Journal of the Arkansas Academy of Science

Volume 50 Article 5

1996

Examination of the Cell Wall of Micrasterias Radiosa Var radiosa (Conjugatophyceae) by Transmission and Scanning Electron Microscopy

Jennifer A. Akin
University of Arkansas, Fayettevile

Richard L. Meyer
University of Arkansas, Fayettevile

Examination of the Cell Wall of *Micrasterias radiosa* Var. *radiosa* (Conjugatophyceae) by Transmission and Scanning Electron Microscopy

Jennifer A. Akin and Richard L. Meyer
Department of Biological Sciences
University of Arkansas at Fayetteville
Fayetteville, AR 72701

Abstract

The cell wall of *Micrasterias radiosa* var. *radiosa* Ralfs 1848 (Conjugatophyceae) was examined by transmission and scanning electron microscopy. Previous electron microscopy of this taxon has not been performed; thus these are new observations. The cell wall was recognized to be of the *Cosmarium* type with complex pores external to the plasma membrane that penetrate the secondary wall and with ornamentation arising from the secondary wall. Subdivided regions of the pore apparatus, the pore head, pore bulb, connecting pore channel, and pore depression were detected. Pores of type 4 were located in the isthmal region and at the division of several quaternary lobes. Previously undescribed ornamentation of an asymmetrical swelling on each semicell face was observed. The *Cosmarium* type cell wall and pores of type 4 are consistent with other investigations upon *Micrasterias* taxa. The presence of the asymmetrical swelling on each semicell face necessitates taxonomic revision of *Micrasterias radiosa* var. *radiosa*.

Introduction

Placoderm desmids, members of the class Conjugatophyceae, comprise a certain group of conjugating unicellular green algae which often possess amazing esthetic geometry and elaborate ornamentation. These desmids are characterized by having a two part overlapping wall which separates the cell into two semicells. The wall is perforated by pores and most cells have a deep constriction, called the isthmus, in the midregion in which lies a centrally located nucleus.

Electron microscopical investigations have revealed that all members of the class Conjugatophyceae possess cell walls with three distinct layers. These layers are an outer mucous sheath-like amorphous layer with two internal fibrilar layers, a primary wall, and a secondary wall (Mix, 1972, 1973).

Two cell wall types are recognized for the placoderm desmids, the *Closterium* type and the *Cosmarium* type (Mix, 1966, 1969). The *Closterium* type wall is described as having simple pores or pore-like gaps which traverse only the outer layer. The primary wall is retained in mature cells and ornamentation is limited to the outer layer. The *Cosmarium* type wall is described as having complex pores consisting of a pore head, connecting pore channel, and pore bulb (Neuhaus and Kiermayer, 1981). These complex pores penetrate the secondary wall and are external to the plasma membrane. The primary wall is shed after the secondary wall material is fully deposited and ornamentation arises from in the secondary layer.

The present study examined the cell wall of the placoderm desmid *Micrasterias radiosa* var. *radiosa* Ralfs 1848 by transmission and scanning electron microscopy to determine the structure and type of cell wall as well as the nature of the pore structure. Previous electron microscopy of this taxon has not been performed, thus these observations are new.

Materials and Methods

Culture of the organism.--The culture was supplied by Carolina Biological Supply Company. Cells were maintained in a 1:40 mixture of Algal Gro: distilled water. Cells from the original culture were distributed into polystyrene centrifuge tubes, maintained at 20° C, and set in a north facing window to receive a natural light/dark cycle.

TEM Fixation.--The cells were fixed by the standard Karnovsky's methods (Karnovsky, 1967), post-fixed in 1% Osmium for one hour, and dehydrated in an ascending ethanol series, 10% to 100% in 10% increments (adding a 95% and two changes of 100%) at two minute intervals. After dehydration, the cells were put into Beem™ capsules and processed through two seven minute changes of propylene oxide. Cells were infiltrated with a 50-50 mixture of propylene oxide to Spurr's medium for two hours, which was then replaced with 100% Spurr's medium. Blocks were sectioned using an MT2-B Ultra Microtome and the sections placed on grids. The sections were stained with 2% uranyl acetate and lead citrate. Sections were examined under a JEOL JEM-100 CX transmission electron microscope.

Pretreatment before SEM fixation.--Cells were pretreated with pectinase for 48 hours to remove mucilaginous secretion from pores and then briefly sonicated.

SEM Fixation.--Cells were collected on Whatman #1 filter paper (technique similar to Marchant's, 1973) and were fixed by the standard Karnovsky's methods and post-fixed in 1% Osmium for one hour. Following dehydration in an ascending ethanol series, 10% to 90% in 10% increments in addition to two changes at 95% at two minute intervals, the filter paper with collected cells was placed into a wire mesh carrier basket and put into a Sorvall critical point dryer. After drying, the filter paper was mounted with double stick tape on a scanner stub and sputter-coated with gold. The cells were examined under an ISI-60 scanning electron microscope.

Results

Micrasterias radiosa var. *radiosa* appears to possess pores of the *Cosmarium* type (Pl. I 1). The primary wall was sloughed and therefore not present in the micrographs. The pore, external to the plasma membrane, can be seen penetrating the secondary wall (Pl. I 1, 2, 4). The complex pore consists of a pore apparatus, subdivided into the pore head (Pl. I 2), pore bulb (Pl. I 1, 4), and connecting pore channel (Pl. I 1, 2, 4). In addition, the plasma membrane shows the characteristic pore depression under the complex pore (Pl. I 1-5).

In the pore depression, two different types of fibrilous materials can be seen (Neuhaus and Kiermayer, 1981). One type observed was the "spider web" fibrilous type (Pl. I 1-5). The horizontal fibrils of the web run parallel to the plasma membrane, whereas the vertical fibrils connect the pore bulb to the plasma membrane and cross the horizontal fibrils. The other type of fibrilous material which forms balls of fibrils also was observed (Pl. I 1, 4, 5).

The secretion of mucilagen through the complex pore can be seen in Pl. I 1-3, 5. The mucilaginous substance appears to radiate outward from the pores as fibrils. The sheath-like amorphous layer also can be seen in close association with the radiating fibrils (Pl. I 1-3, 5).

The general outline of an asymmetrical swelling was visible using light microscopy. This lead to detailed observations with SEM. Ornamentation of an asymmetrical swelling with the apex pointing centripically arising from the secondary wall at the semicell face adaxial to the isthmus was detected by SEM upon *M. radiosa* var. *radiosa* (Pl. II 6). A large amount of mucilagen can also be seen covering a large portion of the cell.

During processing of SEM, many of the cells broke at the isthmus (Pl. II 7). The isthmal lip on this semicell appears to be rolled back. A pore can be seen located next to the isthmus as well as pores at the base of several quaternary divisions. The pore opening was located at the same level as the cell surface with no special differentiated cell area surrounding it (Pl. II 8). Therefore, this variety of *Micrasterias* appears to possess pores of type 4 (Neuhaus and Kiermayer, 1981). This large pore was located in the isthmal region, whereas another smaller pore of pore type 4 was located at the base of a quaternary division (Pl. II 9).

Discussion

Electron microscopy of *Micrasterias radiosa* var. *radiosa* has previously not been performed. Taxa formerly examined by TEM include *M. americana* (Ueda, 1972), *M. denticulata* (Kiermayer, 1964), *M. papillifera* (Kies, 1970), and *M. rotata* (Drawert and Mix, 1961b).

The cell wall of *M. radiosa* var. *radiosa* was determined to be of the *Cosmarium* type, based on the presence of complex pores which were external to the plasma membrane and penetrated the secondary wall (Pl. I 1-5). In addition, the primary wall was not observed in the micrographs. It was shed and dispersed during daughter semicell morphogenesis. Shedding of the primary wall is characteristic of the *Cosmarium* type cell wall. The absence of the primary wall indicates that all sections examined were mature cells.

Within the pore depression, located in the internal surface to the secondary wall, Neuhaus and Kiermayer (1981) observed in *M. denticulata* two different types of fibrilous materials. Both types, the "spider web" fibrilous type and fibrilar ball type were detected in the pore depression of *M. radiosa* var. *radiosa*. Thus, the presence of these two types of fibrilous material in the pore depression is consistent with another *Micrasterias* taxon.

Desmids are known for their secretion of copious amounts of mucilagen through their pores (Mix, 1966, 1969; Kiermayer and Staehelin, 1972). The mucilaginous substance which radiates outwards from the pore as fibrils probably depolymerizes into fragments and into the outer mucous sheath-like amorphous layer (Gerrath, 1969; Drawert and Mix, 1961a).

Pretreatment with pectinase for SEM did not adequately clean the cells of mucilagen for unobstructed viewing. The presence of mucilagen could conceal ornamentational features which could influence the taxonomic placement of the organism. An alternative method of cleaning performed by Pickett-Heaps (1973, 1974) demonstrated that pretreatment with a Glusalase preparation was usually effective in removal of mucilagen, but he indicated that total mucilagen removal is rare (Pickett-Heaps, 1975).

Prescott et al. (1977) have detected by light

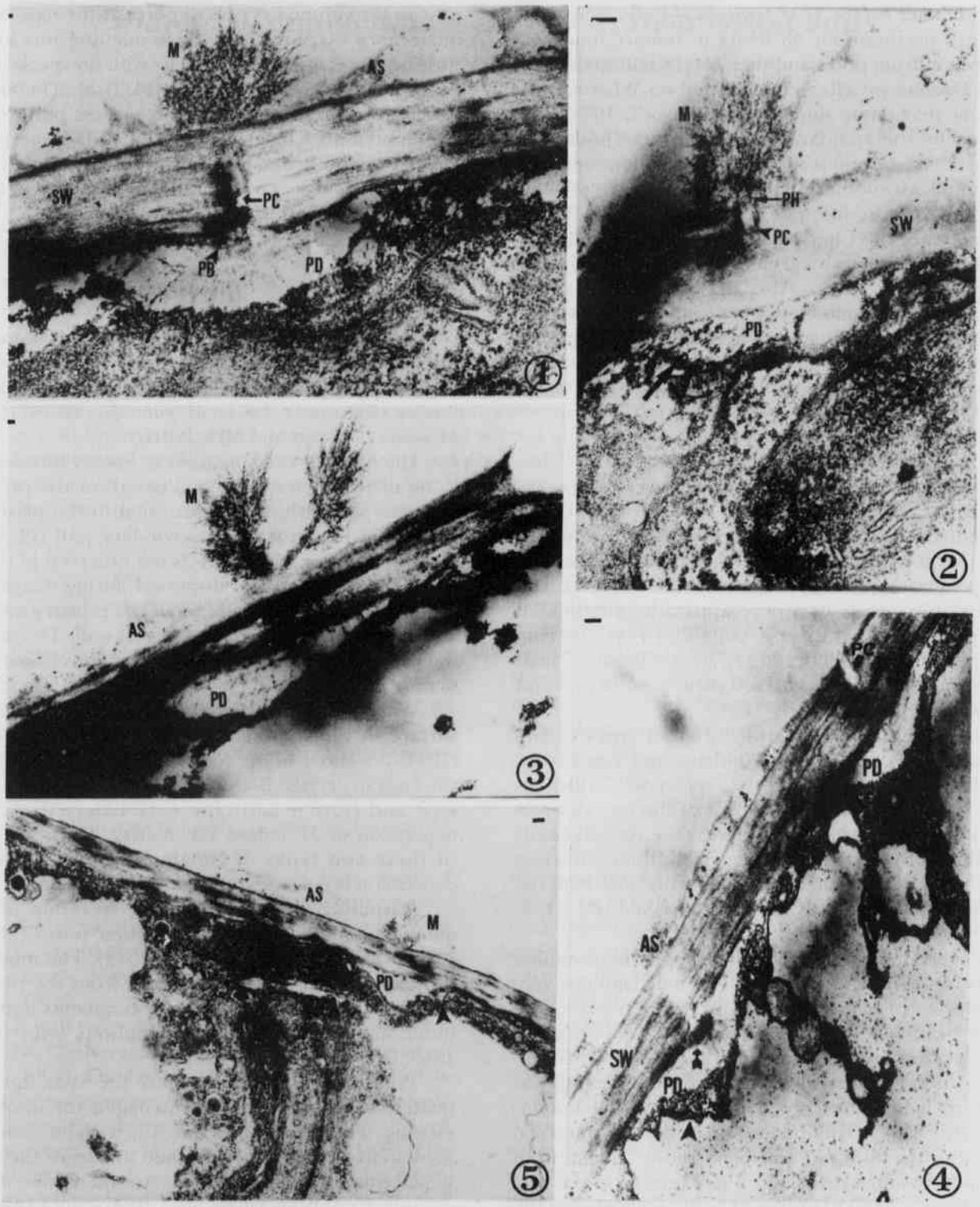

Plate I. TEM micrographs of the cell wall of *Micrasterias radiosa* var. *radiosa*. The two types of fibrilous material, the "spider web" type and the balls of fibrils type can be seen in the PD (1, 4, 5). 1). The pore, external to the plasma membrane (arrow and arrow head), is evident penetrating the SW; PB, PC, and PD can be seen; M radiating out of the pore appearing as fibrils in close association with the AS, 66,000x; 2). PH and PC penetrating the SW; PD; plasma membrane (arrow); M; AS, 66,000x; 3). PD; M; AS, 66,000x; 4). Part of the PC penetrating the SW; PB (thick arrow); PD external to the plasma membrane (arrow head); AS, 52,000x; 5). M in close association with the AS; PD external to the plasma membrane (arrow head), 26,000x.
Lengend: SW = Secondary Wall; PH = Pore Head; PB = Pore Bulb; PC = Pore Channel; PD = Pore depression; M = Mucilaginous Substance; AS = Amorphous Substance.

Examination of the Cell Wall of *Micrasterias radiosa* Var. *radiosa* (Conjugatophyceae) by Transmission and Scanning Electron Microscopy

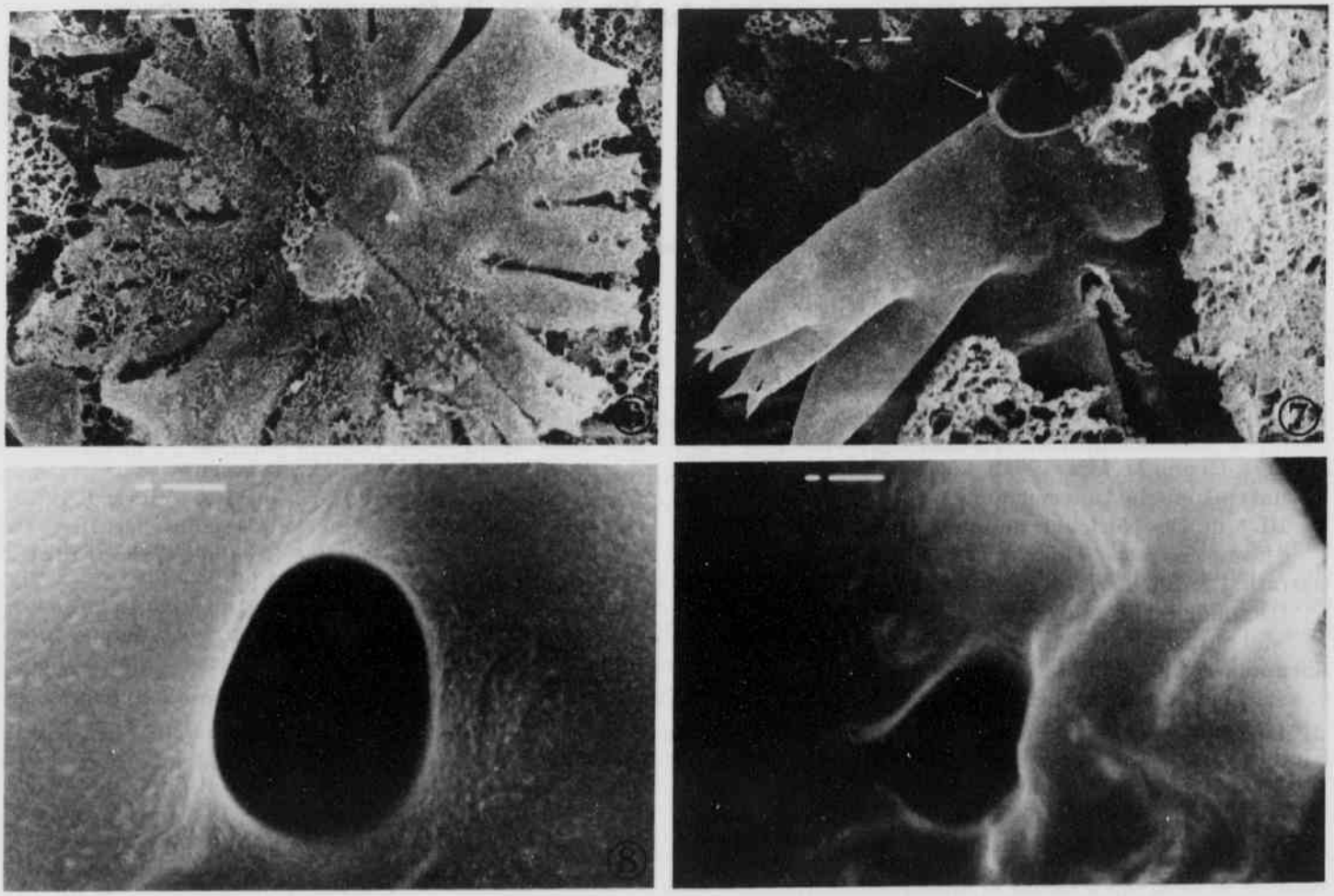

Plate II. SEM micrographs of *Micrasterias radiosa* var. *radiosa*. 6). Arising from the secondary wall, ornamentation of an asymmetrical swelling with the apex pointing centripically on each semicell face adaxial to the isthmus can be seen (arrow); a large amount of mucilagen can be seen covering the cell; 7). Parent semicell of the parent/daughter semicell relationship with the isthmal lip rolled back (white arrow); pores located in the region of the isthmus and at several quaternary divisions (small black arrows); 8). Large pore of pore type 4 located in the isthmal region; 9). Small pore of pore type 4 located at the division of a quaternary lobe.
(Long bars are 10 µm for 6, 7 and 1 µm for 8, 9).

microscopy prominent asymmetrical swellings in other *Micrasterias* species; *M. Borgei, M. denticulata* var. *Taylorii* f. *Taylorii,* and *M. Thomasiana* var. *pulcherrima*. These qualified observers have seen these swellings when present but did not detect the swelling in the nominate *M. radiosa*. This collection which superficially resembles *M. radiosa* contains an additional feature which was easily viewed by light microscopy but the specific morphology was better determined by SEM.

SEM revealed this feature to be ornamentation of an asymmetrical swelling with the apex pointing centripically on each semicell face (Pl. II 6, 7). This previously undescribed feature appears to arise from the secondary wall adaxial to the isthmus. A review of the literature indicates that swellings were not observed in *M. radiosa* var. *radiosa*. Hence, this finding necessitates taxonomic revision upon this variety of *Micrasterias*. The proposed new varietal epithet is *M. radiosa* var. *bulbosa*.

During the processing of SEM, many of the cells broke at the isthmus (Pl. II 7). This was probably due to rough treatment of the delicate cells during critical point drying procedures. The isthmal lip of the semicell appeared to be rolled back (Pl. II 7). Therefore, this semicell is the parent semicell of the parent/daughter semicell relationship (Pickett-Heaps, 1972).

Pores of type 4 were located in the isthmal region and at the division of several quaternary lobes (Pl. II 8, 9). Pore type 4 is characterized by pore openings located at the cell surface with no special differentiated cell area surrounding it (Neuhaus and Kiermayer, 1981). *Micrasterias* taxa have been detected to possess pore type 4 (Neuhaus and Kiermayer, 1981; Drawert and Mix, 1961a). These findings of the *Cosmarium* cell wall type and pores of type 4 upon *Micrasterias radiosa* var. *bulbosa*

are consistent with other findings upon *Micrasterias* taxa.

ACKNOWLEDGMENTS.—The authors would like to thank Dr. K.S. Kim for teaching the technique of electron microscopy. Further appreciation is extended to D.L. Moore for her friendship and help in the processing of the micrographs and special thanks is due to the two anonymous reviewers who provided constructive commentary.

Literature Cited

Drawert, H. and **M. Mix.** 1961a. Licht- und elektronen mikroskopische Untersuchungen an Desmidiaceen II. Hullgallerte und Schleimbildung bei *Micrasterias, Pleurotaenium,* und *Hyalotheca*. Planta 56:237-261.

Drawert, H. and **M. Mix.** 1961b. Licht- und elektronen mikroskopische Untersuchungen an Desmidiaceen. III. Mitt. Der Nucleolus im Interphasekern von *Micrasterias rotata*. Flora 150:185-190.

Gerrath, J.F. 1969. *Penium spinulosum* (Wolle) comb. nov. (Desmidiaceae): a taxonomic correction based on cell wall ultrastructure. Phycologia 8:109-118.

Karnovsky, M.J. 1967. The ultrastructural basis of capill ary permeability studied with peroxidase as a tracer. J. Cell Biol. 35:213-236.

Kiermayer, O. 1964. Untersuchungen uber die Morphogenese und Zellwandbildung bei *Micrasterias denticulata* Breb. Protoplasma 59:76-132.

Kiermayer, O. and **L.A. Staehelin.** 1972. Feinstruktur von Zellwand und Plasmamembran bei *Micrasterias denticulata* Breb. nach Gefrieratzung. Protoplasm 74:227-237.

Kies, L. 1970. Elektronenmikroskopische Untersuchungen uber Bildung und Struktur der Zygotenwand bei *Micrasterias papillifera* (Desmidiaceae) I. Das Exospor. Protoplasm 70:21-47.

Marchant, H.J. 1973. Processing small delicate biological specimens for scanning electron microscopy. Journal of Microscopy 97:369-371.

Mix, M. 1966. Licht- und elektronenmikroskopische Untersuchungen an Desmidiaceen. XII. Zur Feinstruktur der Zellwande und Mikrofibrillen einiger Desmidiaceen vom *Cosmarium* - Typ. Arch. Mikrobiol. 55:116-133.

Mix, M. 1969. Zur Feinstruktur der Zellwande in der Gattung *Closterium* (Desmidiaceae) unter besonderer Berucksichtigung des Porensystems. Arch. Mikrobiol. 68:306-325.

Mix, M. 1972. Die Feinstruktur der Zellwande bei Mesotaeniaceae und Gonatozygaceae mit einer ver gleichenden Betrachtung der verschiedenen Wandtypen der Conjugatophyceae und uber deren systematischen Wert. Arch. Mikrobiol. 81:197-220.

Mix, M. 1973. Die Feinstruktur der Zellwande der Conjugaten und ihre systematische Bedeutung. Beih. Nova Hedwigia 42:179-194.

Neuhaus, G. and **O. Kiermayer.** 1981. Formation and Distribution of Cell Wall Pores in Desmids. In: Cytomorphogenesis in Plants (Kiermayer, O., ed.). Wien-New York: Springer.

Pickett-Heaps, J.D. 1972. Cell division in *Cosmarium botrytis*. J. Phycol. 8:343-360.

Pickett-Heaps, J.D. 1973. Stereo-scanning electron microscopy of desmids. Journal of Microscopy 99:109-116.

Pickett-Heaps, J.D. 1974. Scanning Electron Microscopy of Some Cultured Desmids. Trans. Amer. Micros. Soc. 93:1-23.

Pickett-Heaps, J.D. 1975. The Green Algae. Sinauer Associates, Sunderland, Massachusettes.

Prescott, G.W., Croasdale, H.T. and **W.C. Vinyard.** 1977. A Synopsis of North American Desmids. Part II. Desmidiaceae: Placodermae. Section 2. University of Nebraska Press. Lincoln. 413p.

Ueda, K. 1972. Electron microscopical observation on nuclear division in *Micrasterias americana*. Bot. Mag. (Tokyo) 85:263-271.