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Scanning electron microscopic investigation on the surface topography of *Didymozoon tetragynae*, *Allodidymozoon cylindricum* and *Didymocystis* n. sp of the family Didymozoidae

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Abstract

The scanning electron microscopic investigation clearly revealed the diversified nature of the surface topography of fish trematode parasites *viz., Didymozoon tetragynae, Allodidymozoon cylindricum and Didymocystis n.* sp. These variation are mainly due to the adaptation of the parasites to micro environment. The tegumental folding, ridges and furrows and lamellar network impart considerable stretching capacity to the didymozoids. These structures also increase the surface area of absorption of micro molecular nutrients. Three types of papillae are recorded on the dorsal and dorsolateral surface of *Didymocystis n. sp.* and domed papillae are recorded in *D.tetragynae* and *A. cylindricum*. Each type may perform specific sensory function. The numerous integumental elevations found in *Didymozoon tetragynae and Didymocystis n.* sp suggest that they may help in attachment.

Keywords : *Didymozoon tetragynae*, *Allodidymozoon cylindricum* and *Didymocystis* n. sp., scanning electron microscopy, fish parasites, digenetic trematodes

INTRODUCTION

The digenetic trematodes comprise a bewildering array of families, genera and species that differ in shape, size, number, location, size of suckers, length of intestinal crura, flame bulb arrangement and especially details of the reproductive system (Hyman, 1951). Most digeneans are endoparasitic in the digestive tract and in various other organs of vertebrates, but several species have secondarily adapted to an ectoparasitic way of life (Rohde, 1982). Trematodes of the family *Didymozoidae*, parasitize mainly marine fishes and rarely fresh water ones. They usually occur encysted in pairs on gills, fins, scales and stomach wall of fishes and possess several unique and unusual features by virtue of which they receive special attention.

The knowledge of *Didymozoids* from fish of the Bay of Bengal (Chennai coast) is limited and is restricted to the few species reported by Job (1961 a, b, c & 1966) and (Madhavi, 1982). Hence it was considered worthwhile to record and describe the morphological features of *Didymozoids* collected from the fish barracudas.

The present study describes the surface topographical features of *Didymozoon tetragynae*, *Allodidymozoon cylindricum* and *Didymocystis n.* sp collected from the fish *Sphyraena obtusata*, *S. jello* and *S. bleekeri*, collected from Kasimedu, Rayapuram, Chennai, South India.

MATERIALS AND METHODS

The parasitic species infest different parts of the hosts. For e.g., *Didymozoon tetragynae* is found on fins, *Allodidymozoon cylindricum* on stomach wall and *Didymocystis n.sp.*, on pseudo branch. Fresh specimens were collected from the fins, stomach wall of the host *Sphyraena obtusata* and *Sphyraena jello* and from the pseudobranch of the host *Sphyraena bleekeri*. The worms were washed with phosphate buffer several times to free them from mucus and fixed in 4% glutaraldehyde prepared in 0.1M phosphate buffer of pH 7.2 over night at 4°C. The worms were washed several times again in cold 0.1M phosphate buffer.

The worms were postfixed for 4 hours in 1% osmium tetroxide prepared in 0.1M phosphate buffer at 4° C and were subsequently transferred to 50% ethanol and dehydrated in a graded series of alcohols. Then the specimens were transferred to 100% acetone for one hour, dried and were mounted on aluminum stubs, coated with palladium/gold and examined in a scanning electron microscopy.

RESULTS

The surface topography of adult *Didymozoon tetragynae, Allodidymozoon cylindricum* and *Didymocystis n.sp* as seen by scanning electron microscope are depicted in Figures 1-23.

In *Didymozoon tetragynae* the body surface is not uniform throughout. The dorsal surface appears to be rough with pits (Figures 2 and 3) transverse tegumental folds with microvilli like sctructures on the edges (Figure 4), non- ciliated dome shaped papillae (Figure 5) pits and papillae (Figure 6). In the ventral surface, the ridges and furrows are arranged in a network giving a 'honey comb' appearance (Figure 7). Spine like structures and a prominent pore are also observed (Figure 8). The egg

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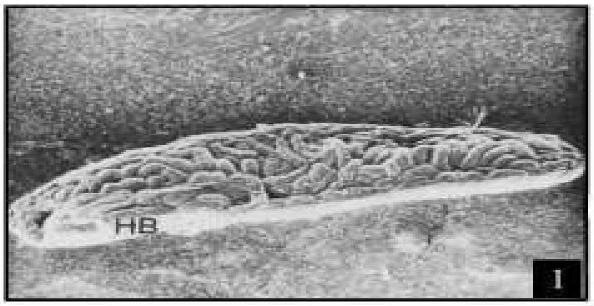
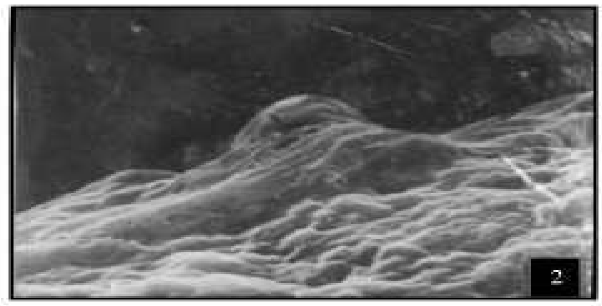
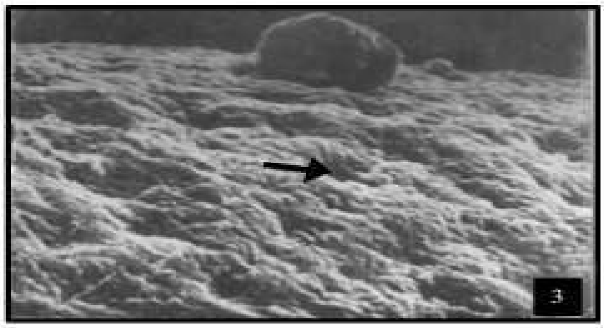
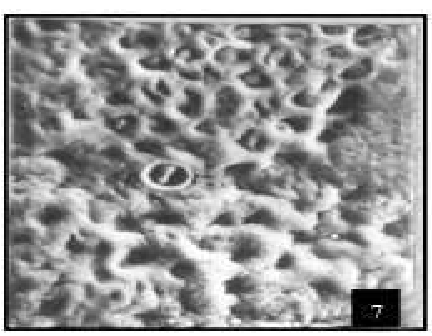








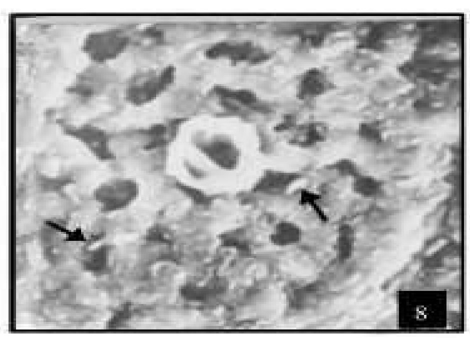


Figure 1. *Didymozoon tetragynae* dorsal view x 15

Figure 2. Rough dorsal surface of *Didymozoon tetragynae* x 512

Figure 3. Rough dorsal surface of *Didymozoon tetragynae showing pits (arrow) x 1125*

Figure 4. Dorsal surface of *Didymozoon tetragynae* showing transverse tegumental folds with microvilli like structure on the edges (arrow) x 512.

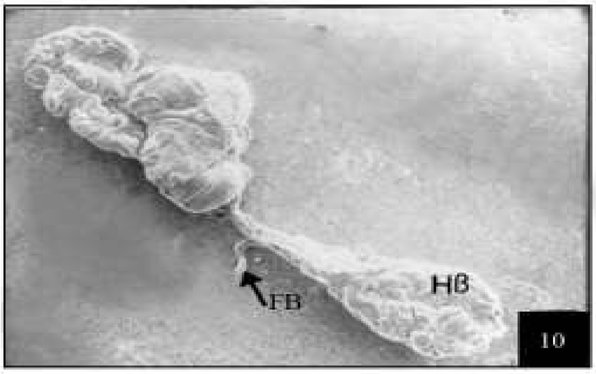
Figure 5. Arrow indicates non-ciliated dome shaped papillae on the dorsal surface of *Didymozoon tetragynae* x 1250

Figure 6. Dorsal surface of *Didymozoon tetragynae* showing the pits and papillae (arrows).

Figure 7. Ventral surface of *Didymozoon tetragynae* showing ridges and furrows arranged in a honey – cumb like appearance x 1125

Figure 8. Ventral surface of *Didymozoon tetragynae* showing small spine like structures (arrow) x 2000 and prominent pores.

Figure 9. Dorsal surface of *Didymozoon tetragynae* showing the transverse furrows and egg (arrows) x 1250



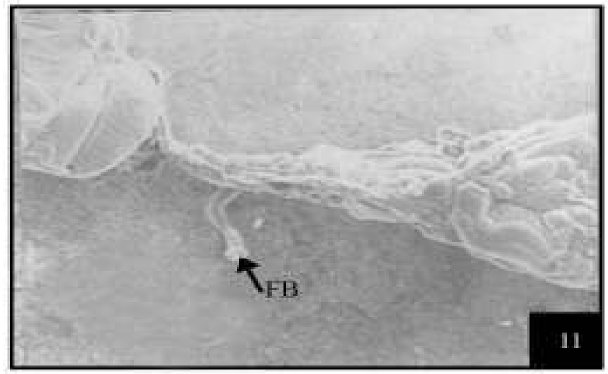


Figure 10. Ventral view of *Allodidymozoon cylindricum* x 10

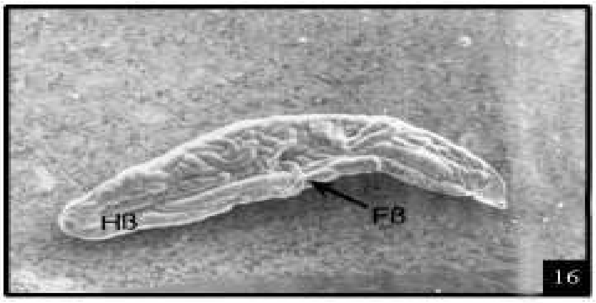
Figure 11. Forebody of *Allodidymozoon cylindricum* x 40

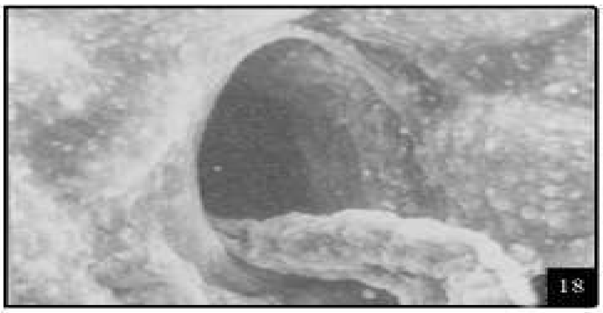
Figure 12. Forebody of *Allodidymozoon cylindricum* showing smooth surface with smaller spine at the base x 512

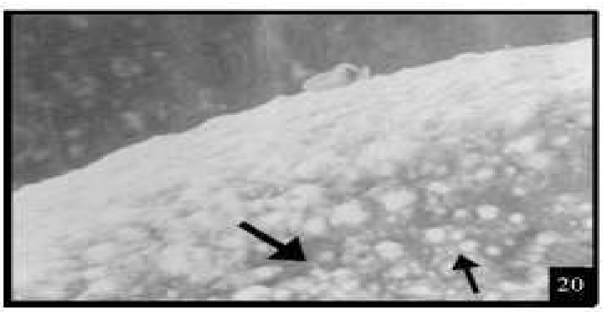
Figure 13. The tip of the forebody of *Allodidymozoon cylindricum* showing rough p itted surface with papillae x 1250

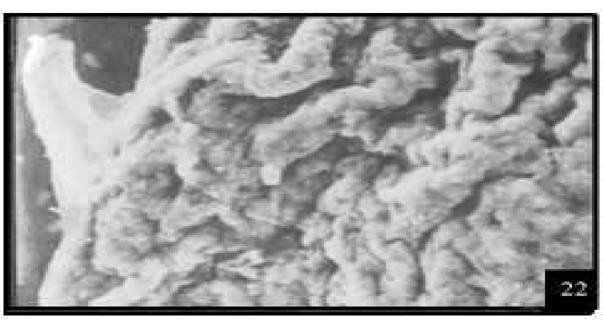
Figure 14. Dorsal surface of ***Allodidymozoon cylindricum*** showing annulation (arrow) x 1250

Figure 15. Egg capsules of ***Allodidymozoon cylindricum*** x 2500









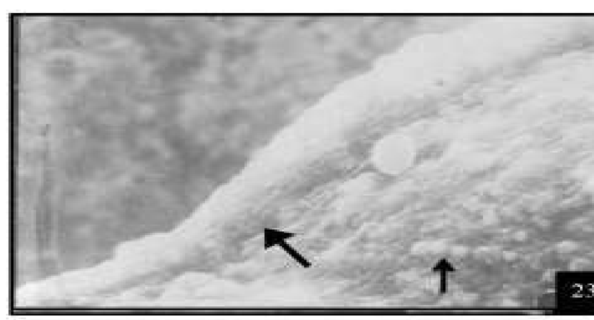


Figure 16. *Didymocystis. n.* sp. ventral view x 25

Figure 17. Ventral surface of *Didymocystis. n.* sp. showing numerous papillae and the origin of forebody through a prominent hole x 240

Figure 18. Fore body of *Didymocystis. n.*sp. magnified x 640

Figure 19. Dorsal surface of *Didymocystis. n.*sp. showing numerous bosses with spine like structure x 1250

Figure 20. Dorsal surface of *Didymocystis n.* sp. showing pits and papille (arrows) x 1250

Figure 21. Dorsal surface of *Didymocystis. n.* sp. showing sensory papillae (long arrow) unciliated papillae (small arrow) and Egg capsula (ES) x 1250

Figure 22. Dorsolateral surface of *Didymocystis. n.*sp. showing irregular ridges and furrows with spine like projections x 1250

Figure 23. The Dorsal surface of *Didymocystis. n.*sp. showing transverse furrow (long arrow) on the edge and the dome shape papillae (small arrow) and sac like egg shell x 1250

shell is ovoid measuring 6.4x4.2 µ (Figure 9).

In *Allodidymozoon cylindricum* the fore body is tubular with broader base and narrower tip (Figure 11). At the base, the surface is smooth with smaller spines (Figure 12). Towards the terminal end the surface is rough with papillae (Figure 13). The dorsal surface shows many undulations with shallow pits, papillae and tubercles (Figure 14). The eggs are more globular measuring 6.2x4.4µ (Figure 15).

In *Didymocystis n.* sp. the ventral surface shows numerous papillae and a prominent hole from which the fore body arises (Figures 17 and 18). In the central part of the dorsal surface numerous bosses with small spines are observed (Figure 19). Sensory papillae and unciliated papillae are also observed on the dorsal surface (Figure 21). The dorso lateral surface shows irregular ridges and furrows with spine like projections (Figure 22). Transverse furrows on the edge and dome shaped papillae are also noticed on the dorsal surface (Figure 23). The egg shell is sac like measuring 6.2x4.6µ (Figure 23).

DISCUSSION

The diversification and modification in the integumental structures of digenetic trematodes can be considered as parasitic adaptations to individual micro–habitats (Abidi *et al.*, 1988). The integument of trematodes is generally considered as a protective sheath. In addition, Morris and Threadgold (1968) reported that the integument aids in absorption. They also suggested a secretory role for the integument. Silk *et al.* (1969) suggested an excretory role for the integument. Sneft *et al.* (1961) suggested that the integument plays an important role in Osmoregulation. Whatever the functions of the integument in digenetic trematodes may be, only incidental attention has been given to the architecture of the integument-medium interface through which any secretion, excretion, absorption or osmoregulation must occur (Miller *et al.*, 1972; Smyth and Halton, 1983).

The present investigation clearly reveals that there is diversification with reference to various surface structures of parasites. These variations may be due to various measures including adaptations of the parasite to microenvironment as the niche of each parasite differs. In this aspect Abidi *et al.* (1988) observed differences in tegument structures of various digeneans occupying various niches and suggested that it is a parasitc adaptation.

The tegumental foldings, ridges, furrows and lamellar net work impart considerable stretching capability to the didymozoids. These structures also increase the surface area for absorption of micro molecular nutrients (Parkening and Johnson, 1969; Nadakavukaren and Nollen, 1975; Smyth and Halton, 1983; Abidi *et al.*, 1988). It is also stated that the ridges on the integument are either due to longitudinal anterior constriction (Bakke , 1978) or due to internal musculature (Nollen and Nadakavukaren, 1974).

Similar tegumental foldings, ridges and furrows observed in all the species of didymozoids in the present study on the dorsal and dorsolateral surfaces suggest that surface structures may also perform the same function like absorption and stretching ability as seen in other trematodes. Since didymozoid parasites occur in pairs, the furrows and ridges on the ventral and dorsal surfaces of the parasites may serve for attachment purpose and they may also help in absorption. Ogbe (1982) described tegumental folds and pits on the surface of adult male and female *Schistosoma margrabousei* and suggested that these may help to increase the surface area of uptake of materials from the blood stream environment. Brennan *et al.* (1991) observed that the extensive in folding of the tegument in *Gastrodiscoides hominis* indicate a role in osmotic and ionic regulation.

Two types of papillae conical with a central cilium and dome shaped without cilium commonly occur in digenetic trematodes (Edwards *et al.*, 1977; Fujino *et al.*, 1979; Font and Wittrock, 1980; Hode and Mitchell, 1981). The domed papillae seem to be of a fundamental type as they have been frequently recorded in trematodes. Domed papillae, smooth, spined or with apical cilia commonly occur in trematodes and it has been suggested that they have a sensory function.

Several types of papillae on the tegument of intestinal flukes have been reported. Domed, oval, bilobed and button types are reported on the tegument of adult *Echinostoma revolutum* and *Isthimophora melis* (Smales and Blankespoor, 1984). Uniciliated papillae were reported in some digeneans such as adult *Echinostoma malayanum* (Tesana *et al.*, 1987) and *Gastrodiescoides hominis* (Brennan *et al.*, 1991).

In the present study, three types of papillae are recorded on the dorsal and dorsolateral surface namely dome shaped, pitted and unciliated in *Didymocystis n.* sp. The domed papillae are numerous. Only the domed papillae are recorded in *Didymozoon tetragynae* and *Allodidymozoon cylindricum*. Each type may represent specific sensory function. Abidi *et al.* (1988) have reported that the non-encysted meta cercariae of *Clinostomum complanatum* is in an active feeding stage and therefore the dome shaped sensory papillae around the oral sucker may act as tango receptors in the location of the food materials. The pit type papillae on the ventral surface of the body of didymozoids may have chemoreceptive function as suggested by Smyth and Halton (1983).

In *D. tetragynae* and *Didymocystis n.* sp. spine like structures are recorded. Similar spines have been reported in *Phyllodistomum conostomum* (Bakke and Lien, 1978), *Urogonimus macrostomus* (Bakke, 1978), *Leucochloridium variae* (Bakke, 1982) and *Opisthorchis pedicellata* (Pandey and Tewari, 1985). But the presence of undentated spines in the present study reveals that they may have a sensory function as suggested by Smyth and Halton (1983). The spines are absent on the ventral surface of the parasite, probably to help the parasites for smooth sealing against the other parasite. In *F. gigantica* the spines are absent around the sucker, which helps the parasites for smooth sealing against the host mucosa (Ahmad *et al.*, 1988) Spines are absent around the suckers in *F. hepatica* (Bennett, 1975 a & b). Bennett (1975 b) suggested that spine like structure when present on the body surface might function in recording pressure changes as the tegument stretches.

Numerous integumental elevations have been recorded in *Didymocystis n.* sp. and *Didymozoon tetragynae* on the dorsal surface which give a rough appearance. It may help in the attachment and also to increase the surface area of absorption. Similar integumental elevation or bosses are recorded in *S. mansoni* (Miller *et al.*, 1972) and *S. haematobium* (Kuntz *et al.*, 1976).

In *Didymozoon tetragynae* microvilli like projections have been recorded on the dorso-lateral sides, which may be to increase the area of absorption.

Thus the various topographical variations observed in *Didymozooids* are mainly for performing various functions like absorption, attachment, sensory and stretching capacity.

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