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TAXONOMIC STUDIES ON THE GENUS ULOTHRIX (ULOTRICHALES, CHLOROPHYCEAE). II

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SUMMARY

In this second report on the taxonomy of the Dutch species of *Ulothrix* Kützing, the morphological and reproductive characters of three other freshwater species, viz. *U. mucosa* Thuret, *U. crenulata* (Kützing) Kützing and *U. verrucosa* Lokhorst (nom. nov.) are discussed. *U. mucosa* shows some relationship with *Binuclearia tectorum* (Kützing) Beger ex Wichmann. These species are shown to differ in several respects, e.g. number of pyrenoids, morphology of the chloroplast, thickness of the H-pieces, morphology of basal and apical cells and structure of the zoosporangium cell wall. Close morphological resemblance exists between *U. crenulata* and *U. verrucosa*. Cell dimensions, morphology of the chloroplast and number of pyrenoids are the most important features for distinguishing these species. Both show taxonomic affinity to *Klebsormidium* Silva, Mattox & Blackwell. On account of chloroplast morphology, number of pyrenoids and shortening of cells the species are classified under *Ulothrix*. Under all photoperiods studied *U. mucosa* is the only species capable of reproduction by means of zoospores, whereas both other species referred to in this paper only show multiplication by fragmentation of filaments. Probably for this reason these species do not show seasonal periodicity in their occurrence in nature.

1. INTRODUCTION

The original descriptions of species and varieties in the genus *Ulothrix* are based mainly on cell dimensions, morphology of the chloroplast and number of pyrenoids. However, for related species, there is considerable overlap in some of these features. In our first paper in this series it is shown that by culturing of the algae many other characters for distinguishing the species, like developmental features and life cycle stages, may be found (LOKHORST & VROMAN 1972).

In the present paper the results of an investigation into the taxonomy of two other species, described in the cell diameter range from 4–14 μ, viz. *U. crenulata* and *U. mucosa*, are presented. A third species, *U. verrucosa*, having larger cell dimensions, is also discussed on account of its close resemblance in morphology and life history to *U. crenulata*.

In a third report data on the taxonomy and life cycle of larger *Ulothrix* freshwater species, viz. *U. implexa* (Kützing) Kützing, *U. tenuissima* Kützing and *U. zonata* (Weber & Mohr) Kützing, will be presented. In a later paper reflections on our concept of the genus, a key for a critical identification of all studied freshwater *Ulothrix* species and a list of doubtful species will be given.

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2. MATERIAL AND METHODS

Several clones of *U. mucosa*, *U. crenulata* and *U. verrucosa* were collected from a number of freshwater habitats in the Netherlands. The salinity of the waters was estimated from water samples from the upper 10 cm and expressed as g Cl′/l (‰ Cl′); 0.3 g Cl′/l was accepted as the upper limit for freshwater, in accordance with general use.

The algae were mostly collected from hard substrata, like stones, sheet-pilings of a bank, submerged stumps of trees, usually around the water level, in more or less sheltered or stagnant waters. Also isolates were made from soft substrata, like sandy bottoms of shallow pools, several cm below the water level in quite transparent and clear waters. In subaereal habitats, like moist soil of ditch sides and wood paths, *U. crenulata* and *U. verrucosa* may also be present. The pH of the waters from which samples were taken ranged from ± 6.0–7.6. Isolating of the filaments was done with aid of a light microscope. A small part of the sample was put on a microscope slide and two drops of aqua dest. were added next to it. With a fine sterile needle, the filaments were taken from the sample portion one by one and drawn through the drops of aqua dest. to remove dirt and contaminating algae. Then they were put into glass boxes, filled with a nutrient solution. If thought necessary, some drops of a GeO_2 solution were added to prevent the growth of diatoms.

For cultivation at first the same photoperiods, culture medium and temperatures as described earlier (LOKHORST & VROMAN 1972) were used. However, this method did not induce asexual or/and sexual reproduction in the species studied. Therefore the algae were subjected to a wider temperature range, viz. 4, 8, 12, 16, 20 and c. 25 °C and for the same reason several other nutrient solutions were tried out, like pure distilled water, DETMER's solution (1888), WICHMANN's solution (1937), HUSTEDE's solutions (1957), modified BRISTOL's solution (DEASON & BOLD 1960), BOLD 3N BRISTOL's solution (STARR 1966), a 2% freshwater Erd-schreiber agar solution and even a marine Erd-schreiber solution and PROVASOLI's enriched seawater (1968). The pH of the solutions was adjusted to the desired value, mostly in the range 6.5–8.5, with some drops of a NaOH or HCl-solution. In an exceptional case the range 3.5–6.5 was used in the freshwater Erd-schreiber solution and WICHMANN's solution. Several photoperiods were used as well, ranging in extremes from absolute darkness to constant lightness for some days. The cultures were illuminated by white fluorescent tubes (Philips de Luxe 20W/33 and 40W/33). Usually the cultures were exposed to a light intensity varying from c. 800–2000 Lux. It was also tried to induce reproduction by changing the light intensity, from practically nought to more than 4000 Lux. These differences were obtained by enclosing the glassboxes with paper or by varying the distance between TL tube and glassbox. The light intensity, falling on the surface of the glassboxes, was measured with a METRAWATT-Luxmeter (type "Metrux-K"). Refreshing of the cultures took place every three weeks. India Ink and a 1% methylene blue-solution in distilled water was employed to determine which of the organisms investigated has a

mucilaginous sheath, if any. A 1 % methylene blue-solution or a 1 % saffranine-solution in distilled water and the chlor-zinc-iodine reaction were used respectively for localisation of pectin and cellulose substances in the cell wall. A 0.01 % solution of neutral red in distilled water was employed to determine the position of vacuoles. Aqueous JKJ aided to establish the presence of starch and to investigate the morphology and number of pyrenoids. By adding this reagent the nucleus could be located easier. Also, volutin was detected by using the method described by GERLACH (1969).

Herbarium material was also studied. This was borrowed from the Jardin Botanique de l'État at Brussels (BR), Botanical Museum and Herbarium at Copenhagen (C), Herbarium Universitatis Florentinae, Istituto Botanico at Florence (FI), Rijksherbarium at Leiden (L), Muséum National d'Histoire Naturelle, Laboratoire de Cryptogamie at Paris (PC), Botanical Department, Naturhistoriska Riksmuseum at Stockholm (S) and Naturhistorisches Museum at Vienna (W). Abbreviations in brackets are according to the Index Herbariorum (LANJOUW & STAFLEU 1964). The herbarium specimens could be made to resume their original habit by treatment with a detergent, which was composed as follows: 50 cc distilled water and 2 drops of a concentrated solution of Teepol. Heating did not aid in this process, moreover by this treatment the cell contents are easily destroyed.

3. SOME REMARKS ON THE RELIABILITY OF TAXONOMIC FEATURES IN HERBARIUM SPECIMENS

Because of the larger dimensions of the algae studied the results were more satisfactory than those of our first study. Moreover, broader use was made of the above-mentioned chemicals. Still, the variation in morphological characters in wild material, caused by environmental conditions and difference in stages of growth, made it difficult to identify the nomenclatural types and other herbarium specimens of the morphologically related genera *Ulothrix*, *Klebsormidium* Silva, Mattox & Blackwell 1972 (= *Hormidium* Kützing sensu Klebs 1896, *Chlorhormidium* Fott 1959, 1960) and *Microspora* Thuret 1850. Left out of consideration were representatives of the other ulotrichacean genera *Geminella* Turpin emend. Lagerheim 1883 and *Gloeotila* Kützing 1843. Usually they are distinguished rather easily in the dried state because the generic characteristics, like cells with rounded ends, which are only slightly adherent to each other or separated, are preserved well in the herbarium specimens. In the above-mentioned group of related genera the shape of the chloroplast is used, among others, as a fundamental criterium for distinguishing these genera. The differences may disappear for a great deal on drying however, caused by the varying nature of the chloroplast in different species of *Microspora* and by the phenomenon that primary reticulate chloroplasts, present in some species of this genus may appear as a parietal plate-like chloroplast in a dried state (*fig.* 1Ca). Moreover the stellate chloroplast in uniseriate *Prasiola* (= *Schizogonium*) species may also sometimes seem to be parietal-like by

extending over the cell lumen (*figs. 1Da, 1Db, 1Dc*). To obtain a better understanding the changes in morphology have been investigated in drying some filaments and studying the effects.

In *Ulothrix* the drying-process usually does not strongly modify the girdle-shaped chloroplast; it remains parietally expanded around the cell lumen (*figs. 1Ae, 1Af, 1Ag*). However, in a few filaments cells may be observed, which respectively show an axial (*figs. 1Aa, 1Ac*), withdrawn (*figs. 1Ad, 1Ai*), granulated (*fig. 1Ab*) or longitudinally folded (*figs. 1Ah, 1Aj*) chloroplast. The latter gives the impression of being composed of connected segments. In older herbarium specimens the chloroplast sometimes is hyalin. In living *Ulothrix*-cells the chloroplast usually is found appressed to the longitudinal cell wall; it may approach cell length and only in exceptional cases, especially under optimal external conditions, there is also a small lobe of the chloroplast extending along the cross walls. In living *Microspora*-cells there is nearly always a remarkable concentration of chloroplast material at the cross walls. This results in striking differences in morphology of dried chloroplasts of both genera (*figs. 1A, 1C*). Cross walls in dried *Ulothrix* cells are usually free of chloroplast material, whereas those in *Microspora* cells are covered with chloroplast material (*fig. 1C*). At the same time this chloroplast contracts to a lesser or greater degree near the centre of the cell lumen (*figs. 1Cb, 1Cc, 1Cd*). The same phenomenon has been noted in *Oedogonium*-species, but in this genus misidentification is precluded by a lot of other distinguishing features, like the ring-like scars on the wall. It is confusing that in *Microspora* the chloroplast may also be broken up into more or less connected longitudinal segments (*figs. 1Ca, 1Cb*).

The size of the chloroplast, important as a generic feature for *Ulothrix* and *Klebsormidium*, when reproductive stages are absent, will provide some difficulties, when it is in an intermediate stage. In that case an arbitrary decision has to be taken. However, in *Klebsormidium* the chloroplasts mostly are withdrawn (*fig. 1Bf*) and may be banana-shaped (*figs. 1Bc, 1Bd*), although the chloroplast plate may be conserved excellently (*figs. 1Ba, 1Bb, 1Be*). In course of time the characteristic morphology of the parietal chloroplast, for example lobed or unlobed, may disappear completely in dried material. Therefore chloroplast morphology must be given a rather low diagnostic value, especially when reproductive stages are present. The chloroplast in dried *Prasiola*-species is usually more concentrated towards the centre of the cell lumen (*figs. 1De, 1Df*), but may still give a plate-like impression (*figs. 1Da, 1Db, 1Dc, 1Dd*).

Pyrenoids, important as a generic feature, are usually not visible in older dried filaments. By adding of JKJ to well-preserved material, the presence of pyrenoids can be detected. Therefore *Prasiola* may be identified easily in the herbarium specimens studied: its pyrenoid, when present, is always found in the centre of the cell. *Microspora* species lack pyrenoids, but one may be deceived by local, dense concentrations of starch. As a matter of course adding of JKJ to *Ulothrix*-cells is less successful when cells are filled by strong accumulation of starch.

By using colouring-reagents the cell wall construction is usually made quite clear. Especially the structure of *Microspora*-cell walls, which are composed of H-shaped pieces, is brought out more prominently. Still this character has to be used with care because some *Ulothrix* species viz. *Ulothrix subtilis*, *U. tenerrima* a.o., under less favourable conditions show a thickened cross wall together with less thickened longitudinal cell walls, thus showing some resemblance to *Microspora*-like H-pieces. In *Prasiola* the cell wall may be conspicuously lamellated; especially the inner layer is striking (*figs. 1De, 1Df*). The structure of the cell wall, smooth or roughened, is unaltered in drying filaments.

When present, specific reproductive stages, like gametangia and zoosporangia, are distinguished without problems. Through the use of JKJ the zoospores and gametes are countable. The basal cell may be shaped rather characteristically. Mostly, in the specimens studied this cell is absent, however due to less careful collecting. To preserve this cell it is necessary to collect, besides the alga, the substratum to which it is attached.

It may be concluded that in spite of occasionally occurring difficulties a detailed investigation of the morphological characters by using colouring reagents, may bring to light the generic attributes. In most cases the whole of these characters ensures a correct identification.

4. INFLUENCE OF CULTURING ON MORPHOLOGY AND LIFE HISTORY

The morphology of the algae in culture is quite identical with that seen in nature. Under both circumstances the length/width ratio in young filaments is generally large and decreases in older plants (*figs. 2A, 2B, 7A, 7B*). Different day-length periods, temperatures and freshwater culture media do not appear to affect the dimensions of the cells in any significant way. The shape of the chloroplast depends on age of the filaments and culture conditions. In young and mature filaments the chloroplast usually is shaped like an unclosed parietal band, generally extending along the full length of the cell; the longitudinal margins may be unlobed or (slightly) lobed (*figs. 2A, 2B, 7A, 7B, 9*). Under highly favourable culture conditions the mature chloroplast may be more strongly developed yet, attaining a perfectly closed band-shape; sometimes it extends lobes along the cross walls or becomes slightly fragmented (*figs. 2B, 7B*). In old filaments, present in exhausted media, the morphology of the chloroplast and the number of pyrenoids is obscured by accumulation of storage products (*figs. 7C, 10A*).

The cell wall is thin in young filaments of *U. crenulata* and *U. verrucosa*, but in exhausted liquid cultures and after a long period of cultivation on agar plates it may be rough and thickened (*figs. 7C, 10A*). The cell wall of one species, to wit *U. mucosa* is surrounded by a mucilaginous sheath, which, however, may sometimes hardly be detectable. Under optimal culture conditions the pyrenoids of *U. crenulata* and *U. verrucosa* are surrounded by a distinct starch sheath, which obscures the contours of the pyrenoids (*figs. 7A, 7B, 9*). During

all stages of the life cycle in the filaments, but especially in more or less exhausted cultures, H-pieces are present, covering the cross walls (*figs. 2A, 2B, 7A, 7B, 9, 10C*). Apical cells are always rounded, never tapering, whereas the morphology of the basal cell is constant as well.

The clones used in the study of *Ulothrix crenulata* and *U. verrucosa* did not produce any zoospores or gametes under the different light regimes. All the other experiments with different culture media and temperatures also yielded negative results. Under short-, intermediate- and long-day conditions, the clones of *Ulothrix mucosa* showed zoosporogenesis, especially after refreshing of the medium (*fig. 3*). All cultures of the three species studied showed infrequent fragmentation of filaments under the above-mentioned photoperiods (*figs. 4D, 8B, 10C*); separation, however, was not always complete. Under extremely exhausted culture conditions the thick-walled filaments of *Ulothrix crenulata* and *U. verrucosa* may become strongly constricted, which suggests a passing into the akinete phase. However, true akinetes, as occurring in *U. zonata* (to be published shortly), never have been observed. After refreshing the medium cell divison takes place in those akinete-like cells and a normal habit is assumed.

5. MORPHOLOGY AND TAXONOMY OF THE SPECIES STUDIED

5.1. Ulothrix mucosa Thuret

Ulothrix mucosa Thuret, 1850 p. 223.

Synonyms:

Conferva zonata Weber & Mohr var. *arachnoidea* Chauvin (nom. nud.), 1831 fasc. 6, no 134;

Ulothrix oscillarina Kützing sensu Stockmayer, ex Crypt. exsic. Mus. Hist. Nat. Vindobon. no 2941.

5.1.1. Living material

Clones were isolated from the following localities: *Gelderland*, on wood in a pool along the river Waal, near Ochten; *Noord-Brabant*, in an old fishing pond near Valkenswaard, on piles and stones at the water level in stagnant water in a rather sheltered place, growing together with *Oedogonium* species; in a ditch near Oisterwijk, on substrata like *Pinus*-stumps and *Quercus*-leaves, intertwined with *Mougeotia*, *Microspora* and *Tribonema* species

5.1.2. Morphology

The straight, in older cultures more or less curved filaments, are always unbranched and consist of uniseriate cells with a parietal, unclosed girdle-shaped chloroplast, which usually covers up to three quarters of the cell circumference. In exceptional cases the girdle is fully closed.

In young filaments the chloroplast is slightly and regularly lobed along the longitudinal margin (*figs. 2A,4B*), but in full-grown cells a more irregular chloroplast could be observed, sometimes covering the cross walls of the cells (*fig. 2B*).

In exhausted culture medium, the cell contents grow denser and show a compact mass (*fig. 2C*).

Under less favourable conditions vacuoles may be observed without colouring. Pyrenoids are present, their number may vary from 1–4. A conspicuous starch sheath could not be demonstrated; only a thin envelope of starch is present, through which the contours of the pyrenoids are clearly distinguishable from the surrounding chloroplast (*figs. 2A, 2B*). Fine volutin-like bodies are distributed throughout the cell.

The cells are mostly cylindrical; in older filaments the ends are more or less rounded, but they always adhere closely to one another (*fig. 2C*). Usually the filaments are surrounded by a layer of mucilage (*figs. 2A, 2B*), the contours may already be visible without colouring reagents, especially in exhausted culture media in which the mucilaginous sheaths consist of a firmer substance (*fig. 2C*). In germlings the sheath is usually only slightly developed (*fig. 4B*), whereas in more full-grown filaments a thicker envelope is to be seen with a width up to 5.6 μ (*figs. 2B, 2C*). However, sometimes in mature cultures this sheath is lacking almost completely and only developed as a very thin layer (not drawn in one of the filaments in *figs. 2A* and *2B*).

In germlings the presence of H-pieces sometimes is observed, but these may be more conspicuous in older filaments (*fig. 2B*).

In young filaments the external limit of the cell wall is easily seen, in older cells, however, it may be rather indistinct. Filaments have a diameter from 9.8–14.0(–15.4) μ and are $\frac{1}{4}$-1 times as long. Young filaments, in which zoosporogenesis usually does not take place, consist of cells varying from 6.3–9.1 μ in diameter and $\frac{3}{4}$-2 times as long. The cell diameters found most often in full-grown cultures under short-day conditions are in the range 9.8–11.9 μ, whereas a range of larger dimensions, viz. 10.5–13.3 μ, is observed in cultures, grown under longer photoperiods (see *table I*).

In wild material this complete range of dimensions could also be observed, with cells usually shorter than wide. The chloroplast of these cells is hardly lobed along its longitudinal margin. The apical cell is rounded and uncapped. Under culture conditions this cell is sometimes seen to behave, after touching the bottom of the glassbox, like the basal cell. The latter, however, never develops into a typical rhizoidal *Ulothrix*-holdfast. Only a slight extension of the cell length and a decrease of the width in its lower part could be observed (*figs. 4B, 5C*).

The basal cell produces a mucilaginous ball, consisting among others of pectin, by which the alga is attached to the substratum. This process is already visible in one- or two-celled germlings (*fig. 4B*). In all developmental stages this firm ball or disc only surrounds the lower part of the basal cell, which phenomenon is characteristic for this species. Sometimes when the zoospores settle closely together, a broad plate may be formed (*fig. 4C*), on which, in course of time, several filaments grow together.

Table 1. Cell diameter frequency-distribution (%) of filaments of *Ulothrix mucosa*, in relation to cultivation-time in weeks, respectively under short- and long-day conditions.

	Short-day								Long-day							
time:	2w.	3w.	4w.	5w.	6w.	7w.	8w.	9w.	2w.	3w.	4w.	5w.	6w.	7w.	8w.	9w.
diameter																
6.3μ	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7.0	25	13	–	–	–	–	–	–	5	1	–	–	–	–	–	–
7.7	45	23	10	1	–	–	–	–	29	3	2	1	–	–	–	–
8.4	20	29	26	9	4	5	–	–	27	20	11	3	1	4	3	–
9.1	3	20	34	27	18	15	11	8	24	19	14	8	6	3	5	–
9.8	3	9	16	25	17	20	21	16	11	24	20	13	11	6	7	4
10.5	1	5	12	26	35	25	30	34	4	19	26	32	24	20	20	18
11.2	–	1	2	11	16	23	23	22	–	10	18	20	19	35	26	24
11.9	–	–	–	1	9	9	12	14	–	3	6	12	13	16	16	16
12.6	–	–	–	–	1	3	2	3	–	1	2	4	15	6	14	22
13.3	–	–	–	–	–	–	1	2	–	–	1	2	5	5	5	11
14.0	–	–	–	–	–	–	–	1	–	–	–	–	3	5	4	3	3
14.7	–	–	–	–	–	–	–	–	–	–	–	1	1	1	1	1
15.4	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1

5.1.3. Reproduction

After transferring filaments from the stock-cultures, preserved at 8 °C under intermediate day-length, into short- and long-day conditions zoosporogenesis takes place in all cells, except the basal one, and leads to the formation of 1–4 zoospores per cell (*fig. 3B*). In nature mostly 1–2 zoospores were seen. The diameter of the zoosporangia usually ranges from 9.8-14.0(–16.1) μ. In young filaments, about 10–15 cells in length, zoosporogenesis only takes place after repeated refreshing of the culture fluid, the dimensions of the zoosporangia then range from 6.3–9.1 μ (*fig. 3A*).

Zoosporangia which contain only one zoospore may be distinguished from the vegetative cells by the conspicuous presence of the eye-spot, the more ellipsoid shape of the cell and the somewhat isolated location of the zoosporangia in the filament (*figs. 3A, 3B*).

Upon the release of the zoospores the longitudinal cell wall becomes partly or completely gelatinised and dissolves (*figs. 3B, 3C*). In the last case the very conspicuous H-pieces may stay behind, held together by the gelatinous sheath (*fig. 3C*). Upon dissolving of the cell wall, the filaments usually become more or less curved. The releasing of the zoospores is delayed, when the mucilaginous layer is well-developed.

The zoospore is more or less pear-shaped (*fig. 4A*), containing a parietal, rather irregular, cup-shaped chloroplast mostly with one pyrenoid (*fig. 4A*). Sometimes more (–3) pyrenoids are observed. The zoospores possess a conspicuous, mostly anteriorly-located, eye-spot and 4 flagella, implanted closely together. With the magnifications used (to 1600×) no apical papilla could be observed.

The zoospores are positively phototactic in swimming. After swarming,

during which the movement is rather quiet, the zoospore at first becomes spherical, than attaches itself to the substratum with its flagellar pole foremost and loses its flagella. Elongation and subsequent division of the zoospore produces a new filament (*fig. 4B*).

In culture also germination of zoospores, which had no contact with the glassbox wall, could be observed. The germination process of attached and free floating germlings is identical, the same cell dimensions being measured among others. The zoospores vary in length from (8.5-)10.2-17.0 μ and from 5.1-10.2 (-11.9) μ in width. Zoospores that are produced singly in a cell, are usually larger than those swarmers, released in groups of 2 or 4. The dimensions of the zoospores are not related to the thickness of the arising germling. Under less optimal conditions aplanospores could be observed (*fig. 3C*), which after refreshing of the culture medium immediately germinated in the mother filament.

Vegetative propagation may take place by fragmentation (*fig. 4D*), which process is accelerated by exhausting of the medium. The arising segments may already be provided with a mucilaginous disc at one end (*fig. 4D*). In culture and in natural material the same zoosporogenesis and aplanosporogenesis could be observed, true akinetes never having been found. Once in nature an intercalary palmella-stage, caused by irregular cell division and gelatinisation, was found.

5.1.4. Taxonomy

Phycologists always have had problems with the identity of *Ulothrix mucosa* Thuret because of the incomplete species diagnosis and scarse drawings (1850, p. 223, pl. 18, figs. 8-11). The author only gave some information on the morphology of the filament and on asexual reproduction, but not on cell length and diameter. RABENHORST (1863) reported a cell diameter range from 1/135–1/112‴ (17.2–20.8 μ), but in 1868 he considered *U. mucosa* to be a synonym of *Ulothrix tenerrima* Kützing. DE-TONI (1889) did not recognize this species either and referred *U. mucosa* as a synonym to *Hormiscia subtilis* (Kützing) De-Toni var. *tenerrima* (Kützing) Kirchner. HEERING (1914) and SKUJA (1956, 1964) reported in their concept of *U. mucosa* that filaments are provided with a stratified mucilaginous sheath and have a cell diameter ranging from 8–10 μ and 5–8 μ respectively. However, SKUJA's drawings differ completely from those of THURET (1850). Regretfully no herbarium material of these two authors was available. Consequently, we got the impression that in course of time a modified *U. mucosa* concept is established.

Recently, even the right of existence for this species is disputed by RAMANATHAN (1964), who reported ". . . that one is still unable to decide whether *U. mucosa* is only a gelatinising condition of some other species . . .".

In our opinion, in spite of the questionable taxonomic history, as described above, *Ulothrix mucosa* Thuret 1850 is to be designated as the name of the present alga. Filaments, preserved in PC, s.n., in a very excellent condition and provided with the annotation "Ulothrix mucosa, Rentilly (S. et M.), Mai, 1846",

written in ink in THURET's handwriting, showed the same cell dimensions as the species studied here. Furthermore there is striking resemblance in the shape of the chloroplast, the small H-pieces, shape and number of pyrenoids, the mucilaginous sheath and the mucilaginous disc of the basal cell in this lectotype.

A specimen in CHAUVIN (1831), Alg. exs. no 134, labelled as *Conferva zonata* var. *arachnoidea* (isotype in L as No. 939.67-782), also showed striking morphological resemblances to our alga. Quite probably CHAUVIN has named this variety "arachnoidea", because the young filament present in the herbarium specimen, collected in free-floating stage, had crowded together by uniting of the gelatinous basal discs. In this way a centrifugal growth was suggested. However, this name must be considered as a *nomen nudum*.

Also, the possibility is considered to indicate the earlier published name *Ulothrix oscillarina* Kützing (1845) to the alga studied.

In several algal flora's the concept of *U. oscillarina* does indicate a similarity in morphological features, here described for *U. mucosa*. For example, the herbarium specimen, named *U. oscillarina* in Cryptog. exs. Mus. Hist. Nat. Vindobon., no 2941 (det. Stockmayer), preserved in BR, could be identified as *U. mucosa*. However, lectotype material of *U. oscillarina* Kützing, preserved in L as No. 939.26-237 and labelled among others respectively as "Conferva compacta Roth, Conferva fugacissima" in ink in an unknown handwriting only shows indistinct characters. For example, no pyrenoids could be demonstrated. KÜTZING's descriptions (1845, 1849) and drawings in Tab. Phyc. II, taf. 88, fig. I (1852) are too vague to permit a conclusion as to its identity, therefore *U. oscillarina* Kützing is considered as a doubtful species. In KÜTZING's herbarium a specimen was found, preserved in L as No. 939.26–238, labelled in KÜTZING's hand as "Ulothrix mucosa Koch, Moorgräben bei Jever, 1/300‴ –aeq.". This combination is recommended to be invalid and illegitimately published. Moreover no date was given and study of the specimen only showed a well-developed mixture of algae, viz. *Klebsormidium*, *Tribonema*, *Microspora* and *Mougeotia* species.

5.1.5. Taxonomic relationships

Studying the descriptions of *Binuclearia tectorum* (Kützing) Beger ex Wichman in WITTROCK (1886), SCHRÖDER (1898), WICHMANN (1937) and LUKAVSKÝ (1970) it appears that there is a taxonomic relationship to *U. mucosa*. Both taxa possess a mucilaginous sheath of varying thickness, a typical *Ulothrix*-like chloroplast and show lack of sexual reproduction. The H-pieces are small or lacking in *U. mucosa* and large, small or lacking in *Binuclearia tectorum*. Zoospores from both species produce a mucilaginous ball during germination. For this reason comparative culture experiments have been carried out, by which several media and low pH-ranges have been used.

Clones of *Binuclearia tectorum* could only be isolated from acid, dystrophic waters; *Drenthe*, Brunstingerveen, on *Juncus effusus*; in a peat-fen near Hijkerveld on *Erica tetralix*; *Noord-Brabant*, Choorven near Oisterwijk on inundated *Pinus*-trunks. From the field-observations and culture experiments it may be

concluded that *Binuclearia tectorum* must be kept separate from *U. mucosa*. The data, which, in our opinion, justify this decision, are summarised in *table 2*.

Table 2. Survey of morphological, growth and reproductive features for distinguishing *Ulothrix mucosa* and *Binuclearia tectorum*.

Ulothrix mucosa	*Binuclearia tectorum*
Apical cell uncapped *(fig. 2A)*	Apical cells usually provided with a cap, protruding from the mucilaginous sheath, *(fig. 5B)*
Basal cell with a firm mucilaginous disc or ball, in which the cell is only partly implanted *(figs. 4B, 5C)*. Basal cell usually tapering *(figs. 4B, 5C)*	Basal cell with a firm mucilaginous ball, which usually surrounds the filament over 1–2 cells above the basal one in older stages *(fig. 5A)*. Basal cell usually not modified to even slightly widened *(fig. 5A)*
1–4 pyrenoids	Only 1 pyrenoid *(figs. 6A, 6B, 6C)*
Chloroplast usually more complexly organised *(fig. 2B)*	Chloroplast usually less complex *(figs. 5A, 6A, 6B, 6C)*
Volutin-bodies always scattered in the cell lumen	Volutin-bodies more pronounced, scattered or located in vacuoles at the distal ends of the cell *(fig. 6A)*
H-pieces small, when present *(fig. 2B)*	H-pieces, especially in nature, conspicuous *(figs. 6A, 6B)*; length to 8 times the width
Growth-optimum at pH values ± 5.6–8.3. Desintegration of filaments at lower values	Growth-optimum at pH, ranging from ± 3.5–6.5. At higher values dying of filaments
Wall of zoosporangia dissolves (nearly) completely before releasing of zoospores *(figs. 3B, 3C)*	Zoosporangium wall conspicuous with a waved, inner wall layer after releasing of the zoospore *(figs. 6A, 6B)*
Number of zoospores 1–4 *(fig. 3B)*	Only 1 zoospore (LUKAVSKÝ 1970, taf. II, *figs. 9, 10)*

5.2. Ulothrix crenulata (Kützing) Kützing

Hormidium crenulatum Kützing, 1845 p. 193; emend. as *Ulothrix crenulata* (Kützing) Kützing, 1849 p. 350.

5.2.1. Living material

Clones were isolated from material, gathered at the following localities: *Groningen*, Zuidlaarder meer, on sheet-piling extending from the water level to 20 cm above, among mosses; *Gelderland*, Aaltensche Slinge, on stones among the moss *Bryum argenteum*, about 10 cm above the water level; Aaltensche Slinge, on stones among *Spirogyra*, *Ulothrix*, *Klebsormidium* and *Oscillatoria* species in slowly running water below the water level; *Limburg*, Ospelse Peel, on the bank of a ditch on sandy soil.

5.2.2. Morphology

The straight or twisted-growing filaments are unbranched. In highly exceptional cases longitudinal division of cells may take place (*fig. 8A*). Filaments

consist of firmly united cells, with a parietal, girdle-shaped chloroplast, which usually covers more than half of the cell lumen. It is quite exceptional that a fully closed girdle-shaped chloroplast is present. In long cells the encircling chloroplast usually is more or less lobed along its longitudinal margins (*figs. 7A, 8B*), but in short ones it is mostly not (*fig. 8A*). The chloroplast may approach cell length; under optimal growing conditions even the cross walls may be covered (*fig. 7B*). In extremely exhausted culture medium the cell contents may shrink (*fig. 7D*).

In most cells only one hyaline pyrenoid is found, embedded in the chloroplast, but in the filaments cells may be present with more, up to three pyrenoids. Mostly the pyrenoid is surrounded by a distinct envelope of starch, which may, however, sometimes be very inconspicuous. Volutin-bodies are distributed throughout the cell lumen.

Cells are pronounced cylindric, in older cultures with more or less rounded ends but always adhering closely to one another. In this case the filaments may become (slightly) constricted at the cross walls (*fig. 7B*). In young and mature cultures in liquid media the cell wall is thin (*figs. 7A, 7B, 8A*), unlamellated. In exhausted liquid cultures and after a long period of cultivation on agar plates the cell wall is thickened, usually becoming rough-walled and somewhat crenulated (*fig. 7C*). At the same time, these cells become filled with storage products like starch and oil-like bodies, by which the shape of the chloroplast may be more or less distorted (*fig.7C*). H-pieces may be present, inconspicuous in a young stage (*fig. 7A*), but more striking in older ones (*figs. 7B, 7D*). Especially in acid culture medium these H-pieces are provided with mucilaginous appendages (*fig. 7B*), but gelatinisation of filaments was never observed. The apical cell is rounded (*fig. 7A*), but never capped, not even when H-pieces were frequently present in the filaments.

The alga is mostly free-floating in culture. Cells, coming into contact with the walls of the glassbox will never develop into a rhizoidal holdfast, unlike what may happen in *Ulothrix tenerrima* and *U. implexa*. The basal cell of segments, formed by fragmentation of the filaments, never produces a typical basal *Ulothrix*-holdfast either. Attachment to the substratum is achieved by means of a soft gelatinous ball secreted by the wall of the undifferentiated basal cell (*fig. 8C*). At the same time the wall of the basal cell may thicken (*fig. 8C*).

Under short-day conditions full-grown filaments have a diameter ranging from 11.2–13.3 μ and are $\frac{1}{3}$–1 (–$1\frac{1}{2}$) times as long. Cell diameters measured most frequently are 11.2 and 11.9 μ. Under long-day conditions individual cells are a little wider, diameters ranging from 11.2–14.0 μ, with highest frequencies at 11.9–12.6 μ. Thick-walled filaments may even reach a diameter up to 15.4 μ. No significant difference existed between measurements of liquid- and agar-growing material. Young filaments, which grow from fragmented older filaments, vary from 9.1–10.5 μ in cell diameter with cells usually as long as wide. In wild material the same cell diameter is measured.

5.2.3. Reproduction

Only vegetative multiplication through fragmentation of filaments is found (*fig. 8B*). However, fragmentation is not as regular as in *Klebsormidium* species, where filaments have been seen to desintegrate into individual cells and few-celled segments. This type of vegetative reproduction especially takes place in older filaments, when brought into fresh agar medium. Under favourable conditions each segment quickly develops into a "young" filament, during the process of which the cells narrow (*fig. 7A*).

5.2.4. Taxonomy

Since this alga is found both in terrestrial and aquatic environment, herbarium specimens described as gathered from both habitats were studied.

In our opinion the name *Ulothrix crenulata* (Kützing) Kützing, proposed in 1845 as *Hormidium crenulatum* and amended in 1849 as *U. crenulata*, respectively with a cell diameter range of 1/280‴ (8.3 μ) and 1/180–1/150‴ (12.9–15.5 μ) is to be designated for this alga. Filaments, preserved in L as No. 939.67–834, among others with the annotation "1/280‴, Hormidium crenulatum, Patavii, Meneghini", written in ink in KÜTZING's hand showed nearly the same cell dimensions as our alga, their diameter being 9.8–12.6(–14.0) μ and their length ⅓–1½ times diameter. In dried filaments cells were observed to be mostly shortened. A conspicuously crenulated cell wall, also present in our cultures and in wild material, 1(–3) pyrenoids, a prominent, band-like chloroplast and uniseriate growth of filaments also could be observed in the lectotype specimen.

GAY (1888), after investigation of only "non-type" herbarium specimens, viz. no 615 in RABENHORST's Alg. Sach., labelled as *Ulothrix crenulata* Kützing and no 637 in WITTROCK & NORDSTEDT's alg. exs., labelled as *Ulothrix crenulata* β *corticola*, transferred this taxonomic unit to the genus *Schizogonium*. In these herbarium specimens GAY (1888) observed a stellate chloroplast, first stated in *Schizogonium* by SCHMITZ (1882). At the same time, an occasional longitudinally divided cell or a short double row of cells was observed now and then in these specimens. According to the International Code of Botanical Nomenclature however, GAY (1888) was not right in transferring a specific epithet to another genus, without analysing the existing original material. Our investigations of the above-mentioned specimens, preserved in L and FI, support GAY's observations on their morphology. RABENHORST and WITTROCK & NORDSTEDT have acted in the sense of KÜTZING's original descriptions in naming the alga they collected *Ulothrix crenulata*: nearly the same cell dimensions were observed. They only have failed to interpret the morphology of the chloroplast, which is not *Ulothrix*-like. Chloroplast morphology must have been a great problem for past-century phycologists, caused probably by lack of refined optics.

HANSGIRG (1888) confirmed GAY's observations, but was of the opinion that the generic name *Hormidium* should be applied to uniseriate filamentous schizogoniacean algae. He re-established the combination *Hormidium crenulatum*, his concept of the morphology of this taxon, however, differing from KÜTZING's. DE-TONI (1889) and WILLE (1890) followed the essence of HANSGIRG's

generic concept. Major changes in the concept of *U. crenulata* were again introduced by BRAND (1913). Studying samples collected from Starnberg (Germany), he applied the name *Ulothrix* (*Hormidium*) *crenulata* to a filamentous green alga with a parietal chloroplast, covering the whole cell wall, but without a pyrenoid. In this alga cell divison usually took place in transversal direction, less frequently in longitudinal direction also, resulting in cell tetrades. It was not possible to compare BRAND's alga with ours, because no material, collected from the above-mentioned place, was available in BRAND's herbarium in W. On account of the phenomena reported by BRAND (1913) *Ulothrix crenulata* (Kützing) Kützing sensu Brand can not be considered to be identical with the alga studied by us. Based on BRAND's observations HEERING (1914) removed this taxon into a separate genus *Hormidiopsis*, but from our study it becomes clear that the generally accepted combination *Hormidiopsis crenulata* (Kützing) Heering is not allowed.

5.2.5. Taxonomic relationships

When comparing the alga in question with the generic descriptions of *Klebsormidium* and *Ulothrix*, its intermediate taxonomic position becomes apparent. The absence of a holdfast-cell and its method of reproduction through fragmentation indicates a relation to the genus *Klebsormidium*. On the other hand the organisation of the chloroplast, number of pyrenoids and shortening of cells in mature filaments are typical *Ulothrix*-like characters. For correct identification of such a species the mode of asexual reproduction must be known, because the two genera can be distinguished infallibly by the morphology of the zoosporangia and zoospores. However, as mentioned before, all attempts to induce this way of reproduction failed and therefore we presume that our alga lost this asexual mechanism. Taking into account that in other *Ulothrix* species studied, for example *Ulothrix subtilis*, *U. implexa*, fragmentation is quite common when (a)sexual reproduction is absent and assuming that also the ability to form a holdfast is lost by the absence of zoospores, which produce this differentiated basal cell during germination, this alga is placed under *Ulothrix*. Moreover, *Klebsormidium*-clones, collected and studied by us, never showed more than one pyrenoid, except in dividing cells.

5.3. Ulothrix verrucosa Lokhorst (*nom. nov.*)

Hormidium mucosum Boye Petersen, 1915 p. 376.

5.3.1. Living material

Clones were isolated from the following localities: *Friesland*, near Galamadammen, in the canal connecting the Morra and Fluessen lakes, on mud among dead stems of *Phragmites australis* around the water level; Eastern side of the Bergumer meer in a rather sheltered place, on bits of wood around the water level; *Gelderland*, Aaltensche Slinge, on stones among the moss *Bryum argenteum* up to $\pm$ 10 cm above the water level; Aaltensche Slinge, on stones in slowly running water among *Spirogyra*, *Ulothrix*, *Klebsormidium* and *Oscilla-*

toria species; *Noord-Brabant*, near Oisterwijk, in a fen, on sandy substratum below the water level; on a humid wood-path near the same fen; *Limburg*, Ospelse Peel on an artificial sandy path between peat-bogs.

5.3.2. Morphology

The straight or twisted growing filaments always are unbranched (*fig. 9*), consisting of uniseriate cells with a parietal, girdle-shaped chloroplast, which usually covers up to two-thirds of the cell circumference. In shortened cells nearly fully-closed chloroplasts girdles may be present (*fig. 9*). The margin of the encircling chloroplast is unlobed or more or less wavy in both short, and long-celled filaments (*fig. 9*).

The chloroplast may reach only three quarters of the cell length or may approach cell length in the case in short cells, but never covers the cross walls (*fig. 9*). In exhausted liquid media and after prolonged cultivation on agar plates the shape of the chloroplast is more or less distorted by accumulation of storage products (*fig. 10A*).

Cells, provided with less-developed chloroplasts, without staining show the hyalin nucleus, usually lying opposite to the pyrenoid (*fig. 9*). Mostly there is only one pyrenoid, embedded in the chloroplast, in an exceptional case 2 were seen; often the pyrenoids are surrounded by a distinct envelope of starch (*fig. 9*). Volutin-like bodies are distributed throughout the cell.

Cells are pronounced cylindric; in older, shortened cells the ends are rounded off somewhat, but always adhering closely to one another (*figs. 9, 10A*). Filaments always lack a gelatinous sheath. In young and mature liquid cultures the cell wall is thin (*fig. 9*), unlamellated. In exhausted liquid cultures and after a long period of growth on agar plates the cell wall thickens, becoming rough-walled to verrucose (*fig. 10A*). H-pieces may be present and are more striking in mature filaments (*figs. 9, 10C*). Mucilaginous appendages, as established in *U. crenulata* have never been observed.

The apical cell is rounded (*fig. 10B*), never capped, despite presence of the H-pieces in the filaments.

The alga is mostly free-floating in liquid culture media. When coming into contact with the walls of the glassbox the cells will never form a differentiated rhizoidal cell, neither does the basal cell of fragmented segments develop into a typical *Ulothrix*-holdfast (*fig. 10B*). Attachment to the substratum is accomplished by means of a soft gelatinous layer secreted by the basal cell (*fig. 10B*), usually only determinable by using India ink.

Under short-day conditions filaments have a cell diameter ranging from 14.0–19.6 μ and are ¼–1½ times as long. Cell diameters most frequently found in full-grown filaments vary from 16.8–18.9 μ.

Under long-day conditions usually more thick-walled cells are present; in that case the cell diameter ranges from 16.1–23.1 μ, often with a highest frequency of 18.2–20.3 μ.

In wild material the filaments show a cell diameter range of 12.6–18.2 (–21.0) μ with rather short cells and mostly a markedly unlobed chloroplast. Especially

in terrestrial isolates roughened, thick-walled filaments, containing conspicuous oil-like bodies, scattered throughout the cell lumen, have been observed. For this alga MARVAN & HINDÁK (1971) established by means of statistical analysis that the outer cells of four-cell groups, arising from one mother-cell, after two subsequent divisions, are somewhat longer than the inner sister cells. The same phenomenon may be observed in our material (*fig. 9*).

5.3.3. Reproduction

Many attempts to produce swarmers have been made, but only vegetative multiplication which may result from accidental breaking of filaments (*fig. 10C*), is observed. Filaments may also break up into short segments of 1–4 or more cells, probably following the desintegration of the middle-lamella of the cross-wall, each segment developing into a new filament.

5.3.4. Taxonomy

As done for *Ulothrix crenulata*, herbarium specimens of *Ulothrix*, *Klebsormidium* and even uniseriate *Prasiola* (*Schizogonium*) species, described as gathered both terrestrial and aquatic habitats, were studied. Following the concept in older works, for instance DE-TONI (1889), HAZEN (1902), MIGULA (1907) and HEERING (1914), on account of its cell diameter our alga may be identified as *Ulothrix aequalis* Kützing. For example, material in DE WILDEMAN's herbarium, preserved in BR, s.n., which proved to be identical with ours, is provided with this name. Modern algologists, viz. RAMANATHAN (1964) and PRINTZ (1964) still adhere to HEERING's concept (1914). His drawings show filaments with vegetative cells as long as wide, suggesting that the alga usually has this habit. Recently, MARVAN & HINDÁK (1971), studying *Ulothrix aequalis*, named so using HEERING's and PRINTZ's descriptions, pointed out the difficulties concerning the identification of their alga.

Ulothrix aequalis Kützing is described in 1845 with the cell diameter range of 1/400–1/300‴ (5.8–7.8 μ) and is amended in 1849, the range mentioned being changed to 1/180–1/150‴ (12.9–15.5 μ).

However, investigation of the lectotype specimen of *Ulothrix aequalis*, from Halle, preserved in L as No. 939.26–235, only showed filaments belonging to the genus *Microspora*. All of the other specimens in KÜTZING's herbarium, which are provided with the annotation "Ulothrix aequalis" in KÜTZING's hand, respectively preserved in L as No. 939.26–236 from Dalmazia (leg. Meneghini), as No. 939.26–270 from Freiburg (leg. A. Braun) and No. 939.26–268 from Falaise (leg. Lenormand) could be recognized as belonging to *Microspora* as well.

Synonyms, mentioned by KÜTZING (1849), for example *Conferva floccosa* Agardh sensu Areschoug, preserved in S in Alg. scand. no 40, must be grouped in *Microspora* as well.

Strangely enough KÜTZING's drawings of *U. aequalis* in Tab. Phyc. II (1852), taf. 89 fig. I, actually represent an *Ulothrix* species. A zonate band-like chloroplast and a typical *Ulothrix* reproduction process is illustrated, from which may

be inferred that younger *Ulothrix zonata* filaments have been drawn. Investigation of the specimen, preserved in L as No. 939.26–269 and labelled in NÄGELI's hand as "585. Ulothrix zonata, Zürich, Nägeli", and "Ulothrix aequalis, Tab. 89 I" in KÜTZING's hand confirmed this surmise.

On the other hand *Ulothrix aequalis* β *pallida* Kützing, described in 1849 as having a cell diameter range of 1/150–1/140‴ (15.5–16.6 μ), upon examination showed filaments which probably belong to *Microspora*. The type specimen is preserved in L as No. 938.174–384 and labelled in LENORMAND's hand as "49. Conferva implexa, Dillw. Zélande".

RABENHORST in 1868 transferred *Ulothrix cateniformis* (Kützing) Kützing, described as *Hormidium cateniforme* in 1847 with the cell diameter of 1/150–1/130‴ (15.5–17.9 μ) to *Hormiscia* (=*Ulothrix*) *aequalis* as a subspecies. In its type specimen however, preserved in L as No. 939.26–266 and labelled in DE BRÉBISSON's hand in ink as "Petalonema limbatum", only *Rhizoclonium* filaments were present. Finally in this historical taxonomic review of *Ulothrix aequalis* it may be mentioned that RABENHORST in 1868 proposed *Conferva austriaca* Stizenberger ex Rabenhorst as a synonym of *U. cateniformis*. The isotype specimen in RABENHORST's Alg. Eur. no 1550, preserved in L as No. 939.23–663, only showed filaments belonging to the genus *Microspora*.

This investigation of herbarium material has indicated that it is wrong to follow HEERING and all others, using the specific epithet "aequalis" within the genus *Ulothrix*. Judging from further examination of herbarium specimens, *Hormidium mucosum* Boye Petersen (1915), preserved in C, s.n., labelled as "Hormidium mucosum n.sp., Road in plantation at Rø, Bornholm, on naked clay soil, 12/10/1912", is to be designated as the lectotype of the alga studied. Nearly the same cell diameter range, viz. 12.6–19.6 μ and shortening of the cells are observed. Moreover the cells contain 1(–2) pyrenoids, a well-developed band-like chloroplast and a very conspicuous, thickened cell wall, being somewhat verrucose. As described above, this last phenomenon is also observed in our material, respectively in nature when growing in terrestrial habitats and under culture conditions among others after prolonged cultivation on agar plates.

However, the combination *Ulothrix mucosa* (Boye Petersen) comb. nov. is not allowed because of the existence of the valid combination *Ulothrix mucosa* Thuret (1850). No other existing names were available and for this reason *Ulothrix verrucosa* is proposed.

5.3.5. Taxonomic relationships

Remarks on taxonomic affinities of this alga are equal to those mentioned for *Ulothrix crenulata*, discussed in paragraph 5.2.5. in this paper.

6. CONCLUSIONS AND DISCUSSION

This study is intended to provide a firm basis for the taxonomy of the genus *Ulothrix*, based on morphological, developmental and life-history attributes, gathered as much as possible from field, culture and herbarium studies. There-

fore both wild and cultivated material is used to show the variation in the morphological characters, like cell diameter, diameter of zoosporangia if present, shape of the chloroplast, number of pyrenoids, morphology of the basal cell etc.. This is only correct when a great number of filaments has been investigated (LOKHORST & VROMAN 1972). As stated for unicellular and colonial algae by VAN DEN HOEK (1963) also within *Ulothrix* morphological investigation of dried nomenclatural types and other herbarium specimens is possible. Suggestions are given to help recognizing taxonomic characters, like morphology of the chloroplast, pyrenoids, cell wall construction, cell wall structure, reproductive stages etc. in dried material. By using colouring reagents more satisfactory results may be obtained.

Of the species studied only *Ulothrix mucosa* produces zoospores but all three species studied show vegetative multiplication through fragmentation. This type of life-history is rather incomplete in comparison with that of other *Ulothrix* species (DODEL 1876, KLEBS 1896, LOKHORST & VROMAN 1972).

However, in these species, morphological characters are sufficient for unequivocal determination. No differences in life-history could be observed among several clones of the species, nor differentiation in the rate of fructification.

The reproductive behaviour of species studied is similar under different photoperiods and temperatures and agrees well with the observations in the field of THURET (1850) concerning *U. mucosa* and with the culturing results of MARVAN & HINDÁK (1971) concerning *U. aequalis*, henceforward called *U. verrucosa*. The cultural results reflect the behaviour of the species in nature; the algae may be present in the filamentous stage throughout the year. Still it is expected that in nature under less favourable conditions true akinetes may be formed, as shown in related ulotrichacean algae. But, in spite of the akinete-like phase of filaments under exhausted culture conditions and the phenomenon of irregular inflating of one or a few cells, observed in exhausted cultures as well, true akinetes were not found to be present. Probably the inflating of cells is caused by some growth disturbance, since it is not followed by typical non-motile spore formation.

It is gathered that zoosporogenesis in *Ulothrix mucosa* is stimulated in spring, probably for securing the possibility to maintain and to disseminate itself.

Ulothrix crenulata and *U. verrucosa* are able to grow under a wide range of conditions. As investigations throughout the year have shown, these species when found in aquatic environments, usually show a thin cell wall, whereas more thick-walled cells usually are observed in material from terrestrial habitats. It may therefore be surmised that these algae do not, in contrast to most *Ulothrix* species, make use of sexual reproduction as a survival mechanism. Within the species studied clear taxonomic characters are demonstrated, which reflect fundamental similarities to other groups of green algae. For example, *Ulothrix mucosa* shows some features, like occasionally the occurrence of a mucilaginous sheath surrounding the filament, which is among others characteristic for the genera *Binuclearia* and *Geminella*. On the other hand *Ulothrix crenulata* and

U. verrucosa are more related in growth habit to the genus *Klebsormidium* because of the absence of a rhizoidal holdfast-cell.

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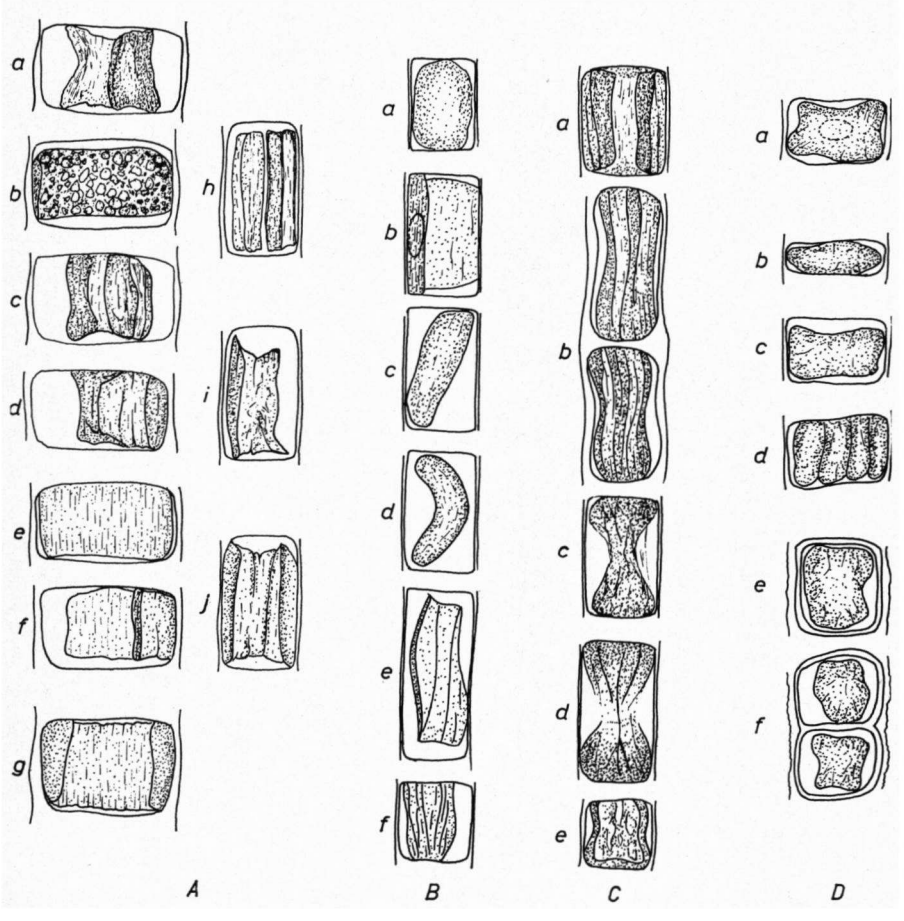

Fig. 1. Schematic representation of chloroplasts in a dried state. A. *Ulothrix*; B. *Klebsormidium*; C. *Microspora*; D. *Prasiola*.

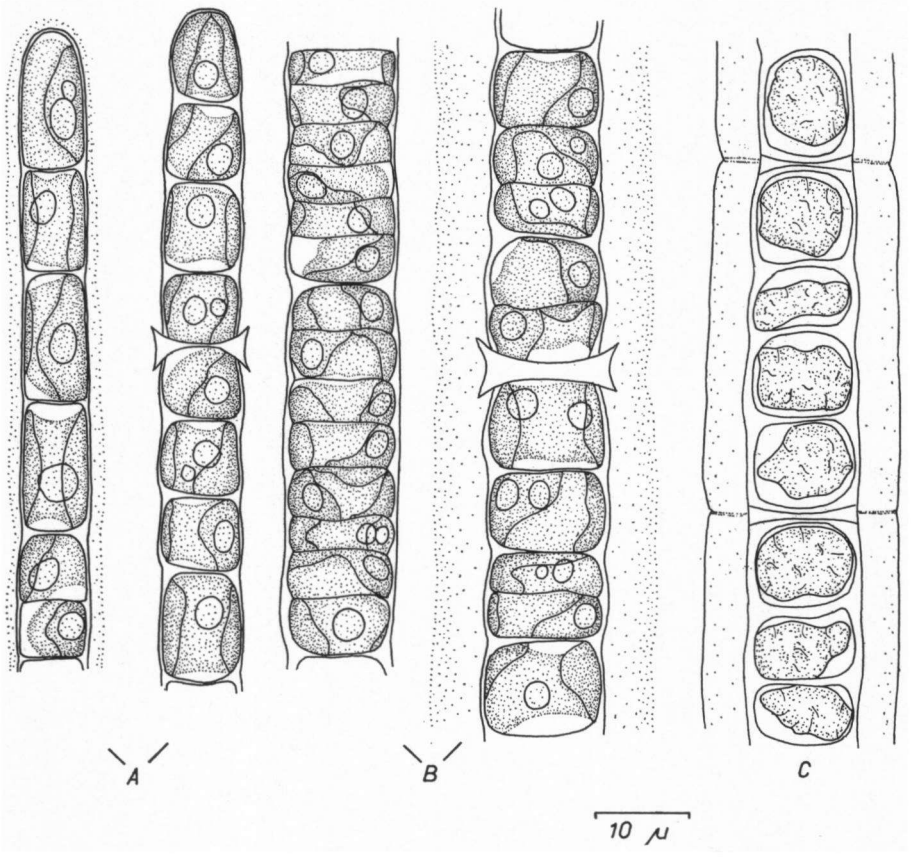

Fig. 2. *Ulothrix mucosa*. A. young filaments; B. full-grown filaments; C. old filament.

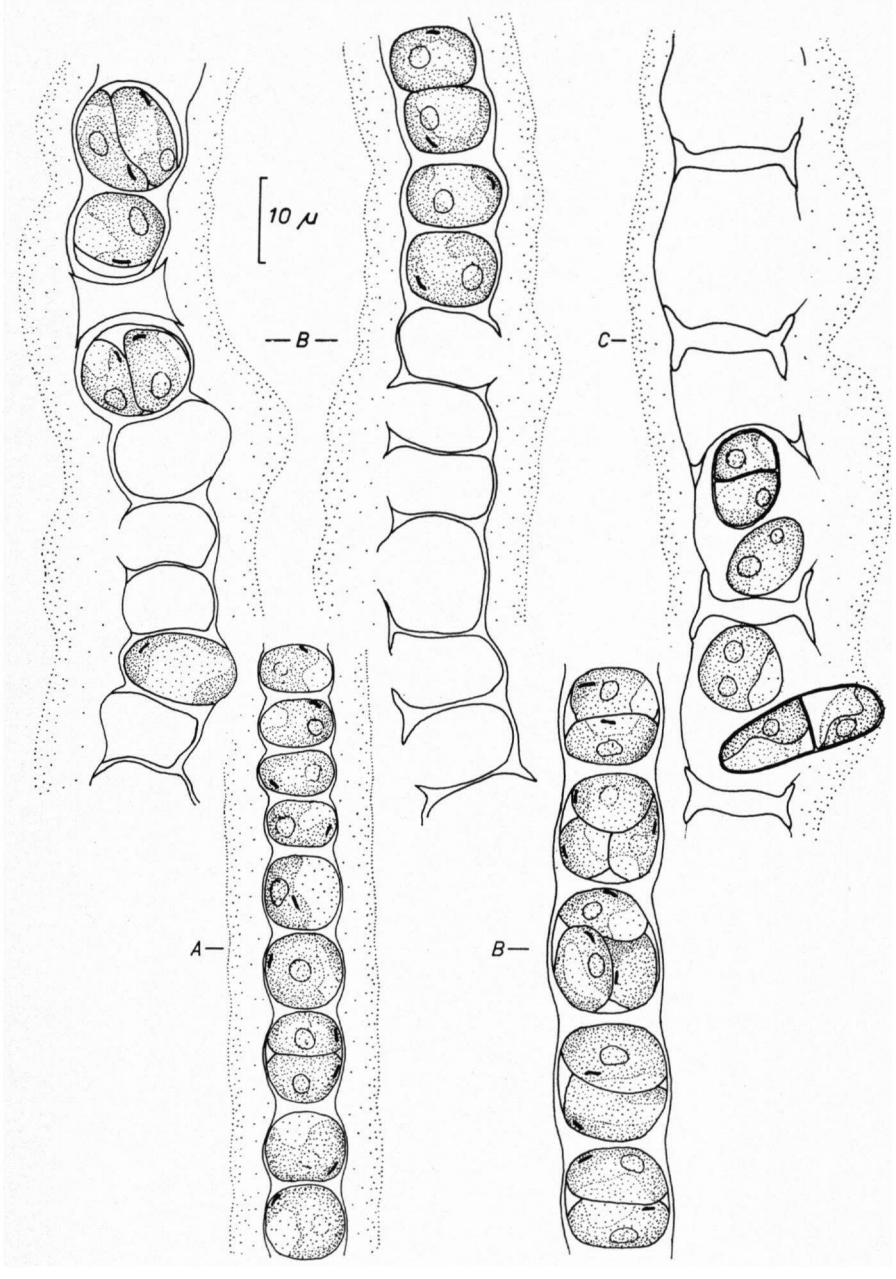

Fig. 3. *Ulothrix mucosa*. A. induced zoosporangia in a young filament; B. full-grown filaments with zoosporangia; C. filament containing aplanospores.

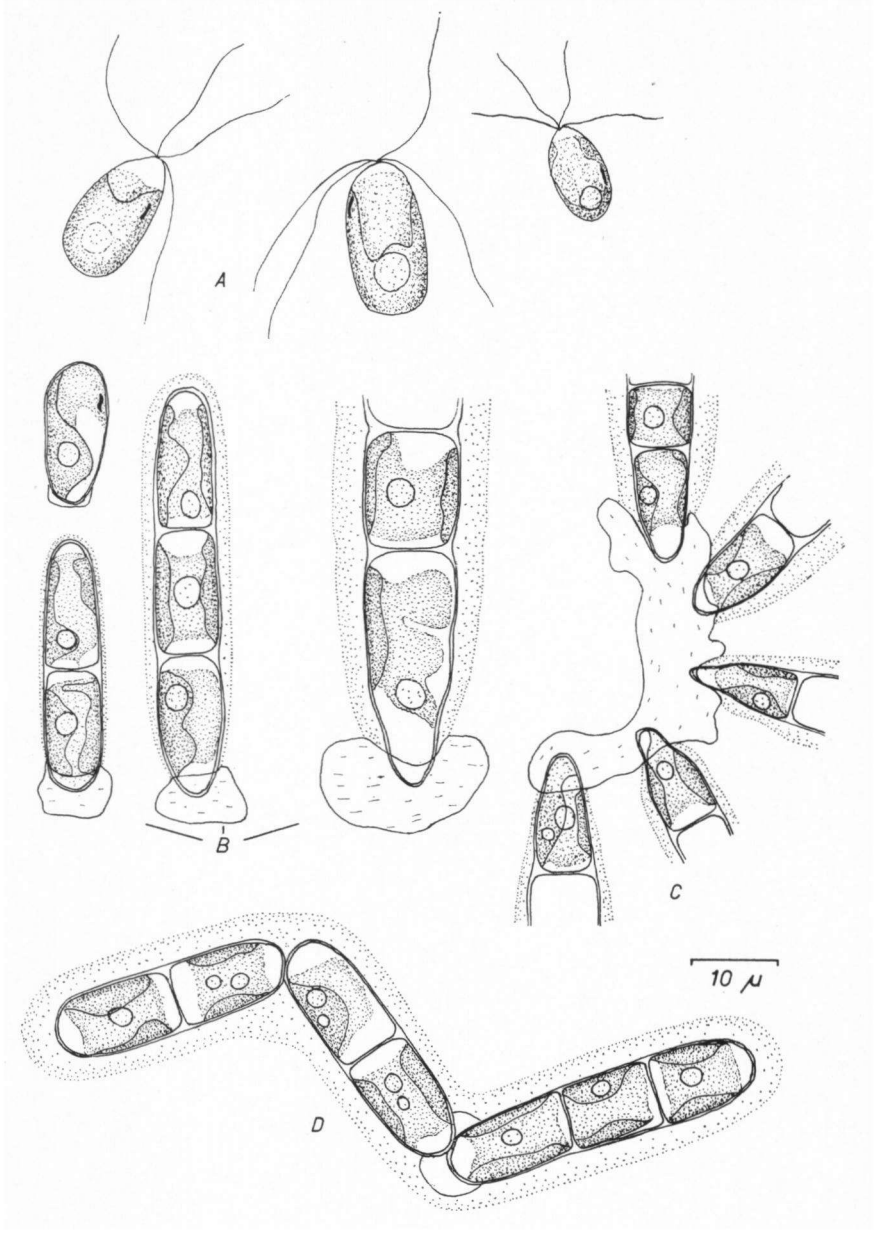

Fig. 4. *Ulothrix mucosa*. A. zoospores; B. germination of the zoospore into a germling; C filaments growing together in a firm mucilaginous plate; D. filament dissociation.

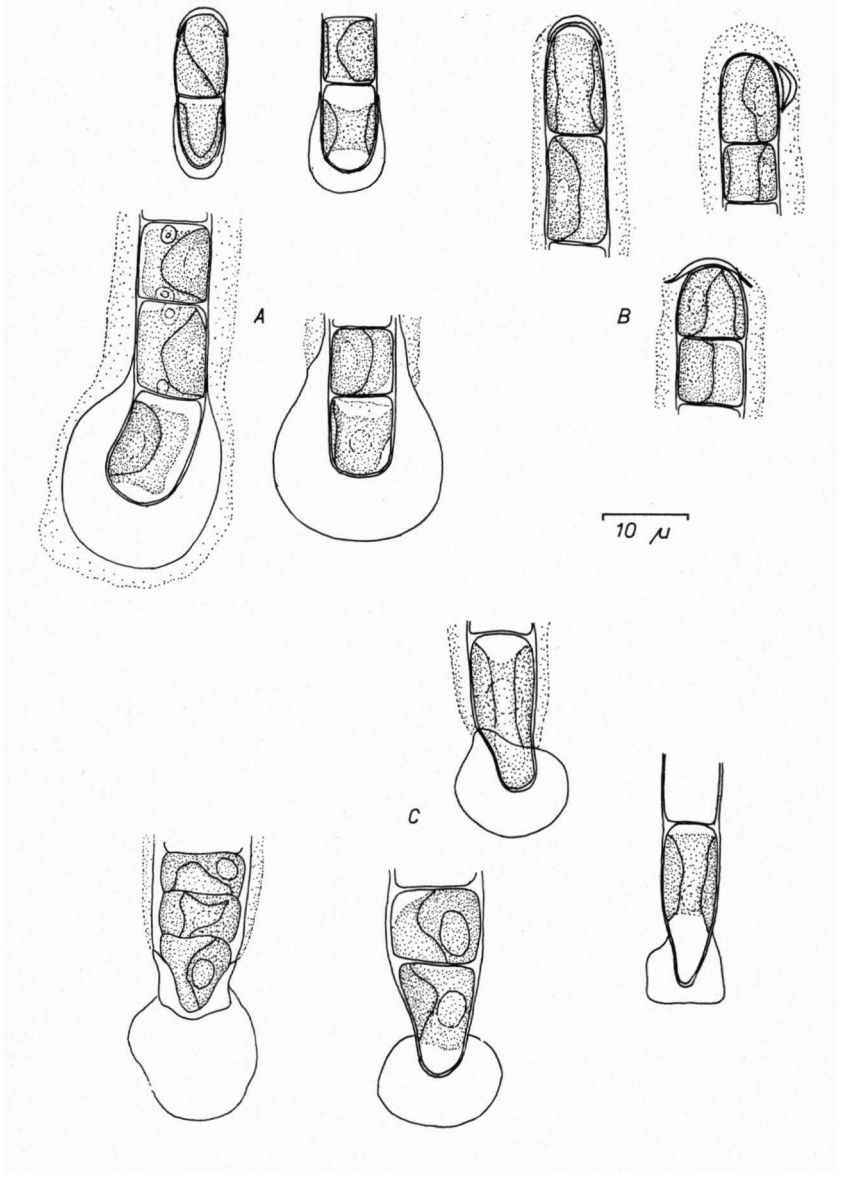

Fig. 5. *Binuclearia tectorum*. A. developmental stages of the basal cell; B. apical cells provided with a cap.
Ulothrix mucosa. C. basal cells.

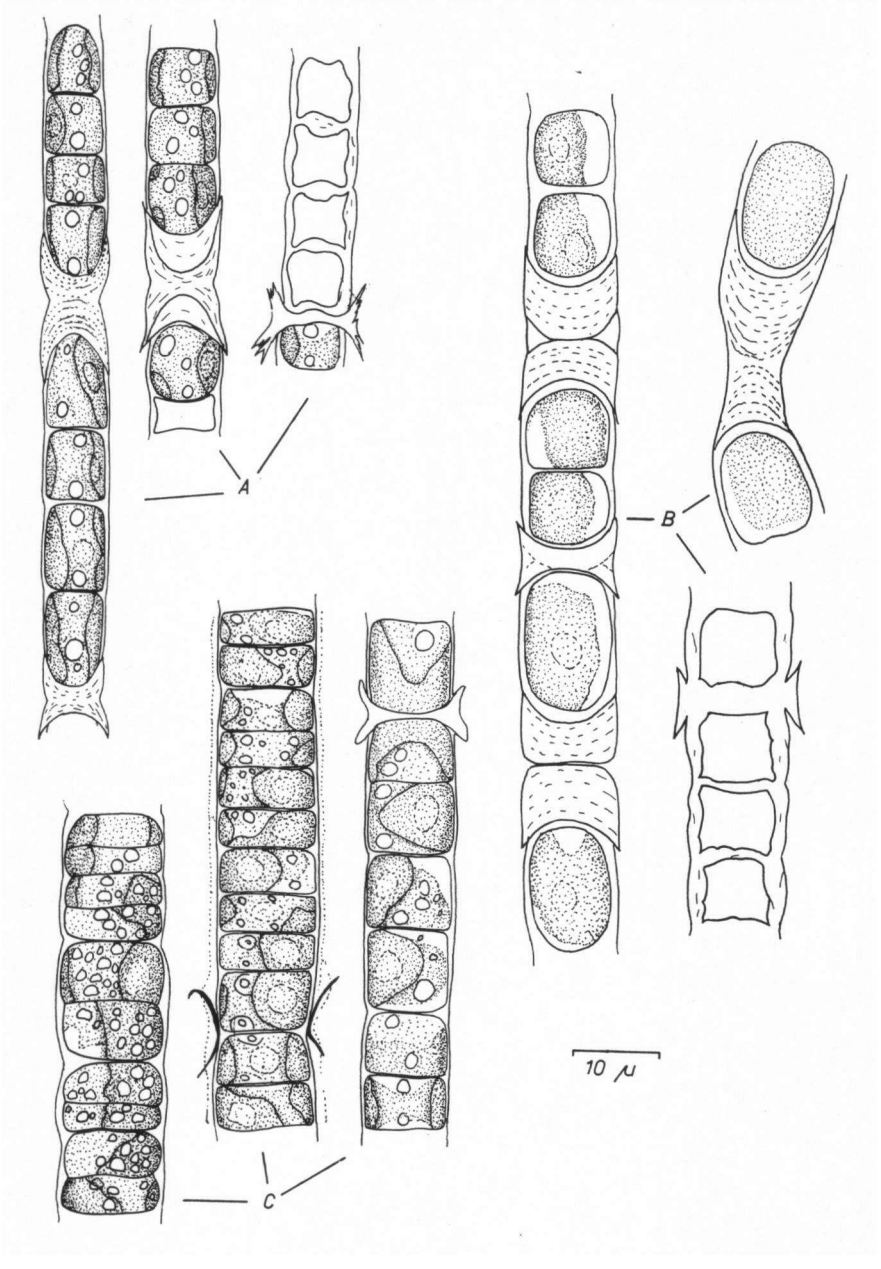

Fig. 6. *Binuclearia tectorum*. A. wild material from a fen near Hijkerveld; B. wild filaments from Oisterwijk; C. cultivated filaments from Brunstingerveen.

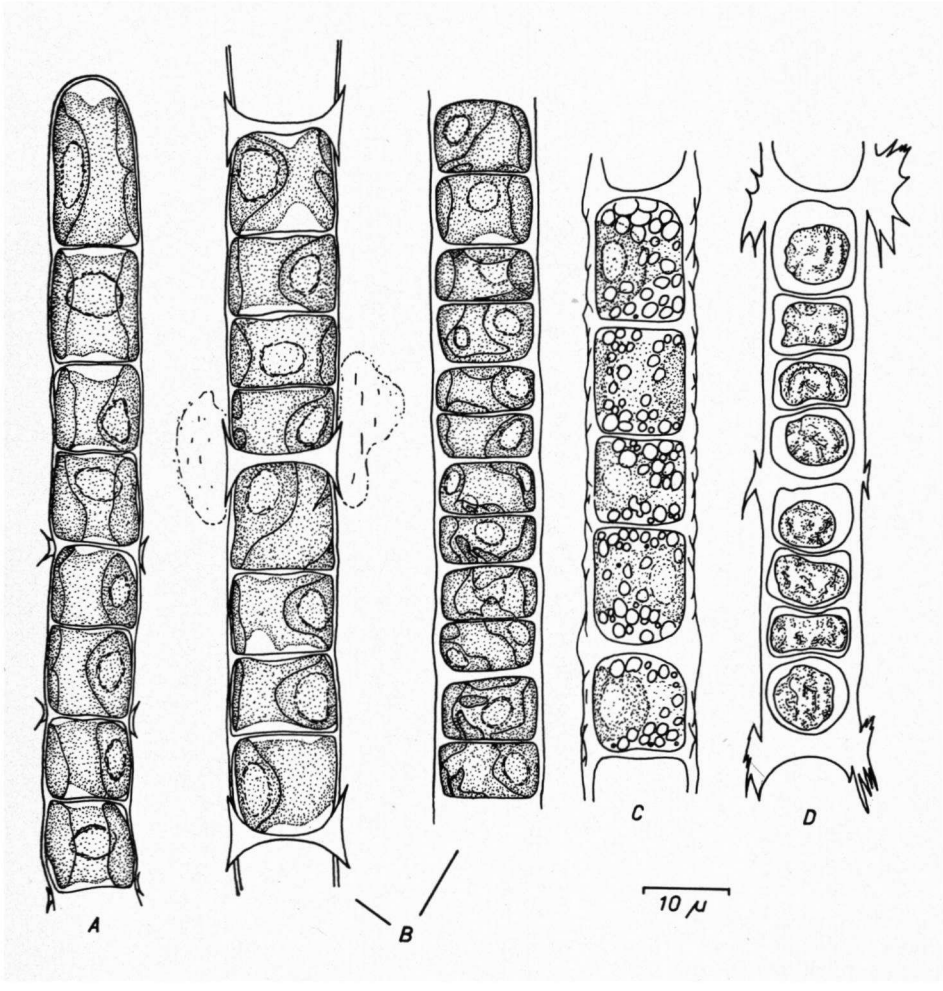

Fig. 7. *Ulothrix crenulata.* A. young filament; B. full-grown filaments; C. older filament with a crenulated cell wall and accumulation of storage products; D. very old filament with dense, withdrawn contents.

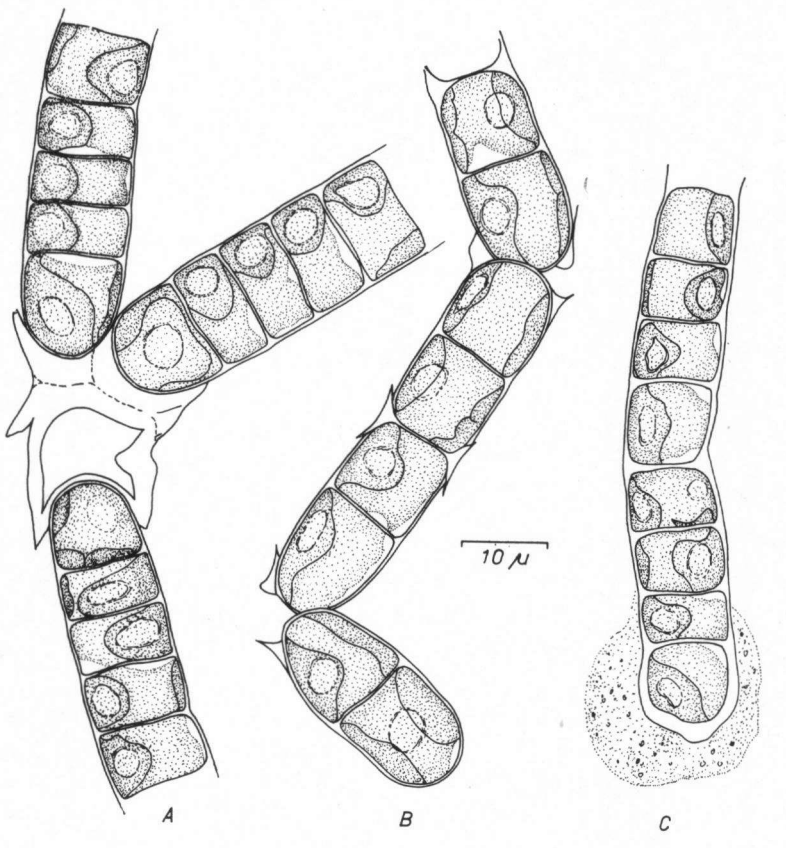

Fig. 8. *Ulothrix crenulata*. A. filament with longitudinal cell division; B. filament dissociation; C. filament with the basal cell attached to substratum by means of a soft, gelatinous ball.

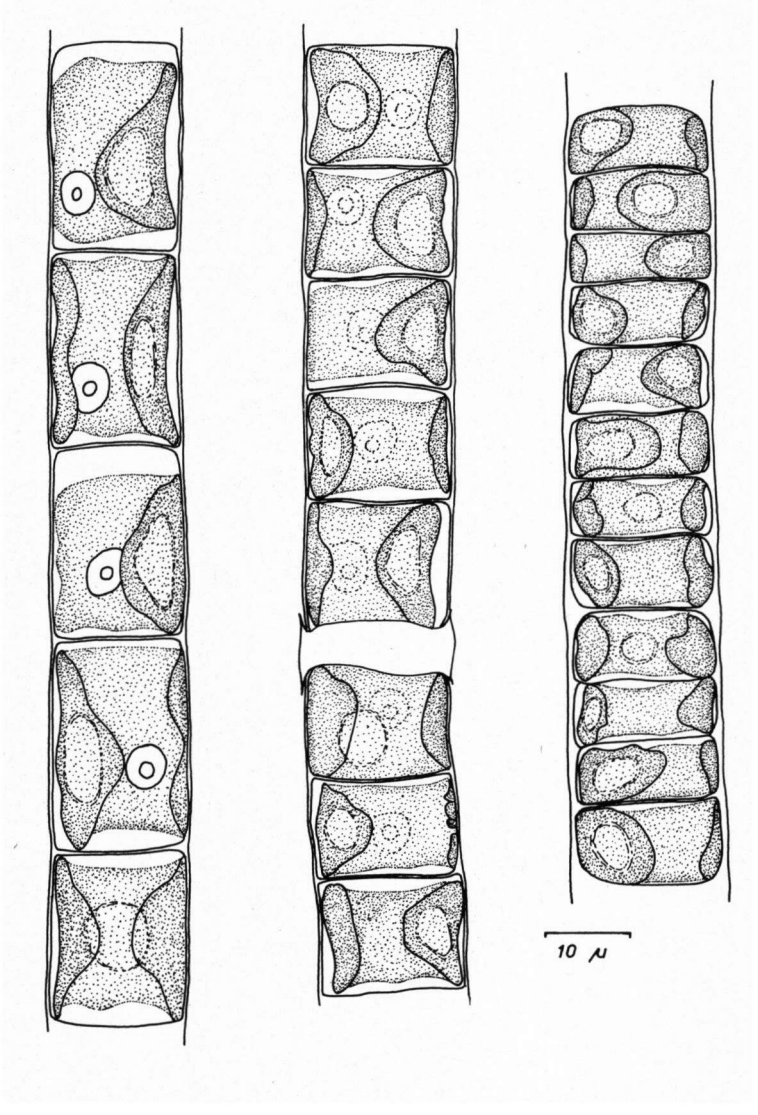

Fig. 9. *Ulothrix verrucosa.* Full-grown filaments.

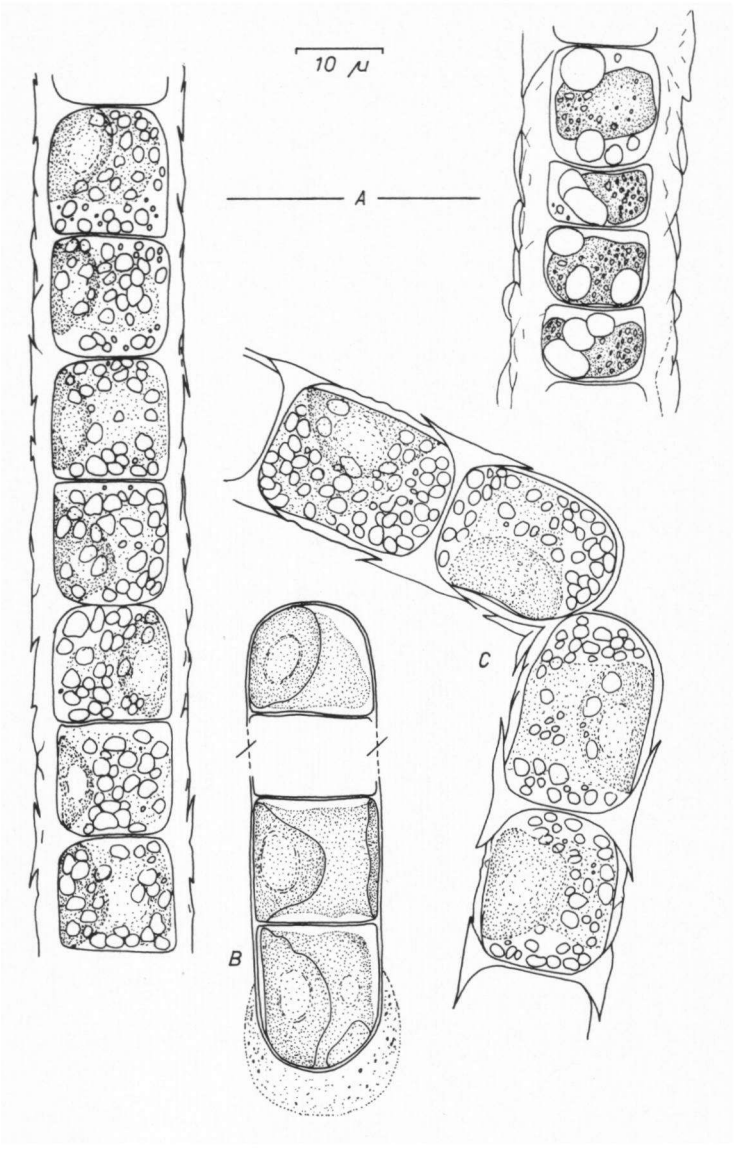

Fig. 10. *Ulothrix verrucosa.* A. older filaments with a roughened cell wall and accumulation of storage products; B. filament with the basal cell attached to substratum by means of a soft, gelatinous ball; C. filament dissociation.