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# Ultrastructure and Taxonomy of *Sporocladopsis novae-zelandiae* (Ulvophyceae, Chlorophyta)

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The ultrastructure of vegetative and reproductive cells in *Sporocladopsis novae-zelandiae* has been studied for the first time to determine the precise taxonomic position of this genus. Vegetative cells contained one parietal chloroplast with 5–8 ulvophycean-type pyrenoids and transverse cell walls with plasmodesmata. The growth of the transverse walls was centripetal by furrow ingrowth. Ovoid zoosporangia initially had one nucleus and a large basal vacuole. Zoospore release occurred by the apical disintegration of the zoosporangial wall and expansion of an apical mucilage plug. Emptied zoosporangia could be occupied by a series of successive new zoosporangia developed from the same mother cell. Zoospores were bi- or quadriflagellate. The flagellar apparatus showed a 180 ° rotational symmetry and overlapped basal bodies with bilobed terminal caps covering their proximal ends. Our study permits us to conclude that *Sporocladopsis* is an ulvophycean genus. This conclusion is based on the occurrence of overlapping basal bodies, cytokinesis by infurrowing, and ulvophycean type of pyrenoids. Bilobed terminal caps and zoospore release by apical disintegration of the cell wall (which is accompanied by expansion of a mucilage plug) in *Sporocladopsis* suggest affinities with the order Ulvales. However, the absence of plasmodesmata and the absence of percurrent proliferation of zoosporangia in all members of the order Ulvales challenge this taxonomical position. The plasmodesmata in *Sporocladopsis* are similar to those in the Trentepohliales and could suggest a relationship with the latter, but the typical features of the Trentepohliales are absent in *Sporocladopsis* and this absence does not support an affinity between *Sporocladopsis* and the Trentepohliales. In conclusion, the fine structural characters of *Sporocladospsis* are paradoxical and it is therefore difficult to relate this genus to an order within the class Ulvophyceae. Molecular data of the genus, which are not available at present, would be necessary before making a final taxonomic decision concerning its ordinal position inside the class Ulvophyceae.

## Introduction

The epiphytic genus *Sporocladopsis* was originally described from the Red Sea on the basis of the type species, *S. erythraea* (Nasr) Papenfuss *et* Fan. by Nasr (1944). V. J. Chapman (1956) considered that the presence of sporangia on prostrate and erect filaments in *Sporocladopsis* marked a significant difference from *Pilinia* Kützing. However, Papenfuss (1962) did not recognise *Sporocladopsis* and interpreted *S. erythraea* as a species of *Pilinia*. The same criterion was adopted by Womersley (1984) who described the marine benthic flora of Southern Australia. Recently, Stegenga *et al.* (1997) resurrected *Sporocladopsis* because the type species for *Pilinia*, *P. rimosa* Kützing, was found to belong to the brown algae by Hooper *et al.* (1987) and O'Kelly (1989). Furthermore, detailed studies at the optical and electron microscopic levels have also made it possible to transfer other species of *Pilinia* to phaeophycean genera. For example, *P. maritima* (Kjellman) Rosenvinge was transferred to the genus *Kolderupia* (Lund 1959, Wilce 1966) because of the presence of phaeophycean hairs. In contrast, *Pilinia earleae* Gallagher *et* Humm (Gallagher and Humm 1980) became the new unique genus *Smithsoniella* (Chlorophyta) and was considered a transitional entity between multicellular filamentous forms of the ulvophycean complex and coenocytic green algae of the Siphonocladales complex (Sears and Brawley 1982).

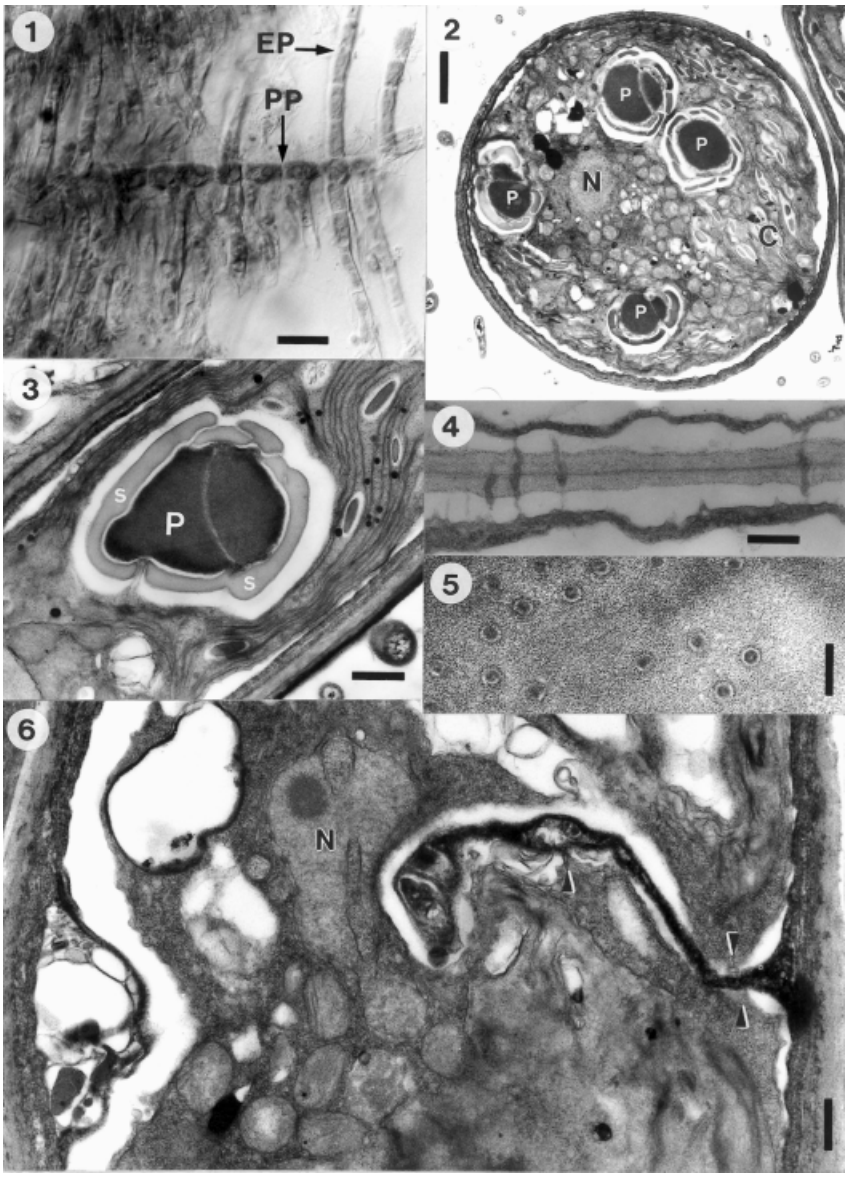
In view of the fact that there are no studies on the fine structure of the genus *Sporocladopsis*, its taxonomical status is far from being clearly determined. The main goal of this study is therefore to characterise the ultrastructure of vegetative and reproductive cells of *S. novae-zelandiae* Chapman to precisely determine the taxonomical position of the genus *Sporocladopsis*.

*Sporocladopsis novae-zelandiae* was erected by V. J. Chapman (1949) for New Zealand specimens. Filaments of *S. novae-zelandiae* grow on sori of the kelps *Lessonia nigrescens* Bory and *L. trabeculata* Villouta

*et* Santelices along the Pacific coasts of South America (Martínez and Correa 1993, Correa and Martínez 1996). The species has been studied in detail at the light microscopic level by Martínez and Correa (1993).

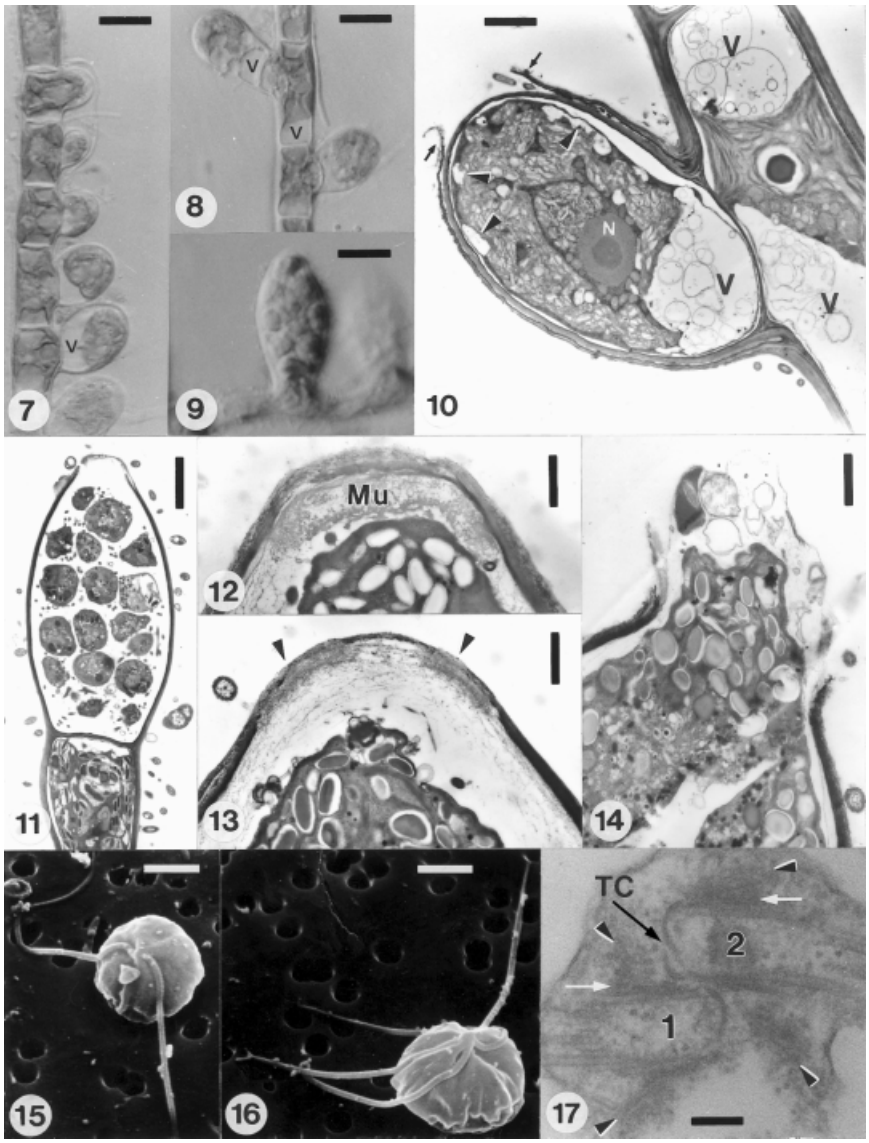
## Material and Methods

Thalli of *Sporocladopsis novae-zelandiae* from heavily infected sori of *Lessonia nigrescens* were collected at Las Cruces (33 ° 30' S, 71 ° 38' W), central Chile. Unialgal cultures were obtained according to Martínez and Correa (1993) using epiphytic filaments separated mechanically from the kelp and maintained in enriched sea water (SFC, Correa 1990) at 15 °C, 45–50 μmol $m^{-2}$ $s^{-1}$ photon flux density and a 12:12 h L:D photoperiod. Freshly collected filaments were also studied. Zoosporogenesis was induced by transferring filaments to fresh medium and by maintaining the same culture conditions. Isolates are maintained in the culture collection of the Ecology Department of the Pontificia Universidad Católica de Chile.




Figs 1–6. Vegetative filament and cell structure of *Sporocladopsis novae-zelandiae.*
Fig. 1. Anoptral phase-contrast micrograph. General view of prostrate and erect systems. Figs 2–6. TEM micrographs. Fig. 2. Transverse section through a cell from a vegetative filament showing the distribution of organelles. Fig. 3. Longitudinal section through a pyrenoid penetrated by a single modified thylakoid. Fig. 4. Detail of plasmodesmata in longitudinal section. Fig. 5. Detail of plasmodesmata in transverse section. Fig. 6. Longitudinal section through a cell where the transverse wall forms centripetally by the furrow. The arrowheads show two incipient plasmodesmata. Abbreviations: EP, erect portion; N, nucleus; P, pyrenoid; PP, prostrate portion; S, starch. Scale bars represent: Fig. 1: 25 μm; Fig. 2: 2 μm; Fig. 3: 0.7 μm, Figs 4–5: 0.4 μm; Fig. 6: 0.7 μm.

For transmission electron microscopy (TEM), filaments were fixed at 5 °C in either a) 2 % glutaraldehyde in 0.2 µm filtered culture medium and postfixed in 1 % osmium tetroxide, or b) 3 % acrolein and 5 % glutaraldehyde in 0.2 µm filtered culture medium, and postfixed in 1 % osmium tetroxide. In both cases, the material was dehydrated in an acetone series and embedded in Spurr's low-viscosity resin (Spurr 1969) using flat embedding (Reymond and Pickett-Heaps 1983). Sections were cut with a diamond knife and stained with uranyl acetate and lead citrate. Sections were examined in a JEOL 100 CX-II electron micro-




Figs 7–17. Light and electron micrographs of the development of zoosporangia and general features of zoospores in *Sporocladopsis novae-zelandiae*.
Figs 7–9 show differential interference contrast (DIC) micrographs. Fig. 7. General view of a portion of a long erect filament with unilateral zoosporangia in different stages of development. Fig. 8. Portion of a long erect filament with bilateral zoosporangia. Fig. 9. Prostrate filament with an apical zoosporangium. Figs 10–14 show TEM micrographs. Fig. 10. Longitudinal section through a zoosporangial mother cell with one nucleus, small peripheral vacuoles (arrowheads) and a large basal vacuole. The arrows show the old zoosporangial cell wall outside the newly formed zoosporangium. Fig. 11. Longitudinal section of an apical mature zoosporangium. Fig. 12. Apical portion of a zoosporangium with condensed mucilage in the upper part, forming a thick plug. Fig. 13. Apical portion of a zoosporangium showing the disintegration of the upper cell wall (arrowheads). Fig. 14. Zoospore liberation by the expansion of mucilage plug. Note that some membranous structures are present. Figs 15–16 show SEM micrographs. Fig. 15. Liberated biflagellate zoospore. Fig. 16. Liberated quadriflagellate zoospore. Fig. 17. Transverse section through the flagellar apparatus in which both basal bodies are longitudinally sectioned. The basal bodies overlap and have bilobed terminal caps. Root insertions were associated with an amorphous material at the anterior-lateral surface of basal bodies (arrows). The roots diverged initially at an angle of about 45–50 ° (arrowheads). Abbreviations: Mu, mucilage plug; N, nucleus; TC, terminal caps; V, vacuole. Scale bars represent: Figs 7–9: 10 µm; Fig. 10: 2.5 µm; Fig. 11: 4 µm; Figs 12– 14: 1 µm; Figs 15–16: 2.5 µm; Fig. 17: 0.15 µm.

scope (Jeol Ltd., Akishima, Tokyo, Japan) at the Centro Regional de Investigaciones Básicas y Aplicadas de Bahía Blanca (CRIBABB), Argentina.

Material for scanning electron microscopy (SEM) was fixed in 3% glutaraldehyde in filtered culture medium, critical-point dried, and coated with a gold-palladium mixture. Observations were carried out in a JEOL JMS-25 S-II (Jeol Ltd., Tokyo, Japan) at the Pontificia Universidad Católica de Chile, Santiago.

## Results

The fine structure of the cells of both prostrate and erect systems (Fig. 1) of *Sporocladopsis novae-zelandiae* was identical. Cells were uninucleated and the parietal chloroplast had 5–8 pyrenoids, which were penetrated by a single modified thylakoid and flanked by a variable number of starch plates (Figs 2–3). Scattered ovoidal starch granules were among the thylakoids (Fig. 3). The longitudinal walls of adult filaments usually consisted of three layers (Fig. 3). The transverse walls normally showed numerous, irregularly distributed plasmodesmata, 40 nm in diameter. (Figs 4–5). During cytokinesis the growth of the transverse walls took place centripetally by ingrowth of a cleavage furrow (Fig. 6).

Zoosporangia developed either unilaterally (Fig. 7) or bilaterally (Fig. 8) from intercallary mother cells from the longer erect filaments. Sometimes they developed apically from the cells of short erect filaments, or less frequently, they developed unilaterally from prostrate filaments (Fig. 9). Zoosporangia were initially ovoid and had one nucleus, small peripheral vacuoles and a large basal vacuole (Fig. 10). The basal vacuole was also visible at the optical level (Figs 7–8) and in vegetative cells (Figs 7, 8, 10). Eventually, each zoosporangium contained 8–32 zoospores (Fig. 11) immersed in mucilage. Apically, the mucilage formed an internal, dense thick plug (Fig. 12). Zoospore release occurred by the apical disintegration of the cell wall (Fig. 13) and the expansion of the mucilage plug. The first released zoospores were accompanied by some membranous structures (Fig. 14). A new zoosporangium developed from the same mother cell and occupied the emptied zoosporangium (Fig. 10, arrows).

Spherical-to-ovoid zoospores, both bi- (Fig. 15) and quadriflagellate (Fig. 16), were produced in a single zoosporangium. The flagellar apparatus had a 180° rotational symmetry, with overlapped basal bodies (Fig. 17). Electron dense, bilobed terminal caps covered the proximal ends of the basal bodies (Fig. 17). Root insertions were associated with an amorphous material at the anterior-lateral surface of the basal bodies (Fig. 17, arrows). The roots diverged initially from the basal bodies at an angle of about 45–50° (Fig. 17, arrowheads). Flagella emerged from an apical papilla and ran proximally into a groove formed laterally in the anterior part of the zoospore (Figs 15–16).

## Discussion

### Fine structure

The chloroplasts of vegetative cells of *Sporocladopsis novae-zelandiae* showed the typical chlorophyte thylakoidal lamellae, intraplastidial starch granules and pyrenoids that were penetrated by a single modified thylakoid and flanked by a variable number of starch plates. This type of pyrenoid has been considered typical of the class Ulvophyceae (Hoek *et al.* 1995). The heterotrichous *Smithsoniella,* a transitional entity among the Ulvophyceae (Sears and Brawley 1982), also has similar pyrenoids.

The transverse walls of *Sporocladopsis novae-zelandiae* regularly were shown to have plasmodesmata. Similar delicate plasmodesmata have also been described in the genera *Cephaleuros*, *Phycopeltis* and *Trentepohlia* (R. L. Chapman and Good 1978, Chappel *et al.* 1978, R. L. Chapman 1984), all representatives of the Trentepohliales, which, on the basis of molecular sequence evidence, are considered an ulvophycean order (R. L. Chapman *et al.* 1995). The same type of plasmodesmata has also been found in *Pilinia rimosa*, which for this reason as well as for the absence of pyrenoids and the presence of 3-thylakoidal lamellae and mitochondria with tubular cristae was transferred to the class Phaeophyceae (O'Kelly 1989). In contrast, in *Smithsoniella earleae* (Gallagher *et* Humm) Sears *et* Brawley transverse walls of adjacent cells lacked plasmodesmata but had septal plugs (Sears and Brawley 1982).

Our observations clearly show that the vegetative cytokinesis in *Sporocladopsis* occurred by a centripetal infurrowing and the synthesis of the cross wall. In the classes Ulvophyceae (Sluiman *et al.* 1983) and Zygnematophyceae (Fowke and Pickett-Heaps 1969a,b), cytokinesis appears as a furrow but without plasmodesmata. Interestingly, the only cases in which plasmodesmata are formed during the ingrowth of a centripetal furrow, as in *S. novae-zelandiae*, are registered in certain members of the class Phaeophyceae (Markey and Wilce 1975, Brawley *et al.* 1977, La Claire 1981). On the contrary, in green algae plasmodesmata are always correlated with a cell plate (R. L. Chapman and Good 1978, R. L. Chapman and Henk 1986, Hoek *et al.* 1995, Cook and Graham 1999). For that reason, the presence in *Sporocladopsis* of plasmodesmata correlated with a centripetal infurrowing would be noteworthy in green algae.

There are no thorough ultrastructural studies on the development of sporangia in related genera. Nevertheless, the preliminary observations on the early development of sporangia in *Sporocladopsis* clearly showed similar patterns to those of immature sporangia in *Smithsoniella earleae* (Sears and Brawley

1982), such as the spatial arrangement of the different organelles.

Zoospore liberation by disintegration of the apical zoosporangial wall and the expansion of a mucilage plug is a common feature in filamentous Ulvales as *Entocladia viridis* Reinke, *Acrochaete operculata* Correa *et* Nielsen, *Endophyton ramosum* Gardner (O'Kelly and Floyd 1983, Correa and Mc Lachlan 1994, Leonardi *et al.* 1997).

To our knowledge, the usual way of formation of new zoosporangia, that is in a repetitive fashion within the emptied walls of previous zoosporangia percurrent found in *Sporocladopsis*, is unique in green algae. An almost identical process takes place in the Oomycota genus *Saprolegnia* (Webster 1986, Alexopoulos *et al.* 1996). The percurrent sporangia have also been observed in the red algae *Audouinella virgatula* (Harvey) Dixon (Boney 1967, Bold and Wynne 1985). The production and release of both biflagellate and quadriflagellate zoospores from the same zoosporangium in *Sporocladopsis novae-zelandiae* were also observed in others filamentous marine green algae, such as *Acrochaete operculata* and *Endophyton ramosum* (Correa and McLachlan 1994, Leonardi *et al.* 1997).

The flagellar apparatus of *Sporocladopsis* exhibited a number of ulvophycean traits, including overlapped basal bodies and bilobed terminal caps identical to representatives of the Ulvales (Floyd and O'Kelly 1984, O'Kelly and Floyd 1984). Further comparisons with the flagellar apparatus of more related genera are not possible because fine structural studies on zooids of genera such as *Pilinia* and *Smithsoniella* have not been carried out so far. Nevertheless, the general fine structure of the basal apparatus of *Sporocladopsis* strongly resembles that of swarmers of other ulvophycean algae such as *Entocladia viridis, Enteromorpha flexuosa* (Wulfen) Agardh and *Endophyton ramosum* (O'Kelly and Floyd 1983, Leonardi and Cáceres 1991, Leonardi *et al.* 1997). Moreover, the anterior grooves along which the flagella run is a characteristic present in zoospores of *Enteromorpha flexuosa* (Leonardi and Cáceres 1991).

### Taxonomic considerations

In view of its heterotrichous thalli and its sporangia clearly different from vegetative cells, *Sporocladopsis* is a genus related to the chlorophycean family Chroolepidaceae (V. J. Chapman 1949, 1956, Stegenga *et al.* 1997). Nevertheless, at ultrastructural level only the presence of plasmodesmata in *Sporocladopsis* is a trait that could in fact favour the idea of a relationship of this genus with Trentepohliales (R. L. Chapman and Good 1978, Thomson and Wujek 1997). However, our results clearly lead us to conclude that *Sporocladopsis* is not related to the Trentepohliales. The following conspicuous features present in the flagellar apparatus of Trentepohliales but absent in *Sporocladopsis* support our conclusion: compressed basal apparatus, no terminal caps, microtubular roots adpressed to the basal bodies, multilayered structures and flattened keeled flagella. Furthermore, no infurrowing but the formation of a cell plate in the vegetative cytokinetic process, as well as the absence of pyrenoids in Trentepohliales do not support the idea of affinities between this order and *Sporocladopsis*.

Our study has made it clear that *Sporocladopsis* is an ulvophycean genus. This conclusion is based on the occurrence of overlapping basal bodies, cytokinesis by furrowing and ulvophycean type of pyrenoids. Within the class Ulvophyceae (O'Kelly and Floyd 1984), the bilobed terminal caps and zoospore release generated by apical disintegration of the cell wall, which is accompanied by expansion of a mucilage plug in *Sporocladopsis*, suggest affinities with the order Ulvales. However the absence of plasmodesmata and the absence of percurrent zoosporangia in all members of the order Ulvales challenge this taxonomical position.

In conclusion, the fine structural characters of *Sporocladopsis* are paradoxical and it is therefore difficult to relate this genus to an order within the class Ulvophyceae. Molecular data for the genus which are not available at present would be necessary before making a final taxonomic decision concerning its ordinal position within the class Ulvophyceae.

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