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Some Ultrastructural Aspects of the Pyrenoids in *Chlorokybus atmophyticus* GEITLER *(Charophyceae, Chlorokybales)**)

By

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With 11 Figures

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Key words: *Charophyceae, Chlorokybus*, green algae. – Crystalline inclusions, plastids, pyrenoids. – Ultrastructure.

Summary

GÄRTNER G. & INGOLIĆ E. 1989. Some ultrastructural aspects of the pyrenoids in *Chlorokybus atmophyticus* GEITLER *(Charophyceae, Chlorokybales)*. – Phyton (Austria) 29 (1): 49–59, 11 figures. – English with German summary.

The ultrastructure of pyrenoids of *Chlorokybus atmophyticus* was studied on a strain isolated from alpine soil. Most of the fine structural aspects of pyrenoid morphology are similar to those reported for other isolates of the monospecific genus. One exception appears to be the presence of crystalline inclusions in the pyrenoid matrix, which are hitherto unreported for this alga.

Zusammenfassung

GÄRTNER G. & INGOLIĆ E. 1989. Beobachtungen zur Ultrastruktur der Pyrenoide von *Chlorokybus atmophyticus* GEITLER *(Charophyceae, Chlorokybales)*. – Phyton (Austria) 29 (1): 49–59, 11 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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*) Dedicated to Prof. Dr. Hellmuth SITTE (Homburg/Saar) on the occasion of his 60th birthday.

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An einem Isolat von *Chlorokybus atmophyticus* aus alpinen Böden wurde die Ultrastruktur der Pyrenoide untersucht. Während die meisten Ultrastrukturmerkmale der Pyrenoide mit den Ergebnissen bei anderen Isolaten übereinstimmen, wurden erstmals kristalline Einschlüsse in der Pyrenoidmatrix nachgewiesen. Diese waren bisher bei den in Kultur befindlichen Isolaten der monospezifischen Gattung nicht bekannt.

Introduction

The pyrenoid is a conspicuous and in many instances easily recognizable structure in, or associated with, the chloroplasts of most algal cells (GEITLER 1926, GIBBS 1962, GRIFFITHS 1970, DODGE 1973, ETTL 1981, HOOBER 1984 and others). Although there are several reports in literature which describe and illustrate the pyrenoid in various algal species, many of the structural and developmental details remain poorly understood (HOLDSWORTH 1968, PEVELING 1968, KOWALLIK 1969, BERTAGNOLLI & NADAKAVUKAREN 1970, BROWN & ARNOTT 1970, FISHER & LANG 1971, STEWART, MATTOX & FLOYD 1973, SILVERBERG & SAWA 1974, CHELUNE & WUJEK 1974, SILVERBERG 1975, WUJEK 1975, WUJEK & GRETZ 1977). Green algae have shown a remarkable display of pyrenoid variation; many of the types were illustrated by GEITLER 1926, 1942, GIBBS 1962, GRIFFITHS 1970, DODGE 1973, PICKETT-HEAPS 1975, ETTL 1980, LOKHORST & STAR 1980. Since GEITLER 1942 the charophycean taxon *Chlorokybus atmophyticus* has been studied several times. The general morphology has been reported by GEITLER in 1942 and 1955, and by RIETH in 1972, the fine structural characteristics of cells during division, development and reproduction were described by ROGERS & al. 1980 and LOKHORST & al. 1988. The conspicuous and unique character of the *Chlorokybus* pyrenoid had already been known and documented by the results of observations from the light microscope (GEITLER 1942, 1955, RIETH 1972, VINATZER 1975, TRENKWALDER 1975). Some ultrastructural details of *Chlorokybus* chloroplast and pyrenoids have already been reported by ROGERS & al. 1980 and LOKHORST & al. 1988. Our study presents additional data on occurrence and fine structure of the different pyrenoid types of a cultured strain of *Chlorokybus atmophyticus* from alpine soils.

2. Material and Methods

A strain of *Chlorokybus atmophyticus* was isolated from calcareous soil samples of alpine areas from the Dolomites (Mt. Pitschberg, 2300 m, South Tyrol, Italy) by G. VINATZER 1975 and deposited in the culture collection of algae at the University of Innsbruck (acronym ASIB, formerly, IBSG, strain number V 202). Cultures were maintained in a controlled environment room at 13°C under a 180 ft-c cool-white fluorescent light in a constant diurnal cycle of 12 h light and 12 h dark. Strains were cultivated in Bold's Basal Medium (BISCHOFF & BOLD 1963). Morphological observations with light microscope were made on cultures grown on 1N BBM-agar or 1N BBM-liquid under the conditions described above.

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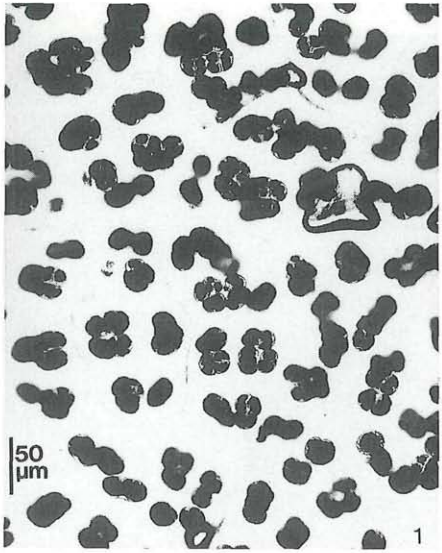

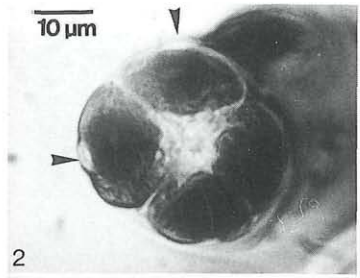

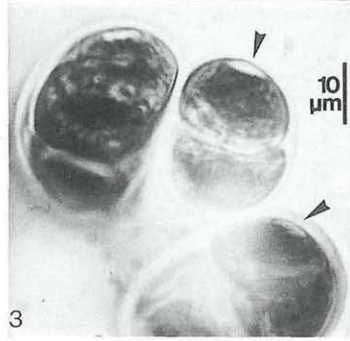

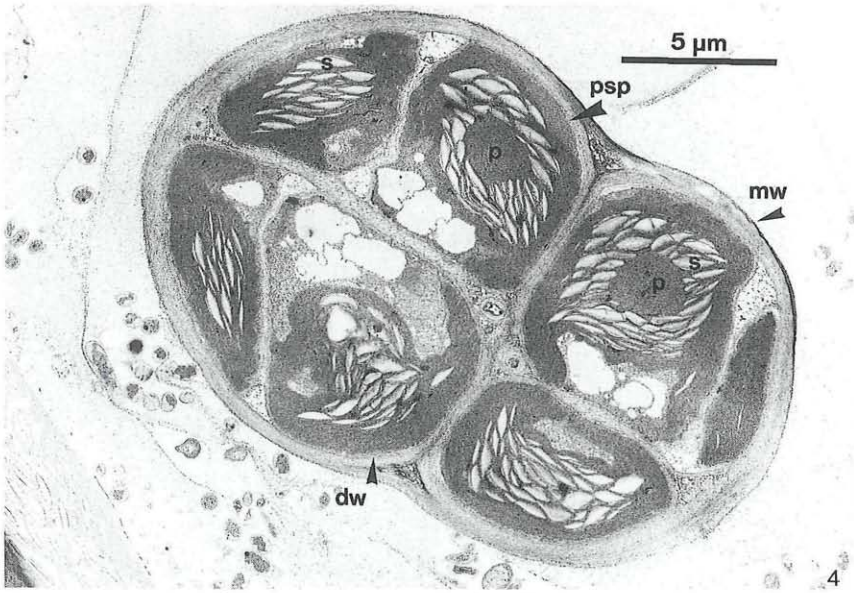

Fig. 1. *Chlorokybus atmophyticus*. – Sarcinoid cell packets of strain V 202 under good growing conditions on 1N BBM-agar; cells stained with JKJ.

Figs. 2, 3. Packets of vegetative cells with parietal chloroplast and closely attached pseudopyrenoids (arrows), cells contrasted with JKJ.

Fig. 4. Vegetative cells after divisions surrounded by the mother cell wall (mw); daughter cells with distinct independent walls (dw); p= pyrenoid, psp = pseudopyrenoid, s = starch grains, more or less parallel arranged to the thylakoids.

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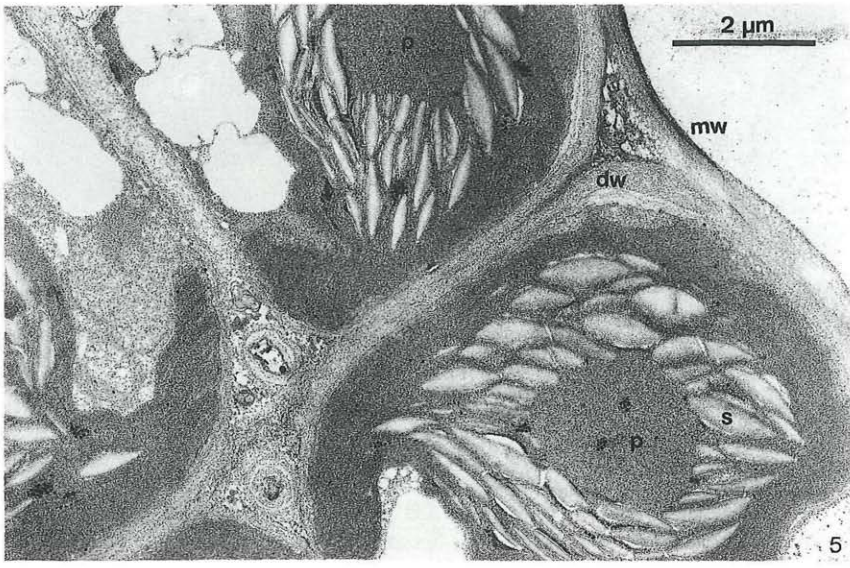

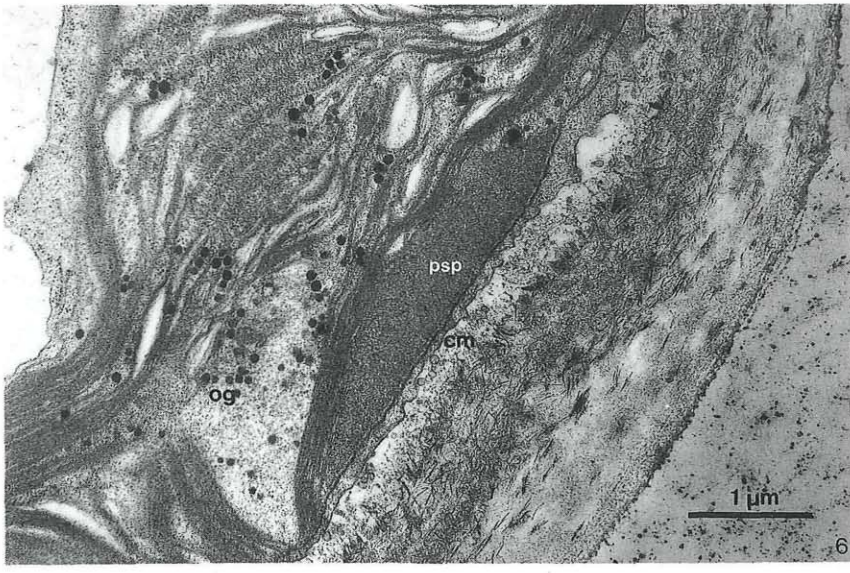

Fig. 5. *Chlorokybus atmophyticus* .– Vegetative cells after divisions. Same details as in Fig. 4 at higher magnification; abbreviations as in Fig. 4.

Fig. 6. Pseudopyrenoid (psp) closely attached to the edge of the chloroplast and enclosed by the chloroplast membrane (cm); some osmiophilic globuli (og) also visible.

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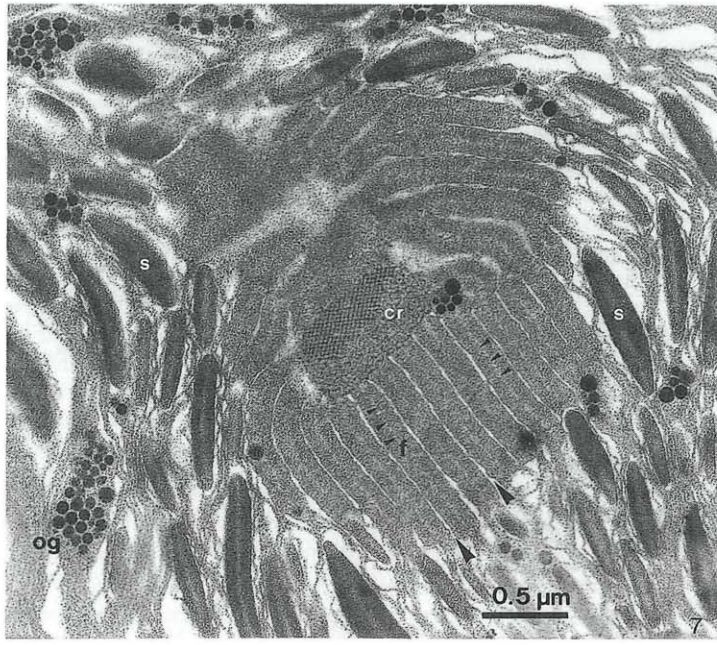

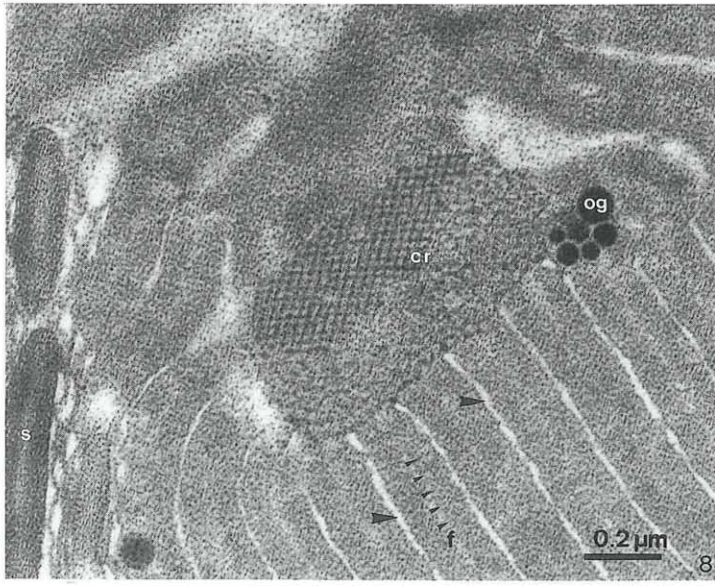

Figs. 7, 8. *Chlorokybus atmophyticus*. – Ultrastructure of the main pyrenoid (Fig. 8 at higher magnification); pyrenoid matrix traversed by single thylakoids, distinct spaces between the matrix components are visible (arrows); within the single matrix components occur faults (small arrows, f); a crystalline inclusion (cr) in the centre of the pyrenoid matrix, with crystal lattice configuration; og = osmiophilic globuli, s = starch.

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For comparison in electron microscopy, two fixation procedures were used. One part of the material was prefixed in 1% glutaraldehyde in 0.1 Na-cacodylate-buffer, pH 7.2 for 1 hr at 4°C. After thorough washing the cells were postfixed in 2% aqueous, unbuffered $KMnO_4$ for 2 hrs at 4°C. The remaining part of the algal material was fixed in 3% glutaraldehyde buffered to pH 7.2 in 0.1 m Na-cacodylate for 2 hrs at 4°C. After fixation the cells were rinsed three times in buffer and then postfixed in 1% OsO_4 in the buffer as mentioned before for 2 hrs at 4°C.

The combination of fixation 2 was used for most of the electron micrographs presented in this paper. Dehydration was carried out in graded dilutions of ethanol at room temperature, followed by treatment with propylene oxide and embedding in Epon 812 (LUFT 1961). Thin sections were cut on a Reichert Ultracut-E, using a diamond knife, and stained with 1% aqueous uranylacetate and lead citrate (REYNOLDS 1963). The algae were examined in a Philips Model "300" electron microscope at 60 kV or a Zeiss EM 9.

3. Results

Mature vegetative cells of the investigated strain V 202 of *Chlorokybus atmophyticus* show characteristic sarcinoid cell packets (Figs. 1–4), resulting from divisions in two directions (desmoschisis or true vegetative cell division after ROGERS & al. 1980). As we suppose, there is not a true vegetative cell division taking place but a modified form of autosporulation (as stated by RIETH 1972), because wall material after division is seemingly deposited around the entire daughter cells (Figs. 4–5, arrows). We never observed a thin septum connected with the mother cell walls, as it was reported by ROGERS & al. 1980. The cells contain a parietal chloroplast with a single massive pyrenoid, lacking a compact starch sheath but surrounded by numerous lenticular starch grains, arranged more or less parallel to the main diameter of the chloroplast (Figs. 4, 5). In transverse sections a second small lenticular additional pyrenoid occurs at the edge of the chloroplast (Figs. 4, 6), first observed by GEITLER 1942 in the type material. This "pseudopyrenoid" in the sense of GEITLER 1942 is not always visible in LM but can be contrastet with JKJ or Millon's reagent (Figs. 2, 3). GEITLER 1955 presumed that this pseudopyrenoid was a real part of the chloroplast. As can be seen in fig. 6, it is closely attached to the edge of the chloroplast and enclosed by the chloroplast membrane. It is characterized by a homogenous matrix, never traversed by thylakoids and obviously not involved in starch deposition. ROGERS & al. 1980 observed in strain CCAP 401/1 (a spontaneous contaminant in cultures, see RIETH 1972), 2 pseudopyrenoids, at the edge of the chloroplast, but this was never visible in our isolate V 202 of *Chlorokybus*. Figs. 7 and 8 demonstrate the ultrastructure of the main pyrenoid. It is traversed by numerous ± regular arrays of single thylakoids, which are running flat or curved, as also observed in the strains investigated by ROGERS & al. 1980 and LOKHORST & al. 1988. The thylakoidal elements dissect the pyrenoid matrix into many flattened compartments, but distinct spaces between them are visible (Figs. 7, 8 arrows), caused by the very close

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association of thylakoids and matrix. No membrane separates the pyrenoid from the rest of the chloroplast, but the granular matrix of the main parts of the pyrenoid with the traversing thylakoids is well distinguishable from the ground substance within the chloroplast stroma. In some cases also osmiophilic globuli are visible in the matrices of the chloroplast and the pyrenoid (Figs. 7, 9). A feature of structural interest is the presence of faults in the granular matrix of the pyrenoid compartments (Figs. 7, 8, smaller arrows, f). These faults pass the matrix components in a linear arrangement and were for the first time reported in cells of *Stichococcus* (SILVERBERG 1975), but are also visible on micrographs of ROGERS & al. 1980 and LOKHORST & al. 1988. As stated by SILVERBERG 1975, these faults may be considered as developmental stages in younger cells, but they are also visible in mature cells of our *Chlorokybus* strain, contrary to observations of SILVERBERG (op. cit.) in cells of *Stichococcus,* where such faults are totally absent in later stages of development. Another unique feature of ultrastructural interest was the presence of "crystalline inclusions" near the centre of the main pyrenoid matrix, where some of the traversing thylakoids seem to extend (Figs. 7, 8, 9, cr). In some instances these inclusions show a crystal lattice configuration over the whole surface of the inclusion (Fig. 9), in other

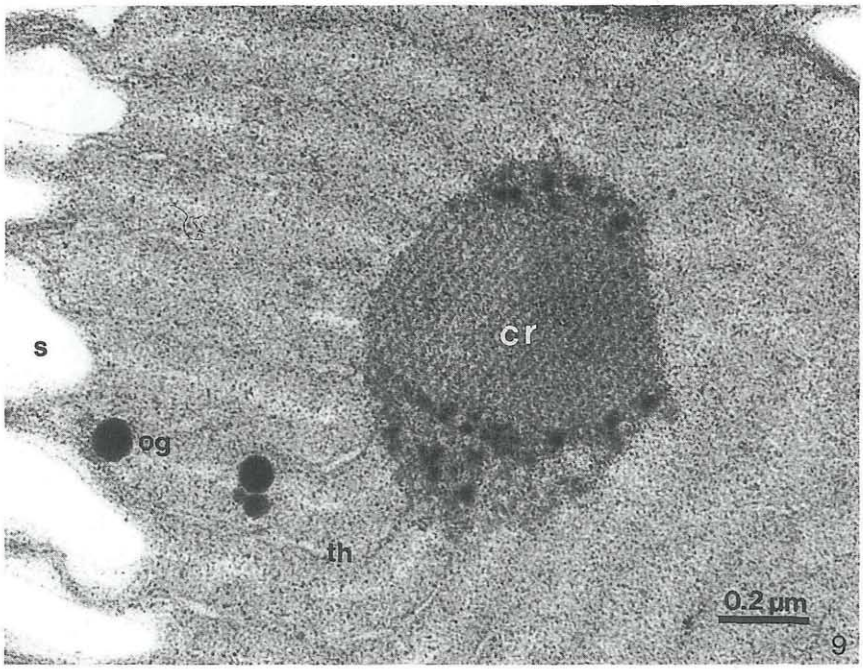

Fig. 9. Crystalline inclusion (cr) in the pyrenoid matrix with crystal lattice configuration over the whole surface; th = thylakoids, og = osmiophilic globuli, s = starch.

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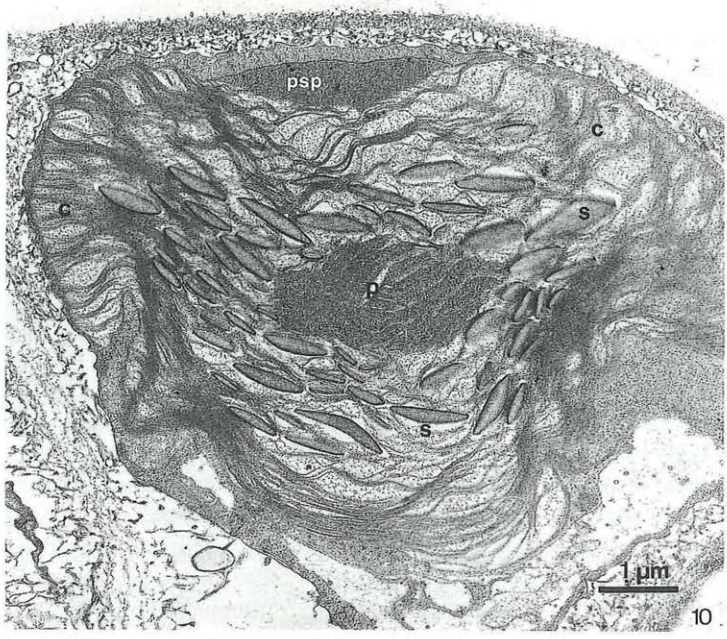

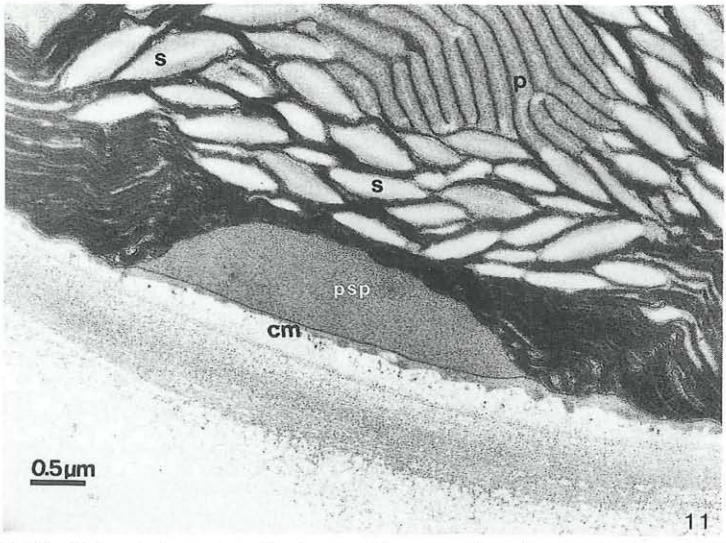

Figs. 10, 11. *Chlorokybus atmophyticus.* – Cross section of a vegetative cell of strain T 106 with the same ultrastructural details as strain V 202; c = chloroplast, p = pyrenoid with curved thylakoids and lenticular starch grains (s); external pseudopyrenoid (psp) within the chloroplast membrane. In Fig. 11 ultrastructural details are recognizable; p = pyrenoid, psp = pseudopyrenoid, s = starch grains, cm = chloroplast membrane.

Fixations: Figs. 1–10 cells fixed with glutaraldehyde/osmiumtetroxide, in Fig. 11 with glutaraldehyde/kaliumpermanganate.

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cases the crystal configuration is only visible on the half of the inclusion (Figs. 7, 8) seemingly caused by different directions of the section, which has a significant influence on the appearance of lattice structures (KOWALLIK 1969).

Such crystalline inclusions were not frequently observed in the pyrenoids of green algae (JOYON & FOTT 1964, BROWN & MCLEAN 1969) and for the first time reported for *Chlorokybus*.

4. Discussion

The presence or absence of pyrenoids and their number in algal chloroplasts have been widely employed as taxonomic parameter and used as characteristic in the classification of genera and species (ETTL 1976, ETTL 1980, ETTL & GÄRTNER 1984). Also the ultrastructure of pyrenoids is an important taxonomic character but it displays a wide range of variation especially on species level (PEVELING 1968, BROWN & MCLEAN 1969, FISHER & LANG 1971, WUJEK 1975, LOKHORST & STAR 1980). The unique character of the main pyrenoid structure in *Chlorokybus atmophyticus* with the presence of superficial pyrenoids is discussed by ROGERS & al. 1980. A similar ultrastructure of the pyrenoid with single traversing thylakoids is known from *Pyramimonas parkeae* and the closely related *Nephroselmis olivacea (Prasinophyceae* or *Micromonadophyceae,* see NORRIS & PEARSON 1975, MATTOX & STEWART 1977, MOESTRUP & ETTL 1979, MATTOX & STEWART 1984). Both green flagellates show primitive characters and are considered as possible representatives of a group of algae which gave rise to the ancestors of *Charophyceae*, bryophytes and vascular plants (MOESTRUP & ETTL 1979). There is no doubt, that similarities in pyrenoid ultrastructure of these organisms should be noticed without further conclusions, as stated also by ROGERS & al. 1980. *Chlorokybus atmophyticus* today exists in only 4 strains in the culture collections of Cambridge respectively Ambleside (CCAP 403/1) and Göttingen (SAG B. 48.80), both isolates from RIETH, and in the collection of the Innsbruck university (V 202, isolated VINATZER 1975, and T 106, isolated TRENKWALDER 1975, see GÄRTNER 1985). Only the strains CCAP 403/1 and SAG B. 48.80 have been hitherto studied with EM (ROGERS & al. 1980 and LOKHORST & al. 1988).

In all investigated strains the ultrastructure of pyrenoids is uniform, the main pyrenoids are traversed by single more or less curved thylakoids and posses superficial marginal lenticular pyrenoids without structure and thylakoids, as can be seen on a section of a vegetative cell of strain T 106 (Figs. 10, 11).

It is known from studies on higher plants and algae that protein storage products are often represented by crystalline inclusions (for literature see THALER 1966, HOLDSWORTH 1968, KOWALLIK 1969). This might be also the situation in *Chlorokybus*. The presence of cyrstalline inclusions in the

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pyrenoids seems to be a stable morphological feature in strain V 202. In all other details of morphology and ultrastructure V 202 is identical with the other three strains of the only known species *C. atmophyticus*.

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