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Ultrastructure of pericarp development in *Gracilaria verrucosa* (Hudson) Papenfuss (Gracilariaceae, Gracilariales, Rhodophyta)

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The ultrastructure of pericarp development in *Gracilaria verrucosa* (Huds.) Papenfuss is described. Anticlinal and successive periclinal divisions of the outer cortical cells result in the formation of a thick multilayer pericarp. Primary pit connections occur between pericarp cells. Mucilage is formed within cytoplasmic concentric membranes, giving thus rise to mucilage sacs. In addition, mucilage sacs seem to arise through local dilations of the nuclear envelope. Mucilage sacs considerably increase in volume resulting in the formation of one or two huge mucilage sacs, which occupy the major part of the cell interior and finally discharge their contents. Thus, inner pericarp cells actually function as secretory cells exhibiting a degenerate appearance after the release of the content of mucilage sacs.

Key words: *Gracilaria*, pericarp, Rhodophyta, ultrastructure.

Abbreviations used in figures: CM = concentric membranes, dNE = dilating nuclear envelope, FS = floridean starch, LB = lipid body, M = mitochondrion, Mb = microbody, MS = mucilage sac, Mu = mucilage, N = nucleus, Nu = nucleolus, P = plastid, PC = pit connection

INTRODUCTION

In the cystocarp of the Florideophyceae, a sterile covering around the carposporophyte is called the pericarp. This envelope of tissue consists of cells, which are of gametophytic origin. Although numerous ultrastructural studies have been carried out on the developing cystocarps and post-fertilization development has almost thoroughly been examined (for references see Pueschel, 1990; Delivopoulos, 2003a, c) no attention has been paid to date to the structure and function of this surrounding wall layer of the gonimoblast. All ultrastructural reports on pericarp cells have been incidental in papers describing carposporogenesis and carposporophyte development (Kugrens & West, 1973; Tsekos, 1983; Tsekos & Schnepf, 1983; Delivopoulos & Tsekos, 1983; Delivopoulos, 2003a, c).

Gracilaria is a commercially valuable agarophyte (Levring *et al*., 1969; Kim, 1970). Because of the presence of about 100 species distributed throughout temperate and tropical waters (Michanek, 1971), it is regarded as one of the more valuable red algae. A pericarp is developed over the gonimoblast filaments, with an ostiole through which the carpospores are released. A complete ultrastructural study of the pericarp development in the species *Gracilaria verrucosa* (Huds.) Papenfuss is presented in this paper in hopes of providing useful information for the elucidation of the functional significance of this important structure.

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MATERIALS AND METHODS

Thalli of *Gracilaria verrucosa* (Huds.) Papenfuss with cystocarps of various sizes were collected at Megalon Emvolon (Gulf of Thessaloniki). They were immediately fixed for 5 h in a 3% formaldehyde 3% glutaraldehyde mixture in 0.1 M phosphate buffer containing 0.25 M sucrose (pH 7.0). The fixed thalli were successively rinsed in decreasing

concentrations of sucrose and buffer and they were finally rinsed in 0.1 M phosphate buffer. The material was post-fixed for 5 h in 0.1 M phosphate buffered 1% osmium tetroxide and then dehydrated in a graded series of methyl cellosolve, ethanol and prophylene oxide. Samples were impregnated for 2 days with a graded series of mixtures of propylene oxide and Spurr's resin and finally embedded in Spurr's resin.

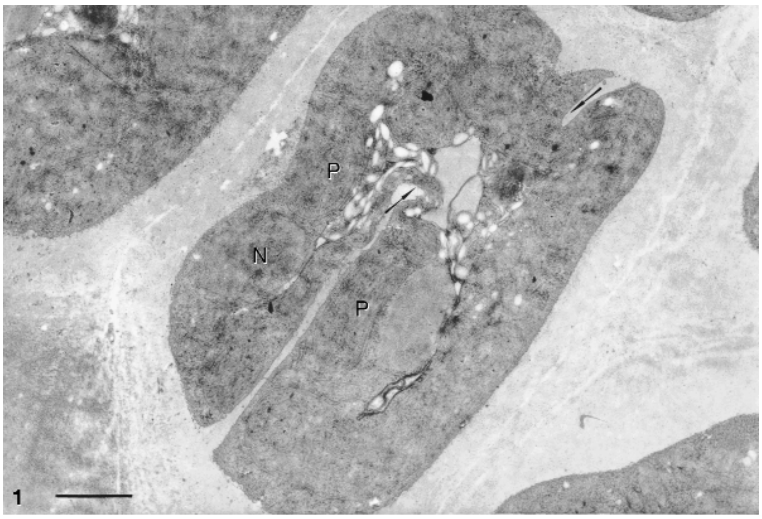
Ultrathin sections were cut on a Reichert OmU_2 ultramicrotome, stained with uranyl acetate and lead citrate and examined with a Jeol 100-B electron microscope.

RESULTS

The outer cortical cells of the gametophytic tissue are initially isodiametrical. Pericarp cells are usually multinucleate. An anticlinal division of each cortical cell (Fig. 1) results in the formation of two elongated daughter cells (Fig. 2). Subsequently, successive periclinal divisions lead up to the formation of a thick multilayer pericarp (see Figs 3B and 3D of Delivopoulos & Tsekos, 1983). The resultant cells are interconnected with primary pit connections (Fig. 3). The movement of a nucleus through the linkup of two neighbouring cells is depicted in Fig. 3. This occurs during the process of a secondary pit connection formation.

One of the first events denoting pericarp cell differentiation is the formation of mucilage sacs (Figs. 5, 6). Concentric membrane bodies occur in various sites of the cytoplasm (Figs 5, 6). During mucilage formation, a dilation between the membranes of the bodies was observed (Figs 5, 6).

The most striking ultrastructural feature during pericarp development is the nuclear envelope activity. Initially, there is a slight enlargement of the per-



FIGS 1-2. *Gracilaria verrucosa*: electron photomicrographs. Scale bars = 1 μm.

FIG. 1. An anticlinally dividing (arrows) pericarp cell.

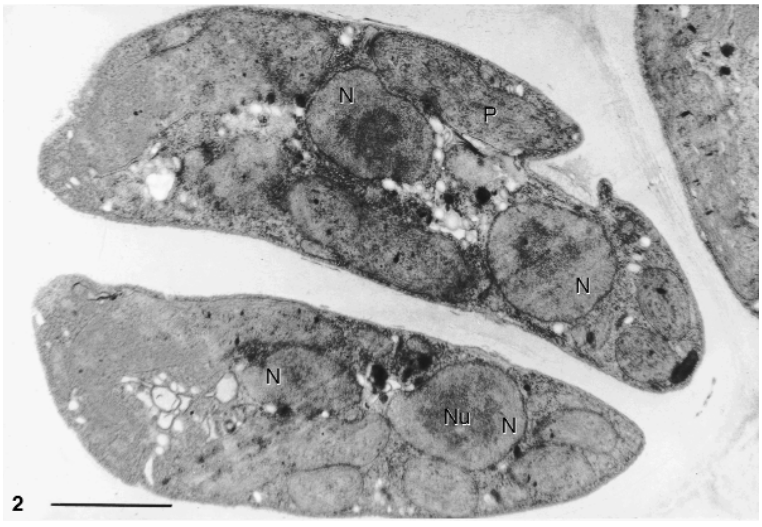


FIG. 2. Two daughter cells after an anticlinal division of a pericarp cell.

FIGS 3-5. *Gracilaria verrucosa*: electron photomicrographs. Scale bars = 1 μm except for Fig. 5 = 0,5 μm.

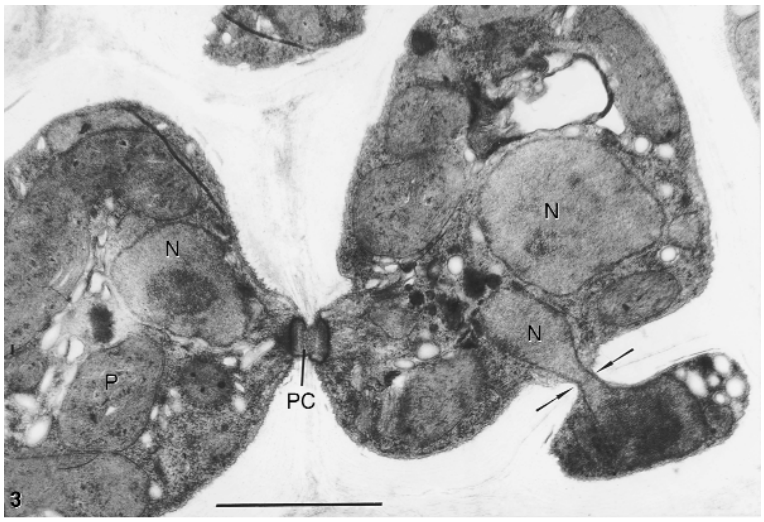


FIG. 3. Secondary pit connection formation at the time of fusion (arrows) of the small cell with the neighbouring one. A nucleus is passing through the linkup between these cells.

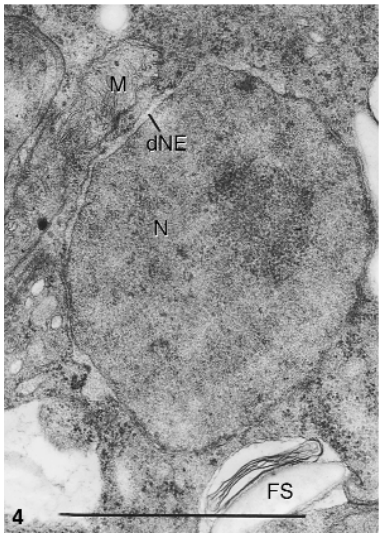


FIG. 4. Beginning of the perinuclear space dilation.

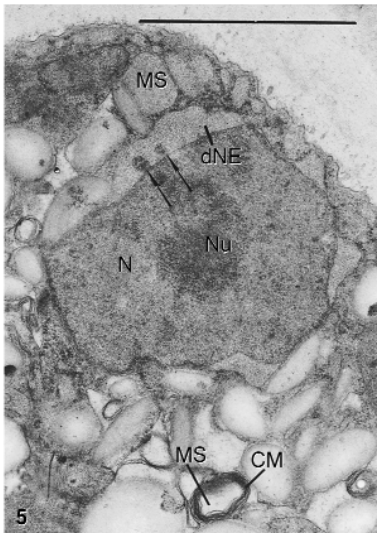
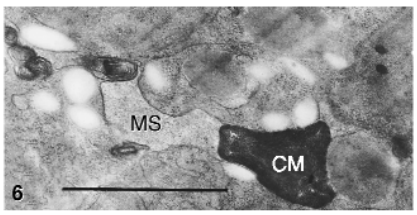


FIG. 5. Gradual increase of the perinuclear space dilation. Note the nucleoplasm protrusions (arrows).

FIGS 6-8. *Gracilaria verrucosa*: electron photomicrographs. Scale bars = 1 μm.



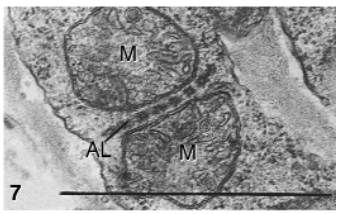


FIG. 6. Concentric membranes in the cytoplasm of a pericarp cell. Mucilage is formed within expanding areas of the membranes.
FIG. 7. Annulate lamellae between two mitochondria.

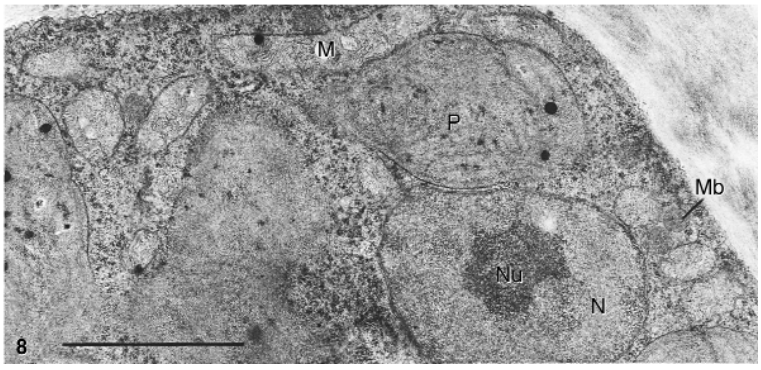


FIG. 8. Close spatial relationship between mitochondria and microbodies.

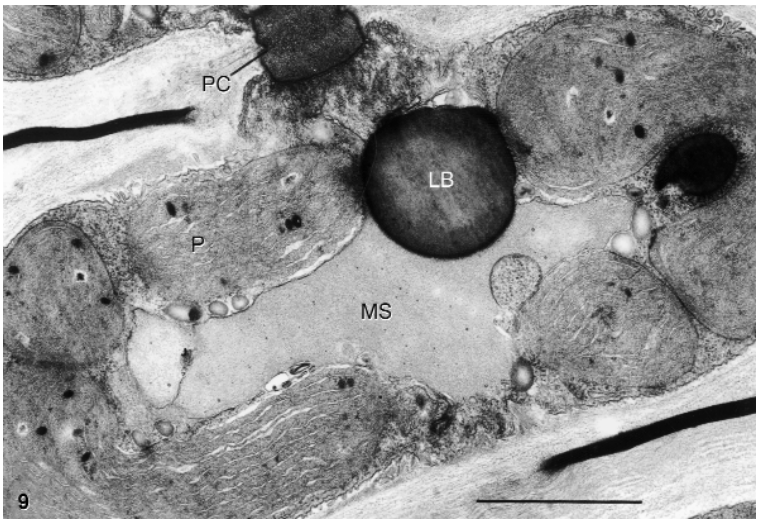
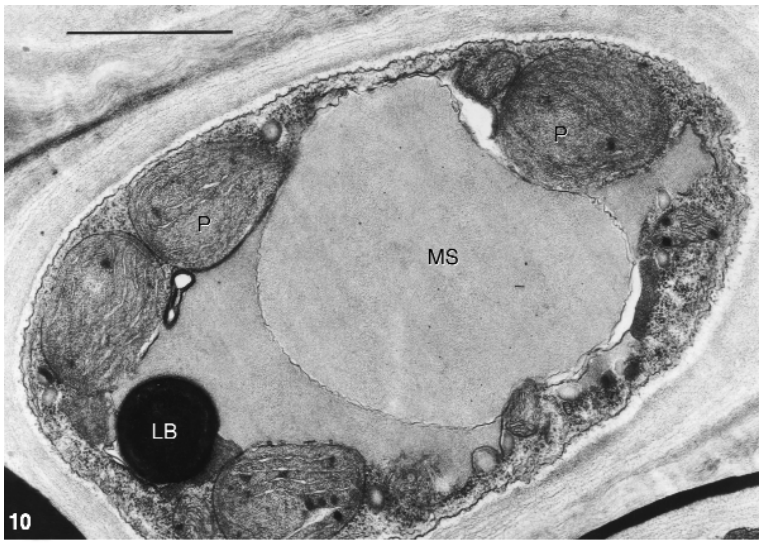


FIG 9. *Gracilaria verrucosa*: electron photomicrographs. Scale bar = 1 μm.

FIG. 9. A large mucilage sac and a large lipid body. Plastids are peripherally distributed.



FIGS 10-11. *Gracilaria verrucosa*: electron photomicrographs. Scale bars = 1 μm.

FIG. 10. Huge mucilage sacs occupying the major portion of a pericarp cell. A lipid body is also present.

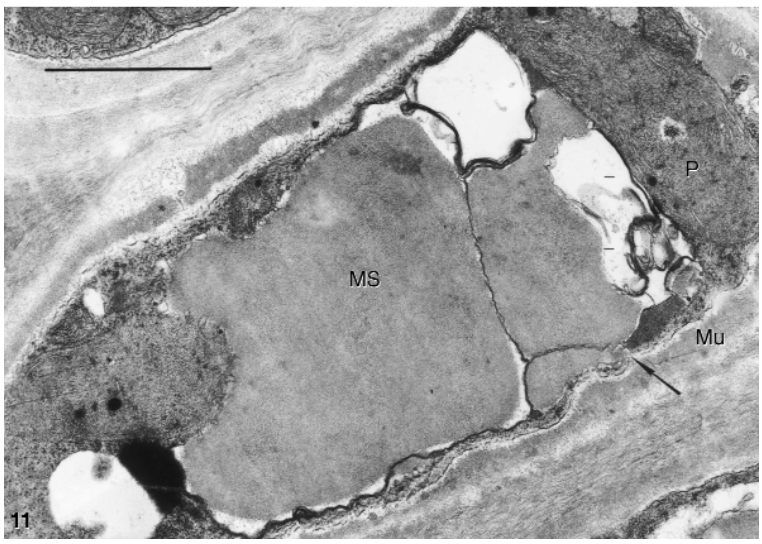


FIG. 11. An inner pericarp cell showing discharging mucilage sacs (arrow).

FIGS 12-13. *Gracilaria verrucosa*: electron photomicrographs. Scale bars = 1 μm.

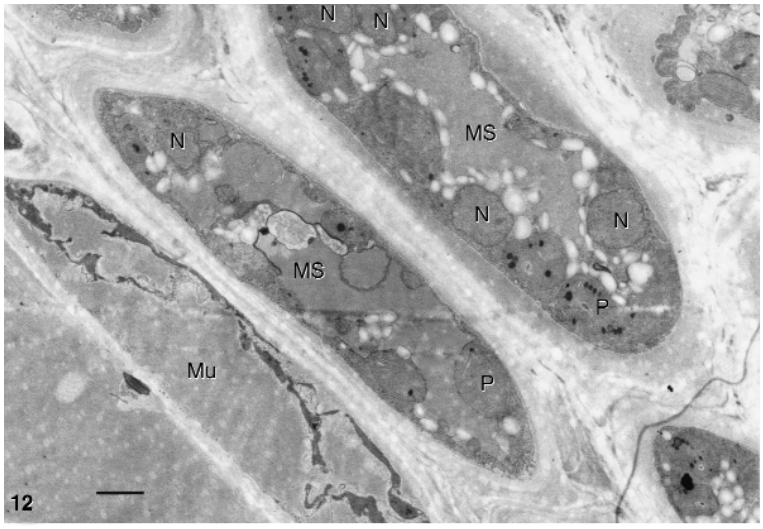


FIG. 12. Two pericarp cells and a degenerating inner pericarp cell.

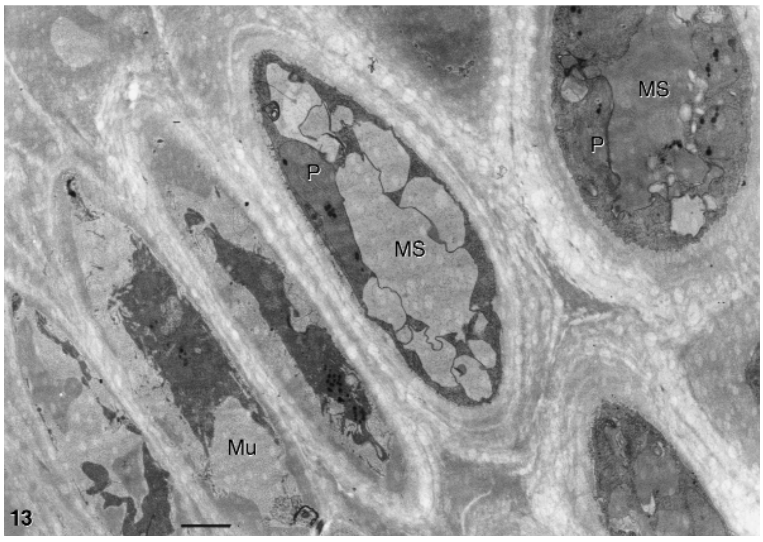


FIG. 13. A pericarp cell with many large mucilage sacs. A series of three degenerating inner pericarp cells after the release of the content of mucilage sacs.

inuclear space (Fig. 4). The gap between the two nuclear membranes gradually increases ranging up to the point of full dilation in some areas (Fig. 5). Protrusions of the nucleoplasm into the dilating perinuclear space are frequently observed (Fig. 5). The material in the perinuclear space is identical in appearance to the mucilage in the nearby occurring mucilage sacs (Fig. 5).

Cytoplasmic annulate lamellae localized between mitochondria were also observed (Fig. 7). Microbodies are frequently present in the cytoplasm of pericarp cells. An interesting feature is the close spatial relationship between mitochondria and microbodies (Fig. 8). Plastids are typical red algal chloroplasts possessing peripheral and internal unstacked thylakoids (Fig. 9). In the cytoplasm of differentiating, internal pericarp cells large osmiophilic bodies also occur (Figs 9, 10). During pericarp cell development, mucilage sacs continue to increase considerably in volume (Fig. 9). This enlargement results in the formation of one or two huge mucilage sacs occupying the major portion of the cell interior. This is the case of the inner pericarp cells lining the cystocarpic cavity (Fig. 11). The membranes of the enlarged mucilage sacs eventually fuse with the plasma membrane discharging their contents to the outside (Fig. 11). Therefore, these cells actually function as secretory cells exhibiting a degenerate appearance afterwards (Figs 12, 13). The entire carposporophyte becomes surrounded by a thick cover of mucilage produced by the inner pericarp cells. These cells are not limited by a distinct wall layer, except for the mucilage. Each cell contains a nucleus, few chloroplasts and occasionally some starch granules (Figs 12, 13).

DISCUSSION

Early after fertilization, the cortical cells near the procarp divide repeatedly and eventually form a thick protective cortical layer named pericarp. This enlarges and ensheathes the gradually swelling cystocarpic cavity and the developing carposporophyte. Pericarp cells are the least investigated structures of the cystocarp. This report is the first ultrastructural study of the pericarp development. In this work, as well as in the previous ones (Kugrens & West, 1973; Tsekos & Schnepf, 1983; Delivopoulos & Tsekos, 1983; Delivopoulos, 2003a, b) it has been demonstrated that pericarps besides being the protective barrier of the developing carposporophyte also function as mucilage suppliers for the cystocarpic cavity.

An interesting feature of the *Gracilaria* pericarp is the function of the inner cells as secretory cells producing large quantities of mucilage. This has previously been reported for *Levringiella gardneri* (Setch.) Kylin (Kugrens & West, 1973), *Gigartina teedii* (Roth) Lamour. (Tsekos & Schnepf, 1983), *Nienburgia andersoniana* (J. Ag.) Kylin (Delivopoulos, 2003a) and *Gelidium robustum* (Gardn.) Hollenb & Abbott (Delivopoulos, 2003b). In the case of *Levringiella*, numerous dictyosome vesicles supply the cystocarpic cavity with large quantities of mucilage, while in *Gigartina*, *Nienburgia* and *Gelidium* mucilage sacs have been considered responsible for the production of mucilage. In *Gracilaria,* it is clearly evident that mucilage sacs are the main source of mucilage for the cystocarpic cavity. Mucilage sacs originate from concentric membrane bodies, as meticulously has been described in *Faucheocolax* (Delivopoulos & Kugrens, 1984) and has been confirmed in a number of red algal species (for references see Pueschel, 1990).

However, *Gracilaria* is the first species where the mucilage sacs of the pericarp cells originate from the nuclear envelope. Accumulation of mucilage within the nuclear envelope space has also been reported for the gonimoblast generative cells of the same species (Delivopoulos & Tsekos, 1985). It must be pointed out that in both kinds of cells, there is a great paucity of dictyosomes and endoplasmic reticulum. Therefore, from the three components of the endomembrane system, only the nuclear envelope is present. The dilated perinuclear space in certain areas of the nuclear envelope has also been reported in differentiating spermatia of *Bangia atropurpurea* (Roth) C. Ag. (Cole & Sheath, 1980), but no specific function has been ascribed to it. Formations similar to those in *Gracilaria* have been observed in the nuclei of carpospores of *Polysiphonia novae-anglia* Taylor (Wetherbee & Wynne, 1973). However, these "fibrillar bodies" have not been found within the perinuclear space, but within outpocketings of the nucleoplasm and the nuclear envelope. In that case, Wetherbee & Wynne (1973) have suggested that the nucleus is actively involved in the synthesis of storage materials. The data of the present study and of a former one (Delivopoulos & Tsekos, 1985) in different types of cells and in a different species than *Polysiphonia* reinforce this idea. Similarly to *Gigartina teedii* (Tsekos & Schnepf, 1983) and *Nienburgia andersoniana* (Delivopoulos, 2003a), the inner pericarp cells of *Gracilaria* degenerate after massive production of mucilage. In *Levringiella gardneri,* these cells have been considered as a special group of secretory cells situated beneath the pericarp cells (Kugrens & West, 1973). It has been suggested that the tremendous amount of mucilage surrounding the red algal carposporophytes has an adhesive and/or antidesiccation function (Kugrens & West, 1973; Kugrens & Arif, 1981). Alternatively, in non-ostiolate cystocarps it has been proposed that mucilage could function as a source of nutrients for the developing carposporophyte (Delivopoulos & Kugrens, 1984). *Gracilaria* has an ostiolate pericarp. However, the presence of lipid bodies in the pericarp cells of *Gracilaria* reinforces the possibility for these cells to be playing a nutritive role, in addition to the adhesive and antidesiccation functions.

Annulate lamellae were observed in pericarp cells of *Gracilaria verrucosa* and they have also been reported to develop during carposporogenesis (Wetherbee & Wynne, 1973; Tripodi, 1974; Wetherbee *et al*., 1974; Delivopoulos & Tsekos, 1986; Kugrens & Delivopoulos, 1986). In both pericarp cells and carpospores of *Gracilaria,* annulate lamellae were found in close relationship with mitochondria. However, their role remains obscure. Plastids of *Gracilaria* pericarp cells are typical red algal chloroplasts possessing a peripheral thylakoid and internal unstacked thylakoids as well. Microbodies are among the most poorly studied organelles of the red algae. Many authors have reported microbodies in red algal cells (for references see Pueschel, 1990).

In conclusion, inner pericarp cells of *Gracilaria* actually function as secretory cells supplying the cystocarpic cavity with large quantities of mucilage.

REFERENCES

Cole K, Sheath RG, 1980. Ultrastructural changes in major organelles during spermatial differentiation in *Bangia* (Rhodophyta). *Protoplasma*, 102: 253-279.

Delivopoulos SG, 2003a. Ultrastructure of post-fertilization development in the red alga *Nienburgia andersoniana* (Delesseriaceae, Ceramiales, Rhodophyta). *Botanical journal of the linnean society*, 142: 289-299.

Delivopoulos SG, 2003b. Ultrastructure of cystocarp development in *Gelidium robustum* (Gelidiaceae : Gelidiales : Rhodophyta). *Marine biology*, 142: 659-667.

Delivopoulos SG, 2003c. Ultrastructure of auxiliary and gonimoblast cells during carposporophyte development in the red alga *Cryptopleura ruprechtiana* (Delesseriaceae, Ceramiales, Rhodophyta). *Biology of the cell*, 95: 383-392.

Delivopoulos SG, Kugrens P, 1984. Ultrastructure of carposporogenesis in the parasitic red alga *Faucheocolax attenuata* Setch. (Rhodymeniales, Rhodynemiaceae). *American journal of botany*, 71: 1245-1259.

Delivopoulos SG, Tsekos I, 1983. A light microscope study of carposporophyte development in *Gracilaria verrucosa* (Hudson) Papenfuss. *Annals of botany*, 52: 317-323.

Delivopoulos SG, Tsekos I, 1985. Nuclear envelope activity in gonimoblast generative cells of the red alga *Gracilaria verrucosa* (Hudson) Papenfuss (Gigartinales). *Flora*, 177: 309-315.

Delivopoulos SG, Tsekos I, 1986. Ultrastructure of carposporogenesis in the red alga *Gracilaria verrucosa* (Gigartinales, Gracilariaceae). *Botanica marina*, 29: 27-35.

Kim DH, 1970. Econonically important seaweeds, in Chile. I. *Gracilaria*. *Botanica marina*, 13: 140-162.

Kugrens P, West JA, 1973. The ultrastructure of carpospore differentiation in the parasitic red alga *Levringiella gardneri* (Setch) Kylin. *Phycologia*, 12, 163-173.

Kugrens P, Arif I, 1981. Light and electron microscopic studies of the fusion cell in *Asterocolax gardneri* Setch. (Rhodophyta, Ceramiales). *Journal of phycology*, 17: 215-223.

Kugrens P, Delivopoulos SG, 1986. Ultrastructure of the carposporophyte and carposporogenesis in the parasitic red alga *Plocamiocolax pulvinata* Setch. (Gigartinales, Plocamiaceae). *Journal of phycology*, 22: 8-21.

Levring T., Hoppe HA, Schmid OJ, 1969. *Marine algae. A survey of research and utilization.* Botanica Marina Handbook. Vol 1, Cramer, de Gruyter and Co, Berlin and New York.

Michanek G, 1971. *A preliminary appraisal of world seaweed resources*. Food and Agriculture Organization of the United Nations, Fisheries Circular No 128, ii + 37 pp.

Pueschel CM, 1990. Cell structure. In Cole CM, Sheath RG, eds. *Biology of the red algae:* Cambridge University Press, 7-41.

Tripodi G, 1974. Ultrastructural changes during carpospore formation in the red alga *Polysiphonia*. *Journal of submicroscopic cytology,* 6: 275-286.

Tsekos I, 1983. The ultrastructure of carposporogenesis in *Gigartina teedii* (Roth) Lamour. (Gigartinales, Rhodophyceae): Gonimoblast cells and carpospores. *Flora*, 174: 191-211.

Tsekos I, Schnepf E, 1983. The ultrastructure of carposporogenesis in *Gigartina teedii* (Roth) Lamour. (Gigartinales, Rhodophyceae): Auxiliary cell, cystocarpic plant. *Flora*, 173: 81-96

Wetherbee R, Wynne MJ, 1973. The fine structure of the nucleus and nuclear association of developing carposporangia in *Polysiphonia novae-angliae* (Rhodophyta). *Journal of phycology*, 9: 402-407.

Wetherbee R, West JA, Wynne MJ 1974. Annulate lamellae in postfertilization development in *Polysiphonia novae-anglia* (Rhodophyta). *Journal of ultrastructure research*, 49: 501-404.