

Surface ultrastructure of a Malaysian *Spirometra* sp. - a preliminary study

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Abstract

The external morphology of the egg, plerocercoid and adult stage of *Spirometra* sp. (Malaysian strain) was studied by scanning electron microscopy (SEM). The egg-shell had scattered deep pits on the surface and the pits varied in size from small to large. The microtriches on the anterior, body and posterior part of the plerocercoid stage were conoid.

Keywords: ultrastructure; *Spirometra* sp.; SEM

Introduction

The cestode surface is covered with numerous microtriches, whose principle function is to absorb nutrients and protect the body from host immune response (Lee, 1972; Lumsden, 1975). The life-cycle of *Spirometra* sp. consists of eggs, coracidium, proceroid stage, plerocercoid (sparganum) stage and adult stage. In this paper, we report the preliminary results of the scanning electron microscopic study on the eggs, sparganum (plerocercoid) stage and the adult stages of *Spirometra* sp. (Malaysian strain).

Materials and methods

Spargana of *Spirometra* sp. (Malaysian strain) were obtained from naturally infected frogs (2nd intermediate hosts) caught in Tanjung Karang, Selangor, Malaysia. Spargana were fed to experimental cats (laboratory model for definitive host) and *Spirometra* sp. eggs were collected from cat's faeces 14 days post infection by sieving fecal suspension.

For EM study, the eggs were kept in physiological saline at 40°C. The eggs were then smeared on a filter paper and coated with gold using the filter paper culture method by Harada & Mori (1955) with modifications, and were observed under low magnification. We did not incubate the eggs before observing it under the low magnification, and they were smeared directly onto the filter paper. Experimental cats were sacrificed 2 - 3 weeks post infection and the adult worms of *Spirometra* sp. were recovered.

The specimens were fixed in 2.5 % glutaraldehyde (GA) and 0.5M PBS (pH. 7.2 - 7.4), then post-fixed with 2.0 % Osmium tetroxide (OsO₄) and 0.5M PBS for 1 hour. It was rinsed a few times with PBS and the specimens were dehydrated through a graded series of ethanol (ascending concentration). They were then dried in a CO₂ critical-point-apparatus, coated with gold, and studied with a Hitachi S430 scanning electron microscope.

Results

The egg is ovoidal shaped (Fig. 1a) with a clear opercular suture (Fig. 1b) and the surface full of scattered deep pits (Fig. 1c). The anterior part of plerocercoid shows a 'spoon-like' structure (Fig. 2a & b,) and on the surface, conoid microtriches were observed (Fig. 2c). Figures 3a and 3b shows the apical view of the anterior end of the plerocercoid stage. The microtriches around the frontal pit of the plerocercoid were modular (Fig. 3c and d). The scolex of the young adult worm was spatulate, and the bothria appears well developed compared to the plerocercoid stage (Fig.4).

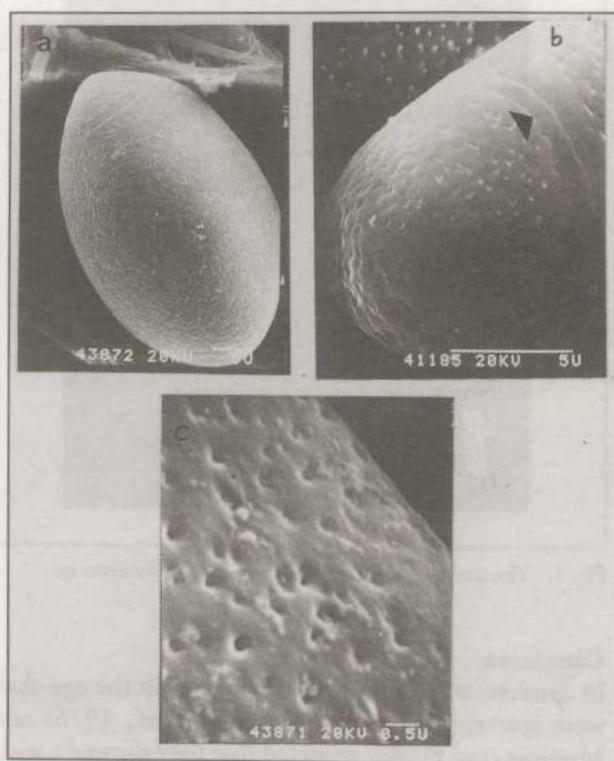


Fig. 1. (a) egg of *Spirometra* sp. under low magnification; (b) arrow-head shows the opercular suture; (c) pits on egg-shell.

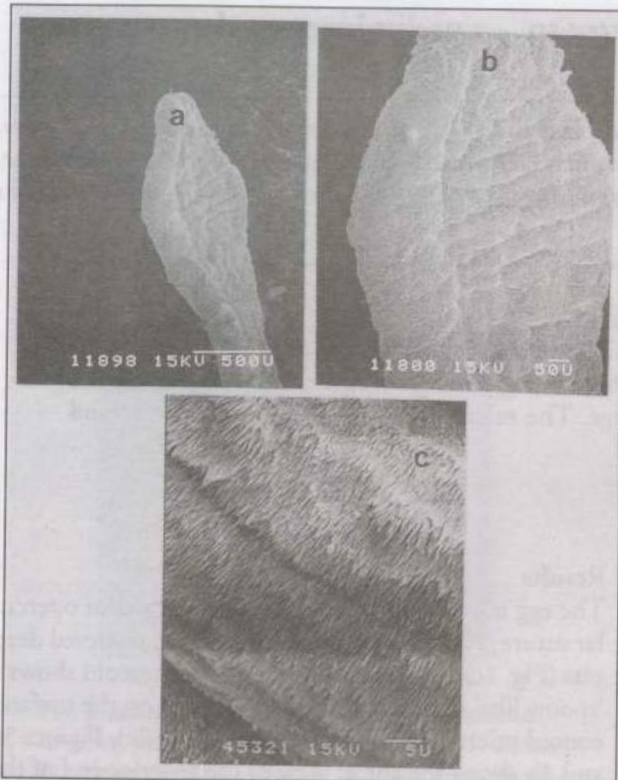


Fig. 2. (a) anterior of the larval stage; (b) higher magnification of anterior end; (c) conoid microtriches covering the anterior end.



Fig. 4. The scolex of a young adult worm of *Spirometra* sp.

Discussion

In *Spirometra erinacei*, the shallow pits on the egg-shell were sparsely distributed (Yamane *et al.*, 1976) and Maejima *et al.* (1983) reported that they showed a wide variety of size, shape and density, without actual measurements. The egg-shell of *Spirometra* sp. (Malaysian strain) had deep pits on the surface and showed a vari-

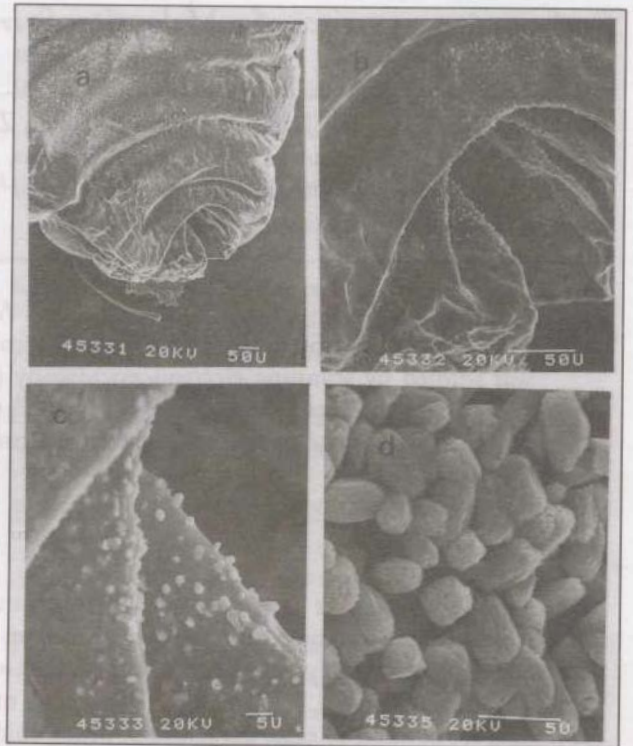


Fig. 3. (a) apical view of anterior end of larval stage covered with microtriches; (b) higher magnification of anterior end; (c) nodular microtriches at anterior end; (d) higher magnification of microtriches.

ety of size and shape (Fig. 1c) resembling *Spirometra erinacei* which have deep pits on the surface ranging from 25-370 μ m (Okino, 1996).

The microtriches of the cestode change from conoid to digitiform through the proceroid to the adult stages (Branten, 1968a, b; Grammelvedt, 1973; Lumsden *et al.*, 1974; Yazaki *et al.*, 1990). The structure and dimensions of the microtriches vary in different regions of the body surface (Thompson *et al.*, 1980, 1982). In *Spirometra erinacei*, filamentous microtriches were restricted to the scolex and seen in 7 day old plerocercoids up to adult worms (Okino, 1996). In *Echinococcus granulosus*, filamentous microtriches were restricted to the scolex and are believed to play an important functional role at the interfacial region between the rostellum of the holdfast organ and the host mucosa (Thompson *et al.*, 1982). From our results, conoid microtriches were observed in the anterior and posterior part of the plerocercoid of the Malaysian strain and we assume that the conoid microtriches play a similar important role in attachment to the host mucosa as the filamentous microtriches.

Yamane *et al.* (1974) found many papillae on the surface of the central region of mature proglottides of *Spirometra erinacei*. Okino & Hatsushika (1994) reported that three types of clumps of papillae were present on the surface of fully gravid proglottides and the papillae were characteristic for *S. erinacei* adult. These can also be a useful index for cestode identification. The scolex of the adult worm of *Spirometra* sp. (Malaysian

strain) have dense papillae on its surface and work is on-going to determine whether it has 3 types of papillae as described by Okino & Hatsushika (1994).

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