Determination of the Distribution of Cilia on the Surface of the Mantle of *Cypraea caputserpentis* utilizing Scanning Electron Microscopy

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ABSTRACT

The distribution of cilia on the mantle of *Cypraea caputserpentis* through the use of scanning electron microscopy will be utilized. The mantle is dissected into regions from the anterior-head region to the posterior region, and each section is systematically scanned for the presence of cilia using preparation techniques outlined. The distribution and abundance of cilia may lead to an explanation of the role of cilia in the physiology of these mollusks.

INTRODUCTION

A recent study utilizing transmission electron microscopy (Yokoi & Hemmes, 1990) has shown that the outer surface of the mantle of *Cypraea caputserpentis* is decorated with numerous cilia interspersed with thin, elongated microvilli. The epithelium has periodic invaginations (30-50um deep) which are filled with these cilia and microvilli. It is not known conclusively what the function of these cilia is on the surface of the cowrie mantle. The cilia may sweep mucous from the mantle surface to help lubricate the foot or simply clear debris from the mantle surface. A first step in understanding the function of these cilia will be to examine their distribution on the outer and inner epithelial layers of the mantle. In order to see the mantle in a three dimensional perspective, techniques for viewing the tissue under the scanning electron microscope need to be developed.

This paper will explore the use of Peldri II (Ted Pella), a sublimation dehydration agent in place of critical point drying for the cilia located on the mantle of the *Cypraea caputserpentis*.

MATERIALS & METHODS

A juvenile specimen (bulla stage) of *Cypraea caputserpentis* was collected in November, 1990 in a tidal pool at Onekahakaha Beach Park on the island of Hawaii. The specimen was immediately immersed in a solution containing 2.5% glutaraldehyde in 0.1M sodium cacodylate, and 5% sucrose with pH 7.2. The specimen was then sectioned and allowed to stay in this solution overnight. The next day, the specimen was washed in a cacodylate buffer made up of 5% sucrose for half an hour. the tissue was then immersed in a solution of 1% osmium tetroxide in 0.1M sodium cacodylate containing 5% sucrose, with pH 7.2 for one hour. It was again washed again in the cacodylate buffer for half an hour. In order to dehydrate the specimen, acetone with concentrations of 30%, 50%, 70%, 90%, 95%, and 100% were used. The specimen was soaked in each solution for ten minutes and saturated in the 100% acetone three times for a total of eighty minutes in acetone. The Peldri II reagent was then warmed and approximately 2 mL of this Peldri II sublimation dehydration solution was poured into a new vial with the specimens. Two milliliters of 100% acetone was also added to the vial and allowed to sit for one hour. The supernatant was discarded and 100% Peldri II was added to the specimens. This new solution containing the specimens was allowed to sit overnight. Within 48 hours, the specimens had absorbed all the Peldri II solution. They were then put in a vacuum evaporator for a few hours. The specimens were now ready for mounting. The tissues were mounted using silver paste on the electron microscope stub and sputter coated with gold palladium in order to decrease charging when viewing. The scanning electron microscope was then warmed up and the specimens viewed and photographed.

Figure 1
Numerous cilia are uniformly distributed on the surface of the mantle. Magnification 5000x.

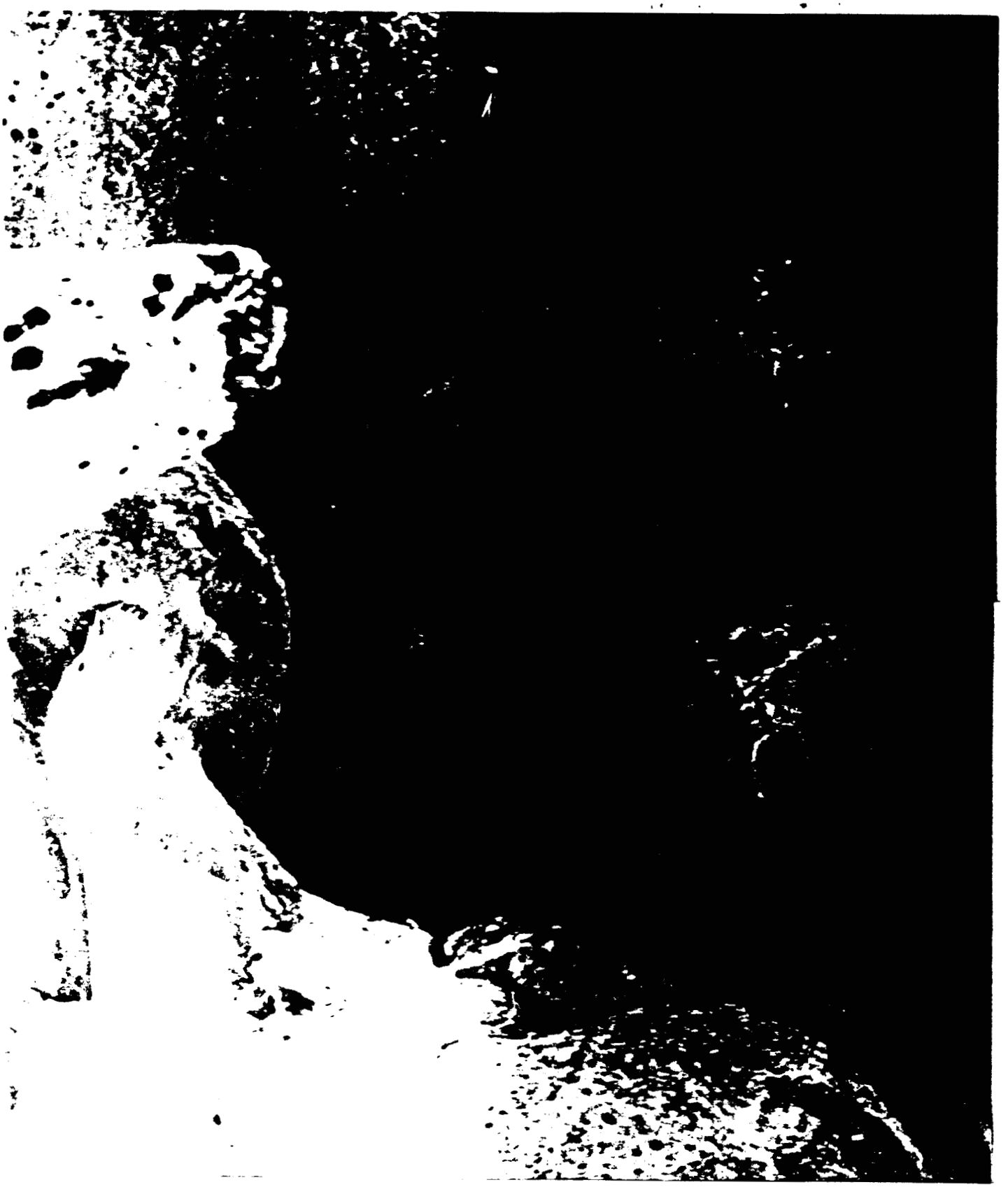

Figure 2
Papillae are shown to be disrupted. Magnification 4800x.

Figure 3
Papillae exhibiting numerous cilia. Magnification 700x.

RESULTS & DISCUSSION

The photographs enclosed show numerous strands of cilia dispersed everywhere on the epithelial layer. In Figure 1, the cilia are shown to be uniformly distributed along the surface. It seems as though, four or five cilia have bound together or are intertwined.

In Figure 2, papillae are shown to be disrupted. Perhaps the disruption may have taken place when the cilia collapsed during the Peldri II dehydration. The papillae seem to have holes on their surfaces. A higher magnification of a papillae is shown in Figure 3. This photograph shows a papillae with numerous cilia on its surface.

The remainder of the photographs taken are located in the appendix including the original scanning electron microscope photographs.

From these photographs, we are able to conclude that there are cilia on the surface of the mantle of the *Cypraea caputserpentis*, and in some areas, there distribution is uniform, as shown in Figure 1. Although the papillae located on the epithelia look smooth in Figure 2, there are many other papillae exhibiting many cilia (Figure 3).

The disruption of the cells seen in many photographs located in the appendix lead us to acknowledge the need for many experiments using different techniques of dehydration and drying of these delicate cells. A good technique which should be tried is the substitution of critical point drying instead of the Peldri II. A comparison of the two would help in determining the best way for preservation of natural looking cells. Another technique which should also be tested is to monitor the specimen when soaking in 100% Peldri II solution, in order to not let the specimen to become dry.

APPENDIX

Magnification 8000x.

Magnification 200x.

Magnification 180x.

Magnification 6000x.

Magnification 6000x.


Magnification 240x.

Magnification 400x.

Magnification 500x.

Magnification 400x.

Magnification 500x.

Magnification 700x.

Magnification 240x.

Magnification 200x.