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SCANNING ELECTRON MICROSCOPY (SEM) OF EGG OF *PROTEOCEPHALUS FILICOLLIS* RUDOLPHI (CESTODA, PROTEOCEPHALIDEA)

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Abstract: Scanning electron microscopic study of eggs of *Proteocephalus filicollis* has demonstrated that the outer surface of the egg has invaginations and gives an irregularly contoured appearance. The external surface of the outer envelope of the embryo has shown to have two forms of surfaces on opposite sides. This is the first study of its type on this fish cestode eggs.
Key words: *Proteocephalus filicollis*,, scanning electron microscopy (SEM) of eggs.

INTRODUCTION

The ultrastructure and the development of the egg and embryonic envelopes of proteocephalid cestode have been little studied. Formation of four main embryonic envelopes, the capsule, outer envelope, inner envelope and oncospheral membrane around the developing embryo of *Proteocephalus longicolis* is described (Swiderski and Subilia, 1978).

Proteocephalus filicollis is a host specific parasite of temperate freshwater fish three-spined stickleback, *Gastersteus aculeatus* L.. The fish is final host of the parasite. Ultrastructure of the egg and embryonic envelopes of *P. filicollis* are well described (Iqbal and Wootten, 2002), Fine structure of spermatozoon of *P. filicollis* is described by (Iqbal, 2003). Present study was aimed to understand the fine and superficial structure of the outer envelop of the egg of *P. filicollis* by histology and Scanning Electron Microscopy.

MATERIALS AND METHODS

Histology

Sampling of fish, procurement of the gravid worms is given by Iqbal and Wootten (2002). For the study of the orientation of the eggs of P. *filicollis* the gravid worm were cut into very small pieces. The pieces from the posterior of the worm were fixed in 10% buffered formalin for at least 24 hrs. The fixed material was automatically

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processed in Histokinette 2000. Sections of wax embedded worms were cut at 5-6 μm. The cut sections were spread and floated on a water bath and then placed on a clean glass slide. The glass slide was placed face down on a hot plate. Sections were stained with hamatoxylin and eosin.

Scanning electron microscopy (SEM)

Proteocephalus ,filicollis eggs were collected in water in a syringe and deposited on a Sartorius polymied filter with a pore diameter of 0.45 μm. The filter membrane with the eggs was put into a Petri dish and flooded with 1% glutaraldehyde buffered with 0.1 M sodium cacodylate and left at 4°C for one hour, after which the solution was replaced with 3% glutaraldehyde buffered with 0.1 M sodium cacodylate at 4°C, in which the specimen was kept for further two days. The eggs were than washed well in sodium cacodylate buffer and post fixed in 1 % osmium tetra oxide in 0.1M sodium cacodylate for two hours at room temperature. The specimen were dehydrated through an acetone series and then transferred to a mixture of 50% Peldri (Ted Pella Inc, Reading California) and 50% acetone in the fume cupboard for an hour. This was replaced by full strength Peldri for an hour, after which the Petri dish was placed on ice to solidify he Peldri. The peldri was sublimed off in the fume cupboard overnight. The filter was then mounted on an aluminum stub and sputter coated with gold in an Edwards 150 B spurt coater, before being examined in Philips 500 scanning electron microscope at 6 Kv.

RESULTS AND DISCUSSION

The uterus of gravid *P. filicollis* consists of a number of diverticula which contain eggs. Eggs are not tightly packed and are distributed through out the diverticula (Fig.1). When viewed by SEM the outer float membrane of *P. filicollis* egg is not swollen but is stretched as a sheath from the outer envelope (Fig.2). The external surface of the outer envelope has an irregularly contoured appearance with small invaginations and pits. In a few cases the external surface of the outer envelope showed two forms of surface sculpturing on opposite side of the embryo, one with broad invaginations and other with more wrinkled appearance (Fig.3, 4).

Scanning electron microscopy *ot P. filicollis* eggs provides little information about the membranes surrounding the oncosphere. The stretched appearance probably represents the capsule, which have obtained this shape during SEM processing. The membranous and somewhat folded structure of *P. filicollis* capsule allows it, to swell to form the float membrane when it comes into contact with water. Similarly, when the outer capsule of *Shipleya inermis* is fully formed it is folded much as a packed parachute, with a very large surface area contained in a small volume (Coil, 1977). An explanation of the invaginations or pits observed on the outer surface of the oncosphere of *P. filicollis* is somewhat problematical. However, it is reported that in cestode having the aquatic phase of their life cycle in freshwater, the egg shell is only superficially pitted (Hillhead, 1972). In the same way, it was found that in the eggs of *Shipleya inermis* the outer surface of the

outer capsule is relatively smooth but has numerous pits (Coil, 1977). The outer envelope of the oncosphere of mice tapeworm *Hymenolepis nana* has been found to have irregular contoured appearance marked by numerous pits (Fairweather and Threadgold, 1981). They also reported that much wider and deeper crests were present in the outer envelope and suggested that this may indicate that the layer is undergoing some degree of degeneration. A similar explanation may account for the surface pits on the eggs of *P. filicollis*. The two types of surface sculpturing on opposite sides of the egg of *P. filicollis* seen in the present study are difficult to explain and they may represent a processing artifact.

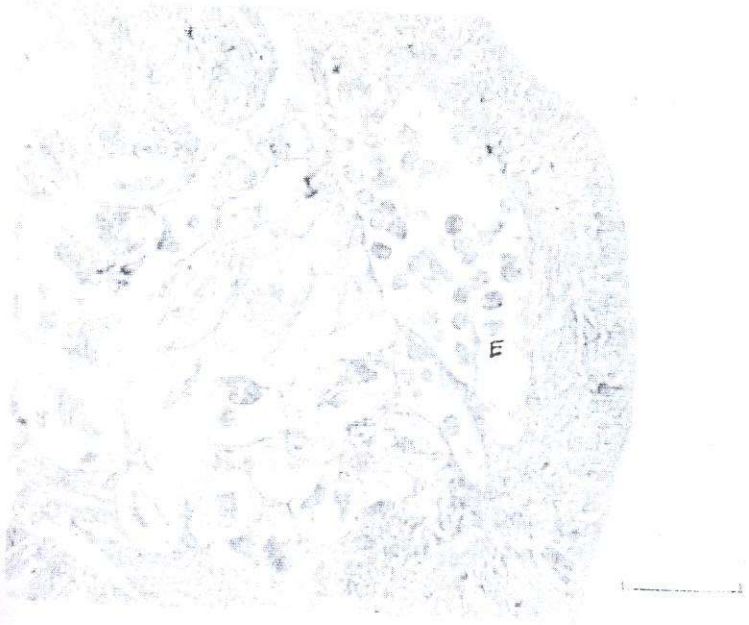

Fig.1: A transverse section of gravid proglottid of *Proteocephalus filicollis* showing eggs (E) in the uterus (H & E). Scale bar = 100 μm).

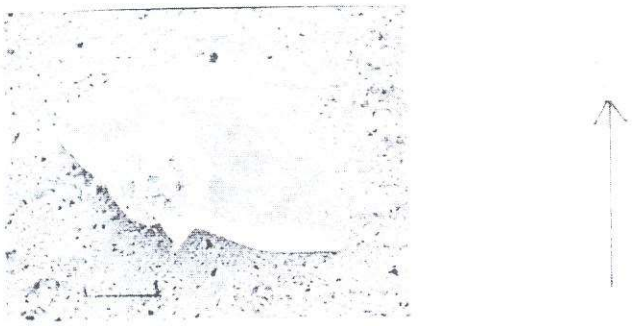

Fig. 2: SEM micrograph of egg of *Proteocephalus filicollis*. The probable capsule (C) is seen stretched as a sheet (Scale bar = 5 μm).

Fig. 3: SEM micrograph of egg of *Proteocephalus filicollis* showing external surface with irregularly contoured appearance (Scale bar = 7.5 μm).

Fig. 4: SEM micrograph of egg of *Proteocephalus filicollis* showing different Sculpturing on opposite sides of the embryo (Scale bar = 5 μm).

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