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Ultrastructure of tetraspore germination in the agar-producing seaweed *Gelidium floridanum* (Gelidiales, Rhodophyta)

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Spore germination is a crucial step in the dispersion and establishment of algae. Here we describe for the first time the ultrastructure of the initial stages of the tetraspore germination of *Gelidium floridanum*, a species considered to be of potential economical value in Brazil. The stages of the germination process of *G. floridanum* tetraspores were studied under light and transmission electron microscopy. When released, the spores had no cell walls and were surrounded by a mucilage envelope that enabled primary attachment of the spore to the substratum. Following spore release, numerous tubular invaginations of the plasma membrane became apparent. These structures appeared to be connected to the endoplasmic reticulum and may play a role in the transport of cell wall material. Small vesicles with fibrillar content, although abundant in the tetraspores, were no longer seen in the sporelings, suggesting that these vesicles contain the precursors of the cell wall material that accumulates during sporogenesis. We observed that, soon after attachment, cellulose deposition began at one pole of the cell and gradually extended over the spore surface in a thin layer. As germination proceeded, a germ tube formed, to which most of the cytoplasmic content of the spore migrated. A cell division and the production of a cross wall separating the tube from the original spore followed. Subsequently, the germ tube elongated and underwent a series of transverse and diagonal septations, producing cells of various sizes. We can conclude that germination of tetraspores in *G. floridanum* depends on a basic process consisting of: attachment, cell wall deposition, activation of dictyosomes, mobilisation of starch accumulated during sporogenesis, polarised vacuolation, germ tube formation, and cell division.

INTRODUCTION

Spore release is the primary means of dispersion employed by red algae and provides the elements linking their life history phases. Although it is well known that the red algae have no motile stages, time-lapse videomicroscopy has shown that freshly released spores may present some kind of movement due to unknown mechanisms (Pickett-Heaps *et al.* 2001).

The spores of red seaweeds are released within a transparent mass of mucilage that is responsible for adhesion of the spores to the substratum (Avanzini 1989). Soon after adhesion to the substratum, a cell wall is produced, and germination takes place (Pueschel & Cole 1985; Ouriques & Bouzon 2003).

Both tetraspore and carpospore germination have long been known to follow well-established patterns of cell division that are characteristic of the order or family of the Rhodophyta (e.g. Thuret 1878).

Germination in the Gelidiales begins with the production of a tube-like outgrowth to which the spore content migrates. The original spore remains as an apparently empty sac attached to the growing germling. Although the sequence of Gelidiales cell division has been known since the time of Chemin (1937) and is well-documented in the literature for different species, including *Gelidium floridanum* W.R. Taylor (Oliveira Filho & Paula 1974), the subcellular organisation of Gelidiales spores and sporelings has not been described.

Here we describe for the first time the ultrastructure of the initial stages of the tetraspore germination of *G. floridanum*, a species considered to be of potential economic value in Brazil (Oliveira 1998).

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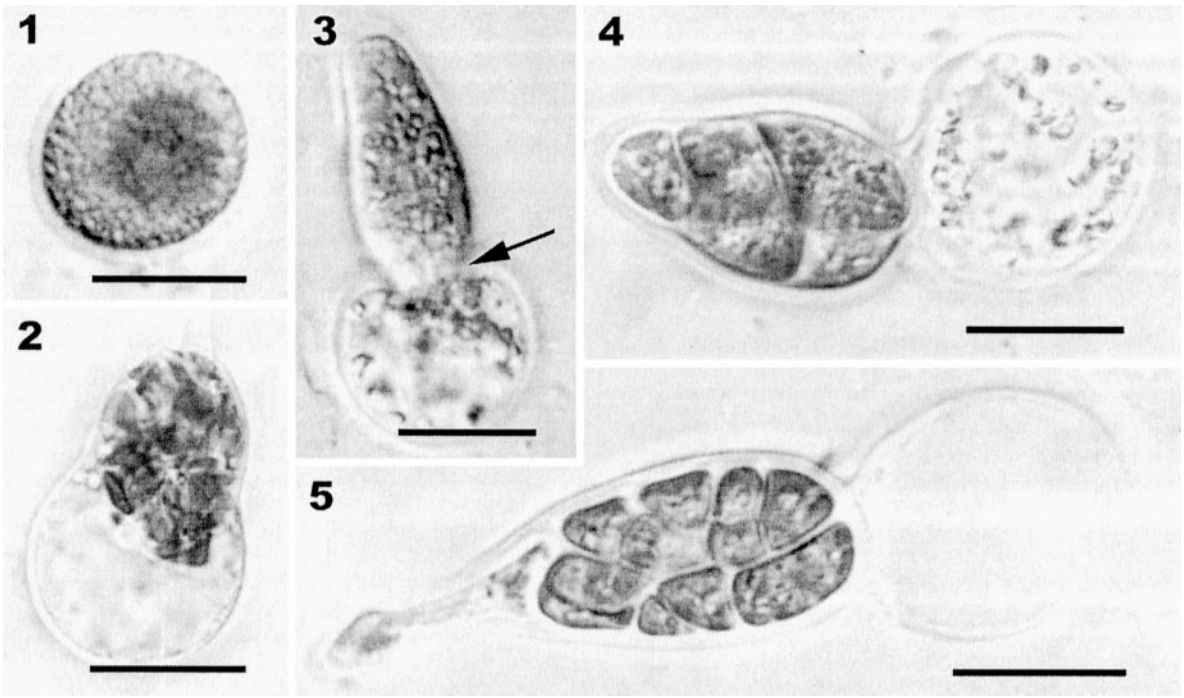
MATERIAL AND METHODS

Tetrasporophytic specimens of *G. floridanum* were collected at Sambaqui beach, Florianópolis, Santa Catarina, in southern Brazil. To release tetraspores, branchlets bearing tetrasporangia were incubated in sterile seawater in Petri dishes, with microscope slides at the bottom. The slides on which live spores had settled and germinated were observed and photographed under light microscopy. For transmission electron microscope (TEM) observation, slides to which tetraspores and sporelings had attached were fixed overnight with 2.5% glutaraldehyde (Merck, Darmstadt, Germany) in 0.1 M sodium cacodylate buffer (pH 7.2) plus 0.2 M sucrose. In order to remove excess fixative solution and unattached spores, the slides were rinsed three times in sodium cacodylate buffer. The settled spores were removed from the slides, transferred to Eppendorf vials, and centrifuged for five minutes. The resulting pellet was embedded in agar (2%, 50°C), postfixed with 1% osmium tetroxide (Sigma, Steinheim, Germany) for five hours, dehydrated in a graded acetone series, and embedded in Spurr's resin (Sigma Co., St Louis, MO, USA). Thin sections were cut and stained with aqueous uranyl acetate (EMS, Fort Washington, PA, USA) followed by lead citrate, as described by Reynolds (1963). The material was observed with a Zeiss EM 900 electron microscope (Zeiss, Oberkochen, Germany).



Figs 1–5. *Gelidium floridanum* tetraspores and tetrasporelings in different stages of development observed in differential interference contrast.
Fig. 1. Tetraspore settled but still lacking cell wall.
Fig. 2. Formation of the germ tube, showing the migration of the cytoplasm to the tube.
Fig. 3. Germ tube forming the first cross wall separating the spore and the tube (arrow).
Fig. 4. Germ tube after many cell divisions.
Fig. 5. Multicellular tetrasporeling showing the differentiation of the primary rhizoid and the differentiating erect thallus. Scale bars = 20 μm.

RESULTS

Observations under light microscopy

The free tetraspores were spherical, measuring 26–30 μm in diameter, and were dark red due to the presence of many small chloroplasts (Fig. 1). When released, the spores had no cell walls and were surrounded by a mucilaginous matrix, which was probably responsible for their rapid adhesion to the substratum. Within a few hours after settlement on the substratum, the spores began germination. Germination started by a polar disorganisation of the cell wall which allowed cell expansion and formation of the germ tube (Fig. 2). Most of the protoplasmic content of the spore migrate to this germ tube (Fig. 2). Concomitant with the protoplasmic migration, a thin cell wall synthesised around the germ tube (Fig. 3). The germ tube then elongated and underwent a series of transverse and diagonal septations, producing progressively smaller cells as the divisions proceeded (Fig. 4). The distal cell became longer and narrower and established the rhizoidal system, whereas the proximal cells, through a series of irregular divisions, became the erect thallus (Fig. 5).

Observations under TEM

The mucilaginous envelope surrounding the naked spore appeared as an amorphous layer of low electron density. Prior to cell wall synthesis, numerous chloroplasts were visible in a perinuclear position and a large quantity of floridean starch and cored vesicles were seen at the cell periphery (Fig. 6). At this stage, numerous tubular invaginations, varying in length and continuous with the plasma membrane, became conspicuous and seemed to be connected to the endoplasmic reticulum (ER) (Figs 7, 8, arrowhead). Soon after attachment, but still prior to germination, a thin cell wall was observed separating the plasma membrane and the mucilage (Fig. 9). The cell wall was composed of microfibrils within a gelatinous matrix, apparently produced by exocytosis (Fig. 10). After cell wall formation, cored vesicles and tubular membranes were no longer observed. At this stage, the chloroplasts and starch granules became randomly distributed within the spore, and an abundance of dictyosomes with enlarged cisternae appeared at the cell periphery (Figs 9, 11).

The initial stages of germination were characterised by reorganisation of the cell content, with displacement of the

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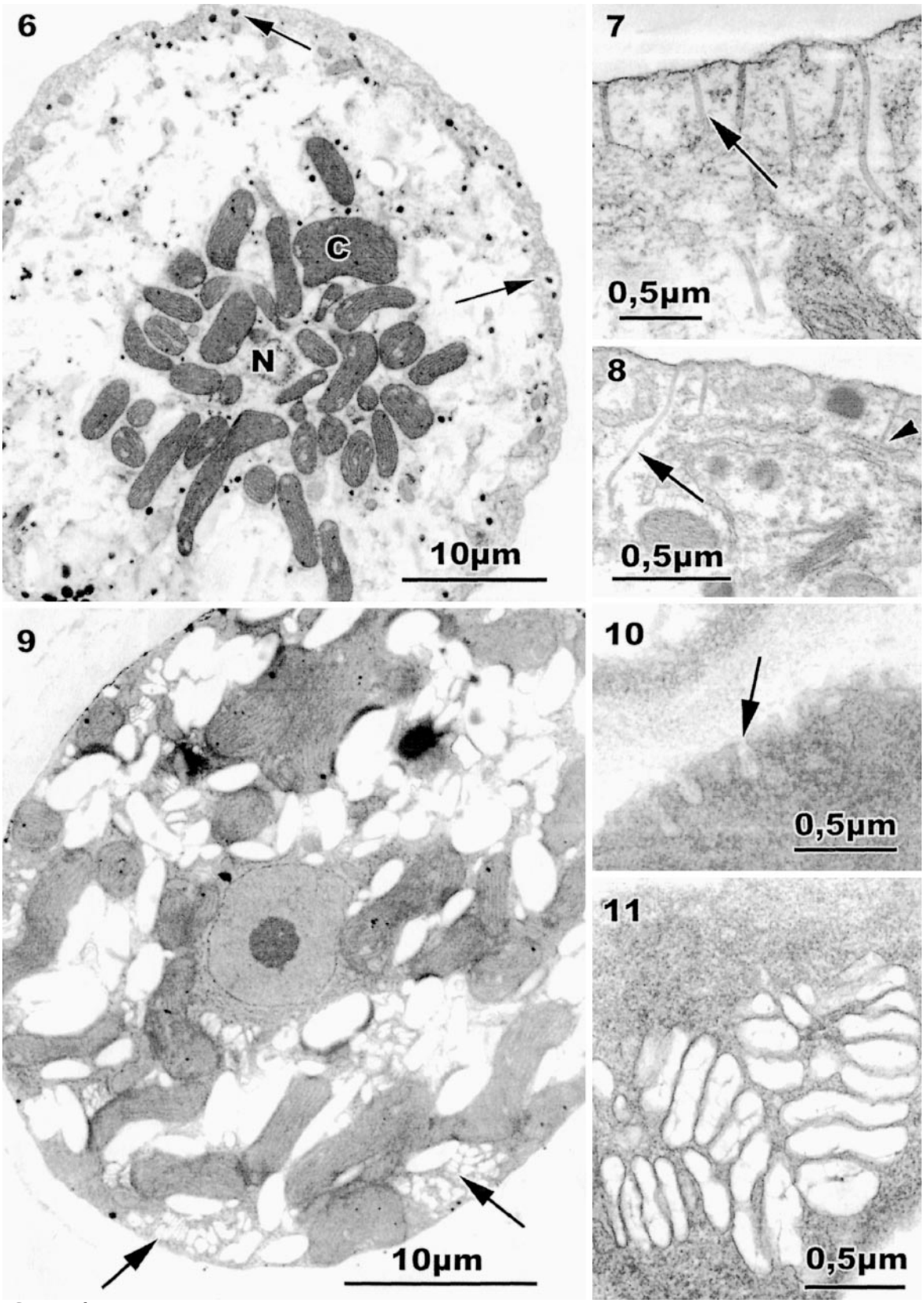
Figs 6–11. TEM micrographs of *Gelidium floridanum* tetraspores. C, chloroplast; N, nucleus.
Fig. 6. Cross section of a nongerminated tetraspore, showing organelle compartmentalisation and the absence of cell wall, with cored vesicles (arrows) and starch grains occupying the peripheral cytoplasm and the chloroplasts surrounding the nucleus.
Figs 7, 8. High magnifications of a tetraspore cytoplasm with the peripheral tubular invaginations, showing tubules of different lengths at right angles to the plasma membrane (arrows). The tubule ends appear to be closed on the ER side (Fig. 8, arrowhead).

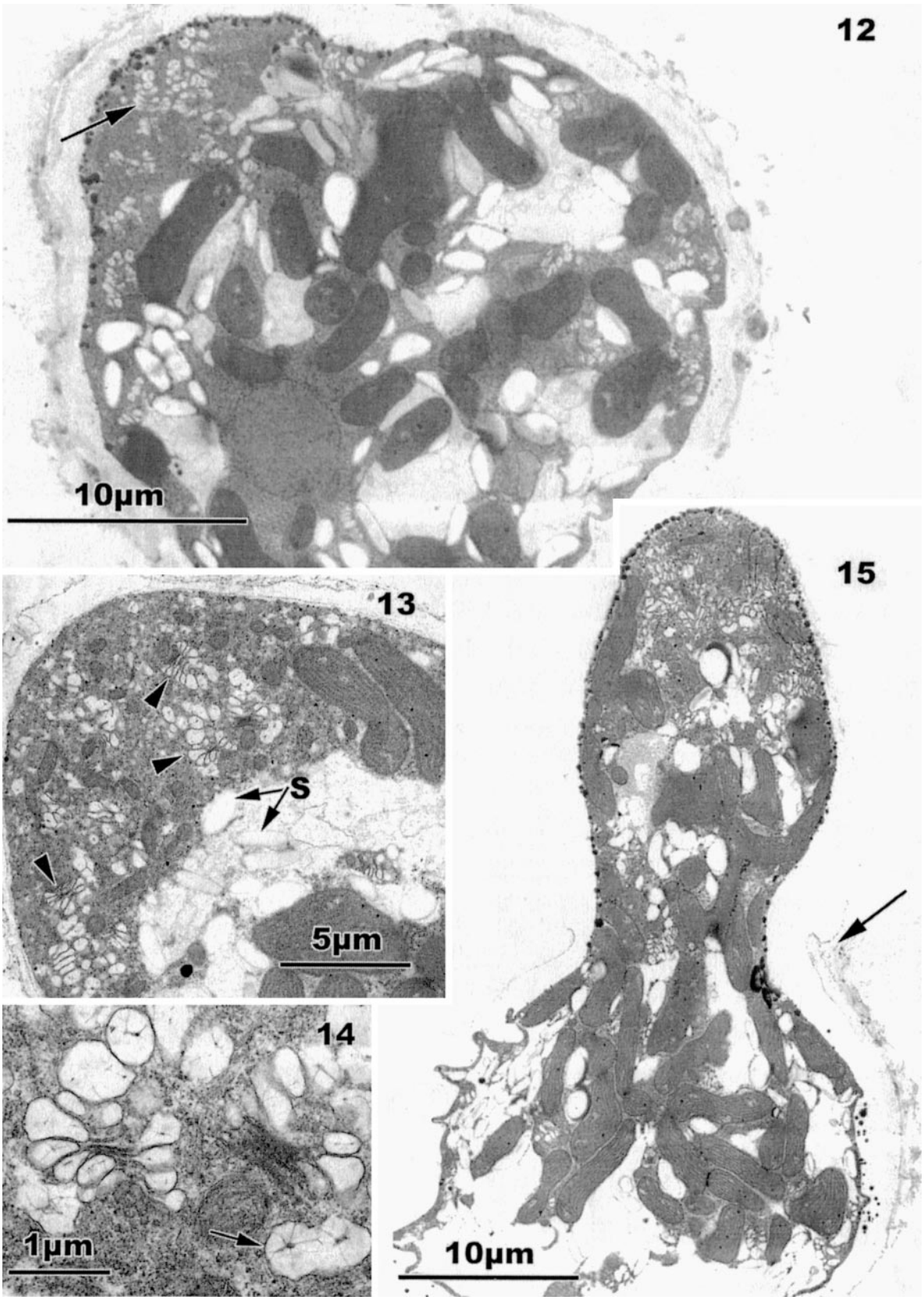


Figs 6–11. Continued.

Fig. 9. Cross section of a nongerminated tetraspore with cell wall. Polarisation is not apparent at this stage; note the large quantities of hypertrophied dictyosomes, especially at the cell periphery (arrows).

Fig. 10. Higher magnification of the cell surface, showing vesicles connected to the plasma membrane; note that a vesicle appears to be discharging its contents into the wall (arrow).

Fig. 11. Detail of hypertrophied dictyosomes showing cisterns with fibrous contents.



Figs 12–15. TEM micrographs of *Gelidium floridanum* tetrasporelings. S, starch grain.

Fig. 12. Tetraspore showing a lump in one of the poles, denoting the start of the germination process; note the concentration of dictyosomes in the lump (arrow).

Fig. 13. Detail of the germination lump showing mitochondria, dictyosomes (arrowheads), and starch grains (arrows).

Fig. 14. Higher magnification view of a dictyosome releasing vesicles with fibrous contents; note the merger of two fibrous vesicles (arrow).

Fig. 15. Tetraspore with an elongating germ tube, showing the migration of dictyosomes and chloroplasts; note that the initial cell wall covers only the spore body (arrow).

organelles towards one pole of the cell. Dictyosomes were abundant in the region where the germination tube would be formed (Figs 12–14) and hypertrophied dictyosomes and derived vesicles appeared at the tip of the germ tube (Figs 13, 14). The dictyosomes were the first organelles to migrate towards the developing tube, followed by the chloroplasts. Wall material, transported by the dictyosomes, was deposited at the tip of the germ tube. The vesicle membranes containing proteins, including those for synthesising wall fibrils, were directed to the proper membrane domain. At the same time, vacuolation began on the opposite side, gradually pushing the cell content into the developing and enlarging germ tube (Fig. 15). When nuclear division was complete, the germ tube emerged from a localised point on the cell surface. In the growing sporelings, mitotically active nuclei were seen in the cells at the tip, below the dictyosomes. The chloroplasts migrated to the interior of the germ tube before the starch grains did, although some starch grains and a nucleus usually remained in the spore body (Figs 16, 17). After most of the cell content had been pushed into the tube, a cross wall was produced, separating it from the old spore body (Figs 17, 18). At this stage, thick mucilage remained around the original spore. The tube cell wall was relatively thick and was composed of two layers: a compact external layer and a layer of microfibrils adjacent to the cell membrane (Fig. 18). Under TEM, we observed that the spore, which appeared empty under light microscopy, contained a large central hyaline vacuole and a parietal cytoplasm with a nucleus and some starch grains (Fig. 17). The spore and the germ tube were connected by a pit connection (Figs 19, 20).

We observed that the tube cell was comprised of a compact cytoplasm and an electron-dense nucleus surrounded by organelles. The chloroplasts were elongated and constituted a considerable portion of the cell volume. The recently formed tube cell first divided obliquely (Fig. 19) and then on several planes, giving rise to the erect thallus.

DISCUSSION

The release of spores without a cell wall is a common feature of the red algae (e.g. Avanzini 1989). Synthesis of the spore wall seems to be triggered by the attachment of the spore to the substratum, as has been reported for other Rhodophyta (e.g. Fletcher & Callow 1992). The germination process is then activated, and cell divisions occur, leading to the type of morphogenesis typically described for the Gelidiales. The mechanism of spore adhesion has been studied in other groups of red algae and has been associated with the production of extracellular polysaccharides and glycoproteins (Chamberlain & Evans 1973; Fletcher & Callow 1992). The participation of the extracellular mucilage and its primary role in the attachment process has also been described (Chamberlain & Evans 1973; Kugrens & West 1973; Boney 1975; Fletcher & Callow 1992). Histochemically, this mucilage is similar to the content of the fibrillar vesicles produced by the dictyosomes during sporogenesis (Chamberlain & Evans 1973). In some genera of red algae, cored vesicles produced by the dictyosomes during sporogenesis can merge with the plasma membrane (Tsekos 1983) or form large fibrillar vacuoles (Scott & Dixon 1973). However, in *G. floridanum,* extensive fusions of those vesicles are not evident and the spores are released with numerous cored vesicles, which are no longer seen in the sporelings. This suggests that these vesicles contain the precursors of the cell wall, which accumulates during the sporogenesis.

Peyrière (1969), in a study of *Griffthsia* tetraspores, Avanzini & Honsell (1984), in tetraspores of *Nitophillum punctatum* (Stackh.) Grev., and Vesk & Borowitzka (1984), in tetraspores of *Haliptilon cuvieri* (Lamouroux) Johansen & Silva, all observed tubular invaginations of the cell membrane similar to those seen in *G. floridanum* in the present study. In all cases, these invaginations were always present prior to the formation of the cell wall, as has been pointed out by Wetherbee (1978), and likely play a role in the transport of cell material. The abundant tubular membranes increase the membrane area considerably and may play other cell physiology roles, such as that of providing more efficient dissemination of the nutrients needed at the beginning of the germination process. The proximity of the internal branches of the invaginations to the ER suggests that they are involved in the transport of enzymes and cell wall compounds produced by the ER.

Each spore is prepared for the rapid succession of cell division that characterises the germination process, during which there is high consumption of the starch and considerable expenditure of energy in order to produce all the organelles necessary for the new cells within a short interkinetic time.

Although the spores have a reasonable number of chloroplasts, the photosynthesis process *per se* is probably unable to supply all of the energy needed for the development of the new thallus. This explains the abundance of starch grains accumulated during sporogenesis and their rapid mobilisation during the initial stages of germination, as previously described by Tsekos (1982) and Vesk & Borowitzka (1984).

The establishment of polarity is required for differentiation of cells and is fundamental to morphogenesis. The highly polarised organisation of *G. floridanum* sporelings is characterised by the creation of three zones: a large basal vacuole, the germ tube (containing the bulk of the cytoplasm and organelles), and the apical region, in which the dictyosomes vesicles are located.

The turgor pressure of the large vacuole is probably responsible for the distension of the germ tube. The abundance of dictyosomes at the extremity of the developing tube may represent a route of secretion and concentration of enzymes that digest the cell wall and allow the expansion of the tube cell. This assumption is derived from TEM observations, in which vesicles were occasionally observed near the plasma membrane at the germ tube tip.

In conclusion, germination of *G. floridanum* tetraspores consists of three basic processes: mobilisation of the starch accumulated during sporogenesis, polarised vacuolation, and dictyosome secretion.

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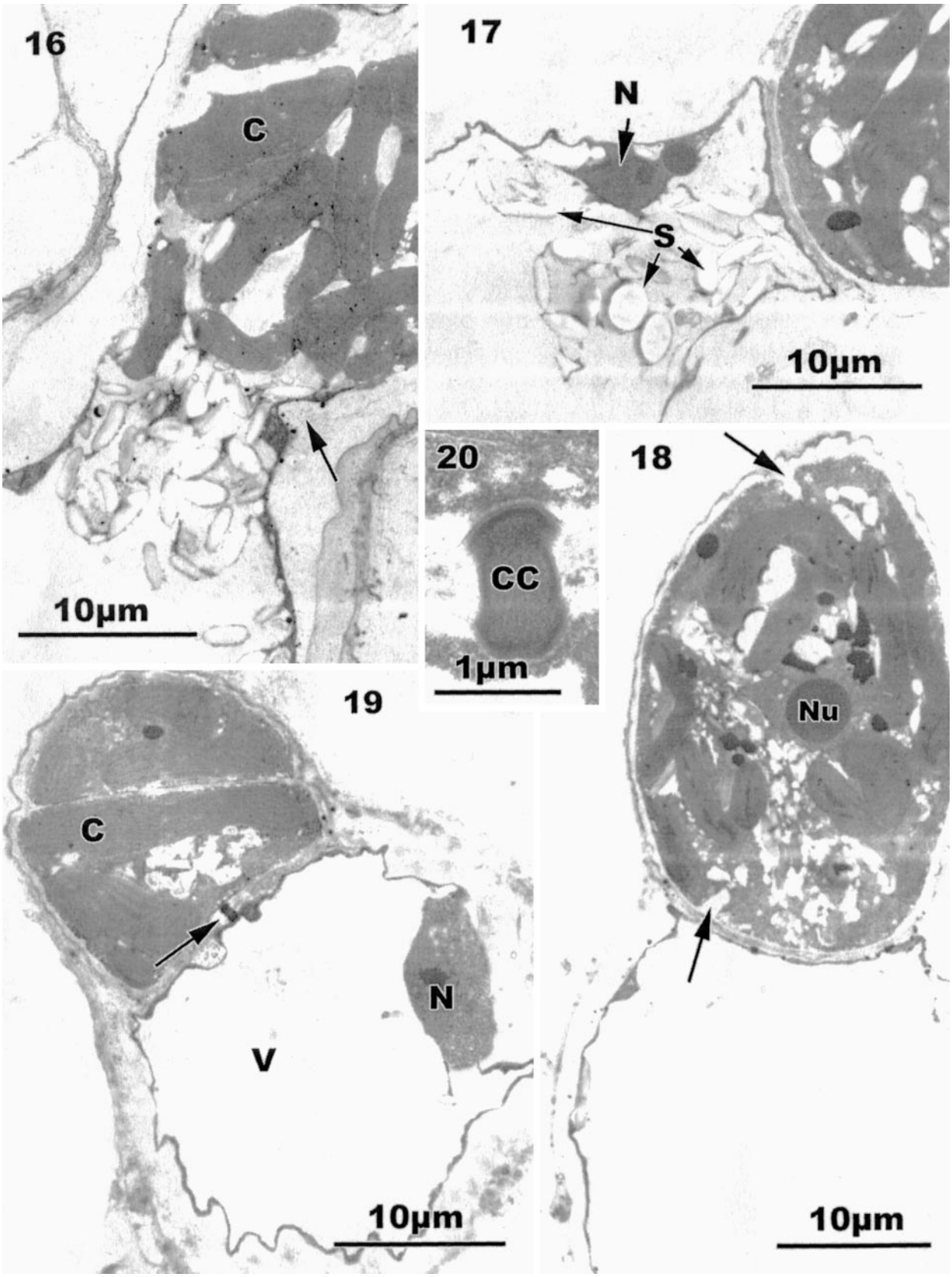

Figs 16–20. TEM micrographs of *Gelidium floridanum* tetrasporelings. C, chloroplast; CC, central core; N, nucleus; Nu, nucleolus; S, starch grain; V, vacuole.

Fig. 16. Longitudinal germ tube section, showing cytoplasmic migration and a cleavage in progress at the base of the tube (arrow).

Fig. 17. Detail of a tetrasporeling in which the cleavage was completed, showing that some starch grains and one nucleus remain in the body of the spore.

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Figs 16–20. Continued.

Fig. 18. A more advanced stage of germination, in which the body of the spore is filled by a large vacuole; note the initiation of the second cleavage in the tube cell (arrows).

Fig. 19. Germ tube composed of two cells surrounded by a thinner cell wall. Note the persistence of a pit connection (arrow).

Fig. 20. High magnification view of the pit connection between the body of the spore and the germ tube.

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