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Surface topography and tegumental morphology of adult digenetic trematode of Indian strain of *Fasciola gigantica* Cobbold

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Abstract

Fasciolosis disease is caused by the most common species *Fasciola hepatica* and *Fasciola gigantica*. Surface topography and some of the important features of morphology of the Indian *F. gigantica* has not yet been described and therefore, the present study was elucidated about the important structural surface topography features of the *F. gigantica*. Adult flukes of *F. gigantica* are flattened and leaf-like in shape and measures approximately 5-6 cm in length, with narrowed anterior and posterior ends and are the presence of uneven surface with alternating surface folds armed with spines. At the anterior end the oral sucker occurs at its tip while the ventral sucker is present at the oral cone-main body junction, they are used to grip the lining of the bile duct of the liver of *Bubalus bubalis* and to assist in the movements of the fluke and the spines are absent around the suckers. The common genital opening for the male and female reproductive systems genital pore which is present just anterior to the ventral sucker. The posterior tip of the body has a prominent excretory pore first time reported with very few spines are present at the posterior end of the body and hence appear smooth.

Keywords: *Fasciola gigantica*, Surface topography, excretory pore

Introduction

Fasciolosis disease is caused by the most common two species *Fasciola hepatica* and *Fasciola gigantica*. *F. gigantica* predominately occurs in tropical regions. *F. gigantica* has been recorded to occur in India, South and Eastern Asia, Africa, the Middle East Europe, America and Australia. In developing countries such as India, treatment is exorbitant and unaffordable, it is normal to accept the damage caused by Fasciolosis (Dumag, *et al*., 1979), or give up ruminant production (Kendall, 1954).

The surface topography of *F. hepatica* and *F. gigantica* are already described in detail by many parasitologists (Bennet, 1975a,b; Fairweather*et al.,* 1999; Dangprasert *et al.*,, 2001; Meaney *et al.,* 2002). Oral sucker and ventral sucker present in the anterior region and tegumental spines were present in all over the body with various size and shape (Fairweather *et al.,* 1999; Dangprasert *et al.,* 2001; Meaney *et al.,* 2002). However, surface topography and some of the important features of morphology of the Indian *F. gigantica* has not yet been described and therefore, the present study was initiated to elucidate the surface topography of the *F. gigantica* and to clarify many of the important structural features.

Materials and methods

Specimen Collection

Fasciola gigantica was collected from the infected liver of the livestock (Buffalo - *Bubalus bubalis*) slaughtered in (Perambur abattoir house) Chennai, Tamil Nadu, India and flukes were rinsed and brought to the laboratory with saline (0.9% NaCl).

Preparation of adult F. gigantica for Scanning Electron Microscopy (SEM) (see Ramasamy, 1996)

Adult *F. gigantica* were fixed for 4 - 6 h at 4°C in 4% (w/v) glutaraldehyde-paraformaldehyde buffered to pH 7.4 with 0.1 M sodium cacodylate/HCl containing 3% sucrose and repeatedly washed in 0.1 M sodium cacodylate buffer (pH 7.4) containing 3% (w/v) sucrose for approximately 24 h at 4°C. *F. gigantica* were post fixed in 1% aqueous osmium tetroxide for 1 h and repeatedly washed in 0.1M sodium caccodylate buffer (pH 7.4) containing 3% (w/v) sucrose for 24 h. Dehydrated in an ascending series of acetone and the flukes were dried in a critical-point dryer using liquid CO_2. Adult *F. gigantica* was mounted on aluminium stubs with double – sided adhesive tape, coated with gold/palladium in a sputter coater and examined in ISI ABT-35 or Joel SEM 6400 scanning electron microscope operating at 10 kv.

Results

Surface topography of F. gigantica

The surface topography of adult *F. gigantica* is described. Adult flukes of *F. gigantica* are flattened and leaf-like in shape and measures approximately 5-6 cm in length, with narrowed anterior and posterior ends (Figs.1a&b). At the anterior end the oral sucker occurs at its tip while the ventral sucker is present at the oral cone-main body junction. The oral and ventral suckers are the main organs of attachment of *F. gigantica* and they are used to grip the lining of the bile duct of the liver of *Bubalus bubalis* and to assist in the movements of the

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fluke. The common genital opening for the male and female reproductive systems genital pore which is present just anterior to the ventral sucker. In light microscopy, a small pinkish colour spot could be seen just behind the ventral sucker representing the Mehlis' gland (Fig.1b).

SEM observations on the surface of *F. gigantica* revealed the presence of numerous spines and surface folding (Fig.2a). Both oral and ventral suckers are spineless and have thick rims covered with transverse folds. The gonopore is located between the oral and ventral sucker (Figs.2b&c). Based on the size, shape and arrangement of the spines, transverse folds, grooves and presumed sensory papillae, the body of *F. gigantica* can be divided into three regions viz., anterior, middle and posterior.

Ventral surface of F. gigantica

Anterior region: The spines are small and closely – spaced at the anterio-ventral surface of the body. Each spine has a serrated edge and measures 20µm in width and 30 µm in height. The surface of the spines appears highly corrugated and invaginated with small ridges and pits (Fig.3a). The surface area between the spines appears corrugated with transverse folds alternating with grooves. At higher magnifications the folds are, in turn composed of a meshwork of interlacing microfolds or small ridges separated from one another by variable-sized pits (Fig.3a). In some areas, there are groups of bulbous papillae like sensory receptors. There are three types of presumed sensory receptors recorded. The first two types of papillae, types 1 and 2, have nipple-like tips, with type 2 also having short cilia on their tips. The third type of papillae, type 3, is fungiform in shape with a smooth top and highly pitted base. Each papilla appears as a small dome, 4-6 µm in diameter at the base. On the anterio-lateral surfaces, type 1 and 2 presumed sensory papillae appear singly or in a group of two to three units. In contrast, both the oral and ventral suckers have thick muscular rims covered with wide transverse folds, surrounded by rows of type 3 papillae in large clusters, and pores of gland cells.

Middle region

On the surface of the mid-ventral region of the body, the spines increase in number and size particularly towards the edges of the body. The large sized spines have blunt rather than sharp serrated edges. The area between the spines appears highly corrugated with ridges separated by pits and slits. This area also contains large groups of presumed sensory papillae with similar characteristics to those found on the anterior region. Towards the lateral aspect of the body, the spines and clusters of presumed sensory papillae become very prominent (Fig.3b).

Posterior Region

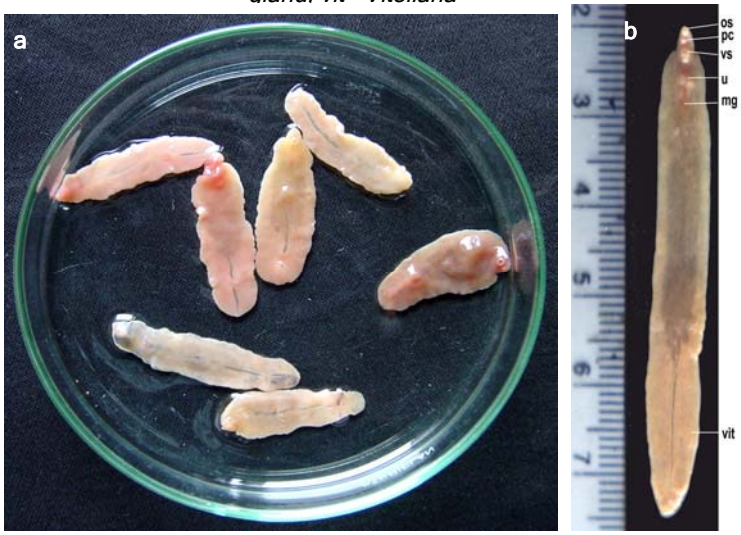
On the surface of the posterio-ventral region of the body, the spines progressively decrease both in size and number and they remain unconnected. The spines are short and covered with highly invaginated surface (Fig.4a). Clusters of presumed sensory papillae are prominent as those on the mid-lateral region. The area between the spines also appears highly folded and invaginated, but the ridges are not as well developed as those on the anterio-middle regions. The posterior tip of the body has a prominent excretory pore very few spines are present at the posterior end of the body and hence appear smooth (Figs.5a&b).

Dorsal surface of F. gigantica

Anterior and Middle regions: Generally, the anterio-middle regions of the dorsal surface exhibit very similar features to those of the ventral surface except that they have smaller-sized spines and fewer presumed sensory papillae. Spines on the anterio- middle regions are still serrated, while those located towards the posterio- lateral regions are smaller and not well serrated. The surface area between the spines appears highly convoluted with folds and grooves, but ridges on each fold tend to be flattened when compared with those on the anterio-mid-ventral surface (Fig.4b).

Posterior region: The posterior region of the dorsal surface possesses fewer, smaller and widely spaced spines. Each spine is short and unserrated. They are covered with a highly invaginated surface. The area between the spines is invaginated with large pits, while the ridges are not well developed. Unlike the ventral surface, the posterior end of the dorsal surface has only a small number of presumed sensory papillae (Fig.4b).

Figs.1 a&b. Adult Fasciola gigantica: os – Oral Sucker; pc – Protruding cirrus; vs – Ventral Sucker; u – Uterus; Mg – Mehlis' gland; vit – Vitellaria



Discussion

The present study has elucidated the surface topography of the *F. gigantica* and clarified many of the important structural features. The surface topography of adult *F. gigantica* resembled that of *F. hepatica*. The most incredible surface topography features of adult *F. gigantica* are the

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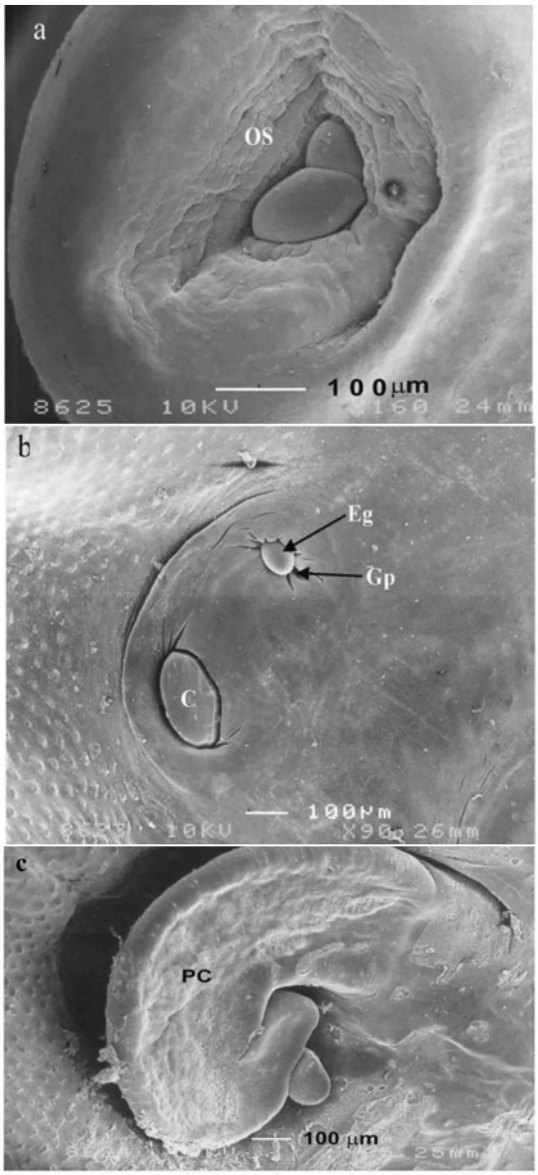
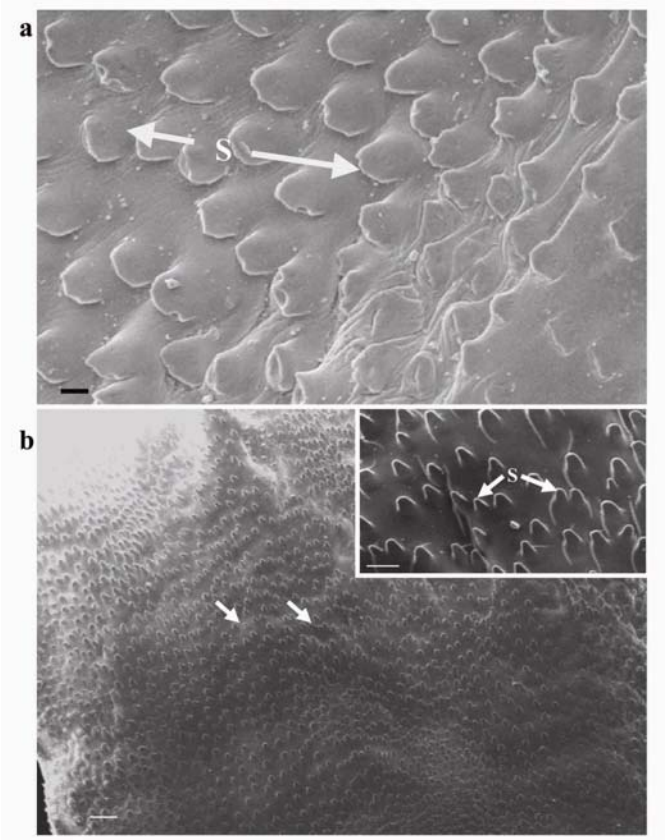
Figs.2a-c. Scanning electron micrograph showing the anterior region and genital pore with egg of the adult F. gigantica. a) SEM of oral sucker; b) genital pore (Gp) of adult F. gigantica. Note the presence of emerging egg via genital pore and c) genital pore with protrusible cirrus and egg. OS-Oral sucker; Eg-Egg; C-Cirrus, PC-Protrusible cirrus.



presence of uneven surface with alternating surface folds armed with spines. The surface folds increase the surface area and could enhance the absorption and exchange of micromolecular nutrients through the tegument, a characteristic feature observed in many other trematodes (Parkening & Johnson, 1969; Nollen *et al.,* 1973; Hockley, 1973; Hockley & McLaren, 1973, 1977; Nadakavukaren & Nollen, 1975; Bennett, 1975b; Jinxin & Yixun, 1981; Fried *et al.,* 1986; Sobhon & Upatham, 1990; Irwin, McCloughlin & Fried, 1991; Kruse *et al.,* 1992; Rosa-Brunet & Fried, 1992; Apinhasmit *et al.,* 1993; Fujino *et al.,* 1994; Ursone & Fried, 1995; Sorensen *et al.,* 1997; Fried *et al.,* 1998; Fried & Reddy, 2000; Nakano, *et al.,* 2003). The tegumental surface annulations, ridges, furrows and lamellar network structures observed in *F. gigantica* may provide flexibility to the worms during motility. There are differences in the occurrence of ridges and pits in different regions of the fluke surface perhaps indicating different degrees of s pecialization of the tegument for various functions including immune responses, ionic and osmotic regulations, absorptive capacities in various regions of the tegument and excretion, a characteristic feature commonly reported from other helminthes (Bennett, 1975b; Hockley &

Figs.3 a&b. Scanning electron micrograph of ventral surface of F. gigantica. a - Anterio-ventral surface showing the closely spaced spines (S). Bar 30 µm. b - Mid-ventral surface showing the increased number of spines (arrows). Bar 200 µm. the insert shows a higher magnification SEM of a portion of Mid-ventral surface spines (S). Bar 80µm.



McLaren, 1977; Smyth & Halton, 1983; Sobhon & Upatham, 1990; Apinhasmit *et al.,* 1993; Ursone & Fried, 1995).

The most unique surface feature of *F. gigantica* is the presence of numerous spines occurring all over the body surface. However, the spines are absent around the suckers and this probably help the parasites for smooth sealing against the host mucosa as suggested by Bennett (1975a) and Bakke (1976a,b). In adult *F. gigantica,* the tegumental spines on the surface may help movement of

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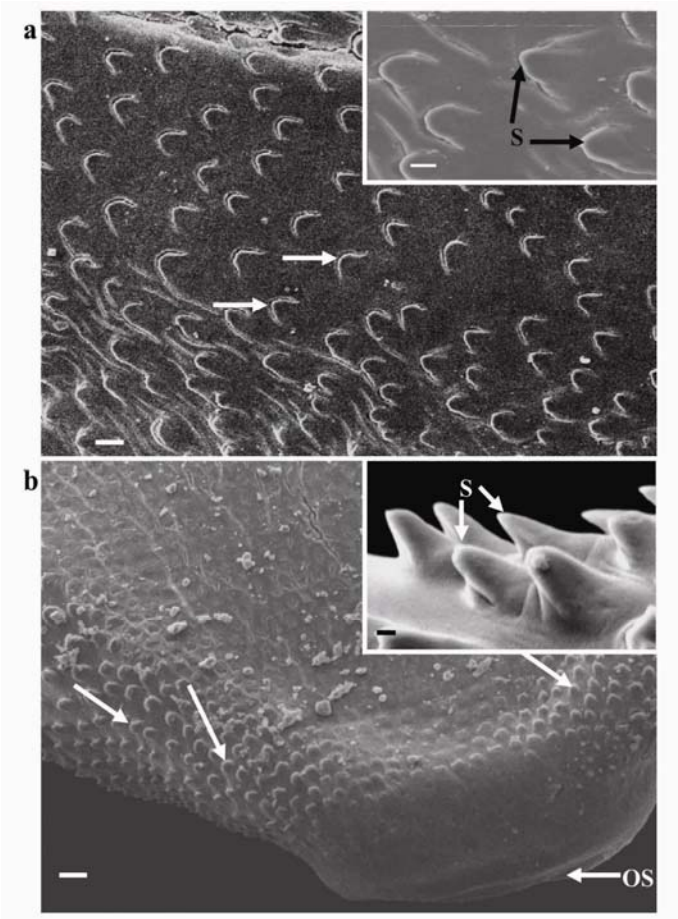
the fluke in the bile ducts of the liver, while the large muscular ventral suckers may help remain to be attached to the wall of the bile ducts and probably prevent the parasite displacement from the host and may further provide resistance to the worm and prevents the dislodgement of the parasite from the bile ducts during the flow of bile juices.

Figs.4 a&b. Scanning electron micrograph of ventral and dorsal surfaces of F. gigantica. a - the posterior-ventral surface showing the wide spaced spines(arrows). Bar 30 µm. The insert shows a portion of magnified SEM view of the posterior-ventral surface spines (S). Bar 18 µm. b-the anterior-dorsal surfaces showing the smooth and serrated spines present in lateral (arrows) and oral sucker region (OS). Bar 30 µm. The insert shows a portion of magnified SEM view of the anterior-dorsolateral surface of the well developed serrated spines (S). Bar 7 µm.



The study has shown regional specialization of the body of *F. gigantica* consisting of three major regions viz., anterior, middle and posterior indicating that, these diversification and modifications in the tegumental structures (size, shape and arrangement of the spines, transverse folds, grooves and presumed sensory papillae) of the flukes help then to be adopted to fit and live in the microhabitat. The presence of larger tegumental papillae on the ventral surface of *F. gigantica* has been reported (Ashour *et al*., 1999). The ventral suckers are shown to be surrounded by presumed fungiform papillae which may act as pressure receptors whereas, other types of papillae that are distributed elsewhere on the surface of the bodies, may act as tango receptors (Sobhon *et al.,* 1994; Srimuzipo *et al.,* 2000; Dangprasert *et al.,* 2001). Smyth & Halton (1983) suggested that these dome shaped papillae and tegumental spines may have sensory functions. However, Bennett (1975b) suggested that these tegumental structures if present on body surface might function in recording pressure changes as the tegument stretches. The external genitalia or the everted cirrus of *F. gigantica* has aggregated sensory papillae and spines and these may help successful delivery and impregnation of sperms and cross fertilization. In genital atrium of *F. gigantica* is shown to be devoid of papillae aggregation of papillae have been observed around the genital pore in *F. hepatica* (Bennett, 1975b). Such variations may indicate surface specialisation that can be ascribed as species specific differences (Ahmad *et al.,* 1988).

References

1. Anantaraman S (1969) The nature and composition nature of the outer layers of the cuticle of *Acanthosentis oligospinus* n.sp. (Acanthocephala) from the fish *Macrones gulio*. *Zeitschrift fur Natureforshung* 24b, 1629-1632.
2. Ahmad M, Nizami WA and Hanna REB (1988) Topographical studies of two digenetic Trematodes of buffalo by scanning electron microscopy. *Zool. Anzeiger*, 220, 59-64.
3. Apinhasmit W, Sobhon P, Saitongdee P and Upatham ES (1993) *Opisthorchis viverrini* changes of the tegumental surface in newly existed juvenile, first week and adult flukes. *Int. J. Parasitol.* 23, 829-839.
4. Ashour AA, Essa Z, Khalil AA and El Sherif EA (1999) Studies on the liver fluke *Fasciola* in Egypt: I-Morphological and morphometrical studies. *J. Egypt. Soci. Parasitol.* 29(3), 979-796.
5. Bakke TA (1976a) Shape, size and surface topography of genital organs of *Leucochloridium* sp. (Digenea), revealed by light and scanning electron microscopy. *Zeitschrift fur Parasitenkunde,* 51, 99-113.
6. Bakke TA (1976b) Functional morphology and surface topography of *Leucochloridium* sp. (Digenea) revealed by scanning electron microscopy. *Zeitschrift fur Parasitenkunde,* 52, 115-128.
7. Bennett CE (1975a) Surface features, sensory structures and movement of the newly excysted juvenile *Fasciola hepatica* L. *J. Parasitol.*, 61, 886-891.
8. Bennett CE (1975b) Scanning electron microscopy of *Fasciola hepatica* L. during growth and maturation in the mouse. *J Parasitol.* 61, 892-898.
9. Dangprasert T, Khawsuk W, Meepool A, Wanicanon C, Viyanant V, Upatham ES, Wongratanacheevin S and Sobhon P (2001) *Fasciola gigantica*: surface topography of the adult tegument. *J. Helmint.* 75, 43-50.
10. Dumag PU, Batolos JA and Gajudo CE (1979) A socioeconomic evaluation of the fascioliasis program of the Bureau of Animal Industry in the provinces of Sorsogon and Leyte. *Philli. J. Anim. Ind.* 34, 13-18.
11. Fairweather I, Threadgold LT and Hanna REB (1999) Development of *Fasciola hepatica* in Mammalian Host. In: *Fasciolosis*. (ed. Dalton JP), CABI Publ, U.K. pp: 47-103.

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12. Fried B and Reddy A (2000). Postmetacercarial changes I *Echinostoma caproni* maintained in a defined medium plus calf serum. *Kore. J. Parasitol.* 38(3), 173-175.
13. Fried B, Frazer BA and Kanev I (1998) Comparative observations on cercariae and metacercaria of *Echinostoma trivolvis* and *Echinoparyphium* sp. *J. Parasitol.* 84(3), 623-626.
14. Fried B, Vates TS, Wisnewski N and Stromberg BE (1986) Scanning electron microscopy and chemical excystation of *Fascioloides magna* (Trematoda) metacercariae. *Zeitschrift fur Parasitenkunde,* 72(5), 631634.
15. Fujino T, Fried B and Hosier DW (1994), The expulsion of *Echinostoma trivolvis* (Trematoda) from ICR mice: etension/retraction mechanisms and ultrastructure of the collar spines. *Parasitol. Res.* 80(7), 581-587.
16. Hockley DJ (1973) Ultrastructure of tegument of *Schistosoma*. *Adv. Parasitol.* 11, 233-305.
17. Hockley DJ and McLaren DJ (1973) *Schistosoma mansoni:* change in the outer membrane of the tegument during development from cercaria to adult worm. *Int. J. Parasitol.* 3, 13-25.
18. Hockley DJ and McLaren DJ (1977). Scanning electro microscopy of eight species of *Schistosoma. Trans. Roy. Soc. Trop. Med. Hyg.* 71, 292.
19. Irwin SWB, McCloughlin TJ and Fried B 1991. Scanning and transmission electron microscopical observations on the tegument of excysted metacercariae and adults of *Zygocotyle lunata*. *J. Helmint.* 65(4), 270-274.
20. Jinxin M and Yixun H (1981), Scanning electron microscopy of Chinese (mainland) strain *Schistosoma japonicum*. *Chin. Med. J.*, 94, 63-70.
21. Kendall SB (1954) Fascioliasis in Pakistan, *Ann. Trop. Med. Parasitol.* 46, 307 313.
22. Kruse DM, Hosier DW and Fried B (1992) The explulsion of *Echinostoma trivolvis* (Trematoda) from ICR mice: scanning electron microscopy of the worms. *Parasitol. Res.* 78(1), 74-77.
23. Meaney M, Fairweather I, Brennan GP, Ramasamy P and Subramanian PB (2002) *Fasciola gigantica*: tegumental surface alterations following treatment *in vitro* with the sulphoxide metabolite of triclabendazole. *Parasitol. Res.* 88(4), 315-325.
24. Nadakavukaren MJ, and Nollen PM (1975) A scanning electron microscopic investigation of the outer surface of *Gorgoderina attenuata*. *Int. J. Parasitol.* 5, 591-595.
25. Nakano T, Fujino T, Washioka H, Tonosaki A, Goto K and Fried B (2003) Tegumentary papillae of *Echinostoma caproni* cercariae, (Trematoda: Echinostomatidae). *Parasitol. Res.* 89(6), 446-450.
26. Nollen PM, Restaino AI and Alberico RA (1973) *Gorgoderina attenuata*: Uptake and incorporation of tyrosine, thymidine and adenosine. *Exp. Parasitol.* 33, 468-476.
27. Parkening TA and Johnson AD (1969) Glucose uptake in *Haemaoloechus medioplexus* and *Gorgoderina* trematodes. *Exp. Parasitol.* 25, 358-367.
28. Ramasamy P (Ed.) (1996)Applications of electron microscopy and cytochemical techniques in the diagnosis of fish diseases – A laboratory manual. Short term training course sponsored by Department of Biotechnology, Government of India, India.
29. Rosa-Brunet LC and Fried B (1992) Growth, development, pathogenicity and transplantation of *Echinostoma caproni* (Trematoda) on the chick chorioallantois. *J. Parasitol.* 78(1), 99-103.
30. Smyth JD and Halton DW (1983) "The physiology of Trematodes". Cambridge University Press, Cambridge, London, New York. pp. 86-114.
31. Sobhon P and Upatham ES (1990) Snail hosts, life-cycle, and tegumental structure of oriental schistosomes. UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. pp. 57-88.
32. Sobhon P, Dangprasert T, Saitongdee P, Wanichanon C and Upatham ES (1994) Surface topography and ultrastructure of the tegument of adult *Fasciola gigantica*. *J. Elec. Micros. Soc. Thai.* 8, 36-45.
33. Sorensen RE, Kaney I, Fried B and Minchella DJ (1997) The occurrence and identification of *Echinostoma revolutum* from North American *Lymnaea elodes* snails. *J. Parasitol.* 83(1), 169-170.
34. Srimuzipo P, Komalamisra C, Choochote W, Jitpakdi A, Vanichthanakorn P, Keha P, Riyong D, Sukontasan K, Komalamisra N, Sukontasan K and Tippawangkosol P (2000) Comparative morphometry, morphology of egg and adult surface topography under light and scanning electron microscopies, and metaphase karyotype among three size-races of *Fasciola gignatica* in Thailand. *South. Asian J. Trop. Med. Pub. Health.* 31(2), 366-373.
35. Ursone RL and Fried B (1995) Light and scanning electron microscopy of *Echinostoma caproni* (Trematoda) during maturation in ICR mice. *Parasitol. Res.* 81(1), 45-51.

Figs.5 a&b. Scanning electron micrograph showing the posterior tip of F. gigantica. a - Lateral region of the posterior end of the body appears smooth with less developed ridges or spines (unlabelled arrows). Bar 10 µm. b - Posterior tip appears smooth with less spines in lateral region, and also show the excretory pore (Ex). Bar 20 µm. The insert shows a portion of magnified SEM view of the posterior tip of the body showing the excretory pore (Ex). Bar 13 µm.