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THE GENERA PHAEOTHAMNION LAGERHEIM, TETRACHRYSIS GEN. NOV. AND SPHAERIDIOTHRIX PASCHER ET VLK (CHRYSOPHYCEAE)*

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SUMMARY

Culturing experiments are described in which the life cycles of several clones of *Phaeothamnion borzianum*, a supposedly palmelloid form of the latter, and *Sphaeridiothrix compressa* are investigated. The three types of algae turned out to be quite distinct taxa, not at all connected by intergrading forms as has been suggested in the literature. A new genus – to be named *Tetrachrysis* – is proposed to accommodate the taxon, formerly regarded as a palmelloid phase of *Phaeothamnion*.

1. INTRODUCTION

Several clones of *Phaeothamnion borzianum* Pascher, *Sphaeridiothrix compressa* Pascher et Vlk, and an alga that has been described as a palmelloid form of a *Phaeothamnion* species by several authors, were isolated from different localities in The Netherlands. Since it has been suggested (GEITLER & SCHIMAN-CZEIKA 1970, GEITLER 1970) from observations on field material that these forms might all be expressions of one extremely plastic species, the opportunity was taken to investigate the life cycle as well as the influence of some environmental factors on the morphology of these algae.

2. MATERIAL AND METHODS

Clones of *Phaeothamnion borzianum* were isolated from glass slides, suspended in the Botshol near Amsterdam (October 1975, for description of this pond see DOP & VROMAN 1976), and the peat pond 't Hol near Hilversum (June 1976). The supposedly "palmelloid" form was isolated from glass slides from the Botshol (October 1975), from a *Phragmites* stem from the same locality (November 1974), and from *Chara* fragments from the broads area in the N.W.-part of the province Overijssel (May 1977; for description of this area see COESEL 1979).

Clones of *Sphaeridiothrix compressa* were isolated from glass slides, submerged in the Botshol (April 1974 and October 1975) and 't Hol near Hilversum (May 1976), and from *Chara* fragments from N.W.-Overijssel (May 1977).

Cultures were maintained in an Erd-Schreiber medium based on Botshol water, and in Wood's Hole artificial freshwater medium (STEIN 1973).

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To induce zoid formation, several methods were tried out, such as varying light/dark regimes of 16/8 and 8/16 hrs., in combination with temperatures from 4 to 20°C. In addition, temperature shock (transfer from 4°C total darkness to room temperature by daylight) and lowering of the medium pH to 4.5 were used.

Morphological variation was tested in crossed temperature-light gradients, varying from 3–25°C and 150–5500 Lux respectively (modified apparatus after EDWARDS & VAN BAALEN 1970), under neutral-day conditions. These experiments (in duplo, with one clone of each species) were evaluated after four weeks. Cell dimensions of five thalli in each Petri-dish were measured, and a combined estimate of growth and reproduction was made and expressed in a five-point scale.

Sphaeridiothrix compressa was also tested for ability to withstand varying chlorinity by culturing it under 12°C, neutral-day conditions in ten different media, with chlorinity varying from zero to $30^0/_{00}$. These media were made up by adding NaCl to Wood's Hole medium. After four weeks, estimates of zoid formation and growth were made and expressed in a four-point scale.

3. OBSERVATIONS

3.1. *Phaeothamnion borzianum* Pascher

3.1.1. Field material

Apart from localities where material was isolated from, this alga was also found in several bêta-mesosaprobic, moderately eutrophic ditches in the province of South Holland.

Thalli are dendroid, and consist of a hemispherical basal cell (8–9 μm diam.) attached to the substratum, from which spring one or more branching systems of oblong cells (4–8 μm × 12–20 μm) (*fig. 1*). Between cells, a short mucilaginous stalk of varying length is sometimes visible, with faint striation parallell to the bordering cell walls (*fig. 2*).

Cell contents comprise one or two, mostly parietal, olive-green chloroplasts, irregularly lobed and incised (*fig. 2*); one or two oil droplets, and a large number of strongly refringent small round and rod-shaped crystals just underneath the cell wall. They exhibit Brownian movement and appear to lie in a peripheral vacuolar system just underneath the cell membrane. A nucleus with nucleolus is sometimes visible in the lower part of the cells.

Zoids or cysts were not observed in field material.

3.1.2. Cultured material

3.1.2.1. Habit and reproduction

In culture, the mucilaginous stalks between cells disappear; chloroplasts become somewhat larger so as to cover almost the entire cell wall. In log-phase cultures, lipid and leucosin droplets and the small peripheral crystals are reduced in

number *(fig. 3)*. Cell dimensions remain the same. Thalli may reach up to 1 mm. in height.

Cell division takes place as follows: nuclei and chloroplasts divide first *fig. 3)*. The two daughter cells then secrete their own wall within the mother cell, and the upper one is extruded from the distal part of the mother cell, stretching its wall around it. Branching is accomplished by sideways extrusion, just below the mother cell apex *(fig. 4)*.

Zoid formation was readily accomplished by both clones upon transfer into fresh medium; no significant influence of photoperiod was detected. Mostly four zoids are produced per cell (occasionally two were observed); all cells are potential zoidangia. Formation of zoids is preceded by chloroplast division; stigmata become visible at chloroplast edges and cell contents are rounded off. Zoids issue singly through an apical pore in the cell wall *(fig. 5)*. After issuing, zoids assume an ovoid shape (6–6,5 × 8–9,5 μm); they have two subapically implanted flagella of respectively 1.5 times and two-third cell length. The longer flagellum is actively used in propulsion, pulling the cell forward in an undulating movement, while the short one is held closely backward against the cell body and only twitches occasionally *fig. 6)*. After settling and shedding the flagella, zoids expand to form a hemispherical basal cell (7–9 μm diam.) attached to the substratum by a mucilage ring. One after another, up to four daughter cells are extruded from the basal cell; each cell in turn can give off up to four branches arising from its apex *(fig. 7)*.

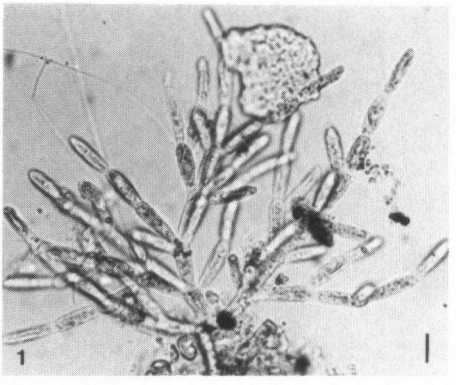
In stationary-phase cultures (4–6 weeks old), thalli consist of shortly ovoid cells, filled with lipid and leucosin globules and with poorly developed chloroplasts. Palmelloid clumps of cells not developed into regular thalli were also present; when transferred into fresh medium, two zoids were observed to issue from several cells *(fig. 8)*.

3.1.2.2. Influence of light and temperature on morphology, growth and reproduction

Results of the crossed light/temperature gradient experiments are presented in *diagrams 1* and *2*. In *diagr. 1*, average cell length is given for 32 out of 36 combinations tested. Those at 25°C/1–4 are left out because only malformed thalli were observed. The table shows a wide range of dimensions, reflecting the variation encountered even within one thallus. While cells did show a tendency to shorten with rising temperature, no significant increase in cell width was observed. Dimension of basal cells did not change either. Thallus structure remained the same as well.

Optimal growth was observed at 13°C/3–5 (c.600–2500 lx), 18°C/3–5 and 22°C/3–6 (c.600–5000 lx) *(diagr. 2)*.

No palmelloid stages were observed.



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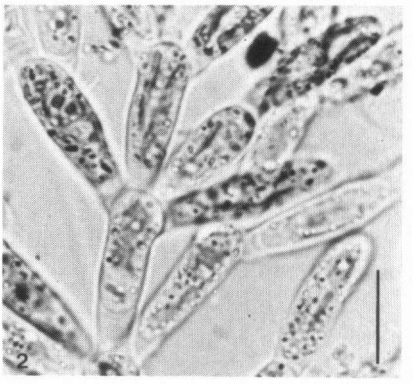

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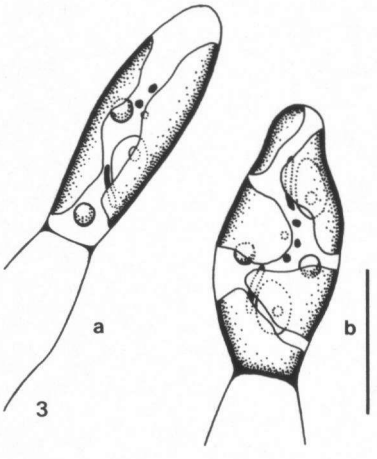

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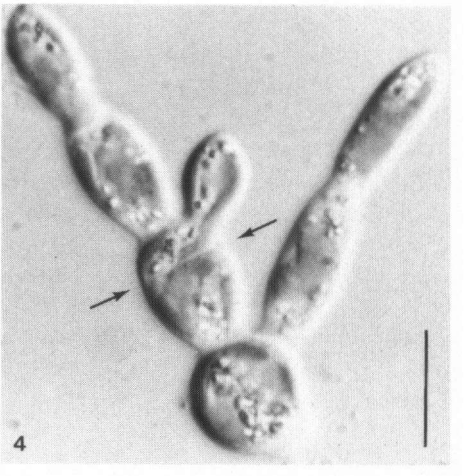

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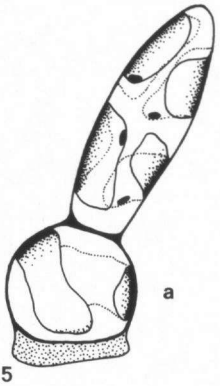

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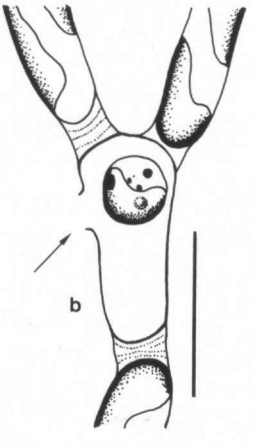

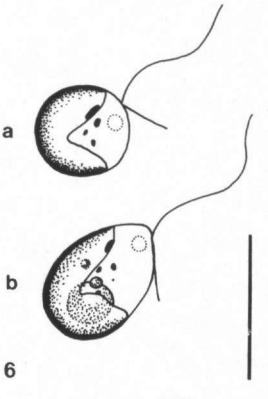

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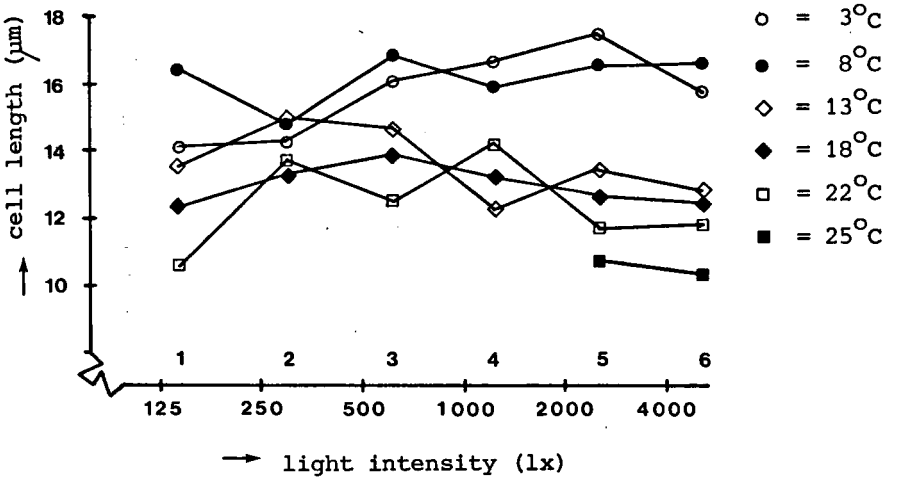


Diagram 1. *Phaeothamnion borzianum.* Cell length at different combinations of temperature and light intensity.
Numerals 1–6 indicate approximate position of rows of culture vessels in the light gradient.

Plate I. *Phaeothamnion borzianum*
Fig. 1. Field material, The Botshol, from glass slide exposed for four weeks. Note oblong, sometimes clavate cell-shape, and bushy habit. Up to four branches per cell are visible.

Fig. 2. Field material, same as in *fig. 1*. Note striation in cross walls. Mostly one chloroplast in each cell, one or two oil droplets; note refringent crystals just underneath the cell membrane.

Fig. 3. Cultured material. In a, two lobed chloroplasts are shown and position of nucleus with nucleolus. Note absence of thickened cross wall. In b, two nuclei with adjacent Golgi bodies and four chloroplasts are present, prior to cell division.

Fig. 4. Cultured material. Young thallus showing mode of branch formation; after a cross wall is formed (arrow), the daughter cell is extruded sideways out of the apical part of the mother cell.

Fig. 5. Cultured material. In a, zoid formation in a two-celled thallus is preceded by chloroplast division: four chloroplasts are observed, with stigmata. In b, one zoid is still within a cell; other(s) have escaped through a pore (arrow).

Fig. 6. Cultured material. a – Zoid just after release, with round shape. A swimming zoid is depicted in b. Note apical contractile vacuole, leucosin vacuole (not always present), oil droplets and single parietal chloroplast with stigma at edge.
Scale bar in all figures equals 10 µm.

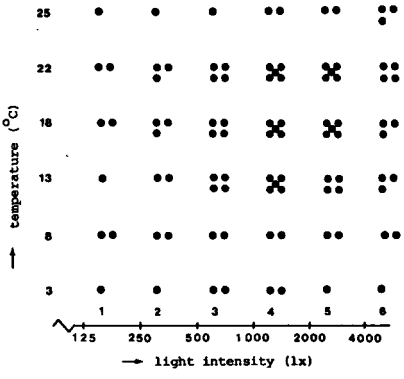


Diagram 2. *Phaeothamnion borzianum*. Evaluation of growth and reproduction at different combinations of temperature and light intensity.
Numerals 1–6 indicate approximate position of rows of culture vessels in the light gradient.
Explanation of symbols:
- • – very little growth or none at all; only a few thalli observed apart from the inoculated ones.
- •• – several new thalli observed apart from the inoculated ones.
- •• • – thalli well-developed, young ones present throughout culture vessel.
- •• •• – Large thalli present: several large patches of young thalli observed.
- •• •• – large clumps of well-developed thalli present; young thalli covering whole bottom of culturing vessel.

3.2. "*Palmelloid form of Phaeothamnion*"

3.2.1. Field material

This alga was rather frequently observed in the waters studied. Its habit is reminiscent of the Chlorophyte genus *Palmodictyon* Kützing, with which it can be confused if observed in preserved material.

Thalli consist of linear or sometimes slightly curved series of four cells, embedded in a firm mucilage sheath and arranged in a tree-like fashion (*fig. 9*). They are attached to the substratum by a slight extension of the lower part of the sheath. The mucilage sheath has a wavy outline; it is absent around the top cells of the branches, and is up to 15 μm thick in the rest of the thallus.

Cells are spherical to slightly oval (7 μm diam. to 7 × 9 μm); in the latter case the long axis of one or more cells in each series of four lies perpendicular to the others (*fig. 9*). Each cell contains one or two brownish-green, lobed chloroplasts, parietal or sometimes centrally located; one or two lipid droplets, a leucosin vacuole, several dark granules, and sometimes the same type of small crystals as reported for *Phaeothamnion borzianum* in a vacuolar system just underneath the cell membrane (*fig. 10*). The cell wall is clearly distinguishable from the surrounding mucilage sheath.

Zoids or cysts were not observed in field material.

3.2.2. Cultured material

3.2.2.1. Habit and reproduction

In culture, habit and cell dimensions remain the same. Chloroplasts are at first parietal; in cultures of four to six weeks old they retract towards the central part of the cell and parietal vacuoles with small crystals become visible as is sometimes the case in field material (*fig. 10*).

The typical thallus form of mostly linear series of four cells arranged in a tree-like fashion, is achieved as follows (see *figs. 11* and *12*). The first cell (settled zoid) undergoes one division, and the daughter cells immediately divide once more along the same axis. Cells of the resulting linear series repeat this process simultaneously. Through slight differences in direction of consecutive series formation, new ones are pushed sideways out of the mucilage sheath of the "mother" series. The two subsequent divisions required for series formation were observed to take place within 2–3 hours. Not every cell undergoes daily division, hence the irregular tree-like habit. Maximum thallus dimension observed was about 1 mm.

Reproduction is by way of zoids. The most reliable way to induce zoid formation proved to be placing cultures for three days at 4°C total darkness followed by transfer to room temperature and full daylight. Within 1–2 hours, zoid release could be observed. One zoid is produced per cell, issuing through a pore in the cell wall and emerging backwards through the mucilage sheath. Zoids are slightly oval, 4.5 × 7 μm and have one or two parietal, lobed chloroplasts, stigma, a contractile vacuole, one or two oil droplets, a leucosin vacuole, two or three dark granules, and two subapically implanted flagella. One flagellum is about 1.5 times cell length, directed anteriorly and shows undulating movement; the other one is two-thirds cell length and directed posteriorly, twitching occasionally (*fig. 13*).

3.2.2.2. Influence of light and temperature on morphology, growth and reproduction

In *diagr. 3*, the results of the crossed light/temperature gradient experiments are given. Morphology of thalli remained constant throughout the experiment. At 3°C, distance between cells was increased slightly (to 5 μm). At 25°C/6 (c.5000 lx), thalli had become palmelloid masses of cells, in which the series of four were not recognisable anymore. Optimal growth and reproduction was observed at 13°3/4–6 (c. 1200–5000 lx), 18°C/4–6 and 22°C/5–6 (c. 2500–5000 lx). The

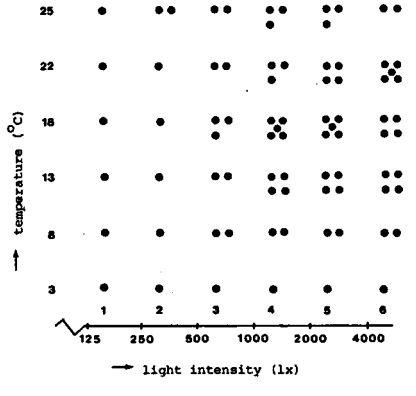
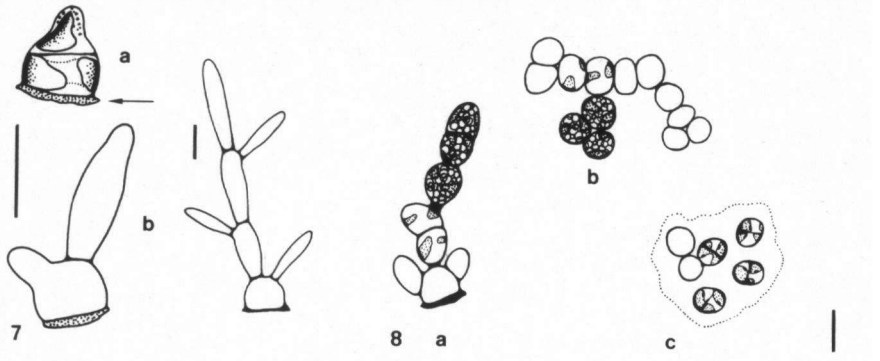


Diagram 3. "Palmelloid form of *Phaeothamnion*". Evaluation of growth and reproduction at different combinations of temperature and light intensity.
Numerals 1–6 indicate approximate position of rows of culture vessels in the light gradient.
See table 2 for explanation of symbols.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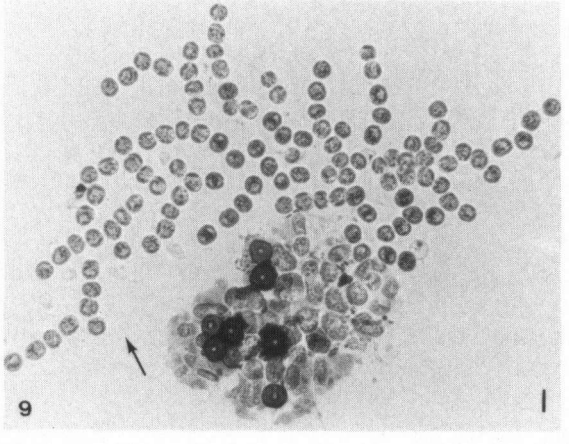
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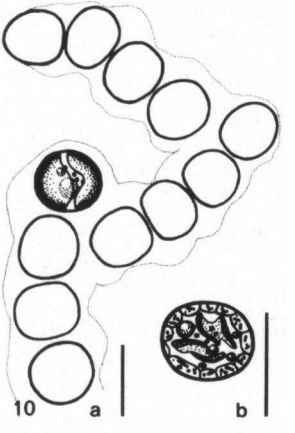
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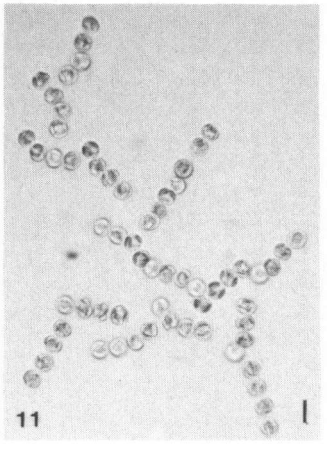
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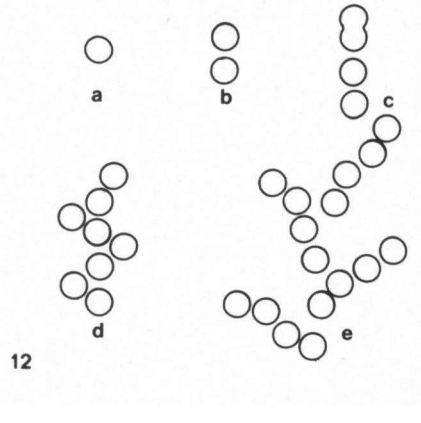
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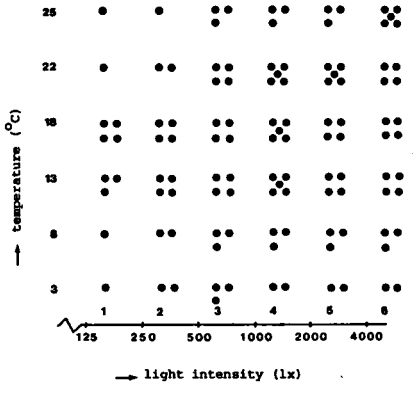


Diagram 4. *Sphaeridiothrix compressa*. Evaluation of growth and reproduction at different combinations of temperature and light intensity.
Numerals 1–6 indicate approximate position of rows of culture vessels in the light gradient. See table 2 for explanation of symbols.

Plate II.

Phaeothamnion borzianum

Fig. 7. Cultured material. In a, basal cell after first division: cross wall has been formed, and daughter cell is extruded from apex. Note mucilage ring (arrow). Young thalli are shown in outline in b and c.

Fig. 8. Cultured material. In a; a thallus from a two month old culture is illustrated, showing cells packed with oil droplets; chloroplasts are reduced to very thin, dissected parietal plates hardly to be distinguished. Note broadly shaped cells. In b, a palmelloid cluster of settled zoids, arrested in development, is shown. Fig. c shows zoid formation in such a palmelloid cluster after transferring into fresh medium.

Tetrachrysis dendroides

Fig. 9. Field material, from slide exposed for four weeks in the Botshol. Note regular series of four cells that seem to branch off from the third cell of lower series; this is especially apparent in young branches. Also visible is the variation in cell shape: spherical to ovoid with slightly flattened sides. The alternation between orientation of long axis of cells is apparent in youngest series (arrow).

Fig. 10. Field material. a – Detail of thallus showing mucilage sheath and cell contents: leucosin and lipid globules, dark granules, and two lobed parietal chloroplasts (depicted in one cell). In b, a cell is illustrated with two, more centrally located, ribbon-like chloroplasts. Also the peripheral vacuoles with crystal-like small bodies are shown.

Fig. 11. Cultured material, young thallus showing regularly alternating series of four cells.

Fig. 12. Schematically represented growth pattern, reconstructed with aid of a water immersion objective in a culture vessel. a–c, Formation of first series of four cells from a settled zoid; d and e show forming of new series by all cells of the first one.

Scale bar in all figures equals 10 μm.

submembranous crystals sometimes present in field material, were observed at 3° and 8°C. Cell shape and dimension remained the same throughout the experiment.

3.3 *Sphaeridiothrix compressa* Pascher & Vlk

3.3.1. Field material

Thalli of this alga consist of an unbranched chain of oval cells (5–8 μm × 10–12 μm), closely appressed or slightly separate, embedded in a firm common mucilaginous sheath (13–25 μm wide). They are attached to the substratum by a slight extension of the basal part of the sheath (*fig. 14, 15*).

Cells contents are one, sometimes two, mostly parietal, variously lobed chloroplasts without pyrenoids, a leucosin vacuole, one or two oil droplets and several small dark granules. When the chloroplast is more centrally located, the same type of small crystals in peripheral vacuoles as reported in the two earlier mentioned algae can be observed (*fig. 15*). A distinct cell wall is present. The mucilage sheath is variously developed from a thin, sharply bordered coat curving closely around the cells to a c. 25 μm wide parallel sheath with diffuse outline. The sheath appears to consist of a diffuse outer layer and a more firm part around and in between cells (*fig. 15*).

Zoids or cysts were not observed in field material.

3.3.2. Cultured material

3.3.2.1. Habit and reproduction

Thalli retain their chain-like habit; cells become more closely appressed, however and become flattened (5 × 13 μm), especially in log-phase cultures. In such cultures, chloroplasts are well-developed, strictly parietal, the lobes sometimes overlapping each other. A nucleus with nucleolus is visible at one end of the oval cells, appressed to a chloroplast; the curved Golgi body can be seen lying close to it. Apart from a few small dark granules, no other inclusions are observed (*fig. 16*). In four to six weeks old cultures (stationary phase) cells become more oval (8 × 10 μm), chloroplasts retreat slightly from the cell walls, and large leucosin and oil droplets appear. The submembranous crystals as sometimes observed in field material are present as well.

Cell division can take place throughout the filament; most activity is observed near the top, however. Upon transferring old filaments into fresh medium, all cells start dividing simultaneously and numerous false branches are formed because daughter cells are pushed sideways out of the mucilage sheath.

Reproduction is by way of zoids; one is formed in each cell. Zoid formation was stimulated by placing cultures under 8 and 12°C short-day conditions; production remained sparse, however. Mass release of zoids could be observed three or four days after lowering pH of culturing medium to 4.5. Zoids are slowly extruded backwards through a slit in the cell wall and through the mucilage (*fig. 17*). They are spherical at first but soon assume a pear shape. Two flagella are

observed, implanted subapically, one twice as long as the cell and undulating; the other one half as long as the cell and held close to the cell body, oriented backwards. Cell contents are the same as for the vegetative cells except for a contractile vacuole, observed near the flagellum implant and an elongate stigma located on a chloroplast lobe in the same vicinity. The nucleus, with a Golgi body visibly appressed to it, is also observed near the basis of the flagella (*fig. 18*). After settling and shedding the flagella, zoids secrete a cell wall and mucilage sheath before developing into new filaments.

3.3.2.2. Influence of light and temperature on morphology, growth and reproduction

In *diagr. 4*, the results of the crossed light/temperature gradient experiments are presented.

Cell shape remained rather variable under all circumstances tested, from only slightly oval to quite flattened: 4–9 μm × 8–12.5 μm, mostly 8 × 12.5 μm. Width of mucilage sheath varied from 14–25 μm, mostly 24 μm.

Optimal growth (straight or only wavy filaments up to two or three cm long) was observed at 13°C/2–6 (c. 300–5000 lx), 18°C/1–6 (c. 150–5000 lx), 22°C/4–6 (c. 1200–5000 lx) and 25°C/6 (c. 5000 lx). At 3° and 8°C, where little growth was observed, the originally inoculated filaments exhibited a lamp-brush habit because new filaments had grown sideways out of the old mucilage sheath.

3.3.2.3. Influence of chlorinity on growth and reproduction

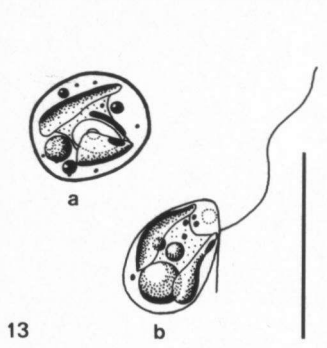
Results are presented in *table 1*.

At Cl^- concentrations of 22 and 30‰, the mucilage sheath became extremely swollen, and filaments died within two weeks. At concentrations of 12 and 16‰ Cl^-, the originally inoculated filaments dissociated and only short new ones were formed. At 4 and 8‰ Cl^- lampbrush-like growth was observed, and at lower concentrations the usual long filaments were formed. Zoids were mostly produced at 2‰ Cl^- and lower. Longest surviving cultures in two simultaneous experiments were those at 4‰ (three months) respectively at 2‰ (four months).

Table 1. Qualitative assessment of influence of chlorinity on growth and zoid production.

‰ Cl^-	0	0.3	0.6	2	4	8	12	16	22	30
growth	++	+++	+++	+++	++	++	++	+	–	–
zoid production	+++	++	++	+++	+	–	–	–	–	–

+++ = abundant; ++ = moderate; + = scarce; – = none.



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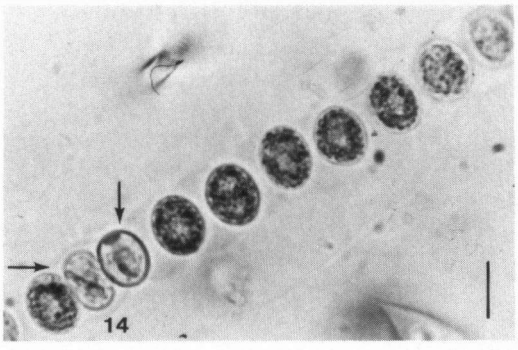

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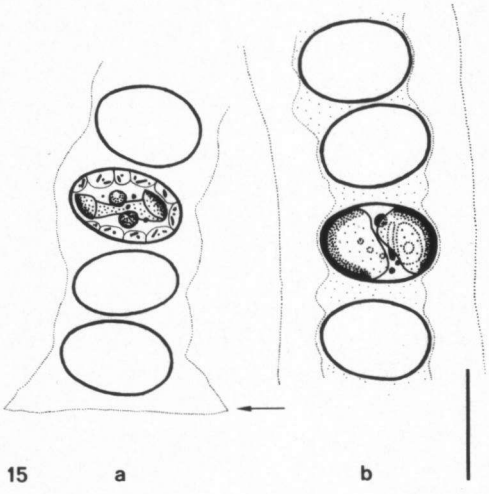

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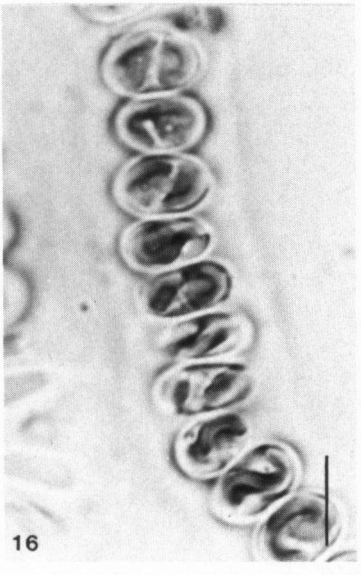

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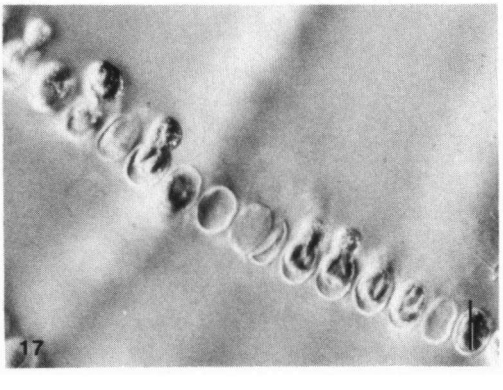

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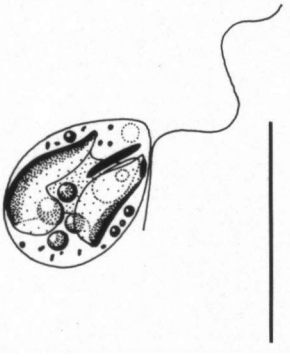

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4. DISCUSSION

4.1. *Phaeothamnion borzianum and its "palmelloid form"*

From the culturing experiments the conclusion may be drawn that our long-celled *Phaeothamnion borzianum* and the alga which has been regarded as its palmelloid form, are distinct taxa. They differ in thallus form, cell shape, the absence of a mucilage sheath in *P. borzianum*, and the presence of a specialised basal cell in the latter. The light-temperature gradient experiments show that both algae are not necessarily of the cold stenotherm type as Chrysophyceae are usually considered to be. Their growth optima, lying at 13–22°C, indicate that other environmental factors may be responsible for their greatest observed presence in spring and autumn.

It is also apparent that cell shape in *Phaeothamnion borzianum* is quite variable. The same is true for *P. confervicolum*, judging by LAGERHEIM's (1884) description. This has apparently led several authors to suppose that this species intergraded with the "palmelloid form" when these two algae were encountered in the field together.

The first mention of a palmelloid stage was by LAGERHEIM (1884), in the description of *P. confervicolum*. In his fig. 19–21 the development of a palmelloid stage is illustrated, but as stated (p. 10), the transformation of the long-celled form into the round-celled palmelloid stage was not observed. His fig. 19 is strongly reminiscent of a young thallus of our tetrad-forming alga.

Plate III.

Tetrachrysis dendroides

Fig. 13. a – Drawing of cell in which zoid formation is in progress. Note nucleus (with nucleolus), Golgi body and stigma at chloroplast edge. In b, a zoid is shown with two subapically implanted heterokont flagella, one lobed chloroplast, contractile vacuole, oil and leucosin globules and dark granules.

Sphaeridiothrix compressa

Fig. 14. Field material, from slide exposed for four weeks in the Botshol. Note irregular outline of mucilage sheath, oval shape of cells, prominent cell wall (arrow), crystal-like rods and globules obscuring cell content in most cells, and axial, ribbon-like single chloroplast in two cells (arrows).

Fig. 15. a – Drawing of field material showing basal (undifferentiated) cell with mucilage extension on substratum (arrow), axial, ribbonlike chloroplast and peripheral crystals. In b, part of a thallus with well-developed parietal lobed chloroplasts (two per cell); nucleus and Golgi body are visible. Note differentiation in mucilage sheath with firm inner part and diffuse outer layer.

Fig. 16. Cultured material. Cell shape varies from flattened to oval; note wide mucilage sheath and lobed parietal chloroplasts.

Fig. 17. Cultured material. Zoids escape slowly by sideways extrusion through slit in cell wall and through mucilage sheath.

Fig. 18. Cultured material. Drawing of a swimming zoid; note pear shape, lobed chloroplast with elongate stigma, contractile vacuole, nucleus with Golgi body appressed to it, and oil and leucosin droplets.

Scale bar in all figures equals 10 μm.

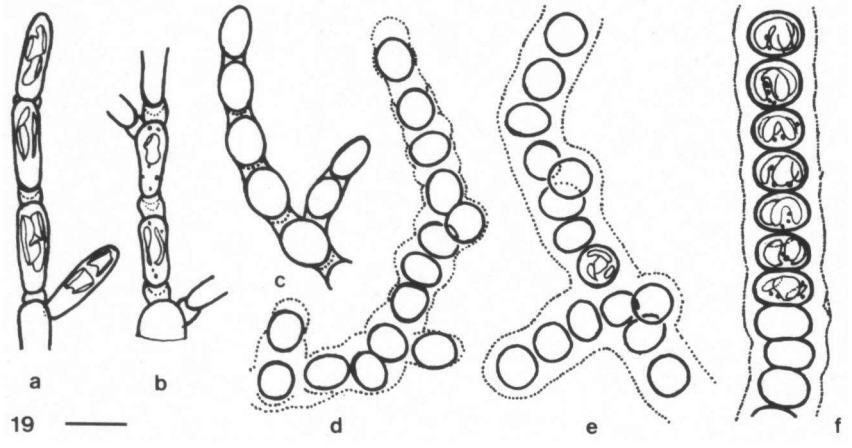

Fig. 19. After GEITLER & SCHIMAN-CZEIKA 1970. Field material.
a – Thallus portion in typical *Phaeothamnion*-form, old cross walls thickened.
b – degenerated form with one or two very pale chloroplasts
c, d – Transition forms to palmelloid stage
e – palmelloid stage with branches
f – part of an unbranched filament, 140 cells long.
In our opinion, a, b and c represent *Phaeothamnion* sp.; d and e are clearly recognisable as *Tetra chrysis dendroides* with its tetrads, mucilage sheath, and oval cells with alternating long axes.
f represents *Sphaeridiothrix compressa.*

Scale bar equals 10 μm.

The next author to illustrate a "palmelloid stage" was BORZI (1892), who assumed to be dealing with the same species. PASCHER (1925) however, interpreted Borzi's find, in connection with observations of his own, as a new separate taxon mainly on account of the presence of a stigma in the zoids: *Phaeothamnion borzianum.*

PASCHER (1925) reproduced Borzi's figures, showing a well-developed, linear tetrad-forming alga, and added some observations of his own. His fig. N represents a palmelloid clump of cells, resulting from division of settled zoids that failed to produce a regular thallus form (with persisting stigmata). In fig. o, "Gallertstadien, hervorgegangen aus den Zellen der verzweigten Fäden" are illustrated. Stigmata are visible, as well as *Gloeocystis*-like stratified mucilage around and between the cells. This remains somewhat of an enigma to us; possibly it is an inhibited zoid-forming thallus.

SMITH (1950) also illustrates a "palmelloid form of *P. confervicolum*" without mentioning a transition of one into the other. In this illustration, four- and eight-celled series are recognisable.

The most carefully documented observations in this matter are those of GEITLER & SCHIMAN-CZEIKA (1970). These authors described field observations from which the transition of "long-celled *Phaeothamnion*" into "palmelloid

stage" and subsequently into *Sphaeridiothrix compressa* is deduced. In subsequent culturing experiments, however, they failed to reproduce this; only some irregular palmella-stages were observed. Their illustrations of field material are reproduced in our *fig. 19. Fig. a, b* and *c* represent variations in the long-celled type that were also observed by us, in the field as well as in culture. The variously shaped cells are shown, as well as differently developed gelatinous stalks between cells and the more centrally located chloroplast in older thalli. *Fig. d* and *e*, however, clearly represent the linear tetrad-forming alga; compare with our *figs. 9* and *10*. In these illustrations, the linear series of four cells are excellently distinguishable as well as the characteristic slightly ovoid cells with differently oriented long axis and the fact that subsequent series seem to spring from the third cell of the lower ones. Attention is also drawn to the fact that the authors observed that all "forms" were not equally represented in field material; mostly or even exclusively only one form was found.

In our opinion, no conclusive evidence has been presented by previous authors of actual transition of any long-celled form of *Phaeothamnion* into the regular round-celled dendroid alga that is traditionally regarded as the "palmelloid form of *Phaeothamnion*". On the other hand, we conclude from the records listed above that our dendroid alga with its characteristic series of four cells is identical with the algae considered to be this palmelloid form. Since we have also demonstrated it to be a distinct taxon, it will be dealt with under 4.4 Taxonomy.

4.2. *Review of described Phaeothamnion species*

Attention is drawn to the fact that the type species of the genus was described by LAGERHEIM (1884) as *Phaeothamnion confervicolum*. PASCHER (1925) changed this into *P. confervicola*, and most subsequent authors have followed his example. This is not correct, however, since *Phaeothamnion* is of neuter gender.

The genus *Phaeothamnion* contains five species and one variety; characters of these are compared in *table 2*. Dimensions for *P. confervicolum* are those given by LAGERHEIM (1884). PASCHER (1925) mentioned larger cells for the same species: "about 6–9 μm wide and up to 20 or 30 μm long". These dimensions would be applicable to all three species described by him: *P. confervicolum*, *P. borzianum* and *P. polychrysis*. Analysis of his figures with aid of the scale bars provided gives the following results however:

P. confervicolum:	4– 9 μm (mean at 6)	× 13–18 μm (mean at 16)
P. borzianum:	4– 7 μm (mean at 5)	× 12–18 μm (mean at 15)
P. polychrysis:	4–12 μm (mean at 7.5)	× 12–24 μm (mean at 20)

This might indicate that the statement "up to 20 or 30 μm" for cell length must be regarded with caution. Our observations on cell size in *P. borzianum* indicate as much, being 4–8 μm (mean at 5.5) × 12–20 μm (mean at 14). The only known record of *P. polychrysis* giving cell dimensions is that of BOURRELLY (1957), giving shorter cells (9–17 μm, mean at 13), while agreeing on cell width. *Phaeothamnion confervicolum* Lagerheim.

The records for this species show much confusion: comparison of *table 2* and PASCHER's (1925) record show that the latter has added liberally to the dimen-

Table 2. Comparison of Phaeothamnion species.

Species	Author	Cell dimensions	Chloroplasts	Zoids	Other characters
P. confervicolum	Lagerheim 1884	4–8 μm × 6–12 μm	One, sometimes two, covering cell walls	Two per cell, 4–5 μm diam., with two equally long apical flagella. No stigma.	Hemispherical basal cell, 5–9 μm diam., attached to substratum with mucilage disc. Thickened stratified walls between cells. Palmelloid stage reported.
P. borzianum	Pascher 1925	6–9 μm wide and up to 20–30 μm long.	One, sometimes two parietal plates	1 or 2 per cell, seldom 4 or 8. Egg- shaped, up to 12 μm long, heterocont, with stigma and two contractile vacuoles.	Hemispherical basal cell 9 μm diam., contents seemingly degenerating with age. Thickened stratified walls between cells. Branches directed upwards. Palmelloid stage reported.
P. polychrysis	Pascher 1925	6–9 μm wide and up to 20–30 μm long.	Several parietal plates	–	Hemispherical basal cell 9 μm diam., contents seemingly degenerating with age. Thickened stratified walls between cells.
P. confervicolum var. brittannica	Godward 1933	3–6 μm (mostly 5) × 10–20 μm (mostly 15)	A single, curved and much lobed structure or consisting of a number of separate pieces	–	Hemispherical basal cell 10 μm diam., differs from type species in the thickened walls at cell apex and especially at cell base. Branches divergent or directed upwards.
P. dichrysis	Villeret 1951	4.5–6 μm × 10–15 μm.	Two, parietal, incised in cells of lower thallus part.	–	Basal cell 8–9 μm diam. Cross walls thickened and stratified in lower part of thallus.
P. articulatum	Ettl 1959	3–5 μm × 8–12 μm	One, centrally located in cell.	1 or 2 per cell, 8–10 μm long, with stigma, contractile vacuole; heterocont.	Cross walls between cells thickened and sometimes swollen. Cell walls possibly consisting of H-shaped segments.

sions in LAGERHEIM's (1884) type description.

Examination of six thalli from the material deposited by Lagerheim in Wittrock et Nordstedt, Algae aquae dulcis exsiccatae fasc. 13, no. 608 (present in the Rijksherbarium at Leiden), provided the following dimensions:

cells 3.5–6 μm (mean at 5) × 8.5–15.5 μm (mean at 11.5). This is slightly different from LAGERHEIM's (1884) type description; it may be due to the fact that according to the label, the material has been cultured before exsiccation. Nothing is stated about culturing conditions, however. It is very well possible that Lagerheim has observed thalli with cells longer than apparent from his description: as stated (LAGERHEIM 1884, p. 4), cells were up to 2.5 times as long as wide; in his illustrations (LAGERHEIM 1884, figs. 3–8) thalli are depicted with cells three and four times as long as wide, however.

Records of *P. confervicolum* in which cell dimensions are given are those of PASCHER (1925), see p. 79; PRESCOTT (1962); "6–11 μm diam., 14–20 μm long", and GEITLER & SCHIMAN-CZEIKA (1970): 5 × 15 μm. Zoids were not observed by these authors and identification is doubtful therefore; especially the dimensions of Prescott and Pascher are rather large.

Since LAGERHEIM (1884) has explicitly stated the zoids of *P. confervicolum* to lack a stigma, and bearing in mind the differences in dimensions of his description and of later records attributed to *P. confervicolum*, the conclusion is drawn that *P. confervicolum* has not unequivocally been recorded since its original description.

Phaeothamnion confervicolum var. brittannica Godward

The distinctive feature of this variety (GODWARD 1933) lies in the thickened cross walls. Because this is a transitory phenomenon, however, it does not merit distinction at the variety level. Godward's record is not referable to *P. confervicolum* with certainty, however, since the cell dimensions given (see *table 2*) are rather large, and no zoids have been observed.

Phaeothamnion dichrysis Villeret

The description of this species (VILLERET 1951) poses yet another problem. Zoids were not observed, and cell dimensions, number and shape of chloroplasts and the thickening of cross walls are conform the description of *P. confervicolum* Lagerheim. The author probably took PASCHER's (1925) circumscription of *P. confervicolum* to be correct, and hence considered his alga to be a new species. Most likely it is conspecific with *P. confervicolum* Lagerheim; since no zoids were observed, it cannot be referred here with certainty.

Phaeothamnion articulatum Ettl

Attention is drawn to the fact that *P. articulatum* Ettl (ETTL 1959) falls within the description of *P. confervicolum* Lagerh. except for the fact that zoids have a stigma; the "articulation" was also mentioned by LAGERHEIM (1884), and the more central location of the chloroplast cannot merit great distinctive value (see our observations on *P. borzianum*). The only other record of *P. articulatum* is that of BOURRELLY (1963), who gives as dimensions 5–5.5 μm × 12–15 μm; zoids were not observed. This record is doubtful, as a consequence.

Conclusion of this review of *Phaeothamnion* species – in our opinion – is that the only "reliable" species within the genus are the following:

P. confervicolum Lagerheim
P. borzianum Pascher
P. polychrysis Pascher
P. articulatum Ettl

Doubtful are the following:

P. confervicolum var. brittannica Godward
P. dichrysis Villeret

A complete list of records is given below.

4.3. *Sphaeridiothrix compressa*

Our field observations and culturing experiments confirm PASCHER & VLK's (1941–42) observations. This species is a uniseriate pseudofilamentous alga with a mucilage sheath of varying thickness. Branching may occur through irregularity in cell division. It has not been established what factor is responsible for this; it can be induced by transferring old thalli into fresh medium (our observations) or by culturing on agar (ANDREWS 1970). Under standard conditions and media, our clones usually grew into straight unbranched filaments of several cm long, but sometimes irregularly curving, slowly growing and occasionally branched thalli were produced.

The presence of contractile vacuoles during cell division, as observed by PASCHER & VLK was not noted by us, nor by other authors (ANDREWS 1970, BOURRELLY 1963, GEITLER & SCHIMAN-CZEIKA 1970). The zoids are largely conform ANDREWS' (1970) description. Our clones produced only one zoid per cell instead of one or two, and a distinct elongate stigma was always visible (sometimes difficult to observe according to Andrews). For other characters all records agree quite well. The small crystals sometimes observed by us just underneath the cell membrane in field material and old cultures, are only mentioned by BOURELLY (1963); They are illustrated by PASCHER & VLK (1941–42) but not described by these authors.

The waters in which *Sphaeridiothrix compressa* was found, were sometimes slightly saline but in most cases slightly alkaline: "salzhaltiges Flachmoor" pH 7.4–8.2 (PASCHER & VLK 1941–42); sal. 1–3‰, Neusiedler See (GEITLER 1970); sal. 2.8–12.6‰, pH 7.0–8.3 (ANDREWS 1970). The Botshol, in which we regularly found this alga, has a chlorinity of 0.4‰ and a pH of 6.8–8.2. ANDREWS (1970) from his experiments draws the conclusion that *Sphaeridiothrix compressa* is a euryhaline species. This is not the case with our material; it might better be termed a halotolerant species.

Our observations confirm ANDREWS's (1970) view that *Sphaeridiothrix globulosa* Pascher 1941–42 and *Sphaeridiothrix brunnea* Pascher 1941–42 are synonymous with *Sphaeridiothrix compressa* Pascher and Vlk 1941–42. The "compressing" phenomenon is not always evident in *S. compressa*, and the cell dimension range covers that of the two other species. We do not agree with Andrews, however, that *Chrysodictyon indicum* Ramanathan 1946 should also be

put into synonymy with *Sphaeridiothrix compressa*. There may be resemblance between thalli, but in *C. indicum* cysts have been observed from which amoeboid zoids issued. Not until this has been recorded for *Sphaeridiothrix compressa* can a decision be taken as to the relation of these algae to one another.

4.4. *Taxonomy*

As we demonstrated, the dendroid alga forming linear series of four cells is a separate taxon; since it cannot be identified with any Chrysophyte described until now, we consider it a new species. In BOURRELLY's (1968) system of the Chrysophyceae, it belongs in the subclass Heterochrysophycidae, order Ochromonadales. There are no existant genera, however, in this order to which this alga can be attributed. Its most likely position would be close to, but not in, the genus *Sphaeridiothrix*, with which it shares the slightly separated, autonomous cells, embedded like strings of beads in a mucilage sheath. The remarkable mode of thallus formation however, with its linear series of four cells branching off from one another, sets it apart from this (in principle) unbranched genus.

For these reasons, we propose to erect a new genus – *Tetrachrysis* – to accommodate this alga, that will be named *Tetrachrysis dendroides*. Its taxonomic position is in the order Ochromonadales, suborder Phaeothamniineae, family Phaeothamniaceae, next to the genus *Sphaeridiothrix*, the taxonomic position of which was already discussed by us (DOP & VROMAN 1976). Other members of this family are the brackish or marine benthic genera *Nematochrysis*, *Chrysonephos* (according to GAYRAL & LEPAILLEUR 1971) and *Apistonema*.

Diagnoses:

Tetrachrysis gen. nov.
Thallus duarum aut quaternarum cellularum seribus compositus strato mucoso variabile tectis. Thallorum cellulae simul divident; series aliae ex aliis ortae. Propagatio per zoosporas typi generis Ochromonadis.
Typus generis: Tetrachrysis dendroides Dop.

Tetrachrysis dendroides spec. nov.
Thallus epiphyticus, arbori similis, quaternarum cellularum seribus strato mucoso angusto tectis compositus. Cellulae globosae vel ovoideae, 7 μm diametro aut 7 × 9 μm, singulo vel duobis chloroplastis lobatis parietalibus aut centralis, globulis leucosinis ac lipidis et membrana firma praeditae. Zoosporae singulae e cellulas ortae, ovoideae (dimensione 4.5 × 7 μm), chloroplasto singulo vel binis, stigmate rubro, vacuola contractibile apicale et globulis leucosinis ac lipidis praeditae; flagella sub apicem implantata quorum unum longitudine 10 μm et unum longitudine 4.5 μm.

Provenit e stagno Botshol prope oppido Abcoude, qua abservatus est in substratis aquaticis diversis.

Iconotypus: figurae nostrae *9*, *10*, *11* et *13*.

Tetrachrysis gen. nov.
Thallus consisting of series of two or four cells, embedded in a variously shaped mucilage sheath. Thallus cells divide simultaneously; series spring from one another. Multiplication by way of Ochromonas-type zoids.

Type species: Tetrachrysis dendroides Dop.

Tetrachrysis dendroides spec. nov.
Thallus epiphytical, tree-like, consisting of series of four cells embedded in a narrow mucilage sheath. Cells spherical or ovoid, 7 μm diam. or 7 × 9 μm, with one or two, parietal or centrally located, lobed chloroplasts, leucosin and lipid globules and a firm cell wall. Zoids one per cell, ovoid, (4.5 × 7 μm) with one or two chloroplasts, stigma, apical contractile vacuole and leucosin and lipid globules; flagella subapically implanted, one 10 μm long, the other 4.5 μm.

From the pond Botshol near the town of Abcoude, where it was observed on several aquatic substrates.

Iconotype: our *figs. 9, 10, 11* and *13.*

Records for the genera *Phaeothamnion, Tetrachrysis* and *Sphaeridiothrix.*

Phaeothamnion confervicolum Lagerheim 1884
Sweden, LAGERHEIM 1884 (type description).

Phaeothamnion borzianum Pascher 1925
Italy, BORZI 1892 (as *P. confervicolum*)
Germany ?, PASCHER 1925 (type description, no place indicated).
The Netherlands, DOP & VROMAN 1976 and this paper.

Phaeothamnion polychrysis Pascher 1925
Germany ?, PASCHER 1925 (type description, no place indicated).
Austria, GEITLER in PASCHER 1925.
France, BOURRELLY 1957.

Phaeothamnion dichrysis Villeret 1951
France, VILLERET 1951 (type description). A doubtful species, see discussion.

Phaeothamnion articulatum Ettl 1959
Tchechoslowakia, ETTL 1959 (type description)

Uncertain records (no zoids observed or mentioned)
Germany ?, PASCHER 1925 (no place indicated) (*P. confervicolum*).
England, GODWARD 1933 (*P. confervicolum var. brittannica*, doubtful, see discussion).
U.S.A., SMITH 1950, PRESCOTT 1962, WHITFORD & SCHUMACHER 1973 (*P. confervicolum*).
France, BOURRELLY 1963 (*P. articulatum*).

Sweden, SKUJA 1965 (*P. confervicolum*).
Austria, GEITLER & SCHIMAN-CZEIKA 1970 (*P. confervicolum*).
Canada, STEIN 1975 (*P. confervicolum*).

Tetrachrysis dendroides n.g., n. sp.
Sweden, LAGERHEIM 1884 as palmelloid stage of *Phaeothamnion confervicolum*.
Italy, BORZI 1892 as palmelloid stage of *Phaeothamnion confervicolum*.
U.S.A., SMITH 1950 as palmelloid stage of *Phaeothamnion confervicolum*.
Austria, GEITLER & SCHIMAN-CZEIKA 1970 as palmelloid stage of *Phaeothamnion confervicolum*.
The Netherlands, DOP & VROMAN 1976 as palmelloid stage of *Phaeothamnion borzianum*, and this paper.

Sphaeridiothrix compressa Pascher and Vlk 1941–42.
D.D.R., PASCHER & VLK 1941–42 (type description), also as *S. globulosa* Pascher and *S. brunnea* Pascher.
France, BOURRELLY 1963.
U.S.A., ANDREWS 1970.
Portugal, FATIMA SANTOS 1976 (as *S. globulosa*).

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