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Ultrastructural observations on five pioneer soil algae from ice denuded areas (King George Island, West Antarctica)

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Abstract: Morphological observations were made using transmission electron microscopy on five species of green soil algae, including *Chlorosarcinopsis* cf. *gelatinosa* Chantanachat & Bold, *Muriella decolor* Visher, Tetracystis aeria Brown & Bold, *Tetracystis pampae* Brown & Bold, and *Stichococcus bacillaris* Nägeli. With an exception of the latter species, they are all new records in Antarctica. These species were the important pioneers in the colonization process of the areas recently denuded of ice. Collection of *Muriella decolor* was the first record from a soil habitat.

key words: algae, Antarctica, soil, taxonomy, ultrastructure

Introduction

Strong evidence has indicated that the climate of the maritime Antarctic has changed appreciably during the 20th century. These rapid changes in regional climate provide a unique opportunity to assess the influences of climate change on the processes of colonization and succession in the areas where ice has vanished in the last few decades (Olech, 1996).

The soil algae constitute one of the major pioneer plants populating deglaciated areas, and possibly play a significant role in preparing the substratum for the colonization and succession of other organisms. The importance of the biodiversity and abundance of terrestrial algae is apparent.

According to Pankow *et al.* (1991) and Broady (1996), it has been estimated that out of 700 taxa of non-marine algae, so far known from Antarctica, about 200 taxa occur in terrestrial habitats. However, the knowledge of terrestrial algae is still far from being complete and the majority of studies concerns the continental Antarctica (Akiyama, 1968; Akiyama *et al.*, 1986; Broady, 1981, 1987, 1989). However, in the maritime Antarctica, fewer studies have been conducted (Broady, 1976, 1979a, b; Longton and Holdgate, 1979; Klaveness and Rueness, 1986; Massalski *et al.*, 1994a, b, 1995, 1999a, b).

The soil algal colonies were conspicuous in areas previously occupied by penguin colonies and seal harem. The soil algae were in most cases very difficult to identify, thus we performed detailed analysis of the ultrastructural features of such organelles as

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pyrenoids and chloroplasts.

The purpose of this study was to conduct careful morphological observations, using transmission electron microscopy, on five soil algal taxa that were the most frequently recorded algae originating from the contemporary glacier moraines. They were presumably characteristic to the soil biotope of the region denuded of ice.

Materials and methods

Field studies were conducted in the years: 1991–1993, 1995–1996 during the XVIth and XXth Polish Antarctic Expeditions organized by the Polish Academy of Sciences to Henryk Arctowski Station.

Investigations encompassed selected terrestrial biotopes from the contemporary moraines of various ages of Ecology glacier on King George Island, South Shetland Islands, West Antarctica. Soil samples were collected in sterile polyethylene bags, stored at 10°C and transported to Poland. In the laboratory, samples were cultured on the agar plates enriched with Bristol's medium, at 20°C under a 8/16 h light/dark cycle at $3000\,\mu Em^{-2}s^{-1}$ provided by 40 W cool fluorescent tubes. The selected materials were fixed and processed for transmission electron microscopy (TEM) as previously described (Massalski *et al.*, 1995). Observations and photographs were made with a TESLA BS 500 transmission electron microscope.

Identification was based on Brown and Bold (1964), Ettl and Gärtner (1995), and Van den Hoek *et al.* (1993).

Observations and discussion

The present studies concerned the green soil algal species: *Chlorosarcinopsis* cf. *gelatinosa, Muriella decolor, Tetracystis aeria, T. pampae* and *Stichococcus bacillaris*. These species belong to the Division Chlorophyta. All taxa, except *S. bacillaris* were recorded from Antarctica for the first time.

Class: Chlamydophyceae
Order: Chlorococcales
Family: Chlorococcaceae
1. ***Tetracystis aeria*** Brown & Bold (Figs. 1–5)
Phycological Studies V, The Univ. Texas Publ. **6417**: 13, Figs. 13–18, 89–92 (1964).

Cells were single, slightly ellipsoidal or spherical measuring 4.5–6 (14.5) μm in diameter. The cell wall was thin in both young and mature cells. The chloroplast was massive, distended in the middle, and it contained a single embedded pyrenoid, which was spherical or ellipsoidal (Fig. 1). The pyrenoid was surrounded by numerous, loosely associated starch grains, and there were a few oil droplets elswhere in the chloroplast. The thylakoids (or tubular extensions of thylakoids) penetrating the pyrenoid had a sinusoidal appearance, and they also seemed to be continuous with the chloroplast thylakoids (Fig. 2). The nucleus, with a relatively large nucleolus, was pressed against the cell wall facing the pyrenoid (Fig. 1). Asexual reproduction was by cell division into tetrads or octads of vegetative cells, the walls of which were closely associated with the parent cell wall (Fig. 3). The progeny vegetative cells within tetrads or octads gradually became more spherical, and they either remained or were released from the parent cell (Fig. 4). The large cells contained numerous starch grains within the chloroplast and had the potential to become a reproductive cell (Fig. 5). In this study asexual reproduction by zoospores and aplanospores was not observed.

The cells in our material were smaller than those originally described by Brown and Bold (1964) from Pampa Texas (USA). *T. aeria* was also found in Japanese soils (Nakano, 1983).

2. ***Tetracystis pampae*** Brown & Bold (Figs. 6–11)

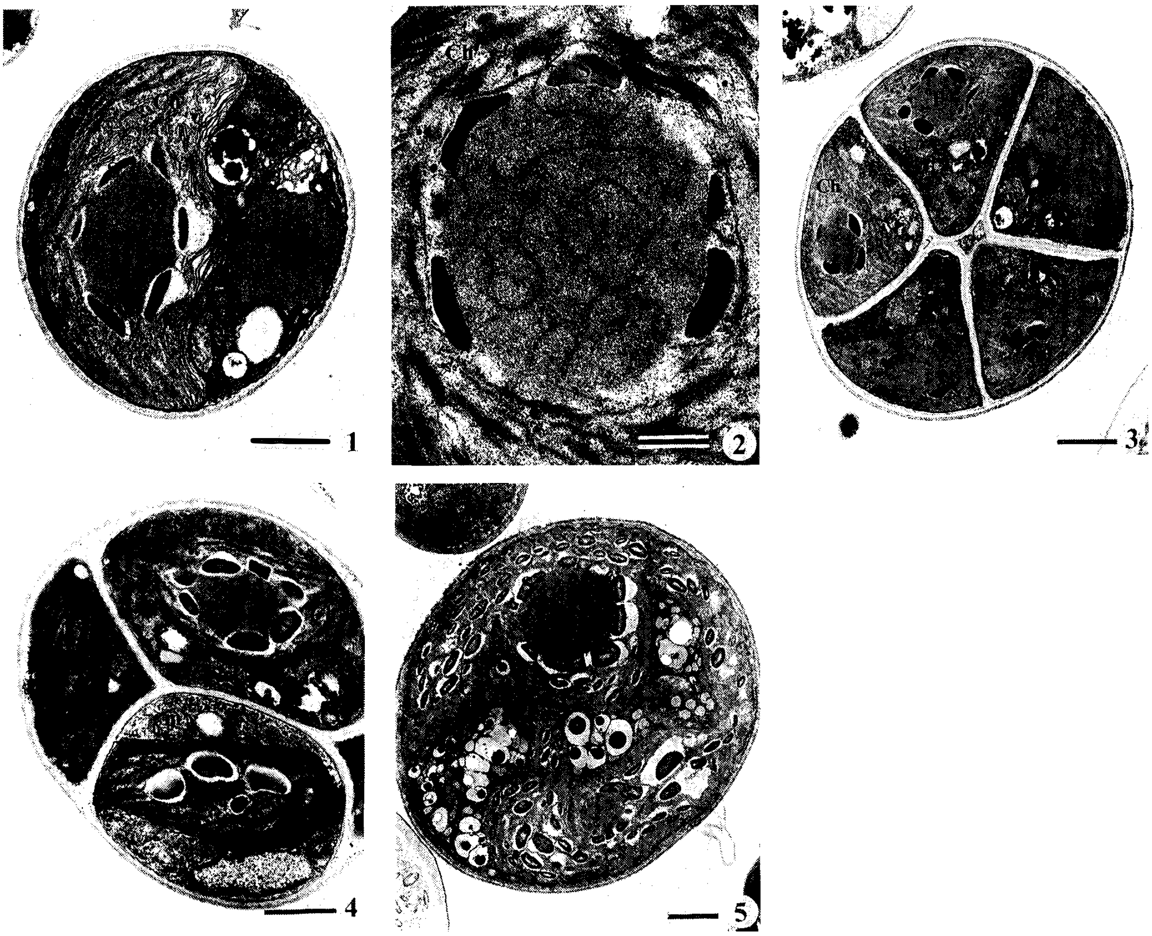
Phycological Studies V, The Univ. Texas Publ. **6417**: 23, Figs. 57–64, 105–106 (1964).

Young cells were elliptical (Fig. 6), and mature cells became spherical measuring 9–11 μm in diameter (Fig. 7). Both young and mature cells had a medium thick cell wall (Figs. 6–7). The chloroplast was single with radial asteroidal extensions containing a number of elliptical starch grains, and broad peripheral invaginations (Fig. 7). The chloroplast lamellae were usually composed of two or three thylakoids, although some of them contained four, five or rarely six thylakoids. Mitochondrial profiles were small, and always parietal. In spherical cells they were situated in the peripheral invaginations of the chloroplast (Fig. 7). In the young elliptical cells, the mitochondrial profiles were narrow and strongly elongated (Fig. 6). The pyrenoid in the young elliptical cells was large, more or less spherical, and it became broadly elliptical to irregularly elliptical in the maturing cells. The pyrenoid was situated in the massive part of chloroplast. The pyrenoid was surrounded by a single starch sheath which appeared to be continuous in most cells, apart from the lamellar discs entry (Fig. 8). Single lamellar discs or flattened tubules penetrated the pyrenoid matrix in a radial fashion, thus in any given section they can be seen sectioned both longitudinally and transversely (Fig. 8). The diameter of these tubular structures appeared to be variable, and not always corresponding to the thickness of chloroplast thylakoids. Several Golgi bodies were situated in the vicinity of nucleus (Fig. 9). The nucleus had a large, strongly electron dense nucleolus (Fig. 7). In spherical cells a number of membrane bound vesicles containing an amorphous electron dense material were observed (Figs. 7, 9). Their function has not been ascertained. Asexual reproduction was by zoospores. Zoosporangia were surrounded by a thick cell wall (Fig. 10). Cylindrical zoospores had a parietal chloroplast in which the pyrenoid was already developed (Figs. 10–11).

Both young ellipsoidal and mature vegetative cells in our material were smaller than those of the type material (Brown and Bold, 1964). The pyrenoid (our material) was penetrated by single lamellar discs or tubules, whereas in the material analyzed by Brown and Bold (1964) there was a triple-disc system penetrating the pyrenoid, showing continuity with the chloroplast lamellar system. Brown and Bold (1964) pointed out that *T. pampae* had the chloroplast lamellae always composed of three thylakoids, whereas in our material the number of thylakoids was variable, ranging from two to six. Nakano (1983) studying soils in Japan also described *T. pampae.*

Figs. 1–23. Transmission electron micrographs.

Abbreviations used in figures: Ch = chloroplast; F = electron dense fibrils; G = Golgi body; m = mitochondrion; N = nucleus; Nc = nucleolus; P = pyrenoid; S = starch sheath (grains) surrounding pyrenoid; s = starch grains in the chloroplast; V = vacuole.



Tetracystis aeria (Figs. 1–5).

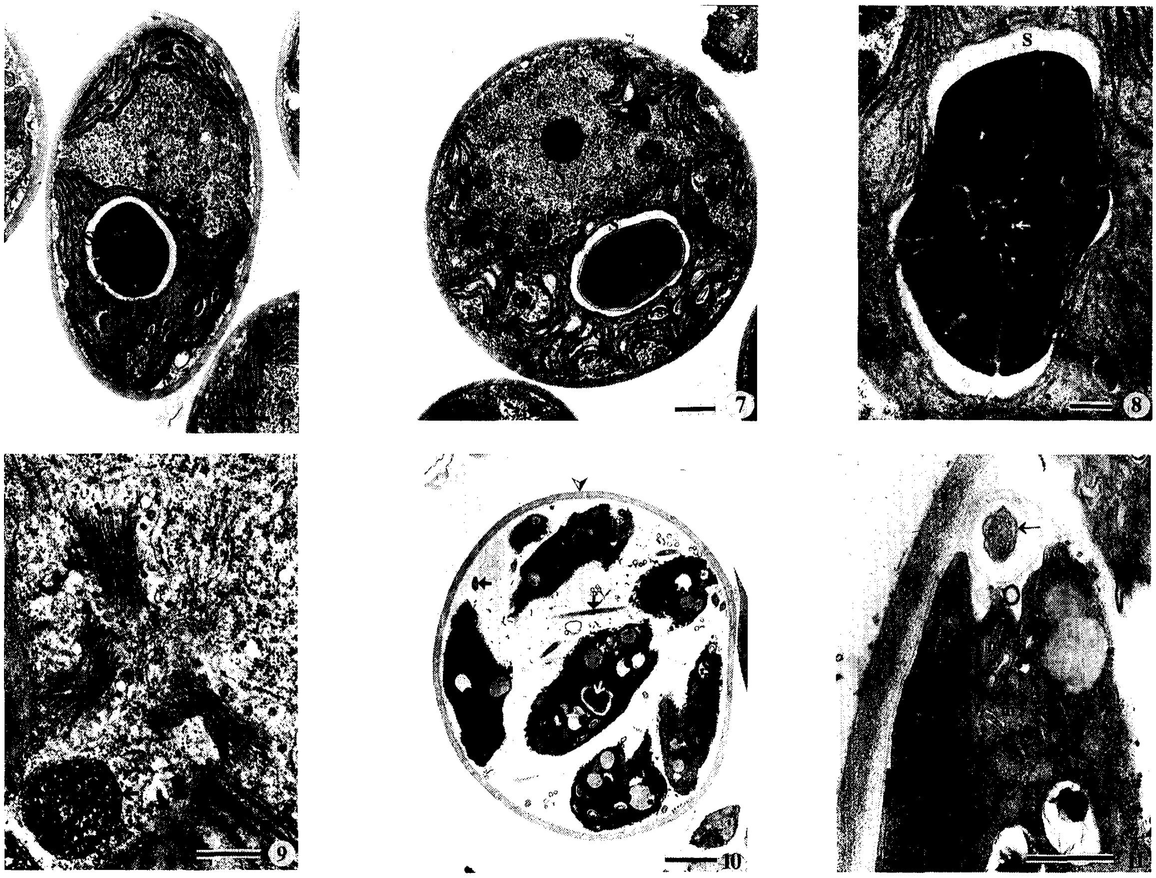
Fig. 1. Vegetative cell. Large pyrenoid penetrated by a single thylakoid or its tubular extension (arrow). Oil droplet (arrowhead). Scale bar = 1 μm.

Fig. 2. Cross section of pyrenoid (P) at higher magnification. Note sinusoidal appearance of thylakoid penetrating pyrenoid (thick arrow), and a connection point of this thylakoid with the chloroplast thylakoids (thin arrow). Scale bar = 0.5 μm.

Fig. 3. Octad of progeny vegetative cells. Only five cells shown in the plane of section. Note the triangular appearance of cells. Scale bar = 1 μm.

Fig. 4. Part of tetrad in which the progeny vegetative cells are gradually becoming more spherical. Note fully developed pyrenoids and conspicuous starch grains surrounding the pyrenoid. Scale bar = 1 μm.

Fig. 5. Large vegetative cell with very numerous starch grains in the chloroplasts. Scale bar = 2 μm.



Tetracystis pampae (Figs. 6–11)

Fig. 6. Young, ellipsoidal vegetative cell. Note a thick cell wall and parietal, elongated mitochondria. Scale bar = 1 μm.

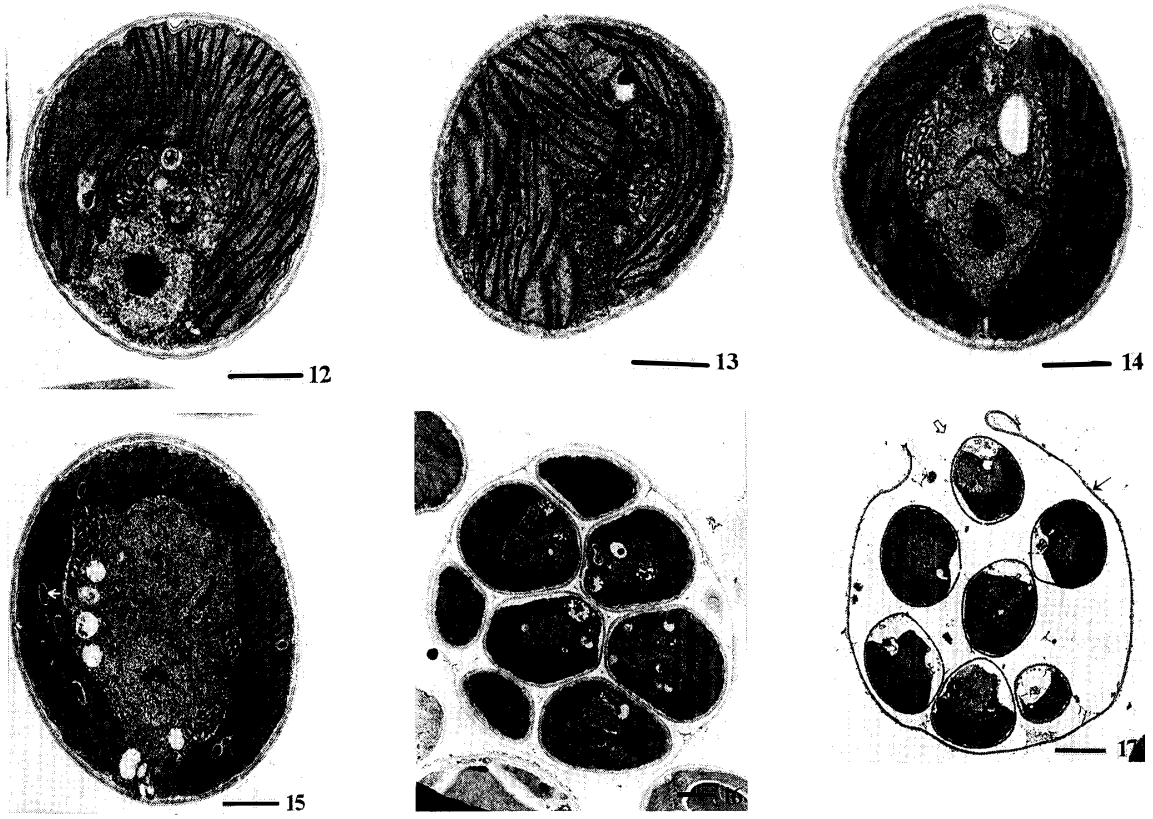
Fig. 7. Spherical vegetative cell. Note a strongly electron dense nucleolus (thin arrow), vesicles filled with an amorphous material (thick arrow), and the peripheral invaginations of chloroplast (arrowheads).

Fig. 8. Cross section of pyrenoid (P) at higher magnification. Note the entry point of single lamellar disc (thin arrow), the cross section of disc (white arrow), and the longitudinal section of disc (thick arrow). Oil droplet (black and white arrow). Scale bar = 0.5 μm.

Fig. 9. Part of vegetative cell at higher magnification showing three Golgi bodies (G), rough endoplasmic reticulum with numerous ribosomes (thin arrow), and membrane bound vesicle filled with amorphous electron dense material (thick arrow). Scale bar = 0.5 μm.

Fig. 10. Zoosporangium containing 8 zoospores. Only 7 zoospores shown in the section plane. Note a thick zoosporangium cell wall (black and white arrowhead), cross and longitudinal sections of flagella (arrows), and the pyrenoid in the longitudinal section of zoospore (white arrow). Scale bar = 2 μm.

Fig. 11. Anterior part of zoospore still enclosed by the zoosporangium cell wall (cw). Cross section through one of flagella (arrow) showing typical 9+2 arrangement of flagellar microtubules. Scale bar = 0.5 μm.



Muriella decolor (Figs. 12–17).

Fig. 12. Vegetative cell showing one cup shaped chloroplast with oil droplets (arrow). Scale bar = 1 μm.

Fig. 13. Vegetative cell displaying a branched chloroplast, and oil droplets (arrow). Note large mitochondria (m). Scale bar = 1 μm.

Fig. 14. Another vegetative cell with two parietal chloroplasts, showing all the major cell organelles. Oil droplet in the chloroplast (arrow). Scale bar = 1 μm.

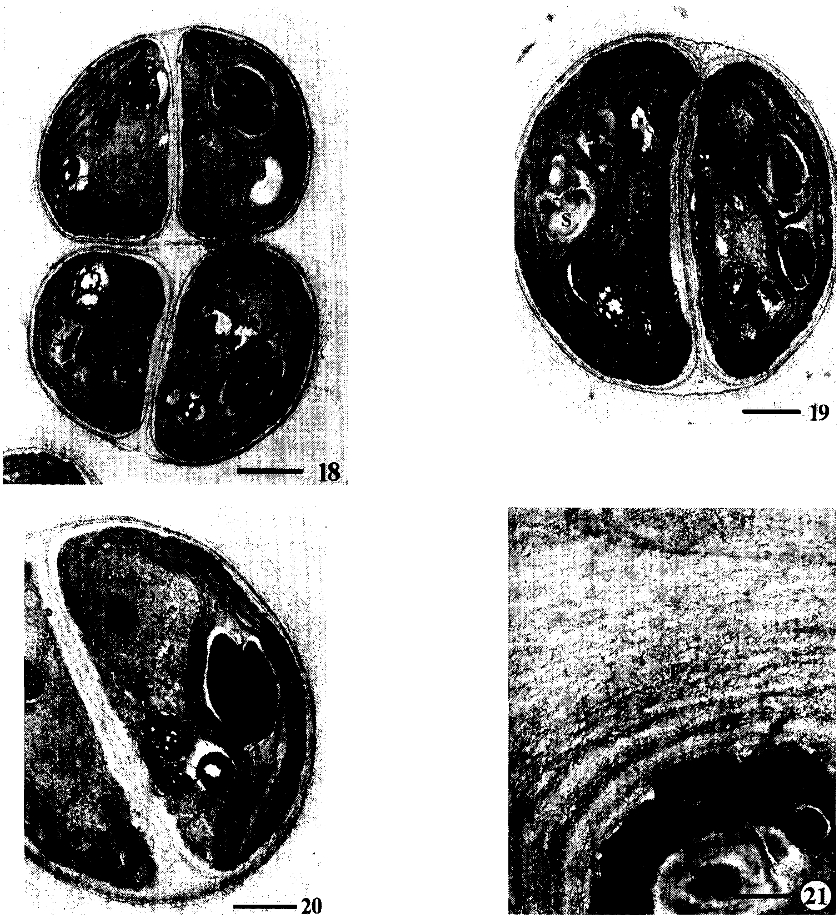
Fig. 15. Large, multinucleate vegetative cell with several chloroplasts. Ellipsoidal starch grains in the chloroplasts (white arrow). Such cells are potential candidates to form autosporangia. Scale bar = 1 μm.

Fig. 16. Autosporangium with 8 autospores. Note close packing of autospores, and large mitochondria (m). Scale bar = 1 μm.

Fig. 17. Autosporangium with a raptured cell wall (black and white arrow) to release the autospores. Apart from the opening the parent cell wall of autosporangium is intact (arrow). Scale bar = 2 μm.

Class: Chlorophyceae
Order: Chlorellales
Family: Chlorellaceae
3. ***Muriella decolor*** Visher (Figs. 12–17)
Ber. Schweiz. Bot. Ges. **45**: 405, Figs. 4–5 (1936).

Cells were single, spherical measuring 5.5–6 μm in diameter, with either one parietal chloroplast (Fig. 12), which in some cells was branched (Fig. 13) or two parietal chloroplasts (Fig. 14). The pyrenoid is not present in this taxon. The chloroplast



Chlorosarcinopsis* cf. *gelatinosa (Figs. 18–21).

Fig. 18. A packet of four cells composed of two units. Each unit consists of two cells. Conspicuous pyrenoid (P) surrounded by two or three starch sheaths. A grazing section through pyrenoid reveals a tripartite character of the starch sheaths (arrow). Scale bar = 1 μm.

Fig. 19. Unit of two cells. Each cell contains two pyrenoids. Three out of four pyrenoids are sectioned tangentially showing only their starch sheaths. Note gelatinous structure of the cell wall. Scale bar = 1 μm.

Fig. 20. Higher magnification of one cell to show an asymmetric position of nucleus (N) with nucleolus (Nc). Note the pyrenoid (P) contained in the distended part of chloroplast. Scale bar = 1 μm.

Fig. 21. An oblique section of cell showing a fibrillar nature of the gelatinous cell wall (F). The fibrillar matrix appears to be stratified (arrow). This stratification becomes less apparent further away from the cell. Scale bar = 0.5 μm.

contained a few starch grains and oil droplets (Figs. 12–14). The nucleus had a large nucleolus (Figs. 12, 14). Each cell contained a single Golgi body (Fig. 14), and several large mitochondria (Figs. 12–14). Asexual reproduction was by autospores. Some large, mature vegetative cells became multinucleate, with more and larger starch grains in the chloroplasts. It is conceivable that such cells at a later stage of development may form autosporangia (Fig. 15). In autosporangia the young autospores were closely packed together, and they contained large mitochondria and the starch grains in chloroplasts (Fig. 16). At a later developmental stage the autospores became more spherical. Finally, they were released through the raptured parent cell wall of the autosporangium (Fig. 17).

Muriella decolor is a typical aerophytic alga, and it was found for the first time growing in a soil habitat. Chloroplast morphology and the number of autospores was used to differentiate the genus *Muriella* from most *Chlorella*-like algae (Kalina and Puncoharova, 1987).

Class: Chlorophyceae
Order: Chlorellales
Family: Chlorosarcinaceae
4. ***Chlorosarcinopsis* cf. *gelatinosa*** Chantanachat & Bold (Figs. 18–21)
Phycological Studies II, The Univ. Texas Publ. **6218**: 34, Figs. 36–42, 105–108 (1962).

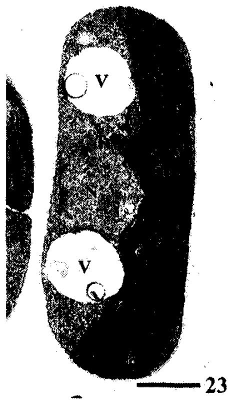
Semicircular cells occurred in groups of 2-4-8 or sometimes more, forming more or less cubical packets. In cross section these packets consisted either of two (Figs. 19, 20) or four cells (Fig. 18). Each group of cells was always embedded into an extensive gelatinous matrix (a sarcinoid level of organization). The gelatinous matrix was composed of irregularly arranged electron dense fibrils which had a stratified structure near the cell, and it became less stratified further away from the cell (Fig. 21). The single, parietal chloroplast was distended in the middle, and contained one or rarely two pyrenoids, which were surrounded by two or three starch sheaths. The chloroplast also contained starch grains, not associated with the pyrenoid, and oil droplets (Figs. 18–20). Two or three thylakoids entered the pyrenoid, giving rise to tubular channels (Figs. 18–20) The nucleus with nucleolus was situated in the corner of cell (Fig. 20). Several large vesicles were partially filled with an amorphous electron dense material (Figs. 18–20). Asexual reproduction was by autospores. Asexual reproduction by zoospores was not observed in this study.

The genera, *Chlorosarcinopsis* and *Chlorokybus*, have similar morphological structures. However, at the ultrastructural level the pyrenoid features of *Chlorosarcinopsis* (Melkonian, 1977, 1978) and *Chlorokybus* (Rogers *et al.*, 1980; Gärtner and Ingolic, 1989) are distinctly different.

Class: Charophyceae
Order: Klebsormidiales
Family: Klebsormidiaceae
5. ***Stichococcus bacillaris*** Nägeli (Figs. 22–23)
Schulthess, Zürich (1849); Ettl H., Gärtner G., 1995 Gustav Fisher Verlag, p. 605, Fig. 204: e, f.

Cylindrical cells, irregularly rounded at the apices, occurred singly or formed short filaments, which easily fell apart into single cells (Figs. 22–23). Two large non-contractile vacuoles were situated at the opposite ends of the cell (Fig. 23). The nucleus was in the middle part of the cell, and the nucleolus was not observed. The mitochondria were small and infrequent.

A single trough-shaped chloroplast without pyrenoid and starch grains, contained only oil droplets. According to Ettl and Gärtner (1995) the pyrenoid in *Stichococcus bacillaris* is naked (without a starch sheath), and difficult to observe. Pickett-Heaps (1974) studied cell division in *S. chloranthus*, and showed a chloroplast embedded with



Stichococcus bacilliaris (Figs. 22–23).

Fig. 22. Two cylindrical cells with irregularly rounded ends. Oil droplets in the chloroplast (arrow). Scale bar = 1 μm.

Fig. 23. A single cell displaying two large, non-contractile vacuoles (V). Note central location of nucleus (N). Scale bar = 1 μm.

numerous starch grains and a naked pyrenoid. *S. chloranthus* is included in *S. bacillaris* as a synonym (Ettl and Gärtner, 1995). The absence of a pyrenoid in this study can be explained, because the material was not serial sectioned.

Stichococcus bacillaris appears to be more ubiquitous occurring in various habitats of the areas where the successional process is more advanced (Broady and Weinstein, 1998; Vinocur and Izaguire, 1994).

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