

TAXONOMIC STUDIES ON THE GENUS *ULOTHRIX* (ULOTRICHALES, CHLOROPHYCEAE) III

G. M. LOKHORST* and M. VROMAN

Vakgroep Plantensystematiek, Biologisch Laboratorium, Vrije Universiteit, Amsterdam

SUMMARY

In this third report of taxonomic studies on the freshwater species of *Ulothrix* Kützing, the morphological and reproductive characteristics of *Ulothrix implexa* (Kützing) Kützing, *U. tenuissima* Kützing and *U. zonata* (Weber & Mohr) Kützing are discussed.

U. implexa is characterised by the lack of gametogenesis whereas the absence of asexual reproduction, occasionally occurring in culture, is accompanied by a very frequent appearance of branched rhizoid-like outgrowths at irregular intervals in the filaments. The chloroplast morphology may show an affinity to the genus *Klebsormidium* Silvia, Mattox & Blackwell. *U. tenuissima* shows both asexual and sexual reproduction. The occurrence of curled filaments containing gametangia and the occasionally pointed zoospores and gametes are characteristic for this species. In a certain growth stage of the chloroplast some morphological resemblance to the genus *Urospora* Areschoug (*Hormiscia* Fries) is present. *U. zonata* shows an identical life cycle as *U. tenuissima*. It is proved that numerous *Ulothrix* species which are described only are growth habits of *U. zonata*.

Both under long- and short-day conditions *U. implexa* only shows reproduction by means of zoospores, whereas the other species, referred to in this paper, show asexual stages under a short-day light regime, and gametogenesis under a long-day photoperiod; sporophytes are observed to be fertile under short-day conditions. The manner of occurrence of the species in nature agrees with the behaviour of the algae under different photoperiods in culture.

1. INTRODUCTION

Earlier the results of an investigation on the taxonomy of six freshwater *Ulothrix* species were reported (LOKHORST & VROMAN 1972, 1974). In these papers it is shown that species distinction is facilitated by culturing the algae. Besides information on developmental and life-history stages, this approach also provides data on the morphological variability.

In the present paper the results of an investigation, based on field-, culture- and herbarium study, into the taxonomy of three other freshwater *Ulothrix* species are treated.

2. MATERIAL AND METHODS

Several clones of *U. implexa*, *U. tenuissima* and *U. zonata* were mainly collected from a number of freshwater habitats in the Netherlands. The species all seem

* Present address: Rijksherbarium, Schelpenkade 6, Leiden.

to be restricted to more or less eutrophic conditions. The algae were collected from hard substrata like stones, sheet-pilings etc. from several cm below to several cm above the water level in the wash zone.

The pH of the visited waters ranged from ± 7.0 –8.0. In acid waters, like peat-moors and fens, these algae proved to be absent. As to the way of isolation and culturing we refer to our previous papers (LOKHORST & VROMAN 1972, 1974). In the present study the cultures sometimes were kept in 50 ml Erlenmeyer flasks, which were aerated.

For herbarium study collections were borrowed from the British Museum (Natural History) at London (BM), the Rijksherbarium at Leiden (L) and the Muséum National d'Histoire Naturelle, Laboratoire de Cryptogamie at Paris (PC). During a visit at the Botanical Museum at Lund, the unbranched Conervae in AGARDH's herbarium were investigated.

3. INFLUENCE OF CULTURING ON MORPHOLOGY AND LIFE CYCLE

The morphology of the algae studied in culture is quite similar to that observed in nature. Among others in both circumstances the length/width ratio is generally large in young filaments and smaller in mature plants (figs. 1A, 1B, 6A, 8C, 13A, 13B). However, in nature the range of cell diameters may be (slightly) larger than in culture. This phenomenon is especially observed in *U. zonata*. Different day-length periods do not affect the morphology and dimensions of the cells. Under long-day regimes cultures become atypical sooner, partly by the faster growth, which exhausts the nutrient medium and partly by the light regime, for the algae are usually only present in the filamentous stage in autumn, winter and spring.

In young filaments the unclosed chloroplast-girdle, containing few pyrenoids, is usually (slightly) lobed along its longitudinal margin (figs. 3B, 8C, 13A). In mature filaments the chloroplast is usually more strongly developed. Then it is shaped like an always-unclosed parietal band in *U. implexa* (fig. 1B), an (ir)regular, unclosed or closed parietal band in *U. tenuissima* (fig. 6A) and a uniformly closed parietal band with proliferations in *U. zonata* (fig. 13B). Under less favourable conditions the morphology of the chloroplast is distorted by accumulation of storage products.

The cell wall is thin in young filaments, but in older ones, especially in *U. zonata* in exhausted culture medium, it may be strongly thickened (fig. 13C) and lamellated. Apical cells are rounded in mature filaments (fig. 6A), but in young stages sometimes narrowed apical cells are observed. In culture the basal cell usually develops into a typical rhizoidal holdfast (figs. 3B, 5A, 8B, 12D, 15C). The basal cell is less complex in wild material. The clones of *U. implexa*, used in this study, only produce zoospores under all day-length regimes (fig. 2). Under short-day conditions the cultures of *U. tenuissima* and *U. zonata* show zoosporogenesis. Also in filaments transferred from intermediate- into long-day conditions zoosporogenesis is found in first instance (figs. 7, 14A, 14B). However, this process soon is replaced by gametogenesis (figs. 9C, 9D, 16A, 16B).

After fusion the zygotes germinate into one-celled sporophytes, which produce zoospores only under short-day conditions (*figs. 11A, 19B*). Non-fusing gametes mostly die off. However, sometimes parthenogenetic development of the gametes into the *Codiolum*-phase is observed (*fig. 17D*), which produces zoospores either. All three species show some degree of filament dissociation under the different photoperiods used, especially when zoosporogenesis and gametogenesis are absent. Only one of the clones of *Ulothrix zonata* shows true akinete formation (*fig. 21C*).

4. MORPHOLOGY, REPRODUCTION AND TAXONOMY OF THE SPECIES STUDIED

4.1. *Ulothrix implexa* (Kützinger) Kützinger

Hormidium implexum Kützinger 1847 p. 177, emend. as *Ulothrix implexa* (Kützinger) Kützinger 1849 p. 349.

Synonym:

Hormiscia implexa (Kützinger) Rabenhorst, 1868 p. 364.

4.1.1. Living material

Clones were isolated from the following localities: *Noord-Holland*, Amsterdam, IJ-meer on stones in the splash zone between *Cladophora* species; *Nieuwe meer* in an exposed place, growing intertwined with *Cladophora*, *Bangia* and *Ulothrix* species; *Utrecht*, Vinkeveense plassen, on stones in an exposed wash zone; *Limburg*, near Geleen in the river Geleen, on piles, at water level in swiftly running water.

4.1.2. Morphology

The straight, in older cultures somewhat curved filaments, are normally unbranched and consist of uniseriate cells with a parietal, unclosed girdle-shaped chloroplast, which usually covers one half to three quarters of the cell circumference (*fig. 1B*). Mostly in young filaments the regularly developed chloroplast is (slightly) lobed along its longitudinal margin (*fig. 3B*); in full-grown cells the chloroplast is still regularly shaped, but usually unlobed (*fig. 1B*). In young filaments the chloroplast may not approach cell length. In exceptional cases the chloroplast only covers half of the cell length (*fig. 1A*) and then the filaments show some morphological similarity to the genus *Klebsormidium*. In older, more shortened cells the chloroplast usually approaches cell length, but never clothes the cross walls (*fig. 1B*). Under less favourable conditions in exhausted culture medium the chloroplasts grow denser and form a compact mass (*fig. 3C*). At the same time a strong accumulation of starch and oil-like bodies may occur. When the chloroplasts are less well-developed the hyaline nucleus and vacuoles are to be seen without using colouring reagents. The nucleus with nucleolus usually lies in a cytoplasmatic bridge opposite to the pyrenoid (*fig. 5C*).

The number of pyrenoids varies from 1–4. However, mostly one conspicuous

[illegible]

Table 2. Frequency-distribution (%) of the diameter of filaments of *Ulothrix implexa* and *U. tenuissima* in cultures, in relation to cultivation-time under long-day conditions.

time:	<i>U. implexa</i>								<i>U. tenuissima</i>							
	2w.	3w.	4w.	5w.	6w.	7w.	8w.	9w.	2w.	3w.	4w.	5w.	6w.	7w.	8w.	9w.
diameter																
4.9 μ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5.6	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6.3	10	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7.0	46	20	1	—	—	—	—	—	1	—	—	—	—	—	—	—
7.7	27	37	4	1	6	—	—	—	23	1	1	—	—	—	—	—
8.4	11	26	18	7	9	—	—	—	19	5	11	2	—	—	—	—
9.1	4	6	19	11	9	—	—	—	24	18	22	2	2	1	2	—
9.8	1	3	14	15	18	1	—	—	17	20	19	4	2	2	—	—
10.5	—	3	22	21	28	2	3	1	13	20	21	8	7	4	1	2
11.2	—	—	9	13	8	5	4	2	3	22	14	16	17	8	2	8
11.9	—	1	8	10	7	9	10	6	—	8	5	16	18	12	5	8
12.6	—	—	4	10	9	17	5	18	—	4	4	17	19	22	7	13
13.3	—	—	1	7	3	27	20	26	—	2	2	16	14	18	17	12
14.0	—	—	—	3	3	33	40	24	—	—	1	15	14	18	16	21
14.7	—	—	—	2	—	6	15	19	—	—	—	3	3	6	16	16
15.4	—	—	—	—	—	—	3	1	—	—	—	1	2	3	16	10
16.1	—	—	—	—	—	—	—	1	—	—	—	—	1	1	7	3
16.8	—	—	—	—	—	—	—	1	—	—	—	—	1	—	1	—
17.5	—	—	—	—	—	—	—	1	—	—	—	—	—	1	4	1
18.2	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	1
18.9	—	—	—	—	—	—	—	—	—	—	—	—	—	2	3	2
19.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
20.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21.0	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	2

and ($\frac{1}{2}$)1–4 times as long. The cell diameters found most often in full-grown cultures under short-day conditions range from 12.6–14.0 μ , whereas somewhat larger diameters, viz. 12.6–14.7 μ , are observed in cultures grown under long-day conditions (see *tables 1* and *2*). In well-developed, and mature wild material this same range of dimensions could be observed, viz. 9.8–14.0(–15.4) μ , with cells usually shorter than wide. The chloroplast in these cells is pronouncedly unlobed along its longitudinal margin and sometimes rather withdrawn.

The apical cell is rounded. Under culture conditions, especially in germlings, this cell sometimes is seen to behave like the basal cell, when touching the bottom of the glassbox. In germlings the basal cell develops into a typical *Ulothrix* holdfast. Already in a one-celled stage a pronounced differentiation may be seen between the future basal and apical part of the filament (*fig. 3B*). In 2–5 celled germlings this process is increased by a strong lengthening of the basal cell, which may be longer than the remaining vegetative cells of the germling. At the same time in this cell the chloroplast becomes irregularly distributed over the cell lumen. In old basal cells chloroplast material even seems to be

absent (*fig. 5A*). The length of the holdfast may reach 260 μ . In young filaments this cell is usually unbranched (*fig. 3B*), but in mature cultures more complex, branched cells are present (*fig. 5A*). Their walls are surrounded by a layer of mucilage, by which the alga is attached to the substratum. This layer is well demonstrated by adding India ink. Secondary rhizoids, growing from intercalary vegetative cells are present, especially when reproduction by zoospores is absent in cultures. This phenomenon is characteristic for this species. These holdfasts are provided with irregularly developed chloroplasts, however, still clearly containing several pyrenoids (*fig. 4A, 4B, 4C, 5B*). The rhizoidal appendages may be present in one separate cell (*fig. 4A*), but also in two (*fig. 4B*) or three (*fig. 4C*) neighbouring cells. The apical part of the secondary rhizoids sometimes may grow into a clinging-foot (*fig. 4C*). The holdfasts may be branched or unbranched (*figs. 4A, 4B, 4C*). Sometimes in long rhizoids cell division may again take place, which leads to the formation of a very complex, branching system (*fig. 5B*). As stated for the basal cell these protuberances are surrounded by a slightly visible, gelatinous sheath as well. Holdfasts are also produced from vegetative cells, neighbouring empty zoosporangia (*fig. 5C*).

4.1.3. Reproduction

Zoosporogenesis takes place under short-, intermediate- and long-day conditions, mostly starting with the apical cell.

Per cell (2-4-)8-16(-32) zoospores (*fig. 2*) are formed. During this process the sporulating filaments may become slightly bent (*fig. 2*). As far as investigated in nature 4-16 zoospores were seen. The diameter of the zoosporangia usually ranges from 9.8-16.1(-17.5) μ . As stated for vegetative cells, also the cell length/width ratio of the zoosporangia in smaller filaments is generally large and decreases in wider plants (*fig. 2*). At the same time a larger number of zoospores is present (*fig. 2*). In young filaments, about 10-15 cells in length, the dimensions of the zoosporangia range from 5.6-9.1 μ . Then the number of zoospores may vary from (2-)4(-8).

During the release of the zoospores the longitudinal cell wall becomes partly dissolved or may burst. The zoospores are usually released while enclosed in a hyaline mucilaginous envelope, which disappears after a few moments. The zoospore is more or less spindle-shaped (*fig. 3A*), containing a parietal, rather regularly cup-shaped chloroplast, mostly with one or two pyrenoids (*fig. 3A*). The zoospore possesses one small, but nevertheless conspicuous median-posteriorly located stigma and 4 flagella, implanted closely together. Sometimes the impression of a pointed apical papilla is given (*fig. 3A*). The zoospores vary in length from (6.8-)8.5-13.6 μ and from 3.4-6.8 μ in width. At all light intensities, varying from low to high, the zoospores are negatively phototactic in swimming. After swarming, which movement is very lively, the zoospore usually at first becomes spherical in shape, then attaches itself to the substratum with its flagellar pole foremost and loses its flagella one by one. Soon it becomes clothed with a thin cellulosic cell wall. It germinates immediately, elongating in both directions, the lower hyaline part growing into the basal cell and the upper

into the vegetative cells (fig. 3B). In culture also germination of zoospores, which had not made contact with the glass box, could be observed. The germination process of attached and free-floating zoospores proved to be identical.

Especially under long-day conditions aplanospores could be observed, which under more favourable conditions germinate directly within the parent filament.

Vegetative propagation may take place by fragmentation (fig. 3C), which process may increase by exhaustion of the culture medium.

True akinetes never have been found.

4.1.4. Taxonomy

In our opinion *Ulothrix implexa* (Kützinger) Kützinger, proposed in 1847 as *Hormidium implexum* without a cell diameter note and amended in 1849 with the cell diameter range $1/180-1/150'''$ ($12.9-15.5\mu$) is to be indicated as the name for this alga. Filaments, preserved in L as No. 939.174-397 (leg. LENORMAND, no 150, Zélande), in a very bad condition and present in a very small quantity together with a *Rhizoclonium* species, which is abundantly present in this type specimen, nearly showed the same cell dimensions as our alga studied, viz. $9.8-16.8\mu$.

In literature (WILLE 1901, SCHUSSNIG 1915), there has been some discussion about the exact nature of *U. implexa*, probably chiefly because the original description and KÜTZINGER's unclear drawing in Tab. Phyc. II (1850-1852) Taf. 94, fig. II do not provide sufficient information. SCHUSSNIG (1915) even doubted, without analysing the original material, however, whether this name has right of existence. The confusion about the identity of this species is also caused by its broad ecological range; the alga is fairly insensitive to salinity. Thereby HAZEN (1902) and RAMANATHAN (1964) stated that this species seems to furnish the link between the series of exclusively freshwater *Ulothrix* species and strictly marine species.

Already KÜTZINGER observed the wide ecological range of *U. implexa*. In the original species description (1847) he mentioned as collecting location "Inter *Rhizoclonium interruptum* prope Goes, in submarinis", whereas the illustrated *Ulothrix implexa* in Tab. Phyc. II is gathered from "Gräben". It is clear that KÜTZINGER did not recognize this species as "marine", because all the marine species, which nowadays belong to *Ulothrix*, were described by the author under *Hormotrichum*.

The wide ecological range of this species is also shown in our studies. The clones are collected from fresh-oligohalinic waters, whereas field-study has indicated the presence of this alga in mesohalinic habitats as well, without appreciable loss of important specific features. Also on account of the phenomenon that morphological features are hardly changed by high or low salinity of the culture medium this species is presented here as a part of this study on the taxonomy of the freshwater *Ulothrix* species; nearly the same range of cell dimensions and number of pyrenoids have been observed in the salinity range $0.02-15\text{‰}$ Cl'.

4.1.5. Taxonomic relationships

This alga may show less well-developed chloroplasts, which in plate-like state are identical to that of *Klebsormidium*. This species is less related to some other studied representatives of *Ulothrix*, since it shows a very complex system of secondary rhizoidal outgrowths (figs. 4A, 4B, 4C, 5B); in some stages one may even speak of false branched filaments (fig. 5B). This phenomenon is very important, since it provides additional evidence concerning the evolutionary position of the genus *Ulothrix* within the ulotrichacean algae.

4.2. *Ulothrix tenuissima* Kützing

Ulothrix tenuissima Kützing 1833 p. 518; 1836 Dec. XV, no. 144; 1843 p. 252.

References and synonyms:

Myxonema tenuissimum (Kützing) Rabenhorst, 1847 p. 99;

Ulothrix tenuis Kützing 1845 p. 197, nov. nom. pro *Ulothrix tenuissima*, 1849 p. 347;

Hormiscia tenuis (Kützing) De-Toni, 1889 p. 165;

Ulothrix tenuis Kützing β *articulis aequalibus* Kützing, 1849 p. 347 (nom. inval.)

Ulothrix rorida Thuret, 1850 p. 223;

4.2.1. Living material

Clones were isolated from material gathered from the following localities: *Groningen*, Paterswolder meer, on a sheet-piling, enclosed in ice, below the waterlevel; *Friesland*, near Dokkum, in the ditch Jaarsloot, in stagnant water; *Overijssel*, Belterwijde, on old stems of *Phragmites* and *Typha*, in a rather sheltered place, several cm below to several cm above the waterlevel; *Utrecht*, the river Lek near Wijk bij Duurstede, in more or less turbulent water on stones in the washzone; *Eempolder*, in a ditch on old stems of aquatic plants in stagnant water; *Noord-Holland*, IJsselmeer near Amsterdam, on stones in the washzone; *Westeinder Plassen*, near Kudelstaart on stones in the washzone in an exposed place; *Zuid-Holland*, Braassemmer meer near Roelofarendsveen on piles in a rather exposed place, around the water level; *Limburg*, Julianakanaal near Berg, in the washzone, in more or less turbulent water.

4.2.2. Morphology

The straight, in older stages more curved filaments are normally unbranched and consist of uniseriate cells with a parietal, girdle-shaped chloroplast. In younger filaments the chloroplast usually does not extend over more than three quarters of the cell circumference (figs. 8B, 8C), whereas in mature filaments more closed chloroplasts are present (fig. 6A). In young filaments the chloroplast is regularly girdle-shaped, (slightly) lobed to unlobed along its longitudinal margin (figs. 8B, 8C) and usually does not approach cell length in long cells (fig. 8C). In full-grown cultures with wider cells a more irregular girdle-shaped chloroplast could be observed, usually occupying the whole cell length (fig.

6A). In strongly shortened cells the impression is given of compressed plate-shaped chloroplasts, which phenomenon is characteristic for this alga (fig. 6A). Under less favourable culture conditions, the chloroplast becomes more or less desintegrated and shows signs of vacuolation (fig. 6B), however, the chloroplast is still parietally expanded over the inner cell wall (fig. 6B). In strongly exhausted culture medium the chloroplast becomes denser, usually completely distorted, which phenomenon is increased by a strong accumulation of storage products. In cells with less well-developed chloroplasts, in normal filaments, the hyaline nucleus and vacuoles can be seen without using reagents.

Pyrenoids are distinct (fig. 6A); their number may vary from 1–4 (–6). The contours of the pyrenoids are clearly distinguishable from the surrounding chloroplast (fig. 6A), only a thin envelope of starchy material is present around the pyrenoid.

Cells are mostly cylindrical; however, in exhausted cultures more barrel-shaped ones are found (fig. 6B). Cells are always closely adherent to each other. As in most *Ulothrix* species some cells in the filament may fail to develop properly; these can be recognized by smaller size, especially in older cultures. In a young stage the cell wall is usually thin, whereas the wall is (scarcely) thickened in older cells. Long-day conditions may increase this process of thickening. H-pieces may be present in young stages as well as in older ones, but are not common.

Cells have a diameter range from 9.8–18.9(–21.0) μ and are $1/6-1\frac{1}{2}$ times as long. The great majority of cells, provided with larger dimensions, is shortened (fig. 6A). Young filaments, in which zoosporogenesis usually does not take place spontaneously, consist of cells, varying in diameter from 4.9–9.1 μ and being 1–5 times as long. The cell diameters found most often in full-grown cultures under short-day conditions are in the range 13.3–15.4 μ , whereas a range 12.6–15.4 μ is observed in cultures grown under long-day conditions (tables 1 and 2). In well-developed wild material almost the same range of cell dimensions could be observed, viz. 9.8–22.4 μ ; the range of diameters found most often may reach to 18.9 μ , however. Mostly these filaments contain 1–2 pyrenoids. In an exceptional case even a cell diameter range up to 50 μ was observed (see below).

In older filaments the apical cell is rounded (fig. 6A), while in germlings the shape may vary from usually rounded to less often more narrowed apical cells. In a highly exceptional case this cell is seen to behave like a basal cell after touching the bottom of the glassbox. In germlings the basal cell develops into a typical *Ulothrix*-holdfast. Already in a one-celled stage a rhizoidal protuberance is to be seen, which elongates in the two-celled stage (fig. 8B). In this stage the basal cell is frequently contracted at the middle (fig. 8B). In young filaments this cell is usually unbranched (fig. 8B), whereas in mature cultures complex, branched cells may be present (fig. 12D). Sometimes in 1–4 cells above the basal cell other outgrowths are formed for attachment to the substratum. During this growing process chloroplast material within these cells becomes more or less irregularly distributed over the whole cell (fig. 12D). Frequently

these basal parts of filaments are less wide. In exceptional cases spontaneous formation of rhizoids, growing from intercalary vegetative cells is observed. Both basal cell and intercalary holdfasts are surrounded by a layer of mucilage, by which the alga is attached to the substratum.

Under less favourable conditions, both in the field and in culture, the alga may show a very characteristic habit (*fig. 6B*). In this case the chloroplast looks reticulate, due to the presence of a large number of vacuoles. At the same time an increase of number of pyrenoids and cell diameter is seen (up to 50 μ). The cells become more or less barrel-shaped and they show some tendency to arrange in pairs. Those filaments show certain morphological similarity to the strictly marine genus *Urospora*. However, upon transferring those filaments into fresh culture medium, cell division is stimulated and the normal growth habit as described above returns. In the newly arising cells the cell diameter strongly decreases and the strictly girdle-shaped chloroplast is again present. Also zoospores, descended from those filaments have been isolated. The germlings arising from these zoospores yielded the same cell dimensions as those arisen from zoospores, formed in filaments with a normal growth habit.

4.2.3. Reproduction

After transferring vegetative filaments from the stock cultures, preserved at 8°C under intermediate photoperiod regime, into short- and long-day conditions, zoosporogenesis takes place in all cells, except the basal ones and leads to the formation of (2-)4-8(-16) zoospores (*fig. 7*). Zoosporogenesis is more frequent under short-day conditions. During this process the filaments may become somewhat constricted (*fig. 7*). In wild material (2-)4-8 zoospores could be observed. The diameter of the zoosporangia usually ranges from 9.8-20.3(-23.8) μ . In young filaments, about 5-15 cells in length, the dimensions of the zoosporangia range from (4.9-)7.7-9.1 μ . The number of zoospores may vary from (2-)4 in these filaments. As stated for vegetative cells the cell length/width ratio of zoosporangia in younger filaments is generally large and decreases in older plants (*fig. 7*), except when 16 zoospores are formed (*fig. 7*).

The zoospores are mostly liberated in a vesicle, protruding through an opening in the side of the zoosporangium wall. The zoospores may vary in shape, from spindle-, globose- to ovoid-like (*fig. 8A*). Sometimes even conspicuously posteriorly pointed zoospores are formed, which again indicates the affinity of this species to the genus *Urospora* (*fig. 8A*). The chloroplast in the zoospores may be cup-shaped, while also unclosed chloroplasts are observed (*fig. 8A*). The zoospores possess a conspicuous, approximately median anteriorly located eye-spot, 1-2 pyrenoids and 4 flagella, implanted closely together. Sometimes, a clear apical pointed papilla is observed. They are relatively robust, varying from (10.2-)11.9-17.0 μ in length and from 5.1-6.8(-8.5) μ in width. The zoospores are positively phototactic in swimming. After this movement, which is regular and lively, they settle down on the substratum and become rounded off; after that the flagella are soon shed, one by one. On germinating they give rise to new plants (*fig. 8B*); in culture the germination pro-

cess of free-floating rounded-off zoospores is identical; the same elongation being observed among others.

Especially under long-day conditions zoosporogenesis may be accompanied by aplanosporogenesis (*fig. 12C*); under more favourable conditions the aplanospores germinate directly within the mother filament (*fig. 12C*).

Only the transfer of vegetative filaments into long-day conditions results in course of time (7–21 days or even more) in gametogenesis. The contents of the cells become olive-green, at the same time the filaments become slightly to strongly curled, which phenomenon is characteristic for this alga (*figs. 9C, 9D*). The number of biflagellate gametes varies from (4–)8–16(–32) (*figs. 9C, 9D*) per cell. They are sized from 6.8–10.2 (–11.9) μ in length and 3.4–5.1 μ in width. The gametes possess a usually medianly located eye-spot, a cup-shaped chloroplast, which sometimes is less well developed and usually contains one pyrenoid (*fig. 9A*). The diameter of the gametangia usually ranges from 9.8–20.3(–23.8) μ , their length from 3.4–17.0 μ , dependent on the cell diameter and the number of produced gametes. In young filaments the cell diameter ranges from 7.0–9.1 μ its length 1–3 times more, whereas usually only 4–8 gametes are formed. The gametes are mostly liberated through a pore in the side wall, surrounded by a mucilaginous envelope which soon bursts after releasing. The gametes may vary in shape (*fig. 9A*): spindle-shaped, globose to ovoid gametes are observed being posteriorly pointed or rounded (*fig. 9A*). They are positively phototactic in swimming.

After swimming, which movement is skittish and very fast in comparison with that of the zoospores, the non-fused gametes may settle down and round off, mostly followed by dying. However, sometimes attached non-fused gametes develop parthenogenetically into uni-celled sporophyte-like plants. Non-attached gametes usually do not loose their flagella and die off.

Fusion of the gametes is isogamous to slightly anisogamous (*fig. 9B*). The alga is monoecious.

The quadriflagellate zygotes swarm for a while, showing a photonegative response in swimming, become spherical, attach themselves to the substratum and soon withdraw their flagella one by one.

Only under short day-conditions they germinate into fertile, one-celled sporophytes of various shape (*fig. 11A*), via stalked or sessile intermediate stages (*fig. 10*). Stalked sporophytes usually have an attaching disc (*figs. 10 and 11A*). During germination the zygote increases in size. The chloroplast becomes irregularly, parietally distributed, containing several pyrenoids (*fig. 10*). Especially under long-day conditions the contents soon become finely granulated by storage products. During maturation the contents may become yellowish-green in colour, while dividing into (4–)8–32 or sometimes more zoospores or aplanospores (*fig. 11A*). Fertile globose sporophytes may reach a diameter up to 70 μ , whereas the more common, pear-shaped to elongated ones, measure up to 84 μ in length and to 55 μ in width. The (weakly) photo-positive quadriflagellate zoospores which arise from the sporophytes vary in shape (*fig. 11B*). When ripened under less favourable conditions, they are

spherical and rather immobile with a cell diameter ranging from (6.8–)8.5–11.9 μ . Normally they are strongly mobile and pear-shaped, their length ranging from 11.9–15.3(–17.0) μ and their width from 5.1–6.8 μ .

These zoospores show the same germination-process as those arising from the filamentous stage (fig. 11C); the same cell dimensions in germlings being observed among others.

Besides biflagellate gametes provided with one eye-spot, sometimes zygotes with 4 flagella and 2 stigmata are observed remaining behind in the gametangium (fig. 12A). In that case the maturation process takes place inside the gametangium (fig. 12B).

Sometimes vegetative multiplication by fragmentation of filaments has been observed.

4.2.4. Taxonomy

Quite probably the alga studied has been described by KÜTZING in 1833. However no material which could have been used for this description was demonstrable in KÜTZING's herbarium. Besides from the above-mentioned description no specific character could be obtained for unequivocal species determination. In 1836 in Decades XV KÜTZING preserved under no. 144 filaments of *U. tenuissima*, collected "In fluvio Murr ad Lebring in Styria", however without giving more information. This material appeared to be identical with ours (see below).

In 1845 KÜTZING for the first time described the cell dimensions, viz. a diameter of 1/300–1/200''' (7.8–11.6 μ) their length being half as long. At the same time he changed the specific name to *Ulothrix tenuis*. The reason for abandoning the original name was not mentioned. In 1849 an amended description was given with a cell diameter range of 1/150–1/110''' (15.5–21.1 μ).

In spite of the confusion, created by KÜTZING himself *Ulothrix tenuissima* is designated as the name for the alga studied. The herbarium specimen of KÜTZING's Decades (1836), preserved in L as No. 910.188–2230 and labelled as "no 144. *U. tenuissima*", proved to be identical to the alga studied, and is hereby designated as the lectotype. The filaments, which are in a very good condition, showed nearly the same cell diameter range, to wit (9.8–)12.6–21.0(–23.8) μ and are 1/5–1 1/2 times that long. Several filaments in this specimen were in the reproductive stage and especially the presence of curled gametangial filaments facilitated an unmistakable identification.

Still in Tab. Phyc. II Taf. 89, fig. I (1850–1852), *Ulothrix tenuis* was only illustrated in the vegetative stage, whereas in wild material usually several filaments are to be found in the reproductive phase. KÜTZING made his drawings from material collected from "Weissenfels", preserved in L as No. 939.26–264. Our investigations show the absence of reproductive stages in this specimen to be purely accidental.

Probably caused by KÜTZING's insufficient description, THURET (1850) did not recognize lively sporulating material collected by him from the washzone of brooks in the neighbourhood of Paris as *Ulothrix tenuissima* and therefore he

established the new name *Ulothrix rorida*. Investigation of the lectotype, preserved in PC, s.n., and labelled in THURET's hand as "*Ulothrix rorida* Thuret, Rentilly (S. et M.) Mars, 1846", showed that this species must be considered as synonymous to *U. tenuissima*.

Ulothrix tenuis KÜTZING β *articulis aequalibus* KÜTZING (1849), established as a nom. inval., also must be relegated to the category of synonyms. Filaments, preserved in L as No. 939.26–263, "from Jever", showed identical morphological characters and the same asexual reproductive stages as our alga.

RABENHORST in his excellent taxonomic studies, sometimes misinterpreted the identity of *Ulothrix* species. For example *U. tenuis*, no. 48 in Alg. Sach. and no. 1539 in Alg. Eur., respectively could be identified as a *Microspora* species and as *Ulothrix zonata*. On the other hand *Ulothrix inaequalis* KÜTZING (1845), considered by us to be a synonym of *Ulothrix zonata*, present as no. 1073 in Alg. Eur., actually represents *Ulothrix tenuissima*.

The same goes for *Ulothrix elongata* Nägeli ex herb. KÜTZING (nom. inval.), filaments of which are preserved in L as No. 939.26–252.

In C. A. AGARDH's collection, preserved at Lund, this alga is also present in several specimens, labelled as *Oscillatoria torta*, namely under nos. 7231, 7233, and 7240, whereas identically named specimens, viz. the numbers 7234, 7236, and 7243, show among others *Ulothrix zonata*, *Enteromorpha* species and the marine *Ulothrix subflaccida* Wille, but no *U. tenuissima*. *Oscillatoria torta* was established by C. A. AGARDH in 1813. The original species description was definitely too vague to establish its identity. Neither was it possible to draw any conclusions from one of the above mentioned numbers, because the annotations in AGARDH's handwriting, written on the sheets, did not make clear what was the original material. The confusion about its identity was increased in 1824, when AGARDH transferred this freshwater species as a synonym to the marine *Conferva contorta*. From the above-mentioned heterogeneous collection it is clear that several unbranched, filamentous algae, which in some respects showed a similar growth habit, such as twisted filaments with clavate tops, were ranked by AGARDH among this species. In our opinion this is quite plausible for curled gametangial filaments of *U. tenuissima* are present in the above mentioned herbarium specimens. To avoid questionable changes in nomenclature it is proposed to apply the already generally accepted and well-known specific epithet "*tenuissima*" to the alga studied, and to consider *Oscillatoria torta* as a *nomen dubium*.

4.2.5. Taxonomic relationships

This alga shows outstanding characters, viz. the parietal, reticulate chloroplast, sometimes observed in nature and also under less favourable culture conditions, together with barrel-shaped vegetative cells and posteriorly pointed zoospores. These features indicate a certain taxonomic relationship to the strictly marine genus *Urospora*.

4.3. *Ulothrix zonata* (Weber & Mohr) Kützing

Conferva zonata WEBER & MOHR 1804 p. 97; *Ulothrix zonata* (WEBER & MOHR) KÜTZING 1833 p. 519; 1843 p. 251; 1845 p. 196; 1849 p. 347.

References and synonyms:

- Myxonema zonatum* (Weber & Mohr) Fries, 1835 p. 329;
Lyngbya zonata (Weber & Mohr) Hassall, 1845 p. 220;
Hormiscia zonata (Weber & Mohr) Areschoug, 1866 p. 12;
Conferva lucens Dillwyn, 1805 pl. 47;
Conferva bicolor Smith & Sowerby, 1814 pl. 2288;
Sphaeroplea crispa Berkeley, 1833 p. 11;
Ulothrix crispa (Berkeley) Kützing, 1849 p. 348;
Ulothrix varians Kützing, 1845 p. 196; 1849 p. 348;
Hormiscia zonata (Weber & Mohr) Areschoug d. *variens* (Kützing) Rabenhorst, 1868 p. 362;
Ulothrix zonata (Weber & Mohr) Kützing var. *ε*) *variens* (Kützing) Hansgirg, 1886 p. 58;
Ulothrix pectinalis Kützing, 1845 p. 196; 1849 p. 347;
Ulothrix zonata (Weber & Mohr) Kützing var. *γ*) *pectinalis* (Kützing) Hansgirg, 1886 p. 57;
Hormiscia zonata (Weber & Mohr) Areschoug var. *pectinalis* (Kützing) De-Toni, 1889 p. 163;
Ulothrix didyma Kützing, 1845 p. 196; 1849 p. 348;
Hormiscia ? *didyma* (Kützing) De-Toni, 1889 p. 170;
Ulothrix inaequalis Kützing, 1845 p. 196; 1849 p. 347;
Ulothrix zonata (Weber & Mohr) Kützing var. *δ*) *inaequalis* (Kützing) Hansgirg, 1886 p. 58;
Hormiscia zonata (Weber & Mohr) Areschoug var. *inaequalis* (Kützing) De-Toni, 1889 p. 163;
Schizogonium latissimum Meneghini ex Kützing, pro. syn., 1845 p. 196;
Ulothrix attenuata Kützing, 1849 p. 348;
Hormiscia zonata (Weber & Mohr) Areschoug b. *attenuata* (Kützing) Rabenhorst, 1868 p. 362;
Ulothrix zonata (Weber & Mohr) Kützing var. *β*) *attenuata* (Kützing) Hansgirg 1886 p. 57;
Ulothrix attenuata concinna De Notaris, Erbar. Crittogam. Ital., Ser. 1, Fasc. 27, no 330(1330), 1866;
Hormiscia zonata (Weber & Mohr) Areschoug c. *concinna* (De Notaris) Rabenhorst, 1868 p. 362;
Ulothrix valida Kützing, 1849 p. 348;
Hormiscia zonata (Weber & Mohr) Areschoug e. *valida* (Kützing) Rabenhorst, 1868 p. 362;
Ulothrix zonata (Weber & Mohr) Kützing b. *valida* (Kützing) Hansgirg, 1886 p. 58.

4.3.1. Living material

Clones were isolated from material, collected from the following localities: *Friesland*, Sneeker meer, at a strongly exposed shore, on stones; Grouw, in the harbour on a sheet-piling, around waterlevel to several cm below; *Overijssel*, Ramsdiep, in the washzone on stones; Belterwijde, on old stems of *Phragmites* and on stones in the washzone in a rather exposed place; Belterwijde, near a swimming pool, on piles in a strongly exposed place; *Gelderland*, IJsselmeer, on stones between *Bangia* species, around the waterlevel in the washzone; *Noord-Holland*, Westeinder Plassen, on branches and stumps of wood, around the waterlevel to approximately 20 cm below, in a rather exposed place in strongly turbulent water; *Zuid-Holland*, Braassemer meer, on old stems of *Phragmites* and *Typha*, around the waterlevel in a rather sheltered place; *Noord-Brabant*, Wilhelminakanaal near Oirschot, on a sheet-piling in the washzone to about 20 cm below the waterlevel; *Limburg*, Maasbracht, in the river Maas on stones in fastly running water, around the waterlevel.

4.3.2. Morphology

The straight, in older cultures more curved, strictly uniseriate filaments are normally unbranched. However, in an exceptional case an irregular branch was observed (fig. 21D). Young filaments consist of cells with a regular, parietal girde-shaped chloroplast, which usually occupies only about three quarters of the cell circumference (fig. 13A). The chloroplast in these cells is usually lobed along its longitudinal margin (fig. 13A). In mature cells fully-closed chloroplasts mostly are present (figs. 13B, 13C), not always occupying the whole length of the cells (figs. 13B, 13C). In this stage the chloroplast may show proliferations into the cell lumen, usually containing one or several pyrenoids (fig. 13B). Under less favourable conditions the chloroplast is withdrawn to the centre of the cell, being still fully-closed (fig. 13C). In that case the chloroplasts uniformity is distorted by accumulation of storage products (fig. 13C). The hyaline nucleus may be seen inside the unclosed chloroplast; vacuoles usually only can be observed after adding colouring reagents.

Pyrenoids are distinct, their number may vary from 1–4 in a young stage, whereas mature cells may contain up to 8(–11) pyrenoids. This number is usually correlated to the width of the vegetative cells (figs. 13A, 13B). Only a thin envelope of starch is present around the pyrenoid.

Cells are mostly cylindric, in older stages some barrel-shaped ones may be present, however. In a young stage the cell wall is thin, whereas under normal circumstances the wall may be slightly thickened (fig. 13B). In exhausted cultures, especially under long-day conditions, mature cell walls become conspicuously lamellated, which phenomenon is characteristic for the species (fig. 13C). Mostly the outer cell layer becomes strongly thickened, whereas the centre of the cross walls, only composed of the inner cell layer may show a sudden narrowing (fig. 13C). H-pieces may be present in all filamentous stages, but are more common in older cultures.

Filaments show a cell diameter from 10.5–37.1 μ and are 1/6–2 times as long.

The great majority of cells, provided with large cell diameters, is short. Young filaments consist of cells, varying from 7.0–9.8 μ in diameter and are 1–4(–6) times as long. The divergency of dimensions in cultures is remarkable, see *table 3*. Therefore it is unsatisfactory only to indicate cell diameters found most often. In well-developed wild material mostly the same complete range of cell dimensions could be observed, however, sometimes in wild material even a cell diameter up to 70 μ could be established. The chloroplast morphology is identical both in wild and cultured filaments.

The apical cell is rounded; in some cases it may be slightly narrowed. After touching the bottom of the glassbox this cell is seen to behave like the basal cell in germlings (*fig. 15D*). Sometimes even in mature filaments the apical cell may thicken, and form a protuberance. In germlings the basal cell develops into a typical *Ulothrix*-holdfast (*fig. 15C*). Already in an one-celled stage a rhizoidal protuberance can be seen, which elongates in two- and three celled stages (*fig. 15C*). In young filaments this cell is usually unbranched (*fig. 15C*), whereas in mature cultures usually complex, branched systems may be present, as drawn for *Ulothrix tenuissima* (*fig. 12D*). Sometimes in 1–5 cells above the basal cell more cell protuberances are formed for attachment to the substratum. A length up to 340 μ has been observed for the basal cell. Sometimes in young stages the basal cell does not elongate and then this cell is not different from other vegetative cells (*fig. 15E*). Attachment is achieved by a thin mucilaginous layer, surrounding the cell. In older stages finger-like protuberances may also be formed, accompanied by a slight elongation of the basal cell. Also several aberrant developmental stages of germlings have been observed (*fig. 15F*) which, sooner or later, may still develop rhizoidal outgrowths (*fig. 15F*).

Especially in non-reproductive filaments, spontaneous formation of simple branched or unbranched rhizoidal holdfasts, growing from intercalary vegetative cells could be observed, but not as frequently as in *Ulothrix implexa*.

4.3.3. Reproduction

The reproduction of *Ulothrix zonata* is already studied intensively (KÜTZING 1843, CRAMER 1871a, 1871b, DODEL 1876, BERTHOLD 1886, STRASBURGER 1892, KLEBS 1896, PASCHER 1907, GROSS 1931, LIND 1932, etc.). Because of the total view the complete results of culture study on this species are reported, however.

After transferring vegetative filaments from the stock-cultures into short- or long-day conditions, zoosporogenesis takes place in ordinary cells including the apical cell. This process mostly starts in the apical cell, proceeds towards the base and leads to the formation of (1)–2–16(–32) zoospores per cell (*figs. 14A, 14B*). During zoosporogenesis the cells may become slightly barrel-shaped (*figs. 14A, 14B*). The diameter of the zoosporangia usually ranges from 10.5–42(–63) μ and they are 1/3–2 times as long. In thinner filaments fewer zoospores are formed, whereas the number is larger in wider filaments (*figs. 14A, 14B*). Zoospores are often located only along the inner cell wall (*fig. 14B*). In young filaments, about 5–15 cells long, zoosporogenesis usually only takes place after repeated refreshing of the culture medium; the dimensions of those zoospor-

rangia range from 7.0–9.8 μ , whereas usually only 1–2 zoospores are formed (fig. 14 A).

The zoospores escape, enclosed in a hyaline mucilaginous envelope, through a lateral opening in the cell wall, usually after gelatinisation of the wall. The zoospores are different in shape, usually varying from regularly ovoid-shaped to globose, but also asymmetrical zoospores are observed with a more or less straight ventral and an arched dorsal surface (fig. 15A). Now and then zoospores are present, which show a strikingly widened anterior part and a narrow posterior part (fig. 15A). The chloroplast is parietally adpressed, varying from incompletely closed to regularly cup-shaped, never occupying the upper anterior part of the zoospore (fig. 15A). They possess an anteriorly-medianly located, conspicuous eye-spot, and 1–7 pyrenoids. There are 4 flagella, implanted closely together (fig. 15A). The zoospores may vary in length from (8.5–)10.2–17.0 (–20.4) μ and from 6.8–11.9 μ in width. After a period of mobility, then showing a remarkable positive phototaxis, the zoospore usually comes to rest on its side or its posterior end (fig. 15B). During the last oscillatory movements the flagella are rejected one by one. When attaching a lateral swelling, perpendicular to the original long axis of the zoospores, is formed (fig. 15B), followed by elongation and subsequent division (fig. 15C).

Sometimes the zoospores stay in the rounded-off phase; it is gathered that in that case germination occurs much more slowly or not at all. Several times aberrant one-celled stages (fig. 15F), arising from zoospores, are observed. As a rule they die off or in course of time they still germinate into normal vegetative filaments. Sometimes both ends may produce protuberances (fig. 15D). During germination the stigma may remain visible in 2–4 celled filaments (fig. 15C). When during cell division cleavage takes place near the location of the stigma, both daughter cells may be provided with eye-spot material (fig. 15C).

Especially under long-day conditions thin-walled aplanospores are formed, which may germinate in the parent cell or may be released upon decay of the filament.

After transferring filaments into long-day conditions in course of time formation of gametes takes place (figs. 16A, 16B). During gametogenesis the contents of the cells may become dark olive-green. At the same time the filament may become slightly curved, not as characteristically, however, as in *Ulothrix tenuissima*. The number of biflagellate gametes varies from (4–)8–32 (–64) (figs. 16A, 16B). Their dimensions are: (5.1–)6.8–11.9 μ in length and 3.4–5.1 μ in width. The gametes are more or less ovoid-shaped and are positively phototactic, apart from a few exceptions. The gametes possess a median-anteriorly located eye-spot, a parietal irregularly cup-shaped chloroplast and mostly 1–2 pyrenoids (fig. 17A). The diameter of the gametangia usually ranges from 10.5–43.4 (–74) μ and they are 1/4–3 times as long, dependant on cell diameter and number of gametes. As stated for the formation of zoospores, frequently in (larger) gametangia the gametes only cover the cell walls (fig. 16B). In young filaments the diameter of the gametangia ranges from 7.0–9.8 μ and they are about 1–3 times as long (fig. 16A).

The gametes upon escaping are enveloped by a clearly distinguishable vesicle, within which they already move about, before being liberated. After a period of activity non-fused gametes will settle and usually die off. Several times, however, parthenogenetic development of the gametes into an one-celled sporophyte-like phase is observed (*fig. 17D*). This applies also for gametes, which are not discharged from the parent cell.

Gametic fusion is generally isogamous (*fig. 17B*), but only gametes from different plants can copulate. After swimming the quadriflagellate zygotes, which show negative phototaxis, attach themselves, round off and usually soon withdraw their flagella one by one. Only under short-day conditions the zygotes germinate and grow directly into fertile, uni-celled sporophytes, via stalked or non-stalked intermediate stages (*fig. 18*). At first the zygote increases slightly in size, by and by protuberances are formed, in which the cell contents shift (*fig. 17C*). In course of this growing-process intermediate stages are observed, which show a hyaline basal part, demonstrating the original contours of the settled zygote and a linear, clavate or irregular-shaped apical part, containing the green, not yet ripened contents (*figs. 17C, 18*). In older stages the stalk may become rather twisted (*fig. 19B*). During ripening of the sporophyte the cell wall usually increases in thickness. The contents divide into larger parts (*fig. 19A*), followed by further cleavage resulting in 4–16 (mostly 8) zoospores, (*fig. 19B*). Fertile globose sporophytes have a size ranging from 18–65 μ , whereas more commonly occurring pear-shaped ones measure 24–80 μ in length and 18–50 μ in width. Only at 4°C short-day conditions mobile zoospores were observed. At 8°C, the original starting-point for temperature ranges in this study, only aplanospores were observed, which ultimately grow into new plants within the sporophyte cell wall (*fig. 20*). The zoospores escape through a conspicuous aperture, formed by gelatinisation of a part of the sporophyte-wall (*fig. 19B*). The zoospores are liberated while still enclosed in a hyaline mucilaginous envelope, which soon disappears. They show positive phototaxis, a limited number, however, shows the opposite reaction. The quadriflagellate zoospores are rather identical to those arising from vegetative filaments. Among others the same range in habit variability and number of pyrenoids could be observed (*fig. 19C*). The ovoid-shaped zoospores measure about 10.2–17.0(–20.4) μ in length and 6.8–10.2(–13.6) μ in width. In course of time the zoospores settle on the substratum; the same mode of attachment and germination process is observed as stated for zoospores arisen from filaments.

Occasionally, during zoosporogenesis and gametogenesis the cleavage of the original cell contents is not completed; in that case within the mucilaginous vesicle besides well-developed reproductive cells, protoplast lumps, provided with several eye-spots and a large number of flagella are present.

Vegetative reproduction takes place in the form of fragmentation of the filaments, especially under less favourable conditions.

In only one of the isolates, from Limburg, in more or less exhausted cultures, besides swelling of cells, the filaments may also show true akinetes, which lie separately or united in series. The contents of these cells is strongly granulated

by accumulation of assimilates (*fig. 21C*). They could not be made to germinate.

In *Ulothrix zonata* KLEBS (1896) and PASCHER (1907) reported the occurrence of macrozoospores, morphologically identical to zoospores as described above, and microzoospores. The latter are narrowly ovoid-shaped, possess a median eye-spot and 2 or 4 flagella. They also have a different temperature sensitivity (KLEBS 1896). The authors described them as morphologically between macrozoospores and gametes.

GROSS (1931) failed to indicate the occurrence of microzoospores. This is comprehensible, since this phenomenon is only rarely present. In our study only in one isolate, from Sneeker meer, microzoospores in the sense of KLEBS and PASCHER have been observed especially under rather unfavourable conditions and a long-day photoperiod. They have a length of 8.5–13.6 μ and a width of 5.1–6.8 μ , possess (1–)2(–3)–4 flagella and 1–3 pyrenoids (*fig. 21B*). The stigma is located about medianly and the parent cells are difficult to distinguish from gametangia (*fig. 21A*). After releasing most of these reproductive cells die off, only a limited number shows a development into sporophyte-like plants whereas a few others are observed to grow into filaments. We presume that these microzoospores are formed under unusual external conditions. PASCHER (1907) observed many microzoospores after thawing of frozen material, whereas KLEBS (1896) mentioned the presence in autumn- and wintertime. Possibly in these cases gametogenesis is oppressed by some unfavourable condition. As a result no gametes are formed, but an intermediate between zoospores and gametes, showing characteristics of both. This hypothesis is supported by the fact that KLEBS (1896) also established the presence of microzoospores in spring, whereas two days later the same material only showed gametangia. We are convinced that an investigation with a broader scope, based on physical and chemical aspects too, may bring more light in these problematic reproductive cells.

4.3.4. Taxonomy

For more than a century this species has been the most intensively studied one of all freshwater *Ulothrix* species. The reason lies in its cosmopolitan distribution and widespread occurrence in eutrophic, more or less turbulent waters in winter and spring and the conspicuous, relatively large filaments.

WEBER & MOHR already in 1804 described this alga as *Conferva zonata*. In their description among others the median-located chloroplast was mentioned, but no cell dimensions were given. Nevertheless, it was not problematic to indicate material, collected by WEBER & MOHR from "Wettern, Sweden" and preserved in AGARDH's herbarium under no. 7296, as the lectotype for this alga. Nearly the same cell dimensions, viz. 13.1–45.1(–63) μ , the same variation in number of pyrenoids and morphological similarity in the habit of zoosporangia was present.

In 1833 for the first time this species was classified under *Ulothrix* by KÜTZING. In 1843 the author reported asexual reproduction. In 1845 a cell diameter up to 1/80''' (29.1 μ) was recorded. KÜTZING did not recognize this

alga as a more or less polymorphic species, showing different growth stages. These forms were described by him as new species, sometimes without giving sufficient arguments, in 1845 as *Ulothrix varians*, *U. pectinalis*, *U. didyma*, *U. inaequalis* and in 1849 *U. crisper* (Berkeley) Kützinger, *U. valida* and *U. attenuata* are added to this series. It was found, however, that all these species show a morphological variability falling within the range of *U. zonata*; moreover, the individual species characters such as strongly shortened cells, presence of coupled cells, thick-walled cells, alternately widened and narrowed parts in the filaments, may be found together in one clone. For these reasons the above-mentioned species must be assigned to *Ulothrix zonata*.

In table 4 the main characters of the above-mentioned species are summarized to indicate that essentially little differences exist in morphology and cell dimensions; this is shown quite clearly in the additional results of the study of herbarium specimens.

From the list of synonyms given by KÜTZING, *Conferva lucens* Dillwyn, type specimen preserved in BM, s.n., *Schizogonium latissimum* Meneghini pro syn. ex KÜTZING, in L as No. 939.67-729, and *Conferva bicolor* Smith & Sowerby (type not seen, but drawings are clear), are also classified under *Ulothrix zonata* on account of their growth habit.

4.3.5. Taxonomic relationships

This study has revealed several conspicuous characters. The branching of the filament, until now only seen in culture and only very exceptionally, may have evolutionary significance. Also the lateral germination of the zoospore is striking. This kind of division reminds of the germination of the zoospores observed in some *Stigeoclonium* species. The polar differentiation however is preserved in the *Ulothrix* germling.

Table 4. Survey of the species, which are classified under *Ulothrix zonata* as a synonym.

Name, and features of original description	Data of preservation	Data from our study and commentary
<i>Ulothrix varians</i> : in 1845 a cell diameter 1/100-1/50''' (23.3-46.5 μ) and 1/2-1 \times as long; in 1849 a range of 1/100-1/60''' (23.3-38.8 μ), 1/2-1 \times as long. Further important species features lack.	Lectotype in L as No. 939.26-247, from Hanau	Cell diameter 12.6-44.8 μ ; length 1/3-2 \times as long. Represents a normal growth habit.
<i>Ulothrix pectinalis</i> : in 1845 a cell diameter range from (1/250-) 1/100-1/80''' (9.5-23.3-29.1 μ) and 1/2-1/4 \times as long; in 1849 1/150-1/100-1/80''' (15.5-23.3-29.1 μ) and 1/2-1/4 \times as long. Comb-shaped habit resulting from the presence of shortened cells.	Lectotype in L as No. 939.26-215, from Timova near Mont-falcone	Cell diameter range 15.4-56.7 μ ; 1/6-1/2 (-1) \times as long. Shortened cells are usually present at low temperatures and low light intensities.

Name, and features of original description	Data of preservation	Data from our study and commentary
<i>Ulothrix didyma</i> : in 1845 a cell diameter range from 1/100–1/90''' (23.3–25.8 μ) and 1/2–1 \times as long; in 1849 1/100–1/60''' (23.3–38.8 μ) and 1/2–1 \times as long. A torulose growth habit, coupled cells, slightly barrel-shaped.	Type in L as No. 939.67–900, from Hirschbach	Cell diameter range from 15.4–52.5 (–70) μ and 1/3–1 \times as long. Represents a typical growth habit under less favourable conditions.
<i>Ulothrix inaequalis</i> : in 1845 a cell diameter range from 1/200–1/90''' (11.6–25.8 μ) and 1/3–1/4 (–1) \times as long; in 1849 1/130–1/60''' (17.9–38.8 μ) and 1/3–1/4 (–1) \times as long. Alternately widened and narrowed filaments.	Type in L as No. 939.26–249, from Hanau	Cell diameter range from 15.4–42.0 μ and 1/6–2 \times as long. This species feature is common both in culture and wild material with less regular growth.
<i>Ulothrix crispa</i> : in 1833 no statement of cell dimensions; in 1849 a cell diameter range from 1/50–1/45''' (46.5–51.7 μ) and 1/2–1/3 \times as long. A cespitose growth habit.	Lectotype in BM ex Herb. Berkeley, s.n., sine loco	Cell diameter range from 14.0–49.0 μ and 1/4–1/2 (–1) \times as long. Represents a normal growth habit.
<i>Ulothrix valida</i> : in 1849 the same dimensions as <i>U. speciosa</i> ; 1/65–1/35''' (35.8–66.4 μ) and 1/2–1/3 \times as long. Rigid filaments.	Type in L as No. 939.67–831, from Zürich	Cell diameter range from 36.4–90.0 μ and 1/5–1 \times as long. A typical growth habit under very unfavourable conditions.
<i>Ulothrix attenuata</i> : in 1849 only cell diameter for the upper part of the filaments is given, viz. 1/60''' (38.8 μ), decreasing in the lower part. At the same time the cell length/width ratio becomes smaller towards the filament top (up to 1/2 times as long).	Type in L as No. 939.67–916, from Offenbach	Cell diameter range from 14.0–42.0 μ and 1/2–2 \times as long. A diminishing cell width towards the base of the filament is not unusual in <i>Ulothrix</i> species.

5. CONCLUSIONS AND DISCUSSION

Two of the species studied, viz. *Ulothrix tenuissima* and *Ulothrix zonata* produce sexual swimmers; all three show asexual reproduction and vegetative multiplication by way of fragmentation. Only one clone of *Ulothrix zonata* shows the presence of akinetes.

The life-history of our clones of *Ulothrix tenuissima* and *U. zonata* is in general

quite identical to that, described by DODEL (1876) and KLEBS (1896) for middle-european forms of *U. zonata*. DODEL (1876) established that non-fusing gametes germinate into narrow filaments, whilst according to KLEBS (1896) these reproductive cells develop into a sporophyte-like stage, which is in agreement with our results. Only in one clone microzoospores in the sense of KLEBS (1896) and PASCHER (1907) are noticed in this study. Within the species the behaviour of the surviving gametes during germination is different from that noticed for the earlier described, smaller *Ulothrix* species (LOKHORST & VROMAN 1972). These species showed gametes, which may laboriously develop into filaments. Probably this phenomenon explains that these species may still be present in the filamentous stage in the Netherlands in summertime, while *U. tenuissima* and *U. zonata* are not.

The behaviour of the species, studied under different photoperiods, reflects seasonal periodicity in nature, expressed in the alternation of life-cycle stages. In winter and spring in the Netherlands *U. zonata* and *U. tenuissima* are abundantly present in the filamentous stage, with zoospores and gametes respectively; in summer it is presumed that they are only present in the sporophytic stage, for, as mentioned above, filamentous stages are completely absent. For *Ulothrix implexa* this periodicity could not be clearly ascertained.

No fundamental difference in the life-history could be noted among the several clones of these species, at most a difference in the rate of reproduction was present. As stated before (LOKHORST & VROMAN 1972, 1974), it is found that both wild and cultured material show constant specific features, among others the limited variation of cell diameter, the number of pyrenoids, zoospores and gametes, morphology of gametangia and zoosporangia, shape of chloroplast, shape of sporophytic stage, phototactic behaviour of zoospores etc.. The identity of wild material is easier and more certainly established, when a large number of filaments is investigated, although on account of the characteristic chloroplast morphology *U. zonata* is usually clearly recognized in mature growth stages.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to the following persons: Drs. W. F. Prud'homme van Reine for his valuable advice in herbarium and nomenclatural problems, Mr. A. P. van Beem for technical assistance, Mr. G. W. H. van den Berg for preparing the drawings for publication, and Mr. A. J. Dop for correction of the English text. The Directors and Curators of the British Museum (Nat. Hist.) at London, the Rijksherbarium at Leiden and the Muséum National d'Histoire Naturelle, Laboratoire de Cryptogamie at Paris for lending herbarium specimens and Dr. O. Almborn for the hospitality at the Botanical Museum at Lund.

The authors are also much indebted to the "Netherlands Organisation for the Advancement of Pure Research (Z.W.O.)", which, by a grant, made it possible for the first author to study AGARDH's herbarium at Lund.

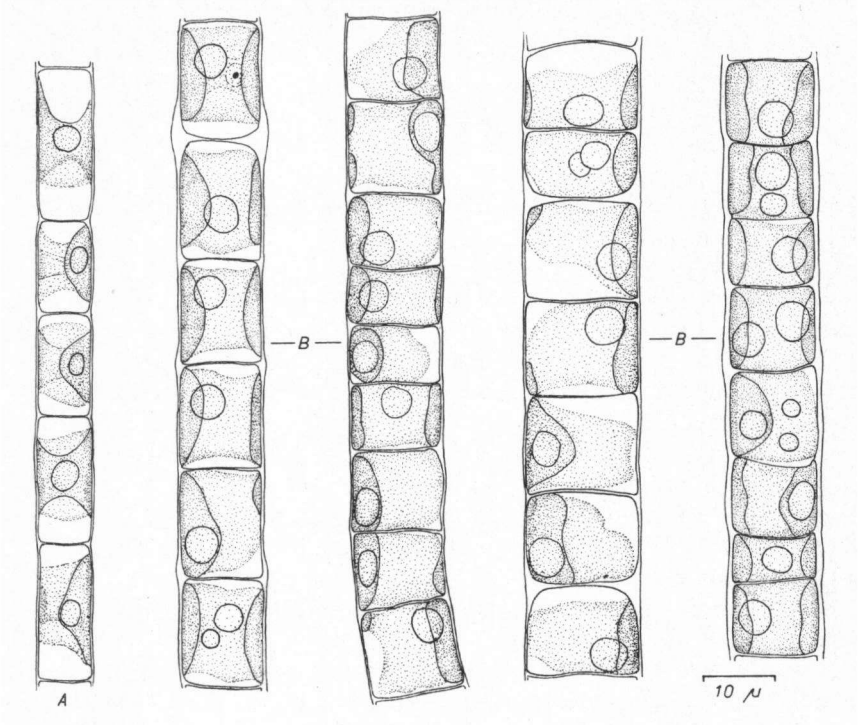


Fig. 1. *Ulothrix implexa*. A. young filament; B. full-grown filaments.

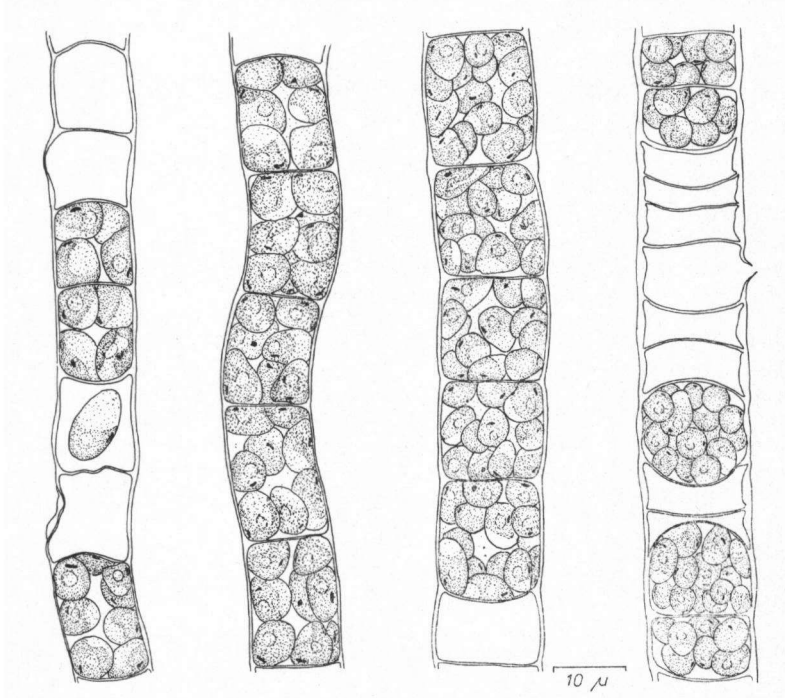


Fig. 2. *Ulothrix implexa*. Filaments with zoosporangia.

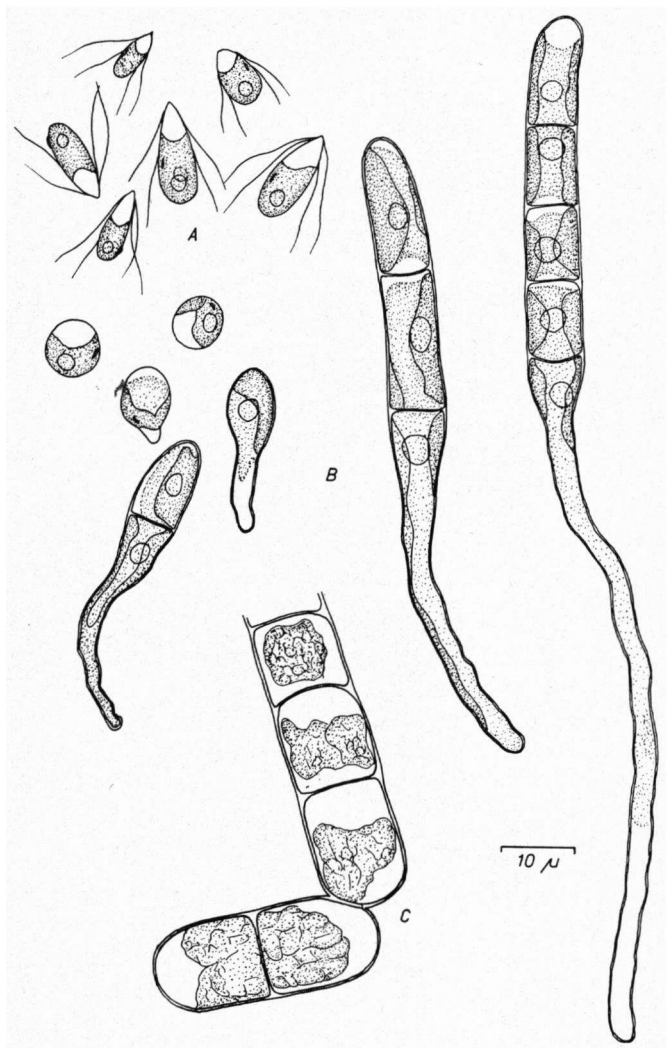


Fig. 3. *Ulothrix implexa*. A. zoospores; B. germination of the zoospore into a germling; C. fragmentation of the filament.

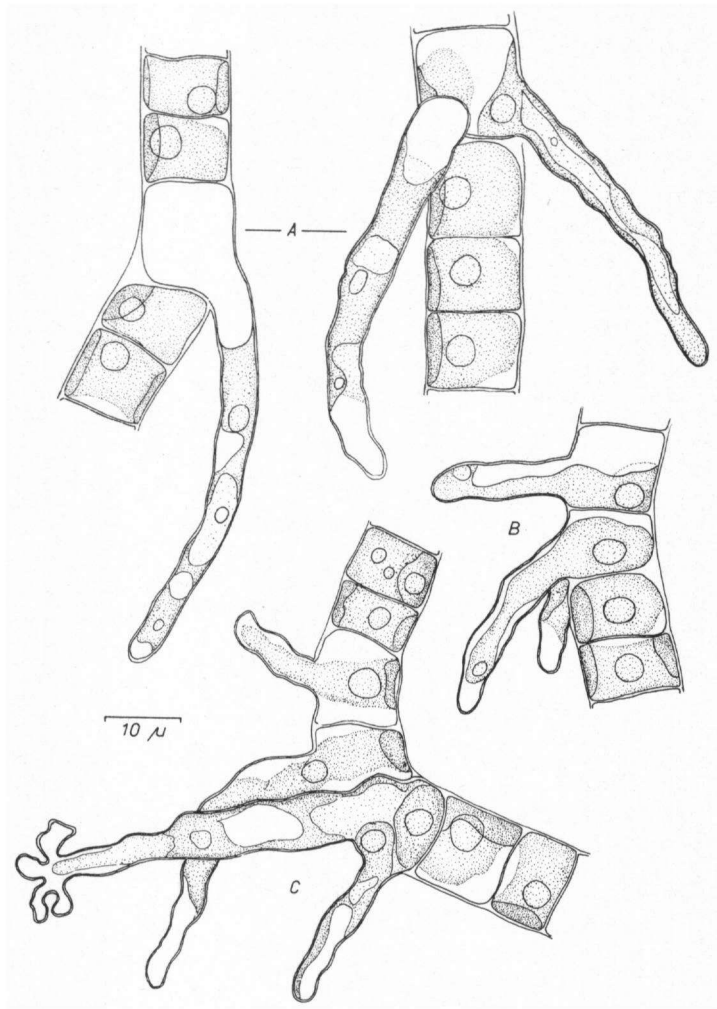


Fig. 4. *Ulothrix implexa*. Formation of rhizoidal appendages. A. in one separate cell; B. in two neighbouring cells; C. in three neighbouring cells.

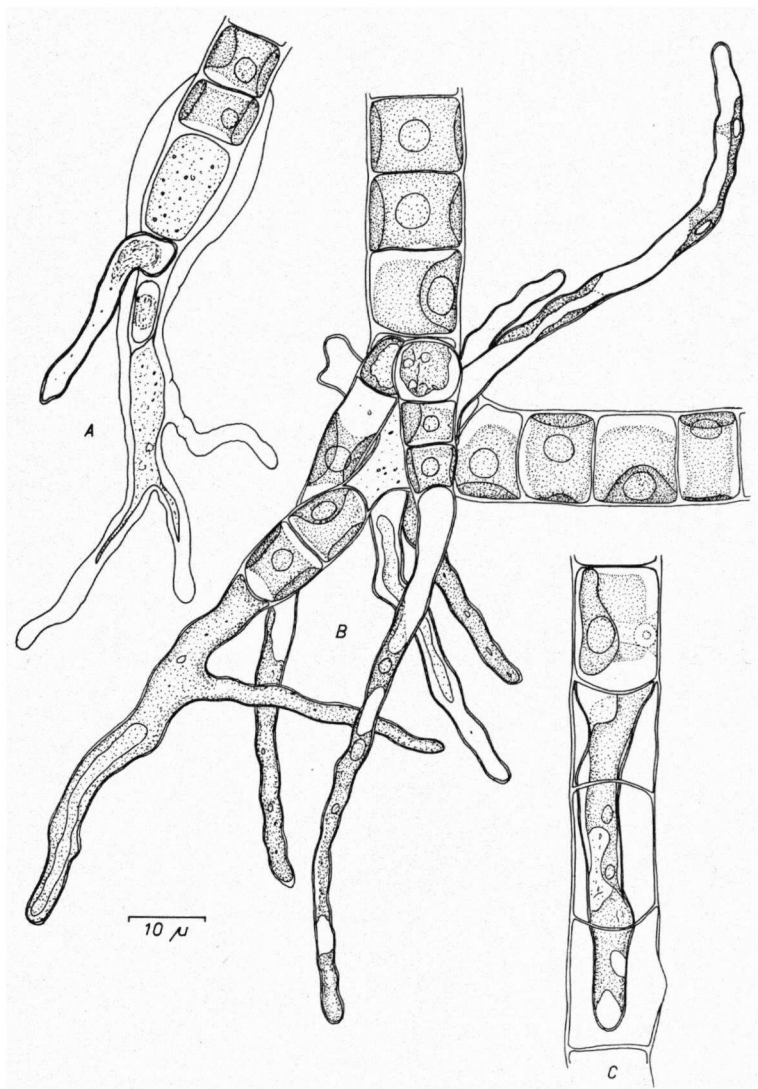


Fig. 5. *Ulothrix implexa*. A. basal part; B. false branching system; C. rhizoidal appendage from a vegetative cell, neighbouring empty zoosporangia.

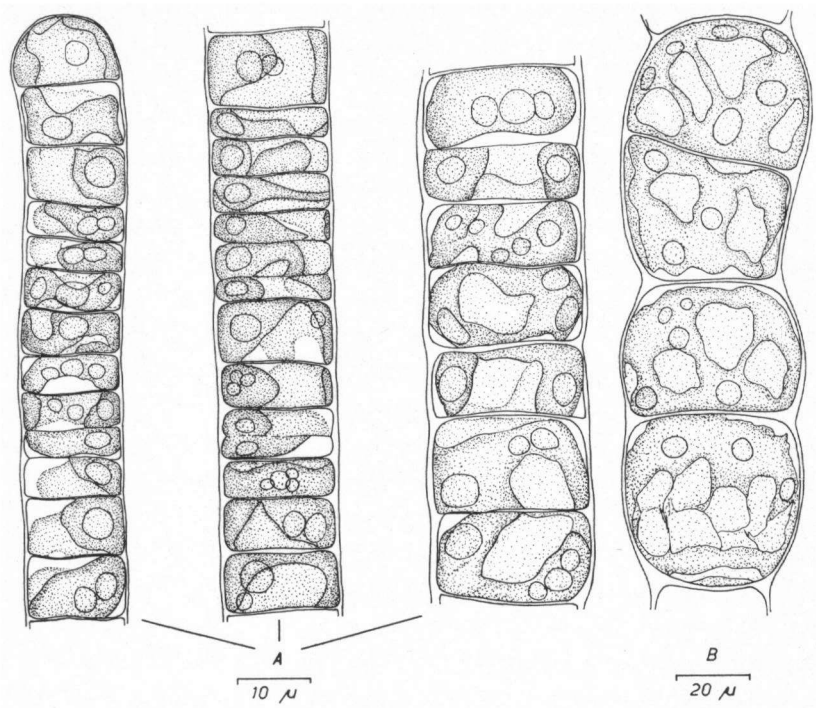


Fig. 6. *Ulothrix tenuissima*. A. full-grown filaments; B. *Urospora*-like filament.

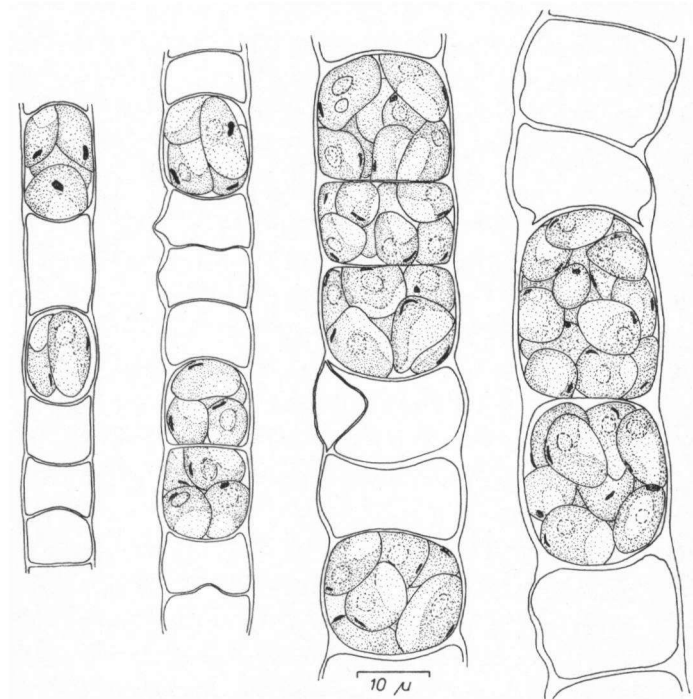


Fig. 7. *Ulothrix tenuissima*. Filaments with zoosporangia.

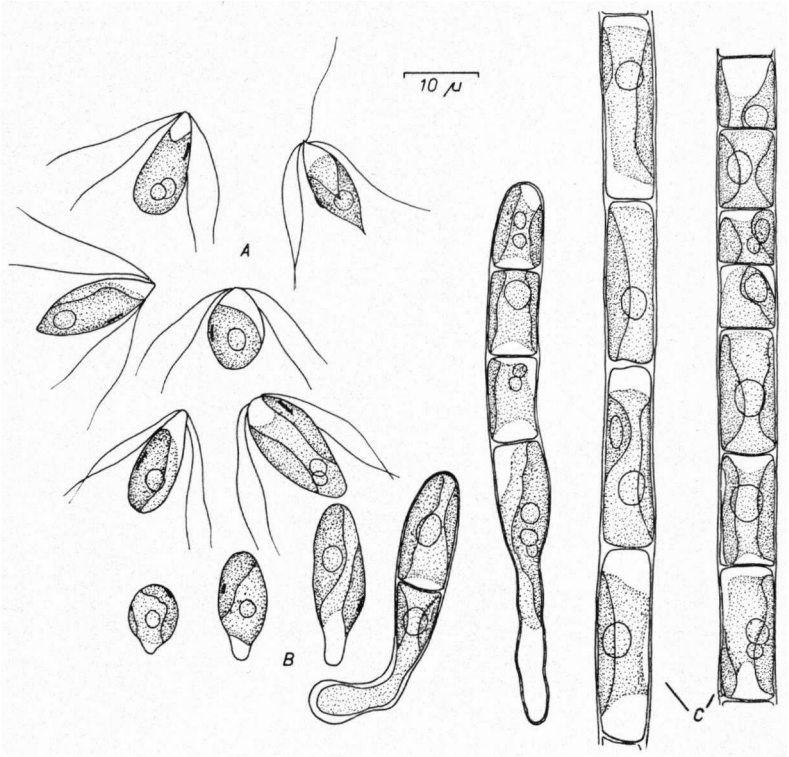


Fig. 8. *Ulothrix tenuissima*. A. zoospores; B. germination of the zoospore into a germling; C. young filaments.

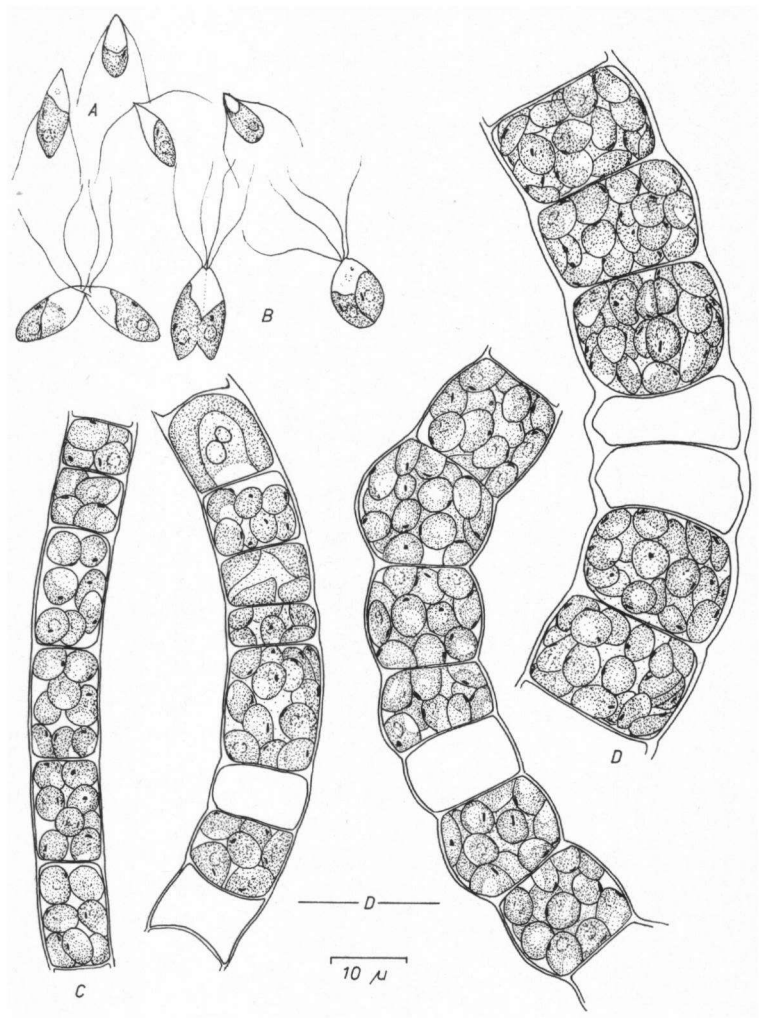


Fig. 9. *Ulothrix tenuissima*. A. gametes; B. zygotes; C. young filament with gametangia; D. full-grown filaments with gametangia.

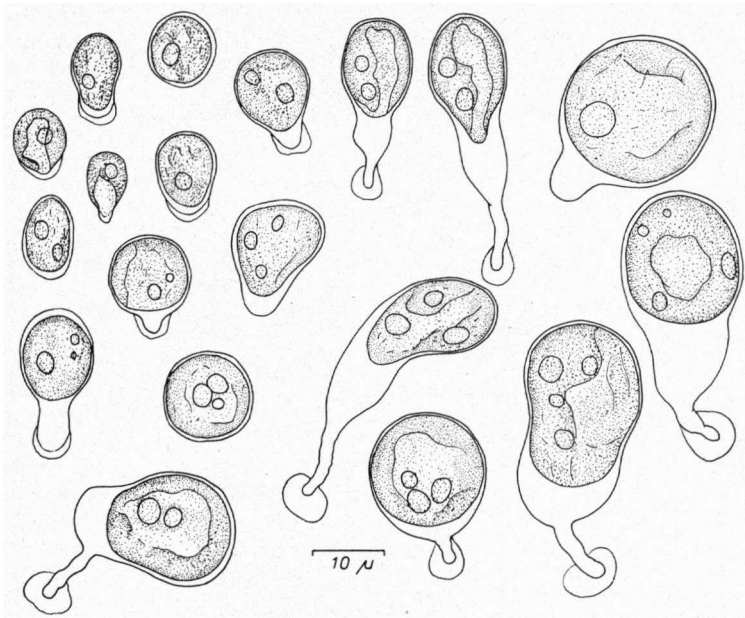


Fig. 10. *Ulothrix tenuissima*. Germination of the zygote via intermediate stages into sporophytes

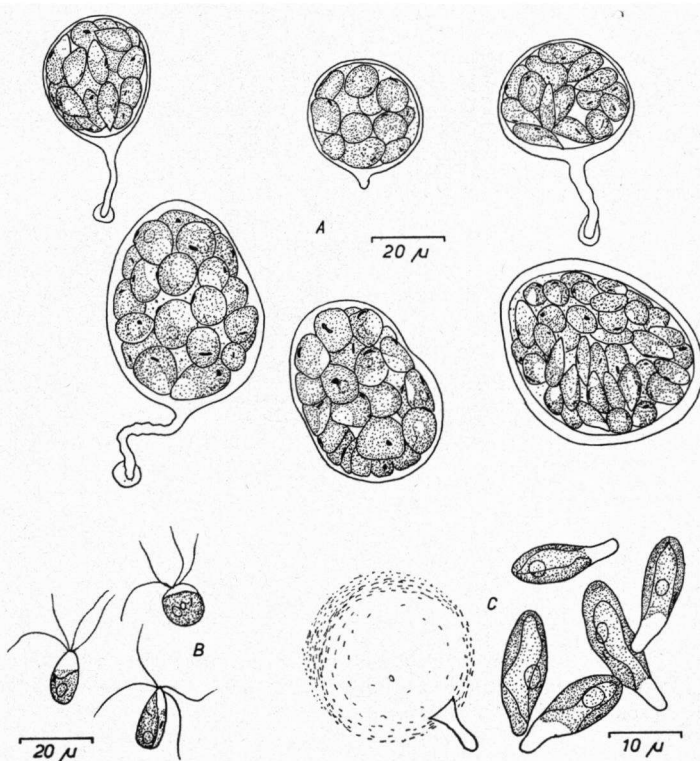


Fig. 11. *Ulothrix tenuissima*. A. fertile sporophytes; B. liberated zoospores from the sporophyte; C. germination of the zoospores into germings with the empty sporophyte.

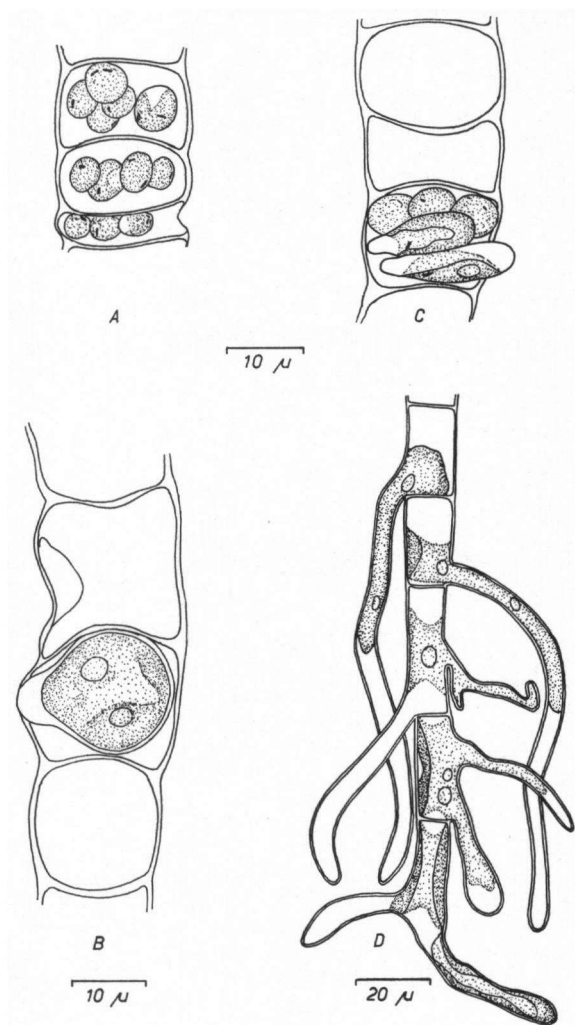


Fig. 12. *Ulothrix tenuissima*. A. filament with zygotes formed internally; B. filament with germination of the zygote inside the gametangium cell wall; C. filament with aplanospores; D. complex, rhizoidal basal part of a filament.

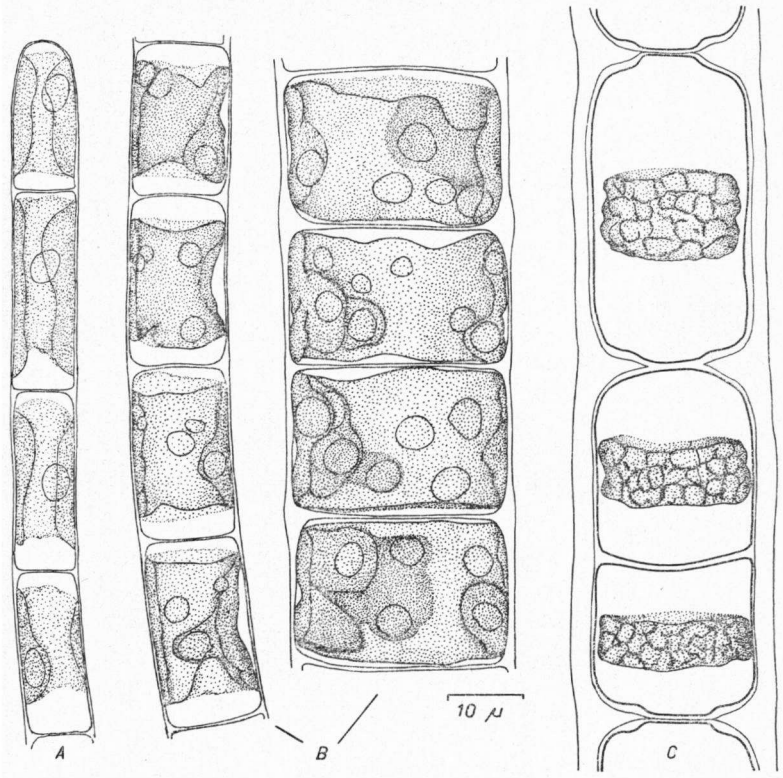


Fig. 13. *Ulothrix zonata*. A. young filament; B. full-grown filaments; C. old filament.

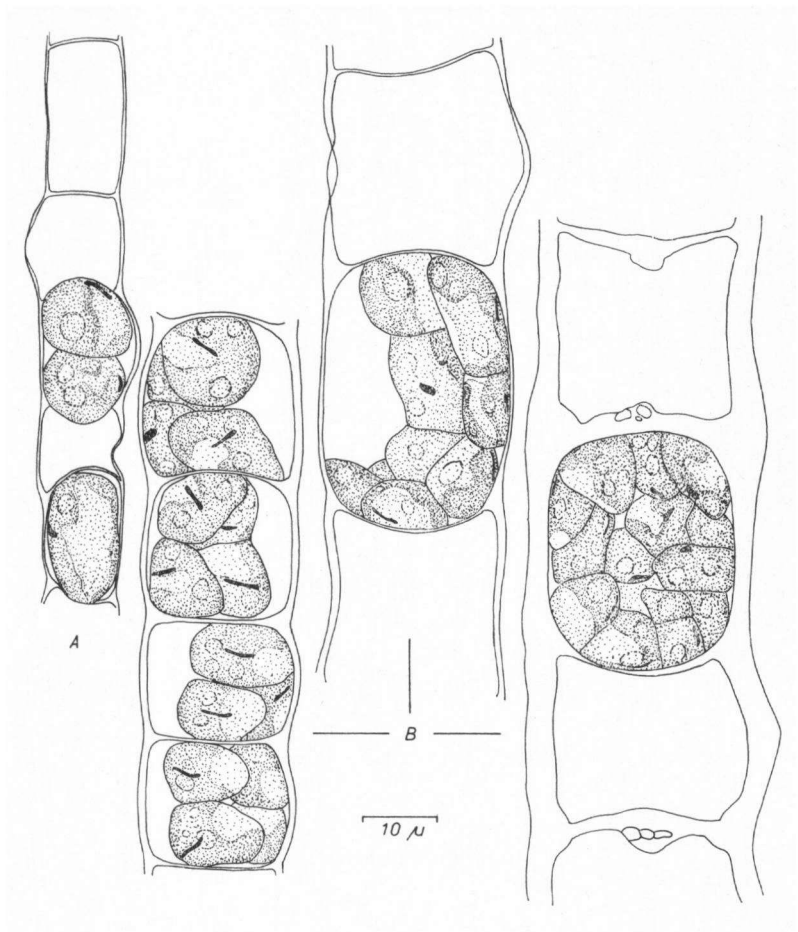


Fig. 14. *Ulothrix zonata*. A. young filament with zoosporangia; B. full-grown filaments with zoosporangia.

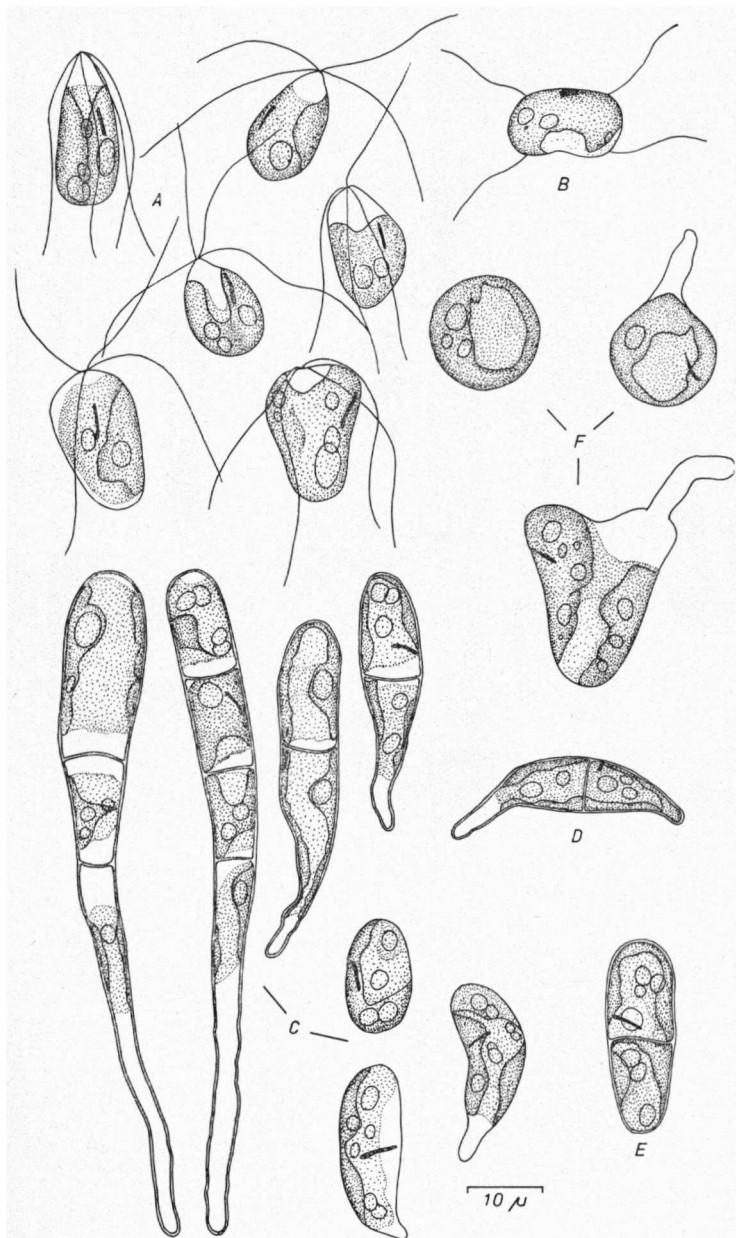


Fig. 15. *Ulothrix zonata*. A. zoospores; B. attachment of the zoospore; C. germination of the zoospores into germings; D. germling producing protuberances at both ends; E. germling without differentiated rhizoidal basal cell; F. aberrant one-celled germling stages.

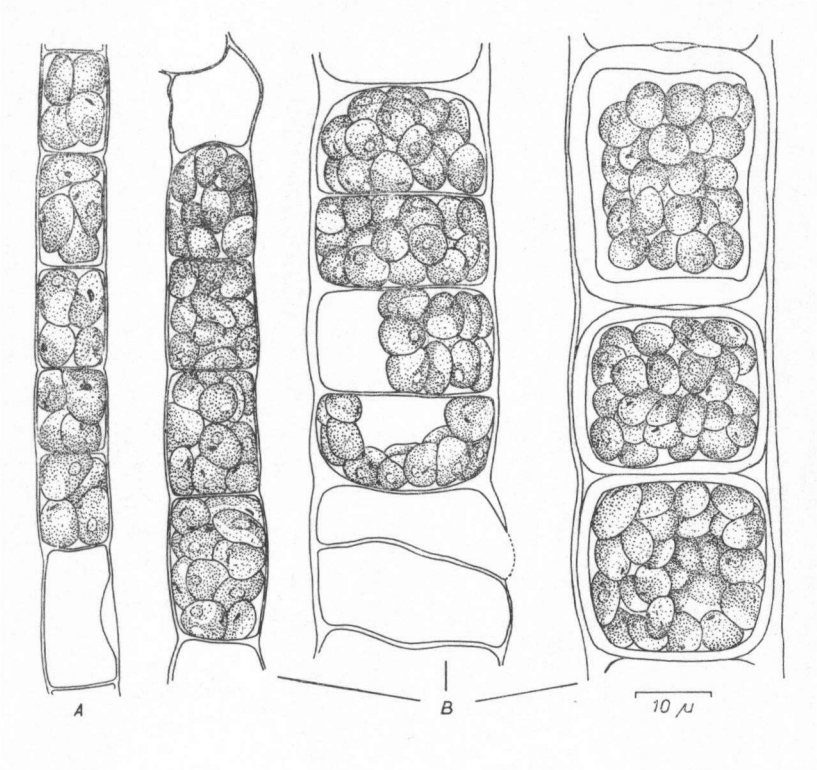


Fig. 16. *Ulothrix zonata*. A. young filament with gametangia; B. full-grown filaments with gametangia.

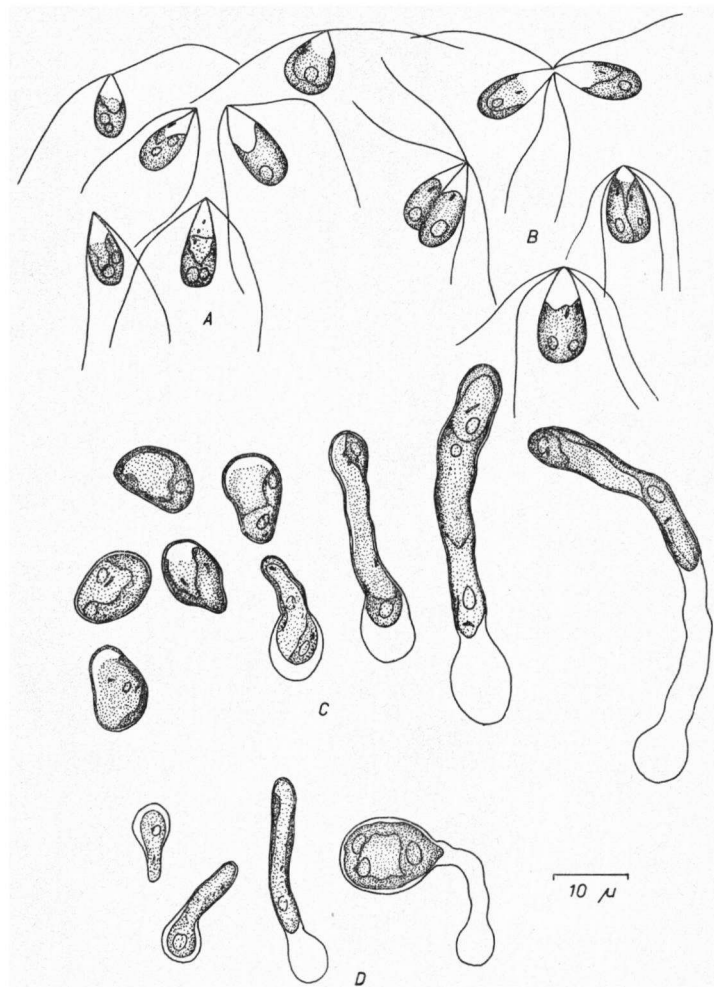


Fig. 17. *Ulothrix zonata*. A. gametes; B. formation of the zygote; C. germination of the zygote into the sporophyte; D. germination of the gamete.

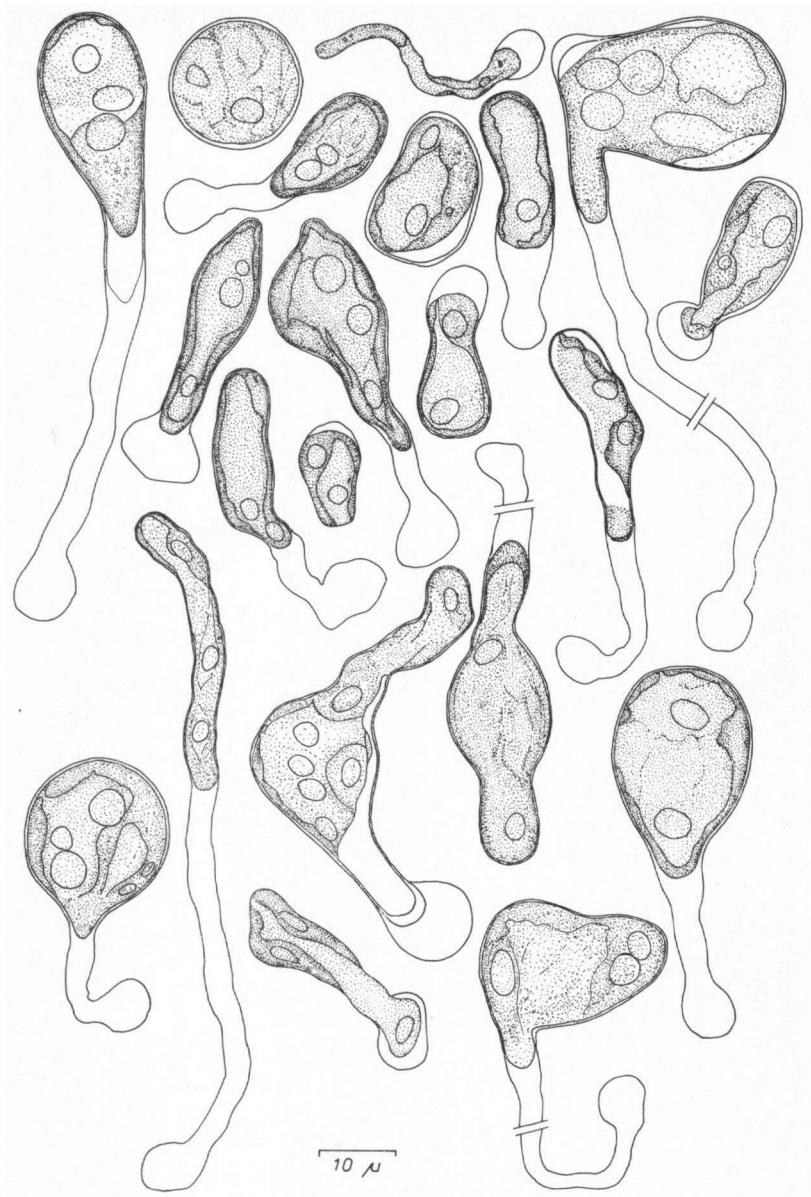


Fig. 18. *Ulothrix zonata*. Non-ripened sporophytes.

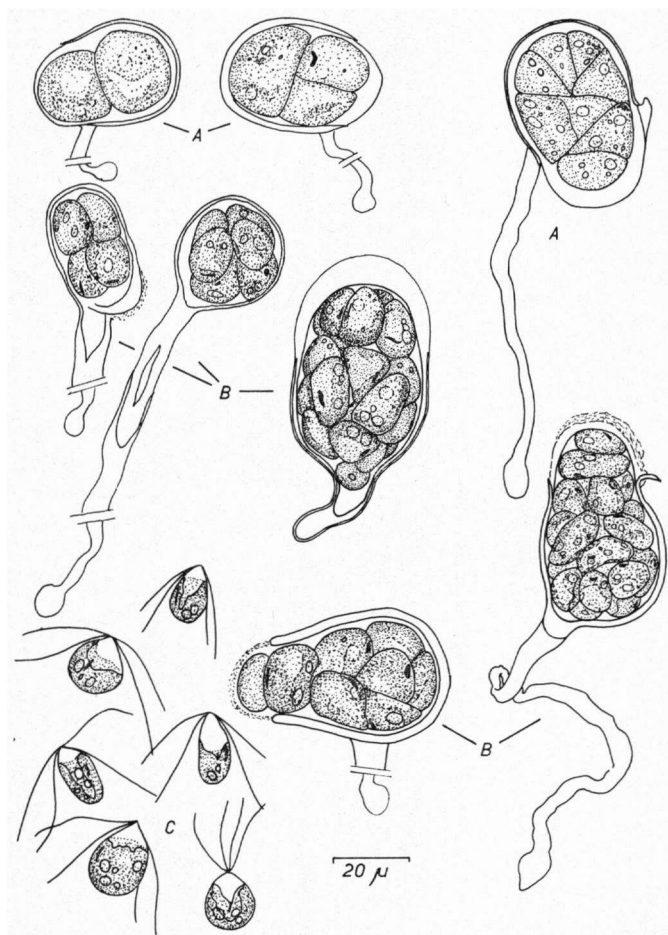


Fig. 19. *Ulothrix zonata*. A. nearly fertile sporophytes; B. fertile sporophytes; C. zoospores arisen from sporophytes.

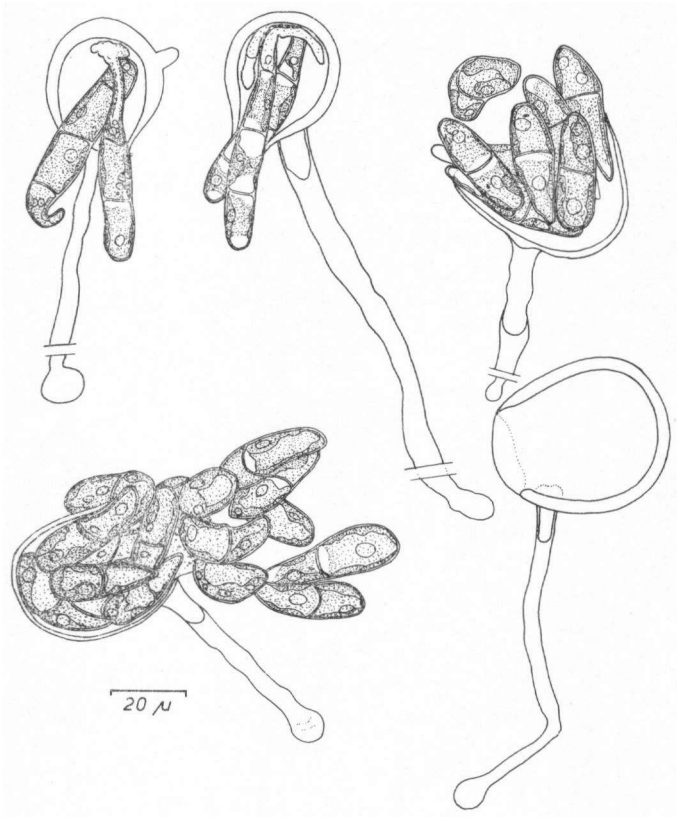


Fig. 20. *Ulothrix zonata*. Germination of aplanospores in sporophytes.

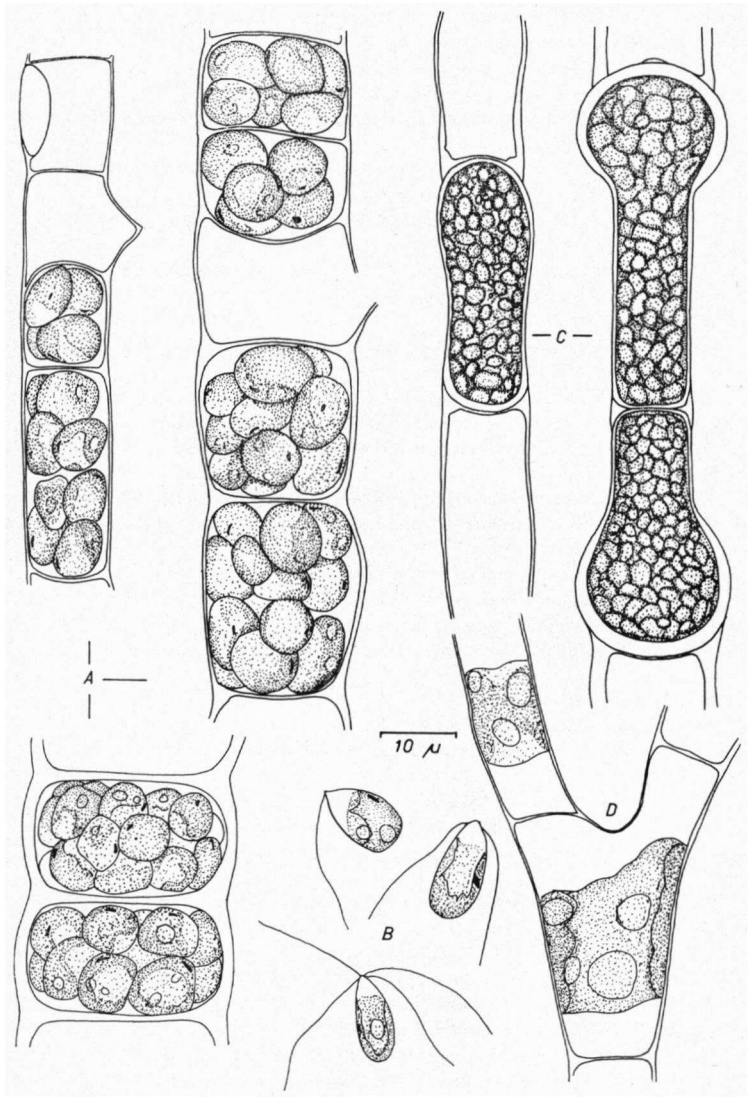


Fig. 21. *Ulothrix zonata*. A. filaments containing microzoospores sensu KLEBS and PASCHER; B. liberated microzoospores; C. akinete formation in filaments; D. a branching.

REFERENCES

- AGARDH, C. A. (1813): *Algarum decas secunda*. Lund.
 — (1824): *Systema algarum*. Lund.
- ARESCHOUG, J. E. (1866): Observationes Phycologicae. Particula prima: De Confervaceis nonnullis. *Act. Reg. Soc. Scient. Upsal.*, ser. III, 6: 1–26.
- BERKELEY, M. J. (1833): *Gleanings of British Algae*. London.
- BERTHOLD, G. (1886): *Studien über Protoplasmamechanik*. Leipzig.
- CRAMER, C. (1871a): Ueber Entstehung und Paarung der Schwärmsporen von Ulothrix. *Bot. Zeit.* 29: 76–80.
 — (1871b): Ueber Entstehung und Paarung der Schwärmsporen von Ulothrix. *Bot. Zeit.* 29: 89–91.
- DE-TONI, G. B. (1889): *Sylloge algarum omnium hucusque cognitarum* I. Padua.
- DILLWYN, L. W. (1802–1809): *British confervae*. London.
- DODEL, A. (1876): Ulothrix zonata. Ihre geschlechtliche und ungeschlechtliche Fortpflanzung. *Jahrb. wiss. Bot.* 10: 417–550.
- FRIES, E. (1835): *Flora scanica*. Uppsala.
- GROSS, I. (1931): Entwicklungsgeschichte, Phasenwechsel und Sexualität bei der Gattung Ulothrix. *Arch. Protistenk.* 73: 206–234.
- HANSGIRG, A. (1886): *Prodromus der Algenflora von Böhmen* I. Prag.
- HASSALL, A. H. (1845): *A History of the British freshwater Algae*. London.
- HAZEN, T. E. (1902): The Ulothrichaceae and Chaetophoraceae of the United States. *Mem. Torrey Bot. Club* 11: 135–250.
- KLEBS, G. (1896): *Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen*. Jena.
- KÜTZING, F. T. (1833): Algologische Mittheilungen II. Ueber eine neue Gattung der Conferveen. *Flora* 16: 517–521.
 — (1836): *Algarum aquae dulcis germanicarum*. Dec. XV.
 — (1843): *Phycologia generalis*. Leipzig.
 — (1845): *Phycologia germanica*. Nordhausen.
 — (1847): Diagnosen und Bemerkungen zu neuen oder kritischen Algen. *Bot. Zeit.* 5: 177–180.
 — (1849): *Species algarum*. Leipzig.
 — (1850–1852): *Tabulae Phycologicae* II. Nordhausen.
- LIND, E. M. (1932): A contribution to the life-history and cytology of two species of Ulothrix. *Ann. Bot.* 46: 711–725.
- LOKHORST, G. M. & M. VROMAN (1972): Taxonomic study on three freshwater Ulothrix species. *Acta Bot. Neerl.* 21: 449–480.
 — (1974): Taxonomic studies on the genus Ulothrix (Ulotrichales, Chlorophyceae) II. *Acta Bot. Neerl.* 23: 369–398.
- PASCHER, A. (1907): Studien über die Schwärmer einiger Süßwasseralgen *Biblioth. Bot.* 67: 1–116.
- RABENHORST, L. (1847): Die Algen Deutschlands. In: *Deutschlands Kryptogamenflora oder Handbuch zur Bestimmung der kryptogamischen Gewächse Deutschlands* II, 2. Abt. Leipzig.
 — (1868): *Flora europaea algarum aquae dulcis et submarinae* III. Leipzig.
- RAMANATHAN, K. R. (1964): *Ulotrichales*. Indian council of agricultural research. New Delhi.
- SCHUSSNIG, B. (1915): Algologische Abhandlungen. Ueber einige neue und seltene Chlorophyceen der Adria. *Sitz. Kais. Akad. Wiss. Wien, Math-nat. Klasse* 1, 124: 425–447.
- SMITH, J. E. & J. SOWERBY (1814): *English Botany*. Vol. 36. London.
- STRASBURGER, E. (1892): *Histologische Beiträge*, Heft IV. Jena.
- THURET, G. (1850): Recherches sur les zoospores des algues et les anthéridies des Cryptogames. *Ann. Sci. nat. Bot. Ser. III*, 14: 214–260.
- WEBER, F. & D. M. H. MOHR (1804): *Naturhistorische Reise durch einen Theil Schwedens*. Göttingen.
- WILLE, N. (1901): Studien über Chlorophyceen. I–VII. *Vidensk. Skr. I. Math.-naturv. Klasse* No. 6: 1–46.