Ettliella tetraspora (Chlorophyta, Chlorophyceae): life cycle and taxonomy

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Abstract: The morphology and mode of reproduction in *Ettiella tetraspora* Hindák 1988, a green coenobial alga described from the Czech Republic and Finland, were studied from the plankton of the water reservoir Förmitzspeicher in NE Bavaria, Germany. The morphology of cells and coenobia of the species in this population was in agreement with published data for the species, but its reproduction was not of the *Oocystis* – type as it has been declared in the relevant literature. The protoplast of mother cells does not divide simultaneously into 4 autospores as in many coccal green algae, but always in two autospores. The first division of the mother cell is perpendicular to the longitudinal axis of the cell and two walled daughter cells arise. Subsequently, the newly formed daughter cells divide again, but this time perpendicular to the new formed cell cross wall. Ultimately, in a new coenobium all four daughter cells are arranged in parallel. Daughter cells remain inside a slightly enlarged mother cell wall (well visible only near the cross wall) and are released by its gelatinisation. Such subsequent production of four autospores from the mother cell represents a special type of multiplication in the green coccal algae known also in some other species [e.g. *Tetrastrum komarekii* Hindák 1977*, Willea irregularis* (Wille) Schmidle 1900, *Makinoella tosaensis* Okada 1949].

Key words: autospores subsequent division, coenobial green algae, taxonomy, Oocystaceae, Scenedesmaceae, Germany

Introduction

During a workshop devoted to the determination of planktic cyanophytes and chlorophytes of lakes and reservoirs organised by the Bavarian Regional Office for the Environment (Bayerisches Landesamt für Umwelt) in Wielenbach, Germany, in July 2010, samples from several local water bodies were analysed. In the phytoplankton sample collected from the water reservoir Förmitzspeicher a rare coccal green alga, *Ettliella tetraspora*, was observed.

The monotypic genus *Ettliella* Hindák 1988 including *E. tetraspora* Hindák 1988 was described on the basis of two observations. Ettl (1965) was the first to find the alga in a fishpond near Svitavy in Moravia, the Czech Republic, but designated it as *Tetrachlorella* cf. *alternans* Korshikov (?nov. var.) (cf. also Komárek & Fott 1983; Hindák 1988). Later, Hindák (1988) observed it in a lake near Jyväskylä, Central Finland. The morphology of cells and colonies and the formation of autospores were similar in material from both localities. Ettl was of the opinion that the mother cell wall expands during autosporulation and therefore he assigned the alga to the family Oocystaceae. When Hindák (1988) established this alga as a new genus and species, *Ettliella tetraspora*, he followed this classification, although the release of autospores and remnants of mother cell walls were not observed in detail. It was noticed, however, that the regular *Tetradesmus* – like clustering of cells of *Ettliella* is reminiscent of some representatives of the family Coelastraceae or Scenedesmaceae.

In one phytoplankton sample from Bavaria, coenobia of *Ettliella tetraspora* occurred quite abundantly, thereby offering an opportunity to study the process of autosporulation in all its stages. These observations led to the conclusion that the mother cell wall does not expand before the liberation of autospores as in the Oocystaceae. Probably Ettl (1965) did not depict expanded mother cell walls around daughter coenobia, but mucilaginous envelopes newly formed around autocoenobia.

Material and Methods

Plankton samples were collected from the water

reservoir Förmitzspeicher (Förmitzsee) near Schwarzenbach a.d. Saale, NE Bavaria, Germany, pH 7.9, July 5, 2010, by Mrs C. Hauenschild, Hof, using a plankton net with 10 µm mesh size (Fig. 1).

Cyanophytes and algae were determined according to KORSHIKOV (1953), HINDÁK (1977, 1980, 1984, 1988, 1990, 2008), KOMÁREK & FOTT (1983), KRAMMER & LANGE–BERTALOT (1986, 1988, 1991a, b), KOMÁREK & ANAGNOSTIDIS (1998, 2005), KOMÁREK & ZAPOMNĚLOVÁ (2007), LENZENWEGER (1999) and RAJANIEMI et al. (2005). Identifications were performed on living material. Light microscope observations were carried out with a Leitz Diaplan microscope, and photomicrographs were taken with a Wild Photoautomat MPS45. Preserved material in formaldehyde is stored at the Institute of Botany SAS, Bratislava. Attempts to cultivate this organism under laboratory conditions were unsuccessful.

Results

***Ettliella tetraspora* HINDÁK (Figs 2–9)**

Coenobia free floating, solitary, usually 4–8(16)–celled, ovoid to spherical, 40–60 µm in diameter, or cells solitary before reproduction, embedded by wide homogeneous hyaline mucilaginous envelopes. Cells in coenobia clustered into tetrads as in *Tetradesmus* G.M. SMITH, i.e. positioned (but not joined) in parallel next to each other forming a square when viewed from above, with a central opening (Figs 2–4). Cells broadly ovoid or short cylindrical, before division slightly asymmetrical to kidney–shaped, 8–12(18) × 6–8(10) µm, ends broadly oval. Cell walls thick, smooth, without incrustations or conspicuous polar thickenings. Chloroplasts single, bright green, parietal, transversely pot–shaped, with a large central starch–associated pyrenoid, and in cells in tetrads oriented toward the external side. Reproduction by autocoenobia with 4 autospores. Coenobia before autosporulation mostly disintegrating into solitary cells, losing their tetrahedrical arrangement of cells (Figs 5–8). When autospores are formed, colonies arise temporarily with tetrads of young cells, so that composite colonies exhibit cell tetrads of a similar clustering (Figs 7–9).

Although the product of autosporulation is one 4

Fig. 1. Locality where *Ettiella tetraspora* was collected: the water reservoir Förmitzspeicher in NE Bavaria, Germany (photo C. Hauenschild, summer 2010).

Table 1. List of cyanophytes and algae found in the phytoplankton of the water reservoir Förmitzspeicher, Germany.

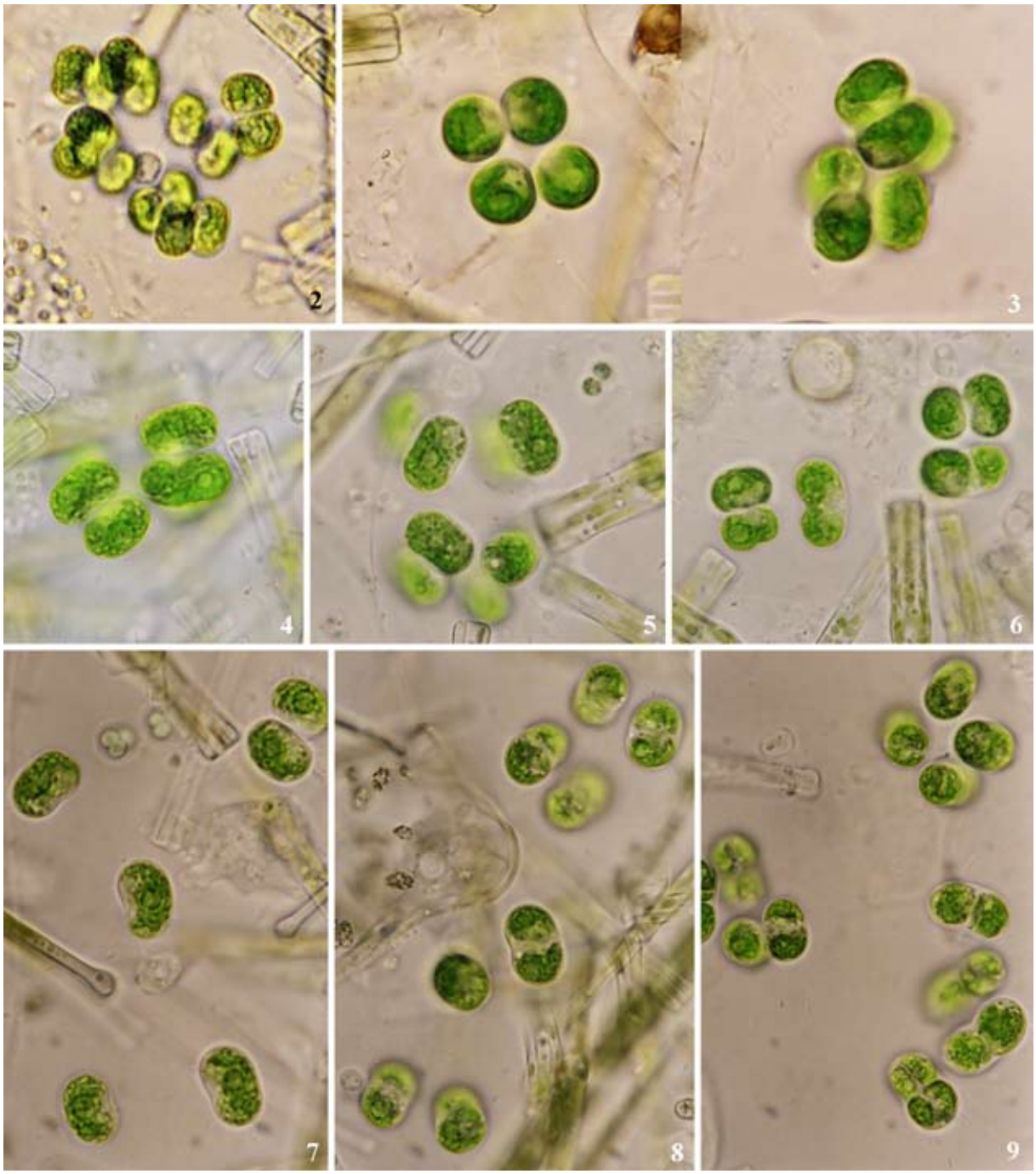
Cyanophyceae
Aphanocapsa delicatissima W. et G.S. West
Aphanocapsa planctonica (G.M. Smith) Komárek et Anagnostidis
Aphanothece clathrata W. et G.S. West
Aphanothece nidulans Richter in Wittrock et Nordstedt
Chroococcus limneticus Lemmermann
Chroococcus minutus (Kützing) Nägeli
Eucapsis minor (Skuja) Elenkin
Merismopedia glauca (Ehrenberg) Kützing
Merismopedia minima Beck
Microcystis aeruginosa (Kützing) Kützing
Microcystis flos–aquae (Wittrock) Kirchner
Snowella litoralis (Häyrén) Komárek et Hindák
Synechococcus endophyticus (W. et G.S. West) Joosten
Woronichinia naegeliana (Unger) Elenkin
Oscillatoria limosa Agardh ex Gomont
Pseudanabaena catenata Lauterborn
Pseudanabaena mucicola (Naumann et Huber–Pestalozzi) Schwabe
Anabaena mucosa Komárková–Legnerová et Eloranta
Anabaena planctonica Brunnthaler

Bacillariophyceae
Aulacoseira ambigua (Grunow) Simonsen
Cyclotella balatonis Pantoczek
Discostella pseudostelligera (Hustedt) Houk et Klee
Melosira varians C. Agardh
Stephanodiscus medius Håkansson
Achnanthes laevis Oestrup
Achnanthes lanceolata ssp. *frequentissima* Lange–Bertalot
Achnanthes minutissima Kützing
Achnanthes cf. *petersenii* Hustedt
Achnanthes cf. *saccula* J. R. Carter
Caloneis silicula (Ehrenberg) Cleve
Cymbella gracilis (Ehrenberg) Kützing
Cymbella silesiaca Bleisch
Eunotia bilunaris (Ehrenberg) Mills
Eunotia praerupta var. *bidens* (Ehrenberg) Grunow
Fragilaria crotonensis Kitton
Fragilaria tenera (W. Smith) Lange–Bertalot
Fragilaria ulna var. *acus* (Kützing) Lange–Bertalot
Frustulia vulgaris (Thwaites) De Toni
Gomphonema angustatum (Kützing) Rabenhorst
Gomphonema parvulum (Kützing) Kützing
Navicula capitata Ehrenberg
Navicula capitatoradiata H. Germain
Navicula cryptocephala Kützing
Navicula cryptotenella Lange–Bertalot
Navicula detenta Hustedt
Navicula rhynchocephala Kützing
Nitzschia angustiforaminata Lange–Bertalot
Nitzschia fonticola Grunow
Nitzschia hantzschiana Rabenhorst
Nitzschia cf. *liebetruthii* Rabenhorst
Nitzschia palea (Kützing) W. Smith
Nitzschia valdestriata Aleem et Hustedt
Pinnularia divergens var. *media* Krammer
Tabellaria flocculosa (Roth) Kützing

Chlorophyceae
Carteria globosa Korshikov ex Pascher
Chlamydomonas passiva Skuja
Eudorina elegans Ehrenberg
Asterococcus limneticus G.M. Smith
Ankistrodesmus falcatus (Corda) Ralfs
Ankistrodesmus spiralis (W.B. Turner) Lemmermann
Botryococcus braunii Kützing
Closteriopsis acicularis (G.M. Smith) J.H. Belcher et Swale
Coelastrum pseudomicroporum Korshikov
Coenochloris polycoccus (Korshikov) Hindák
Coenocystis planctonica Korshikov
Crucigeniella apiculata (Lemmermann) Komárek
Desmodesmus abundans (Kirchner) E.H. Hegewald
Desmodesmus armatus (Chodat) E.H. Hegewald
Desmodesmus brasiliensis (Bohlin) E.H. Hegewald
Desmodesmus communis (E.H. Hegewald) E.H. Hegewald
Desmodesmus dispar (Brébisson) E.H. Hegewald
Desmodesmus maximus (W. et G.S. West) E.H. Hegewald
Desmodesmus serratus (Corda) An, Friedl et E.H. Hegewald
Desmodesmus subspicatus (Chodat) E.H. Hegewald et A. Schmidt
Dictyosphaerium tetrachotomum Printz
Ettliella tetraspora Hindák
Komarekia appendiculata (Chodat) Fott
Monoraphidium griffithii (Berkeley) Komárková–Legnerová
Oocystella borgei (J. Snow) Hindák
Oocystella lacustris (Chodat) Hindák
Oocystella marssonii (Lemmermann) Hindák
Pediastrum biradiatum Meyen
Pediastrum boryanum (Turpin) Meneghini
Pediastrum duplex Meyen
Pediastrum tetras (Ehrenberg) Ralfs
Pseudodictyosphaerium minusculum Hindák
Pseudodidymocystis inconspicua (Korshikov) Hindák
Pseudokirchneriella contorta (Schmidle) Hindák
Pseudokirchneriella irregularis (G.M. Smith) Hindák
Scenedesmus acuminatus (Lagerheim) Chodat
Scenedesmus arcuatus (Lemmermann) Lemmermann
Scenedesmus obliquus (Turpin) Kützing
Scenedesmus obtusus Meyen
Selenastrum bibraianum Reinsch
Tetraedron caudatum (Corda) Hansgirg
Willea irregularis Schmidle

Zygnematophyceae
Cosmarium circulare Reinsch
Cosmarium hornavanense Gutwinski
Staurastrum cuspidatum Brébisson
Staurastrum mucronatum Ralfs ex Ralfs
Staurastrum planctonicum Teiling
Staurastrum punctulatum Brébisson

Euglenophyceae
Trachelomonas planctonica Swirenko



Figs 2–9. Coenobia of *Ettiella tetraspora* from natural material: (2) coenobia at low magnification; (3) coenobia from the top and lateral view; (4) coenobia from the lateral view; (5, 6) disintegration of coenobia; (7 – 9) division of cells into two daughter cells.

–celled coenobium, the protoplast of mother cells and subsequently daughter cells divides always into two steps. First division of the mother cell is perpendicular to the longitudinal axis of the cell and two walled daughter cells arise (Figs 7, 8). Subsequently, new daughter cells divide again, but perpendicular to the new cell wall. Daughter cells remain in a slightly enlarged mother cell wall near the cross wall and are released by its gelatinisation (Fig. 9). Finally, in a new coenobium all four daughter cells are arranged in parallel (Fig. 3).

Our material does not differ significantly from published descriptions of the species except for the size of cells and coenobia, which are larger than mentioned by Ettl (1965) or Hindák (1988). In contrast to a previous observation of the alga from Finland, 2–celled coenobia were not found in the German material.

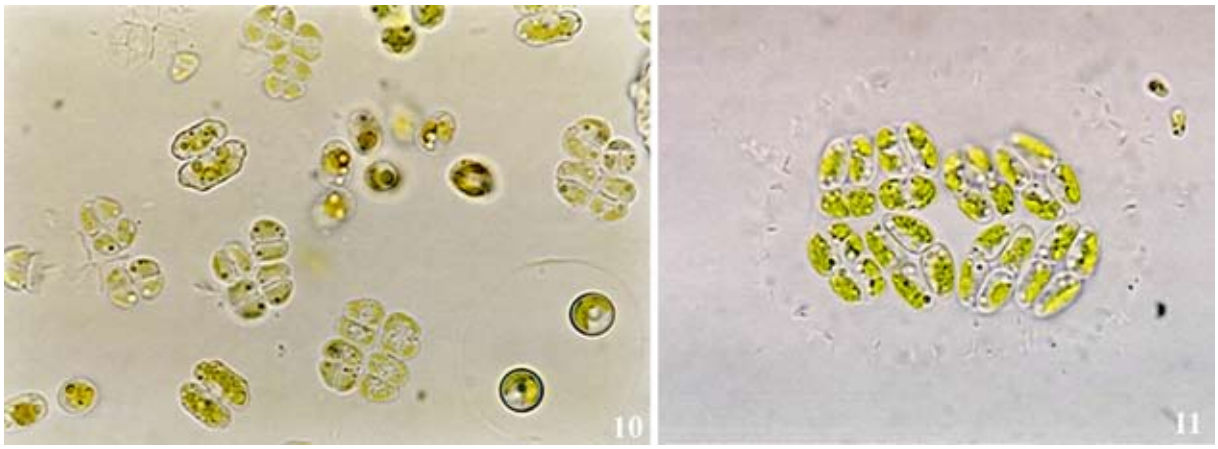


Fig. 10. *Tetrastrum komarekii*, formation of 4–celled coenobia. Fig. 11. *Willea irregularis*, formation of 4–celled coenobia.

Coenobia of *E. tetraspora* occurred quite abundantly among other members of the phytoplankton assemblage of the water reservoir Förmitzspeicher. The cyanophycean water bloom consisted mainly of colonies of *Woronichinia naegeliana* (Unger) Elenkin with endogloeic *Synechococcus endophyticus* (W. et G.S. West) Joosten (Hindák 2008), *Microcystis aeruginosa* (Kützing) Kützing and filaments of *Anabaena mucosa* Komárková–Legnerová et Eloranta (Komárek & Anagnostidis 1998; Rajaniemi et al. 2005; Komárek & Zapomnělová 2007). The diatoms *Fragilaria crotonensis* Kitton and *F. tenera* (W. Smith) Lange–Bertalot were amongst the dominant phytoplankton species. In the following list of found taxa we used the classification system by Hindák & Hindáková (1998), which has been adopted for the algal flora databank at our institute.

The occurrence of *Ettliella tetraspora* in the plankton of stagnant mesotrophic water basins seems to be typical for the species. In the Czech Republic it was found in a fishpond near Svitavy (Ettl 1965), and we have reported this species from the lake Patalakti near Jyväskylä in Finland (Hindák 1988). However, Stoyneva (2000) recorded *E. tetraspora* from the Alepou swamp, a wetland on Southern Black Sea coast, Bulgaria, but did not provide a description or figure.

Discussion

The taxonomic position of *Ettliella tetraspora* [as *Tetrachlorella* cf. *alternans* Korshikov (?nov. var.)] in the Chlorococcales was originally determined by Ettl, who placed it in the family Oocystaceae, but Komárek & Fott (1983) classified the genus *Tetrachlorella* Korshikov (together with *T.* cf. *alternans* nov. var. (?) sensu Ettl) in the family Scenedesmaceae. However, the latter authors noted that the parallel arrangement of cells in a coenobium precludes the classification of this alga in the genus *Tetrachlorella* Korshikov 1953 which is characterised by flat coenobia, and suggested that it might be considered as a new species and genus. Hindák (1988) on the basis of material from a Finnish lake accepted this opinion and established *Ettliella tetraspora* as a new member of the family Oocystaceae, although the release of autospores and remnants of mother cell walls were not observed in detail.

Successive cell division of mother cells into 4 autospores is character that is found also in other coccal green algae, e.g. *Tetrastrum komarekii* Hindák (Fig. 10), *Willea irregularis* (Wille) Schmidle (Fig. 11) (Korshikov 1953; Hindák 1977, 1980, 1988; Komárek & Fott 1983) and *Makinoella tosaensis* Okada (Hindák & Hindáková 2010). Although the final product of autosporulation in these genera is a 4–celled coenobium, mother cells and subsequently daughter cells do not divide simultaneously into 4 autospores, but always into two parts/autospores, not in four or more parts/autospores like in many coccal green algae. Similarly, the chloroplast divides before the protoplast division only in two portions, not in four.

The placement of the genus *Ettliella* into a higher taxonomic unit is not yet clear. The mode of reproduction, together with the feature that mother cell wall does not significantly enlarge before the release of autospores, precludes the placement of *Ettliella* in the family Oocystaceae. The method of

coenobium formation is suggestive of a taxonomic affinity with the family Scenedesmaceae sensu Komárek & Fott (1983), particularly the subfamily Crucigeniodeae and the genus *Willea* Schmidle. However, there are some arguments to let *Ettliella tetraspora* placed in the Oocystaceae. Members of the other genera with similar propagation pattern as *Ettliella* like *Makinoella* and *Tetrachlorella* are already molecular–phylogenetic characterized and confirmed members of the Oocystaceae (Pažoutová et al. 2010). It was also shown that several similar in mode of reproduction crucigenoid green algae such as *Crucigeniella* belong to Oocystaceae (Krienitz et al. 2003). A final taxonomical decision on *Ettliella* must await the results of an ultrastructural investigation of the cell wall and molecular taxonomic studies.

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