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STRUCTURE AND CHEMICAL COMPOSITION OF THE SPORE WALL IN SPIROGYRA (ZYGNEMATACEAE, CHLOROPHYCEAE)

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SUMMARY

Spores of twenty species of *Spirogyra* were treated by acetolysis and viewed by scanning electron microscopy. The terms exo-, meso- and endospore should be maintained and based on chemical composition. Exo- and endospore are cellulose and/or pectin containing membranes. The mesospore is acetolysis-resistant and presumably contains sporopollenin.

As basic pattern the spore wall in *Spirogyra* is composed of four layers: one exo- and endospore layer and two mesospore layers. The outer mesospore layer is mostly thin and hyaline, the inner layer thick and brown or yellow coloured and often sculptured. The germination suture is located in the inner mesospore layer. Some variations on this basic pattern were observed in *Spirogyra bellis*, *S. cleveana* and *S. majuscula*. Details of mesospore sculpture were observed and some taxonomic implications are discussed.

The observed basic pattern is assumed to be valid for the *Zygnemataceae* as a whole.

1. INTRODUCTION

From recent literature it appears that spore walls of Zygnematacean algae have gained renewed attention. We are dealing with studies on ecology and systematics of *Spirogyra* species occurring in The Netherlands. During identification of species we often remarked inconsistencies and vague illustrations concerning spore wall descriptions in the existing flora's (TRANSEAU 1951, RANDHAWA 1959, KADLUBOWSKA 1972). By applying a technique of acetolysis in combination with scanning electron microscopy (SEM), we did some observations concerning the number of wall layers and details of wall structures. Some standard histochemical tests were used to determine the chemical composition of the different wall layers.

In literature no unanimity exists concerning the basic number of wall layers. According to most authors the wall of *Spirogyra* spores generally is composed of three layers; the exo-, meso-, and endospore.

Recently ASHRAF & GODWARD (1980) confirmed this conception. They studied the wall structures of three *Spirogyra* species with the aid of acetolysis and scanning and stated that the walls are composed of three layers. The one-layered exospore would sometimes be sculptured and contain cellulose, pectin and in one occasion chitin. The one-layered mesospore is sometimes sculptured and would contain sporopollenin. The endospore in a thin colourless inner layer.

However, TRANSEAU (1951) stated that the wall is really a complex of three to five walls, one or more may variously be sculptured. According to this author the outer one or two layers are of cellulose, the median one or two layers would contain chitinous deposits in or on cellulose, and the inner wall again is of cellulose.

HOSHAW (1980) published light-microscopial and scanning observations on spores of species of *Sirogonium*, a genus closely allied with *Spirogyra*. He stated that the spores possess two mesosporium layers which would be composed of chitin.

From all this it will be clear that there is unanimity only with regard to the endospore. In this contribution we intend to clarify some matters by defining a basic pattern.

2. MATERIALS AND METHODS

Spores originated from field samples fixed in F.A.A., or from material which was induced to sexuality under conditions of light intensities between 5000 and 8000 Lux and N-depletion of the culture medium. The field samples contained ripened spores of *Spirogyra* species, in a few occasions mixed with spores of *Mougeotia, Zygnema* and *Oedogonium*. One field sample contained pure material of *Sirogonium sticticum*. The cultured material always contained ripened spores of *Spirogyra*. As culture medium a Woods Hole medium (STEIN 1973) was used. Living or F.A.A.-fixed spores were pressed and gently moved under a coverslip in order to detect different wall layers.

For determination of cellulose, pectin, and chitin the histochemical tests described by JENSEN(1962) were used. At one occasion a Kjeldahl test was carried out on acetolysed spore material to determine total organic N-content (LAURO 1931).

To release and clean spores from cell walls, material containing ripened spores was washed twice, in acetic acid centrifugating during 5 min. at 3000 r.p.m. Acetolysis was carried out according to the method described by ERDTMAN (1969). The acetolysed material was in part mounted in Euparal on slides for light-microscopic (LM) observations, and in part was prepared for scanning electron-microscopic (SEM) analysis.

For scanning the spores were dried and coated with gold-palladium. The specimens were studied in the ISI 40 scanning electron microscope at 10 kV at magnifications between 500 and 7000.

3. OBSERVATIONS

3.1. Test on the occurrence of chitin and sporopollenin

Freshly produced spores of *Spirogyra teodoresci, S. weberi* and F.A.A.-fixed spores of *Sirogonium sticticum* were tested for chitin. Control tests were carried out on the elytron of the beetle *Forficula lesnei* with fresh and F.A.A.-fixed material. Both controls were positive for chitin.

In all trials on *Spirogyra* and *Sirogonium* the tests appeared to be negative, so that we can conclude that chitin is not present in the wall layers of *Spirogyra* and *Sirogonium* spores.

It appeared that spores which were acetolysed as preparation for scanning, always contained at least two acetolysis-resistant layers. In our conception these layers make up the mesospore. According to ÀTKINSON et al. (1972) resistance against acetolysis would be sufficient evidence for sporopollenin. As it is known that sporopollenin does not contain any nitrogen (SHAW 1971), contrary to chitin which contains about 6% weight nitrogen (TAYLOR 1975), we applied a Kjeldahl test on acetolysed spore material of *Spirogyra weberi*. It appeared that the material contained 1.08% nitrogen, which could be ascribed to rest material from the spore content and/or microorganisms associated with the spore walls.

Spores of species of *Mougeotia, Zygnema* and *Oedogonium*, which were mixed in an acetolysed field sample containing *Spirogyra acanthophora*, appeared also to be acetolysis-resistant.

Resuming we can state that the mesospore of *Spirogyra, Sirogonium, Zygnema, Mougeotia*, and also *Oedogonium* most likely contains sporopollenin instead of chitin.

3.2. Analysis of wall layers

Spores of twenty species of *Spirogyra* were treated by acetolysis and scanned. Four species were treated with chemical tests. It appears that the species can be arranged in two categories, viz. a group with spores possessing four wall layers, and a group with more than four spore wall layers.

3.2.1. Spores with four wall layers

Seventeen species belong to this group: *Spirogyra acanthophora* (Skuja) Czurda, *S. endogranulata* Bock et Bock, *S. fluviatilis* Hilse, *S.* cf. *franconica* Bock et Bock, *S. granulata* Jao, *S. hassalli* (Jenner) Petit, *S. kuusamoensis* Hirn, *S. lagerheimii* Wittrock, *S. lenticularis* Transeau, *S. maxima* (Hassall) Wittrock, *S. megaspora* (Lagerheim) Transeau *S. nodifera* Bock et Bock, *S. singularis* Nordstedt, *S. teodoresci* Transeau, *S. varians* (Hassall) Kützing, *S.* cf. *verruculosa* Jao, *S. weberi* Kützing.

All these species possess one thin hyaline exospore and endospore containing cellulose and pectic substances, and two acetolysis-resistant middle-layers. From the latter two layers, the outer one is thin and hyaline, and the inner one thicker and pigmented. We call these two acetolysis-resistant middle layers the mesospore. The two mesospore layers are shown in *S. granulata (fig. 7), S. varians (fig. 1)*, and *S. weberi (fig. 4)*. The thin outer mesospore layer appeared to be easily loosened and thrown off by the acetolysis treatment.

Under LM spores of many species appear as smooth, and are described as such in the flora's. Yet the scanning pictures often reveal a more or less rough outer surface of the inner mesospore layer. In *S. varians* remarkable little bulbs occur on the inner mesospore layer (*fig. 1*), which are not seen with LM. In the allied species *S. teodoresci* which also shows smooth spores with LM (*fig.*

13), the scanning picture shows a clear reticulate sculpture on the inner mesospore (*fig. 14*).

When visible, the germination suture is always located in the inner mesospore layer.

Many species have sculptured spores when viewed with LM. Scanning pictures from this category are shown for *S. acanthophora* (*figs. 5, 6*), *S. fluviatilis* (*fig. 10*), *S. lenticularis* (*figs. 18–20*), *S. maxima* (*fig. 15*), *S. megaspora* (*fig. 16*), *S. granulata* (*figs. 7, 8*), and *S. lagerheimii* (*fig. 9*). These pictures reveal details allowing a refinement of the often vague descriptions and pictures. Of course best correspondence between LM and SEM is seen in species with pronounced wall structure as in *S. acanthophora*. Yet in SEM views it appears that spore sculpture in *S. acanthophora* is not a true network with spines as stated in the original description, but a structure with star-like projections with round depressions in the centre of the "stars" (*fig. 6*). For *S. fluviatilis* spore sculpture is described in the flora's as "corrugate or finely wrinkled". The SEM picture (*fig. 10*) reveals a finely reticulate sculpture. The inside of the inner sculptured mesospore layer shows a remarkable spongy structure (*fig. 10*). In *S. granulata* the sculpture of the inner mesospore, as viewed by SEM (*fig. 8*) is finely reticulate-granulate, and not only granulate as in the flora description. The inner mesospore layer is loosely surrounded by the thin outer mesospore layer (*fig. 7*) causing a wrinkled structure of that layer which is also mentioned in the floras. For *S. lagerheimii* the SEM sculpture on the inner mesospore (*fig. 9*) can be described as densely granulate instead of finely punctate as seen with LM. The inner mesospore wall of the species *S. maxima* and *S. megaspora,* both with large lenticular spores, shows a labyrinthine reticulated structure (*figs. 15* and *16*) which is very much alike between the two species, suggesting a close taxonomic affinity.

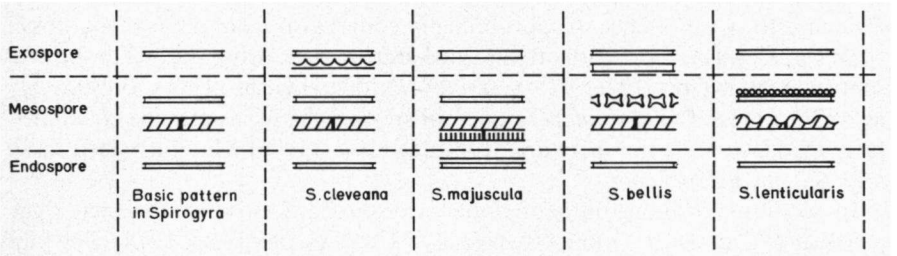
In another species with large lenticular spores, *S. lenticularis,* the two mesospore layers differ from each other in sculpture. On the surface of the thin outer layer a rough finely verrucose crust can be seen (*fig. 18*), which was often washed off by the acetolysis-treatment. The sculpture itself is acetolysis-resistant and belongs to the mesospore in our view. The inner mesospore layer is thick, coloured and sculptured causing a grooved brain-like view (*fig. 20*). In side-view this inner layer appears to be very thick and shows a transition from an inner loose structure to a compact outer consistency (*figs. 20* and 21). The exospore is thin and hyaline and contains cellulose and pectin, as is the case with the membranic endospore.

In some occasions (e.g. at *S. acanthophora* and *S. maxima*), we observed variation in density of sculpture, depending on the ripening-stage of the spores.

3.2.2. Spores with more than four wall layers

Three species belong here: *S. cleveana* Transeau, *S. bellis* (Hassall) Cleve, and *S. majuscula* Kützing.

S. cleveana: Spores are described as possessing two exospore layers of which the inner one shows a regular reticulate sculpture (*fig. 11*). In the acetolysis-



Scheme 1. Schematic representation of spore wall structure in the investigated species of *Spirogyra*. The thick vertical line in the inner mesospore layer represents the germination suture. The obliquely hatched layer is the coloured layer.

preparations we saw only spores with two smooth mesospore layers, the outer thin and hyaline, the inner firm and coloured (*fig. 12*). From this we can conclude that the sculpture is indeed located in the non-acetolysis-resistant exospore.

S. bellis: The exospore consists of two sublayers: one thin hyaline cellulose containing outer membrane, and one relatively thick shiny pectic layer (*fig. 25*). it is this layer on which the statement in the flora's "a thick exospore" is based. The mesospore consists of one outer hyaline rather thin layer with a very pronounced scrobiculate sculpture (*figs. 26–28*) and one thick coloured inner layer with suture (*fig. 26*). The one-layered endospore is a pectic membrane.

S. majuscula: The exospore of this species consists of a rather thick hyaline pectic layer. The acetolysis-resistant mesospore consists of three layers: a thin hyaline outer layer, and a set of two coloured layers narrowly connected with each other (*fig. 22–24*). The inner sublayer shows a finely striated structure in side-view (*fig. 22*), which appears to be a loose spongy structure under SEM (*figs. 23, 24*). In the SEM picture the two-layered nature of the inner mesospore is clearly visible (*fig. 24*). The endospore consists of two layers: a very thin outer cellulose-containing membrane, and an inner thicker pectic layer. To summarize all observations *scheme 1* is presented.

4. DISCUSSION

As the results presented cover a fairly large random sample of species, some generalisations can be made.

The terms exo-, meso-, and endospore should be maintained and can be based on chemical composition. Exo- and endospore contain cellulose and/or pectic substances, and the mesospore is acetolysis-resistant and presumably contains sporopollenin. Exo- and endospore are mostly hyaline, but the mesospore is nearly always brown or yellow coloured presumably by carotenoid substances.

Contrary to the statement of HOSHAW (1980), the mesospore of *Sirogonium* does not contain chitin. Like *Spirogyra* the mesospore of *Zygnema* and *Mougeotia* spores are acetolysis-resistant and are assumed to contain sporopollenin.

Evidence for the presence of sporopollenin comes also from the fact that fossil spores of *Zygnemataceae* are often recorded from the Carboniferous to the present time (VAN GEEL 1976; VAN GEEL & VAN DER HAMMEN 1978). Presumably also the spores of *Oedogoniaceae* contain sporopollenin, as may be concluded from evidence presented here and from VAN GEEL (1976) who illustrated fossil spores from *Bulbochaete*.

In general sporopollenin is of rather widespread occurrence in green algae (ATKINSON et al. 1972; GOOD & CHAPMAN 1978; VAN DEN HOEK 1978). The biological significance for the zygospores of *Zygnemataceae* is undoubtedly to give protection against desiccation and fungal parasitism as Zygnematacean algae are often growing in small temporary water bodies.

In all spores observed we detected at least four wall layers: one exo- and endospore layer and two mesospore layers, so that we regard this as a basic pattern in *Spirogyra* spores. This is in accordance with the observations of HOSHAW (1980) in *Sirogonium*, a genus closely allied with *Spirogyra*. This basic pattern was also observed at *Mougeotia laevis* (Kütz) Archer and *Zygnema cruciatum* (Vauch.) Agardh. On account of all observations we suppose that it is valid for the *Zygnemataceae* as a whole. The exospore of *Spirogyra* spores is mostly one-layered, but sometimes two layers are present of which the inner one may be sculptured as in *Spirogyra cleveana*. The mesospore is at least two-layered, the outer layer mostly being thin and hyaline, the inner one being firm and brown or yellow coloured and often sculptured. When present, the suture is always located in the inner mesospore layer. Variations in the mesospore concern the number of layers (e.g. three in *Spirogyra majuscula*) and the location of sculpture (e.g. only in the outer layer in *Spirogyra bellis*, or in both layers as in *S. lenticularis*). When the two mesospore layers are clearly separated from each other, the spores were described as having two mesospore layers. A good example offers the description of *Spirogyra quadrilaminata* Jao by RIETH (1972). In most cases however, the two layers are in close apposition to each other and then the mesospore looks one-layered with LM, as in most species.

We cannot confirm the statement of ASHRAF & GODWARD (1980) that in the inner surface of the mesospore pits should always be present. In some cases as in *Spirogyra fluviatilis* we detected a remarkable spongy structure on the inner surface of the inner mesospore layer, and also in other species the inner part of this layer was often of a looser structure.

The SEM technique offers good opportunity to study mesospore sculptures more detailed than with LM, which may be useful in taxonomic studies. In *Spirogyra maxima* and *S. megaspora* for instance, spore sculpture is almost identical, suggesting that the two species may be conspecific. *S. megaspora* differs from *S. maxima* only in larger dimensions of the filaments and spores. In *Spirogyra teodoresci* spores under LM are smooth, but when scanned these are clearly sculptured. Presumably *S. teodoresci* belongs to a species complex, as one sample of *S. teodoresci*, identified with LM, did not show any spore sculpture with SEM.

The endospore is always one-layered, except in *Spirogyra majuscula* where it is two-layered.

Resuming we can state that the analysis of spore wall layering in *Spirogyra* by TRANSEAU (1951) was correct except for the presence of chitin in the mesospore. The present study has demonstrated that the mesospore is not generally one-layered, but at least two-layered, which is supposed to be a basic feature in the genera *Spirogyra, Sirogonium* (HOSHAW, 1980), and presumably also in *Mougeotia* and *Zygnema.*

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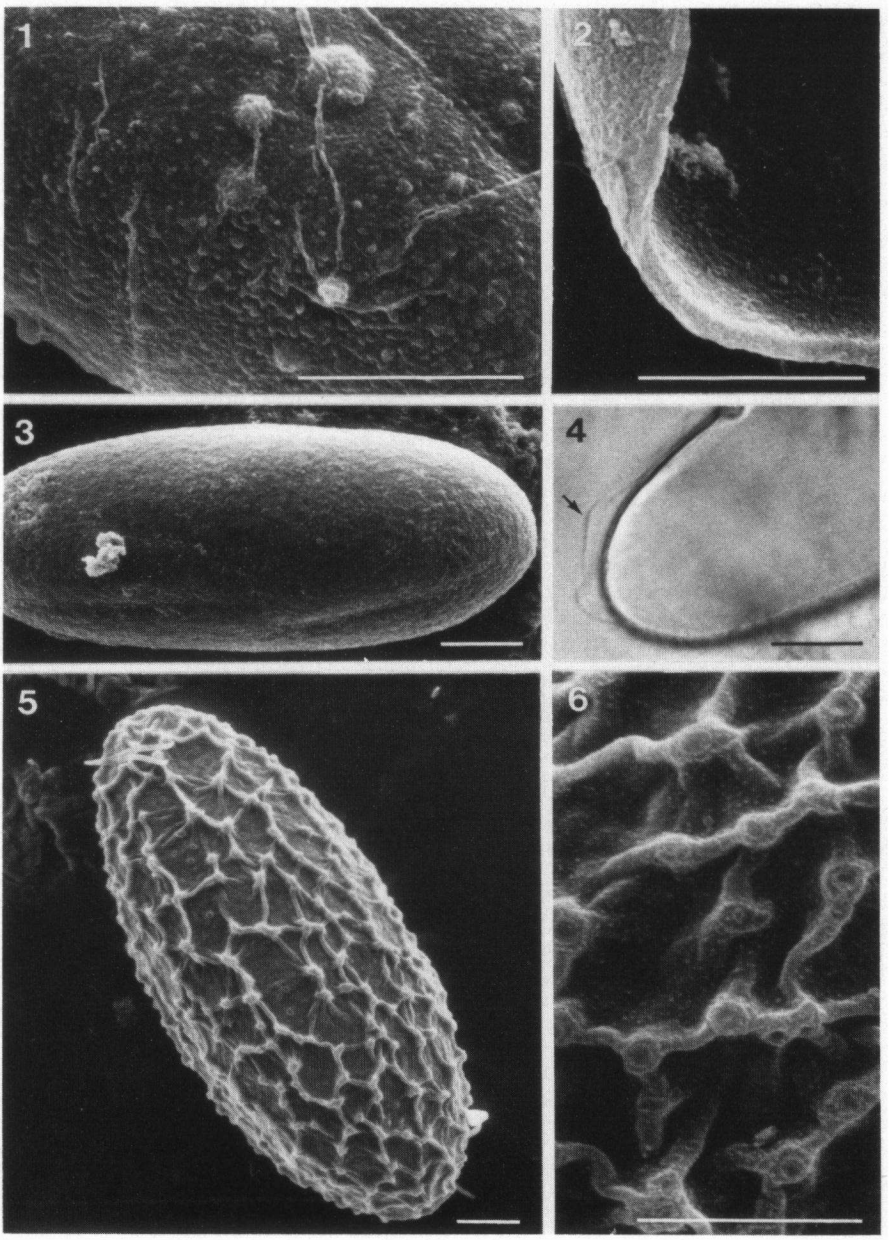

Plate I. Fig. 1: *Spirogyra varians,* detail of mesospore with the thin outer layer wrapped around the thick inner layer; fig. 2: *S. singularis,* inner surface and side view of inner mesospore layer; fig. 3: *S. weberi;* fig. 4: *S. weberi,* LM view of acetolysed spore, the arrow points to the outer mesospore layer; fig. 5: *S. acanthophora;* fig. 6: *S. acanthophora,* detail of mesospore sculpture. Scale bar = 10 μm.

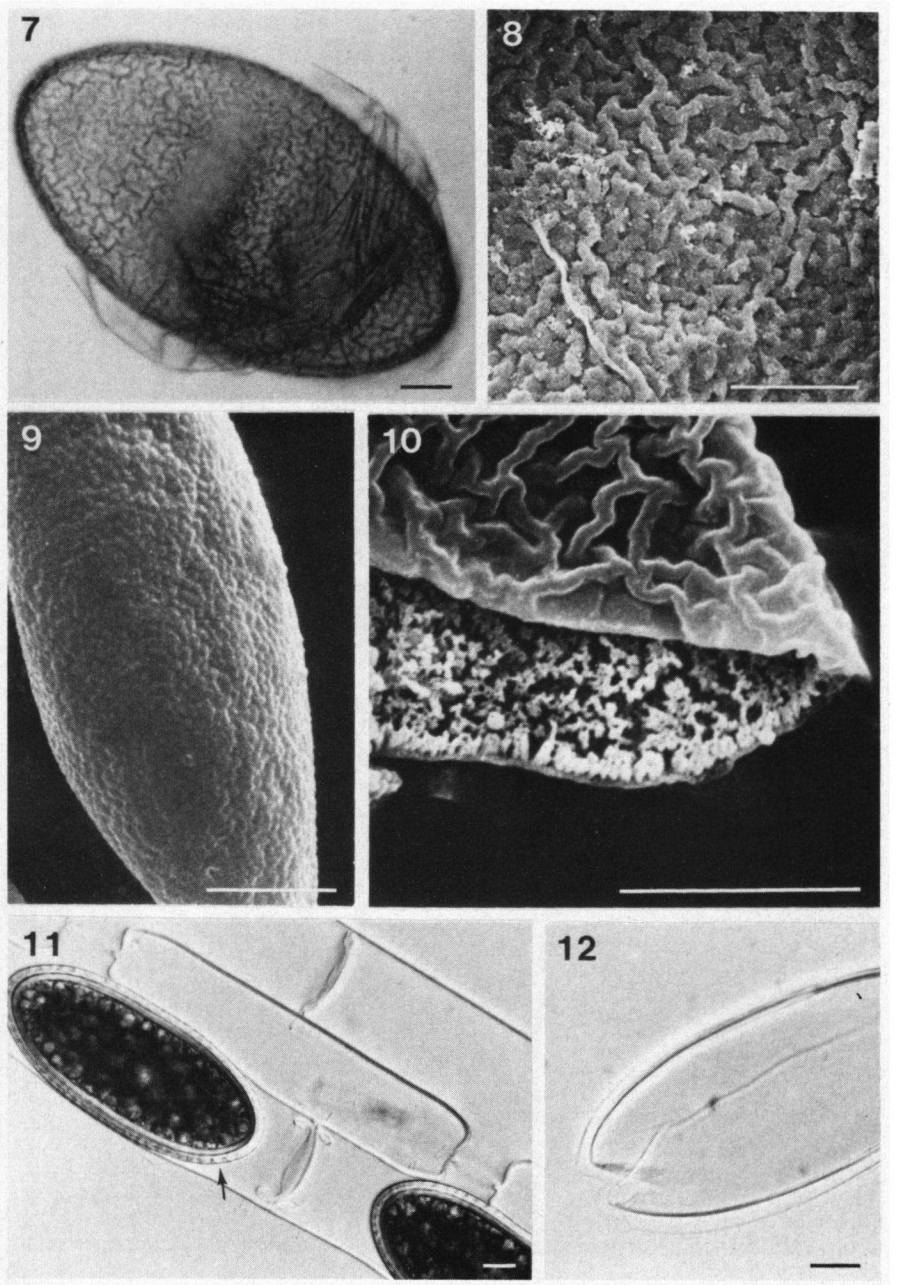

Plate II. Fig. 7. *Spirogyra granulata*, LM view of acetolysed spore with two mesospore layers; fig. 8: *S. granulata*, sculpture on the inner mesospore layer; fig. 9: *S. lagerheimii;* fig. 10: *S. fluviatilis*, outer and inner surface of inner mesospore layer; fig. 11: *S.cleveana*, the arrow points to the structure on the inner exospore layer; fig. 12: *S. cleveana*, LM view of acetolysed spore showing the two mesospore layers and suture in the inner layer. Scale bar = 10 μm

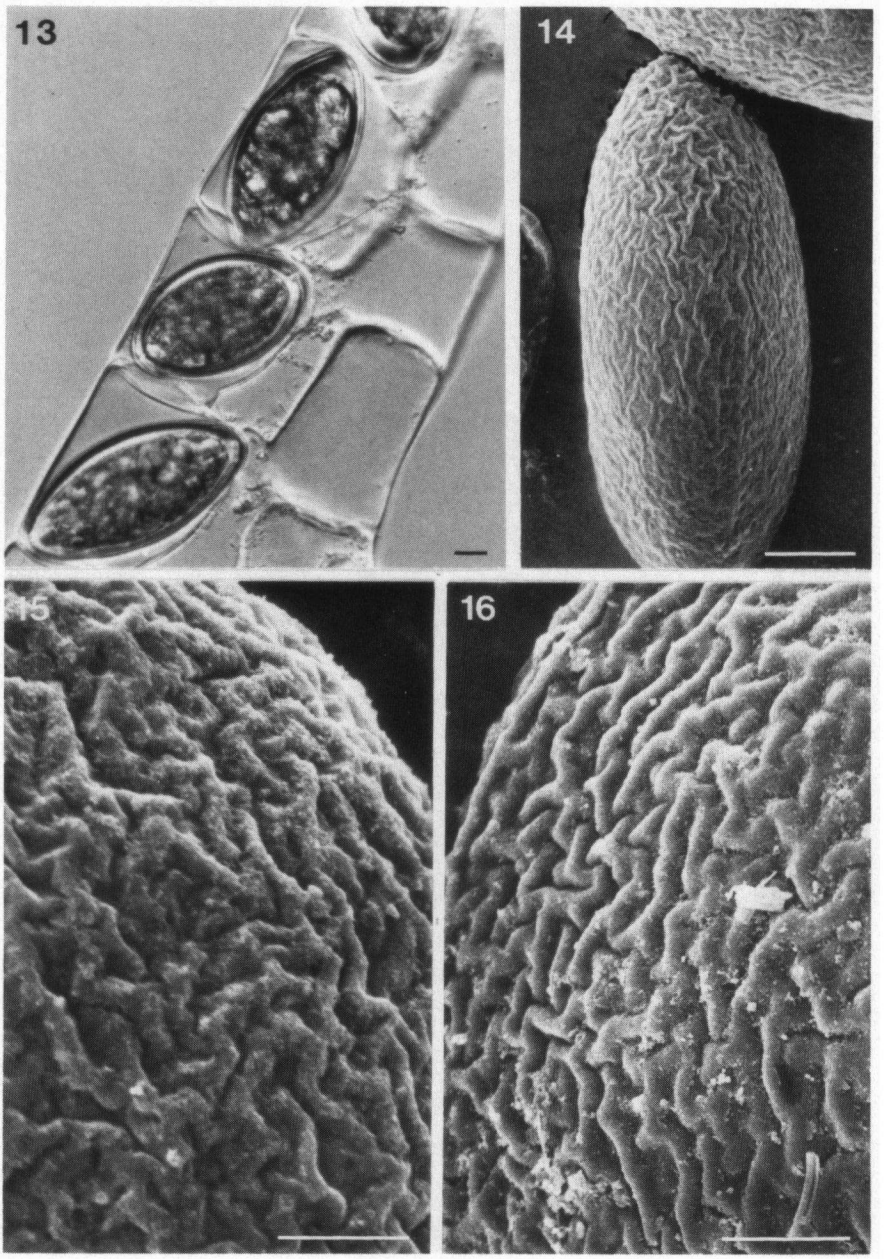

Plate III. Fig. 13: *Spirogyra teodoresci*, LM view; fig. 14: *S. teodoresci*, SEM view; fig. 15: *S. maxima;* fig. 16: *S. megaspora*. Scale bar = 10 μm.

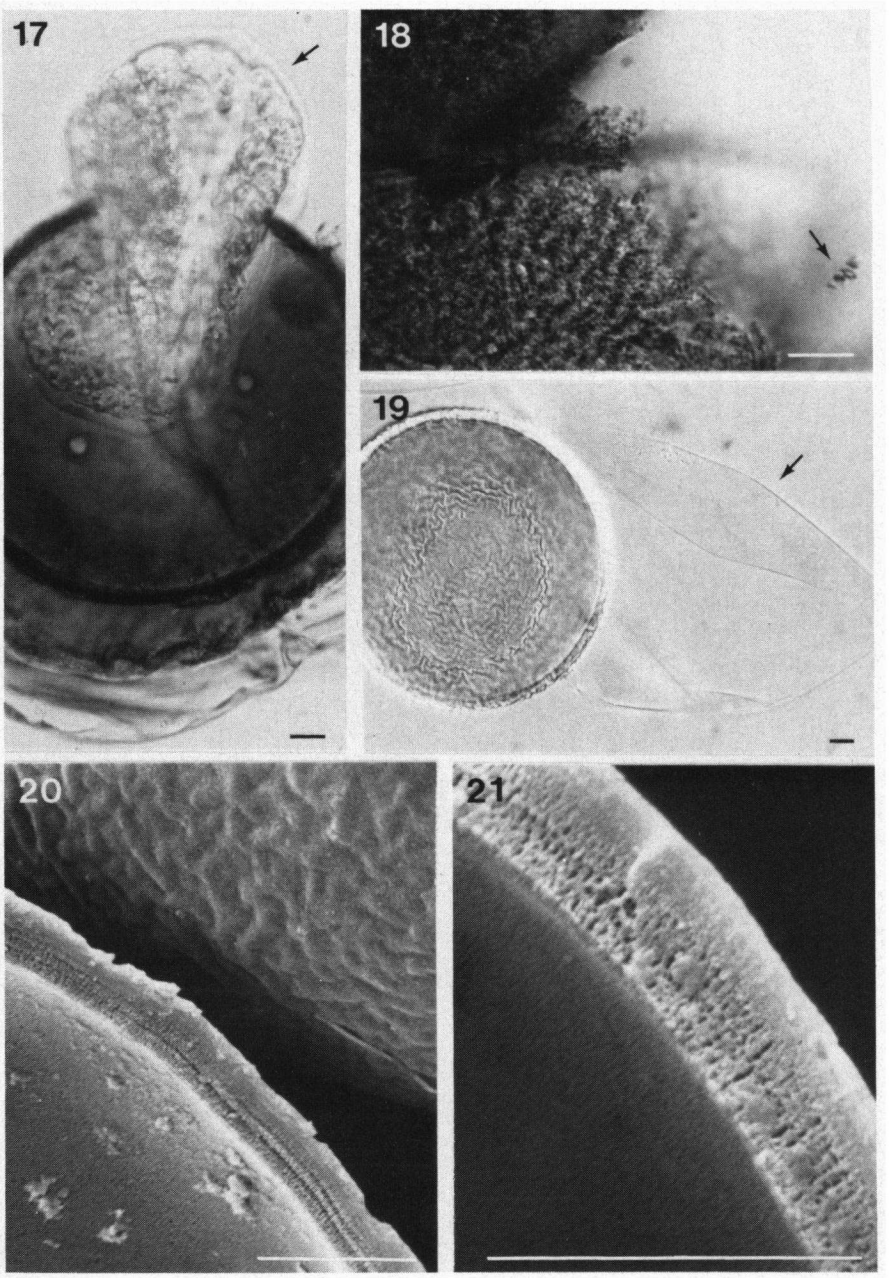

Plate IV. *Spirogyra lenticularis*. Fig. 17: LM view of pressed spore, the arrow points to the endospore membrane; fig. 18: LM view of the sculpture on the outer mesospore layer, the arrow points to material loosened from the surface crust; fig. 19: LM view of acetolysed spore, the arrow points to the outer mesospore layer from which the surface structure is washed off; fig. 20: surface and side view of inner mesospore; fig. 21: detail of side view of inner mesospore. Scale bar = 10 μm.

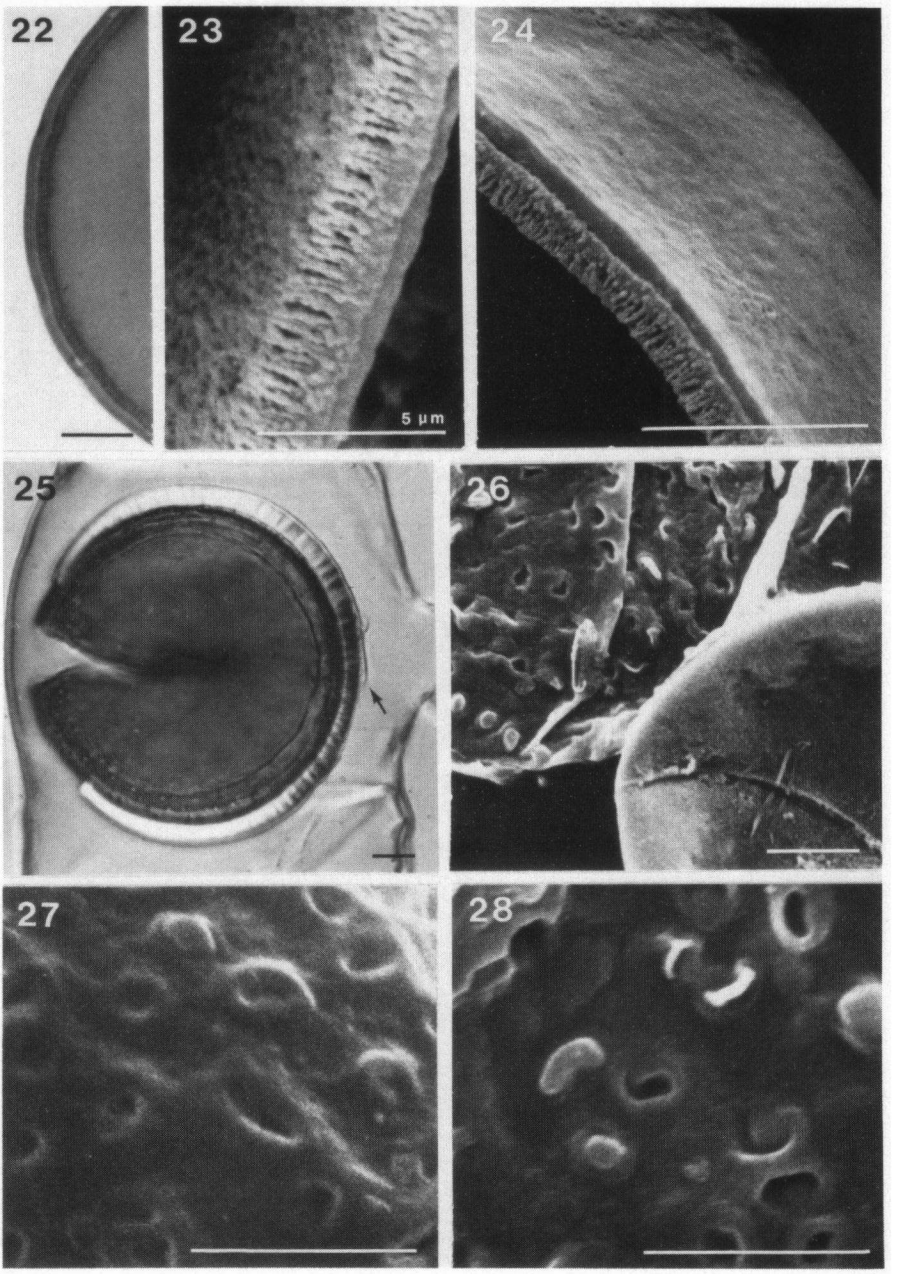

Plate V. *Spirogyra majuscula*. Fig. 22: LM view of acetolysed spore with the two inner mesospore layers; fig. 23: SEM view of side and inner surface of the two inner mesospore layers; fig. 24: idem, side and outer surface view. Figs. 25–28: *S. bellis*. Fig. 25: LM view of pressed spore, the arrow points to the thin outer exospore layer; fig. 26: outer mesospore layer (left), and inner mesospore layer with suture (right); fig. 27: surface of mesospore with the outer layer lying around the inner one; fig. 28: detail of loosened outer mesospore layer. Scale bar = 10 µm.