LIGHT AND SCANNING ELECTRON MICROSCOPIC OBSERVATIONS OF THE LARVAL STAGES AND ADULT OF SPHAERIDIOTreMA GLOBuLUS (TREMATODA: PSILOSTOMIDAE)1

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ABSTRACT

Sphaeridiotrema globulus (Rudolphi, 1814) is a digenetic trematode occurring in river and lentic aquatic systems in North America and has been identified as the cause of ulcerative hemorrhagic enteritis in waterfowl. The purpose of this study was to describe the stages of S. globulus using light (LM) and scanning electron microscopy (SEM). Elimia virginica snails infected with S. globulus were collected from Lake Musconetcong, NJ. Daughter rediae and cercariae were collected from the infected snails and LM and SEM microscopy were used to describe these stages with particular emphasis on the tegumentary surface. The tegument of S. globulus daughter rediae and cercariae is aspinose. The daughter rediae and cercariae showed simpler tegumentary structures than similar stages described in several species of echinostomids. [J PA Acad Sci 86(1): 61-68, 2012]

INTRODUCTION

Sphaeridiotrema globulus (Rudolphi, 1814) (Psilostomidae) is a cosmopolitan digenean that infects the digestive tract of waterfowl. It causes severe hemorrhagic enteritis that may be fatal to the host. Price (1934) reported three fatal outbreaks of S. globulus in the lesser scaup (Marisa affinis) on the Potamac River in Washington, DC. Huffman and Roscoe (1989) and Mucha and Huffman (1991) studied the pathogenic effects of this fluke in experimentally infected mute swans (Cygnus olor), Canada geese (Branta canadensis), and mallard ducks (Anas platyrhynchos). Sauer et al. (2007) investigated waterfowl die-offs from S. globulus in the Upper Mississippi River National Wildlife and Fish Refuge. The larval stages of S. globulus occur in Elimia virginica (Pleuroceridae) snails (Huffman and Fried 1983).

Huffman (1986) examined the structure and composition of the metacercarial cyst wall of S. globulus by light and transmission electron microscopy. McLaughlin et al. (1993) reported on the scanning electron microscopy (SEM) of adult of S. pseudoglobulus. The purpose of this study was to describe the stages of S. globulus using light (LM) and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Elimia virginica snails were collected from Lake Musconetcong (40045'N, 74042'W) located in Morris County, New Jersey. Snails were collected by hand and transported to the laboratory in coolers. Infected snails were identified and cercariae collected following previously described methods within 24 hr of collection (Huffman and Fried 1983). All cercariae were examined using a compound microscope at 100x and 400x. Cercariae of S. globulus were identified based on the specific characteristics given in Macy and Ford (1964).

Four adult Pekin ducks were each infected with 500 S. globulus metacercariae. The ducks were necropsied on days 4 and 6 postinfection. Intestinal tissue with worms attached, and isolated worms were collected, washed in phosphate buffered saline (PBS), fixed in cold 3% gleraldehyde in 0.2 M phosphate buffer (pH 7.0) and used for SEM. Some adult worms were teased with needles to collect eggs. Some of the eggs were fixed in 3% gleraldehyde, and others were placed in spring water and incubated at 250C to study development. Eggs were randomly selected, and observed for development, at 4 day intervals for the first 16 days postembryonation, and daily after day 16.

Sphaeridiotrema globulus metacercariae, cercariae and daughter rediae were fixed in 10% neutral buffered formalin for 1-2 hr for observations by LM. Fixed cercariae and rediae were mounted on slides in a drop of water, covered with a coverslip, and measured under a compound microscope with the aid of a calibrated ocular micrometer. For the rediae, the total length, total width, and the pharyngeal width were measured, and the number of cercariae in the daughter rediae was recorded. For cercariae, the body length, body width, and tail length, were measured. The mean, standard deviation (SD), and range were determined for each measurement using Microsoft Excel.

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Free swimming *S. globulus* cercariae released from snails, and daughter rediae dissected from snails were fixed in 3% gluteraldehyde in 0.2 M phosphate buffer (pH 7) for 24 hr for SEM. The samples were dehydrated in a graded ethanol series (70%, 85%, 90%, and 100%) and post fixed with 1% osmium tetraoxide for 1-2 hr. The samples were again dehydrated in a graded ethanol series (70%, 85%, 90%, and 100%), and placed in examethyldisilazane (HMDS) (Polysciences, Inc., W arrington, PA) for 15 min and then allowed to air dry. Samples were attached to stainless steel stubs using double sided tape and sputter coated with gold palladium. All samples were examined with an Amray 1810 Scanning Electron Microscope at an acceleration of 20kV at varying magnifications.

**RESULTS**

The length of the adult worm (Figure 1A) ranged from 850 to 940 μm with a mean ± SD of 900.4 ± 4.5 μm. The width ranged from 350 to 560 μm with a mean ± SD of 487 ± 7.3 μm. The oral sucker length ranged from 56 to 81 μm with a mean ± SD of 73.3 ± 0.7 μm. The width ranged from 46 to 60 μm with a mean ± SD of 53.5 ± 0.5 μm. The length of the acetabulum ranged from 361 to 402 μm with a mean ± SD of 379 ± 1.2 μm. The width ranged from 79 to 90 μm with a mean ± SD of 84.9 ± 0.5 μm. The length of the tegumentary spines ranged from 27 to 31 μm with a mean ± SD of 28.4 ± 0.13 μm. The aciliate papillae diameter ranged from 27 to 31 μm with a mean ± SD of 28.4 ± 0.09 μm.

SEM of two adult *S. globulus* (Figure 1B) attached to the intestine illustrates the erosion of the intestinal villi. The outer surface of the adult *S. globulus* was covered with tegumental protuberences with both deep and shallow irregular transverse grooves that encircled the body (Figure 2 A & B). Numerous aciliate papillae surrounded the oral sucker (Figure 3A). The diameter of the papillae was 8.2 ± 0.09 μm (Figure 3B). The lip of the ventral sucker was comprised of rows of spines in an alternating pattern (Figure 3C). The acetabular spines were 2.84 ± 0.13 μm long (Figure 3D). The excretory pore was located dorso-posteriorly (Figure 4A). Protuberances with transverse grooves appeared more compact in this area than elsewhere on the tegument. Papillae were present on the dorsal surface measuring 2.75 ± 0.09 μm in diameter (Figure 4B).

Fifty eggs dissected from worms were measured (Figure 5A & B). The egg shell length ranged from 77 to 99 μm with a mean ± SD of 89 ± 0.7 μm. The width ranged from 70 to 79 μm with a mean ± SD of 73.1 ± 0.28 μm. The opercular diameter ranged from 24 to 31 μm with an average of 26.4 ± 0.2 μm. The abopercular knob diameter ranged from 24 to 32 μm with a mean ± SD of 26.4 ± 0.23 μm. The topography of the egg shell and operculum was smooth (Figure 6A). The abopercular knob was uneven with an apparent ring (Figure 6B & C), and had no folds.

Fifty daughter rediae (Figure 7A) of *S. globulus* dissected from *E. virginica* snails were measured. The total length of the daughter rediae ranged from 672 to 976 µm with a mean ± SD of 810.8 ± 10 μm. The width of the daughter rediae ranged from 288 to 400 μm with a mean ± SD of 366.8 ± 3.6 μm. The pharyngeal diameter of the rediae ranged from 9 to 13 μm with a mean ± SD of 10.5 ± 1.2 μm. The number of cercariae within the redia was 6-8 with a mean ± SD of 6.4 ± 0.1 per redia.

The SEM of daughter rediae of *S. globulus* had a papilliform process located at the posterior end of the redia (Figure 7B & C), and two ambulatory buds located on the
posterior third of the redia (Figure 7D). An excretory pore (Figure 8A) was located centrally on the redia; a mouth containing a muscular pharynx was located at the anterior end (Figure 8B), and a birth papilla was located just posterior to the mouth (Figure 8C).

Figure 9A shows the numerous microvilli and uniciliate papillae of the daughter redia of *S. globulus*. Figure 9B shows a uniciliate papillae. The mouth of the daughter redia was surrounded by a collar containing numerous concentric tegumental folds (Figure 9C). The birth pore was 15 ± 2.0 μm in length, and 4 ± 0.25 μm in width. The papilliform process had irregular tegumental folds (Figure 9D). The tegument was covered with protuberances (Figure 10A). Deep or shallow transverse grooves encircled the redia (Figure 10B).

Fifty *S. globulus* cercariae obtained following isolation of *E. virginica* were measured by LM. The body length of the cercariae (Figure 11) ranged from 158 to 212 μm with a mean ± SD of 190.8 ± 1.8 μm. The body width ranged from 106 to 172 μm with a mean ± SD of 136.1 ± 2.3 μm. The tail length ranged from 272 to 380 μm with a mean ± SD of 328.8 ± 4.2 μm. The length of the ventral sucker ranged from 48 to 72 μm with a mean ± SD of 60.8 ± 0.8 μm. The width of the ventral sucker ranged from 64 to 84 μm with a mean ± SD of 73.8 ± 1.3 μm. The spines on the ventral sucker ranged in length from 0.7 to 1.3 μm with a mean ± SD of 1.04 ± 0.05 μm. The aciliate papillae were 0.50 μm in length and the uniciliate papillae were 1.0 μm in length.

SEM of the cercariae (Figure 12A) showed the cup-shaped structure of the cercarial body. The cercarial tail...
lacked fin folds (Figure 12A). A lateral groove extended the length of the tail. The tail tegument consisted of numerous protuberances that occurred in a repeated rectangular pattern (Figure 12B).

The tegument of the body was surrounded by protuberances and shallow or deep transverse grooves which were covered with minute spines (Figure 12C). Dispersed randomly among the spines were uniciliate papillae. The uniciliate papillae were short and extended just above the tegument; the diameter of the papillae was 0.50 μm (Figure 12C). The sensory papillae varied in size. The cercarial acetabulum was located medially (Figure 13A). Small spines were present on the tegument of the acetabular lip, pointing away from the ventral opening (Figure 13B). The spines measured 1.04 ± 0.05 μm in length. Inside the acetabular lip, surrounding the opening, was a ring of tightly packed knob-like tubercles with smooth surfaces (Figure 13C).

The interior of the oral sucker (Figure 14A) was surrounded by aciliate papillae and uniciliate papillae (Figure 14B). Six aciliate papillae appeared to form a crown close to the oral opening. These papillae contained cilia of varying lengths, and were scattered throughout the tegument. The aciliate papillae measured 1 μm in diameter, and showed a pattern of circular rows surrounding the body.

The metacercariae of *S. globulus* are spherical in shape, with a diameter that ranged from 119 to 137 μm with a mean ± SD of 125.4 ± 0.7 μm (Figure 15A). SEM of the cysts indicated that the outer layer is smooth with a wrinkled surface (Figure 15B & C).

**DISCUSSION**

In the adult the oral sucker is surrounded by numerous aciliate papillae. These papillae occur without a distinct pattern and may serve a sensory function, aiding the adult worm in locating highly vascularized areas in the host gut. A second type of papilla (ciliate) was observed on the adult *S. globulus*. It occurred on both the dorsal and ventral surfaces, and did not have any regular pattern. It had a dome-like region located close to the tegumentary surface with a short cilium extending just above the tegument; this type of papillae may have a sensory function as noted in studies on various echinostomatids (Fried and Fujino 1987).
The acetabulum of *S. globulus* is surrounded by spines which occur in rows extending in an alternating pattern. The spines point away from the acetabulum as described by Barber and Caira (1995) for *Austrobilharzia variglandis*. The excretory pore of the *S. globulus* adult was oval in shape, and numerous protuberances surrounded the pore.

McLaughlin et al. (1993) compared the morphology and morphometrics of adult *S. globulus* and *S. pseudoglobulus* grown experimentally in domestic ducklings from metacercariae obtained from *E. virginica* from Lake Musconetcong in Netcong, NJ and from *Bithynia tentaculatum* collected from the Riviere du Sud, near Lacolle, Quebec. Differences in morphologic measurements of the worms were noted between the McLaughlin et al. (1993) study and this one. The McLaughlin et al. (1993) study made measurements using LM and this study used SEM.

Many radial measurements based on LM and SEM have been done on various species of the family Echinostomatidae. By comparison the radial length of *S. globulus* is smaller than that reported for *Echinostoma trivolvis* and *E. caproni* (Fried and Awatramani, 1992; Krejei and Fried, 1994). The radial width of *E. trivolvis* and *E. caproni* is larger than that of *S. globulus* (Fried and Awatramani, 1992; Krejei and Fried, 1994). The pharyngeal diameter of *S. globulus* was smaller (10.5 ± 1.2 μm) than measurements obtained for *E. caproni* (56 ± 10 μm) and for *E. trivolvis* (40.2 ± 1.1 μm) (Fried and Awatramani, 1992; Krejei and Fried, 1994). The number of mature cercariae for *E. caproni* was 4.8 ± 1.4, and for *E. trivolvis* it was 3.2 ± 1.1 (Fried and Awatramani, 1992; Krejei and Fried, 1994). The number of cercariae for *S. globulus* was greater with a mean of 6.4 ± 0.1.

The tegumentary structure of the rediae of *S. globulus* has not been previously described. Transverse folds were seen along the length of the daughter rediae of *S. globulus* as seen in various other trematodes (Fujino and Ichikawa, 2000). Numerous uniciliated papillae were seen surrounding the mouth of the rediae of *S. globulus*. The uniciliated papillae are probably sensory structures and have been seen in several other trematode species (Valkounova et al. 1989; Fujino and Ichikawa, 2000). The distal portion of the lobed collars and the ambulatory buds were observed to have a ridged tegumentary structure, which was devoid of other structural features. The lack of transverse folds at the distal portion of these structures may relate to use of these structures for locomotion and positioning within the snail.

Spines did occur around the margin of the ventral sucker of *S. globulus*. Transverse ridges or folds were observed along the body of the cercariae. Unciliated papillae were observed over the entire cercariae. The cilia observed on the cercariae were longer than those observed around the mouth of the rediae. The uniciliated papillae probably have a sensory function and aid the cercariae in its search of a second intermediate host and possibly an encystment site in that host. Small spherical bodies were also
Figure 9. A. *S. globulus* redia illustrating microvilli (MV) and uniciliate papillae (P). Bar = 1 μm. B. uniciliate papilla. Bar 1 μm. C. Redial collar with concentric folds. Bar = 10 μm. D. Irregular folds of papilliform process. Bar = 10 μm.

Figure 10. A. Protuberances on the surface of the redia. Bar = 1 μm. B. Deep transverse grooves of the redial tegument. Bar = 10 μm.

Figure 11. Light micrograph of *S. globulus* cercaria. Stained with Mayer’s carmine.

Figure 12. A. Cercarial tail lacking fin fold. Bar = 100 μm. B. Lateral groove and rectangular protuberences. Bar = 10 μm. C. Protuberances and minute spines on the cercarial tegument. Bar = 1 μm.

Figure 13. A. Cercarial acetabulum. Bar = 10 μm. B. Acetabular lip with spines and uniciliate papillae. Bar = 1 μm. C. Uniciliate papillae. Bar = 1 μm.
observed on the tegument of the cercariae. These spherical bodies have been observed in several other species of trematodes but the exact function is unknown (Fujino and Ichikawa, 2000).

Although McLaughlin et al. (1993) did not find acetabular spines in the *S. globulus* specimens he examined, we report the presence of spines in worms of this species. Reasons for this difference are not apparent at this time.

The measurements of the rediae and cercariae presented here represent the average values for *S. globulus* found in *E. virginica* snails in Lake Musconetcong. These measurements should aid in a better understanding of distinguishing characteristics of species of the genus *Sphaeridiotrema* or for identifying potential geographic differences where closely related species of *Sphaeridiotrema* occur.

**LITERATURE CITED**


