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# Scanning electron microscopic observations on the tegumental surfaces of two rumen flukes (Trematoda: Paramphistomata)

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**ABSTRACT**

The surface microtopography of *Calicophoron papillosum* (Stiles & Goldberger) and *C. calicophorum* (Fischoeder), inhabiting the rumen of sheep, has been studied by SEM. In *C. papillosum*, conspicuous transverse ridges encircle the mid-body, gradually lessening dorsally towards the posterior end. The general body surface is aspinose and smooth except for minute bead-like tubercles in the circumoral area. Two types of putative sensory structures—domed aciliate papillae and tegumental pits—are present. Their characteristic aggregation in certain regions and their distribution in other parts of the body is described. A unique pattern of ridges, confined only to the base of the genital papilla and the genital atrium, has been observed. In *C. calicophorum*, tegumental folds encircle the body, these are more numerous near the anterior end and fewer posteriorly. The oral area has numerous concentric folds with domed aciliate papillae; between or on the papillae are tegumental elevated pits from the centre of which a knob-like structure protrudes. These sensory structures occur to a lesser extent in the acetabular area.

## INTRODUCTION

The outer covering or the tegument of digenetic trematodes is a physiologically important interface in the host-parasite relationship (ERASMUS, 1970). With the recent use of scanning electron microscopy (SEM), a new approach has been possible elucidating the ultrastructure and surface features of this interface and to newly interpreting its functional morphology and significance in taxonomy. A few digenetic trematodes have been subject to SEM studies for visualization and description of their surface features (TANDON & MAITRA, 1981).

As part of our studies on the surface microtopography of the members of the digenetic family Paramphistomidae Fischoeder, 1901, the present paper illustrates the surface characteristics of *Calicophoron papillosum* (STILES & GOLDBERGER, 1910) NÄSMARK, 1937 and *C. calicophorum* (FISCHOEDER, 1901) NÄSMARK, 1937.

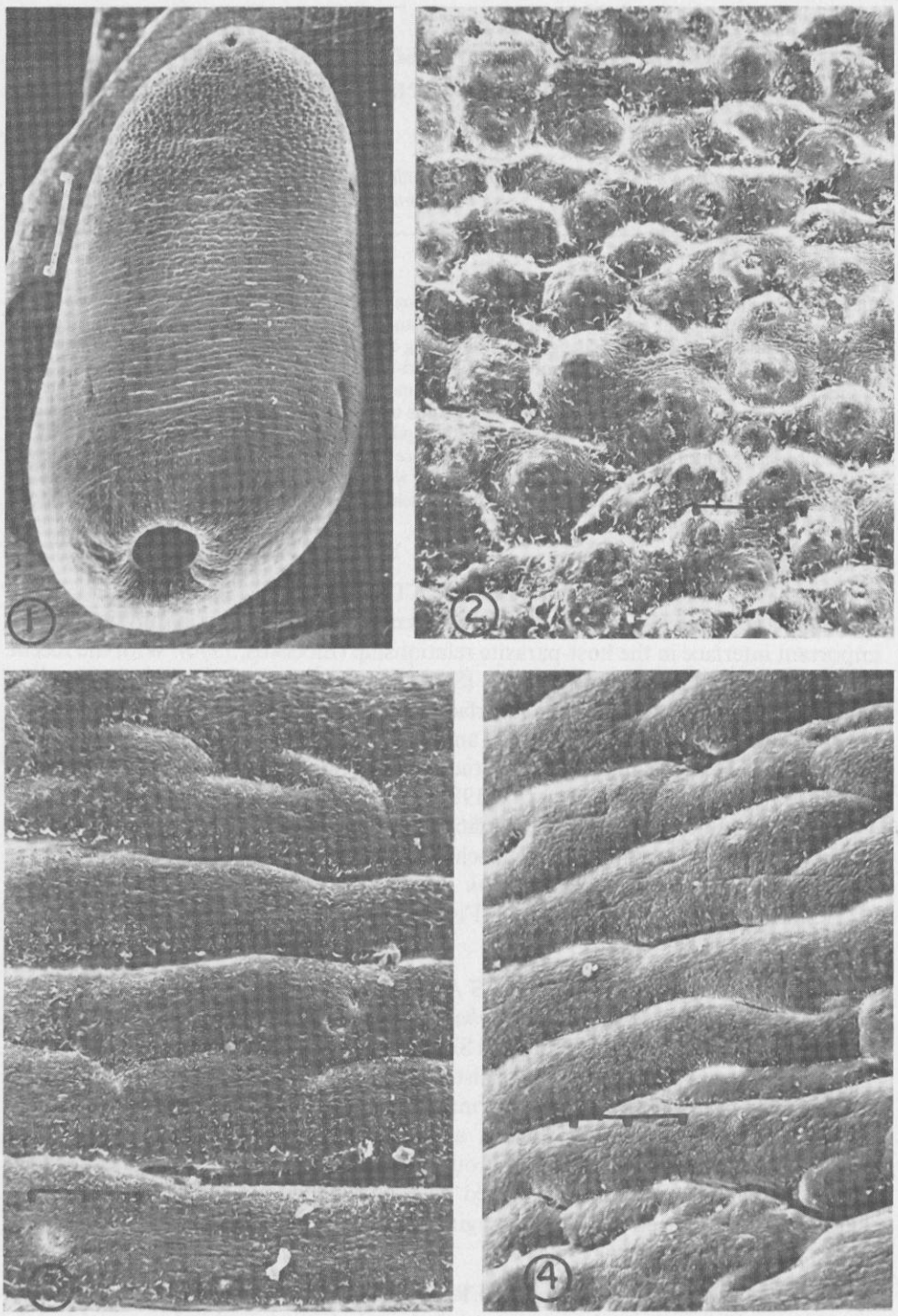
## MATERIALS AND METHODS

Living *C. papillosum* and *C. calicophorum* were collected from the rumen of sheep slaughtered at the abattoirs of Kulu and Simla (Himachal Pradesh, India), respectively. These species were identified on their histology, following NÄSMARK (1937). For SEM studies, after thorough washing in normal saline, the flukes (n = 10 for each species) were fixed in acetic-alcohol-formalin and preserved in 70% alcohol till further processing. They were dehydrated through an ascending series of ethyl alcohol to critical point drying with $CO_2$, coated with gold-palladium and observed with a Stereoscan 180 at electron voltage ranging from 15 to 20 kv.



## OBSERVATIONS

In *C. papillosum* the oral aperture and the acetabulum are both subterminal and the genital pore lies ventrally in the middle of the anterior third of the body (Fig. 1). The tegument of the posterior two thirds of the body shows transverse folds or a wrinkled appearance both ventrally and dorsally; the ventral side of the anterior third reveals an aggregation of dome-shaped papillae (Figs. 2, 3, 4). These papillae are very densely



FIGS. 1–4. *Calicophoron papillosum*, scanning electron microscopy: 1. Full worm (ventral view) showing the oral, genital and acetabular apertures. (Scale bar = 1 mm.) 2. A portion of the anterior ventral surface of the body showing densely arranged domed papillae. (One division of scale bar = 30 $\mu$m.) 3. Tegument in the midventral region of the body. (One division of scale bar = 30 $\mu$m.) 4. Dorsal surface of the body showing wrinkles. (One division of scale bar = 30 $\mu$m.)

arranged towards the oral end, gradually become less so posteriad and finally diminish and merge with the wrinkled surface pattern in the mid-body. The dorsal surface is more wrinkled anteriorly, less so in the middle and very little near the posterior end where it is almost smooth. No tubercles or spines were revealed on the general tegumental surface of the flukes even under higher resolutions, except in the area surrounding the oral aperture. Pits arranged in between the roughly concentric rings of tegumental folds were seen evenly scattered in the circumoral area (Fig. 5). In higher magnifications, these pits appeared to be deep holes in the tegument, oval or rounded in contour (Fig. 6) with the surrounding tegument clearly revealing tubercle-like minute elevations.

Around the acetabular aperture the tegument has folds irregularly radially directed (Fig. 1) which give the rim of the sucker a cobblestone-like appearance, with prominent domed papillae arranged singly or in groups of a few between the folds (Figs. 7, 8). Pits, similar to those in the circumoral area but fewer in number, were also observed in the acetabular zone. No folds or craters of tegument could be observed within the acetabular cavity.

The genital pore lies at the tip of a genital papilla which emerges from the genital atrium (Fig. 9). The tegument covering the dome-shaped protruding part of this papilla has domed papillae scattered over-all but near the base the surface microtopography changes to a pattern of filamentous, sinuous, narrow ridges which may anastomose in places (Fig. 10). This pattern is also continued to and apparent on the tegument lining the genital atrium (Fig. 11), the only difference being that the ridges on the genital atrial tegument are wider apart.

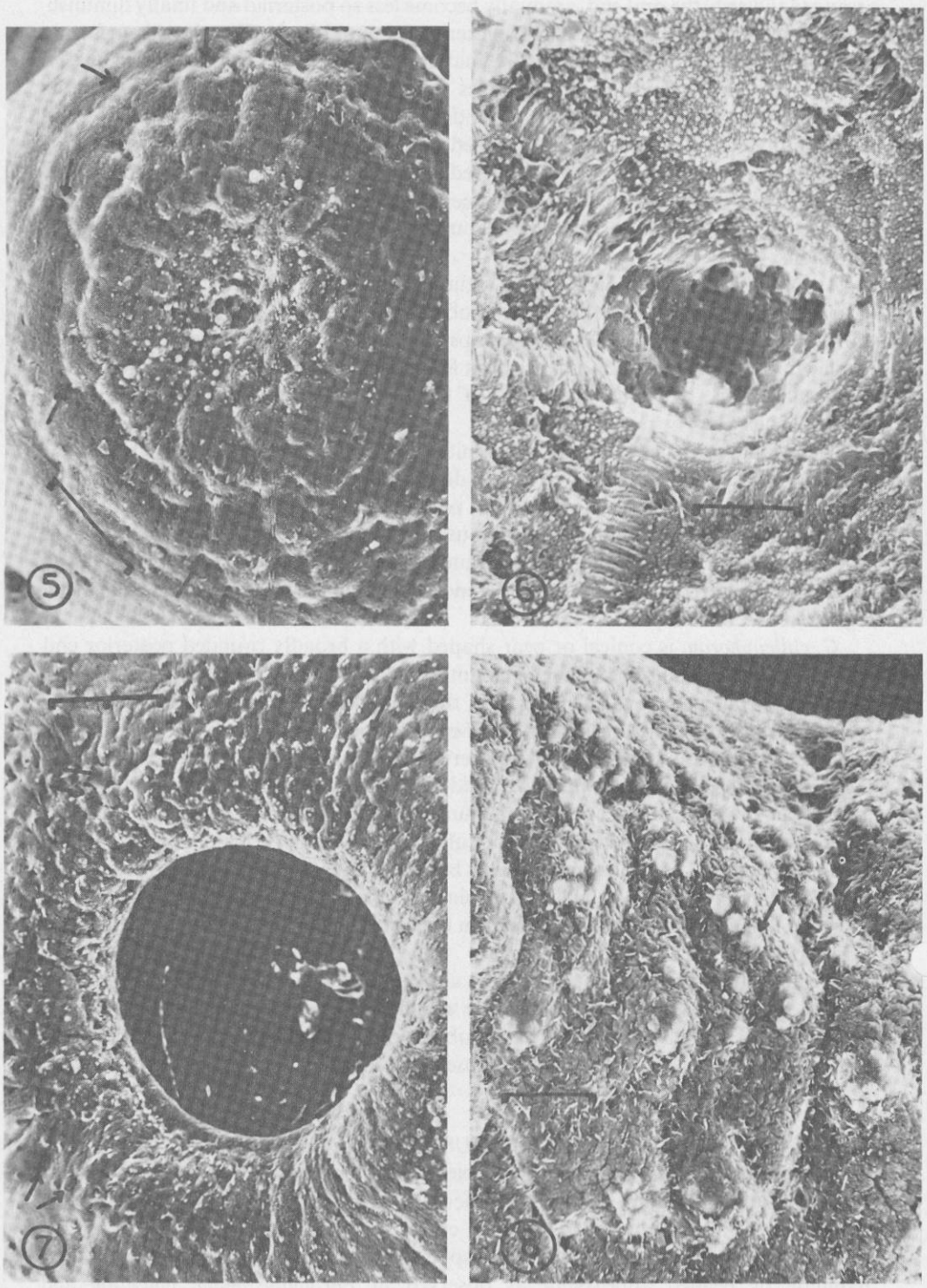
*C. calicophorum* is conical or pear-shaped with a broadly rounded posterior end with terminal acetabulum and narrower anterior end (Fig. 12). The tegument covering the body is transversely folded or ridged, the folds being closer and more numerous near the anterior end, decreasing in numbers posteriorly. In the posterior region, the tegumental folds are directed antero-posteriorly. The surface microtopography of the tegument both on the ventral and dorsal sides appears to be similar (Fig. 15). The area around the oral aperture is encircled by numerous concentric ridges or folds (Figs. 15, 16), between and on which are two types of structure, one type being domed papillae without apical cilia or knobs and the other being tegumental raised areas with a central pit-like depression through which irregular knob or spine-like structures protrude (Fig. 17). There is no apparent regularity in the distribution pattern of these papillae or raised pits.

Around the acetabular aperture there are radially arranged surface corrugations. On the lateral sides of the acetabulum the tegument is drawn into small and irregular ridges but ventro-subterminally it has a cobblestone-like appearance (Fig. 18). Knob-like protuberances (Fig. 19), similar to those at the anterior extremity, are scattered all around the acetabular area.

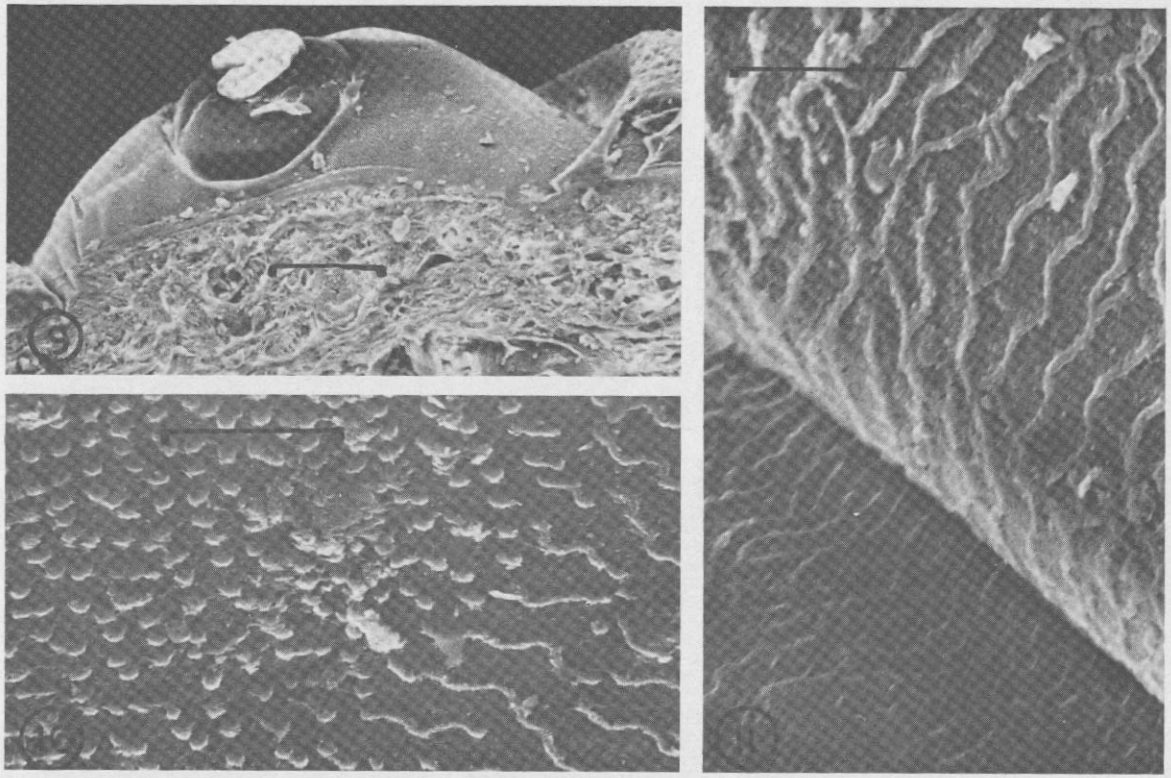
## DISCUSSION

The present SEM studies of these species revealed a number of differences in their surface microtopography which also differentiate them from the other paramphistomid rumen flukes (EDUARDO, 1980a, b, c; TANDON & MAITRA, 1981).

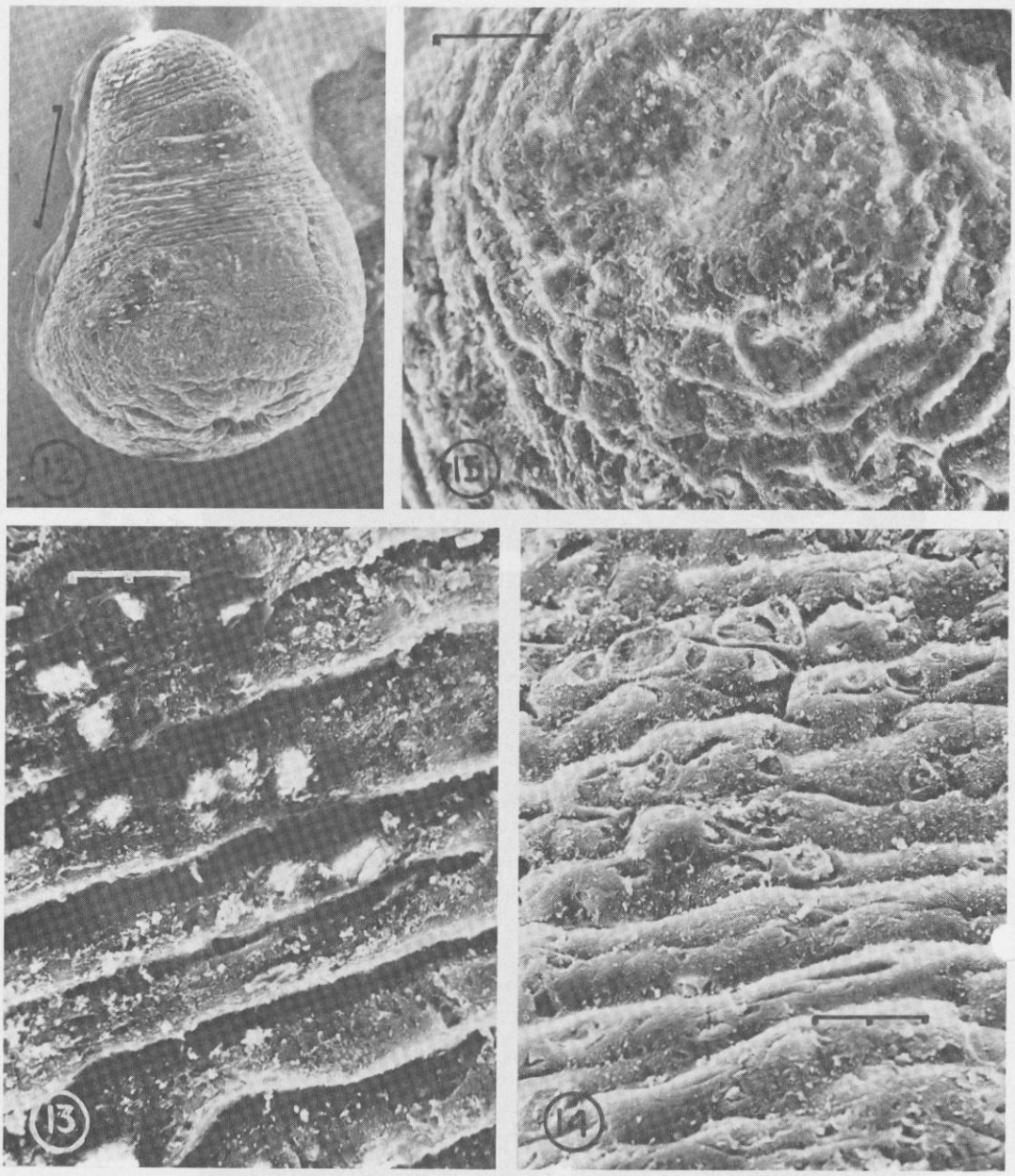
The tegument in both *Calicophoron* spp. studied in this work shows no general papillate or beaded appearance as observed in *Paramphistomum epiclitum* and *Gastrothylax crumenifer* (see TANDON & MAITRA, 1981). In *C. papillosum* there is characteristic dense aggregation of domed papillae on the anterior third of the ventral side of the tegument and these are also present at other sites on the body. Domed papillae, smooth, spined or with apical cilia, commonly occur in trematodes and it has



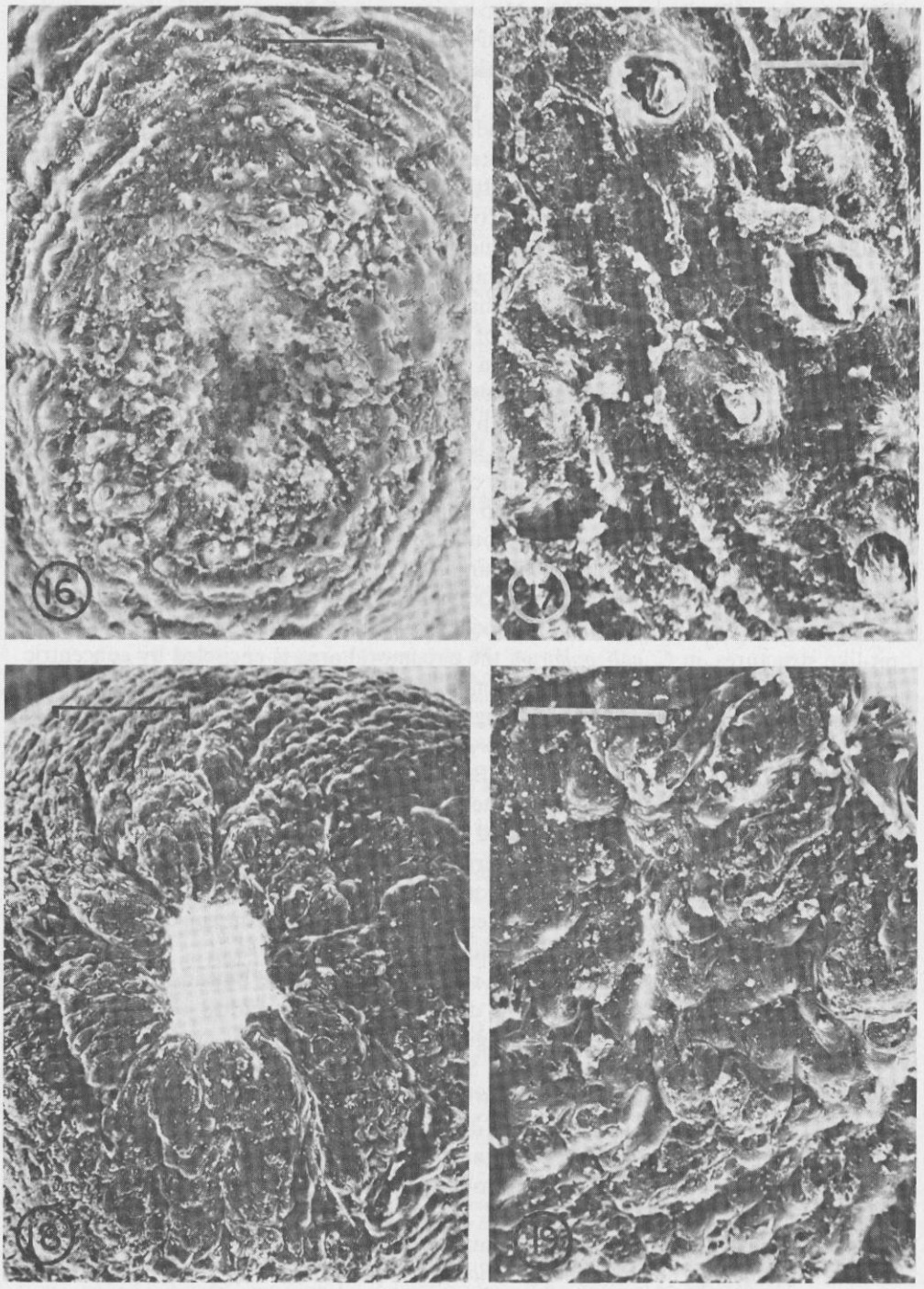
FIGS. 5–8. *Calicophoron papillosum*, scanning electron microscopy: 5. Mouth opening (front view) encircled by tegumental folds with pits (arrows). (Scale bar = 100 $\mu$m.) 6. A single pit (under high magnification) in the finely tuberculated tegument. (One division of scale bar = 3 $\mu$m.) 7. Acetabular aperture showing aggregation of papillae and pits (arrows) around it. (One division of scale bar = 100 $\mu$m.) 8. A portion of the acetabular rim under higher magnification showing domed papillae (arrows). (Scale bar = 30 $\mu$m.)



FIGS. 9–11. *Calicophoron papillosum*, scanning electron microscopy: 9. A sagittal section through the genital region revealing the genital pore, genital papilla and genital atrium. (Scale bar = 100 $\mu$m.) 10. Tegument on the basal region of the genital papilla showing domed papillae and ridges. (Scale bar = 10 $\mu$m.) 11. Pattern of ridges at the base of the genital papilla and inner border region of the genital atrium. (Scale bar = 10 $\mu$m.)



FIGS. 12–15. *Calicophoron calicophorum*, scanning electron microscopy: 12. Entire worm (ventral view). (Scale bar = 1 mm.) 13. Ventral surface of the body showing tegumental folds. (One division of scale bar = 30 $\mu$m.) 14. Tegument on the dorsal surface of the body. (One division of scale bar = 30 $\mu$m.) 15. Mouth opening surrounded by tegumental folds. (Scale bar = 100 $\mu$m.)




FIGS. 16–19. *Calicophoron calicophorum*, scanning electron microscopy: 16. Circumoral area (front view) showing concentric tegumental folds. (Scale bar = 100 $\mu$m.) 17. Tegumental elevated pits with spine-like structure inside them. (Scale bar = 30 $\mu$m.) 18. Surface topography of the tegument in the acetabular region. (Scale bar = 300 $\mu$m.) 19. A portion of the acetabular rim under higher resolution. (Scale bar = 100 $\mu$m.)

been suggested that they have a sensory function (SILK *et al.*, 1970; MILLER *et al.*, 1972; MORRIS, 1973; BENNETT, 1975a; NADAKAVUKAREN & NOLLEN, 1975; KUNTZ *et al.*, 1976; SAKAMOTO & ISHII, 1977; BAKKE, 1978; BAKKE & LIEN, 1978). Amongst the rumen amphistomes, in *Bilatorchis papillogenitalis* papillae arranged in concentric rings have been observed in the oral area, each papilla having 14 to 22 apical knob-like projections; around the genital pore and the acetabulum smooth, club-shaped and dome-like papillae, respectively, are present (EDUARDO, 1980a). In *Orthocoelium indonesiense* and *Leiperocotyle okapi*, only the anterior quarter or third of the body is papillate. In the latter, the papillae are covered with hair-like projections (EDUARDO, 1980b, c). These papillae, when present near the mouth could be involved in contact reception during food detection or feeding; those found on the general body surface might function in recording pressure changes as the tegument stretches (BENNETT, 1975a). Domed papillae in the region of the genital pore, which occur all over the surface of the protruding genital papilla of *C. papillosum*, probably have a specific sensory function during the pairing for sexual reproduction (BAKKE, 1976a, b). Similar aciliate papillae have also been reported by BAKKE (1976a) in the genital pore region of *Leucochloridium* sp. and also appear to be present in *O. indonesiense* (see EDUARDO, 1980b, Plate I). In the present studies, no consistency in the number or arrangement of papillae in the oral or acetabular region could be demonstrated. Their number and arrangement on the suckers has been found to be constant in adult *Phyllodistomum conostomum* (see BAKKE & LIEN, 1978) and also during the developmental stages of some other *Phyllodistomum* spp. (see GOODCHILD, 1943; THOMAS, 1958).

Whereas in *C. papillosum* the oral opening is surrounded by tegumental folds and pit-like structures, in *C. calicophorum* the circumoral area is encircled by concentric ridges studded with two types of tegumental sensory structures. Ridges encircling the anterior end are reported in many digeneans, e.g., *Megalodiscus temperatus* (see NOLLEN & NADAKAVUKAREN, 1974), *Gorgoderina attenuata* (see NADAKAVUKAREN & NOLLEN, 1975), *Fasciola hepatica* (see BENNETT, 1975a, b) and *P. conostomum* (see BAKKE & LIEN, 1978) but these ridges lack the studded pattern of papillae seen in *C. calicophorum*. Pits, similar to those found in the circumoral area of *C. papillosum*, also occur in the ventral unspined area posterior to the oral sucker in *Leucochloridium* sp. (see BAKKE, 1976b) but in the latter they are not arranged concentrically. Tegumental depressions or pits have also been reported by BAKKE (1978) on the rim of, and close to, both suckers in *Urogonimus macrostomus*.

As in *C. calicophorum*, two types of raised structure—one button-like patches of tissue and the other appearing as a depression with a central small knob-like structure—have been observed in the tegument of the oral sucker in *G. attenuata* (see NADAKAVUKAREN & NOLLEN, 1975). In *Schistosoma mansoni* pit-like structures with 'integumental papillae-like elevation with central protuberance' have also been observed (RACE *et al.*, 1971; MILLER *et al.*, 1972).

A pattern of narrow ridges, which anastomose infrequently, covering the basal portion of the genital papilla and continuing on the surface of the depression of the genital atrium, is found to be unique to *C. papillosum*. Anastomosing small ridges have been described in studies of the surface microtopography of the cirrus in *Leucochloridium* sp. and *Urogonimus macrostomus* by BAKKE (1976a, 1977) but the functional significance is not yet known.

As the general body surface in *C. papillosum* and *C. calicophorum* lacks spines, the radial folds of the acetabular tegument, probably reflecting the strong radial musculature of the sucker (NOLLEN & NADAKAVUKAREN, 1974), may function in drawing the rumen wall tissue of the host into the acetabular cavity and in providing a strong hold for the parasite.

In spite of the dissimilarity in type, distribution and arrangement of tegumental papillae in the various amphistome species of ruminants so far studied, the general occurrence of papillae in all of them and the unarmed dorsal and ventral surfaces are indicative of similar adaptation to a similar microenvironment, i.e., the rumen (BAKKE, 1978).

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