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## ***Micrasterias* – Little Stars**

### **Part 2: Dictyosomes**

The genus *Micrasterias* is very interesting for phycologists. That can be concluded from the fact that the section „phycology“ of the German Botany society proclaimed *Micrasterias* as „Alga of the Year 2008“. To us as hobby biologists with the restriction on the optical microscope, the genus *Micrasterias* offers interesting views into the cell. The first part of this report discussed, apart from the general taxonomy and morphology, the observations of mitochondria. The emphasis of the second part is appropriate on a type of organelles, which can be observed extremely rarely in simple ways with the light microscope, i.e. the dictyosomes.

During photomicrography of *Micrasterias rotata* using high aperture objectives (Planapo 40/1.0 Oil, Planapo 63/1.4 Oil), I recognize a larger number of round particles just beneath the cytoplasm layer situated under the central part of the cell wall in the vicinity of the nucle-

us. With DIC optics I was able to differentiate an internal structure in this parts, starting from an aperture of appr. 0.8. (fig. 1). They showed concentric interior bodies with differing refractive index. Approx. 25 to 40 such body per cell were visible with size between 2.5 and 5  $\mu\text{m}$ . Could that be spherosomes, i.e. oil droplets? It is far common with desmids to store oil as storage substances. However, their internal structure (interleaved spheres) did not fit this interpretation (fig. 2a to c). Soon afterwards, further particles in the cytoplasm were found which fit substantially better the appearance of oil drops (fig. 2a, lower picture part). In addition it was to be stated that the oil droplets were arbitrarily far shifted by the current of the cytoplasm, while the large round objects remained territorial and oscillated only slightly around a virtually central point. Which cell structure could fit these features? After discussions with friends and researches in appropriate literature, the suspicion substantiated that the observed particles were part of the Golgi apparatus, the dictyosomes.



Fig. 1: Overview of *M. rotata*. The circular particles are dictyosomes. As the picture shows, the occurrence of dictyosomes is not limited to the vicinity of the nucleus. Scale bar indicates 50  $\mu\text{m}$ .

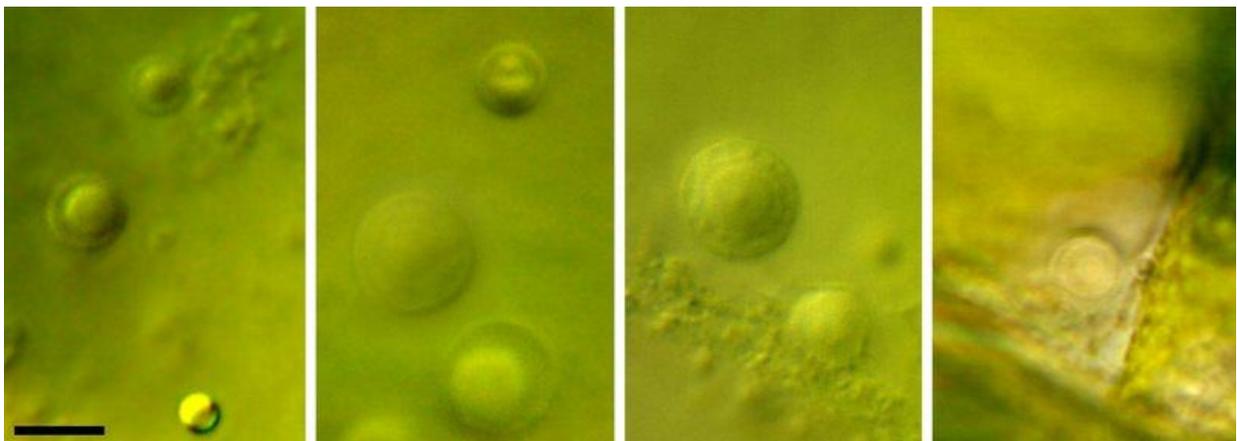


Fig. 2: Mucilage producing dictyosomes of *M. rotata*. a: With oil droplet (bottom). b: Upper particle could be state of binary fission. c: Sometimes an internal vesicular structure were visible. d: Bright field shot. Scale bar indicates 5  $\mu\text{m}$ .

Electron-optical photographs show the fact that dictyosomes are surrounded by small membrane enclosed blisters, the Golgi vesicles (fig. 3). Considering this fact, the smoothly edged objects from figure 2a and 2b did not fit the expected picture. But: Because of the limited resolution of the optical microscope, the tiny Golgi vesicles are not be distinguished from the central body of the dictyosome. If these structures were closely situated to the coverslip, sometimes I was able to observe even whiffs of vesicle shapes (fig. 2c). Even after switching to bright field, some dictyosomes remained visible if they were in auspicious position (fig. 2d). A single dictyosome showed constriction in the central range (fig. 2b). In the literature (Menge and Kiermayer, 1977; Noguchi, 1978) there were electron micrographs which showed that this picture could correspond to a phase of dictyosome's binary fission.

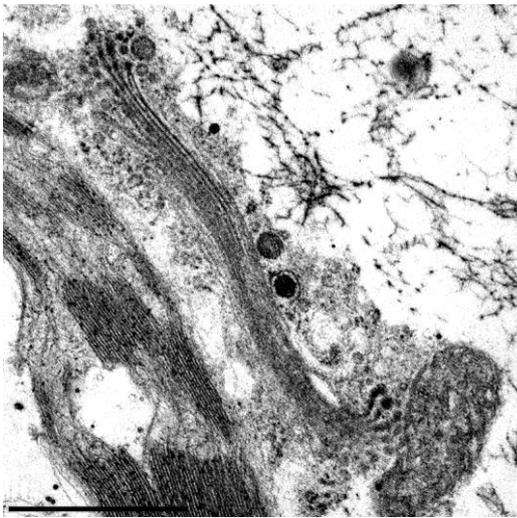


Abb. 3: Cross section of a dictyosome of *Micrasterias denticulata*. In addition the picture shows a cut through a mitochondrion (right down) as well as through a chloroplast (diagonally running from on the left of above to the center down). Preparation and micrograph by Dr. Detlef Kramer, TU Darmstadt, Germany. Scale bar indicates 1  $\mu\text{m}$ .

### **The Golgi apparatus**

The Golgi apparatus is an important cell organelle for synthesis. It produces/modifies proteins and polysaccharides in co-operation with the endoplasmic reticulum (ER) for many different purposes (e. g. enzymes, membrane proteins, basic substances for cell wall construction). After a substance is synthesized, Golgi vesicles are pinched off from the dictyosome. Then these are shifted by the transport system of the cell (microtubules and motor proteins) to the appropriate places (Kleinig and Mayer, 1999; Plattner and Hentschel, 2006; Meindl et al, 1992). Dictyosomes also produce silicate scales, known for example of the gold algae, the Euglyphida (Testacea) or the Heliozoa.

Dictyosomes are usually too small and delicate to be identified by the optical microscope. Not so the species of *Micrasterias* with large cells (diameter 200  $\mu\text{m}$  and larger). Such large and high-contrasted dictyosomes were already described in the 1960's. However, the micrographs in the articles cited were produced electron-optically. Without these confirmations a assured interpretation of the light-microscopically observed phenomena would be difficult. For mitochondria something similar is valid. The appropriate confirmations concerning interpretation of tiny cell structures are often supplied solely by electron microscopy (Drawert and Mix, 1961; Kiermayer, 1965, 1967, 1970; Staehelin and Kiermayer, 1970). Differential interference contrast and phase contrast (if the preparation is thin and pale) however permit observation under in-vivo conditions and, in the case of *M. rotata*, also an assured correlation.

### **Dictyosomes as producers of mucilage**

Desmids possess a special type of dictyosomes, which produce polysaccharide mucilage for locomotion. Their diameter of approximately 5  $\mu\text{m}$  is disproportionately large compared with those in other groups of algae and ordinary plant cells. By means of this mucilage, the desmids can move autonomously, in order to adjust themselves toward the light direction and even to move towards the light source. If it becomes dark, then *Micrasterias* cells place themselves perpendicularly (fig. 4b). So they build the maximized sensor area on the direction of arrival of the light which can be expected at the beginning of the dawn of the new day. With dawn they align themselves standing at right angle to the light incidence. A movie of IWF (C1496, Wenderoth, 1985) shows this in an impressive way. If one sees the bulky output of mucilage to induce movement, it is unclear how the cells can produce such an abundance of material. The procedure becomes only understandable if one conceives that Golgi vesicles bear dehydrated mucilage with a high swelling property. Measurements on root tips of maize resulted in the swelling factor of 1.000 (Steer, 1985).

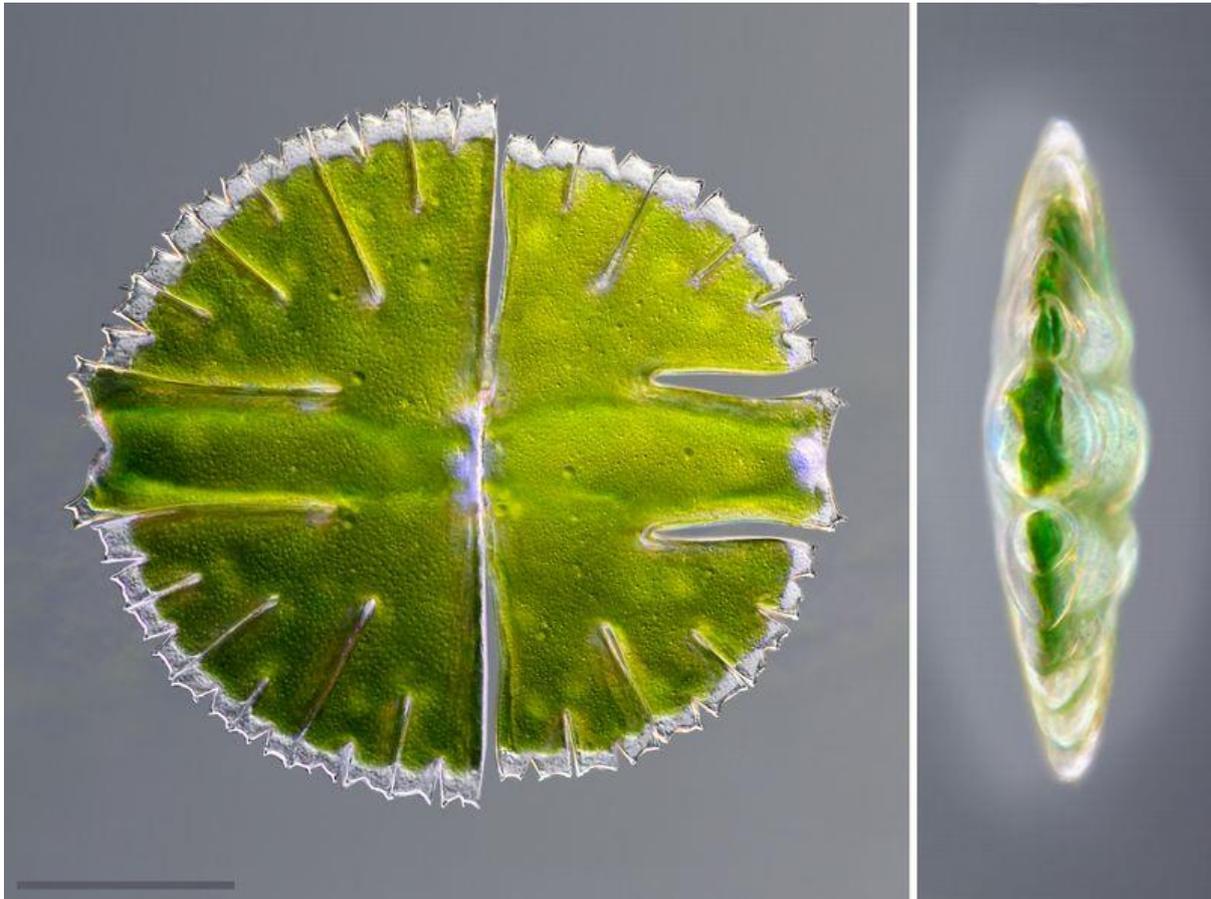


Fig. 4: *Micrasterias rotata*. a: Synoptic picture of shape, surface texture and mucilage producing dictyosomes. This picture is manually stacked using 40 frames.  
 b: *M. rotata*, taken up with an inverse microscope, standing more or less perpendicularly on the coverslip. Manually stacked using 27 frames. Scale bar indicates 50  $\mu\text{m}$ .

### **Separation of vesicles with *Micrasterias rotata***

Further observations on the focus level of the dictyosomes showed sometimes the separation of huge vesicles on their equatorial plane (fig. 5). Here a sketch of the observation: First the edge of the outline was bulging (look at fig. 5, first frame). Then the bulge was expanded to a blister and then became a dumbbell-shaped thing with unequal ball diameters. Gradually the connecting strand was protracted and developed thin. After separation the vesicles disappeared in the current of the cytoplasm. In each case such procedure lasted approximately four minutes.

Noguchi (1978) examined mucilage producing dictyosomes of *Micrasterias americana* by electron microscope, she called them curved dictyosomes. They have an elliptical cross section, the membrane-bound stacks are tub-shaped curved and huge vesicles with diameters of 1,2 - 1.8  $\mu\text{m}$  are segregated.

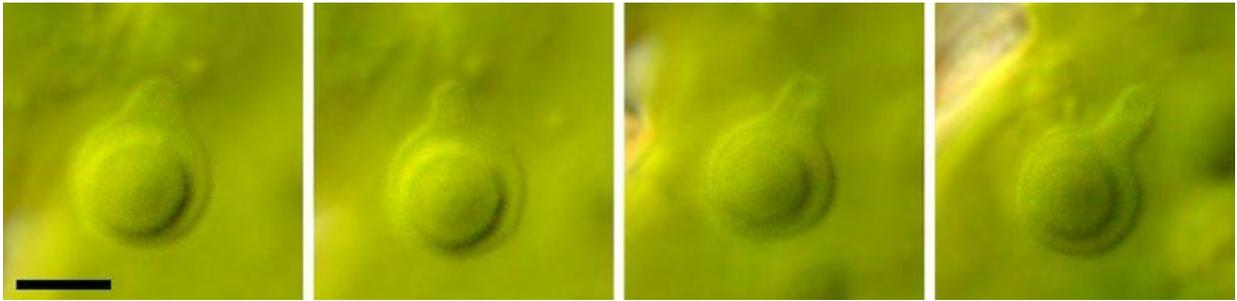


Fig. 5: *M. rotata*. Debonding of a huge vesicle from a dictyosome. Scale bar indicates 5  $\mu\text{m}$ .

### The comparison to *Micrasterias apiculata*

*M. apiculata* bears two kinds of particles with similar size and refractivity as the Golgi stacks seen at *M. rotata*. Figure 6a shows the first type, situated mostly at the periphery of the cells. They have a high optical density and a good visibility in bright field observation. They don't exhibit the manner of oscillation which features dictyosomes. But their brightness is low than that of most spherosomes (oil droplets). It would be necessary to use TEM to determine. But: In the vicinity of the nucleus there were structures known from *M. rotata*, and these should be dictyosomes, too. Figure 6b and 6c illustrates the two types of particles situated in the same area. Arrow heads point to dictyosomes whereas arrows indicate the other type.

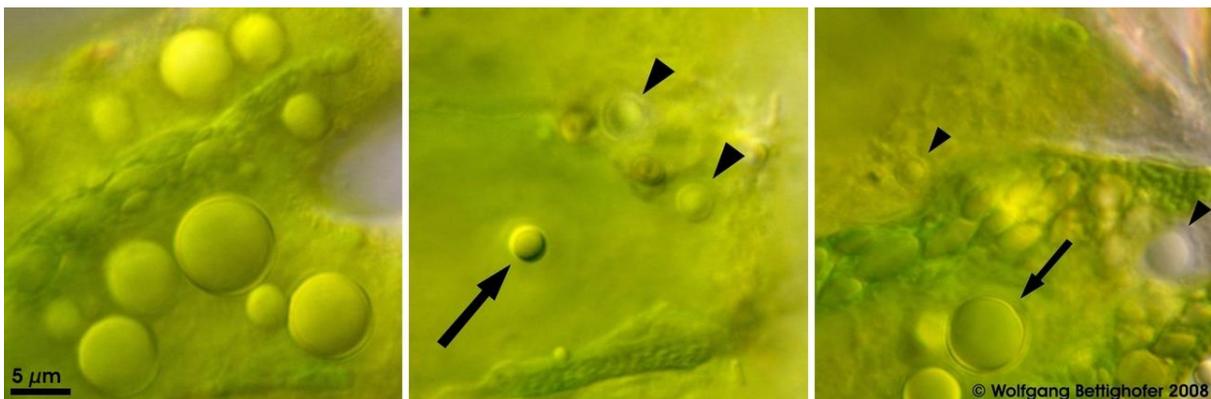


Fig. 6: *Micrasterias apiculata*. a: Particles of uncertain classification. b and c: Dictyosomes and parts of the other type close together in the vicinity of the nucleus. Scale bar indicates 5  $\mu\text{m}$ .

### The ordinary type of dictyosomes

With *Micrasterias denticulata* und *M. thomasiana* var. *notata* (fig. 7 and 8) as object I succeeded in finding a further type of rounded bodies with a diameter of 2.5 to 5  $\mu\text{m}$ . They oscillated slightly, too. Sometimes they were shifted a short distance. Now and then they tilted and could be seen in a lateral view. Surface and edge appeared as if they were covered with small pellets or warts (fig. 9 and 10).

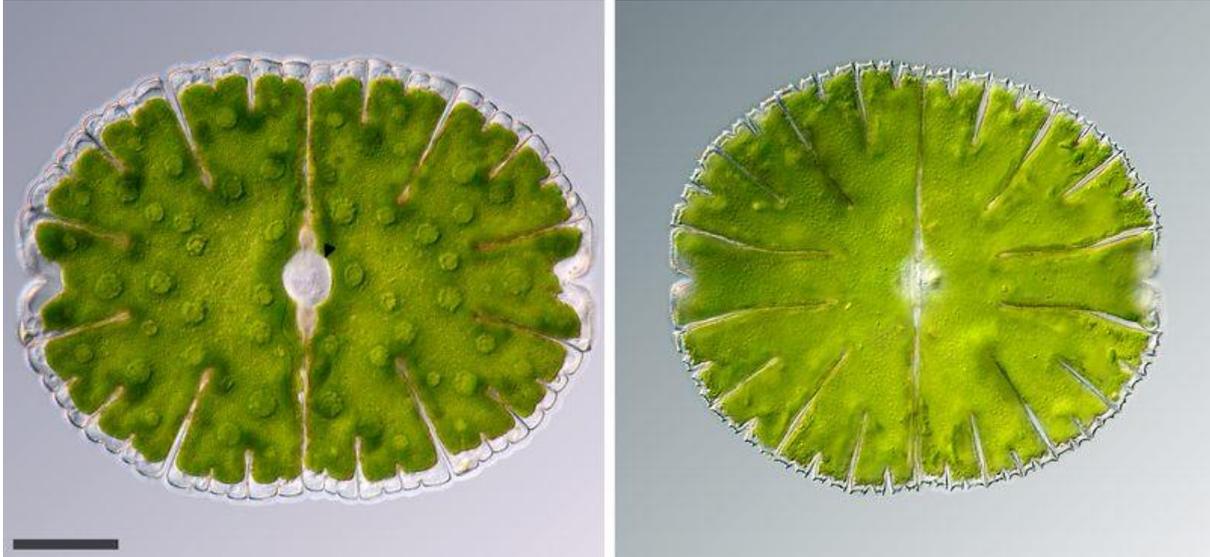


Fig. 7: *Micrasterias denticulata*: Synoptic image of shape, chloroplasts with pyrenoids plus nucleus (arrow head). 20 frames manually stacked. - Fig. 8: *Micrasterias thomasiانا* var. *notata*, showing shape and surface structure. 35 frames manually stacked. . Scale bar indicates 50  $\mu\text{m}$ .

I had the opportunity to discuss these observations with Professor Dr. Werner Herth, cell biologist at the University of Heidelberg. He stated that this pictures show most likely the ordinary dictyosomes (the ones which don't produce mucilage for motility) of the examined algae.

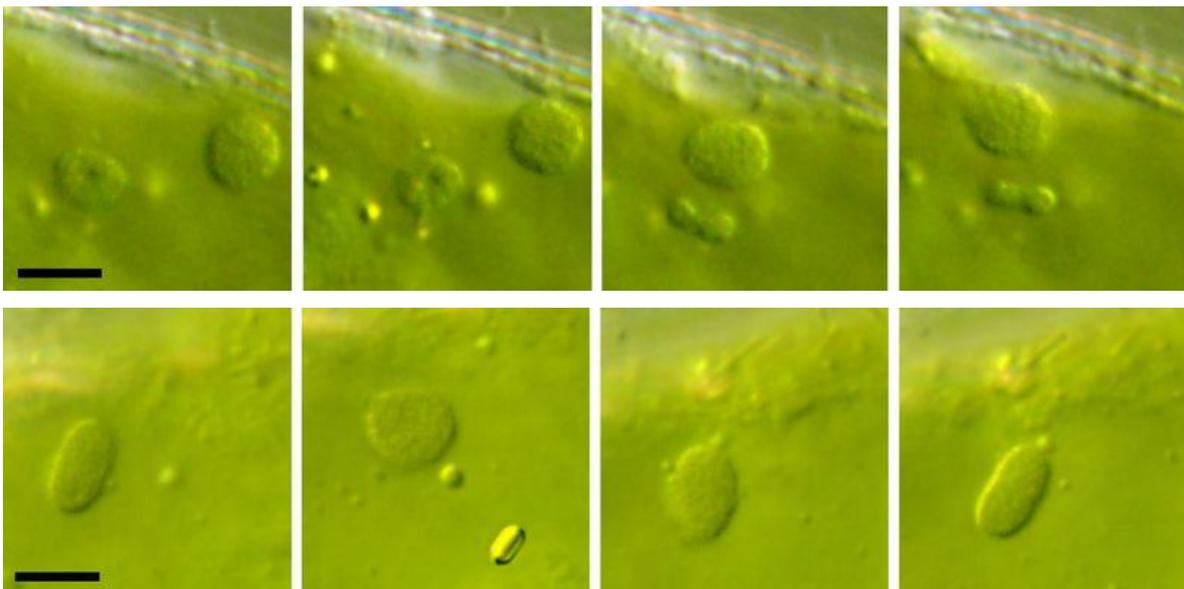


Fig. 9 and 10: Continuous shootings of ordinary dictyosomes of *M. denticulata* (9) and *M. thomasiانا* var. *notata* (10). The typical tilting motions mentioned in the literature are recognizable. Scale bar indicates 5  $\mu\text{m}$ .

## Summary and acknowledgement

The large-cellular *Micrasterias* species provided interesting discoveries of cell details. Never before, I had the ability to observe mitochondria with such distinctness. For the observations the following devices were used: Zeiss Universal with DIC optics, Planapochromats 40/1,0 oil and 63/1,4 oil, photo eyepieces Mipro  $f = 63\text{mm}$  (4x) and S-KPL 10x, digital compact camera Olympus C7070.

On the way from the first step of the discoveries up to the finished report described above, there were many prolific discussions and a number of shared observations with Dr. Detlef Kramer, cytologist at the University of Darmstadt, who accounted valuable contributions to this report. To him I am primarily grateful. He discussed our observations with the cytologists Prof. Dr. Eberhard Schnepf and Prof. Dr. Werner Herth (University of Heidelberg). I am also grateful for their critical evaluations and encouragement. Furthermore, many thanks to Prof. Rupert Lenzenweger for his support in taxonomic and ecological questions referring desmids and delivery of desmid specimen. In addition I am grateful to Dr. Jens Hallfeldt for specimen of *Micrasterias thomasiana* var. *notata*.

Last but not least a statement of Dr. Detlef Kramer:

„The large-celled species of *Micrasterias* show a number of small and smallest cell components in the optical microscope in unusual clarity, which usually can only be depicted clearly with the electron microscope. It is fascinating to accomplish these observations at living organisms and to experience their characteristic dynamics. This is impossible after preparation for the electron microscope. And: These observations are possible for the ambitious amateur with an equipment, which is available today on the second-hand market to a fractional amount of the value as new. The differential interference contrast microscopy by Normarski offers possibilities, which turn out in life science more and more into oblivion. This can be a bridge from the purely static cytology, which employs the electron microscopy, to a fascinating alive view of the cell's interior life. After 37 years working on the electron microscope I am enthusiastic of having been pushed into this fascinating topic!”

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