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A COMPARISON OF TWO SPECIES OF *TRACHELOMONAS* (EUGLENOPHYCEAE) WITH SCANNING ELECTRON MICROSCOPY

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ABSTRACT

Scanning electron microscopy was used for examination of the surface of *Trachelomonas variabilis* Singh and *Trachelomonas volvocinopsis* var. spiralis Pringsheim. Specimens of both species were examined with air dried and critical point dried (CPD) techniques. In *T. variabilis*, the CPD specimens revealed greater resolution o-structures comprising the lorica, whereas the air dried specimen displayed distortion and obscurement of finer details of the structure. Comparison of *T. variabilis* with *T. volvocinopsis* revealed that both species possess ovoid, papillate, punctate loricas with a small collar. The spines of *T. variabilis* appear tapered and at right angles to the lorica, while the spines of *T. volvocinopsis* are rounded and are not set perpendicular to the lorica.

INTRODUCTION

Organisms of the genus *Trachelomonas* are found as free-swimming forms with a flagellum extending through a circular pore in the lorica. The lorica is a rigid mucilaginous coating which, in nature, may become brown because of impregnation with ferric hydroxide and manganese compounds (Zipkin, 1973; Leedale, 1975). The surface of the lorica may be smooth or ornamented with spines and pores (punctate); minute papillae may be seen at the higher magnifications with Scanning Electron Microscopy (SEM) (Rosowski, 1975). The protoplast is not attached to the lorica, and the cell may lose its flagellum and rotate inside the lorica (Smith, 1950; Leedale, 1967). Examination of a non-loricate cell with SEM reveals the euglenoid pellicle and pellicular striations. The pellicle immediately substends the plasmalemma and consists of flat interlocking strips which surround the cell. Hence, the pellicle is not equivalent to a cell wall since the latter lies external to the plasmalemma (Leedale, 1967). The purpose of this study is to examine the differences between *T. variabilis* and *T. volvocinopsis* and compare air dried specimens with critical point dried (CPD) specimens.

MATERIALS AND METHODS

Cultures of *Trachelomonas variabilis* Singh were obtained from Verzeichnis der Sammlung von Algenkulturen am Pflanzenphysiologischen Institut der Universität Göttingen, Germany, and cultures of *Trachelomonas volvocinopsis* var. *spiralis* Pringsheim were obtained from the culture center of Algae and Protozoa, Cambridge, England. These cultures were maintained in biphasic soil-water media with the addition of two pea varieties and a barley grain to the soil-water medium. Growth took place at 18°C in a Sherer environmental growth chamber, Model AT18 B-SE, and the organisms were exposed to 1300 lux of light in a 16 to 8 L/D hour diurnal photoperiod. The cultures were allowed 2–3 weeks to multiply before the cells were processed and examined as described below.

Cells were concentrated to a pellet by centrifugation at speeds of less than 1000 × g. The pelleted cells were washed and then fixed in 1.5% aqueous OsO_4 at ca. 4°C for 30 minutes. Afterward, the cells were washed four times in a phosphate buffer solution at pH 7.0 and were then placed on a glass cover slip coated with a 0.1% solution of poly-l-lysine in water.

Cells were either air dried or critical point dried (CPD). The air dried samples were placed on aluminum SEM pedestals. For CPD, the cells were transferred through a graded series of acetone solutions to effect dehydration. The specimens were then CPD in a Bomar Critical Point Drier, No. SPC-900 Ex, using CO_2, and were finally placed on aluminum SEM pedestals and coated with ca. 200 angstroms of Au/Pd in a Denton DV 515 Vacuum evaporator.

All samples were observed and photographed with an ETED Autoscan Scanning Electron Microscope operated at 20 KV, with the specimens examined at various angles of specimen tilt. Images were recorded on Polaroid 55 Positive-Negative Film.

RESULTS

Trachelomonas variabilis

The mature lorica of this species is ovoid with tapered spines papillae each about 0.10–0.16 μm in diameter and punctae about .06 μm (Plate I, Figure 1).

PLATE I

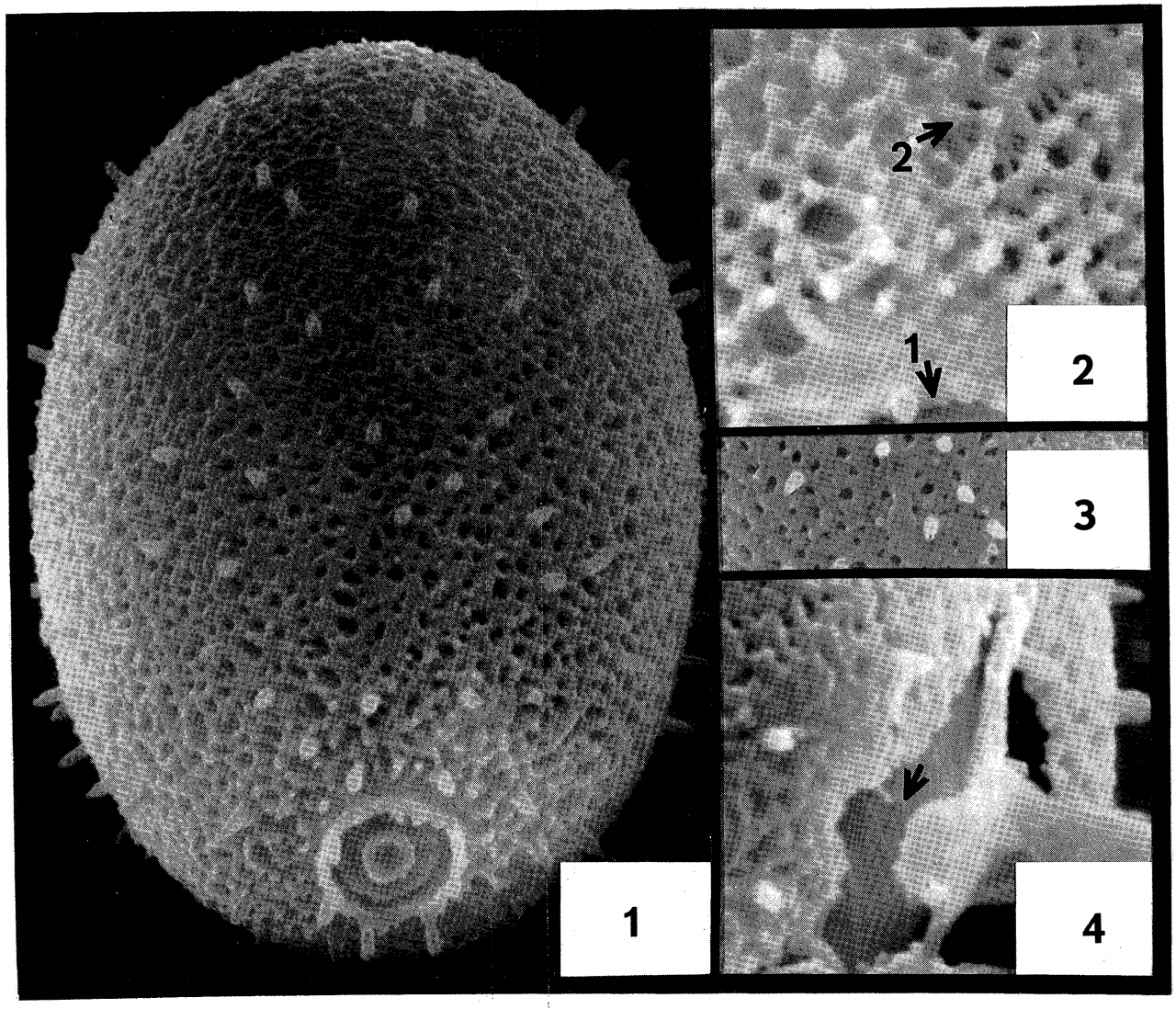

Figure1. *Trachelomonas variabilis* CPD preparation as seen by SEM. This view shows the entire mature lorica with spines and the punctate surface. Note the collar also has spines projecting from the rimed elevation. The punctae show variation in size and distribution. X12,000. **Figure 2.** *T. variabilis* CPD preparation. This view near the collar (note pointer 1) shows anastomosing strands (note pointer 2) and papillae forming a layer of uneven thickness. X18,000. **Figure 3.** *T. variabilis* air dried. this specimen shows distinct pores or punctae, spines and minute papillae. X6,000. **Figure 4.** *T. variabilis* air dried. This micrograph displays a damaged specimen showing the pellicle (note pointer). X14,000.

PLATE II

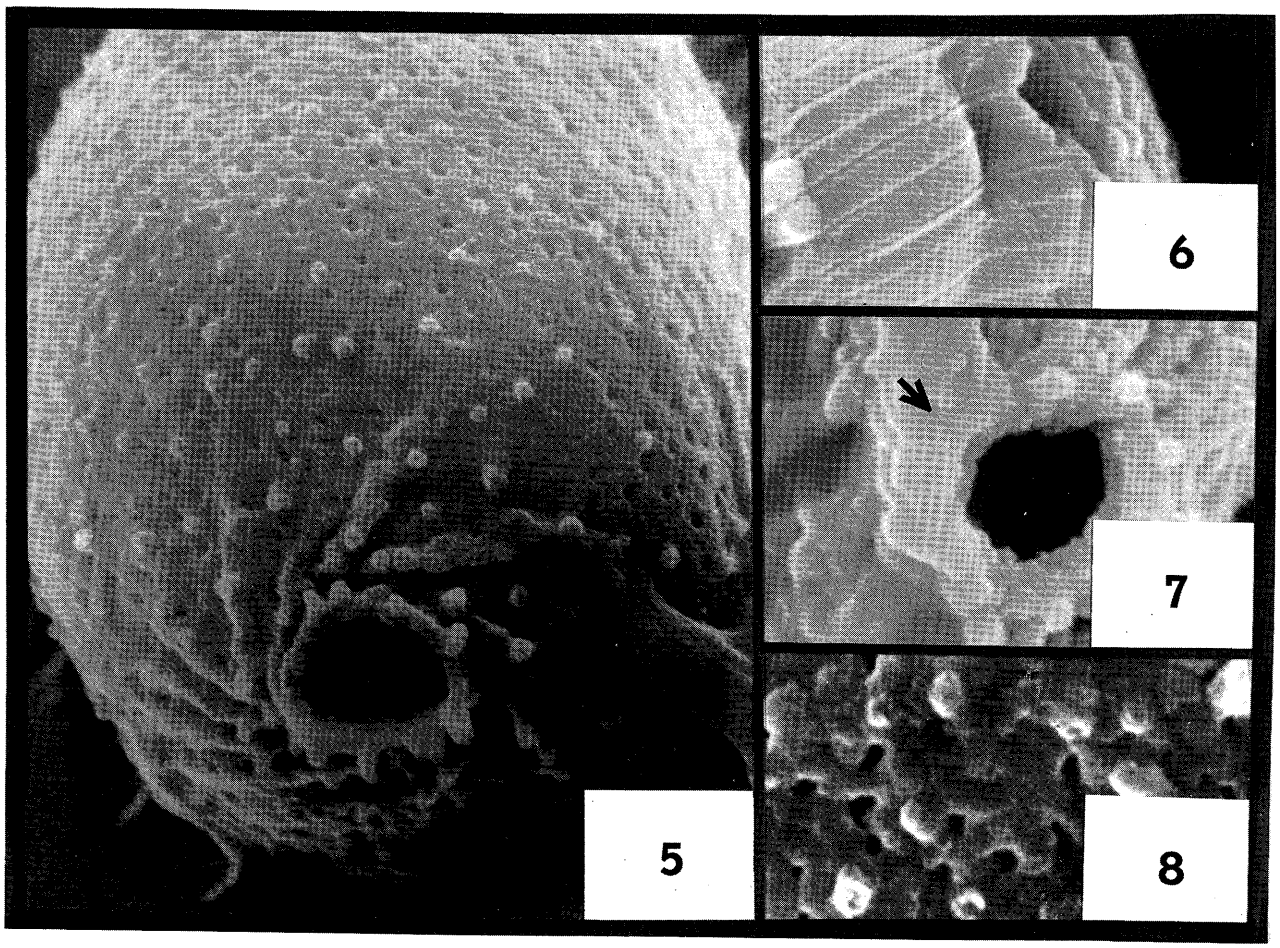

Figure 5. *Trachelomonas volvocinopsis* CPD preparation. This view shows the ovoid lorica with blunt and rounded spines. Note the collar also has spines projecting from the rimed elevation. The punctae shows a general uniformity in size and distribution. X12,000. **Figure 6.** *T. volvocinopsis* CPD preparation. Micrograph of cell without lorica showing the pellicle which has flat interlocking strips and pellicular striations. X14,000. **Figure 7.** *T. volvocinopsis* air dried preparation. This view of the lorica shows the collar with presumably mucilaginous aggregations (note pointer) on the papillae and spines. X14,000. **Figure 8.** *T. volvocinopsis* air dried preparation. Details of lorica showing blunt and rounded spines. X9,000.

The organism possesses a small elevated collar with spines around the opening at the anterior end (Plate I, Figure 3), distinct pores or punctae, spines and minute papillae are visible at a magnification of 6,000x. A damaged specimen, revealing the pellicle (arrow) is shown in Figure 4 on Plate I. Spines were observed on the lorica of both air-dried and critically point dried specimens. At higher magnification the interwoven strands of material that make up the lorica and papillae were observed forming a layer of uneven thickness (Plate I, Figure 2). Immature loricas were lacking spines and general surface characteristics (not shown). In specimens that were critically point dried the surface of the lorica appeared more intricate, and small papillae were observed with low magnification. (Plate I, Figure 2).

Trachelomonas volvocinopsis

The mature lorica of this species is ovoid with blunt and rounded spines about 0.16–0.25 μm in diameter. The rigid and highly ornamented lorica has a collar surrounding the anterior pore with spines (Plate II, Figure 5). The pellicle is visible on some specimens and pellicular striation are clearly observed in this species (Plate II, Figure 6). In both the air-dried (Plate II, Figure 7) and CPD specimens (Plate II, Figure 5) distinct pores or punctae and spines are observed. Air-dried specimens appeared to be covered with a presumptive mucilaginous aggregation (Figure 7). In the CPD specimens the lorica appeared more intricate and small papillae were observed at low magnification (Plate II, Figure 8). Immature loricas were lacking spines and general surface characteristics (not shown).

Discussion

The ornamental features of the loricas of the two species differed greatly. *Trachelomonas variabilis* has tapered spines which are set at right angles to the lorica surface in the mature state (Plate I, Figure 1). *Trachelomonas volvocinopsis* has blunt and rounded spines which are not set at right angles to the lorica in the mature form (Plate II, Figure 5). Both species have ovoid loricas with minute papillae. The number of pores or punctae are greater and more variable in size on the lorica of *T. variabilis* than on *T. volvocinopsis* (Plate I, Figure 1 and Plate II, Figure 5). The loricas of both species seemed to consist of anastomosing stands of material which could be seen at higher magnifications (Plate I, Figure 2 and Plate II, Figure 8).

In comparison of air-dried and CPD techniques, there is a close similarity between general features revealed by both methods. General features of ornamentation are employed for most taxonomic work; thus air-drying may be sufficient for most of this work. However, air-drying tends to distort or obscure finer features (Plate I, Figure 3 and Plate II, Figure 7). This study serves to reinforce the experimental findings of Rosowski et al. (1975). The Rosowski et al. study of *Trachelomonas* showed that the strands of mucilage which make up the lorica become indistinct or aggregated when air-dried. This phenomena was not observed in CPD specimens. However, since the more general features of the loricae such as size, shape and degree of ornamentation are similar in both air-dried and CPD preparations, air-drying is adequate for most taxonomic studies of *T. variabilis* and *T. volvocinopsis* .

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Works Cited

Leedale, G. F., 1967. Euglenoid Flagellates. Prentice-Hall Inc. Englewood Cliffs, N.J.

Leedale, G.F., 1975. Envelope formation and structure in the euglenoid genus *Trachelomonas*. Br. Phycol. J. 10: 17–41.

Pringsheim, E. G., 1953. Observations on some species of *Trachelomonas* grown in culture. New Phytol. 52: 93–113, 238–266.

Rosowski, J R., P.L. Walne and L.K. West, 1975. Comparative effects of critical point and air drying on the morphology of the rigid mucilaginous coating (lorica) of *Trachelomonas* (euglenophyceae). Micron 5: 321–339.

Rosowski, J. R., R. L. Vadas and P. Kugrens, 1975. Surface configuration of the lorica of the euglenoid *Trachelomonas* as revealed with scanning electron microscopy. Amer. J. Bot. 62 (1): 48–57.

Smith, G. M., 1950.The Freshwater Algae of The United States. McGraw-Hill Book Company, New York.

Zipkin, I., 1973. Biological Mineralization. Zipkin, I. (ed) John Wiley and Sons, New York.