

## Fine structure of the utricles and gametogenesis of *Codium decorticans* (Cauleriales, Chlorophyta)

## Ultraestructura de los utrículos y gametogénesis en *Codium decorticans* (Cauleriales, Chlorophyta)

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### ABSTRACT

*Codium decorticans* utricles exhibited a large central vacuole surrounded by a thin parietal layer of cytoplasm. Nuclei and other organelles were placed in the outermost portion of the cytoplasm whereas chloroplasts protruded into the vacuole. The utricle cell wall was formed by a single stratum covered by a rugose cuticle. Only gametangia producing a single type of gametes were observed. Progametangia consisted of an apical vacuolate portion and a basal portion with partitioned cytoplasm. The clear apical portion of immature gametangia revealed abundant endoplasmic reticulum, dictyosomes and electron translucent vacuoles. Most of the gametangium volume was occupied by spherical nuclei and small chloroplasts. Gametes were delimited by the activity of electron translucent vesicles. Mature gametes were pear-shaped with a prominent papilla occupied by the basal apparatus. An anterior nucleus was anteriorly flanked by a large mitochondrion. Spherical non-flagellate cells with a discrete cover were also observed in the gametangia. The gametes of *Codium decorticans* resemble the male gametes of other siphonous green algae, in the flagellar apparatus morphology, in the appearance of the capping plate and the structure and location of the terminal caps, and in the presence of one large mitochondrion. We can assert that the Atlantic Argentinian populations produce only male gametes. Therefore, agamic germination of male gametes would be the only asexual reproduction mechanism of the Argentinian populations. Further studies are necessary to confirm the hypothesis that these populations reproduce asexually by germination of only one type of cell, male gametes that in this case may be functionally considered zoospores.

**Key words:** *Codium decorticans*, gametogenesis, siphonous green algae, ultrastructure, utricle.

### RESUMEN

Los utrículos de *Codium decorticans* presentaron una gran vacuola central rodeada por una delgada capa de citoplasma parietal. Núcleos y otras organelas se localizaron en la porción más externa del citoplasma parietal, mientras que los cloroplastos hicieron protrusión en la vacuola. La pared del utrículo presentó un único estrato cubierto por una cutícula aserrada. Sólo se observaron gametangios produciendo un solo tipo de gametas. Los progametangios presentaron una porción apical vacuolada y una basal con el citoplasma particionado. La porción apical clara del gametangio inmaduro reveló abundante retículo endoplásmico, dictiosomas y vacuolas electrónicamente translúcidas. La mayor parte del gametangio estaba ocupado por núcleos esféricos y pequeños cloroplastos. Las gametas maduras piriformes presentaron una prominente papila. Un núcleo anterior estaba flanqueado anteriormente por una gran mitocondria. En los gametangios se observaron células esféricas aflageladas con una discreta cubierta. En general, las gametas de *Codium decorticans* se asemejan

a las gametas masculinas de otras algas verdes sifonales, como lo indican la morfología del aparato flagelar ("capping plate" y "terminal caps") y la presencia de una gran mitocondria. De acuerdo a estas observaciones, concluimos que las poblaciones atlánticas argentinas producen sólo gametas masculinas. Por lo tanto, la germinación agámica de gametas masculinas sería el único mecanismo de reproducción asexual de las poblaciones argentinas. Posteriores estudios son necesarios para confirmar la hipótesis de que estas poblaciones se reproducen asexualmente por germinación de gametas masculinas que en este caso pueden ser consideradas zoósporas.

**Palabras claves:** *Codium decortcatum*, gametogénesis, algas verdes sifonales, ultraestructura, utrículo.

## INTRODUCTION

The genus *Codium* Stackhouse is characterized by thalli composed by interwoven coenocytic filaments which form a loose and colorless medulla and an utricle palisade cortex (Borden and Stein, 1969b). Gametangia are borne laterally on utricle protuberances (Silva, 1960). The genus has been described as having a pronounced anisogamy (Borden and Stein, 1969b); however, Feldmann (1956), Dangeard and Parriaud (1956), Churchill and Moeller (1972), Rico and Pérez (1993) have indicated the presence of only one type of gametes in *Codium fragile*, described as female gametes which germinate parthenogenetically.

The available information on *Codium* species is fragmentary. Reproduction and differentiation at the optical microscope level in *Codium fragile* (Suringar) Hariot have been studied (Arasaki *et al.*, 1956; Borden and Stein, 1969a,b; Churchill and Moeller, 1972; Ramus, 1972; Prince, 1988; Park and Sohn, 1992; Rico and Pérez, 1993). Schussnig (1950) studied gametogenesis at the optical microscope level in *Codium decortcatum* (Woodward) Howe and also there are caryological studies of this species (Schussnig, 1950; Kapraun and Martin, 1987, Kapraun *et al.*, 1988). The only ultrastructural studies done in the genus consist of brief observations on chloroplasts and nuclei (Hori and Ueda, 1967; Roth and Friedmann, 1980).

The aim of this research is to study at the optical and ultrastructural level the utricle morphology and the gametogenesis process in field-collected thalli of *Codium decortcatum* populations growing along the Atlantic Argentinian coast.

## MATERIALS AND METHODS

Thalli of *Codium decortcatum* bearing gametangia in different stages of development were collected in Puerto Madryn, Province of Chubut (42° 46' S, 65° 03' W), in Las Grutas, Province of Río Negro (40° 44' S, 64° 56' W) and in Bahía San Blas, Province of Buenos Aires (40° 34' S, 62° 14' W).

Light microscopy studies were carried out with a Leitz SM Lux microscope and a Carl Zeiss Axiolab microscope with anoptical phase-contrast. For transmission electron microscopy studies, utricles and gametangia in different stages of development were fixed in 2% glutaraldehyde in 0.1M cacodylate buffer, postfixed in 1% OsO<sub>4</sub>, dehydrated in an acetone series, and embedded in Spurr's low viscosity resin (Spurr, 1969) by the flat embedding method (Reymond and Pickett-Heaps, 1983). Sections were cut with a diamond knife and stained with uranyl acetate and lead citrate. Sections were observed with a JEOL 100 CX-II electron microscope at the Centro Regional de Investigaciones Básicas y Aplicadas de Bahía Blanca (CRIBABB), Bahía Blanca, Argentina.

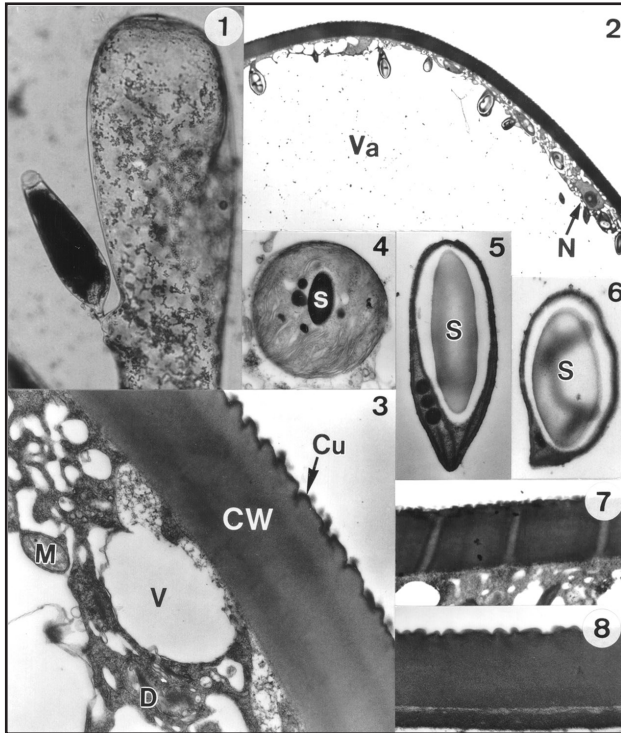
## RESULTS

### Utricle structure

*Codium decortcatum* utricles were cylindrical, 1 mm long and somewhat dilated at the apex, measuring 250 µm wide (Fig. 1). In transverse sections two zones were recognized: a large central vacuole and a circumvacuolar thin parietal layer of cytoplasm (Fig. 2). The vacuolar contents were homogeneous.

Nuclei were adjacent to the cell wall in the outermost portion of the cytoplasm (Fig. 2). In interphase, they were polymorphic with the main axis parallel to the utricle cell wall (Fig. 2). They were 4 - 6 µm long and 1.5 - 3 µm wide. They had scarce heterochromatin distributed in the nucleoplasm and one or two prominent nucleoli. Dictyosomes, endoplasmic reticulum and mitochondria were also located in this region (Fig. 3). Vesicles containing either electron translucent contents or fuzzy material were adjacent to the plasmalemma (Fig 3).

The chloroplasts placed in the innermost portion of the parietal cytoplasm protruded into the vacuole; they were fusiform and were oriented predominantly perpendicular to the utricle cell wall (Fig. 2). Chloroplasts with various features were observed in the same utricle: some of them presented stroma mainly occupied by thylakoids, small starch granules



Figures 1-8. *Codium decorticatum* vegetative structure. 1. Light micrograph of utricle with a mature gametangium. X500. 2-8. TEM micrographs. 2. Transverse section of utricle. X2000. 3. Detail of a portion of cell wall and cytoplasm. X14000. 4-6. Different chloroplast features. X14000. 4. Transverse section through a chloroplast with the stroma mainly occupied by thylakoids. 5. Longitudinal section through a chloroplast with reduced thylakoids and one large starch granule. 6. Longitudinal section through a plastid almost completely occupied by a starch granule. 7. Detail of the clear bands crossing the medial wall portion. X6700. 8. Detail of two-strata portion of wall. X14000.

and lipid globules (Fig. 4); and others presented reduced thylakoids, lipid globules and one or more oval starch granules (Fig. 5); other plastids were almost completely occupied by a large starch granule (Fig. 6).

The utricle cell wall was formed by a single 1.5 - 3.5  $\mu\text{m}$  wide stratum (Fig. 3). In the medial wall portion, there were clear bands surrounded by an electron dense sector (Fig. 7) that crossed the whole wall transversally at regular intervals. A thin rugose cuticle covered the utricle cell wall (Figs 3, 7, 8).

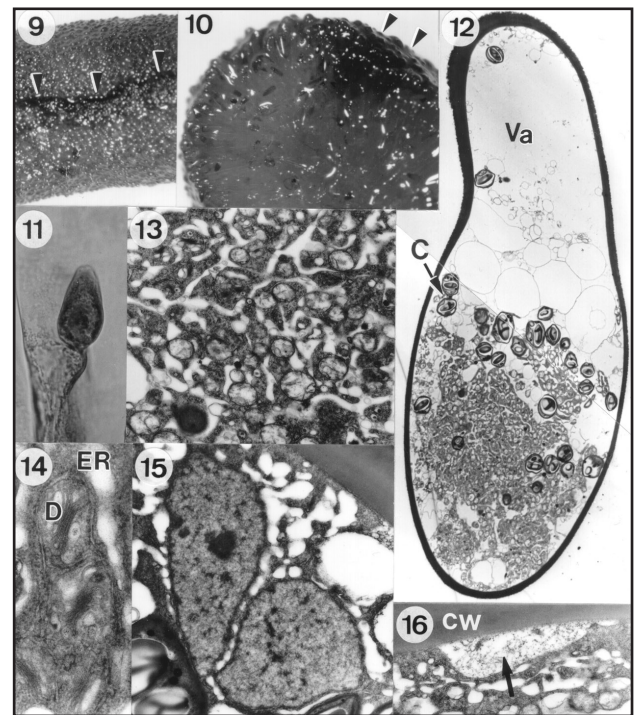
Only one type of gametangium producing one type of gametes was observed. The gametangia were located singly or in pairs on the side of each utricle on conspicuous protuberances and they were separated from the utricle by a septum (Fig.1). Near the union between the gametangium and the utricle, there was a second fibrillar 0.15 - 0.28  $\mu\text{m}$  wide stratum in

the wall (Fig. 8). Gametangia were disposed mainly in lines of utricles situated in the inner side of dichotomies (Figs 9, 10).

### Gametogenesis

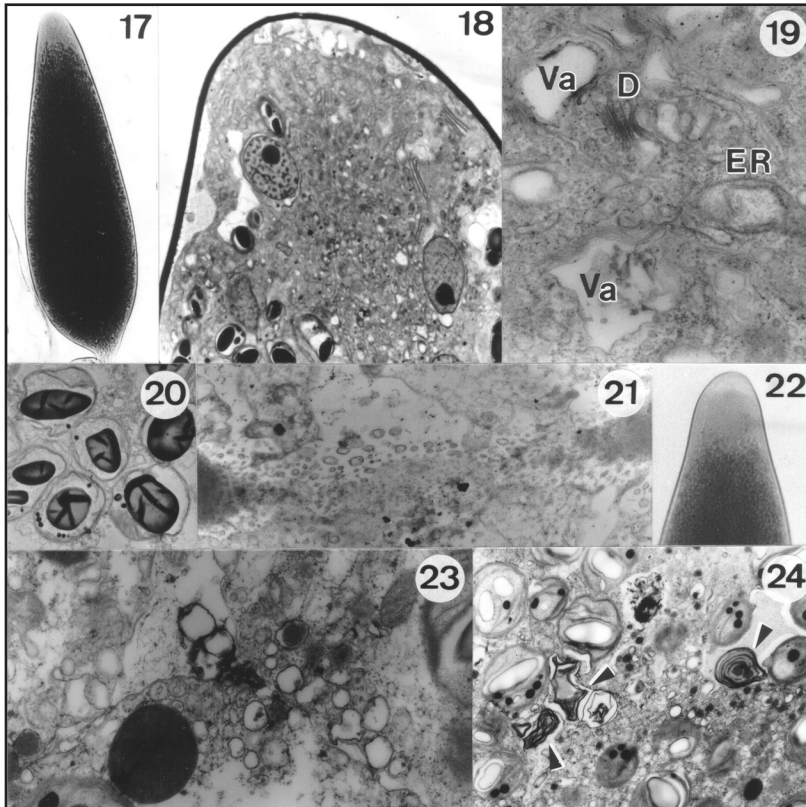
Progametangia were ovoid and pale green at the optical microscope level (Fig. 11). Their fine structure showed an apical vacuolate portion (Fig. 12) and a basal portion with the cytoplasm partitioned by a system of vesicles forming a fenestrated reticulum (Figs 12, 13). The cytoplasm contained dictyosomes with their cis face in association with rough endoplasmic reticulum (Fig. 14), recently divided nuclei (Fig. 15) and scarce small ovoid chloroplasts mainly occupied by starch granules and reduced thylakoids (Fig. 12). Fuzzy material was observed between the plasmalemma and the cell wall (Fig. 16).

Immature gametangia had a refringent apical portion and bright green homogeneous contents at optical level (Fig. 17).



Figures 9-16. *Codium decorticatum* gametogenesis. 9-11. Light micrographs. 9. General branch view with gametangia disposed in a line (arrowheads). X175. 10. Transverse section of a branch with gametangia (arrowheads). X280. 11-16. Progametangia. 11. Progametangium. X850. 12-16. TEM micrographs. 12. Longitudinal section of progametangium with the vacuolated apical portion. X2000. 13. Detail of the basal cytoplasm. X8000. 14. Detail of dictyosomes associated with reticulum endoplasmic. X2700. 15. Recently divided nuclei. X10000. 16. Detail of portion of cytoplasm showing the fuzzy material between plasmalemma and cell wall (arrow). X10000.





Figures 17-24. *Codium decorticatum* gametogenesis. Immature gametangia. 17. Light micrograph. X850. 18-21. TEM micrographs. 18. Gametangium apex. X2700. 19. Detail of a portion of gametangium. X27000. 20. Detail of small chloroplasts. X4000. 21. Detail of centripetal growth of wall separating the gametangium from the utricle. X6700. 22. Light micrograph of a gametangium showing the apical mucilage. X850. 23-24. TEM micrographs. 23. Detail of aligned electron translucent vesicles delimiting the future gametes. X14000. 24. Detail of the concentric membranes and mucilage between the gametes (arrowheads). X5000.

They were twice as long as progametangia. The fine structure of the clear apical portion (Fig. 18) revealed abundant both smooth and rough endoplasmic reticulum, dictyosomes with vesicles and electron translucent vacuoles (Fig. 19). Nuclei were spherical and exhibited scattered heterochromatin (Fig. 18). Most of the gametangium volume was occupied by numerous small chloroplasts, originating by division (Fig. 20). The gametangium wall was thinner than the utricle wall - 0.6 - 0.7  $\mu\text{m}$  wide - (Fig. 18). At this developmental stage a ring of wall started to form centripetally by vesicle congregation (Fig. 21) separating the gametangium from the utricle.

In the next stage the translucent contents increased in the apical zone (Fig. 22). Portions of cytoplasm with one nu-

cleus, many chloroplasts and the rest of organelles were delimited by electron translucent vesicles, initiating the separation of the developing gametes (Fig. 23). Concentric membranes and mucilage were found between gametes (Fig. 24).

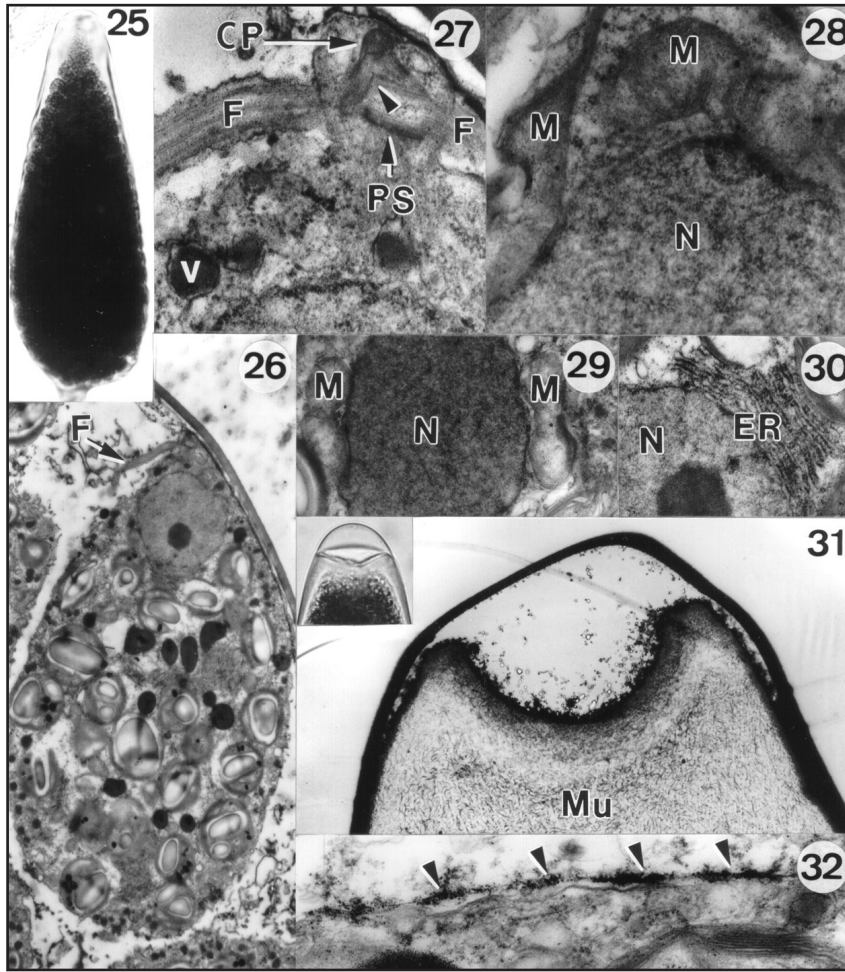
Mature gametangia were dark green and the gametes were individualized at optical level (Fig. 25). Gametes about to be discharged were pear-shaped (Fig. 26) and measured 12 - 16  $\mu\text{m}$  long and 8 - 11  $\mu\text{m}$  wide. The basal apparatus occupied a prominent papilla and the flagella emerged backwards (Fig. 27). A continuous electron dense, non-striated capping plate with an enlarged central region connected the basal bodies (Fig. 27). Terminal caps consisting of two subunits disposed orthogonally to each other were associated with each basal body; one subunit was closely appressed to the anterior surface of the basal body while the other covered its proximal end (Fig. 27). A proximal sheath with constant thickness subtended the proximal end of each basal body (Fig. 27). A spherical nucleus occupied the anterior portion of the cell (Figs 26, 28) and was flanked anteriorly by a large inverted V-shaped mitochondrion (observed in serial sections, Fig. 28) whose arms, in transverse section, had biconcave shape (Fig. 29). Several stacks of five to ten sacs of rough endoplasmic reticulum were associated with the nuclear envelope (Fig. 30). Electron dense vesicles of 0.18 - 0.37  $\mu\text{m}$  diam were observed near the nucleus and next to the plasmalemma (Fig. 27). Numerous chloroplasts were found in both the median and the posterior portions of the gametes (Fig. 26).

In the apical tip of mature gametangia, a prominent mucilage cap was formed, then it retracted from the apex forming a concavity first (Fig. 31 and inset) and eventually the exit canal when the gametes were released.

Spherical non-flagellate cells with a discrete cover (Fig. 32) were also observed in the same gametangium.

Spherical non-flagellate cells with a discrete cover (Fig. 32) were also observed in the same gametangium.

Abbreviations used in figures: C: chloroplast; Cu: cuticle; CP: capping plate; CW: cell wall; D: dictyosome; ER: endoplasmic reticulum; F: flagellum; M: mitochondrion; Mu: mucilage; N: nucleus; PS: proximal sheath; S: starch; V: vesicle; Va: vacuole.



Figures 25-32. *Codium decorticans* gametogenesis. Mature gametangia and male gametes. 25. Light micrograph. X650. 26-32. TEM micrographs. 26. Mature gamete with an anterior nucleus and many chloroplasts. X5000. 27. Detail of an apical portion of a gamete showing the prominent papilla, the capping plate, the terminal cap (arrowhead) and the proximal sheath. X27000. 28. Longitudinal section through the anterior portion of a gamete showing the large mitochondrion adjacent to the nucleus. X27000. 29. Transverse section through nucleus and mitochondrion. X10000. 30. Rough endoplasmic reticulum associated with nuclear envelope. X16000. 31. Detail of mucilage at gametangium apex. Note it is partially retracted. X2700. Inset: light micrograph. X1400. 32. Spherical aflagellate cell with discrete covering (arrowheads). X27000.

## DISCUSSION

### Utricle structure

This is the first comprehensive ultrastructural study carried out in the genus *Codium*. In general, the ultrastructural organization of *C. decorticans* utricles does not differ from

the pattern present in other siphonous algae, such as *Bryopsis hypnoides* Lamouroux, *Caulerpa prolifera* (Forsskål) Lamouroux, *Udotea petiolata* (Turra) Børgesen, *Derbesia tenuissima* (De Notaris) Crouan, *Halimeda tuna* (Ell. et Sol.) Lamouroux, *Penicillus capitatus* Lamarck (Burr and West, 1970; Sabnis, 1969; Dawes and Barilotti, 1969; Mariani Colombo, 1978; Wheeler and Page, 1974; Palandri, 1972; Turner and Friedmann, 1974).

However, there are some aspects worth mentioning with respect to the ultrastructure of the members of the order Caulerpales. The cell wall in *Codium decorticans* has a homogeneous aspect, such as that in *Penicillus capitatus* and *Udotea petiolata* (Turner and Friedmann, 1974; Mariani Colombo, 1978); while in *Bryopsis hypnoides*, *Caulerpa sertularioides* (Gmelin) Howe and *Derbesia tenuissima* it has fibrillar aspect (Burr and West, 1970; Mishra, 1969; Wheeler and Page, 1974). The number and disposition of the wall strata also vary: whereas in *Penicillus capitatus* the wall is stratified, in *Udotea petiolata* it has a single stratum, as in the *Codium decorticans* utricles for

the most part. Nevertheless, in the latter, a thin additional stratum is observed near the union of the gametangium and the utricles. Something similar occurs in *Bryopsis hypnoides*, which also has a single stratum over most of the thallus and a longitudinal wall with layers adjacent to the plug (Burr and West, 1970). The cell wall of *Derbesia tenuissima* has a thick inner layer composed of several strata and an outer electron dense layer (Wheeler and Page, 1974).

A cuticle is generally present in Caulerpales (Roth and Friedmann, 1987). In *Bryopsis hypnoides* (Burr and West, 1970) and *Udotea petiolata* (Mariani Colombo, 1978) it is rugose, as the one described here in *C. decorticans*; on the contrary, in *Derbesia tenuissima* the cuticle is smooth (Wheeler and Page, 1974).

In *Bryopsis hypnoides* two rather distinct layers can be distinguished in the mature parietal cytoplasm: an outer layer or ectoplasm next to the cell wall which contains most of the organelles and an inner layer or endoplasm where the chloroplasts are located (Burr and West, 1970). Even though we



found a similar disposition of the organelles in *Codium decorticutum*, the ecto and endoplasm differentiation was not so obvious.

Chloroplasts with numerous thylakoids and scarce starch granules, similar to those described by Hori and Ueda (1967) in *Codium fragile* and *C. repens*, were observed in *C. decorticutum*. Moreover, we also observed in the same utricle chloroplasts with reduced thylakoids and one or more oval starch granules and plastids almost completely occupied with a large starch granule. Although this variation can not be considered a heteroplasty as occurs in *Caulerpa*, *Dichotomosiphon*, *Avrainvillea*, *Chlorodesmis*, *Halimeda* and *Udotea* (Hori and Ueda, 1967; Borowitzka, 1976; Roth and Friedmann, 1987), the great variation observed in the number of thylakoids and the amount of starch in different plastids in the same utricle in *Codium decorticutum* is noteworthy.

### Gametogenesis

In 1950, Schussnig studied gametogenesis of *C. decorticutum* at optical level. The present report is the first ultrastructural study of the gametogenesis in the genus. The development of gametangia has been studied ultrastructurally only in two species of Caulerpales: *Derbesia tenuissima* and *Bryopsis hypnoides* (Wheeler and Page, 1974; Burr and West, 1970), whereas in *Caulerpa racemosa* there are studies only at the optical microscope level (Enomoto and Ohba, 1987).

The disposition of gametangia in lines of utricles situated in the inner side of dichotomies in *C. decorticutum* is described for the first time in the genus. In other species of *Codium* the gametangia are disposed mainly at random.

Gamete formation in *C. decorticutum* occurred in the cytoplasm of the basal portion of the gametangium; in *Bryopsis hypnoides*, the differentiation of gametes begins in the cytoplasm remaining in the periphery of the gametangium (Burr and West, 1970).

In *C. decorticutum* nuclear division took place in the pro-gametangia followed by chloroplast division. On the contrary, in *Bryopsis hypnoides*, the first indication of gamete formation is the simultaneous multiplication of chloroplasts and nuclei (Burr and West, 1970).

In *C. decorticutum* the portions of protoplasm that will give rise to the gametes are initially delimited by spherical electron translucent aligned vesicles. In *Bryopsis hypnoides* cleavage takes place also through vesicles but they are large, flattened and aligned around the nucleus (Burr and West, 1970). In the case of *Derbesia tenuissima* and *D. marina* pro-

toplasm cleavage occurs by proliferation of vacuoles between the organelles (Wheeler and Page, 1974).

Gamete discharge in *C. decorticutum* took place through an operculum. The gametes were released in a stream of a slimy substance as happens in other species of the genus, such as *C. fragile*, *C. tomentosum*, *C. elongatum* and *C. bursa* (Borden and Stein, 1969b). In other Caulerpales, such as *Derbesia*, *Bryopsis* and *Caulerpa*, gamete release takes place in the area under a papilla after the dissolution of the wall (Burr and West, 1970; Wheeler and Page, 1974; Enomoto and Ohba, 1987).

In general, the flagellar apparatus of the gametes of *Codium decorticutum* resembles that of male gametes of *Derbesia tenuissima* (Roberts *et al.*, 1981), in the morphology of the capping plate, the structure and location of the terminal caps and the presence of proximal sheaths. The last feature is neither described by the authors in *D. tenuissima* nor in *Pseudobryopsis* sp. (Roberts *et al.*, 1982), although electron dense proximal sheaths subtending the proximal end of the basal bodies were observed in their figures 12 and 8. Terminal caps formed by two orthogonally disposed subunits are also present in male gametes of *Bryopsis maxima* and *Pseudobryopsis* sp. (Hori, 1977; Roberts *et al.*, 1982). The last two genera also possess a capping plate similar to that of *C. decorticutum*; however, in those cases each capping plate half is distally attached by a fibrous connective (Roberts *et al.*, 1982) instead of by an electron dense material.

Just as in all male gametes studied in the Caulerpales (Burr and West, 1970; Gori, 1979; Hori, 1977; Roberts *et al.*, 1981, 1982), *C. decorticutum* gametes presented an anterior large mitochondrion and no eyespot. On the contrary, the female gametes of the Caulerpales studied, possess several small mitochondria and generally an eyespot (Hori, 1977; Roberts *et al.*, 1982).

Schussnig (1950) observed male and female gametangia in *C. decorticutum* growing in Mediterranean Sea (Naples Gulf); Kapraun and Martin (1987) presumed also sexual reproduction by anisogametes in the same species in the North Atlantic coast (North Carolina); however, we found only one type of gametangium in the South Atlantic coast. Even though Schussnig (1950) does not mention gamete measurements, the nucleus size of South Atlantic coast gametes agrees with that described by Schussnig for *C. decorticutum* male gametes. Considering also that the fine structure features of *C. decorticutum* gametes agree with those of male gametes of other siphonous green algae, we can assert that the Atlantic Argentinian populations of *C. decorticutum* produce only one type of gametes, and that these belong to the male sex.

The rounding of the cells inside the gametangium concomitant with the loss of the flagella and a synthesis of a discrete covering by the cells, observed in our study, suggests an intragametangial germination. Therefore, agamic germination of male gametes would be the only asexual reproduction mechanism of the Argentinian populations. Parthenogenetical germination of microgametes has been predicted for *C. decorticaum* by Schussnig (1950) who inferred their zoosporic potentiality, from an analysis of the gametogenesis. The gametes of *C. fragile* populations of the Atlantic coast have been described as female gametes capable of parthenogenetic development by Feldmann (1956), Dangeard and Parriaud (1956), Dangeard (1958), Churchill and Moeller (1972), Rico and Pérez (1993). Further studies are necessary to confirm the hypothesis that *Codium decorticaum* populations reproduce asexually by germination of male gametes, that in this case may be functionally considered zoospores.

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