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SURFACE FEATURES OF *TETRAHYMENA ROSTRATA* AS VIEWED WITH SCANNING ELECTRON MICROSCOPY

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ABSTRACT

Trophonts and cysts of *Tetrahymena rostrata* (ANL) were examined with scanning electron microscopy in order to reveal minute surface morphological features for the first time in this species. Measurements of surface organelles from electron micrographs provided the following average values: ciliary widths (0.31 μm), ciliary lengths (4.7μm), alveolar pit diameters (3.7μm), buccal overture (6.7 $\times$ 5.4μm). The number of ciliary units in a typical somatic kinety was 40, while that of a post-oral kinety was 30. The external wall of encysted individuals is irregular and featureless; the average diameter of cysts was 24μm.

Morphological descriptions of stages in the life cycle of the hymenostome ciliate, *Tetrahymena rostrata* (Kahl, 1926; Corliss, 1952); have been confimed so far to investigations with the light microscope (Stout, 1954; Corliss, 1973) or to the analysis of thin-sectioned material viewed with the transmission electron microscope (Zebrun et al., 1967; McArdle et al., 1980). Although methods for the preparation of ciliate protozoans for scanning electron microscopy have been available for some time (Small and Marszalek, 1969) there have not been any previously published reports on the external features of *T. rostrata* using this informative and valuable method. This investigation undertakes to provide information on the surface features of the cortex of trophic organisms and on the cyst wall of encysted representatives of this polymorphic species (Corliss, 1973).

MATERIALS AND METHODS

The trophonts (feeding stages) and cysts used in this study were isolated by the author from moss plants growing on the grounds of the Argonne National Laboratory (ANL) and identified as *Tetrahymena rostrata*. Some subcultures were rendered axenic with antibiotics for laboratory culture (Holz and Thomp-

son, 1955) and maintained on an enriched proteosepeptone broth medium (Dobra et al., 1980). Other subcultures were periodically administered raw beefsteak while being cultured in 0.01 % Knop's solution in glass three-hole depression slides in a humidity chamber. Between periods of feeding, the lack of food would induce cyst-formation (McArdle et al., 1980). Organisms were withdrawn from these cultures at appropriate intervals of their life cycle and fixed according to the methods described by Small and Marszalek (1969). Following fixation, they were washed several times in distilled water before being dropped by pipette onto the surface of an aluminum "boat" floating on liquid nitrogen. The frozen droplets that formed were transferred to a metal stub and subjected to drying by sublimation in a Baltzer vacuum evaporator for approximately 12 hours. Following drying the specimens were gold-coated and examined with a Cambridge Stereoscan Mark IIA scanning electron microscope made available by the Division of Biological and Medical Research of ANL.

RESULTS AND DISCUSSION

The surface features of *Tetrahymena rostrata* strain ANL as revealed by SEM are pictured in Figures 1-5. Trophonts (feeding stages) of the ciliate are depicted in Figs. 1-3 and 5. Fig. 1 shows the ventral surface of the typical body form of a trophont (49μm long $\times$ 26μm wide). The cilia on such forms are characteristically arranged in regular rows (kineties) extending from the subapical pole of the cell to the subterminal pole. The cilia vary only slightly in width in this preparation (0.27-0.36μm) and are generally rather uniform in length, with a range of from 3.6 - 6.1μm (average 4.7μm; n = 10). The cilia in Fig. 3 can be seen to emerge on the surface of the ciliate from shallow, ovoid-shaped depressions that have an average diameter of 3.7 μm (n = 26). On favorably oriented specimens the number of individual cilia in a single kinetal row can be counted. For example on one such organism, the exact number of cilia in a full anterior-posterior kinety was 40, while the shorter, post-oral kinety that extends from the posterior border of the buccal overture to the sub-terminal pole was 30 ciliary units. The buccal overture is ovoid in shape (see Fig. 2), being slightly longer (6.7μm) than it is wide (5.4μm). Details of the interior of this cavity are obscured by the dense packing of the cilia comprising the undulating membrane.

Several "resting" or "resistant" cysts are shown in Fig. 4. These generally ovoid and elliptical-shaped forms were obtained from a "bacterized meat" culture. They show the characteristic aggregation that occurs prior to encystment when small groups of trophont ciliates congregate together while secreting a cystic wall. The finished wall is an un-ornamented, irregular sheet of secreted mucus completely enclosing the organism. In this figure the cyst walls are covered with bacterial organisms that were associated with them in the medium at the time of fixation. The measured diameters of the cysts varied from 22-27 μm (with an average value of 24μm).

The posterior pole of a trophont is illustrated in Fig. 5. The kineties converge toward the posterior end, but stop just short of it. The membranous area at the posterior pole possesses a single (sometimes double) cilium that has been termed a "caudal cilium" (Corliss, 1973). In this specimen it has been sheared off close to the surface membrane and is seen only as a short stub.

The results of this SEM investigation essentially corroborate the observations made to date on *T. rostrata* by both light microscopy and transmission electron microscopy. In addition, it contributes new information on the length and width of individual cilia and on the number of discrete ciliary units in somatic and post-oral kineties. The surface features of bacterized cysts are shown for the first time as is the precise location of the "caudal" cilium at the posterior pole of the organism. These provide a basis for comparison with the surface features of other tetrahymenid species.

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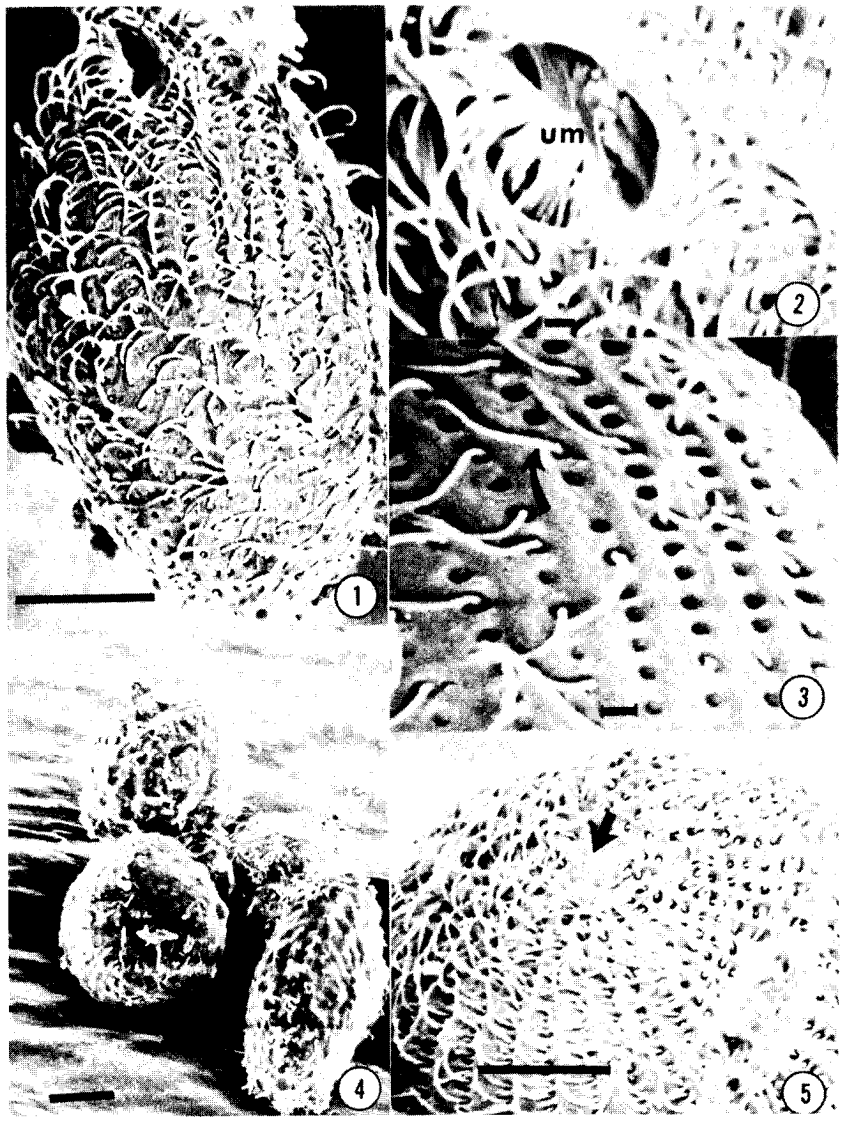

Fig. 1. Ventral view of a trophont of *T. rostrata* showing the subterminal position of the buccal overture at the anterior end and the holotrichous arrangement of the ciliary rows. The opaque material at the periphery of the organism is likely agglomerated mucoid material secreted by the organism's mucocyst organelles. The scale bar indicates 10 μm.

Fig. 2. The buccal overture is depicted at higher magnification. It shows the position of the undulating membrane (um) on the organism's right side of the buccal opening. The oral area is surrounded by somatic ciliation. The scale bar indicates 1 μm.

Fig. 3. A partially de-ciliated surface of a trophont. The cilia that remain (arrow) are relatively uniform in size and emerge from aveolar depressions in the cortex. The scale bar indicated 1 μm.

Fig. 4. Four encysted individuals of *T. rostrata*. The secreted cyst wall is uneven and irregular in its appearance but conforms to the general shape of the organism within. The small, light-colored rods on the surface are environmental bacteria adhering to the outer face of the cyst wall. The scale bar represents 10 μm.

Fig. 5. A view of the posterior end of a trophont showing the convergence of the ciliary rows and the location of a characteristic polar cilium (arrow). The scale bar denotes 10 μm.