Biomineralization of calcified skeletons in three Pacific coralline demosponges – an approach to the evolution of basal skeletons

With 3 plates

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Abstract

Biomineralization of calcareous basal skeletons in coralline sponges is a strongly phylogenetically convergent character. However, the basic mineralization process is ancestral and exhibits similarities with mineralization processes seen in bacterial biofilms and organomineralization via a controlled taphonomy. The main biocalcification events in the phylogenetically distinct taxa Vaceletia sp., Astrosclera willeiana, and Spirastrella (Acanthochaetetes) wellsi are discussed. Vaceletia, a demosponge with a thalamid basal skeleton, exhibits the most ancestral way to build a calcareous skeleton via controlled taphonomy. Archaeocyaths exhibit the same skeletal forming mode as seen in Vaceletia. The stromatoporoid Astrosclera willeiana forms intracellularly eggshaped aragonitic aster in a first step which grow together via an epitactical process. This mode of calcification is realized in many late Permian and Triassic coralline sponges with different phylogenetic origins. The chaetetid S. (Acanthochaetetes) wellsi, phylogenetically the most evolved coralline sponge taxon, forms its unique calcitic skeleton in extracellularly acidic organic mucilages in the presence of collagen. In all cases the mineralization is controlled iby acidic matrix proteins.

Keywords: Biomineralization, coralline demosponges, Astrosclera, Acanthochaetetes, Vaceletia, Archaeocyatha, evolution of basal skeletons.

Introduction

Calcified sponges have been dominant reef building organisms since the beginning of the Phanerozoic. Replaced in their reef-building function by scleractinian corals in modern reefs, the living relatives of these calcified sponges ("coralline sponges") can be found in cryptic niches of almost all Recent coral reefs. They were the first metazoans producing a carbonate skeleton and their microstructural features have remained completely unchanged during this very long period of time. The biomineralization processes are extremely conservative and still present in extant calcified sponges. Within this group of organisms very little is known about the modalities of the formation of the basal skeleton. Biomineralization events have been investigated in three different taxa of coralline sponges from the Pacific and Indo-Pacific realm. The secondary calcareous skeleton of the agelasid stromatoporoid Astrosclera is made of aragonite spherulites (AYLING 1982; REITNER 1992; WÖRHEIDE et al., in press, a, b). The hadromerid chaetetid Spirastrella (Acanthochaetetes) produces a high-Mg calcite skeleton beside the spicular skeleton (HARTMAN & GOREAU 1975; REITNER & ENGESER 1987; REITNER & GAUTRET 1996; WÖRHEIDE et al., in press, b). The taxon Vaceletia exhibits no primary spicular skeleton and builds an aragonitic secondary basal skeleton (REITNER 1992).
Material and Methods

The investigated specimens of Astrosclera, S. (Acanthochaetetes), and Vaceletia were all collected by SCUBA diving during several field trips from 1990 to 1996 in shallow water reef caves near Lizard Island Section and Osprey Reef (Great Barrier Reef & Queensland Plateau, Australia), from Satao Island (Sumbawa, Indonesia), and from Mactan Island (Cebu, Philippines).

The procedures of specimen fixation, preservation, histological and chromatographic analyses, and the following methods of investigation were described by Reitner (1993) and Gautret et al. (1996).

Basal skeleton forming processes

Ancestral demosponge taxon Vaceletia crypta (Vacelet 1977) with affinities to the Archaeocyatha

The primary organic skeleton of Vaceletia is non-spicular (pl. 1). It has a trabecular organisation and is overlayed by a hemi-spherical top-layer (“dermal-layer”) (pl.1/fig.2). The trabecles consist of irregular, organic filaments with a very thick central filament. This central filament has a supporting function and could be seen as an „organic spindle“. A network of very thin fibres surrounds this central filament. The calcification of the secondary aragonitic skeleton starts between this organic fibres. This secondary skeleton consists of irregular aragonitic micrite. The central filament will not be fully calcified due to its highly acidic nature. The formation of the secondary skeleton is not a continuous process, but happens step by step in the following order (cf. Reitner 1992). The sponge bears numerous symbiotic bacteria (ca. 50% of the total biomass) within its mesohyde. They control most of the physiological processes of the sponge (nutrition, dewasting etc.) (Reitner 1993).

Formation of skeletal-pillars and chambers

A new, non calcified chamber with a hemi-spherical dermal top-layer and a trabecular organisation and organic skeletal-pillars containing a thick central filament is formed. These skeletal-pillars are filled with thin fibres. The entire space inside the pillar is filled up with acidic glycoprotein/proteoglycanic mucus. This space is filled up successively during the ontogenesis by aragonite crystals. The mineralization starts at the inner margins of the organic pillars. Further on, the whole fibrous insoluble saccharid-rich matrix of a newly formed pillar is successively substituted by aragonite crystals. The acidic mucus (proteic macromolecules) substances are reduced, respectively. The thick central filament will not be mineralized. This central filament has only a primary initial supporting function, because the irregular fibres inside the pillars are not able to support the choanosome on their own.

Newly formed chambers never show the complete structure and size of the later calcified ones. Such a chamber in statu nascendi is increasing slowly in size. The crystalization seems to start from the borders of the uncalcified skeletal elements. An initial, prismatic layer of aragonite crystals is observed. Larger crystals overlay this layer forming a loose network. The density of calcification is higher in the central part and at the border of the pillars, in between the calcification is slower.

Macromolecular analyses of the soluble matrices of the active growing portion have shown ca. 60% glucidic and 40% proteic substances which are incorporated in the basal skeleton (0.03 mg/g skeleton carbonate). The proteic phase exhibits 5.6 mol% asp and 12.4 mol% glu only. This weak acidic character of the macromolecules is typical for small matrix proteins (30 kD) which directly control the calcification. The glucidic phase is enriched in fucose (12.7 mol%), galactose (20.2 mol%) and glucose (40.1 mol%). Fucose is often observed in calcifying mucus substances and probably part of bacterial EPS (exopolymer substances).

Calcification of the inactive parts of the skeleton-controlled taphonomy

In the older parts of the skeleton, a second calcification phenomenon is observed (pl.1/fig.2-5). The upward moving soft-tissue is able to form organic phragmas via the basopinacoderm. These phragmas separate chambers which are filled up with solubile acidic glycoproteinic mucus (0.05 mg/g carbonate). This organic mucus shows high concentrations of Asp (7-10 mol%) and Glu (11,42-14,5 mol%) in combination with high values of the glucidic phase (75% of the organic matter) (Reitner 1992; Gautret et al. 1996). The soluble matrix is interspersed by polymerised mucus fibres (remains of bacterial EPS) which act as the insoluble organic matrix (IOM). The steps of mineralization are the same as with the pillars (Reitner 1992).

In Vaceletia five acidic matrix proteins were isolated with molecular weights of 37, 36, 33, 30, and 18 kDa which clearly show the various types of Ca²⁺-binding matrix protein in this phylogenetically ancestral taxon. Each protein is responsible for a special type of biomineeralization. Presently, we are not able to localize the various proteins. But it is evident that the second order of calcification in Vaceletia is a product of Ca waste elimination (Ca detoxification) via controlled taphonomy. The organic remains of the mesohyde bacteria, mostly EPS (pl.1/fig.5), sponge cell relics, and intercellular soluble acidic mucus eliminate toxic surplus of Ca²⁺. The Ca²⁺-binding glycoproteinic mucus exhibits a strong tetracycline and/or calcein induced fluorescence.

This phenomenon is often observed in similar formed fossil sphinctozoans [the taxon Vaceletia has been known since the middle Triassic (Stylothalamia)] and the observed mineralization processes of the Vaceletia-type
could be a model for all irregular, micritic-granular basal skeletons of stromatoporoid and thalamid organisation.

Of special interest is that all types of Archaeocyatha, the oldest known coraline sponges, exhibit this very specific type of calcification mode (pl.1/5-6). The modern *Vaceletia* thus may be a modern “Archaeocyath” sponge.

**Stromatoporoid *Astrosclera willeyana* Lister 1900**

**A modern stromatoporoid sponge**

Stromatoporoids are polyphyletic coraline demosponges which exhibit a thick soft tissue layer combined with a complex exhalant canal system. On the surface of the sponges, so called astorphiza patterns exist which have one large oscular opening (pl.2/fig.1). The basal skeleton types are highly variable (e.g. REITNER 1992; WOOD 1987). Only two modern sponges with a stromatoporoid grade of soft tissue organisation are existing, *Astrosclera willeyana* and *Calcituberospanga actinostromorioides* HARTMAN 1979. First representatives of this type are known from the Lower Cambrian, but they became highly diverse in Middle Ordovician. In the Devonian they are the most important reef building organisms.

First taxa of the Astroscleridae have been known since the Late Permian. *Astrosclera willeyana* exhibits a unique intracellular mode of basal skeletal formation. The soft tissue of *Astrosclera willeyana* is organized in a dermal zone, a choanosomal part, and a prominent exhalant system (LISTER 1900) (pl.2/fig.2). The soft tissue itself occupies only a few millimeters of the youngest portion of the basal skeleton. The inner choanosomal layer is characterised by a more or less dense mesohyle with numerous microbes (mostly *Vibrio*-types) and very small choanoceyte chambers (5-20 μm). The microbes may represent 30-50 % of the biomass within this zone. The root-shaped exhalant canal system ends in so-called astrophizae patterns often located on superficial mamelons. The basal skeleton is made of aragonitic spherulites (pl.2/figs.2-8).

The dermal layer and related mesohyle is free of microbes and enriched in motile cells. Most of them have an archaeocyte character (pl.2/fig.5). The mesohyle is formed by a dense network of EDTA insoluble fibres where the mobile cells are moving. Within the studied specimens, up to 60 % of the mobile cells are spherulite forming (large vesicular cells = LVC) (REITNER 1992, 1993; WORHEIDE et al., in press, a, b) (pl.2/figs.5-7). The size of the LVC ranges from 3-5 μm to about 20 μm, depending on the ontogenetic stage. The outer shape is round to egg shaped (pl.2/fig.8). They possess a large nucleus with a nucleolus, abundant mitochondria, and a lot of small vacuoles with reserve granules and/or phagocytised bacteria. The granules are extremely electron dense (osmophile) and, therefore, enriched in lipids. During successive stages, the LVC increase in size (pl.2/figs.5-6). Ontogenetically early stages are characterised by one large vacuole. It includes a minimum of 50 % of the total volume of the entire cell. At the last stage, the volume of the vacuole is more than three times larger than that of the remaining cell.

The vacuole is primarily filled up with a three dimensional network of fibres and sheets, probably formed under control of the electron dense reserve bodies. Sheets and fibres are forming small containers (30-50 nm) in which the first seed crystals are formed (pl.2/figs.5-6). The entire vacuole is filled up with Ca^2+/-binding glycoproteic mucus which exhibits a strong tetracycline and/or calcein induced fluorescence (pl.2/figs. 3,4,7).

The seed crystals are euhedral and randomly orientated in the early stages (2-3 μm). In the later stages of development (3-10 μm), the seed crystals get more orientated in direction of a c-axis of an aragonite crystal. All observed aragonite fibre crystals of the spherulites are compounds of 30-50 nm sized seed crystals (REITNER 1992). The aragonite fibre crystals are now more or less radially orientated and form aster shaped spherulites with a large remaining space filled up with acidic organic mucus. The aster spherulites are released normally in the 15 μm stage. At this stage, the LVC is lysing, the membranes are broken, and the aragonite asters are free in the mesohyle. In some sections, an enrichment of small amoebocytes was observed which probably transport the asters to certain places. The isolated asters grow together by epitalical processes. The spherulite-fibres, not embedded in a cell anymore, grow in direction of the c-axis of the aragonite crystal. When the fibres are get in contact with the fibres of other spherulites, they interfinger with them and stop to grow. The fibres not disrupted in growth grow until they interfinger with other spherulites. Due to this growth obsolescence, the spherulites get an asymmetrical shape in the older part of the basal skeleton. Mostly they show one elongated part and get an “egg-like” shape (GAUTRET 1986). After enzymatic proteolysis, which destroys the organic envelope, distinctive concentric growth lines are visible (GAUTRET 1986). The fibres are about 0,5-1 μm in diameter and are composed of parallely arranged smaller fibres of about 50-70 nm in diameter (GAUTRET 1986; REITNER 1992). The surface of the growing spherulites is covered by basopinacocytes. The space between the top of the fibres and the basopinacocytes is filled with acidic mucus, which exhibits a strong calcene induced yellow epifluorescence. This mucus functions as a buffer for Ca^2+/-ions and, therefore, controls the speed and direction of the epitalical growth of the aragonite fibres.

The amino acid and monosaccharide composition were studied on an approximate 400 years old specimen from Ribbon Reef No. 10 (Lizard Island Section, Great Barrier Reef, Australia) (WORHEIDE et al., 1997 in press, a). Amino acid and monosaccharide compositions of the insoluble intracrystalline matrix are very stable in all portions of the skeleton. No strong diagenetic effect on the insoluble organic matrix (IOM) is visible due to the stable composition. The IOM is dominated by proteins and is represented by the intravacuole fibres and sheets form-
ing the containers for the seed crystals. Collagen was not detected in the IOM.

The soluble organic matrix (SOM) is characterised by acidic glycoproteins, high amounts of proline which is needed for the synthesis of glutamic acid, and high amounts of aminosugars. The glucids are the dominant fraction of the SOM. Two distinct intracrystalline matrix proteins of 37 and 120 kD were detected. These results show the molecular weights are remarkable. It is unknown which protein is restricted to the intracellularly formed aragonitic spherulites. We assume that 37 kD is responsible for Ca\(^{2+}\)-binding and forming the seed crystals. The very large one is probably responsible for the epitaxial growth and extracellular formation of the basal skeleton. The character of the SOM is very typical for Ca\(^{2+}\)-binding mucus substances. A strong diagnostically effect is visible in the SOM, both in composition of amino acids and monosaccarides and in the quantity (Worheide et al. 1997 in press, a).

*Spirastrella (Acanthochaetes) wellsi* (Hartman & Goreau 1975) – phylogenetically modern type of a chaetetid coralline sponge

Chaetetids are also a polyphyletic grouping of organisms with affinities to cnidarians and sponges. Most chaetetids with sponge affinities are related to the demosponge taxon Hadromerida (Reitner 1992). *Spirastrella (Acanthochaetes) wellsi* is the most evolved chaetetid sponge. Its high-Mg calcite basal skeleton is unique and an excellent autapomorphy of this taxon (pl.3/fig.1,2). It has been known since the Aptian (Reitner 1989; Reitner & Engeser 1986) and is part of cryptic benthic communities in Cretaceous, Tertiary and modern Indo-Pacific reefs (Reitner 1989).

Only the 0.5 - 1 mm thick youngest generation of the calcites of the chaetetid-type basal skeleton is occupied by the living soft tissue (pl.3/fig.2). Soft tissue and basal skeleton exhibit a vertical anatomy divided in six major zones. The basal skeleton is made of high-Mg Calcite (15-19 mole% MgCO\(_3\)).

The formation of the basal skeleton can be summarized as below (Reitner & Gautret 1996):

At the uppermost position, a thick crust layer of spiraster microscleres (dermal area, zone I) and tylostyle megascleres settle which are arranged in clear plumose bundles proving the close phylogenetic relationship to *Spirastrella* (pl.3/fig.2). Below the outer dermal area, the internal dermal area (zone II) formed by mesohyle tissue, devoid of choanocyte chambers, enriched in mobile cells. Large inhalant chambers (lacunae) and distributing canals cross this zone, serving the choanosome with water filtered through the ostia. The mesohyle is characterized by large cells (ca. 10 \(\mu\)m) containing numerous inclusions (LGC: large cells with granules) and directly lying upon the calcareous skeleton (Reitner 1992) (pl.3/fig.2). These highly mobile cells are undoubtedly responsible for the secretion of collagen fibres and they probably derive from a special type of lophocytes. Collagen fibres are forming strong bundles which cross through the basal pinacocyte layer and anchor into the rigid skeleton (Vacelet & Garrone 1985). Thin collagenous fibres produced by non-modified lophocytes are widely sprayed in the intercellular mesohyle. They condense and become organized into a frame-building matrix at the top of the walls and they will stay entrapped inside skeletal structures after calcification. Calcite formation occurs as soon as these two types of fibres are present, proving their ability to attract divalent cations. However, the accicular shape of crystals and the highly organized microstructure, both characteristic for the S. (Acanthochaetes) skeleton, were never observed in these places. Skeletal formation starts inside the uppermost fibre template in the form of a soft structure, the elements of which have the shape and size of the future characteristic S. (Acanthochaetes) crystals, but these are not rigid and they look like “cooked spaghetti” (Reitner & Gautret 1996). This random structure becomes calcified and organized, when a mucus is secreted in the narrow space between the basopinacoderm and the calcified skeleton, by the pinacocytes which are forming the most basal continuous cell layer. This mucus is highly soluble making direct observations difficult to perform with electron microscopy. It is not preserved in TEM preparations and, at best, it can be recognized with the SEM through the collapsed clumps which are closely related with growing crystals in very well fixed specimens (pl.3/figs.3-6).

The central part of the tubes (zone III) is characterized by the choanosome which exhibits large choanocyte chambers (30-35 \(\mu\)m) leading to large oscular channels. Few tylostyles are normally present. Typical of a chaetetid skeletal type is the occurrence of tabulae stepping the tubes (zone IV). These are formed by the basopinacoderm, first as a thin organic phragma or sheet. Below the choanosomal zone, LCG cells become enriched and cause the mineralization of the organic sheet. Continuous upward moving basopinacoderm is forming a space filled with Ca\(^{2+}\)-binding and mineralizing organic mucus. This mineralizing process happens only when LCG cells are present (Reitner 1992). Within the closed spaces between tabulae, they contain accumulations of modified archaeocytes with numerous storage granules (thecocyte-like cells) and few spiraster microscleres (zone V). These cells should play a role in regeneration processes (Vacelet 1985, 1990) making the sponge able to start rising again when it has been drastically damaged.

The soluble matrix extracted from the superficial part of the skeleton contains high amounts of glycine, proline and hydroxyproline-rich compounds (collagenous affinity). Amino sugars are enriched in this zone. The presence of highly concentrated materials with collagenic or glucidic affinities results in the fact that relative amounts of acidic amino acids (Asp and Glu) appear less repre-
sented here than in the immediately underlaying older part. Only one matrix protein of 44 kDa was detected in S. wellsi which controls the seed nucleation of the Mg calcite crystals. However, absolute quantities of these two amino acids should be at least 3 to 5 times higher in the uppermost part of the skeleton (Gautret et al. 1996). The transformation starting from the area immediately below the active mineralizing zone exhibits a regular tendency with decreasing acidic amino acids as the most obvious feature, whereas aromatic amino acids (Tyr and Phe), serine and amino sugars also decrease. The increasing constituents are basic and aliphatic amino acids (mainly glycine).

Insoluble matrices exhibit quite similar, collageng amino acid compositions in all parts of the skeleton. Only the quantity of insoluble matrix changes in an important way, decreasing considerably from the surface to the base. This matrix completely differs from soluble compounds, with much less acidic amino acids, less 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 & Gautret 1996).

Discussion

The different types of formation of the rigid calcareous basal skeleton in three different taxa of coralline sponges demonstrate certain evolutionary steps. Leading to a comparable endproduct, a rigid calcareous skeleton, each taxa forms the basal skeleton in its highly specialised way using different modifications of calcium carbonate (Astroscera and Vaceletia = Aragonite; Acanthochaetes = High-Mg Calcite).

The most ancestral way to form a basal skeleton is realised in taxon Vaceletia which is explained as a process of "controlled taphonomy". This modified organomineralization is the first step to an organism controlled biomineralization. The first calcified sponges, the Archaeocyath, exhibit the same mode of calcification process (pl.1/figs.6-7). The calcification starts in a separated space inside organic pillars and is not under direct control of the sponge cells. Also in the later stage, when the chambers of the ontogenetically oldest parts of the skeleton are mineralized, the process does not happen under direct control of the sponge. Five different acidic matrix-macromolecules control the calcification process.

In the cases of Astroscera and S. (Acanthochaetes), modified lophocytes act for the initialisation of the mineralization. In Astroscera, the LVC's form a template of 3 dimensional fibres inside a large vacuole (pl.2/figs.5-6). The vacuole is filled with highly acidic mucus, rich in Asp and Glu. The fibres and the mucus act as the organic matrix for seed crystallization (Wörheide et al., in press, a, b). This process could be described as "biologically controlled calcification". In S. (Acanthochaetes), the lophocytes secrete collagenous fibres which also act as a template for the initial nucleation of the seed crystals (pl.3/figs.2,4,6). At a later stage, modified basopinacocytes control the mineralization process. In Astroscera, basopinacocytes cover the released and fused spherulites. The epitaxial growth of the spherulites is controlled by a highly acidic mucus between the crystal surface and the basopinacocytes. This mucus is highly soluble and exhibits a strong calcin induced epifluorescence. In S. (Acanthochaetes), the crystal growth is also controlled by acidic mucus substances, which fill up the space between the collagenous fibres and the basopinacocytes. The later stages in both cases could be described as "biologically induced calcification".

Very important in all cases is the presence of an organic matrix, forming a template for crystal nucleation (Simkiss 1986; Mann et al. 1989). The presence of a soluble organic mucus, rich in Asp and Glu, is controlling the crystal growth in the earliest and latest stages of mineralization. The mineral preference, aragonite or Mg-Calcite, is controlled by the structure of the organic macromolecules, forming β-sheets (Simkiss 1986), and acting as the Ca²⁺ attractors. The distances of the carboxyl-groups of the macromolecules control the crystallographic base plane of the initial calcite or aragonite crystal (Mann et al. 1989; Reitner 1993; Wörheide et al, in press, b).

The three described modern coralline sponges represent various evolutionary stages of the evolvement of basal skeletons. The thalamid taxon Vaceletia has strong affinities to the Archaeocyath. The taxon itself is known since the Middle Triassic. First representatives of the stromatoporoid Astroscera have been known since the Late Permian, Spirastrella (Acanthochaetes) wellsi originated in the early Cretaceous.

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Plate 1

Vaceletia - Archaeocyatha

Fig. 1: New species of colonial form of the taxon Vaceletia; Osprey Reef, northern Queensland Plateau, Australia.

Fig. 2: Vertical section of a single branch of Vaceletia crypta (Vacelet 1977) from Ribbon Reef No.10 (Lizard Island Section, northern Great Barrier Reef, Australia). The older part of the basal skeleton is completely filled up with CaCO₃ via controlled taphonomic calcification. The sponges are forming Ca-waste deposits (CWD) in the inactive portions of the basal skeleton.

Fig. 3: Backscatter image of a CWD chamber in Vaceletia crypta. SEM image of polished section.

Fig. 4: Critical Point dried specimen of a fixed portion of a CWD chamber. Within the acidic organic mucus (wurm-like fabrics), small aragonite crystals are growing. Acidic matrix proteins are controlling the fill up of the CWD chambers.

Fig. 5: TEM image of EPS fibres with seed nuclei of aragonite crystals. The seeds are dark stained using OsO₄. The lipid rich organic matrices are stained with OsO₄.

Fig. 6: CWD chambers of an archaeocath (Warrioottacyathus wilkawillnensis) of the Lower Cambrian (Atdabanium) of the Flinders Ranges (South Australia).

Fig. 7: CWD chambers of a further archaeocyath Ardrossacyathus grandis from the same locality which exhibit a close relationship to the modern ones in the taxon Vaceletia.
Plate 2

*Astrosclera willeiana* LISTER 1900

Fig. 1: Very large specimen of *Astrosclera willeiana* from 25 m water depth of Ribbon Reef No.10 (see Pl.1, fig.2).

Fig. 2: Vertical section of the upper portion of the living part of the basal skeleton. The uppermost part of the sponge exhibits isolated aragonitic spherulites which grow together in the deeper portions to a rigid skeleton. Soft tissue of the sponge is red stained using basic fuchsins.

Fig. 3: Calcein (Ca-sensitive fluorochrome dye) stained isolated spherulites of the upper dermal layer. Ca$^{2+}$-rich acidic organic matrices are orange/yellow stained.

Fig. 4: Epifluorescence image (wide-band pass filter 17 Zeiss green) of the same anatomic part (fig.3). The active calcifying process is detected by a strong yellow fluorescence within the spherulites.

Figs. 5 & 6: TEM sections of large vesicle cells (LVC) which form the primary aragonitic spherulites. This type of cells forms extremely large vacuoles and radially arranged, insoluble organic fibres which control the aragonite fibre growth. Fig.5 demonstrates a young LVC, fig.6 shows the mature stage shortly before releasing the entire spherulite.

Fig. 7: Calcein stained young spherulites within the soft tissue.

Fig. 8: SEM images of newly released aragonitic spherulites.
Plate 3

Spirastrella (Acanthochaetetes) wellsi (Hartman & Goreau 1975)

Fig. 1: Underwater images of a living specimen from Lizard Island Bommie Bay reef cave (Great Barrier Reef), 10 m water depth.

Fig. 2: Uppermost portion of a calicle wall of the chaetetid sponge. The upper part of the mineralized skeleton is weakly red stained due to remaining acidic organic matter. The skeleton is surrounded by large cells (lcg - large cells with granules) which are forming collagen fibres. As a by-product, these cells secrete the only Ca²⁺-binding matrix protein of this sponge. Mineralization happens between the exopinacoderm and the already calcified skeleton.

Fig. 3: Initial mineralizing zone. On the left hand remains of the pinacoderm are visible. SEM image.

Fig. 4: Detailed magnification of fig.3. The spaghetti-fibres are organic templates for the elongated high-Mg calcites crystals.

Fig. 5: Newly formed crystal network. Soft tissue is removed. SEM image.

Fig. 6: TEM image of the spaghetti-fibres. They exhibit the typical black staining of the lipid rich intracrystalline organic matter when OsO₄ is used.
Spirastella (Acanthochaetetes) wellsi