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CILIA POLYMORPHISM AMONG SCHISTOSOME SNAIL HOSTS AS REVEALED BY SCANNING ELECTRON MICROSCOPY

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INTRODUCTION

Finding a suitable and susceptible snail host is one of the most important functions of Schistosome miracidia (Chernin, 1974; Pan, 1980). A number of behavior type studies have been carried out on Schistosoma mansoni miracidia utilizing simple quantitative and biochemical assays in relation to host-finding (Chernin, 1962, 1968, 1970, 1971, 1972).

There are indications that the swimming behavior of miracidia and contact with a suitable and susceptible snail host might be due to chemical substances being emitted from snails. Such substances have been termed "mixaxones" (Chernin, 1974). These substances are present in snail mucus and are thought to be potential attractants for miracidia (Chernin, 1974).

However, few studies have directed their attention to investigating structures on the surface of snail hosts that may aid directly or indirectly in miracidial host-location.

In this preliminary study, the distribution, pattern orientation, surface morphology and morphometric analyses of cilia were made of the following snail hosts: Biomphalaria glabrata, B. straminea, B. tenagophila and Bulinus truncatus.

MATERIALS AND METHODS

Scanning Electron Microscopy

Snails (8-12 mm in diameter) were obtained from the Tropical Disease Research Center, University of Lowell, Lowell MA. Snails were deshelled, washed several times in distilled water and fixed for two hours in 2.5% glutaraldehyde buffered with Sorenson's sodium phosphate. Organisms were

rinsed in several changes of the same buffer for one hour and then post-fixed in 1% buffered osmium tetroxide for two hours.

Specimens were rinsed extensively in buffer and dehydrated in the following ascending concentrations of ethyl alcohol: 30, 50 70, 83, 95 and 100%, and then two 10 minute changes of 100% alcohol. Snails were then placed in 7% amyl acetate for 15 minutes and then three changes of 100% amyl acetate, 15 minutes each. Specimens were critical point dried in liquid carbon dioxide, sputter coated with gold-palladium and observed in an Etec Omniscan Scanning Electron Microscope. Photographs were taken at desirable magnifications. A method was devised by Prewitt and Jones (1984) for morphometrical measurements.

RESULTS AND DISCUSSION

To our knowledge this is the first evidence of an investigation concerned with the three-dimensional surface topography of cilia on snail hosts that transmit schistosomiasis. The basic anatomical features of snail hosts consisted of the head-foot region, tentacles, mantle collar, body and tail region. Figure 1 shows the surface topography of the head-food region, tentacles and mantle collar of the snail host, Biomphalaria glabrata. At higher magnifications the mouth region showed the presence of irregular folds and short-stubbed, densely compacted masses of cilia. Food material was observed in the center of the mouth opening (Figure 2).

The inner surface of the mantle collar of B. glabrata consisted of distinct tufts of cilia similar to ciliary tufts observed in B. tenagophila (Figures 3 and 4). In B. tenagophila, ocassionally, irregular-sized blebs were seen collecting at the end of the ciliary tufts (Figure 4). Such structures were believed to be condensations of snail mucus.

One of the problems encountered in examining the surface topography of snail hosts was the presence of considerable amounts of mucus on the surface. In this study, special attention was made to developing a protocol which allowed extensive rinsing of the snail surface with a standard electron microscopy buffer for mucus removal.

Ciliary tufts on the surface of the snail host, Bulinus truncatus appeared short, distinct and regularly distributed (Figures 5 and 6). Figure 7 shows an electron scan of the head-foot region of Biomphalaria straminea. The specimen was orientated to a lateral angle to reveal the bottom surface of the foot area. The tentacles of B. straminea consisted of numerous, densely compacted tentacles of cilia, in comparison to B. glabrata, B. tenagophila and B. truncatus. Cilia on the tentacles of B. straminea showed a multi-directional orientation (Figure 8.) However, the

Figure 1. Scanning Electron Micrograph of the Head-Foot Region of the Snail Host, Biomphalaria glabrata: (h), Head; (t), Tentacles; (mc), Mantle Collar; (f), Foot. Marker, 100 µm.

Figure 2. Mouth Region of B. glabrata showing Irregular Folds and Densely Compacted Bosses of Cilia. Note the ingestion of food (lettuce) by the organism, arrows. Marker, 100 µm.

Figure 3. Inner Surface of Mantle Collar of B. glabrata showing Tufts of Cilia. Marker, 10 μm.

Figure 4. Inner Mantle Collar of the Biomphalaria tenagophila showing Tufts of Cilia. Marker, 1 μm.

Figure 5. Top Surface of the Head Region of Bulinus truncatus showing Tufts of Cilia. Marker, 100 μm.

Figure 6. Higher Magnification of Cilia of Bulinus truncatus. Marker, 10 μm.

anterior-lateral head-region of B. straminea consisted of densely packed cilia uni-directional (Figure 9). Cilia on the lower mantle collar of B. straminea revealed a similar orientation and appearance (Figure 10).

Morphometric quantitative analyses of the relative lengths of cilia on the surface of snail hosts were made by reconstructing a schematic diagram of cilia based on the appearance of scanning electron micrographs (Figure 11). The schematic reconstruction developed by Prewitt and Jones (1984) utilizes the following parameters on measuring cilia lengths: scanning electron microscope voltage, working distance, the conversion factor, magnification and negative number (Figure 11).

The morphometric measurements for cilia length were somewhat in accord to standard methods of morphometry (Eisenberg, 1985; Weibel, 1969; Weibel et al., 1967; Weibel, 1980; DeHoff et al., 1968; and Underwood, 1970). Two methods were devised for quantitative morphometric analyses of cilia (Prewitt and Jones, 1985). In method one, the length of a cilium in microns was equal to the length of a cilium measured in millimeters divided by the magnification of the micrograph. In the second method, the length of cilium in microns was equal to the length of cilium measured in millimeters multiplied by the scale marking conversion factor on the micrograph. Slight differences and a small percent error existed between the two methods (Table 1). Even though morphometric analyses were made of all snails, computations for B. straminea and B. Glabrata were provided in this report.

The relative minimum length of cilia or the upper mantle collar of B. straminea using method one was 4.23 microns and 4.45 microns using method two (Table 1). In all morphometric computations at least five different measurements were made on a specific host surface area or anatomical region from numerous scanning electron micrographs. The differences between the two given methods could possibly be due to some of the following: surface structures on the snails in which cilia was present, the pattern of cilia orientation, the surface morphology of cilia, and the appearance of cilia origin. Cilia that was present on the lower mantle collar of B. straminea showed a relative minimum length of 9.05 microns using method one and 9.57 microns using method two (Table 3). The relative minimum length of cilia present on the tentacle was 5.88 microns using both methods (Table 4).

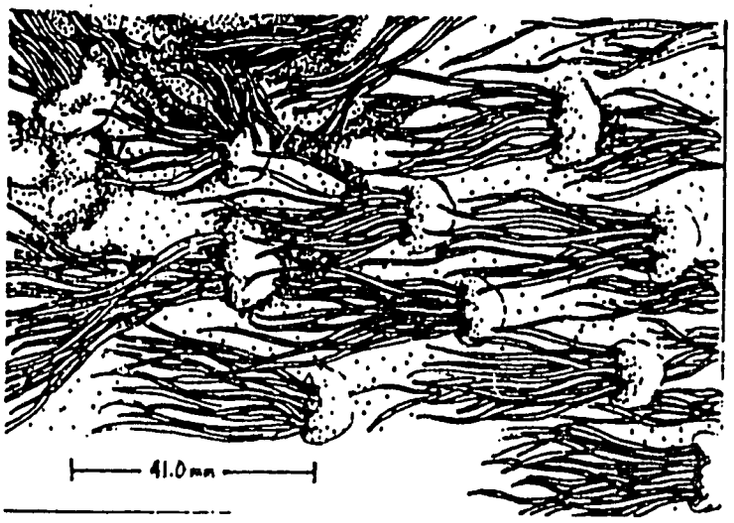
Cilia on the anterior-lateral head surface region of B. straminea showed a relative-minimum length of 13.9 microns based on computations using method one and 14.3 microns from method two (Table 5). The medial foot region of B. straminea revealed a relative-minimum length of cilia

Figure 7. Scanning Electron Micrograph of the Head-Foot Region of Biomphalaria straminea: (h), Head; (t), Tentacles; (mc), Mantle Collar; (f), Foot. Marker, 100 μm.

Figure 8. Cilia on the Tentacles of B. straminea showing Tangles and a Multi-directional Orientation. Marker, 40 μm.

Figure 9. Densely Compacted Cilia on the Anterior-Lateral Head Surface of *B. straminea*. Marker, 30 μm.

Figure 10. Laterally-oriented Cilia on the Lower Mantle Collar of *B. straminea*. Marker, 27 μm.



20.1 KV 12 MM 7.27 02.83 KX 12683

Figure 11. Representative Schematic Diagram of Cilia on the Surface of Biomphalaria straminea Primarily based on Scanning Electron Micrographs (Prewitt and Jones, 1984) 20.1 KV = Scope Voltage; 12 mm = Working Distance; 7.27 μ = Conversion Factor (7.27 μ is equal to 20.0 mm at a magnification of 2.83 KX); 02.83 KX = Magnification Number in kilotimes (2830X); 12683 = Negative number for micrograph.

Table 1. Methods of Quantitative Morphometric Analysis.

METHOD I

$$\text{Length of a cilium in } \mu = \frac{(\text{Length of cilium measured in mm})}{(\text{Magnification number})} \times \frac{(1000\ \mu)}{(1.00\ \text{mm})} = \underline{X}\ \mu$$

METHOD II

$$\text{Length of a cilium in } \mu = (\text{Length of cilium measured in mm}) \times \text{Scale-marking Conversion Factor} = \underline{X}\mu$$

Example:

Question: How can one measure the length, in μ, of cilium which measures 41.0 mm in a photomicrograph having a magnification of 2830X with a scale-marking of 7.27 μ/20.0 mm?

METHOD I

$$\text{Length of the cilim in } \mu = \frac{(41.0\ \text{mm})}{(2830)} \times \frac{(1000\ \mu)}{(1.00\ \text{mm})} = 14.5\ \mu$$

METHOD II

$$\text{Length of the cilium in} = 41.0\ \text{mm}\ \frac{(7.27\ \mu)}{(20.0\ \text{mm})} = 14.9\ \mu$$

Table 2. Morphometric Computations for the Cilia Present on the Upper Mantle Collar of *Biomphalaria straminea*.

Method I	Method II
$\frac{(13.0\ mm)}{(2840)}\frac{(1000\ \mu)}{(1.00\ mm)} = 4.58\ \mu$	$13.0\ mm\ \frac{(7.42\ \mu)}{(10.0\ mm)} = 4.82\ \mu$
$\frac{(14.0\ mm)}{(2840)}\frac{(1000\ \mu)}{(1.00\ mm)} = 4.93\ \mu$	$14.0\ mm\ \frac{(7.42\ \mu)}{(20.0\ mm)} = 5.19\ \mu$
$\frac{(8.00\ mm)}{(2840)}\frac{(1000\ \mu)}{(1.00\ mm)} = 2.82\ \mu$	$8.00\ mm\ \frac{(7.42\ \mu)}{(20.0\ mm)} = 2.97\ \mu$
$\frac{(10.0\ mm)}{(2840)}\frac{(1000\ \mu)}{(1.00\ mm)} = 3.52\ \mu$	$10.0\ mm\ \frac{(7.42\ \mu)}{(20.0\ mm)} = 3.71\ \mu$
$\frac{(15.0\ mm)}{(2840)}\frac{(1000\ \mu)}{(1.00\ mm)} = 5.28\ \mu$	$15.0\ mm\ \frac{(7.42\ \mu)}{(20.0\ mm)} = 5.56\ \mu$
Relative-minimum length of cilia = 4.23 μ	Relative-minimum length of cilia = 4.45 μ

Table 3. Morphometric Computations for Cilia Present on the Lower Mantle Collar of *Biomphalaria straminea*.

Method I	Method II
$\frac{(23.0\ mm)}{(2540)}\frac{(1000\ \mu)}{(1.00\ mm)} = 9.05\ \mu$	$23.0\ mm\ \frac{(8.32\ \mu)}{(20.0\ mm)} = 9.57\ \mu$
$\frac{(21.0\ mm)}{(2540)}\frac{(1000\ \mu)}{(1.00\ mm)} = 8.27\ \mu$	$21.0\ mm\ \frac{(8.32\ \mu)}{(20.0\ mm)} = 8.74\ \mu$
$\frac{(24.0\ mm)}{(2540)}\frac{(1000\ \mu)}{(1.00\ mm)} = 9.45\ \mu$	$24.0\ mm\ \frac{(8.32\ \mu)}{(20.0\ mm)} = 9.98\ \mu$
$\frac{(22.0\ mm)}{(2540)}\frac{(1000\ \mu)}{(1.00\ mm)} = 8.66\ \mu$	$22.0\ mm\ \frac{(8.32\ \mu)}{(20.0\ mm)} = 9.15\ \mu$
$\frac{(25.0\ mm)}{(2540)}\frac{(1000\ \mu)}{(1.00\ mm)} = 9.84\ \mu$	$25.0\ mm\ \frac{(8.32\ \mu)}{(20.00\ mm)} = 10.40\ \mu$
Relative-minimum length of cilia = 9.05 μ	Relative-minimum length of cilia = 9.57 μ

Table 4. Morphometric Computations for the Cilia Present on the Tentacles of *Biomphalaria straminea*.

Method I

$$\frac{(80.0\ mm)\ (1000\ \mu)}{(15000)\ (1.00\ mm)} = 5.33\ \mu$$

$$\frac{(99.0\ mm)\ (1000\ \mu)}{(15000)\ (1.00\ mm)} = 6.60\ \mu$$

$$\frac{(98.0\ mm)\ (1000\ \mu)}{(15000)\ (1.00\ mm)} = 6.53\ \mu$$

$$\frac{(70.0\ mm)\ (1000\ \mu)}{(15000)\ (1.00\ mm)} = 4.67\ \mu$$

$$\frac{(94.0\ mm)\ (1000\ \mu)}{(15000)\ (1.00\ mm)} = 6.27\ \mu$$

Relative-minimum length of cilia = 5.88 μ

Table 5. Morphometric Computations for the Cilia Present on the Anterior-Lateral Head of *Biomphalaria stramines*.

Method I		Method II	
$\frac{(41.0\ mm)}{(2830)} \frac{(1000\ \mu)}{(1.00\ mm)}$	= 14.5 μ	$41.0\ mm \frac{(7.27\ \mu)}{(20.0\ mm)}$	= 14.9 μ
$\frac{(35.0\ mm)}{(2830)} \frac{(1000\ \mu)}{(1.00\ mm)}$	= 12.4 μ	$35.0\ mm \frac{(7.27\ \mu)}{(20.0\ mm)}$	= 12.7 μ
$\frac{(45.0\ mm)}{(2830)} \frac{(1000\ \mu)}{(1.00\ mm)}$	= 15.9 μ	$45.0\ mm \frac{(7.27\ \mu)}{(20.0\ mm)}$	= 16.3 μ
$\frac{(39.0\ mm)}{(2830)} \frac{(1000\ \mu)}{(1.00\ mm)}$	= 13.8 μ	$39.0\ mm \frac{(7.27\ \mu)}{(20.0\ mm)}$	= 14.2 μ
$\frac{(37.0\ mm)}{(2830)} \frac{(1000\ \mu)}{(1.00\ mm)}$	= 13.1 μ	$37.0\ mm \frac{(7.27\ \mu)}{(20.0\ mm)}$	= 13.4 μ
Relative-minimum length of cilia = 13.9 μ		Relative-minimum length of cilia = 14.3 μ	

averaging 2.94 microns and 4.16 microns using methods one and two, respectively, (Table 6).

Using both methods, the relative-minimum length of cilia on the tail region of B. straminea averaged 1.37 microns. Computations provided in the text only reflect measurements from method one (Table 7). A summary of morphological scanning electron microscopic data, and computations from morphometrical analyses of B. straminea can be found in Table 8. The distribution of cilia on a specific region of the snail host surface varies in addition to cilia orientation and length (Table 8). Table 9 shows the average relative-minimum length of cilia on given regions of B. glabrata. Distinct variations and differences in cilia lengths were found on different body regions.

The combined morphological and quantitative morphometrical data showed that cilia polymorphism exists in not only cilia structure but cilia lengths, pattern of orientation and distribution. Similar scanning electron microscopic observations were reported on microvilli (microtriches) in Hymenolepid tapeworms (Berger and Mettrick, 1971). These investigators reported that various species of Hymenolepis revealed polymorphic microvilli with differencies in orientation and it was suggested that these structures might play a role in increasing the surface area of worms for nutrient absorption.

The role(s) of cilia present on the surface of snail hosts remains to be elucidated. However, such structures, as microvilli in tapeworms, may aid in increasing the overall absorptive surface area and thus create a more adequate environment for snail mucus which contains substances that are potential attractants for miracidia.

SUMMARY

The distribution, pattern of orientation, surface morphology and morphometric analyses were made of the following snails: B. glabrata, B. straminea, B. tenagophila and Bulinus truncatus. In the upper lip and mouth regions of B. glabrata and B. straminea, cilia was sparsely distributed and appeared as short bud-like projections. Whereas, in B. tenagophila dense populations of cilia were observed in these regions. In all species, dense populations of long cilia were exhibited in the upper and inner mantle collar. In B. glabrata and B. tenagophila, cilia was arranged in tufts. Average morphometric analyses were made of all snail hosts and variations were observed in all species.

Table 7. Morphometric Computations for the Cilia Present on the Tail Region of *Biomphalaria straminea*.

Method I

$$\frac{(4.00\ mm)\ (1000\ \mu)}{(\ 2825\ )\ (1.00\ mm)} = 1.41\ \mu$$

$$\frac{(3.00\ mm)\ (1000\ \mu)}{(\ 2825\ )\ (1.00\ mm)} = 1.06\ \mu$$

$$\frac{(4.50\ mm)\ (1000\ \mu)}{(\ 2825\ )\ (1.00\ mm)} = 1.59\ \mu$$

$$\frac{(3.80\ mm)\ (1000\ \mu)}{(\ 2825\ )\ (1.00\ mm)} = 1.34\ \mu$$

$$\frac{(4.10\ mm)\ (1000\ \mu)}{(\ 2825\ )\ (1.00\ mm)} = 1.45\ \mu$$

Relative-minimum length of cilia = 1.37 μ

Table 8. Summary of Morphological and Morphometrical Data of *Biomphalaria straminea*.

Region Observed	Distribution of Cilia	Orientation of Cilia	Relative-Minimum Length of Cilia
Upper Mantle Collar	Densely covered with cilia	Multi-directional	4.23 microns
Lower Mantle Collar	Densely covered with cilia	Laterally-directed orientation	9.05 microns
Tentacles	Densely covered with cilia	Multi-directional	5.88 microns
Anterior-Lateral Head	Moderately concentrated with cilia	Anteriorly-directed orientation with respect to the tail	13.9 microns
Medial Foot	Densely populated with cilia	Posteriorly-directed orientation with respect to the head	2.94 microns
Tail	Sparsely	Multi-directional	1.37 microns

Table 9. Morphometric Computations for Cilia Present on the Surface of *Biomphalaria glabrata*.

Upper Mantle Collar	$\frac{13\ mm}{2850} \left(\frac{1000\ \mu}{1\ mm}\right) = 4.56\ \mu$
Lower Mantle Collar	$\frac{17\ mm}{1200} \left(\frac{1000\ \mu}{1\ mm}\right) = 14.16\ \mu$
Head	$\frac{55\ mm}{6000} \left(\frac{1000\ \mu}{1\ mm}\right) = 9.16\ \mu$
Tentacle	$\frac{70\ mm}{3000} \left(\frac{1000\ \mu}{1\ mm}\right) = 23.30\ \mu$
Mouth	$\frac{10\ mm}{2680} \left(\frac{1000\ \mu}{1\ mm}\right) = 3.73\ \mu$
Foot	$\frac{10\ mm}{2640} \left(\frac{1000\ \mu}{1\ mm}\right) = 3.78\ \mu$

REFERENCES

Berger, J. and Mettrick, D. (1971). Microtrichial polymorphism among Hymenolepid tapeworms as seen by scanning electron microscopy. Trans. Amer. Micros. Soc., 90, 393-403.

Chernin, E. and Dunavan, C. (1962). The influence of host-parasite dispersion upon the capacity of Schistosoma mansoni miracidia to infect Australorbis glabratus. Amer. J. Trop. Med. Hyg., 11, 455-471.

Chernin, E. (1968). Interference with the capacity of Schistosoma mansoni miracidia to infect the mulluscan host. J. Parasitol., 54, 509-516.

Chernin, E. (1970). Behavioral responses of miracidia of Schistosoma mansoni and other trematodes of substances emitted by snails. J. Parasitol., 56, 287-296.

Chernin, E. (1972). Penetrative activity of Schistosoma mansoni miracidia stimulated by exposure to snail-conditioned water. J. Parasitol., 58, 287-296.

Chernin, E. (1974). Some host-finding attributes of Schistosoma mansoni miracidia. Amer. J. Trop. Med. Hyg., 23, 320-327.

DeHoff, R. and Rhines, F. (1968). Quantitative Microoscopy, p. 18, McGraw Hill, NY.

Eisenberg, B. (1985). Quantitative Analysis of Electron Micrographs, In: Jones: Electron Microscopy by 17 Scientists, pp. 357-377, second ed., Library Research Associates, NY.

Jones, B. and Prewitt, D. (1984). Surface ultrastructure and a morphometric analysis of cilia on Biomphalaria straminea. 12th NIH-MBRS Symp., p. 19 (abstr.), Washington, D.C.

Pan, S. (1980). The fine structure of the miracidium of Schistosoma mansoni. J. Invertebr. Path., 36, 307-372.

Underwood, E. (1970). Quantitative Stereology, pp. 72-94, Addison-Wesley Co., Readings, MA.

Weibel, E. and Elias, H. (1967). Quantitative methods in morphology. In: Proc. Sympos. Quant. Methods in Morph., p. 32-40, Springer-Verlag, NY.

Weibel, E. (1969). Stereological principles for morphology in electron microscopic cytology. Int. Rev. Cytol., 26:235.

Weibel, E. (1970). Stereological techniques for electron microscopic morphometry. In: Principles and Techniques of Electron Microscopy, edited by M. A. Hayat, Van Nostrand Reinhold Co., NY.

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