

TAXONOMIC STUDY ON THREE FRESHWATER ULOTHRIX SPECIES

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SUMMARY

The present study, based on rearing experiments and herbarium investigation, deals with the taxonomy of three freshwater *Ulothrix* species; *Ulothrix subtilis* Kützing, *U. tenerrima* Kützing, and *U. albicans* Kützing. Laboratory cultures have to be used for unequivocal determination. Of special importance are developmental features like the shape of the basal cell, and life-history features like the limited variation in number of zoospores and gametes. When many filaments are present and when culturing is impossible, the limited variation of the cell diameter of vegetative cells, zoosporangia and gametangia can also be used, with care. The behaviour of the species under different photoperiods is correlated with the way the algae appear in nature in winter and summer time.

1. INTRODUCTION

The genus *Ulothrix*, a group of unbranched uniseriate filamentous green algae, was established by KÜTZING in 1833 with the description of the freshwater species *U. tenuissima*. At the same time KÜTZING (1833a) transferred 4 freshwater species from the genus *Conferva* (*C. zonata* Weber & Mohr, *C. dissiliens* Dillwyn, *C. capillaris* C. Agardh and *C. compacta* Roth) and the terrestrial species *C. muralis* Dillwyn to this new genus. In *Species Algarum* (1849) KÜTZING mentioned 44 species, the large number caused by the fact that the genus *Hormidium*, which included only terrestrial species, was also regarded as a section of *Ulothrix*. RABENHORST in 1863 listed 16 species of *Ulothrix*. In 1868, however, for the purely aquatic and thick-walled species he had changed that genus name to *Hormiscia*, whereas *Ulothrix* was maintained for thin-walled species which may occur in aquatic, marshy and terrestrial habitats.

HAZEN (1902) clearly established that *Ulothrix* has priority over *Hormiscia* and that the two genera must not be treated as synonymous. The description given by Hazen for the genus *Ulothrix* was accepted by nearly all phycologists (HEERING 1914, SMITH 1950, PRINTZ 1964, PRESCOTT 1951, RAMANATHAN 1964 etc.). Only MATTOX & BOLD (1962), after studying the taxonomy of *Ulothrix* and *Hormidium* with the culture method, proposed a modified concept for *Ulothrix*. The present authors have some objections against this genus concept, based on the size and morphology of the chloroplast and the number of pyrenoids. The characters used by Mattox & Bold are strongly variable within a number of species. On close examination the two genera can only be distinguished by the morphology of the zoosporangia and zoospores (LOKHORST 1969).

The present paper gives the result of a study of the life-history of the fresh-

water species *Ulothrix subtilis* Kützing, *U. tenerrima* Kützing, and *U. albicans* Kützing. In our investigations of the taxonomy of the Dutch species of *Ulothrix* these species were studied first and foremost because there have been many species described in the cell diameter range of 4–14 μ . Phycologists always have had many problems about the identity of *Ulothrix* species in this range. This is caused by the insufficient indication of morphological features, like the width of the filaments and cell-length, in the original descriptions. By culturing of the algae many characters for distinguishing the species, like developmental features and life cycle stages, may be found.

The need of unialgal cultures for elucidating the taxonomy of *Ulothrix* has also been emphasized by KORNMANN (1963, 1964) and PERROT (1968, 1970, 1971) for the marine species *U. subflaccida* Wille, *U. pseudoflaccida* Wille, and *U. flacca* (Dillwyn) Thuret. The same was concluded for freshwater *Ulothrix* and *Hormidium* species by MATTOX & BOLD (1962) and FAROOQUI (1969).

The life-history of the studied freshwater species is shown in fig. 1.

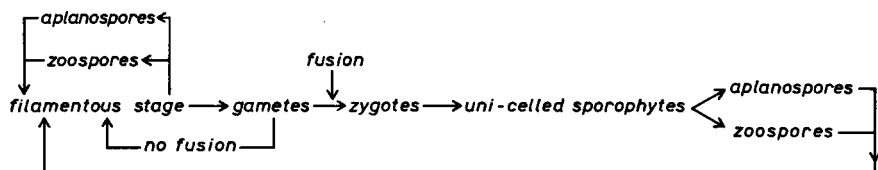


Fig. 1. Diagram of the life-history of *Ulothrix*

2. MATERIAL AND METHODS

Several clones of *Ulothrix subtilis*, *U. tenerrima*, and *U. albicans* were isolated from a number of freshwater habitats in the Netherlands.

The algae were collected from hard substrates like sheet-pilings of a bank, stones, stems of *Phragmites australis*, mostly 0–25 cm below water level in rather quiet water. Sometimes, when the water was turbulent or when the edge of the waters was regularly inundated by shipping turbulence, the species could be collected several cm above the water level.

The pH of the waters ranged from \pm 7.0–8.0. In acid habitats, like peat-moors and fens, these species were usually absent.

From the isolates uni-algal stock-cultures were made, which were kept in a culture room at c. 8°C in glass boxes (diameter 5 to 15 cm) containing a freshwater Erdschreiber solution composed as follows: KNO₃ 10 mg, K₂HPO₄ 20 mg, CaCO₃ 10 mg, Fe-EDTA 0.1 cc, soil extract 50 cc, fresh water 1000 cc.

The stock-cultures were illuminated by white fluorescent tubes at a distance of 30 cm and exposed to a daily photoperiod of 12 hours and a light intensity of about 1500 lux.

For studying the influence of the photoperiod on the life-history, vegetative filaments were taken from the stock-cultures, transferred to glass boxes with

fresh medium, and exposed to photoperiods of 8 hours ("short-day conditions") or 16 hours ("long-day conditions"). For the investigation of changes in the diameter of filaments in relation to the cultivation-time under different photoperiods, vegetative filaments were taken from the stock-cultures and transferred to glass boxes with fresh medium. On the bottom a slide was put. After a short time zoosporogenesis took place and many zoospores settled on the slide. From then till 9 weeks later (full-grown filaments) the diameter of 100 germlings was measured once a week (see *tables 1 and 2, fig. 2*). Renewal of the medium took place every 3 weeks.

The reproductive cells were picked up by the capillary method, then brought into a drop of medium on a slide. Evaporation of the drop was prevented by bringing the slide into a petri-dish provided with a wet filtering-paper on the bottom. After a few hours the free-swimming reproductive cells (zoospores, gametes and zygotes) attach themselves to the slide. These cells were located by means of the mechanical stage of the light microscope and then the slides were put into petri-dishes filled with fresh medium. In this way their development could be followed.

For studying their morphology the reproductive cells could be almost immobilized and easily kept under observation in a 1% solution of Na-alginate in tap-water (VAN DEN HOEK & FLINTERMAN 1968).

Herbarium material was also studied. This was borrowed from the Botanical Museum and Herbarium at Copenhagen (C), the Rijksherbarium at Leiden (L), and the British Museum (Nat. Hist.) at London (BM). The herbarium specimens could be made to resume their original habit by treatment with a detergent. The results were not very satisfactory, however, due to poor preser-

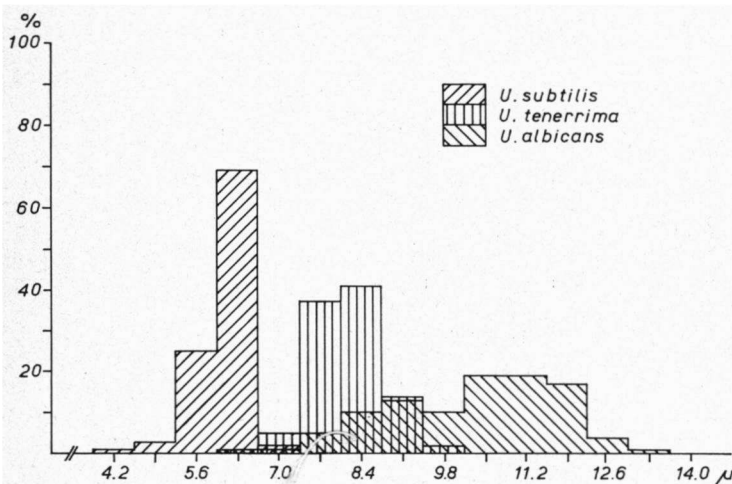


Fig. 2. Frequency-distribution of the cell diameter of 9 weeks old germlings of *Ulothrix subtilis*, *U. tenerrima* and *U. albicans* cultivated under short-day conditions.

vation of the material. It was difficult to obtain from the herbarium specimens valuable information on the morphology of the chloroplast, the number of pyrenoids, etc., features which are essential for the distinction of the genera *Ulothrix* and *Hormidium*. This conclusion has also been drawn by HAZEN (1902).

3. INFLUENCE OF CULTURING ON MORPHOLOGY AND LIFE CYCLE

3.1. Effect of photoperiod on the morphology

In young filaments the length of the cells is generally greater than their width (figs. 5A, 10A). In older cultures the cell-length of non-dividing cells is mostly equal to or shorter than the cell-diameter (figs. 5B, 10B). Different day-length periods do not appear to have a marked influence on the length-width ratio of the cells. However, in young cultures under long-day conditions an evident tendency to grow faster could be observed. In young cultures (3 weeks old, see tables 1 and 2) reared under long photoperiods thicker filaments are present.

In young filaments the chloroplast, containing few pyrenoids, is mostly regularly lobed along its longitudinal margin (figs. 5A, 10A). In older cultures the chromatophore is more strongly developed and therefore more irregularly organized (figs. 5B, 10B). In old (10–12 weeks and more) cultures without renewal of the culture medium the chloroplast often becomes atypical, being strongly granulated (figs. 3B, 10C), sometimes withdrawn (fig. 3B). At the same time the pyrenoids disappear. This happens earlier in long-day cultures, where the faster growth exhausts the nutrient medium sooner. Sometimes, at regular intervals, the filaments are somewhat constricted. In these places the cells are slightly elongated (fig. 10B).

In nature mostly pronounced square cells containing an unlobed chloroplast could be observed.

The number of pyrenoids may vary. In thicker cells they are greater in number (figs. 5B, 10B). The top cells are always bluntly pear-shaped (figs. 3E, 5A, 10A, 10B). Never were *Uronema*-like top cells seen, as reported by MATTOX & BOLD (1962).

The morphology of the basal cell, which is rather characteristic, is discussed below.

3.2. Influence of short-day conditions on the life-history

Several days after transferring vegetative filaments to short-day conditions the contents of nearly all cells of the filaments, except the basal ones, start to divide and zoosporogenesis takes place. The zoospores possess a median-posterior eye-spot, a cup-shaped chloroplast with one pyrenoid, and 4 flagella (figs. 3D, 6C, 11C). The swimmers are liberated through an irregular opening in the cell wall (fig. 3C), which is mostly only visible with the phase-contrast microscope. After swarming, which movement is regular and rather quiet, the zoospores become spherical, attach themselves to the substratum, and then germinate into new filaments (figs. 3E, 6D, 11E). After several weeks these

germlings again show the described asexual reproduction, especially after renewal of the medium.

Zoospores varying in size are sometimes present in one sporangium. However, usually small zoosporangia provide small zoospores; in large zoosporangia small and large zoospores may be produced side by side. Therefore it is difficult to make a distinction between micro- and macrozoospores with 4 flagella for the *Ulothrix* species studied, as KLEBS (1896) and PASCHER (1907) established for *U. zonata* strains.

3.3. Influence of long-day conditions on the life-history

Several days after transferring vegetative filaments to long-day conditions also zoosporogenesis takes place. However, the number of cells dividing into zoospores is much smaller than under short-day conditions. This zoosporogenesis is probably caused by transferring the filaments into a fresh medium. Then for a few days strong vegetative growth can be observed. After 7–14 days, sometimes more, the contents of the cells become slightly light green to pronouncedly yellowish green and gametogenesis takes place.

The length of the gametes in one gametangium may vary strongly. It is difficult, however, to make a distinction between micro- and macrogametes, as no discontinuity is present in the length and width of these reproductive cells.

The gametes are pear-shaped and always possess a cup-shaped chloroplast with one pyrenoid and a mostly approximately median eyespot (*figs. 4B, 7B, 13C*). They move rather irregularly (skittishly) and very fast in comparison with the zoospores. The gametes are strictly photo-positive. Fusion always takes place by isogamous gametes and may be monoecious or dioecious. The quadriflagellate zygote swarms for a while, being strictly photo-negative. Later the zygote becomes spherical, attaches itself to the substratum and sheds the flagella. The zygote germinates into a uni-celled sporophyte, which only under short-day conditions produces zoospores (*figs. 4D, 9A, 14B*).

The gametes may also germinate into filaments, but much more slowly than zoospores do. After one week, when zoospores under the same conditions would have formed multi-celled filaments, gametes only have produced an elongated body of at most two cells. Gradually, however, they develop into filaments, which are narrower than those arisen from zoospores. This is in agreement with the results of DODEL (1876), LIND (1932) and WILLE (1900) for, respectively, *U. zonata* and *U. pseudoflaccida*.

When long-day cultures are brought under short-day conditions, the long-day effect for some time still is perceptible in these cultures. Gametogenesis still continues, but in the course of time the liberated gametes are no longer able to fuse and develop into filaments. These reproductive cells were probably considered biflagellate microzoospores by KLEBS (1896), PASCHER (1907) and LIND (1932).

3.4. Germination of the zygote

Under short-day conditions the zygote increases in size. The chloroplast becomes irregularly lobed and contains several pyrenoids (figs. 4C, 8A, 14A). Afterwards the contents become finely granulated. During the maturation phase the sporophyte usually becomes more or less spherical in *U. albicans* (fig. 14B) and pear-shaped or spherical to irregularly shaped in *U. subtilis* (fig. 4D) and *U. tenerrima* (fig. 9A). The zygotes in our experiments never germinated into a filamentous stage, as PERROT (1968, 1970, 1971) established in the life-history of French strains of *U. flacca*, *U. subflaccida*, and *U. pseudoflacca*.

In the three freshwater species studied it is quite exceptional that a stalked sporophyte (fig. 9B) is formed, as described for *U. zonata* (DODEL 1876) and *U. subflaccida* (KORNMAN 1964).

After 2 weeks to 3 months under short-day conditions, depending on the time the zygote was under long-day conditions, the contents of the sporophyte divide into 8, 16, 32, 64, or sometimes more zoospores or aplanospores (figs. 4D, 9A, 14B). During this ripening process the contents of the sporophyte commonly become yellowish-brown in colour.

4. RESULTS

4.1. *Ulothrix subtilis* Kützing

Ulothrix subtilis Kützing, 1845 pg. 197; emend. 1849 pg. 345.

References and synonyms:

Ulothrix subtilis Kützing a. *genuina* Kirchner, 1878 p. 77;

Hormiscia subtilis (Kützing) De-Toni var. *genuina* Kirchner, De-Toni 1889, p. 160;

Ulothrix thermanum Wartmann in Rabenhorst (1857) Alg. Sach., no 655 (non *U. thermanum* Wartmann in Rabenhorst, Alg. Sach., no 457);

Ulothrix subtilis Kützing c. *thermarum* (Wartmann) Rabenhorst, 1868 p. 365;

Hormiscia subtilis (Kützing) De-Toni var. *thermarum* (Wartmann) Rabenhorst, De-Toni 1889 p. 160;

Ulothrix subtilissima Rabenhorst, 1863 p. 263;

Ulothrix subtilis Kützing b. *subtilissima* Rabenhorst, 1868 p. 365;

Hormiscia subtilis (Kützing) De-Toni var. *subtilissima* Rabenhorst, De-Toni 1889 p. 160.

4.1.1. Living material

Clones were isolated from the following localities: *Groningen*, Schildmeer, on a sheet-piling in a rather exposed place; canal near Zuidlaarder Meer, on stones. *Utrecht*, ditch in the Eempolder on stones; Loosdrechtse plassen, on stones, in a rather sheltered place; Botshol, on hard substrates like tree roots and old stems of *Phragmites australis*. *Zeeland*, ditch near Wolphaartsdijk.

4.1.2. Morphology

The straight filaments are unbranched and uniseriate. Each cell contains a parietal, always typically band-like chromatophore, that mostly, especially in older filaments, encircles more than half of the cell lumen and contains 1–2 pyrenoids, commonly depending on the length of the cells (figs. 3A, 3C).

The encircling chloroplast is lobed along the longitudinal margin in long cells but is mostly not lobed in short ones (*figs. 3A, 3C*). In wild material *Ulothrix subtilis* mostly has rather short cells and then the chloroplast encircles only about half the cell lumen and usually contains one pyrenoid.

Individual cells have a diameter of 4.9–7.0 (–7.7) μ and are 3/4–3(–5) times as long. Young filaments, in which zoosporogenesis usually does not take place, have a diameter of (3.5–)4.2 μ .

The development of the cell diameter in relation to the cultivation-time is expressed in *tables 1* and *2* and *fig. 2*. In full-grown cultures the cell diameter measured most often is 6.3 μ . Furthermore it is remarkable that long-day cultures already become atypical after 6–7 weeks, in spite of renewal of the medium every 3 weeks. This results in the already described degeneration of the chloroplast and a lower average of the cell diameter (*table 2*). In wild material the cell diameter is usually about 0.7 μ less than in cultivated material.

In wild and culture material the basal cell of the germlings hardly ever develops into a typical *Ulothrix* holdfast. Attachment to the substratum is usually achieved by means of a gelatinous layer secreted by the wall of the basal cell (*fig. 3E*). However, in an exceptional case in a very old culture a differentiation of the basal cell into a holdfast could be observed (*fig. 3B*).

4.1.3. Reproduction

After transferring vegetative filaments to short- and long-day conditions zoosporogenesis takes place in ordinary cells and leads to the formation of 2–4 zoospores per cell (*fig. 3C*). In nature mostly two zoospores were seen. The diameter of the zoosporangia ranges from 4.9–7.0 μ . The zoospore is slightly photo-positive and measures (6.8–)8.5–11.9 μ in length and 3.4–5.1 μ in width.

After swarming the zoospore becomes spherical, attaches itself to the substratum and germinates without directly producing a typical *Ulothrix* holdfast (*fig. 3E*). Sometimes in old cultures formation of aplanospores (*fig. 3F*) and a distinct fragmentation process of the filaments could be observed.

Gametogenesis takes place under long-day conditions. The contents of the cells become somewhat lighter in colour, not yellowish-green as in most other *Ulothrix* species. The entire cell functions as a gametangium, producing 4–8 biflagellate gametes (*fig. 4A*). In nature mostly a number of 2–4(–8) could be observed. The filaments containing gametangia are never curled up as in most other *Ulothrix* species. The gametes measure 5.1–8.5 μ in length and are 1.7–3.4 μ wide, whereas the diameter of the gametangia varies from (4.9–)5.6–7.0 μ . The cells containing 8 gametes are $1\frac{1}{2}$ – $2\frac{1}{2}$ times as long as wide.

Fusion of the gametes is strictly isogamous and mainly monoecious. The quadriflagellate zygote swarms for a brief period, showing a clear photo-negative response in swimming, whereas the behaviour of the gametes is just the opposite. Then the zygote attaches itself to the substratum and casts off the flagella.

Under short-day conditions the zygote germinates into a uni-celled sporophyte (*fig. 4C*) which during the maturation mostly becomes irregularly spheri-

cal, ovoid to bluntly pear-shaped (*fig. 4D*). The pear-shaped sporophyte is 20–75 μ long and 15–45 μ wide. The globose ones vary from 15–55 μ . The quadriflagellate zoospores formed by the sporophyte vary in shape and are usually immobile and spherical with a diameter from (5.1–)6.8–10.2 μ . Sometimes these zoospores are strongly mobile and pear-shaped with a length ranging from 8.5–11.9(–13.6 μ) and a width of 5.1–6.8 μ .

Aplanospores could occasionally be observed.

4.1.4. Taxonomy

Presumably the species studied has been described by Kützing in 1845. In 1849 the author gave an emended description still based on indistinct vegetative characters, however. The cell diameter mentioned ranges from 1/450–1/400'' (5.2–5.8 μ) with the same cell-length or a little longer. However, there is no specimen under this name in Kützing's herbarium. Therefore, *U. subtilis* Kützing, preserved in L as No. 910.188–2225 and labelled "Rabenhorst, Algen Sachs, resp. Mitteleuropa's. 657. *Ulothrix subtilis* Ktz. Spec. 345, 1856", is designated as the neotype for the described species. After measuring the filaments from the herbarium of Rabenhorst, which rendered it rather easy to confirm that the specimen did belong to the genus *Ulothrix*, we could conclude that the filaments show nearly the same cell width (4.9–7.0 μ) and cell length ($\frac{1}{2}$ – $2\frac{1}{2}$ times the width) as our material does.

Other *Ulothrix* species with a cell diameter to 7 μ , which have to be considered as synonymous, are *U. subtilissima* Rabenhorst (1863), in L as No. 910.188–2236 (Rabenhorst, Algen Sach., no 656), and *U. thermarum* Wartmann (1857) in L as No. 910.188–2227 (Rabenhorst, Algen Sach., no 655. Not Algen Sach., no 457!, which could be determined as *U. zonata*).

Measuring the isotype exsiccatae of Rabenhorst provided cell diameter ranges of 4.9–6.3 μ (no 656) and 4.9–7.0 (–7.7) μ (no 655).

4.2. *Ulothrix tenerrima* Kützing

Ulothrix tenerrima Kützing, 1845 p. 197; emend. 1849 p. 346.

References and synonyms:

- Ulothrix subtilis* Kützing e. *tenerrima* (Kützing) Kirchner, 1878 p. 77;
- Hormiscia subtilis* (Kützing) De-Toni var. *tenerrima* (Kützing) Kirchner, De-Toni, 1889 p. 160;
- (non *Conferva tenerrima* Kützing, 1833b p. 361; 1833c Dec. VI, no 55; non ? *Ulothrix tenerrima* Kützing, 1843 p. 253; non *Myxonema ? tenerrimum* (Kützing) Rabenhorst, 1847 p. 99);
- Conferva compacta* Roth sensu Kützing, pro syn., 1843 p. 245; 1845 p. 197; 1849 p. 345;
- (non *Ulothrix compacta* (Roth) Kützing, 1833c, Dec. V, no 48; which could be determined as an *Oedogonium* species);
- Gloeotila compacta* (Roth) Kützing, 1843 p. 245;
- Ulothrix compacta* (Roth) Kützing, 1845 p. 197; 1849 p. 345;
- Ulothrix subtilis* Kützing f. *compacta* (Roth) Hansgirg, 1886 p. 59;
- Hormiscia subtilis* (Kützing) De-Toni var. *compacta* (Roth) Hansgirg, De-Toni, 1889 p. 160;
- Ulothrix pallescens* Kützing, 1845 p. 197; 1849 p. 346;
- Ulothrix pallide virens* Kützing, 1845 p. 197; 1849 p. 346;
- Hormidium tenue* Kützing, 1845 p. 192;

- Ulothrix tenuis* Kützing, no 4 in 1849, p. 346;
Hormidium variabile Kützing, 1845 p. 192;
Ulothrix variabilis (Kützing) Kützing, 1849 p. 346;
Ulothrix subtilis Kützing c. *variabilis* (Kützing) Kirchner, 1878 p. 77;
Hormiscia subtilis (Kützing) De-Toni var. *variabilis* (Kützing) Kirchner, De-Toni, 1889 p. 160.

4.2.1. Living material

Clones were isolated from the following localities: *Friesland*, canal Hindeloopen-Bolsward, near Parrega, on stems of *Phragmites australis* in a rather sheltered place, about 10 cm below water level; Sneekermeer, near Sneek, on stones in a rather exposed place. *Overijssel*, Ketelmeer, on stones at the water level in a sheltered place. *Noord-Brabant*, Wilhelminakanaal, on a sheet-piling at 15 cm below water level. *Limburg*, river Geul near Mechelen, in swiftly running water on bits of wood around the water level.

4.2.2. Morphology

The straight, sometimes curved filaments are always unbranched and consist of uniseriate cells with a parietal chloroplast, which mostly has a regularly lobed margin in young filaments (fig. 5A). In full-grown filaments a more irregular chloroplast could be observed (fig. 5B). Sometimes the cells show vacuoles (figs. 5A, 5B).

The number of pyrenoids may vary from 1–3(–4) (figs. 5A, 5B). Individual cells have a diameter from 7.0–9.1 μ , even to 10.5 μ under optimal conditions and are $\frac{1}{2}$ – $1\frac{1}{2}$ times as long. Young filaments, in which zoosporogenesis usually does not take place, consist of cells varying from (4.9–)5.6–6.3(–7.7) μ in diameter and $\frac{1}{2}$ –3 times as long. These filaments resemble those of *U. subtilis*.

The cell diameters found most often in full-grown cultures are 7.0, 7.7, and 8.4 μ (tables 1 and 2). In wild material mostly a diameter near 7.0–8.4(–9.8) μ is found, with cells usually as long as wide. The chloroplast of these cells is hardly lobed.

The basal cell of the filaments always consists of a holdfast which is mostly characteristically unbranched and attached to the substratum by a thin gelatinous layer (fig. 6D).

4.2.3. Reproduction

Zoosporogenesis mostly takes place under short-day conditions.

Entire cells function as a sporangium containing 2–4 zoospores (fig. 6B). The diameter of the zoosporangia usually ranges from 7.0–9.1 μ , sometimes to 9.8 μ . In young filaments, with a diameter varying from 5.6–6.3 μ and 5–10 cells long, the zoosporangia (fig. 6A) look like those of *U. subtilis*. The zoospore is pear-shaped, possesses a cup-shaped chloroplast with one pyrenoid (fig. 6C), and is strictly photo-positive. The zoospores measure 8.5–15.3 μ by 3.4–6.8 μ .

The germling produces a typical *Ulothrix* holdfast (fig. 6D) which is usually unbranched. Sometimes 1–2 cells above the basal cell in the filament another cell-bulge is formed for attachment to the substratum.

Sometimes aplanospores could be observed.

Gametogenesis takes place in curled filaments (*fig. 9D*), which phenomenon is characteristic for this species. The contents of the cells divide and turn light yellowish-green. The number of the biflagellate gametes varies from 4–8 (*fig. 7A*). The diameter of the gametangia usually ranges from 7.0–9.1 μ , sometimes to 10.5 μ , whereas the gametes vary from 5.1–8.5 (–10.2) μ in length and 1.7–3.4 μ in width. Fusion of the gametes is isogamous and monoecious to dioecious. The gametes are weakly photo-positive.

Under short-day conditions the zygote germinates (*figs. 8A, 8B*) into a fertile uni-celled, mostly pear-shaped sporophyte (*fig. 9A*), which may be stalked (*fig. 9B*). Pear-shaped sporophytes measure 20–65 μ by 11–35 μ . The globose ones vary from 21–51 μ . The quadriflagellate zoospores, formed by the sporophyte, vary in shape. Mostly the zoospores are globose and rather immobile with a diameter varying from 5.1–10.2 μ . Sometimes these zoospores are pear-shaped and strongly mobile. These zoospores measure 8.5–13.6 μ by 3.4–6.8 μ .

Aplanospores could also be observed (*fig. 9C*).

4.2.4. Taxonomy

In 1833 Kützing described *Conferva tenerrima* (1833b) without a diameter note. He only gave a description of the morphology of the filaments. In 1843, 1845 and 1849 for *Ulothrix tenerrima*, cell diameters ranging respectively from 1/600–1/500''' (3.9–4.7 μ), 1/600''' (3.9 μ) and 1/240''' (9.7 μ) were reported by the author. The description of 1849 is considered as emended.

However, investigation of the type specimen of *Conferva tenerrima* (1833b), preserved in L as No. 939.67–730 and labelled in Kützing's hand as "Conferva tenerrima, Weissenfels im Schlosshofe", showed filaments which for the greater part belong to the genus *Microspora*. Sometimes a filament which could belong to *Ulothrix* was to be found in these exsiccatae. This fact was already noticed by Kützing himself. In 1843 the author referred *Conferva tenerrima ex parte* as a synonym to ? *Ulothrix tenerrima*.

From the description of 1843 and 1845 no material could be found in Kützing's herbarium. Therefore *Ulothrix tenerrima* (1849), preserved in L as No. 939.67–914 and labelled in Kützing's hand as "10. Ulothrix tenerrima, Nordhausen", is designated as the lectotype for the studied alga. Measuring the cell diameter yielded almost the same dimensions, 6.3–9.1(–10.5) μ , as our material did. After studying the type specimen of *Conferva tenerrima* Kützing β *stagnorum* Kützing (1833c, Dec. VI, no 56), preserved in L as No. 910.185–410, it could be concluded that all filaments belong to the genus *Microspora*.

Herbarium material of *Ulothrix tenuis* (no 4 in *Species Algarum*, p. 346) with a cell diameter range 1/340–1/300''' (6.8–7.8 μ) must be considered to have been mislaid. However, the number 4 is written on a part of the sheets of *Ulothrix tenuis* Kützing (no 18 in *Species Algarum*, p. 347, preserved in L as No. 939.26–265). Measuring all these specimens showed that they all belong without any exception to *U. tenuis*, no 18. On account of the cell diameter reported in

the species diagnosis *U. tenuis* (no 4) must be considered as synonymous.

The names *Ulothrix compacta* (Roth) Kützing (1845, p. 197; not 1833c! in Dec. V, no 48), with a cell diameter, ranging from 1/700–1/680''' (3.3–3.4 μ) and clearly emended in 1849 with a proposed cell width of 1/350''' (6.6 μ), and *U. variabilis* (Kützing) Kützing (1849), proposed as *Hormidium variabile* Kützing in 1845 with filaments of 1/400–1/300''' (5.8–7.8 μ) in diameter, give rise to very difficult taxonomic problems. The cell diameter range, recorded in both species diagnoses, shows a clear overlap with the dimensions of our material of *U. subtilis* and *U. tenerrima*. On account of the values measured in the specimen called *Conferva compacta* Roth in Kützing's herbarium, preserved in L as No. 939.26–300 and in the type specimen *Hormidium variabile* (in very poor condition in L as No. 939.26–299), respectively for *U. compacta* (5.6–)6.3–9.1 μ and for *U. variabilis* 7.0–10.5 μ , it was decided to consider these species as synonymous with *U. tenerrima*.

Ulothrix pallide virens (1845), emended in 1849 with the cell diameter range 1/300–1/240''' (7.8–9.7 μ) showed dimensions ranging from 6.3–10.5 μ . The dried filaments from Hanau (West Germany) are preserved in L as No. 939.26–204. The type of this species, indicated by KÜTZING (1849), is *Conferva brachymelia* Lyngbye (1819, no 1), preserved in Lyngbye's herbarium in C, s.n. These filaments showed a cell diameter of 7.0–21.0 μ . Only *U. pallide virens* is considered as a synonym.

The same is proposed for *U. pallescens* Kützing (1845), emended in 1849 and with a cell diameter of 1/300''' (7.8 μ). Investigation of Kützing's type (in L as No. 939.26–16, with the following notes in Kützing's hand "5. *Ulothrix pallescens*, Salona, am Gerinne des Eisenhammers, 27.3–1835") showed a cell diameter range of 6.3–9.8 μ .

4.3. *Ulothrix albicans* Kützing

Ulothrix albicans Kützing, 1845 p. 197; emend. 1849 p. 346.

References and synonyms:

- Ulothrix subtilis* Kützing h. *albicans* (Kützing) Hansgirg, 1886 p. 59;
- Hormiscia subtilis* (Kützing) De-Toni var. *albicans* (Kützing) Hansgirg, De-Toni, 1889 p. 161;
- Conferva compacta* Roth sensu Jürgens, pro syn., 1817 Dec. IV, no 8;
- Ulothrix jürgensii* Kützing, 1849 p. 347;
- Hormiscia* ? *jürgensii* (Kützing) De-Toni, 1889 p. 170;
- Hormidium moniliforme* Kützing, 1843 p. 244; 1845 p. 192;
- Ulothrix moniliformis* (Kützing) Kützing, 1849 p. 347;
- Hormiscia moniliformis* (Kützing) Rabenhorst, 1868 p. 361;
- (non *Sphaeroplea* ? *vermicularis* Hassall, 1843 p. 436; non *Lyngbya vermicularis* (Hassall) Hassall, 1845 p. 224; in BM, s.n. which could be determined as *Microspora* species);
- Ulothrix braunii* Kützing, 1849 p. 346;
- Ulothrix moniliformis* Kützing var. β *braunii* (Kützing) Rabenhorst, Hansgirg, 1886 p. 59;
- Hormiscia moniliformis* (Kützing) Rabenhorst var. *braunii* (Kützing) Rabenhorst, De-Toni, 1889 p. 166;
- (non *Ulothrix lacustris* Hilse in Rabenhorst (1863),⁹ Alg. Eur. no 1540).

4.3.1. Living material

Clones were isolated from the following localities: *Friesland*, Prinses Margrietkanaal near Irnsum, on a sheet-piling below the water level in periodical turbulence caused by shipping. *Gelderland*, a pool along the river Waal near Varik. *Zuid-Holland*, Eastern side of the Braassemermeer, on stones in a rather exposed place; in a ditch near Dirksland, on stems of *Phragmites australis* below the water level.

4.3.2. Morphology

The straight filaments are always unbranched and consist of uniseriate cells with a parietal chloroplast, which in young filaments mostly has a lobed margin (*fig. 10A*). In older filaments a more irregularly shaped chloroplast (*fig. 10B*) could be observed, in very old cultures it had sometimes crumbled to pieces (*fig. 10C*). This phenomenon does not usually appear in wild material. The number of pyrenoids may vary from 1–5. Individual cells range from 7.7–12.6 (–13.3) μ , in very old cultures under exhausted conditions to 14 μ in diameter and $\frac{1}{2}$ –2 times as long. Young filaments, in which zoosporogenesis usually does not take place, consist of cells varying from (5.6–)6.3–7.0 μ in diameter and to 4 times as long. In young filaments vacuoles may be observed (*fig. 10A*).

The development of the cell diameter in relation to the cultivation-time is shown in *tables 1* and *2*. In full-grown cultures most values measured are in the range from 8.4–11.9 μ . These dimensions could also be observed in wild material usually containing cells of equal length and width and provided with a poorly developed, unlobed chloroplast, and one pyrenoid.

In wild material and especially in cultures the basal cell of the filaments always consists of a typical holdfast, which is mostly branched rather complexly (*fig. 12*) and attached to the substratum by a gelatinous layer.

4.3.3. Reproduction

Zoosporogenesis takes place under short- and long-day conditions. In *U. albicans* this process is rather independent of the day-length. Entire cells function as a sporangium containing 2–8 zoospores (*fig. 11B*). The diameter of the zoosporangia ranges from 7.7–11.9 (–13.3) μ . Most dimensions vary from 9.1–11.9 μ . When 8 zoospores are formed the zoosporangia mostly are twice as long as wide (*fig. 11B*).

Sometimes zoosporogenesis takes place in young filaments, with a diameter varying from 6.3–7.0 μ . Then the zoosporangia (*fig. 11A*) resemble those of *U. subtilis*.

The zoospore is nearly always pear-shaped and usually has a cup-shaped, sometimes irregularly shaped chloroplast with one pyrenoid (*fig. 11C*). In contrast to those of *U. subtilis* and *U. tenerrima*, the zoospores show mostly a photo-negative reaction. A fraction of them, however, is always photo-positive. Sometimes in clones rather flattened zoospores could be detected (*fig. 11D*). Sometimes even a longitudinal groove could be distinguished on the bottom side of the zoospores, which continues in ventral and dorsal direction (*fig. 11D*).

This phenomenon is probably caused by less favourable culture conditions or by the process of inbreeding. Most zoospores possess a median eye-spot, but its location may vary. The zoospores are 10.2–18.5 μ long and 3.4–8.5 μ wide. The germling usually promptly produces a long basal cell with a very complex system of branches (*fig. 12*).

Sometimes under less favourable conditions aplanospores could be observed.

Gametogenesis, like zoosporogenesis, is not dependent in the same way on the day-length as in *U. subtilis* and *U. tenerrima*. However, most gametogenesis processes could be observed under long-day conditions. The number of the pear-shaped biflagellate gametes varies from (4)–8–16 (*fig. 13A*). The diameter of the gametangia usually ranges from 7.7–11.9(–14.0) μ , the length from 7.7–17.0 μ , whereas the gametes vary from 8.5–13.6 μ in length and 1.7–5.1 μ in width. Under non-optimal conditions the gametangia show abnormal sizes. Then the diameter ranges from 5.6–7.0 μ by 4 times that long.

Fusion of the gametes is isogamous and monoecious. The quadriflagellate zygotes are clearly photo-positive. Fusion of gametes does not always take place outside the gametangium. This could be concluded from the fact that besides biflagellate gametes provided with one eyespot, reproductive cells containing 4 flagella and 2 stigmas are often liberated from the gametangia (*fig. 13D*). Both types of zygotes germinate into a uni-celled sporophyte. Sometimes the zygote formed inside does not leave the gametangium and then maturation takes place within the cell (*fig. 13B*).

Only under short-day conditions do the zygotes germinate, via stalked or non-stalked intermediate stages (*fig. 14A*), into sporophytes of various shape. Globose sporophytes, which are usually present in cultures, vary from 21–65 μ in diameter, whereas the less common pear-shaped ones measure 25–65 μ in length and 21–45 μ in width.

The weakly photo-positive quadriflagellate zoospores vary in shape and are mostly spherical and immobile with a cell diameter ranging from (5.1)–6.8–11.9 μ . Sometimes they are strongly mobile, and are then pear-shaped with a length ranging from 8.5–15.3 μ and a width of 5.1–6.8 μ . Sometimes aplanospores could be observed.

4.3.4. Taxonomy

In our opinion *Ulothrix albicans* Kützing, proposed in 1845 and emended in 1849, respectively with a cell diameter range 1/300–1/280''' (7.8–8.3 μ) and 1/300–1/200''' (7.8–11.6 μ), is to be designated as the type of this alga. Filaments preserved in L as No. 939.26–224, provided with the annotation "Flotow, 1841, no 31, Hirschberg" in Kützing's hand, showed the same cell dimensions as our species studied.

Ulothrix jürgensii Kützing (1849) must be considered as synonymous.

According to the author this taxon has a cell diameter of 1/180''' (12.9 μ), whereas the isotype specimen, in Kützing's herbarium (No. 939.26–218) as *Conferva compacta* Roth sensu Jürgens (1817), showed a cell diameter range of 8.4–13.3(–14.0) μ .

The systematic position of *Ulothrix moniliformis* (Kützing) Kützing (1843) is not quite clear. This species shows filaments constricted at the cross walls. For that reason the cells are barrel-shaped. At the same time the cell wall is very thick and the chloroplast lies on one side of the cell. These characters are probably caused by extremely averse environmental conditions. However, after bringing these filament from nature into culture, we could observe in due course that they show normal *Ulothrix* features, such as square cells and a well-developed chloroplast. At the same time the cell diameter decreases.

The type specimen of *U. moniliformis*, proposed by Kützing as *Hormidium moniliforme* in 1843 and emended in 1849, respectively with a cell diameter of $1/300''$ (7.8μ) and $1/200-1/180''$ ($11.6-12.9 \mu$) showed a cell diameter range of $9.1-14.0 \mu$. The type is preserved in L as No. 939.26-219. Therefore *U. moniliformis* is considered with reserve as a synonym.

Ulothrix mucosa Thuret (1850), with nearly the same cell dimensions as *U. albicans* but showing other distinctive species characters (which will be described in a following publication), sometimes shows the same phenomena under extremely averse environmental conditions, like barrel-shaped cells etc. Therefore *U. moniliformis* also must be considered with reserve as a synonym of *U. mucosa*.

Ulothrix lacustris Hilse (1863) was considered to be a synonym of *U. moniliformis* (RABENHORST 1868, DE-TONI 1889). This must be rejected because the isotype, preserved in L as No. 939.26-13 (Rabenhorst, Algen Eur., no 1540), predominantly shows akinete stages and several vegetative filaments and belongs to the genus *Microspora*.

Ulothrix braunii Kützing (1849), preserved in L as No. 939.26-220 (leg. Braun, Titisee), is also considered to be a synonym on account of the cell diameter of the type specimen, which shows a range of $(7.7-8.4-12.6(-14.0) \mu$. This species was described in 1849 with a cell diameter of $1/260-1/200''$ ($8.9-11.6 \mu$).

5. CONCLUSIONS AND DISCUSSION

The life-history of the three freshwater *Ulothrix* species studied is almost identical with that of *U. zonata* as described by DODEL (1876) and KLEBS (1896). Only the biflagellate microzoospores, described by KLEBS (1896), must very probably be considered to be gametes formed under less favourable conditions. In our opinion it is difficult to make a distinction between micro- and macrogametes, as WILLE (1912) did, because small and large gametes may be formed in a single gametangium.

GROSS (1931) is of the opinion that the life-history of *U. zonata* consists of an alternation of a haploid multicellular filamentous generation with a unicelled diploid generation. This is in accordance with our view of the life cycle of the three species. Our conclusions have not yet been confirmed by cytological experiments, like those of GROSS (1931), but are based on the fact that in filaments, under the influence of a different photoperiod, zoospores and gametes may be formed.

Our results are in contrast to the experiments of KORNMAN (1964) and PERROT (1968, 1970 and 1971) on marine *Ulothrix* species, which show either an incomplete life-history or a very intricate life cycle. Perhaps only marine *Ulothrix* species show these special characters.

No difference within the life-history could be observed for the different clones of the species studied, only a difference in the rate of fructification was noted. Several clones gave a lively reproduction with many empty cells as a result, others, after induction, only showed a laborious reproduction. From our study it is clear that the three *Ulothrix* species investigated in their life-history show specific constant features, among others the limited variation of zoospores and gametes, the shape of the filament containing gametangia. The basal cell also yields species-distinguishing marks. This cell is hardly differentiated in *U. subtilis*, rather well developed but hardly ever branched in *U. tenerrima*, whereas *U. albicans* has a well developed, sometimes very complex, branched basal cell.

The sporophytes of *U. subtilis* and *U. tenerrima* are (irregularly) pear-shaped to globose, whereas the fertile diploid phase of *U. albicans* is mostly globose. Lastly a typical species feature can be observed in the phototactic reaction of the zoospores. *U. subtilis* and *U. tenerrima* have zoospores with a remarkably positive phototactic reaction in swimming. Zoospores of *U. albicans* usually show an opposite reaction.

By studying the species in culture an impression of the limited variation of the morphological species-distinguishing features was obtained, like cell diameter, diameter of zoosporangia, shape of chloroplast, number of pyrenoids, etc. These impressions are of course only correct when many filaments have been investigated (± 25). The same can be said for the interpretation of wild material. When only a few filaments have been isolated, no conclusion may be drawn regarding the identity of the taxon and certainty may only be obtained by culturing.

The behaviour of the species studied under different photoperiods agrees with the results of DODEL (1876), LIND (1932) and HYGEM (1948) for *U. zonata* and *U. flacca*. The freshwater species produce zoospores under short-day conditions, gametes under long-day conditions, and germination of the zygote takes place under a short-day light regimen. These results reflect the behaviour of the species in nature. In winter and spring in the Netherlands these algae are abundantly present in the filamentous stage, respectively with zoospores and gametes, in summer they are predominantly present in the sporophytic stage.

COLLINS (1909) reported that external conditions may induce modifications of the normal growth process. Filaments may break up into individual cells and these may change by copious formation of gelatine into a *Palmella* and a *Gloeocystis* condition. These processes could never be observed in our algae, however.

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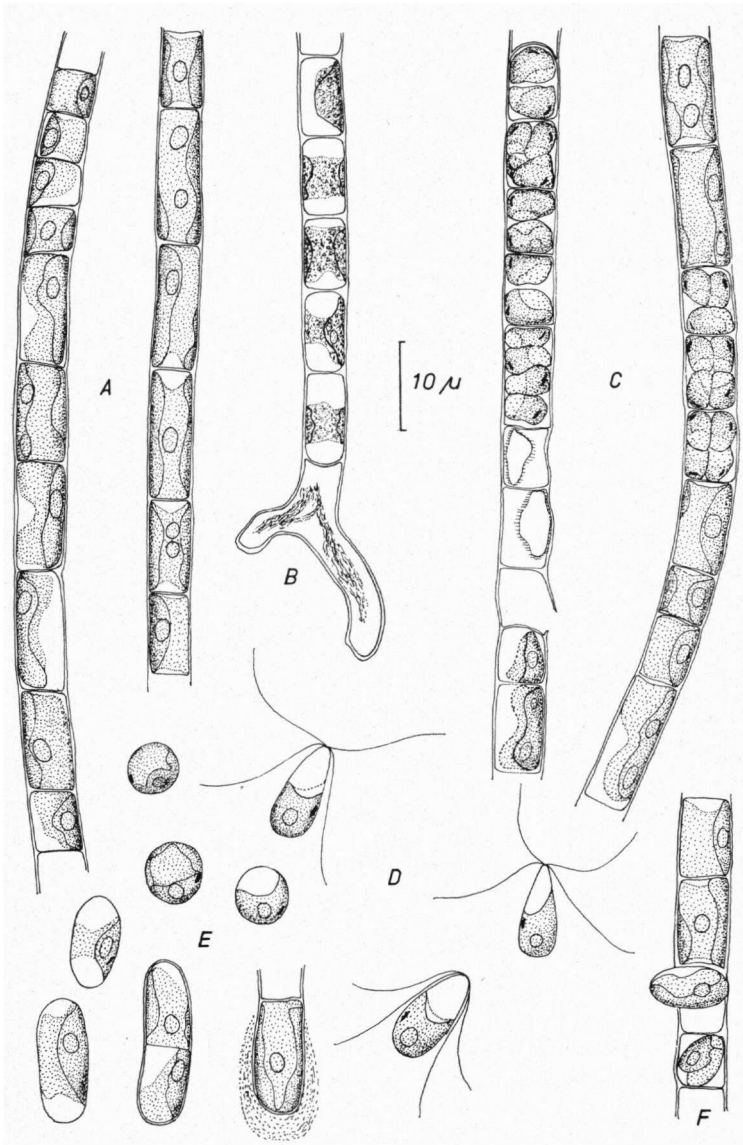


Fig. 3. *Ulothrix subtilis*. A. vegetative filaments; B. old filament; C. filaments filled with zoosporangia; D. free-swimming zoospores; E. germination of the zoospore; F. filament containing aplanospores.

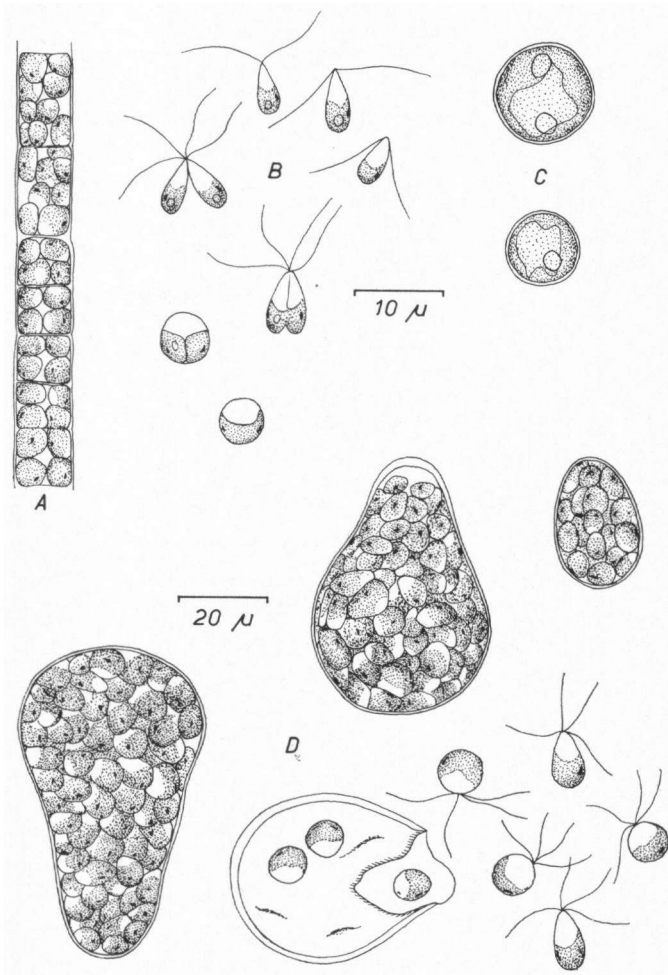


Fig. 4. *Ulothrix subtilis*. A. filament filled with gametangia; B. gametes and zygotes; C germination of the zygote; D. fertile sporophytes with zoospores.

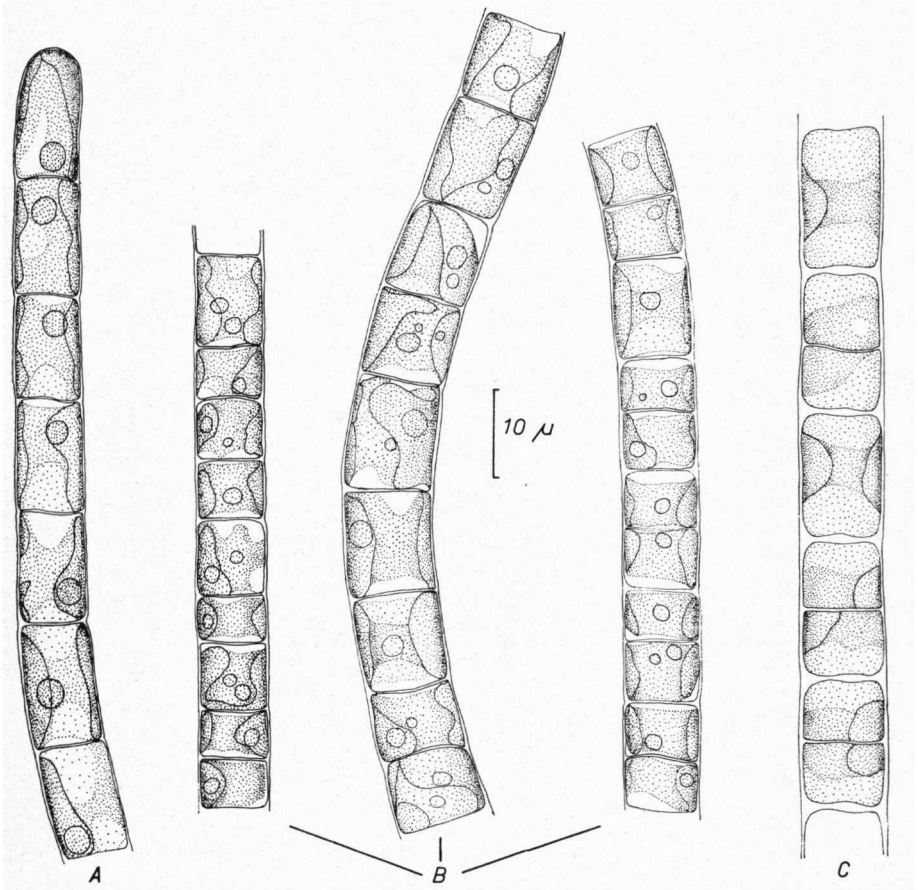


Fig. 5. *Ulothrix tenerrima*. A. young filament; B. full-grown filaments; C. old filament.

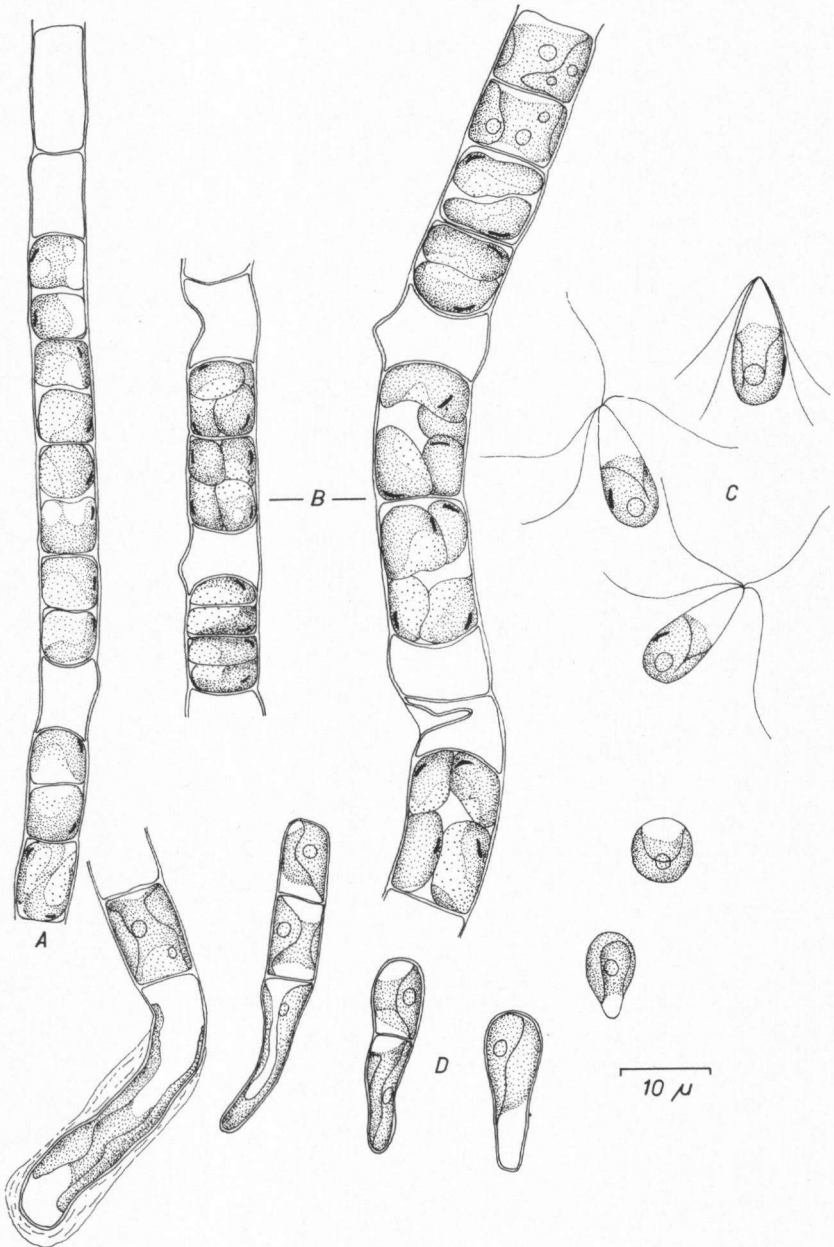


Fig. 6. *Ulothrix tenerrima*. A. induced zoosporangia in a young filament; B. full-grown filaments with zoosporangia; C. zoospores; D. germination of the zoospore into a germling.

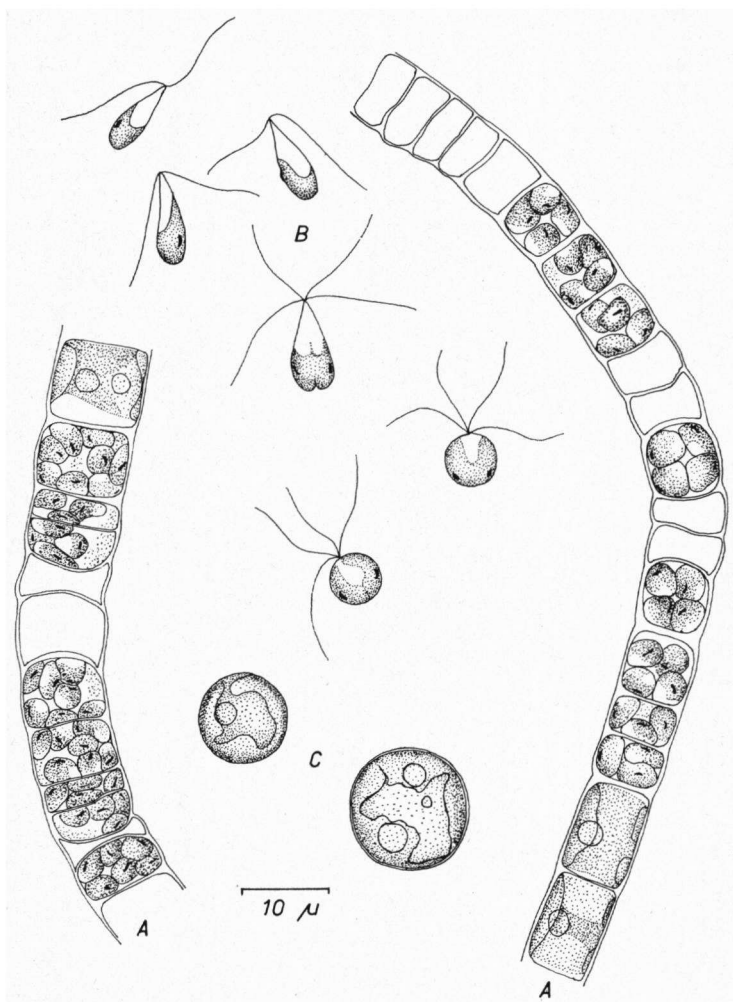


Fig. 7. *Ulothrix tenerrima*. A. filaments with gametangia; B. gametes and zygotes; C. germination of the zygote.

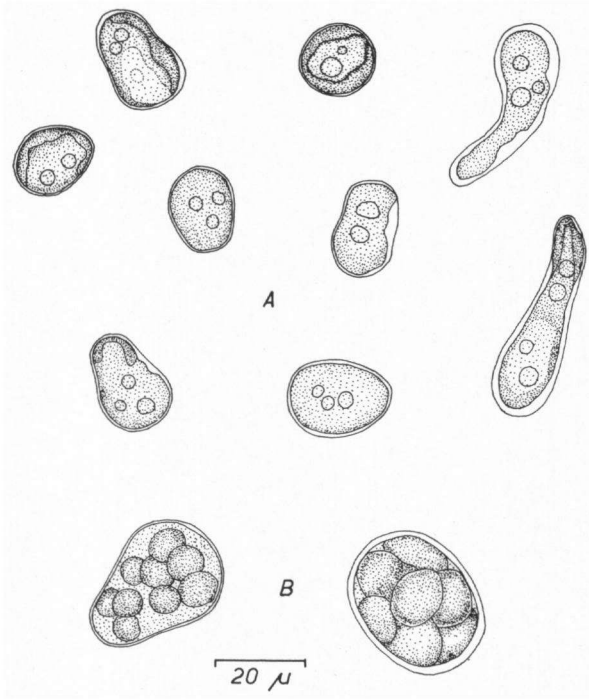


Fig. 8. *Ulothrix tenerrima*. A. germination of the zygote via intermediate stages; B. nearly fertile sporophytes.

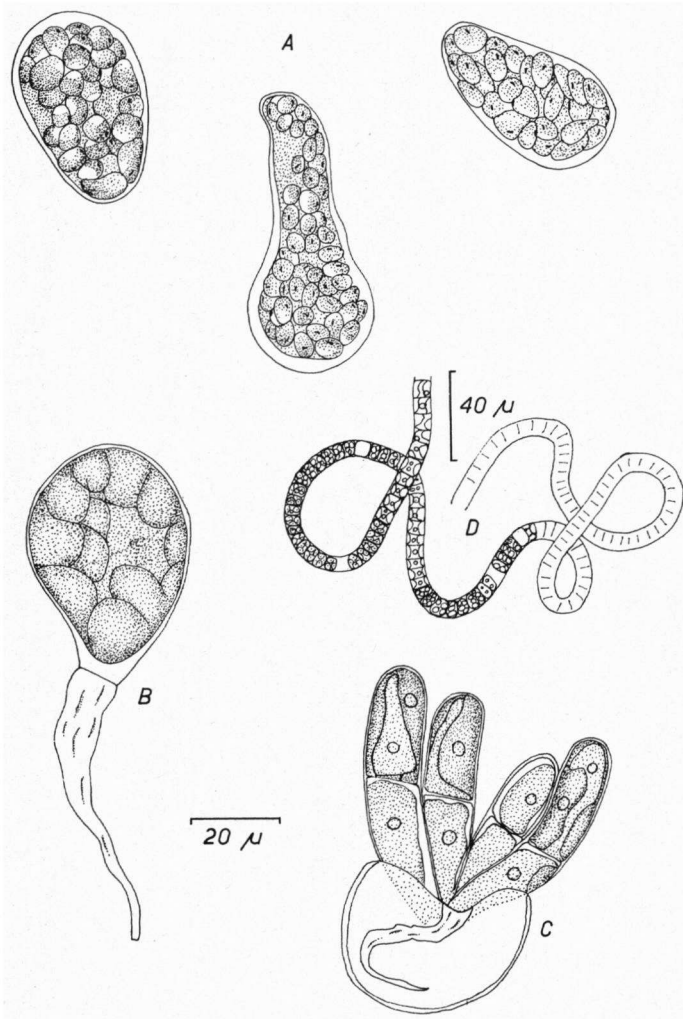


Fig. 9. *Ulothrix tenerrima*. A. fertile sporophytes; B. stalked, nearly fertile sporophyte; C. sporophyte with germinating aplanospores; D. curled filament with gametangia. Bar indicates 20 μ in A and B, but 10 μ in C.

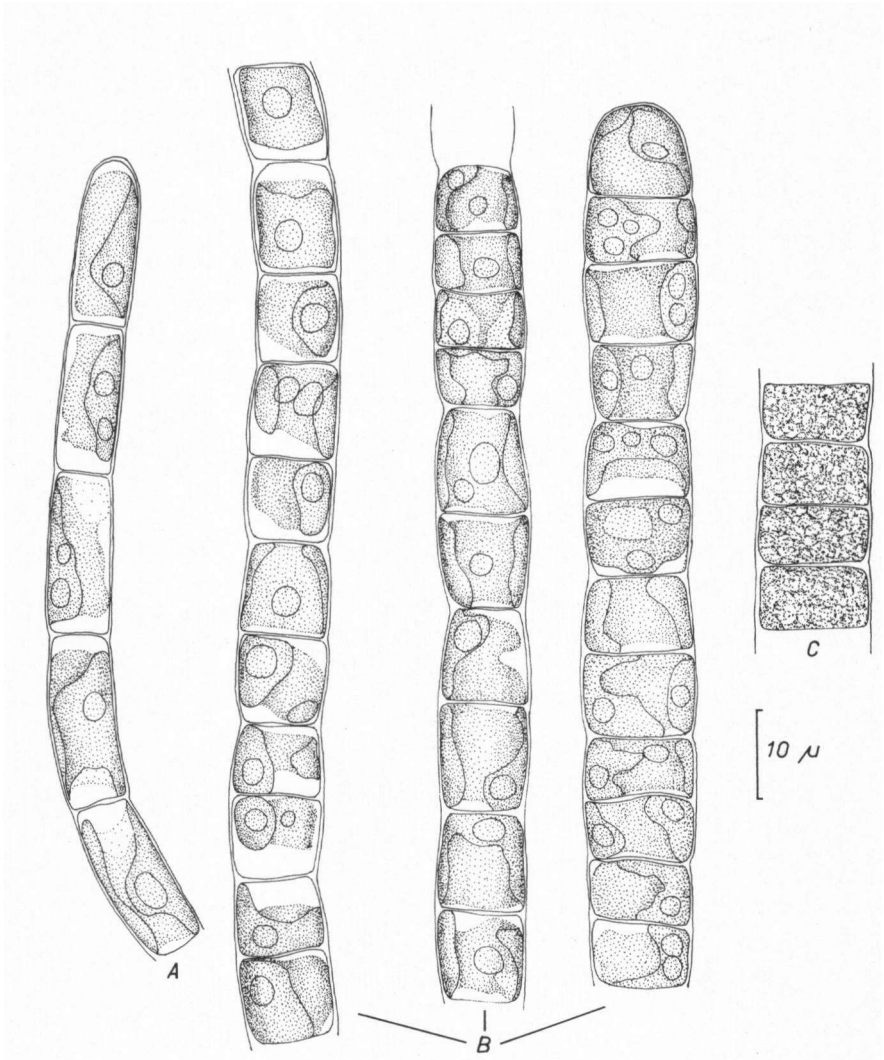


Fig. 10. *Ulothrix albicans*. A. young filament; B. full-grown filaments; C. old filament.

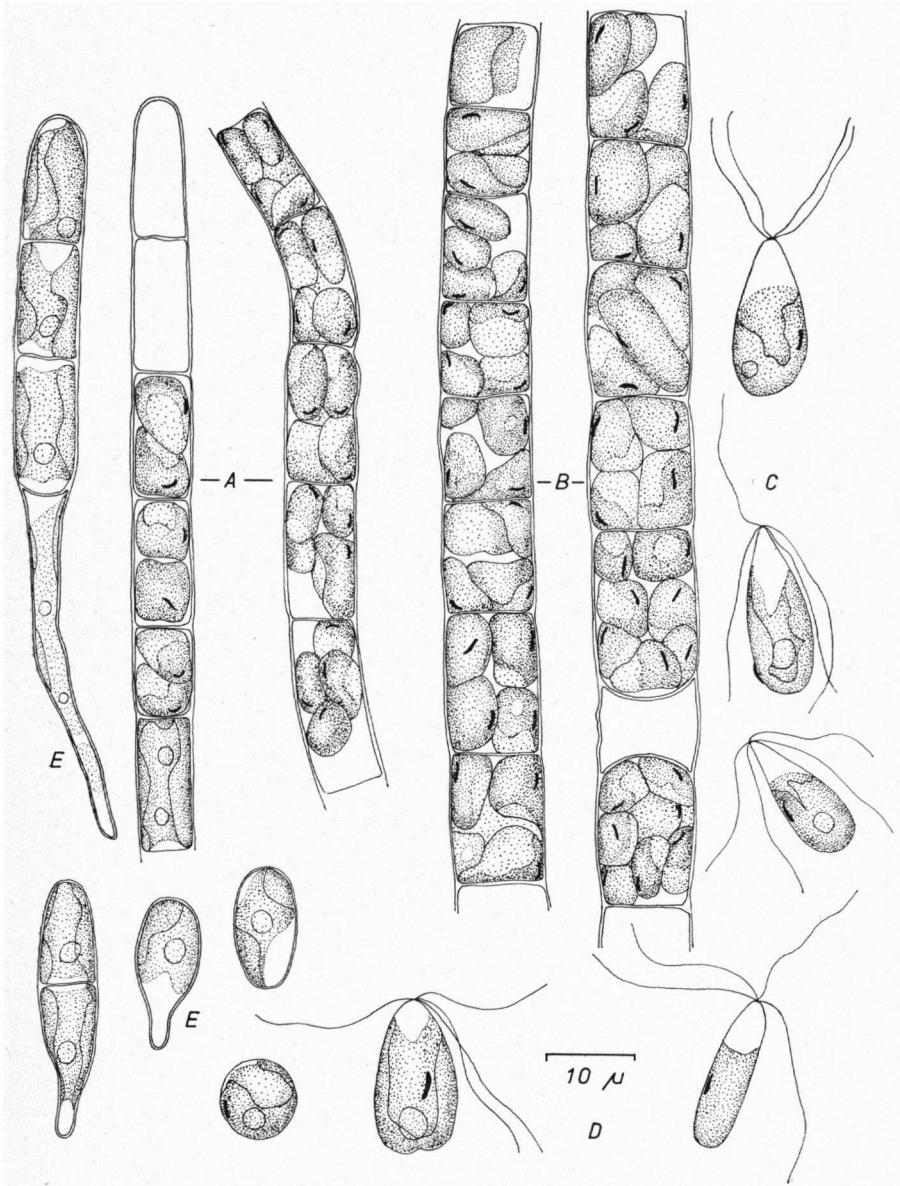


Fig. 11. *Ulothrix albicans*. A. induced zoosporangia in a young filament; B. full-grown filaments with zoosporangia; C. pear-shaped zoospores; D. flattened zoospores; E. germination of the zoospore into a germling.

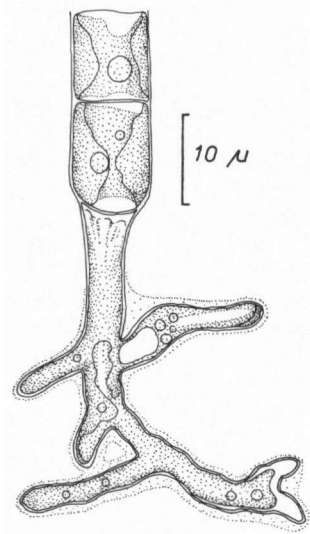


Fig. 12. *Ulothrix albicans*. basal cell.

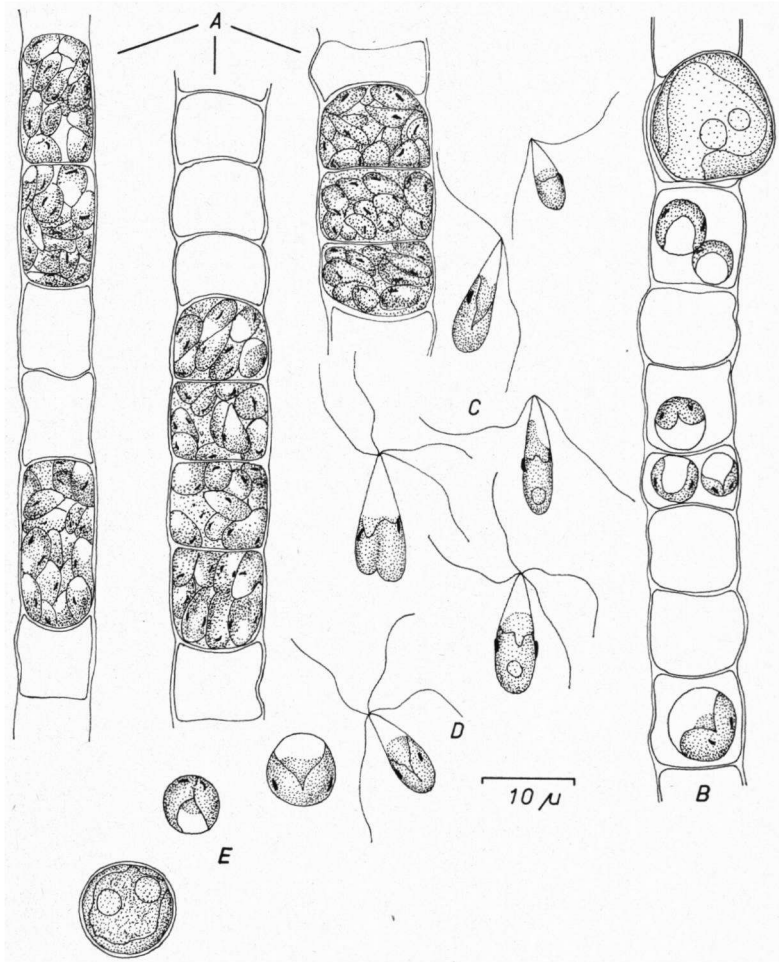


Fig. 13. *Ulothrix albicans*. A. filaments with gametangia; B. filament with zygotes formed internally; C. gametes and zygote formed externally; D. liberated zygotes formed within the gametangium cell wall; E. germination of the zygote.

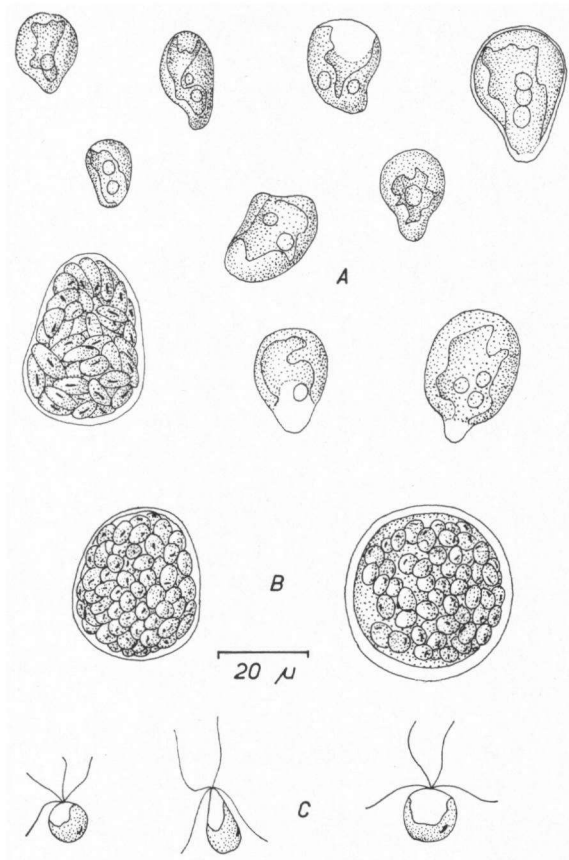


Fig. 14. *Ulothrix albicans*. A. germination of the zygote via intermediate stages; B. fertile sporophytes; C. liberated zoospores.

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