Cysts and their formation in some neustonic *Euglena* species

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Morphology, ultrastructure and formation of resting cysts of *E. agilis* H.J. Carter and *E. anabaena* Mainx are described. The SEM micrographs of cysts of *E. anabaena* and *E. agilis* are presented for the first time. Information on the neuston film formed by these species is given. An ecological database and list of accompanying species is also included.

Les kystes chez quelques espèces neustoniques du genre *Euglena*

Mots-clés : *Euglena agilis*, *E. anabaena*, *E. viridis*, *Euglena*-neuston, kystes, structure, MEB, Slovaquie.

La morphologie, l'ultrastructure et la formation des kystes chez *Euglena agilis* H.J. Carter et *E. anabaena* Mainx sont décrits. Des photographies de ces deux espèces, réalisées au microscope électronique à balayage (MEB), sont présentées pour la première fois. Des informations sur le film neustonique constitué par ces deux espèces sont données. Des précisions sur leur écologie ainsi que la liste des espèces accompagnatrices sont également fournies.

1. Introduction

Many representatives of the genus *Euglena* Ehrenb. are able to transform their bodies from characteristic unicellular motile stages into non-motile mucilaginous colonies. Euglenoid monads during the encystation change their original shape into ovoid, oval up to spherical forms, losing flagella. Subpellicular mucus bodies eject mucilage (which consists of polysaccharide and/or glycoproteins) that forms layers around the *Euglena* cell (Cogburn & Schiff 1984). Mucilaginous envelopes (cysts) are usually conspicuous, spherical, flask-shaped (*E. tuba* H.J. Carter), or pentagonal (*Distigma* Ehrenb.). In the phycological literature (e.g. Skuja 1948, Gojdics 1953, Huber-Pestalozzi 1955, Starmach 1983) these mucilaginous colonies are usual-

ly called palmelloid or gloeocystis stages. Three basic types of cyst are formed by *Euglena* species : (i) protective — generally have a heavy, sometimes stratified, wall and containing one cell ; (ii) reproductive (palmella formation) — have a thin, elastic, permeable membrane that increases in diameter as the cells divide, palmella formation occurs among large number of cells (Gojdics 1953), the cells are nonflagellated ; (iii) temporary, resting, or transitory cysts — have a wall that is impervious to water, but which contains a small pore.

Some species of *Euglena* may form more than one of the cysts during the life cycle (Buetow 1968). According to Jahn 1951 (after Buetow 1968), *E. gracilis* G.A. Klebs can form a reproductive cyst or a temporary cyst. The cells embedded in mucilaginous colonies are able to divide and move slightly in their own mucilage capsule changing position of the cell; and also reform the flagella and leave the mucilage. Palmella stage occurs among large numbers of cells (Gojdics 1953). Such colonies are free-floating in the plankton or fall into the metaphyton/benthos. In *E. gracilis* and *E. viridis* Ehrenb. the cyst may contain 32-64 cells (Jahn, after Buetow 1968)).

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Several *Euglena* species create a neuston film — a red neustonic film is caused by *E. sanguinea* Ehrenb., a green one by *E. polymorpha* P.A. Dang., *E. granulata* (G.A. Klebs) Schmitz, *E. agilis* H.J. Carter, *E. viridis*, *E. anabaena* Mainx — (see cited monographs). A unique neustonic bloom of *E. granulata* var. *polymorpha* (P.A. Dang.) T.G. Popova (= *E. polymorpha*) was observed in an experimental fishpond at Laskowa near Zator, S Poland, by Bednarz (1974). On the surface of the water a pectin-like hyaline net was formed, in the mesh of which moved *Euglena* cells with flagella always directed downwards the water (Figs. 1-4).

Another type of resting cysts has been observed in *Euglena agilis* (= *E. pisciformis* G.A. Klebs) by the first author of this article (Hindák 1982, 1986). Motile cells of *Euglena* entered a colonial mucilage of planktic species of the genus *Microcystis* [*M. aeruginosa* (Kütz.) Kütz., *M. minutissima* W. West] and *Anabaenopsis elenkinii* Miller, rarely also of other cyanophytes, changing into oval up to spherical bodies and producing a brownish to dark brown envelope layer around. Because of some similarities in the shape and colour of these envelopes with loricae of the genus *Trachelomonas* Ehrenb., the endogloeic cysts were called *Trachelomonas*-like resting cysts (Figs. 5, 6). These cysts did not exhibit any conspicuous tendency to wait out the unfavourable life conditions, as is characteristic of other types of resting stages, since the protoplast of cysts divided after a definite, sometimes relatively short time, and the daughter protoplasts were able to emerge from the *Trachelomonas*-like brown loricae by an intensive changing cell motion and, sometimes, also with the help of a long flagellum, and move. The new daughter protoplasts then remain either in the same host colony, or leave it, moving freely in the water and/or passing into the mucilage of another host colony. The wall of *Trachelomonas*-like cysts was irregularly granulated, but no regular structure and no opening for the flagellum was observed as in the genus *Trachelomonas* (Fig. 5). In contrast to the mentioned palmelloid stages in *Euglena*, these cysts occurred in cyanophyte colonies mostly solitary or, in small groups, never forming large colonies.

In recent years, similar cysts with a brown *Trachelomonas*-like envelope were found in neuston films formed by *E. agilis*, *E. anabaena* and *E. viridis*. However, in *E. agilis* these neustonic cysts (Figs. 7-9, 23-27) differed from those found in planktonic cyanophyte colonies (Figs. 5, 6), both, in the envelope structure and the ecology. In this article the formation of resting cysts of the mentioned two species and morphology of cysts

under the light and electron microscopy are described and discussed.

2. Study area, material and methods

Euglena neuston bloom was studied from two places, both located within the city limits of Bratislava. One locality is a small forest fishpond in Zelezná Studienka, situated ca 2 km northwest of the State sanatorium, while the other one a big garden flower-pot with accumulated rainwater placed in the park of the Slovak National Uprising Square.

The investigated forest fishpond is about 0.5 km², and has a slightly prolonged rectangular shape, max. depth about 2 m, pH 7.6-7.8, temperature 18-21°C (in the second half of July 1994). The macroscopic neuston bloom was observed only in July and August 1994. The fishpond was discharged in the previous autumn and in the late spring and summer 1994 refilled but only to half the usual water level. Because of favourable conditions, i.e. a relatively hot summer that year, low water level — and thus partly protected strong wind — the neuston film developed enormously on the surface of the fishpond, at first sight resembling a cyanophyte water bloom (Fig. 22). The neuston bloom covered the water surface in the form of a fine film, but when the neuston was blown toward the shore it became up to several mm thick and deep green in colour. The neuston film took about a third of the water surface of the fishpond, with the maximum near the shore and outlet. Under unfavourable conditions, e.g. by strong wind or heavy rain, the neuston film was mixed with the plankton water and *Euglena*-cells left the mucilage capsules, transformed into motile stages with flagella and swam in the water. However, under favourable conditions the neuston film formed again. During neustonic bloom also the water of the fishpond itself was green because of the dominance of motile cells of *Euglena anabaena* and *E. agilis* in the planktonic community. The neuston bloom disappeared in autumn and since then has never again been formed in this locality.

The second place where an euglenoid neuston was observed is from ecological point of view quite different from the previous one. In a small park in the Slovak National Uprising Square, in a garden flower-pot about 50x50x70 cm in size (at the time the pot was without flowers but still contained garden soil), rainwater had accumulated. On the surface of the pot water layer a conspicuous green neuston film developed at the end of September 1995.

For LM observations a Diaplan Leica microscope was used, and for SEM studies the REM JEOL JSM-

Figs. 1-19. 1, 2 : *Euglena granulata* var. *polymorpha*, free swimming cells ; 3 : network with empty meshes and those with cells ; 4 : scheme of distribution of the *Euglena* cells in the network (from Bednarz 1974) ; 5-9, 14-19 : cysts of *E. agilis* : 5, 6 : protective cysts living in the mucilage of *Microcystis minutissima* (from Hindák 1982, 1986) ; 7-9 : resting cysts ; 14-19 : free swimming cells (after Hindák 1986) ; 10-13 : *E. anabaena* : 10, 13 : protective cysts, 11, 12 : free swimming cells. Scales = 10 μ m.

Figs. 1-19. 1, 2 : *Euglena granulata* var. *polymorpha*, cellules libres ; 3 : vue du réseau visible à la surface de l'eau avec kystes encore présents et enveloppes vides (d'après Bednarz 1974) ; 4 : schéma de la distribution des cellules d'*Euglena* dans le réseau (d'après Bednarz 1974) ; 5-9, 14-19 : kystes de *Euglena agilis* : 5, 6 : kystes vivants à l'intérieur du mucilage de *Microcystis minutissima* (d'après Hindák 1982, 1986) ; 7-9 : kystes en phase de repos ; 14-19 : cellules libres (d'après Hindák 1986) ; 10-13 : *E. anabaena* : 10, 13 : kystes, 11, 12 : cellules libres. Echelles = 10 μ m.

6300. Material from the fishpond was fixed with glutaraldehyde, dehydrated with acetone or alcohol series, critical point dried and sputtered with gold.

3. Results and discussion

3.1. Neuston of the forest fishpond in Bratislava—Železná Studienka

3.1.1. Dominant species of the neuston film

As already mentioned, the neuston film of the forest fishpond consisted predominantly of resting cysts of *Euglena anabaena* (Figs 11, 12, 20, 21, 28) and *E. agilis* (Figs 14-19, 38). Under favourable climatic conditions, the motile cells of both species, abundantly occurring in the plankton of the fishpond that summer, moved upward to the water surface, attached to the water film, and transformed into resting cysts. From the beginning of the neuston development, the film resembled a hyaline honeycomb or a network, i.e. the motile cells were fixed to the water film and joined by the mucilage very tightly, so that the neighbouring cells created a one-layered compact or perforated film (Figs. 7, 13, 22). The motile cells were fixed to the surface water film by their antapical part, having their apical part with the flagellum being submerged in the water (Figs. 23, 24, 27). The attached cells gradually produced irregular mucilage layers, and created a special envelope directly round their bodies. The envelopes were at the beginning hyaline and thin, but later on thicker and brownish.

The neustonic network was mainly formed by the cysts of *E. anabaena* (Figs. 10, 13, 28-31) in which vegetative cells were conspicuously larger than those of *E. agilis* (Figs. 38-41). The cysts of the latter species usually filled empty gaps between those of the former one. Owing to strong wind and other forms of mechanical movement of the water surface (and also during the transportation of the samples of the neuston film into the laboratory), the *Euglena* cells in the neuston were able to move from it very quickly, leave the film layer, and swim again in free water by means of flagella (Figs. 11, 12, 14-19, 20, 21, 28, 38).

— Morphology of cysts of *Euglena anabaena*

The cysts of *E. anabaena* (Fig. 31) were from the top view, circular to irregularly circular or broadly oval, from the side view, oval and slightly flattened (Figs. 29, 30). Developed cysts were completely covered by an envelope (Figs. 38-41). The surface of envelopes was without any regular structures, mostly smooth or finely rough and porous. Some envelopes were allusively concentrically layered or spirally twisted (as a

fine replica of a spiral construction of *Euglena* cells), only rarely with a central porus in the top (Figs. 36, 37). The envelope was composed of fine fibrils (Fig. 35). The upper part of the envelopes (obviously being in contact with the open air) was thicker than the submerged lower part, thus forming a cap as is known in other neustonic microorganisms (Pascher 1942, Korshikov 1953).

— Morphology of cysts of *Euglena agilis*

Morphology of cysts of *Euglena agilis* (Figs. 38, 39) exhibited many features of those of *E. anabaena*, namely in origin, structure and composition. However, there were some differences (see Hindák 1982, 1986). The cysts were protective with a heavy, sometimes stratified wall (Figs. 40, 41).

3.1.2. Accompanying species of the neuston film

Apart from strongly dominant species of *Euglena anabaena* and *E. agilis*, other cyanophyte and algal species were found as a part or an admixture of the neuston film. This is only to enumerate accompanying species, because they will be treated in detail elsewhere. From Cyanophyta it was mainly *Trichormus* sp., while other species, such as *Merismopedia tenuissima*, *Lyngbya* sp. occurred only sporadically. From other groups of algae were present *Heterothrix ulotrichoides* Pascher (Xanthophyceae), *Amphora libyca* Ehrenb., *Nitzschia palea* (Kütz.) W. Sm., *Navicula cryptotenella* Lange-Bert., *N. pupula* Kütz. (Bacillariophyceae), but mainly green flagellates [*Chlamydomonas petasus* H. Ettl, *C. mantonii* (H. Ettl) H. Ettl, *C. epibiotica* H. Ettl, *C. spinifera* H. Ettl, *Carteria asterochloris* H. Ettl, *Diplostauron angulosum* Korshikov, *Pedinomonas minor* Korshikov] and chlorococcal algae (*Nautococcus mammilatus* Korshikov, *Ferricystis mucicola* Hindák). Taxa published from the territory of Slovakia for the first time are marked with asterisk (*) (Hindák & Hindáková 1998).

List of taxa found in the neuston film :

Cyanophyta

Lyngbya sp., *Merismopedia tenuissima* Lemmerm., *Trichormus* sp.

Xanthophyceae

**Heterothrix ulotrichoides* Pascher

Bacillariophyceae

Amphora libyca Ehrenb., *Navicula cryptotenella* Lange-Bert., *N. pupula* Kütz., *Nitzschia palea* (Kütz.) W. Sm.

Figs. 20-27. 20, 21 : *Euglena anabaena* ; 22 : neustonic network with cysts of *E. anabaena* and *E. agilis* ; 23-27 : cysts of *E. agilis*.
Scales = 10 μ m.

Figs. 20-27. 20, 21 : *Euglena anabaena* ; 22 : film neustonique avec kystes de *E. anabaena* et *E. agilis* ; 23-27 : kystes de *E. agilis*.
Echelles = 10 μ m.

Figs. 28-31. 28 : *Euglena anabaena*, free swimming cell ; 29, 30 : cysts with clearly visible flagellum ; 31 : group of resting cysts. SEM. Scales = 10 μ m.

Figs. 28-31. 28 MEB : *Euglena anabaena*, cellules libres ; 29, 30 : kystes avec flagelle bien visible ; 31 : groupe de kystes. Echelles : 10 μ m.

Figs. 32-35. *Euglena anabaena* : 32-34 : resting cysts ; 35 : fragment of protective cyst. SEM. Scales = 1 μ m.

Figs. 32-35. MEB : *Euglena anabaena* : 32-34 : kystes ; 35 : fragment de kyste. Echelles = 1 μ m.

Figs. 36-37. Envelope as fine replica of spirally striated *Euglena* cells. SEM. Scales = 1 µm.

Figs. 36-37. MEB : Enveloppes de kystes apparaissant comme un replicat de stries hélicoïdales de la cuticule des cellules de *Euglena*. Echelles = 1 µm.

Chlorophyceae

Volvocales : *Carteria asterochloris* H. Ettl, *Chlamydomonas epibiotica* H. Ettl, **C. mantonii* (H. Ettl) H. Ettl, **C. petasus* H. Ettl, *C. spinifera* H. Ettl, *Diplostauron angulosum* Korshikov, *Pedinomonas minor* Korshikov

Chlorococcales : *Ferricystis mucicola* Hindák, **Nautococcus mammilatus* Korshikov

Euglenophyceae

Euglena anabaena Mainx, *E. agilis* H.J. Carter

3.1 3. Phytoplankton species composition of the forest fishpond during the neustonic bloom

The plankton of the forest fishpond was dominated by two species of the genus *Euglena* : *E. agilis* and *E. anabaena* which also dominated in the neuston film. Other cyanophytes and groups of algae (except diatoms) occurred relatively in small number of species and quantity.

List of taxa found in the plankton :

Cyanophyta

Merismopedia tenuissima Lemmerm., *Microcystis wesenbergii* (Komárek) Komárek, *Snowella lacustris* (Chodat) Komárek et Hindák

Chrysophyceae

Dinobryon divergens O.E. Imhof

Bacillariophyceae

Aulacoseira granulata (Ehrenb.) Simonsen, *Cyclotephanos dubius* (Fricke) Round, *Cyclotella atomus* Hust., *C. meneghiniana* Kütz., *C. pseudostelligera* Hust., *C. quadrijuncta* (Schröt.) Keissl., *C. stelligera* Cleve et Grunow, *Stephanodiscus binderanus* (Kütz.) Willi Krieg., *S. hantzschii* Grunow, *Thallasiosira pseudonana* Hasle et Heimdal, *Achnanthes lanceolata* (Bréb. ex Kütz.) Grunow, *A. minutissima* Kütz., *A. subatomoides* (Hust.) Lange-Bert., *Amphora lybica* Ehrenb., *A. montana* Krasske, *A. pediculus* (Kütz.) Grunow, *Asterionella formosa* Hassal, *Caloneis bacillum* (Grunow) Cleve, *C. silicula* (Ehrenb.) Cleve, *Cy-*

Figs. 38-41. *Euglena agilis* : 38 : free swimming cell ; 39 : palmella stage of *E. agilis* (small cell arrow) and large one of *E. anabaena* ; 40, 41 : protective cysts. SEM. Scales = 10 μ m (38), 1 μ m (39-41).

Figs. 38-41. MEB : *Euglena agilis* : 38 : cellule libre ; 39 : stade palmella de *E. agilis* (la petite cellule, flèche) et de *E. anabaena* ; 40, 41 : kystes. Echelles = 10 μ m (38), 1 μ m (39-41).

matopleura solea (Bréb.) W. Sm., *Cymbella cistula* (Ehrenb.) Kirchn., *C. microcephala* Grunow, *C. minuta* Hilse, *C. sinuata* W. Greg., *Diploneis oculata* (Bréb.) Cleve, *Fragilaria crotonensis* Kitton, *F. parasitica* var. *subconstricta* Grunow, *F. ulna* var. *ulna* (Nitzsch) Lange-Bert., *F. ulna* var. *acus* (Kütz.) Lange-Bert., *Frustulia vulgaris* (Thwaites) de Toni, *Gomphonema augur* var. *turris* (Ehrenb.) Lange-Bert., *G. parvulum* (Kütz.) Kütz., *G. sarcophagus* W. Greg., *G. truncatum* Ehrenb., *Gyrosigma acuminatum* (Kütz.) Rabenh., *G. attenuatum* (Kütz.) Rabenh., *Meridion circulare* (Grev.) C. Agardh, *Navicula accomoda* Hust., *N. capitata* Ehrenb., *N. capitatoradiata* H. Germ., *N. cryptotenella* Lange-Bert., *N. elginensis* (W. Greg.) Ralfs, *N. gregaria* Donkin, *N. libonensis* Schoeman, **N. medioconvexa* Hust., *N. menisculus* Schum., *N. phyllepta* Kütz., *N. pupula* Kütz., *N. pygmaea* Kütz., *N. subminuscule* Manguin, *N. tripunctata* (O.F. Müll.) Bory, *N. trivialis* Lange-Bert., *N. veneta* Kütz., *Neidium ampliatus* (Ehrenb.) Krammer, *Nitzschia acicularis* (Kütz.) W. Sm., *N. acidoclinata* Lange-Bert., *N. amphibia* Grunow, *N. capitellata* Hust., *N. fonticola* Grunow, *N. hungarica* Grunow, *N. inconspicua* Grunow, *N. intermedia* Hantzsch, *N. levidensis* (W. Sm.) Grunow, *N. palea* (Kütz.) W. Sm., *N. pusilla* Grunow, *N. recta* Hantzsch, *N. sigmoidea* (Nitzsch) W. Sm., *N. subacicularis* (Kütz.) Hantzsch, **N. supralitorea* Lange-Bert., *N. tubicola* Grunow, *Pinnularia gibba* Ehrenb., *P. neomajor* Krammer, *P. obscura* Krasske, *P. subcapitata* W. Greg., *P. viridis* (Nitzsch) Ehrenb., *Rhoicosphenia abbreviata* (C. Agardh) Lange-Bert., *Stauroneis legumen* (Ehrenb.) Kütz., *Surirella brebissonii* var. *brebissonii* Krammer et Lange-Bert., *S. brebissonii* var. *kuetzingii* Krammer et Lange-Bert., *S. gracilis* Grunow, *S. linearis* W. Sm., *S. minuta* Bréb., *S. splendida* (Ehrenb.) Kütz., *S. visurgis* Hust.

Dinophyceae

Peridinium cinctum (O.F. Müll.) Ehrenb.

Chlorophyta

Volvocales : *Carteria asterochloris* H. Ettl, *Chlamydomonas aepibiotica* H. Ettl, **C. mantonii* (H. Ettl) H. Ettl, **C. petasus* H. Ettl, *C. spinifera* H. Ettl, *Chlamydomonas* spp., *Chlorogonium acutiforme* Bourr., *Diplostauron angulosum* Korshikov, *Pedinomonas minor* Korshikov, *Phacotus lenticularis* (Ehrenb.) F. Stein

Chlorococcales : *Actinastrum hantzschii* Lagerh., *Coelastrum astroideum* De Not., *C. cambricum* Arch., *Desmatractum delicatissimum* Korshikov, *Golenkinia radiata* Chodat, *Kirchneriella lunaris* (Kirchn.)

Schmidle, *Micractinium pusillum* Fresen., *Monoraphidium contortum* (Thur.) Komárk.-Legn., *Pediastrum boryanum* (Turpin) Menegh., *Oocystella parva* (W. et G.S. West) Hindák, *Polyedriopsis spinulosa* (Schmidle) Schmidle, *Pseudodidymocystis inconspicua* (Korshikov) Hindák, *P. planctonica* (Korshikov) E.H. Hegew. et Deason, *Scenedesmus abundans* (Kirchn.) Chodat, *S. acuminatus* (Lagerh.) Chodat, *S. communis* E.H. Hegew., **S. protuberans* Fritsch, *Tetraedron caudatum* (Corda) Hansg., *Tetrastrum staurogeniaeforme* (Schröd.) Lemmerm.

Ulotrichales : *Elakatothrix acuta* Pascher

Conjugatophyceae

Cosmarium sp., *Staurastrum chaetoceros* (Schröd.) G.M. Smith, *Staurastrum* sp.

Euglenophyceae

Euglena agilis H.J. Carter, *E. anabaena* Mainx, *Trachelomonas stokesii* Drezep.

3.2. Neuston in the garden pot

The neuston film on the water surface in the garden pot situated in a small park in the main square of Bratislava, the Slovak National Uprising Square, was intensively dark green in colour, hence apparent, but in limited quantity, however. In this neuston, the dominant species were *E. agilis* and *E. viridis*. In contrast to the neuston of the forest fishpond, the envelopes of cysts of both species were not so intensively brownish but distinctly thick. The garden pot was mostly shadowed by the neighbouring trees and exposed to direct sunlight only for some time.

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