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COCCOID AND PALMELLOID BENTHIC CHRYSOPHYCEAE FROM THE NETHERLANDS*

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SUMMARY

The life cycle of several clones of *Phaeoschizochlamys mucosa*, *Tetrachrysis minor* spec. nov., and *Chrysosphaera botryoides* spec. nov. is described. Zoids were found to occur in the life cycle of *Phaeoschizochlamys;* as a consequence, this genus is removed from the subclass Acontochrysophycidae to the Heterochrysophycidae, order Chromulinales. The new *Tetrachrysis* species differs from the type species in this genus in mode of cell division and its copious mucilage investment; its systematic position is discussed. The new *Chrysosphaera* species differs from other algae in this genus in that it has *Ochromonas*-like zoids instead of the *Chromulina* type described for the genus *Chrysosphaera*.

1. INTRODUCTION

In the course of a study on benthic Chrysophyceae, the authors compiled a large collection of uni-algal cultures of coccoid and palmelloid species from various ponds and ditches in the Western part of The Netherlands.

The palmelloid algae proved difficult to be identified in field material. Among these, only *Phaeoschizochlamys mucosa* Lemmerman could easily be identified. Zoids were not known to occur in its life cycle; the formation of these was induced in culture. A second palmelloid alga could not be identified with any Chrysophyte described thus far. On account of its growth habit, consisting of linear or slightly curved series of four cells, embedded in an irregular mucilage matrix, it is described here as a new species in the genus *Tetrachrysis* Dop.

Two more palmelloid algae were observed: one turned out to be a representative of the Prymnesiophycean genus *Chrysotila*, and the other has not yet been identified but provisionally referred to the genus *Chrysocapsa*.

Only one coccoid species was found in our collections. Judging by its habit, it can be referred to the genus *Chrysosphaera* Pascher. Since knowledge of the zoids and the range of cell size was necessary for identification, it was submitted to some culturing experiments.

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2. MATERIALS AND METHODS

Most algae were gathered by exposing artificial substrates (glass microscope slides) to algal growth for several weeks. Observations on field material were obtained by placing these slides under the microscope. Uni-algal cultures were started from algae detached from these slides and sometimes also natural substrates.

Six cultures of *Phaeoschizochlamys mucosa* were started from material gathered in January and February 1978 in the Botshol, a peat pond near Amsterdam (for description of this area, see DOP & VROMAN 1976).

Seven cultures of *Tetrachrysis minor* were obtained from the same area; two in December 1975, one in January 1977, and four in February 1978.

Five cultures of *Chrysosphaera botryoides* were available; four were obtained from the Botshol (one in April 1974, three in October 1975) and one from the pond of the Hortus Botanicus of the Vrije Universiteit.

Cultures were maintained at 12 °C, 1500 Lux fluorescent lighting, neutral day conditions in Wood's Hole artificial freshwater medium (STEIN 1973) or in a Botshol-water based Erd-Schreiber medium.

3. OBSERVATIONS

3.1. *Phaeoschizochlamys mucosa* Lemmerman

3.1.1. Field material

Apart from in the Botshol, this species was also observed in ditches in the central Western part of The Netherlands (near Zegveld and Nederhorst den Berg, on glass slides, August 1977 and February 1978 respectively) and in the broads area in the N.W. part of the province of Overijssel (on *Chara* stems, August 1977; for description of this area see COESEL 1979). This alga was mostly found among detritus, suspended among other algae growing on the glass slides, or simply entangled between filamentous algae.

Thalli consist of cells distributed in a mucilage matrix of various shape, up to 0.5 mm large, mostly globular and lobed. Cells are single, in groups of (mostly) two, or four. Each cell divides to form two or four autospores; the mother cell wall is shed and can be observed as a "cap" near the daughter cells. Thalli vary extremely in distance between cells or cell groups. In *fig. 1* a thallus is shown with distances between cell groups as much as 8–10 times cell diameter and with a diffuse mucilage border. *Fig. 2* shows a compact thallus in which the groups of cells are quite close and apparently have separate mucilage investments giving the thallus a lobed appearance.

Cells are spherical or slightly oval; dimensions are variable. Newly released autospores have a diameter of 6 μm and cells about to divide measure up to 9.5 μm.

Cell contents are one or two, bilobed, light or dark olive-green chloroplasts that may be appressed to the cell wall, or retracted towards the cell interior, and

one or two leucosin vacuoles and lipid droplets. A large number of variously shaped, strongly refringent small crystals and muciferous bodies (which stain with brilliant cresyl blue) are visible just underneath the cell membrane (*fig. 2*), when chloroplasts are not in parietal position.

3.1.2. Cultured material

In culture, thalli do not exhibit the extreme range of distances between cell groups as in field material. They may reach up to 1 or 2 mm diameter, are irregularly lobed and seem to be an assembly of several smaller globular units. Cells lie alone or in groups of two or four; the mother cell walls are visible as "caps", *figs. 3* and *4*.

The reproductive cycle of *Phaeoschizochlamys* is summarized in *fig. 5*. Cells form two or four autospores; instead of the autospores, four zoids may also be formed.

Zoid formation was only induced by slightly modifying the Wood's Hole culturing medium (substituting TRIS buffer with HEPES 250 mg/l, omitting the Na_2SiO_3 and adding $NaHCO_3$, 200 mg/l). Four days after transferring thalli into this medium, zoids were observed. They issue singly through a large pore in the cell wall (*fig. 5*). Zoids are pear-shaped, 4.5–5 μm long, with one subapical long flagellum. They have one bilobed parietal chloroplast with a ventral stigma. A contractile vacuole is observed near the flagellum implant; two or three dark granules are observed at the posterior end; a leucosin vacuole is sometimes present as well (*fig. 6*). In swimming, the long flagellum seems to pull the cell forward in an undulating movement. After settling on a substratum the zoids secrete a cell wall and a thallus is formed.

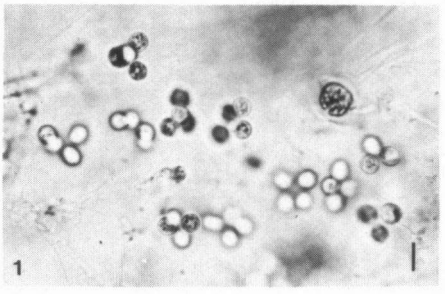
3.2. *Tetrachrysis minor* spec. nov.

3.2.1. Field material

Like *Phaeochizochlamys mucosa*, this alga was found among detritus between epiphytic algae. Apart from in the Botshol, it was also found, together with *Phaeoschizochlamys mucosa* and *Tetrachrysis dendroides*, epiphytic on *Chara* stems in the broads area of N.W.-Overijssel.

Thalli consist of groups of two cells, or sometimes linear or slightly curved series of four cells, embedded in a common spherical, lobed or irregularly shaped mucilage investment (up to 250 μm large) with a diffuse outline. The groups of cells are slightly separate; they can be randomly distributed or be so closely placed that a dendroid habit is suggested (*figs. 7* and *8*).

Cell shape is spherical or slightly oval (5.5 × 4.5–6 μm); one or two, bilobed, pale olive-green chloroplasts are observed, either parietal or centrally located. The chloroplasts are relatively small; if only one is present in parietal position, it covers slightly less than half of the prominent cell wall. Other cell contents observed are two or three leucosin vacuoles and lipid droplets; if chloroplasts are in central position, peripheral vacuoles with small, strongly refringent crystal-like bodies are observed (*fig. 9*).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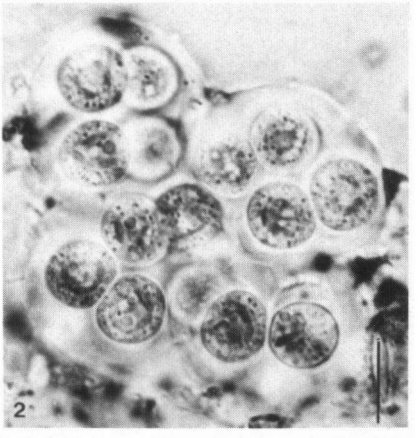
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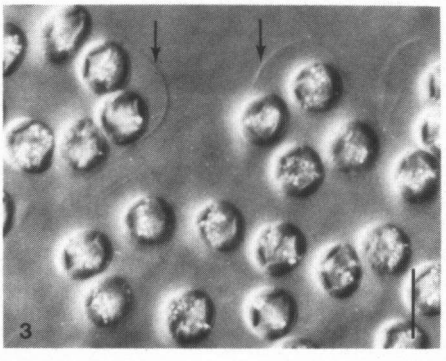
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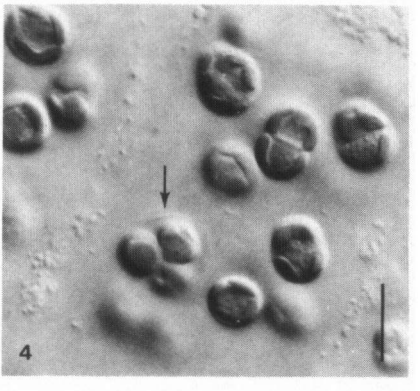
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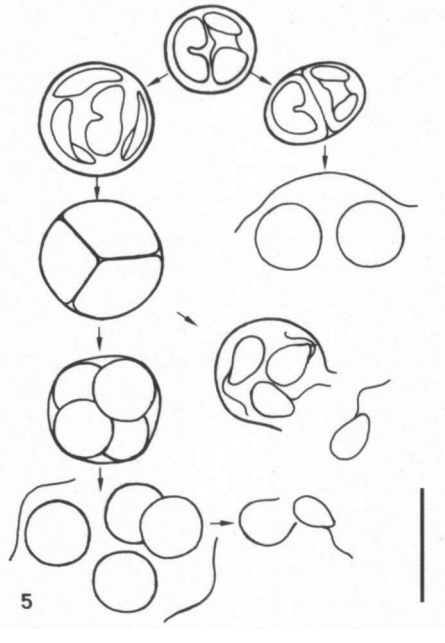
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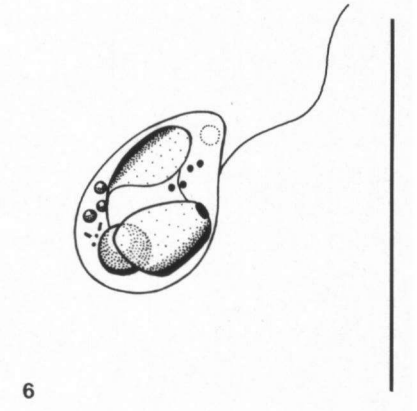
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3.2.2. Cultured material

In culture, cell dimensions remain the same as in field material. In log-phase cultures, one or two parietal and bilobed chloroplasts are observed. In stationary-phase cultures (4–6 weeks old) they retreat towards the cell centre, and oil and leucosin droplets and the peripheral crystals as observed in field material, appear. As in field material, the single chloroplast in recently divided cells covers less than half of the prominent cell wall; in older cells two chloroplasts are observed (*fig. 10*).

Habit of cultured thalli is slightly different from that in field material. While in the latter the diffuse outline of the thallus mucilage may be irregular, it mostly is globular or hemispherical in culture, up to 1.5 mm large.

Cells are grouped in pairs and just as often in series of four; the latter condition was rare in field material. In *fig. 10* part of a cultured thallus is shown with cells arranged in this way. The remarkable way of cell grouping was analysed with time-lapse photography of young thalli with aid of a water-immersion objective; it is achieved as follows (see also *fig. 11*). The first cell (settled zoid) divides once; after this, the resulting daughter cells simultaneously divide once more. When these divisions take all place along the same axis, a linear series of four results. This is not always the case, however; the direction of division changes irregularly and in oval cells is reflected in the orientation of the long axis. All cells within one thallus mostly divide synchronously; time between divisions varies from a few hours to 24 hours. The groups of two or series of four cells are pushed apart by secretion of mucilage, the rate of which determines whether loosely built or compact thalli are observed.

In *fig. 12*, four photographs are shown from a series showing the development of a young thallus. In *fig. 12a* two parallel series of four cells are formed; *12b* and *c* were taken two days later, 1.5 hours apart. In this period, almost all cells had undergone division. *Fig. 12d* is from two days later; at the thallus edge, linear

Plate I. *Phaeoschizochlamys mucosa*

Fig. 1. Field material, on a slide exposed in a ditch near Zegveld. Loose cell tetrads are widely distributed in amorphous mucilage matrix. Caps of old maternal cell walls are close to cells, only visible at higher magnification.

Fig. 2. Field material from the Botshol. Very compact thallus; note lobed appearance due to spherical mucilage of cell tetrads. Chloroplasts are small and ribbon-like; crystals in peripheral vacuoles and dark muciferous bodies are also visible.

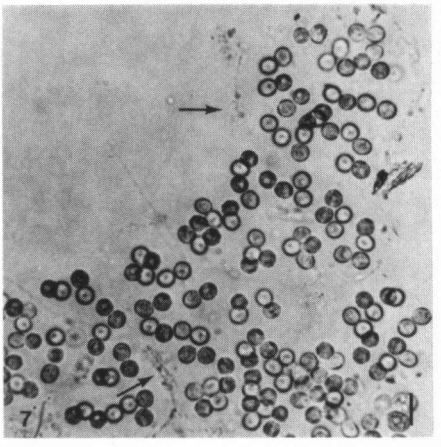
Fig. 3. Cultured material. Note wel-developed parietal chloroplasts and cap-like maternal cell walls (arrows). Interference-contrast photograph.

Fig. 4. Cultured material. Part of a large thallus; outline of mucilage lobe is indicated by bacteria. Several cells undergo division; arrow points to tetrad of autospores with maternal cell wall visible around it. Note well-developed, lobed chloroplasts. Interference-contrast photograph.

Fig. 5. Reproductive cycle of *Phaeoschizochlamys mucosa*. Two or four autospores may be formed, or four zoids. Maternal cell wall may remain behind as two caps or also as one.

Fig. 6. Drawing of zoid showing single, subapical undulating flagellum, single lobed chloroplast with stigma, apical contractile vacuole, leucosin vacuole and dark granules.

Scale bar in all figures equals 10 μm.



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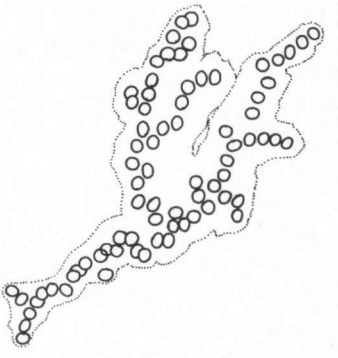

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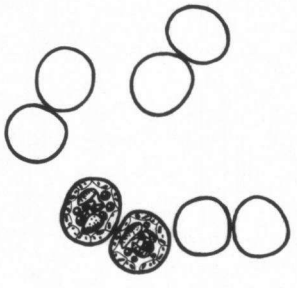

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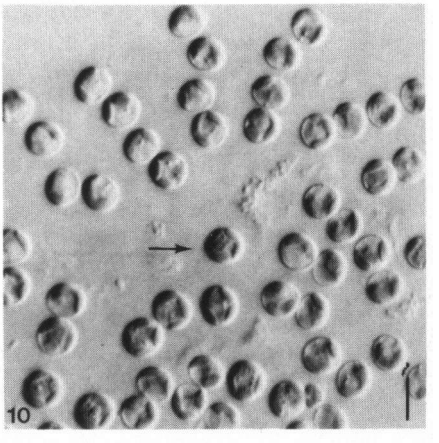

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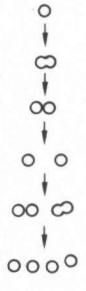

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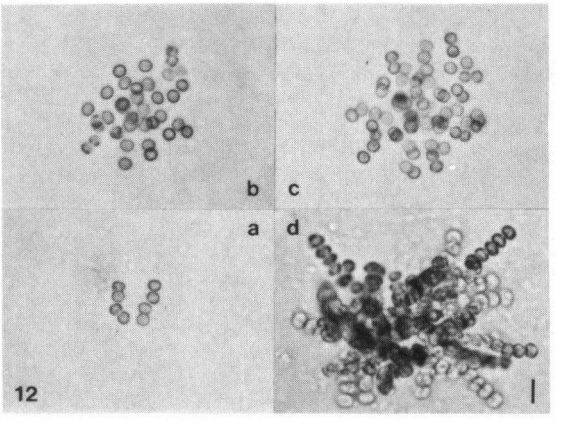

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series of four are prominent and seem to radiate from the thallus centre (also visible in *fig. 10*).

Zoids were readily produced in culture within three days after inoculating stationary-phase thalli into fresh culturing medium. Zoids are spherical to pear-shaped, 5.5–6 µm diameter, with one lobed, parietal chloroplast, a stigma, one or two leucosin vacuoles, several dark granules, and a contractile vacuole near the flagellum implant. They have two unequally long flagella, implanted subapically of which the long one is approximately 1.5 times cell length, pulling the cell forward in an undulating movement. The short flagellum is half as long as the cell, and is held backwards close to the cell body (*fig. 13*).

3.3. *Chrysophaera botryoides* spec. nov.

3.3.1. Field material

Apart from the waters mentioned, this species was also observed in ditches in the Northern part of the province North-Holland (near Noord-Scharwoude, January 1977), in ditches around Amsterdam (December 1976, October and November 1977, quite commonly, and August 1977, sparsely). It probably is a rather common epiphyte on filamentous algae (*Cladophora spp.*, *Oedogonium spp.*, and Chaetophoralean algae) and other substrates as *Phragmites* stems, in eutrophic or moderately eutrophic lakes and ditches.

Young thalli (approximately up to 16 cells) appear as regular rectangular, flat packets (*fig. 15*). Later, colonies consist of an irregular mass of cells with circular outline and a multilayered centre (*fig. 14*), up to 150 µm in diameter.

Plate II. *Tetrachrysis minor*

Fig. 7. Field material, from a slide exposed in the Botshol. Irregularly lobed thallus; borders of lobes marked by bacteria (arrows). Cells all in groups of two. Cultured material of this thallus is shown in *figs. 10* and *12*.

Fig. 8. Field material. Drawing of a thallus found on a *Chara* stem from N.W.-Overijssel, accompanied by *T. dendroides* and *Phaeoschizochlamys mucosa*. Note suggested dendroid habit comparable to that of *T. dendroides*.

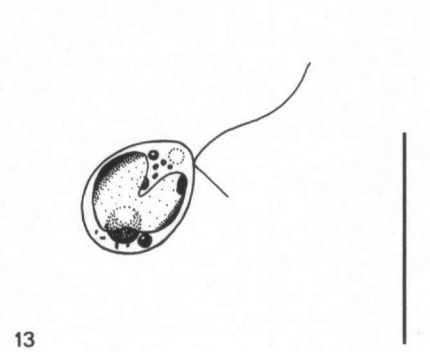
Fig. 9. Field material. Drawing of cells with centrally located, small and ribbon-like chloroplasts, and crystals in peripheral vacuoles.

Fig. 10. Cultured material, the same as in *fig. 7*. Edge of thallus showing cell series. The one at upper right is clearly linear; toward the upper left they seem to dissociate in pairs. Note also the single lobed chloroplast in most cells, covering less than half of the cell wall, and a few cells having two (arrow). Interference contrast photograph.

Fig. 11. Diagram of cell division in *T. minor*. First cell (settled zoid) divides once. If second division follows quickly, a series of four cells may result as in *T. dendroides*. Otherwise, pairs of cells are formed, gradually pushed apart by mucilage secretion.

Fig. 12. Time-lapse photography of developing thallus. In 12a, a young thallus is shown (15-4-'78); in b. the same thallus on 17.4.'78 at 11.30 am and in c. at 1.00 pm. Note that nearly all cells (except in upper right) have divided. In d., the thallus is shown on 20-4-'79; note rows of four cells radiating from the thallus centre.

Scale bar in all figures equals 10 µm.



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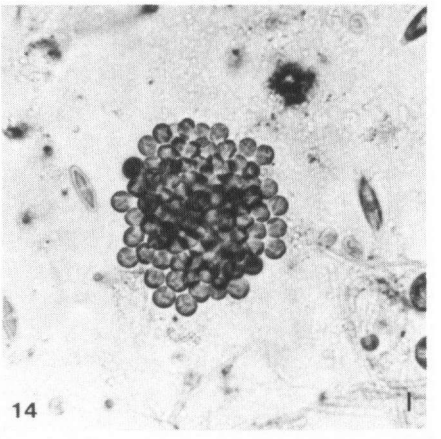

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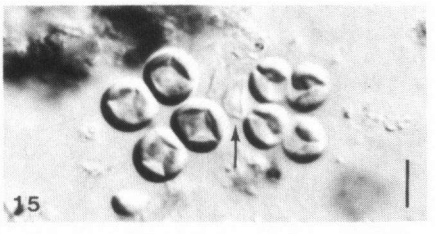

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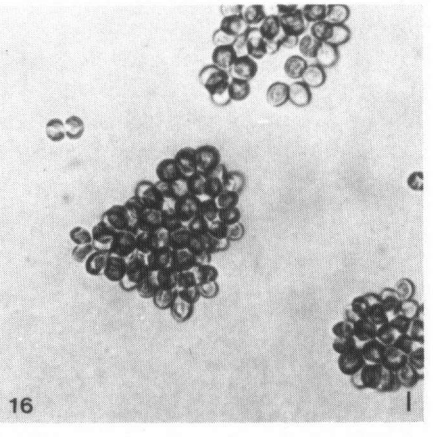

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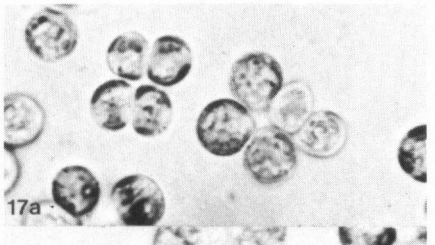

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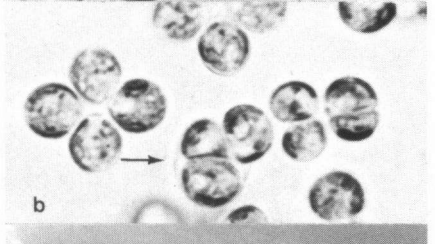

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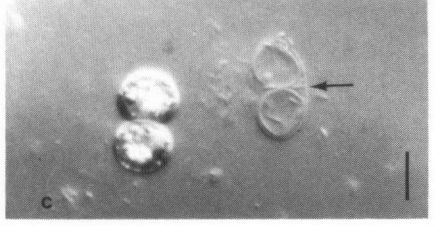

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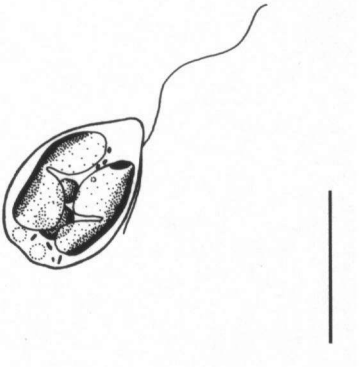

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Cells are spherical or oval, and slightly flattened when lying close to one another. Cell size varies from 6.5–11.5 μm. A distinct cell wall is present; after sporulation it is left behind as empty shell. Two pale golden-brown or dark brown parietal, deeply lobed chloroplasts are present, covering most of the cell wall or sometimes only part of it. Due to the large lobes, they often appear to be four in number. One or two leucosin vacuoles and a varying number of oil droplets are observed in the cell lumen. Just underneath the cell membrane several small globules are visible; they stain with brilliant cresyl blue, and might be muciferous bodies (BOURRELLY 1957).

Zoids or cysts were not observed in field material.

3.3.2. Cultured material

In culture, thalli are rarely attached to the walls of the culturing vessels; they are shaped like a bunch of grapes and mostly float around or lie on the bottom (*fig. 16*). The rather wide range of cell size observed in the field was also found in thalli, cultured under standard conditions. Cells of all five clones varied in shape from oval (young cells) to spherical; diameter 6.5–12 μm. In log-phase cultures two (sometimes four) deeply lobed golden-brown or dark brown, parietal chloroplasts, one or two leucosin vacuoles, and one or two lipid droplets were observed. In stationary-phase cultures the chloroplasts appear smaller and paler, and the cells are filled with globules of reserve material. Cells multiply by autosporulation. Two autospores are formed in each cell; they form their own cell wall and in expanding, the mother cell wall is stretched and dissociated, holding the initially oval young cells together at their bases (*fig. 17a* and *b*). In *fig. 17c*, the empty cells of a two-celled thallus are shown after zoids have issued; the old mother-cell wall can be seen to stretch partly around the autospore cell walls.

Plate III. *Tetrachrysis minor*

Fig. 13. Drawing of zoid, showing single lobed parietal chloroplast; stigma, leucosin and lipid globulus, dark granules, apical contractile vacuole and two flagella.

Chrysosphaera botryoides

Fig. 14. Field material. Thallus growing on slide exposed for four weeks in the pond of the Hortus Botanicus, Vrije Universiteit. Note multilayered centre of aggregate.

Fig. 15. Field material, same locality as *fig. 14*. Note two lobed parietal chloroplasts in four large cells. In this case, four autospores have apparently formed; this is an exception. Maternal cell wall is visible (arrow); note single lobed chloroplast in young cells. Interference contrast photograph.

Fig. 16. Cultured material. Note grape-bunch aspect of these floating thalli. Cells are mostly oval.

Fig. 17. Cell division in C. botryoides. In a, two young autospores are shown; note oval shape and the fact that the cells are held together at their bases. In b, somewhat irregular autospore formation is shown (arrow). In c, an empty two-celled thallus is shown after zoid formation. Note maternal cell wall (arrow) extending around large part of autospore walls, holding these together.

Fig. 18. Drawing of zoid showing two parietal, lobed chloroplasts, leucosin vacuoles, basal contractile vacuoles and unequal, subapically implanted flagella, of which the short one is rather delicate and held close to the cell body.

Scale bar in all figures equals 10 μm.

Zoid formation was sparsely observed; only one clone readily produced zoids upon transferring stationary-phase thalli into fresh culturing medium. In the other clones it could be induced by transferring after two days of culturing under standard conditions to total darkness, 4°C. After 10–15 hrs under these circumstances, a few zoids could generally be observed. One zoid is formed per cell, which is pear-shaped, 8–11 μm long, with two (rarely four) parietal, lobed chloroplasts, one to three leucosin vacuoles and two or three contractile vacuoles at the posterior, slightly metabolic end. Zoids have two slightly subapically implanted flagella of different length. The long one is 1.5 times to twice as long as the cell and in swimming pulls the cell forward in an undulating movement. The short flagellum, three quarters cell length, is held close to the cell body, directed backwards. Due to the fact that it is held close to the cell body, it is difficult to discern in swimming zoids. After settling and shedding the flagella, the zoid secretes a cell wall and a new thallus is formed (*fig. 18*).

4. DISCUSSION

4.1. *Phaeoschizochlamys mucosa* Lemmerman

The identification of our material did not pose much problems. The only Chrysophytes having a mucilaginous thallus in which the cells are distributed in groups of two or four and where the mother cell walls remain visible after autosporulation are *Phaeogloea mucosa* Chodat and two species in the genus *Phaeoschizochlamys: P. mucosa* Lemmerman and *P. delicatula* (West) Bourrelly. *Phaeogloea mucosa* is distinguished by the heterokont zoids, lacking a stigma. Habit and dimensions of our clones are completely in accordance with the type species of the genus *Phaeoschizochlamys*, *P. mucosa* Lemmerman. This author found the algae on the walls of culturing vessels in which freshwater algae from the Frisian isle Wangerooge were maintained. The illustrations in LEMMERMAN 1898, plate V fig. 1 and 2 show a lobed mucilage investment with single cells and groups of two, rarely four, cells surrounded by caps consisting of the old mother-cell walls; dimensions are stated to be 4–8 μm. In our clones, dimensions vary from 6–9.5 μm.

Since this is the first time that zoids are reported in the life cycle of the type species of *Phaeoschizochlamys*, the genus should be removed from the Subclassis Acontochrysophycidae to the Subclassis Heterochrysophycidae, Ordo Chromulinales, Subordo Chromulineae (in which the palmelloid genera are found) in the Fam. Chrysocapsaceae, where the palmelloid or gloeocystoid Chrysophytes that lack pseudocilla or a specialized growth zone, are grouped (systematics according to BOURRELLY 1968).

4.2. *Tetrachrysis minor* sp. nov.

From our field observations and culturing experiments the conclusion can be drawn that this is a palmelloid species with extreme variation in thallus form. Especially with field material, description proves to be very difficult. Cells can be single (rarely, only just before division) or in groups of two or series of four. This

variation in cell groups is also seen in *Phaeoschizochlamys mucosa.*

An outstanding character of this alga is formed by the synchronous divisions of the cells in one thallus. This results in the regular pairs of cells; if divisions succeed one another quickly enough to prevent the secretion of mucilage from pushing cells apart too soon and the subsequent divisions take place in the same direction, series of four cells as illustrated in *figs. 8, 10* and *12* may result. This aspect is strongly reminiscent of *Tetrachrysis dendroides* (DOP 1980b). It differs from this species not only in cell size however, but also in the mode of cell division. In *T. dendroides*, cells are only observed in linear cell series because two (also synchronous) cell divisions take place directly after one another. In the present alga, there may be a pause between subsequent divisions, and mucilage secretion is more copious (only a narrow sheath is present around *T. dendroides* cell series); as a consequence, cells in *T. minor* may be observed in pairs as well as in linear series of four.

The taxonomic position of the present alga is difficult to determine. On account of the heterokont zoids, it should be placed in the Subclassis Heterochrysophycidae, Ordo Ochromonadales. It could possibly be placed in the Fam. Ruttneraceae, in the genus *Chrysocapsella* Bourrelly (BOURRELLY 1957), consisting of algae with homogeneous or gloeocystoid mucilaginous thalli, ellipsoid or irregularly shaped, in which cells are centrally located in groups of 2,4 of 8. Cell shape is ovoid or ellipsoid; two parietal chloroplasts are observed. Zoids have two unequally or almost equally long flagella. Type species is *Chrysocapsella paludosa* (West) Bourr., basionym: *Phaeococcus paludosus* W. et G.S. West. A second species, *Chrysocapsella granifera* (Mack) Bourr. was proved to be the benthic phase of a *Pavlova* species: *P. granifera* (Mack) Green (GREEN 1973). Recently a new species, *Chrysocapsella mucophila* Starmach, (STARMACH 1972), was described: no zoids were observed, so the position of this species is doubtful.

We hesitate to place our alga in the genus *Chrysocapsella* however, since in our opinion the type species of this genus is poorly known and may very well prove to be the benthic phase of a Prymnesiophycean species, since its zoids have subequal flagella. Because our alga is reminiscent of *Tetrachrysis dendroides* Dop (Subordo Phaeotheamniineae, Fa. Phaeothamniaceae, DOP 1980b), in several aspects of thallus formation, we propose to place it next to this alga as the second species in this genus. In this view, the family Phaeothamniaceae covers a wide range of thallus forms: two- or four-celled rows (*Tetrachrysis*), mucilaginous unbranched filaments of separate cells (*Sphaeridiothrix*), unbranched truly filamentous (*Nematochrysis*), and branched filamentous (*Phaeotheamnion, Apistonema, Chrysonephos*). The formal description of *Tetrachrysis minor* will be given below.

4.3. *Chrysosphaera botryoides* spec. nov.

Apart from the fact that the zoids are heterokont, this species fits perfectly within the description of the genus *Chrysosphaera* Pascher. It is also quite similar to most of the species described in this genus until now. Several characters of these

Table 1. Comparison of *Chrysosphaera* species.

Species	Author	Cell dimensions	Chloroplasts	Multiplication	Other characters
C. nitens	Pascher 1914	12–24 μm, round or oval.	Two, parietal, lobed; four in dividing cells.	Two autospores per cell; 6–8 uniflagellar zoids.	Prominent cell walls. Small granula at chloroplast inside. Dimensions are those of Pascher 1925.
C. paludosa	Korshikov 1924 (as Phaeocapsa paludosa)	8–10 μm, round.	One, parietal, large, with edges turned in.	Up to 4 autospores per cell.	Contractile vacuole observed. Pascher (1925) mentions palmelloid stage, cysts and zoids with probably one flagellum for this species.
C. parvula	Pascher 1925 (as Chrysobotrys parvula)	8 μm, round.	One, parietal, cup-shaped; two in dividing cells.	2–4 autospores per cell; mostly one zoid with one? flagellum.	Rather prominent cell wall; thin mucilage investment.
C. melosirae	Meyer 1930 (as Epichrysis melosirae)	Up to 10 μm, round.	One, discoid, with edge turned outward.	2–8 uniflagellar zoids per cell.	Epiphytic on Melosira; thin cell wall.
C. nitellae	Geitler 1928 (as Epichrysis nitellae)	Up to 12 μm; ellipsoid	4–8 mostly lobed parietal small discs.	Two autospores per cell.	Very thin cell wall.
C. marina	Schussnig 1940	7–10 μm, round.	Two, parietal, large and plate-like.	Two autospores per cell.	Pyrenoid observed; prominent cell wall. Marine habitat.
C. pygmaea	Schussnig 1940 (as Chrysobotrys pygmaea)	3.5–7.5 μm, round.	Two, parietal, plate-like.	Regular cell division.	Thin cell wall. Marine habitat.
C. feldmannii	Bourr. & Magne, 1953 (as Chrysobotrys feldm.)	8–20 μm, round.	Up to 12, parietal, discoid.	–	Pyrenoid observed; prominent cell wall. Polygonal granula in cell. Marine habitat.

Table 1. (continued)

Species	Author	Cell dimensions	Chloroplasts	Multiplication	Other characters
C. gallica	Bourrelly 1957	8–12 µm, round.	3–5, parietal, disc-shaped.	–	Thin cell wall, with "corps mucifères" beneath it.
C. epiphytica	Starmach 1966	5.6–7.8 µm, round.	Two, parietal, cup-shaped.	2–4 autospores per cell.	Distinct cell wall; contractile vacuole observed.
C. stigmatica	Starmach 1972	8–12 (–14) µm, round.	One, parietal, band-like, covering approx. half of cell wall.	Up to 32 autospores per cell; also by cell division.	Distinct cell wall; stigma observed.
C. magna	Belcher 1974	Up to 10 µm, variously shaped.	One, lobed, parietal.	Up to 256 zoids, with one long flagellum and a second very short one, only visible under E.M.	Colonies 5–60 µm large, consisting of a mucilaginous wall (up to 15 µm thick) surrounding cells provided with active flagellar apparatus. Does not belong in this genus (see Dop 1980c).

Note: All new combinations were published by Bourrelly (1957).
Thalli of all species consist of uni- of multilayered aggregates of cells, epiphytical on various aquatic substrates.

species are described in *table 1*. Our alga might be identified with *C. gallica* Bourrelly or *C. nitellae* (Geitler) Bourr. if assumed that the larger number of chloroplasts of these species are due to a misinterpretation of a smaller number of deeply lobed ones. This is doubtful however, since both of the above mentioned authors are careful observers.

If *C. magna* Belcher is excluded from the genus *Chrysosphaera* (see DOP 1980c), only two out of the eleven species remain in which zoids were observed with certainty: the type species *C. nitens* Pascher (PASCHER 1914) and *C. melosirae* Meyer (MEYER 1930). *C. paludosa* (Korsh.) Bourrelly was observed by PASCHER (1925) to have zoids with probably one flagellum; palmelloid stages and cysts were also observed by this author. In the description of *C. parvula* (PASCHER 1925), the zoids are stated to have a single flagellum, but the possibility that a second one is also present, is not excluded by the author on account of the way of swimming. ETTL (1965) records *C. nitens* to have two uniflagellar zoids per cell, or two autospores. The cell dimensions given are quite different from those of *C. nitens* however: 10–12 μm (see *table 1*).

Our conclusion is that until more is known about the motile cells of other representatives of the genus *Chrysosphaera*, it is preferable to include our alga as a new species in this genus. The second, short flagellum of the zoids was very difficult to distinguish in our clones (it was rather thin and held close to the cell body); possibly it has escaped detection by some authors. The alternative, to create a new genus exactly similar to *Chrysosphaera* in habit and vegetative reproduction, to be placed in the order Ochromonadales, is not preferable. This would be necessary however, since no existing genus can accommodate this type of alga. Besides, we think that there are developments in Chrysophyte taxonomy that render the distinction between algae with *Chromulina*-type and *Ochromonas*-type zoids less important than might be gathered from BOURRELLY's (1968) classification; see discussion on *Mucosphaera magna* gen. et spec. nov. (DOP 1980c).

Diagnoses:

Tetrachrysis minor spec. nov.
Thallus epiphyticus, duarum aut quattuor cellularum seribus in matrice mucosa irregulare et lobata distributis compositus. Cellulae globasae aut ovoideae (dimensione 5.5 μm × 4.5–6 μm) singulo vel duobis chloroplastis lobatis parietalibus vel centralis, cellulam haud replentibus, blobulis leucosinis ac lipidis et membrana firma praeditae. Zoosporae singulae ex cellulas ortae, ovoideae aut sphaericae (5.5–6 μm diametro), chloroplasto singulo, lobato, stigmate rubro, globulos leucosinis ac lipidis et vacuola contractibile apicale praeditae; flagella sub apicem implantata quorum unum longitudine 8 μm et unum longitudine 3 μm.

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

Iconotypus: figurae nostrae *7*, *9*, *10* et *13*.

Tetrachrysis minor spec. nov.

Thallus epiphytical, consisting of rows of two or four cells distributed in an irregular, lobed mucilaginous matrix. Cells are spherical or ovoid (5.5 μm × 4.5–6 μm) with one single or two lobed, parietal chloroplasts, not filling the cells, leucosin and lipid globulus and a firm cell wall. One zoid issues from each cell, ovoid or spherical (5.5–6 μm diameter), with a single, lobed chloroplast with red stigma, leucosin and lipid globulus and an apical contratile vacuole; the flagella are implanted subapically, of which one is 8 μm long and the other 3 μm.

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

Iconotype: our *figs. 7, 9, 10* and *13.*

Chrysosphaera botryoides spec. nov.

Thallus epiphyticus, cellularum congregatione irregulare sine tegmento mucoso compositus. Cellulae ovoideae vel sphaericae, diametro 6.5–11.5 μm, duobis chloroplastis ochraceis parietalibus et lobatissimis, globulis leucosinis ac lipidis et membrana firma praeditae. Propagatio per autosporas quae binae formantur, aut zoosporas quae singulae ex cellulas ortae. Zoosporae sunt ovoideae, longitudine 8–11 μm, duobis (rarius quaternis) chloroplastis parietalibus ac lobatis, globulis leucosinis et lipidis et duobis aut tres vacuolis basalibus praeditae. Flagella sub apicem implantata, quorum unum longitudine 12–18 μm et unum circa 6 μm longitudine.

Observata est in fossis et stagnis Hollandiae septentrionalis, epiphytica in substratis aquaticis diversis.

Iconotypus: figurae nostreae *14, 15, 16* et *18.*

Chrysosphaera botryoides spec. nov.

Thallus epiphytical, consisting of an irregular aggregate of cells, without surrounding mucilage. Cells are ovoid or spherical, 6.5–11.5 μm diameter, with two brownish, parietal and much lobed chloroplasts, leucosin and lipid globulus and a prominent cell wall. Multiplication by way of autospores (two per cell), or zoids that are formed singly. The zoids are ovoid, 8–11 μm long, with two (seldom four) parietal, lobed chloroplasts, leucosin and lipid globulus and two or three basal, contractile vacuoles. Flagella are implanted subapically, of which one is 12–18 μm long and the other about 6 μm long.

Observed in ditches and ponds in the Western part of The Netherlands, epiphytic on various aquatic substrates.

Iconotype: our *figs. 14, 15, 16* and *18.*

Since zoids were observed for the first time in *Phaeoschizochlamys mucosa* Lemmermann, the diagnosis of genus and species should be augmented. They should now read as follows:

Phaeoschizochlamys Lemmermann 1884, p. 501.

Cellulae solitariae vel 2–4 in familias gelatinosas consociatae. Chlorophora brunnea, parietalia. Propagatio per zoosporas typus generis Chromulinae, vel

bipartitione cellularum in duas directiones; membrana cellularum matricalium in 2 fragmenta muco hyalino diutius cohaerentia disrupta.

Phaeoschizochlamys mucosa Lemmermann 1884, p. 502.

Cellulae globosae, 4–8 μm crassae, plerumque singulae vel geminatim (rarius quaternis) approximatae. Zoosporae quaternae formantur, ovoideae, chloroplasto singulo, lobato, cum stigmate, et vacuola contractibile apicale praeditae; flagellum sub apice implantatum, circa 8 μm longum.

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