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TAXONOMIC STUDIES ON THE MARINE AND BRACKISH-WATER SPECIES OF ULOTHRIX (ULOTRICALES, CHLOROPHYCEAE) IN WESTERN EUROPE

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CONTENTS

SUMMARY

In this fourth report on the taxonomy of *Ulothrix* Kützing a new classification of the marine and brackish-water species in western Europe is proposed. Comparative studies on field collections, uni-algal cultures, herbarium collections and sections prepared for electron microscopy lead to the recognition of three marine species, viz. *Ulothrix speciosa* (Carmichael *ex* Harvey in Hooker) Kützing, *U. flacca* (Dillwyn) Thuret in Le Jolis, and *U. palusalsa* Lokhorst (*nov. nom.*), and two brackish-water species, viz. *U. implexa* (Kützing) Kützing and *U. subflaccida* Wille. The vegetative anatomy, the life history, the fine structure of the vegetative thallus and the distributional pattern in nature are amply discussed. Salient, reliable characters proved to be, *e.g.*, the nature and construction of the cell wall, the texture of the cell wall's surface, the fine structure of the pyrenoid, the developmental stages of germinating zoospores, the coalescense of filaments, the shape of the gametangial filament, and the limited variation of the number of zoospores and gametes. A brief discussion is given of the ecological status of the individual species. In addition there is a brief comment on the taxonomic affinity of *Ulothrix* with the morphologically related genus *Urospora* Fries and on the phyletic relationship of *Ulothrix* with the progenitors of the higher land plants. The reproductive behaviour of the species under different photo periods in culture appeared to be correlated with the seasonal periodicity expressed by the algae in nature.

INTRODUCTION

The genus *Ulothrix*, which embraces a group of unbranched, uniseriate, filamentous, green algae containing one parietal girdle-shaped chloroplast per cell, was established by Kützing in 1833. The complicated taxonomic history of this genus has been comprehensively reviewed previously (Lokhorst, 1974). At first *Ulothrix* was considered to be mainly restricted to freshwater and terrestrial habitats (Kützing, 1833; 1845; 1849) and the marine species now ranked under *Ulothrix* were classified in *Conferva* and *Lyngbya* (Harvey in Hooker, 1833), *Hormidium* (Kützing, 1843), and *Hormotrichum* (Kützing, 1845; 1849). In 1863 marine species were for the first time assigned to the genus *Ulothrix* (Le Jolis). Later, however, some authors again transferred marine *Ulothrix* species to for example *Urospora* and *Hormiscia* (Rabenhorst, 1868; Areschoug, 1872; De-Toni, 1889). This frequent change of taxonomic position shows the considerable confusion which existed in the 19th century with respect to the circumscription of genera of green algae. In 1901 Wille, in a lengthy report, described marine *Ulothrix* representatives from the Oslofjord. Amongst these, three species were presented as new. The present-day classification of marine *Ulothrix* species is mainly based on his findings. The specific descriptions of Wille (1901), based largely on vegetative characters are, however, fairly vague, incomplete, inadequate, and as a result insufficiently distinctive. Furthermore, since all species were described directly from nature, many features of the organisms, for example those concerning the life history, were not ascertained. More recently, Kornmann (1964) and Perrot (1968, 1970, 1971) emphasized the necessity of studies on life history in uni-algal cultures in the laboratory for the elucidation of the taxonomy of the marine *Ulothrix* species.

Within the scope of comparitive studies in the genus *Ulothrix* in western Europe, the present study was devoted to the brackish water and marine group. The principles underlying this examination are similar to those described earlier for the freshwater members of *Ulothrix* (Lokhorst, 1974). However, the methods have been enriched by the use of the transmission electron microscope.

MATERIAL AND METHODS

Numerous clones of the species studied were collected from hard as well as soft substrates in a large number of brackish-water and marine areas, situated at irregular intervals along the Dutch coast line, in the vicinity of the biological stations of Ambleteuse and Roscoff (France), and near Dröbak and Espegrend (Norway). Several localities in the Netherlands were inspected periodically over a period of at least one year. The algal strata were mostly scratched off; sometimes chipped substrate debris with contaminating algae were also taken to the laboratory. The techniques for isolation of filaments into uni-algal cultures, for maintenance of stock cultures and the use of diurnal photo periods, and of staining reagents have been described earlier (Lokhorst & Vroman, 1972, 1974a). Cultures were kept at 4° and 8 °C respectively. In order to determine the capacity of the individual cell walls to produce inflations some drops of JKJ (1% in 5% lactophenol) were added to the filaments. Normally the marine species were grown in Provasoli's enriched sea water (1968) or sometimes in Erdschreiber medium (Föyn, 1934). The brackish-water species were inoculated in half-concentrated Provasoli's enriched

sea water. Dilution was carried out by adding distilled water or pure fresh water from a lake origin. In order to answer the question whether vegetative and reproductive characters are affected by environmental factors like the salinity the algae were studied in culture under different salinity values, ranging from 2 to $16^0/_{00}Cl^-$. At times, Gorham medium ($0^0/_{00}Cl^-$) and a solution of $45^0/_{00}Cl^-$ were added to this series. In order to imitate the tidal effect, algae derived from intermittently flooded coastal areas were kept in a tide-simulating apparatus. From the great number of isolates in uni-algal cultures, fifty two clones were chosen for further intensive studies. The development of young germlings and sporophytic stages were studied *in situ* on the glass surfaces in culture. One method employed to observe these stages was to examine the plants from the bottom side of the culture vessel with a microscope equipped with a plunge objective (32 ×). A second method has been described previously (Lokhorst & Vroman, 1972). In order to encourage the growth and maturation in sporophytes the algae were sometimes given a cold shock by keeping them for some days below 0°C.

Several different methods were tried for preparing *Ulothrix* for ultrastructural studies. The filaments were sampled for fixation 2—6 hours after entrance of the diurnal dark cycle but also at several points of time in the light period. The algae, two clones per species, were harvested during stages of active growth so that accumulation of starch was at a minimum. Occasionally, freshly collected material was involved in these studies. Small tufts of vegetative filaments were cut from the cultures and placed into the fixative. Two types of fixative were applied. In the one fixation method filaments were kept for 3—24 hours at 4°C in 1—4% glutaraldehyde in 0.1M cacodylate buffer, with osmolalities of the fixing solution adjusted to approximately that of sea water or brackish water by the addition of 0.7M or 0.35 M NaCl. The other fixative consisted of 2% glutaraldehyde and 2% acroleine in 0.1 M cacodylate buffer. For purification the glutaraldehyde was agitated with active C and $BaCO_3$ for about 12 hours at room temperature. Prior to the fixation in OsO_4, the plants were rinsed for ten minutes in 0.1M cacodylate buffer solutions with stepwise decreases in osmolality, respectively enriched with (3.5%), 2.6%, 1.9%, 0.7% and 0% NaCl. Each buffer had a pH of 7.5. Next, the plants were fixed in 1% OsO_4 in 0.1M cacodylate buffer (pH = 7.5) for two hours at 4°C. The OsO_4-fixed material was subsequently washed for ten minutes respectively in 0.1 M cacodylate buffer and pure distilled water and before ethanol dehydration embedded in a 1% agar solution to rearrange the filaments; then rinsed twice in propylene oxide and infiltrated and embedded in Epon. Polymerisation lasted 48 hours at 60°C. Sections were cut with a diamond knife and stained with uranyl acetate and lead citrate (Reynolds, 1963). Occasionally, only lead citrate was used. They were examined with a Philips EM-300 electron microscope.

Herbaria, from which material was studied, are enumerated in the following list. Abbreviations are according to the Index Herbariorum (Holmgren & Keuken, 1974). BR – Jardin botanique national de Belgique, Brussels; C – Botanical Museum and Herbarium, Copenhagen; CGE – Botany School, University of Cambridge, Cambridge; CN – Laboratoire de Botanique, Faculté des Sciences, Caen; TCD – School of Botany, Trinity College, Dublin; E – Royal Botanic Garden, Edinburgh; FI – Herbarium Universitatis Florentinae, Istituto Botanico, Florence; HBG – Institut für Allgemeine Botanik, Hamburg; KIEL – Botanisches Institut der Universität, Kiel; L – Rijksherbarium, Leiden; BM – British Museum

(Natural History), London; LD – Botanical Museum, Lund; PC – Muséum National d'Histoire Naturelle, Laboratoire de Cryptogamie, Paris; S – Section for Botany, Swedish Museum of Natural History (Naturhistoriska Riksmuseet), Stockholm; UPSV – Växtbiologiska Institutionen, Uppsala Universitat, Uppsala. Some herbarium specimens were from the herbarium collection of Dr. Kornmann; these are cited as HELGOLAND. Prior to microscopy observations the herbarium specimens could be made to resume their original habit by treatment with a synthetic detergent. The detergent consisted of 50cc distilled water, to which about two drops of a concentrated solution of TEEPOL was added.

KEY TO THE SPECIES

1a. Filaments usually with a soft cell wall, smoothly surfaced but occasionally sparsely studded with fouling organisms and/or micro-particles. (Local) inflation of the cell wall occasionally present, certainly so after adding a 1% JKJ solution in 5% lactophenol 2

b. Filaments usually with a firm cell wall, smoothly or roughly surfaced and often (densely) contaminated with fouling organisms and/or micro-particles. (Local) swelling of the cell wall absent, even after adding JKJ 3

2a. Diameter of vegetative cells (9.7—)14.8—63.6(—85.8) μm, cell height (3.6—)4.8—15.6(—22.9) μm. Zoosporangia absent. On the gametophytic filaments (8—)16—128 and possibly more gametes per cell. Dioecious plants. Predominantly in intertidal areas, both on hard substrates and soft soils.
1. Ulothrix speciosa

b. Diameter of vegetative cells (8.4—)12.1—25.8(—28.9)μm, cell height (3.6—)4.8—16.9(—20.4) μm. Zoospores 4—16 per cell. Gametes (8—)16—32 per cell. Monoecious plants. Soils both in intertidal and inland salt marshes.
3. Ulothrix palusalsa

3a. Growth habit mostly as a complex basal-erect system, filaments often coalescent. Diameter of vegetative cells (4.8—)14.4—32.6(—44.2)μm, cell height (3.6—)4.8—9.6(—15.7)μm. Zoospores (4—)8—32 per cell, gametes (4—)8—64(—128) per cell. Monoecious plants. Hard substrates, predominantly in open intertidal areas . . . . . **2. Ulothrix flacca**

b. Growth habit fundamentally consisting of one upright filament with a basal cell (slightly) modified for attachment, filaments never coalescent 4

4a. Diameter of vegetative cells (3.6—)9.6—15.4(—26)μm, cell height (3.6—)4.8—10.9(—15.6)μm. Zoospores (2—)4—16(—32) per cell. Gametes (4—)8—32 per cell. Upon ripening of the gametangia cell contents olivaceous. In absence of zoosporogenesis or gametogenesis secondary rhizoids frequently present. Wide-spread in all kinds of brackish-water localities, and sometimes invading fresh water in considerable quantities. Never observed on soils in salt marshes.
4. Ulothrix implexa

b. Diameter of vegetative cells (4.8—)7.6—12.1(—13.2) μm, cell height (3.6—)4.8—15.7(—18.1) μm. Zoospores (1—)2—4(—8) per cell. Gametes (2—)4—16 per cell. Upon ripening of the gametangia no change of colour of cell contents. In the absence of reproductive stages secondary projections not obvious. Wide-spread in all kinds of brackish-water habitats, also on soils in salt marshes.
5. Ulothrix subflaccida

1. Ulothrix speciosa (Carmichael *ex* Harvey in Hooker) Kützing — **Figs. 1—5; Plates 1—4; Table 1.**

Lyngbya speciosa Carmichael *ex* Harvey in Hooker (1833) 371; Harvey (1841) 161; (1849) 227; Berkeley (1849) Pl. 2926; Harvey (1851) Pl. 186 B; Johnstone & Croall (1860) 175. — *Ulothrix speciosa* (Carmichael *ex* Harvey in Hooker) Kützing (1849) 348; (1852) 29, Tab. 93 (*p.p.*); Le Jolis (1863) 57; De-Toni (1889) 233 (*pro syn.*); Batters (1891) 8; (1902) 13; Newton (1931) 56; Chapman (1937) 228; Blackler (1951) 123; Kornmann (1964) 28; Pankov (1971) 74 (*p.p.*); von Wachenfeldt (1975) 233. — *Conferva speciosa* (Carmichael *ex* Harvey in Hooker) Areschoug (1850) 204. — *Hormotrichum speciosum* (Carmichael *ex* Harvey in Hooker) Crouan & Crouan (1852) no. 349. — *Hormiscia speciosa* (Carmichael *ex* Harvey in Hooker) Rabenhorst (1868) 363. — L e c t o t y p e : Great Britain, Appin, as *Lyngbya speciosa, Carmichael* (BM, holo; TCD).

Urospora penicilliformis (Roth) Areschoug (1874) 4 (*p.p., typo excl.*); Kjellmann (1883) 386 (*p.p.*); De-Toni (1889) 232 (*p.p.*).

Ulothrix isogona auct. non (Smith & Sowerby) Thuret in Le Jolis: Hauck (1885) 442 (*p.p.*).

Urospora penicilliformis (Roth) Areschoug *β vermicularis* (Kützing) Hauck & Richter (1893) 102. — T y p e : not indicated.

U. flacca auct. non (Dillwyn) Thuret in Le Jolis: Foslie (1890) 144 (*p.p.*); Wille (1901) 18; Børgesen (1902) 497; Jónsson (1903) 358 (*p.p.*); Cotton (1912) 109; Printz (1926) 231 (*p.p.*); Hamel (1930) 20 (*p.p.*); Carter (1933) 129; Feldmann (1937) 39 (*p.p.*); Levring (1937) 16 (*p.p.*); Kylin (1949) 12 (*p.p.*); Sundene (1953) 139 (*p.p.*); Feldmann (1954) 11 (*p.p.*); Perrot (1968) 1954 (*p.p.*).

~~*U. flexuosa* Kornmann (1964) 31, *nom. inval., non* Schussnig (1915) 435~~; Van den Hoek & Donze (1966) 315. — T y p e : Heligoland, on a jetty near the Helgoländer Düne, *Kornmann*, 15/3/1963 (HELGOLAND).

Marine gametophytic plants up to 12 cm long, (bright) green. *Thalli* flaccid and glossy. *Filaments* solitary or gregarious, often tuft-like. In a young stage filaments straight; later on, particularly during reproduction, (partially) bent or twisted and entangled. Filaments always unbranched and normally uniseriate. At gelatinisation cells sometimes multi-seriate, enclosed by a common cell wall; at times filamentous arrangement even completely distorted then. *Cell wall* both in young and mature stages flaccid, in young cells thin and smoothly surfaced, soon becoming thickened with a permanently smooth outer surface; only occasionally covered by micro-organisms and/or micro-particles. Sometimes, both in vegetative and reproductive cells the cell wall spontaneously swollen at one or more places. Occasionally, however, the cell walls entirely swollen over the whole length of the filament, which then exhibits a very conspicuous sheath-like habit with the uniseriate arrangement of the cells sometimes passing into a zig-zag pattern, in which the individual cells still adhere to one another. *Cells* pronounced cylindrical, their ends more swollen in older stages. Young cells closely appressed; later on, especially at gelatinisation resulting in multiseriate rows, occasionally slightly separated. Cells in the filaments sometimes arranged in pairs or fours. Transverse walls occasionally wrapped by ruptured pieces of the mother cell wall, appearing as H-pieces. Cell diameter (9.7—) 14.8—63.6(—85.8) μm, cell height (3.6—)4.8—15.6(—22.9) μm. Young filaments, in which gametogenesis rarely occurs, 9.7—14.8 μm wide. From the basal cell to 20—40 cells above it, the cell width often gradually increasing and cell length decreasing.

Chloroplast parietal and varying in shape with age and environmental conditions. In young cells always a pronounced open girdle, unlobed to lobed along its longitudinal margin; occasionally somewhat delicately incised and wrinkled, usually covering 2/3—3/4 of the cell circumference; not always approaching cell length. In older cells, the chloroplast usually extended towards the cross walls; now and then coarsely reticulate due to the chloroplast bridges, which connect chloroplast clumps present on both cross walls. Less commonly in mature cells an open to fully

closed chloroplast ring present, only expanded in the middle of the cell, hence showing an *Ulothrix zonata*-habit. In older stages the chloroplast sometimes withdrawn from the lateral and cross walls. Upon disintegration, the chloroplast distorted by assimilates, abundant and scattered both in chloroplast matrix and cell lumen. Cells uninucleate; *nucleus* conspicuous when chloroplast little developed. *Pyrenoids* 1—6, especially in compressed cells usually somewhat ellipsoidally elongated; now and then a decrease in the average dimension of the individual pyrenoids coinciding with the increase in number of pyrenoids per cell; their contours clearly distinct, normally covered with a thin layer of starch grains, except in chloroplasts strongly laden with assimilates.

In germlings the *basal cell* varying in length and width, often terminating in a rounded widened extreme end becoming variously differentiated. Attachment to the substratum by a simple mucilaginous layer. Reinforced attachment to the substratum by secondary rhizoids produced from intercalary cells especially in the basal portion of the filament. Chloroplast material with embedded pyrenoids randomly distributed in the basal cell, disappearing in older cells. Length of the basal cell up to 170 μm. Apical cell rounded in young as well as in older filaments, never behaving like the basal cell. Occasionally one to several intercalary cells smaller.

Gametogenesis occurring in ordinary cells, including the apical cells, not in the basal cell and intercalary cells producing outgrowing rhizoidal projections, usually proceeding from the terminal cells downwards; rarely gametogenesis restricted to some intercalary cells in the middle of the filament. Filaments with gametangia usually strongly curled. Mature *gametangia* olivaceous, especially in wide filaments inflated. Cell dimensions gametangia: (9.7—)14.8—52.8(—75.7) μm wide, 1/7—1 times as long as wide. At times, only gelatinisation and subsequent disappearance of the transverse walls of proximate gamete-producing cells visible, giving rise to a random dispersal of gametes inside the delicate sheath-like outer cell wall. *Gametes* issued all enclosed in a hyaline mucilaginous vesicle through an irregular shaped lateral aperture in the gametangial cell wall, the ensheathed gametes already active. Infrequently, in the sheath-like filaments, upon release of the gametes, there is only a rupture occurring in the inner layer of the lateral wall, leading to a discontinuous distribution of free-moving gametes in the gelatinized cell wall which is apparently partly hollow. Gametes (8—)16—128 (and possibly more) per cell, 5.8—8.6(—10.4) by 2.9—3.8 μm. Occasionally in wide cells the production of gametes less abundantly than might be expected and gametes partially covering the inner cell walls. Biflagellate gametes spindle-shaped to ovoid, sometimes globose at release. Now and then posteriorly terminating in a short point; no anterior papilla present. Parietal chloroplast of the gametes usually lining about half of the cell wall, irregularly cup-shaped and with one embedded pyrenoid and the relatively large stigma approximately median-posterior, conspicuous. Gametes positively phototactic when swimming, with a few exceptions. After relatively long, swift swimming movements, usually attachment of the non-fused gametes, commonly followed by parthenogenetic development to one-celled sporophytes. Germination of non-discharged gametes inside the gametangium similar to that of non-fused discharged sexual cells. Gametic mating isogamous to slightly anisogamous, dioecious. Quadriflagellate zygotes strictly negatively phototactic, rounding off, subsequently settling and developing into non-stalked, one-celled, subglobose or ovoid to ellipsoid sporophytes. Mature sporophytes nearly always elongated. Less frequently, upon

development, the zygotes form a protuberance, containing the moved up cell contents, ultimately giving rise to stalked sporophytes; these are linear, ellipsoid, clavate, or irregular-shaped with a basal attaching disc formed by the original contours of the zygote wall. The continuous, irregularly lobed, parietal chloroplast, containing 1—4 embedded pyrenoids, occasionally with accumulation of assimilates. Upon maturation the sporophytic contents brownish-yellowish green, dividing entirely or sometimes only partially into 32—128 or even more *zoospores* or *aplanospores*. Prior to and with ripening there is gelatinisation and ultimate disappearance of the stalk, and at the same time the thick-walled sporophyte body becomes elongated.

Fertile sporophytes up to 132 μm long and 52 μm wide. Release of zoospores through a randomly located irregularly shaped aperture, caused by preceding gelatinisation. *Zoospores* 7.2—13.8 by 4.2—6.3 μm, four-flagellate, ovoid-globose, mostly quite sluggish in locomotion, chloroplasts regularly cup-shaped, generally with one $\pm$ posterior pyrenoid, and with one stigma. Zoospores feebly positively phototactic, with few exceptions. Settlement on both hard and soft substratum, with the flagellar pole foremost, associated with the loss of the flagella. *Germination of the zoospores*, at times, consistently bipolar, ultimately giving rise to one filament of several cm long. Mostly, however, germination of the zoospores only bipolar in the beginning, the lower hyaline portion producing a short unmodified or slightly elongated basal cell and simultaneously the upper portion an uniseriate row of vegetative cells, often stretching horizontally. In 3—10-celled germlings not only transversal cytokinesis, but also with cell cleavages in longitudinal direction, directly leading to several erect, strictly unbranched, uniseriate daughter filaments, normally basi-apically differentiated and never coalescent. Organization of arising tufts simple-stellate, depending on the frequency and the place of the longitudinal cell divisions. At times the sporophyte issuing aplanospores, varying in the number as the zoospores do. *Germination of aplanospores* mostly starting inside the sporophyte, soon followed by a rupture of its cell wall, later on growing into filaments identical to those developing from zoospores. *Vegetative propagation* of filaments by fragmentation, incidentally accompanied by gametogenesis in one or more fragmented cells. After a long period of arrested growth, occasionally a peculiar developmental process in the filaments may occur caused by the outgrowth of the individual cells into independent daughter filaments, perpendicular to the original longitudinal axis of the mother filament.

NORWAY. Kvalsund, as *Ulothrix flacca, Foslie*, 13/7/1891 (O); Saltdalen, on stones in littoral, as *Ulothrix flacca, Sommerfeldt*, April (O); Molde, on *Fucus vesiculosus*, mixed with *Ulothrix flacca, Wille*, 28/7/1918 (S); Bergen, as *Ulothrix flacca, Boye*, 19/4/1893 (O); Espegrend, near Biological Station on iron, *Lokhorst*, 24/4/1975 (L); Dröbak, as *Ulothrix flacca, Simmons*, 15/4/1898 (S); as *Ulothrix flacca, Lagerheim*, 9/4/1898, *ex Wittrock, Nordstedt & Lagerheim-Algae exsiccatae 1446* (BM, L sheet 910.200–455); as *Ulothrix flacca, Wille*, 10/4/1898 (O); as *Ulothrix flacca*, on stones in the littoral zone near the Biological Station, *Wille, ex Kryptogamae exsiccatae 2147* (BM, BR, C, FI, L sheet 920.13–300. LD, S, UPSV); as *Ulothrix subflaccida, Hylmö*, 3/4/1912 (LD); as *Ulothrix pseudoflacca*, 14/4/1912 (C, S); as *Ulothrix flacca, Printz*, 4/1912 (L sheet 960.81–009); as *Ulothrix flacca, Sjöstedt*, 4/4/1917 (L, LD); Storskjaer and Smaskjaer, on stones intermingled with *Urospora penicilliformis, Lokhorst*, 21/4/1975 (L).

SWEDEN. Kristineberg, as *Ulothrix flacca, Kylin*, 9/4/1906 (LD); Varberg, as *Ulothrix subflaccida* and *Ulothrix flacca, Hylmö*, 26/4/1912, 13/3/1914, 16/5/1917, 13/3/1927 (LD, O); Kullen, Arild, as *Ulothrix flacca, Levring*, 17/3/1934 (GB); Kullen, Mölle, as *Ulothrix flacca, Hylmö*, 20/7/1921 (LD); Malmö, as *Ulothrix flacca, Sjöstedt*, 10/5/1917 (LD); Malmö, Limhamn, as *Ulothrix flacca, Sjöstedt*, 21/2/1928 (L, LD).

GERMANY. Flensburg, on piles in the harbour, as *Codiolum penicilliforme, Weidemann 52*, 10/4/1879

(L sheet 939.26–78); Kiel, as *Hormotrichum vermiculare* and *Urospora penicilliformis, Reinbold* (BM); Kieler Föhrde, as *Urospora penicilliformis β vermicularis, Reinbold*, 6/1888, *ex Hauck und Richter-Phykotheka universalis 528* (BM, HBG, KIEL, L sheet 920.13–253, PC); Wismar, as *Ulothrix flacca, Raettig* (O); Heligoland, western part, on granite, *Kornmann*, 10/3/1964 (HELGOLAND).

THE NETHERLANDS. Den Helder, on dike-slope on stones, as *Urospora isogona, Den Hartog 218, 278*, 29/3/1951, 30/3/1951, 1/4/1952 (L sheet 953.44–353, 956.313–551, 956.313–552); Ooltgensplaat, on basalt rocks and wood, *Lokhorst*, 13/3/1975 (L); Vlissingen, on boulders, intermingled with *Urospora penicilliformis, Lokhorst*, 13/3/75 (L).

GREAT BRITAIN. Orkneys, Stronsay, on stones at high water mark, intermingled with *Ulothrix flacca, Sinclair 580*, 16/3/1940 (E); Appin, as *Lyngbya speciosa* and *Oscillatoria speciosa, ex herb. Griffiths* (BM); Gourock, intermingled with *Ulothrix flacca*, as *Lyngbya speciosa*, 24/4/1852, 3/5/1852 (E); Granton, on wooden posts, intermingled with *Urospora penicilliformis*, 9/4/1881, *ex herb. Traill* (E); Joppa, as *Ulothrix flacca*, intermingled with *Ulothrix flacca, Traill 8593*, 3/4/1880 (E); Berwick, *Batters*, 10/1884, 1/1887, 5/2/1887, 1/1888, 3/2/1888 (BM, E, KIEL, L sheet 939.26–279); Scolt Head, Cockle Big Island, *Chapman*, 2/1933 (BM); Scolt Head, Hills Marsh, as *Urospora isogona, Chapman*, 3/1933 (BM); Gillingham, Copperhouse Marshes, mats on mud-sand-shingle-spit, as *Ulothrix flacca, Tittley*, 10/3/1970 (BM); East Rainham, River Medway, Otterham Quay, mat over mud, *Tittley*, 10/3/1970 (BM); Teynham, The Swale, Conyer Creek, over mud, between *Spartina* and *Halimione*, as *Ulothrix flacca, Tittley*, 11/3/1970 (BM); Budleigh Salterton, as *Lyngbya cutleriae* and *Ulothrix implexa, Griffiths & Cutler*, 5/1851, 7/1851, 6/1852 (BM, E); Torbay, as *Lyngbya speciosa, Griffiths*, 5/1849, *Wyatt-Algae Danmoniensis 196* (BM, CN, E, KIEL, L sheet 910.202–866, TCD); Plymouth, as *Lyngbya carmichaelii. Harvey*, 1851, *ex herb. Greville* (E); Penzance, *ex herb. Kützing* (L sheet 939.67–915); Anglesey, Penmon, as *Ulothrix flacca, Holmes*, 9/4/1881 (BM, L sheet 959.313–938, LD).

IRELAND. Clare Bay, on flat muddy shores, *Cotton*, 2/1911 (BM); Giants Causeway, as *Ulothrix flacca, ex herb. Dickie* (BM).

FRANCE. Cap Gris Nez, on boulders intermingled with *Enteromorpha, Lokhorst*, 20/3/1975 (L); Cherbourg, intermingled with *Ulothrix flacca, Bornet 79*, 4/4/1853 (L sheet 939.26–277); 9/4/1853, *ex herb. Gomont* (PC); as *Hormotrichum speciosum, Thuret 113*, 9/4/1853 (PC); intermingled with *Urospora* and *Enteromorpha* spec., *Bornet*, 9/4/1853, *ex herb. Thuret* (UPSV); as *Hormotrichum speciosum* and *Urospora penicilliformis, Le Jolis 1632*, 6/4/1860 (BM, CN, E); as *Ulothrix flacca, Bornet*, 27/3/1873, *ex herb. Thuret* (PC); Fermanville, on rocks, as *Hormotrichum speciosum, ex herb. Lebel 2059* (PC); Brest, as *Conferva flacca* and *Urospora penicilliformis*, 1836, *ex herb. Crouan* (BM); Finistère, as *Urospora speciosa, Ulothrix flacca* and *Ulothrix carmichaelii, ex herb. Lebel and Crouan* (PC); Biarritz, Port de Pêcheurs, on *Patella* grazing in high littoral vegetation of blue-green algae, *Enteromorpha* and *Blidingia* spec., as *Ulothrix flexuosa, Van den Hoek*, 18/8/1964 (L sheet 965.172–016).

Nomenclature and historical aspects

This species has been misinterpreted for a long time. It was described in 1833 and the superficial knowledge of the algal taxonomy at that time is well expressed in the treatment of this species under the blue-green algal genus *Lyngbya*, probably prompted by the occasional occurrence of sheath-like conditions of the cell wall. Obviously, Harvey (1833) characterized the margin of the filaments as crenate, which might suggest a similarity with the typical coarse outer cell wall texture of *Ulothrix flacca*. However, it may be concluded from Harvey's comments accompanying the specific description that he attributed the crenate appearance of filaments to the ripening and concurrent protrusion of the gametangia. Harvey's description was based on material collected by Captain Carmichael from the coast near Appin (Scotland). The specimen marked as 'Appin, Capt. Carmichael', preserved in BM, is hereby designated as the lectotype. In 1849, Kützing transferred this species to the genus *Ulothrix*, but unfortunately the confusion as to its identity was rather increased as a result of its addition to the list of pure freshwater *Ulothrix* species. Furthermore, Kützing classified all marine species, which nowadays belong to *Ulothrix*, in the genus *Hormotrichum*. During the latter half of the 19th century the systematic position and the concepts of this species were rather open to question.

In 1852, Crouan & Crouan, following Kützing, classified it under *Hormotrichum*. In 1863, however, Thuret reduced this genus again to *Ulothrix*, but his misunderstanding of the generic delimitation of *Ulothrix* is expressed by the presence of species, now to be ranked in the genus *Urospora*. In 1868 Rabenhorst assigned *U. speciosa*, which he supposed to be a brackish-water species, to *Hormiscia*, which should have the ability to occur in fresh water. It is the writer's suggestion that the resemblance of a particular growth stage of the chloroplast of *U. speciosa* to that of the freshwater species *Ulothrix zonata*, must have caused these incorrect field observations. The erroneous interpretation of the systematic position of *U. speciosa* is demonstrated by its addition to the list of synonyms, compiled for *Ulothrix isogona* (Hauck, 1885) and for *Urospora penicilliformis* (De-Toni, 1889). In 1901, Wille summarized his systematic observations on the marine *Ulothrix* representatives in the Oslofjord. The species in that paper reported under the name *Ulothrix flacca* is, in fact, *Ulothrix speciosa*. For more than 50 years most phycologists on the west European continent named this species in the sense of Wille (1901). It seems, however, that the name *Ulothrix speciosa* was maintained in the majority of contemporaneous reports from the British Isles, its native country. In 1964, Kornmann, after studies on the marine *Ulothrix* species of Heligoland, reintroduced this species in the right sense and reported additional, valuable features on the reproduction.

The systematic status of *Ulothrix flexuosa* Kornmann (1964) is intriguing. It is morphologically similar to *U. speciosa*, distinguishable from it by the formation of only aplanospores in the sporophytes, and in a more fundamental way by the monoecious reproduction. In the writer's opinion *U. flexuosa* has to be assigned as a synonym to *U. speciosa*, despite the reported distinctive characters, which might only be used for the distinction of infraspecific taxa, however. For evaluation of the features monoecious and dioecious, see under Conclusions.

It is highly probable that in Perrot's studies of *Ulothrix flacca* (1968) the present species *Ulothrix speciosa* is concerned as well. One of the reported types of life history almost fits that of the present species.

Morphology

The *general habit* seen in culture is quite identical to that observed in nature. In cultures, after dissemination of motile zoospores matured in the sporophytes, simple filaments or mostly tufts, grow up individually from one zoospore, these randomly distributed on the bottom and sometimes also on the upright inner side of the culture vessel. The morphological divergence in growth habit could not be correlated with any circumstantial factor. Commonly in culture it is seen as a rapid-growing alga with the mature filaments fairly equal in length and width as compared to those grown under natural circumstances. Free floating plants have only been seen in culture, exhibiting an appearance similar to that of attached organisms. Local swellings of the cell wall or even inflation over the entire filament length may occur spontaneously; these phenomena can be induced artificially by adding JKJ in lactophenol or a dilute acid solution.

It is remarkable that this species lacks a gelatinous outer *cell wall* surface. It is highly probable that this wall type accounts for the usual absence of fouling micro-organisms and micro-particles on it. By staining reagents a predominant cellulose

concentration in the inner layer of the longitudinal cell wall, which is continuous in the transverse wall was detected. The outer wall layer proved to consist chiefly of pectinic substances. In the present studies the precise external factor, which accounts for extreme gelatinisation of filaments resulting in multiseriate or even disordered cell rows, could not be detected, although this process shows a tendency to intensify at extremely low salinity values ($0^0/_{00}Cl^-$). In young filaments the cells are closely adherent to one another, but they may become spaced in dilapidated cultures or after spontaneous strong gelatinisation of the filaments.

In natural populations the *cell dimensions* may vary considerably. For example, measurements, carried out on plants collected in the Oslofjord near Dröbak (*c.* $10–12^0/_{00}Cl^-$), showed a cell diameter range up to 32.6 μm, while in herbarium material from Berwick and in a fresh collection from the vicinity of Kats (Netherlands) (*c.* $16^0/_{00}Cl^-$) cell diameters up to 77.5 μm and 85.8 μm respectively could be ascertained. For diameters of reproductive cells, the above mentioned differences are mostly smaller. For example, plants in Dröbak had gametangia up to 45 μm in width. On the other hand, in cultures initiated from plants collected from various localities in western Europe, the differences in cell dimensions of vegetative filaments became also smaller with the exception of plants from Dröbak, which showed a lower average cell width. In general, in cultures the cell diameter tended to diminish at lower salinities. The various photo periods, the sea water media, and temperatures used did not strikingly affect the cell dimensions. Both on solid and in liquid media approximately the same cell diameter range could be determined in the individual clones. In general, the cell length/width ratio is larger in young filaments, but this larger ratio may also persist in more aged plants growing in salt marshes.

Commonly the *chloroplast* has a similar appearance both in culture and in nature. In natural populations with lower salinities (*c.* $8^0/_{00}Cl^-$), the chloroplast is frequently lighter green in colour and showing an extension only over the middle of the inner lateral wall, which is morphologically similar to a certain growth stage of the chloroplast of *Ulothrix zonata*. Often, in dilapidated cultures, the chloroplast is obscured by a strong accumulation of assimilates; a phenomenon rarely seen in plants under natural conditions.

In young filaments the number of *pyrenoids* is usually reduced, and the same applies to mature cells with less expanded chloroplasts. The size of the pyrenoid may vary unaccountably.

The shape of the *basal cell* seems more or less determined by external circumstances. In nature the basal cells are (slightly) narrowed, often only showing a small elongation; they are usually thick-walled and overgrown with micro-organisms like fungi. Their contents is hyaline. Generally they are flattened at base, ensuring a close touch with the substratum. The ability of the basal cell to develop into a complex rhizoidal system seems for the present species to be restricted mainly to cultures. In all plants examined the apical cells were similar in shape.

Reproduction and life history

Compared with the remaining *Ulothrix* species, with the exception of *U. implexa*, certain aspects of reproduction and life history of *Ulothrix speciosa* have been studied quite intensively in the past. As early as 1833 Harvey reported marginal protrusion of the "sporidia" by which the threads have a crenate appearance. A few

years later, Berkeley (1849) described the endochrome as finally separated into lenticular spores. Afterwards, several authors depicted filaments with gametangia without giving much detail on e.g. the nature of the reproductive cells or the type of life history (Kützing, 1852; Foslie, 1890; Rosenvinge, 1893). Wille's comprehensive studies on *U. flacca* (1901) provided additional information on the shape of the gametangial filament and the mating of the gametes.

In 1948, in a preliminary account, Hygen communicated that biciliate zoids, formed under different photo regimes, are morphologically alike. Zoids produced under long-day conditions form zygotes; if the zygotes are subsequently transferred to a short-day regime germination starts within a few weeks. Zoids, arisen in filaments under short-day conditions, behave as asexual spores and germinate directly into new filaments.

In 1964, Kornmann emphasized the alternation of heteromorphic generations in *U. speciosa.*

In 1968, Perrot in a paper dealing with *U. flacca* (apparently *U. speciosa* in part) reported two fundamentally different types of sexual reproduction in this species. One seems to be associated with organisms distributed only in the lower level of the littoral zone. It is characterized as anisogamous and dioecious with a heteromorphic, mono- or digenetic life history. The other one is only found in plants from the upper level of the littoral zone. It is defined as monoecious and isogamous with an isomorphic, digenetic life history. The first type almost corresponds with our knowledge of *U. speciosa.* In the present study the second type of reproduction has not been found, while the first type's pronounced preference for a special zonation level in the intertidal belt could not be affirmed. In the present paper much new information concerning the reproduction of this species is communicated, e.g. the number of gametes, sporophytic zoospores, developmental stages of zoospores, dimensions.

After transferring vegetative plants preserved at 8 °C to different photo periods the filaments commenced to produce gametes spontaneously. Under short-day conditions, in the course of 1—2 weeks this process is induced in only a few filaments, but under long-day photo periods sexual plants were observed in large quantities. In cultures, the production of gametes decreases distinctly at lower salinity ($< 8^0/_{00} Cl^-$). No obvious difference in frequency could be noted between the numerous clones from various types of localities in western Europe. Parallel with the first mitotic divisions, the filaments become usually strongly curled, generally starting in the apical cells. Consequently, the demarcation between the vegetative and reproductive portion can easily be observed with the naked eye. On maturation the gamete-producing cell is olivaceous and often inflated. The colour of the chloroplasts of discharged gametes is greenish and the cause of this colour change is for the moment unexplained.

Gametogenesis may be induced in liquid culture medium as well as on firm agar plates. In the individual cultures, initiated by inoculation of one filament, the discharged gametes without exception failed to mate, which confirms the strict dioecism of the species. The absence of zygotes does not impede the completion of the alga's life history in culture. Most gametes develop parthenogenetically to sporophytes, which under short-day conditions produce zoospores in course of time. Only occasionally discharged gametes do not develop any more and eventually die.

The results of cross-fertilization experiments are summarized in table 1. These

	Espegrend	Grevelingen dam	Cap Gris Nez	Roscoff
Espegrend		+	±	−
Grevelingen dam	+		+	+
Cap Gris Nez	±	+		−
Roscoff	−	+	−	

Table 1. Survey of cross-fertilization experiments amongst four clones of *Ulothrix speciosa*. Fusion of gametes leading to true zygotes is indicated by the symbol +, absence of the mating process is symbolized by the mark −, whilst beginning of pairing of gametes but not resulting in true zygotes is indicated by the symbol ±.

results show the definite mating type of the individual clones. Attempts to induce zygotes from joined gametes of clones from Espegrend and Cap Gris Nez sometimes gave rise to a normal first contact and subsequent start of fusion of the flagellar poles, the process coming to a stop half way down, resulting in four-flagellate swarmers with one mated anterior end but with separated posterior ends. Fusion of the nuclei apparently did not occur in these cells, which may be explained by an almost equal hereditary make-up of the individuals concerned. Only four clones were involved with these cross fertilization experiments, and the negative results with gametes, descended from Espegrend and Roscoff repectively, are not sufficient to postulate a genetic barrier between Norwegian and French populations of *U. speciosa*.

The mucilagination and subsequent disappearance of only the cross walls in a series of proximate gamete-producing cells in filaments has been seen both in culture and in the field. This phenomenon could not be connected with any environmental condition.

After mating of the gametes, the zygotes may swim for a couple of hours, later on attaching themselves and germinating into sporophytes. They distinctly prefer to mature under short-day conditions. In most cultures the attached zygote simply enlarges, which ruptures its envelope. The remnants of this envelope may remain visible as an attaching disc for a long time. Particularly in cultures from Vlissingen and Dröbak, besides the process described above, attached zygotes were seen to form a protuberance in which the cell contents were shifted, ultimately giving rise to stalked sporophytes. Remains of the zygote wall were also seen here to persist as a sort of a husk, in which the base of the sporophytic stalk is anchored. Mostly the ripening of the sporophyte contents coincided with partial or complete mucilagination of its stalk. Occasionally, the cell contents do not cleave completely upon ripening.

The zoospores are mostly globose in shape. In cultures they sometimes remain buoyant. A fundamentally similar germination process is seen both in attached and free-floating zoospores. Differing varying temperatures, daylength and light intensities used did not markedly affect the developmental stages in any incipient plant.

Aplanosporogenesis, as a result of arrested zoosporogenesis, occurs spontaneously in sporophytes. After protrusion of germinated aplanospores, conspicuous remnants of the sporophyte cell wall are seen adhering to the clump of these spores for some time.

In exhausted cultures from Espegrend once some aberrant reproductive cells were produced under short-day conditions. These were globose-ovoid, relatively

large in size, up to 12.1 by 6.0 μm, mostly four-ciliate, and with two stigmata. Their locomotion was sluggish and the development to sporophytes was the same as described for non-fused gametes and zygotes. Ripening of their contents was not ascertained, however. Presumably, the occurrence of these peculiar reproductive cells is connected with suboptimal external circumstances. Young as well as older sporophytic stages were not encountered in nature or in herbarium specimens. Presumably, the negative phototactic response of the zygotes accounts for this.

The simultaneous outgrowth of a series of proximate cells into daughter cells, arising perpendicular to the axial length of the parent filament, was only found in clones from Cap Gris Nez and Ooltgensplaat. This phenomenon might be interpreted as a particular sort of vegetative propagation, unique within the marine and brackish-water *Ulothrix* species studied. It was seen only in cultures on agar plates, after a period of arrested growth.

Vegetative reproduction by fragmentation of filaments of one or more cells is usually associated with the entire disappearance of the outer-most layer of the cell wall. It is not clear which external factors are in fact involved here, although the frequency of this phenomenon tends to increase in culture media with extremely low or high chlorinity.

Ultrastructure

Cell division. Without furnishing clear evidence, it can be concluded from some photographs that after nuclear division the new transverse cell wall appears as an annular ingrowth from the plasmalemma and the lateral cell wall. This furrow grows across the cell lumen, cleaving completely through the central vacuole. No microtubuli were found in the vicinity of the developing septum.

Cell wall. The surface of the cell wall is obviously smooth and never clothed with an outer mucilaginous layer. It is composed of two membranes, which could be well demonstrated in the filaments of plants from the field as well as from cultures. In nature, it may be slightly studded by some fouling organisms, of which it appeared that they did not damage the membranes. The surface may show slight depressions. Underneath the double membrane there is an outer layer which consists of randomly arranged microfibrils embedded in an amorphous matrix. It may be concluded from the micrographs that especially this layer is engaged in swelling events, for instance when induced in this species by adding fixatives. The middle layer, which is electron denser and mainly consisting of parallel running microfibrils, is relatively thin. The inner microfibrillar layer may vary considerably in thickness, particularly in the cross walls. Its microfibrils may be arranged randomly as well. Lomasome structures were observed in young transverse cell walls. Lee & Fultz (1970) proposed the possibility that in *Porphyra leucosticta* these structures are involved in cell wall synthesis, as first suggested by Marchant & Robards (1968) for fungi. The lomasome was assumed to be a transitional stage during the incorporation of cell wall precursors. This presumed function can neither be confirmed nor denied in the present study.

Chloroplast. The chloroplast may partially or completely clothe the cross wall, but a direct contact is prevented by the presence of a thin layer of cytoplasm and the

plasmalemma. Mostly the chloroplast is regularly extended over the cross wall; at times, however, it may be very strongly lobed. The chloroplast is covered by a continuous two-membrane envelope. It seems that particularly the inner membrane can be studded with ribosomes. The intervening cavity does not appear to be equally spaced throughout. In some areas the individual membranes undulate. Unlike the nuclear envelope, the chloroplast membranes are not continuous with any cell organel. The thylakoids may occur singly, but mostly several, up to seven, adhere together in long lamellae. One or more thylakoids may pass from one lamella into another, hence showing a reticulate appearance. Compared with the closely related *U. palusalsa*, it sometimes seems that in *U. speciosa* the lamellae-network is relatively less-developed. In chloroplast lobes the thylakoids terminate abruptly, mostly in close proximity to one another. The two membranes of an individual thylakoid frequently are not parallel, particularly in single thylakoids and in those lying in the lamellae exteriorly. Furthermore, the individual thylakoid terminates somewhat dilated. The chloroplast stroma contains ribosomes, sometimes lying closely together. Between the thylakoid lamellae there are osmiophilic lipid plastoglobuli in varying numbers in the chloroplast matrix. They also vary in size and are approximately globose-ellipsoid. Spheroidal or elongated starch grains are usually distributed in the chloroplast. It seems that the mode of interpolation of these grains between the lamellae is responsible for the splicing of the latter. The marginal zone of the individual starch grain usually appears denser than the inner area. Most of the starch is normally present as segmental grains in the starch shell surrounding the pyrenoid. External conditions may cause a variation in the amount of starch grains. For example, pyrenoids in filaments collected from nature mostly show a poorly developed starch envelope as compared with those grown up in culture.

The *pyrenoid* is positioned between the lamellae of the chloroplast. Occasionally it may protrude from it into the cell lumen, but even then it is still bordered by some chloroplast stroma and its envelope. The finely granular pyrenoid matrix is penetrated by tubular chloroplast invaginations on all sides. The extent of development of the starch shell does not affect their number. These invaginations normally contain 3–5 thylakoids and mostly they terminate before reaching the centre of the pyrenoid. Their ends are somewhat swollen. The outer side of their membrane in the pyrenoid is frequently occupied with osmiophilic bodies, the pyrenoglobuli, which are fairly constant in size and shape.

Nucleus. In longitudinal section the nucleus is generally circular. In much shortened cells it is (slightly) flattened, however. Often, one side of the nucleus is closely adjacent to the chloroplast membranes, separated from it only by a small layer of cytoplasm. The rest of the nuclear envelope may be bounded respectively by one or several vacuoles and in presumably highly active cells by a complex network of mitochondria, dictyosomes and accompanying vesicles. The single nucleolus, variable in size and shape, lies in the central part of the nucleus. Its matrix seems to exist of granules and fine filaments, which are not equally dispersed. The nuclear stroma seems to contain equally dispersed finely filamentous euchromatin and more densely granular heterochromatin in dense randomly dispersed clusters. The inner membrane of the nuclear envelope is sometimes rough, while the outer seems to be continuous with the cytoplasm ER. Generally, the outer membrane is covered with ribosomes. Both membranes may have a wavy appearance. The perinuclear space,

which separates both membranes, is dilated at irregular intervals. The nuclear envelope is regularly perforated by pores.

Mitochondria. The amount of mitochondria may fluctuate in the individual sections. A specific spatial relationship of mitochondria to any other component of the cell could not be ascertained. They may be situated very close to the chloroplast membrane. Sometimes an individual mitochondrion appears to be enclosed within a chloroplast cavity. In presumably highly active cells a rim of mitochondria is found around the nuclear envelope, but coalescence of mitochondria with the nucleus as seen in higher plants (Mota, 1964) has never been observed. Mitochondria may also lie adjacent to the plasma membrane. In general, there is a thin layer of cytoplasm between the adjoining cell organel and the mitochondrion. It is apparent, that round as well as oval mitochondrial profiles predominate, but also (sinuous) elongate figures are observed repeatedly.

Tubulate cristae appear to be almost evenly spaced and located parallel to one another in some sections. In other longitudinal sections they are irregularly distributed in the mitochondrial lumen. It seems that their base is slightly constricted. In all cells, all mitochondria are generally of the same type, which, according to Öpik (1968), might be named the narrow cristate form, associated with a relatively light electron-dense matrix.

Golgi apparatus. The Golgi apparatus usually consists of a series of numerous dictyosomes. It may cluster round the nucleus, particularly when it is located in front of pores in the nuclear envelope. The apparatus may also be situated above the nucleus and just beneath one of the transverse cell walls. The single dictyosome consists of a stack of 3—7 cisternae, a relatively small number. The produced vesicles become separated from the cisternae by budding off at the edges of the dictyosome. They are found freely scattered both inside and outside the Golgi ring. Its association with cell wall formation is frequently suggested in our material.

Cytoplasm inclusions. Only one type of osmiophilic bodies, the so-called fat droplets, frequently reside in the cytoplasm, singly or in clusters. At times they protrude into the vacuole, which might be an indication for a discharge of their lipid contents. On the other hand, this might equally well imply a reverse process. The droplets are variable in size and spherical, ovoid, or elongate. Once a droplet was found entirely surrounded by a diffuse osmiophilic secretion. Occasionally, simply-elongated or complexly-organized peroxisomes were found localized near the chloroplast envelope. These contain a finely granular substance and are bounded by a single membrane. Amongst other cell components, peculiar membraneous inclusions of unknown function were observed in the cytoplasm. These internal structures were not consistent in size and number. They were found to consist of an irregular membrane system, varying from single simple rings of membranes to complexly organized whorls of concentric rings of lamellae. At times, these structures seem to be bounded by, or continuous with membranes which generally run out into cytoplasmic ER-strands, enclosed by chloroplast lobes. Since remnants of these membraneous inclusions were encountered in vacuoles, it is surmised here that they represent sequestered degraded ER-clumps, which are released in the cell vacuole where they are broken down. These vacuoles can particularly be recognized by their internal local myelin-like figure formation. In addition it can be mentioned that the

occurrence of these myelin-like inclusions is an established symptom of protoplasmic degeneration (Gomez *et al.*, 1974).

Vacuole. In general, mature cells contain one single large central vacuole, enclosed by a continuous tonoplast. Sometimes, the vacuole is divided into numerous vesicles, which are delimited individually by distinct membranes. Such divided vacuoles seem to be distributed randomly in mature vegetative cells. Non-active vacuoles appear empty and structureless, except for a flocculent precipitate. The vacuolar basic substance may be more differentiated. The matrix of the lomasomal structures earlier referred to, representing invaginations of the plasmalemma and containing vesicular or membraneous inclusions, strikingly resembles the cytoplasm vacuoles. Sometimes, the dictyosomes are coated with relatively large vesicles, which, after separation, resemble vacuolar portions.

Ecology

In winter and spring *Ulothrix speciosa* is fairly common in the littoral zone of the coasts of western Europe. On more southern coasts the alga disappears sooner, apparently coinciding with the earlier rise of water- and air temperatures. It mostly resides in the upper zone of the inter-tidal belt of natural and artificial rocky shores, but, particularly in France, it is also found in lower littoral zones. Plants found in different parts of the inter-tidal zone are almost of the same shape. Its seasonal cycle overlaps with that of *U. flacca*, but field observations showed an earlier appearance and disappearance of filaments of *U. speciosa*. Its relatively wide ecological amplitude is clearly demonstrated by its ability to constitute algal populations in brackish-water inland lakes, such as for instance in the Veerse Meer (Netherlands), which has an annual fluctuating chlorinity of 8—11 $^0/_{00}$ Cl^-. In these areas it is only sparsely distributed and the plants have a cell diameter of only up to 32 μm, while the chloroplast is frequently only expanded in the middle of the cell.

In general, this species prefers sheltered localities. Strong wave action washes the alga away, as noticed on a usually protected dike-slope in Bruinisse (Netherlands), which became exposed to the wind. It does not only grow on hard substrata but may also be luxuriantly distributed in salt marshes on soft bottom where it may play an important role in the salt marsh algal flora, as a predominant species growing in separate, hibernal or vernal vegetation units in the mosaic algal communities. Also, the almost bare soil in open clayey or sandy spots in salt marshes may be covered by a thin green, relatively homogeneous *U. speciosa* stratum. Finally, at times it can also be encountered under halophytes like *Spartina townsendii*, *Puccinellia maritima*, *Salicornia europaea* and *Aster tripolium*. The species is found epilithic on granite, on limestone boulders, and at times on concrete. No remarkable deposition of sand grains could be ascertained in *U. speciosa* sheets as observed for *U. flacca* in these spots. Apparently, no kind of natural substratum can be found, on which *U. speciosa* can not grow. It has not yet been found on bitumen, presumably due to poisons added to this substratum, to prevent algal development. In contrast with the field observations in salt marshes, this alga does not occur in the undergrowth of larger algae on hard substrates. It is hardly ever found on animals and *Fucoid* algae.

The origin of the material in culture

Uni-algal cultures were started from plants collected from the following localities. NORWAY: Oslofjord, in the harbour at Dröbak, near the high water mark; Maripollen, near the Biological Station (Espegrend), on concrete slabs in high littoral. THE NETHERLANDS: Friesland, Holwerd, near landing-stage on clayey soil in a salt marsh; Zuid-Holland, Goeree Overflakkee, Ooltgensplaat, on a wooden pile; Zeeland, Schouwen Duiveland, eastern side Grevelingen dam near Bruinisse, on large concrete tiles in high littoral; Zuid Beveland, Sas van Goes, on the limestone dike-slope, near sluice gates, high littoral; Walcheren, Vlissingen, middle and high littoral, on boulders. GREAT BRITAIN: Norfolk, Morston, in salt marsh in the *Puccinellietum maritimae* (material collected by Mr. P. Polderman). FRANCE: Cap Gris Nez, on boulders, high littoral; Roscoff, in midlittoral on stones and boulders.

Notes on herbarium material

The study of herbarium material of this species was very useful. Almost all specimens examined, whether mounted on microglass slides, mica or paper, proved to be well-preserved and in consequence regained their original habit after soaking in some drops of detergent.

The species could be fairly readily identified, particularly by its soft cell wall. The cell wall of prolongedly dried filaments did usually not show (local) swellings upon soaking, but their identity became unequivocally clear after adding JKJ, which mostly prompted a strikingly inflated wall or even its disintegration. Furthermore, the very characteristic spiralization of the gametangial filament persists in the dried state and is visible in herbarium collections. There was never any doubt as to the identity of young plants of this species. Field and culture observations have revealed that the smaller, closely related *U. palusalsa* is, when exhibiting overlapping cell dimensions, at a mature age and consequently can be distinguished by its (a)sexuality.

2. Ulothrix flacca (Dillwyn) Thuret in Le Jolis* — Figs. 6—10; Plates 5—7.

Conferva flacca Dillwyn (1805) Pl. 49; Smith & Sowerby (1808) Pl. 1943; Lyngbye (1819) 144; Agardh (1824) 102, Jürgens (1824) no. 5; Harvey in Hooker (1833) 354; Harvey (1841) 131; Areschoug (1850) 205. — *Hormidium flaccum* (Dillwyn) Kützing (1843) 244; Rabenhorst (1847) 96. — *Hormotrichum flaccum* (Dillwyn) Kützing (1845) 204; (1849) 381; (1853) 20, Tab. 63; Crouan & Crouan (1852) no. 347. — *Lyngbya flacca* (Dillwyn) Harvey (1849) 227. — *Lyngbya ? flacca* (Dillwyn) Harvey (1851) Pl. 300; Johnstone & Croall (1860) 177. — *U. flacca* (Dillwyn) Thuret in Le Jolis (1863) 56; Hauck (1885) 442; Traill (1885) 16; De-Toni (1889) 233 (*pro syn.*); Foslie (1890) 144 (*p.p.*); Batters (1891) 8; Reinbold (1891) 129; Traill (1891) 305; ? Foslie (1892) 16; Rosenvinge (1893) 935 (*p.p.*); ? Boye (1894–95) 44; Batters (1902) 13; Børgesen (1902) 497 (*p.p.*); ? (1903) 4; Jónsson (1903) 358 (*p.p.*); ? Hariot (1912) 13; Van Goor (1923) 105; Printz (1926) 231 (*p.p.*); Hamel (1930) 20 (*p.p.*); Newton (1931) 56; ? Hamel & Lami (1934) 11; Chapman (1937) 228; Feldmann (1937) 39 (*p.p.*); Levring (1937) 16 (*p.p.*); Kylin (1949) 12 (*p.p.*); Blackler (1951) 123; ? Parke (1952) 12; Sundene (1953) 139 (*p.p.*); Feldmann (1954) 11 (*p.p.*); Blackler (1956) 46; ? Grenager (1957) 48; ? Breivik (1958) 35; Van den Hoek (1958) 204; ? Moss (1959) 108; ? Burrows (1960) 23; (1963) 245; ? Jorde & Klavestad (1963) 76; Jaasund (1965) 12; ? Perrot (1968) 1953. — *Hormiscia flacca* (Dillwyn) Areschoug (1872) 91; (1874) 5 (*pro syn.*). — *Urospora penicilliformis* (Roth) Areschoug f. *flacca* (Dillwyn) Reinke (1889) 83; Svedelius (1901) 80; Lakowitz (1929) 170. — Lectotype: Swansea, on red alga, *Dillwyn* (BM, holo; C).

Bangia ? *laetevirens* Harvey in Hooker (1833) 317; Harvey (1841) 173. — *Schizogonium laetevirens* (Harvey in Hooker) Kützing (1843) 246; (1845) 194; Rabenhorst (1847) 98; Kützing (1849) 351; (1852) 32, Tab. 100. — *Schizogonium laetevirens* (Harvey in Hooker) Kützing *β majus* Kützing (1849) 351;

* This species has been studied in cooperation with Mr. G. J. Kranenburg

Suringar (1854) 55. — T y p e : Ireland, Miltown Malbay, near high water mark, as *Bangia laetevirens, Harvey* (TCD).

Lyngbya carmichaelii Harvey in Hooker (1833) 371; Harvey (1841) 161; Berkeley (1849) Pl. 2927; Harvey (1849) 226; (1851) Pl. 186 A; Johnstone & Croall (1860) 173. — *Hormotrichum carmichaelii* (Harvey in Hooker) Kützing (1849) 382; (1853) 20, Tab. 64. — L e c t o t y p e : Torbay, as *Hormotrichum carmichaelii* and *Ulothrix flacca* var. *carmichaelii, Wyatt, Algae Danmoniensis 230* (TCD, h o l o ; BM, E, L sheet 910.202–876, 939.26–67).

Schizogonium crispatum Kützing (1843) 246; Rabenhorst (1847) 98; Kützing (1852) 32, Tab. 100. — *Schizogonium crispum* Kützing (1845) 194. — *Schizogonium laetevirens* (Harvey in Hooker) Kützing γ *crispum* (Kützing) Kützing (1849) 351. — T y p e : lost.

Hormotrichum fasciculare Kützing (1847) 166; (1849) 382; (1853) 20, Tab. 64. — T y p e : Heligoland, as *Hormotrichum fasciculare, ex herb. Kützing* (L sheet 939.67–722).

Hormotrichum affine Kützing (1849) 381. — T y p e : Heligoland, *ex herb. Kützing* (L sheet 939.67–727).

Hormotrichum didymum Kützing (1849) . . . . . .

Hormotrichum didymum Kützing (1849) 381; (1853) 20, Tab. 63. — T y p e : Spikerooge, mixed with *Rhizoclonium* spec., *Koch 128*, 8/1844, *ex herb. Kützing* (L. sheet 939.67–723).

Hormotrichum vermiculare Kützing (1849) 382; (1853) 20, Tab. 64. — T y p e : Nordsee, *Jürgens, ex herb. Kützing* (L sheet 939.67–713).

U. speciosa auct. non (Carmichael *ex* Harvey in Hooker) Kützing: Kützing (1852) 29, Tab. 93 (*p.p.*); Pankov (1971) 74 (*p.p.*).

Urospora penicilliformis auct. non (Roth) Areschoug: Areschoug (1874) 4 (*p.p., type excl.*); Kjellman (1883) 386 (*p.p.*); De-Toni (1889) 232 (*p.p.*); Van Goor (1920) 17.

U. submarina auct. non Kützing: ? Gobi (1878) 88; ? Kjellman (1883) 385.

Urospora penicilliformis (Roth) Areschoug f. *hiemalis* Wittrock & Nordstedt (1882) no. 418. — L e c t o t y p e : Göteborg, Varhomen, *Åkermark*, 4/1872, *ex Wittrock & Nordstedt's Algae Exsiccatae 418* (L sheet 939.26–99, h o l o ; BM).

Bangia virescens Foslie (1890) 62. — T y p e : Norway, Omgang, *Foslie*, 8/7/1887 (C).

U. pseudoflacca Wille (1901) 22 (incl. f. *major* Wille and f. *minor* Wille); Børgesen (1902) 498; Jónsson (1903) 357; Wille (1910) 284; Cotton (1912) 109; Hariot (1912) 13; Hylmö (1916) 4; Printz (1926) 232; Lakowitz (1929) 108; Hamel (1930) 22; Hamel & Lami (1934) 11; ? Lund (1934) 23; Chapman (1937) 228; Feldmann (1937) 40; Levring (1937) 17; (1940) 2; Kylin (1949) 12; Sundene (1953) 139; Feldmann (1954) 11; Blackler (1956) 46; ? Breivik (1958) 35; Van den Hoek (1958) 204; ? Burrows (1960) 23; (1963) 245; ? Jorde & Klavestad (1963) 76; ? Perrot (1971) 858; ? Pankov (1971) 77; Starmach (1972) 47; Von Wachenfeldt (1975) 232. — L e c t o t y p e : Dröbak, in the littoral zone near the Biological Station, *ex Kryptogamae Exsiccatae 2146* (L sheet 920.13–382, h o l o ; BM, BR, C, FI, LD, S, UPSV).

U. consociata Wille (1901) 25; Børgesen (1902) 498; Cotton (1912) 109; Hariot (1912) 13; Printz (1926) 230; Hamel (1930) 24; Levring (1937) 17; Sundene (1953) 139; Blackler (1956) 46; Von Wachenfeldt (1975) 232. — L e c t o t y p e : Dröbak, on stones in the harbour, *Wille*, 10/4/1898 (O, h o l o ; S).

U. consociata Wille var. *islandica* Jónsson (1903) 354. — *Ulothrix islandica* (Jónsson) Printz (1926) 230. — T y p e : specimen not seen, but drawings with the original publication are obvious.

Marine gametophytic plants up to 6 cm long, bright to dark green. *Thalli* flaccid. *Filaments* solitary or tufted, often coalescent; in young stages erect, later on erect to twisted, unbranched and uniseriate, very rarely one cell in the filament becoming biseriate by longitudinal cell division. In young stages the firm cell wall thin and smooth, soon becoming thickened and mostly rough by micro-organisms and/or micro-particles anchored in the continuous adhesive mucilaginous outer layer. *Cells* pronouncedly cylindrical, their ends somewhat rounded; usually closely adherent to one another except in very aged stages, sometimes arranged in pairs. *Cell wall* often varying in thickness in one cell. Cell diameter (4.8—) 14.4—32.6(—44.2) μm, height (3.6—)4.8—9.6(—15.7) μm; cell width usually gradually diminishing and cell height increasing towards the base of the filament. Young filaments 9.6—14.4 μm wide, rarely with zoosporogenesis or gametogenesis.

Chloroplast strictly parietal, varying in shape according to age; usually girdle-shaped, unlobed to slightly lobed along its margin, sometimes, especially in young stages, somewhat incised, usually equalling about 1/2—3/4 of the cell circumference; in young cells open, in mature cells infrequently partially closed, caused by the

presence of one or several chloroplast bridges between the lateral chloroplast lobes. In young filaments the chloroplast not always approaching cell length, in mature cells the transverse walls frequently covered. Chloroplast sometimes reticulate due to the presence of a large number of vacuoles; in very aged cells sometimes withdrawn; upon disintegration distorted by storage products, which are abundantly present in the chloroplast as well as in the cell lumen and occasionally showing a *Microspora*-like habit. Cells *uninucleate*. *Pyrenoids* 1—3(—8), usually globose, in short cells ellipsoid. The average size of the individual pyrenoid decreasing with increasing number of pyrenoids per cell, their contours usually clearly distinguishable, except in disintegrating chloroplasts.

Basal cell slightly elongated or, particularly in mature plants, differentiated as a simple or complex rhizoidal branching system. The apical part of the rhizoids sometimes developing into a simple clinging-foot. Attachment to the substratum by means of a thin gelatinous layer; chloroplast of basal cell with the embedded pyrenoids irregularly distributed over its whole cell lumen, in very aged cells disappearing. Secondary attachment to the substratum by newly formed projections protruding from vegetative intercalary cells. Length of the basal cell up to 100 μm. Apical cell rounded, sometimes developing like the basal cell. Occasionally one to several filament-cells not properly developing; these smaller, deeper green, and with the chloroplast apparently uniformly distributed.

Zoosporogenesis and *gametogenesis* occurring in ordinary cells, including the apical cell, not in the basal cell and intercalary cells from which secondary rhizoids develop; both processes may occur in one filament. Zoosporogenesis often starting in the apical part of the filament. *Zoosporangia* usually yellowish-green, mostly slightly inflated. Cell dimensions: (9.6—)14.4—26.6(—38) μm wide, 4.8—14.7(—24.2) μm long. Cell length/width ratio of wider zoosporangia notably higher than of vegetative cells with comparable cell diameter. *Zoospores* liberated one by one or together enclosed in a hyaline mucilaginous envelope through an irregular-shaped lateral opening in the cell wall; sometimes one to several zoospores failing to leave the sporangium. Number of zoospores (4—)8—32, size 6.6—10.6 μm by 2.1—6.4 μm. The fourflagellate zoospores ovoid-spindle-shaped and sometimes apically pointed. Chloroplast regularly cup-shaped with one pyrenoid and a ± median stigma. Zoospores positively phototactic with few exceptions, relatively fast swimming; after release, the free swimming zoospores often soon becoming spherical in.shape. Attachment to hard substratum with the flagellar pole foremost and concurrent loss of the flagella. Germination of the attached zoospore various, either bipolar, by cell division, and the lower hyaline part developing into the basal cell and the upper part into a uniseriate row of vegetative cells, or during germination often after one cell division, the daughter cells independently developing uniseriate filaments, usually coalescent for some time (twin-filaments), or frequently the attached zoospore developing into an unbranched prostrate filament without obvious basal-apical differentiation, or into a small creeping pseudoparenchymatic base. Later on, following cell division perpendicular to the original length axis, the individual cells independently giving rise to erect uniseriate daughter filaments of indeterminate length, single or up to six filaments coalescing. The growth of the daughter filaments starting in the central cells. *Aplanospores* occurring, sometimes abundantly, together with zoospores, numbering 1—32 per sporangium. Germination in the parent cell or after release with the decay of the filament; its subsequent growth to filaments identical with that

of zoospores. Filaments heavily loaded with germinating aplanosporangia showing a woolly habit.

Ripe gametangia light olive-brown, sometimes slightly inflated. Filaments with gametangia straight or slightly to strongly curled. Cells (9.6—)14.7—25.4(—40) μm wide and 7.2—13.2(—15) μm long. *Gametes* released all together, (4—)8—64(—128), enclosed in a hyaline mucilaginous vesicle, through an irregular shaped lateral opening in the cell wall, 4.2—6.4 (—8.5) μm by 2.1—4.2 μm, biflagellate, ovoid to spindle-shaped, sometimes posteriorly acutely pointed, usually becoming spherical soon after release or occasionally, upon protruding the vesicle, rounded off. No pointed anterior papilla present. Chloroplast of the gametes less developed, regularly cup-shaped with one pyrenoid and one median-posterior stigma; positively phototactic with a few exceptions. After skittish swimming movements there is loose attachment of the non-fused gametes, mostly dying or sometimes parthogenetically developing into one-celled sporophytes. Gametic fusion isogamous, monoecious. Quadriflagellate zygotes negatively phototactic, soon becoming rounded off followed by attachment, the loss of the flagella, and subsequent development into one-celled sporophytes; this occasionally by simple enlargement but mostly by forming a protuberance containing the shifted cell contents. This growing process gives rise to sporophyte stages, each either sessile or with shortly stalked hyaline basal part with an attaching disc, formed by the original contours of the zygote-envelope and a linear, or clavate, or irregular shaped apical part, containing greenish contents. Non-stalked sporophytes subglobose, suboval, ellipsoid to irregular in shape. The continuous parietal chloroplast irregularly lobed, with 1—3 pyrenoids. Secondary stalk protuberance rarely formed in immature sporophytes. Upon maturation the brownish yellowish contents dividing into 4—16 zoospores or aplanospores.

Fertile sporophytes subglobose, either with a cell diameter of up to 23 μm or more commonly elongated, up to 41 μm in length and 16 μm in width. Zoospores formed in the sporophyte identical in behaviour, as well as in cell dimensions, and mode of attachment, and germination to those from filaments. Initiation of growth of aplanospores mostly inside the sporophyte cell wall. Occasionally *vegetative reproduction* by fragmentation of the filament.

NORWAY. Pasvik, epiphytic on *Fucus vesiculosus*, *Foslie*, 8/1889, *ex Hauck & Richter-Phykotheka universalis 381b* (C, BM, HBG, KIEL, L sheet 940.130–63, PC); Svaerholt, as *Bangia virescens*, *Foslie*, 13/7/1887 (C); Trondheim, littoral near Biological Station, as *Ulothrix consociata*, *Wille*, 7/7/1906 (O); Trondheim, littoral, as *Ulothrix consociata*, *Wille*, *ex Kryptogamae Exsiccatae 2144* (C, BM, BR, FI, L sheet 920.13–379, LD, S, UPSV); Molde, littoral, as *Ulothrix pseudoflacca* f. *minor*, *Wille*, 28/7/1918 (O); Ålesund, littoral, mixed with *Urospora mirabilis*, as *Ulothrix pseudoflacca*, *Wille*, 14/8/1902 (O); mixed with *Enteromorpha spec.*, as *Ulothrix pseudoflacca* f. *pachyderma*, *Wille*, 19/5/1902 (O); Steinviksholm, on a wooden pile of a bridge, as *Ulothrix consociata*, *Wille*, 10/7/1906 (O); on boulders, as *Ulothrix consociata*, *Wille*, 20/7/1906 (O); Espegrend, Kviturdvikpollen, on *Fucus serratus*, intertidal pool, mixed with *Enteromorpha* and *Monostroma* species, *Lokhorst*, 24/4/1975 (L); Dröbak, as *Ulothrix pseudoflacca*, *Wille*, 30/3/1912 (S); as *Ulothrix pseudoflacca*, *Printz*, 4/1912 (L sheet 960.81–103); as *Ulothrix pseudoflacca*, *Hylmö*, 14/4/1912 (LD, O): on cliffs in the upper part of the foreshore near Biological Sation, as *Ulothrix consociata*, *Wille*, 15/5/1920 (O); as *Ulothrix pseudoflacca*, *Sjöstedt*, 6/4/1917 (LD); in the harbour on *Fucus vesiculosus* and in high littoral level of the isles Storskjaer and Smaskjaer, *Lokhorst*, 21/4/1975 (L).

SWEDEN. Fiskebäckskil, on *Fucus vesiculosus*, *Kylin*, 8/7/1948 (LD); Kristineberg, as *Ulothrix pseudoflacca*, *Kylin*, 5/4/1905 (LD); the harbour, *Kylin*, 28/6/1948; Kristineberg, Blabarsholmen, *Kjellman*, 9/4/1890 (UPSV); *Kylin*, 9/4/1906 (UPSV); as *Ulothrix pseudoflacca*, *Kylin*, 9/4/1906 (LD, UPSV); Bondhalet, *Kylin*, 9/4/1906 (LD); Mansholmen, *Kylin*, 29/4/1906 (LD); Göteborg, on *Fucus vesiculosus*, as *Hormiscia flacca* and *Urospora penicilliformis*, *Åkermark 342*, June (BM, L sheet 938. 86–482, LD);

Varberg, on *Fucus*, as *Ulothrix pseudoflacca*, *Hylmö*, 7/5/1922 (C, LD); *Hylmö*, 20/2/1928 (S); Hallands Väderö, Kapellhamn, as *Ulothrix pseudoflacca*, *Sjöstedt*, 6/1919 (LD); Arild, as *Ulothrix pseudoflacca*, *Levring*, 17/3/1934 (GB, LD, S); Mölle, as *Ulothrix pseudoflacca*, *Levring*, 17/3/1934 (GB); Barseback, as *Ulothrix pseudoflacca*, *Sjöstedt*, 5/7/1915 (LD); *Hylmö*, 5/3/1916 (LD); *Hylmö*, 23/3/1921 (LD); Malmö, Limhamn, as *Ulothrix pseudoflacca*, *Hylmö*, 23/3/1910 (LD, S); *Sjöstedt*, 26/3/1910 (UPSV); as *Ulothrix pseudoflacca*, *Sjöstedt*, 3/1920, 21/2/1928 (LD); Malmö, Ribersborg, as *Ulothrix pseudoflacca*, *Sjöstedt*, 20/3/1916 (L, LD, O); Ystad, as *Ulothrix pseudoflacca*, *Sjöstedt*, 20/8/1917 (LD); Käseberga, as *Ulothrix pseudoflacca*, *Sjöstedt*, 11/6/1927 (LD); Skillinge, *Sjöstedt*, 12/6/1927 (LD); Wartsholmen, as *Urospora penicilliformis*, *Akermark* (L sheet 939.26–72); Agnö, on *Patella vulgata*, as *Ulothrix consociata*, *Levring*, 1/8/1935 (GB).

GERMANY. Norderney, on *Fucus*, as *Conferva flacca* and as *Hormotrichum affine*, *Jürgens 4*, *Algae aquaticae XVIII* (L sheet 910. 200–462, 939.26–64); Heligoland, as *Schizogonium laete virens*, *ex herb. Kützing* (L sheet 938.86–342); as *Hormotrichum affine β gracilius*, *ex herb. Kützing* (L sheet 939.67–728); as *Ulothrix maritima* (*nom. inval.*), *Sonder*, 8/1873 (L sheet 939.26–290); as *Schizogonium laetevirens*, *ex herb. Hauck* (L sheet 939.26–21); Kriegshafen, as *Ulothrix pseudoflacca*, *Hylmö*, 25/7/1922 (LD); near the Biological Station, on *Polysiphonia urceolata*, *Schiffner*, *Algae marinae no. 450*, 4/1928 (BM); on rocks, as *Ulothrix carmichaelii*, *Sahling*, 16/3/1964 (HELGOLAND); on cliffs, stones and on *Fucus*, *Sahling*, 9/4/1970 (HELGOLAND); Nathurn, on *Fucus serratus*, *ex herb. Kornmann* (HELGOLAND), Wilhelmshafen, *Hylmö*, 16/6/1922 (LD, O); Flensburg, as *Hormotrichum vermiculare*, *Weidemann*, 5/4/1879, 17/4/1879 (L sheet 939.26-79, 939.26–89); on the beach on stones, *Weidemann 115*, 5/4/1879 (L sheet 939.26–79); on wall in the harbour, as *Bangia*, *Weidemann 117* (L sheet 939.26–80); Kiel, on *Fucus vesiculosus*, *Reinbold*, 5/1888, *ex Hauck & Richter-Phykotheka universalis 381a* (BM, C, HBG, KIEL, L sheet 939.26–29, 920.13–133, PC).

THE NETHERLANDS. Delfzijl, on *Fucus*, *Weber 282*, 20/6/1885 (L sheet 942.253–36); on dike-slope, strongly exposed, *Kranenburg*, 9/3/1976 (L); Oostmahorn, on a stone, *Den Hartog 1114*, 31/5/1952 (L sheet 956.313–509); Ameland, pier near Ballum, *Den Hartog 1399*, 10/4/1953 (L sheet 956.313–508); Harlingen, near the harbour on *Fucus vesiculosus*, *Kranenburg*, 8/3/1976 (L); Breezanddijk, on dike-slope in the high-littoral zone near Noorder haven, *Kranenburg*, 17/11/1976 (L); Vlieland, *Weber-Van Bosse 1212*, 17/5/1891 (L sheet 942.253–92); Wieringen, Normerven, on stones in high-littoral zone, *Kranenburg*, 1/3/1976 (L); Den Helder, Harsens, on a stone, *Van Goor*, 2/5/1917 (L); Buitenhaven, Nieuwediep, on *Fucus vesiculosus*, mixed with *Ectocarpus siliculosus*, *Van Goor*, 2/5/1917 (L); sea bank, *Den Hartog*, 22/4/1955 (L sheet 955.151–102); Camperduin, on basalt block, mixed with *Urospora penicilliformis*, *Koster 6137*, 10/5/1957 (L sheet 957.142–233); Ymuiden, Noordzeekanaal, epiphytic on *Fucus spiralis*, strongly intermingled with *Urospora penicilliformis*, *Kranenburg*, 9/6/1977 (L); Noordwijkerhout, on a wooden pile, mixed with *Bangia atropurpurea*, *Blidingia minima* and *Urospora mirabilis*, *Koster 6985*, 19/2/1961 (L sheet 961.26–102); Katwijk, on banks, as *Schizogonium laetevirens* f. *majus*, *Suringar 101*, 6/1854 (L sheet 928.174–351); on stones, as *Schizogonium laetevirens*, *ex herb. De Vriese* (L sheet 938.174–349); mouth of River Rhine, on stones in high-littoral zone, mixed with *Enteromorpha* and *Urospora penicilliformis*, *Kranenburg*, 9/6/1977 (L); Scheveningen, on *Fucus* near northern pier, *Koster 99*, 7/6/1936 (L sheet 939.294–123); near the harbour on *Fucus vesiculosus*, *Kranenburg*, 30/3/1976 (L); Hoek van Holland, on stones, *Van den Hoek 385*, 15/3/1953 (L sheet 956.314–061); pier, *Van den Hoek 1138*, 23/1/1954 (L sheet 959.212–672); pier, as *Ulothrix pseudoflacca*, *Van den Hoek 2027*, 10/4/1954 (L sheet 959.212–791); on basalt blocks, as *Ulothrix pseudoflacca*, *Koster 4278*, 10/4/1954 (L sheet 954.125–199); on basalt block mixed with *Urospora penicilliformis* and *Petalonia fascia*, as *Ulothrix pseudoflacca*, *Koster 4293*, 10/4/1954 (L sheet 954.125–188); Rozenburg, Nieuwe Waterweg, on *Fucus*, mixed with *Blidingia minima* and *Urospora penicilliformis*, *Mulder*, 28/5/1956 (L sheet 958.045–714); De Grevelingen, near Bruinisse, on concrete blocks, forming a green belt in the high-littoral zone, *Lokhorst*, 13/3/1975 (L); on basalt blocks in high-littoral zone, *Lokhorst*, 13/3/1975 (L); Schouwen, Rengerskerke, on basalt blocks, mixed with *Enteromorpha compressa*, *Koster 4354*, 23/4/1954 (L sheet 954.125–112); on wood, mixed with *Urospora penicilliformis*, *Koster 4375*, 23/4/1954 (L sheet 954.125–130); Tholen, *ex herb. Suringar 971* (L sheet 941.310–741); Strijenham, on *Fucus vesiculosus*, as *Ulothrix pseudoflacca*, *Koster 5860*, 15/5/1956 (L sheet 956.182–336); Mosselhoek, on basalt blocks in high-littoral zone, *Kranenburg*, 17/2/1976 (L); Zuid-Beveland, Yerseke, oyster-pond, *Den Hartog 2245*, 28/3/1955 (L sheet 956.313–734); Zuid-Beveland, Kanaal van Zuid-Beveland, Postbrug, in wash-zone with *Porphyra leucosticta*, as *Urospora penicilliformis*, *Van den Hoek 3146*, 14/5/1956 (L sheet 956.182–229); on wooden pile in wash-zone, as *Ulothrix pseudoflacca*, *Koster 5869*, 14/5/1956 (L sheet 956.182–332); Zuid-Beveland, Wemeldinge, on a stone, in *Pelvetia*-association. *Den Hartog 1554*, 6/6/1954 (L sheet 956.313–507); on dike-slope in the high-littoral zone, *Kranenburg*, 24/12/1976 (L); Zuid-Beveland, Kattendijke, on stones, mixed with *Enteromorpha*, *Brakman 220*, 14/3/1941 (L sheet 939.69–1159); on *Ascophyllum nodosum*, *Brakman 240*, 14/3/1941 (L sheet 939.69–1180); on *Ascophyllum nodosum*, *Brakman 242*, 14/3/1941 (L sheet 939.69–1179); on young *Fucus* plants in *Pelvetia canaliculata* zone,

Koster 5867, 14/5/1956 (L sheet 956.182–334); in *Ascophyllum nodosum* zone, *Koster 5873*, 15/5/1956 (L sheet 956.182–338); on young *Fucus vesiculosus* plants, *Koster 5898*, 15/5/1956 (L sheet 956.182–344); Zuid-Beveland, Sas van Goes, on Vilvordian limestone, mixed with *Ulothrix speciosa*, *Kranenburg*, 17/2/1976 (L); on basalt blocks, *Kranenburg*, 17/2/1976 (L); Zuid-Beveland, Wolphaartsdijk, on *Ascophyllum nodosum*, *Brakman 274*, 12/3/1941 (L sheet 939.69–1178); on *Ascophyllum nodosum* and *Fucus spiralis*, *Brakman 203*, 12/3/1941 (L sheet 939.69–1182); Walcheren, sea banks near Arnemuiden on *Ascophyllum nodosum*, *Brakman 201*, 8/3/1941 (L sheet 939.69–1181); Walcheren, Vlissingen, on sea dike on bitumen in high-littoral zone, *Kranenburg*, 17/2/1976 (L); Zuid-Beveland, Kaloot near Borssele, on stems of *Spartina townsendii*, *Beeftink*, 13/5/1952 (L sheet 953.44–316); Zuid-Beveland. Borssele, on *Salicornia herbacea*, as *Ulothrix pseudoflacca*, *Brakman*, 23/11/1940 (L sheet 939.294–45); on *Ascophyllum nodosum*, *Koster 5868*, 14/5/1956 (L sheet 956.182–333); Zuid-Beveland, pier Noordnol, near Borssele, on *Fucus spiralis*, *Van den Hoek 3140*, 14/5/1956 (L sheet 956.182–254); on stones, on *Fucus* and *Ascophyllum nodosum*, as *Ulothrix pseudoflacca*, *Koster 5874*, 14/5/1956 (L sheet 956.182–923); in *Fucus vesiculosus* zone, *Van den Hoek 3166*, 14/5/1956 (L sheet 956.182–238); Zuid-Beveland, Ellewoutsdijk, the harbour, on a stone, *Koster 5904*, 14/5/1956 (L sheet 956.182–328); Zuid-Beveland, Hoedekenskerke, on basalt blocks in the high-littoral zone, *Lokhorst*, 13/3/1975 (L); Zuid-Beveland, Biezelinge, on stones, *Walrecht*, 12/5/1952 (L sheet 952.210–347).

GREAT BRITAIN. Orkneys, Stronsay, on *Ceramium rubrum* in rock pools, *Sinclair 591*, 23/3/1940 (E); on rocks, mixed with *Ulothrix speciosa*, *Sinclair 590*, 23/3/1940 (E); Iona, Camas Cuil An T-Saimh, *Mull Survey*, 25/5/1967 (BM); Kilereggan, as *Lyngbya carmichaelii*, 17/10/1852 (E); Gourock, as *Lyngbya speciosa*, *Arnott* (TCD); Gourock, McInroy's Point, 24/4/1852 (E); as *Lyngbya carmichaelii*, 3/5/1852, 4/5/1852, 14/6/1852 (E); Millport, 6/5/1853 (E); Cumbrae, *Arnott*, 5/1853, *ex herb. Greville* (E); as *Urospora isogona*, *Landsborough*, 5/1853 (E); Peterhead, as *Conferva flacca*, *Arnott* (TCD); Aberdeen, on *Gigartina purpurescens*, as *Lyngbya carmichaelii*, 6/1843, *ex herb. Dickie* (BM); Kinghorn, 6/1855, *ex herb. Greville* (E); Granton, strongly intermingled with *Urospora*, *Traill*, 27/4/1881 (BM); Leith, Black rocks, 1855, *ex herb. Greville* (E); Joppa, *Traill*, 8/7/1883 (BM); on *Fucus vesiculosus*, as *Urospora penicilliformis*, 1/6/1884 (BM); Edinburgh, Caroline Park, on *Halidrys*, as *Urospora penicilliformis*, *Traill 9258*, 31/5/1884 (E); on *Fucus vesiculosus*, as *Urospora penicilliformis* and *Ulothrix isogona*, *Traill 9259*, 31/5/1884 (E); Firth of Forth, as *Lyngbya carmichaelii*, *Anon.* (E); 9/4/1881 (TCD); Dunbar, *Traill*, 22/5/1881 (BM); Berwick, as *Ulothrix flacca* var. *carmichaelii* and *Lyngbya carmichaelii*, *Batters*, 5/1882, 8/1883, 20/5/1895, 25/5/1895 (BM, E); Mablethrope, *Price*, *Honey*, and *Tittley*, BML 25 (BM); Scarborough, *Anon.* (BM); Filey, on *Ceramium rubrum*, as *Lyngbya flacca*, *Gatty*, 7/1852 (BM); Sutton-on-Sea, on *Fucus*, *Wallace*, 7/12/1969 (BM); River Medway, as *Ulothrix ? subflaccida*, *Tittley*, 10/3/1970 (BM); Teynham, Conyer Creek, on *Fucus*, *Tittley*, 11/3/1970 (BM); Hythe, *Tittley*, 14/7/1969 (BM); Hastings, on *Fucus spiralis*, *Tittley*, 26/8/1969 (BM); Eastbourne, *Batters*, 6/1889 (BM); Ventnor, *Holmes*, 3/1880 (BM); Isle of Wight, Steephill Bay, intermingled with *Ulothrix speciosa*, *Foslie*, 2/1/1886, *Wittrock & Nordstedt-Algae exsiccatae 1070* (BM, KIEL, L sheet 910.200–465, O); as *Lyngbya carmichaelii*, *ex herb. Lenormand* (L sheet 939.26–56); Yarmouth, *Arnott* (TCD); as *Lyngbya carmichaelii*, 4/5/1852 (BM); as *Urospora isogona*, *Batters*, 5/1888 (BM); Swanage, Dwilston Head, *Cotton*, 18/4/1916 (BM); Budleigh Salterton, as *Ulothrix implexa*, *Cutler*, 27/4/1850 (BM); as *Lyngbya carmichaelii*, *Cutler* (BM); Sidmouth, as *Lyngbya flacca*, *Holmes*, 18/8/1880 (UPSV); Weymouth, *Holmes* (BM); Plymouth, as *Lyngbya carmichaelii*, *Wyatt*, 18/5/1832 (BM, L sheet 939.26–95, LD, TCD); Falmouth, harbour, as *Lyngbya flacca*, 25/5/1897, *ex herb. George* (BM); Penzance, as *Lyngbya carmichaelii*, *Robinson*, 26/2/1842 (BM); Shores of Devon & Cornwall, on *Fucus*-species, as *Lyngbya carmichaelii*, *Cocks Algarum fasciculae 1855.60* (BM); as *Lyngbya carmichaelii*, *Griffiths*, 6/1852 (BM); as *Conferva littoralis*, *Turner 2*, *ex herb. Davies* (BM); as *Ulothrix implexa*, *Jellam*, 30/3/1880, *ex herb. Holmes* (BM); on *Fucus*, as *Lyngbya carmichaelii* and *Oscillatoria majuscula*, *Arnott* (TCD); as *Lyngbya carmichaelii*, *Griffiths*, 1862 (E); Jersey, as *Lyngbya speciosa*, *Anon.* (BM).

IRELAND. Roonak, on exposed boulders, as *Ulothrix consociata*, *Cotton*, 2/1911, *Clare Island Survey Marine Algae 197* (BM); Clare Island, as *Ulothrix pseudoflacca*, *Cotton*, 4/1911, *Clare Island Survey Marine Algae* (BM); Balbriggan, on *Polysiphonia spec.*, as *Lyngbya flacca* and *Hormotrichum flaccum*, *Harvey*, 5/1847 (BM, TCD).

FRANCE. Cap Gris Nez, on boulders near high tide level, *Lokhorst*, 20/3/1975 (L); Ambleteuse, on a stone, *Den Hartog 1916*, 2/8/1954 (L); Le Havre, on a wooden pile, 5/1881 (PC); Arromanches, as *Conferva flacca* and as *Schizogonium laetevirens*, *Lenormand 1836* (L sheet 939.26–62); as *Schizogonium laetevirens*, *Lenormand*, 1836 (L sheet 910.200–453); as *Schizogonium laetevirens*, *Lenormand* (L sheets 939.26–39, 939.26–41, 939.26–38, 910.26–38, 910.200–444, 910.200–454, 939.26–84); as *Schizogonium laetevirens* and *Ulothrix laetevirens*, *Lenormand* (PC); as *Schizogonium laetevirens*, *Bangia laetevirens*, *Lyngbya carmichaelii*, and *Urospora penicilliformis*, *Lenormand* (E); as *Conferva flacca*, *ex herb. Chauvin* (L sheet 939.26–60, PC, TCD); as *Bangia laetevirens*, *Lenormand*, 1841 (PC, S); as *Schizogonium laetevirens*, *ex Hohenackers Algae Marinae Siccatae 105* (BM, L sheets 910.200–452, 939.26–40); as

Schizogonium (*Bangia*) *laetevirens* β *majus*, *Lenormand 694* (L sheet 939.26–85); as *Bangia laetevirens* and *Urospora penicilliformis*, *Lenormand*, *ex Algues de France 493* (BM); as *Ulothrix youngana* and *Urospora mirabilis*, *ex herb. Lebel 694* (PC); as *Lyngbya speciosa* and *Hormotrichum speciosum*, *De Brébisson 1870* (PC); Côtes du Calvados, as *Bangia* ? *laetèvirens* and *Urospora penicilliformis*, *Lenormand* (BM); Ile de Tatihou, as *Ulothrix pseudoflacca*, *Hariot*, 16/7/1909 (PC); as *Ulothrix pseudoflacca*, *Hariot*, 14/4/1911 (PC, O); Saint Vaast-la-Hougue, as *Ulothrix consociata*, *Hariot*, 13/4/1911 (PC); Cherbourg, *Bornet*, 23/2/1853, *ex herb. Gomont* (PC); on *Zostera*, *Bornet*, 7/3/1853, *ex herb. Gomont* and *herb. Thuret* (PC); on *Fucus*, *Bornet 77*, 27/3/1853 (L sheet 939.26–96, PC); mixed with *Ulothrix speciosa*, as *Ulothrix speciosa*, 9/4/1853, *ex herb. Gomont* (PC); on *Fucus*, as *Conferva flacca* and *Hormotrichum flaccum*, *ex herb. Lenormand* (L sheet 939.26–66); as *Hormotrichum flaccum*, *ex herb. Lebel 208* and *herb. Roussel* (PC); on *Zostera*, *Cystoseira* and *Ceramium rubrum*, as *Hormotrichum flaccum*, *ex Desmazières*, *Plantes Cryptogames de France 144* (BM); on *Fucus serratus*, as *Hormotrichum carmichaelii*, *ex Desmazières*, *Plantes Cryptogames de France 145* (BM); as *Hormotrichum flaccum*, 29/3/1854, *ex herb. Le Jolis 408* (BM); as *Hormotrichum flaccum*, 4/1856, *ex herb. Le Jolis 579* (O): as *Hormotrichum carmichaelii*, *ex herb. Le Jolis 587* and *herb. Kützing* (L sheet 939.67–710); as *Hormotrichum carmichaelii*, *Hormotrichum fasciculare*, and *Urospora penicilliformis*, 29/3/1858, *ex herb. Le Jolis 1294* (E, KIEL, L sheet 939.67–722, PC); as *Lyngbya flacca*, *Hormotrichum flaccum*, and *Urospora penicilliformis*, 5/4/1860, *ex herb. Le Jolis 1623* (E, KIEL, L sheet 939.26–92); on *Fucus tuberculatus*, as *Hormotrichum flaccum*, *Hormotrichum carmichaelii*, *Lyngbya flacca*, and *Lyngbya carmichaelii*, 25/4/1861, *ex herb. Le Jolis 1736*, *Algues marines de Cherbourg 113* (BM, C, KIEL, L sheets 942.227–222, 939.67–724, 939.26–94, PC, S); 7/3/1863, *ex herb. Le Jolis 2029* (C, LD); as *Urospora penicilliformis*, 17/3/1863, *ex herb. Le Jolis 2028* (BM, C, L sheets 939.26–108, 939.26–91); as *Hormotrichum flaccum* and *Urospora penicilliformis*, 3/1863, *Le Jolis*, *Algues marines de Cherbourg 169* (BM, C, KIEL, L sheet 942.227–23, PC); on rocks near high tide level, *Le Jolis*, *ex herb. Weber-Van Bosse* (L sheet 939.26–91); on rocks near high water mark, *Le Jolis*, *Rabenhorst Algen Europa 2135* (BM, C, HBG, L sheets 939.26–28, 939.26–90, O, UPSV); as *Hormotrichum flaccum* and *Urospora penicilliformis*, ? *Lenormand* (TCD); Anse Sainte Anne, on *Ceramium rubrum*, as *Hormotrichum flaccum*, *Thuret 111*, 30/3/1853 (PC); Saint-Malo, mixed with *Ulothrix subflaccida*, as *Urospora mirabilis*, *Hamel*, 13/3/1922 (PC); as *Ulothrix consociata*, *Hamel*, 9/4/1922 (PC); Saint Servan, as *Urospora mirabilis*, *Hamel*, 3/1921 (PC); *Hamel*, 12/3/1922 (PC); as *Ulothrix pseudoflacca*, 9/4/1926 (PC); Dinan, on a stone, *Petelier*, 14/4/1957 (L sheet 957.142–115); Saint-Lunaire, as *Ulothrix pseudoflacca*, *Lami*, 1940 (PC); Roscoff, on stones in mid-high littoral zone, *Lokhorst*, 8/4/1975 (L); Ile de Batz, on boulders in low-littoral zone, *Lokhorst*, 10/4/1975 (L); Finistère, as *Hormotrichum flaccum*, *Crouan*, *Algues marines Finistère 347* (PC); Saint Nazaire, on rocks and several *Fucus*-species, as *Lyngbya carmichaelii* and *Ulothrix flacca*, 3/6/1847, *ex Algues de l'Ouest de la France 73* (PC).

Nomenclature and historical aspects

In 1805, Dillwyn described this alga as *Conferva flacca* from the neighbourhood of Swansea (Great Britain), as a delicate parasite on *Confervae*, on the smaller *Fuci*, and on the sides of boats and other wood exposed to sea water. The specimen marked as "*Conferva flacca*, L. W. Dillwyn", preserved in BM, is hereby designated as the lectotype. Its general macroscopic habit and that of the anchored host fits well with Dillwyn's drawings. Dillwyn (1805) reported that he was unable to discover reproductive stages. In the lectotype specimen zoosporangia could be detected, however.

During the nineteenth century the systematic position of this species frequently changed, due to the very wide and unstable concepts of the various genera. In 1863, Thuret placed it for the first time in *Ulothrix*. In 1889, De-Toni considered *Ulothrix flacca* to be a synonym under *Urospora penicilliformis*. Unfortunately, this opinion was held for a long time and a remarkable number of *Ulothrix flacca* specimens are still kept in many herbarium collections under the name *Urospora penicilliformis*.

Dillwyn's description of *Conferva flacca* (1805) is very meagre: only the unbranched character, the delicate thallus, the short "articulations", and the colour of the cross walls are emphasized. In 1845 Kützing recorded arbitrary cell dimensions for the first time, though in 1849 he did not recognize this alga as a rather

polymorphic species, which, as a consequence, may show different growth stages. Kützing raised these to specific rank and, besides describing this alga as *Hormotrichum flaccum*, he recorded a series of (new) species viz. *Hormotrichum affine*, *H. carmichaelii*, *H. didymum*, *H. didymum β gracile*, *H. fasciculare* and *H. vermiculare*. These species were individually defined, mainly by means of inadequate characters, such as for instance cell length, cell width, general habit of filaments, colour, and occurrence of secondary projections. Kützing's splitting of *Hormotrichum flaccum* did not find general acceptance, however.

Later phycologists of the 19th century (e.g. Le Jolis, 1863; Hauck, 1885) also failed to amend the poor specific concept of *Ulothrix flacca* or to gain a better insight into the species delimitation of the genus. For example, Dodel-Port (1883) amply studied the morphology and several stages of the reproduction of an alga reported as *Ulothrix flacca*. In fact, *Ulothrix implexa* is recorded in his clear drawings. Or also, despite his comprehensive studies, Wille (1901) did not elucidate the confused taxonomy of *Ulothrix*, partly because no attention was paid to dried type specimens. It was rather surprising that Wille's newly described *Ulothrix pseudoflacca*, which nowadays has become a current name, proved in the present study to be identical with Dillwyn's original material of *Ulothrix flacca*. The frequent occurrence of *Ulothrix flacca* with smaller cell dimensions, particularly when growing epiphytic or when present in water with lower salinity (Oslofjord), may have deceived Wille.

In the past, the frequent occurrence of coalescence of two or more filaments has not been recognized as a typical growth stage of *Ulothrix flacca*. For that reason it is not surprising to find specimens with this growth habit under the multi-seriate genus *Schizogonium*, as *Schizogonium laetevirens* (Kützing, 1843, 1845, 1849). Nevertheless Kützing (1845) did point out the resemblance between simple filaments of *Schizogonium laetevirens* and *Hormotrichum* (= *Ulothrix*) *flaccum*, without realizing the systematic consequences. The coalescence of filaments led Wille to distinguish his new species *Ulothrix consociata*. As has been mentioned before, Wille's views on the taxonomy of the genus *Ulothrix*, up to now generally accepted, cannot be shared any longer.

Morphology

The *general habit* of *Ulothrix flacca* is less characteristically developed in cultures, especially when growing in stagnant culture solution. About 14 days after dissemination of zoospores in cultures, simple germlings or mostly differentiated tufts, each arisen from one zoospore, appeared regularly distributed on the inner glass wall of the culture vessel. Further growth was often slow, but ultimately the plants did reach a length of up to *c.* 3 cm. In aerated cultures growth was faster and the prostrate-erect differentiation of the growth habit was more pronounced, which agrees with the alga's ability to colonize natural habitats exposed to wave action. Free-floating plants were only found in stagnant cultures. The stretching of the basal cells in these plants may have been delayed. Swellings in the cell wall, as found in the marine species *Ulothrix speciosa* and *U. palusalsa*, were not observed in this alga under normal conditions.

In nature the *cell wall* of aging plants is usually conspicuously rough because of adhering micro-particles; it may also be heavily studded with micro-organisms. In

these cases observation of the cell contents is strongly obscured. This sticking of micro-particles to the alga is caused by a thin, continuous, gelatinous layer on the cell wall, which is detectable with the aid of Indian Ink. Plants grown up in cultures are smoother, which can be attributed to fewer micro-organisms (only bacteria) and the absence of micro-particles in the culture solution. Cells in filaments are usually closely adpressed to one another, but especially in neglected cultures the mature vegetative cells were sometimes seen to be more loosely arranged, a phenomenon which is only rarely seen in natural material.

In various natural populations the *diameter* range of *cells* of filaments may vary greatly. For instance, *Ulothrix flacca* in the Oslofjord near Dröbak and in the Kviturdpollen near Espegrend (Norway) only had a cell diameter up to 25.8 μm. In plants from Cherbourg and Roscoff, especially when collected from the lower littoral, a cell diameter of up to 44 μm was found. In cultures, however, these striking differences were annulled and in general cell diameters were found chiefly up to 27 (—36.1) μm. At lower salinity ($2^0/_{00}Cl^-$) the cell diameter tended to be even smaller. Different day-length periods and temperatures did not affect the dimensions of the cells in a significant way. In material from culture and from natural environments, the length/width ratio of vegetative cells mostly becomes larger in very aged plants.

In general the *chloroplast* has a healthy appearance both in material from nature and from cultures. In low salinities the chloroplast is less dark green in colour and shows a tendency to reduce its extension around the cell contents. The reticulate growth stage of the chloroplast in mature cells is less commonly present in material from natural locations, in which the vegetative cells presumably do not have the opportunity to age, because of earlier reproduction. Under less favourable culture conditions the contours of the chloroplast often become obscured; at the same time it may line the transverse cell walls to the same degree as it does the lateral wall. These cells show a certain morphological resemblance to those of the freshwater genus *Microspora*.

As a rule there are 1—3 *pyrenoids* per cell present in material from nature; in material from cultures, however, this number may rise to eight. Their size is nearly constant within the range 1—3, but an increase in number negatively affects their volume.

The shape of the *basal cell* is more or less dependent on external circumstances. In nature the basal cells of simple filaments, twin-filaments, and of those protruding from a prostrate filament or base respectively, usually show a slight elongation and terminate slightly narrowed. The cell wall of the basal cell and sometimes that of various cells above it, is covered by a dense sediment, consisting of yellowish-brown micro-particles. The more complexly shaped basal cell seems to be confined to material from cultures. The apical cell is surrounded only by a thin cell wall.

Reproduction and life history

Despite its widespread occurrence along the coasts of western Europe, the reproduction of *Ulothrix flacca* has only been studied to a limited extent (e.g. Wille, 1901; as *Ulothrix pseudoflacca*). Zoospores varying from 2—8 in number, and gametes were reported. The present study confirms the presence of zoospores and gametes. However, there are (4—)8—32 zoospores per cell, and (4—)8—64(—128)

gametes per cell. Furthermore, its full life history has now been accomplished, including the ascertainment of sporophytes.

After transferring vegetative filaments, preserved at 8 °C, to both short- and long-day conditions, zoosporogenesis takes place spontaneously. No striking differences in frequency of zoosporogenesis could be noticed amongst the considerable number of clones studied. The zoospores are relatively small, which is probably why their swimming movements are faster in comparison with those of the remaining *Ulothrix* species studied. It is very likely that the small dimensions enable the zoospores to attach on hard substrata in exposed localities more easily. Upon attachment the zoospores shed off their flagella one by one. During this process the flagellum may become shortened and strongly curled. The different patterns of germination of the zoospores, as described before, are seemingly performed independently of the temperature, day-length and light intensity employed.

The formation of aplanospores is strongly induced by less favourable external conditions. Filaments, nearly completely laden with undischarged germinating aplanospores, gradually show a distinct woolly appearance, which is characteristic of this species. It is not possible to determine which mode of germination will be produced by the aplanospores not yet liberated from the parent cell.

Gametogenesis is induced after transferring filaments to long-day conditions. In comparison with zoospores the production of gametes in cultures occurs less frequently and apparently more laboriously. This corresponds well with the field observations. Sexual reproduction could even not be induced at all in some cultures, grown from material obtained from different localities in western Europe. In nature, filaments containing zoospores could be harvested during a rather long vegetation period, whilst the occurrence of gametes seems to be restricted to a shorter period. It is assumed that in nature several successive filamentous generations, which only produce zoospores, occur in one season.

The filaments of *Ulothrix flacca* which contain gametes are straight or curled, whereas in the remaining species studied the filaments in this reproductive stage are rather constant of shape. A relatively large number of gametes failed to mate in cultures, and eventually died; sometimes, however, a parthenogenetic outgrowth to one-celled sporophytes was formed. Zygotes obtained in cultures swim for only a few minutes, followed by settlement and subsequent development into relatively small sporophytes, which have a pronounced preference to ripen under short-day conditions. Occasionally, the cell contents of the sporophyte did not entirely divide, resulting in the only formation of four zoospores. This phenomenon could not be related to any external condition. Upon maturation in culture, the produced zoospores were often not discharged from the sporophyte, but passed into aplanospores; after the protrusion of the young germinating filaments the old husks remained visible as conspicuous remnants of the sporophyte cell wall. The ripening of the sporophyte cell contents coincided only accidentally with the gelatinisation of the hyaline stalk. Sometimes the gametes were not liberated from the gametangia. An initiation to a parthenogenetic development of gametes, staying together in close proximity, could then be observed. Young and mature sporophytic stages were once found in nature (Grevelingen Dam), but never in herbarium specimens.

Both in nature and in cultures vegetative propagation may take place by fragmentation; a process which increased in cultures as the culture medium became exhausted.

Ultrastructure

Cell division. The process of cytokinesis could only be studied in one example. Judging from Plate 5, 1, which depicts pyrenoids just separated and one pyrenoid ready for cleavage, one may reasonably assume that this process takes place by furrowing. Unfortunately, no indications on possible involvement of longitudinally or transversely oriented microtubules could be detected.

Cell wall. As mentioned earlier, the texture of the surface of the cell wall is affected by its environment. The outer double membrane could only be well demonstrated in filaments from cultures, when the plants are only sparsely overgrown with bacteria, while micro-particles are absent. The double membrane is usually covered by a mucilaginous amorphous layer, with a flocculent appearance. In contrast, in plants from natural habitats, a dense occupancy by micro-particles and micro-organisms embedded in this mucilaginous layer obscures the contours and the visibility of the outer membranes. The surrounding coat of attached or embedded material is not always homogeneously developed; at times it is strikingly thickened on one side or along the periphery of the filaments, or larger accumulations are only locally.

The present preliminary ultrastructural study has revealed a continuous outer microfibrillar layer, a thin middle microfibrillar layer, and an inner microfibrillar layer which may vary considerably in thickness. Microfibrils seem to be more densely arranged in the middle layer, but seem to be more or less parallel throughout the cell wall. Lomasomes were found frequently.

Chloroplast. In agreement with light microscopy observations the chloroplast may line the transverse wall, separated from it by only a thin layer of cytoplasm and the plasmalemma. The chloroplast is enclosed by a fairly smooth two membrane envelope, which has neither obvious pores nor association with any cell organel. The thylakoids appear singly or there are up to five, arranged in elongate lamellae. In lobes of complexly shaped chloroplasts the thylakoids terminate abruptly in close proximity to one another. Short lamellae are mostly absent. The two membranes of a thylakoid, either single or adhering together in lamellae, are not always parallel. Adjacent thylakoids appeared to be not very closely adpressed together, inasmuch as interthylakoidal gaps are in between them. In various algal groups, especially the *Cryptophyceae* and *Phaeophyceae* (Dodge, 1973), also distinct spaces are found between the thylakoids; it is suggested that this phenomenon is related to the fixation procedure used. In the present study, however, the use of different preparation techniques did not reveal fundamental differences in the pattern of arrangement of thylakoids and the gaps in between them. Frequently thylakoids pass from one lamellae into another, hence showing a reticulate appearance. The chloroplast has a finely granular stroma, containing fairly equally dispersed ribosomes. Osmiophilic plastoglobuli generally occupy randomly located areas in the chloroplast, but at times dense concentrations are found near the starch sheath of the pyrenoid. These globules vary in size and are approximately globose. Spherical or elliptical starch grains are frequently present in the chloroplast, lying between the thylakoid-lamellae and usually distorting them. The outer rim of the individual starch grains sometimes appears slightly denser than the inner area. However, most of the starch is found in segmental grains around the pyrenoid. They may either form a continuous envelope around the pyrenoid or, on the other hand, only sparsely clothe

it. This variation almost certainly depends on external conditions such as the time of exposure to darkness, intensity of light, nutrition in the culture solution, etc.

The pyrenoid may protrude into the cell lumen or may approach very close to the transverse cell wall; in both cases it is still surrounded by a (thin) layer of the chloroplast stroma. The matrix of the pyrenoid is not traversed by chloroplast invaginations; however, one or two chloroplast thylakoids may invade the starch shell through intergranular gaps and approach the surface of the pyrenoid. If so, they do not enter the pyrenoid, but continue between the adjoining starch grain and the pyrenoid. A direct contact exists between the matrix of the pyrenoid and the chloroplast thylakoids, and between the chloroplast stroma and the starch sheath, respectively. In pyrenoids capped by a reduced starch shell the interposing thylakoids are only present in areas where the starch grains are still retained.

Nucleus. In longitudinal sections the nucleus is variously shaped: squarish, elongate, or irregularly amoeboid-like. One (or sometimes both) lateral side(s) of the nucleus is usually adjacent to the chloroplast, and separated only by a thin layer of cytoplasm. The single nucleolus, various of shape and size, usually lies in the centre of the nucleus. As mostly in the nucleus, its matrix possesses two types of chromatin. The inner membrane of the nuclear envelope is smooth, the outer membrane seems to be continuous with the cytoplasm ER and is ribosome-studded. The outer membrane is undulating in some areas. The intervening cavity between the two membranes is variable in dimension. At irregular intervals the nuclear surface is perforated by fenestrations.

Mitochondria. The number of mitochondria is variable. They can be found adjacent to the plasmalemma and chloroplast membranes, usually only separated by a thin layer of cytoplasm. Circular and oval forms predominate. The individual cristae appear to be arranged parallel to one another in most sections. They are not very numerous and leave relatively large spaces between them. In contrast with the flattened cristae in mitochondria of zoospores of *Microthamnion* (Watson & Arnott, 1973), the shape of the cristae in cross section is strictly circular in the present species.

Golgi apparatus. The Golgi apparatus, situated opposite the nucleus and often near one of the transverse walls, consists of an interrupted ring of dictyosomes. Each dictyosome consists of a stack of 6—9 cisternae. The numerous produced vesicles cluster freely inside and outside the ring, respectively. Incipient vesicles are also found as buds on the lateral ends of the individual cisternae. The distinct undulation of the plasmalemma, where it is adjacent tot the apparatus, is an indication of the fusion of Golgi vesicles with the membrane.

Cytoplasm inclusions. Lipid droplets are normally present. They are delimited by a dense marginal layer and possess a finely granular matrix. Their shape is globose, or slightly elongate, or ovoid. They are often situated beneath the plasmalemma near the transverse walls, or at the end of the lateral lobes of the chloroplast. A second very peculiar sort of inclusion consists of relatively large, irregular shaped, osmiophilic bodies which are scattered in the cytoplasm. They are also seen in close association with a regular packing of vesicular subunits, which have a scum-like appearance. It seems likely that both sorts of inclusions pass into one another. This

phenomenon presumably indicates a lytic process. In very diverse plant species, examples of enclaves with disintegrating contents have been found (e.g., Sievers, 1966; Thornton, 1967). On the other hand, the possibility cannot be ruled out that this phenomenon, which is characteristic in this alga, merely is an artefact.

A single elongated peroxisome lies adjacent to the chloroplast envelope.

Vacuole. In fully developed cells usually one or several vacuoles are present, mutually dissimilar in shape and size, and limited by a single membrane, the tonoplast. Prominent small vacuolar bladders of low density, containing a flocculent matrix and resembling vacuoles are occasionally found just beneath the plasmalemma near the transverse walls. It is not clear at present, whether they do actually represent vacuoles or not, as it is just possible that only shallow invaginations of the cell wall have been seen.

Ecology

In winter, spring and sometimes in autumn, *Ulothrix flacca* is very common in the littoral zone of the coasts of western Europe. However, in the northernmost part of Norway, the optimal vegetation period of the filamentous stage apparently falls in spring and summer. On coasts in the middle and northern part of western Europe this species seems to thrive only in the middle-upper part of the littoral belt, whereas in France it also flourishes luxuriantly in the lower littoral fringe. High-littoral plants are generally more slender than those growing on a lower level in the littoral zone.

The wide ecological amplitude of *Ulothrix flacca* is expressed by its ability to invade the wash-zone of canals with saline water, as observed in the Kanaal van Zuid Beveland (Netherlands). Furthermore, it may penetrate brackish waters with low salinity, as seen in the Nieuwe Waterweg, the entrance to Rotterdam harbour. It is also able to tolerate considerable salinity fluctuations, as it was found near Zandvlietsluis in the sea arm Wester Schelde, in the estuary of the Schelde River, where a daily fluctuation in the chlorinity of about 0.9—6.8 $^0/_{00}Cl^-$ may occur; however, it was rare there and it had a very impoverished appearance.

Localities exposed to wave action proved to be a convenient habitat for this alga, but in sheltered habitats it may also occur abundantly. It is strictly absent along the beaches of western Europe on mud and sand, as well as in the undergrowth of the halophytes in salt marshes and on gently sloping creek banks. The physical structure of soil possibly prevents its settlement and growth there.

Ulothrix flacca has been found as an epilithic alga on granite boulders, basalt blocks, pebbles, concrete sewer pipes discharging into the sea, Vilvordian limestone, and on bitumen. The individual plants usually had a tufted appearance and were united in expanded continuous carpets. Considerable amounts of sand are often accumulated in such mats, and this apparently does not interfere with the alga's growth. It does not occur in caves, crevices, fissures, or in other dark places, in which other algal species find protection against insolation. Sometimes the present species has been found as an epiphloeodic alga. It may also thrive when growing on plants and animals, mainly Moluscs. It was detected on larger brown and red algae, such as *Fucus serratus*, *Fucus spiralis* and *Fucus vesiculosus*, less frequently on *Ascophyllum nodosum*, on *Halidrys siliquosa* and *Cystoseira species*, and a few times on *Ceramium*

rubrum and *Polysiphonia species*. In salt marshes it may be widely distributed on perennial stems of *Spartina townsendii* and *Salicornia europaea*. On animals it was recorded as a habitant of *Patella* species and barnacles.

The origin of the material in culture

Uni-algal clones were initiated from plants collected from the following localities. NORWAY: Oslofjord, Storskjaer, near high water mark; Espegrend, Kviturdpollen, on *Fucus spiralis*. THE NETHERLANDS: Groningen, Delfzijl, from a pier near the harbour; Friesland, Harlingen, near the harbour, epiphytic on *Fucus spiralis*; West Terschelling, on a pier and on a wooden pile; Normerven, on stones; Zuid Holland, Scheveningen, on stones near the harbour and epiphytic on *Fucus spiralis*; Zeeland, Schouwen Duiveland, De Grevelingen, near Bruinisse, on concrete paving stones; Tholen, near Mosselhoek, on the sea dike slope; Zuid Beveland, Sas van Goes, on the dike slope near the sluice gates; Walcheren, Vlissingen, on bitumen on the sea dike. FRANCE: Cap Gris Nez, on boulders, exposed to wave action; Roscoff, on a sewer pipe, near the high water mark; Ile de Batz, on boulders, in the low littoral zone, strongly exposed.

Notes on herbarium material

The results obtained by study of the herbarium material of this alga were very satisfactory; almost without exception the dried plants resumed their original natural habit after adding a detergent solution. The rough surface of the cell walls proved to be very well preserved, which consequently facilitated the identification. Occasionally, however, plants in herbarium collections showed a smooth cell envelope, for instance in those gathered from the low littoral belts in France. In addition, these specimens mostly had larger cell dimensions and for that very reason, particularly when reproductive stages were lacking, such plants could easily have been incorrectly identified as *Ulothrix speciosa*. Their identity became unequivocally clear, however, with the help of a diluted acid or a solution of JKJ in lactophenol, which detected the firm nature of the cell wall in these plants.

Young filaments of *Ulothrix flacca* and *Urospora penicilliformis* may strongly resemble each other in herbarium collections. It was found that the help of JKJ was indispensable, since the number of pyrenoids, in this case the most obvious differentiating character, was usually difficult to count in untreated dried cells.

3. Ulothrix palusalsa Lokhorst, *nom. nov.* — Figs. 11—14; Plates 8—11.

U. pseudoflacca auct. non Wille: Carter (1933) 131. — *U. pseudoflacca* Wille var. *salina* Chapman (1946) 292. — Lectotype: Great Britain, Cheshire, Malpas Brine Pits, (CGE).

Marine gametophytic plants up to 6 cm long, green to bright green. *Thalli* flaccid and glossy. *Filaments* solitary or gregarious, often tuft-like, at times forming rope-like bundles, straight when young, later on more bent or irregularly whirled, always unbranched, usually uni-seriate, rarely biseriate and then still sheathed by a common cell wall. Both in young and advanced growth stages *cell walls* flaccid; in young filaments cell walls remarkably thin and smooth-surfaced, later on thickened, but still almost smooth, rarely contaminated with fouling organisms and/or micro-particles, or ornamented with warts. Occasionally, in vegetative and reproductive filaments, the cell wall slightly or strongly spontaneously swollen at one site or at several places

at irregular intervals, either on one or on all sides of the cells. Sometimes, however, the cell envelope almost entirely swollen resulting in a sheath-like habit, and then often simultaneously showing a zig-zag appearance in the uni-seriate cell row. *Cells* mostly cylindrical, their ends closely adpressed to one another in the young stage; sometimes in mature threads the ends more rounded and the cells only meeting at their poles. Upon gelatinisation, cells occasionally inflated and slightly separated (*Geminella*-like stage). At times, cells in the filaments tending to be arranged in pairs or in fours. Hyaline cylindrical remnants of mother cell walls sometimes wrapping daughter cells. Cell diameter (8.4—)12.1—25.8(—28.9) μm, cell height (3.6—)4.8—16.9(—20.4) μm; young filaments 8.4—12.1 μm wide; the upper and lower cells often gradually slightly narrowing and usually higher.

Chloroplast parietal, variously shaped, among others depending on their age; in young cells always regularly girdle-shaped, usually extending to 1/2–3/4 of the cell lumen, sometimes lobed along its longitudinal margin, sometimes slightly incised or constricted. Sometimes the chloroplast not reaching over the whole length of the cell; sometimes young chloroplasts variable in height, at maturity often with an extension towards the cross wall. As an exception, the chloroplast clothing the whole inner cell wall, causing in these cells the *Microspora*-chloroplast. In very old cells the chloroplast often withdrawn; upon disintegration of the cells the chloroplast obscured by accumulation of storage products. Reticulate chloroplasts present only in strongly vacuolated cells. *Nucleus* often opposite the pyrenoid, containing one nucleolus; nucleus conspicuous in cells with less developed chloroplasts. *Pyrenoids* 1—2(—5), almost globose, in strongly flattened or lengthened cells more or less ellipsoid, smaller in cells with more pyrenoids. Pyrenoid with distinct contour, normally ensheathed by a thin layer of starch, except in cells with many assimilates.

In germlings, the basal cell hardly differentiated or only somewhat elongated in the early stages, later on mostly developing into simple or branched rhizoids; these variable in width, attenuate or bulbous, or swollen at the end, the surface with a mucilaginous layer and often developing irregular-shaped bulges, particularly at the rhizoidal tip. In germlings, besides transversal cell division, there frequently is also cytokinesis in longitudinal direction, ultimately leading to the formation of several filaments radiating in all directions, rendering the basal portion of such plants more complex. Frequently, there is reinforced attachment to the substratum by newly developed secondary projections, sometimes springing from proximate vegetative cells in the lower portion of the filament. The cell walls of these projections often uneven and wavy. In rhizoids the chloroplast is parietal, containing one to several pyrenoids; sometimes there are massive chloroplast clumps discernable. Young and mature *apical cells* rounded, sometimes simply capped or entirely enclosed in a prominent gelatinous appendage. Occasionally, one to several cells failing to grow properly, remaining smaller, and showing etiolated massive chloroplasts, considerable amounts of starch, and appreciably thickened cell walls.

Asexual reproduction by zoospores and aplanospores, which arise in ordinary cells, including the apical cell, but not in basal cells and in cells producing secondary projections. *Zoosporogenesis* normally commencing in the uppermost cell and proceeding downwards, but also seen starting from more centrally located cells of the filaments. *Zoosporangia* in early stages of development greenish, subsequently sometimes becoming yellowish-green, and mostly slightly inflated. Asexual filaments often more or less curved. Zoosporangia 12.1—24.2 μm wide, 12.1—23.4 μm long. The cell length/width ratio of wide zoosporangia markedly greater than of

vegetative cells of comparable diameter. Zoospores issued together in a common hyaline mucilaginous enveloping vesicle, through an irregular-shaped lateral pore. *Zoospores* 4—16 per cell, 9.6—13.7 μm by 4.8—8.4 μm, four-flagellate, usually spindle-shaped to broadly pyriform, mostly with rounded posterior ends, later on becoming spherical; chloroplast parietal, cup-shaped with a single, large, conspicuous posterior pyrenoid and a large median to slightly posterior stigma; positively phototactic, with few exceptions, movement occasionally sluggish, or rotating, particularly just prior to attachment. Upon attachment follows a one by one abscission of the flagella. *Germination of the zoospores* bipolar, the lower part forming a short basal cell, later on usually differentiating into a more pronounced rhizoidal basal system; the upper part simultaneously producing a uniseriate row of cells, finally resulting in a filament of several cms long. In horizontally stretched 3—10 celled bipolar germlings, one to several proximate centrally-located cells often show cell division perpendicular to the length axis, which ultimately gives rise to more or less stellate, tufted germlings with up to nine radiating, never coalescing, daughter branches. These finally developing into an erect unbranched filament of several cms long, usually basi-apically differentiated. *Aplanospores* occurring simultaneously with zoospores, especially with increasing age; these at times abundantly present, 1—16 per sporangium, either germinating in the parent cell or after discharge from it by decay of the filament. Germlings arisen from aplanospores similar to those from zoospores. Sometimes, in sheath-like filaments, a pore only in the inner layer of the cell wall is formed, resulting in an accumulation of zoospores in an apparent lumen in the cell wall. At times, gelatinisation and subsequent disappearance of the partition cell wall of proximate cells occurs, ultimately giving to a random distribution of zoospores (or aplanospores), wrapped by the outer delicate sheathlike cell wall. Gametes produced in intercalary cells as well as in the apical cells; not in the basal cells and intercalary cells forming secondary rhizoids. Commonly the uppermost cells in the filament sporulating first, or rarely restricted to some intercalary cells, located more or less centrally in the filament, and without appreciable modification of their size and shape. Filaments containing gametangia usually distinctly curled. *Mature gametangia* olivaceous and generally slightly inflated; 12.1—22(—27.2) μm wide, (4—)7.3—13.6(—19.3) μm long. In general, the average length/width ratio of the more widened gametangia relatively large. Gametes discharged all together in a delicate gelatinous vesicle through an irregular aperture in the lateral cell wall. Upon liberation of the vesicle from the gametangium, the still enveloped gametes are mobile already; after dissipation of the vesicle the gametes become free. As stated for zoospores, in sheathed plants only a rupture in the inner cell wall layer may incidentally be formed resulting to a random distribution of free-moving gametes in the inflated hollow cell lumen. *Gametes* (8—)16—32 per cell; (4.8—)5.8—9.6(—10.6) μm by 2.9—4.8 μm, equally biflagellate, spindle-shaped to ovoid, rarely with a slender spine-like posterior projection, no anterior papilla present; at times the gametes already spherical upon discharge. Chloroplast parietal, situated in the posterior part of the gamete, (irregularly) cup-shaped, sometimes their margins with delicate incisions. The single pyrenoid central posteriorly situated, embedded in the chloroplast; sometimes not distinct. Stigma median or posterior in the cell body. Gametes positive phototactic, with a few exceptions. Gamete movement skittish. On quiescence usually becoming spherical and subsequently attached, simultaneously shedding the flagella. Subsided gametes occasionally remaining free-floating for a while, without the loss of flagella and without modification of their original shape. All

types of gametes, including those arrested within the parent cell, usually parthenogenetically developing into one-celled sporophytes. Gametic fusion predominantly isogamous, monoecious. Quadriflagellate zygotes showing a distinct negative phototactic reaction; upon settlement becoming almost spherical, the flagella being shed. Germination simple, by enlargement; sporophytes sessile or very rarely short-stalked. Usually non-ripened sporophytes with a massive chloroplast closely adpressed to the inner cell wall, irregularly lobed along its margin, containing 1—3(—7) pyrenoids and occasionally filled with storage products. On maturity, the sporophyte contents showing complete cleavage to 32 or more zoospores or aplanospores. *Fertile sporophyte* globose, up to 76.6 μm in diameter. *Zoospores* discharged in a vesicle, after disintegration of the sporophyte cell wall; these showing identical phototactic behaviour, and the same pattern of settlement and subsequent germination as in those originating from filaments. Dimensions of sporophyte zoospores slightly deviating, 7.7—13.7 μm in length, 4.8—6.7 μm in width. *Chlorella*-like aplanospores may be produced because of arrested zoosporogenesis, the aplanospores mostly cohering in the first developmental stages.

Filaments able to reproduce by fragmentation. This *vegetative multiplication* of the filaments often occurs by separation of the cells enclosed within a common mucilaginous filamentous envelope. Occasionally this leading to amorphous *palmelloid*-stages with a gelatinous matrix of undetermined extent, containing irregularly distributed (sub-)globose vegetative cells. The separate cells have a massive chloroplast, partly clothing the cell wall and containing one embedded pyrenoid.

Senescent cells, either isolated or united, may form characteristic thick-walled *akinetes*; these are smooth-walled and laden with heavy accumulations of food reserve.

THE NETHERLANDS. Terschelling, Boschplaat, in a halophytic vegetation, in winter time periodically flooded, *Lokhorst*, 28/1/1976 (L).

GREAT BRITAIN. Cheshire, Malpas Brine Pits, as *Ulothrix pseudoflacca* var. *salina*, 30/1/1944 (CGE).

FRANCE. Terenez, in and near halophytic vegetation and near the mouth of freshwater streams in intertidal salt marsh, *Lokhorst*, 27/4/1975 (L).

Nomenclature and historical aspects

In the literature the present species, only occurring on soft substrates, was hardly recognized as an independent taxon. Nevertheless, a review of algal floristic studies, particularly of those carried out in salt marshes, revealed adequate illustration of this species in Carter's (1933) comparitive studies on the algal flora of Canvey and Ynyslas (Great Britain). The figures depicted are undertitled with the name *Ulothrix pseudoflacca*, however, a species presently known to be synonymous with *Ulothrix flacca* which is characteristic for hard substrates. They show filaments with smooth-walled cells, local swellings, and *Geminella*-like stages. In the accompanying description, Carter reports that the cell wall shows essentially the same features as the alga nowadays known as *Ulothrix speciosa*. This fits well with our observations; in both species the cell wall is smoothly surfaced, flaccid, and occasionally sheath-like.

It was not until 1946 that Chapman described this alga as the new variety *salina* under *U. pseudoflacca*. It was found at the edge of an inland saline pit on damp mud, south of Malpas, England. Since this alga seems to be restricted to soft substrates in salt marshes the new, more applicable, epithet *palusalsa* is here proposed when raising

the taxon to the specific rank (*palus salsa* = salt marsh). I could hardly trace the species in the available herbarium collections. The reasons for this apparently are its rather narrow ecological range and its sparse occurrence. In addition, the relatively late start of intensive exploration of algae in salt marshes may also account for the late discovery of this species.

Morphology

The *general habit* of the individual filaments in the field is also observed in cultures. In cultures after dispersal of zoospores, whether discharged from filaments or from sporophytes, single filaments or stellate bunches of filaments, each arisen from one zoospore, colonized the bottom and the upright side of the culture vessel in course of time. In liquid media this species is found to grow rapidly into algal masses of several cms long, particularly when the culture solution is replenished. On the other hand, on firm or soft agar plates the development of the filaments was rather poor and the appearance of uni-algal coats was comparable to their poor appearance in nature. It is inexplicable why the alga grows so luxuriantly in liquid media, while it is absent as a continuously immersed alga in pools and basins in salt marshes.

Occasionally, in cultures, floating plants evidently have longer rhizoids as compared to those filaments firmly attached to the smooth wall of the culture vessel. The explanation of this more pronounced development at the base of the plants as being an effort to reach vast substrate is strengthened by the observation that floating plants develop secondary rhizoids more readily.

In both young and mature filaments, the *cell wall* may swell locally, or, even profusely so, along its whole length. Attempts to determine the environmental factors which promote these swellings were unsuccessful. The cell wall inflations can be induced artificially by adding a solution of JKJ in lactophenol, or by adding a diluted acid solution to the filaments. Only on one occasion the filaments had a partial "naked" appearance by local absence of the outer layer of the cell wall. The cell wall is not coated with a thin gelatinous layer as in *Ulothrix flacca*; it has a smooth, continuous surface. This apparently explains the sparseness of fouling organisms and/or micro-particles on filaments in field collections. Staining reagents detected a considerable quantity of pectin and cellulose in the firm, thin, inner cell wall layer, while the outer layers, which are able to swell, proved mainly to consist of a pectin-like substance.

In nature, vegetative cells in the filaments of *Ulothrix palusalsa* usually attain a *cell diameter* ranging up to 25.8(—28.9) μm. Occasionally, however, a cell diameter variation of only up to 21 μm could be measured in plants gathered from a single locality in nature. In cultures, comparable cell diameter ranges were apparent. The different culture conditions employed, such as the use of solid and liquid culture media, various photo periods and temperatures, did not affect the cell diameter variation significantly. Studies performed with salinity series revealed only a slight increase (up to 2 μm) of the average cell width in plants grown at higher salinity (8—16 $^0/_{00}Cl^-$).

Under less favourable culture conditions, and usually after a prolonged period of desiccation in nature, the cells become etiolated. In such plants the cell division rate becomes almost negligible, resulting in an higher cell length/width ratio. At the

same time the chloroplast changes of colour to yellowish-green and subsequently becomes loaded with an overwhelming number of starch grains.

Commonly, in normal cells, the *chloroplast* has a similar shape and size both in culture and in nature. At lower salinities, the chloroplast is usually lighter green in colour and obviously tends to become less expanded around the cell lumen. A limitation of the chloroplast, only reaching to the middle of the cell, as stated for the closely related *U. speciosa*, has not yet been noted.

In young filaments in culture, the number of *pyrenoids* per cell is reduced. In field material usually only one pyrenoid is found per cell, irrespective of the diameter of cells.

Basal cells were hardly encountered in field collections. The apical cell is sometimes covered with an almost hyaline cap, which presumably can be regarded as a remnant of the ruptured parent cell wall. Below the tip of the filament there are ornaments of persisting hyaline remnants of parent cells, which appear as H-pieces wrapping the cross walls; these have been seen by pure accident. On the bottom of the culture vessel the apical cells of horizontally expanded filaments appeared to be capable of functioning as basal cells. They are anchored in a bulbous, gelatinous appendage.

Reproduction and life history

Little is known of the reproductive attributes. Chapman (1946) counted in field material the number of zoospores as 4(—8) per cell. These cells were regarded by him as macrozoospores, because, when his material had been kept in tap water for some time, some of the cells became in the process of division and appeared to produce microzoospores. Their nature was not further clarified, however. Although these spores were mostly produced in numbers of four per cell they are regarded as sexual cells, generated under adverse external conditions. Chapman (1946) observed the (macro)zoospore germination when attached to a microscope slide. Further details concerning the subsequent germination of these cells were meagre and inadequate. It cannot be explained why the zoospores observed by Chapman developed only one simple germling, which is different from the germination as recorded in the present study.

In the present study it appeared that after transferring vegetative plants to short-day conditions asexual spores arose in 1—3 weeks. This induction of asexuality was successful in only four clones, however; no traces of asexual stages were found in five other clones. The sporulation process occurred vicissitudinously in the four clones. Zoospore formation could not be related to the salinity of the various media used. Zoospores were never seen in plants collected from nature. Asexual cells were produced freely in both liquid and solid cultures, in which, shortly after the attachment of the individual zoospores, a similar mode of subsequent germination and growth into an incipient plant occurred. The outgrowth of both attached and suspended quiescent zoospores in liquid media was also similar. Furthermore, it appeared that different culture temperatures and photo periods did not affect their development.

When growing on agar plates conditions proved to be conducive to the development of a mass of aplanospores. This phenomenon was sometimes accompanied by the increase of cell diameter and subsequent gelatinisation of only the transverse

walls of proximate aplanosporangia, followed by their ultimate disappearance.

The zoospores were occasionally quite variable in shape, presumably related with the number produced per zoosporangium. Besides the common spindle-shaped to broadly pyriform ones, zoospores were observed to be flattened on one lateral side, or they were rounded anteriorly and elongated posteriorly with the ends often attenuated to a short point.

The sexual process was common in all isolates studied. Long photo periods were found to influence the induction of sexuality considerably, but also under short-day illumination gametangia were present, although often in small quantities. In the filaments gametic sporulation may occur in cells next to zoosporangia. Gametes were produced both in liquid and in agar cultures. Because of the mutually different behaviour of the clones studied, no significant relationship between the effect of salinity and the development of sexual stages could be detected. For example, the clone from Terenez had a frequent production of gametes in the salinity range 0—$16^0/_{00}Cl^-$, whilst profuse sexuality in the Boschplaat isolate was only incubated in a relatively high saline medium (8—$16^0/_{00}Cl^-$). No obvious differences in frequency were noted within the nine clones studies. Sexual stages were also frequently observed in plants collected from nature. Upon attachment, the gametes showed a preference to colonize in a rather close aggregation.

The various successive developmental stages of sporophytes, generated from both non-fused gametes and from zygotes, were similar. Vegetatively the sporophytes are characterized by a consistent (almost) globose shape and the extensive parietal chloroplast, usually containing 1—3(—7) pyrenoids. It was determined from cultures that sporophytes are only capable of producing zoospores if kept for two or more weeks under short-day conditions. Sporophytes kept under long-day conditions only showed enlargement of their cells, and eventually their chloroplasts became strongly vacuolate, and concurrently occupied by a strong accumulation of storage products masking the pyrenoids. It is likely that the stickyness of the sporophyte cell wall accounts for its attachment to a hard substrate. In contrast with *U. speciosa*, the mature sporophytes did not undergo any change in shape on ripening of the zoospores; only the colour of their cell contents sometimes changed to olivaceous. Most of the zoospores formed were discharged from the sporophytes; if not so, the remaining zoospores were seen to become (almost) globose aplanospores, at the same time losing their stigmas and increasing in size.

The peculiar fragmentation process in the filaments, only met with in culture by pure accident, is up to now unique within the group of *Ulothrix* species studied. Cells, after dissociation of the cell row, may remain embedded in a common sheath-like filamentous envelope. With increasing age, the common envelope may widen greatly and if so, the alga assumes a *Palmella*-like growth stage. When returned to a fresh culture solution the individual cells develope again into uni-seriate, multi-celled filaments; these still enveloped by the common sheath, however, and finally growing into a very tangled growth habit. One may only guess whether this fragmentation process and subsequent regrowth might also occur in nature, for instance, when the alga was subjected to a long period of adverse environmental conditions. It is conceivable that in nature the chance of liberation of aplanospores is augmented by a mechanical injury.

Smooth, thick-walled, cylindrical akinetes were observed only in cultures kept on agar plates. Efforts to induce germination by refreshing the culture medium, for example, failed. It might be queried as to whether these sorts of cells may be

considered as being true akinetes or not, inasmuch as they apparently do not serve in reproduction.

Ultrastructure

Cell division. Prior to the observations with the electron microscope it was found to be very difficult to obtain sufficiently large numbers of actively dividing cells. Therefore, the nature of cell wall formation of *Ulothrix palusalsa* is not yet completely understood. In various micrographs only a thin, apparently just completed, transverse wall is depicted between the daughter cells. Its lateral ends, adjacent to the inner surface of the longitudinal wall, are strongly inflated and that is why it is assumed that cell wall formation is initiated from the inner periphery of the lateral walls and proceeds to the cell lumen. Micrographs depicting newly completed cell walls as a result of cleavage furrow, show similar cross walls (Floyd *et al.*, 1972). When cytokinesis occurs by cell plate formation, the almost complete, daughter cross walls are usually thicker in the middle than in the immediate vicinity of the inner periphery of the lateral cell wall (McBride, 1967). Neither microtubuli nor plasmodesmata were observed near or in the cross walls.

Cell wall. Electron microscopy observations on the cell wall surface confirmed the observations with light microscopy. The cell wall is bordered by a continuous smooth envelope. This apparently consists of a double membrane system, which could be demonstrated in both filaments gathered from nature as well as in those grown in the laboratory. It may be sparsely covered with fouling organisms. These may injure the membranes, especially as seen when studded with bacteria. At irregular intervals, the cell wall may have slightly depressed cavities. The membranes usually have a straight or sometimes somewhat undulated fringe. Occasionally, the exterior envelope showed a frayed appearance, suggestive of a sloughing off process, which is in accordance with the light microscopy observations. Three layers are present in the lateral cell wall underneath the outer membranes. The outermost layer of these is fairly electron lucent and built up of randomly arranged microfibrils, probably intermixed with small granules. Both components are embedded in a hyaline amorphous matrix. The thin middle layer is strongly electron dense and consists of microfibrils which often seem to run parallel. The moderately electron dense innermost layer may vary in thickness and mostly has microfibrils, generally on all sides parallel to the cell contents. As for *U. speciosa*, it is assumed that particularly the outer layer is responsible for the possible swelling of the cell wall. Both middle and innermost layers also contribute in the formation of the transverse wall. Lomasomes are often found in the transverse cell wall.

Chloroplast. EM-data support the observations obtained with light microscopy. Besides the inner lateral wall, the chloroplast may partially or entirely occupy the cross wall as well, only separated from it by a thin layer of cytoplasm and the plasmalemma. Generally, the chloroplast extends as a regular continuous mass over the inner cross wall, but at times it appears as with fissured lobes. Its ultrastructure is essentially similar to that found in the other *Ulothrix* species studied. The intervening space between the double chloroplast membrane does not seem to be equally

wide throughout, and now and then the membrane has a rolling appearance. It does not have connections with other cell organelles. Sometimes it seems to be contaminated with ribosome-like structures, but one may wonder whether these globules actually represent true ribosomes or should be interpreted as staining deposits. In lobed areas of the chloroplast the thylakoids lie with their long dimension almost parallel to that of the chloroplast. Consequently, they end abruptly in the chloroplast lobes. The thylakoid may be single, but usually there are several, up to eight, adpressed in long, sometimes slightly undulating lamellae. One or more thylakoids may detach from one lamella and pass into another, thus causing a reticulate appearance. As has been pointed out for *Ulothrix speciosa*, also in the present species the intrathylakoid lumen may vary markedly in size. The chloroplast shows a matrix with fairly homogeneously distributed ribosomes. The osmiophilic, lipid plastoglobuli occur in varying numbers embedded in its stroma; at times they are situated closely adjacent to the periphery of the thylakoid-lamellae. The plastoglobuli may vary slightly in size and are almost circular in shape. They may appear in fairly large quantities (up to 16 observed in one section) in the immediate vicinity of the starch cap of the pyrenoid. They may even approach the pyrenoid by invading, in small numbers, through the gaps of the starch shell. Starch grains are scattered between the chloroplast lamellae, appearing as oval-ellipsoid condensations of a homogeneous structure. It is likely that the majority of the starch in the chloroplast is present as ellipsoid-oval or almost square grains in the starch shell about the pyrenoid. The number and position of the grains may vary, apparently associated with environmental conditions.

The pyrenoid is enveloped by the lamellar chloroplast. Its homogeneous, granular stroma is repeatedly protruded by tubular chloroplast invaginations, which traverse the starch shell, on all sides. Usually these invaginations are not very deeply into the pyrenoid matrix, their ends may be strikingly inflated. Generally they contain 2—5 chloroplast thylakoids. Their membranes are exteriorly closely set with osmiophilic globuli, which are fairly constant in size, but on the average smaller than those in the chloroplast stroma. In some sections it seems that the individual thylakoids terminate on the inner surface of the limiting membrane of the chloroplast invaginations, just opposite an externally situated globule. Pyrenoids, with or (partially) without starch grains, are mostly spherical, or sometimes elliptic.

Nucleus. In longitudinal sections the nucleus is usually globose, but occasionally it has a slightly elongated appearance, with folds along the periphery. It mostly has a more or less central position in the cell lumen, separated from the chloroplast on one side and vacuoles on the other by only a thin layer of cytoplasm. In apparently active cells, the nucleus may also be surrounded by some mitochondria and dictyosomes. The shape of the single nucleolus depends on that of the nucleus. The nucleolus matrix, the nuclear stroma, and envelope are of similar construction as described for *U. speciosa.*

Mitochondria. Mitochondria are always present in the cytoplasm, the number per section fluctuating, up to nine, and without fixed position or shape. In only one out of numerous photographs cross sections of tubular cristae were seen. The bases of the individual cristae often seem to be slightly constricted. The infoldings appear to be almost equally spaced and parallel to one another in some sections, in other sections they appear to be irregularly arranged and the intracristal space occasionally seems slightly dilated at irregular intervals.

Golgi apparatus. This consist of a rim of several dictyosomes, seemingly associated with the nucleus. In vertical section the individual dictyosomes consist of a stack of 6—12 dissimilar cisternae. In one stack there mostly is a gradual change of diameter of this cisternae, from one pole to the other. This pattern corresponds well with that described for higher plants (Ledbetter & Potter, 1970). Its involvement in the assemblage and package of precursor wall materials in vesicles is clearly confirmed by several electron micrographs. These vesicles arise from the edges of the Golgi-cisternae by budding off and then pass to the cell wall where they have been observed to fuse with it. There is evidence to assume an unusual sequence of vesicle formation. Vesicles derived from the dictyosomes are believed to aggregate sometimes, subsequently producing spherical multivesicular bodies, probably delimited by one membrane. Similarly constructed structures have also been seen continuous with the transverse walls. Although one must be cautious in correlating ultrastructure components, it is assumed that these active pinocytotic profiles are of dictyosomal origin. Marchant & Robards (1968) have named these pinocytotic structures paramural bodies.

Cytoplasm inclusions. Osmiophilic droplets are apparently scattered in the cytoplasm. At times they protrude into the vacuole. They are relatively robust, mostly elongated, and sometimes notched in the middle. The same peculiar membranous, myelin-like inclusions as fully described for *U. speciosa*, are present in *U. palusalsa* where they sometimes adjoin the transverse cell wall. Strange multivesicular bodies are also sometimes found close to the transverse cell wall. They are ensheathed and bounded by a pronounced double membrane, which encloses numerous aggregated vesicles. It is not yet clear whether this bordering double membrane is derived from existing cytoplasmic membranes. As judged from their organization these peculiar bodies might be incipient paramural bodies, although fusion with the plasmalemma is not yet ascertained.

Vacuole. In general, mature vegetative cells contain one relatively large vacuole, which is enclosed by one simple membrane. Besides a flocculent precipitate in an electron lucent underground, also plasm intrusions of different nature are found. Sometimes there are membrane-bounded sub-compartments inside the main vacuole, discernable by their less electron-dense matrix. One may wonder whether these enclosures are fusing with the main vacuole or in turn have just been separated from it.

Ecology

Like other *Ulothrix* species, this alga shows seasonal periodicity; the filamentous stage is common in winter and spring. Its distributional pattern includes a relatively small variety of habitats, however. It seems to be chiefly confined to soft saline substrates, and, moreover, never grows so abundant as the other *Ulothrix* species studied. Often, in intertidal areas in open halophilous vegetations, it grows as occasional threads in algal carpets, which may predominantly consist of *Ulothrix subflaccida*, *Rhizoclonium riparium*, *Percursaria percursa*, *Enteromorpha prolifera*, numerous *Vaucheria* spp., and blue-green algae.

Tiny, almost uni-algal sheets of *U. palusalsa* have been encountered on bare

damp soils, which were predominantly clayey or sandy e.g. in the high littoral part of the shores of creeks intersecting salt marshes, or in horizontal zones between salt marshes and dunes.

Occasionally, in intertidal environments, it also participates in the algal undergrowth of dense vegetations of phanerogams, e.g. those composed of *Spartina townsendii*, *Puccinellia maritima*, *Salicornia europaea*, and *Juncus* spp. Infrequently it may clothe the basal portions of perennial stems of halophytes. In almost bare clayey land reclamations outside the dikes, this species has been found as a pioneering alga.

In non-tidal regions it is frequently found on bare damp spots, or in swampy grassy vegetations on the shores of inland saline basins or pools. Recently reclaimed polders also appear to be a convenient habitat, and this species was also once found near the mouth of a tidal freshwater stream. It was not found growing on other algae.

The origin of the material in culture

Uni-algal cultures were initiated from filaments gathered from the following localites. THE NETHERLANDS: Friesland, Terschelling, Boschplaat, in a halophilous vegetation in winter periodically flooded; Lauwerszee Polder, on vast sandy moist soil; Noord-Holland, Texel, Mokbaai, in the undergrowth of phanerogams; Balgzand, on an eroded escarpment, with halophytes; Zeeland, Schouwen Duiveland, Suzanna's Inlaag, an inland salt marsh, on soft bottom; Zuid Beveland, Deesche Watergang, near Kattendijke, in inland salt marsh on bare, sandy and clayey damp soil; Sas van Goes, intertidal salt marsh, on bare sandy spots amongst *Spartina townsendii*, Zeeuws Vlaanderen, Baalhoek, in open bare spots (material collected by Drs. W. F. Prud'homme van Reine). FRANCE: Terenez, near Roscoff, in and near a halophilous vegetation proximate to the mouth of a freshwater stream.

Notes on herbarium material

Ulothrix palusalsa has been encountered only once in the herbarium material consulted. It could be identified with certainty with the help of JKJ, which gives an inflated appearance to the vulnerable cell wall.

4. Ulothrix implexa (Kützing) Kützing — Figs. 15—17; Plates 12—15; Table 2.

Hormidium implexum Kützing (1847) 177. — *U. implexa* (Kützing) Kützing (1849) 349; (1852) 30, Tab. 94; Hauck (1885) 440; Reinke (1889) 83; Foslie (1890) 143; Reinbold (1891) 129; Batters (1902) 13; Lakowitz (1929) 107; Newton (1931) 55; Printz (1964) 14; Pankov (1971) 76; Lokhorst & Vroman (1974b) 563. — *Hormiscia implexa* (Kützing) Rabenhorst (1868) 364; De-Toni (1889) 168; ? Van Goor (1923) 104; Ollivier (1929) 91. – Type: The Netherlands, Zeeland, Goes, *Lenormand* (L sheet 938.174–397).

?*U. submarina* Kützing (1849) 349. — *Hormiscia implexa* (Kützing) Rabenhorst *b. submarina* (Kützing) Rabenhorst (1868) 364. — Type: Nordsee, *Kützing* (L sheet 939.67–830).

Lyngbya (Hormotrichum) cutleriae Harvey (1851) Pl. 336; ? Johnstone & Croall (1860) 179. — *U. cutleriae* (Harvey) Thuret in Le Jolis (1863) 56. — ? *Hormiscia cutleriae* (Harvey) Rabenhorst (1868) 363. — Type: Great Britain, Budleigh Salterton, near mouth of stream, *Cutler*, 30/5/1850, *ex herb. Harvey* (TCD).

U. flacca auct. non (Dillwyn) Thuret in Le Jolis: Dodel-Port (1883) 148.

U. subflaccida auct. non Wille: Wille (1901) 27 (*p.p., typo excl.*); Wille (1913) 20; ? Printz (1926) 233 (*p.p.*); ? Hamel (1929) 86; ? Hamel (1930) 23; Carter (1933) 131; ? Hamel & Lami (1934) 11; ? Chapman (1937) 228; Feldmann (1937) 40; ? Levring (1937) 17; Kornmann (1964) 33; Jaasund (1965) 12; Starmach (1972) 47; Von Wachenfeldt (1975) 233 (*p.p.*).

U. acrorhiza Kornmann (1964) 37; Von Wachenfeldt (1975) 231. — T y p e : not available.
? *U. pseudoflacca auct. non* Wille: Perrot (1971) 358.

Gametophytic plants, mainly from brackish waters, up to 4(—20) cm long, green to bright green. *Thalli* flexible, glossy, with the *filaments* solitary or growing together in tufts or clews, straight when young, later on more tortuous, unbranched and uni-seriate. Both in young and in mature growth stages the *cell wall* relatively firm and thin, occasionally (slightly) thickened and warty, concurrently covered with fouling organisms and/or micro-particles. Mature cross walls occasionally wrapped by hyaline H-shaped remnants of the mother cell walls. *Cells* in the filaments sometimes arranged in pairs or in fours. Cells always distinctly cylindrical, closely adpressed to one another without appreciable rounded ends. Cell diameter (3.6—)9.6—15.4(—26) μm, cell height (3.6—) 4.8—10.9(—15.6) μm; young cells 3.6—9.6 μm wide. Occasionally the lowermost cells slightly and gradually attenuated, and usually higher.

Chloroplast parietal, variously shaped according to age and environmental conditions. In young cells the chloroplast always regularly girdle-shaped, usually extending over 1/2—2/3 of the cell circumference, frequently not approaching the transverse walls; mostly, in longer cells, lobed along its longitudinal fringe. At maturity the chloroplast often extending towards the transverse walls, straight to slightly lobed along its longitudinal margin, sometimes incised; sometimes with small separate randomly distributed chloroplast fragments. Chloroplast girdle rarely closed with a bridging part, mostly reduced in the length-axis of the filament. In very aged cells the chloroplast assuming a massive form, sometimes withdrawn. Upon disintegration the chloroplast obscured by deposition of storage products, or by intensified vacuolation of the cell contents. *Nucleus* one per cell, situated often opposite the pyrenoid. *Pyrenoids* 1(—4), spherical, in much shortened cells slightly flattened, in long cells sometimes slightly elongated and compressed at one side; pyrenoids smaller if more per cell. Contours of pyrenoids distinct, usually enveloped by a thin cap of starch, except in cells with an abundant production of assimilates.

In germlings the basal cell differentiated into a simple, rather conspicuously lengthened rhizoid, in mature plants the *basal cells* sometimes (profusely) branched. Individual rhizoids variable in width, inflated, or tapered at the end, its cell wall occasionally wavy or with bulges, often surrounded by a delicate gelatinous sheath. Chloroplast in the germling rhizoid as an open girdle, later on falling to pieces, concurrently becoming pale green, and disappearing in very aged rhizoidal cells. Lateral secondary rhizoids simple or branched, rare in young plants, occasionally present in older thalli, sometimes as very compound ramifications. Secondary projections sometimes also present inside the lumen of former reproductive cells. Apical cells usually rounded; upon contact with hard substrates capable of differentiating into basal-cell like rhizoids, after which the filaments may assume a loop-shaped habit. Occasionally, one to several cells in the filaments not well developed, flattened and with hyaline contents.

Asexual reproduction by zoospores and aplanospores, initiated in all ordinary vegetative cells, including unmodified apical cells, but not in differentiated basal cells and those producing secondary rhizoids. Generally, first occurrence of asexual reproduction in the apical part of the filament progressing onto the more centrally situated cells. Asexual filaments sometimes slightly bent. Mature *zoosporangia* light

green, hardly inflated, 9.7—16.9(—23.4) μm wide, 8.4—18.8 μm long. The cell length/width ratio of wide zoosporangia usually markedly greater than in vegetative cells with comparable cell width. Zoospores discharged in a hyaline vesicular envelope, through an irregular shaped or oval lateral pore. Within a few seconds after discharge the vesicle bursts, followed by scattering of the zoospores. *Zoospores* (2—)4—16(—32) per cell, (6.8—)8.5—13.6 μm by (3.4—)4.8—8.4 μm, fourflagellate, usually spindle-shaped or with rounded posterior and attenuated anterior poles or occasionally asymmetric and flattened on one side or with a crooked posterior end; sometimes a pointed papilla occupying the zoospore apex. Chloroplast parietal, cup-shaped or reduced at one side, sometimes with incised lobed or unlobed margins; pyrenoids 1—2, embedded in the posterior part, and with one median-posterior stigma. Zoospores positively as well as negatively phototactic; locomotion fairly rapid, prior to settlement being a sluggishly spinning against the substratum with the anterior pole foremost; shape concurrently becoming spherical and followed by abscission of the flagella on quiescence. Germination of the zoospore bipolar, starting with enlargement, later on with repeated partitioning of the cells, followed by stretching of daughter cells, ultimately leading to the formation of a filament of several cms. long. Sometimes, as a manifestation of arrested zoosporogenesis, *aplanospores* are formed, 1—8(—16) per sporangium, without any significant modification in size or shape of the sporangial cells. Aplanospores elongated, (5—)7—8 μm by (4—)5—6 μm, when spherical (4—)5—7(—8) μm; able to germinate in the parent cell.

Gametogenesis possible in all vegetative cells, including unmodified apical cells, but not in basal and differentiated intercalary cells. Gametangial areas commonly first developing in the apical portion of the filament, later on filaments entirely filled with gametangia, straight or slightly curved. *Mature gametangia* olivaceous, hardly inflated, (7.8—)9.7—18.8(—23.4) μm wide, 7.2—19.3 μm long. In general, the average length/width ratio of wide gametangia relatively large. *Gametes* discharged enclosed in a tiny hyaline vesicle through a lateral pore; the gametes being already lively mobile, later on (suddenly) released by bursting of the vesicle. Gametes (4—)8—32 per cell, 4.2—8.5(—10.6) μm by 2.1—4.2 μm, biflagellate, usually ovoid, sometimes asymmetric by a slight inflation of the dorsal side, or rarely the anterior pole slightly drawn out to form a flexible neck, sometimes notched; sometimes, however, gametes spherical with a hardly distinguishable stigma. Gamete chloroplast parietal, occupying the posterior portion, cup-shaped or open at one side with a hardly discernable pyrenoid. Stigma, if traceable, median or posterior in the cell body. Gamete movement skittish, positively phototactic, prior to quiescence mostly showing irregular rotation movements, concurrently becoming spherical and often followed by only a loose attachment to the substratum and subsequent detachment of the flagella. Sometimes quiescent gametes also remaining suspended without a prompt abscission of both flagella and without marked modification of their shape. Both sorts of gametes and those prevented form escaping from the parent cell, mostly parthenogenetically developing into one-celled sporophytes. Mating of the gametes generally isogamous and dioecious. Quadriflagellate zygotes strictly negatively phototactic, upon settlement flagella abscised and usually becoming spherical. *Germination* occurs either by enlargement, leading to spherical and slightly elongated forms, or by the development of a lengthened protuberant bulge, which later on contains the removed zygote contents, finally leading to young stalked sporophytes with a straight or curved linear, or spherical, or pyriform shape,

or with an irregularly shaped anterior part. The original zygote-envelope becomes transformed to serve as an attaching disc. Vegetative sporophytes with a parietal chloroplast, not wholly occupying the cell wall, lobed or strongly fissured along its margins, and with 1—3 pyrenoids; occasionally having massive homogeneously structured cell contents by abundant accumulation of storage products. Prior to ripening, the sporophytes assume an approximately, globose or pyriform habit; simultaneously the cell contents become cleaved into a number of large chloroplast clumps and the stalk breaks off close to the sporophyte by gelatinisation. *Mature sporophytes* up to 70 μm in diameter, olivaceous, cell wall often reddish-brown, containing 16 or more *zoospores*; these positively phototactic, similar in shape and developmental behaviour to those arisen from filaments.

Vegetative reproduction by dissociation of the filament. Smooth-walled, spherical or slightly flattened *akinetes* were seen once. Chloroplast of akinete green, massive, and starch-laden.

NORWAY, Espegrend, Maripollen near Lønninghamn, high littoral on sheet-piling or roofing-tiles, intermingled with *Ulothrix subflaccida*, *Lokhorst*, 25/4/1975 (L); Drammenfjord, Hurum, Verket, in fast streaming water on stones, intermingled with *Ulothrix subflaccida*, *Lokhorst*, 22/4/1975 (L); Dröbak, on stones in the littoral zone near the Biological Station, intermixed with several *Ulothrix* species, *Wille*, April, *ex Kryptogamae exsiccatae no 2145* (BM, L, LD).

SWEDEN. Fiskebäckskil, as *Ulothrix flacca*, *Kylin*, 7/7/1948 (LD); Malmö, Limhamn, in the littoral zone as *Ulothrix pseudoflacca*, *Sjöstedt*, 21/2/1928 (BM); Malmö, Ribersborg, as *Ulothrix pseudoflacca*, *Sjöstedt*, 7/4 (LD); Malmö, kanalen vid Gamla Kyrkogården, as *Ulothrix pseudoflacca*, *Sjöstedt*, 26/4/1919 (LD); Malmö, mynningen av turbindammen, as *Ulothrix pseudoflacca*, *Sjöstedt*, 18/4/1919 (LD); Trelleborg, as *Ulothrix pseudoflacca* and *Ulothrix subflaccida*, *Sjöstedt*, 6/5/1917 (BM, C, L, LD); Vardö, epiphytic on *Dictyosiphon*, *Foslie*, 8/1887, *ex Hauck & Richter-Phykotheka universalis 530* (BM, C, GB, HBG, KIEL, L sheet 920.13–251, PC).

GERMANY. Warnemünde, on rocks near the high water level, *Heiden*, 15/4/1888 (BM, C, HBG, KIEL, L, PC).

THE NETHERLANDS. Amsterdam, dike near Zeeburg, *Weber-van Bosse* 77, 24/2/1885 (L sheet 942.253–35); Amsterdam, 't IJ, on wood, intermingled with *Bangia atropurpurea*, *Den Hartog & Moeliono*, 17/5/1953 (L sheets 956.209–004, 956.313–524); De Grevelingen near Grevelingen Dam, on stones in the wash zone, *Lokhorst*, 13/3/1975 (L); Veerse Meer, Kortgene, on basalt blocks in the wash zone, intermingled with *Urospora penicilliformis* and *Ulothrix subflaccida*, *Lokhorst*, 22/2/1976.

GREAT BRITAIN. Budleigh Salterton, intermingled with *Ulothrix speciosa*, *Cutler*, 1851 (E).

BELGIUM. Antwerp, Scheldekanaal, near Berendrecht, in the wash zone, intermingled with *Urospora penicilliformis*, *Lokhorst*, 10/4/1975 (L).

FRANCE. Terenez, on stones near the mouth of a stream, intermingled with *Ulothrix subflaccida*, *Lokhorst*, 9/4/1975 (L); Cherbourg, as *Ulothrix cutleriae*, *Bornet*, 30/3/1853 (L sheet 939.67–829); Equeurdreville, as *Ulothrix cutleriae*, *Thuret 102*, 30/3/1853 (L sheet 939. 67–829).

Nomenclature and historical aspects

As stated previously (Lokhorst & Vroman, 1974b), the identity of this species has frequently been disputed in the literature (e.g., Wille, 1901; Hazen, 1902; Schussnig, 1915). The uncertain position of the species was probably due to Kützing's vague and meagre original description (1847) and its indistinct illustration in Tab. Phycol. II (1852). Also the ecological fact that *U. implexa* proves to be fairly insensitive to daily fluctuating salinity has been an additional factor in its misidentification. Carter (1933) and Chapman (1937, 1954) preferred to retain the name *U. implexa* for the freshwater forms, using the name *U. subflaccida* only for the corresponding marine forms.

Actually, *U. implexa* was already figured adequately in Dodel-Port's illus-

trations, 1883, though unfortunately under the wrong name *U. flacca*, which added again to the considerable confusion in the specific delimitation within the genus *Ulothrix*. Hauck (1885) copied part of these illustrations and correctly named it *U. implexa*. However, Wille (1901), when judging both Kützing's and Dodel-Port's figures, concluded that the two depicted forms could not be identical. His conclusion is understandable because the filaments figured by Kützing (1852) were assumably drawn from dried material. His conclusions caused Wille (1901) to avoid using the name *U. implexa*, but he did not exclude the possibility that further study of the original material might clarify its controversial identity. One may wonder why he did not actually study the available original material; this may have avoided a lot of confusion. Furthermore, Wille emphasized that Kützing in 1852 intended to use the name *U. implexa* only for a species occurring in ditches, and not for marine forms. As a result of this statement, Wille (1901) described and redescribed several marine forms of the genus. With the same arguments, Schussnig (1915) even advocated the rejection of the name *U. implexa*.

The erection of the substitute name *Ulothrix subflaccida* for *U. implexa* by Wille, proved to increase rather than to reduce the taxonomic and nomenclatural confusion in the group of smaller brackish-water and marine *Ulothrix* species. For example, by some authors later on the older name *U. implexa* was placed in the synonymy of *U. subflaccida* (Hamel, 1930; Levring, 1937; Ramanathan, 1964), whilst others still accepted *U. implexa* (Hazen, 1902; Printz, 1964); other authors, as mentioned above, maintained both names for the forms occurring in habitats with differing salinity (Carter, 1933; Chapman, 1937, 1954).

A comparison of the overall cell diameter range, viz. 3—26 μm, for the two presently recognized brackish-water species, with that recorded by Wille (1901) for *U. subflaccida* (5—26 μm), leads one to assume that he was unaware of the existence of two brackish-water species, often growing intermingled. Examination of Wille's drawings confirmed this assumption. Besides vegetative filaments of the genuine *U. subflaccida*, also asexual filaments of the alga defined in the present paper as *U. implexa*, are depicted. Strangely enough, in a foot-note Wille (1901) expressed his doubts about the identity of this depicted reproductive stage. Both species were also found together on Wille's herbarium sheets. Finally, it was very surprising to conclude from the present herbarium study, that both controversial names, discussed above, could be legally applicated for two closely related brackish-water *Ulothrix* species; both species being difficult to distinguish, also because they often occur intermingled.

The type specimen, preserved in L, is in a very bad condition; it is present as only two filaments, together with abundantly *Rhizoclonium* species. For this reason, material collected in the Netherlands (Grevelingen Dam, Lokhorst, 13/3/1975, L) is recommended here as illustrating specimen for future reference.

Morphology

The *general habit* of the plants collected in diverse habitats in nature is largely similar to that observed under favourable culture conditions. However, the plants in nature attain only a length of up to 4 cm while in cultures threads were found as long as 20 cm. In cultures, after dispersal and settlement of zoospores, simple erect filaments arose on the bottom and sides of the culture vessels, ultimately giving rise

to a dense flexible algal carpet of several cm thick. In liquid media, particularly under long photo periods, this species was found to be a very rapidly growing alga. In etiolated cultures the mature filaments became detached from the bottom, particularly when the culture medium had not been refreshed on time. When inoculated on agar plates the alga started to grow again, but then with greatly reduced development.

The *cell wall* is firm and generally remarkably thin, but unfavourable external conditions induce a thickening of the wall, up to 4 μm. The cell wall of filaments may undergo slight deformations such as the appearance of a warty surface when, in nature, the plants were periodically or continuously washed by (turbid) water. Concurrently, the cell wall surface may become contaminated with micro-organisms and/or micro-particles, facilitated by the presence of a very delicate gelatinous outer layer, detectable only with the aid of an electron microscope.

In the field, the vegetative cells usually attain a *cell diameter* of up to 15.4(—26) μm. Incidentally, however, a cell diameter of only up to 13.5 μm was noted. In cultures, variations in photo period and temperature did not affect the absolute cell diameter range significantly. At higher salinities, a larger average cell diameter was usually induced. The culture experiments did also reveal an influence of salinity on the development of the *chloroplast*. In filaments under short-day conditions in Gorham medium ($0^0/_{00}Cl^-$) or sometimes in $2^0/_{00}Cl^-$ medium, the chloroplast in the mature cells usually was in the form of a prominent, regular-shaped incomplete band, extending over about half of the cell circumference and often not approaching the transverse cell wall. Fully developed chloroplasts usually occurred in the salinity range $4—8^0/_{00}Cl^-$, and they often showed growth disturbances after a very prolonged stay at $16^0/_{00}Cl^-$ without refreshment of the medium. In this latter case the chloroplast withdrew from the lateral wall and showed a wrinkled texture. At low salinities the chloroplast was paler green. In field collections a fairly regular, fully-developed chloroplast was usually seen. In less favourable conditions in culture, such as a prolonged stay at long-day conditions without regular refreshment or replenishment of the culture solution, the filaments usually began to grow more poorly. Concurrently, the individual cell contents grew denser, showing signs of vacuolation and an increased synthesis of assimilates. Eventually in these cells the chloroplast became withdrawn. In addition, it can be marked that in these depleted cultures the pyrenoid(s) in the cells could only be traced with the help of JKJ. One *pyrenoid* per cell could commonly be detected in field collections.

In general, the formation of secondary projections tended to be augmented by Gorham medium and also when the alga was exposed to excessive salinity in the culture solution (*c.* $45^0/_{00}Cl^-$) for some time. Rhizoidal outgrowths were accordingly observed in field material from fresh-water origin. A development of these appendages on any place in the filament appeared also possible, however, at moderate salinities, for instance as a result of cell injury, or, when there was only a limited (a)sexuality. In cultures, the apical cell is usually rounded in all stages of development. Regardless of age or degree of development of plants, this cell was occasionally seen to differentiate like the *basal cell*, when in contact with the bottom of the glass tank. In these cases, firstly the apex of this cell seemded to elongated, then a narrow protuberance was formed, eventually developing into a simple or complex rhizoidal system. This phenomenon has never been observed in nature.

Apparently due to less favourable environmental factors, it was seen that some cells randomly located in the filaments became considerably lengthened and

inflated, with a concurrent crumbling of their cell contents; hence the filaments showed *akinete*-like stages.

Reproduction and life history

Some aspects of the reproduction of *U. implexa* have already been described by several authors. In view of the previously discussed nomenclatural confusion of this species, it is not surprising that these reports on the reproduction have been published under different specific names. In 1883, Dodel-Port depicted fairly adequately some attributes of the sexual reproduction under the name *U. flacca*. The illustrated gametes were described as microzoospores. He also figured the mating process and immature spherical sporophytes, showing germination of the gametes inside the gamentangial cell wall. These stages of development were described as *palmelloid*-stages. The asexual reproduction of this alga was somewhat further clarified by Wille's findings (1913) on what he called the more robust form of *U. subflaccida*. Precise information on the number of zoospores per cell, shape of zoospores, and their cell body dimensions, as well as additional remarks with respect to the sexuality were given. He also detected the peculiar secondary rhizoidal outgrowths from proximate vegetative cells into the empty cell lumen of former reproductive cells. Kornmann (1964) apparently accomplished the life history of this species in culture for the first time, under the name *U. subflaccida*. Although the illustrations are excellent, detailed information in the accompanying text is fairly meagre. No data were published on the number and dimensions of zoospores and gametes, nor on shape and measurements of zoosporangia and gametangia. Kornmann's reported findings differ in several respects from the data regarding *U. implexa* as given in the present paper. It is unfortunuate, that actual cultures or herbarium specimens of the alga studied by Kornmann were not available for comparison. Unfortunately, also material of *U. acrorhiza* Kornmann (1964), regarded in this study as synonymous to *U. implexa*, was not available. This growth form does not differ in any vegetative feature from *U. subflaccida sensu* Kornmann, but it was nevertheless described as a new species on account of the lacking of sexuality. This very interesting topic will be dealt with in detail later in this chapter.

The asexual reproduction of fresh-water clones of U. implexa has previously been discussed by the present author (Lokhorst, 1974).

Asexual spores were formed after transferring vegetative plants under short-day conditions. In general, the introduction of asexuality was successful in all clones studied. The rate of sporulation differed, however. In the clones initiated from fresh-water material, this type of reproduction was slow, whilst the clone from Limburg, collected at c. 100 km distance from the sea, could hardly be induced to produce zoospores. This alga accordingly usually had a frequent production of secondary outgrowths. Moderate salinity tended to increase the production of zoospores, whilst in some clones, especially those collected from oligohalinic or freshwater habitats, the process of asexuality stagnated in the Gorham medium ($0^0/_{00}Cl^-$). These facts all point to the conclusion that in nature a prolonged stay in fresh water decreases the ability to produce zoospores.

The zoospores proved to be quite similar in shape in all clones studied, although the zoospores of fresh water origin were faster swimming, and had smaller dimensions on the average. Moreover, the zoospores from freshwater specimens had a

photonegative response while swimming, whereas those originating from brackish-water and marine habitats just had the opposite reaction to light. There is no explanation for this mysterious phenomenon. In liquid culture solution the germination of attached and suspended zoospores was similar. Different day lengths and temperatures did not affect their development into incipient plants. Aplanospores were particularly formed under long-day conditions; they germinated directly within the parent cell wall when brought to more favourable conditions.

In comparison with the marine *Ulothrix* species, the germination of both zoospores and aplanospores of *U. implexa* is different. Each of them invariously grows into only one filament, which entirely corresponds with the observations for all freshwater *Ulothrix* species (Lokhorst, 1974). Therefore we may presume that *U. implexa* descended from freshwater species. On the other hand, its requirements for the induction of sexuality in filaments is an argument in favour of its origin from species from marine habitats. Sexual reproduction only occurred, and then rarely, in clones initiated from plants collected in brackish-water and marine areas. The only exception found was in a clone from Verket (Norway), where sexuality could not been initiated at all. Growth of the alga in a medium with salinity of 2 to $16^0/_{00}Cl^-$ and long photo periods were found to be prerequisites for sexual reproduction. Because of this it is inferred that *U. acrorhiza* Kornmann (1964) merely represents a race of *U. implexa* in which the genetic capacity for sexual reproduction has been masked in some way.

During differentiation of vegetative cells into gamete-producing cells the general habit of the filaments is not drastically transformed. Only some filaments may become slightly bent. In cultures with sexual plants the discharged gametes consequently failed to mate, thus showing their strict dioecious character in culture. However, the absence of zygotes in these cultures did not prevent the alga of accomplishing its life history. Most gametes parthenogenetically developed into sporophytes, which in course of time issued zoospores under short-day conditions. The results of some cross fertilization experiments are summarized in table 2.

	Veerse Meer	Ooltgensplaat	Terenez
Veerse Meer		+	−
Ooltgensplaat	+		+
Terenez	−	+	

Table 2. Survey of cross-fertilization experiments amongst three clones of *Ulothrix implexa.*

Fusion of joined gametes, from Veerse Meer and Ooltgensplaat respectively, led to the formation of considerable numbers of zygotes. Contrary, a meagre production of zygotes was found upon assembling sexual spores derived from Ooltgensplaat and Terenez. After pairing of the gametes, the zygotes may remain mobile for a period of a few minutes to up to several hours. It was not possible to determine the requirements for the development of a specific sporophyte shape, since the various forms appeared together in any sexual culture observed. In the Veerse Meer culture, however, the stalked sporophytes were in the majority, whereas in the Grevelingen culture the spherical forms predominated. Maturation of sporophytes was only achieved after transporting non-fused gametes or zygotes into short-day conditions.

The breaking of the stalk of the sporophyte, in the immediate vicinity of its cell body, usually occurs at the beginning of the first cleavage of its cell contents, prior to ultimate ripening. Probably the same happens in nature. Here the sporophytes originate from negatively phototactic zygotes and consequently grow in darker places. In order to accomplish the final cleavage processes, eventually leading to the formation of zoospores, the plants assumably need more light, which can be reached by breaking of the stalk. Young sporophytes continuously kept under long-day conditions, in fact only showed an enlargement of their cell body. Simultaneously, the contents became strongly vacuolated, or heavily loaded with an accumulation of assimilates. Upon maturation at short-day conditions the cell contents, after successive dividing processes, ultimately displayed a stunted ripening process, resulting in sixteen or more zoospores or aplanospores. Sporophytes in nature expectedly produce zoospores profusely, in order to guarantee the species dissemination and maintenance. Possibly, only the daily fluctuating external factors in its natural habitat affect the ripening process of the sporophyte contents.

The vegetative propagation by dissociation of filaments is increased in etiolated cultures. On one occasion, smooth thick-walled akinetes were found in a culture, which by pure accident had been kept at $\pm$ 40°C for a short period. Efforts to induce germination of these akinetes thus far failed.

Ultrastructure

Cell division. In *U. implexa*, during formation of the transverse cell wall, the initiated septum extends into the cell lumen as an annular ingrowth from the existing lateral wall, whereby the chloroplast is cleaved. In this furrowing process, no microtubules are discernable with the developing furrow; thus the phycoplast does not appear. The in-growing septum is surrounded by a highly active cytoplasm holding (in-) direct participants, such as numerous mitochondria, dictyosomes, ribosomes, strikingly extended ER, and finally vesicular structures or strands; probably the latter represent precursory wall material. The cell division in *U. implexa* is fundamentally similar to that observed in *Klebsormidium* (Floyd et al., 1972; Pickett-Heaps, 1972) and in *Stichococcus* (Pickett-Heaps, 1974). Differences are in the organization of the final stage of the telophase cell. In *U. implexa*, no collection of vacuoles in the interzone area between the daughter nuclei can be detected, and the chloroplast cleavage is not initiated until this telophase. In the above mentioned genera, the division of the chloroplast apparently has already commenced in the prophase. In *U. implexa*, the daughter nuclei remain in proximity during cytokinesis, whilst for the other genera remains of daughter nuclei near the ends of the dividing cell have been found until the new transverse strand is completed.

Cell wall. The composition of the cell wall of *U. implexa* differs somewhat from that observed in the marine *Ulothrix* species. It consists of an inner zone of less compactly, more or less parallel arranged microfibrils and a thinner outer zone of electron denser material. Besides circumferent arrangement in the lateral cell wall, both layers also contribute in the constitution of the mature transverse wall, but the electron denser layer mostly predominates here. In *U. implexa* the cell wall surface has never been seen to consist of a double membrane system as in other species. Both in plants collected from nature and in those cultivated at the laboratory the cell wall

seems to be clothed by a thin gelatinous cuticular layer, on or in which epiphytes and micro-particles may anchor. The presence of this layer apparently obscures the visibility of the double membranes. Occasionally, it shows a local thickening with flocculose fringes. The outer layer of the cell wall is sometimes somewhat frayed, particularly when injured by bacteria. At irregular intervals the cell wall may appear as slightly wavy, which never has been seen in light microscopy studies. Possibly this is caused by the fairly drastic fixation procedure used prior to electron microscopic examination. On one occasion, remnants of H-pieces, which proved to be firmly attached to the lateral wall in the immediate vicinity of the transverse cell wall, were seen. The transverse wall may show swellings, mostly centrally located. Lomasomes were seldom found.

Chloroplast. EM observations support those obtained with the light microscope. The chloroplast may partially or entirely cover the transverse wall, where it is separated from it only by a thin layer of cytoplasm and the plasmalemma. The chloroplast is compact, hardly incised or at the most irregularly undulated. Its micro-anatomy is fundamentally identical to that in the marine *Ulothrix* species. The double membrane chloroplast envelope and the granular stroma with embedded thylakoids are distinctly discernable. The thylakoids, ending abruptly in the chloroplast lobes, may run singly, but they are mostly aggregated, up to eight, in straight or undulating lamellae. One or more thylakoids may leave from one lamella and subsequently associate with another, hence having a reticulate arrangement. More frequently as compared to the marine *Ulothrix* species, the longitudinal sections of the chloroplast of *U. implexa* also have thylakoids which are seemingly arranged in short lamellae, by which it resembles somewhat to the thylakoid arrangement in grana and intergrana regions in *Chara* (compare the photograph by Crawford in Dodge, 1973). In comparison with the marine *Ulothrix* species, the thylakoid network of the present species is often relatively denser per square unit of chloroplast matrix. This latter fact cannot be associated with light intensity, because all clones studied were exposed to the same external conditions. When observing the cytokinesis, one may express the view that the chloroplast propagate only by fissuring and its division is clearly linked with that of the cell. Besides ribosomes, the chloroplast stroma contains the plastoglobuli, which may appear singly or tightly packed together between the chloroplast lamellae, particularly proximate to the pyrenoid. They may also be located fairly close to the periphery of slightly starch-capped pyrenoids. They are fairly constant in size and are almost sperical or oval. Near the pyrenoid sometimes also larger clumps of osmiophilic character are seen, but it is not clear whether these bodies are formed by fusion of plastoglobuli. Globular, ovoid, and ellipsoid starch grains are located in larger areas amongst the elements of the lamellar system, by which these are apparently split. The relatively thin marginal area of the individual starch grains appears denser at times than the inner zone. Most of the starch is accumulated as segmented grains in the starch shell coating the pyrenoid. Their number and size may vary from filament to filament, most probably depending on the external environmental conditions. Occasionally, within one filament their number per cell may also vary considerably.

The pyrenoid is always part of the chloroplast, even when it seems to protrude from it. Generally, however, it lies somewhere about the centre of the chloroplast. In section it is mostly circular; sometimes oval or slightly compressed. No lamellae enter the pyrenoid; however, 1—3(—4) thylakoid lamellae may intrude the starch

shell, but on reaching the pyrenoid proper, they branch off and continue between the pyrenoid matrix and the starch cap inside. In sections where the starch shell is superficially cut through, it is clear that the thylakoids may also pass only between the starch grains, without approaching the periphery of the pyrenoid.

Nucleus. In longitudinal section this is almost circular in shape, or less frequently somewhat elongated. In cells with a fully developed chloroplast the nucleus seems to be sandwiched in between the lateral chloroplast ends. It often lies opposite the pyrenoid. It is also seen to be bounded by several small vacuoles, or one large vacuole, and in apparently highly active cells, prior to or just after cell division, by a network or a ring of mitochondria, dictyosomes, and vesicular structures. The interphase nucleus is filled with one central nucleolus and two types of chromatin respectively, condensed in demarcated clumps and equally dispersed in the nucleus. The delimiting nuclear envelope consists of a double membrane, which is continuous with the ER-system. The narrow perinuclear space may be slightly expanded at irregular intervals. The nuclear membranes are frequently perforated by fenestrations.

Mitochondria. These cytoplasmic organelles may be situated in rather large quantities in the proximity of the transverse wall of the interphase cell. On one occasion a mitochondrion was found near the corners of the cell lumen, with its long axis parallel to the lateral wall and pinched between the plasmalemma and the outer chloroplast envelope. The mitochondria may also be found fairly closely adjacent to the chloroplast or to the nuclear envelope. In general, they are discrete ovoid bodies, while at times their form is elongate or irregularly long-stretched. The cristae are tubular, and particularly in elongated mitochondria they are situated, parallel to one another. In some other sections these infoldings appear to be irregularly arranged, while the intracisternal space occasionally seems slightly widened at irregular intervals. Sometimes, the base of the individual cristae seems to be slightly constricted.

Golgi apparatus. The Golgi apparatus consists of an array of several dictyosomes. It is usually associated with the nucleus. Individually, the dictyosomes may be situated very closely adjacent to the nuclear envelope. In vertical section the individual dictyosomes consist of a stack of 6—9 cisternae; however, this number is based on only a small number of micrographs. In the immediate vicinity of the furrowing septum, besides simple vesicles, also multi-vesicular bodies, composed of aggregated vesicles bounded by a common membrane, can be seen. As already discussed for *U. palusalsa*, these bodies are highly probably derived from the Golgi apparatus and subsequently implicated in the formation of the cell wall.

Cytoplasm inclusions. Besides the osmiophilic lipid droplets, two peculiar other types of inclusions are noted in the periphery of the cell lumen, close to the transverse cell wall. The first are apparently minute vesicles containing several stellate osmiophilic piths. They are sinuous and compressed in shape. The second type is very peculiar because of its architecture, resembling the nucleus in some respects. Its stroma appears similar to the nucleus, and it is bounded by a double membrane, which is apparently interrupted at times by irregular fenestrations. Several of these oval or stretched bodies group together. Their function and origin is

unknown. Their position near the transverse cell wall is suggestive of that they are somehow involved with the cell wall formation. The presence of peroxisomes could not unequivocally be demonstrated in the present study.

Vacuole. The vacuole usually occupies only a small part of the cell lumen. In consequence, either no vacuoles, or alveolate vacuoles are present, each delimited by a simple membrane. The ground plasma is generally remarkably electron lucent with an occasional flocculent deposit.

Ecology

Ulothrix implexa shows a seasonal periodicity, particularly when occurring in brackish-water and marine habitats. The filamentous stage is predominant in late autumn, winter and (early) spring. The species has a fairly wide ecological range. The alga grows commonly along the west European coasts, particularly in places transitional between sea and fresh water, where the environmental factors such as salinity, temperature, oxygen content, irradiation, precipitation, evaporation, emersion and immersion, may show fairly extreme (daily) variation. For example, *U. implexa* can frequently be found near the mouth of intertidal freshwater streams, where the plants are covered at high tide with sea water, in estuaries or rivers subjected to tides, on (artificial) rocks in the vicinity of the high-water mark, in supralittoral pools on rocky shores within the reach of spring tides and storm waves, and in places in the supralittoral exposed to drip of fresh water. The distribution of the alga may also extend to inland non-tidal areas like meso-oligohalinic pools, lakes and canals. Den Hartog (1967, 1970) recognized all such locations as well-defined brackish-water habitats having their own individual specific ecological features and biocoenoses. The species is also encountered as a resident of the wash-zone of freshwater lakes, many kilometers from the coast. Once it was seen sparsely dispersed at about the water level of a swift-flowing, heavily polluted freshwater stream.

The alga proved to be confined to hard substratum, and consequently in salt marshes it is only found on perennial stems of phanerogams immersed in (temporary) tidal pools. In open areas it prefers fairly sheltered localities, although it can stand (moderate) wave action for some time.

U. implexa may flourish on any hard substratum, while wooden piles, roots, stems, and leaves of (aquatic) plants also afford a convenient base for anchorage.

In the transitional zone between sea- and fresh water, this alga often forms almost bi-algal tufts with *U. subflaccida.* In these areas it has also been found as a companion alga residing in algal mats or bunches consisting of e.g. *U. speciosa, U. flacca, Percursaria percursa, Urospora penicilliformis* and *Rhizoclonium riparium.* In freshwater lakes it may form rope-like tufts of *c.* 3 cm long, which were seen growing together with *Bangia atropurpurea* and *Cladophora glomerata.* In a freshwater stream it was found associated with *U. tenerrima* and *Prasiola velutina.*

The origin of the material in culture

Uni-algal cultures were initiated from plants collected from the following localities. NORWAY: Drammenfjord, Buskerud, Verket, on stones, about the water level; Espegrend, Maripollen near Lønninghamn, high littoral, on sheet-piling and roofing tiles. THE NETHERLANDS: Utrecht, Vinkeveense Plassen, on stones in exposed wash zone; Zuid-Holland, Goeree Overflakkee, Ooltgensplaat, hig littoral,

on concrete face near the sluice, Zeeland; Schouwen Duiveland, De Grevelingen, in the wash zone, on stones; Noord Beveland, Veerse Meer, near Kortgene, in wash zone on stones; Limburg, near Geleen, in stream Geleen, on wooden piles, at the water level in swiftly flowing water. FRANCE: Ile de Batz, near Roscoff, in supratidal pool, about the water level; Terenez, near the mouth of a freshwater stream discharging into an intermittently flooded salt marsh.

Notes on herbarium material

The identification of the herbarium specimens was sometimes difficult, especially when the material was sparse, or mixed with other algae. When the dried filaments were not reproductive one had to rely on the available cell dimensions. Identifications could be made with certainty only when the collections contained (a-)sexual plants. Particularly the presence of finely split cell contents of the gametangia warranted an indisputable identification.

5. Ulothrix subflaccida Wille — Figs. 18—19; Plate 16.

Ulothrix subflaccida Wille (1901) 27, *p.p., type incl.*; ? Jónsson (1903) 357; Cotton (1912) 109; Hylmö (1916) 4; Jørstad (1919) 61 (? *p.p.*); ? Printz (1926) 233 (*p.p.*); Lakowitz (1929) 107; Carter (1933) 131; Lund (1934) 23; Levring (1940) 2; Kylin (1949) 13; ? Parke (1952) 12; ? Sundene (1953) 139; Van den Hoek (1958) 204; ? Jorde & Klavestad (1963) 76; ? Perrot (1970) 932; Pankov (1971) 76. — Lectotype: Norway, Dröbak, on stones in the littoral zone near the Biological Station (intermingled with *Ulothrix implexa*), *Wille, ex Kryptogamae Exsiccatae 2145* (UPSV, holo; BM, BR, FI, L sheet 920.13-380, LD).

Gametophytic plants, mainly from brackish waters, up to 3(—5) cm long, mostly bright green. *Thalli* flaccid and upon moistening glossy. *Filaments* solitary or regularly grouped together in tufts or crowded and entangled to form dense patches; straight when young, later on sometimes slightly curled, or geniculately bent, strictly unbranched and uni-seriate. *Cell wall* usually firm and thin, with increasing age sometimes slightly thickened and somewhat verrucose or crenate, occasionally studded with fouling organisms and/or micro-particles. *Cells* usually cylindrical, their polar ends closely appressed to one another, at times somewhat inflated with more rounded ends. Transverse cell walls sometimes tunicated by H-shaped remnants of parent cell walls. Cells often arranged in pairs or in groups of several cells. Cell diameter (4.8—)7.7—12.1(—13.2) μm, cell height (3.6—) 4.8—15.7 (—18.1) μm; young cells 4.8—7.7 μm wide. The lowermost cells occasionally gradually slightly tapering towards the filament base.

Chloroplast parietal, variously shaped mainly according to age and environmental conditions. Chloroplast in young cells regularly shaped, usually extended over half of the cell circumference, particularly in longer cells not extending over the whole length; its margins in both short and longer cells hardly lobed. When mature, particularly in compressed cells, the chloroplast usually extended towards the transverse cell wall, with a straight or slightly lobed margin, or sometimes delicately fissured. Besides the main chloroplast, sometimes with additional smaller chloroplast lumps. Chloroplast girdle rarely closed, under less favourable conditions bleaching and simultaneously withdrawn to cover only the lateral wall, or withdrawn to a corner of the cell; then the chloroplast with a slightly notched or fimbriate fringe, laden with assimilates; upon heavy accumulation the chloroplast contours fading. Cells with one *nucleus*, often situated opposite the pyrenoid(s). *Pyrenoids* 1(—4) per cell, almost globose, in very short cells somewhat compressed,

in long cells slightly narrowed towards the polar cell ends. Average size of the individual pyrenoids decreasing with the increasing number of pyrenoids per cell. Pyrenoid contour generally distinct, enclosed by only a delicate cap of starch.

Basal cell in germling mostly differentiated to a simple lengthened rhizoidal cell, (slightly) dilated at irregular intervals and usually narrowed towards its end; sometimes only becoming slightly inflated, or somewhat elongated and curved halfway. Mature basal cells usually lengthened, sometimes sparsely branched, forming local proliferations or bulges. Some cells above the basal cell occasionally developing rhizoidal outgrowths serving for a firm grip on the substratum. Infrequently, in germlings the 1—4 lowermost cells slightly elongated, and the next cell forming a well-developed simple or sparsely branched rhizoidal appendage, sometimes also present in the central and upper part of the filament. As an exception, secondary rhizoidal projections also growing inside into the empty cell lumina of former reproductive cells. Chloroplast in germling rhizoid cells as an open band, nearly completely occupying the cell girth, upon lengthening of the basal cell extending in the length axis of the cell and producing more pyrenoids; in very aged basal cells the chloroplast becoming bleached and sometimes withdrawn to the cross wall, and ultimately disappearing. *Apical cells* normally rounded, in young plants at times slightly attenuate or bent, very rarely developing rhizoidal projections as in the basal cells and then the filaments assuming a loop-shaped growth habit. Sometimes one or more cells in the filaments failing to develop properly; or much inflated.

Asexual reproduction by zoospores or aplanospores, formed in ordinary vegetative cells, unmodified apical cells included; not in basal cells and in those producing secondary projections. *Zoosporogenesis* usually initiated in the apical cell, often progressing downwards and finally over the whole thallus. When vegetative propagation was present in the apical part of the filament the more centrally located cells remained capable of inducing reorganization of the cytoplasm preparatory to zoospore formation. On maturation, neither zoosporangia nor aplanosporangia showing a pronounced change of colour. *Zoosporangia* (6.7—)7.7—13.6 μm wide, 5—20 μm long, hardly inflated. The cell length/width ratio of wide zoosporangia usually larger than in comparable vegetative cells. *Zoospores* liberated sometimes one by one, but mostly together in a hyaline vesicular envelope, through an irregular pore. Only seconds after release the enveloping vesicle collapsing, followed by violent swimming movements of the zoospores. Zoospores (1—)2—4(—8) per cell, 7.2—16.3(—17.2) μm by 5.7—9.6 μm, fourflagellate, normally spindle shaped to ovoid, sometimes asymmetric by inflation of the anterior pole or by flattening of the longitudinal side; no apical papilla observable. Zoospore chloroplast normally cup-shaped or partially open, with tiny incisions, sometimes crumbled; provided with a single large conspicuous posterior pyrenoid and a distinct relatively coarse median to posterior stigma; positively phototactic, showing rapid gyrate movements, often jerky and with abrupt changes of direction. Prior to settlement of the zoospores there is sluggishly spinning close to the substratum with the anterior pole foremost; before or just after settlement becoming spherical, and shedding of the flagella. *Germination* bipolar, at the beginning simply by enlargement and subsequent elongation, soon followed by transverse cell division into two cells, the lower cell producing the basal cell, the upper cell dividing again, and finally developing into a filament of several cms long. Cells infrequently converted into sometimes inflated aplanosporangia, as a result of arrested zoosporogenesis. *Apla-*

nospores 1—8 per cell, 7.7—15.6 μm long, 5.8—7.7 μm wide, if spherical 5.8—8.6 μm in diameter, at germination bursting through the lateral aplanosporangial cell wall.

All vegetative cells able to produce gametes, the unmodified apical cell included; not the basal and differentiated intercalary cells. *Gametangial areas* commonly firstly occurring in the uppermost cells of the filament and extending downwards in the plant; sometimes gametangial areas alternated by zoospore-producing cells. Filaments almost straight when entirely filled with gametangia. *Mature gametangia* (7.0—)7.7—12.5 μm wide and 5—12(—16) μm long; no change of colour and hardly inflated. In general the average length/width ratio in wide gametangia more than in comparable vegetative cells. Gametes usually discharged from the parent cell all together in a delicate common envelope. Upon release of the vesicle, just after dehiscence of the lateral cell wall, the still enclosed gametes are often already mobile; the vesicle soon collapsing. *Gametes* (2—)4—16 per cell, 4.3—8.7(—10.9) μm by 2.2—4.3 μm, biflagellate, slenderly spindle-shaped to ovoid, sometimes bilaterally asymmetric or posteriorly pointed. Chloroplast of gametes parietal, cup-shaped, occupying 1/2—3/4 of the gamete cell body, with an often hardly discernable pyrenoid and a median to (slightly) posterior usually distinct stigma. Gamete movements usually lively, erratic-skittish, positively phototactic. Prior to quiescence, the gamete often becoming spherical, concurrently with a slow gyratory movement and with their flagella laterally stretched out in one line; at quiescense, the gametes occasionally still elongate with randomly stretched flagella, often followed by loose attachment to the substratum and subsequent loss of the flagella. Quiescent gametes sometimes remaining suspended without showing a prompt detachment of one or both flagella. Both suspended and attached gametes, and those not released from the parent cell, mostly showing germination into one-celled *sporophytes*. Gametic fusion principally isogamous and dioecious. Quadriflagellate zygotes negatively phototactic, upon attachment becoming spherical and showing subsequent disappearance of the flagella. Germination simply by enlargement to form circular, pyriform, or irregular bent sporophytes, containing a much extended parietal chloroplast with 1—3 embedded pyrenoids; these sometimes with a reticulate appearance when vacuolated. Sporophytes laden with hyaline assimilate grains, when kept under prolonged adverse conditions. Mature sporophytes predominantly spherical, exceptionally somewhat pyriform; cell diameter up to 51 μm, containing 16 or more zoospores or aplanospores. These similar in phototactic behaviour, size, shape, and subsequent developmental stages as zoospores originating in filaments.

Vegetative propagation by disintegration of the filaments. Plants after prolonged stay under adverse conditions showing an *akinete*-like phase, characterized by distinctly inflated thick-walled cells, up to 18 μm in diameter and completely filled with starch.

NORWAY. Steinviksholm, *Wille*, 10/7/1906 (O); Espegrend, Lønninghamn, on stones in the wash zone, intermingled with *Ulothrix implexa*, *Lokhorst*, 25/4/1975 (L); Drammenfjord, near Hurum, Verket, in fast-flowing water on stones, intermingled with *Ulothrix implexa*, *Lokhorst*, 22/4/1975 (L); Dröbak, *Wille*, 10/4/1898, 3/4/1912, 27/4/1918 (O, S, UPSV); *Hylmö*, 3/4/1912 (LD).

SWEDEN. Kullen, Arild, intermingled with *Urospora penicilliformis*, *Levring*, 26/4/1933, 17/3/1934 (GB); Kullen, Foglaviken, on *Fucus serratus*, *Levring*, 14/5/1934 (LD, GB, S); Barsebäck, *Sjöstedt*, 19/2/1928 (LD); Malmö, Limhamn, *Sjöstedt*, 23/7/1912, 16/6/1913 (LD, O); Karlshamn, intermingled with *Urospora penicilliformis*, *Levring*, 6/11/1939 (GB); Ronneby, in a littoral basin, *Levring*, 8/4/1939 (GB); Torhamn, growing at 0.5 m depth, *Levring*, 8/5/1937 (GB).

GERMANY. Kiel, as *Hormiscia implexa*, *Reinbold 46*, 1889, *ex herb. Holmes* (BM); Kieler Föhrde, Schilksee, on balks and stones, *Hoffman*, 28/4/1939 (C);

THE NETHERLANDS. Den Helder, in the harbour on stones among the high water mark, in algal vegetation, *Den Hartog 2075*, 6/12/1954 (L sheet 956.313–527); Noordzee Kanaal, South-side near Velserpoort, in the wash zone, as *Codiolum penicilliforme*, *Mulder*, 6/7/1954 (L sheet 956.312–937); Hellevoetsluis, harbour, in the high littoral zone, among halophylous phanerogams, *Van den Hoek 4036*, 13/5/1957 (L sheet 959.212–715); Veerse Meer, on stones in the wash zone, *Prud'homme van Reine*, *Algae Zeelandicae 81*, 24/2/1977 (L); Kanaal door Zuid-Beveland, on stones, together with *Rhizoclonium riparium* and young *Enteromorpha* plants, *Den Hartog 1067*, 25/10/1951 (L sheet 956.313–523); Terneuzen, in the outer harbour, on chasers, below water level, *Koster 1180*, 7/4/1948 (L sheet 947.160–65); Braakmanpolder, in the Westgeul, *De Visser*, 21/1/1959 (L sheet 959.41–033).

BELGIUM. St. Anna near Antwerp, on concrete stones, near the wash zone, *Lokhorst*, 7/3/1975 (L).

GREAT BRITAIN. Point of Ayr Flints, as *Ulothrix cutleriae* and *Lyngbya cutleriae*, *Batters*, 20/7/1886, *ex herb. Traill* (BM, E); Berwick, as *Ulothrix implexa* and *Ulothrix cutleriae*, *Batters*, 28/1/1888, 3/2/1888 (BM, E, KIEL, L sheet 939.26–24).

FRANCE. Le Havre, sur les vases marines, as *Hormotrichum penicilliformis*, *Dupray*, May, *ex Algues des Eaux Douces de la France 1089* (L sheet 939.26–59, PC); Environs of Le Havre, in stagnant brackish water as *Hormiscia implexa submarina*, *Dupray*, *ex Algues de France 590* (BM, L sheet 939.26–37); Tatihou, as *Ulothrix pseudoflacca*, *Hariot*, 4/1911 (PC); Cherbourg, as *Ulothrix implexa*, *Bornet*, 30/3/1853, *ex herb. Thuret* (UPSV); Saint Malo, intermingled with *Cyanophyceae* and *Enteromorpha*, as *Ulothrix implexa* and *Urospora mirabilis*, *Hamel*, 13/3/1922, 9/4/1922 (PC); Saint Lunaire, Pointe du Decoltè, as *Ulothrix pseudoflacca*, *Lami*, 1940 (PC); Saint Servan, as *Ulothrix subflaccida*, *Hamel*, 3/1921 (PC); Terenez, on stones near the mouth of a freshwater stream discharging into an intertidal salt marsh, intermingled with *Ulothrix implexa*, *Lokhorst*, 9/4/1975 (L); Banyuls sur Mer, *Feldmann 2118*, 24/3/1932 (C, PC); Port Vendres, as *Ulothrix pseudoflacca*, *Feldmann 2182* (L sheet 940.130–140); Étang de l'Arnel, Herault, on *Ruppia*, *Van den Hoek 3742*, 28/7/1957 (L sheet 959.151–705); Étang de Prévot, among *Ruppia spiralis*, *Chaetomorpha linum*, and *Monostroma oxyspermum*, *Van den Hoek 3727*, 19/8/1957 (L sheet 959.151–704).

Nomenclature and historical aspects

As stated earlier in this paper, the delimitation of the two closely related brackish-water *Ulothrix* taxa, in this study accepted at specific level as *U. implexa* and *U. subflaccida*, has presented many problems in the past. Doubts concerning the taxonomic status of *U. subflaccida* started in 1901, because of Wille's original description which was not conclusive as to its identity. It is clear that Wille was not aware of the existence of two closely related taxa. As a consequence, his description of *U. subflaccida* also included *U. implexa*, and therefore it was not surprising to find both taxa together among Wille's herbarium specimens, *U. implexa* in the great majority and the smaller *U. subflaccida* only in small quantities. In spite of this scarcity these latter specimens are hereby designated as the holotype (*Kryptogamae Exsiccatae no 2145*, preserved in UPSV). Almost uni-algal *U. subflaccida* sheets have been collected by Batters from Point of Ayr Flints in 1886. This herbarium sheet, preserved in BM and E, and labelled as *Ulothrix* (*Lyngbya*) *cutleriae*, is hereby recommended as good illustrating specimen.

Some later authors, like Hylmö (1916), Carter (1933), Levring (1940), Kylin (1949), and Pankov (1971) reported more proper diagnostic characteristics for *U. subflaccida*, such as the cell diameter range of *c.* 5—12 μm and the presence of 1(—2) pyrenoids. In the light of the knowledge revealed in this study, the reported features on the vegetative stages by these authors may pertain to mature vegetative filaments of *U. subflaccida* as well as to younger filaments of *U. implexa*, however.

Morphology

The *general habit* of plants grown in cultures is quite similar to that of plants collected from all sorts of natural habitats. The length of the threads varies, however. In field observations plants have only been seen to attain a length of up to 3 cm, while they may grow up to 5 cm in culture. It is conceivable that natural conditions favour a more vigorous and spontaneous reproduction in mature filaments, which may start at an earlier growth stage, than might happen under artificial circumstances. In plants in nature this will result in an earlier complete disarticulation of the entirely empty filaments. In stagnant cultures, after dissemination and subsequent attachment of the zoospores, simple filaments appeared on the inner walls of the culture vessel, in course of time producing a dense flexible algal stratum, several cms. in length. Sometimes, zoospores remained buoyant on quiescence, following the same developmental stages as observed on attached zoospores. In liquid media, especially under long-day conditions, this alga was found to be a rapid grower. In neglected cultures the filaments sometimes became detached from the walls. Free-floating or detached plants were never found in considerable quantities in nature, however. After inoculation on agar plates this alga appeared to grow less rapidly.

The *cell wall* is usually firm and smooth, but after prolonged stay in a culture medium of $16^0/_{00}Cl^-$ and in nature, especially when growing on soft substrates, the cell wall becomes slightly verrucose or crenate. Extreme salinities ($0^0/_{00}Cl^-$ and $45^0/_{00}Cl^-$) in culture solution occasionally led to seemingly soft cell walls, which may finally decay in the vicinity of the transverse cell wall, followed by entire disintegration of the plants into slightly inflated and lengthened cells. Filaments with little inflated cells and with apparently soft cell walls were also found once in plants which were found continuously submerged in tidal pools in the salt marsh of the Balgzand (Netherlands). The culture of these filaments produced firm cell walls in time. The factor responsible for this phenomenon in nature is unknown. The surface of the filaments is sometimes contaminated with micro-epiphytes and/or micro-particles.

In the field collections vegetative *cells* have been seen up to 12—13 μm wide, but also several populations were found in which the width of the filaments did not exceed 10 μm. The various culture conditions, like different photo periods, salinity amounts, and temperatures, did not affect the cell diameter range significantly. At lower salinities (0%—$2^0/_{00}Cl^-$) the germlings tend to be backward at the beginning of their development, although after 9 weeks under unchanged external culture conditions, the absolute cell diameter range of plants grown in the overall salinity series (0%—$16^0/_{00}Cl^-$) has become similar.

The development of the *chloroplast* is slightly inhibited at low salinity. Furthermore, after inoculation of filaments in such culture solutions, the smaller nutritional value is reflected in an apparently earlier withdrawal and simultaneous bleaching of the chloroplast, often accompanied by the accumulation of storage grains. In general, healthy-looking plants, with a cylindrical outline of the cells and showing a regular fully-developed chloroplast girdle, are most frequently encountered in the salinity range 4—8(—16)$^0/_{00}Cl^-$ and in nature chiefly in almost ever moist habitats. After a long period of desiccation in the field, for instance caused by lowering of the water level or by change of the prevailing wind direction, the thallus becomes yellowish green, the cells with an unhealthy-looking chloroplast packed with

assimilates and with a hardly traceable pyrenoid. The transfer of such plants to liquid culture medium soon results in the normal growth habit. In plants from nature, usually only one *pyrenoid* per cell is observed.

This alga was found to form intercalary projections, especially when growing on soft substrates, for example in salt marshes. In liquid cultures no special factor or condition responsible for the sprouting of these appendages could be detected. The *lowermost cell* in germlings mostly develops into a rhizoidal base, which apparently anchors on any substratum. A firm attachment is achieved by the forming of a thin mucilaginous layer, although this is not always produced along its whole length. Sometimes, some germlings, particularly those grown in freshwater culture solution, do not develop a differentiated base. Then only a slightly swollen or somewhat elongated cell develops which may persist in mature filaments, such as was also frequently observed in plants carefully scratched off from natural substrates. In cultures, the apical cell of germlings is very rarely elongated like the rhizoidal basal cell. This phenomenon has never been noticed in mature plants in culture, nor in any stage of development in nature.

Reproduction and life history

In the light of the confusion on the taxonomy and nomenclature, and position of both *U. implexa* and *U. subflaccida* as discussed earlier, it is not surprising that little reliable information was known about the reproduction of this alga. Most of the knowledge of the reproduction reported in literature for *U. subflaccida*, apparently concerns *U. implexa* (Wille, 1901, 1913; Kornmann, 1964). However, in 1919 Jørstad reported observations on zygotes and their developmental stages in *U. subflaccida*, collected with the filamentous phase from natural habitats. Unfortunately, he did not perform extensive culture experiments to link these stages. Perrot (1970) accomplished the life history of *U. subflaccida* from the coast of La Manche. Besides the statement that the morphology of the habit of her species used was identical with that described by Wille (1901; 1913), neither figures nor obvious comments on the taxonomic status of the alga were given. Consequently, it cannot conclusively be determined whether the alga studied was actually *U. implexa* or the genuine *U. subflaccida*.

In general, after inoculation of vegetative *U. subflaccida* plants into a fresh culture solution, the cells readily begin to produce zoospores. Short-day conditions tend to induce a more vigorous reproduction than long-day conditions do. Furthermore, short-day conditions apparently cause sustained reproduction in consecutive generations in one culture vessel. Zoospore formation occurs in all salinity amounts used. However, it became obvious that three week old plants already commonly showed asexuality under short-day conditions at $8\,^0/_{00}Cl^-$, while in plants grown in 0, 2 or $16\,^0/_{00}Cl^-$ the zoosporogenesis remained backward and did not occur before the filaments were 4—5 weeks old. In general, the rate of asexual reproduction lagged behind most obviously in plants grown in Gorham medium. The observations on the asexual reproduction in filaments cultivated under long-day conditions, were fairly variable, and not suitable for commentary.

Zoospores in cultures show quite the same variability in form as those in field material. After detachment of the flagella from the zoospore body the individual flagella may display irregular jerky movements for a while, concurrently showing a

balloon-like swelling at the base. The zoospores are usually released all together from the parent cell ensheathed by a common delicate hyaline vesicle. Rarely, in filaments grown at $16^0/_{00}Cl^-$, the zoospores are released one by one, and very laboriously, however. In that case the zoospore wriggles through a lateral pore which is much smaller than the zoospore, by which it becomes, when half-way, dumb-bell shaped. This manner of release is essentially identical to that observed in *Klebsormidium* (Cain *et al.*, 1973). Aplanospores were seen in aged cultures grown under long-day conditions and at times in material from natural habitats.

Unlike that established for the true marine *Ulothrix* species, the zoospore germination of this alga exclusively gives rise to only one filament per zoospore as do the freshwater *Ulothrix* species (Lokhorst, 1974).

Abundant sexuality in this species was only present under long-day conditions in moderately saline culture solutions ($2—8^0/_{00}Cl^-$). In Gorham medium ($0^0/_{00}Cl^-$) sexual reproduction is rarely seen, and then this possibly should be regarded as the reaction of the alga to adverse conditions. Furthermore, it was obvious that in the cultures initiated from filaments derived from Verket, Espegrend, Ouddorp, and Terenez, respectively, the sexual reproduction disappeared, either immediately or in course of time. Filaments from Espegrend in particular, had been seen to produce gametes profusely at the time of collecting. During the differentiation of vegetative filaments to gametangia the uniform habit of the filaments did not drastically change. The gametangia were often almost isodiametric and hardly inflated at all. Upon ripening, the gamete-producing cell did not undergo any colour change, differing in this respect from the other *Ulothrix* species studied. Both zoosporangia and gametangia may be formed in the same plant. In cultures this species exclusively has a dioecious reproduction. As an exception, on one occasion, gametes released from plants, collected in the Balgzand, showed monoecious fusion. The lack of forming of zygotes in cultures was compensated by the ability of the gametes to germinate freely parthenogenetically to a sporophyte, which discharged zoospores in course of time under short-day conditions only. An extensive field collection of sexual plants was made in the Veerse Meer, presently an inland brackish-water lake, since 1961 cut off from the open sea-arm Oosterschelde. None of the thousands of lively gametes observed showed copulation tendencies in the laboratory, however. These observations were made in both concentrated and weak gamete-suspensions. A plausible inference would be to assume the presence of a sequestered population of *U. subflaccida* in this lake, but it is difficult to accept that the recent closure of the lake separated a population of plants of one sex only. Further investigations on *U. subflaccida* populations in this lake are needed.

After the disappearance of sexuality in several clones, the chance of achieving zygotes in cultures was considerably reduced. Nevertheless, one attempt finally succeeded with the joining of gametes derived from clones from the Veerse Meer and from Vlissingen. The zygotes remained mobile for a period of a few minutes to several hours. The sporophytes were never seen to be stalked as in *U. implexa*. Maturation of these sporophytes could only be accomplished after transfer of the zygotes to short-day conditions.

In nature this species may very probably produce a sporophytic generation two or more times during one optimal vegetation period. In the Veerse Meer, besides a mass of sexual plants, also young and (nearly) fertile sporophytes could be collected in February, during the mild winter of 1977. The peak in the presence of sexual plants in this species usually is not reached before March or April.

Filaments with akinete-like cells were found to be produced in relation with adverse external conditions; attempts to promote the growth of true akinetes failed.

Ultrastructure

Cell division. Despite several attempts, occurrence of cytokinesis could not be studied. It is assumed, however, that the cell division in this species closely resembles that of the much related *U. implexa.*

Cell wall. The composition of the cell wall differs from that described for the other *Ulothrix* species studied. For the greater part it consists of one major component, in which the microfibrils often seem to be arranged more or less parallel along the whole circumference of the cell lumen. Local stratification in this layer, occasionally observed halfway along the lateral wall of an individual cell, seems to be due to a different arrangement of the microfibrils, which causes that the middle portion in the cell wall appears to be more compact. Only in the vicinity of the transverse wall this major component is sometimes laterally wrapped by a thin electron denser layer which may intrude the transverse cell wall slightly. The cell wall is usually smoothly surfaced and seems to be delimited outside by a double membrane system, not visible in most of the micrographs, however. On the contrary, the lateral cell wall is frayed at times, which may be caused by bacterial injury or the result of a prolonged drastic fixation procedure.

Although the present ultrastructural study only concerned the substructure of vegetative cells, as a matter of course also the reproductive stages underwent the fixation procedure and subsequently were longitudinally sectioned. The organization of the cell wall of the zoosporangium appeared quite similar to that of the vegetative cells. Furthermore, it was seen in nearly zoospore-releasing cells that the set-in of the disintegration of the lateral wall prior to zoospore release started in its peripheral area and subsequently proceeded inwards. The middle portion of the transverse cell wall may be obviously swollen. Lomasomes were usually found.

Chloroplast. EM-observations support the observations made with the light microscope. The chloroplast may occupy partly or entirely the transverse wall in isodiametric cells or in cells wider than long, whereas in (strongly) lengthened cells the transverse cell wall usually is not covered by the chloroplast. The chloroplast extensions towards the transverse cell wall are fairly compact, without distinct fissures. Its ultrastructure is fundamentally identical to that in the marine *Ulothrix* species. The thylakoids terminate all abruptly in the tapered ends of the chloroplast lobes. They occasionally are single, but usually there are up to seven assembled into straight or slightly undulated lamellae. The course of adjacent lamellae is more or less parallel, though these fuse at intervals, hence showing a reticulate appearance. As ascertained in *U. implexa*, the chloroplast of the present *U. subflaccida* shows thylakoids which are often arranged in conspicuous short lamellae. The space between adjacent lamellae is filled by a ground substance, in which approximately equally dispersed ribosomes can be seen. Plastoglobuli may appear singly or crowded, sometimes proximate to the pyrenoids. They are fairly equally spaced and are almost circular to oval of shape. Starch grains in the stroma appear as white mottles, sometimes with a faint grey margin, between the lamellae. They are often

situated in the immediate vicinity of the pyrenoid. In longitudinal section they have an almost oval, elliptic, circular, or even a bent appearance. Most of the starch, however, is present as segmental grains in the starch shell enclosing the ubiquitous pyrenoid.

The pyrenoid fills a considerable portion of the chloroplast volume. It is situated in the mid-region of the cell, mostly opposite the nucleus. In longitudinal section it mostly has a circular shape, or sometimes it is slightly stretched. The organization of the pyrenoid recalls that in *Ulothrix flacca* and *U. implexa*. No thylakoidal rows invade the pyrenoid, though 1—6 thylakoid lamellae may traverse the starch cap; close to the pyrenoid matrix they branch off and subsequently become pinched in between the pyrenoid and the starch cap. In gaps in the starch envelope 1—6 thylakoid lamellae may be closely parallel to the pyrenoid.

Nucleus. In longitudinal section the nucleus is circular or slightly elongated. In cells with a nearly completely closed chloroplast the nucleus seems to be pinched between the lateral chloroplast lobes. It is also observed as bordered by a ring of mitochondria or a mass of dictyosomes and vesicular structures. The nuclear organization is fundamentally identical to that described previously for other species.

Mitochondria. The mitochondria are localized proximate to the transverse cell wall and adjacent to both chloroplast and nucleus proper. In general, they are ovoid bodies, although sometimes they are elongated or with an irregular shape. Their ultrastructure is like that of *U. implexa*.

Golgi apparatus. Since the dictyosomes are only sparsely present in the sections, no details can be given on their ultrastructure morphology. Judged from the sparse observations, however, they probably agree with those of *U. implexa*.

Cytoplasm inclusions. Osmiophilic droplets are found randomly in the cytoplasm. They have a strongly variable form. They appear mostly single, but at times they are also seen to be clotted together. The presence of the minute vesicular bodies, containing one to several stellate osmiophilic piths underlines the close affinity of this species with *U. implexa*. Peroxisomes are localized close to the lateral chloroplast lobe.

Vacuole. In longitudinal sections vacuoles are hardly present in the cell lumen. As in *U. implexa*, the cytoplasm of healthy-looking filaments is apparently not forced to a great autophagic activity.

Ecology

U. subflaccida has seasonal periodicity. Its filamentous stage is very common in winter and spring, whereas in the remaining seasons threads are only sparsely encountered in nature. Its distributional pattern illustrates a fairly wide ecological range and for the most part coincides with that of *U. implexa*.

Den Hartog (1967, 1970) distinguished nine main types of brackish-water habitats with instable salinity conditions, according to the continuity and the discontinuity of the transition between sea and fresh water and the rate of

periodicity of fluctuations in salinity. During the present study *U. subflaccida* has been found in six of these habitats, namely: in estuaries, in canal (river) mouths not directly subjected to the tides, in so-called shock-habitats such as fresh water streams and trickles in the intertidal zone which are periodically submerged by sea water, in supralittoral pools reached by spring tides and storm floods, in inland brackish water as in the wash zone of brackish-water lakes, canals, ponds and ditches, and finally in the upper eulittoral and lower supralittoral zone of (artificial) rocky shores. In view of this wide-spread occurrence in brackish waters, *U. subflaccida* cannot be regarded as a reliable indicator species, characteristic of one of these brackish habitat types. It might only be considered as a general indicator of the presence of brackish water, and actually this alga does not invade pure sea and pure fresh water in large quantities.

In open habitats it prefers fairly sheltered localities, although it can assumably withstand wave action for some time. Several times it was observed that the alga could stand long periods of desiccation. It does not seem to have definite requirements as regards the substratum, since wooden piles, roots, stems and leaves of higher plants, all sorts of stones, iron-wire, and even plastics proved to be a suitable anchorage. Its occurrence is not confined to hard substrates, and it has also been found thriving on soft sandy or clayey soils in both inland and open salt marshes. Furthermore, it has been found as a pioneering alga in open land reclamation regions and in recently reclaimed polders. In the tidal salt marshes it may occur in pure sheets where there is plenty of bare soil not colonized by phanerogams. In these areas it may also inhabit vertical eroded walls of hummocks, and steep banks of intersecting creeks. Besides of forming pure algal sheets on the surface of the soil, this species may occur as a companion species in flappy algal vegetations mainly comprising *Enteromopha* species, or in firm algal carpets consisting of *Rhizoclonium riparium*, *Vaucheria* spp., *Ulothrix speciosa*, *U. palusalsa*, and blue-green algae. It also frequently occurs on soft substrates overshadowed by halophytic phanerogam growth. In these localities, it is also found festooning (perennial) stems and roots of halophytic plants.

The origin of the material in culture

Uni-algal cultures were initiated from plants collected from the following localities. NORWAY: Drammenfjord, Buskerud, Verket, on stones at about water level; Espegrend, Maripollen near Lønninghamn, high littoral, on sheet-piling and roofing-tiles. THE NETHERLANDS: Friesland, Wierum, on eroded escarpment in the marginal zone of salt marsh; Noord-Holland, Balgzand, in tidal pool of salt marsh; Zuid-Holland, Goeree Overflakkee, De Grevelingen, near Ouddorp, in the wash zone; Zeeland, Noord Beveland, Veerse Meer, near Kortgene, in the wash zone, on stones; Zuid Beveland, salt marsh near Sas van Goes, on bare moist sandy soil; Walcheren, Vlissingen, high littoral on a wooden landing stage. FRANCE: Ambleteuse, near the mouth of the river Slack, discharging onto beach; Terenez, near the mouth of a freshwater stream discharging into the intertidal salt marsh.

Notes on herbarium material

The identification of the herbarium specimens was sometimes difficult, particularly when the species occurred in strongly intermingled algal sheets. Identifications have been made largely on vegetative characters and zoosporangia since sexual plants were rarely seen in herbarium collections. The chance for an incorrect

identification of young filaments of *U. implexa* was reduced by measuring the average cell diameter and average cell height of many filaments.

CONCLUSIONS

The present study has revealed the existence of five species occurring in marine and brackish-water habitats in western Europe, namely, *Ulothrix speciosa*, *U. flacca*, *U. palusalsa*, *U. implexa* and *U. subflaccida*. It appeared to be possible to maintain the names *U. speciosa* and *U. flacca*, as well as *U. implexa* and *U. subflaccida*. Their controversial status under *Ulothrix* was mainly caused by inadequate original descriptions, and also, as it became clear from review of the literature, because successive later taxonomists modified the original species concept without carrying out herbarium studies, by which the species became entirely unrecognizable.

The relatively wide range in cell diameter found in the vegetative filaments of each species studied, makes the main use of this character for the identification of the species rather inadequate; traditionally this was done in several algal floras. Modern taxonomic study of *Ulothrix* should be amplified by the study of the development, the reproduction, the life history, the ultrastructure, and the geografical and ecological distribution. Unfortunately, for a collector who wishes to identify *Ulothrix* samples to the specific level in a collection from nature, all features for a reliable identification are not always present, particularly when the material is scarse. Therefore, especially with respect to the smaller *Ulothrix* species, cultures have to be initiated for unequivocal identification. When many filaments are available and when culture cannot be performed, one has to rely on the morphological/reproductive features, these being the least variable. The most reliable and helpful characters for identification of this group of *Ulothrix* species studied, is mentioned here in some detail.

Two species, viz. *U. speciosa* and *U. palusalsa*, are quite distinct from the other species by the peculiar soft nature of their cell wall, which is clearly manifested in both field and culture material. These two species show a close morphological relationship to one another, and form a clear-cut taxomonic group. Mutually, they can be distinguished by the limited variation of cell diameter of the mature vegetative cells and the gamete-producing cells, the variation in number of gametes, and their ecological distributional pattern. Also on the basis of the ultrastructure *U. speciosa* and *U. palusalsa* appeared closely related. The pyrenoid and the cell wall, for example, are fundamentally similar in construction, but still there are specific differences such as in the length and form of the terminative end of the chloroplast invaginations in the matrix of the pyrenoid.

By the firm nature of the cell wall, the remaining *Ulothrix* species studied, namely *U. flacca*, *U. implexa* and *U. subflaccida* can be distinguished as a second clear-cut taxonomic group. In nature the cell wall is mostly roughly surfaced in *U. flacca*, whereas it is only occasionally so in *U. implexa* and *U. subflaccida*. Furthermore, *U. flacca* frequently has coalescence of the filaments and a typical growth habit, differentiated in a basal and an erect system. The limited variation of the diameter of mature vegetative cells, zoosporangia, and gametangia are reliable and useful features in this species. The identification of *U. implexa* and *U. subflaccida* may be

very troublesome especially when little material is available. These species are hardly to identify reliably when the filaments have a cell diameter of *c.* 10—12 μm, the main transitional cell diameter range. Initiation of cultures is inevitable in such cases. On the other hand, a luxuriant growth of these algae, particularly when occurring solely, facilitates their specific identification. Useful characters of these species in nature are the limited variation of cell width of mature filamentous plants, the number of zoospores and gametes produced, the change of colour during ripening of the gametangia, the range of cell diameter of zoosporangia, especially of those of gametangia, and finally the ecological distributional pattern, although the two species often grow intermingled. Additionally, *U. implexa* rapidly produces secondary projections inside the empty cell lumen of former reproductive cells. It is of special interest to note here the striking resemblance of the ultrastructure of the pyrenoid in the three species of the second group. Their specific delimitation can also be justified by specific ultrastructural dissimilarities of the thylakoid arrangement and the micro-anatomy of the cell wall.

The morphology of the chloroplast has been extensively described and it is obvious that its shape cannot be considered to be a constant specific feature. It may vary from almost half-encircling the cell lumen to forming a complete girdle. Furthermore, it may extend towards the transverse walls, though this is only occasionally so in long(er) cells in *U. subflaccida*. It became obvious that environmental factors are the main cause of the variation in the chloroplast.

The number of pyrenoids has been often cited in literature as an important species-distinguishing feature. Species with a cell diameter of up to *c.* 15 μm would possess only one pyrenoid per cell, while the presence of more pyrenoids per cell would be restricted to the larger species. This study has revealed that actually also this feature shows a remarkable plasticity. For example, the larger species *U. flacca* and *U. speciosa* may have in nature filaments containing only one pyrenoid in a cell. Consequently, the number of pyrenoids cannot be used as a reliable character for the identification of *Ulothrix* species in natural collections.

Both the shape of the basal cell and apical cell are also considered as of little diagnostic value. Generally, in natural material the mature basal cell is slightly lengthened, usually thick-walled, and occasionally tapered towards the substratum. In cultures, in contrast, the plants usually have more complex basal cells.

Besides a number of features found to be useful in the identification of *Ulothrix* samples from nature, the present studies have also put forward reliable developmental and other reproductive attributes, which are mostly better demonstrated in cultures. First of these, the developmental pattern of the zoospores is found to be of particular taxonomic significance. Zoospores of the two brackish-water species germinate consistently bi-polar, consequently giving rise to only one mature filament per zoospore. In the three other, marine, species the germination of the zoospores ultimately may also lead to the formation of one mature filament per zoospore. However, in these marine species, in the 3—10 celled bipolar germlings, individual cells may also show successive divisions perpendicular to their length axes. In *U. speciosa* and *U. palusalsa* this development eventually gives rise to simple-stellate tufts, which consist of an erect system of several (not one) unbranched filaments of indeterminate length. Each filament develops its own basal cell, modified for attachment. The differentiation of germlings is most advanced in

U. flacca. Besides simple and twin-filaments, the zoospores of this species may even ultimately germinate into tufts composed of a small compact prostrate base of restricted growth and an extensive erect portion consisting of frequently coalescent filaments of indeterminate length. The above described growth habits are unique to the genus *Ulothrix*, although hardly to be seen in scratched off natural material. One can observe these growth habits only if the plants are collected together with the substratum and then only after careful analysis of the basal part of the algal stratum with the aid of a stereo-microscope.

Additional useful data in the group studied, which are clearly revealed in the cultures, are reproductive features such as the number of zoospores per zoosporangium, their size and morphology, and phototactic behaviour, the size and shape of the zoosporangia and aplanosporangia (the preceeding types of characters do not apply to *U. speciosa*), the number of gametes per gametangium, and their shape and size, the shape and size of the sporophytes, the number of zoospores per sporophyte, the pattern of fragmentation of the filaments, etc.

In principle, all species studied show a heteromorphic alternation of generations, in which the zygote develops into an independent unicellular sporophyte. The produced asexual spores develop into the gametophytic filamentous stage. Meiosis is assumed to take place in the sporophytes, prior to the cleavage of their cell contents into zoospores. As regards the life history only *U. speciosa* is distinct from the other species in that its gametophytic phase does not produce zoospores which evoke the same generation. Besides the above mentioned type of life history, Perrot (1968, 1970) postulated for marine species of *Ulothrix* from the French coast an additional isomorphic alternation of generations. However, though a considerable number of clones were studied by the present author, not a single example of this type of life history could be detected.

In only two species, namely *U. flacca* and *U. palusalsa*, the sexual reproduction was found to be monoecious in cultures. It is dubious, however, whether this character can be considered as being of major taxonomic importance, since in one field population of *U. subflaccida*, a species which is normally dioecious, only monoecious sexual plants were encountered. Kornmann (1964) apparently found the same plasticity in clones of *U. speciosa*, which were initiated from plants collected from different habitats on Heligoland. In the author's opinion there is no valid reason for giving these clones different specific status, as was done by Kornmann (1964). It is interesting to note that *U. palusalsa* readily produces sexual spores, while asexuality in filaments was restricted to only four of the clones studied. Asexual filamentous plants, moreover, have never been seen by the writer in nature. In the gametophytes of the closely related *U. speciosa*, asexual spores were never found. Phylogenetically it is interesting to question whether this asexuality is disappearing in *U. palusalsa* or whether this feature should be regarded as newly acquired in this alga. In view of the reproductive characters of *U. speciosa* and the fact that asexual filamentous plants could only be examined under favourable culture conditions, the author is inclined towards the first supposition. The remaining species show a preponderance of asexual plants both in cultures and in nature, whilst the induction of sexuality in plants in cultures proved to be difficult and not be achieved in all of the laboratory clones used. Although in *U. implexa* the occurrence of sexuality is clearly correlated with the salinity, it is believed that besides long-day conditions also a number of other factors may influence the sexual behaviour of the species. In this respect one should keep in mind that precise

imitation of all physical and chemical conditions to which the algae are daily subjected in natural habitats, cannot be imitated in the laboratory, and this may be responsible for the occasional lack of sexual plants in cultures.

In handbooks *Ulothrix* is often represented as the prototypical example for an alga with a haplobiontic life history, as also e.g. in *Chlamydomonas* (Scagel *et al.*, 1965; Strasburger, 1971). The zygote is regarded as the diploid phase, which, after a period of dormancy and subsequent meiosis, gives rise to four zoospores, which finally grow into the gametophytic filamentous phase. It is now clear that the life history of *Ulothrix* has to be defined as heteromorphic diplobiontic, because the zygotes germinate by enlargement into an independent, occasionally stalked generation, which is individually able to anchor on a substratum. Upon maturation of the plants it is assumed that the first nuclear division follows meiosis and subsequently the individual daughter nuclei mitosis, eventually resulting in numerous zoospores.

A number of additional data on the ultrastructure strengthens the subdivision in the presently treated species of *Ulothrix*. The cell wall surface, its mode of stratification, and the orientation of microfibrils in the sublayers, as far as could be well interpreted, differ within this group, and are proved to be specific features. The same holds for the micro-anatomy of the pyrenoid. Depending on the species, it may be invaded by chloroplast invaginations on all sides. The differences in the number of thylakoids in the chloroplast lamellae of the various species studied are too minute to consider that as a diagnostic character. By the frequent arrangement of thylakoids in short(er) lamellae, *U. implexa* and *U. subflaccida* have a special position. In the other species the orientation of the thylakoids is usually in long lamellae. On the specific level the organization of the dictyosomes in the Golgi-apparatus shows an obvious variation in number of cisternae. This suggests that this system would also have taxonomic significance but further study is needed to ascertain this. There are a considerable number of chloroplast- and cytoplasmic inclusions described, such as plastoglobuli, peroxisomes, peculiar vesicular bodies, and myelin-like figures. It seems likely that most of these structures are mainly associated with the general cell metabolism and their diagnostic and systematic value is not rated very high as yet. A central vacuole is commonly present in the larger species *U. speciosa*, *U. flacca* and *U. palusalsa*, in both senescing as well as in healthy-looking filaments. Probably its common occurrence in these species is also connected with its function to prevent cell collapse, as also known in higher plants. The nucleus in the species studied is fundamentally similar in ultrastructure.

It is likely that only one type of cytokinesis occurs, the transverse wall formed as an annular ingrowth from the existing lateral walls inward the parent cell. On the contrary, as pointed out by Stewart *et al.* (1973), the cytokinesis in various freshwater *Ulothrix* species would follow a different pattern. The mode of cell division as revealed in the present study in the marine/brackish-water *Ulothrix* species is essentially similar to that described for *Klebsormidium* and *Stichococcus* (Floyd *et al.*, 1972; Pickett-Heaps, 1972, 1974). The mitochondria in the present *Ulothrix* species show a quite identical variable size and shape. In some species they sometimes have very bizarre forms. Since their presence and shape seem to be associated with the activity in the individual cells, it is unlikely that mitochondria provide reliable, useful, specific features. Öpik (1968) defined the type of mitochondrion as in the present group studied, as narrow cristate associated with a relatively light electron dense matrix. Future comparative studies in the group of the *Ulo-*

trichales will possibly prove whether the anatomy of the mitochondria has taxonomic significance on the generic/specific level or not.

The main character for the distinction of *Ulothrix* from *Urospora*, which is in the growth habit the most closely related genus in marine and brackish-water habitats, include the chloroplast morphology and some reproductive features. The chloroplast in *Ulothrix* is essentially continuous. In *Urospora* the chloroplast is girdle-shaped only in young filaments, with advancing maturity it becomes distinctly reticulate. In *Ulothrix* the zoospores are principally ovoid-spindle shaped, whereas those of *Urospora* are characteristically pyramidal and terminate posteriorly in an acute tail.

In 1961, Kornmann reported his findings on *Urospora speciosa*, a species which, due to the almost similar growth habit of filaments, much resembles *Ulothrix speciosa* (in his paper reported as *U. flacca*). Kornmann placed his alga studied in the genus *Urospora*, on the basis of the shape of the zoospores. Unfortunately, the present author did not find any evidence in *Ulothrix speciosa* of asexual filamentous plants, which might clarify the alga's arbitrary position. Furthermore, the asexual sporophytes of *Ulothrix speciosa* produced almost globose-ovoid, not pyramidal zoospores. Because of these findings, some doubts are raised on the existence of *Urospora speciosa*. In all clones collected of *Ulothrix speciosa* its close taxonomic affinity to *U. palusalsa* was apparent in detail. Additionally, the genus *Urospora* is characterized by multinucleate cells. The clones of *Ulothrix speciosa*, used in the present study consistently had only uninucleate cells, which is an *Ulothrix*-feature.

In the account by Islam (1963), three different types of zoospore germination were enumerated for the freshwater genus *Stigeoclonium*. It is noteworthy that zoospores of the marine *Ulothrix* species essentially conform to two of the three reported categories, whilst the fresh and brackish-water *Ulothrix* species do apply to only one type described. It is argued that, in view of the mode of zoospore germination, the marine *Ulothrix* representatives take an intermediate position between the fresh and brackish-water *Ulothrix* species on one side, and the genus *Stigeoclonium*, with branched filaments, on the other. The evolutionary development of the marine forms has presumably been stagnant while, on the contrary, some similarly shaped progenitors in fresh water presumably succeeded to differentiate into the forms nowadays known as *Stigeoclonium*. Anyhow, the particular zoospore germination in *Ulothrix* provides additional evidence for its position in the evolutionary lines of advance which gave ultimately rise to higher plants.

According to Den Hartog's classification of brackish-water habitats (1967, 1970) all *Ulothrix* species, dealt with in the present paper might be regarded as brackish-water algae. I only agree for *U. subflaccida* and *U. implexa*. Both species actually have an optimal distribution and growth in habitats with unstable salinity, caused by (diurnal) change of the sea and fresh water compound. Furthermore, it is accordingly proved in culture experiments that their general habit and the range of the cell dimensions are hardly affected by different salinity concentrations. Consequently, these species can be considered as true indicator algae for brackish-water. It is interesting to note that *U. subflaccida* may also inhabit soils in salt marshes where *U. implexa* proved to be strictly absent. On the contrary, *U. implexa* is able to flourish plentifully in fresh water.

Though *U. speciosa* and *U. flacca* may invade brackish waters, they only grow luxuriantly in the (lower), middle, and upper littoral of open coastal areas, that is where the immersion by pure concentrated sea water occurs twice daily, which

seems a prerequisite for optimal growth. It is apparently not necessary for the condition of these two algal species to be soaked daily in fresh water. The fact that these algae inhabit coastal belts assumably refers to adaption to withstand the sudden changes of the osmotic pressure, as, on emersion, occasional rainfall is not harmful to their thallus condition. It is, in passing, necessary to note that cell diameters of *U. speciosa* and *U. flacca* tend to decrease with prolonged stay at consistent low salinities in cultures. Finally, it was found that a constant immersion in 16‰ Cl^- culture solution does not have a negative influence on the morphology of these species. All these facts indicate that these species rather can be termed euryhaline marine algae.

Since *U. palusalsa* is virtually only found in both inland salt marshes as well as in marshes open to the sea, its ecological status is arbitrary. It appeared difficult to correlate culture and field observations. In nature it only occurs on soils as an companion alga, or at most uni-algally in small strata. In liquid cultures it shows a mass-appearance. The absence of competition with other organisms and their secretory products seems to stimulate its growth in culture. Its absence in tidal pools in salt marshes is possibly explained by the fact that this species is extremely sensitive to all, or some, of the physical and chemical factors, which fluctuate daily in these habitats. The effect of these parameters is difficult to determine and this was beyond the scope of the present study. As yet, the author cannot regard *U. palusalsa* as a pure brackish-water species. Its occurrence in its natural habitat is not yet clearly seen associated with (strong) fluctuation of the factor salinity; one can say that it occurs in natural situations where external factors cause instability, but apparently the marine element is prerequisite. Therefore, since autecological studies and accompanying laboratory experiments have not yet performed, this alga is presently, for convenience, regarded as euryhaline marine.

Both herbarium and field studies have revealed a rather common distribution of all species studied in western Europe. The assumed wide-spread presence of *U. palusalsa* has still to be confirmed in salt marshes in more northerly portions in western Europe, however.

The mode of reproductive response of the algae studied to the different photo periods used essentially agrees with that of the freshwater *Ulothrix* species (Dodel, 1876; Lind, 1932; Lokhorst, 1974). In general, the algae studied produce zoospores both in gametophytic and/or sporophytic stages under short-day conditions, while the initiation of gametes is strongly promoted only under long-day conditions. These findings correspond with the periodicity of the species in nature in western Europe. In general, in winter and spring, and to a lesser extent in autumn, they are predominantly present in their filamentous stage, subsequently with zoospores and/or gametes, while in summer they are mainly represented by their sporophytic stage. It is also found in nature, however, that *U. subflaccida* and *U. flacca* may have simultaneously an alternation of several generations during the yearly optimal vegetation period of the gametophytic filamentous phase.

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EXPLANATION OF FIGURES AND PLATES

For all plates the following abbreviations are used: b—bacterium; c—chloroplast; ce—chloroplast envelope; cf—cleavage furrow; ci—chloroplast invagination; cp—plastoglobule; er—endoplasmic reticulum; f—flagellum; g—Golgi body or dictyosome; gv—vesicle derived from the Golgi apparatus; l—lipid droplet in the cytoplasm; lo—lomasome; m—mitochondrion; mi—membraneous, myelin-like inclusion; mp—micro-particle; mvb—multivesicular body; n—nucleus; nc—nucleolus; ne—nuclear envelope; p—peroxisome; py—pyrenoid; pyg—pyrenoglobule; s—just completed septum; st—starch; t—thylakoid; v—vacuole; vi—virus-like inclusion; w—cell wall; wa—adhesive mucilaginous layer on the surface of the cell wall; wm—cell wall membrane.
In the figures the bar drawn represents a length of 20 μm.

FIGURES

Fig. 1. *Ulothrix speciosa.* — a. vegetative filaments; b. vegetative filament with a sheath-like habit and cells arranged in groups; c. vegetative filament with an *Ulothrix zonata*-like growth habit, grown at lower salinity; d. vegetative filaments, grown in adverse conditions, with chloroplasts studded with assimilates.

Fig. 2. *Ulothrix speciosa.* — a. filaments with gametangia; b. gametes and zygotes; c. reproductive filament with peculiar reproductive cells provided with two stigmas, originating from Espegrend; d. these fourciliate reproductive cells when liberated.

Fig. 3. *Ulothrix speciosa.* — a. filament with parthenogenetic developmental stages of gametes; b. germination of the quiescent zygote by forming a protuberance; c. stalked sporophytes, not yet ripened; d. germination of the quiescent zygote by simple enlargement. Note the persistent remnant of the zygote envelope. The final stage is laden with assimilates; e. fertile sporophytes.

Fig. 4. *Ulothrix speciosa.* — a. sporophytes with aplanospores in subsequent stages of development; b. zoospores, liberated from the sporophytes; c. bipolar germination of the attached zoospore; d. germlings with filaments developing in diverse directions as a result of additional cell division, perpendicular to the original length axis of the bipolar germling; e. more advanced stages of such germlings; f. similar developmental stages as a result of germination of assembled aplanospores.

Fig. 5. *Ulothrix speciosa.* —a. gelatinisation of the cell wall of the whole filament, resulting in a disorganized cell arrangement; b. portion of this growth habit in detail; c. fragmentation of vegetative filaments; d. fragmenting filament with gametangia; e. outgrowth of the individual cells into independent daughter filaments as seen in a mature filament after a long period of arrested growth on agar plates.

Fig. 6. *Ulothrix flacca.* — a. vegetative filaments. The roughly surfaced threads are from natural origin; b. coalescence of filaments; c. portion of coalesced filaments in detail; d. vegetative filament, after prolonged period of adverse conditions.

Fig. 7. *Ulothrix flacca.* — a. filaments with aplano- and zoosporangia; b. zoospores; c. bipolar germination of the attached zoospore; d. germination of the attached zoospore, giving rise to twin-filaments.

Fig. 8. *Ulothrix flacca.* — a. germination of the attached zoospore, giving rise to a growth habit differentiated in a prostrate portion of restricted growth and an erect portion of indeterminate length; b. initiation of the fragmentation process in a vegetative filament; c. filament with (germinating) aplanospores; d. advanced stages of germination of aplanospores in a filament.

Fig. 9. *Ulothrix flacca.* — a. filaments with gametangia; b. gametes and zygotes; c. germination of quiescent zygotes into stalked and non-stalked sporophytes; d. sporophyte partially with fertile contents.

Fig. 10. *Ulothrix flacca.* — a. fertile sporophytes; b. zoospores liberated from a sporophyte; c. sporophytes with aplanospores; d. advanced stages of germination of aplanospores in sporophytes; e. parthenogenetic developmental stages of gametes in a filament.

Fig. 11. *Ulothrix palusalsa.* — a. vegetative filaments; b. filaments with *Geminella*-like growth habit; c. local swelling of the cell wall in a vegetative filament, protruding in one direction; d. curved vegetative filament with pronouncedly inflated cell wall, which may finally result in a disorganization of the cell row, as drawn for *Ulothrix speciosa* in fig. 5a; e. vegetative filament with biseriate cell row; f. initiation of fragmentation of a filament. Note the cylindrical remnants of the parent cell walls, wrapping the daughter cells.

Fig. 12. *Ulothrix palusalsa.* — a. filaments with zoosporangia; b. filament with germinated aplanospores; c. zoospores; d. bipolar germination of the attached zoospore; e. germlings with (incipient) filaments developing in diverse directions as a result of additional cell division, occurring perpendicular to the original length axis of the bipolar germling; f. ditto, drawn at lower magnification.

Fig. 13. *Ulothrix palusalsa.* — a. filaments with gametangia; b. filament with sheath-like envelope, containing gametangia. Note the release of gametes only through a pore in the inner cell wall, resulting in the occurrence of gametes and copulants in the lumen in the cell wall; c. gametes and zygotes; d. germination of the quiescent zygote into a sporophyte; e. first gross cleavage of the cell contents of a sporophyte, prior to its final ripening into zoospores; f. a fertile sporophyte; g. zoospores, released from the sporophyte.

Fig. 14. *Ulothrix palusalsa.* — a. filament with parthenogenetic developmental stages of gametes; b. filament with sheath-like envelope, enclosing aplanospores. Note the disappearance of only the transverse cell walls; c. filament with intercalary akinetes; d. fragmentation of a vegetative filament by separation of cells enclosed inside the common mucilaginous envelope of the filament; e. filament with a capped apical cell; f. amorphous *palmelloid*-stage as the final result of the fragmentation process drawn in d.; g. detail of the inset in f.; h. advanced developmental stage of a basal cell.

Fig. 15. *Ulothrix implexa.* — a. vegetative filaments; b. filament grown at low salinity. Note the less extended chloroplast in the individual cells; c. growth habit of a filament under adverse conditions; d. filaments with zoosporangia; e. filament with (germinating) aplanospores.

Fig. 16. *Ulothrix implexa.* — a. zoospores; b. germination of the attached zoospore into a bipolar germling; c. advanced stage of the basal portion of a vegetative filament; d. loop-like habit of a vegetative filament, caused by the differentiation of the original apical cell in a rhizoidal appendage; e. filament with akinetes; f. filaments with gametangia; g. gametes and zygotes; h. outgrowth of vegetative cells into the lumen of the proximate former reproductive cells.

Fig. 17. *Ulothrix implexa.* — a. germination of quiescent zygotes into stalked and non-stalked sporophytes; b. sporophytes with cell contents heavily laden with assimilates. Their stalks are already broken down; c. sporophyte with an unimpaired stalk; d. first gross cleavage of the sporophyte cell contents, prior to its final ripening into zoospores; e. ditto. Note the initiated gelatinisation of its stalk, see arrow; f. fertile sporophytes; g. released zoospores; h. remnant of the sporophyte with germinating zoospores; i. filament with parthenogenetic development of gametes.

Fig. 18. *Ulothrix subflaccida.* — a. vegetative filaments; b. vegetative filament under adverse conditions. Note the withdrawal of the chloroplast in the individual cells; c. vegetative filament with slightly inflated cells, from a specimen collected on Balgzand; d. filaments with zoosporangia; e. filament with (germinating) aplanospores; f. zoospores.

Fig. 19. *Ulothrix subflaccida.* — a. germination of the attached zoospore into a bipolar germling; b. germlings with a slightly differentiated basal cell, as appearing in pure freshwater medium; c. advanced stages of the basal portion of vegetative filaments; d. filaments with gametangia; e. filament with parthenogenetic developmental stages of gametes; f. gametes and zygote; g. young sporophytes; h. fertile sporophytes.

PLATES

Plate 1. *Ulothrix speciosa.* — 1. longitudinal section of the cell wall. Note its three-layered composition and the smooth surface, × 11.715; —2. chloroplast lobe with abruptly terminating thylakoids, × 36.300; —3. part of the cytoplasm showing a mitochondrion with some transversely sectioned cristae (arrows) and the slipper-shaped peroxisome, × 57.750; —4. dictyosome in detail, × 57.750; —5. a complexly proliferated peroxisome proximate to the chloroplast envelope, × 29.700.

Plate 2. *Ulothrix speciosa.* — 1. pyrenoid with invaginating chloroplast strands, × 18.150; -2. detail of chloroplast invagination in the pyrenoid matrix. Note the electron-opaque pyrenoglobuli studding the outer surface of the tubular chloroplast invagination, × 57.750.

Plate 3. *Ulothrix speciosa.* —1, 2, and 3. peculiar membraneous, myelin-like inclusions in the cytoplasm, which are continuous with the ER (arrows), × 29.700.

Plate 4. *Ulothrix speciosa.* — Longitudinal section of a part of the cell contents with a seemingly autophagic vacuole. The transformation of the sequestered ER-clumps is apparent (arrows). Note also the extension of the chloroplast towards the transverse cell wall, × 11.715.

Plate 5. *Ulothrix flacca.* — 1. cell division by furrowing. Note the just bisected pyrenoids and the pyrenoid ready for cleavage, × 8085; —2. the pyrenoid. Note the chloroplast thylakoids traversing the starch shell and subsequently continuing their way between the adjoining starch grain and the pyrenoid matrix (arrows), × 29.700.

Plate 6. *Ulothrix flacca.* — 1. detail of the cell wall of a filament, grown in culture. Its surface proves to consist of a double membrane (arrow), × 29.700; —2. ditto, but here the surface is distinctly covered with a mucilaginous layer, in which a bacterium is embedded, × 29.700; —3. cell wall of a filament collected from a natural habitat near Sas van Goes (Netherlands). Its outer surface is densely studded with microparticles, × 29.700.

Plate 7. *Ulothrix flacca.* — 1. lipid droplets, × 29.700; —2. lomasomes near the transverse cell wall, × 29.700; —3, 4, 5, and 6. subsequent stages of presumed distintegration of the peculiar cytoplasm inclusions (arrows), × 29.700.

Plate 8. *Ulothrix palusalsa.* — Longitudinal section of the filament, × 8085.

Plate 9. *Ulothrix palusalsa.* — 1. detail of the cell wall showing a slight depression (arrow). Note the distinct three layered composition and the smooth surface, × 14.850; —2. detail of the occasionally frayed appearance of the cell wall, × 8085; —3. multivesicular body, bounded with a double membrane system, × 57.750; —4. just completed cell division, × 8085.

Plate 10. *Ulothrix palusalsa.* — 1. pyrenoid with invaginating chloroplast strands, × 29.700; —2. ditto, in detail, × 57.750; —3. reticulate arrangement of the thylakoid-lamellae, with interlying starch grains, × 29.700.

Plate 11. *Ulothrix palusalsa.* — 1. myelin-like inclusions adjacent to the transverse cell wall, × 14.850; — 2. dictyosomes and apparently produced multivesicular bodies, × 57.750; — 3. fusion of a multivesicular body with the transverse cell wall, × 57.750.

Plate 12. *Ulothrix implexa.* — 1. longitudinal section of the filament. Note the presence of only minute vacuoles (arrows), × 8085; —2. peculiar cytoplasm inclusions (arrows), limited by a double membrane in which fenestrations may be present, × 29.700.

Plate 13. *Ulothrix implexa.* —1. the pyrenoid, × 29.700; —2. detail of the cell wall. Note its two-layered composition and the thin dense gelatinous layer embedded with particles, × 29.700; —3. detail of the frayed surface of the cell wall, presumably caused by anchored bacteria, × 29.700; —4. cytoplasm inclusion (arrow) with strongly osmiophilic piths, × 29.700.

Plate 14. *Ulothrix implexa.* —1 and 2. detail of the ingrowing septum. Note the assemblage, package, and fusion of cell wall material. No microtubuli are present, × 29.700.

Plate 15. *Ulothrix implexa.* — Longitudinal section of a part of a cell. Note the arrangement of thylakoids in short lamellae (arrows), × 29.700.

Plate 16. *Ulothrix subflaccida.* — 1. longitudinal section of a vegetative cell. Note the absence of the chloroplast towards the transverse cell walls, × 9900; —2 and 3. detail of the cell wall, × 29.700 and × 23.100; —4. detail of a ruptured cell wall, × 29.700, —5. gelatinisation of the cell wall (arrows), prior to release of the zoospores, × 57.750.

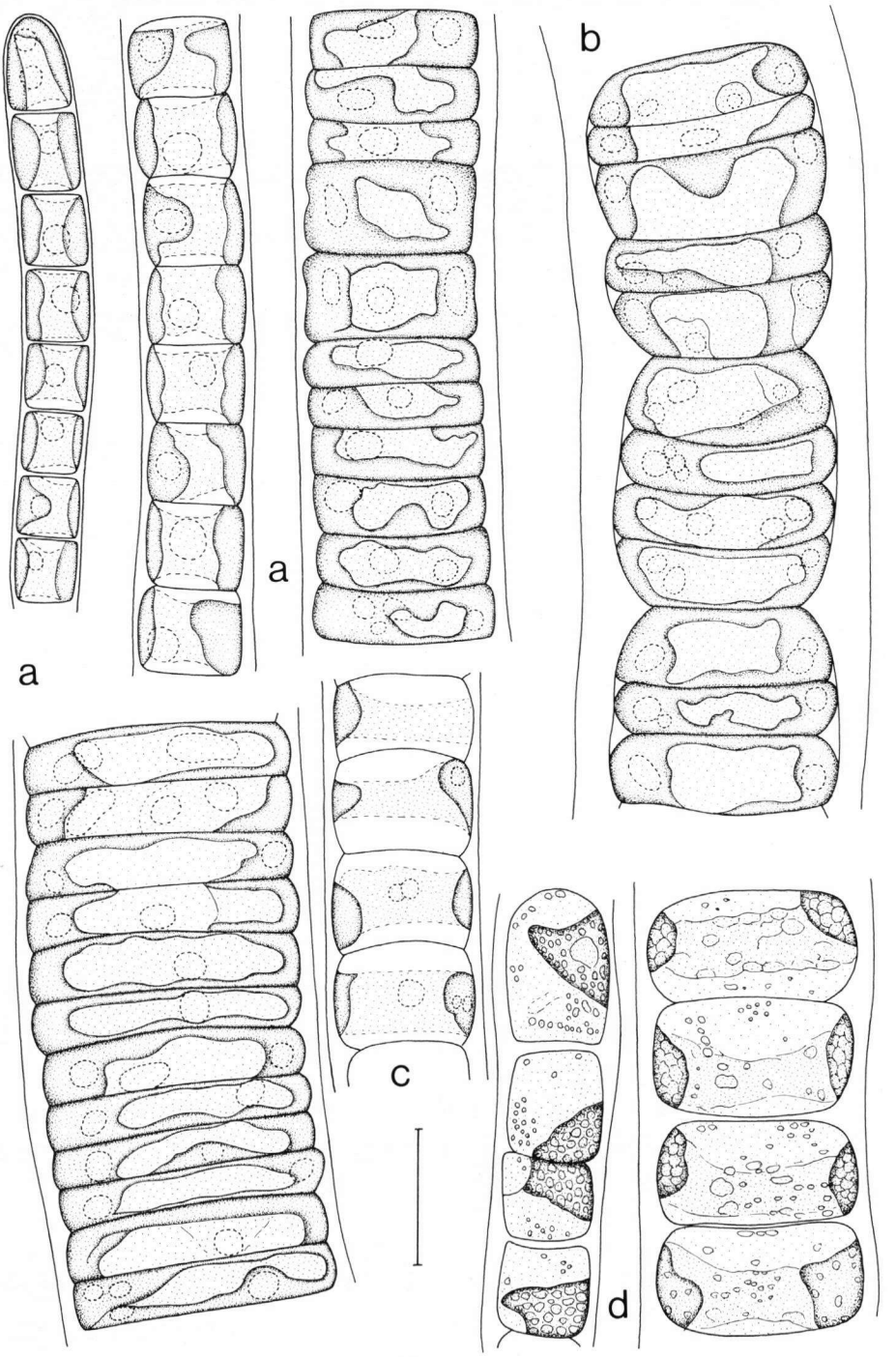
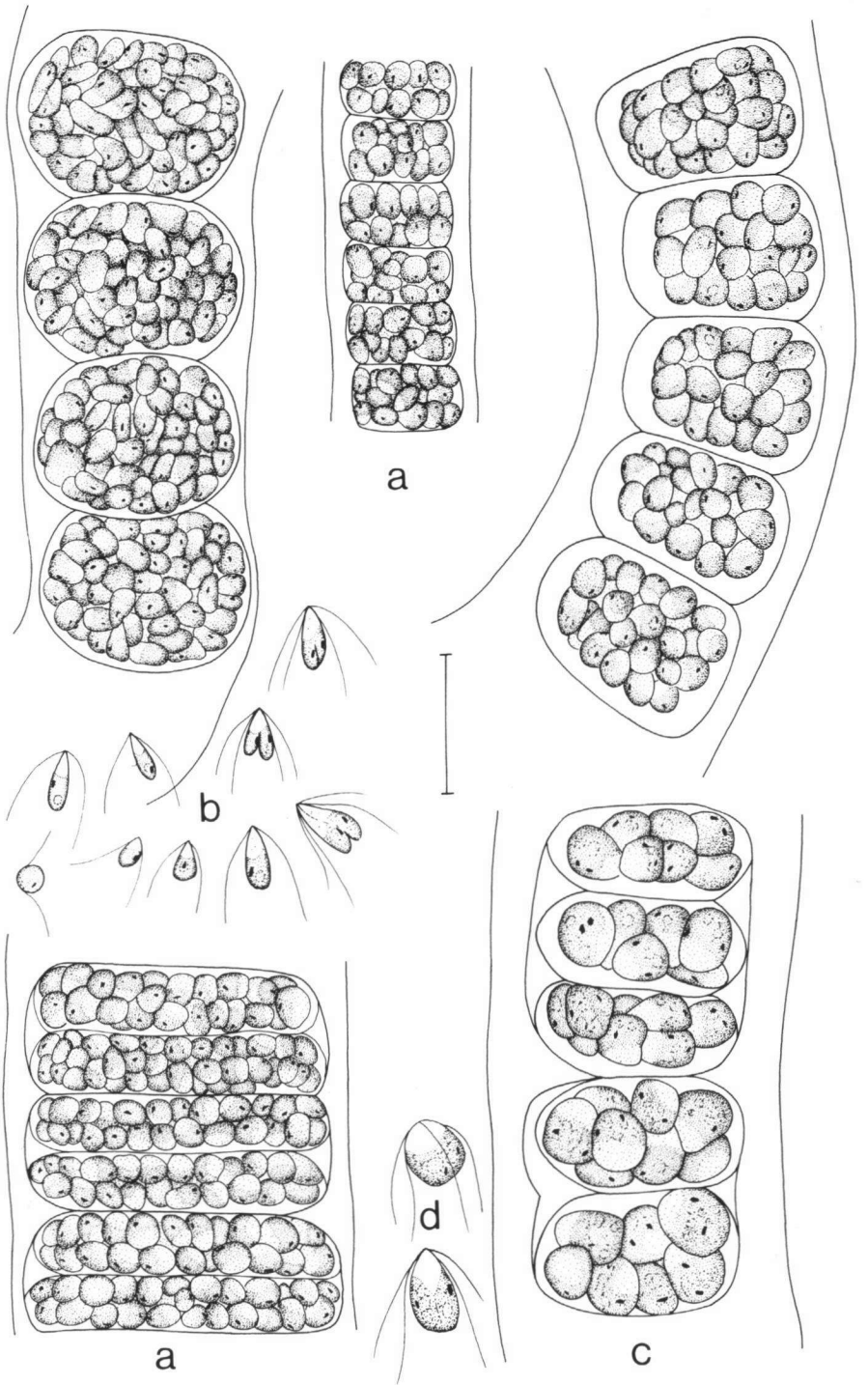


Figure 1



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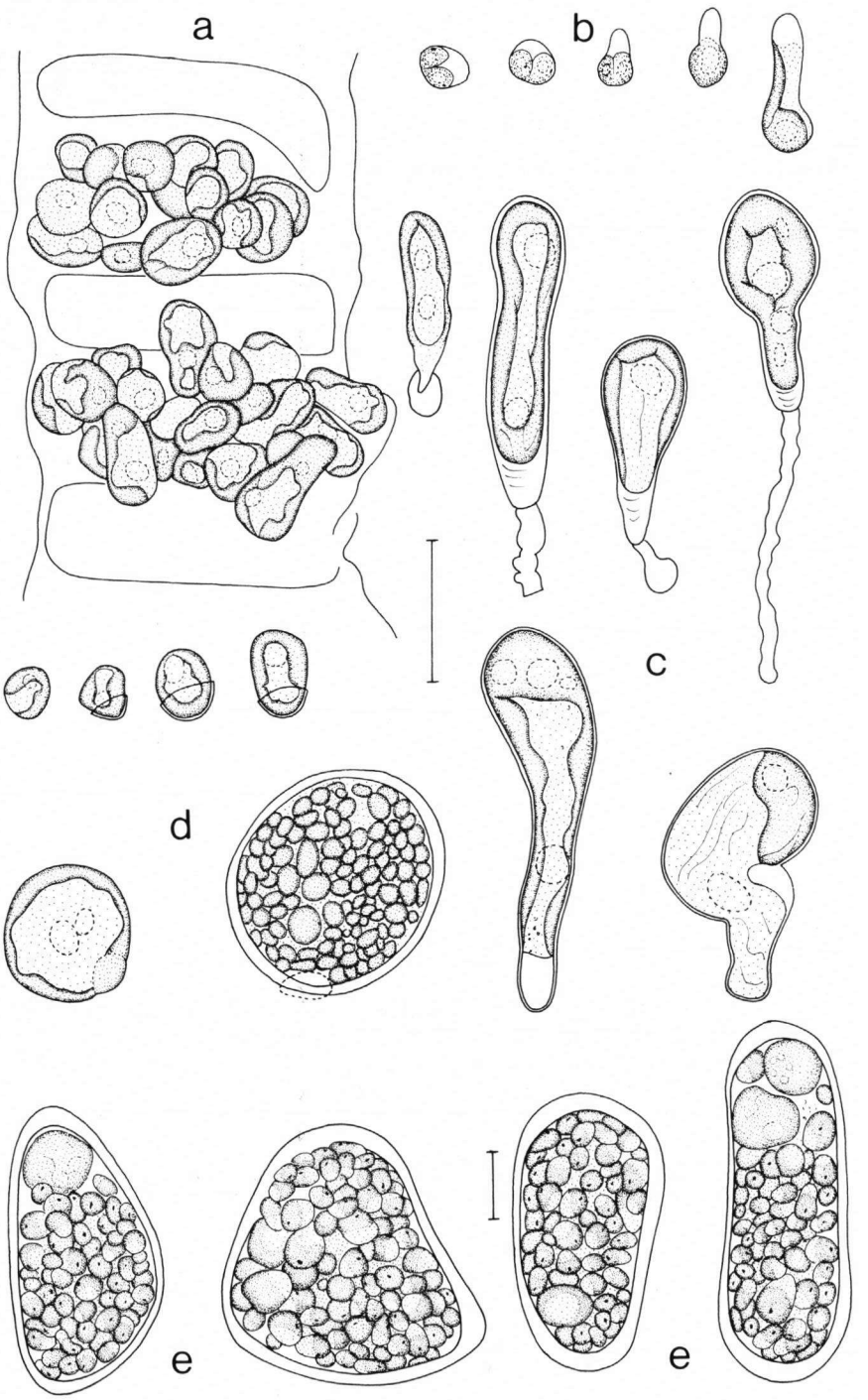

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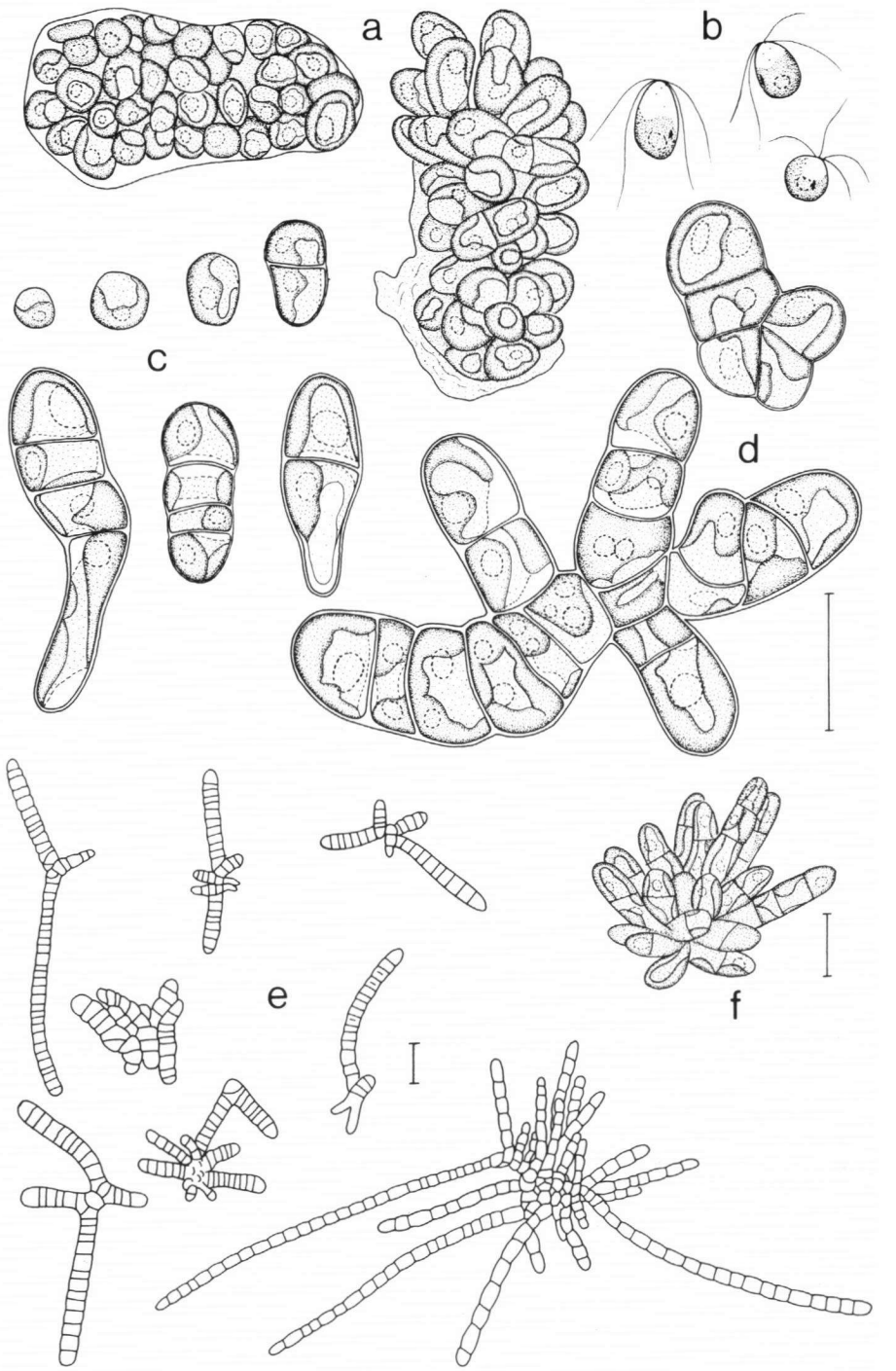

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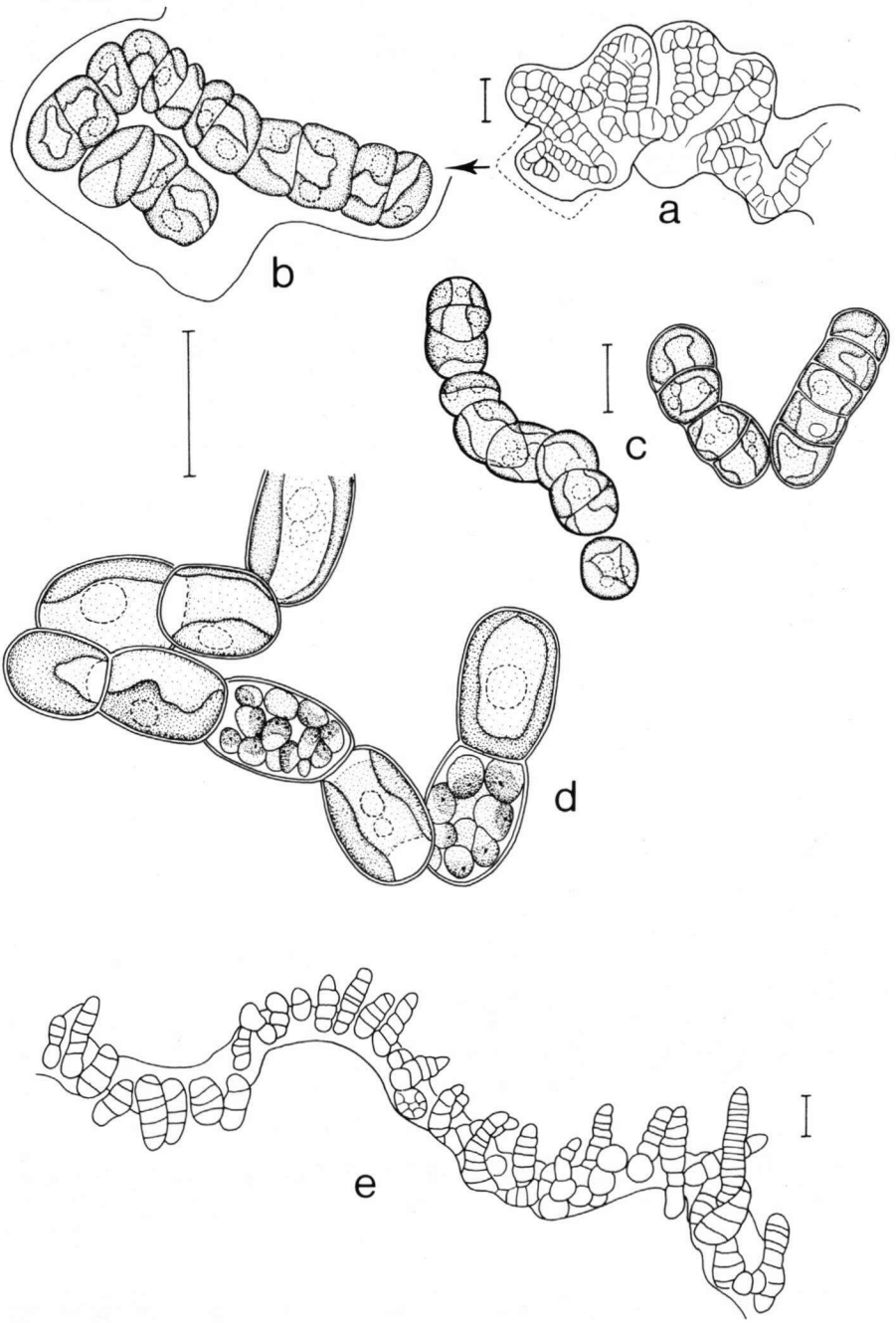

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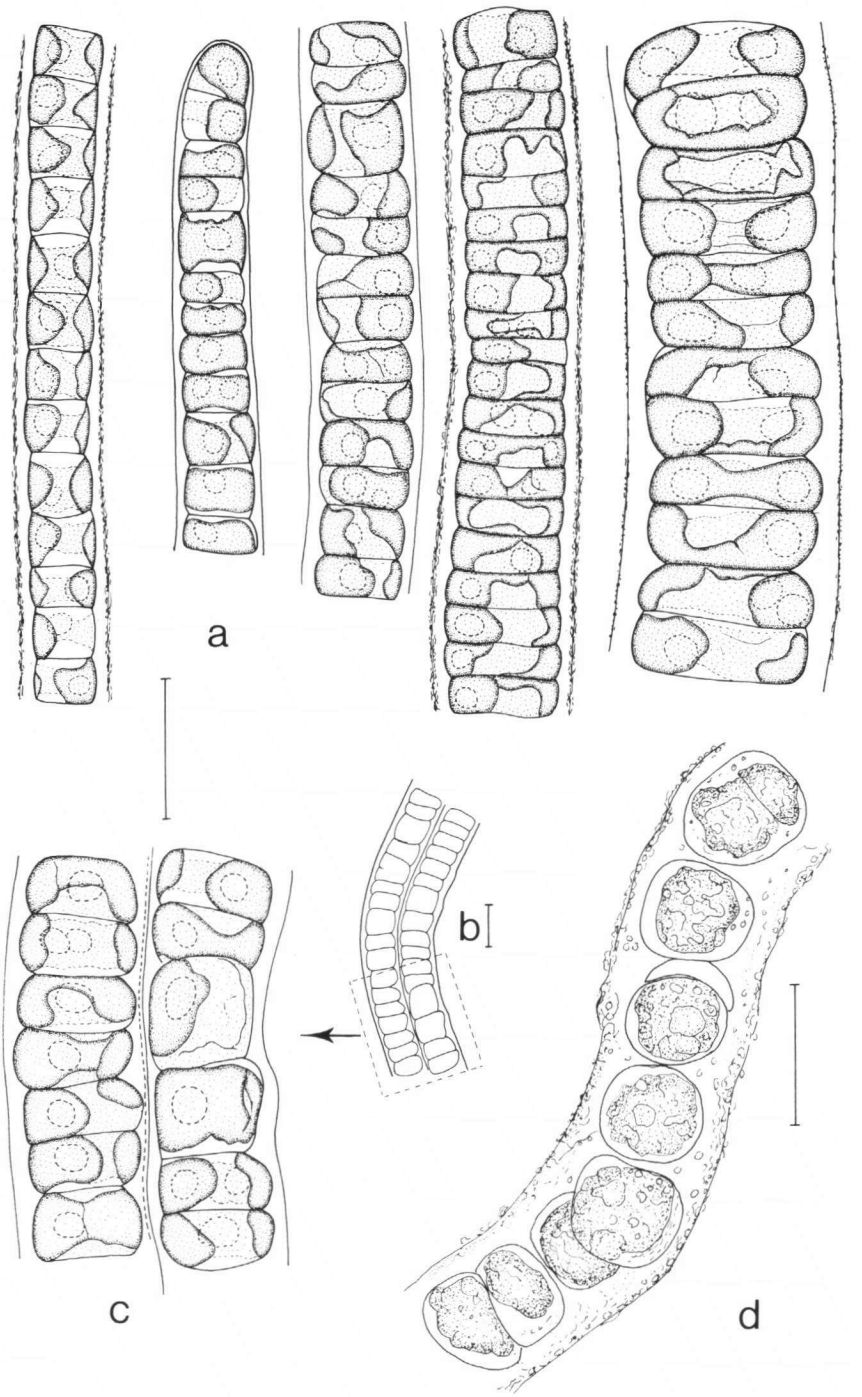

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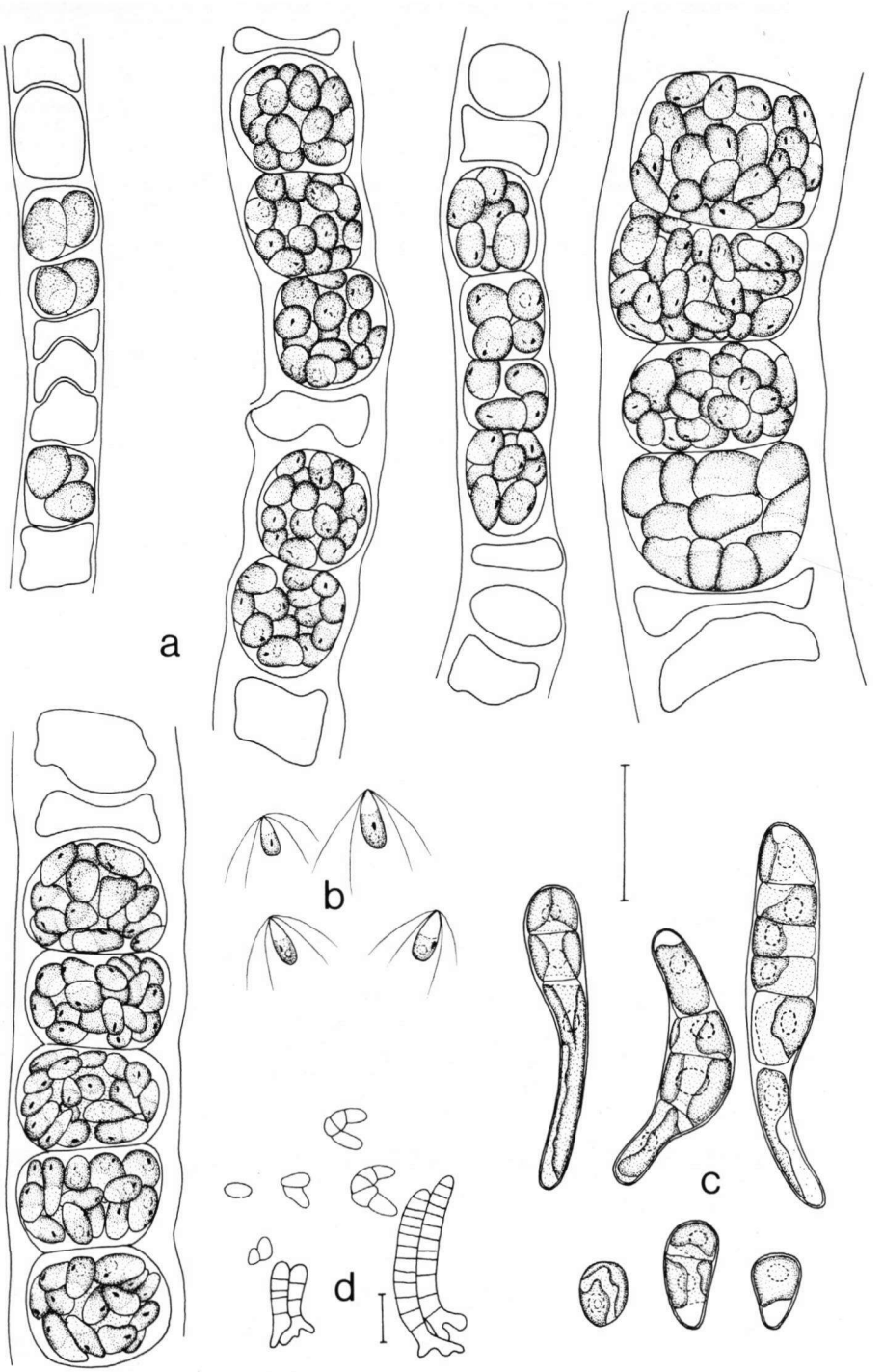

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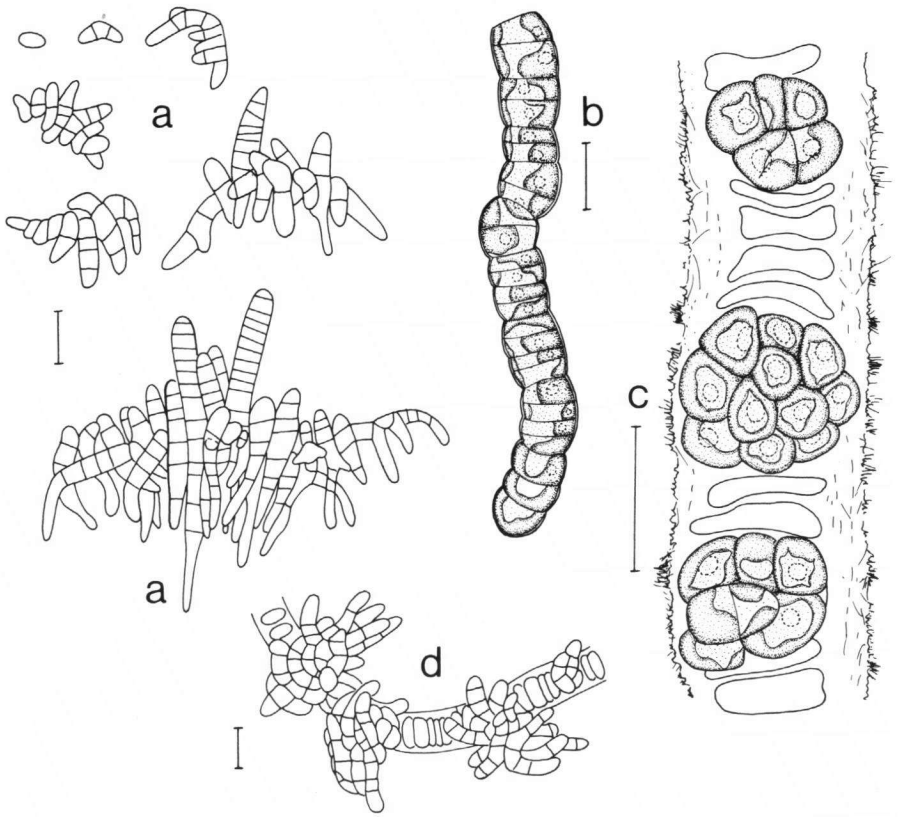

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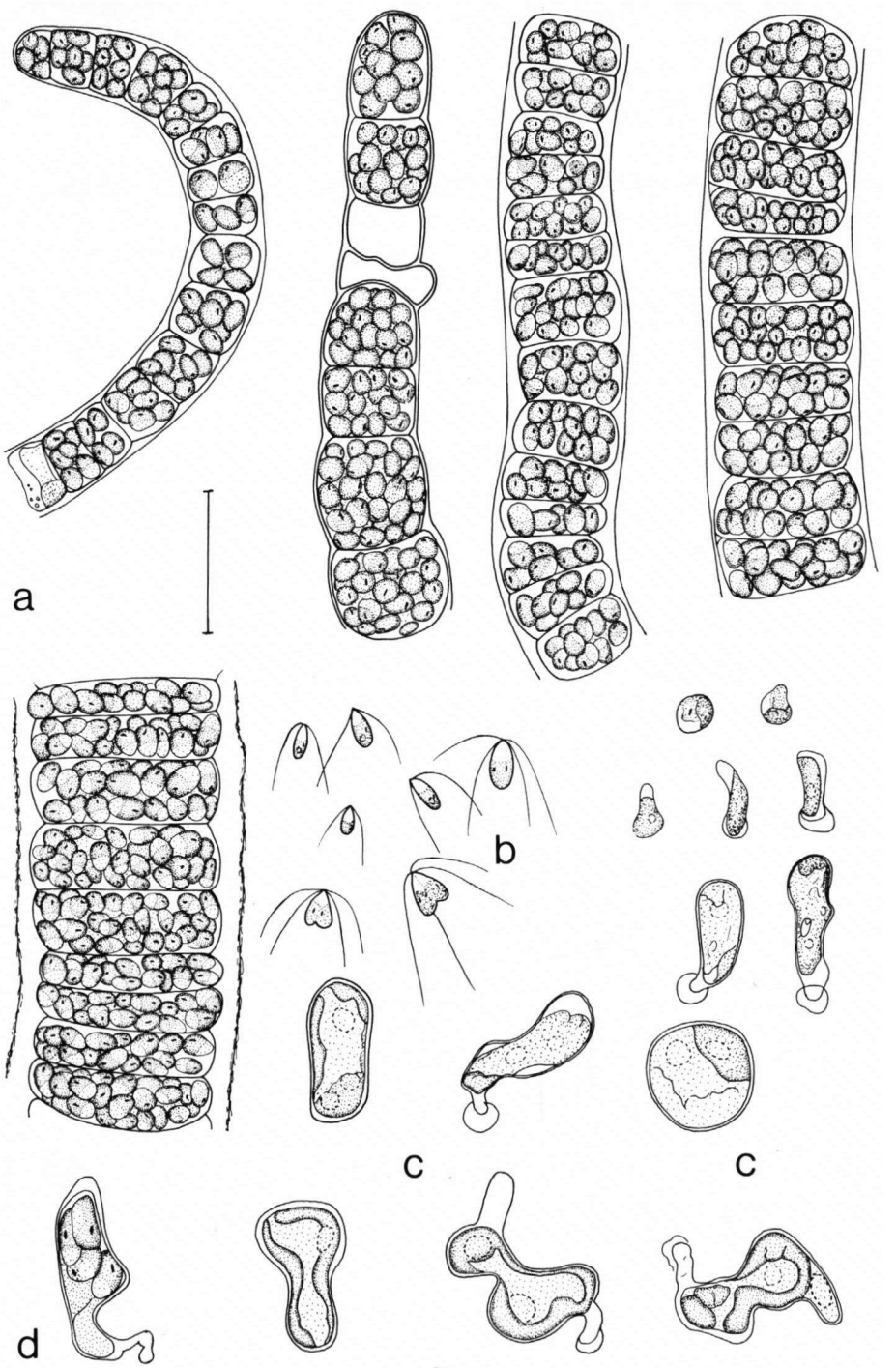

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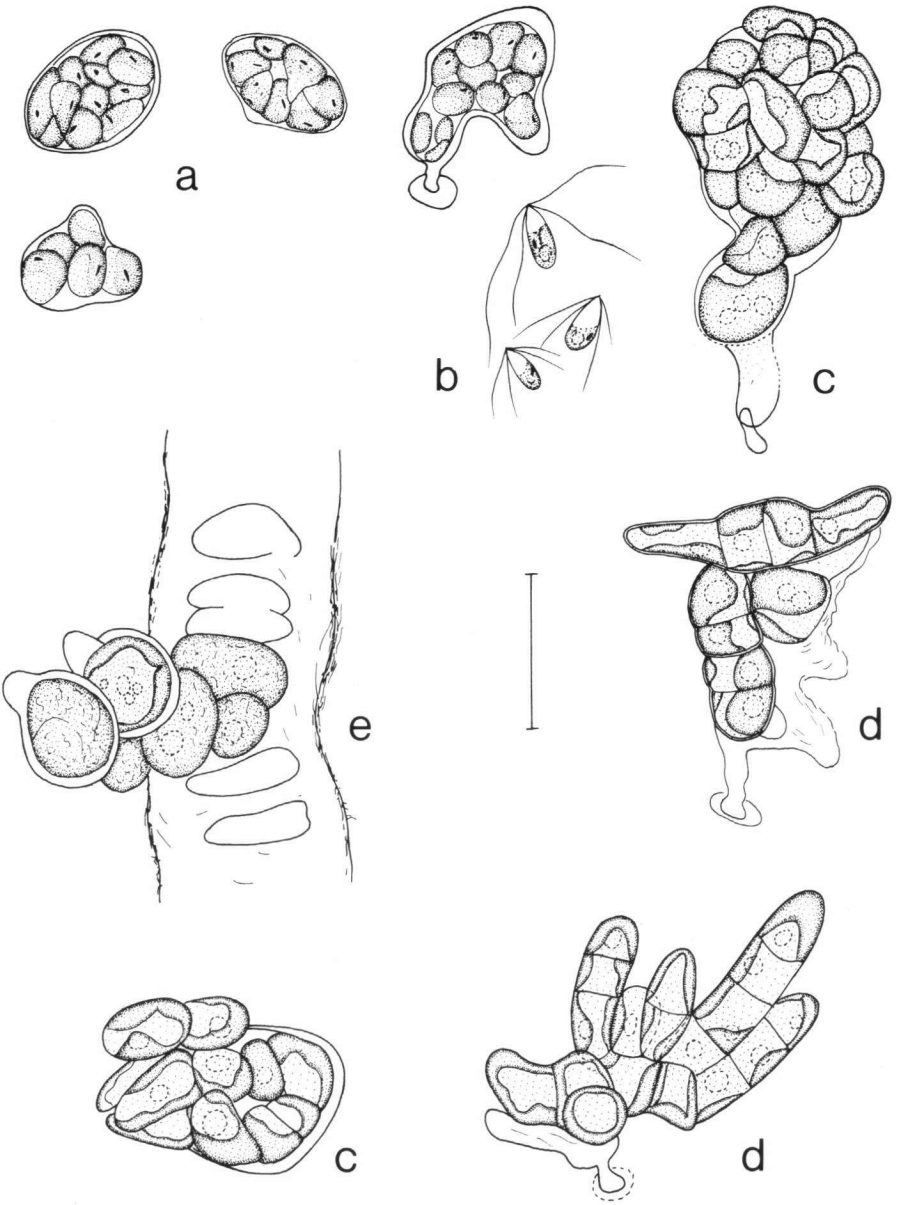

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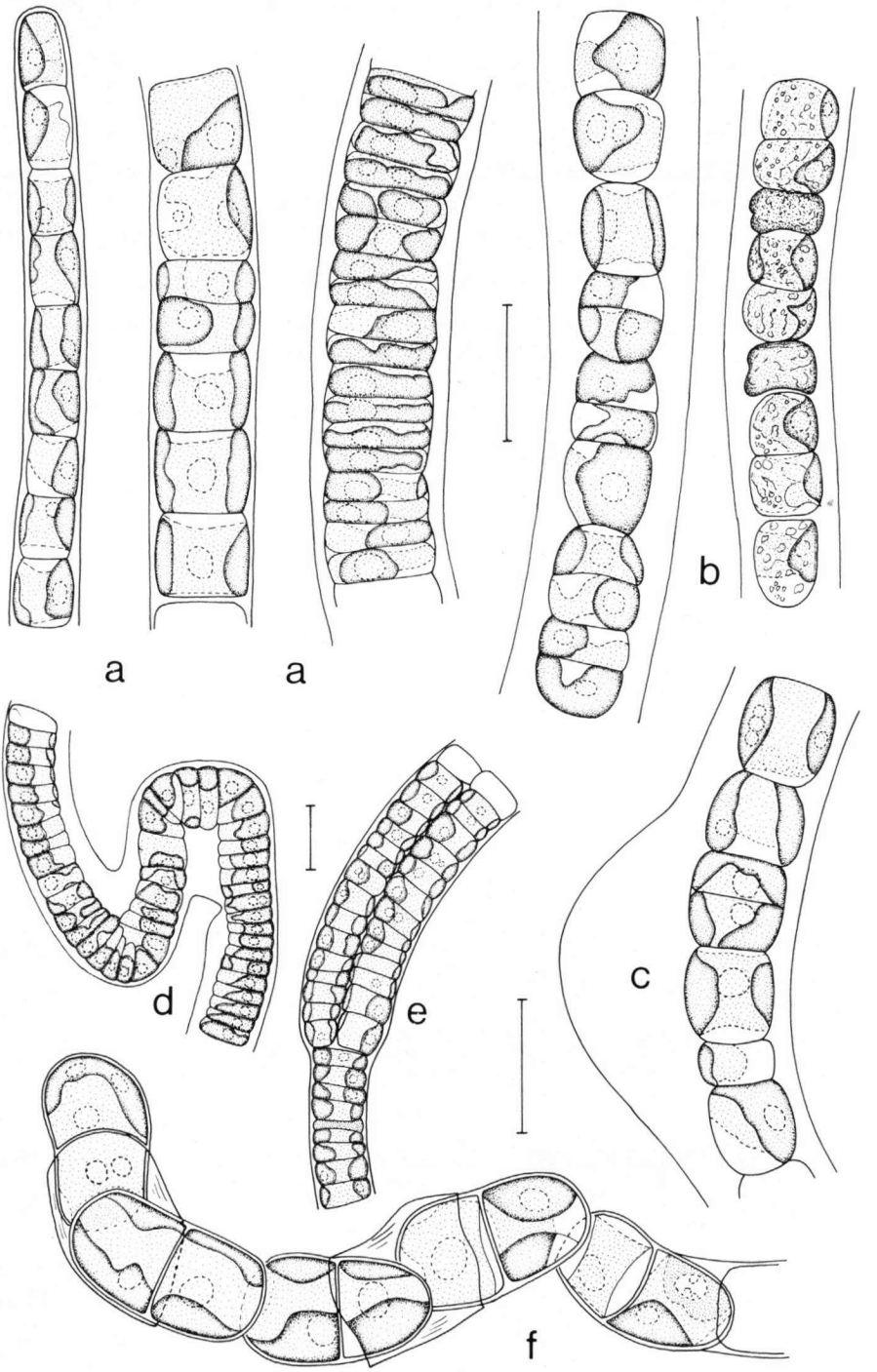

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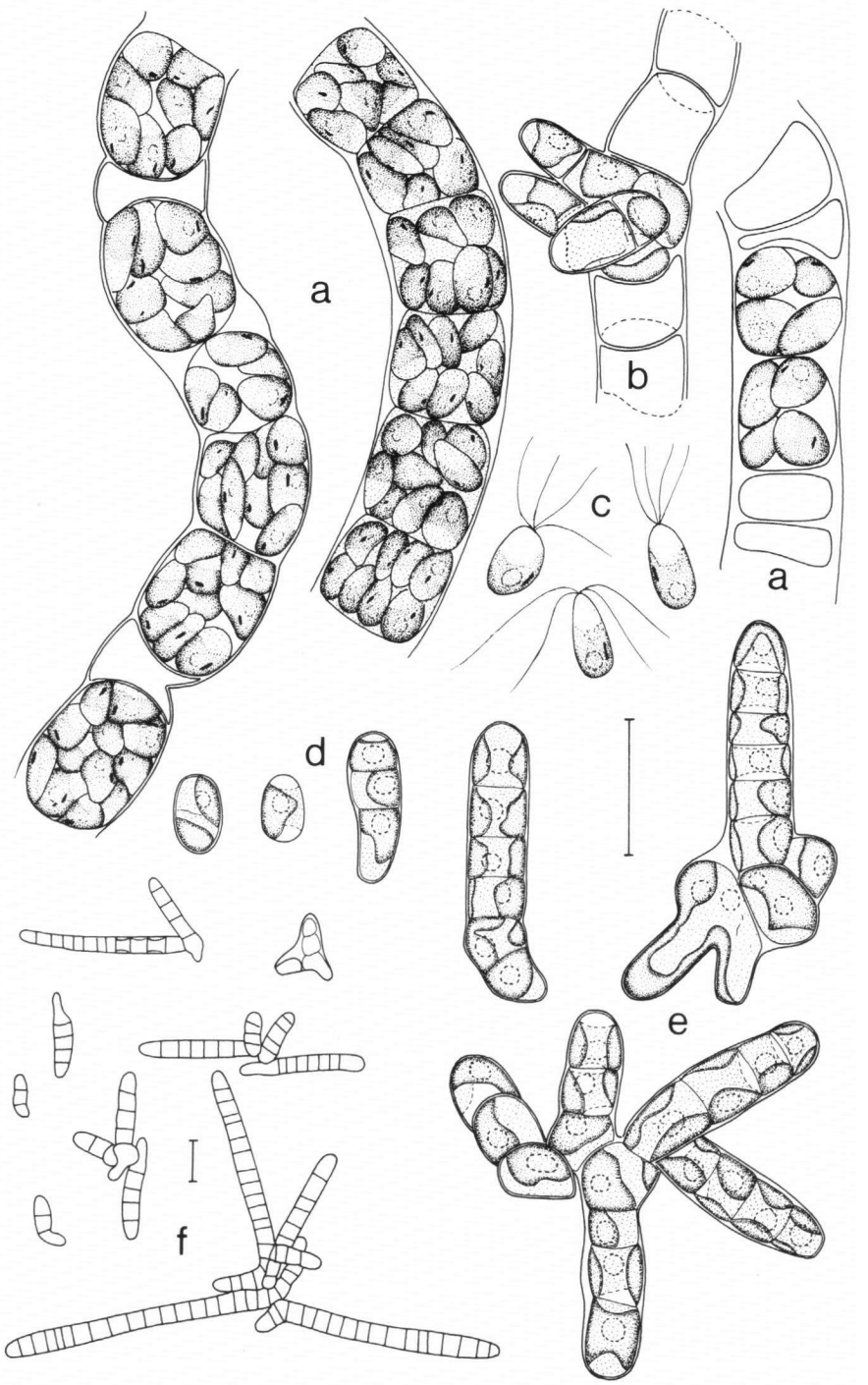

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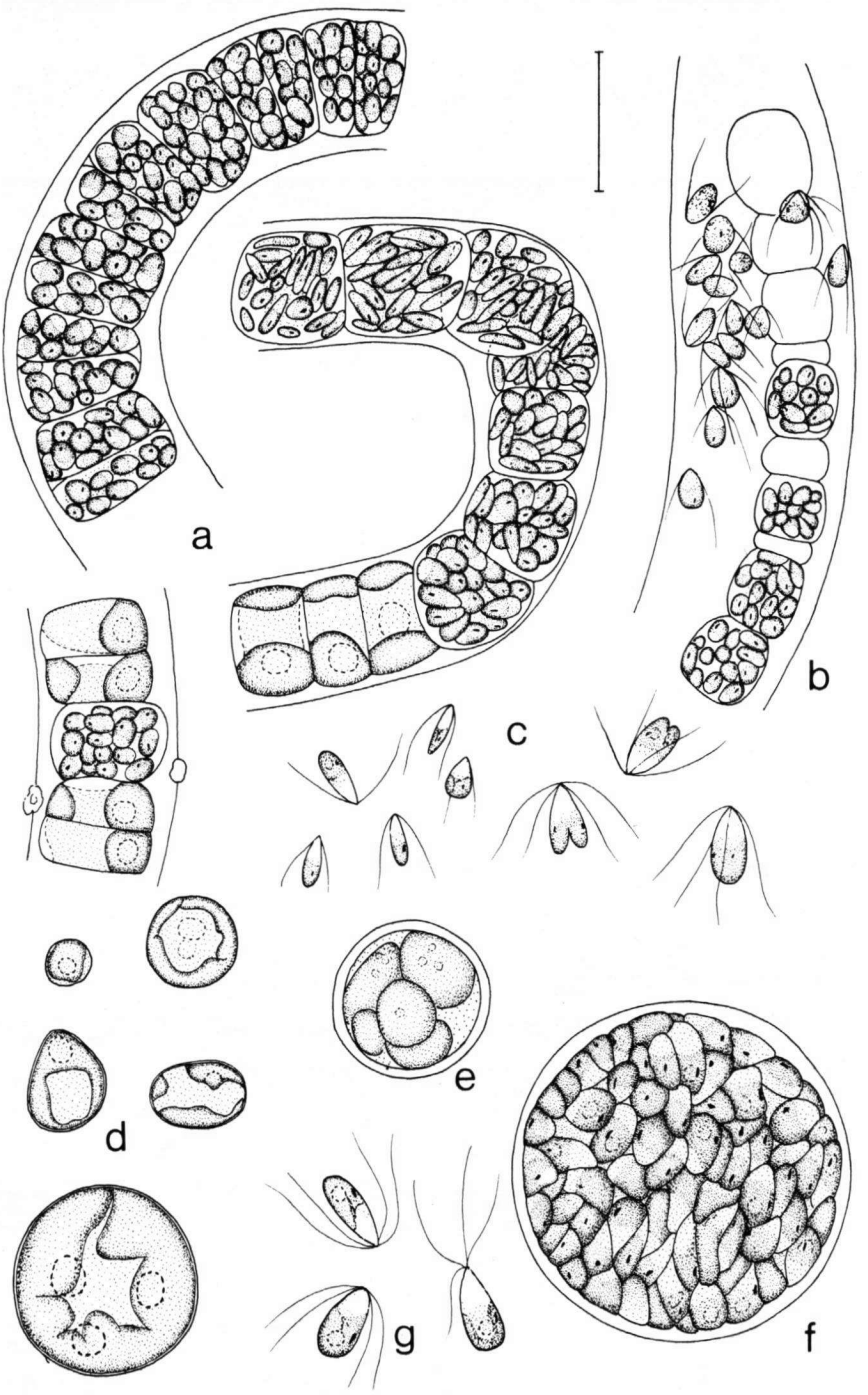

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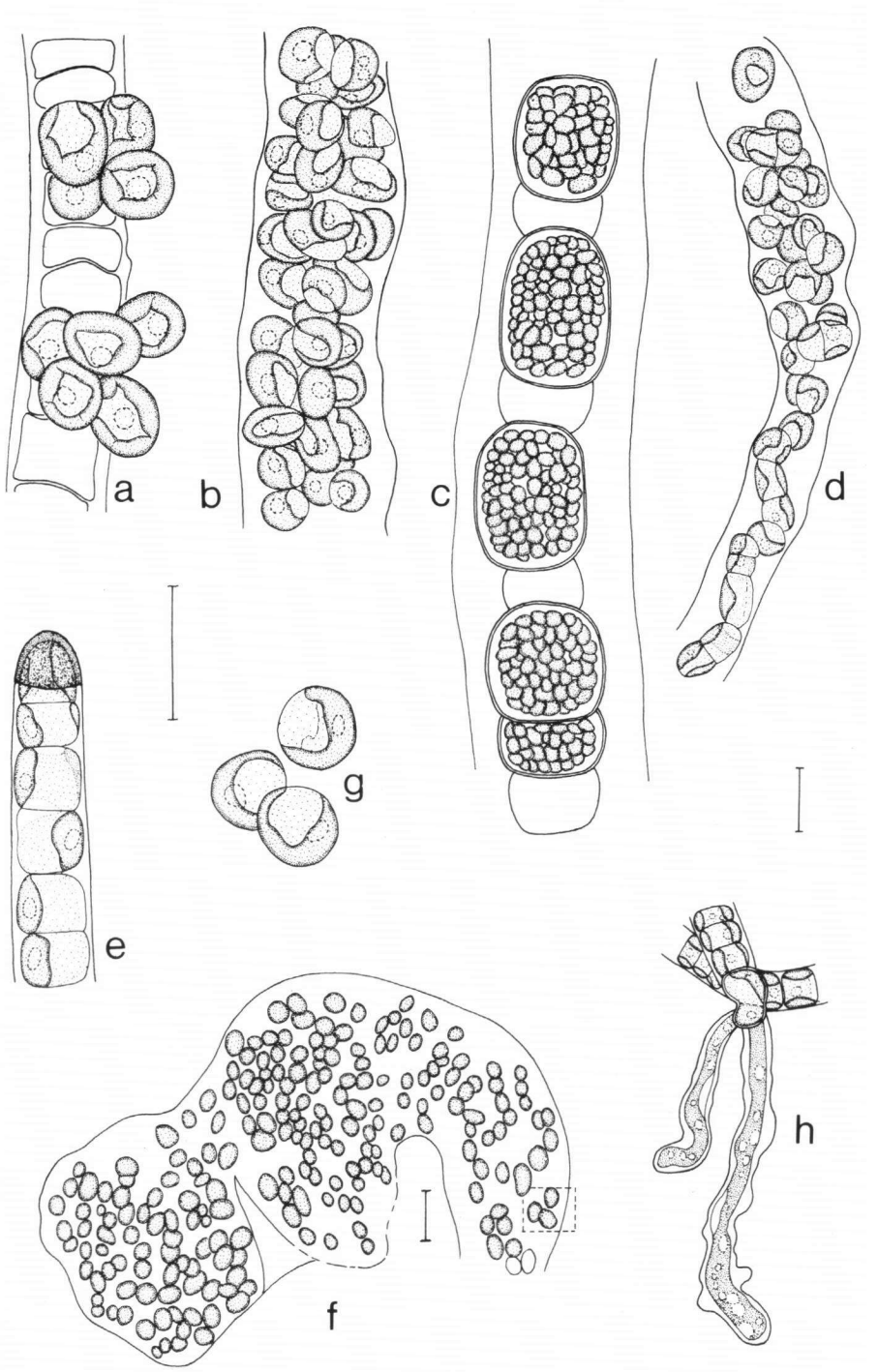

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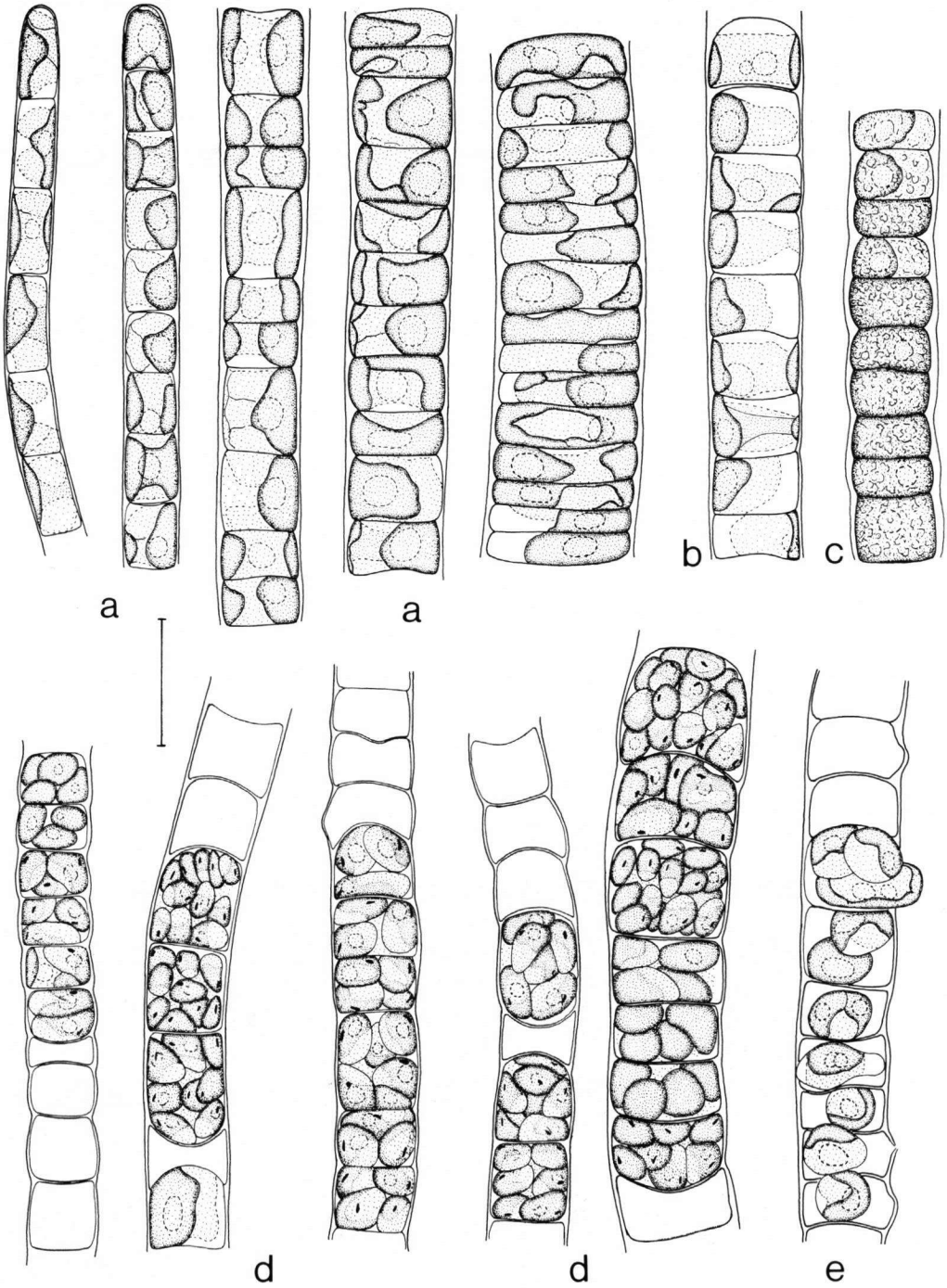

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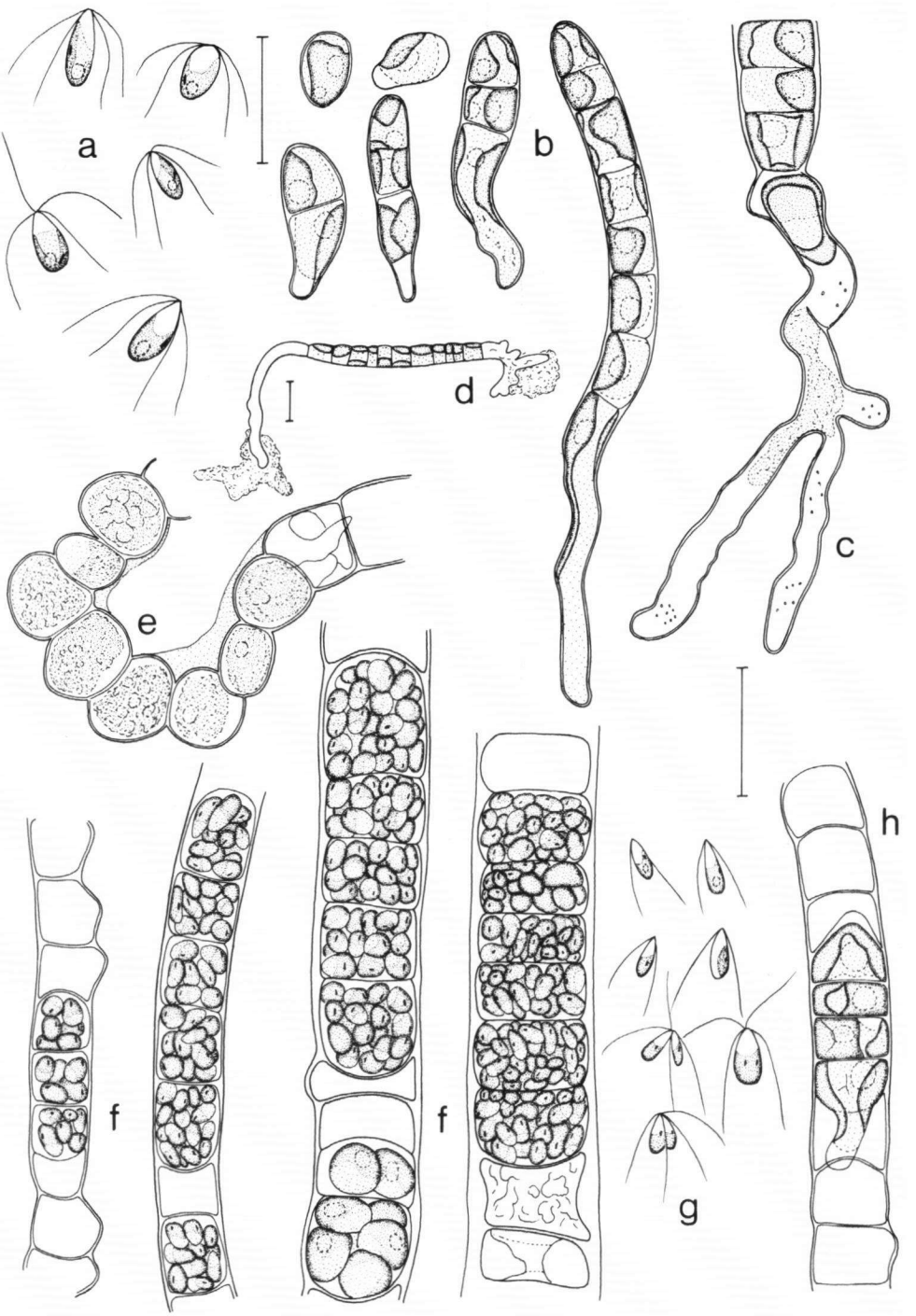
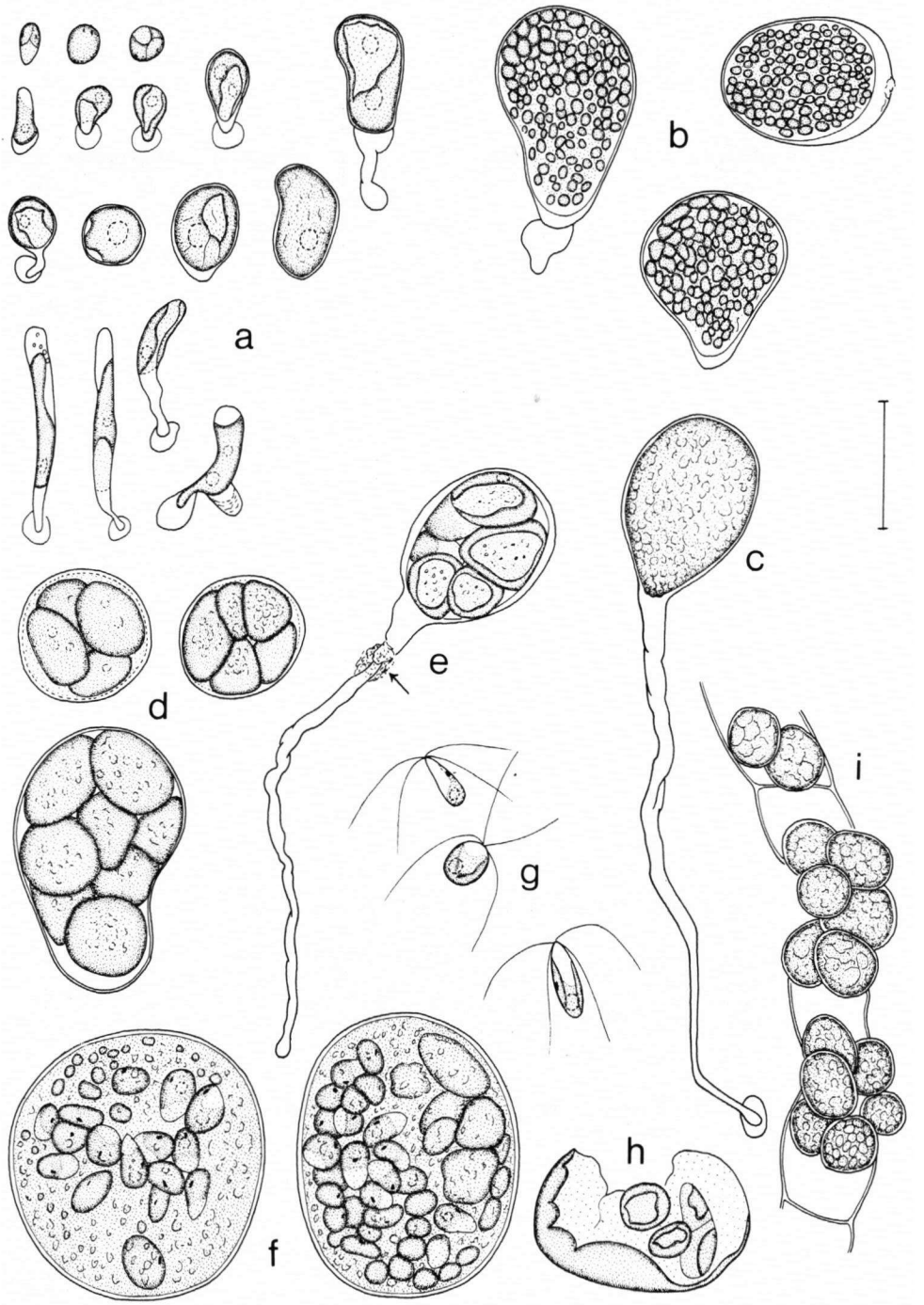


Figure 16



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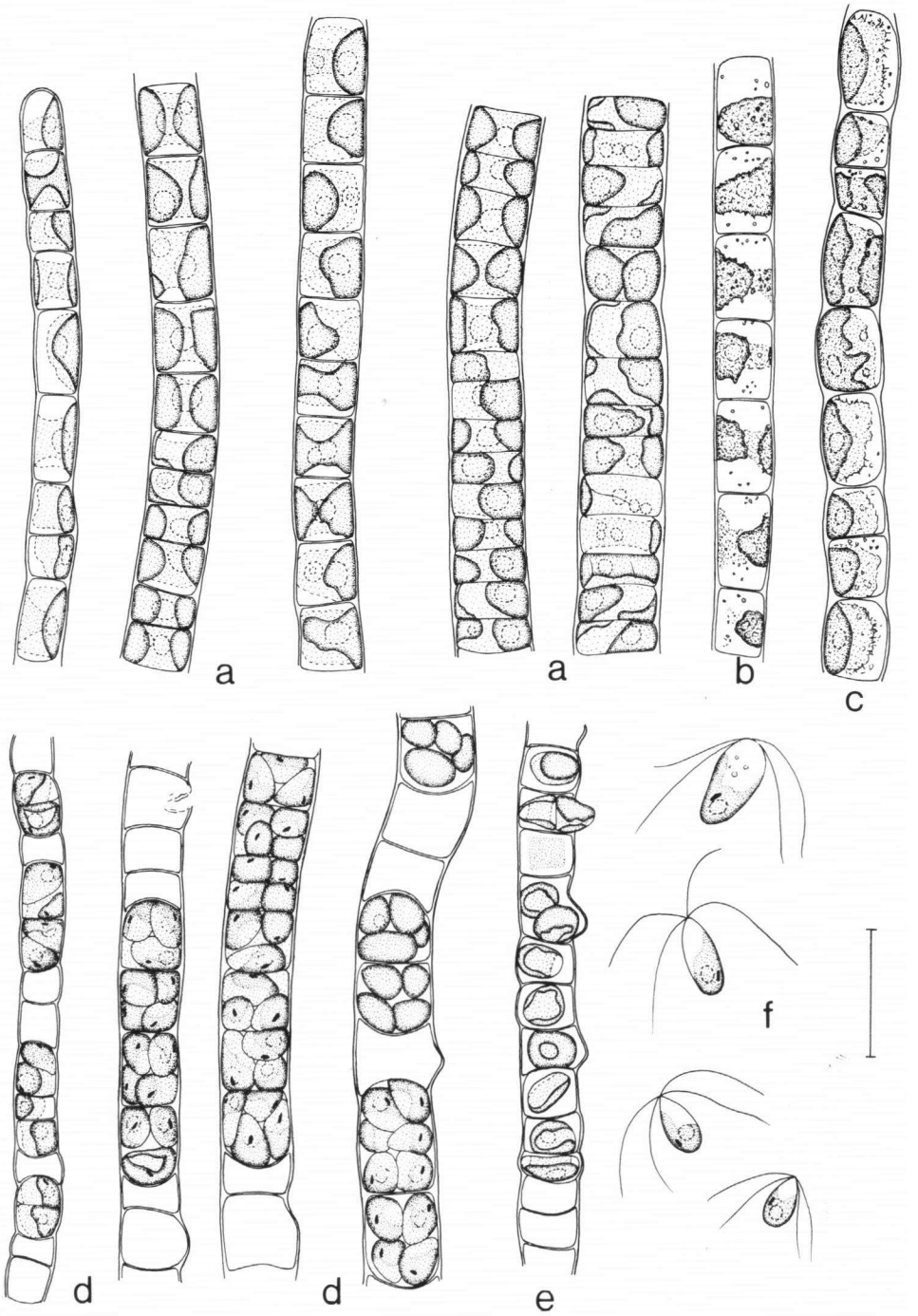

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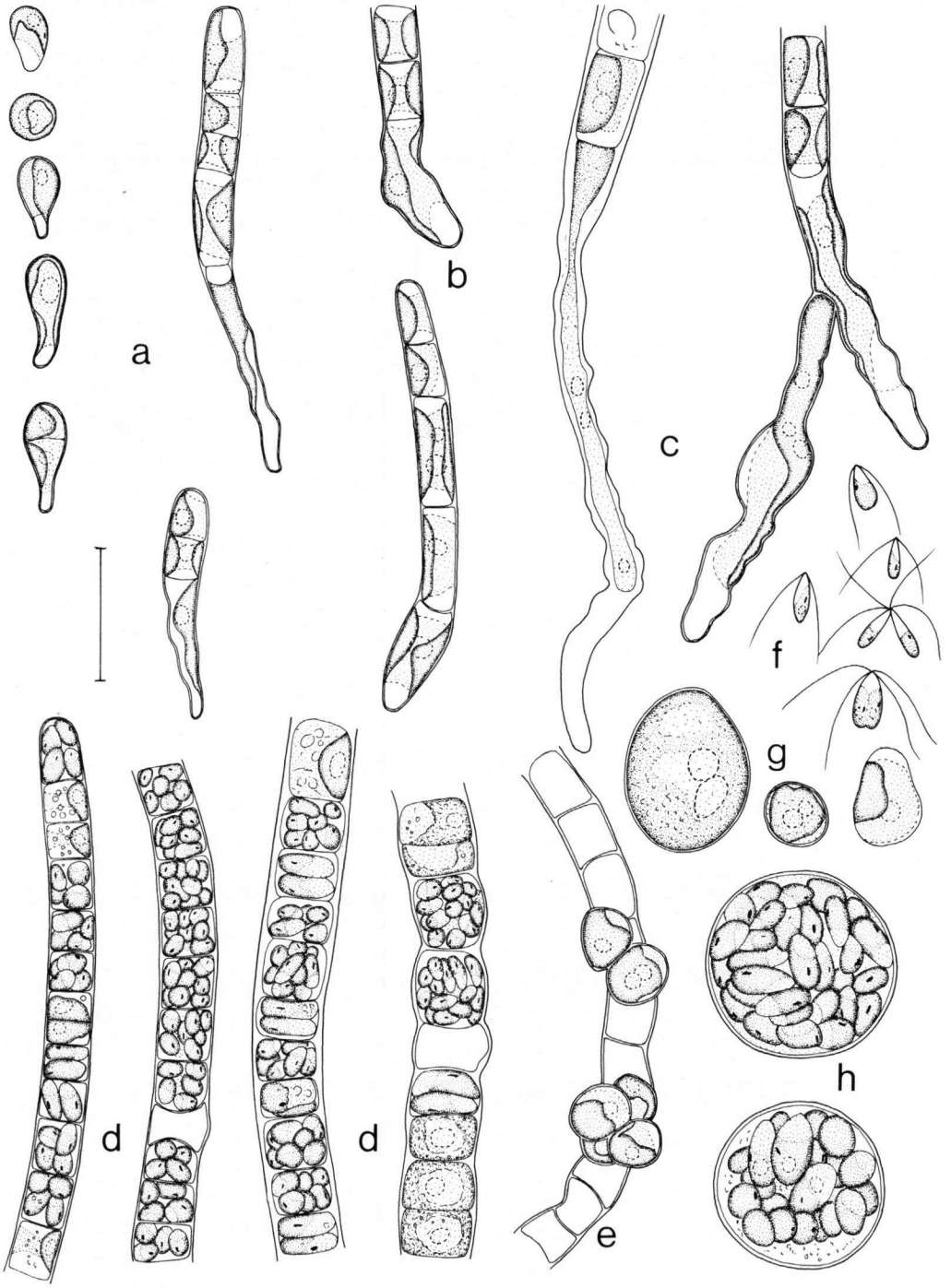
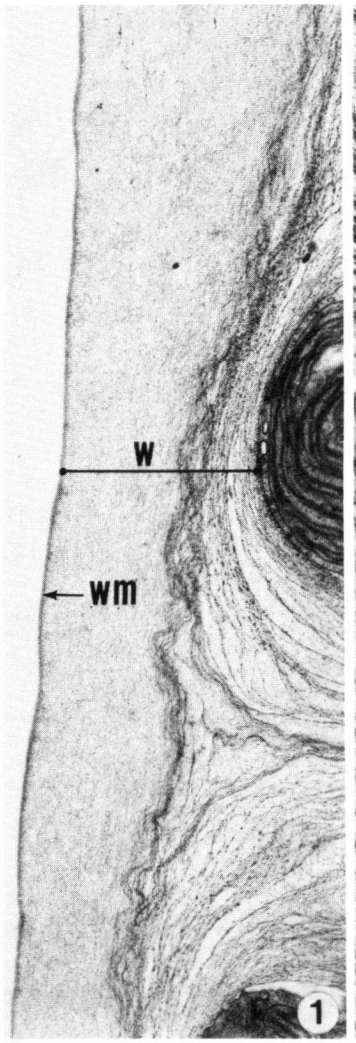
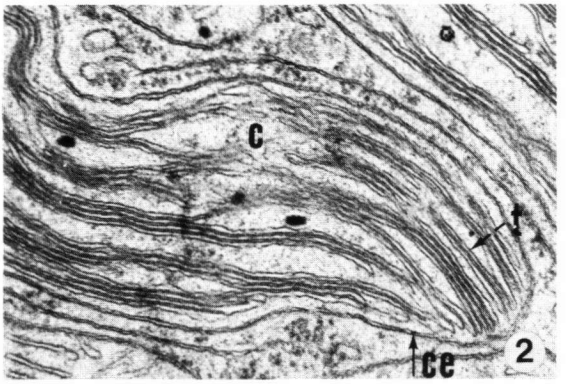
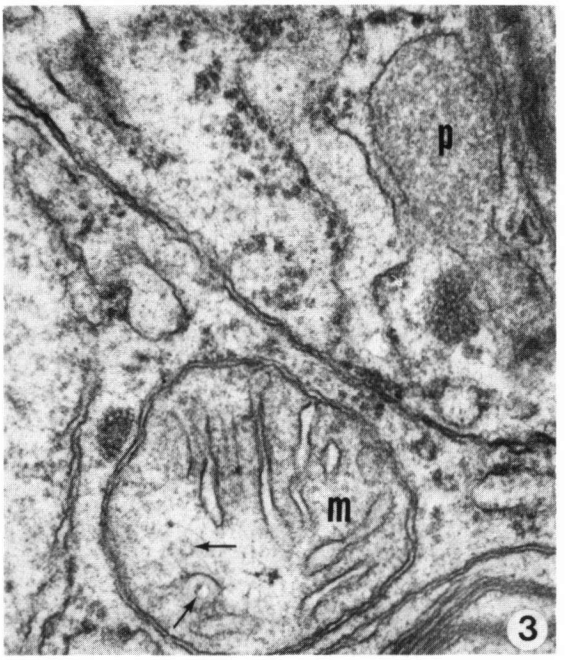
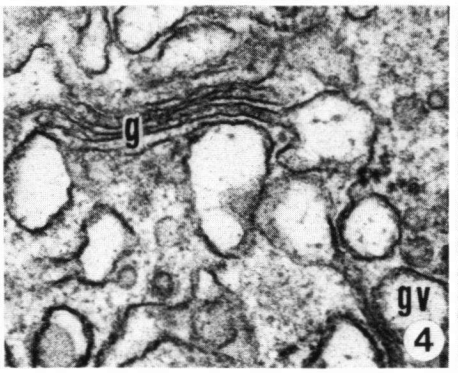


Figure 19









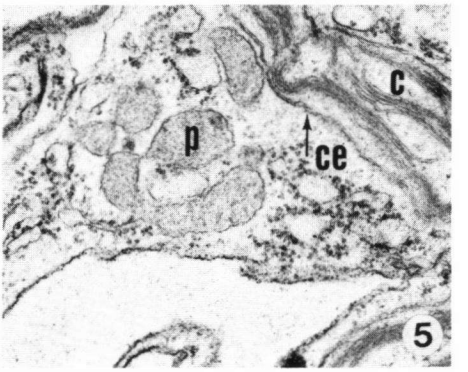


Plate 1

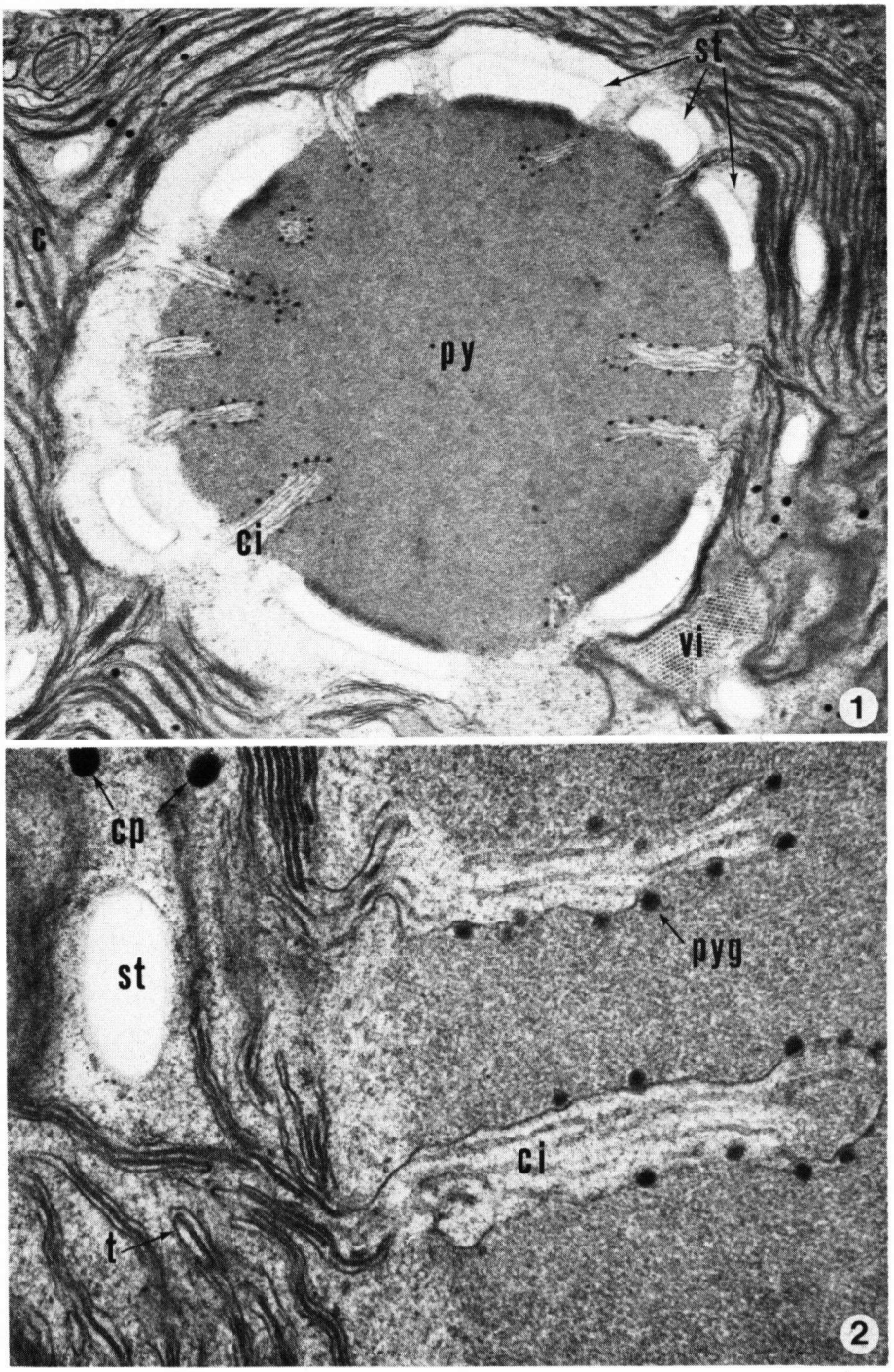


Plate 2

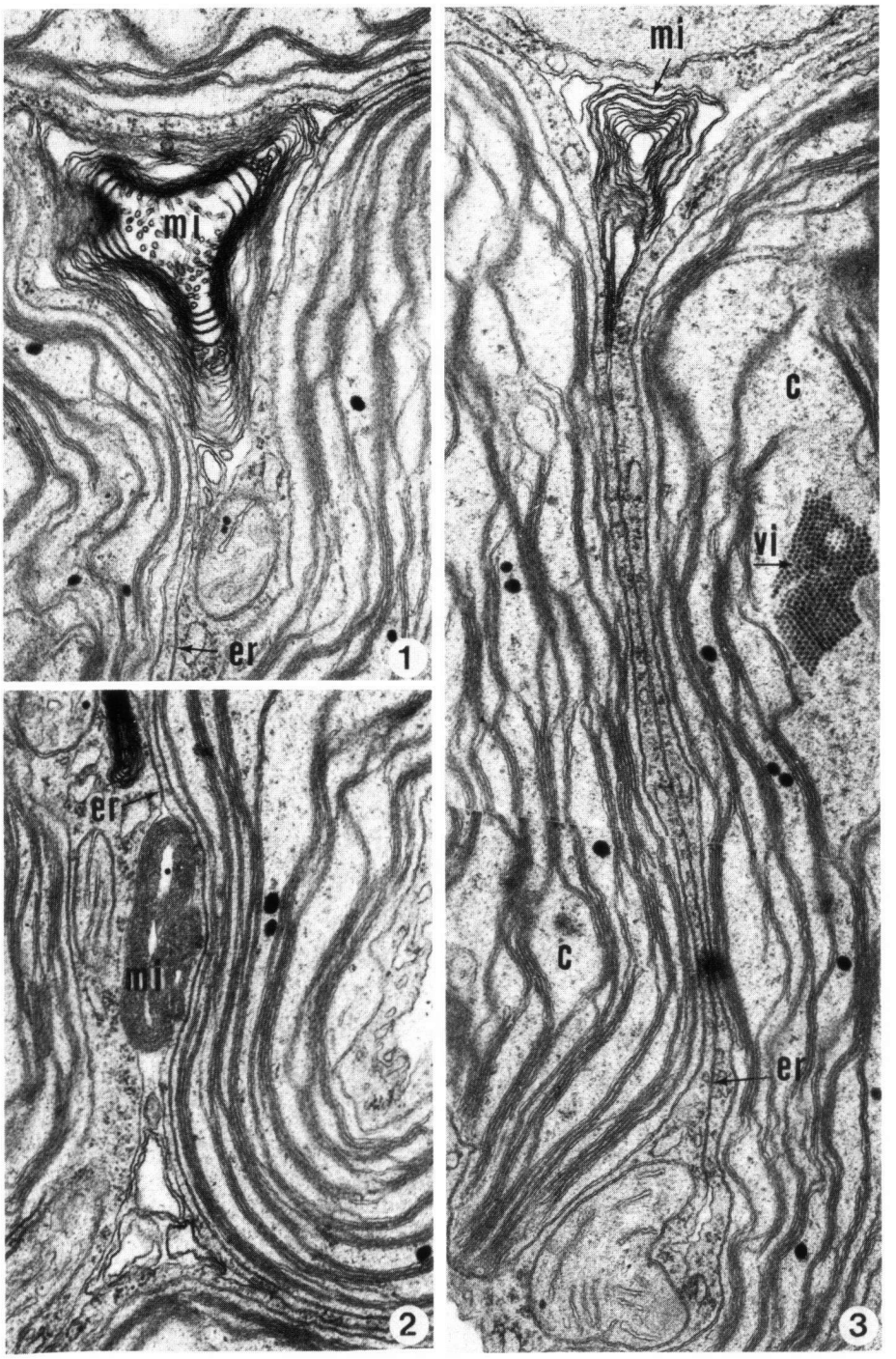
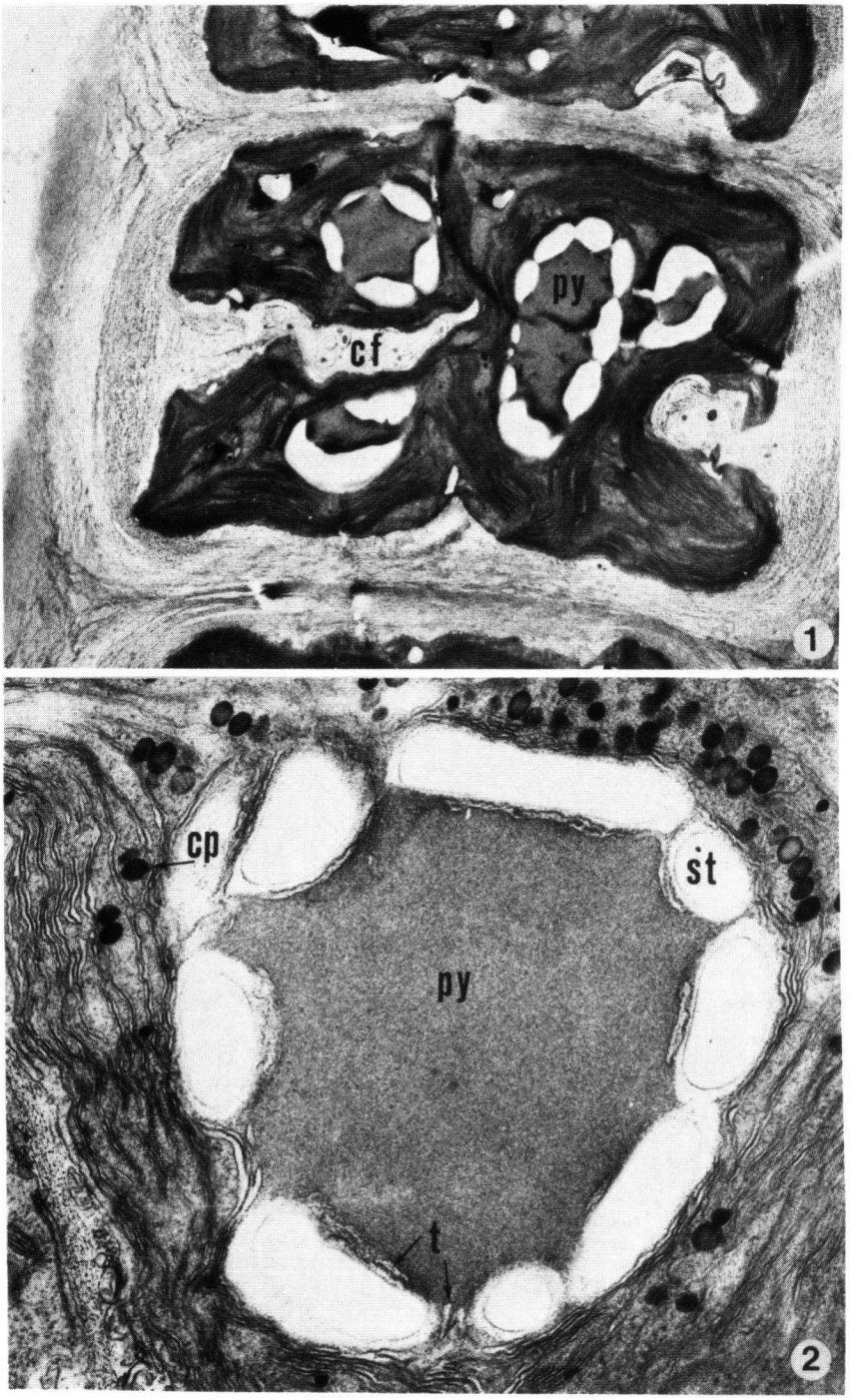


Plate 3

Plate 4



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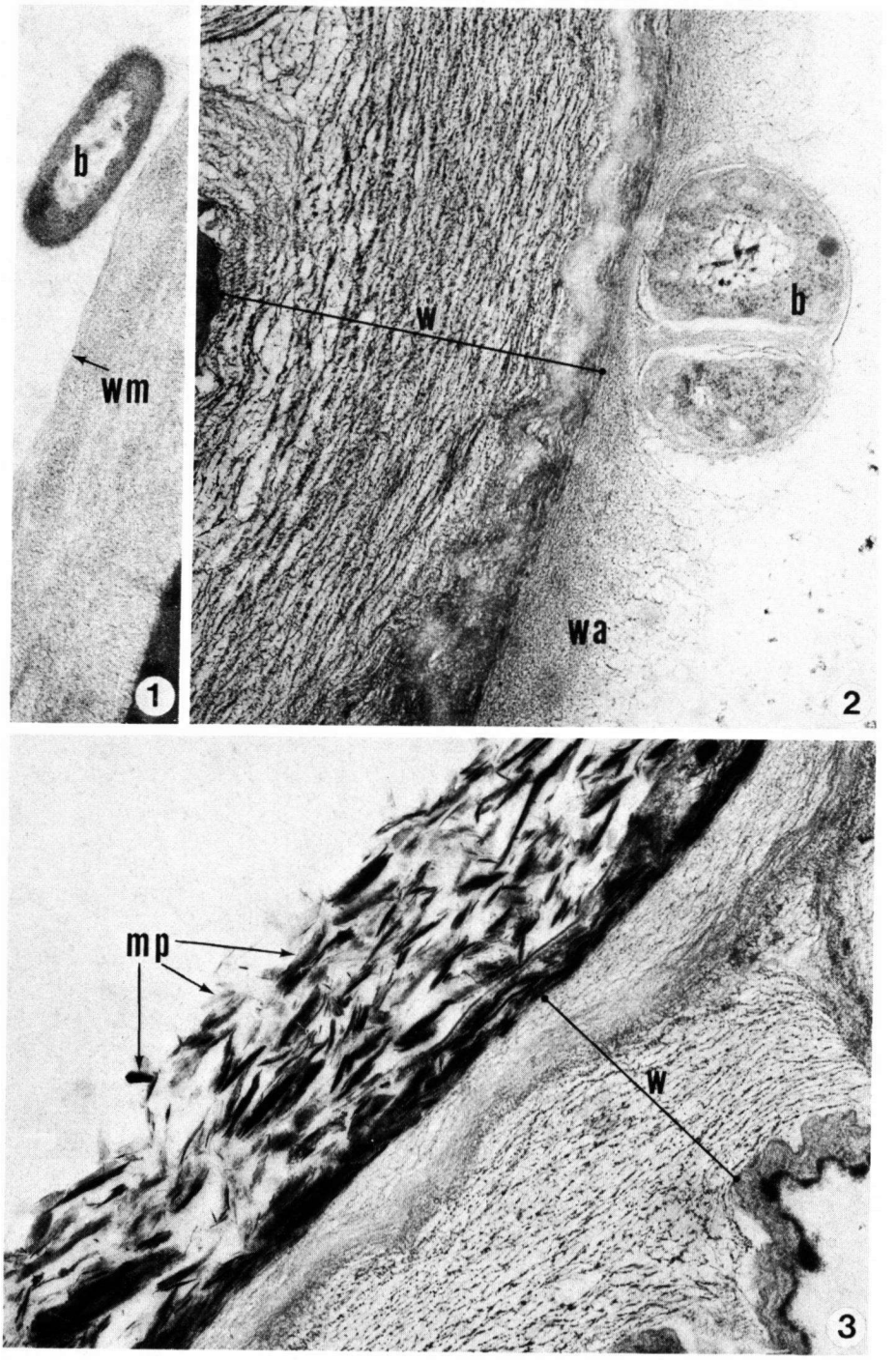
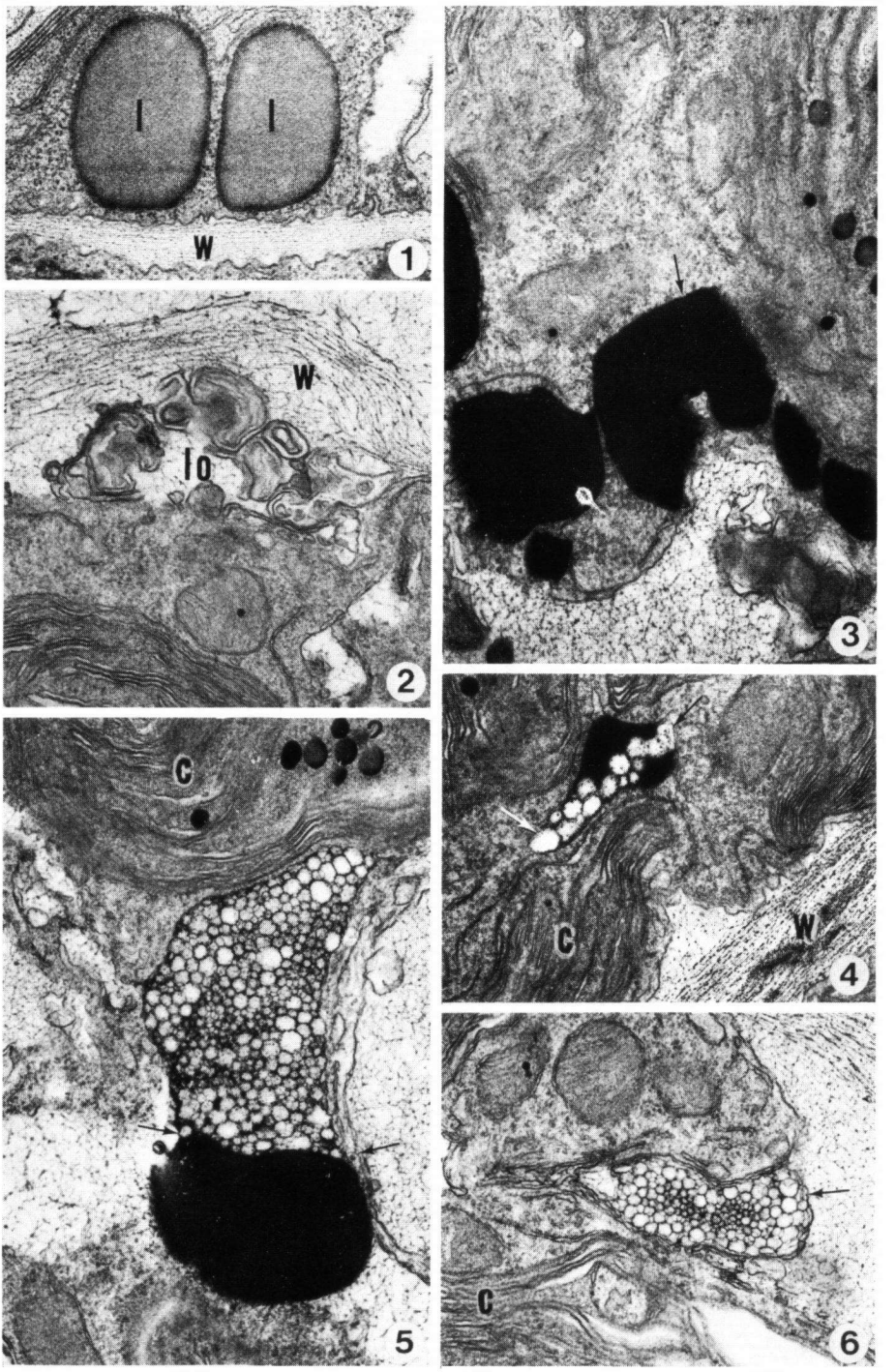


Plate 6



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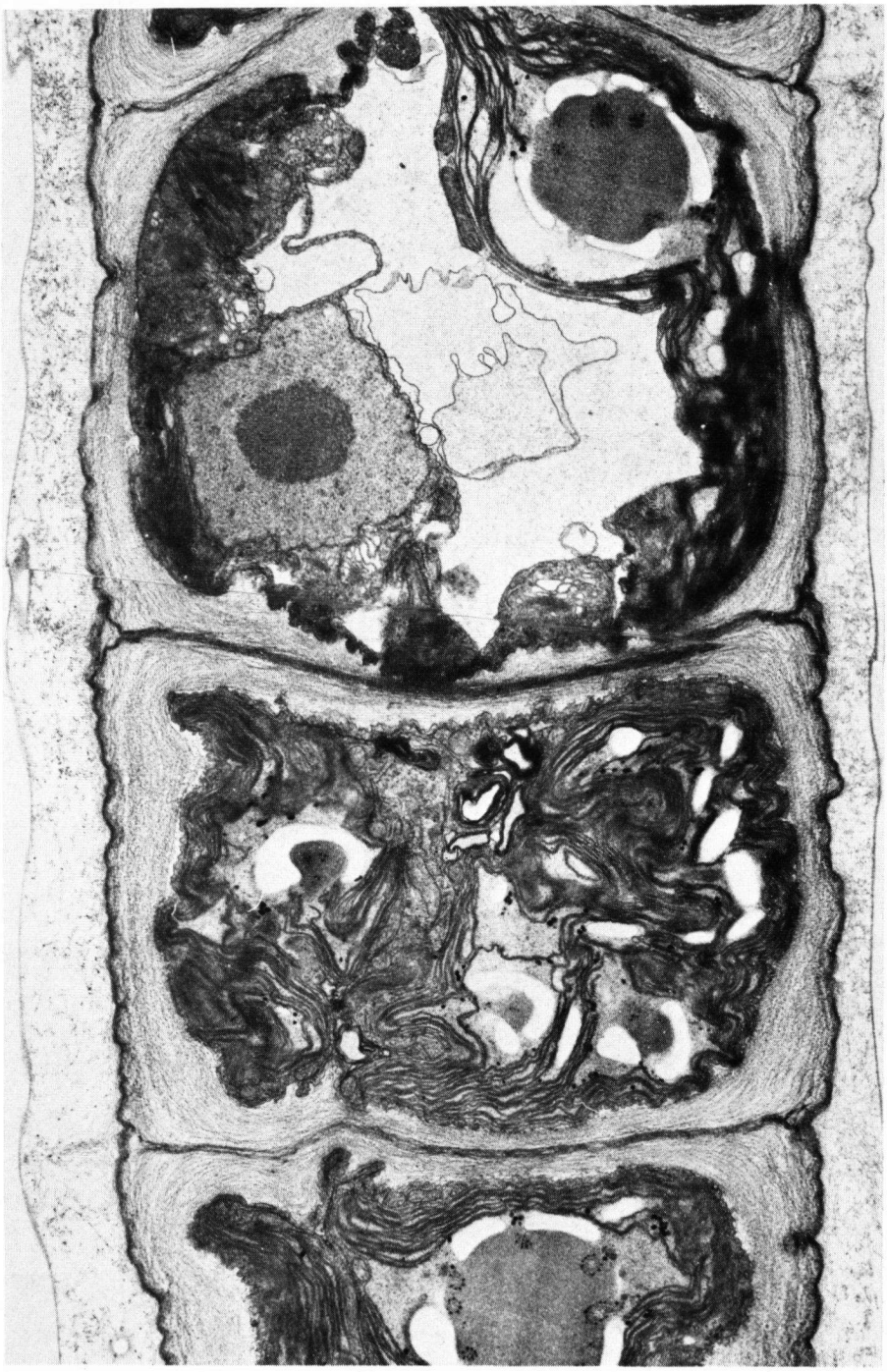


Plate 8

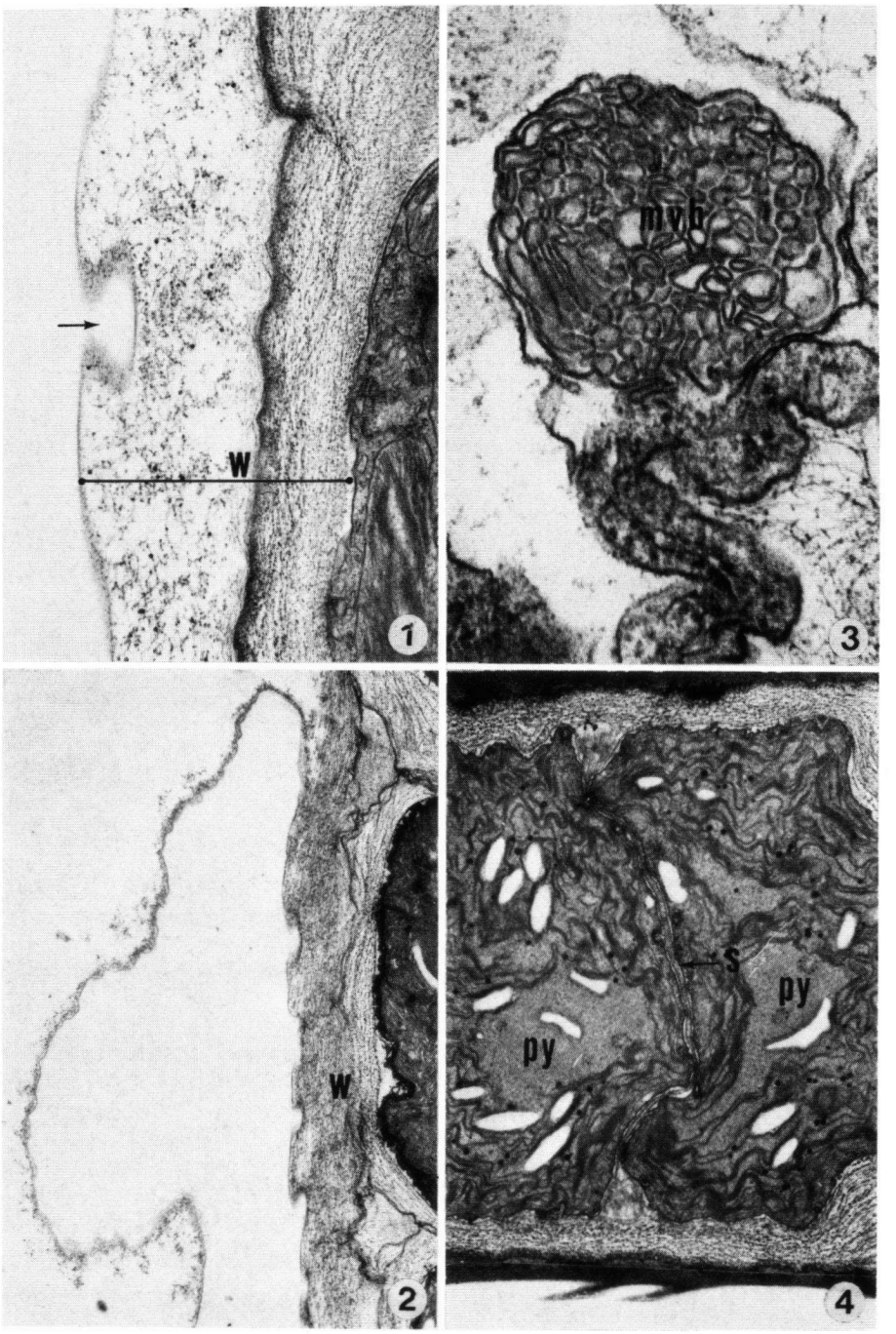
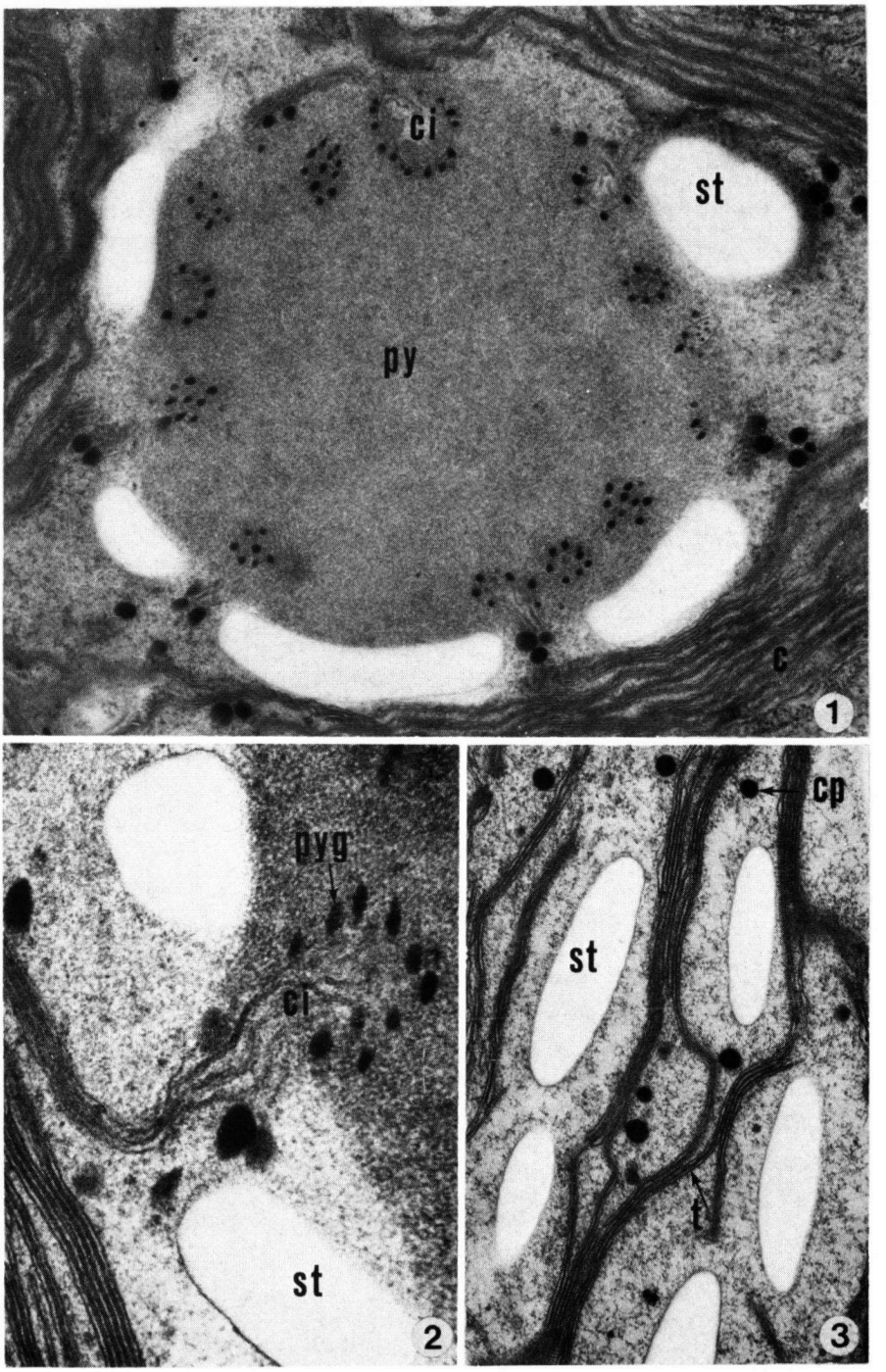


Plate 9



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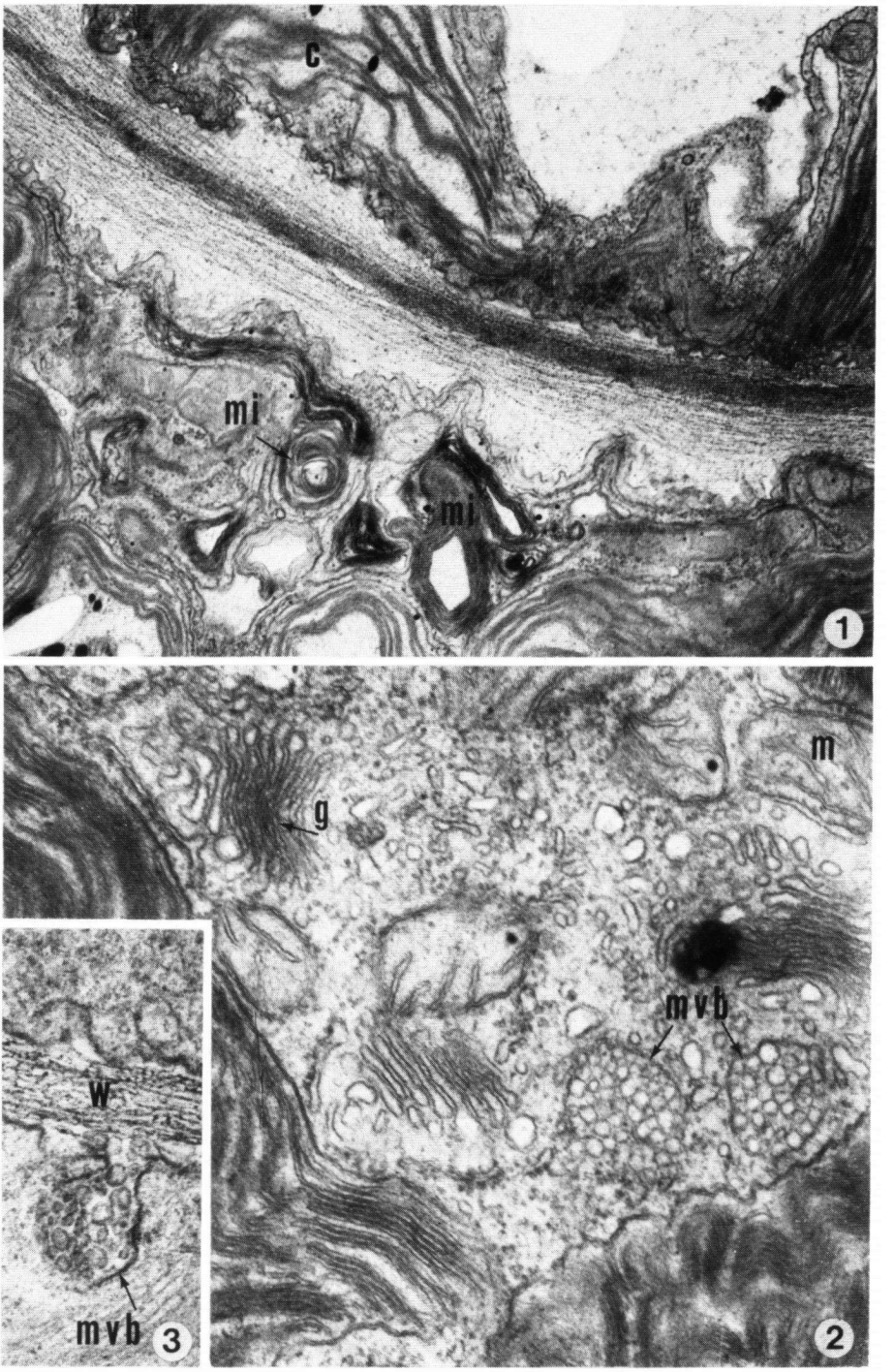


Plate 11

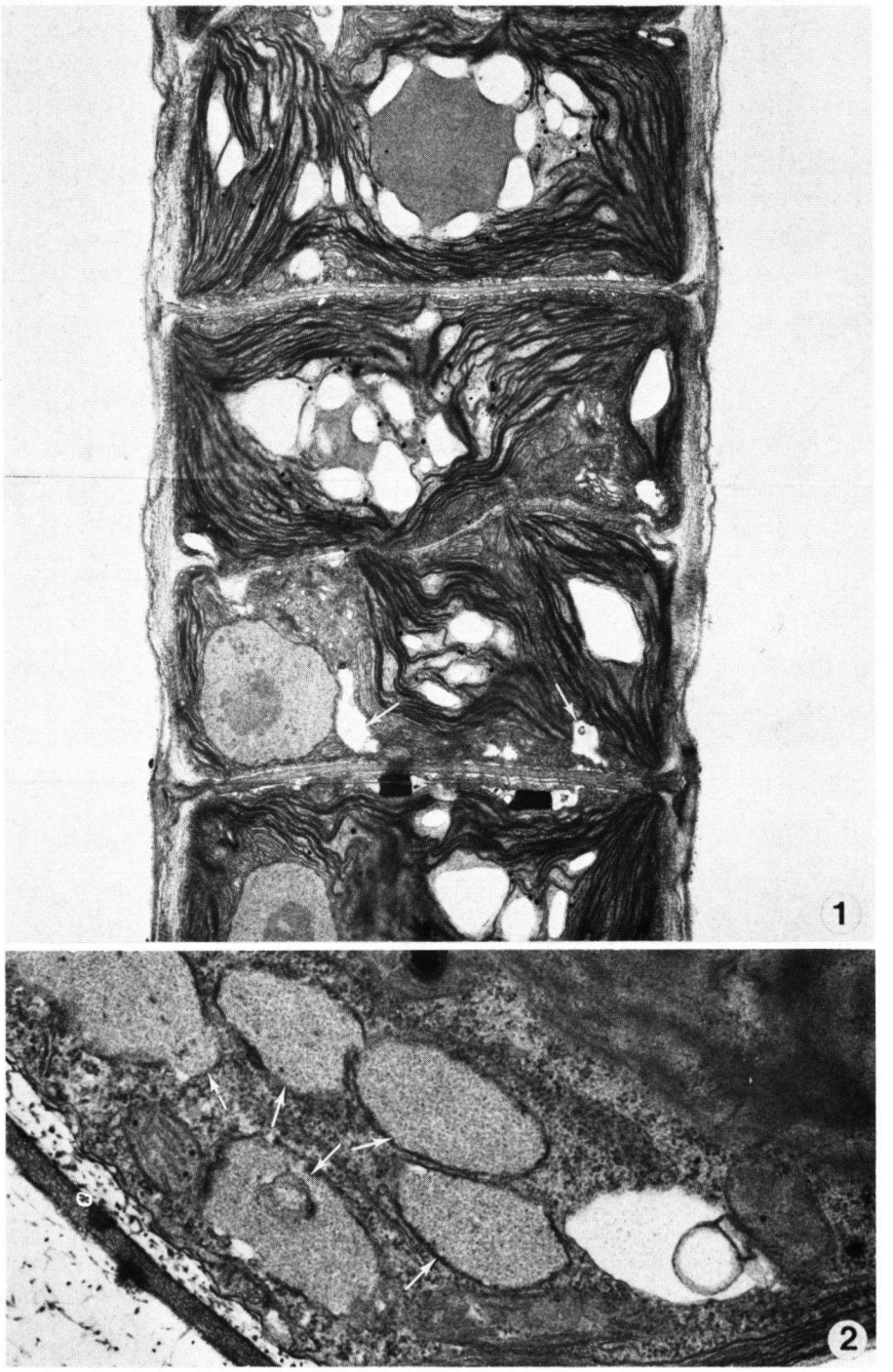


Plate 12

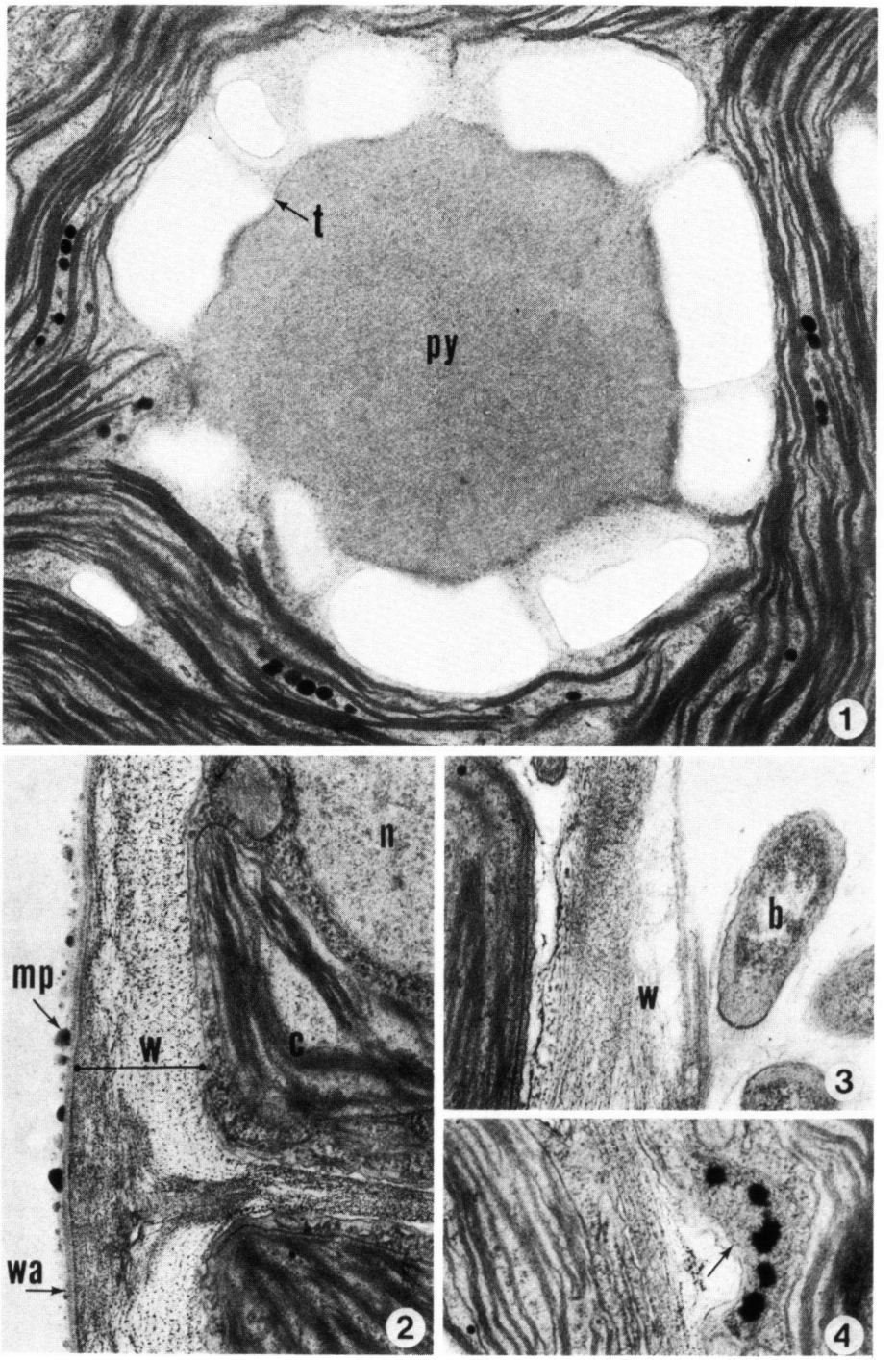
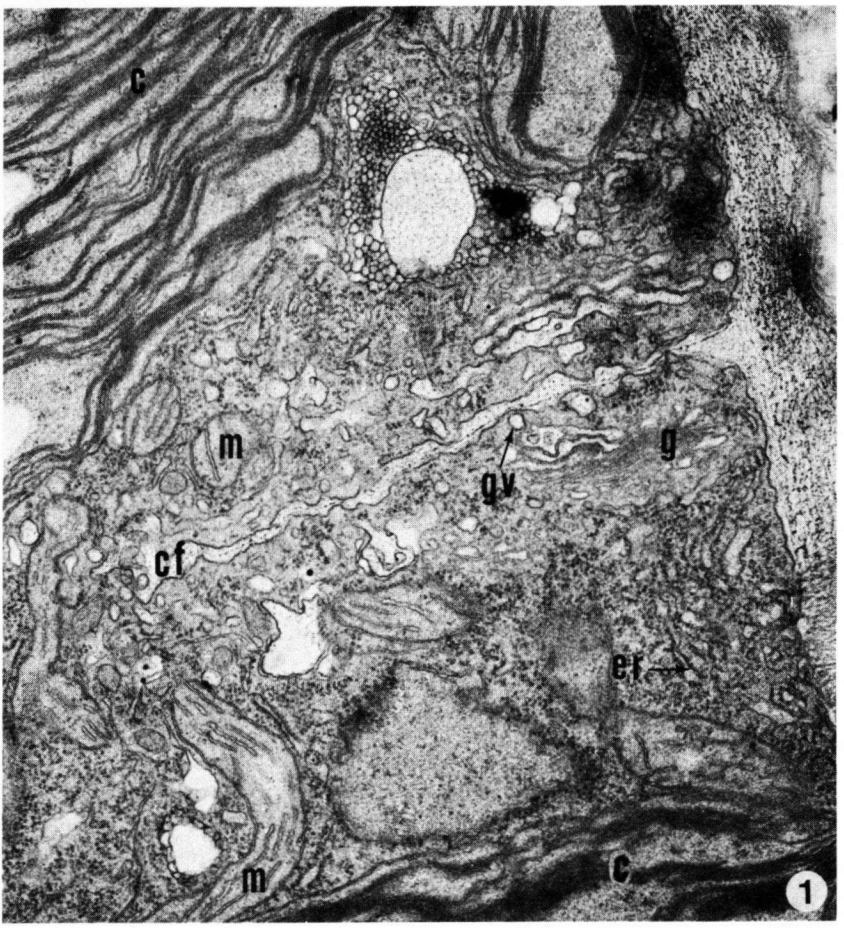


Plate 13



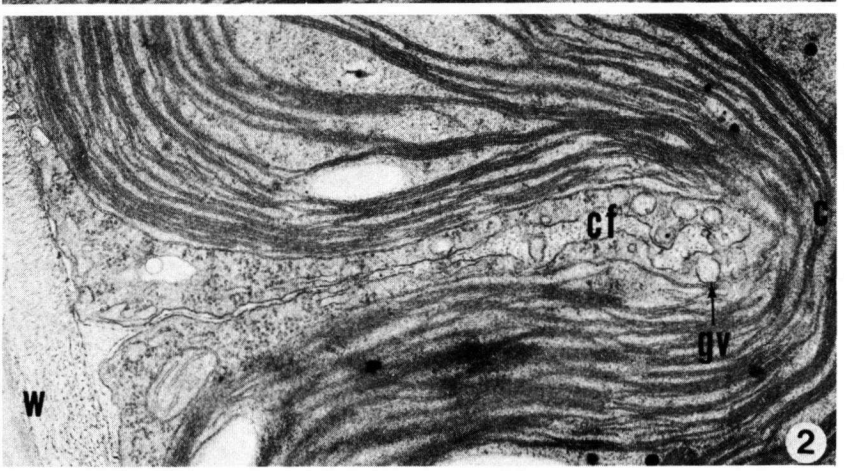


Plate 14

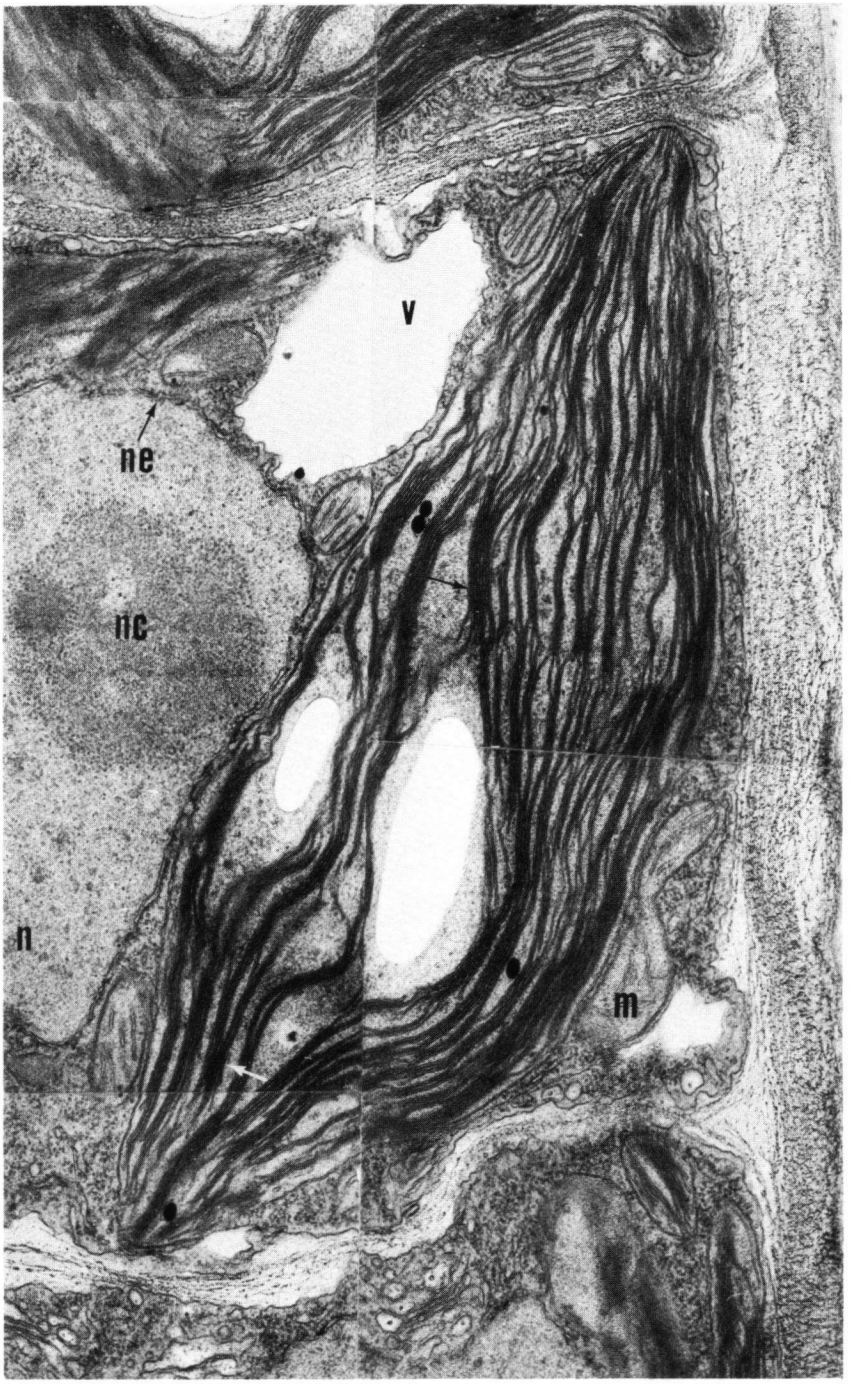


Plate 15

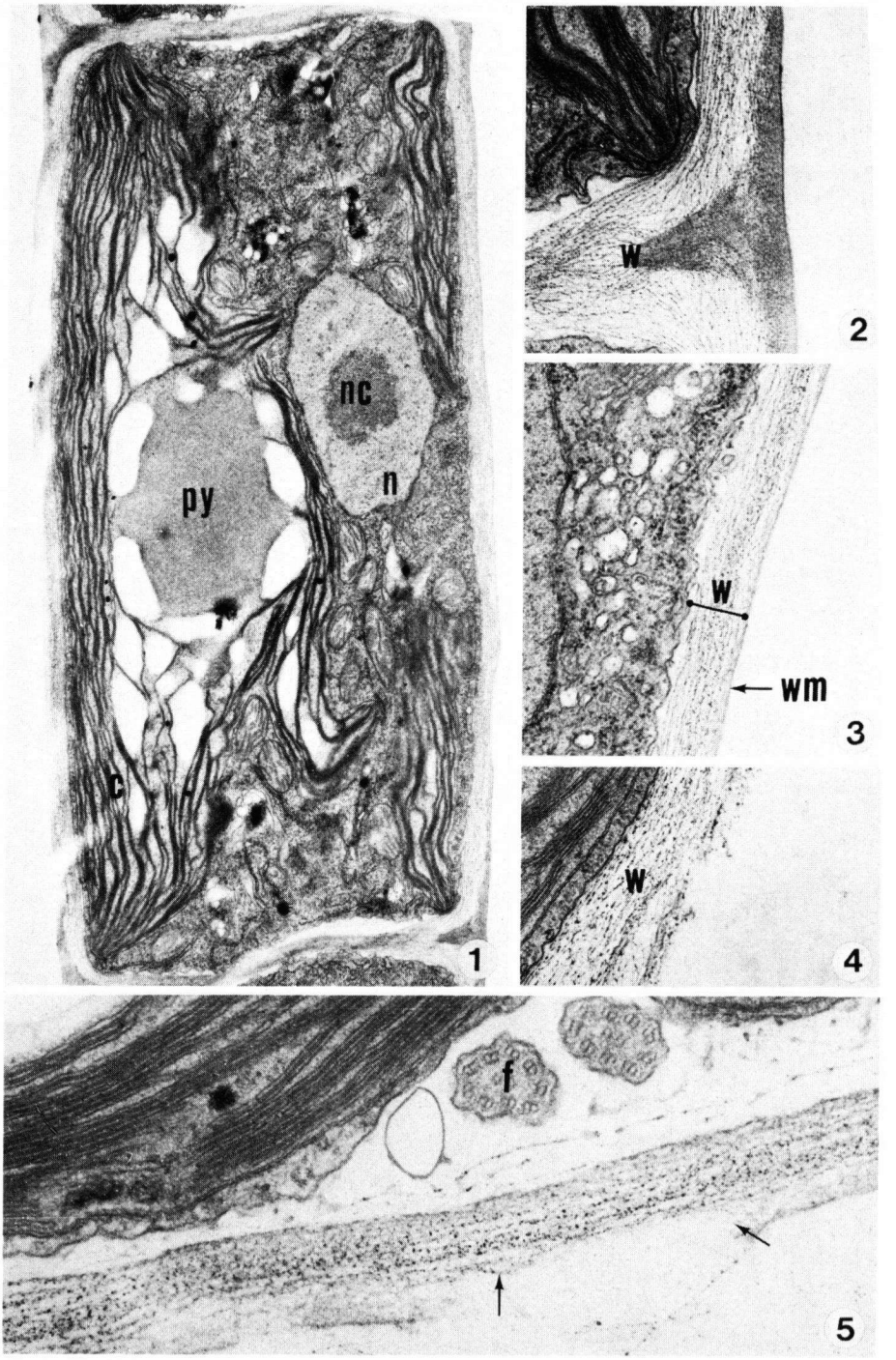


Plate 16