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PYRENOID ULTRASTRUCTURE IN ULOTHRIX (CHLOROPHYCEAE)

G. M. LOKHORST and W. STAR

Rijksherbarium, Schelpenkade 6, 2313 ZT Leiden

SUMMARY

The ultrastructure of the pyrenoid in the gametophytic phase of the species of *Ulothrix* shows a large diversity. This was revealed by a study of the morphology of the pyrenoid matrix, the associated starch cap and the pattern of the intrapyrenoidal chloroplast strands. From these features, eight types of pyrenoid structure were recognized in the genus *Ulothrix*. The closely related species *Ulothrix crenulata* and *U. verrucosa*, which only propagate by fragmentation, also have a common, very characteristic pyrenoid morphology. In contrast, *Ulothrix mucosa*, only capable of reproduction by means of zoospores and possessing a peculiar soft cell wall, has a pyrenoid structure overlapping with that in the not very closely related *U. zonata*, which is characterized by a complete life history and a firm cell wall. The findings reported here support the recently proposed classification of the genus *Ulothrix*, especially that of the problematic group of species having cell diameters ranging from 3.5–12.6 µm.

1. INTRODUCTION

The number of pyrenoids in the chloroplast of *Ulothrix* species as detected with the light microscope has been used as an important species distinguishing feature in the past (HEERING 1914, PRINTZ 1964, RAMANATHAN 1964, and others). Species with cell diameters up to about 10–15 µm generally were reported to have only one pyrenoid, whereas the presence of more pyrenoids was said to be restricted to the larger species. Recent taxonomic studies on *Ulothrix* species in western Europe revealed that this feature shows a considerable plasticity. Species with small diameters, e.g. *Ulothrix subtilis*, *U. tenerrima*, *U. albicans* and *U. subflaccida*, often possess more pyrenoids per cell. The number of pyrenoids generally increases in the larger species, except in *Ulothrix verrucosa* (LOKHORST 1974, 1978). Consequently, the number of pyrenoids cannot be used as a reliable character for the identification of *Ulothrix* species. On the other hand, the taxonomic significance of the micro-anatomy of the individual pyrenoid has already been emphasized for the brackish-water and marine *Ulothrix* species (LOKHORST 1978). Depending on the species, the pyrenoid matrix is invaded by intrapyrenoidal chloroplast strands on all sides. Since the earlier studies on the freshwater species of *Ulothrix* were only performed with the light microscope, the present study was devoted to the ultrastructure of the pyrenoid.

The findings here substantiate the reported subdivision of freshwater *Ulothrix* (LOKHORST 1974).

2. MATERIAL AND METHODS

From the large number of uni-algal freshwater cultures at the Rijksherbarium, eight clones were isolated for further ultrastructural studies. The algae were routinely maintained under a regime of 16 hours darkness and 8 hours light at 8 °C. Culture methods were not essentially different from those described in earlier studies on *Ulothrix* (LOKHORST 1974, 1978).

The culture medium was changed every three weeks and the plants were grown in glass boxes (diameter 10 cm). The algae were prepared for the ultrastructural studies during stages of active growth so that accumulation of starch in the chloroplast was at a minimum.

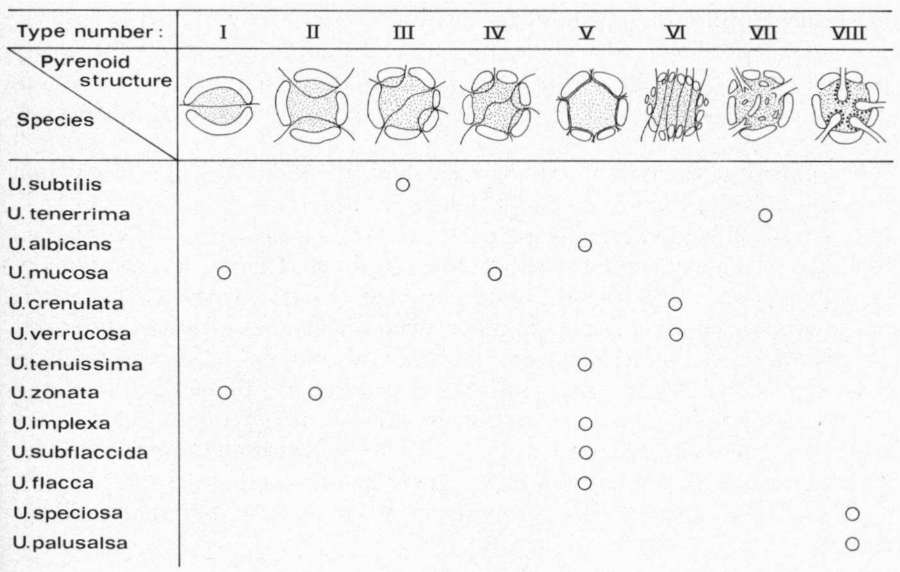
The techniques of preparation for electron microscopy have been previously described (LOKHORST 1978). Two types of fixatives were applied. In one fixation method, filaments were kept for 1–4.5 hours in 0.5–2 % glutaraldehyde in 0.1 M cacodylate buffer. The other fixative, in which the algae were kept for only 30 minutes, consisted of 1 % $KMnO_4$ in distilled water. Prior to fixation in 1 % OsO_4 in 0.1 M cacodylate buffer, the plants were rinsed in a pure solution of 0.1 M cacodylate buffer in distilled water. In some cases, the material was first fixed in a 1 % glutaraldehyde-solution and subsequently postfixed in 1 % $KMnO_4$. In that case, the fixation in OsO_4 was omitted. All fixation treatments were carried out at 4 °C. Occasionally, the filaments were stained with uranyl acetate during dehydration in graded ethanol series. The material was sectioned with a diamond knife and examined with a Philips EM-300 electron microscope. In some cases, the thin sections were poststained with uranyl acetate and lead citrate.

3. OBSERVATIONS

DODGE (1973) divided the pyrenoid types in the algae into a number of (somewhat arbitrary) categories. He pointed out that the type of pyrenoid found in most members of the Chlorophyceae usually has a starch sheath and is entirely embedded in a starch-containing chloroplast. The results of the present study show that the pyrenoids in *Ulothrix* fit into this common chlorophycean type. The eight variants of this type (Type I–VIII) encountered in the genus *Ulothrix* are schematically depicted in *fig. 1*, and referred to in the specific descriptions.

3.1. *Ulothrix subtilis* Kützing --- Cell diameter (3.5–)4.2–7.0(–7.7) μm, length/width ratio 3/4–3(–5). 1–2 pyrenoids per cell --- *Fig. 3.*

In longitudinal sections, the globose pyrenoid of *U. subtilis* is usually positioned in a lobe of the chloroplast. Starch grains are generally randomly located in the chloroplast, but dense concentrations are often found in the vicinity of the starch sheath of the pyrenoid. A network of chloroplast lamellae, each consisting of a pair of thylakoids, is frequently found in these dense starch concentrations. Their presence does not disturb the course of the thylakoid bands in the pyrenoid matrix. The pyrenoid core is usually cleaved by 3 sinuous intrapyrenoidal chloroplast lamellae (Type III). CHADEFAUD (1941) defined this kind of pyrenoid as



Type number:	I	II	III	IV	V	VI	VII	VIII
Pyrenoid structure / Species								
U. subtilis			○					
U. tenerrima							○	
U. albicans					○			
U. mucosa	○			○				
U. crenulata						○		
U. verrucosa						○		
U. tenuissima					○			
U. zonata	○	○						
U. implexa					○			
U. subflaccida					○			
U. flacca					○			
U. speciosa								○
U. palusalsa								○

Fig. 1. Schematic representation of the pyrenoid types in the genus *Ulothrix*. The framework of this figure is after HORI (1973).

the zonate type. In some cases, these intrapyrenoidal chloroplast lamellae, reduced to 1–2 thylakoids per lamella, appear to be rejoined in the center of the pyrenoid. This phenomenon is probably due to the manner of sectioning the pyrenoid matrix. In section, at least four starch particles appear to cover the surface of the pyrenoid. It is interesting to note here that at approaching the starch shell, the chloroplast lamellae which run through the pyrenoid already have been reduced to one or two thylakoids. In other species studied, this reduction appears at the moment of intrusion into the pyrenoid. In one section only, there was an indication of the presence of a peroxisome.

3.2. *Ulothrix tenerrima* Kützing --- Cell diameter (4.9–)7.0–9.1(–10.5) μm, length/width ratio ½–1½(–3). 1–3(–4) pyrenoids per cell --- *Figs. 4–6.*

In longitudinal sections, the globose pyrenoid of *U. tenerrima* is often localized in the corner of the cell. Dense concentrations of starch plates are not found in the immediate vicinity of the starch shell of the pyrenoid. On all sides, the pyrenoid is characterized by tubular chloroplast invaginations which soon after penetration of its matrix branch out in all directions, giving the pyrenoid a typical vesicular appearance (Type VII). The tubular chloroplast strands consist of 2–5 thylakoid membranes. In exceptional cases, a chloroplast strand invades the starch sheath through an intergranular gap and approaches the surface of the pyrenoid but then continues between the adjoining starch grain and the pyrenoid periphery. In some cases, the tubular invagination takes a distinctly swollen appearance, but it is not clear whether this is the result of the fixation procedure used. The number of grains covering the pyrenoid varies from 4–8 in a longitudinal section. In pyrenoids capped by a reduced starch shell, the thylakoid lamellae may lie closely parallel to the periphery of the pyrenoid where the starch grains have disappeared. In none of the sections could the presence of a peroxisome be detected.

3.3. *Ulothrix albicans* Kützing --- Cell diameter (5.6–)7.7–13.6(–14.0) μm, length/width ratio $\frac{1}{2}$–2(–4). 1–5 pyrenoids per cell --- *Fig. 7.*

In longitudinal section, the globose pyrenoid in *U. albicans* is usually positioned in a lobe of the chloroplast in the middle of the cell. Dense concentrations of starch are never observed in the immediate vicinity of the pyrenoid. The matrix of the pyrenoid is not traversed by chloroplast lamellae; its periphery, however, is frequently covered with a single thylakoid or by a bundle of 2(–3) thylakoids in certain portions. These thylakoids extend between the pyrenoid matrix and fragments of the surrounding starch shell (Type V). In pyrenoids enclosed by a reduced starch shell, the interposing thylakoids are only found near the retained starch grains. The number of starch grains covering the pyrenoid varies from 4–10 in a longitudinal section. A peroxisome structure seems to be absent in this species.

3.4. *Ulothrix mucosa* Thuret --- Cell diameter (6.3–)9.8–14.0(–15.4) μm, length/width ratio 1/4–1(–2). 1–4 pyrenoids per cell --- *Figs. 8–9.*

In longitudinal section, the pyrenoid in *U. mucosa* occupies a relatively large portion of the chloroplast. Dense concentrations of starch are never found surrounding the pyrenoid. *U. mucosa* usually has a pyrenoid with a matrix maximally divided into 5 compartments by intrusion of several intrapyrenoidal lamellae, each consisting of a single thylakoid (Type IV). CHADEFAUD (1941) defined this kind of pyrenoid as the polypyramidal type. Occasionally, pyrenoids are observed which are bisected by only one chloroplast strand, consisting of 1(–2) thylakoids (Type I). This kind of structure has been called "bilenticular" (CHADEFAUD loc. cit.). In this case, the pyrenoid is only surrounded by a pair of starch grains, which are hemispherical in shape. In both pyrenoid types, bundles of thylakoids mostly five in number, succeed in invading the starch shell through the intergranular gaps. Half-way across these gaps or at the surface of the pyrenoid, however, four thylakoids terminate abruptly in close proximity to one another and only one invades the pyrenoid proper. Depending on the number of intrusions of intrapyrenoidal bands, the starch shell consists of 2–8 starch grains in a longitudinal section. Peroxisomes were not seen.

3.5. *Ulothrix crenulata* (Kützing) Kützing --- Cell diameter (9.1–) 11.2–14.0(–15.4) μm, length/width ratio 1/3 – 1(–1.5). 1(–3) pyrenoids per cell --- *Fig. 10.*

In longitudinal section, the slightly elongated pyrenoid in *U. crenulata* almost completely fills the longitudinal lobe of the chloroplast. As has been observed with the light microscope, the pyrenoid is often surrounded by a distinct envelope of starch, which may even consist of 2–3 layers of grains. The position of these starch fragments differs from the pyrenoid types described above. In longitudinal sections, almost all grains are oriented with their long axis more or less in the long axis of the filament. As a consequence, the boundary of the pyrenoid matrix is difficult to demarcate. The pyrenoid is transected by numerous intrapyrenoidal chloroplast lamellae, which lie approximately parallel to the longitudinal cell wall and extend the entire length of the pyrenoid (Type VI). Each strand is composed of one or 2–3 closely appressed thylakoids. Although they are usually evenly distributed, the strands are not always completely separated from one another. In pyrenoids with a reduced starch cap, the number of traversing chloroplast strands is approximately the same. Normally, the pyrenoid is covered with numerous starch grains in a longitudinal section. A single, greatly elongated peroxisome is usually positioned between the nucleus and the chloroplast proper.

3.6. *Ulothrix verrucosa* Lokhorst --- Cell diameter 12.6–20.3(–23.1) µm, length/width ratio 1/4–1½. 1(–2) pyrenoids per cell --- *Figs. 11–13.*

In longitudinal section, the embedded pyrenoid in *U. verrucosa* takes up a large part of the lateral lobe of the chloroplast. The slightly elongated pyrenoid is usually surrounded by a very distinct envelope of starch, which consists of a compact mass of grains. The ultrastructure of the pyrenoid stroma shows traversing intrapyrenoidal chloroplast lamellae and is essentially the same as described for *Ulothrix crenulata* (Type VI). In *U. verrucosa*, however, the pyrenoid matrix is only traversed by single thylakoids. One peroxisome per cell is found. It lies clamped between the chloroplast membrane and the nucleus or the centrally located vacuole. In dividing cells, the pyrenoid is cleaved by the ingrowing furrow.

3.7. *Ulothrix tenuissima* Kützing --- Cell diameter (4.9–)9.8–22.4(–50) µm, length/width ratio 1/6–1½(–5).1–4(–6) pyrenoids per cell --- *Figs. 14–15.*

The pyrenoid in *U. tenuissima* can take any position in the chloroplast. The single layered starch shell is never surrounded by dense concentrations of starch plates. *U. tenuissima* shows the same pyrenoid ultrastructure as described above for *U. albicans* (Type V). Occasionally, the matrix of the pyrenoid varies in electron density. In some cases, light-coloured spots are found in the center of the pyrenoid and these sometimes extend to the periphery. In one case, however, a shallow intrusion of thylakoids into the pyrenoid was found. In this respect *U. tenuissima* takes an intermediate position between the *Ulothrix* species with appressed thylakoid membranes and those with traversing thylakoids in the pyrenoid matrix. The number of starch grains covering the pyrenoid varies from 4–10 in longitudinal section. They are not always equal in length and not always closely connected. An elongated peroxisome is located in the vicinity of the pyrenoid, more or less parallel to the long axis of the filament.

3.8. *Ulothrix zonata* (Weber & Mohr) Kützing --- Cell diameter (7.0–) 10.5–37.1(–70) μm, length/width ratio 1/6–2(–6). 1–8(–11) pyrenoids per cell --- *Figs. 16–18.*

The pyrenoid is usually found in a lateral lobe of the chloroplast. As described above for *U. tenuissima*, no loosely dispersed starch grains are found near the pyrenoid. In several sections, the pyrenoid is elongated rather than globular. The pyrenoid matrix is usually penetrated by one (Type I), in some cases by two, curved intrapyrenoidal chloroplast strands (Type II), each consisting of a single thylakoid. In the intergranular gaps of the starch shell, these strands still may contain lamellae consisting of five thylakoids. The starch shell is composed of two (hemispherical) or four starch plates in the longitudinal sections. No peroxisomes could be detected.

The pyrenoid ultrastructures of the brackish-water species *Ulothrix implexa* (Kützing) Kützing and *U. subflaccida* Wille and of the marine species *U. speciosa* (Carmichael ex Harvey in Hooker) Kützing, *U. palusalsa* Lokhorst and *U. flacca* (Dillwyn) Thuret in Le Jolis have been earlier described in detail (LOKHORST 1978). *Ulothrix speciosa* and *U. palusalsa* have pyrenoids with a matrix penetrated by tubular chloroplast invaginations on all sides (Type VIII). These invaginations normally contain 3–5 thylakoids that terminate in most cases before reaching the center of the pyrenoid. The outer side of their membrane is often occupied with pyrenoglobuli which are fairly constant in size and shape (*Figs. 19–20*) *Ulothrix implexa*, *U. subflaccida* and *U. flacca* have the pyrenoid type as described above for *U. albicans* and *U. tenuissima* (Type V).

4. DISCUSSION AND CONCLUSIONS

Comparative studies of the chloroplast fine structure in the Chlorophyta have frequently emphasized that the ultrastructure of the pyrenoid is of taxonomic significance. BROWN & BOLD (1964) were among the first to comparatively describe the fine structural organisation of the pyrenoid at the species level. In the green alga *Tetracystis*, five different types of pyrenoids could be differentiated at the ultrastructural level. Criteria included the lamellar position, the number of lamellar structures which invade the pyrenoid core, their interconnecting pattern, the number and position of starch grains and pyrenoid shape and size.

During the last decade there has been a tremendous increase in the knowledge of taxonomic relationships in pyrenoid morphology in a wide variety of green alga genera. Ultrastructural variations in pyrenoid morphology have been demonstrated in species of *Chlamydomonas* (ETTL 1966, 1976), *Trebouxia* (PEVELING 1968), *Chlorococcum* (BROWN & MCLEAN 1969), *Monostroma* (HORI 1973), *Caulerpa* (HORI 1974, CALVERT et al. 1976), *Pyramimonas* (NORRIS & PIENAAR 1978) and representatives of Cladophorales, Siphonocladales, Siphonales, and Dasycladales (HORI & UEDA 1967). The present study has revealed that by comparing the morphology of the pyrenoid core, the associated starch cap and the pattern of the intrapyrenoidal strands, eight types of pyrenoids can be recognized in the 13 species of *Ulothrix* in western Europe. It must be emphasized here that only the filamentous generation of the life history was studied . HORI (1973) found that in *Monostroma nitidum* and in *M. angicava* the pyrenoid structure varies with the alternation of generations. In other *Monostroma* species, e.g. *M. groenlandicum*, *M. zostericola*, *M. undulatum*, the basic pattern of pyrenoid structure remains essentially unchanged in the whole life history. This study revealed that there were no essential differences in pyrenoid structure between the material from nature and from laboratory cultures. Infrequently, however, pyrenoids were observed (partially) without starch grains. This phenomenon is probably due to different environmental conditions.

In studies on the taxonomy of the freshwater representatives of *Ulothrix*, based primarily on light microscopical observations, the heterogeneous nature of this group of *Ulothrix* species has been analysed (LOKHORST 1974). The classification of the species in the cell diameter range up to ± 12 μm appeared to be especially problematic, in part due to the strongly overlapping cell diameters. It was therefore argued that controlled laboratory cultures must be used for unequivocal identification. The results in this paper support the view that *Ulothrix subtilis*, *U. tenerrima* and *U. albicans* are distinct species. The pyrenoids in their filamentous generations differ clearly.

A close relationship between *Ulothrix crenulata* and *U. verrucosa* was suggested by studies on their life history (LOKHORST & VROMAN 1974). The present study of pyrenoid structure also confirms the similarity of these algae. If the two species are compared with the generic descriptions of *Klebsormidium (Hormidium)* and *Ulothrix* (RAMANATHAN 1964, PRINTZ 1964, BOLD & WYNNE 1978), their intermediate taxonomic position becomes apparent. Features such as the organisation of the chloroplast and the number of pyrenoids have earlier been used to justify their classification into the genus *Ulothrix* (LOKHORST & VROMAN loc. cit.). FLOYD et al. (1972), PICKETT-HEAPS (1972) and MARCHANT et al. (1973), however, found in *Klebsormidium subtilissimum* and *K. flaccidum* approximately the same pyrenoid ultrastructure as shown in the present study for *Ulothrix crenulata* and *U. verrucosa*. Therefore, it may be necessary to reconsider the conflicting taxonomic position of these species in the heterogeneous *Ulothrix*-complex. The results here suggest that in pyrenoid fine structure the affinity lies nearer to *Klebsormidium*. A similar electron microscopic inspection of the pyrenoid in the remaining *Klebsormidium* species is needed before a decision can be made on the removal of these species from the genus *Ulothrix*. It would be interesting to see whether other cytological features, used by STEWART & MATTOX (1975) as the basis for their newly proposed reclassification of some green algae, also necessitate the assignment of *U. crenulata* and *U. verrucosa* to the new family *Klebsormidiaceae*.

A rather close taxonomic affinity between the species *Ulothrix albicans*, *U. tenuissima*, *U. subflaccida*, *U. implexa* and *U. flacca* has been revealed by life history studies (LOKHORST 1974, 1978). The pyrenoids in all these species lack thylakoid elements in their matrices (Type V). This reinforces the association of them into a clear-cut taxonomic group. Since this relatively large group of species includes the type species *Ulothrix tenuissima*, it may be expected that this type of pyrenoid will be inseparably associated with the genus *Ulothrix*. The pyrenoid of *U. tenuissima* is proposed here as the standard type of pyrenoid. The basal cell of *U. subtilis* hardly ever develops into a differentiated holdfast (LOKHORST & VROMAN 1972). In this respect, *U. subtilis* shows a relatively close affinity to the genus *Klebsormidium*. This taxonomic relationship can not be confirmed by the present study. The pyrenoid of *U. subtilis* is fundamentally different in its ultrastructure from that found for *Klebsormidium* (PICKETT-HEAPS 1972 etc.).

The bilenticular type of pyrenoid is met with in both filamentous stages of *Ulothrix zonata* and *U. mucosa*. The discovery of this phenomenon is rather surprising in that the two species differ in several morphological respects. These features include the nature of the cell wall, the organisation of the basal part of the filament and the mode of zoospore germination.

It is worthwhile to note that the membranes of the chloroplast invaginations in the closely related marine species *Ulothrix speciosa* and *U. palusalsa* are covered on their outside with osmiophilic pyrenoglobuli. It often appears that the individual chloroplast thylakoids terminate on the inner surface of the limiting membrane of the invagination, just opposite an externally situated pyrenoglobule. It may be possible that these globules are involved in the conduction of photosynthetic products from the photosynthetic thylakoids to regions specialized in the synthesis of storage material.

In a previous attempt to classify chaetophoralean algae on the basis of comparative cytology, STEWART et al. (1973) indicated that the pyrenoid is usually conservative in structure. All genera with plasmodesmata have pyrenoids, characterized by the lack of traversing chloroplast membranes, but usually show membranes appressed to the periphery of the pyrenoid matrix. For this reason they considered the fine structure of the pyrenoid as a valuable generic characteristic. Later STEWART & MATTOX (1975) demonstrated that the pyrenoid structure is even uniform at the family level, for instance in the Chaetophoraceae and in the Aphanochaetaceae. This is in contrast with the results of the present study. Furthermore, *Ulothrix implexa* possesses a pyrenoid lacking traversing membranes, but its cell wall is not penetrated by plasmodesmata. However, the present study shows that the detailed structure of the pyrenoid is of great help in the delimitation of taxa at the species level in the genus *Ulothrix*.

The basic type of pyrenoid in *Ulothrix* normally has a starch sheath and is entirely embedded in the chloroplast. Two main variants of this basic type can be distinguished. The first is characterized by a simple homogeneous matrix. Thylakoid membranes are only present close to the periphery, clamped between the starch plates and the pyrenoid matrix (Type V). The second variant has a more compound structure with lamellae traversing the pyrenoid matrix. Two clear-cut units can be discerned within this group. In the pyrenoid types I, II, III, IV and VI, the traversing system apparently consists of invading chloroplast thylakoid lamellae only. In the types VII and VIII, in contrast, the tubular invaginations into the pyrenoid matrix seem to contain a relatively large amount of chloroplast ground substance in addition to the thylakoid lamellae. Furthermore, it is peculiar that the invaginations are delimited by a one-membrane system. It is arbitrary, however, to conclude from the micrographs whether this limiting membrane is composed of a split thylakoid or originates from an unknown membrane system.

HORI & UEDA (1967) have proposed a hypothetical scheme of phylogenetic relationships of chloroplasts and pyrenoids in thirty-one species of siphonous green algae based on their fine structure. They considered the non-grana structured chloroplast, present e.g. in *Urospora, Rhizoclonium, Cladophora,* to be more primitive than the grana structure found in *Acetabularia* and *Neomeris.* This is not surprising, since most of the higher plants have this particular grana specialization, with a few exceptions such as *Zea mays* and some other grasses. Moreover, HORI & UEDA (1967) found that the occurrence of grana(-like) structured chloroplast coincides with the disappearance of the pyrenoid. Although the thylakoid lamellae in the chloroplast of the brackish-water species *Ulothrix implexa* and *U. subflaccida* are usually reticulately arranged, their thylakoids are sometimes associated in short lamellae (LOKHORST 1978). This somewhat resembles the thylakoid arrangement into grana and intergrana regions of *Chara.* Thus, from an evolutionary point of view, these two species can be considered to be more advanced in chloroplast fine structure. A concomittant disappearance of the pyrenoid could not be detected in these species, however.

A hypothetical scheme of phylogeny of the pyrenoid ultrastructure in the genus *Ulothrix* derived from this study is depicted in *fig. 2.* At least two lineages come into consideration. In the first line, the complex polypyramidal pyrenoid (Type IV) is regarded to be more primitive than the bilenticular form (Type I). The relationship of types V and VI with the evolutionary lineage 1 is considered merely hypothetical. Notwithstanding that, these two types can be considered more easily to be descendants than predecessors of the polypyramidal form. In this respect, the advancements in the ultrastructure of the pyrenoids of *Ulothrix implexa* and *U. subflaccida* parallel the advancements in the fine structure of the chloroplast. Following lineage 2, the pyrenoid with tubular invaginations which

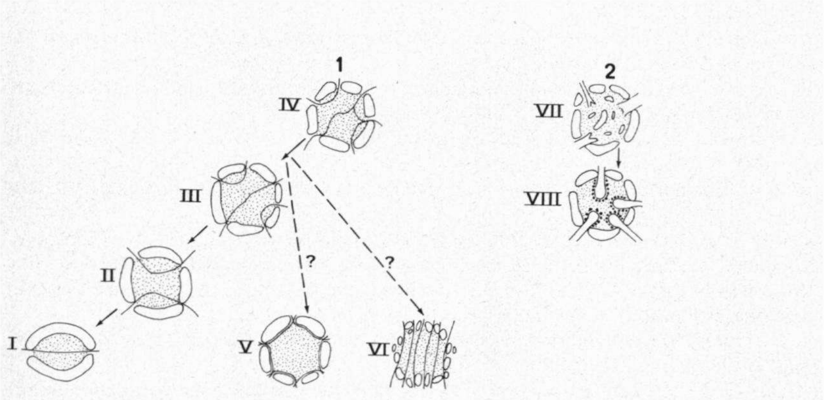


Fig. 2. Hypothetical scheme of the phylogenetic relationship of pyrenoids in the genus *Ulothrix.*

terminate before reaching the center of the pyrenoid (Type VIII) is considered to be more advanced than the pyrenoid form with a rather chaotic arrangement of tubular invaginations in its matrix (Type VII).

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EXPLANATION OF THE PLATES

For all plates the following abbreviations are used: c – chloroplast; ci – chloroplast invagination; n – nucleus; p – peroxisome; py – pyrenoid; pyg – pyrenoid globule; st – starch; t – thylakoid; v – vacuole.

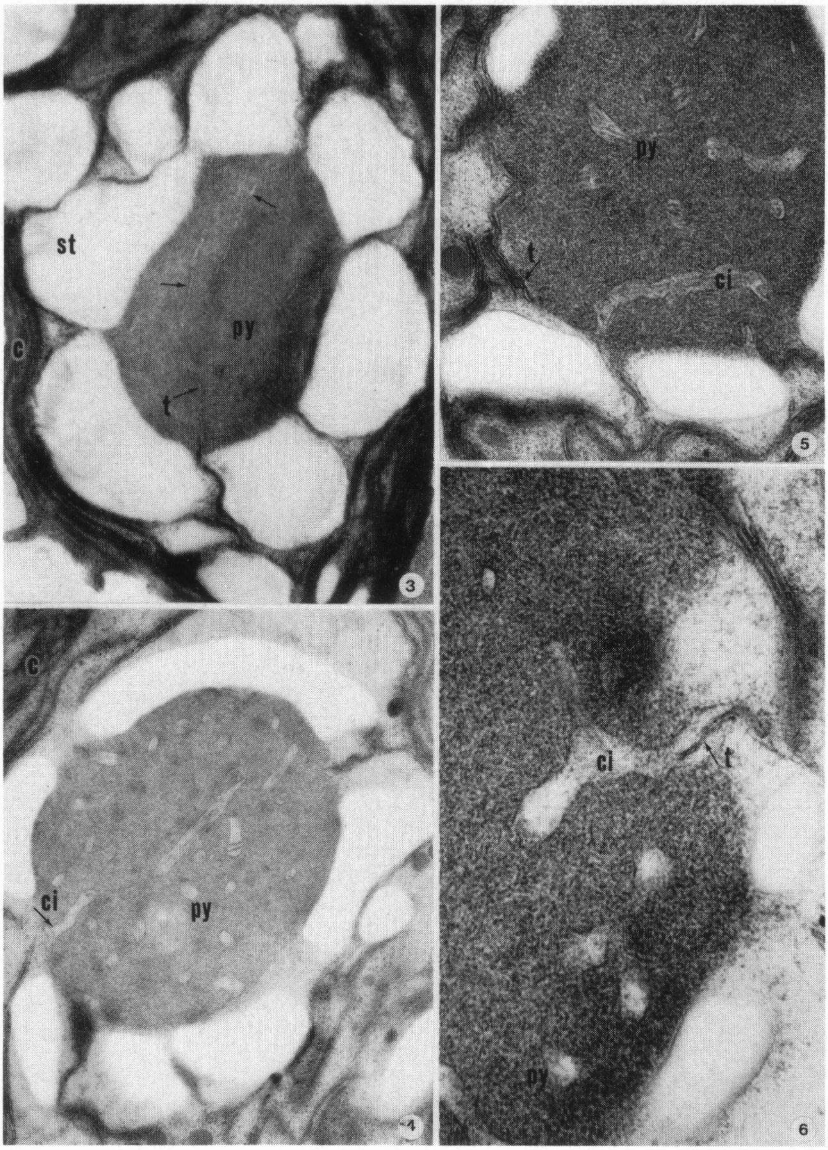

PLATE I. Fig. 3. *Ulothrix subtilis* – pyrenoid penetrated by three winding intrapyrenoidal thylakoid lamellae (arrows). As viewed in this section, the starch shell is divided into 6 starch plates; glut.–OsO_4 fixation, × 20,100. Figs. 4–6. *Ulothrix tenerrima*. Fig. 4. – pyrenoid with tubular chloroplast invaginations, glut.–OsO_4 fixation, × 20,100. Fig. 5.–pyrenoid with reduced starch sheath. Note the chloroplast lamellae close to the periphery of the pyrenoid (arrow), glut.–OsO_4 fixation, × 31,260. Fig. 6. – detail of a chloroplast invagination, glut.–OsO_4 fixation, × 50,250.

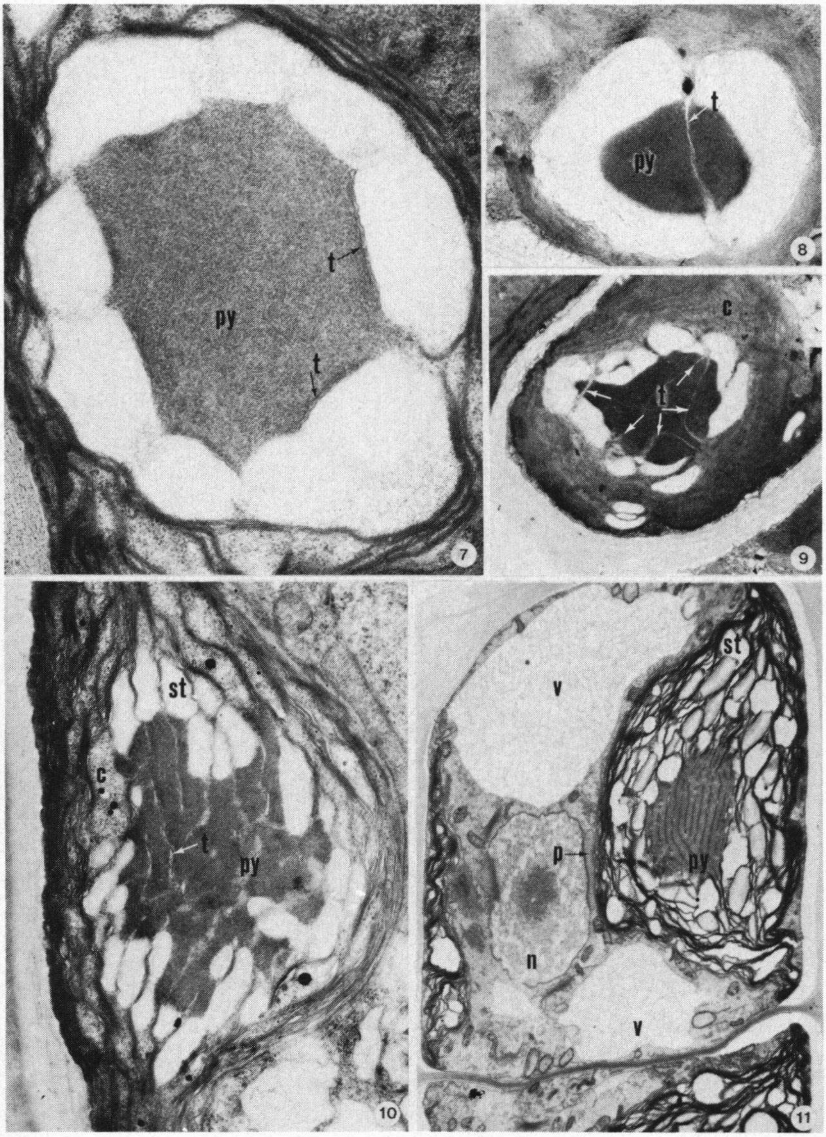

Plate II. Fig. 7. *Ulothrix albicans* – pyrenoid with thylakoid membranes appressed to the periphery of its matrix (arrows), glut.–OsO_4 fixation, × 20,100. Figs. 8 and 9. *Ulothrix mucosa*. Fig. 8. – the bilenticular type of pyrenoid with two hemispherical starch plates, glut.–OsO_4 fixation, × 15,630. Fig. 9. – the polypyramidal type of pyrenoid with a number of smaller discoidal starch grains, glut.–OsO_4 fixation, × 20,100. Fig. 10. *Ulothrix crenulata* – pyrenoid, traversed by numerous thylakoid lamellae, which are positioned more or less parallel to the longitudinal cell wall, glut.–OsO_4 fixation. × 12,280. Fig. 11. *Ulothrix verrucosa* – longitudinal section of a cell. Note multi-layered starch sheath and the peroxisome, positioned between the chloroplast lobe and the nucleus (arrow), $KMnO_4$–OsO_4 fixation, × 4,470.

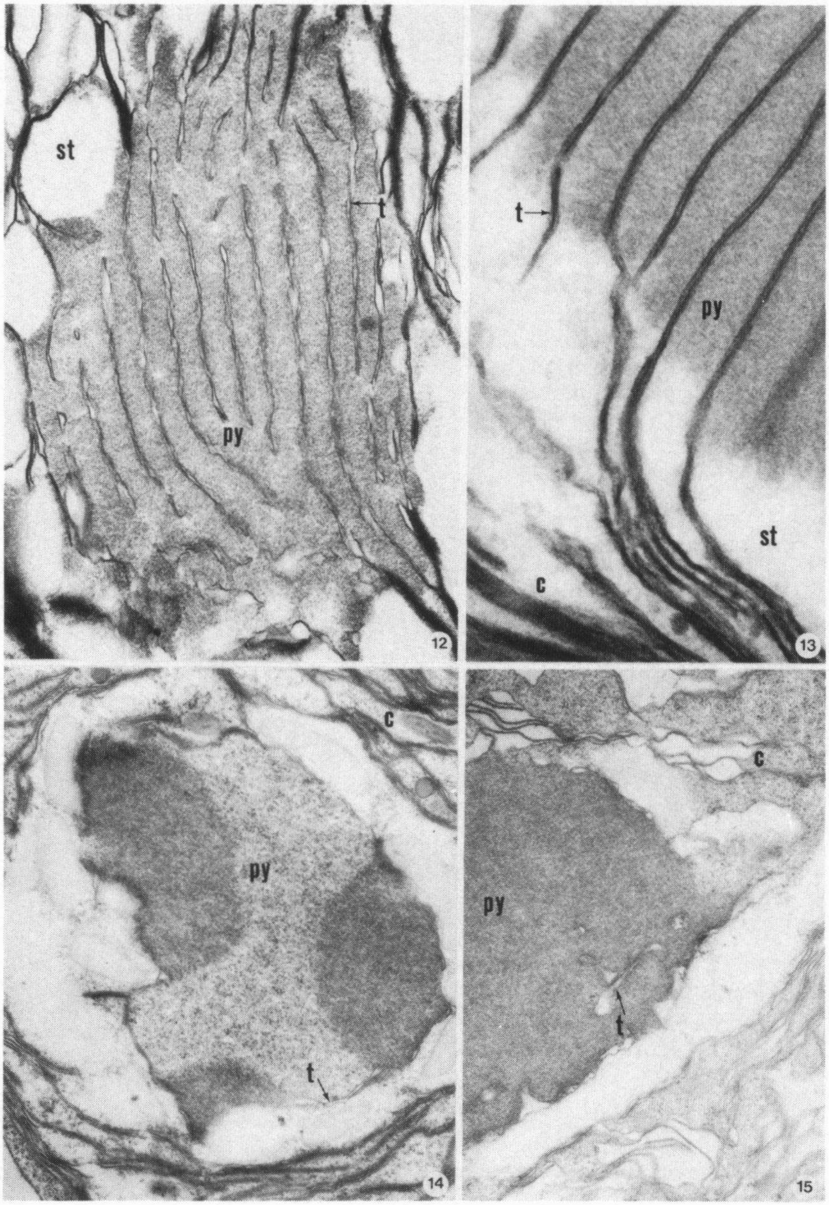

Plate III. Figs. 12 and 13. *Ulothrix verrucosa*. Fig. 12. – pyrenoid with traversing thylakoid lamellae, lying parallel to the length of the filament, $KMnO_4$–OsO_4 fixation, × 20,100. Fig. 13. – detail of the entrance of thylakoids into the matrix of the pyrenoid, glut.–$KMnO_4$ fixation, × 39,850. Figs. 14 and 15. *Ulothrix tenuissima*. Fig. 14. – pyrenoid with a matrix varying in electron density and thylakoids only appressed to its periphery, glut.–OsO_4 fixation, × 12,280. Fig. 15. – detail of a pyrenoid with thylakoids, penetrating a short distance into its matrix, glut.–OsO_4 fixation. × 20,100.

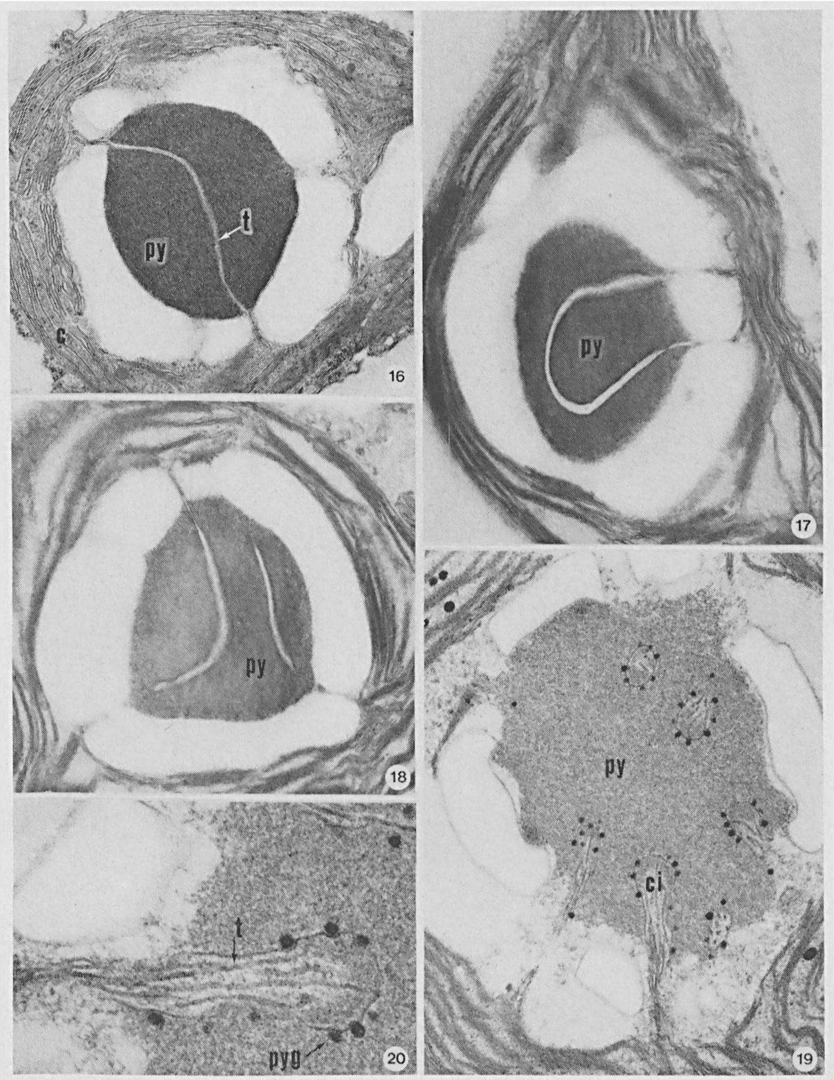

PLATE IV. Figs. 16–18. *Ulothrix zonata.* Fig. 16. – the bilenticular type of pyrenoid, glut.-OsO_4 fixation, × 20,100. Fig. 17. – a variant of the foregoing type, glut.-$KMnO_4$ fixation, × 20,100. Fig. 18. – a longitudinal section of a pyrenoid from which the matrix is cleaved by two winding thylakoid lamellae, glut.-$KMnO_4$ fixation, × 20,100. Figs. 19 and 20. *Ulothrix speciosa.* Fig. 19. – pyrenoid with invaginating chloroplast strands, glut.-OsO_4 fixation, × 20,100. Fig. 20. – detail of a chloroplast invagination. Note the electron-opaque pyrenoglobuli covering the exterior surface of the tubular chloroplast invagination, glut.-OsO_4 fixation, × 39,850.