Fine structural study of the red seaweed *Gymnogongrus torulosus* (Phyllophoraceae, Rhodophyta)

José M. Estevez* and Eduardo J. Cáceres**

* Departamento de Química Orgánica (CIHIDECAR-CONICET), FCEyN-Universidad de Buenos Aires, Ciudad Universitaria-Pab. 2, 1428 Buenos Aires, Argentina.
** Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, 8000 Bahía Blanca, Argentina.

**Key words:** anomalous chloroplasts, cell wall, cystocarpic thalli, fine structure, *Gymnogongrus torulosus*, pit plugs, Rhodophyta.

**ABSTRACT:** The present study analyzed several characters of the red seaweed *Gymnogongrus torulosus*, such as cellular structure of the thallus, cuticle, pit plug and cell wall ultrastructure, and morphology of some organelles like plastids, Golgi bodies and mitochondria. Also, anomalous chloroplasts with thylakoid disorganization were found in medullary cells. The significance of this thylakoid disposition is still unclear. This is one of the first studies focused on the fine structure of a red alga recorded in Argentina.

**Introduction**

*Gracilaria verrucosa* (Hudson) Papenfuss, *Gigartina skottsbergii* Setchell and Gardner and *Sarcothalia crispata* (Bory) Leister (= *Iridaea undulosa* Bory), are the most important red seaweeds used in the hydrocolloid industry of Argentina (Boraso et al., 1998). Another local red seaweed with a potential commercial interest is *Gymnogongrus torulosus* (Hook. F. et Harv.) Schmitz (Phyllophoraceae, Gigartinaceae), since it is known to produce mainly iota- and kappa/iota-carragheenan (Furneaux and Miller, 1985; Estevez et al., 2001).

Studies on the genus *Gymnogongrus* have been principally focused on morphology (Masuda et al., 1979; André, 1978), molecular sequences (Maggs et al., 1992; Fredericq and Ramírez, 1996), life history (Cordeiro-Marino and Candia Poza, 1981; DeCew and West, 1981; Lewis et al., 1991), ecological aspects (Romanello et al., 1983), and chemistry of the cell wall (McCandless et al., 1982; Furneaux and Miller, 1985). In contrast, information about fine structural aspects of the genus is scarce. A brief comment on the pit plugs structure of *G. chiton* (Howe) Silva et DeCew (Pueschel, 1989) and the study of Santelices et al. (1999) on the fine structure of germinating carpospores and tetrasporophytes in two species of *Ahnfeltiopsis*, a new genus segregated from *Gymnogongrus* (Silva and DeCew, 1992; Masuda, 1993), are the only references on this matter.

In this work we present the investigation on the fine structure of cystocarpic thalli for the first time in the genus *Gymnogongrus*. This study was made on field growing thalli from natural populations of *Gymnogongrus torulosus* from Argentina.
Materials and Methods

Cystocarpic thalli of *Gymnogongrus torulosus* (Hooker *et al.*) Schmitz were collected in March 1998, from Cabo Corrientes, Mar del Plata (38° 03' S, 57° 31' W), Buenos Aires Province, Argentina. The specimens were fixed in 4% formalin/sea water and were deposited under the number 35707 in the herbarium of the Museo Bernardino Rivadavia, Buenos Aires, Argentina (BA). Thalli of the same material were sent dry, in silica gel, to Susan Fredericq for the taxonomic identification based on molecular sequence analysis of the chloroplast gene *rcbL* (Fredericq, pers. com.). For the electron microscopy cystocarpic thalli were fixed at room temperature in 2% glutaraldehyde in sodium cacodylate buffer (0.05M), postfixed in 2% OsO₄ in the same buffer, dehydrated through a graded acetone series and embedded with Spurr’s low viscosity resin (Spurr, 1969) by the flat embedding method (Reymond and Pickett-Heaps, 1983). All sections were cut with a diamond knife (Diatome Ltd., Bienne, Switzerland) in a Reichert-Jung Ultract ultramicrotome (C. Reichert Optische Werke, Wien, Austria). Semithin sections (1-3 µm) were mounted on glass slides and then observed with a Carl Zeiss Axiolab microscope (Carl Zeiss, Jena, Germany). Thin sections were mounted on Formvar coated grids, stained with uranyl acetate (40 min) and lead citrate (40 sec) and then observed with a Jeol 100 CX-II electron microscope (Jeol Ltd., Akishima, Tokyo, Japan) at the Centro Regional de Investigaciones Básicas y Aplicadas de Bahía Blanca, Argentina (CRIBABB).

Results

Morphology

Cystocarpic thalli of *G. torulosus* were gelatinous, dark purplish, up to 8 cm high, irregularly branched and sometimes with apical dichotomy. Main axes were cylindrical or flattened (1-3 mm in diameter), and one or more cystocarps were observed (Fig. 1) in the terminal branches (0.33-0.5 mm in diameter). Multiaxial thalli were composed by several layers of compact and densely colored cortical cells and a well developed medullary cells (Fig. 2). The cortex was formed by 2-5-celled filaments, anticlinally disposed, and connected by primary and secondary pit plugs to internal, interconnecting cells, which were disposed in 1-3 layers (Fig. 2). Cortical cells of the filaments were ellipsoidal (3-8 µm in diameter), well pigmented and non-vacuolated (Fig. 3). Similar characteristics were observed in the interconnecting cells of the thallus. In contrast, medullar zone consisted of several layers of colorless and highly vacuolated spherical cells (Fig. 2), that were the largest in the algal thallus (15.4-20 µm in diameter).

Fine structure of the cortical filaments

Cell walls of the cortical filaments (2 µm in width) (Fig. 3) were composed by an inner fibrillar matrix and an outer clear-amorphous matrix. In the fibrillar zone, the microfibrils were deposited in layers irregularly crosslinked by interconnecting ones, adopting a net like appearance (Figs. 4-5), and the amorphous matrix was well developed (Fig. 5). A single cuticle layer (0.5-0.75 µm in wide) was located in the outter zone of the thallus (Fig. 7). Discoid chloroplasts, that occupied almost the entire volume of the cell (Figs. 4-5), had a single peripheral encircling thylakoid and a few inner parallel thylakoids (Fig. 5). This plastids were parietally arranged, in close association with the mitochondria, which were located near the pit plugs (Fig. 5). Several floridean starch grains were observed (Fig. 4) surrounding the pericentral nucleus (1.2-2.2 µm in diameter).

Pit plugs

Primary and secondary pit plugs (Figs. 3-6) exhibited a core of 370 nm in length, 300 nm in minimal diameter (at the central zone), and 570 nm in maximal diameter (at the ends). The plug showed an homogeneously high electron opacity at the ends and sides, with a markedly decrease in opacity in the central part of the plug core. Cap membranes were observed in the pit plugs of all cells but, they were not clearly defined in the flat surfaces of this structure (Fig. 6, arrowheads). Immediately underneath, there was a well defined and clear layer (25 nm in width), conspicuously distinct from the rest of the core (Figs. 5, 6). Outside the cap membrane, no outer cap layer was found (Fig. 6).

Interconnecting cells

In this cells the walls were well developed and displayed the same arrangement and structure as described before in the outer cortical cells (Fig. 8). The chloroplasts (2-5) were peripheral (0.9 µm x 4.4 µm in size) (Figs. 8-9), with many central thylakoids (4-10) and one encircling thylakoid. Floridean starch storage was less abundant as compared with the outer cortical cells (Figs. 8-9). Large vesicles derived from Golgi bodies were located
Figs. 1-7. Cystocarpic thallus of Gymnogongrus torulosus.

**FIGURE 1.** General aspect of the cystocarpic plant with cystocarps (arrowheads). Scale bar = 0.3 cm.

**FIGURE 2.** Light photomicrograph of a transversal semithin section of the thallus. Cortical filaments show pit plugs between contiguous cortical cells and between cortical cells and interconnecting cells (arrowheads). Scale bar = 10 µm.

**Figs. 3-6.** Electron microscopy of cortical cell and cuticle.

**FIGURE 3.** General aspect of the cortex. Cortical and interconnecting cells are connected by primary (small arrowhead) and secondary (large arrowhead) pit plugs. The fibrillar and the amorphous matrix of the cell wall are both clearly discernible. Scale bar = 3.7 µm.

**FIGURE 4.** Three-celled cortical filament. Scale bar = 2.5 µm.

**FIGURE 5.** Details of two contiguous cortical cells sectioned at the level of a primary pit plug. Scale bar = 0.7 µm.

**FIGURE 6.** Details of a pit plug. Cap membranes are ill defined at the ends of the plug core (arrowheads); there are conspicuous clear zones underneath the cap membranes. Scale bar = 0.25 µm.

**FIGURE 7.** Outer region of the thallus covered by a single layered cuticle. Scale bar = 1.25 µm. a, amorphous matrix; c, cortical cell; ch, chloroplast; ct, cuticle; f, fibrillar stratum; i, interconnecting cell; m, medullary cell; mi, mitochondria; n, nucleus; s, starch; v, vacuole.

Figs. 8-9. Interconnecting cell.

FIGURE 8. General aspect of the cell. The cell wall is well developed with both fibrillar and amorphous strata. The chloroplasts are peripheral and well developed. Note the significant dictyosomic activity: flat vesicles are adjacent to the plasma membrane and mainly located in the zone free of chloroplasts. Scale bar = 1.25 µm.

FIGURE 9. Details: the chloroplast shows a typical arrangement of the thylakoids. The content of the vesicles is electron clear and some of them appear fused with the plasmalemma (small arrowheads). Also, flat cisternae of the Golgi body were observed (large arrowheads). Scale bar = 0.7 µm.

Figs. 10-12. Medullary cells.

FIGURE 10. General aspect of the cell. Note that the large, central vacuole occupies most of the cell volume, and the few and small chloroplasts and nuclei are peripheral. The content of the vacuole is clear and there are numerous vesicles placed close to the periphery (arrowheads). Scale bar = 2.5 µm.

FIGURE 11. Detail of the peripheral vesicles. They are fused with the plasmalemma and possibly force out their content into the cell wall (arrowheads). Scale bar = 0.25 µm.

FIGURE 12. Detail of a portion of the cell wall. Periclinal microfibrils are irregularly crosslinked by other fibrils anticlinally disposed. Scale bar = 0.1 µm. a, amorphous matrix; c, cortical cell; ch, chloroplast; ct, cuticle; f, fibrillar stratum; gv, Golgi derived vesicles; i, interconnecting cell; m, medullary cell; mi, mitochondria; n, nucleus; s, starch; v, vacuole.
against the plasmalemma (Fig. 8), and also, some of them were found fusing with the plasma membrane (Fig. 9).

*Medullary cells*

The medullary cells had more than one nucleus and showed a large, central vacuole and the rest of the organelles were located in the cell periphery (Fig. 10). Large and marginal vesicles had a clear content and were fused with the plasmalemma (Figs. 10-11). The cell wall reached the maximal thickness in the thallus (Fig. 10) and showed an irregular crosslinked appearance (Fig. 12). Normal and functional chloroplasts had the typical red algal structure (Fig. 13) but, also anomalous small chloroplasts were found (3.5 µm x 1.5 µm). They had thylakoids in different degree of disorganization (Figs. 14-17). Some chloroplasts had the thylakoids separated, making their lumen larger and irregular, but they still showed the presence of phycobilisomes (Fig. 14). In other plastids, the phycobilisomes were no longer discernible (Figs. 15-17) and the membranes of contiguous thylakoids fused, forming tubules or sacs with irregular disposition, size and number. Also, at least in a few chloroplasts, were observed a totally disorganized thylakoid system (Fig. 17).

**Discussion**

Cystocarpic thallus of the red alga *Gymnogongrus torulosus* displayed a multiaxial organization with a clear distinction of cortical and medullar zones like in other genera of Gigartinales (Bold and Wynne, 1985; Coomans and Hommersand, 1990; van den Hoek *et al*., 1995).

Cuticle is a widespread feature in red algae (Hanic and Craigie, 1969; Gerwick and Lang, 1977; Craigie *et al*., 1992; Flores *et al*., 1997) and protects the thallus.

---

**Figs. 13-17.** Cystocarpic thallus of *Gymnogongrus torulosus*. Details of the progressive disorganization of the thylakoids in some chloroplast of medullary cells.

**FIGURE 13.** Normal chloroplast. Scale bar = 0.7 µm.

**FIGURE 14.** The thylakoid membranes are separated (arrows) making their lumen ample and irregular. Phycobilisomes are still present (arrowhead). Scale bar = 0.5 µm.

**FIGURE 15.** Membranes of contiguous thylakoids fuse to form irregularly shaped tubules or sacs (arrowheads). Scale bar = 0.5 µm.

**FIGURE 16.** Chloroplast with a reduced number of irregularly disposed sacs (arrowheads).

**FIGURE 17.** Chloroplasts with an almost totally disorganized thylakoid system. The tubules are scarce and the chloroplast envelope is still present (arrowheads). Scale bar = 0.37 µm.
during adverse conditions, such as desiccation, herbivore grazing and bacterial degradation (Gerwick and Lang, 1977). Usually, the cuticle is composed by a multilayered structure (Brawley and Wetherbee, 1981; Homersand and Fredericq, 1990; Foltran et al., 1996), but G. torulosus presents a single and unstratified one, like in Porphyra umbilicalis (L.) J. Agardh (Hanic and Craigie, 1969). One possibility of the adaptive advantage, if any, of the multilayered cuticle could be a more resistant to the marine environment than the monolayered one.

Primary and secondary pit plugs in G. torulosus showed both the same fine structure, like those described in G. chiton (Pueschel and Cole, 1982). Also, the pit plugs of the red algae Ahnfeltiopsis furcellata (C. Agardh) Silva et DeCew (Santelices et al., 1999), Ahnfeltia gigartinoides J. Agardh (Pueschel, 1989) and Rhodymenia pertusa (Post. & Rupr.) J. Agardh (Pueschel and Cole, 1982) strongly resemble those of G. torulosus. The presence of a clear layer underneath the cap membrane observed in the pit plugs of G. torulosus could indicate a different chemical composition compared with that of the rest of the core (Pueschel, 1980). A similar layer has been considered as inner cap layer in Gelidiella acerosa (Försskål) J. Feldmann et Hamel (Pueschel, 1989). In contrast, a layer found in the pit plugs of Rhodymenia californica Kylin (Pueschel and Cole, 1982), Gracilaria foliifera (Försskål) Borgesen (Pueschel, 1989), Dumontia contorta (S. G. Gmelin) Ruprecht (Pueschel, 1989) and Iridaea cordata (Turner) Bory (Foltran et al., 1996) was not considered as an inner cap layer in any case. According to Pueschel (1989), this character has limitations for taxonomic descriptions since there is no diagnostic procedure to demonstrate the presence or absence of the inner cap layer. In consequence, in the present work we are not able to interpret the clear layer as an inner cap layer in G. torulosus.

According to Esteve et al. (2001), more than 50.0% of the dry weight of the thallus in G. torulosus is composed by sulphated polysaccharides and they are located in the amorphous matrix of the cell wall and intercellular spaces (unpublished results) using rutemin red method (Krishnamurthy, 2000). Also, similar sulphated polysaccharides were found in the amorphous matrices of other red algae (Diannelidis and Kristen, 1988). In the red seaweeds, the cells synthesize wall polysaccharides in cytoplasmatic vesicles derived from the Golgi body (Ramus, 1972; Ramus and Robins, 1975; Tveten-Gallagher et al., 1981; Gretz et al., 1990) and discharge their contents into the intercellular spaces (Tsekos and Schnepf, 1991). The presence of similar vesicles found in G. torulosus could be related with an active cell wall biosynthesis.

The thylakoid system of many chloroplasts present in medullary cells of G. torulosus, suffered a notable disorganization like in other red algae (Hara and Chihara, 1974; Hara, 1975; Borowitzka, 1978; Pueschel and van der Meer, 1984). In Porphyra leuosticta, a similar phenomenon was caused by low levels of illumination, both in field and in culture conditions (Sheath et al., 1977). However, in the case of Griffithsia pacifica Kylin (Koslowsky and Waaland, 1987) the chloroplast deterioration was explained as a result of a cytoplasmatic incompatibility reaction. The significance of the thylakoid fragmentation and the presence of tubular units is still unclear in G. torulosus. Additional studies of this red seaweed focused on growth under controlled conditions are needed, in order to understand the origin of this degenerative process of the plastids.

Acknowledgements

This work was carried out with the support of a grant of the Secretaría de Ciencia y Tecnología de la Universidad Nacional del Sur, Argentina, PGI CSU-24/B043 to E.J.C. J.M.E is fellow of the Consejo Nacional de Investigaciones Científicas de la República Argentina (CONICET). E.J.C. is member of the Comisión de Investigaciones Científicas de la Provincia Buenos Aires, Argentina (CIC). We thank to Dr. Dieter G. Müller, University of Konstanz, Germany, for reading the manuscript and helpful comments as well as Dr. Susan Fredericq for providing the taxonomic identification of the material.

References


