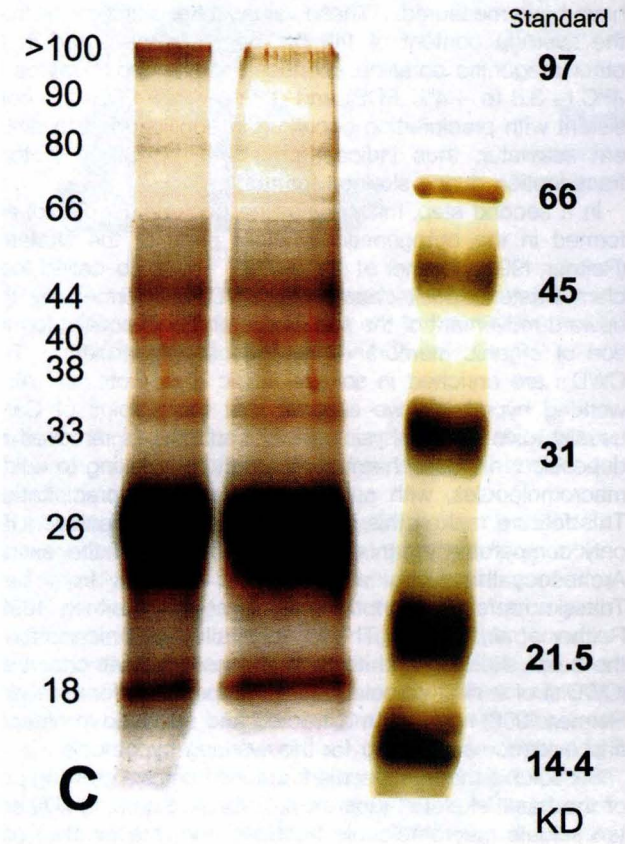
In a first step, basal skeleton is formed by aragonite precipitation between the thin organic fibers associated with the central filaments. Millimeter-sized chambers are the result, subdivided by pillars of compact, irregular, microcrystalline aragonite. Trace element analyses of this aragonite show high Sr (8,000-10,000 ppm) and U (4-6 ppm) contents. In addition, unusually large amounts of Mg (3,500-1,752 ppm) have been measured. These values differ significantly from the average content of 140-500 ppm Mg measured in all other aragonitic coralline sponges investigated. Values of $\delta^{13}C$ (+3.8 to +4‰ PDB) and $\delta^{18}O$ (−1.3‰ PDB) are consistent with precipitation occurring in equilibrium with ambient seawater, thus indicating no vital effect on isotope fractionation during skeletal formation.

In a second step, microcrystalline pockets (Figure 2d) are formed in the ontogenetically older parts of the skeleton (Reitner, 1992 ; Reitner *et al*., 1997b). These so-called "calcium waste deposit chambers" (CWD) are formed by the upward movement of the soft-tissue and subsequent formation of organic membranes by the basopinacoderm. The CWD's are enriched in soluble acidic glycoproteins. As a working hypothesis we suggest that the surplus of Ca^{2+}, usually toxic under physiological conditions, is removed by deposition in these chambers through complexing to acidic macromolecules, with subsequent aragonitic precipitation. This feature makes this coralline sponge truly unique ; the only comparable organisms known are taxa of the extinct Archaeocyatha along with coralline sponges from Late Triassic reefs (*Cassianothalamia, Uvanella*) (Reitner, 1992 ; Reitner *et al*., 1997b). The intracrystalline organic matrix of the basal skeleton and its calcium waste deposit chambers (CWD's) of a new colonial *Vaceletia* species (Wörheide and Reitner, 1996) have been extracted and analyzed in order to find evidence supporting for this working hypothesis.

Six soluble macromolecule fractions from the growing part of the basal skeleton (approx. 120, 95, 50, 33, 25, 16 kD), and ten soluble macromolecule fractions from the inactive part (CWD's) (approx. 120, 90, 80, 66, 44, 40, 38, 33, 26, 18-16 kD) were isolated using SDS gel electrophoresis (Figure 2c). The negatively charged character of the intracrystalline organic matter was confirmed by isoelectric focusing.







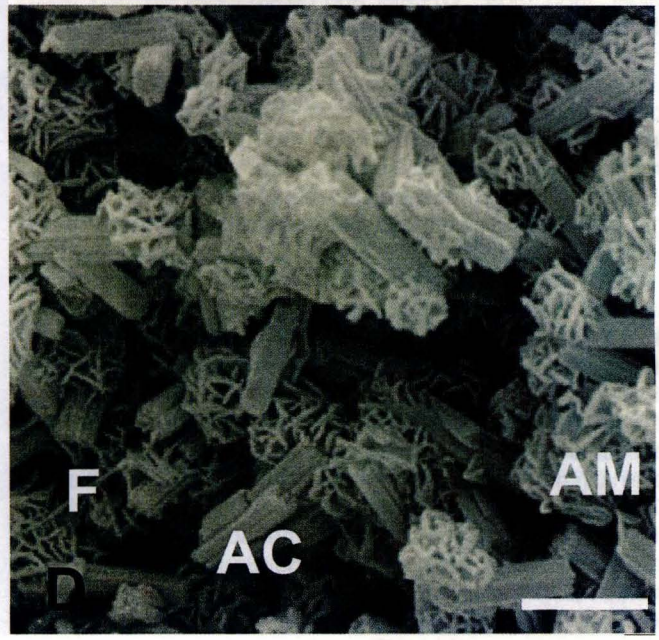


Figure 2. *Vaceletia*—a modern archaeocyathid sponge. **a.** *Vaceletia* n.sp. of the branching type, from Osprey reef. Living and dead portions. **b.** Tetracycline stained waste chambers. **c.** SDS PAGE of waste chamber organic mucus with 10 different macromolecules (indicated on the left). **d.** SEM micrograph of newly formed aragonite crystals in calcium waste chambers (CWD).

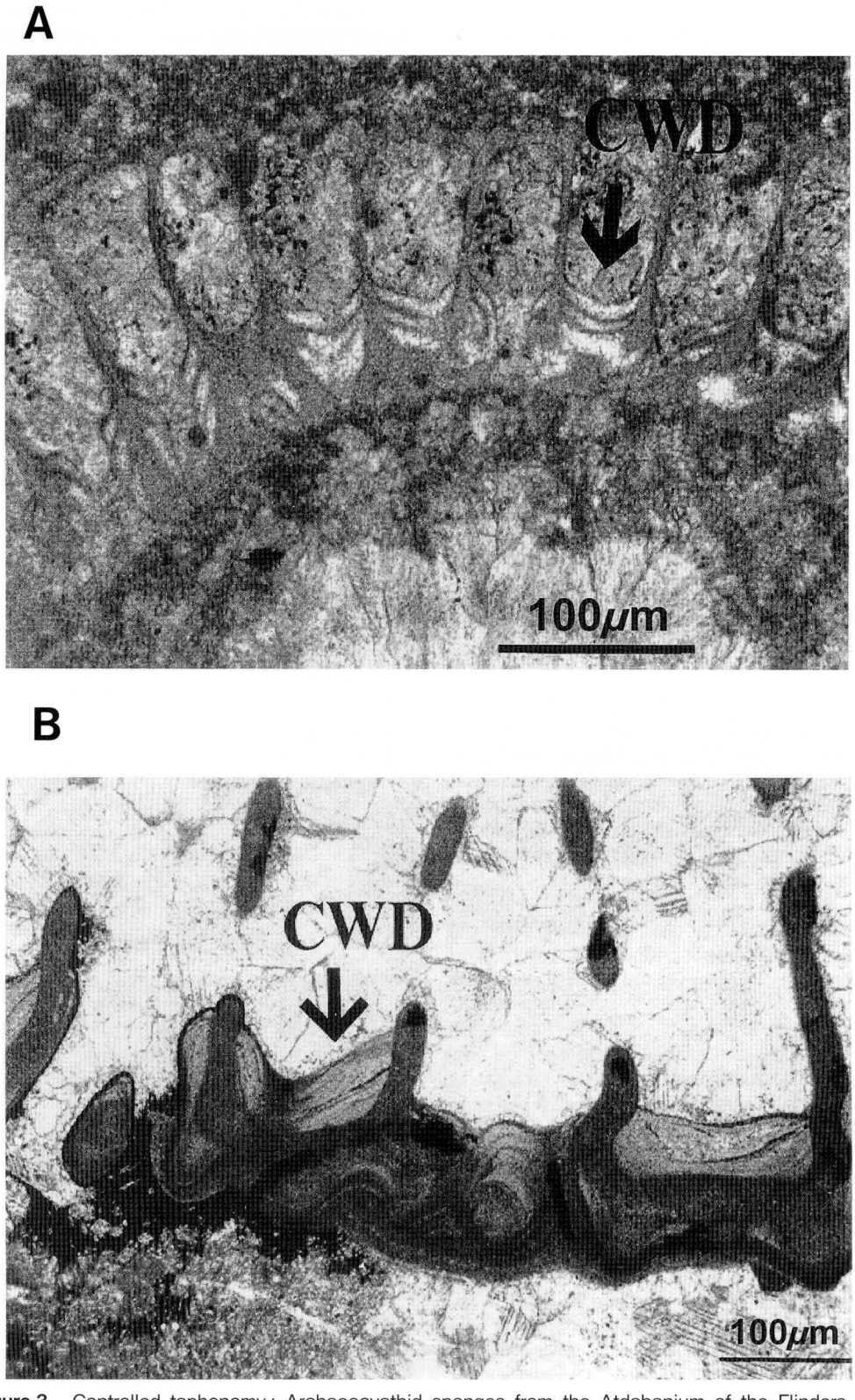


Figure 3. Controlled taphonomy : Archaeocyathid sponges from the Atdabanium of the Flinders Ranges (Australia). **a.** *Warriootacyathus* with organo-mineral deposits along the base of the inner wall. **b.** *Ardrossacyathus* with organo-mineral deposits along the inner zone of the outer wall.

HPLC (High Performance Liquid Chromatography) analyses of the upper living part of the basal skeleton and the Ca^{2+} waste chambers from the inactive deeper part of the basal skeleton show an enrichment of glutamic acid (12–14 mol%) in the EDTA soluble organic matter. The medium sized molecules (80–38 kD) are restricted to calcium waste chambers and probable degradation products. The smaller molecules (33–16 kD) are the most acid ones. Aspartic acid is well-represented, with amounts between 6–15 mol% in the entire organic phase. The proteinaceous materials are, on the average, 15–20 mol% of entire organic matter. The sacchariferous components constitute the dominant phase which can be explained by the high amount of EPS (exopolymeric substances) from symbiotic bacteria. One part of the intracrystalline proteins are glycoproteins, supported by the detection of the amino-sugar N-galac, which is probably bound to serine. In calcium waste chambers the amount of polysaccharidic EPS and the total amount of intracrystalline organic matter (ca. 50 μg/g carbonate) is clearly enriched in contrast to the living portions of the sponge skeleton (35 μg/g carbonate).

Inhibition tests of the bulk intracrystalline organic matter (see Gunthorpe *et al.*, 1990 ; Wheeler *et al.*, 1981 for methods) demonstrate the Ca^{2+} binding property of the acidic macromolecules of both the tissue-supporting skeleton and the calcium waste deposit chambers. The standardization of absolute amounts of organic matter subject to the experiments is in progress and will allow drawing further conclusions.

Although our present data are incomplete and not all molecular mechanisms are fully understood, the geochemical and biochemical characteristics of the basal skeleton and its Ca^{2+} waste chambers point to matrix-controlled biomineralization here being involved to form a defined skeleton (via moderately acidic high-molecular-weight substances) along with matrix-mediated surplus Ca^{2+} removal as calcium waste deposits (via highly acidic low-molecular weight substances). The latter process represents the most ancient way to build a calcareous skeleton, via controlled taphonomy (Reitner *et al.*, 1997b). On the other hand, stable $\delta^{13}C$ isotopes in equilibrium with ambient seawater indicate that no enzymatic system to cause isotopic fractionation is directly involved in this precipitation (Reitner, 1992 ; see also Böhm *et al.*, 1996). Therefore in terms of phylogeny, biomineralization in *Vaceletia* may be regarded as a first step towards more strictly biologically controlled biomineralization of higher Metazoa (Reitner *et al.*, 1997b).

The earliest fossil taxa of the *Vaceletia*-type (*Stylothalamia*) are recorded from the Ladinian (Middle Triassic) ; since then a continuous record is known (Reitner, 1992). This type of extant "sphinctozoan" is restricted to small caves and other dark reef environments in present day seas. As discussed by Reitner *et al.* (1997b), the genus *Vaceletia* is ultraconservative and, therefore, is comparable in some skeletal features (CWD's) with certain taxa of Archaeocyatha from the Lower Cambrian which had a similar mode of biomineralization (Figure 3) (see also Pickett, 1985).

***Ceratoporella* (Hickson 1911)—a coralline sponge with a heavy aragonitic basal skeletal crust** (Figure 4)

Ceratoporella forms an isotopically heavy, aragonitic basal skeleton with small calicles on the top in which the soft tissue of the sponge is located (Figure 4a). This soft tissue is also characterized by large amounts of symbiotic bacteria (ca. 60% of the entire biomass) (Santavy *et al.*, 1990 ; Willenz and Hartman, 1989). In contrast to *Vaceletia*, the aragonite is orientated in clinogonal fibers ("water jet" structure). Shortly after removal of soft tissue during growth, the calicles are closed by rapid epitaxial growth of aragonitic fibers. The calcification fronts stain easily with calcein and are therefore well suited for *in situ* measurements of growth (Willenz and Hartman, 1985, 1999). These sponges grow extremely slowly, with an average of 200–500 μm yearly growth (Böm *et al.*, 1996 ; Willenz and Hartman, 1999). The entire basal skeleton represents a thick aragonitic crust. The aragonite has characteristically high Sr amounts (10,000 ppm), and additionally, extraordinarily high amounts of U (7–8 ppm), which allow excellent age determinations using the U/Th method. The carbon used for skeletal formation is heavy and in equilibrium with ambient seawater ($\delta^{13}C$ +5 to +3,8). Isotopic analyses of entire basal skeletons demonstrate the rapid change of CO_2 amounts and the increase of light carbon due to an intense combustion of fossil carbon since 1820 (Böhm *et al.*, 1996) (Figure 5).

The internal parts of the basal skeletons have no apparent function as is seen in the taxa *Acanthochaetetes* and *Vaceletia*. The oldest representatives of *Ceratoporella* are known from the Permian of Djebel Tebaga (Tunisia) (Reitner, 1992).

However, only little information is available about the specific amounts of intracrystalline organic matter in *Ceratoporella*. In the uppermost zone of the basal skeleton, 200–500 μg/g carbonate organic matter was measured, with 20–50 μg/g carbonate in deeper older portions. The proteinaceous portions of this organic matter show a medium acidic character, as determined by isoelectric focusing. HPLC analysis have shown that asp and glu have about 25 mol% which fits well with our isoelectric focussing data. SDS PAGE (polyacrylamide gel electrophoresis) has shown eight, sometimes 9 bands (i.e., macromolecules). At the moment we believe that one small protein (18 kD) and one heavy protein (ca. 80 kD) play the central role as matrix proteins (Figure 4d). Inhibition experiments demonstrate that the bulk organic matter has only a weak inhibition potential compared to water (used as comparison). Matrix proteins from the initial (oldest) parts of the basal skeleton (a very thin layer, ca. 1 mm) inhibit calcification somewhat more strongly than those isolated from the mature portion of the basal skeleton. However, both inhibition curves run more or less parallel. The weaker inhibition character of the mature skeleton is related to the smaller amount of organic matter and the loss of such functional groups as COO^- during early diagenesis of organic matter. This coincides well with the less acid character of the organic matter as tested by isoelectric focusing. *Ceratoporella* still has considerable reef-building potential in deep parts of forereef areas on Caribbean islands.

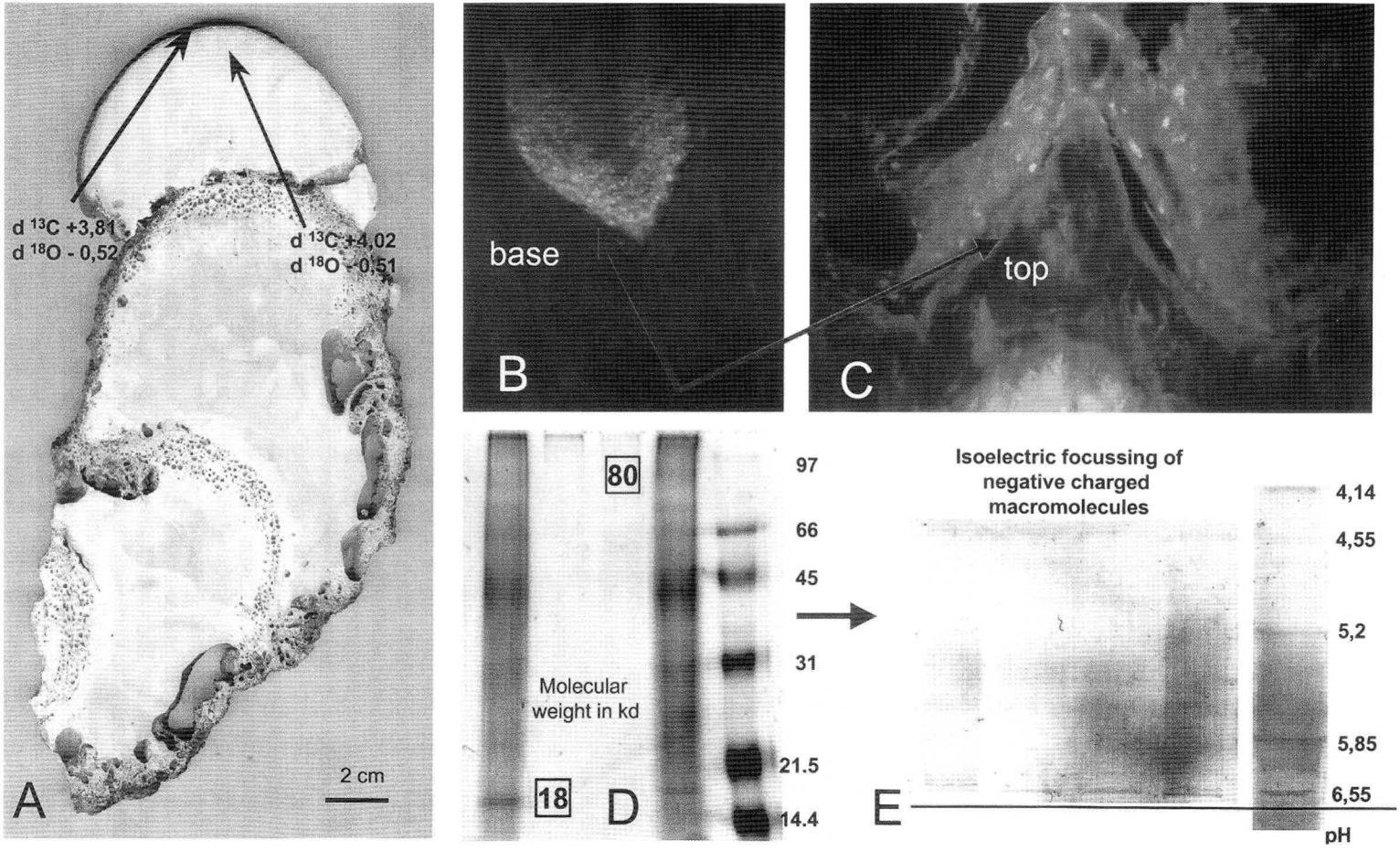


Figure 4. *Ceratoporella nicholsoni* with an aragonitic basal skeleton crust. **a.** Section of a specimen from Jamaica with stable isotope values indicated from the upper part. The uppermost growing zones exhibit relatively light delta ^{13}C 3.8 and delta ^{18}O −0.4 values on average. **b.** Tetracycline stained base of a calicle. **c.** Tetracycline stained top of a calicle. **d.** SDS gel with two detected macromolecules (18 kD, 80 kD). In *Ceratoporella* two acidic Ca^{2+}-binding matrix proteins are observed which are enriched in the amino acids asp (20 mol%) and glu (15 mol%). **e.** Isoelectric focusing confirms the acidic character of both detected macromolecules.

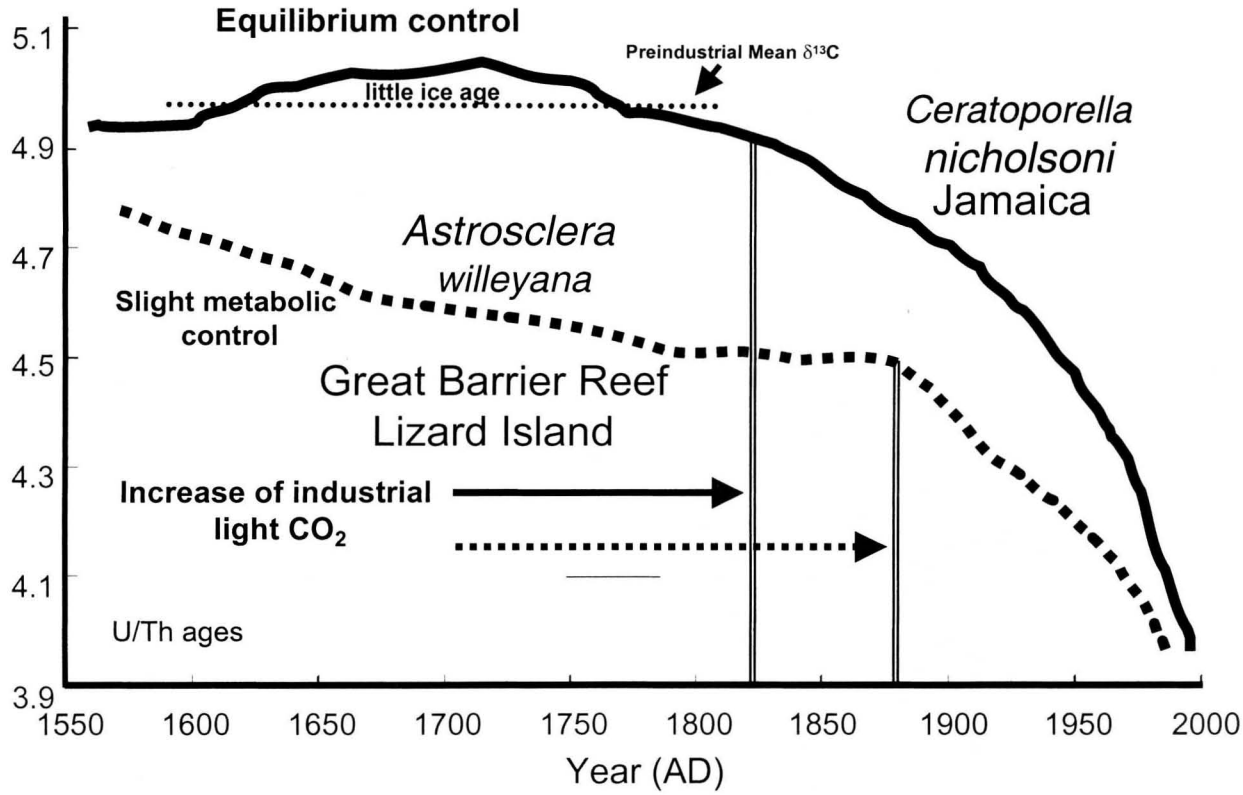


Figure 5. The Carbon isotope history of the Jamaican *Ceratoporella* and the Pacific *Astrosclera* since 1550 clearly shows a slight metabolic control of the calcification in *Astrosclera*. The drop of values indicating the increased industrial input of light CO_2 due to burning of fossil fuel starts some years later in the Pacific Astrosclera than in Ceratoporella, but with a similar slope. The $\delta^{13}C$-curve of Ceratoporella nicholsoni exhibits clearly the litte ice age event.

***Astrosclera willeyana* Lister 1900—a stromatoporoid coralline sponge**

The main features of soft tissue as well as processes of formation of basal skeleton in *Astrosclera* have recently been described in detail, therefore only a summary is given here. The reader is referred to Wörheide (1998) for details:

The living tissue of *Astrosclera* penetrates the basal skeleton to a maximum depth of 50%, depending on specimen size. The soft tissue shows a stromatoporoid grade of organization (e.g., Wood, 1987) and can be divided into three major zones. The ectosome is the area directly beneath the exopinacoderm, with a thickness of up to 100–300 μm. This zone is characterized by the absence of choanocyte chambers, an enrichment of storage and supporting cells (SSC's), and archaeocyte-like large vesicle cells (LVC's) responsible for the initial formation of aragonitic spherulites. Megasclerocytes have only rarely been observed, but show a remarkable number of cytoplasmatic digitates. The choanosome, contiguous to the ectosome with a more-or-less sharp transition, comprises the major part of the living tissue of *Astrosclera* and is characterized by small choanocyte chambers ($\pm$15 μm diameter) and a high density of bacterial symbionts. Other cellular components of the choanosomal mesohyl are archaeocytes, typically pluripotent, phagocytic demosponge cells, SSC's, and rare fiber cells. Bacteriocytes, which contain up to 30 bacteria in a large vacuole, were described (Wörheide, 1998) for the first time in coralline sponges. He additionally noted a new cell type identified as a "waste cell", characterized by a large vacuole filled with a fluffy substance, probably the non-digestible remains of bacteria (membranes, fibrilar EPS). These cells are probably derived from archaeocytes or bacteriocytes and are often observed close to excurrent canals. Most of the ground substance of the choanosome lacks collagen and spongin, but has abundant polysaccharide mucus (EPS) produced by the symbiotic bacteria. The zone of epitaxial backfill (ZEB) is considered to be a subzone of the choanosome due to its important role in the *syn vivo* cementation of the lowest parts of the basal skeleton. It is characterized by a reduced number (or absence) of choanocytes and bacteria and sometimes an enrichment of SSC's. *Astrosclera* has a viviparous mode of reproduction and its bacterial symbionts are transferred from the parent sponge in the parenchymella larvae. A large proportion of the bacterial population inhabits the choanosome, where bacteria can reach up to 70% of total biomass in some areas. The ectosome is nearly free of bacteria. Four major bacterial morphotypes are recognized. Bacteria act either

as a direct food source for the sponge (archaeocytes), or the sponge benefits from certain metabolic products of the bacteria (bacteriocytes). At the least, these bacteria are in part facultatively anaerobic, eliminating metabolic waste products of the sponge through fermentative processes during times of reduced or halted pumping, or at any time. The aragonitic calcareous skeleton of *Astrosclera* is thus formed by the combination of three processes. In the first, spherulites are formed in LVC's in the ectosome. LVC's possess a large vesicle which is filled with a three dimensional network of sheets and fibers and acidic mucus. Sheets and fibers act as the insoluble organic matrix (IOM) and mucus as soluble organic matrix (SOM) for seed crystal nucleation. In the first stage, seed crystals are randomly oriented, later they are oriented in the direction of the aragonite c-axis. When they attain a size of $\pm 15\,\mu m$ spherulites are released from the cell and enveloped by basopinacocytes. In the second process, basopinacocytes transport the spherulites to the tips of skeletal pillars where they fuse together by epitaxial growth. This epitaxial growth is controlled by acidic mucus substances in the extracellular space (ECS) between the growing aragonitic fibers and the basopinacoderm. The mucus is produced by basopinacocytes and acts as a buffer for Ca^{2+} ions. The ECS-mucus is thought to have a different composition than the mucus inside vesicles of the LVC's. The third process involves withdrawal of soft tissue during upward growth, when it is pulled upwards from the lowermost parts of skeletal cavities. The space remaining is subsequently filled by the epitaxial growth of aragonite fibers. The zone of epitaxial backfill is characterized by an absence of choanocyte chambers and a reduced number of bacteria, but sometimes also by an increased number of SSC's. The ECS in the ZEB is also filled with acidic mucus, which controls the speed and direction of epitaxial growth. The number of SSC's is sometimes increased, indicating again their importance in nutrient supply during skeletal elaboration.

It was suggested by Wörheide (1998) that the absence of bacterial symbionts in certain areas of the sponge (ectosome, parts of the ZEB) may result in the accumulation of metabolic waste products (e.g., ammonia). This occurs in these areas at times of reduced or halted pumping (i.e., the bacteria do not function in the elimination of metabolic waste products here). These areas, with anaerobic conditions and enriched ammonia, thus provide an environment with increased pH and increased alkalinity, facilitating biocalcification.

Wörheide (1998) further postulated that LVC's in *A. willeyana* are probably derived from choanosomal bacteriocytes and/or late stages of waste cells. Bacteriocytes were described as "farming" bacteria within large vacuoles, and it is most likely that these bacteria would be phagocytized inside vacuoles by bacteriocytes at some stage. This is even more probable, as shown by the waste cells containing irregular, "fluffy" substances noted above, the supposed non-digestible remains of bacteria. Waste cells seem to represent an advanced stage of bacteriocytes, perhaps subsequently transforming into spherulite-forming LVC's. As a working hypothesis it is proposed that *Astrosclera* uses the fibrilar content of the vacuole/vesicle as the first inorganic template (IOM) for seed crystal nucleation. If the assumption of a cellular cycle (bacteriocyte to waste cell to LVC) can be further substantiated, this cycle, and the use of bacterial EPS in the first stages of skeletal elaboration would be truly remarkable and once again demonstrate the sophisticated interaction between symbiotic bacteria and the sponge.

Amino acid and monosaccharide composition of the intracrystalline organic matrix were studied (Wörheide *et al*., 1996) on an approximately 400 year old specimen from Ribbon Reef No. 10 (Lizard Island Section, Great Barrier Reef, Australia). Amino acid and monosaccharide composition of the insoluble intracrystalline matrix is very stable in all portions of the skeleton. No strong diagenetic effect on the Insoluble Organic Matrix (IOM) was noted, due to the stable composition. The IOM is dominated by proteins and is represented by the intravacuolar fibers and sheets forming substrate for the seed crystals.

The soluble organic matrix (SOM) is characterized by acidic glycoproteins, large amounts of proline needed for the synthesis of glutamic acid, as well as large amounts of aminosugars. Glucids are the dominant fraction of the SOM. The character of the SOM is typical for Ca^{2+}-binding mucus. A strong diagenetic effect is visible in the SOM, both in the composition of amino acids and monosaccharides and in their quantity. Stromatoporoids with aragonitic spherulites formed within intracellular vacuoles are known since the Permian and the first occurrence of *Astrosclera* is known in the late Triassic of southern Turkey (*Astrosclera cuifi* Wörheide 1998). An important problem is still the lack of a record during younger times until the Holocene (thus, a "Lazarus"-taxon). This gap is poorly understood and may be related to diagenetic processes.

Astrosclera is a very slow growing species, the mean growth rate of *Astrosclera* is $230\,\mu m$ per year, with an average growth rate of $0.63\,\mu m$ per day, as determined by *in vivo* staining with Calcein-Na_2, AMS, ^{14}C and U/Th data (Wörheide, 1998). The oldest known modern specimen has an individual age of more then 500 years (Wörheide, 1998).

Besides *Astrosclera*, another extant "stromatoporoid" taxon is known, *Calcifibrospongia actinostromarioides* from the Bahamas (Hartman, 1979; Reitner, 1992). This type is phylogenetically related to the taxon *Haliclona* (Haplosclerida) and the spicular skeleton is constructed of long thin strongyles. The basal skeleton is of extracellular construction, built of irregular spherulites of aragonite. The shape of the entire sponge is head-like and living tissue covers only the uppermost portion of the basal skeleton, with a maximum thickness of 1cm. At the base of the soft tissue horizontal laminae are observed that are comparable with those seen in many fossils. However, close fossil relatives of this genus are unknown.

Astrosclera differs in many aspects from the phylogenetically closely related *Ceratoporella*. Newly formed aragonite clusters are depleted in ^{13}C ($\delta^{13}C$: 3.5), as compared with mature basal skeleton. Spherulites are enriched in Sr, P, Li and Mo. In the younger cemented area

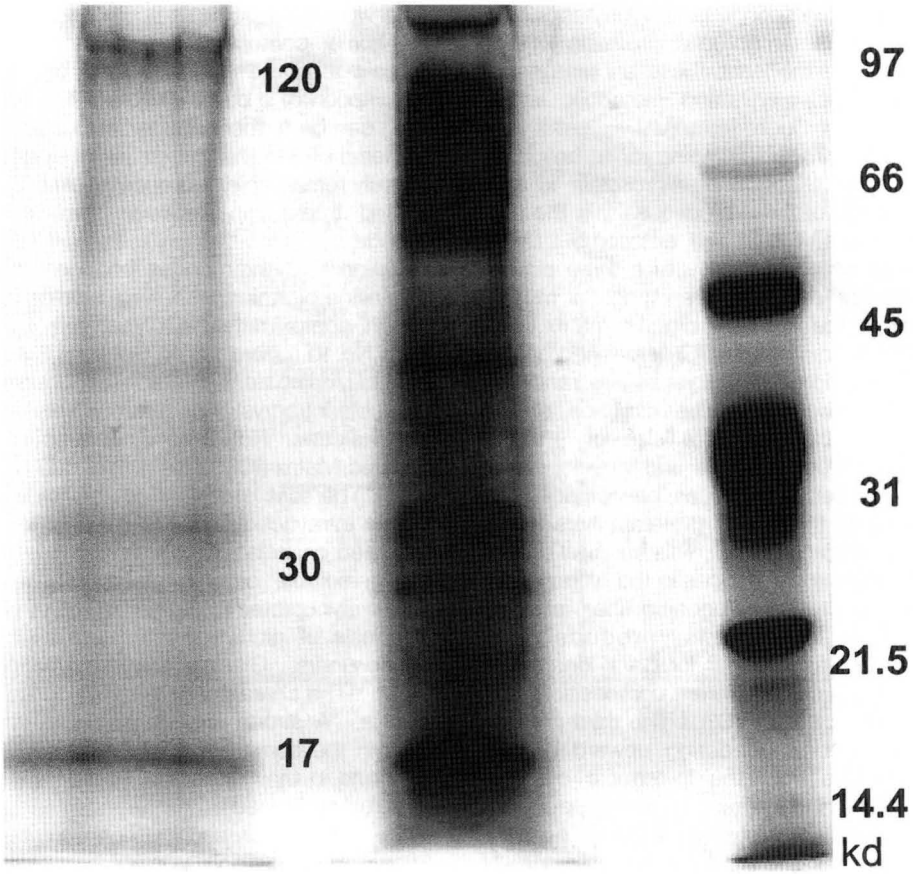


Figure 6. *Astrosclera willeyana*, a stromatoporoid coralline sponge. SDS gel showing three detected macromolecules (17 kD, 30 kD, and 120 kD) in the intracrystalline soluble organic matrix.

of the skeleton the content of ^{13}C increases to a $\delta^{13}C$ value of 4.03. *Astrosclera* has three acidic matrix proteins (SOM), a small one (17 kd) with an as yet unknown function, a medium sized one (30 kd) which probably controls spherulite growth, and a large one (120 kd), which probably is involved in the epitaxial cementation process in ZEB (Figure 6). The initial skeletal formation process shows a vital growth effect (with respect to $\delta^{13}C$) in contrast to the epitaxially formed parts of the skeleton (under equilibrium conditions).

***Spirastrella* (*Acanthochaetetes*) *wellsi* (Hartman and Goreau 1975) : a chaetetid coralline sponge** (Figure 7)

Only the 0.5–1 mm thick, youngest portion of the calicles of the chaetetid-type basal skeleton is occupied by living soft tissue. Soft tissue and basal skeleton can be divided in six major zones based on anatomical differences. The basal skeleton consists of high-Mg calcite (15–19 mole% $MgCO_3$). Formation of the basal skeleton is summarized below (but, see also Reitner *et al.*, 1997b ; Reitner *et al.*, 1996 ; Wörheide *et al.*, 1996 ; Wörheide *et al.*, 1997b for further detail).

The uppermost position is formed by a thick crust of spiraster microscleres (dermal area, Zone I) and tylostyle megascleres arranged in clearly plumose bundles, reflecting a close phylogenetic relationship to *Spirastrella*.

Underneath the external dermal area, the internal dermal area (Zone II) contains mesohyl tissue, devoid of choanocyte chambers but enriched in mobile cells. Large inhalant chambers (lacunae) and distributing canals criss-cross this zone, providing the choanosome with water filtered through the ostiae. The choanosome is characterized by very large choanocyte chambers (80–100 μm). The mesohyl is also characterized by large cells (ca. 10 μm) that contain numerous inclusions (LCG : large cells with granules) which lie directly upon the calcareous skeleton (Reitner, 1992 ; Reitner and Gautret, 1996). Mesohyl bacteria are rare (about 5% of mesohyl biomass) and they are very small (500 nm). The highly mobile cells are undoubtedly responsible for secretion of collagen fibers and probably derive from a special type of lophocytes. Collagen fibers form strong bundles which penetrate the basopinacoderm and anchor into rigid skeleton (Vacelet and Garrone, 1985). Thin collageneous fibers produced by unmodified lophocytes are widely distributed within intercellular mesohyl. They condense and become organized into a frame-building matrix at the top of the walls and remain trapped within skeletal structures after calcification. Calcite deposition occurs as soon as these two types of fibers are present, suggesting that they have the potential to attract divalent cations. However, the acicular shape of

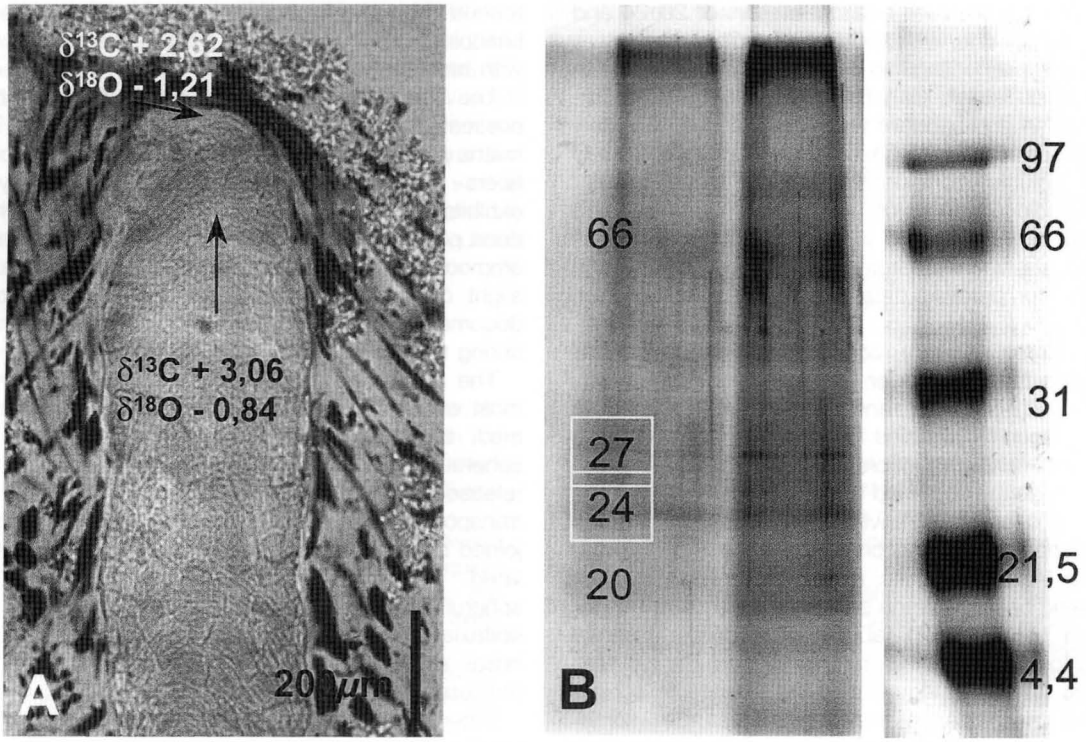


Figure 7. *Spirastrella* (*Acanthochaetetes*) *wellsi*, a chaetetid coralline sponge. **a.** The uppermost growing zone of *Acanthochaetetes* (top of a tube wall) with stable isotope values from different growth zones. **b.** SDS gel showing four detected macromolecules (20 kD, 24 kD, 27 kD, and 66 kD). The standard ladder on the right is for comparison of molecule size.

crystals and highly organized microstructure are both characteristic for *S.* (*Acanthochaetetes*), but were never observed (in this study). Skeleton formation begins inside the uppermost fiber template in the form of a soft structure displaying the shape and size of the future crystals characteristic of *S.* (*Acanthochaetetes*). These elements, however, are not rigid, but rather show the appearance of "cooked spaghetti" (Reitner and Gautret, 1996). This structure then becomes calcified and organized when mucus is secreted into the narrow space between the basopinacoderm and the calcified skeleton by basopinacocytes forming the basal cell layer above the calcareous skeleton. This mucus is highly soluble, thus direct observation is difficult with electron microscopy. Mucus is not preserved in TEM preparations (as a result of preparation techniques) and at best is recognized using SEM, where collapsed amorphous lumps closely associated with growing crystals can be observed in well fixed specimens.

The central part of the tubes (Zone III) is characterized by choanosome with large choanocyte chambers (80–100 μm) leading to large oscular channels. Normally, few tylostyles are present here.

The occurrence of tabulae is typical of the chaetetid type of skeleton, forming steps in the tubes (Zone IV). These are formed by the basopinacoderm, first as a thin organic diaphragm or sheet. Below the choanosomal zone, LCG cells become enriched in calcium and cause mineralization of the organic sheet. Continuously upward moving basopinacoderm forms a space filled with Ca^{2+}-binding and mineralizing organic mucus. This mineralization process happens only when LCG cells are present (Reitner, 1992).

The closed spaces between tabulae contain accumulations of modified archaeocytes with numerous storage granules (thesocyte-like cells) and few spiraster microscleres (Zone V). These cells play a role in regenerative processes (Reitner, 1992 ; Vacelet, 1985, 1990) enabling the sponge to start growing again when it has been drastically damaged.

The soluble matrix (SOM) extracted from the superficial part of the skeleton contains large amounts of glycine, proline and hydroxyproline-rich compounds (collageneous affinity). Amino sugars are also enriched in this zone. Due to the presence of highly concentrated materials with collagenic or glucidic affinities, the relative amounts of acidic amino acids (asp and glu) appear to be less abundant here than in immediately underlying older skeleton. However, absolute quantities of these two amino acids are generally at least 3 to 5 times higher in the uppermost part of the skeleton (Gautret *et al.*, 1996). Starting from the area immediately below the active mineralizing zone, there is a general transformation of SOM detectable, most obvious with the decrease of acidic amino acids, but aromatic amino acids (tyr and phe), serine, and amino sugars also decrease. Increasing constituents are basic and aliphatic amino acids (mainly glycine). Four macromolecules have been isolated

using SDS PAGE three small ones with sizes of 20, 24 and 27 kD, and a larger one, with 66 kD (Figure 7b).

IOM exhibits similar collagenic amino acid compositions in all parts of the skeleton. Only the quantity of the insoluble matrix changes in an important way, it decreases considerably from the surface to the base. This matrix completely differs from soluble compounds, having much smaller amounts of acidic amino acids, serine and threonine, and almost no amino sugars. It is strongly enriched in all aliphatics (gly, ala, val, leu), aromatics (phe, tyr), proline and hydroxyproline (for detailed data see Reitner and Gautret, 1996).

Acanthochaetetes is very conservative; its first fossil record is known from the Lower Cretaceous (Fischer, 1970; Reitner, 1982, 1989; Reitner and Engeser, 1983), and in contrast to aragonitic species of coralline sponges, this taxon has a continuous record since then. During the Cretaceous the genus occupied three ecological niches, the shallow marine open water environment (*Acanthochaetetes ramulosus*), deep forereef environment (*Ac.* cf. *seunesi*), and cryptic niches (*Ac. seunesi, Ac.* n. sp.).

The chaetetid basal skeleton serves for protection and for resting space between two tabulae in a calicle (internal "gemmulae") for special omnipotent cell types (thesocytes-archaeocytes). This strategy allows the sponge to survive environmental crises. As a result, these sponges sometimes show a numerous buds, with their point of origin on one calicle. This feature might also help explain the extremely slow growth rates of basal skeleton, with 50 μm/a (Reitner and Gautret, 1996). The oldest known living specimen from a deep submarine cave of Cebu (Philippines) has an age of more then 600 years (Reitner and Wörheide, unpublished data).

Conclusions

All known coralline sponges form their basal skeleton via organic matrix molecules. The CO_2 source is seawater, and resulting calcification is more or less in equilibrium with ambient seawater. Therefore, coralline-demosponge basal skeletons provide excellent proxy archives for measurement of CO_2 accumulations during the last one thousand years. The type of basal skeleton formation realized in coralline demosponges first occurs at the evolutionary beginning of matrix-controlled biomineralization. Coralline sponges evolved first in the Tommotian, with Archaeocyatha representing controlled taphonomic calcification. The sphinctozoan demosponge *Vaceletia* is the probable modern representative of this group, based on similar skeletal features. Important here are Ca^{2+}-waste deposits in dead portions of the basal skeleton, readily comparable with those seen in various types of Cambrian Archaeocyatha. A mixture of at least 10 types of Ca^{2+}-binding intracrystalline matrix proteins were detected in *Vaceletia*. The primary aragonitic skeleton is extracellular, formed within acidic mucus of pillar and wall compartments. *Ceratoporella* possesses a crust-type aragonitic basal skeleton, and only one distinct skeleton-forming matrix protein (18 kD) was detected, with asp- and glu- rich peptides. The basal skeleton is formed by a thin acidic mucus veneer beneath the basopinacoderm. Skeletal growth is in strict equilibrium with ambient seawater.

The hadromerid chaetetid *Spirastrella* (*Acanthochaetetes*) possesses a high-Mg calcite basal skeleton. The Ca^{2+}-matrix proteins are linked to the formation of collageneous fibers produced by LCG-cells. The primary skeleton exhibits four small, weakly acidic matrix proteins. Within dead portions of the basal skeleton organomineralization via ammonification is observed. Stable isotope data show a slight disequlibrium with ambient seawater and therefore documents a small vital effect which causes fractionation during formation of the basal skeleton.

The agelasid stromatoporoid *Astrosclera* represents the most evolved biomineralization of basal skeleton, with two modi of basal skeletal formation developed. Aragonitic spherulites grow in large intracellular vacuoles until they are released at a size of 15 to 50 μm. These spherulites are transported by "invaded" basopinacocytes and later are joined by epitaxial growth. Three matrix proteins were isolated. The small one (30 kD) is probably responsible for spherulite growth, the large one (>100 kD) controls the epitaxial growth. Isotope analyses have shown that the basal skeleton growth is not strictly in equilibrium with ambient seawater.

Different evolutionary grades of basal skeleton development are seen in the four coralline sponges studied. The most ancient grade is present in *Vaceletia* and *Ceratoporella*. *Spirastrella* (*Acanthochaetetes*) differs in many aspects and the stable isotope signatures show a clear vital effect. *Astrosclera* is the most highly evolved one due to the intracellular formation of initial spherulites, which clearly show a vital effect in their $\delta^{13}C$ values. Basal skeleton formation in coralline sponges represents one first and important step towards completely biologically controlled biomineralization. The first metazoans with a complex biomineralisation in the fossil record are *Cloudina* from the Neoproterozoic of Namibia, forming calcified tubes. These calcified tubes of *Cloudina* show some morphological similarities with modern Pogonophorida, dwelling at hydrocarbon seeps (*Vestimentifera* with sulphide oxidizing symbiotic bacteria) or hydrothermal vents (*Pogonophora* with methanotrophic bacteria) (Peckmann *et al.*, 1999; Sibuet and Olu, 1998; Young *et al.*, 1996). Probably calcification events in *Cloudina* and some modern vestimentiferan worms can also interpreted as controlled taphonomic processes as shown above, and apparently mark the evolutionary beginning of biologically controlled biomineralization in the late Neoproterozoic and early Cambrian time. The onset of Metazoan biomineralization is one of the major geobiological events in earth history.

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