

SURFACE FINE TOPOGRAPHY OF *DIPLODISCUS* SPP. (TREMATODA: PARAMPHISTOMIDAE) PARASITIC IN ANURAN FROGS

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ABSTRACT: Stereoscan studies on the surface topography of the paramphistomid digeneans, *Diplodiscus amphichrus* Tubangui, 1933 and *D. mehrai* Pande, 1937, both parasitizing the rectum of anuran frogs, revealed differences in the type and distribution pattern of the surface fine features. In *D. amphichrus* the general body tegument has randomly distributed domed papillae and elevated papillae abound in the circum-oral region. In *D. mehrai* the domed papillae on the general tegument and papillate ornamentation in the region surrounding the mouth are not present.

Keywords: *Diplodiscus* spp., Surface topography, SEM, Paramphistomidae, amphistome, frog parasite.

INTRODUCTION

Fine features of surface topography of digenean parasites as revealed by scanning electron microscopy (SEM) have provided newer information and inferences regarding the functional morphology of this important host-parasite interface. With regard to amphistomid flukes, SEM studies have revealed species-specific features of tegumental topography (Eduardo, 1985; Tandon and Maitra, 1982, 1983, 1987; Sey, 1984; Roy and Tandon, 1992); the majority of these parasites so studied represent the species occurring in ruminant hosts.

Diplodiscus occurs as a common paramphistomid parasite in the rectum of anuran Amphibia. Of the two species of the genus reported from India, *D. amphichrus* Tubangui, 1933 has a wide distribution, having been reported from many localities such as Uttar Pradesh, West Bengal, Maharashtra, Tamil Nadu, Kerala and Meghalaya; the other species, *D. mehrai* Pande, 1937 has so far been recorded from Kumaon Hills (Uttar

Pradesh) and Kashmir (Agrawal, 1966; Mukherjee and Ghosh, 1972; Nama and Khichi, 1973; Singh, 1977; Diengdoh, 1989). While Mukherjee (1966) and Agrawal (1966) considered *D. mehrai* a synonym of *D. amphichrus*, other workers did not support their view (Nama and Khichi, 1973; Mehra, 1980). We describe herein the surface fine features of the two species, *D. amphichrus* and *D. mehrai*, the specimens of which were morphologically differentiated on the basis of the size of the body, the position of the genital pore and the egg size.

METHODOLOGY

Mature specimens of *D. amphichrus* and *D. mehrai* were collected from the rectum of naturally infected frogs in Nagaland (India), the former from the rhacophorid frogs, *Polypedates leucomystax* and *Rhacophorus nigropalmatus* at Mokochung and the latter from *Euphlyctis cyanophlyctis* (= *Rana cyanophlyctis*) at Kohima and *Rana* sp. at Dimapur. A few specimens of both species were processed for whole mount preparation, using 70% ethanol as a fixative and borax carmine for staining.

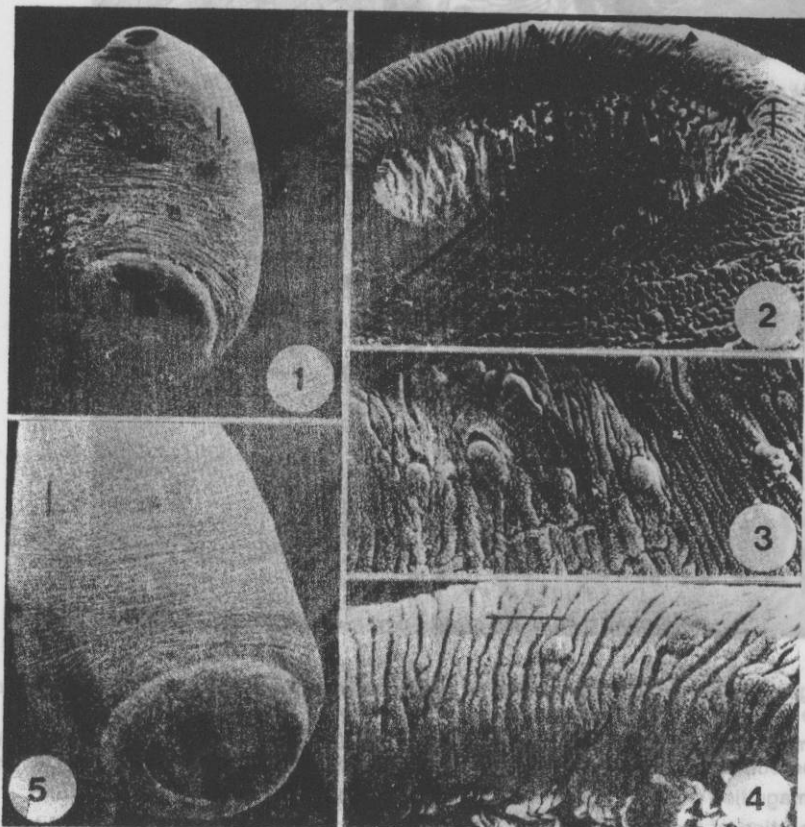
For SEM, after thorough washing in 0.7% saline, the flukes were fixed in cold 10% neutral buffered formalin. The specimens, dehydrated through a graded series of ethanol, were further dried using tetramethylsilane following the procedure described by Roy and Tandon (1991), metal coated and viewed under a JSM 35 CF (Jeol) scanning electron microscope at accelerating voltage of 10-15 kv.

RESULTS AND DISCUSSION

D. amphichrus

The flattened flukes measure 4.83-6.34 mm in length and 1.46-1.89 mm in maximum width, possess post-bifurcal genital pore and their eggs are 130-140 x 63-71 μm in size. The fine topography of the fluke as revealed by SEM shows fine transverse foldings of the surface tegument. The latter, at high resolution presents a finely beaded texture throughout. The oral rim as well as the inner wall of the buccal cavity which have fine radial and longitudinal tegumental foldings, respectively, are studded with button-like elevated papillae. The tegument covering the acetabular region has similar ornamentation. The tegument of the genital pore region also has fine and dense foldings and densely beaded tuberculate papillae are present all along the pore rim and also in the

circumgenital pore region, though there is no discrete pattern of their distribution. The domed papillae abounding on the general body surface are scattered randomly, each being distinctly delimited from the surrounding by concentric circular folds of the tegument (Figs. 1-10).



Figs. 1-10: *Diplodiscus amphichrus*, scanning electron micrographs

- Fig. 1: Full worm, ventral view (bar = 100 μ m).
- Fig. 2: A magnified view of the oral end, showing the oral rim as well as the inner wall studded with button-like elevated papillae (arrows; bar = 10 μ m).
- Fig. 3: A magnified view of the oral rim, showing button-like papillae (bar = 10 μ m).
- Fig. 4: Anterodorsal rim of the oral opening under higher resolution, showing radial foldings studded with button-like papillae and below it a part of the inner wall of the pharynx (bar = 10 μ m).
- Fig. 5: Another specimen, showing a protuberance in the acetabulum (bar = 10 μ m).

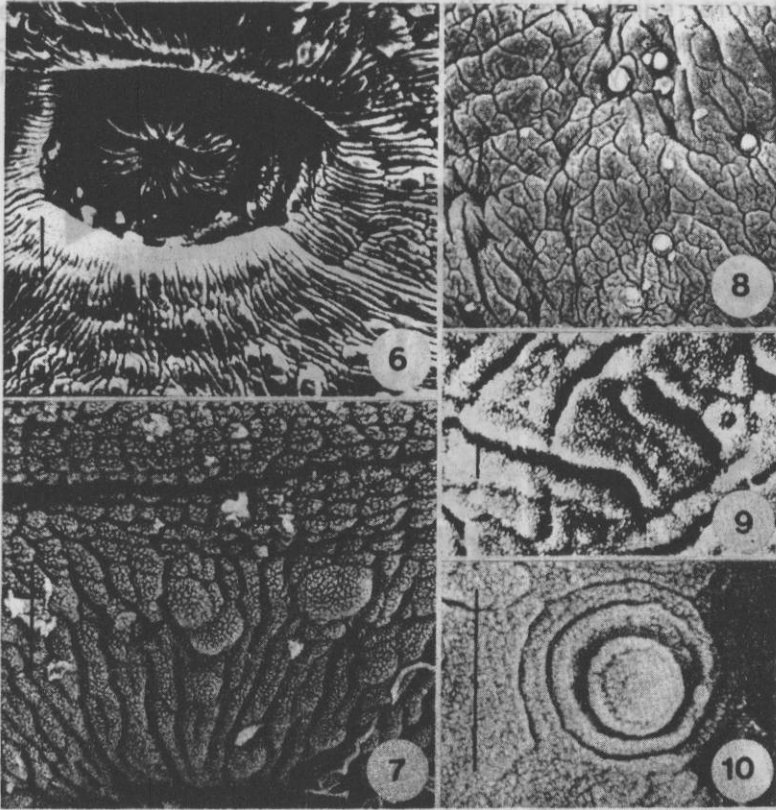
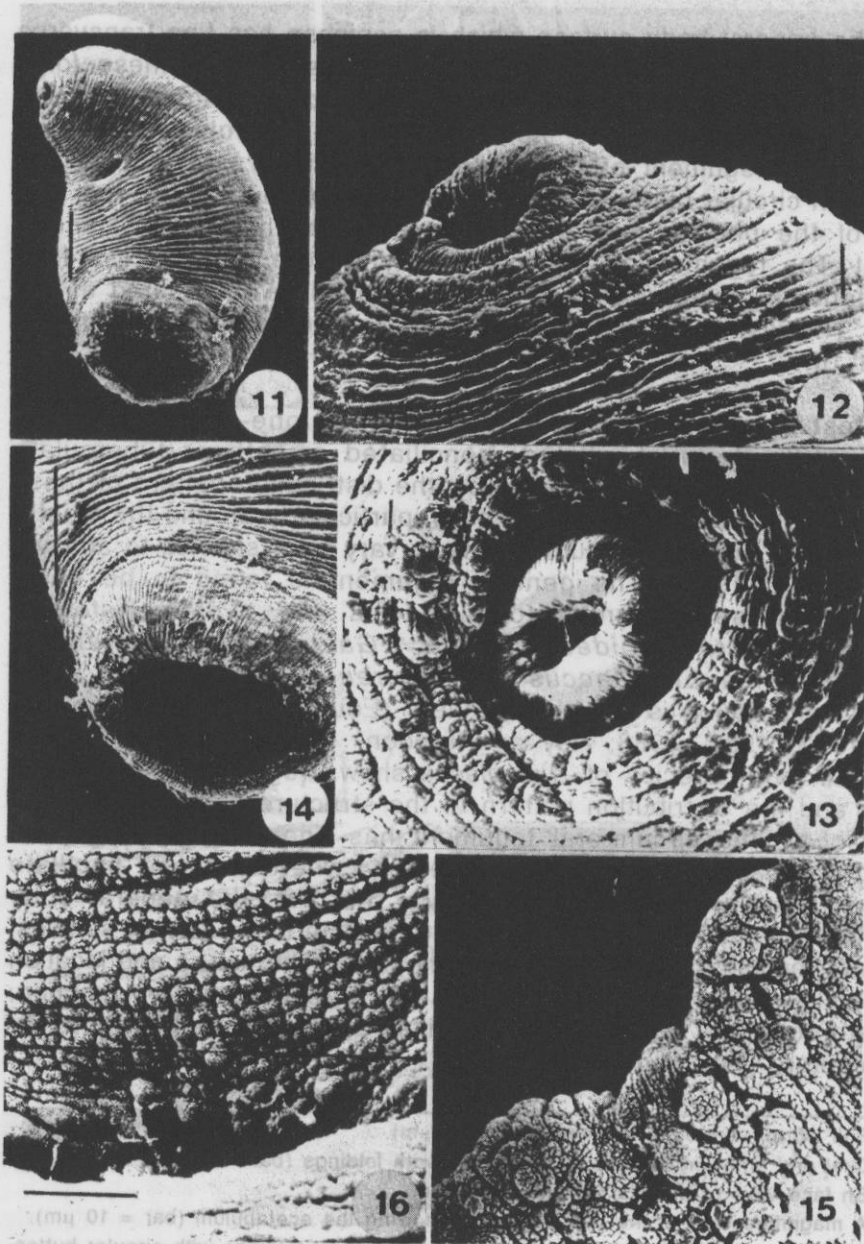


Fig. 6: Tegument in the circum-genital pore region, showing fine radial foldings with button-like papillae in between them (bar = 10 μ m).
Fig. 7: A magnified view of the rim of the genital pore in another specimen, showing fine tuberculate texture of the tegument and elevated papillae (arrows; bar = 10 μ m).
Fig. 8: A magnified view of the general body tegument with papillae (bar = 10 μ m).
Fig. 9: A portion of the tegument under higher resolution, showing densely beaded texture (bar = 10 μ m).
Fig. 10: A domed papilla in a closer view (bar = 10 μ m).

D. mehrai

The flukes have a small body, 1.66-2.64 mm in length and 0.85-1.13 mm in maximum width; the genital pore lies at the junction of oesophagus and intestinal caeca or just anterior to it and the eggs measure 90-110 x 50-60 μ m in size.



Figs. 11-16: *Diplodiscus mehrai*, scanning electron micrographs

Fig. 11: A lateral view of the whole organism. Fig. 12: A close-up view showing concentric ridges in the anterior region. Fig. 13: A close-up view showing the circular button. Fig. 14: A close-up view showing the circular button with a scale bar. Fig. 15: A magnified view showing domed papillae in the upper inner rim of the pore. Fig. 16: A magnified view showing domed papillae in the upper inner rim of the pore with a scale bar.

The general body surface which is aspinose has fine transverse folds of tegument both on dorsal and ventral sides and these folds acquire a concentric arrangement at the oral end. The circum-oral and circum-acetabular tegument, however, has radial folds. At higher resolution, the tegument of the acetabulum exhibits a texture of fine foldings with circular button-shaped papillae abounding near its rim. The surface of the papillae has a texture similar to that of the general tegument. The tegument of the circum-genital pore region presents a cobble-stone-like texture, with large domed papillae aggregated at the pore rim. The rest of the body tegument appears to be devoid of papillae (Figs. 11-16).

Most amphistomes possess more than one type of surface features in the form of ciliated, nonciliated smooth or tuberculate tegumental papillae, and pits. However, the distribution pattern of these structures in various regions of the body, particularly around the mouth, genital opening and acetabulum seems to vary from species to species (Eduardo, 1982, 1991). The general tegument is found to be finely tuberculated in several amphistome species (e.g., *Paramphistomum epiclitum*, *Gastrodiscoides hominis*, *Gastothylax crumenifer*, *Velasquezotrema brevisaccus*, *V. tripurensis* and *Leiperocotyle meghalayansis*) but it is smooth in others (Eduardo, 1981; Tandon and Maitra, 1981, 1982, 1983; Roy and Tandon, 1990a, b). Although the tegument in both species of *Diplodiscus* shows regional modifications, the type and the distribution pattern of the structures present differ. In *D. amphichrus* the circum-oral tegument has fine radial foldings and elevated papillae abounding in the region, whereas these papillate structures are not present in *D. mehrai*. Further, the general body tegument has randomly distributed domed papillae in the former species, while such papillae are not observed in the latter. The nature and location of the papillate structure is indicative of their probable sensory function, as aciliate domed or button-shaped papillae are suggestedly proprio- or tangoreceptors (Roy and Tandon, 1992).

Fig. 11: Full worm, ventrolateral view (bar = 100 μ m).

Fig. 12: Oral end, showing circum-oral tegument with foldings (bar = 10 μ m).

Fig. 13: En face view of the oral end (bar = 10 μ m).

Fig. 14: A magnified view of the posterior end, showing the acetabulum (bar = 10 μ m).

Fig. 15: Acetabular rim in a closer view, showing tegumental foldings with circular button-like papillae (arrows; bar = 10 μ m).

Fig. 16: A magnified view of the genital pore region, showing the cobble-stone-like arrangement and domed papillae in the upper inner rim of the pore (bar = 10 μ m).

On the basis of their surface fine topography, *D. amphichrus* and *D. mehrai* appear distinct species.

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