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ON THE TAXONOMIC POSITION OF PHAEOBOTRYS SOLITARIA ETTL (CHRYSOPHYCEAE)

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SUMMARY

Phaeobotrys solitaria Ettl was found at several localities in The Netherlands; details of its morphology, including ultrastructure, are presented. Culturing experiments revealed its life cycle to contain *Ochromonas*-like zoids. It is therefore removed from the subclass Acontochrysophycidae to the Heterochrysophycidae, order Ochromonadales.

1. INTRODUCTION

Phaeobotrys solitaria was described by ETTL (1966) from a meadow drainage ditch, filled with water from the spring thaw, in North Moravia, where it occurred together with *Tribonema* spp. This solitary coccoid Chrysophyte showed an astonishing growth: small young cells (6 to 7 μm diam.) with delicate walls and three or four discoid, parietal chloroplasts grew into large thickwalled multinucleate cells (22 to 28 μm diam.) with numerous chloroplasts. Multiplication was accomplished by autospores (8 to 32), liberated through diffluction of the parent cell wall.

In November 1973 this alga was found in a small ditch in Bergen, The Netherlands; in January 1977 in a ditch near Noord Scharwoude, and in November 1976 and December 1977 in ditches in the Amsterdamse Bos. It was collected from submerged glass microscope slides, reed stems or other algae (*Vaucheria* spp., *Cladophora glomerata*). Main accompanying organism in most cases was *Vorticella* sp. The waters concerned may all be classified as eutrophic.

2. MATERIALS AND METHODS

A clone was started from the material found in Bergen and cultured in Wood's Hole artificial freshwater medium (STEIN 1973) at 12 C, ± 1500 Lux fluorescent lighting under a 12 hrs light/12 hrs darkness regime. Three to five days after inoculating large, thick-walled old cells into fresh medium, formation of autospores and zoids could be observed. Light microscopical observations were made with normal and phase- and differential interference contrast optics.

For electron microscopy, cells were fixed in a cacodylate-buffered mixture of osmium tetroxide (final conc. 1%) and glutaraldehyde (final conc. 0.8%) at pH 7.4, for two hours on ice. After rinsing in distilled water the material was taken up in 1% agar and cut in small fragments before dehydrating in a graded alcohol

series. Embedding was done in Epon 812. Ultrathin sections were cut on a Reichert ultramicrotome using glass knives and picked up on formvar-coated copper grids. Sections were not stained; examination was done on a Zeiss EM9A electron microscope.

3. OBSERVATIONS

3.1. Light microscopy

3.1.1. Field material

In the field, *P. solitaria* was found as rather conspicuous brown, sometimes greenish-brown, solitary or loosely clustered spherical to ovoid cells of varying dimensions (10 to 20 μm).

In *fig. 1*, a group of cells growing on a glass slide is illustrated. Chloroplast shape is discoid or oval; the number varies from two in small cells to at least twenty in large ones. In small cells of about 10 μm in diameter, two to five chloroplasts are arranged parietally; in larger cells they are also observed in the central part of the cell lumen. Other cell contents such as lipid and leucosin vacuoles and nuclei are very difficult to observe in large cells on account of the chloroplasts. Zoids nor autospores have been observed in field material.

3.1.2. Cultured material

3.1.2.1. Habit

Cell dimensions vary from 5 μm for recently settled zoids or newly released autospores to 30 μm for thick-walled older cells. Cell shape is spherical to ovoid.

In *fig. 2* a group of cells picked up from the meniscus of a culturing vessel illustrates the increase in cell size from autospore to ripe sporangium as well as differences in chloroplast arrangement.

Chloroplast shape is discoid or oval, sometimes lobed. The number of chloroplasts varies with cell size: zoids and autospores may have two, while up to at least twenty have been counted in large cells. In these, stigmata are often observed on the edges of the chloroplasts. Lipid droplets and leucosin vacuoles (stainable with resp. Sudan black and Cresyl blue) were also present but in large cells difficult to observe due to the numerous chloroplasts. For the same reason, nuclei (one or two) were only visible in small cells (up to 10 μm). At the cell

Scale bar equals 10 μm.

Fig. 1. Field material; cluster of cells on glass slide from ditch in the Amsterdamse Bos. Note slightly irregular outline of cells.

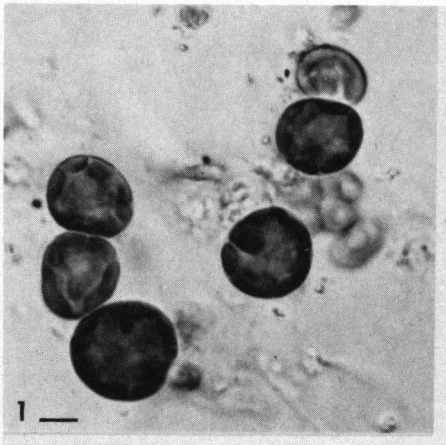
Fig. 2. Cultured material, two weeks old. Extremes in cell size are shown here from young cell to ripe zoosporangium (note several stigmata visible at chloroplast edges in the largest cell; arrows). Also note the large, irregular shape of the chloroplasts in the cell at the lower right-hand side.

Fig. 3. Cultured material; differential interference contrast photograph showing subsurface vesicles.

Fig. 4. Cultured material; autospores are released in clusters.

Fig. 5. Extreme thickening of parent cell wall just before release of autospores.

Fig. 6. Drawing of zoid, showing heterokont flagella, two chloroplasts one of which carries a stigma, contractile vacuole and lipid globules.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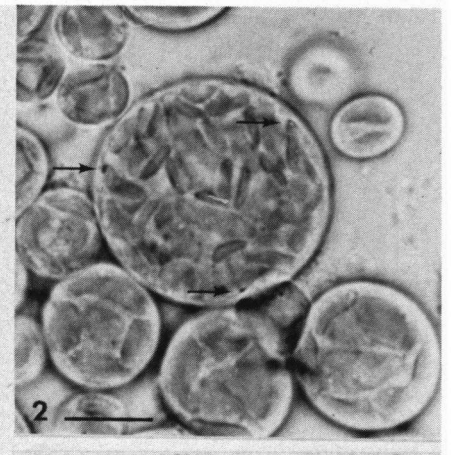
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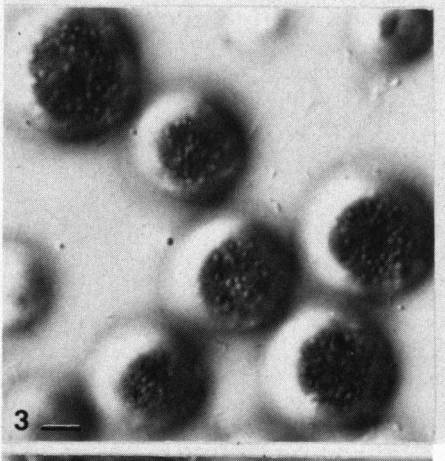
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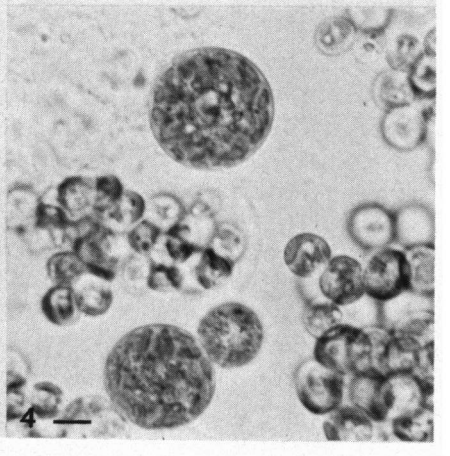
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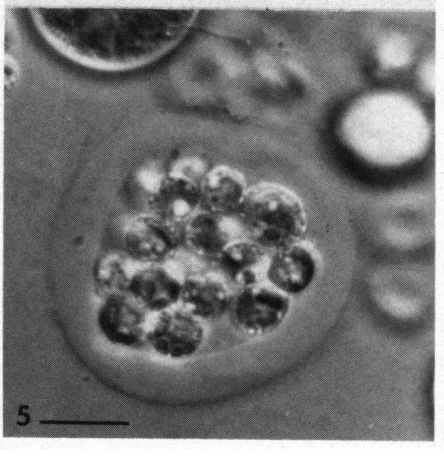
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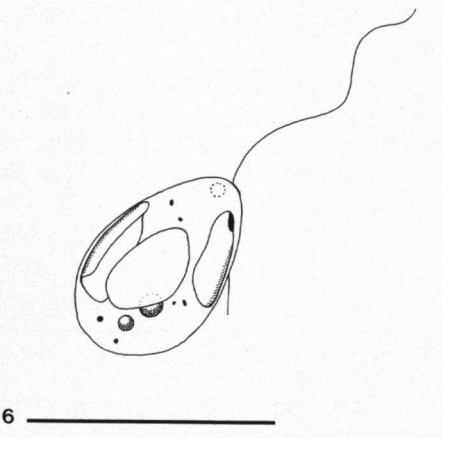
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periphery, directly under the cell wall, small vesicles of about 1 μm diameter can be observed with the aid of differential interference contrast optics; they are interpreted as "corps mucifères" (see *fig. 3*).

The cell wall is delicate in small cells while in cells of 20 to 30 μm in diameter a thickness up to 1 μm is found.

3.1.2.2. Reproduction

Our clone of *Phaeobotrys solitaria* reproduced by autospores as well as by zoids, both of which are formed by cells of 20 μm or larger.

These cells, that might be termed autosporangia or zoosporangia, are not distinguishable from vegetative cells in culture except when shortly before liberation of reproductive cells the zoid eyespots or the individual autospores become visible. Stigmata may appear and disappear again in cultures; this is especially the case when three weeks after starting a subculture all reproductive activity comes to a halt.

Autospores are spherical, about 7 μm in diam. and have two lobed chloroplasts. Up to 32 in number are released in a cluster after the parent cell wall has swollen to three or four times its original thickness and ruptured, see *figs. 4* and *5*.

Zoids are also liberated through a rupture in the swollen parent cell wall; they escape singly, however. Up to 32 have been observed to issue from one cell. The zoids are pear-shaped, 5 to 8 μm long, with two subapically inserted heterokont flagella. The long flagellum is directed anteriorly and is twice as long as the cell; the short one, approximately as long as the cell, is directed obliquely towards the zoid posterior end. Zoids swim in the usual heterokont fashion, the long flagellum pulling the cell forward in an undulating movement while the short one is carried close to the cell body. Two lobed parietal chloroplasts are present (sometimes three or four small discoid ones); a stigma lies on the outer edge of a chloroplast near the flagellum insertion point. In this region a single contractile vacuole is situated.

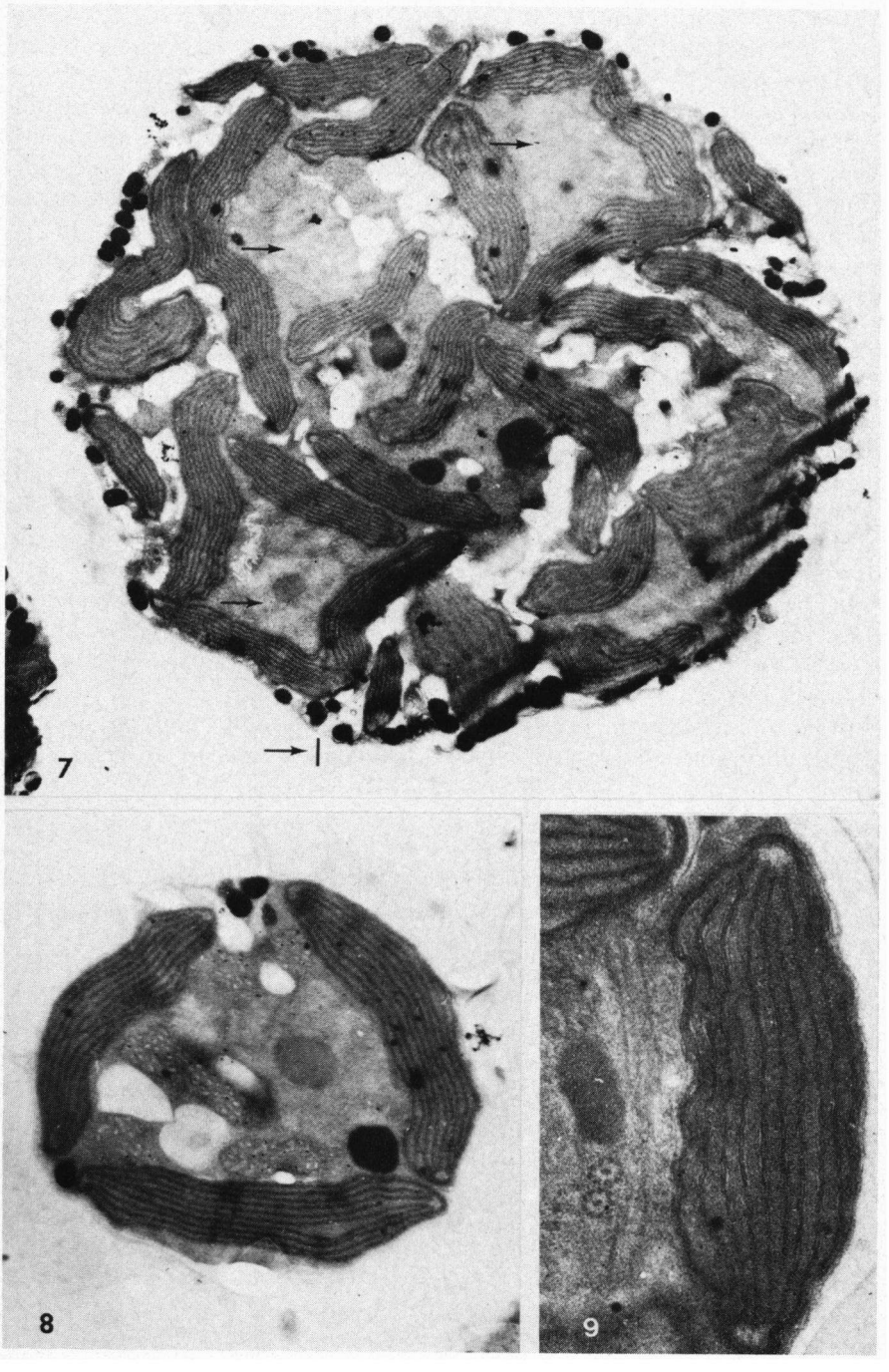
Two or three small lipid globules are observed near the cell posterior, see *fig. 6*.

Zoids keep swimming only for a very short period after issuing (two minutes at the most). They settle on the substratum with a vibrating movement and quickly resorb their flagella; this coincides with an undulating movement of the cell membrane before the cell assumes a globular shape.

Fig. 7. Survey section of large cell, in process of spore formation. Note small chloroplasts with girdle lamellae, numerous small vacuoles and subsurface osmiophilic droplets. Three nuclei are visible (arrows), one with dark nucleolus, which are surrounded by chloroplasts. Thick cell wall is visible in lower left-hand corner (arrow). ▶

Fig. 8. Young cell showing chloroplasts with girdle lamellae, thin cell wall, nucleus with dark nucleolus, elongate mitochondria with tubular cristae, leucosin vacuoles and osmiophilic globules.

Fig. 9. Part of a young cell showing one longitudinal and two cross sections of retracted flagella, next to dark body of unknown nature.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3.2. Electron microscopy

Large cells proved difficult to fix; some shrinkage occurred and the sections were sometimes wavy.

A survey section of a large cell (20 μm diam.) is illustrated in *fig. 7*. The cell wall is distinct, finely fibrous and varying in thickness. Just underneath the cell membrane, numerous small osmiophilic droplets are visible, assumed to correspond with the "corps mucifères" reported under light microscopical observations. Dimensions do not quite correspond, however, probably due to processing. Chloroplasts consist of eight to twelve three-thylakoid lamellae, one of which is a girdle lamella. Plastoglobuli are observed between the lamellae. In young cells (*fig. 8*), the chloroplasts are arranged parietally; in older cells they move toward the cell centre as well. In *fig. 7* the partitioning of cell contents by several chloroplasts preliminary to zoid or autospore formation is apparent.

Mitochondria are sausage-shaped with tubular cristae. They are found in the cell lumen and never directly under the cell membrane.

In young cells, one or two nuclei are observed (*fig. 8*), while in older cells several are present during the process of zoid or autospore formation (*fig. 7*). The chromatin in the nuclei is evenly distributed; one dark nucleolus is present. No observations have been made on Golgi body and E.R.

In the cell lumen, osmiophilic globules and empty vacuoles (presumably leucosin vacuoles) are observed. The latter are especially numerous in sections of large cells where they seem to be positioned mainly around the spores in process of formation (*fig. 7*).

In *fig. 9* part of a young cell is shown, in which several sections of absorbed flagella are visible, next to a dark body of unknown nature.

4. DISCUSSION

The light microscopical observations are in agreement with Ettl's original description of *Phaeobotrys solitaria* except for the supplementary observation of the zoids.

The life cycle of *P. solitaria* as reconstructed in culture is as follows: zoids or autospores develop into large unicellular auto- or zoosporangia that release reproductive cells upon gelatinisation of the cell wall. A comparable cycle is found in the Xanthophycean genus *Botrydiopsis* Borzi.

We observed the alga in The Netherlands during the winter months only; apparently it is a cold-adapted species.

The ultrastructural features observed are consistent with the position of this alga in the Chrysophyceae.

BOURRELLY (1968) placed *Phaeobotrys* in the subclass Acontochrysophycidae, order Stichogloeales, family Stichogloeaceae. *Stichogloea* Chodat, the type genus of this family, has recently been shown to possess *Ochromonas*-like zoids in its life cycle (NORRIS 1977). In this paper, Norris discusses the taxonomic position of *Stichogloea* and the order Stichogloeales. Following FOTT's (1971) classification rather than BOURRELLY's (1968), he stresses the vegetative aspect of the algae as

being more important than the type of flagella of motile cells in Chrysophyte taxonomy. Therefore, Norris proposes to abandon the order Stichogloeales and to provisionally move the Stichogloeaceae to the order Chrysosphaerales sensu FOTT (1971). This order would then include the Chrysosphaeraceae, Stichogloeaceae and Chrysapiaceae, all having coccoid, walled vegetative cells.

If one adheres to Bourrelly's classification, the genus *Stichogloea* would fit in the subclass Heterochrysophycidae, order Ochromonadales, suborder Chrysapiineae, family Chrysapiaceae (the last two names being orthographic changes of Bourrelly's Chrysapionineae and Chrysapionaceae, respectively).

The suborder Chrysapiineae contains coccoid, solitary or colonial Chrysophytes, multiplying by way of anisokont zoids as well as by simple vegetative division or autosporulation.

The Chrysapiaceae constitute its only family, with the monotypic genera *Chrysapion* Pascher et Vlk, *Koinopodion* Pascher and *Phaeogloea* Chodat, of which only the last one has ever again been recorded after its original description. Following this classification, *Stichogloea* would fit in close to *Phaeogloea*, a genus of colonial Chrysophytes in which the globular cells are irregularly distributed throughout a spherical, homogeneous mucilage matrix, in which after autosporulation the maternal cell walls remain visible.

The findings presented in this paper justify the placing of *Phaeobotrys solitaria* among the Chrysapiaceae, just like *Stichogloea*. We propose to place it close to the solitary, pyriform *Chrysapion*.

For the moment we prefer to adopt the abovementioned systematic arrangement for *Stichogloea* and *Phaeobotrys*. Several members of the Stichogloeaceae (and other families in the Acontochrysophycidae) need further investigation and may prove to possess motile cells in their life cycles. This may lead to a gradual adaptation of Bourrelly's classification.

The evidence of zoids in the life cycle of *Phaeobotrys solitaria* also necessitates augmentation of Ettl's description of genus and species. The description of *Phaeobotrys* Ettl 1966 should be enlarged as follows:

Propagatio fit 8–32 autosporis vel zoosporis typus generis Ochromonadis.

The following sentence describing the zoids should be added to the description of *Phaeobotrys solitaria* Ettl 1966:

Zoosporae ovatae vel sphaericae, 5–8 μm longae, chloroplastis binis disciformibus, membrane adpressis, stigmate rubro et duobis flagellis inaequalibus anteriore emergentis.

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