Chapter 8
Interspace Mineralization Within Bilayered Organic Matrix of Deep-Sea Bamboo Coral (Anthozoa: Gorgonacea: Isididae)

Abstract Bamboo corals are the representatives of the Gorgonacea order within Isidids octocorals. Opposed to corals from the Scleractinia order, in which the biomineralization process has been well evaluated, the mineralization in the Isididae corals is still not fully understood. The interspace mineralization process in the deep-sea bamboo corals bilayered organic matrix is described and analyzed.

It is well known that, the mineralization phenomena in corals is a process where the organism is able to create an organic structure that provokes the nucleation and development of crystals, together with the framework microarchitecture and appropriate crystallographic orientation (Goldberg 2001). Opposed to corals from the Scleractinia order, in which the biomineralization process has been well evaluated (Cuif and Sorauf 2001; DeCarlo 2018; Tambutte et al. 2011), the mineralization in the Isididae corals is still not fully understood. These are known as long-lived psychrophilic gorgonin octocorals (Frenkel et al. 2017).

Bamboo corals are the representatives of the Gorgonacea order within Isidids octocorals (for overview see Etnoyer 2008; Watling and France 2011; Watling et al. 2011; Alderslade and McFadden 2012; Dueñas et al. 2014; Frenkel et al. 2017). They are the deepest occurring habitat-forming deep-sea coral family, with a maximum depth of over 3800 m. Over 50% of Northeast Pacific coral organisms are usually located at a depth higher than 1000 m (Etnoyer and Morgan 2003). In the Isididae family, 138 species within 38 genera have been described by S. Cairns and F. Bayer from the Smithsonian Institute in Washington D.C. (Bayer 1990). The unbranched as well as abundantly branched taxa can be easily found. In some species, branching might appear at the internodes, as well as at the nodes in another species (Fig. 8.1a). The skeleton is coated by the coenenchyme which is the colonial living tissue comprised of calcareous sclerites and epidermal layers surrounding mesoglea, similar to polyps. Sanchez (2002) characterized Isidids as classic representatives of Calcaxonia suborder with branching tree-like networks, calcareous joints and continuous multilayered axis (Fig. 8.1c, d). In 2006 we conducted research to understand the nanotopography of gorgonin intersurfaces and node surfaces, of the coral axial internode together with the morphology of the nanoscale mineralized as well as observed patterns. We evaluated the function of the gorgonin

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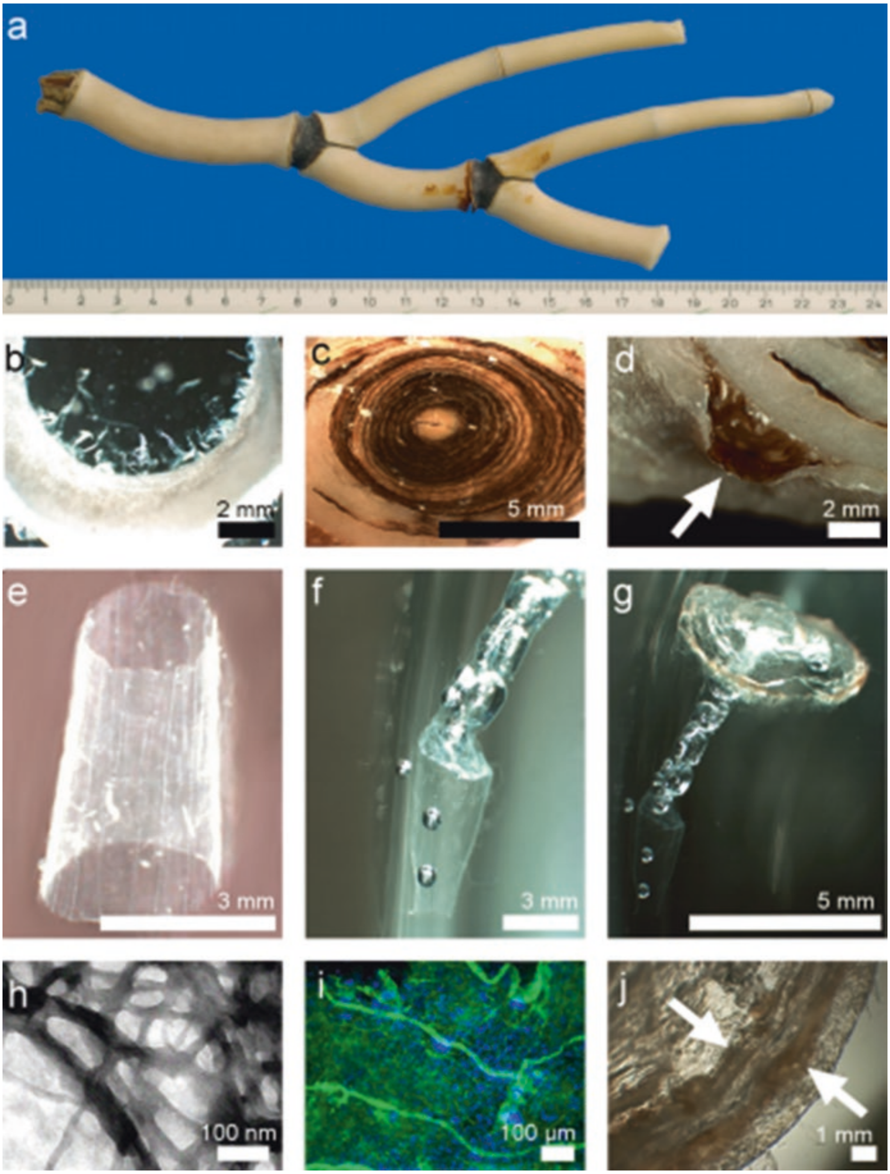

Fig. 8.1 Finger-like architecture is typical for the bamboo corals of Isididae family (**a**). The cutted surface of the skeletal nodes helps to visualize the continous multilayered axis (**c**, **d**). Osteosoft treatment leads to the isolation of the organic matrix (**b**, **e**, **f**, **g**). It possesses noncollagenous, but nanofibrillar structure (**h**). Layers made of gorgonin are usually visible in fluorescence (**i**) as well as light (**j**) microscopy

mineralization, as well as the biomimetic capability of the bamboo corals for the possible progress in obtaining novel biological materials for the biomedical purposes (Ehrlich et al. 2006).

In order to understand the nanostructure as well as nature of the axial organic matrix obtained from corals, the process of decalcification with Osteosoft™ was applied (Fig. 8.1b). After 7 days of decalcification, the total lysis of the calcite axis internode, and the presence of a transparent gelatinous pellicle were observed (Fig. 8.1e–g). The fibrillar protein behaviour of the organic matrix was confirmed using SEM, TEM (Fig. 8.1h) and AFM, respectively. The amino acid analysis shows that the proline and glutamine (24.0, 28.9% and respectively) are the main building blocks of this matrix (Ehrlich et al. 2006). The low glycine content (2.5%) excludes the eventuality that the fibrillar structure has collagen origin. This is contrary to the collagen detection within the organic matrix obtained from *Corallium rubrum* (Allemand et al. 1994). Thus, we found that the Isididae's axial internode organic matrix can be assumed as an acidic fibrillar protein. These findings are in the line of the popular Lowenstam and Weiner theory, which stated that these proteins are essential during the biomineralization (Lowenstam and Weiner 1989). It is worth to notice that, in a distal gorgonin-containing node, any disruption effects were observed, even after 3 months of the analogous decalcification procedure with Osteosoft™. Interestingly, after 72 h of alkali treatment the fragmentary disruption and dissolution this sample was achieved.

The light microscopy analysis of the dark brown Isidid node from the distal part of sample (Fig. 8.1i, j) indicates the existence of yearly alternating rings of mineral and horny (gorgonin) origin.

Data of the chemical characterization of sample, performed with use of EDX/ESEM, demonstrate the existence of C-57.73, N-20.91, O-10.94, Br-2.54, S-1.96, Na-1.86, P-1.05, Cl-1.65, Mg-0.87, Ca-0.21, Fe-0.20 Zn-0.08 (At/%). Recently, gorgonin (known also as chitinous- iodine- bromine-, mucopolysaccharide-containing protein or tanned-collagen (Ehrlich et al. 2003)) had no determined chemical composition. In 1855 Valenciennes first noticed that the Mediterranean Gorgonia internal skeleton consisted of a horn-like protein, therefore it receives the name gorgonin (Block and Bolling 1939). In 1939, Block and Bolling demonstrated the abnormally large amounts of phenylalanine (6.5%), cystine (9%) and tyrosine (13%) in gorgonin obtained from *Gorgonia flabellum*.

The coating of the organic matrix middle channel from the gorgonin distal node observed using SEM (Fig. 8.2a, b) is brownish under light microscopy. Differences among these two structures emerge from the unique organization of the nanostructure (Figs. 8.3a, b and 8.4a, b). In the bilayered epithelium of the gorgonin-comprising middle channel node, the mineralized fibrous structures could be observed on both interfaces (thickness of the layer about 150 nm). The spherical formations of calcium carbonate, about 70 nm large, are closely spread on microfibrils to form the intersurface of gorgonin (Fig. 8.5a, b). Within the microfibrils-free surface of the gorgonin layers, no formation of the mineral phase was observed. Therefore, gorgonin layers themselves can act as patterns for the formation of proteinaceous microfibrils, which can be used as a template for the biomineralization. These results

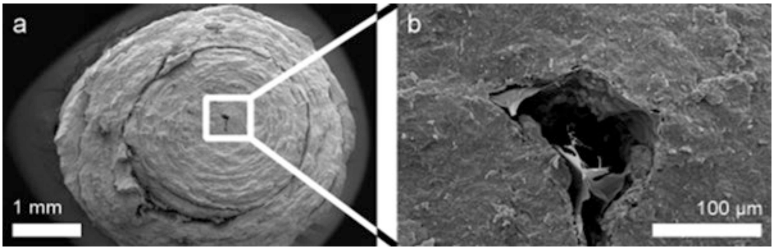

Fig. 8.2 SEM image: transverse view of the distal gorgonin-containing node

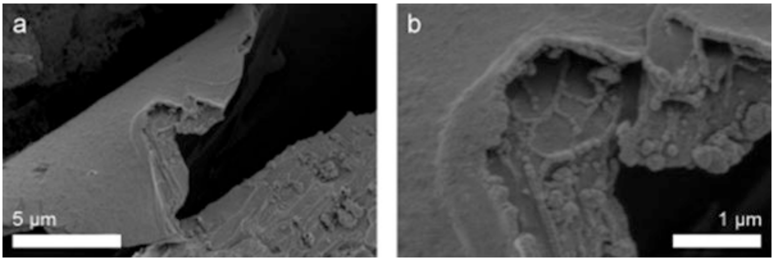

Fig. 8.3 SEM images of the bi-layered gorgonin-containing epithelium covering the middle channel of distal node

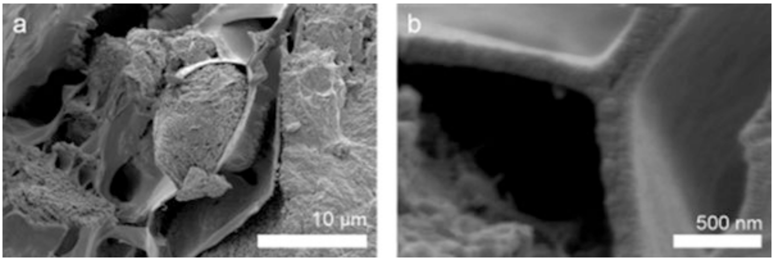

Fig. 8.4 SEM images of the bi-layered gorgonin-containing epithelium covering the middle channel of distal node

corresponds with those obtained by Bayer and Macintyre (2001). They described the submicron spheres of carbonate-comprising hydroxylapatite found in some gorgonian species within their chambered axial core. We supposed that on the nanolevel the interspace biomineralization process can be typical for octocorals.

The pigment recognition amenable for brownish gorgonin-comprising internodes was conducted using FTIR and UV/Vis- spectroscopy. FTIR spectra (Ehrlich et al. 2006) accurately correlate with the IR-spectra obtained for the quinone-tanned Mefp-1 ft protein received from the *Mytilus edulis* sea mussel by Suci and Geesey (2001). Results of the UV/Vis analysis of the yellow, sticky supernatant (Fig. 8.6, right below) from the alkali-treated gorgonin node (data not shown) received after

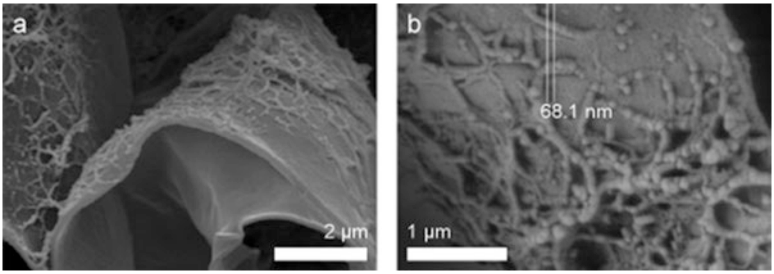

Fig. 8.5 (**a**) SEM Image of gorgonin-containing epithelium that function as a template for microfibrils on which mineralization occur (**b**)

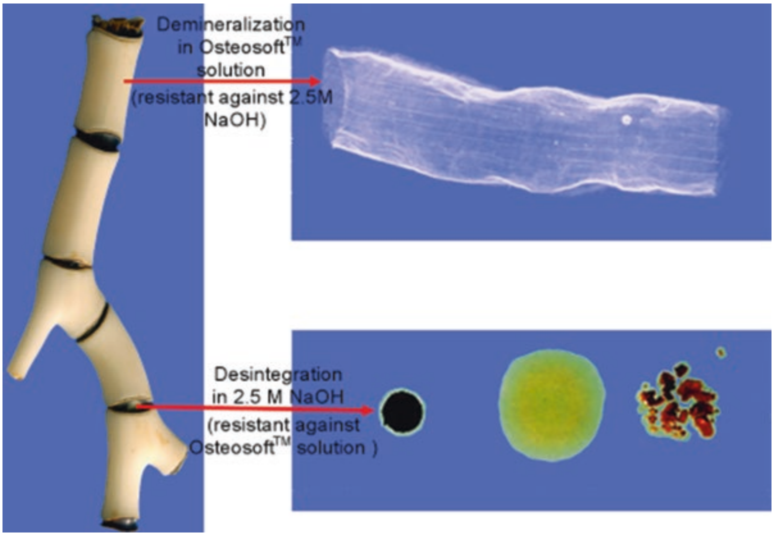

Fig. 8.6 Both fibrillar organic matrix (right above) and dark colored gorgonin-based extracts (right below) can be isolated after selective chemical treatment of Isididae sp. coral fragment

centrifugation (15,000 g, 30 min) were alike to the spectra of the quinone compounds isolated from the dark pigment of the capsules skate egg, what was reported by Koob and Cox (1990).

A gorgonin tanning potential mechanism can be determined as follows: a stable cross-linked protein is formed by the reaction of the quinones with sulphydryl and terminal amino groups. The polymerisation of quinones during the synthesis of the melanin-like biopolymers is responsible for the brow colour of the tanned Isidid coral nodes. A graphical representation (Fig. 8.7) of the likely polyphenolic compounds of the gorgonin, elucidate the unavailability of these non-reactive oxygen residues for any interaction with gorgonin's Ca-ions. The detailed comparison of the other references similar to various quino-proteins of marine origin like antipathin (Goldberg et al. 1994) or byssus (Coyene and Waite 2000; Zhao and Wait 2005)

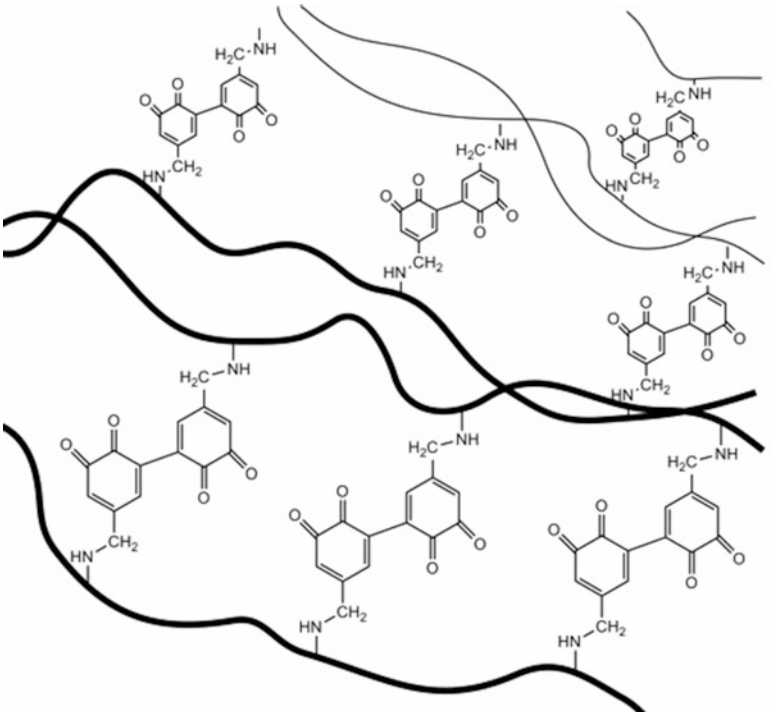

Fig. 8.7 Schematic view of the possible gorgonin-based cross-links in Isididae corals (image courtesy Denis Kurek)

validates our hypothesis that majority of the proteins comprising polyphenol are basic and hydrophobic, thus the mineralization under natural conditions is impossible. Therefore, in Isididae corals, the presence of gorgonin nodes is related to their flexibility, not to hardness, as opposed to calcite-containing axial internodes.

8.1 Conclusion

Thus, for the first time, we showed that within the Gorgonacean family: Isididae, the quinones-containing compounds play important role in the gorgonin nodes. A versatile knowledge about gorgonin-containing nodes and their structure, composition and mineralization can be used to create new pathway of biomimetic formation of hybrid materials with characteristic bioelastomeric features essential for their application in biomedicine. The biopolymers based on polyphenolic compounds can be applied in the formation of novel blood vessel implants and calcification resistant biomaterials.

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