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Stereoscan observations on the surface topography of *Gastrothylax crumenifer* (Creplin, 1847) Poirier, 1883 and *Paramphistomum epiclitum* Fischoeder, 1904 (Trematoda: Digenea)

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ABSTRACT

The critical-point-dried specimens of *Gastrothylax crumenifer* (Creplin, 1847) Poirier, 1883 and *Paramphistomum epiclitum* Fischoeder, 1904, both recovered from the rumen of sheep, were studied by scanning electron microscopy at magnifications ranging from 10 to 10,000× to reveal the structural differences of the tegument between the two species.

In *G. crumenifer*, both dorsal and ventral surfaces have tubercle-like tegumental elevations, devoid of spines. A row of prominent, regularly arranged, button-like protuberances encircle the rim of mouth. It is suggested that these are sensory in nature. The lining of the buccal tube also bears papillae, some of which appear balloon-like. The tegument near the outer acetabular rim is thrown into prominent ridges bearing groups of six to eight small papilla-like elevations. Towards the interior of the acetabular cavity this pattern merges into one with stout, finger-like projections with groups of papillae at their blunt tips. From their structure, these projections appear to aid strong anchorage to the host's surface and the papillae to be secretory.

P. epiclitum has a similar tuberculated pattern on the dorsal and ventral surfaces. In the oral region, the tegument has some irregular, patch-like elevated areas. The acetabular surface is thrown into a pattern of deep folds and craters.

INTRODUCTION

Few adult digenetic trematodes have been subjected to scanning electron microscopy (SEM) for surface topography studies. These include *Diplostomum phoxini* (see ERASMUS, 1970), *Schistosoma mansoni* (see SILK *et al.*, 1970; MILLER *et al.*, 1972), *Megalodiscus temperatus* (see NOLLEN & NADAKAVUKAREN, 1974), *Gorgoderina attenuata* (see NADAKAVUKARAN & NOLLEN, 1975), *Fasciola hepatica* (see BENNETT, 1975 a, b), *Leucochloridium* sp. and *Urogonimus macrostomus* (see BAKKE, 1976 a, b, 1978), *S. haematobium* and *S. intercalatum* (see KUNTZ *et al.*, 1976, 1977; HICKS & NEWMAN, 1977), *S. japonicum* (see SAKAMOTO & ISHII, 1977) *S. matthei* (see TULLOCK *et al.*, 1977) *Phyllodistomum conostomum* (see BAKKE & LIEN, 1978), *S. bovis* (see KUNTZ *et al.*, 1979) and *Clonorchis sinensis* (see FUJINO *et al.*, 1979). Few SEM studies had been reported for amphistomes from mammals until Eduardo's work on *Bilatorchis papillogenitalis*, *Orthocoelium indonesiense* and *Leiperocotyle okapi* (see EDUARDO, 1980 a, b, c). Amphistomes from mammals are difficult to identify because they are apparently morphologically similar. The histology of the pharynx, acetabulum and genital atrium has been, so far, the most widely used criterion in the systematic studies of these parasites (NÄSMARK, 1937). We have used SEM to illuminate the surface

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topography and reveal any specific structural characteristics of the tegument in two members of this group. *Gastrothylax crumenifer* (Creplin, 1847) Poirier, 1883 and *Paramphistomum epiclitum* Fischoeder, 1904 are both common parasites of sheep and goats and occupy the same habitat in their host and were chosen for the present study.

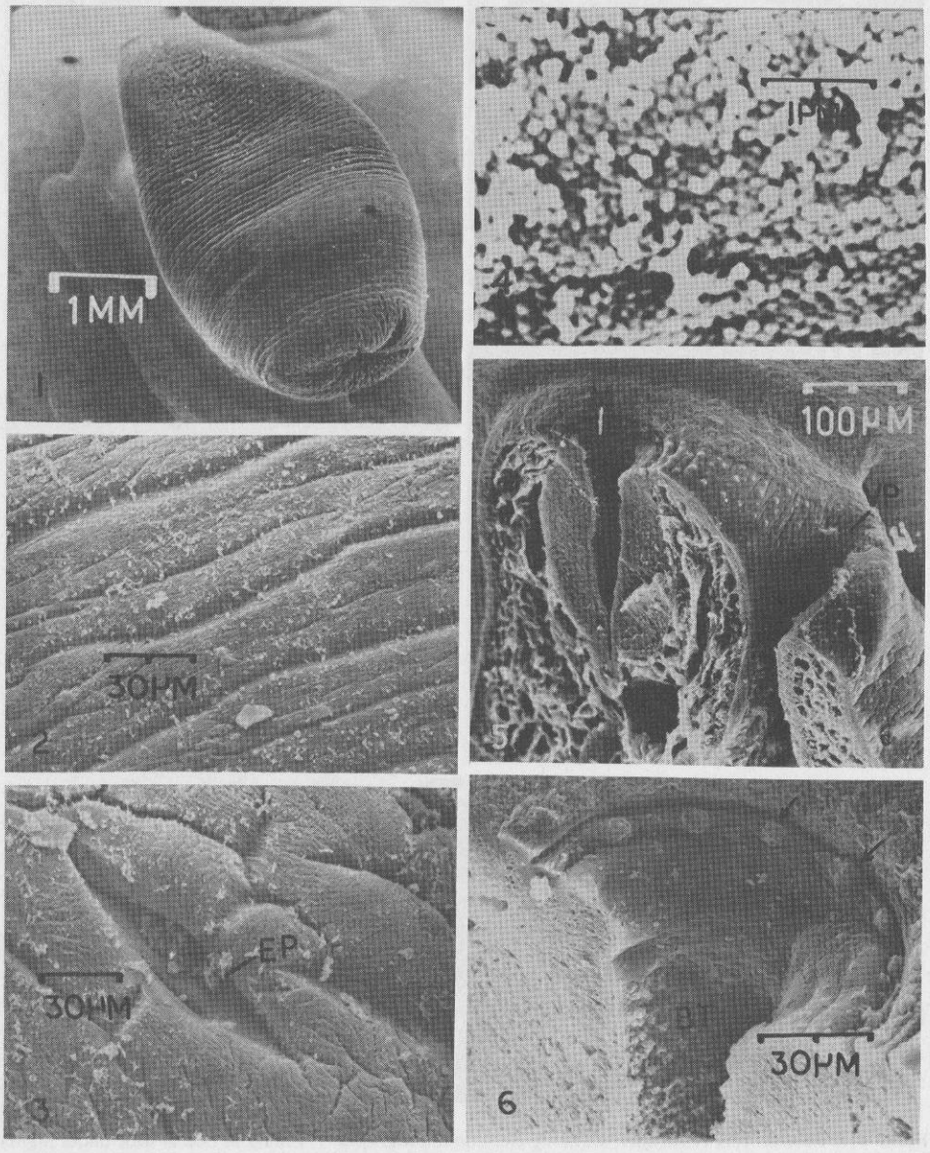
MATERIALS AND METHODS

Live *G. crumenifer* and *P. epiclitum* were obtained from the rumen of freshly slaughtered sheep at the local abattoir. After thorough washing, the worms were fixed in 10% buffered formalin or 70% alcohol and preserved in 70% alcohol until further processing. They were dehydrated in a graded series of ethanol-amyl acetate mixtures to pure amyl acetate. The specimens were dried in a critical point drier (Balzers Union) using liquid carbon dioxide, oriented and attached with colloidal silver to aluminium stubs, coated with gold-paladium in a 'Polaron' SEM coating unit E 5000, and finally observed in a Stereoscan 180 at 10 to 10,000 × magnification at electron accelerating voltages ranging from 15 to 20 KV. 15 specimens of each species were processed for SEM studies.

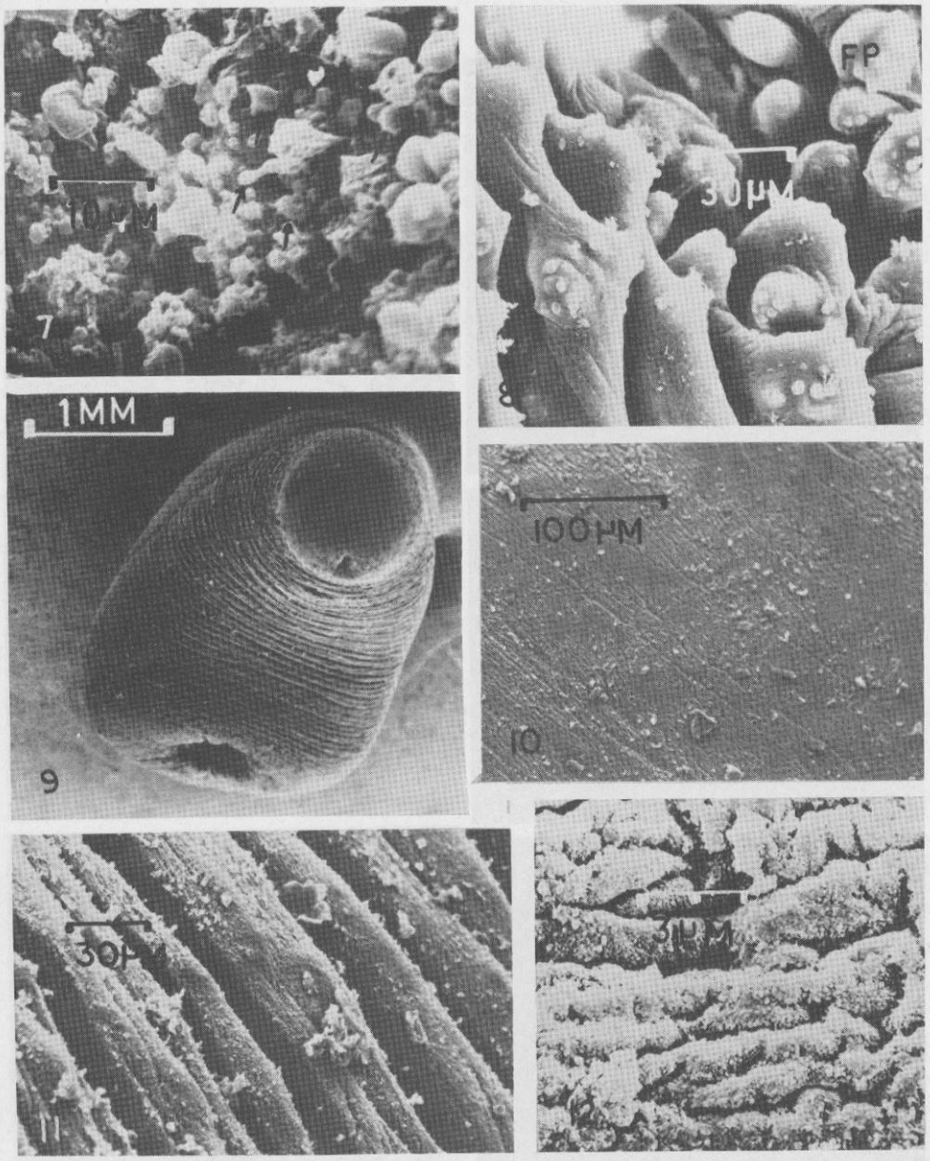
OBSERVATIONS

In *G. crumenifer* both the dorsal and ventral surfaces of the body appear to have wrinkled, but otherwise smooth, contours. The wrinkles are more prominent in the anterior half and lateral sides of the body (Fig. 1). Under a high resolution the tegument is revealed as having tubercle-like elevations all over the dorsal and ventral surfaces (Figs. 2–4) giving it a beaded appearance. No spines were observed to be associated with these elevations. The rim of the mouth opening is encircled by a single row of button-like prominent protuberances which are regularly arranged, almost equidistance from one another (Figs. 5, 6). Similar conspicuous elevations, but more in number, are also present in the region of the opening of the ventral pouch (Fig. 5). The interior of the buccal tube also bears papillae like the outer tegument (Figs. 6, 7) but some are larger than the rest and appear balloon-like. A few host red blood corpuscles are also seen attached to the buccal lining. The tegument near the outer acetabular rim does not exhibit a tuberculated pattern as in the other parts of the body but is thrown into prominent, overlapping ridges. On these, groups of six to eight conspicuous, but minute, papillae are situated. Towards the interior of the acetabular cavity this pattern of ridges merges into one with stout, stumpy, finger-like projections with groups of small papillae, on the blunt tips. These papillae are similar to those observed near the outer rim of this anchoring organ (Fig. 8).

The tegument in *P. epiclitum* is also wrinkled (Fig. 9) but less so on the dorsal (Fig. 10) than the ventral surface (Figs. 11, 12). There are finer wrinkles near the anterior end of the body. However, under the higher resolution, both surfaces are observed to have small bead-like distinct protuberances arranged compactly throughout (Fig. 13). The rim of the mouth, which is subterminal in position, lacks a definite pattern of patch-like structures as present in *G. crumenifer* but numerous similar elevations are arranged irregularly in the region around the mouth (Fig. 14). The acetabular tegument exhibits a pattern of craters and folds, the latter becoming deeper and more conspicuous towards the interior of the acetabular aperture (Figs. 15, 16).

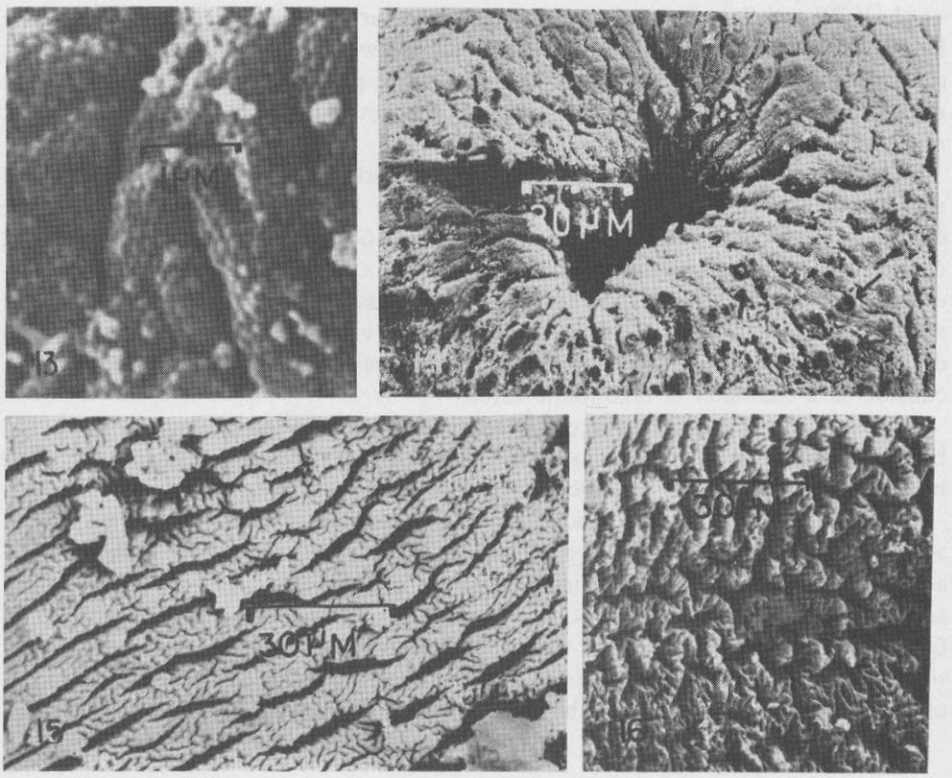


FIGS. 1–6. *Gastrothylax crumenifer*, scanning electron micrographs. 1. Entire worm (ventral view). 2. Dorsal surface of body showing wrinkles. 3. Portion of dorsal surface showing excretory pore (EP). 4. Tuberculated elevations on the tegument under a high resolution. 5. Protuberances at the rim of mouth (M) and near opening of the ventral pouch (VP). 6. High magnification of oral region showing protuberances (arrows) arranged in a regular pattern at rim of the mouth and in interior of buccal tube (BT).



FIGS. 7, 8. *Gastrothylax crumenifer*, scanning electron micrographs. 7. Lining of buccal tube under higher magnification showing "balloon-like" papillae (arrows). 8. Pattern of ridges and finger-like projections (FP) in acetabular area.

FIGS. 9–12. *Paramphistomum epiclitum*, scanning electron micrographs. 9. Entire worm in ventral view. 10. Tegument on dorsal surface of the body. 11. Ventral surface showing relatively more wrinkles. 12. Ventral surface under higher magnification showing tegumental folds.



FIGS. 13–16. *Paramphistomum epiclitum*, scanning electron micrographs. 13. Bead-like protuberances on tegument under higher resolution. 14. Circum-oral area showing patch-like elevations (arrows). 15. Craters and folds in acetabular tegument. 16. Intricate pattern of tegumental foldings towards interior of acetabular aperture.

DISCUSSION

A papillate or beaded tegumental surface, as observed in *G. crumenifer* and *P. epiclitum*, has also been reported for *Posthodiplostomum minimum* (see MITCHELL & CRANG, 1976), *Gorgoderina attenuata* (see NADAKAVUKAREN & NOLLEN, 1975) and *Phyllodistomum constomum* (see BAKKE & LIEN, 1978). It has been suggested that these raised protuberances increase the absorptive surface of the trematode (NADAKAVUKAREN & NOLLEN, 1975). A similar function has also been assigned to the spineless microtriches in the posterior part of the strobila of the caryophyllidean cestode, *Hunterella nodulosa* (see HAYUNGA & MACKIEWICZ, 1975) and also in the pseudophyllidean, *Diphyllobothrium* sp. (see ANDERSON, 1975). The papillate elevations on the general tegument of the amphistomes studied herein must be primarily involved in food absorption since anchorage seems to be entirely by the acetabulum and the anterior muscular thickening, i.e., pharynx. This is suggested because the tegument lacks spines. The spines have been observed in many trematode species, e.g. in *Fasciola hepatica* by BENNETT (1975 a, b), *Leucochloridium* sp. by BAKKE (1976 a, b), male *Schistosoma haematobium* by HICKS & NEWMAN (1977), *S. japonicum* by SAKAMOTO & ISHII (1977), *Urogonimus macrostomus* by BAKKE (1978), *Clonorchis sinensis* by FUJINO

et al. (1979) and *S. bovis* by KUNTZ *et al.* (1979). Further, in the *Schistosoma* spp. the female has a relatively smooth surface or possesses fewer spines than the male because it does not have to attach to the host's surface and the spined gynaecophoric canal of the male provides an effective means of clasping the female (MILLER *et al.*, 1972; KUNTZ *et al.*, 1976, 1979; SAKAMOTO & ISHII, 1977).

The occurrence of small papillae or patch-like structures studding the circum-oral area, as observed in the present study, has also been reported in *Megalodiscus temperatus* by (NOLLEN & NADAKAVUKAREN (1974), *G. attenuata* by NADAKAVUKAREN & NOLLEN (1975), *Urogonimus macrostomus* by BAKKE (1978) and *Phyllodistomum constomum* by BAKKE & LIEN (1978). These authors assigned a sensory function to these structures on the grounds of their location in the vicinity of the oral opening. The difference in the pattern of the arrangement of these patches or papillae could be considered a character of taxonomic value.

The papillae with a ballon-like appearance observed on the luminal surface of the buccal tube in *G. crumenifer* seem to be fluid-filled structures which suggests that the buccal lining may also be involved in food absorption.

The main difference in the topography of the tegumental surface of the two species studied lies in the pattern of the acetabular surface. Whereas in *P. epiclitum* the acetabular tegument is thrown into a pattern of deep folds and ridges, as in *M. temperatus* see (NOLLEN & NADAKAVUKARAN, 1974), and serves as a holdfast organ, in *G. crumenifer* the ridges and finger-like projections on the acetabular region seem to aid strong anchorage to the intervillous areas of the host's rumen with the added assistance of the group of small papillae which they bear. The latter may have a secretory function.

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